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ANNEX B3.1

Environmental Assessment Sampling Methodology Report

Grand Union Canal Transfer SRO Affinity Water, Severn Trent Water, Canal & River Trust



Grand Union Canal Strategic Resource Option

Sampling Methodology Report

January 2022

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Grand Union Canal Strategic Resource Option

Sampling Methodology Report

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Glossary

Acronym	Definition
ACWG	All Company Working Group
AWB	Artificial Waterbody
EAR	Environmental Assessment Report
EU	European Union
GEP	Good Ecological Potential
GES	Good Ecological Status
GUC	Grand Union Canal
HMWB	Heavily Modified Waterbody
INNS	Invasive Non Native Species
MI/d	Megalitres per day
POM	Programme of Measures [WFD measures required to improve waterbody status]
PS	Pumping station
RAPID	Regulators' Alliance for Progressing Infrastructure Development
RBMP	River Basin Management Plan
RNAG	Reason for Not Achieving Good [WFD status]
SRO	Strategic Resource Option
WFD	Water Framework Directive
WRSE	Water Resources South East
WSR	Water supply reservoir
WSW	Water Supply Works
WwTW	Water Treatment Works

Executive Summary

This Sampling Methodology Report presents the proposed methodology for the various surveys to be carried out as part of the Gate 2 submission to Regulators' Alliance for Progressing Infrastructure Development (RAPID) for the Grand Union Canal (GUC) Strategic Resource Option (SRO). The aim of the report is to agree the proposed sampling methodology with stakeholders and regulators.

The aim of the GUC SRO (hereafter referred to as 'the Scheme') is to investigate options for transferring available water from Severn Trent Water's Minworth wastewater treatment works (WwTW) into the GUC, to supplement Affinity Water's supply. From the GUC, it is proposed to transfer the additional resource southwards towards Affinity Water's supply area using Canal and River Trust assets.

In order to inform the production of the Gate 2 assessments and the latter Environmental Impact Assessment (EIA) for the Scheme, a number of surveys are required, as follows:

- Electro-fishing
- Fish eDNA
- Invasive Non-native Species
- Sediment sampling

The aims, timing and methodologies for these surveys are set out in this report.

1 Introduction

1.1 Overview

This Sampling Methodology Report presents the proposed methodology for the various surveys to be carried out as part of the Gate 2 submission to Regulators' Alliance for Progressing Infrastructure Development (RAPID) for the Grand Union Canal (GUC) Strategic Resource Option (SRO) (referred to as 'the Scheme').

Tale 1.1 below shows the surveys carried out as part of the Gate 1 submission following the All Companies Working Group (ACWG) guidance¹ and the proposed sampling for the Gate 2 submission.

Survey	Gate 1 surveys carried out	Gate 2 proposed surveys
CPET	Yes	No ²
Electrofishing	Yes	Yes
Fish eDNA	No	Yes
Fish scales	No	Yes
INNS	Yes	Yes
Sediment sampling	No	Yes
Habitat	Yes	Yes
Protected species	Yes	Yes

Table 1.1: GUC Options

1.2 Severn Trent to Affinity Transfer - Grand Union Canal Options

The outputs of the Gate 1 engineering workstream identified three viable transfer routes to get the water from Minworth in to the canal network, and reviewed and short-listed three potential abstraction locations for the GUC transfer as shown in Table 1.1 and Figure 1.1. The transfer would be reliant on the operation of the Minworth SRO as the source of additional water. Further details on the Scheme description are included in Chapter 2.

Table 1.2: GUC Options

Option	GUC route	Abstraction location	Option description
1A	1 Grove		Treated wastewater transfer from Minworth WwTW to The Grove via Route 1 (Birmingham to Fazeley, Leicester Line and Grand Union Canals)
1B	3	Grove	Treated wastewater transfer from Minworth WwTW to The Grove via Route 3 (Pipeline from Minworth to Atherstone then transfer via Leicester Line and Grand Union Canals)
1C	6	Grove	Treated wastewater transfer from Minworth WwTW to The Grove via Route 6 (Pipeline from Minworth to Learnington Spa then transfer via Grand Union Canal)
2A	1	Hemel Hempstead	Treated wastewater transfer from Minworth WwTW to Hemel Hempstead via Route 1 (Birmingham to Fazeley, Leicester Line and Grand Union Canals)

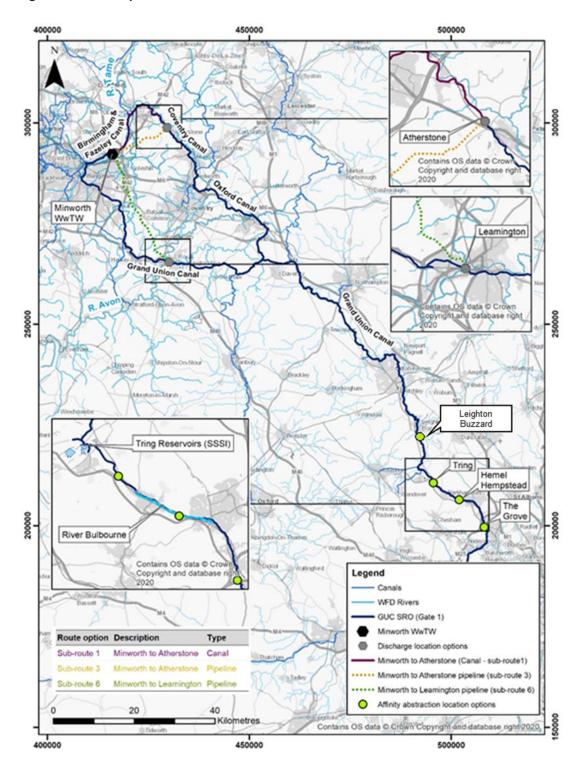
¹ All Companies Working Group, WRMP environmental assessment guidance and applicability with SROs, October 2020. The Gate 1 monitoring programme was designed by the Project Management Board (PMB) and used to inform the ACWG methodologies.

² While CPET surveys will not be carried out by Mott MacDonald at Gate 2, they will form part of the Gate 2 surveys to be carried out by APEM under the ecological monitoring workstream. Results of the Gate 1 CPET surveys will be used in the Mott MacDonald Gate 2 environmental assessments.

Option	GUC route	Abstraction location	Option description			
2B	3	Hemel Hempstead	Treated wastewater transfer from Minworth WwTW to Hemel Hempstead via Route 3 (Pipeline from Minworth to Atherstone then transfer via Leicester Line and Grand Union Canals)			
2C	6	Hemel Hempstead	Treated wastewater transfer from Minworth WwTW to Hemel Hempstead via Route 6 (Pipeline from Minworth to Leamington Spa then transfer via Grand Union Canal)			
3A	1	Tring	Treated wastewater transfer from Minworth WwTW to Tring via Route 1 (Birmingham to Fazeley, Leicester Line and Grand Union Canals)			
3B	3	Tring	Treated wastewater transfer from Minworth WwTW to Tring via Route 3 (Pipeline from Minworth to Atherstone then transfer via Leicester Line and Grand Union Canals)			
3C	6	Tring	Treated wastewater transfer from Minworth WwTW to Tring via Route 6 (Pipeline from Minworth to Leamington Spa then transfer via Grand Union Canal)			
4A*	1	Leighton Buzzard	Treated wastewater transfer from Minworth WwTW to Leighton Buzzard via Route 1 (Birmingham to Fazeley, Leicester Line and Grand Union Canals)			
4B*	3	Leighton Buzzard	Treated wastewater transfer from Minworth WwTW to Leighton Buzzard via Route 3 (Pipeline from Minworth to Atherstone then transfer via Leicester Line and Grand Union Canals)			
4C*	6	Leighton Buzzard	Treated wastewater transfer from Minworth WwTW to Leighton Buzzard via Route 6 (Pipeline from Minworth to Leamington Spa then transfer via Grand Union Canal)			

*Leighton Buzzard abstraction location added post Gate 1 studies

Figure 1.1: GUC options



Further post-G1 optioneering has been carried out to investigate the feasibility of an abstraction location at Leighton Buzzard. This builds on feedback received from the Environment Agency at Gate 1 and would have the benefit of avoiding the Chilterns Area of Outstanding Natural Beauty (AONB), reducing pumping costs and therefore also reducing carbon, and taking the abstraction point away from the most sensitive area of interconnected chalk streams.

1.3 Report Purpose

This Sampling Methodology Report presents the proposed methodology for the various surveys to be carried out as part of the Gate 2 submission for the Scheme. The aim of the report is to agree the proposed sampling methodology with stakeholders and regulators.

The sampling proposals initially cover all route options and abstraction locations, and the locations have been prioritised in relation to the wide spatial scale involved and are considered to be appropriate for this stage in the RAPID gated assessment process. Should any options be discounted, the sampling locations will be reviewed and may be revised and adapted accordingly.

1.4 Assumptions and Limitations

Information provided by third parties, including publicly available information and databases, is considered correct at publication. Due to the dynamic nature of the environment, conditions may change in the period between the preparation of this report and the undertaking of the proposed works. Any uncertainties and the limitations of the assessment process are acknowledged and highlighted.

2 GUC Scheme Description

2.1 Overview

The Scheme aims to investigate options for transferring available water from Severn Trent Water's Minworth WwTW into the GUC to supplement Affinity Water's supply. The Scheme proposes to transfer the additional resource southwards towards Affinity Water's supply area using Canal and River Trust assets.

At this stage, there are twelve proposed options, comprising three separate routes to get the water in to the canal network and four separate Affinity Water abstraction locations. However, it is important to note that downstream of the Braunston junction, the three sub-routes converge and follow the same route. Each route option is being considered for a transfer volume of either 50MI/d or 100MI/d. A map of the three potential route options and three Affinity Water abstraction locations is given in Appendix A. The ultimate solution will be a single route and abstraction location. i.e. the routes and abstraction locations are mutually exclusive with one another.

2.2 Route Sub-Options

Each of the proposed transfer options utilises one of three proposed routes:

- Route 1 (all 'A' options): Birmingham to Fazeley, Leicester Line and Grand Union Canals
- Route 3 (all 'B' options): Pipeline from Minworth to Atherstone then transfer via Leicester Line and Grand Union Canals
- Route 6 (all 'C' options): Pipeline from Minworth to Learnington Spa then transfer via Grand Union Canal

2.3 Affinity Water Abstraction Location Options

Four potential abstraction locations could be applied to any transfer route option:

- The Grove (options 1A, 1B, 1C): The proposed Grove abstraction site is located near Abbots Langley and Hunton Bridge, downstream of GUC interactions with the River Gade and Bulbourne and upstream of the River Colne.
- Hemel Hempstead (options 2A, 2B, 2C): The proposed Hemel Hempstead abstraction site is in the GUC stretch adjacent to the village of Bourne End in Hertfordshire, between Berkhamsted and Hemel Hempstead, within reach of GUC interactions with the River Bulbourne and upstream of the River Gade.
- Tring (options 3A, 3B, 3C): The proposed Tring abstraction site is located between Tring and Berkhamsted, downstream of Tring WwTW (Thames Water) and upstream of GUC interactions with the River Bulbourne or Gade.
- Leighton Buzzard (options 4A, 4B, 4C): This builds on feedback received from the Environmental agency at Gate 1 and would have the benefit of avoiding the Chilterns AONB, reducing pumping costs and therefore also reducing carbon, and taking the abstraction point away from the most sensitive area of interconnected chalk streams.

3 Survey overview

3.1 Overview

In order to inform the production of the Gate 2 assessments and the likely future EIA for the Scheme, several surveys are required. These have been identified as a result of the Gate 1 assessments. The aims, timing and methodologies for these surveys are set out below and in sections 4 to 8 of this report.

3.2 Electro-fishing

The proposed fish survey programme includes a survey at selected waterbody connections with the GUC (at locations where connectivity with the GUC is relevant), in addition to the drawdown surveys in the canal. Survey methods will follow the Environment Agency sampling e-fishing depletion methods and allow an understanding of the current fish community diversity and population age structure. The latter will be complemented by fish scale analysis.

3.3 Fish eDNA

In addition, the electro-fishing will be complemented with the use of e-DNA sampling at each survey point. This approach is an effective way of maximising the efficiency of aquatic surveys, increasing the success in identifying all relevant fish species in the study area.

3.4 Invasive Non-native Species

Surveys will be undertaken at target locations in the GUC and a representative sub-set of connected waterbodies to identify the presence of high-impact Invasive Non-native Species (INNS), and the potential for transfer via these connections. Due to the project timeframe, the focus will be on non-native aquatic invertebrates, which can normally be captured through most or all of the year. Species of particular interest include zebra mussel, quagga mussel, demon shrimp, killer shrimp and signal crayfish.

Site locations will be informed by the gap analysis report findings with sampling points proposed at a representative sub-set of connected waterbodies and a maximum of six points in the GUC. At each site, signs of the presence of these invasive species will be collected together with e-DNA samples, noting that e-DNA will detect the presence of signal crayfish, zebra mussel and quagga mussel only. This follows recommendations in the Gate 1 Ecology Gap Analysis report.

Non-native fish will be targeted through the electro-fishing and fish sampling e-DNA programme.

The project timeframe is outside of the peak growth period (typically June to September) of most non-native aquatic and riparian plants. However, some species may still be observable and where this is this case, these species will be recorded encountered during these surveys. In order to inform the proposal, it is recommended that plant surveys be undertaken during the peak growth period during subsequent stages of the RAPID gated process.

INNS surveys may be updated as considered appropriate for the later stages of the assessment, including the EIA.

3.5 Sediment sampling

Sediment sampling will be carried out in each canal pound and adjacent watercourses (where these are connected directly to the canal or via known sluices and overspills) to identify the main sediment issues effectively. The sediment sampling will be targeted to understand existing

variations in bed sediment and quality throughout the canal and to what extent pathways for movement into connected rivers already exist. Existing datasets (such as the BGS stream sediment geochemistry database) will be used where appropriate to ensure the monitoring effort is efficient and focuses on key issues.

Given the wide spatial scale of the route options, the sediment sampling proposed is considered sufficient to provide a high-level characterisation of the sediments and associated potential contamination throughout the routes, e.g. to identify whether there is a spatial variation in sediment quality between canal pounds and whether changes are linked to specific sources. The proposals are outlined in more detail in Section 7. This sampling will assist in the understanding of the potential risk of mobilising contaminated sediments due to the proposed transfer options.

This analysis is required for Gate 2 to enable the Project Management Board (PMB) to understand this risk, discuss any issues and potential mitigation with stakeholders, and efficiently plan for any intensive sampling in the future on the main risk areas if required.

4 Electro-fishing Methodology

4.1 Purpose of the sampling

The purpose of the fisheries surveys is to understand the current fish community, species diversity, population age structure within the GUC and a representative sub-set of the connected waterbodies. This understanding will determine the feasibility of the Scheme and influence the optioneering and design.

It is important to have high-quality quantitative baseline fish data prior to the Scheme commencing to be able to assess potential impacts and identify mitigation measures. This data will inform the baseline prior to the scheme works and will allow for comparisons with post-construction monitoring data.

4.2 Sampling methodology

All surveys will be undertaken using Water Framework Directive (WFD) (2000/60/EC) compliant fully quantitative electro-fishing, three-run catch depletion methodology. All sampling using electric fishing equipment will be completed following BS EN 14011:2003, BS 6068-5.32:2003 (Water quality: Sampling of fish with electricity). Electro-fishing is the primary survey method used to assess the WFD status of fish populations throughout England and Wales

Survey methods will be in accordance with Environment Agency sampling electro-fishing depletion methods. It uses a direct current of electricity flowing between a submerged cathode and anode; stunning fish can then be easily and safely captured, details recorded, and then returned unharmed to the same waterbody.

All electro-fishing surveys will be led by a trained and highly experienced fisheries surveyor certified by the Environment Agency or the Game and Wildlife Conservation Trust (GWCT). All additional staff are provided with bespoke in-house electric fishing training to Environment Agency standards.

The equipment used will be a combination of backpack and standard multiple anode PDC (Pulsed Direct Current) control box units that can be bank-based or towed in a boat by staff wading upstream. However, it should be noted that the selection of methodology will be undertaken on a site-by-site basis by an experienced fisheries ecologist taking into consideration individual site conditions, channel depth and width, flow rate and health and safety considerations. The process for the section of appropriate sampling methods will be compliant with BS EN14962 (Water quality – Guidance on the scope and selection of fish sampling methods).

Each 100m site is isolated using stop-nets set across the channel to prevent fish entering or exiting the fixed area, then a minimum of three passes or 'runs' are made moving in an upstream direction, and depletion in numbers should be encountered; where poor depletions are observed additional passes will be made to ensure a catch depletion is achieved. A depletion is required to allow for fully quantitative absolute population metrics to be calculated using the method described by Carle and Strub (1978)³.

Upon capture, fish are stored in aerated holding tanks, with the catch of each run stored separately. Processing of the catch involves species identification and measuring each fish to the fork in the caudal fin, known as fork length, to the nearest millimetre and returned alive to

³ Carle & Strub (1978) Carle FL, Strub MR. A new method for estimating population size from removal data. *Biometrics*. 1978;34:621–630.

the water. If high numbers of fish are encountered, or there are limitations on time (e.g. daylight), then sub-sampling of the catch will be undertaken following the Environment Agency's Operational Instruction 150_03 protocol for WFD compliant surveys.

In addition to catch data, information on the water quality and habitat character will be recorded on a standardised proforma. Field-based water quality parameters including temperature ($^{\circ}$ C), pH, dissolved oxygen (DO; Mgl⁻¹ and % saturation), and conductivity (μ Scm⁻¹) will be recorded using a hand-held calibrated YSI Pro-Plus meter. This data will also complement the wider water quality monitoring undertaken for this study which collects more detailed and frequent water quality data. Habitat characteristics recorded include water depth, site length, river wetted width, turbidity, macrophyte cover (%), flow type (pool, riffle, run, glide), substrate composition, cover for fish, shade (%).

4.2.1 Guidance and legislation

- All surveys will be undertaken using WFD (2000/60/EC) compliant fully quantitative electric fishing, three-run catch depletion methodology.
- All sampling using electric fishing equipment will be completed in accordance with BS EN 14011:2003, BS 6068-5.32:2003 (Water quality: Sampling of fish with electricity).
- An Environment Agency 'Authorisation to use fishing instruments other than rod & line in England' is required prior to surveying with electro-fishing equipment. These authorisations are issued under Section 27A of the Salmon & Freshwater Fisheries Act, 1975 (as amended).
- The Canal and River Trust site permit issued under the Keeping and Introduction of Fish Regulations (2015), will determine what fish can be kept in their waters. There will be conditions about not returning Zander (*Sander lucioperca*) to the water once caught.
- There is a duty to report any other non-native species which may be caught, including but not limited to:
 - Wels Catfish (*Silurus glanis*), Channel Catfish (*Ictalurius punctatus*), Sunbleak;
 (*Leucaspius delineatus*), Bitterling (*Rhodeus sericeus*), Topmouth Gudgeon;
 (*Pseudorasbora parva*), Grass carp (*Ctenpharyngodon idella*), Silver Carp; and
 (*Hypophthalmichthys molitrix*), Pumpkinseed (*Lepomis gibbosus*) and nonnative/ornamental Sturgeon/Sterlet (*Acipenser spp.*).
 - The WFD Technical Advisory Group has created an "alarm list" for the UK, a list of species that are not currently known to be in the UK, but if they are observed, then a rapid response to eradicate them will be initiated. This includes freshwater gobies and weatherfish.

4.3 Sample locations

Figure 4.1 and Table 4.1 shows proposed sample locations for fish surveys. These sites were chosen taking in consideration access and health and safety considerations as well as being representative of the wider river habitats. A site walkover will be undertaken prior to the surveys to confirm site suitability and propose new sites if required.

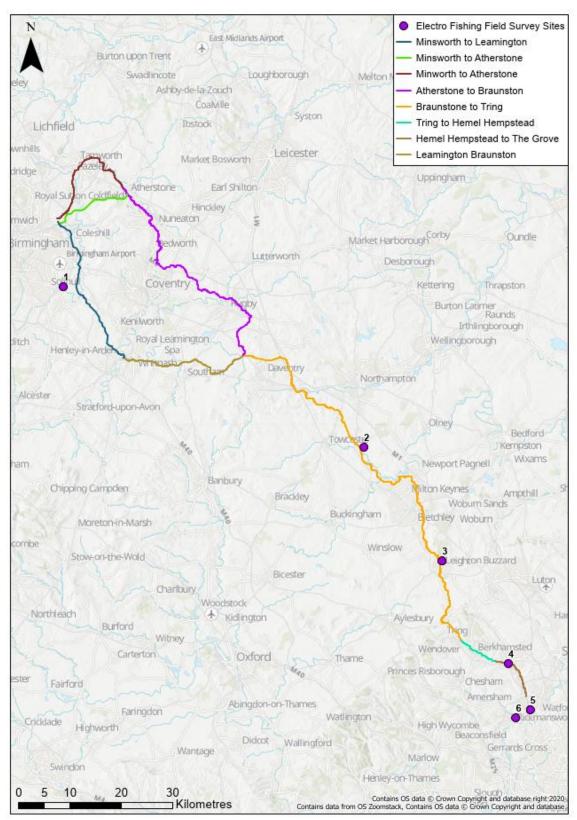


Figure 4.1: Electro-fishing field survey sites

Grid references for continued monitoring locations redacted

Table 4.1: Electro-fishing field survey sites

Ref	Site	NGR	Likely timing	Expected fish community
1	River Blythe at Henwood Lane		June 2022	Expected coarse community, although trout may be present
2	River Tove at Mill Farm		June 2022	Expected coarse community
3	River Ouzel at Monarchs Way		June 2022	Expected coarse community
4	River Bulbourne at Boxmoor		June 2022	Expected coarse community
5	River Gade at Cassiobury Park		June 2022	Expected coarse community
6	River Chess at Scotsbridge Mill		June 2022	Expected coarse community

4.4 Sample frequency

A single round of electrofishing surveys will be carried out for the Gate 2 assessments, although these may be updated for the later stages of the assessment (including the EIA).

4.5 Sample analysis

All the field data will be entered onto a bespoke fish population survey recording and analysis tool, which converts fish fork length to weight (g) using the Environment Agency length-weight factors used for the National Fisheries Monitoring Programme (NFMP).

- From the depletion in numbers, observed estimated population metrics are calculated using the method described by Carle and Strub (1978) to provide total estimated biomass (g/100 m²) and density (No./100 m²) for each species.
- From the fish numbers, estimated weights and known sampled area values for observed fish biomass (g/100 m²) and density (No./100 m²) are calculated for each species.
- Fish scale analysis will be used to confirm age class structure, growth rates and insights into the fishery's performance. Fish lay down seasonal calcified annuli (or rings) on their scales. These rings can then be counted, and the fish can be aged in a comparable way to ageing a tree. This information will give insights into fish growth rates, including if the fish are recruiting well, growing as expected, or stunted (i.e. fish are older than they should be for a given size).
- Recording the current extent and population structure of invasive non-native fish is an important outcome of the surveys. It will be essential to assess whether the Scheme could result in their spread and what protocols might need to be implemented to manage them or even locally eradicate them. Zander is of immediate concern, but other invasive non-native fish will need to be considered.

4.6 Assumptions and standard best-practice mitigation measures

- If the opportunity arises, we will carry out a full fish survey, recording all of the fish, within
 approximately 500 metres of the Grand Union Canal (length may vary depending on what is
 possible). A full survey of what fish are present can only be undertaken if the canal gets
 drawn-down. This will give us an 'absolute biomass' and data about the fish community for
 the length surveyed. This fully quantitative data will be analysed with the qualitative fish data
 (collected in Gate 1), so that we can understand the level of confidence and limitations of the
 qualitative data.
- However, drawdowns are significant stoppages and are a major disruption to the navigation and they need to be carefully managed by the Canal and River Trust. The Trust will not draw-down the canal for a fisheries survey, but if they have any planned maintenance work

which requires a draw-down then we will be able carry out a survey in parallel. We have not been supplied with dates or locations of these drawdowns, but discussion are ongoing with the Canal and River Trust and we will continue to work closely with The Trust to fully understand their planned maintenance schedule and attempt to work alongside to maximise opportunities. Final site selection will be agreed with the Canal and River Trust's National Fisheries & Angling Manager,

Individual's name redacted

- It is assumed that a suitable section of canal will be identified and de-watered by the Canal and River Trust to approximately 600mm deep (knee depth) in the centre of the channel over a 500m stretch. If the stretch of watercourse that is dewatered varies from this length, this will affect the catch size.
- The Canal and River Trust maintenance programme is over the winter months, to get the canals ready for Easter at the start of the boating season.
- Any water management will be carried by the Canal and River Trust. They will undertake the de-watering and they will tell us when it is safe to work in the canal. This is the same procedure for their Fisheries Framework Contractors, who carry out fish rescues on behalf of the Trust during routine engineering works.

Refer to Section 9 for biosecurity measures to be implemented to prevent the spread of diseases and INNS between survey sites.

5 INNS Survey Methodology

5.1 Purpose of the sampling

This sampling aims to generate positive biological records of INNS to improve understanding of their distribution and dispersal within the canal network and a representative sub-set of connected waterbodies. This understanding will contribute towards the INNS risk assessment work needed to determine the feasibility of the GUC SRO and influence optioneering and design. As surveys are required in late 2021 and early 2022, this activity will focus on non-native aquatic invertebrates, using the following techniques to maximise the probability of detecting species:

- Manual search for non-native aquatic invertebrates using a pond net
- Collection of environmental DNA (eDNA) samples
- Recording of any aquatic and riparian non-native plants encountered

Non-native fish will be assessed in the electro-fishing and fish eDNA programme.

5.2 Sampling methodology

5.2.1 Macroinvertebrates

Macroinvertebrates will be sampled with a standard pond net. The sampling will be targeted to represent the range of habitats present at each site, including natural and artificial features, the latter of which are often favoured by non-native species.

This element will therefore involve walking along a section of the watercourse and collecting a number of samples, covering each habitat type present. Samples will be collected from within a reach length of 50m minimum in order to capture localised habitat variability; however longer reaches may be sampled if required to include specific habitat features.

Individual sample size will be determined by professional judgment based upon the nature of the habitat features being sampled. It is estimated that individual samples will involve 15-60 seconds active net sampling depending on the size of habitat feature being sampled and the need to collect an appropriate sample volume to be examined in a tray. Due to the nature of net sampling, sample volume cannot be specifically defined, though individual samples will be limited to the amount of detritus which it is judged can be reasonably searched in the field.

Upon collection, individual samples will be examined in the field in a white plastic tray until the analyst is sufficiently confident in the likely presence or absence of INNS. Non-native invertebrates identifiable in the field by eye or hand lens will be recorded, and potential non-native species which cannot be confirmed in the field will be retained for microscopic examination.

Total sampling effort will not be standardised and will be based on professional judgment based on habitat variability. Sampling will continue at any given location until the range of habitats is judged to have been adequately sampled, and repeated sampling fails to yield new species. It is anticipated that a minimum of 45 minutes of active sampling and field analysis will be undertaken per location, though this time will be extended as required.

5.2.2 Environmental DNA

At each site, up to 1 litre of sampled water will be filtered through an encapsulated disk filter immediately upon collection using a syringe to monitor the volume of water sampled. As per the

sampling instructions provided by NatureMetrics, less than 1 litre of water may be filtered if the filter becomes clogged. A preservative solution will then be added to the filter units, and they will be promptly sent to the specialist laboratory of NatureMetrics for analysis.

The sampling instructions provided by NatureMetrics do not include a protocol for canals; however assuming that water may be poorly mixed, canal sampling will involve collection and subsequent mixing of around 20 sub-samples from the water's edge at a range of locations; these will be throughout the 50m sample reach used for macroinvertebrates.

The lake sampling protocol will be followed for the three Tring reservoirs, and will involve the collection of around 20 sub-samples of water at approximately evenly-spaced points around the perimeter, where access allows.

For river reaches, the river sampling protocol will be followed. This will involve collecting five water samples from different parts of the flow within the river, throughout the 50m minimum reach sampled for macroinvertebrates. This will be mixed in a bucket before being passed through the filter.

5.2.3 Plants

The project timeframe is outside the peak growth period for most aquatic and riparian plants, which is typically June to September inclusive. However, some species may still be observable from late autumn to spring. Therefore, any aquatic or riparian non-native plant species observed within the 50m minimum reach for macroinvertebrates will be recorded. A grapnel will be taken on site to aid collection of any potential non-native aquatic species which require closer examination. Although it is likely that any species found will be identifiable in the field, specimens which cannot confirmed in the field will be retained for microscopic examination.

In order to inform the proposal, it is recommended that plant surveys are undertaken during the peak growth period at a later stage in the assessment.

5.2.4 Guidance and legislation

The following legislation is relevant to INNS:

- Wildlife and Countryside Act 1981, as amended by the Wildlife and Countryside Act 1981 (Variation of Schedule 9) (England and Wales) Order 2010, the Natural Environment and Rural Communities Act 2006 and the Countryside and Rights of Way Act 2000
- The Invasive Alien Species (Enforcement and Permitting) Order 2019
- Import of Live Fish (England and Wales) Act 1980
- Keeping and Introductions of Fish Regulations, 2015

It is intended that the results of these surveys will feed into the SRO Aquatic INNS Risk Assessment Tool (SAI-RAT) developed by the EA in 2021.

5.3 Sample locations

It is proposed to undertake INNS sampling at the sites detailed in Figure 5.1 and Table 5.1 below. These points have been chosen as a representative sub-set of possible connections between the canal network and other watercourses, or at key junctions in the canal network. Work to understand the connectivity of the canal network is ongoing, and it is likely that further survey will be need at later stages in the Scheme assessment process (e.g. Gate 3 and EIA).

The National Grid References (NGRs) denote a point when a canal crosses or connects to another watercourse. Unless otherwise stated, sampling will be undertaken on the canal and the co-located watercourse, downstream of the canal. It is planned to visit two nearby points at which the GUC crosses watercourses within the River Blythe catchment due to uncertainties

about the possible location of canal overflows. Sites due for survey in April 2022 may be subject to change based on an evolving understanding of the preferred water transfer route option.

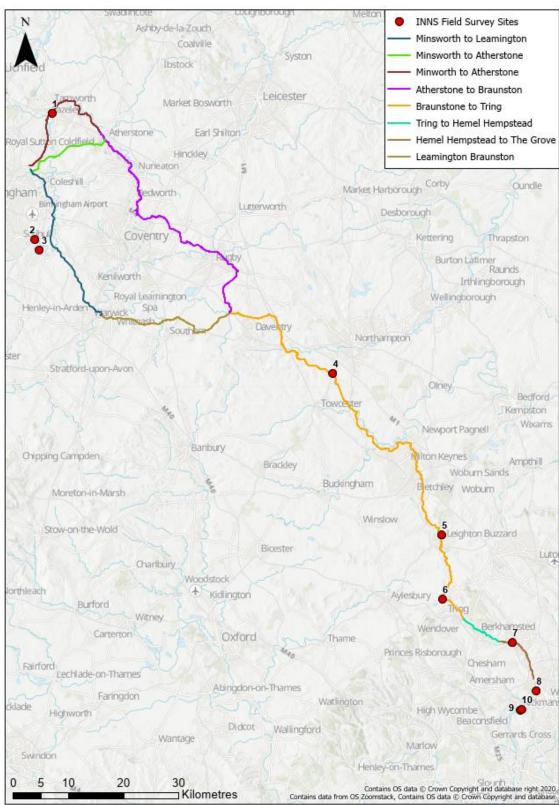


Figure 5.1: INNS Survey Sites

Grid references for continued monitoring locations redacted

Table 5.1: INNS field survey sites

Ref.	Site	NGR	Likely timing
1	River Tame & Coventry Canal		April 2022
2	River Blythe & GUC site 1		April 2022
3	River Blythe & GUC site 2		April 2022
4	GUC at Northampton arm intersection (GUC only)		November 2021
5	River Ouzel & GUC		November 2021
6	Tring reservoirs & GUC		November 2021
7	River Bulbourne & GUC		November 2021
8	River Gade & GUC		November 2021
9	River Colne & GUC		November 2021
10	River Chess & GUC		November 2021

5.4 Sample frequency

A single round of eDNA surveys will be carried out for the Gate 2 assessments, although these may be updated for the later stages of the assessment (including the EIA). At these stages, it would be advised to sample in at least two seasons (e.g. spring and autumn) to increase the probability of detecting species.

eDNA sampling can be undertaken throughout the year though is considered to be most effective when species are more active. Activity is likely to be lowest during the winter period therefore the optimal period is considered to be March to November inclusive, with unusually cold periods avoided where possible. Timing of sampling (November 2021 and April 2022) will therefore take place within the optimal period.

5.5 Sample analysis

Macroinvertebrate and plant specimens that cannot be identified in the field will be retained and later examined by microscopy.

The laboratory analysis of eDNA samples will aim to detect the following species:

- Signal crayfish Pacifastacus leniusculus
- Zebra mussel Dreissena polymorpha
- Quagga mussel Dreissena rostriformis

Individual species will be targeted to maximise sensitivity and therefore the probability of detecting those which present the highest risk. eDNA analysis has not yet been developed to detect all species of concern, particularly crustaceans, though these can typically be found by net sampling. The metabarcoding assay used to detect Dreissenid mussels may also detect additional non-native bivalve molluscs.

5.6 Assumptions and standard best-practice mitigation measures

DNA sampling is susceptible to contamination from other sources. The potential for this to occur will be reduced by following sampling instructions provided by NatureMetrics.

Biological records generated by eDNA surveys will be sense-checked, and assumed to be correct for risk assessment and reporting purposes if they accord with current understanding. However, records which may be unexpected or have significant implications (in particular, the quagga mussel) will be raised at the earliest opportunity so that appropriate actions can be determined.

As instruments will not be used to capture fish physically, an Environment Agency authorisation under Section 27A of the Salmon & Freshwater Fisheries Act, 1975, (as amended) is not required. These authorisations are required when using electro-fishing equipment, traps, and nets. Section 9 details biosecurity measures to be implemented to prevent the spread of diseases and INNS between survey sites.

6 Fish eDNA Methodology

6.1 Purpose of the sampling

The electro-fishing results will be complemented by eDNA metabarcoding techniques (whole fish communities). This technique estimates fish community diversity at sampling locations by sequencing collected DNA in water samples. By this method, species of fish that were not captured during the quantitative electric-fishing survey will still get recorded.

Fish are wild animals, and they can move freely within the watercourse. They are not uniformly distributed throughout the length of a watercourse, and at different ages, they will have specific habitat preferences. A good example of this is when fish have migrated out of the area for spawning or are in deep inaccessible pools. In these cases, they will either be missed or underrepresented in the survey data. The advantage of using eDNA to support the physical survey data is that fish DNA will still be present in the water, and fresh eDNA will still be flowing downstream from upstream sources. This also reduces the need to carry out an extensive survey programme throughout the catchment. As a result, we can have confidence in our understanding of the fish community prior to the Scheme commencing, and this will assist us in determining what mitigation and compensation measures may be necessary.

In addition, e-DNA sampling will be undertaken to test for the presence of invasive non-native species as discussed in Section 5.

6.2 Sampling methodology

At the same sampling location of the invasive species surveys, a water sample will be taken, filtered on-site and sent to the laboratory for analysis.

The sampling methodology will be identical to that described for aquatic INNS in Section 5.2.2. In summary, up to 1 litre of sampled water will be filtered through an encapsulated disk filter, though a smaller volume may be sampled if the filer becomes clogged. For canal sites, around 20 sub-samples will be collected from the water's edge at a range of locations throughout an approximate 50m reach. For river samples, due to greater mixing, five water samples will be collected from different parts of the flow within a 50m river reach. For the Tring reservoirs, around 20 sub-samples will be collected at evenly-spaced points around the perimeter.

6.2.1 Guidance and legislation

DNA (eDNA) methodology for still waterbodies has been developed by the EA in collaboration with Nature metrics. Surveys will be undertaken following Nature Metrics protocols (both for still and running waterbodies) and samples will be analysed in Nature Metrics laboratories.

It should be noted that Natural England has recognised the use of eDNA as a rapid and costeffective survey technique to establish Great Crested Newt presence or absence since 2014, although not for other species.

6.3 Sample frequency

A single round of eDNA surveys will be carried out for the Gate 2 assessments, although these may be updated for the later stages of the assessment (including the EIA). At these stages, it would be advised to sample in at least two seasons (e.g. spring and autumn) to increase the probability of detecting species.

eDNA sampling can be undertaken throughout the year though is considered to be most effective when species are more active. Activity is likely to be lowest during the winter period therefore the optimal period is considered to be March to November inclusive, with unusually cold periods avoided where possible. Timing of sampling (November 2021 and April 2022) will therefore take place within the optimal period.

6.4 Sample analysis

Samples will be analysed by eDNA metabarcoding techniques (whole fish communities). This technique can estimate fish community diversity at sampling locations by sequencing collected DNA in water samples.

The testing procedure involves the use of the quantitative polymerase chain reaction (PCR) procedure, where DNA from each filtered sample is extracted and amplified. PCRs are performed under a negative and positive sample (mock community with a known composition). This technique uses universal primers, which can work across a range of species to amplify specific short regions of DNA. The amplified DNA is then sequenced to identify the diversity of species present.

Caution is required with the interpretation of the DNA analysis as the sampling methodology inevitably captures DNA from upstream reaches, not just that which is specific to the sampling location. However, the information collected is valuable in identifying the potential presence of fish species in the different canal pounds, as well as in nearby lakes. This information will be analysed in light of the habitats present in the study area. In addition, the information collected is of value for understanding the potential use of the watercourse by migratory fish species (for example, eels) and informing mitigation and compensation measures.

6.5 Assumptions and standard best-practice mitigation measures

As instruments will not be used to capture fish physically, an Environment Agency authorisation under Section 27A of the Salmon & Freshwater Fisheries Act, 1975, (as amended) is not required. These authorisations are required when using electro-fishing equipment, traps, and nets.

Refer to Section 9 for biosecurity measures to be implemented to prevent the spread of diseases and INNS between survey sites.

7 Sediment Sampling Methodology

7.1 Purpose of the sampling

The WFD assessment undertaken at Gate 1 identified risks associated with water quality, changes in flow and mobilisation of sediment and potential contamination concerns. Sampling and analysis are being undertaken to characterise the physical and chemical properties of the canal and connected watercourse sediments. This work will enable interpretation of impacts arising from any increase in flow velocity associated with the scheme, resulting in greater mobility of what might be contaminated sediments. This interpretation will be undertaken in parallel with the numerical modelling studies of flows in the canal network. The sediment sampling will be sufficient in spatial scale to understand existing variations in bed sediment quality and to what extent pathways for a movement already exist.

Sediment sampling results will be combined with the water quality (including suspended sediments) and modelling workstreams undertaken by Atkins and JBA, respectively. We have established links with the ongoing modelling and water quality workstreams. These interactions will facilitate integration with the predicted changes to hydraulics.

7.2 Sampling locations

Mott MacDonald proposes to obtain sediment samples from the GUC bed at the 55 locations along the length of the canal and in key interconnected rivers. The samples will be collected by our sub-contractor Partrac and analysed by a UKAS accredited laboratory. Sampling locations have been determined using outputs from an existing numerical model and the location of canal pounds and canal overflow/connection points.

7.2.1 Numerical model

Output from a hydraulic model developed using *Flood Modeller Pro*⁴ software has been provided to Mott MacDonald by JBA and guided the sediment sampling locations. Specifically, the model outputs provide minimum and maximum bed shear stress values that indicate locations where flow velocities are likely to increase during water transfer through the network.

Results from the model provided by JBA are shown in Figure 7.1. These data indicate that throughout the GUC network, maximum bed shear stress τ_{max} , is less than 1N/m² and reflect low flow velocities in the canal. Higher τ_{max} values are predicted in the canal section between Royal Learnington Spa and Stockton (see inset in Figure 7.1).

It is understood that JBA is currently refining numerical modelling of the GUC, and new results may emerge. For now, it is considered that the τ_{max} values from the initial modelling do not provide a definitive guide to the regions of the canal likely to be affected by increased flows attributable to water transfer. Therefore, our selection of sampling points has been based on the location of pounds and interconnections with watercourses rather than areas of perceived higher bed erosion/transport. These are considered the most suitable for characterising the sediment properties along the GUC and at sites with potential for canal sediments to enter watercourses.

⁴ https://www.floodmodeller.com/

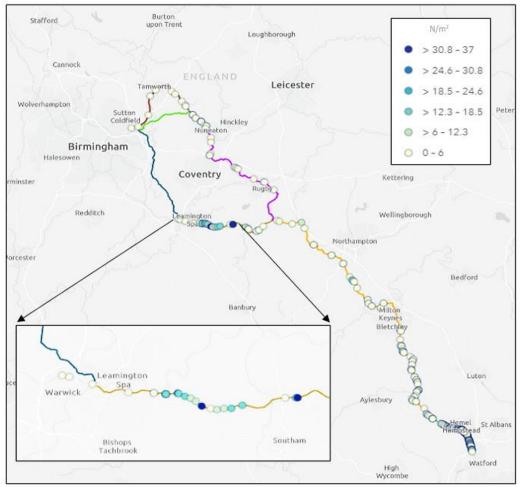


Figure 7.1: Predicted maximum bed shear stress from *Flood Modeller Pro* during an initial water transfer simulation

Source: Mott MacDonald (2021) with shear stress data provided by JBA (2021)

7.2.2 Pounds and connected water bodies

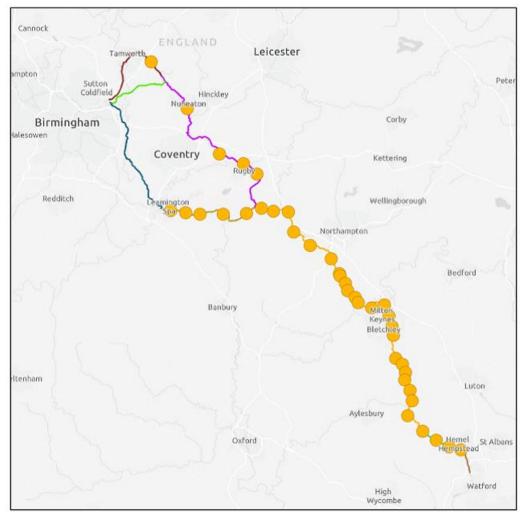
Information provided by PMB to Mott MacDonald concerning the location of modelled pounds, locks, sluices and interconnected rivers has been incorporated into a GIS and has been used as the primary means of selecting the sampling locations. At least one sample will be obtained in each canal pound and in the primary watercourse connections for: River Tame south of Tamworth; River Anker from Atherstone to Tamworth; Withy Brook east of Coventry; Tributary of River Leam; Tributary of River Nene; River Tove; Great Ouse north of Milton Keynes; River Ouzel through Milton Keynes; Bulbourne; and River Gade. The location of proposed sampling points was assessed using Google Earth to assess potential access issues. Some refinement may be required as more information becomes available.

The proposed location of sediment samples in each canal pound is summarised in Table 7.1 and illustrated in Figure 7.2. The location of sediment samples in connected watercourses is summarised in Table 7.2 and illustrated in Figure 7.3. Images of each proposed sample location in the canal pounds and connected watercourses are provided in Appendix B and Appendix C.

Grid references for
continued monitoring
locations redacted

Sample No.	Latitude	Longitude	Longitude	Easting	Northing
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2					
3					
4					
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7					
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38					

Table 7.1: Location of sediment samples for each canal pound





Source: Mott MacDonald (2021)

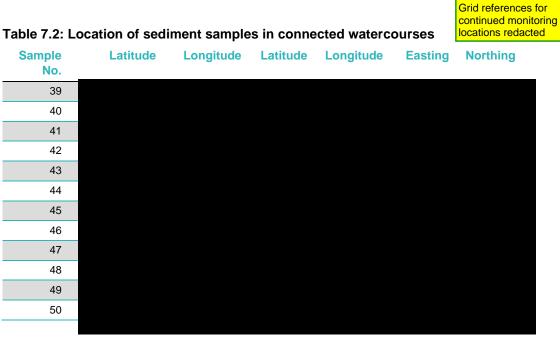
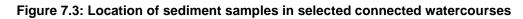


Table 7.2: Location of sediment samples in connected watercourses

Sample No.	Latitude	Longitude	Latitude	Longitude	Easting	Northing	Grid refere
51							locations r
52							
53							
54							
55							
							4

Grid references for continued monitoring locations redacted





7.3 Sampling methodology

Bed sediment samples will be obtained by piston core with grab sampling as a backup method., All sampling operations will be undertaken using a well-established 'clean hands - dirty hands' approach to avoid any grab sample cross-contamination:

- 'Dirty hands' are responsible for the preparation of the sampler ancillaries (i.e., except the sample container itself), operation of any machinery (e.g., winch), and for all other activities that do not involve direct contact with the sample.
- 'Clean hands' are responsible for all operations involving contact with the sampler, preparing and priming the sampler and transferring the sample[s] from the grab to the sample containers.

Only 'Clean hands' are permitted to contact the sampler, collect the sediment sample and inspect it. If the sample is acceptable, 'Dirty hands' record an assessment of sample quality and take a digital photograph. The following is recorded:

- Sample volume (approx.)
- Sediment description following BS5930:2015, which will include:
 - Texture
 - Particle shape (if apparent to the naked eye)
 - Consistency
 - Colour (according to the Munsell Colour System)
 - Smell/odour
 - Stratification
 - Presence of debris
 - Presence of surface biology (in/epifauna)

'Clean hands': Take care to avoid material loss; carefully collect and transfer to sample container and seal; and rinse the sampler free of sediment and wash with water. Following collection and completion of a DPR Log entry, samples are kept cool and in the dark.

7.3.1 Sample Storage and Analysis

7.3.1.1 Storage

- Transfer samples to secure, cool and darkened storage location
- Check Sample Registration Form matches the Sample Master Sheet
- Check labels have not been lost/damaged

7.3.1.2 Sample Labelling

Samples will be labelled as follows: SS (for Sediment Sample) / Location – Time, Day, Month and Year. Locations will be defined using Lat./Long. and OSGB coordinates.

7.4 Sample frequency

A single round of sediment surveys will be carried out for the Gate 2 assessments, although these may be updated for the later stages of the assessment (including the EIA).

7.5 Sample analysis

All analyses defined below will be undertaken by a UKAS accredited laboratory for the following:

- Laser particle size analysis (PSA)
- Setting velocity
- Heavy metals (As, Cd, Cr, Cu, Hg, Pb, Ni and Zn)
- PCBs (ICES 7)
- PAHs (EPA16)
- Total Petroleum hydrocarbons (>C8-C40)

The surficial shear strength/entrainment threshold of the collected samples will be provided in units of N/m^2 .

PSD analyses will be conducted following the NBMAQC scheme best practice guidance (NMBAQC, 2016⁵). The analyses will be conducted using a combination of laser diffraction and sieving techniques. Sediments <1 mm will be analysed via laser diffraction. Larger sized material will be dry sieved on a range of sieves between 1 mm and 63 mm, at 0.5 phi intervals.

The following data outputs will be provided:

- Equipment checks performed during mobilisation
- Time, location, water depth where a sample was taken
- Sample description
- Sediment sample images
- All sample analysis results
- Weather and canal boat traffic conditions during the survey period

7.6 Assumptions and standard best-practice mitigation measures

Refer to section 9 for biosecurity measures to be implemented to prevent the spread of diseases and INNS between survey sites.

⁵ NMBAQC's Best Practice Guidance Particle Size Analysis (PSA) for Supporting Biological Analysis (2016) available at http://www.nmbaqcs.org/media/1255/psa-guidance_update18012016.pdf

8 Ecological survey methodology

8.1 Purpose of the surveys

The purpose of the surveys is to:

- Provide initial baseline information on the habitats and species present or likely to be present along the canal routes and connected waterbodies.
- Make an assessment of the nature conservation value of the above habitats and species which have the potential to be present (ecological receptors).
- Identify potential constraints that could influence design, programme, construction timing, methods and working areas on site.
- Identify what additional surveys are required, if any, to inform appropriate mitigation measures.

8.2 Survey methodology

A desk based assessment using publicly available data will be carried out, to refresh our knowledge of statutory and non-statutory designates sites, habitats of principal importance etc.

The following data sources will be used for the desk based assessment:

- The Government's 'Multi-agency Geographic Information for the Countryside' (MAGIC) website for statutory designations
- Joint Nature Conservation Committee (JNCC) and Natural England websites for descriptions of statutory designated site

In addition to this, we will produce a first pass habitat map to the UKHabs classification system. This will be carried out in GIS using OS mastermap data (to be provided by the client) to provide an initial habitat map of the pipeline route (and appropriate buffer), and of those locations along the canal where INNS surveys have already been carried out.

This map will then be reviewed, to identify those areas where:

- Protected species are likely to be present, and so may require detailed surveys later in the design and assessment programme.
- Targeted site visit would be useful, to verify the findings of the first pass habitat map, and to add more certainty to the potential for protected species to be present.

A number of targeted site visits will then be undertaken. Note that these visits will be dependent on the availability of land access, and so may be restricted to being carried out from public highways, public rights of way etc, which will limit the effectiveness, coverage and robustness of the data collected.

A review of the data and background information collected by the INNS team will also be carried out, to identify whether visits to the samples canal sites would also be useful.

This mapping, the initial analysis and subsequent targeted site visits will be used to inform the project team as to the likely ecological constraints that the project will be required to address as it moves through its various subsequent stages.

A separate ecological monitoring workstream is being undertaken by APEM, which will also be used to inform the Gate 2 environmental assessments. This workstream will carry out surveys for the following:

- Canal CPET
- INNS targeted samples for bivalves and amphipods
- Chalk stream macrophytes
- Chalk stream habitat transects at flow sensitive locations

8.3 Survey frequency

A single round of surveys will be carried out for the Gate 2 assessments, although these may be updated for the later stages of the assessment (including the EIA).

9 Biosecurity protocols

Biosecurity measures will be implemented to prevent the spread of diseases and INNS between survey sites. The following specific measures will be taken:

- For river sites, sites were surveyed in an upstream-to-downstream direction.
- Multiple pond nets and net bags will be taken to reduce the risks of transferring attached organisms.
- Substrate (for example, silt or sand) and plant fragments will be removed from survey equipment and personal protective equipment (including waders) between visits to different survey locations.
- Brushes to clean off the equipment.
- Equipment will be disinfected using Virkon[®] Aquatic disinfectant sites, following the manufacturers' instructions.

10 Conclusions

This Sampling Methodology Report presents the proposed methodology for the various surveys to be carried out as part of the Gate 2 submission for the GUC SRO. The aim of the report is to agree the proposed sampling methodology with stakeholders and regulators.

The following surveys are required to inform the production of the Gate 2 assessments and the EIA for the Scheme: Electrofishing, fish eDNA and fish scale analysis, INNS and sediment sampling. These will be carried out in accordance with current legislation and policy.

A. Maps

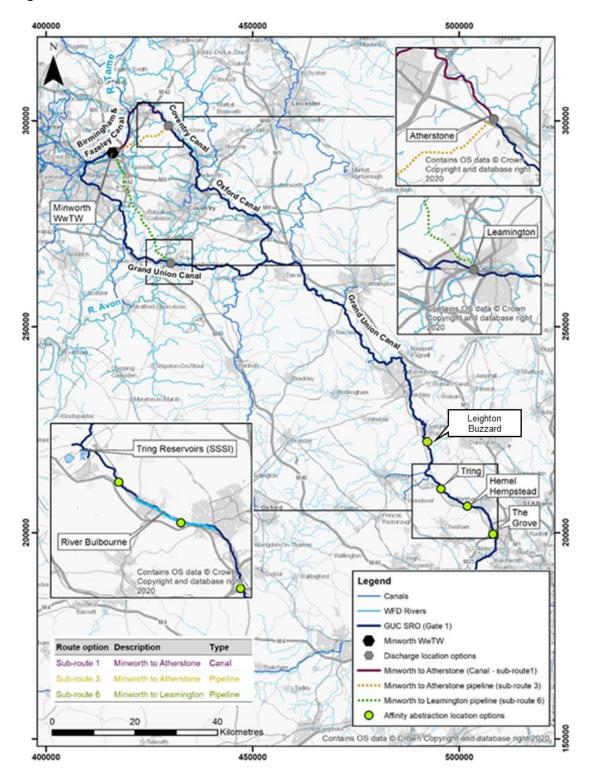
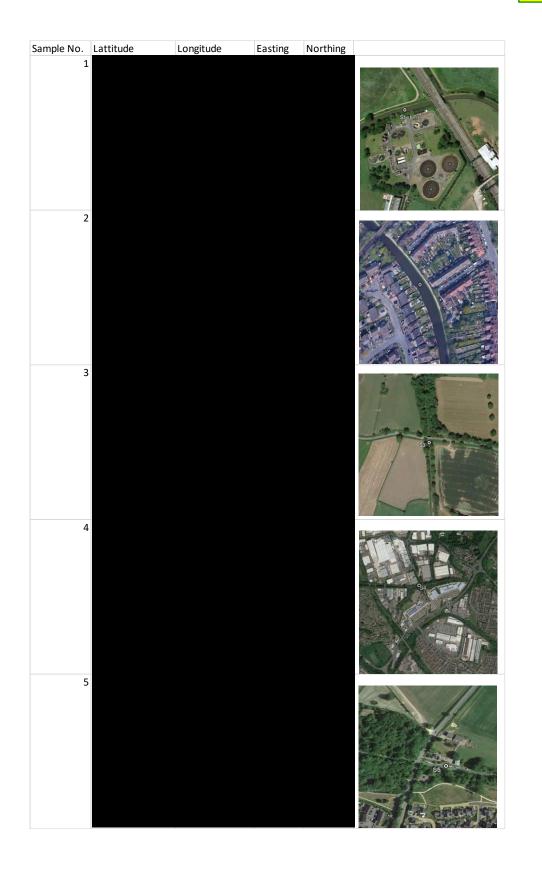
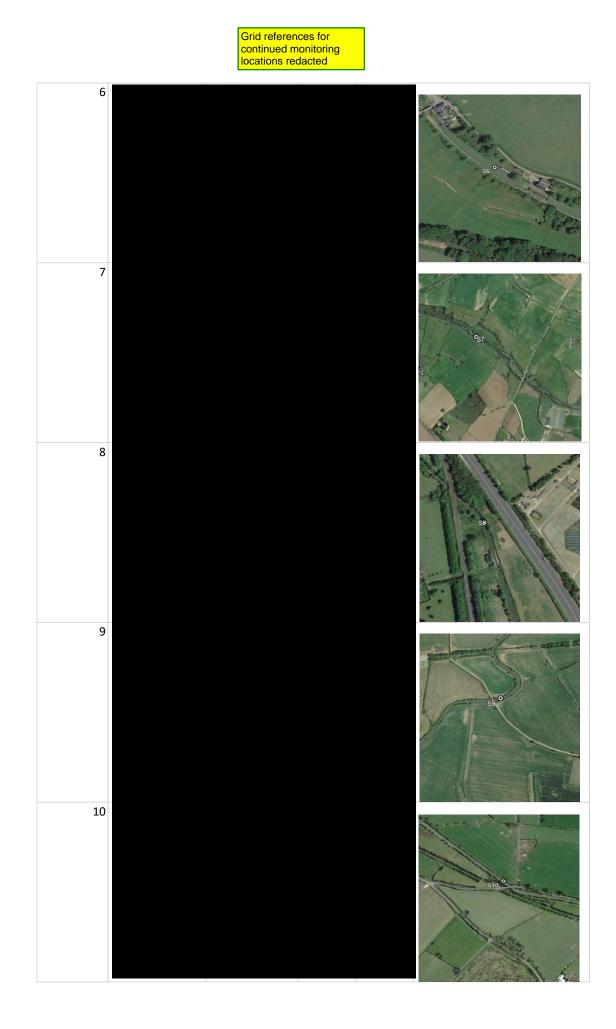


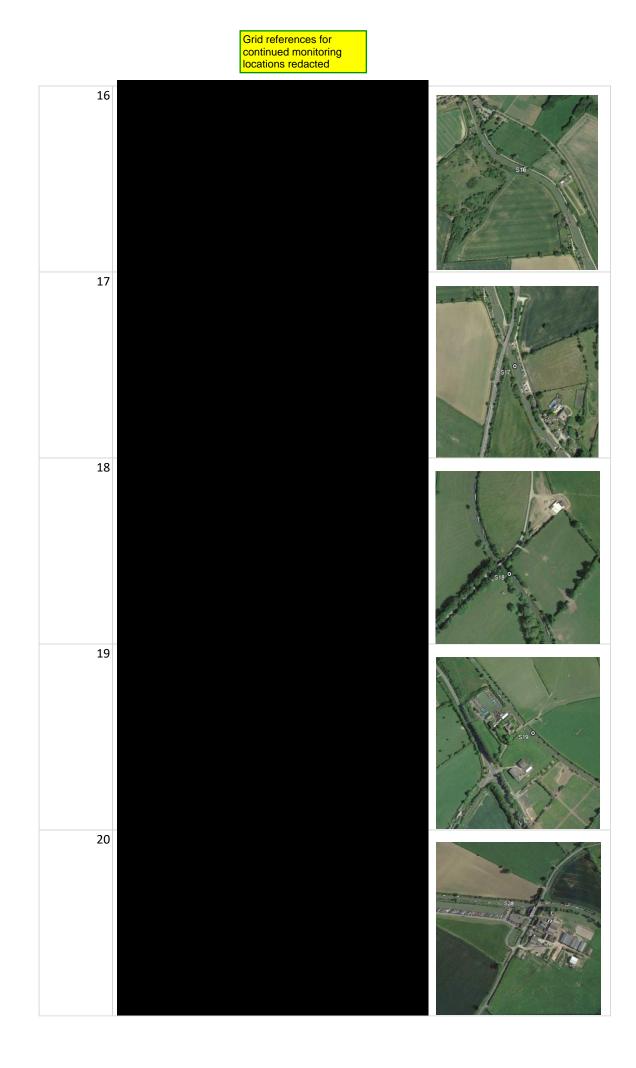
Figure A.1: Overview of the GUC Scheme

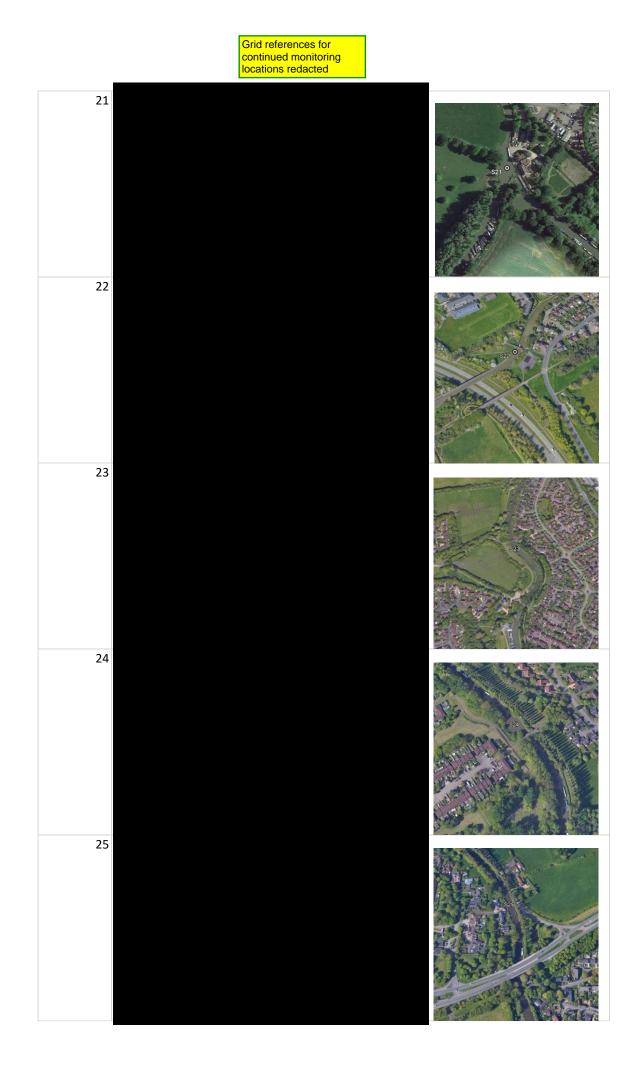
B. Sediment sampling: Canal pounds

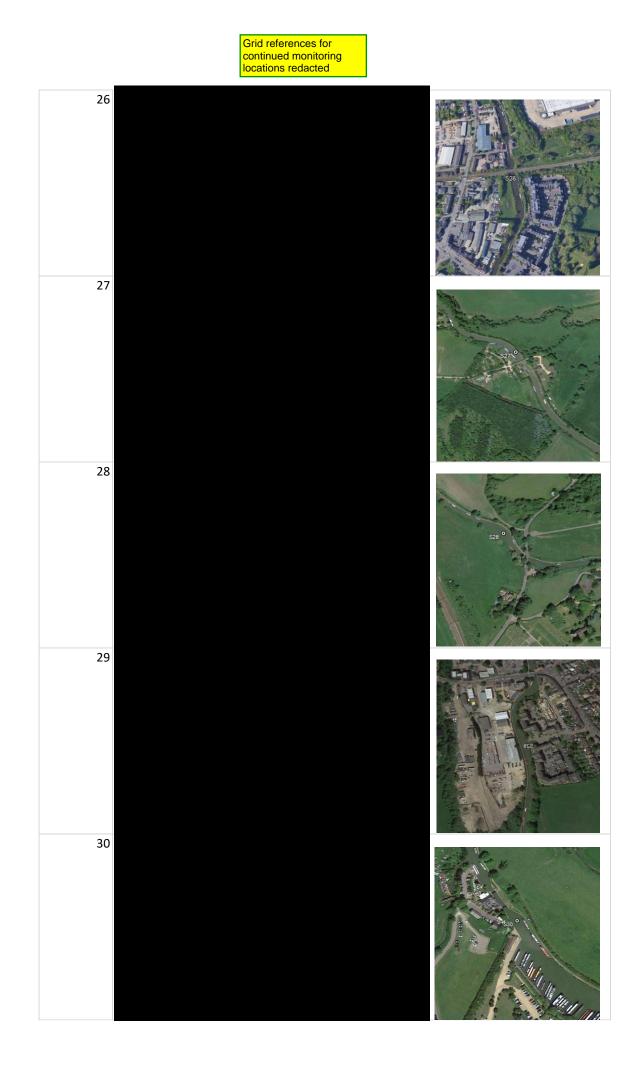
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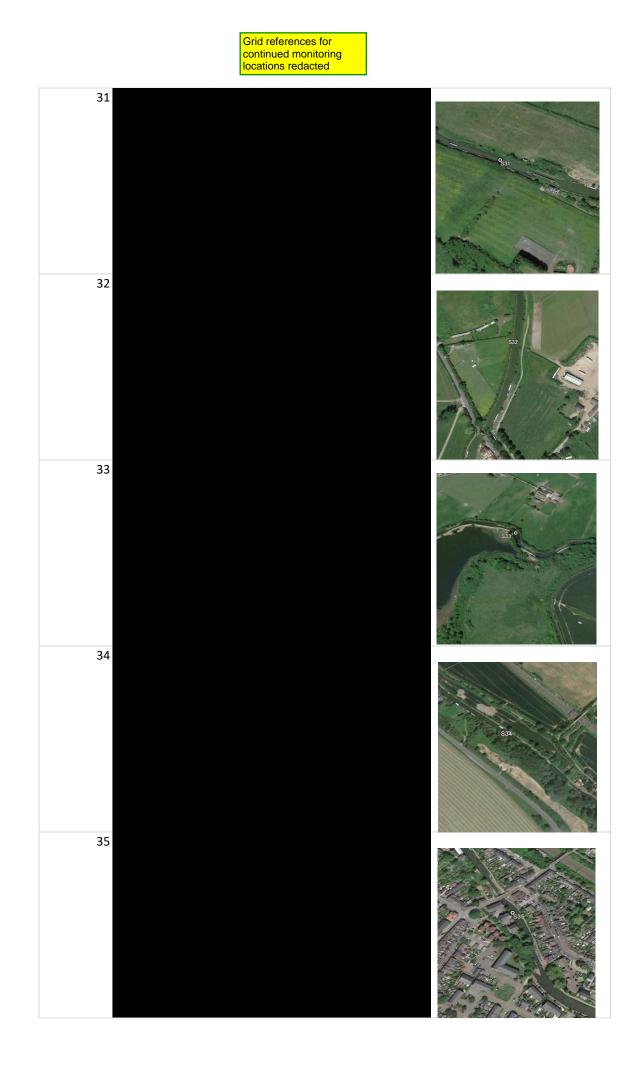


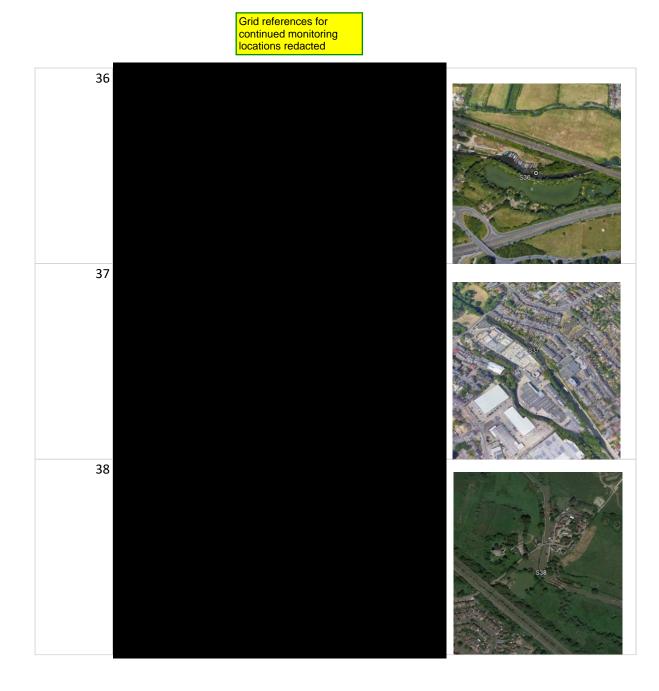








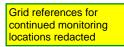




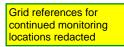
C. Sediment sampling: Connected watercourses

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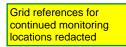
								Grid references for continued monitoring locations redacted
Sample No.	Description	Connectivity with Canal (Sluice GIS reference if available)	Comments	Lattitude	Longitude	Easting	Northing	
39	River Tame south of Tamworth	Coventry Canal CC-043-002 Sluice 25 River Tame Indirect connections further upstream via Langley Brook and Bourne Brook	Upstream to the south, before indirect connections via tributaries.					50
40	River Tame south of Tamworth	Coventry Canal CC-043-002 Sluice 25 River Tame Indirect connections further upstream via Langley Brook and Bourne Brook	Downstream of Sluice 25 (Coventry Canal aqueduct crossing R Tame).					540
41	River Anker from Atherstone to Tamworth (particularly around Polesworth)	Coventry Canal; CC- 036-002 Sluice 21; CC- 033-001 Sluice 18 (Bed Valve); CC-030-005 Sluice 17A. Note feeders also present from tributaries within this waterbody.	Upstream to the south, before Sluice 17A: (access maybe difficult)					
42	River Anker from Atherstone to Tamworth (particularly around Polesworth)	Coventry Canal; CC- 036-002 Sluice 21; CC- 033-001 Sluice 18 (Bed Valve); CC-030-005 Sluice 17A. Note feeders also present from tributaries within this waterbody.	Downstream of Sluice 21					
43	Withy Brook east of Coventry	Oxford Canal; OX-008- 005; Sluice 2, Nettle Hill	Downstream of Oxford Canal					



	Tributerus f		Diversion		
44	Tributary of River Leam east of Leamington Spa / Offchurch Lane	GUC; GU-046-001; Sluice 32, Radford Bottom Flood Paddle. Connection into tributary flowing east to west, joining River Leam just downstream.	River Leam downstream of GUC connection		
45	Welton Village tributary (of River Nene)	GUC; GU-075-007; Sluice 37, Offside Braunston Summit. Also feed from Daventry Reservoir upstream?	Downstream of the GUC crossing/sluice		45
46	Tributary of River Nene near Bugbrooke	GUC; GU-094-003; Sluice 45, Banbury Lane Flood Paddle	Downstream of sluice		Sib
47	River Tove, Stoke Bruerne to Grafton Regis	GUC – various sluices; GU-105-015 Sluice, Flood Paddle GU-106- 002 Sluice, Flood Paddle GU-106-004 Sluice GU-108-001 Sluice, Flood Paddle	Upstream of the four sluices		
48	River Tove, Stoke Bruerne to Grafton Regis	GUC – various sluices; GU-105-015 Sluice, Flood Paddle GU-106- 002 Sluice, Flood Paddle GU-106-004 Sluice GU-108-001 Sluice, Flood Paddle	Downstream of the four sluices		



49	Great Ouse, north of Milton Keynes, Stanton Low Park	GUC; GU-120-005 Sluice, Target Turn Sluice	Downstream of the sluice	
	50 River Ouzel through Milton Keynes	GUC; GU-129-010 Sluice, Tinkers Br. Flood Paddle GU-132- 004 Sluice	Upstream of the two sluices. Note highly urbanised area including major roads likely to contribute sediment quality	
	51 River Ouzel through Milton Keynes	GUC; GU-129-010 Sluice, Tinkers Br. Flood Paddle GU-132- 004 Sluice	Downstream of the two sluices. Note highly urbanised area including major roads likely to contribute sediment quality	
	52 Bulbourne through Berkhamsted to Hemel Hempstead	Direct flow connections with GUC	Upstream. Note, watercourse is ephemeral in headwaters and is heavily culverted in places.	
	53 Bulbourne through Berkhamsted to Hemel Hempstead	Direct flow connections with GUC	Downstream. Note, watercourse is ephemeral in headwaters and is heavily culverted in places.	





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