

|                           |                                                                                                                                                                                                 |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Site:</b>              | Hinckley College, Leicestershire                                                                                                                                                                |
| <b>Client:</b>            | Gleeds                                                                                                                                                                                          |
| <b>Job Number:</b>        | 784-B039151                                                                                                                                                                                     |
| <b>Survey Type(s):</b>    | GCN eDNA and Habitat Suitability Index (HSI) Assessment                                                                                                                                         |
| <b>Date of Survey(s):</b> | 15 <sup>th</sup> June 2022 and 24 <sup>th</sup> June 2022                                                                                                                                       |
| <b>File Location:</b>     | \\lds-dc-vm-101\Data\Projects\784-B026224 - Gleeds - DfE - Support\784-B039151 Gleeds Additional Works\60 Project Output\61 Work in Progress\Hinckley - Ecology\Hinckley Academy eDNA\Reporting |

## INTRODUCTION

Tetra Tech was commissioned by Gleeds in April 2022 to undertake great crested newt *Triturus cristatus* (GCN) eDNA surveys of the site known Hinckley College in Hinckley, Leicestershire. The location of the site is shown on Figure 1.

The 'site' is located at Hinckley in Leicestershire and is centred at Ordnance Survey National Grid Reference SP 43998 94547 – see Figure 1. It comprises a school with associated buildings, hardstanding, scattered broad-leaved trees, amenity grassland, one accessible waterbody, and intact species-poor hedgerows on the boundary.

The site is located within an urban setting with residential housing to the north and the west of the site, Hinckley golf course to the east, and arable fields to the south.

This report has been prepared by Project Ecologist Thomas Cumberland BSc (Hons) and the conditions pertinent to it are provided in Appendix A.

## METHODOLOGY

### 1.1 HABITAT SUITABILITY INDEX ASSESSMENT

A Habitat Suitability Index (HSI) Assessment was carried out on the waterbodies shown on Figure 1. The HSI approach provides an objective method for assessing the suitability of a waterbody as habitat for GCN (Oldham *et al.*, 2000; Herpetological Conservation Trust, 2008). The system provides an index between 0 and 1, with 0 indicating unsuitable habitat and 1 optimal habitat. Ten suitability indices are used to calculate the index score, each representing a factor considered to affect GCN. These factors are listed and briefly explained below:

1. **Location:** i.e. where the pond is located in the British Isles. Lowlands are generally thought to be most suitable; suitability declines with increases in altitude
2. **Pond area:** i.e., the water surface area of a pond. Suitability peaks at approximately 800 m<sup>2</sup>;
3. **Pond drying:** how often a particular pond dries out. Ponds which dry out more frequently are less suitable;
4. **Water quality:** an indication of water quality based on the invertebrate diversity present. High invertebrate diversity indicates high water quality and suitability;
5. **Shade:** an estimate of the total shaded perimeter of a pond. Shoreline shade below 60% is optimal;
6. **Fowl:** indication of impact by waterfowl. High waterfowl numbers are generally considered detrimental;
7. **Fish:** indication of fish abundance. High fish numbers are generally considered detrimental;

8. *Pond count*: based on the density of ponds occurring within 1km of a particular pond. Suitability is positively correlated with pond density;
9. *Terrestrial habitat*: based on the availability of suitable habitat in the pond vicinity, e.g. rough grassland, scrub and woodland. For this assessment, the categories provided in the NARRS Survey Pack (Herpetological Conservation Trust, 2008) were used. This differs from the assessment criteria by Oldham *et al.* (2000); and
10. *Macrophytes*: based on an estimate of the percentage cover by emergent and aquatic vegetation. Suitability peaks at between 70% and 80% cover.

The results are then compared against a categorical scale developed by Lee Brady (unpublished) to give an overall rating. Therefore, the result for each waterbody is categorised as follows:

- <0.5 = **Poor**
- 0.5 – 0.59 = **Below Average**
- 0.6 – 0.69 = **Average**
- 0.7 – 0.79 = **Good**
- >0.8 = **Excellent**

The HSI assessment was applied to the waterbodies shown on Figure 1, using data that was collected during site visits made in June 2022.

## 1.2 eDNA SURVEYS

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### 1.2.1 Sample Collection Protocol

Surveyors attended site on 15<sup>th</sup> June 2022 and 25<sup>th</sup> June 2022 to undertake eDNA sampling. Collection of the eDNA samples was undertaken by Project Ecologist Thomas Cumberland Bsc (hons) (Natural England GCN Class 1 survey licence number - 2022-10253-CL08-GCN) and Consultant Ecologist Sophie Lunn.

Waterbodies WB1, WB4, WB10 and WB13 were surveyed on 15<sup>th</sup> June 2022.

Waterbodies WB2, WB3, WB5, WB6, WB7, WB8, WB9 and WB14 were surveyed on 24<sup>th</sup> June 2022.

Waterbodies WB11 and WB12 were not surveyed due to access constraints (see Limitations section).

The eDNA sampling was carried out in accordance with the survey methodologies defined within Natural England's accepted protocol (Biggs *et al.*, 2014 - WC1067 Appendix 5. *Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA*).

The sampling was carried out using a Surescreen Scientifics eDNA testing kit, with the samples collected and handled in accordance with their approved methodology (Surescreen Scientifics Ltd., 2021).

### 1.2.2 eDNA Analysis

The eDNA testing was carried out by Surescreen Scientifics Ltd.

## LIMITATIONS

The optimum period for undertaking GCN HSI assessments is generally considered to be spring. The period for undertaking GCN eDNA surveys is between the 15<sup>th</sup> April and the 30<sup>th</sup> June. The surveys were all conducted within June which is just outside the optimum season for HSI but is within the optimum season for eDNA. This is not considered to be a limitation to the results of the surveys due to aquatic vegetation present within the waterbody and water still present before waterbodies may dry up in later summer.

Access was provided to the entire site at the time of the survey and all waterbodies outside the site with the exception of WB11 and WB12 which were located within private land.

At the time of the survey, WB4 was dry therefore no eDNA analysis was possible during the survey on 15<sup>th</sup> June 2022. This is not considered to be a limitation to the results of the survey.

The results of the survey will remain valid for 12 months (June 2023) from the date of the surveys, after this time the development proposals and the results of the survey should be reviewed to determine if further updates are required. It will also be necessary to update the surveys if any changes are made to the red line boundary or development proposals on which this survey was designed.

## RESULTS

A GCN eDNA survey was undertaken on 15<sup>th</sup> and 24<sup>th</sup> June 2022. The eDNA results of this survey are documented in Appendix B and shown on Figure 2.

Waterbodies WB1 and WB3 returned a positive eDNA result for GCN.

Waterbodies WB2, WB5, WB6, WB7, WB8, WB9, WB10, WB13 and WB14 returned a negative eDNA result for GCN.

The HSI scores for all waterbodies are presented below in Table 1. Photographs and descriptions of the waterbodies are provided in Appendix B.

**Table 1 – HSI scores for WB1 – WB10, WB13 and WB14.**

| <u>Suitability Index</u> | <u>WB1</u>       | <u>WB2</u>           | <u>WB3</u>   | <u>WB5</u>   | <u>WB6</u>   | <u>WB7</u>   | <u>WB8</u>   | <u>WB9</u>       | <u>WB10</u>  | <u>WB13</u>  | <u>WB14</u>  |
|--------------------------|------------------|----------------------|--------------|--------------|--------------|--------------|--------------|------------------|--------------|--------------|--------------|
| Field location           | 1.00             | 1.00                 | 1.00         | 1.00         | 1.00         | 1.00         | 1.00         | 1.00             | 1.00         | 1.00         | 1.00         |
| Pond area                | 1.00             | 1.00                 | 0.85         | 0.90         | 0.85         | 1.00         | 1.00         | 1.00             | 0.10         | 0.10         | 0.05         |
| Pond drying              | 1.00             | 0.50                 | 0.90         | 0.90         | 0.90         | 0.90         | 1.00         | 0.50             | 0.90         | 0.90         | 0.10         |
| Water quality            | 0.67             | 0.33                 | 0.67         | 0.67         | 0.67         | 1.00         | 0.67         | 0.33             | 1.00         | 0.01         | 0.01         |
| Shade                    | 1.00             | 1.00                 | 1.00         | 1.00         | 1.00         | 1.00         | 1.00         | 1.00             | 0.80         | 0.60         | 0.60         |
| Fowl                     | 1.00             | 1.00                 | 0.01         | 0.01         | 0.01         | 0.01         | 1.00         | 1.00             | 1.00         | 1.00         | 1.00         |
| Fish                     | 1.00             | 1.00                 | 0.01         | 0.01         | 0.01         | 0.01         | 1.00         | 1.00             | 0.67         | 1.00         | 1.00         |
| Ponds                    | 0.40             | 0.10                 | 1.00         | 1.00         | 1.00         | 1.00         | 0.40         | 0.10             | 1.00         | 1.00         | 1.00         |
| Terrestrial habitat      | 1.00             | 1.00                 | 1.00         | 1.00         | 1.00         | 1.00         | 1.00         | 1.00             | 1.00         | 1.00         | 1.00         |
| Macrophytes              | 0.80             | 0.20                 | 0.30         | 0.30         | 0.60         | 0.60         | 0.80         | 0.20             | 1.00         | 0.80         | 0.80         |
| SCORE:                   | 0.162000         | 0.000069             | 0.000015     | 0.000016     | 0.000031     | 0.000054     | 0.000054     | 0.119394         | 0.048240     | 0.000432     | 0.000024     |
| <b>HSI SCORE:</b>        | <b>0.834</b>     | <b>0.384</b>         | <b>0.330</b> | <b>0.332</b> | <b>0.354</b> | <b>0.374</b> | <b>0.374</b> | <b>0.809</b>     | <b>0.738</b> | <b>0.461</b> | <b>0.345</b> |
| <b>Pond Suitability:</b> | <b>Excellent</b> | <b>Below average</b> | <b>Poor</b>  | <b>Poor</b>  | <b>Poor</b>  | <b>Poor</b>  | <b>Poor</b>  | <b>Excellent</b> | <b>Good</b>  | <b>Poor</b>  | <b>Poor</b>  |

Following the confirmation of GCN presence through eDNA sampling in waterbody 1 (WB1) located 330m and waterbody 3 (WB3) located 280m from the proposed development site location, within the grounds of the adjacent golf course (see Figure 2). As both waterbodies are located between 250m and 500m buffer for potential impacts from development, the GCN rapid risk assessment tool has been used below to calculate the need for an EPS licence.

## **NE RAPID RISK ASSESSMENT – WB1, WB3, WB11 AND WB12**

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NE have developed a 'rapid risk assessment' tool which can be used to determine the requirement for an EPS development licence under Regulation 53(2)(e). The rapid risk assessment tool takes into account five different factors to assess the overall likelihood of an offence occurring which are as follows:

- The effect on a GCN breeding waterbody (i.e. whether any breeding ponds will be damaged or destroyed by the works),
- The area of land lost (including habitat not suitable for GCN) within 100m of a known GCN breeding waterbody,
- The area of land lost (including habitat not suitable for GCN) between 100m – 250m of a known GCN breeding waterbody,
- The area of land lost (including habitat not suitable for GCN) >250m of a known GCN breeding waterbody,
- The effect on individual GCN (i.e. level of disturbance, likelihood of obstructing dispersal, likelihood of killing or injuring GCN).

The rapid risk assessment operates on a Red, Amber, Green basis, whereby:

**"Green: offence highly unlikely"** indicates that the development activities are of such a type, scale and location that it is highly unlikely any offence would be committed should the development proceed. Therefore, no licence would be required.

**"Amber: offence likely"** indicates that the development activities are of such a type, scale and location that an offence is likely. In this case, the best option is to redesign the development (location, layout, methods, duration or timing as described in Table 8 below).

**"Red: offence highly likely"** indicates that the development activities are of such a type, scale and location that an offence is highly likely. In this case, you should attempt to re-design the development location, layout, timing, methods or duration in order to avoid impacts (see Table 8). If the offences, cannot be avoided then a EPSL should be applied for.

This tool has been applied to the current proposals in relation to positive waterbodies WB1 and WB3 for a 250m to 500 m buffer from the assumed breeding pond. This tool has also been applied to the current proposals in relation to ponds WB11 and WB12 which were not accessed, for a 250m to 500 m buffer from the assumed breeding pond as follows:

**Table 2: NE Rapid Risk Assessment Score**

| 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)  | No effect                                                                                                                                           | 0                                  |
| Land >250m from any breeding pond(s)     | 0.1 – 0.5 ha Lost or damaged                                                                                                                        | 0.03                               |
| Individual great crested newts           | No effect                                                                                                                                           | 0                                  |
| Maximum:                                 |                                                                                                                                                     | 0.03                               |
| Rapid risk assessment result:            | <b>GREEN: OFFENCE HIGHLY UNLIKELY</b>                                                                                                               |                                    |

For this assessment, it is assumed that WB1 and WB3 are a breeding ponds following a positive GCN eDNA result. For this assessment, it is assumed that WB11 and WB12 are breeding ponds following the ponds not being accessible during the eDNA survey (see limitations section).

The current proposals for the development result in a rapid risk assessment of Green (see above) with the development unlikely to cause any offences to the breeding ponds, which are located roughly 280m to 500m away from the development. The majority of the development is hardstanding which is not considered as suitable habitat for GCN.

Therefore, it is considered that under the current proposals an **EPSL would not be required** to facilitate the development.

It is recommended that a Non-Licensed Method Statement (NLMS) is produced prior to the start of the development works, to mitigate for any GCN that might be found within the development footprint, during the works.

The results should be assessed against the final development design to determine the requirement for an EPS licence for the works to proceed, should development plans change following the GCN assessment.

It is considered that GCN are not a constraint to the development.

## SUMMARY

- eDNA analysis confirmed GCN as being present in WB1 and WB3.
- eDNA analysis confirmed GCN as absent in waterbodies WB2, WB5, WB6, WB7, WB8, WB9, WB10, WB13 and WB14.
- An EPS would not be required to facilitate the development. Following the rapid risk assessment tool, it is considered that WB1, WB3, WB11 and WB12 are unlikely to be impacted by the development proposals, with all four of the waterbodies located over 250m away from the proposed area of development on the site.
- It is recommended that a Non-Licensed Method Statement (NLMS) is produced prior to the start of the development works, to mitigate for any GCN that might be found within the development footprint, during the works.
- It is considered that GCN are not a constraint to the development.

| Document Control                                                            |                                                                              |                                                                                      |              |
|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------|
| <b>Revision:</b>                                                            | <b>V1</b>                                                                    | <b>Status:</b>                                                                       | <b>Final</b> |
| <b>Date:</b>                                                                | 16.09.2022                                                                   |                                                                                      |              |
| <b>Prepared by:</b><br><b>Project Ecologist</b><br><b>Thomas Cumberland</b> | <b>Checked by:</b><br><b>Senior Ecologist</b><br><b>Richard Brown ACIEEM</b> | <b>Approved By:</b><br><b>Principal Ecologist</b><br><b>Elizabeth Sanders MCIEEM</b> |              |

## FIGURES

**Figure 1 – Waterbody Location Plan**

**Figure 2 – eDNA Results Plan**



# Waterbody Location Plan

Hinckley



Gleeds

## Legend

-  Site boundary
-  Site boundary 500m buffer
-  Waterbodies

## Notes:

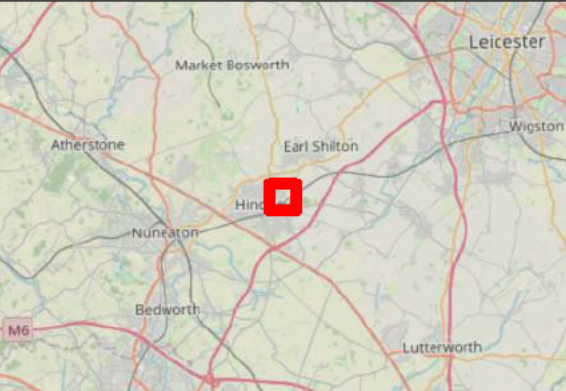
Drawn by: EC  
Checked by: TC  
Office: Southampton

Figure No. 1  
Revision No. B

0 50 100 150 200 250 Meters  
Scale 1 6,000 @A3

16 September 2022  
NGR: 443881E 294420N

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# eDNA Results Plan

Hinckley

Gleeds

## Legend

- Site boundary
- Site boundary 500m buffer
- Positive
- Negative
- Dry
- No Access

## Notes:

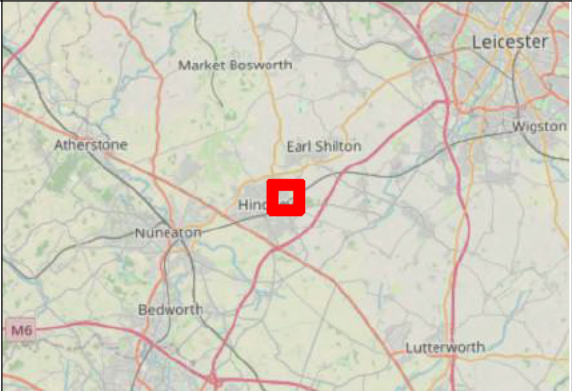
Drawn by: MS  
Checked by: TC  
Office: Southampton

Figure No. 2  
Revision No. A

0 50 100 150 200 250 Meters  
Scale 1 6,000 @A3

16 September 2022  
NGR: 443881E 294420N

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## APPENDIX A – REPORT CONDITIONS

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The whole of the report must be read as other sections of the report may contain information which puts into context the findings in any executive summary.

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The performance of environmental protection measures and of buildings and other structures in relation to acoustics, vibration, noise mitigation and other environmental issues is influenced to a large extent by the degree to which the relevant environmental considerations are incorporated into the final design and specifications and the quality of workmanship and compliance with the specifications on site during construction. Tetra Tech accept no liability for issues with performance arising from such factors.

## APPENDIX B – TARGET NOTES

| Waterbodies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Photograph                                                                         |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| <p><b>WB1</b></p> <p>WB1 is located roughly 350m from the sites' northern boundary. It is likely that given the housing development in 2014, this waterbody, along with WB4 were created as mitigation ponds for GCN. Upon a search of Magic, license EPSM2012-4412 was granted in 2012 for the destruction of a GCN resting place within this area.</p> <p>WB1 was roughly 210m<sup>2</sup> in size. The depth of the waterbodies was estimated as being between 0.5m to 1m. The waterbody was absent of fish and was assumed as likely being absent of waterfowl as none were seen during the survey. Terrestrial habitat surrounding the waterbody was good with hedgerows and other waterbodies located close by to the east of the waterbody, within the golf course. The waterbody had some duckweed <i>Lemna minor</i> present on the surface of the water but was largely absent of macrophyte cover.</p> <p>Species present surrounding the waterbody and included within the waterbody consisted of bull rush <i>Scirpoides holoschoenus</i>, creeping bent <i>Agrostis stolonifera</i>, Yorkshire fog <i>Holcus lanatus</i>, Common rush <i>Juncus effusus</i>, cow slip <i>Primula veris</i>, buttercup <i>Ranunculus bulbosus</i>, willow herb <i>Epilobium hirsutum</i> and yellow flag iris <i>Iris pseudacorus</i>.</p> <p>The HSI score for WB10 returned a score of 0.83 indicating excellent suitability to support breeding GCN.</p> <p>eDNA analysis returned a positive result for GCN presence in the waterbody.</p> |  |
| <p><b>WB2</b></p> <p>This waterbody was a large, 1,375m<sup>2</sup> waterbody which was located within the center of the Hinckley golf club, roughly 280m from the sites north-eastern corner.</p> <p>The waterbody was heavily covered in largely water lillies <i>Nymphaea spp</i>, frog bit</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                    |

*Hydrocharis morsus-ranae* and bull rush. There is an island in the middle of the waterbody largely dominated by bull rush with occasional water mint *Mentha aquatica* and some duckweed. Koi carp were a major presence in the waterbody with a minimum of 20 individuals identified during the survey, all sized between 30cm and 60cm. Waterfowl in the form of mallards *Anas platyrhynchos* and coot *Fulica atra* were present in the waterbody in significant numbers, with evidence of breeding and ducklings of both species.

The waterbodies eastern shoreline was heavily shaded with the remainder of the waterbody less shaded in comparison.

WB2 was located outside of the site boundary. The HSI score for WB2 returned a score of 0.38 indicating poor suitability to support breeding GCN.

eDNA analysis returned a negative result for GCN presence in the waterbody.



### WB3

This waterbody was a large, 1,560m<sup>2</sup> waterbody which was located within the center of the Hinckley golf club, roughly 285m from the sites north-eastern corner.

Evidence of breeding mallard were present on the site with the possibility of fish being present within the waterbody, although no fish were identified at the time of the survey. The waterbody also holds a wooden structured platform which is used for golf. The waterbody was limited in shade and absent of significant macrophyte cover.

Species located within the waterbody and surrounding the banks included: large amount of bullrush, some lily pads common rush *Juncus effusus*, purple leaved willowherb *Epilobium ciliatum*, common sow thistle *Sonchus oleraceus*, water figwort *Scrophularia auriculata*, water mint and common nettle *Urtica dioica*.

WB3 was located outside of the site boundary. The HSI score for WB3 returned a score of 0.33 indicating poor suitability to support breeding GCN.



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|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <p>eDNA analysis returned a positive result for GCN presence in the waterbody.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                      |
| <p>WB4</p> <p>Waterbody was dry at the time of the survey.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                      |
| <p>WB5</p> <p>This waterbody was a large, 1,650m<sup>2</sup> waterbody which was located within the center of the Hinckley golf club, roughly 290m from the sites south-eastern corner.</p> <p>Evidence of breeding mallard were present on the site. Koi carp were a major presence in the waterbody with a minimum of 15 individuals identified during the survey, all sized between 20cm and 70cm. The waterbody was limited in shade and absent of significant macrophyte cover.</p> <p>Species located within the waterbody included: bull rush, lily pad and some water mint. Macrophyte cover was limited, likely due to the presence of fish in the waterbody.</p> <p>The HSI score for WB5 returned a score of 0.33 indicating poor suitability to support breeding GCN.</p> <p>eDNA analysis returned a negative result for GCN presence in the waterbody.</p> |    |
| <p>WB6</p> <p>This waterbody was a large, 2,700m<sup>2</sup> waterbody which was located within the eastern corner of the Hinckley golf club, roughly 450m from the sites south-eastern corner.</p> <p>Evidence of breeding mallard and coot were present on the site. Carp were a major presence in the waterbody with a minimum of 10 individuals identified during the survey, all sized between 20cm and 40cm. The waterbody was significantly shaded on the southern shoreline and absent of significant macrophyte cover.</p> <p>Species located within the waterbody included: dominant bullrush, white willow <i>Salix alba</i>, curly waterweed <i>Lagarosiphon major</i> which was dominant, willow herb and small amount of duckweed. Frogs <i>Rana</i></p>                                                                                                   |  |

*temporaria* were also identified in the waterbody.

The HSI score for WB6 returned a score of 0.35 indicating poor suitability to support breeding GCN.

eDNA analysis returned a negative result for GCN presence in the waterbody.

#### WB7

This waterbody was a large, 432m<sup>2</sup> waterbody which was located within the southern boundary of the Hinckley golf club, roughly 315m from the sites south-eastern corner.

Evidence of breeding mallard were present on the site. Fish were a presence in the waterbody identified during the survey. The waterbody was limited in shade and absent of significant macrophyte cover, with some frog bit present.

Species located within the waterbody included: curly waterweed *Lagarosiphon major*, hydrilla *Hydrilla verticillata*, water lily, bullrush and water mint.

The HSI score for WB7 returned a score of 0.37 indicating poor suitability to support breeding GCN.

eDNA analysis returned a negative result for GCN presence in the waterbody.



#### WB8

This waterbody was a large, 910m<sup>2</sup> waterbody which was located within the southern boundary of the Hinckley golf club, roughly 255m from the sites south-eastern corner.

Evidence of breeding mallard were present on the site. Fish were a presence in the waterbody identified during the survey. The waterbody was limited in shade and was roughly 60% covered in macrophytes, with frog bit and algae present.

Species located within the waterbody included: watercress *Nasturtium officinale*, curly waterweed and water crowfoot *Ranunculus aquatilis*.



|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                      |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <p>The HSI score for WB8 returned a score of 0.37 indicating poor suitability to support breeding GCN.</p> <p>eDNA analysis returned a negative result for GCN presence in the waterbody.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                      |
| <p><b>WB9</b></p> <p>This waterbody was a large, 910m<sup>2</sup> waterbody which was located within the southern boundary of the Hinckley golf club, roughly 255m from the sites south-eastern corner.</p> <p>Evidence of breeding mallard were present on the site. Fish were absent during the survey. The waterbody was heavily shaded and absent of significant macrophyte cover, with some algae present within the eastern area of the waterbody.</p> <p>Species located within the waterbody was limited to large amounts of dominant bullrush.</p> <p>The HSI score for WB9 returned a score of 0.81 indicating excellent suitability to support breeding GCN.</p> <p>eDNA analysis returned a negative result for GCN presence in the waterbody.</p>                                                     |    |
| <p><b>WB10</b></p> <p>WB10 is located to the center of the site. The waterbody is located within the west of the woodland held water at the time of the survey. The water depth was approximately 0.3 m at the time of the survey. The waterbody is roughly 50m<sup>2</sup> and dominated by common bulrush with perennial ryegrass <i>Lolium perenne</i>, bulrush, yellow flag iris, hydrilla, duckweed, pennywort <i>Centella asiatica</i> and water starwort <i>Callitriche heterophylla</i> also present. Small fish, likely stickleback <i>Gasterosteus aculeatus</i>, were observed within the waterbody.</p> <p>The HSI score for WB10 returned a score of 0.74 indicating good suitability to support breeding GCN.</p> <p>eDNA analysis returned a negative result for GCN presence in the waterbody.</p> |  |
| <p><b>WB11</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                      |

No access to survey waterbody on 24<sup>th</sup> June 2022. eDNA samples were not taken.

#### WB12

No access to survey waterbody on 24<sup>th</sup> June 2022. eDNA samples were not taken.

#### WB13

WB13 is located within the site boundary, located in the north-eastern corner of the site, behind building block B1. The waterbody is very small at 2m<sup>2</sup> and is lined. The waterbodies surface was completely covered in duckweed with no other vegetation noted. The depth of the waterbody was roughly 0.25m. The waterbody was absent of fish and was considered unlikely to support waterfowl.

The HSI score for WB13 returned a score of 0.46 indicating poor suitability to support breeding GCN.

eDNA analysis returned a negative result for GCN presence in the waterbody.



#### WB14

WB14 is located within the center of the golf course, roughly 300m from the eastern boundary of the site. The waterbody held a minimum amount of water roughly 0.15m in depth, enough for an eDNA sample with the waterbody largely dry. The waterbody was significantly covered in bull rush and flag iris with duckweed also present to the north area of the waterbody which held water. The waterbody was 0.5m<sup>2</sup> in area. No vegetation was present within the area of the waterbody which held water at the time of the survey.

The HSI score for WB14 returned a score of 0.35 indicating poor suitability to support breeding GCN.

eDNA analysis returned a negative result for GCN presence in the waterbody.



## **APPENDIX B – SURESCREEN EDNA REPORT**

Folio No: E14311  
Report No: 1  
Purchase Order: 7006496  
Client: Tetra Tech  
Contact: Thomas Cumberland

## TECHNICAL REPORT

### ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

#### SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

#### RESULTS

**Date sample received at Laboratory:** 20/06/2022  
**Date Reported:** 29/06/2022  
**Matters Affecting Results:** None

| Lab Sample No. | Site Name                | O/S Reference | SIC  | DC   | IC   | Result   | Positive Replicates |
|----------------|--------------------------|---------------|------|------|------|----------|---------------------|
| 2480           | WB13<br>Hinckley Academy |               | Pass | Pass | Pass | Negative | 0                   |
| 2485           | WB10<br>Hinckley Academy |               | Pass | Pass | Pass | Negative | 0                   |
| 2494           | WB1 Hinckley Academy     |               | Pass | Pass | Pass | Positive | 10                  |

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)

**Reported by:** Chris Troth

**Approved by:** Gabriela Danickova



## **METHODOLOGY**

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

## **INTERPRETATION OF RESULTS**

**SIC:** **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

**DC:** **Degradation Check** [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

**IC:** **Inhibition Check** [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

**Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]

**Positive:** GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

**Positive Replicates:** Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. 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. 0/12 indicates negative GCN presence.

**Negative:** GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E14476  
Report No: 1  
Purchase Order: 7006496  
Client: Tetra Tech  
Contact: Thomas Cumberland

## TECHNICAL REPORT

### ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

#### SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

#### RESULTS

**Date sample received at Laboratory:** 24/06/2022  
**Date Reported:** 06/07/2022  
**Matters Affecting Results:** None

| Lab Sample No. | Site Name                | O/S Reference | SIC  | DC   | IC   | Result   | Positive Replicates |
|----------------|--------------------------|---------------|------|------|------|----------|---------------------|
| 2490           | WB14<br>Hinckley Academy |               | Pass | Pass | Pass | Negative | 0                   |
| 6295           | WB2                      |               | Pass | Pass | Pass | Negative | 0                   |
| 6299           | WB5                      |               | Pass | Pass | Pass | Negative | 0                   |
| 6300           | WB6                      |               | Pass | Pass | Pass | Negative | 0                   |
| 6301           | WB8                      |               | Pass | Pass | Pass | Negative | 0                   |
| 6302           | WB9                      |               | Pass | Pass | Pass | Negative | 0                   |
| 6303           | WB3                      |               | Pass | Pass | Pass | Positive | 12                  |
| 6304           | WB7                      |               | Pass | Pass | Pass | Negative | 0                   |

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)



**Reported by:** Chris Troth

**Approved by:** Gabriela Danickova

## **METHODOLOGY**

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If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

## **INTERPRETATION OF RESULTS**

- SIC:**                    **Sample Integrity Check** [Pass/Fail]  
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:**                    **Degradation Check** [Pass/Fail]  
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:**                    **Inhibition Check** [Pass/Fail]  
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:**                **Presence of GCN eDNA** [Positive/Negative/Inconclusive]  
**Positive:** GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.  
**Positive Replicates:** Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. 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. 0/12 indicates negative GCN presence.  
**Negative:** GCN eDNA was not detected or is below the threshold detection level and the test result



should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

