Spawning Success and Population Structure of Shad (*Alosa* spp.) in the River Teme, 2015: with supplementary note on Sea Lamprey spawning

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EXECUTIVE SUMMARY

Focussing on the River Teme between Powick Weir and the River Severn confluence, the primary objective of this project was to establish a baseline of spawning activity of twaite shad (*Alosa fallax*). A secondary objective which evolved during the project was to quantify the spawning activity of sea lamprey (*Petromyzon marinus*) within the same reach.

Due to the complex spatial ecology of anadromous *Alosa* spp., and in particular, the limited duration that larvae reside at natal sites, monitoring temporal and spatial trends in population recruitment success using standard sampling methods presents considerable challenges. The sampling of egg abundance and distribution has however been shown to provide a cost-effective method to gather semi-quantitative information on spawning activity, distribution and habitat utilisation.

Over the course of two days' fieldwork a total of 111 kick samples were conducted across five sites located downstream of Powick Weir. Using a combination of size and transparency as the key distinguishing features of shad eggs, both shad and non-shad eggs were enumerated. A total of 430 non-shad eggs and 128 shad eggs were recorded, with 108 shad eggs retained for DNA analysis.

In order to (a) validate whether collected eggs belonged to *Alosa* spp. and (b) determine the relative proportion of *A. fallax* and *A. Alosa* eggs, two different genetic markers – mitochondrial DNA and nuclear DNA: nif1-nDNA were applied to the analysis. Where shad specific DNA amplification was not achieved from an individual egg, universal primers were subsequently applied to define species identity. Of the 108 eggs identified as belonging to shad in the field, only one egg was misidentified, thus confirming field identification accuracy as greater than 99 percent. The application of universal primers confirmed this individual egg as chub *Squalius cephalus*. With regard to shad eggs, DNA analysis has confirmed the stock (at the time of the survey) to be dominated by *A. fallax*, with a high frequency of hybridisation (~26%) with *A. alosa*

Throughout the survey sub-samples of eggs were periodically examined using a magnifying hand lens to determine the developmental phase of embryos. While on the 28 May all eggs were observed to be in the earliest stages of development (e.g. epiboly), by 1 June, development ranged from epiboly (pre- organogenesis) through to well-developed embryos. This provides evidence that egg deposition was ongoing with spawning occurring over a period of several nights.

The homogeneity of habitat characteristics between Sites A-E and the observed presence of shad eggs at all sites confirms that several areas of quality functional spawning habitat currently exist throughout the 3.3 km reach of the River Teme between Powick Weir and the River Severn. The application of CPUE analysis which revealed considerably higher egg abundance immediately downstream of Powick Weir (Section A), suggests that the weir is functioning as a migratory bottleneck, with aggregations of spawning shad becoming concentrated on the first available habitat downstream of this structure.

During a subsequent survey of the river for sea lamprey on 8 and 9 July, a total of 94 nests distributed throughout the same sections surveyed for shad, confirmed the current importance of habitats downstream of Powick for the recruitment of sea lamprey to the Severn Estuary

EMS. A brief summary report of lamprey spawning activity, including the enumeration of nests and observations of adult activity is presented in Appendix I of this report.

The current project has been successful in qualifying the functionality of spawning habitat for both twaite shad and sea lamprey utilising the River Teme downstream of Powick Weir. In addition, the results also provide a semi quantitative temporal baseline of adult population size for sea lamprey within the same reach. Survey results are discussed within the context of data limitations and future monitoring requirements to reliably monitor future population trends for condition assessment and species conservation management.

CONTENTS

1 INTRODUCTION

1.1 Background

In addition to Ramsar and Special Protection Area (SPA) designations, the Severn Estuary European Marine Site also includes a Special Area of Conservation (SAC) (Appendix III). Under the latter, three species of anadromous fish qualify as 'Interest Features': twaite shad (*Alosa fallax*), sea lamprey (*Petromyzon marinus*) and river lamprey (*Lampetra fluviatilis*). Despite the freshwater reaches of the River Severn and its tributaries falling outside the SAC boundary, they serve an essential function as spawning and nursery habitats for the designated species which depend on the SAC to facilitate migratory passage between freshwaters and the marine environment. Under the Habitats Regulations, Natural England (NE) has a statutory duty to assess the condition of each site feature against Conservation Objectives set. This provides the necessary data to monitor population trends and inform appropriate regulation of development schemes which might impact on the interest features.

Due to the presence of major instream barriers in the lower Severn catchment (e.g. Diglis Weir on the Severn and Powick Weir on the Teme), the spatial migratory potential of the fish species of interest is compromised; and with specific reference to shad, considered to be exclusively restricted to habitats downstream of these structures. Following a walkover survey conducted in September 2014, APEM (2014) identified the lower River Teme as providing several areas of potential spawning habitat for both shad and migratory lampreys; species which also represent qualifying features of the Teme Site of Special Scientific Interest (SSSI).

Due to the complex spatial ecology of anadromous *Alosa* spp., and in particular, the limited duration that larvae reside at natal sites, monitoring temporal and spatial trends in population recruitment success using standard sampling methods presents considerable challenges. In accordance with the specific attributes listed in the Regulation 33 conservation advice package, the sampling of egg abundance and distribution has been shown to provide a cost-effective method to gather semi-quantitative information on spawning activity and distribution (Thomas & Dyson 2012a, 2012b). Furthermore, the distribution of eggs also facilitates the detailed characterisation of habitat utilisation (Pinder *et al*., in press).

Due to similarities in size, colouration and morphology, positive field identification of eggs between some fish species can be problematic. In such cases, genetic markers can provide a relatively cost-effective method to identify fish eggs to species level. This genetic toolkit is particularly useful in the case of the shad species (*A. fallax* and *A. alosa)* as there are no visible criteria on which the eggs of each species can be distinguished. For shad, genetic markers were developed by Alexandrino and Faria (2004) and have been used with varying success in distinguishing between the two species (see Hardouin *et al.,* 2013). Although the genetic markers available are not able to distinguish each individual to species level with 100 % accuracy, they can provide important insight into the genetic composition of spawning populations.

Focussing on the River Teme between Powick Weir and the River Severn confluence, the primary objective of this project was to establish a baseline of spawning activity of twaite shad (*A. fallax*). A secondary objective which evolved during the project was to quantify the

spawning activity of sea lamprey (*P. marinus*) within the same reach. Reporting of the latter objective has been included within this report as an appendix.

1.2 Specific project aims and objectives

- Qualify whether the lower River Teme currently functions as a spawning ground for twaite shad
- Establish a baseline of temporal and spatial spawning activity and relative abundance of eggs
- Use genetic markers to determine the community structure of spawning shad, specifically to detect the presence of *A. alosa* and frequency of hybridisation
- Using nest counts, quantify the abundance and spatial distribution of sea lamprey.
- Provide preliminary indication of the current condition of the Twaite shad spawning distribution for the Severn Estuary
- Identify potential threats and associated management measures that could protect the spawning grounds

2 STUDY AREA

Due to the potential for Powick Weir to act as a comprehensive barrier to shad migration and its further potential to compromise the migratory performance of other fish species (e.g. sea lamprey), this study was focused on the 3.3 km of the River Teme between Powick Weir and its confluence with the River Severn, downstream of Worcester. Survey sites were predetermined by a walkover survey conducted during September 2014 and guided by the mapped outputs of habitat patches considered suitable to function as spawning habitat for both shad species and migratory lampreys (APEM, 2014). Based on the locations suggested in the Invitation to Tender (ITT) (Table 2.1), Figure 2.1 provides spatial representation of the distribution of the five survey sites examined during the current investigation. Figure 2.2 provides further context of the relative location of the River Teme, the Severn Estuary SAC (Appendix III) and other major rivers.

Table 2.1 Suggested sampling locations for Twaite shad eggs on the River Teme (NE, 2015)

Location	Type	Comments
SO 83254 52363	Shallow spawning	First spawning habitat identified downstream of Powick Weir. Access is located on the right hand bank.
SO 83521 52471	Shallow spawning	Spawning location in close proximity to pools for adults to hold prior to spawning. Easy access possible from both banks.
Ca. 100m stretch from SO 83650 52375 to SO 83733 52340	Mixed shallow and deep spawning	Significantly large stretch of suitable habitat spanning entire channel width. Access possible from both banks, but left hand bank likely to be the most appropriate.
SO83916 52382	Shallow spawning	*Ca*. 25m long stretch of suitable habitat, spanning entire channel width. Access possible from both banks.
SO 84634 51823	Shallow spawning	*Ca*. 25m long stretch of suitable habitat, spanning entire channel width. Most downstream area of suitable habitat identified before confluence with the River Severn. Access possible from both banks.

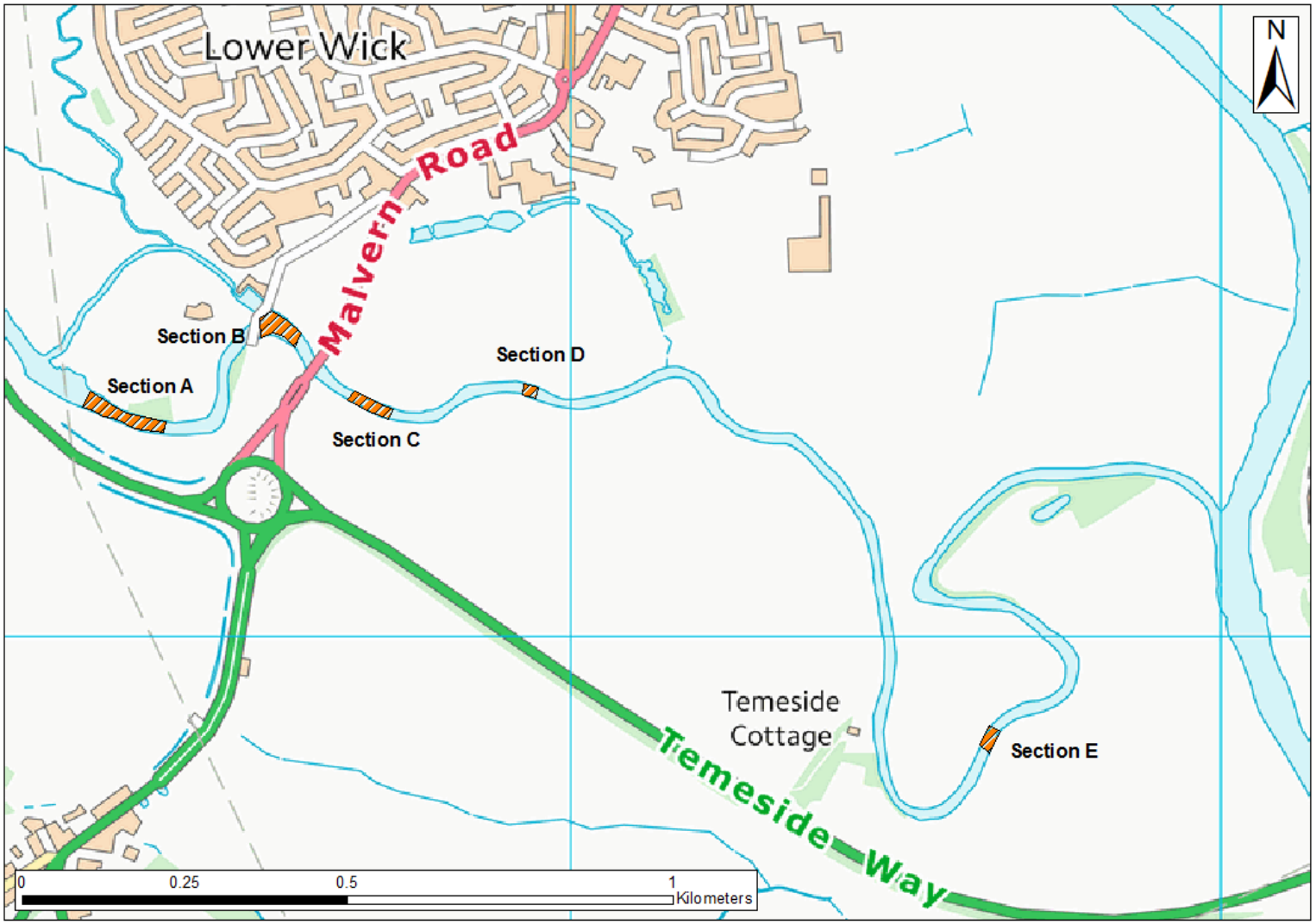

Figure 2.1 Map showing the 3.3km of River Teme between Powick Weir and the River Severn. Sections A – E represent areas of survey focus.

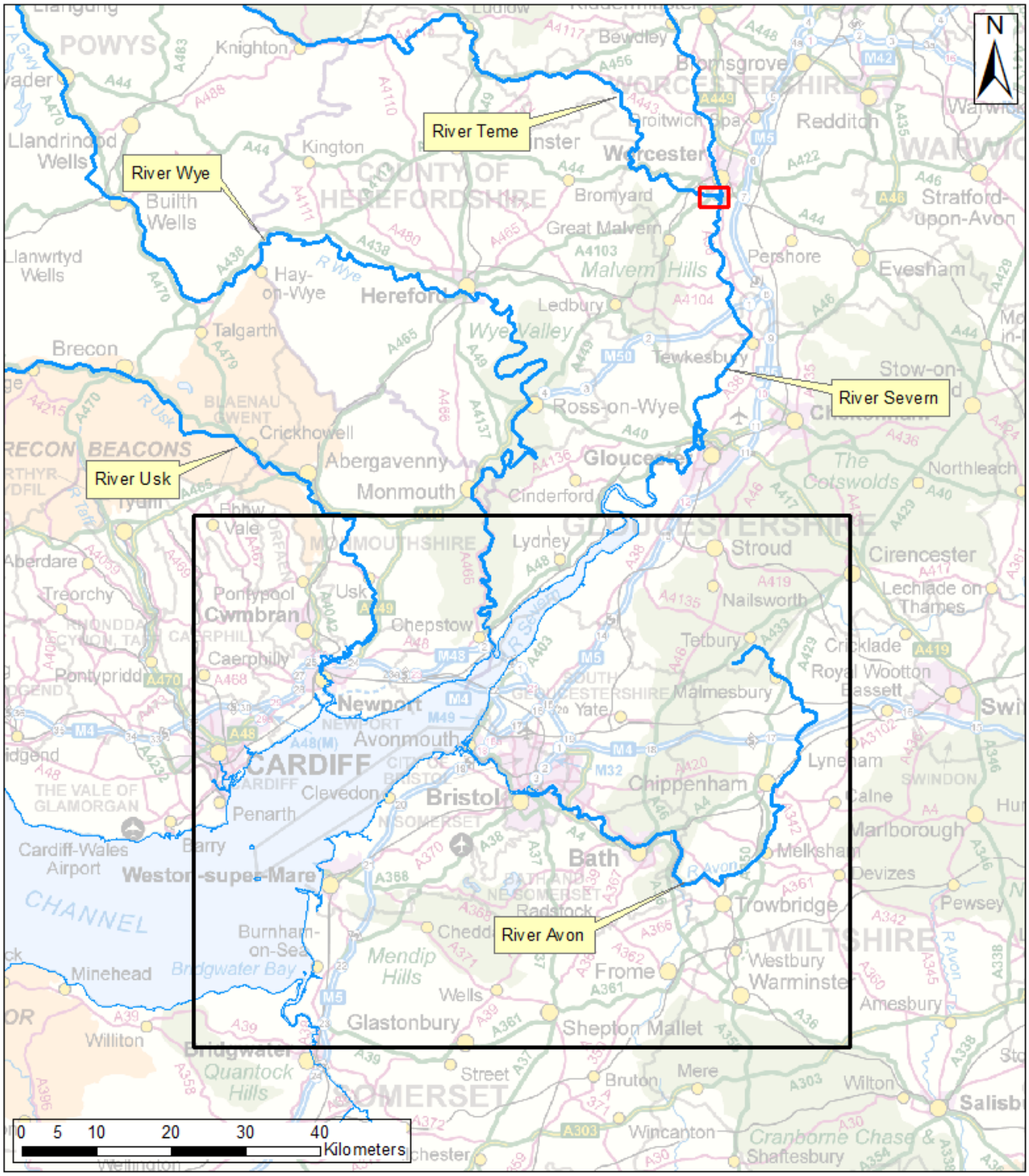

Figure 2.2 Map showing the relative location of the River Teme study site (red box) (Figure 2.1), the Severn Estuary SAC (black box) (Appendix III) and other major rivers.

3 METHODS

3.1 Egg collection, identification and fixation

Following the capture of three individual suspected shad eggs in a drift net deployed to sample barbel eggs on 28 May 2015, an immediate survey response was considered prudent to reduce the risk of freshly laid shad eggs being washed out by heavy rain forecast over the coming days. Following the successful collection of shad eggs from Sections A and B on 28 May and the continuation of favourable river levels over the weekend, Adrian Pinder and Catie Gutmann-Roberts of BUG, returned to site on Monday 1 June to survey the three remaining sections (C, D and E). Section A (downstream of Powick Weir) was also revisited on 1 June, and additional shad eggs collected.

On arrival at each site, an initial walkover survey was conducted to identify the patches of previously identified spawning habitat (APEM, 2014) and to update dynamic risk assessments including the scoping of safe points for access and egress to the river.
Working areas of suitable habitat in a downstream to upstream direction, samples were semi-randomly distributed (ignoring small patches of sand, silt etc.) across the full width of river. In accordance with the method described by Thomas and Dyson (2011), a standard 250 µm macroinvertebrate hand net was used to collect materials (Figure 3.1) dislodged by kicking upstream of the net for approximately 15 seconds. The contents of the net were then washed into a white sorting tray to check for the presence of eggs.

Figure 3.1 Examining kick net contents below Powick Weir (left) and collected shad eggs (right)

Using a combination of size and transparency as the key distinguishing feature of shad eggs (see Figure 3.1), both shad and non-shad eggs were enumerated. While non-shad eggs were visually identified to species before returning to the river, using a combination of tweezers and a pipette, shad eggs were placed in a petri dish of distilled water, before being individually fixed in pre-labelled Eppendorf tubes containing ATL buffer. Preserved eggs were then put on ice ready for transport to BU, where they were cold stored at -70^{0}C prior to molecular analysis.

With a view to targeting the collection of a total of 100 shad eggs across five sites, kick sampling was repeated until either a total of 20 eggs had been collected or a maximum of 25 samples had been conducted at each site.

3.2 Habitat assessment

Regardless of the presence/absence of eggs, each sampling location was georeferenced using a handheld GPS unit (Garmin 60 Csx) and the following physical habitat characteristics recorded: stream depth (cm), stream width (m), distance to bank (m), stream velocity (cm/s - Valeport 002 wading set) and substrate composition (% cobble (64-246mm), pebble (32-64mm), gravel (2-32mm), sand (0.063-2mm), silt (<0.063m)). Channel position was also recorded as a ratio of total stream width, where a channel position of 0.5 would represent mid channel width. At each of the five sampling sites a single suite of the following physicochemical parameters was also recorded using a YSI Pro+ handheld multi-probe: dissolved oxygen (% and mg/l), conductivity (µS), temperature (^{0}C) and pH. Digital photographs were also captured to record general site characteristics at each of the five sampling sites.

3.3 Spatial analysis and GIS

As a proxy for spatial egg abundance, Catch-Per-Unit-Effort (CPUE) was calculated as a ratio of the number of shad eggs to the number of samples collected within each section (A-E). In analysing spatial variance between CPUE and longitudinal distance from Powick Weir, distances were measured using www.digimap.edina.co.uk.

Locations of all individual sampling sites were mapped onto a satellite layer using basemaps provided by Bing within ArcGIS 10.1. The satellite basemap was transformed to British National Grid using OSGB_1936_To_WGS_1984_1. Samples lacking shad eggs have been plotted as green circles, with the relative size of purple circles corresponding with categories of shad egg abundance (number). Hard copy outputs are presented in Section 4 of this report (Figs 4.2, 4.4, 4.6, 4.8 & 4.10) with shapefiles referenced to the British National Grid and corresponding attribute tables supplied on the accompanying DVD.

3.4 Molecular analyses

In order to (a) validate whether collected eggs belonged to *Alosa* spp. and (b) determine the relative proportion of *A. fallax* and *A. Alosa* eggs, two different genetic markers – mitochondrial DNA and nuclear DNA: nif1-nDNA were applied to the analysis. DNA was extracted from whole eggs using the DNEasy extraction kit from Qiagen using the manufacturer instruction. Where shad specific DNA amplification was not achieved from an individual egg, universal primers were subsequently applied to define species identity. Sequencing conditions for each of the three methods of analyses are provided in Sections 3.4.1 – 3.4.3 and summarised in a 'quick look-up' Table 3.1.

3.4.1 Mitochondrial Cyt B sequencing:

A segment of the 401 nucleotides of the mitochondrial CytB gene was amplified using the primers from Alexandrino *et al*. (2006). PCR conditions included, PCR buffer (1x), 2 mM of MgCl2, 0.2 mM of dNTPs, 0.25 µM of each primer and 0.5 units of Taq polymerase (Promega flexi Taq) per sample. DNA concentration per PCR reaction ranged from 50-100 ng and the

reaction volume was 32 µl. Cycling conditions consisted of an initial denaturation at 94 °C for 2 minutes followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 60 °C for 45 seconds and extension 72 °C for 45 seconds followed by a final extension at 72 °C for 2 minutes.

3.4.2 Ninf1-nDNA sequencing conditions:

In order to develop the method, ninf1-nDNA was sequenced using primers described in Faria *et al*. (2004) for a sub-sample of 15 eggs to ensure the presence of the polymorphic sites in the Teme populations. Once the presence of the polymorphism was confirmed, all shad individuals were screened at the ninf1-nDNA locus.

PCR conditions included, PCR buffer (1x), 2 mM of MgCl2, 0.2 mM of dNTPs, 0.4 µM of each primer and 0.5 units of Taq polymerase (Promega flexi Taq) per sample. DNA concentration per PCR reaction ranged from 50-100 ng and the reaction volume was 12 µl. Cycling conditions consisted of an initial denaturation at 94 °C for 2 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds and extension 72 °C for 30 seconds followed by a final extension at 72 °C for 2 minutes.

The restriction enzyme digestion reaction conditions were 1x buffer, 10 units of Hae III per sample and 12 µl of template PCR in a total volume of 50 µl. The enzymatic reaction lasted for one hour at 37°C followed by 20 min at 80°C to deactivate the enzyme.

The restriction digests were migrated through an agarose gel and scored. Individuals were scored as homozygous CC or heterozygous (one allele CG and one CC) at the enzymes restriction site.

3.4.3 Non-shad eggs identification:

A sequence of 553 bp of COI was amplified on all non-shad eggs samples using the cocktail primers C_FishF1t1 – C_FishR1t1 from Ivanova *et al*. (2007). PCR conditions included, PCR buffer (1x), 2 mM of MgCl2, 0.2 mM of dNTPs, 0.25 µM of each primer and 0.5 units of Taq polymerase (Promega flexi Taq) per sample. DNA concentration per PCR reaction ranged from 50-100 ng. Cycling conditions consisted of an initial denaturation at 94 °C for 2 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 48 °C for 40 seconds and extension 72 °C for 60 seconds followed by a final extension at 72 °C for 10 minutes.

3.4.4 Phylogenetic analysis:

Cyt B sequences were then cleaned and aligned using Codon Code Aligner (CodonCode Corp.), Mega (Tamura *et al*. 2007) and BioEdit (Hall 1999), to determine the *Alosa* haplotypes present. A phylogenetic tree was drawn using MEGA (Tamura *et al*. 2007). The substitution model was tested with Mega and the K2 (Kimura 2 parameter model) was used using maximum likelihood and 1000 bootstraps.

Table 3.1 Summary of sequencing conditions across all three methods

	Mitochondrial Cyt B	Ninf1-nDNA	Non-shad eggs
Buffer	1x		
MgCl2	2mM		
dNTPS	0.2mM		
Primers (forward and reverse)	0.25 µM	0.4 µM	0.25 µM
Taq	0.5Unit (Promega Flexi Taq) per sample		
DNA concentration	50-100 ng		
Total volume	32 µl	12 µl	-
	PCR Cycling Conditions		
	94°C for 120s		
	94°C for 45s 60°C for 45s 72°C for 45s 35 cycles	94°C for 30s 54°C for 30s 72°C for 30s 40 cycles	94°C for 30s 48°C for 40s 72°C for 60s 35 cycles
	72°C for 120s		72°C for 600s

4 RESULTS

4.1 Egg collection

Over the course of two days' fieldwork a total of 111 kick samples were conducted across five sites (A–E). Of this total, 61 samples (55%) contained a total of 430 non-shad eggs and 29 (26%) contained a total of 128 shad eggs. Of the 128 shad eggs recorded, 108 were retained for DNA analysis. A spatial and temporal breakdown of sampling activity and eggs recorded is summarised in Table 4.1. Note: while barbel eggs could be reliably identified in the field using colour and size as diagnostic features, due to similarities, identification of chub and minnow to individual species was not possible.

Table 4.1 Summary of temporal and spatial sampling activity and eggs recorded.

Site	Date	Total Kick samples	No. shad eggs	No. non-shad eggs	Notes on no-shad eggs
A	28/5/15	10	31	137	1 barbel, remainder chub and minnow
A Extra	1/6/15	6	31		
B	28/5/15	25	20	157	11 barbel, remainder chub and minnow
C	1/6/15	25	4	40	Mix of chub and minnow
D	1/6/15	25	15	8	Mix of chub and minnow
E	1/6/15	25	7	88	Mix of chub and minnow
TOTALS		**116**	**108**	**430**	

4.2 Spatial distribution of samples and habitat assessment

The following section provides general physical description of sites A – E, summarises the microhabitat characteristics of sample points where shad eggs were recorded and incorporates GIS outputs to present the spatial distribution of all sampling points and the spatial abundance of shad eggs.

4.2.1 Site A

Site A represents the most upstream site surveyed and was located downstream of Powick Weir. Despite the substrate immediately downstream of the weir pool being relatively fine (small gravel), as the river narrowed to a mean width of ~9 meters, water velocities accelerated to a maximum of 0.72 m/sec, resulting in a patches of larger cobbles and pebbles which extended approximately 200 metres downstream of the weir. Throughout the section of appropriate habitat to support shad spawning, both banks were high with a steep gradient and densely covered with herbaceous vegetation. Along the north bank, several willow trees provided both high level canopy shading and some areas of low level/in stream cover for fish refuge (Figure 4.1).

Figure 4.1 Example of typical habitat in Section A. Note patches of pebble/gravel substrate and in-stream cover provided by overhanging willow. Insert show close up of microhabitat utilised by spawning shad.

Figure 4.2 Spatial distribution of sampling points (green dots) and relative abundance of shad eggs (purple circles) in Section A.

Shad eggs were collected from seven of a total of 17 sample points surveyed in Section A. A descriptive summary of habitat characteristics for the sites containing shad eggs is provided in Table 4.2

Table 4.2 Habitat characteristics of sample points containing shad eggs at Site A

Site A			Substrate (%)						
	Depth (cm)	Velocity (cm/s)	cobble	pebble	gravel	sand	silt	Width (m)	Channel position*
Mean	42.14	38	4.285	50	38.57	2.85	4.28	9.42	0.36
SD	15.356	17	5.34	28.284	25.448	4.879	5.34	0.534	0.096
Min	24	21	0	20	20	0	0	9	0.2
Max	68	72	10	70	80	10	10	10	0.5
N=	7	7	7	7	7	7	7	7	7

*Channel position recorded as a ratio of total stream width, where a channel position of 0.5 represents mid channel width. Measurement always refers to nearest bank.

4.2.1 Site B

Site B was located between Powick Bridge and the A449 road bridge. Within this relatively short section, the river formed a wide, deep pool immediately below Powick Bridge which then narrowed to a mean width of approximately 12 metres. Through this narrower section larger substrate and elevated flows considered suitable for spawning extended over a distance of approximately 40 metres. Throughout the area surveyed, both banks were steeply sloping, with the South bank populated with herbaceous vegetation and the North bank populated by dense willows which extended cover approximately three metres into the channel.

Figure 4.3 Example of typical habitat in Section B. Looking downstream from Powick Bridge

Figure 4.4 Spatial distribution of sampling points (green dots) and relative abundance of shad eggs (purple circles) in Section B.

Shad eggs were collected from seven of a total of 25 sample points surveyed. A descriptive summary of habitat characteristics for the sites containing shad eggs is provided in Table 4.3.

Table 4.3 Habitat characteristics of sample points containing shad eggs at Site B

Site B			Substrate (%)						
	Depth (cm)	Velocity (cm/s)	cobble	pebble	gravel	sand	silt	Width (m)	Channel position*
Mean	45	55	17.14	34.28	38.57	4.28	5.71	12.28	0.36
SD	5.41	17	17.04	25.07	29.68	5.34	5.34	1.79	0.076
Min	36	38	0	0	10	10	0	11	0.25
Max	53	83	40	70	90	30	10	16	0.45
N=	7	7	7	7	7	7	7	7	7

*Channel position recorded as a ratio of total stream width, where a channel position of 0.5 represents mid channel width. Measurement always refers to nearest bank.

4.2.1 Site C

Of all five survey sites, Section C provided the greatest length (~100m) of suitable spawning habitat, extending from approximately 35 metres below the A449 road bridge to the first downstream bend in the river. Under the relatively low flows at the time of the survey, the North bank was typically characterised by shallow gravel beach, while the steeper South bank was incised with channel depths of 30 – 40 cm immediately against the bank. With the exception of two willow trees which overhung the channel and provided some low level cover on the South bank additional in stream or riparian cover was limited.

Figure 4.5 View of Section C taken from the South bank looking upstream towards the A449 road bridge.

Figure 4.6 Spatial distribution of sampling points (green dots) and relative abundance of shad eggs (purple circles) in Section C.

Shad eggs were collected from just three of a total of 25 sample points surveyed. A descriptive summary of habitat characteristics for the sites containing shad eggs is provided in Table 4.4.

Table 4.4 Habitat characteristics of sample points containing shad eggs at Site C

Site C			Substrate (%)						
	Depth (cm)	Velocity (cm/s)	cobble	pebble	gravel	sand	silt	Width (m)	Channel position*
Mean	35	60	13.3	36.66	30	13.33	6.66	8.66	0.42
SD	9.539	9.1	15.27	5.77	17.32	7.63	7.63	0.57	0.08
Min	26	52	0	30	20	5	0	8	0.33
Max	45	70	30	40	50	20	15	9	0.5
N=	3	3	3	3	3	3	3	3	3

*Channel position recorded as a ratio of total stream width, where a channel position of 0.5 represents mid channel width. Measurement always refers to nearest bank.

4.2.2 Site D

Located approximately 400 metres downstream of Section C, suitable spawning habitat at Section D was limited to a short section of approximately 20 metres. Optimal spawning habitat was concentrated towards the North bank where velocities were greater and substrate typically larger. While the North bank was extremely steep and densely covered with herbaceous vegetation, the south bank consisted of a shallow scalloped beach with relatively fine sediment and better suited to function as nursery rather than spawning habitat.

Figure 4.7 View of Section D from the top of the South bank. Note potential shad nursery habitat in foreground.

Figure 4.8 Spatial distribution of sampling points (green dots) and relative abundance of shad eggs (purple circles) in Section D.

Shad eggs were collected from eight of a total of 25 sample points surveyed. A descriptive summary of habitat characteristics for the sites containing shad eggs is provided in Table 4.5.

Table 4.5 Habitat characteristics of sample points containing shad eggs at Site D

Site D			Substrate (%)						
	Depth (cm)	Velocity (cm/s)	cobble	pebble	gravel	sand	silt	Width (m)	Channel position*
Mean	48.75	37	8.75	50.62	23.12	7.5	11.25	10.62	0.38
SD	5.36	6	8.34	17.82	13.35	5.34	6.94	0.52	0.05
Min	43	30	0	20	10	0	0	10	0.3
Max	58	50	20	70	40	15	20	11	0.45
N=	8	8	8	8	8	8	8	8	8

*Channel position recorded as a ratio of total stream width, where a channel position of 0.5 represents mid channel width. Measurement always refers to nearest bank.

4.2.1 Site E

Representing the most downstream survey area, Site E was located approximately one kilometre upstream of the River Severn confluence and approximately 350 metres downstream of Temeside Cottage. Access to the river proved challenging at Site E with attempts to descend the near vertical gradient of the South bank abandoned in favour of negotiating a path through the dense border of herbaceous vegetation on the North bank. Spawning habitat was limited to a short length of approximately 15 metres where depths ranged between 42 and 65cm and pebble dominated the sediment matrix. Water velocity was relatively high throughout this section and ranged between 0.58 and 0.66 m/s^{-1} at sites where shad eggs were recorded. Throughout the reach, overhanging willows lined the North bank, providing low level shade and some instream cover for fish.

Figure 4.9 View of Site E from the bottom of Site E looking upstream.

Figure 4.10 Spatial distribution of sampling points (green dots) and relative abundance of shad eggs (purple circles) in Section E.

Shad eggs were collected from four of a total of 25 sample points surveyed. A descriptive summary of habitat characteristics for the sites containing shad eggs is provided in Table 4.6.

Table 4.6 Habitat characteristics of sample points containing shad eggs at Site E

Site E			Substrate (%)						
	Depth (cm)	Velocity (cm/s)	cobble	pebble	gravel	sand	silt	Width (m)	Channel position*
Mean	50	62	15	52.5	25	5	2.5	7.75	0.29
SD	10.23	4	19.14	9.57	12.91	5.77	5	0.5	0.11
Min	42	58	0	40	10	0	0	7	0.19
Max	65	66	40	60	40	10	10	8	0.44
N=	4	4	4	4	4	4	4	4	4

*Channel position recorded as a ratio of total stream width, where a channel position of 0.5 represents mid channel width. Measurement always refers to nearest bank.

4.3 Habitat summary across all sites

Throughout the survey, the semi-random distribution of sampling points was restricted within those habitats considered, to represent optimal spawning habitat; visually characterised by large (pebble/gravel) clean substrate and depths not exceeding 65 cm. Figure 4.11 provides a summary of data pooled from a total of 116 sampling points and distinguishes the characteristics of both sites containing shad eggs (n=29) and those sites where no shad eggs were recorded (n=87). Based on mean values and their associated 95% confidence intervals, it is evident there were no observed significant differences to indicate subtle preferences in habitat selection within the areas of habitat identified as 'potential' spawning habitat (APEM, 2014).

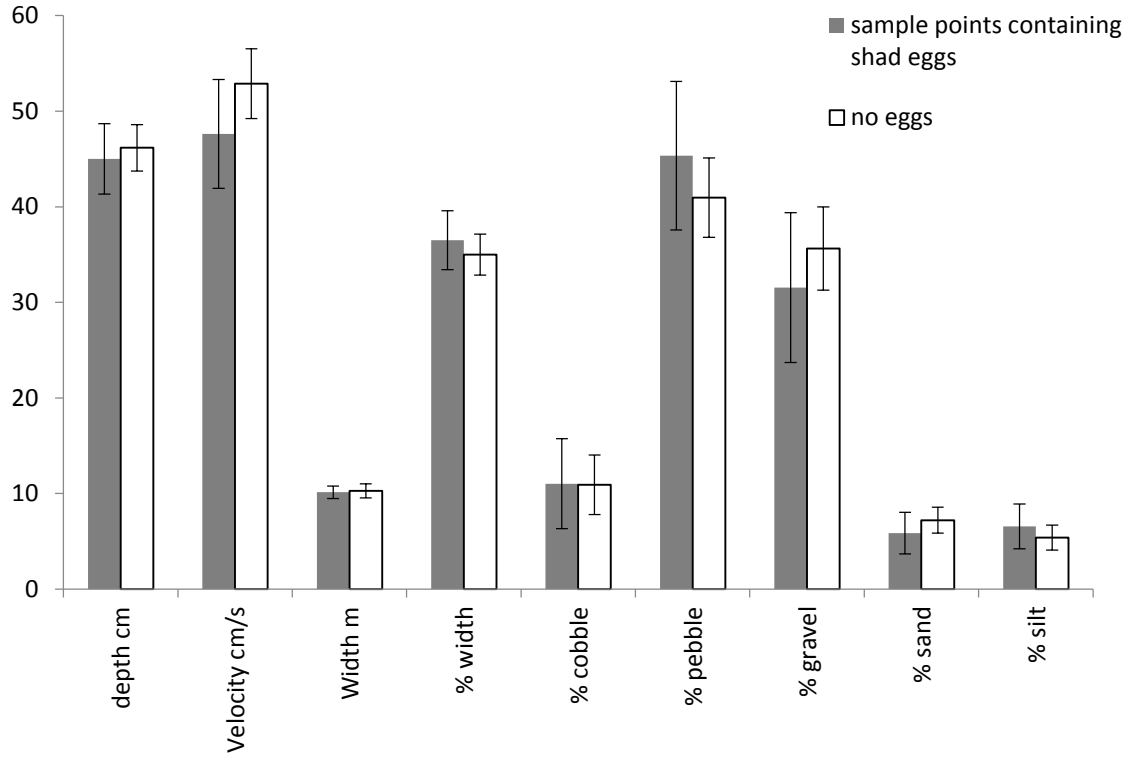

Figure 4.11 Summary of habitat data pooled from a total of 116 sampling points. Mean values and 95% CL are provided for sites containing shad eggs (grey) and not containing shad eggs (white)

4.4 Spatial summary of relative abundance of shad eggs

To examine the relative abundance of shad eggs between the five survey sites (A-E), Catch-Per-Unit-Effort (CPUE) was calculated as a ratio of the number of shad eggs to the number of samples collected within each section. These data were then plotted against downstream distance from Powick Weir (Figure 4.12). The Highest CPUE (3.81) was observed in Section A, with CPUE ranging between 0.16 and 0.8 across the remaining sites B-E).

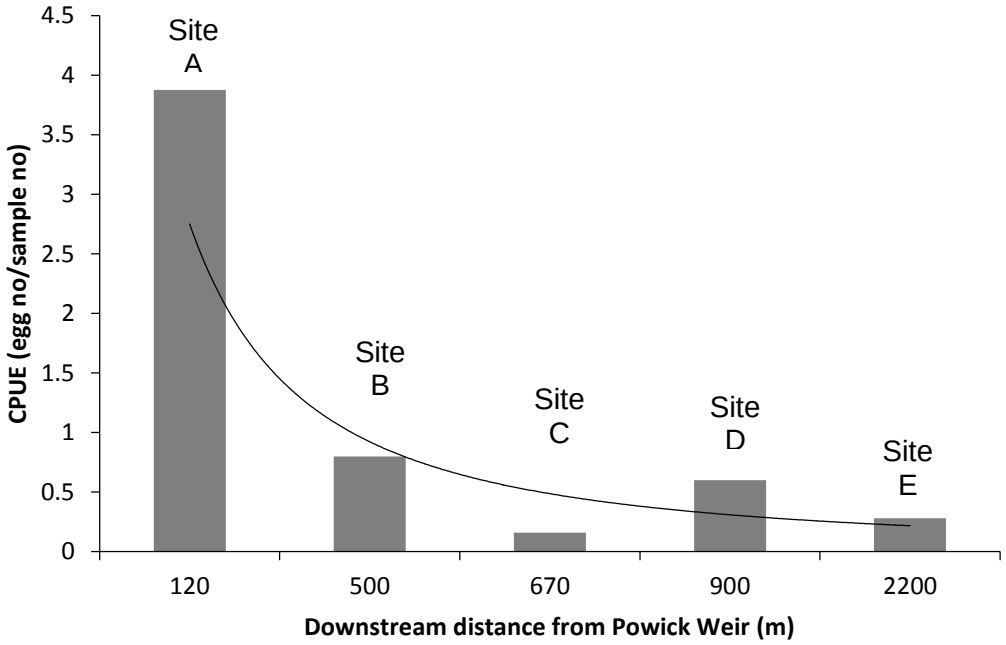

Figure 4.12 Relative Catch-Per-Unit-Effort (CPUE) versus downstream distance from Powick Weir.

4.5 Temporal variations in river flow

To characterise the timing of spawning in relation to river flows, discharge data were collated from Knightsford Bridge gauging station (OS grid: SO 73506,55737). Figure 4.13 presents 15 minute data intervals (m^3/s) between 1 May and 31 July 2015. The temporal survey period for shad eggs (28 May – 1 June) is highlighted with green shading. The grey shading corresponds to the timing of the lamprey nest count survey, as reported in Appendix I of this document. It is noteworthy, that heavy rainfall on 2 June led to a peak ($13.3m^3/s$) in the hydrograph immediately after shad eggs were collected.

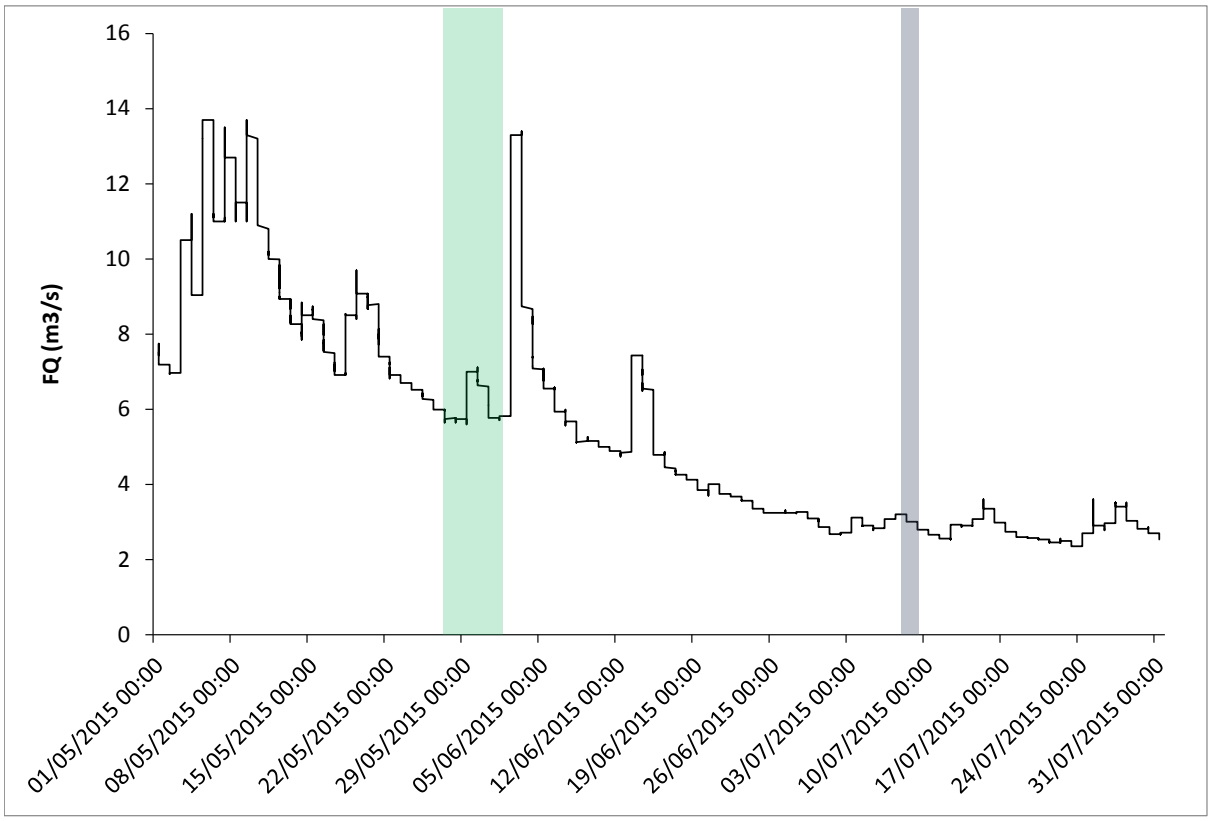

Figure 4.13 River discharge (m^3/s) at Knightsford Bridge between 1/5/15 and 31/7/15. Green and grey shading represents timing of shad and lamprey surveys respectively. Flow data courtesy of Environment Agency.

4.6 Ontogenetic development of shad eggs

Throughout the survey sub-samples of eggs were periodically examined using a magnifying hand lens to determine the developmental phase of embryos. While on the 28 May all eggs were observed to be in the earliest stages of development (e.g. epiboly), by 1 June, development ranged from epiboly (pre- organogenesis) through to well-developed embryos (Figure 4.14). This provides evidence that egg deposition was ongoing with spawning occurring over a period of several nights.

Figure 4.14 Shad eggs collected 1 June 2015 exhibiting advanced stages of development. Note the well pigmented eyes on the left specimen and deformed notochord in lower specimen.

4.7 Molecular results

Out of the 100 eggs, 87 sequences were successful for 420 bases of the Cytochrome B. Any sample, for which there was no amplification, had the PCR repeated. 13 samples spread through all five sites yielded no amplification following two independent PCR reactions. This could be due to low DNA qulality and/or the presence of inhibitors. A total of eight different haplotypes were found in the Teme (Haplotype diversity = 0.723 (SD=0.035); Nucleotide diversity = 0.00791 (SD= 0.00057)). A test of neutrality was also performed on the dataset using Tajima's D statistical analysis. Tajima's D was equal to -0.04537 and was found not to be significant (P>0.10). This test predicts that all mutations are selectively neutral.

Haplotype diversity was in accordance with the results from a previous study focused on three Welsh rivers; the Tywi, the Usk and the Wye, which found a haplotype diversity of 0.739 (SD= 0.041), 0.655 (SD= 0.051) and 0.465 (SD= 0.056) respectively (Hardouin *et al.,* 2013).

In the River Teme, no geographical distribution of haplotypes was observed across the five sites (Figure 4.15). All sites had a mixture of *A. alosa* (32.2%) and *A. fallax (*67.8%) *haplotypes* (Figure 4.16).

Interestingly, the percentage of *A. alosa* mitochondrial haplotype in the Teme was higher than previously reported. Indeed, Alexandrino & Faria (2004) described an increase in the percentage of *A. alosa* haplotypes from the east to the west of Wales, with the group Wye-Teme-Severn having approximately 20-26% *A. alosa*, Usk 49% and Tywi 71% (Alexandrino & Faria, 2004).

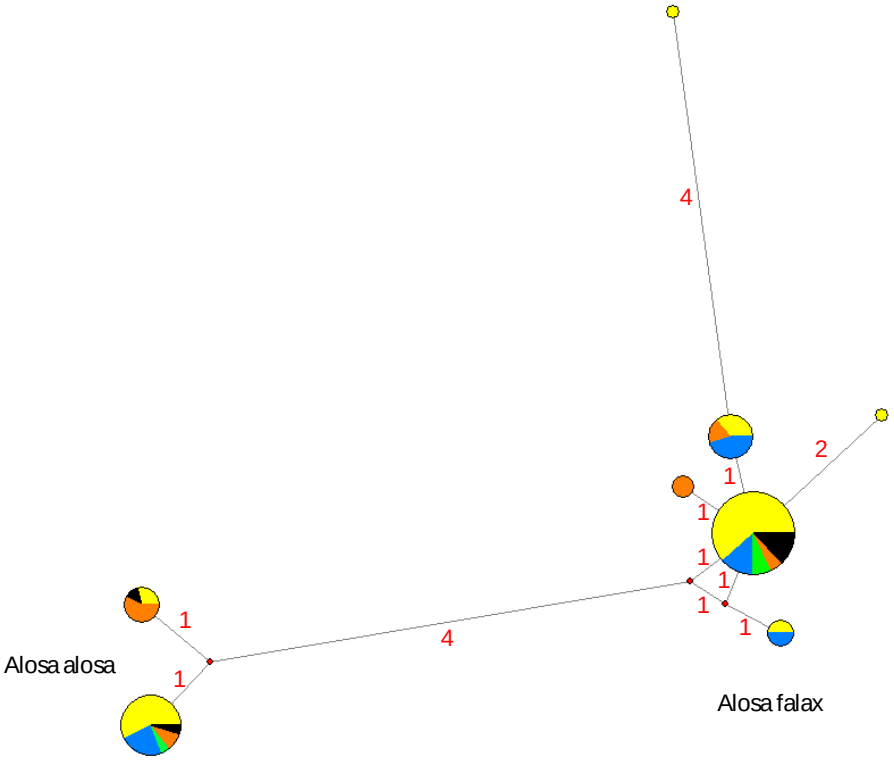

Figure 4.15 Haplotype genetic network calculated using Median Joining. The size of the circles represent the frequency of the different haplotypes. The number of mutational step between node is shown in red. Small red circles represent branch splits. The colour code is as follow: Site A in yellow, Site B in blue, Site C in green, Site D in Orange and Site E in black.

4.7.1 Site A-E

46 eggs were successfully sequenced from Site A, with the highest proportion assigning to *A. fallax* from both the Cytochrome B and ninf1-nDNA (Figures 4.16 and 4.17). When the 2 genetic regions were combined, 55 % of the eggs were assigned to *A. fallax* and 45 % were identified as hybrids. At Site B,17 eggs were successfully genotyped with the highest proportion assigned to *A. fallax* using both genetic regions (cytochrome B and ninf1-nDNA). When both regions were combined, 64.3 % were identified as *A. fallax*, 7.1% as *A. alosa* and 28.6 % as hybrids (Figures 4.16 and 4.18).

Sites C and D had low egg numbers. Four eggs were genotyped for Site C, with the highest proportion assigned to *A. fallax* using both the Cytochrome B and ninf1-nDNA genetic regions. Due to low amplification of the ninf1-nDNA genetic region, hybrids between the two species were not detected hybrids (Figures 4.16 and 4.19).but are expected to be present based on the cytochrome B sequences. At Site D, 13 eggs were genotyped with the highest proportion assigned to *A. fallax* using both the cytochrome B and ninf1-nDNA genetic regions. Due to low amplification of the ninf1-nDNA genetic region, *A. alosa* were not identified (Figures 4.16 and 4.20) but are again expected to be present based on the cytochrome B sequences.

At Site E, seven eggs were successfully genotyped with the highest proportion assigned to *A. fallax* using both the cytochrome B and ninf1-nDNA genetic regions. Due to low amplification of the ninf1-nDNA genetic region, *A. alosa* were not identified (Figures 4.13 and 4.21) but are expected to be present based on the cytochrome B sequences.

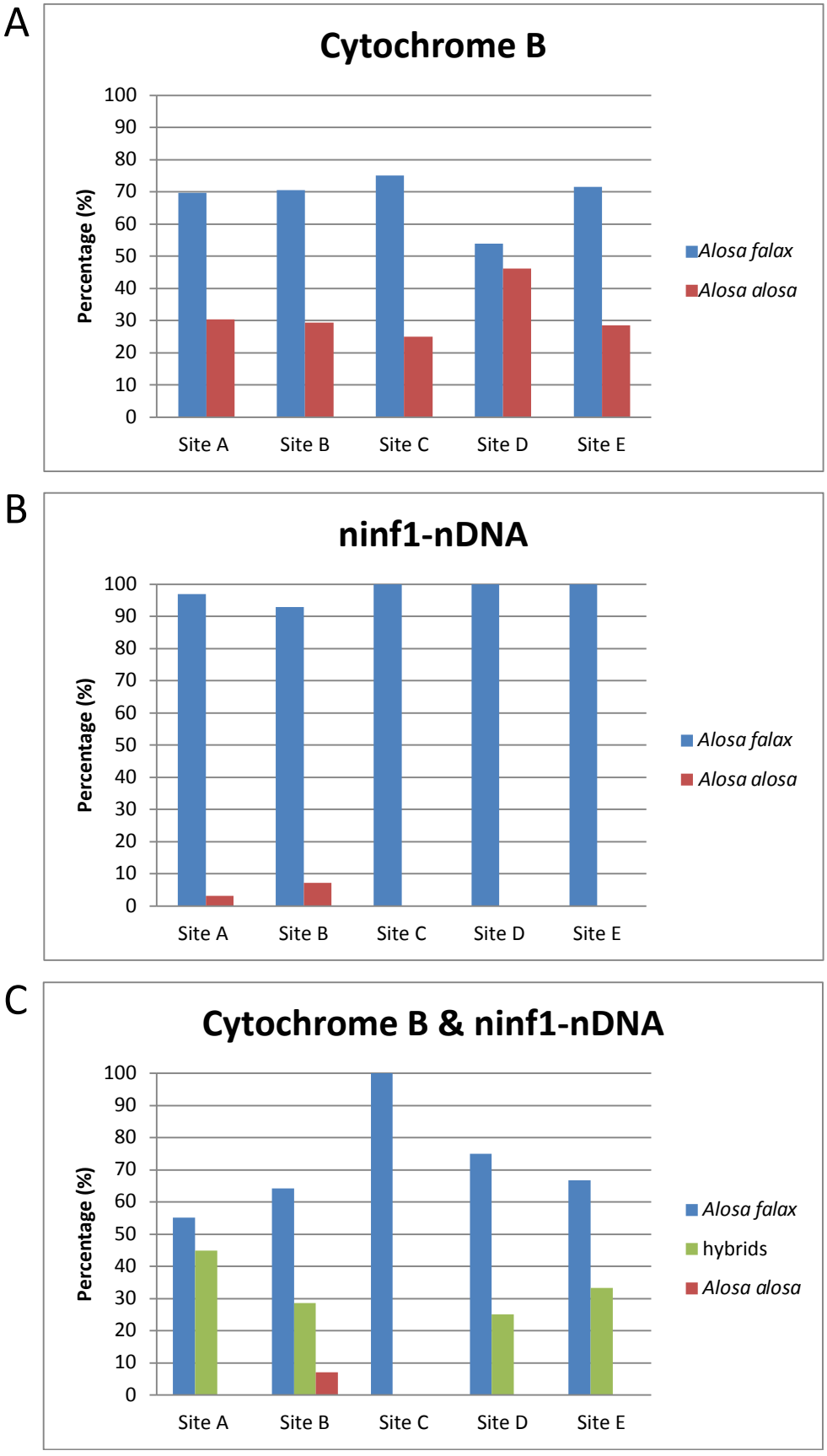

Figure 4.16 Percentage of *Alosa fallax*, *A. alosa* and hybrids between the two species using (A) only the Cytochrome B data; (B) only the ninf1-nDNA and (C) using both markers in combination. Note the small sample size for Site C (n=4).

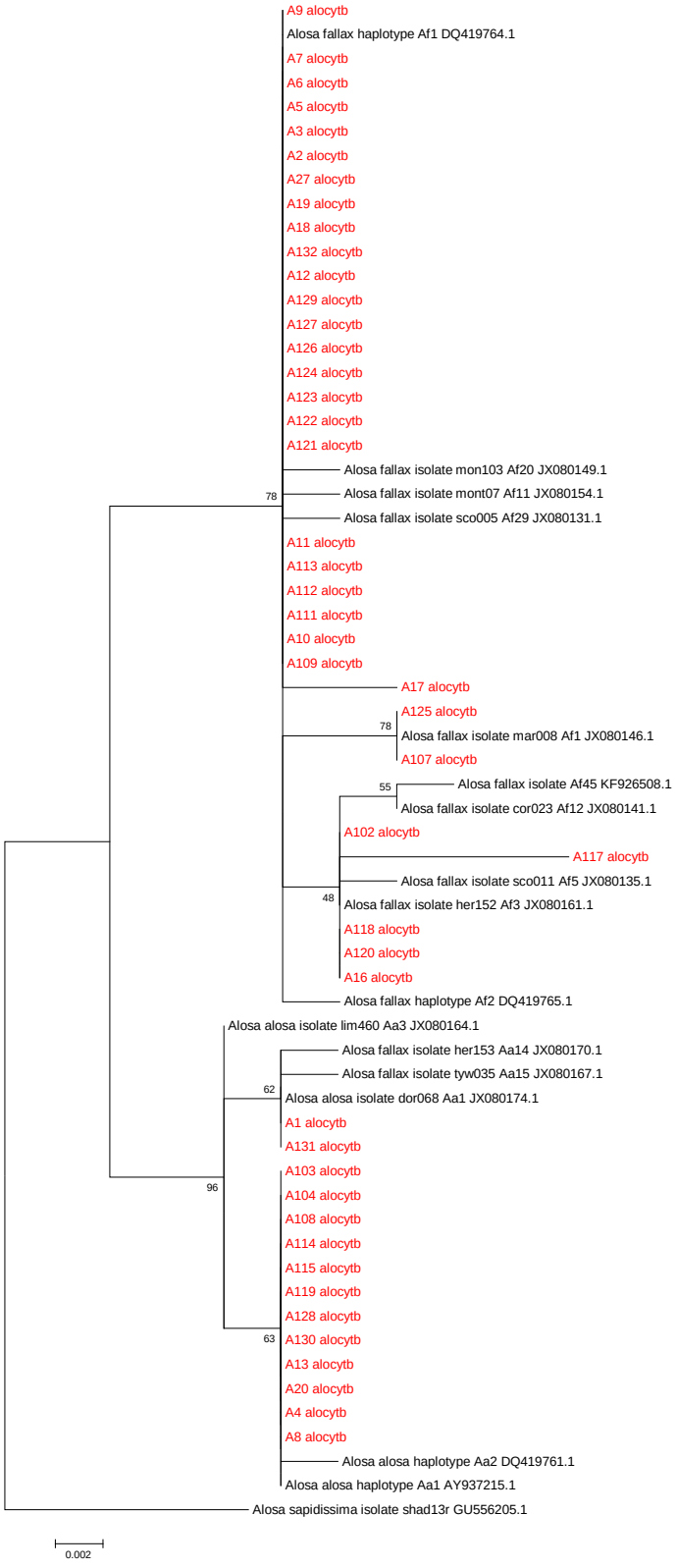

Figure 4.17 Site A: neighbour-joining consensus tree with 1000 bootstrap replicates drawn using MEGA (red text represents the phylogenetic position of individual eggs sampled). Genbank accession numbers are also provided within the phylogenetic tree. 46 haplotypes were identified; 32 as *Alosa fallax* and 12 as *A. alosa.*

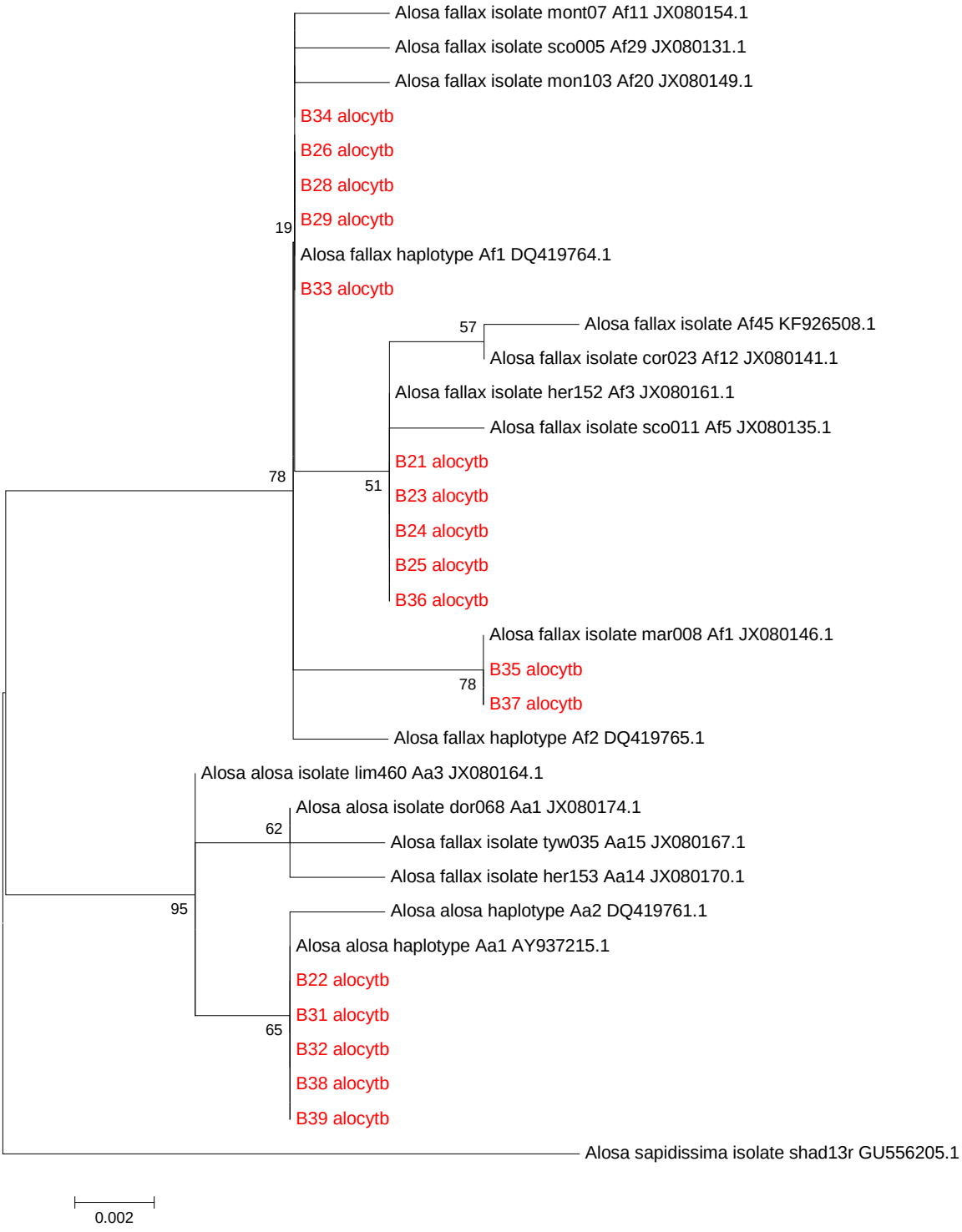

Figure 4.18 Site B: neighbour-joining consensus tree with 1000 bootstrap replicates drawn using MEGA (red text represents the phylogenetic position of individual eggs sampled). Genbank accession numbers are also provided within the phylogenetic tree. 17 haplotypes were identified; 12 as *Alosa fallax* and 5 as *A. alosa.*

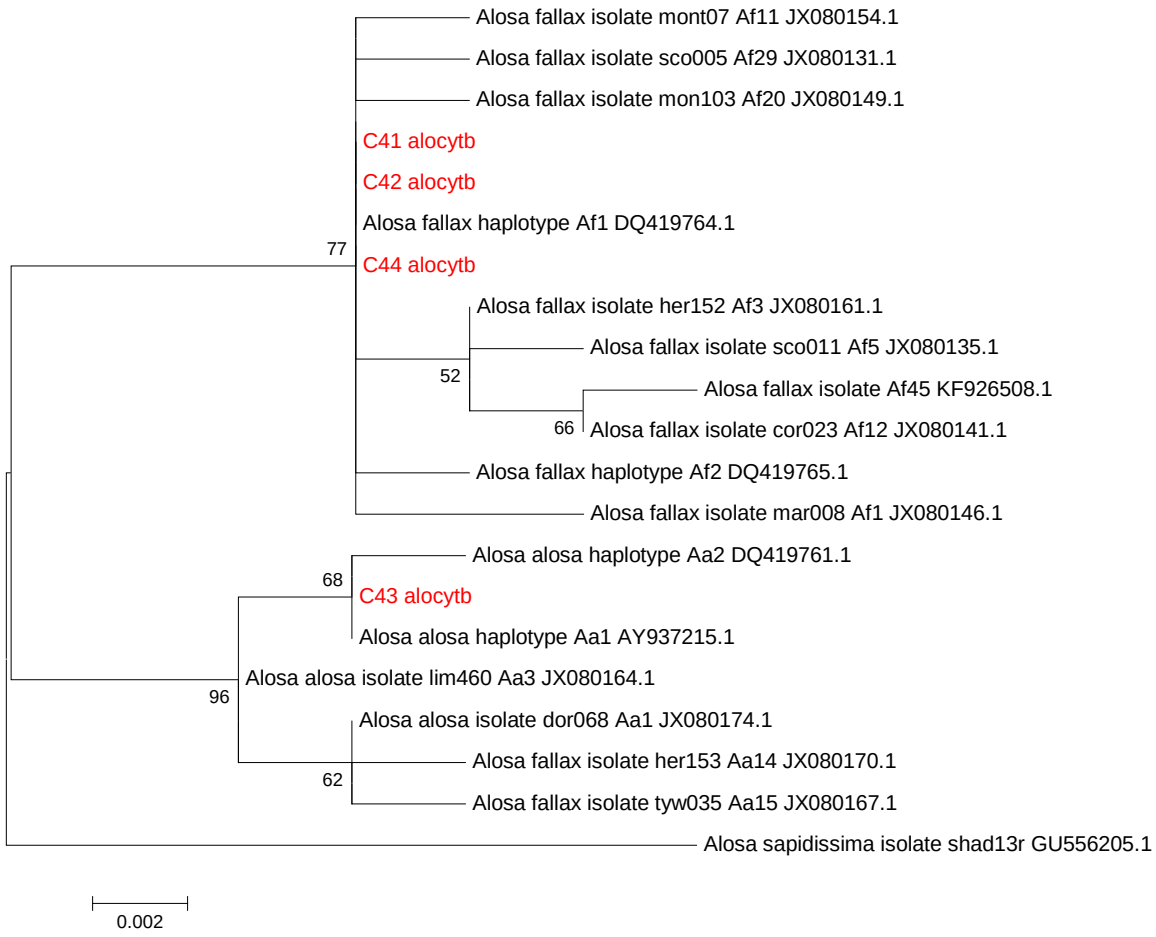

Figure 4.19 Site C: neighbour-joining consensus tree with 1000 bootstrap replicates drawn using MEGA (red text represents the phylogenetic position of individual eggs sampled). Genbank accession numbers are also provided within the phylogenetic tree. 4 haplotypes were identified; 3 as *Alosa fallax* and 1 as *A. alosa.*

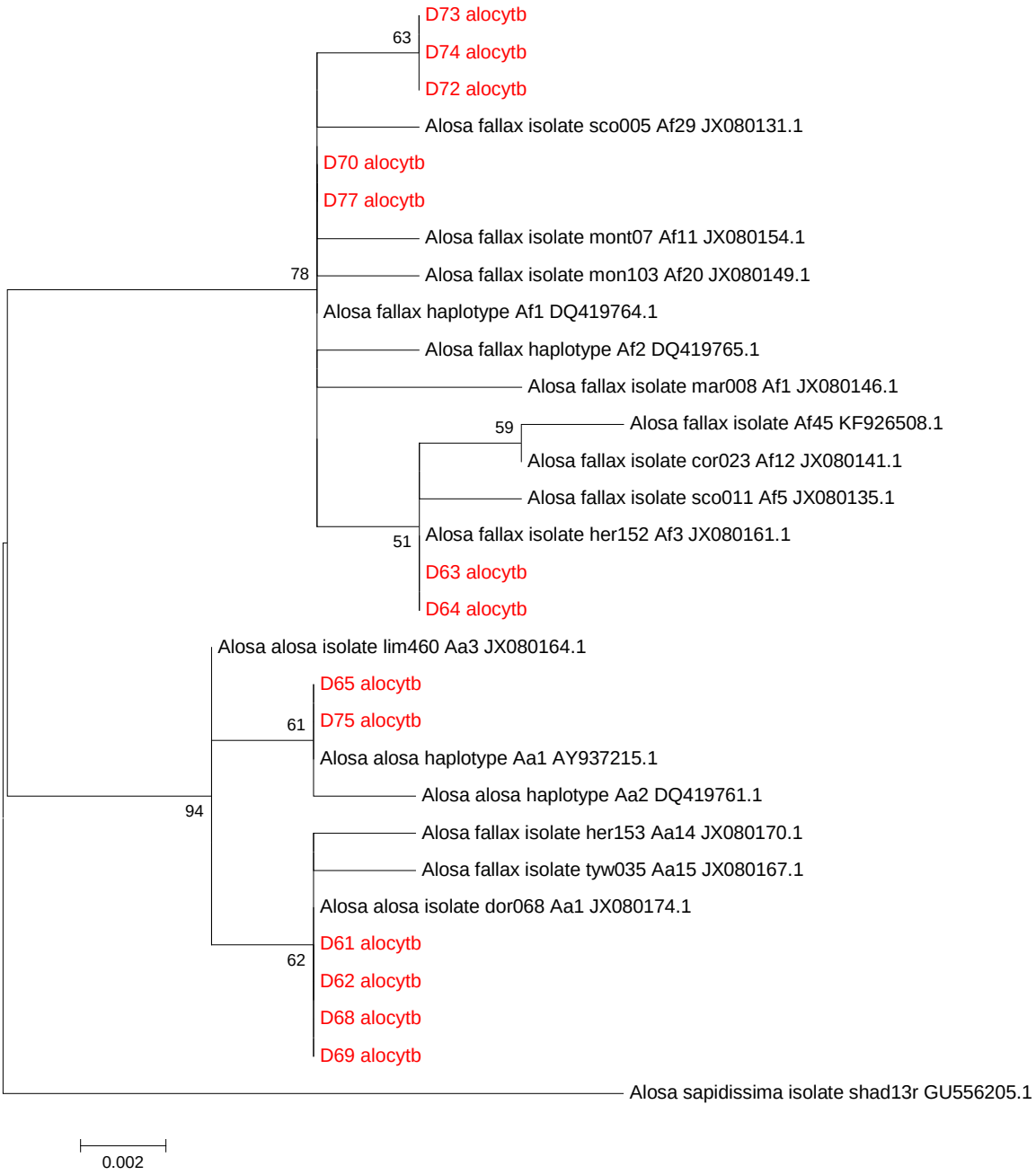

Figure 4.20 Site D: neighbour-joining consensus tree with 1000 bootstrap replicates drawn using MEGA (red text represents the phylogenetic position of individual eggs sampled). Genbank accession numbers are also provided within the phylogenetic tree. 13 haplotypes were identified; 7 as *Alosa fallax* and 6 as *A. alosa.*

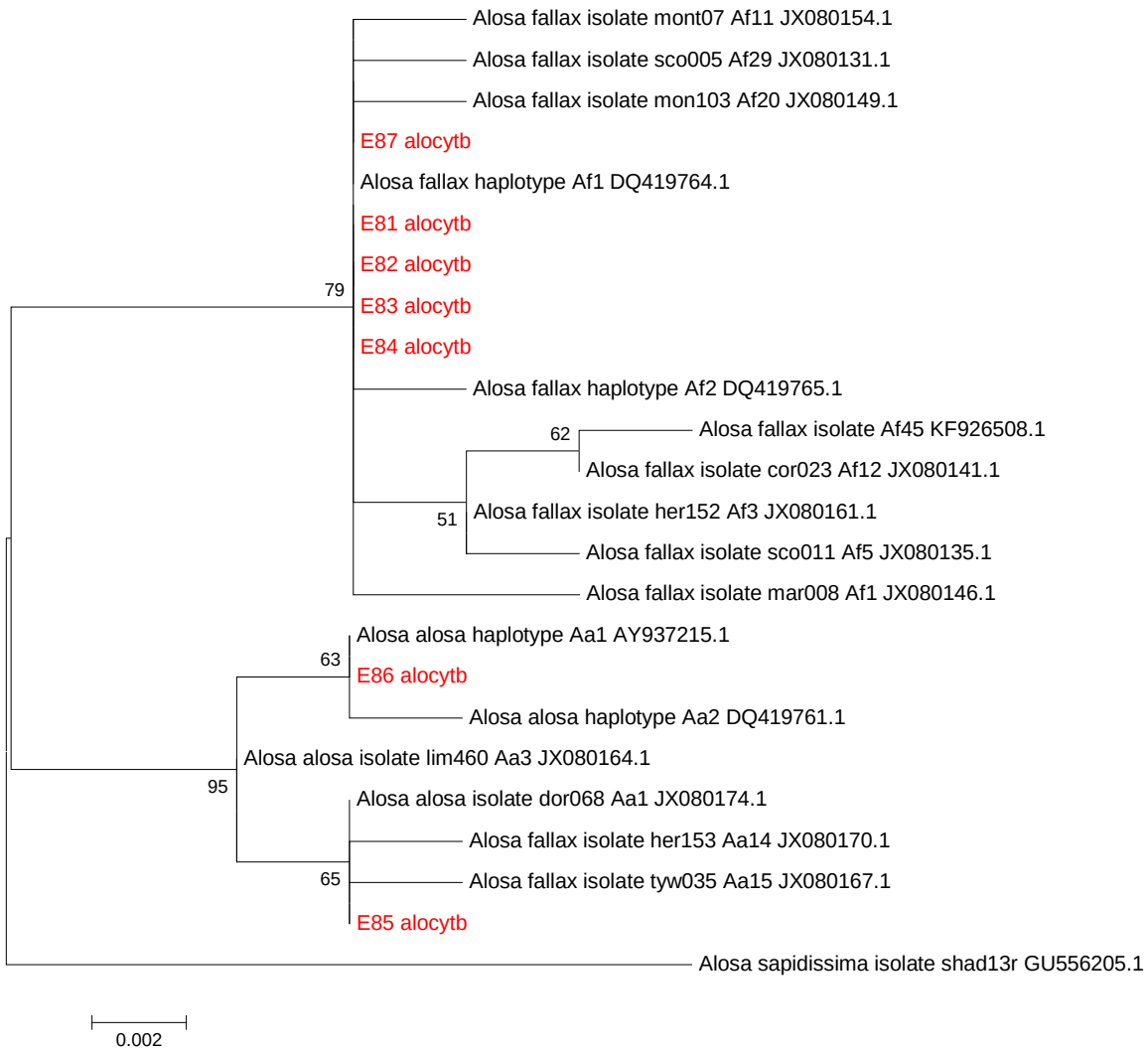

Figure 4.21 Site E: neighbour-joining consensus tree with 1000 bootstrap replicates drawn using MEGA (red text represents the phylogenetic position of individual eggs sampled). Genbank accession numbers are also provided within the phylogenetic tree. 7 haplotypes were identified; 5 as *Alosa fallax* and 2 as *A. alosa.*

5 DISCUSSION AND CONCLUSIONS

Despite the challenges associated with timing a single survey to correspond with spawning activity, the on-site presence of a BU PhD student provided continuous surveillance of the river which facilitated a rapid survey response (<18 hours) to the onset of shad spawning activity (as evidenced by the by-catch of three shad eggs in drift nets intended to sample barbel eggs). Over the course of two days in the field (28 May and 1 June), a total of 108 suspected shad eggs were collected of which molecular analysis confirmed all but one to belong to *Alosa* spp. While all eggs recorded on 28 May were observed to be in the earliest stages of development, the ontogenetic staging of eggs collected on 1 June varied from recently fertilised through to well-developed stages of embyogenesis, thus indicating that spawning had not been restricted to a single night but extended over a period of no less than five nights. On both survey days (28 May and 1 June), river flow was approximately 3.8cm^3/s, however, over the weekend separating these dates, flows increased to 4.81cm^3/s. Following sampling, a more significant spate was recorded with flows peaking at 9.07cm^3/s on 3 June 2015. Due to no further field sampling, it is not known what impact these elevated flows would have had on the survival of eggs, freshly hatched free embryos and the further spawning behaviour of adults.

Across all sites, a total of 430 non-shad eggs were recorded and returned to the river. While these were almost exclusively dominated by chub and minnow, a total of 12 barbel eggs (identified by their size and colour) were also recorded.

Comparisons of microhabitat between those sites containing and lacking shad eggs revealed no significant differences. This demonstrates that the general habitat characteristics identified as being suitable for spawning during the earlier walkover surveys (APEM, 2014) provided sound guidance on where egg sampling should be focused.

The homogeneity of habitat characteristics between Sites A-E and the observed presence of shad eggs at all sites confirms that several areas of quality functional spawning habitat currently exist throughout the 3.3 km reach of the River Teme between Powick Weir and the River Severn. The application of CPUE analysis which revealed considerably higher egg abundance immediately downstream of Powick Weir (Section A), therefore suggests that the weir is functioning as a migratory bottleneck, with aggregations of spawning shad becoming concentrated on the first available habitat downstream of this structure.

The main objective of the genetic analysis was to identify if *A. fallax* and/or *A. alosa* were spawning in the River Teme. Out of the 108 eggs collected, only one sample was not a shad and was identified as chub, *Squalius cephalus*, thus demonstrating that BUG scientists were more than 99% accurate in their visual identification of shad eggs in the field. Overall there were more individuals identified as *A. fallax* across sites, although rates of hybridisation were observed to be high. This is consistent with previous studies (e.g. Faria *et al*. 2012; Jolly *et al.,* 2012; Hardouin *et al*., 2013). Only a single individual was assigned as *A. alosa* (at Site C) using both genetic markers (Cytochrome B and ninf1-nDNA).

It is important to note that the efficacy of the genetic markers available to distinguish between the two shad species is not 100% accurate. For example, Alexandrino & Faria (2004) identified the two shad species using morphological characters and then compared the morphological identification to the Cytocrome B gene. Their morphological analysis did not identify any *A. alosa*, however, based on the Cytochrome B gene the genetic data identified 37.8% of their samples as "*A. alosa*" haplotype. This result can only be explained by present or past

hybridization between *A. alosa* female(s) and *A. fallax* male(s) (Alexandrino & Faria, 2004). The percentage occurrence of both species haplotypes are also similar to the ones identified during the present study.

In addition, the observed frequency difference of the nif1-nDNA allele between the two species has demonstrated high discriminative power compared to the Cytochrome B (Faria *et al*. 2011). However, ninf1-nDNA was not 100% accurate. Currently, by only using ninf1-nDNA and the mitochondrial Cytochrome B, the degree of hybridisation is likely to represent an underestimate as the only hybrids detectable were the animals displaying markers of both species, or the heterozygote for ninf1-nDNA. That said, hybridization can occur at every locus in the genome. Furthermore, as neither of these markers are 100% accurate, the identification of hybrids is even more uncertain. In order to advance reliable species identification from shad eggs, further investment (both time and financial) would need be applied to the development of new species specific markers.

5.1 Condition Assessment

5.1.1 Migratory access (barriers to migration)

Taking into account the attributes given in the favourable condition table (see Appendix II), the data collected during this study confirm that in 2015 Powick Weir functioned as a barrier to the upstream migration of adult shad. This is further evidenced by a study conducted by the Environment Agency during the 2014 migration season (B. Morris pers. com.). Here, drift nets were set both upstream and downstream of Powick Weir to intercept drifting eggs. The collection of ~800 eggs from the nets downstream of Powick and absence of eggs in the nets upstream of the weir suggests that Powick Weir currently represents a total barrier to shad migration.

While there are no physical barriers to interrupt or delay the downstream migration of juvenile shad within the River Teme downstream of Powick Weir, the impact of the flow regulation structure ‘High Lode’ on the River Severn remains unassessed.

5.1.2 River population (size of populations)

With respect to establishing a baseline of ‘river population’ size against which future trends can be monitored and assessed against the 50 percent reduction threshold (see Appendix II), the data collected in 2015 are not considered adequate for this purpose. Indeed, due to limited temporal coverage it is not known whether the eggs recorded between 28 May and 1 June are indicative of peak spawning activity. To establish a baseline against which to track inter-annual (and spatial) population trends, data collection would need to cover the entire spawning period.

The data collected during 2015, does however provide a robust baseline of spatial spawning activity of shad. This will facilitate important future temporal (inter-annual) comparison of spatial spawning activity and assist in evaluating the population response of *Alosa* spp. to planned catchment management actions (e.g. weir removal).

5.1.3 Condition attributes beyond the scope of the current project

While the abundance and distribution of shad eggs has potential to provide a proxy for inter-annual trends in the number of adult shad ascending the River Teme, the quantification of **population size (using returning adults)** lies beyond the scope of the current project. The assessment of **prey species** abundance also lies beyond the scope of the current project.

5.2 Threats

With the exception of the migratory blockage currently caused by Powick Weir, no other obvious threats were identified which were considered likely to impact on the population performance of shad. This said, eggs will be sensitive to mechanical damage during incubation and as such, caution should be applied by anyone wading in the river at this time of year (e.g. electric fishing surveys and recreational anglers). While water pollution poses a continuous risk to aquatic biota, no potential high risk point sources of pollution were observed within the survey reach; however, it must be noted that such lines of investigation lie beyond the scope of the current project.

5.3 Conclusions

In conclusion, the study has confirmed that:

- In 2015 the River Teme functioned as an important spawning ground for *Alosa* spp.
- BUG field scientists were able to distinguish the eggs of *Alosa* spp. from other coarse fishes with more than 99% accuracy. This confirms that were budgets are limited, cost-effective surveys to qualify and quantify shad spawning activity and habitat utilisation is achievable with a high degree of confidence.
- DNA analysis has confirmed the stock (at the time of the survey) to be dominated by *A. fallax*, with a high frequency of hybridisation with *A. alosa*.
- The observed concentration of spawning activity immediately downstream of Powick Weir indicates that this structure currently presents an impermeable barrier to shad migration.
- The planned removal of Powick Weir has important geomorphological implications for the quality and longitudinal distribution of shad spawning habitat and has considerable potential to impact (either positively or negatively) on the future performance of twaite shad as an Interest Feature of the Severn SAC.

6 RECOMMENDATIONS

With the planned removal of Powick Weir, it is important to stress that the current study has been limited to qualifying the functionality of spawning habitats with the lower River Teme. Having confirmed the extensive spawning activity of shad within this reach, it is now essential to establish a baseline understanding of total egg deposition and the value of early nursery habitat within the same reach. The latter should be achieved using a small mesh seine net within marginal low flow habitats during the early summer of 2016. The characterisation of functional nursery habitats and their temporal utilisation also represents an important component of this study.

The genetic health of the population can be further assessed using polymorphic genetic markers such as microsatellites that are available for these species. These markers can be used to track changes in population genetic health on a year to year basis and can be used to calculate the effective population size for shad which is important in assessing the sustainability of these populations.

The legacy of genetic introgression and the potential for further F1 hybridisation of *A. fallax* and *A. alosa* remains unclear. Genetic tools such as singly nucleotide polymorphisms could be developed in order to better discriminate between the two species and evaluate the degree of hybridisation. Such investigation may be usefully complemented with some focus applied to the adult stock. The ability to observe the adult population would facilitate the non-destructive collection of morphometric/meristic (squamation pattern) and genetic data.

Following the removal of Powick Weir, habitat walkover surveys should be undertaken to re-map the distribution of potentially important habitats. This information will be needed to redesign the spatial survey design needed to track the population response to the biological baselines now under development.

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8 APPENDIX I SEA LAMPREY

8.1 Introduction

Due to the continuous field presence of a BU PhD student, BUG was alerted in early July to sea lamprey (*Petromyzon marinus*) spawning activity within the same reaches surveyed for shad eggs. To capitalise on the opportunity to record a baseline of the spatial distribution of spawning and the numbers of adults in this section of the river, a project extension was agreed with the NE project manager to conduct a nest count survey.

8.2 Methods

Focusing on the same sections (A – E) surveyed for shad eggs (see Section 2), each section was accessed at the downstream end by the surveyor, with a buddy also based on top of the bank. The purpose of the buddy was to ensure the safety of the surveyor and to assist in spotting nests from an elevated position. Wading upstream in a 'zig-zag' from bank to bank, nests were counted and the dimensions of each nest depression either estimated (to the nearest 10cm) or measured using a metal rule (to the nearest cm). Polarised glasses were worn throughout the survey to reduce surface glare and assist with sub-surface visibility. Live video footage and still photographs of lamprey were captured using a pole mounted underwater video camera (GoPro 3).

Surveys of nests were conducted on 8 and 9 July 2015.

Figure AI1 Examining nests (red circles) in Site A, downstream of Powick Weir.

8.3 Results

Across all five sites (A – E) a total of 94 individual nests were recorded. Of this total 19 nests contained spent males, with spawning pairs (or in some cases two females and a single male) still actively spawning on five nests. Nests were typically circular 'crater' shaped, with excavated stones deposited variable distances downstream of the nest in accordance with local water velocities. Across all sections, nest dimensions ranged between 30 – 150 cm (mean = 78.85 ± 2.58) for nest width and 30 – 150 cm (mean = 80.42 ± 2.56) for nest length. A summary of total nest counts and numbers of adult lamprey observed during the survey is provided in Table AI1.

Table AI1 Number of nests recorded in each section and associated observation of adult lamprey

Date	Section	US Grid Ref	Total number of nests	Total number of adults counted	No. of nests with spawning activity
08/07/15	A	SO 83254 52363	16	5	1
08/07/15	B	SO 83521 52471	16	1	0
08/07/15	C	SO 83650 52375	23	11	2
09/07/15	D	SO83916 52382	18	4	1
09/07/15	E	SO 84634 51823	21	10	1
TOTALS			**94**	**31**	**5**

Figure AI2. Two females and a single male observed constructing a nest and spawning in Section C.

8.4 Summary

The nest count survey was successful in qualifying the functional importance of habitat downstream of Powick Weir for supporting the spawning activity of sea lamprey. The results collected during this survey also provide baseline quantification of the abundance of nests excavated during the 2015 spawning season. Due to the survey being conducted towards the end of the spawning season, coupled with the fact that adults had vacated the majority of nests, it is considered that the majority of spawning had already taken place and the numbers of nests counted represent a reliable measure of spawning activity for the 2015 spawning season.

Despite the known semi-permeability of Powick Weir to migrating sea lamprey, the distribution of nests observed during 2015 (i.e. no lamprey nests observed upstream of Powick during extensive surveys for barbel fry) confirms that in 2015, the vast majority of (if not all) spawning occurred downstream of this structure.

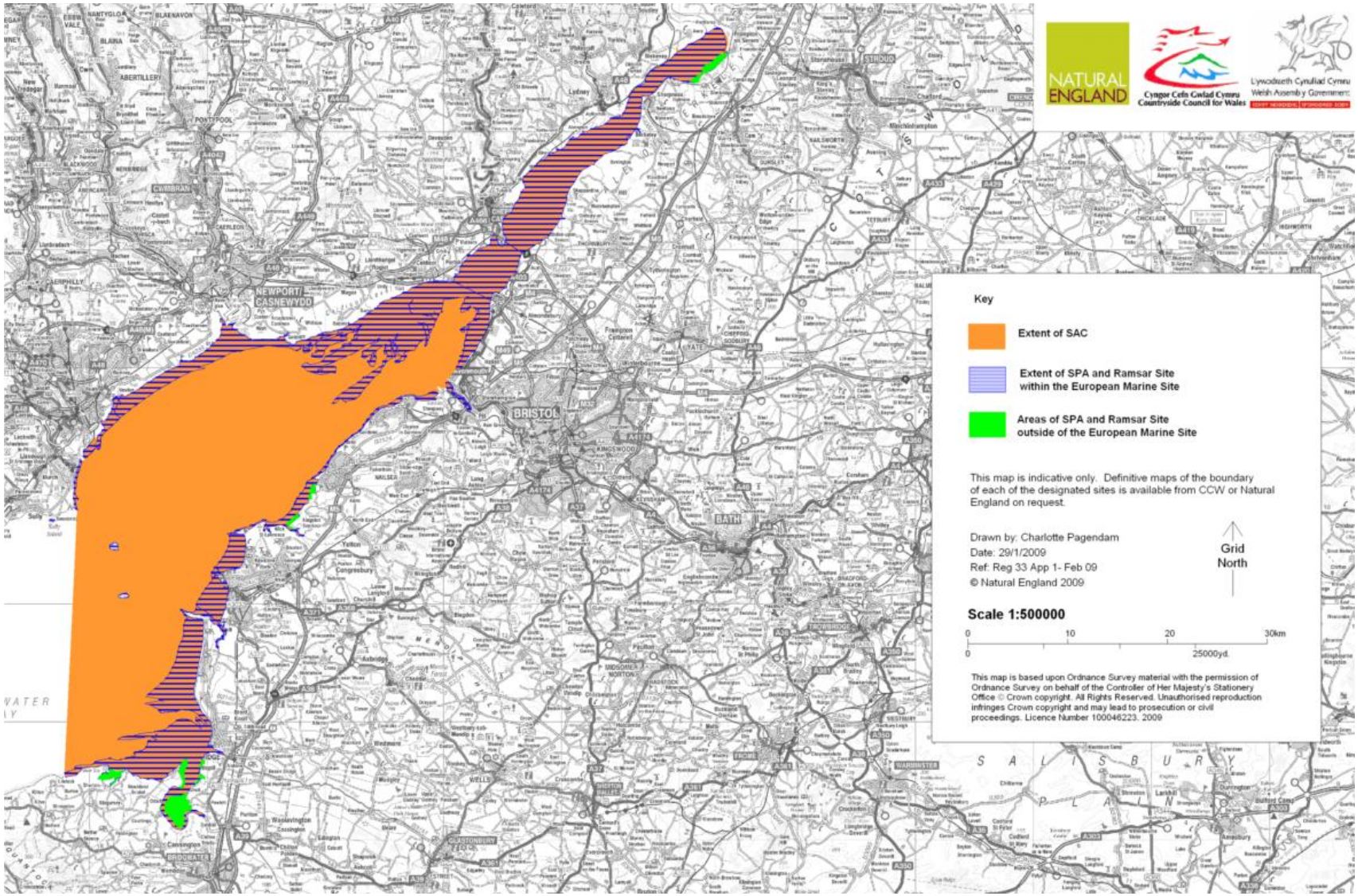
The distribution of nests throughout all areas of suitable habitat (as identified by APEM, 2014), highlights the current importance of habitats downstream of Powick for the recruitment of sea lamprey to the Severn Estuary EMS. Accordingly, any instream works which have the potential to impact on flow dynamics and habitat characteristics throughout this reach (e.g. the proposed removal of Powick Weir), should be critically assessed to ensure the spatial extent of functional habitat abundance provides overall benefits for all fish species, with particular focus on the migratory Annex II species such as lamprey and shads.

Due to the inevitable natural inter-annual variability in adult migrant numbers, it is strongly recommended that annual nest count surveys are continued to enhance the current baseline and to quantify changes mediated through future habitat improvement/restoration schemes.

9 APPENDIX II CONDITION TABLE FOR TWAITE SHAD

Favourable condition table for the "twaite shad" feature of the Severn Estuary SAC			
Attribute	**Measure**	**Target**	**Comment**
Migratory access (Barriers to migration)	Water quality measured regularly throughout the reporting cycle in the Bristol Channel, Severn Estuary, River Wye SAC, River Usk SAC and River Severn. Water flows measured regularly throughout the reporting cycle (frequency to be determined) in the River Wye SAC, River Usk SAC and River Severn Physical barriers Mapping and quantification of potential obstructions in relation to height, type and water depth below obstruction once during the reporting cycle.	Water quality is sufficient to support migratory passage. Levels (for temperature, salinity, turbidity, pH, and dissolved oxygen) should comply with targets established under the EA Review of Consents and the Water Framework Directive. Flows from the river into the estuary must be sufficient to allow migration. No artificial barriers significantly impairing, adults from reaching existing and historical spawning grounds, or juveniles from moving downstream.	Significant variation in these physico-chemical parameters may act as barriers to migration. For example, the timing, duration and consistency of their upstream migration are believed to be closely related to temperature changes . Peak migration usually coincides with river temperatures that remain above 10°C and continues until temperatures reach 18°C. Dissolved oxygen can also be significantly reduced in stretches receiving significant BOD inputs, or through the resuspension of organic rich sediments. Toxic contaminants may act as a barrier to migration. Environmental Quality Standards (EQSs) are set for dangerous substances as defined under the Dangerous Substances Directive or Government Policy for freshwater and marine environments .
Population size (returning adults)	Number of returning adults measured using fish counters on the Usk and Wye rivers during the migratory period.	No drop in the annual run size greater than would be expected from variations in natural mortality alone. Baseline is yet to be established - fish counter data may be able to provide a baseline in future years. Noble et al. (2007) provides historical information on returning adults for the River Wye	Fish counter technology is being developed to monitor adult shad but is not yet installed on the feeding rivers of the Severn Estuary. Fish counter technology should be further developed to monitor migrating adult shad.
River population (size of populations)	Seine netting for juveniles in the lower rivers and upper estuaries and monitoring of shad eggs by kick sampling	River population targets for the Usk and Wye must be met Baseline yet to be established. Noble et al. (2007) provides some information on juvenile densities.	Seine netting should occur in lower rivers and upper estuaries. Netting should be carried out in late summer early autumn (July-October). For each river, juvenile densities should exceed a specified minimum target at least two years in six. The extent of spawning should be monitored by kick sampling for eggs at a proportion of known spawning sites. A reduction in the spawning distribution of more than 50 % compared with the baseline will indicate an adverse change. Kick sampling should occur during May and June.
Prey species (abundance of prey)	The abundance of key prey species measured by EA in their routine monitoring of the rivers and estuary	No significant reduction in abundance of key prey species against an established baseline Baseline is yet to be established through fish surveys in estuary and rivers	Twaite shad require a variety of invertebrates including crustacean, mysids and copepods, small fish and fish eggs particularly in that section of the estuary where saline and freshwaters meet.

10 APPENDIX III MAP SHOWING THE EXTENT AND RELATIONSHIP OF THE SEVERN ESTUARY SAC, SPA AND RAMSAR SITE



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