Aquaterra

Environmental DNA monitoring

A not-for-profit citizen science organisation supported by the Darwin Tree of Life Consortium

River Ivel eDNA baseline survey

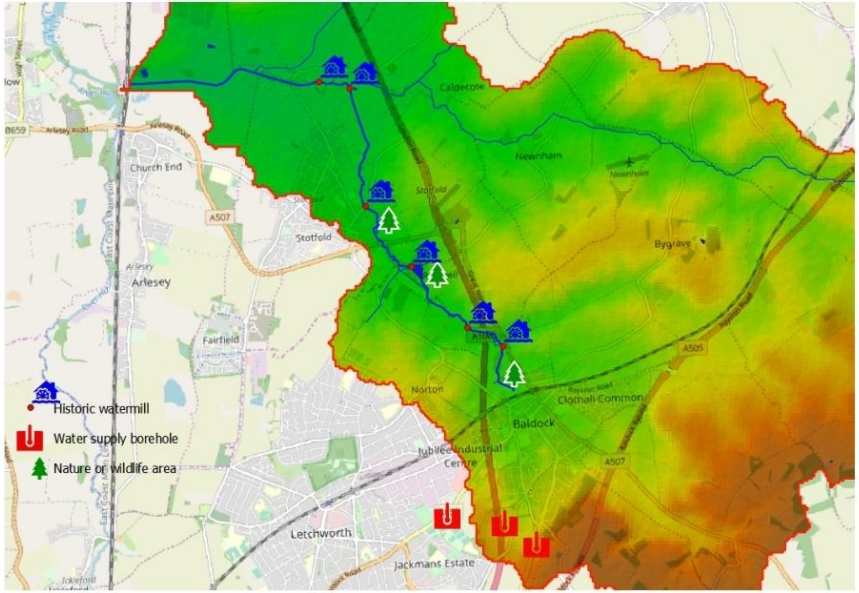
Funded by University of Hertfordshire

Why do we exist?

The upper reaches of the River Ivel are dying. We are a volunteer-based community organisation and we intend to do something about it, by informing and educating the local population, raising awareness of the sources of the problem and tackling the issues directly wherever we can.

The source of the Ivel is just north of Baldock. It flows through Hertfordshire and Bedfordshire joining the River Ouse at Tempsford before flowing into the North Sea north of Kings Lynn.

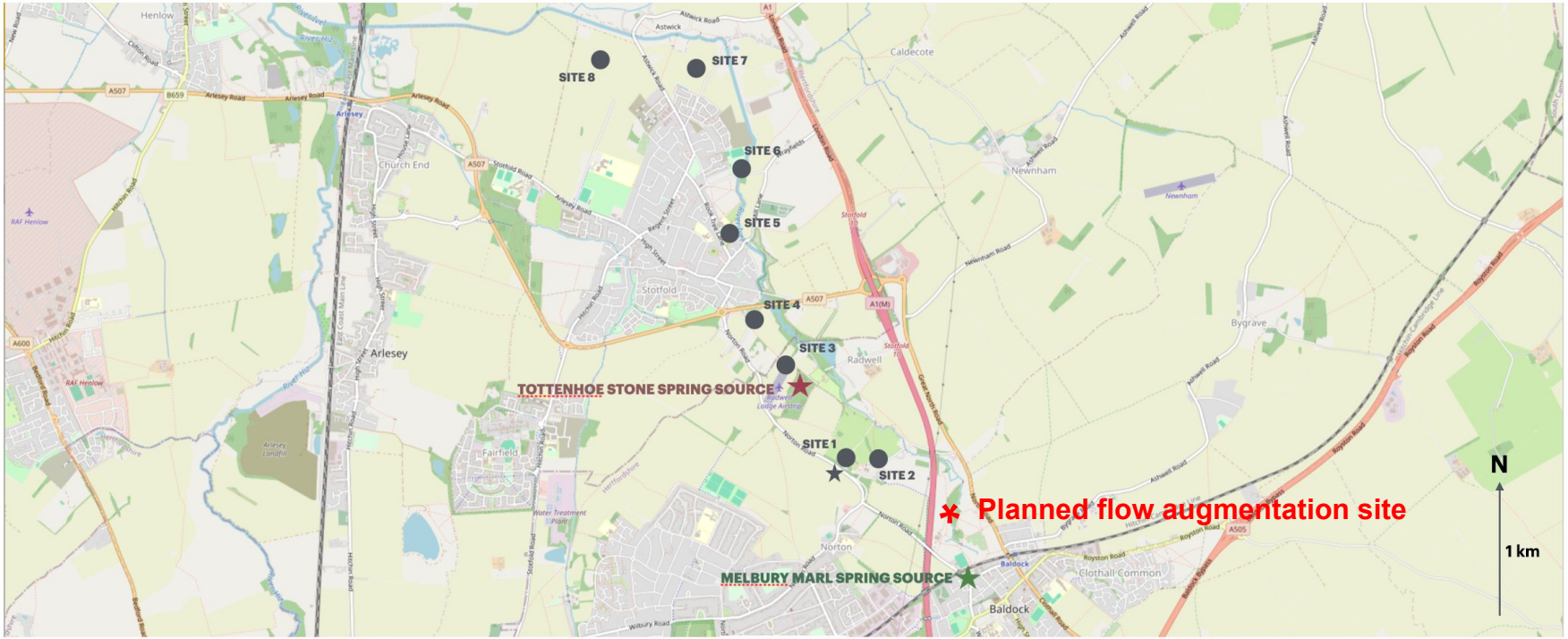
Our goal is for the restoration of sufficient flow in the upper River Ivel to sustain brown trout all year round.



ABOUT US →

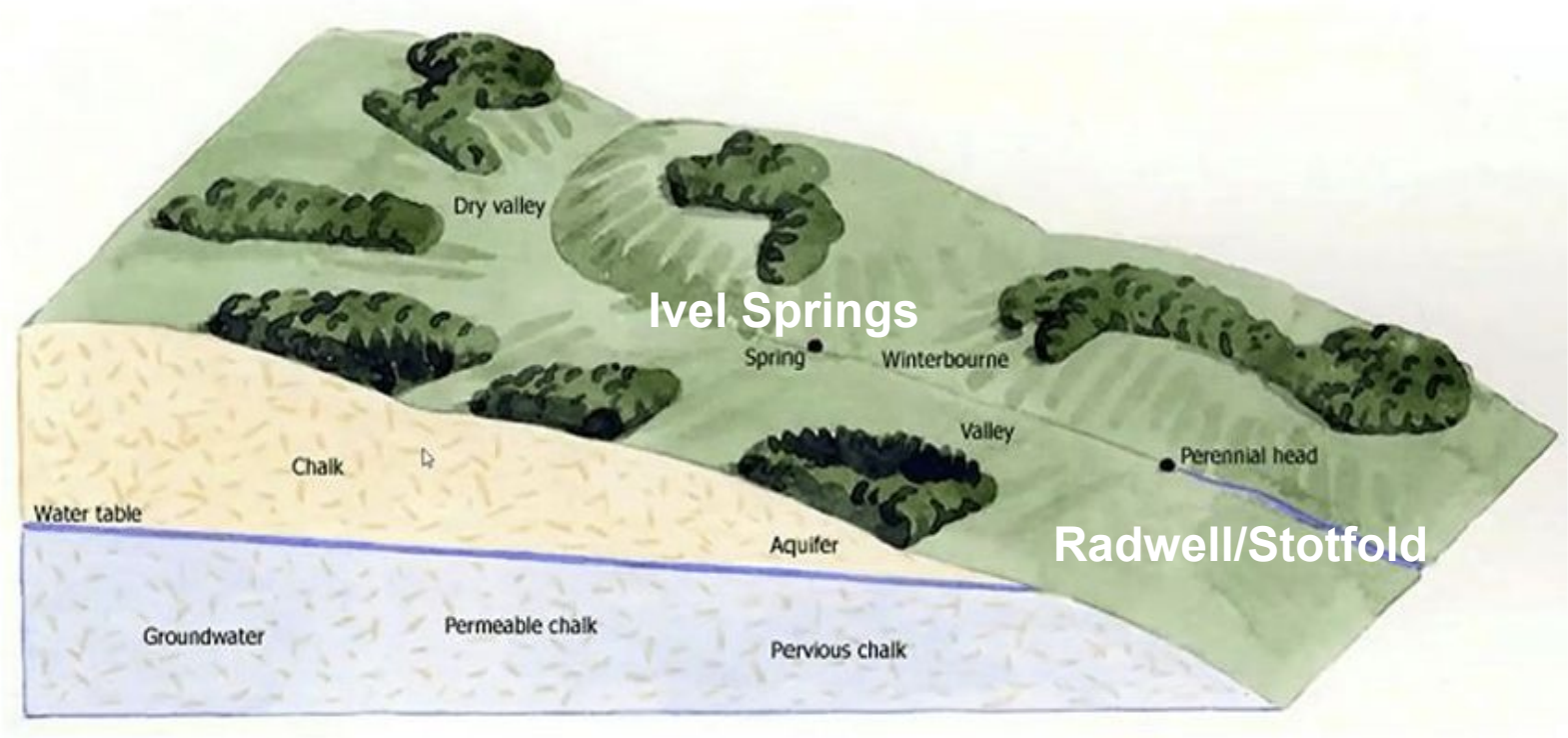
One of hundreds of local community conservation groups across the UK

River Ivel Project Location Map



Uppermost section of river (sites 1, 2) is stressed due to overabstraction - aquifer is dry. Further down stream additional aquifer springs augment river flow.
This was a baseline survey ahead of a flow augmentation project (now in progress)

Upper Ivel Schematic



END TO END WORKFLOW

Can be conducted by anyone over ~16 years old

Can be conducted by anyone with a STEM A-level

To be evaluated

Sampling

Key info

0.45um Sterivex filters

>200ml of representative water at each site. Sample different parts of the river to maximise diversity

1-2 days

Labwork

Key info

Qiagen B&T extraction kit
MiniPCR kit
FwhF1/FwhR2n primers

4-8 days

Sequencing

Deep Seq:

Key info

Currently send offsite to Nottingham Uni

Investigating uss of Minion to greatly speed up workflow

1 month

Bioinformatics

BRAVE

Key info

First results within hrs of receipt of FASTQ files

1 week

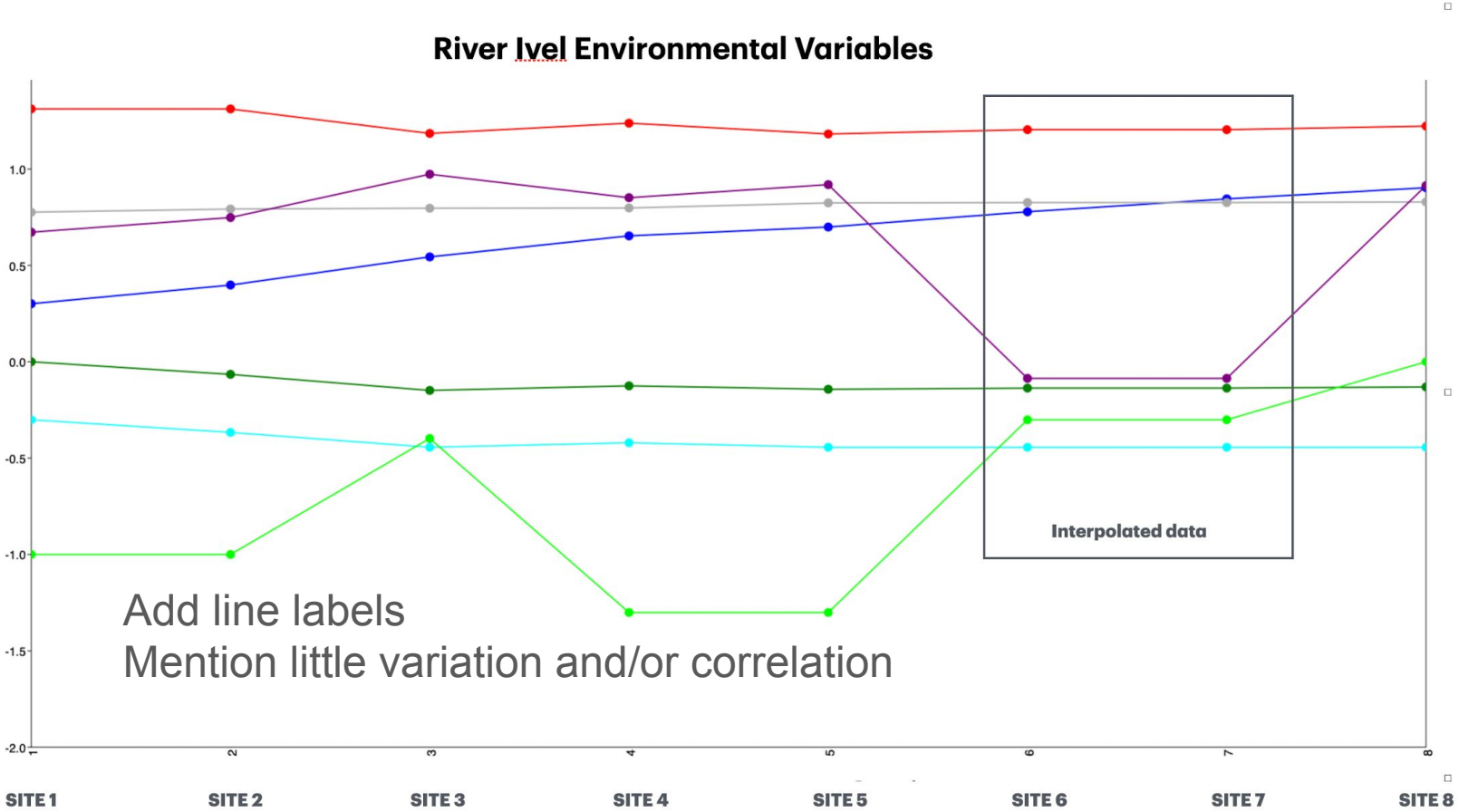
Final analysis

PAST4

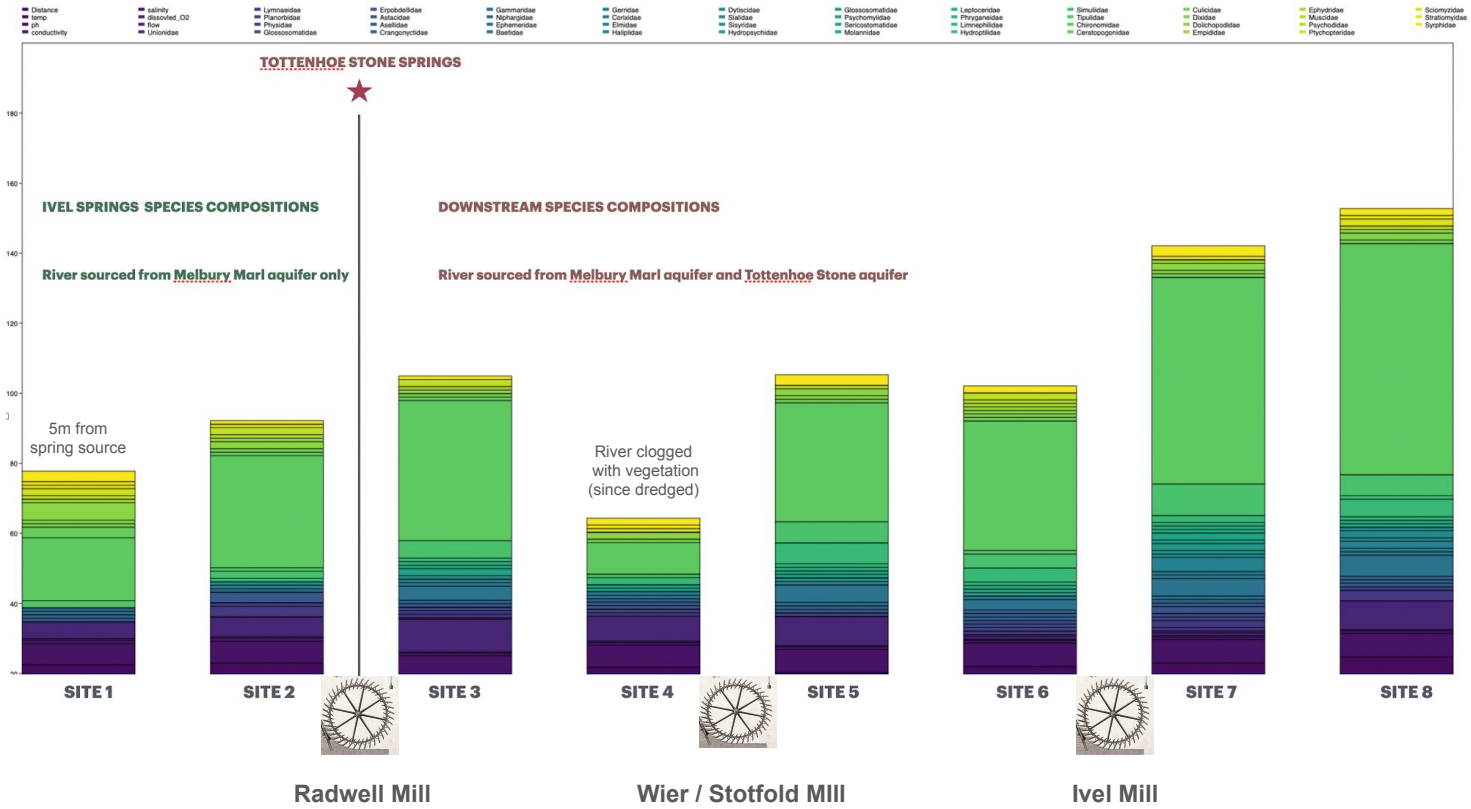
Key info

Intuitive spreadsheet based app.from NHM.

1 week


River Ivel Environmental Variables
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
1
2
3
4
5
6
7
8
Interpolated data
Add line labels
Mention little variation and/or correlation
SITE 1
SITE 2
SITE 3
SITE 4
SITE 5
SITE 6
SITE 7
SITE 8

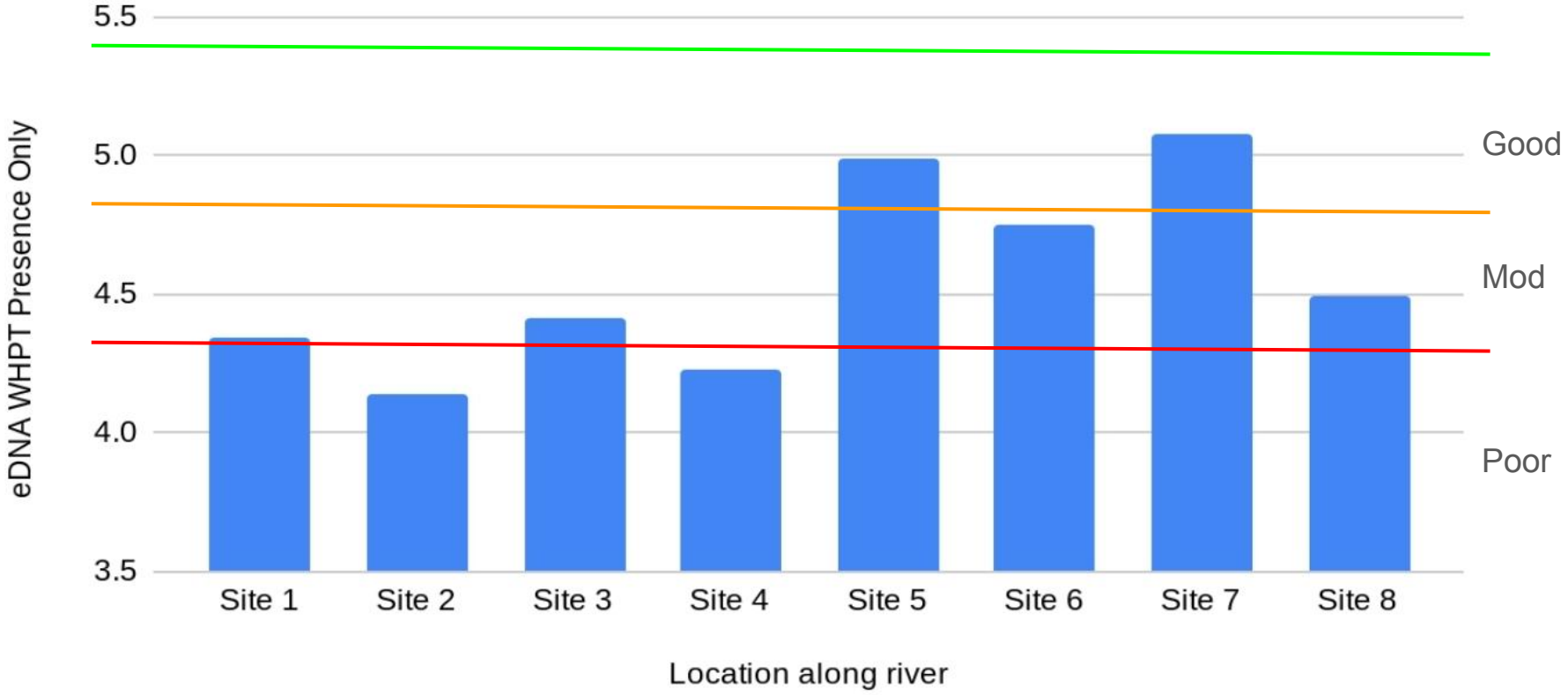
WHPT FAMILY COMPOSITION PROFILE



eDNA WHPT Presence Only

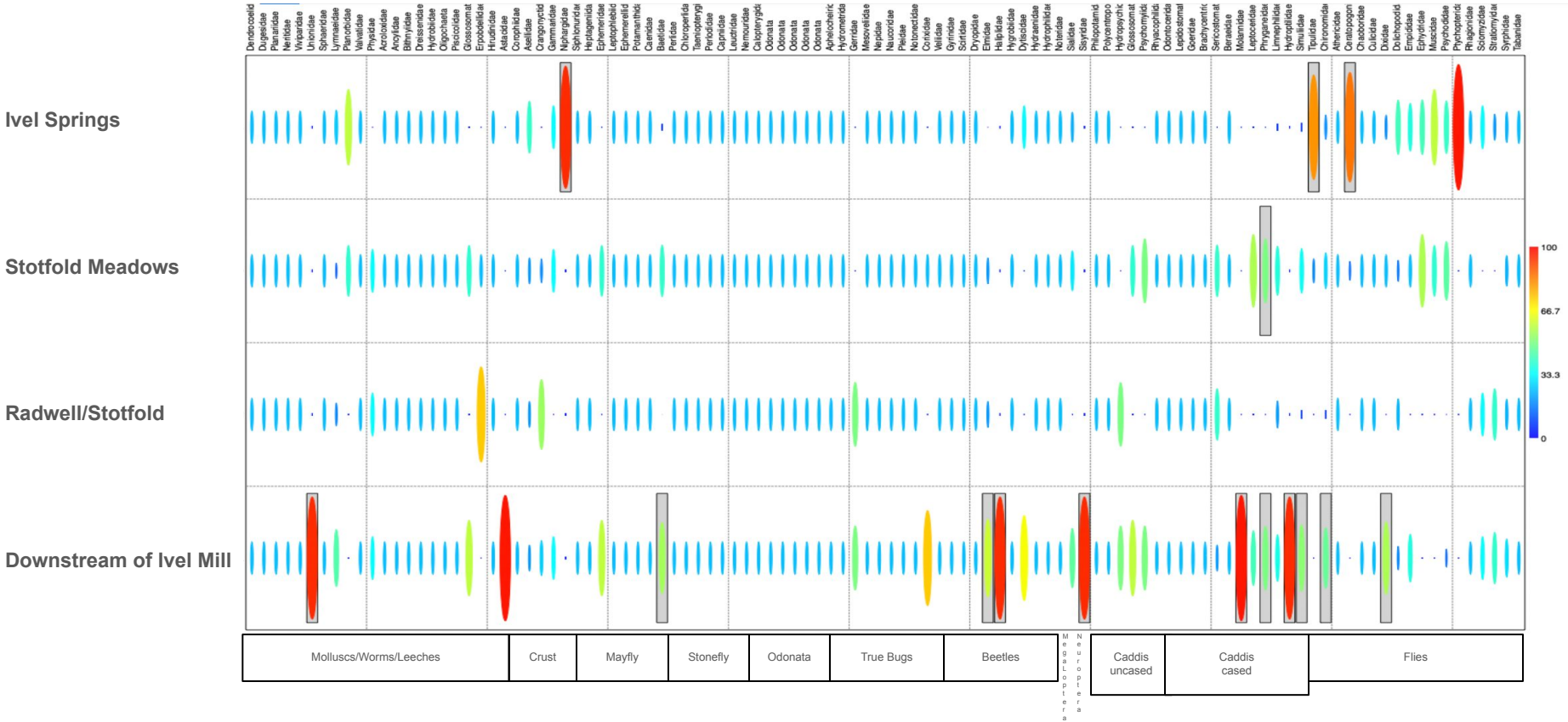
Proxy for WHPT ASPT

ASPT	Interpretation
<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



Upper Ivel characteristic WHPT taxa

Statistically significant



Upper Ivel characteristic WHPT taxa

Significant
Statistically significant

October 15th 2023

IVEL SPRINGS

Niphargidae

Subterranean amphipod

Tipulidae

Crane flies

Ptychoptera

Phantom crane Flies

Ceratopogonidae

Biting midge

RADWELL / STOTFOLD

Phryganeidae

Ephemeridae

Glossomatidae

Caddis flies

STOTFOLD MEADOWS

Erpobdellidae

Leeches

DOWNSTREAM OF IVEL MILL

Astacidae

Unionidae

Elmidae

Haliplidae

Baetidae

40% higher diversity

Sisyridae

Molannidae

Hydroptilidae

Phryganeidae

Dixidae

Chironomidae

Ephemeridae

Glossomatidae

Corixidae

Simuliidae

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Significant
Statistically significant

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STOTFOLD MEADOWS

Erpobdellidae

Leeches

DOWNSTREAM OF IVEL MILL

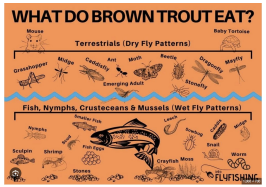
Astacidae

Unionidae

Elmidae

Haliplidae

Baetidae



Sisyiridae

Molannidae

Hydroptilidae

Phryganeidae

Dixidae

Chironomidae

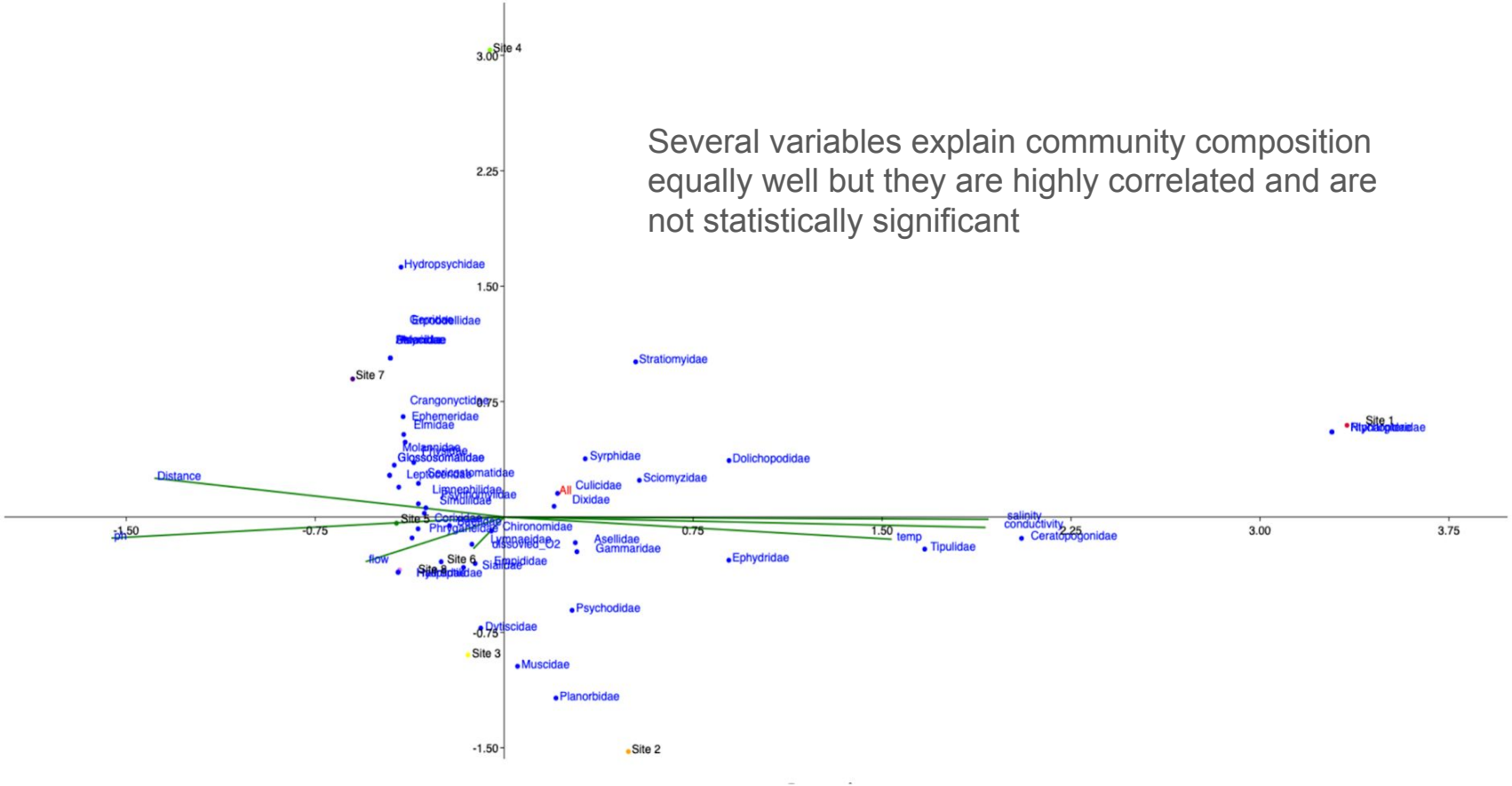
Ephemeridae

Glossomatidae

Corixidae

Simuliidae

CCA coordination plot



Several variables explain community composition equally well but they are highly correlated and are not statistically significant

Project successes:

User friendly end to end workflow. First to combine these freely available tools.

Open access “packaged pipelines” (mBRAVE and PAST4). No need for command level programming.

Quite easy to generate WHPT Presence-Only profiles along the river.

Able to demonstrate that upper and lower rivers had statistically significant differences in community composition

Able to identify characteristic taxa for different river flow regimes

Able to identify the environmental factors that most explain taxa composition variation although they are were not statiscally insignificant

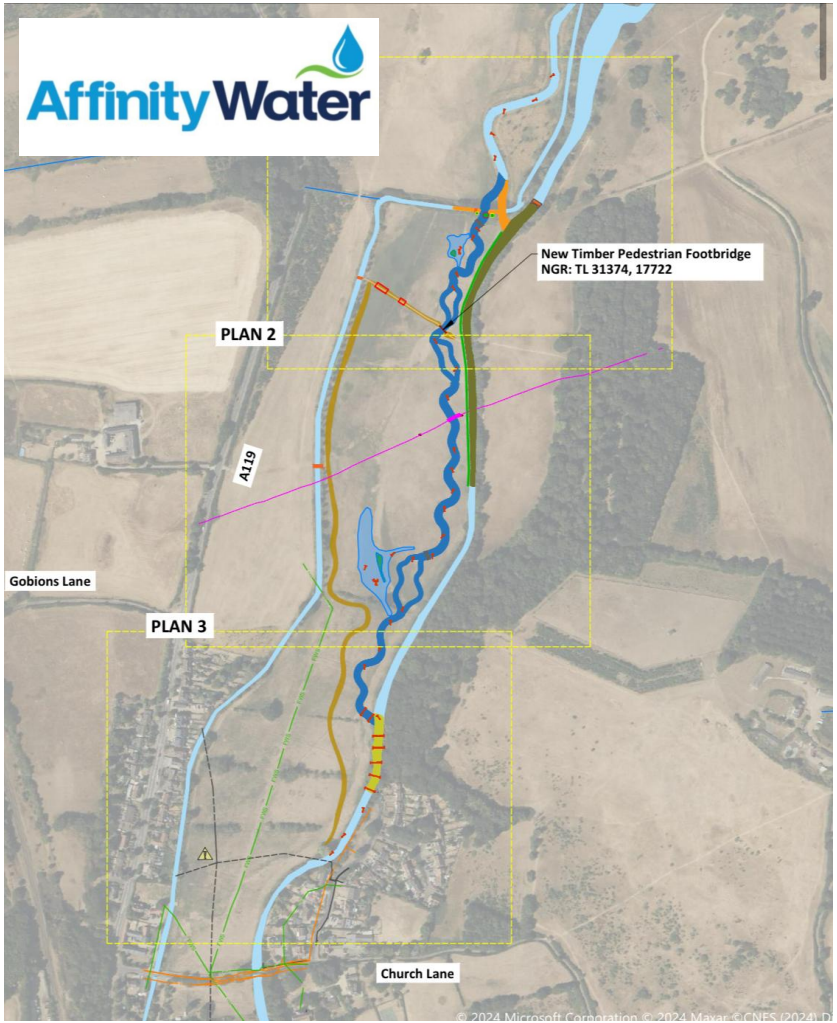
Areas for improvement :

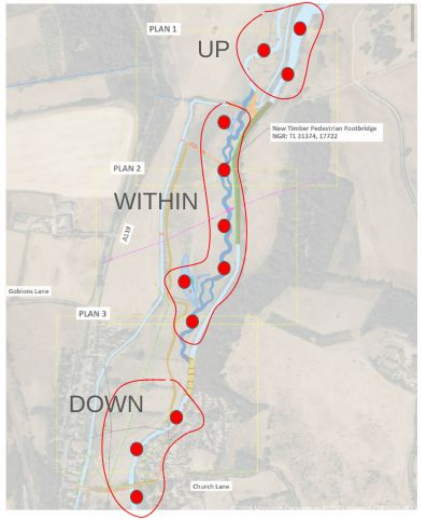
There may be better ways to utilise the data than WHPT presence only analysis but it seemed to be a good framework to start with.

Contamination was present from a terrestrial investrebrate study the week before. By focussing on WHTP family analysis the data could still be used although the sequencing depth of the aquatic taxa DNA will have been highly compromised.

Next time we will collect more environmental information. Marcophyte diversity and nutrient levels to name just two which may help to explain composition variation.

In 2025/26 we plan to build on this by conducting baseline and repeat surveys on a section of thr River Beane which is being restored (see overpage).





eDNA Survey Design

12 sites upstream, within and downstream of new channel and adjacent wetlands

April/May 2025

eDNA all sites plus three Riverfly sites

April/May 2026

eDNA all sites plus three Riverfly sites

Study Hypothesis

eDNA collected and analysed by community scientists can monitor invertebrate community changes resulting from river intervention projects

Welcome to the website of the Lea Catchment Partnerships – bringing together people and projects for the benefit of the River Lea and its tributaries.

The River Lea and its tributaries - the Mimram, Beane, Ash, Rib, Quin and Stort - drain over 1000 km² of south-east England. These rivers include rare and precious chalk streams, canals and navigations, and urban watercourses. To find out more about the rivers, the catchment partnerships, and to view the catchment plans, please navigate using the map opposite or the menu bars above.

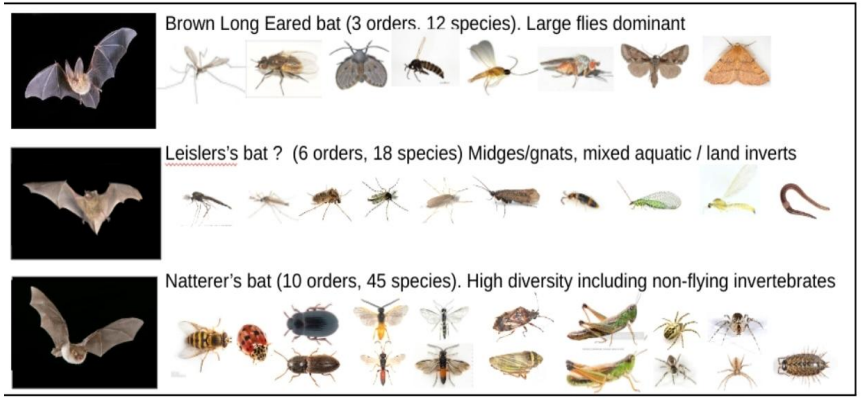
A fantastic citizen science scheme helping local volunteers monitor the water quality of their local river

The Rivers Trust

The Riverfly Partnership

sanger institute
Earlham Institute
EMBL-EBI
UNIVERSITY OF OXFORD
THE UNIVERSITY of EDINBURGH
Marine Biological Association
UNIVERSITY OF CAMBRIDGE
NATURAL HISTORY MUSEUM
Royal Botanic Garden Edinburgh
Kew

Other 2023/4 projects



igure 4 : Summary of the three wild bat species and their prey (full taxa lists in Appendix A)

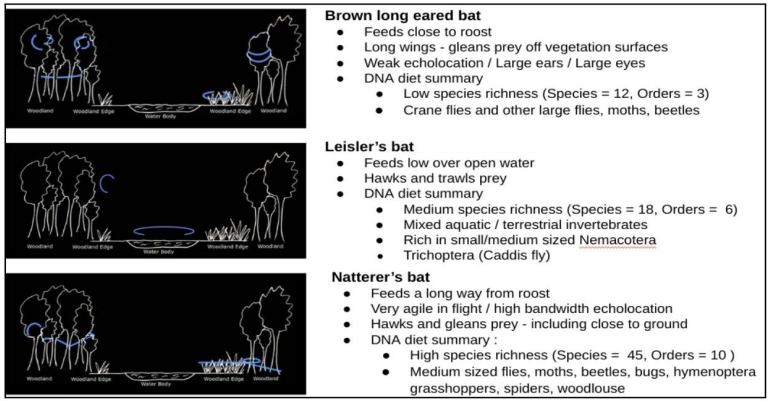
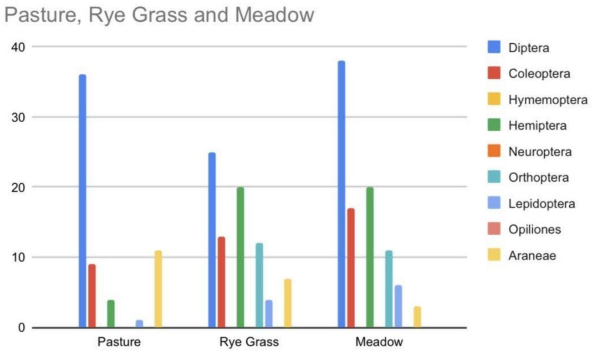


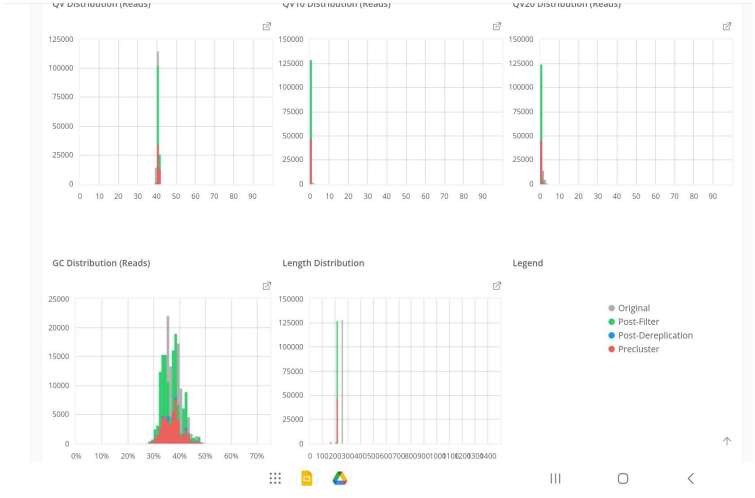
Figure 5 : Bat species foraging behaviour and diet summary from this study

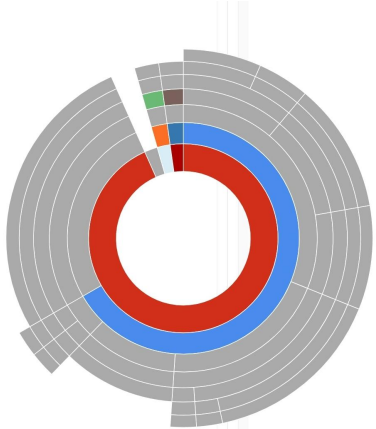


mBRAVE processing log and QC displays

Read Breakdown

	Reads	Mean Length	Mean QV	Mean GC Composition
Uploaded	129521	253.21bp	40.42	38.11%
Post Filter	128441	213.25bp	40.76	36.81%
Dereplicated	46703	213.26bp	40.76	37.11%
Preclustered	46703	213.26bp	40.76	37.12%
Reads in BINs	83625	213.76bp	n/a	36.17%
Reads in OTUs	39734	212.17bp	n/a	38.14%
Reads in Chimeras	5082	n/a	n/a	n/a





91 % of matched reads from order Arthpoda of which

65 orders
207 families
456 genera
643 species/species groups

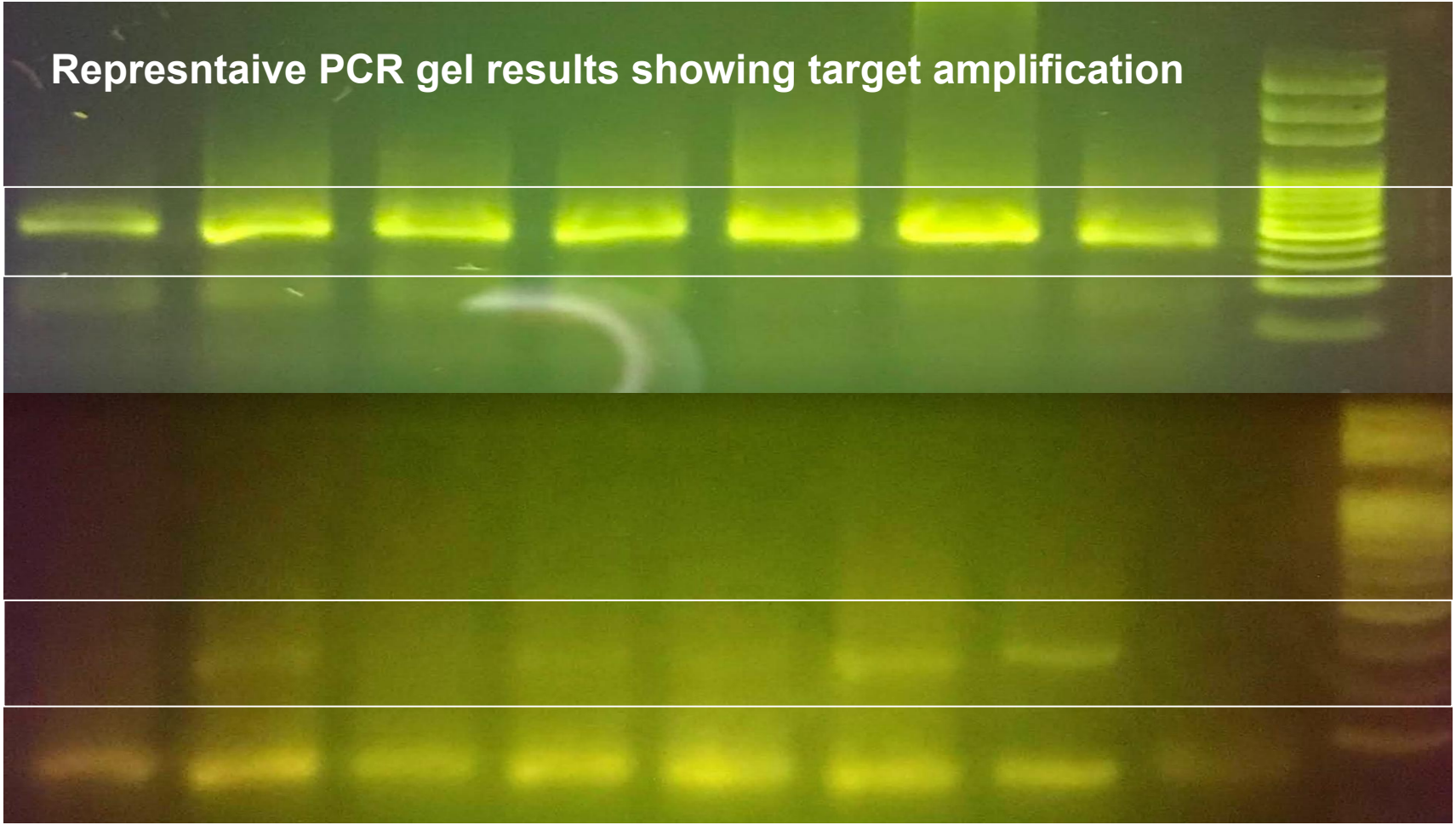
Abundant reads from several pecies not associated with water.
These were present in samples processed I the lab the day before

Chorthhipus sp

Galeruca ponoma

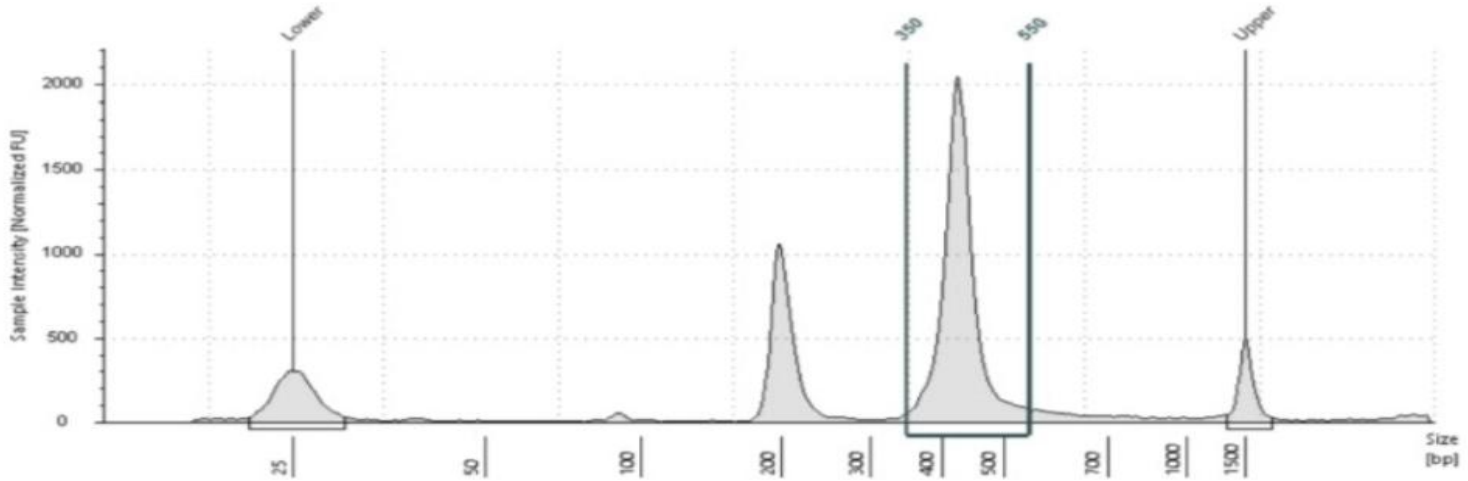
Tenebrio molitor

Represntaive PCR gel results showing target amplification



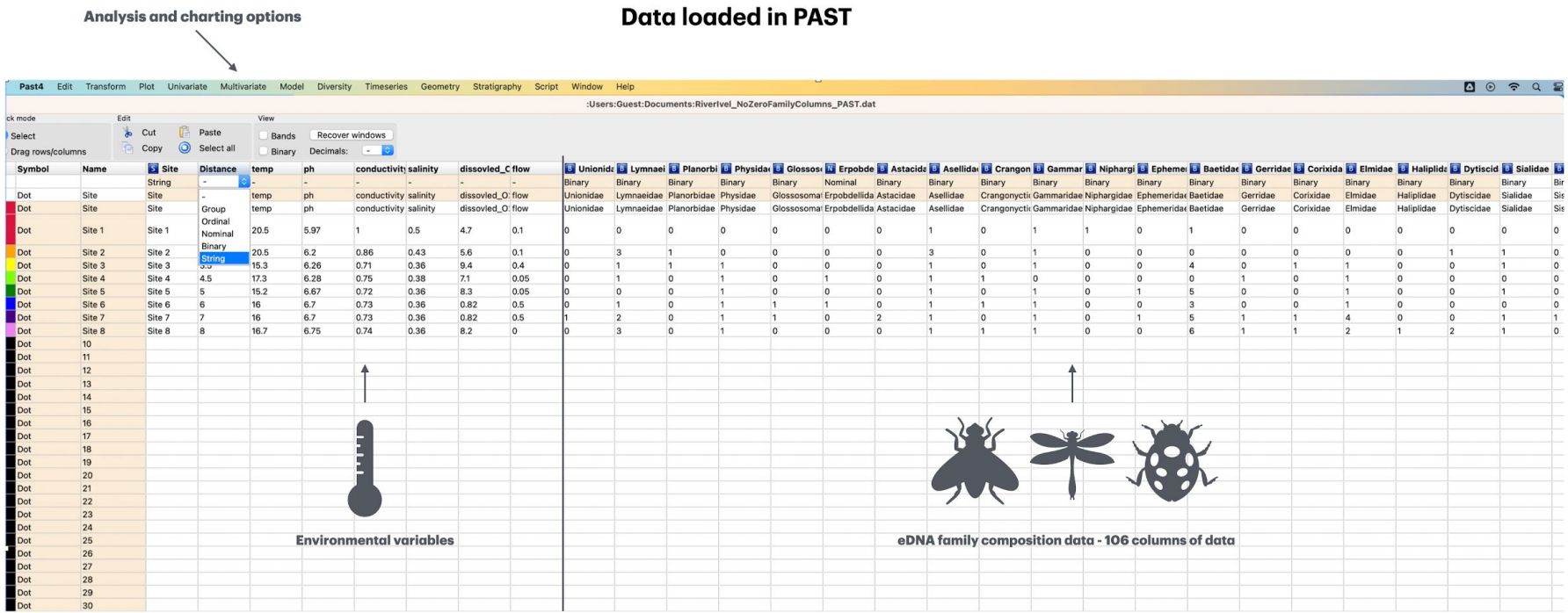
Amplicon cuts on multiplexed sample pool

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment	Observations
25	455	-	28000	-		Lower Marker
198	803	-	6240	29.96		
429	1880	-	6740	70.04		
1500	250	250	256	-		Upper Marker



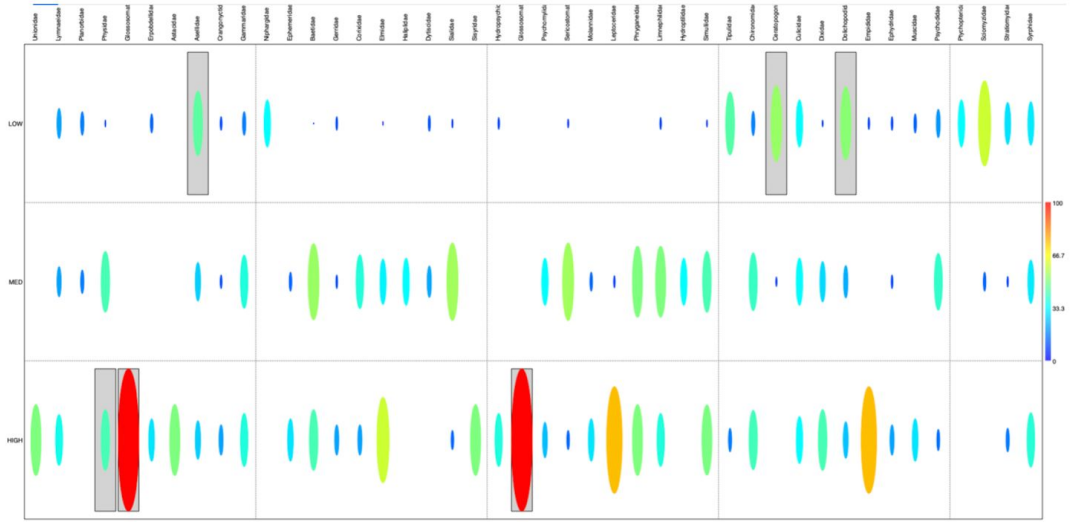
Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
350	550	432	2040	7340	62.60		



PAST4 is very user friendly once spreadsheet is set up for DNA taxa composition analysis and environmental factor hypothesis testing.

WHPT family composition analysis



ownstream ?

positions but this

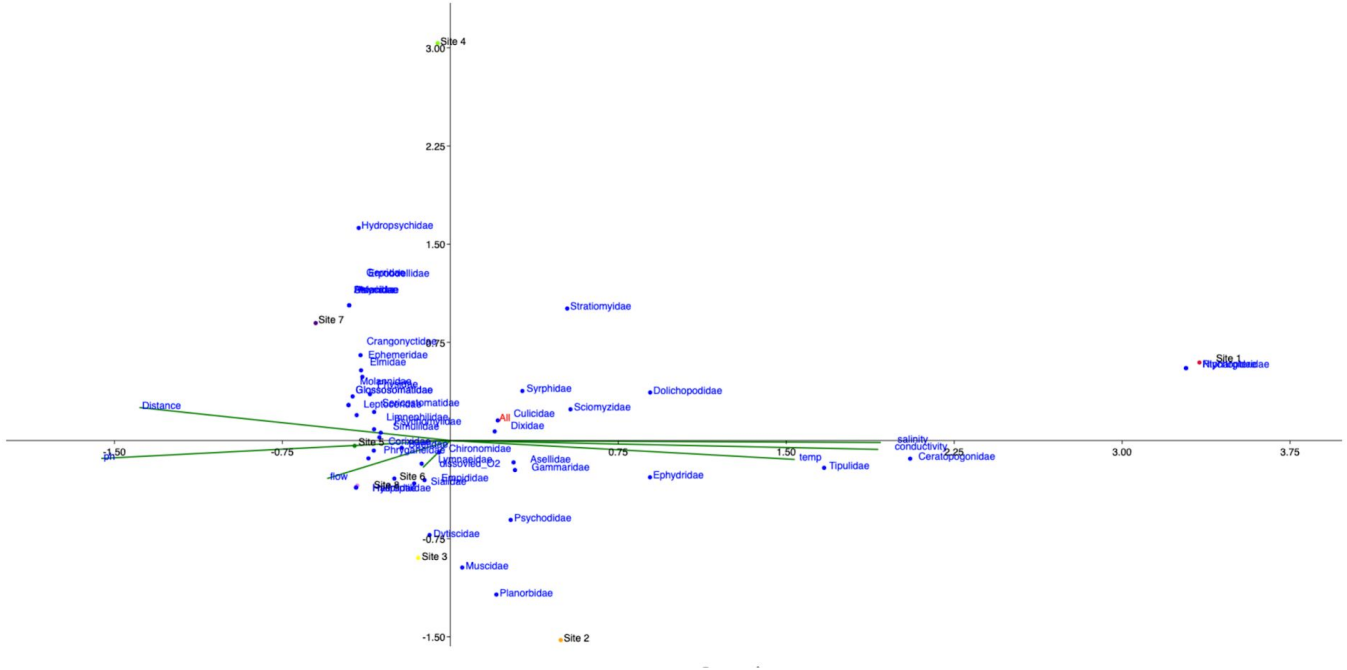
f downstream

Which families are most characteristic of different levels of River flow ?

Glossosomatidae (Caddis Fly) is a statistically significantly indicators of higher river flow

Dolicopodidae (long legged flies) and Ceratopogonidea (biting midges) are statistically significant indicators of low river flow

CCA ORDINATION PLOT (showing WHPT family clustering)

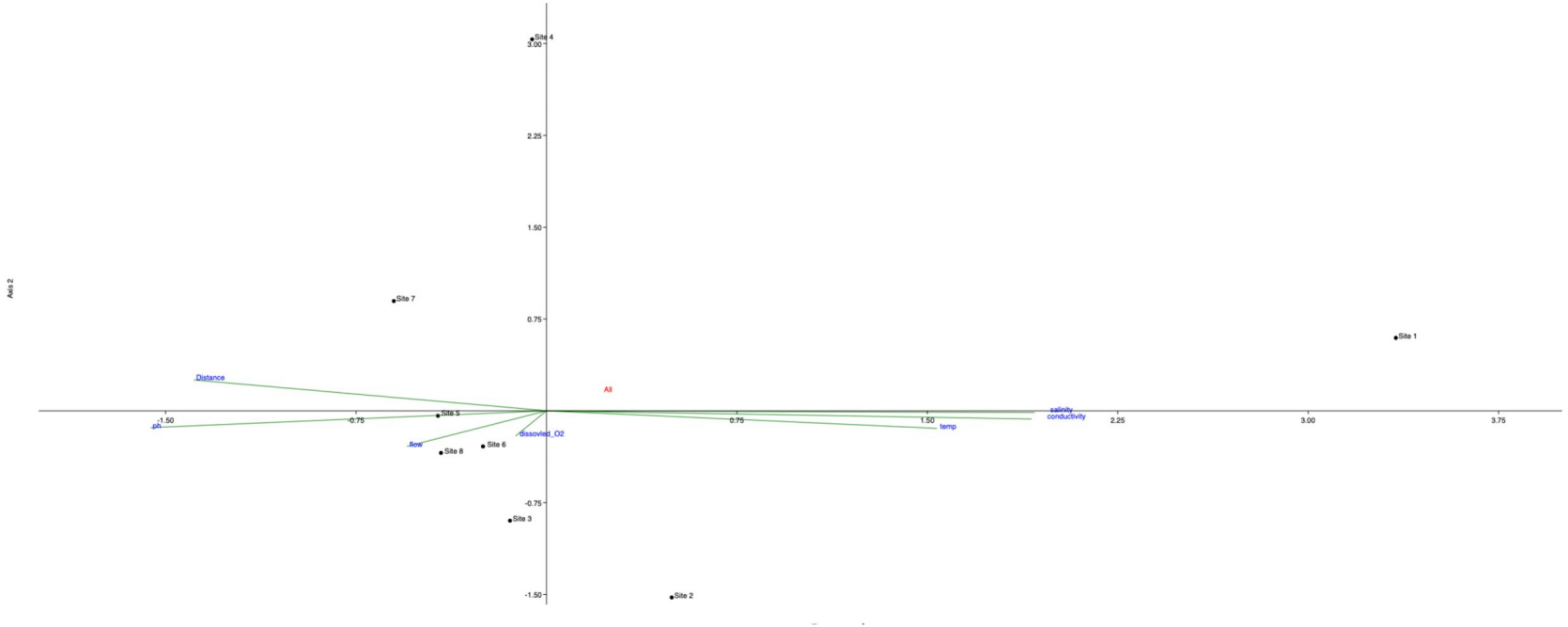


Which environmental variables, if any, are potentially impacting species composition variation (correlation does not mean causation) ?

The longest vectors are distance from source, temperature, salinity, conductivity and pH although these variables are themselves highly correlated.

Flow does not seem to significantly explain species composition variation.

CCA ORDINATION PLOT



Which environmental variables, if any, are <u>potentially</u> controlling species composition variation (correlation does not mean causation) ?

The longest vectors are 'distance from source', temperature, salinity, conductivity and pH although these variables are themselves highly correlated.

Flow does not significantly explain species variations.