

**Benthic Macroinvertebrate Assemblage in relation to
Environmental and Hydrological Variables in a Chalk
Stream, Upper Colne Catchment**

By

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Abstract

Chalk streams are a rare and valuable river type that are mostly confined to southeast England. They are known for their clear waters flowing over gravel beds and array of flora and fauna, including specially adapted macroinvertebrates that can survive periods of drought. This study aimed to determine the benthic macroinvertebrate community composition in the upper course of Colne Catchment in Hertfordshire and to establish its hydrological regime and water quality status. Previous studies have shown that year-round monitoring is important and that the diversity of macroinvertebrates in chalk streams is related to a number of factors. However, many of these have not been researched in a cohesive manner. Therefore, this study identified the influence the chalk stream environment had on the family richness and diversity of macroinvertebrate taxa. The study was carried out on four sites in the Upper Colne catchment in Hertfordshire over two annual seasons between April 2023 to February 2025. There were significant differences in the macroinvertebrate community composition among the sites, which were indicative of certain environmental conditions. For example, the Ver site, which had the most consistently high flow velocity had greater prevalence of *Gammaridae*, whereas upstream sites were dominated by *Limnephilidae* which preferred slower flowing waters. All sites on in the Upper Colne Catchment experienced instances where the water nutrient levels (phosphate, ammonium, nitrates) exceeded the recommended WFD guidelines for surface waters, which suggested that the sites could be at risk from eutrophication, coupled with low flows and high biological oxygen demand levels. In addition, the biometric indices indicated that the sites were mostly of poor environmental quality and associated with low flows. Whilst chalk streams are expected to have lower stream power than other river types, the anthropogenic causes of impeded flow have altered their hydrological functioning. Therefore, with the combination of future warming from climate change and the neglect from authorities and stakeholders, the upper courses of chalk streams could be at a greater risk of negatively being impacted by increasing nutrient levels, pollution events and groundwater abstraction, all of which would alter the macroinvertebrate community and damage the fragile ecosystem along the whole river course. Long-term monitoring of the aquatic macroinvertebrates and environmental parameters will be crucial in preserving the chalk streams ecosystem.

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Chapter 1: Introduction to Chalk Streams and Literature Review

1.1. The Hydrology of Chalk Streams

While chalk rivers adhere to fundamental hydrological principles and processes, such as those governing the water cycle and water balance, they possess distinct characteristics setting them apart as lowland river systems. This chapter delves into these unique attributes, including the intermittent flow regime of the headwaters and drying of the riverbed during warmer temperatures.

The crucial distinction between chalk streams and other lowland river types is the aquifer type that supplies the baseflow of these streams. The conditions required for the formation of chalk streams developed during the mid-to-late Pleistocene when repeated glaciations across Great Britain led to changes in the location of river networks and drainage basins (Belshaw *et al.*, 2014), shifting the river channels towards the upper cretaceous limestone bedrock of the southern England Chalk Formation. This led to most chalk streams in the UK being ill-defined braided channels that relied heavily on groundwater sources for their baseflow (Harrison & Harris, 2002; Pacioglu & Robertson, 2017). Typically, a main characteristic of a chalk stream is that the majority of its baseflow is provided by groundwater contributions, rather than surface runoff, although with modifications this may not always be the case in the modern day and the braided feature has been lost to the majority of channels being straightened during the agricultural revolution. Only a few small stretches of river systems, such as 500m reach of the Bere Stream (a tributary of the Piddle, Dorset) and the Upper Wylfe (tributary of the River Avon) display a similar environment to what chalk streams originally flowed as, through willow and alder carr, with fen and swamp species on the river banks due to the highly shaded nature of the river valleys (Mainstone *et al.*, 1999). Nowadays, the characteristic feature of a chalk stream is large areas of light-dependent *rannunculus* and an open water meadow on the floodplain.

The main pathway of water from the chalk aquifer to the river network is through percolation into the aquifer and discharge via springs in addition to the perennial head and winterbourne sections. Mainstone *et al.* (1999) conducted several studies on the

flow properties and hydrology of chalk rivers in the UK. On average, it has been found that baseflow from the chalk aquifer makes up 80% of any given river's flow, meaning overland flow is a minor component of the stream discharge, which is uncommon amongst other river types, such as alluvial (Mainstone *et al.*, 1999). The constant release of filtered chalk-enriched water to the rivers from the aquifer is what would naturally maintain the chalk streams' stability and reduces the sudden responses to precipitation, meaning a stable hydrological regime with a characteristic annual cycle is developed shown in figure 1.1 (Mainstone *et al.*, 1999). There is a strong dependence on the level of instream flow on the aquifer recharge through rainfall. During winter, when there is a higher amount of precipitation falling in the catchments, the aquifer begins to recharge towards ground level and continues to rise until spring. During this period, the winterbournes reactivate after being dry during summer, and the perennial stretches of river become stronger, before flows decline in summer and autumn. Then, during the following winter, the rainfall recharges the aquifer once more. Due to the reliance on groundwater for their baseflow, this means these rivers are not as 'flashy', although the peak of discharge often remains higher than non-chalk river networks, e.g. spring-fed channels were found to exceed bank full flow by 30% of the time, compared to five percent for run-off dominated streams (Mainstone *et al.*, 1999; Whiting and Stam, 1995). There are some instances when the catchment's aquifers are full and soil moisture deficits are low where moderate flashy events can occur, as well as some catchments having drift deposits of materials such as clay which influence the amount of run-off (Mainstone *et al.*, 1999).

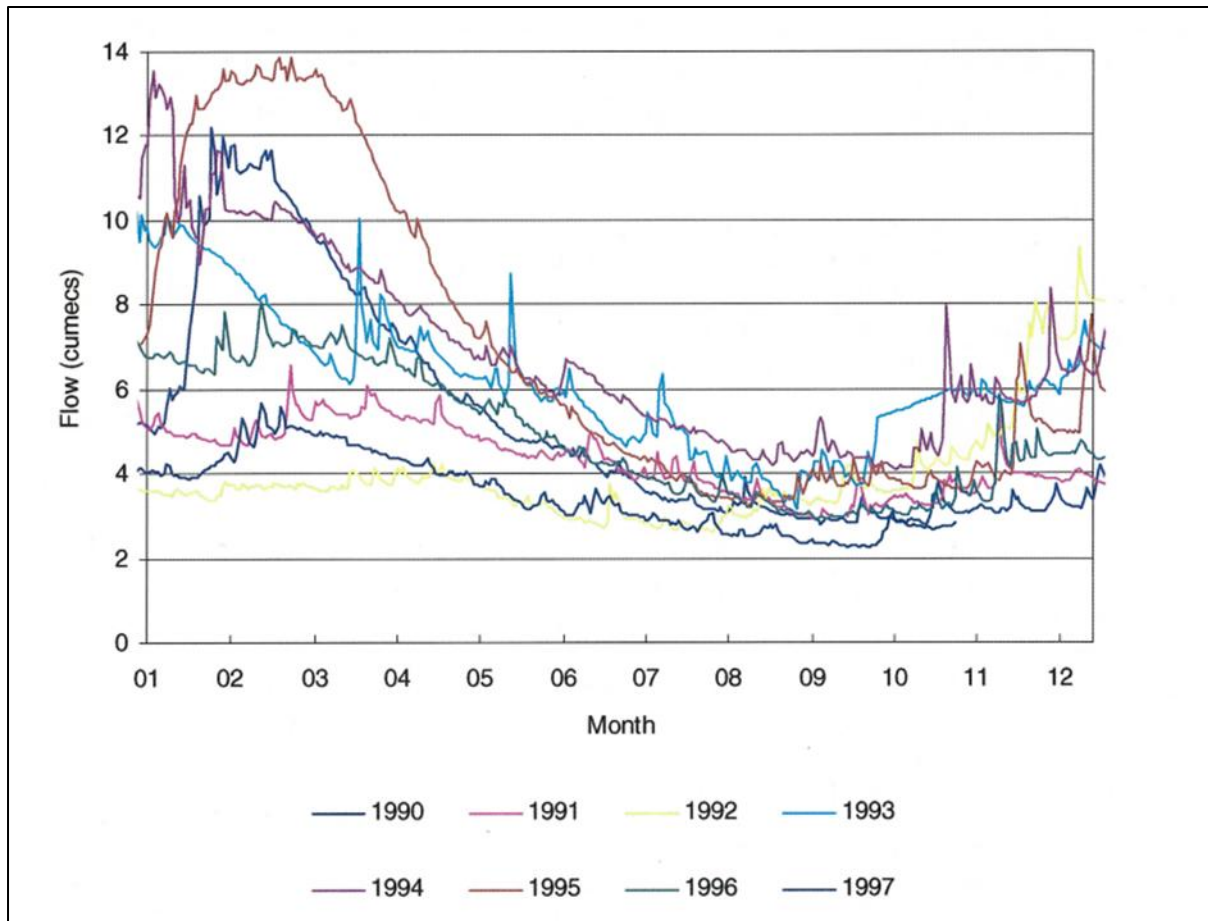


Figure 1. 1: An example of an annual chalk stream hydrograph taken from a study on the River Test in Chilbolton, Hampshire (Mainstone et al., 1999). This displays the typical cycle of flow in a chalk stream, with high flow in the winter and spring months, and low flow during the summer and autumn, when the aquifer water level is lowered.

1.1.2. The Chalk aquifer flow regime

The flow regime of chalk rivers is dominated by groundwater supplies which make them less variable than surface water-fed streams, as in some chalk rivers only two percent of streamflow is made up from rainfall contributions (Wheater & Peach, 2004). The Base Flow Index (BFI) was developed by the UK Centre for Ecology and Hydrology as part of the Low Flow Study (Gustard *et al.*, 1992) to assess the low flow characteristics of rivers in the UK (UK Centre for Ecology & Hydrology, 2023). It is the proportion of the river's flow that is derived from stores such as the aquifer. If there is more permeable bedrock and highly permeable superficial deposits and soils within the catchment, the BFI is higher and the river will be more likely to experience flow even with dry weather (UK Centre for Ecology & Hydrology, 2023). Due to the high permeability of chalk bedrock, chalk streams have a higher BFI of around 0.9 as groundwater is a larger component of the river discharge, compared to a clay catchment which may only have a BFI of 0.15 – 0.35 (UK Centre for Ecology & Hydrology, 2023). This creates a more stable river discharge with less variation in characteristics like water temperature which ranges between 10 – 12 °C and a slightly alkaline pH as the chalk stream has a stronger influence from baseflow compared to rainfall contributions (Wheater & Peach, 2004).

The relationship between the flow of groundwater to baseflow in a river is dependent on the hydraulic gradient and degree of hydraulic connection, which is affected by the geology of the catchment, recharge rates, channel geomorphology and the riverbed sediments and stage (Parkin *et al.*, 2007). For example, fine sediments can lead to more resistance to the flow of water between a river and its aquifer due to smaller pore spaces between the sediment particles and this can cause the gap between the riverbed and the water table to become unsaturated, leading to a disconnected river (a river not connected to its groundwater supply) (Rushton, 2003). However, over-abstraction of groundwater leads to a reduced amount of baseflow provided to rivers, altering the ratio of baseflow to surface water flow comprising the discharge of a chalk river.

Therefore, it is critical to understand the functioning of the chalk streams and identify their current hydrological status, as this will allow the contribution of the groundwater

from the chalk aquifer to be identified and to observe the impacts that environmental changes will have on the baseline hydrological functioning of this unique river type.

1.1.3. The annual hydrological cycle of chalk streams in the UK

Many chalk streams experience periods of low to non-existent flow, usually during the warmer summer months in the UK, and this is known as an intermittent or temporary stream, due to the fluctuation in river flow (figure 1.2) (Boulton, 2003). The upper courses of chalk streams are also referred to as winterbournes, as they flow during the winter months when the chalk aquifer is recharging, higher water tables and there are higher levels of precipitation that increase the flow into the river (Boulton, 2003). However, due to this unique dynamic of chalk stream hydrology caused by the close functional relationship between baseflow and fluctuating aquifer levels, this means they are very vulnerable to small environmental changes, such as prolonged drought (Arnell, 1996). This vulnerability is due to the fact that catchment scale flow occurs through the chalk aquifer with seasonal discharge to the river channel and floodplain as groundwater rises in autumn and winter (Rangley-Wilson, 2020). Chalk streams also experience laterally discontinuous aquifer contributions and local flow occur through alluvial aquifer in the valley bottom downstream (figure 1.3). Therefore, the aquifer upwelling becomes more diffuse further downstream and relies more on surface runoff and the gravel aquifer than the chalk aquifer. This is because seepage is harder to maintain when the gravel becomes thicker downstream (Grapes *et al.*, 2006). This indicates another way that chalk streams can be vulnerable to hydrological changes, as the dominant substrate of the aquifer will alter the water chemistry and quality of the aquatic environment.

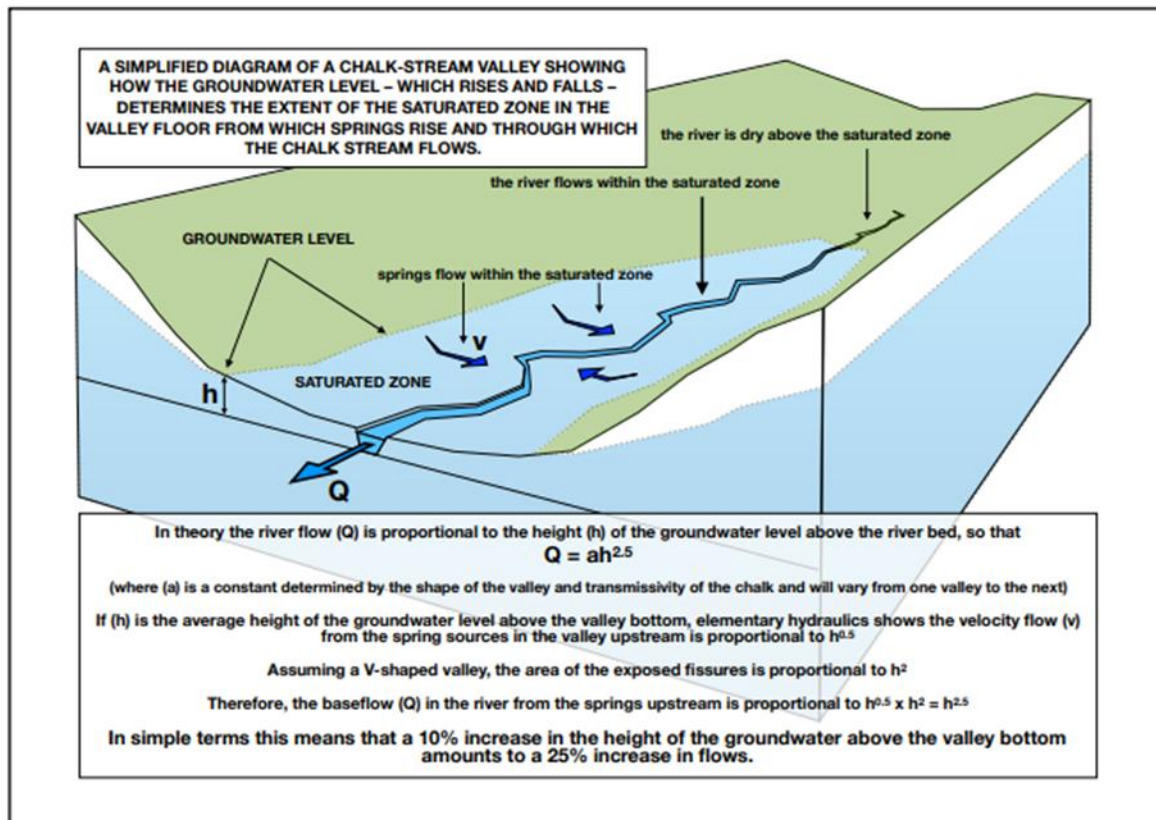


Figure 1. 2: The dynamics of a chalk stream and its interaction with groundwater level. The river flow is proportional to the groundwater level that is above the valley floor and there is a 25% increase in river flow with a 10% rise in the height of the groundwater level. As the groundwater level in the unconfined chalk aquifer rises, the saturated zone on the floodplain in the river valley increases and moves upstream, allowing the winterbourne section of the chalk stream to rise and flow during times of aquifer recharge (usually winter and spring). The saturated zone then decreases during the summer months, when the aquifer level is lower due to a reduction in rainfall, shortening the river's length. (Rangely-Wilson, 2020).

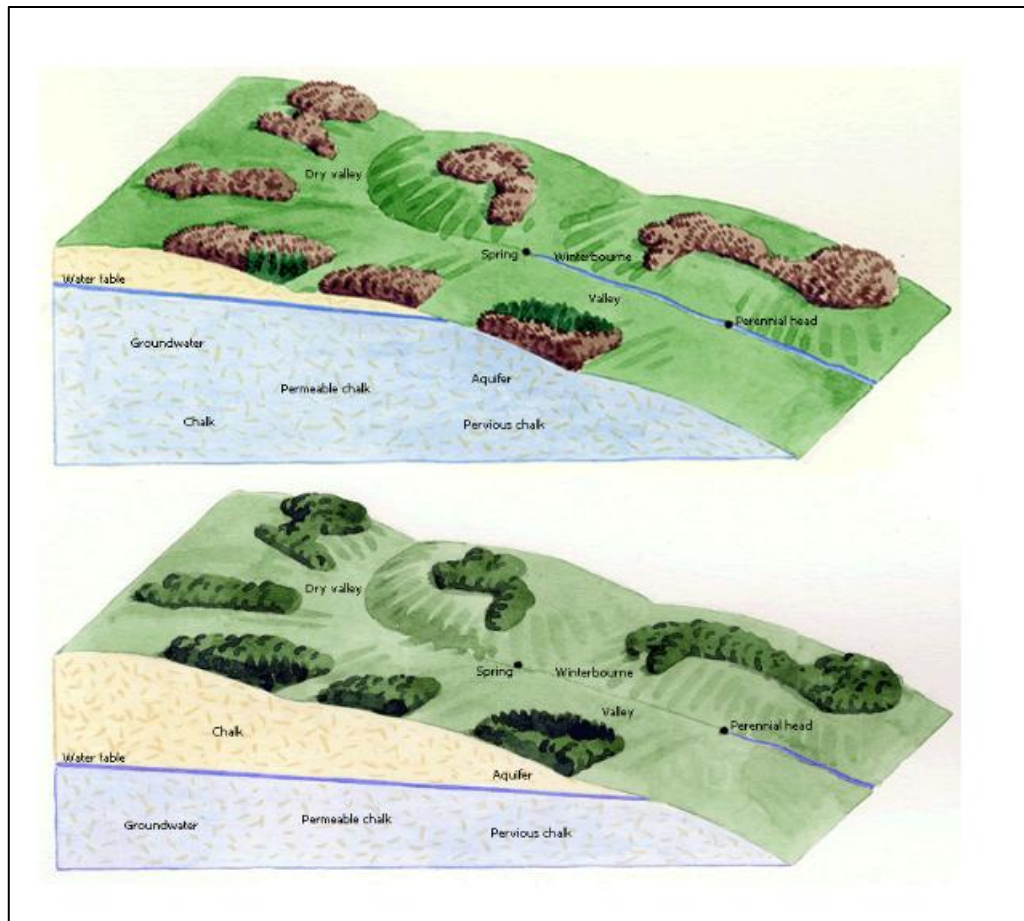


Figure 1. 3: Top – When there is heavy rainfall, usually during winter, the chalk aquifer is higher causing the water table to rise and the head of the stream moves up the valley. In the bottom figure, the water table has lowered due to lower rates of percolation into the aquifer from evaporation and evapotranspiration. This means the headwater moves downstream, leaving the upper stream dry. (Chilterns Chalk Streams Project, 2023).

Chalk streams can also be classified as temporary streams in their upper courses, since they temporarily cease flowing or have flow intermittence when the groundwater level drops below the saturated zone and they vary between having total absence of surface water, or having persistent pools, going from providing an aquatic environment to a terrestrial one. The predictability of the intermittence depends upon the climate – in the UK the chalk streams depend upon seasons (Bunting *et al.*, 2020; Stubbington *et al.* 2017). Chalk stream headwaters (winterbournes) have been found to have sharp land-water gradients and maximal landscape interface (the area where the land and water meet) (Westwood *et al.*, 2006). This means that the chalk stream headwaters

can be used as a natural laboratory to monitor the rate of environmental change as they can allow responses from drought to be predicted through monitoring the rates of recolonisation of aquatic and terrestrial species and to identify the factors determining the composition and changes of biological communities (Westwood *et al.*, 2006).

Intermittent rivers and ephemeral streams (IRES) are streams that do not flow permanently, and this characteristic makes them particularly vulnerable to the risk of deterioration from climate change and anthropogenic activity (Sefton *et al.*, 2019). Intermittent streams are important for biodiversity and provide an ecologically diverse environment within a river system that relies on the periods of drought to determine the biodiversity of the streams (Sefton *et al.*, 2019). Activities such as water abstraction, impoundment, sewage effluent and augmented flow alter the natural variation of the flow and thus change the hydrological behaviour of the stream, along with the ecosystem services they provide (Sefton *et al.*, 2019; De Girolamo *et al.* 2015; Datry *et al.*, 2017). Previous research on the hydrological studies of the intermittent streams has either focussed on temporal or spatial changes, but rarely both in conjunction. For example, flow duration curves were calculated to show the characterisation of flow intermittence (flow permanence) and the duration, frequency and timing of the drought period (Sefton *et al.*, 2019; Costigan *et al.*, 2017). These methods provided temporal data on the hydrologic regime at a daily or monthly resolution but were restricted to individual sites in the spatial resolution. Other studies collected longitudinal hydrological data at various sites on a river, but only on a few occasions in the peak flow and drought conditions (Sefton *et al.*, 2019; Turner & Richter, 2011). Therefore, to get a comprehensive observation of the hydraulic regime of intermittent streams, both temporal and spatial data should be recorded (an example of one management scheme that has tried to achieve this is mentioned in section 1.1.4.2.).

Therefore, understanding the annual hydrological regime of chalk streams is critical to quantifying the environments and habitats provided by the changes in the hydrology and extent of the chalk river system during different seasons, and to predict how the combination of aquatic and terrestrial environments may vary in the future with more variations in meteorological weather patterns and extreme weather events.

1.1.4. The hydrological regime of the River Colne and Colne Catchment

1.1.4.1 The Chalk aquifer

There are a total of 11 principal aquifers in England and Wales and the Chalk is the major aquifer in southeast England shown in figure 1.4 (although it is also present in southeast Yorkshire and into Lincolnshire) and it extends across central southern England through the Thames Basin, Norfolk, Kent, towards the south coast in Dorset and the Isle of Wight (BGS, 2024a). It is the second youngest aquifer in the UK and is formed from microporous white limestone with a low matrix permeability with well-developed interconnected network of fractures (BGS, 2024a; BGS, 2024b).

The chalk aquifer in Hertfordshire was investigated in 1992 by Price *et al.*, to determine the impact of a pollution incident entering the chalk aquifer from runoff on the newly constructed M25/ M1 interchange (figure 1.5). The heterogeneity of the chalk means that both fast and slow paths are taken by the tracer, meaning it could be difficult to determine the longevity of any pollution that enters the chalk aquifer and its future impact on the chalk streams. The hydraulic gradient of the groundwater levels in the Chalk is suggested to be towards the south-south-east, towards the interchange in Bricket Wood. This means that the pollution entering the chalk near the M1/M25 interchange would be carried towards Bricket Wood. However, in 1976 during water level data collection after the 1975 drought, the flow paths within the aquifer are only coincident with the direction of maximum hydraulic gradient when the aquifer is isotropic (when the hydraulic conductivity (K) is equal for flow in all directions) (Price *et al.*, 1992). This would mean that the flow would only travel towards Bricket Wood if the aquifer is heterogenic in material, which chalk often is not due to irregularities in its structure and fissures.

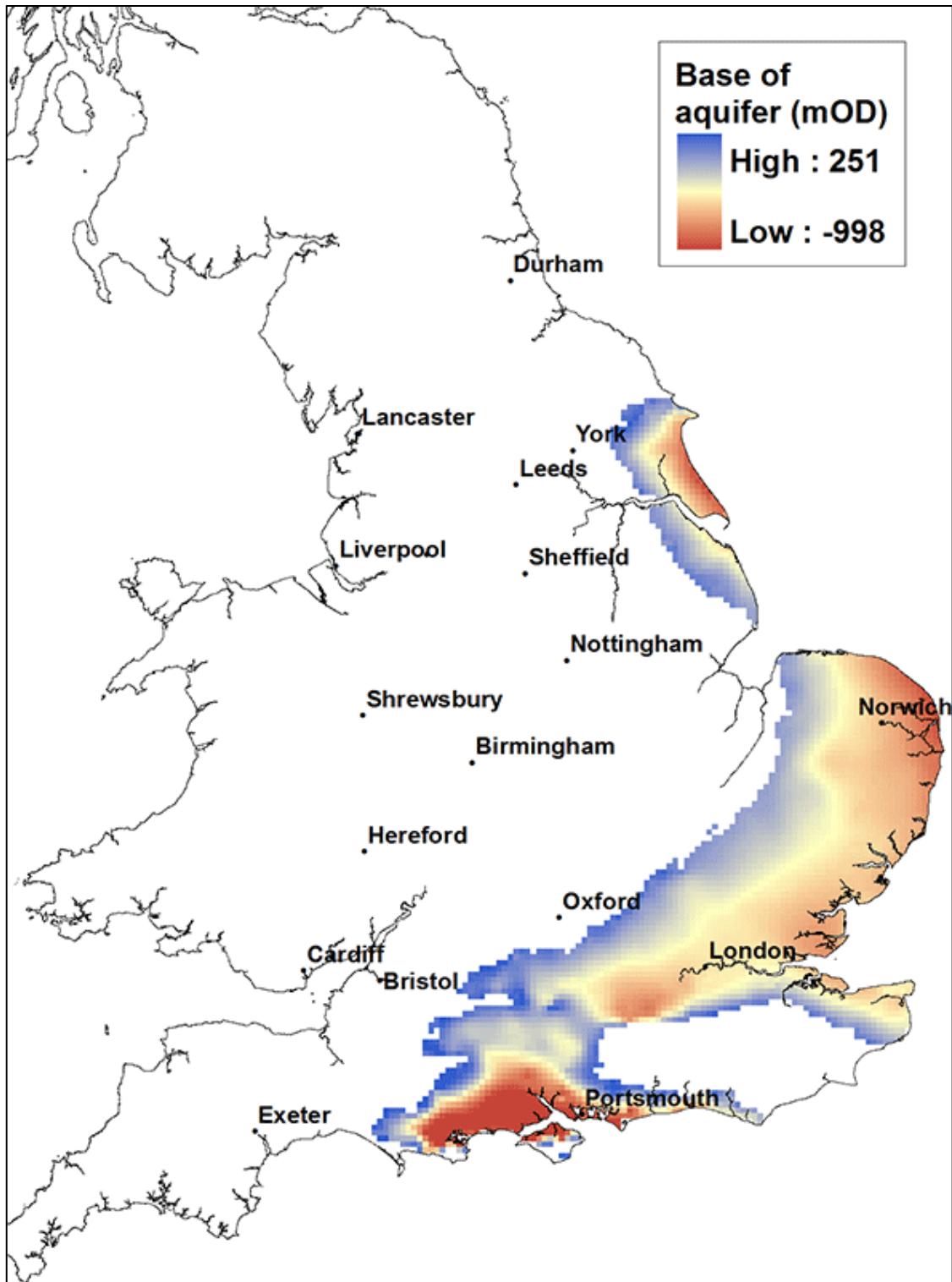


Figure 1. 4: A map showing the Chalk aquifer that is located in southern England and is responsible for the formation of the chalk stream springs. (BGS, 2024a). The measurement is in mOD which stands for meters above ordnance datum (or sea level (EA, 2025a). The shallower aquifer is to the north of the Chalk, where the chalk is more exposed and closer to the surface geology.

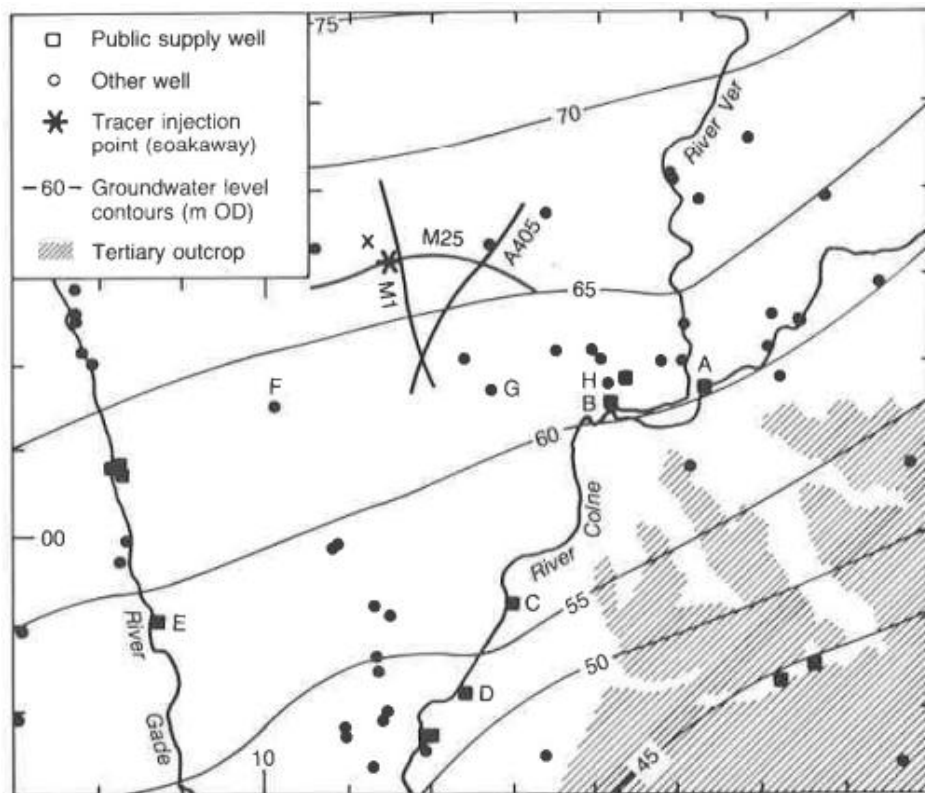
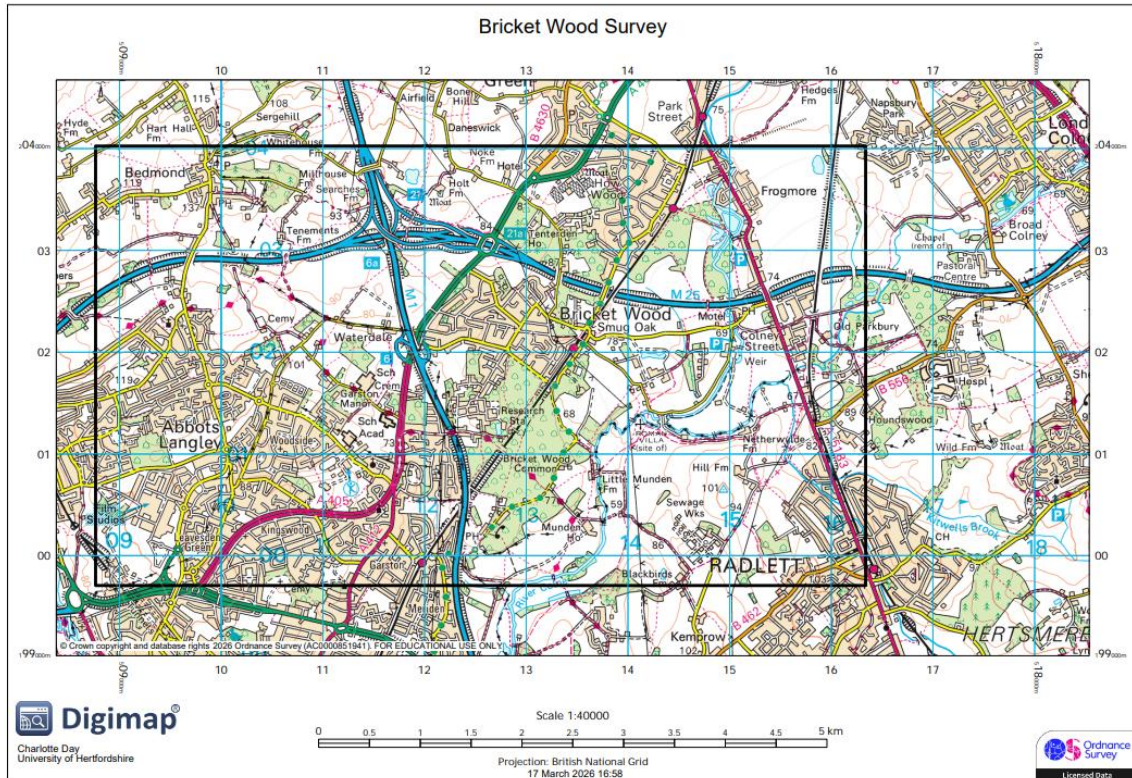


Figure 1. 5: The location of the study by Price et al. (1992) (outlined in black above) for the hydrogeological investigation of the chalk aquifer in Bricket Wood. Sampling sites are indicated by the letters and located at pumping stations at different depths to detect the tracer dye to determine flow through the aquifer.

The Chalk aquifer in Bricket Wood is known to be a highly productive aquifer and its transmissivity has been assumed to be not less than 2000m² /day and is most concentrated in the top 50m of the saturated aquifer (Price *et al.*, 1992).

The average flow speed (v) through a groundwater system is given by:

$$v = \frac{Tl}{bnp} \quad (1)$$

where T is the transmissivity of the effective aquifer; l is the hydraulic gradient; b is the thickness of the effective aquifer; and np is the dynamic porosity (porosity which flow is occurring).

In summer 1976 the hydraulic gradient was between 0.002 and 0.003 and most secondary fissures are near-horizontal where the gradient exists. The primary fissures were found to have a hydraulic gradient of 6m/day and 150m for the secondary fissures (Price *et al.*, 1992). These calculations suggest that flow could travel from the soakaways to Bricket Wood within five days with an average flow speed of 600m/day to be harmful which led to Price *et al.* (1992) modelling the travel time of a solute to increase reliability. The first tracer study showed that a tracer entering the chalk aquifer near the M1/M25 arrived as two pulses (figure 1.6): one within three days of injection and a second stronger concentration within four weeks which shows that the tracer travelled through a fissure connection at an average of 1km/day. The second tracer test showed a similar pattern with the bacteriophages showing a three day and four-week trace detected, although none of the Rhodamine was found to be present after injecting into the chalk aquifer. Overall, the majority of the tracers were not recovered, which may be explained by the sampling sites being located on the edge of a plume or one of several plumes (although this is unlikely as the modelling suggested that highly transmissive systems with high flow speeds have a narrow plume).

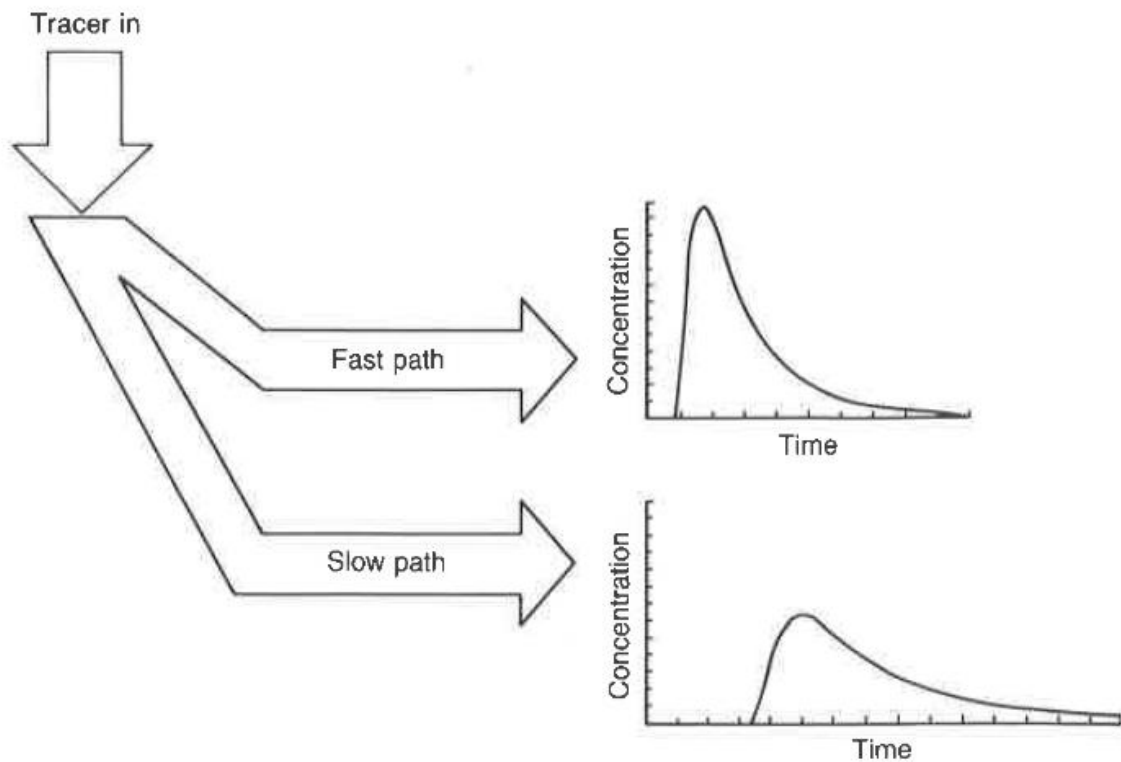


Figure 1. 6: A diagram showing the different hydraulic pathways within the chalk aquifer of the Chalk at Bricket Wood. This shows the peak of the pulse-input tracer dye (although the dilution during the study by Price *et al.* (1982) was heavily diluted and only detectable in small amounts due to the dispersion through fissures within the chalk).

From this study, it could be inferred that the majority of the tracer was travelling slowly through the aquifer in a primary fissure component and the tracer may be recovered at site B in low concentrations over the following year. However, since the Chalk aquifer is heterogeneous, the flow paths will vary, with some following the rapid transport through a fissure system without significant exchange of tracer between the fissure and water in the matrix pore space model and some following another model involving diffusion of the tracer into the matrix pore space, diluting the tracer (Price *et al.*, 1992). Therefore, flow could also be assumed to be predominantly through large fissures and rapid enough to inhibit retarding effects of diffusion, with some smaller fissures creating a retarding and diluting effect from diffusion. Flow speeds of 2km/day were indicated from this test, but the recoveries were very low, which hinted at the high dispersal within the chalk due to fissures where the tracer was adsorbed or moved too slowly to be detected within two months (Price *et al.*, 1992).

This heterogeneity will make it difficult to determine the exact longevity of any pollutants within the chalk aquifer water and how it will later impact the chalk stream discharge quality, as it is not easy to predict when the affected groundwater will once again rise, due to the variation in flow paths taken through the fissures. This means it is essential that all precautions are taken to minimise the pollutants entering the chalk aquifer and groundwater supply as it is unknown how the pollutants will be detrimental on the future water quality of the stream.

1.1.4.2. A summary of the Colne Catchment Hydrology

Many sites along the River Colne in its upper reaches have been suffering from low flows and this has been investigated to see the impact on the ecology from licensed abstractions. The Integrated Catchment Management Report for the Environment Agency in 2008 highlighted that there was strong evidence that the upper Colne was once a typical chalk stream before major water abstraction was undertaken. The Colne now loses water along all its course and only experiences groundwater contributions when the groundwater levels are very high (Morgan, 2008).

The Colne catchment has six main rivers and tributaries: the Colne, Ver, Gade, Bulbourne, Chess and Misbourne and these rivers range from chalk rivers in the upper catchment to clay-based urban systems in the lower catchment (figure 1.7). The rivers in this catchment all rise from the Chiltern Hills Area of Outstanding Natural Beauty. The main tributaries of the Colne depend on the chalk groundwater for its baseflow, and this can fluctuate seasonally with the amount of rainfall within the catchment and the subsequent recharge of the chalk aquifer which provides the groundwater source of the river flow (Morgan, 2008). The Upper Colne also has several tributaries east of the A1(M) such as Mimmshall Brook, Ellen Brook and Nast. Mimmshall Brook is a chalk stream and rises southwest of Boreham wood where it flows north and enters the River Colne east of the A1(M). This river has resulted in serious flooding to properties on five occasions since 1928, resulting in the creation of the Mimmshall Brook Flood Alleviation Scheme against 2% of an annual fluvial flood event.

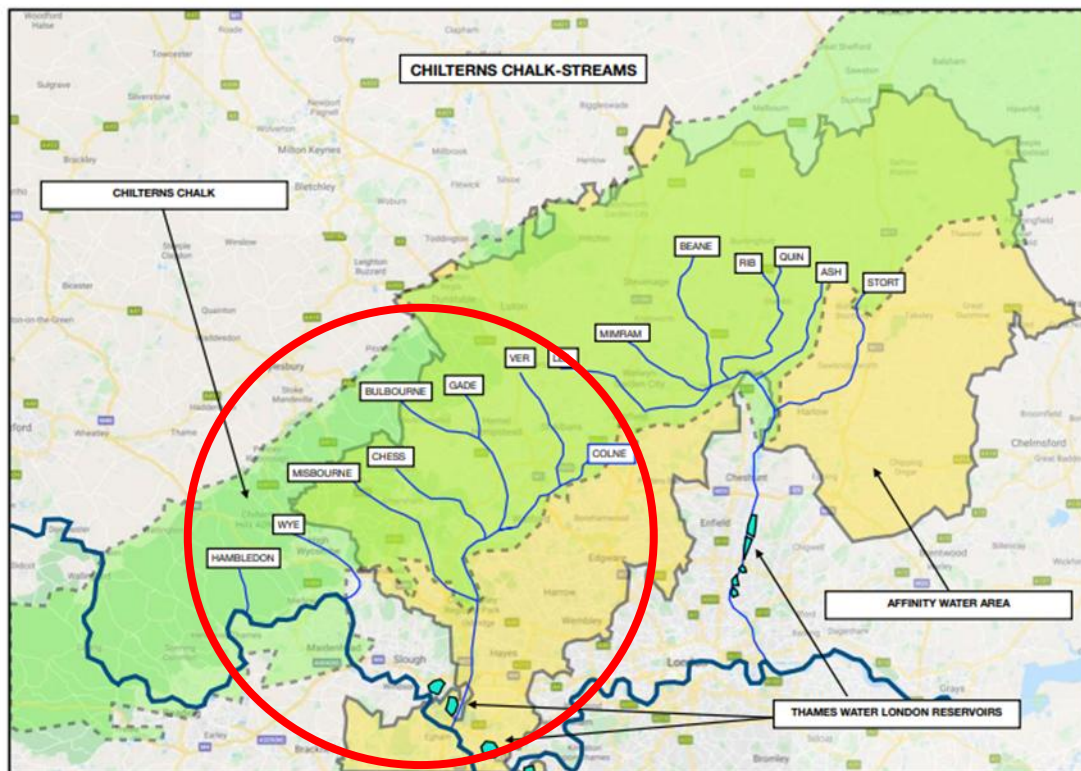


Figure 1. 7: The Chilterns chalk streams including the Colne (red circle) and Lee catchments in relation to the water demand areas of Thames Water and Affinity Water. The green is the chalk aquifer and yellow is the area the water company can drain from within the area (Rangley-Wilson, 2020).

The middle reaches of the Colne, between the Gade confluence and Denham Green (including the lakes, one of which is a designated Site of Special Scientific Interest (SSSI) are currently on the Restoring Sustainable Abstraction programme as they may be suffering from low flow and low lake levels. The Environment Agency concluded that further investigation was needed to determine the abstraction related impacts on the rivers to gain a better understanding of the hydrology and hydrogeology of the lakes and River Colne, as well as how lake management and other water bodies like the Grand Union canal impact the flow (Morgan, 2008).

The main reason for water abstraction within the Colne catchment is for potable drinking water. This is predominantly supplied by Three Valleys Water which heavily relies on groundwater for its supply. The per capita demand is 177l/h/d, which is one of the highest in the country compared to the national average of 155l/h/d (Morgan, 2008; Hertsmere Council, 2025). Due to contamination issues within the catchment

area, this has restricted where abstraction can take place, and although no new licenses have been granted in recent years, the area still experiences over-abstraction, as past licenses were licenses of right (no time-limit) (Morgan, 2008).

A monitoring programme was set up in southern England that investigated the intermittent headwaters of ten chalk rivers within the Colne and Lee catchments that shows a monthly resolution of longitudinal data of multiple hydrological states over the past 20 years (figure 1.8) (Sefton *et al.*, 2019). The hydrological state was observed and photographed, and the dataset was put into four ecologically relevant flow states: dry, ponded, moderate flow and high flow (bank full or over bankfull) (Gallart *et al.*, 2012). The Gade, a tributary of the Colne, displayed intermittent behaviour in its upper reaches and perennial flow in the lower reaches. The lowest extent of the flowing reaches occurred during the drought years of 1997-1998 and 2005-2006 and showed highest flow during the 2001 and 2013- 2014 winters when groundwater levels had risen (Sefton *et al.*, 2019). Transitioning between the high and low flow states was found to be slow, with many reaches remaining dry or moderately flowing for two or more years after a drought period. The distance between the headwater of the tributary and confluence of the Colne also impacted the flow observed, with moderate flow being more common closer to the confluence. Most upper reaches were found to flow at least once during the early months of the year (January to April) in the 10-year study period and the driest times occurred in the lower reaches at least once in between June to November (Sefton *et al.*, 2019).

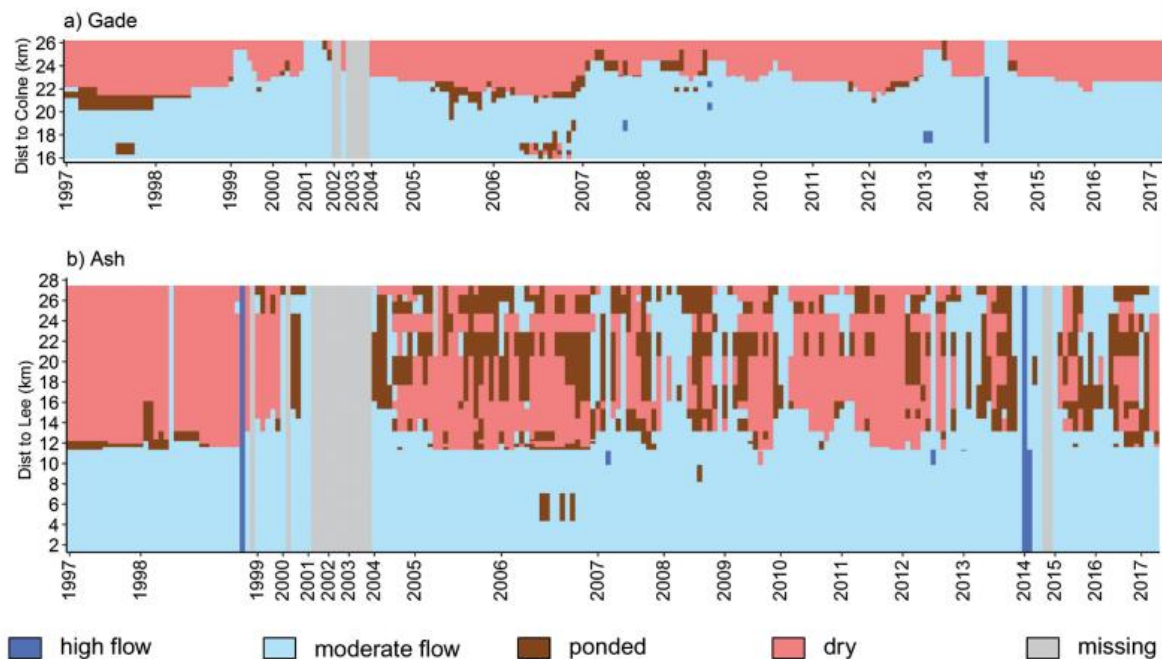


Figure 1. 8: *The hydrological circumstances that occurred at each reach of the rivers Gade and Ash between April 1997 and April 2017. It shows a mosaic of high and moderate flow and ponded and dry periods. Both rivers experienced more periods of dry and ponded areas with increased distances from the confluence with the trunk stream, and there were few high flow states recorded. (Sefton et al., 2019).*

However, more extreme weather events and altering weather patterns and seasons are causing changes to the stability of the hydrological regimes of these rivers. After months of 50-70% lower than average rainfall in the winter of 2016 to 17 and early Spring of 2017, many Hertfordshire rivers struggled to sustain their flow. The Colne was dry in London Colney, leaving the upper Colne completely dry such as its tributary Ellen Brook (Lewis, 2017). This was detrimental to the invertebrate life and those that relied on them as a food source such as birds and fish also suffered. Previously in 2012, it took another chalk stream, the Mimram, five years to recover to pre-drought conditions, but it was then halted with the 2017 drought.

Therefore, the Colne catchment does display a typical pattern for a chalk stream with its annual hydrograph. However, with the recent more extreme weather events, with a particularly hot summer in 2022, followed by a very wet winter in 2023-24, continuous monitoring of the flow within the catchment will be essential in maintaining the riverine environment and protecting the ecology that relies on the specific hydrological conditions and variations to survive.

1.1.5. Unique hydrological features of the Colne Catchment

The Colne catchment is also the site of some unique hydrological features that are scarce and not fully understood. These are the swallow holes (a type of sinkhole) at Water End, North Mimms, Hertfordshire shown in figure 1.9 and they are some of the largest and more accessible swallow holes in southern England. Sinkholes are depressions in the ground that capture overland flow, normally found in chalk or limestone landscapes connecting to the underlying chalk aquifers. They are a permanent feature of the landscape and are a drainage outlet for the karstic basin. The solid geology of the area of the Upper Colne and its tributaries is the Upper Chalk in the north with the Lambeth Group outcropping through the Welwyn-Hatfield borough. The area is influenced by the unconfined Upper Chalk which is a major aquifer and provides a significant water source for the towns and villages in the area. Dye tests from the 1920s showed that there is a considerable divergence of surface from subsurface drainage from the swallow holes. During the spring and autumn, Mimms Hall Brook and Potterells Stream have a modest flow to the swallets at Water End, which are confined to the surface flow (Walsh & Ockenden, 1982). During the summer months, when there is less precipitation, no drainage reaches the swallow holes as the rainfall is absorbed into the streambeds on the higher ground in Potters Bar, Barnet and Borehamwood. However, during winter and early spring, when the rainfall is at its highest, the swallow hole complex becomes unable to absorb the discharges from the two feeder streams, causing a lake to form in the depression surrounding the swallets (Walsh & Ockenden, 1982). This lake then discharges through an overflow channel under the A1(M), flowing north-west to join the permanent reaches of the River Colne (Walsh & Ockenden, 1982). The complex of swallow holes contains two types: funnel shaped holes that directly drain into fissures in the Chalk aquifer (with the chalk bedrock sometimes visible at the bottom of the swallow hole);

and soakaway basins where water percolates slowly into the alluvium before draining into the Chalk.



Figure 1. 9: *A swallow hole at Water End, a Site of Special Scientific Interest because it is the only area with 15 swallow holes in chalk that are permanent in the landscape (Hertfordshire Geological Society, 2024; Hertfordshire Walker, 2024).*

The water table at Water End falls in two directions perpendicular to each other. The steepest hydraulic gradient is towards the southeast where it exceeds 1:100. However, this merely suggests the direction of flow – there is a relative barrier of low permeability around the Shenley-Broxbourne anticline where the isopiestic contours are particularly close between Borehamwood and Potters Bar. The groundwater has been found to flow north-eastwards from Water End along the trough of the Colney Heath – Hoddesdon Syncline at around 550m/day. This suggests that an open fissure system is present here where the joints have been widened by solution erosion, in spite of this structure being a syncline and having a low hydraulic gradient (Walsh & Okenden, 1982; Morris & Fowler, 1937). This shows that the Colne Catchment, whilst being important for the chalk streams it poses, also has other features which are considered a priority to protect and are rare.

1.2. The Morphology of Chalk Streams

1.2.1. The Basics of River Morphology

The channel morphology of a river is determined by the hydrological regime, sediment supply and the drift geology that the river flows through (Mainstone *et al.*, 1999). Rivers reshape their channels continuously through erosion of the channel boundary, including the bed and banks, and the reworking and deposition of sediments. The balance between the erosive power of a river's flow and the resistance of the bed and bank material is critical in determining the morphology and channel form. The energy needed for geomorphological work is provided by the flow of water through the channel (Charlton, 2007). The ways a river may reshape their channels depends upon the stream energy, which determines the threshold of erosion and the sediment transfer and deposition (Knighton, 1998). The erosion and undermining of the riverbanks leads to the widening of the channel, scouring of the beds leads to channel deepening, and deposition of sediments and material reduces the depth and leads to formation of bars and other channel formations. Channels that are comprised of unconsolidated material offer less resistance to erosion than those of bedrock, but there is also variability in this resistance dependent on the exact sedimentary composition of bed and banks. For example, sandy alluvium is easy to erode, whereas clays are harder to detach and entrain due to the cohesive electro-chemical forces between particles. Vegetation will also influence erosive resistance, through the ability of root structures to bind and strengthen soils and sediments. Irrespective of channel composition, it is often the case that only extreme floods significantly alter channel morphology, particularly in the case of bedrock channels.

1.2.2. Chalk Stream Morphology

Chalk streams geomorphology is not thoroughly understood and the majority of chalk rivers in the UK are no longer in their natural formation, rather they have experienced heavy modification from historical uses (Sear *et al.*, 1999; English Nature, 2002). Therefore, most of the information on what a semi-natural chalk stream should display have been predicted from palaeoecological information. Chalk rivers generally have a low hydraulic energy due to their shallow valley gradients, which are often too low to transport larger sediments yields, such as those consisting of larger gravel clasts,

which means that chalk rivers have less ability to reshape their channel morphology compared to other river types (Mainstone *et al.*, 1999). A report by French *et al.* (2005) surveyed a chalk stream River Test in Dorset, England and it was suggested that the upper course displayed a wide, shallow, low sinuosity meandering channel with low stream power per unit catchment area (English Nature, 2002). The riverbed substrate is normally dominated by Pleistocene river gravels, overlaid by peat and tufa (a form of limestone that is precipitated from water in unheated conditions) and capped by silt-clay alluvium (Sear *et al.*, 2006). The specific measurements for geomorphological features of chalk streams compared to other stream types are detailed in the table 1.1. below.

Table 1.1.: *The geomorphological features that are associated with the main UK aquifer types (Sear et al. 1999).*

Aquifer/Lithology	Power (W/km^2)	Bankfull width (m)	Width: depth ratio	Rifle spacing/ bankfull width	Mean number VSSF / 500m	Mean number DSSF / 500m	Sinuosity	n
Chalk	6.1 (17.8)	8.7 (4.4)	18.4 (14.0)	51.0	0.1	0.1	1.29 (0.47)	21
Soft Limestones	15.6 (31.6)	7.7 (4.5)	11.0 (7.1)	27.2	0.8	1.0	1.24 (0.41)	15
Permo-Triassic Sandstone	6.8 (13.9)	10.8 (8.3)	9.2 (6.3)	20.9	0.6	0.6	1.25 (0.24)	24
Clay	6.3 (19.3)	6.1 (3.8)	7.8 (5.4)	31.0	0.4	0.5	1.31 (0.33)	29
Hard limestones	72.1 (236)	9.2 (7.9)	11.7 (8.5)	15.4	0.7	1.2	1.22 (0.26)	35
Impermeable lithology	25.4 (81.8)	10.3 (10.6)	10.0 (9.3)	15.1	1.4	1.5	1.20 (0.25)	352

Figures in brackets are standard deviations of the sample population.

VSSF = Vegetated sediment storage features (point bars, mid channel bars and side bars)

DSSF = Dynamic sediment storage features (point bars, mid channel bars and side bars)

Some common features of lowland semi-natural groundwater dominated streams include: low stream power per unit catchment area; large width : depth ratios; limited in-channel coarse sediment storage (bars); high residence time of large organic matter such as woody debris; woody debris islands and few debris dams; high floodplain water tables with rich organic floodplain soils; low rates of lateral channel adjustment; limited accumulation of fine sediment on river bed in undisturbed catchments; higher duration of flows at bank-full or overbank discharge compared to runoff dominated streams; high density of aquatic macrophytes.

Chalk rivers often have a fairly consistent depth morphology, due to a poorly defined pool-riffle sequence and the substrates tend to have a smaller particle size consisting of gravels, sand and silts, rather than larger rocks and boulders associated with highland rivers (Acornley & Sear, 1999). Chalk rivers typically have wide, shallow channels with substrates dominated by gravel and cobble sized clasts, usually with superficial bed siltation (Bunting *et al.*, 2021). Using the Modular River Surveys (MoRPH) indices (a survey technique to assess and monitor river channel characteristics, resulting in a preliminary condition score for the river section), the study by Bunting *et al.* (2021) found that the channel physical habitat complexity was low, as was riparian physical habitat and riparian vegetation structural complexity, which reflects the surrounding land uses of recreational grassland and livestock pasture.

1.2.3. Chalk Stream Sediments and its Impact on Channel Morphology

The gravel bed is a crucial component of chalk stream morphology, and the gravel bed sediment budget has been proposed to be a controlling factor of the accumulation of fine sediment in chalk streams (Mondon *et al.*, 2021). Typically, it would be expected that chalk streams have a higher rate of sediment accumulation, due to the low sediment storage and a high sinuosity, as well as the presence of a high width : depth ratio. However, since chalk streams tend to rise from discrete springs rather than highland valleys, this has led to a lack of sediment available for transport and less energy available for bank erosion in the upper courses due to low bank-full discharge capacity, shallow valley gradients and low bank-full stream power (Sear *et al.*, 2006; Whiting & Moog, 2001). This leads to a rather static riverbed, formed of larger gravel particles that do not frequently move due to the low stream power (the energy available per unit area of river bed available to transport sediment), and armouring of the river bed by tufa will further cement the river bed substrate in place, restricting bedload transport (Sear *et al.*, 2006).

Instead, fine silts and sands that are stored in low powered streams have been found to dominate the sediment storage in stretches of chalk streams with glides and pools and marginal dead water areas (Sear *et al.*, 2006). This makes the transport and accumulation of fine sediment an important factor in chalk stream geomorphology,

particularly as the limited sediment supply and transport load leads to a lack of bank erosion and lateral migration of the channel (Whiting & Moog, 2001). Coupled with many chalk rivers having high densities of aquatic macrophytes in channel creating sediment traps and leading to the accumulation of fine sediment on the riverbed (figure 1.10), this adds to the importance of fine sediments playing a larger part in available material for transportation and storage.

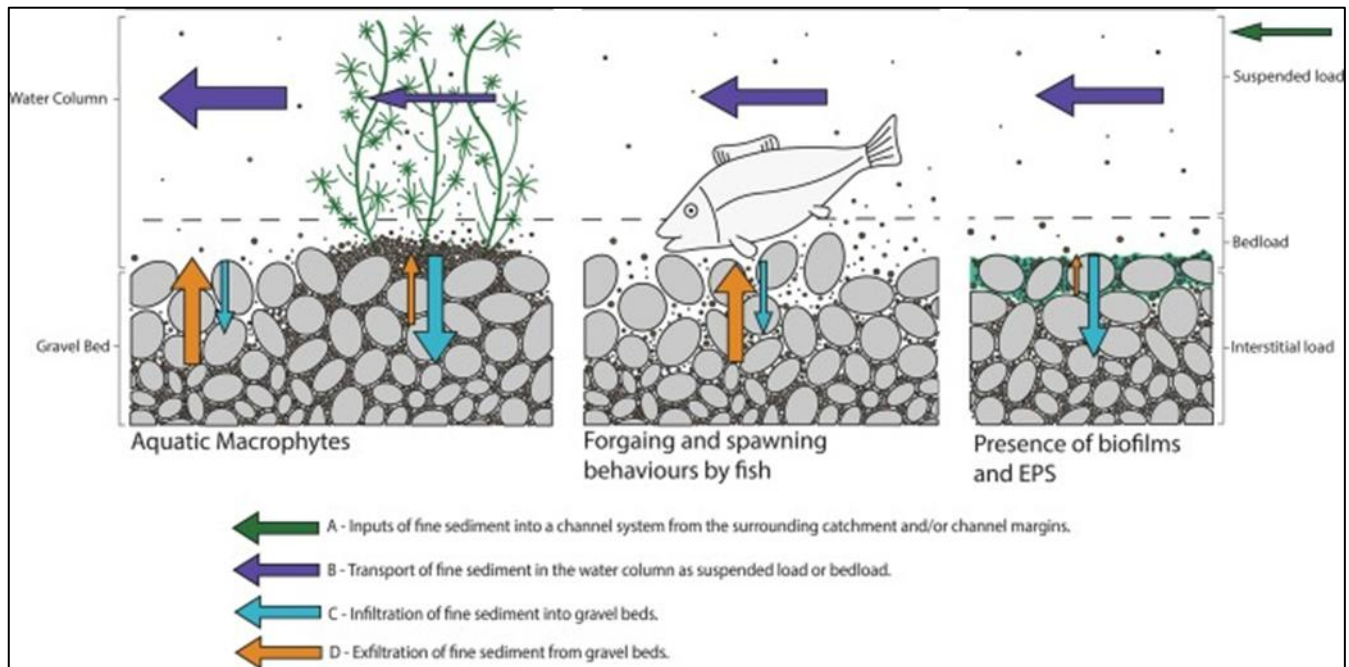


Figure 1. 10: The different amounts of collimation on the gravel bed of chalk streams in the presence of different ecosystem engineers and biological processes. Plants and macrophytes leads to infiltration of the sediment into the gravel bed whereas foraging and spawning from fish leads to the exfiltration of sediment from the gravel bed. (Mondon *et al.*, 2021).

The lack of flushing of the fine sediments from the gravel bed leads to a relatively large quantity stored within the gravel matrix and this has been perceived to be of ecological significance in the relation of spawning salmonids, bullhead and lamprey, but also leading to an impoverished invertebrate fauna (Sear *et al.*, 2006; Woods & Armitage, 1999). The downstream movement of fine sediments can be shown in figure 1.11, as the river corridor survey taken in 1981 on the River Nar, King's Lynn, showed that the upper reaches of the chalk stream are dominated by visible gravel clasts, whereas the lower reaches have a higher proportion of silt and fine sediments visible.

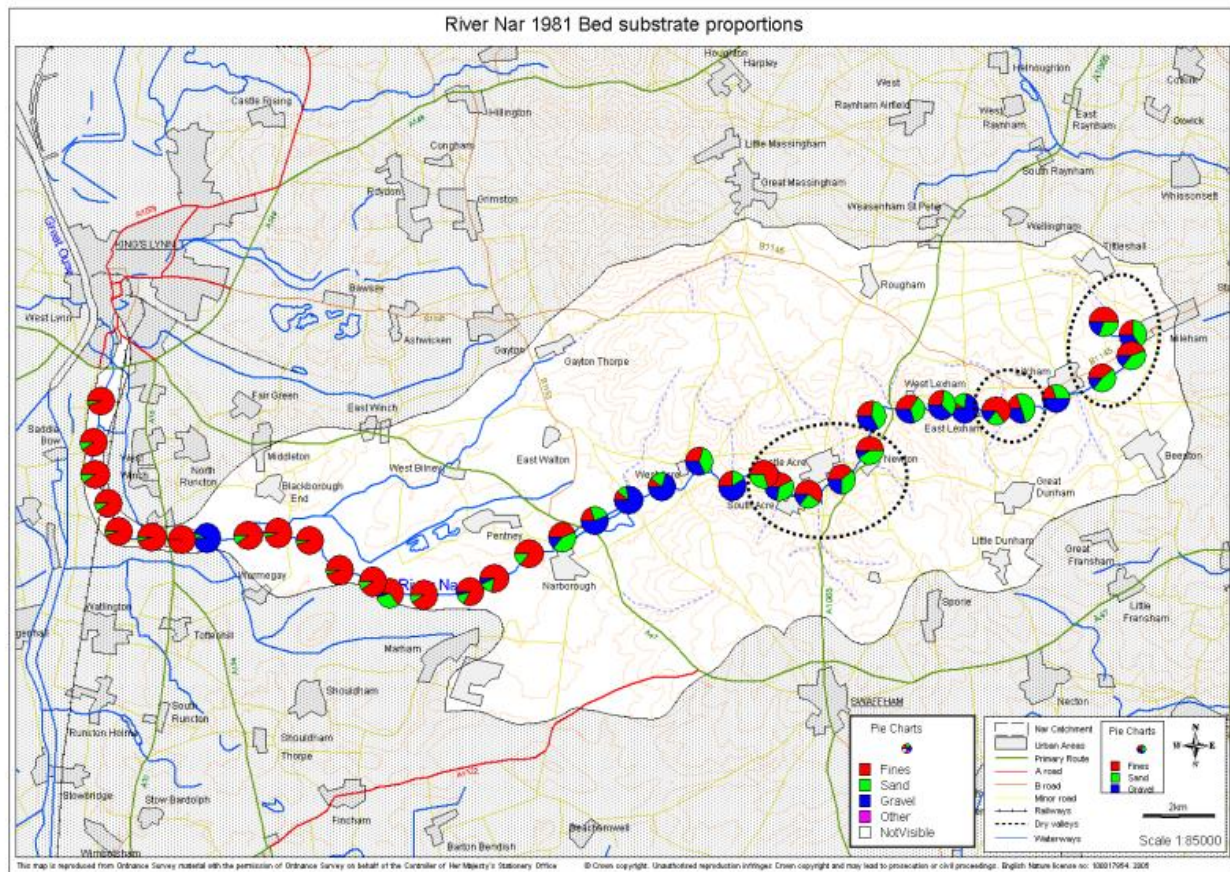


Figure 1. 11: The variation of bed substrate proportions in 1981 on the River Nar, King's Lynn, Norfolk (Sear *et al.*, 2006). The sediments are indicated as follows: red is fine materials; green is sand; blue is gravel; pink is other.

Chalk stream gravel beds are formed and controlled themselves by physical, chemical and biological factors within the stream and overall catchment, which can be split into four mechanisms. These are shown in figure 1.11 and are as follows: a) inputs of fine sediment into the channel system from the catchment; b) transport of fine sediment as suspended load in the water column; c) infiltration of sediment into gravel beds; and d) exfiltration of fine sediment from the gravel beds (Mondon *et al.*, 2021). These processes determine the sediment budget which controls the amount of fine sediment accumulating on the gravel bed, potentially leading to negative impacts on chalk stream biota (Mondon *et al.*, 2021).

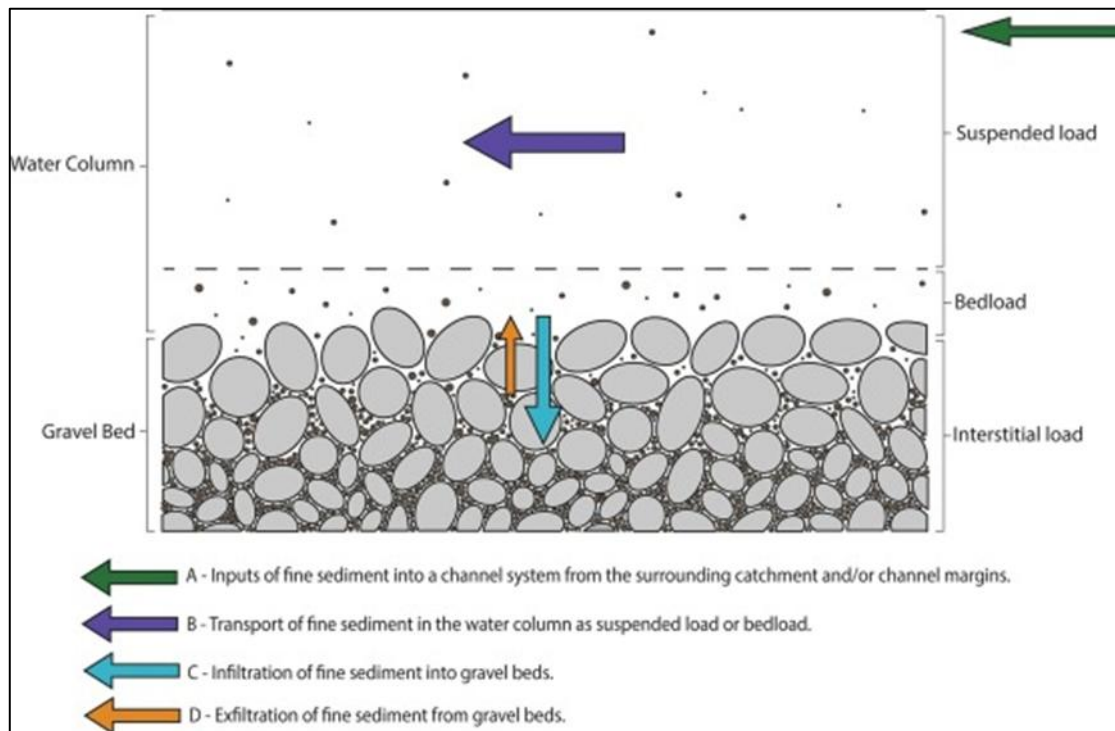


Figure 1. 12: A diagram of a chalk stream gravel bed, showing the four interacting mechanisms that control the amount of gravel bed collimation (Mondon *et al.*, 2021). The four mechanisms are: a) inputs of fine sediment into the channel system from the catchment; b) transport of fine sediment as suspended load in the water column; c) infiltration of sediment into gravel beds; and d) exfiltration of fine sediment from the gravel beds.

Another important input of material into chalk stream systems is organic matter in the form of woody debris. This is often discounted as a component of chalk stream morphology, but woody debris has been found to create local scour and habitat diversity, as well as channel-floodplain connectivity on the upper Nar Chalk Stream, near Kings Lynn (Sear *et al.*, 2006). However, the woody debris or other organic material inputs such as fallen trees can cause obstructions on the river channel, leading to further sediment accumulation shown in figure 1.13.

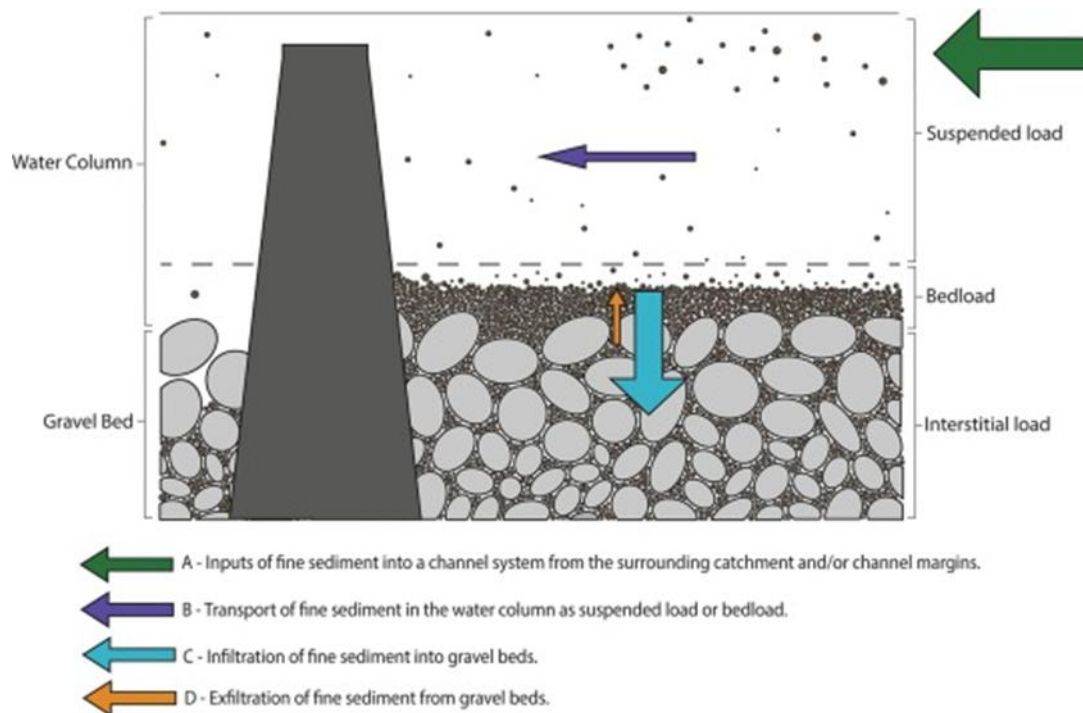


Figure 1. 13: This diagram shows how the mechanisms interacting in a chalk stream gravel bed are influenced by the increased amount of fine sediment input from agricultural land use and reduced flow from channel modifications such as weirs (Mondon *et al.*, 2021).

Chalk streams are difficult to generalise, but a sinuous channel could be expected, with a distinct riffle-pool structure being absent due to the low hydraulic energy, with gravels featuring extensively in the bed sediment and low fine sediment loads in unimpacted circumstances, with gravel shoals and exposed sediment occurring infrequently, but providing a valued ecological habitat for invertebrates (Mainstone *et al.*, 1999).

1.2.4. External Influences on Channel Morphology

It is important to understand how the river systems and the surrounding landscape morphology changes overtime in relation to the river's processes and external influence, in order to be able to predict how these circumstances could change in the future, since with climate change, these external factors will shift, altering the riverine environment. These predictions can be made using past historical data and historical scenarios, combined with current hydrological and morphological information, to gauge how a river system would reflect changes in the wider environment. Climate change usually refers to changes in air temperature and meteorological patterns in regions, and this, along with precipitation levels and vegetation cover, exerts a major control on a river's flow regime and influences the sediment load of a river system. So, an understanding of the environment type and the past environmental conditions is crucial in predicting how future climatic changes will alter the present environment.

Other factors contributing to change in the river basin, such as tectonic activity creating cross-valley tilting or disturbances such as external factors like climate, vegetation, land use and base-level are a major cause of instability in the fluvial system. Internal change within the basin can also be initiated from the inputs to the channels such as the amount of precipitation occurring and energy in a localised area causing higher levels of erosion once the thresholds of the normal functioning of the river have been overcome (Shumm, 1973).

Therefore, there are several natural influences on river systems, but these natural processes often have a much longer time scale for the impact to be noticeable overall. Therefore, whilst still important, the human influence on chalk streams has been more immediate and has caused a larger shift in the morphology and functioning of the chalk streams over the past 300 years or so, which is discussed in the next section.

In particular, the last 300 years has seen an increasing human impact on drainage basins and their constituent channels. The scale of climatic fluctuation has been much less during that period of 300 years, but human modification of the physical environment may have induced similar changes that would have naturally taken years as described in table 3 (Knighton, 1998; Shaw, 1983). Direct modification has taken place on the majority of rivers in the UK to alleviate the impact of flooding on residential, industrial and agricultural locations. Indirect changes result from activity in

extra-channel areas that modifies the discharge or sediment load of the stream and ultimately results in stream channel response, such as dredging to remove channel bed sediment to generate faster channel flow. River regulation has also occurred through constructing dams and there are around 2,800 dams across England, Scotland and Wales currently (Knighton, 1998, McKie, 2019).

Chalk streams have experienced major alterations to their natural form. For example, water meadows are a common feature found adjacent to chalk stream channels on the floodplain, and even up to the headwaters (some existed as far up in the winterbourne section to take advantage of the winter flow, although these had less success). These meadows were mostly used for grazing of sheep and cattle, as the flooding of the floodplain would be controlled to grow a flush of new grass (Mainstone *et al.*, 1999). To create the water meadows, large areas of alder and willow trees were cleared, particularly on larger chalk streams like the River Test and River Itchen, which permanently altered the character and morphology of the chalk stream landscape, with only a few small stretches of river remaining untouched today. The removal of the trees increased the amount of light available to the river channel and floodplain, allowing increased plant growth. The majority of this meadow formation took place in the 1600 – 1800s during the agricultural revolution, as previously only small sections were cleared in the Roman and Saxon times. This also involved the establishment of larger river channels, removing the complex braided network to suit the needs of farming and settlements (Mainstone *et al.*, 1999).

However, since the majority of chalk streams are now protected in their current state by the UK BAP, most conservation organisations do not aim to return to this state as it would be hugely detrimental to the current flora and fauna that the modified chalk streams are valued for. Therefore, the conservation of chalk streams focusses on preserving the low-intensity, meadow-dominated catchments with a high-water table and frequent winter flooding of the floodplain, rather than a return to the woodland carr that originally surrounded the streams (Mainstone *et al.*, 1999).

1.3. The Geology of Chalk Streams

This section contains a brief overview into the geology of chalk streams as a whole. Section 3.1.2. provides specific detail on the Colne catchment that has been used in this study.

Chalk streams take their namesake from the underlying geology of the area and it is where the aquifer that feeds the chalk stream is situated. Cretaceous Chalk is the dominant bedrock in many lowland areas of Great Britain, especially in southeast England. This means that many of the aquifers in southern England are situated in chalk bedrock and outcrops of calcareous rock and are a major water supply aquifer (Wharton *et al.*, 2006; BGS, 2023). Chalk is a dual permeability, dual porosity medium with a fine-grained, high porosity, low-permeability matrix and a fracture network that allows the transfer of water and the fracture porosity is 1% (Price *et al.*, 1993). Seasonal fluctuation in water table elevation can be large e.g. 10 -1 20m across interfluvial areas at the start of the winter recharge season, and the variation between years can also be significant (Parker, Butler & Jackson, 2016). This variation in water table elevation means that chalk river systems are characterised by *bourne* behaviour where the river's source migrates up and down the river valley seasonally.

Chalk aquifers provide a large groundwater resource in Europe, especially in the UK and they are responsible for maintaining the stable flow in chalk rivers during periods of low rainfall (Allen *et al.*, 2010). The chalk aquifer in Britain, commonly referred to as the Chalk is the most important aquifer in southeast England and is part of the larger Thames Basin, which also contains the Colne catchment. It is formed from a dual porosity medium with groundwater flow occurring within both the matrix and through fractures and most of the aquifer's storage is from these fractures (BGS, 2023). These fractures are parallel to the bedding plane within the upper 60 metres and have a decrease in permeability with depth. This creates a strong topographical control on the transmissivity, where there are high transmissivity values within the valleys and lower towards the interfluvial areas (BGS, 2023). It is a valuable groundwater resource and needs to be managed sustainably to ensure the water table is maintained to allow for sufficient baseflow into the chalk streams, as well as being critical for the water supply for London and the home counties.

Chalk rivers are often controlled by the aquifers, and their hydrology responds to the contributions of groundwater. The chalk outcrop is where the unconfined chalk is drained by intermittent streams in periglacial valleys, which are where the winterbourne stretches of chalk streams rise. Here, groundwater baseflow contributions are high (BFI 0.95) and spring lines are well developed (Figure 1.14) (BGS, 2023). This leads to relatively large seasonal water table variations, and the upper headwater of the stream can migrate up dry valleys due to the high fracture permeability and low effective matrix porosity. Through the London Basin, the chalk becomes confined by Palaeogene deposits within the syncline, and this leads to the formation of karstic features within the aquifer due to the acidic runoff from these deposits (BGS, 2023).

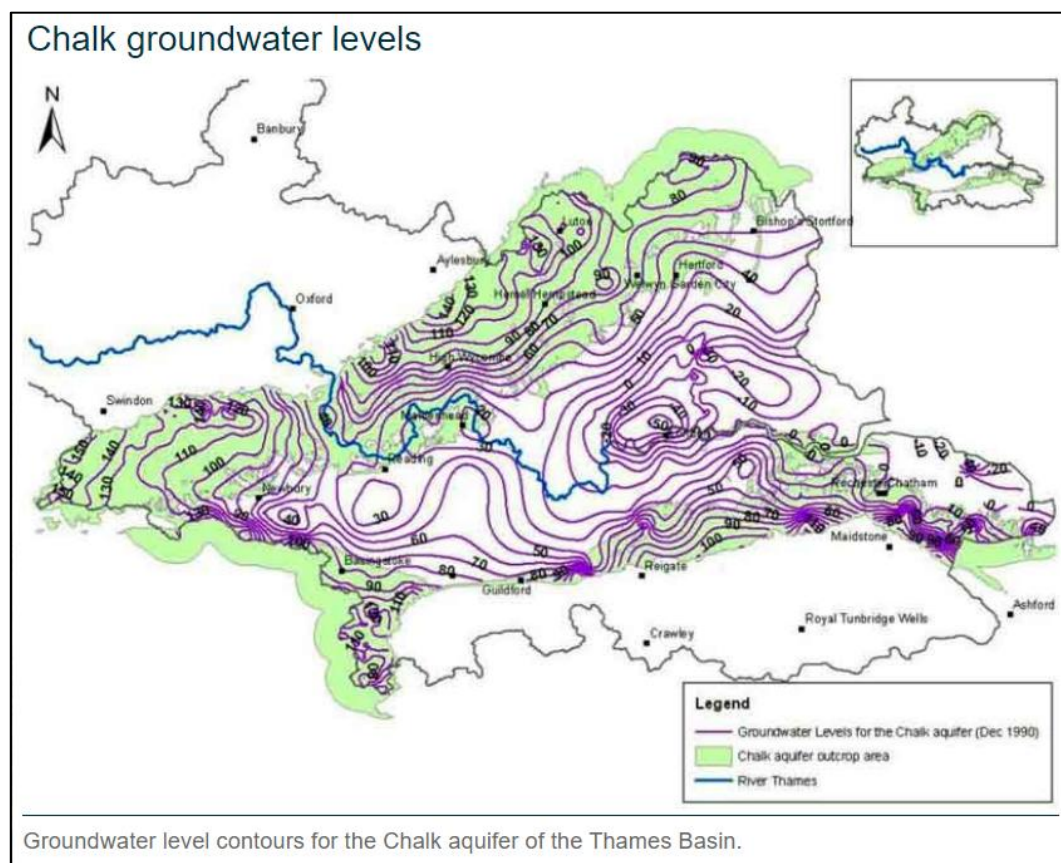


Figure 1. 14: The chalk groundwater levels across the Chalk aquifer and London Basin. (BGS, 2023). The River Colne's source is within the chalk aquifer outcrop area with a ground water level of 60 mAOD (Meters Above Ordnance Datum) in 1990.

1.4. The Water Chemistry of Chalk Streams

Water chemistry is the chemical makeup of the water in a river system or waterbody and involves any pollutants or organic and inorganic compounds that end up dissolved in the water. Not all chemicals found in water present a risk to the water environment, but if they are at or above a threshold level, this may lead to detrimental impacts on aquatic life and those that rely on these waterbodies to survive. Chemicals can be persistent, which means they accumulate in the environment or through the food chain, and some substances can cause issues with the endocrine system in fish, and lead to increased risk of cancer, reproductive problems and damage DNA (EA, 2021).

Chemicals found in water can either be from point or diffuse sources. Diffuse sources are those such as road runoff, agricultural runoff, mining activity and sewage overflow (Defra, 2012). Common point sources are misconnections of sewage pipes in houses and outputs from businesses and industries. These chemicals can range from fertilisers, solvents, hormones, pharmaceuticals, heavy metals and others (Defra, 2012; EA, 2021). Without human activity, most of these chemicals would not be entering the river network, so therefore it is important to understand what they do and how they alter the environment and functioning of the river ecosystem. This chapter discusses the common chemicals found in both the groundwater and surface water systems of rivers and chalk streams and the mitigation that has been put in place.

1.4.1. Groundwater and Chalk Aquifer Chemistry

About 35% of public water supply in England and Wales is from groundwater resources, but this average is exceeded in the southeast on England where it is 70% (Shand *et al.*, 2003). Groundwater seepage, where the water from the aquifer uprises into the springs of the river, is essential for summer river flows and other river environments, such as maintaining nearby wetland habitats on the floodplains. Therefore, the water quality and quantity is very important to sustain the water supply of these environments (Shand *et al.*, 2003). Until the middle of the 20th century, groundwater was a predominantly clear water source, but with increased abstraction and industrial activities, these groundwater sources have had increased amounts of a

range of chemicals (EA, 2021). Most of the compounds found are industrial compounds, pesticides, pharmaceuticals, personal care products, halogenated solvents and plasticisers, and some of these substances are toxic, although most are only present in low concentrations (EA, 2021). However, due to the slow flow rates of some aquifers, these chemicals have a long residency time and can still be detected decades after the initial pollution incident. For example, in 2003 the pesticides atrazine and simazine were removed from use, but they are still found in groundwater today (EA, 2021).

The Chalk aquifer in the London basin accounts for more than half of all groundwater use in England (Shand *et al.*, 2003). Therefore, the chemical makeup of the water in the aquifer is important to ensure it does not have negative impacts on the environment and, in turn, the human population that relies on rivers for clean, unpolluted water. The water chemistry is controlled by the natural reactions within the chalk matrix. Groundwater rapidly reaches saturation with calcite during the infiltration due to the fine-grained nature of the matrix and unconfined aquifer are of a Ca-HCO₃ type (Shand *et al.*, 2003). Dissolution then releases impurities in the chalk (Mg, Sr) which increases in concentration with the age of the groundwater. The water is also mixed with formation water (water that has resided within the confined aquifer) that has not been flushed from the aquifer, giving rise to higher concentrations of chloride (Cl), and some ion-exchange occurs, releasing sodium (Na) from clays which is exchanged with dissolved calcium (Ca) deep within the chalk matrix into the water from aquifer freshening (where the water goes from being brackish to freshwater) (Shand *et al.*, 2003; Lipparini *et al.*, 2023).

The most significant changes happen when the chalk becomes confined by the Palaeogene rocks at the redox boundary of the aquifer. This is where oxygen cannot be detected and nitrate is removed from the system by denitrification and iron (Fe) and Manganese (Mn) levels increase (Shand *et al.*, 2003). This shows how the water chemistry can vary throughout the aquifer due to various chemical processes that occur. Chalk groundwater chemistry has also been found to have high bicarbonate, calcium and pH levels and the greatest difference is between unconfined and confined chalk. Where the Chalk is concealed beneath superficial deposits of low permeability

material, the aquifer is confined as the low permeability prevents direct recharge and the water is under pressure (figure 1.15A). Confined chalk therefore has been found to have low oxygen and nitrate levels, as it is not well connected to other recharge areas and the residency times are longer (Maurice *et al.*, 2016).

Dissolved carbon dioxide is found in chalk aquifers because of calcium carbonate (calcite) acid dissolution brought about by microbiological activity (Griffith *et al.*, 2007). The rate of the reactions depends on the CO₂ concentrations and degree of mineral unsaturation in the water. The Upper and Middle Chalk formations (Figure 1.15B) have relatively pure calcium carbonate but may still have impurities such as Magnesium (Mg), strontium (Sr), manganese (Mn) and iron (Fe). The chemistry of rainwater percolating through soils and bedrock will change as it passes through different subsurface layers. Chalk soils are thin (around one metre) and are well drained, however, they have a slow infiltration time of about 1m-year. The biological activity in the soil leads to an increase in CO₂ concentration from around 340 ppm to 6000ppm in the soil (Edmunds *et al.*, 1992). The rate of CO₂ production is linearly related to temperature and also the type of vegetation on the soil e.g. more CO₂ production on unfarmed soils. After the soil layer, the water percolates into the dual-porosity medium of the unsaturated zone of Chalk. Due to the biological reactions in the soils, the water held in the soil layer is nearly at equilibrium with groundwater CO₂ concentrations. Therefore, the water is displaced downwards due to gravity allowing bicarbonate and calcium ions to transfer and be liberated from the chalk matrix. In the saturated zone, calcite saturation is rapidly established near bedrock outcrops (Edmunds *et al.*, 1992). The Chalk continues to experience diagenetic changes, where older stored water comes into contact with younger water, leading to the slow chemical modification of the groundwater. Deeper groundwater may experience mineral dissolution, precipitation and cation exchange at a slower rate than near-surface groundwater. The salinity will also be higher at deeper levels. This shows that there is a geochemical zonation of groundwater with depth (Griffith *et al.* 2007). Higher concentrations of CO₂ in groundwater entering surface-water drainage systems will lead to CO₂ degassing which can affect stream-water chemistry. CO₂ production and consumption from biological and photosynthetic activity was not found to be enough to mask the groundwater-surface water difference in dissolved CO₂ levels. This means that the groundwater CO₂ level will have a detectable impact on the environment created from

the upwelling of this groundwater source, such as increasing the acidity of the water. Therefore, if CO₂ levels rise too high in the soil and percolate into the aquifer, this could impact organisms such as those who have high calcium contents.

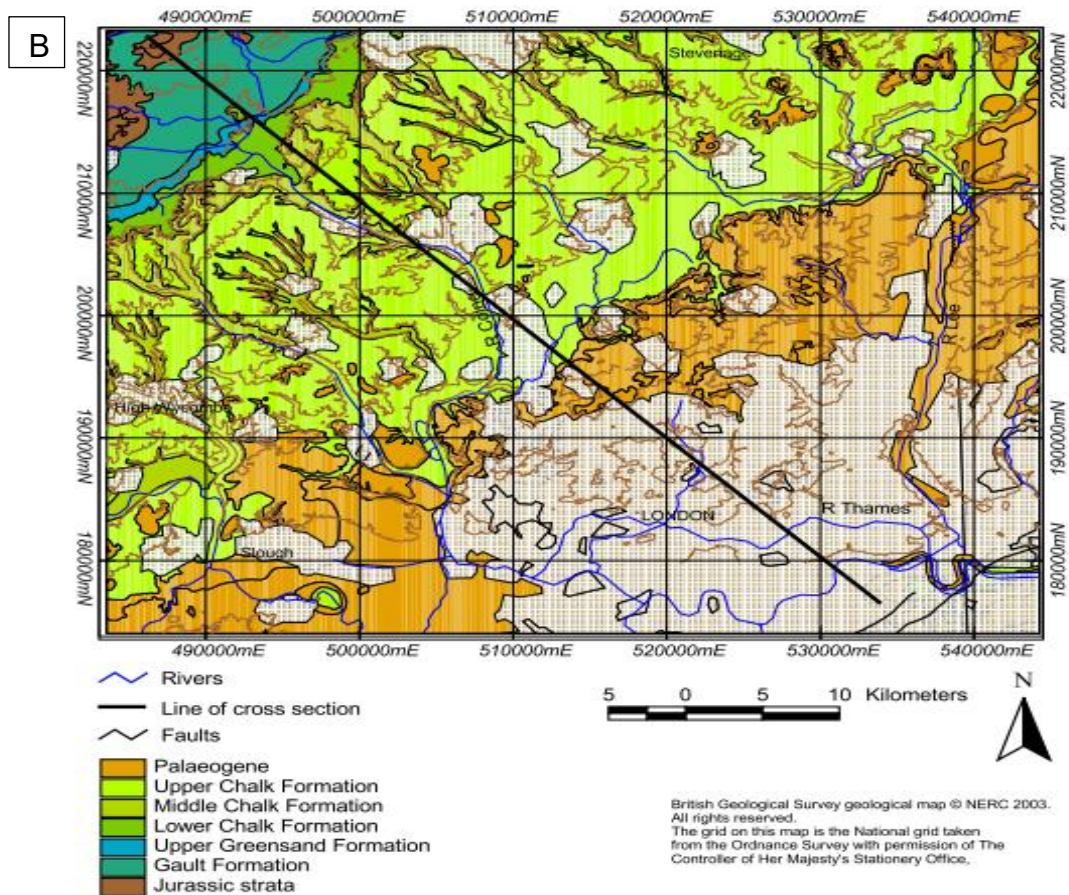
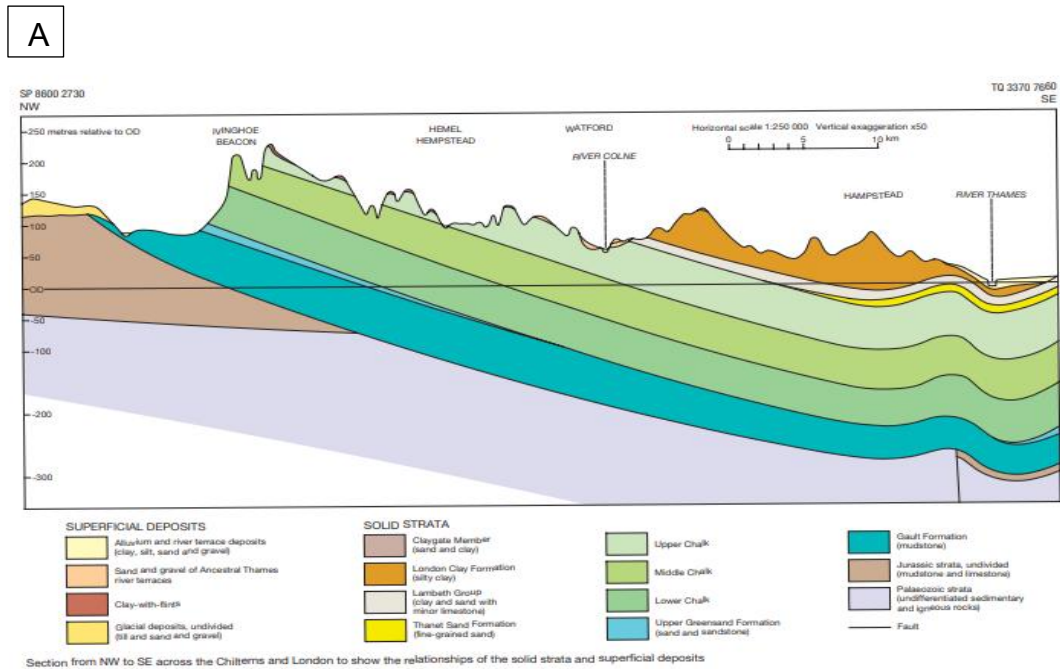


Figure 1. 15: A) The geological cross section of the Chalk aquifer, going from north to South across the Colne Valley catchment. The River Thames is confined by the London Clay formation, whilst the River Colne dips into the Upper Chalk formation, allowing it access to the unconfined aquifer (Shand et al., 2003). B) The geology of

the chalk aquifer in the Thames Basin and the different chalk formations that make up the Chalk. The black line represents the cross section in figure 1.15A (Shand et al., 2003).

1.4.2. Surface Water Chemistry

The water chemistry of riverine environments is dependent upon biological, chemical and physical inter-related components that are influenced by natural and human processes (Casey, 1976). Therefore, an understanding of water chemistry is crucial to decipher the ecological community too, as many organisms require specific environmental conditions. As previously discussed, groundwater chemistry is particularly important in chalk stream catchments, as the groundwater provides a significant proportion of the baseflow that sustains the chalk streams, in comparison to the surface runoff contribution that usually sustains other alluvial river types. However, the contribution of surface runoff in chalk streams cannot be discounted, particularly with more impermeable surfaces being created and the likelihood of more extreme flood events occurring, generating increased surface runoff conditions in chalk streams. It has also been found that the ecology of chalk streams changes depending on the land use within the catchment, such as changing from grassland to arable farming with increased fertiliser usage (Casey, 1976) as this impacts the water chemistry of the surface water entering the chalk streams.

A study conducted by Casey (1976) investigated two chalk streams in Dorset between 1965 and 1966: the Bere Stream (a smaller chalk stream) and the River Frome (a larger chalk stream). They took measurements of rainfall, discharge, dissolved oxygen and water temperature and chemical parameters. The smaller stream had a fairly constant chemical composition, whereas the River Frome displayed larger fluctuations. An inverse relationship between bicarbonate alkalinity and calcium, and discharge was found, where the concentrations of alkalinity and calcium decreased when there were higher rainfall inputs into the river system, diluting the chalk-influenced groundwater contributions (Casey, 1976).

Phosphates, silicate and potassium had a more varied, unpredictable variation in both the small and larger chalk streams. In the Bere Stream, there could have been influence from the fertiliser use at the watercress beds upstream, where the

concentration varied from $41 \mu\text{g l}^{-1}$ to $660 \mu\text{g l}^{-1}$ of dissolved reactive phosphorus ($\text{PO}_4\text{-P}$) with a mean of $204 \mu\text{g l}^{-1}$ during working days, but at the weekends would only range between 17 to $50 \mu\text{g l}^{-1}$ with a mean of $37 \mu\text{g l}^{-1}$ (Casey, 1976; Westlake *et al.*, 1972). Magnesium variations were shown to have little variability in the River Frome, whilst alkalinity and calcium concentrations decreased in times of flood and nitrate concentrations were usually highest at peak discharges, whilst sodium concentration were highest after snowmelt off the roads (Casey, 1976). Phosphate-phosphorus and potassium had the greatest fluctuation, and phosphorus showed a reduction between February and May, which the study suggests was due to diatom growth (Casey, 1976). Potassium concentration was at its lowest during high stream-bed cover of macrophytes. Discharge and nitrate level were found to correlate in one of the winterbourne tributaries of the River Frome and can have a significant influence onto the channels they flow into. For example, at peak flow $1.51 \text{ m}^3 \text{ s}^{-1}$ the winterbourne was contributing 8.9% of the total flow of the River Frome at East Stoke (Casey, 1976).

A further investigation into this winterbourne stream, named South Winterbourn, was undertaken (Casey & Ladle, 1976) which found that the changes in flow and chemical composition were related to the biological and physical conditions of the environment. Only two out of eight sites completely dried up in the summer, and as the flow decreased the pollution from phosphate and potassium became more obvious (Casey & Ladle, 1976). Nitrate levels were shown to be the highest when the discharge of the river was in a state of flux between drought or low flow and high flow, which was thought to be brought about by the leaching of nitrates, as this has not been common in other chalk stream catchments such as the Piddle or Frome (Casey, 1976). Since the alkalinity and calcium concentration remained relatively stable, these chemicals must come from the chalk groundwater and not run-off.

Phosphate was found to largely be dependent upon sewage effluent as the flow of this increased from $0.054 \text{ m}^3 \text{ s}^{-1}$ in 1965 to $0.070 \text{ m}^3 \text{ s}^{-1}$ in 1972. Nitrate was found to be fairly stable in the Frome catchment, but this deviated from the trend from other chalk streams in the UK which could be due to the farming style in Dorset or the geological structure of the chalk (Casey, 1975). The nutrient content of watercourses has been found to link to macrophyte growth, although there is often an excess in the water compared to the macrophyte growth requirements and it had not yet been understood

if the macrophytes can utilise the nutrients directly from the water or if it must be obtained from the bottom sediments (Casey, 1976; Casey & Newton, 1973).

A study by Jarvie *et al.* (2006) also found that soluble reactive phosphorus (SRP) and boron concentrations in chalk streams like the Pang and Lambourn are heavily influenced by point-source inputs, rather than only diffuse sources, and the effects of dilution and accretion further downstream. Soluble reactive phosphorous (SRP) accounted for 66% of the total phosphorous concentration under baseflow conditions. Nitrate accounted for 87%+ of the total dissolved nitrogen in the Pang and Lambourn. Downstream, it was found that SRP increased after the sewage inputs. SRP showed negative relationships with flow under low flow conditions (Jarvie *et al.*, 2006). Overall, this shows that both point and diffuse sources of pollution or specific chemicals in surface runoff can have a significant impact on the water chemistry of chalk streams.

1.4.3. Water Chemistry of the Colne Catchment

The Environment Agency's Report on the Colne (1997) used the General Quality Assessment scheme to regularly monitor the water quality of rivers in the area using four components of general chemistry, nutrients, aesthetics and biology, but only general chemistry was used in the report (the other components were under development). The GQA has six grades (A to F, with A and B being good, C and D fair, and E and F poor quality) defined by the standards for dissolved oxygen, Biological Oxygen Demand (BOD) and total ammonia (EA, 1997). The majority of the rivers within the Colne catchment were either C or D, being "fair" quality, with higher quality streams being found in the west of the catchment area with tributaries of the Colne like the Chess, Misbourne and Alderbourne achieving a "good" status (EA, 1997). The River Colne achieved a grade C along its upper reaches from its source in North Mymms to Bricket Wood, where the quality increases to a grade B towards Watford, before deteriorating to a grade C again towards Rickmansworth and London (EA, 1997). The River Colne experiences high turbidity due to large numbers of individual particles becoming suspended in the channel discharge by heavy rainfall transporting silt and sediment from agricultural land (Louis, 2019).

1.4.4. The Management of the Water Chemistry of Chalk Rivers

The Water Framework Directive (UK Technical Advisory Group, 2013) sets out the status objectives for water bodies in the UK and include the following: “To prevent the deterioration in status of the water bodies; to restore water bodies to good status by 2015”. Standards are set to aid the achievement of the objectives and these can set limits on the amount of water abstracted from a water course, or how much of a pollutant is deemed acceptable to enter the water body, in order to protect the environmental quality (UKTAG, 2013). There are no clear thresholds across Europe for nutrients in surface waters (Poikane *et al.*, (2019). Nitrates have a 50mg/l threshold in the EC Nitrates Directive and EC Drinking Water Directive. Rivers should not exceed 0.1mg/l annual mean phosphate concentrations (Muscutt & Withers, 1996). The threshold for UK rivers is 0.1mg/l for phosphates but the majority of rivers are more than 1.0mg/l. These thresholds are outlined in table 1.2.

Table 1.2: The standards for the water quality parameters outlined in the Water Framework Directive (UK TAG, 2013).

Parameter	River Type	High	Good	Moderate	Poor	Bad
Dissolved oxygen (% saturation)	Upland, low alkalinity	80	75	64	50	<50
	Lowland, high alkalinity	70	60	54	45	<45
Total ammonia (mg/l)	Upland, low alkalinity	0.2	0.3	0.75	1.1	>1.1
	Lowland, high alkalinity	0.3	0.6	1.1	2.5	>2.5
Biological Oxygen Demand (mg/l)	Upland, low alkalinity	3	4	5	7.5	>7.5
	Lowland, high alkalinity	4	5	6.5	9	>9
Water Temperature	Salmonid waters	20	23	28	30	NA
	Cyprinid waters	25	28	30	32	NA
pH	Clear waters with a dissolved organic carbon value of 10mg/l or less	6.6	5.95	5.44	4.89	NA
	Humic waters with DOC value of more than 10mg/l	5.1	4.55	4.22	4.03	NA

Managing the chemicals that enter waterbodies is a complex situation that requires a range of mitigation and interventions – ranging from voluntary schemes to regulations that require safer use or withdrawal of certain chemicals. Most of the legislation relating to chemical risks is from the EU and international level, meaning that in the UK, the initiatives to reduce chemicals in the environment are put in place to deliver on the international agreements. The European REACH Regulation, the Plant Protection Products Regulations 2011 and the Biocidal Products Regulations (EU) (528/2012) are regulatory instruments that come from the EU to control the manufacturing of chemicals and ensure safer use of the chemicals (EA, 2021). Another method is Environmental Permitting Regulations which require users of certain chemicals to obtain a permit and to adhere to limits on the releases of chemicals and reduce the risk of pollution incidents.

Understanding the impact a chemical could potentially have on the environment, including the hazardous nature of the chemical(s) and the nature and extent of the exposure on the environment, is critical to protect the aquatic life, human health and water supplies from chemical contamination. The Environment Agency (2021) does this through a strategic approach that identifies chemicals on a national, local or emerging scale and provides sustainable management that is appropriate to meet the WFD objectives. Local concern chemicals are those that have only a few isolated issues that would be best managed at the water body or catchment level with partnerships between local authorities, water companies, NGOs and agricultural, environmental and government agencies. This may be seen as water companies engaging with stakeholders to reduce certain chemicals entering the watercourse through sewage outputs. Chemicals that have a national concern are more widespread and usually involves the output of emissions and their pathways into the water system, rather than remedial action after a pollution incident. Chemicals of emerging concern do not have a statutory Environmental Quality Standard (EQS), but the Environment Agency is part of a network of organisations that are analysing data on newer chemicals to identify their risk to the aquatic environment. The EU already has a Watch List of potential polluting substances that may need future designation, to decide on their level of concern and if they are a priority substance, and are compared against the Environmental commissions Predicted NO Effect Concentrations (PNECs), of which three substances in 2018 have exceeded these

thresholds: Imidacloprid (an insecticide); 17 α -ethinylestradiol (EE2) (a hormone); and Tri-allate (a herbicide) (EA, 2015; EA 2021).

The Europe's state of water 2024 report found that the chemical and ecological aims for good status within the Water Framework Directive had not been achieved by the 2015 deadline and in 2021 only 37% of surface waters achieved good or high ecological status, and 29% achieved good chemical status (EEA, 2024) and only 14% of the rivers in the UK have achieved good status (Environment Agency & Defra, 2023). However, there are still plans to improve upon the ecological and chemical status of the chalk streams and meet the WFD thresholds. The 25 Year Environment Plan establishes the goals the Environment Agency has to improve water chemistry, committing to safely use and manage the use of chemicals and significantly reduce the levels of harmful chemicals entering the environment (EA, 2018A). The four goals include to eliminate the use of Polychlorinated Biphenyls (PCBs) by 2025; to reduce land-based emissions of mercury by 50% by 2030; to increase the destruction or irreversible transformation of Persistent Organic Pollutants (POPs) by 2030; and to fulfil the Stockholm Convention commitments.

Since the River Colne is largely surrounded by agricultural land in its upper course, this project will mainly focus on the nitrates, phosphates and ammonia levels that are found in the river's discharge, as other studies on chalk streams have also focused on these chemicals (Bowes, Leach & House, 2005). A report by the Colne Valley Fisheries Consultative in 2023 highlighted the issue of micropollutants in the River Colne (CVFC, 2023) which is further discussed in the pollution section 1.6.

1.5. The Ecology of Chalk Streams

Chalk streams provide unique habitats that vary along the longitudinal profile in response to changing hydrological conditions. For example, winterbourne species exhibit traits that enable resilience against intermittent flow, such as drought-resistant eggs and high dispersal potential (Mandon *et al.*, 2021; Bunting *et al.*, 2021). The rich diversity means that many chalk streams are protected by national and international legislation, such as the designations of Sites of Special Scientific Interest (SSSI) and Special Areas of Conservation (SAC). An overview of priority species found in chalk streams are listed in table 1.3.

Table 1.3: The designated priority species that are characteristic to chalk streams and the chalk stream fluvial habitat they are most commonly found in (SAC: Special Area of Conservation, BAP: Biodiversity Action Plan Priority Species) (Mandon *et al.*, 2021).

Group	Species	Winterbourne	Perennial	Designation
Plants	Pond water-crowfoot (<i>Ranunculus peltatus</i>)	Y	Y	SAC - Annex I habitat
	Stream water-crowfoot (<i>Ranunculus penicillatus</i> subsp. <i>Pseudofluitans</i>)		Y	SAC - Annex I habitat
	River water-crowfoot (<i>Ranunculus fluitans</i>)		Y	SAC - Annex I habitat
Macroinvertebrates	Southern damselfly (<i>Coenagrion mercuriale</i>)		Y	SAC - Annex II species, BAP
	White-clawed crayfish (<i>Austropotamobius pallipes</i>)		Y	SAC - Annex II species, BAP
	Desmoulin's whorl snail (<i>Vertigo moulinsiana</i>)	Y	Y	SAC - Annex II species, BAP
Fish	Atlantic Salmon (<i>Salmo salar</i>)	Y	Y	SAC - Annex II species, BAP
	Brown Trout (<i>Salmo trutta</i>)	Y	Y	BAP
	Brook lamprey (<i>Lampetra planeri</i>)	Y	Y	SAC - Annex II species
	River lamprey (<i>Lampetra fluviatilis</i>)	Y	Y	SAC - Annex II species, BAP
	Sea lamprey (<i>Petromyzon marinus</i>)		Y	SAC - Annex II species, BAP
	Bullhead (<i>Cottus gobio</i>)	Y	Y	SAC - Annex II species
	Spined loach (<i>Cobitis taenia</i>)	Y	Y	SAC - Annex II species, BAP

The flora and fauna of chalk streams play a key role in maintaining the overall health of these aquatic systems by supporting various ecological processes, including nutrient cycling and the oxygenation of the water (Westwood *et al.*, 2006). These organisms also help regulate biological oxygen demand and provide essential refuges and breeding sites for fish and invertebrate species. The presence of aquatic plants and animals also adds structural diversity to the water column by contributing material that helps to stabilise the streambed and offer surfaces for colonisation by algae and diatoms (Westwood *et al.*, 2006; Holmes, 1999).

This chapter therefore discusses the different ecological communities that inhabit chalk streams in the winterbourne and perennial sections and how this diversity contributes to the unique functioning of the chalk stream ecosystem.

1.5.1. The Biodiversity of Chalk Streams

1.5.1.1. The Biodiversity of Intermittent Streams

Chalk rivers have always hosted a diverse and unique assemblage of flora and fauna, from their original state through willow and alder meadows, to the more well-known meandering streams through water meadows which supports a variety of birds such as kingfishers catching fish and aquatic invertebrates for their food and watercress thriving in the stream flow (Pacioglu & Robertson, 2017). Many species that inhabit chalk streams have unique adaptations to ensure their survival in this dynamic environment, such as being able to survive periods of extended drought. This is particularly evident in the headwaters of chalk streams, or winterbournes, which usually only flow during the autumn and winter before drying up in the summer. During the spring months, when the river's flow is at its highest, chalk streams support an abundance of aquatic macroinvertebrates which are able to recolonise the environment after the drought period due to their unique adaptations and strategies to survive (Boulton, 2003; Vowles & Kemp, 2019). During the dry spell, when the rivers flow ceases and a mosaic of pools is created on the riverbed, aquatic invertebrates that were not mobile enough to reach downstream reaches provide food for the newly colonising terrestrial invertebrate species and other organisms such as beetles and bird species (Pacioglu & Robertson, 2017). This shows that the wet and dry phases have an influence on biodiversity, as a wider range of species can inhabit one site at different hydrological stages in the annual hydrological cycle of a chalk stream.

Although the chalk stream environment has contributions from both aquatic and terrestrial species, temporary and intermittent streams generally support fewer species than perennial streams (when comparing the taxonomic richness of aquatic communities (Datry *et al.*, 2014)). The total Walley, Hawkes, Paisley & Trigg (WHPT) (Paisley, Trigg & Walley, 2014) score has been found to increase with flow permanence with increasing taxa richness, but WHPT-ASPT (Average Score per Taxa) scores were

similar at perennial and long flowing sites, but lower at intermittent sites (Bunting *et al.*, 2020). The ASPT scores in this study were relatively stable and this means that it is appropriate to assess the ecological quality of winterbournes (Wilding *et al.*, 2018). However, there are some adaptations to the model that could be made, such as excluding the lotic species that are not present at temporary sites (Bunting *et al.*, 2020; Stubbington *et al.*, 2018). However, as the hydrological regime fluctuates and potentially alters in the future, the extent of the drought timing could significantly alter the food web and have impacts further downstream on fish stocks and mammals.

1.5.1.2. The Biodiversity of the Chalk Aquifer

The ecosystem within the chalk aquifer is poorly understood. A study by Maurice *et al.* (2016) found that stygobitic macro-invertebrates were common in unconcealed chalk aquifer and most species were found in shallow boreholes. Only two species were found in chalk aquifers in northern England compared to six in southern England. However, two of the species present in the south were not found in the north, suggesting they did not survive glaciations during the Devensian where glaciers were generally more extensive and had a colder environment for longer. Invertebrate species that live in groundwater are known as syngobites and they are crucial to the biodiversity of groundwater and have a role in the biogeochemical cycles and contaminant attenuation through the interaction with microbial communities (Maurice *et al.*, 2016). In Britain, there are eight stygobitic macro-invertebrate species which are crustaceans, however this is a low diversity compared to other areas of the world due to local extinctions during glaciations (Proudlove *et al.*, 2003; Robertson *et al.*, 2009). Stygobitic invertebrates are absent from concealed chalk where dissolved oxygen is low, but are common in unconcealed chalk, where there are deep water tables, suggesting there is a vertical ecosystem of invertebrate and microbial communities. Whilst these species are not present in the surface water, their contribution to biogeochemical cycles within the aquifer has an impact on the water quality that the chalk aquifers provide to the surface chalk streams and should still be noted as part of the ecosystem's biodiversity.

1.5.2. The Macroinvertebrate Communities in Chalk Streams

1.5.2.1. The Benthic Macroinvertebrates in Chalk Streams

One of the major communities of organisms inhabiting chalk streams are invertebrates. Freshwater invertebrates within all stream types have precise requirements for stream velocities, flow ranges, water chemistry and pH which means that certain taxa could be used as indicators of stream characteristic change within chalk streams and also monitor the health of these rare rivers (Extence, Balbi & Chadd, 1999). The use of macro-invertebrates (defined as invertebrates of size greater than 0.5 mm) as indicators of river quality have been used for at least the past fifty years. (Westwood *et al.*, 2017). Originally, the presence or absence of a particular species was thought to characterise the pollutant level in a river, but this has since been found to be an unreliable method to monitor water quality as part of river management (Davies, 2001). It has been replaced by biological quality of rivers and of other surface waters, which encompasses not only macro-invertebrates but many other facets of river quality. Due to their relatively sedentary lifestyle and long lifespans of benthic macroinvertebrates, they allow the possibility of detecting changes in water quality at the time of sampling and over longer time periods too (Rosenberg and Resh, 1996, Schwoerbel, 1999). They also show sensitivity towards many factors that are responsible for changes in water quality and are less expensive to use than some equipment (Stein, Springer & Kohlmann, 2008). The taxonomic diversity of a benthic invertebrate community reflects the water quality conditions, since generally a high diversity indicates a high water quality (Hynes, 1984). Secondly, specific taxonomic groups can be used as bioindicators, which are characteristic for certain habitat and water quality conditions (Stein, Springer & Kohlmann, 2008). Replicated, quantitative sampling in both riffles and pools interpreted through a variety of biological attributes provides the strongest assessment of biological condition based on invertebrates (Kerans, Karr & Ahlstedt, 1991).

Many of these macroinvertebrates and crustaceans found in chalk streams depend upon the mineral rich waters that the chalk streams provide, such as the higher levels of calcium (above 5 mg/l) and pH values of 7 - 9 that they use to develop their shells and exoskeletons (Mainstone *et al.*, 1999). Studies have found that the invertebrates that have been documented to make up the chalk benthic communities exhibit strong seasonal patterns in their colonisation and were influenced by increased water levels in winter, unlike those species within the hyporheic zone which were relatively stable throughout the year in perennial zones (Pacioglu & Robertson, 2017). The use of benthic invertebrates in the environmental assessment of aquatic habitats is well established within the literature, yet there are few studies on how the hydrological regime of a river relates to the structure of the benthic community (Extence, Balbi & Chadd, 1999).

Temporary stream communities are dominated by generalist species, but there are also resilient taxa which colonise certain habitats that become available (Datry *et al.*, 2014). In chalk streams particularly, there are some specialist caddisfly larvae (*Limnephilus vittatus* and *Glyptotendipes pallidus*) that inhabit intermittent karst network springs and they also support characteristic plant communities (Mainstone *et al.*, 1999), stonefly and mayfly nymphs. These species display life cycle adaptations associated with colonisation of temporary aquatic habitats, such as an extended flight period from spring into autumn or aestivation as adults during the drier periods (e.g. *Limnephilus auricula*, *Limnephilus lunatus* and *Micropterna sequax*) (Smith, Wood & Gunn, 2003). Some species are particularly restricted to chalk winterbournes too, such as the blackfly *Metacnephia amphora*. At intermittent sites, the aquatic community diversity is lower compared to similar perennial sites. Temporary streams have important ecosystem functions such as navigation corridors for vertebrates, habitat for specialist biota, refuge for lotic taxa, carbon cycling and organic matter processing (Stubbington *et al.*, 2017).

1.5.2.2. Specialist Macroinvertebrate Communities in Chalk Streams

The species that inhabit the winterbourne sections of chalk streams can be divided into permanent, facultative and specialised species. Permanent species are those that have migrated from the perennial sections of river to the temporary areas and are able to do so because of their high tolerance to a variety of conditions, displayed by the freshwater shrimp *Gammarus pulex* who can survive short periods of water loss. However, these species are not highly specialised and cannot survive longer periods of drought (Mainstone *et al.*, 1999). Facultative species are well designed for colonisation through the use of an aerial phase in their life cycles, such as species of Coleoptera and Hemiptera (Mainstone *et al.*, 1999; Cooling 1981) meaning they can exist in a wider range of water bodies. This means that intermittent sections may support more species than strictly perennial sections, but the community composition varies between areas and is not as consistent (Mainstone *et al.*, 1999). Specialised species of intermittent streams may be restricted to only those intermittent areas due to their highly adapted nature. For example, they may have life cycle strategies that make them resistant to drought or have the ability to use specific areas of refugia like the interstitial spaces within the gravel bed (Mainstone *et al.*, 1999; Stubbington *et al.*, 2017; Datry *et al.*, 2014; England *et al.*, 2019). Some species that are resistant to drying out are the pea mussels *Pisidium casertanum* and *P. personatum* and the gastropod *Anisus leucostoma*. Other species, such as the mayfly *Paraletophlebia wernerii* and Stonefly *Nemoura cinerea*, and blackfly *Metacnephia amphora*, lay eggs that can survive prolonged drought in the dry sediments of the riverbed, before hatching once the flow has resumed (Mainstone *et al.*, 1999; Wallace & Wallace, 1990). The caddis fly *Limnephilus auricula* has a reproductive diapause where ovarian maturation is delayed until springtime, as well as a long adult flight time which favours the set-up of temporary streams. Other species are able to bury themselves in the riverbed and use the deep gravel substratum to their advantage (such as the flatworm *Phagocata vitta*, crustacean *Niphargus aquilex*, and dytiscid beetles *Hydroporus marginatus* and *Agabus biguttatus*) (Mainstone *et al.*, 1999; Macadam, Stubbington & Wallace, 2022).

Some of these specialist species are rare, such as the blackfly *Metacnehia amphora*, mayfly *Paraleptophlebia weneri* and stonefly *Nemoura lacustris*. The presence of these species in winterbourne chalk rivers increases the conservation status of these rivers, compared to perennial rivers (White *et al.*, 2018). There are also notable terrestrial species that inhabit the dry riverbed, but these communities still remain largely poorly documented (Stubbington *et al.*, 2019a). The benthic community taxonomic richness in chalk rivers is found to be higher at perennial reaches, compared to intermittent sites (Bunting *et al.*, 2020) and previous research has shown that the absence of sensitive lotic species reduces invertebrate richness in non-perennial streams (Datry *et al.*, 2014; White *et al.*, 2018). However, these studies also showed that even though intermittent streams had overall lower total richness, they still supported taxa that is not present in perennial sites (Bunting *et al.*, 2020; Datry *et al.*, 2014). These species included stonefly *Nemoura lacustris* (Nemouridae). This species has been defined as a temporary stream specialist because its eggs remain dormant in dry sediments and later hatch when water is available during the cooler seasons where river flow has resumed (Bunting *et al.*, 2021). Seedbanks of desiccation-tolerant invertebrate life stages can support the recolonisation of species after a dry event (Stubbington & Datry, 2013). Other species that have been found only in the temporary sites include 15 *Leptophlebiidae* (Ephemeroptera) juveniles and 275 *Simuliidae* (Diptera) larvae, although these do not have species level identification, so their distribution is not fully known (Bunting *et al.*, 2020). There were also 97 beetle taxa recorded in the study by Bunting *et al.* (2020), including ground beetle species that were found in the terrestrial invertebrate community. These beetles are very mobile and colonise the dry riverbed from the marginal and riparian habitats to exploit newly exposed resources, including dead aquatic invertebrates (Mascagni, 2014). Four nationally rare or scarce species: *Amara montivaga*, *B. unipustulatus*, *B. peltatus* and *P. anthracinus* (*Carabidae*) were found. However, this study only focussed on beetles, meaning the diversity of other terrestrial invertebrate species, such as spiders and ants, was overlooked. A study by England *et al.* (2019) aimed to create an index based on the flow preferences of both aquatic and terrestrial invertebrates within the chalk aquifer (the Colne and Lee catchments) and another focused on the use of invertebrates as bioindicators for various measures such as the WHPT, Lotic-invertebrate Index for Flow Evaluation (LIFE) metrics and within the Thames basin (Tough *et al.*, 2020).

1.5.2.3. Macroinvertebrate diversity within Chalk Streams

Taxonomic diversity of an assemblage is its species richness and evenness, which means the number of taxa present and the relevant contribution each species makes to its total abundance (Stubbington *et al.*, 2017). Alpha diversity is the taxa present locally in one sampling unit, and beta diversity is the heterogeneity between sites. The temporary presence of different habitats at the same location enables sequential use of lentic, lotic and terrestrial taxa to share the same environment and this sequence is shown in figure 1.16 (Stubbington *et al.*, 2017).

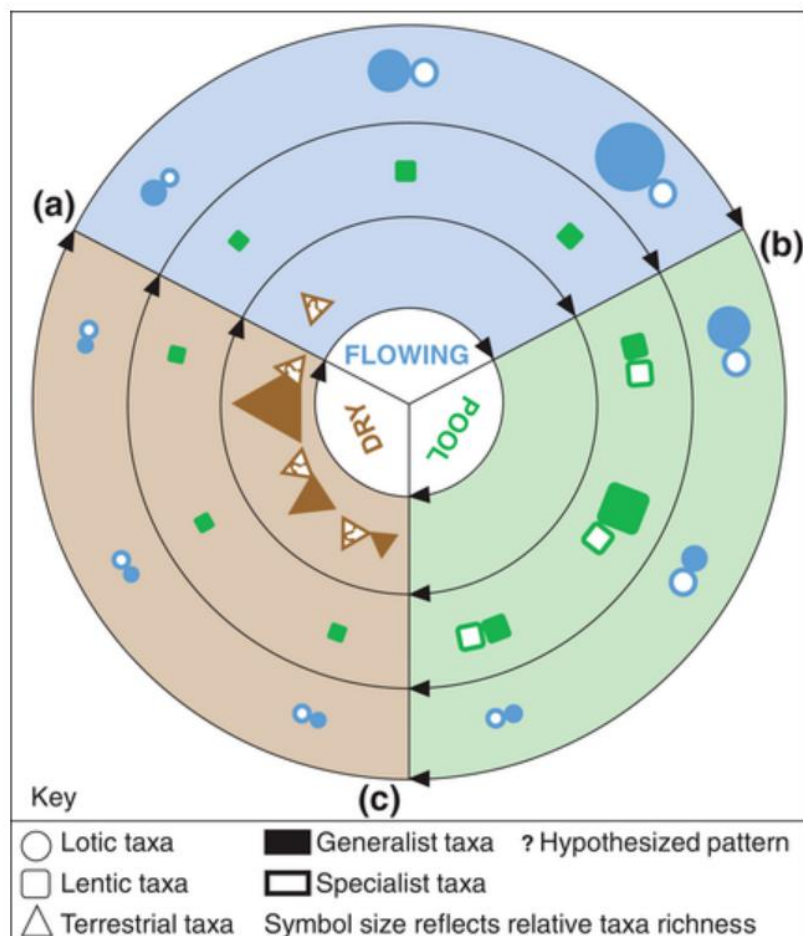


Figure 1. 16: The turnover of lotic, lentic and terrestrial taxa during flowing, pool and dry habitat phases in a temporary stream reach. Arrows indicate the typical annual cycle of environmental changes, reversals and omissions (Stubbington *et al.*, 2017).

If flow is resumed after a period of drought in the summer, the lotic taxa richness increases rapidly as the species hiding in refuges in the intermittent reach rise in number and colonists increase too. Generalists normally dominate this population, with a few specialists present. The inundation-tolerant terrestrial taxa will have a short-lived persistence in the wet-phase (Stubbington *et al.*, 2017). When flow stops, pools may form and the richness in these pools may increase due to the colonisation of lentic species alongside lotic refugees, but due to poor habitat stability, and lower water quality, the richness then decreases. During the dry phases, generalist taxa colonise from the riparian zone and catchment, increasing in richness as the dry phase goes on. While the aquatic richness decreases as desiccation-sensitive species are lost, the seedbank of desiccation-tolerant aquatic life stages in the sediment allows a moderate richness to exist (Stubbington *et al.*, 2017). The annual hydrological cycle of chalk streams determined by the groundwater levels and recharge of the chalk aquifer that supplies the winterbourne headwaters leads to greater temporal variation in environmental conditions and means that intermittent sites can have a higher biodiversity than perennial sites with the result that there is more spatial diversity due to more variance in habitats, compared to perennial reaches (Figure 1.17). This leads to greater regional scale aquatic diversity due to more spatial and temporal heterogeneity.

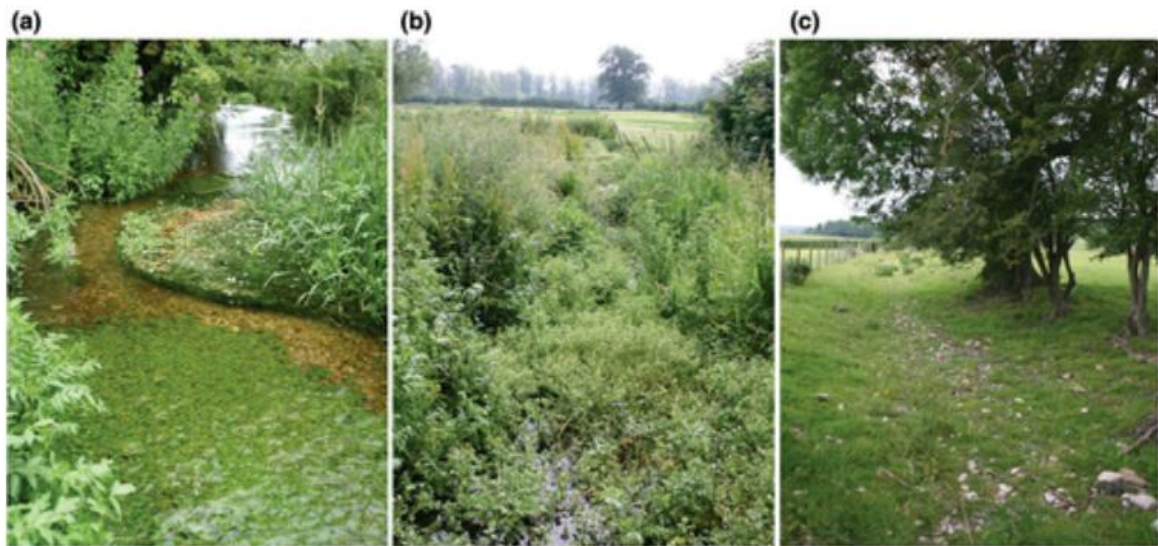


Figure 1. 17: The River Misbourne has longitudinal zonation of macrophyte species during the summer months. a) perennial flow, b) shorter dry period (2-3 months), c) longer dry period (4-8 months). (Stubbington *et al.*, 2017).

It has been found through kick sampling and pitfall trap use, that both wet and dry channels support a diverse invertebrate community that contributes to temporary stream biodiversity, with 54 aquatic and 120 terrestrial taxa recorded (Bunting *et al.*, 2020). The Whalley, Hawkes, Paisley & Trigg Score (WHPT) score (Water Framework Directive, 2021) was higher in areas with flow permanence, along with increasing taxa richness, whilst the WHPT-ASPT scores were comparable at perennial and long flowing sites, but lower at intermittent sites. This shows that the stability of the Average Score Per Taxon (ASPT) score could be appropriate to assess the ecological quality of winterbournes (Wilding *et al.*, 2018; Bunting *et al.*, 2021). The taxonomic richness of benthic communities was higher at perennial sites compared to intermittent sites and the taxonomic richness of hyporheic communities was higher at perennial sites too and included *C. luctuosa/macrura* and the caddisflies *Agapetus fuscipes* (Glossosomatidae) and *Odontocerum albicorne* (Odontoceridae) (Bunting *et al.*, 2021). This was found to be similar to the results of research conducted by Datry *et al.*, (2014) and White *et al.* (2018) who also found that sensitive lotic species are not present in temporary or winterbourne streams, with a consequent reduction in aquatic invertebrate richness (Bunting *et al.*, 2021). Despite this, the winterbourne

temporary sites did support rare taxa not found elsewhere, such as 22 juveniles of the stonefly *Nemoura lacustris* (Nemouridae) being found at all four winterbourne sites, but not at any perennial site, indicating it is a temporary stream specialist (Bunting *et al.*, 2021). However, despite these findings, the study found overall that hyporheic communities among flow permanence regimes, difference between the permanent and intermittent sections, but this could have been due to low replication of the study (Bunting *et al.*, 2021).

Lancaster & Ledger (2015) ran an experiment in the stream mesocosm where they tested short-term drying events (5-6 days) which resulted in population losses of macroinvertebrates. They conducted 10 replicate drying events over a year and found a range of 12 core taxa in the community including *Oligochaeta*, *Gastropoda*, *Gammaridae*, *Diptera*, *Ephemeroptera* and *Trichoptera*. The responses to drying were site-specific and there were three different response types: half the taxa were drying resistant (no mortality even in total loss of water); density-independent mortality; and density-dependent mortality. The 12 core taxa are as follows: two molluscs (*Potamogyrgus antipodarum*, *Radix balthica*), one Gammaridae (*Gammarus pulex*), one Ceratopogonidae (*Bezzia sp.*), one *Ephemeroptera* (*Baetis scambus*) and one net-spinning Trichoptera (*Polycentropus flavomaculatus*). Four taxa of Chironomidae were also identified, namely Chironomini (mainly *Cryptochironomus*, *Microtendipes*), *Orthoclaadiinae* (*Cricotopus*, *Heterotrissocladius*, *Synorthoclaadius*), *Tanypodinae* (*Macropelopia*, *Pentaneura*, *Procladius*) and *Tanytarsini* (principally *Micropsectra*). This also links to another study by Whitehead (1935) who studied the different environments available in chalk streams and how this affects the taxonomic richness of the streams.

Invertebrate drift also often shows significant temporal and spatial variation. There is a well-documented diel pattern, with more invertebrates present at dusk and during the night (Miller, 1963), and a higher density of invertebrates in summer than spring (Neal *et al.*, 2008). Low energy streams were found to have horizontal and vertical stratification of drifting invertebrates and since there is less turbulence in low-energy streams there is reduced mixing of the invertebrates in the channel (Neal *et al.*, 2008). Therefore, if there is greater turbulence of the streams, such as during high rainfall events, or a reduction in channel area during periods of drought, this will increase the

invertebrate mixing, which could alter the taxonomic richness of the benthic communities, potentially leading to greater impacts in the diversity downstream.

Chalk streams can provide a variety of refugia and different habitats for the invertebrates to live in, such as the hypohoric zone, the riverbed itself and the banks and margins. On the stony gravel bed, the chalk river had a composition of fauna that varied throughout the year (Whitehead, 1935). *Agapetus* was common during the late summer and autumn, while *Gammarus* and *oligochaeta* are common throughout the year, typical of the gravel bed. *Chironomids* and *Simulium* are less common in this stoney area, and this area as a whole is less prolific in animal life. The still water areas such as above mill weirs or deep water tend to be dominated by silt and mud. In March, *Valvata piscinalis* was found in large quantities and *Pisidium*, *Chironomidae* and *Oligochaeta* are characteristic of these habitats (table 13) (Whitehead, 1935). Many stream insects have more than one brood per annum and the nymphal stages can be short, ranging from a few days (although some can live for up to two years such as some species of Mayfly) (Hamrsky, 2024). For example, *Baetis* have two hatching periods in early June and mid-July and temperature has been found to be a controlling factor in the hatching of aquatic macroinvertebrates (Whitehead, 1935). The number of insect larvae diminish rapidly in autumn and the majority spend the winter as small larvae or eggs. During the autumn floods, there is a redistribution of insects across the environment, with fewer animals found in the stony regions and a rise in the submerged plants.

Chalk riverbeds can be less stable than other river types. For example, the stony or gravel bed material is not as stable as a limestone river, shown by Whitehead (1935) in the comparison between the Driffeld chalk stream and the River Wharfe. Some species adapt differently to this instability, such as the *Simulium* being lithophile in the River Wharfe and phytophile in Driffeld, preferring the more stable submerged phanogams.

1.5.3. Fish, Mammals and Birdlife in Chalk Streams

Chalk streams also provide a range of fish habitats, ranging from the smaller, sheltered sections of the upper courses to the larger chalk river sections, which has allowed the development of a species-rich fish community when the whole catchment is regarded. It is important to consider the fish population of chalk streams as they form a large component of the ecological community and can have direct and indirect effects on the invertebrate populations. Common and notable fish species characteristic of chalk streams include grayling *Thymallus thymallus*, barbel *Barbus barbus* (originally confined to SE England, later distributed through angling communities), Atlantic salmon *Salmo salar* and three lamprey species. However, these species are under concern as the Salmon use chalk streams as their main salmonid habitat in lowland Britain, and most of the above species are a priority species under the EU Habitats Directive (Mainstone *et al.*, 1999).

The intermittent headwaters of chalk streams are an unsuitable habitat for fish, so when flow resumes, only a few species are able to colonise these stretches, such as the brown trout *Salmo trutta* which migrate upstream in the early winter when the springs have emerged to spawn, accompanied by minnows *Phoxinus Phoxinus* and three-spined sticklebacks *Gasterosteus aculeatus*. When the headwaters begin to dry in the summer months, the adults of these species then migrate towards the perennial sections (Mainstone *et al.*, 1999). These juveniles may contribute a good proportion of the population and must not be discounted, even if some do become trapped in pools upstream during the drying. The winterbourne portion of the population can vary significantly between years, depending on when the springs break through - if the winter is particularly dry, the springs flows in the headwaters may not rise, meaning there is no additional spawning in this location (Mainstone *et al.*, 1999). The perennial stretches contain the typical native fish community of those listed above, as well as Bullhead *Cottus gobio*, Pike *Esox Lucius*, Eel *Anguilla Anguilla* and stone loach *Noemachilius barbatulus*. (Mainstone *et al.*, 1999). It has also been noted that around 60% of the fish production in the smaller chalk streams comes from smaller species, such as the bullheads in the Bere Stream (LeCren, 1969; Mainstone *et al.*, 1999). Further downstream, especially in larger chalk stream sections, there is often a wider variety of species that prefer lower flow velocities and deeper waters, such as dace, roach *Rutilus rutilus*, chub *Leuciscus cephalus*, gudgeon *Gobio gobio*, barbel and perch

Perca fluviatilis. Some of these species migrate upstream into the chalk stream reaches to spawn because on the gravel beds making suitable spawning habitat (Mainstone *et al.*, 1999). Table 1.4 identifies some of the most common fish species in chalk streams.

Table 1.4: *The spawning seasons and the preferred substrates inhabited by the key species of fish found in chalk streams (Mainstone et al., 1999).*

Species	Season	Substrate Need
Atlantic salmon	Dec - Feb	Coarse gravel, fast flow
Brown trout	Dec - Feb	Gravel, fast flow
Brook lamprey	April - May	Sand or gravel
Sea lamprey	May - June	Sand and gravel
River lamprey	April - May	Sand and gravel
Grayling	Mar - April	Gravel
Minow	April - May	Gravel shallows
Bullhead	Mar - June	Large stones
Dace	Feb - April	Gravel shallows
3-spined sickleback	Mar - June	Weeds
Stone loach	May - July	Gravel and vegetation
Pike	Feb - April	Vegetation and sticks

Trout in particular, are an important component of the chalk river community and provide a high recreational value for angling. These fish aggregate in pools and choose to remain in the same site, with only some diurnal variations at dawn and dusk (Kemp *et al.*, 2017). Where the abiotic parameters in a habitat are stable, such as water temperature, flow velocity, depth and substrate size, the highly seasonal growth of macrophytes may explain the variations in habitat use. A study conducted by Vowles & Kemp (2018) found that brown trout had a strong relationship between fish density and depth in winter and spring, and contrastingly, a lack of association in September (Figure 1.18), when the vegetation cover is respectively low and high. Therefore, depth becomes a more important habitat characteristic when there is less vegetation cover. Trout exhibit a risk-minimising strategy where they seek refuge in deeper pools during

winter when there is less macrophyte cover to hide from predators, whereas in summer they inhabit shallower areas and move less distance. This is to ensure they are hidden from predators by either the shade of the overhanging and in-channel vegetation, and the lack of movement reduces the chance of attracting attention, as well as keeping cooler in the shallower water. This study also emphasised the need for consideration of vegetation cutting in river restoration projects to provide suitable cover for brown trout in rivers with limited pool systems (Vowles & Kemp, 2019).

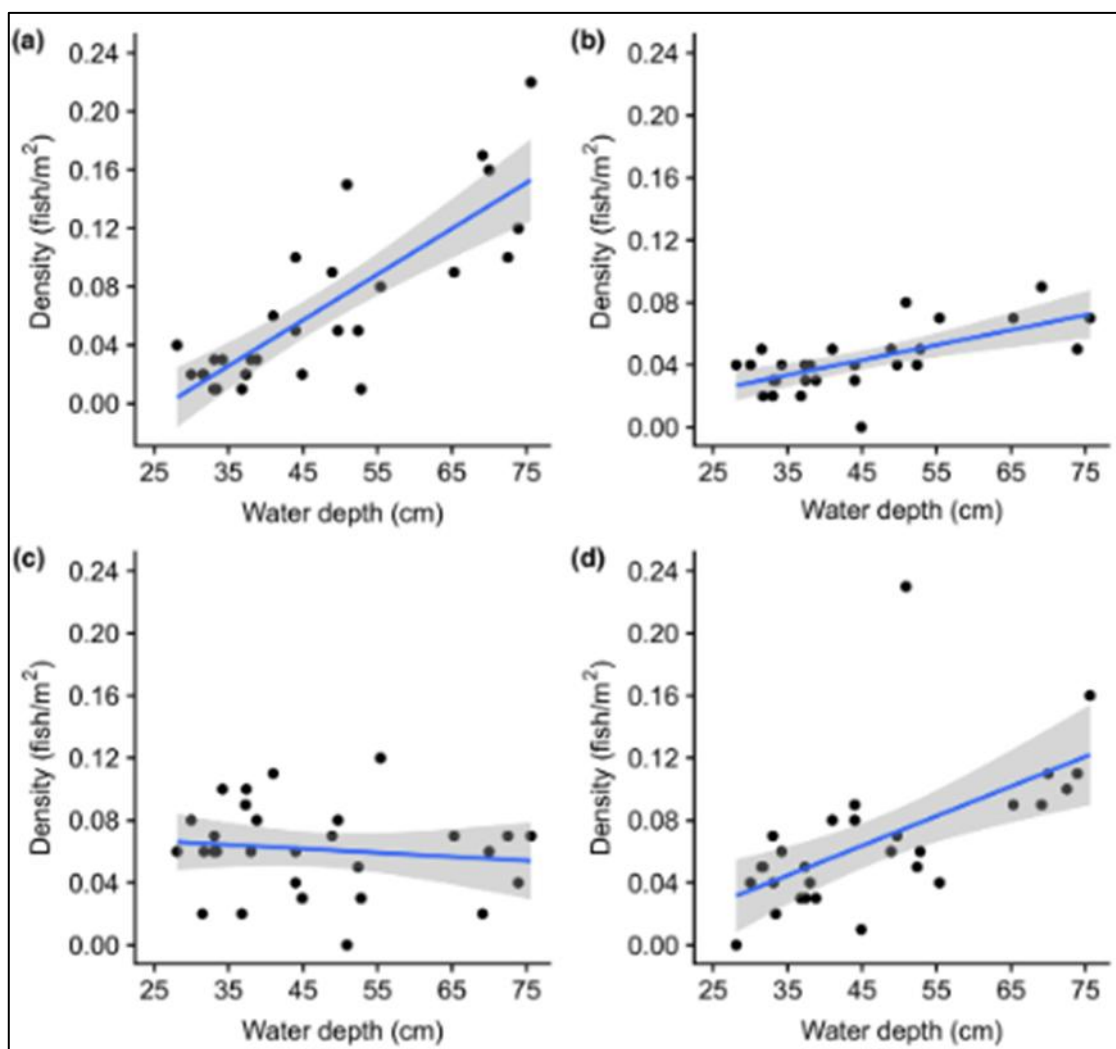


Figure 1. 18: The density of fish per metre squared in relation to the mean water depth in the River Lambourn in a) Feb 2011; b) May 2011; c) September 2011; and d) May 2021 with fitted linear regression lines. Shading denotes the 95% confidence interval (Vowles & Kemp, 2019).

Overall, fish are an important component of the chalk stream community as they have both direct (predation) and indirect (nutrient levels) impacts on the macroinvertebrate communities, and they can also indicate the health of the chalk stream environment by their presence.

Chalk streams in the winterbourne sections do not tend to foster as strong a bird community as the perennial sections, as many require flowing water as part of their habitat for feeding, such as grey wagtails *Motacilla cinerea* and Kingfishers *Alcedo atthis*. The birds that tend to use the winterbourne sections are not specifically related to riverine environments and instead use it as a water source. There has been some experience of the remnants of water meadows in the upper courses remaining, leading to a permanent waterlogged environment that wetland birds can use, even if the winterbourne is not flowing, such as on the Bourne tributary of the river Avon, the redshank, lapwing, mallard and coot nested on the floodplain of a dry winterbourne between 1898 to 1992 (Mainstone *et al.*, 1999).

The watercress beds that are associated with headwaters of chalk streams have a distinct bird community, such as being a wintering site for water pipits *Anthus spinoletta* and green sandpipers *Tringa ochropus*. The bird communities of chalk streams and rivers varies with distance downstream due to the reduced channel gradient and larger river size. For example, disturbed water with riffles and weirs are more suitable for grey wagtails upstream, and downstream with flood meadow habitat are more suitable for overwintering species for large flocks of white-fronted geese *Anser albifrons*, lapwing, snipe and redshank, although many species move downstream during the winter (kingfishers, grey wagtails, little grebes (Mainstone *et al.*, 1999).

1.5.4. Aquatic Macrophytes in Chalk Streams

Aquatic macrophytes have a major contribution to the overall health of chalk streams because they are involved in providing oxygen to the aquatic environment, cycle nutrients, provide refugia for fish and invertebrates. They also provide the bridge between the air-land-water for invertebrates to complete their life cycles, create structural diversity within the water column, particularly where the channel is fairly uniform and supplies surfaces for smaller microorganisms to inhabit such as algae and diatoms (Westwood *et al.*, 2005; Holmes, 1999). The shallow depth of chalk rivers,

along with the high nutrient level and low turbidity leads to high levels of macrophyte growth, particularly in spring and early summer, before declining in autumn (figure 1.19). This shows a seasonal pattern of growth of macrophytes which provides the hydrological and physical system with some heterogeneity, in a system that is generally fairly stable (Biggs *et al.*, 2018). There is a significant link to flow and the macrophyte community, as most of the macrophyte community has the ability to adapt to the typical ephemeral hydrological conditions of chalk rivers (Westwood *et al.*, 2005). This means that the presence and absence of species can be predicted using stream-flow records up to 24-months ago, as well as records of hydro-climatic variables, local topography, fluvial geomorphology and rates of water abstraction (Westwood & Teeuw, 2001).

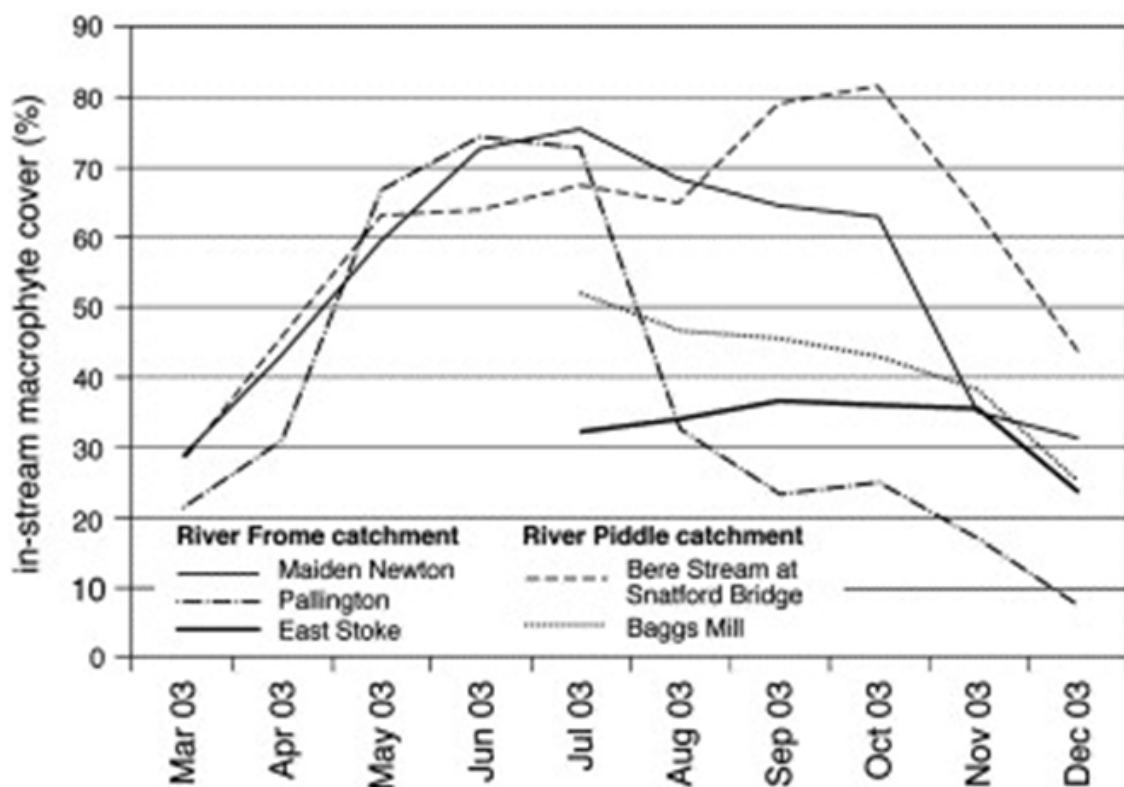


Figure 1. 19: The percentage of vegetation cover within the River Frome and Piddle catchments at the 3 sites from each river (Westwood *et al.*, 2020). The percentage of vegetation cover increases during summer and decreases during winter.

Chalk rivers often have extensive macrophyte cover and the most common plant is *Ranunculus* plants which provide food and habitat for many species of invertebrates, fish and birds (Wharton *et al.*, 2006). These plants grow in the channel on the gravel beds and have an impact on the flow and sediment dynamics within the stream. Green (2005) found that the flow velocity was significantly reduced with coverage of the plants on the riverbed, leading to greater retention of sediments and more deposition of material compared to open areas of channel. This allows the water to remain clear and have a low turbidity with suspended solids being reportedly below 5mg l⁻¹ throughout summer (Wharton *et al.*, 2006; Farr & Clarke, 1984).

One example of how macrophytes are crucial for the ecological functioning of chalk streams is the relationship between a species of blackfly and the water crowfoot *ranunculus*. Larval blackflies (*Diptera Simuliidae*) feed on *Ranunculus* and attach to the plant during periods of high flow velocity for refuge. They feed on the small particles and dissolved organic matter filtered from the water column above (Wotton & Malmqvist, 2001). Due to very little ingested material being assimilated into the blackfly larvae, much of the matter is released from the organism in faecal pellets (>100 pellets per day) and they are carried away and deposited downstream in areas of low flow (Wharton *et al.*, 2006; Malmqvist *et al.*, 2001). This is an important transfer of sediment and nutrients from the water column to the riverbed and occurs during the summer when other detritus has already been decomposed, providing an input to the substratum (Wharton *et al.*, 2006).

The composition of macrophytes that inhabit a chalk stream is determined by a number of different interlinked environmental variables including catchment rainfall, water availability, geology and soil type, riverbank profiles, agriculture and livestock, and management practices (Westwood *et al.*, 2006). The main factor controlling the vegetation in a stream is the flow regime, but the relationship between streamflow, channel morphologies and plant communities is complicated by the interactions between the plants themselves, such as competition, as well as with the sediment and flowing water (Westwood *et al.*, 2020). Vegetated flow velocities are lower at all times of year than open flow velocities (figure 1.19). For example, mean vegetated flow ranged between 0.02 and 0.19m/ s⁻¹ whereas open flow was 0.30 and 0.70 m /s⁻¹ on

the River Piddle, Dorset. Sediment loads and concentrations of macronutrients have increased within chalk streams and this is linked to land use changes, increased water abstraction, sewage outflows, channel modification and management practices (Wharton *et al.*, 2006). The macrophytes create distinct meso-habitats through the modification of flows and the trapping and retention of fine sediments (<2mm diameter), and they also reduce the flow velocity within plant stands and increased the velocity in open channels due to funnelling (Wharton *et al.*, 2006). The faecal component of these sediments was found to be 60% of the total mass, which shows the importance of the biogenic component of fine sediments. The impact of vegetation such as *Ranunculus* should be considered when applying management strategies to chalk streams, as weed cutting could cause changes in the flow regime and sediment flows (Wharton *et al.*, 2006).

1.6. Challenges and threats to Chalk Streams

All rivers, including the rare chalk streams are facing increasing pressures from climatic and anthropogenic causes. Many chalk streams flow through industrialised and farmed parts of the UK, with three chalk streams flowing through the capital London and many of the others passing through the heavily farmed countryside (Rangeley-Wilson, 2021) and this in combination with changes to the natural environment will exemplify any challenges the environment is currently facing.

There are many ways that chalk streams, and rivers in general are monitored to assess the pressures facing them. The EU and UK Water Framework Directive use several ecological and chemical parameters to determine the overall health status of rivers across the UK. These include priority substances like heavy metals, pharmaceuticals, pesticides, biological indicators such as invertebrates and fish, the hydrological regime, physical-chemical parameters like ammonia, nitrates, BOD and phosphate levels and supporting elements like non-native species (The Rivers Trust, 2022). There are three pillars of the ecological health of chalk streams: water quantity; water quality and physical habitat quality (the physical shape of the river and factors such as invasive species). One way that rivers are assessed for their condition is through the use of the Good ecological status (GES) metric. This assesses the health of the water environment through using various flow, habitat and biological quality tests (DEFRA,

2021). This assessment has been retained in UK law since the departure from the EU and is the framework to assess the ecological and chemical quality, and therefore health, of the UK rivers. This indicator, and the statutory framework that supports it, have been retained in UK law after the UK's departure from the EU (DEFRA, 2021). In the last assessment in 2019, only 14% of rivers have been found to be of good ecological status (GES) and zero are achieving good chemical status (House of Commons, 2022). The main reasons for rivers not achieving Good Status from the Water Quality in Rivers Report (2022) are outlined in the table 1.5 below. Acting on these issues individually will lead to a greater understanding of how they interact together and how the network would most benefit from attention. Unfortunately, the majority of chalk streams are deteriorating, with now 77% of the chalk rivers across the UK failing to reach the 'good' water and biological quality status (Defra, 2004; O'Neill & Hughes, 2014).

Table 1.5: *The reasons why rivers in England are not achieving Good Status (Reasons for Not Achieving Good Status (RNAGs)). The three biggest drivers of poor water quality are highlighted under water management issue. The data set includes 15,029 individual reasons which have been classified into the five topics. (Environment Agency, 2021).*

Water Management Issue	Changes to natural flow and water level	Physical modifications	Pollution from rural areas	Pollution from urban areas	Pollution from waste water	Totals
Agriculture and rural land management	116	820	5148	8	3	6095
Water Industry	289	365	0	130	3355	4139
Urban and Transport	0	779	0	892	33	1704

1.6.1. Modification of Chalk Streams

Rivers have been modified for centuries by humans for various activities, such as to increase agricultural productivity or lessen the impact of flooding in a particular area. Many chalk rivers have faced extensive modification through human activities over the centuries. For example, during the agricultural revolution, many river channels were altered by riparian vegetation being removed and the bed dredged to deepen the channel, and the levels of fine sediments within the flow of the channel increased from

discharges from mill systems, water meadows and agricultural runoff (Westlake *et al.*, 1972; Mandon *et al.*, 2021; Evans, 2017). The removal of hedgerows and the increased use of heavier farming equipment has led to increased surface runoff and fine sediment erosion, increasing the connectivity between agricultural land and chalk streams (Mandon *et al.*, 2021). The impact of cattle and grazing animals on bank stability is also notable, as well as damaged road verges, watercress farming and sewage effluent is exacerbating the diffuse pollution problems associated with fine bed sediment storage in chalk streams (Neal *et al.*, 2000; Mandon *et al.*, 2021).

Chalk streams have often been historically heavily modified by deforestation, water mills flash locks, water meadows, ditching/drainage of floodplains, dredging and canalisation. Invasive non-native species like Himalayan balsam *Impatiens glandulifera* and American signal crayfish have an adverse effect on the ecological health and physical integrity of streams. Chalk streams are gentle, low-energy rivers shaped by glaciation; they cannot self-repair once the damage is done. Chalk streams are more dependent on ecological processes than high-energy streams, such as on macrophytes interacting with the flow, treefall, spawning salmonids mobilising gravel and blackfly larvae in *ranunculus* beds filtering diatoms in water. Chalk stream restoration should focus on processes that enable stream slow, intact gravel bed, dynamic interaction with fallen trees and floodplain.

The modification of the river channel through channel widening and meander removal and embankment creation has led to a disconnect between the river channel and its floodplain. Floodplain ecosystems strongly rely on the disturbance caused by regular flooding events. River floodplains have many different habitats present and a high primary productivity and biodiversity, which is created by the hydrological connectivity between rivers and floodplains (Cliverd *et al.* 2013). The regular flooding events provide an exchange of nutrients, sediment, water and energy between the floodplain and river channel which leads to a variety of floodplain habitats being created in the dynamic physical environment (Robertson *et al.*, 2001). Therefore, floodplains are crucial for the maintenance of high biodiversity, as well as a store for flood water and downstream flood peak attenuation (Cliverd *et al.*, 2013). They are also used to mediate the flow of water and energy between aquatic and terrestrial environments, which is key for most food webs and biogeochemical processes like aerobic respiration, denitrification and methanogenesis (Cliverd *et al.*, 2013). The occurrence

of flooding onto the floodplain is also important for the deposition of sediment and nutrients for plants species to use, which can increase the species richness on the banksides (Nilsson *et al.*, 2010). (Cliverd *et al.*, 2013).

Another action taken to improve agricultural productivity on the chalk stream flood plain was the creation of water meadows. Water meadows were often created in the south of England in many chalk catchments to improve the productivity of the land, and whilst this was originally for agricultural gain, these habitats have also been essential in providing some of the characteristic chalk stream ecosystems (Mainstone *et al.*, 1999; Buglife, 2019). However, as these areas are not as valuable nowadays, the majority have been left to decline leaving behind relict features such as weirs and artificial channels (Mandon *et al.*, 2021), and other actions have to be taken to maintain the water meadows to preserve the wildlife that relies upon this habitat. There is also some evidence that some modern meadows have been shown to act as sediment sinks (Cook *et al.*, 2017) and the artefacts from the modifications, like weirs, have been found to alter the hydrogeomorphology, by homogenising flow conditions, trapping sediment and increasing residency times (Mandon *et al.*, 2021). This shows that whilst these structures are now largely defunct, they still have an impact on the functioning of the chalk streams and may still cause impacts that could be detrimental to the ecosystems and hydrological functioning of the chalk streams, especially as they are no longer being monitored through the use of water meadows, particularly as chalk streams are low energy rivers and are unable to revert their course through natural processes alone (Mandon *et al.*, 2021).

Rivers have also experienced modification for industrial reasons, and chalk streams and rivers are no exception. Their flow has been capitalised for activities such as milling, and transportation. For example, Green Mill in St. Albans optimises flow from the River Ver, a tributary of the River Colne, and the Grand Union Canal diverts water away from the River Colne and its tributaries to be used in the canal network for transport of goods and materials from London to the Midlands. One of the most damaging activities undertaken to modify the chalk rivers was the dredging carried out to landscape the river banks and deepen the channels to reduce the flood risk (Rangeley-Wilson, 2021). , which dredge the gravel river-bed which is important for chalk aquatic life, to deepen the channels and reduce flooding on the flood plain (Rangeley-Wilson, 2021). Dredging is usually done to improve the flow of the river and

reduce the likelihood of flooding, although it is known to be severely ecologically damaging as it removes the valuable gravel riverbed which is important for aquatic life. Dredging has been occurring on the Gaywood River, near Kings Lynn, annually as farmers pay the King's Lynn Internal Drainage Board to dredge the river to reduce the flooding onto agricultural land and it also protects the Anglian Water Sewage plant in Grimston (Sennitt, 2023; Gaywood River Revival, 2023). However, the machines that are used to clear plants are more destructive as they disturb the silt on the riverbed too, which can smother invertebrates and plants downstream (Sennitt, 2023), as well as directly removing the gravel bed that many fish and invertebrates rely on as a source of refugia and breeding habitat. This destruction and cleansing of the dredging of the River Gaywood can clearly be seen in figure 1.20, which shows the barren riverbanks void of vegetation and the more turbid water flow from the disturbed silt and sediments.



Figure 1. 20: *The Gaywood River after vegetation removal on the banks and in-channel (Sennitt, 2023).*

Watercress (*Nasturtium officinale*) farms have also been a focal point of chalk stream history and are one of the things heavily associated with the headwaters of this

environment (Dixon & Shaw, 2011). Watercress tends to thrive in chalk streams due to the more stable water temperatures which avoids frosts during the winter and has a high nutrient concentration, meaning they often dominate the macrophyte assemblage in the summer months (Dixon & Shaw, 2011). In the 19th century, with advancements in the railway network allowing for the wider transportation of goods, watercress farms began to spring up along many chalk streams in England and led to the formation of the Watercress Line which supplied watercress from farms to London and south England. Chalk streams were diverted to irrigate gravel beds where the watercress was grown, before the water was returned to the rivers (White, 2017). Whilst the majority of the process is now mechanised, the modern watercress farms still abstract water from chalk streams and discharge it after the washing process, once it has been filtered to remove excess sediment. However, there is also the concern of the chemicals released from washing the watercress, as it has been found that the compound the plant naturally releases as a defence mechanism (Phenethyl isothiocyanate (PEITC)) may be toxic to aquatic life in the larger amounts that are being released from farming practices (White, 2017; Dixon & Shaw, 2011). One significant impact on the invertebrate community is the lower frequency of the fairly tolerant species, freshwater shrimp, which is a major food source of salmonid fish (White, 2017). For example, on a tributary of the River Test, there was a reduction in *G. pulex* numbers after the watercress farm due to the elevated PEITC levels, although it is not clear if the chemical causes immediate death of the invertebrate, or if it impacts the reproductive system (acute or sublethal ecotoxicological response) (Dixon & Shaw, 2011). The study undertaken by Dixon & Shaw (2011) showed that the exposure to PEITC at concentration and wash levels (to stimulate watercress washing discharge from farms) led to pair separation during the *Gammarus* pair mating behaviour, which indicated that watercress farms would lead to impaired reproduction of the species when exposed to the chemical. This shows that mass production and exploitation of chalk streams and its naturally occurring resources can be detrimental to the environment and its functioning.

1.6.2. Climate Change and altered weather patterns across Chalk Stream Catchments

Streams and rivers are one of the most sensitive environments to climate change, with the increase in average air temperatures being the leading cause of concern (Durance & Ormerod, 2009). This is especially the case in shallower riverine environments, such as those in the ephemeral or intermittent zones of chalk streams (Bunting *et al.*, 2020). In these areas, the relationship between water temperature and air temperature is even more apparent, with the water temperature being strongly controlled by the energy and hydrological fluxes at the interfaces between the air and water surface, as well as the riverbed and channel flow (Watts *et al.*, 2015; Durance & Ormerod, 2009). Climate change is not only important to the seasonal variation in water temperature but also to diurnal fluxes, due to the difference in the amount of solar radiation hitting the water's surface at different times of the day (Bunting *et al.*, 2020; Datry *et al.*, 2014). For example, the temperature of the water usually peaks during midday, when the sun is at its highest point in the sky, and is coolest during the nighttime. This leads to cyclical variations in key physicochemical parameters within the stream environment, such as dissolved oxygen concentration and rates of production within the ecosystem (Durance & Ormerod, 2009). This will significantly alter the physical and also chemical environment provided by the streams, especially as many aquatic organisms are exothermic and are highly sensitive to temperature changes. The rise in air temperatures and its subsequent impact on water temperature may have many profound effects on the phenology, growth, distribution and population size of many species (Durance & Ormerod, 2009). Warmer water also mobilises certain toxic substances and increases the acidity of the water too, which could have negative ecological impacts (Watts *et al.*, 2015; IPCC, 2014).

With the rising temperatures meaning that the summers are hotter and winters are milder, there will be a greater influence of air temperature on surface waters. Despite chalk streams having a characteristic stable water temperature of between 10 – 12 degrees Celsius brought about by the groundwater contributions (Robinson *et al.*, 2024; EA, 2014), recently many chalk streams are displaying warmer surface temperatures due to increased solar radiation (Rangley-Wilson, 2020). This will have

a major impact on some of the species that inhabit chalk streams, particularly those that are cold-water sensitive, such as Atlantic Salmon and Grayling, or that rely on temperature changes for the timing of their lifecycle events like invertebrates such as mayflies, stoneflies and caddisflies (Stubbington *et al.*, 2022). These increasing temperatures and more extreme flow and drought scenarios are predicted to lead to population declines and local extinctions of many fish species such as migratory salmonids, eel and lamprey, as well as invertebrates that have life cycles associated with wet and dry phases and the already rare white clawed crayfish will decline too (Stubbington *et al.*, 2022). The temperature change may however be beneficial to some species, allowing them to populate areas that would previously be unsuitable, such as some species of dragonflies and damselflies which have been migrating northwards (Stubbington *et al.*, 2022). The warmer environment could also be more suitable for invasive species, which will change the ecosystem dynamics and lead to the potential extinction of many species that are unique to chalk stream habitats.

Rainfall patterns are also predicted to shift in the future (IPCC, 2014) and more frequent extreme meteorological and hydrological events, such as floods, will occur (figure 1.21). Since 1988, there have been six of the ten wettest years on record, and the winter storms of 2015 were 40% more likely due to climate change (Met Office, 2024a). It is predicted that by 2070, the intensity of rainfall will be up by 20% in the summer and 25% in winter and the days when rainfall will exceed 30mm per hour will be twice as likely (Met Office, 2024b). The changing rainfall patterns will alter the flow regimes in these streams, such as future decreases in summer discharge and the potential for drought may lead to increased pressure on these streams, although it is difficult to predict accurately (Watts *et al.*, 2015). The loss of hydrological variation poses a threat to organisms that have adapted to fluctuating conditions (Stubbington *et al.*, 2017). During dry periods, there is already a low baseflow within the aquifers and within the rivers, so this means there is a reduced ability to dilute any pollutants that enter the water, increasing the nutrient concentration within the water. The high water residency times, brought about by low gradient and low energy stream systems, make chalk stream environments particularly susceptible to pollution and sensitive to nutrient inputs and eutrophication (Jarvie *et al.*, 2004).

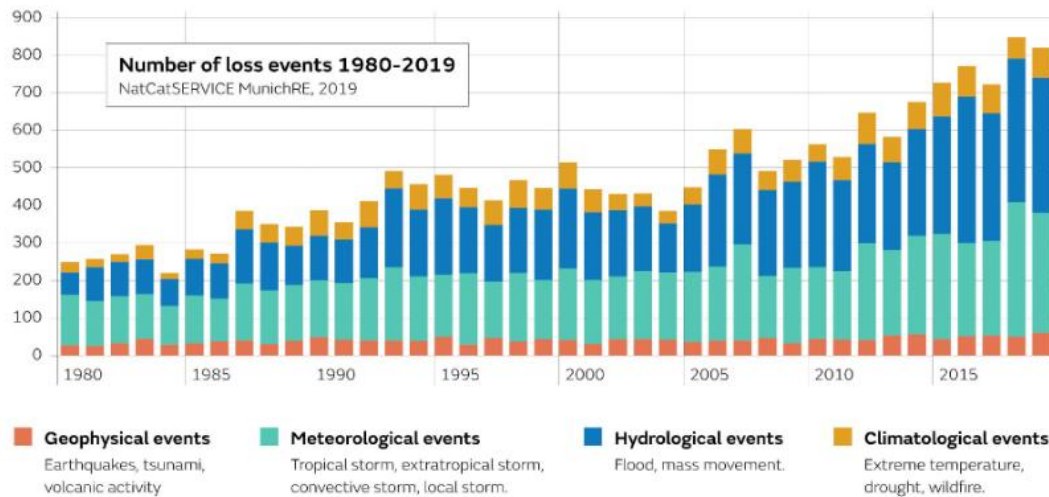


Figure 1. 21: The increase in extreme events from 1980 to 2019 which lead to significant environmental and human loss (Met Office, 2024a).

The 1989 – 1992 ground water drought occurred in southern England, when there was a temporary shift northwards of the rain-bearing winter weather systems, which increased the northwest-southeast climatic divide across the UK (Westwood *et al.*, 2005; Price 1996). This meant that southern and eastern England received up to 50% less rain, causing the groundwater levels to be the lowest on record (Price, 1996). This had a significant impact on chalk rivers as the baseflow decreased, causing the stream network to recede, leading to the widespread loss of aquatic habitat. This meant that water-dependent biotic communities simplified themselves or were replaced with terrestrial species (Giles *et al.*, 1991; Westwood *et al.*, 2005). It is predicted that some ephemeral valleys in the upper courses of chalk streams will remain dry for years due to lower amounts of rainfall in these areas, leading to a decline in subsurface sediments which will impact the species that have adapted to have a period of dormancy in these sediments, leading to fewer surviving.

For the streams that still maintained some flow, the usually clear gravel bed became silted up which allowed species more suitable for wetland environments to colonise, compared to aquatic floras (Westwood *et al.*, 2005). The recovery of the streams was monitored post 1992 after the drought, which showed the rate of recovery of the in-channel aquatic wetland macrophytes and the ecological impacts this would have. The survey by Holmes (1996) on 12 sites within the Thames basin showed that

winterbourne macrophyte communities and their species have a measurable variability in relation to streamflow which is due to their adaptation to typical ephemeral hydrological conditions. In the perennial sections, the water depth of the chalk streams may decrease, leading to the loss of fast flowing riffles and the inundated marginal habitats and water meadows (Stubbington *et al.*, 2022). It is suggested that the more frequent occurrence of extreme drought may lead to the perennial reaches of chalk streams to move further downstream and winterbourne reaches may extend further upstream, encroaching on near-perennial reaches and their location may fluctuate more often, due to lower water tables from lower aquifer recharge (Stubbington *et al.*, 2022). There is also the problem of “false starts” in river flow, where short, fast rainfall events lead to a short-term flow that occurs before proper flow resumption of a river, which interrupts the flow dynamic and ecosystem, potentially impacting the lifecycles of some species that rely on the return of the flow period (Stubbington *et al.*, 2022).

1.6.3. Water Abstraction in Chalk Stream Catchments

Groundwater abstraction from the chalk aquifers increased drastically after the wars, peaking in the mid-1980s when over half of the water available was abstracted or all of it during dry years (Rangeley-Wilson, 2021). The National Rivers Authority identified 15 chalk streams suffering from over-abstraction and launched the Alleviation of Low Flows initiative. To combat this the Environment Agency’s Restoring Sustainable Abstraction programme was created and delivered alterations to 124 abstraction licences on chalk streams, returning 105 Ml/d. However, 75 chalk streams still do not support GES flow. The Water Framework Directive has Environmental Flow Indicators to show what levels of flow are needed to support GES, yet most are below this e.g. in the Colne catchment, the Ver is 77% below natural flow and Chess 41% when it should be only be 10% below natural flow levels (figure 1.22) (Rangeley-Wilson, 2021). With the increasing population and the growing demand for water resources, this will only put further pressure on the groundwater resources and also the surface water resources. Therefore, educating the population about reducing their water use will be essential to managing the water usage within the catchment and reduce the pressure on these streams, along with more stringent abstraction licenses and monitoring of water companies.

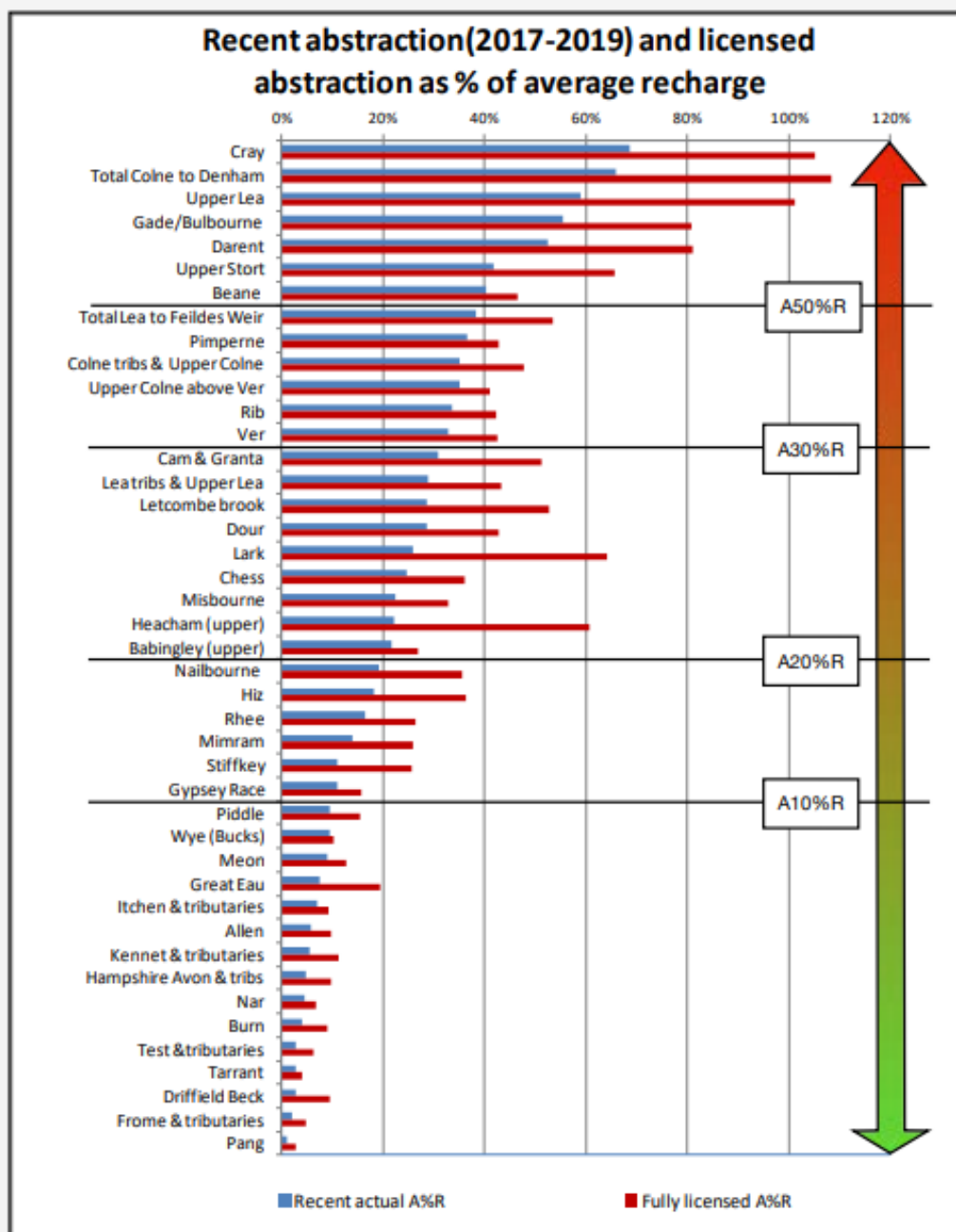


Figure 1. 22: The amount of abstraction of chalk groundwater compared to the recharge rate. A10%R is that 10% of the aquifer recharge can be abstracted. Some of the licences are above the recharge rate, meaning the river flows will decrease annually if the full amount is abstracted. (Lawson, 2022).

Some local communities have taken action to address the abstraction issues. The Ver Valley society set up by the local people of St Albans to protect the River Ver, which joins the Colne at Bricket Wood, campaigned against the increasing abstraction of water from their chalk stream during the 1970s by lobbying the Thames Water Authority, local water companies and MPs. In 1989 a report by the National Rivers Authority addressed the low flow problems and in 1992 the Friars Wash Pumping Station was closed and only used for emergency only, the water demand instead coming from a new pump in Luton to bring water to the Grafham reservoir. This led to the river Ver recovering its natural flow initially, although over two thirds of the river remain dry during summer due to drought and over abstraction (Ver Valley Society, 2023). This shows that whilst some mitigation measures have been implemented and campaigned for, there is still a long way to go to ensure the abstraction levels are manageable and not detrimental to the chalk stream system.

1.6.4. Water Pollution in Chalk Stream Catchments

Water quality as well as quantity is also crucial. The main impacts on water quality in chalk stream catchments are from sediment input and nutrient loading, both of which enter the watercourses from diffuse and point sources. For example, agricultural runoff from fields is high in nitrates and phosphates, run-off from roads contains rubber and other micropollutants and developed land and septic tanks create negative impacts on the river's water quality. Increased phosphate levels lead to eutrophication of the rivers, leading to excess growth of weeds and algae, which chokes the rivers and lowers the oxygen levels, leading to a toxic environment unable to support aquatic life. To help achieve an improvement in water quality we must work with local industries, agriculture, and households to help them understand how to best store, use and dispose of chemicals to support the environment. The Water Framework Directive is an important piece of legislation in the monitoring of streams and rivers and the Environment Agency uses it to measure water chemistry and ecological health as well as other parameters (The Rivers Trust, 2021). No river currently achieves an overall good status, 77% are moderate and 22% are poor or bad status in 2021 (EEA, 2024). Water pollution has become a crisis in recent years, with no river in England or Wales

achieving a good status in overall health in 2020 (The Rivers Trust, 2022). 14% of England's rivers were found to be of good ecological health, yet they all fail the chemical standards set (House of Commons, 2022; The Rivers Trust 2021). There are several causes of river pollution, most of which are diffuse pollution sources, including agricultural runoff, industrial pollution, urban pollution and most notably recently sewage outlets (House of Commons, 2022; The Rivers Trust, 2022). This section will discuss the various pollutants and their impacts on chalk streams in more detail.

1.6.4.1. Pollution from Water and Sewage Treatment Works

Chalk streams are an important source of water for the public water supply and therefore it is essential that good water quality standards are maintained. However, once the water is abstracted from the chalk aquifer it exits the natural system, and when it is returned it is not in the same condition it was found – often it has higher nutrient loads from sewage treatment and other human activities, exacerbating the eutrophic conditions now seen in many rivers (Rangeley-Wilson, 2021).

With an ever-increasing population and housing demand, the situations that lead to contamination of rivers flowing in urban areas are becoming more common and encroaching on the rural areas too. For example, poorly constructed plumbing or overwhelmed sewage networks in built environments have led to increased sewage contamination of rivers, especially when combined with increased runoff overwhelming the outdated sewage systems. The Sewage Map 2021 (The Rivers Trust, 2022) shows all the incidents where the sewage network discharged treated and untreated sewage and storm waters into the river network. The range of counted spills using the 12-24 hour counting method varies between 20 to over 100 spills, most of which are occurring close to the water company sewage discharges, suggesting there is an overall issue in the treatment of waste. Over 1600 rivers or waterbodies were affected by discharges of treated sewage effluent in 2020 and there were 403,171 raw sewage spills in 2020 (The Rivers Trust 2021). This has led to 53% of the rivers failing to achieve good status, with the water sector being blamed as the main reason why. Agriculture also has a negative impact on the health of rivers, with nutrient runoff from fertilisers having the biggest impact or livestock waste being washed into the rivers (The Rivers Trust 2021; Jarvie *et al.*, 2006).

Majority of EU rivers are impacted by sewage treatment works and sewage effluent can make up between 20 – 70% of the total river flow (Halliday *et al.*, 2015). The increases in water abstraction from rivers also concentrates the amount of sewage in the rivers, increasing the dominance of the effluent (Johnson *et al.*, 2009).

Sewage treatment works effluent poses risks to the environment, ecosystems and eventually humans, but it is difficult to quantify the threshold of sewage effluent that is harmful to a river because the amount and content of the effluent varies everyday (Lehmann *et al.*, 2016). One of the most notable impacts is that on riverine biofilms which control the biogeochemical cycles and food webs and can be impacted by having increased growth rates from added nutrients, harmful algae blooms, lowered photosynthetic and metabolic efficiency, decreased bacterial biodiversity, decreased colonisation rates and spread of antibiotic resistant genes (Hill & Fanta, 2008; Wakelin *et al.*, 2008; Ricciardi *et al.*, 2009; Amos *et al.*, 2014). These effects can also get passed through the food chain, such as antibiotic resistance or hormones being released into the rivers from effluent which is affecting the functioning of different species and could also lead to impacts within the human population through consumption. There has been some development of sewage treatment methods to remove bacteria, pharmaceuticals, macronutrients and contaminants from the effluent but unfortunately it is a costly process and is not always achievable – additionally, it is very hard to remove the contaminants from the rivers flow once they are released (Lehmann *et al.*, 2016).

Sewage effluent is a major source of phosphorous in many lowland rivers and is one of the nutrients responsible for the excessive growth of aquatic plants and decreased dissolved oxygen levels in rivers during summer (Neal *et al.*, 2000). Combined with the reduction in rainfall in summer and the high evapotranspiration rates in southeast England reduces the capacity for sewage to be diluted in rivers, creating higher nutrient concentrations. This means that the reduction of point sources of phosphorus is a key objective in the Urban Wastewater Treatment Directive and EC Water Framework Directive (Neal *et al.*, 2000). There is also the issue of pharmaceuticals being released into the river systems through sewage outputs. Lehmann *et al.* (2016) conducted a study on two chalk rivers in the UK comparing the release of sewage effluent on a river with a STW (the Lambourn) and one without (the Kennet). They found that samples exposed to effluent had a significant impact on the number of

functional genes, leading to more antibiotic resistance, even if there were no taxonomic changes.

However, in recent years, with the increasing anthropogenic pressures on all river systems in the UK, this environment has become threatened and high quality chalk streams have become extremely rare, with large sections suffering from the pollution of sewage outfalls for hundreds of hours, and good sections are restricted to isolated stretches of river. Unfortunately, the majority of chalk streams are experiencing degradation, appearing muddy and polluted with sewage outputs, making uninhabitable for many species, leading to a loss of biodiversity, which is why it is crucial to understand the baseline functioning of the upper courses to understand how a chalk stream should function with limited sewage input, to give organisations a goal in standards to aim for.

1.6.4.2. Pollution from Farming and Agricultural Practices

The Environment Agency has identified farming activities as one of the major contributors of water pollution (House of commons, 2022). One of the direct ways of pollution is soil being washed into the rivers and streams which leads to an increased sediment load of the discharge. Sediment enters the rivers from arable land from livestock trampling riverbanks, from floods washing sediment into the rivers and from highways (ColneCAN, 2022c). This pollutes the river by infilling gravel on the riverbed, which prevents fish from spawning and reduces the viability of their eggs (Vowles & Kemp, 2019). It also increases the turbidity of the stream discharge, which lowers light levels and affects the productivity of macrophytes in the channel, as well as interfering with invertebrate life cycles and diurnal light patterns (Environment Agency, 2019a).

Chemical pollution is also an impact from agriculture on aquatic environments. In particular, phosphorus and nitrogen are common nutrients that are largely derived from farmland from chemical and organic fertilisers and slurry, which although beneficial in moderate amounts, can lead to the contamination of soil and water when they exceed the amount taken up by the crops and can be a major source of diffuse pollution to

watercourses (House of commons, 2022). High levels of phosphate have been found to be the biggest reason for the failure of achieving good ecological status of many water bodies (55%) (Environment Agency, 2019b). This is because it can lead to algal blooms and lower the oxygen levels which is harmful to aquatic life and phosphorus is the main cause of eutrophication, particularly due to its environmental persistence. Whilst regulations have reduced the use of fertiliser in the past 30 years there is still an annual phosphorus surplus in the UK, leading to its accumulation in soils and leach into water courses (House of Commons, 2022). For example, in the River Wye catchment was found to have an agricultural input of phosphorus that exceeds the crop uptake by 3000 tonnes ever year, (17kg per hectare), contributing to devastating algal blooms along the river (The RePhoKUs Project, 2021). This is a particularly high phosphorus concentration as it is 60% above the national average of 3.2kg per hectare (DEFRA, 2019), but this is because of the poultry dominated agriculture situated in the catchment (42% poultry) where the faeces is washed directly into the watercourses from the ground the birds live on (House of Commons, 2022).

Natural chalk streams have low nutrient levels, low sediment levels and clear water and stable temperatures, around 10-11°C at the spring source. Pollution from point sources like sewage and diffuse sources from agriculture and road runoff increase levels of nutrients, sediment and toxic chemicals. Excess sediment penetrates gravel and directly impacts the plants, invertebrates and fish, smothering eggs and clogging interstitial spaces in the gravel where invertebrates live. Nutrients and toxins accumulate in silts. Excess sediment comes from agricultural run-off 77% in wet weather. Phosphorous and nitrogen are two chemicals normally found at very low levels in chalk streams. Leads to accelerated plant production, inhibits root growth, reduces resilience, limits biodiversity and leads to the dominance of algae and extreme oxygen depletion. Targeting reductions in phosphorous yields the greatest short-term results as the trigger level for phosphorous means nitrogen is already several times above the trigger level. Urban Wastewater Treatment Directive and Water Framework Directive have lowered phosphorous. Small sewage works must be improved as they still discharge lots of phosphorous to rural chalk streams. Larger works have been improved, usually in lower river reaches. Chalk binds to phosphorous so it is a big problem in aquifers. Sustainable Farming Incentives should have rules to address runoff in chalk catchments and Environmental Land Management schemes enable

changes to land management at catchment scales to prioritise headwaters. Storm overflows significant problem (Rangeley-Wilson, 2021).

1.6.4.3. Micropollutants in Chalk Streams

A newer area of research is into micropollutants and microplastics that are now found in most environments, including chalk streams. For example, 85 out of 267 substances with an Environmental Hazard classification were found throughout the River Colne in a study conducted by the Colne Valley Fisheries Consultative (2023) and 150 had a Human hazard classification, being damaging to fertility or carcinogenic and none were naturally occurring in the river system. Misconnections have been found to be a major contributor to the pollution in the Colne, where the misconnected outfalls discharge untreated sewage into the Ver and Colne, along with road and agricultural runoff. Industrial and pharmaceutical substances were also found at high levels but there was no clear point source of this pollution. Another issue is the level of PAHs, where there are particularly high levels in the sediment above the MAC-EQS levels. Their findings suggest that the environment and water is being irrevocably damaged and the drinking water supply is at serious risk of contamination, posing a risk to human life too (CVFC, 2023). This shows that micropollutants also have a significant contribution to the pollution of the chalks streams, along with the more majorly identified sources, so should not be discounted.

1.6.5. Invasive and Non-natural Species in Chalk Streams

Another threat that is not only restricted to chalk streams are invasive plant and animal species. Along the Colne, over 11 invasive non-native species inhabit the aquatic environment, such as Signal Crayfish and Floating Pennywort which are common invasive species in many of the UK's chalk streams.

1.6.6. Urban Development in Chalk Stream Catchments

Many chalk stream catchments have already experienced a great deal of development throughout their histories, but now with the rising pressure for housing and urban encroachment, the land use surrounding the river channels is changing. More impermeable materials are being laid alongside the river channels and areas that had previously been left alone are now being modified for flood defences or facing channel alterations to reduce the flood risk of new housing developments. One example is the recent planning application by Vistry housing developments for 150 properties to be built on the floodplain in Colney Heath just north of the River Colne (figure 1.23) which was refused at the local council meeting on grounds that stakeholders, locals and agencies such as the EA who were not happy to have it so close to the “precious chalk stream”. Not only is it a major flood risk to the development, but the additional runoff created by impermeable materials, along with debris and pollution created during construction, would be damaging to the Colne, especially so far upstream where the river does not often have enough flow to flush pollutants away. This could lead to potential accumulation of pollutants or micropollutants creating a poor-quality environment for flora and fauna (Colne Valley Fisheries Consultative, 2023). The additional road traffic would also increase the heavy metals and materials available to enter the river through surface runoff. This is just one of many planning applications in Hertfordshire which could have an impact on the chalk rivers in the county, and generally across the country, as the majority of chalk streams are in the southeast which is coincidentally where the majority of the demand for new housing is.



Figure 1. 23: The amended plan from the Planning Application 5/2022/1988 Land To The Rear Of 42-100 Tollgate Road & 42 Tollgate Road Colney Heath St Albans Hertfordshire. Outline application (access sought) - Demolition of existing house and stables and the construction of up to 150 dwellings including affordable and custom-build dwellings together with all ancillary works (Vistry Homes Ltd, 2022).

Other developments, such as the greater need for transportation links are also putting additional pressure on the chalk streams. Historically, chalk rivers had their flow diverted for the use of the canal networks which has led to some low flow in the rivers in these areas. However, more recently, the demand for high-speed rail, in the form of HS2, is putting the natural landscape of the chalk streams in southern England at risk. The Colne Valley is under threat from HS2 and HS2 Ltd has currently taken possession of the nature reserve and removed public access during works. One company that was affected from the closure was the Hillingdon Outdoor Activity Centre which now wants to relocate to Broadwater Lake due to the closure of their original premises by HS2, but this would cause irreversible damage to this nationally important site through the introduction of water sports to this previously undisturbed area and could be significantly detrimental to bird and mammal life already present (Herts and Middlesex

Wildlife Trust, 2023b). Therefore, this shows that human activity that is currently ongoing and potentially occurring has the opportunity to cause long term damage to the hydrological and ecological functioning of the chalk stream catchments.

1.7. Chalk River System Response to Environmental Change

There are large variations in a river system's response to change and this often depends on the balance between opposing and resisting forces and the degrees of freedom a channel has (Charlton, 2007). For example, the material of the bed and banks determines how resistant a channel would be to hydraulic forces, with harder bedrock materials being more resistant, while alluvial sediments are easily manoeuvred. There is also the factor of the length of the event or disturbance, such as short-term episodic floods where the river channel shortly returns to its original state, or longer-term changes to the inputs of a river system which gradually change the environment (Charlton, 2007). Chalk streams are notably low energy catchments as they are a lowland river type and do not tend to have high volumes of discharge. This means that they tend to experience slower rate of change to the morphology of the river system. However, with the modifications mentioned in section 5 and the predicted changes in the climate, the chalk stream systems have been and will be experiencing larger magnitude events which the chalk streams will struggle to recover from, leading to a new equilibrium to be reached, which could be detrimental to the ecosystem that relies on this low energy environment. Chalk stream Malaise is a term that means the general deterioration of classic chalk stream habitats, linked to the loss of macrophytes and excessive growth of benthic and filamentous algae, as well as the increased turbidity and siltation of gravel beds (Defra, 2003; Jarvie *et al.*, 2006).

1.7.1. Mitigation and Chalk Stream Restoration Projects

The news coverage and availability of information about the state of the UK's rivers has led to many movements from community groups and charitable organisations to invest their time into campaigning for cleaner rivers and carrying out voluntary work to

clean up the rivers and re-introduce species that have been ousted from their homes, such as the water vole, in an attempt to revive these unique habitats. While there has been some success with this, much more needs to be done to bring these rivers back to their former rich biodiversity. In 2022, untreated sewage was released into rivers in the Hampshire and Isle of Wight area for 38,525 hours (Lancaster & Palmer, 2023). For example, a recent report on the River Colne in Hertfordshire has shown that it has become polluted with many micropollutants, while the nearby River Ver has often faced exacerbated drying, creating a fractured ecosystem that struggles to recover from the channel drying. The Chilterns chalk streams are under major threat from the over-abstraction of their water courses (Salter & Singleton-White, 2019) and the River Darent in Kent still suffers from low flows from historic abstraction, despite the Darent's Action Plan to reduce the levels of abstraction (Salter & Singleton-White, 2019). All of these issues have founded local communities to lobby larger organisations and stakeholders such as the water companies and government to pledge to protect the chalk streams before irreversible damage is done, and some have taken action themselves as they do not feel enough action is happening. Some of these projects are described below.

1.7.2. Improving Flood Plain Connectivity

A study on the River Glaven, Norfolk was undertaken to explore the floodplain-river hydrological connectivity before and after a restoration scheme which removed river embankments. The river-flood plain restoration was found to cause more frequent, short-duration flood events on the floodplain, leading to improved flood storage and an enhanced soil-water regime. Embankments were found to be a major barrier of river-floodplain interactions, limiting the horizontal subsurface flow as over-bank flow was not possible (Cliverd *et al.*, 2011). During high-flow summers after the embankments were removed, it was found that the macrophyte abundance was lowered. This is because in the spring and summer, plants tend to be in their growth phase and if the plants become submerged in water during this time, it leads to aeration stress as they cannot have a sufficient exchange of gases like O₂ and CO₂. However, this had a positive effect on limiting the growth of more dominant species and increasing the diversity of macrophyte species on the floodplain, as well as

providing new spaces for colonisation (Cliverd *et al.*, 2011; Nilsson *et al.*, 2010). The river water was found to be more alkaline, whereas water that had flowed through the floodplain substrate became more acidic due to the sandy loam soil (Cliverd *et al.*, 2011). There was also an increase in the groundwater level at the river-floodplain interface due to enhanced river intrusion. The river floodplain also experienced increased nutrient enrichment from sediment and river water during flood events. This has the potential to protect adjacent ecosystems from nutrient loading but may affect plant species richness leading to monocultures. Therefore, further management needs to be undertaken.

1.7.3. Controlling and Removing Invasive Species

To date, the majority of the work undertaken to control the invasive species has been carried out by organisations like the Chilterns Chalk Streams Project, Wildlife Trusts and the Impress the Chess project has yet to fully restore flow in the River Chess (ColneCAN, 2022a; Chilterns Conservation Board, 2023). However, this creates a lack of overall action as the efforts are disjointed, making it difficult to manage these areas. The management of this largely involves citizen scientists, such as encouraging the use of the PlantTracker app (now closed) (PlantTracker, 2023) to record and share data on these species, while simultaneously looking to establish a catchment co-ordinated approach connected to the wider river health with the landowners, borough and parish councils (ColneCAN, 2022a). Projects to restore flow regimes, habitats and communities may be crucial in preserving these communities. This has seen some success in winterbourne chalk stream reaches following flow restoration in over-abstracted reaches, allowing macrophyte communities to replace terrestrial grasses and rare aquatic invertebrate recolonised (Holmes, 1999; Stubbington *et al.*, 2017). The Hertfordshire's Living Rivers Network Project has been set up to address the threats facing chalk rivers and is government funded by the Green Recovery Challenge Fund. The Project focuses on the River Lea and its tributaries (the Mimram, Beane, Ash, Rib, Quin and Stort) covering more than 1000 km². Hertfordshire's chalk rivers are now in better connection due to targeted works and invasive species removal and volunteers now have more knowledge and skills for monitoring chalk rivers. Heritage has been improved through recording and water voles are back on the

river Beane, giving a real time picture of river health (Herts and Middlesex Wildlife Trust (2023a).

In conclusion, this chapter identifies the major contributors to chalk stream degradation and highlights how climate change, agriculture, urban development and sewage pollution can all directly affect the hydrological and ecological systems, but also exacerbate the impacts of each other, compounding the issues. It is clear that more management is needed to preserve these chalk stream environments. That is why it is crucial to understand the baseline hydrological and ecological functioning and annual cycles of the chalk streams in England, so that predictions can be made and standards set to improve and mitigate impacts against.

Chapter 2: Project Rationale and Aims

2.1. Project Rationale

With the emergence of recent environmental initiatives such as the Chalk Streams Restoration Strategy (2021) (Rangeley-Wilson, 2021) and the Chalk Streams (Protection) Bill 2024 (UK Parliament, 2024), the importance of chalk streams as a rare and highly sensitive freshwater habitat is becoming more apparent than ever before. Chalk rivers are one of the most botanically biodiverse of all English rivers (Rangeley-Wilson, 2021), supporting a wider variety of flora and fauna than other river types, such as alluvial rivers, with many species specifically adapted to the conditions provided. Chalk rivers are recognised as a priority habitat under the UK Biodiversity Action Plan (BAP) and the EU Habitats Directive (Council of European Communities, 1992). Due to the unique properties of chalk rivers some chalk rivers in southern England have also been given environmental designations such as Sites of Special Scientific Interest (SSSI) under the Wildlife and Countryside Act 1981 and at international levels under the Special Areas of Conservation (EC Directive [92/43/EEC] (Vowles & Kemp, 2019). Within the Colne catchment there are a few SSSIs. The water end swallow holes in Welham Green are a designated SSSI which is just upstream of the headwaters of the River Colne and during times where the water table is high, they can contribute flow to the Colne as they did in winter 2024. Frogmore Meadows is another SSSI which occurs on the floodplain of the River Chess which is a tributary of the Colne and it is recognised for its marshy, damp grassland areas and fauna diversity, including the presence of Priority Species in UK such as the water voles. The Broadwater Lakes in the Mid Colne Valley is a SSSI designated site, but unfortunately this site has been disrupted and is at risk from the HS2 construction (HMWT, 2025). These SSSI designations exemplify how important the Colne valley chalk system is to biodiversity, conservation and ecological functioning in the UK. This makes it critical that their ecological functioning is thoroughly understood and managed effectively, ensuring their preservation for generations to come.

The changes in the external environment and climate also pose a threat to the functioning of chalk streams, particularly in the sensitive headwaters that are susceptible to temporary drying (Rangeley-Wilson, 2020). With predicted changes in

weather patterns and the likelihood of more extreme weather events, along with rising global air temperatures (IPCC, 2014), the fragile stability of the chalk stream environment, shaped by its distinct seasonal flow patterns, is highly dependent on the delicate balance between groundwater level within the drainage basin and streamflow (Pacioglu & Robertson, 2017). This equilibrium is increasingly under pressure from factors such as climate change, over-abstraction, and land use changes, all of which threaten to disrupt the natural hydrological rhythms that sustain these unique ecosystems. For example, the drought period during summer could be extended, which would have a drastic impact on the habitats provided and could lead to further, more serious fragmentation of the habitats within the river systems' ecosystem (Extence, Balbi & Chadd, 1999). There are also consequences for the hydrogeomorphology of the chalk streams too. During times of low flow, the rate of siltation is enhanced, altering the channel morphology and the composition of aquatic vegetation, and impacting the water chemistry (Boulton, 2003). This means a drastically different habitat is created during periods of drought, which is then recolonised months later when constant flow resumes (Boulton, 2003). Although chalk streams have many species with well-developed and suitable adaptations, the future changes and the rate of change in the hydrological cycle and its impact on the extent of drought within the drainage basins will greatly impact the success and biodiversity of these invertebrate community assemblages (Watts *et al.*, 2015).

Another factor alongside climate change is the anthropogenic impact on chalk rivers becoming more apparent. With the increased pressure for housing demand, particularly in the southeast of England where population density is high, there has been a growing effort to protect the chalk streams from the adverse impacts of proposed developments on their surrounding floodplains. The population density already ranges between 900 persons per square kilometre in the Upper Colne and 5000 persons per square kilometre in the mid and lower Colne Valley (ONS, 2022) and this will likely increase with the rise in housing demand within Hertfordshire. Hertfordshire as a whole is expected to have a 19% increase in housing (CPRE, 2024) and St Albans County council has been required to build 15,000 new properties to meet the demand and there are growing concerns that these developments will be on green belt land, as recent developments have been passed on the basis that the need for housing is greater than protecting the green spaces (St. Albans City and District

Council, 2024). For example, within the upper Colne catchment, there are several development plans that are encroaching on the greenbelt land. A notable example is the application for a new residential development in Colney Heath situated just eight metres away from the River Colne's channel and within its floodplain (St Albans City and District Council, 2022). This development would potentially remove much-needed floodplain and alter the surface flow that enters the chalk stream. It may also have the potential to pollute the groundwater supply, depending on the activities, particularly as surface runoff from the development would be directly discharged into the Colne (St Albans City and District Council, 2022). The Thames Water company affirmed that the sewage network would feasibly be able to withstand the increase in sewage from the proposed development but the combined increase from this and other developments within the Colne catchment particularly in the upper course such as the 100 houses at Colney Manor and a further 155 houses at Roundhouse farm (Day, 2025), would likely lead to more pollution events and goes against the already increasing number of sewage outfalls within the network.

Over-abstraction of water is also a major issue within chalk stream catchments, as this can deplete the chalk aquifers and reduce the aquifer recharge, which reduces the baseflow of the chalk streams, hence there will be less water flowing within the chalk streams (EEA, 2024). The Colne Catchment has been assessed as having no available water for further abstraction under any flow scenario, as the annual river flows consistently fall below the Environmental Flow Indicator thresholds (EA, 2018A). The abstraction from the Colne chalk aquifer is only suggested to occur for three days a year to protect this system (EA, 2018A). This indicates that the catchment is already under significant hydrological stress, and any additional water extraction would risk further ecological degradation. Hertfordshire is one of the driest counties in the UK and the average consumption per person is 8% higher than the national average with between 150 – 160 l/h/d (Hertsmere Council, 2025). Therefore, the southeast has more than half of the UK's total predicted water demand by 2050 (Environment Agency, 2024a). The population of the Colne catchment continues to grow, placing increasing pressure on water demand and intensifying stress on already overdrawn groundwater resources (ColneCAN, 2022b). Along with the residential requirement for water supply accounting for about 96% of the water use, agriculture, horticulture and quarrying use the remainder (ColneCAN, 2022b). The households within the

catchment use around 182 litres of water a day per person which is the highest in Europe, around 32 litres above the national average and 42 litres above the Government's target (ColneCAN, 2022b). This escalating demand is likely to exacerbate issues in the headwaters of the River Colne, where desiccation and persistently low flows are becoming more frequent. Without sustainable water management, these pressures could lead to long-term ecological degradation and the loss of critical habitats.

There is also the issue of the reduction in resources being diverted into caring for the rivers in the UK, and there has been a spike in pollution events such as the release of raw sewage into chalk streams like the River Ver, as well as the overall urbanisation of the chalk river floodplains which generates increased amounts of road runoff. This is likely to have a detrimental impact on the water quality of the chalk streams and directly impacting the habitats that many species are relying upon (Richards, 1982; Ward & Robinson, 2000). Therefore, it is crucial to understand what the main factors are affecting the water quality, as well as the hydrology, to determine how environmental and human activities affect the chalk stream environment.

Hydrological models and climate predictions suggest that rivers in the southeast of England, where the chalk streams are situated, will become drier and warmer in the future, and will have higher nutrient levels, which could lead to more concerns regarding the water quality (Johnson *et al.*, 2009). Whilst chalk rivers are slightly protected from reductions in rainfall due to the larger contribution from groundwater, these reserves will likely become depleted faster and may have a longer-term impact on the functioning of these rivers. The UK Climate Impacts Programme 2002 (UKCIP02) highlighted that the southeast of England as a particular area of concern for climate change and this will increase the opportunities for chalk streams in this area to be even more exploited for their resources. With an increase in water demand and therefore water scarcity, there would be a rise in pressure to preserve the riverine systems we have and ensure they are of a suitable quality for both the environment and also for human gain. Otherwise, if the environmental quality of these chalk streams is allowed to further decline, it will require more economic costs to meet the requirements of the WFD and European Union Habitat Directive to ensure site integrity and good ecological status of water bodies.

Another critical concern is that over-exploited and degraded river systems have lower economic and social value. Rivers that are highly polluted, visually unappealing and lacking in biodiversity are less attractive to a wide range of stakeholders. This includes residents and businesses who may be deterred from settling nearby, as well as tourists who wish to visit these special rivers for recreation such as angling. In contrast, well-maintained rivers contribute significantly to local economies, community well-being and cultural identity. For example, angling can contribute £1.18 billion to the economy and providing 37,000 jobs in 2005 (EA, 2007). Therefore, if the water quality and biodiversity decline too drastically, the financial loss from angling would be huge (EA, 2007; Johnson *et al.*, 2009).

Previously, there has been lack of sufficient knowledge and data regarding the functioning of chalk streams and how they might respond to changing climatic and environmental stimuli (Eastman *et al.*, 2021). This knowledge gap underscores the importance of studying the current status of these chalk streams to establish a baseline for their ecological health. By doing so, we can better predict the future changes and implement effective management strategies that preserve their environmental and human value. However, there have been several monitoring schemes on the chalk rivers in the UK to try and determine their health status. The Environment Agency and Defra are responsible for acting upon the deliverables of the legislation in place, such as the WFD and UK BAP, such as the maintenance of macrophyte and invertebrate communities (Wharton *et al.*, 2006). Despite these actions, the Europe's state of water 2024 reported that only 37% of surface waters achieved good or high ecological status, and 29% achieved good chemical status (EEA, 2024). The major contributors to poor ecological and chemical water quality were from atmospheric pollution, changes to the hydrogeomorphology, and diffuse and point source pollution (EEA, 2024). These factors influence the resilience of the water bodies to the changing climate too, with disrupted weather patterns increasing the pressure on river flows. However, there is a discrepancy in their monitoring based on the individual parameters achieving a "good status, such as flow regime achieving 88% and invertebrates achieving 76% good ecological status, and 77% of individual tests having good status, yet only 14% of overall rivers in the UK have achieved the good status (Environment Agency & Natural England, 2023; Newson, 2024; Tough *et al.*, 2020). The Environment Agency has also admitted to not measuring certain

aspects of the water environment that will be likely to change and could not measure progress against the DEFRA 25 Year Plan to improve water courses (Environment Agency & Natural England, 2023). The House of Commons Environmental Audit Committee (2022) also confronted this point, stating that the monitoring regimes are outdated, underfunded and inadequate, and have a narrow focus on certain parameters such as water nutrients, without considering the environment as a whole (Newson, 2024).

Government funding is also not available to carefully monitor pollution incidents and ensure that environmental policy is being upheld. This explained the 36% drop in the EA attending pollution incidents between 2018 and 2023 and the number of prosecutions for environmental damage fell from 800 to 17 between 2007 to 2021 (O'Brien, 2024). There has also been an investigation by the Office for Environmental Protection as to whether the Environment Agency and Defra have failed to comply with the WFD regulations (Neil, 2025). In 2024, they found that both bodies failed to protect rivers from sewage discharges and allowed discharges to occur beyond the exceptional circumstances outlined (Neil, 2025). The Ver, a tributary of the Colne has had over 2000 hours of sewage discharge from the Thames Water Markyate Sewage Treatment Works between December 2024 and March 2025, and it continued to experience regular discharges even in dry weather when it should not be necessary for the combined storm overflows to be active (Ver Valley Society, 2025; Thames Water, 2025a). Furthermore, the River Colne in the upper catchment has been designated as either in a state of bad or poor ecological health, which is the same as the remainder of its course towards Watford and eventually the Thames, part of the 23% of rivers that are classed as poor or bad (The Rivers Trust, 2024). Therefore, it is important to investigate the headwater of the River Colne, as this area is largely untested by the Environment Agency which has focused more on the mid and lower Colne valley and the tributaries. However, the upper Colne still contributes to the overall health of the Colne and the previous work appears to be assumption based as classified areas have been mis-mapped where the Gade has been mis-identified as the Colne (The Rivers Trust, 2024; Shand *et al.*, 2003). Therefore, there remains a significant data gap concerning the water quality and baseline hydrological and ecological functioning on the upper Colne catchment. Addressing this gap is essential

for developing informed management strategies and ensuring the long-term sustainability of this sensitive chalk stream system.

Now the responsibility to better protect the UK's waterbodies, including the chalk streams has fallen to local communities and activists who want to see change in environmental quality. For example, Rediscovering the River Colne project in Watford encourages local people to explore the River Colne and understand what makes a healthy chalk stream. The project organises volunteering work to clean up the river by removing invasive species such as Himalayan Balsam and monitoring the water quality using riverfly's (Watford Borough Council, 2025) There is little faith in the agencies abilities to ensure improvements and the regulator doubts the 2027 water improvement targets will be fulfilled due to generic management plans and low confidence in success (Gurnell *et al.*, 2019; Neil, 2025). ; There are many benefits to being surrounded by nature and want to protect it to preserve it for the future. Therefore, citizen science has become a necessary part of monitoring chalk stream environments through utilising volunteers who may or may not have been scientifically trained to collect and analyse data through schemes such as the Riverfly Network or Modular river surveys (MoRPh) (Gurnell *et al.*, 2019). However, there are concerns regarding the accuracy of data collected by untrained volunteers, although this has been rectified by training schemes to improve reliability (Crall *et al.*, 2011; Gurnell *et al.*, 2019). There are numerous schemes in place which all monitor separate aspects of the river environment such as water nutrients, macroinvertebrates or water chemistry and often to only a basic requirement like identifying macroinvertebrates to the family level or using nutrient strips to determine nitrogen and phosphate in water. While a substantial amount of data is being collected on the various parameters of chalk streams, there is a lack of coordination and integration among the various stakeholders, which limits the effectiveness of this information and there could be interactions missed due to the nuanced preferences of macroinvertebrates at the species level, compared to the family level. Therefore, there are gaps in the understanding of how the hydrology, chemical and ecological conditions interact and influence the overall health of chalk streams.

One widely used method of studying the water quality of aquatic environments is the through the use of biotic indices that rely on benthic macroinvertebrates composition. The use of macroinvertebrates to monitor aquatic environmental change has been

used successfully across the globe e.g. Europe (WHPT index), Africa (SASS5, TARSIS ,ZISS). Since chalk benthic invertebrate communities were found to exhibit strong seasonal patterns and have precise requirements for hydrological parameters such as velocity, they have the potential to be used as indicators of stream characteristic change within chalk streams (Extence *et al.*, 1999). However, the current macroinvertebrate based biotic indices only identify the macroinvertebrates to family level, meaning that fine-tuned interactions could be missing from interpreting the water quality and interactions with the environment (Moolna *et al.*, 2019). With climate change likely to cause significant shifts within the hydrology and water quality of chalk streams, by potentially increasing the periods of drought and increasing water pressures on aquifers and altering the dilution of chemicals such as nitrates and phosphates, as well as other pollutants, it is important to understand how these communities respond temporally to different hydrological regimes and water quality states, to predict the future ecological health and stability of chalk streams (Extence *et al.*, 1999; Vowles & Kemp, 2019).

Chalk stream headwaters are particularly interesting in terms of monitoring environmental change because they can support rarer species that are specialised to survive the absence of flow and can therefore indicate the influence of drought by their presence or absence in the headwaters, rates of recolonisation and the factors that determine the makeup of the ecological community (Westwood *et al.*, 2005). This essentially makes the headwaters a natural laboratory that can provide information on the rate and format of environmental change at spatial scales across the river catchment (Westwood *et al.*, 2005; Westwood & Teeuw, 2001).

However, in the upper Colne catchment, most of the river fly surveys are concentrated downstream, with only one site at Otterspool with intermittent data for the past five years (Riverfly data, 2025). Most of the sampling instead focused on the tributaries of the chalk stream and further downstream in the Colne Valley park (Riverfly, 2025). This has left a gap in data on the macroinvertebrate communities in the the upper Colne catchment (Riverfly Network, 2025). The previous standard riverfly surveys relies on eight specific indicator species and of these, only cased caddis and caseless caddis, *Ephemeraidae*, *Baetidae* and *Gammaridae* were recorded. This current dataset only provides a very limited and basic insight into the macroinvertebrate community within the Colne's headwaters. Another study that aimed to identify indicator species based

of flow rates was also undertaken within the Colne and Lee valleys over the summer and autumn seasons between 1993 and 2017) but this did not include any sites on the upper Colne and the sites sampled varied between years (England *et al.*, 2019). The relative inaccessibility and dispersed location of the headwaters of chalk streams has led to limited characterisation of these environments, both ecologically and environmentally (Kabir *et al.*, 2024). Previous research work in the headwaters of chalk rivers is largely based on data from a single season. This means there is the potential for considerable temporal variability in community composition which makes it important for multi-season sampling to generate a more comprehensive understanding of site-specific biodiversity (Kabir *et al.*, 2024). Multiple surveys that cover more than one season often provide a more comprehensive picture of total biodiversity (Berlajolli *et al.*, 2019). Therefore, sampling the upper Colne catchment near its source in the reach of the Colne that experiences temporary drying and intermittent flow is important in understanding how the ecological community responds to changes in the aquatic environment where there is already a stress on resources. Monthly sampling of the benthic macroinvertebrates throughout all intra-annual seasons is also important to understand the influence of environmental changes on the communities and diversity, rather than just a snapshot in time. As the majority of chalk rivers are located in Southeast England, an area facing both increasing population pressures and the potential for significantly altered rainfall patterns due to climate change, it is vital to prioritise the study of these ecosystems. Understanding their current ecological status and biodiversity, particularly the composition of invertebrate communities, is essential. By analysing how these communities respond to present environmental conditions, we can better predict future changes under shifting climatic and hydrological regimes. This knowledge will be crucial for informing evidence-based management strategies aimed at protecting the unique habitats of chalk streams and the diverse flora and fauna that depend on them for survival.

An investigation into the River Colne at Colney Heath common was undertaken in 2023 and this characterised the habitat types, flows and macrophytes present, along with bird species and mammals. However, this study did not include specific measurements of the environmental parameters such as flow velocities, water temperatures and nutrient levels. It also did not link this specific site on the River Colne

to the wider catchment in the Upper Course. Therefore, whilst there have been individual studies on the River Colne and its downstream course, there is a need to establish the trends across the wider upper Course of the Colne chalk stream and how the longitudinal changes may affect the hydrological and ecological functioning and interactions between the riverine and wider environment. Previous work has also focused on the micropollutants on the Colne, rather than the three major water nutrients (CVFC, 2023). It is also important to study upstream from any Sewage Treatment Works or water abstraction plants to understand the natural hydrological cycle of the intermittent chalk streams so that restoration projects can know what goals to set for restoration of the natural flow regime.

2.2. Aims and Objectives

Therefore, this study aims to (i) establish a comprehensive baseline of the ecological and hydrological functioning of the Upper Colne catchment by adopting a multidisciplinary approach. The study will focus on characterising macroinvertebrate communities, and their diversity while identifying temporal and spatial changes within the headwaters and (ii) to understand how abiotic factors (flow regime, water chemistry, and physical-chemical parameters) influence the biological communities, thereby informing future management strategies for the conservation of this sensitive chalk stream ecosystem.

The specific objectives of the project are:

1. To establish a baseline of the environmental and ecological health of the River Colne in its upper catchment, via ecological, chemical, and hydrological monitoring (Chapters 5, 6, 7, 8).
2. To determine the macroinvertebrate ecological community of the upper River Colne Catchment at four different sites and to understand how the environmental parameters of the river influence these communities (Chapter 7).
3. To fully understand the temporal and spatial variation of macroinvertebrate diversity and hydrological regime in the Upper Colne Catchment's, this study will determine the relative influence of key environmental factors driving ecological responses throughout the annual hydrological (Chapters 5 and 7).

4. To apply the current theory of ecological bioindicator measures relating to community and diversity relations with environmental parameters in chalk stream catchments to the upper Colne catchment in order to advise the best management strategies for stakeholders and other interested parties (Chapters 7 and 8).

Chapter 3: The Field Study Area

The UK hosts the majority of the world's chalk streams, with over 200 identified across the UK (Defra, 2004). Chalk streams derive their names from their geology. These rivers and streams are situated following the bands of chalk bedrock shown in figures 3.1 and 3.2 which are mostly located in the south and east of England, although there are a few located in the east midlands along the coast. The headwaters of chalk streams are fed by underground chalk aquifers which were formed from and maintained by rainwater percolation through the ground and chalk rock via fissures towards the water table and into the permeable chalk bedrock where the water is stored (Berrie, 1992). Chalk rivers are a unique type of lowland river as they are dominated by groundwater discharge from chalk geology, including those that flow over non-chalk surface geologies at different locations in their reach, particularly further downstream (Mainstone *et al.*, 1999). Due to the specific geological conditions, this also creates a distinctive hydrochemistry and flow regime, which relies on the seasonality of the weather and its influence on the chalk aquifers. The headwater of a chalk stream can vary between summer and winter, as when the chalk aquifer is recharged with percolated rainfall, the headwater of the stream can move up the valley, but in summer the headwater migrates down the valley as the water table is lower due to greater evaporation and evapotranspiration, leaving the upper stream dry (Chilterns Chalk Streams Project, 2024). As there are large areas of chalk outcropping in south England, particularly in the Chilterns, south Downlands and white cliffs of Dover, this is where most of Europe's chalk streams are situated, and due to their international importance for biodiversity and ecological and socio-economic services, such as hosting the habitats for rare invertebrate species in the headwaters, or providing suitable conditions for watercress farms, they are a recognised key habitat in the UK BAP (UK Biodiversity Steering Group, 1995).

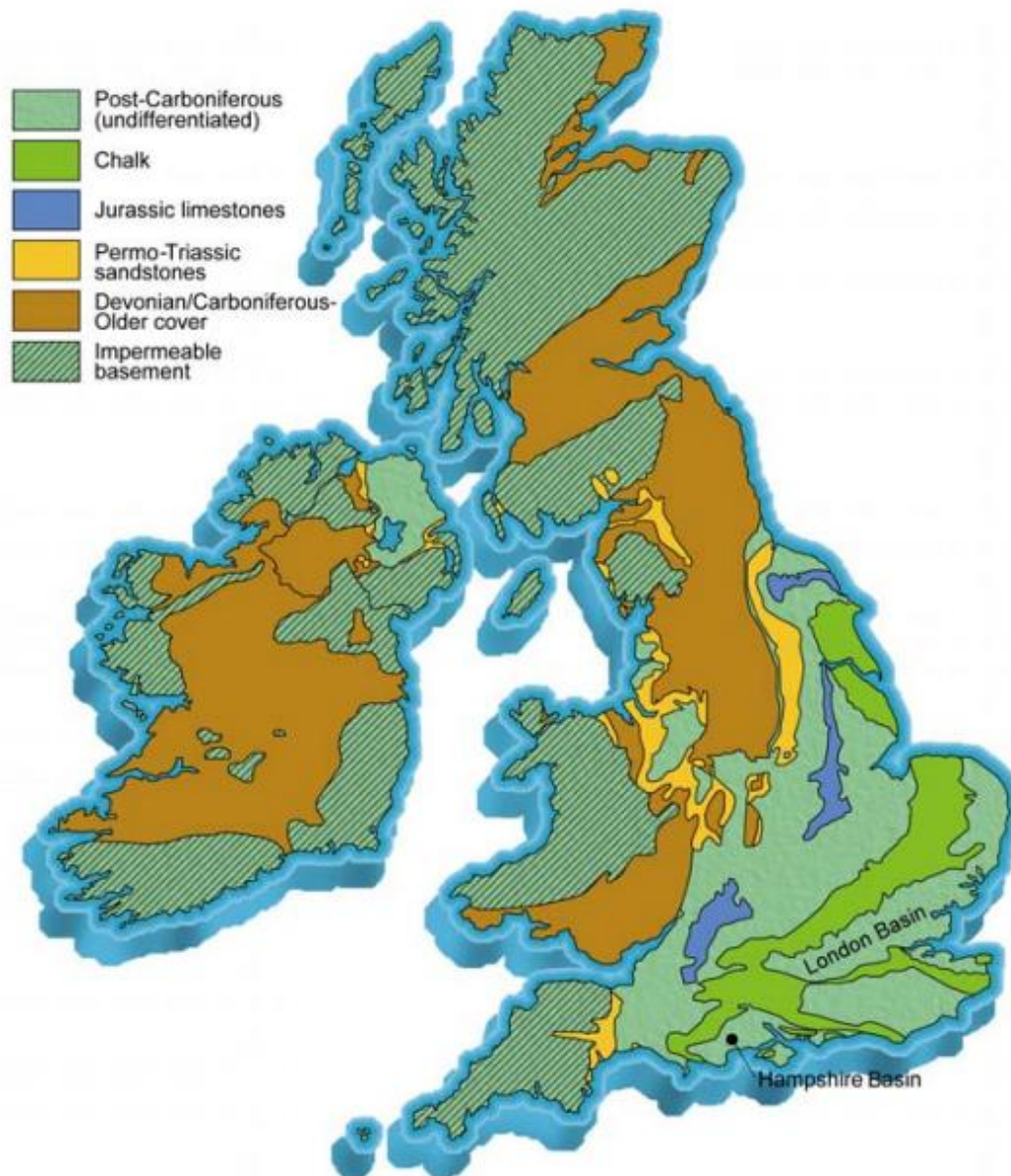


Figure 3. 1: Distribution of the principal aquifers in Britain and Ireland. (Downing, 1998). The green band of chalk runs primarily east to southwest in southern England.

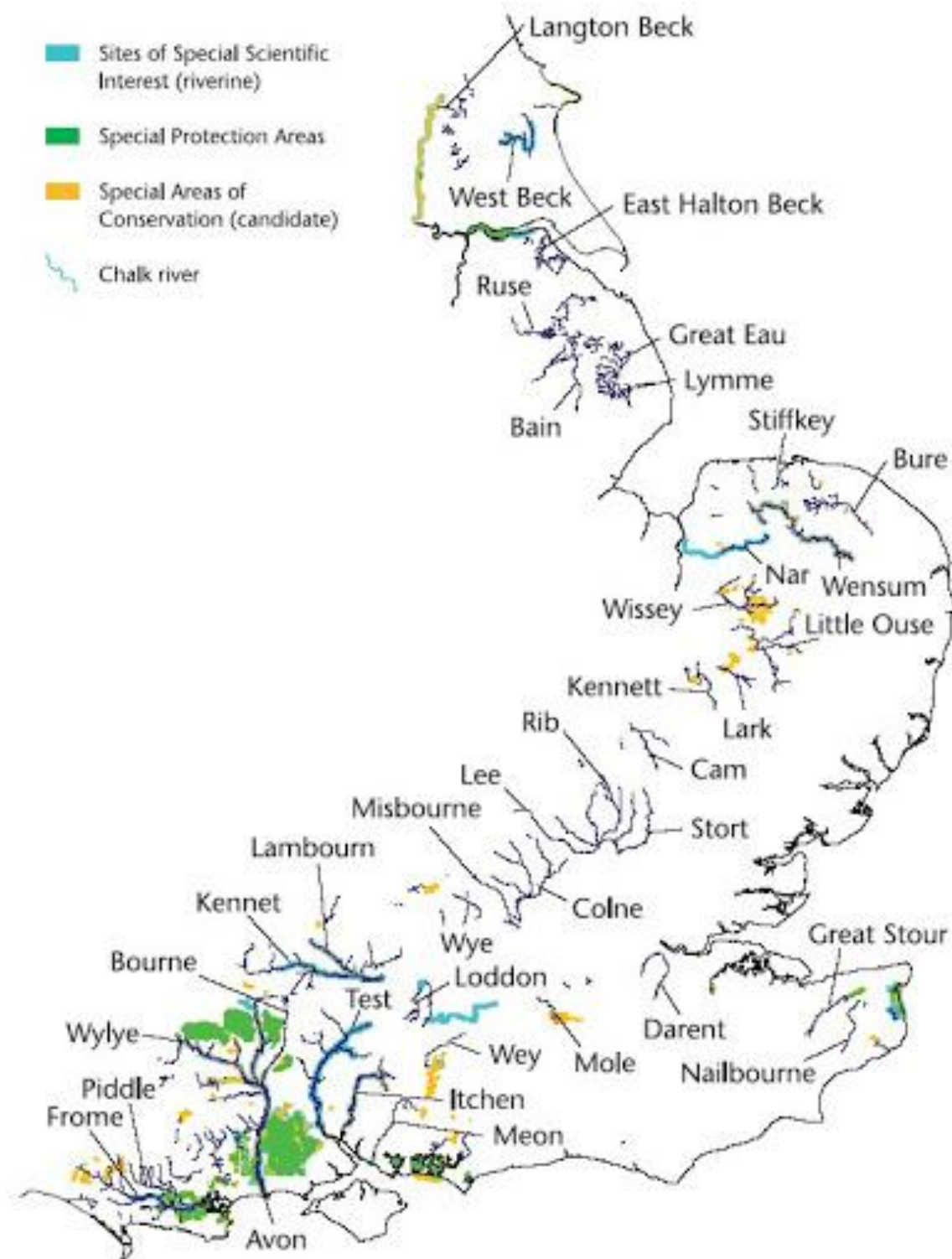


Figure 3. 2: A map of the designated chalk river in southern England and their respective protected statuses (Ranzetta, 2020). The Colne catchment does not currently have any designations either within the Riverine environment or in the surrounding areas. However, there are a few local SSSI's upstream of the Colne at the Swallow Holes in North Myms and the Water Meadows at Frogmore.

3.1.1. The Colne Catchment

The River Colne catchment covers approximately 1018 km² across the three counties of Hertfordshire, Buckinghamshire and Middlesex (figure 3.3). This catchment contains eight individual catchments: The Upper River Colne and Tributaries; River Ver, Rivers Gade and Bulbourne; River Chess; Rivers Misbourne and Alderbourne; Colne Valley Park North; Colne Valley Park South; and the Rivers Pinn, Ash and the Twin Rivers. These rivers drain in a south-easterly direction, before the Colne flows into the River Thames just upstream of Teddington Lock in Staines Upon-Thames. (River Colne CAN, 2024). The source of the River Colne is in North Mymms (figure 3.4), and its tributaries such as the Ver, Gade and Chess, rise in the Chilterns chalk river catchment (see figure 3.3 below) (Tomkins, 1966).

The Colne is considered a chalk stream as it is fed by the unconfined chalk aquifer in the Chalk (detailed further in section 1 and 4). The River Colne is also a classified certain chalk river by the under the UK Biodiversity Action Plan (Freshwater Biological Association, 2023) and is a type A chalk stream which rises directly from the chalk and flows over Tertiary deposits (Rangley-Wilson, 2014). Most is a C2.3 EUNIS code low energy river, as classified by the EUNIS Habitat classification 2012 and UK BAP with a >0.85 baseflow index (typical of a classic chalk stream) (Rangley-Wilson, 2023), although there are some characterisations of moderate and high flow on the database, but this is largely due to fluctuations in available runoff throughout the year, rather than its baseflow (Natural England, 2022). Originally, only chalk rivers were protected under the UK BAP, but it has since expanded to include active shingle rivers and headwaters, which means that even some smaller tributaries of the Colne, such as the Ellenbrook are now protected too, even if they rise outside of the Chalk aquifer boundary. It is important that rivers have protection and are considered by environmental and governmental initiatives as they are a driver for several hydrological, ecological, chemical, physical and biological processes (FBA, 2023). They also play a crucial role in the connectivity of different habitats such as the water meadows and alder meadows heavily associated with chalk streams (table 18), as well as the more obvious link of the habitat transition between aquatic and terrestrial environments in the winterbourne headwaters.

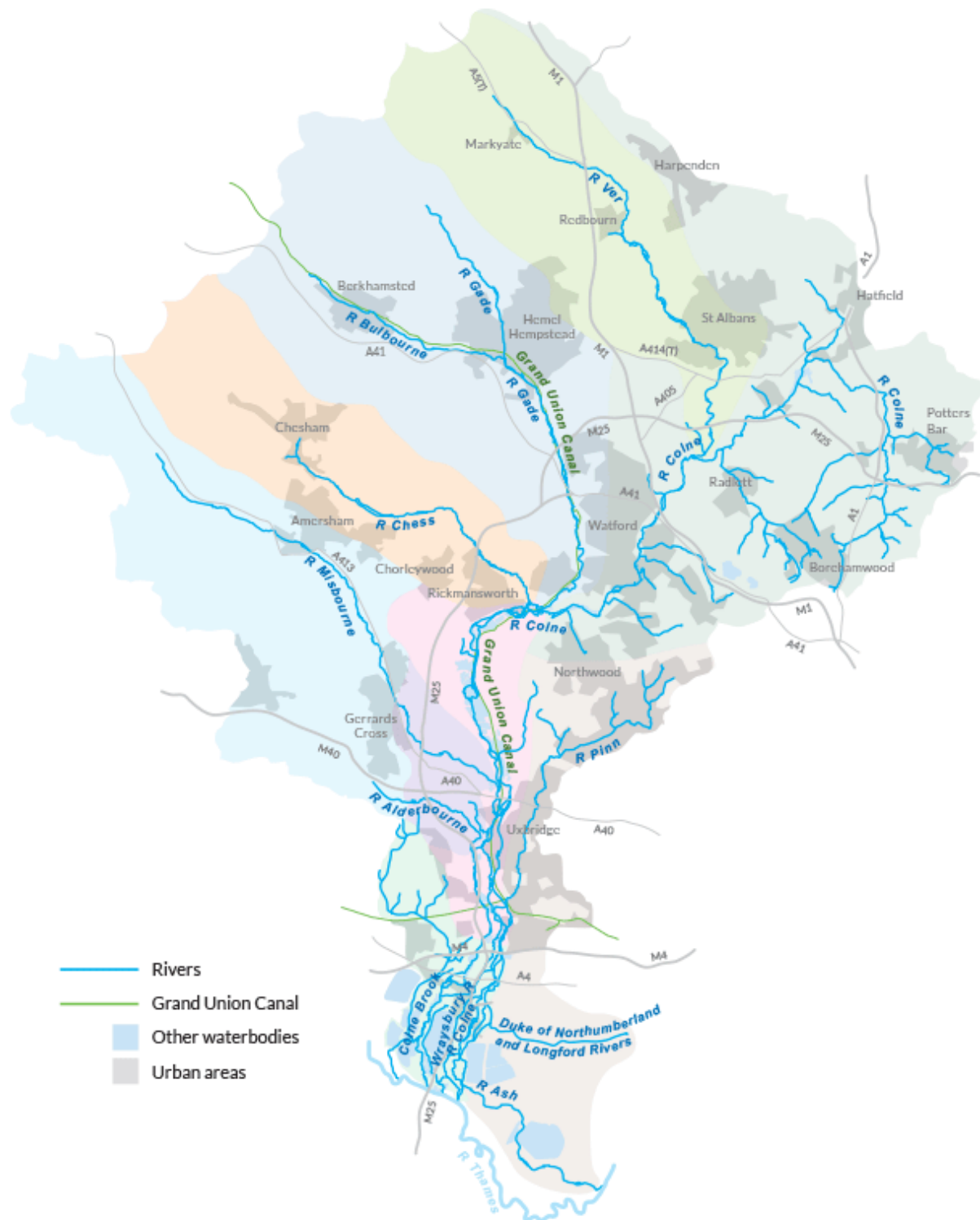


Figure 3. 3: A map showing the River Colne Catchment. This catchment has eight individual catchments that comprise the Colne's tributaries and the main river (River Colne CAN, 2024).



Figure 3. 4: *The source of the River Colne in Flood at North Mymms on the 3rd April 2024 after heavy rainfall the night before. Chalk water has risen up the swallow holes at Water End, and the water has flushed through towards the headwater of the River Colne at North Mymms, which is often dry during the summer (Tomkins, 1996). Above is upstream; Below is downstream.*

Table 3. 1: *The key environmental assets on the Colne Valley Park (Baxter, 2011). These sites are all located within the borough of Hillingdon and are on the mid and lower course of the Colne, before the Colne converges with the Thames.*

Environmental Asset	Notes
Lower Colne Valley	A system of Colne, Wraysbury and Fray chalk streams which supports diverse aquatic and marginal flora, including pennyroyal (only area present within London).
Mid Colne Valley	A system of chalk streams that flows through several SSSIs including Frays Farm Meadows and Denham Woods.
Little Britain	A system of lakes and neutral grassland, formerly gravel pits and wasteland, with a high bat population.
Harefield Chalk Pit	An oak woodland developed over an old chalk pit.
Springwell and Stocker's Lakes	The lakes are on the site of a flooded gravel workings and are sites of ornithological importance and an important London heronry.
Fray's River at Uxbridge Moor	A diverse area of wetland plants and adjacent recreational fields.
Canals	Important for reeds and waterfowl habitats with trees and shrubs on towpaths.

3.1.2. Geology of the Colne Catchment

In south-eastern Hertfordshire, the Chalk is divided into the Lower Chalk (Grey Chalk Subgroup) and the Middle and Upper Chalk (White Chalk Subgroup) (figure 3.5) with the major boundary occurring at an unconformity surface at the base of the Plenium Marl (Hertfordshire Geological Society, 2024). The two units create a 680ft thick layer. The Lower Chalk (180ft thick) is found at the base of the Chiltern scarp and projects north-westwards, as seen near Whipsnade and Ivinghoe in Bedfordshire, and the two formations (Westbury and Zig Zag) are separated by Totternhoe Stone (which has been quarried and used in local buildings such as the St Albans Abbey Gateway) (Hertfordshire Geological Society, 2024). The majority of the chalk in Hertfordshire and the Colne Catchment is the Upper chalk which is 500ft thick and has four distinct formations present (figure 3.6). The Holywell Nodular Formation is flint-less and its base is formed from very hard, shelly Melbourne Rock. The New Pit Chalk is non-nodular and massively bedded with regular marl (argillaceous clay) seams between 1 -10cm thick. The Lewes Nodular Chalk is a hard nodular chalk which is locally iron stained and flinty and this is the unit that is most common along the Colne Valley where the tributary chalk streams are present. These chalk streams are likely to be following

fault lines in the chalk which developed further via erosion during the ice age. For example, the River Bulbourne is thought to follow a series of northwest – southeast trending faults at Tring Gap (Hertfordshire Geological Society, 2024). Towards the south of the county and where the upper Colne and tributaries rise is where the Seaford Formation is situated. This is the youngest chalk formation and is a typical uniform, soft white chalk with regular flint bands and local shell-rich horizons with a high microporosity, leading it to being one of the major aquifers in southern England (Hertfordshire Geological Society, 2024). The boundary of the chalk aquifer is denoted by the clay deposits of the Lambeth Group and London Clay which confines the chalk aquifer. The deposition of any newer chalk deposits in the Chalk aquifer ceased around 66 Ma ago and up to 500m of chalk could have been eroded over the following 10 Ma by regional structural uplift, such as that that led to the deposition of the London Clay in the Paleogene (Bailey, 2023). The Anglian Glaciation (480 to 425 ka ago) led to glacial ice sheets spreading southwards from the north of the British Isles and the repeated freeze-thaw cycles would have broken down any chalk at the surface outcrops in the Chilterns. This led to the formation of deep incised valleys which created the Chiltern dip-slope, as well as the plateau Drift sediments and clay with flints to be present from the mixing of chalk with upper sediments (Bailey, 2023). The Seaford Chalk formation is also highly fractured from these freeze-thaw cycles and is therefore the most porous aquifer in the upper chalk unit, although this could also mean it has the highest potential for contamination through pollution due to its highly fractured nature.

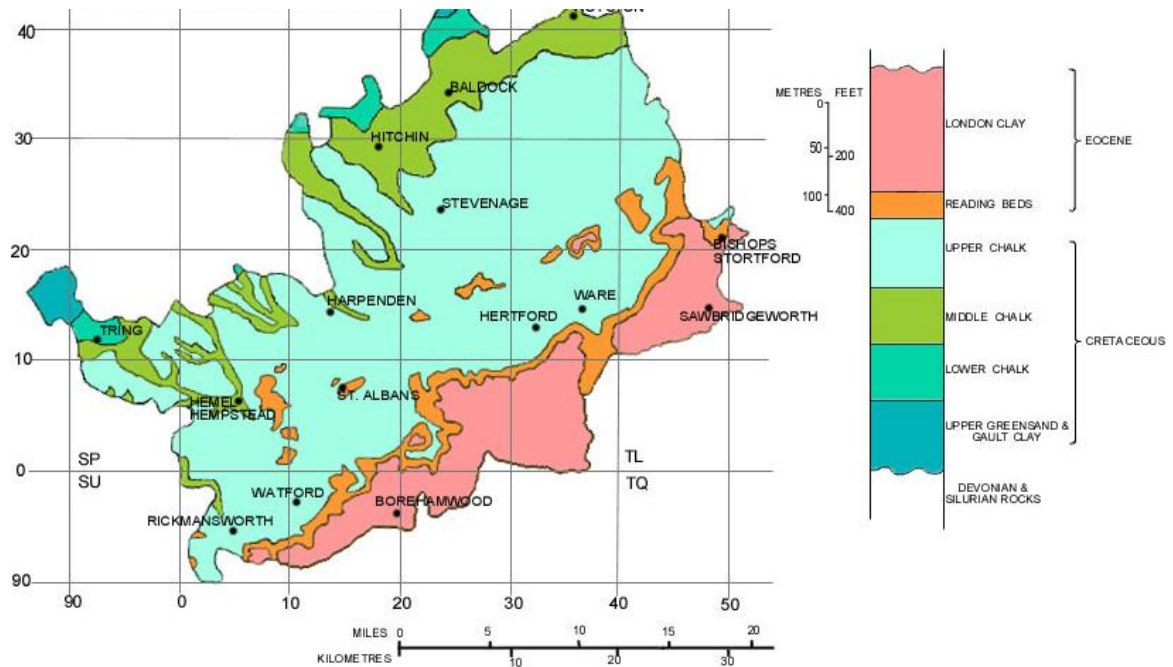


Figure 3. 5: The solid geology of Hertfordshire. The majority is Upper chalk, with middle chalk to the south, and London Clay in the south of the county, on the border of London and the Thames catchment. (Catt, 2021). The chalk is of Upper Cretaceous age about 100 million years old. The chalk is around 680ft thick in south east Hertfordshire. This is due to the uplifting and tilting of the bedrock at the end of the Cretaceous period (Catt, 2021).

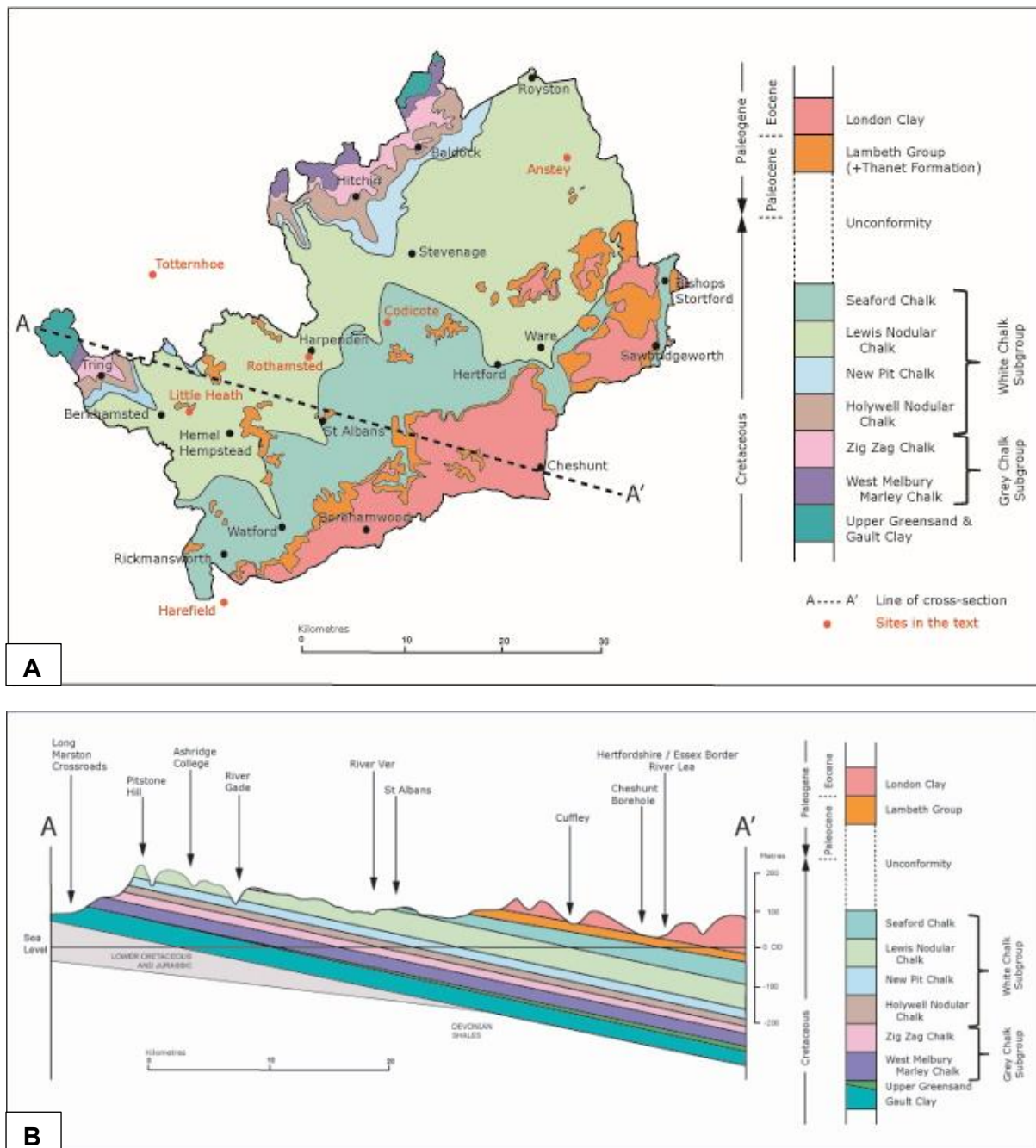


Figure 3. 6: A map of the solid geology formations in Hertfordshire(A) and the cross section of the formations (B). The River Colne and its upper catchment are located where the upper chalk (White Chalk subgroup) is present (Hertfordshire Geological Society, 2024). The chalk aquifer is constrained by the clay deposits in the south of the county.

3.1.3. Resource Availability within the Colne Catchment

Flow statistics have been used by the Environment Agency (2018) to show how the flow can vary throughout the catchment throughout the year. This flow statistic is shown as the percentage of time that flow is exceeded at four different flows Q95 (where the flow is exceeded for 95% of the time, a low flow), Q70 (summer flow), Q50 (mean flow) and Q30 (the highest flow, winter flow). However, across the catchment in all flow scenarios, there is no water available for abstraction (figure 3.7) within the Colne catchment. This means that water is not available for licensing and recent actual flows are below the Environmental Flow Indicator (EFI), meaning the flow of the water body is below the indicative flow requirement to help support a healthy ecology in our rivers (EA, 2018A). Water availability including the groundwater resource availability is the same as the surface water for the Colne Abstraction Licensing Strategies (ALS), with the exception of the southeast chalk aquifer which is overlain by clay (figure 3.8), meaning the aquifer is confined and is part of the London ALS. However, the River Colne and the majority of its tributaries, with the exception of Ellens Brook, all rise in the unconfined chalk aquifer, meaning they are under the Colne ALS. Therefore, there is no reliable water resource available for abstraction in the Colne Catchment Area, as it is available less than 30% of the time. This data and designation were concluded from surface water flows at Assessment Points (significant points on rivers such as confluences or gauging stations) and as groundwater abstraction directly impacts surface flows in chalk rivers, the surface water assessment points are monitored (EA, 2018A). The river flow is below the EFI at all assessment points on the Colne, with AP10 Lower Colne being the most critical. At this point, the license is set to 1% or approximately 3 days of an average year to protect the ecology of the river (EA, 2018A). Groundwater licenses are also closed to consumptive licenses.

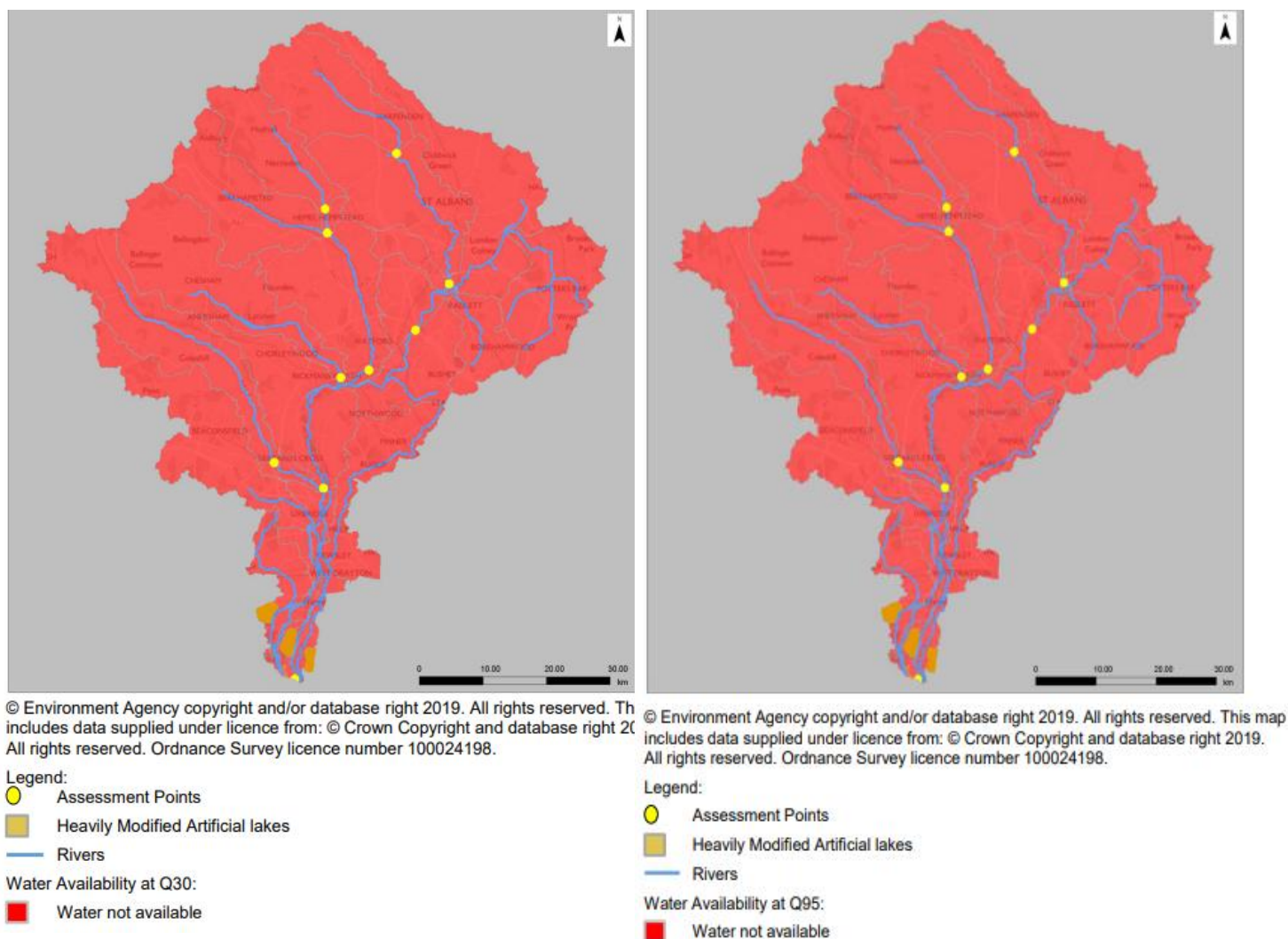
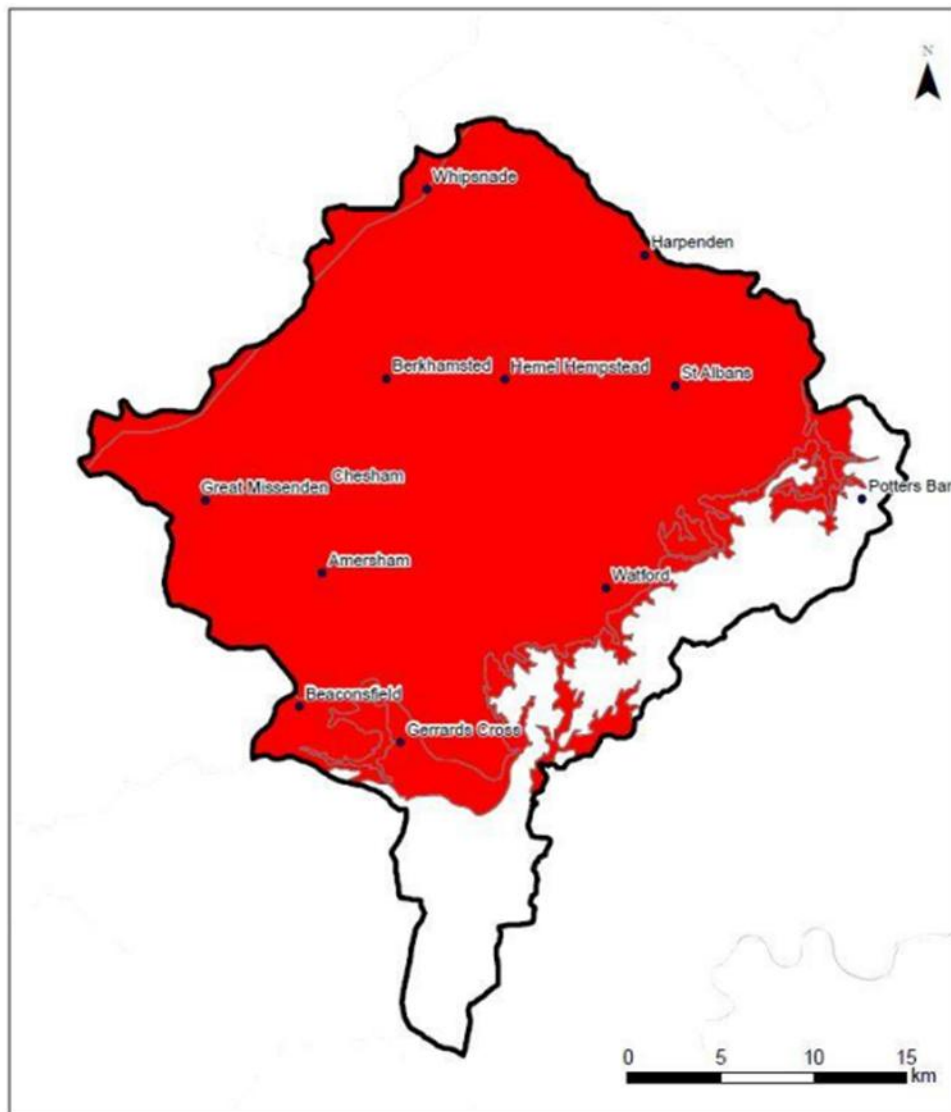


Figure 3. 7: The water resource availability of the Colne catchment. Left: Q30 (winter flow). Right: Q95 (drought) (EA, 2018A).



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Legend:

- Unconfined Chalk Aquifer
- Confined Chalk Aquifer

Figure 3. 8: A map showing the groundwater aquifer boundary between confined and unconfined chalk aquifer. To the southeast the chalk aquifer becomes confined by London Clay (EA, 2018A).

3.1.4. The Ecology of the Colne Catchment

Colne Valley regional park is located in Rickmansworth in the north and Staines and the Thames in the south with over 200km of river and 60 lakes. It was established in 1967 to preserve the area for leisure, recreation and conservation with many habitats and wildlife corridors. The wetlands provide habitats for nationally significant populations of waterbirds (Gadwall, Shoveler, Pochard, Tufted Duck, Smew, Great Crested Grebe and Cormorant). The Mid-Colne Valley SSSI has Trust's Broadwater Lake Nature reserve and also Stocker's Lake is a refuge for wintering birds. The Broadwater Lake in the SSSI was man-made by dredging gravel in the 1960s and takes up 60% of the SSSI (Herts and Middlesex Wildlife Trust, 2023b).

The River Colne is important for bat species, particularly the Daubentons and Pipistrelle with many maternity roosts. Water margins are good habitat for dragonflies and damselflies, vegetated margins are used by Reed and Sedge warblers. Stocker's Lake is home to the largest heronry in Hertfordshire, with 30 pairs breeding in the summer (Herts and Middlesex Wildlife Trust, 2023b). Fish are also a crucial part of the ecosystems, with the presence of Chubb *Squalius cephalus*, barbel *Barbus barbus*, Bream *Abramis brama*, Carp *Cyprinus carpio*, Brown Trout *Salmo trutta*, Trench *Tinca tinca*, Eels *Anguilla anguilla*, Pike *Esox lucius*, Perch *Perca fluviatilis* and Dace *Leuciscus leuciscus* (Herts and Middlesex Wildlife Trust, 2023b). The Herts and Middlesex Wildlife Trust works in partnership with the Colne Valley Regional Park, the Colne Valley Fisheries Consultative and the National Lottery Heritage Fund to train interested parties in wetland ecology and water vole surveying and has funded conservation works within 23 fisheries (HMWT, 2023b).

3.2. The Upper Colne Catchment

The headwater of the River Colne is known to be dry during the end of a rainless season - for example during the summer of 1893, the river could be crossed on foot near Tyttenhanger House, downstream of Colney Heath (Tomkins, 1966). This area tends to only be in flow when there has been heavy rainfall, such as in April 2024 where 111.4mm of rain fell on average during the month (Met Office, 2024c), leading to the rising of the water table to become high enough to generate flow at the springs

(figure 3.4 in Section 3.1.1.) (Environment Agency, 2024), which then flow towards to more perennial sections of the Colne at Colney Heath and Watford. The River Colne is approximately 58 km long, and its confluence is with the River Thames at Staines-upon-Thames. Due to the historical drying up of the upper areas of the River Colne (figure 3.9), it is important to study the hydrology and macroinvertebrate assemblages of this location. Due to climate change, it is highly likely that more frequent periods of drought and extreme flooding will occur and this will have consequences on the quality of the environment (figure 3.10) and habitats provided further downstream at locations such as Watford, Rickmansworth and Uxbridge, as well as in the Colne Valley Park (Shand *et al.*, 2003).

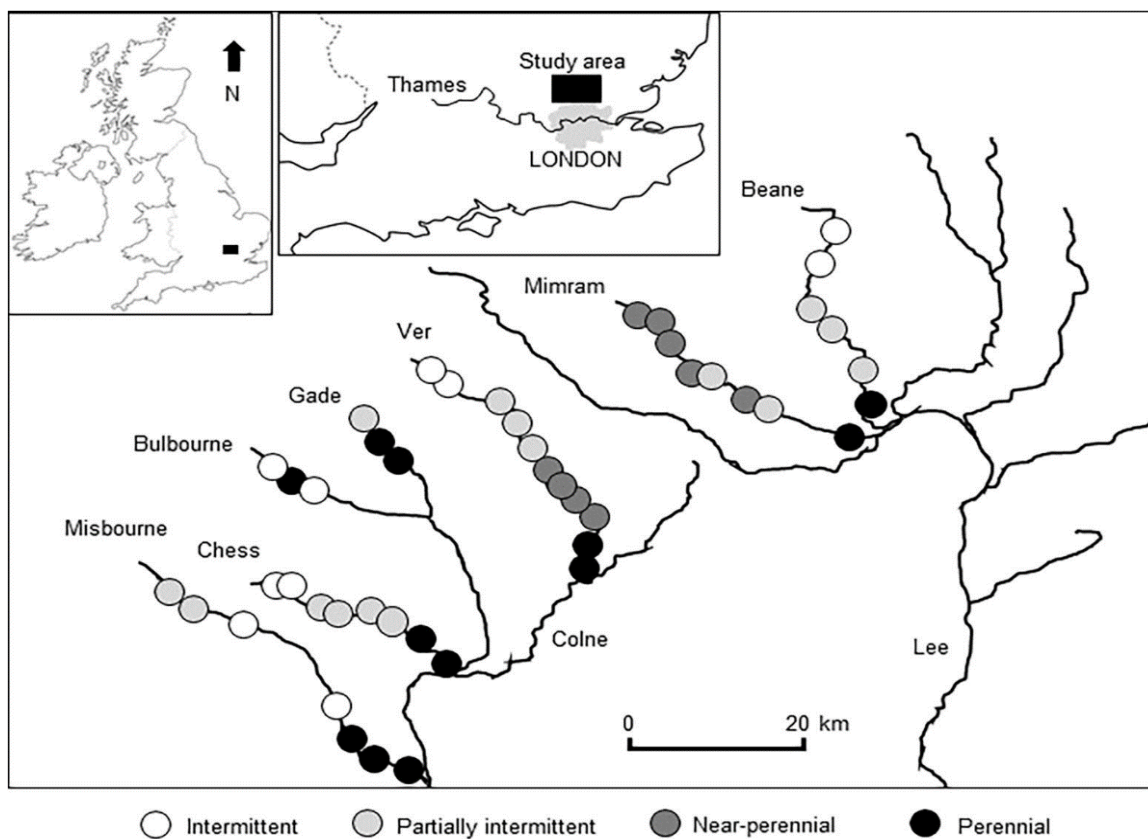


Figure 3. 9: The River Colne catchment and the flow designation from England *et al.*, 2019. The upper Colne has not been previously studied. It is presumed that the site would either be intermittent (winterbourne) or partially intermittent in Colney Heath (England *et al.*, 2019).



Figure 3. 10: *The ecological health of rivers in Hertfordshire (The Rivers Trust, 2021). The majority of the Colne is moderate, with some poor areas, and further east in the minor upper Colne tributaries classified as bad.*

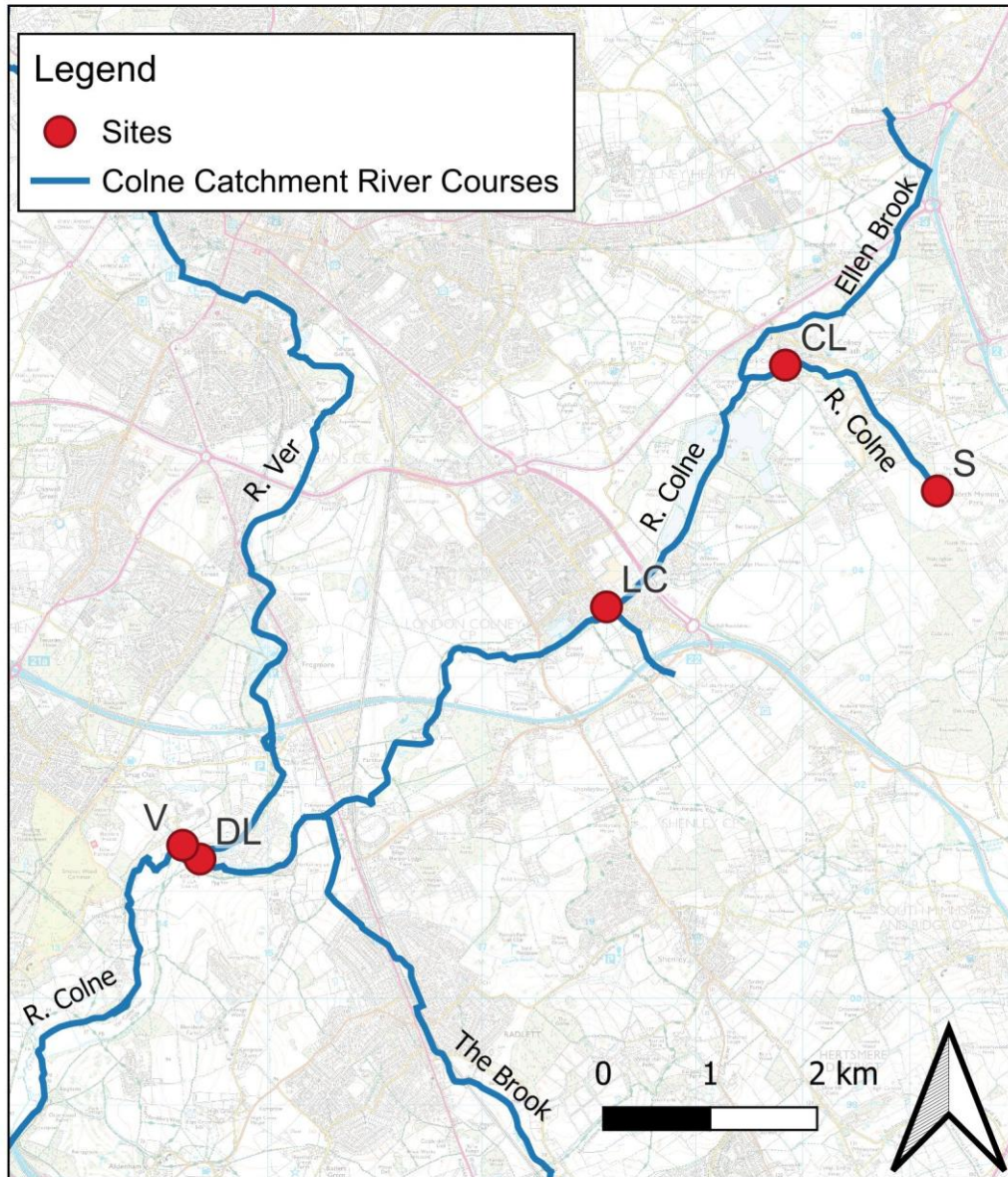
3.3. Study Sites

There are four sites (Table 3.2; Figure 3.11, 3.12) that have been chosen due to their accessibility and distance downstream on the upper Colne, before the Colne flows through more urbanised environment in Watford. These locations also do not currently have any previous monitoring on them, making them interesting sites to establish a baseline of functionality and quality. The sites that were chosen for this study overlaps with some of the sites chosen for the micropollutants report on the River Colne. While that report primarily focused on the River Colne, it also included a comparative analysis involving the River Ver at Drop Lane (Colne Valley Fisheries Consultative, 2023). Two permanent sites were chosen for hydrological monitoring due to their ease of accessibility and higher permanence of flow in Church Lane and Drop Lane. Both sites are predominantly rural, flowing through areas of common and agricultural land, although both sites have roads nearby used for access to residential areas (figure 3.13).

Table 3. 2: *The four sites (1 – 4) chosen for this study along with the abbreviations that are used to refer to throughout this study. The grid reference location is included, along with the distance of the site from the source (0) and the distance between the sites in the Upper Colne.*

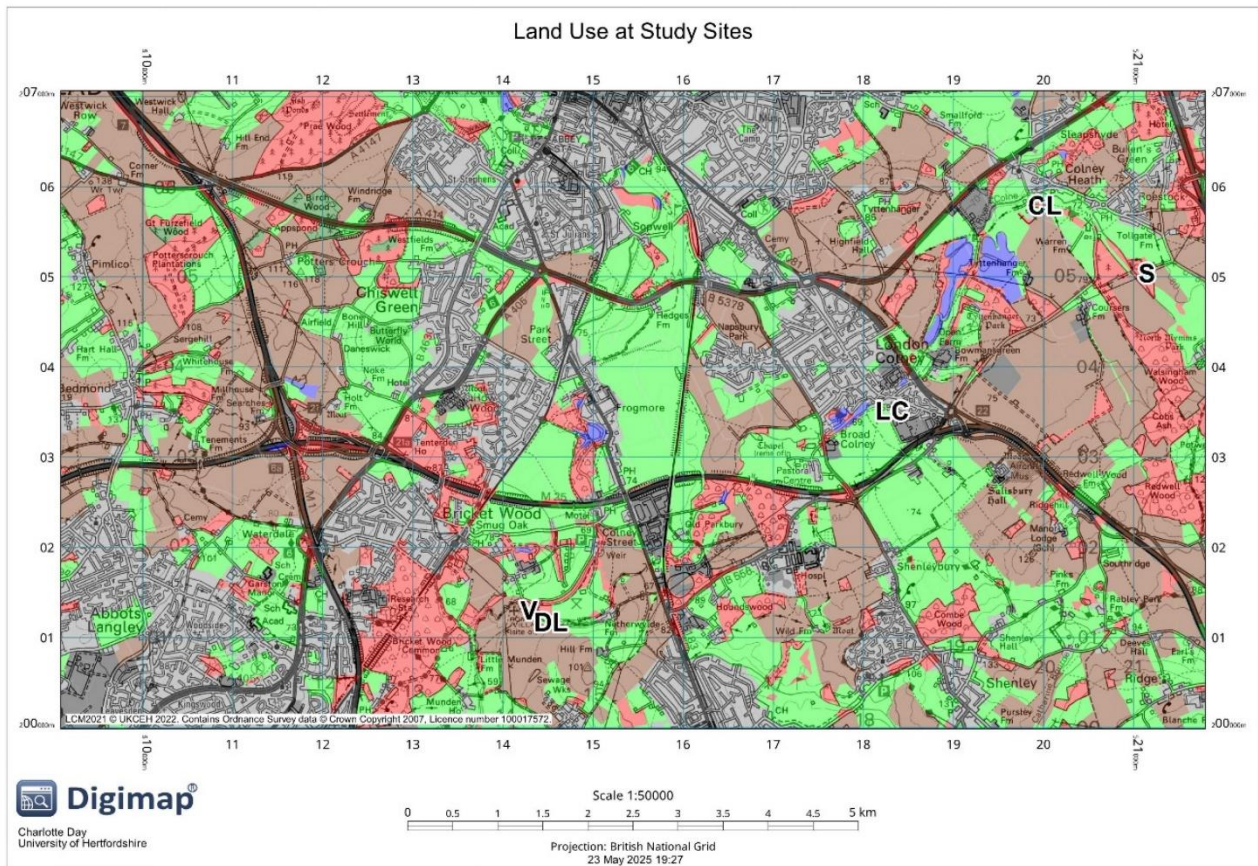
Site Number	Site Name	Abbreviation	Location	Distance From Source (m)	Distance Between Sites (m)
0	Source	S	TL21270473	0	0
1	Church Lane	CL	TL19830592	2703.3	2702
2	London Colney	LC	TL18210365	6304.1	3600.8
3	Drop Lane	DL	TL14360130	12287.4	5983.3
4	Ver	V	TL14200143	12618.9	331.5

The Study Sites in the Upper Colne Catchment



Map sources taken from Digimap (2025) and the Environment Agency Catchment Data Explorer (2025). GB Overview [TIFF geospatial data], Scale 1:5000000, Tiles: GB, Updated: 4 December 2024, Ordnance Survey (GB), Using: EDINA Digimap Ordnance Survey Service, <<https://digimap.edina.ac.uk>>, Downloaded: 2025-06-07 18:23:35.462

Figure 3. 12: The four study sites (CL, LC, DL, V) and their relative positions within the Upper River Colne and Upper Colne Catchment, and the distance from the source (S) (Digimaps, 2023). CL and DL were the locations with the permanent gauging station for monitoring and all four sites had monthly sampling.



Legend

Broadleaved Woodland	■
Coniferous Woodland	■
Arable and Horticulture	■
Improved Grassland	■
Neutral Grassland	■
Calcareous Grassland	■
Acid Grassland	■
Freshwater	■
Urban	■
Suburban	■

Figure 3. 13: The land use of the Upper Colne, showing a patchwork of agricultural, improved grassland, broadleaved woodland and suburban uses (Digimap, 2023). The crosses mark the sites and the source of the River Colne.

3.3.1. The River Colne at Church Lane, Colney Heath

One of the sites is located on Colney Heath Common TL 19847 05916, in Colney Heath, Hertfordshire (Figure 3.12, Figure 3.14). This Heath Common is one of the few acid heathlands in Hertfordshire and is a designated Hertfordshire Heritage Site and a Local Nature Reserve, recognised under the Natural Environment and Rural Communities Act (2006) (CHPC, 2019). It provides a variety of different habitat types including improved grassland and broadleaved woodland. The site is currently managed by Colney Heath Parish Council which have implemented a 5-year management plan to protect and increase the biodiversity of the heathland on the Common and protect the grade 1 River (CHPC, 2019). By 2024, they aimed to cut back woodland and exposed scrub growth in zones 3 and 4 by the riverside by 20-30% (CHPC, 2019). This would alter the immediate vegetation surrounding the river and also impact the amount of canopy cover over the river, changing the habitat provided. Here, the river flows from east to west, bisecting the flat common ground, before flowing towards the gravel extraction pits and then south, down towards Tyttenhanger, the quarry lakes and further onto London Colney and Watford. The river plays an important role in the ecology of the Heath Common as during the winter, the upstream swallow holes overflow, leading to flooding on the common (CHPC, 2019). There is also scrub and woodland nearby to the River Colne, and pasture farmland is located to the east of the Common. To the south of the river is Fredericks Wood (forestry) and the Warren is heathland that is now grazed by livestock in the summer. At the Church Lane site, the flood risk is of a medium concern around the bridge and road where the site was identified. This means that the floodplain has a risk of flooding of 1% (1 in 100 each year), but is not of a major concern (EA, 2022). The majority of the common is of a low risk of flooding and further upstream is mostly low risk of flooding, with some small areas of medium risk along the river channel.

While previous research has focused on these tributaries or the lower Colne, such as the area in the Colne Valley Park and urban areas, there has been less research on the upper Colne. Therefore, this project focuses on the River Colne starting from its source in North Mymms, as it flows east to west through Colney Heath, London Colney and Bricket Wood.



Figure 3. 14: The sampling site at Church Lane. Left: The bridge at Church Lane looking in a south easterly direction. The river's flow was fairly low and it is surrounded by marginal vegetation, with good access to the banks of the river and the weir at low flow. The stage gauge and white box was left by the Environment Agency from a temporary monitoring station they set up. (16th November 2022).

Right: The view upstream of the River Colne at the site on Church Lane in Colney Heath. Above: The stream at the end of summer (27th September 2023) where there is large areas of overhanging vegetation and marginal vegetation within the stream.

3.3.2. The River Colne at Telfords Bridge, London Colney

The River Colne flows through fishing lakes and willows farm before reaching London Colney TL 18191 03664. Before it reaches the Telford Arches Bridge, the river flows through parkland and a lake (figure 3.12; figure 3.15). There is an Environment Agency monitoring station upstream of the bridge which is monitored regularly. The river channel is deep prior to the bridge, but the flow is funnelled through the bridge into the ford which has shallow sloped banks and a gravel bed. The land use is mixed, with this site having the most urban setting (figure 3.13). There are willow trees lining the banks of the river and grassland. Through the village is one main road that bisects the river at the bridge and there are a few residential areas backing onto the river next to the ford. There are footpaths and footbridges further down, but this part of the channel is dry for most of the year as the bed is heavily vegetated. Further downstream are more fishing lakes with houses at the lake edge. The channel at the ford can dry up and become fragmented during summer, but in winter the flow of the river can exceed bank full and flow nearly to the top of the bridge. Downstream at London Colney, the flood risk is mostly contained to the floodplain, with areas of medium risk around the old quarry pits which are now wildlife lakes. At the site in London Colney at Telfords Bridge, the flood risk is medium around the main channel, with an area of low risk from a tributary from the north into the Colne.



Figure 3. 15: *The River Colne looking upstream towards Telfords Arches Bridge in summer (left) and after heavy rainfall (right). The water tends to pool in the disused*

ford which often silts up. During high flow, the river level can be seen on the markings on the bridge, at least halfway up the bridge.

3.3.3. The River Colne at Drop Lane, Bricket Wood

The river Colne at Drop Lane TL 14363 01300 flows parallel to the River Ver, just before its confluence after the Thames Water owned sewage pumping station upstream (figure 3.12). The fields surrounding the river are arable and pastureland for cattle (figure 3.13) grazing for long horn cattle. There are some farm buildings nearby and a temporary generator nearby to the pool on the River Ver. The road is used for domestic and Thames Water vehicles, as well as horse riders, dog walkers and ramblers. The site has gated access through arrangements with Thames Water where the monitoring station has been secured to the bridge over the Colne. Upstream of the bridge there is a lot of debris in the river, with a fallen tree damming the flow. The river pools downstream of the bridge, and the riverbed has a 2–4-inch layer of silt over the gravel bed. The river does not flow very well in this location and it is wide. The banks of the river are relatively steep and vegetated, with litter. An electric fence is found 100m downstream from the bridge, where the channel narrows and becomes shallower, exposing the gravel bed and some small riffles, before the river continues towards the confluence. The electric fence is in place to keep the cattle in the neighbouring fields and providing a passageway between the two fields and the majority of the land use is for agriculture and livestock (figure 3.16). The flood risk at Drop Lane, Bricket Wood has a wider area of low-risk flooding on the floodplain onto the agricultural land just after the confluence of the Colne and its tributary the Ver. This has a wider margin than the flooding of the other sites, which could be due to the additional discharge being supplied from the tributary.



Figure 3. 16: *The River Colne at the Drop Lane site with permission from Thames Water in April 2024. The view downstream (left) with a very silty river bank and a barbed wire fence across the channel. This is a crossing for the cattle in the adjacent fields. The view upstream shows a significantly obstructed flow (right), with organic debris across the channel.*

3.3.4. The River Ver at Drop Lane, Bricket Wood

The River Ver site TL 14213 BNG 01431 is located also on Drop Lane next to the Ver Valley Walk 8 (Ver Valley Society, 2018). The site is approximately 100m from the confluence of the Ver and the Colne (figure 3.12), whereafter the Colne continues to flow west. About 300m upstream, the River Ver narrows before it pools at the site location (figure 3.17). The pool is fairly shallow and stepping stones have been placed into the channel to cross between banks. The site is frequented by visitors such as children, dog walks and used for recreational activities such as swimming, yoga, and a hand-made swing sits over the southerly bank. The riverbed is gravel and the southerly bank is vegetated, whilst the northern bank is shallow gradient and more silty with fine material. A small area next to the meander traps debris in the slower-flowing area of the channel, and the middle of the channel is relatively fast-flowing and unobstructed. The stepping stones create pockets of deposition behind them and funnel the flow between them. The River Ver joins the Colne at its confluence and the river continues towards Little Munden as the River Colne. This area flows through arable fields and the channel banks are steep and inaccessible (the land use is the same as at the Drop Lane site, figure 3.13). Himalayan Balsam has become a problem

in this area, and much of the banks are covered in this plant, which is managed by charity volunteers who do regular clearing along the walks on the Ver. This site is the furthest downstream in the Upper Colne Catchment of the four sites and the only site on the Ver (a tributary of the Colne). The upper course of the Ver begins in Markyate, Bedfordshire and has two sewage treatment works on its course as well as five pumping stations for groundwater abstraction (Ver Valley Society, 2023; Thames Water, 2025).



Figure 3. 17: *The River Ver site in summer (21 June 2023) looking downstream with the stepping stones visible in the stream that children play on (left). On the right is the River Ver after heavy rainfall in 13th March 2024 looking upstream, where the Ver had burst its banks and had flooded onto the flood plain and has joined the Colne further upstream of the confluence.*

Chapter 4: Methodology

4.1. Hydrological Monitoring

4.1.1. Hydrological Gauging Stations

To obtain hydrological data for the upper Colne, two continuous gauging stations were set up on the River Colne at the Church Lane and Drop Lane sites. The frames of the two stations were similarly constructed out of rigid aluminium poles (~25mm diameter) secured together with jubilee clips (hose clips). The Church Lane station was affixed to the base of the concrete weir by drilling screw holes into the concrete (with permission from the landowner) and screwing base flange handrail fittings (size 5) into the base. The aluminium poles were then secured by tightening the base using the screws. At the Drop Lane site, there were no suitable hard surfaces to attach any hardware, so the aluminium poles were zip tied to the railings of the bridge using heavy duty cable ties. All fixings were additionally secured by wrapping with duct tape to ensure the stations stayed together, in the event of any screws dislodging due to vibrations during high flows. For both stations, the hydrological sensors (detailed below) were secured to the aluminium poles using zip ties and duct tape, and the wires were run along the poles, secured with more tape, towards the weather-proof box containing the logger. The sensors were then wired into the Campbell CR10X control (figure 5.1) (Campbell Scientific, 2022) and measurement devices (hereafter referred to as datalogger(s)) set to measure at 10 second intervals with an average value sent to the datalogger memory every two minutes.

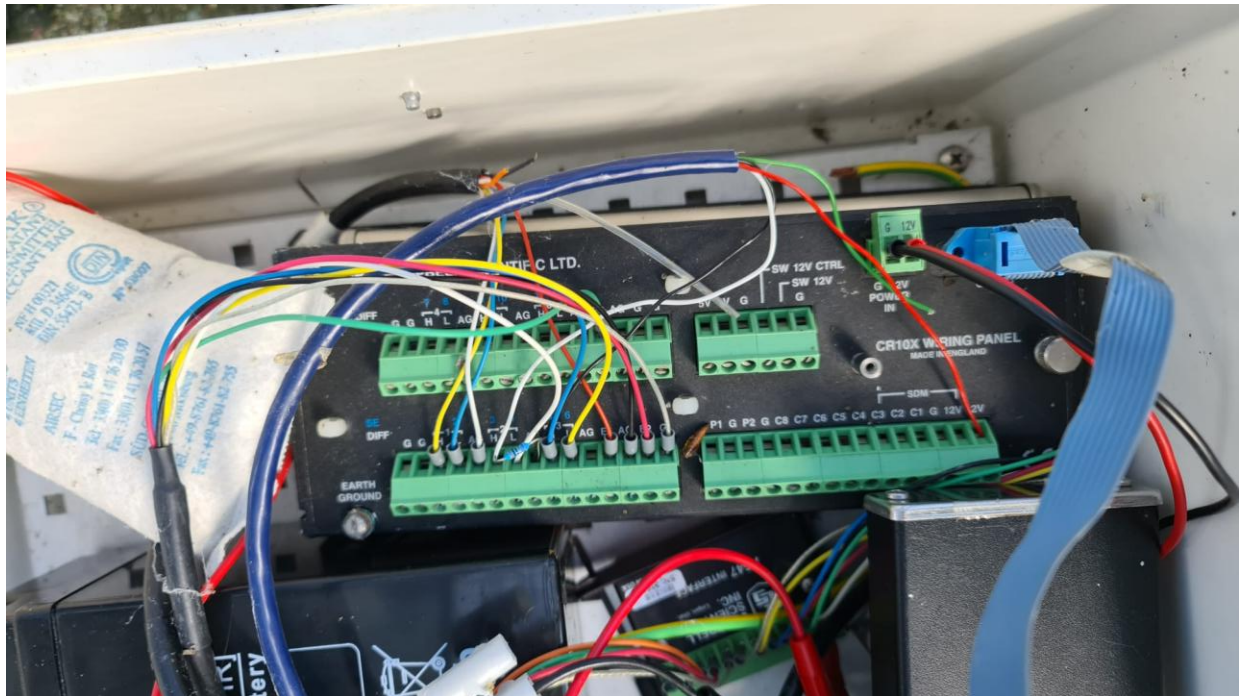


Figure 4. 1: Above: The wiring of the CR10x gauging station at Drop Lane with the pressure, conductivity and water temperature and turbidity sensors plugged in. Below: The stage and turbidity sensors secured to the metal pole using zip ties and tape, with the wiring directed and secured along the poles to the box (25th November 2022).

At the Church Lane site, the PDCR 800 series pressure transducer probe with 15m head (up to 15m water depth) and the Partech IR15C infrared sensor for turbidity were secured to the weir under the bridge using metal clamps that are drilled into the concrete weir (with permission from the local Colney Heath Parish Council) (figure 5.2). The probes were then attached to the vertical stay using tape and zip ties to secure them (figure 66), before the wires were carefully laid up the bank to the box containing the data logger. The box containing the data logger at this site was fitted into the original Environment Agency box affixed to the side of the bridge, which permission had been sought to use (John Thurlow) (figure 67). The box containing the data logger was padlocked for security reasons and well hidden inside the Environment Agency box.

The site in Colney Heath at Church Lane was visited every seven to ten days to swap the battery so the instruments have a constant power supply. During the visits, the wires were checked to see that they are all still attached to the logger and the data is downloaded and dated. The stay attached to the concrete weir was also checked for debris, to allow better functioning of the probes. Unfortunately, this site did experience a high level of debris and leaf litter material within the channel flow, meaning the site often became obstructed, despite regular maintenance. In July 2023, there was a major setback of the site, where the aluminium poles had been tampered with and snapped, resulting in the stays becoming loosen and free to move. This has made it hard to stabilise the instruments, which affected some of the readings. However, a temporary fix of securing a wooden stake to the two aluminium poles gave extra stability and meant the station remained upright in high flows. This was finally fully corrected in April 2024 (Plate 6), but unfortunately only lasted a week before the site was vandalised once more, leaving the probes out of the river channel (Plate 7). This was once again re-drilled and fixed in June 2024. In total, the station monitoring stage and turbidity at CL ran between April 2023 to September 2024.



Figure 4. 2: *Left: The initial temporary station hammered into the banks of the Colne just before the bridge, with the stage and turbidity sensor attached to it and wired to the box kept within the EA box on the side of the bridge. (25 November 2022).*

Right: A new steel pole was installed and the remaining aluminium poles were repurposed to create a more stable structure. When tested, there was very little mobility of the vertical pole meaning the instruments were kept in place securely in the river just before the weir. (24 April 2024)

At the Drop Lane site in Bricket Wood, a similar set up was installed with the addition of a conductivity and water temperature flow (the CL site was not secure enough to install this sensor). The PDCR 800 series pressure transducer probe with 15m head (up to 15m water depth) and the Partech IR15C infrared sensor for turbidity and Campbell scientific CS547A-L combined conductivity and temperature probe were attached to a stay constructed from metal and wooden poles hammered into the silt and gravel riverbed and banks (figure 5.3). The cables were then laid up the bank to the bridge railings where the box containing another CR10X data logger and battery was secured to the railings using zip ties. The Drop Lane site was located on Thames

Water owned land and property, meaning approval had to be granted for access every four to six weeks and a member of the team had to be on site.



Figure 4. 3: *The stage, turbidity, conductivity and water temperature gauge were installed in May 2023 at Drop Lane by affixing an aluminium pole with the probes secured to it to the railings of the Thames Water owned bridge. This is the view looking upstream of the River Colne with the vertical pole of the gauge inserted into the river channel. (27 September 2023).*

On 26 July 2023, a high-resolution programme was installed to the CR10X data logger with the resistor for the turbidity sensor installed. In December 2023, after collating all the data that has currently been collected from Drop Lane, it was noticed that there were large gaps where the data has not been stored, coupled with a recharging issue from a malfunctioning solar panel. To mitigate this, a new solar panel was installed, alongside an additional storage module and a newer CR 1000 logger uploaded with the programme and installed. Despite additional visits arranged with Thames Water, data gaps still remained, and the quality of the data was not meeting the requirements. Therefore, this station collected data between May 2023 to July 2024 (slightly shorter than the Church Lane site due to logistics).

4.1.2. In-situ hydrological measurements

At each of the four sampling states, monthly measurements of channel width, average channel depth, average flow velocity, river cross-sectional channel area and total discharge were taken. The channel width was measured using a tape measure at the widest section of the wetted perimeter of the river channel. At equal intervals along this width, measurements of the channel depth were taken using a metre rule. At the same locations flow velocity was measured using the Geopacks Advanced Flowmeter (Geopacks, 2025). This value was then averaged for the average flow velocity at each site. Calculations for the river cross-sectional channel area were made by multiplying the channel width and depth.

Information on rainfall levels and air temperature levels for the sampling period between April 2023 to February 2025 were requested from the Met Office (Met Office, 2024).

4.2. Water Quality Monitoring

4.2.1. Water Quality Field Methods

From each site two 100ml labelled bottles of river water were collected in the field each month between April 2023 to February 2025. Each bottle was pre-rinsed with river water, before being filled to the top with the lid screwed on tightly to ensure minimal trapped air. One bottle was kept in a climate controlled dark room for five days. Then a second reading using the Lutron Dissolved Oxygen Meter (Model PDO-519) (nhbs, 2023) was taken and recorded. This measurement is then subtracted from the original DO reading to give the biological oxygen demand. The second bottle for each site was then transported to the lab for water nutrient testing for ammonium, phosphates and nitrates (detailed in section 4.2.2).

4.2.2. Laboratory testing for Water Quality

The remaining bottles of river water from each site were used to carry out the chemical analysis using the HACH machine at Bayfordbury Laboratory. Before carrying out this work, a risk assessment for Bayfordbury Field Site and Lab was completed along with the COSHH form for each chemical test (appendices). For each sample a test for nitrate, ammonia and phosphate was prepared in the laboratory and then the cuvette was placed into the HACH machine to carry out the analysis. The chemical tests were staggered in the lab to ensure efficient time usage and kept in separate racks to avoid mixing up the sites (figure 4.4).



Figure 4. 4: Left: The laboratory set up for the HACH machine cuvettes for ammonia, phosphate and nitrates. Right: The HACH machine and balance machine to calibrate the pipettes.

To test for nitrate levels, 2ml of the water samples were pipetted into the LCK 339 Nitrat/Nitrate Sample cuvette and 0.2ml of the reagent A was added to the cuvette. The cap was screwed on and the cuvette was inverted before inverting a few times and left for 15 minutes. The outside is wiped clean before inserting into the cell holder of the spectrophotometer HACH machine where an automatic reading takes place via reading a barcode. The reading is then stored on the HACH machine and recorded manually in the results for the site. The range for the LCK 339 test is 0.23 - 13.5 mg/L $\text{NO}_3\text{-N}$. The pH of the sample must be between pH 3 – 10 and the temperature of the water sample and reagents must be between 20 - 24 °C (HACH Company, 2026).

The Ammonium HACH test LCK 303-1 sample cuvette was used to analyse the ammonia content of each water sample. The foil from the LCK 303-2 Ammonium DosiCapZip was removed and 5ml of the water sample was pipetted into the cuvette. The dosicap was screwed onto the cuvette and the cuvette was inverted for a few seconds to dissolve the white powder before being inverted a few more times. The cuvette was then left for 15 minutes before the outside is wiped clean before inserting into the cell holder of the spectrophotometer HACH machine where an automatic reading takes place via reading a barcode. The reading is then stored on the HACH machine and recorded manually in the results for the site. The measuring range for LCK 304 was 0.015 - 2.0 mg/L $\text{NH}_4\text{-N}$ and the water sample had to be between pH 4 – 9 and 20 °C (HACH Company, 2026).

The Phosphate LCK 249 alternative method was used. This involved unscrewing the lid of the cuvette leaving the foil lid intake and pipetting 0.5ml of the water samples into the cuvette and then replacing the lid and inverting the cuvette a few times. 0.2ml of reagent B was then pipetted into the cuvette and the DOSICAP was screwed onto the cuvette before inverting a couple of times. This method does not involve heating of the sample. After 10 minutes has elapsed, the cuvette was then inverted a few more times and the outside is wiped clean before inserting into the cell holder of the spectrophotometer HACH machine where an automatic reading takes place via reading a barcode. The reading is then stored on the HACH machine and recorded manually in the results for the site. The measuring range was between 0.05 - 1.5 mg/L $\text{PO}_4\text{-P}$ and water sample needed to have a pH between 2 – 10 and a temperature between 15 - 25 °C (HACH Company, 2026).

The error was reduced during the testing of water chemistry parameters by adhering to the requirements of the HACH Cuvettes and using the correct cuvette test for the ranges that were identified from preliminary water samples. If any samples produced an error in the range, the test was rerun to determine accuracy and the sample within the correct range was recorded.

4.2.3. In-situ Physical-Chemical Monitoring

In-situ measurements were taken each month at each of the four sites for the physical-chemical variables including pH, water temperature, dissolved oxygen (DO) levels, electrical conductivity and turbidity levels. The Hanna Instruments Combo pH & EC Probe (HI198129/HI198130) was used to take measurements of pH, EC and Total Dissolved Solids (a proxy for turbidity) and water temperature (Hanna Instruments, 2023). The probe was submerged into the river at each site and allowed to stabilise before the reading for each parameter was recorded. The dissolved oxygen was also recorded in mg/l by dipping the sensor of the Lutron Dissolved Oxygen Meter (Model PDO-519) (Nhbs, 2023) into the river water and allowing it to stabilise. The calibration of the DO meter and Hanna Combo instrument was carried out once a month to ensure the accuracy of the monthly readings. Calibration was carried out according to the user manuals (Nhbs, 2023; Hanna Instruments, 2023).

4.3. Ecological Sampling Methods

4.3.1. Macroinvertebrate Kick-Sampling

Each month between April 2023 to February 2025 (with the exception of May 2024 when the heavy rainfall made it unsafe), macroinvertebrates were sampled at each of the four sites using the standard 3-minute kick sampling method. The kick net had a 250mm frame with a 1.5mm mesh. This net was positioned downstream and the candidate kicked the riverbed next to the net opening to dislodge the gravel and benthic macroinvertebrates. Each sample was taken for three minutes, with consideration taken for the channel bed substrate to ensure an even sample. After the 3-minute sampling period is up, the net was swirled in the water to collect the sample together, before this was placed into a labelled plastic ziplock bag already containing some river water from the site. This was then followed by a one-minute hand search for macroinvertebrates on riverbed material and individuals were also placed into the ziplock bag. This bag was then sealed and placed into a bucket with a lid for transportation to the laboratory for analysis.

4.3.2. Laboratory Macroinvertebrate Identification

For each month, the macroinvertebrates from the kick sample were then taken to Bayfordbury Laboratory on the same day. Each site sample was then analysed separately. The contents of the ziplock bag were poured into a white tray, ensuring all individuals were decanted. Large pieces of vegetation were removed and placed on the side of the tray to allow any individuals to crawl into the water. Macroinvertebrates were picked out using forceps and plastic spoons and placed into petri dishes with a shallow layer of water. Predatory taxa were separated from other taxa. An optical microscope and field guides were used to identify the macroinvertebrates to family or species level. In the first season (March 2023 – January 2024), the macroinvertebrates were mostly identified to family level as this was sufficient for the original aims of the project. However, during the study, the project became more ecologically focused, and the need to identify to species level became greater. The second season (February 2024 – February 2025) had more taxa identified to species level. If not all analysis was possible on the day, the individual macroinvertebrates were stored in labelled containers in 70% IMS to preserve the specimens. These were then analysed over several weeks to species level for Crustacea, Ephemeroptera, Mollusca and Trichoptera. The macroinvertebrate taxa chosen to be identified to species level depended upon the field guides available and the supervisory expertise. Therefore, certain groups were not identified further than family level, such as the Chironomidae, but it is noted that identifying taxa to a higher level would potentially influence the community dynamics and diversity. However, it was decided that for the scope of this study and compared to previous studies which largely only summaries into family level, the invertebrate identification was sufficient. A selection of FBA field guides were used at Bayfordbury to identify the invertebrates to the lowest taxa resolution. The FBA Guide to Freshwater invertebrates for Biotic Assessment (FBA, 2011), Guide to Aquatic Invertebrates of the Upper Midwest (Bouchard, 2004), The Key to the case-bearing caddis larvae of Britain and Ireland (Wallace, Wallace & Philipson, 1990), Caseless caddis larvae of the British Isles (Edington & Hildrew, 1981), Adults of the British aquatic *hemiptera heteroptera*: A key with ecological notes (Savage, 1989), A key to the nymphs of British *ephemeroptera* (Macan, 1979), A key to the freshwater triclads of Britain and Ireland with notes on their ecology (Reynoldson & Young, 2000),

Dragonflies. Naturalists' Handbooks 7 (Miller, 1987). A Key to the British Fresh- and Brackish-water Gastropods (Macan, 1994) were used to identify the macroinvertebrate to family and/or species level. The number of each family/species was then recorded into a database for the site and month.

4.4. Statistical Analysis Methods

4.4.0. Testing for Normality and Multicollinearity of Parameters

The variables were tested for collinearity using the Pearson's Correlation Rank in Past4. If the p-value was greater than 0.05, the two variables were significantly correlated which may affect the statistical analysis. This test allowed for an informed choice to be made when including certain variables and which would be most suited, based on their relations to each other. This was performed on the independent variables in the 2023/24 season and 2024/25 season.

To test if the hydrological, physical-chemical, water quality and ecological variables had a normal distribution, each variable was tested by the Shapiro-Wilks Test in R (R Core Team, 2023). If the p-value was greater than 0.05, a normal distribution was assumed. If $p < 0.05$, the data was deemed to not be normally distributed. These variables were then log transformed or square-root transformed to meet the assumptions of parametric statistical tests. The transformed variable was then re-tested using the Shapiro-Wilks test and if $p > 0.05$, a normal distribution was assumed. However, if the p-value remained below 0.05, non-parametric alternatives were used for the analysis of those variables. The outcomes of the normality testing can be found in table 4.1 and the test type used on that variable for the 2023/24 and 2024/25 seasons.

Table 4. 1: The outputs of the Shapiro-Wilks test performed on each variable in R to test whether the variable is normally distributed. If $p < 0.05$, the variables was assumed to have a normal distribution. If $p > 0.05$, the variable was transformed and retested. If the variable remained non-normal, the non-parametric alternative statistics test was used (variables shaded in grey).

2023/24						
Variable	W-Value	p-value	Normal?	Transformation	p-value	Test
pH	0.73	2.05E-07				Non-Parametric
Electrical Conductivity	0.75	4.34E-07				Non-Parametric
Turbidity	0.71	5.67E-08				Non-Parametric
DO	0.94	3.50E-02	No	Sqrt	0.07	Parametric
BOD	0.98	4.80E-01	Yes			Parametric
Water Temperature	0.98	4.90E-01	Yes			Parametric
Channel Width	0.87	2.00E-04				Non-Parametric
Average Channel Depth	0.94	4.98E-02	No	log	0.24	Parametric
Average Flow Velocity	0.81	5.39E-06				Non-Parametric
Channel Area	0.87	2.00E-04				Non-Parametric
Discharge	0.75	2.86E-07				Non-Parametric
Phosphate	0.22	8.40E-14				Non-Parametric
Nitrate	0.96	1.15E-01	Yes			Parametric
Ammonium	0.62	2.42E-09				Non-Parametric
Daily Total Rainfall	0.84	3.09E-05				Non-Parametric
Max Temperature	0.9122	3.00E-03				Non-Parametric
Minimum Temperature	0.922	6.00E-03				Non-Parametric
2024/25						
Variable	W-Value	p-value	Normal?	Transformation	p-value	Test
pH	0.73	3.76E-07				Non-Parametric
Electrical Conductivity	0.75	8.46E-07				Non-Parametric
Turbidity	0.7	1.37E-07				Non-Parametric
DO	0.94	3.00E-02	No	log	0.12	Parametric
BOD	0.97	4.30E-01	Yes			Parametric
Water Temperature	0.97	4.30E-01	Yes			Parametric
Channel Width	0.85	1.00E-04				Non-Parametric
Average Channel Depth	0.76	3.00E-02	No	log	0.51	Parametric
Average Flow Velocity	0.76	1.62E-06				Non-Parametric
Channel Area	0.85	2.00E-04				Non-Parametric
Discharge	0.68	5.79E-08				Non-Parametric
Phosphate	0.23	4.37E-13				Non-Parametric
Nitrate	0.96	1.70E-01	Yes			Parametric
Ammonium	0.55	9.74E-10				Non-Parametric
Daily Total Rainfall	0.76	1.39E-06				Non-Parametric
Max Temperature	0.91	5.00E-03				Non-Parametric
Minimum Temperature	0.93	1.30E-02				Non-Parametric

4.4.1. Statistical Analysis of the Hydrological variables

The stage measurements from the gauging stations at Church Lane and Drop Lane were combined with the monthly stage and flow measurements and the cross-sectional area measurements at the respective sites to calculate stage-discharge relationships for the sites. The channel area measurement and average flow velocity measurements were used to calculate the river discharge river discharge using the following formula:

$$Q = vA \quad (1)$$

Where Q is the discharge (m^3/s), v is the flow velocity (m/s) and A is the cross-sectional channel area.

A linear regression model was determined for the two-minute average pressure stage (x) with the simultaneous discharge measurements (y) to calculate the stage-discharge relationship at the sites. If there were cases of no flow measurements taken in situ, these results were removed from the calculations, to avoid skewing the relationship. For the London Colney and Ver sites, stage data, channel measurements (width, depth) and the discharge calculations from the monthly measurements were used to compute the stage-discharge relationships. The stage and discharge measurements were transformed using the log-log transformation before calculating the regression equation. This gave the following equation:

$$\log Q = a \log d + b \quad (2)$$

Where $\log Q$ is the logarithmic version of discharge, a is the slope (rate of change of discharge), $\log d$ is the stage or river depth (m) and b is the bias.

The equations were then exponentiated in the following format:

$$Q = ad^b \quad (3)$$

Where Q is the river discharge (m^3/s) for the cross-sectional area, a is the scaling coefficient, d is the stage (or river depth) and b is the exponent.

To show the variation in the stage level throughout the inter-annual seasons and to observe the annual hydrological cycle, the daily average stage measurements from the gauging stations were plotted for the CL and DL sites. To understand the interaction between the river discharge and rainfall levels, the logged monthly average discharge measurements ($\log Q$) were compared graphically to the monthly average total daily rainfall levels for each of the four sites. To establish if there was a correlation between the discharge level and total daily rainfall within the catchment, a spearman's rank correlation coefficient was performed on the log transformed ($\log_{10}$) discharge and log transformed rainfall data for the Church Lane site to establish if there was a significant relationship. The rainfall and discharge parameters were log-transformed to provide clearer results. The graph for the outcome was produced using the ggplot package in R Version 4.3.1 (R Core Team, 2023). A Cross-correlation for the rainfall and discharge measurements was performed in R by using a Spearman's rank correlation with the rainfall data shifted -24 hours to determine if there was a lag in the hydrological response to rainfall events.

Box plots were generated in MS Excel for each hydrological parameter (average river stage, average river channel width, average river channel depth, average river flow velocity, average river discharge, average cross-sectional channel area, river water temperature, total daily rainfall, average daily maximum air temperature and average daily minimum air temperature) using the monthly recordings to display the range and variation in the variables across the intra-annual seasons (spring, summer, autumn, winter), the sites (CL, LC, DL, V) and the inter-annual seasons (2023/24 and 2024/25).

The software R was used to statistically analyse the hydrological parameters with the following packages (Base R, FSA). Firstly, the variables were tested for normality using the Shapiro wilks function and if $P > 0.05$, a normal distribution was assumed, and the

parametric tests were used. If the data remained non-normal even after using log and root transformations, the variable would be assumed to have a non-normal distribution, and non-parametric alternatives were used. To identify the differences in the hydrological parameters across the sites and seasons, Two-Way ANOVA (Fisher, 1925) was performed on the parametric variables. If there was a significant difference ($p < 0.05$) between either the sites, seasons or both the post hoc Tukey HSD test (Tukey, 1949) was used to determine the specific differences. For the non-parametric variables, the alternative Kruskal-Wallis test (Kruskal & Wallis, 1952) was performed twice for the site and season, and if there was a significant difference ($p < 0.05$), the post-hoc Dunn test (Dunn, 1964) with Bonferroni correction was used to show which site(s) and/or season(s) were different from each other. To compare the two inter-annual seasons (2023/24 and 2024/25) for each hydrological variable, the t-test (Student, 1908) was used if the variable was parametric, and the Wilcoxon signed rank test (Wilcoxon, 1945) was used if the data was non-parametric. If the p-value was greater than 0.05, the null hypothesis that there was no significant difference between the sites/seasons was accepted.

The hydrological variables were also presented in boxplots to show the range of the variables at the different sites in different seasons. The average of each of the three measurements for each intra-annual season (spring, summer, autumn and winter) were taken and these three measurements were used to generate the box plots in Excel. This was repeated for both the 2023/24 and 2024/25 seasons, as well as a inter-annual comparison across the 2023/24 and 2024/25 seasons. Observations of the mean averages and ranges were made, and any outliers noted and described in the results section.

4.4.2. Statistical Analysis of the Water Chemistry and Physical-Chemical Variables

The water quality variables, which include water nutrients, referred to as water chemistry variables, and the physicochemical variables were tested to determine if there were any significant differences spatially and temporally. The water chemistry variables were the phosphate, ammonium and nitrate levels and the physicochemical variables were the electrical conductivity of the water, the turbidity of the water, the pH, dissolved oxygen levels and the biological oxygen demand levels. These variables were compared between the intra-annual seasons (spring, summer, autumn and winter) and the inter-annual seasons (the 2023/24 season and the 2024/25 season) and also between the four sampling sites (Church Lane, London Colney, Drop Lane and the Ver). To do this, the data was analysed in R (R Core Team, 2023). The individual variables were tested for normality using the Shapiro-Wilks test with confidence limits of 95%. If the p-value was more than 0.05, the data was considered normal and if $P < 0.05$, the data was assumed to not be normally distributed. If this was the case, transformations, such as log, square roots and inverse logs were used to try and transform the variables into a normal distribution. If this was still unsuccessful, non-parametric tests were performed instead.

The water chemistry variables that were normally distributed were analysed for differences between the sites and the intra-annual seasons using Two-Way ANOVA in R (using the package car). If the p-value was less than 0.05, the result was deemed to be significantly different. The post-hoc Tukey HSD test was then performed on any significant results from the Two-Way ANOVA, and if the output p-values from the post-hoc test was < 0.05 , the difference was statistically significant. If the water chemistry variable was not normally distributed, the non-parametric Kruskal-Wallis test was run separately for both the site and intra-annual season independent variables in R, and if $p < 0.05$, the result was statistically significantly different. The significant results were then tested again using the Dunn post-hoc test with Bonferroni correction (Dunn, 1964) using the FSA package in R (Ogle, Wheeler & Dinno, 2023). If the result of this test was $p < 0.05$, the difference between the specific sites or specific seasons was statistically significant. These methods were repeated on the physical-chemical variables to check for differences between the sites and intra-annual seasons.

Statistical testing to make comparisons between the inter-annual seasons on the water quality and physical-chemical variables was also performed in R. If the variable was normally distributed, a paired t-test was performed and the non-parametric alternative Wilcoxon Signed rank sum test with continuity correction was used. If the p-value was less than 0.05, the null hypothesis was rejected and the difference between the 2023/24 and 2024/25 season was determined.

The water chemistry variables and physicochemical variables were also presented in box-plots to show the range of the variables at the different sites in different seasons. The average of each of the three measurements for each intra-annual season (spring, summer, autumn and winter) were taken and these three measurements were used to generate the box plots in excel. This was repeated for both the 2023/24 and 2024/25 seasons, as well as an inter-annual comparison across the 2023/24 and 2024/25 seasons. Observations of the mean averages and ranges were made, and any outliers noted and described in the results section.

Principal Component Analysis (PCA) was performed on the water quality variables, and also the hydrological variables (channel width, average channel depth, average channel flow, average channel area, total channel discharge, maximum and minimum air temperatures and water temperature) data in R using the packages factextra and ggplot2 to reduce the dimensionality of the multivariate data and understand how the different variables explained the variation in the inter-annual seasons (2023/24 and 2024/25 seasons).

4.4.3. Macroinvertebrate Diversity Statistical Analysis

The diversity indices (Shannon Diversity, Simpson Diversity and Shannon Evenness) were calculated for the 2023/24 and 2024/25 seasons at the family level and at the species level for 2024/25 season. This was performed in Past 4.17 (Hammer, Harper & Ryan, 2001). These indices scores were then plotted in box plots in Excel to see the variation across the sites and seasons for the 2023/24 and 2024/25 season. The indices were statistically tested for differences between the sites and intra-annual seasons in R using the non-parametric Kruskal-Wallis test and the post-hoc Dunn test to determine which site(s) or season(s) had significantly different diversity. The

Wilcoxon Test was then used to test for inter-annual differences between the 2023/24 seasons for each of the indices. The difference in Shannon and Simpson Diversity between the family and species level was also tested using the Wilcoxon test for the 2024/25 season. For all of the above tests, if the p-value was less than 0.05, the difference between the independent variables was deemed significant.

To assess the relationships between the environmental parameters and the diversity measures (Family richness, number of individuals, Shannon Diversity and Simpson diversity), multiple linear regression was undertaken in R. The family richness and number of individuals data was Hellinger-transformed and any non-normal variables were log-transformed. For the hydrological model, all variables were included initially, before removing highly correlated variables (maximum and minimum air temperature, channel area) and using the most relevant variables instead. The water quality model included the phosphate, ammonium and nitrate levels. The physical-chemical model used the pH, water turbidity, electrical conductivity, dissolved oxygen and biological oxygen demand variables. If the multiple linear regression model was significant and had significant individual predictors, a linear regression model was then performed on the significant predictors to understand how much they contributed to the relationship of the diversity measure.

4.4.4. Macroinvertebrate Community Composition Analysis

The macroinvertebrate family level data was analysed alongside the water chemistry, physical-chemical and hydrological variables to test for relationships between the community structure and the environment. The family/species dependent variables were transformed using the Hellinger transformation prior to any analysis in R. The community analysis was performed in R, with the following packages installed: Vegan (Oksanen *et al.*, 2022) and ggplot2 (Wickham, 2016). A Non-Metric Multidimensional Scaling method (NDMS) was run on the macroinvertebrate community data to visualise the community patterns and clustering based on Bray-Curtis dissimilarity (Kruskal, 1964). The Permutational Multivariate Analysis of Variance (PERMANOVA) test was then used to determine if the community composition significantly differs between the clustered groups ($p < 0.05$) (Anderson, 2001). This was followed by

applying the Analysis of Similarity (ANOSIM) (Clarke & Green, 1988) to the NDMS and the site or seasonal groups to identify if there were significant dissimilarities between the sites and/or seasons. If the result was significant ($p < 0.05$), a paired ANOSIM was then used to identify which sites/seasons were significantly different from each other. Then the Similarity Percentage Analysis (SIMPER) was used to identify species or families that have been contributing to the dissimilarity between the groups (Clarke, 1993). The SIMPER results were then visualised in a plot with an overlay of the top 10 contributing species/ families and the NMDS ordination was overlaid onto the SIMPER. This process was carried out for the family community data in the 2023/24 season and 2024/25 season, and on the species community level data in the 2024/25 season.

A redundancy analysis (RDA) was used to model the effect of the environmental parameters onto the invertebrate composition. Redundancy Analysis (RDA) is an extension of multiple regression, where it builds upon the general multiple regression model to model the effect of an explanatory matrix on a response matrix (Legendre & Legendre, 2012). In the case of this dataset, it was used to model the effect of the hydrological, water chemistry and environmental variables on the whole invertebrate community, rather than only using species or family richness. This was performed in R using the *vegan* and *packfor* packages (R Core Team, 2023; Oksanen *et al.*, 2024; Legendre & Blanchet, 2016). Three models were made: Model 1 for all environmental parameters; model 2 for the hydrological variables; and model 3 for the water quality/physical-chemical variables and the model variables are detailed in table 4.2. The variables were selected based upon their collinearity with one another and their importance in determining the ecological community. Forward selection of the most significant variables was used in each model to determine which variable had the greatest impact on the ecological community in the Upper Colne Catchment. These models were then tested for significance using ANOVA. Variance partitioning was then carried out using the forward selected variables in R using the *Vegan* package and “*varpart*” function to determine how much variance the hydrological and physicochemical models explained in the macroinvertebrate community for the 2023/24 and 2024/25 seasons.

Table 4. 2: *The three models for the RDA and their respective variables used to generate the effect on macroinvertebrate community composition in the Upper Colne Catchment.*

Model	Variables
Model 1 (all parameters)	pH, Electrical Conductivity, Turbidity, Dissolved Oxygen, Biological Oxygen Demand, Channel Width, Average Channel Depth, Average Flow Velocity, Cross-Sectional Channel Area, Discharge, Phosphate, Ammonium, Nitrate, Daily Total Rainfall, Maximum Air Temperature, Minimum Air Temperature
Model 2 (Hydrological)	Channel Width, Average Channel Depth, Average Flow Velocity, Cross-Sectional Channel Area, Discharge, Daily Total Rainfall, Maximum Air Temperature, Minimum Air Temperature
Model 3 (Water Quality)	pH, Electrical Conductivity, Turbidity, Dissolved Oxygen, Biological Oxygen Demand, Phosphate, Ammonium, Nitrate

4.4.5. Assessing the Water Quality Using Biological Indices

The Whalley, Hawkes, Paisley & Trigg (WHPT) Metric scoring system (based on the Biological Monitoring Working Party (BMPW) System (Hawkes, 1997) uses the sensitivity of macroinvertebrate families to organic pollution to assign a numerical value to around 100 different WHPT-Scoring taxa and the abundance of the different scoring taxa (WFD-UKTAG, 2014). There are four taxa abundance classes and a score of Pressure Sensitivity Scores (PSs) is then assigned to each taxa based on the taxa sensitivity and abundances recorded. The total WHPT score for a sample is the sum of PSs and the Average Score Per Taxon (ASPT) scores are the sum of PSs divided by the number of scoring taxa (NTaxa). The WHPT, WHPT-ASPT scores were calculated for each site based on the scoring families present using the WHPT calculator Version 3.2 (Murray-Bligh, 2015) and the interpretation of the WHPT-ASPT scores can be found in table 4.3. These values were then plotted in box plots to observe the variation in WHPT and WHPT-ASPT scores across the four sites and the four intra-annual seasons in the 2023/24 and 2024/25 seasons, as well as inter-annually to compare the two seasons. The scores were then tested for differences across the sites and seasons using the Two-Way ANOVA in R, and the accompanying

Tukey HSD post hoc test to show which site(s) and season (s) were different from each other. For comparing the 2023/24 and 2024/25 season a paired T-test was performed in R to determine if the scores were different.

Table 4. 3: The WHPT-ASPT Score interpretation for the ecological health of the aquatic environment (SSE Thermal, 2021)

ASPT Score	<u>Interpretation</u>
<3.0	Very poor, heavily polluted
3.0-4.3	Poor, polluted or impacted
4.3-4.8	Moderate, moderately impacted
4.8-5.4	Good, clean but slightly impacted
>5.4	Very good, unpolluted, unimpacted

The LIFE Index was developed by Extence *et al.* (1999) and summarises how invertebrate communities respond to changes in instream habitat conditions by assigning certain taxa to numeric variables that associate them to a flow group (fast, moderate, slow-flowing, standing waters or drying or drought impacted conditions (table 4.3) and to an estimated abundance class (table 4.4) . The flow group and abundance class are then combined to give a flow score to each taxon (table 4.5), and the mean flow score for all scoring taxa provides the LIFE score from 0 to 12 for a sample (Extence *et al.*, 1999; Sarremejane *et al.*, 2019). LIFE is calculated for a sample by calculating the average of the flow scores ($f_{(Si)}$) for each of the n taxa present in the sample.

$$LIFE = \sum_{i=1}^n \frac{f_{Si}}{n} \quad (4)$$

The assigned flow groups for the most common taxa used in the WHPT metric for river water quality assessment (table 4.6). The Environment Agency has recognised the value of the LIFE index in monitoring the ecological response to changes in flow conditions in rivers (Clarke *et al.*, 2003). However, LIFE was found to vary between different river types and therefore needed to be calibrated for different river types, such as chalk streams. Therefore, this study aimed to calculate the LIFE index for the upper Colne, as previous work had only focused on the tributaries of the Colne (Misbourne, Chess, Bulbourne, Gade and Ver) (Sarremejane *et al.*, 2019). The LIFE Scores were

then plotted in box plots to observe the variation in across the four sites and the four intra-annual seasons in the 2023/24 and 2024/25 seasons, as well as inter-annually to compare the 2023/24 and 2024/25 seasons. The scores were then tested for significant differences across the sites and seasons using the Two-Way ANOVA in R, and the accompanying Tukey HSD post hoc test to show which site(s) and season (s) were different from each other. For comparing the 2023/24 and 2024/25 season a paired T-test was performed in R to determine if the scores were different.

Table 4. 4: *The flow groups used to classify benthic macroinvertebrate taxa according to their mean flow velocity primary preference (Extence et al., 1999).*

Flow Group	Ecological Flow Association	Mean Flow Velocity (m/s)
I	Rapid Flows	>1 m/s
II	Moderate to fast flows	0.2 - 1 m/s
III	Slow to sluggish flows	< 0.2 m/s
IV	Slow flowing and standing waters	NA
V	standing waters	NA
VI	Drying or drought impacted sites	NA

Table 4. 5: *The macroinvertebrate abundance categories (Extence et al., 199).*

Category	Estimated Number of Individuals in a sample
0	0
A	0 - 9
B	10 - 99
C	100 - 999
D	1000 - 9999
E	10000+

Table 4. 6: The flow scores (fs) (1 – 12) calculated using the abundance categories of taxa (A – E) and its corresponding assigned flow group (I – VI) (Extence et al., 1999).

Flow Group		Abundance Categories			
		A	B	C	D/E
I	Rapid	9	10	11	12
II	Moderate	8	9	10	11
III	Slow	7	7	7	7
IV	Flowing/standing	6	5	4	3
V	Standing	5	4	3	2
VI	Drought Resistant	4	3	2	1

Table 4. 7: LIFE flow group (I-VI) BMWP score and WHPT Score for all families included in the BMWP system. Adapted from Extence et al. (1999), Clarke et al. (2003) & Murray-Bligh (2015).

Family	BMWP Score	WHPT Score (Presence Only)	LIFE Flow Group
Triclada (flatworms)			
Planariidae (inc. Dugesidae)	5	4.9	IV
Dendrocoelidae	5	3	IV
Mollusca (snails, limpets and mussels)			
Neritidea	6	6.4	II
Viviparidae	6	5.7	III
Valvatidae	3	3.2	IV
Hydrobiidae (incl. Bithyniidae)	3	4.2	IV
Physidae	3	2.4	IV
Lymnaeidae	3	3.3	IV
Planorbidae	3	3.1	IV
Ancylidae (inc. Acroloxidae)	6	5.7	II
Margaritiferidae	NA	NA	II
Unionidae	6	5.3	IV
Sphaeriidae	3	3.9	IV
Dreissenidae	NA	3.7	IV
Oligochaeta (worms)			
Oligochaeta	1	2.7	NA
Hirudinea (leeches)			
Piscicolidae	4	5.2	II
Glossiphoniidae	3	3.2	IV
Hirudinidae	3	-0.8	IV
Erpobdellidae	3	3.1	IV
Crustacea (crayfish, slaters and shrimps)			
Astacidae	8	7.9	II
Asellidae	3	2.8	IV
Corophiidae	6	5.8	III
Gammaridae (incl. Crangonyctidae & Niphargidae)	6	4.4	II
Ephemeroptera (mayflies)			
Siphonuridae	10	11.5	IV
Baetidae	4	5.5	II
Heptageniidae	10	9.7	I
Leptophlebiidae	10	8.8	II
Potamanthidae	10	10	III
Ephemeridae	10	8.4	II
Ephemerellidae	10	8.2	II
Caenidae	7	6.5	IV
Plecoptera (stoneflies)			
Taeniopterygidae	10	11.3	II
Nemouridae	7	9.3	IV
Leuctridae	10	10	II
Capniidae	10	9.6	I
Perlodidae	10	10.8	I
Perlidae	10	12.7	I
Chloroperlidae	10	11.6	I
Odonata Zygoptera (damselflies)			
Platycnemididae	6	6	IV
Coenagriidae	6	3.5	IV
Calopterygidae	8	6	III

Family	BMWP Score	WHPT Score (Presence Only)	LIFE Flow Group
Odonata Anisoptera (dragonflies)			
Gomphidae	8	NA	II
Cordulegasteridae	8	NA	II
Aeshnidae	8	4.7	IV
Libellulidae	8	4.1	IV
Hemiptera (bugs)			
Mesovelidae	5	4.7	V
Hydrometridae	5	4.3	IV
Veliidae	NA	4.5	IV
Gerridae	5	5.2	IV
Nepidae	5	2.9	V
Naucoridae	5	3.7	IV
Aphelocheiridae	10	8.5	II
Notonectidae	5	3.4	IV
Corixidae	5	3.8	IV
Coleoptera (beetles)			
Halipidae	5	3.6	IV
Dytiscidae (incl. Noteridae)	5	4.5	IV
Gyrinidae	5	8.2	IV
Hydrophilidae (incl. Hydraenidae)	5	6.2	IV
Scirtidae (=Helodidae)	5	6.9	IV
Dryopidae	5	6	NA
Elmidae	5	6.6	II
Megaloptera (alderflies)			
Sialidae	4	4.3	IV
Osmylidae	NA		II
Sisyridae	NA	5.7	IV
Trichoptera (caddisflies)			
Rhyacophilidae (inc. Glossosomatidae)	7	8.4	I
Hydroptilidae	6	6.2	IV
Philopotamidae	8	11.2	I
Psychomyiidae (incl. Ecnomidae)	8	5.8	II
Polycentropodidae	7	8.1	IV
Hydropsychidae	5	6.6	II
Phyrganeidae	10	5.5	IV
Brachycentridae	10	9.5	II
Lepidostomatidae	10	10.1	II
Limnephilidae	7	6.2	IV
Goeridae	10	8.8	I
Beraeidae	10	8.7	II
Sericostomatidae	10	9.1	II
Odontoceridae	10	11	I
Molannidae	10	6.6	IV
Leptoceridae	10	6.7	IV
Diptera (True Flies)			
Tipulidae	5	5.9	IV
Ptychopteridae	NA	6.4	II
Chaoboridae	NA	3	V
Culicidae	NA	2	V
Chironomidae	2	1.1	NA
Simuliidae	5	5.8	II
Syrphidae	NA	1.9	V

These WHPT and LIFE scores were then plotted in box plots in Excel to see the variation across the sites and seasons for the 2023/24 and 2024/25 season. The scores were statistically tested for differences between the sites and intra-annual seasons in R using the Two-Way ANOVA test and Tukey HSD post-hoc test on the parametric variables and the Kruskal-Wallis and the post-hoc Dunn test for non-parametric variables to determine which site(s) or season(s) had significantly different diversity. The T-test (parametric) or Wilcoxon Test (non-parametric) was then used to test for inter-annual differences between the 2023/24 and 2024/25 seasons for each of the scores. For all of the above tests, the confidence limit was 95% and if the p-value was less than 0.05, the difference between the independent variables was deemed significant.

Chapter 5: The hydrological cycle and the variation of the hydrological variables within the Upper Colne Catchment

5.0. Introduction

Hydrological processes are one of the primary drivers of change in riverine environments, and the interactions are particularly important within chalk stream catchments due to the greater influence of groundwater sources from the highly permeable chalk aquifers (Homan *et al.*, 2024). Chalk streams have a distinctively higher groundwater contribution to flow, which accounts for around 90% of annual discharge, and the baseflow index can range between 0.53 to 0.98 (Holman *et al.*, 2010; Mainstone, 1999; Johnes, 2007; Shaw, Leung & Clarke, 2021). Combining the groundwater flow dynamics with the surface water dynamics has been a challenge when developing hydrological models to identify differences in the respective contributions of the water sources to a chalk river's discharge (Homan *et al.*, 2024) and chalk rivers can be hydrologically variable, with both perennial and temporary streams (seasonally intermittent streams) (Kabir *et al.*, 2024). However, it has been demonstrated that during dry periods, rivers often have an open conduit system with point-source inputs. The majority of chalk streams are semi-natural which means that some modifications have taken place to change their form and hydraulics, but these characteristics have become important for sustaining the flora and fauna that have developed (Sear *et al.*, 1999; Sear *et al.*, 2006). The key hydrological characteristics of these semi-natural chalk streams include a low drainage density network, low stream power, relatively large width-depth ratios, limited in-channel storage (bars), limited accumulation of fine sediments, and irregular or straight channel planforms with some variable sinuosity (Sear *et al.*, 2006). The groundwater contribution to baseflow also helps to minimise the influence of irregularities in streamflow from rainfall and can stabilise the water temperature to around 10°C (Berrie, 1992).

Hydrological variables, including the in-stream flow velocity, stream discharge, water electrical conductivity, turbidity, water temperature, channel width and channel depth (stage), can all impact the aquatic environment and interact with the ecological communities that inhabit the chalk stream (Sarremejane *et al.*, 2024; Jackson *et al.*, 2016). One of the largest challenges in chalk stream catchments is the frequency and severity of droughts and dry periods, and these are often unpredictable disturbances

that have short-term ecological responses (Sarremejane *et al.*, 2024; Stubbington *et al.*, 2021). Despite the upper courses of chalk streams having experienced periodic drought, more extreme events could lead to different impacts on the hydrological and ecological functioning (Sarremejane *et al.*, 2024). With the changing weather patterns and anthropogenic influences such as abstraction from both surface water and groundwater sources, it is critical to understand the functioning the Upper Colne Catchment, where there is less influence from abstraction, to gain an insight into the baseline hydrological functioning of a upper chalk stream.

This chapter therefore, sets out the continuous observations made on the stage levels at two sites on the Upper Colne in the 2023/24 and 2024/25 seasons and the monthly measurements allowed for the calculation of stream discharge levels at the four sites (CL, LC, DL, V). The stage-discharge relationships allowed a greater insight into the flow dynamics of the stream to be identified and the relationship between the discharge and rainfall levels was investigated to see how large the influence of surface runoff within the upper catchment was. The hydrological variables were compared intra-annually to establish if there were any differences in the hydrological functioning of the upper Colne catchment across the intra-annual seasons and also between the sites. An inter-annual comparison of the hydrological variables was also undertaken to show if there were any difference between the 2023/24 and 2024/25 seasons.

This chapter examines the patterns in the hydrological cycle of the Upper Colne Catchment (using the river stage level from the gauging stations at Church Lane and Drop Lane as a metric of the hydrological functioning) and develops the stage-discharge relationships for each of the four sites from April 2023 to September 2024 (Church Lane, London Colney, Drop Lane and Ver). The total daily rainfall and average daily air temperatures are also incorporated into the stage measurements to understand the cycle's patterns. The spatial and temporal (intra-annual seasonal variation) variation in stage is also explored. The other hydrological variables (average channel depth, river channel width, average flow velocity, river channel discharge, river water temperature, river channel cross-sectional area, total daily rainfall, average maximum air temperature and average minimum air temperature) were analysed spatially (between the four sites) and seasonally (referred to as intra-annual variation). The hydrological variables are also subject to inter-annual variation between the two sampling seasons (April 2023 – February 2024 and March 2024 to February 2025).

5.1. The annual hydrological cycle of the Upper Colne Catchment

In the 2023/24 season, the average stage at the Church Lane site (0.50 ± 0.1 m) was consistently greater than the Drop Lane site (0.26 ± 0.04 m) (figure 5.1A). The river stage at Church Lane was greatest on day 89 at 1.2m and highest in spring (0.55 ± 0.15 m), and lowest during the summer months (0.49 ± 0.02 m, decimal day 121 to 243: May to August 2023). The stage at the DL site was more consistent across the seasons Spring: 0.26 ± 0.03 m; Summer: 0.026 ± 0.008 m; autumn 0.27 ± 0.02 m, winter 0.21 ± 0.08 m). There was a high stage level at DL on day 78 at 0.24m and between days 126 to 136 (0.24 ± 0.002 m). Day 263 had increased rainfall (figure 5.1A), but stage on increased in day 291 in 0.62 m and 302 1.02m after a more continuous period of rainfall.

In the 2024/25 season, (day 60 1 March – 264 22 September) the stage at CL had its highest peak on day 141 (21 May 2024) at 1.27m (Figure 5.1B). The month of May had a higher stage (0.50 ± 0.13 m) than the rest of spring (0.47 ± 0.12 m) in 2024/25 season and this corresponded with the higher rainfall levels in May (3.54 ± 10.34 mm) (figure 5.1B). During the summer months (day 153 to 244), there was a rise in stage at CL between days 187 to 199 (0.52 ± 0.07 m) brought on by the period of corresponding rainfall in the catchment (6.42 ± 10.70 mm). However, levels then declined again until September, when they began to rise once more (figure 5.1B).

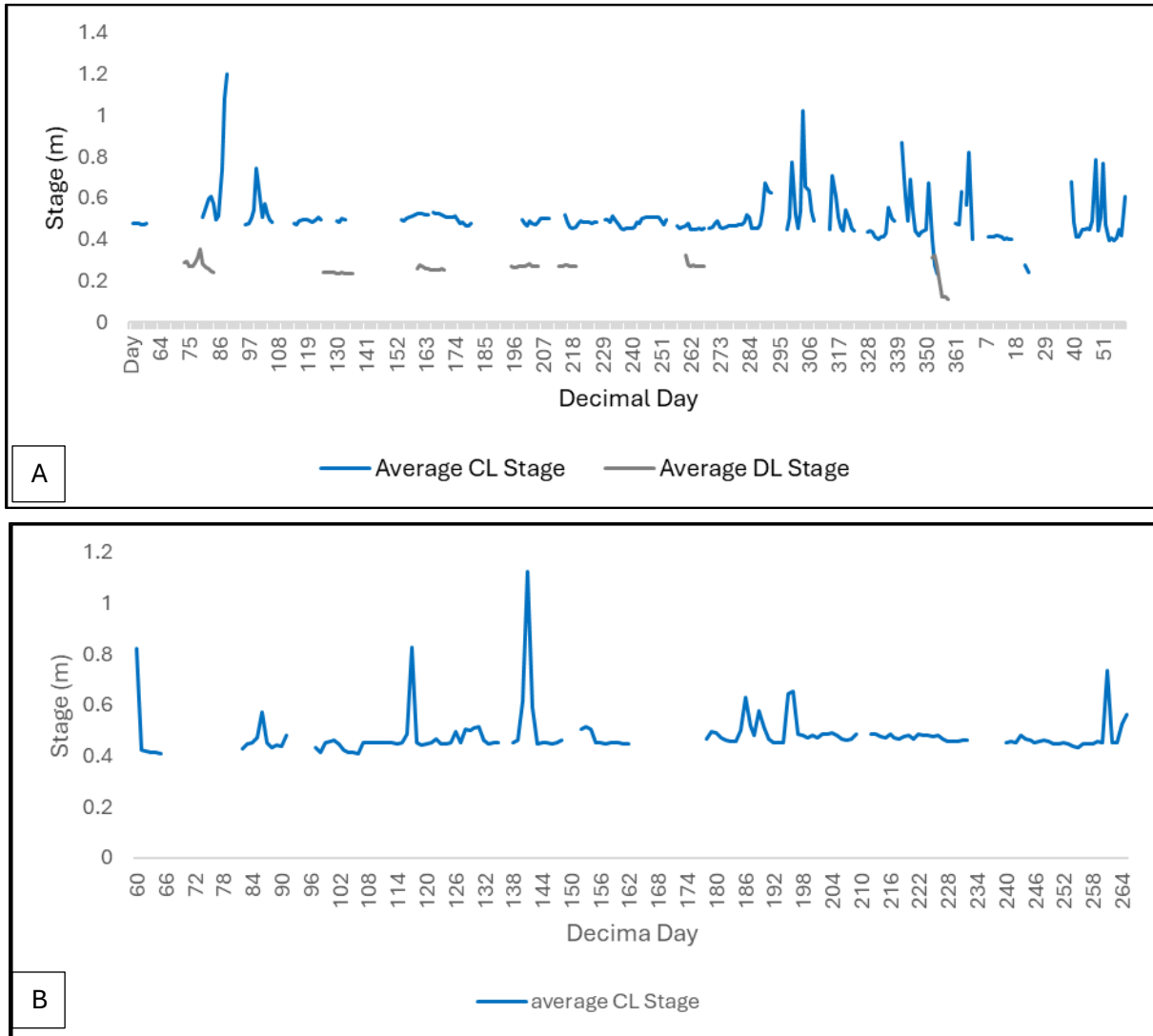


Figure 5. 1: The average daily river stage measurements collected from the gauging stations at: (A) Church Lane (CL) and Drop Lane (DL) in the 2023/24 season (April 2023 – February 2024); (B) Church Lane (CL) in the 2024/25 season (March 2024 – September 2024). Decimal days are used to denote the time of year (Spring 60 – 151, summer 152 – 243, autumn 244–334, winter 335 – 59).

The stage-discharge relationship was calculated for each of the four sites on the upper Colne catchment (table 5.1). The stage-discharge relationship ($Q=652.83 \times d^{6.8507}$) at the Church Lane site based on the monthly readings had the steepest gradient (figure 5.2B). The scaling coefficient (652.83) indicated that for a 1 metre increase in stage, the discharge would be 652.83 m³/s. The exponent (6.85) indicated that discharge increases exponentially with stage and the model was significant ($p=1.6e-10$). The R² value (0.93) showed that stage explained 93% of the variation in river channel discharge which was the largest variation in discharge explained by stage across the four sites.

Table 5. 1: The stage-discharge relationship exponentiated equations calculated using the stage and discharge relationships at each of the four sites, along with the R² value to explain the strength of the equation and the 95% confidence limits.

Site	Equation	R ²	Lower 95% Confidence	Upper 95% Confidence
CL	$Q=652.83 \times d^{6.8507}$	0.923	$Q=157.76 \times d^{5.834}$	$Q=2691.53 \times d^{7.87}$
LC	$Q = 16.7996 \times d^{3.0674}$	0.766	$Q=3.524 \times d^{2.30}$	$Q=79.43 \times d^{3.834}$
DL	$Q=26.2724 \times d^{4.118}$	0.775	$Q=7.755 \times d^{2.94}$	$Q=88.92 \times d^{5.293}$
V	$Q= 11.2435 \times d^{1.8909}$	0.602	$Q=5.24 \times d^{1.18}$	$Q=23.99 \times d^{2.598}$
CL Guage	$Q = 41.2 \times d^{8.823}$	0.28	$Q=0.082 \times d^{0.297}$	$Q=20417.37 \times d^{17.35}$

In contrast, the stage-discharge relationship at the CL gauging station ($Q = 41.2 \times d^{8.823}$) (figure 5.2A) had the smallest small R² value (0.35) which showed that stage did not explain a large amount of the variation in total channel discharge based on these readings. However, the model was significant ($p=0.04$) and the scaling coefficient (41.2) indicated that for a stage of 1m, the discharge would be 41.2 m³/s which is a steep increase in flow with depth. The exponent (8.825) indicated that discharge increased exponentially with stage.

The London Colney site had a scaling coefficient of 16.80 which indicated that for a 1 metre increase in stage, the discharge would be 16.80 m³/s ($Q = 16.7996 \times d^{3.0674}$) (figure 5.2C). The exponent (3.07) indicated that discharge increased exponentially with stage. The model was significant ($p=6e-08$) and the R² value (0.78) shows that stage explained 78% of the variation in river channel discharge. Similarly, at the Drop Lane

site ($Q=26.2724 \times d^{4.118}$), the variation in stage was found to explain 79% of the variance in river channel discharge ($R^2 = 0.79$) and the model was significant ($p=2e-06$) (figure 5.2D). The scaling coefficient (26.27) indicated that for a 1 metre increase in stage, the discharge would be 26.27 m³/s. The exponent (4.12) indicated that discharge increased exponentially with stage. The Ver site ($Q= 11.2435 \times d^{1.8909}$) had a lower R^2 value (0.62), showing that only 62% of the variation in river discharge was explained by the stage of the river. However, the model was significant ($p=1.6e-10$) and the scaling coefficient (11.24) indicated that for a 1 metre increase in stage, the discharge would be 11.24 m³/s. The exponent (1.89) indicated that discharge increases exponentially with stage (figure 5.2E). The stage-discharge relationship at the Ver also had the shallowest gradient.

There was a significant relationship between the average daily discharge and the total daily rainfall at the Church Lane site across the two years ($p=0.0001225$, $S=8233344$, $\rho = 0.1923157$) (figure 5.3A). However, this was a weak positive correlation. The relationship was weaker when a lag of 1 day was introduced for the spearman's rank correlation between discharge and rainfall ($p=0.01$, $\rho=0.12$) (figure 5.3B). The average river discharge level did tend to follow the pattern of being greater when the rainfall level was higher, as shown across the sites in figure 5.4. In the 2024/25 season, the discharge level at CL was fairly constant across the months, which was reflected similarly in the rainfall level with the exception of September (figure 5.4A). The Drop Lane and Ver sites exhibited very similar patterns in the discharge levels throughout the inter-annual seasons (figure 5.4CD) and were more responsive to changes in rainfall levels than LC (figure 5.4B) and CL. This suggested that the upper Colne catchment is quick to respond to changes in surface runoff and rainfall events.

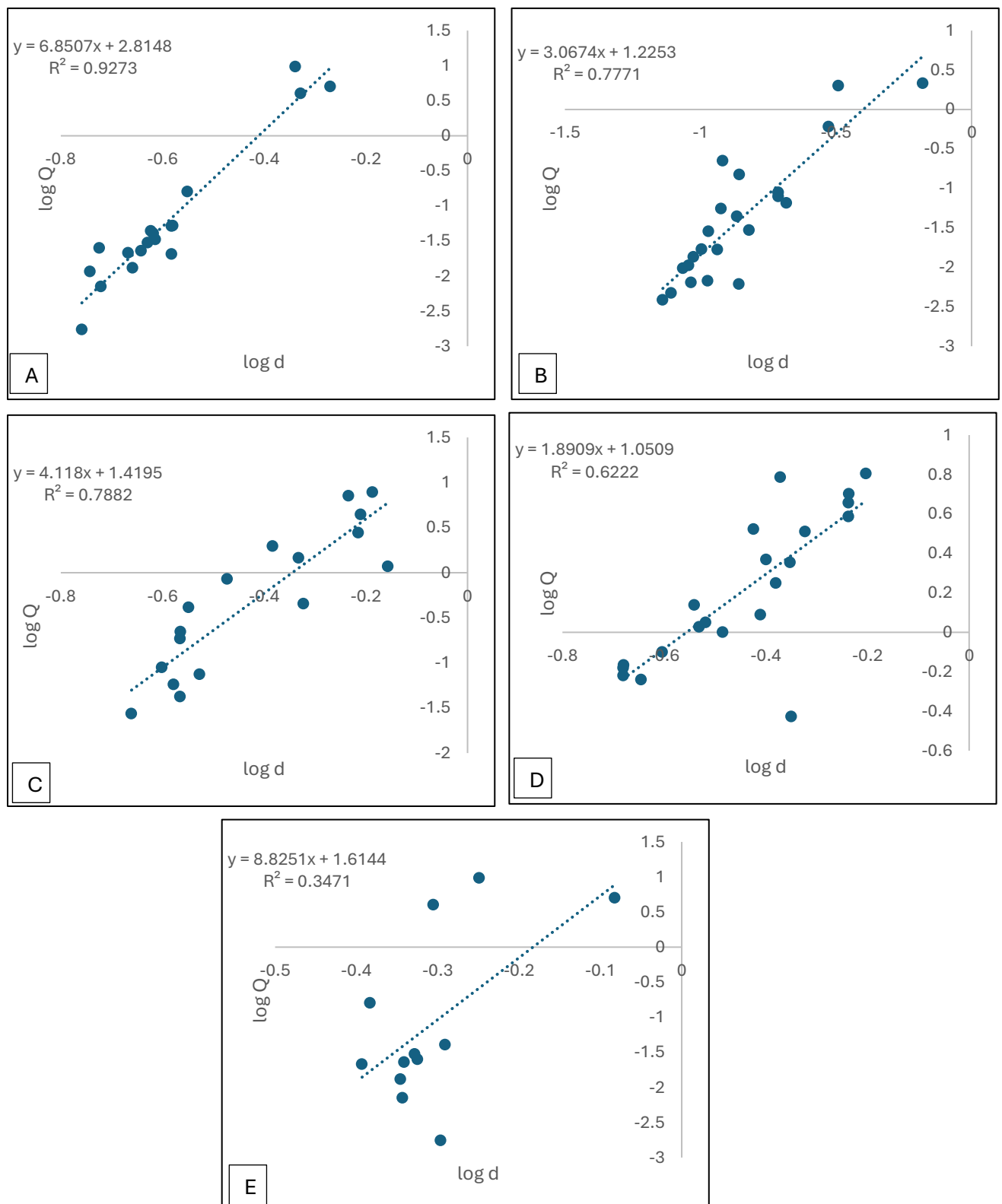


Figure 5. 2:The stage-discharge relationships for each of the four sites in the upper Colne catchment where (A) is CL (based on monthly readings); (B) is LC, (C) is DL, (D) is V; and (E) is CL based on gauging station readings. The regression was calculated using the log transformed stage and discharge readings. The exponentiated stage-discharge equations can be found in table 5.1.

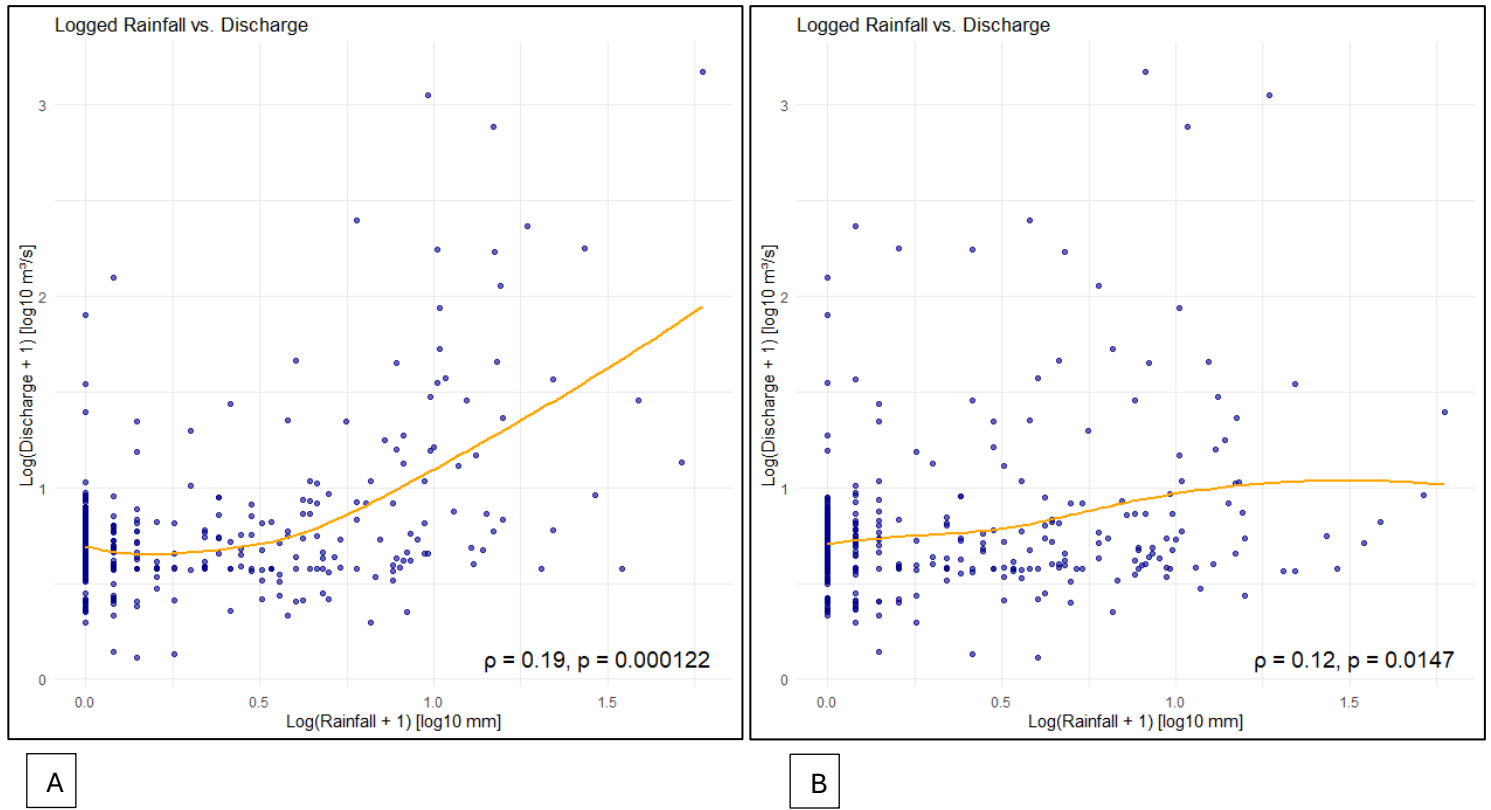


Figure 5. 3: The Spearman's Rank Correlation relationship between the river discharge m^3/s and total daily rainfall (mm) at the Church Lane site in the 2023/24 season. The relationship based on comparative dates (A) had a stronger relationship than the induced lag relationship (B) (where discharge data was one day later than rainfall data) was weaker.

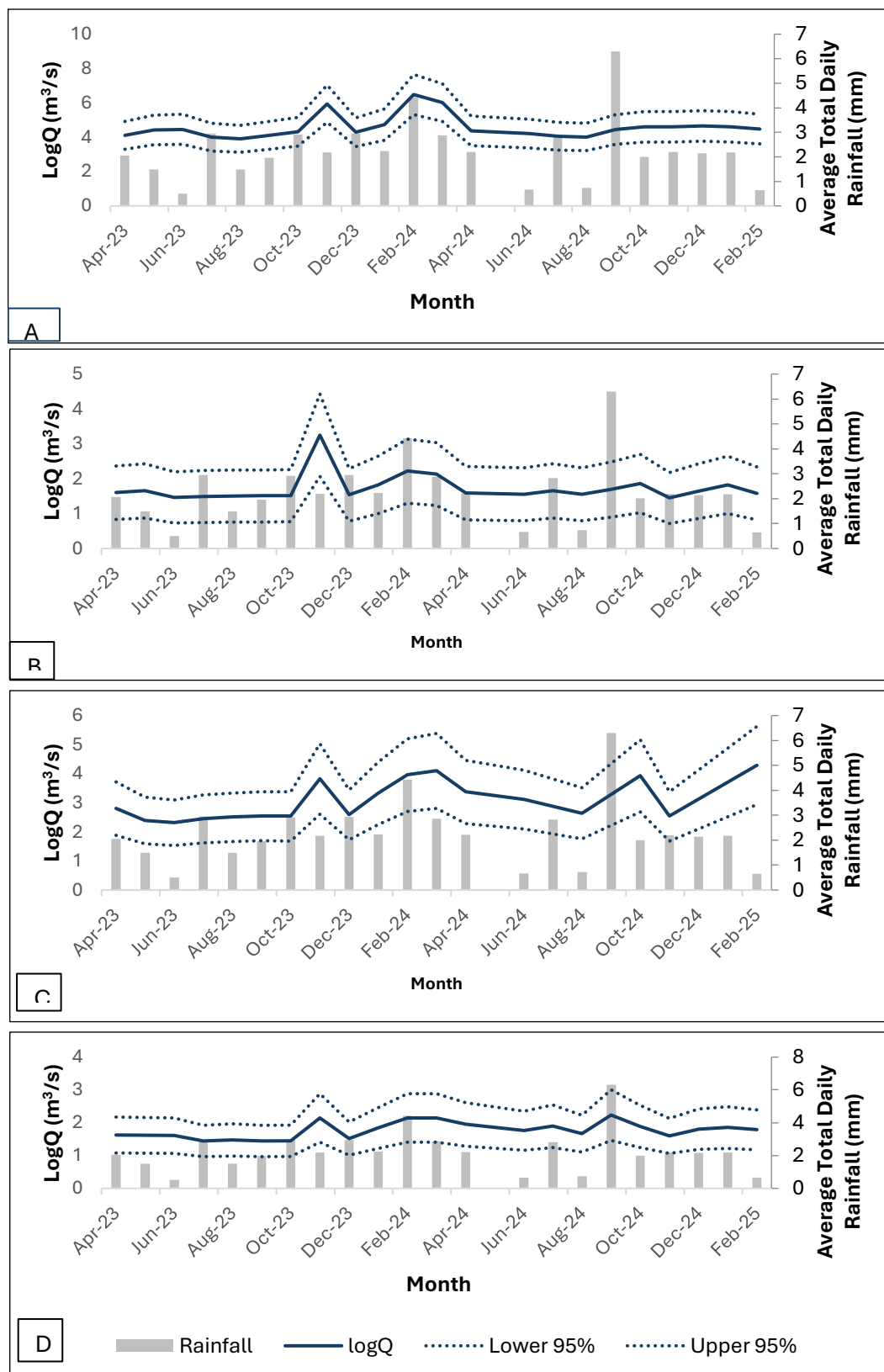


Figure 5. 4: The amount of river discharge throughout the 2023/24 and 2024/25 seasons at the four sites (A) CL, (B) LC; (C) DL; (D) V and the 95% confidence limits of the equations (dotted lines). On the right axis is the average total daily rainfall per month (mm) throughout 2023 - 2025.

In the 2023/24 season, the stage was greatest at the CL site ($0.51 \pm 0.06\text{m}$) and lowest at the LC site ($0.19 \pm 0.16\text{ m}$) (figure 5.5A). However, the LC site did have the largest range in stage in autumn, ranging from 0.10 to 0.66m. There was a significant difference in the river stage level in the 2023/24 season across the sites (Kruskal-Wallis: $H(3)=24.368$, $p=2.093\text{e-}05$) (table 5.2). The post hoc Dunn test showed that both CL had a higher stage than both LC ($p=5.58\text{e-}06$) and V ($p=0.03$) (table 5.3). Across the four intra-annual seasons, the stage was consistent with similar means for all seasons (figure 5.5A) and there was not a significant difference across the seasons (Kruskal-Wallis: $H(3)=2.2796$, $p=0.5164$).

In the 2024/25 season, the variation in stage across the sites was similar to the previous season, where CL had the highest average stage level ($0.48 \pm 0.01\text{ m}$) and LC had the lowest average river stage level ($0.13 \pm 0.03\text{ m}$). This was reflected in the statistical test, which showed there was a significant difference in stage level across the sites (Kruskal-Wallis: $H(2)=16.482$, $p=0.0002636$). The post hoc Dunn test also showed that CL was higher than LC ($p=1.54\text{e-}04$). The ranges in stage level at each site were fairly even and the stage level across the seasons was consistent (figure 5.6B). Therefore, there was not a significant difference across the seasons (Kruskal-Wallis: $H(2)=0.092352$, $p=0.9549$).

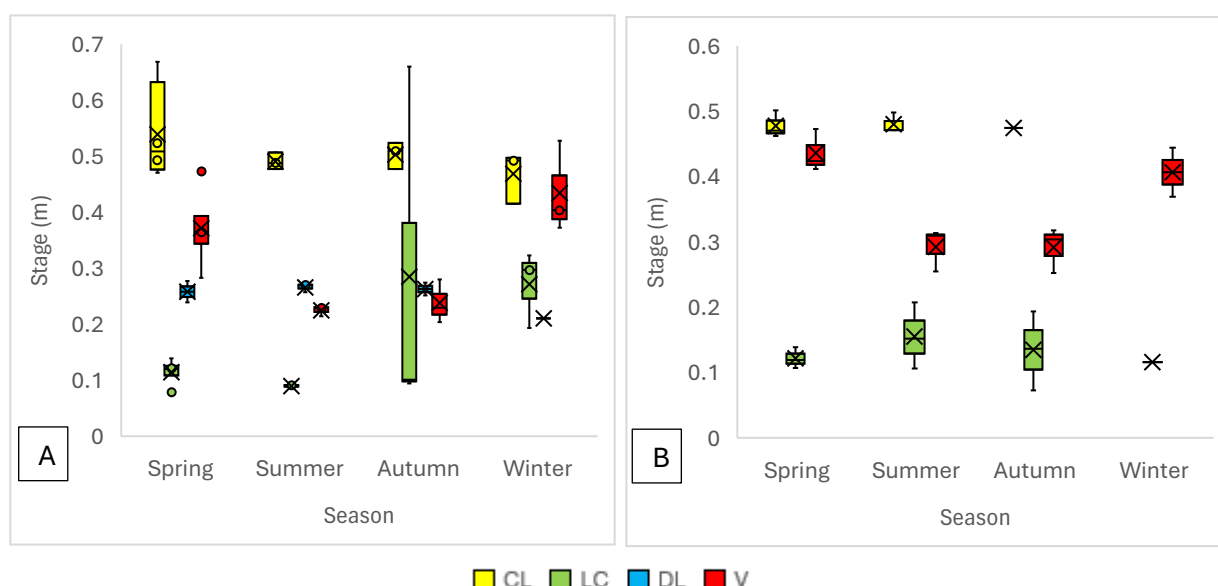


Figure 5. 5: The intra-annual variation in river stage levels across the four sites (CL, LC, DL, V) and the four seasons (spring, summer, autumn, winter) for the (A) 2023/24 season and (B) 2024/25 season.

Table 5. 2: The Kruskal-Wallis results for the differences in river stage across the sites and seasons in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p < 0.05$, the difference was significant (grey highlight).

Year	Nutrient	Independent Variable	Chi-Squared	df	P-Value
2023/24	Stage	Site	42.54	4	1.29E-08
		Season	0.98	3	8.07E-01
2024/25	Stage	Site	29.47	3	1.78E-06
		Season	4.03	3	2.58E-01

Table 5. 3: The post hoc Dunn test results for the significant differences in river stage from the Kruskal-Wallis test to determine which sites/ seasons were significantly different from each other in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p < 0.05$, the difference was significant (grey highlight).

Year	Dependent	Comparison	Z	P.unadj	P.adj
2023/24	Stage	CL - DL	3.21	0.00	0.013495
		CL - LC	5.46	0.00	4.83E-07
		DL - LC	1.56	0.12	1.00E+00
		CL - V	2.94	0.00	0.032594
		DL - V	-0.64	0.52	1.00E+00
		LC - V	-2.52	0.01	1.19E-01
		CL - W	3.93	0.00	0.000848
		DL - W	0.23	0.82	1.00E+00
		LC - W	-1.53	0.13	1.00E+00
		V - W	0.99	0.32	1
2024/25	Stage	CL - LC	5.33	0.00	6.01E-07
		CL - V	2.50	0.01	0.074116
		LC - V	-3.24	0.00	0.007195
		CL - W	2.49	0.01	0.07764
		LC - W	-3.26	0.00	0.006748
		V - W	-0.02	0.99	1

The river stage level was consistent across the 2023/24 and 2024/25 seasons, with the highest stage being at CL ($0.49 \pm 0.04\text{m}$) and the lowest at LC ($0.16 \pm 0.13\text{m}$) (figure 4.6). This meant there was no significant difference in river stage between the 2023/24 and 2024/25 season (Wilcoxon Test: $W=518$, $p=0.752$). However, there were more outliers at the CL site in 203/24, which showed there was more unusual stage levels outside of the normal range in this season. The Ver site also had a larger range in 2023/24 and the DL site was absent in the 2024/25 season.

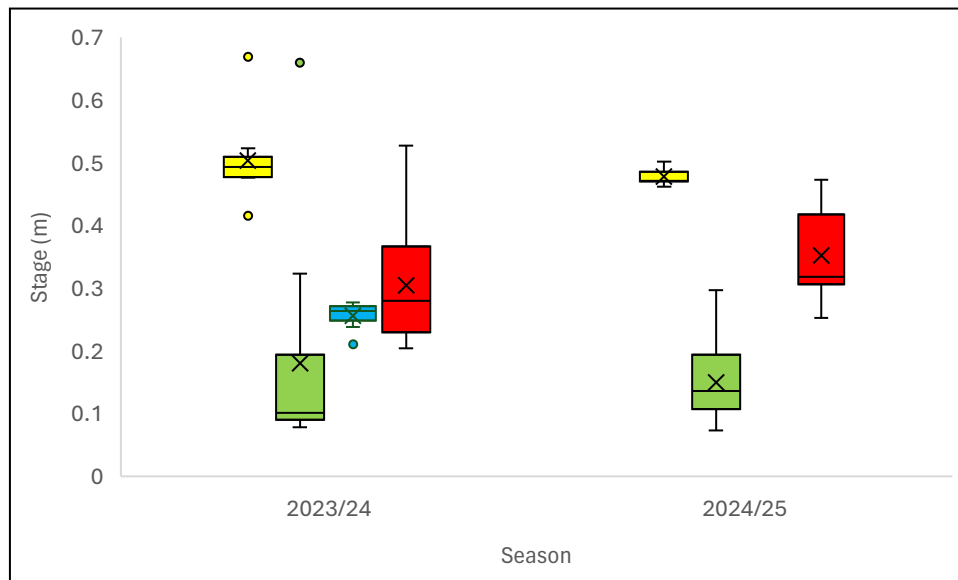


Figure 5. 6: The intra-annual variation in river stage levels across the two seasons (2023/24 and 2024/25). CL and DL measurements were taken from the gauging stations, and the LC and V levels were from monthly measurements. DL is absent from 2024/25 as the gauging station did not run during this period.

5.2. Intra-annual variation of the hydrological variables in the Upper Colne Catchment

The average river channel depth in the Upper Colne Catchment was deepest during the autumn (0.32 ± 0.19 m) and winter (0.36 ± 0.16 m) and shallowest during the summer (0.19 ± 0.07 m) in the 2023/24 season (figure 5.7A). There was a significant difference between the intra-annual seasons across the 2023/24 season (ANOVA: $F(3)=3.36$, $p=0.03$) (table 5.4) and the post hoc Tukey HSD test showed that winter was significantly deeper than summer ($p=0.03$) (table 5.5). LC had the largest variation in channel depth in autumn (0.09 – 0.66m) and the smallest range in summer (0.02 – 0.04m). The channel depth was deeper at DL (0.35 ± 0.14 m) and V (0.33 ± 0.14 m) sites compared to the CL (0.26 ± 0.12 m) and LC (0.18 ± 0.17 m) sites. There was a significant difference in the average channel depth across the sites (ANOVA: site $F(3)=7.02$, $p=0.001$) and the post hoc Tukey HSD test showed that LC had a shallower average depth than CL ($p=0.05$), DL ($p=0.002$) and V ($p=0.005$).

Table 5. 4: The Two-Way ANOVA results for the hydrological variables in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p<0.05$, the difference was significant (grey highlight).

Year	Variable	Independent Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
2023/24	Average Depth	Site	3	4.80	1.60	7.02	0.001222
		Season	3	2.30	0.77	3.36	0.033223
		Residuals	27	6.16	0.23		
	Channel Area	Site	3	17.35	5.78	13.44	1.49E-05
		Season	3	4.34	1.45	3.36	0.033188
		Residuals	27	11.62	0.43		
2024/25	Water Temperature	Site	3	8.08	2.69	0.35	0.788109
		Season	3	605.43	201.81	26.38	8.99E-08
		Residuals	24	183.59	7.65		
	Average Depth	Site	3	0.71	0.24	28.99	3.77E-08
		Season	3	0.09	0.03	3.52	0.030362
		Residuals	24	0.20	0.01		

Table 5. 5: The post hoc Tukey HSD results for the significant differences in hydrological variables from the two-way ANOVA test to determine which sites/ seasons were significantly different from each other in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p < 0.05$, the difference was significant (grey highlight).

Season	Hydrology Variable	Significant Difference	Difference	Lower	Upper	P-Value
2023/24	Average Depth	LC-CL	-0.56	-1.12	-0.01	0.0463961
		LC-DL	-0.84	-1.40	-0.29	0.0015985
		V-LC	0.78	0.21	1.35	0.0047169
		Winter-Summer	0.59	0.06	1.13	0.0254551
	Channel Area	DL-CL	0.90	0.13	1.66	0.0169405
		V-CL	0.84	0.06	1.63	0.0316809
		LC-DL	-1.53	-2.29	-0.76	4.95E-05
		V-LC	1.47	0.69	2.26	0.0001164
		Winter-Summer	0.82	0.09	1.56	0.0229856
2024/25	Water Temp	Summer-Autumn	3.35	0.08	6.62	0.0430846
		Winter-Autumn	-6.85	-10.26	-3.44	0.00006
		Summer-Spring	5.95	2.47	9.43	0.0004698
		Winter-Spring	-4.25	-7.87	-0.63	0.0171673
		Winter-Summer	-10.20	0.00	-6.93	0
	Average Depth	DL-CL	0.23	0.11	0.35	0.0000937
		V-CL	0.18	0.74	0.29	0.0005028
		LC-DL	-0.34	-0.46	-0.22	0.0000003
		V-LC	0.28	0.18	0.39	0.0000007
		Summer-Spring	-0.13	-0.24	-0.02	0.0207821

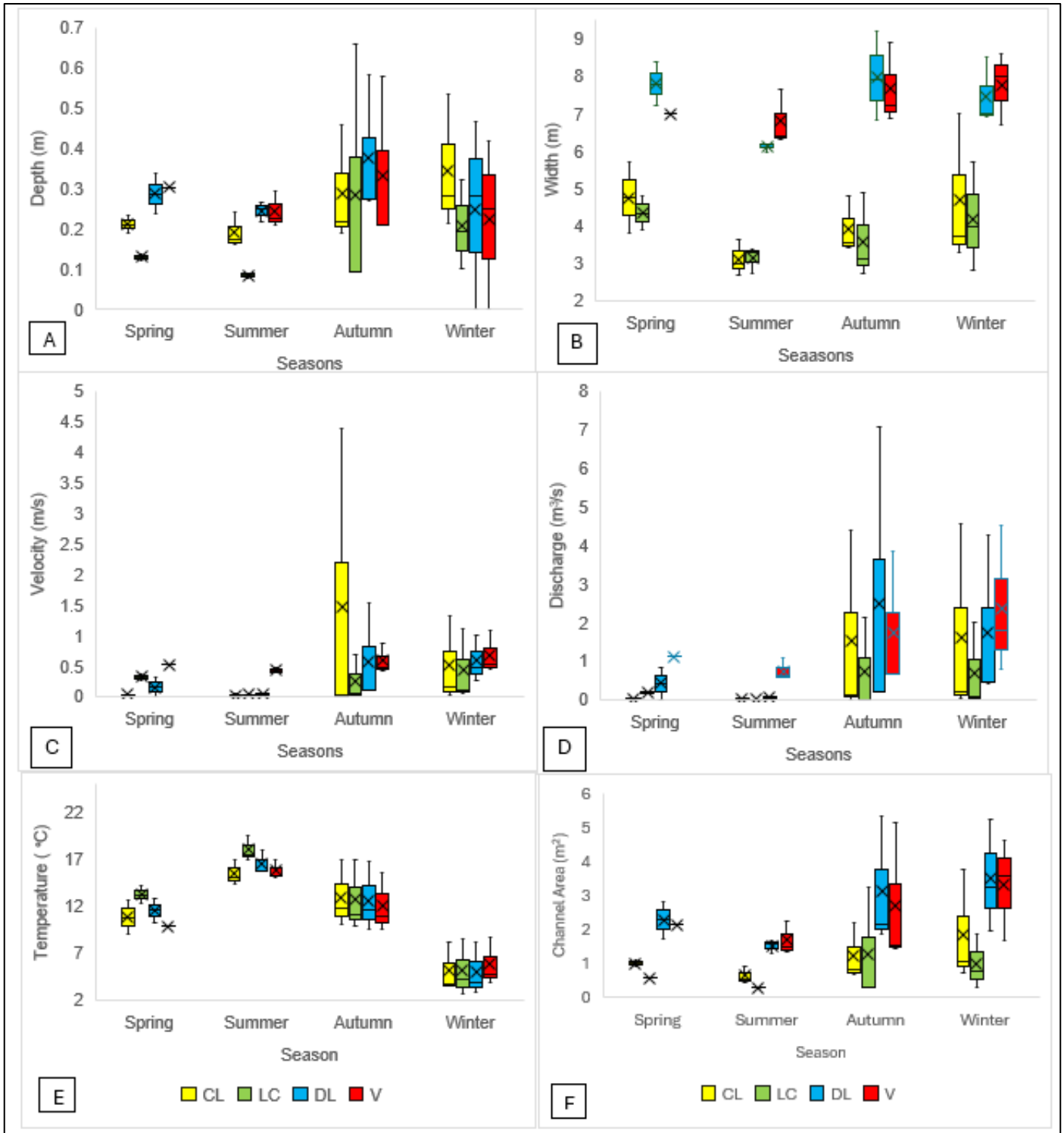


Figure 5. 7: The variation in the hydrological variables across the sites (CL, LC, DL, V) and the seasons (spring, summer, autumn, winter) in the 2023/24 season. (A) is the average channel depth; (B) is the channel width; (C) is the average river flow velocity; (D) is the total river discharge; (E) is the river water temperature; and (F) is the river channel cross sectional area of the site.

The river channel width was observed to be consistently higher at DL ($7.30 \pm 1.02\text{m}$) and V sites ($7.36 \pm 0.84\text{m}$) than both the CL ($4.05 \pm 1.22\text{ m}$) and LC sites ($3.76 \pm 0.95\text{ m}$) (Figure 5.7B). The largest range in channel width was at the CL site in winter ($3.31 - 7\text{m}$) and the smallest range in channel width was at LC in summer ($2.75 - 3.37\text{m}$). LC site had the narrowest width, and the widest width was at V, apart from autumn where the widest width was at DL ($7.98 \pm 0.97\text{ m}$). This difference in channel widths between the sites was significant (Kruskal-Wallis: $H(3)=29.06$, $p=2.17\text{e-}06$) (table 5.6) and the post hoc Dunn test showed that CL was significantly lower than DL ($p=0.002$), DL was significantly wider than LC ($p=0.00044$) and CL and LC were both narrower than the Ver site (CL -V : $p=0.0016$; LC – V : $p=0.0003$) (table 5.7). The channel widths were lowest in summer ($4.79 \pm 1.72\text{ m}$) and did increase throughout the 2023/24 season with the largest width in winter ($6.02 \pm 1.98\text{ m}$) (figure 5.7B) However, there was no significant difference across the seasons in the 2023/24 season (Kruskal-Wallis: $H(3) = 3.77$, $p=0.29$).

Table 5. 6: The Kruskal-Wallis test results for the significant differences between the sites and seasons for the hydrological variables in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p < 0.05$, the difference was significant (grey highlight).

Year	Dependent Variable	Independent Variable	Chi-Squared	df	P-Value
2023/24	Channel Width	Site	29.06	3	2.17E-06
		Season	3.77	3	0.287
	Average Flow Velocity	Site	10.42	3	1.53E-02
		Season	8.81	3	0.03191
	Discharge	Site	10.67	3	1.30E-02
		Season	8.22	3	0.04166
	Daily Total Rainfall	Site	0.12	3	9.89E-01
		Season	16.46	3	0.009112
	Min Temp	Site	0.00	3	9.90E-01
		Season	20.41	3	0.00014
	Max Temp	Site	0.01	3	1.00E+00
		Season	29.21	3	3.29E-06
	Water Temp	Site	0.47	3	9.25E-01
		Season	32.22	3	4.71E-07
2024/25	Width	Site	29.96	3	1.41E-06
		Season	2.95	3	4.00E-01
	Average Flow	Site	19.26	3	2.42E-04
		Season	6.06	3	1.09E-01
	Discharge	Site	22.68	3	4.70E-05
		Season	4.57	3	2.06E-01
	Daily Total Rainfall	Site	0.56	3	9.05E-01
		Season	17.20	3	6.42E-04
	Min Temp	Site	0.01	3	9.97E-01
		Season	31.10	3	8.11E-07
	Max Temp	Site	0.05	3	9.97E-01
		Season	33.27	3	2.82E-07
	Channel Area	Site	30.51	3	1.08E-06
		Season	2.71	3	4.39E-01

Table 5. 7: The post hoc Dunn test results for the significant differences in hydrological variables from the Kruskal-Wallis test to determine which sites/ seasons were significantly different from each other in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $p < 0.05$, the difference was significant (grey highlight).

Year	Dependent Variable	Independent Variable	z	P unadj	P value
2023/24	Channel Width	CL - DL	- 3.54	0.00	0.002389
		DL - LC	3.97	0.00	0.000439
		CL - V	- 3.64	0.00	0.001642
		LC - V	- 4.05	0.00	0.000303
	Average Flow Velocity	CL - V	- 3.15	0.00	0.009961
		Summer - Winter	- 2.80	0.01	0.030252
	Discharge	CL - V	- 2.77	0.01	0.0334
		LC - V	- 2.81	0.00	0.029385
		Summer - Winter	- 2.76	0.01	0.035155
	Daily Total Rainfall	Spring - Winter	- 3.52	0.00	0.002605
		Summer - Winter	- 3.33	0.00	0.005222
	Minimum Air Temperature	Spring - Summer	- 3.09	0.00	0.012134
	Maximum Air Temperature	Autumn - Winter	3.07	0.00	0.012918
		Summer - Winter	5.29	0.00	7.42E-07
	Water Temperature	Autumn - Winter	3.41	0.00	0.003943
		Spring - Winter	2.64	0.01	0.049121
		Summer - Winter	5.63	0.00	1.05E-07
2024/25	Channel Width	CL - DL	- 2.81	0.01	0.03
		DL - LC	3.34	0.00	0.005061
		CL - V	- 4.05	0.00	0.000306
		LC - V	- 4.65	0.00	1.96E-05
	Average Flow Velocity	CL - V	- 4.14	0.00	0.000208
		LC - V	- 3.10	0.00	0.011576

Year	Dependent Variable	Independent Variable	z	P unadj	P value
	Discharge	CL - V	- 3.96	0.00	0.000453
		LC - V	- 3.79	0.00	0.000889
	Daily Total Rainfall	Spring - Summer	3.22	0.00	0.007627
		Spring - Winter	3.49	0.00	0.002849
	Minimum Air Temperature	Spring - Summer	- 2.75	0.01	0.035542
		Autumn - Winter	3.16	0.00	0.009492
		Summer - Winter	5.54	0.00	1.80E-07
	Maximum Air Temperature	Autumn - Summer	- 2.68	0.01	0.043795
		Spring - Summer	- 2.94	0.00	0.019684
		Autumn - Winter	2.93	0.00	0.02042
		Summer - Winter	5.74	0.00	5.62E-08
	Channel Area	DL - LC	4.20	0.00	0.000162
		CL - V	- 3.01	0.00	0.01572
		LC - V	- 4.81	0.00	8.85E-06

The river experienced its fastest average flow velocity at the Ver site throughout the 2023/24 season (0.56 ± 0.22 m/s), except for autumn where it was the CL site (1.46 ± 2.05 m/s) (figure 5.7C). This difference in flow velocity between the sites was significant (Kruskal-Wallis: Site H (3) =10.42, $p=1.53e-02$) and the post-hoc Dunn test showed that CL had a lower flow velocity than V ($p=0.009$). The flow velocity increased throughout the 2023/24 season, with faster average flow velocities in autumn (0.72 ± 1.19 m/s) and winter (0.55 ± 0.44 m/s). The lowest flow velocity was in summer (0.13 ± 0.18 m/s). This difference in flow velocity between the seasons was significant (Kruskal-Wallis: H (3) =8.81, $p=0.03$), and summer had a significantly lower average river flow velocity than winter ($p=0.03$).

The discharge of the rivers in the Upper Colne Catchment increased in autumn (1.61 ± 2.21 m³/s) and winter (1.60 ± 1.76 m³/s) and were lowest in the summer (0.21 ± 0.33 m³/s). There was a significant difference in river discharge across the seasons (Kruskal-Wallis: H(3)=8.22, $p=0.04$) and the post hoc test showed that summer had a significantly lower river discharge than winter ($p=0.035$). The largest range in river discharge was in autumn at the DL site (0.18 – 7.08 m³/s). However, the Ver site had

the highest discharge across the sites ($1.57 \pm 1.27 \text{ m}^3/\text{s}$). The discharge increased with distance downstream too, with the exception of LC in autumn and winter (Figure 5.7D). This difference in discharge between the sites was significant (Kruskal-Wallis: $H(3)=10.67$, $p=1.30\text{e-}02$) and the post hoc test showed that CL had a lower discharge than V ($p=0.03$) and LC had a lower discharge than V ($p=0.03$).

The river water temperature was greatest in summer ($16.42 \pm 1.45 \text{ }^\circ\text{C}$) and lowest in winter ($5.24 \pm 2.27 \text{ }^\circ\text{C}$) (figure 5.7E). This difference in water temperature between the seasons was significant (Kruskal-Wallis: $H(3)=32.22$, $p=3.29\text{e-}07$) and the post hoc test showed that all other seasons (autumn ($p=0.003$), spring ($p=0.05$) and summer ($p=1.05\text{e-}07$)) were significantly higher than winter. The water temperature between the sites was consistent and there was no significant difference in water temperature across the sites (Kruskal-Wallis: $H(3)=0.47$, $p=0.93$).

The river cross-sectional channel area was greatest at the Drop Lane site ($2.62 \pm 1.37 \text{ m}^2$) and lowest at the London Colney site ($0.78 \pm 0.89 \text{ m}^2$) in the 2023/24 season. The DL and V sites were consistently higher than the CL and LC sites (figure 5.7F). The difference in the river channel area was significant (ANOVA: $F(3)=13.43$, $p=1.49\text{e-}05$). The post hoc test showed that both the V and DL sites had a significantly larger channel area than CL (DL-CL $p=0.02$; V-CL $p=0.03$). DL and V also had a significantly larger channel area than LC (DL-LC $p=4.95\text{e-}05$; V-LC $p=0.0001$). The channel area also increased in autumn ($2.07 \pm 1.65 \text{ m}^2$) and winter ($2.39 \pm 1.58 \text{ m}^2$) and was lowest in summer ($1.01 \pm 0.64 \text{ m}^2$). There was a significant difference in the river channel area across the seasons (ANOVA: $F(3)=3.36$, $p=0.03$) and the post hoc test showed that Winter had a greater channel area than summer ($p=0.02$).

The daily total rainfall in the Upper Colne Catchment increased across the seasons in the 2023/24 season, with spring having the lowest rainfall in the catchment ($1.78 \pm 0.28 \text{ mm}$) and winter having the highest rainfall level ($3.20 \pm 0.91 \text{ mm}$) (figure 5.8A). This difference in total daily rainfall in the catchment between the seasons was significant (Kruskal-Wallis: $H(3) = 16.46$, $p=0.009$). The post hoc test showed that spring was significantly lower than winter ($p=0.002$) and summer also had significantly lower total daily rainfall levels than winter ($p=0.005$). The maximum and minimum air temperatures in the catchment were highest in summer (Max: $21.91 \pm 1.09 \text{ }^\circ\text{C}$; Min: $11.88 \pm 0.50 \text{ }^\circ\text{C}$) and lowest in winter (Max: $9.1 \pm 1.52 \text{ }^\circ\text{C}$; Min: $3.63 \pm 0.28 \text{ }^\circ\text{C}$) (figure

5.8BC). There was a significant difference in the mean daily maximum and minimum air temperatures across the seasons (Kruskal-Wallis: Max Temperature $H(3) = 29.21$, $p = 4.71 \times 10^{-4}$; Min Temperature $H(3) = 20.41$, $p = 0.00014$). The post hoc Dunn test showed that spring had a lower minimum temperature than summer ($p = 0.01$) and summer had a lower minimum temperature than winter ($p = 0.0002$). For the maximum air temperature, the post hoc test showed that autumn ($p = 0.01$) and summer ($p = 0.0002$) were higher than winter in the 2023/24 season.

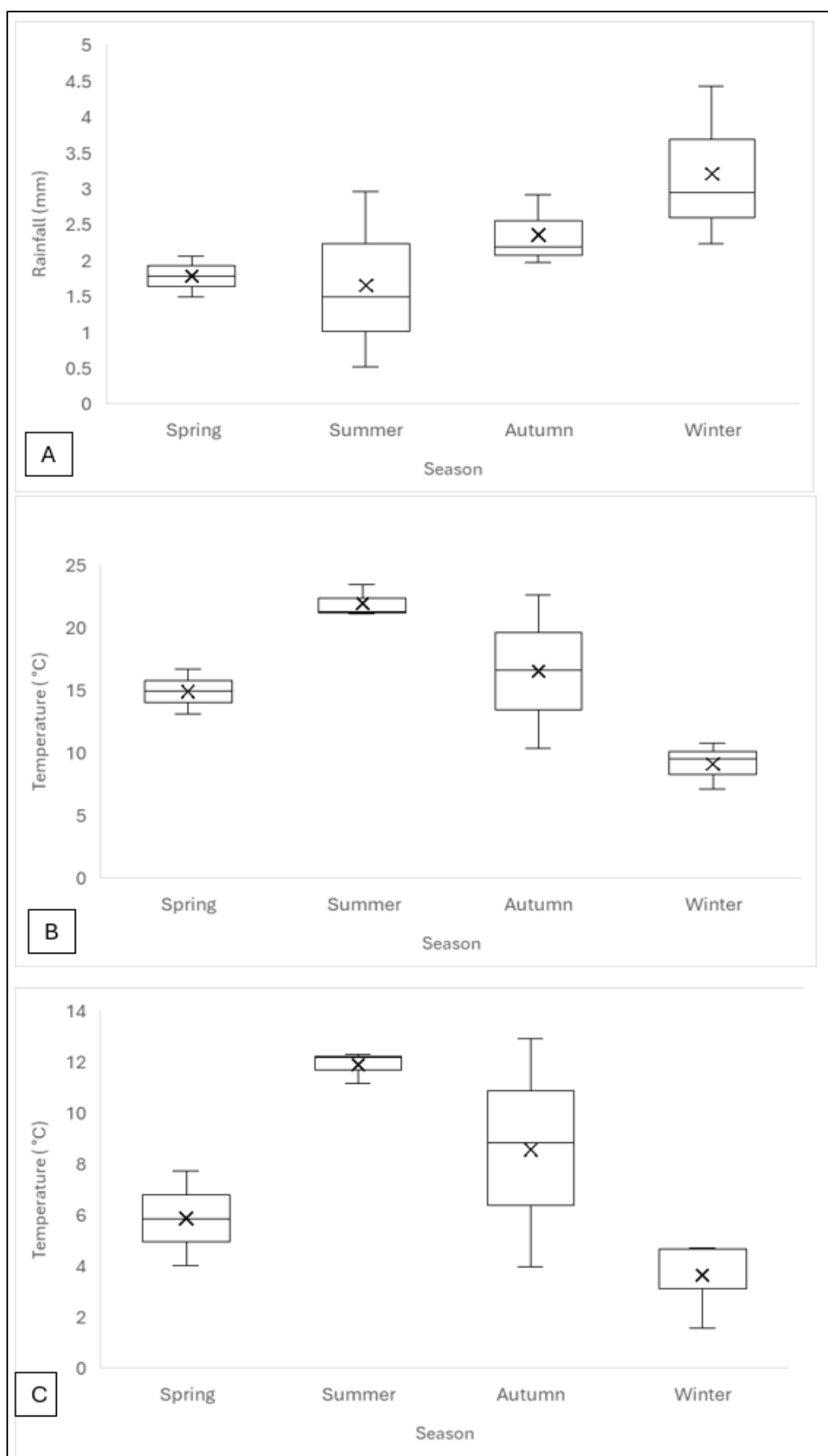


Figure 5. 8: (A) Total daily rainfall (mm) from the Upper Colne Catchment. (B) Maximum daily temperature. (C) Minimum daily temperature. The climatic data is from

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In the 2024/25 season the river channel was deepest at Drop Lane (0.48 ± 0.15 m) and Ver (0.43 ± 0.09 m) sites, and lowest at the CL (0.25 ± 0.07 m) and LC sites (0.15 ± 0.06 m). The LC site consistently had the shallowest depth (figure 5.9A). The difference between the sites was significant (ANOVA: $F(3) = 28.99$, $p = 3.77 \times 10^{-8}$) and the post hoc Tukey HSD test showed that CL had a significantly shallower depth than both the DL ($p = 0.000093$) and V ($p = 0.0005$) sites. LC was also significantly shallower than both DL ($p = 0.0000007$), and V ($p = 0.0000007$). For the 2024/25 season, summer had the lowest river channel depth (0.25 ± 0.12 m) but the other seasons had a similar average depth (spring, autumn winter). There was a significant difference in the river channel depth across the seasons (ANOVA: $F(3) = 3.52$, $p = 0.03$) and the post hoc test showed that summer had a lower river channel depth than spring ($p = 0.02$).

The river channel width was wider at the DL (7.36 ± 1.9 m) and V (8.16 ± 1.0 m) sites and lowest at the LC site (3.11 ± 0.54 m) (figure 5.9B). This variation in channel widths between sites was significant (Kruskal-Wallis: $H(3) = 29.96$, $p = 1.41 \times 10^{-6}$) and the post hoc test showed that CL was significantly lower than both the DL ($p = 0.03$) and V ($p = 0.003$) sites. It also showed that LC had a narrower width than both DL ($p = 0.005$) and V ($p = 1.96 \times 10^{-5}$). The channel width increased slightly at the DL and V sites in autumn and winter, but there was not a significant difference across the seasons in the 2024/24 season (figure 5.9B) (Kruskal-Wallis: $H(3) = 2.94$, $p = 0.4$).

The V site had the fastest average flow velocity in the Upper Colne Catchment in the 2024/25 season (0.90 ± 0.42 m/s) and the CL site had the slowest flow velocity (0.15 ± 0.39 m/s). There was a significant difference in the flow velocities across the sites (Kruskal-Wallis: $H(3) = 19.26$, $p = 2.42 \times 10^{-4}$) and the post hoc Dunn test showed that the Ver site had a significantly higher velocity than both CL ($p = 0.0002$) and LC ($p = 0.1$). The average flow velocity increased with distance downstream, except for the spring season where there was more variation in flow velocities (figure 5.9C). However, there was little variation in the average flow velocity between the seasons and there was no significant difference across the seasons (Kruskal-Wallis: $H(3) = 6.05$, $p = 0.11$).

The river channel discharge was greatest in spring (2.70 ± 2.7 m³/s) and lowest in summer (0.62 ± 1.04 m³/s) (figure 5.9D). However, there was no significant difference

between the seasons in the 2024/25 season (Kruskal-Wallis: $H(3)=4.57$, $p=0.21$). The Ver site had the largest mean discharge across the sites (2.97 ± 1.97), except for DL in spring ($4.13 \pm 3.68 \text{ m}^3/\text{s}$). The LC site had the smallest discharge ($0.09 \pm 0.17 \text{ m}^3/\text{s}$) (figure 5.9D). This difference in river discharge between the sites was significant (Kruskal-Wallis: $H(3) = 22.68$, $p=4.70\text{e-}05$) and the post hoc Dunn test showed that CL was significantly lower than both DL ($p=0.05$) and V ($p=0.00045$), and that LC had a significantly lower discharge than V ($p=0.0089$).

The river water temperature was similar across the sites in the 2024/25 season, although CL was slightly lower than the other sites ($10.43 \pm 4.34 \text{ }^\circ\text{C}$) (figure 5.9E) but there was not a significant difference across the sites (ANOVA: $F(3)=0.35$, $p=0.79$). The river water temperature declined throughout the seasons in the 2024/25 season, with the highest river water temperature in summer ($15.89 \pm 2.90 \text{ }^\circ\text{C}$) and the lowest in winter ($5.36 \pm 2.84 \text{ }^\circ\text{C}$). This was a significant difference across the seasons (ANOVA: $F(3) = 26.38$, $p=8.99\text{e-}08$) and the Tukey HSD test showed that summer had significantly higher water temperatures than autumn ($p=0.04$), spring ($p=0.0005$) and winter ($p=0.00$). Winter also had significantly lower water temperatures than autumn ($p=0.00006$) and spring ($p=0.017$).

The river channel cross-sectional area was largest at the DL site ($3.74 \pm 1.69 \text{ m}^2$) and smallest at the LC site ($0.48 \pm 0.27 \text{ m}^2$). There was a significant difference in the river channel areas in the 2024/25 season (Kruskal-Wallis: $H(3) = 30.51$, $p=1.08\text{e-}06$). The post hoc Dunn test showed that DL had a larger river channel area than LC ($p=0.0002$) and the V site had a larger channel area than both CL ($p=0.016$) and LC ($p=8.85\text{e-}06$). However, there was not much variation in the river channel areas across the seasons (figure 4.9F) and there was no significant difference between the seasons (Kruskal-Wallis: $H(3) = 2.71$, $p=4.39\text{e-}01$).

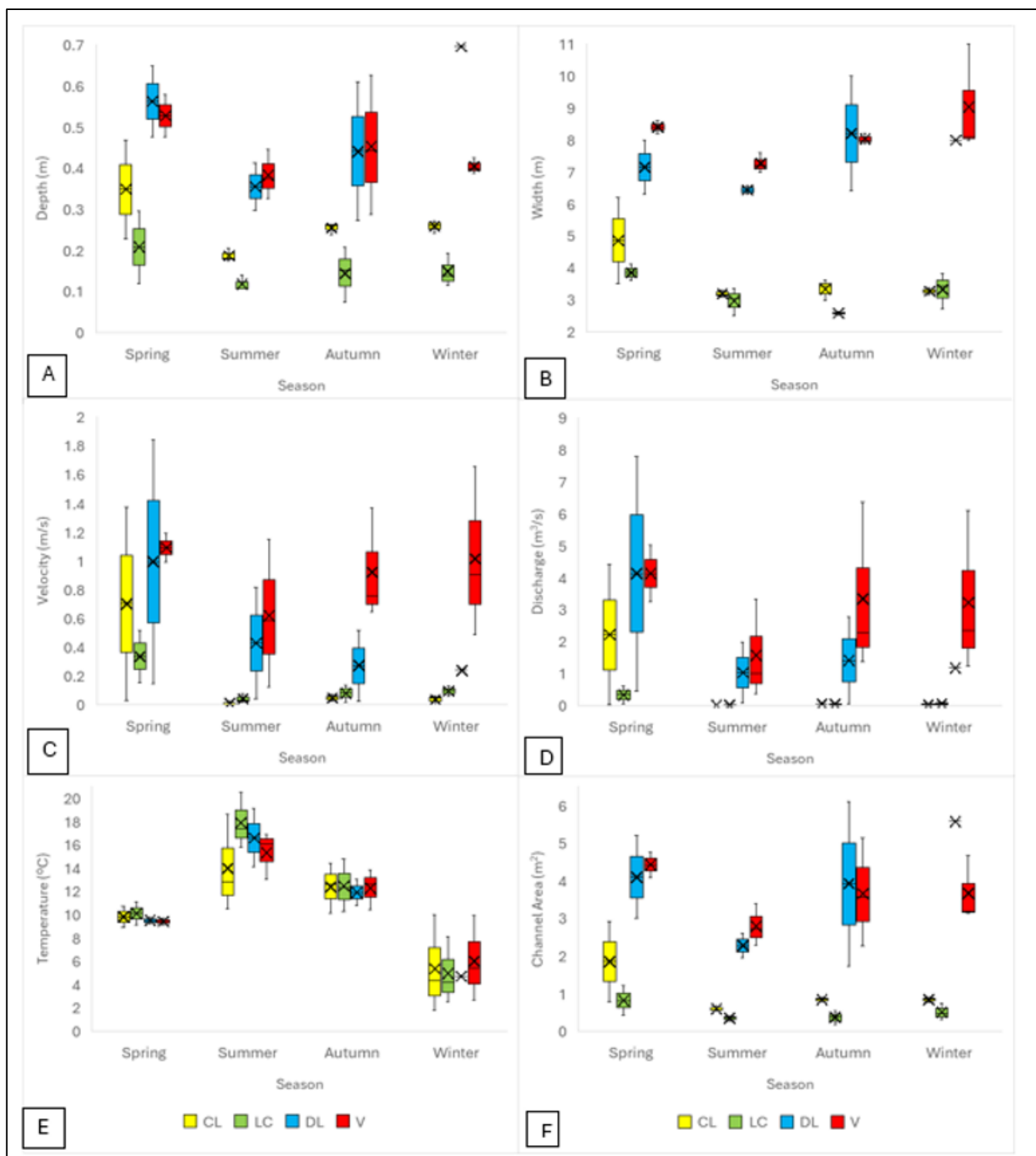


Figure 5. 9: The variation in the hydrological variables across the sites (CL, LC, DL, V) and the seasons (spring, summer, autumn, winter) in the 2024/25 season. (A) is the average channel depth; (B) is the channel width; (C) is the average river flow velocity; (D) is the total river discharge; (E) is the river water temperature; and (F) is the river channel cross sectional area of the site.

The total daily rainfall in the Upper Colne catchment in 2024/25 was highest in autumn (3.24 ± 1.98 mm), followed by spring (2.53 ± 0.33 mm) and lowest in the summer (1.28 ± 1.00 mm) (Figure 5.10A). This difference between the seasons was significant (Kruskal-Wallis: $H(3) = 17.20, p = 6.42 \times 10^{-4}$). The post hoc test showed that spring had higher total daily rainfall levels than both summer ($p = 0.008$) and winter ($p = 0.003$) had significantly lower total daily rainfall levels than spring. The maximum and minimum air temperatures were highest in summer (Max temp: 21.23 ± 1.26 °C; Min temp: 11.12 ± 1.60 °C) and lowest in winter (Max temp: 6.98 ± 1.13 °C; Min temp: 1.72 ± 1.81 °C) (figures 5.10BC). There was a significant difference in air temperatures across the seasons (Kruskal-Wallis: Max Temp: $H(3) = 33.27, p = 2.82 \times 10^{-7}$; Min Temp: $H(3) = 31.09, p = 8.11 \times 10^{-7}$). The post hoc Dunn test showed that maximum air temperatures in autumn ($p = 0.04$) and spring ($p = 0.02$) were significantly lower than summer, and autumn ($p = 0.02$) and summer ($p = 5.62 \times 10^{-8}$) were significantly higher than the winter season. For the minimum air temperature, spring ($p = 0.04$) and winter ($p = 1.80 \times 10^{-7}$) were significantly lower than summer, and autumn had significantly higher minimum air temperatures than winter ($p = 0.009$).

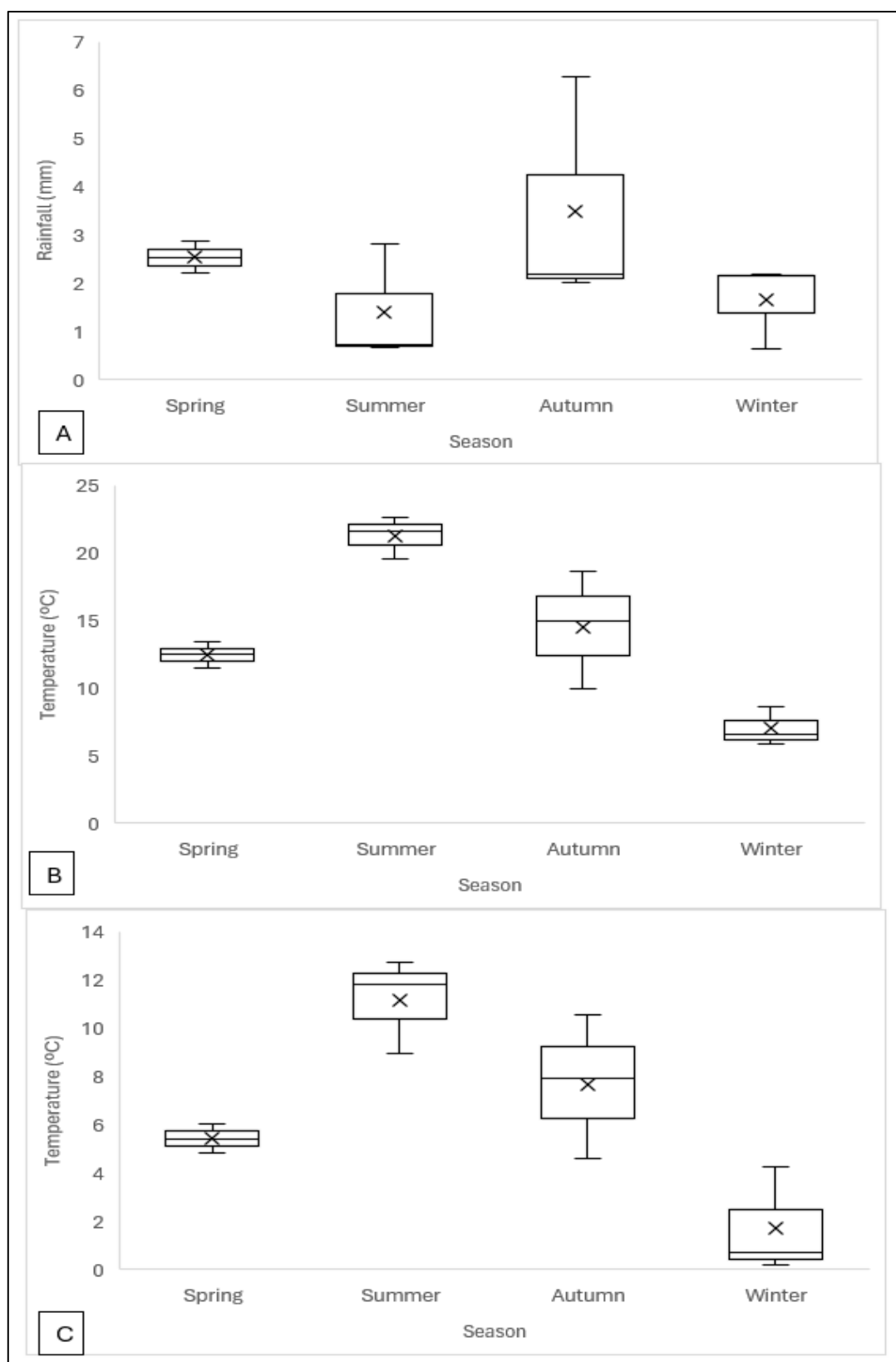


Figure 5. 10: (A) Total daily rainfall (mm) from the Upper Colne Catchment. (B) Maximum daily temperature. (C) Minimum daily temperature. The climatic data is from the Met Office (2025). © Crown Copyright [2025]. Information provided by the National Meteorological Library and Archive – Met Office, UK.

5.3. Inter-annual variation of the hydrological variables in the Upper Colne Catchment

The average channel depth was comparable across the seasons (2023/24: 0.28 ± 0.16 m; 2024/25: 0.31 ± 0.16 m) and there was no significant difference (T-Test: $t=1.063$, $p=0.29$). However, the DL and V sites had a higher mean in 2024/25 (DL: 0.48 ± 0.15 m; V: 0.43 ± 0.09 m) than 2023/24 (DL: 0.35 ± 0.14 m; V: 0.33 ± 0.14 m) (Figure 5.11A). The channel width was consistent across the 2023/24 (5.58 ± 1.99 m) and 2024/25 (5.36 ± 2.45 m) seasons, with the CL and LC sites having a narrower width than DL and V (figure 5.11B). The V site had more outliers in the 2024/25 season with two instances of wider and narrower channel widths. However, there was not a significant difference in the channel widths between the seasons (Wilcoxon Test: $W=933$, $p=0.5087$). The average flow velocity was similar across the 2023/24 and 2024/25 seasons (2023/24: 0.43 ± 0.72 m/s; 2024/25: 0.41 ± 0.52 m/s) and there was no significant difference (Wilcoxon Test: $W=829$, $p=0.78$). However, the flow velocity had a higher mean in 2024/25 at DL and V (DL: 0.52 ± 0.60 m/s; V: 0.90 ± 0.42 m/s) (figure 5.11C). Similarly, the river channel discharge had a higher mean at DL and V in the 2024/25 season (1.31 ± 2.02 m³/s) compared to the 2023/24 season (1.01 ± 1.65 m³/s), but this was not significant (Wilcoxon Test: $W=837$, $p=0.84$) (figure 5.11D). The river water temperature was consistent across the seasons (2023/24: 11.42 ± 4.78 °C; 2024/25: 11.03 ± 4.52 °C) although there were more outliers at the CL site in 2024/25, indicating two instances of lower water temperature and one of high water temperature outside the normal range (figure 5.11E). However, there was not a significant difference across the seasons (Wilcoxon Test: $W=922$, $p=0.58$). The river channel cross-sectional area was consistent across the seasons (2023/24: 1.76 ± 1.41 m²; 2024/25: 2.03 ± 1.73 m²) and there was not a significant difference between the seasons (Wilcoxon Test: $W=822$, $p=0.73$). However, the average river channel area was greater at DL (3.74 ± 1.69 m²) and V (3.57 ± 0.94 m²) in the 2024/25 season (figure 5.11F).

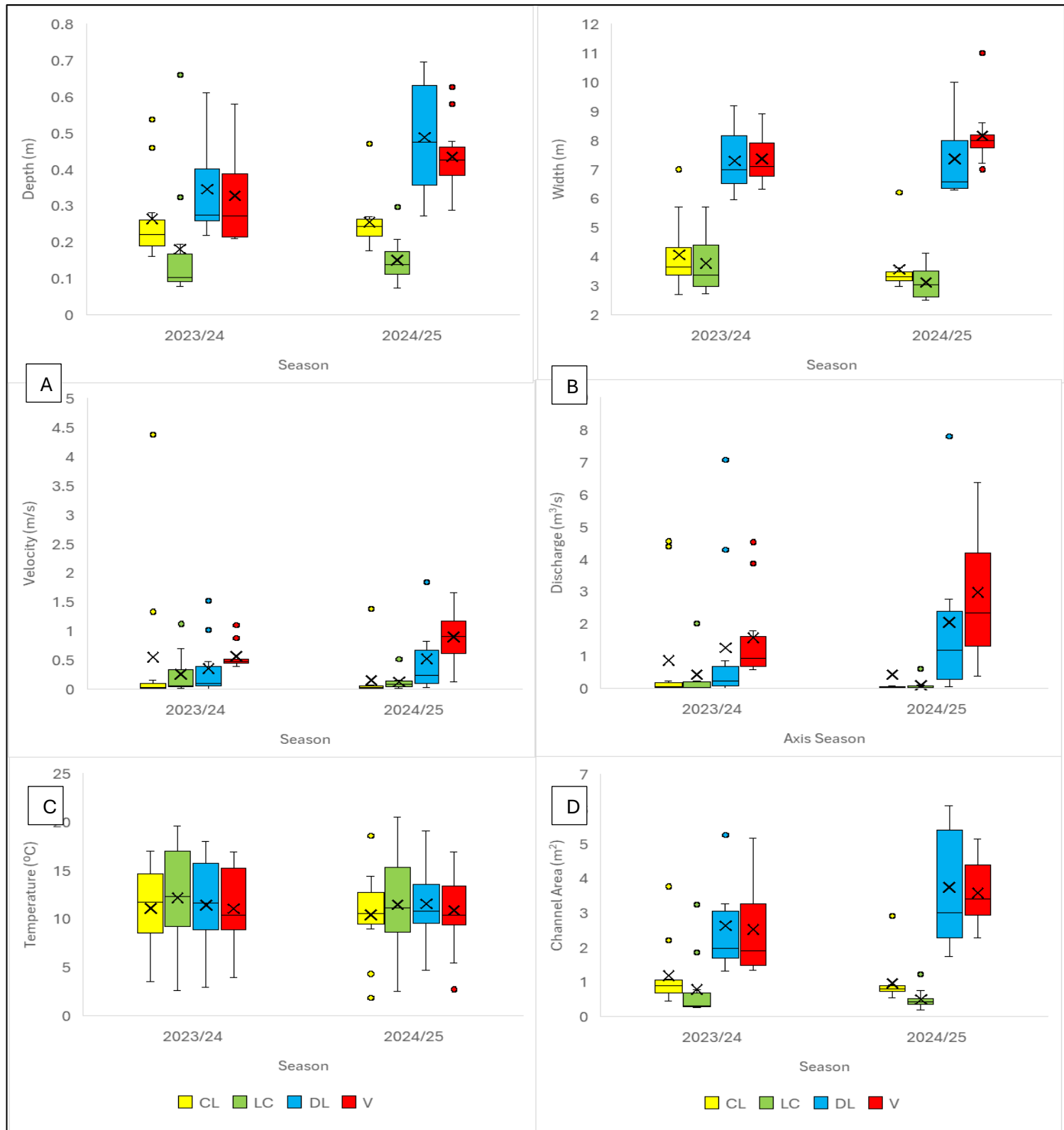


Figure 5. 11: The variation in the hydrological variables across the sites (CL, LC, DL, V) and the seasons (2023/24 and 2024/25 seasons). (A) is the average channel depth; (B) is the channel width; (C) is the average flow velocity; (D) is the total river discharge; (E) is the river water temperature; and (F) is the river channel cross sectional area of the site.

The total daily rainfall in the Upper Colne Catchment was consistent across the seasons (2023/24: 2.87 ± 0.97 mm; 2024/25 2.25 ± 1.50 mm) but the 2023/24 season had a larger range (0.51 – 2.94 mm) (figure 5.12A). However, there was not a significant difference in the total daily rainfall between the seasons (Wilcoxon Test: $W=996$, $p=0.21$). The maximum air temperature in the catchment was similar across the seasons, with a slightly lower mean in 2024/25 (2023/24: 15.66 ± 5.56 ; 2024/25: 13.94 ± 5.71) (figure 5.12B). The minimum air temperature was also consistent across the seasons, slightly lower in 2024/25 (2023/24 7.63 ± 3.87 ; 2024/25 6.0 ± 4.01) (Figure 5.12C). However, there was not a significant difference across the seasons in either the maximum (Wilcoxon Test: $W=1002$, $p=0.19$) or minimum (Wilcoxon Test: $W=938$, $p=0.47$) air temperatures.

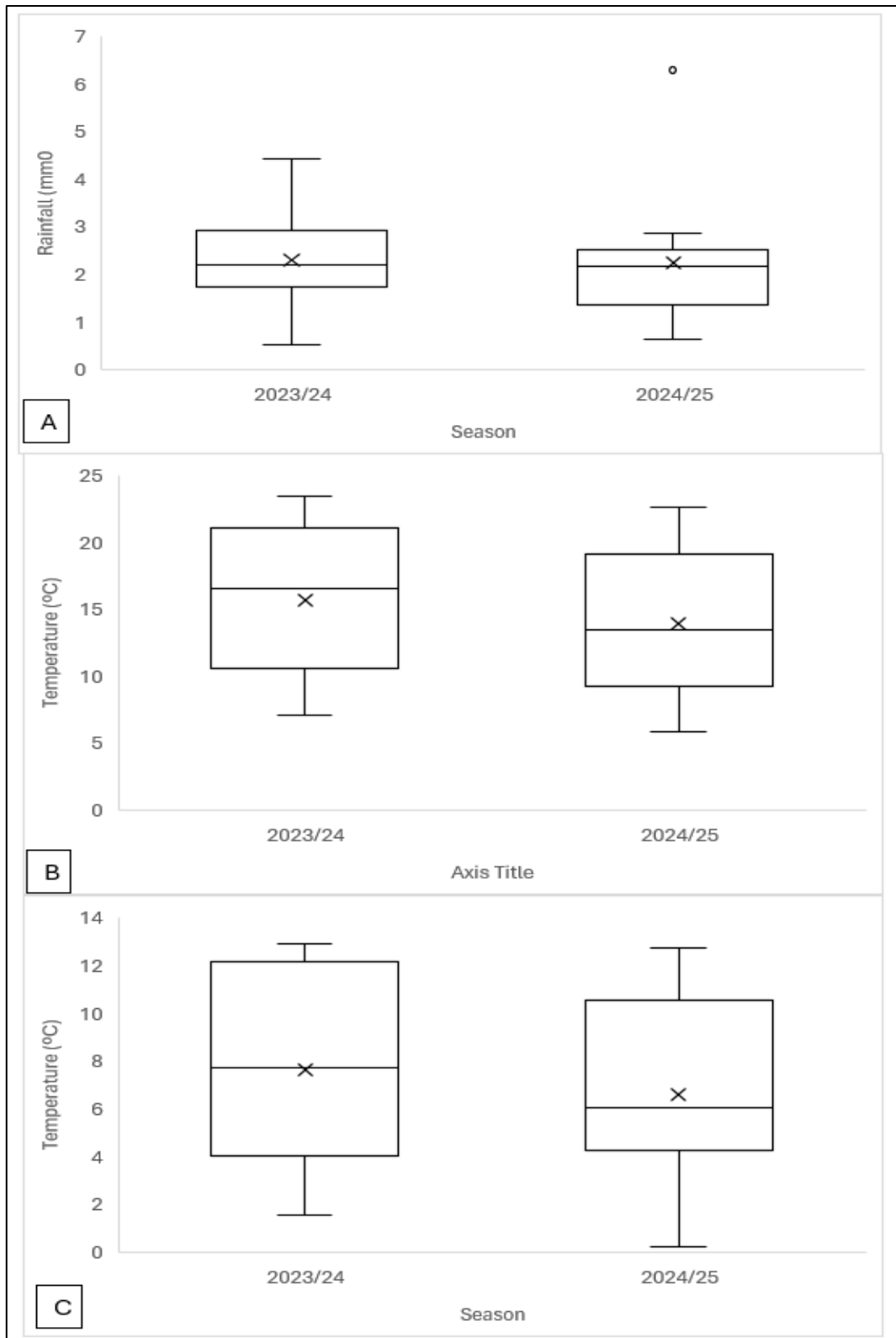


Figure 5. 12: The variation in the climate data across the 2023/24 and 2024/25 seasons. (A) Total daily rainfall (mm) from the Upper Colne Catchment. (B) Maximum daily temperature. (C) Minimum daily temperature. The climatic data is from the Met Office (2025). © Crown Copyright [2025]. Information provided by the National Meteorological Library and Archive – Met Office, UK.

5.4. Discussion

This study showed that the Upper Colne Catchment displayed the assumed hydrological cycle of a chalk stream with higher river stage levels in the autumn and winter, and lower stage levels in the summer (Mainstone *et al.*, 1999), although throughout the two-year period, there was no cessation of flow as seen in previous years (summer of 2021 and 2023 where the upper course had a dry river bed with pools of water) (Herts & Middlesex Wildlife Trust, 2023). The continuous stage monitoring at the two sites on the Colne showed that the CL site had a higher stage level than the DL site, although this could be due to the placement of the stage monitoring equipment, as observational data and the monthly in-situ depth measurements often showed that DL had a higher stage than CL in the 2023/24 season. The CL site also had more variability in the stage levels as there were higher levels in spring and the lowest stage levels were in summer 2024. However, the DL site had a consistent stage level throughout the seasons. This could be due to the narrower nature of the river channel at the CL site and the freer draining floodplain that is unobstructed by debris and vegetation, whereas the DL site had a major blockage just upstream of the sensor which would limit the flow of the river and subsequently impede the stage levels.

5.4.1. Stage and Discharge Relations

The discharge levels were strongly related to the stage levels at all four sites in the Upper Colne Catchments, although the monthly measurements were more reliable than the calculations based on gauging station measurements. This was likely due to the consistency of the hand-held measurements and the accessibility, compared to the station which was fixed and depended on the amount of water available at the sensor. Despite this, all relationships between stage and discharge were significant, meaning that stage measurements could potentially be used to predict the river discharge at the sites. The CL site had the largest response in discharge levels to an increase in stage and this was due to the open nature of the site, where surface runoff could easily enter the river channel without upstream obstruction. The river channel at CL also had the potential to contain larger amounts of discharge compared to more constrained sites such as LC (which had a shallower valley gradient) and DL (which

had an obstruction upstream of the site). The discharge rate here could be slowed as just downstream of the site was the concrete weir which slowed the flow of the water, confining it to the site more than the LC, DL and V sites. The stage at the Ver site had the lowest impact on the discharge, but this site was also furthest down in the Upper Colne catchment, so the effect could be more diluted, than upstream where a normally smaller discharge occurs and can be easily modified by additional discharge inputs from rainfall or snowmelt.

5.4.2. Rainfall and Discharge Relations

There was a significant correlation between the rainfall and discharge in the catchment. At the CL site, the response from the rainfall events was almost instantaneous with the discharge rising quickly with an increase in rainfall, and also quickly returning to pre-event levels once the rainfall levels had decreased. This showed that the catchment was quick to respond to changes in surface runoff. However, after a longer-term period of rainfall, such as throughout the month of May 2024, the river levels remained higher after the rainfall stopped, due to the influence of groundwater. Despite this, the correlation between discharge and rainfall was still relatively weak, which is to be expected within a chalk stream catchment, as there is more influence from groundwater contributions. Another study also found that heavy periods of rainfall during the winter period did not impact river flow rates (Robinson *et al.*, 2024). The higher groundwater levels during the study combined with higher rainfall levels in spring could be why there was no drought period of the channel in these seasons, compared to prior years (Met Office, 2024b).

5.4.3. Hydro-geomorphological and Environmental Changes to the Chalk Stream Environment

The hydro-geomorphological parameters showed variation between the sites, and occasional variation between the intra-annual seasons, but were consistent between the inter-annual seasons, showing that the upper catchment of the chalk stream was resilient to outside factors (i.e. rainfall levels) at the time. Most of the hydrological variables were similar to those expected in a semi-natural chalk stream environment (Sear *et al.*, 2006). The channel depth varied across the four sites, with depth generally increasing downstream. The deepest channel was at the DL site, which was where the flow pooled after the bridge and accumulated debris upstream. In the 2023/24 season

the depth was fairly consistent, although after the heavy rainfall in May 2024, the riverbed morphology shifted at the DL and CL sites. The DL site at the point of monthly measurements became shallower as sediments and gravels from upstream had been deposited just before a small ridge in the channel bed (where the farmer's fields were linked). The CL site also experienced a downstream shift in the bed sediments, deepening the channel at the site as material had been removed. This sediment transportation also removed the silty layer on top of the riverbed, exposing the gravel beneath the silt. Therefore, at sites that had more consistent flow levels, such as the V and LC sites, the gravel bed was more exposed, compared to CL and DL which were often slow or non-flowing. The availability of exposed gravel bed is critical in chalk streams for refuges for macroinvertebrate and fish species and streams that lack this often suffer ecologically (Neal *et al.*, 2008). The seasonal variation in channel depth showed that the summer months had significantly lower depths than the winter months, which was to be expected with lower rainfall amounts and the lower aquifer levels. The similar levels in channel depth and width across the remainder of the year, reflected the consistent baseflow provided by the chalk aquifer and the relatively quick response to surface runoff and rainfall events. River discharge was greatest in the springtime after the aquifer recharge and additional rainfall inputs, and lowest in the summer when the aquifer levels lowered and there was less rainfall. Discharge was found to be closely linked to mean channel depth, flow velocity and sediment particle size. At the sites with lower discharge levels, there were usually lower flow velocity rates and more silty sediment layers on the river bed, but the channel depth was not as affected (e.g. at the DL site there was a deeper average channel depth but low discharge rate due to low flow velocity) (Stelzer, Shupryt & Pillsbury, 2022). These measurements of variations in average channel depth, river discharge rates, average flow velocity and their seasonal variations align with previous work in other chalk stream catchments. For example, the River Itchen and Test also had greater flow in the winter season, and flow was lowest in the spring season (Robinson *et al.*, 2024; Mainstone *et al.*, 1999). River depth also varied in line with changes in river flow and groundwater levels in the Colne catchment, similar to the Rivers Itchen and Test (Robinson *et al.*, 2024; Mainstone, 1999). The flow rate stability is crucial for maintaining habitat diversity and ecological functioning and in chalk streams the flow rate varies between 0.05m/s to 0.5m/s which was the case for the four sites in the Colne Catchment. This flow rate provides sufficient oxygenation and removes

deposited material from the riverbed, clearing the gravel and supporting macroinvertebrate mobility (Cambridge City Council, 2025, England *et al.*, 2019). Chalk streams are also especially susceptible to prolonged drought due to their reliance on groundwater for around 80% of their annual discharge and how the flow is related to rainfall in the preceding months (Berrie, 1992). The lower reaches of chalk streams usually have high nutrient levels, stable flow regimes, dense macrophyte cover and diverse macroinvertebrate communities (Boulton, 2003; Wright, 1992). Reductions in flow can lead to reduced water quality, higher temperatures and lower dissolved oxygen levels which can negatively impact the ecosystem (Boulton, 2003). Therefore, the varied hydrological regime of the Upper Colne catchment showed how susceptible chalks streams can be to changes in the rainfall and groundwater levels and how the hydrological parameter can vary seasonally and longitudinally.

The water temperature in the Upper Colne Catchment experienced fluctuations alongside seasonal air temperature changes. CL had the lowest water temperature at 10.43 ± 4.34 °C and this was the closest to the expected 10 °C for a chalk stream (EA, 2014). However, the other sites, such as the Ver were more exposed to solar radiation and the furthest site downstream, which meant that solar radiation could enter the stream and increase the water temperature. The DL site was also warmer due to the stagnation of the water, allowing the water to heat up for longer, and LC was the shallowest site, which meant that solar radiation would have a greater effect on the water temperature. Water temperatures above 11°C have been found to reduce dissolved oxygen levels and favours the growth of bacteria and fungi over aquatic species, altering the species distributions in chalk streams (Jacobsen, 2008; Cambridge City Council, 2025). However, the range for water temperature in chalk rivers can be between 5 to 21°C (Mainstone *et al.*, 1999) and the Upper Colne remained within this range. Another study found that water temperature ranged between 8°C in autumn to 19°C in summer with no large variations (Robinson *et al.*, 2024). This warming of the water temperature is one of the major impacts of climatic warming that could affect the chalk streams, as the lower river flows and increase in water temperature will alter the aquatic environment and could potentially impact temperature-sensitive invertebrates (Author, Zischke & Ericksen, 1982; Acornley, 1999) and other fauna which is especially the case as air and water temperatures were highly correlated. The hydrological parameters were consistent between the inter-

annual seasons, suggesting that there were no major changes to the upper Colne catchment and the river channel morphology and hydrological functioning remained stable, with only minor fluctuations. However, this study period did not account for the extreme weather patterns that have been observed in the past 10 years, such as the heatwave of 2022 (Kendon, 2022), or the increasing rainfall events. Therefore, whilst the changes in the river hydrology and morphology have been minimal, these influences could be exacerbated if more drastic external influences occur and change the environment in the upper Colne.

5.4.4. Summary

Overall, this study characterised the hydrological cycle and hydrological variability of the river channel environment throughout the different intra-annual seasons and established the baseline functioning of the Upper Colne catchment during periods where there were high and low flows, but not during the times of extreme drought. Therefore, longer term monitoring of the upper Colne catchment would be required to identify the frequency and extent of the river channel drying and the impact this has on the hydrological functioning in a long-term scenario.

Chapter 6: The temporal and spatial variation of water quality parameters in the Upper Catchment of the River Colne

6.0. Introduction

Since most chalk streams in the UK are situated in the south of England, they are at greater risk of being affected by anthropogenic activity. Their characteristic cycle of high and low flows can leave them susceptible to small changes in nutrient levels, such as phosphate, nitrates and ammonium levels. Due to the nature of their location often being in the most populated areas of England and also flowing through large areas of agricultural land, over-abstraction combined with diffuse and point-source pollution of nutrients can be a major contributor to the decline in the ecological health of the chalk streams (Homan *et al.*, 2024; Johnson *et al.*, 2009). There has been an increase in nutrient loading from point sources such as Wastewater Treatment Works and Sewage Treatment works in highly populated areas which is detrimental on lowland chalk river environments. Despite the EU Urban Wastewater Treatment Directive (UWWTD) (91/271/EEC) requiring phosphorous stripping in Wastewater Treatment Works, phosphorous loading is still a major issue as many rivers flow through agricultural land and fertilisers are one of the major contributors to water pollution (Yates & Johnes, 2013; Johnes, 2007). This pollution is often exacerbated by an inability to dilute pollutants brought about by lower river flows from over-abstraction of watercourses, and leading to higher concentrations of nitrate, ammonium and phosphates (Yates & Johnes, 2013).

If the nutrient levels are high in chalk rivers, it can lead to a condition called chalk stream malaise, where key macrophytes such as *Ranunculus spp.* decline, macroinvertebrate diversity and abundance reduce, and there is excessive growth of filamentous algae and increased siltation of the gravel beds, all of which contribute to the deterioration of the chalk stream environment (Yates & Johnes, 2013; Neal *et al.*, 2008). Currently, only 14% of the UK's chalk streams are in Good Ecological Health and none have achieved good chemical status based upon the Water Framework Directives requirements, which is a stark contrast from 20 years ago in 2006 when 82% of the chalk rivers were in good chemical quality (O'Neill, 2014; Talks, 2008).

Recently, a report on the Micropollutants in the River Colne (2023) found that 267 different micropollutants were present in the Colne in Watford and upstream in the Upper Catchment (Colne Valley Fisheries Consultative, 2023). The majority of these micropollutants were from sewage misconnections and sewage treatment work discharges, road runoff and agricultural runoff, along with industrial and pharmaceutical chemicals. However, an understanding of the current levels of the main water nutrient levels (nitrate, ammonium and phosphate) is still limited in the Upper course of the River Colne and its catchment. Therefore, it is critical to understand the nutrient levels in the Upper Colne catchment, as higher nutrient levels upstream will have compound impacts on the water quality and furthermore on the ecological health of these rare chalk streams further downstream.

This chapter examines the relationships between the water nutrient variables (phosphate, nitrate and ammonia), referred to as water chemistry, and the physical-chemical variables (pH, water turbidity, electrical conductivity, dissolved oxygen and biological oxygen demand). The analysis compares the spatial variation of these parameters across the four sites (Church Lane, London Colney, Drop Lane and the Ver at Bricket Wood). Additionally, the seasonal variation (spring, summer, autumn and winter) of water nutrient and physical-chemical variables referred to as intra-annual variation is explored. This chapter also compares the water chemistry and physical-chemical variables between two sampling seasons here defined as inter-annual seasonal variation (April 2023 – February 2024: 2023/24 season) and March 2024 – February 2025: 2024/25 season). The hydrological parameters (daily rainfall, total discharge, average flow, depth, channel width, total channel area, and water temperature) are included in the Principal Component Analysis (PCA) to get a better understanding of how the hydrological variables relate to water chemistry and physical-chemical variables in the Colne Catchment. Full details of the data analysis can be found in section 4.4.2.

6.1. Results

6.1.1. Intra-annual variation of water quality in the Rivers Colne and Ver

6.1.1.2. Intra-annual variation of water chemistry variables

In the 2023/24 season, phosphate levels increased across all sites, peaking at 0.558 mg/l PO_4^{3-} at the Drop Lane site. The lowest phosphate concentration was also recorded at Drop Lane during summer 2023/24, at 0.178 mg/l. However, despite these fluctuations, seasonal variation in phosphate levels was not statistically significant (ANOVA $F(3,25) = 1.95$, $p = 0.15$). The phosphate levels were also similar across the sites, particularly in the autumn and winter seasons (figure 6.1A) and there was no significant difference in phosphate levels among the sites (ANOVA: $F(3,25) = 2.11$, $p = 0.12$).

In contrast, the nitrate levels in the upper Colne Catchment significantly varied between the intra-annual seasons in the 2023/24 season (ANOVA $F(3,24) = 4.72$, $p=0.01$) and between the sites (ANOVA $F(2,24) = 8.52$, $p=0.0016$). At the Church Lane site on the River Colne the nitrate levels showed the greatest variation between the seasons ranging from 0.04 mg/l in Spring to 6.45 mg/l in autumn 2023 (figure 6.1. B). The London Colney site had the highest nitrate levels of 9.94 mg/l in autumn 2023. The post-hoc Tukey HSD test showed that the Ver site had significantly higher nitrate levels (5.24 ± 1.06 mg/l) than both the Church Lane site and Drop Lane site (table 6.2). The nitrate levels in the summer season were significantly lower than autumn while the winter season had higher levels (table 6.2).

The ammonium concentrations exhibited at Drop Lane were consistently the highest, peaking in autumn at 0.298 mg/l (figure 6.1C). Meanwhile, the Ver site maintained the lowest ammonium levels, with a seasonal mean of $0.0272 \text{ mg/l} \pm 0.017$. This was reflected in the significant difference found in ammonium levels between the four sites (ANOVA $F(3,24) = 7.99$, $p = 0.00073$). A post-hoc Tukey HSD test revealed that ammonium concentrations at the Ver site were significantly lower than those at the other Upper Colne sites (Church Lane, London Colney, and Drop Lane) (Table 6.2). The ammonium levels were comparable across the seasons (0.07 ± 0.06 mg/l) but the

levels appeared to increase slightly throughout the seasons, with higher levels in winter (0.08 ± 0.07 mg/l), compared to summer (0.07 ± 0.05 mg/l) (Figure 6.1C). However, there were no significant seasonal differences (ANOVA $F(2,24) = 0.026$, $p = 0.97$) (Table 6.1).

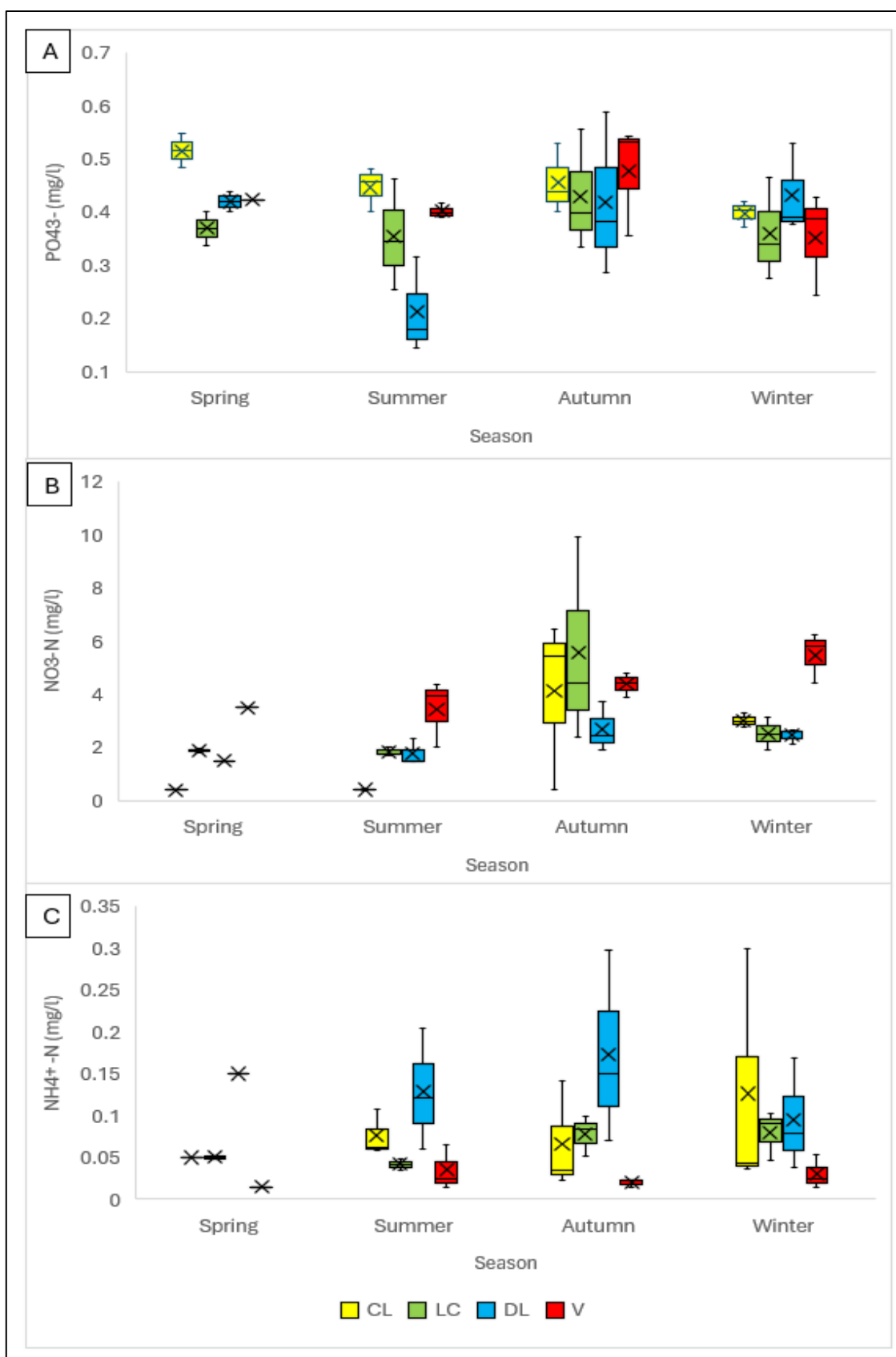


Figure 6. 1: The range of the water chemistry variables in the Rivers Colne and Ver in 2023/24 season: (A) phosphate levels, (B) nitrate levels and (C) ammonium levels. The crosses indicate the mean average and any dots represent the outliers.

Table 6. 1: Two-Way ANOVA results for the water chemistry parameters in the Upper Colne Catchment in the 2023/24 and 2024/25 seasons.

Year	Variable	Independent Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
2023/24	Nitrate	Site	3	2.46	0.82	4.72	0.01001
		Season	2	2.96	1.48	8.52	0.0016
		Residuals	24	4.17	0.17		
	Phosphate	Site	3	0.06	0.02	2.11	0.12373
		Season	3	0.05	0.02	1.95	0.14783
		Residuals	25	0.23	0.01		
	Ammonia	Site	3	10.44	3.48	7.99	0.00073
		Season	2	0.02	0.01	0.03	0.97371
		Residuals	24	10.45	0.44		
2024/25	Nitrate	Site	3	8.68	2.89	5.42	0.00544
		Season	3	0.39	0.13	0.24	0.86481
		Residuals	24	12.81	0.53		

Table 6. 2: Post-Hoc Tukey HSD results for the water chemistry parameters in the Upper Colne Catchment in the 2023/24 and 2024/25 seasons.

Season	Water Chemistry Variable	Significant Difference	Difference	Lower	Upper	P-Value
2023/24	Nitrate Level	V-CL	0.68	0.14	1.22	0.01029
		V-DL	0.58	0.04	1.12	0.03212
		Summer-Autumn	-0.67	-1.09	-0.24	0.00173
		Winter-Summer	0.52	0.09	0.94	0.01483
	Ammonia Level	V-CL	-0.97	-1.83	-0.11	0.02255
		V-DL	-1.50	-2.35	-0.64	0.00037
		V-LC	-0.92	-1.78	-0.06	0.32739
2024/25	Nitrate Level	V-CL	1.23	0.37	2.09	0.00316

In the 2024/25 season, there was little variation in the phosphate level across the sites (figure 6.2A) and there was no significant difference in the phosphate level across the sites (Kruskal-Wallis: $H(3) = 2.32$, $p = 0.51$) (table 6.3). The phosphate level decreased slightly throughout the seasons, with the highest average phosphate level in summer (0.47 ± 0.03 mg/l) and the lowest in winter (0.42 ± 0.06 mg/l). However, there was also no significant difference across the seasons (Kruskal-Wallis: $H(3) = 7.42$, $p = 0.06$). The nitrate levels were greatest in autumn (4.69 ± 2.68 mg/l) in the 2024/25 season, but the other seasons were comparable (figure 6.2B). There was no significant difference in the nitrate levels across the intra-annual seasons (ANOVA: $F(3,24) = 0.24$, $p = 0.86$) (table 6.1). The Ver site had a higher average nitrate level (5.23 ± 1.07 mg/l) compared to the other sites and CL had the lowest average level (2.63 ± 2.80 mg/l), with the exception of autumn (figure 6.2B). There was a significant difference in the nitrate levels in the 2024/25 season across the sites (ANOVA: $F(3,24)$

= 5.42, $p = 0.005$). The Tukey HSD test showed that the Ver site had significantly higher nitrate levels than the CL site ($p = 0.003$) (table 6.2). The ammonium levels in the 2024/25 season were greatest in spring (0.17 ± 0.18 mg/l) and decreased throughout the year and were the lowest in autumn (0.03 ± 0.02 mg/l). However, the ammonium levels were comparable across the sites, although CL and LC had a larger range in spring than other seasons. Therefore, there was no significant difference in ammonium levels across the sites (Kruskal-Wallis: $H(3) = 6.52$, $p = 0.09$) or the intra-annual seasons (Kruskal-Wallis: $H(3) = 3.77$, $p = 0.29$) in the 2024/25 season (table 6.3).

Table 6. 3: *The Kruskal-Wallis results for the non-parametric water chemistry variables in the Upper Colne Catchment in the 2024/25 season.*

Year	Nutrient	Independent Variable	Chi-Squared	df	P-Value
2024/25	Phosphate	Site	2.32	3	0.5092
		Season	7.42	3	0.05966
	Ammonium	Site	6.52	3	0.08901
		Season	3.77	3	0.2871

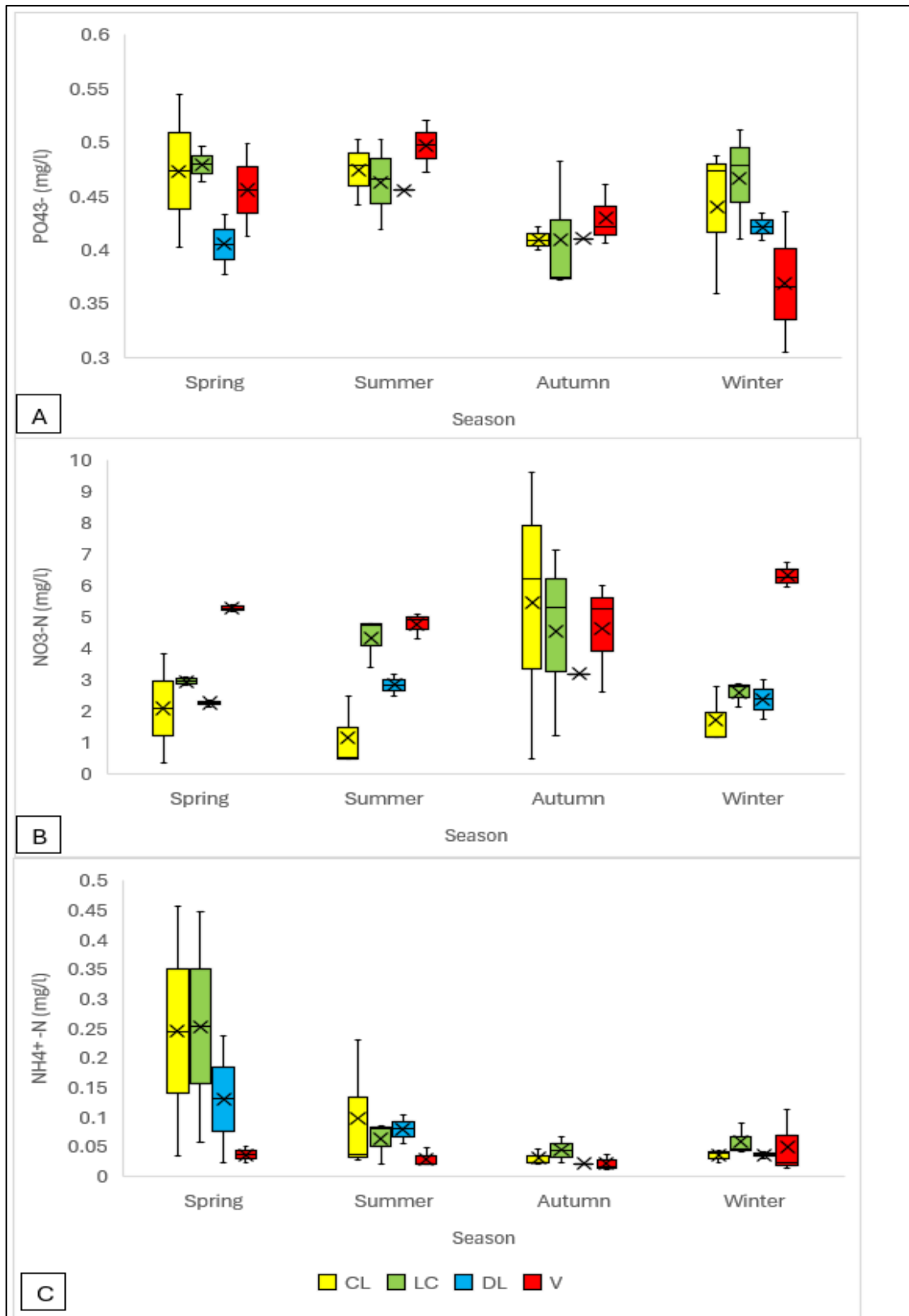


Figure 6. 2: The range of the water chemistry variables in the Rivers Colne and Ver in 2024/25 season: (A) phosphate levels, (B) nitrate levels and (C) ammonium levels. The crosses indicate the mean average, and any dots represent the outliers.

6.2.1.2. Intra-annual variation of physical-chemical variables

The water pH in the Rivers Colne and Ver increased across the seasons (spring to winter) in the 2023/24 season (figure 6.3A). The intra-annual season had a significant effect on the pH variation in the river water (ANOVA: $F(3,24) = 9.31$, $p = 0.0002$) in the Upper Colne Catchment (table 6.4). The post-hoc Tukey HSD test showed that there was a significant difference in pH between spring and all other seasons (table 6.5). The lowest or most acidic pH (5.4) was recorded at Church Lane in spring and the most alkaline pH (10.28) was at Church Lane in winter at 10.28 (Figure 6.3A). However, the reading of 5.4 is exceptionally low and is likely a measurement error as chalk streams range between 7 and 9. However, the average mean pH was similar across the sites (7.58 ± 1.05) and there was no significant difference in the pH of the water across the sites (ANOVA: $F(3,24) = 9.31$, $p = 0.93$).

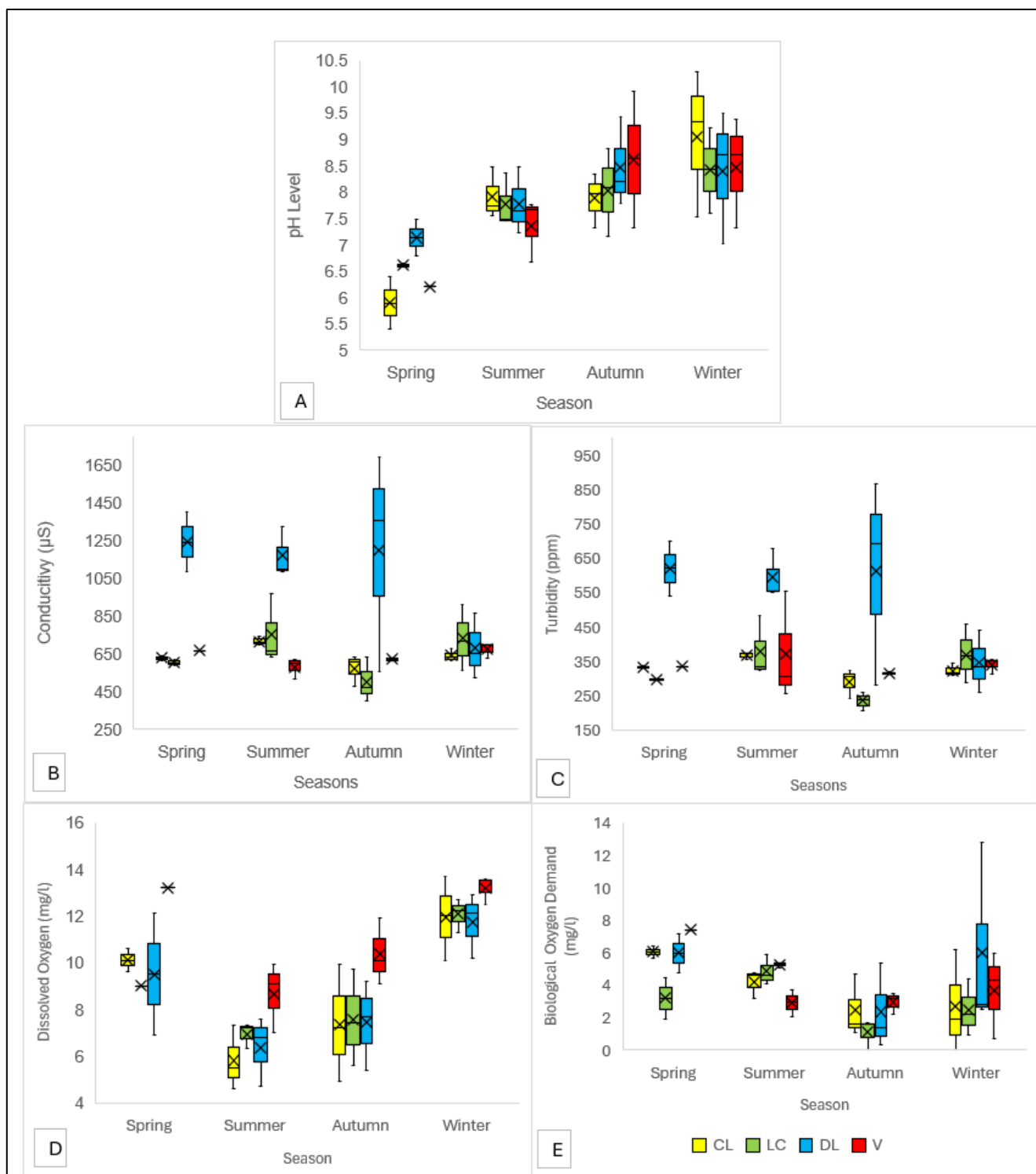


Figure 6. 3:The range of the physicochemical variables in the Rivers Colne and Ver in 2023 - 2024. A is the pH levels, B is the electrical conductivity, C is the turbidity levels, D is the dissolved oxygen level and E is the biological oxygen demand. The crosses indicate the mean average, and any dots represent the outliers.

Table 6. 4: Two-Way ANOVA on 2023/24 and 2024/25 seasons for physical-chemical variables

Year	Physical-Chemical Variable	Independent Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
2023/24	pH	Site	3	0.33	0.11	0.14	0.93348
		Season	3	21.34	7.11	9.31	0.00022
		Residuals	27	20.62	0.76		
	DO	Site	3	38.85	12.95	4.87	0.00779
		Season	3	192.21	64.07	24.10	8.53E-08
		Residuals	27	71.79	2.66		
2024/25	DO	Site	3	0.32	0.11	0.59	0.62518
		Season	3	0.93	0.31	1.72	0.19041
		Residuals	24	4.34	0.18		

Table 6. 5: Post-Hoc Tukey HSD test on physical-chemical variables for 2023/24 and 2024/25 seasons.

Season	Physical-Chemical Variable	Significant Difference	Difference	Lower	Upper	P-Value
2023/24	pH	Spring - Autumn	-1.74	-2.88	-0.61	0.001413
		Summer-Spring	1.20	0.07	2.34	0.035248
		Winter--Spring	2.08	0.94	3.22	0.000166
	DO	V-CL	2.32	0.37	4.27	0.015272
		V-DL	2.30	0.35	4.25	0.016234
		V-LC	2.11	0.16	4.06	0.030394
		Winter-Autumn	4.06	2.24	5.88	9.40E-06
		Summer-Spring	-3.36	-5.48	-1.23	0.001006
		Winter-Summer	5.29	3.47	7.11	1.00E-07

The Drop Lane site had the highest electrical conductivity (EC) levels ranging between 522 to 1694 μS and the highest turbidity levels throughout the season ranging between 261 to 865 ppm in the 2023/24 season (figure 6.3BC). The average EC level was ($636.91 \mu\text{S} \pm 103.25$) and the average turbidity level was ($321.14 \text{ ppm} \pm 52.90$). The EC levels were similar across the CL, LC and V sites and there were higher levels at DL (figure 6.3B) and this difference amongst the sites was significant in the 2023/24 season (Kruskal-Wallis: $H(3) = 9.63, p = 0.02$) (table 6.6). The EC levels were similar across the seasons and were not significantly different in the 2023/24 season (Kruskal-Wallis: 2023/24 $H(3) = 5.56, p = 0.13$). Turbidity also had a similar pattern to EC, where the levels at CL, LC and V were comparable and DL had higher turbidity levels and there was a statistically significant difference in turbidity levels in the 2023/24 season (Kruskal-Wallis: $H(3) = 9.65, p = 0.002$). However, there was not much variation between the seasons and this difference was not significant (Kruskal-Wallis: $H(3) = 5.49, p = 0.14$) (table 6.6). However, the post hoc test showed that both the EC and turbidity levels were significantly lower at the DL site compared to the LC site (EC: $p=0.032$; turbidity: $p = 0.02$), and there was no significant difference in the CL and V sites.

Both the levels of dissolved oxygen (DO) and biological oxygen demand (BOD) varied between the sites and seasons in 2023/24 (figure 6.3DE). The largest variation in BOD occurred at Drop Lane in winter, ranging from 10.2 to 12.9 mg/l although the mean was negatively skewed ($6.03 \text{ mg/l} \pm 4.79$). The BOD level was highest at the Ver site in spring, autumn and winter (figure 6.3E). There was a significant difference in the BOD across the sites (Kruskal-Wallis: ($H(3) = 27.55, p = 4.46\text{e-}06$) (table 6.6) but not amongst the seasons (Kruskal-Wallis: $H(3) = 3.049, p = 0.38$). The post-hoc Dunn Test showed that CL had significantly higher BOD levels to DL ($p = 1.24\text{e-}05$), LC ($p = 9.63\text{e-}05$) and V ($p = 0.029$) (table 6.7).

The DO level was similar across the sites in autumn 2023 averaging $8.18 \text{ mg/l} \pm 2.07$ (figure 6.3E). The highest DO level ($11.73 \text{ mg/l} \pm 1.13$) was recorded in winter 2023/24 (figure 6.3E). There was a significant difference in the DO levels amongst the sites (ANOVA: $F(3,9) = 4.87$, $p = 0.0079$) and seasons (ANOVA: $F(3,9) = 24.098$, $p = 8.53\text{e-}08$) (table 6.4). The Tukey HSD test (table 6.5) showed a significant difference in DO levels between the Ver site and the other three sites (CL $p = 0.015$, LC $p = 0.030$, DL $p = 0.016$) where the DO level was higher than the other sites. The DO levels in winter were also significantly higher to both autumn ($p = 0.0000094$) and summer ($p = 0.0000001$) while summer DO levels were significantly lower to spring ($p = 0.001$).

Table 6. 6: *Kruskall-Wallis test for non-parametric physical-chemical variables 2023/24 and 2024/25 seasons.*

Year	Physical-Chemical Variable	Independent Variable	Chi-Squared	df	P-Value
2023/24	EC	Site	9.63	3	0.02197
		Season	5.56	3	0.1351
	Turbidity	Site	9.65	3	0.02181
		Season	5.50	3	0.1388
	BOD	Site	27.58	3	4.46E-06
		Season	3.05	3	0.3841
2024/25	pH	Site	4.28	3	0.2325
		Season	6.63	3	0.08444
	EC	Site	7.77	3	0.05108
		Season	1.08	3	0.7813
	Turbidity	Site	16.18	3	0.001043
		Season	1.13	3	0.7695
	BOD	Site	21.85	3	7.02E-05
		Season	3.08	3	0.3797

Table 6. 7: The post-hoc Dunn test on the significant variables from the Kruskal-Wallis test on the physical-chemical variables in the 2023/24 and 2024/25 season.

Year	Physical-Chemical Variable	Independent	z	P unadj	P value
2023/24	EC	DL - LC	2.78	0.01	0.032125
	Turbidity	DL - LC	2.83	0.00	0.02766
	BOD	CL - DL	4.75	0.00	1.24E-05
		CL - LC	4.31	0.00	9.63E-05
		DL - LC	-0.43	0.66	1
		CL - V	2.82	0.00	0.02911
		DL - V	-1.82	0.07	0.416534
		LC - V	-1.39	0.16	0.981666
2024/25	Turbidity	CL - DL	-3.72	0.00	0.0012
		CL - LC	-0.38	0.70	1
		DL - LC	3.38	0.00	0.004331
		CL - V	-0.65	0.52	1
		DL - V	3.15	0.00	0.009865
		LC - V	-0.26	0.79	1
	BOD	CL - DL	3.96	0.00	0.000451
		CL - LC	3.75	0.00	0.001065
		DL - LC	-0.65	0.51	1
		CL - V	3.41	0.00	0.003875
		DL - V	-0.95	0.34	1
		LC - V	-0.34	0.74	1

In the 2024/25 season, the Church Lane site experienced the largest variation in pH level ranging from 7.51 to 11.96 across the seasons (figure 6.4A). However, there was no significant difference in pH across the sites (Kruskal-Wallis: $H(3) = 4.28$, $p = 0.235$) or season ($H(3) = 6.62$, $p = 0.084$) (table 6.5). The EC at Drop Lane was relatively higher than the other sites in summer, autumn and winter, where the mean was $1232.8 \mu S \pm 371.21$, compared to the other sites (figure 6.4B). However, electrical conductivity was not significantly different between the sites ($H(3) = 7.77$, $p = 0.051$) nor the seasons ($H(3) = 1.08$, $p = 0.78$) which is reflected in figure 3.4B. Similarly, turbidity was higher in spring, summer and winter (figure 6.4C) with the highest turbidity level recorded in winter (1060 ppm). There was a significant difference in turbidity amongst the sites (Kruskal-Wallis: $H(3) = 16.17$, $p = 0.001$) but not between the seasons ($H(3) = 1.13$, $p = 0.769$) (table 6.6). The post-hoc test showed that DL had significantly higher turbidity levels compared to the other three sites and (CL $p = 0.0012$, LC $p = 0.0043$, V $p = 0.0098$) (table 6.7).

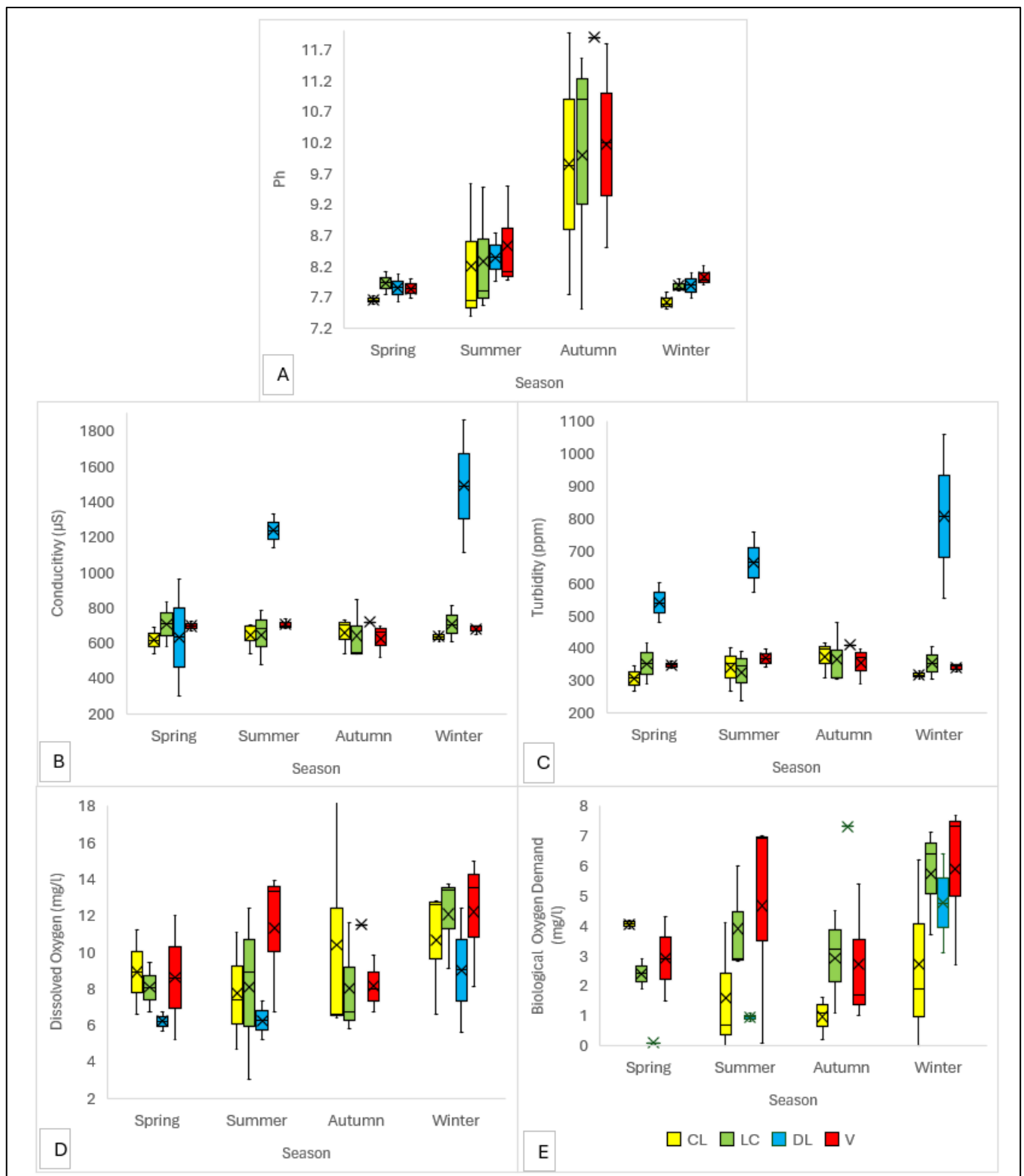


Figure 6. 4: The range of the physical chemical variables pH (A), conductivity (B), turbidity (C), dissolved oxygen (D) and biological oxygen demand level (E) in the 2024/2025 season. The crosses indicate the mean average and any dots represent the outliers.

The dissolved oxygen level was similar across the seasons, although CL had a large range in autumn 2024 (6.4 to 18.2mg/l) (figure 6.6D). The mean average of the DO at the Ver site ($10.2 \text{ mg/l} \pm 3.29$) was higher than the other sites although there was not a significant difference in DO across the sites (ANOVA: $F(3,24) = 0.594$, $p = 0.625$) or the seasons (ANOVA: $F(3,24) = 1.716$, $p = 0.190$). The BOD was significantly different across the sites (Kruskal-Wallis: $H(3) = 21.84$, $p = 7.02 \times 10^{-5}$), with the highest level at the Ver (figure 6.6E). The post hoc test showed that the CL site had a significantly higher BOD compared to DL ($p = 0.000451$), LC ($p = 0.0011$) and V ($p = 0.0039$) (table 6.7).

6.2.2. Inter-annual variation of the water chemistry and physical-chemical variables

The phosphate levels were significantly different between the 2023/24 and 2024/25 seasons (Wilcoxon: $W = 509$, $p = 0.005$) (table 6.8) with higher levels observed in the 2023/24 season (figure 6.5A). Phosphate had its largest range at the DL site in 2023/24 (0.145 – 0.455 mg/l) whereas the average phosphate level ($0.441 \text{ mg/l} \pm 0.051$) was more consistent across the sites in 2024/25. The nitrate levels were consistent at the different sites between the 2023/24 and 2024/25 seasons (figure 6.5B) and therefore there was not a significant difference across the seasons (Wilcoxon: $W = 599$, $p = 0.21$). This was also the case for the ammonium levels which did not have a significant difference between the 2023/24 and 2024/25 seasons (Wilcoxon: $W = 904$, $p = 0.06$) (table 6.8), although the DL site did have a higher mean in 2023/24 compared to 2024/25 (figure 6.5C).

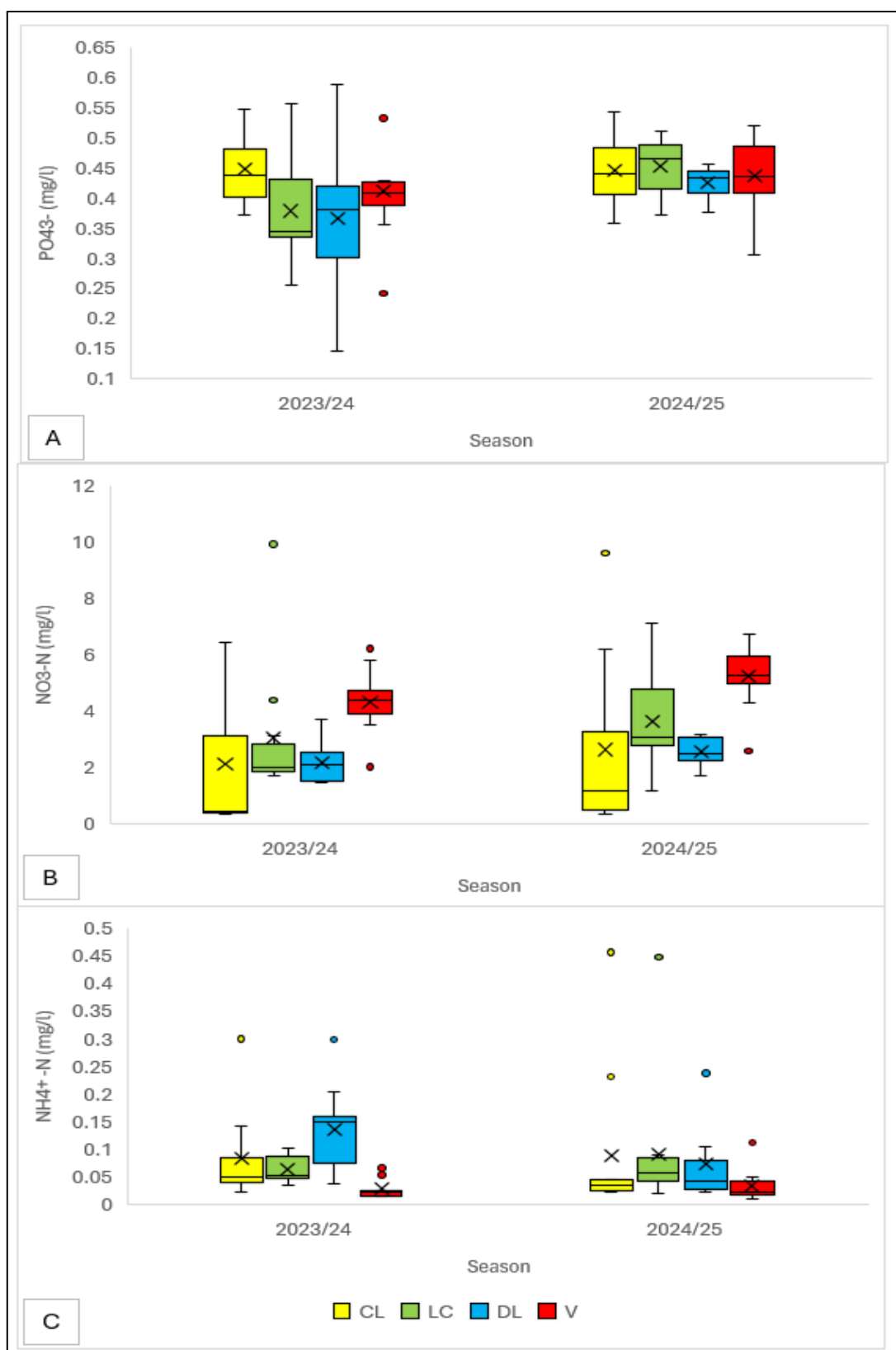


Figure 6. 5: The range of the water chemistry variables in the Rivers Colne and Ver between the two seasons (2023/24 and 2024/25). A is the phosphate levels, B is the nitrate levels and C is the ammonium levels. The crosses indicate the mean average and any dots represent the outliers.

Table 6. 8: *The Wilcoxon Signed Rank Test for the comparison of the two inter-annual seasons.*

Variable	W	p-value
Phosphate	509	0.005
Nitrate	599	0.21
Ammonium	904	0.06
pH	616	0.026
EC	767	0.4
Turbidity	691.5	0.17
DO	894	0.76
BOD	764	0.384

pH was slightly lower in the 2023/24 season (7.91 ± 1.04) compared to the 2024/25 season (8.56 ± 1.34) season and there was a significant difference across the seasons (Wilcoxon: $W = 616$, $p = 0.03$) (table 6.8). The pH levels of the river water were slightly lower in 2023/24 compared to 2024/25 (figure 6.6A). The EC levels were consistent across the 2023/24 and 2024/25 seasons with higher levels at DL compared to the other sites (figure 6.6B). The range in EC was slightly lower at the DL site in 2024/25 (301 – 757 ppm) (figure 6.6B). However, there was not a significant difference across the 2023/24 and 2024/25 seasons (Wilcoxon: $W = 767$, $p = 0.4$). Overall, the turbidity levels were consistent across the 2023/24 and 2024/25 seasons, with higher turbidity levels at the DL site compared to the other sites (figure 6.6C). The range in turbidity was smaller at the DL site in 2024/25 (480 – 1060ppm) compared to 2023/24 (261 – 865 ppm) , although the mean turbidity at DL was consistent across both seasons (2023/24: 536.18 ± 183.59 ppm ;2024/25: 633 ± 200.91 ppm) and there was not a significant difference between the 2023/24 and 2024/25 seasons (Wilcoxon: $W = 691.5$, $p = 0.13$). The DO levels were consistent across the 2023/24 seasons (figure 6.6D). CL had a larger range in DO in the 2024/25 season (4.7 – 18.2 mg/l) and the mean DO at DL was lower in 2024/25 (7.77 ± 2.73 mg/l) than 2023/24 (8.69 ± 2.68 mg/l). The mean DO levels at the Ver were the highest across the sites in the 2023/24

(10.99 ± 2.15 mg/l) and 2024/24 (10.2 ± 3.29 mg/l) seasons but the range was larger in 2024/25 (7.0 – 13.6 mg/l) (figure 6.6D). However, there was no significant difference between the seasons (Wilcoxon: $W = 894$, $p = 0.76$). The BOD levels were mostly consistent between the seasons. The Ver site had the largest range (0.1 – 7.7 mg/l) in the 2024/25 season, but overall, the ranges at each site varied more in 2024/25. The DL site had the highest mean in 2023/24 (4.82 ± 3.19 mg/l) and the lowest mean in 2024/25 (2.7 ± 2.79 mg/l). However, there was not a significant difference in BOD levels between the 2023/24 and 2024/25 season (Wilcoxon: $W = 764$, $p = 0.38$).

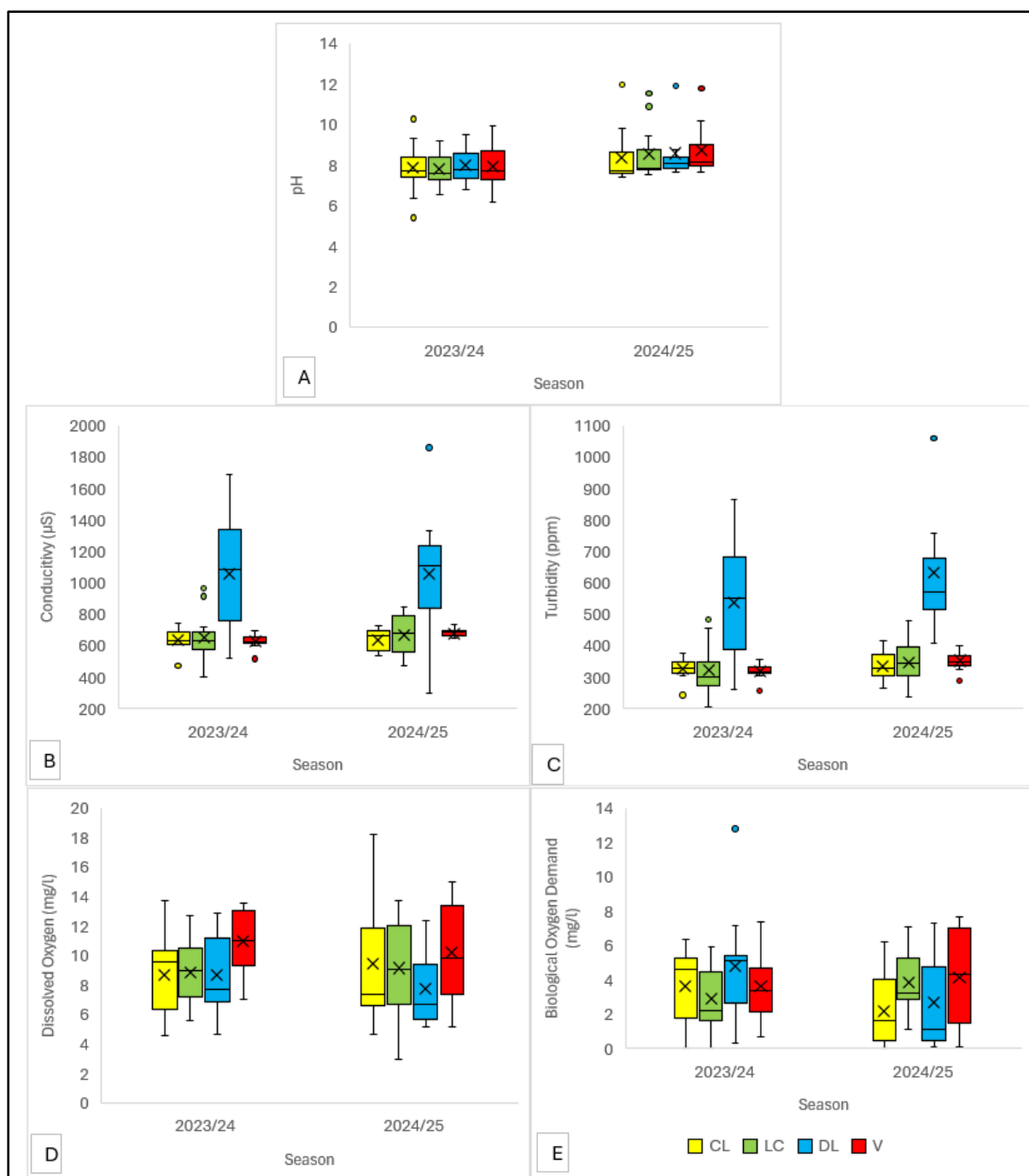


Figure 6. 6: The range of the physical-chemical variables in the Rivers Colne and Ver between the two seasons (2023/24 and 2024/25). A is the phosphate levels, B is the nitrate levels and C is the ammonium levels. The crosses indicate the mean average and any dots represent the outliers.

6.2.3. Patterns and relations between water chemistry and physical-chemical variables

In the 2023/24 season, Principal Component Axis 1 (PCA 1) and the Principal Component Axis 2 (PCA 2) together accounted for 56.8% of the total variance in the water chemistry, physical-chemical and hydrological variables (figure 6.7 A). The PCA 1 explained 38.4% of the variance and was strongly positively related to the water temperature, air temperature and negatively related to the amount of rainfall, total river discharge and the depth of the river channel. The amount of daily rainfall and the average channel depth were significantly positively correlated (Pearson $r = 0.37$ and $p < 0.05$) and had a large negative effect on PCA 1. PCA 2 explained 18.4% of the variance in the dataset and was strongly negatively related to the dissolved oxygen (DO) level in the water and Biological Oxygen Demand (BOD), and then negatively related to the ammonia level, electrical conductivity (EC) and turbidity of the water in the Colne Catchment (Figure 6.7A). The pH level had a medium negative effect on both PCA 1 and PCA 2 and was highly significantly correlated to water temperature, but the relationship was weak ($p = 0.021$, Pearson $r = -0.33$) (Figure 6.8). The nitrate levels were significantly and positively correlated to DO ($p = 0.020$, Pearson $r = 0.38$) and significantly negatively correlated to conductivity ($P = 0.041$, Pearson $r = -0.34$) and turbidity ($p = 0.043$, Pearson $r = -0.34$) (Figure 6.8.). DO also had a significant positive correlation to several hydrological parameters such as width ($p = 0.0042$, Pearson $r = 0.43$) average depth ($p = 0.042$, Pearson $r = 0.49$), average flow ($p = 0.028$, Pearson $r = 0.34$), daily rainfall ($p = 0.0006$, Pearson $r = 0.50$), channel area ($p = 0.00078$, Pearson $r = 0.49$), total discharge ($p = 0.013$, Pearson $r = 0.37$) (Figure 6.8.). However, the phosphate levels had a small effect on PCA 1, but a larger effect on PCA 2, but did not have any significant correlations to the other chemical, physiochemical or hydrological variables. Site had a small effect on both PCA 1 and PCA 2 in the 2023/24 season.

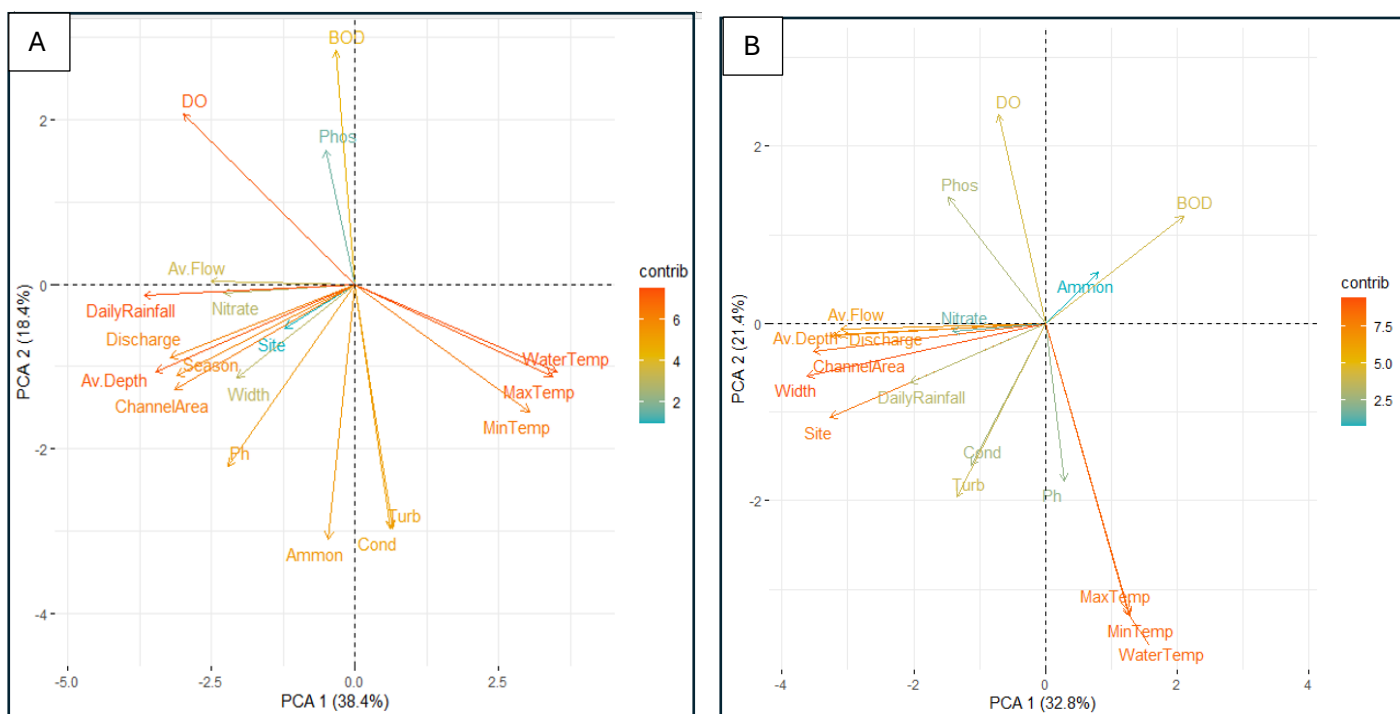


Figure 6. 7: The Principal Component Analysis of the two datasets to show the amount of variance the water chemistry, physiochemical and hydrological variables explained. A is the 2023/24 season and B is the 2024/25 season. In 2023/24 PCA 1 and PCA 2 explain 56.8% of the variance and in 2024/25 PCA 1 and PCA 2 explain 54.2% of the variance. The contribution legend indicates how much of an effect the variables have on the PCA 1 or PCA 2 and the axis describe whether it is a positive or a negative effect.

In the 2024/25 season the PCA showed similar relations in the variation of dissolved oxygen, phosphate level, average depth, discharge, average flow and site, to the 2023/24 season (Figure 6.7B). However, the PCA 1 and PCA 2 together explained only 54.2% of the variation which was less than the 2023/24 season. The PCA 1 explained 32.8% of the variance and was strongly positively related to the minimum and maximum air temperatures and water temperatures and negatively related to the channel width and average channel depth. PCA 2 explained 21.4% of the variance and was strongly negatively related to dissolved oxygen and phosphate level and positively related to the air and water temperatures (Figure 6.7B). Phosphate levels were not significantly correlated to other parameters. DO was significantly negatively correlated to turbidity ($P=0.044$, Pearson $r = -0.31$) and water temperature ($P=0.0097$, Pearson $r = -0.38$) and positively correlated to nitrate levels ($P<0.0036$, Pearson

$r=0.42$) (Figure 6.9.). Both pH level and ammonia had a small positive contribution to PCA 1 and a small negative contribution to PCA 2 compared to the 2023/24 season. The water pH was significantly and positively correlated to water temperature ($P=0.021$, Pearson $r=0.36$), and both maximum air temperature ($P=0.049$, Pearson $r=0.32265$) and minimum air temperature ($P=0.014$, Pearson $r=0.39$), as well as to nitrate levels ($P=0.006$, Pearson $r=0.41$). Season had a large positive effect on PCA 2 whereas it was a lesser negative relation in the 2023/24 season. The nitrate levels were less related to PCA 1 and PCA 2 compared to the season 2023/24.

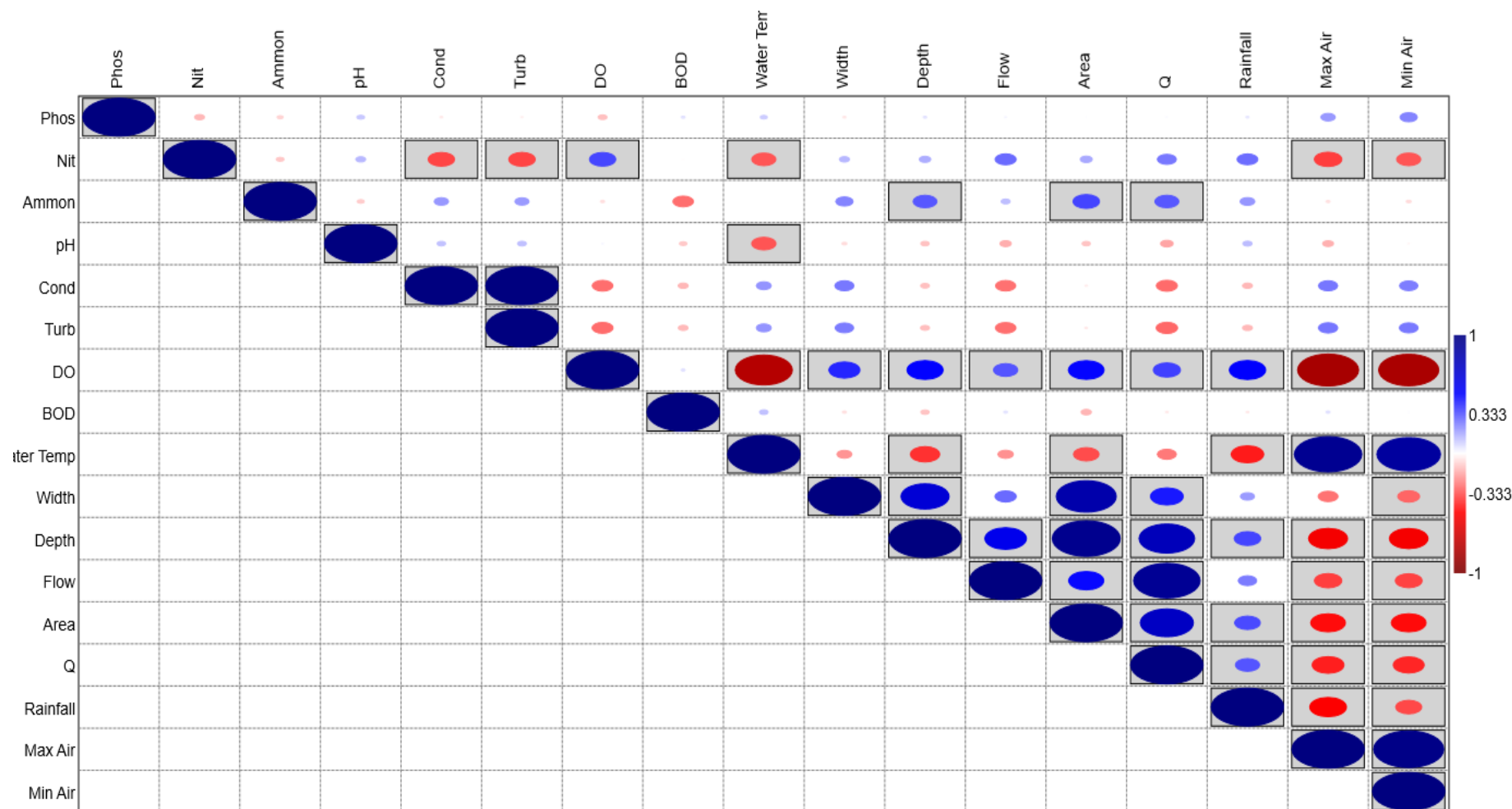


Figure 6. 8:The graphical output from the Past4 software depicting the correlation between the 17 water chemistry, physiochemical and hydrological variables in the 2023/24 season. The larger the circle, the higher the multicollinearity between the two variables, and if there is a grey box that indicates they are significantly correlated ($P < 0.05$). The colour grading represents the direction of the relationship i.e. red is a negative correlation and blue is a positive correlation and the darker the colour, the closer the Pearson's r value is to 1.00 or -1.00

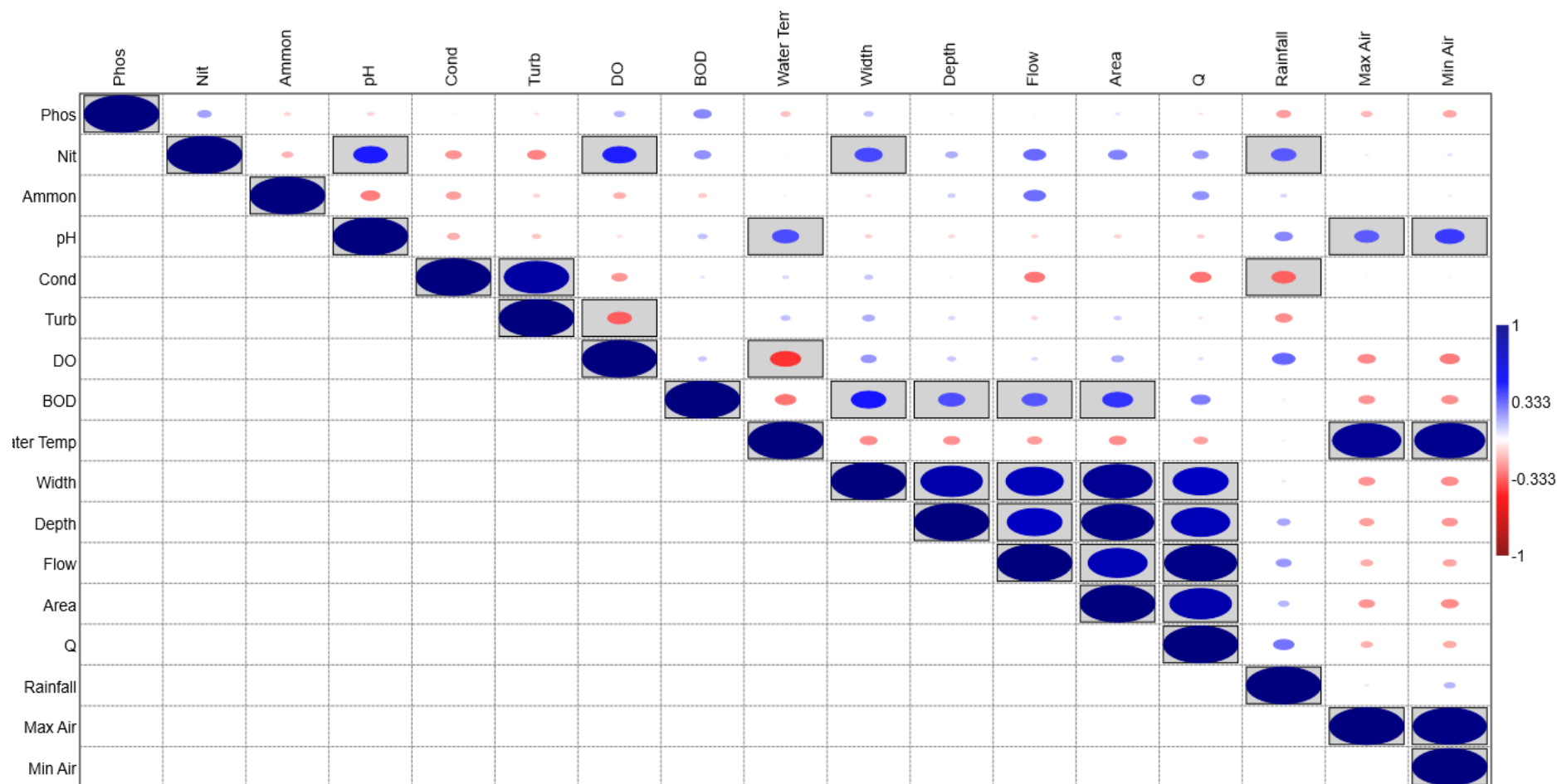


Figure 6. 9: The graphical output from the Past4 software depicting the correlation between the 17-water chemistry, physiochemical and hydrological variables in the 2024/25 season. The larger the circle, the higher the multicollinearity between the two variables, and if there is a grey box that indicates they are significantly correlated ($P < 0.05$). The colour grading represents the direction of the relationship i.e. red is a negative correlation and blue is a positive correlation and the darker the colour, the closer the Pearson's r value is to 1.00 or -1.00.

6.3. Discussion

6.3.1. Variation in Water Nutrient Levels in the Upper Colne Catchment

All the sites in the Colne Catchment displayed high levels of the three nutrients at some stage during the study, which indicates that this chalk stream is experiencing high levels of nutrient inputs and water pollution, which is particularly degrading for chalk streams (Pokaine *et al.*, 2019). The nitrate levels had the most variability out of the three water nutrients across both the sites and the seasons. In the 2023/24 season, there was a significant difference between both the sites, where the nitrate levels were higher at the Ver site than the CL and DL sites further upstream. Despite the V and DL sites' close proximity, the difference in nitrate levels must be influenced by factors within the river water, rather than the immediate land use (which is pastoral farming with cattle in the adjacent fields). The nitrate levels were also higher in the autumn and winter than in the summer, which means that despite higher river discharges, there was little dilution effect on the nitrate levels (Yates & Jones, 2013). However, in the second inter-annual season, the nitrate levels were similar across all seasons, except for in autumn where it was higher once again. This could be from point sources of nitrates, such as fertilisers being applied during this time period, or from harvesting disturbing the soil, and an increase in surface runoff leaching the nitrates into the watercourse (Yates & Johnes, 2013). Again, the Ver had the highest nitrate level, and this site was the furthest downstream, which suggests that inputs from upstream sources could become more concentrated further downstream with additional inputs. The average nitrate level across the four sites for both inter-annual seasons was 14.35 ± 9.04 mg/l which is below both the 50mg/l WFD limit and the 30mg/l 95 percentile limit of the EC Drinking Water Directive which is important to consider as the Upper Colne Catchment is in a Drinking Water Safeguard Zone (Defra, 2012; EA, 2025b). However, when the nitrate level peaked at LC in 2023/24 and CL in 2024/25 both were above the 30mg/l limit. Contrastingly, despite the Ver site having the highest average nitrate levels of 4.81 ± 1.17 mg/l NO₃-N (or 21.31 ± 5.18 mg/l nitrate) among the sites, its levels were below both of the annual nitrate limits. The WFD recommends no more than 5-6 mg/l of nitrate-N to achieve a good ecological status, which is greater than the nutrient tolerance of most chalk stream flora and fauna, which require only 2mg/l

Nitrate N (Cambridge City Council, 2025; Defra, 2012). Therefore, the nitrate levels within the Upper Colne catchment are not of immediate concern, despite occasional spikes, but these levels are currently manageable.

The phosphate levels in the upper Colne were relatively stable across the four sites and the four intra-annual seasons. The average phosphate levels for the two seasons were $0.13 \text{ mg/l} \pm 0.03$ which was above the government guidance for mean annual concentrations of 0.01 mg/l of phosphate (Defra, 2012). The phosphate-P levels should not exceed 0.1 mg/l for a good ecological status according to the WFD but chalk streams specifically have limits of 0.05 mg/l (Natural England, 2009). There was a minimal decrease throughout intra-annual seasons the 2024/25 season, with higher levels in summer than in winter although this was not found to be significant.. Therefore, the lack of variation in the phosphate levels suggests that there were not any specific point sources of this nutrient and that the levels remained consistent throughout the Upper Colne. This suggests that the phosphate was also coming from the agricultural land surrounding the Upper Colne catchment as 26% of phosphate levels in English water courses arise from agricultural sources (Defra, 2012). The phosphate levels were significantly higher in the second season and the highest phosphate level recorded was in autumn in 2023/24 at DL at 0.19 mg/l ($0.59 \text{ mg/l PO}_4^{3-}$) and in the 2024/25 season it was highest in spring at CL site at 0.17 mg/l (0.544 PO_4^{3-}) which is higher than that 0.1 mg/l limit (Defra, 2012) and also above the limit for chalk stream headwaters of 0.04 mg/l (Natural England, 2009). This shows that the nutrient limit was exceeded both up and downstream on the river Colne's course. This suggested that there is a limited dilution effect from surface water and that the input of phosphate is constant throughout the seasons. Interestingly, sewage treatment works are a source of phosphates, and can enter the water via binding to soil particles, which would have occurred on the agricultural land adjacent to the river channel (Maidment & Fones, 2023). This high phosphate level could lead to vigorous and excessive algal growth, which in turn would lower oxygen concentrations and cause the process of eutrophication to occur (Defra, 2012; Maidment & Fones, 2023). Therefore, it is crucial to continue to monitor the levels of phosphate within the Upper Colne, as if the levels are exceeded upstream, this issue will only be compounded by additional inputs from sewage treatment works further downstream on the river network (Maidment & Fones, 2023).

The ammonium levels were significantly lower at the Ver site in the 2023/24 season compared to the other sites, but in the 2024/25 season the levels at the Ver were comparable to the other three sites. This suggests that there may have been a point influence on the Ver site that was then removed in the 2024/25 season. The DL site had the highest level of ammonium present, which suggested that the difference between the sites was not based on a diffuse source from the surrounding area, as again the V and DL sites were in the closest proximity. The ammonium levels were similar across the seasons in 2023/24, but had a peak in spring of 2024/25 at CL, LC and DL, but not at the V site. However, the levels decreased to the average range for the remaining season in 2024/25 which meant that there was not much of a response to the changing climate or rainfall events, and the changes were more local to each site. Ammonia levels NH₃-N are required to be less than 0.05mg/l for a healthy chalk stream system (Natural England, 2009; Cambridge City Council, 2025) and in this study the ammonium levels were below this threshold. Therefore, ammonium levels are not of an immediate concern in the Upper Colne Catchment, unlike further downstream.

To summarise, nitrate levels had the largest variation between the intra-annual seasons and among the sites, ammonium levels did show some variation between the sites and phosphate only showed intra-annual variation. This was similar to a previous study on the Winterbourne chalk stream in Dorset which showed that nutrient levels were found to be consistent across several sites, with only temporal changes occurring over decades, rather than within the year (Armitage & Bass, 2013). For example, nitrate levels increased between 1981 to 2010 and phosphate levels decreased over the same time period. This means that this study may not have been long enough for temporal differences in nutrient levels to be observed. However, the smaller nuanced changes make it important to monitor the variability of the nutrients within the Upper Colne Catchment, as the nutrients all respond to the riverine environment and external environment in different ways e.g. with increases in water temperature or discharge levels and rainfall patterns. This is especially the case in the Upper Colne Catchment, as there was already evidence of nutrient enrichment leading to accelerated algal growth, and reducing the productivity of aquatic plants such as *Ranunculus* sp. which macroinvertebrates require for feeding and refuge in chalk streams (Herts & Middlessex Wildlife Trust, 2023). Of particular importance to monitor are the

phosphate levels which did exceed the WFD limits on occasion. This could potentially exacerbate the achievement of nutrient limits further downstream, which will impact the overall water quality of these chalk streams (Defra, 2012). The WFD recommendations have been suggested not to be stringent enough to consider the unique needs of the chalk stream environment (Cambridge City Council, 2025). Furthermore, the water quality monitoring strategy for organic contaminants in the UK is based on spot water samples which only provides an estimate of the pollutants present and may miss stochastic events (Robinson *et al.*, 2024) which was also the case for the monthly measurements in this study. For most freshwater river systems in the UK, there is no information on the total nutrient flux from land to water and only partial results are available for catchments (Johnes, 2007). However, the information on the nutrient fluctuation in the Upper Colne from this study will add to the understanding of nutrient sources and cycling in the upper courses of chalk streams and can assist planning and mitigation efforts for the Upper Courses of chalk streams in southern England, particularly in high water demand locations.

6.3.2. Variation in the physical-chemical parameters in the Upper Colne Catchment

There was also some variation in the physical-chemical parameters within the riverine environment of the chalk stream. The pH of the water was similar across the sites, but the water became more alkaline in the autumn and winter in 2023/24 but in 2024/25 there was not a significant difference between the sites or seasons, although pH was slightly higher in 2024/25. The increase in pH is likely due to the influence of the chalk bedrock from the greater contribution of water from the chalk aquifer in winter times, when the water table is higher. However, this differed from previous research on another chalk stream (the River Itchen) where the pH was greater in summer at 8.6 than winter at 7.3 (Robinson *et al.*, 2024). This suggests that there may be other influences such as road runoff within the Upper Colne Catchment that may contribute to the lower pH level in summer. The pH levels of the chalk stream environment is important at determining the reactions that take place within the river water, such as how soluble nutrients are (Berrie, 1982; England *et al.*, 2019). Chalk streams are typically slightly alkaline which was the case in the Upper Colne, and an increase in acidity can increase the solubility of heavy metals (while the Upper Colne already experiences issues with) and can enhance ammonium toxicity (CVFC, 2022; Berrie,

1982; Mainstone *et al.*, 1999). Since ammonium levels were higher at the CL site, and this was shown to be related to pH levels, there seems to be a relationship there which could influence the water quality and ecology of the Upper course. Therefore, monitoring the alkalinity of the Upper Course of the Colne can help to predict its vulnerability to nutrient levels and other pollutants.

The electrical conductivity and turbidity levels were closely related and experienced similar patterns throughout the Upper Colne Catchment. Chalk streams typically have a very low turbidity <5 NTU (Nephelometric Turbidity Units) which allows the typical clear waters to flow and ensures enough sunlight to reach aquatic macrophytes for photosynthesis (Mondon *et al.*, 2021; Cambridge City Council, 2025). High turbidity levels can be caused by pollution, erosion and sediment disturbances and if the turbidity level is too high with an increase in suspended sediments, there can be a detrimental impact on the ecology. For example, an increase in the amount of silt within the watercourse can lead to siltation of the gravel bed which chalk streams struggle to flush out, impacting the invertebrates and fish populations that rely on these interstitial habitats for their life cycles (Natural England, 2009). In the Upper Colne catchment, the highest turbidity was experienced at the DL site, where just upstream was a dam formed of woody debris and fallen trees, and there was significant silt deposits over the gravel, which when disturbed by flow or other movement increased the turbidity. However, in shallower areas of the channel at this site, the water appeared clearer and flowed over clean gravel beds. Therefore, this site appeared to be a depositional pool with decreased flow. Heavy rainfall events would also lead to greater amounts of sediment being washed into the water course from the floodplain at CL and from downstream at DL. EC can act as a proxy for pollution based on dissolved ions in the water and is influenced by urban runoff, agricultural inputs, industrial discharges and can help to identify pollution sources (Mondon *et al.*, 2021; Heywood & Walling, 2003) e.g. from fertilisers, road salts and industry, and there was a spike in EC at the CL site during the winter months due to the salting of the residential road that bisects the River Colne at this site. Although the EC levels were consistent across the seasons, if there are greater variations in flow or discharge, the EC levels could become concentrated, as higher EC levels have been recorded in summer than in winter in chalk stream catchments in previous studies (Robinson *et al.*, 2024). This could also interact with other variables, such as altering the pH of the river, and leading to changes in solubility

and leaching of toxic metals and nutrients into water courses, as well as salt retention which could impact reproductive cycles of fauna (Szklairek, Górecka & Wojtal-Frankiewicz, 2022; Kelly *et al.*, 2025). Consistent monitoring can allow for measures to protect stream health to be implemented by understanding where they arise from.

The dissolved oxygen levels and biological oxygen demand did not have much variation across the intra-annual seasons. DO was significantly higher in winter than autumn, summer was lower than spring and winter. DO increased in spring and summer due to photosynthesis and fish farming and shallower depths had created a decline in DO levels (Robinson *et al.*, 2024). Water temperature also influences the amount of dissolved oxygen available and cooler water can hold more oxygen than warmer, which explains the difference between intra-annual seasons. The BOD levels were highest at CL compared to the LC, DL and V sites, and V had the highest DO out of the four sites. Chalk streams often have high levels of DO, particularly in areas where there is flow over exposed gravel beds in riffles, and the saturation is typically 85% which is important for many oxygen-demanding species, such as the macroinvertebrates, and fish species like brown trout and brook lamprey (Cambridge City Council, 2025, EA, 2014; Chapman, 1998). In unpolluted waters, DO levels are around 10mg/l and concentrations below 5mg/l may negatively affect biological communities and below 2mg/l fish populations begin to die (Chapman, 1998). If there are waste discharges or high nutrient levels, DO levels usually decline due to increased microbial activity and (EA, 2014). The sites with the higher BOD levels such as CL mean that there was activity occurring that required more oxygen demand. At the Ver site, there were higher nutrient levels of nitrates, phosphates and ammonium and this could increase microbial activity, increasing the BOD (Natural England, 2009; EA, 2014). This could also be due to the sewage treatment works and effluent discharges in the River Ver, compared to directly upstream of the Colne, where there are no STWs upstream of the three sites. At the DL site, there were higher turbidity and EC levels, which again could indicate more microbial activity.

6.3.3. Summary

Overall, this study has shown that the nutrient and physical-chemical dynamics within the Upper Colne Catchment can vary in response to both spatial and temporal factors and that these interactions reflect the complexity of the relations between water quality

variables and the in-river environment. Whilst the water nutrients were mostly within the regulatory thresholds for chalk streams, the periodic exceedances and sporadic increases in certain physical-chemical parameters such as pH and EC, suggest that there is a need for continuous, fine-scale monitoring, along with implementation of management strategies, to secure improvements in the water quality and move towards good water quality status. These management strategies should prioritise phosphorus and nitrate reduction, improve point source mitigation such as reducing effluent outfalls from STWs, and consider chalk stream-specific ecological requirements, which may not be fully addressed by current broader regulatory standards. However, with the Chalk streams bill having been recently rejected by parliament, this may take longer to achieve (Laville & Horton, 2025). Long-term, site-specific data will be essential to detect meaningful trends and inform sustainable water quality management in sensitive headwater systems like the Upper Colne.

Chapter 7: Macroinvertebrate taxa diversity and composition within the Upper Colne Catchment and its relations to environmental parameters

7.0. Introduction

Due to the inaccessibility and isolation of many chalk stream headwaters, taxa diversity at the landscape-scale (beta diversity) can be relatively high compared to other river types or further downstream of the rivers, even though their local-scale (alpha) diversity may be lower (Kabir *et al.*, 2024). The varying hydrological regimes of these headwaters (or winterbournes) can have a different aquatic macroinvertebrate community to the perennial reaches with more drought-tolerant species (Stubbington *et al.*, 2017). For example, specialist species such as the rare blackfly *Metacnephia amphora*, mayfly *Paraleptophlebia wernerii* and stonefly *Nemoura lacustris* were found in the winterbourne headwaters of a chalk stream and the stonefly species had only been recorded at 16 other sites, making it nationally rare (Bunting *et al.*, 2020; Macadam, 2015; Armitage & Bass, 2013). However, the biodiversity and conservational value of smaller waterbodies such as the upper courses, has only recently been recognised and many are not characterised (Kabir *et al.*, 2024; Stubbington *et al.*, 2017). The macroinvertebrate communities of low-gradient winterbourne headwaters of the major chalk rivers in the south of England have been characterised, but the biodiversity within the headwaters has been relatively unknown, which means that there is still the opportunity to investigate how site-specific environmental characteristics influence headwater biodiversity (Kabir *et al.*, 2024).

There are many measures to measure the diversity of a habitat. The Shannon Diversity index measures the diversity of taxa in a particular community, with higher values indicating that there is high taxa richness (multiple taxa present). The complimentary Shannon Evenness measure allows for the similarity in taxa abundances to be measured, and if the score is closer to one, the taxa abundances are evenly distributed, whereas a smaller score means a particular taxon is dominant. Similarly, the Simpson's diversity Index combines both the taxa richness and abundances of the taxa, to produce a diversity score between 0 and 1, where the higher the value, the greater the diversity of the habitat.

Macroinvertebrates are a critical component of the riverine ecosystem and have an intermediate role in the river food web. The community composition is known to be sensitive to changes in environmental conditions, and certain species are used as indicators of water quality such as the presence of mayfly *Ephemeroptera* species or stonefly *Plecoptera* species indicating higher unpolluted water quality, such as in the WHPT, LIFE and RIVPACs measures and models (Johnson *et al.*, 2009). Environmental parameters that have an influence on the macroinvertebrate community composition and abundance are water temperature, increases and decreases in flow velocities, oxygen concentration, turbidity and conductivity levels and pH level (Durance & Ormerod, 2007; Durance & Ormerod, 2009; Johnson *et al.*, 2009; Everall *et al.*, 2019). However, the interactions between these parameters and the macroinvertebrate community can vary depending on the time of year and outside influences (Everall *et al.*, 2019), and due to the intermittent phases that occur in the upper courses of chalk streams, it is important to gauge how the macroinvertebrate communities respond in these locations.

Since the upper courses of chalk rivers experience periods of drought and low flows, and this is expected to be exacerbated by rising air temperatures and changing weather patterns in the UK with lower rainfall levels in future summers, slower flowing water impacts other parameters of the aquatic environment (Johnston *et al.*, 2009). For example, slower flowing water would have lower oxygen levels, and lead to greater sedimentation of the gravel beds, along with more concentrated nutrient levels (Boulton, 2003; Everall *et al.*, 2019). This could lead to the presence of more limnophilic invertebrate taxa increasing in the upper courses of chalk streams and the reduction of rheophilic species. Also, even though aquatic invertebrates have been shown to recolonise after periods of drought, the community may not always be consistent (Boulton, 2003). This means that any changes in the environment will likely impact the macroinvertebrate community composition and diversity. Since, the biological water quality indices favour macroinvertebrates that prefer high oxygenated water and low organic nutrient levels, it can be presumed that the future water quality scores will decline with these changes in the environmental parameters (Johnson *et al.*, 2009). Therefore, it is crucial to understand the baseline community composition and diversity in the upper catchments of chalk streams and to establish which

environmental parameters have the greatest influence on the taxa diversity so that any changes can be monitored alongside the changing aquatic environment.

This chapter explores the macroinvertebrate taxon diversity within the Upper Colne Catchment at family level in the 2023/24 season and at species level in the 2024/25 season. The differences in family abundance (the number of families present) and number of individuals were compared spatially and seasonally. Family and species richness indices (Shannon Diversity Scores, Simpson Diversity scores and Shannon Evenness scores) were calculated for the family level in the 2023/24 season and at family and species level in the 2024/25 season. The macroinvertebrate data was analysed to compare the spatial taxon diversity among the four sites (Church Lane, London Colney, Drop Lane and the Ver at Bricket Wood). The seasonal variation (spring, summer, autumn and winter) defined here as intra-annual variation, in taxon diversity was explored too. A comparison of the macroinvertebrate family taxon diversity is also made between the two seasons here defined as the inter-annual variation (April 2023 – February 2024 (referred to as the 2023/24 season) and March 2024 – February 2025 (referred to as 2024/25 season). A comparison between the diversity indices at the family and species level was also made in the 2024/25 season to identify if there were any significant differences between family and species level analysis.

Section 4.4.4 describes the methods used to investigate the macroinvertebrate community and the significance of the differences in the temporal and longitudinal assemblages. The methods of determining the impact of the hydrological, physicochemical and chemical variables on the community are also described in section 4.4.4. The significant environmental variables were identified and these were further tested to understand the amount of variation they contribute by using the variance partitioning. In section 5.3, to establish the health of the upper Colne Catchment using the biological indices, the WHPT score and LIFE scores were applied to the macroinvertebrate communities in the 2023/24 and 2024/25 season and the measures were compared across the four sites and the four intra-annual seasons, along with a comparison of the inter-annual season. This allowed a full understanding of the macroinvertebrate diversity to be devised, along with the macroinvertebrate community composition across the sites to be established and to gauge how this

contributes to the overall health of the Upper Colne Catchment using pre-existing models and biological indices.

7.1 Macroinvertebrate taxa diversity in the Upper Colne Catchment

7.1.1 Intra-annual taxa diversity

In the 2023/24 season, a total of 4917 individual specimens from 46 families were identified across the four sites (Church Lane (CL), London Colney (LC), Drop Lane (DL) and Ver(V)) throughout the four seasons (spring, summer, autumn, winter). Table 7.1. shows the mean average abundances for each family across the four sites and four intra-annual seasons. The London Colney site (LC) had the highest family richness (29) throughout the 2023/24 season, whilst the Ver had the lowest family richness (7) (Figures 7.1A, 7.2). However, the largest total number of individuals (1920) were collected from the Ver site (figure 7.1B). This was supported by the significant difference in family richness across the four sites (ANOVA: $F(3)=3.99$, $p=0.01$) (table 7.2). The post hoc Tukey HSD test (table 7.3) showed that LC had a significantly higher family richness than DL ($p=0.021$). *Gammaridae* was the most common family across all the sites and season in the 2023/24 season. The other common families at different sites were *Asellidae* (CL), *Oligochaeta* (LC), *Gammaridae* (DL and V) (figure 7.2). The least common families with only one individual present throughout the 2023/24 season were *Tipulidae* (V), *Sialidae* (DL), *Planariidae* (CL), *Glossosomatidae* (V), *Beraeidae* (V), *Corduliidae* (LC) and *Dendrocoelidae* (CL) (figure 7.2). The largest taxa in the 2023/24 season was *Mollusca* with five families, followed by *Trichoptera* with five families, although two of these families *Beraeidae* and *Glossosomatidae* were only present at the Ver site (table 7.1). *Gammaridae* had its highest frequency in winter 2023/24 and *Asellidae* had its highest frequency in autumn (figure 7.3). Spring was dominated by *Oligochaeta*, and *Chironomidae* and *Caenidae* were the most abundant family in summer 2023/24 (figure 7.3).

Similarly, there was a significant difference in the family richness among the four seasons (ANOVA: $F(3)=6.52$, $p=0.001$) (figure 7.1A, table 7.2). The post hoc Tukey HSD test showed that family richness in spring ($p=0.0056$) and summer ($p=0.041$) was significantly higher than autumn. The winter season also had significantly lower family

richness than spring ($p=0.01$). However, there was not a significant difference between the number of individuals found between the sites (ANOVA: $F(3)=1.92$, $p = 0.15$) or the seasons (ANOVA: $F(3)=1.73$, $p = 0.18$) (Figure 7.1B).

Table 7. 1: The taxon families with mean average abundance and standard error for 2023/24 season.

Taxon	Family	CL	LC	DL	V		Spring	Summer	Autumn	Winter
Anomopoda	<i>Daphniidae</i>	14.55 ± 21.89	20.9 ± 44.41	19.64 ± 57.20	0.00 ± 0.00		0.00 ± 0.00	46.25 ± 62.02	4.25 ± 13.80	0.00 ± 0.00
Coleoptera	<i>Dytiscidae</i>	0.18 ± 0.57	0.18 ± 0.57	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.33 ± 0.74	0.00 ± 0.00	0.00 ± 0.00
	<i>Elmidae</i>	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.57	0.03 ± 0.90		0.29 ± 0.70	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.83
	<i>Gyrinidae</i>	0.64 ± 1.49	0.27 ± 0.62	0.00 ± 0.00	0.00 ± 0.00		0.57 ± 0.90	0.50 ± 1.38	0.00 ± 0.00	0.00 ± 0.00
	<i>Hydrophilidae</i>	1.27 ± 2.56	0.18 ± 0.39	0.45 ± 0.99	0.10 ± 0.03		1.14 ± 1.88	1.08 ± 2.18	0.08 ± 0.28	0.00 ± 0.00
Crustacea	<i>Assellidae</i>	31.64 ± 20.69	9.73 ± 11.93	19.64 ± 18.29	0.4 ± 0.92		9.00 ± 7.78	13.58 ± 16.91	19.59 ± 22.44	17.83 ± 20.74
	<i>Astacidae</i>	0.00 ± 0.00	0.09 ± 0.29	0.09 ± 0.29	0.00 ± 0.00		0.14 ± 0.35	0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00
	<i>Gammaridae</i>	0.09 ± 0.29	7.63 ± 6.53	20.00 ± 13.86	159.9 ± 129.90		42.86 ± 81.52	44.91 ± 89.17	35.17 ± 68.39	53.58 ± 110.92
Diptera	<i>Ceratopogonidae</i>	0.18 ± 0.57	0.54 ± 0.99	1.36 ± 4.31	0.20 ± 0.50		3.00 ± 4.99	0.33 ± 0.85	0.00 ± 0.00	0.00 ± 0.00
	<i>Chironomidae</i>	2.18 ± 2.59	7.63 ± 9.36	6.73 ± 9.50	1.10 ± 1.51		12.43 ± 10.03	5.67 ± 8.09	2.50 ± 4.23	0.67 ± 0.75
	<i>Culicidae pupa</i>	0.00 ± 0.00	2.09 ± 4.81	0.00 ± 0.00	0.00 ± 0.00		2.71 ± 5.87	0.33 ± 0.85	0.00 ± 0.00	0.00 ± 0.00
	<i>Simuliidae</i>	1.09 ± 2.87	2.18 ± 4.02	0.27 ± 0.86	0.40 ± 0.92		1.57 ± 1.40	0.00 ± 0.00	0.00 ± 0.00	2.67 ± 4.44
	<i>Tipulidae</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.30		0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
Ephemeroptera	<i>Baetidae</i>	4.45 ± 7.05	12.54 ± 12.86	1.18 ± 1.90	5.40 ± 8.13		5.00 ± 8.70	8.50 ± 11.57	4.08 ± 6.91	5.67 ± 9.07

Taxon	Family	CL	LC	DL	V		Spring	Summer	Autumn	Winter
	<i>Caenidae</i>	0.45 ± 1.44	1.00 ± 1.54	3.09 ± 9.77	0.60 ± 1.50		7.00 ± 11.26	0.42 ± 0.55	0.00 ± 0.00	0.17 ± 0.55
	<i>Ephemeridae</i>	0.00 ± 0.00	5.27 ± 8.55	0.18 ± 0.39	0.60 ± 0.92		3.43 ± 7.61	0.08 ± 0.28	3.17 ± 6.52	0.25 ± 0.60
Hemiptera	<i>Corixidae</i>	0.18 ± 0.57	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.25 ± 0.60	0.00 ± 0.00	0.00 ± 0.00
	<i>Pleidae</i>	0.55 ± 1.16	0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00		0.85 ± 1.36	0.08 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
Hirudinea	<i>Erpobdellidae</i>	3.73 ± 4.00	3.72 ± 8.57	0.00 ± 0.00	0.00 ± 0.00		0.29 ± 0.70	0.58 ± 1.44	5.58 ± 8.40	0.50 ± 1.66
	<i>Glossipholiidae</i>	2.91 ± 4.12	2.09 ± 3.65	0.09 ± 0.29	0.00 ± 0.00		0.43 ± 0.73	0.75 ± 2.20	1.00 ± 3.32	2.67 ± 3.84
	<i>Piscicolidae</i>	0.18 ± 0.39	0.00 ± 0.00	0.36 ± 1.15	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.50 ± 1.12	0.00 ± 0.00
Megaloptera	<i>Sialidae</i>	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00		0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
Mollusca	<i>Bithyniidae</i>	0.00 ± 0.00	0.18 ± 0.57	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.17 ± 0.55	0.00 ± 0.00	0.00 ± 0.00
	<i>Hydrobiidae</i>	0.00 ± 0.00	0.27 ± 0.62	0.00 ± 0.00	10.80 ± 23.79		3.29 ± 6.86	6.83 ± 22.06	0.00 ± 0.00	0.50 ± 1.38
	<i>Lymnaeidae</i>	0.36 ± 0.88	0.81 ± 1.59	0.00 ± 0.00	6.00 ± 12.00		4.71 ± 10.37	2.75 ± 8.26	0.00 ± 0.00	0.58 ± 1.38
	<i>Neritidae</i>	0.45 ± 0.89	1.18 ± 2.85	0.00 ± 0.00	0.50 ± 1.50		0.14 ± 0.35	0.92 ± 1.55	0.92 ± 2.75	0.00 ± 0.00
	<i>Physidae</i>	0.00 ± 0.00	0.09 ± 0.29	0.18 ± 0.57	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.60
	<i>Planorbidae</i>	0.64 ± 0.98	0.36 ± 0.64	0.00 ± 0.00	2.50 ± 6.02		1.14 ± 1.73	2.00 ± 5.49	0.08 ± 0.28	0.25 ± 0.60
Odonata Anisoptera	<i>Corduliidae</i>	0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
Odonata Zygoptera	<i>Coenagrionidae</i>	1.36 ± 1.92	0.18 ± 0.57	0.09 ± 0.29	0.00 ± 0.00		0.86 ± 1.74	0.58 ± 1.44	0.25 ± 0.83	0.17 ± 0.37

Taxon	Family	CL	LC	DL	V		Spring	Summer	Autumn	Winter
Oligochaeta	<i>Oligochetes</i>	12.09 ± 16.03	24.1 ± 25.03	11.55 ± 14.88	0.50 ± 1.50		19.29 ± 14.75	15.00 ± 15.30	13.50 ± 27.65	4.50 ± 6.08
Trichoptera	<i>Beraeidae</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.30		0.14 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	<i>Glossosomatidae</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.30		0.14 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	<i>Leptoceridae</i>	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.29	0.10 ± 0.03		0.00 ± 0.00	0.08 ± 0.28	0.08 ± 0.28	0.00 ± 0.00
	<i>Limnephilidae</i>	2.82 ± 4.34	0.36 ± 0.77	0.91 ± 2.27	1.80 ± 2.09		0.57 ± 1.05	0.17 ± 0.37	0.42 ± 1.11	4.33 ± 3.99
	<i>Rhyacophilidae</i>	0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00	0.10 ± 0.30		0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00	0.08 ± 0.28
	<i>Dendrocoelidae</i>	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.08 ± 13.80	0.00 ± 0.00
	<i>Planariidae</i>	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.28	0.00 ± 0.00
Triclada										

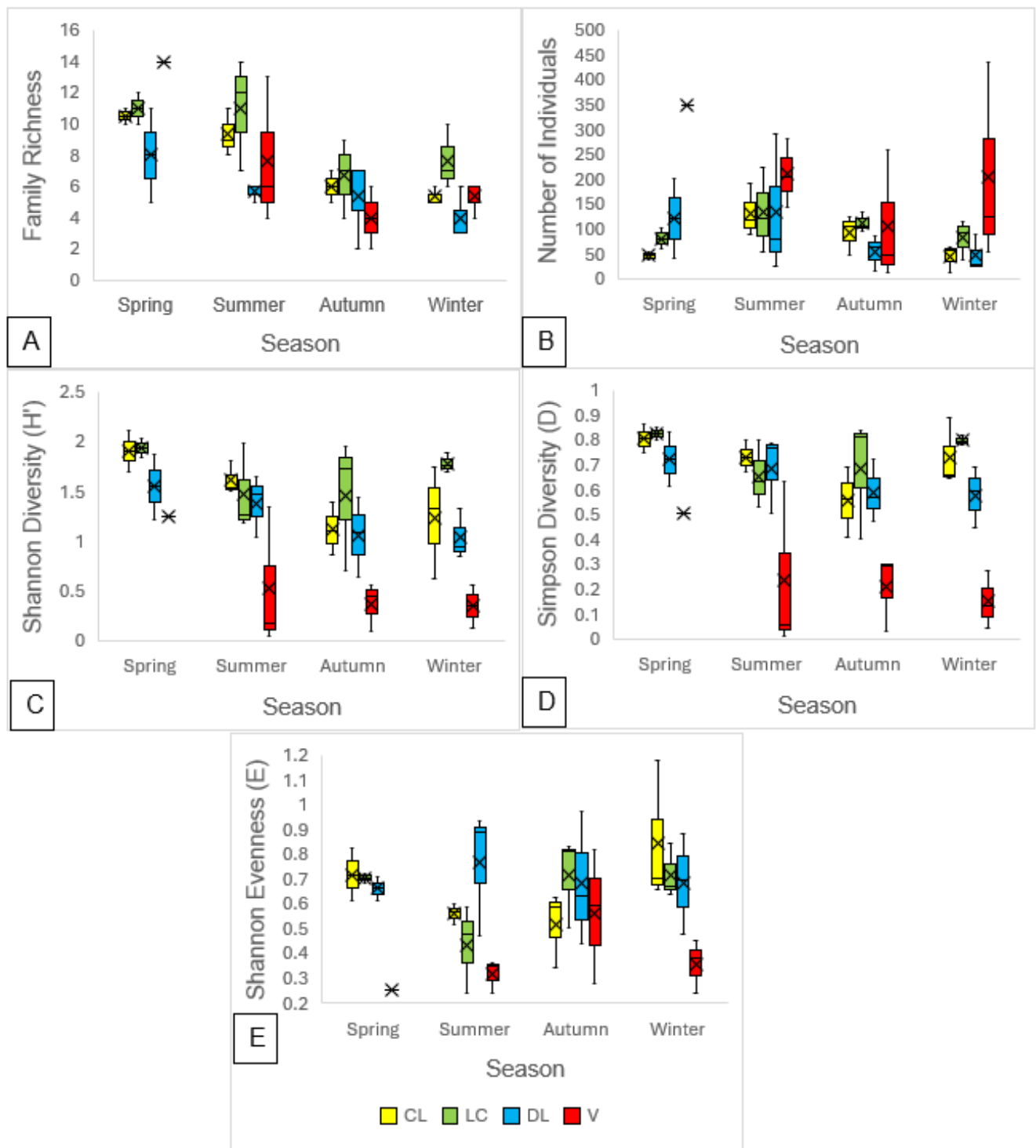


Figure 7. 1: The range of family richness (A) and number of individual macroinvertebrates (B) present at the four sites across the four intra-annual seasons. The Shannon (H') (C) and Simpson Diversity (D) (D) indices and the Shannon Evenness Score (E) for the four sites (CL, LC, DL, V) in the 2023/24 season.

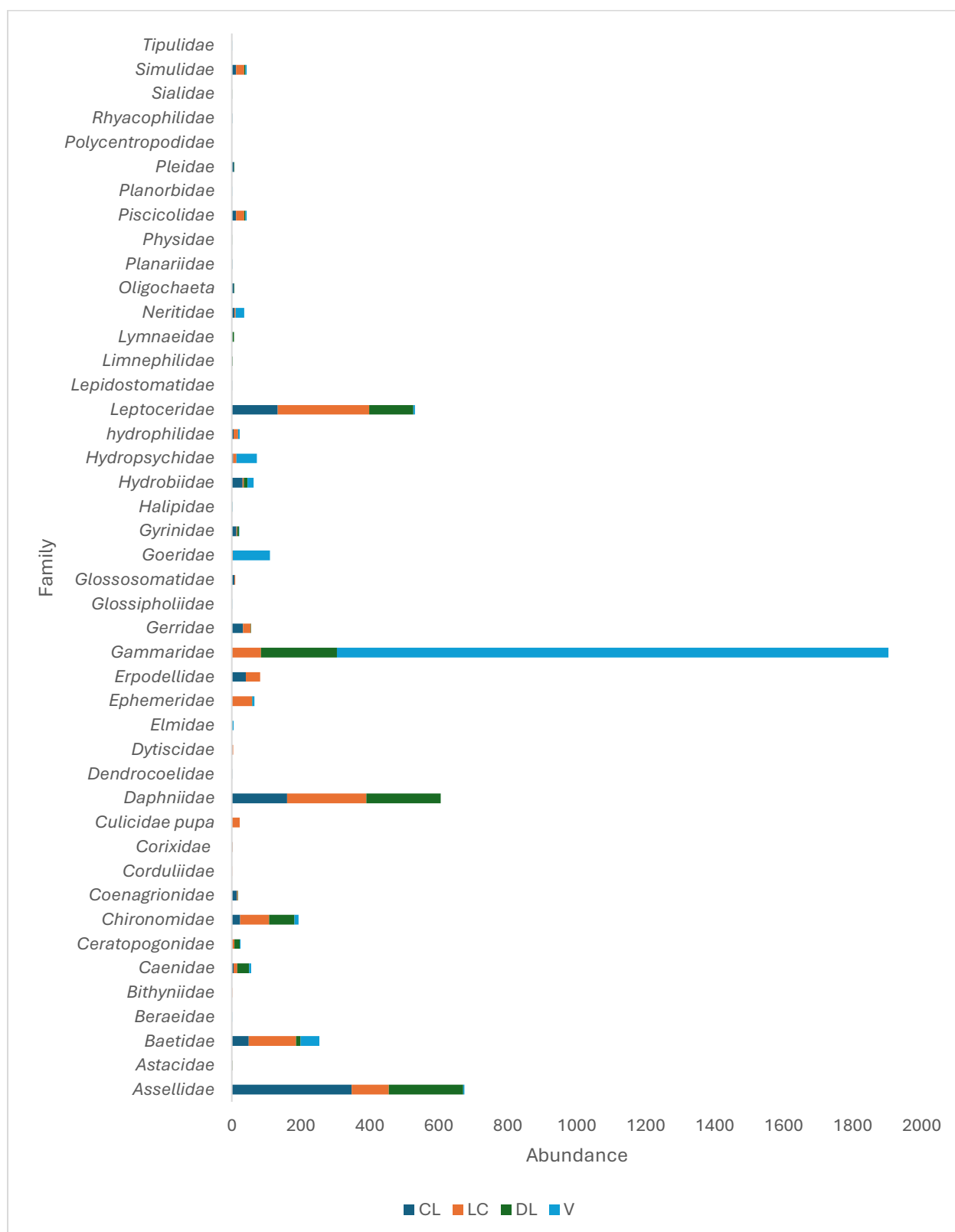


Figure 7. 2: The total abundance of each of the families found in the Upper Colne Catchment in the 2023/24 season and the relative abundance at each of the four sites.

Table 7. 2: The Two-Way ANOVA on the family richness, the total number of invertebrates and Shannon Evenness at the family and species level in the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $P < 0.05$, the difference is significant (results highlighted in grey).

Season	Variable	Independent Variable	Df	Sum Sq	Mean Sq	F value	p-value
2023/24	Family Richness	Site	3	1.79	0.60	3.99	0.017871
		Season	3	2.93	0.98	6.53	0.001835
		Residuals	27	4.04	0.15		
	Total Number of Individuals	Site	3	4.01	1.34	1.92	0.15027
		Season	3	3.63	1.21	1.73	0.183643
		Residuals	27	18.83	0.70		
	Shannon Evenness Family Level	Site	3	0.58	0.19	5.61	0.004019
		Season	3	0.11	0.04	1.09	0.368194
		Residuals	27	0.94	0.03		
2024/25	Family Richness	Site	3	246.66	82.22	40.27	1.59E-09
		Season	3	30.88	10.29	5.04	0.007522
		Residuals	24	49.00	2.04		
	Total Number of Individuals	Site	3	192.27	64.09	10.75	0.000114
		Season	3	89.93	29.98	5.03	0.007623
		Residuals	24	143.13	5.96		
	Species Richness	Site	3	15.35	5.12	25.96	1.04E-07
		Season	3	4.43	1.48	7.50	0.001045
		Residuals	24	4.73	0.20		
	Shannon Evenness Family Level	Site	3	1.09	0.36	17.59	3.72E-06
		Season	3	0.23	0.08	3.65	0.027525
		Residuals	23	0.48	0.02		
	Shannon Evenness Species Level	Site	3	2.49	0.83	11.83	3.96E-05
		Season	3	0.65	0.22	3.11	0.042941
		Residuals	27	1.89	0.07		

Table 7. 3: The Tukey HSD test on the significantly different parameters from the Two-Way ANOVA for 2023/24 and 2024/25 seasons with a 95% confidence limit. If $P < 0.05$, the difference is significant (results highlighted in grey).

Season	Dependent Variable	Independent Variable	Comparison	Difference	Lower	Upper	P-Value
2023/24	Family Richness	Site	LC-DL	0.51	0.06	0.97	0.021186
		Season	Spring - Autumn	0.67	0.17	1.18	0.0055661
			Summer - Autumn	0.45	0.01	0.88	0.0414929
			Winter - Spring	-0.62	-1.12	-0.11	0.011887
	Shannon Evenness Family Level	Site	DL-CL	0.05	-0.17	0.26	0.9381533
			LC-CL	-0.02	-0.24	0.2	0.9955741
			V-CL	-0.26	-0.48	-0.04	0.017086
			LC-DL	-0.06	-0.28	0.15	0.8503543
			V-DL	-0.31	-0.53	-0.08	0.0042617
			V-LC	-0.24	-0.47	-0.02	0.0289326
2024/25	Family Richness	Site	DL-CL	-3.57	-5.48	-1.67	0.00015
			V-CL	-4.27	-5.95	-2.59	0.0000017
			LC-DL	5.21	3.3	7.11	0.0000005
			V-LC	-5.91	-7.59	-4.23	0
	Season	Season	Summer-Spring	2.24	0.41	4.07	0.0126745
			Winter-Spring	1.97	0.1	3.84	0.0361681
	Number of Individuals	Site	DL-CL	-4	-7.26	-0.75	0.0120712
			LC-DL	5.51	2.25	8.76	0.0005315
			V-DL	6.34	3.09	9.6	0.0000902

Season	Dependent Variable	Independent Variable	Comparison	Difference	Lower	Upper	P-Value
		Season	Summer-Spring	4.08	0.95	7.21	0.0073966
	Species Richness	Site	DL-CL	0.02	-0.57	0.61	0.9996739
			LC-CL	0.14	-0.39	0.66	0.8884654
			V-CL	-1.33	-1.85	-0.8	1.75E-06
			LC-DL	0.12	-0.48	0.71	0.9487708
			V-DL	-1.35	-1.94	-0.75	9.95E-06
			V-LC	-1.46	-1.98	-0.94	3.38E-07
		Season	Summer-Autumn	0.86	0.33	1.38	7.46E-04
			Winter-Autumn	0.65	0.12	1.19	1.32E-02
	Shannon Evenness Family Level	Site	DL-CL	0.39	0.18	0.59	0.0001254
			LC-CL	-0.01	-0.18	0.16	0.999195
			V-CL	-0.14	-0.31	0.03	0.140746
			LC-DL	-0.39	-0.6	-0.19	9.62E-05
			V-DL	-0.52	-0.73	-0.32	1.49E-06
			V-LC	-0.13	-0.3	0.04	0.1777775
		Season	Spring-Autumn	0.11	-0.08	0.3	0.4145365
			Summer-Autumn	-0.11	-0.29	0.06	0.3092579
			Winter-Autumn	-0.03	-0.2	0.15	0.9762979
			Summer-Spring	-0.22	-0.4	-0.03	0.0163463
			Winter-Spring	-0.13	-0.32	0.06	0.2335155
			Winter-Summer	0.09	-0.09	0.26	0.5368677
	Shannon Evenness Species Level	Site	DL-CL	0.43	0.09	0.77	0.0101843
			LC-CL	-0.04	-0.33	0.26	0.987904
			V-CL	-0.32	-0.61	-0.02	0.0319205
			LC-DL	-0.47	-0.81	-0.12	0.0050902
			V-DL	-0.75	-1.09	-0.4	1.41E-05
			V-LC	-0.28	-0.58	0.01	0.0652528
		Season	Spring-Autumn	0.17	-0.17	0.52	0.5258725
			Summer-Autumn	-0.19	-0.5	0.13	0.3802314
			Winter-Autumn	-0.09	-0.39	0.21	0.8493004

Season	Dependent Variable	Independent Variable	Comparison	Difference	Lower	Upper	P-Value
			Summer-Spring	-0.36	-0.7	-0.02	0.0321951
			Winter-Spring	-0.26	-0.58	0.06	0.1415252
			Winter-Summer	0.1	-0.19	0.39	0.7870556

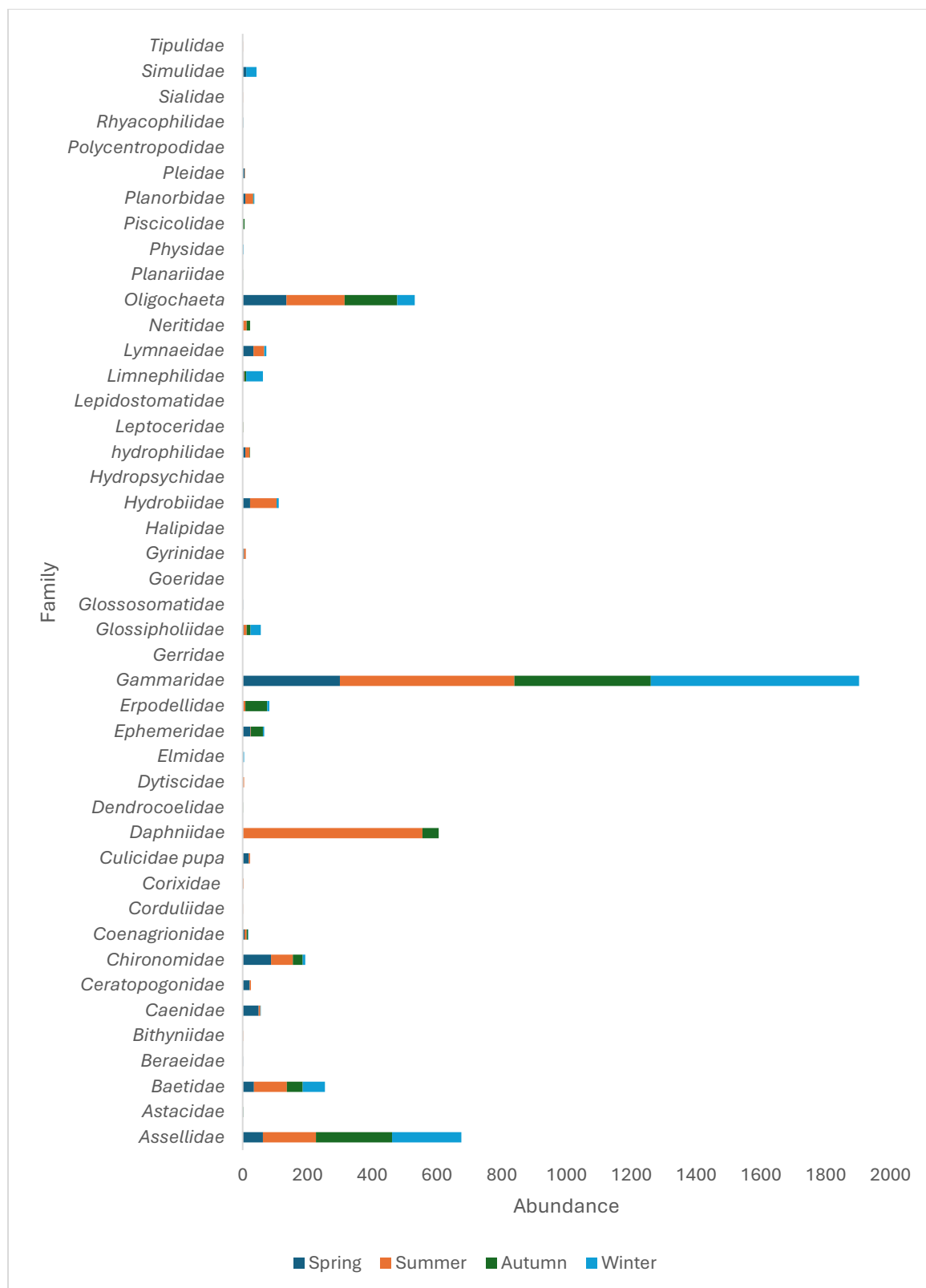


Figure 7. 3: The total number of individuals per family collected in the Upper Colne Catchment in the 2023/24 season and the relative abundance across all intra-annual seasons.

A similar pattern was observed in the diversity of macroinvertebrates, where the Ver site had the lowest family diversity measures across the four sites and throughout the four seasons. Both the Shannon diversity and the Simpson diversity measures were the lowest at the Ver site ($H' = 0.496 \pm 0.438$ and $D = 0.230 \pm 0.203$) (figure 7.1CD) while the London Colney site had the highest family diversity in spring ($H' = 1.94 \pm 0.0899$, $D = 0.827 \pm 0.027$), autumn ($H' = 1.46 \pm 0.55$, $D = 0.69 \pm 0.20$) and winter ($H' = 1.79 \pm 0.082$, $D = 0.801 \pm 0.016$). In summer of the 2023/24 season, the Church Lane ($H' = 1.62 \pm 0.136$) and Drop Lane ($D = 0.69 \pm 0.13$) sites had higher diversity. There was a significant difference in the Shannon and Simpson measures of diversity across the sites (Shannon: $H(3) = 20.28$, $p = 0.0001$; Simpson: $H(3) = 20.71$, $p = 0.0001$) (table 7.4). The post hoc test showed that the Shannon diversity measure was significantly higher at the CL ($p = 0.052$) and LC ($p = 9.87e-05$) sites compared to the V site (table 7.5).

The Simpson diversity score was also significantly higher at the LC ($p = 9.87e-05$) and CL ($p = 0.0052$) sites compared to the V site (table 7.4). The Shannon diversity measure slightly decreased throughout the 2023/24 season (figure 7.1C), However, there was not a statistically significant difference in the Shannon Diversity ($H(3) = 7.62$, $p = 0.055$) nor the Simpson Diversity measures ($H(3) = 5.28$, $p = 0.15$) among the seasons (table 7.5).

The Shannon evenness scores were higher in winter (0.65 ± 0.24) and lowest in summer (0.52 ± 0.21) (figure 7.1E) meaning winter had fewer dominant species (figure 7.3). However, there was not a significant difference in the Shannon Evenness scores across the intra-annual seasons (ANOVA: $F(3) = 1.09$, $p = 0.37$). The Ver site had the lowest evenness scores (0.40 ± 0.18 E) due to the dominance of the Gammaridae family (figure 7.2) and there was a significant difference in Shannon Evenness across the sites (ANOVA: $F(3) = 5.61$, $p = 0.004$). The post hoc test showed that V had a significantly lower Shannon Evenness score than CL ($p = 0.02$), LC ($p = 0.03$) and DL ($p = 0.004$) (table 7.1).

Table 7. 4: The Kruskal-Wallis test performed on the Shannon Diversity and Simpson Diversity measures for the 2023/24 and 2024/25 seasons to identify differences in the family and species diversity between the sites and seasons with a 95% confidence limit. If $P < 0.05$, the difference is significant (results highlighted in grey).

Year	Dependent Variable	Independent Variable	Chi-Squared	df	P-Value
2023/24	Shannon Diversity Score Family Level	Site	20.28	3	0.000148
		Season	7.62	3	0.05461
	Simpson Diversity Family Level	Site	20.71	3	0.000121
		Season	5.28	3	0.1525
2024/25	Simpson Diversity Family Level	Site	21.61	3	7.86E-05
		Season	1.60	3	0.6599
	Shannon Diversity Family Level	Site	27.72	3	4.16E-06
		Season	2.00	3	0.5733
	Simpson Diversity Species Level	Site	20.72	3	1.20E-04
		Season	2.19	3	0.5341
	Shannon Diversity Species Level	Site	26.43	3	7.76E-06
		Season	3.06	3	0.3818

Table 7. 5: The post hoc Dunn test performed on the significant outcome of the Kruskal-Wallis test on the 2023/24 and 2024/25 seasons with a 95% confidence limit. If $P < 0.05$, the difference is significant (grey highlight).

Season	Dependent Variable	Comparison	Z	P.unadj	P.adj
2023/24	Shannon Diversity	CL - DL	1.02	0.31	1.00E+00
		CL - LC	-1	0.32	1.00E+00
		DL - LC	-2.02	0.04	0.25997
		CL - V	3.33	0	0.0052
		DL - V	2.34	0.02	0.116829
		LC - V	4.31	0	9.87E-05
	Simpson Diversity	CL - DL	1.02	0.31	1
		CL - LC	-1	0.32	1
		DL - LC	-2.02	0.04	0.25997
		CL - V	3.33	0	0.0052
		DL - V	2.34	0.02	0.116829
		LC - V	4.31	0	9.87E-05
2024/25	Simpson Diversity Family	CL - DL	-0.46	0.65	1
		CL - LC	-1.11	0.27	1
		DL - LC	-0.52	0.6	1
		CL - V	3.2	0	0.008224
		DL - V	3.28	0	0.006201
		LC - V	4.31	0	9.65E-05
	Shannon Diversity Families	CL - DL	1.15	0.25	1
		CL - LC	-1.7	0.09	0.539161
		DL - LC	-2.65	0.01	0.048886
		CL - V	3.42	0	0.003762
		DL - V	1.87	0.06	0.372422
		LC - V	5.12	0	1.87E-06
	Sp. Simpson	CL - DL	-0.04	0.97	1
		CL - LC	-0.55	0.58	1
		DL - LC	-0.44	0.66	1
		CL - V	3.53	0	0.002502
		DL - V	3.15	0	0.009635
		LC - V	4.08	0	2.75E-04
	Sp. Shannon	CL - DL	1.68	0.09	0.559469
		CL - LC	-0.88	0.38	1
		DL - LC	-2.45	0.01	0.085577
		CL - V	3.88	0	0.000638
		DL - V	1.74	0.08	0.49177
		LC - V	4.75	0	1.21E-05

In the 2024/25 season, a total of 2809 individual macroinvertebrates were identified to 47 families and 51 species (table 7.6). The Ver site had the lowest range of family richness in the 2024/25 season, ranging between one to five families across the seasons. The most common family was Gammaridae, and the most common species was *Gammarus pulex* (figures 7.5 and 7.7). In contrast, the London Colney site had the highest family richness out of the four sites across the seasons (figure 7.4A) and the largest range of family richness of 8 to 14 families, which occurred in winter 2024/25 (figure 7.4A). The LC and CL sites had higher family richness than both the DL and V sites throughout the 2024/25 season (figure 7.4A) and this difference in family richness between the sites was significantly different (ANOVA: $F(3) = 40.27$, $p = 1.59 \times 10^{-9}$). The post hoc Tukey test showed that DL ($p = 0.0002$) and V ($p = 1.7 \times 10^{-6}$) sites were significantly lower than, and LC was higher than both the DL ($p = 5.0 \times 10^{-7}$) and V ($p = 0.0001$) sites (tables 7.1 and 7.2). The family richness also slightly increased throughout the 2024/25 season, with autumn and winter having greater family abundances than spring and summer (figure 7.4A) and this difference in family abundance was significant among the seasons (ANOVA: $F(3) = 5.04$, $p = 0.0075$). The post hoc Tukey test revealed that family richness in summer was significantly higher than spring ($p = 0.013$) and that family richness in winter was higher than spring ($p = 0.036$) (tables 7.1 and 7.2.). The two largest taxon's were the same as in 2023/24, but *Trichoptera* had greater family richness than 2024/25, with five families and 18 species, 12 of which belonged to the Limnephilidae family. The Mollusca taxon had three families and six species, with the majority being part of the *Lymnaeidae* or *Planorbidae* families (table 7.6).

Table 7. 6: The taxon families with mean average abundance and standard error for 2024/25 season.

Taxon	Family	Species	CL	LC	DL	V		Spring	Summer	Autumn	Winter
Anomopoda	<i>Daphniidae</i>	<i>Daphina pulex</i>	1.81 ± 5.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	2.00 ± 6.00	0.00 ± 0.00
Chironomidae	<i>Chironomidae</i>	<i>Sp.</i>	2.72 ± 4.33	9.73 ± 22.37	2.33 ± 2.49	0.00 ± 0.00		1.75 ± 2.38	9.73 ± 22.63	1.50 ± 2.80	1.50 ± 1.91
Coleoptera	<i>Dytiscidae</i>	<i>Sp.</i>	0.18 ± 0.39 0.09	0.00 ± 0.00	0.33 ± 0.75 0.00	0.00 ± 0.00		0.00 ± 0.00	0.18 ± 0.39	0.20 ± 0.40	0.00 ± 0.00
	<i>Elmidae</i>	<i>Sp.</i>	0.29 ± 0.36	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.30
	<i>Haliplidae</i>	<i>Sp.</i>	0.64 ± 0.09	0.00 ± 0.09	0.00 ± 0.33	0.00 ± 0.00		0.13 ± 0.33	0.27 ± 0.62	0.00 ± 0.00	0.00 ± 0.00
	<i>Hydrophilidae</i>	<i>sp.</i>	0.29 ± 0.29	0.09 ± 0.29	0.33 ± 0.75	0.00 ± 0.00		0.00 ± 0.00	0.27 ± 0.62	0.00 ± 0.00	0.10 ± 0.30
Crustacea	<i>Assellius</i>	<i>Asellus aquaticus</i>	28.18 ± 14.31	8.27 ± 5.61	2.67 ± 3.25	0.00 ± 0.00		6.38 ± 10.92	9.72 ± 13.00	12.40 ± 17.02	13.50 ± 13.27
	<i>Gammaridae</i>	<i>Gammarus pulex</i>	1.81 ± 2.44	12.45 ± 13.38	3.67 ± 2.81	107.91 ± 92.21		3.13 ± 3.26	63.91 ± 95.14	18.70 ± 27.16	45.10 ± 76.13
	<i>Astacidae</i>	<i>Pacifastacus leniusculus</i>	0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00
Diptera	<i>Culicidae</i>	<i>Sp.</i>	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.37	0.00 ± 0.00		0.00 ± 0.00	0.09 ± 0.29	0.00 ± 0.00	0.00 ± 0.00

Taxon	Family	Species	CL	LC	DL	V		Spring	Summer	Autumn	Winter
	<i>Simuliidae</i>	<i>Sp.</i>	0.18	1.36	0.17	0.00 ±		0.13 ±	0.00 ±	0.00 ±	1.70 ±
			±	±	±	0.00		0.33	0.00	0.00	3.52
			0.57	3.42	0.37						
	<i>Tipulidae</i>	<i>Sp.</i>	0.00	0.09	0.00	0.00 ±		0.00 ±	0.09 ±	0.00 ±	0.00 ±
			±	±	±	0.00		0.00	0.29	0.00	0.00
			0.00	0.29	0.00						
Ephemeroptera	<i>Baetidae</i>	<i>Baetis rhondani</i>	2.63	8.91	0.50	3.18 ±		8.13 ±	2.45 ±	2.40 ±	4.90 ±
			±	±	±	7.04		10.64	3.77	4.45	3.99
			4.18	6.76	1.12						
	<i>Caenidae</i>	<i>Caenis rivulorum</i>	0.00	1.55	0.17	0.09 ±		0.00 ±	0.81 ±	0.20 ±	0.80 ±
			±	±	±	0.29		0.00	1.34	0.40	1.17
			0.00	1.37	0.37						
	<i>Ephemeridae</i>	<i>Ephemera vulgata</i>	0.00	0.00	0.00	0.27 ±		0.00 ±	0.00 ±	0.00 ±	0.30 ±
			±	±	±	0.86		0.00	0.00	0.00	0.90
			0.00	0.00	0.00						
		<i>Ephemera danica Mull</i>	0.00	0.00	0.33	1.73 ±		1.00 ±	0.55 ±	0.40 ±	0.30 ±
			±	±	±	2.34		2.65	1.23	0.80	0.64
			0.00	0.00	0.75						
Hemiptera	<i>Gerridae</i>	<i>Sp.</i>	0.45	0.09	0.00	0.00 ±		0.00 ±	0.45 ±	0.10 ±	0.00 ±
			±	±	±	0.00		0.00	1.16	0.30	0.00
			1.16	0.29	0.00						
	<i>Veliidae</i>	<i>Microvelia reticulata</i>	0.18	0.00	0.00	0.00 ±		0.00 ±	0.18 ±	0.00 ±	0.00 ±
			±	±	±	0.00		0.00	0.57	0.00	0.00
			0.57	0.00	0.00						
	<i>Pleidae</i>	<i>Pleidae</i>	0.00	0.18	0.00	0.00 ±		0.00 ±	0.18 ±	0.00 ±	0.00 ±
			±	±	±	0.00		0.00	0.57	0.00	0.00
			0.00	0.57	0.00						
Hirudinea	<i>Erpobdellidae</i>	<i>Erpobdella octoculata</i>	1.45	1.36	0.00	0.00 ±		0.63 ±	1.45 ±	0.60 ±	0.40 ±
			±	±	±	0.00		0.99	2.74	1.02	0.80
			1.23	2.67	0.00						
	<i>Glossipholiidae</i>	<i>Glossiphonia complanata</i>	2.91	9.45	0.67	0.00 ±		2.25 ±	1.18 ±	8.10 ±	2.80 ±
			±	±	±	0.00		2.59	1.80	9.38	5.13
			3.45	8.52	1.49						

Taxon	Family	Species	CL	LC	DL	V		Spring	Summer	Autumn	Winter
	<i>Piscicolidae</i>	<i>Piscicola geometra</i>	0.09 ± 0.29	1.18 ± 2.08	0.00 ± 0.00	0.09 ± 0.29		0.00 ± 0.00	0.73 ± 1.35	0.20 ± 0.60	0.50 ± 0.92
Megaloptera	<i>Sialidae</i>	<i>Sp.</i>	0.00 ± 0.00	0.45 ± 1.44	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.45 ± 1.44	0.00 ± 0.00	0.00 ± 0.00
Mollusca	<i>Lymnaeidae</i>	<i>Lymnaea (Galba) truncatula</i>	0.36 ± 0.77 0.00	1.55 ± 1.62 0.64	0.00 ± 0.00	0.00 ± 0.00		0.13 ± 0.33	0.55 ± 1.23	1.10 ± 1.58	0.30 ± 0.64
		<i>Lymnaea (Leptolimnaea) glabra</i>	0.00 ± 0.00	1.37 ± 2.09	0.00 ± 0.00	0.00 ± 0.00		0.50 ± 1.32	0.27 ± 0.86	0.00 ± 0.00	0.00 ± 0.00
		<i>Lymnaea peregra</i>	0.00 ± 0.09	3.09 ± 0.73	0.00 ± 0.00	0.00 ± 0.00		0.13 ± 0.33	1.55 ± 3.20	0.30 ± 0.90	0.20 ± 0.60
	<i>Physidae</i>	<i>Physa heterostrophia</i>	0.09 ± 0.29	0.27 ± 1.35	0.00 ± 0.00	0.00 ± 0.00		0.13 ± 0.33	0.73 ± 1.35	0.00 ± 0.00	0.00 ± 0.00
		<i>Planorbis complanatus</i>	0.09 ± 0.29	0.27 ± 0.86	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.92
	<i>Planorbidae</i>	<i>Planorbis planorbis</i>	0.64 ± 1.23	0.27 ± 0.86	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.36 ± 0.88	0.60 ± 1.28	0.00 ± 0.00
Odonata Zygoptera	<i>Calopterygidae</i>	<i>Calopteryx splendens</i>	0.36 ± 1.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 1.20
		<i>Enallagma cyathigerum</i>	0.18 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.60
	<i>Coenagrionidae</i>	<i>Ischnura elegans</i>	0.45 ± 0.78	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.60	0.30 ± 0.64

Taxon	Family	Species	CL	LC	DL	V		Spring	Summer	Autumn	Winter
Oligochaeta	<i>Oligochetes</i>		7.45	16.09	2.83	0.00 ±		1.75 ±	8.82 ±	9.60 ±	6.90 ±
			±	±	±	0.00		1.92	11.37	17.32	7.42
		<i>Sp.</i>	6.32	17.25	3.08						
Trichoptera	<i>Goeridae</i>		0.00	0.00	0.00	0.55 ±		0.13 ±	0.36 ±	0.00 ±	0.10 ±
			±	±	±	1.16		0.33	1.15	0.00	0.30
		<i>Goera pilosa (Fabricius)</i>	0.00	0.00	0.00						
	<i>Hydropsychidae</i>		0.00	0.09	0.00	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.10 ±
			±	±	±	0.00		0.00	0.00	0.00	0.30
		<i>Hydropsyche siltalia</i>	0.00	0.29	0.00						
	<i>Leptoceridae</i>		0.00	0.00	0.17	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.10 ±
			±	±	±	0.00		0.00	0.00	0.00	0.30
		<i>Mystacides longicornis</i>	0.00	0.00	0.37						
			0.09	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.10 ±
			±	±	±	0.00		0.00	0.00	0.00	0.30
		<i>Halesus sp.</i>	0.29	0.00	0.00						
	<i>Limnephilidae</i>		0.00	0.18	0.00	0.00 ±		0.00 ±	0.00 ±	0.20 ±	0.00 ±
			±	±	±	0.00		0.00	0.00	0.40	0.00
		<i>Limnephilidae Incisus</i>	0.00	0.39	0.00						
			0.45	0.45	0.00	0.27 ±		0.25 ±	0.18 ±	0.30 ±	0.60 ±
			±	±	±	0.45		0.66	0.39	0.64	0.92
		<i>Limnephilidae Instar III</i>	0.66	0.99	0.00						
			0.64	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.40 ±	0.30 ±
			±	±	±	0.00		0.00	0.00	1.20	0.90
		<i>Phacopteryx brevipennis</i>	1.37	0.00	0.00						
			0.55	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.40 ±	0.20 ±
			±	±	±	0.00		0.00	0.00	1.20	0.60
		<i>Potomophylax circulatus</i>	1.23	0.00	0.00						
			0.09	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.10 ±
			±	±	±	0.00		0.00	0.00	0.00	0.30
		<i>Limnephilus flavicornus</i>	0.29	0.00	0.00						
			0.55	0.00	0.00	0.00 ±		0.63 ±	0.00 ±	0.00 ±	0.10 ±
			±	±	±	0.00		1.65	0.00	0.00	0.30
		<i>Limnephilus decipiens</i>	1.44	0.00	0.00						

Taxon	Family	Species	CL	LC	DL	V		Spring	Summer	Autumn	Winter
		<i>Limnephilus fuscicornis</i>	0.00	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.00 ±
			±	±	±	0.00		0.00	0.00	0.00	0.00
		<i>Limnephilus Lunatus</i>	0.00	0.00	0.00	0.00 ±		1.25 ±	0.00 ±	0.00 ±	0.10 ±
			1.00	0.00	0.00	0.00		3.31	0.00	0.00	0.30
		<i>Limnephilus Instar II</i>	0.27	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.30 ±
			±	±	±	0.00		0.00	0.00	0.00	0.64
		<i>Limnephilus rhombicus Instar IV</i>	0.62	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.20 ±	0.50 ±
			0.64	0.00	0.00	0.00		0.00	0.00	0.40	1.20
		<i>Melampophylax mucoreus Instar III</i>	±	±	±	0.00 ±		0.00 ±	0.00 ±	0.00 ±	0.00 ±
			0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
	<i>Polycentropodidae</i>	<i>Plectrocnemia Conspersa</i>	0.00	0.09	0.00	0.00 ±		0.13 ±	0.00 ±	0.00 ±	0.00 ±
			±	±	±	0.00		0.33	0.00	0.00	0.00
		<i>Sp.</i>	0.09	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.10 ±	0.00 ±
			±	±	±	0.00		0.00	0.00	0.30	0.00
	<i>Psychomyiidae</i>	<i>Tinodes waeneri</i>	0.29	0.00	0.00	0.00 ±		0.00 ±	0.00 ±	0.10 ±	0.00 ±
			±	±	±	0.00		0.00	0.00	0.30	0.00

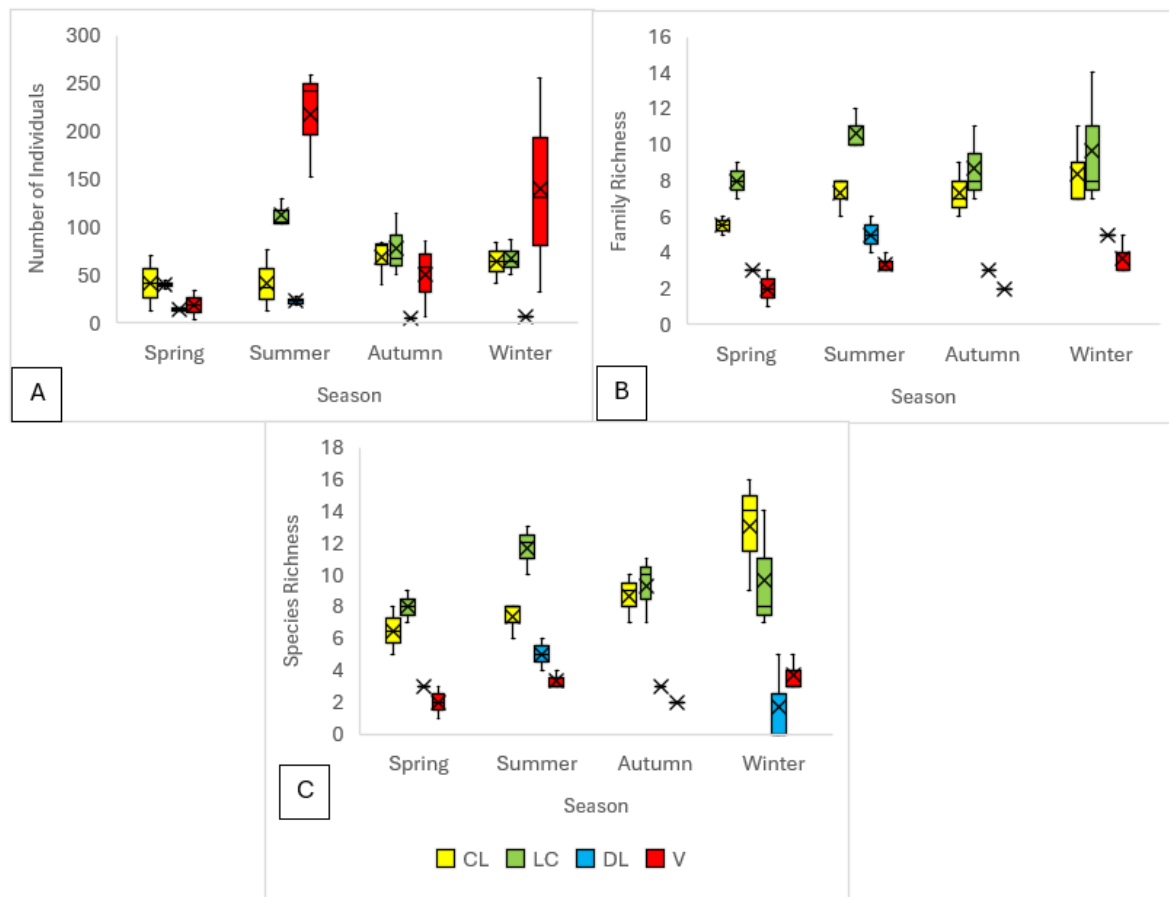


Figure 7. 4: The 2024/25 season family abundance (A), number of individual macroinvertebrates (B) and species abundance (C).

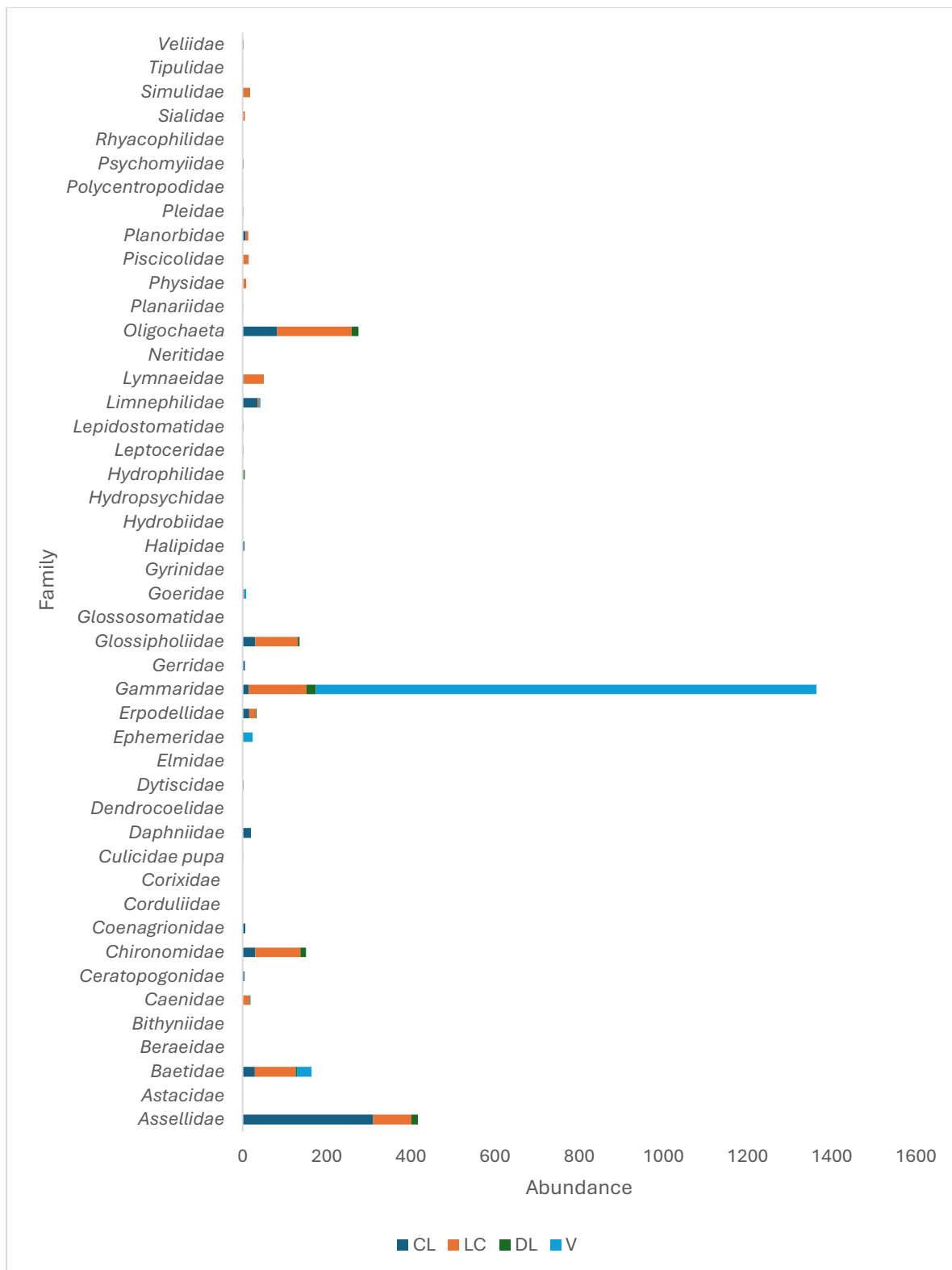


Figure 7. 5: The total number of individual macroinvertebrates per family and the relative abundance at the four sites in the 2024/25 season.

The Ver site had the highest abundance of macroinvertebrates (114.45 ± 1.03), particularly during both the summer and winter (figure 7.4B). Additionally, the Ver site had the largest range in number of individuals with counts varying between 32 and 258. In spring, CL had the highest number of individuals and in autumn it was LC. Drop Lane was found to have the lowest number of individuals across the 2024/25 season with an average of 14 ± 2 spring, 23 ± 5 in summer, 5 ± 0 in autumn and 7 ± 0 in the 2024/25 season (figure 7.4B). This difference in the number of individuals found between the four sites was shown to be significant (ANOVA: $F(3)=10.74$, $p = 0.000114$) and the post-hoc Tukey test showed that DL had significantly lower number of individuals than CL ($p=0.012$), LC had more number of individuals than DL ($p=0.00053$), and V had more individuals than DL ($p=9.02e-05$) (tables 7.1 and 7.2). However, the lowest number of individual macroinvertebrates was found at the Ver in spring with only 4 individuals found belonging to the Gammaridae family. The number of macroinvertebrates was found to be higher in summer (105.63 ± 81.22), autumn (59.4 ± 33.47) and winter (81.9 ± 66.34) compared to spring (28.63 ± 20.72) (figure 7.4B) and this was supported by the significant difference in the number of individuals across the seasons (ANOVA: $F(3)=5.03$, $p = 0.0076$). The post hoc test showed that only summer had a significantly higher number of individuals than spring ($p=0.0074$), meaning the other seasons (summer, autumn and winter) were similar.

The family that was most abundant at the Ver and Drop Lane sites was Gammaridae, and this was the same for the species *Gammarus pulex* (figure 7.5 and 7.7). Church Lane was dominated by Asellus (*Asellius aquaticus*) and *Oligochaeta* were most abundant at London Colney (these could not be identified to species level) (figure 7.7). The Baetidae family was the most abundant at LC and these specimens were identified to the species *Baetis rhondani*. The Limnephilidae family was most abundant at Church Lane and the most common species were *Limnephilidae instar III* (15 individuals), *Limnephilus lunatus* (12 individuals) and *Limnephilus rhombicus* (8 individuals) (figure 7.4 and 7.7). The *Limnephilidae*, Baetidae and Asellidae families were most common in winter 2024/25, Gammaridae was most dominant in spring and *Oligochaeta* was the most abundant family in summer and autumn (figure 7.6, figure 7.8).

The species richness also varied between the sites in the 2024/25 season and there was a significant difference in species richness among the sites (ANOVA: $F(3)=25.96$, $p = 1.04e-07$). The highest species richness was in winter at the CL site with 16 species found (table 7.5, figure 7.4C, 7.7). CL and LC both had higher species richness than the DL and V sites (figure 7.4C). The post-hoc test found that there was a significant difference in species richness where the richness at the Ver site was lower than the other three sites (V-CL $p=1.75e-06$; V- DL $9.95e-06$; V- LC $p=3.38e-07$) (table 7.2). The species richness increased throughout the 2024/25 season, with the lowest species richness in spring (4.88 ± 2.67) and highest species richness was in winter (7.00 ± 5.18) (figure 7.3C). This difference in species richness between the seasons was significant (ANOVA: $F(3) = 7.49$, $p = 0.001$). However, the post hoc test showed that both summer and winter had higher species richness than autumn (Summer-Autumn $p=1.32e-02$; Winter-Autumn $=1.32e-02$), but the comparisons with spring were not significant.

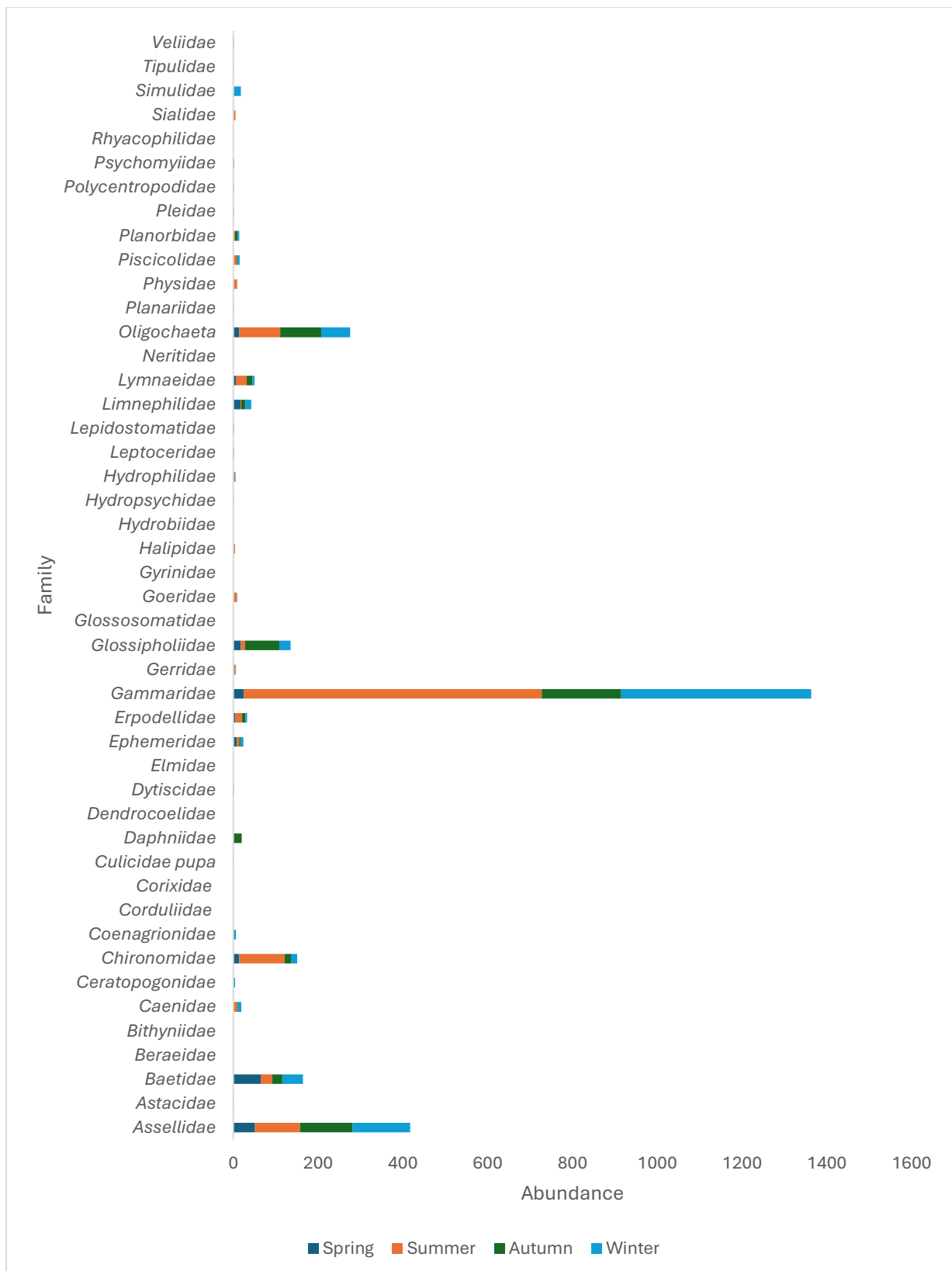


Figure 7. 6: The total number of individual macroinvertebrates per family and the relative abundance at the four intra-annual seasons in the 2024/25 season.

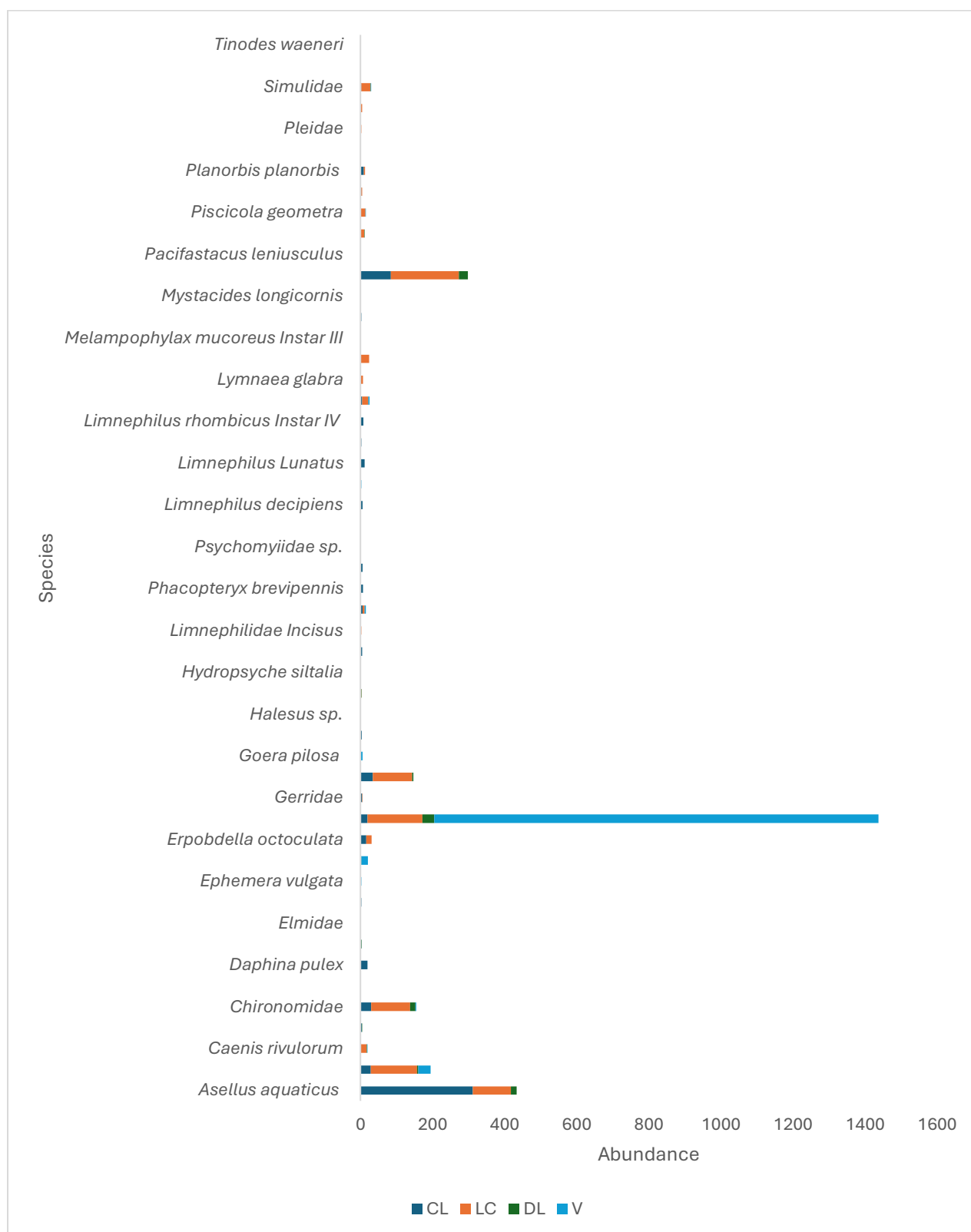


Figure 7. 7: The total number of individual macroinvertebrates per species and the relative abundance at the four sites in the 2024/25 season.

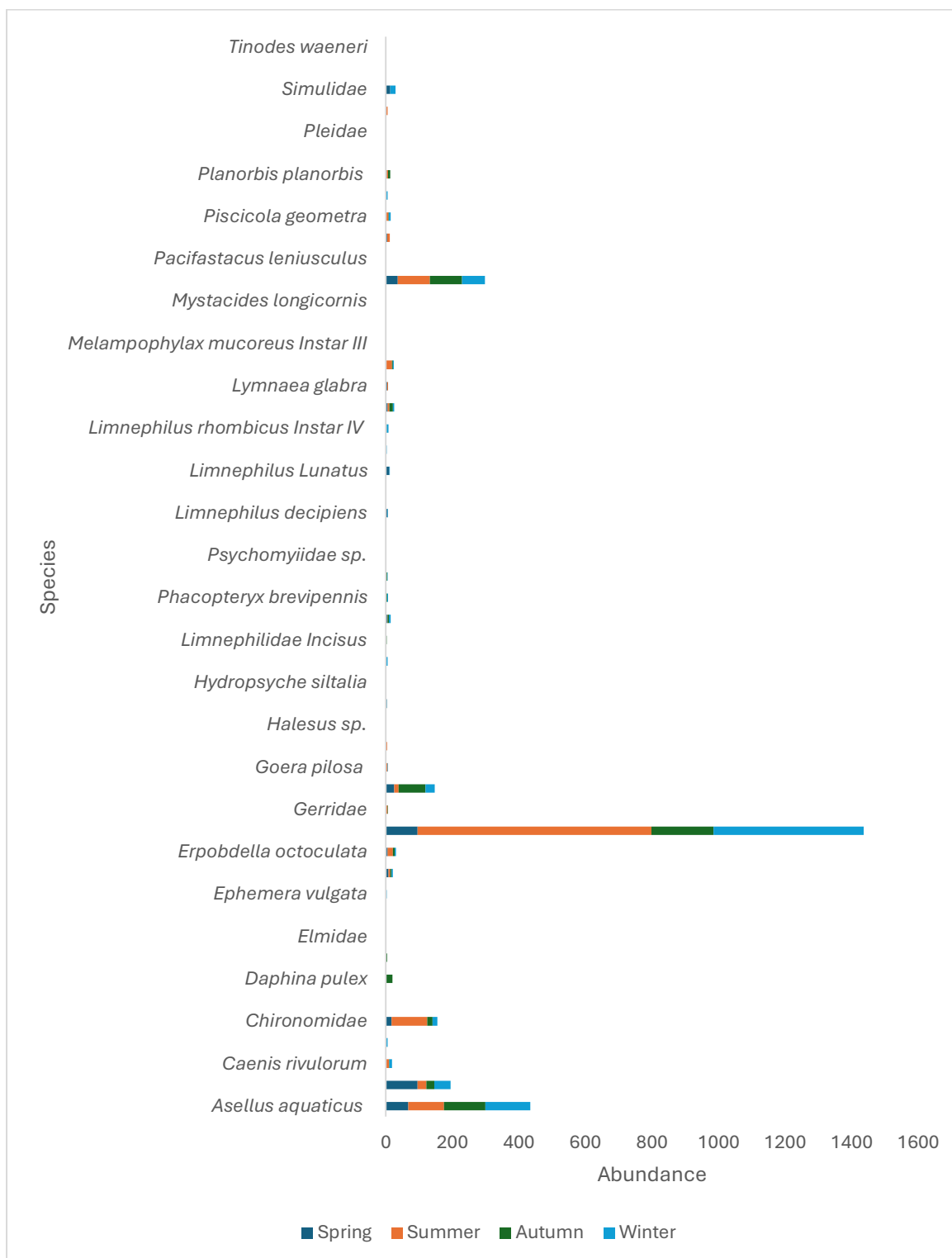


Figure 7. 8: The total number of individual macroinvertebrates per species and the relative abundance at the four sites in the 2024/25 season.

The Ver had the lowest family diversity across the seasons as shown by the Shannon Diversity ($0.22 \pm 0.19 H'$) and Simpson Diversity ($0.12 \pm 0.13 D$) measures (figure 7.9AC). LC had the highest diversity across the 2024/25 season reaching a maximum of $1.92 H'$ and $0.89 D$ in winter 2024/25. However, DL had the largest range in family diversity ($0 - 1.05 H'$). There was a significant difference in the family diversity measures among the sites (H' : Kruskal-Wallis: $H(3)=27.72$, $p=4.16e-06$; D : Kruskal-Wallis: $H(3)=21.61$, $p=7.86e-05$). The post hoc test showed that the Shannon diversity measure was lower at DL than LC ($p=0.049$), and CL and LC were higher than V (CL-V $p=0.0038$; LC-V $p=1.87e-06$). The Simpson diversity measure also showed that diversity was significantly higher at CL, LC and DL than V (CL- V $p=0.008$; LC- V $p=9.65e-05$; DL- V $p=0.0062$).

The family diversity was highest in winter at LC and the Shannon diversity score in winter at LC had a mean of $1.92 \pm 0.28 H'$ (figure 7.9D). However, the Simpson diversity was highest at DL in summer ($0.79 \pm 0.03 D$), autumn ($0.8 \pm 0 D$) and winter ($0.85 \pm 0 D$) (figure 7.9C). However, as the patterns shown in each of the sites were similar between the seasons, with CL, LC and DL being higher than the V in Shannon Diversity and Simpson diversity (figure 7.9AC), there was not a significant difference between the seasons detected (H' : Kruskal-Wallis: $H(3)=1.99$, $p = 0.57$; D : Kruskal-Wallis: $H(3) = 1.60$, $p=0.66$).

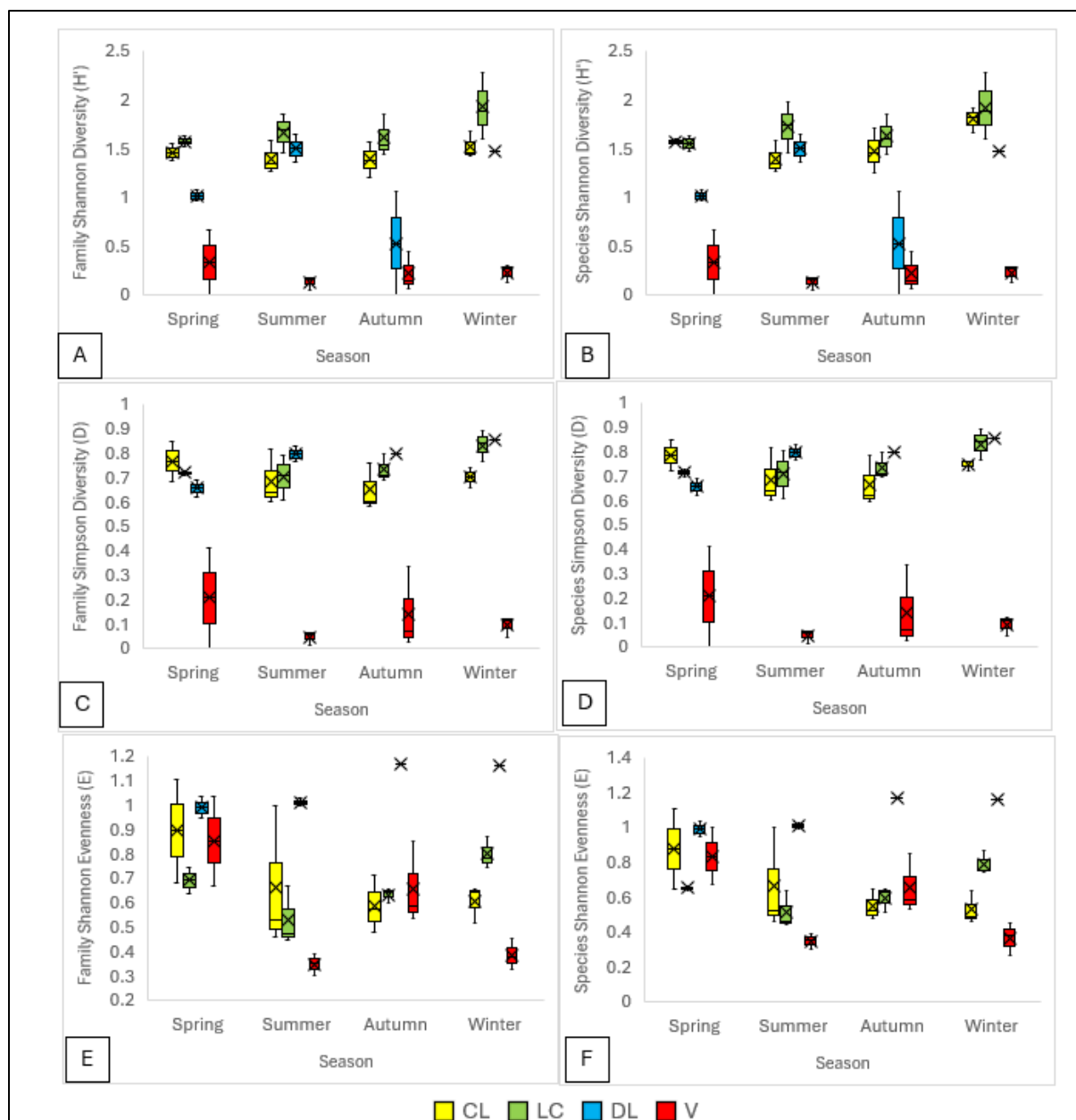


Figure 7. 9: The Shannon diversity index scores for the family level (A) and species level (B) and the Simpson diversity index scores for family level (C) and species level (D) and the Shannon Evenness measures for family (E) and species level (F) in the 2024/25 season.

For the species level in 2024/25, species diversity was highest at the LC site across the 2024/25 season (figure 7.9BD) and reached its peak in winter with a mean of 1.92 ± 0.28 H' for the Shannon Diversity score. The lowest diversity was at the Ver site in summer with a mean of 0.13 ± 0.06 H'. This was also shown by the Ver having the lowest Simpson diversity measure for species out of all the sites and it had its lowest score in summer 0.047 ± 0.02 D. The species diversity measures were significantly different between the sites (H': Kruskal-Wallis: $H(3)=26.43$, $p=7.76e-06$; D: Kruskal-Wallis: $H(3)=20.72$, $p=1.20e-04$). The post hoc test showed that the Ver site had lower diversity than CL (H': $p=0.00064$, D: $p=0.003$) and LC (H': $p=1.21e-05$; D: $p=1.21e-05$) and DL (D: $p=0.001$). However, despite this difference between the species diversity among the sites, the species diversity was similar between the seasons (figure 7.9BD) and there was not a significant difference in the both the Shannon (Kruskal-Wallis: $H(3)=3.06$, $p=0.38$) and Simpson diversity (Kruskal-Wallis: $H(3)=2.19$, $p=0.53$) measures across the seasons.

The Shannon evenness scores in the 2024/25 season were shown to decrease across the intra-annual seasons, where spring had the highest evenness score (0.86 ± 0.18) and winter had the lowest scores (0.65 ± 0.24). This was supported by the significant difference in evenness across the seasons (ANOVA: $F(3)=3.65$, $p=0.03$). The Tukey HSD test showed that summer had a significantly lower evenness score than spring ($p=0.01$). The DL site was found to have the highest evenness score (1.06 ± 0.08) and there was a significant difference across the sites too (ANOVA: $F(3)=17.59$, $p=3.72e-06$). The post-hoc Tukey HSD test also showed that DL had a significantly higher evenness score than both LC ($p=9.62e-05$) and V ($p=1.49e-06$).

For the species level, the Shannon evenness score was greatest in spring (0.86 ± 0.18) and lowest in winter (0.65 ± 0.24). There was a significant difference in Shannon evenness at the species level across the seasons (ANOVA: $F(3)=11.83$, $p=3.96e-05$) and the post hoc Tukey HSD test showed that summer had a significantly lower evenness score than spring ($p=0.03$). DL had consistently the highest Shannon evenness score (1.06 ± 0.08), which was similar to the family level scores. The two-way ANOVA showed that this difference between sites was significant ($F(3)=3.11$, $p=0.04$) and the post-hoc Tukey HSD test showed that DL was higher than both CL

($p=0.01$) and LC ($p=0.005$) and V ($p=1.41e-05$), and that V was significantly lower than CL ($p=0.03$).

The family and species level diversity scores, whilst similar and having only minor differences in the ranges and means (figures 7.9A-D) were shown to be significantly different when tested using the Wilcoxon test (table 7.6).

Table 7. 7: Family vs. Species level Wilcoxon signed rank test on 2024/25 family and species diversity scores.

Diversity Measure	Family vs. Species Level 2024/25	
	V	p-value
Simpson	78	0.02533
Shannon	99.5	0.02673

7.1.2. Inter-annual taxa diversity in the Upper Colne

Family richness displayed a similar pattern in both the 2023/24 and 2024/25 seasons, where CL and LC was higher than DL and V, although LC had a larger variation in 2023/24 and V had more outliers indicating higher family richness in 2024/24 than in 2024/25. The inter quartile range (IQR) was also smaller for all the sites in 2024/25 compared to 2023/24, indicating there was less variance in the family richness (figure 7.10A). However, there was not a significant difference in the family richness between the two years (Wilcoxon Rank Sum Test: $W=1064.5$, $p=0.061$). There were fewer individual macroinvertebrates identified in the 2024/25 season, compared to the 2023/24 season. This difference in the number of individual macroinvertebrates between the two inter-annual seasons was significantly (T-Test: $t(81) = 2.74$, $p = 0.0074$). The family diversity also showed a similar pattern where CL, LC and DL had similar diversity measures, whereas V had much lower Shannon and Simpson diversity measures across both inter-annual seasons (figure 7.10AB). The variation in family diversity was also smaller in 2024/25 than in 2023/24 (figures 7.10AB) although overall, there was not a significant difference in the diversity measures across the seasons (Wilcoxon Rank Sum Test: H': $W=928.5$, $p = 0.54$, D: $W=891.5$, $p=0.78$).

There was more variation in the Shannon Evenness scores in the 2024/25 season compared to the 2023/24 season. The Ver had consistently the lowest evenness score across the intra-annual seasons (0.47 ± 0.21). However, the average evenness scores were consistent across the seasons (2023/24: 0.60 ± 0.22 ; 2024/25: 0.69 ± 0.24) and there was no significant difference in the scores between the seasons (T-test: $p=0.10$).

There was a significant difference in Shannon evenness species scores across the sites (ANOVA: $F(3)=11.83$, $p=3.96e-05$) and the post-hoc Tukey HSD test showed that DL was significantly higher evenness score than both LC ($p=0.005$) and V ($p=1.41e-05$), and that V had a lower evenness score than CL ($p=0.03$). There was also a significant difference in evenness scores across the seasons (ANOVA: $F(3)=3.11$, $p=0.04$) and the post-hoc Tukey HSD test showed that summer was significantly lower than spring ($p=0.03$) which is similar to the family evenness scores for 2024/25.

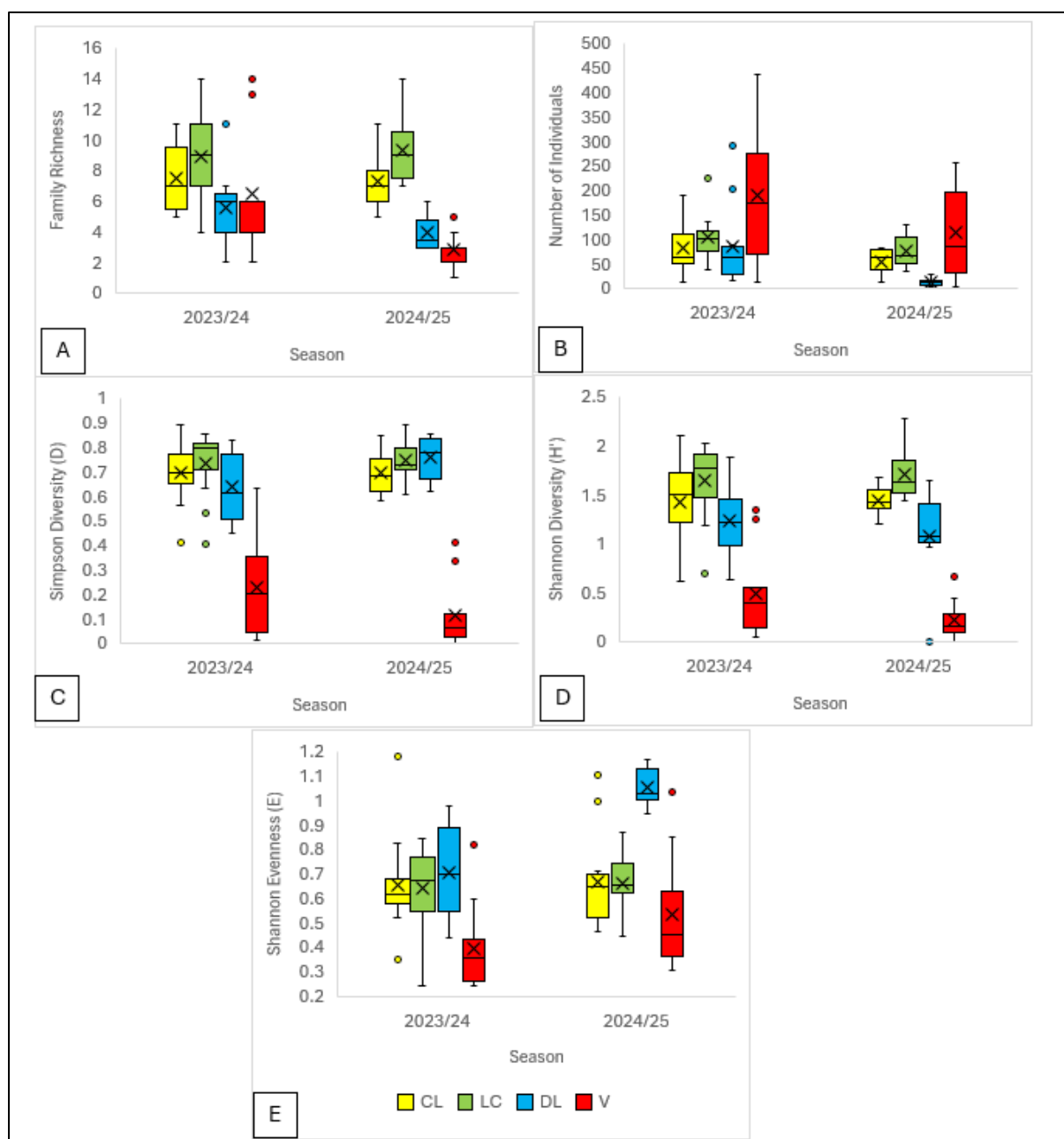


Figure 7. 10: Inter-annual variation in the Shannon (A) and Simpson diversity (B) index scores at family level between 2023/24 and 2024/25 seasons.

7.1.3. Relations between the taxa diversity and explanatory variables

The multiple linear regression model showed that the water quality variables (phosphate, ammonium and nitrate levels) had a significant effect on family richness, number of individuals, Shannon diversity and Simpson diversity. Water quality explained the largest amount of variation in the Simpson diversity ($\text{Adj } R^2 = 0.25$). In this model, nitrate had a significant negative effect on the Simpson diversity ($B = -0.05$, $p = 5.79 \times 10^{-5}$) explaining 22.6% of the variance although this was a weak relation while ammonium had a significant and stronger positive effect on Simpson Diversity ($B = 0.68$, $p = 0.04$) explaining 7.3% of the variance in Simpson Diversity (figure 7.11AB). Similarly, the nitrate level was also a significant predictor for the number of individuals ($\text{Adj } R^2 = 0.08$, $B = 9.09$, $p = 0.04$), and Shannon diversity ($\text{Adj } R^2 = 0.16$, $B = -0.12$, $p = 0.001$). The linear regression showed that nitrate explained 5.8% of the variance in the number of individual macroinvertebrates and 15.8% of the variance in Shannon Diversity (figure 7.11CD). Whilst the multiple linear regression model for predicting family richness was significant ($p = 4.65 \times 10^{-14}$) and explained 0.6% of the variance ($\text{Adj } R^2 = 0.006$), none of the individual water quality predictors had a significant effect on the family richness in the Upper Colne catchment. The graphs below are the linear regression outputs for the effect that nitrate had individually on the dependent variables.

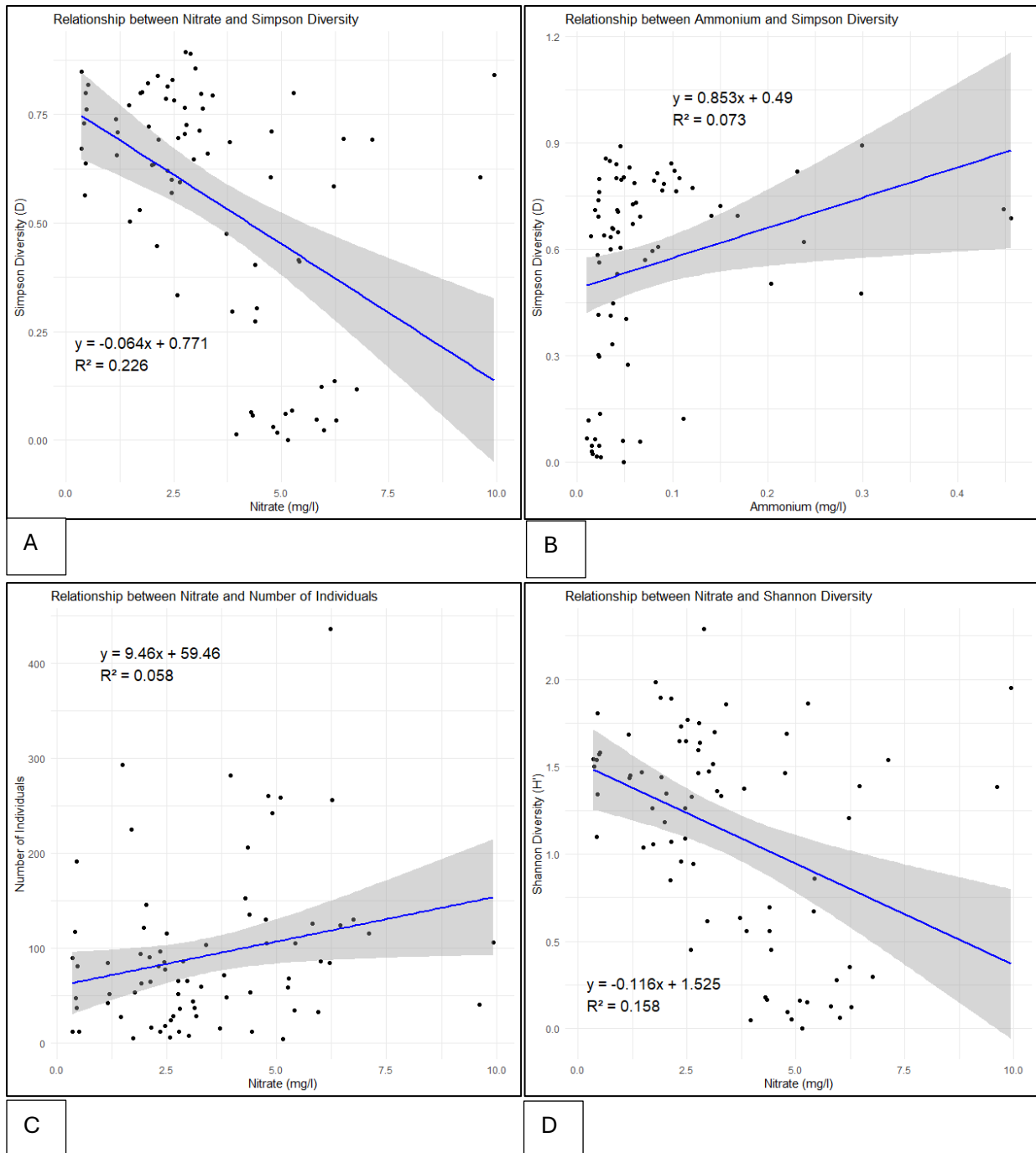


Figure 7. 11: The linear regression outputs for the significant variables from the multiple linear regression of the water quality variables on the diversity variables. (A) is the relationship between nitrate levels and Simpson diversity; (B) is the relationship between Ammonium and the Simpson diversity measure; (C) is the relationship between nitrate level and number of individuals and (D) is the relationship between nitrate levels and Shannon Diversity.

The hydrological variables significantly explained 42.21% of the variation in family richness (Adj $R^2=0.422$, $p=0.03$). Both channel width ($B=-0.41$, $p=0.02$) and average channel depth ($B=-0.82$, $p=0.01$) had a significant negative effect on family richness, but channel depth had a stronger influence. The linear regression showed that individually, channel width explained 35.1% and channel depth explained 41.6% of the variance in family richness (figure 7.12AB). Overall, the hydrological variables did not have a significant effect on the number of individual macroinvertebrates present ($p=0.19$). However, the model showed that the channel width had a significant positive effect on number of individual macroinvertebrates ($B=19.59$, $p=0.001$) and average channel depth had a significant negative effect on number of individual macroinvertebrates ($B=-251.20$, $p=0.02$). The hydrological model had a significant effect on the diversity measures (Shannon: $p = 6.95e-13$, Adj $R^2 = 0.41$; Simpson: $p = 4.70e-12$, Adj $R^2 = 0.34$) and for both models channel width had a slightly negative significant effect on diversity (Shannon: -0.18 , $p=3.04e-06$; Simpson: -0.08 , $p = 1.75e-06$) and was found to explain 34.7% of the variation in both Shannon and Simpson diversity indices (figure 7.12CD).

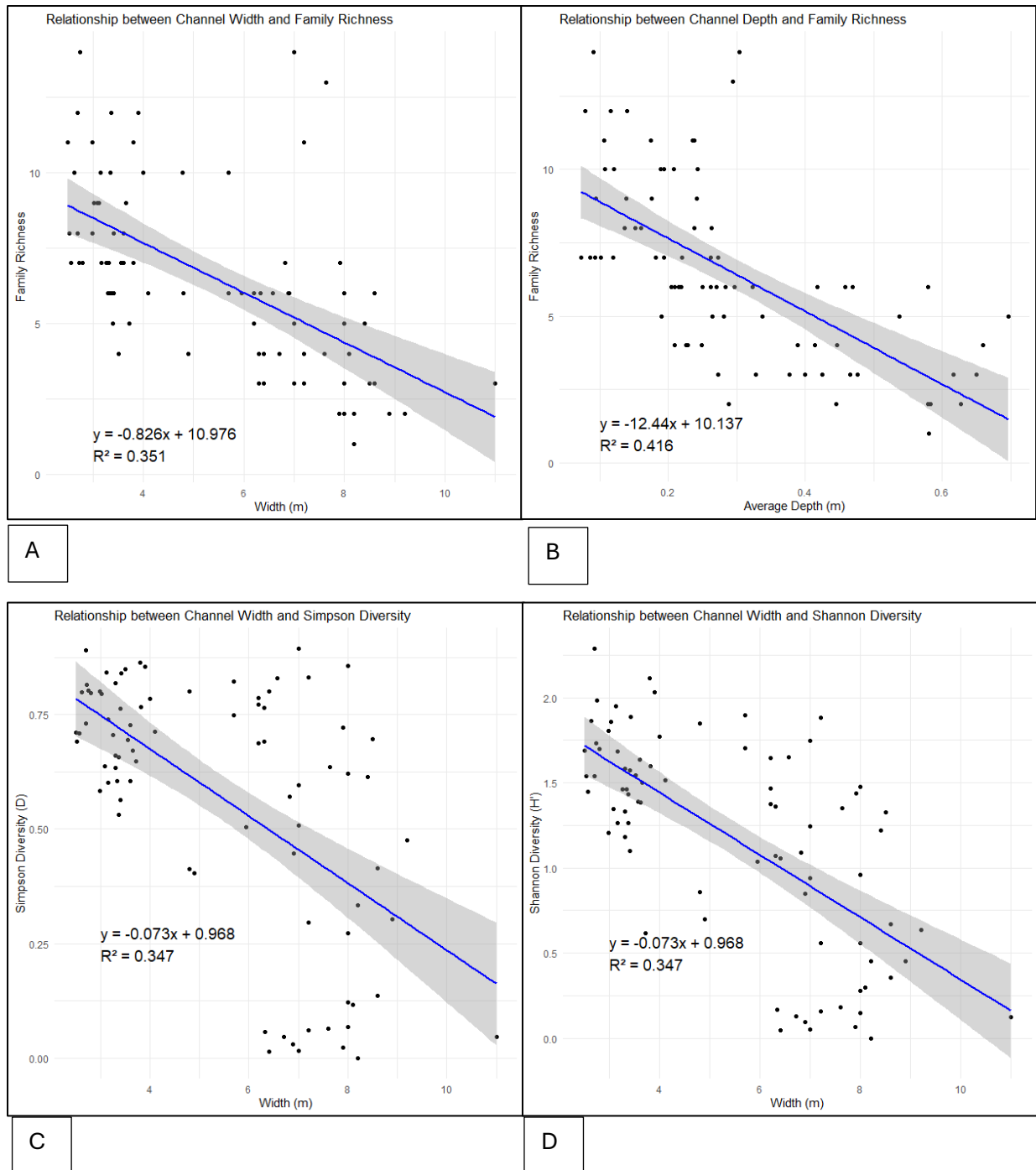


Figure 7. 12: The linear regression outputs for the significant variables from the multiple linear regression of the hydrological variables on the diversity variables. (A) is the relationship between channel width and family richness; (B) is the relationship between average channel depth and family richness; (C) is the relationship between channel width and Simpson diversity and (D) is the relationship between channel width and Shannon Diversity.

The physical-chemical model explained most of the variance in the number of individual macroinvertebrates ($p = 1.88\text{e-}34$, $\text{Adj } R^2 = 0.10$). However, the only significant predictor in this model was turbidity which had a very small negative influence on the number of macroinvertebrates ($B=-1.94\text{e-}04$, $p=0.04$) (figure 7.13A). BOD also had a small significant negative effect on Simpson diversity ($B=-0.03$, $p=0.03$) (figure 7.13B).

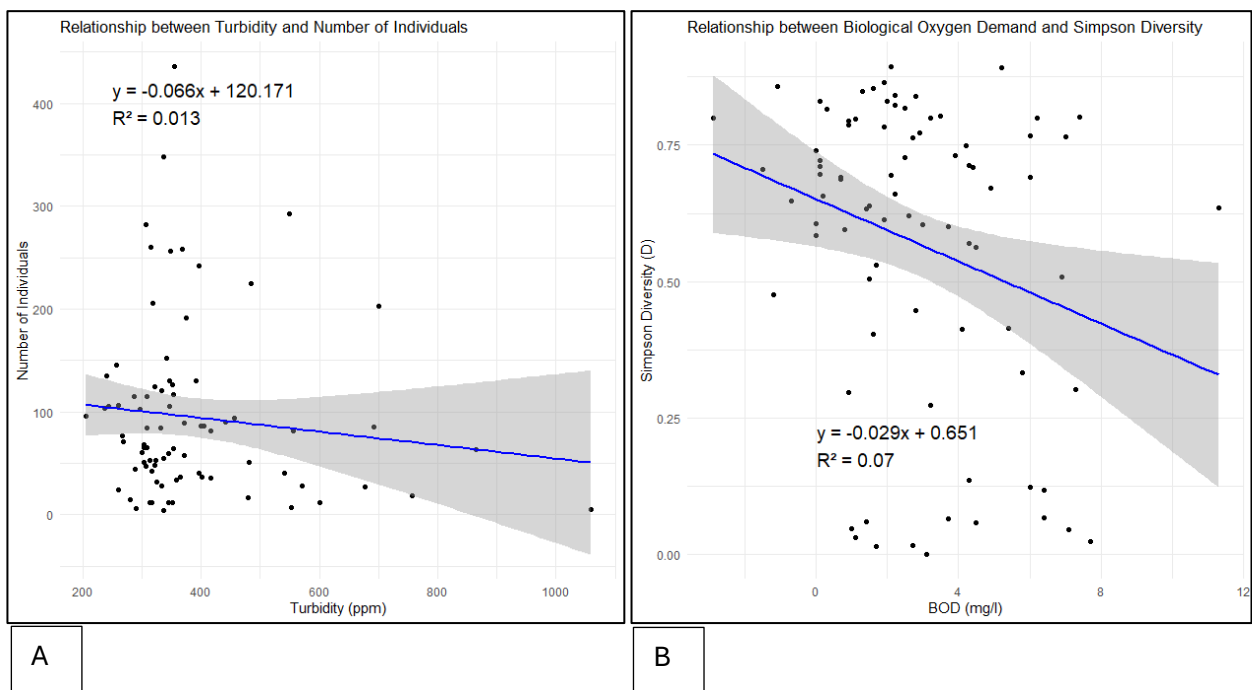


Figure 7. 13: The linear regression outputs for the significant variables from the multiple linear regression of the physical-chemical variables on the diversity variables. (A) is the relationship between turbidity and number of individuals; (B) is the relationship between biological oxygen demand and Simpson diversity.

7.2. Macroinvertebrate taxa community composition in the Upper Colne Catchment

In the 2023/24 season the non-metric multidimensional scaling analysis (NMDS) had a stress value of 0.187 which indicated a fair representation of the community data at the sites and the solution was stable after 6 random starts out of 20 attempts. However, as the stress value is just below the 0.20 threshold, interpretation of the NMDS ordination was acceptable but not perfect and over-interpretation of small differences between points was avoided. The NMDS determined that the family *Gammaridae* was strongly positively associated with NMDS 1 (0.99, $p=0.001$) and *Hydrobiidae* was strongly negatively associated with NMDS 1 axis (-0.93, $p=0.001$). The family *Asellidae* was strongly positively associated with axis NMDS 2 (0.8, $p=0.001$) and *Erpodeiidea* was strongly negatively associated with NMDS 2 (-0.84, $p=0.001$). This means that these families, along with those shown in table 7.8 were influencing the ordination of the site clusters in the NMDS ordination plot (figure 7.14A). Summer and winter significances were furthest apart meaning the macroinvertebrate communities occurring in these seasons were different. The NMDS plot clearly separated the winter and summer seasons, thereby indicating a difference in the species composition in these seasons. This was confirmed by the ANOSIM ($R=0.17$, $p=0.001$) where the dissimilarity ranks between the seasons (median =477.5) was greater than the seasons (Spring = 312, Summer =337, Autumn = 465, Winter = 358). However, each season overlapped with at least one other, suggesting similarities in the community composition across seasons. The pairwise ANOSIM did not find any statistically significant differences between the seasons which also suggests that the macroinvertebrate community composition between the seasons were similar. The NMDS suggested that the families negatively associated with NMDS2 influenced summer and spring, whilst the species that positively influenced NMDS2 influenced autumn and winter. The sites had clear groupings of macroinvertebrate communities in the 2023/24 season shown in figure 7.14B. The V and CL sites did not overlap with any other sites, suggesting they had significant community structures. There was some overlap with the LC and DL significance ellipses, suggesting that they share some similarities in the community composition. The differences between the sites was more influenced by NMDS1 axis and associated with the relationships those axes

have with certain species (table 7.8). The macroinvertebrate community at the V site was more aligned with the *Gammaridae* family, and the CL had more influence from the *Assellidae*, *Erpodeiidae* and *Glossiphoniidae* families. The ANOSIM supported this difference too ($R = 0.57$, $p = 0.001$). The dissimilarity rank between the sites (median = 531) was greater than within the sites (CL = 272, LC = 278, DL = 166, V = 37) which suggests that there were differences between the macroinvertebrate assemblages between the sites. In particular, V was very different to the other three sites and CL and LC were most similar. The pairwise ANOSIM also indicated that each site was significantly different from each other ($p = 0.006$) with CL and V having the strongest dissimilarity ($R = 0.914$).

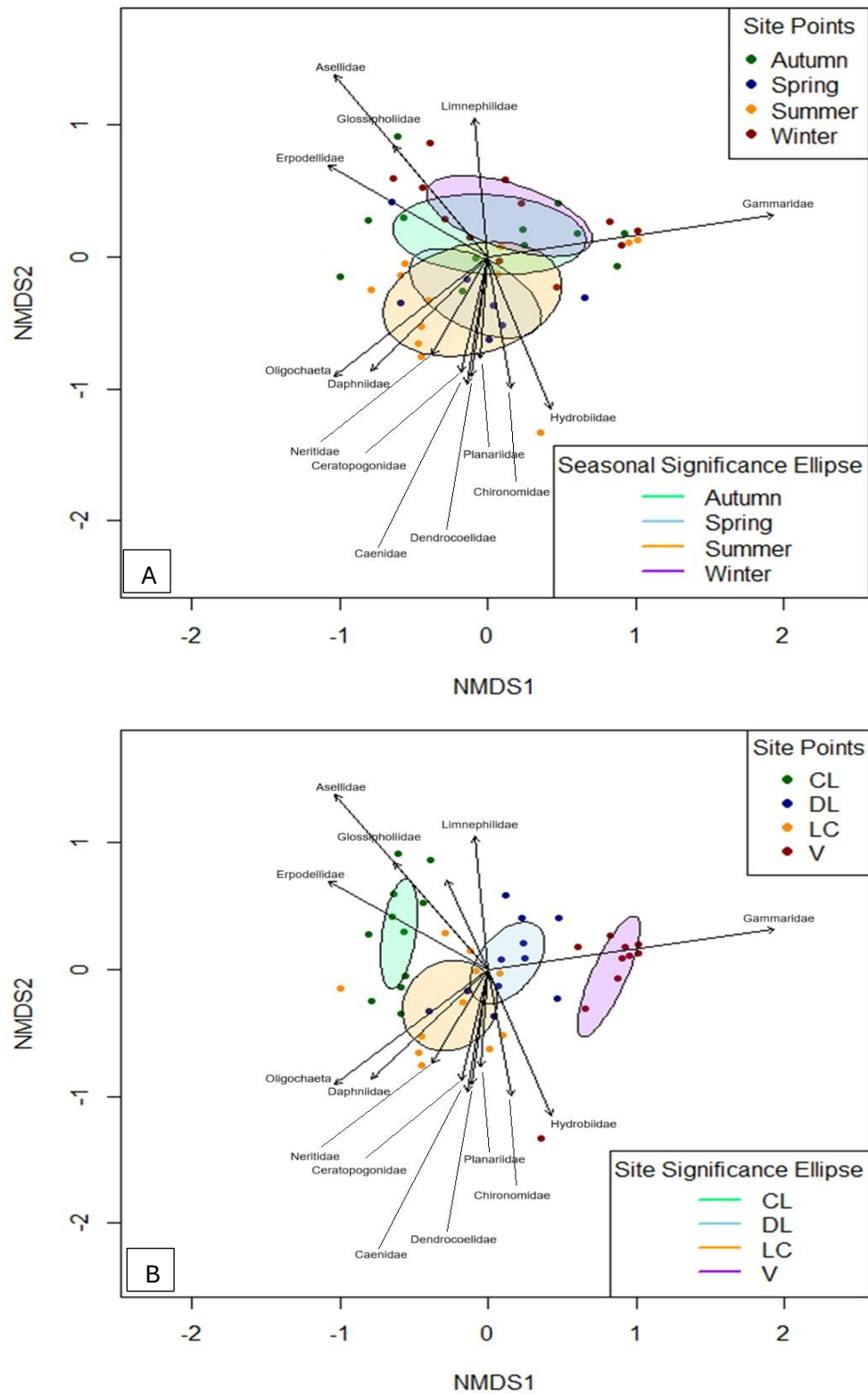


Figure 7. 14:The NMDS ordination plot with the top 10 families contributing to the NMDS 1 and NMDS2 axis, with the 95% significance limits for the seasonal groupings (A) and the site groupings (B) in the 2023/24 season.

Table 7. 8: The significant macroinvertebrate families and their contributions to the NMDS axes for the 2023/24 season. There was a 95% confidence limit and if $p < 0.05$, the family was a significant contributor to the axis.

2023/24 Significant Families	NMDS1	NMDS2	pvals
<i>Assellius</i>	-0.60	0.80	0.001
<i>Daphniidae</i>	-0.68	-0.74	0.001
<i>Erpodeiidae</i>	-0.84	0.54	0.001
<i>Gammarus</i>	0.99	0.16	0.001
<i>Hydrobiidae</i>	0.35	-0.94	0.001
<i>Oligochetes</i>	-0.75	-0.66	0.001
<i>Glossiphoniidae</i>	-0.60	0.80	0.002
<i>Limnephilidae</i>	-0.09	1.00	0.004
<i>Chironomides</i>	-0.15	-0.99	0.007
<i>Caenidae</i>	-0.13	-0.99	0.01
<i>Ceratopogonidae</i>	-0.20	-0.98	0.016
<i>Tipulidae</i>	0.16	-0.99	0.026
<i>Neritidae</i>	-0.45	-0.89	0.033
<i>Dendrocoelidae</i>	-0.37	0.93	0.038
<i>Planariidae</i>	-0.37	0.93	0.038
<i>Culicidae.pupa</i>	-0.07	-1.00	0.043

In the 2023/24 season, there was a significant difference in the macroinvertebrate community across the sites (PERMANOVA: $F(3,42)=9.900$, $p=0.001$) and the sites were shown to explain 43.23% of the variance in the macroinvertebrate community ($R^2=0.43$). The CL and V sites had a 90.03% largest dissimilarity in community composition Gammaridae contributed most to the observed dissimilarity, accounting for 22.4% (table 7.9). This difference was statistically significant and consistently observed (ratio = 2.65, $p=0.001$), with average abundances of 0.8 at CL and 0.85 at V. Assellidae also explained 15.2% of the dissimilarity between the sites ($p=0.001$) with higher abundance at site V compared to CL. Other families (Limnephilidae, Erpodeiidae, Glossiphoniidae and Hydrobiidae) also contributed significantly to the dissimilarity, though to a lesser extent, with individual contributions, ranging between

4.39 and 2.93%. The LC and V sites had the next largest dissimilarity with 76.98% dissimilarity. Here, Gammaridae explained 16.03% of the dissimilarity between the two sites, with a significantly higher abundance at LC than V ($p=0.001$). The most similar sites were LC and DL with a 58.08% dissimilarity. Gammaridae explained the most dissimilarity but only contributed 7.15% to the dissimilarity and this was not significant ($p=0.99$). However, there were only two families with significant dissimilarity scores for LC and DL. *Baetidae* explained 5.78% with a lower abundance at DL than LC ($p=0.007$) and Ephemeridae explained 2.76% with a slightly higher abundance at LC than DL ($p=0.03$).

Table 7. 9: The top 10 families for each site comparison for the SIMPER analysis on the 2023/24 family communities. The families are ranked with the highest contributor to dissimilarity at the top, and the lowest contributor in the top 10 at the bottom for each site comparison. The p-value indicates whether the contribution of the family is significant in the dissimilarity. If $p < 0.05$, the dissimilarity is significant.

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
CL_LC	<i>Assellidae</i>	0.07	0.63	0.05	1.55	0.61	0.28	0.11	0.711
CL_LC	<i>Daphniidae</i>	0.06	0.63	0.06	0.90	0.19	0.19	0.80	0.276
CL_LC	<i>Oligochaeta</i>	0.05	0.63	0.05	1.11	0.30	0.45	1.00	0.965
CL_LC	<i>Gammaridae</i>	0.05	0.63	0.03	1.52	0.01	0.27	0.92	1
CL_LC	<i>Baetidae</i>	0.05	0.63	0.04	1.35	0.15	0.31	0.29	0.275
CL_LC	<i>Chironomides</i>	0.04	0.63	0.03	1.30	0.12	0.21	0.62	0.788
CL_LC	<i>Erpodellidae</i>	0.04	0.63	0.03	1.18	0.16	0.09	0.91	0.033
CL_LC	<i>Glossiphiliidae</i>	0.03	0.63	0.03	1.03	0.15	0.10	0.95	0.049
CL_LC	<i>Limnephilidae</i>	0.03	0.63	0.04	0.84	0.15	0.03	1.00	0.307
CL_LC	<i>Ephemeridae</i>	0.02	0.63	0.04	0.65	0.00	0.12	0.89	0.094
CL_DL	<i>Gammaridae</i>	0.13	0.64	0.05	2.65	0.01	0.55	0.95	0.02
CL_DL	<i>Oligochaeta</i>	0.06	0.64	0.05	1.29	0.30	0.30	1.00	0.728
CL_DL	<i>Daphniidae</i>	0.06	0.64	0.07	0.86	0.19	0.12	0.86	0.324
CL_DL	<i>Assellidae</i>	0.05	0.64	0.04	1.26	0.61	0.47	0.20	0.998
CL_DL	<i>Chironomides</i>	0.04	0.64	0.03	1.37	0.12	0.20	0.70	0.703
CL_DL	<i>Limnephilidae</i>	0.04	0.64	0.04	0.93	0.15	0.06	1.00	0.054
CL_DL	<i>Erpodellidae</i>	0.04	0.64	0.03	1.16	0.16	0.00	0.94	0.013
CL_DL	<i>Baetidae</i>	0.03	0.64	0.03	1.13	0.15	0.09	0.38	0.977

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
CL_DL	<i>Glossipholiidae</i>	0.03	0.64	0.04	0.88	0.15	0.01	0.97	0.036
CL_DL	<i>Coenagrionidae</i>	0.02	0.64	0.03	0.85	0.09	0.02	0.75	0.006
CL_V	<i>Gammaridae</i>	0.22	0.90	0.08	2.65	0.01	0.85	0.96	0.001
CL_V	<i>Assellidae</i>	0.15	0.90	0.06	2.57	0.61	0.03	0.25	0.001
CL_V	<i>Oligochaeta</i>	0.07	0.90	0.06	1.31	0.30	0.01	1.00	0.187
CL_V	<i>Daphniidae</i>	0.05	0.90	0.07	0.71	0.19	0.00	0.88	0.547
CL_V	<i>Limnephilidae</i>	0.04	0.90	0.04	1.02	0.15	0.08	1.00	0.011
CL_V	<i>Erpodellidae</i>	0.04	0.90	0.04	1.14	0.16	0.00	0.95	0.001
CL_V	<i>Glossipholiidae</i>	0.04	0.90	0.04	0.85	0.15	0.00	0.97	0.007
CL_V	<i>Baetidae</i>	0.04	0.90	0.03	1.23	0.15	0.11	0.50	0.923
CL_V	<i>Chironomides</i>	0.03	0.90	0.03	1.03	0.12	0.09	0.73	0.95
CL_V	<i>Hydrobiidae</i>	0.03	0.90	0.05	0.65	0.00	0.13	0.99	0.009
LC_DL	<i>Gammaridae</i>	0.07	0.58	0.05	1.44	0.27	0.55	0.93	0.996
LC_DL	<i>Oligochaeta</i>	0.06	0.58	0.05	1.17	0.45	0.30	1.00	0.704
LC_DL	<i>Assellidae</i>	0.06	0.58	0.05	1.33	0.28	0.47	0.12	0.975
LC_DL	<i>Baetidae</i>	0.06	0.58	0.04	1.44	0.31	0.09	0.33	0.007
LC_DL	<i>Daphniidae</i>	0.06	0.58	0.07	0.77	0.19	0.12	0.84	0.325
LC_DL	<i>Chironomidae</i>	0.04	0.58	0.03	1.33	0.21	0.20	0.68	0.62
LC_DL	<i>Ephemeridae</i>	0.03	0.58	0.04	0.74	0.12	0.02	0.91	0.028
LC_DL	<i>Erpodellidae</i>	0.02	0.58	0.04	0.52	0.09	0.00	0.92	0.724
LC_DL	<i>Glossipholiidae</i>	0.02	0.58	0.03	0.71	0.10	0.01	0.95	0.713
LC_DL	<i>Caenidae</i>	0.02	0.58	0.03	0.75	0.07	0.04	0.60	0.145
LC_V	<i>Gammaridae</i>	0.16	0.77	0.07	2.30	0.27	0.85	0.96	0.001
LC_V	<i>Oligochaeta</i>	0.11	0.77	0.06	1.83	0.45	0.01	1.00	0.001

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
LC_V	<i>Assellidae</i>	0.06	0.77	0.04	1.72	0.28	0.03	0.21	0.944
LC_V	<i>Baetidae</i>	0.06	0.77	0.05	1.32	0.31	0.11	0.43	0.003
LC_V	<i>Daphniidae</i>	0.05	0.77	0.08	0.59	0.19	0.00	0.86	0.554
LC_V	<i>Chironomidae</i>	0.05	0.77	0.04	1.27	0.21	0.09	0.71	0.089
LC_V	<i>Ephemeraidae</i>	0.03	0.77	0.04	0.75	0.12	0.02	0.94	0.007
LC_V	<i>Hydrobiidae</i>	0.03	0.77	0.04	0.70	0.03	0.13	0.99	0.008
LC_V	<i>Erpodeiidae</i>	0.02	0.77	0.05	0.52	0.09	0.00	0.95	0.553
LC_V	<i>Glossiphiliidae</i>	0.02	0.77	0.03	0.67	0.10	0.00	0.97	0.564
DL_V	<i>Assellidae</i>	0.13	0.63	0.07	1.78	0.47	0.03	0.21	0.001
DL_V	<i>Gammaridae</i>	0.11	0.63	0.06	2.04	0.55	0.85	0.97	0.146
DL_V	<i>Oligochaeta</i>	0.08	0.63	0.07	1.17	0.30	0.01	1.00	0.063
DL_V	<i>Chironomidae</i>	0.05	0.63	0.04	1.32	0.20	0.09	0.80	0.03
DL_V	<i>Baetidae</i>	0.04	0.63	0.03	1.10	0.09	0.11	0.52	0.891
DL_V	<i>Daphniidae</i>	0.03	0.63	0.07	0.47	0.12	0.00	0.90	0.895
DL_V	<i>Hydrobiidae</i>	0.03	0.63	0.05	0.65	0.00	0.13	0.99	0.002
DL_V	<i>Limnephilidae</i>	0.03	0.63	0.03	0.98	0.06	0.08	1.00	0.609
DL_V	<i>Lymnaeidae</i>	0.02	0.63	0.03	0.48	0.00	0.07	1.00	0.429
DL_V	<i>Caenidae</i>	0.01	0.63	0.03	0.46	0.04	0.02	0.71	0.677

There was a significant difference in the macroinvertebrate community composition in the 2023/24 season (PERMANOVA: $F(3,42) = 2.77$, $p = 0.002$). The model explained 17.56% of the variance in the community composition (Adjusted $R^2 = 0.18$) in this season. The dissimilarity between summer and winter was the greatest at 68.85% and this, along with the other seasonal comparisons is detailed in table 7.10. Gammaridae had the highest dissimilarity score for summer-winter with 10.71% and a greater abundance in winter than summer, but this was not significant ($p = 0.34$). However, *Daphniidae* contributed the next largest share to the dissimilarity, explaining 9.25%. This was statistically significant ($p = 0.001$), with higher abundance in summer and complete absence in winter. The *Limnephilidae* family significantly explained 5.14% of the dissimilarity between summer and winter ($p = 0.001$) with a greater average abundance in summer. Summer and autumn had the next highest dissimilarity at 66.5%. *Gammaridae* explained the most of the variation but this was not significant ($p = 0.13$). Therefore, *Daphniidae* could be an indicator between the seasons with a significant contribution to dissimilarity of 9.40% ($p = 0.001$) and greater abundance in summer (0.41) than winter (0.06). These were the only two significant families for the summer-autumn comparison. The smallest dissimilarity between macroinvertebrate communities was between autumn and winter in 2023/24 at 62.4%. *Limnephilidae* and *Glossiphonidae* had a higher abundance in winter than in autumn and were significant in explaining the dissimilarity (*Limnephilidae*: 5.49%, $p = 0.001$; *Glossiphonidae*: 3.51%, $p = 0.006$). *Erpodeiidae* also significantly explained 3.93% of the dissimilarity between the autumn and winter seasons, with a greater abundance in autumn than winter ($p = 0.003$).

Table 7. 10: The top 10 families for each seasonal comparison for the SIMPER analysis on the 2023/24 family communities. The families are ranked with the highest contributor to dissimilarity at the top, and the lowest contributor in the top 10 at the bottom for each site comparison. The p-value indicates whether the contribution of the family is significant in the dissimilarity. If $p < 0.05$, the dissimilarity is significant.

Comparison	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
Spring_Summer	<i>Daphniidae</i>	0.08	0.65	0.06	1.24	0.00	0.41	0.78	0.019
Spring_Summer	<i>Gammaridae</i>	0.08	0.65	0.06	1.20	0.31	0.32	0.92	0.982
Spring_Summer	<i>Oligochaeta</i>	0.06	0.65	0.05	1.26	0.44	0.29	1.00	0.738
Spring_Summer	<i>Assellidae</i>	0.05	0.65	0.04	1.28	0.31	0.26	0.12	0.996
Spring_Summer	<i>Chironomidae</i>	0.05	0.65	0.03	1.48	0.36	0.16	0.63	0.106
Spring_Summer	<i>Baetidae</i>	0.04	0.65	0.03	1.28	0.13	0.22	0.33	0.891
Spring_Summer	<i>Caenidae</i>	0.03	0.65	0.03	1.06	0.16	0.03	0.54	0.003
Spring_Summer	<i>Hydrobiidae</i>	0.03	0.65	0.04	0.69	0.07	0.07	0.98	0.193
Spring_Summer	<i>Simulidae</i>	0.02	0.65	0.02	0.96	0.11	0.00	1.00	0.2
Spring_Summer	<i>Ceratopogonidae</i>	0.02	0.65	0.02	1.03	0.10	0.02	0.59	0.002
Spring_Autumn	<i>Gammaridae</i>	0.09	0.66	0.07	1.37	0.31	0.49	0.92	0.816
Spring_Autumn	<i>Oligochaeta</i>	0.08	0.66	0.06	1.38	0.44	0.22	1.00	0.08
Spring_Autumn	<i>Assellidae</i>	0.06	0.66	0.05	1.39	0.31	0.42	0.14	0.892
Spring_Autumn	<i>Chironomidae</i>	0.06	0.66	0.04	1.68	0.36	0.12	0.64	0.005
Spring_Autumn	<i>Baetidae</i>	0.03	0.66	0.03	1.19	0.13	0.14	0.36	0.95

Comparison	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
Spring_Autumn	<i>Caenidae</i>	0.03	0.66	0.03	0.99	0.16	0.00	0.55	0.001
Spring_Autumn	<i>Erpodellidae</i>	0.03	0.66	0.03	0.94	0.03	0.15	0.90	0.155
Spring_Autumn	<i>Ephemeridae</i>	0.03	0.66	0.04	0.75	0.08	0.09	0.88	0.096
Spring_Autumn	<i>Simulidae</i>	0.03	0.66	0.03	0.97	0.11	0.00	1.00	0.13
Spring_Autumn	<i>Ceratopogonidae</i>	0.02	0.66	0.02	0.98	0.10	0.00	0.60	0.001
Spring_Winter	<i>Gammaridae</i>	0.09	0.66	0.06	1.33	0.31	0.48	0.95	0.925
Spring_Winter	<i>Oligochaeta</i>	0.07	0.66	0.06	1.29	0.44	0.21	1.00	0.299
Spring_Winter	<i>Assellidae</i>	0.07	0.66	0.05	1.44	0.31	0.40	0.13	0.723
Spring_Winter	<i>Chironomidae</i>	0.06	0.66	0.03	2.00	0.36	0.08	0.66	0.004
Spring_Winter	<i>Limnephilidae</i>	0.04	0.66	0.03	1.33	0.04	0.22	1.00	0.026
Spring_Winter	<i>Baetidae</i>	0.03	0.66	0.03	1.20	0.13	0.16	0.35	0.945
Spring_Winter	<i>Caenidae</i>	0.03	0.66	0.03	1.02	0.16	0.02	0.56	0.004
Spring_Winter	<i>Simulidae</i>	0.03	0.66	0.03	1.16	0.11	0.10	1.00	0.028
Spring_Winter	<i>Glossipholiidae</i>	0.03	0.66	0.03	0.96	0.04	0.14	0.98	0.203
Spring_Winter	<i>Ceratopogonidae</i>	0.02	0.66	0.02	0.98	0.10	0.00	0.61	0.004
Summer_Autumn	<i>Gammaridae</i>	0.11	0.67	0.09	1.24	0.32	0.49	0.94	0.129
Summer_Autumn	<i>Daphniidae</i>	0.09	0.67	0.08	1.22	0.41	0.06	0.87	0.001
Summer_Autumn	<i>Assellidae</i>	0.08	0.67	0.06	1.26	0.26	0.42	0.17	0.483
Summer_Autumn	<i>Oligochaeta</i>	0.07	0.67	0.06	1.21	0.29	0.22	1.00	0.319
Summer_Autumn	<i>Baetidae</i>	0.05	0.67	0.04	1.29	0.22	0.14	0.43	0.104

Comparison	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
Summer_Autumn	<i>Chironomidae</i>	0.04	0.67	0.03	1.26	0.16	0.12	0.76	0.546
Summer_Autumn	<i>Erpodeiidae</i>	0.04	0.67	0.04	0.93	0.03	0.15	0.93	0.008
Summer_Autumn	<i>Ephemeriidae</i>	0.02	0.67	0.03	0.61	0.01	0.09	0.92	0.334
Summer_Autumn	<i>Hydrobiidae</i>	0.02	0.67	0.05	0.37	0.07	0.00	0.98	0.513
Summer_Autumn	<i>hydrophilidae</i>	0.02	0.67	0.02	0.68	0.06	0.01	0.99	0.131
Summer_Winter	<i>Gammaridae</i>	0.11	0.69	0.09	1.25	0.32	0.48	0.95	0.342
Summer_Winter	<i>Daphniidae</i>	0.09	0.69	0.08	1.22	0.41	0.00	0.87	0.001
Summer_Winter	<i>Assellidae</i>	0.08	0.69	0.06	1.26	0.26	0.40	0.16	0.263
Summer_Winter	<i>Oligochaeta</i>	0.06	0.69	0.05	1.21	0.29	0.21	1.00	0.868
Summer_Winter	<i>Limnephilidae</i>	0.05	0.69	0.04	1.37	0.01	0.22	1.00	0.001
Summer_Winter	<i>Baetidae</i>	0.05	0.69	0.04	1.32	0.22	0.16	0.41	0.179
Summer_Winter	<i>Glossipholiidae</i>	0.03	0.69	0.04	0.89	0.03	0.14	0.96	0.054
Summer_Winter	<i>Chironomides</i>	0.03	0.69	0.02	1.27	0.16	0.08	0.73	0.995
Summer_Winter	<i>Simuliidae</i>	0.02	0.69	0.03	0.65	0.00	0.10	1.00	0.229
Summer_Winter	<i>Hydrobiidae</i>	0.02	0.69	0.05	0.44	0.07	0.02	0.99	0.398
Autumn_Winter	<i>Gammaridae</i>	0.11	0.62	0.09	1.26	0.49	0.48	0.98	0.176
Autumn_Winter	<i>Assellidae</i>	0.09	0.62	0.07	1.30	0.42	0.40	0.18	0.017
Autumn_Winter	<i>Oligochaeta</i>	0.07	0.62	0.06	1.03	0.22	0.21	1.00	0.542
Autumn_Winter	<i>Limnephilidae</i>	0.05	0.62	0.04	1.33	0.03	0.22	1.00	0.001
Autumn_Winter	<i>Baetidae</i>	0.05	0.62	0.04	1.14	0.14	0.16	0.44	0.361

Comparison	Family	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	ratio (average contribution / sd) Higher = more consistent	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity (up to 70%)	p-value
Autumn_Winter	<i>Erpodellidae</i>	0.04	0.62	0.04	0.90	0.15	0.03	0.97	0.003
Autumn_Winter	<i>Chironomidae</i>	0.04	0.62	0.03	1.05	0.12	0.08	0.78	0.906
Autumn_Winter	<i>Glossipholiidae</i>	0.04	0.62	0.04	0.84	0.03	0.14	0.98	0.006
Autumn_Winter	<i>Simuliidae</i>	0.02	0.62	0.04	0.65	0.00	0.10	1.00	0.15
Autumn_Winter	<i>Ephemeridae</i>	0.02	0.62	0.03	0.68	0.09	0.01	0.96	0.199

In 2024/25 season, the macroinvertebrate family communities showed substantial overlap across the four intra-annual seasons (figure 7.15A). The two-dimensional solution had a stress value of 0.142, indicating a fair representation of the data reduced dimensions. The solution was stable and achieved after 2 random starts out of 20 attempts. The stress value of 0.142 is just below the 0.20 critical value, meaning that the interpretation of the NMDS ordination was acceptable but not perfect and over-interpretation of small differences between points should be avoided. For the axis, The *Gammaridae* family had a strong positive association (0.93, $p=0.001$) with NMDS1 while *Oligochaeta* had a strong negative association (-0.99, $p=0.001$) (table 7.11). *Baetidae* was strongly positively associated with the NMDS2 axis, (0.99, $p=0.001$) while the *Chironomidae* showed a strong negatively association (-0.94, $p=0.001$) (table 7.11).

Spring overlapped with all of the other three seasons and did not appear to be influenced particularly by either NMDS1 or NMDS2 (figure 7.15A). The summer significance ellipse was more negatively influenced by NMDS2, suggesting a weak relation to the *Chironomidae* family. The winter season was slightly positively aligned with NMDS1 axis, suggesting an influence by *Baetidae*. There was also a cluster of points outside of the significance ellipses more aligned with NMDS1, suggesting that these points were more influenced by the *Gammaridae* family. However, there was no significant difference in the macroinvertebrate family community composition across the intra-annual seasons (PERMANOVA: $F(3,38) = 0.75$, $p = 0.69$) and only explained 6% of the variance in community composition (Adjusted $R^2 = 0.06$). The ANOSIM also showed there was not a statistically significant difference in macroinvertebrate community structure between the seasons ($R=-0.03$, $p=0.69$). There were distinct macroinvertebrate communities displayed at the four different sites in the 2024/25 season. The Ver site was especially distinct and was more associated with NMDS1 and the *Gammaridae* family had a strong influence on the community at this site (figure 7.15B). The CL site was the most different to V, and more associated negatively with NMDS 1 and families such as *Oligochaeta* and *Assellidae*. The LC and DL sites were the only sites to have overlap, suggesting that the community composition at these sites was similar, although due to the larger significance ellipse of the DL site, this suggests that it was more influenced by the top species from the NMDS than LC. This difference between the communities were significant (PERMANOVA: $F(3,38) = 12.994$, $p=0.001$) and the site was found to explain 56.69% of the variation in community (Adjusted $R^2 = 0.53$). This difference between the sites was also supported by the ANOSIM ($R=0.81$, $p=0.001$) and the difference between the sites (median = 442) was greater than within the sites (CI = 127, LC = 132, DL = 466, V=28). The Ver site was

the most different macroinvertebrate community between the sites once again, and CL and LC were most similar. The pairwise ANOSIM also showed that all the sites were significantly different from each other ($p=0.001$) and that CL and V were most dissimilar ($R=0.93$) and LC and DL were most similar ($R=0.44$).

Table 7. 11: The significant macroinvertebrate families and their contributions to the NMDS axes for the 2024/25 season.

2024/25 Significant Families	NMDS1	NMDS2	p-value
<i>Asellidae</i>	-0.90	-0.44	0.001
<i>Baetidae</i>	-0.12	0.99	0.001
<i>Chironomidae</i>	-0.34	-0.94	0.001
<i>Ephemeraeidae</i>	0.14	0.99	0.001
<i>Gammarus</i>	0.93	-0.38	0.001
<i>Oligochaeta</i>	-0.99	-0.13	0.001
<i>Erpodeiidae</i>	-0.66	0.75	0.002
<i>Halipidae</i>	-0.63	-0.77	0.013
<i>Goeridae</i>	0.20	0.98	0.016
<i>Dytiscidae</i>	-0.58	-0.81	0.029

The largest overall dissimilarity in macroinvertebrate family communities between sites in the 2024/25 season was between CL and V with 92.93 % (table 7.12). *Gammaridae* contributed the largest proportion of dissimilarity at 23.58%, and having an average abundance score of 0.69 at CL and 0.89 at V, and this was significant ($p=0.001$). The two following largest contributing families were *Asellidae* (19.55% contribution, 0.69 average abundance at CL, 0 at V, $p=0.001$) and *Oligochaeta* (9.28% contribution, 0.33 average abundance at CL, 0 at V, $p=0.002$), which suggested that these three families were drivers of community dissimilarity between these two sites. LC and V had the next highest dissimilarity at 78.59%, followed by DL and V with 72.63% and they had the same top three families contributing to the dissimilarity (*Gammaridae*, *Oligochaeta* and *Asellidae*), although *Asellidae* was not a significant family in either comparison (LC-V: $p=0.732$, DL-V: $p=0.427$). Instead, for LC-V,

Baetidae contributed to 7.95% of the dissimilarity and had greater abundance at LC than V ($p=0.01$) and for DL – V, Chironomidae contributed to 7.43% of the dissimilarity and had a greater abundance at DL ($p=0.009$). The site comparison with the lowest dissimilarity was CL-LC at 58.39%. In this comparison, only one of the top contributors were significant and this was *Lymnaeidae* at 3.6% ($p=0.002$) and there was a greater abundance of *Lymnaeidae* at LC than CL (table 7.12).

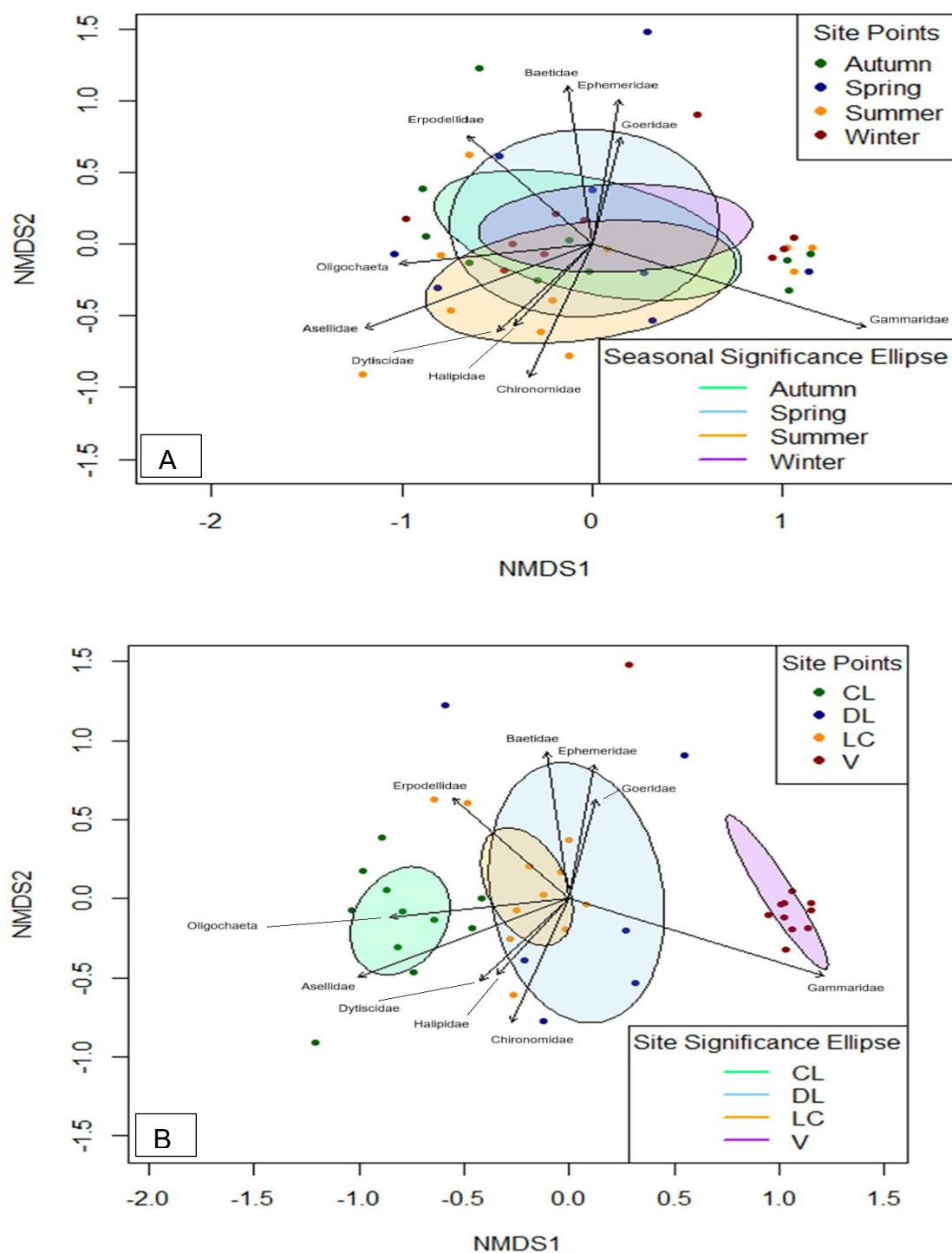


Figure 7. 15: The NMDS ordination plot with the top families contributing to the NMD S 1 and NMDS2 axis, with the 95% significance limits for the season groupings (A) and the site groupings (B) in the 2024/25 season.

Table 7. 12: The top 10 families for each seasonal comparison for the SIMPER analysis on the 2024/25 family communities. The families are ranked with the highest contributor to dissimilarity at the top, and the lowest contributor in the top 10 at the bottom for each site comparison. The p-value indicates whether the contribution of the family is significant in the dissimilarity. If $p < 0.05$, the dissimilarity is significant (grey highlighted boxes).

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity	sd	ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity	p-value
CL_LC	Assellidae	0.08	0.58	0.03	2.51	0.70	0.31	0.13	0.893
CL_LC	Gammaridae	0.06	0.58	0.04	1.56	0.07	0.35	0.92	1
CL_LC	Baetidae	0.05	0.58	0.04	1.23	0.15	0.34	0.32	0.821
CL_LC	Glossipholiidae	0.05	0.58	0.04	1.29	0.16	0.30	0.94	0.44
CL_LC	Chironomidae	0.04	0.58	0.04	1.03	0.15	0.21	0.65	0.829
CL_LC	Oligochaeta	0.04	0.58	0.03	1.24	0.33	0.38	1.00	1
CL_LC	Lymnaeidae	0.04	0.58	0.02	1.54	0.03	0.20	0.99	0.002
CL_LC	Erpodellidae	0.03	0.58	0.03	1.09	0.16	0.07	0.90	0.548
CL_LC	Limnephilidae	0.03	0.58	0.03	0.81	0.13	0.03	0.99	0.413
CL_LC	Caenidae	0.02	0.58	0.02	1.31	0.00	0.11	0.54	0.083
CL_DL	Assellidae	0.10	0.67	0.06	1.63	0.70	0.26	0.15	0.069
CL_DL	Gammaridae	0.09	0.67	0.06	1.50	0.07	0.43	0.94	0.934
CL_DL	Chironomidae	0.06	0.67	0.04	1.29	0.15	0.24	0.70	0.183
CL_DL	Oligochaeta	0.05	0.67	0.04	1.41	0.33	0.33	1.00	0.903
CL_DL	Erpodellidae	0.05	0.67	0.05	1.06	0.16	0.11	0.93	0.052
CL_DL	Baetidae	0.05	0.67	0.04	1.08	0.15	0.11	0.37	0.796
CL_DL	Glossipholiidae	0.04	0.67	0.04	1.16	0.16	0.08	0.96	0.655
CL_DL	Limnephilidae	0.03	0.67	0.04	0.70	0.13	0.00	1.00	0.312

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity	sd	ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity	p-value
CL_DL	<i>Ephemeridae</i>	0.03	0.67	0.06	0.44	0.00	0.11	0.91	0.411
CL_DL	<i>Halipidae</i>	0.02	0.67	0.03	0.54	0.07	0.00	0.99	0.139
CL_V	<i>Gammaridae</i>	0.24	0.93	0.08	2.99	0.07	0.89	0.96	0.001
CL_V	<i>Assellidae</i>	0.20	0.93	0.03	7.16	0.70	0.00	0.25	0.001
CL_V	<i>Oligochaeta</i>	0.09	0.93	0.04	2.08	0.33	0.00	1.00	0.002
CL_V	<i>Baetidae</i>	0.05	0.93	0.05	0.93	0.15	0.13	0.56	0.789
CL_V	<i>Glossipholiidae</i>	0.05	0.93	0.04	1.06	0.16	0.00	0.98	0.57
CL_V	<i>Erpodellidae</i>	0.04	0.93	0.04	1.02	0.16	0.00	0.95	0.022
CL_V	<i>Chironomidae</i>	0.04	0.93	0.04	0.97	0.15	0.00	0.80	0.763
CL_V	<i>Limnephilidae</i>	0.04	0.93	0.05	0.84	0.13	0.05	1.00	0.003
CL_V	<i>Ephemeridae</i>	0.03	0.93	0.04	0.77	0.00	0.11	0.95	0.315
CL_V	<i>Halipidae</i>	0.02	0.93	0.04	0.54	0.07	0.00	1.00	0.001
LC_DL	<i>Baetidae</i>	0.07	0.62	0.05	1.60	0.34	0.11	0.32	0.134
LC_DL	<i>Glossipholiidae</i>	0.06	0.62	0.05	1.29	0.30	0.08	0.97	0.04
LC_DL	<i>Assellidae</i>	0.06	0.62	0.03	2.16	0.31	0.26	0.12	0.998
LC_DL	<i>Chironomidae</i>	0.06	0.62	0.04	1.41	0.21	0.24	0.72	0.147
LC_DL	<i>Gammaridae</i>	0.06	0.62	0.05	1.26	0.35	0.43	0.96	1
LC_DL	<i>Oligochaeta</i>	0.05	0.62	0.04	1.35	0.38	0.33	1.00	0.942
LC_DL	<i>Lymnaeidae</i>	0.04	0.62	0.03	1.68	0.20	0.00	1.00	0.004
LC_DL	<i>Erpodellidae</i>	0.03	0.62	0.05	0.70	0.07	0.11	0.96	0.375
LC_DL	<i>Caenidae</i>	0.03	0.62	0.02	1.34	0.11	0.06	0.59	0.02
LC_DL	<i>Ephemeridae</i>	0.02	0.62	0.05	0.44	0.00	0.11	0.95	0.453
LC_V	<i>Gammaridae</i>	0.16	0.79	0.06	2.71	0.35	0.89	0.97	0.003
LC_V	<i>Oligochaeta</i>	0.10	0.79	0.05	2.08	0.38	0.00	1.00	0.001

Site	Family	Average Contribution to dissimilarity	Overall dissimilarity	sd	ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution to overall dissimilarity	p-value
LC_V	<i>Assellidae</i>	0.08	0.79	0.04	2.26	0.31	0.00	0.20	0.732
LC_V	<i>Baetidae</i>	0.08	0.79	0.06	1.41	0.34	0.13	0.43	0.011
LC_V	<i>Glossipholiidae</i>	0.08	0.79	0.06	1.29	0.30	0.00	0.98	0.001
LC_V	<i>Chironomidae</i>	0.06	0.79	0.06	0.98	0.21	0.00	0.80	0.134
LC_V	<i>Lymnaeidae</i>	0.05	0.79	0.03	1.69	0.20	0.00	1.00	0.001
LC_V	<i>Caenidae</i>	0.03	0.79	0.02	1.31	0.11	0.01	0.70	0.001
LC_V	<i>Ephemeridae</i>	0.03	0.79	0.03	0.77	0.00	0.11	0.96	0.404
LC_V	<i>Piscicolidae</i>	0.02	0.79	0.02	0.76	0.07	0.02	1.00	0.01
DL_V	<i>Gammaridae</i>	0.17	0.73	0.08	2.02	0.43	0.89	1.00	0.006
DL_V	<i>Oligochaeta</i>	0.10	0.73	0.07	1.39	0.33	0.00	1.00	0.002
DL_V	<i>Assellidae</i>	0.09	0.73	0.09	0.99	0.26	0.00	0.23	0.427
DL_V	<i>Chironomidae</i>	0.07	0.73	0.08	0.95	0.24	0.00	0.84	0.009
DL_V	<i>Baetidae</i>	0.06	0.73	0.08	0.76	0.11	0.13	0.49	0.421
DL_V	<i>Ephemeridae</i>	0.06	0.73	0.07	0.80	0.11	0.11	1.00	0.009
DL_V	<i>Erpodellidae</i>	0.04	0.73	0.08	0.44	0.11	0.00	1.00	0.336
DL_V	<i>Glossipholiidae</i>	0.02	0.73	0.05	0.44	0.08	0.00	1.00	0.998
DL_V	<i>Caenidae</i>	0.02	0.73	0.04	0.49	0.06	0.01	0.76	0.327
DL_V	<i>Leptoceridae</i>	0.02	0.73	0.04	0.44	0.06	0.00	1.00	0.014

For the macroinvertebrate communities at the species level, the sites in the 2024/25 season also displayed differences. The two-dimensional solution had a stress value of 0.18, indicating a fair representation of the data reduced dimensions. The solution was stable and achieved after 1 random starts out of 20 attempts. The stress value of 0.18 is below the 0.20 critical value, meaning that the interpretation of the NMDS ordination was acceptable but not perfect and over-interpretation of small differences between points should be avoided. For NMDS1, *Gammarus pulex* was the most significantly positively associated species (0.99, $p = 0.001$) and *Limnephilus Rhombicus* was most negatively associated (-0.99, $p = 0.037$). For NMDS2 axis, *Ephemera dancia* was most positively associated (0.95, $p = 0.01$) and *Microvelia reticulata* was most negatively associated (-0.97, $p = 0.02$) (table 7.13). The Ver site was once again separate and more aligned with NMDS2 axis and the *Gammarus pulex* species. The sites appeared to have a gradient along NMDS1, with CL being more negatively associated and V more positively associated (figure 7.16A). The Ver and the Drop Lane sites had more influence from *Ephemera dancia* and *Gammarus pulex*, whereas CL and LC had more influence from the other species negatively associated with NMDS2 (table 7.13). There was a significant difference between the species communities across the sites in the 2024/25 season (PERMANOVA: $F(3,42) = 12.99$, $p = 0.001$) and the sites explained 49.98% of the variance between communities at the species level ($R^2 = 0.49$). The ANOSIM also showed that there was a statistically significant difference in macroinvertebrate community structure between the sites ($R=0.70$, $p=0.001$) and this was the strongest difference out of the tests. The between dissimilarity was greater (median = 536.5) than within group dissimilarity (CL = 222, LC = 142.5, DL = 567, V = 34.5). The pairwise ANOSIM showed that all the sites were significantly different from each other ($p=0.001$) and that CL and V had the greatest dissimilarity ($R=0.93$) and CL and LC were most similar ($R=0.51$).

In contrast, the intra-annual seasons did not show any clear differences in the macroinvertebrate community at the species level (figure 7.16B). There was a significant overlap between all the seasons and the significance ellipses were central to the NMDS axis, suggesting that none of the seasons favoured any particular species. Summer was slightly more aligned negatively to NMDS 2 axis, suggesting that it could be more influenced by the presence of *Chironomidae* species. Spring and autumn had a similar alignment and may be more influenced by the presence of

Erpobdella octoculata and *Baetis rhondani*. Winter had the opposite alignment to the other seasons, suggesting it could be more influenced by *Asellus aquaticus*, *Limnephilus lunatus* and *Oligochaeta*. However, there was no significant difference in the macroinvertebrate community composition at the species level amongst the intra-annual seasons (PERMANOVA: $F(3,42) = 0.73$, $p=0.73$) and season only explained 5.31% of the variation in species community composition ($R^2 = 0.05$). Similarly, the ANOSIM showed that there was not a significant difference in the macroinvertebrate species community composition among the seasons ($R=0.0006$, $p=0.41$) and the between median dissimilarity score (451.5) was similar to the within group score (Spring =431, Summer = 446, Autumn = 584, Winter = 420).

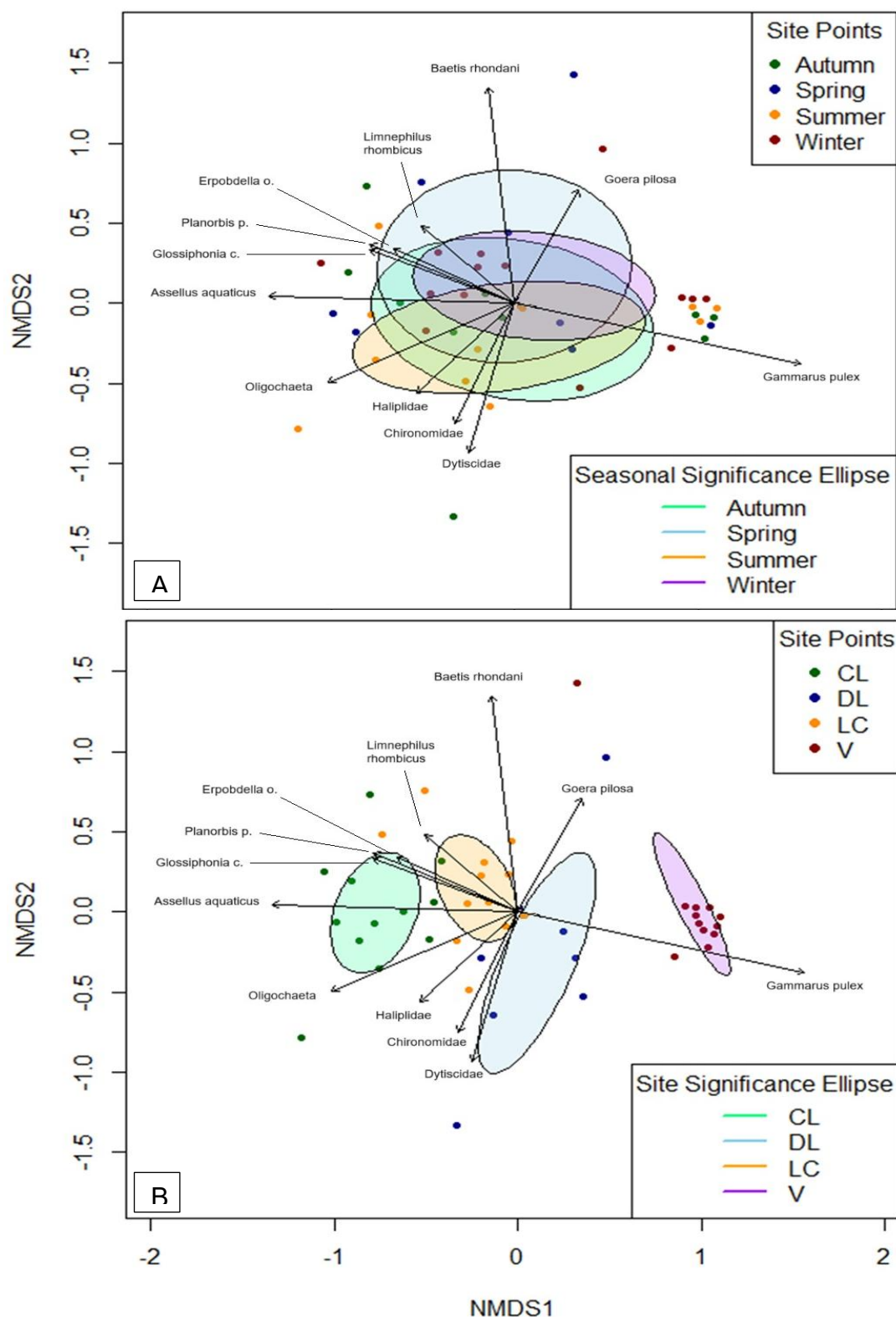


Figure 7. 16: The NMDS ordination plot with the top species contributing to the NMDS 1 and NMDS2 axis, with the 95% significance limits for the site groupings (A) intra-annual seasonal groupings (B) in the 2024/25 season.

Table 7. 13: The significant macroinvertebrate species and their contributions to the NMDS axes for the 2024/25 season.

2024/25 Sp.	NMDS1	NMDS2	pvals
<i>Asellus.aquaticus</i>	-0.99	0.034	0.001
<i>Baetis.rhondani</i>	-0.10	0.99	0.001
<i>Chironomidae</i>	-0.39	-0.91	0.004
<i>Dytiscidae</i>	-0.25	-0.96	0.001
<i>Erpobdella.octoculata</i>	-0.90	0.42	0.004
<i>Gammarus.pulex</i>	0.97	-0.23	0.001
<i>glossiphonia.complanata</i>	-0.92	0.39	0.002
<i>Goera.pilosa..Fabricius.</i>	0.44	0.89	0.013
<i>Haliplidae</i>	-0.68	-0.72	0.017
<i>Limnephilus.rhombicus.Inst. IV</i>	-0.72	0.69	0.033
<i>Oligochaeta</i>	-0.89	-0.43	0.001
<i>Planorbis.planorbis</i>	-0.88	0.46	0.016

The largest dissimilarity between the sites was again between CL and V at 92.76%. The species that contributed the most to this dissimilarity was *Gammarus pulex* (0.22, $p = 0.001$) which had greater abundance at V than CL, followed by *Asellus aquaticus* (0.17, $p=0.001$) which had greater abundance at CL and was absent at V (table 7.14). Other significant species to the dissimilarity between these sites were *Oligochaeta*, *Erpobedella octoculata*, and *Planorbis planorbis*, all of which were present at CL, but not at V. The next highest dissimilarity was between LC and V sites with 78.13% and the species with the highest contributor to dissimilarity was *Gammarus pulex* with a 15.08% contribution ($p=0.001$) and had greater abundance at V. *Baetis rhondani* and *Oligochaeta* were also significant contributors to the differences with 9.42% and 8.23% respectively and both with a greater abundance at LC. The site with the smallest dissimilarity was CL and LC at 59.6% and only one species was significantly different between the sites. This was *Lymnaea peregra* with a contribution of 1.87% ($p=0.002$) and a higher abundance at LC and absent at CL. LC was more similar to DL (62.46% dissimilarity) than CL was to DL (70.89% dissimilarity).

Table 7. 14: The top 10 families for each seasonal comparison for the SIMPER analysis on the 2024/25 species level communities. The species are ranked with the highest contributor to dissimilarity at the top, and the lowest contributor in the top 10 at the bottom for each site comparison. The p-value indicates whether the contribution of the family is significant in the dissimilarity. If $p < 0.05$, the dissimilarity is significant (grey highlighted boxes).

Comparison	Species	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	Ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution	p-value
CL_LC	<i>Asellus.aquaticus</i>	0.07	0.60	0.03	2.10	0.66	0.32	0.11	0.978
CL_LC	<i>Gammarus.pulex</i>	0.06	0.60	0.04	1.54	0.09	0.35	0.73	1
CL_LC	<i>Baetis.rhondani</i>	0.05	0.60	0.04	1.30	0.13	0.36	0.21	0.702
CL_LC	<i>glossiphonia.complanata</i>	0.04	0.60	0.03	1.26	0.20	0.31	0.77	0.753
CL_LC	<i>Chironomidae</i>	0.04	0.60	0.04	1.02	0.13	0.20	0.43	0.845
CL_LC	<i>Oligocheta</i>	0.03	0.60	0.03	1.18	0.33	0.38	0.99	1
CL_LC	<i>Erpobdella.octoculata</i>	0.03	0.60	0.03	1.00	0.14	0.06	0.71	0.102
CL_LC	<i>Lymnaea..Galba..truncatula</i>	0.02	0.60	0.02	1.17	0.05	0.10	0.97	0.167
CL_LC	<i>Caenis.rivulorum</i>	0.02	0.60	0.02	1.17	0.00	0.10	0.29	0.152
CL_LC	<i>Lymnaea.peregra</i>	0.02	0.60	0.02	1.03	0.00	0.10	0.98	0.002
CL_DL	<i>Asellus.aquaticus</i>	0.10	0.71	0.06	1.68	0.66	0.22	0.14	0.013
CL_DL	<i>Gammarus.pulex</i>	0.09	0.71	0.06	1.57	0.09	0.46	0.75	0.926
CL_DL	<i>Oligocheta</i>	0.05	0.71	0.04	1.42	0.33	0.36	1.00	0.933
CL_DL	<i>Chironomidae</i>	0.05	0.71	0.04	1.21	0.13	0.23	0.47	0.259
CL_DL	<i>glossiphonia.complanata</i>	0.05	0.71	0.03	1.34	0.20	0.07	0.79	0.518

Comparison	Species	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	Ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution	p-value
CL_DL	<i>Baetis.rhondani</i>	0.04	0.71	0.04	0.98	0.13	0.09	0.27	0.901
CL_DL	<i>Erpobdella.octoculata</i>	0.03	0.71	0.04	0.90	0.14	0.00	0.73	0.058
CL_DL	<i>Dytiscidae</i>	0.03	0.71	0.05	0.54	0.04	0.09	0.61	0.078
CL_DL	<i>Ephemera.danica.Mull</i>	0.02	0.71	0.05	0.40	0.00	0.09	0.71	0.482
CL_DL	<i>Planorbis.planorbis</i>	0.02	0.71	0.03	0.64	0.08	0.00	1.00	0.125
CL_V	<i>Gammarus.pulex</i>	0.22	0.93	0.08	2.87	0.09	0.89	0.84	0.001
CL_V	<i>Asellus.aquaticus</i>	0.17	0.93	0.04	4.56	0.66	0.00	0.23	0.001
CL_V	<i>Oligocheta</i>	0.09	0.93	0.04	2.16	0.33	0.00	1.00	0.001
CL_V	<i>glossiphonia.complanata</i>	0.05	0.93	0.04	1.28	0.20	0.00	0.87	0.189
CL_V	<i>Baetis.rhondani</i>	0.04	0.93	0.05	0.87	0.13	0.12	0.42	0.917
CL_V	<i>Erpobdella.octoculata</i>	0.04	0.93	0.04	0.90	0.14	0.00	0.83	0.001
CL_V	<i>Chironomidae</i>	0.04	0.93	0.04	0.89	0.13	0.02	0.62	0.893
CL_V	<i>Ephemera.danica.Mull</i>	0.02	0.93	0.03	0.69	0.00	0.09	0.81	0.348
CL_V	<i>Limnephilidae.Instar.III</i>	0.02	0.93	0.03	0.80	0.07	0.05	0.94	0.052
CL_V	<i>Planorbis.planorbis</i>	0.02	0.93	0.03	0.65	0.08	0.00	1.00	0.002
LC_DL	<i>Baetis.rhondani</i>	0.08	0.62	0.05	1.64	0.36	0.09	0.23	0.049
LC_DL	<i>glossiphonia.complanata</i>	0.06	0.62	0.05	1.43	0.31	0.07	0.88	0.015
LC_DL	<i>Asellus.aquaticus</i>	0.06	0.62	0.03	2.15	0.32	0.22	0.12	0.989
LC_DL	<i>Gammarus.pulex</i>	0.06	0.62	0.04	1.31	0.35	0.46	0.84	1
LC_DL	<i>Chironomidae</i>	0.05	0.62	0.04	1.31	0.20	0.23	0.50	0.164
LC_DL	<i>Oligocheta</i>	0.05	0.62	0.04	1.40	0.38	0.36	1.00	0.917
LC_DL	<i>Caenis.rivulorum</i>	0.03	0.62	0.02	1.19	0.10	0.05	0.32	0.015
LC_DL	<i>Simulidae</i>	0.02	0.62	0.03	0.78	0.09	0.05	1.00	0.105

Comparison	Species	Average Contribution to dissimilarity	Overall dissimilarity between groups	sd	Ratio	Average Abundance in Group A	Average Abundance in Group B	Cumulative contribution	p-value
LC_DL	<i>Lymnaea.peregra</i>	0.02	0.62	0.02	1.03	0.10	0.00	1.00	0.005
LC_DL	<i>Lymnaea..Galba..truncatula</i>	0.02	0.62	0.02	1.09	0.10	0.00	1.00	0.167
LC_V	<i>Gammarus.pulex</i>	0.15	0.78	0.05	2.82	0.35	0.89	0.90	0.001
LC_V	<i>Oligocheta</i>	0.09	0.78	0.04	2.22	0.38	0.00	1.00	0.001
LC_V	<i>Baetis.rhondani</i>	0.08	0.78	0.06	1.49	0.36	0.12	0.31	0.003
LC_V	<i>Asellus.aquaticus</i>	0.08	0.78	0.03	2.34	0.32	0.00	0.19	0.324
LC_V	<i>glossiphonia.complanata</i>	0.08	0.78	0.05	1.44	0.31	0.00	0.93	0.001
LC_V	<i>Chironomidae</i>	0.05	0.78	0.05	0.95	0.20	0.02	0.62	0.169
LC_V	<i>Caenis.rivulorum</i>	0.02	0.78	0.02	1.17	0.10	0.01	0.42	0.002
LC_V	<i>Lymnaea..Galba..truncatula</i>	0.02	0.78	0.02	1.13	0.10	0.02	1.00	0.007
LC_V	<i>Lymnaea.peregra</i>	0.02	0.78	0.02	1.03	0.10	0.00	1.00	0.001
LC_V	<i>Ephemera.danica.Mull</i>	0.02	0.78	0.03	0.69	0.00	0.09	0.86	0.387
DL_V	<i>Gammarus.pulex</i>	0.16	0.71	0.08	1.91	0.46	0.89	0.92	0.001
DL_V	<i>Oligocheta</i>	0.11	0.71	0.07	1.55	0.36	0.00	1.00	0.001
DL_V	<i>Asellus.aquaticus</i>	0.07	0.71	0.08	0.86	0.22	0.00	0.22	0.743
DL_V	<i>Chironomidae</i>	0.07	0.71	0.07	1.01	0.23	0.02	0.66	0.003
DL_V	<i>Baetis.rhondani</i>	0.06	0.71	0.08	0.71	0.09	0.12	0.38	0.523
DL_V	<i>Ephemera.danica.Mull</i>	0.05	0.71	0.07	0.73	0.09	0.09	0.89	0.004
DL_V	<i>Dytiscidae</i>	0.03	0.71	0.07	0.40	0.09	0.00	0.80	0.036
DL_V	<i>glossiphonia.complanata</i>	0.02	0.71	0.05	0.41	0.07	0.00	0.95	1
DL_V	<i>Simuliidae</i>	0.02	0.71	0.04	0.47	0.05	0.01	1.00	0.36
DL_V	<i>Caenis.rivulorum</i>	0.02	0.71	0.04	0.45	0.05	0.01	0.48	0.361

7.3. Relationships between ecological community against water chemistry, physicochemical and hydrological variables

7.3.1. Environmental quality based on ecological measures

The WHPT score was highest in the spring season in the 2023/24 season (46.53 ± 16.92) and decreased across the seasons, where it was lowest in winter (23.75 ± 7.29) (Figure 7.17A). The statistical test showed that there was a significant difference between the seasons (ANOVA: $F(3,27) = 4.37$, $p = 0.01$) (table 7.15) and the post-hoc Tukey HSD test showed that spring had a significantly higher WHPT score than autumn ($p=0.008$) (table 7.16). The WHPT score was more consistent across the sites in the 2023/24 season, but the LC site did have a relatively higher WHPT score (36.88 ± 15.03). However, there was no significant difference across the sites (ANOVA: $F(3,27) = 2.50$, $p = 0.08$).

The WHPT-ASPT score was similar across the intra-annual seasons, although it was slightly lower in summer (3.87 ± 0.71) (Figure 7.17B). However, there was no significant difference across the intra-annual seasons (ANOVA: $F(3,27) = 1.24$, $p = 0.31$). The WHPT-ASPT score was highest at the Ver site (4.56 ± 0.80) but the other sites were similar to each other. The Two-Way ANOVA showed that there was a significant difference in WHPT-ASPT scores across the sites (ANOVA: $F(3,27) = 3.16$, $p = 0.04$) and the post hoc Tukey HSD test showed that V had a significantly higher WHPT-ASPT score than CL ($p = 0.04$).

The LIFE score increased across the intra-annual seasons, with the lowest LIFE score in summer (5.14 ± 1.27) and the highest in winter (6.28 ± 0.87) (Figure 7.17C). The difference in LIFE scores between the seasons was significant (ANOVA: $F(3,27) = 5.05$, $p = 0.007$). The post hoc Tukey HSD test showed that the summer season had a significantly lower score than autumn ($p = 0.04$) and winter ($p = 0.04$). The Ver site had the highest average LIFE score (6.49 ± 1.12) and Drop Lane site had the lowest average LIFE score (5.07 ± 1.32).

There was a significant difference in LIFE scores across the sites (ANOVA: $F(3,27) = 3.81$, $p = 0.02$) and the post hoc Tukey HSD test showed that V had a significantly higher LIFE score than DL in the 2023/24 season ($p = 0.01$).

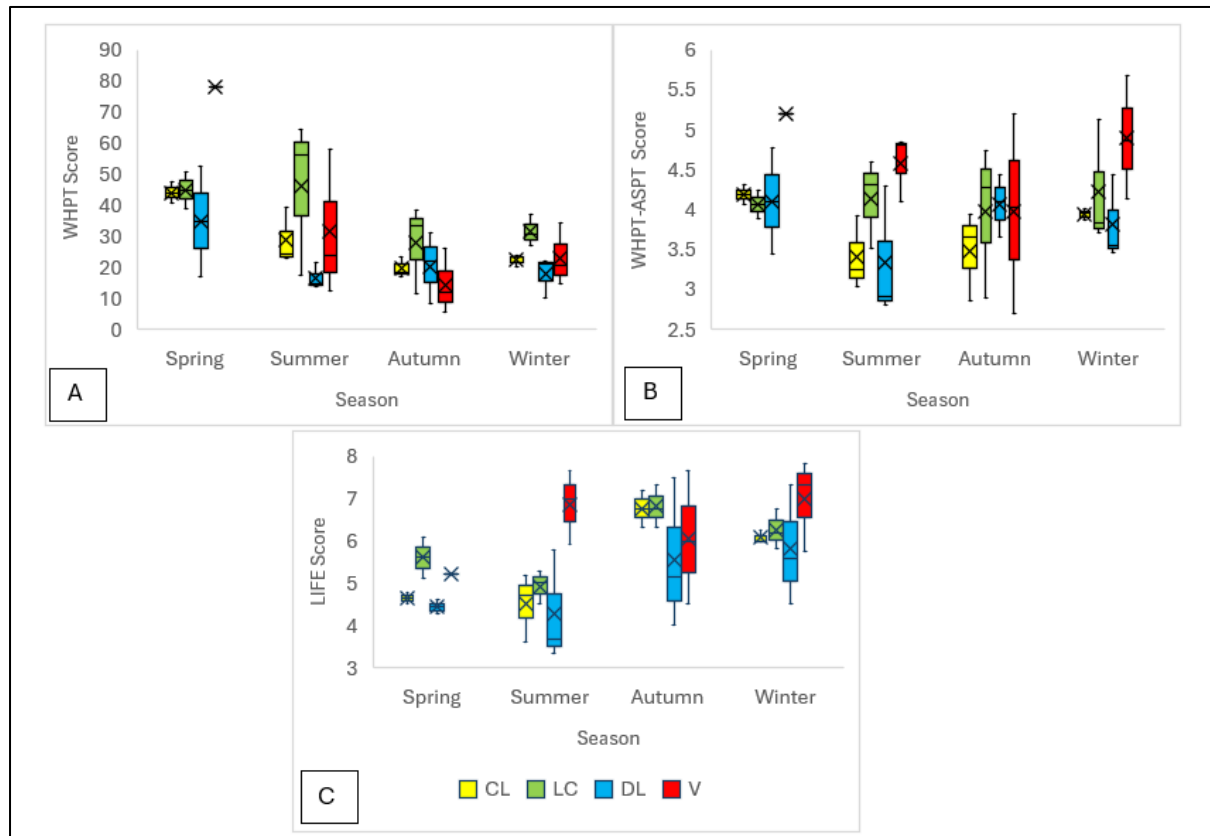


Figure 7. 17: The WHPT (A), WHPT-ASPT (B) and LIFE (C) scores for the 2023/24 season.

Table 7. 15: The results from the Two-Way ANOVA on the WHPT, WHPT-ASPT and LIFE scores for the 2023/24 and 2024/25 season. The confidence limit was 95% and if $p < 0.05$, the result was significant

Season	Variable	Independent Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
2023/24	WHPT	Site	3	1.97	0.66	2.50	0.080568
		Season	3	3.44	1.15	4.37	0.012411
		Residuals	27	7.08	0.26		
	WHPT_ASPT	Site	3	4.46	1.49	3.16	0.040617
		Season	3	1.75	0.58	1.24	0.313399
		Residuals	27	12.69	0.47		
	LIFE	Site	3	11.22	3.74	3.81	0.021374
		Season	3	14.90	4.97	5.05	0.0066
		Residuals	27	26.54	0.98		
2024/25	WHPT	Site	3	2745.75	915.25	15.65	9.20E-06
		Season	3	420.15	140.05	2.39	0.094456
		Residuals	23	1345.33	58.49		
	LIFE	Site	3	65.98	21.99	22.06	5.88E-07
		Season	3	0.76	0.25	0.25	0.857711
		Residuals	23	22.93	1.00		

Table 7. 16: The results from the post-hoc Tukey HSD test on the significant Two-Way ANOVA results performed on the WHPT, WHPT-ASPT and LIFE scores for the 2023/24 and 2024/25 season. The confidence limit was 95% and if $p < 0.05$, the result was significant.

Season	Dependent Variable	Independent Variable	Significant Difference	Diff.	Lower	Upper	P-Value
2023/24	WHPT	Season	Spring-Autumn	0.86	0.19	1.53	0.007624
			Summer-Autumn	0.38	-0.19	0.96	0.280595
			Winter-Autumn	0.23	-0.34	0.81	0.683458
			Summer-Spring	-0.48	-1.14	0.19	0.226428
			Winter-Spring	-0.63	-1.29	0.04	0.069995
			Winter-Summer	-0.15	-0.72	0.42	0.889341
	WHPT-ASPT	Site	DL-CL	0.09	-0.71	0.89	0.989572
			LC-CL	0.39	-0.41	1.19	0.556535
			V-CL	0.84	0.02	1.66	0.043069
			LC-DL	0.3	-0.5	1.1	0.742269
			V-DL	0.75	-0.07	1.57	0.082063
			V-LC	0.45	-0.37	1.27	0.444051
	LIFE	Site	DL-CL	-0.51	-1.67	0.65	0.629317

Season	Dependent Variable	Independent Variable	Significant Difference	Diff.	Lower	Upper	P-Value
			LC-CL	0.35	-0.81	1.51	0.84111
			V-CL	0.91	-0.28	2.1	0.178438
			LC-DL	0.86	-0.3	2.02	0.201585
			V-DL	1.42	0.23	2.6	0.014424
			V-LC	0.56	-0.63	1.75	0.574781
		Season	Spring-Autumn	-1.25	-2.54	0.04	0.060202
			Summer-Autumn	-1.15	-2.26	-0.04	0.03951
			Winter-Autumn	-0.01	-1.12	1.1	0.999994
			Summer-Spring	0.1	-1.19	1.39	0.996635
			Winter-Spring	1.24	-0.05	2.53	0.063137
			Winter-Summer	1.14	0.03	2.25	0.041866
2024/25	WHPT	Site	DL-CL	-9.42	-20.16	1.33	0.100162
			LC-CL	10.41	1.38	19.43	0.019707
			V-CL	-9.95	-18.98	-0.93	0.026938
			LC-DL	19.82	9.08	30.57	0.000197
			V-DL	-0.54	-11.28	10.2	0.999013
			V-LC	-20.36	-29.39	-11.34	1.28E-05
		Season	Spring-Autumn	-1.76	-11.79	8.28	0.961934
			Summer-Autumn	5.3	-3.95	14.55	0.405516
			Winter-Autumn	6.05	-3.42	15.52	0.31323

Season	Dependent Variable	Independent Variable	Significant Difference	Diff.	Lower	Upper	P-Value
			Summer-Spring	7.06	-2.78	16.89	0.222289
			Winter-Spring	7.81	-2.23	17.84	0.167071
			Winter-Summer	0.75	-8.5	10	0.995926
	LIFE	Site	DL-CL	-0.49	-1.9	0.91	0.765145
			LC-CL	1.17	-0.01	2.35	0.052307
			V-CL	2.94	1.77	4.12	2.72E-06
			LC-DL	1.66	0.26	3.07	0.016072
			V-DL	3.44	2.04	4.84	3.66E-06
			V-LC	1.77	0.6	2.95	0.00196

In the 2024/25 season, the LC site had the highest WHPT score (35.89 ± 10.10) and the V site had the lowest (15.53 ± 6.08) (figure 7.17A). This difference in WHPT scores across the sites was significant (ANOVA: $F(3,23) = 15.64$, $p = 9.30 \times 10^{-6}$) (table 7.15) and seemed to decrease downstream. The post-hoc Tukey HSD test showed that LC had a significantly higher WHPT score than the three other sites (CL: $p = 0.02$; DL: $p = 0.0002$; V: $p = 1.28 \times 10^{-5}$) (table 6.16). The WHPT score at CL was also significantly higher than V ($p = 0.03$). The WHPT score was similar across the seasons (24.16 ± 11.03) and there was not a significant difference across the seasons in the 2024/25 season (ANOVA: $F(3,23) = 2.39$, $p = 0.09$).

Contrastingly, the WHPT-ASPT score was highest at the Ver site (5.57 ± 1.11) and the other sites had similar means (Figure 7.17B). There was a significant difference in the WHPT-ASPT scores across the sites (Kruskal-Wallis: $H(3,23) = 19.34$, $p = 0.0002$) (table 7.17) and the post-hoc Dunn test showed that V had a significantly lower WHPT-ASPT score than the other three sites (CL: $p = 0.0002$; LC: $p = 0.05$; DL: $p = 0.02$) (table 7.18). The WHPT-ASPT score was found to decrease throughout the intra-annual seasons, with the highest average score in spring (4.33 ± 1.34) and the lowest average

score in summer 4.33 ± 1.22). There was also no significant difference in WHPT-ASPT scores across the seasons (Kruskal-Wallis: $H(3,23) = 0.64$, $p=0.88$) (table 7.17).

The LIFE score was greatest at the Ver site (8.18 ± 0.57) and lowest at the DL (4.74 ± 1.01) and CL sites (5.24 ± 1.00) (Figure 7.17C). This difference in LIFE scores across the sites was significant (ANOVA: $F(3,23) = 22.06$, $p = 5.88e-07$) and the post-hoc Tukey HSD test showed that V was higher than CL ($p = 2.72e-06$), LC ($p=0.002$) and DL ($p=3.66e-06$). It also showed that LC had a significantly higher LIFE score than DL ($p=0.02$). However, the LIFE scores did not show much variation between the seasons and there was no significant difference in LIFE scores across the intra-annual seasons (ANOVA: $F(3,23) = 0.25$, $p = 0.86$) (table 7.15).

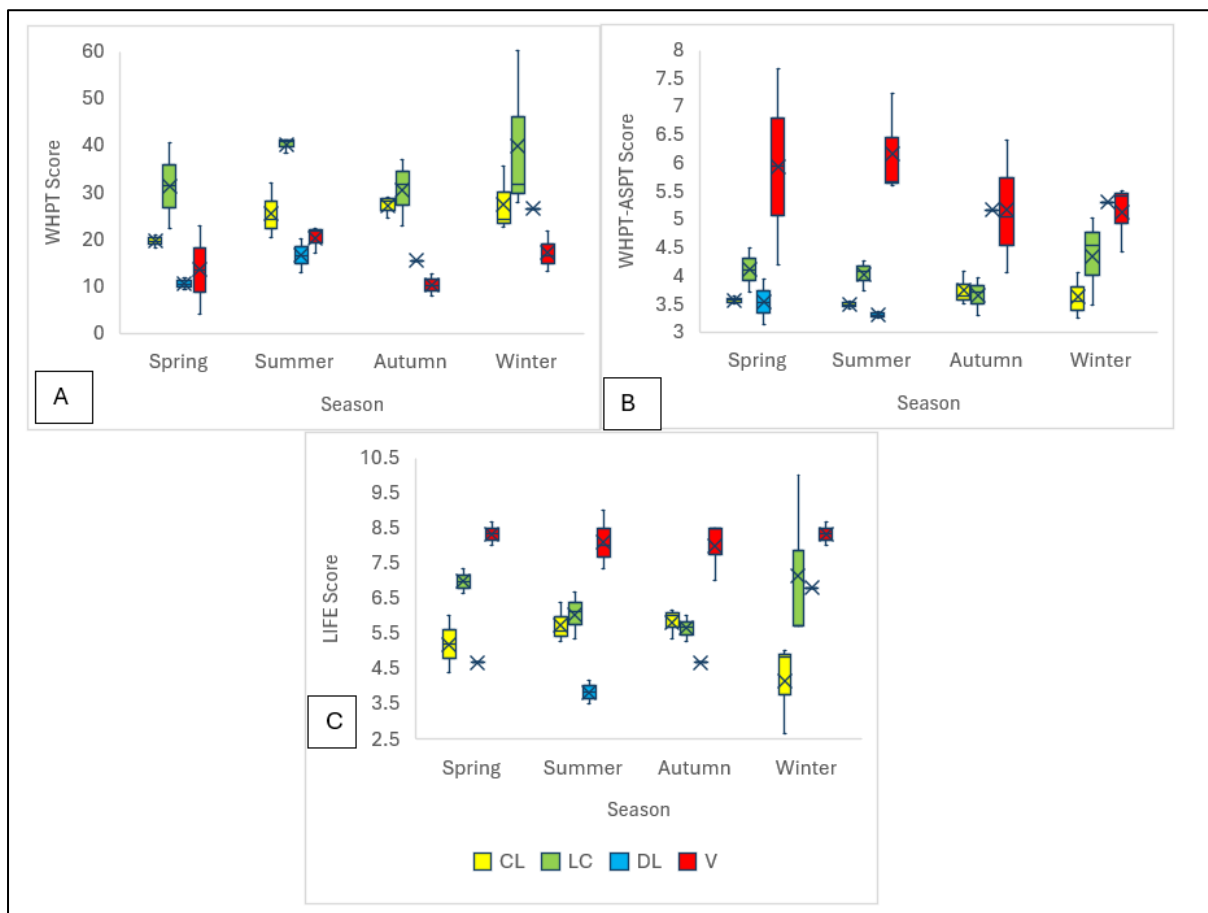


Figure 7. 18: The WHPT (A), WHPT-ASPT (B) and LIFE (C) scores for the 2024/25 season.

Table 7. 17: The results from the non-parametric Kruskal-Wallis on the WHPT-ASPT scores for the 2024/25 season. The confidence limit was 95% and if $p < 0.05$, the result was significant.

Season	Dependent Variable	Variable	Chi-Squared	p-values
2024/25	WHPT-ASPT	Season	0.63504	0.8884
		Site	19.329	0.000234

Table 7. 18: The post-hoc Dunn test results on the WHPT-ASPT scores from the 2024/25 season. The confidence limit was 95% and if $p < 0.05$, the result was significant.

Season	Dependent Variable	Comparison	Z	P.unadj	P.adj
2024/25	WHPT-ASPT	CL - DL	0.58^{-}	0.56	1
		CL - LC	1.53^{-}	0.13	0.751078
		DL - LC	0.71^{-}	0.48	1
		CL - V	4.19^{-}	0.00	0.000168
		DL - V	2.94^{-}	0.00	0.019822
		LC - V	2.66^{-}	0.01	0.047531

The WHPT score was lower in the 2024/25 season (28.52 ± 16.03) compared to the 2023/24 season (24.16 ± 11.03) and there was a wider range at the Ver and LC sites in the 2023/24 seasons with more outliers indicating higher WHPT scores (Figure 7.19A). However, this difference in WHPT scores across the inter-annual seasons was not found to be significant (T-test: $p=0.29$) (table 7.19).

The WHPT-ASPT scores were consistent across the seasons (2023/24: 4.03 ± 0.70 ; 2024/25: 4.34 ± 1.08) , although the score at the Ver site was slightly higher in the 2024/25 season than the 2023/24 season (Figure 7.19B). However, there was no

significant difference between the inter-annual seasons in the WHPT-ASPT scores (T-test: $p=0.17$). There was more variation in the LIFE score in the 2024/25 season with the sites having smaller ranges compared to the 2023/24 season (Figure 7.19C). The Ver site also had a higher average LIFE score in 2024/25 (8.18 ± 0.57) than 2023/24 (6.49 ± 1.11) and there were more outliers suggesting more variation. However, there was no significant difference in the LIFE scores across the inter-annual seasons (T-test: $p=0.08$) and the two season's means were comparable (2023/24: 5.75 ± 1.20 ; 2024/25: 6.32 ± 1.62) (table 7.19).

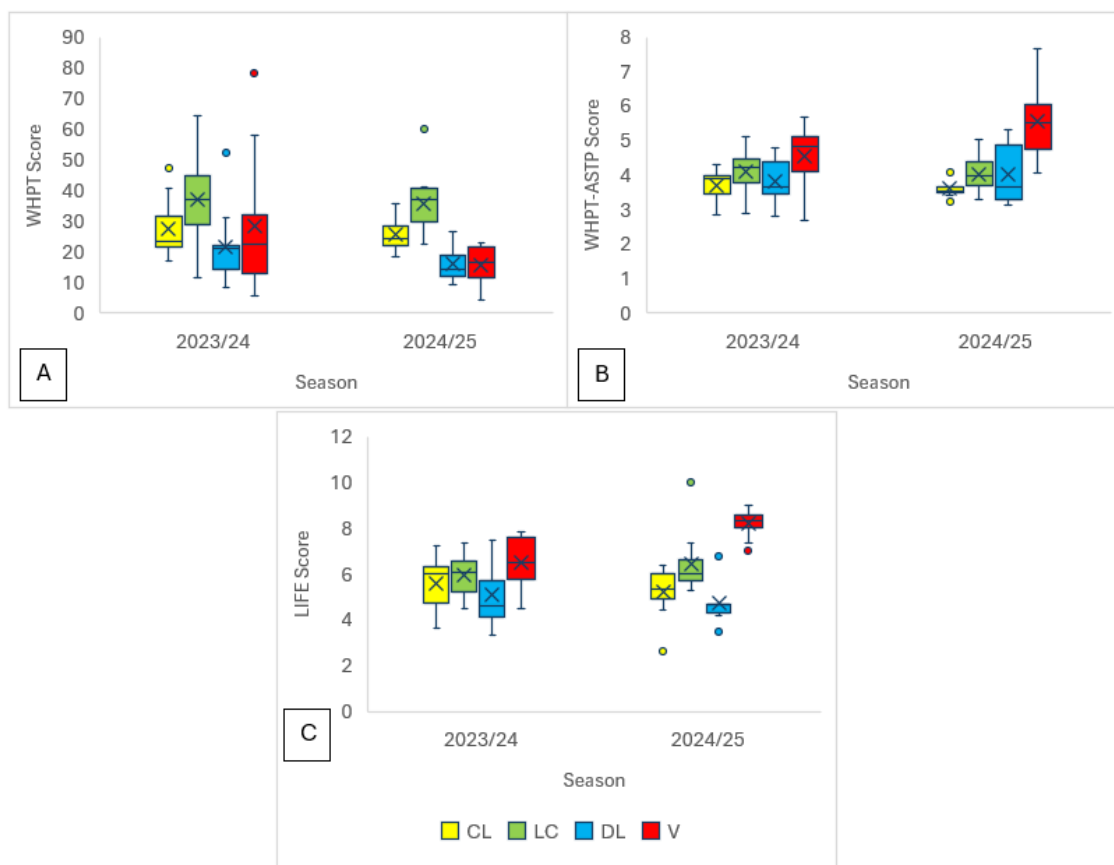


Figure 7. 19: The intra-annual comparison for the WHPT (A), WHPT-ASPT (B) and LIFE (C) scores for the 2023/24 and 2024/25 seasons.

Table 7. 19: The T-test results from the comparison of the WHPT, ASPT, and LIFE scores between the 2023/24 and 2024/25 seasons. The confidence limit was 95% and if $p < 0.05$, the result was significant.

Variable	t	df	p-value
WHPT	1.0536	80	0.295
ASPT	-1.3707	80	0.1743
LIFE	-1.7979	80	0.07597

7.3.2. Assessing the Influence of Environmental Variables on Community Composition

In 2023/24 season, the environmental variables had a significant effect on the macroinvertebrate community (ANOVA: $F(17,25) = 2.583$, $p = 0.001$). pH did have a significant effect in the model ($p = 0.014$) but did not have a large effect on the RDA 1 (-0.10) or RDA 2 (0.40) axes. DO ($p = 0.001$), Water Temp ($p = 0.003$), Width ($p = 0.001$), depth ($p = 0.02$), nitrate ($p = 0.019$) were also all significant variables within the model and the model contributed to explaining 39% of the variance in the macroinvertebrate communities in the 2023/24 season (Adjusted $R^2 = 0.39$). RDA 1 explained 30% of variance, and RDA 2 explained 11.51% within the model. The channel width had the largest negative effect on the RDA 1 axis (-0.75) and maximum air temperature had the largest positive effect although this was relatively low (0.19). Water temperature had the largest negative effect on RDA 2 (-0.70) and DO had the largest positive effect of RDA 2 (0.43). The forward selected variables that were deemed most important in the model were channel width, water temperature, nitrate level, minimum temperature, dissolved oxygen and average flow velocity (table 7.20). From figure 7.20a., sites 2 (LC) and 3 (DL) were most negatively influenced by RDA 2 and positively by RDA 1, meaning that they were mostly affected by the temperature variables (maximum temperature, minimum temperature and water temperature), and least influenced by DO and hydrological variables. Site 4 (V) was negatively influenced by RDA 1, and channel width, channel area and nitrates levels affected this site the most (figure 7.20a). Site 1 (CL) was positively influenced by RA 1 and RDA 2 and had a larger

influence from the water quality and physical-chemical variables, such as pH, ammonium and phosphate levels. The key families for each site were as follows: *Asellus* at CL, *Oligochaeta* at LC, *Daphniidae* at DL and *Gammarus* at V.

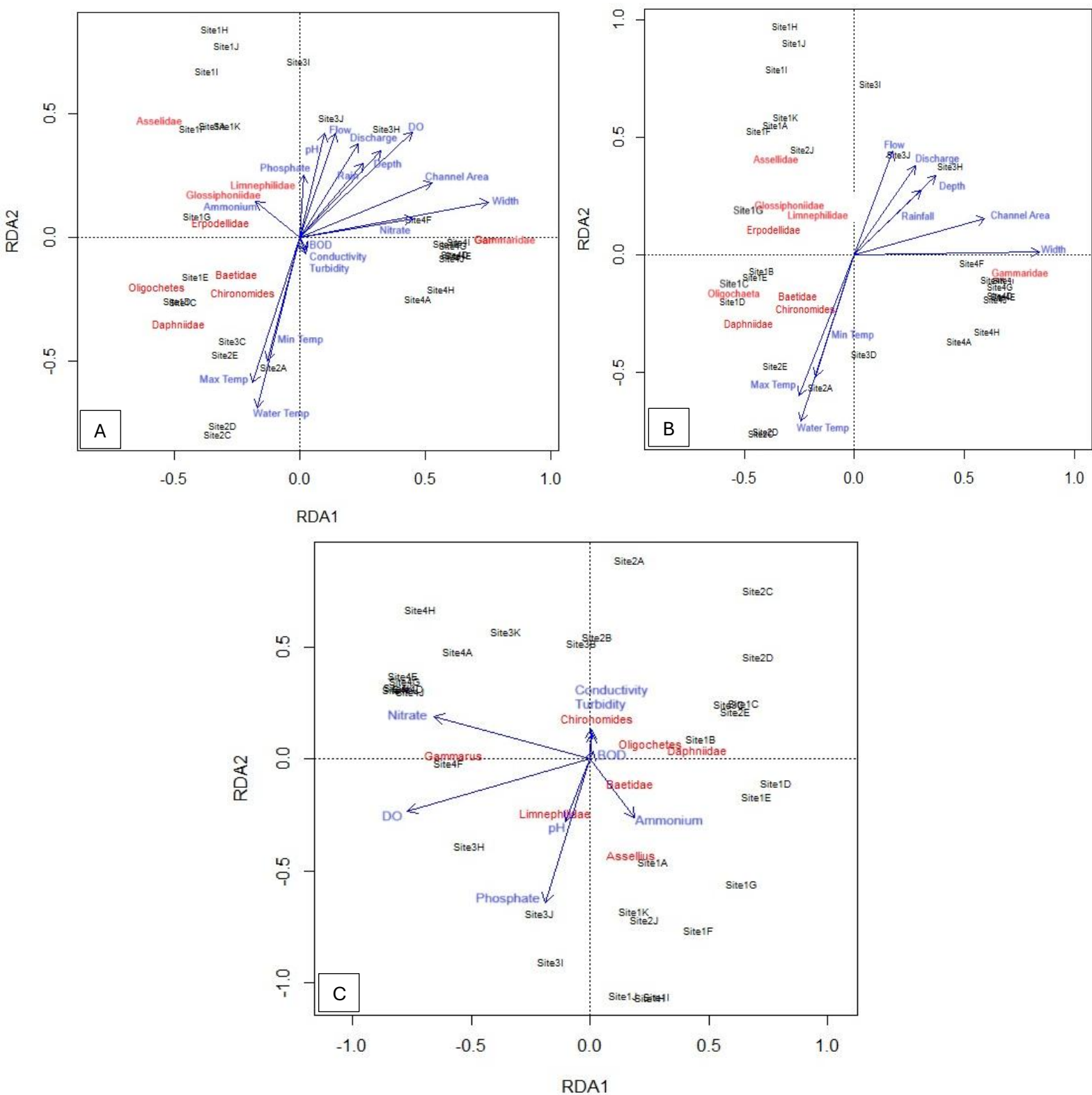


Figure 7. 20: The RDA plots showing the relationships between the environmental variables and the sites and macroinvertebrate families in the 2023/24 season. The

variables are in blue and the length and direction of the arrow signifies the strength of the variable and its relation to the RDA axes and sites and families. Site 1 is CL, Site 2 is LC, Site 3 is DL, Site 4 is V. The months are denoted as letters (A is April, B = May, C = June, D = July, E = August, F = September, G = October, H = November, I = December, J = January, K = February). (a) is the plot for model 1 (all environmental parameters), (b) is model 2 (hydrological parameters) and (c) is model 3 (the water quality parameters including water nutrients and physical-chemical variables).

Table 7. 20: The significances of the variables in each RDA model in both the 2023/24 and 2024/25 season. There was a 95% confidence limit and if $p < 0.05$, the variable is considered to have a significant effect on the macroinvertebrate community (grey highlight).

Season	Model	Variable	Df	Variance	F	p-value
2023/24	Model 1 (All variables)	pH	1	0.02	2.86	0.014
		Conductivity	1	0.02	1.96	0.063
		Turbidity	1	0.01	1.52	0.175
		DO	1	0.06	7.03	0.001
		BOD	1	0.01	0.71	0.649
		WaterTemp	1	0.04	5.42	0.003
		Width	1	0.06	7.35	0.001
		Depth	1	0.02	2.70	0.018
		Flow	1	0.01	1.78	0.082
		ChannelArea	1	0.01	1.54	0.131
		Discharge	1	0.01	1.12	0.327
		Phosphate	1	0.01	0.92	0.44
		Nitrate	1	0.02	2.70	0.019
		Ammonium	1	0.01	1.82	0.074
		DailyTotalRainfall	1	0.01	0.84	0.506
		MaxTemp	1	0.01	0.97	0.405
		MinTemp	1	0.02	2.66	0.026
		Residual	25	0.20		
	Model 2 (Hydrological Variables)	Depth	1	0.03	3.54	0.005
		Width	1	0.09	9.74	0.001
		Discharge	1	0.01	0.98	0.444
		ChannelArea	1	0.02	1.73	0.082
		Flow	1	0.01	1.05	0.363
		DailyTotalRainfall	1	0.02	1.77	0.101
		MaxTemp	1	0.02	2.18	0.051
		MinTemp	1	0.04	4.13	0.002

Season	Model	Variable	Df	Variance	F	p-value
	Model 3 (Water Quality Variables)	Residual	34	0.31		
		pH	1	0.02	2.02	0.065
		Conductivity	1	0.02	1.39	0.172
		Turbidity	1	0.01	1.07	0.352
		DO	1	0.06	4.96	0.001
		BOD	1	0.01	0.50	0.85
		Phosphate	1	0.01	0.74	0.64
		Nitrate	1	0.03	2.91	0.012
		Ammonium	1	0.01	1.05	0.395
		Residual	34	0.38		
2024/25	Model 1 (All variables)	pH	1	0.01	1.14	0.291
		Conductivity	1	0.02	2.22	0.059
		Turbidity	1	0.01	0.84	0.594
		DO	1	0.01	1.01	0.35
		BOD	1	0.11	11.08	0.001
		WaterTemp	1	0.01	1.18	0.294
		Width	1	0.07	7.40	0.001
		Depth	1	0.02	1.73	0.11
		Flow	1	0.01	0.73	0.622
		ChannelArea	1	0.02	1.93	0.083
		Discharge	1	0.02	1.85	0.114
		Phosphate	1	0.00	0.29	0.994
		Nitrate	1	0.01	0.58	0.737
		Ammonium	1	0.01	0.73	0.592
		DailyTotalRainfall	1	0.01	0.99	0.393
		MaxTemp	1	0.01	1.32	0.235
		MinTemp	1	0.01	0.58	0.728
		Residual	21	0.21		
	Model 2 (Hydrological Variables)	Depth	1	0.08	7.66	0.001
		Width	1	0.08	8.18	0.001
		Discharge	1	0.01	0.81	0.543
		ChannelArea	1	0.03	2.66	0.029
		Flow	1	0.02	2.09	0.065
		DailyTotalRainfall	1	0.01	0.49	0.84
		MaxTemp	1	0.01	1.20	0.268
		MinTemp	1	0.01	1.37	0.204
		Residual	30	0.31		
	Model 3 (Water Quality Variables)	pH	1	0.01	0.97	0.408
		Conductivity	1	0.02	1.88	0.094
		Turbidity	1	0.01	0.71	0.694
		DO	1	0.01	0.86	0.519
		BOD	1	0.11	9.37	0.001
		Phosphate	1	0.01	0.45	0.922
		Nitrate	1	0.03	2.58	0.032
		Ammonium	1	0.01	1.04	0.375
		Residual	30	0.35		

The hydrological variables (model 2) also had a significant effect on the macroinvertebrate communities (ANOVA: $F(8,34) = 3.14$, $p = 0.001$) and the hydrological variables were found to explain 29% of the variance in the macroinvertebrate communities (Adjusted $R^2 = 0.29$). There were only two significant variables within the model which were average channel depth ($p = 0.005$) and channel width ($p = 0.001$) (table 7.20). Channel width had the strongest association with the RDA 1 axis (-0.84) and flow had the strongest positive association (0.44). Maximum air temperature had the largest positive impact on RDA 2 axis, although this was still relatively small (0.25) and the other variables were all negatively associated, with water temperature having the largest negative effect (-0.72). The RDA 1 axis explained 24.24% of the variance in the macroinvertebrate community and RDA 2 explained only 8.40%, with a total of both axis explaining 32.64% of the variance. The forward selected variables that were most important in the model were channel width, water temperature and minimum air temperature. Site 1 (CL) was not closely related to any of the variables as shown in figure 7.20b. Site 2 (LC) was more aligned positively with RDA 1 and negatively with RDA 2 and had more of an influence from water temperature and the maximum and minimum air temperatures. Site 3 (DL) was positively aligned with RDA 2 and negatively with RDA 1, and had more influence from the average flow velocity, total channel discharge, average channel depth and total daily rainfall. Site 4 (V) was negatively aligned with both RDA 1 and RDA 2 and was more influenced by channel width and channel area.

The water chemistry and physicochemical variables (water quality variables in model 3) were significant (ANOVA: $F(8,34) = 1.8305$, $p = 0.005$) and the water quality and physical-chemical variables explained 14% of the variation in the macroinvertebrate community composition (Adjusted $R^2 = 0.14$). The significant variables in the model were DO ($p = 0.001$) and nitrate ($p = 0.012$) (table 7.20). Ammonium had a positive influence on RDA 1 axis (0.18) and DO had a negative influence (-0.78). For the RDA 2 axis, nitrate had a positive influence (0.21) and pH had a larger negative influence (-0.68). The RDA 1 axis explained 14.52% of the variance and RDA 2 axis explained a further 6.07% of variation in the macroinvertebrate community. CL (site 1) was positively associated with RDA 1 and negatively with RDA 2 and was more influenced

by ammonium levels figure 7.20c). LC was not aligned positively with both RDA 1 and RDA 2 but was not associated with any particular variables. DL was negatively aligned with RDA 1 and RDA 2, and had more influence from pH and phosphate levels, and the Ver site was more associated with nitrate levels. Gammaridae was more affected by nitrate levels and DO levels, whereas Chironomidae were affected by turbidity and EC. Assellus was more positively associated with ammonium levels and Limnephilidae was positively associated with phosphate levels. The forward selected models deemed most important from this model were DO, nitrate and turbidity (table 7.21).

Table 7. 21: The forward selected variables from each model in the RDA models from the 2023/24 and 2024/25 season. These variables were deemed most significant from each model and were used in the variance partitioning. There was a 95% confidence limit and if $p < 0.05$, the variable was significant (grey highlight).

Forward Selection of Variables								
Season	Model	variables	order	R2	R2Cum	AdjR2Cum	F	pval
2023/24	Model 1 (All variables)	Width	10	0.18	0.18	0.16	9.03	0.001
		WaterTemp	9	0.06	0.24	0.20	3.28	0.004
		Nitrate	2	0.06	0.30	0.24	3.13	0.004
		MinTemp	17	0.05	0.35	0.28	2.69	0.005
		DO	7	0.04	0.39	0.31	2.72	0.008
		Flow	12	0.03	0.42	0.33	2.13	0.05
	Model 2 (Hydrological Variables)	Width	2	0.18	0.18	0.16	9.03	0.001
		WaterTemp	1	0.06	0.24	0.20	3.28	0.002
		MinTemp	9	0.04	0.29	0.23	2.34	0.022
	Model 3 (Water Quality Variables)	DO	7	0.10	0.10	0.08	4.52	0.001
		Nitrate	2	0.04	0.14	0.10	2.10	0.038
		Turbidity	6	0.04	0.19	0.13	2.09	0.043
2024/25	Model 1 (All variables)	Width	10	0.26	0.26	0.24	12.99	0.001
		BOD	8	0.06	0.32	0.28	3.11	0.001
		Conductivity	5	0.04	0.36	0.31	2.38	0.019
	Model 2 (Hydrological Variables)	Width	2	0.26	0.26	0.24	12.99	0.001
	Model 3 (Water Quality Variables)							
		BOD	8	0.19	0.19	0.17	8.88	0.001

The variance partitioning model combined the forward selected variables from models 2 and 3 and the result was significant (ANOVA: $F(3,36) = 2.5583$, $p=0.001$) (figure 7.21). The hydrological variables (channel width, water temperature, minimum air temperature) explained 18.8% and the water quality variables (DO, nitrate levels and turbidity) explained 8.2% of the variance in macroinvertebrate communities. The shared effect of the two models explained 4.3% of the variance in the macroinvertebrate community, leaving 68.7% of the variance unexplained.

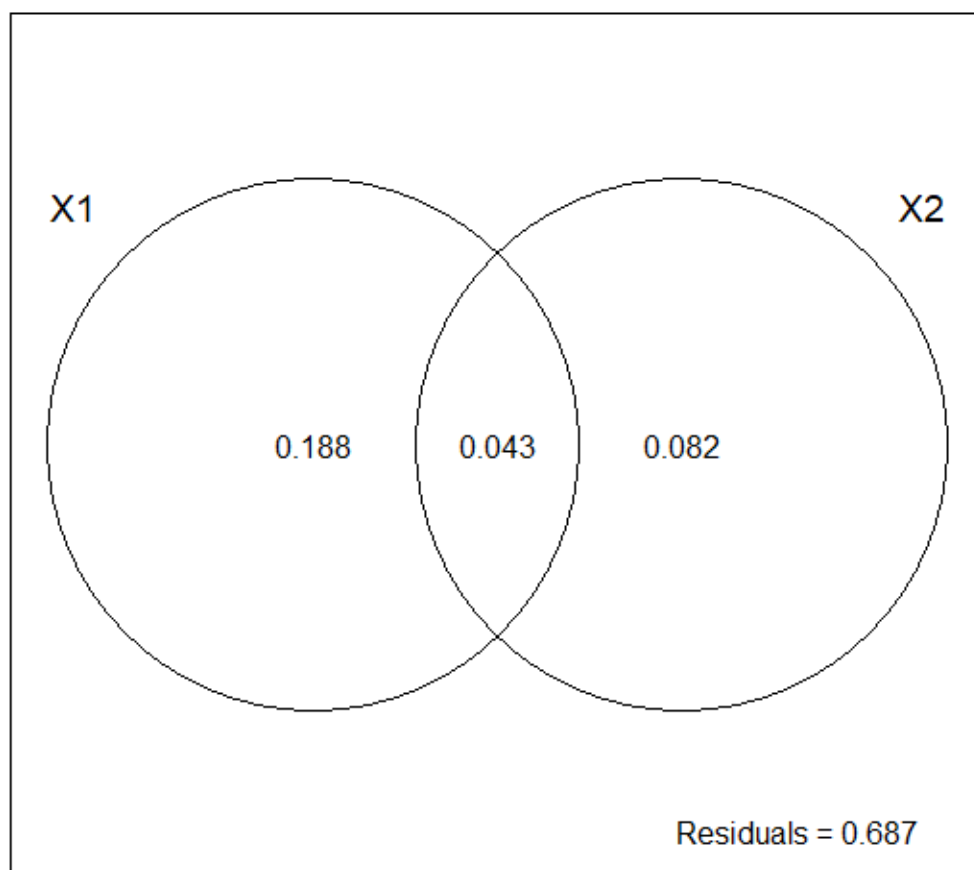


Figure 7. 21: The variance partitioning of the amount of variance in the macroinvertebrate community explained by the forward selected models from the 2023/24 season. X1 is the model2 (hydrological) and X2 is model 3 (water quality variables). The amount each individual model contributes to the variation is in the separate venn diagram section and the middle section represents the combined effect

of the two models. The residuals is the remaining variance that is unexplained by the models.

In 2024/25 season, the environmental parameters were found to significantly explain 33% of the variance in the macroinvertebrate community (ANOVA: $F(17,21)=2.09$, $p=0.001$, adjusted $R^2 = 0.33$). The RDA 1 axis explained 34.80% of the variation within the model and was positively influenced by ammonium (0.21) and negatively by channel width (-0.34). The RDA 2 axis explained a further 7.61% and was positively affected by the minimum air temperature (0.24) and negatively by EC (-0.34). However, only BOD and channel width were significant predictors within the model (table 5.20). Site 1 (CL) and the asellus family were closely associated positively with both the RDA 1 and RDA 2 axes, and were influenced more by the ammonium levels, air and water temperature and daily total rainfall levels (figure 7.22a). Site 2 (LC) was more associated positively with RDA 2 and negatively with RDA 1, and the site experienced more influence from turbidity and conductivity. Site 3 (DL) was more associated with pH and air and water temperature variables and site 4 (V) and Gammaridae were more influenced by channel width, average flow velocity and discharge. The seasons did not appear to have any particular influence from the variables and were randomly dispersed across the RDA axes. The forward selected variables were channel width, BOD and electrical conductivity (table 7.21).

The hydrological variables (model 2) significantly explained 29% of the variation in the macroinvertebrate community in the 2024/25 season (ANOVA: $F(9,30) = 2.97$, $p = 0.001$, adjusted $R^2 = 0.29$). The RDA 1 axis explained 29.44% of the variance and was strongly positively influenced by channel width (0.92) and negatively by maximum air temperature (-0.12). The RDA 2 axis explained a further 6.59% and was positively associated with channel discharge (0.40), but did not have any negative associations (figure 7.22b). However, average channel depth ($p = 0.001$), channel width ($p = 0.001$) and channel area ($p = 0.029$) were the significant variables within the model (table 7.20). CL and DL were more associated positively with RDA 2 and negatively with RDA 1 and the air temperature, water temperature and daily total rainfall levels had a positive influence on the macroinvertebrate community at these sites (figure 7.22b). LC and *Baetidae* did not show any strong relations to the hydrological variables. V was positively associated with RDA 1 and RDA 2 and were positively influenced by average

channel depth, channel width, channel area and discharge. The seasons appear to be randomly distributed and not have any alignment to the RDA axes or variables. However, for model 2 only channel width was selected in the forward selection (table 7.21).

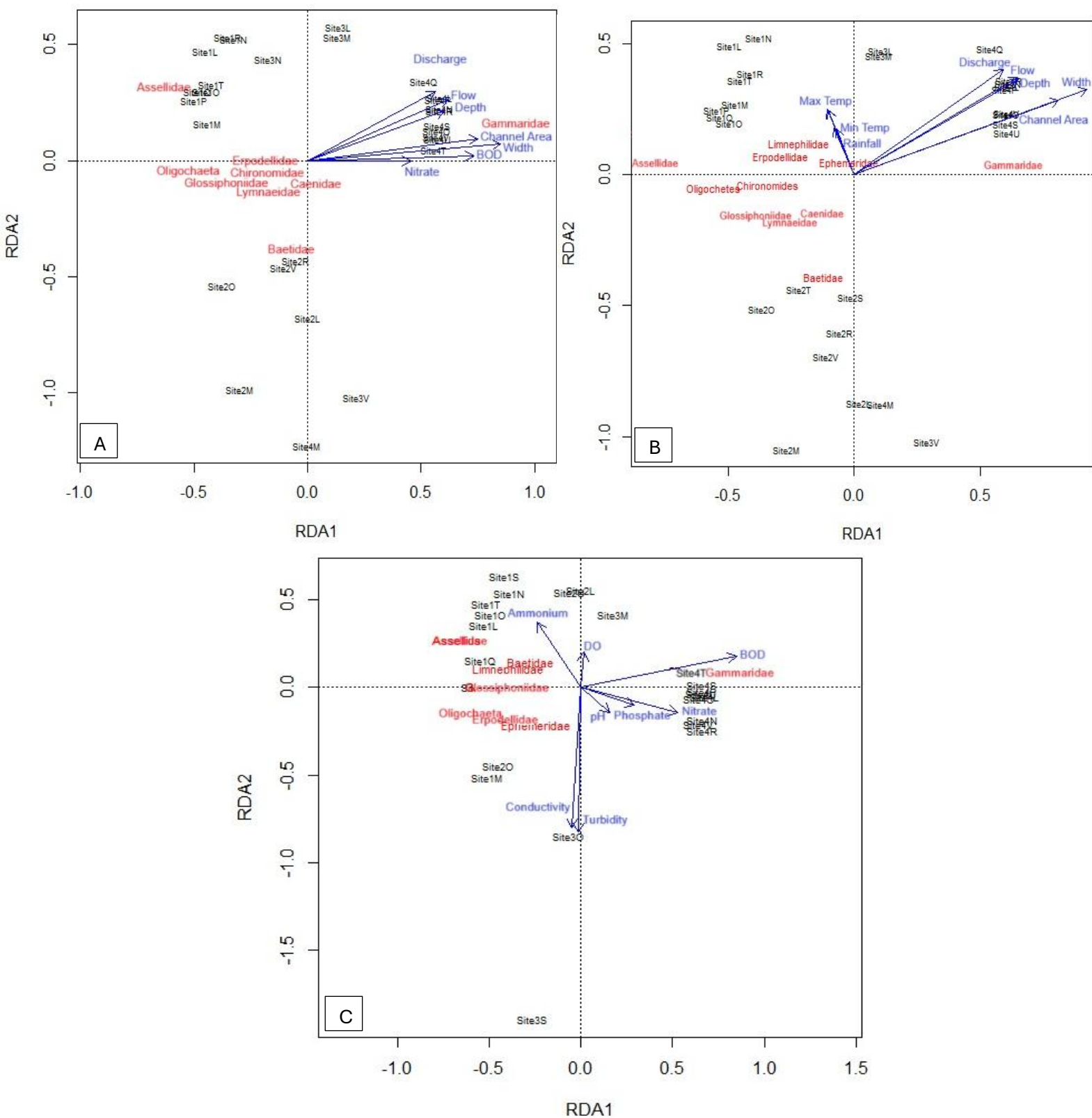


Figure 7. 22: The RDA plots showing the relationships between the environmental variables and the sites and macroinvertebrate families in the 2024/25 season. The

variables are in blue and the length and direction of the arrow signifies the strength of the variable and its relation to the RDA axes and sites and families. Site 1 is CL, Site 2 is LC, Site 3 is DL, Site 4 is V. The months are denoted as letters (A is April, B = May, C = June, D = July, E = August, F = September, G = October, H = November, I = December, J = January, K = February). (a) is the plot for model 1 (all environmental parameters), (b) is model 2 (hydrological parameters) and (c) is model 3 (the water quality parameters including water nutrients and physical-chemical variables).

The water quality variables were also significant for the 2024/25 season and explained 21% of the variance in the macroinvertebrate community composition (ANOVA: $F(8,30) = 2.23$, $p = 0.001$, adjusted $R^2 = 0.21$). The significant variables were BOD ($p = 0.001$) and nitrate levels ($p = 0.032$) (table 5.20). The RDA 1 axis explained 26.69% of the variance within the model and was positively associated with ammonium (0.24) and strongly negatively associated with BOD (-0.85) (figure 7.22c). RDA 2 explained 4.76% of the variance and was positively associated with ammonium levels (0.37) and negatively with turbidity (-0.82). At the CL site, ammonium had a strong influence on the community and Asellus was a key species at the site that was positively associated with ammonium levels (figure 7.22c). In summer, the LC and DL sites had more influence from turbidity and EC. DO had a stronger influence on LC in spring and winter months. V was negatively associated with RDA 1 and had a positive relationship with BOD, nitrate and phosphate and Gammaridae was a key species. However, the only forward selected variable was DO (table 7.21).

The variance partitioning model combined the forward selected variables from models 2 and 3 and the result was significant (ANOVA: $F(2,36) = 8.42$, $p = 0.001$) (figure 7.23), although only two variables were used as only one was significant from each model. The hydrological variables (channel width) explained 10.9% and the selected water quality variables (BOD) explained 4.1% of the variance in macroinvertebrate communities. The shared effect of the two models explained 13.1% of the variance in the macroinvertebrate community, leaving 71.9% of the variance unexplained.

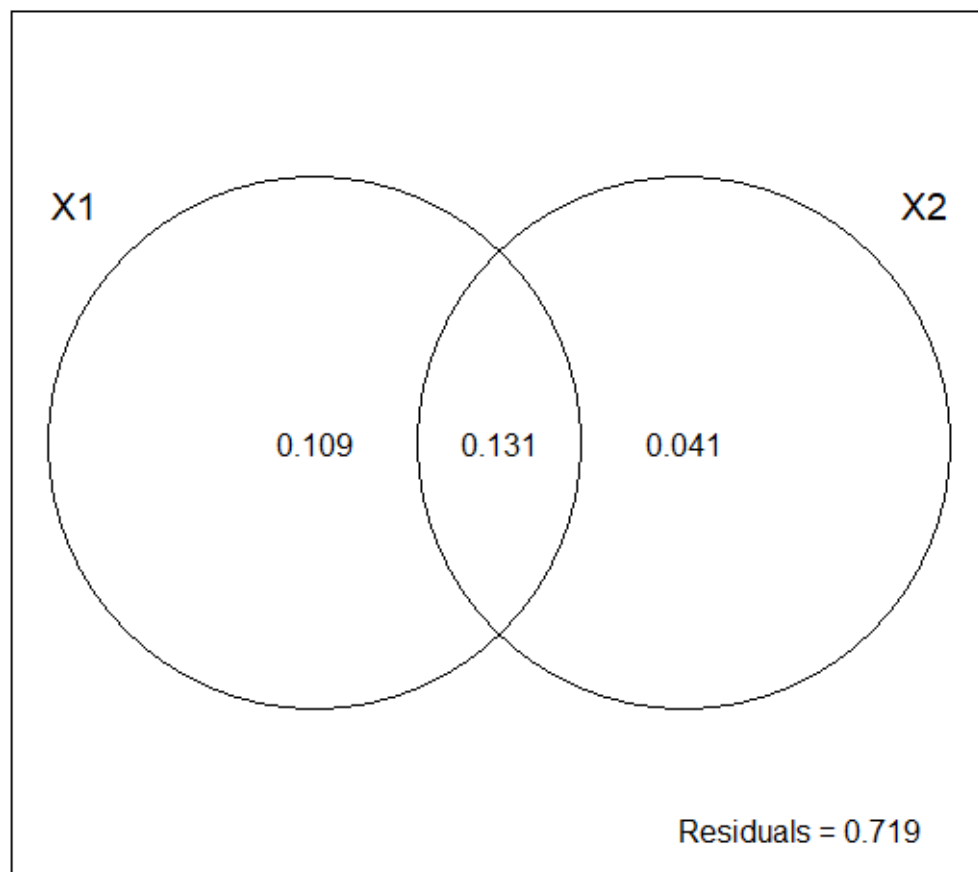


Figure 7. 23: The variance partitioning of the amount of variance in the macroinvertebrate community explained by the forward selected models from the 2024/25 season. X1 is the model2 (hydrological) and X2 is model 3 (water quality variables). The amount each individual model contributes to the variation is in the separate venn diagram section and the middle section represents the combined effect of the two models. The residuals is the remaining variance that is unexplained by the models.

7.4. Discussion

7.4.1. Changes in taxa diversity within the Upper Colne Catchment

The patterns of diversity remained consistent across the two inter-annual seasons, with minimal variation observed in diversity measures at both the family and species levels. This indicates that, for the Upper Colne catchment, family-level monitoring is sufficient to detect changes in diversity. Among the sites, London Colney consistently exhibited the highest family richness, species richness, and diversity scores (Simpson and Shannon indices), making it the most ecologically diverse site. In contrast, the Ver site consistently had the lowest family richness, species richness, and diversity scores. However, it recorded the highest number of individuals, attributed primarily to the high abundance of *Gammarus pulex*. This dominance was further reflected in the Ver site's lower Shannon Evenness scores, indicating a skewed community structure dominated by the Gammaridae family. These findings suggest that diversity tends to decline further downstream, where generalist taxa become more prevalent, as opposed to the upper reaches, which may host more resilient communities in response to changing environmental conditions. In the 2024/25 season, the Drop Lane site exhibited the lowest Shannon Evenness score, implying that while *Gammaridae* was the most abundant taxon, it did not dominate other families to the extent observed at the Ver site.

While family richness varied between seasons, the number of individuals remained relatively stable throughout 2023/24, but differed in 2024/25. In 2023/24, family richness was higher in spring and summer than in autumn, potentially due to macroinvertebrates being airborne during autumn and resuming activity or hatching in spring due to rising temperatures (Wallace *et al.*, 1990; Bunting *et al.*, 2020). Species richness followed a similar trend, being greater in winter and summer than in autumn. However, diversity scores showed little seasonal variation across both 2023/24 and 2024/25, suggesting that although species composition may have changed, family richness remained relatively stable, meaning certain species may dominate during certain seasons, but resulting in the same number of taxa being present. Shannon Evenness was slightly lower in summer, suggesting the presence of a few dominant species—likely those adapted to low-flow conditions such as *Erpodeiidae*,

Glossiphonidae and *Limnephilidae* (Extence *et al.*, 1999). This exemplifies how seasonal variations in taxa diversity can be complex and how temporal variations can influence the diversity. This was also the case in a previous study that found that spring and autumn diversity were similar at one site but differed at another, possibly due to the low detection of some taxa (Aspin & House, 2022; Kabir *et al.*, 2024).

Due to the hierarchical structure of river systems, the number of organisms generally increases downstream. This pattern was observed in the current study, with the highest number of invertebrates recorded at the Ver site compared to the upstream locations. However, compositional turnover, or beta diversity, tends to be greatest in headwaters, where environmental conditions are more variable (Sarremejane *et al.*, 2017). Headwater communities typically contain fewer species, and the isolation of habitats can lead to limited dispersal, increasing the chances of stochastic extinctions and variability between communities (Sarremejane *et al.*, 2017).

Despite the general downstream increase in abundance, the reduced number of invertebrates and families at Drop Lane suggests that the relationship between invertebrate abundance and spatial location is more complex. Habitat type can also play a role in shaping family richness which may be something for future studies to investigate. For instance, Wright and Symes (1999) found that family richness per sample was higher in December than in June, and higher on macrophytes than on gravel or silt. Nevertheless, over a nine-year period, a large proportion of invertebrate families were found across all biotope types (Wright & Symes, 1999). This shows that temporally, diversity levels can remain stable if there are no external pressures. Therefore, the relatively consistent level of diversity throughout the seasons in the Upper Colne may be expected.

7.4.2. Relationships between taxa diversity and environmental parameters within chalk stream environments

Among environmental variables, hydrological factors had the most significant influence on diversity. Channel width and average channel depth significantly affected the number of individuals present, as well as Simpson and Shannon diversity measures. Specifically, both channel width and depth had a negative effect on family richness, indicating that wider and deeper channels supported fewer taxa - consistent with the low diversity observed at the Ver site. Channel width, however, was positively correlated with the number of individuals, which aligns with the high individual counts at the Ver site, particularly of *Gammarus pulex* which prefers faster flows (and therefore wider and deeper channels) and lower counts at the narrower CL and LC sites. These sites instead had families or species that preferred more lotic conditions, such as the *Limnephilidae*, *Erpodellidae* and *Glossiphonidae*, as well as the mollusc families. In a similar study, taxon richness was observed to increase steadily with rising flow rates, boosting diversity among *Chironomidae*, *Diptera*, dragonflies, water mites, lentic stoneflies, and caddisflies (Boulton, 2003). However, with flow cessation, average taxa richness declined sharply - sometimes by half (Boulton, 2003). This would correlate with the presence of debris dams upstream of the Drop Lane site and downstream of the weird at the Church Lane site. These sites often had impeded flow, and whilst debris dams have been found to benefit macroinvertebrate diversity, in this study the flow cessation was more of a controlling factor that had a negative influence on the macroinvertebrate diversity, particularly at DL where there was increased siltation. The increased siltation would be detrimental as naturally chalk streams are low power and would experience lower levels of sediment transport and deposition (Acornley *et al.*, 1999), which may indicate the decrease in macroinvertebrate diversity as the taxa are adapted to exposed gravel riverbeds (Acornley *et al.*, 1999; Sear, Armitage & Dawson, 1999). This decline also coincided with reduced DO, increased electrical conductivity, and higher water temperatures, all of which favour predatory taxa (Boulton, 2003). Furthermore, a study by England *et al.* (2024) also showed that macroinvertebrate diversity increased post weird removal, showing that in chalk stream catchments, mechanically impeded flow has a detrimental impact on the

macroinvertebrate diversity. Previous studies also found that intermittent streams in the upper reaches of chalk streams had lower taxon richness and abundance than perennial streams but higher than longer-flowing temporary reaches (Boulton, 2003; Stubbington *et al.*, 2017). This higher taxon richness was contributed by Trichoptera, *Mollusca*, Ephemeroptera, Coleoptera, and *Annelida*. Permanent sites were dominated by *Gammarus pulex* and Ephemeroptera (including *Baetis rhodani*, *Serratella ignita*, *Caenis luctuosa*), while winterbourne sites hosted more *Diptera* (Armitage & Bass, 2013). However, this was the opposite for the Upper Colne catchment, where the sites furthest upstream exhibited greater taxa richness and abundance. Also, certain species adapted to temporary streams - such as *Halipplus lineatocollis*, *Agabus biguttatus*, *Agabus guttatus*, and limnephilid caddisflies (*Limnephilus vittatus*, *Limnephilus bipunctatus*) (Armitage & Bass, 2013) - were absent from all Upper Colne sites with the exception of only one *Simuliidae* specimen that was identified to the family level. This shows that each river catchment, even though both are classified as chalk streams, can exhibit unique diversity and community traits.

Nitrate levels were another significant predictor: higher nitrate concentrations were associated with reduced diversity but increased individual abundance. Conversely, ammonium levels were positively associated with Simpson diversity, suggesting that higher ammonium may support greater diversity, albeit with dominant taxa present such as *Assellidae*, *Gammaridae* and *Oligochaeta* at DL where ammonium levels were relatively higher. Both turbidity and BOD had small negative impacts on Simpson diversity, implying that more turbid waters and lower BOD levels support fewer tolerant taxa. Community composition and taxon richness showed limited response to flow permanence due to substantial compositional variability within and among communities. This variability was driven by infrequent taxa, geographical isolation of headwaters (which supports distinct community assemblages), and habitat heterogeneity. Sites with the highest biodiversity and conservation value were those with high environmental heterogeneity, as this supports rare and specialist taxa. Conversely, unshaded, slow-flowing sites had lower diversity, often as a result of human activity such as riparian vegetation removal, water abstraction, land drainage, and impoundment (Allan, 2004; Kabir *et al.*, 2024).

Despite this study including a wide range of environmental parameters that influence the taxa richness and diversity, the amount of variance explained by the significant variables such as channel width and nitrate levels was still relatively low. This means that there are other factors which are influencing the macroinvertebrate diversity dynamics in the Upper Colne catchment. A previous study based on land-use parameters rather than riverine environment conditions found that taxonomic richness and diversity were higher in freshwater systems bordered by woodland (Broadmeadow & Nisbet, 2004) and declined with increased anthropogenic activity (Suurkuukka *et al.*, 2014). Biodiversity metrics were responsive to shading, influenced by local riparian canopy cover and overhanging vegetation. Taxa richness was generally greater at shaded sites, which provided optimal conditions for macroinvertebrates, including sufficient light to sustain macrophytes and biofilms that serve as food for grazing and shredding macroinvertebrates (Wright & Symes, 1999; Riley *et al.*, 2009; Kabir *et al.*, 2024). Therefore, this suggests that the interactions between the riverine environment and the wider surrounding environment and passive interactions on macroinvertebrate taxa are important in understanding the full extent of the patterns in diversity within chalk stream catchments.

7.4.3. Establishing the macroinvertebrate community composition within the Upper Colne Catchment

The macroinvertebrate community recorded in the Upper Colne catchment during this study did reflect assemblages typically associated with chalk streams, aligning with the findings of previous research on such systems (e.g. Bunting *et al.*, 2020, Datry *et al.*, 2014, Mainstone, 1999). However, it diverged notably from the only other macroinvertebrate survey conducted specifically on the Upper Colne from 1993 (Gunn & Blackburn, 1993). The site that was closest to the field sites at Munden House from the 1993 study (downstream of the current Drop Lane site) found 11 taxa present, with only one overlapping with this study (*Ischnura elegans*). None of the previous 11 taxa were Red Data Book or Notable species, nor occurred in less than 5% of RIVPACS III reference sites and this was also the case in the 2023/24 and 2024/25 seasons. However, this difference in macroinvertebrate taxa assemblage potentially highlighted the long-term change in community structure over the past three decades within the

Upper Colne macroinvertebrate community. This change in community composition, particularly at the Drop Lane site, could be due to the change in environment, particularly the influence of the debris dams (explained in section 7.4.2.), but also the relative warming of the climate in the UK since the 1993 study and other chalk streams have experienced community composition changes during this period (Durance & Ormerod, 2009).

In terms of community composition, there was not much variation across the intra-annual seasons, due to the large overlaps in the NMDS plots which signify that the seasons largely shared macroinvertebrate communities in both the 2023/24 and 2024/25 season. In 2023/24, the winter season was more associated with *Limnephilidae*, *Asellus*, and *Glossiphoniidae* and the summer season was associated with *Hydrobiidae*, *Oligochaeta*, *Chironomidae* and *Daphniidae*. In 2023/24 there was a significant difference in the community composition between the seasons. However, the season only explained 17.56% of the differences in community composition and all taxa contributed to less than 10% of the differences in the macroinvertebrate communities. Summer and winter had the largest difference and *Gammaridae* was the strongest indicator species, followed by *Daphniidae* (more abundant in summer than winter). *Limnephilidae* also had a higher abundance in summer than in winter, but this could be due to higher flows in winter reducing their available habitat preference (Brophy, 2025). These were minor changes in the seasonal macroinvertebrate assemblages, and this was not replicated in the following 2024/25 season, when season did not have a significant effect on macroinvertebrate community composition. This finding has been found previously where the community structure has remained similar across decades and only found to vary longitudinally (Armitage & Bass, 2013).

In contrast, the macroinvertebrate community composition did vary longitudinally across the four sites. The Ver site was strongly dominated by *Gammaridae* which are a keystone species of chalk streams (Chamout *et al.*, 2015). CL was strongly associated with *Asellidae*, *Erpodeellidae*, *Glossiphoniidae* and slightly with *Limnephilidae*. LC was associated with *Oligochaeta*, *Chironomidae*, *Daphniidae* and *Neritidae*. DL was associated with *Gammaridae*, but not strongly and negatively associated with the presence of *Oligochaeta*, *Neritidae*, *Hydrobiidae*, *Chironomidae*. The site location was found to explain 43.23 % of the variation between the

macroinvertebrate communities and CL and V had a 90% dissimilarity. Between these sites, *Gammaridae* and *Asellidae* were the largest contributors to the differences, as well as *Limnephilidae*, *Erpodeiidae*, *Glossiphonidae* and *Hydrobiidae*, all of which had greater abundance at CL than V. Overall, *Gammaridae* were a big indicator of differences between all four sites at varying degrees. This difference was replicated between the sites in the 2024/25 season too, with *Gammaridae* being dominant at the Ver site, and CL had a more diverse community assemblage including *Assellidae* and *Oligochaeta*. CL and V were again the most different to each other. LC and DL did have an overlap and shared taxa composition, meaning their community composition was more similar. CL and LC had the most similar community compositions in both inter-annual seasons, where the only significantly different family was *Lymnaeidae* being more abundant at CL. Some indicator taxa for the sites could potentially be presence of *Baetidae* for LC, *Gammaridae* at V, *Assellidae* at CL and *Chironomidae* at DL.

The species level of macroinvertebrate taxa added another layer of complexity to the macroinvertebrate community composition models. Whilst *Gammarus pulex* was still a significant contributor to determining the macroinvertebrate community, more specialist species such as *Limnephilus Rhombicus*, *Ephemera dancia* and *Microvelia reticulata* became more influential on the composition, whereas these families tended to play minor roles in the previous model. However, this was only significant at the community composition differences between the sites, not the seasons. Summer was marginally influenced by a greater presence of *Chironomidae* species and spring and autumn had a similar composition and influence from *Erpobedella octoculata* and *Baetis rhondani*. Winter had more influence from *Asellus aquaticus*, *Limnephilus lunatus* and *Oligochaeta*. In contrast, the sites did show significant differences in their community composition at the species level and the largest dissimilarity was between CL and V with *Gammarus pulex* being a determining species. Only *Lymnaea peregra* made a slight difference between CL and LC. LC was also more similar to DL than CL was to DL and there was greater overlap with the species present (similar to family level) i.e. *Gammarus pulex*, *Chironomidae*, *Oligochaeta*, *Coernagrionidae* and *Ceratopogonidae*. The community composition of the upper course being significantly different to the DL and V sites could also be due to the intermittent nature of the chalk stream headwaters, where the communities are often markedly different to their

perennial sections (Boulton, 2003). During droughts, certain species such as *Gammarus pulex* (the dominant crustacean in English chalk streams) disappear which may be why they are less common upstream (Boulton, 2003).

The most frequently encountered taxa in the present study across both sampling seasons did align with taxa commonly found in chalk streams (Bunting *et al.*, 2020) and the most abundant taxa were *Gammaridae*, *Asellidae*, and *Oligochaeta*, with *Mollusca* and *Trichoptera* representing the most taxonomically diverse groups at the family level. Notably, the family *Limnephilidae* (Trichoptera) exhibited particularly high diversity, with twelve species recorded in the 2024/25 season, consistent with their known adaptability to the hydrological variability of temporary or intermittent systems (Wallace, Wallace & Philipson, 1990). These caddisflies exhibit larval development during wetter periods and metamorphose into adults before river flows diminish, with oviposition occurring in late summer and autumn. Species with larval diapause tend to be restricted to perennial systems, where females can access aquatic substrates for egg-laying. The spatial distribution of *Limnephilidae* further indicated site-specific preferences, with almost all individuals recorded at the Church Lane site and only larval instars present at the three downstream locations. This suggests that environmental conditions at Church Lane were particularly favourable. For example, *Limnephilus incisus* is known to inhabit slow-flowing or stagnant ditches and pools with neutral to alkaline pH; *L. flavicornis* prefers marginal pond habitats and exhibits summer diapause behaviour; while *L. decipiens* prefers detritus-rich, slow-flowing waters (Brophy, 2025). These ecological requirements correspond closely to conditions observed at Church Lane, including its slow, stagnant flow, alkaline pH, and availability of particulate organic matter from marginal vegetation and floodplain interactions. Additionally, one specimen of *Mystacides longicornis* was recorded at Drop Lane in winter. This species is a more generalist limnephilid species, capable of exploiting a wider range of habitats and is more broadly distributed than those previously mentioned (Brophy, 2025). These findings suggest a general preference for the more protected, slower-flowing upper reaches of the Colne among *Limnephilidae*, likely driven by the hydrological conditions of the upper reaches of chalk streams.

Out of the 13 sites studied along the Colne, only 26 species overlapped with the current study. In the 1993 survey (Gunn & Blackburn, 1993), only one limnephilid

species (*Limnephilus lunatus*) was identified, and both *Polycentropodidae* and *Leptoceridae* were absent from all sites. Additionally, only one snail species (*Lymnaea peregra*) was found, and several taxa (*Elmidae*, *Astacidae*, *Culicidae*, *Caenis rivulorum*, *Ephemera vulgata*, and *Pleidae*) that were absent in 1993 were recorded in the 2023/24 and 2024/25 investigation. Among the more widely distributed taxa, *Baetis rhodani* was found at all four sites. This highly adaptable species is associated with both fast-flowing streams and vegetation-rich chalk streams and is among the most common mayflies in Europe (Gattolliat & Sartori, 2008). *Caenis rivulorum* was recorded at all sites except Church Lane and is the most common representative of *Caenidae* in the UK. It has a relatively localised distribution in southeast England and exhibits an annual life cycle, overwintering as nymphs with adults emerging between May and September (Elliott, Humpesch & Macan, 1988). The absence of this species from CL could be due to the very slow flowing nature of the site, along with the lack of available stony river margins, compared to LC and V.

Other ecologically significant taxa observed included members of the *Goeridae* and *Ephemeridae* families. *Goera pilosa* was the only *Goeridae* species recorded, with larval forms present in spring, summer, and winter, but absent in autumn—consistent with adult emergence between May and September (Wallace *et al.*, 1990). It was also only present at the Ver site, where the gravel bed was more exposed, providing more material for the construction of its cases. *Ephemera danica* was found at the Drop Lane and Ver sites and is normally associated with unpolluted, fast-flowing, sandy or gravel-bed rivers, where the nymphs burrow into the substrate (Wallace *et al.*, 1990). Although traditionally assumed to have a two-year life cycle, recent studies suggest this may be reduced to a single year in the warmer waters of southeastern England (Macadam & Bennett, 2010) which may be why this species was present throughout sampling. *Ephemera vulgata* was recorded only at the Ver site in winter and is known to inhabit organically rich sediments and to bioaccumulate heavy metals (Jop, 1991), which may be a particular concern due to the recent concerns regarding heavy metals within the Colne Catchment (CVFC, 2023).

These findings are broadly consistent with previous studies on chalk stream macroinvertebrate assemblages. For instance, Bunting *et al.* (2020) reported dominance by *Oligochaeta*, *Gammarus pulex*, *Chironomidae*, *Ceratopogonidae*,

Limnephilidae, *Serratella ignita*, and *Caenis* spp. in hyporheic communities. Benthic assemblages were similarly dominated by *Gammarus pulex*, *Limnephilidae*, *Oligochaeta*, *Serratella ignita*, and *Hydroptila*, collectively accounting for over 60% of individuals. Taxon richness was found to be generally higher at perennial sites further downstream in previous studies, although overall abundance did not vary significantly across hydrological regimes (Bunting *et al.*, 2020; Kabir *et al.*, 2024; Stubbington *et al.*, 2017). However, this was contradicted by this study where there was higher family and species richness at the two most upstream sites (CL and LC) compared to further downstream, although other factors could be influencing the dynamics more than the flow regime discussed in section 7.4.3. Despite this, the most common taxa were still similar when compared to past studies with *Trichoptera*, *Diptera*, and *Coleoptera* being the most diverse orders, representing 24%, 22%, and 14% of total richness, respectively (Kabir *et al.*, 2024). The taxa found further upstream at CL and LC were also still generalist taxa and there were not many temporary specialists macroinvertebrates present, other than some of the *Limnephilidae* species at CL, but this is also the case in other chalk streams, where resilience to changing environments is more key than specific adaptations to survive lower flow levels (Datry *et al.*, 2014).

Overall, the macroinvertebrate communities displayed marked differences across the sites and became dominated by *Gammaridae* with increases downstream. Upstream, the community had more variation, and the other 3 sites had the largest difference with the Ver site. The differences between the seasons were minimal, and due mostly to the presence of more specialist taxa such as *Limnephilidae*. CL and LC were the most similar in terms of their community composition and this could be due to their similar environmental conditions, such as narrower channel width and shallower depth, and slow flow velocity. The increase in family/species richness and diversity further upstream was notable, as usually sites that experience intermittent flow or cessation have been found to have lower taxa richness and taxa diversity (Datry *et al.*, 2014). However, due to the consistent rainfall levels throughout the summer seasons in 2023/24 and 2024/25, the aquifer levels did not decrease significantly to allow for the intermittent flows that are often experienced in the upper courses of a chalk stream, as seen previously in 2022. Macroinvertebrate taxa composition was found to not be consistent across the sites and inter-annual seasons. If there was flowing-water consecutive years, the taxa composition remained similar, but if there was a year of

drying the composition could be different due to decreased densities of various taxa throughout the drought (Boulton, 2003). The findings of the present study contribute to a growing body of evidence that macroinvertebrate communities in chalk stream systems are shaped by a complex interplay of hydrology, water chemistry and physicochemical characteristics, sediment characteristics, and habitat heterogeneity. Temporal shifts in community composition and site-specific patterns of taxon richness underline the importance of long-term and spatially resolved monitoring to detect and interpret ecological change in these sensitive and dynamic systems.

7.4.4. The use of bioindicies in the Upper Colne Catchment to determine ecological and water quality

The Upper Colne Catchment mostly experienced poor ecological quality status across the sites and intra-annual seasons, according to the WHPT-ASPT scores, meaning that the taxa present did not indicate good ecological standards or good water quality. This is in line with the current state of the chalk streams largely being in poor ecological health and poor water quality status (O'Neill, 2014; Talks, 2008). Interestingly, compared to the diversity scores, the Ver site had the highest WHPT-ASPT score which indicated that the families that inhabit this site (mostly *Gammaridae*, and the occasional *Limnephilidae* and *Goeridae* family) were all indicative of higher water quality, compared to the sites further upstream. Of particular note was the CL site which had greater diversity but the lowest WHPT-ASPT scores and was categorised as poor for most months. The high numbers of *Gammarus pulex* at the Ver site may also have skewed the WHPT-ASPT score more so than at the other sites which had lower number of individuals present. However, it had been previously found that the total WHPT score and WHPT-ASPT scores increase with flow velocity and permanence, so the upper reaches having a lower WHPT score compared to further downstream where the flow rate is more stable is not unusual (Bunting *et al.*, 2020). This normally coincides with an increase in taxa richness, although that was not the case in the Upper Colne catchment (Bunting *et al.*, 2020). This could mean that there are more complex interactions occurring in the Upper Colne catchment determining

the taxa present and the quality of the taxa found at each site. However, the overall poor-quality status of the Upper Colne means that not enough has been done to improve the water courses and if the status is already poor in the upper courses, this will only be impacted further downstream, with additional impacts from STWs, agriculture and urban land uses.

The LIFE score was different between the sites and seasons. It was higher during winter, when there were greater flows at all the sites, meaning that macroinvertebrates that are more suited to high flow tolerances were found such as *Gammaridae*, *Ephemeraeidae*, *Glossosomatidae*, *Physchomyiidae* and *Goeridae* (Extence *et al.*, 1999; Stubbington *et al.*, 2017). In the summer, more slow flowing species were found which coincides with a reduction in total daily rainfall levels (Met Office, 2025) and the reduction in the groundwater levels leading to a lower flow rate within the Upper Colne Catchment (EA, 2024). The Ver had the highest LIFE index, and this was the most constantly flowing site, whereas DL had the lowest along with CL and there was often very little to now flow at these sites, where *Caenidae*, *Erpobdellidae*, *Planorbidae*, *Lymnaeidae*, *Physidae*, *Planariidae* were present. The LC site had an intermediate LIFE score and this was the second most consistently flowing site. Therefore, the flow velocity does appear to depict what macroinvertebrates are inhabiting a site and having a mixture of flow and refuges along a chalk stream is important for maintaining a diverse ecosystem (Extence *et al.*, 1999). However, despite the differences in the LIFE index between the sites and the preference for more sensitive species being present at the sites with faster flow velocities, the use of the WHPT in conjunction indicates that flow velocity is not the only useful indicator of good aquatic health. Therefore, section 7.4.5. investigates the other relations between macroinvertebrate diversity and assemblages with the hydrological, chemical and physicochemical variables.

7.4.5. The relations between the macroinvertebrate community composition and the chalk stream environment

This study showed that there were several hydrological and water quality variables (including water nutrients and physical-chemical variables) that were significantly influencing the macroinvertebrate community composition in the Upper Colne Catchment. Channel width, water temperature, nitrate level, minimum air temperature, dissolved oxygen level and average flow velocity were the forward selected variables for importance out of all the environmental parameters which explained 39% of the variance in community composition. As previously mentioned, the Ver site was largely dominated by *Gammaridae* which was associated with faster average flow velocities and wider channel widths, both of which were factors at this site. The macroinvertebrate community at DL was affected by water and air temperatures, where if the temperatures were higher, there was greater variance in community, and certain taxa thrived, such as *Daphiidae*. *Glossiphonidae* was more associated with ammonium as leeches often prefer nutrient rich waters (Pohle & Hamburger, 2005). *Gammaridae* was also associated positively with nitrates (as they are somewhat tolerant to non-lethal levels (Stelzer & Joachim, 2010), but *Chironomidae* *Baetidae* and *Oligochaeta* are negatively associated with nitrate, channel width, average flow velocity and dissolved oxygen levels. Therefore, the large numbers of *Gammarus pulex* present at the Ver site could be that they are better adapted to nitrate pollutants, which may have come from point sources from the sewage treatment works along the Ver's course. This pattern was reflected in the separate models for water quality and hydrology, with channel width, water temperature and minimum air temperature being the most important variables. However, average flow velocity was not included as a significant variable despite high correlation to channel width and was an original significant variable in the model. This shows how the interactions between the variables and the effects on the community are complex and multifaceted. Most of the species within the communities were centralised in the RDA plots, meaning they had intermediate responses or a broader tolerance for the hydrological conditions. *Assellidae* was more associated with flow and negatively with warmer temperatures and *Gammaridae* was strongly associated with channel width again. For the water

quality model, the majority of the macroinvertebrate families did not show a major preference. The most important variables were nitrate levels, dissolved oxygen and turbidity levels. *Chironomidae* seemed to be positively associated with conductivity and turbidity. *Asellidae* has a positive association with ammonium and negative association with nitrate, and *Limnephilidae* with phosphates and pH level. *Gammaridae* was associated with nitrates. *Oligochaeta* were associated with a decrease in DO. The separate models allowed for the more intricate responses of the families to the variables to be identified, even if they were not significant.

The environmental variables in the 2024/25 season appeared to explain slightly less of the community composition at 33% compared to 39% and only two parameters (BOD and channel width) were significant. The hydrological variables explained more variation at 29% and channel depth, channel width and channel area were the significant variables. The selected water quality variables for the 2024/25 season were BOD and nitrate levels. The associations were similar to previous year, although more families like *Baetidae*, *Glossiphonidae*, *Chironomidae* and *Lymnaeidae* were negatively associated with an increase in width, depth, discharge and flow. Only *Gammaridae* was positively associated with the hydrological variables. This suggests other species are more adapted to slower flowing, shallower river channels typical of the upper course of a chalk stream, and when there is a steep increase in flow, the family composition reduces to only those that can tolerate the conditions. This could potentially indicate why the hydrological variables sometimes explained more of the variance in macroinvertebrate community assemblages compared to water quality parameters as the taxa found across the sites were more sensitive to differences in flow which had more variation across the sites than the water quality.

The hydrology and water quality variance partitioning models made with the forward selected variables only explained around 30% of the variability in community composition, suggesting that there are other factors at play that affect the macroinvertebrate community. These could include factors such as substrate, sediment availability, macrophytes, land use, shade and vegetation clearance, micro pollutants from road runoff, predation. Community-level responses to change are usually due to responses in flow levels (Lancaster and Ledger., 2015). Some core taxa such as *Oligochaeta*, gastropods and Diptera are resistant to drying at all times of year

and are therefore more likely to be present in streams that have a history of drying, such as those sites further upstream on the Colne (Lancaster and Ledger., 2015). However, despite relatively high NMDS stress values (>0.2 ; Clarke, 1993), Kabir *et al.* (2024) found no evidence that macroinvertebrate community composition differed significantly across gradients of flow regime, land use, velocity, shade, or coarse woody debris. Instead, taxa richness increased with mean sediment size—being higher in gravel-dominated substrates than silt—and with faster flow velocities. Interestingly, local contributions to beta diversity (LCBD) were highest at non-flowing sites, suggesting that unique assemblages may be associated with stagnant or intermittently flowing habitats. Water quality, water quantity, and habitat quality are widely recognised as key drivers of macroinvertebrate community structure (England, 2004). Within these categories, factors such as water temperature, flow regime, river management practices, habitat heterogeneity, light availability, and density-dependent biological interactions play significant roles (Westlake *et al.*, 1972; England, 2004). Agricultural land use, in particular, can negatively impact macroinvertebrate communities by introducing fine sediment into streams, which homogenises the substrate and reduces habitat heterogeneity (Wood & Armitage, 1997; Kabir *et al.*, 2024). Fine sediments may also impair access to subsurface refuges critical for survival during dry periods. Conversely, coarse sediments offer larger interstitial spaces, promoting biodiversity by providing stable microhabitats (Kabir *et al.*, 2024). This study affirmed the importance of both flow and water temperature in shaping community composition. However, the relatively low proportion of variance explained by these parameters suggests that other unmeasured factors—such as river management, substrate type, and shading—may also be significant. Notably, the study focused on directly quantifiable variables like water nutrients, which also showed significant ecological influence. Future research would benefit from incorporating broader environmental parameters such as land use, sediment composition, habitat complexity, and vegetation management to develop a more holistic understanding of macroinvertebrate community dynamics in chalk streams.

7.4.6 Summary

This study affirmed the importance of both flow and water temperature in shaping community composition. However, the relatively low proportion of variance explained by these parameters suggests that other unmeasured factors—such as river management, substrate type, and shading—may also be significant. Notably, the study focused on directly quantifiable variables like water nutrients, which also showed significant ecological influence. Future research would benefit from incorporating broader environmental parameters such as land use, sediment composition, habitat complexity, and vegetation management to develop a more holistic understanding of macroinvertebrate community dynamics in chalk streams.

Chapter 8: General Discussion

This study was able to determine the benthic macroinvertebrate community in the Upper Colne Catchment and provide a cohesive insight into the temporal and spatial differences in macroinvertebrate assemblages and diversity, water quality and the chalk stream hydrological regime. The key takeaways were that macroinvertebrate taxa diversity and community structure showed more longitudinal variation, showcasing differences at the four different sites. The temporal differences between the intra-annual seasons, did not have as much of an influence on the chalk stream macroinvertebrate communities and diversity. The intra-annual seasons did have an effect on the hydrological regime and hydrological parameters, along with the physicochemical parameters. The macroinvertebrate communities were distinct at each site, having similar taxa observed at their specific sites, and this was consistent throughout the year. The diversity indices and family richness were also similar throughout the seasons, although this only indicated a similar number of families being present, not necessarily the same families (Kabir *et al.*, 2024). The difference between diversity at the family and species level was significant, suggesting that finer-level monitoring is critical in understanding the nuanced changes in diversity within chalk streams, as specific species, such as some *Limnephilidae* have certain adaptations to enable their survival (Brophy, 2025).

8.1. The Hydrology of the Upper Colne Catchment

This study established that the hydrological regime of the Upper Colne reflected that of other chalk streams, with low or non-flowing areas in the headwaters in the summer months and early autumn, to higher flow velocity between November to April when the groundwater and water table levels are high or perched (Sear *et al.*, 2006; Mainstone *et al.*, 1999; Costigan *et al.*, 2017). However, there was no total drying of the channel experienced in the 2023/24 or 2024/25 seasons, unlike in recent previous years in 2021, 2022 and 2023 (HMWT, 2023). Despite this, there were often instances when the sites on the upper Colne such as CL and DL were stagnant or had very slow flow. Although it is natural for flow to pool in some locations, at CL this was due to the

concrete weir of the bridge impacting flow and creating a dam for the buildup of organic leaf litter and woody debris. This is also the case for DL where the fallen trees upstream of the bridge have created a cross-channel blockage and the environment was distinctly different to the Ver site. This could be why these two sites experience different physical environments and nutrient levels, despite being the closest spatially out of all the sites. However, the Ver has a history of management along its course before this site, indicating that maintenance of the rivers is crucial in creating a prosperous flow dynamic. Constant flow impediment could be detrimental to the chemical and ecological health of the river at these sites, allowing pollution to buildup and stay in-situ for longer periods of time than if the flow was unobstructed. Long term monitoring of the headwaters of the Colne could be important for understanding the changes in the flow regime along with climatic changes and channel modification which has altered the flow level. Since flow was found to be an important factor in determining the macroinvertebrate community and influencing the taxa diversity, which is supported by previous studies where flow was also one of the most important factors in defining macroinvertebrate assemblages and diversity (Durance & Ormerod, 2009; Westwood & Leming, 2019; Extence *et al.*, 1999). The LIFE index also indicated low flow scores across the sites, so a key takeaway is that it is crucial that flow restoration is considered and actioned by the management plans. The presence of *Daphniidae* at the site furthest upstream at Church Lane also indicated that flow was extraordinarily slow for a chalk stream (Belaidi, Taleb & Gagneur, 2004). Potentially the chalk stream in the Upper Colne could be behaving more like a slow slowing ditch rather than a river system, which would impact upon the water quality and therefore the macroinvertebrate community that is able to thrive there. The presence of *Daphniidae* also indicates that their food source, phytoplankton is also readily available, which is a clear indicator of degradation. Restoring flow would improve the biodiversity in the macroinvertebrates and encourage colonisation of other invertebrates and fish species, leading to an overall greater biodiversity of chalk streams and their surrounding environment. Success at restoring flow has happened in other chalk stream catchments such as the River Nar in Norfolk (Norfolk Rivers Trust, 2025; CaBC, 2022). However, the current focus of the Colne Catchment Action Plan is to monitor micropollutants, remove invasive species, study micropollutants and pollution incidents and all of these actions are being carried out further downstream towards

Watford (ColneCAN, 2025). Although a recent survey was conducted on the upper Colne and some management strategies were suggested (HMTW, 2023), these have not been included in the local management plans and there were no mention of any specific monitoring strategies for improving the health of the Upper Colne (HMWT, 2022). Therefore, there is still a need for management plans and action to be taken in the Upper Colne Catchment as the quality of the river upstream will be compounded further downstream, regardless of downstream management practices.

8.2. The Water Quality of the Chalk Streams in the Upper Colne Catchment

The water nutrient levels in the Upper Colne catchment suggested that it is not in immediate danger from nutrients such as phosphates, nitrates and ammonium, although there are times when the WFD limits are exceeded. However, this could become more of an issue with potential lower flows as these nutrients can become concentrated (Defra, 2012). Some species are more sensitive to nutrient levels and this would affect them such as caddisflies, mayflies and stoneflies.

The water quality and flow indices also proved useful in indicating the quality of the chalk stream environment. The majority of the LIFE index scores were relatively low, due to the low power of chalk streams, and thus the species found were more tolerant of low flows. The LIFE index was more suited in showing temporal differences as the flow velocity had significant variation throughout the seasons, with lower flows in summer and higher flows in winter (Johnson *et al.*, 2009; Stubbington *et al.*, 2017). Therefore, if the species that are likely to appear in one site change or become more aligned with slower or faster flows, this would suggest that there has been a shift in the flow regime. The WHPT scores were useful in identifying the water quality along the upper Colne and did show a general decline in water quality, where most sites had a poor score by the end of the study. However, the WHPT measure does not detect dominance of species which could bias the results at the Ver which was generally higher quality than the other sites, where not many different taxa found, compared to upstream. Therefore, the WHPT and LIFE indices need to be used in combination with other measurements and diversity measures to generate a full picture of the aquatic environment and ecology. A particularly notable species in this study was *Gammarus pulex* which is often used as an indicator of good water quality in chalk streams (Dixon

& Shaw, 2011; Chaumot *et al.*, 2015.) *Gammaridae* became more dominant further downstream, particularly at the Ver site and this is due to their preference for a more perennial flow site, compared to the upper sites such as CL and LC where *Gammaridae* were largely absent. Therefore, the presence of *Gammaridae* could tell how long a site and flow has been established, which would be important going forward, with the likelihood of more frequent drying or drought events in the chalk stream headwaters. However, this species is largely unaffected by nitrate levels, so it would not be beneficial to use their presence to monitor water chemistry. The presence of Limnephilidae species in the upper courses has been shown to indicate higher dissolved oxygen levels (Nebeker *et al.*, 1996), and this was the case for the Colne catchment where Limnephilidae species were more common at the CL site where DO levels were relatively high. Therefore, there are certain species present within the Colne that can still be indicative of certain environmental conditions and their presence and absence can be used to mark changes in the aquatic environment (Westwood *et al.*, 2017; Stein, Springer & Kohlmann, 2008).

Whilst this study focused on the three main nutrients that macroinvertebrates and biodiversity is affected by, it did not cover all pollutants as this has been monitored elsewhere (such as the micropollutants report (CFVC, 2021)). There is a gap here to establish the impact of micropollutants and small changes in water chemistry. This is especially true if upper course starts to experience higher levels of nutrients, as this will be compounded further downstream with outfalls from sewage treatment works and water works (Neal *et al.*, 2000; Durance & Ormerod, 2009), or other sources of pollution, such as illegal dumping of sewage next to the river as in the common at Warren in Colney Heath, or at the Ver where there was oil pollution running in from a drain in November 2023.

8.3. The macroinvertebrate community assemblages and diversity and their relations to the aquatic environment

This study showed that the macroinvertebrate community and diversity levels can be maintained and remain stable if the aquatic environment conditions also remain stable. This suggests that the macroinvertebrate community in the Upper Colne can be resilient to minor changes in flow regime and water quality over short periods of time and recover well after short periods of drought or low flow, as stated by previous work (Eastman *et al.*, 2021; England *et al.*, 2019). However, comparing the macroinvertebrate community found in the Upper Colne in this study to the study from 30 years ago suggests that there has been longer-term changes to the diversity and community composition, as most of the species found in 1993 were not featured in this study (Gunn & Blackburn, 1997). This longer term change has also occurred in other chalk stream catchments, where unlike other surface-fed streams where the macroinvertebrate assemblages are constant, chalk stream assemblages experienced significant change over decades. Therefore, investigating only seasonal changes may not be sufficient to fully understand the temporal pressures that macroinvertebrates face (Durance & Ormerod, 2009).

The mix of water nutrients, physicochemical parameters and hydrological variables were found to be important in determining the macroinvertebrate community structure. This shows how interlinked the changes in the community are to their specific environment which would suggest why the spatial variation was greater than the seasonal. Seasonal changes were experienced at the catchment scale, rather than the local scale. Therefore, changes to the immediate chalk stream environment will be very likely to influence the macroinvertebrate community structure, moreso than larger climatic changes in the upper catchment (Durance & Ormerod, 2009). For example, the management of macrophytes and bank and in-channel vegetation influence the amount of sunlight that can enter the chalk stream and influence the rate of BOD turnover. Diversity was greater at sites with more shading such as CL. The flow velocity and the depth and width of the channel was also crucial. Turbidity and electrical conductivity was also important as the higher the turbidity and EC, the lower the Shannon and Simpson diversity. This could be due to higher nutrient levels and

sediment levels reducing the suitable environment for more sensitive macroinvertebrates that require interstitial spaces between gravel for refuge (Mondon et al., 2021). At highly turbid sites, *Asellidae* was more common as they are capable of existing in low quality water environments, whereas *Limnephilidae*, *Baetidae* prefer less turbid waters. With the extreme weather events in May 2024 and April 2024, where there were high levels of rainfall, this also impacted the macroinvertebrate communities, as the higher flow washed the invertebrates downstream and only those that preferred higher flow velocities could stay. The timing of the sampling also affected the macroinvertebrates found, as if there had been recent high flow, the site may not have had sufficient time to recover. However, the number of macroinvertebrates did not appear to differ greatly between seasons, suggesting that this did not impact the population dynamics too greatly. However, further understanding of temporal changes in community structure could be gained by sampling multiple times per month, to give a fuller picture of this, rather than a specific moment in time. Temporal variation in macroinvertebrate communities was less pronounced than spatial differences. Despite significant rainfall events and fluctuations in flow and discharge, macroinvertebrate diversity indices remained relatively stable across seasons. This suggests a degree of resilience in community structure, though the dominance of certain taxa may have shifted in response to short-term disturbances such as scouring flows in spring 2024.

Whilst this study did determine the relationships between the macroinvertebrate communities and taxa diversity with the riverine environment and identified the variables with the most significant impact such as flow, nitrates, channel width and depth and turbidity, it only explained 30% of the variation. This suggests that a study that also included external environmental parameters such as macrophyte type, substrate type, land use, other micropollutants use to create an interdisciplinary monitoring network in the Upper Colne. It is important to look after all sections of river, not just those advocated for by communities. For example, the River Ver has intensive cleaning, restoration and invasive species removal practices carried out by volunteers. In contrast, the Colne adjacent to the Ver has had obstructed flow for two years and management has been minimal further upstream too with only some bank vegetation removal once a year. Reviews and studies of the upper Colne and management reports need to be more interconnected and share data and ideas to have a holistic idea of the changes experienced in the upper Colne and the drivers of change.

Furthermore, the presence of *Daphniidae* in the Upper Colne indicates that this area of chalk stream is severely degraded as *Daphniidae* usually inhabit slow flowing lentic environments with a readily available supply of phytoplankton (Carotenuto & Lampert, 2004), which indicates the high possibility of the river becoming eutrophic, which has already been shown to be the case by the study by the Herts and Middlesex Wildlife Trust (HMTW, 2023). In another study on the River Nar by Marker & Garbutt (1993) the presence of these diatoms indicated that the river had the potential for eutrophication, particularly in summer during low flows, which was a similar case for the Colne in summer at Church Lane. Therefore, more needs to be done to ensure the flow of the River Colne is improved and not stagnant even during periods of low flow, to prevent further environmental degradation. Options such as river bed management and removing debris dams that are preventing flow would be paramount as these are the factors largely causing issues and leading the build-up and siltation of the river bed, particularly as the superficial deposits in the Colne are largely clay over gravel, which puts the catchment at a greater risk of smothering the gravel bed with clay deposits and creating a decline in the fine balance for aquatic macroinvertebrates and other species, such as fish species.

8.4 Future monitoring and management suggestions

Whilst this study has established the current status of the hydrological regime, water quality levels and macroinvertebrate communities and diversity, it is important to note that more work still needs to be done. One key takeaway is the interconnectedness of the interactions between the macroinvertebrate community and its surrounding environment. However, whilst this study aimed to include most riverine environmental parameters, other variables such as vegetation cover and land-use changes, or pollution incidents were not taken into consideration. Therefore, a more holistic approach to studying the macroinvertebrate communities would be beneficial, as the riverine environment only explained 30% of the variance in ecological communities.

Therefore, future monitoring should focus on integrating several aspects of a chalk stream environment to determine the largest impact, be those direct changes within

the aquatic environment, or wider changes from urban developments, wider catchment abstraction and pollution events or river modifications. Sampling multiple times a month or event-based sampling would offer greater resolution on post-disturbance recovery and seasonal dynamics, ensuring the repeatability of the results, even though the ecological community, diversity and environmental parameters did not differ between the inter annual seasons.

Also, through contrasting the Ver and the Colne within the upper catchment, the value of management practices has been shown. Where the river is cared for by local volunteers, more pollution incidents are reported and more understanding and knowledge is generated, meaning more people take an interest and are involved in preserving and cleaning the river. Unfortunately, for the upper Colne, there has been little maintenance work carried out, as the flow is often impeded, with debris entering the river from the roadsides, and organic debris remaining in-channel for far longer than at other river locations. Whilst chalk streams are a natural resource, humans have modified them for centuries and to preserve their characteristic nature, human intervention is sometimes needed to maintain them. That is why it is crucial that more awareness is raised, and that wider legislation is implemented to protect chalk streams at a larger scale. While climate change is a pressing matter for aquatic environments (Woodward *et al.*, 2010), what is a more imminent threat to chalk streams is anthropogenic activity, such as the release of effluent into streams, pollution events, urban runoff, micropollutants water abstraction and channel modification (Horváthová *et al.*, 2024). The Chalk Stream Restoration Strategy (2021) has aimed to set out recommendations and integrated policies, such as development rules for chalk streams, distinct designation for chalk streams and abstraction goals (CaBA, 2022). However, there has been rebuttal from the government, Environment Agency and policy makers, which makes change difficult. If the chalk streams are not managed properly by the stakeholders through the collaboration of local communities, land users, scientists and governments do not collaborate, it is certain that only a further decline in the ecological, chemical and environmental health of the chalk streams will occur, potentially eradicating red list species and irreversibly damaging the chalk stream environment. Therefore, a regular monitoring scheme set up across all stretches of the Upper Colne could help to understand changes in health across the wider catchment and the implementation and development of management plans and

legislation such as the Chalk Stream Bill (2024) would be hugely beneficial to the success of these chalk streams and securing their future.

Also, since this chalk stream catchment has displayed some unique characteristics, such as the frequent pooling of flow and the high risk of siltation due to the clay deposits over the chalk bedrock on the flood plains, it is even more paramount that any future management plans consider this. Comparable strategies implemented within the catchment, such as those on the River Gade in Hemel Hempstead, aim to reduce the impact of the weir and also reduce the pooling on the flood plain caused by the perched river channel (EA, 2018Ab). This would be particularly relevant at the Church Lane site, where the flood water often remains on the common land after heavy flooding and frequently experiences slow flow. Reestablishing the flow and hydro-morphological conditions has also been a key theme in the restoration projects within the wider Colne catchment, such as in the Chess Restoration Strategy (Thames Water, 2025b). This would also be of major benefit to the upper Colne areas investigated in this study which has had historical channel straightening. The reintroduction of meanders could potentially improve the flow (EA, 2018Ab), and this would in turn improve the water quality by reducing the build-up of pollutants and improve the habitat conditions, potentially leading to improved biodiversity. Therefore, there is clear potential to extend and implement proven restoration from the wider catchment into the Upper Colne too. This would offer a critical opportunity to mitigate current environmental pressures and actively restore the long-term resilience, ecological and hydrological integrity of this chalk stream.

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Appendix A: The risk assessment for fieldwork and laboratory work signed off on the 17 April 2023.

**SCHOOL OF LIFE AND MEDICAL SCIENCES
UNIVERSITY OF HERTFORDSHIRE**

Life and Medical Sciences Risk Assessment

Ref No.	
Date	16/3/23
Review Date	3/24
	OFFICE USE ONLY

The completion of this is an integral part of the preparation for your work, it is not just a form to be completed, but is designed to alert you to potential hazards so you can identify the measures you will need to put into place to control them. You will need a copy on you when you carry out your work

General Information				
Name	Charlotte Day	Email address	lottieday@hotmail.co.uk	Contact number
Supervisor's name (if student)	Dr Phil Porter	Supervisor's e-mail address	p.r.porter@herts.ac.uk	Supervisor's contact number
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Activity	
Title of activity	<i>The Current and Future Health of Chalk Rivers in Southeast England under the Influence of a Changing Climate and Increasing Human Pressures. PhD Project.</i>
Brief description of activity	Fieldwork on the River Colne in Drop Lane, Bricket Wood and Church Lane, Colney Heath to collect the hydrological parameters stage, turbidity and conductivity via a permanent monitoring station (2-years) and intermittent readings of temperature, pH, chemical samples of nitrates and phosphates and kick sampling to collect benthic invertebrate samples at regular intervals. Data analysis for these samples and a written report. Driving and walking to and from the field site with staff from UH and/or Thames Water present when we are on site at all times. The monitoring station involves a CR10X data logger attached via wiring to a turbidity sensor and pressure sensor. The instruments will then be affixed to a station built from metal poles which are hammered/drilled into the bank of the river/ the concrete weir. The data logger will be either stored in the Environment Agency box in Colney Heath, or secured to the bridge in Bricket Wood in a water tight box with appropriate signage.
Location of activity	The bridge over the River Colne on Drop Lane owned by Thames Water and the river banks and bed. The bridge over the River Colne at Church Lane in Colney Heath, owned by the Colney Heath Parish Council, the river banks and bed. Other sites to be determined for kick sampling and point measurements. The University of Hertfordshire
Who will be taking part in this activity	PhD student, members of fieldwork team (e.g. members of LMS staff, LMS undergrad/masters students, my parents/brother) . Supervisor, Thames Water Team.

Types of Hazards likely to be encountered					
☒ Computers and other display screen	☐ Falling objects	☐ Farm machinery	☐ Fire	☒ Cuts	
☐ Falls from heights	☒ Manual handling	☐ Hot or cold extremes	☐ Repetitive handling	☒ Severe weather	
☒ Slips/trips/falls	☒ Stress	☒ Travel	☒ Vehicles	☐ Workshop machinery	
☐ Psychological distress (to interviewer or interviewee)	☐ Aggressive response, physical or verbal	☒ Cattle/Livestock	☒ Working near water	☒ Electric fence	
Other hazards not listed above					

Risk Control Measures						
List the activities in the order in which they occur, indicating your perception of the risks associated with each one and the probability of occurrence, together with the relevant safety measures. Describe the activities involved. Consider the risks to participants, research team, security, maintenance, members of the public – is there anyone else who could be harmed? In respect of any equipment to be used read manufacturer's instructions and note any hazards that arise, particularly from incorrect use.						
Identify hazards	Who could be harmed?	How could they be harmed?	Control Measures – what precautions are currently in place?	What is the residual level of risk after the control measures have been put into place?	Are there any risks that are not controlled adequately?	Is more action needed to reduce/manage the risk? for example, provision of support/aftercare, precautions to be put in place to avoid or minimise risk or adverse effects
Travel/Vehicles	Fieldwork team Other Drivers on the road Thames Water Team	Potential hazard from vehicles on journey at crossings. Dogs from dog walkers Car accident	Are there standard operating procedures or rules for the premises. Are there any other local codes of practice/local rules which you are following, eg Local Rules for the SHE labs? Have there been agreed levels of supervision of the study? Will trained medical staff be present? Etc	Low Medium or High	No	Yes, do not travel if conditions become too dangerous.

Working near water	Fieldwork Team Thames Water Team	Weil's disease Drowning	GEP Code of practice First aid kit, mobile phone carried at all times. Be cautious of footing, don't tread on unstable banks. River depth will be checked using a kicknet and work will not take place in water above knee height. Training in the equipment to be used for kick sampling and fluvial measurements. Make sure all vaccinations (tetanus etc) are up to date. Carry sanitiser and ensure hands are clean before touching face or eating. Wear a life jacket and gloves during the visits and clean hands after work has been completed. Wear high visibility jackets to ensure visibility if an accident happens. Preparation and briefing for team each morning. No lone working	Low	No	Yes, if water level becomes too high or fast moving, abandon fieldwork
Slips, trips and falls	Fieldwork Team/ Office workers	Physical injury	GEP Code of practice First aid kit, mobile phone carried at all times. Wear appropriate footwear e.g. walking boots/ waders/ wellies depending on the terrain. Wear a hard hat when working underneath or near the overhang of the bridge. If in an office environment, make sure computer equipment is neatly organised and any additional hardware is stored appropriately and not left to cause a trip hazard to other users.	Low	No	No

Computers and display screen equipment	PhD Student	Repetitive strain injury Eye strain	GEP Code of Practice Regular Breaks taken. Adjust any office space e.g. PC monitor and keyboard, office chair to feel comfortable and ergonomic.	Low	No	No
Severe weather	Fieldwork Team Members of the public at the same location	Physical injury Illness	GEP Code of practice First aid kit, mobile phone carried at all times. Weather checked each morning, activities rescheduled if appropriate. Wear high visibility jackets when onsite. Wear weather appropriate clothing.	Low	No	Yes, if weather becomes too severe to work in, abandon data collection.
Cattle/ Livestock	Fieldwork Team Farmer Thames Water Team	Cattle could harm the teams or become frightened.	GEP Code of practice Countryside Code Do not approach cattle. If livestock appears unhappy, postpone activities until it is safe to resume.	Low	No	No
Electric fence surrounding field at Drop Lane	Fieldwork Team Farmer Thames Water Team	Electric fence could stun team members.	Do not attempt to cross the electric fence barrier until the fence has been deactivated/ disconnected. Avoid having to go into areas fenced off by the electric fence.	Low	No	No
Manual Handling	Fieldwork Team Supervisor	Physical injury	GEP Code of Practice Never work alone if carrying out manual work. Communicate clearly to other team members what is happening and stop procedure is necessary. First aid kit, mobile phone carried at all times.	Low	No	No

Appendix B: The COSHH forms for the water nutrient testing at Bayfordbury laboratory.

		Health Safety & Sustainability COSHH Risk Assessment Form					Page 1 of 6 Ref: Date:	
Title		HACH testing of water samples from river Colne						
Assessor		Charlotte Day						
Substance(s) Information								
Outline of Task/Method		Water samples being tested for ammonia, nitrite and phosphate using the HACH machine. Water samples analysed for Biological Oxygen Demand (BOD).						
Location of Activity		Bayfordbury Laboratory (H08)						
Product/ Substance	Hazard	Form	Quantity Used or Stored	Duration of Exposure	Route of Entry	Frequency of Exposure	Workplace Exposure Limit UH Guidance EH40	Risk Level ¹ (RAG rating)
River Water samples	 Biohazard	Liquid	100ml	<15 minutes	Skin contact Eye contact Ingestion	3- 6 times monthly	None	
Ammonium HACH test LCK 303-1 sample cuvette Sodium hydroxide (1310-73-2)	 Corrosive to metals. May cause severe skin burns and eye damage.	Liquid Colour less, odourless	Low mg	<15 minutes	Skin contact Eye contact Ingestion Inhalation	3- 6 times monthly	Sodium Hydroxide 2 mg/m ³	Low
LCK 303-2 Ammonium DosiCapZip Sodium nitroferri- cyanide (14402-89-2) Dichloroisocyanuric acid, sodium salt (2893-78-9)	 Harmful is swallowed. Serious Eye irritation. May cause respiratory irritation. Contact with acids liberates toxic gas  Toxic to aquatic life with long- lasting effects.	Solid, white, odourless	Low mg	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3-6 times monthly	Sodium nitroferri- cyanide 5mg/ m ³	Low

¹ Risk level is based on the likelihood of exposure and the severity of the health effects. For example, a high-risk substance could cause serious health effects and the level of exposure makes it likely that these effects could occur. The risk level will determine the level of controls required to control exposure.

LCK 349 Phosphat/P hosphate, Sample cuvette sulphuric acid 11 % (7664-93-9) di-Sodium hydrogen phosphate (7558-79-4)	 May be corrosive to metals. Causes severe burns and eye damage.	Liquid, colour less, odourless	Low mg	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3-6 times monthly	Sulphuric acid 0.05mg/m ³	Low
LCK 349 Phosphat/P hosphate, LCK 349 Reagent B Sulphuric acid 16 % (7664-93-9)	 May be corrosive to metals. Causes severe burns and eye damage.	Liquid, colour less, odourless	Low mg	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3—6 times monthly	Sulphuric acid 0.05 mg/m ³	Low
LCK 349 Phosphat/P hosphate; LCK 349 DosiCap C Sodium metaborate, Tetrahydrate (10555-76-7)	  Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation. Suspected of damaging fertility or the unborn child.	Solid (lyophilised) White Odourless	Low mg <25%	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3 -6 times monthly	-	Low
LCK 339 Nitrat/Nitrate, Sample cuvette sulphuric acid (7664-38-2) phosphoric acid (7664-93-9)	 May be corrosive to metals. Causes severe skin burns and eye damage.	Liquid, colour less, odourless	Low mg 50-60% 25-33%	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3-6 times monthly	Sulphuric acid 0.05 mg/m ³ Orthophosphoric acid 1 mg/m ³	Low
LCK 339 Nitrat/Nitrate, LCK 339 A propan-2-ol; isopropyl alcohol; isopropanol (67-63-0)	 Causes serious eye irritation. May cause drowsiness or dizziness.	Liquid, colour less, aromatic	Low mg 20-30%	<15 minutes	Skin Contact Eye contact Ingestion Inhalation	3-6 times monthly	Pentyl acetates: Isopentyl acetate 270 mg/m ³ Propan-2- ol 999 mg/m ³	Low

University of Hertfordshire UH		Health Safety & Sustainability COSHH Risk Assessment Form				Page 3 of 6 Ref: Date:	
 Flammable liquid and vapour.							
Do any of the substances require a licence or notification to purchase? COSHH Tables		No					
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?		All fully water soluble. LCK303-2: If in contact with acids, it can liberate toxic gas. LCK339 Solution A: vapour pressure at 20 °C: (2-Prop.) 43 hPa LCK349 sample cuvette: 10°C: 15 hPa LCK349 Reagent B: vapour pressure at 20°C: 20 hPa					
Who will be Harmed and How?							
Who may be exposed to the substance(s)?		PhD Student, Staff at Bayfordbury, fieldwork assistants e.g. parents of PhD student/family/undergraduate students					
What are the general consequences of exposure?		LCK339 solution A: repeated exposure may cause skin dryness or cracking. Vapour may cause dizziness and drowsiness and could cause long-term adverse effect in the aquatic system. Toxic if swallowed or on the skin. LCK339 cuvette: Cause burns. LCK349 sample cuvette: May be corrosive to metals. Causes severe skin burns and eye damage. LCK 349 Dosicap C: skin and eye irritation. May cause respiratory irritation or damage to unborn child. LCK303 Dosicap D: serious eye irritation, skin conditions, toxic to aquatic life with long term effects. Can cause toxic gas.					
Do any of the substances pose additional risks to certain individuals (e.g. expectant mothers)		Yes – expectant mothers: LCK 349 DosicapC. Yes – asthmatics/respiratory issues: LCK 349 DosicapC.					
Control Measures							
Does the substance(s) require authorisation to retain? (i.e. is it used very infrequently, or age poses additional risks) Consult your SBU safety contact if unsure.						Yes	No
Can a less hazardous substance be used to do the same job? (if you do not know, please contact your supplier for additional information)						Yes	No
Physical or Engineering Controls						Technical measures and appropriate working operations should be given priority over the use of personal protective equipment (LCK 349 sample cuvette). Use only in well-ventilated areas.	
Administrative Controls						The substances must be stored in a locked cabinet when not in use. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Other workers, staff and students in the lab should also be notified of the potential risk to fertility or the unborn child.	
Personal Protective Equipment Specify which type and when they are to be worn						Use barrier skin cream. Chemical resistant gloves made of butyl rubber or nitrile rubber category III according to EN 374. Lab coat and safety glasses to be worn. Face mask when needed.	
Storage Requirements						Keep substances tightly closed in their containers in a dry, cool and well-ventilated place (LCK339 cuvette and solution A). LC349, LC303-1 and LC303-2 need to be kept away from metals. LC339 should be kept in tightly sealed container as is explosive when exposed to air. Keep in a dry, cool place. Storage temperatures 15- 25 (LCK349 sample cuvette, LCK349 reagent B, LCK 349 DosicapC, LCK303 DosicapD, LCK303 sample cuvette)	

Transport of the substance(s)	<i>HACH Cuvettes and kit – transport in original package and by an approved supplier.</i> <i>Water samples – 100ml transfer from site via 100ml bottles.</i>			
Disposal (Refer to local procedures)	Do not flush into surface water or sanitary sewer system at Bayfordbury. It must be disposed of in accordance with local and national regulations. The method of disposal for the tests is by replacing the chemicals in their original packaging and labelling them as “used”. They will be stored and transported to be disposed of by a contractor.			
Health Surveillance required? (UH Guidance)	Does this substance(s) require any user health surveillance? if yes, please state which type & level and who it is conducted by:	Yes	No	
			x	
Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? (Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).		Yes	No	
		x		
Emergency Procedures				
First Aid Measures If substance(s) reacts with water this must be mentioned first (Refer to local procedures)	<i>Usual first aid measures are:</i> <i>Call a first aider or Security.</i> <i>If ingested: Rinse mouth with water .</i> <i>LC303-2, LC349, LC339 Do not induce vomiting if ingested. Rinse mouth and seek medical assistance.</i> <i>If inhaled: Move person into fresh air.</i> <i>If in contact with eyes: Flush eyes with water. Consult a physician. If eye irritation persists: Get medical advice/attention.</i> <i>If in contact with skin: Wash off with plenty of water for 5-20 minutes, repeat up to twice more if necessary. Consult a physician.</i> <i>If not applicable, list required first aid measures here.</i>			
Fire Fighting Measures (Refer to local procedures)	<i>Use extinguishing measures appropriate to circumstances and the surrounding environment. Do not use water spray as this will contaminate local sewer systems. Instead use CO2, alcohol-resistant foam or dry powder to contain fires.</i>			
Accidental Release Measures (Refer to local procedures)	<i>Use personal protective equipment. Do not flush into surface water or sanitary sewer system. Soak up with inert absorbent material and dispose of as hazardous waste.</i>			
DSEAR Risk Assessment (Dangerous Substances & Explosive Atmospheres Regulations)				
Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? i.e. flammable, explosive, compressed gas, corrosive to metal (HSE guidance)	Yes	No		
If yes, then please contact your SBU Safety Adviser or Contact Health, Safety and Sustainability .	x			
Are there any hazards not relating to substances that need to be considered for this activity?				
Hazard	What harm	Who	Risk Level	Controls
Slips, trips and falls	Minor injury	Staff/student	low	LMS and GEP Codes of practice Risk Assessment
Spillages	Minor injury	Staff/student	Low	LMS and GEP Codes of practice Risk Assessment
Weil's Disease (Leptospirosis)	Illness	Staff/student	Low	LMS and GEP Codes of practice Risk Assessment
Glass breakages	Cuts	Staff/student	Low	LMS and GEP Codes of practice Risk Assessment
Manual Handling	Minor injuries	Staff/student	low	LMS and GEP Codes of practice

				Risk Assessment	
Assessment of Risk					
Are all the controls detailed above currently in place?				Yes	No
				x	
If these controls are not in place or additional controls are required, please state action to be taken. Please note – the work CANNOT take place if adequate control measures are not in place.					
Remedial Actions Required			By who?	By when?	
Lab use will be pre-arranged with the staff at Bayfordbury to ensure all protective equipment and measures are in place before any activities are carried out.			Myself	Before each lab visit.	
Are all hazards to health adequately controlled with all control measures?				Yes	No
				x	
Assessor Name		Assessor Signature		Date	
Charlotte Day				14/04/2023	
Has an assessment been made previously? If so, please state the details of the assessment below:					
Previously Assessed by				Date	
The line manager or supervisor should sign below to show that the assessment is a correct and reasonable reflection of the hazards and of the control measures and actions required.					
Line Manager/Supervisor Name		Line Manager/Supervisor Signature		Date	
Lab or Workshop Manager/Health and Safety Adviser					
Name		Job Title		Signature	
Aiden Bygrave		Lab Manager			
Date of Next Review				5/24	

Section 1: Risk	
Risk of fire, explosion or energetic event <i>Describe how the activity can lead to fire, explosion or thermal runaway or cause an explosive atmosphere</i>	Flammables incorrectly stored or exposed to source of ignition, spillages releasing fumes near sources of ignition. Corrosives incorrectly stored in a non-corrosive resistant container can lead to leakage and destroy metal casings protecting other equipment which can lead to electrical shortages.
Ignition sources <i>What potential sources of ignition are present e.g. Bunsen burners, hot plates, ovens, furnaces or heating equipment?</i>	Electrical equipment and plug sockets

Section B: Specific DSEAR controls

What steps have been taken to control releases at source or reduce the quantity of the dangerous substance stored or used to a minimum?	Activities using the substance are undertaken away from any electrical equipment or plug sockets in a well-ventilated room.		
What steps have been taken to avoid releases that may lead to formation of explosive atmosphere under normal and accidental conditions?	Only the minimum required substance is dispensed for activities before being returned to storage.		
A full DSEAR risk assessment may be required if:		Yes	No
	The work activity involves the use or storage of pressurised flammable gas cylinders such as acetylene or hydrogen;		x
	Very large quantities of flammable substances are involved, for where the recommended limits for storage of flammable substances are exceeded (50 litres for extremely or highly flammable substances and flammable liquids with a flashpoint below the maximum ambient temperature of the working area, 250 litres for other flammable liquids with a higher flashpoint of up to 55°C.);		x
	The work activity involves the use of explosives;		x
	The work activity is still likely to create an explosive atmosphere even when using the controls specified above;		x
	The work activity involves the use of flammable/oxidising/explosive substances or compressed gases only and not substances hazardous to health as defined by COSHH.		x

Appendix C: The environmental parameter data between April 2023 – February 2025.

Season	Site Name	Month	Site Coding	Phosphate PO43- (mg/l)	Nitrate NO3-N (mg/l)	Ammonium NH4+-N (mg/l)	pH	Conductivity (uS)	Turbidity (ppm)	Dissolved Oxygen (mg/l)	Biological Oxygen Demand (mg/l)	Water Temperature (°C)	Channel Width (m)	Channel Depth (m)	Average Flow Velocity (m/s)	Channel Area (m ²)	Discharge (m ³ /s)	Daily Total Rainfall (mm)	Max. Air Temperature (°C)	Min. Air Temperature (°C)
2023/24	CL	April	SiteA	0.4	2.276735	0.0625	5.4	640	333	10.6	4.2	9	5.7	0.188667	0.023333	1.0754	0.024016	2.06	13.1	4.01666667
2023/24	CL	May	SiteB	0.547	2.402143	0.08325	6.39	614	337	9.6	1.9	12.6	3.8	0.235	0.033333	0.893	0.0329333	1.490323	16.66129032	7.706451613
2023/24	CL	June	SiteC	0.481	0.361	0.058	7.55	745	372	7.3	4.9	14.3	3.65	0.241	0.046033	0.87965	0.0410631	0.506667	23.44	11.17333333
2023/24	CL	July	SiteD	0.401	0.45	0.107	8.47	710	375	5.5	7.4	17	2.98	0.174333	0.003333	0.519513	0.001937	2.943387	21.0516129	12.17096774
2023/24	CL	August	SiteE	0.457	0.419	0.062	7.73	695	354	4.6	3.9	15	2.7	0.16	0	0.432	0	1.49375	21.23125	12.3
2023/24	CL	September	SiteF	0.528	0.435	0.023	7.98	606	306	4.9	4.5	16.9	3.4	0.19	0.010982	0.646	0.0071566	1.96	22.58333333	12.92
2023/24	CL	October	SiteG	0.437	6.45	0.141	8.35	634	322	7.2	2.1	11.7	3.56	0.219333	0.016732	0.780827	0.0133745	2.909677	16.5483871	8.806451613
2023/24	CL	November	SiteH	0.4	5.42	0.035	7.31	475	243	9.9	4.1	10.1	4.8	0.458333	4.3792	2.2	9.170208	2.186667	10.36	3.94666667
2023/24	CL	December	SiteI	0.372	3.28	0.037	9.34	676	345	10.1	2.2	3.5	3.31	0.215	0.029946	0.71165	0.022539	2.941935	9.503225806	4.64516129
2023/24	CL	January	SiteJ	0.419	2.97	0.043	10.28	612	309	12	0.7	3.7	3.72	0.281	0.152918	1.04532	0.2222649	2.232258	7.058064516	1.54516129
2023/24	CL	February	SiteK	0.403	2.78	0.299	7.54	633	316	13.7	2.1	8.1	7	0.536667	1.330486	3.76687	4.550841	4.427586	10.72413793	4.706896552
2024/25	CL	March	SiteL	0.402	3.8	0.456	7.59	538	288	11.2	0.7	8.9	6.2	0.469333	1.374104	2.909667	4.4019139	2.870968	11.54516129	4.825806452
2024/25	CL	April	SiteM	0.544	0.357	0.034	7.72	691	345	6.6	1.3	10.7	3.51	0.228	0.028343	0.80028	0.0204787	2.206667	13.4266667	6.04
2024/25	CL	June	SiteN	0.441	0.507	0.231	7.65	704	351	4.7	2.5	12.8	3.3	0.204333	0	0.6743	0	0.666667	19.56	8.973333333
2024/25	CL	July	SiteO	0.478	2.46	0.035	7.4	537	267	11.1	3.7	10.5	3.16	0.181	0.020257	0.57196	0.0144419	2.819355	21.61612903	11.84516129
2024/25	CL	August	SiteP	0.502	0.45	0.028	9.54	693	401	7.4	1.5	18.6	3.08	0.175	0	0.539	0	0.729032	22.60645161	12.71612903
2024/25	CL	September	SiteQ	0.4	9.62	0.045	9.83	699	397	18.2	3	14.4	3.61	0.238333	0.051023	0.860383	0.0707339	6.3	18.63	10.36333333
2024/25	CL	October	SiteR	0.421	6.21	0.021	11.96	536	308	6.6	0	12.6	2.98	0.263	0.066618	0.78374	0.0421449	2.006452	14.98387097	7.906451613
2024/25	CL	November	SiteS	0.408	0.477	0.023	7.75	731	416	6.4	2.7	10.1	3.41	0.261667	0.022983	0.892283	0.0326168	2.193333	9.933333333	4.613333333
2024/25	CL	December	SiteT	0.473	1.17	0.038	7.78	635	316	6.6	0.2	10	3.37	0.27	0	0.9099	0	2.148387	8.638709677	4.261290323
2024/25	CL	January	SiteU	0.359	2.76	0.043	7.59	609	304	12.8	1.5	1.8	3.26	0.261667	0.060423	0.853033	0.0375117	2.180645	5.841935484	0.193548387
2024/25	CL	February	SiteV	0.487	1.16	0.022	7.51	665	331	12.6	0	4.3	3.15	0.243	0.04344	0.7666	0.0458	0.644444	6.58888889	0.7
2023/24	LC	April	SiteZA	0.089	3.693333	0.0525	6.57	618	300	9	3.2	12.3	4.79	0.121	0.38	0.57959	0.2235493	2.06	13.1	4.01666667
2023/24	LC	May	SiteZB	0.337	3.931667	0.06675	6.65	589	296	9	1.6	14.1	3.9	0.139333	0.27	0.5434	0.149604	1.490323	16.66129032	7.706451613
2023/24	LC	June	SiteZC	0.462	1.71	0.042	7.45	967	484	6.3	1.7	16.9	3.37	0.078	0.0164	0.26286	0.0047222	0.506667	23.44	11.17333333
2023/24	LC	July	SiteZD	0.255	1.99	0.035	8.37	665	334	7.2	1.4	19.6	3.31	0.086333	0.033723	0.285763	0.0096749	2.948387	21.0516129	12.17096774
2023/24	LC	August	SiteZE	0.344	1.77	0.049	7.48	630	323	7.3	3.5	17.5	2.75	0.090333	0.042231	0.248417	0.0105057	1.49375	21.23125	12.3
2023/24	LC	September	SiteZF	0.556	2.36	0.084	8.08	403	205	5.6	0.3	17	2.73	0.092333	0.025207	0.25207	0.0063839	1.96	22.58333333	12.92
2023/24	LC	October	SiteZG	0.333	9.94	0.099	8.83	512	261	7.4	2.2	11.1	3.12	0.094333	0.044674	0.29432	0.0134599	2.909677	16.5483871	8.806451613
2023/24	LC	November	SiteZH	0.397	4.39	0.051	7.16	471	240	9.7	1.6	9.9	4.9	0.86	0.687225	3.234	2.1354094	2.186667	10.36	3.94666667
2023/24	LC	December	SiteZI	0.34	3.14	0.046	8.42	719	365	11.3	1.1	4.2	2.8	0.101	0.057233	0.2828	0.0169107	2.941935	9.503225806	4.64516129
2023/24	LC	January	SiteZJ	0.274	2.51	0.091	9.21	563	287	12.7	1.9	2.6	4	0.193667	0.11224	0.774667	0.078816	2.232258	7.058064516	1.54516129
2023/24	LC	February	SiteZK	0.464	1.9	0.102	7.61	914	457	12.2	2.2	8.5	5.7	0.323	1.117811	1.8411	2.0014467	4.427586	10.72413793	4.706896552
2024/25	LC	March	SiteZL	0.496	3.09	0.448	7.74	579	288	6.7	4.3	9.1	4.1	0.296667	0.516942	1.216333	0.6022603	2.870968	11.54516129	4.825806452
2024/25	LC	April	SiteZM	0.463	2.8	0.058	8.12	833	417	9.4	2.5	11.1	3.6	0.119	0.151725	0.4284	0.0552529	2.206667	13.4266667	6.04
2024/25	LC	June	SiteZN	0.503	4.75	0.095	7.81	763	391	8.9	0	15.8	3.34	0.107	0.076104	0.35738	0.0283384	0.666667	19.56	8.973333333
2024/25	LC	July	SiteZO	0.466	3.4	0.081	7.56	474	237	12.4	0.9	17.4	3.02	0.136667	0.014832	0.418773	0.0060957	2.819355	21.61612903	11.84516129
2024/25	LC	August	SiteZP	0.419	4.78	0.019	9.47	681	347	3	0.1	20.5	2.5	0.106333	0.028245	0.265833	0.0066851	0.729032	22.60645161	12.71612903
2024/25	LC	September	SiteZQ	0.482	7.11	0.066	10.9	543	309	11.6	0.7	14.8	2.53	0.151333	0.081361	0.382873	0.0296032	6.3	18.63	10.36333333
2024/25	LC	October	SiteZR	0.374	5.28	0.023	11.55	535	304	6.7	6.2	12.3	2.63	0.207333	0.136322	0.545287	0.0651676	2.006452	14.98387097	7.906451613
2024/25	LC	November	SiteZS	0.372	1.19	0.042	7.51	844	481	5.8	4.4	10.3	2.57	0.072667	0.01671	0.186753	0.0038488	2.193333	9.933333333	4.613333333
2024/25	LC	December	SiteZT	0.511	2.13	0.041	7.8	705	353	9.1	2.8	8.1	3.42	0.136333	0.09908	0.46626	0.0442797	2.148387	8.638709677	4.261290323
2024/25	LC	January	SiteZU	0.41	2.76	0.089	7.85	608	303	13.4	6	2.5	3.81	0.193667	0.125438	0.73787	0.0884659	2.180645	5.841935484	0.193548387
2024/25	LC	February	SiteZV	0.478	2.88	0.045	8	808	404	13.7	5.2	4.2	2.7	0.1157	0.0553	0.3123	0.016655	0.644444	6.58888889	0.7

Season	Site Name	Month	Site Coding	Phosphate PO43- (mg/l)	Nitrate NO3-N (mg/l)	Ammonium NH4+-N (mg/l)	pH	Conductivity (µS)	Turbidity (ppm)	Dissolved Oxygen (mg/l)	Biological Oxygen Demand (mg/l)	Water Temperature (°C)	Channel Width (m)	Channel Depth (m)	Average Flow Velocity (m/s)	Channel Area (m²)	Discharge (m³/s)	Daily Total Rainfall (mm)	Max. Air Temperature (°C)	Min. Air Temperature (°C)
2023/24	DL	April	Site3A	0.3065	1.756667	0.13375	6.79	1085	541	12.1	1.9	10.3	8.4	0.336667	0.306667	2.828	0.85204	2.06	13.1	4.016666667
2023/24	DL	May	Site3B	0.439	1.9	0.1005	7.48	1402	700	6.9	0.1	12.8	7.2	0.237333	0	1.7088	0	1.490323	16.66129032	7.706451613
2023/24	DL	June	Site3C	0.178	1.49	0.204	7.65	1100	550	4.7	1.5	18	5.95	0.218333	0.020704	1.299083	0.0272945	0.506667	23.44	11.17333333
2023/24	DL	July	Site3D	0.145	2.32	0.06	7.22	1088	556	7.6	0.9	15.7	6.2	0.250333	0.05654	1.552067	0.0883006	2.948387	21.0516129	12.1798774
2023/24	DL	August	Site3E	0.315	1.46	0.121	8.47	1327	677	6.8	2.9	15.8	6.2	0.264333	0.034826	1.638867	0.0573889	1.49375	21.23125	12.3
2023/24	DL	September	Site3F	0.588	1.92	0.15	9.43	1694	865	5.4	0.1	16.7	7.91	0.272667	0.099997	2.156793	0.2194481	1.96	22.58333333	12.92
2023/24	DL	October	Site3G	0.381	2.45	0.071	8.21	1357	692	7.7	4.3	11.6	6.82	0.272	0.098549	1.85504	0.1642768	2.909677	16.5483871	8.806451613
2023/24	DL	November	Site3H	0.286	3.72	0.298	7.78	553	280	9.2	1.2	9.5	9.2	0.583333	1.520556	5.366667	7.0816877	2.186667	10.36	3.946666667
2023/24	DL	December	Site3I	0.377	2.11	0.038	9.49	867	442	12.1	2.8	3.9	6.9	0.263	0.276633	1.9627	0.4112701	2.941935	9.503225806	1.64516129
2023/24	DL	January	Site3J	0.389	2.65	0.079	8.7	653	334	12.9	0.8	2.9	7	0.465	0.47175	3.255	1.4520314	2.232258	7.058064516	4.54516129
2023/24	DL	February	Site3K	0.528	2.6	0.168	7.03	522	261	10.2	0.1	8.2	8.5	0.616667	1.012595	5.241667	4.3798278	4.427586	10.72413793	4.706896552
2024/25	DL	March	Site3L	0.377	2.36	0.238	7.63	301	601	5.7	2.6	9.2	8	0.65	1.841667	5.2	7.8033778	2.870968	11.54516129	4.825806452
2024/25	DL	April	Site3M	0.433	2.14	0.022	8.08	960	480	6.7	6	9.8	6.3	0.475667	0.145554	2.9667	0.449286	2.206667	13.42666667	6.04
2024/25	DL	June	Site3N	0.455	3.18	0.104	7.95	1142	571	7.3	7	14.1	6.3	0.413333	0.8126	2.604	1.97064	0.666667	19.56	8.973333333
2024/25	DL	August	Site3P	0.455	2.47	0.055	8.74	1329	757	5.2	2	19.1	6.57	0.297	0.041465	1.95129	0.0740995	0.729032	22.60645161	12.71612903
2024/25	DL	November	Site3S	0.408	1.73	0.041	7.69	1860	1060	5.6	1.1	10.8	6.4	0.272	0.024661	1.7408	0.0419806	2.193333	9.933333333	4.613333333
2024/25	DL	February	Site3V	0.434	3	0.03	8.1	1114	553	12.4	6.9	4.7	8	0.6967	0.23687	5.5733	1.17296	0.644444	6.588888889	0.7
2023/24	V	April	Site4A	0.394	4.145	0.03225	6.2	688	336	13.2	11.3	14.1	7	0.303333	0.516667	2.123333	1.12	2.06	13.1	4.016666667
2023/24	V	June	Site4C	0.418	2.03	0.014	7.67	516	257	7	4.5	16.9	7.63	0.294667	0.476667	2.248307	1.0652497	0.506667	23.44	11.17333333
2023/24	V	July	Site4D	0.397	4.34	0.066	6.67	623	318	9.9	1.7	15	6.33	0.208667	0.43182	1.32086	0.6021642	2.948387	21.0516129	12.17098774
2023/24	V	August	Site4E	0.391	3.95	0.025	7.75	602	306	9.1	0.9	15.3	6.4	0.226333	0.384767	1.448533	0.5743661	1.49375	21.23125	12.3
2023/24	V	September	Site4F	0.532	3.86	0.023	8.63	629	321	9.1	1.1	15.5	7.2	0.208667	0.42568	1.5024	0.6592268	1.96	22.58333333	12.92
2023/24	V	October	Site4G	0.542	4.81	0.015	9.91	619	315	10.1	7.3	11	6.89	0.209	0.470773	1.44001	0.681466	2.909677	16.5483871	8.806451613
2023/24	V	November	Site4H	0.355	4.43	0.022	7.31	614	313	11.9	1	9.5	8.9	0.579333	0.876531	5.156067	3.8633452	2.186667	10.36	3.946666667
2023/24	V	December	Site4I	0.387	5.81	0.015	8.7	695	352	12.5	4.3	4.8	6.71	0.249	0.448177	1.67079	0.7936606	2.941935	9.503225806	4.64516129
2023/24	V	January	Site4J	0.242	6.23	0.024	9.39	696	355	13.5	3.2	3.9	8.6	0.417	0.519068	3.5862	1.7754217	2.232258	7.058064516	1.54516129
2023/24	V	February	Site4K	0.428	4.4	0.053	7.32	629	314	13.6	3.1	8.6	8	0.578667	1.093037	4.629333	4.5269464	4.427586	10.72413793	4.706896552
2024/25	V	March	Site4L	0.499	5.15	0.049	7.88	671	336	5.2	5.4	9.1	8.2	0.58	1.189	4.756	5.0239487	2.870968	11.54516129	4.825806452
2024/25	V	April	Site4M	0.412	5.4	0.022	8	720	359	12	1.4	9.7	8.6	0.476	0.99416	4.0936	3.2384971	2.206667	13.42666667	6.04
2024/25	V	June	Site4N	0.52	5.09	0.048	7.97	736	368	6.7	3.7	13	7.2	0.376333	1.15158	2.7096	3.325468	0.666667	19.56	8.973333333
2024/25	V	July	Site4O	0.498	4.29	0.019	8.12	683	341	13.3	2.7	16.1	7.6	0.446667	0.120228	3.394667	0.3730007	2.819355	21.61612903	11.84516129
2024/25	V	August	Site4P	0.472	4.9	0.02	9.5	694	396	13.9	5.8	16.9	7	0.327333	0.58429	2.291333	1.0019782	0.729032	22.60645161	12.71612903
2024/25	V	September	Site4Q	0.406	2.58	0.037	10.2	516	290	9.8	6.4	13.8	8.2	0.626667	1.364958	5.138667	6.3685035	6.3	18.63	10.56333333
2024/25	V	October	Site4R	0.46	5.25	0.01	11.8	659	372	6.7	7.7	12.6	8	0.44667	0.755933	3.557333	2.2619067	2.006452	14.98387097	7.906451613
2024/25	V	November	Site4S	0.421	6	0.016	8.5	698	399	8	6	10.4	7.9	0.288	0.64464	2.2752	1.3761665	2.193333	9.933333333	4.613333333
2024/25	V	December	Site4T	0.435	5.94	0.112	7.97	649	325	8.1	7.1	9.9	8	0.399	0.9044	3.192	2.3393632	2.148387	8.638709677	4.261290323
2024/25	V	January	Site4U	0.305	6.27	0.023	8.22	696	349	15	6.4	2.7	11	0.425	1.655729	4.675	6.1038618	2.180645	5.841935484	0.193548387
2024/25	V	February	Site4V	3.66	6.75	0.012	7.9	692	346	13.5	7.3	5.4	8.1	0.3883	0.48707	3.1455	1.224591	0.644444	6.588888889	0.7

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Site	Site Name	Month	Site Coding	Assilius	Astacidae	Baridae	Bethynidae	Cerastidae	Corabopogonidae	Chironomidae	Coarsigranidae	Coridiidae	Cuticidae pupa	Daphniidae	Dendrocoelidae	Dyscidae	Elmidae	Ephemeridae	Eropelidae	Gammarus	Gerridae	Glossiphiliidae	Glossosomatidae	Goridae
2023/24	CL	April	Sie7A	18	0	3	0	0	5	0	7	0	0	0	0	0	0	0	0	2	0	0	2	0
2023/24	CL	May	Sie7B	11	0	0	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
2023/24	CL	June	Sie7C	14	0	0	0	0	0	5	0	5	0	2	0	10	0	0	0	0	0	0	0	0
2023/24	CL	July	Sie7D	51	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	
2023/24	CL	August	Sie7E	30	0	4	0	0	0	1	0	0	0	0	0	50	0	0	0	0	1	0	0	
2023/24	CL	September	Sie7F	30	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	October	Sie7G	30	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	November	Sie7H	49	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	12	0	0	0	
2023/24	CL	December	Sie7I	32	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	
2023/24	CL	January	Sie7J	35	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	6	0	0	0	
2023/24	CL	February	Sie7K	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	March	Sie7L	35	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	April	Sie7M	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	May	Sie7N	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	
2023/24	CL	June	Sie7O	46	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	July	Sie7P	21	0	1	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	August	Sie7Q	26	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	
2023/24	CL	September	Sie7R	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	October	Sie7S	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	
2023/24	CL	November	Sie7T	30	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	December	Sie7U	25	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5	0	0	
2023/24	CL	January	Sie7V	30	0	5	0	0	0	3	0	0	0	0	0	0	0	0	0	0	6	0	0	
2023/24	CL	February	Sie7W	37	0	2	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	March	Sie7X	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	
2023/24	CL	April	Sie7Y	6	0	2	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	May	Sie7Z	3	0	4	0	0	0	2	0	1	0	0	3	150	0	0	0	0	12	0	0	
2023/24	CL	June	Sie2C	3	0	4	0	0	0	3	0	2	1	0	3	150	0	0	0	0	1	0	0	
2023/24	CL	July	Sie2D	1	0	40	0	0	0	0	0	0	1	0	80	0	0	0	0	0	1	0	0	
2023/24	CL	August	Sie2E	5	0	12	0	0	0	1	3	0	0	0	20	0	0	0	0	0	1	0	0	
2023/24	CL	September	Sie2F	10	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	5	20	0	0	
2023/24	CL	October	Sie2G	12	1	4	0	0	0	0	13	0	0	0	0	0	0	0	0	6	10	0	0	
2023/24	CL	November	Sie2H	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	December	Sie2I	5	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	30	1	0	0	
2023/24	CL	January	Sie2J	45	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	
2023/24	CL	February	Sie2K	15	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	
2023/24	CL	March	Sie2L	4	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	
2023/24	CL	April	Sie2M	2	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	
2023/24	CL	May	Sie2N	10	1	6	0	0	0	80	0	0	0	0	0	0	0	0	0	0	7	0	0	
2023/24	CL	June	Sie2O	10	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	July	Sie2P	6	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	9	0	0	0	
2023/24	CL	August	Sie2Q	12	0	5	0	0	0	9	0	0	0	0	0	0	0	0	0	4	52	1	0	
2023/24	CL	September	Sie2R	3	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	10	13	0	
2023/24	CL	October	Sie2R	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	November	Sie2S	15	0	6	0	0	0	4	0	0	0	0	0	0	0	0	0	1	15	0	0	
2023/24	CL	December	Sie2T	20	0	8	0	0	0	3	0	0	0	0	0	0	0	0	0	0	12	0	0	
2023/24	CL	January	Sie2U	8	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	February	Sie2V	8	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	
2023/24	CL	March	Sie2V	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	
2023/24	CL	April	Sie2W	22	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	May	Sie2X	42	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	49	0	0	
2023/24	CL	June	Sie2X	10	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	
2023/24	CL	July	Sie2C	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	
2023/24	CL	August	Sie3E	7	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	
2023/24	CL	September	Sie3E	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	October	Sie3G	25	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	CL	November	Sie3H	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	
2023/24	CL	December	Sie3I	65	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	15	0	0	
2023/24	DL	January	Sie3J	15	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	
2023/24	DL	February	Sie3K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	
2023/24	DL	March	Sie3L	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	
2023/24	DL	April	Sie3M	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	DL	May	Sie3N	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	
2023/24	DL	June	Sie3P	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	5	0	0	
2023/24	DL	August	Sie3P	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	2	0	0	
2023/24	DL	November	Sie3S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	
2023/24	DL	February	Sie3V	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
2023/24	V	April	Sie4A	0	0	26	1	0	0	2	5	0	0	0	0	0	0	0	0	0	240	0	1	
2023/24	V	June	Sie4C	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	V	July	Sie4C	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	200	0	0	
2023/24	V	August	Sie4E	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	280	0	0	
2023/24	V	September	Sie4F	3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0	0	
2023/24	V	October	Sie4G	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2023/24	V	November	Sie4H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256	0	0	
2023/24	V	December	Sie4I	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	10	0	0	
2023/24	V	January	Sie4J	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	123	0	0	
2023/24	V	February	Sie4K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	405	0	0	
2023/24	V	March	Sie4L	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	45	0	0	
2023/24	V	April	Sie4M	0	0	25																		

Appendix E: The macroinvertebrate species identified between February 2024 to February 2025.

Season	Site Name	Site Month	Site Coding	Asellus aquaticus	Baetis rhodani	Caenis rivulorum	Calopteryx splendens	Chironomidae	Culiidae	Daphnia pulex	Dytiscidae	Elmidae	Enallagma cyathigerum	Ephemera vulgata	Ephemera danica	Epobdella octoculata	Gammarus pulex	Gerridae	Glossiphonia complanata	Coera pilosa	Halipidae	Halesus sp.	Hydrophilidae	Hydropsyche silvatica	Ischnura elegans	Phaenocarpa brevipennis	Potomophylax circulator
2023/24	CL	February	SiteK	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
2024/25	CL	March	SiteL	35	0	0	0	6	0	0	0	0	0	0	0	0	1	0	0	7	0	0	0	0	0	0	0
2024/25	CL	April	SiteM	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2	0	1	0	0	0	0	0
2024/25	CL	June	SiteN	5	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
2024/25	CL	July	SiteO	46	2	0	0	15	0	0	0	0	0	0	0	0	0	0	4	0	0	1	0	0	0	0	0
2024/25	CL	August	SiteP	21	1	0	0	1	0	0	1	0	0	0	0	0	0	3	0	0	1	0	0	1	0	0	0
2024/25	CL	September	SiteQ	26	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0
2024/25	CL	October	SiteR	52	0	0	0	0	0	0	0	0	0	0	0	0	2	4	1	10	0	0	0	0	0	0	0
2024/25	CL	November	SiteS	30	15	0	0	0	0	20	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	2	4
2024/25	CL	December	SiteT	25	3	0	4	0	0	0	0	0	0	0	0	0	2	5	0	0	0	0	0	0	0	0	0
2024/25	CL	January	SiteU	30	5	0	0	3	0	0	0	1	0	0	0	0	2	5	0	1	0	0	0	0	1	0	0
2024/25	CL	February	SiteV	37	2	0	0	4	0	0	0	0	2	0	0	0	0	6	0	2	0	0	1	0	0	0	3
2023/24	LC	February	SiteX	15	30	0	0	1	0	0	0	0	0	0	0	0	0	15	0	5	0	0	0	0	0	0	0
2024/25	LC	March	SiteL	4	22	0	0	3	0	0	0	0	0	0	0	0	0	7	0	5	0	0	0	0	0	0	0
2024/25	LC	April	SiteM	2	18	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0	0	0	0	0
2024/25	LC	June	SiteN	10	6	4	0	80	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
2024/25	LC	July	SiteO	10	13	3	0	0	0	0	0	0	0	0	0	0	9	0	0	3	0	0	0	0	0	0	0
2024/25	LC	August	SiteP	6	3	1	0	1	0	0	0	0	0	0	0	0	4	52	1	5	0	0	0	0	0	0	0
2024/25	LC	September	SiteQ	12	0	1	0	9	0	0	0	0	0	0	0	0	0	10	0	13	0	0	0	0	0	0	0
2024/25	LC	October	SiteR	3	5	1	0	2	0	0	0	0	0	0	0	0	1	15	0	25	0	0	0	0	0	0	0
2024/25	LC	November	SiteS	1	2	0	0	4	0	0	0	0	0	0	0	0	0	12	0	24	0	0	0	0	0	0	0
2024/25	LC	December	SiteT	15	6	3	0	3	0	0	0	0	0	0	0	0	0	8	0	16	0	0	0	0	0	0	0
2024/25	LC	January	SiteU	20	8	3	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	1	0	0	0
2024/25	LC	February	SiteV	8	15	1	0	5	0	0	0	0	0	0	0	0	0	14	0	9	0	0	0	1	0	0	0
2023/24	DL	February	SiteX	0	0	0	1	1	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0
2024/25	DL	March	SiteL	3	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
2024/25	DL	April	SiteM	4	0	0	0	5	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
2024/25	DL	June	SiteN	9	0	0	0	6	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
2024/25	DL	August	SiteP	0	0	0	0	3	1	0	0	0	0	0	0	0	0	2	0	4	0	0	0	2	0	0	0
2024/25	DL	November	SiteS	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
2024/25	DL	February	SiteV	0	3	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
2023/24	V	February	SiteK	0	0	0	0	2	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0
2024/25	V	March	SiteL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
2024/25	V	April	SiteM	0	25	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	1	0	0	0	0	0	0
2024/25	V	June	SiteN	0	0	0	0	0	0	0	0	0	0	0	0	4	0	280	0	4	0	0	0	0	0	0	0
2024/25	V	July	SiteO	0	2	0	0	0	0	0	0	0	0	0	0	2	0	147	0	0	0	0	0	0	0	0	0
2024/25	V	August	SiteP	0	0	1	0	0	0	0	0	0	0	0	0	0	0	240	0	0	0	0	0	0	0	0	0
2024/25	V	September	SiteQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
2024/25	V	October	SiteR	0	0	0	0	0	0	0	0	0	0	0	0	2	0	56	0	0	0	0	0	0	0	0	0
2024/25	V	November	SiteS	0	1	0	0	0	0	0	0	0	0	0	0	0	0	85	0	0	0	0	0	0	0	0	0
2024/25	V	December	SiteT	0	1	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0
2024/25	V	January	SiteU	0	5	0	0	0	0	0	0	0	0	0	0	1	0	250	0	0	0	0	0	0	0	0	0
2024/25	V	February	SiteV	0	1	0	0	0	0	0	0	0	0	0	3	2	0	120	0	0	1	0	0	0	0	0	0

