

Ecology Report



Great Crested Newt Survey, Solar Farm on Land South of Berrington

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Where field investigations have been carried out, these have been restricted to a level of detail required to achieve the stated objectives of the work.

This work has been undertaken in accordance with the quality management system of RSK ADAS Ltd.

Revision History

Revision	Date	Amendment
MPT69105-501(00)	October 2021	INITIAL REPORT

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1 Summary

ADAS was commissioned by Eenergy International Ltd. to undertake a Preliminary Ecological Appraisal (PEA) in March 2021 of a plot of land to the south-west of Berrington for the construction of a solar farm. The site was found to contain suitable terrestrial habitats to support great crested newts (GCN) (*Triturus cristatus*).

A desktop review of mapping and aerial photographs identified 21 ponds within 500m. In March 2021, as part of the PEA survey, Habitat Suitability Index (HSI) assessments (Oldham et al, 2000) were undertaken of all accessible ponds on or within 500m of the site and not separated from the site by barriers to GCN movement, to determine their suitability to support GCN.

Following this, five ponds (Ponds 2, 3, 5, 13 and 15) were identified as requiring further surveys in the form of eDNA analysis.

Following the eDNA surveys, three of the ponds (Ponds 3, 13 and 15) returned positive results for the presence of GCN DNA. As a result, further population estimate surveys of these ponds were carried out following best practice guidance (English Nature, 2001). Despite the positive eDNA result for all three ponds, no GCN were identified during any of the population estimate surveys in Ponds 3 or 15. A 'small' population of GCN was recorded in Pond 13, with a peak count of ten newts recorded. It is assumed that the presence of GCN DNA in Pond 15, but absence of newts recorded during the population estimate surveys is due to the presence of newts in Pond 13 utilising Pond 15 but in such small numbers, they could not be recorded using traditional presence/likely absence methods. The positive eDNA result in Pond 3 but lack of GCN during subsequent population estimate surveys is assumed to be a false positive.

With consideration to the scale of the proposed work and size of the newt population, a European protected species mitigation (EPSM) licence is not required. Instead, a precautionary approach has been recommended and a non-licensed method statement (NLMS) should be prepared by a suitably experienced ecologist prior to the commencement of works on site, incorporated appropriate mitigation measures including supervision of the proposed works on site by a suitably licensed ecologist acting as Ecological Clerk of Works (ECoW).

In following the recommendations outlined in this report, the proposed works will comply with current legislation.

2 Introduction

2.1 Background

Econergy International Ltd proposes to construct a solar farm site on arable land located to the south-west of Berrington, Shrewsbury (Grid Reference: SJ 52741 07125).

In March 2021 ADAS was commissioned by Econergy International Ltd to undertake a Preliminary ecological appraisal (PEA) (ADAS, 2020) to assess the suitability of the habitats in and around the site for protected species. The site was found to contain suitable terrestrial habitats for great crested newts (GCN) (*Triturus cristatus*). A desk-study review of local maps and aerial photographs identified a total of 21 waterbodies within 500m of the site, with two located within the site boundary.

The construction works and vegetation clearance work at the crossing has the potential to disturb GCN should they be present and so a further Habitat Suitability Index (HSI) assessment of the ponds was recommended to evaluate their suitability to support GCN. Any ponds with an HSI score of 'Average' suitability or better will require further surveys including eDNA surveys. Ponds with positive eDNA results will need six traditional survey visits to determine the size of the population of GCN.

This report documents the methodology and results of the HSI assessment, eDNA and GCN population estimate surveys.

2.2 Survey Objectives

The objectives of GCN eDNA and subsequent population estimate surveys were to:

- identify any waterbodies which are currently being used as aquatic habitat by GCN;
- where present, identify if GCN are breeding in any waterbodies;
- estimate the population size, if GCN are recorded in any waterbodies; and
- propose mitigation measures required to ensure any proposed works do not negatively affect GCN.

The survey effort and techniques were carried out in accordance with the Great Crested Newt Mitigation Guidelines (English Nature (now Natural England), 2001).

2.3 Site Description

The site was located c. 1.36km south-west of Berrington, Shrewsbury (Grid Reference: SJ 52741 07125). The site was approximately 44.09 ha. The site was bound by narrow single-track roads along the eastern, northern and western boundary which led to arable fields in the east, livestock fields to the north and a small woodland to the south that concealed Cound Brook which is approximately 3m

wide and relatively fast flowing. The wider area generally consisted of farmland with a settlement to the north east (as shown in Figure 1).



Figure 1. Site location and wider landscape (site indicated by red line boundary)
Imagery taken from Microsoft Virtual Earth (Esri). March 2021.

2.4 Proposed Works

The site is to be developed into a solar farm. At this time no further details of the scheme are known but it is anticipated that boundary features, trees and the ponds on site will be retained. This report assumes that all internal aspects (including the ponds) will be kept and that the surrounding hedgerows (and centre track) will be retained.

3 Legislative Background

The domestic legislation protecting GCN arises largely from the Habitats Directive, which has a central aim to restore scheduled species to a favourable conservation status.

GCN are protected by UK and European legislation. The Wildlife and Countryside Act 1981 (as amended) makes it an offence to:

Intentionally kill, injure or take a GCN,

Possess or control any live or dead specimen or anything derived from a GCN,

Intentionally or recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a GCN, and

Intentionally or recklessly disturb a GCN while it is occupying a structure or place which it uses for that purpose.

In addition, The Conservation of Habitats and Species (Amendment) Regulations 2017 make it an offence to:

Deliberately capture or kill a GCN,

Deliberately disturb a GCN,

Damage or destroy a breeding site or a resting place of a GCN, and

Keep, transport, sell or exchange or offer for sale or exchange a live or any part of a GCN.

GCN are a species of principle importance under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006. In England, all public bodies must have regard for species and habitats of principle importance when carrying out their duties.

4 Methodology

4.1 Habitat Suitability Index

Accessible Ponds located within 500m of the site and not separated by significant barriers to dispersal were assessed for their suitability for supporting GCN using the standard Habitat Suitability Index (HSI) methodology as described by Oldham et al. (2000). The HSI assessment was conducted on the 25th of March at the same time as the PEA survey by ADAS surveyor Katherine Coope BSc, MSc, ACIEEM and assisted by Seasonal Ecologist Rachel Richards BSc (hons) qualifying member of CIEEM.

An HSI is a numerical index, between 0 and 1, 0 representing unsuitable habitat and 1 representing optimal habitat. The HSI for the great crested newt incorporates ten suitable indices, all of which are factors thought to affect great crested newts. The 10 indices include:

1. Geographical location;
2. Pond area;
3. Permanence (how regularly does the pond dry out);
4. Water quality;
5. Shade;
6. Water fowl (population density);
7. Fish (stocking density);
8. Pond count (number of ponds within 1km);
9. Terrestrial habitat (quality of terrestrial habitat local to the pond); and
10. Macrophytes (% cover of vegetation cover during the newt breeding season March-May).

Each of the indices are given a score ranging from 0.01-1 and incorporated into the formula below which give an overall score for the pond:

$$\text{HSI} = (\text{SI1} \times \text{SI2} \times \text{SI3} \times \text{SI4} \times \text{SI5} \times \text{SI6} \times \text{SI7} \times \text{SI8} \times \text{SI9} \times \text{SI10})^{1/10}$$

The Calculated HSI score will range between 0-1 and the score indicates different habitat suitability:

<0.5 = poor

0.5-0.59 = below average

0.6-0.69 = average

0.7-0.79 = good

>0.8 = excellent

4.2 eDNA Survey Method

GCN will travel up to 500m from their breeding ponds, although distances vary depending on several factors including the quality of their terrestrial habitat. Therefore, any populations of newts using the ponds within 500m of the works area could potentially be impacted by the works. eDNA testing is used to determine the potential current or recent presence of great crested newts in ponds. However, following the HSI assessment of accessible ponds within 500m of the site (Ponds 1, 2, 3, 4, 5, 6, 7, 9, 11 and 13) and given the limited scale of the proposed works, the survey area was limited to accessible ponds within 250m of the site and not beyond.

The ADAS eDNA survey protocol involves collecting 20 water samples from each pond, then using a pipette to fill 6 conical tubes containing 35ml of preserving fluid with 15ml of pond water. These conical tubes are then sent to the laboratory for eDNA testing using Polymerase Chain Reaction (PCR). This is in accordance with Technical advice note for field and laboratory sampling of GCN environmental DNA (Biggs et al. 2014). If the results of the eDNA test indicate presence of great crested newts, six visits will then be required to produce a population class estimate.

Following the HSI assessment access to Pond 15 was granted and an eDNA survey of Ponds 2, 3, 5, 13 and 15, situated within 250m of the works area, was conducted by ADAS Ecological Consultants Oli Bulpitt BSc (Hons) MSc (2019-42924-CLS-CLS) and Seasonal Ecological Consultant Rachel Richards BSc (hons) and qualifying member of CIEEM, on 15th April 2021.

4.3 Great Crested Newt Survey Method

The GCN surveys were undertaken based on the standard methodology described in the Great Crested Newt Mitigation Guidelines (English Nature, 2001). The surveys were carried out by licensed GCN surveyors Rebecca Sambrook (survey licence: 2016-23319-CLS-CLS), Mark Benson, Chris Gosset (2017-28794-CLS-CLS), Luke Osman and Clare Christian.

Six visits were undertaken to all ponds which returned positive eDNA scores for the presence of GCN eDNA (Ponds 3, 13 and 15) between the 15th of May and 28th of June 2021, with at least one week between survey visits, to determine the presence/absence of GCN. A minimum of three survey methods were used for each pond on every visit. The survey methods used included a combination of:

Bottle trapping ;

Egg searching;

Torching; and

Netting.

The vegetation along the water margins was searched for the presence of newt's eggs. If GCN eggs were identified, egg searches and netting ceased in the breeding pond.

A dip net with a 2-4mm mesh was used to sweep the waterbody for 15 minutes per 50m of shoreline. High power torches (1,000,000 candle power) were used after dusk by surveyors who walked slowly around the waterbody shining torches into the water searching for newts. Particular attention was paid to any marginal vegetation or areas where newts could congregate. Any amphibians seen were identified, counted and where possible sexed.

Standard 2L bottle traps were installed at intervals of 2m around the shore of the pond (pond 3 – 10 bottle traps). The canes were secured to the bottle traps either with elastic bands or thread through holes on either side of the bottle trap. The traps were checked to ensure that at least one third of the trap contained an air to prevent the risk of newts drowning. The traps were left over night and were checked between 07:30 and 09:00 the following morning. All newts were recorded to the level of species, sex, life stage (adult, subadult, larvae etc) and were released back into the pond. All bottle traps and canes from the pond were counted in and counted out of the pond.

4.4 Population Size Class Method

The assessment of population size and class was carried out following the standard guidance described in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

This method stipulates that the maximum adult count per pond per night gained through torch survey or bottle-trapping can be used and expressed as 'Peak counts' per pond. This count enables the populations to be classified as:

'low' for maximum counts up to 10;

'medium' for maximum counts between 11 and 100; and

'large' for maximum counts over 100.

4.5 Limitations and Constraints

The wildlife and wider ecological value of a site can change. The report presented here is a statement of the findings of surveys carried out from May to June 2021. Any appreciable delay in making reference to this report may necessitate a re-survey.

During the HSI not all ponds could be assessed due to surveyors being denied access by landowners, Ponds 8, 12, 14, 15 or 16 were not surveyed at this time. Access to Pond 15 was granted for eDNA surveys and populations surveys.

All surveys took place during the optimal time of year and under suitable weather conditions.

During the 5th of May overnight temperature dropped below 5°C so conditions were unsuitable for bottle trapping. Other survey methods were used during this survey so three methods were used during this survey.

Throughout the surveys for Pond 3 it was noted that there was a high-level water duckweed (*Lemna* sp.) covering the surface of the pond. This affected the visibility of the pond during torching, during the surveys the surveyors would use net to clear area of pond. This was done 15 minutes before torching so not stir up sediment.

5 Results

5.1 Habitat Suitability Index

Twenty-one ponds were identified on or within 500m of the site during the PEA (ADAS, 2021) and HSI assessments were carried out for all ponds on and within 500m of the site that were accessible at the time of survey and not beyond impassable barriers to GCN movement. Table 1 below, provides a summary of the HSI scores. For pond locations see Appendix 1.

Pond 10 was dry at the time of the HSI assessment, and no access was possible to Ponds 8, 12, 14, 15 or 16. Ponds 17, 18, 19, 20 and 21 were all separated from the site by Cound Brook which was considered to be a barrier to GCN movement.

Table 1: Summary of Habitat Suitability Index results

Pond Number	Distance and direction from site	HSI Score (0-1)	Suitability
1	On Site	0.46	Poor
2	On Site	0.57	Below Average
3	124m North	0.68	Average
4	238m North	0.34	Poor
5	231m North East	0.78	Good
6	490m North West	0.53	Below Average
7	447m North East	0.53	Below Average
9	387m North East	0.29	Poor
11	365m North East	0.78	Good
13	219m East	0.50	Below Average

5.2 eDNA survey

Following the HSI, owing to the anticipated impact of the proposed works, the survey area for further surveys was reduced to 250m from the site. In addition, following the HSI surveys, access to Pond 15 was granted. Therefore, eDNA surveys were carried out on all accessible ponds within 250m of the site that scored 'Below Average' or higher in the HSI assessments (Ponds 2, 3, 5 and 13) as well as Pond 15. The eDNA surveys showed that only three ponds surveyed had a positive result for GCN (Ponds 3, 13 and 15). A summary of the eDNA result can be found in the table below and the full results can be found in Appendix 3.

Table 2: eDNA results for waterbodies within 500m of the works area

Pond / Ditch Number	eDNA Result	Traditional Surveys Required?
2	Negative	No
3	Positive	Yes
5	Negative	No
13	Positive	Yes
15	Positive	Yes

5.3 Population Assessment

Six surveys were undertaken at all ponds which returned a positive eDNA result (Ponds 3, 13 and 15). During these surveys and despite the positive eDNA result, no GCN were identified in Ponds 3 or 15 and no GCN eggs were recorded in these ponds either. GCN and GCN eggs were recorded in Pond 13, confirming that Pond 13 is a breeding pond.

Low numbers of smooth newts (*Lissotriton vulgaris*) were noted during surveys to Pond 13. Weather conditions were suitable during all surveys.

Estimates of GCN population size were derived from the survey results provided in Appendix 4. Table 3 below summarises the GCN peak numbers recorded.

Table 3: GCN peak count of ponds surveyed

Pond	Peak Count	Population Size Class
Pond 3	0	None
Pond 13	10	Small
Pond 15	0	None

6 Impact Assessment

6.1 Rapid Risk Assessment

Despite the positive eDNA results, traditional surveys found no GCN in Ponds 3 and 15. Given the close proximity of Pond 15 to Pond 13, where GCN were identified using traditional survey techniques, it is assumed that the positive eDNA result for Pond 13 reflects a transient population of GCN in this pond present in such low numbers that they could not be recorded using traditional techniques. The positive eDNA result for Pond 3 is considered likely to be a false positive considering it is not within close proximity to a pond where GCN presence has been confirmed.

In Pond 13 a low population of GCN and eggs were also found during the surveys, identifying it as a GCN breeding pond. In order to assess the risk of committing an offence in relation to GCN the Natural England Great Crested Newt Licence rapid assessment tool was used. This assessment assumes no mitigation measures are put in place for the works.

Table 4: Rapid risk assessment of the likely impact to GCN assuming no mitigation

Component	Likely effect (select one for each component; select the most harmful option if more than one is likely; lists are in order of harm, top to bottom)	Notional offence probability score
Great crested newt breeding pond(s)	No effect	0
Land within 100m of any breeding pond(s)	No effect	0
Land 100-250m from any breeding pond(s)	0.1 - 0.5 ha lost or damaged	0.1
Land >250m from any breeding pond(s)	0.01 - 0.1 ha lost or damaged	0.001
Individual great crested newts	Minor disturbance of newts	0.5
	Maximum:	0.5
Rapid risk assessment result:	AMBER: OFFENCE LIKELY	

Given the lack of GCN and eggs found during surveys it is assessed that Ponds 3 and 15 do not constitute GCN breeding ponds. This means that the likelihood of an offence in relation to GCN is low/negligible for these ponds. However, Pond 13 is a confirmed breeding pond and is located within 250m of the proposed works. GCN typically migrate 250m from a breeding pond. Terrestrial movement of GCN between Pond 13 and to the Site is possible via connective habitat in the form of field boundary hedgerows. However, given the distance of the works to the pond (219m) and the limited impact of the proposed works a European protected species mitigation (EPSM) is not required for the works however, a precautionary approach should be taken, and works should be undertaken under a non-licensed method statement (NLMS). Natural England advocates the use of avoidance measures to minimise the impact of a proposed activity on wildlife (Natural England 2012). Licensing

should be seen as the last resort where all other alternative ways of avoiding impacts on the species have been discounted.

The NLMS will outline mitigation and working methods in order to manage impacts and ensure the works do not result in an offence. In order to mitigate the potential risks to GCN the following avoidance measures are recommend.

Prior to the start of the works, the ecologists and contractors will agree on a proved access route to the work area to avoid on minimise tracking through habitats where GCN might be present.

Vegetation clearance and any ground excavations to be kept to a minimum required to facilitate access and enable works.

Any excavations should be backfilled on the same day (preferably) or securely capped overnight to prevent possible entrapment of GCN.

Other measure that should be considered are:

As the works will not disturb hibernation habitat works should be designed to be carried out during the winter months when GCN are in hibernation (November to February inclusive).

A suitably qualified and experienced ecologist should provide a toolbox talk to contractors on site prior to the commencement of works to ensure contractors can readily identify GCN and understand the legal protection afforded them.

During the works a suitably qualified and experienced ecologist will undertake a fingertip search of the habitat to be removed for great crested newt. If any GCN are identified works will cease.

To minimise disturbance to any great crested newts utilising adjacent habitats, works should take place only during daylight hours and movement and storage of equipment and machinery should be kept as far away from woodland habitat as possible.

The above measures combined with the short-term, localised nature of the works means that, in the balance of probability it is considered unlikely that GCN will be adversely affected by the proposed works and that the risk of committing an offence is highly unlikely. In these circumstances undertaking significant mitigation measures, such as fencing, trapping and re-location, would be considered unnecessary and disproportionate. However, in the unlikely event a GCN is encountered during the works, all works should cease immediately, and the appointed ecologist will be consulted on how works may proceed, and an EPSM licence may be required.

7 Conclusion

A low population of GCN was found to be present in Pond 13, within 250m of the site. The surveys were undertaken at the appropriate time of year and in suitable weather conditions and the findings are considered to be an accurate representation of the GCN population within this area.

The eDNA results were positive for pond 3 and 15 but the population estimate surveys recorded no GCN or GCN eggs during the surveys. Pond 15, given its proximity to Pond 13, was deemed likely to support a transient population of GCN in low numbers; whilst the eDNA result for Pond 3 was deemed likely to be a false positive. Neither pond was deemed to be a GCN breeding pond.

Owing to the presence of GCN, there is the potential that the proposed works could result in the disturbance of GCN. However, given the limited scale of the proposed works and the distance between the site and Pond 13 (219m) a EPSM licence for GCN is deemed unnecessary. Instead, works should be carried out following a NLMS. The method statement will detail appropriate mitigation measures to avoid /minimise the risk of disturbing GCN. Works under the NLMS should be supervised on site by a suitably qualified ecologist acting as Ecological Clerk of Works

In the unlikely event a GCN is encountered during the works, all works should cease immediately, and the appointed ecologist consulted on how works may proceed, and an EPSM may be required.

Following the recommendations outlined in this report, the proposed works will comply with current legislation and reasonably protect GCN and their habitats from the impacts of the permitted development.

8 References

ADAS. (2021). Preliminary Ecological Appraisal: Solar Farm on Land South of Berrington, Shrewsbury MPT69105-501(00).

English Nature. (2001). Great Crested Newt Mitigation Guidelines. English Nature, Peterborough.

Oldham, R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000) Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*). *Herpetological Journal*. 10 (4), 143-155.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., 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. Freshwater Habitats Trust, Oxford

Natural England (2012) European Protected Species: Mitigation Licensing -How to get a licence WML-G12.

Appendix 1: Pond Location Map

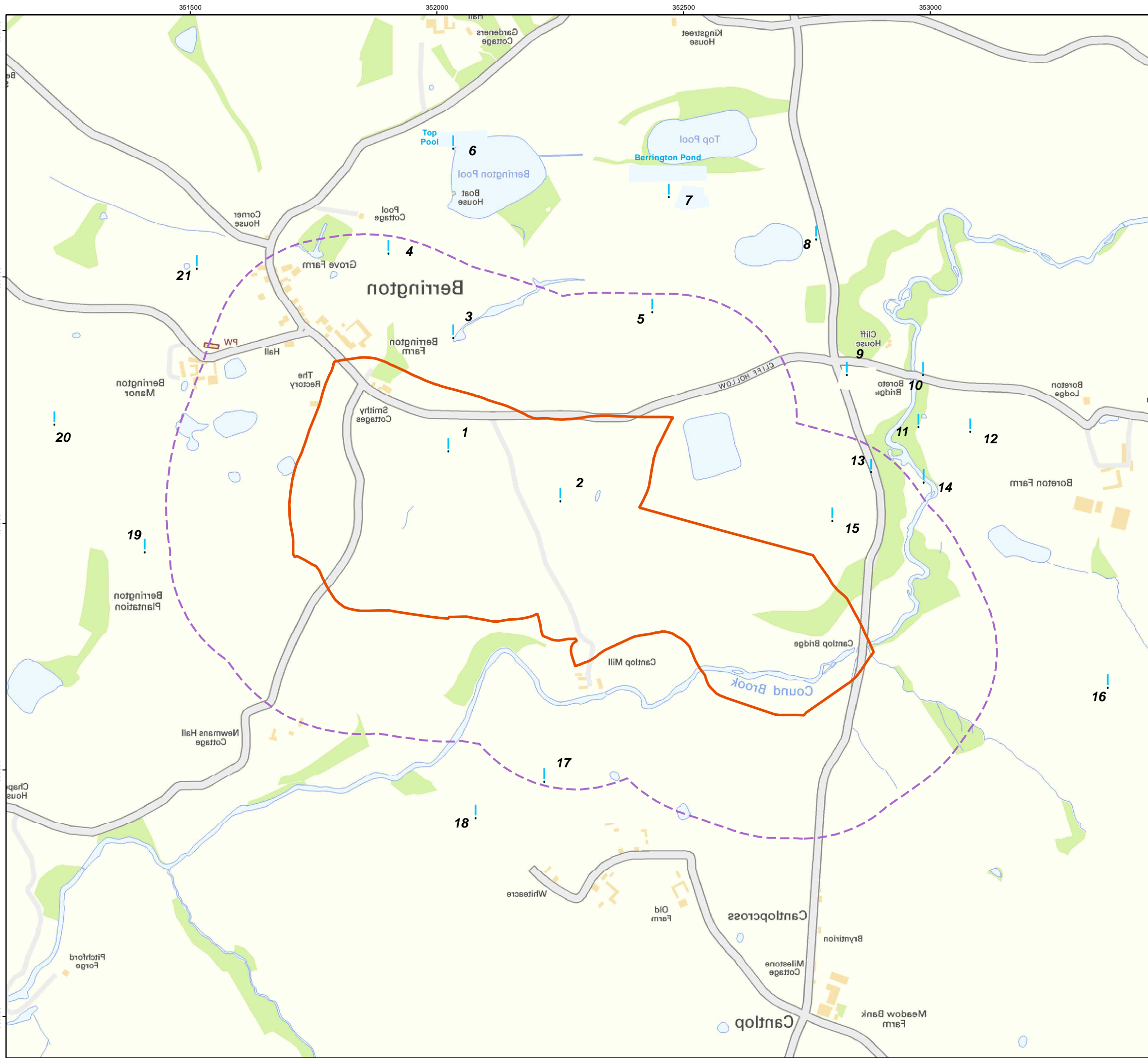
See following page.

Econergy International Ltd

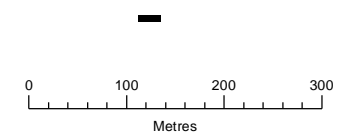
Solar PV Development,
Berrington Farm, Shropshire

Map of ponds within 500m of site

-  Site boundary
-  250m buffer
-  Pond location



Drawn by Paul Taylor 23/09/2021, Verified by Joseph Dyson 23/09/2021



Scale 1:7,750 at A3 size

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Appendix 2: HSI Results

Suitability Indices	Criteria	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Pond 9	Pond 11	Pond 13
Sl ₁ Location	Location within GCN range	1	1	1	1	1	1	1	1	1	1
Sl ₂ Pond area	Pond surface area	na	0.6	0.6	na	1	na	na	0.2	na	1
Sl ₃ Pond drying	Frequency of pond drying, annually to never	0.9	0.5	0.9	0.1	0.9	0.9	0.9	0.1	0.9	0.5
Sl ₄ Water quality	Invertebrate diversity, and submerged plants	0.33	0.33	0.33	0.33	0.77	1	1	0.1	0.67	0.67
Sl ₅ Shade	Estimate of percentage perimeter shaded	1	0.4	0.6	0.2	0.6	1	1	1	1	1
Sl ₆ Fowl	Any waterfowl present, or signs of impact	0.01	1	1	0.01	0.67	0.01	0.01	0.67	0.67	0.01
Sl ₇ Fish	Any evidence of fish present	0.67	1	1	1	1	0.67	0.67	1	1	1
Sl ₈ No. of Ponds	Number of ponds within 1km (excluding pond surveyed)	1	1	1	0.95	1	1	1	1	1	1
Sl ₉ Terrestrial habitat	Quality of terrestrial habitat	0.67	0.33	0.67	1	1	1	1	0.01	0.67	1
Sl ₁₀ Macrophytes	Percentage of pond surface area occupied by macrophyte cover	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	HSI Score	0.46	0.57	0.68	0.34	0.78	0.53	0.53	0.29	0.78	0.50

Appendix 3: eDNA Results

See following page.

Client: Rachel Richards,
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Sample ID: ADAS-0486 Condition on Receipt: Good Volume: Passed
Client Identifier: p13 Berrington Description: pond water sample
Date of Receipt: 19/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control [§]	2 of 2	Real Time PCR	21/04/2021
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	21/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 22/04/2021 Date of issue: 22/04/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-0487 Condition on Receipt: Good Volume: Passed
Client Identifier: 5 Berrington solar farm Description: pond water sample
Date of Receipt: 19/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	20/04/2021
Degradation Control [§]	2 of 2	Real Time PCR	20/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 22/04/2021 Date of issue: 22/04/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Rachel Richards,
ADAS



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Sample ID: ADAS-0488 Condition on Receipt: Good Volume: Passed
Client Identifier: 2 Berrington solar farm Description: pond water sample
Date of Receipt: 19/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control [§]	2 of 2	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-0489 Condition on Receipt: Good Volume: Passed
Client Identifier: 15 Berrington solar farm Description: pond water sample
Date of Receipt: 19/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	20/04/2021
Degradation Control [§]	2 of 2	Real Time PCR	20/04/2021
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	20/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 22/04/2021 Date of issue: 22/04/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-0490 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: p3 Berrington Description: pond water sample
Date of Receipt: 19/04/2021 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control [§]	2 of 2	Real Time PCR	21/04/2021
Great Crested Newt*	10 of 12 (GCN positive)	Real Time PCR	21/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 22/04/2021 Date of issue: 22/04/2021

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

Appendix 4: Pond Survey Results

Great Crested Newt Record Form

Site & Pond Identification

3

Map Ref (OS)

Dates		05/05/2021	12/05/2021	17/05/2021	24/05/2021	02/06/2021	28/06/2021
Temp at Start (°C)		6.00	13.00	11.00	11.00	18.00	16.00
GCN BT Catch	M	NA	0	0	0	0	0
	F	NA	0	0	0	0	0
	Juv.	NA	0	0	0	0	0
	Sum	NA	0	0	0	0	0
GCN Net Catch	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Refugia Search	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Eggs	Y/N	N	N	N	N	N	N
GCN Torch Survey	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
Turbidity (0 = clear, 5 = very turbid)		1	1	1	1	1	1
Veg Cover (0 = no veg, 5 = water completely obscured)		5	5	4	5	4	5
Peak Total Adult		0	0	0	0	0	0
Surveyor(s) Initials		RS, MB	RS, MB	CG, CC	RS, MB	CG, LO	RS, MB
Notes		Pond shallow with high duckweed cover, too cold to BT	High duckweed cover	High duckweed and algae cover	High duckweed cover		High duckweed cover

Great Crested Newt Record Form

Site & Pond Identification

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Map Ref (OS)

Dates		05/05/2021	12/05/2021	17/05/2021	24/05/2021	02/06/2021	28/06/2021
Temp at Start (°C)		6.00	13.00	11.00	11.00	18.00	16.00
GCN BT Catch	M	NA	0	7	6	2	1
	F	NA	0	3	4	0	1
	Juv.	NA	0	0	0	0	0
	Sum	NA	0	10	10	2	2
GCN Net Catch	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Refugia Search	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Eggs	Y/N	Y	NA	NA	NA	NA	NA
GCN Torch Survey	M	0	0	1	0	0	1
	F	0	0	1	1	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	2	1	0	1
Turbidy (0 = clear, 5 = very turbid)		1	1	2	1	2	1
Veg Cover (0 = no veg, 5 = water completely obscured)		1	1	1	1	1	1
Peak Total Adult		0	0	12	11	2	3
Surveyor(s)		RS, MB	RS, MB	CG, CC	RS, MB	CG, LO	RS, MB
Notes		Too cold to BT overnight		1male smooth newt, 2 common frogs	1 female smooth new t	1male smooth, 1 unknown smooth, 6 common frogs	2 female smooth new ts

Great Crested Newt Record Form

Site & Pond Identification

15

Map Ref (OS)

Dates		05/05/2021	12/05/2021	17/05/2021	24/05/2021	02/06/2021	28/06/2021
Temp at Start (°C)		6.00	13.00	11.00	11.00	18.00	16.00
GCN BT Catch	M	NA	0	0	0	0	0
	F	NA	0	0	0	0	0
	Juv.	NA	0	0	0	0	0
	Sum	NA	0	0	0	0	0
GCN Net Catch	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Refugia Search	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
GCN Eggs	Y/N	N	N	N	N	N	N
GCN Torch Survey	M	0	0	0	0	0	0
	F	0	0	0	0	0	0
	Juv.	0	0	0	0	0	0
	Sum	0	0	0	0	0	0
Turbidity (0 = clear, 5 = very turbid)		1	1	1	1	1	1
Veg Cover (0 = no veg, 5 = water completely obscured)		5	1	1	1	1	1
Peak Total Adult		0	0	0	0	0	0
Surveyor(s)		RS, MB	RS, MB	CG, CC	RS, MB	CG, LO	RS, MB
Notes		Too cold to BT overnight		A lot of algae around the pond margins			