

Aquatic Invertebrate eDNA Report



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Compiled by:

Lowri Watkins – Evidence Officer

Gwent Wildlife Trust

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Aquatic Invertebrate eDNA

Client:	Monmouthshire County Council – LEADER Funding
Surveyor(s):	Andy Karran MSc, BSc, MIEEM; Peter Hunt
Author(s):	Lowri Watkins MSc, BSc
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Checked by:	Andy Karran MSc, BSc, MIEEM	30/9/22
Approved by:	Andy Karran MSc, BSc, MIEEM	30/9/22

1. Introduction

The overarching purpose of this eDNA study is to seek cost effective, innovative solutions to make rapid assessments of biodiversity with potential future applications in Gwent Wildlife Trust's work, and more widely in that of NRW.

Our initial intention was to sample for dung beetle eDNA, but due to unforeseen circumstances with resourcing at the eDNA testing lab, we switched our focus to sampling the aquatic invertebrate fauna of the reens and ditches in and around Magor Marsh Nature Reserve, on the Gwent Levels.

The Gwent Levels are one of the most extensive areas of reclaimed wet pasture in Great Britain, and the largest in Wales. The reens and ditches, which characterise the area, are home to rare aquatic flora and fauna, including many nationally rare and scarce invertebrate species. This impressive diversity of life and its importance has been recognised through the designation of much of the landscape as a series of Sites of Special Scientific Interest.

With over 800 miles of waterways and such a rich invertebrate assemblage, however few specialist entomologists are currently able to fully assess and report on the condition of the aquatic wildlife found below the surface. The development of eDNA technology could vastly improve our understanding of this important landscape, allowing us to produce more rapid and frequent assessments of the condition of protected sites.

This report will present the results of our first ever aquatic invertebrate eDNA survey and we will discuss the limitations and draw conclusions on the success of this sampling technique.

2. Methodology

2.1 Sample sites selection

Ten ditches/reens were selected for eDNA sampling. These were chosen to represent a range of habitats and conditions. A number of the sample points were also selected to correspond with sampling points from a traditional invertebrate field survey by Peter Kirby in 2010. Details can be found in [Table 1](#) and the locations can be seen on the map, [Figure 1](#).

Table 1. Details of ditch/reen sample points

Location	Sample no.	Name (if exists)	Type	Kirby?	Description
Bridewell Common	01	N/A	Ditch		Shaded ditch, large willows
Magor Marsh	02	Cwrta Well Reen	Ditch	Y	Open end of reen
Magor Marsh	03	N/A	Ditch	Y	Smaller, open and good condition
Bridewell Common	04	N/A	Ditch		Open. Recently cleared and recast '21
Barecroft Common	05	Rushwall North Reen	Reen		Open, good condition. 100m W of 09
Magor Marsh	06	Whitewall Reen	Reen	Y	Open, good cond. Recently re-cast
Barecroft Common	07	Rushwall South Reen	Reen		Reen opposite to sample 09
Bridewell Common	08	West Mead Reen	Reen		Open and good condition
Barecroft Common	09	Rushwall North Reen	Reen		Open, good condition. 100m E of 05
Bridewell Common	10	Mill Reen	Reen		Large main reen. Open, good cond.

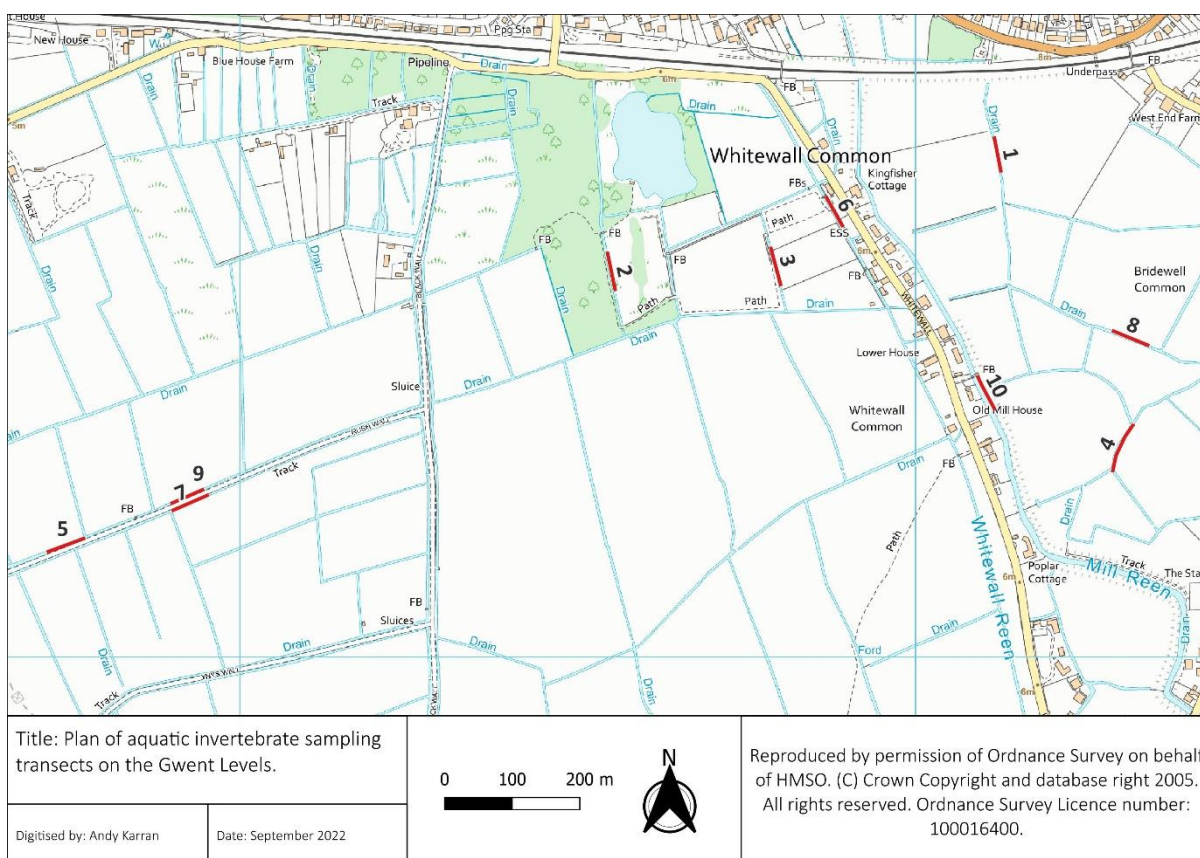


Figure 1. Location of sample points

2.2 Sample collection

All samples were collected on the 8th July 2022, in warm, sunny conditions. The surveyors were Andy Karran (GWT Evidence Manager) and Peter Hunt (GWT Volunteer).

Sampling kits for each of the sample points were received via post from the testing laboratory – Nature Metrics. Instructions for collecting samples were sent by Nature Metrics as a YouTube video: [Aquatic eDNA sampling instructions with NatureMetrics - YouTube](#). The steps were as follows:

1. Using the sterile collection bag (or a bottle in difficult conditions) we took a water sample. As eDNA doesn't mix well in still waters, we took a number of subsamples to get a representative sample and mixed the subsamples into the collection bag.
2. Using the syringe provided we drew up 100ml of water from the bagged sample, attached the filter and pushed the water through. This step was repeated to capture as much DNA as possible. The amount of water filtered was recorded in the Nature Metrics survey app.
3. Next, we expelled the water from the filter by detaching it, filling the syringe with air and reattaching it to the filter, before pushing the air through. This forced the last of the water out.
4. Next, to preserve the DNA in the filter, we uncapped the small syringe provided and attached it to the filter. Pointing the filter upwards, we pushed the preservative solution in the small syringe into the filter until a small bubble of preservative appeared at the top. We capped the end of the filter, turned it upside down, pushed the remaining solution in and capped the other end.
5. The samples were then sealed in the transport bag and posted to Nature Metrics.



Figure 2. Photo demonstrating water sample filtering process. (c) Andy Karran

2.3 eDNA analysis

The eDNA analysis was carried out by Nature Metrics. The methodology for this can be seen in the accompanying pdf "[NM-UMJ907.SO00871.Macroinvertebrate](#)".

3. Results

KEY RESULTS

- A total of **194 taxa** were detected
- Average **taxonomic richness** per sample site was **43.9** and ranged from **13** to **86**
- **Most abundant sequences:** *Chaoborus pallidus* (a phantom midge/glassworm species)
- **Most commonly detected taxa:** *Acricotopus lucens* (non-biting midge species), *Coryneura scutellata* (non-biting midge species) and *Caenis robusta* (a small square-gill mayfly species)
- Possible new records (UK)*: **7 taxa**
- Possible new records (Wales)*: **17 taxa** (9 excluding those new to UK)
- Possible new records (Gwent)*: **25 taxa** (8 excluding those new to Wales)
- UK IUCN Red List taxa detected: **11 taxa** (1 x Near Threatened; 10 x Least Concern)

* Taxa recorded as new to UK/Wales/Gwent are yet to be confirmed with specialist recording groups and are based on record searches of Aderyn and NBN only.

3.1 eDNA Amplification

High-quality macroinvertebrate sequence data were obtained for 8 of the 10 eDNA samples. eDNA metabarcoding of macroinvertebrates was not successful for sample points '02 Cwrta Well Reen (Open)' and '10 Mill Reen', which failed to amplify despite troubleshooting. All laboratory controls behaved as expected.

A total of 194 taxa were detected (see accompanying spreadsheet "[NM-UMJ907.S000871.Macroinvertebrate](#)"), with 40.2% (78 taxa) at least 99% similar to species in the global reference databases used by Nature Metrics. Accordingly, species names were suggested. For the remaining taxa, they were identified to the lowest possible taxonomic level: 30.4% to genus (59 taxa), 19.1% to family (37 taxa), 6.7% to order (13 taxa), 2.1% to class (4 taxa), 0.5% to phylum (1 taxon) and the remaining 2 to kingdom. Overall, the taxa detected belonged to 15 orders, 49 families and 85 genera.

3.2 Summary by taxa

A total of 194 taxa were detected across the eight successful samples. The full list of all taxa can be seen in the accompanying spreadsheet - "[NM-UMJ907.S000871.Macroinvertebrate](#)". Table 1 of this spreadsheet lists the full taxa, by sample and includes percentage match and the relative proportion of total sequence reads. Nature Metrics notes in their accompanying pdf that the abundance of taxa cannot be directly inferred from the proportion of total sequence reads; "While the proportion of sequence reads is a consequence of abundance, it

is also impacted by biomass, activity, surface area, condition, distance from the physical sample, primer bias, and species-specific variation in the genome.”

Species detected

Chaoborus pallidus (a phantom midge/glassworm species), which accounted for 15.4% of the total sequence reads, was among the most abundant in terms of sequences. Interestingly, this species was found to only have 4 Welsh records (all within Gwent), according to Aderyn searches. The genus, *Chaoborus* are opportunistic eaters, preying predominantly on copepods and cladocerans. Their main predator is fish.

Among the most commonly detected species were *Acricotopus lucens* (a non-biting midge species), *Corynoneura scutellata* (a non-biting midge species), and *Caenis robusta* (a small square-gill mayfly species), which were detected in 6, 5 and 4 samples, respectively. *Caenis robusta*, is a mayfly species in the Caenidae family, which scores highly in the Biological Monitoring Working Party assessment of water quality using macroinvertebrates. This is discussed further below under section 3.5.

A. lucens and *C. scutellata*, both hail from the Chironomidae family, the taxon of which were detected in all samples, with a staggering 33 different species identified. Many of these were found to have very low numbers of records for Gwent, Wales and the UK, including some that had no records at all. It’s likely that due to the difficulty in identifying these tiny organisms, they are under-recorded.

Families detected

The most frequently detected families i.e. those detected at the greatest number of sample sites were: the **non-biting midge/bloodworm family** (Chironomidae) - detected at all 8 sites; the **copepod family** (Cyclopidae) – 7/8 sites; the **mosquito family** (Culicidae) – 6/8 sites; the **long-legged flies** (Dolichopodidae) – 6/8 sites; and the **pond snails** (Lymnaeidae) – 6/8 sites.

The least frequently recorded families, each detected at only 1/8 sample sites, were: the **microdrile oligochate family** (Lumbriculidae); the **soldier beetles** (Cantharidae); the **June bugs** (Melolonthidae); the **darkling beetles** (Tenebrionidae); an **acalyptate fly family** (Asteiidae); a **fly family** (Dryomizidae); the **hybotid fly family** (Hybotidae); the **scuttle flies** (Phoridae); the **lesser dung flies** (Sphaeroceridae); the **horseflies** (Tabanidae); the **water boatmen** (Corixidae); the **shore bugs** (Salididae); the **green lacewings** (Chrysopidae); the **long-horned caddisflies** (Leptoceridae); an **ostracod family** (Candodidae); a **rotifer family** (Brachionidae).

It is hard to make conclusions about whether these lists accurately represent the composition of invertebrates at these sites due to the limitations of the sampling technique and eDNA analysis (discussed later). A number of the families listed appear to be terrestrial families, so not the target groups of this study.

Possible new records

Records searches of all taxa identified to the species level where undertaken via a range of online databases – NBN, the record database for the UK, which links to Local Environmental Record Centres; Aderyn, the record database for the Welsh Local Environmental Record Centres; and where no records were found in the former, or the species name was not found, GBIF was checked. Tables are provided below for the species which returned no records in these databases, split into UK, Wales and Gwent. These records are yet to be verified via specialist recording groups and should not be taken as certain at this point.

Possible new to UK records		
Species name	Common name	No. samples
<i>Chaetogaster diastrophus</i>	A clitellate oligochaete worm	2
<i>Chironomus melanotus</i>	A non-biting midge/bloodworm family	4
<i>Cricotopus relucens</i>	A non-biting midge/bloodworm family	1
<i>Cricotopus cylindraceus</i>	A non-biting midge/bloodworm family	1
<i>Cricotopus caducus</i>	A non-biting midge/bloodworm family	1
<i>Psychoda lativentris</i>	A moth/drain fly	1
<i>Satchelliella trivialis</i>	A moth/drain fly	1

Figure 3 List of species detected which may represent records new to the UK.

Possible new to Wales records		
Species name	Common name	No. samples
<i>Chaetogaster diastrophus</i>	A clitellate oligochaete worm	2
<i>Corynoneura carriana</i>	A non-biting midge/bloodworm family	3
<i>Cryptochironomus albofasciatus</i>	A non-biting midge/bloodworm family	1
<i>Kiefferulus tendipediformis</i>	A non-biting midge/bloodworm family	2
<i>Limnophyes natalensis</i>	A non-biting midge/bloodworm family	1
<i>Parachironomus parilis</i>	A non-biting midge/bloodworm family	3
<i>Paraphaenocladus impensus</i>	A non-biting midge/bloodworm family	2
<i>Pseudosmittia trilobata</i>	A non-biting midge/bloodworm family	1
<i>Philotarsus picicornis</i>	A booklouse	3

Figure 4 List of species detected which may represent records new to Wales.

Possible new to Gwent records		
Species name	Common name	No. samples
<i>Pegomya flavifrons</i>	A root maggot fly	1
<i>Chironomus annularius</i>	A non-biting midge/bloodworm family	1
<i>Chironomus luridus</i>	A non-biting midge/bloodworm family	2
<i>Chironomus riparius</i>	A non-biting midge/bloodworm family	2
<i>Dicrotendipes lobiger</i>	A non-biting midge/bloodworm family	3
<i>Parachironomus arcuatus</i>	A non-biting midge/bloodworm family	1
<i>Paratendipes albimanus</i>	A non-biting midge/bloodworm family	1
<i>Psychoda phalaenoides</i>	A moth/drain fly	1

Figure 5 List of species detected which may represent records new to Gwent.

3.3 Summary by sample point

The taxon richness and assemblage of species detected, varied considerably between sample sites. Figure 6 and 7 below show the taxon-richness between sites, with sample site 04, a newly cleared section of ditch on Bridewell Common scoring highest for both number of taxa and number of taxa identified to species level. Site 06, Whitewall Reen at the Magor Marsh entrance, scored lowest.

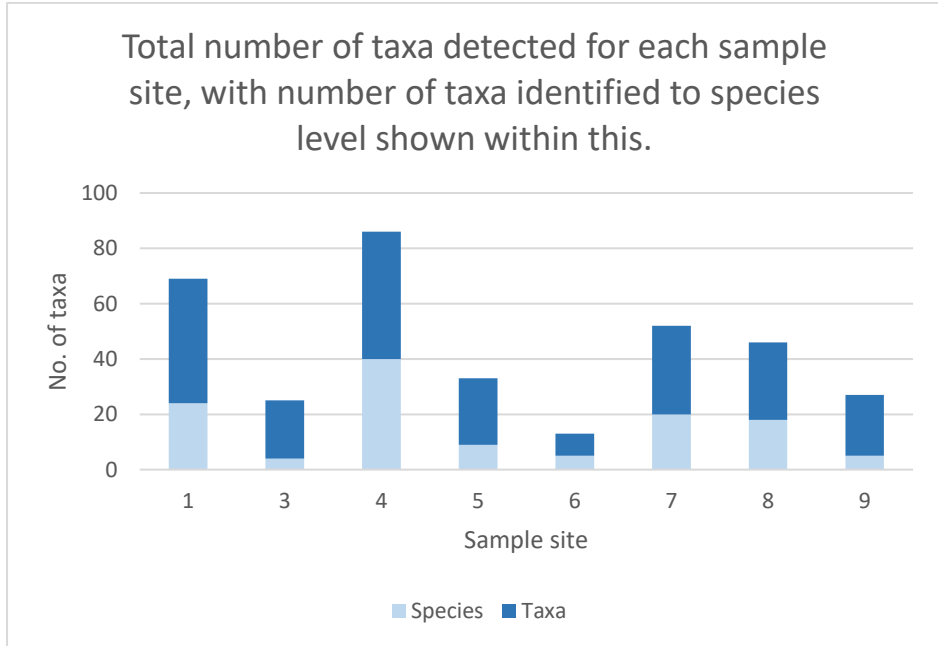


Figure 6 Number of taxa for each sample site

Sample ID	Class	Order	Family	Genus	Taxa (Species)
01 Bridewell un-named Reen (Shaded)	5	9	20	30	69 (24)
03 Un-named	7	9	13	11	25 (4)
04 Un-named Reen	5	9	29	46	86 (40)
05 Blackwall Reen	3	5	12	17	33 (9)
06 Whitewall Reen	4	4	7	8	13 (5)
07 Across track from 9 Blackwall Reen	5	9	24	29	52 (20)
08 West Mead Reen	5	9	21	27	46 (18)
09 Blackwall Reen	4	8	11	14	27 (5)

Figure 7 Taxon richness by sample site

A table follows on the next page. For each sample point, an indicative photo is provided, along with a description of the habitat and species detected. Following this a full list of taxa detected is provided, along with details on the number of sample points they were detected in (out of the 8 that that were successful) as well as notes about whether the species has a conservation designation, notable numbers of records etc. Numbers of records will be commented where they are less than 25 for the UK or less than 5 for Wales or Gwent. As stated earlier in the report, record numbers have come from checking online databases – NBN for the UK, Aderyn for Wales and Gwent, with GBIF checked for any not found in NBN or Aderyn. These have therefore not been verified yet with specialist recording groups, this step could not be achieved in the time available, but will progress after our report submission.

Sample point 01

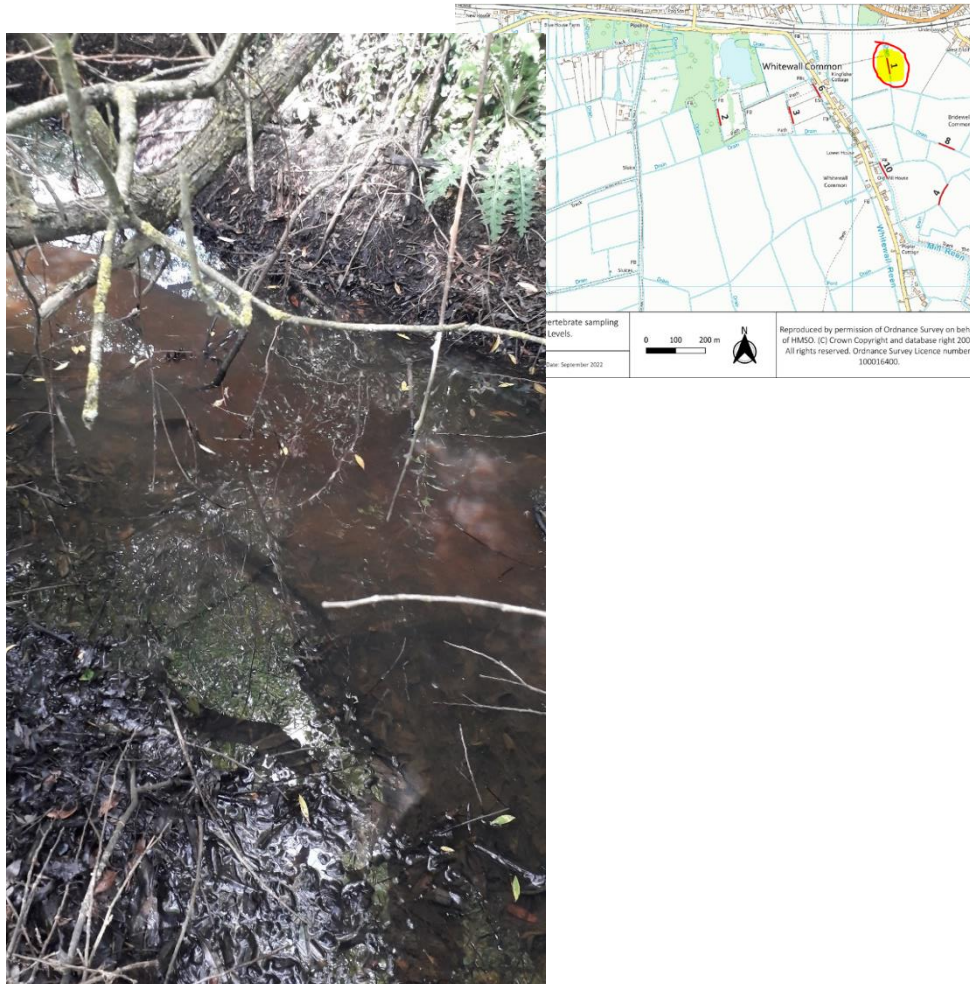


Figure 8 Photo of sample point 01. Shaded ditch, Bridewell Common. © Andy Karran)

Description

Sample point 01 was taken from a shaded ditch in the northwest of GWT’s Bridewell Common Nature Reserve. The water here is shallow and silty, with much leaf litter. The ditch is lined with large overgrown willow trees on both sides, with some bramble. Aquatic vegetation is limited at the immediate bankside. The ditch is sandwiched between the two most species-rich marshy grasslands on the site, two fields that are assigned as Local Wildlife Sites. The site has in the past been subject to mixed levels of management, with missed haycuts and patchy grazing. The large sprawling bankside trees would have limited cattle access to the ditch, meaning poaching is minimal, or concentrated in limited locations along the ditch.

The results for this sample point are possibly the most surprising. We would assume that being shaded, shallow and lacking much in the way of emergent and bank-side vegetation, this ditch would lack some of the more optimal conditions for aquatic invertebrate life. However, this sample point was the second most taxon-rich, with 69 taxa, of which 24 were identified to species level. Two of the species identified are listed as **Least Concern** according to UK IUCN Red List assessment.

Several taxa detected were from four of the higher scoring families in the Biological Monitoring Working Party (BMWP) assessment of water quality using macroinvertebrates and this was calculated to be the second highest scoring site for good water quality.

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Agabus sturmii</i>	(a predaceous diving beetle)	Detected in 2 sample sites.	This species is in the family Dytiscidae which has a BMWP water quality rating of 5/10.
<i>Hydroporus figuratus</i>	(a predaceous diving beetle)	Only detected at this sample site.	There is only a single Welsh record for this species, from Nash on the Gwent Levels. There are 61 UK records. This species is in the family Dytiscidae which has a BMWP water quality rating of 5/10.
<i>Hydroporus planus</i>	(a predaceous diving beetle)	Only detected at this sample site.	This species is in the family Dytiscidae which has a BMWP water quality rating of 5/10.
<i>Nartus grapii</i>	(a predaceous diving beetle)	Detected at 3 sample sites.	Nature Metrics listed this sequence as a 99.3% match for <i>Rhantus grapii</i> . Searching online suggests that the accepted name is <i>Nartus grapii</i> . Searching the various databases for the latter showed many UK, Wales and Gwent records (whereas <i>Rhantus grapii</i> was not found). This species is in the family Dytiscidae which has a BMWP water quality rating of 5/10.
<i>Palpomyia lineata</i>	(a biting midge)	Detected in 4 sample sites.	This species has only 1 Gwent record, 3 Welsh records and 23 UK records.
<i>Chaoborus pallidus</i>	(a phantom midge/glassworm)	Detected in 2 sample sites.	This species has only 4 Welsh records , 3 of them from Gwent. Interestingly, this species was the most abundant sequence picked up across all samples, representing 15.4% of all sequences, but found at only 2 sample points.
<i>Chironomus luridus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are only 3 Welsh records for this species, none in Gwent.

<i>Chironomus riparius</i>	(a non-biting midge)	Detected in 2 sample sites.	<u>No previous records in Gwent.</u>
<i>Chironomus melanotus</i>	(a non-biting midge)	Detected in 4 sample sites.	This species has <u>no known UK records.</u> Limited GBIF records are localised to Finland. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Coryoneura scutellata</i>	(a non-biting midge)	Detected in 5 sample sites.	This species has <u>only 5 Gwent records.</u>
<i>Limnophyes minimus</i>	(a non-biting midge)	Detected in 2 sample sites.	This species has <u>only 5 Gwent records.</u>
<i>Paraphaenocladus impensus</i>	(a non-biting midge)	Detected in 2 sample sites.	This species has <u>no Gwent or Welsh records.</u>
<i>Psectrotanypus varius</i>	(a non-biting midge)	Detected in 3 sample sites.	This species has <u>only one Gwent record.</u>
<i>Pseudosmittia trilobata</i>	(a non-biting midge)	Only detected at this sample site.	This species has <u>no Gwent or Welsh records</u> and <u>only 16 records for the UK.</u>
<i>Culiseta annulata</i>	(a mosquito)	Detected in 2 sample sites.	
<i>Campsicnemus scambus</i>	(a long-legged fly)	Only detected at this sample site.	
<i>Chrysotus femoratus</i>	(a long-legged fly)	Detected in 2 sample sites.	This species has <u>only one Gwent record.</u>
<i>Dryomyza anilis</i>	(a fly)	Only detected at this sample site.	
<i>Erioptera squalida</i>	(a crane fly)	Only detected at this sample site.	
<i>Molophilus obscurus</i>	(a crane fly)	Only detected at this sample site.	
<i>Psychoda lativentris</i>	(a moth/drain fly)	Only detected at this sample site.	This species has <u>no UK records.</u> Limited GBIF records have a N. American and N. Europe distribution.

<i>Satchelliella trivialis</i>	(a moth/drain fly)	Only detected at this sample site.	This species was not found on NBN or Aderyn, GBIF has the accepted name as <i>Pneumia trivialis</i> and displays many UK occurrences, however this name was also not found in NBN or Aderyn. No other synonyms were listed.
<i>Ptychoptera albimana</i>	(a phantom crane fly)	Only detected at this sample site.	
<i>Ptychoptera contaminate</i>	(a phantom crane fly)	Detected in 3 sample sites.	
<i>Tipula oleracea</i>	Marsh Cranefly	Detected in 2 sample sites.	This species is in the family Tipilidae, which has a BMWP water quality rating of 5/10.
<i>Hesperocorixa sahlbergi</i>	(a water boatman)	Only detected at this site.	This species is Least Concern on the UK Red List. There are many Gwent, Welsh, UK records. It was detected only at this sample point, with a 99.3% similarity to this species in the DNA databases. This species is in the family Corixidae, which has a BMWP water quality rating of 5/10.
<i>Nepa cinerea</i>	Water Scorpion	Detected in 2 sample sites.	This species is Least Concern on the UK Red List. There are many Gwent, Welsh, UK records. This species is in the family Nepidae, which has a BMWP water quality rating of 5/10.
<i>Philotarsus picicornis</i>	(a booklouse)	Detected in 3 samples sites.	This species has only 4 UK records, none in Gwent/Wales , however the aggregate species listed in Aderyn shows 12 Welsh records, with two of these within Gwent.
Family: Chrysomelidae	Leaf beetle family		Two sequences for this family which is rated 5/10 in the BMWP water quality assessment were detected, however they could not be identified to species or genus level.

Sample point 03



Figure 9 Photo of sample site 03, ditch at Magor Marsh. Not taken at time of survey.

Description

Sample point 03 was taken from an internal ditch within Magor Marsh Nature Reserve. It is a small ditch, only 1-2 metres wide, with a water depth of about 50cm-1m. It is open and in good condition, with a fringe of c. 1-2m of vegetation flanking either bank, and some emergent vegetation within the ditch. The water is relatively clear here.

At the northern end there is a dipping platform and area of thicker common reed, at the southern end a sluice pens in water to the north, flowing south through a shadier patch of willow and scrub and out into the ditch at the reserve’s boundary.

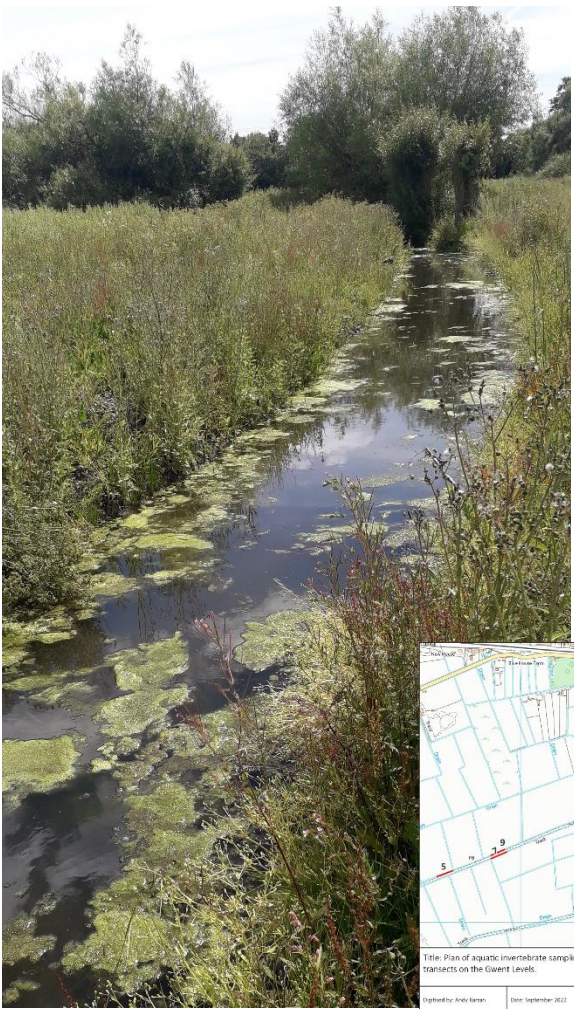
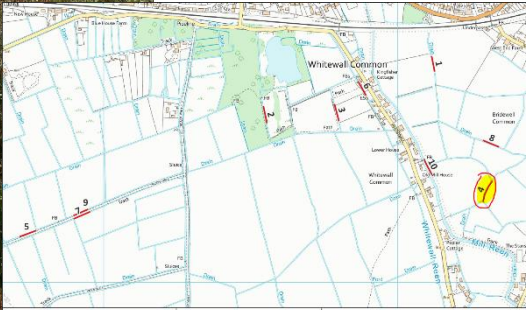
The ditch is bound on both sides by grassland within the nature reserve, to the west a large sedge field and to the east a neutral-marshy grassland that is pony-grazed for much of the year. A fence on this side prevents grazing and poaching within 1-2m of the ditch. This field undergoes a haycut every year. The sedge field is cut sporadically, depending on conditions. It either receives a cut to half of the field each year, rotated with quarter overlaps, or in years when it is too wet it a quad is used to cut some paths through the vegetation.

This sample point was the second least taxon-rich, with 25 taxa, of which 6 were identified to species level. This site had taxon from two of the higher scoring BMWP families, which score 5/10.

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Lumbricus rubellus</i>	Red Worm (a terrestrial worm)	Detected in 3 sample sites.	
<i>Chaetogaster diastrophus</i>	(a clitellate oligochaete worm)	Detected in 2 sample sites.	This species has no known UK records on NBN , and no Welsh/Gwent records, however GBIF shows 2 possible records, in Anglesey and one near the Chilterns.
<i>Nartus grapii</i>	(a predaceous diving beetle)	Detected in 3 sample sites.	Nature Metrics listed this sequence as a 99.3% match for <i>Rhantus grapii</i> . Searching online suggests that the accepted name is <i>Nartus grapii</i> . Searching the various databases for the latter showed many UK, Wales and Gwent records (whereas <i>Rhantus grapii</i> was not found). This species is in the family Dytiscidae, which has a BMWP water quality rating of 5/10.
<i>Enochrus testaceus</i>	(a water scavenger beetle)	Detected in 2 sample sites.	This species is in the family Hydrophilidae, which has a BMWP water quality rating of 5/10.
<i>Psectrotanypus varius</i>	(a non-biting midge)	Detected in 3 sample sites.	This species only has 1 Gwent record and 15 in Wales.
<i>Culiseta annulata</i>	(a mosquito)	Detected in 2 sample sites.	
<i>Eucyclops</i>	(copepod genus)		Two sequences were detected for this genus, but neither could be identified to species level. There are a few species and records recorded in Wales, within this genus.
<i>Chironomus</i>	(non-biting midge genus)		Four sequences were detected for this genus, but none could be identified to species level.

<i>Coryoneura</i>	(non-biting midge genus)		Two sequences were detected for this genus, but neither could be identified to species level. Other sample sites detected sequences from this genus which were identified to the species level.
<i>Paracladopelma</i>	(non-biting midge genus)		A single sequence was detected for this genus, but could not be identified to species level. This genus of non-biting midges has one species recorded in Wales, with two records.
<i>Candona</i>	(ostracod genus)		A single sequence was detected for this genus, but could not be identified to species level. This genus of crustaceans has only 3 species recorded in Wales, in low numbers.
Family: Psychodidae	(moth/drain fly family)		One sequence was detected for this family, which is represented at the species level at other sample sites.
Family: Lymnaeidae	(pond snail family)		One sequence was detected for this family, it was not identified to a lower taxonomic value elsewhere.
Family: Brachionidae	(rotifer family)		One sequence was detected for this family, it was not identified to a lower taxonomic value elsewhere.

Sample point 04	Description
 <p data-bbox="815 264 1167 373"><i>Figure 10 Photo of sample point 04. Newly cleared and re-cast ditch on Bridewell Common. (C) Andy Karran</i></p>  <p data-bbox="631 1206 1155 1265">Title: Plan of aquatic invertebrate sampling transects on the Gwent Levels. Reproduced by permission of Ordnance Survey on behalf of HMSO. (C) Crown Copyright and database right 2005. All rights reserved. Ordnance Survey Licence number: 100016100. Digitized by Andy Karran. Date: September 2022.</p>	<p data-bbox="1205 276 2022 592">Sample point 4 was taken from an internal ditch at the southern end of GWT's Bridewell Common Nature Reserve. This ditch is open. Satellite photography shows this ditch was formerly double-hedged for at least two decades, and holding only minimal water. It was cleared of large overgrown willows and scrub, and re-cast in autumn 2021, linking it between two reens – Mill Reen and West Mead Reen. The aquatic vegetation is still recovering on the banks and in-channel, currently comprising an abundance of terrestrial plants, indicative of disturbed ground.</p> <p data-bbox="1205 639 2022 842">Another surprising result with this ditch, as it was found to be the most taxon-rich of all sample points, despite only recently being cleared. Sequencing identified 86 taxa in total, with a staggering 40 taxa identifiable to species level. Two of the species identified are listed as Least Concern according to UK IUCN Red List assessment, these were both terrestrial species.</p> <p data-bbox="1205 890 2022 1062">Several taxa detected were from higher scoring families in the Biological Monitoring Working Party (BMWP) assessment of water quality using macroinvertebrates. These included: Dytiscidae (predaceous diving beetles); Haliplidae (crawling water beetles); Tipulidae (crane flies); and Naucoridae (saucer bugs).</p>


Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Lumbricus rubellus</i>	Red Worm (a terrestrial worm)	Detected in 3 sample sites.	Nature Metrics lists that this species identification was based on limited reference matches.
<i>Rhagonycha fulva</i>	Common Red Soldier Beetle	Only detected at this site.	This species is Least Concern on the UK Red List. There are many Gwent, Welsh, UK records.
<i>Agabus sturmii</i>	(a predaceous diving beetle)	Detected in 2 sample sites.	This species is in the family Dytiscidae which has a BMWP water quality rating of 5/10.
<i>Haliphus heydeni</i>	(a crawling water beetle)	Only detected at this site.	Nature Metrics listed this sequence as a 99.3% match. This species is in the family Haliplidae which has a BMWP water quality rating of 5/10.
<i>Amphimallon solstitiale</i>	Summer Chafer	Only detected at this site.	This species is Least Concern on the UK Red List. There are 13 Gwent records, 40 Welsh records, 365 UK.
<i>Phytomyza ranunculi</i>	(a leaf miner fly)	Only detected at this site.	
<i>Delia platura</i>	(seedcorn maggot/bean seed fly)	Only detected at this site.	
<i>Asteia amoena</i>	(an acalyprate fly)	Only detected at this site.	This species has <u>only 5 Gwent records</u> .
<i>Mallochohelea nitida</i>	(a biting midge)	Only detected at this site.	This species has <u>only 2 Welsh records</u> , both in Gwent and <u>only 22 UK records</u> . Nature Metrics lists that this species identification was based on limited reference matches.
<i>Chaoborus flavicans</i>	(a phantom midge/glassworm)	Only detected at this site.	
<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Chironomus annularius</i>	(a non-biting midge)	Only detected at this site.	There are <u>no Gwent records</u> , <u>only 3 Welsh records</u> and <u>only 8 UK records</u> for this species.

<i>Chironomus cingulatus</i>	(a non-biting midge)		There are <u>no Welsh records</u> for this species and only <u>21 records for the UK</u> . Nature Metrics lists that this species identification was based on limited reference matches.
<i>Chironomus luridus</i>	(a non-biting midge)	Detected in 2 sample sites.	This species has <u>no Gwent records</u> and <u>only 3 Welsh records</u> .
<i>Chironomus riparius</i>	(a non-biting midge)	Detected in 2 sample sites.	This species has <u>no Gwent records</u> .
<i>Chironomus melanotus</i>	(a non-biting midge)	Detected in 4 sample sites.	This species has <u>no UK records</u> . Limited GBIF records are localised to Finland.
<i>Corynoneura carriana</i>	(a non-biting midge)	Detected in 3 sample sites.	There are <u>no Welsh records</u> for this species and only <u>13 records for the UK</u> .
<i>Corynoneura scutellata</i>	(a non-biting midge)	Detected in 5 sample sites.	This species has <u>only 5 records in Gwent and 9 in Wales</u> . It was one of the most commonly detected species, occurring in 5/6 sample sites.
<i>Cryptochironomus albofasciatus</i>	(a non-biting midge)	Only detected at this site.	There are <u>no Welsh records</u> for this species and only <u>5 records for the UK</u> . Nature Metrics lists that this species identification was based on limited reference matches.
<i>Kiefferulus tendipediformis</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>no Welsh records</u> for this species and only <u>20 records for the UK</u> .
<i>Limnophyes minimus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>only 5 Gwent records</u> for this species.
<i>Macropelopia nebulosa</i>	(a non-biting midge)	Only detected at this site.	There is <u>only 1 Gwent records</u> for this species.
<i>Micropsectra lindrothi</i>	(a non-biting midge)	Only detected at this site.	There are <u>only 6 UK records</u> for this species, with <u>3 records in Wales</u> and <u>2 records in Gwent</u> .

<i>Parachironomus arcuatus</i>	(a non-biting midge)	Only detected at this site.	This species names was not found in Aderyn/NBN, however a synonym – <i>P. gracilior</i> – was searchable. There were only 3 Welsh records for this species, there were no records in Gwent .
<i>Parachironomus parilis</i>	(a non-biting midge)	Detected in 3 sample sites.	There are no Welsh records for this species and only 20 records for the UK .
<i>Paratendipes albimanus</i>	(a non-biting midge)	Only detected at this site.	There were no records in Gwent for this species and only 1 Welsh record and 30 UK records.
<i>Polypedilum nubeculosum</i>	(a non-biting midge)	Only detected at this site.	There was only 1 Gwent record for this species.
<i>Psectrotanypus varius</i>	(a non-biting midge)	Detected in 3 sample sites.	There was only 1 Gwent record for this species.
<i>Tanytus punctipennis</i>	(a non-biting midge)	Only detected at this site.	There were only 3 Gwent records for this species and 23 Welsh records.
<i>Tanytarsus eminulus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are only 5 UK records for this species, with 1 record in Wales (in Gwent) .
<i>Tanytarsus pallidicornis</i>	(a non-biting midge)	Only detected at this site.	There were only 5 records in Gwent .
<i>Tanytarsus usmaensis</i>	(a non-biting midge)	Only detected at this site.	There were only 2 records in Wales, both in Gwent .
<i>Dicraeus vagans</i>	(a glass fly)	Detected in 3 sample sites.	
<i>Chrysotus femoratus</i>	(a long-legged fly)	Detected in 3 sample sites.	There was only 1 Gwent record for this species.
<i>Hydrellia maura</i>	(a shore fly)	Only detected at this site.	
<i>Notiphila cinerea</i>	(a shore fly)	Only detected at this site.	

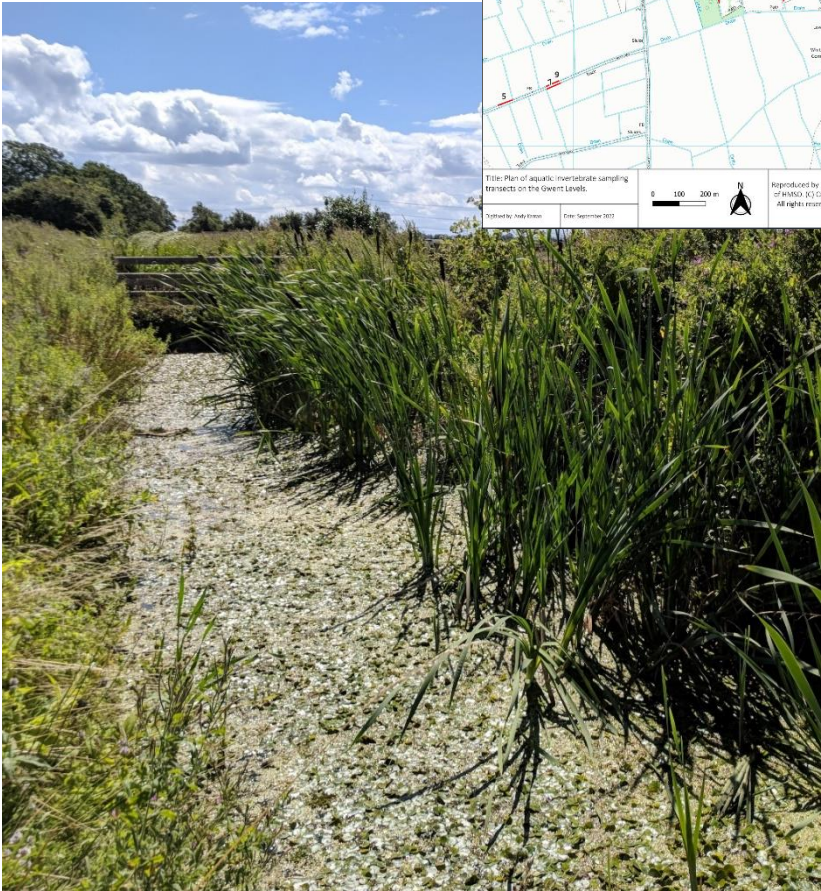
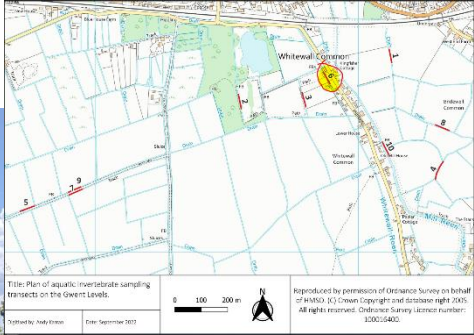
<i>Muscina prolapsa</i>	(a house fly)	Only detected at this site.	
<i>Psychoda phalaenoides</i>	(a moth/drain fly)	Only detected at this site.	There were <u>no records in Gwent</u> for this species.
<i>Tipula oleracea</i>	Marsh Crane fly	Detected in 2 sample sites.	
<i>Cunctochrysa albolineata</i>	(a lacewing)	Only detected at this site.	
<i>Valenzuela flavidus</i>	(a booklouse)	Detected in 2 sample sites.	
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			

Sample point 05	Description
 <p data-bbox="203 1297 1151 1353"><i>Figure 11 Photo of sample point 05. Rushwall N Reen (west end, looking east). Photo not taken at time of survey.</i></p>	<p data-bbox="1205 276 2027 555">Sample point 05 was taken from a rean on Barecroft Common – Rushwall North. This rean forms the southern boundary for four of the most westerly fields of GWT’s Magor Marsh Nature Reserve (left side of the photo opposite). The rean is approximately 3-5m wide and with a similar depth from the bank top. It is open along its full length, with typical rean vegetation flanking both banks. The northern bank is part fenced and has scattered scrub and trees, whereas the southern bank is absent of any scrub/trees, forming part of a long access track.</p> <p data-bbox="1205 600 2027 807">This sample was one of three in this area, to test variation in aquatic invertebrate fauna within close proximity. The second sample was taken from the same rean, 100m to the east, and a third sample on the rean opposite this – Rushwall South. These two reans run adjacent to each other for almost 3km, either side of a c.5m rough farm track. The final photo in this table shows the context of these two reans.</p> <p data-bbox="1205 852 2027 1166">This sample point was the 5th most taxon-rich (out of 8), with 33 taxa detected, 9 of which were identifiable to the species level. Of the families detected, 3 of these were from higher scoring families in the Biological Monitoring Working Party (BMWP) assessment of water quality using macroinvertebrates. A species was identified in the family Caenidae (the small square-gill mayflies), which has a value of 7. Two further taxa were identified only to family level for the families Chrysomelida (leaf beetles) and Naucoridae (saucer bugs), both of which are values at 5/10.</p>

Species detected at this sample point:

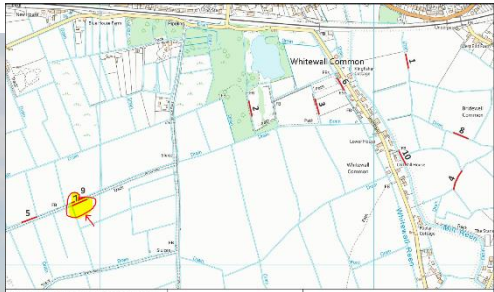

Species name	Common name	Sample distribution	Notes
<i>Palpomyia lineata</i>	(a biting midge)	Detected in 4 sample sites.	This species <u>has only 1 Gwent record, 3 Welsh records and 23 UK records.</u>
<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Chironomus melanotus</i>	(a non-biting midge)	Detected in 4 sample sites.	This species has <u>no UK records.</u> Limited GBIF records are localised to Finland. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Corynoneura scutellata</i>	(a non-biting midge)	Detected in 5 sample sites.	This species has <u>only 5 records in Gwent and 9 in Wales.</u> It was one of the most commonly detected species, occurring in 5/6 sample sites.
<i>Cricotopus cylindraceus</i>	(a non-biting midge)	Only detected at this site.	This species has <u>no UK records.</u> Limited GBIF records x37 were shown from Alaska, Netherlands, Norway, Sweden, ~Belarus. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Dicrotendipes lobiger</i>	(a non-biting midge)	Detected in 3 sample sites.	There were <u>no Gwent records</u> for this species and <u>only 3 Welsh records.</u>
<i>Parachironomus parilis</i>	(a non-biting midge)	Detected in 3 sample sites.	There are <u>no Welsh records</u> for this species and <u>only 20 UK records.</u>
<i>Dicraeus vagans</i>	(a glass fly)	Detected in 3 sample sites.	
<i>Symplecta stictica</i>	(a crane fly)	Only detected at this site.	
<i>Chrysops viduatus</i>	Square-spot Deerfly	Only detected at this site.	There are 10 Gwent records for this species, which has been assigned the IUCN category Least Concern , as well as Nationally Scarce.

			Nature Metrics reported that the sequence was a 99.3% match for this species.
<i>Caenis robusta</i>	(a small square-gill mayfly)		There are 105 Gwent records for this species, which has been assigned the IUCN category Least Concern . This species is in the family Caenidae which has a value of 7/10 in the BMWP water quality metric, suggesting good water quality at this sample site.
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			

Sample point 06	Description
<p data-bbox="203 277 1126 331">Figure 12 Photo of sample point 06. Whitewall Reen, Magor Marsh. Photo not taken at time of survey.</p>  	<p data-bbox="1200 277 2027 491">Sample point 06 was taken from a reen on the eastern boundary of Magor Marsh SSSI, between two bridges in front of the Derek Upton Centre. The reen is open and approximately 3-5m wide and 2-3m deep from the banktop. The banks are fringed with vegetation and <i>Typha latifolia</i> readily colonises the reen at this location, as can be seen in the photo (figure 7), though this was not taken at the time of survey.</p> <p data-bbox="1200 529 2027 810">This reen was selected as one that had been recently cleared and strimmed by Natural Resources Wales in November 2021. The reen banks had revegetated well with typical plants by the time of the survey. The reen is bound to the east by the Magor Marsh Nature Reserve car park, and to the north by a marshy grassland field which receives an annual haycut but no grazing. As such there is no poaching along this section of reen. This reen undergoes quite extreme seasonal fluctuations in water levels, at times holding only a few inches of water.</p> <p data-bbox="1200 849 2027 1027">This was the least taxon-rich sample point of all 8 samples, with only 13 taxa detected, 5 of which were identified to species level. This site had taxon from two of the higher scoring BMWP families, including two taxa from the family Caenidae (small square-gill mayflies), which scores 7/10.</p>

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Enochrus testaceus</i>	(a water scavenger beetle)	Detected in 2 sample sites.	This species is in the family Hydrophilidae, which has a BMWP water quality rating of 5/10.
<i>Chaoborus pallidus</i>	(a phantom midge/glassworm)	Detected in 2 sample sites.	This species has only 4 Welsh records , 3 of them from Gwent. Interestingly, this species was the most abundant sequence picked up across all samples, representing 15.4% of all sequences, but found at only 2 sample points.
<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Caenis robusta</i>	(a small square-gill mayfly)	Detected in 4 sample sites.	There are 105 Gwent records for this species, which has been assigned the IUCN category Least Concern . This species is in the family Caenidae which has a value of 7/10 in the BMWP water quality metric, suggesting good water quality at this sample site.
<i>Caenis luctuosa</i>	Angler's Curse	Only detected at this site.	There are 21 Gwent records for this species, which has been assigned the IUCN category Least Concern . This species is in the family Caenidae which has a value of 7/10 in the BMWP water quality metric, suggesting good water quality at this sample site.
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			

Sample point 07	Description
<div data-bbox="206 242 698 593">  <p data-bbox="206 534 698 593">Title: Plan of aquatic invertebrate sampling transects on the Great Levels. Digitized by: A. D. Karim Date: September 2022 Reproduced by permission of Ordnance Survey on behalf of HMSO. © Crown Copyright and Database right 2020. All rights reserved. Ordnance Survey Licence number: 100015400.</p> </div> <div data-bbox="206 274 1104 1157">  </div> <p data-bbox="206 1173 1176 1225"><i>Figure 13 Photo of sample point 7. Rushwall South Reen (eastern end looking west). Photo not taken at time of survey.</i></p>	

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Lumbricus rubellus</i>	Red Worm (a terrestrial worm)	Detected in 3 sample sites.	
<i>Chaetogaster diastrophus</i>	(a clitellate oligochaete worm)	Detected in 2 sample sites.	This species has no known UK records on NBN , and no Welsh/Gwent records, however GBIF shows 2 possible records, in Anglesey and one near the Chilterns.
<i>Nartus grapii</i>	(a predaceous diving beetle)	Detected in 3 sample sites.	Nature Metrics listed this sequence as a 99.3% match for <i>Rhantus grapii</i> . Searching online suggests that the accepted name is <i>Nartus grapii</i> . Searching the various databases for the latter showed many UK, Wales and Gwent records (whereas <i>Rhantus grapii</i> was not found). This species is in the family Dytiscidae, which has a BMWP water quality rating of 5/10.
<i>Dytiscus marginalis</i>	Great Diving Beetle	Only detected at this site.	This species is in the family Dytiscidae, which has a BMWP water quality rating of 5/10.
<i>Liopterus haemorrhoidalis</i>	(a predaceous diving beetle)	Only detected at this site.	This species is in the family Dytiscidae, which has a BMWP water quality rating of 5/10.
<i>Hydrophilus piceus</i>	Great Silver Water Beetle	Only detected at this site.	There are 116 Welsh records for this species, with 103 of these being Gwent records. It has been assigned the IUCN category Near Threatened . This species is in the family Hydrophilidae, which has a BMWP water quality rating of 5/10.
<i>Scirtes hemisphaericus</i>	(a marsh beetle)	Detected in 2 sample sites.	
<i>Palpomyia lineata</i>	(a biting midge)	Detected in 4 sample sites.	This species has only 1 Gwent record, 3 Welsh records and 23 UK records .

<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Corynoneura carriana</i>	(a non-biting midge)	Detected in 3 sample sites.	There are <u>no Welsh records</u> for this species and only <u>13 records for the UK</u> .
<i>Corynoneura scutellata</i>	(a non-biting midge)	Detected in 5 sample sites.	This species has <u>only 5 records in Gwent and 9 in Wales</u> . It was one of the most commonly detected species, occurring in 5/6 sample sites.
<i>Cricotopus caducus</i>	(a non-biting midge)	Only detected at this site.	There are <u>no UK records</u> for this species. Limited GBIF records (8) are localised to Finland and Sweden. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Dicrotendipes lobiger</i>	(a non-biting midge)	Detected in 3 sample sites.	There are only <u>3 Welsh records</u> and <u>no records in Gwent</u> .
<i>Kiefferulus tendipediformis</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>no Welsh records</u> for this species and only <u>20 records for the UK</u> .
<i>Limnophyes natalensis</i>	(a non-biting midge)	Only detected at this site.	There are <u>only 10 UK records</u> for this species, <u>no records in Wales/Gwent</u> . Nature Metrics listed the sequence as a 99.3% match for this species.
<i>Parachironomus parilis</i>	(a non-biting midge)	Detected in 3 sample sites.	There are <u>only 20 UK records</u> for this species, <u>no records in Wales/Gwent</u> .
<i>Paraphaenocladus impensus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>no Welsh records</u> for this species.
<i>Ptychoptera contaminata</i>	(a phantom crane fly)	Detected in 3 sample sites.	
<i>Copromyza stercoraria</i>	(a lesser dung fly)	Only detected at this site.	

<i>Ranatra linearis</i>	Water Stick-insect	Only detected at this site.	There are 153 Welsh records for this species, with 40 of these being Gwent records. It has been assigned the IUCN category Least Concern . This species is in the family Nepidae, which has a BMWP water quality rating of 5/10. Nature Metrics reported that the sequence was a 99.29% match for this species.
<i>Chartoscirta cincta</i>	(a true bug)	Only detected at this site.	There are 378 Welsh records for this species, with 55 of these being Gwent records. It has been assigned the IUCN category Least Concern .
<i>Philotarsus picicornis</i>	(a booklouse)	Detected in 3 samples sites.	This species has <u>only 4 UK records, none in Gwent/Wales</u> , however the aggregate species listed in Aderyn shows 12 Welsh records, with two of these within Gwent.
<i>Trienodes bicolor</i>	(a caddis fly)	Only detected at this site.	There are 217 Welsh records for this species, with 46 of these being Gwent records. It has been assigned the IUCN category Least Concern . This species is in the family Leptoceridae, which is one of the highest rated families in the BMWP water quality metric – 10/10.
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			
Sample point 08		Description	



Figure 14 Sample point 08, West Mead Reen. Bridewell Common. (C) Andy Karran

This sample point was taken from a reen that bisects Bridewell Common Nature Reserve, West Mead Reen. It runs west to east and is open along its length. It is approximately 3m wide and 2-3m deep, being shallower at the western end and deeper at the eastern end.

The reen banks are lush with typical water-loving plants and trees are scattered in places, a mix of pollarded willow, poplar and hawthorn, appearing on one bankside only, wherever present. The water level is generally stable throughout the year, with a depth of approximately 0.5-1.5m. The site was acquired by the Trust in 2019/20 and has since been brought into regular management, with late summer haycuts and aftermath grazing through autumn/winter. These practices were more sporadic prior to GWT's acquisition of the site.

In 2021 a number of very large poplar trees at the far western end of the reen were pollarded. This area used to suffer with shading and considerable leaf litter accumulation, but is now much more open and light.

This sample site was the fourth most taxon-rich site, with 46 taxon detected, of which 18 were identifiable to the species level. This site had taxon from four of the higher scoring BMWP families, including one taxon which scores 7/10.

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Haliphus ruficollis</i>	(a crawling water beetle)	Only detected at this site.	Nature Metrics reported that the sequence was a 98.59% match for this species.
<i>Scirtes hemisphaericus</i>	(a marsh beetle)	Detected in 2 sample sites.	
<i>Pegomya flavifrons</i>	(a root maggot fly)	Only detected at this site.	There were <u>no Gwent records</u> for this species and only 10 in Wales.
<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Corynoneura carriana</i>	(a non-biting midge)	Detected in 3 sample sites.	There are <u>no Welsh records</u> for this species and only <u>13 records for the UK.</u>
<i>Corynoneura scutellata</i>	(a non-biting midge)	Detected in 5 sample sites.	This species has <u>only 5 records in Gwent and 9 in Wales.</u> It was one of the most commonly detected species, occurring in 5/6 sample sites.
<i>Chironomus cingulatus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>no Welsh records</u> for this species and only <u>21 records for the UK.</u> Nature Metrics reported that the species identification was based on limited reference matches.
<i>Cricotopus sylvestris</i>	(a non-biting midge)	Only detected at this site.	
<i>Cricotopus relucens</i>	(a non-biting midge)	Only detected at this site.	There were <u>no UK records</u> for this species. Limited (21) GBIF records were localised to Finland and Montenegro. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Dicrotendipes lobiger</i>	(a non-biting midge)	Detected in 3 sample sites.	There were <u>no Gwent records</u> for this species and <u>only 3 Welsh records.</u>

<i>Eukiefferiella claripennis</i>	(a non-biting midge)	Only detected at this site.	There was <u>only 1 record in Gwent</u> .
<i>Limnophyes prolongatus</i>	(a non-biting midge)	Only detected at this site.	There were <u>no UK records</u> for this species name, however searching the synonym <i>Limnophyes pentaplastus</i> showed <u>3 records in Wales, 1 record for Gwent</u> and <u>24 records for the UK</u> .
<i>Tanytarsus eminulus</i>	(a non-biting midge)	Detected in 2 sample sites.	There are <u>only 5 UK records</u> for this species, with <u>1 record in Wales (in Gwent)</u> .
<i>Dicraeus vagans</i>	(a glass fly)	Detected in 3 sample sites.	
<i>Chrysotus femoratus</i>	(a long-legged fly)	Detected in 2 sample sites.	This species has <u>only one Gwent record</u> .
<i>Ptychoptera contaminata</i>	(a phantom crane fly)	Detected in 3 sample sites.	
<i>Caenis robusta</i>	(a small square-gill mayfly)	Detected in 4 sample sites.	There are 105 Gwent records for this species, which has been assigned the IUCN category Least Concern . This species is in the family Caenidae which has a value of 7/10 in the BMWP water quality metric, suggesting good water quality at this sample site.
<i>Nepa cinerea</i>	Water Scorpion	Detected in 2 sample sites.	This species is Least Concern on the UK Red List. There are many Gwent, Welsh, UK records. This species is in the family Nepidae, which has a BMWP water quality rating of 5/10.
<i>Philotarsus picicornis</i>	(a booklouse)	Detected in 3 samples sites.	This species has <u>only 4 UK records, none in Gwent/Wales</u> , however the aggregate species listed in Aderyn shows 12 Welsh records, with two of these within Gwent.
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			
Sample point 09		Description	

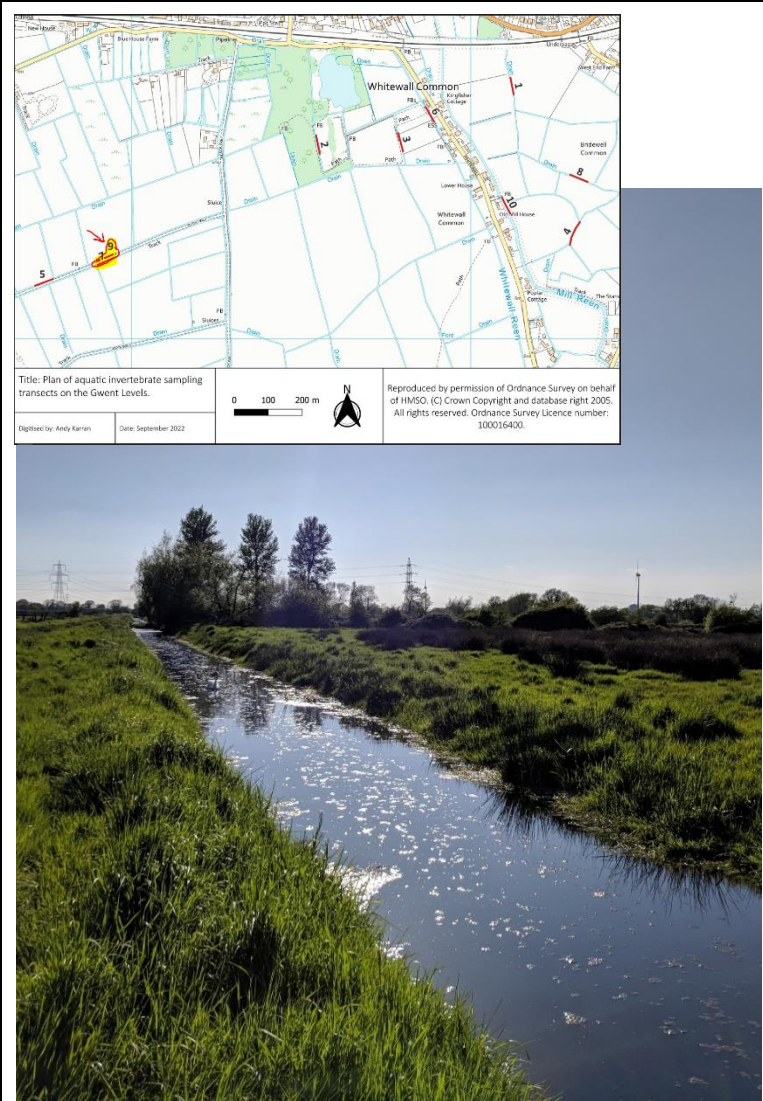


Figure 15 Photo of sample point 09. Rushwall North Reen (eastern end, looking west). Photo not taken at time of survey.


Sample point 09 was taken from Rushwall North reen, out on Barecroft Common. This sample was taken just 100m east of sample point 05, on the same reen and opposite, sample point 07 which is just across a vegetated access track. The reen is approximately 3-5m wide and with a similar depth from the bank top. It is open along its full length, with typical reen vegetation flanking both banks. The northern bank (right in the photo) is part fenced and has scattered scrub and trees, whereas the southern bank (left in the photo) is absent of any scrub/trees, forming part of a long access track.

The land visible on the right side of the reen in this photo is GWT’s Magor Marsh Nature Reserve and is a rush-dominated marshy grassland, with scattered bramble scrub. It is grazed by cattle for part of the year and the banks have intermittent poaching.

Sample point 09 was the third least taxon-rich sample out of nine sites, with 27 taxa identified, of which 5 could be identified to the species level. This site had taxon from two of the higher scoring BMWP families, including one taxon from the family Caenidae, which scores 7/10.

Species detected at this sample point:

Species name	Common name	Sample distribution	Notes
<i>Palpomyia lineata</i>	(a biting midge)	Detected in 4 sample sites.	This species <u>has only 1 Gwent record, 3 Welsh records and 23 UK records.</u>
<i>Acricotopus lucens</i>	(a non-biting midge)	Detected in 6 sample sites.	This was one of the most commonly detected species, across all sample sites.
<i>Chironomus melanotus</i>	(a non-biting midge)	Detected in 4 sample sites.	This species has <u>no known UK records.</u> Limited GBIF records are localised to Finland. Nature Metrics reported that the sequence was a 100% match for this species.
<i>Psectrocladius limbatellus</i>	(a non-biting midge)	Only detected at this site.	
<i>Caenis robusta</i>	(a small square-gill mayfly)	Detected in 4 sample sites.	There are 105 Gwent records for this species, which has been assigned the IUCN category Least Concern . This species is in the family Caenidae which has a value of 7/10 in the BMWP water quality metric, suggesting good water quality at this sample site.
<i>Valenzuela flavidus</i>	(a booklouse)	Detected in 2 sample sites.	
A number of further taxa were detected, identifiable only to the Genus or Family level. Please refer to the attached spreadsheet for the full list.			

Context for points 05, 07 and 09	Description
 A landscape photograph showing a narrow access track between two reens (Rushwall South and Rushwall North) under a bright sun. The track is a narrow strip of land, approximately 5m wide, running between two larger bodies of water. The sun is high in the sky, creating a bright glare. The surrounding area is grassy and appears to be a rural or agricultural setting. In the distance, there are power lines and a few trees.	<p>This photo (not taken at the time of survey) has been included to give context to the proximity of sample points 05, 07 and 09. The reen on the left of the photo is Rushwall South and to the right is Rushwall North. The land between the reens (approx. 5m wide) is an infrequently used access track, which runs for 3km between Blackwall track in the east and North Row in the west.</p>

3.4 Comparison with Kirby Survey (2010)

Of the three overlapping sample points between Kirby’s 2010 survey and our 2022 eDNA survey, only two were successfully amplified – sample points 03 and 06.

Our sample from point 03 detected a total of 23 taxa, six identified to species level. Kirby’s sample at this location detected 43 taxa, 42 were to species level. There were two species recorded by both Kirby’s 2010 sample and the 2022 eDNA sample. These were both beetles - *Nartus grapiii* (a predaceous diving beetle) and *Enochrus testaceus* (a water scavenger beetle). Additionally, Kirby recorded two pond snail species at this location and the eDNA sampling detected the pond snail family (Lymnaeidae), however failed to identify to any lower taxonomic level.

Our sample from point 06 detected a total of 13 taxa, five identified to species level. Kirby’s sample at this location detected 21 taxa, 19 identified to species level. There were no direct matches in the taxa recorded between the two surveys. Kirby recorded two pond snail species at this location and the eDNA sampling detected the pond snail family (Lymnaeidae), however failed to identify to any lower taxonomic level.

3.5 Biological Monitoring Working Party (BMWP) taxa

Seventeen species were detected from 10 invertebrate families that score 5 or more in the BMWP macroinvertebrate scoring system for the assessment of water quality. A further two taxa were detected to family level only. The families detected and their BMWP values were: Chrysomelidae (leaf beetles) (5); Dytiscidae (predaceous diving beetles); Haliplidae (crawling water beetles); Hydrophilidae (water scavenger beetles); Tipulidae (crane flies); Caenidae (small square-gill mayflies) (7); Corixidae (water boatmen); Naucoridae (saucer bugs); Nepidae (water scorpions and water stick-insects); Leptoceridae (caddis flies) (10).

Although our sampling approach was not consistent with that of BMWP system, I have applied the scoring system to the taxa detected through eDNA analysis. The individual species and total BMWP scores for each sample site can be seen in [Table 2](#) below. The scores for families detected but not identified to species level are included in brackets but not in the calculation. An alternative calculation known as the ASPT is also included, this takes the BMWP total and divides by the number of species identified. Both scoring systems are reported to have limitations.

Table 2 BMWP scoring

Sample no.	Species No.	Family no.	Species BMWP values	BMWP total	ASPT total
01	7	4	5 + 5 + 5 + 5 + 5 + 5 + 5 (+ 5)	35	5
03	2	2	5 + 5	10	5
04	3	4	5 + 5 + 5 (+ 5)	15	5
05	1	3	7 (+ 5 + 5)	7	7
06	3	2	7 + 7 + 5	19	6
07	7	6	10 + 5 + 5 + 5 + 5 + 5 + 5 (+ 5 + 5)	40	6
08	3	4	7 + 5 + 5 (+ 5)	17	6
09	1	2	7 (+ 5)	7	7

3.6 Taxa with Conservation Designations

Eleven taxa detected in the eDNA samples were listed in the JNCC [Conservation Designations for UK Taxa spreadsheet](#), which collates conservation designation listings, such as Red Lists, UK Priority Species etc. All eleven of the species were listed for their UK IUCN Red List statuses, with one species also listed as Nationally Scarce.

Ten species had been assessed and placed in the lowest threat category, **Least Concern**. A taxon is Least Concern when it has been evaluated against the criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category. The species were: *Rhagonycha fulva* (Common Red Soldier Beetle), *Amphimallon solstitiale* (Summer Chafer), *Chrysops viduatus* (Square-spot Deerfly), *Caenis luctuosa* (Angler's Curse), *Caenis robusta* (a small square-gill mayfly), *Hesperocorixa sahlbergi* (a water boatman), *Nepa cinerea* (Water Scorpion), *Ranatra linearis* (Water Stick-insect), *Chartoscirta cincta* (a true bug), and *Triaenodes bicolor* (a caddis fly).

Additionally, *Chrysops viduatus*, was also listed as **Nationally Scarce**. This means it occurs in only 16-100 hectads in Great Britain. This designation was made in 2017, based on an assessment in 'A Review of the status of the Larger Brachycera flies of Great Britain'.

One species, *Hydrophilus piceus*, (Great Silver Water Beetle), a water scavenger beetle, was assessed and assigned to the second lowest threat category, **Near Threatened**. This means it does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future. This species was detected at only one sample point – 07 Rushwall NE. Sample point 07 was the 3rd most taxon-rich location and one of the highest scoring locations when assessed using the Biological Monitoring Working Party metric.

The species, their designations and the number of sample points they were detected in can be seen in [Table 3](#). Further information on *Chrysops viduatus* and *Hydrophilus piceus* is provided below.

Table 3 Species detected listed in the JNCC Conservation Designations for UK Taxa spreadsheet.

Species	Common name	Taxon type	UK Designation	No. of samples
<i>Rhagonycha fulva</i>	Common red soldier beetle	A soldier beetle	Least Concern	1
<i>Hydrophilus piceus</i>	Great silver water beetle	A water scavenger beetle	Near Threatened	1
<i>Amphimallon solstitiale</i>	Summer chafer	A chafer	Least Concern	1
<i>Chrysops viduatus</i>	Square-spot deerfly	A deerfly	Least Concern, Nationally Scarce	1
<i>Caenis luctuosa</i>	Angler's curse	A small square-gill mayfly	Least Concern	1
<i>Caenis robusta</i>		A small square-gill mayfly	Least Concern	4
<i>Hesperocorixa sahlbergi</i>		A water boatman	Least Concern	1
<i>Nepa cinerea</i>	Water scorpion	A water scorpion	Least Concern	2
<i>Ranatra linearis</i>	Water stick-insect	A water stick-insect	Least Concern	1
<i>Chartoscirta cincta</i>		A true bug	Least Concern	1
<i>Triaenodes bicolor</i>		A caddis fly	Least Concern	1

Chrysops viduatus or the Square-spot Deerfly, is a species of horsefly in the Family Tabanidae. The species favours wet habitats, such as marshy grassland, mires, fens and wet woodland. The larvae feed on organic material on wet, peaty detritus and the adults feed on large mammals. The *Review of the status of the Larger Brachycera flies of Great Britain* reports that the species has been "Recorded in 71-99 hectads since 1990. Moved beyond NS under Criterion 7".



Figure 16 Photo of *Hydrophilus piceus*, showing silvery underside. Sampled by freshwater ecologist at Magor Marsh, Oct 2017.

Hydrophilus piceus or the Great Silver Water Beetle, is a species of predatory diving beetle in the family Hydrophilidae. The species favours drainage ditches, particularly those choked with vegetation such as ivy-leaved duckweed and with a fringe of common reed. The larvae feed on water snails and so conditions with an abundance of molluscs are needed. *H. piceus* has been recorded from 50 hectads in England and Wales from 1980 onwards. It appears to have contracted in range and threats listed in the *Review of the scarce and threatened Coleoptera of Great Britain. Part 3: Water beetles of Great Britain* are: “Loss of traditional grazing fen may result in loss of this species, particularly if drains are destroyed or become overgrown. Drainage of the Cambridgeshire Fens and the London Marshes in the 19th Century must have resulted in the greatest reductions in this species in Britain, indicating the potential for further decline.”

4. Limitations

This report represents the findings of what we consider to be a pilot study of the accessibility and efficacy of eDNA analysis in assessing the aquatic invertebrate interest of the reens and ditches of the Gwent Levels, and what this may be able to inform us about their condition. As such, there is much to discuss in the way of limitations, but much that has been learned through this exercise, allowing it to be improved upon in future.

We chose aquatic invertebrates to replace our planned dung beetle eDNA analysis, which could no longer be supported by the testing lab within the funding period. As such, we didn't have as long to research and plan this survey. We feel that our recording could be improved upon as a result, as we failed to record enough site data, which could have been used run analyses against the various features and management approaches of the different sample points. In future we would seek to accurately record variables, such as bank dimensions, slope, water depth, vegetation cover, bare ground cover, cutting and casting regime, grazing regime, weather conditions etc.

Another key limitation of this survey was the relatively small sample size. The sample size was dictated by the scale of funding available. In future we would like to roll out the survey on a far bigger scale, with many more sample points. This would allow for more robust data analyses and more meaningful conclusions to be drawn. Ideally these would look to sample in a more systematic way, perhaps applying the methodology discussed in Buglife's field survey manual "*A manual for the survey and evaluation of the aquatic plant and invertebrate assemblages of grazing marsh ditch systems*". We would like to incorporate a wider range of watercourse types and conditions, with more spread across the Gwent Levels landscape, and SSSIs.

We had minimal crossover between our sample points and those of past field surveys, this meant that we were unable to undertake much in the way of comparison between the two sampling techniques. The two sites that did crossover between our sampling and that of Peter Kirby's, did not yield many interesting findings, with only a couple of species in common. A larger sample size would allow us to undertake a more robust analysis and we would look to approach NRW, local authorities and others to identify past sampling sites, which we could replicate. An even more robust study would seek to undertake eDNA sampling and traditional field-survey sampling by an entomologist *at the same time*, allowing for direct comparison of the two methodologies.

Another limitation, was the time we had available to analyse the data. While surveying was carried out in July, the eDNA results did not arrive until part way through September. With our funding deadline and other work commitments, this did not leave much time to analyse and write up the report. Consequently, we have not been able to approach invertebrate specialists to verify our records and gain more context on their importance at the national, regional and local scales. This is something which we will complete after our submission of the report to the funder, so that we may report more widely on the findings and deliver the verified records to the South East Wales Biodiversity Record Centre.

The eDNA analysis is also limited in the scope of organisms it can detect. Hard bodied invertebrates, such as beetles shed less eDNA, so the results may represent an underestimate for this taxon group, among others. The technique we were instructed to use also meant sampling only took place from mid-water samples. While many macroinvertebrates use this niche, many more can be found elsewhere, such as the silty ditch bottom or amongst marginal plants. It is likely we missed out on sampling some taxon groups as a result. The eDNA technology is continually being developed, and though other

methods aren't yet ratified they may be available to us in future, allowing for more representative sampling.

As was mentioned in the methodology section, eDNA doesn't mix as well in still waters. All of our sample points were in still-sluggish water, as is typical of ditches and reens of the Gwent Levels. While we followed the instructions to take subsamples and mix these, it's possible that we ended up with a less representative sample of the aquatic invertebrate interest at our sample sites.

5. Conclusions

The purpose of this eDNA study was to seek cost effective, innovative solutions to make rapid assessments of biodiversity with potential future applications in Gwent Wildlife Trust's work, and more widely in that of NRW. This was our first experience using eDNA sampling to detect aquatic invertebrates in the ditch and reed system of the Gwent Levels. We consider this to have been a successful pilot study, as we were able to test the methodology and better understand the limitations and results that it can yield.

eDNA was successfully amplified from eight out of ten of our sampling sites, though the reason for the failure of two of these samples is unknown. The analysis detected 194 taxa, of which 78 were at least 99% similar to species in the global reference databases and were able to be identified to species level, with confidence. We discovered that bloodworms featured most heavily in the eDNA sequences, and were detected at all sample sites. A number of the taxa detected across samples may be newly recorded in the UK and require verification.

Additionally, a number of taxa from high scoring families in the Biological Monitoring Working Party macroinvertebrate scoring system for the assessment of water quality, including one species of long-horned caddisfly, which is attributed the highest score of 10/10. Adding up the full scores, revealed some sample sites scored much higher than others, but we must highlight that this was an improper application of the assessment, which requires a standardised field survey. Our eDNA results would have been much more skewed to soft-bodied invertebrates and so, unable to detect many of the hard-bodied organisms that are also indicative of high quality waters.

Due to various limitations discussed in the previous section, it is difficult to make many conclusions on what the species identified, the numbers and assemblages can tell us about the levels of biodiversity and condition of each of the samples sites. The overarching conclusion, is that we need to increase our sample size and record a wider range of standardised metrics in the field, to allow more complex analysis of the data. Future advances in the eDNA technology, may also further our ability to sample for a wider and more representative range of the important aquatic invertebrates of this region.

6. References

The following resources have been referenced in this report:

[Palmer, M., Drake, M., Stewart, N. A manual for the survey and evaluation of the aquatic plant and invertebrate assemblages of grazing marsh ditch systems. Version 6. Buglife.](#)

[A review of the status of Larger Brachycera flies of Great Britain. Species Status No.29 \(2017\). Natural England.](#)

[A review of the status of the beetles of Great Britain: The stag beetles, dor beetles, dung beetles, chafers and their allies. Species Status No. 31. \(2016\). Natural England.](#)

[Conservation Designations for UK Taxa – designations spreadsheet. JNCC.](#)

[Aderyn](#)

[GBIF](#) Global Biodiversity Information Facility