

Heckington Fen Solar Park

EN010123

Environmental Statement | Volume 3: Technical Appendices

Appendix 8.11: Great Crested Newts – Energy Park and Cable Route Corridor

Applicant: Ecotricity (Heck Fen Solar) Limited

Document Reference: 6.3.8.11

Pursuant to: APFP Regulation 5(2)(a)

February 2023



APPENDIX 8.11 – GREAT CRESTED NEWTS – ENERGY PARK AND CABLE ROUTE CORRIDOR

Document Properties		
Regulation Reference	Regulation 5(2)(a)	
Planning Inspectorate Scheme Reference	EN010123	
Application Document Reference	6.3.8.11	
Title	Appendix 8.11 – Great Crested Newts – Energy Park and Cable Route Corridor	
Prepared By	Heckington Fen Energy Park Project Team (RSK Biocensus)	
Version History		
Version	Date	Version Status
Rev 1	February 2023	Application Version



Ecotricity (Heck Fen Solar) Ltd

Heckington Fen Energy Park

Great Crested Newt Survey Report

2483649

NOVEMBER 2022

RSK
biocensus
EXPERTS IN ECOLOGY

RSK GENERAL NOTES

Project No.: 2483649

Title: Heckington Fen Energy Park – Great Crested Newt Survey Report

Client: Ecotricity (Heck Fen Solar) Ltd

Date: 10th November 2022

Office: Coventry

Status: Rev01

Author	Charlotte Rose	Technical and quality reviewer	Mark lang – Associate Director MCIEEM, CEcol, CEnv
Signature	CR	Signature	ML
Date:	10/11/2022	Date:	14/11/2022
Project Manager	Charlotte Rose	Project Director	Mark lang – Associate Director MCIEEM, CEcol, CEnv
Signature	CR	Signature	ML
Date:	10/11/2022	Date:	14/11/2022

RSK Biocensus Ltd (RSK) has prepared this report for the sole use of the client, showing reasonable skill and care, for the intended purposes as stated in the agreement under which this work was completed. The report may not be relied upon by any other party without the express agreement of the client and RSK. No other warranty, expressed or implied, is made as to the professional advice included in this report.

Where any data supplied by the client or from other sources have been used, it has been assumed that the information is correct. No responsibility can be accepted by RSK for inaccuracies in the data supplied by any other party. The conclusions and recommendations in this report are based on the assumption that all relevant information has been supplied by those bodies from whom it was requested.

No part of this report may be copied or duplicated without the express permission of RSK and the party for whom it was prepared.

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 Biocensus Ltd.

EXECUTIVE SUMMARY

1. Heckington Fen, near Boston, Lincolnshire (grid reference: TF 20591 43674) (hereafter known as the 'site'). The surveys were undertaken to inform an ecological impact assessment of the Proposed Development of a new energy park. The Heckington Fen Energy Park will comprise the following three elements: the Energy Park, cable route to, and above ground works at, the National Grid Bicker Fen Substation referred to within this report as 'the Proposed Development'.
2. The Proposed Development site is located between Sleaford and Boston in Lincolnshire just east of the village of East Heckington. The site lies to the north and south of the A17.
3. This report includes the results and evaluations of a Habitat Suitability Index (HSI) assessment, environmental DNA analysis and presence/absence surveys conducted within the red-line boundary.
4. Five water features suitable for Great Crested Newt (GCN) breeding were identified previous surveys. This included two ponds and three ditches. One of the ponds and the three ditches were on the site of the proposed solar park and the second pond was to the south on the proposed cable route.
5. eDNA analysis yielded a negative result for pond 2 and an inconclusive result for pond 1 and ditches 1, 2, and 3. Presence/absence surveys therefore took place on all indeterminate waterbodies.
6. No GCN were recorded in pond 1 or ditches 1, 2, or 3 and so are considered to be likely absent from the site.
7. The terrestrial habitat in the immediate vicinity of all water features was assessed as part of the Habitat Suitability Index provide sub-optimal foraging habitat for GCN. The areas around the peripheries of the site and along the boundaries additionally provide sub-optimal terrestrial habitat. The areas within the Proposed Development site comprise large arable fields and are thus considered to be of negligible to sub-optimal suitability for GCN.

CONTENTS

1 INTRODUCTION	4
1.1 Purpose of this Report.....	4
1.2 Ecological Context.....	4
1.3 Project Background	4
1.4 Survey Validity.....	4
2 METHODS	5
2.1 General.....	5
2.2 Background Data Search	5
2.3 Habitat Suitability Index.....	5
2.4 Environmental DNA Surveys	6
2.5 Presence/Absence Survey	7
2.6 Survey Constraints	9
3 RESULTS	10
3.1 Background Data Search	10
3.2 Habitat Suitability Index.....	10
3.3 Environmental DNA Analysis.....	10
3.4 Presence/Absence Surveys	11
4 EVALUATION AND CONCLUSIONS	12
4.1 Habitat Assessment.....	12
4.2 Presence/Absence Assessment and Evaluation	12
4.3 Potential Impacts.....	12
5 REFERENCES	13
6 FIGURES	14
APPENDIX A – DETAILED SURVEY RESULTS	15
APPENDIX B – EDNA RESULTS	17
APPENDIX C – RELEVANT LEGISLATION	18
Great Crested Newt.....	18

TABLES

Table 1 : Weather conditions during HSI assessment	6
Table 2 : Survey dates	7
Table 3 : Waterbodies habitat suitability index results.	10
Table 4. Summary of the results of GCN population class surveys	11
Table 5. Detailed survey results of the GCN population surveys conducted in 2022.	15

1 INTRODUCTION

1.1 Purpose of this Report

- 1.1.1 This report presents the results of great crested newt (GCN) (*Triturus cristatus*) surveys carried out at Heckington Fen, near Boston, Lincolnshire (grid reference: TF 20591 43674) (hereafter known as the 'site'). The surveys were undertaken to inform an ecological impact assessment of the three elements of the proposed development: the Energy Park, cable route to, and above ground works at, the National Grid Bicker Fen Substation (hereafter known as the 'Proposed Development'). The Proposed Development site is shown in Figure 1. RSK Biocensus carried out the surveys on behalf of Ecotricity (Heck Fen Solar) Ltd.
- 1.1.2 Previous great crested newt surveys in relation to the wind park were conducted in 2010 and 2017. The results of these surveys are now considered to be out of date and an updated great crested newt survey of the site is required, so that potential impacts from the Proposed Development can be accurately assessed.

1.2 Ecological Context

- 1.2.1 The Proposed Development site is located between Sleaford and Boston in Lincolnshire just east of the village of East Heckington. The site lies to the north and south of the A17. The surrounding habitat is dominated by arable farmland, with fields surrounding the site to the north, east, south and west. There are numerous wet and dry ditches within this arable landscape.
- 1.2.2 Results from the GCN surveys conducted between 2010 and 2017 showed GCN were likely absent from site following presence/absence surveys, however, the eDNA results in 2017 were inconclusive. It is likely this was due to the presence of calcium compounds in the water causing a precipitate in the samples. During the 2010 surveys smooth newts were found on the site.

1.3 Project Background

- 1.3.1 In 2013, 22 wind turbines and associated infrastructure were approved by the Secretary of State on the Energy Park Site.

1.4 Survey Validity

- 1.4.1 The data from these surveys are generally considered valid for a maximum of two years. Therefore, if more than two years elapse prior to commencement of the Proposed Development, additional updated surveys may be required to ensure up-to-date information is considered.

2 METHODS

2.1 General

2.1.1 GCN surveys were first conducted in 2010 and 2017. These surveys included Habitat Suitability Index assessments, traditional presence/absence surveys and eDNA surveys. RSK Biocensus was commissioned by Ecotricity to undertake GCN surveys on the selected waterbodies present on site. These round of surveys act as an update with an aim to identify and confirm GCN presence or absence and assess any populations that may exist.

2.2 Background Data Search

2.2.1 The Lincolnshire Environmental Records Centre were consulted in June 2022 for records of protected and noteworthy amphibians within 1 km of the site boundary. Furthermore, previous GCN surveys were used as a reference to inform this year's surveys.

2.3 Habitat Suitability Index

2.3.1 Water features were assessed to determine their suitability for Great Crested Newt using a Habitat Suitability Index (HSI) developed by Oldham *et al.* (2000), which is derived from assessment systems developed by the US Fish and Wildlife Service. It is a numerical index, between 0 and 1, where 0 indicates unsuitable habitat and 1 represents optimal habitat. The HSI for the Great Crested Newt uses ten factors (suitability indices (SI) 1 to 10), which are thought to affect Great Crested Newts as follows:

- geographic location (SI 1);
- surface area (SI 2);
- hydrology (drying) (SI 3);
- water quality (SI 4);
- shade (SI 5);
- presence of water fowl (SI 6);
- presence of fish (SI 7);
- number of adjacent water features (SI 8);
- terrestrial habitat (SI 9); and
- macrophyte cover (SI 10).

2.3.2 Each factor is scored, and the scores are converted to SI scores on a scale from 0.01 to 1 from graphs given in Oldham *et al.* (2000). The HSI result is calculated using the following formula:

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

2.3.3 Further research by Brady (unpublished) has developed a system for using HSI scores to define pond suitability for Great Crested Newts according to the following categories:

- HSI <0.5 = poor
- HSI 0.5 – 0.59 = below average
- HSI 0.6 – 0.69 = average
- HSI 0.7 – 0.79 = good
- HSI > 0.8 = excellent

2.3.4 There is a positive correlation between HSI scores and presence and abundance of Great Crested Newts in ponds. Generally, ponds with high HSI scores are likely to support larger populations. However, the relationship is not sufficiently precise to conclude that a pond with a high HSI will definitely have a large newt population, or that a pond with a low HSI score will only have a small newt population or no newts at all.

2.3.5 The HSI assessments were carried out in April 2022 by RSK Biocensus ecologists Sophie Elliot and Charlotte Rose. Sophie is an experienced consultant with a Level 1 GCN Class Licence.

Table 1 : Weather conditions during HSI assessment

Date	Air Temperature (°C)	Cloud Cover (Octas)	Wind Speed (Beaufort)	Precipitation
21.04.2022	21	2	3	Dry

2.4 Environmental DNA Surveys

2.4.1 This method investigates whether GCNs have been in a pond by analysing the water for their DNA (which can be shed in skin secretions, excrement etc). Using kits from approved suppliers, three samples were taken from the suitable waterbodies following the HSI assessments using strict protocols (Biggs *et al.* 2014) approved by Natural England, which - among other things - ensure that samples do not get cross-contaminated. Sampling took place at the recommended time, i.e. between mid-April and June. Subject to safety of access, sample spacing was regular, except in so far as it targeted aquatic vegetation that might be used for egg-laying. The 20 samples from each waterbody were finally collected into a single sample bag and gently homogenized, after

which 6 sub-samples were preserved in an ethanol-based preservative and sent to the ADAS laboratory for analysis.

2.4.2 The water samples were collected by Sophie Elliot and Charlotte Rose on the 21st April 2022.

2.5 Presence/Absence Survey

2.5.1 Standard surveys to assess the presence/absence of GCN involve four repeat visits to survey the site using several methods (netting, egg searching, torching, and bottle trapping).

2.5.2 The surveys were led by Natural England licenced surveyors Anna Burnham, Emma Powell and Sam Spicket of RSK Biocensus, all of whom are associate members of the Chartered Institute of Ecology and Environmental Management (CIEEM), and hold Level 1 GCN Class licences with Natural England.

2.5.3 Surveys were carried out according to Natural England survey guidelines (Natural England 2001), which outline the survey standards as follows:

- four survey visits between mid-March and mid-June;
- survey in suitable weather;
- two of the four survey visits between mid-April and mid-May; and
- survey using (where practical) a minimum of three methods, including egg searching, netting, torching and bottle trapping.

2.5.4 The survey dates for each pond are provided in *Table 2*.

Table 2 : Survey dates

Water Feature reference	Survey 1 date	Survey 2 date	Survey 3 date	Survey 4 date
Ditch 1	11.05.2022	12.05.2022	17.05.2022	26.05.2022
Ditch 2	11.05.2022	12.05.2022	17.05.2022	26.05.2022
Ditch 3	11.05.2022	12.05.2022	17.05.2022	26.05.2022
Pond 1	11.05.2022	12.05.2022	17.05.2022	26.05.2022

Field methods

Egg search

2.5.5 This method involved searching the leaves of aquatic vegetation for the distinctive eggs of GCN, which is very effective for detecting presence. However, egg searching can be

difficult or unreliable in deep water or heavily vegetated ponds with small newt populations. The eggs are characteristically laid in the leaves of submerged aquatic vegetation, which are then folded over. When examined, the eggs of great crested newt are found to be large and white/yellow in colour, and this makes them easily distinguishable from the eggs of smooth newt (*Lissotriton vulgaris*) and palmate newt (*L. helveticus*) which are smaller and off-white, brown, or grey in colour. This method was used in daylight during bottle-trap retrieval or netting when surveyors were by the pond edge and could easily see folded vegetation.

Netting

- 2.5.6 Netting was sometimes carried out using a long-handled dip-net with a fine mesh. The perimeter of the water feature was walked, and 15 minutes of netting was carried out for every 50m of water-margin. The method is less effective than bottle trapping and torching when surveying for adult great crested newts but is very useful when searching for larvae. This method was used as an alternative or extra method of survey when weather or other constraints did not allow efficient or safe bottle trapping. If a pond had significant quantities of dead leaf litter on the bottom, netting was not carried out due to the amount of disturbance that would be caused and subsequent impact on the water quality of the pond.

Torching

- 2.5.7 This technique was carried out at night when newts are most active. Negative results are only meaningful if the surveys are carried out in suitable weather. Ideal weather conditions are given by Natural England (2001) as a night-time air temperature of more than 5°C, little or no wind, and no rain. Torchlight surveys involved walking slowly around the edge of the pond and scanning the water with a high-powered spotlight (Clulite, 1,000,000 candle power) where access and safety permitted. Using this method, great crested newts can be easily identified from smooth or palmate newts in clear water and counted. Bright light may cause newts to seek cover where they will be undetected, but the technique is appropriate to establish presence and for estimating populations. The species, sex (if possible), number of newts, and survey times were determined and recorded.

Bottle trapping

- 2.5.8 This method involves trapping newts at night and if not carried out correctly, can be harmful to the trapped newts. Because of this, strict guidelines from Natural England were followed.
- 2.5.9 The method is reliable for detecting presence of great crested newt and is especially useful in weedy or turbid water where visibility is poor, or the vegetation is too dense for torch surveys to give reliable results. 'Funnel traps' constructed from plastic bottles

attached to bamboo canes were immersed in the pond after dusk and removed early the following morning. Newts enter through the funnel entrance but cannot find their way back out again. The recommended density of traps is one trap for every 2m of margin, placed 2m from the edge, though this depends on habitat suitability and substrate as well as the shore incline and depth of the pond. Traps were checked in the early morning before the temperature rose, and the trapped newts were sexed, counted, and released.

- 2.5.10 A cautionary approach to this method was used because there is a risk that newts may be harmed, even following standard trapping protocol as described by Natural England (2001), and questions have therefore been raised regarding the safety of this technique. The technique is also unsuitable during periods of hot weather when dissolved oxygen levels in water decrease markedly, where water-levels were too low, or where there was a risk of vandalism. To reduce the risks, all bottles were deployed with an air bubble but were only left for the window that is allowed when an air bubble is not included: 12 hours in March and April, 10 hours in May and 8 hours in June. None of the survey nights were considerably warm and there was considered to be a low risk of vandalism due to the site's security enclosed status. Trapping was not used when ponds were deemed unsafe or unable to contain them, for example due to steep banks or concrete lined ponds.

2.6 Survey Constraints

- 2.6.1 The ditches could not be bottle trapped or netted due to health and safety concerns as the ditched had steep sides which would have made accessing the water unsafe. Where this was the case much more time was spent torching than average.

3 RESULTS

3.1 Background Data Search

3.1.1 The background data search returned a record of GCN within 100m of the cable route in 2015, this was the only record of GCN found in the 1km search area. Surveys carried out in 2010 and 2017 both found GCN to be likely absent from the Energy Park site.

3.2 Habitat Suitability Index

3.2.1 Five water features suitable for GCN breeding were identified for surveying from the previous surveys. This included three ditches and a pond within the solar park site and a pond within the cable route site. These five waterbodies were assessed using the Habitat Suitability Index, with results in *Table 3* below.

Table 3 : Waterbodies habitat suitability index results.

Water Feature Reference	HSI Score	GCN Suitability	eDNA survey carried out?
Ditch 1	0.46	Poor	Yes
Ditch 2	0.44	Poor	Yes
Ditch 3	0.30	Poor	Yes
Pond 1	0.51	Below average	Yes
Pond 2	N/A	Below average	Yes

3.3 Environmental DNA Analysis

3.3.1 Following the HSI assessments, water samples were collected from pond one, two, and ditches one, two and three and were subject to eDNA analysis.

3.3.2 The eDNA results for pond two returned negative for GCN presence in the pond. Pond one and ditches one, two and three returned inconclusive for the presence of GCN in the waterbody. This inconclusive result was likely due to the presence of calcium compounds in the water. Traditional population size-class survey was required for all four of the inconclusive waterbodies to confirm likely presence/absence and if needed, to assess their potential population sizes.

3.3.3 See *Appendix B* for full eDNA results.

3.4 Presence/Absence Surveys

3.4.1 *Table 4* summarises the results of the population-class surveys for GCN. All of the survey results are included within *Appendix A*. All surveys were undertaken during suitable weather conditions, as advised by Natural England.

Table 4. Summary of the results of GCN population class surveys

Water Feature Reference	Surveying Techniques Used	Amphibians Recorded
Ditch 1	Torching, egg searching	Common Frog
Ditch 2	Torching, egg searching	None
Ditch 3	Torching, netting, egg searching	Smooth Newt, Common Frog
Pond 1	Torching, bottle trapping, netting, egg searching	Smooth Newt, Common Frog, Common Toad

4 EVALUATION AND CONCLUSIONS

4.1 Habitat Assessment

- 4.1.1 The terrestrial habitat in the immediate vicinity of all water features assessed as part of the Habitat Suitability Index provide limited foraging habitat for GCN. The site and the wider landscape provides sub-optimal terrestrial habitat for GCN.

4.2 Presence/Absence Assessment and Evaluation

- 4.2.1 Evidence of GCN was absent from all waterbodies surveyed so it can be assumed GCN are likely absent from the site. This is the same result as the previous surveys in 2010 and 2017.

4.3 Potential Impacts

- 4.3.1 No GCN breeding waterbodies or high-quality terrestrial habitat will be lost as a result of the Proposed Development. No potential impacts on GCN by the Proposed Development are anticipated.

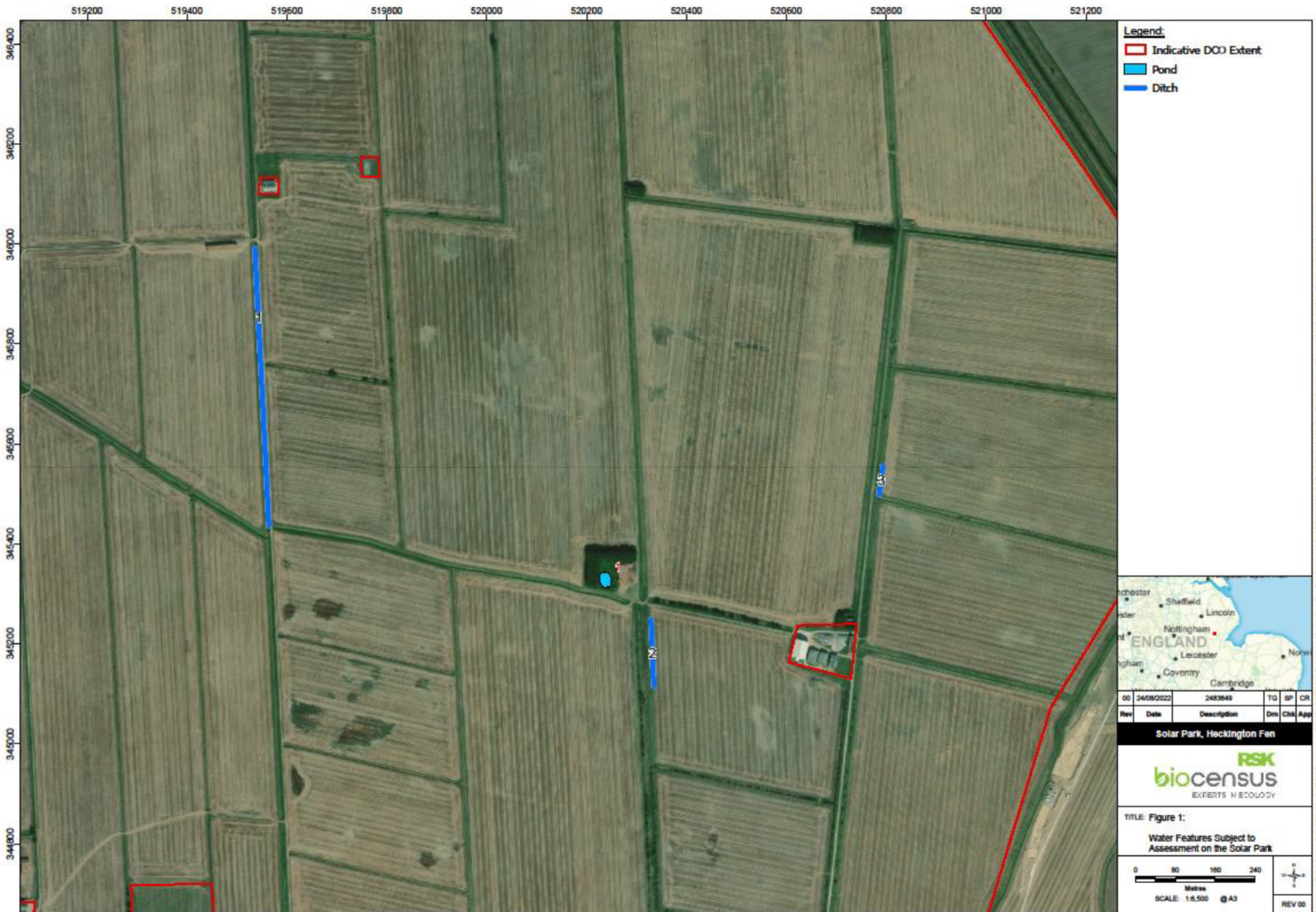
5 REFERENCES

- Beebee T.J.C. & Griffiths, R.A. (2000), *Amphibians and Reptiles – A Natural History of the British Herpetofauna*. HarperCollins, London.
- Biggs, J., Ewald N., Valentini A., Gaboriaud C., Griffiths R.A., Foster J., Wilkinson J., Arnett A., Williams P. & 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.
- English Nature (2001). *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough
- Froglife (2001) *Great Crested Newt Conservation Handbook*. Natural England.
- 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.

6 FIGURES

Figure 1. Water features subject to assessment on the Energy Park Site

Figure 2. Water features subject to assessment on the cable route.



Legend:

- Indicative DCO Extent
- Pond
- Ditch

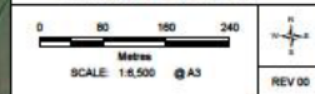


Rev	Date	Description	Dm	Chk	App
00	24/09/2022	2483649	TG	SP	CR

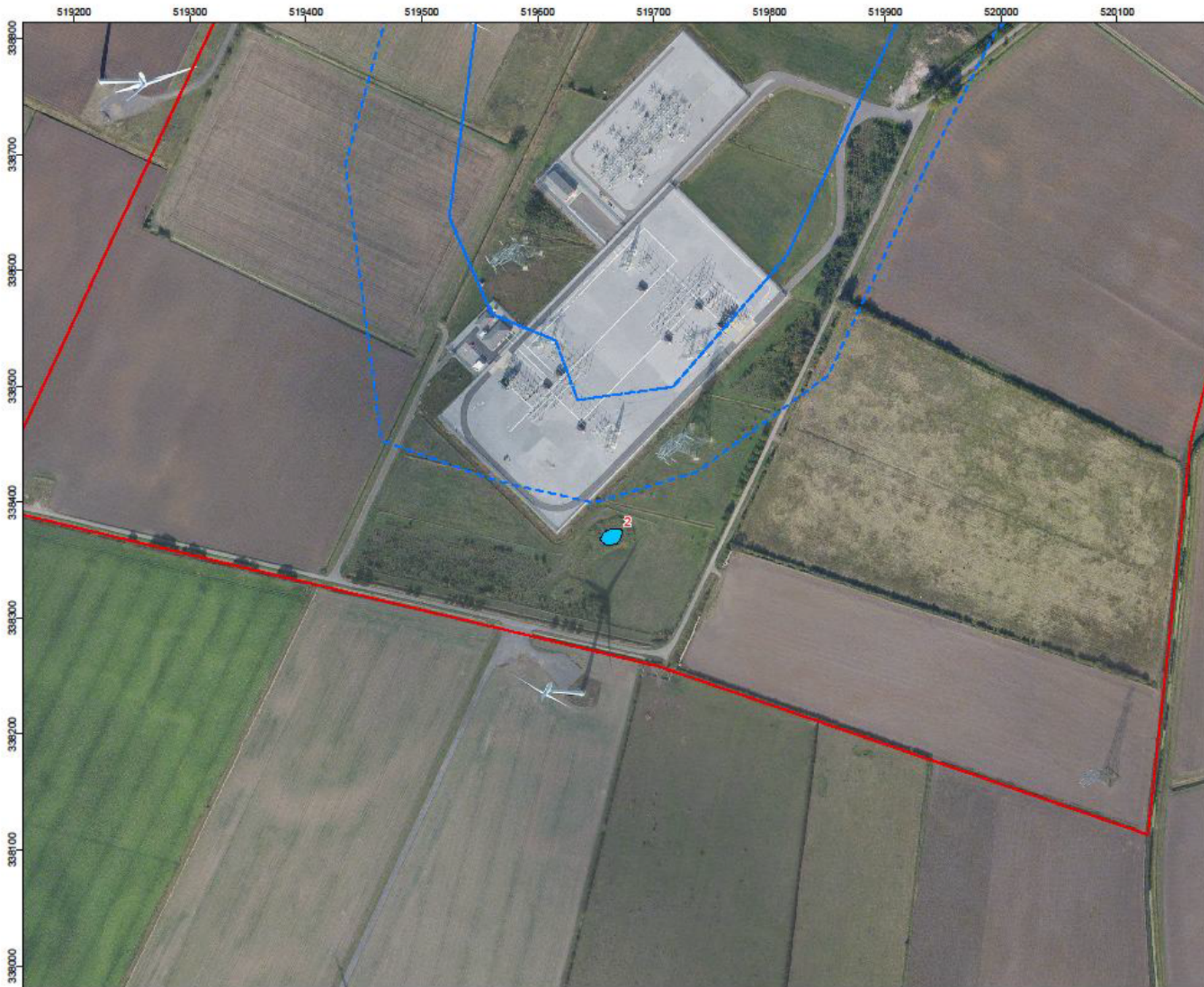
Solar Park, Heckington Fen



TITLE: Figure 1:
Water Features Subject to Assessment on the Solar Park



Document Path: C:\OneDrive\RSK Group\RSK Biocensus - 01 GIS - 24(884) - Solar Park\Heckington Fen Eco Surveys\03 - APPROX\03049 - Solar Park\Heckington Fen Surveys - Fig 1 - Solar Park Water Features Subject to Assessment.aprx



- Legend:**
- Indicative DCO Extent
 - Cable Route
 - Pond



Rev	Date	Description	Dim	Chk	App
00	24/08/2022	2483649	TG	SP	CR

Solar Park, Heckington Fen



TITLE: Figure 2:
Water Features Subject to Assessment on the Cable Route



Contains Ordnance Survey data © Crown copyright and database right 2022
World Imagery (Clarifai): This work is licensed under the Earthstar License Agreement View Summary | View Terms of Use (if you have a user account) This layer is not intended to be used to support files for offline. Data Collection and Editing: This layer may be used to verify ArcGIS apps to support data collection and editing, with the results used internally or shared with others, as described for these use cases.
World Imagery: Microsoft
© Open Restream. Contains OS data © Crown Copyright and database right 2022

APPENDIX A – DETAILED SURVEY RESULTS

Table 5. Detailed survey results of the GCN population surveys conducted in 2022.

Survey visit: 11 th May 2022					
Waterbody Number	Torch Survey	Bottle Trap Survey	Netting	Egg Search	Notes
Pond 1	0	0	0	0	Smooth newts and common frog recorded.
Ditch 1	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	
Ditch 2	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	
Ditch 3	0	N/A – Cannot bottle trap due to steep banks	0	0	
Survey visit: 12 th May 2022					
Pond 1	0	0	0	0	Smooth newts and common toad recorded
Ditch 1	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	Common frog recorded.
Ditch 2	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	
Ditch 3	0	N/A – Cannot bottle trap due to steep banks	0	0	
Survey visit: 17 th May 2022					
Pond 1	0	0	0	0	Smooth newts and common frog recorded.
Ditch 1	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	
Ditch 2	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	Drying out and becoming very shallow.

Ditch 3	0	N/A – Cannot bottle trap due to steep banks	0	0	Single smooth newt recorded. Drying out and becoming very shallow.
Survey visit: 26th May 2022					
Pond 1	0	0	0	0	Smooth newts recorded
Ditch 1	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	
Ditch 2	0	N/A – Cannot bottle trap due to steep banks	N/A – Cannot net due to steep banks	N/A – Cannot egg search due to steep banks	Almost completely dry
Ditch 3	0	N/A – Cannot bottle trap due to steep banks	0	0	Almost completely dry

APPENDIX B – EDNA RESULTS

Client: Charlotte Rose,
 RSK Biocensus



ADAS
 Spring Lodge
 172 Chester Road
 Helsby
 WA6 0AR

Tel: 01159 229249
 Email: [REDACTED]@adas.co.uk

Sample ID: ADAS-3752 Condition on Receipt: White Precipitate Volume: Passed
 Client Identifier: Ditch 1 Heckington Fen Description: pond water samples in preservative
 Date of Receipt: 25/04/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control ¹	2 of 2	Real Time PCR	03/05/2022
Degradation Control ³	Evidence of degradation	Real Time PCR	03/05/2022
Great Crested Newt [*]	Indeterminate	Real Time PCR	03/05/2022
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: 04/05/2022 Date of issue: 04/05/2022

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.*

¹ 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.

³ 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 C – RELEVANT LEGISLATION

Great Crested Newt

Triturus cristatus (Great Crested Newt) is listed on *Schedule 5* of the *Wildlife and Countryside Act 1981* (as amended), and receives full protection under *Section 9*. Great Crested Newts are also European Protected Species listed on *The Conservation of Species and Habitats Regulations 2010* (as amended). This legislation makes it an offence to:

- deliberately capture, injure or kill a Great Crested Newt;
- deliberately disturb a Great Crested Newt (in such a way as to be likely to significantly affect, (i) the ability of a significant group of Great Crested Newt to survive, breed or rear/nurture their young; and (ii) the local distribution or abundance of the species concerned);
- deliberately take or destroys the eggs of such an animal;
- damage or destroy a breeding site or resting place of a Great Crested Newt; and
- possess, control, transport, sell, exchange a Great Crested Newt, or offer a Great Crested Newt for sale or exchange.

All resting and breeding places of Great Crested Newts receive legal protection even when Great Crested Newts are not present.