River Chew fish eDNA investigation report
BART-20222-REP
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Contents

1.0 Report Summary

Fish are keystone species of the river environment and a useful biological indicator of the long-term health of a river system. Improving a river system, such that it holds healthy natural populations of native fish, is one of the most effective indicators of a healthy of water environment. The Bristol Avon and its tributaries has had a long history of being detrimentally impacted by industrial development, leaving large areas of disconnected and degraded habitats. Many pollution events over the years have left some watercourses with fragmented fish populations where natural recovery is very difficult. If the current situation was to be left without investment, there would be little or no recovery of fish populations.

Bristol Avon Rivers Trust (BART) is working in partnership with the Bristol Avon Catchment Partnership and the Environment Agency (EA) to carry out a pilot DNA analysis for individual fish species and communities by testing samples collected from the water column at 30 sites across the catchment. BART undertook the sampling. Whilst developing the project scope and sampling methodology, BART worked closely with Nature Metrics who specialise in DNA analysis and provided this service for the River Chew pilot project.

The environmental DNA (eDNA) survey has provided a valuable fish species distribution dataset for the entire River Chew catchment that will build upon and complement existing EA fisheries datasets derived from electrofishing surveys. eDNA could provide a complementary cost-effective technique for future monitoring of fish and can be used to provide a snapshot of the presence of fish. The data collected during this project provides a useful tool for future monitoring and for aiding prioritisation of future river improvements.

Key findings:

- eDNA is an economic methodology for collecting fish species presence and absence data; particularly in areas that have not been surveyed by traditional methods recently if at all – such as tributaries.
- The River Chew eDNA survey has indicated locations where **Atlantic salmon** appear to be and points to where further monitoring might be helpful. The data also indicates those tributaries that need protection and improvement as a priority.
- The majority of species recorded during the EA's electrofishing surveys (2019) were detected in eDNA samples.

The eDNA dataset helps to improve our understanding of whether fish are accessing spawning tributaries and illustrates the presence or absence of species throughout the catchment. This information could be used as part of a plan to help prioritise future habitat improvements and fish passage works. Within this report, eDNA data has been overlaid with survey data collected via BART's walkover assessments. This includes habitat, barrier (weirs & sluices etc) and sediment pathway / diffuse pollution data which can be viewed in the interactive StoryMap (link below). Layers can be filtered in order to compare sets of data within the mapping tool that we have generated.

As a method for displaying eDNA data, a Story Map has been created to visualise the findings. The Story Map provides an interactive display, through which users can access details for each sampling data point. The River Chew eDNA story map can be found by following this link:
https://bristolrivers.maps.arcgis.com/apps/MapSeries/index.html?appid=8b69405fd7a542ddbbcc42261bb33489#

2.0 Introduction to the River Chew fish Environmental DNA (eDNA) project

Natural rivers features have been substantially modified across the UK, and locally throughout the Bristol Avon. For example, the River Chew is impounded by weirs and reservoirs for water supply, has undergone channel straightening, bank reinforcement, felling of floodplain woodland for pasture, removal of riparian vegetation and woody debris, landscape drainage and formation of embankments to prevent connection of floodplains. What we are left with, is a river catchment that has become fragmented, formed of small-scale disjointed mosaic habitats rather than a free-flowing watercourse. In turn, this has impacted wildlife, including recruitment and population distributions of fish. As a result of fish being relatively elusive and not visible to people, local populations are often poorly understood. The Environment Agency (EA) deliver statutory fish monitoring programmes, but this tends to be sporadic (delivered every 3 years) and at limited locations along the main river. It is unclear whether this monitoring programme will continue as frequently or be as extensive in future years.

Many of the Chew's tributaries have not been surveyed in 15 or 20 years, and the EA are unlikely to have the resources to repeat the surveys. So, collectively we need to find new techniques to monitor the fish populations of many of our rivers and streams. At the very least, we need to find out if species are still present to determine the status of these fish populations; hence the importance of exploring techniques such as eDNA.

Why is there a need for better understanding fish populations?

- Fish populations declining within the catchment/globally
- Under investment in protecting our rivers and fish
- Fish and healthy rivers underpin a healthy ecosystem and provide vital natural services
- Rivers provide many socio-economic benefits-recreation, tourism, angling, etc

Better understanding of our fish is important, not only in terms of the way that they enrich our ecosystems but also in our ability to value and protect our natural world. It is important to overcome the historic perception of separateness between freshwater fish and other wildlife and the role of fish in natures recovery. What lies beneath the surface of water is significant and often the neglected part of our ecosystem.

In many cases, the tributaries that support the spawning of migratory anadromous species (those living as adults at sea but returning freshwater to breed i.e., UKBAP sea trout and Atlantic salmon) have become disconnected and inaccessible due to modifications. Likewise, catadromous fish (those living in freshwater but returning to sea to spawn such as the critically endangered eel) are also impacted, as are resident fish that need to move shorter distances to feed, spawn and shelter from high flows. Pollutions including poor nutrient management, sewage discharge and abstraction add even more pressure the freshwater ecosystem.

The River Chew which is the focus of this project, supports many species of fish and other aquatic life, including coarse species (grayling, barbel, roach, chub and bream) and migratory species (brown trout, salmon, eels and lamprey). Rainbow trout and grayling are also fished throughout the catchment. There is angling interest in the catchment, with several angling clubs leasing the fishing rights through the upper to lower reaches of the catchment. Angling clubs include: Keynsham Angling Association (lower reaches), Bathampton Angling Association (lower), Chew Fly Fishing Club (middle reaches) and Knowle Angling Association (middle & formerly upper reaches, including fishing on Chew Magna reservoir).

To better understand the distribution of fish populations within the River Chew, BART have delivered a pilot project to produce a methodology for using eDNA to provide a visual snapshot of the fish species present throughout the River Chew Catchment. This report summarises findings and where possible, provides suggestions behind patterns in the data.

2.1 What is Environmental DNA (eDNA)?

eDNA analysis is a service which uses metabarcoding of the 12S gene to characterise fish species diversity from eDNA samples. Sources of eDNA include secreted faeces, mucous, gametes, shed skin, scales, hair and carcasses. Metabarcoding is a highly validated and widely used analysis that has been shown to outperform electrofishing for fish community assessment. Semi-quantitative data is obtained from this analysis and fish can be surveyed year-round.

eDNA offers a cost-effective and supplementary monitoring technique that will improve our understanding of the distribution of fish throughout the catchment. This technology also provides an indication of relative abundance, which can be useful for suggesting relative differences in abundance of a particular genus between two points in a river network. This is particularly useful for comparing relative abundance up and downstream of a barrier, where results can strongly indicate where genus are prevented passage due to the presence of a river obstruction or impact to habitat.

The Environment Agency is also using eDNA for monitoring fish communities, and is working on a tool that would generate a Water Framework Directive (WFD)-compliant index score for lake fish communities, based on the work carried out in collaboration with the University of Hull (e.g. Lawson-Handley et al., 2019).

2.2 Why the need for Environmental DNA (eDNA) analysis?

Currently, fish data is restricted to 5 fixed locations along the main river. Therefore, we do not fully understand which species colonise the wider tributaries.

2.3 Project Aims and Objectives

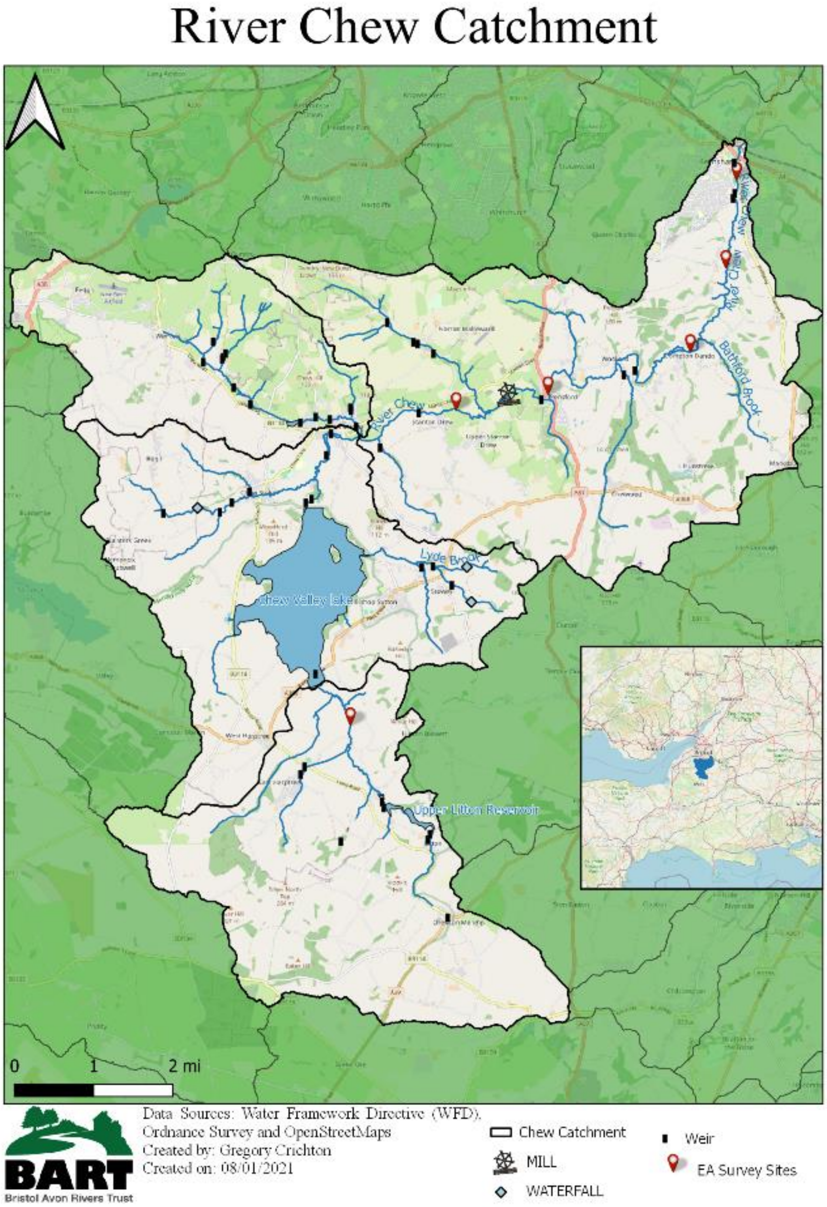
The main aims and objectives for this project are:

1. Deliver eDNA analysis for the River Chew at 30 sites;
2. Provide mapped outputs of fish distributions throughout the catchment;
3. Provide a comparison with previous Environment Agency electrofishing data;
4. Demonstrate learning and determine any limitations with applying eDNA analysis for fish.

2.4 Project Area

The River Chew (Figure 1) is a small rural lowland water course rising in the Mendip Hills in the southwest of England and discharging into the River Avon at Keynsham, midway between the cities of Bristol and Bath. The River Chew catchment covers an area of approximately 145 km^2 and is hydrologically complex owing to the permeability and the presence of reservoirs, particularly the Chew Valley Lake which supplies potable water to the City of Bristol. The catchment experienced extensive flooding during the summer flood event of July 1968 during which the historic bridge at Pensford was completely destroyed.

Figure 1. Map showing the project area.



2.5 River Chew Water Framework Directive Status

The WFD aims to protect and improve the water environment by setting objectives to meet ‘Good Status’ for surface and groundwater bodies in terms of biological, physical and chemical elements, as described in River Basin Management Plans (RBMPs). The Severn RBMP includes the study area of the River Chew (ID GB109053021852). The River Chew is failing to meet good status.

The River Chew has Water Framework Directive failures for fish and phosphate, plus other reasons for failure (rural land management, fish barriers, water industry infrastructure and sewerage discharge), details summarised in Table 1. Through field investigations, BART has identified issues impacting river habitat including barriers which are impeding fish passage throughout the catchment. During an initial appraisal of sites, BART has estimated that there are fifteen significant barriers to fish passage and estimated that approximately one third of the River Chew catchment downstream of Chew Valley Lake is impounded by man-made obstructions including weirs and sluice gates which historically used to divert rivers to mills. It is likely that these structures (primarily weirs and sluice gates) are impeding, delaying or reducing the passage of a percentage of wild fish populations from moving freely throughout the catchment and accessing suitable spawning habitat and tributaries.

Not only do these structures impede passage of fish in search of food and spawning grounds, but obstructions also change habitat type and flow conditions. Consequently, due to the impounding effect of obstructions such as weirs and sluice gates, natural riffle-pool habitat has been transformed to slower flowing "canalised" sections of water which are unnaturally straight and lack cross-sectional habitat diversity that is necessary to support a variety of species.

Sewage discharge into the River Chew has occurred on multiple occasions in 2020 (Rivers Trust, 2021), particularly along the main river at multiple sites throughout the catchment. Sewage problems arising in the River Chew are likely to have a significant impact upon the biodiversity of the whole of the catchment, as well as degrading its attraction as a recreational focus point for the public. This is a catchment-wide issue and needs to be addressed at catchment level.

Urban development throughout the catchment adds to the existing pressures facing the River Chew catchment. New development needs to ensure Biodiversity Net Gain, giving due consideration to all aquatic wildlife; this is currently not considered during planning consultations.

There are also current failures for phosphates which leach into watercourses and impact on water quality status of the river. BART has delivered several surveys throughout the catchment to identify areas of sediment input, poor farming practices and modified sections of river with unfavourable habitat and barriers to fish. These failures are likely to be impacting upon recruitment and population distributions of fish within the catchment.

Considerable quantities of water are abstracted form the River Chew. The River Chew is classified by the EA as a catchment with low water supply reliability. Over abstraction will lead to poor ecological conditions in the local catchment and further downstream, and evidence suggests that the main river experiences low flows in the dry months. The EU Water framework Directive work done by the EA in 2019 lists water abstraction as one of the reasons that the River Chew is not achieving Good Ecological Status.

Table 1. Water Framework Directive overview of the River Chew waterbodies

WATERBODY	CLASSIFICATION 2019 cycle	Reason for not achieving good status & reasons for deterioration (RNAG)
Chew - source to Chew Valley Lake	POOR (Status deteriorated – moderate in 2016 cycle)	Physical modification - Local and Central Government & urban & transport; Failures for fish Flow – water industry Point source pollution - Abandoned mine / quarry
Chew - Chew Valley Lake to conf Winford Brook	MODERATE	Failures for Phosphate: Poor nutrient management - Agriculture & rural land management; Sewerage discharge & physical modification - Water Industry; Physical modification – urban & transport, local & central government
Chew - conf Winford Bk to conf R Avon (Brist)	MODERATE	Sewage discharge (continuous) – water industry – impact to macrophytes and phytobenthos combined. Failures for phosphate.
Winford Brook - source to conf R Chew Overview	POOR	Physical modification, barriers, ecological discontinuity - water industry; Regulating reservoir flow regime – water industry; Fish stocking - other

2.5 River Chew Fish Species

The River Chew and its tributaries, whose headwaters arise from the Mendip AONB, act as a precious wildlife corridor for the many pollution sensitive species. There are populations of brown trout (UKBAP), grayling, chub, roach, eels (IUCN Red List Critically Endangered species), brook lamprey (limited distribution), Atlantic salmon (UKBAP) plus invertebrates including various species of mayflies. Water Vole (UKBAP) and Otter are known to frequent the river, as well as Dipper and Kingfishers.

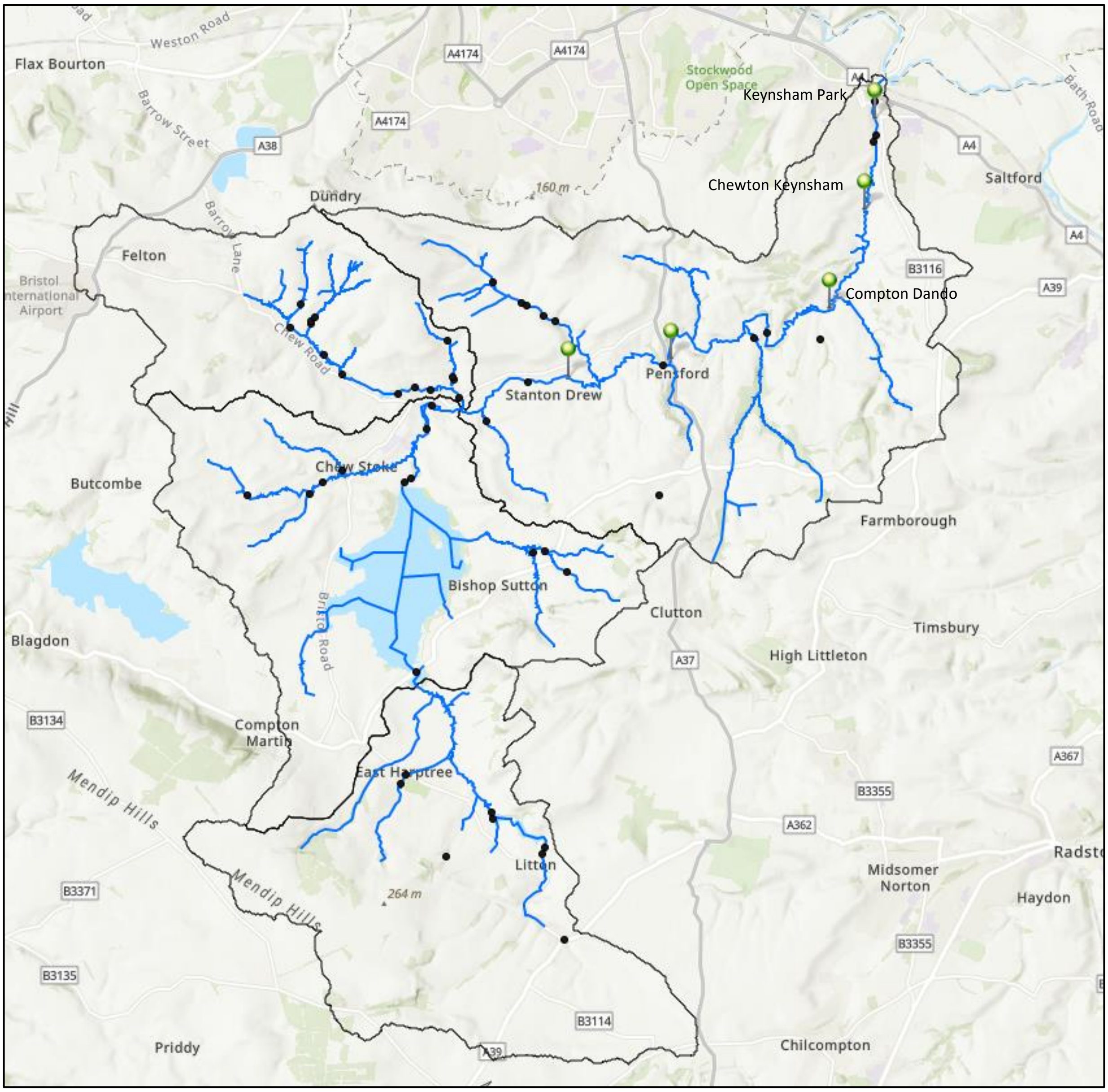
There is angling interest in the catchment, with several angling clubs leasing the fishing rights through the upper to lower reaches of the catchment. Angling clubs include: Keynsham Angling Association (lower reaches), Bathampton Angling Association (lower), Chew Fly Fishing Club (middle reaches) and Knowle Angling Association (middle & formerly upper reaches, including fishing on Chew Magna reservoir). Bristol Water also mange fisheries on Chew Valley Lake. Each angling club will obtain a catch record, but this will be limited to those species of fish that are caught by anglers (game and coarse fish).

One of the most frequent terms that you may encounter when discussing fish is ‘game’ and ‘coarse’. These terms came into play in the late nineteenth century. In a nutshell, ‘game’ fish refer to those species which can be hunted via fly fishing such as brown trout and salmon, as distinct from ‘coarse’ fishes which are generally caught by anglers in a more sedentary means – examples include Roach, Chub, Tench and wild-caught Bream. We have distinguished between the two terms for the species identified during the eDNA analysis which has been summarised in Table A1 (Appendix 1). During this project, we’ve also included a third term ‘minor’ which includes those species which do not have an angling interest such as European Bullhead (UKBAP) and Three-Spined Stickleback.

2.5.1 Environment Agency (EA) River Chew Electrofishing Data

The EA carries out fish population surveys every three years in the River Chew catchment. The surveys are carried out using electrofishing, which is a technique commonly used by scientists and fisheries biologists to survey rivers and assess what fish are present. When done properly, it stuns fish for a short period allowing them to be removed carefully with a net, examined and then returned alive with no permanent harm done. By using information from electrofishing samples, the agency can collect data about the number of species and the conditions in a body of water.

Figure 2. Map showing the location of the Environment Agency electrofishing sampling sites (green pins). Black dots represent potetnial barriers to fish movement, such as weirs and sluices



The effectiveness of electrofishing is influenced by numerous biological, logistical, and environmental factors. Fish size, water visibility, level of stunning, and crew experience are all factors that can have an impact on catches. In addition, fish that live in deep areas, often don't float up in time and recover before they can be netted.

Fish at the edge of the electrical field are often not completely stunned and may escape capture. Since not all fish can be collected, electrofishing provides a sample of the river's population.

2.5.2 Environment Agency 2016 & 2019 fish population data

During 2016, the Environment Agency delivered a fish population survey (electrofishing) of five sites (Figure 2) throughout the lower- middle reaches of the River Chew. A total of fifteen species of fish were recorded (out of the 542 fish that were caught), with brown trout the most widespread species (recorded at all 5 survey sites). Four of the sites surveyed were downstream of Chew Valley Lake except for the Shrowl Bridge site, which was the uppermost survey site, approximately 1.8km upstream of Chew Valley Lake.

In 2019, the Environment Agency repeated the fish population surveys at four of the sites and delivered a fifth at Chewton Keynsham (instead of the Shrowl Bridge site), 2.5 km upstream from Keynsham Park, in the lower reaches of the catchment. A total of twelve native fish species were recorded (out of the 445 fish captured), with Brown trout being the most widespread and Dace being the most abundant (114 individuals).

A comparison between the EA's electrofishing data from 2016 and 2019 surveys has been undertaken and summarised below: summarised below:

- *Overall decrease in the estimated population densities of key fish species at three of the four sites.*

- *At Stanton Drew, fish population density (number of fish per 100m2) of key fish species decreased by 84%. This site had little refuge for fish but was dense with vegetation, so this may affect the number of fish caught as well as the efficiency of the survey.*

- *Pensford and Compton Dando fish population densities decreased by 62% and 70% respectively, but brown trout numbers were higher at both sites in 2019 than in 2013.*

- *Declines in the population density of adult and elver European eel, Brown trout and Grayling between 2016 and 2019 across these three sites are the main species declines contributing to the overall population density decline here.*

- *At Keynsham Park there has been a 60% increase in key species population densities, largely due to the increase in Dace and Roach. Being a deep, impounded reach, this site is better suited to coarse fish and this is reflected in the catches. It is also less likely to be impacted by the lower than usual water levels/flows as a result of low rainfall experienced in 2018 & 2019 that may have impacted fish survey data at other sites due to fish moving out of the survey area in search of better refuge habitat.*

To provide a more holistic context, the EA provided the following summary:

- *The EA survey results from 2019 are comparable to historic year's surveys in the mid-2000's, with 2016 having better catch results. However, catches are still lower than expected given the habitat available.*
-
- *The EA's 2016 survey report concluded that large barriers located along the River Chew, are impacting on fish stocks, particularly of migratory species such as European eels, Brown trout and Atlantic salmon.*
-
- *As a next step, the Environment Agency advised, that it would be important to look for project opportunities in the catchment to improve fish passage as a primary measure, whilst also looking to restore habitat in less favourable stretches.*

Whilst the EA delivers electrofishing surveys throughout the River Chew every 3 years at 5 fixed sites along the main river, there is not any existing data that provides information about fish species within the tributaries and smaller streams. Tributaries provide fundamental habitat for fish refuge and spawning. Tributaries can also be impacted most quickly and severely to pollution events, but they're also quickest to respond and recover. In our view, it is therefore important that we begin to incorporate smaller waterbodies within our interpretation of catchment fish populations.

This project has explored the use of eDNA analysis to identify which species colonise the main river and smaller tributaries which will help us to fill in the gaps and provide a presence and absence dataset. Population densities (relative abundance) via eDNA have also been estimated, but this data has limitations which have been summarised within a section of this report.

3.0 eDNA Sampling Methodology

The sampling method for eDNA is relatively simple: requiring a syringe and filter. BART identified a total of 30 sites to sample, which include the EA electrofishing sites. During February 2021, at each site, water was collected from the waterbody at several locations throughout the cross-section (in one sampling bag) in order to collect data from each habitat type. The water was then pushed through a filter via a syringe (as per title page image), and the filter containing biological fragments were sent to the supplier for them to run DNA sequencing and analysis. Results were issued to BART in the form of a report (Appendix A2).

Image credit: Nature Metrics

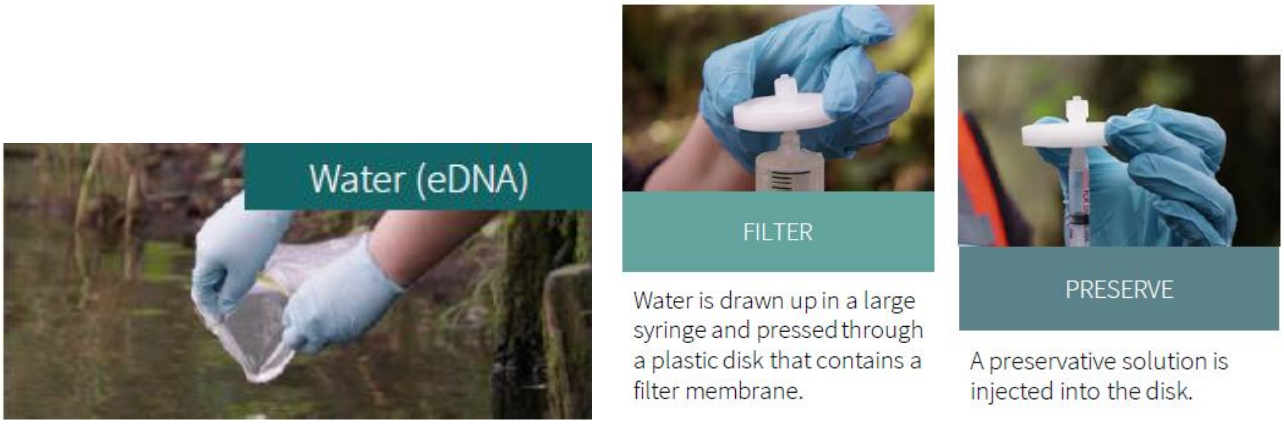
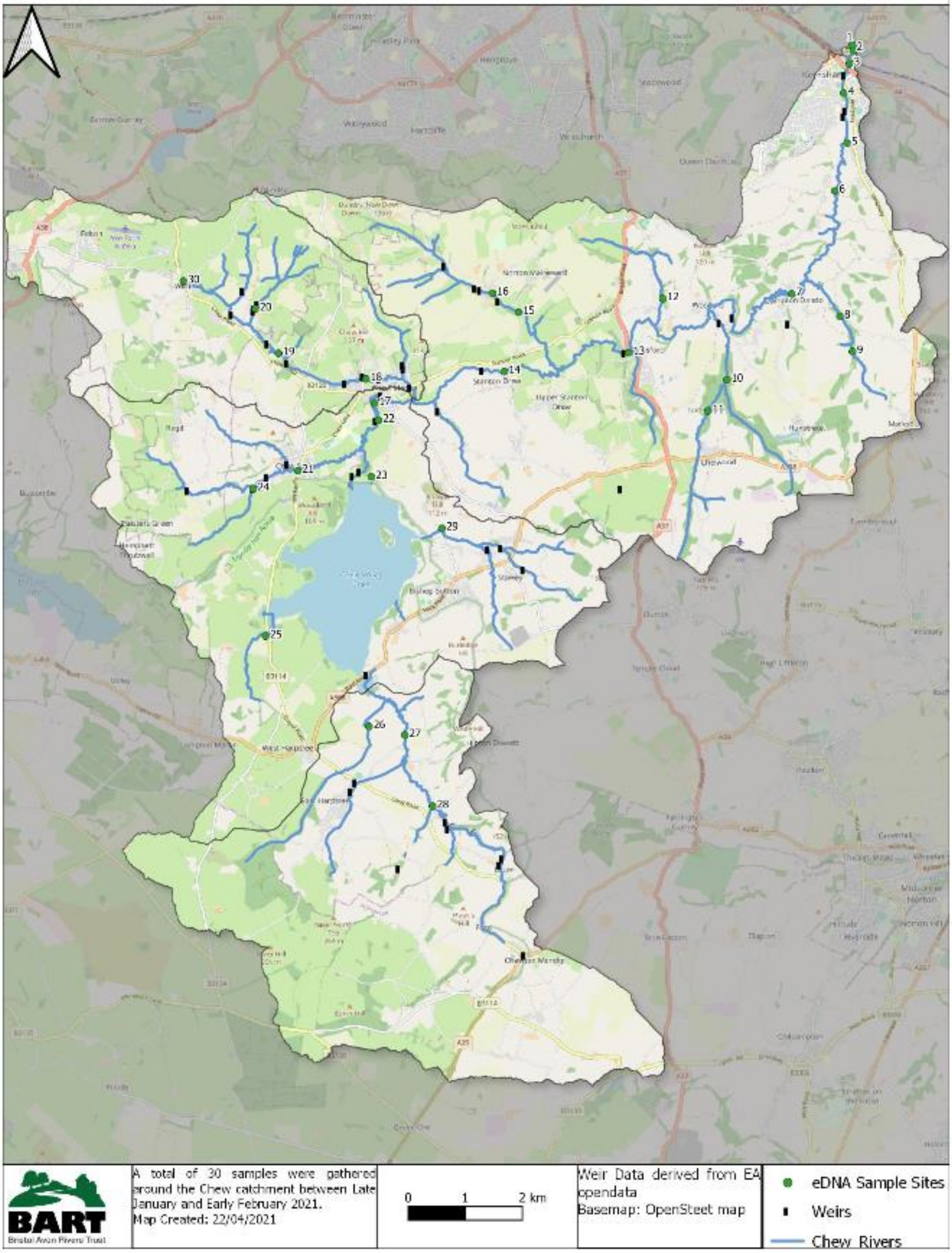


Figure 3. Map showing the project area and eDNA sampling sites

Chew Catchment eDNA Sample Sites



The analysis (Metabarcoding) identifies the presence of organisms to 'genus' level, providing a qualitative data set, because the analysis does not provide precise counts for each genus. The results are useful at giving a strong indication of the presence of a genus, including some species that do not always get picked up using traditional sampling methods such as electrofishing.

The distance downstream that eDNA may still be detected depends on flow rate and volume of water. For this project we have estimated that the data for each sample is relevant to 1km upstream, but this is reduced if there is a barrier within the section of river above the sampling point.

4.0 eDNA Results

30 eDNA samples were collected throughout the catchment (sampling sites identified in Figure 2), with 19 species of fish identified through the metabarcoding analysis. Further mapping for the distribution of each species is provided in the Appendix A1. A high-level summary has been provided within this section of the report.

4.1 eDNA Overview:

- *A total of 19 fish taxa were detected*
- *Average taxonomic richness (no. of species present) per sample was 4.2 and ranged from 1 to 12*
- *Most abundant sequences: European bullhead (Cottus gobio) and three-spined stickleback (Gasterosteus aculeatus)*
- *Most commonly detected species: three-spined stickleback (Gasterosteus aculeatus), European bullhead (Cottus gobio) and trout (Salmo trutta)*
- *Species of note include European eel (Anguilla anguilla) - critically endangered, UKBAP), European bullhead (Cottus gobio - SAC), Atlantic salmon (Salmo salar - SAC & UKBAP), trout (Salmo trutta - UKBAP), and lamprey (Lampetra sp. - SAC & UKBAP).*
- *No fish species were detected in sample 20*

4.2 eDNA Sample composition

A total of 19 unique fish taxa were detected during the sampling. 84.2% (16 taxa) were at least 99% similar to a species in the global reference databases, and species names are suggested for these taxa. The remaining taxa were identified to genus (3 taxa). Species of note include European eel (*Anguilla anguilla*) - critically. endangered, UKBAP), carp (*Cyprinus carpio* - vulnerable), European bullhead (*Cottus gobio* - SAC), Atlantic salmon (*Salmo salar* - SAC & UKBAP), trout (*Salmo trutta* - UKBAP), and lamprey (*Lampetra sp.* - SAC & UKBAP).

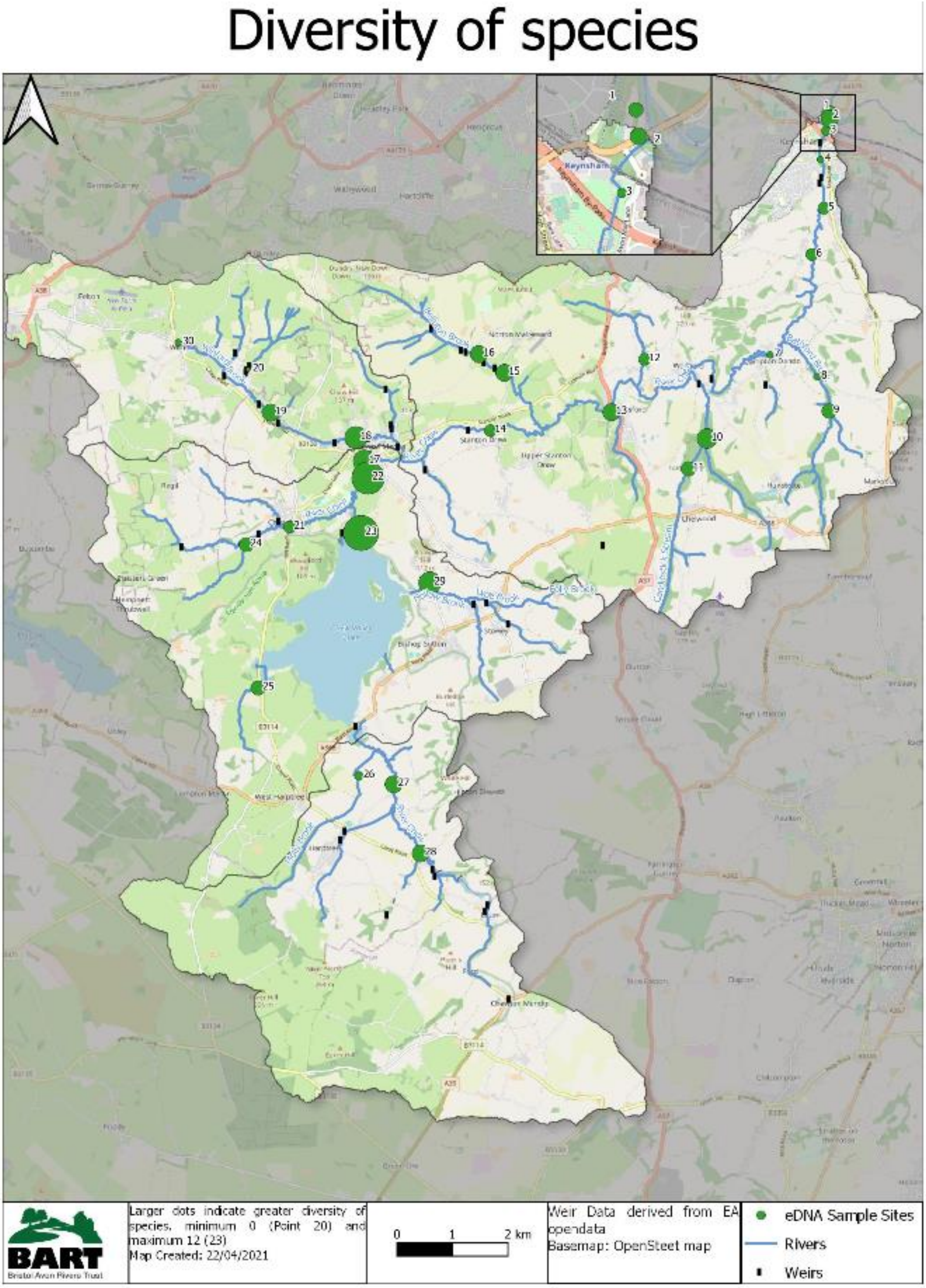
The 19 species detected in the 30 samples:

European eel (*Anguilla anguilla*)
Common bream (*Abramis brama*)
Crucian (*Carassius* sp.)
Carp (*Cyprinus carpio*)
Gudgeon (*Gobio gobio*)
Dace/orfe (*Leuciscus leuciscus/idus*)
Minnow (*Phoxinus phoxinus*)
Roach (*Rutilus rutilus*)
Rudd (*Scardinius erythrophthalmus*)
Tench (*Tinca tinca*)

Stone loach (*Barbatula barbatula*)
Northern pike (*Esox lucius*)
Three-spined stickleback (*Gasterosteus aculeatus*)
Perch (*Perca fluviatilis*)
Rainbow trout (*Oncorhynchus mykiss*)
Atlantic salmon (*Salmo salar*)
Brown trout (*Salmo trutta*)
European bullhead (*Cottus gobio*)
River lamprey (*Lampetra* sp.)

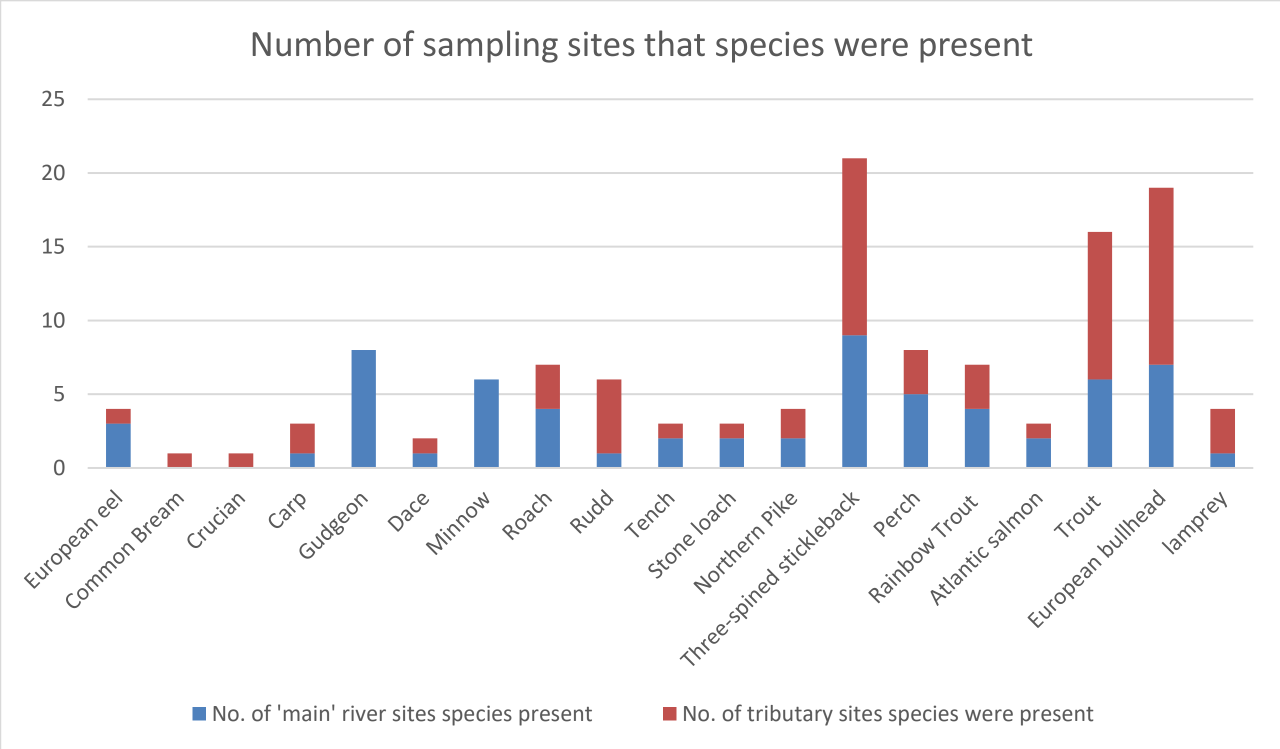
European bullhead (*Cottus gobio*), which accounted for 19% of the total sequence reads, was among the most abundant in terms of sequences. Among the most commonly detected species were the three-spined stickleback (*Gasterosteus aculeatus*), European bullhead (*Cottus gobio*) and trout (*Salmo trutta*), which were detected in 21, 19, and 16 of the samples, respectively.

Figure 3. Map showing the species diversity at each site (1-30) – bubble relative to number of sequences found in each smaple.



A breakdown of the species present at each site has been presented in Table 1 for ‘main’ river & ‘tributary’ sites.

Table 1. Number of smapling sites that species were present



4.3 Summary of eDNA fish data

The following summarising statements have been provided following analysis of eDNA data:

Highest relative abundance in the catchment, based on the number of DNA detected within samples:

- *Three-spined stickleback (21)*
- *European bullhead (19)*
- *Brown trout (16)*

Lowest relative abundance within the catchment:

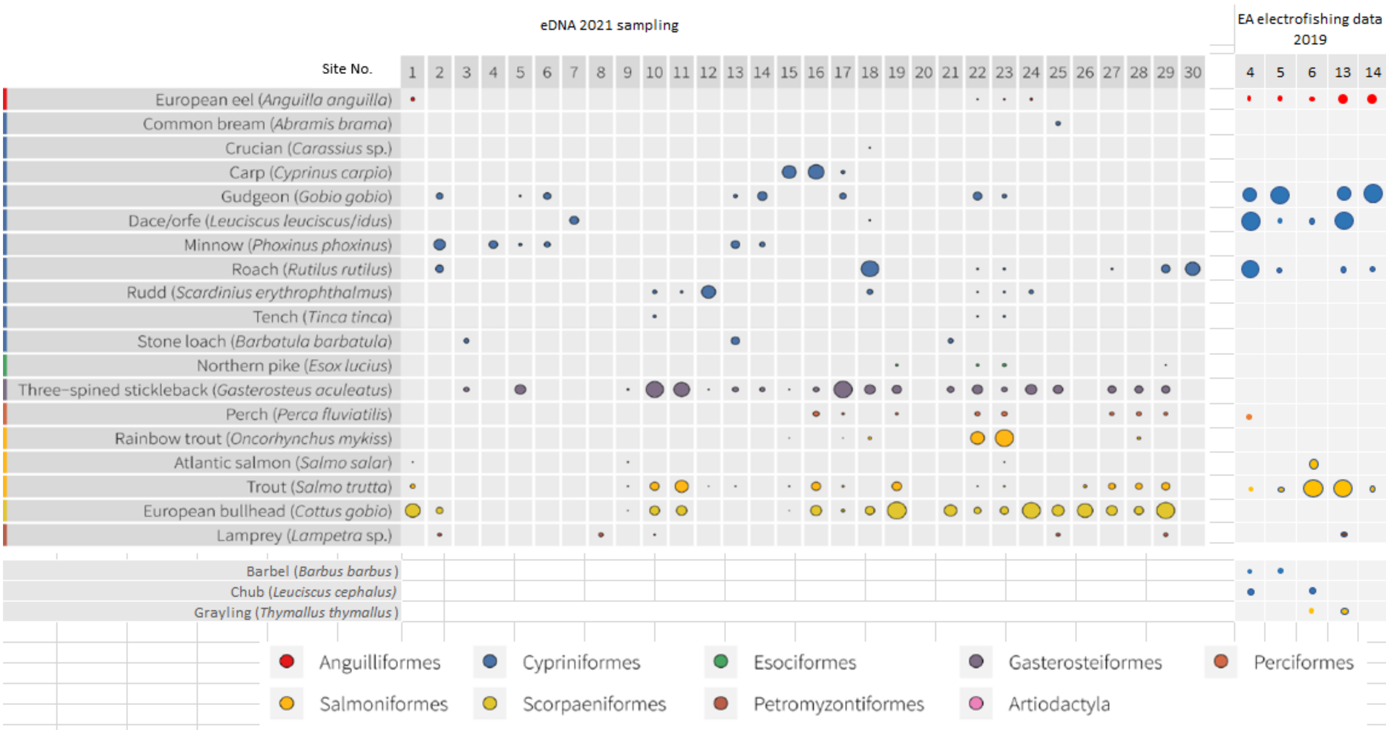
- *Common Bream (1)*
- *Crucian carp (1)*

- ***The most diverse sampling point is site 23 with 12 species and site 22 with 11** species recorded. These sites are located downstream of Chew Valley Lake and therefore it is possible that these samples include DNA from species which inhabit the lake.*
- ***The River Chew's tributaries support greatest diversity of minor species** including bullhead, stickleback, lamprey, which thrive in habitats that have clean gravels and well oxygenated water.*
- ***Winford Brook sample site 20 contained no fish DNA**. The adjacent land is agriculture – predominantly pastoral farming and the sample contained high levels of cow DNA which could distort the other reads. Several sediment issues have been identified here (see sediment pathway data on [Story Map]). The sample was also collected between two weirs which are likely to provide a barrier to fish movement.*
- ***Winford Brook Site 30 only contained Roach** which is tolerant to poor water quality. This urban section near the headwaters has been artificially straightened and culverted in sections. Ongoing sediment pollution issue caused by a local development. We were surprised to not find minor species such as European Bullhead and Stone Loach here.*
- ***Minnow and Gudgeon were present only at sites on the main river** within sections that are characteristic of slow flowing habitats.*
- ***Perch were found in the upper catchment, above Pensford only.** Perch colonise flowing freshwater habitat and tend to shoal when hunting at dusk. The eDNA data has indicated that Perch were identified along some of the upper catchment tributaries & above Chew Valley Lake.*
- ***Rainbow Trout were identified in high relative abundance directly below Chew Valley Lake and the reach above the lake.** Data implies that there are escapees from the lake which has been stocked with Rainbow Trout for angling. Or it may suggest that the samples collected below the lake contains the DNA from the fish within the lake, which could distort the data for sites 22 & 23. Previous electrofishing surveys have identified rainbow trout above the lake.*
- ***BAP Atlantic salmon found at 3 sites in low abundancy** – one on the Bristol Avon below the confluence of the Chew, one on the Bathford Brook (Chew tributary) and one site below Chew Valley Lake. Mature male Atlantic salmon run rivers from the sea to spawn but live out their adult lives at sea, returning after between one and four years to their natal rivers to spawn. EA electrofishing surveys has also identified Atlantic Salmon within the catchment.*
- ***BAP European eels were only present at 3 locations and in low abundancy**; one site on the Bristol Avon near to the confluence of the River Chew in Keynsham, and two sites below Chew Valley Lake and one site along the Chew Stoke arm. This could suggest that barriers are inhibiting eel movement within the catchment as you would expect a much more widespread population throughout the catchment. Particularly as populations were identified below Keynsham Park weir and within the confluence of the Avon, but not within the reaches above the weir. During the time of sampling in February 2021, you would expect eels to be moving throughout the river network. However, as Eels were picked up at all of the EA survey sites in 2019, this might indicate that the eDNA survey method doesn't pick up the eel DNA so well.*
- ***BAP Brown trout were located at a number of sites**, with approx. 2/3 of their presence found within tributaries. Brown Trout prefer cleaner and clearer rivers and spawn in clean well oxygenated riverbed gravel, which is in keeping with its presence within the catchment*
- ***BAP / SAC European Bullhead were abundant throughout the catchment**, with approx. 2/3 of their presence found within tributaries. They were not detected within deeper, slower flowing impounded reaches. This is in keeping with their preferred habitat, which includes living in the gravel or rocky bed of rivers in shallower faster flowing sections where they feed on invertebrates and fish fry.*
- ***BAP / SAC Lamprey were most abundant within the tributaries**. No presence detected in the main river, apart from the Bristol Avon at the confluence of the Chew, where they are known to spawn. It was interesting that Lamprey had a relatively high abundance on the Bathford Brook, suggesting that this tributary (most downstream tributary) could provide valuable spawning and refuge for this species.*

4.4 Comparison to Environment Agency fish survey data

The following summary provides a comparison of BART's eDNA datasets with the existing EA electrofishing data. Table 2 illustrates the presence and relative abundance, in relation to species identified within the sample. We have also displayed the latest set of EA electrofishing data from 2019 as a comparison. Sites 4, 5, 6, 13 & 14 are the EA monitoring sites which we sampled using eDNA analysis.

Table 2. The proportion of the sequencing output allocated to the different species (rows) within each sample (columns). Each bubble per sample represents the proportion of DNA for each species for that sample. The size of the bubble is relative to the number of sequences from all species for that sample



The majority of species recorded during the EA's electrofishing surveys (2019) were detected in eDNA samples. However, Grayling, Barbel and Chub were not identified within the eDNA analysis but were present during the 2019 electrofishing surveys. It is unclear whether these species are absent from the catchment or that their DNA was not picked up. We would recommend collecting eDNA samples at the same time as electrofishing in order to make a direct comparison.

We also found that several species not picked up by electrofishing are consistently detected by eDNA such as Three-spined Stickleback, Minnow and Stone Loach. These species are often under-recorded during electrofishing due to the size and difficulty of assessing minor species via electrofishing. Historically the drivers behind the surveys were different to more recent surveys and minors were not recorded and therefore are not represented fully in historic datasets. This is rarely the case now and they will be recorded, but there may be differences in how they are recorded (i.e., abundance noted only 1-99 or first 50 of each species measured then counted thereafter.

4.5 eDNA Limitations

There were a high number of reads corresponding to cow (*Bos taurus*) in all samples (ranging from 1.3% to 100%), which could indicate the sampling location had a large amount of cow DNA. Although the primers used are designed to target fish, they can also amplify DNA from some other vertebrates especially when that DNA is abundant in the sample. An excess of DNA from one species could result in it outcompeting amplification of DNA from other species during PCR. Species richness may be reduced in samples where there is an overwhelming quantity of DNA from one species, this is particularly true for sample 20 where only cow DNA was detected. This is still very interesting, because it proves how widespread an issue there is regarding nutrient enrichment from agriculture. We already know this from the walkover surveys, but it illustrates quite well how it affects the whole catchment.

When comparing the eDNA results against the EA electrofishing data for 2019, we are effectively comparing datasets from two years apart, so in terms of direct comparison, we can conclude very little. However, the eDNA results correspond well with previous electrofishing datasets, demonstrating that eDNA analysis has identified the same or similar species at each of the EA fish sampling sites.

The number of species identified at sites below the lakes could be misleading and include those species which colonise the lake. This could be true in the most abundant sample at site's 23 & 22 which will likely include DNA from Chew Valley Lake. There are a small number of stocked fisheries in the catchment which include some of the species recorded like crucian carp, common bream, common carp, rudd that wouldn't normally be permitted for stocking to rivers.

Rates of DNA shedding may vary between species and life events and fish behaviours may increase/decrease shedding rates which will influence relative abundance. For example, reproduction events or fish species that are very active will tend to shed more DNA. In rivers, concentrations are stronger when the sampling site is closest to the fish, as DNA degrades quite quickly in the water as it travels downstream meaning it will become more dilute.

5.0 Conclusion & Recommendations

In summary, the project has provided an approach in using eDNA to provide an illustrative output for presence and absence data of fish across the entire Chew catchment. The evidence provided within this report alongside the wider eDNA research being undertaken across the world makes a strong case that eDNA can help collect a baseline for fish. eDNA can supplement existing EA electrofishing data and offers a more complete picture for distributions of fish within the catchment.

This study provides a useful approach in showing how eDNA can be applied to help 'fill in the gaps' to collect a baseline for smaller waterbodies which provide valuable habitat and refuge for fish. The study demonstrated that the River Chew's tributaries support the greatest diversity of minor species including bullhead, stickleback, lamprey, which thrive in habitats that have clean gravels and well oxygenated water. It also shows which tributaries are supporting populations of migratory Brown Trout and Atlantic Salmon, which could help targeted restoration to improve spawning substrate and supporting habitat.

The eDNA samples didn't provide us with the level of data to determine whether a particular barrier provides complete or partial fish passage issues, but the data does suggest that barriers could be fragmenting species including the European eel which was found in very low abundance at limited sites. Sampling closer together could potentially provide this level of detail. Limitations of eDNA sampling have also been highlighted within this report. One of those being the sample site downstream of Chew Valley Lake reservoir has picked up DNA from species present within the reservoir, which has very likely skewed results for the reaches directly downstream. The rates of DNA shedding may vary between species and life events and fish behaviours may increase/decrease shedding rates which will influence relative abundance; therefore, we conclude that eDNA is most useful to determine presence and absence within a watercourse, including identifying invasive non-native species (potentially as an early warning) that thankfully were not recorded in this survey.

BART supports the development of a Bristol Avon Fish Recovery Plan to improve our catchment partnerships understanding of the species distributions within our local rivers. Rather than focussing on short-term fixes, we promote the proactive approach of ecosystem management to build up the resilience of fish stocks through measures such as habitat enhancement. This strategic approach will address the needs of mixed fish populations and the ecosystems of which they are part of.

6.0 Glossary

species (s./pl.) - A group of individuals capable of interbreeding. This is the most important taxonomic unit defined by scientists and the population trends of individual species are a key indicator in judging the effect of conservation programs. Related species are grouped together into progressively larger taxonomic units, from genus to kingdom. Homo sapiens (human) is an example of a species.

genus (s.) / genera (pl.) - A group of closely related species. Each genus can include one or more species. Homo is an example of a genus.

UKBAP species UK Biodiversity Action Plan species have been identified as being the most threatened and requiring conservation action under the UK Biodiversity Action Plan.

7.0 References

Environment Agency (2012) Bristol Avon and North Somerset Streams WFD Management Area Abstraction Licensing Strategy.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/292758/LIT_7605_cbc33b.pdf

JNCC, UK BAP Priority Habitats, (2019).
https://jncc.gov.uk/our-work/uk-bap-priority-habitats/#background

Holden J *et al* (2015) Agriculture's impacts on water quality.
https://www.foodsecurity.ac.uk/?s=agriculture%27s+impact+on+water+quality

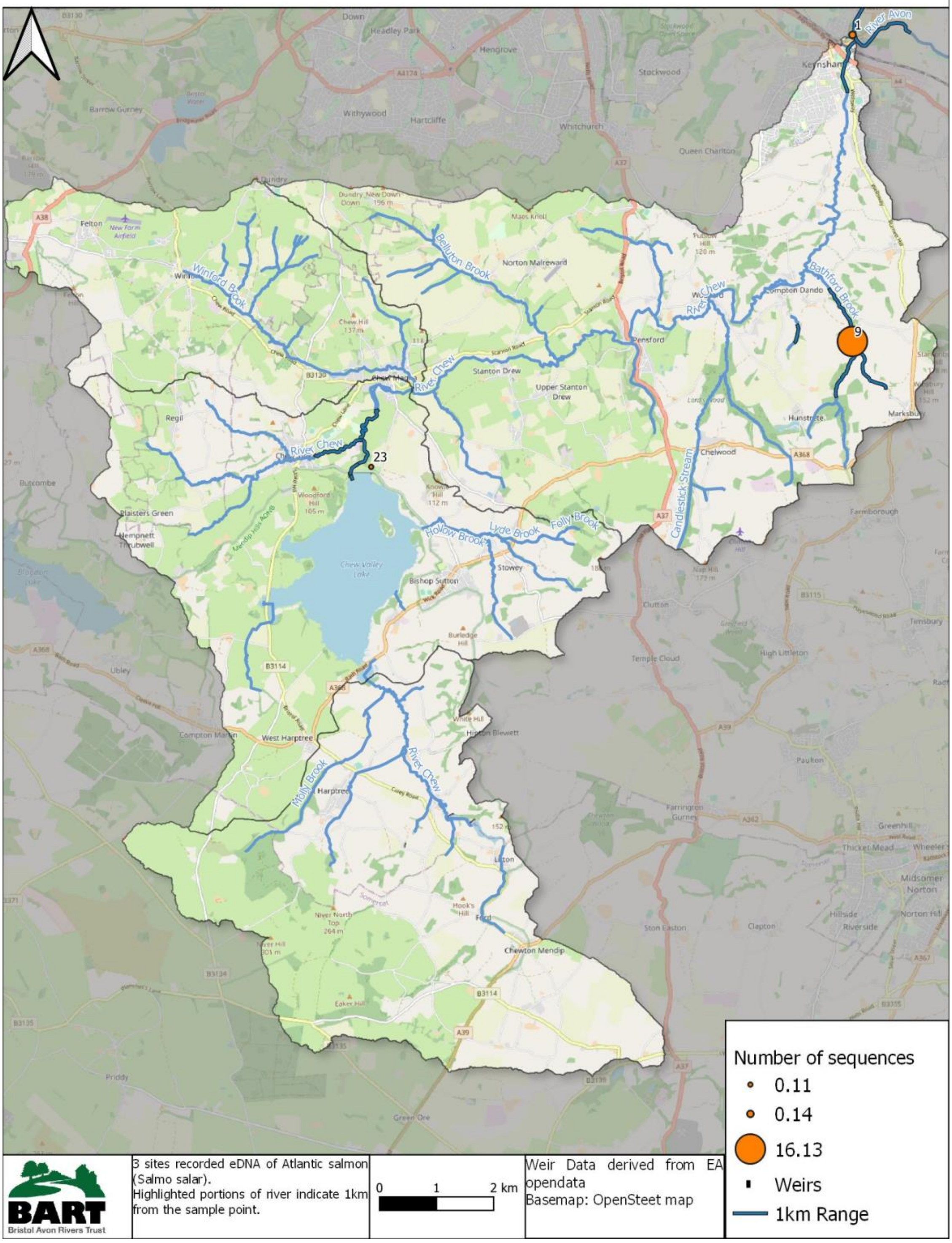
The Rivers Trust: Sewage Update (April 2021)
https://www.theriverstrust.org/2021/04/07/sewage-update-2020-spill-data-added-to-our-map/

Nature Metrics (April 2021), METABARCODING RESULTS, Bristol Avon Rivers Trust, River Chew Catchment

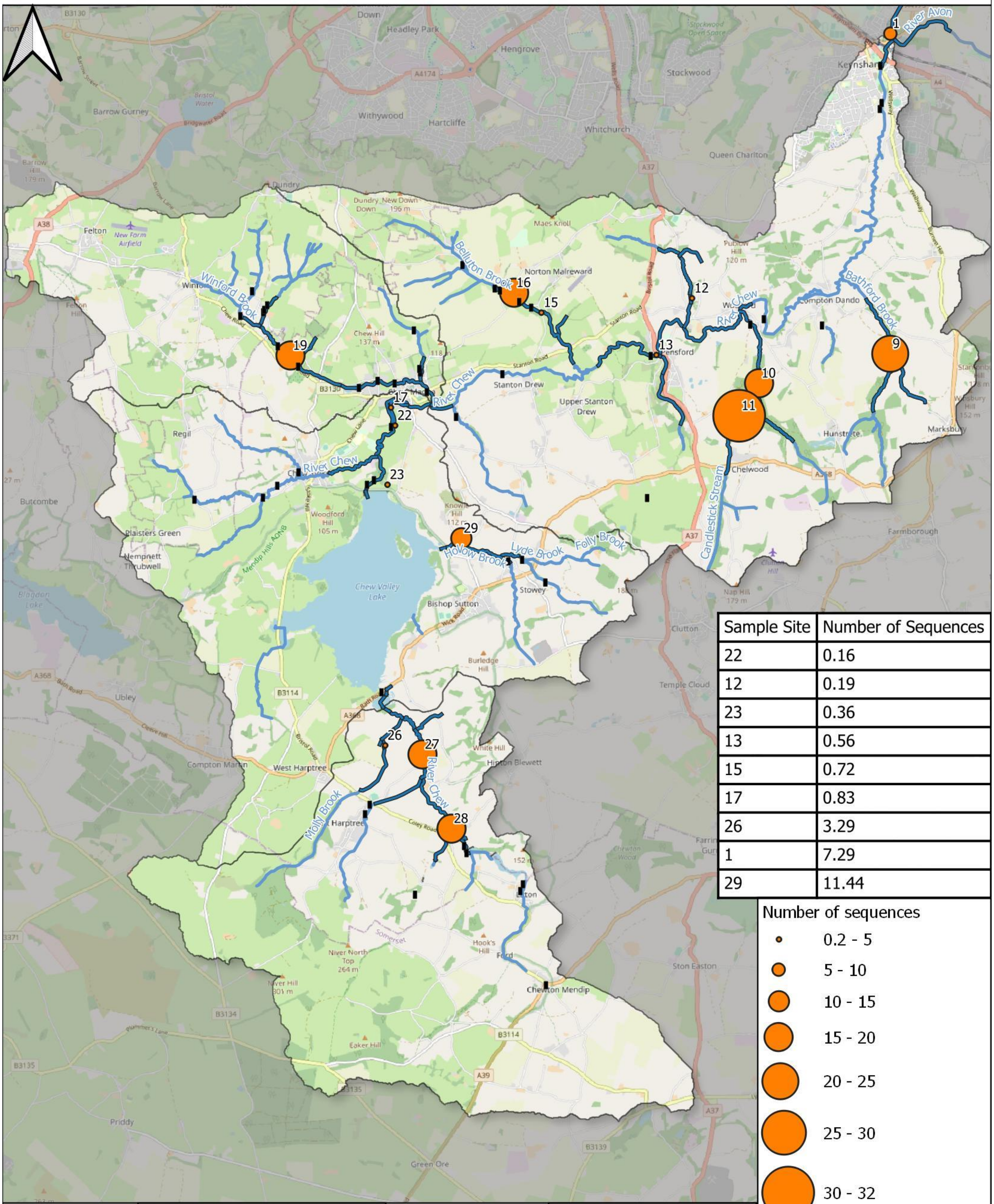
Appendix A.1 – Survey Findings

Where applicable, relative abundance has been demonstrated by the number of sequences identified within the sample (coloured circles on the maps). However, please note that rates of DNA shedding may vary between species and life events and fish behaviours may increase/decrease shedding rates which will influence relative abundance. For example, reproduction events or fish species that are very active will tend to shed more DNA. In rivers, concentrations are stronger when the sampling site is closest to the fish, as DNA degrades quite quickly in the water as it travels downstream meaning it will become more dilute.

Atlantic salmon (Salmo salar)



Brown trout (Salmo trutta)

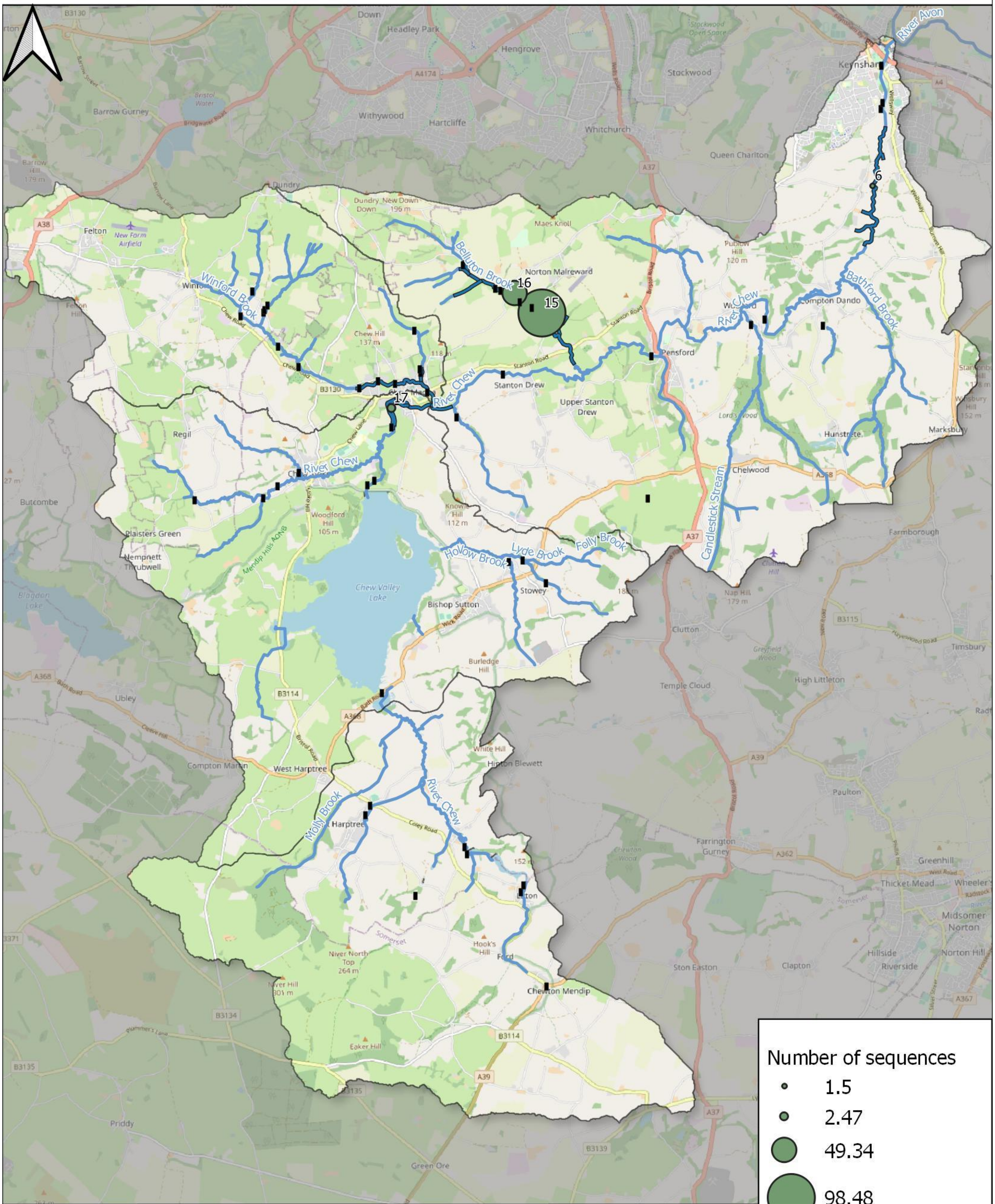


Sample Site	Number of Sequences
22	0.16
12	0.19
23	0.36
13	0.56
15	0.72
17	0.83
26	3.29
1	7.29
29	11.44

Total of 14 sites recorded eDNA of Brown trout (Salmo trutta).
Highlighted portions of river indicate 1km from the sample point.

Weir Data derived from EA opendata
Basemap: OpenSteet map

Carp (Cyprinus carpio)

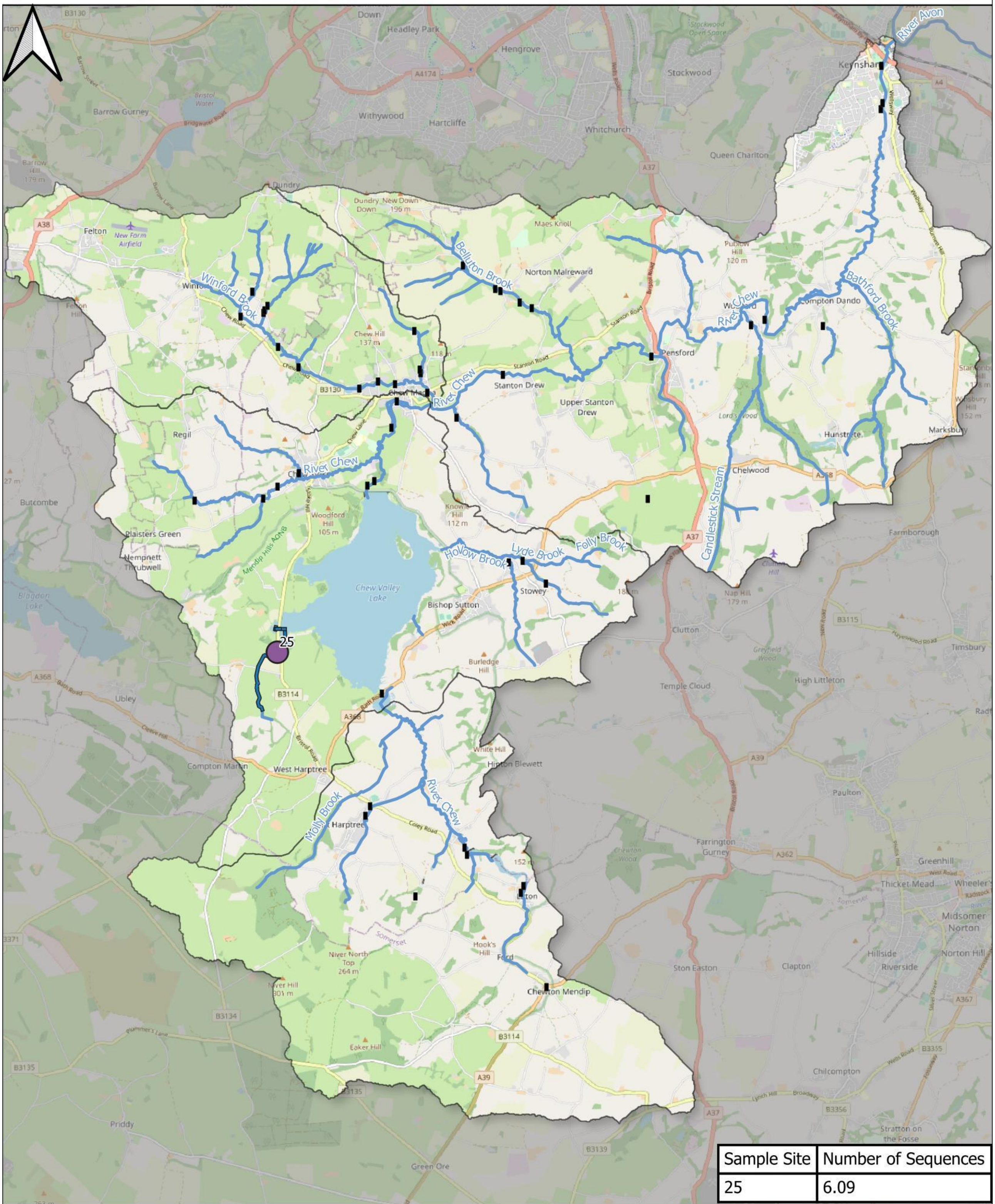


Total of 4 sites recorded eDNA of Carp (Cyprinus carpio).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map
Image: iStockphoto

Common bream (Abramis brama)



Sample Site	Number of Sequences
25	6.09

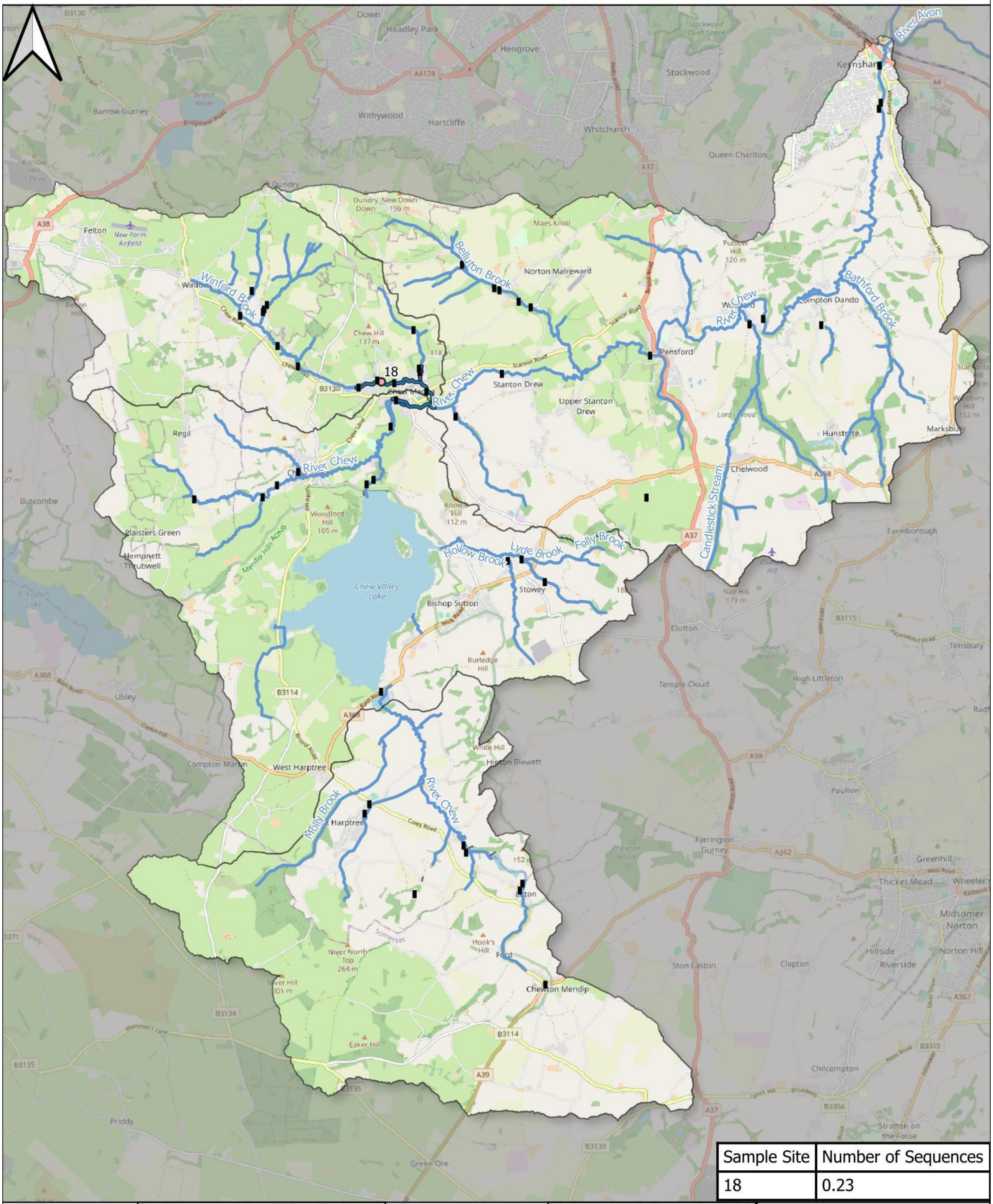
Total of 1 site recorded eDNA of Common Bream.
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

Weirs
1km Range

Crucian (Carassius sp.)



Sample Site	Number of Sequences
18	0.23

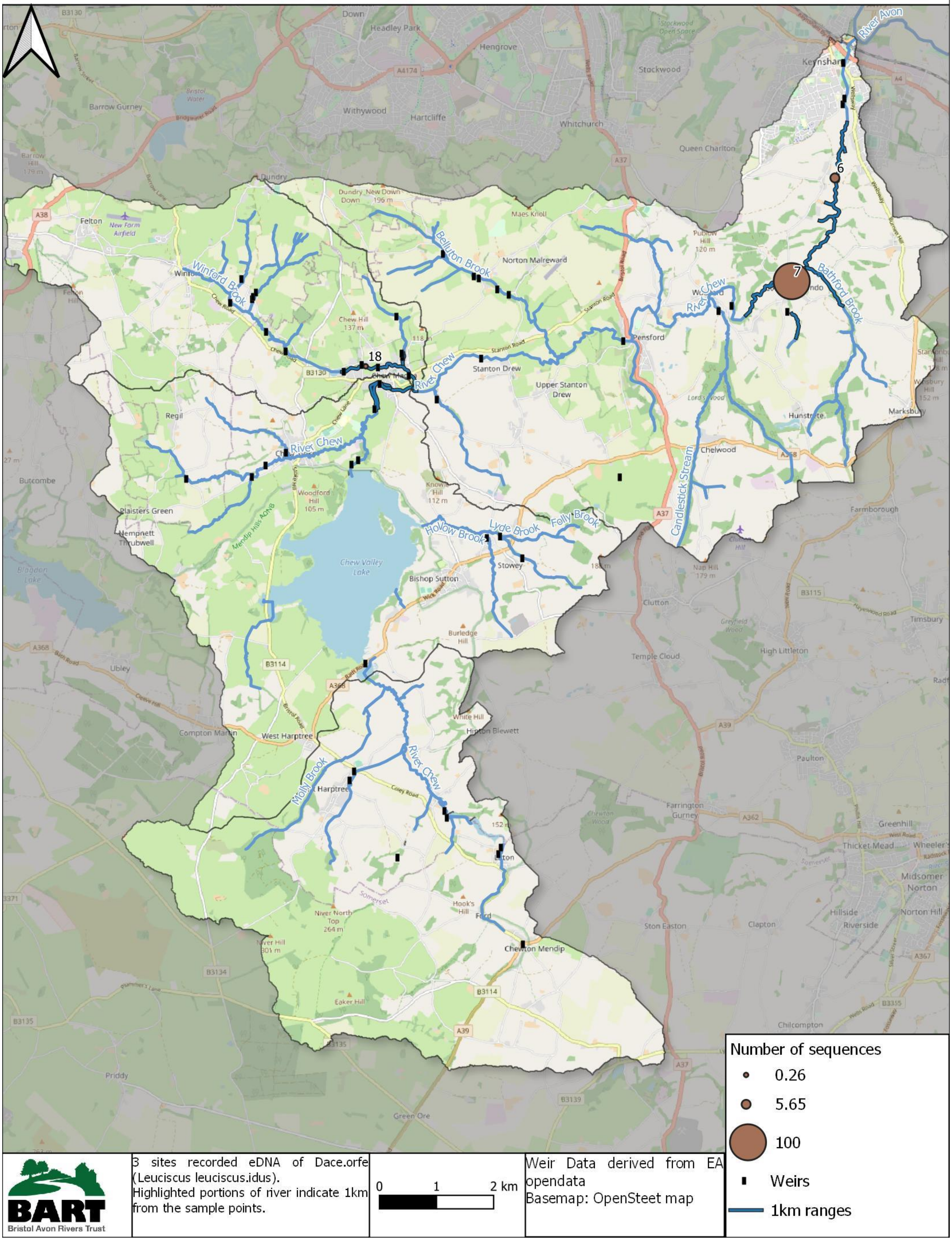
1 site recorded eDNA of Crucian (Carassius sp.).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

Weirs
1km range

Dace.orfe (Leuciscus leuciscus.idus)

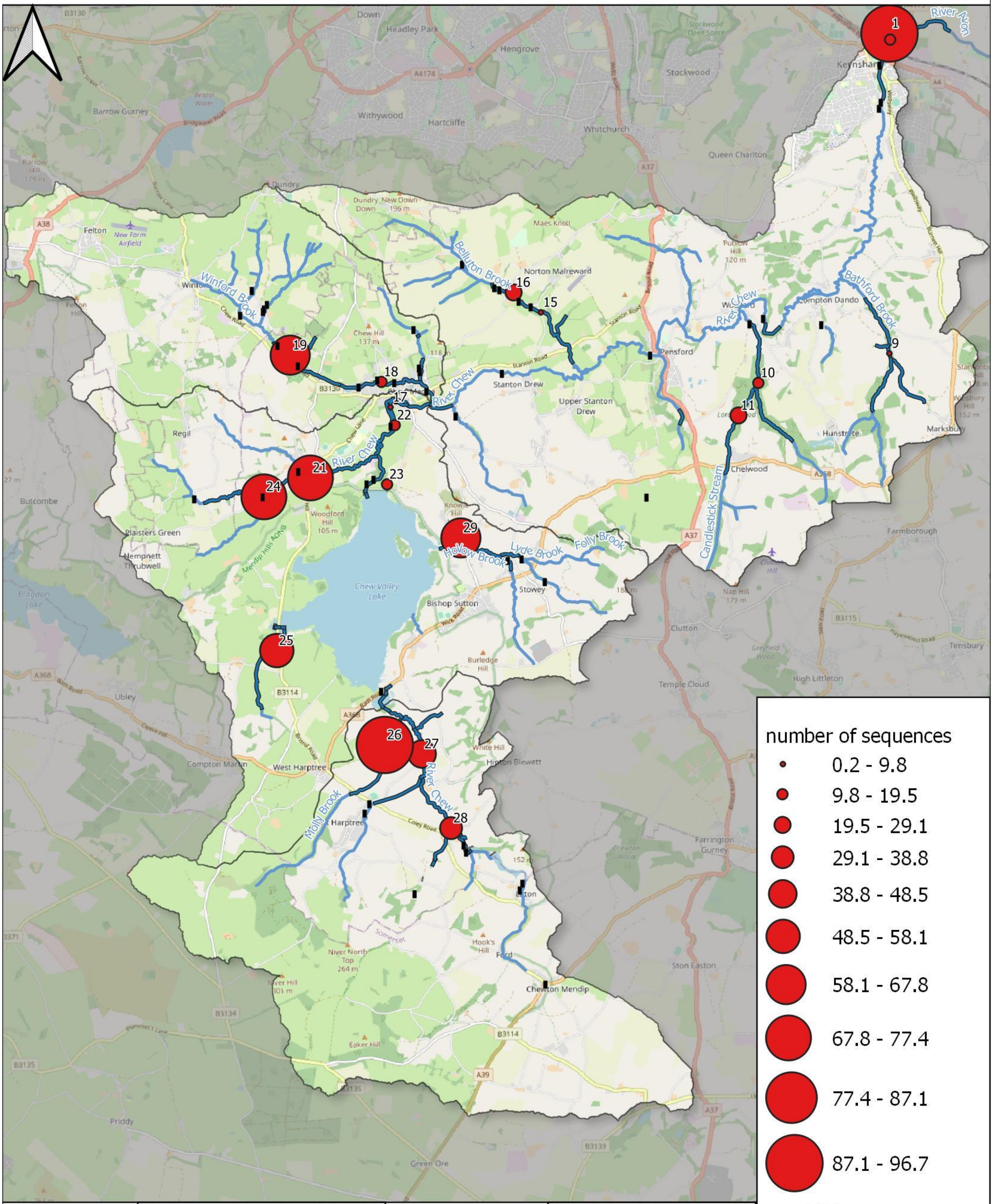


3 sites recorded eDNA of Dace.orfe (Leuciscus leuciscus.idus). Highlighted portions of river indicate 1km from the sample points.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

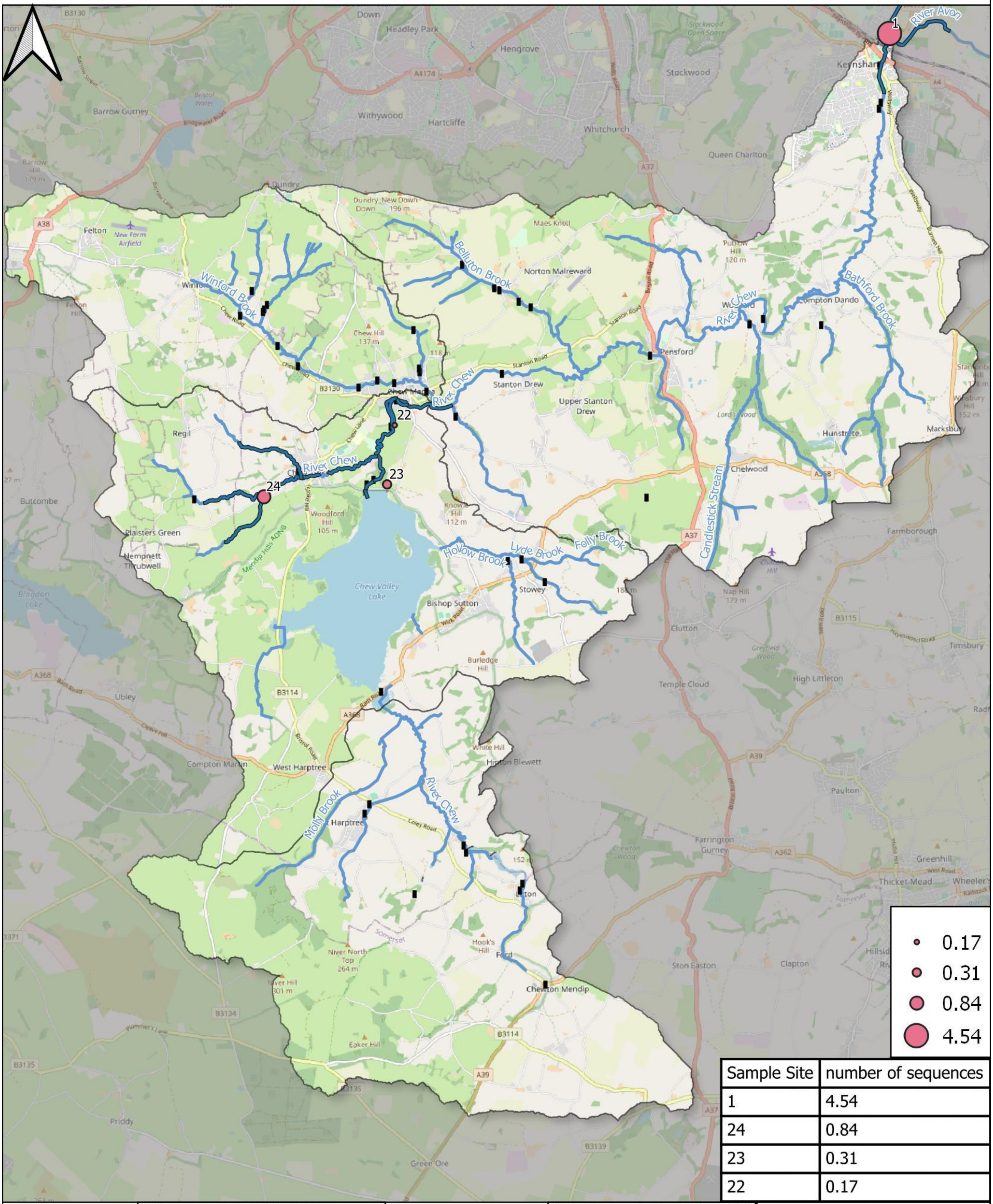
European bullhead (Cottus gobio)



18 sites recorded eDNA of European bullhead (Cottus gobio)
Highlighted portions of river indicate 1km from the sample point.

Weir Data derived from EA opendata
Basemap: OpenSteet map
Image: Alamy

European eel (Anguilla anguilla)



Sample Site	number of sequences
1	4.54
24	0.84
23	0.31
22	0.17

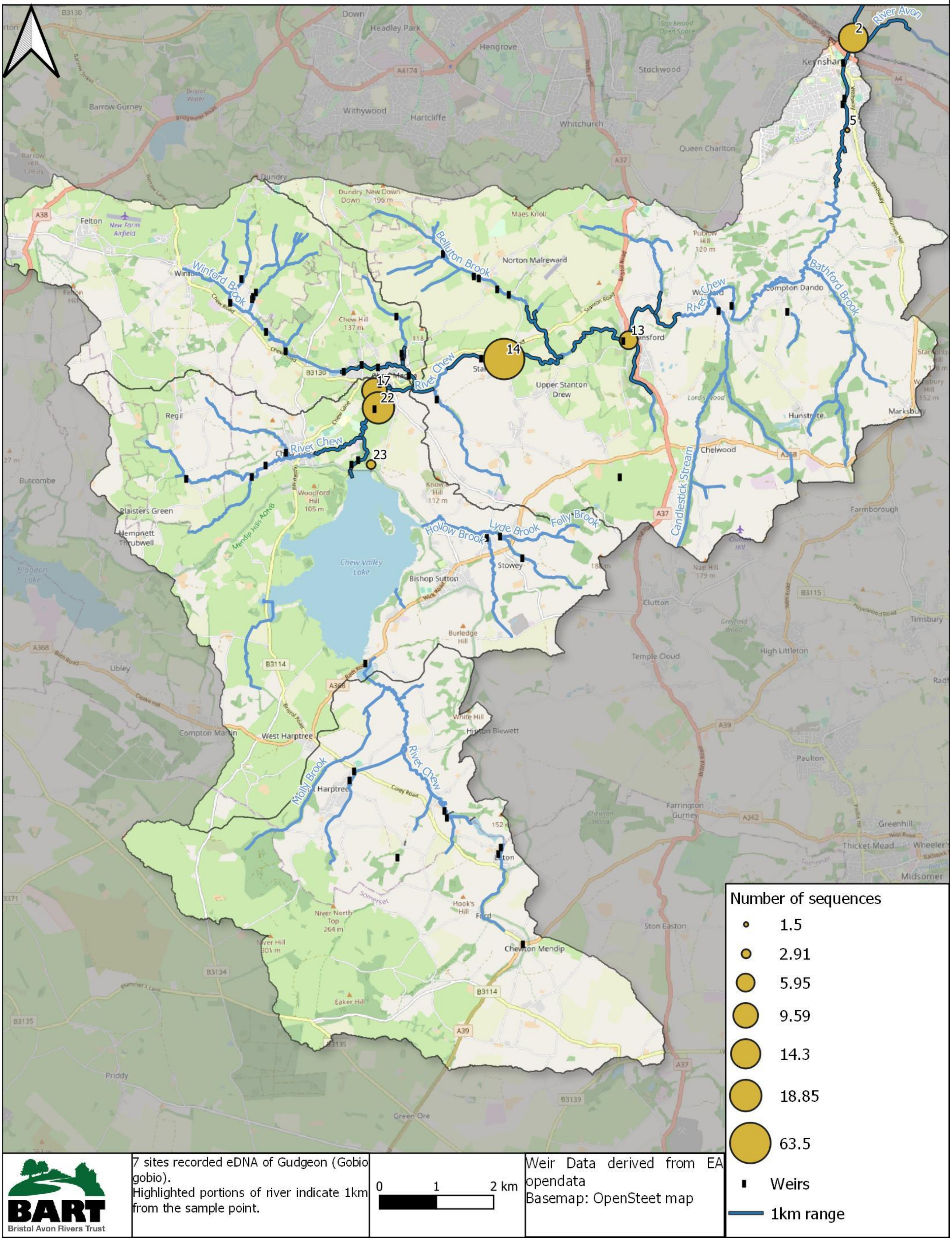
4 sites recorded eDNA of European eel (Anguilla anguilla).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

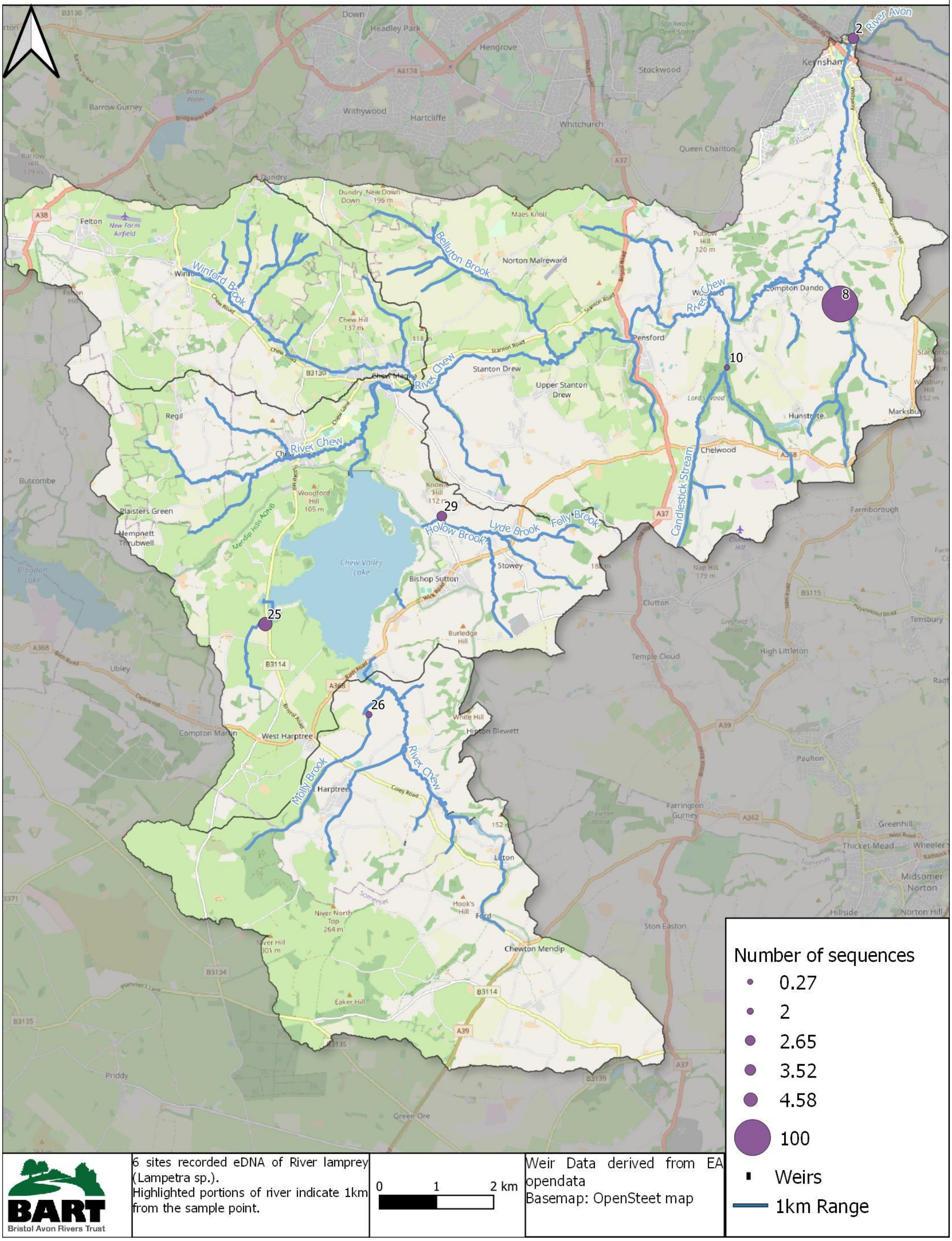
Weir Data derived from EA opendata
Basemap: OpenSteet map
Image: Alamy

Weirs
1km range

Gudgeon (Gobio gobio)

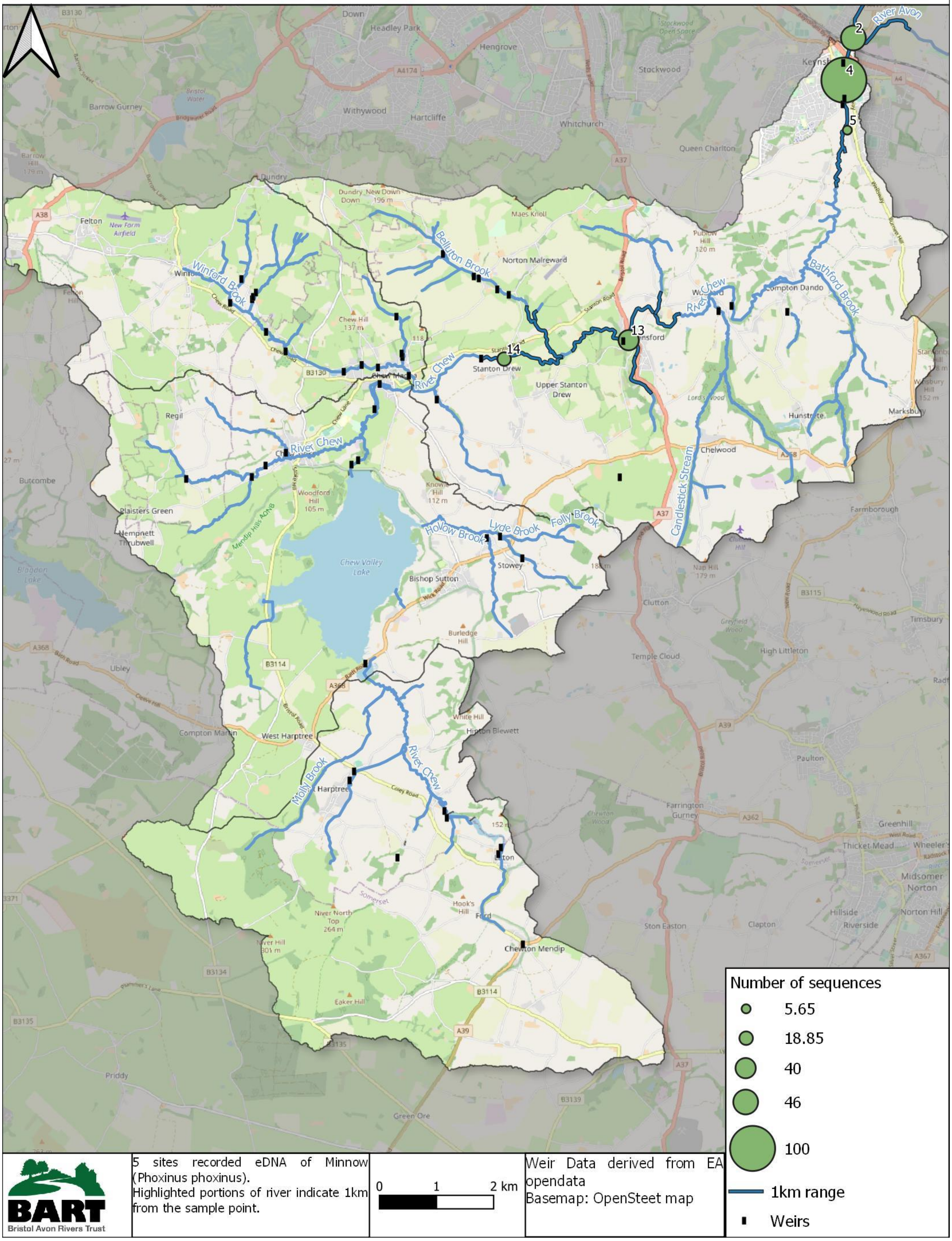


River lamprey (Lampetra sp.)

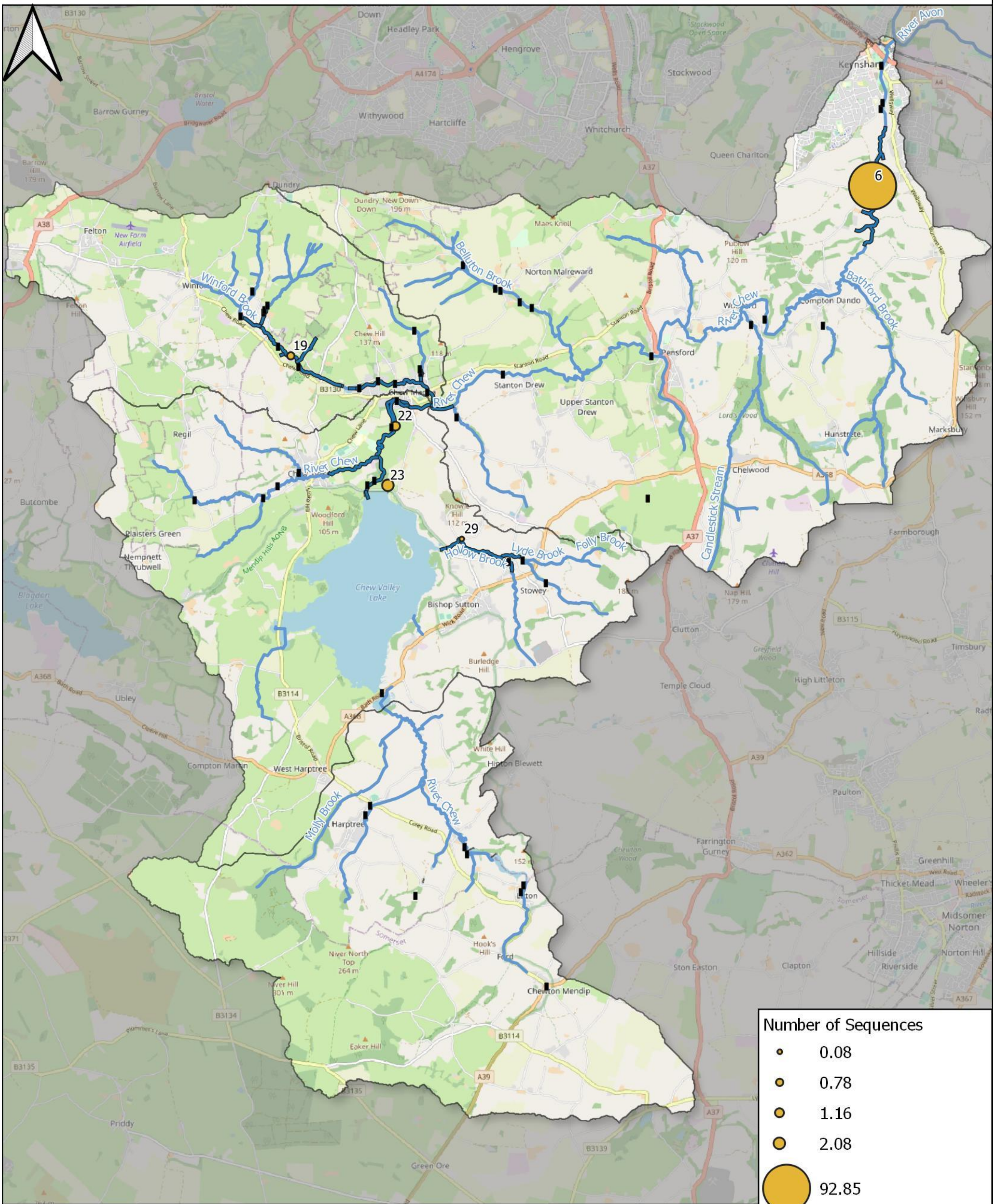


6 sites recorded eDNA of River lamprey (Lampetra sp.). Highlighted portions of river indicate 1km from the sample point.	0 1 2 km	Weir Data derived from EA opendata Basemap: OpenSteet map	

Minnow (Phoxinus phoxinus)



Northern pike (Esox lucius)

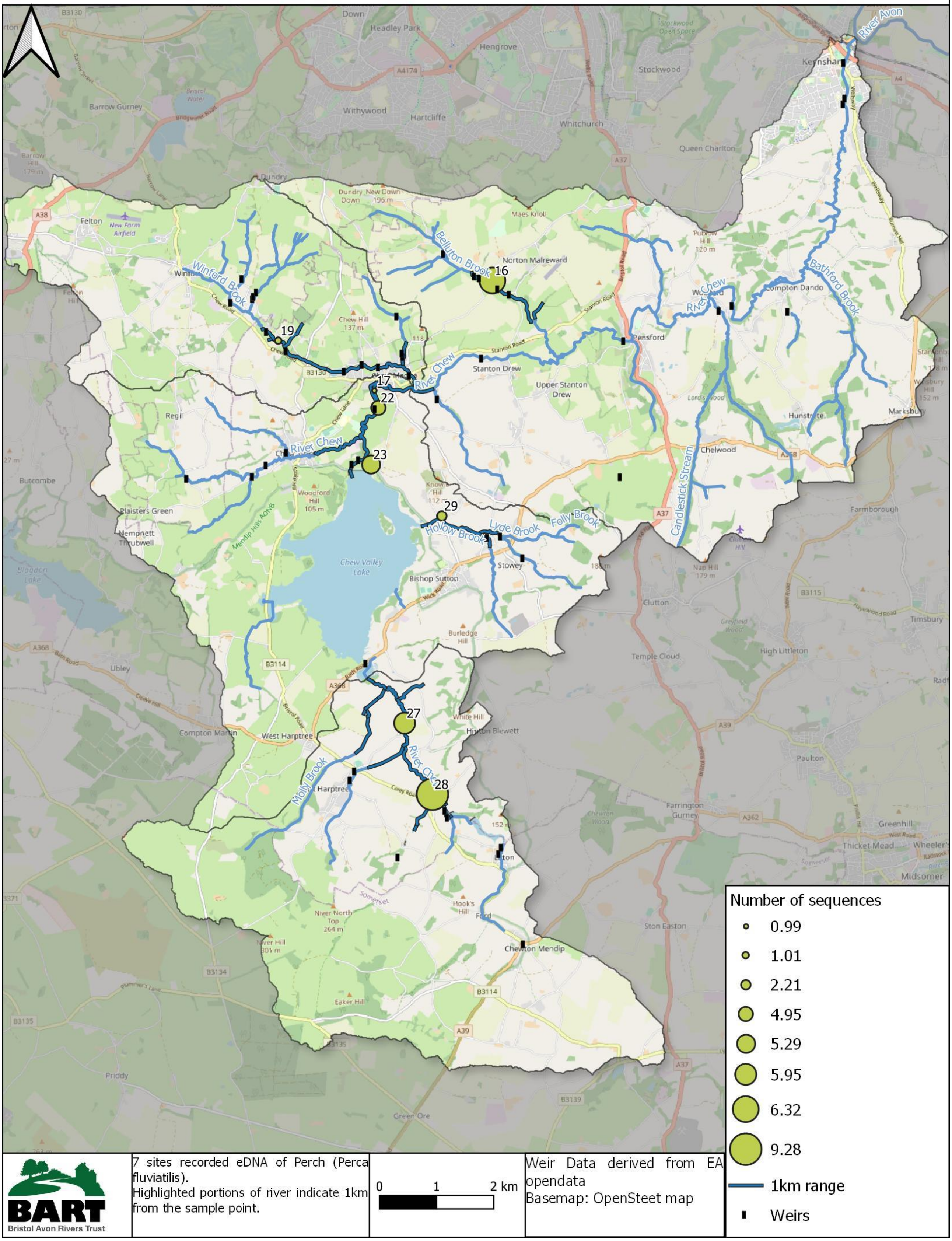


5 sites recorded eDNA of Northern pike (Esox lucius).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

Perch (Perca fluviatilis)

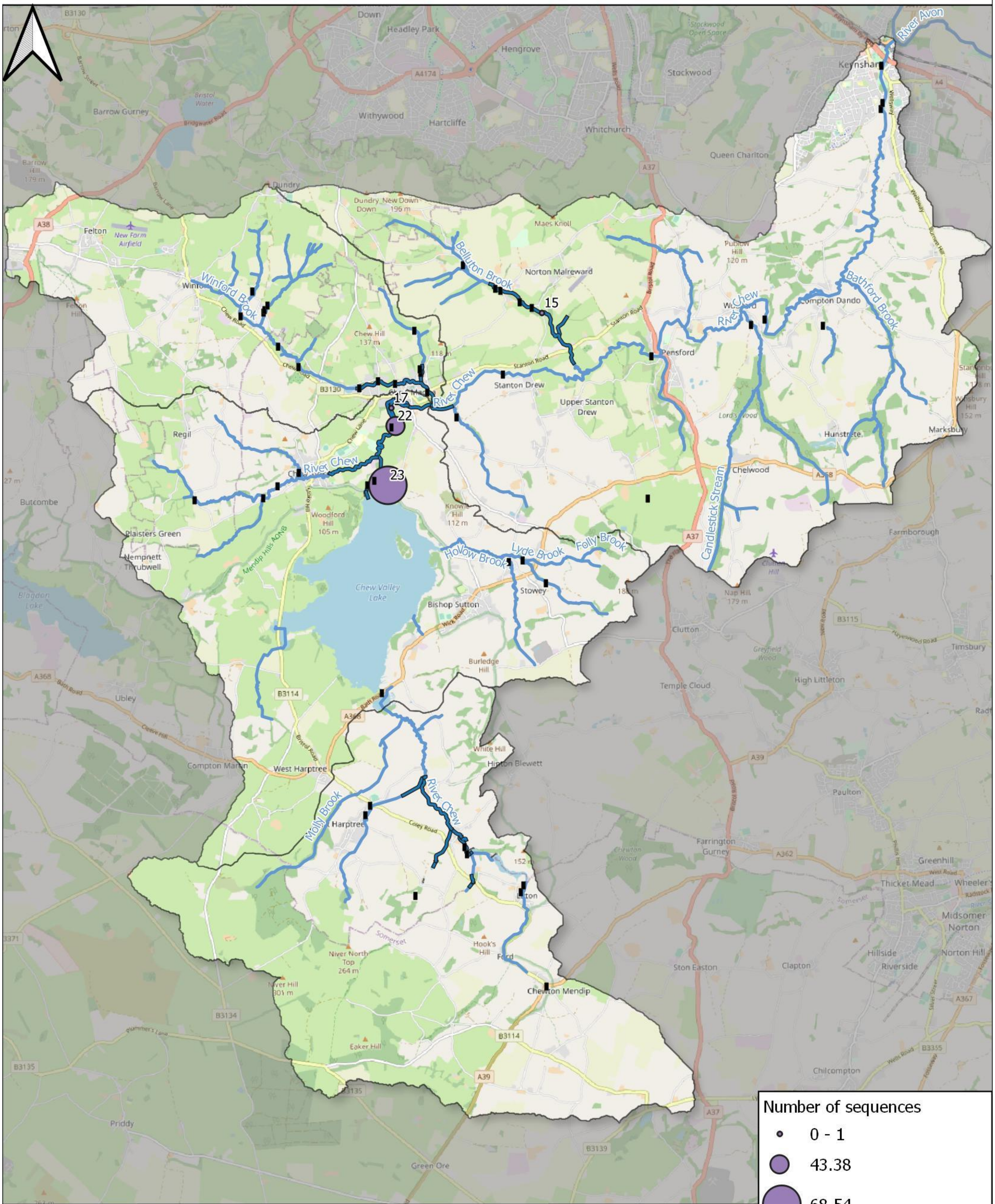


7 sites recorded eDNA of Perch (Perca fluviatilis).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

Rainbow trout (Oncorhynchus mykiss)



Number of sequences
- 0 - 1
- 43.38
- 68.54
- 1km range
- Weirs

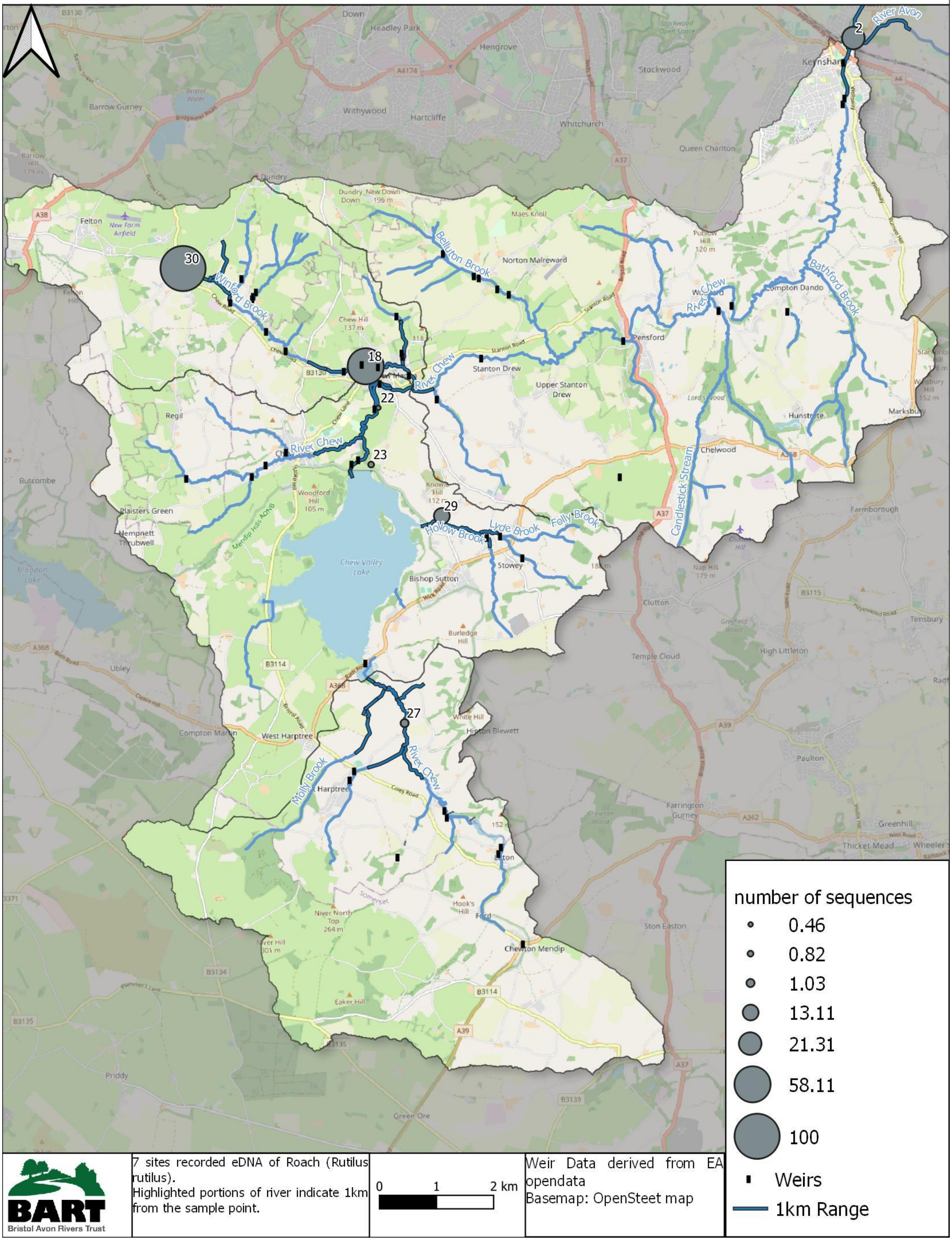
BART
Bristol Avon Rivers Trust

5 sites recorded eDNA of Rainbow trout (Oncorhynchus mykiss).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map
Image: Alamy

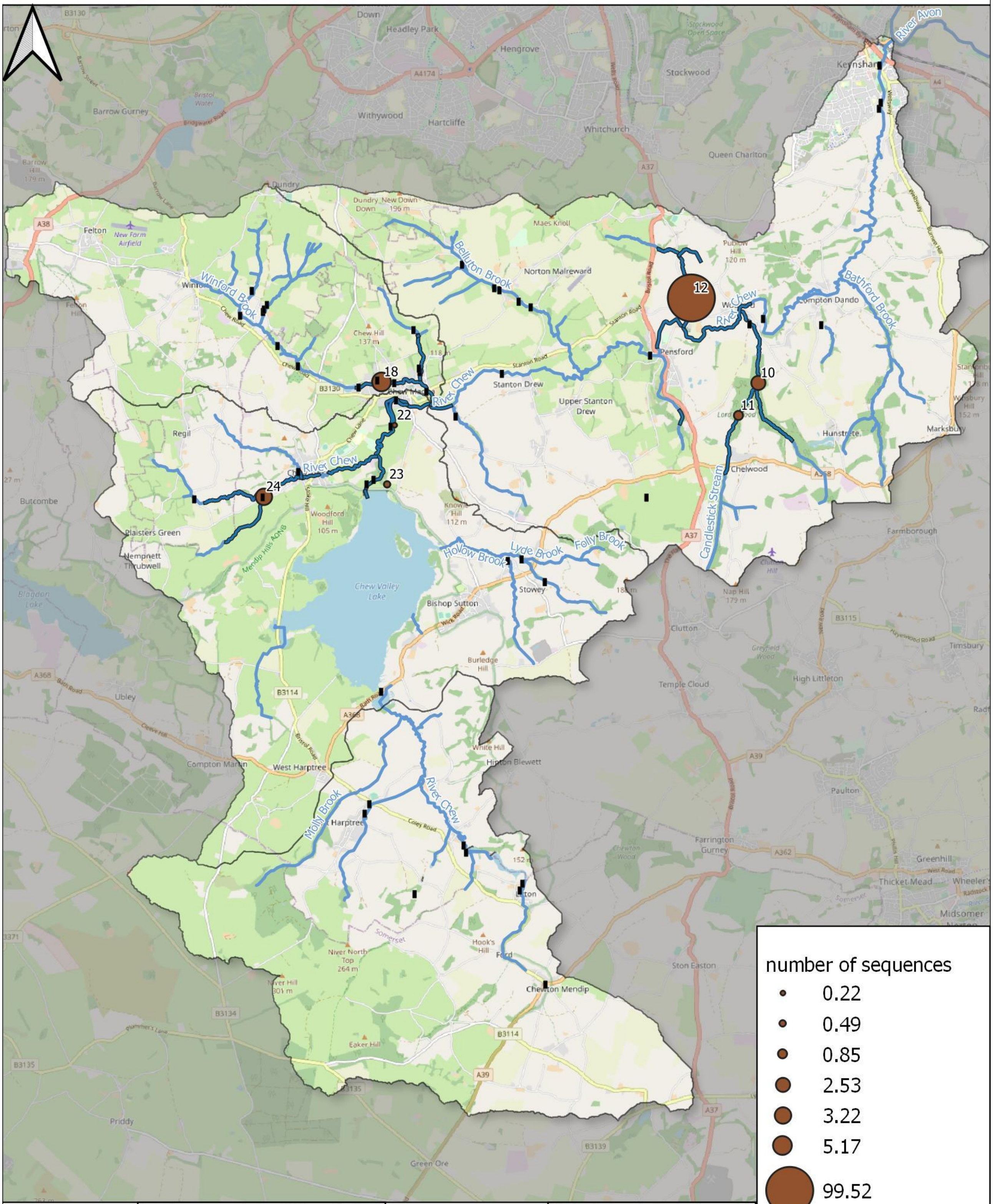
Roach (Rutilus rutilus)



7 sites recorded eDNA of Roach (Rutilus rutilus).
Highlighted portions of river indicate 1km from the sample point.

Weir Data derived from EA opendata
Basemap: OpenSteet map

Rudd (Scardinius erythrophthalmus)

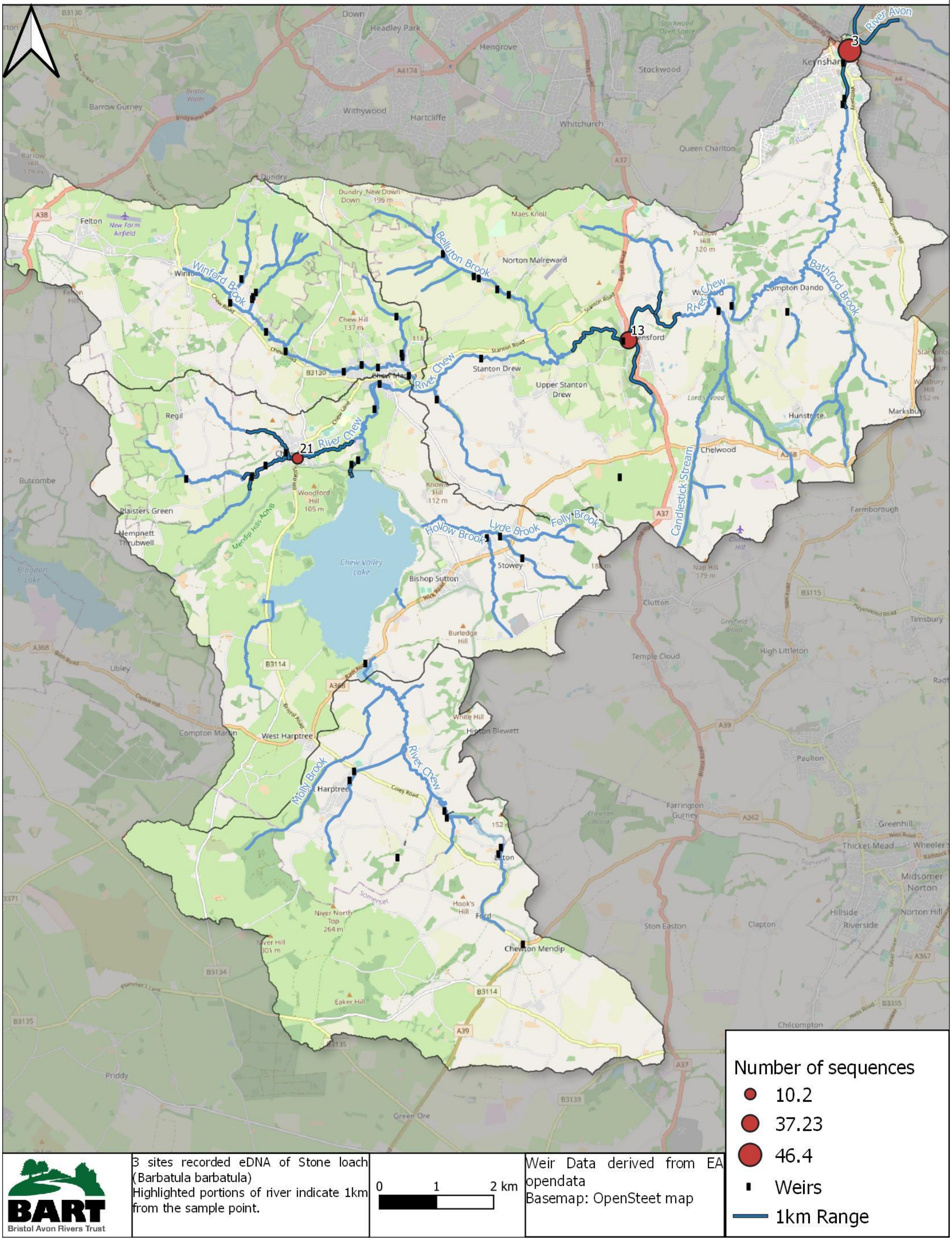


7 sites recorded eDNA of Rudd (Scardinius erythrophthalmus). Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

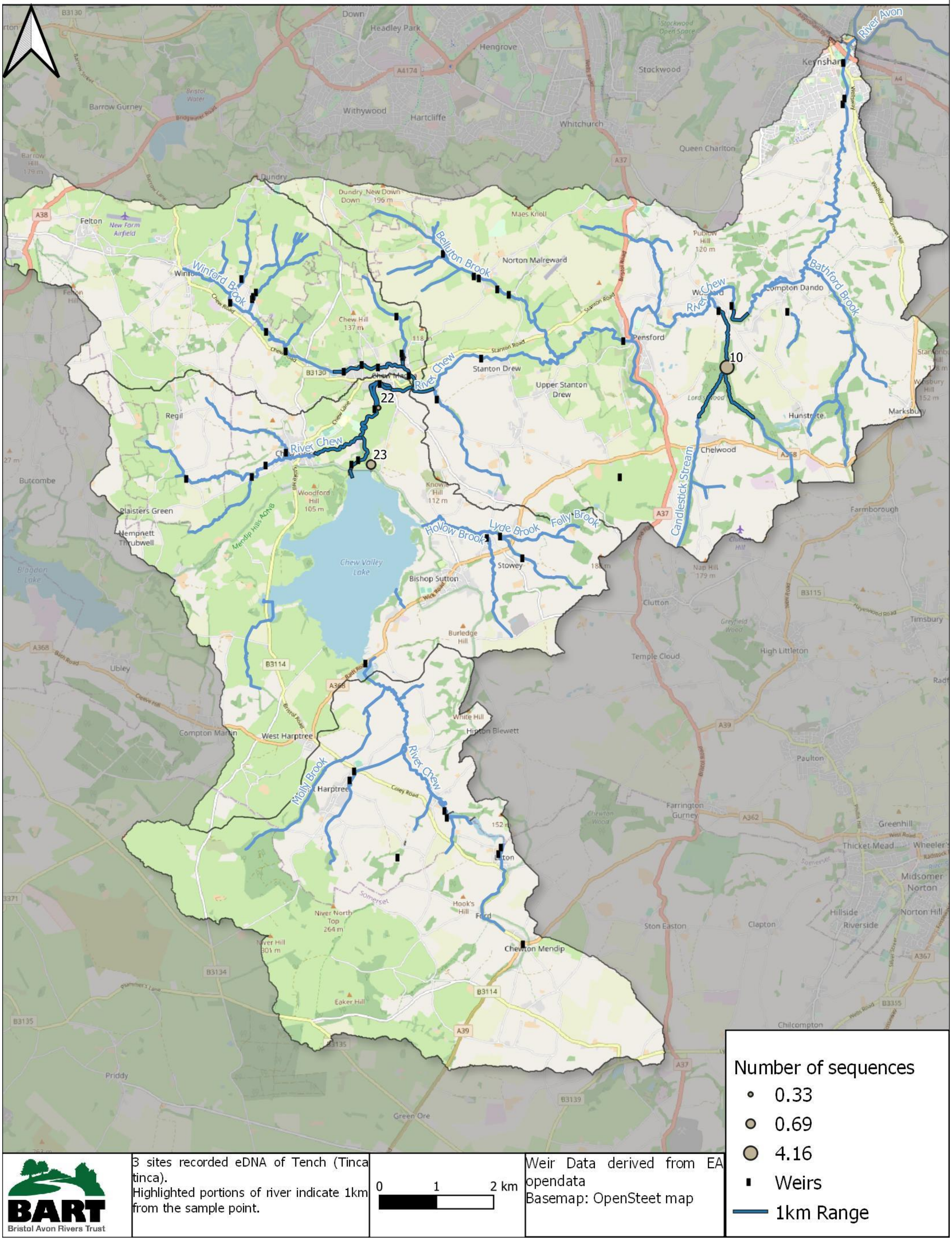
Weir Data derived from EA opendata
Basemap: OpenSteet map

Stone loach (Barbatula barbatula)



BART Bristol Avon Rivers Trust	3 sites recorded eDNA of Stone loach (Barbatula barbatula) Highlighted portions of river indicate 1km from the sample point.	0 1 2 km	Weir Data derived from EA opendata Basemap: OpenSteet map	

Tench (Tinca tinca)



3 sites recorded eDNA of Tench (Tinca tinca).
Highlighted portions of river indicate 1km from the sample point.

0 1 2 km

Weir Data derived from EA opendata
Basemap: OpenSteet map

Three-spined stickleback (Gasterosteus aculeatus)

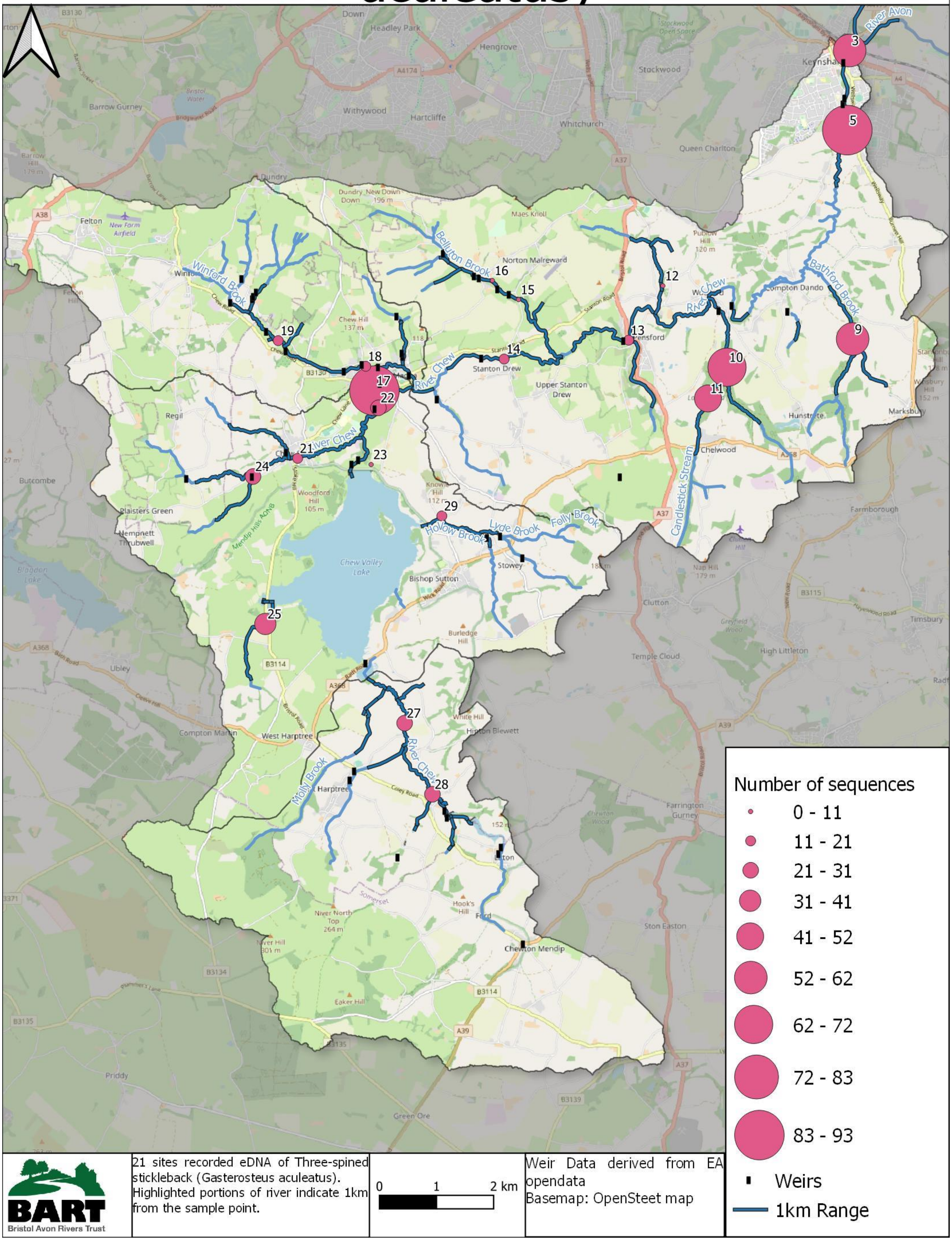


Table A1. Species presence - 'Main' River sampling sites

Site no.	Angling waters (Y/N)	Species present	Species fished (Y/N)	Game / Coarse / Minor species	Endemic / Introduced
1	Y	European eel Atlantic salmon Trout European bullhead	N Y Y N	n/a Game Game Minor	Endemic Endemic Endemic Endemic
2	N	Gudgeon Minnow Roach European bullhead Lamprey	Y N Y N N	Coarse Minor Coarse Minor Minor	Introduced Endemic Endemic Endemic Endemic
3	N	Stone loach Three-spined stickleback	N N	Minor Minor	Endemic Endemic
4	Y	Minnow	N	Minor	Endemic
5	Y	Gudgeon Minnow	Y N	Coarse Minor	Introduced Endemic
6	Y	Gudgeon Minnow	Y N	Coarse Minor	Introduced Endemic
7	Y	Dace	Y	Coarse	Endemic
13	Y	Gudgeon Minnow Stone loach Three spined stickleback Trout	Y N N N Y	Coarse Minor Minor Minor Game	Introduced Endemic Endemic Endemic Endemic
14	Y	Gudgeon Minnow Three-spined stickleback	Y N N	Coarse Minor Minor	Introduced Endemic Endemic
17	Y	Carp Gudgeon Three spined stickleback Perch Rainbow trout Trout European bullhead	Y Y N Y Y Y N	Coarse Coarse Minor Coarse Game Game Minor	Introduced Introduced Endemic Endemic Introduced Endemic Endemic
22	Y	European eel Gudgeon Roach Rudd Tench Northern pike Three spined stickleback Perch Rainbow trout Trout European bullhead	N Y Y Y Y Y N Y Y Y N	N/a Coarse Coarse Coarse Coarse Coarse Minor Coarse Game Game Minor	Endemic Introduced Endemic Endemic Endemic Endemic Endemic Endemic Introduced Endemic Endemic

23	Y	European eel	N	N/a	Endemic
		Gudgeon	Y	Coarse	Introduced
		Roach	Y	Coarse	Endemic
		Rudd	Y	Coarse	Endemic
		Tench	Y	Coarse	Endemic
		Northern pike	Y	Coarse	Endemic
		Three-spined stickleback	N	Minor	Endemic
		Perch	Y	Coarse	Endemic
		Rainbow trout	Y	Game	Introduced
		Trout	Y	Game	Endemic
		Atlantic salmon	Y	Game	Endemic
		European bullhead	N	Minor	Endemic
27	Former angling waters	Roach	Y	Coarse	Endemic
		Three spined stickleback	N	Minor	Endemic
		Perch	Y	Coarse	Endemic
		Trout	Y	Game	Endemic
		European bullhead	N	Minor	Endemic
28	N	Three spined stickleback	N	Minor	Endemic
		Perch	Y	Coarse	Endemic
		Rainbow trout	Y	Game	Introduced
		Trout	Y	Game	Endemic
		European bullhead	N	Minor	Endemic

Table A2. 'Tributary' sampling sites

Site no.	Angling waters (Y/N)	Species present	Species fished (Y/N)	Game / Coarse / Minor species	Endemic / Introduced
8	N	Lamprey	N	Minor	Endemic
9	N	Three spined stickleback	N	Minor	Endemic
		Atlantic salmon	Y	Game	Endemic
		Trout	Y	Game	Endemic
		European bullhead	N	Minor	Endemic
10	N	Rudd	Y	Coarse	Endemic
		Tench	Y	Coarse	Endemic
		Three-spined stickleback	N	Minor	Endemic
		Trout	Y	Game	Endemic
		European bullhead	N	Minor	Endemic
		Lamprey	N	Minor	Endemic
11	N	Rudd	Y	Coarse	Endemic
		Three-spined stickleback	N	Minor	Endemic
		Trout	Y	Game	Endemic
		European bullhead	N	Minor	Endemic
12	N	Rudd	Y	Coarse	Endemic
			N	Minor	Endemic

		Three-spined stickleback Trout	Y Y	Minor Game	Endemic Endemic
15	N	Carp Three-spined stickleback Rainbow trout Trout European bullhead	Y N Y Y N	Coarse Minor Game Game Minor	Introduced Endemic Introduced Endemic Endemic
16	N	Carp Three-spined stickleback Rainbow trout Trout Perch European bullhead	Y N Y Y Y N	Coarse Minor Game Game Coarse Minor	Introduced Endemic Introduced Endemic Endemic Endemic
18	N	Crucian Dace Roach Rudd Three-spined stickleback Rainbow trout Trout European bullhead	Y Y Y Y N Y Y N	Coarse Coarse Coarse Coarse Minor Game Game Minor	Introduced Endemic Endemic Endemic Endemic Introduced Endemic Endemic
19	N	Northern pike Three-spined stickleback Perch Trout European bullhead	Y N Y Y N	Coarse Minor Coarse Game Minor	Endemic Endemic Endemic Endemic Endemic
20	N	-	-	-	-
21	N	Stone loach Three-spined stickleback European bullhead	N N N	Minor Minor Minor	Endemic Endemic Endemic
24	N	European eel Rudd Three-spined stickleback European bullhead	N N N N	Minor Minor Minor Minor	Endemic Endemic Endemic Endemic
25	N	Common bream Three-spined stickleback European bullhead Lamprey	Y N N N	Minor Minor Minor Minor	Endemic Endemic Endemic Endemic
26	N	Trout European bullhead	Y N	Game Minor	Endemic Endemic
29	N	Roach Northern pike Three-spined stickleback	Y Y N	Coarse Coarse Minor	Endemic Endemic Endemic

		Perch Trout European bullhead Lamprey	Y Y N N	Game Coarse Minor Minor	Endemic Endemic Endemic Endemic
30	N	Roach	Y	Coarse	Endemic

Appendix A2

Nature Metric metabarcoding results report Bristol Avon Rivers Trust - 101820 - River Chew Catchment - Report.pdf