West Winch Housing Access Road

Environmental Statement Chapter 8: Annex 8.5 Aquatic Ecology Survey Report

Author: WSP

Document Reference: NCC/3.08.09

Version Number: 01

Date: November 2023

Contents

Tables

1 Aquatic Ecology Survey Report

1.1 Methodology

Desk Study

1.1.1 A desk study was undertaken in July 2022 for the Aquatic Ecology Scoping Report (WSP, 2022). The data gathered in that desk study was reviewed and confirmed to be up to date for the purpose of this report. The desk study was conducted to review relevant existing ecological baseline information available in the public domain, to obtain information held by relevant third parties and confirm the Survey Area. For the purpose of the desk-based exercise, records were collected from various radii as detailed below, based on hydrological connectivity to the assessed watercourses. Hydrological connectivity was determined using maps and aerial imagery.

Designated Sites

1.1.2 An online desk study of information relating to statutory sites within 5km of the Site was undertaken. Information was obtained from Multi Agency Geographical Information for the Countryside (MAGIC) website (Natural England, 2023).

Water Framework Directive

1.1.3 The current Water Framework Directive (WFD) status for the relevant watercourses was obtained from the Environment Agency's Catchment Data Explorer website (Environment Agency, 2023a).

Environment Agency Records

1.1.4 A search of the Environment Agency's Ecology and Fish Data Explorer was completed to identify any existing aquatic ecology survey data within 10km of the Proposed Scheme (Environment Agency, 2023b).

Fish Survey

1.1.5 The 'Survey Area', as it is referred to hereafter, includes Pierpoint Drain, located within or in close proximity to the Scheme Boundary.

Environmental DNA (e-DNA)

1.1.6 Traditional quantitative electric fishing surveys were scoped out due to the channel profile, steep banks and bankside vegetation cover constraining access to the watercourse (WSP, 2022). Instead, to gain an indicative understanding of the fish populations within this watercourse, water samples were taken at three strategic locations within the watercourse and analysed for fish environmental DNA (e-DNA) against an extensive reference library.

1.1.7 In aquatic environments, animals shed cellular material into the water via reproduction, saliva, urine, faeces, and skin cells. This DNA will persist for several weeks and can be collected through a water sample, which is then analysed to determine if the target species of interest are present.

1.1.8 Studies have shown this approach to be effective for inventorying fish in lakes and rivers (Civade *et al*., 2016; Hänfling *et al*., 2016; Olds *et al*., 2016; Valentini *et al*., 2016; Nakagawa *et al*., 2018).

Sample Collection

1.1.9 e-DNA samples were collected from three locations within Pierpoint Drain by suitably qualified and experienced aquatic ecologists. The sampling site national grid references (NGR) are provided in Table 1-1.

Table 1-1 – Fish e-DNA sampling locations

Site	National Grid Reference (NGR)
Pierpoint Drain Upstream	TF 63924 18148
Pierpoint Drain Ditch 10 Confluence	TF 63877 18154
Pierpoint Drain Downstream	TF 63713 18513

1.1.10 Each sample consisted of 2 litres of water collected from sub-sampling different habitat and flow types present within each watercourse sampled. The water was collected by a surveyor entering the margins of the watercourse and collecting water upstream of their position. The sample was collected using nitrile gloves, collecting as little sediment as possible, to avoid contamination.

1.1.11 The sample was filtered until 2 litres of water was sampled or to the point where no more liquid could be pushed through the filter. The amount of liquid filtered was recorded. The filter was then removed, a preservative added and capped before being returned to the laboratory for analysis.

1.1.12 This methodology follows NatureMetrics' standard operating procedure, which is consistent with the current draft of the BS EN 17805. Water sampling for capture of macrobial environmental DNA in aquatic environments (European Standard, 2023).

e-DNA Sample Analysis

1.1.13 The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample.

1.1.14 The extracted sample is then tested via real time polymerase chain reaction (PCR) (also called q-PCR) for each of the species selected in the analysis. This process amplifies a select part of DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this reaction is measured for fast and objective data analysis.

1.1.15 Consensus taxonomic assignments were made for each taxon using sequence similarity searches against the NCBI nt (GenBank) reference database. Assignments were made to the lowest possible taxonomic level where there was consistency in the matches. Conflicts were flagged and resolved manually. Minimum similarity thresholds of 99%, 97%, and 95% were used for species-, genus- and higher-level assignments respectively. In cases where there were equally good matches to multiple species, public records from the Global Biodiversity Information Facility (GBIF) were used to assess which were most likely to be present in the United Kingdom. Higher-

level taxonomic identifications or multiple potential identifications were reported in cases that could not be resolved in this way.

1.1.16 Taxa with low abundance (<0.02% or <10 reads) were removed, as was any unidentified, non-target, and common contaminant sequences.

1.1.17 The proportion of sequence reads per detected taxon is calculated. This metric is not an indicator of relative taxon abundance, as whilst it is a consequence of abundance, it is also impacted by factors such as biomass, activity, surface area, condition, distance from the physical sample, primer bias, and species-specific variation in the genome. High proportion of sequence reads can however be interpreted as lending greater confidence in detection.

1.1.18 There is lower support for the taxonomic identification when there are fewer than three matches to sequences in the reference database (NCBI nt GenBank), and/or limited geographic occurrence records for the taxon. Where this has occurred, confidence in the taxonomic assignment, and consequently absence/presence of the taxa, has been determined by habitat suitability/viability of the proposed taxa, supplemented by professional experience.

1.1.19 True positive controls, negatives and blanks are included in every analysis, and these have to be correct before any result is declared, therefore acting as additional quality control measures.

Aquatic Macroinvertebrate Survey

1.1.20 Aquatic macroinvertebrate surveys were undertaken on 15 November 2022 (autumn) and repeated on 15 March 2023 (spring). The sampling site NGRs are provided in Table 1-2.

Table 1-2 -Aquatic macroinvertebrate sampling locations

Site	NGR
Pierpoint Drain Upstream	TF 63924 18148
Pierpoint Drain Downstream	TF 63713 18513

1.1.21 Aquatic macroinvertebrate samples were collected using standard three-minute bankside sweep sampling methodology. These surveys were carried out using a standard sampling net (1mm mesh), with a one-minute timed hand search following the Environment Agency (2017) procedure. This sampling method conforms to BS EN ISO 10870:2012 Water Quality – Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters (British Standards Institution, 2012).

1.1.22 A standardised field sheet was completed to record details of channel and bank physical habitat (bank material, substrate, flow types, channel features, bank structure), riparian land use and potential sources of anthropogenic stress.

1.1.23 Samples were placed in one-litre sample pots, preserved in Industrial Denatured Alcohol (IDA) on site and transported to the laboratory for sorting and identification to Taxonomic Level 5, in adherence with Environment Agency (2014) procedures.

Biological Metrics

1.1.24 The use of biological metrics allowed the assignation of ecological values to the aquatic macroinvertebrate communities observed, and an assessment of pressures on those communities to be made.

River Invertebrate Classification Tool

1.1.25 The River Invertebrate Classification Tool (RICT) determines the ecological condition of a given watercourse based on a comparison of aquatic macroinvertebrate communities observed at each sampling location, with the aquatic macroinvertebrate communities observed at reference sites (Davy-Bowker *et al.*, 2007). RICT reference sites are deemed to be as close as

possible to pristine conditions and not impacted by environmental stressors such as pollution, habitat modification or flow stress. Reference sites provide an expected aquatic macroinvertebrate community score for that river type. The observed aquatic macroinvertebrate community score at a given watercourse is divided by the expected community score. Reference and bias adjustments are then applied to obtain the Ecological Quality Ratio (EQR). RICT can derive EQR scores for a number of biological metrics. These metrics are discussed further below.

Whalley, Hawkes, Paisley and Trigg

1.1.26 The Whalley, Hawkes, Paisley and Trigg (WHPT) metric (WFD UKTAG, 2014a) is based on the tolerance of different aquatic macroinvertebrates to organic pollution. Each aquatic macroinvertebrate family is assigned a score from -1.6 to 13, depending on their tolerance to pollution and abundance category (on a continuous scale, -1.6 is for highly abundant pollution-tolerant taxa, 13 is for highly abundant pollution-intolerant taxa) and an overall score is produced from the total. The WHPT index is widely used to determine the ecological water quality of running waters and specifically the detection of organic pollution. As such, any extrapolation of other water quality pressures should be undertaken with caution.

1.1.27 The Average Score Per Taxon (ASPT) is derived from the WHPT index. By dividing the total WHPT score by the number of scoring taxa present (NTAXA), the average score per taxon can be calculated. This metric is more easily comparable with other sites and enables an assessment of biological water quality that is less influenced by the presence of a greater proportion of low scoring taxa or sampling effort than the overall WHPT score. In both the case of WHPT score and ASPT, higher scores indicate better ecological quality.

Lotic-invertebrate Index for Flow Evaluation

1.1.28 Aquatic macroinvertebrates have specific requirements for flow conditions and can be used to determine not only predominant flow types (Extence *et al*.,

1999), but also changes in flow character. The Lotic-invertebrate Index for Flow Evaluation (LIFE) metric uses abundance data to assign a flow preference score to aquatic macroinvertebrate families present in a sample and an overall score for the sampling site can be interpreted as an abundance-weighted average score per taxon metric. The family-level LIFE score is calculated in RICT as a ratio of the observed/expected at reference sites (O/E) for the sample.

1.1.29 There are currently no WFD-related class boundaries for LIFE EQRs, but a threshold of 0.94 is used to indicate the presence of flow stressed aquatic macroinvertebrate communities (Environment Agency, 2012).

Proportion of Sediment-sensitive Invertebrates

1.1.30 The Proportion of Sediment-sensitive Invertebrates (PSI) metric acts as a proxy for the quantity of fine sediment at a site (Extence *et al.*, 2011). Aquatic macroinvertebrate species are assigned a fine sediment sensitivity rating that ranges from highly insensitive to highly sensitive to fine sediment. The PSI score is calculated as the percentage of sensitive taxa in the sample and used to indicate how sedimented a watercourse is, from minimally sedimented/un-sedimented to heavily sedimented (Table 1-3).

Table 1-3 – Proportion of sediment sensitive invertebrates (PSI) scores and interpretation

PSI Score	River bed condition
81 – 100	Minimally sedimented / un-sedimented
61 – 80	Slightly sedimented
41 – 60	Moderately sedimented
21 – 40	Sedimented
0 – 20	Heavily sedimented

1.1.31 There are currently no WFD-related class boundaries for PSI EQRs, but a threshold of 0.70 is used to indicate the presence of low stressed aquatic macroinvertebrate communities (Turley *et al.*, 2016).

Community Conservation Index

1.1.32 The diversity and conservation interest of an aquatic macroinvertebrate community at each sampling site can be represented by analysing species level data through the Community Conservation Index (CCI). The CCI incorporates elements of taxon rarity and richness to summarise the conservation value of aquatic macroinvertebrate communities (Chadd and Extence, 2004). Scores defined within Chadd and Extence (2004) are assigned to species within the sample to derive a total sample conservation score which infers a conservation value from the criteria listed in Table 1-4.

Table 1-4 – Community conservation index (CCI) scores and classification descriptions

Conservation Score	Conservation Classification	Description
0 ≤ 5	Low	Sites supporting only common species and/or a community of low taxon richness.
5 ≤ 10	Moderate	Sites supporting at least one species of restricted distribution and/or a community of moderate taxon richness.
10 ≤ 15	Fairly high	Sites supporting at least one uncommon species, or several species of restricted distribution and/or a community of high taxon richness.
15 ≤ 20	High	Sites supporting several uncommon species, at least one of which may be nationally rare and/or a community of high taxon richness.
> 20	Very high	Sites supporting several rarities, including species of national importance, or at least one extreme rarity (such as taxa included in the British RDBs) and/or a community of very high taxon richness (potentially of national significance and may merit statutory protection).

Water Framework Directive Aquatic Macroinvertebrate Classification

1.1.33 The WFD uses the pollution sensitivity (WHPT ASPT) and aquatic macroinvertebrate richness (WHPT NTAXA) EQR scores to determine

whether a watercourse meets Good Ecological Status, as required under the WFD.

1.1.34 There are five ecological status classes: Bad, Poor, Moderate, Good and High. Where an aquatic macroinvertebrate community is recorded at, or above Good Ecological Status, then biological or physical pressures including flow and anthropogenic pollution are not assumed to be affecting aquatic ecology.

1.1.35 Watercourses failing to meet Good Ecological Status for aquatic macroinvertebrates may be influenced by a variety of stressors, and EQRs can be interrogated to determine the likely cause of failure to meet Good Ecological Status. For WFD classification the lower scoring of these EQR scores determines the aquatic macroinvertebrate classification of a given site.

1.1.36 A relative WFD class was calculated from the aquatic macroinvertebrate community identified at each of the five sample locations for comparison purposes.

Macrophyte Survey

Field Survey

1.1.37 A macrophyte survey was undertaken on 08 June 2023. The sampling site grid references of the 100m surveyed reach are provided in Table 1-5.

Table 1-5 – Macrophyte survey location

Site	Upstream NGR	Downstream NGR
Pierpoint Drain	TF 63686 18364	TF 63706 18477

1.1.38 All surveys were carried out using the Water Framework Directive UK Technical Advisory Group's methodology for assessing macrophytes in rivers (WFD UKTAG, 2014b). This method conforms with CEN 14184: 2003 Water Quality – Guidance standard for the surveying of aquatic macrophytes in running waters. The methodology specifies that a 100m stretch of the

watercourse should be sampled between 01 June and 30 September and that sampling should not be completed during or immediately after high flows.

1.1.39 The presence of all macrophytes present with the Survey Area were recorded to species level where possible. Where this was not possible species were recorded under its genus or other aggregate taxon level.

1.1.40 The percentage of the river channel (up to the height of bank that would typically be submerged for >50 % of the year) covered by each species was estimated by assigning it an appropriate taxon cover value, as detailed in Table 1-6.

Table 1-6 – Cover values for lotic macrophyte taxa

Percentage cover range (% of channel area)	Taxon cover level	Mid-point percentage
<0.1	1	0.05
0.1<1	2	0.5
1<2.5	3	1.7
2.5<5	4	3.8
5<10	5	7.5
10<25	6	17.5
25<50	7	37.5
50<75	8	62.5
≥75	9	87.5

Biological Indices

1.1.41 The condition of the Pierpoint Drain macrophyte community within the surveyed reach was assessed by calculating various indices using data recorded during the field survey. These indices are detailed in the following paragraphs within this section.

River Macrophyte Nutrient Index (RMNI)

1.1.42 The RMNI is a measure of the plants that grow in the river and their association with high nutrient levels. It is measured on a scale from 1-10. Each scoring macrophyte taxon was assigned its corresponding RMNI species score. RMNI was then calculated using the equation:

$$RMNI = \frac{\sum_{j=1}^{n}(C_j x\ R_j)}{\sum_{j=1}^{n} C_j}$$

where:

- 'Rj' is the river macrophyte nutrient index score for taxon 'j';
- 'j' represents a scoring taxon and has a value of 1 to 'n' indicating which taxon it represents; and,
- 'Cj' is the taxon cover value for taxon 'j'.

Number of Macrophyte Taxa (NTAXA)

1.1.43 NTAXA is the number of truly aquatic (non-helophyte) scoring taxa recorded in the field survey, which is used as a measure of diversity.

Number of Functional Groups (NFG)

1.1.44 NFG is a diversity metric calculated by assigning all truly aquatic (non-helophyte) scoring taxa to one of 24 'functional groups'. The NFG value is given by the sum of the number of different functional groups of taxa that were identified as being present in the river.

Cover of Green Filamentous Algae (ALG)

1.1.45 ALG is the percentage cover of green filamentous algae over the whole survey section. This was calculated by adding up the mid-point percentage cover values for all algae species identified as being present.

1.1.46 The value for the parameter ALG represents the total coverage of the riverbed by green filamentous algae and will range from 0-100. This metric is used as a measure of nutrient enrichment.

River Predictions and Classification Systems for Macrophytes (LEAFPACS2)

1.1.47 The River LEAFPACS2 classification tool was used to contextualise RMNI, NTAXA, NFG, and ALG metric scores. Ecological Quality Ratios (EQRs) are derived from these metrics based on observed data and site-specific predicted reference values derived from the physical and chemical parameters listed in Table 7.

1.1.48 EQRs are normalised so they fit the same scale and combined to provide an overall EQR representing an ecological status class as defined by the WFD ('High', 'Good', 'Moderate', 'Poor' and 'Bad'). The class boundaries are outlined in Table 1-8 below.

Table 1-7 – Predictive reference parameters for LEAFPACS2

Invariant data	**Variant data**
NGR	Alkalinity
Slope	-
Distance from source	-
Altitude	-

Table 1-8 – River LEAFPACS2 class boundaries

Status class boundary	**EQR**
High/Good	0.8
Good/Moderate	0.6
Moderate/Poor	0.4
Poor/Bad	0.2

Ellenberg Light Indicator Values

1.1.49 Ellenberg light indicator values score flora along gradients reflecting various habitat preferences (Ellenberg *et al*., 1991). The values and associated tolerances are described in Table 1-9 below.

Table 1-9 – Ellenberg light indicator values and descriptions (Ellenberg *et al.*, 1991)

Value	Description
1	Plant in deep shade
2	Between 1 and 3
3	Shade plant, mostly less than 5% relative illumination, seldom more than 30% illumination when trees are in full leaf
4	Between 3 and 5
5	Semi-shade plant, rarely in full light, but generally with more than 10% relative illumination when trees are in leaf
6	Between 5 and 7
7	Plant generally in well-lit places, but also occurring in partial shade
8	Light-loving plant rarely found where relative illumination in summer is less than 40%
9	Plant in full light, found mostly in full sun

Notes and Limitations

1.1.50 Every effort has been made to provide a comprehensive description of the Study Area; however, the following specific limitations apply to this assessment:

- Ecological survey data is typically valid for 12 to 18 months unless otherwise specified. The likelihood of surveys needing to be updated increases with time and is greater for mobile species or in circumstances where the habitat or its management has changed significantly since the surveys were undertaken. Factors to be considered include (but are not limited to): whether a site supports, or may support, a mobile species which could have moved on to site, or changed its distribution within a site (CIEEM, 2019).
- e-DNA data cannot provide information on the age structure or provide information on the size of fish populations within a water body; however, they can provide information of the species composition of a

fish community. These data provide valuable information on the presence of protected and notable fish species. As such, the use of e-DNA data to determine the fish baseline condition and inform the impact assessment and necessary mitigation measures were considered a reasonable alternative to traditional electric fishing surveys.

- The aquatic macroinvertebrate sampling methods used were selected to provide the data necessary for the calculation of a range of biological quality indices. It is not intended that the sampling methods will capture a full list of all species present within the watercourses, which will vary according to season and abundance of individual species. Identification to species level is not always possible where juvenile or damaged specimens are present in the sample, or where identification to species level is not standard practice. Nevertheless, through the calculation of appropriate indices, it is possible to evaluate the biological quality of the water body in relation to others.
- Records held by local biological record centres and local recording groups are generally collected on a voluntary basis. Therefore, the absence of records does not demonstrate the absence of species, it may simply indicate a gap in recording coverage.

1.2 Results

Desk Study

Designated Nature Conservation Sites

1.2.1 There is one statutory designated site with aquatic species as a primary reason for selection or as a qualifying feature, within 2km of the Study Area:

- River Nar SSSI.

1.2.2 Designation details relating to the River Nar SSSI are summarised in Table 1-10.

Table 1-10 – Statutory designated sites within 2km of the Study Area

Site	Designation	Size (ha)	Approximate Distance and Orientation from Site	Description
River Nar	SSSI	233.43	1.30km west	The River Nar originates as a spring-fed stream, west of Mileham in Norfolk and flows for 42km before joining the River Great Ouse at Kings Lynn, where a sluice prevents the penetration of seawater at high tide. The River combines the characteristics of a southern chalk stream and an East Anglian fen river. Together with the adjacent terrestrial habitats, the Nar is an outstanding river system of its type. The upper Nar has a wide range of natural physical features incorporating riffles, pools, gravel beds and meanders, whilst the lower reaches below Narborough are embanked and steep sided with water flowing sluggishly through a predominantly arable flood plain. The variation in physical features and the influence of the underlying chalk give rise to a rich and diverse flora. Amongst the 78 species of riverine and bankside plants are many eutrophic and mesotrophic species, including 5 pondweeds and 8 bryophytes. The flora of the first 10km of the river, to West Lexham, is typical of a calcareous, lowland ditch community with an abundance of Starwort *Callitriche* spp. and Reed Sweetgrass, *Glyceria maxima*. The next 12km of the River, to Narborough Mill, is fast flowing over stoney substrates and is rich in chalk stream plants including Narrow-leaved Water Parsnip, *Berula erecta*; Mare's-tail, *Hippuris vulgaris*; Greater Tussock-sedge, *Carex paniculata*; Water Crowfoot, *Ranunculus pseudofluitans var. vertumnus* and Opposite-leaved Pondweed, *Groenlandia densa*. The wet margins, with a constantly high-water table typical of chalk streams, support a wide range of emergent plants. The final 18.5km is embanked and although less physically diverse than the upper reaches, it possesses a contrasting flora with several species not found in the upper river. These plants are characteristic of sluggish flows and include 3 pondweeds, *Potamogeton spp*.; 2 Water Crowfoots, *Ranunculus* spp.; Hornwort, *Ceratophyllum demersum*; Water-milfoil, *Myriophyllum spicatum*; and River Water-dropwort, *Oenanthe aquatica*. The Nar is well-known locally for its Brown Trout, *Salmo trutta*. Since 1985, Trout numbers have increased steadily; Pike, *Esox lucius*, numbers have remained fairly stable whilst Roach, *Rutilus rutilus*, and Eel, *Anguilla anguilla*, have continued to be the dominant species in the river. A further 11 species have been recorded in the Nar although they contribute only a small amount to the total fish biomass.

Water Framework Directive

1.2.3 There are three WFD-designated water bodies located within 2km of the Study Area, the Nar downstream of Abbey Farm Water Body (GB105033047792), Middleton Stop Drain Water Body (GB105033047670) and County Drain Water Body (GB105033047770) (Environment Agency, 2022a).

1.2.4 The 2019 WFD ecological status of the Nar downstream of Abbey Farm Water Body was Moderate overall. Fish and invertebrates were both classified as High. Macrophytes/phytobenthos were not assessed.

1.2.5 The 2019 WFD physico-chemical status of the Nar downstream of Abbey Farm Water Body was Moderate overall. Ammonia, phosphate and pH were classified as High. Temperature was classified as Good, and dissolved oxygen as Moderate. The reasons for the dissolved oxygen quality element not reaching Good status were not listed.

1.2.6 The 2019 WFD ecological status of the Middleton Stop Drain Water Body was also Moderate overall. Invertebrates and macrophytes/phytobenthos were classified as High and Moderate, respectively. The reason for the macrophytes/phytobenthos combined element not achieving Good status was listed as physical modification from land drainage.

1.2.7 The 2019 WFD physico-chemical status of Middleton Stop Drain Water Body was classified as Good. Acid neutralising capacity, phosphate, temperature and pH were classified as High. Ammonia and dissolved oxygen were classified as Good.

1.2.8 The 2019 WFD ecological status of the County Drain Water Body was Poor overall. Invertebrates and macrophytes/phytobenthos were classified as High and Poor, respectively. The reason for the macrophytes/phytobenthos combined element not achieving Good status was listed as point source pollution from continuous sewage discharge.

1.2.9 The 2019 WFD physico-chemical status of the County Drain Water Body was classified as Moderate. Ammonia, temperature and pH were classified as High. Phosphate was classified as Good and dissolved oxygen as Moderate. The reasons for the dissolved oxygen quality element not reaching Good status were not listed.

Environment Agency Fish Survey Data

1.2.10 A search of the Environment Agency's Ecology and Fish Data Explorer returned data from a single catch survey conducted in 2017 on the River Nar at NGR TF 67090 13498, approximately 3.7km south east of the Scheme Boundary.

1.2.11 A total of 67 fish, across nine species, were caught during the survey. The data are detailed in Table 11.

1.2.12 Three species of conservation interest were captured in the survey. Brown/sea Trout *Salmo trutta* is listed under Section 41 of the Natural Environment and Rural Communities (NERC) Act (2006) (HMSO, 2006) as a Species of Principal Importance (SPI). European Eel *Anguilla anguilla* is a migratory species listed under Section 41 of the NERC Act (2006) as a SPI and is further protected under The Eels (England and Wales) Regulations (2009) (HMSO, 2009). The species is also listed on the IUCN Red List of Threatened Species as being critically endangered (Jacoby and Gollock, 2014). River Lamprey *Lampetra fluviatilis* is a migratory species listed under Section 41 of the NERC Act (2006) as a SPI. The species is also afforded protection under Schedule 4 of The Conservation of Habitats and Species Regulations (2019) (HMSO, 2019).

Table 1-11 – Environment Agency fish survey data from the River Nar (NGR TF 67090 13498) conducted on 21/03/2017

Common Name	Latin Name	No. of Individuals
Gudgeon	*Gobio gobio*	27
Brown/sea Trout (Note 1)	*Salmo trutta*	10
European Eels > elvers (Note 1)	*Anguilla anguilla*	10
3-spined Stickleback	*Gasterosteus aculeatus*	9
Stone Loach	*Barbatula barbatula*	4
Perch	*Perca fluviatilis*	2
Bullhead	*Cottus gobio*	2
Pike	*Esox lucius*	2
River Lamprey (Note 1)	*Lampetra fluviatilis*	1
Total	Null	67

Note 1: Denotes protected/notable species.

Environment Agency Aquatic Macroinvertebrate Data

1.2.13 A search of the Environment Agency's Ecology and Fish Data Explorer returned results from aquatic macroinvertebrate surveys carried out in spring 2021 on the River Nar, at NGR TF 63588 13456, approximately 1.2km south of the Scheme Boundary.

1.2.14 Results from the most recent survey carried out in spring 2021 are detailed below in Table 12.

1.2.15 No legally protected aquatic macroinvertebrate species were recorded in the spring 2021 sample. Two invasive non-native species (INNS), the New Zealand Mudsnail *Potamopyrgus antipodarum* and the freshwater amphipod *Crangonyx pseudogracilis/floridanus* were identified in the sample.

Table 1-12 – Environment Agency biological metrics for samples collected on the River Nar (NGR TF 63588 13456) during spring 2021

Date	WHPT-ASPT	WHPT-NTAXA	LIFE (TL5)	PSI (TL5)	CCI
12/03/2021	4.73	27	6.65	22.45	7

1.2.16 The WHPT-ASPT score of 4.73 indicates that neither pollution tolerant nor intolerant taxa dominated the assemblage.

1.2.17 The LIFE score of 6.65 indicates the predominant presence of taxa primarily associated with slow to moderate flows.

1.2.18 The PSI score of 22.45 classifies the River Nar at this sampling location as Sedimented.

1.2.19 The CCI score of 7 classifies the River Nar at this sampling location as having an aquatic macroinvertebrate community of Moderate conservation value.

Environment Agency Macrophyte Survey Data

1.2.20 A search of Environment Agency's Ecology and Fish Data Explorer returned data from a survey conducted in 2019 from a location on Country Drain at NGR TF 67716 14049, approximately 4.2km east of the Scheme Boundary.

1.2.21 A total of 16 taxa were recorded in the survey; 13 flowering macrophyte species, two algal species, and one Horsetail species. No protected macrophyte taxa, nor any INNS were recorded (Table 1-13).

Table 1-13 – Environment Agency macrophyte survey taxon list for County Drain (NGR TF 67716 14049) on 19/07/2019

Common Name	Latin Name	Percentage Cover Bands
Green Algae	*Cladophora glomerata/Rhizoclonium hieroglyphicum*	9
Branched Bur-reed	*Sparganium erectum*	7

Common Name	Latin Name	Percentage Cover Bands
Reed Sweet Grass	*Glyceria maxima*	6
Common Duckweed	*Lemna minor*	6
Arrowhead	*Sagittaria sagittifolia*	6
Unbranched Bur-reed	*Sparganium emersum*	5
Watercress	*Rorippa nasturtium-aquaticum agg.*	5
Lesser Water Parsnip	*Berula erecta*	4
Reed Canary Grass	*Phalaris arundinacea*	4
Clasping-leaf Pondweed	*Potamogeton perfoliatus*	3
Bentgrass	*Agrostis sp.*	2
Flexuous Gutweed	*Enteromorpha flexuosa*	2
Hairy Willowherb	*Epilobium hirsutum*	2
Pink Water-speedwell	*Veronica catenata*	2
Fool's Water-cress	*Apium nodiflorum*	1
Common Horsetail	*Equisetum arvense*	1

Fish Survey

Pierpoint Drain Upstream

1.2.22 The e-DNA of a total of two fish taxa was detected at the Pierpoint Drain upstream sampling location. The relative proportion of the sequences found in the sample is detailed in Table 14. No e-DNA of any legally protected or otherwise notable fish species, nor any invasive non-native fish species, was detected in the sample.

Table 1-14 – The proportion of sequencing output allocated to the fish taxa identified at Pierpoint Drain Upstream

Common Name	Latin Name	Percentage Composition (%)
Stickleback species (Note 1)	Pungitius sp.	60.38
Three-spined Stickleback	Gasterosteus aculeatus	39.62

Note 1: Likely to be the native Nine-spined Stickleback *Pungitius pungitius*

Pierpoint Drain Ditch 10 Confluence

1.2.23 The e-DNA of a total of seven fish taxa was detected at the Pierpoint Drain downstream sampling location. The relative proportion of the sequences found in the sample is detailed in Table 15. No e-DNA of any legally protected or otherwise notable fish species, nor any invasive non-native fish species, was detected in the sample.

1.2.24 Common Carp *Cyprinus carpio* is not regarded as an INNS, however, the species is not native to England.

Table 1-15 – The proportion of sequencing output allocated to the fish taxa identified at Pierpoint Drain Ditch 10 Confluence

Common Name	Latin Name	Percentage Composition (%)
Stickleback species (Note 1)	*Pungitius sp.*	55.03
Three-spined Stickleback	*Gasterosteus aculeatus*	43.29
Common Roach	*Rutilus rutilus*	0.97
Common Rudd	*Scardinius erythrophthalmus*	0.45
Carp species	*Cyprinus sp.*	0.12
Gudgeon	*Gobio gobio*	0.09
Common Carp	*Cyprinus carpio*	0.04

Note 1: Likely to be the native nine-spined stickleback.

Pierpoint Drain Downstream

1.2.25 The e-DNA of a total of eight fish taxa was detected at the Pierpoint Drain downstream sampling location. The relative proportion of the sequences found in the sample is detailed in Table 1-16**.** No e-DNA of any legally protected or otherwise notable fish species, nor any invasive non-native fish species, was detected in the sample.

Table 1-16 – The proportion of sequencing output allocated to the fish taxa identified at Pierpoint Drain Downstream

Common Name	**Latin Name**	**Percentage Composition (%)**
Stickleback species (Note 1)	*Pungitius sp.*	65.98
Three-spined Stickleback	*Gasterosteus aculeatus*	32.28
Common Rudd	*Scardinius erythrophthalmus*	0.83
Common Dace (Note 2)	*Leuciscus leuciscus*	0.27
Common Roach	*Rutilus rutilus*	0.26
Northern Pike	*Esox lucius*	0.18
Gudgeon	*Gobio gobio*	0.12
European Perch	*Perca fluviatilis*	0.09

Note 1: Likely to be the native Nine-spined Stickleback.

Note 2: There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon

Aquatic Macroinvertebrate Survey

Biological Metrics

1.2.26 The full aquatic macroinvertebrate taxon list is presented in Appendix A. Images of sampling locations are displayed in Appendix B.

1.2.27 The biological metrics calculated for each site based on the aquatic macroinvertebrate communities present in autumn 2022 and spring 2023 are displayed in Table 1-17.

Table 1-17 – Biological metrics for the two aquatic macroinvertebrate sampling sites in autumn 2022 and spring 2023

Site	Season	WHPT-ASPT (TL2)	WHPT-NTAXA (TL2)	LIFE (O) (TL5)	LIFE (E) (TL5)	LIFE EQR	PSI (O) (TL5)	PSI (E) (TL5)	PSI EQR	CCI (TL5)
Pierpoint Drain Upstream	Spring	4.27	18	5.67	5.80	0.98	2.50	5.15	0.49	17.77
Pierpoint Drain Upstream	Autumn	3.67	18	5.88	5.70	1.03	2.86	4.89	0.58	11.50
Pierpoint Drain Downstream	Spring	4.55	31	5.77	5.79	1.00	4.76	4.95	0.96	17.82
Pierpoint Drain Downstream	Autumn	3.52	22	5.68	5.69	1.00	0.00	4.73	0.00	4.80

1.2.28 Across both sites, 54 different taxa were identified. Of these, the Pierpoint Drain downstream sampling location displayed the greatest diversity of WHPT scoring taxa.

1.2.29 The observed LIFE scores suggest the predominant presence of taxa associated with standing to slow flowing water at all sites, in both autumn 2022 and spring 2023.

1.2.30 The observed PSI scores classify both sampling locations within Pierpoint Drain as being heavily sedimented in both autumn 2022 and spring 2023.

1.2.31 The observed CCI scores classify the Pierpoint Drain upstream sampling location as having an aquatic macroinvertebrate community of Fairly High conservation value in autumn 2022 and of High conservation value in spring 2023. The observed CCI scores classify the Pierpoint Drain downstream sampling location as having an aquatic macroinvertebrate community of Low conservation value in autumn 2022 and of High conservation value in spring 2023.

River Invertebrate Classification Tool

1.2.32 RICT analysis was performed to produce indicative WFD classification scores for aquatic macroinvertebrates; outputs are summarised in Table 1-18.

Table 1-18 – RICT output for the five aquatic macroinvertebrate sampling sites in spring and autumn 2022

Site	Index	Spring EQR	Autumn EQR	Combined EQR	Overall classification	Confidence of class (%)
Pierpoint Drain Upstream	WHPT-ASPT	1.05	0.94	0.99	Good	59.04
Pierpoint Drain Upstream	WHPT-NTAXA	0.73	0.75	0.74	Good	59.04
Pierpoint Drain Downstream	WHPT-ASPT	1.10	0.90	1.00	High	67.22
Pierpoint Drain Downstream	WHPT-NTAXA	1.20	0.90	1.05	High	67.22

1.2.33 The aquatic macroinvertebrate community at the Pierpoint Drain upstream sampling location was indicative of Good WFD status, whilst the community at the downstream sampling location was indicative of High WFD status.

Aquatic Macroinvertebrate Assemblage and Conservation Status

1.2.34 The Pierpoint Drain upstream spring sample was dominated by the amphipod *Crangonyx pseudogracilis/floridanus* and the Faucet Snail *Bithynia tentaculata*. The autumn sample was dominated by non-biting midge Chironomidae and Pea Clam *Pisidium* sp.

1.2.35 The Pierpoint Drain downstream spring and autumn samples were dominated by the Pond Olive Mayfly *Cloeon dipterum*, with the autumn sample also comprising large numbers of annelid worms.

1.2.36 One INNS, the amphipod *Crangonyx pseudogracilis/floridanus* agg, was recorded in all samples taken from both the upstream and downstream sampling locations.

1.2.37 Three species of note under CCI scoring, the Water Scavenger Beetles *Berosus affinis* and *Helochares lividus* and the Black Moss Beetle *Hydraena nigrita* were identified in the samples, detailed in Table 1-19.

1.2.38 Three individuals of the Water Scavenger Beetle *Berosus affinis* were identified in the spring 2023 upstream sample, with a further eight individuals recorded in the autumn 2023 downstream sample. The beetle has a conservation score of 7 and as such is Notable (scarce in Great Britain but not of Red Data Book status).

1.2.39 Two individuals of the Water Scavenger Beetle *Helochares lividus* were identified in the spring 2023 downstream sample. The beetle has a conservation score of 7 and as such is Notable (scarce in Great Britain but not of Red Data Book status).

1.2.40 Three individuals of the Black Moss Beetle *Hydraena nigrita* were identified in the spring 2023 downstream sample. The beetle has a conservation score of

7 and as such is Notable (scarce in Great Britain but not of Red Data Book status).

Table 1-19 – Aquatic macroinvertebrates identified with a Conservation Score of six or greater

Latin name	Common name	Conservation Score	Status
Berosus affinis	Water Scavenger Beetle	7	Notable (but not Red Data Book status)
Helochares lividus	Water Scavenger Beetle	7	Notable (but not Red Data Book status)
Hydraena nigrita	Black Moss Beetle	7	Notable (but not Red Data Book status)

Macrophyte Survey

1.2.41 Pierpoint Drain within the Survey Area had a mean width of approximately 4m and had a water depth greater than 1m for 100% of the surveyed reach.

1.2.42 Images of the surveyed stretch of Pierpoint Drain are displayed in Appendix D.

1.2.43 A total of 10 macrophyte taxa were recorded, eight of which are LEAFPACS2 scoring taxa. The majority of the Survey Area was dominated by macrophytes with an Ellenberg light indicator value of 7.

1.2.44 No protected or otherwise notable macrophyte species were recorded in the survey, however, one INNS, Nuttall's Waterweed *Elodea nuttallii* was recorded. The full macrophyte taxon list is presented in Appendix C.

1.2.45 Rigid Hornwort *Ceratophyllum demersum* was the most dominant species, accounting for 40% of the Survey Area's total macrophyte cover (Table 1-20). Broad-leaved Pondweed *Potamogeton natans* accounted for 30% of the total macrophyte coverage, followed by Nuttall's Waterweed (15%) and Shining

Pondweed *Potamogeton lucens* (7%). Filamentous Green Algae was present throughout much of the surveyed reach of Pierpoint Drain.

Table 1-20 – Macrophyte species with taxon cover of 4 or above recorded during the macrophyte survey of Pierpoint Drain

Common Name	Latin Name	Taxon Cover Value	% Cover Range	Ellenberg Light Indicator Value
Greater Pond Sedge	*Carex riparia*	4	2.5 < 5	7
Shining Pondweed	*Potamogeton lucens*	5	5 < 10	7
Nuttall's Waterweed	*Elodea nuttallii*	6	10 < 25	6
Broad-leaved Pondweed	*Potamogeton natans*	7	25 < 50	7
Rigid Hornwort	*Ceratophyllum demersum*	7	25 < 50	7

1.2.46 The overall EQR for the surveyed stretch of Pierpoint Drain was 0.60, which is indicative of a 'Good' WFD class for the macrophyte quality element (Table 1-21).

Table 1-21 – Pierpoint Drain LEAFPACS2 indicative WFD class calculator results

Parameter	Observed Value	Expected Value	Raw EQR	Adjusted EQR	Final EQR	WFD Class
RMNI	8.35	7.65	0.70	0.60	0.60	Good
NTAXA	8.00	10.03	0.80	0.76	0.60	Good
NFG	5.00	6.30	0.79	NULL	0.60	Good
ALG	1.70	NULL	0.98	0.87	0.60	Good

1.3 References

- British Standards Institution, (2012). BS EN ISO 10870:2012 Water Quality – Guidelines for the selection of sampling methods and Devices for Benthic Macroinvertebrates in Freshwaters. London, BSI.
- Chadd, R. and Extence, C. (2004). The Conservation of freshwater macroinvertebrate populations: a community-based classification scheme. Aquatic Conservation, 14, 59-624.
- CIEEM, (2019). Advice note on the lifespan of ecological reports and surveys. Available at: https://cieem.net/wp-content/uploads/2019/04/Advice-Note.pdf. [Accessed July 2022].
- Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J-C., Bonin, A., Taberlet, P. and Pont, D. (2016). Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. PLoS ONE, 11 (6).
- Davy-Bowker, J., Clarke,R., Corbin, T., Vincent, H., Pretty, J., Hawczak, J., Blackburn, J., Murphy,J. (2007). River Invertebrate Classification tool. SNIFFER Project WFD72C.
- Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W. and Paulissen, D. (1991.) Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica, 18, 1–248.
- Environment Agency, (2012). Hydroecological validation using macroinvertebrate data: Operational Instruction 318_10. Environment Agency, Bristol.
- Environment Agency, (2014). Freshwater macroinvertebrate analysis of riverine samples: Operational Instruction 024_08. Issued 28/01/14. Environment Agency, Bristol.

- Environment Agency, (2023a). Catchment Data Explorer. [Online]. Available at: https://environment.data.gov.uk/catchment-planning/WaterBody/GB105033042690 [Accessed July 2023].
- Environment Agency, (2023b). Ecology and Fish Data Explorer. Available online: https://environment.data.gov.uk/ecology/explorer/ [Accessed July 2023].
- European Standard, (2023). CEN EN 17805:2023 Water quality - Sampling, capture and preservation of environmental DNA from water [Accessed March 2023].
- Extence, C.A., Balbi, D.M., and Chadd, R.P. (1999). River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. Regulated Rivers: Research and Management, 15, 543-574.
- Extence, C.A., Chadd, R., England, J., Wood, P.J. and Taylor., E. (2011). The assessment of fine sediment accumulation in rivers using macro-invertebrate community response. River Research and Applications, 29, 17-55.
- Hänfling, B., Lawson Handley, L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A. and Winfield, I.J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Molecular Ecology, 25, 3101–3119.
- His Majesty's Stationery Office (HMSO) (1975). The Salmon and Freshwater Fisheries Act. HMSO, London.
- HMSO, (1981). The Wildlife and Countryside Act. HMSO, London.
- HMSO, (2006). Natural Environment and Rural Communities (NERC) Act. HMSO, London.
- HMSO, (2009). The Eels (England and Wales) Regulations. HMSO, London.

- HMSO, (2017). The Water Environment (Water Framework Directive) (England and Wales) Regulations. HMSO, London.
- HMSO, (2019). The Conservation of Habitats and Species Regulations (Amendment) (EU Exit). HMSO, London.
- Jacoby, D., and Gollock, M. (2014). Anguilla anguilla. The IUCN Red List of Threatened Species. Available online: https://www.iucnredlist.org/species/60344/45833138 [Accessed July 2023].
- Nakagawa, H., Yamamoto, S. Sato, Y., Sado, T., Minamoto, T. and Miya, M. (2018). Comparing local- and regional-scale estimations of the diversity of stream fish using e-DNA metabarcoding and conventional observation methods. Freshwater Biology, 63 (6), 569–580.
- Natural England, (2023). MAGIC website. [Online]. Available at: http://www.natureonthemap.naturalengland.org.uk/home.htm [Accessed July 2023].
- Olds, B.P., Jerde, C.L., Renshaw, M.A., Li, Y., Evans, N.T., Turner, C.R., Deiner, K., Mahon, A.R., Brueseke, M.A., Shirley, P.D., Pfrender, M.E., Lodge, D.M. and Lamberti, G.A. (2016). Estimating species richness using environmental DNA. Ecology and Evolution, 6 (12), 4214–4226.
- Turley, M. D., Bilotta, G. S., Cadd, R. P., Extence C. A., Brazier, R. E., Burnside, N. G., Pickwell, A. G. G. (2016). A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. Ecological Indicators 70, 151-165.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J., Peroux, T., Crivelli, A.J., Olivier, A.,

Acqueberge, M., Brun, M.L., Møller, P.R., Willerslev, E. and Dejean T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology, 25, 929-942.

- Water Framework Directive UK Technical Advisory Group (WFD UKTAG), (2014a). Invertebrates (General Degradation): Whalley, Hawkes, Paisley and Trigg (WHPT) metric in River Invertebrate Classification Tool (RICT). Stirling, Scotland.
- Water Framework Directive UK Technical Advisory Group (WFD UKTAG), (2014b). UKTAG River Assessment Method Macrophytes and Phytobenthos: Macrophytes (River LEAFPACS2): Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG).
- WSP, (2022). West Winch Relief Road Aquatic Ecology Scoping Report. WSP UK Ltd.