Cyfoeth Naturiol Cymru
Natural Resources Wales

Afon Tywi SAC shad egg surveys 2013 & 2014.

Garrett HM, Thomas Rh, & Hatton-Ellis TW.

Report No. NRW Evidence Report No. 48

Date: February 2015

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Our purpose is to ensure that the natural resources of Wales are sustainably maintained, used and enhanced, now and in the future.

Published by:
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Report series:	Natural Resources Wales Evidence Report.
Report number:	NRW Evidence Report No. 48
Publication date:	February 2015
Contract number:	N/A
Contractor:	N/A
Contract Manager:	N/A
Title:	**Afon Tywi SAC shad egg surveys 2013 & 2014.**
Author(s):	Garrett HM, Thomas Rh, & Hatton-Ellis TW.
Technical Editor:	[Enter initial and surname here.]
Peer Reviewer(s)	[Enter name and surname here]
Approved By:	[Enter initial and surname here]
Series editor(s):	N/A.
Restrictions:	None.

Distribution List (core)

Harding, Alex
Higgins, Jenny
Gjerlov, Charlotte
Hyatt, Paul
Thornton, Leila
Carpenter, Gideon
Hatton-Ellis, Tristan
Thomas, Rhian.

Distribution List (others)

NRW Library (Paper copy)

Recommended citation for this volume:

Garrett, HM, Thomas, Rh, & Hatton-Ellis, TW. 2014. Afon Tywi SAC shad egg surveys 2013 & 2014. NRW Evidence report no 48 37pp, Natural Resources Wales, Bangor.

Cyfoeth Naturiol Cymru
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Mae herlod a gwangod yn ddwy rywogaeth o bysgod sydd dan fygythiad rhyngwladol ac mae'r ddau yn silio ar dair afon yn Ne Cymru, yn cynnwys Afon Tywi. Rhwng Mai a dechrau Gorffennaf yn 2013 a 2014, bu staff Cyfoeth Naturiol Cymru (CNC) yn profi am wyau gwangod o nifer o safleoedd ar hyd yr afon drwy ddefnyddio methodoleg cic samplo yn unol â phrotocol safonol y DU.

Mae'r adroddiad interim hwn yn dadansoddi data 2013 a 2014 fel cyfraniad tuag at yr asesiad cyflwr terfynol yn erbyn yr amcanion cadwraeth ar gyfer poblogaethau gwangod Afon Tywi. Caiff yr asesiad cyflwr terfynol ei gwblhau tuag at ddiwedd trydydd cylch monitro chwe blynedd y Gyfarwyddeb Cynefinoedd (2013 - 2018) a phan fydd mwy o ddata ar wyau gwangod wedi'i gasglu.

- **2013:**

Roedd wyau gwangod yn bresennol yn saith o 13 safle'r arolwg (54%). Ymwelwyd â phob un o'r 13 safle arolwg unwaith. Arolygwyd yr 13 safle rhwng 11 Mehefin ac 1 Gorffennaf dros dri diwrnod gan wyth arolygwr.

Roedd maint y sampl wyau'n amrywio o 1 - 100+ o wyau. Terfyn y silio i lawr yr afon oedd y terfyn Llanw (safle 16) a'r terfyn i fyny'r afon oedd Maenordeilo (safle 11). Cofnodwyd cyfanswm o 177+ o wyau. Cadarnhawyd mai wyau gwangod oeddynt drwy ddadansoddiad genetig yn nau o'r tri safle samplu a ddewiswyd.

- **2014:**

Cofnodwyd wyau gwangod yn wyth o'r 14 safle arolygu (57%). Ymwelwyd â saith o'r safleoedd samplu ddwy waith (54%) ac ymwelwyd â phedwar safle dair gwaith (29%). Arolygwyd yr 14 safle rhwng 16 Mai ac 11 Mehefin dros 6 diwrnod gan 13 o arolygwyr.

Roedd maint y sampl wyau yn amrywio o 1 - 370 o wyau. Terfyn y silio i lawr yr afon oedd y terfyn Llanw (16) a'r terfyn i fyny'r afon oedd Llandeilo (10). Cofnodwyd cyfanswm o 1218 o wyau. Cadarnhawyd fod 99% o'r wyau a anfonwyd i'w dadansoddi yn rhywogaethau gwangen (dim ond 2 wy o sampl o 162 oed heb fod yn rhai gwangod).

Canfuwyd un wy heb fod yn un gwangen yn safle Penddaulwyn (safle 2) ond cadarnhawyd presenoldeb gwangod (*Alosa fallax*) gan yr wyau oedd ar ôl yn y sampl. Cadarnhawyd gwangod hefyd mewn dau safle: safle 4a Cynefin 8 a safle 5 pont Nantgaredig.

Cadarnhawyd rhywogaeth math *Alosa* yn safleoedd eraill y terfyn Llanw (safle 16), Glantowylan (safle 3) a chydlifiad y Cothi (safle 6). Yn wahanol i ddadansoddiad 2013, yn 2014 ni luniwyd y dadansoddiad i ganfod cymysgrywiau ond dim ond i gadarnhau presenoldeb rhywogaethau gwangod.

Cafodd samplau o 2013 a 2104 eu sicrhau o ran ansawdd hefyd drwy ddefnyddio technegau genetig i wneud yn siŵr mai gwangod oeddynt ac i benderfynu pa rywogaethau oedd yn bresennol; cyflwynir y dystiolaeth hon mewn adroddiadau ar wahân (Hardouin *et al*. 2013; Stone, 2014). Cyn 2013 ni wnaed dadansoddiad genetig ar wyau gwangod Afon Tywi ac mae'r dechneg ddadansoddi ychwanegol hon wedi galluogi gwerthuso i ba raddau y gellir cael ffydd yn yr adnabyddiaeth yn y maes. Roedd lefel yr adnabyddiaeth yn dda yn 2013 (85%) ac yn ardderchog yn 2014 (99%).

Mae graddfa'r silio ar Afon Tywi yn 2013 a 2014 yn dilyn patrwm tebyg i'r dosbarthiad a ganfuwyd yn ail rownd monitro'r Gyfarwyddeb Cynefinoedd (2007 - 2012).

Executive summary

Allis and twaite shad are two internationally threatened fish species which spawn on three rivers in South Wales, including the Afon Tywi. Between May and early July in 2013 and 2014, Natural Resources Wales (NRW) staff sampled for shad eggs from numerous sites along the river using a kick sampling methodology according to a UK standard protocol.

This interim report analyses the 2013 and 2014 data as a contribution towards the final condition assessment against the conservation objectives for the Afon Tywi populations of shads. The final condition assessment will be completed near the end of the third Habitats Directive six year monitoring cycle (2013 – 2018) and when more shad egg data has been collected.

- **2013:**

Shad eggs were present at seven of the 13 survey sites (54%). Each of the 13 survey sites were visited once. The 13 sites were surveyed between 11 June and 1 July over three days by eight surveyors.

The egg sample size ranged from 1 – 100+ eggs. The downstream limit of spawning was Tidal limit (site 16) and the upstream limit was Manordeilo (site 11). A total of 177+ eggs were recorded. The identification of shad eggs were confirmed through genetic analysis at two of the three selected sample sites.

- **2014:**

Shad eggs were recorded at eight of the 14 survey sites (57%). Seven of the sample sites were visited twice (54%) and four sites were visited three times (29%). The 14 sites were surveyed between 16th May and 11 Junes over 6 days by 13 surveyors.

The egg sample size ranged from 1 – 370 eggs. The downstream limit of spawning was Tidal limit (16) and the upstream limit was Llandeilo (10). A total of 1218 eggs were recorded. 99% of the eggs sent for analysis were confirmed as a shad species (only 2 eggs from a sample of 162 were not shad).

One non-shad egg was found in the Penddaulwyn site (site 2) but the presence of twaite shad (*Alosa fallax*) was confirmed by the remaining eggs in the sample. Twaite shad was also confirmed at two sites: site 4a Habitat 8 and site 5 Nantgaredig bridge.

An *Alosa* type species was confirmed at the remaining sites of Tidal Limit (site 16), Glantowylan (site 3) and Cothi confluence (site 6). Unlike the 2013 analysis, in 2014 the analysis was not designed to identify hybrids but only to confirm the presence of shad species.

Samples from 2013 and 2014 were also quality assured using genetic techniques to ensure that they were shad and to determine which species was present; this evidence is

presented in separate reports (Hardouin *et al*. 2013; Stone, 2014). Prior to 2013 no genetic analysis had been conducted on Afon Tywi shad eggs and this additional analysis technique has allowed the degree of confidence in field identification to be evaluated. The level of identification was good in 2013 (85%) and excellent in 2014 (99%).

The extent of spawning on the Afon Tywi in 2013 and 2014 follows a similar pattern to the distribution identified in the second Habitats Directive monitoring round (2007 – 2012).

1.0 Introduction

1.1 Background

Two species of shad occur in Britain; allis (*Alosa alosa*) and twaite (*Alosa fallax*). Shads are anadromous members of the family *Clupeidae*, which also includes herrings. Although allis shad are the larger of the two species, they can only reliably be differentiated by counting gill rakers. Both *A. alosa* and *A. fallax* are listed in Annexes II and V of the EC Habitats Directive (EC Directive on the Conservation of Natural Habitats and of Wild Flora and Fauna (92/43/EEC)), Appendix III of the Bern Convention, Schedule V of the UK Wildlife and Countryside Act (1981) (as amended) and as priority species in the UK and Wales Biodiversity Action Plans. The Usk, Wye and Tywi rivers in South Wales hold the main UK populations of shad (Aprahamian *et al.* 1999; JNCC, 2012). Both species are found in the Afon Tywi, Carmarthenshire and the main river corridor of the Tywi has been designated as a Special Area of Conservation (SAC) for shads.

The Joint Nature Conservation Committee (JNCC) published generic guidance on monitoring freshwater fauna' (JNCC, 2005) outlining the attributes (mandatory and discretionary) and targets for monitoring freshwater fish. These include targets for shad population, flow, water quality and river morphology. At the time of writing, the CSM freshwater fauna guidance is being reviewed by the country agencies and the attribute targets may be amended.

The distribution of spawning shad within each river catchment is assessed using a kick sampling technique for eggs and is conducted in the spring. This report is a brief summary of the 2013 and 2014 egg survey data. This interim report covers only the 'spawning distribution' within the population attribute. A full condition assessment of the attribute will be made nearer the end of the third reporting cycle (2013 – 2018) when more egg survey data is available.

Molecular techniques were used for quality assurance and confirming the identification of the egg samples as belonging to the *Alosa* family (Hardouin*, et al.,* 2013; Stone, 2014).

1.2 Conservation status of shad species

Mature shad migrate into fresh water during late spring (April to June), thus giving the shad the name of 'May Fish'. Shad eggs are clear, non-adhesive, semi-buoyant and 1.5–4.5 mm in diameter. They tend to drift downstream, with most falling to the river bed and remaining there in crevices until the fry hatch out four to eight days later. A more detailed account of shad ecology is given in Maitland & Hatton-Ellis (2003).

Figure 1. The Afon Tywi SAC boundary (shown in dark blue).

1.3 Condition assessment & monitoring of shad species

NRW is responsible for monitoring the condition of shad populations within SACs and to ensure that they reach and are maintained in favourable condition. The JNCC have published generic guidance on 'Common Standards Monitoring Guidance for Freshwater Fauna' (JNCC, 2005) outlining the attributes (mandatory and discretionary) and targets for monitoring freshwater fish. These include targets for shad population, flow, water quality and river morphology. Assessments are made on a 6 year cycle. We are currently in the third monitoring cycle (2013-2018). Both allis and twaite shad have been reported as unfavourable overall in the first (2001-2006) and second (2007-2012) reporting cycles.

Although the JNCC guidance gives details for hydroacoustic counters to assess the 'adult run size' target this method was not successful in monitoring adult shads (Gregory, 2012). In addition the seine netting suggested for monitoring juvenile densities has proved to be uneconomic (Noble *et al.* 2007). Hence these two targets have not been assessed in this second monitoring cycle and this report covers the 'spawning distribution' target within the population attribute only.

The CSM guidance for the population attribute calls for 'no decline in spawning distribution', which is assessed by kick sampling during May and June. For further details of using CSM targets to undertake shads' condition assessment on the Afon Tywi SAC refer to Garrett *et al.* (2013).

CSM freshwater fauna guidance is currently under review and a revised version is in preparation, but in the meantime, the shad egg data collected in 2013 and 2014 will be assessed against the existing targets (JNCC, 2005). It is assumed that neither the spawning target nor protocol will not alter significantly.

1.4 Quality assurance of egg samples 2013

This investigation was conducted on behalf of NRW by the University of Bournemouth and it focussed on the collection of eggs from 12 sampling sites in 2013 from the three Welsh rivers where shad spawn (Wye, Usk and Tywi). The objectives were to determine:

- Whether the collected eggs belonged to *Alosa spp.*
- The proportion of *A. fallax* and *A. alosa* using three different genetic markers.

Full details are shown in Hardouin *et al.* (2013), but the significant results will also be discussed in conjunction with the spawning distribution analysis for 2013 in this report (section 4.0 Results).

1.5 Quality assurance of egg samples 2014

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) analysed genetic material from shad eggs collected during 2014 on the Afon Tywi. The objective was to determine:

- Whether the collected eggs belonged to *Alosa species* (Stone, 2014).

Full details are shown in Stone (2014), but the significant results will also be discussed in conjunction with the spawning distribution analysis for 2014 in this report (section 4.0 Results).

2.0 Objectives

The aim of this interim report is to contribute to the assessment the condition of the shad population in the Afon Tywi SAC based on 2013 and 2014 egg surveys. The objectives of this study are to;

- Conduct a shad egg survey on the Afon Tywi in 2013 and 2014.
- Analyse and present the results for spawning distribution of the population attribute only.
- Use the data obtained from the genetic analysis of shad egg tissue to give additional context to the above analysis.

3.0 Sampling methodology

3.1 Spawning distribution sampling methodology

In general, the sampling method described in Hillman *et al*. (2003) was followed but with modifications; the Hillman method is quantitative which involves counting shad eggs in 25 kick samples per site. The usefulness of this data, which is time consuming to collect in the field, is limited. For example, it is not possible to estimate the number of shad which have spawned from the number of eggs found. In 2012, the Hillman methodology was modified to a minimum of 10 kick samples were undertaken at each site with the number of eggs found in each kick recorded. If 20 or more shad eggs were recorded after 10 kicks the survey was terminated, however, if fewer than 20 shad eggs were recorded, kick sampling continued either until 20 eggs were found or 25 kick samples had been completed. Copies of the CCW shad egg monitoring protocol were given to each survey team (Thomas, 2012b).This modified methodology has already been successfully adopted on the Rivers Usk and Wye (Thomas & Dyson, 2010; Thomas & Dyson, 2011). Kick sampling should be undertaken over an area judged to be potentially suitable spawning habitat; these were fast-flowing, shallow, areas of unconsolidated gravel/pebble and/or cobble substrate (Caswell & Aprahamian, 2001; Maitland & Hatton-Ellis, 2003). A standard macroinvertebrate hand net (250µm) was used to collect material dislodged by kicking (or ‘shuffling’) upstream of the net for approximately 15 seconds. The net was checked for the presence of shad eggs after each kicking interval and any detritus or channel substrate removed before kicking was resumed. The number of shad eggs were counted after each kick sample and recorded on the field sheet. Sampling started at the downstream end of the potential spawning site to prevent the possible re-recording of eggs dislodged in previous kicks. If possible, sites where no eggs were found were re-visited at a later date

in the spawning season, however, this was dependant on weather conditions and availability of the survey team.

In 2014 the majority of sample sites were revisited to obtain genetic samples as the required vials were not available in time for the start of the survey or to monitor the duration of spawning.

In 2013 the 3 survey days were spread between 11 June and 1 July. In 2014 the seven survey days were extended between 16th May and 11 June (Table 3).

The established survey sites are spread throughout the SAC boundary and were based on locations where eggs had previously been recorded. Site names and numbers were originally set up by EAW and subsequently adopted by CCW and NRW. In line with the methodology used on the River Usk, any sites within 500m of the original EA site were subsequently given the same name & number (Thomas & Dyson, 2010). In 2012 one additional survey site (site code 16) was set up by CCW at Tidal Limit near Abergwili (SN448204) (Table 1 & Figure 2).

3.2 Field collection methodology for quality assurance of egg samples

Egg samples for molecular analysis (Table 1) were carefully transferred using forceps to sample pots pre-loaded with either buffer solution (2013) or 95% ethanol (2014). The samples were kept cool initially in a cool box and refrigerated overnight before being delivered by courier to the laboratory the following day. In 2013, three samples were sent for molecular analysis by Bournemouth University (Hardouin *et al,* 2013). In 2014, nine samples were determined by CEFAS (Stone, 2014). Table 2 gives the locations where eggs for molecular determination were sampled.

NGR	Site Name	Site Code	Sampling Date
SN4930020300	Nantgaredig Bridge	T1	11/06/2013
SN4670021400	White Mill	T2	11/06/2013
SN5000022000	Cothi Confluence	T3	13/06/2013
SN4623120402	Penddaulwyn	2 (& 2a)	20 & 28/05/2014
SN4690021000	Glantowylan	3 (& 2b)	20, 28 & 29/05/2014
SN4714621161	Habitat 8	4a	20/05/2014
SN4930020300	Nantgaredig Bridge	5	21/05/2014
SN5003820096	Cothie confluence	6	28/05/2014
SN6265321991	Llandeilo Bridge	10	21/05 & 11/06/2014
SN4480020400	Tidal limit	16	16, 20 & 28/05/2014

Table 1. Locations of sample sites where eggs were selected for genetic analysis

3.3 Sample site locations and naming convention

Three new locations were sampled in 2014; the surveyors recorded one new location as "2a" but it was within 500m of site 2 so according to the agreed protocol, .(Thomas & Dyson, 2010) the results were subsumed into site 2. Similarly the surveyors recorded another new site as 2b but it was within 500m of site 3 and so the results have also been combined as site 3. The third new location 4a is upstream of Glantowylan by more than 500m so it was treated as a new sample site, however, there was no nearby identifiable location so the surveyors didn't give this site a geographical name. this site is referred to as "Habitat 8" in this report. The new locations were sampled to help inform the impact assessment of Nantgaredig abstraction.

In this report site numbers will follow the site name in brackets e.g. Nantgaredig bridge (site 5). This will help the reader to put the location of the site into the context of the catchment. i.e. a low site number indicates that the sample site is in the lower part of the catchment (with the exception of Tidal limit (site 16).

3.4 Protected species legislation

The surveyors undertook this survey using licence number 38048:CCW:SA:2012.

4.0 Results

4.1 NRW shad egg survey results 2013 & 2014

Site No.	Site name	NGR	Date surveyed 2013	Shad present or absent	Date surveyed 2014	Shad present or absent
1	Llangunnor	SN4240020300	Not surveyed	Not surveyed	Not surveyed	Not surveyed
2 (& 2a)	Penddaulwyn	SN4623120402	Not surveyed	Not surveyed	20-May	**Present**
					28-May	**Present**
					29-May	**Present**
3 (& 2b)	Glantowylan	SN4690021000	13-Jun	Absent	20-May	**Present**
					28-May	**Present**
					29-May	**Present**
4	White Mill	SN4674021494	11-Jun	**Present**	20-May	**Present**
					28-May	**Present**
4a	Habitat 8	SN4714621161	Not surveyed	Not surveyed	20-May	**Present**
5	Nantgaredig Bridge	SN4930020300	11-Jun	**Present?**[1]	16-May	**Present**
					21-May	**Present**
					28-May	**Present**
					11-Jun	**Present**
6	Cothie confluence	SN5003820096	13-Jun	**Present**	21-May	**Present?**[2]
					28-May	**Present**
6a	Cothie Bridge*	SN5050421250	13-Jun	Absent	21-May	Absent
					28-May	Absent
7	Llanegwad	SN5150021200	13-Jun	Absent	Not surveyed	Not surveyed
8	Dryslwyn	SN5503120345	13-Jun	Absent	21-May	Absent
					28-May	Absent
9	Cilsan Bridge	SN5916821463	13-Jun	**Present**	21-May	Absent
					29-May	Absent
10	Llandeilo Bridge	SN6265321991	11-Jun	**Present**	21-May	**Present**
					29-May	Absent
					11-Jun	**Present?**[2]

Table 2. Shad egg survey results 2013 & 2014.

[1] Sub-sample of eggs identified as minnow, see genetic analysis discussion in section 4.4.
[2] Poor DNA yield so unable to confirm species as shad, see genetic analysis discussion in section 4.4.
* Sample site outside of the SAC boundary at sites 6a, 14 & 15.

Site No.	Site name	NGR	Date surveyed 2013	Shad present or absent	Date surveyed 2014	Shad present or absent
11	Manordeilo	SN6875926802	11-Jun	**Present**	21-May	Absent
					11-Jun	Absent
12	Llanwrda	SN7180931006	Not surveyed	Not surveyed	21-May	Absent
					11-Jun	Absent
13	Lwynjack	SN7548833138	01-Jul	Absent	21-May	Absent
14	Llandovery*	SN7613435746	Not surveyed	Not surveyed	Not surveyed	Not surveyed
15	Dolauhirion*	SN7620036100	01-Jul	Absent	Not surveyed	Not surveyed
16	Tidal limit	SN4480020400	13-Jun	**Present**	16-May	**Present**
					20-May	**Present**
					28-May	**Present**

Table 3. Shad egg survey results for 2013 & 2014.

Details of egg counts are shown in Appendix 2.

To summarise:

- **2013:** Shad eggs were present at seven of the 13 survey sites (54%). Each of the 13 survey sites were visited once.
- **2014:** Shad eggs were recorded at eight of the 14 survey sites (57%). 7 of the sample sites were visited twice (54%) and four sites were visited three times (29%).

4.2 Frequency of sampling at sites in 2014.

The majority of sites were sampled more than once (table 4).

Sample site no.	Sample site name	*No. site visits in 2014*
01 07 14 15	Llangunnor Llanegwad Llandovery Dolauhirion	*0*
4a 13	Habitat 8 Llwynjack	*1*
04 06 6a 08 09 11 12	White Mill Cothie confluence Cothie bridge Dryslwyn Cilsan bridge Manordeilo Llanwrda	*2*
02 03 10 16	Penddualwyn Glantowylan Llandeilo bridge Tidal Limit	*3*
05	*Nantgaredig bridge*	*4*

Table 3. Frequency of visits to each survey site in 2014.

In previous years most survey sites were visited once. The repeat visits in 2014 did not substantially add to the assessment because the presence or absence of eggs at a sample site remained consistent with the result of the first visit i.e. if eggs were present during the first site visit then they were also found during any subsequent ones and *vice versa.*

4.3 Presence and absence of eggs at sample sites 2013 and 2014.

4.3.1 Sites with negative results

In 2013 and 2014 no eggs were found at three sample sites: Cothie bridge (site 6a) (outside of SAC boundary), Dryslwyn (site 8) and Llwynjack (site13).

In addition to the above three negative sites, no eggs were found at two additional sample sites, Llanegwad (site 12) and Dolauhirion (site 15) but both of these sites were only surveyed in 2013.

The two most notable negative sites are those in the upper Tywi catchment at Llwynjack (site 13) and Dolauhirion (site 15) which are the most upstream sample sites within the SAC boundary (Figure 2).

4.3.2 Sites with positive results

Eggs were present at five sites in both 2013 and 2014; White Mill (site 4), Nantgaredig Bridge (site 5), Cothie confluence (site 6), Llandeilo Bridge (site 10) and the Tidal limit (site16). The most upstream site is at Llandeilo Bridge which is located in the mid Tywi catchment (Figure 2).

Eggs were present at Cilsan bridge (site 9) and Manordeilo (site 11) in 2013 but they were absent in 2014. Eggs were absent at Glantowylan (site 3) in 2013 but they were present in 2014.

4.3.3 Extent and limits of spawning

In 2013 the downstream limit of spawning was at the Tidal limit, near Abergwili (1 egg, Site 16) and the upstream limit of spawning was at Manordeilo bridge (2 eggs, Site 11). The small sample size and the fact that the eggs were not verified by genetic testing so there is low confidence in this assessment. Only two sample sites were verified by genetic analysis and the downstream limit of spawning can be confirmed as White Mill (site 4) and the most upstream site is the Cothie confluence (6a)

Shad eggs were recorded as present at sample sites spread over approximately 45km of meandering river corridor although the species was only determined at two sample sites.

In 2014 the downstream limit of spawning was Tidal Limit (112 eggs, site 16) but the upstream limit was Llandeilo Bridge (31 eggs, site10). This site is downstream of Manordeilo (site 11) and the distribution of shad eggs was spread over approximately 34km of river corridor, although the eggs at Llandeilo Bridge did not yield sufficient DNA material for a strongly confirmed species determination. .

In 2013 the sample size ranged from 1 to 100+ eggs and the two samples with just one egg were found at the Tidal limit (site 16) and Cilsan bridge (site 9). The total number of eggs recorded was 177+ (the largest sample of over 100 eggs was not counted)

In 2014 the sample size ranged from 1 to 370 eggs and a total of 1,218 eggs were recorded; the greater number was counted at Glantowylan (site 3) on 28th May after 10 kick samples. There were two more visits on the 20th & 29th May and a further count of 201 eggs was obtained. One egg was found at Nantgaredig Bridge (site 5) on the 16th May, however, the same site was subsequently sampled on three more occasions up until 11 June and a further total of 52 eggs were recorded.

4.5 Presence and absence at sample sites 2014.

In 2014, no eggs were recorded at Llanwrda (12).

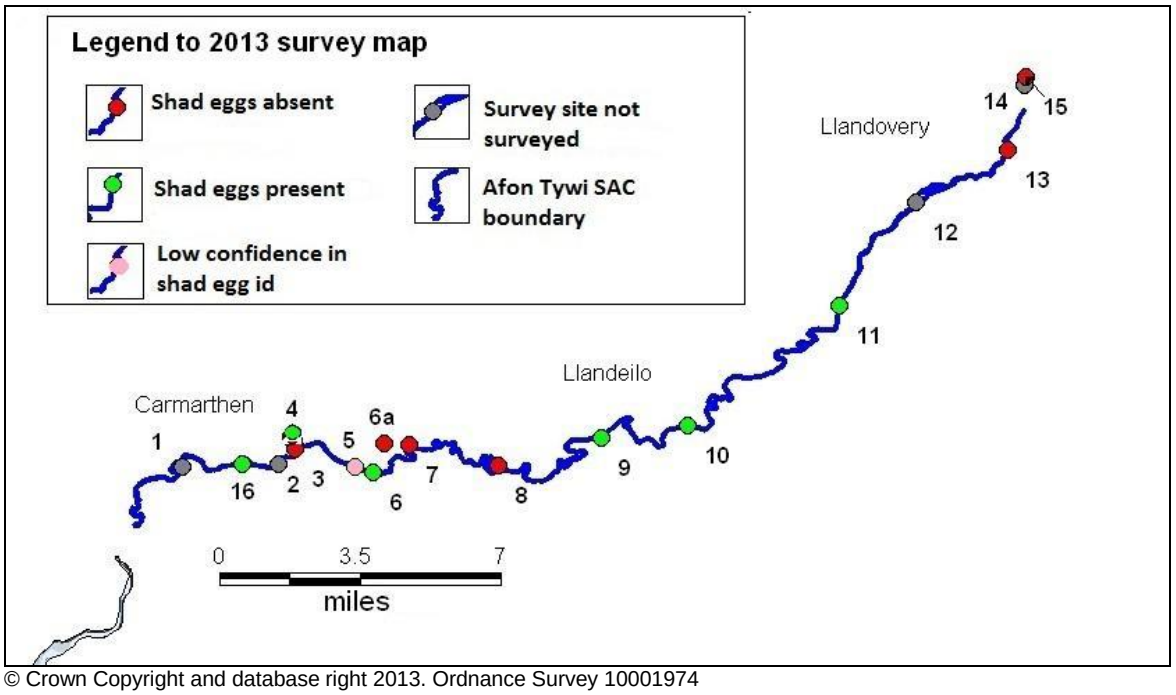

Figure 2. Map showing presence of shad eggs in 2013 survey.

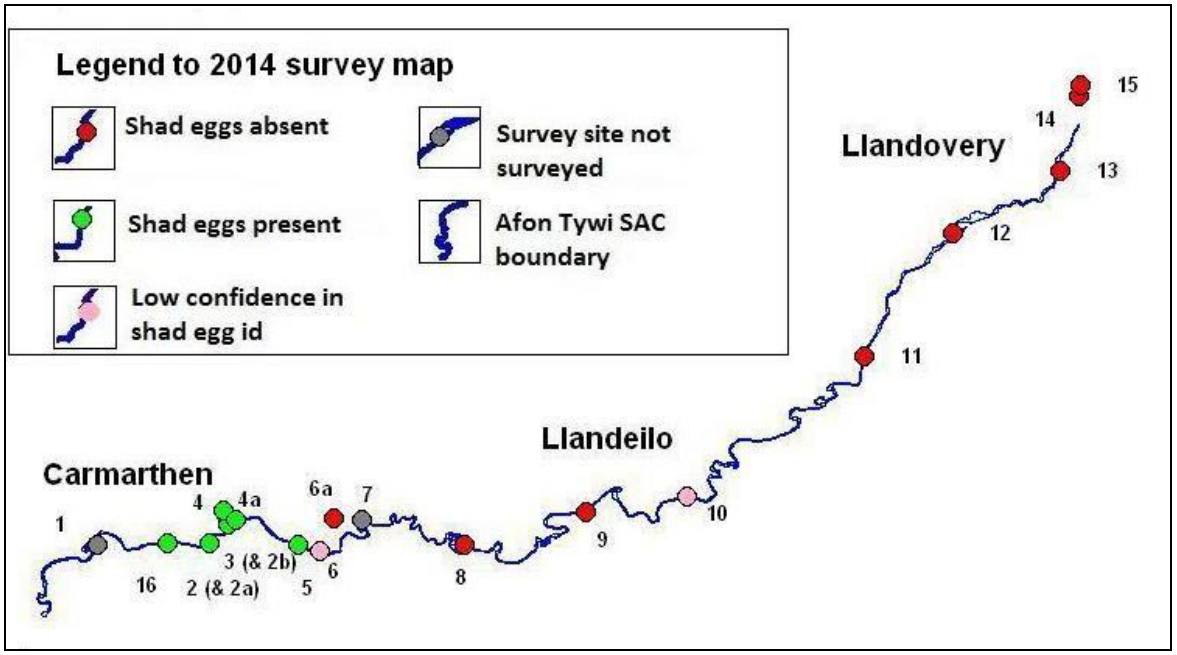

Figure 3. Map showing presence of shad eggs in 2014 survey.

4.4 Results of DNA genetic analysis 2013 & 2014.

Twenty eggs were collected from three sample sites for DNA analysis in 2013. The three sites were site 4 White Mill, site 5 Nantgaredig bridge and site 6 Cothie confluence. The greatest number of eggs (100+) was recorded at Nantgaredig Bridge (Site 5) and 20 of these were sent for DNA analysis but they were found to be minnow (*Phoxinus phoxinus).* This result casts doubt on the identification of the remaining eggs in the sample and the field record sheets and spreadsheets have been amended to record low confidence in the species identification. The field surveyors also collected 20 eggs at Whitemill (Site 4) and the Cothi confluence (Site 6) and these were determined as shad species by DNA analysis. The DNA analysis of eggs sampled from three Welsh rivers demonstrated that field identification was correct for 85% of the total 2013 sample (Hardouin *et al.*, 2013).

As Hardouin *et al.* (2013) observed that "due to overlapping habitat utilisation, it is not unexpected that other lithophilic spawning species are present within the samples of shad eggs. Indeed, spawning activity of one species can often stimulate other species to spawn. This is commonly observed in minnow (*Phoxinus phoxinus*) which often opportunistically capitalise on the disturbance and cleaning of gravels by larger species (A. Pinder *pers. com.*)". In 2013 85% of the eggs collected for analysis on the Tywi proved to be of shad origin. Both species of SAC freshwater shad fish, allis shad (*Alosa alosa)* and twaite shad (*Alosa fallax)* have historically been recorded on the Afon Tywi, but none of the eggs collected on the Tywi could unequivocally be identified as allis shad (Hardouin *et al*. 2013).

In 2014, 99% of the eggs were confirmed as a shad species (only 2 eggs from a sample of 162 were not shad). Each of the six eggs recorded at Llandeilo Bridge were processed for genetic analysis. One of these eggs did not yield a strong match with the Alosa primer, however, Stone (2014) proposes that it was not a different fish species but rather a shad egg that produced poor DNA material for analysis. A further 19 eggs from other different sites also yielded poor DNA material and it was also suggested that the eggs were of shad origin.

One non-shad egg was found in the Penddaulwyn site (site 2) but the presence of twaite shad (*Alosa fallax*) was confirmed by the remaining eggs in the sample. Twaite shad was also confirmed at two sites: site 4a Habitat 8 and site 5 Nantgaredig Bridge.

An *Alosa* type species was confirmed at the remaining sites of Tidal Limit (site 16), Glantowylan (site 3) and Cothi confluence (site 6). Unlike the 2013 analysis, in 2014 the analysis was not designed to identify hybrids but only to confirm the presence of shad species.

4.5 Weather patterns and temperatures during 2013 & 2014

In 2013 the mean temperature over the UK for spring was 6.0 °C, which is 1.7 °C below the long-term average and May was 0.8 °C below the long term average for this month (MetOffice, 2014a). Overall May was also wetter than average for the UK.

The summer months of 2013 in the UK were the warmest summer since 2006, but not exceptionally so, with a mean temperature of 15.2 °C, which is 0.8 °C above the long-term average. June was the only relatively cool month at 0.2 °C below the long-term average, although rainfall in Wales was slightly below average during this period (MetOffice, 2013).

Minimum Spring temperatures were above average in May 2014 although the month was wetter than average for most areas (MetOffice, 2014b).

Summer 2014 saw several spells of fine, settled weather in both June and July but no major heat-waves. The UK mean temperature for summer 2014 was 14.8 °C which is 0.5 °C above the 1981-2010 average - although it was not as warm as summer 2013 although June was slightly above average. Rainfall totals were below average in Wales and June was a sunny month (MetOffice, 2014c).

To summarise the two periods for Wales: May 2013 was cooler and wetter than average and May 2014 was warmer and wetter than average. June 2013 was cooler than average but with below average rainfall in Wales; June 2014 was slightly warmer than average but with below average rainfall.

4.6 Afon Tywi water temperature readings

Surveyors experienced problems with the reliability of temperature data loggers in both 2013 and 2014 and so readings were not taken for every kick sample.
Three temperature recordings were made at three sites during 2013 between 11 June and 1 July. 23 temperature recordings were made at twelve sites in 2014 between 16 May and 24 June 2014. The readings are shown in Table 5.

	Water temperature ºC	2013 dates. 3 survey days	2014 dates. 7 survey days	Water temperature ºC
Lowest temperature recorded	12	11 June	29 May	12.7
Highest temperature recorded	14	11 & 13 June	21 May	19.4
Mean temperature	13			15.5

Table 4. Afon Tywi water temperature readings during shad egg surveys 2013 – 2014.

A total of three temperature readings were recorded in 2013 and two of the survey days were in mid-June. 23 readings were taken in 2014 and the majority of the sampling took place in late May. In 2013, the mean temperature was 13 ºC (*n* = 3) and 15.54 ºC (*n* = 23) in 2014. These results reflect the weather trends described by the Meteorology Office for these two periods (MetOffice, 2013; 2014a; 2014b).

4.7 Monthly flow patterns in May & June on the Afon Tywi

There are four flow gauge stations (FGS) on the Afon Tywi and table 6 shows the variation in mean and minimum monthly flows at each one. 2013 did not have exceptional mean monthly or minimum flows for May or June although on the mean monthly flows were generally lower at all the FGS in June. The mean monthly flows during in 2014 were very similar to those recorded in 2013 (Figure 4).

	Capel Dewi SN4853320603				Manorafon SN6572524004				Dolau Hirion SN7616336232				Ystradffin SN7854747239			
	Mean monthly flow **m³/s**		Min monthly flow **m³/s**		Mean monthly flow **m³/s**		Min monthly flow **m³/s**		Mean monthly flow **m³/s**		Min monthly flow **m³/s**		Mean monthly flow **m³/s**		Min monthly flow **m³/s**	
Year	May	June	May	June	May	June	May	June	May	June	May	June	May	June	May	June
2000	20.80	31.60	8.48	9.35	12.00	17.50	5.86	6.50	5.96	7.95	2.59	2.56	2.47	3.33	0.44	0.68
2001	16.40	7.77	7.65	2.44	9.27	5.62	5.20	4.02	5.05	3.80	2.06	2.09	2.87	3.26	0.94	1.28
2002	60.30	29.30	10.50	8.01	29.30	14.80	6.22	5.06	10.60	6.11	2.57	2.17	2.79	2.56	0.79	0.92
2003	34.80	12.80	6.72	4.04	20.00	6.99	6.22	4.79	9.18	3.70	2.39	2.47	1.78	2.23	0.86	0.97
2004	16.60	6.08	4.94	3.14	11.80	5.96	5.20	4.27	6.39	4.29	2.29	2.85	3.37	3.33	0.94	1.90
2005	17.20	9.74	7.71	5.44	9.75	6.95	4.99	5.14	5.06	4.17	1.96	2.17	1.86	2.43	0.80	0.88
2006	47.60	10.50	8.96	4.15	28.30	7.70	6.63	4.83	13.30	3.34	2.50	1.81	4.82	2.05	0.96	0.89
2007	22.60	13.80	4.20	4.63	14.30	8.85	4.06	4.06	5.83	5.54	1.92	3.25	1.69	2.55	0.96	0.99
2008	11.80	9.48	5.43	3.27	7.33	6.25	4.49	4.24	3.68	3.79	1.95	2.14	2.37	2.58	0.91	1.07
2009	24.40	15.70	7.70	5.93	13.70	10.20	5.63	4.70	6.29	4.72	2.57	2.39	2.29	2.77	1.16	1.17
2010	7.41	5.23	3.56	2.02	5.54	4.92	3.79	3.59	3.36	3.43	3.11	3.08	2.67	2.73	2.61	2.66
2011	8.27	19.80	3.80	4.93	6.12	12.20	3.94	4.95	4.12	5.74	2.40	2.46	2.81	1.91	0.74	0.77
2012	32.60	61.20	7.85	8.22	19.40	36.40	5.57	5.69	8.31	19.60	2.58	3.32	3.34	7.73	0.89	0.96
2013	29.20	12.00	8.62	7.28	16.00	7.58	5.36	5.54	7.23	4.04	2.32	2.43	3.16	2.11	0.76	0.99
2014	29.1	14.1	12.4	5.96	17.2	9.07	6.92	5.02	8.58	4.29	2.91	2.14	3.80	2.41	1.06	1.06

Table 5. Monthly mean and minimum flows for May and June at the four FGS on the Afon Tywi, 2000 – 2014.

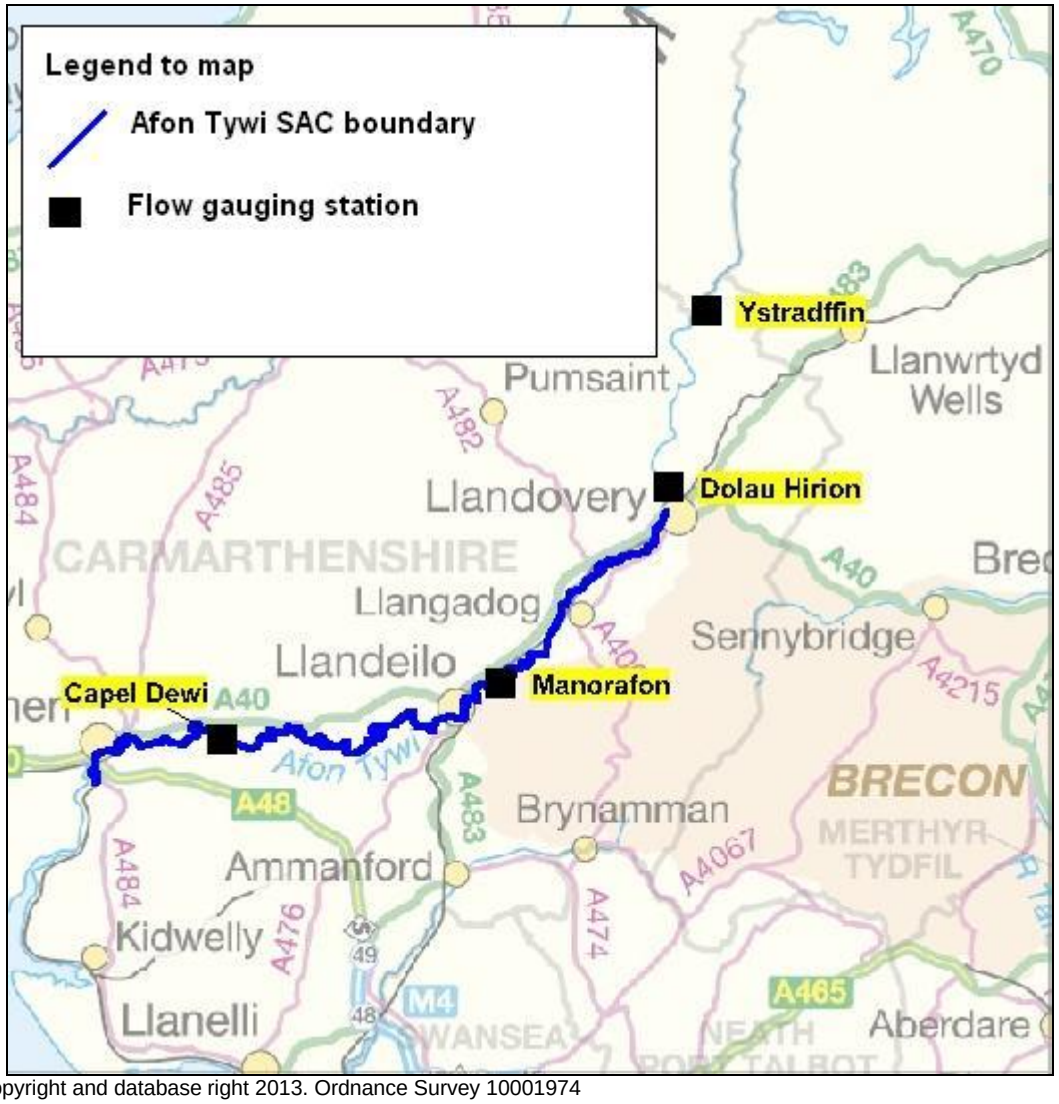

Figure 4. Location of the gauging stations. Note that Ystradffin is outside of the SAC boundary.

The average of the May and June 2014 mean monthly minimum flows was calculated for each FGS and compared with the average for the same months in the previous decade (long term mean for 2000 – 2012) (Table 7). The mean minimum flow in 2013 & 2014 was similar to the long term mean for three of the FGS, but the minimum flow was higher than the long term mean at Capel Dewi.

Mean minimum flow	Capel Dewi	Manorafon	Dolau Hirion	Ystradffin
May – June 2014	9.18	5.97	2.53	1.06
May – June 2013	7.95	5.45	2.38	0.88
May - June 2000 - 2012	6.50	5.41	2.63	1.15

Table 6. Comparison of mean minimum flow for 2013 & 2014 with long term minimum mean for 2000 – 2012.

More detailed analysis of flows and temperature has been completed by Carpenter (2011) and he concluded that the period 2006 to 2011 has been wetter than average with average annual mean flows being 10 to 22% higher than the 1984 to 2004 longer term average. See Carpenter's report for further details of the influence of Llyn Brianne reservoir releases and abstractions on the flow patterns in the Afon Tywi.

4.8 Additional fish species

Surveyors are requested to record any other fish species that they observe during their kick sampling surveys (Table 5). Bullheads were frequently recorded at sites throughout the catchment. Juvenile bullhead are relatively small and so the kick sampling technique is more likely to dislodge them from under large cobbles and small boulders. The Afon Tywi is designated as a SAC for bullhead and all three native lamprey species.

	Sample site no.	
Non-target fish species	**2013**	**2014**
Bullhead	9,11,13, 15	16, 5, 6a, 9, 10, 11, 12, 13
Lamprey species	3,11,15	5
Brown trout parr	15	
Stickleback species	3	

Table 7. Non-target fish species observed on the Afon Tywi

5.0 Discussion

5.1 Data analysis

The extent of shad spawning is usually fully assessed once in each six year monitoring cycle using CSM freshwater fauna monitoring guidance (JNCC, 2005). The third monitoring cycle runs between 2013 and 2018 and so this report is only a short interim analysis of the 2013 and 2014 survey data.

In this report distribution of spawning has not been analysed against CCW or EAW management units because, as part of the Afon Tywi SAC management plan revision, there are plans to harmonise the two unitisation systems but to date this work has not been completed. In addition, the JNCC CSM guidance for freshwater fauna is also being reviewed, although it is not anticipated that the targets or protocols for egg sampling will alter significantly.

In 2014 the most upstream spawning was detected at site 10 (Llandeilo bridge), which is 12km downstream of the most upstream 2013 spawning site (site 11 at Manordeilo). The Manordeilo site was not selected for genetic analysis so the identification could not be confirmed. The identification skills of the 2013 survey team were thought to be good because 85% of the eggs analysed were found to be of shad origin. The 2014 sample at Llandeilo bridge was analysed and one egg from the batch (*n*= 6) was found to be another fish species. The poor DNA yield from the remaining 5 eggs did not produce a strong match to the Alosa primer but the analyst still considered these eggs to be of shad origin.

5.2 *Sampling methods*

For a full discussion of the field methodology, see Garrett *et al.* (2013). Prior to 2013 no genetic analysis had been conducted on Afon Tywi shad eggs and this additional analysis technique has allowed the degree of confidence in field identification to be evaluated. The level of identification was good in 2013 (85%) and excellent in 2014 (99%). A greater number of samples were tested in 2014 and the spread of samples along the river corridor was greater than in 2013. If resources allow then it would be beneficial to repeat this type of analysis on the river Usk and the river Wye which are the other two Welsh shad rivers.

6.0 Conclusion & recommendations

The extent of spawning on the Afon Tywi in 2013 and 2014 follows a similar pattern to the distribution identified in the second monitoring round (2007-2012).

Prior to 2014 survey sites were usually only sampled once during the spawning season, however, in 2014 the majority of sites received repeat visits. The additional visits did not appear to provide any additional information. In future, the survey teams should consider whether repeat visits are required for sites where eggs are found during the initial visit.

Resource efficiencies could be made by just repeat sampling negative sites, especially those higher up in the catchment.

For detailed recommendations relating to survey data analysis and sampling methods see Garrett *et al.* (2012). For further in-depth recommendations regarding developing genetic analysis, see Hardouin *et al.* (2013) and Stone (2014).

The Afon Tywi SAC core management plan (Lovering, 2008) is due for revision and the spawning attribute target should be reviewed in-line with the revised CSM Freshwater fauna guidance (JNCC, *in prep*) and any evidence arising from the on-going investigations into the response of shad to temperature regimes (Knights, 2014; Carpenter, *in prep*).

Acknowledgements

The Freshwater Team are grateful to the field surveyors;
Alex Harding
Alison Baird
Emma Keenan
Hilary Foster
Iestyn Evans
James Moon
Jill Howells
Julie Gething
Kerry Rogers
Leila Thornton
Meryl Tandy
Mike Jenkins
Nicola Broadbridge
Paul Hyatt
Richard Lewis

Special thanks to Alex Harding and Leila Thornton for organising the field surveys and to Gideon Carpenter and Tony Brown for providing hydrological data. We also wish to thank all the landowners who gave permission to our teams to survey on their land.

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APPENDIX 1: DATA ARCHIVE APPENDIX

Data outputs associated with this project are archived as Project No. 459 and Media No. 1520 on server–based storage.
The data archive contains:]

[A] A report in .doc and .pdf format (see Ffynnon NRW-13-071629) also DMS https://cyfoethnaturiolcymru.sharepoint.com/sites/smar/fm/Running%20Waters%20Habitat%20Directive/Forms/NRW%20View.aspx?RootFolder=%2Fsites%2Fsmar%2Ffm%2FRunning%20Waters%20Habitat%20Directive%2FShad%20Fish%20Species%20%2D%203rd%20Reporting%20Cycle&FolderCTID=0x012000BDE997E6142E2E41832764EF95CDBC2C&View=%7B5B29CB1C%2D815E%2D4BF1%2DB48E%2D3744D288F8B2%7D

[B] A series of GIS layers in MapInfo .Tab format on which the maps are based

[C] A set of spreadsheets for the raw data and worked up data in xls format (NRW-13-054339, NRW-13-054417)

[D] Field notes in paper format (held in Llandarcy office, copies held in Evidence and Analysis Team, Maes y Ffynnon, Bangor)

Metadata for this project is publicly accessible through Natural Resources Wales Library Catalogue url TBC/ by searching ‘Dataset Titles’. The metadata is held as record no 115889.

APPENDIX 2

Table of results from 2013 & 2014

Site no	Site name	NGR	2013				2014			
			Date surveyed	Shad present	No. kick samples (max 25)	No. shad eggs	Date surveyed	Shad present	No. kick samples (max 25)	No. shad eggs
1	Llangunnor	SN4240 020300	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed
2 (& 2a)	Penddaulwyn	SN4623 120402	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	20-May	**Present**	10	14
							28-May	**Present**	25	8
							29-May	**Present**	10	31
3 (& 2b)	Glantowylan	SN4690 021000	13-Jun	**Absent**	25	0	20-May	**Present**	10	95
							28-May	**Present**	10	370
							29-May	**Present**	10	106
4	White Mill	SN4674 021494	11-Jun	**Present**	17	20	20-May	**Present**	17	18
							28-May	**Present**	10	123
4a	Habitat 8	SN4714 621161	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	20-May	**Present**	10	250
5	Nantgaredig Bridge	SN4930 020300	11-Jun	**Present**	22	100	16-May	**Present**	25	1
							21-May	**Present**	10	33
							28-May	**Present**	25	7
							11-Jun	**Present**	13	12
6	Cothie confluence	SN5003 820096	13-Jun	**Present**	10	25	21-May	**Present?**	25	1
							28-May	**Present**	25	6
6a	Cothie Bridge	SN5050 421250	13-Jun	**Absent**	25	0	21-May	**Absent**	25	0
							28-May	**Absent**	25	0
7	Llanegwad	SN5150 021200	13-Jun	**Absent**	9	0	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed
8	Dryslwyn	SN5503 120345					21-May	**Absent**	25	0
			13-Jun	**Absent**	25	0	28-May	**Absent**	25	0
9	Cilsan Bridge	SN5916 821463	13-Jun	**Present**	25	1	21-May	**Absent**	25	0
							29-May	**Absent**	25	0
10	Llandeilo Bridge	SN6265 321991	11-Jun	**Present**	14	28	21-May	**Present**	25	14
							29-May	**Absent**	25	0
							11-Jun	**Present**	10	17
11	Manordeilo	SN6875 926802	11-Jun	**Present**	25	2	21-May	**Absent**	25	0
							11-Jun	**Absent**	13	0
12	Llanwrda	SN7180 931006					21-May	**Absent**	25	0
							11-Jun	**Absent**	25	0
13	Lwynjack	SN7548 833138	01-Jul	**Absent**	25	0	21-May	**Absent**	25	0
14	Llandovery	SN7613 435746	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed
15	Dolauhirion	SN7620 036100	01-Jul	**Absent**	25	0	Not Surveyed	Not Surveyed	Not Surveyed	Not Surveyed
16	Tidal limit	SN4480 020400	13-Jun	**Present**	25	1	16-May	**Present**	11	13
							20-May	**Present**	10	75
							28-May	**Present**	10	24