## 1. Contestant profile

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| ▪ Contestant name: | **Jacob Ball** |
| ▪ Contestant occupation: | Student (Undergraduate) |
| ▪ University / Organisation | University of the West of England |
| ▪ Number of people in your team: | **5** |

## 2. Project overview

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| Title: | Current and Potential Value of Limestone Quarry Habitats for the Critically Endangered European eel |
| Contest: (Research/Community) | Research |
| Quarry name: | Chipping Sodbury |

# Current and Potential Value of Limestone Quarry Habitats for the Critically Endangered European eel


## Abstract

The European eel (*Anguilla anguilla*) is classified as Critically Endangered with a global population decrease of 95% (Freyhof & Kottelat, 2010), yet conservation efforts are hampered due to survey limitations. We used the QLA project to develop a new in-field sampling method for environmental DNA (eDNA) in addition to significantly improving our laboratory methods. We sampled five water bodies within Chipping Sodbury Quarry, Gloucestershire and a further two control sites. Invertebrate and water quality samples were taken from each quarry site to provide an assessment of habitat suitability. DNA extractions were performed on each water sample and PCR analysis carried out to test for the presence of eels within the waterbodies. Results showed an absence of *A.anguilla* from the quarry water bodies, however habitat suitability analysis suggests that restored quarry voids could provide suitable habitat for eels. Recommendations are given for how quarry restoration plans could provide opportunities to improve habitat for *A.anguilla*. Opportunities for further study are discussed with development of quantification for eDNA with European eels.

## Introduction

The European eel (*Anguilla anguilla*) is classified as Critically Endangered (Jacoby & Gollock, 2014), with a global decline of 95% since the 1980s (Freyhof & Kottelat, 2010). The Seven Estuary is one of the largest migratory routes in the world for the European eel and a key site for eel populations (Jacoby & Gollock, 2014). A study in Bridgewater Bay, Somerset, has highlighted the rapid decline of this species, showing a decline of 15% per year in population size since 1980 (Henderson, *et al.*, 2011).

European eels have several life stages. After hatching in the Sargasso Sea[1] they travel eastwards on currents such as the gulf stream as leptocephali larvae. Upon reaching the shores around Europe and North Africa they undergo the first of many metamorphoses, developing into glass eels where they travel upstream (Ibbotson, *et al.*, 2002). After migrating upstream to a suitable water body where they become largely sedentary 'yellow eels', remaining in that area for up to twenty years (Aprahamian, 1988). They finally metamorphose into silver eels and migrate back downstream, towards the Sargasso Sea to spawn (Righton, *et al.*, 2016). Eels are threatened from habitat loss, overharvesting, migratory barriers and a lack of knowledge (Deelder, 1958). Eels have been shown to mostly eat benthic invertebrates, whilst also scavenging on dead organic matter (Mann & Blackburn, 1990).

The restoration of quarries to waterbodies may provide the opportunity to increase eel habitat provision. Eels could be well suited to the deep waterbodies created when some limestone quarries are restored as they are thought to have a greater tolerance for deep water than many native freshwater fish. This project was designed to assess whether eels were already present within waterbodies within an active quarry and whether restoration plans could be adapted to increase the chance of quarries becoming important landscape elements for eels.

Chipping Sodbury Quarry's management plan currently aims to flood the five quarry voids on site. This would provide an estimated $5.315 \times 10^{10}$L of water as potential habitat for European eels. Chipping Sodbury Quarry also has a stream running through the site, which is linked to the River Frome providing the wildlife corridor needed to allow colonization and migration of eels (Ibbotson, *et al.*, 2002). However, assessing water bodies for the eels is traditionally difficult, involving expensive, invasive and potentially destructive sampling techniques. We adopted the emerging technique of environmental DNA (eDNA sampling) and modified in-field and laboratory procedures to develop a robust and sensitive, non-invasive survey technique. This project was aimed at not only developing a new method, but developing an effective, reliable and cost-effective method. eDNA sampling involves extracting DNA molecules from environmental samples, e.g. water, and using PCR (polymerase chain reaction) techniques to identify whether the DNA of a target species is within the sample (Ficetola *et al*. 2008; see also Tillotson, *et al.*, 2018). This is a repeatable and affordable method allowing ease of surveying around any water body, providing a new method that can be implemented within all organizations.

This project provides recommendations for quarry habitat with potential for eel colonization via migratory corridors. It highlights management to improve habitat for eels, easing access for immigrating and emigrating individuals.

[1] This is the presumed breeding ground of *A. anguilla*, but definitive proof has yet to be obtained.

## Methods

Prior to in-field sampling DNA collection rates were tested against filter sizes (figure 1). The sample was collected from a tank at Bristol Zoo containing *A.anguilla* ensuring it was a positive sample. 1L of sample water was pulled through various filters using a vacuum pump. Filters were then processed assessing filter limitations and optimum filter size, a 3µm filter was chosen.

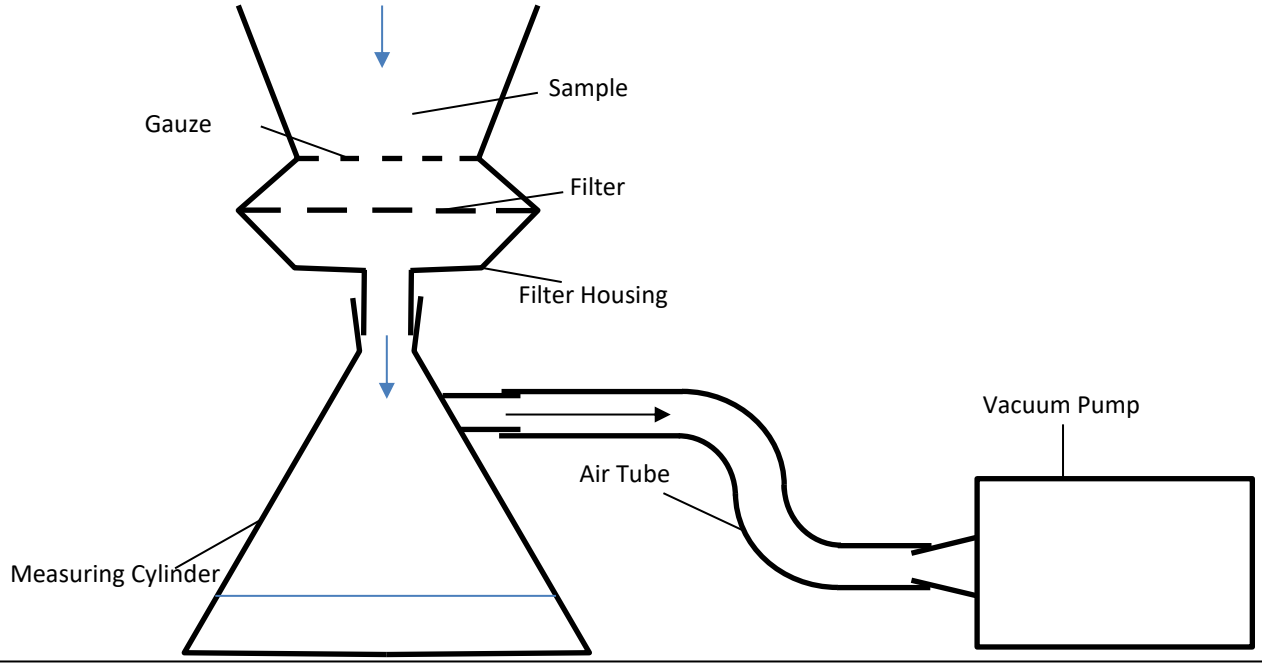


Figure 1; aparatus used to test differences in filter size compared to quantity of DNA collected, targetting *Anguilla anguilla*. Water from a tank containing *A.anguilla* was used, ensuring sample was positive. 1 L of sample water was passed through each filter.

Five water bodies at Chipping Sodbury were sampled (figure 2). Invertebrate samples were collected at each site using a standardized netting procedure for 10 mins. The specimens were collected from each site were identified to Family level and released. Nitrate, Phosphate, pH, Dissolved oxygen and Temperature was measured, at each site using hand held probes and Palin tests. Control samples were also taken from the River Frome (51.542476, -2.440176) to test whether eels could be detected in the adjoining waterbodies. Samples were collected from Westhay Moor, Somerset as a positive control. All samples were taken on 16th July 2018, apart from the control samples which were taken on 11th September 2018 and 12th September 2018. Chipping Sodbury samples were process in the laboratory on 18th July 2018. Control samples and positive samples where processed together in the laboratory on 15th September 2018.

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Figure 2; a site map of Chipping Sodbury quarry, the numbers highlighting the 5 sample points on the site and the red line showing the boundary of Chipping Sodbury quarry. Water quality, invertebrates samples and eDNA samples were collected from each site.

eDNA degrades rapidly, so a rapid and effective in-field collection system is required. We built on MSc research from UWE (Weldon *et al*. in prep) to develop a new in-field sampling technique based on in situ filtration of samples across a 3µm filter (see figure 3). This allows samples to be taken and pre-processed on the same day before storage, preventing degradation of DNA in the water sample. Five 200 ml samples from each waterbody were combined and pumped through a single filter. The filter was removed from the housing and stored in a 500µl of Longmire buffer, which has been shown to be the most effective way of preserving eDNA samples prior to DNA extraction (Weldon *et al*. in prep), allowing samples to be stored for at least two months.

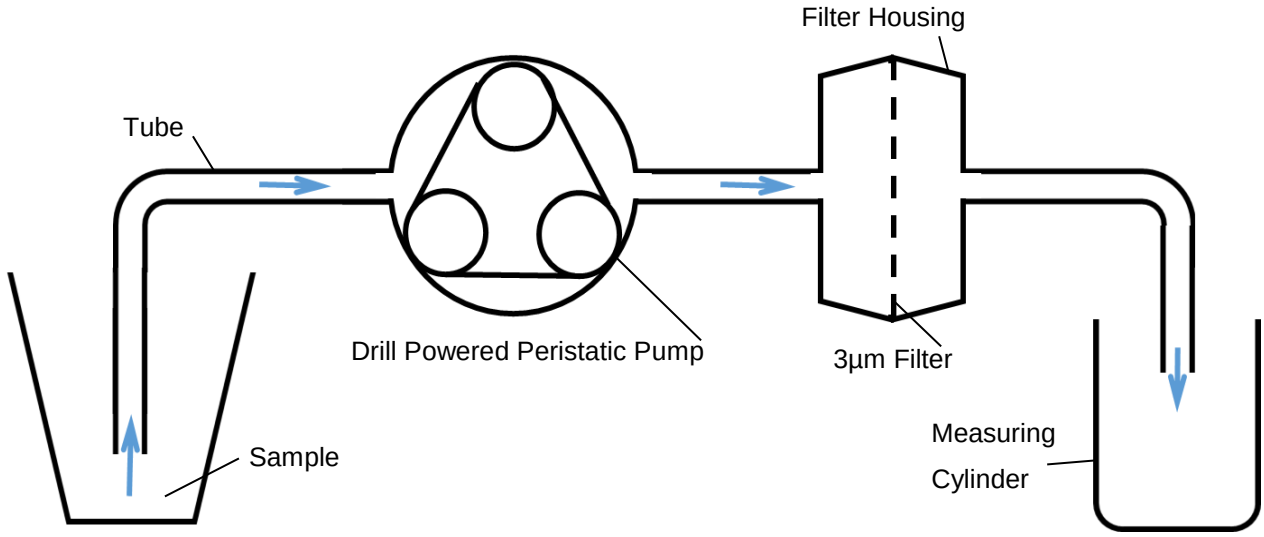


Figure 3; a schematic showing the in-field filtration system developed for sampling environmental DNA (eDNA), specifically for detection of European eel (*Anguilla anguilla*). A 3µm filter is used, 1L of water is passed through the filter. Filter is then removed and stored in Longmire's buffer.

The filter and buffer had proteinase-k added breaking open cellular material and digesting proteins retained on the filter. Filters were incubated overnight at 55°C and DNA extracted from solution by addition of a phenol chloroform mix. Centrifugation at 12,500rpm for 30 minutes moves any DNA to the aqueous phase. DNA was then isolated by precipitation and cleaned by cycles of centrifuging at 12,500rpm for 5 minutes and washing with sterile distilled water. After the sample was clean it was dried in a heating block at 38°C for 30 minutes and then rehydrated in 100 µl sterile distilled water. A unidirectional workflow was adopted in preparing the qPCR plates to prevent cross contamination. Using UV treated equipment and consumables, PCR plates were prepared 30 minutes prior to set up. Each 15µl reaction contained; 1.5 µl sample, 1 x qPCRBIO probe mix (PCR Biosystems, London, UK), 0.2 pmol Aangcytb1F, 0.2 pmol Aangcytb1R (Eurofins Genomics, Germany) and 0.1 pmol fluorescently labelled probe Aangcytb1P (Integrated DNA Technology, Belgium). Three no template controls were used in each sample containing 1.5µl of sterile distilled water. A set of five standards containing *A. anguilla* DNA were included in a 1 in 5 sequential series of dilution from 20ng DNA to 32pg Samples were amplified in triplicate. Standard concentrations were confirmed using a nanodrop prior to qPCR. Reactions were carried out on *a* StepOne Plus™ real time PCR System (ThermoFisher Scientific, UK). PCR cycling parameters of 3 m denaturation at 95 °C, followed by 40 cycling steps of 5 s at 95 °C and 20 s at 59 °C were used.

## Results

The results show a wide range of body sizes within all invertebrate samples from 2mm to 55mm (figure 4). Site 3 had little invertebrate life with only 1 family of invertebrate being found possibly due to the small amount of water and no vegetation present at the site. Site 5 had the highest species richness with 12 different families being found. Site 5 also had the highest average body size of invertebrate at 24.1mm. Site 5 had the most vegetation on site, explaining potential for highest diversity of species. Site 3 had no vegetation and the water source was pumped out, preventing the development of a pond.

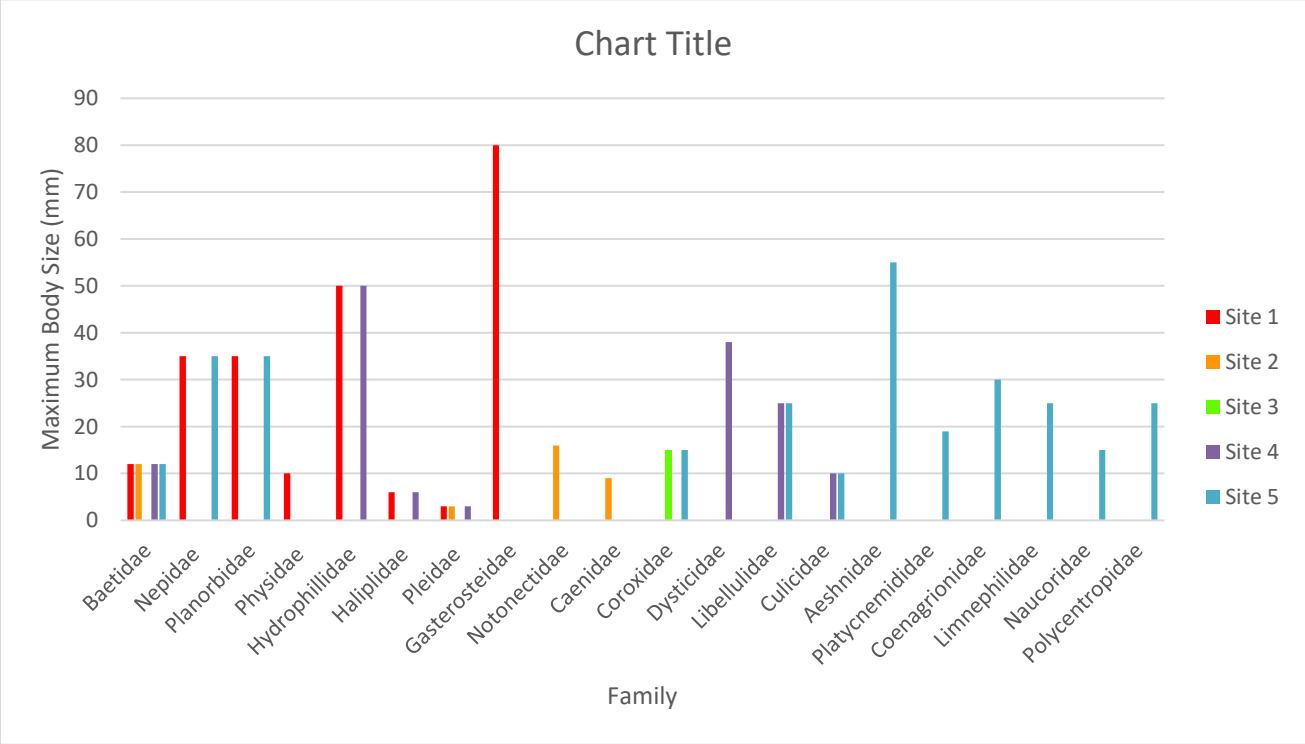


figure 4; graph showing the comparision between invertebrate families found at each sample site on Chipping Sodbury quarry and there maximum body size (mm)

The water quality was comparable between all sites (figure 5). Site 4 had the highest dissolved oxygen at 18.39mg/L, whilst site 3 had the lowest at 7.25mg/L. The highest pH was found at site 4 at 8.9, the lowest was found at site 3 at 6.9. Site 5 had the highest level of $NO^3$ at 7.2mg/L, site 3 had the lowest at 3.2mg/L. The results indicate good water quality on each site, therefore showing the current suitability for European eel

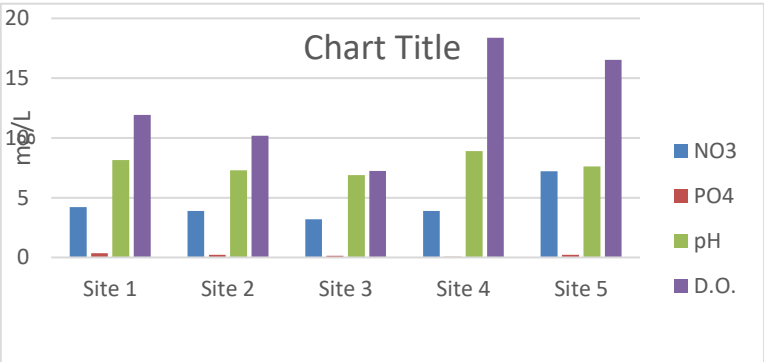


Figure 5; graphs showing water quality found at each sample site on Chipping Sodbury quarry in mg/L.

Figure 6 shows the results from the eDNA sampling. The amplification plot shows a negative result on all sample sites. The River Frome result was also negative. Figure 6 also shows results from a positive control at Westhay Moor, Somerset, and River Frome. Although the level of DNA at Westhay Moor was low, the threshold of presence was passed and shows a working method.

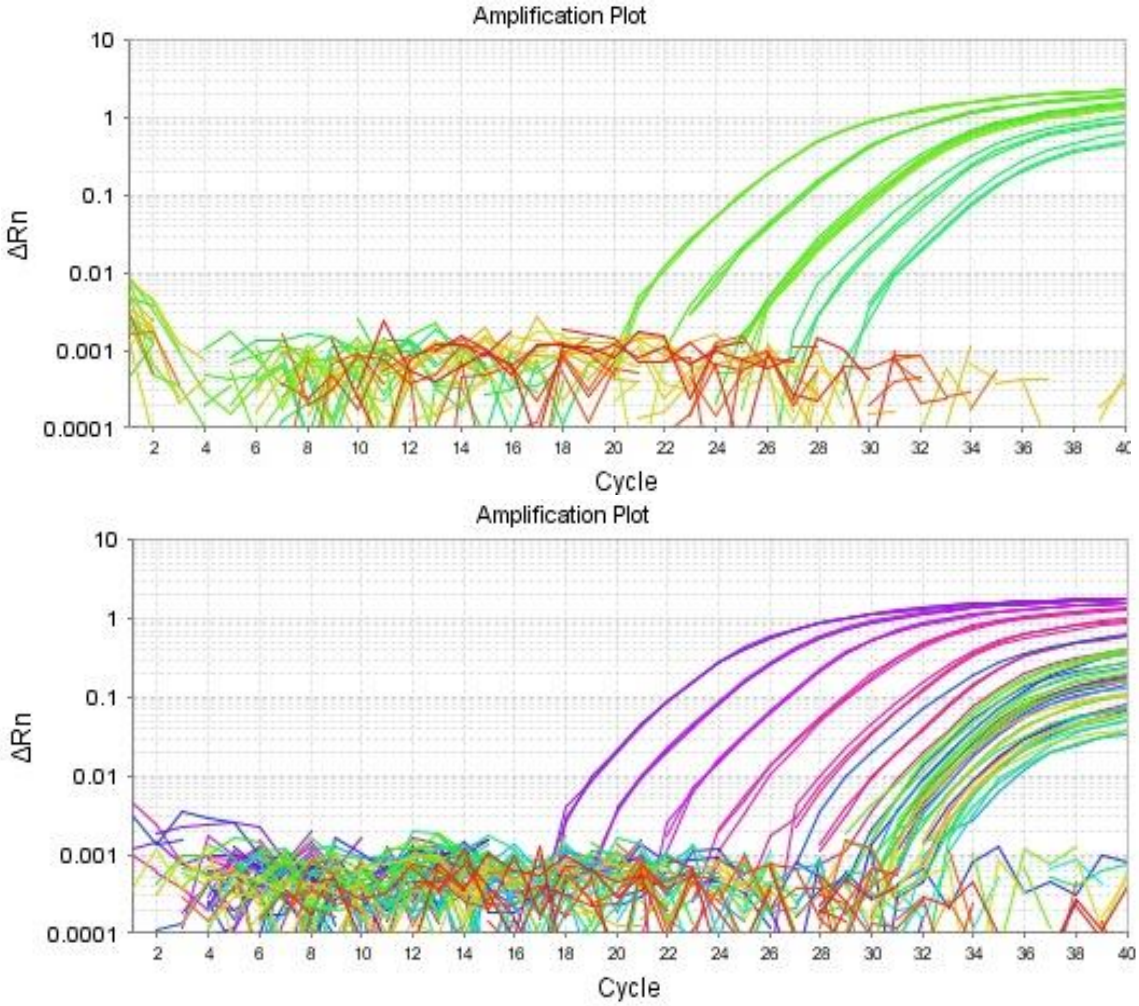


figure 6; amplification plots from PCR reactions, testing samples collected at Chipping Sodbury Quarry (above), River Frome and Westhay Moor (below) targetting *Anguilla anguilla*. Standards are shown in light green (above) purple (below) as standard curves in a 1 in 5 sequential series of dilution from 20ng DNA to 105.The light blue standard curve represents a positive result coming from site 1 (above). Standard curves to the right are positive results from Westbay Moors (below). Parameters of 3 m denaturation at 95 $^{o}$C, followed by 40 cycling steps of 5 s at 95 $^{o}$C and 20 s at 59 $^{o}$C.

## Discussion

The results collected throughout the project have highlighted the current potential for Chipping Sodbury Quarry to support European eel. The good range of invertebrate life indicates the ability to provide forage and support yellow eel. The water quality results have furthered the evidence to support current potential for European eel. With high dissolved oxygen and low levels of phosphates and nitrates the current water within Chipping Sodbury quarry is of adequate quality to support populations. However, further management is needed to provide greater connection between water bodies in quarry voids and to the Frome tributary as a migratory corridor. With the current management plans to flood quarry voids, there is future potential for Chipping Sodbury to support large populations of European eel. Further management may be needed to maximize the amount of emergent vegetation. Habitat links between water bodies would need creating, either through the use channels or planting of tall grasses where channels are not applicable. Eels have been known to migrate over land where tall vegetation exists. The current tributary to the Frome would need to be connected through the same management methods to the quarry voids, allowing migratory individuals to access the habitat. The current project run by The Severn Rivers Trust – *'Unlocking the Severn'* - increases the potential benefit of Chipping Sodbury quarry for all migratory fish species not just European eel. The project is focused on removing historic barriers within streams of the Severn estuary, notably the river Frome, to improve migratory fish populations and biodiversity by 2040 (Severn Rivers Trust, 2014). The management required would also need methodical and yearly monitoring to ensure habitat quality. The monitoring should also include the monitoring of the water bodies using eDNA methods developed in this project to assess for presence absence of European eel and in the future population densities. With habitat management and development being carried out, there is also the potential for reintroduction. This would involve co-operation with numerous organizations such as; Severn Rivers Trust, Bristol Avon Rivers Trust, Sustainable eel group, Bristol water, Severn Trent water and the Bristol Fish Project. After habitat had been established these organizations can provide individuals to release into Chipping Sodbury. This is a potential for Chipping Sodbury to protect and preserve a Critically Endangered Species.

The management recommendations and the methods used in this project are applicable to any quarry with similar traits. European eels have strong tolerance to poor water quality and have been shown to be a ubiquitous species. This highlights the potential for European eel to establish populations in any quarry with a water body and a migratory corridor in the Severn estuary area. Some examples of suitable quarries are Whatley quarry, Tytherington Quarry, Machen Quarry, Lithalun Quarry and Forest Wood Quarry. These quarries are all within the catchment of the Severn estuary with wildlife corridors on the border. One of the projects aims was too show the potential value of any quarry for Critically Endangered European eel, this has highlighted some of the available potential in the Severn catchment.

This in turn is addressing a conservation crisis and providing the skills and resources for Hanson and Heidelberg Cement to become a leading name for the European eel. By increasing the availability of good quality habitat for this species one of the major threats is address and the protection of this species secured.

The results from the eDNA samples indicate that there are currently no eel on site, despite the apparent suitability of some of the habitats. However, this is not defining of the project, but highlights the ease and effectiveness of this survey technique developed in partnership with UWE and Hanson & Heidelberg Cement. The method that has been developed from this project is not only time effective, but highly cost effective and the best current method of eDNA analysis and surveying for European eel. The method has been proven to work through samples Westhay Moor (figure 5). This has achieved our aim of the project by providing a method for surveying European eel that is non-invasive, reliable and cheap, thus making it an applicable survey technique for all organizations. This ultimately will help to improve the knowledge and understanding of the European eel, not only within the Severn estuary, but globally. This method can also be repeated for other species. Other migratory species of conservation concern are found within the Severn estuary, such as; Twaite shad (*Alosa fallax*), Atlantic salmon (*Salmo salar*) and Lamprey (*Lampetra sp. & Petromyzon sp.*). With a field filtration method developed, DNA primers have to be developed for the target species and this survey method is repeatable for the designated species. This illustrates the versatility of the method developed and the possible benefit to not only European eel, but all freshwater species.

The development of this project and the funding provided has allowed further study and opportunity to be developed. Hanson & Heidelberg cement are influential, by providing funding for a Qubit® which will allow accurate readings of DNA to be carried out, in turn providing a method for quantification. Using a Qubit® the standard curves produced on an amplification plot (figure 5) when carrying out PCR reactions will be to highly accurate dilutions. When positive results are recorded the measurement of DNA can then be clearly classified into sections of concentrations and thus provide a scale on which to measure the sample concentration. This allows the quantification of eDNA and develops the method further by showing population densities from sampled areas. This would then provide a quick, cost effective, reliable and repeatable survey method for determining population size and therefore health for the European eel, but also potentially for other targeted species. The conservation and protection value of this method is of great value and will help to preserve this Critically Endangered species.

Recommendations;

1. Carry out assessments of individual quarries to understand their connection to potential eel migratory routes and whether those routes hold significant man-made migratory obstructions such as weirs.
2. Improve the future habitat for European eel on suitable quarries by including the planting of emergent vegetation and ensuring links between the migratory route for eel and other species on the quarry site.
3. Once management has been undertaken, carry out surveys through the use of eDNA to assess for colonization and potential population size on each water body within Chipping Sodbury quarry, whilst also assessing the habitat.
4. Provide the possibility of creating an ark site for the European eel (*Anguilla anguilla*) by introduction of individuals into quarry voids at Chipping Sodbury quarry and other applicable quarries, with continued monitoring to assess population densities and health.

## Conclusions

Although no eels were found in Chipping Sodbury Quarry or within the river Frome, the project has developed a method for surveying of this species whilst also providing easily achievable recommendations to benefit the European eel (*Anguilla anguilla*). The recommendations provided are applicable to all quarries with suitable migration corridors and suitable water bodies on site.

A new method of eDNA sampling and European eel surveying has been developed, with the possibility of applying the current method to other target species. A new method of quantification is being developed due to the work and resources provided by this project, which shall provide information on population densities within eel samples.

Chipping Sodbury quarry as a proxy for limestone quarries has been proven to have the water quality and prey abundance needed to support a population of European eel. Whilst management is needed to benefit this species within limestone quarries, it shows the conservation potential of disused quarries for the European eel.

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Laura Weldon, BSc., PhD student at University of the West of England

Westhay Moors, Somerset Wildlife Trust, Somerset

**Project tags (select all appropriate):**

This will be use to classify your project in the project archive (that is also available online)

Project focus:

- ☒Beyond quarry borders
- ☒Biodiversity management
- ☐Cooperation programmes
- ☐Connecting with local communities
- ☐Education and Raising awareness
- ☐Invasive species
- ☐Landscape management
- ☐Pollination
- ☒Rehabilitation & habitat research
- ☒Scientific research
- ☐Soil management
- ☒Species research
- ☐Student class project
- ☐Urban ecology
- ☐Water management

Flora:

- ☐Trees & shrubs
- ☐Ferns
- ☐Flowering plants
- ☐Fungi
- ☐Mosses and liverworts

Fauna:

- ☐Amphibians
- ☐Birds
- ☐Insects
- ☒Fish
- ☐Mammals
- ☐Reptiles
- ☐Other invertebrates
- ☐Other insects
- ☐Other species

Habitat:

- ☐Artificial / cultivated land
- ☐Cave
- ☐Coastal
- ☐Grassland
- ☐Human settlement
- ☐Open areas of rocky grounds
- ☐Recreational areas
- ☐Sandy and rocky habitat
- ☐Screes
- ☐Shrub & groves
- ☐Soil
- ☐Wander biotopes
- ☒Water bodies (flowing, standing)
- ☐Wetland
- ☐Woodland

Stakeholders:

- ☐Authorities
- ☐Local community
- ☐NGOs
- ☐Schools
- ☒Universities