# Determining causes and solutions for harmful algal blooms on the Wye River: Update report 06/24.

**Report prepared for the Wye and Usk Foundation (WUF), 10th June 2024 by:**

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**Summary of key findings (at time of report writing):**

- Levels of Phosphorus on the Wye River for the duration of sampling for this project are currently within SAC targets for orthophosphate.
- Levels of orthophosphate for the duration of sampling for this project are currently lower than those historically reported.
- Levels of total phosphorus for the duration of sampling for this project are within recommended levels at most locations on the Wye
- The headwaters of the Ithon are subject to large phosphorus spikes causing SAC target failures.
- Phosphorus levels for the duration of sampling for this project are routinely higher than target levels on the river Ithon and river Lugg at Disserth bridge and Mordiford Bridge respectively.
- Levels of nitrate for the duration of sampling for this project are currently higher than historically reported on the Wye
- No algal blooms were observed at sampling locations during the duration of this project.
- Preliminary eDNA data analysis reveals a diverse community composition, no species dominance observed.
- eDNA data is concordant with historical algal surveys conducted on the Wye
- Cyanobacteria were not problematic on the river Wye during 2022
- Data currently suggests that algal blooms are not being driven by the levels of phosphorus within the catchment.
- Transient nutrient spikes observed are currently being investigated utilising a metagenomic approach to microbial source tracking.

*Overarching conclusion to date from this project is that:*

> Phosphorus concentrations in the River Wye are likely not the primary cause of algal blooms in the river itself (this does not include its tributaries). It is suggested that nitrogen forms are of significant importance, however the likelihood is that river flows (low summer flows and high peak winter flows), combined with high summer temperature are of high importance. This emphasizes the need for an holistic management approach addressing flow rate riparian zone management, as well as all nutrient fractions is essential, and that a focus purely on phosphorus management will not address the needs for river water quality improvement in the River Wye.

# Determining causes and solutions for harmful algal blooms on the Wye River: Update report 06/24.

**Project Background**

The river Wye and its tributaries span an area of England and Wales of ~ 4,285km, starting at its source at Plynlimon in mid Wales and ending at the Severn estuary. The Wye River is over 200km in length, making it the fifth largest in the U.K., and is considered both a Site of Special Scientific Interest (SSSI) and a Special Area of Conservation (SAC). Following a prolonged algal bloom covering approximately 140 miles of the river Wye during the summer of 2020, there has been an increased scrutiny of the health of the Wye River, with a particular focus on the level of phosphorus (P) finding its way into the waters from various sources, and how this phosphorus loading has impacted, and continues to impact the river's ecology. Much of the concern has centered around the levels of orthophosphate (OP) in the river, as this is understood to be the limiting nutrient in freshwater environments. The current narrative and perception amongst the public is of a river with permanently elevated levels of OP, which has resulted in increasing levels of eutrophication. Of particular concern is the perceived growth in the number and severity of algal blooms resulting from this eutrophication, and in turn the subsequent harm to the river's ecology, wildlife, and stakeholder livelihoods.

Phosphorus has traditionally been viewed as the limiting nutrient in freshwater aquatic systems, its availability influencing the speed at which planktonic and macroalgae species can grow and proliferate. Urbanisation and agriculture have seen an increase in the use of P and consequently an increase in the nutrient enrichment, or eutrophication, of freshwater systems. Eutrophication can lead to lasting ecological impacts due to increased algal and aquatic plant growth, which in turn can result in oxygen depletion and hypoxic "dead zones" within waterbodies. When discussing levels of phosphorus in a freshwater body, OP is often considered of most importance as this is the fraction that is most readily bioavailable to organisms (Correll, 1999). In freshwater, phosphorus can also be found as condensed phosphates (Con-P) and organic phosphates (Org-P), which can both become bioavailable under the right conditions (Darsch et al., 2014). Therefore, it is important to monitor the total phosphorus (TP) concentration of any freshwater system to understand the impact of P on algal growth rates and the system's ecology. Due to historical and current high levels of agricultural land use and increased development within the Wye catchment (Withers et al., 2022), which necessitates increased wastewater treatment and disposal, levels of phosphorus have come under increasing scrutiny due to a need to comply with the European Community (EC) Habitats Directive designation of the catchment as a Special Area of Conservation (SAC). Using the EC Water Framework Directive (WFD) guidelines (European Commission, 2014), each waterbody within the Wye catchment has a targeted maximum level of OP, and these are outlined in the Wye Nutrient Management Plan. Levels of OP are regularly tested within each waterbody, and a three-year rolling average is used to decide whether they pass their respective target (Natural Resources Wales/The Environment Agency, 2021). No such targets are designated within the Wye Nutrient Management Plan for the levels of TP within the catchment, but governmental guidance recommends that rivers should not exceed annual mean concentrations of 0.1mg/L (DEFRA., 2012).

Previous work has shown that phosphorus in isolation cannot be used to predict the occurrence of algal blooms, high phosphorus levels does not mean algal blooms are a certainty and is not directly related to algal biomass (McCall et al., 2017). High levels of phosphorus can facilitate algal blooms, but other chemical and physical factors must be in conjunction for this to occur. These include: the presence of various nitrogen (N) fractions (nitrate ($NO_3$), nitrite ($NO_2$), ammonium ($NH_4$)) (Davis et al 2015), the ratios between the P and N fractions present (Harris et al., 2016; Klausmeier and Litchman, 2004), levels of sunlight available to algae (Litchman et al., 2003), and the temperature of the water (Shi et al., 2011).

A project currently underway at Cardiff University in conjunction with the Wye and Usk foundation (WUF) has sought to understand and address the issues currently faced. In both 2022 and 2023 between the months of June and November a bi-monthly sampling regime was undertaken at 14 sites within the Wye catchment, covering the main body of the river Wye but also the tributaries of the rivers Irfon, Ithon, and Lugg. A total of 365 of paired samples were collected over the two sampling seasons. One of each paired sample was subject to chemical nutrient analysis to determine the levels of different P (OP, Con-P, and TP) and N ($NO_3$, $NO_2$, $NH_4$, and total nitrogen (TN)) fractions. Alongside this analysis, the second water sample collected was passed through specialised filters capable of capturing the environmental DNA (eDNA) contained within the water at each location (Thomas et al., 2019). Following extraction, this DNA was sequenced, and the components of the algal and bacterial communities elucidated using a process known as metabarcoding. The project aims to use the data nutrient and eDNA data to establish the current risks and likely causes of algal community dynamics within the Wye catchment, with the objective of enabling the identification of intervention strategies that may be implemented to improve the ecology of the river.

Table 1. Concentrations of orthophosphate, total phosphorus, nitrate, and total nitrogen at 14 sites within the Wye catchment during the months June – November 2022 and 2023.

| Site Name | Orthophosphate Range mg/L All sites | Orthophosphate 2022 Av. mg/L | Orthophosphate 2023 Av. mg/L | Orthophosphate Two-year Av. mg/L | NMP Target mg/L | Total Phosphorus Range mg/L All sites | Total Phosphorus 2022 Av. mg/L | Total Phosphorus 2023 Av. mg/L | Total Phosphorus Two-year Av. mg/L | Nitrate Range mg/L All sites | Nitrate 2022 Av. mg/L | Nitrate 2023 Av. mg/L | Nitrate Two-year Av. mg/L | Total Nitrogen Range mg/L All sites | Total Nitrogen 2022 Av. mg/L | Total Nitrogen 2023 Av. mg/L | Total Nitrogen Two-year Av. mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Esgairdraenllwyn Bridge | <LOD - 0.271 | 0.024 | 0.028 | 0.026 | 0.01 | 0.001 - 0.859 | 0.158 | 0.107 | 0.132 | <LOD - 24.21 | 3.26 | 3.37 | 3.32 | <LOD - 40.65 | 6.69 | 6.95 | 6.82 |
| Llananno Bridge | | 0.020 | 0.010 | 0.015 | 0.01 | | 0.121 | 0.086 | 0.104 | | 2.99 | 5.19 | 4.09 | | 6.33 | 6.87 | 6.60 |
| Crossgates | | 0.022 | 0.015 | 0.019 | 0.015 | | 0.107 | 0.104 | 0.105 | | 2.82 | 4.88 | 3.85 | | 6.07 | 7.20 | 6.63 |
| Pentybont Bridge | | 0.022 | 0.019 | 0.020 | 0.015 | | 0.105 | 0.068 | 0.087 | | 3.10 | 5.11 | 4.10 | | 6.03 | 6.53 | 6.28 |
| Newbridge-on-Wye | | 0.019 | 0.004 | 0.012 | 0.015 | | 0.081 | 0.039 | 0.060 | | 1.93 | 1.63 | 1.78 | | 2.87 | 2.73 | 2.80 |
| Disserth Bridge | | 0.042 | 0.021 | 0.031 | 0.01 | | 0.144 | 0.185 | 0.165 | | 5.71 | 5.93 | 5.82 | | 8.89 | 8.71 | 8.80 |
| Irfon/Wye Junction | | 0.020 | 0.004 | 0.012 | 0.015 | | 0.106 | 0.091 | 0.099 | | 2.33 | 1.73 | 2.03 | | 4.52 | 3.68 | 4.10 |
| Hay on Wye | | 0.020 | 0.008 | 0.014 | 0.03 | | 0.131 | 0.058 | 0.094 | | 2.57 | 3.07 | 2.82 | | 5.04 | 4.32 | 4.68 |
| Brewardine | | 0.023 | 0.008 | 0.015 | 0.03 | | 0.101 | 0.080 | 0.091 | | 2.70 | 3.39 | 3.05 | | 4.62 | 5.36 | 4.99 |
| Hereford Bridge | | 0.025 | 0.007 | 0.016 | 0.03 | | 0.097 | 0.060 | 0.079 | | 3.58 | 4.41 | 4.00 | | 6.31 | 6.54 | 6.42 |
| Holme Lacy Bridge | | 0.046 | 0.037 | 0.042 | 0.039 | | 0.155 | 0.091 | 0.123 | | 9.06 | 8.68 | 8.87 | | 10.20 | 12.11 | 11.16 |
| Wilton Bridge | | 0.039 | 0.025 | 0.032 | 0.05 | | 0.145 | 0.060 | 0.102 | | 8.84 | 9.18 | 9.01 | | 10.33 | 12.14 | 11.24 |
| Bigsweir | | 0.044 | 0.024 | 0.034 | 0.039 | | 0.148 | 0.086 | 0.117 | | 8.56 | 10.34 | 9.49 | | 9.30 | 12.81 | 11.12 |
| Mordiford Bridge | | 0.128 | 0.087 | 0.068 | 0.05 | | 0.256 | 0.132 | 0.186 | | 19.45 | 16.63 | 17.86 | | 18.32 | 19.62 | 19.06 |

# Determining causes and solutions for harmful algal blooms on the Wye River: Update report 06/24.

**Summary of Project Results to date**

Between 17th June and the 31st of November 2022, and 7th June and 15th November 2023 a bi-monthly sampling regime was undertaken at 14 sites within the Wye catchment, capturing the main body of the river Wye but also the Rivers Lugg, Ithon, and Irfon. A total of 365 samples were collected across 26 sampling trips which were subsequently processed to determine the levels of phosphorus and nitrogen nutrient fractions, physiological attributes (pH, conductivity, and turbidity), and the bacterial algal community composition of each site utilising eDNA.

**Nutrient Analysis**

Analysis has revealed that during the sampling period concentrations of OP and TP within the catchment ranged from <LOD – 0.271mg/L and 0.001 – 0.859mg/L respectively (Table 1.). Mean concentrations for both P fractions were higher during the 2022 sampling period compared to the 2023 sampling period. Within the main body of the Wye River during both sampling periods, OP and TP concentrations can be seen to gradually increase as you move downstream (Figure 1.). Holme Lacy Bridge had the highest mean concentrations of OP during both sampling periods and the

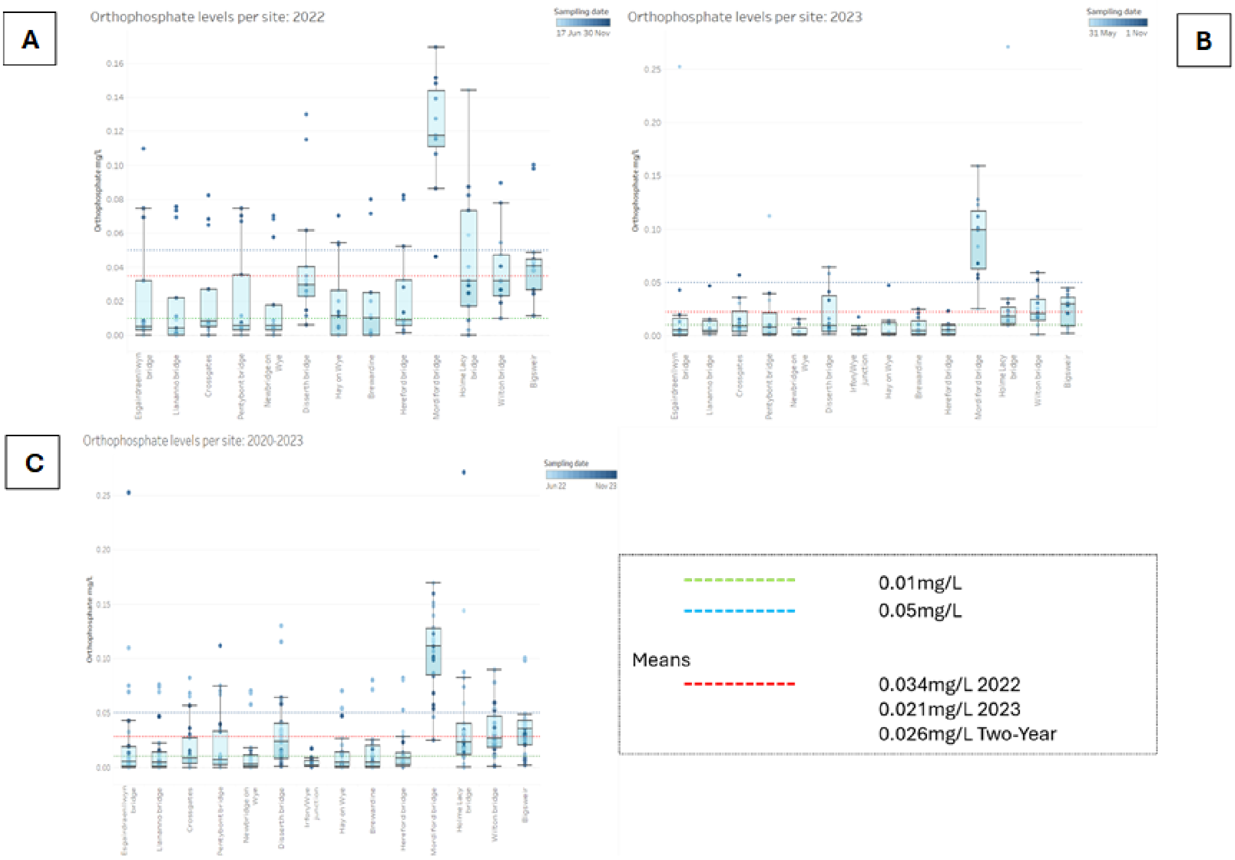


Figure 1. Orthophosphate concentrations within the Wye River catchment during the months June to November for A) 2022, B) 2023, and C) both years. Sites on the Wye River met their SAC targets during the two-year sampling period. Sites at the headwaters of the Ithon experienced spikes of OP which can skew their mean concentrations causing them to largely fail SAC targets. Disserth Bridge on the river Ithon and Mordiford Bridge on the river Lugg consistently failr to meet their SAC targets

highest two-year rolling average (0.046mg/L, 0.037mg/L, and 0.042mg/L). Holme Lacy also experienced the largest range of readings during both the 2022 sampling season (<LOD – 0.144mg/L), and 2023 season (0.009 – 0.271mg/L). Esgairdraenllwyn Bridge experienced the highest mean TP concentrations over both sampling seasons, with 0.158mg/L being recorded during 2022 season and 0.107mg/L during the 2023 season, but also the highest two-year rolling mean (0.132mg/L). The largest range of TP readings observed for each season were Hay-on-Wye (<LOD – 0.638mg/L) and (0.0021 – 0.578mg/L), for 2022 and 2023 respectively. Disserth Bridge experienced consistently higher readings of OP (Figure 1.) and TP (Figure 2.) when compared to sites on the main

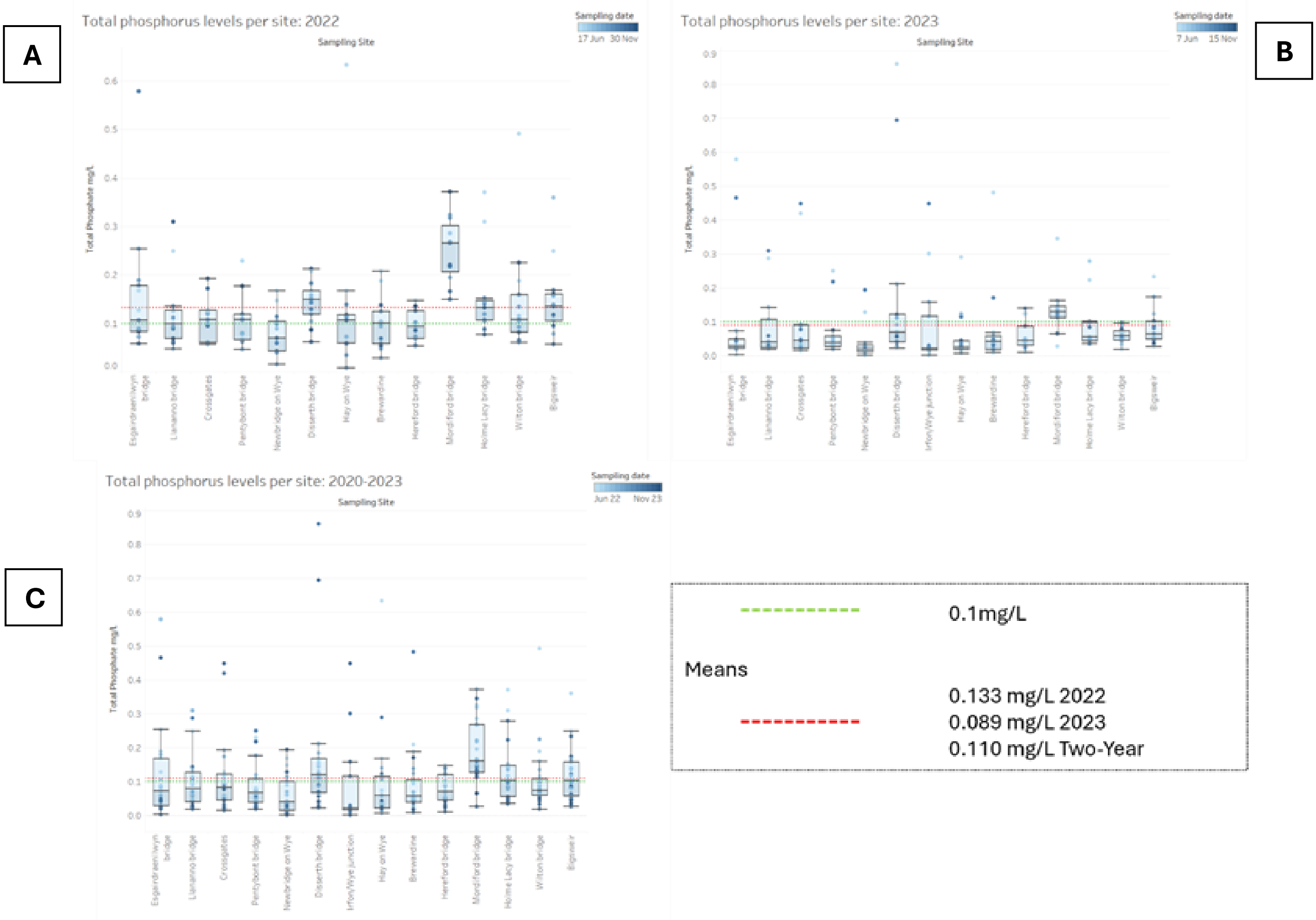


Figure 2. Total Phosphate concentrations for the Wye catchment during the months June to November for A) 2022, B) 2023, and C) both years. Sites at the top and middle of the Wye River remained at or were lower than the recommended 0.1mg/L target. Sites on the Wye at the lower end of the catchment failed to meet this target during the 2022 season, causing them to fail to do so over the two-year sampling period. TP concentrations exceed 0.1mg/L at a small number of sites at the headwaters of the Ithon, but also experience large transient spikes that cause this failure. TP levels are are consistently elevated at Disserth bridge on the river Ithon and at Mordiford Bridge on the river Lugg

body of the river Wye (excluding Holme Lacy Bridge, Wilton Bridge, and Bigsweir Bridge); the largest TP reading of both sampling periods was recorded at Disserth (0.859mg/L). This indicates that the river Ithon had elevated levels of all P fractions compared to the Wye itself. Similarly, Mordiford Bridge on the river Lugg experienced consistently elevated levels of all P fractions compared to all sites for the duration of the sampling periods, this being particularly pronounced during the 2022 sampling season. Again, this indicates that the river Lugg at Mordiford has elevated levels of P compared to the river Wye.

During the sampling period $NO_3$ and TN within the catchment ranged from <LOD – 24.21mg/L and <LOD – 40.65 respectively. Interestingly, unlike the P fractions which saw a drop in concentration during the two sampling periods, mean levels of both N fractions either stayed approximately the same between both years, or slightly increased at a small number of sites (Table 1.). Mean $NO_3$ concentrations increased at 3 sites at the top of the catchment, namely Llannano Bridge, Crossgates, and Pentybont Bridge. Conversely, TN concentrations increased slightly between

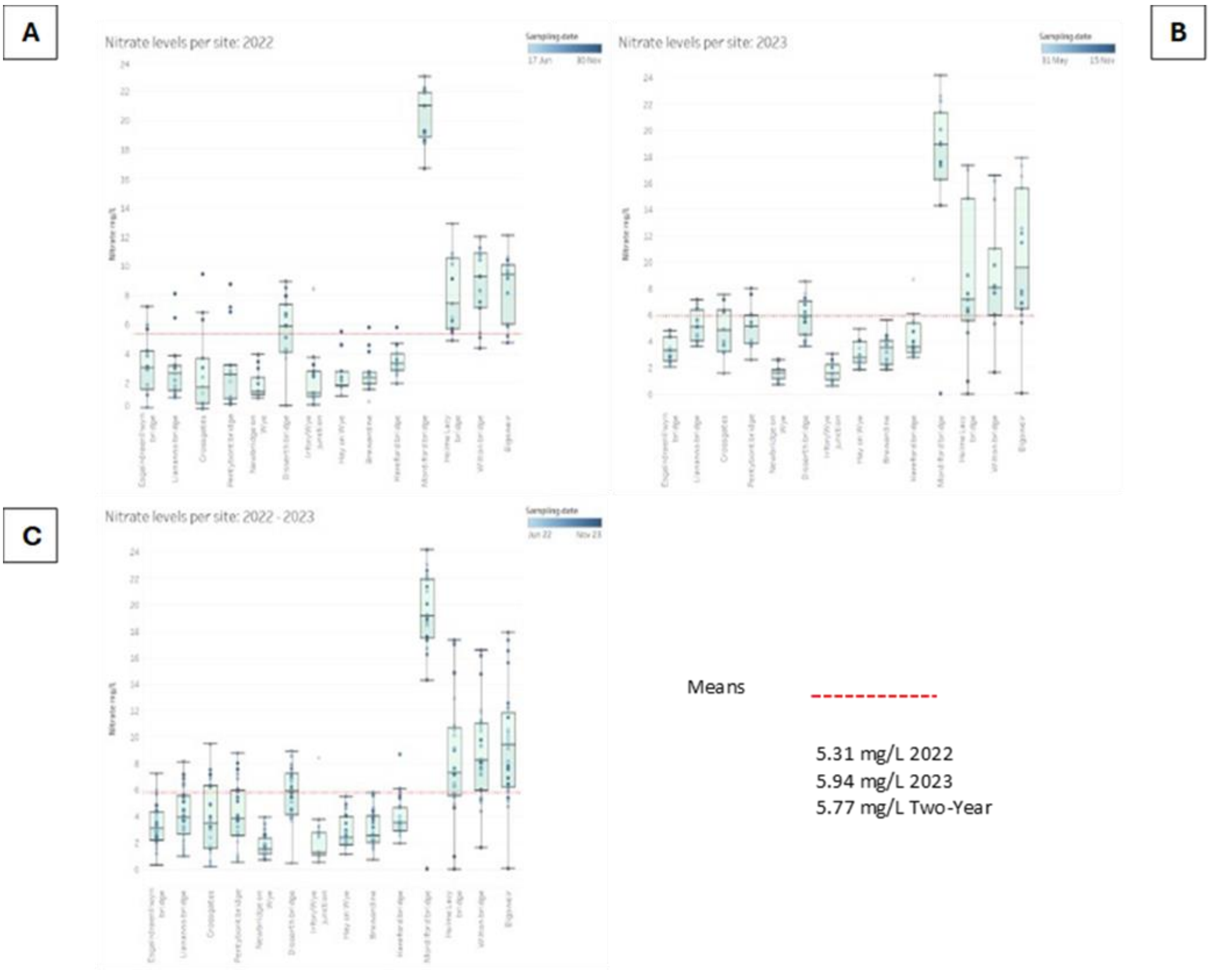


Figure 3. Nitrate concentrations for the Wye catchment during the months June to November for A) 2022, B) 2023, and C) both years. $NO_3$ concentrations remained relatively unchanged during the sampling period. $NO_3$ levels increased gradually along the length of the Wye. Concentrations of $NO_3$ are highest at Mordiford Bridge but were high throughout much of the catchment and were higher than historically recorded.

2022 and 2023 at the three sites nearest the river mouth, Holme Lacy Bridge, Wilton Bridge, and Bigsweir. Figure 3. and Figure 4. demonstrate how $NO_3$ and TN fractions follow the pattern observed for OP and TP, with mean concentrations gradually increasing as you move downstream. On the main body of the river Wye, Holme lacy Bridge experienced the highest mean concentrations of $NO_3$

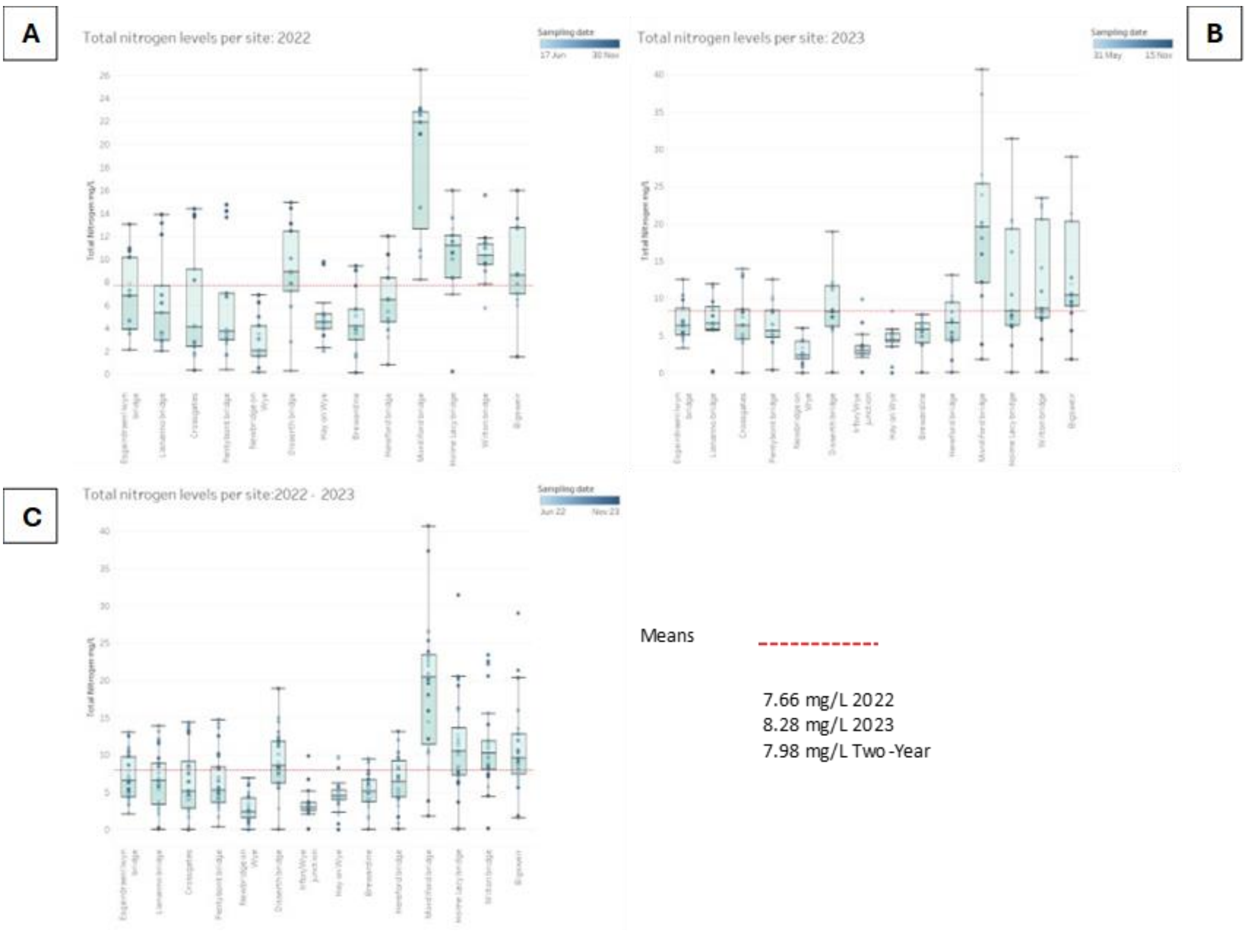


Figure 4. concentrations for the Wye catchment during the months June to November for A) 2022, B) 2023, and C) both years. TN concentrations remained relatively unchanged during the sampling period. TN levels increase gradually along the length of the Wye. Concentrations of TN were highest at Mordiford Bridge.

during the 2022 sampling season (9.06mg/L), with Bigsweir experiencing the highest during 2023 and over the two seasons combined (10.34mg/L and (9.49mg/L).

When considering TN, Wilton Bridge experienced the highest mean concentrations in 2022 and over both years combined (10.33mg/L and 11.24mg/L), with Bigsweir experiencing the highest mean concentration during 2023 (12.81mg/L). Similarly to both P fractions, Disserth bridge on the river Ithon and Mordiford on the river Lugg experienced consistently elevated readings compared to those on the Wye, again indicating that these tributaries of the Wye generally have higher concentrations of N. It is worth noting that $NO_3$ readings are closely correlated with OP readings

($r(363) = .52$, $p = < .001$) and TN readings are closely correlated to TP readings ($r(363) = .25$, $p = < .001$), following similar patterns in their relative concentrations (Figure 5.), indicating they may share common sources of ingress into the Wye and its tributaries.

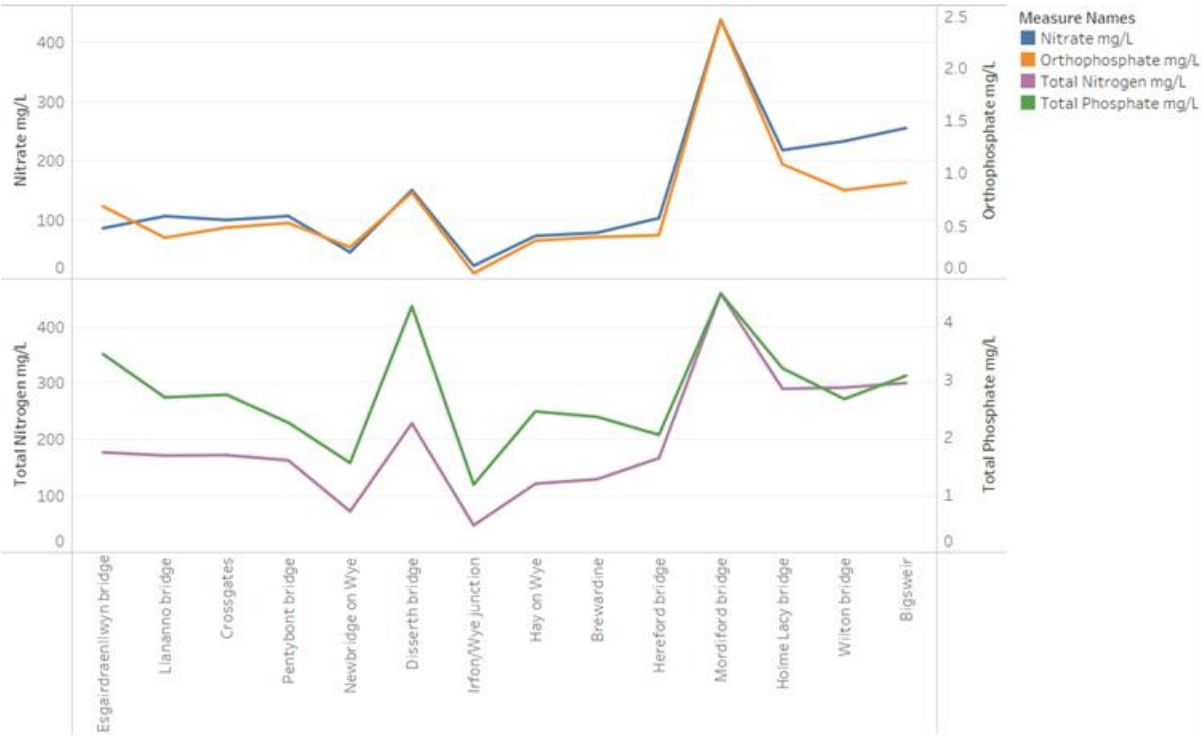


Figure 5. Over the two-year sampling period, concentrations of $NO_3$ correlated with OP, and concentrations of TP correlated with TN, at all sampling locations.

**Algal and bacterial community composition**

Following extraction and sequencing of the eDNA collected during the sampling period, bioinformatic approaches were utilised to determine the composition and relative abundance of the chlorophyte (green algae), Bacillariophyte (diatoms), and bacterial communities present at each location and time point. Currently all data generated is of relative abundance and as such is qualitative; the abundance of any genera or phyla is expressed as a percentage of the total community but is not currently indicative of biomass. Future work will utilise qPCR techniques to provide a quantification of the biomass of the community present. Data represents the eDNA samples collected during the 2022 sampling period, eDNA data for the 2023 period is currently being processed and will be available shortly. No algal blooms were observed at any of the sampling locations at any time point during the duration of the sampling period.

Table 2. contains the 5 most abundant phyla of the bacterial community observed during the sampling period. Cyanobacteria comprised approximately 1% of the total reads generated during sequencing for the entire sampling period. The highest abundance of cyanobacteria was observed at Newbridge-on-Wye during July (4.9%). Data indicates that cyanobacteria were not problematic at any of the sampling sites monitored for this project during the 2022 sampling period.

Table 2. The 5 most abundant Chlorophyte (green algae) and Bacillariophyte (diatoms) genera, and the most abundant bacterial phyla observed during 2022 following eDNA analysis

| Chlorophyta Genera | % of Total Read Abundance |
|---|---|
| Desmodesmus | 28.2 |
| Tetradesmus | 16.7 |
| Mychonastes | 14.9 |
| Chlamydomonas | 11.4 |
| Carteria | 5.6 |
| | |
| Bacillariophyta Genera | % of Total Read Abundance |
| Stephanocyclus | 21.9 |
| Nitzschia | 18.1 |
| Melosira | 17.9 |
| Navicula | 8.8 |
| Fistulifera | 6.9 |
| | |
| Bacteria Phyla | % of Total Read Abundance |
| Bacteroidota | 34 |
| Proteobacteria | 30 |
| Actinobacteria | 15.9 |
| Firmicutes | 6.8 |
| Verrucomicrobiota | 3.8 |
| Cyanobacteria | 1 |

Table 2. contains the 5 most abundant chlorophyte genera observed during the sampling period. Based on current data analysis, the chlorophyte community appears diverse during the duration of the sampling period, no one genera appear to dominate and outcompete the other members of the community. Figure 6. reveals the relative abundance of each genera observed monthly at each site during 2022. At sites near the top of the catchment on the river Ithon and its tributaries (Llannano Bridge, Crossgates, Pentybont Bridge, and Disserth Bridge) the predominant genera present throughout much of the sampling season are *Desmodesmus* and *Tetradesmus.* The exception to this pattern was Esgairdraenllwyn Bridge whose community was predominantly composed of *Tetradesmus* sp. (41.6 %) during June, before shifting to predominantly *Chlamydomonas* sp. from July until August (35.9% and 47.7%), returning to *Tetradesmus* sp. predominance during September (32.8%), before finally returning to *Chlamydomonas* sp. during October and November (23.9% and 59%). Disserth Bridge, also on the Ithon, followed a pattern that is distinct from the sites previously mentioned, the community being predominantly composed of *Tetradesmus* sp. and *Desmodesmus* sp. for the entirety of the sampling period. Sites on the main body of the Wye above Bredwardine (Newbridge-on-Wye, Irfon/Wye Junction, and Hay-on-Wye) appeared to have a distinct chlorophyte community composition compared to those below Bredwardine (Hereford Bridge, Holme Lacy Bridge, Wilton Bridge, and Bigsweir). Sites above Bredwardine oscillated between predominance by *Tetradesmus* sp. and *Desmodesmus* sp. for much

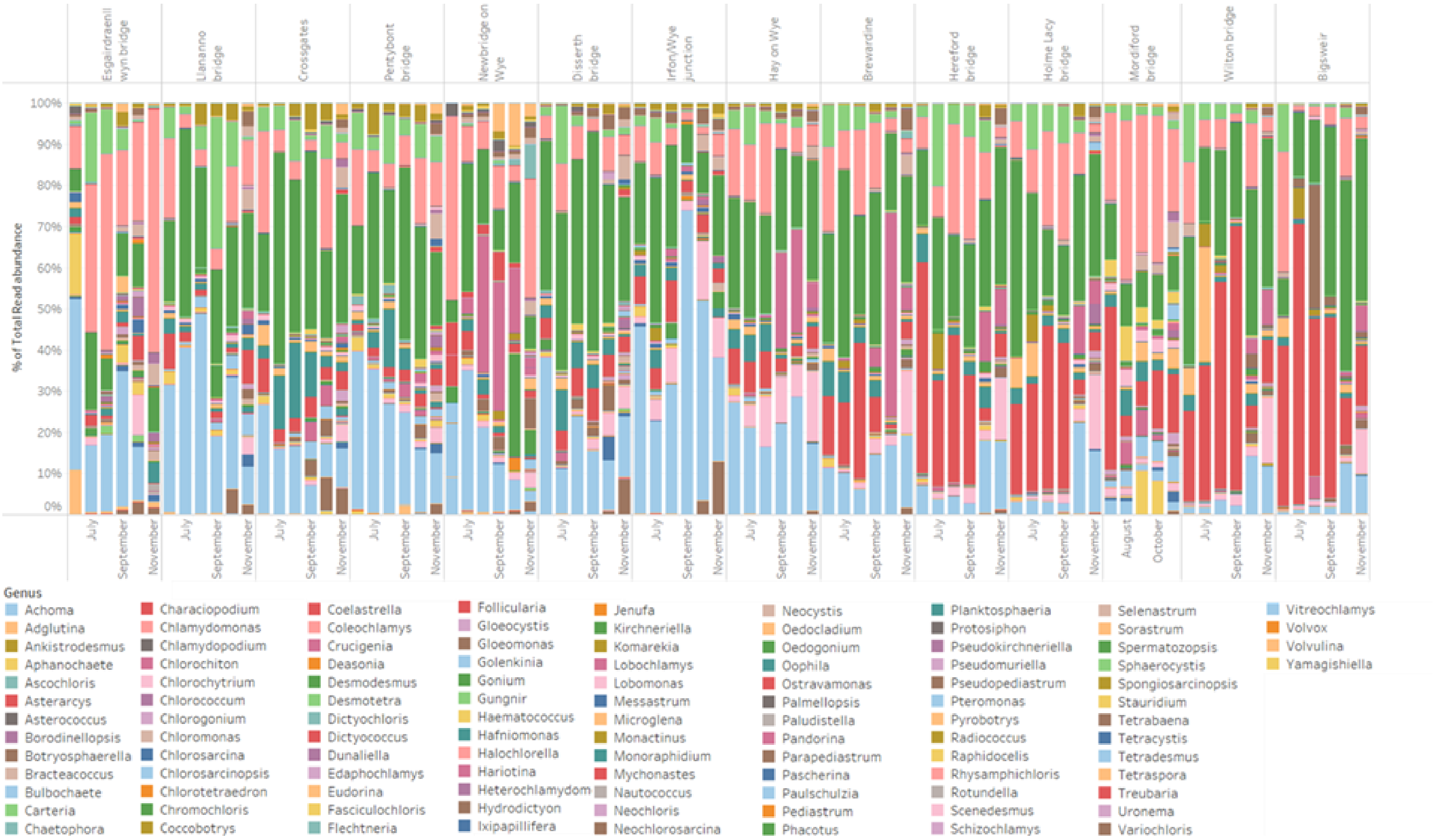


Figure 6: The composition of the chlorophyte community present each month at each sampling location during the 2022 sampling period. Genera are represented as a percentage of the overall community abundance.

of the sampling period but with a pronounced increase in the number of *Hariotina* sp. observed between August and October. The chlorophyte community at sites below Bredwardine was primarily composed of *Mychonastes* sp. between the months of June and September, with the highest abundance being observed at Bigsweir during the month of July (68%), with *Desmodesmus* sp. and *Chlamydomonas* sp. making up a large proportion of the remaining community. The community shifted in composition at each site during the months of October and November with *Desmodesmus* sp. returning to predominance, the highest abundance being recorded at Bigsweir during October (46.5%). Finally, the chlorophyte community observed at Mordiford Bridge on the river Lugg was predominantly composed of *Mychonastes* sp. during June (40%) before being dominated by *Chlamydomonas* sp (38.8% - 20%) for the remainder of the sampling period.

Table 2. contains the 5 most abundant Bacillariophyte genera observed during the 2022 sampling period. Current analysis (Figure 7.) shows that for the majority of the sampling season the community appeared diverse with no one genera dominating. The exception to this was at Disserth bridge during June when *Melosira* sp. were observed to comprise 92% of all diatom species observed. This kind of species dominance is often associated with bloom events (Egerton, 2014),

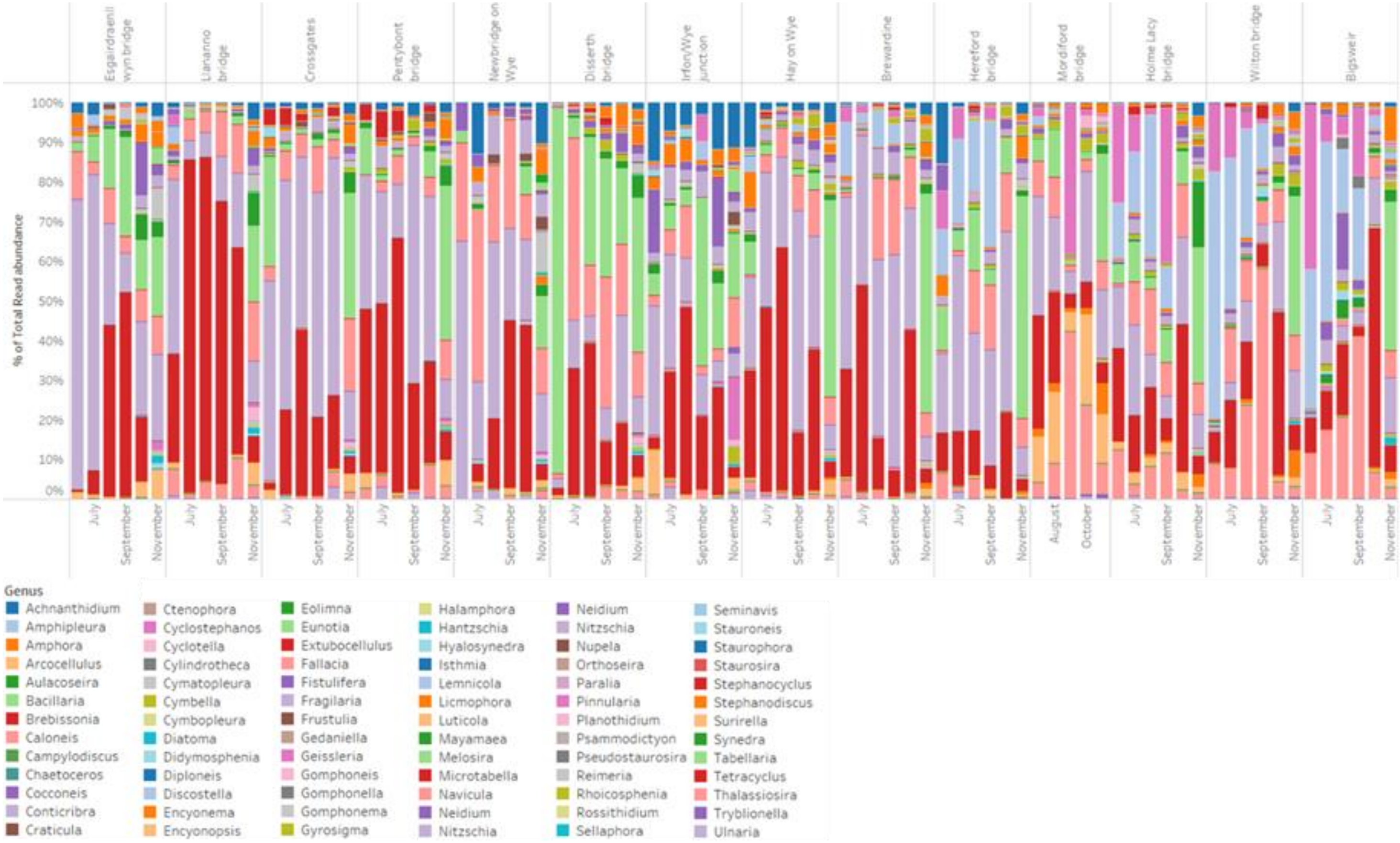


Figure 7. The composition of the bacillariophyte community present each month at each sampling location during the 2022 sampling period. Genera are represented as a percentage of the overall community abundance.

however, as previously stated, no algal blooms were observed at any location during the sampling period. Additionally, once that abundance is considered within the context of the full phytoplankton community, it only accounts for 59.7% of the total abundance. Between the months of July and October the predominant genera present at Disserth Bridge were *Navicula* sp. (45.9% - 12.9%), *Stephanocyclus* sp. (47.4% - 11.1%), and *Melosira* sp. (32.2% - 19%), before becoming predominantly composed of *Melosira* sp. (38.97) during November. A similar pattern emerged in the Bacillariophyte community to that of the chlorophyte community with sites close to the top of the catchment on the river Ithon having a distinct community compared to those on the Wye. These sites had a community comprised mostly of *Nitzschia* sp. during June and July before a shift to predominantly *Stephanocyclus* sp. between August and October before finally moving to *Melosira* sp. being the most abundant. Sites on the river Wye above Bredwardine from Newbridge-on-Wye down to Hereford Bridge followed a similar pattern with an abundance of *Nitzschia* sp. between June and July, with *Stephanocyclus* sp. becoming dominant during August to October before *Melosira* sp. became predominant during November. However, abundance of *Navicula* sp. was high during June and July (24.7% and 43.7%) at Newbridge-on-Wye. Sampling sites below Hereford Bridge (Holme Lacy Bridge, Wilton Bridge, Bigsweir) also followed a similar pattern to those above Hereford Bridge but *Stephanocyclus* sp. comprised less of the overall community here and abundances of *Cyclostephanos* sp., *Thalassiosira* sp., and *Discotella* sp. increased.

**Conclusions**

Data generated thus far in this project is beginning to indicate that the current public perception surrounding levels of phosphorus and its effect on algal dynamics within the river Wye may be misleading. All sampling sites located on the main course of the river Wye met their SAC targets for OP during the two-year sampling period. 4 sites (Newbridge-on-Wye, Irfon/Wye junction, Home Lacy Bridge, and Bigsweir) failed to meet their targets during the 2022 sampling season but Figure 1. demonstrates that these SAC targets were largely met but mean data has been skewed by a small number of time points. Sites on the Ithon at the headwaters of the catchment (Esgaridraenllwyn Bridge, Llananno Bridge, Crossgates, and Pentybont Bridge) failed to meet their SAC targets during the 2022 season but passed them, except for Esgairdraenllwyn Bridge, during the 2023 season. Again, large spikes of OP at a small number of sampling points give the impression that levels of OP may be consistently higher than they are. Levels of OP were however, elevated at two sites within the catchment on tributaries of the Wye, Disserth Bridge and Mordiford Bridge; they consistently failed to meet their targets during the sampling period. It is worth noting that historical studies of the nutrient dynamics of the Wye and its tributaries found levels of OP to be higher than (Jones, 1984), or of a similar level (Oborne, 1980) to those reported here, even following P stripping measures introduced at wastewater treatment sites within the catchment (Jarvie et al., 2003). Similar trends were observed in each of the studies, with OP concentrations gradually increasing along the length of the Wye from the headwaters to the mouth of the river, but the authors also reported noticeably larger readings observed on the tributaries of the Ithon and the Lugg, both of which are consistent with the findings reported here. Work undertaken by McCall et al. (2017) to investigate the effects of light intensity and phosphorus concentration on periphytic algal biomass, demonstrated that increasing the concentration of OP from a background level of 0.049mg/L to 0.155mg/L had no discernible effect on community biomass, but it did cause shifts in the composition of the community. The same work found that at these concentrations, decreasing the light intensity available to the periphytic community caused the biomass to decrease by 40%. They report phosphorus and light colimitation at concentrations at ~0.033mg/L and concentrations as low as ~0.023mg/L for OP levels to be truly limiting. They suggested it may be impractical to achieve concentrations as low as this in lowland stretches of rivers due to various factors and suggest light limitation may be an effective strategy to help control algal growth and improve ecological status. This factor may be of importance regards the Wye catchment where a large proportion of the OP within the river comes from diffuse agricultural sources, and that even if immediate changes are made to agricultural practices within the catchment, legacy P found in agricultural soils may cause a lag between changes and actual reductions in water concentrations (Withers et al., 2022)

Both Oborne (1980) and Jones (1982) investigated the concentrations of $NO_3$ found within the waters of the catchment. Both studies reported lower concentrations of $NO_3$ in both the main body of the river Wye and the tributaries when compared to the results generated here. Increasingly it is being understood that controlling nitrogen inputs may be as important as controlling phosphorus inputs to freshwaters to improve or maintain good ecological status. Mebane et al. (2021) report that different communities (e.g. phytoplankton, periphyton, and macrophyte) within the larger algal community, experienced differing nutrient requirements and differing limiting nutrients. They report however, that nitrogen limitation or co-limitation (N and P) were most common. Work by Klausmeier and Litchman (2004) demonstrated the importance of monitoring and understanding the relative nitrogen and phosphorus ratios, and not just absolute values, when considering the effect of nutrient limitation on algal dynamics. They showed that different species within the community have specific cell stoichiometry, or cellular ratios, and thus different growth responses and different optimum nutrient ratios. By reducing the P load to rivers whilst not considering the concentrations of N availability may have the unintended consequence of selecting for problematic species, particularly those with in the cyanobacterial community that are often nitrogen limited (Mebane et al., 2021).

It has been reported with regularity that the waters of the river Wye have in recent times been observed to turn "pea-soup green" during the summer months (McKie, 2020; Gatten, 2023); this would imply proliferation by either cyanobacterial species or planktonic chlorophyte species in the water column. As stated previously, no algal blooms were observed at any of the sampling locations during the sampling period. This is not to say algal blooms did not occur on the river Wye during the sampling period, just that none were observed by this author. To be clear, The Environment Agency report a short lived (~two days) bloom located at Monmouth during 2023 (The Environment Agency, 2023), and this author has received a private correspondence identifying a short-lived bloom (duration and location unclear) during the 2023 season. It is worth considering that there currently appears to be no clear definition of the term "algal bloom". The definitions most often used are either some approximation of "the rapid excessive growth of algae", without quantification, or the one devised by the World Health Organisation describing cell densities at ~20,000 cells/ml (WHO, 1999). Review of the WHO definition indicates that this definition was devised specifically to describe the risk to health associated with toxin producing cyanobacteria, rather than cell densities that can be observed. As previously stated, cyanobacteria composed a small proportion of the bacterial community present at each site during the 2022 sampling period and as such, were not problematic on the Wye during this period. eDNA data for the 2023 sampling period is currently being processed and will be reported in due course. Additionally, despite *Melosiral* sp. dominating the diatoms community during the month of June 2022, no bloom was observed, indicating that despite conditions being suited to *Melosira* sp. outcompeting other diatom species, conditions may not have been suitable for uncontrolled proliferation.

Jones (1982) conducted a survey of, and the variables most impacting the phytoplankton algal community dynamics of the Wye over two years in 1980 and 1981. The author reports peak cell densities of 251 x $10^3$ $ml^{-1}$ (251,000 cells/ml), indicating that chlorophyte phytoplankton can reach large biomasses within the river Wye. It was found that the phytoplankton community was predominantly composed of *Cyclotella* sp. and *Scenedesmus* sp.. Since publication *Cyclotella* sp. have been reclassified to be contained within *Stephanocyclus* (Kulikovskiy et al., 2022), and *Scenedesmus* sp. reclassified to either the genus *Desmodesmus* (Friedl and Hegewald, 1998) or *Tetradesmus* (Wynne and Hallan, 2015), with *Scenedesmus obliques* being synonymously described as *Tetradesmus obliques* (Oliveira et al., 2021). These observations are concordant with the most observed chlorophyte and Bacillariophyte species observed to be present following sampling during the 2022 season. Jones reports that algal dynamics on the river Wye did not overly correlate with levels of nutrients available but most closely correlated with low river flow rates and high solar radiation. This agrees with a study conducted by Bae and Seo (2021) on the Nakdong river, which found algal dynamics were linked to decreased flow rate and water retention time within the river following the construction of artificial weirs. Lower reaches of the river became susceptible to algal blooms following construction of these weirs as water was retained for longer in the system in a stretch of the river that had was wide and received high levels of solar radiation due to decreased riparian cover. Similarly. Jarvie et al. (2004) report that following P stripping utilised at wastewater treatment sites, epiphytic algal growth in locations immediately adjacent to wastewater treatment works became sensitised to large spikes in OP concentrations during base river flow conditions, and experienced temporary increases in biomass (when a threshold of 0.1mg/L was passed), but this effect was seen to disappear entirely when similar spikes were observed during periods of high flow. Most conceptual understanding of algal bloom dynamics is informed by investigations in Lentic systems. Hilton et al. (2006) suggest this is not applicable to rivers in most instances, especially in rivers with short retention times or at the upper reaches of rivers where initial cell inoculum will be small, flow rates will be high, and a large number of cell doublings will be necessary to reach nuisance levels. Conceptually, rivers with a retention time shorter than the planktonic algae doubling time, will rarely see problematic algal biomass irrespective of nutrient conditions. More investigation of the flow dynamics is needed to understand how applicable this conceptualisation is to the Wye.

Algal data for 2023 is currently being processed and further analysis of nutrient dynamics will include environmental data such as precipitation and flow rates within the catchment, which will begin to build a bigger picture of the threats of pollution and eutrophication that the Wye catchment currently faces. As stated, it is becoming apparent that the focus on the levels of P alone within the catchment, and the focus on algal blooms may be detracting from a holistic overview of river management, and that other factors may need to be considered, such as levels of N inputs into the river, changes to the course of the river that may have unduly affected flow rate during times of dry weather and thus increasing retention time, and the changes to riparian cover that mean algae are receiving increased solar radiation. Further work has been undertaken to investigate the spikes of P that were witnessed during the sampling season utilising a method of microbial source tracking that may begin to give insight into sources of nutrient pollution into the river Wye.

**References**

Bae, S. and Seo, D. 2021. Changes in algal bloom dynamics in a regulated large river in response to eutrophic status. *Ecological Modelling* 454(1). doi:10.1016/j.ecolmodel.2021.109590

Correll, D. 1999. Phosphorus: a rate limiting nutrient in surface waters. *Poultry Science,* 78(5), pp. 674-682. doi:10.1093/ps/78.5.674.

Darsch, T., Blackwell, M., Hawkins, J., Haygarth, P. and Chadwick, D. 2014. A meta-analysis of organic and inorganic phosphorus in organic fertilizers, soils, and water: implications for water quality. *Critical Reviews in Environmental Science and Technology*, 44(19). pp. 2172-2202. doi: 10.1080/10643389.2013.790752.

Davis, T., Bullerjahn, G., Tuttle, T., McKay, R. and Watson, S. 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during Planktothrix blooms in Sandusky Bay, Lake Erie. Environmental Science Technology 49, pp. 7197-7207. doi: 10.1021/acs.est.5b00799.

Department for Environment, Food and Rural Affairs (DEFRA). 2012. *Observatory monitoring framework – indicator data sheet*. Available at: https://assets.publishing.service.gov.uk/media/5a7b1f23ed915d3ed90624fc/defra-stats-observatory-indicators-da3-120224.pdf [Accessed: 10 March 2024].

Egerton, T., Morse, R., Marshall, H. and Mullholland, M. 2014. Emergence of algal blooms: The effects of short-term variability in water quality on phytoplanklton abundance, diversity, and community composition in a tidal estuary. *Microorganisms* 2(1), pp. 33-57. doi:10.3390/microorganisms2010033

The Environment Agency. 2023. River Wye Special Area of Conservation 2023 growing season monitoring summary report. Available at: https://ehq-production-europe.s3.eu-west-1.amazonaws.com/1046d320f248fbb13d6d754463a73da810eae445/original/1705311483/4805e557af47ba24a774ef742128454c_Growing_Season_Monitoring_Summary_Report_2023_.pdf. [Accessed 03 May 2024].

European Commission, Directorate General for Environment. 2014. *The EU Water Framework Directive.* Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02000L0060-20141120 [Accessed 10th April 2024].

Friedl, S. and Hegewald, E. 1999 Phylogenetic relationships of *Scenedesmus and Scenedesmus*-like Coccoid Green Algae as inferred from ITS-2 rDNA sequence comparisons. *Plant Biology* 1(4), pp. 418-428. doi:/10.1111/j.1438-8677.1999.tb00724.x

Gatten, E. 2023. River Wye ecological status downgraded as sewage turns water into “pea soup”. The Telegraph 30 May. Available at: https://www.telegraph.co.uk/news/2023/05/30/river-wye-ecological-status-downgraded-sewage. [Accessed 25 May 2024].

Harris, T., Smith, V., Graham, J., Van de Waal, D., Tedesco, L. and Clercin, N. 2017. Combined effects of nitrogen to phosphorus and nitrate to ammonia ratios on cyanobacterial metabolite concentrations in eutrophic Midwestern USA reservoirs. Inland Waters 6(2), PP. 199-210. doi: 10.5268/IW-6.2.938.

Hilton, J., O'Hare, M., Bowes, M. J. and Jones, I. 2006. How green is my river? A new paradigm of eutrophication in rivers. *Science of the Total Environment* 365(3), pp. 66-83. doi:10.1016/j.scitotenv.2006.02.055

Jarvie, H., Neal, C., Withers, P., Robinson, A. and Salter, N. 2003. Nutrient water quality of the Wye catchment, UK: exploring patterns and fluxes using the Environment Agency data archives. *Hydrology and Earth System Sciences* 7(5), pp. 722-743. doi:10.5194/hess-7-722-2003.

Jarvie, H., Neal, C. and Williams, R. 2004. Assessing changes in phosphorus concentrations in relation to in-stream plant ecology in lowland permeable catchments: bringing ecosystem functioning into water quality monitoring. *Water, Air, and Soil Pollution: Focus* 4(1), pp. 641-655.

Jones, F. H. 1984. The dynamics of suspended algal populations in the lower Wye catchment. *Water Research* 18(1), pp. 25-35. doi:10.1016/0043-1354(84)90044-7.

Klausmeier, C. and Litchman, E. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. *Limnology and Oceanography* 49(4), pp. 1463 -1470.

Kulikovskiy, M., Genkal, S., Maltsev, Y., Glushchenko, A., Kuznetsova, I., Kapustin, D., Gusev, E., Martynenko, N. and Kociolek J. 2022. Resurrection of the diatom genus *Stephanocyclus* (Coscinodiscophyceae: Stephanodiscaceae) on the basis of an integrated molecular and morphological approach. *Fottea Olomouc* 22(2), pp. 181-191. doi: 10.5507/fot.2021.025

Litchman, E., Steiner, D. and Bossard, P. 2003. Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength. *Freshwater Biology* 48, pp. 2141-2148.

McCall, S., Hale, M., Smith, J., Read, D. and Bowes, M. J. 2017. Impacts of phosphorus concentration and light intensity on river periphyton biomass and community structure. *Hydrobiologia* 792(1), pp. 315-330. doi:10.1007/s10750-016-3067-1.

Mckie, R. 2020. 'Its like pea soup': poultry farms turn Wye into wildlife death trap. Guardian 20 June. Available at: https://www.theguardian.com/environment/2020/jun/20/its-like-pea-soup-poultry-farms-turn-wye-into-wildlife-death-trap [Accessed: 10 June 2023].

Mebane, C., Ray, A. and Marcarelli, A. 2021. Nutrient limitation of algae and macrophytes in streams: Integrating laboratory bioassays, field experiments, and field data. *PlosOne* 16(6), Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8213151. [Accessed 29 April 2024].

Natural Resources Wales, The Environment Agency and Natural England. 2021. *River Wye SAC nutrient management action plan.* Available at: https://www.herefordshire.gov.uk/downloads/file/23069/river-wye-sac-nutrient-management-plan-phosphate-action-plan-november-2021. [Accessed: 10th April 2024].

Oborne, A., Brooker, M. and Edwards, R. 1980. The chemistry of the river Wye. *Journal of Hydrology* 45, pp. 233-252. doi10.1016/0022-1694(80)90022-0.

Oliveira, C. Y. B., Oliveira, C. D. L., Prasad, R., Ong, H., Araujo, E., Shabnam, N. and Galvez, A. 2021. A multidisciplinary review of *Tetradesmus obliques*: a microalga suitable for large-scale biomass production and emerging environmental applications. *Reviews in Aquaculture* 13(3), pp. 1594-1618. doi:10.1111/raq.12536.

Shi, L., Cai, Y., Kong, F. and Yu, Y. 2011. Changes in abundance and community structure of bacteria associated with buoyant Microcystis colonies during the decline of cyanobacterial bloom (autumn-winter transition). International Journal of Limnology 47, pp. 355-362. doi:10.1051/limn/2011047.

Thomas, A., Nguyen, P., Howard, J., Goldberg, C. and Jentoft, S. 2019. A self-preserving partially biodegradable eDNA filter. *Methods in Ecology and Evolution* 10(1), pp. 1136-1141. doi: 10.1111/2041-210X.13212

Withers, P., Rothwell, S., Forber, K. and Lyon, C. 2022. *RePhokus: re-focusing phosphorus use in the Wye catchment.* Available at: https://councillors.herefordshire.gov.uk/documents/s50101856/RePhoKUs_Wye_Report_310522.pdf [Accessed: 10th April 2024].

WHO, 1999. Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. WHO, New York.

Wynne, M. and Hallan, J. 2015 Reinstatement of *Tetradesmus* G. M. Smith (Sphaeropleales, Chlorophyta). *Fedde Repertorium* 1(126), PP. 83-86. doi: 10.1002/fedr.201500021.

**Appendix – Overview of methods used.**

Samples were collected fortnightly from 14 sites within the Wye catchment (Table 1, Figure 1.) between June and November 2022 and 2023. Paired samples were collected at each sample location and time point to enable chemical nutrient analysis and environmental DNA (eDNA) analysis. Water for chemical analysis was collected in 500ml wide mouth polypropylene bottles from the surface waters at each site, then immediately stored at 4°C.

**Phosphate analysis**

All samples were analysed to determine the levels of orthophosphate (OP), total phosphorus (TP), condensed phosphates (Con-P) and organic phosphorus (Org-P). Sample OP was quantified using the ammonium molybdate method for the determination of phosphorus as described in Standard Methods for the Examination of Water and Wastewater, 22nd edition (Rice et al., 2012). To determine Con-P samples were digested for 30mins in water at 100°C with 3M sulfuric acid before undergoing the ammonium molybdate method. TP was determined by digesting samples with potassium persulfate at 15 psi and 121°C for 60 minutes and subsequently subjected to the ammonium molybdate method. Org-P was determined by subtracting the OP and Con-P fractions from the TP value for each sample.

**Nitrogen Analysis**

All samples were analysed to determine the levels of total nitrogen (TN), nitrite ($NO_2$), and nitrate ($NO_3$). Sample nitrite concentrations was determined by subjecting samples to colorimetric analysis using the Griess Assay as described in Standard Methods for the Examination of Water and Wastewater, 22nd edition (Rice et al., 2012). To determine nitrate concentrations, samples were first diluted 1 in 3 before enzymatic digestion to nitrite utilising nitrate reductase. Samples then underwent the Griess assay, and sample nitrite concentration subtracted to reveal final sample nitrate concentrations. TN was determined by digesting samples with potassium persulfate at 15 psi and 121°C for 60 minutes, converting all N species present to nitrite, before being subjected to the Griess assay.

**eDNA metabarcoding analysis**

Prior to sampling glass collection bottles were sterilised by autoclaving them at 121°C for 60 minutes. Samples were collected at each sampling location and immediately stored at 4°C. . Following completion of field work and upon return to Cardiff University, samples were filtered using individual Smith-Root 1.2µm self-preserving eDNA filters (Thomas et al., 2019) and a Smith-Root citizen science diaphragm air pump. Filters were then stored in the dark at room temperature until processing.

**DNA extraction**

Whilst using sterile methods, each filter was removed from its casing and cut into two pieces; one half being placed in a sterile 50ml falcon tube for archiving and storage, and the other half was cut into small pieces before being transferred to a sterile 15ml falcon tube. 460ul of ATL buffer (Qiagen) and 40ul of Proteinase K were added to each 15ml sample tube, each tube was vortexed, briefly centrifuged, before being incubated in a water bath for 30 minutes at 56°C. Samples were then placed into storage at -20ºC overnight. Each sample was thawed in a water bath for 1 minute at 65ºC before being flash frozen by being dipped into liquid nitrogen for 10 seconds. Samples were again thawed in the water bath at 65ºC before being cooled for being placed in ice for 2 minutes to cool. 0.4ml of 1.0mm and 0.1mm beads (Thistle scientific LTD, UK) were added to each sample, followed by 800ul of extraction buffer (5M NaCl, 3mM NaEDTA and 70mM tris), 800ul chloroform and 100ul of 10% DTAB. Each sample was twice subjected to bead beating at 5m/s for 30 seconds with a 5-minute rest step in between. Samples were centrifuged at 4250rpm for 10 minutes, before the supernatant was removed to a sterile 15 ml Falcon tube, and equal parts Buffer AL (Qiagen) and 100 % ethanol were added to it. Following a brief vortex, all samples were passed through DNA mini spin columns (Qiagen), being centrifuged for 1 minute at 14,000 rpm. Subsequently, the remaining extraction steps followed those outlined in the DNeasy Blood and Tissue.