

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



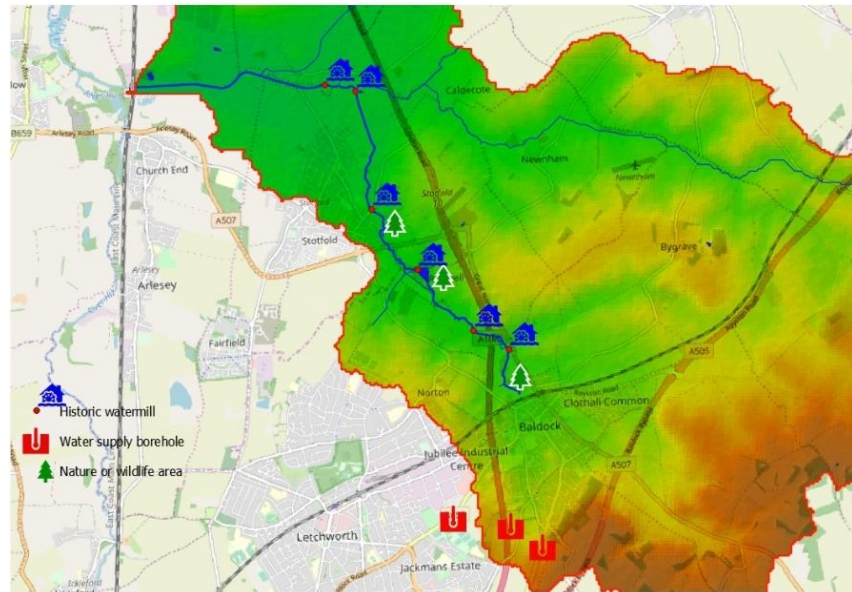
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Why do we exist?

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.

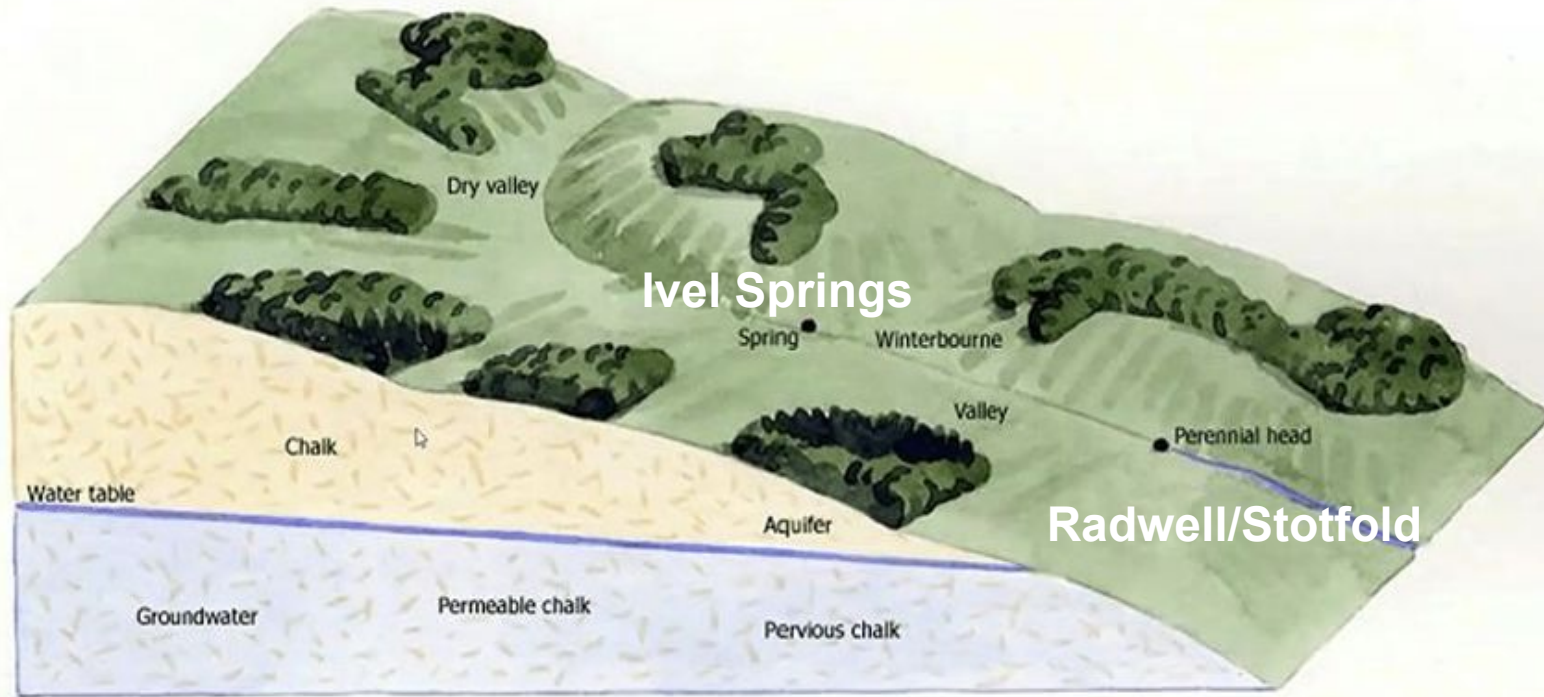
The source of the Ivel is just north of Baldock. It flows through Hertfordshire and Bedfordshire joining the River Ouse at Tempsford before flowing into the North Sea north of Kings Lynn.

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

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



Labwork



Sequencing



Deep Seq:

Bioinformatics



Final analysis



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

Key info

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

4-8 days

Key info

Currently send offsite to Nottingham Uni

Investigating use of Minion to greatly speed up workflow

1 month

Key info

First results within hrs of receipt of FASTQ files

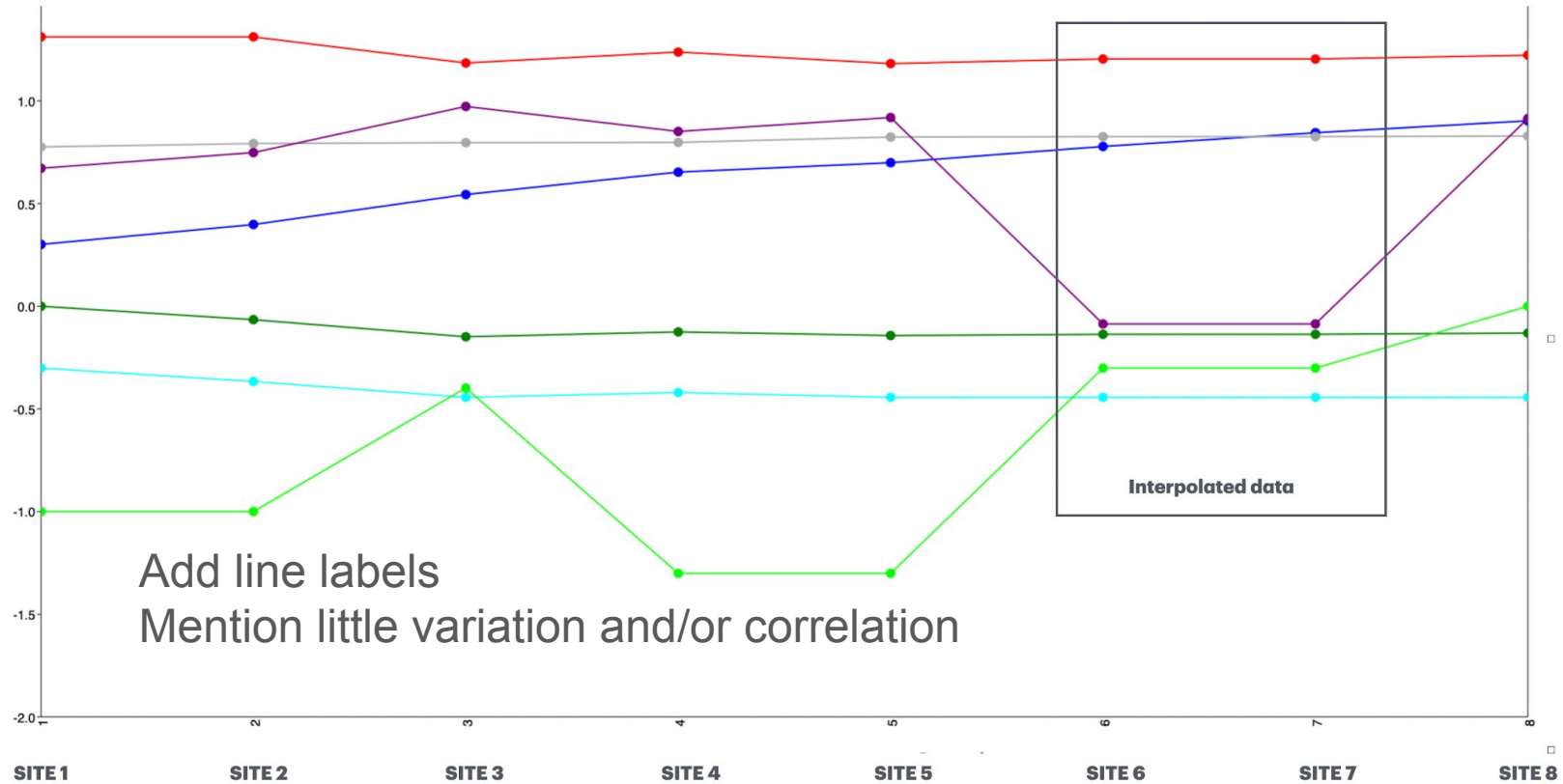
1 week

Key info

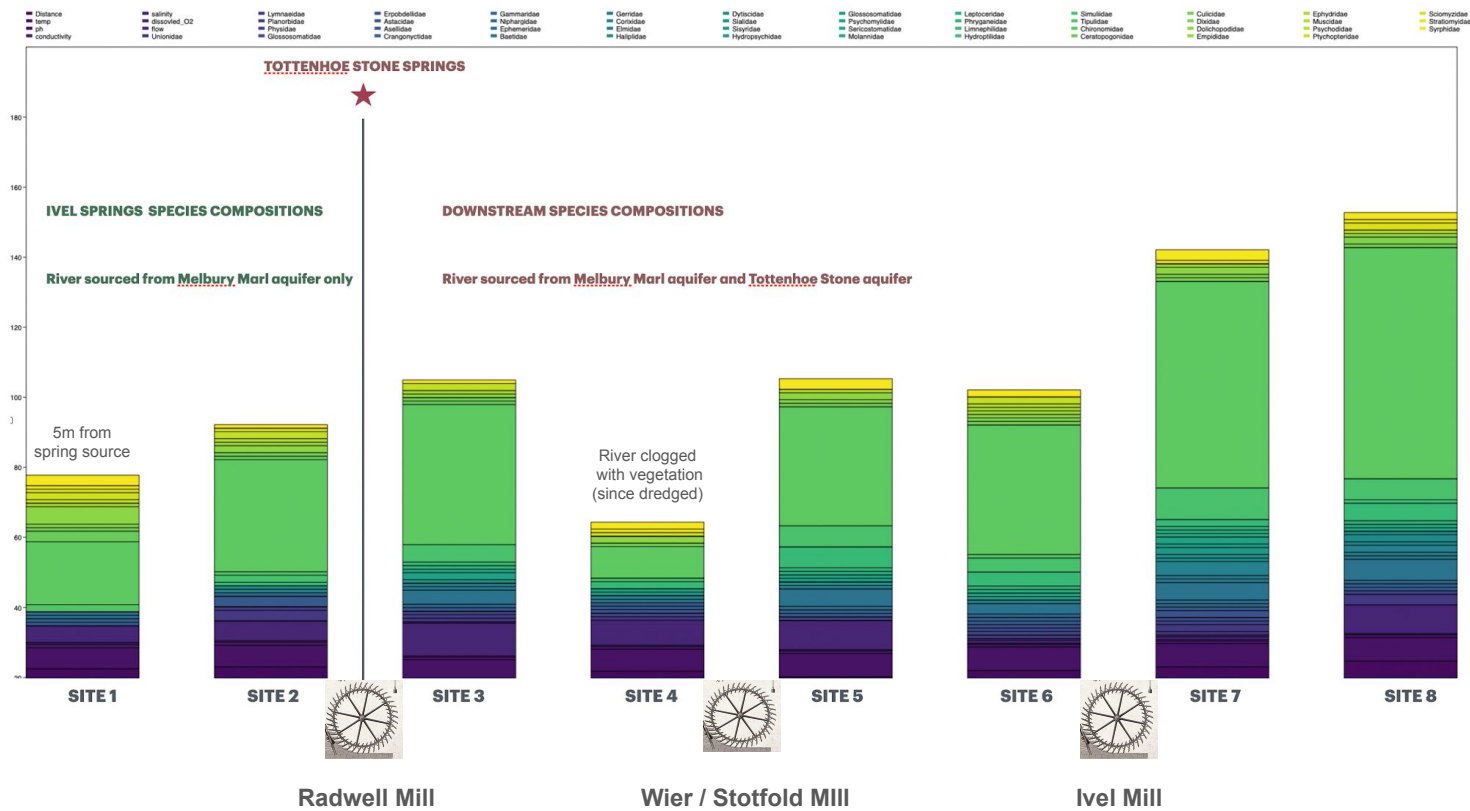
Intuitive spreadsheet based app.from NHM.

1 week

River Ivel Environmental Variables



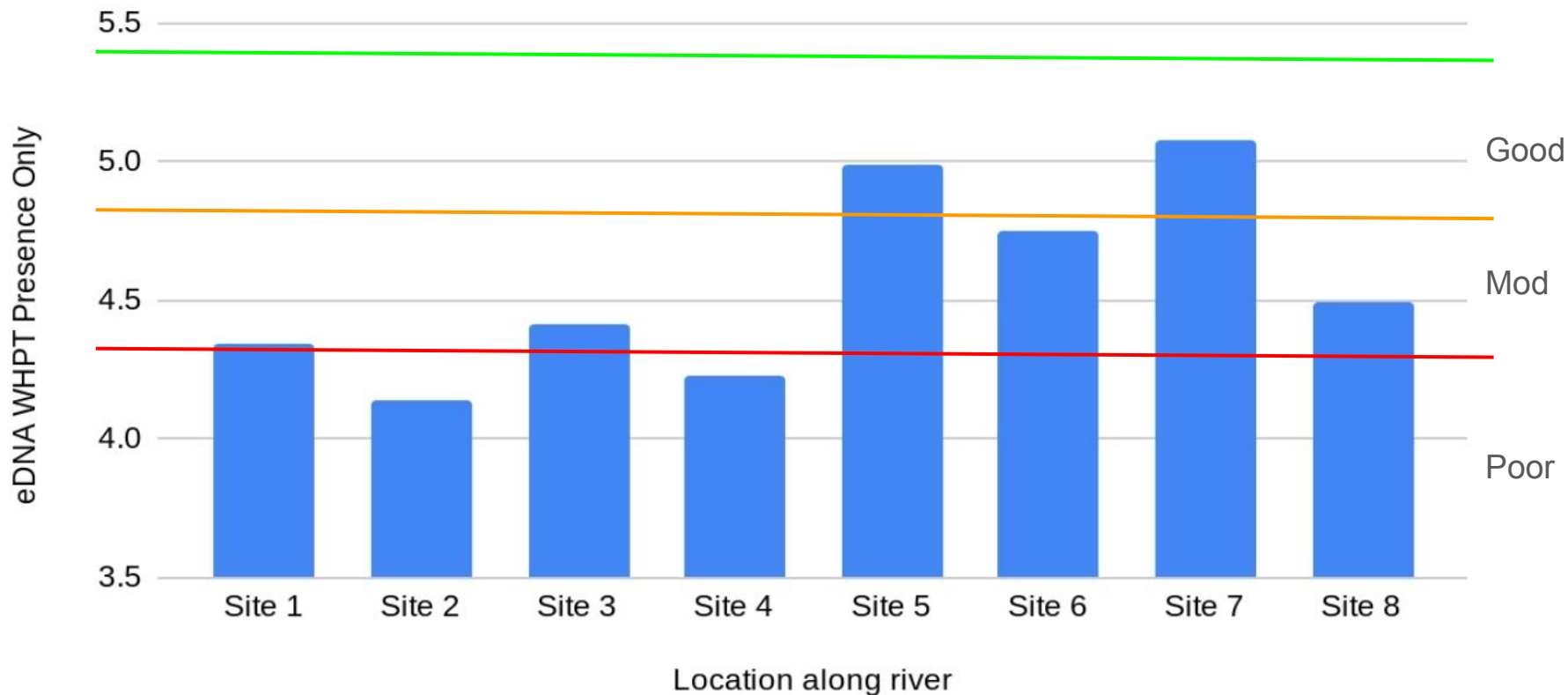
WHPT FAMILY COMPOSITION PROFILE

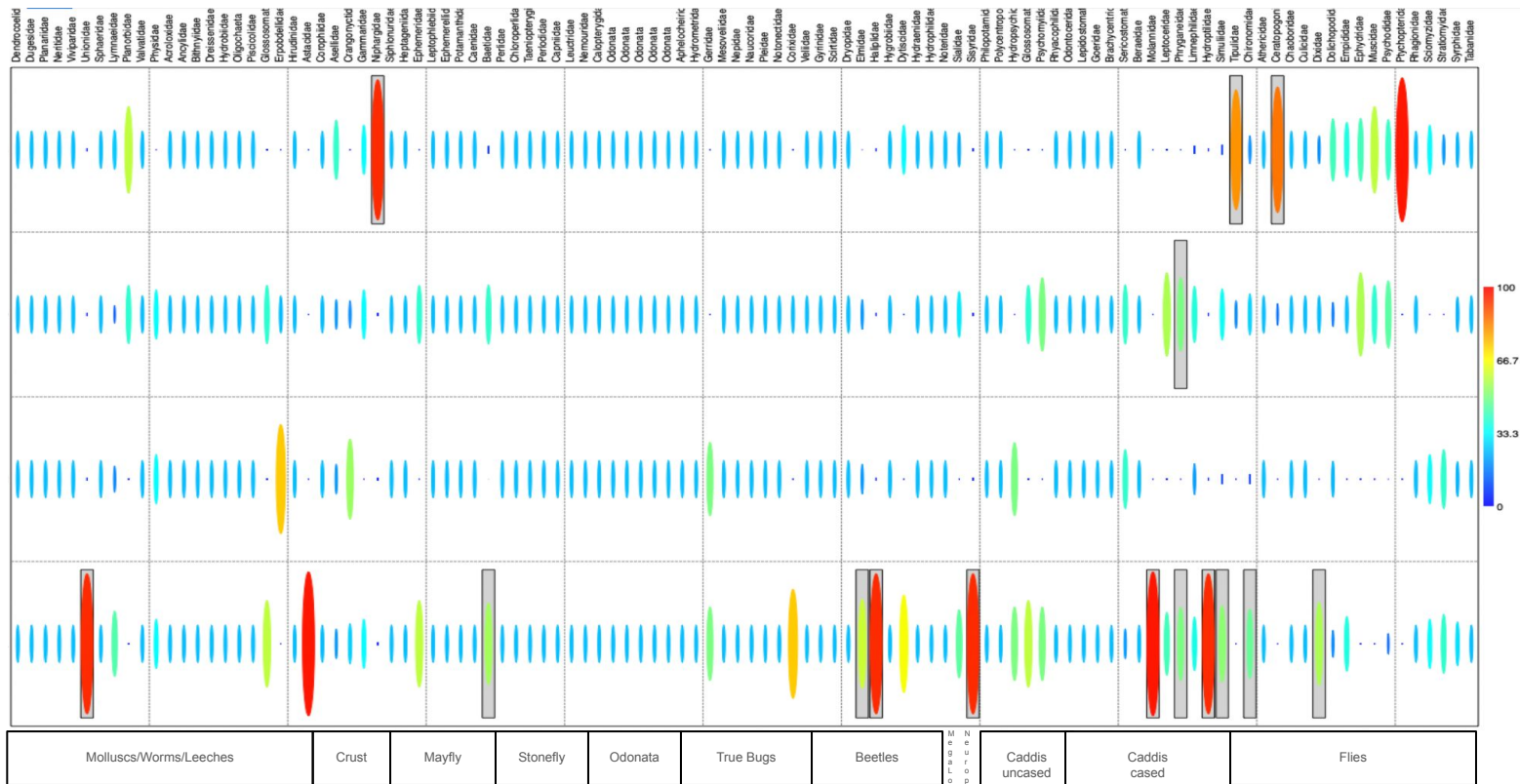


eDNA WHPT Presence Only

Proxy for WHPT ASPT

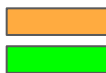
ASPT	Interpretation
<3.0	Very poor, heavily polluted
3.0-4.3	Poor, polluted or impacted
4.3-4.8	Moderate, moderately impacted
4.8-5.4	Good, clean but slightly impacted
>5.4	Very good, unpolluted, unimpacted



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Upper Ivel characteristic WHPT taxa

October 15th 2023



Significant



Statistically significant

IVEL SPRINGS

Niphargidae



Subterranean
amphipod

Tipulidae



Crane flies

Ptychoptera



Phantom crane
Flies

Ceratopogonidae



Biting midge

RADWELL / STOTFOLD

Phryganeidae



Ephemeridae



Glossomatidae



STOTFOLD MEADOWS

Erpobdellidae



Leeches

Caddis flies

DOWNSTREAM OF IVEL MILL

Astacidae



Unionidae



Elmidae



Halipidae



Baetidae

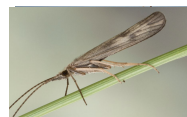


40% higher
diversity

Sisyridae



Molannidae



Hydroptilidae



Phryganeidae



Dixidae



Chironomidae



Ephemeridae



Glossomatidae



Corixidae



Simuliidae



Upper Ivel characteristic WHPT taxa

October 15th 2023



Significant



Statistically significant

IVEL SPRINGS

Niphargidae



Subterranean
amphipod

Tipulidae



Crane flies

Ptychoptera



Phantom crane
Flies

Ceratopogonidae



Biting midge

RADWELL / STOTFOLD

Phryganeidae



Ephemeridae



Glossomatidae



STOTFOLD MEADOWS

Erpobdellidae



Leeches

Caddis flies

DOWNSTREAM OF IVEL MILL

Astacidae



Unionidae



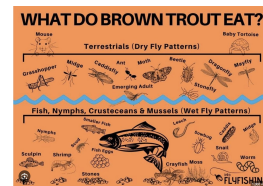
Elmidae



Halipidae



Baetidae



Hydroptilidae



Phryganeidae



Dixidae



Chironomidae



Ephemeridae



Glossomatidae



Corixidae

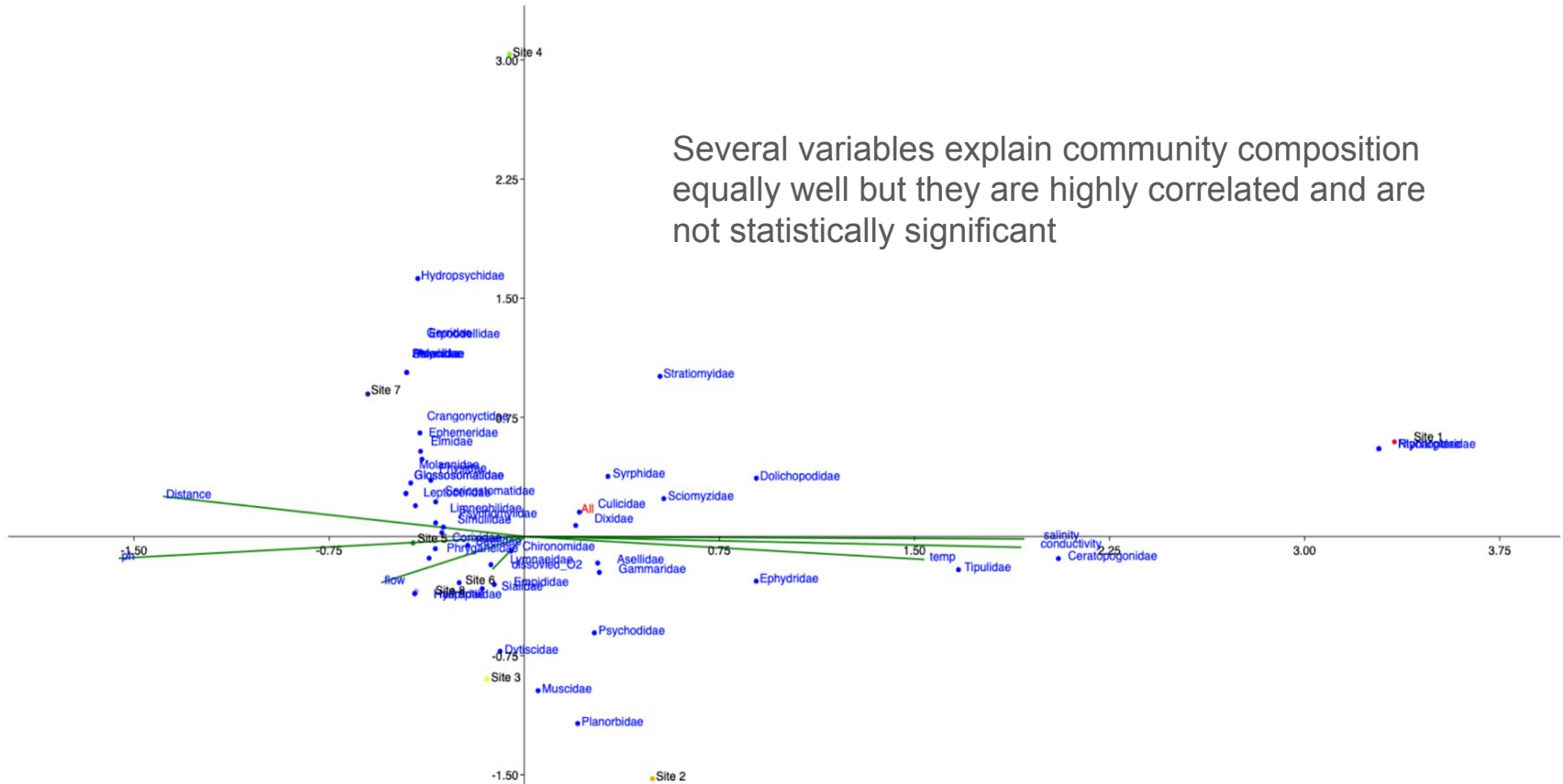


Simuliidae



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 were not statistically insignificant

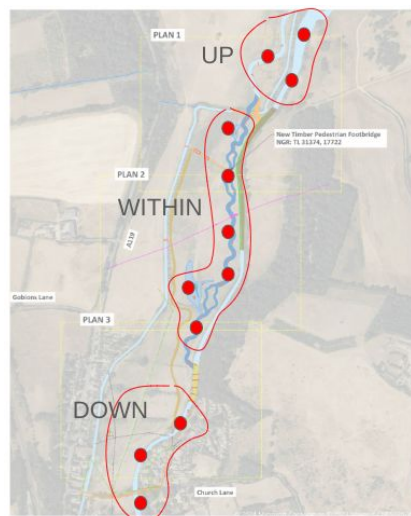
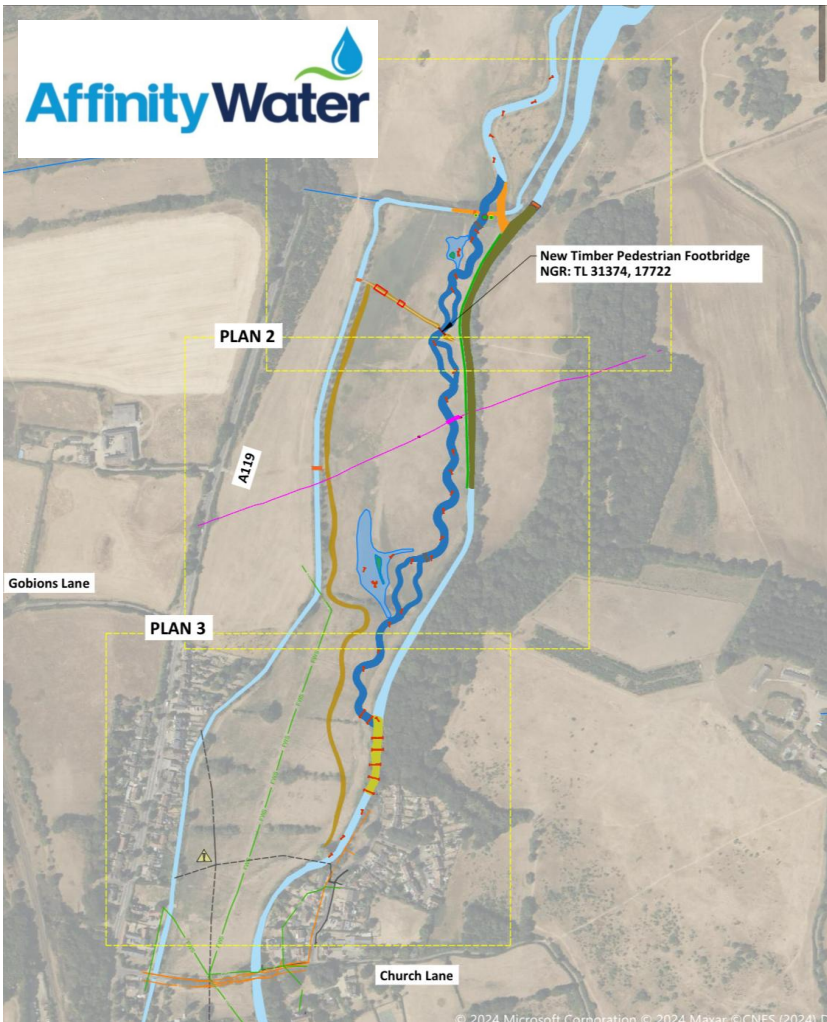
Areas for improvement :

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 invertebrate study the week before. By focussing on WHPT 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. Macrophyte 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 the 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 M4, M25, M3, M1, M6, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, M25, M26, M27, M28, M29, M30, M31, M32, M33, M34, M35, M36, M37, M38, M39, M40, M41, M42, M43, M44, M45, M46, M47, M48, M49, M50, M51, M52, M53, M54, M55, M56, M57, M58, M59, M60, M61, M62, M63, M64, M65, M66, M67, M68, M69, M70, M71, M72, M73, M74, M75, M76, M77, M78, M79, M80, M81, M82, M83, M84, M85, M86, M87, M88, M89, M90, M91, M92, M93, M94, M95, M96, M97, M98, M99, M100, M101, M102, M103, M104, M105, M106, M107, M108, M109, M110, M111, M112, M113, M114, M115, M116, M117, M118, M119, M120, M121, M122, M123, M124, M125, M126, M127, M128, M129, M130, M131, M132, M133, M134, M135, M136, M137, M138, M139, M140, M141, M142, M143, M144, M145, M146, M147, M148, M149, M150, M151, M152, M153, M154, M155, M156, M157, M158, M159, M160, M161, M162, M163, M164, M165, M166, M167, M168, M169, M170, M171, M172, M173, M174, M175, M176, M177, M178, M179, M180, M181, M182, M183, M184, M185, M186, M187, M188, M189, M190, M191, M192, M193, M194, M195, M196, M197, M198, M199, M200, M201, M202, M203, M204, M205, M206, M207, M208, M209, M210, M211, M212, M213, M214, M215, M216, M217, M218, M219, M220, M221, M222, M223, M224, M225, M226, M227, M228, M229, M230, M231, M232, M233, M234, M235, M236, M237, M238, M239, M240, M241, M242, M243, M244, M245, M246, M247, M248, M249, M250, M251, M252, M253, M254, M255, M256, M257, M258, M259, M260, M261, M262, M263, M264, M265, M266, M267, M268, M269, M270, M271, M272, M273, M274, M275, M276, M277, M278, M279, M280, M281, M282, M283, M284, M285, M286, M287, M288, M289, M290, M291, M292, M293, M294, M295, M296, M297, M298, M299, M300, M301, M302, M303, M304, M305, M306, M307, M308, M309, M310, M311, M312, M313, M314, M315, M316, M317, M318, M319, M320, M321, M322, M323, M324, M325, M326, M327, M328, M329, M330, M331, M332, M333, M334, M335, M336, M337, M338, M339, M340, M341, M342, M343, M344, M345, M346, M347, M348, M349, M350, M351, M352, M353, M354, M355, M356, M357, M358, M359, M360, M361, M362, M363, M364, M365, M366, M367, M368, M369, M370, M371, M372, M373, M374, M375, M376, M377, M378, M379, M380, M381, M382, M383, M384, M385, M386, M387, M388, M389, M390, M391, M392, M393, M394, M395, M396, M397, M398, M399, M400, M401, M402, M403, M404, M405, M406, M407, M408, M409, M410, M411, M412, M413, M414, M415, M416, M417, M418, M419, M420, M421, M422, M423, M424, M425, M426, M427, M428, M429, M430, M431, M432, M433, M434, M435, M436, M437, M438, M439, M440, M441, M442, M443, M444, M445, M446, M447, M448, M449, M450, M451, M452, M453, M454, M455, M456, M457, M458, M459, M460, M461, M462, M463, M464, M465, M466, M467, M468, M469, M470, M471, M472, M473, M474, M475, M476, M477, M478, M479, M480, M481, M482, M483, M484, M485, M486, M487, M488, M489, M490, M491, M492, M493, M494, M495, M496, M497, M498, M499, M500, M501, M502, M503, M504, M505, M506, M507, M508, M509, M510, M511, M512, M513, M514, M515, M516, M517, M518, M519, M520, M521, M522, M523, M524, M525, M526, M527, M528, M529, M530, M531, M532, M533, M534, M535, M536, M537, M538, M539, M540, M541, M542, M543, M544, M545, M546, M547, M548, M549, M550, M551, M552, M553, M554, M555, M556, M557, M558, M559, M560, M561, M562, M563, M564, M565, M566, M567, M568, M569, M570, M571, M572, M573, M574, M575, M576, M577, M578, M579, M580, M581, M582, M583, M584, M585, M586, M587, M588, M589, M590, M591, M592, M593, M594, M595, M596, M597, M598, M599, M600, M601, M602, M603, M604, M605, M606, M607, M608, M609, M610, M611, M612, M613, M614, M615, M616, M617, M618, M619, M620, M621, M622, M623, M624, M625, M626, M627, M628, M629, M630, M631, M632, M633, M634, M635, M636, M637, M638, M639, M640, M641, M642, M643, M644, M645, M646, M647, M648, M649, M650, M651, M652, M653, M654, M655, M656, M657, M658, M659, M660, M661, M662, M663, M664, M665, M666, M667, M668, M669, M670, M671, M672, M673, M674, M675, M676, M677, M678, M679, M680, M681, M682, M683, M684, M685, M686, M687, M688, M689, M690, M691, M692, M693, M694, M695, M696, M697, M698, M699, M700, M701, M702, M703, M704, M705, M706, M707, M708, M709, M710, M711, M712, M713, M714, M715, M716, M717, M718, M719, M720, M721, M722, M723, M724, M725, M726, M727, M728, M729, M730, M731, M732, M733, M734, M735, M736, M737, M738, M739, M740, M741, M742, M743, M744, M745, M746, M747, M748, M749, M750, M751, M752, M753, M754, M755, M756, M757, M758, M759, M760, M761, M762, M763, M764, M765, M766, M767, M768, M769, M770, M771, M772, M773, M774, M775, M776, M777, M778, M779, M780, M781, M782, M783, M784, M785, M786, M787, M788, M789, M790, M791, M792, M793, M794, M795, M796, M797, M798, M799, M800, M801, M802, M803, M804, M805, M806, M807, M808, M809, M810, M811, M812, M813, M814, M815, M816, M817, M818, M819, M820, M821, M822, M823, M824, M825, M826, M827, M828, M829, M830, M831, M832, M833, M834, M835, M836, M837, M838, M839, M840, M841,



Other 2023/4 projects

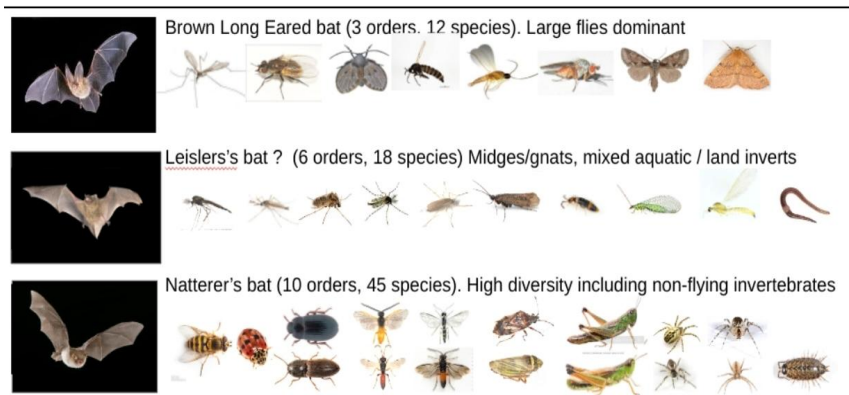


figure 4 : Summary of the three wild bat species and their prey (full taxa lists in Appendix A)

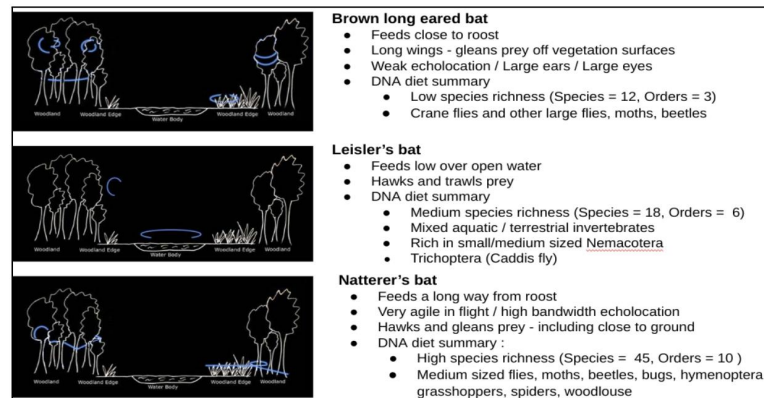
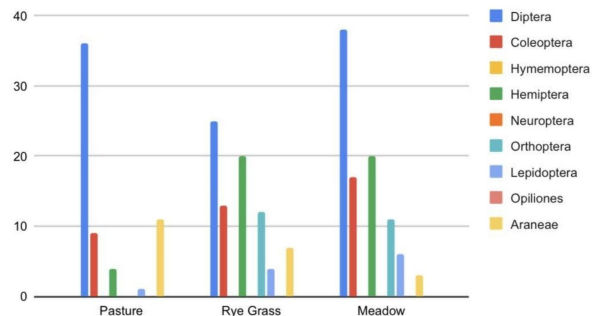


Figure 5 : Bat species foraging behaviour and diet summary from this study

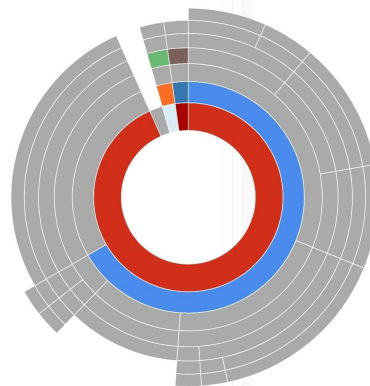
Pasture, Rye Grass and Meadow



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 Arthropoda of which

65 orders
207 families
456 genera
643 species/species groups

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

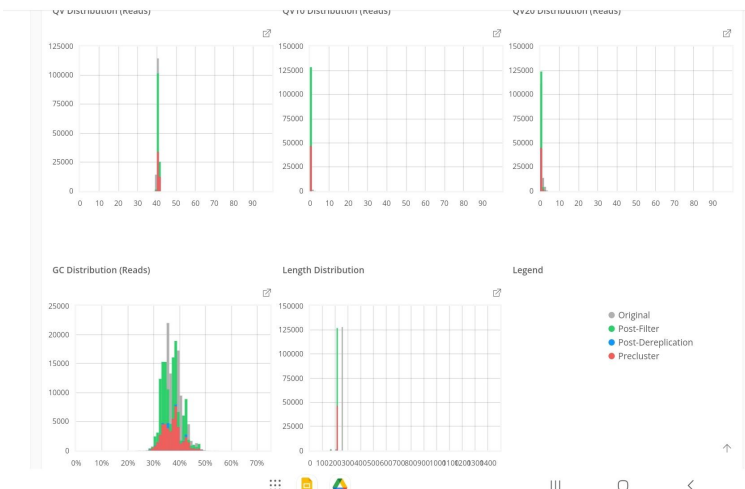
Chorthippus sp



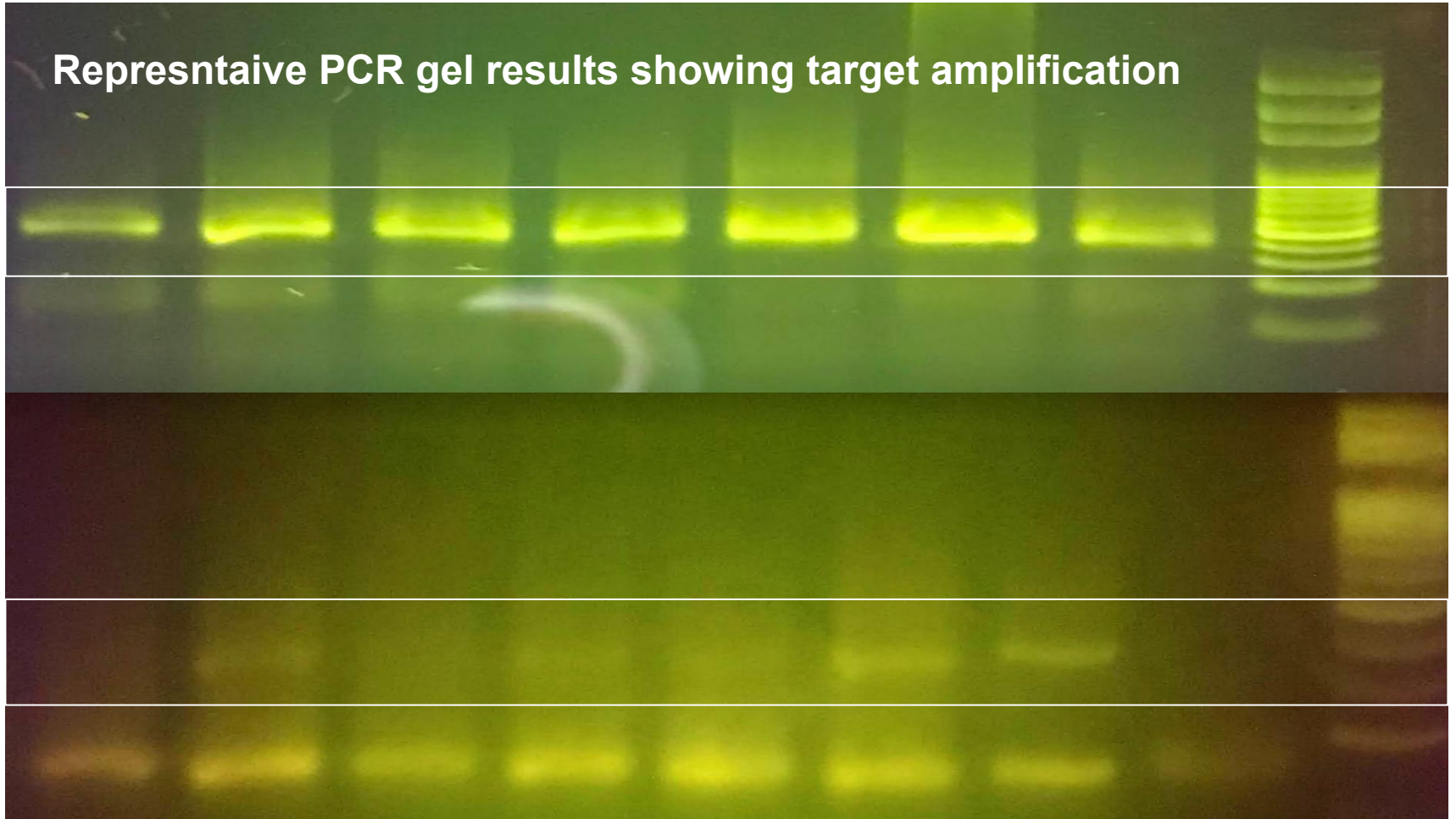
Galeruca ponomai



Tenebrio molitor

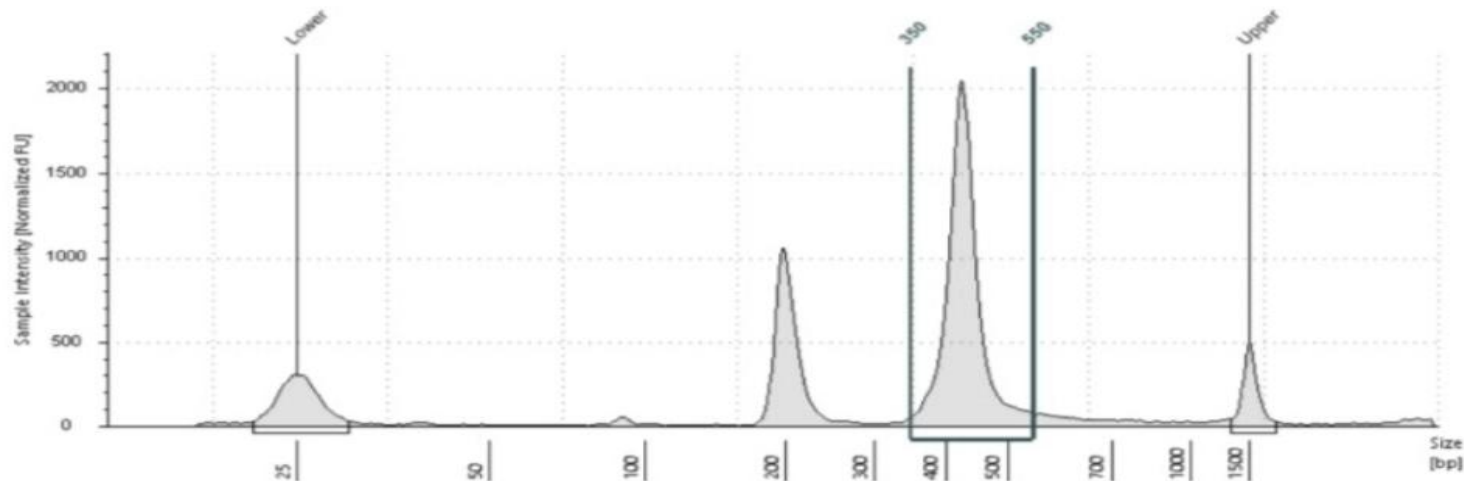


Representative PCR gel results showing target amplification



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		

Analysis and charting options

Data loaded in PAST

Past4 Edit Transform Plot Univariate Multivariate Model Diversity Timeseries Geometry Stratigraphy Script Window Help

:Users\Guest\Documents\Rivervel_NoZeroFamilyColumns_PAST.dat

ck mode Edit View

Select Drag rows/columns

Cut Copy Paste Select all

Bands Recover windows

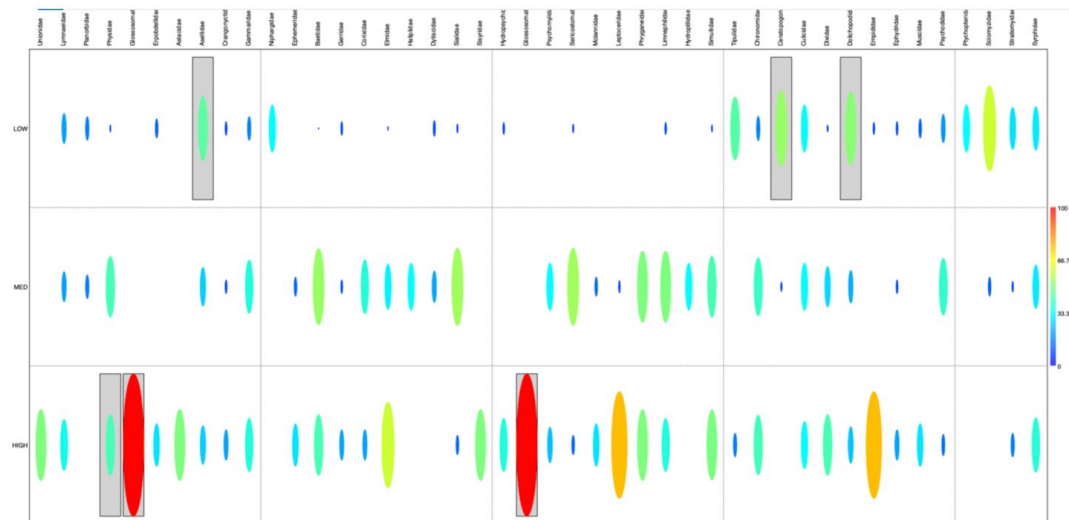
Binary Decimals: -

Symbol	Name	Site	Distance	temp	ph	conductivity	salinity	dissolved_C_flow	Unionids	Lymnaei	Planorbi	Physida	Glossos	Erpobde	Astacidae	Asellidae	Crangon	Gammar	Niphargi	Ephemer	Baetidae	Gerridae	Corixidae	Elmidae	Halipidae	Dytiscidae	Sialidae	Sis
Dot	Site	Site	-	temp	ph	conductivity	salinity	dissolved_O_flow	Unionidae	Lymnaeidae	Planorbidae	Physidae	Glossosoma	Erpobdellida	Astacidae	Asellidae	Crangonycti	Gammaridae	Niphargidae	Ephemeridae	Baetidae	Gerridae	Corixidae	Elmidae	Halipidae	Dytiscidae	Sialidae	Sis
Dot	Site	Site	Group	temp	ph	conductivity	salinity	dissolved_O_flow	Unionidae	Lymnaeidae	Planorbidae	Physidae	Glossosoma	Erpobdellida	Astacidae	Asellidae	Crangonycti	Gammaridae	Niphargidae	Ephemeridae	Baetidae	Gerridae	Corixidae	Elmidae	Halipidae	Dytiscidae	Sialidae	Sis
Dot	Site 1	Site 1	Ordinal	20.5	5.97	1	0.5	4.7	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0
Dot	Site 2	Site 2	Binary	20.5	6.2	0.86	0.43	5.6	0	3	1	0	0	0	0	3	0	1	0	0	0	0	0	0	0	1	1	0
Dot	Site 3	Site 3	String	15.3	6.26	0.71	0.36	9.4	0	1	1	1	0	0	0	1	0	1	0	0	4	0	1	1	0	0	1	0
Dot	Site 4	Site 4	4.5	17.3	6.28	0.75	0.38	7.1	0	1	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0
Dot	Site 5	Site 5	5	15.2	6.67	0.72	0.36	8.3	0	0	0	1	0	0	0	1	0	1	0	1	5	0	0	1	0	0	1	0
Dot	Site 6	Site 6	6	16	6.7	0.73	0.36	0.82	0	1	0	1	1	1	0	1	1	1	0	0	3	0	0	1	0	0	0	0
Dot	Site 7	Site 7	7	16	6.7	0.73	0.36	0.82	1	2	0	1	1	0	2	1	0	1	0	1	5	1	1	4	0	0	1	1
Dot	Site 8	Site 8	8	16.7	6.75	0.74	0.36	8.2	0	3	0	1	0	0	0	1	1	1	0	0	6	1	1	2	1	2	1	0
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Environmental variables

eDNA family composition data - 106 columns of data

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



Downstream ?

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

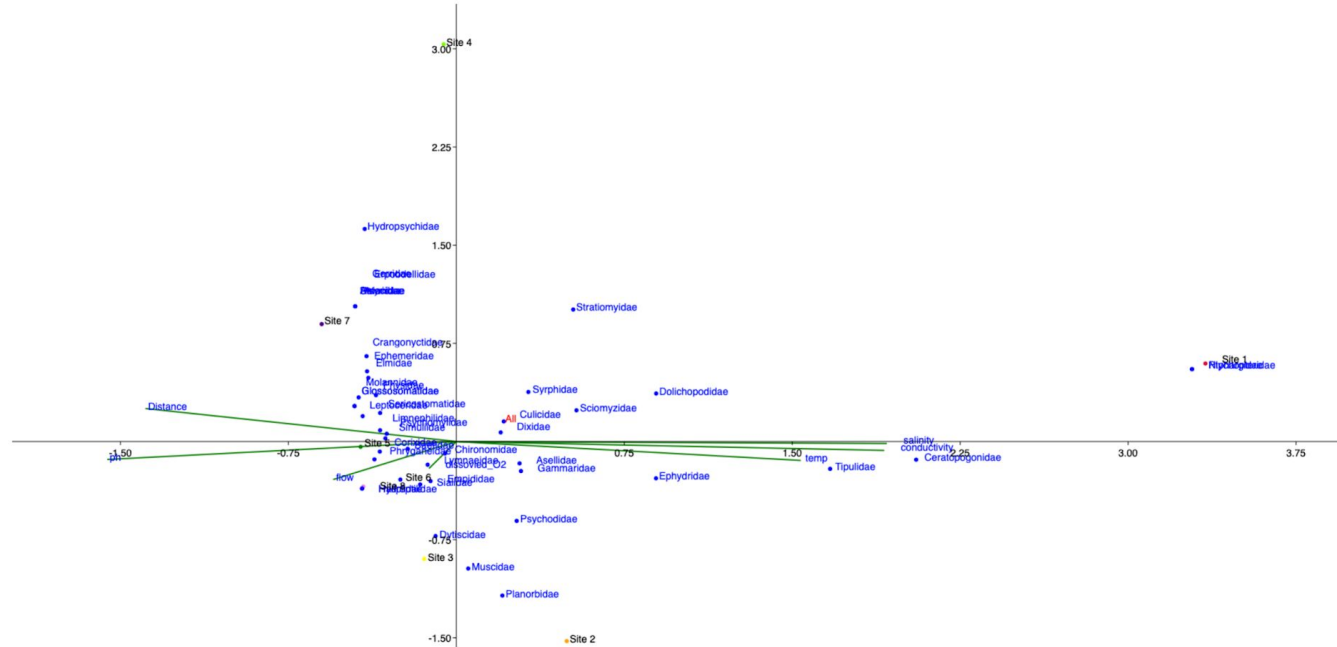
positions but this

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

of downstream

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

CCA ORDINATION PLOT (showing WHPT family clustering)

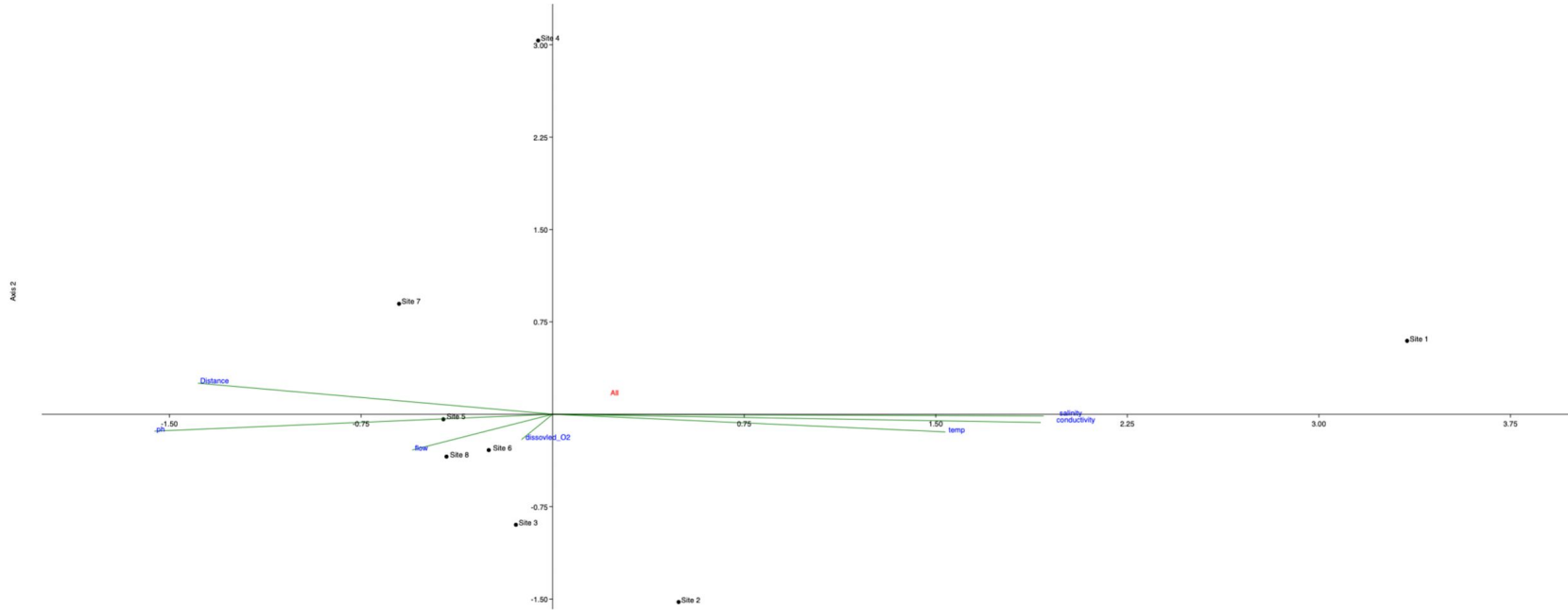


Which environmental variables, if any, are potentially impacting species composition variation (correlation does not mean causation) ?

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 potentially 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.