**Supplementary material**

**Appendix A**

The following appendix displays outputs from different analyses characterising the environmental and biological properties of the study region and the ‘Ecohydrological Classification’ (EHC) groups (summarized in Table 1 of the manuscript). Firstly, the water quality of the studied headwater streams are described and physico-chemical measurements are presented. Secondly, the statistical variation accounted for by the EHC groups on macroinvertebrate community compositions was examined in relation to alternative hydrological groupings and clustering techniques. Finally, the predominance of specialist ‘Winterbourne’ taxa (reported within Table 2 of the manuscript) across alternative EHC groups is examined.

The chalk headwater streams examined in this study typically possess comparable water qualities due to strong geological controls and similar land uses, whereby all rivers are predominantly surrounded by arable agriculture. However, one of the studied rivers (Nine Mile River) flows through areas of unimproved grassland due to the presence of a military range within the catchment. All sites are typically characterized by high oxygen levels and alkalinity, low phosphate levels, but higher nitrate levels which can be predominantly attributed to surrounding agriculture. Table S1 summarizes some of the key water quality parameters measured along six of the streams sampled within this study. Measurements were taken from sites positioned furthest upstream by the Environment Agency (the statutory environment regulator within the United Kingdom) and averaged (where observations were available) across the study period.

**Table S1** – Average water quality measurements from six headwater streams studied between 2002-2007.

|  |  |
| --- | --- |
| **Water quality parameter** | **River** |
| **Wylye** | **Ebble** | **Fonthill Brook** | **Till** | **Nine Mile River** | **Bourne** |
| Ammonia (mgN/l) | 0.041 | 0.038 | 0.025 | 0.020 | 0.016 | 0.019 |
| Dissolved oxygen (% saturation) | 105.59 | 97.71 | 96.54 | 102.65 | 93.91 | 104.33 |
| Nitrates (mg/l) | 28.47 | 30.55 | 23.92 | 23.80 | 20.92 | 30.17 |
| Phosphates (mg/l) | 0.095 | 0.045 | 0.030 | 0.030 | 0.015 | 0.165 |

In addition, the following environmental characteristics were recorded for each sample analysed within this study: (i) survey altitude; (ii) distance from source; (iii) channel slope; (iv) channel width; (v) average depth (3 measurements were taken across each channel cross-section); (vi) water conductivity (some missing values for <6% of samples) and (vii) substrate composition. Water conductivity was measured using a ‘Hanna HI 98311’ digital meter and the substrate composition was measured by surveyors visually assessing the proportion of different sediment groups. Differences in these environmental characteristics between EHC groups is displayed in Table S2.

**Table S2** – Mean average (±1 standard deviation) values of various environmental parameters across each EHC group.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **EHC group** | **Distance from source (km)** | **Channel slope (%)** | **Altitude (m asl)** | **Width (m)** | **Depth (m)** | **Conductivity (µS cm-1)** |
| P | 11.04 (10.51) | 4.72 (5.21) | 80.36 (24.29) | 6.18 (2.21) | 0.27 (0.14) | 205.14 (267.41) |
| T | 8.64 (9.7) | 3.87 (3.55) | 75 (14.05) | 5.8 (3.15) | 0.34 (0.19) | 338.36 (269.97) |
| S | 4.64 (8.76) | 6.69 (3.33) | 99.25 (19.67) | 3.99 (1.83) | 0.21 (0.15) | 428.15 (258.42) |
| W | 3.47 (1.74) | 2.88 (1.5) | 85.69 (10.41) | 4.35 (2.2) | 0.33 (0.15) | 504 (153.75) |
| I | 8.07 (9.92) | 3.96 (2.22) | 101.93 (21.25) | 3.29 (1.42) | 0.28 (0.14) | 343.05 (275.27) |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **EHC group** | **Bedrock (%)** | **Cobbles (%)** | **Pebbles/ Gravels (%)** | **Sand (%)** | **Silt (%)** | **Clay (%)** |
| P | 1.84 (13.36) | 10.84 (17.11) | 63.29 (23.38) | 12.68 (11.31) | 11.36 (17.11) | 0.00 (0.00) |
| T | 0.00 (0.00) | 4.00 (5.24) | 64.00 (20.52) | 13.08 (12.47) | 18.77 (17.91) | 0.15 (0.55) |
| S | 0.00 (0.00) | 2.95 (3.07) | 73.40 (20.78) | 9.75 (7.82) | 12.65 (16.87) | 1.25 (5.59) |
| W | 0.00 (0.00) | 7.9 (13.40) | 58.48 (22.94) | 14.6 (11.80) | 19.02 (15.42) | 0.00 (0.00) |
| I | 2.50 (15.81) | 2.65 (5.30) | 35.63 (32.48) | 6.98 (8.90) | 50.25 (37.50) | 2 (12.65) |

Table 2 (continued – Substrate composition)

The multivariate composition of the family- and species/genus-level community abundances were analysed in relation to various environmental groupings and clustering techniques. The EHC groups were derived from ‘Two-Way Indicator Species Analysis’ (TWINSPAN – Hill, 1979), which was performed on the family-level community dataset examined within this research. Five clusters were subsequently used within this research (see Table 1 within the manuscript) and these groups were assigned to all samples within both the family- and species-level datasets. The antecedent flow duration was grouped into 2, 3, 4 and 6 month intervals, while the distance from the perennial source and groundwater abstraction influences were grouped into categories based on the minimum and maximum values, as well as the inter-quartile range (minimum value – 25th percentile; 25th percentile – 50th percentile; 50th percentile – 75th percentile; 75th percentile – maximum value). Two clustering techniques were also used: Hierarchal cluster analysis and K-means cluster analysis (see Oksanen, 2016). Both were performed in R studio (version 3.3.1), with the former being conducted *via* the ‘*hclust*’ function within the ‘Vegan’ package (Oksanen et al., 2017) on a Bray-Curtis dissimilarity matrix and the latter being calculated using the ‘kmeans’ function within the base package. The optimal number of clusters were ascertained from visually assessing gap (obtained from the ‘clusGap’ function within ‘cluster’ package – Maechler et al., 2017) and sum of squares scree plots (for k-means analysis). In all cases, the optimal number of clusters was deemed to be 2, but to improve the robustness of this analysis 2 through to 6 clusters were established for both techniques on both family- and species-level datasets. The significance and statistical variation accounted for by all independent variables was assessed *via* a ‘Permutational Multivariate Analysis of Variance’ (PERMANOVA), which was conducted using the ‘*adonis*’ function in Vegan. The results of these analyses are displayed in Table S3 and highlight that macroinvertebrate community compositions differed significantly between EHC groups and accounted for the highest amount of statistical variation (denoted by the r2 value) compared to all other clustering and grouping techniques.

**Table S3.** Results from PERMANOVA testing the significance of different hydrological controls and clustering techniques on the multivariate composition of family- and species-level community abundance data. NS = non-significant; \*\*\*p ≤ 0.001; \*\*p ≤ 0.01;\*p ≤ 0.05.

|  |  |  |
| --- | --- | --- |
| **Grouping variable** | **Family** | **Species / genus** |
| **r2** | **F** | ***p-value*** | **r2** | **F** | ***p-value*** |
| TWINSPAN | 0.24 | 13.11 | *0.001\*\*\** | 0.24 | 8.72 | *0.001\*\*\** |
| 2-month antecedent flow duration intervals | 0.18 | 7.40 | *0.001\*\*\** | 0.19 | 5.10 | *0.001\*\*\** |
| 3-month antecedent flow duration intervals | 0.16 | 10.32 | *0.001\*\*\** | 0.16 | 7.27 | *0.001\*\*\** |
| 4-month antecedent flow duration intervals | 0.16 | 15.73 | *0.001\*\*\** | 0.15 | 10.28 | *0.001\*\*\** |
| 6-month antecedent flow duration intervals | 0.13 | 24.32 | *0.001\*\*\** | 0.13 | 16.74 | *0.001\*\*\** |
| Distance from perennial source divisions | 0.19 | 9.76 | *0.001\*\*\** | 0.18 | 6.00 | *0.001\*\*\** |
| Mean abstraction when flowing divisions | 0.04 | 1.62 | *0.015\** | 0.04 | 1.22 | *0.147*(NS) |
| Loss of modelled flows divisions | 0.02 | 1.50 | *0.059*(NS) | 0.02 | 1.31 | *0.146*(NS) |
| Hierarchal 2 clusters | 0.01 | 2.13 | *0.012\** | 0.02 | 1.77 | *0.008\*\** |
| Hierarchal 3 clusters | 0.01 | 2.03 | *0.001\*\*\** | 0.03 | 3.75 | *0.001\*\*\** |
| Hierarchal 4 clusters | 0.18 | 36.72 | *0.001\*\*\** | 0.03 | 3.06 | *0.002\*\** |
| Hierarchal 5 clusters | 0.15 | 30.74 | *0.001\*\*\** | 0.11 | 14.52 | *0.001\*\*\** |
| Hierarchal 6 clusters | 0.13 | 24.73 | *0.001\*\*\** | 0.14 | 19.15 | *0.001\*\*\** |
| K-means 2 clusters | 0.13 | 25.07 | *0.001\*\*\** | 0.06 | 7.92 | *0.001\*\*\** |
| K-means 3 clusters | 0.13 | 25.32 | *0.001\*\*\** | 0.05 | 5.41 | *0.001\*\*\** |
| K-means 4 clusters | 0.05 | 8.53 | *0.001\*\*\** | 0.05 | 5.83 | *0.001\*\*\** |
| K-means 5 clusters | 0.16 | 32.57 | *0.001\*\*\** | 0.09 | 11.14 | *0.001\*\*\** |
| K-means 6 clusters | 0.12 | 23.99 | *0.001\*\*\** | 0.11 | 13.38 | *0.001\*\*\** |

The prevalence of ‘Winterbourne’ specialists across different EHC groups was found to vary between taxa. For example, some species (notably those of higher conservation values) were rarely sampled from samples clustered within alternative EHC groups, such as ‘*Paraleptophlebia werneri*’ (Ephemeroptera) and ‘*Aplexa hypnorum*’ (Gastropoda). Other taxa were more evenly distributed across EHC groups, such as ‘*Radix balthica*’ (Gastropoda) and ‘*Galba truncata*’ (Gastropoda). These results have been summarized in Table S4.

**Table S4.** The percentage occurrence of different winterbourne specialists across EHC groups.

|  |  |  |  |
| --- | --- | --- | --- |
| **Taxonomic order** | ***Genus/species*** | **Conservation score** | **EHC groups** |
| **P** | **T** | **S** | **W** | **I** |
| Ephemeroptera | *Paraleptophlebia werneri* | 8 | 0.0 | 0.0 | 6.7 | 86.7 | 6.7 |
| Trichoptera | *Limnephilus bipunctatus* | 5 | 0.0 | 0.0 | 7.7 | 84.6 | 7.7 |
| Plecoptera | *Nemoura cinerea / lacustris* | 1a | 4.3 | 0.0 | 12.8 | 66.0 | 17.0 |
| Plecoptera | *Isoperla grammatica* | 2 | 17.6 | 5.9 | 2.9 | 70.6 | 2.9 |
| Gastropoda | *Aplexa hypnorum* | 5 | 8.3 | 0.0 | 0.0 | 91.7 | 0.0 |
| Coleopetera | *Dryops sp.* | NAb | 14.3 | 0.0 | 0.0 | 85.7 | 0.0 |
| Gastropoda | *Anisus leucostoma* | 5 | 7.1 | 1.8 | 8.9 | 53.6 | 28.6 |
| Coleopetera | *Agabus sp. / Ilybius sp.* | NAb | 5.5 | 5.5 | 12.7 | 40.0 | 36.4 |
| Amphipoda | *Niphragus aquilex* | 6 | 15.2 | 3.0 | 6.1 | 60.6 | 15.2 |
| Trichoptera | *Limnephilus vittatus* | 3 | 0.0 | 9.1 | 0.0 | 90.9 | 0.0 |
| Gastropoda | *Radix balthica* | 1 | 34.8 | 3.0 | 12.1 | 42.4 | 7.6 |
| Coleopetera | *Hydroporus sp.* | NAb | 0.0 | 0.0 | 0.0 | 70.0 | 30.0 |
| Gastropoda | *Galba truncata* | 3 | 2.7 | 0.0 | 10.8 | 48.6 | 37.8 |

a *Nemoura lacustris* has been assigned an initial conservation score of 7 following its recent discovery within the UK (House and Tapia, 2014).

b High variability of conservation scores within each of these genera.

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**Appendix B**

This appendix summarises all taxa recorded sampled across the study period (2002-2007) from all sites across the Hampshire Avon (Southern England, UK).

|  |  |  |
| --- | --- | --- |
| **CNIDARIA** | **INSECTA** | **Trichoptera (continued)** |
| Hydra sp. | **Ephemeroptera** | *Plectrocnemia conspersa* (Curtis, 1834) |
| **PLATYHELMINTHES** | Baetidae | *Polycentropus flavomaculatus* (Pictet, 1834) |
| **Microturbelleria** | *Rhithrogena semicolorata* (Curtis, 1834) | *Hydropsyche angustipennis* (Curtis, 1834) |
| **Seriata** | *Heptagenia sulphurea* (Müller, 1776) | *Hydropsyche pellucidula* (Curtis, 1834) |
| *Planaria torva* (Müller, 1774) | *Ecdyonurus* sp. | *Hydropsyche siltalai* (Döhler, 1963) |
| *Polycelis feline* (Dalyell, 1814) | *Leptophlebia* sp. | *Phryganea bipunctata* (Retzius, 1783) |
| *Polycelis nigra/tenius* | *Paraleptophlebia submarginata* (Stephens, 1835) | *Crunoecia irrorata* (Curtis, 1834) |
| *Phagocata vitta* (Duges, 1830) | *Paraleptophlebia werneri* (Ulmer, 1919) | *Lasiocephala basalis/ Lepidostoma hirtum* |
| *Dugesia lugubris /polychroa* | *Habrophlebia fusca* (Curtis, 1834) | *Drusus annulatusa* (Stephens, 1837) |
| *Dugesia tigrine* (Girard, 1850) | *Ephemera danica* (Müller, 1764) | *Halesus digitatusa* (Schrank, 1781) |
| *Bdellocephala punctata* (Pallas, 1774) | *Serratella ignita* (Poda, 1761) | *Halesus radiatusa* (Curtis, 1834) |
| *Dendrocoelum lacteum* (O.F.Müller, 1774) | *Caenis* sp. | *Melampophylax mucoreusa* (Hagen, 1861) |
| **ANNELIDA** | **Plecoptera** | *Micropterna sequaxa* (McLachlan, 1875) |
| **Oligochaeta** | *Nemurella picteti* (Klapálek, 1900) | *Potamophylax cingulatusa* (Stephens, 1837) |
| **Rhynchobdella** | *Nemoura avicularis* (Morton, 1894) | *Potamophylax latipennisa* (Curtis, 1834) |
| *Piscicola geometra* (Linnaeus, 1761) | *Nemoura cambrica* (Stephens, 1836) | *Potamophylax rotundipennisa* (Brauer, 1857) |
| *Theromyzon tessulatum* (O.F.Müller, 1774) | *Nemoura cinerea / lacustris* | *Stenophylax permistusa* (McLachlan, 1895) |
| *Glossiphonia complanata* (Linnaeus, 1758) | *Nemoura dubitans* (Morton, 1894) | *Chaetopteryx villosaa* (Fabricius, 1798) |
| *Helobdella stagnalis* (Linnaeus, 1758) | *Leuctra* sp. | *Anabolia nervosaa* (Curtis, 1834) |
| *Haemopsis sanguisuga* (Linnaeus, 1758) | *Siphonoperla torrentium* (Pictet, 1841) | *Glyphotaelius pellucidusa* (Retzius, 1783) |
| *Erpobdella octoculata* (Linnaeus, 1758) | *Isoperla grammatica* (Poda, 1761) | *Limnephilus auriculaa* (Curtis, 1834) |
| *Erpobdella testacea* (Savigny, 1812) | **Odonata** | *Limnephilus bipunctatusa* (Curtis, 1834) |
| *Trocheta subviridis* (Dutrochet, 1817) | Coenagrionidae | *Limnephilus centralisa* (Curtis, 1834) |
| **NEMERTEA** | *Calopteryx splendens* (Harris, 1782) | *Limnephilus lunatusa* (Curtis, 1834) |
| **NEMATODA** | Libellulidae | *Limnephilus marmoratusa* (Curtis, 1834) |
| **MOLLUSCA** | **Hemiptera** | *Limnephilus rhombicusa* (Linnaeus, 1758) |
| **Gastropoda** | *Velia caprai* (Tamanini, 1947) | *Limnephilus vittatusa* (Fabricius, 1798) |
| *Theodoxus fluviatilis* (Linnaeus, 1758) | *Notonecta maculata* (Fabricius, 1794) | *Goera pilosa* (Fabricius, 1775) |
| *Valvata* sp. | *Micronecta* sp. | *Silo nigricornis* (Pictet, 1834) |
| *Bithynia tentaculata* (Linnaeus, 1758) | *Sigara dorsalis* (Leach, 1817) | *Silo pallipes* (Fabricius, 1781) |
| *Potamopyrgus antipodarum* (J.E.Gray, 1843) | *Sigara nigrolineata* (Fieber, 1848) | *Beraea* sp. |
| *Aplexa hypnorum* (Linnaeus, 1758) | *Sigara venusta* (Douglas & Scott, 1869) | *Beraeodes minutus* (Linnaeus, 1761) |
| *Physa fontinalis* (Linnaeus, 1758) | **Hymenoptera** | *Sericostoma personatum* (Spence, 1826) |
| *Physella acuta* (Draparnaud, 1805) | *Agriotypus armatus* (Curtis, 1832) | *Odontocerum albicorne* (Scopoli, 1763) |
| *Lymnaea stagnalisa* (Linnaeus, 1758) | **Coleoptera** | *Molanna angustata* (Curtis, 1834) |
| *Galba truncatulaa* (O.F. Müller, 1774) | *Brychius elevates* (Panzer, 1793) | *Athripsodes* sp. |
| *Stagnicola palustrisa* (O.F. Müller, 1774) | *Haliplus* sp. | *Ceraclea* sp. |
| *Radix balthicaa* (Linnaeus, 1758) | *Hygrotus confluens* (Fabricius, 1787) | *Mystacides* sp. |
| *Planorbis carinatus* (O.F. Müller, 1774) | *Hydroporinae* sp. | *Adicella reducta* (McLachlan, 1865) |
| *Anisus leucostoma* (Millet, 1813) | *Hydroporus* sp. | *Triaenodes bicolor*/ *Ylodes* sp. |
| *Anisus vortex* (Linnaeus, 1758) | *Nebrioporus* sp. | *Oecetis testacea* (Curtis, 1834) |
| *Bathyomphalus contortus* (Linnaeus, 1758) | *Oreodytes sanmarkii* (C.R. Sahlberg, 1826) | *Apatania muliebris* (McLachlan, 1866) |
| *Gyraulus albus* (O.F. Müller, 1774) | *Platambus maculatus* (Linnaeus, 1758) | Pyralidae |
| *Gyraulus crista* (Linnaeus, 1758) | *Colymbetinae* sp | **Lepidoptera** |
| *Hippeutis complanatus* (Linnaeus, 1758) | *Agabus* sp. / *Ilybius* sp. | **Diptera** |
| *Ancylus fluviatilis* (O.F. Müller, 1774) | *Dytiscus* sp. | Tipuloidae |
| *Succinea* *putris* (Linnaeus, 1758) | *Orectochilus villosus* (O.F. Müller, 1776) | *Tipula* sp. |
| *Zonitoides nitidus* (O.F. Müller, 1774) | *Helophorus* sp. | *Nephrotoma* sp. |
| **Bivalvia** | *Hygrotus confluens* (Fabricius, 1787) | Limoniidae |
| *Pisidium* sp. | Hydrophilidae | *Dicranota* sp. |
| **HYDRACARINA** | *Hydraena rufipes* (Curtis, 1830) | *Tricyphona* sp. |
| **ORIBATEI** | *Elodes* sp. | Psychodidae |
| **CLADOCERA** | *Dryops* sp | *Ptcychoptera* sp. |
| **ARTHROPODA** | *Elmis aenea* (P.W.J. Müller, 1806) | *Dixa* sp. |
| **Ostracoda** | *Limnius volckmari* (Panzer, 1793) | Ceratopogonidae |
| **Copepoda** | *Oulimnius* sp. | Simuliidae |
| **Isopoda** | *Riolus subviolaceus* (P.W.J. Müller, 1817) | Chironomidae |
| *Asellus aquaticus* (Linnaeus, 1758) | Curculionidae | Stratiomyidae |
| *Asellus meridianus* (Racovitza, 1919) | **Megaloptera** | Dolichopodidae/ Rhagionidae |
| **Amphipoda** | *Sialis lutaria* (Linnaeus, 1758) | *Chrysophilus* sp. |
| *Crangonyx pseudogracilis* (Bousfield, 1958) | **Trichoptera** | Tabanus sp. |
| *Gammarus pulex (*Linnaeus, 1758) | *Rhyacophila* sp. | Empididae |
| *Niphargus fontanus* (Bate, 1859) | *Agapetus* sp. | Sciomyzidae |
| *Niphargus aquilex* (Bate, 1859) | *Allotrichia pallicornis* (Eaton, 1873) | Syrphidae |
| **Collembola** | *Hydroptila* sp. | Ephydridae |
| Isotomidae | *Oxyethira* sp. | Limnophora riparia (Fallén, 1824) |
|  | *Ithytrichia* sp. | Lonchopteridae |
|  | *Lype* sp. | Fanniidae |
|  | *Psychomyia pusilla* (Fabricius, 1781) | Scatopsidae |
|  | *Tinodes unicolor* (Pictet, 1834) | Bibionidae |
|  | *Tinodes waeneri* (Linnaeus, 1758) | Sarcophagidae |
|  |  | Agryotipidae |

a The taxon was included within the presence/absence species/genus dataset, but was aggregated to a coarser taxonomic resolution for the species/genus community abundance dataset.