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10 October 2022

Our reference: 2483510 – Worsborough Treatment Works (TW) GCN eDNA Survey Results

Dear Andrew,

Worsborough Treatment Works (TW)

This letter presents the results and conclusions following an environmental DNA (eDNA) survey of ponds at the Worsborough treatment works. This survey was conducted to inform decisions concerning the proposed installation of ground mounted solar photovoltaic (PV) panels on the Worsborough treatment works site. The proposed solar panel installation is expected to cover *c.*0.6ha of the 7ha site, however the exact location of the development is still to be confirmed.

A preliminary ecological appraisal (PEA) of the Worsborough TW site was conducted by BL Ecology in May 2020. The PEA identified five ponds (Ponds 1-5) either on or close to the site boundary, offering suitable aquatic habitat. A habitat suitability index (HSI) assessment was carried out by BL Ecology to establish the suitability of the ponds for great crested newts (GCN) (*Triturus cristatus*), with Ponds 1 and 2 found to have 'poor', Pond 3 'average', Pond 4 'good' and Pond 5 'excellent' suitability for GCN, Mackenzie (2020[1]). To establish the likely presence or absence of GCN in the ponds, it was recommended that an environmental DNA (eDNA) survey should be undertaken.

RSK Biocensus undertook eDNA surveys of Ponds 1 and 5 on 19 April 2022 and Ponds 2 and 3 on 25 April 2022. Pond 4, previously sampled by BL ecology has already returned a negative result. Detailed methods and results of the HSI assessment and eDNA survey are provided below.

[1] Mackenzie E., (2020). Solar PV site screening project, site 49 – Worsborough treatment works, preliminary ecological appraisal report, BL Ecology, Clayton.

Habitat Suitability Index (HSI) Assessment

The suitability of Ponds 1-5 for GCN was assessed by BL Ecology on 20 May 2020. This was done using an HSI assessment developed by Oldham *et al*. (2000[2]). It is a numerical index, between 0 and 1, where 0 indicates unsuitable habitat and 1 represents optimal habitat. The HSI for the GCN uses ten factors (suitability indices (SI) 1 to 10), which are thought to affect GCN, 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).

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:

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

Further research by Brady (unpublished) has developed a system for using HSI scores to define pond suitability for GCN 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

There is a positive correlation between HSI scores and presence and abundance of GCN 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 have a large newt population, or that a pond with a low HSI score will only have a small newt population or no newts present at all.

[2] 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.

The findings of the HSI assessments are detailed in Table 1 below:

Table 1: Habitat Suitability Index (HSI) Summary

Pond number	Description	HSI score	Suitability	eDNA Result
1	Large fishing lake bordered by scattered trees. The lake is stocked with coarse fish species including carp.	0.49	Poor	Indeterminate
2	A small, lined, artificial pond within the water treatment works boundary. The pond was seen to be used regularly by waterfowl and as such there was little macrophyte presence.	N/A	Ruled out as unsuitable	Negative
3	A shallow pond, heavily shaded by willow scrub. The pond was bordered by common reed (*Phragmites australis*) on the northern aspect, lying adjacent to rough grassland and tall ruderal vegetation to the west.	0.64	Average	Negative
4	A relatively large pond bordered by short grassland and willow scrub. The pond contains small areas of common reed and is situated within a local wildlife site adjacent to the proposed development.	0.75	Good	Negative (Sampled by BL Ecology)
5	A small pond split into two distinct areas of standing water. The pond is surrounded by scrub and tall ruderal vegetation. The water depth was no greater than 0.3m, with numerous small fish noted during the eDNA survey.	0.80	Excellent	Negative

eDNA survey

eDNA samples were taken from Ponds 1 and 5 by ecologists Kallum Buxton and Sam Spickett on 19 April 2022, and from Ponds 2 and 3 by ecologists Elizabeth Fenn and Rob Regan on 25 April 2022 using a kit purchased from approved suppliers. Water samples were collected according to strict protocols approved by Natural England and described in Biggs *et al*. (2014[3]) and sent to labs for analysis. The results for Ponds 2, 3 and 5 came back negative for GCN, with Pond 1 returning an indeterminate result (see Appendix A for full details).

Sample analysis for Pond 1 indicated degradation of the sample had occurred before analysis. This may be an indication that the water chemistry in Pond 1 is unsuitable for the reliable extraction of eDNA. However, as Pond 1 was found to offer poor quality habitat it is considered unlikely that GCN would be present, particularly when considering negative tests on the other nearby ponds. On this

[3] 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.* Defra Project WC1067. Freshwater Habitats Trust: Oxford.

basis it was determined that there was no requirement for further presence/absence and population size-class assessment surveys.

Conclusions

The GCN eDNA survey completed at Ponds 2, 3 and 5 provided a negative result, indicating GCN are not present. Pond 4 was previously sampled for eDNA by BL Ecology and returned a negative result, also indicating GCN are not present. The sample collected at Pond 1 provided an indeterminate result, however Pond 1 is a large fishing lake stocked with species which are likely to predate on GCN, therefore the pond is unlikely to be used by GCN. Whilst the results are not proof of the absence of GCN, it does indicate that they are likely to be absent from the ponds, or only present so rarely as to be undetectable. Consequently, it is unlikely that GCN will be present on the Worsborough TW site, however due to the presence of suitable terrestrial habitats on site, GCN presence in their terrestrial life stage cannot be conclusively ruled out.

As the risk to GCN is low it is recommended the works are completed under a precautionary working method statement (PWMS). The PWMS should be produced once the final site plans are provided.

We hope you find these results and recommendations useful. If you require any further information, please contact me on 07584 314385 or email joseph.mould@rskbiocensus.com.

Yours sincerely,



Joseph Mould, Assistant Ecologist

Technical review by



Will Holden, Principal Consultant
On behalf of RSK Biocensus

Figures

Figure 1 – GCN Survey Map

435800 435900 436000 436100 436200 436300 436400 436500 436600 436700 436800 436900 437000

403900 403800 403700 403600 403500 403400 403300 403200 403100 403000 402900

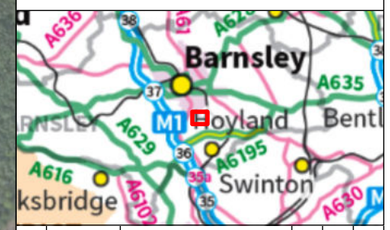
P1 P2 P3 P4 P5

Legend:

- Site Boundaries
- Site Buffer - 250m

GCN Ponds

- Negative eDNA Result
- Indeterminate eDNA Result



Rev	Date	Description	Drn	Chk	App
02	10/10/2022	2483510	RPH	RH	JM

Yorkshire Water Solar Sites

RSK biocensus EXPERTS IN ECOLOGY

TITLE: Figure 1:

Worsborough
Great Crested Newt Survey

0 50 100 150

Metres

SCALE: 1:4,000 @ A3

N W E S

REV 00

Document Path: C:\OneDrive\RSK Group\RSK Biocensus - 06 GIS - 2483510 - Yorkshire Water Solar Sites Eco Surveys\2483510 - Yorkshire Water Solar Sites - GCN Surveys - Figure 1 - Worsborough.aprx

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World Imagery (Clarity): This work is licensed under the Esri Master License Agreement.View Summary | View Terms of UseExport: This layer is not intended to be used to export tiles for offline. Data Collection and Editing: This layer may be used in various ArcGIS apps to support data collection and editing, with the results used internally or shared with others, as described for these use cases.
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Appendix A – eDNA Results

Client: Lewis Wright,
RSK Biocensus

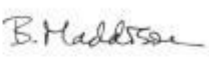
ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3027	Condition on Receipt: Good	Volume: Passed
Client Identifier: Yorkshire Water Worsborough P1	Description: pond water samples in preservative	
Date of Receipt: 19/03/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	0 of 2	Real Time PCR	21/04/2022
Degradation Control[§]	Evidence of degradation or residual inhibition	Real Time PCR	21/04/2022
Great Crested Newt*	Indeterminate	Real Time PCR	21/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-6} 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:	22/04/2022	Date of issue:	22/04/2022

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.*

[†] 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^{-5}, 10^{-4}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Spring Lodge, 172, Chester Road, Helsby, Frodsham, England, WA6 0AR, UK
Registered number: 04364279

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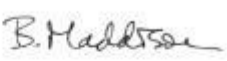
Client: Lewis Wright,
RSK Biocensus

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3019	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Worsborough Pond 2 Yorkshire Water	Description: pond water samples in preservative	
Date of Receipt: 27/04/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	03/05/2022
Degradation Control[§]	Within Limits	Real Time PCR	03/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	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.*

† 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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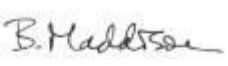
Client: Lewis Wright,
RSK Biocensus

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3018	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Worsborough Pond 3 Yorkshire Water	Description: pond water samples in preservative	
Date of Receipt: 27/04/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	29/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/04/2022
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

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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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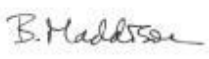
Client: Lewis Wright,
RSK Biocensus

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3024	Condition on Receipt: Good	Volume: Passed
Client Identifier: Yorkshire Water Worsborough PS	Description: pond water samples in preservative	
Date of Receipt: 19/03/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2022
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:	22/04/2022	Date of issue:	22/04/2022

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