SP MANWEB

Reinforcement to the North Shropshire Electricity Distribution Network

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Amphibian Surveys

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Reinforcement to the North Shropshire Electricity Distribution Network

on behalf of SP Manweb

Appendix 7.6: Amphibian Surveys

DCO Document 6.7.6

avian ecology

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The Planning Act 2008

The Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009

Regulation 5(2)(a)

Reinforcement to the North Shropshire Electricity Distribution Network

Environmental Statement: Appendix 7.6 – Ecology and Biodiversity Amphibian Survey

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1 INTRODUCTION

1.1.1 This Appendix presents the results of amphibian surveys undertaken to inform the Ecological Impact Assessment (EcIA) and Environmental Statement for the Proposed Development.

1.1.2 The survey areas were identified through an iterative process, drawing upon early route corridor option studies, professional judgement in relation to the extent and nature of the Proposed Development, standing advice published by Natural England[1] and consultation engagement with Shropshire Council, Natural England, RSPB, the Canal and Rivers Trust and Shropshire Wildlife Trust (**DCO Document 5.1**).

1.1.3 Great crested newts *Triturus cristatus* (GCN) are European Protected Species (EPS) and they and their habitat are fully protected under the Wildlife & Countryside Act 1981 (as amended)) and the Conservation of Habitats and Species Regulations 2017. The combined legislation makes it illegal to:

- intentionally or deliberately capture, kill or injure a great crested newt;
- intentionally or recklessly damage, destroy or obstruct access to any place used for shelter and protection including resting and breeding places, whether occupied or not;
- deliberately, intentionally or recklessly disturb a great crested newt when in a place of shelter;
- possess a great crested newt, or any part of it, unless acquired lawfully;
- sell, barter, exchange or transport or offer for sale great crested newts or parts of them.

1.1.4 Activities which may affect EPS must consider the presence of EPS, their breeding sites and resting places.

2 METHODOLOGY

2.1 Desk Study

2.1.1 A data request was submitted to SEDN and Shropshire Wildlife Trust (SWT) for amphibian records within 1km of the Proposed Development.

2.2 Habitat Suitability Index

2.2.1 Pond locations are shown on Figure 7.7 (**DCO Document 6.14**). Ponds were identified within approximately 50m either side of the Order Limits, with additional ponds also visited in the wider area. Ponds were accessed and subject to a Habitat Suitability Assessment. The survey covered all ponds in proximity to route options considered during the evolving line design. As the alignment of the Proposed Development was amended over time, a number of these ponds were subsequently

[1]https://www.gov.uk/guidance/protected-species-how-to-review-planning-applications#standing-advice-for-protected-species

excluded from further survey, lying well beyond the potential zone of influence of the Proposed Development.

2.2.2 30 ponds were subject to a Habitat Suitability Index (HSI) assessment in 2017/2018 in order to provide an indication of their potential suitability for great crested newts (Annex AN7.6.1). Potentially suitable ponds were highlighted for follow-up presence-absence survey based on their proximity to the evolving alignment of the Proposed Development.

2.2.3 The HSI assessment methodology followed the Amphibian and Reptile Groups of the United Kingdom (ARG UK) methodology (ARG UK, 2010[2]), which is a refined version of the Oldham et al. (2000[3]) method. The assessment calculates a habitat suitability score for each pond based on a series of indices generated from variables including pond size and the presence/absence of wildfowl. Final scores relate to suitability and range from 'poor' to 'excellent' suitability.

2.2.4 The HSI assessment involves the measurement of ten different indices which, when combined, have been found to provide a good indication of the general suitability of ponds for great crested newts. Each of the indices is scored (between 0.01-1) using a series of graphs and figures within the guidance notes (ARG UK, 2010). These scores are then used to calculate an overall Habitat Suitability Score for each pond. Final scores relate to pond suitability for great crested newt and range from 'poor' to 'excellent'.

2.2.5 The results of the HSI assessment can be used to provide a useful indication of potential newt presence and help assess any likely impacts of a development, but do not represent a substitute for full surveys. In some cases, ponds that were identified from HSI assessment early in the year to have potential were found to be dry by the spring breeding season and therefore unsuitable for great crested newt breeding or for presence/absence survey.

2.3 Environmental DNA Survey

2.3.1 Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, depending on the conditions (Biggs *et al*., 2014a[4]). The technique for determining presence/absence of great crested newt uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

2.3.2 Recent research by the Department for Environment Food and Rural Affairs (Defra) Project WC1067, concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether great crested newts are

[2] ARG UK (2010) ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.
[3] Oldham R.S., Keeble J., Swan M.J.S. and Jeffcote M. (2000) Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). Herpetological Journal, 10(4), pp. 143-155.
[4] Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al.,* 2014).

2.3.3 Natural England accepts the use of environmental DNA surveys as evidence of presence or absence of great crested newts, provided samples are taken when newts are likely to be present (this depends on location and conditions like the weather). Generally this is considered to be between mid-April and 30th June; however in ponds which have been used for breeding there is also some potential to record efts/larvae in July and August. Surveys in these months cannot prove absence, but can provide useful information for confirmation of breeding.

Field Sampling Technique

2.3.4 Amphibian eDNA surveys were undertaken by suitably trained and experienced surveyors Ms C Baldock MRes ACIEEM (Licence No. 2016-19849-CLS-CLS), Mr T Winter BSc Grad CIEEM (Licence no. 2017-27525-CLS-CLS), Mr A Hulme BSc (Licence No. 2018-33563-CLS-CLS), Mr Graham Burns and Mr Z Hinchcliffe BSc. (Licence No. 2018-33560-CLS-CLS) Surveys were undertaken in May and June 2017, with a small number of additional ponds surveyed in May 2018 to reflect the evolving detailed design of the proposed route. Surveys were undertaken during suitable weather conditions. Photographs of typical ponds are provided in Annex AN7.6.2.

2.3.5 The protocol for sampling followed that outlined within Biggs *et al*., 2014b[5] , which required the collection of 20 x 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

2.3.6 Each sample was then placed within a Whirl-Pak bag and shaken for 10 seconds, before a 15ml sample was pipetted from the bag and placed in a specimen tube for laboratory analysis. Samples were refrigerated prior to laboratory dispatch. This process was repeated for each sampled pond.

Laboratory Analysis

2.3.7 Laboratory analysis was undertaken by SureScreen Scientifics. The laboratory follows the analysis methodology outlined within the Defra Project WC1067 research note (Biggs *et* al., 2014) using the q-PCR test conducted in two phases.

2.3.8 The sample first goes through an extraction process to acquire as much eDNA as possible to produce a pooled sample. The pooled sample is then tested via 1-PCR.

2.3.9 Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification. Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

Survey Limitations

[5] Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA.

2.3.10 No significant survey limitations were encountered. Sufficient ponds could be surveyed to provide a suitable indication of GCN presence along the whole of the Proposed Development to inform the assessment of effects and appropriate avoidance or mitigation measures where required.

3 RESULTS

3.1 Desk Study

3.1.1 Very few records were returned for amphibians, restricted to four records for frog *Rana temporaria* and two for toad *Bufo bufo*, and 13 records for great crested newt *Triturus cristata*. This scarcity is considered to reflect a lack of survey information for the area.

3.2 Habitat Suitability Index

3.2.1 The results of the HSI assessment are presented in Annex AN7.6.1 of this appendix. Habitat suitability ranged from Poor to Excellent.

3.2.2 The majority of ponds within the survey area are field ponds in managed agricultural land, generally arable or improved grassland. A number of these ponds are isolated within open fields, lacking good habitat connectivity or extensive areas of high quality foraging or refuge habitat nearby apart from a narrow border of trees and scrub around their banks. Field ponds lying at the boundaries of fields adjacent to hedgerows or woodland copses have better habitat connectivity and higher quality terrestrial habitat nearby for great crested newts and other amphibians.

3.2.3 It is considered that the dominant habitats (arable and grazed improved grassland farmland) provide low value amphibian terrestrial habitat, with higher value habitats provided by woodland copses, hedgerows and associated ditches, unmanaged scrub and ruderal vegetation present in limited areas, mainly along watercourse banksides.

3.3 Environmental DNA

3.3.1 eDNA survey results are summarised in Table 7.6.1. Laboratory reports are provided in Annex AN 7.6.3.

3.3.2 Of the 21 ponds that could be surveyed, nine tested positive for the presence of great crested newts. There was generally good correlation between HSI and eDNA results with positive eDNA results from ponds whose HSI score ranged from Good-Excellent.

3.4 Works in Proximity to Ponds

3.4.1 There will be no loss of any ponds as a result of the Proposed Development. Poles are mainly located well away from pond banks, thereby reducing the potential for indirect effects on aquatic habitats. The underground sections of the Proposed Development do not lie within 50m of any pond.

3.4.2 Poles in proximity (within approximately 50m of ponds) are shown on Table 7.6.1.

Table 7.6.1: Surveyed Pond eDNA results.

Pond Number	Summary description	eDNA tested: Presence (P) Likely absence (A)	Nearest Pole # or Access within approx. 50m
0a/0b	P0a an oxbow shaped pond in corner of field linked to P0b a shallow pond filled with macrophytes. Good refuge habitat of stone piles and potential hibernacula nearby.	A	2 Access
1	Pond on edge of improved grassland field.	A	15/16
2	Turbid, shallow. Good vegetation cover.	A	26/27
3	Open, well vegetated pond. Willows, alder and oak around the perimeter but plenty of light reaching water. Marginal vegetation included flag iris, branched bur-reed and water milfoil	P	44
3a	Shallow field pond, dry at time of survey	N/A DRY	45
4	Open pond fringed with *Typha* and rushes.	No access*	None
5	Shaded pond surrounded by mature oaks, hawthorn, sycamore.	No access*	None
6/7	Adjoining shaded ponds with a deep layer of mud and debris, overhanging scrub and alder oak and hawthorn. Water turbid and lacking macrophytes	A	81/82
8/9	Two ponds linked by a central channel. Bank edges were either heavily poached or steep sided. Bank vegetation comprised mainly common grasses and several large mature oaks.	A	86
10	Pond surrounded by mature trees and scrub. A large percentage of the margin overhung by willow scrub. Limited macrophyte presence in water.	A	90
11	Field pond/depression (dry by early April 2017) isolated position in arable field.	N/A DRY	96
11a&11b	Two heavily shaded ponds in woodland surrounded by arable fields. Lacking emergent or aquatic vegetation.	A	Access
11c&11d	Two newly created ponds on farm surrounded by lawn and pasture. Macrophytes (mainly reeds) present. Some waterfowl and fish. Not shaded.	P	Access
12	Pond situated on the field edge with dense hedgerow surrounding it, as well as tall oaks which left the entire bank in shade.	P	104

Pond Number	Summary description	eDNA tested: Presence (P) Likely absence (A)	Nearest Pole # within approx. 50m
13	An open shallow waterbody with no defined banks located centrally within an improved grassland field. The pond was heavily poached by cattle.	A	104
14	Heavily shaded pond, overhung by large area of dense scrub including hazel, willow, aspen.	P	120
15	Pond in arable field. Large stand of marginal vegetation with water horsetail, willow, hawthorn shrubs around edge.	A	121
16	Open lagoon. Marginal vegetation included water mint, spike rush and soft rush	A	123
17	Pond surrounded by hawthorn, dogrose, ash scrub. Enclosed by vegetation but plentiful light penetration. Plentiful invertebrates including dragonflies.	P	123
18	Adjacent to roadway and well shaded by oak, alder, blackthorn, ash. Pond shallow and largely lacking aquatic vegetation.	P	123
19	Partially shaded pond with livestock access and surrounded by alder shrubs. Marginal vegetation included hard rush.	P	125
20	Large ornamental / fishing pond in small woodland. Irregular shape with central island. Shaded with deep layer of leaf litter and limited marginal vegetation (flag iris). Trees around pond included oak, alder, ash, hazel, willow.	No access*	127
21	Field pond at margin of improved grassland field adjacent hedgerow.	No access*	None
22	Field pond at margin of improved grassland field adjacent hedgerow.	No access*	146
23/24	Two ponds combined to form a large pond located on the edge of an arable field with heavily shaded areas by alder and oak. Some areas along its banks were heavily poached by cattle	P	147
25	Dry pond/depression situated within an arable field.	N/A DRY	148
26	Dry pond/depression situated within an arable field. Small area of bulrush denotes occasional flooding.	N/A DRY	148
27	A pond situated within an arable field with heavy poaching on one end. 2/3 of the pond is shaded by alder, hawthorn and bramble.	P	150

28	A large reservoir surrounded by improved grassland. Very little shading around its banks and very little macrophytes.	A	152
29	Dry field depression with a dense growth of grasses. May flood occasionally.	N/A DRY	152/153
30	Large pond situated within a dense woodland. The entirety of the pond was shaded by the dense woodland canopy	A	153

**No access permission to undertake eDNA survey*

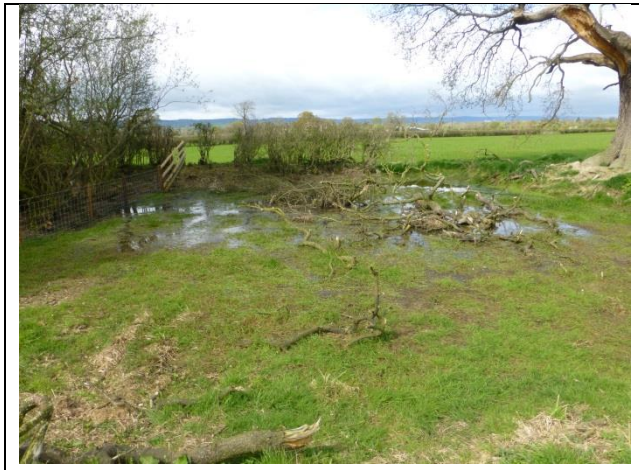
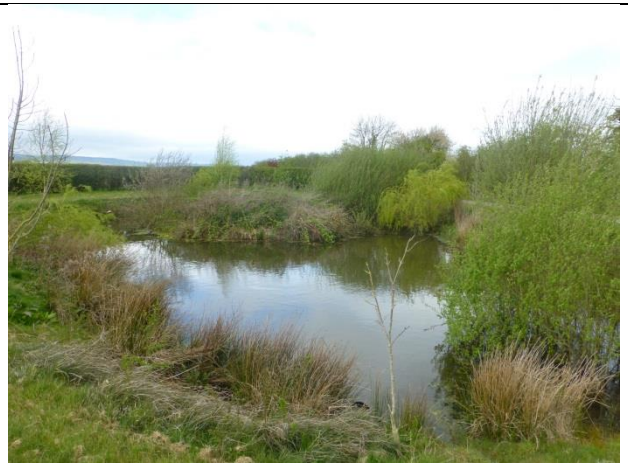
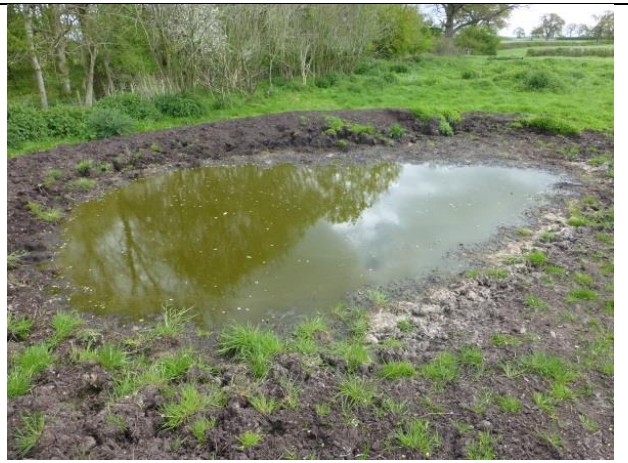
Annex AN7.6.1– Habitat Suitability Index Parameters

Pond Number																
Indices	**P0a**	**P0b**	**P1**	**P1a**	**P1b**	**P1c**	**P2**	**P3**	**P3a**	**P4**	**P5**	**P6**	**P7**	**P8 & P9**	**P10**	**P11**
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	0.2	0.2	0.8	0.8	0.2	0.2	0.8	0.4	0.2	0.9	0.4	0.4	0.4	1	1	1
S3	0.1	0.1	1	0.9	0.1	0.1	1	0.9	0.1	0.9	1	1	1	1	0.9	1
S4	1	1	1	1	0.33	0.67	1	1	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
S5	0.6	1	1	1	1	1	0.6	0.6	0.6	1	0.6	1	1	0.6	0.6	1
S6	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
S7	1	1	1	1	1	1	1	1	1	0.67	1	1	1	0.67	0.67	1
S8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S9	0.67	0.33	0.67	0.67	1	1	1	0.33	0.33	0.33	0.33	0.33	0.33	0.67	0.67	0.67
S10	1	1	1	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Suitability	Below Average	Below Average	Excellent	Excellent	Good	Good	Excellent	Good	Below Average	Below Average	Average	Good	Good	Average	Below Average	Good

Pond Number														
Indices	**P11a, P11b**	**P11c, P11d**	**P12**	**P13**	**P13a**	**P14**	**P15**	**P16**	**P17**	**P18**	**P19**	**P20**	**P21**	**P22**
S1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S2	0.5	0.4	0.8	0.4	0.2	1	1	0.9	1	1	1	1	0.8	0.8
S3	0.9		0.9	0.9	0.9	1	1	0.9	1	1	1	0.9	0.9	0.9
S4	0.01		0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
S5	0.2		0.6	0.6	1	1	0.6	0.6	1	1	0.6	1	1	0.6
S6	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
S7	1	0.33	1	1	1	1	0.67	0.33	1	1	1	0.67	1	1
S8	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S9	1	0.67	0.67	0.33	0.33	1	0.33	0.1	0.67	1	0.33	1	0.33	0.33
S10	0.3	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Suitability	Poor	Good	Poor	Poor	Poor	Excellent	Average	Poor	Good	Excellent	Excellent	Average	Good	Below Average

Pond Number								
Indices	**P23**	**P24**	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**
S1	1	1	1	1	1	1	1	1
S2	0.8	1	0.8	0.8	1	0.9	0.8	1
S3	0.9	0.1	0.1	0.9	1	0.9	0.9	0.9
S4	0.67	0.33	0.33	0.67	0.67	0.67	0.67	0.67
S5	0.6	0.6	0.6	1	0.6	1	0.6	0.6
S6	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
S7	0.67	0.67	1	0.67	1	1	1	0.67
S8	1	1	1	1	1	1	1	1
S9	0.67	0.67	0.1	0.1	0.67	0.1	0.67	1
S10	0.7	0.7	0.5	0.5	0.9	0.5	0.5	1
Suitability	Good	Good	Below Average	Poor	Good	Good	Poor	Excellent

Annex AN7.6.2 – Selected Pond Photographs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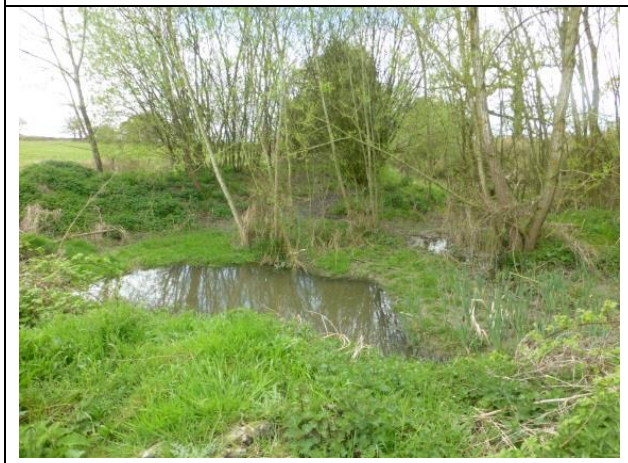	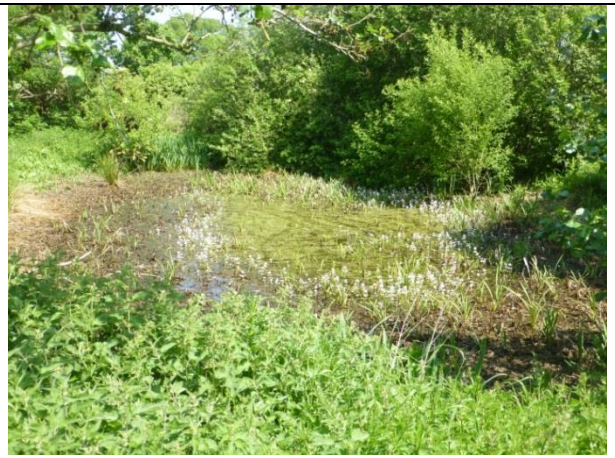	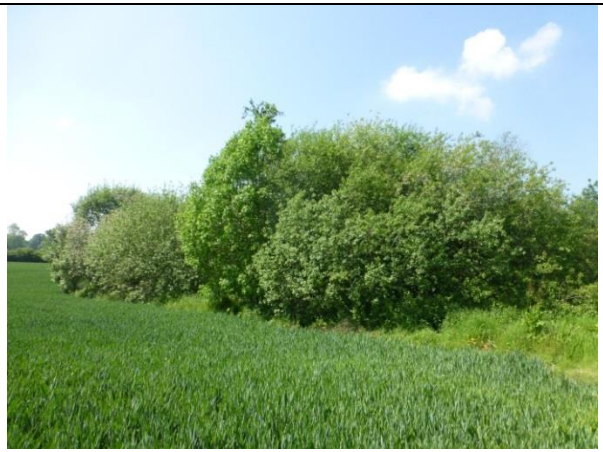
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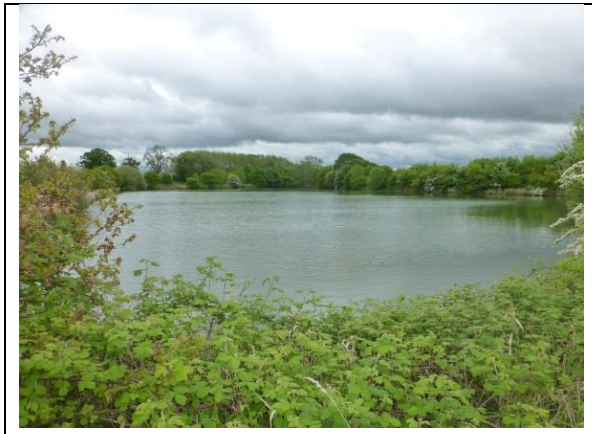	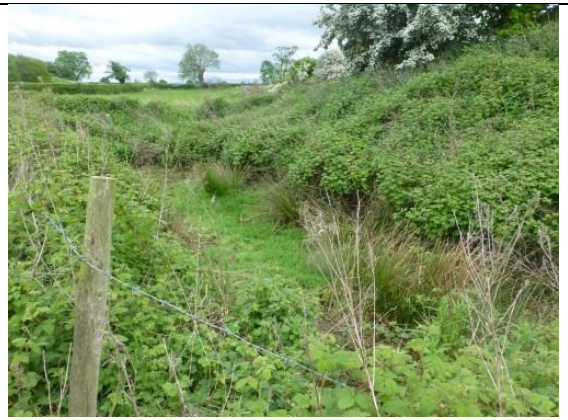	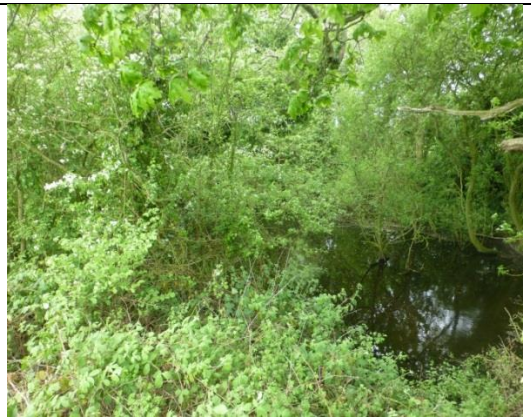
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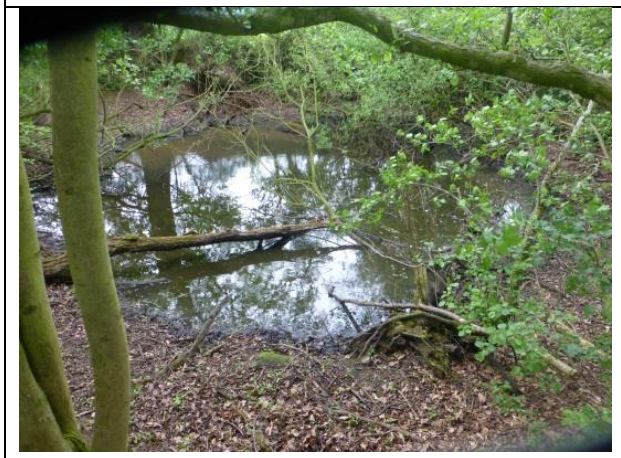		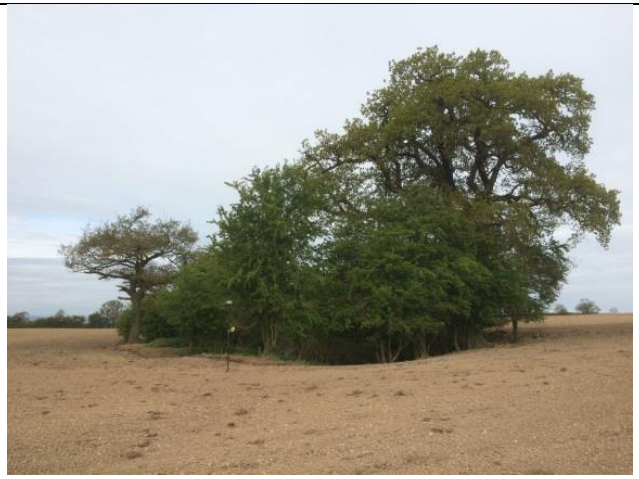
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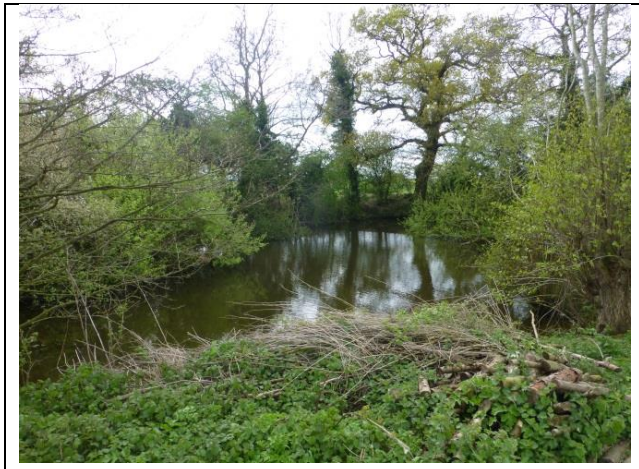	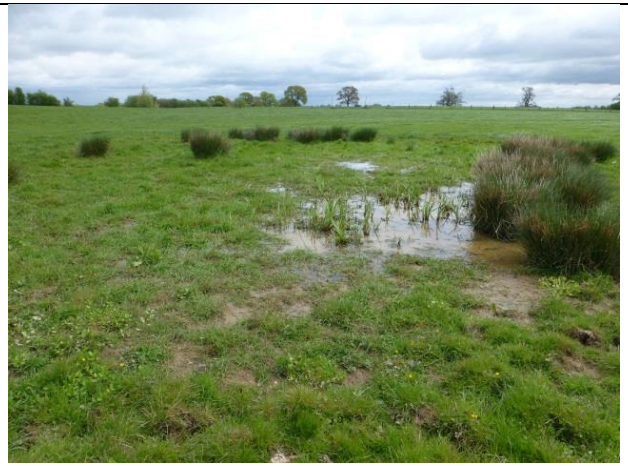	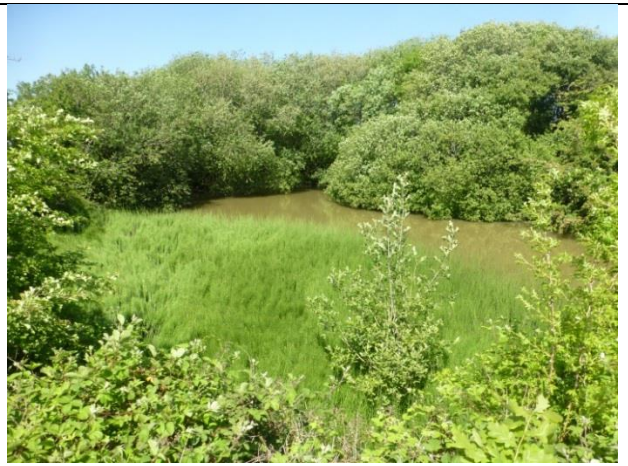
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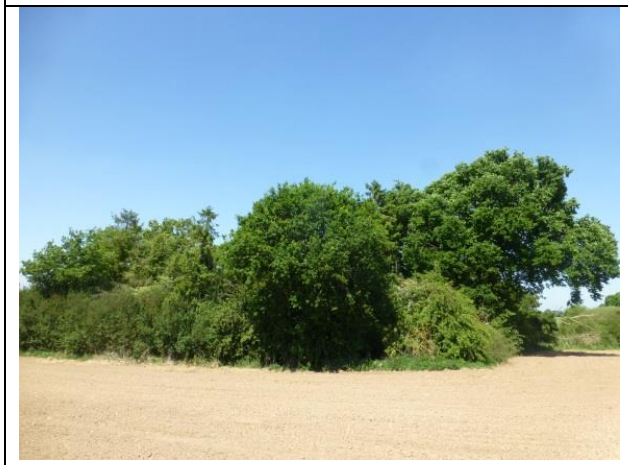
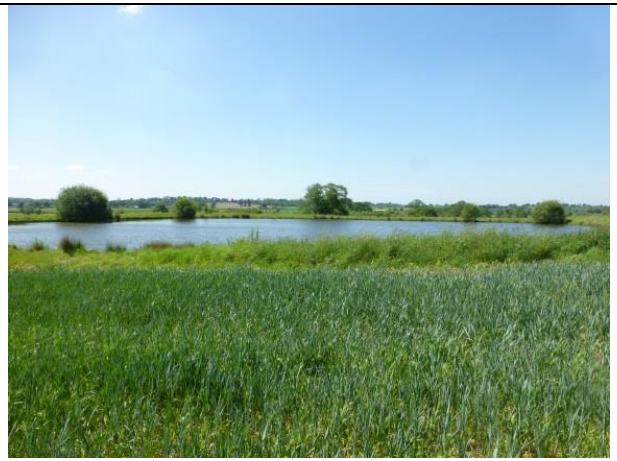
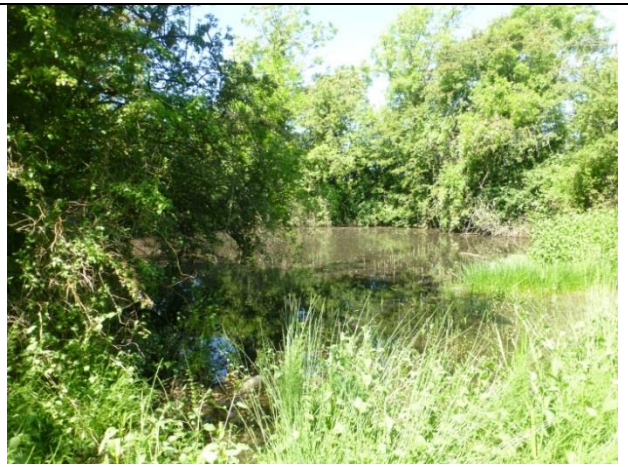
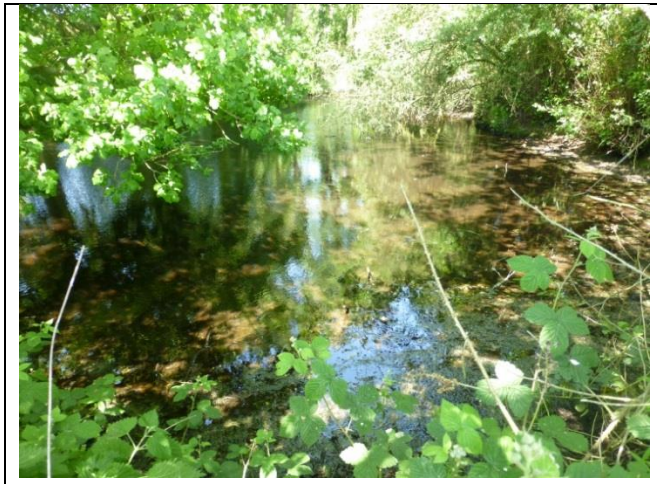
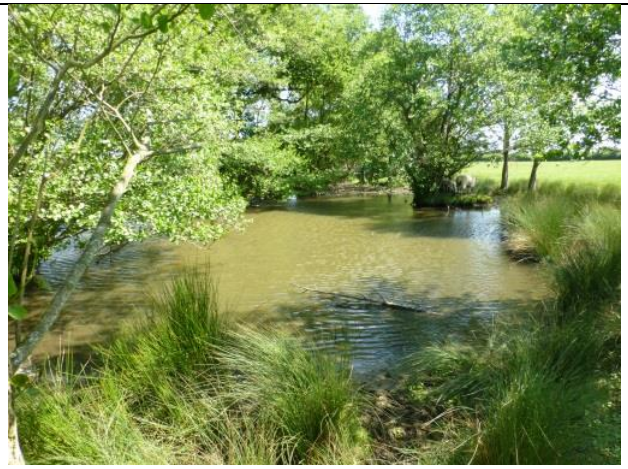
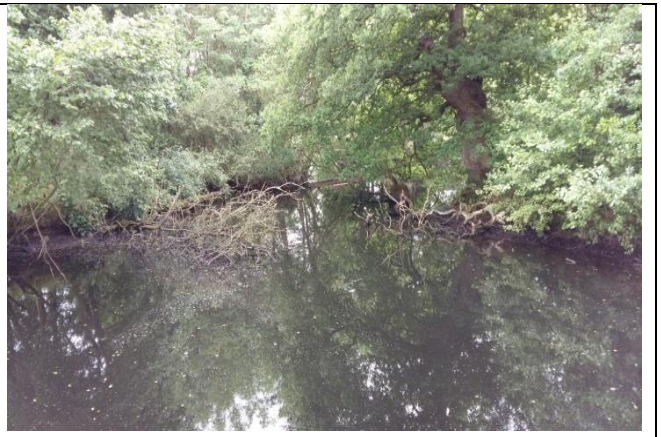
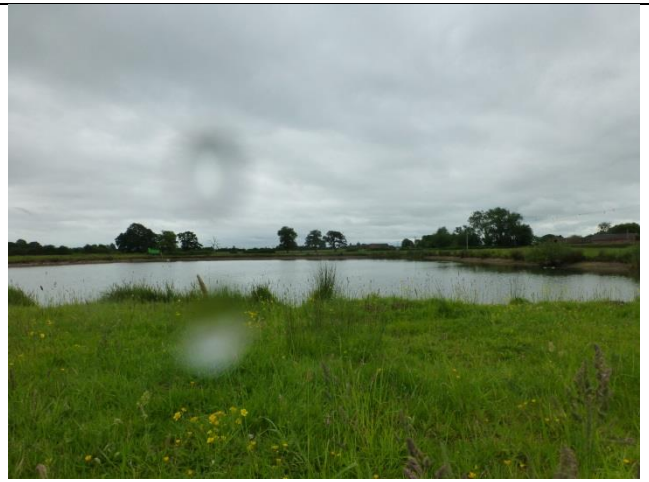
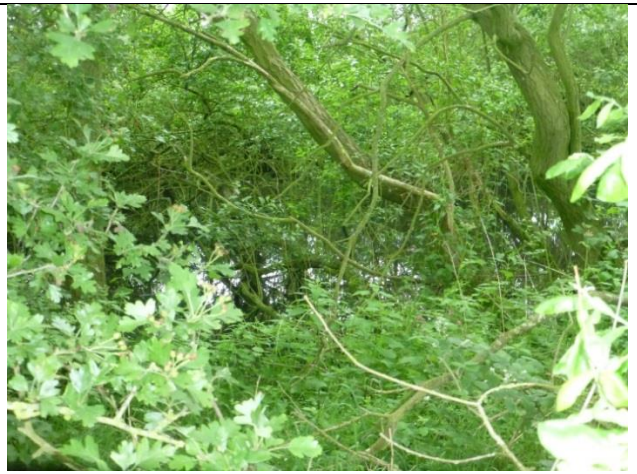
		
Scrub around P15	P16	P17

		
P18	P19	P20
		
P25 DRY	P28	P30

ANNEX AN7.6.3 – eDNA Laboratory Reports

SureScreen Scientifics

Folio No: E0515
Report No: 1
Order No: [No PO Received on Paperwork]
Client: Avian Ecology
Contact: Tom Winter
Contact Details: tom.winter@avianecology.co.uk
Date: 10/05/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 03/05/2017
Date Reported: 10/05/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
31392	Pond 889 Inked	N/A	Pass	Pass	Pass	Negative	0
31394	Pond 12	N/A	Pass	Pass	Pass	Positive	1
31396	P2	N/A	Pass	Pass	Pass	Negative	0
31397	P1	N/A	Pass	Pass	Pass	Negative	0
31398	Pond 13	N/A	Pass	Pass	Pass	Negative	0
31399	Pond 10	N/A	Pass	Pass	Pass	Negative	0
31403	P0a	N/A	Pass	Pass	Pass	Negative	0

SureScreen Scientifics

Folio No:	E0816
Report No:	1
Order No:	AE17_012
Client:	Avian Ecology
Contact:	Zac Hinchcliffe, Catherine Baldock
Contact Details:	zac.hinchcliffe@avianecology.co.uk, cathy.baldock@avianecology.co.uk
Date:	25/05/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 23/05/2017
Date Reported: 25/05/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
31401	North Shropshire Line	-	Pass	Pass	Pass	Negative	0

SureScreen Scientifics

Folio No:	E0969
Report No:	1
Order No:	AE-17-040
Client:	Avian Ecology
Contact:	Tom Winter, Catherine Baldock
Contact Details:	tom.winter@avianecology.co.uk, cathy.baldock@avianecology.co.uk
Date:	07/06/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 05/06/2017
Date Reported: 07/06/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
31828	North Shropshire	-	Pass	Pass	Pass	Positive	1
32894	Pond 23 & 24, Shropshire Lines	-	Pass	Pass	Pass	Positive	8

SureScreen Scientifics

Folio No: E0968
Report No: 1
Order No: AE-17-043
Client: Avian Ecology
Contact: Tom Winter
Contact Details: tom.winter@avianecology.co.uk
Date: 16/06/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 05/06/2017
Date Reported: 16/06/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
32889	Pond 16, Shropshire Lines	-	Pass	Pass	Pass	Negative	0
32890	Pond 15, Shropshire Lines	-	Pass	Pass	Pass	Negative	0
32893	Pond 27, Shropshire Lines	-	Pass	Pass	Pass	Positive	12
32895	Pond 28, Shropshire Lines	-	Pass	Pass	Pass	Negative	0
32896	Pond 14, Shropshire Lines	-	Pass	Pass	Pass	Positive	10
32897	Pond 30, Shropshire Lines	-	Pass	Pass	Pass	Negative	0

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32898	Pond 19, Shropshire Lines	-	Pass	Pass	Pass	Positive	1
32899	Pond 17, Shropshire Lines	-	Pass	Pass	Pass	Positive	11
32900	Pond 18, Shropshire Lines	-	Pass	Pass	Pass	Positive	5

SureScreen Scientifics

Folio No: E2982
Report No: 1
Order No: ae18043
Client: Avian Ecology
Contact: Rachel Hughes
Contact Details: rachel.hughes@avianecology.co.uk
Date: 04/06/2018

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 21/05/2018
Date Reported: 04/06/2018
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1242	Pond 11a	-	Pass	Pass	Pass	Negative	0
1243	Pond 11b	-	Pass	Pass	Pass	Negative	0
1255	Pond 11d	-	Pass	Pass	Pass	Positive	4
1256	Pond 11c	-	Pass	Pass	Pass	Positive	4

SUMMARY

When Great Crested Newts (GCN); Triturus cristatus inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the

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Company Registration No. 08950940

unique sample identity.

RESULTS INTERPRETATION

Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference – Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. It may be assumed that small fractions of positive analyses suggest low level presence but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in

our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Sam Humphrey **Approved by:** Harry Neal

End Of Report