



Technical Appendix 9-4

Great Crested Newt and Amphibians

Scout Moor II Wind Farm

Cubico Wind Development (Wind 1) Ltd



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Document Prepared For

Cubico Wind Development (Wind 1) Ltd

Document Prepared By

Rebekah Booth

Ecologist

rebekah.booth@atmosconsulting.com

Jessica Hosier

Principal Ecologist

jessica.hosier@atmosconsulting.com

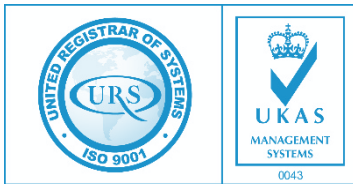
Document Approved By

Alexander Hatton

Technical Director, Ecology and Environmental Management

alex.hatton@atmosconsulting.com

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1. Introduction

1.1 Terms of Reference

In April 2024, Atmos Consulting Ltd (Atmos) were commissioned by Cubico Wind Development (Wind 1) Ltd to undertake survey work with respect to great crested newt (GCN) *Triturus cristatus* and subsequent technical appendix to inform the planning application for the development of a wind farm known as Scout Moor II. This planning application includes the creation of the wind farm, associated infrastructure and access, moorland restoration and works on the Common Land as well as the electrical grid connection. Collectively, these components are hereafter referred to as the 'Project for Assessment'.

Surveys were initially completed in 2023, however in 2024 the red line boundary was amended, requiring additional ponds to undergo surveys. Furthermore, population size class assessment surveys were completed during the 2024 season for any ponds where GCN presence had been confirmed.

1.2 Site Location and Description

The Assessment Boundary comprises c.1024 ha of land that is located to the north of Rochdale, Greater Manchester, England. The Assessment Boundary has an approximate central national grid reference (NGR) of SD 85570 18022.

The Assessment Boundary is an upland habitat, predominately moorland with fields to the south of the Assessment Boundary that are often utilised by farmers. A series of existing wind turbines lie adjacent west of the Assessment Boundary with some present in the north-west extent of the Assessment Boundary itself. The environment surrounding the Assessment Boundary is mostly urban, with Rochdale to the south of the Assessment Boundary and further housing developments being undertaken to the east.

1.3 Objectives

The objective of the survey and subsequent technical appendix is to determine the potential presence of GCN within the Assessment Boundary and appropriate buffer.

2. Relevant Legislation

Conservation of Habitats and Species Regulations (2017)

The Conservation of Habitats and Species Regulations 2017, (the Habitats Regulations) as amended, consolidate the Conservation of Habitats and Species Regulations 2010 with subsequent amendments. The Regulations transpose Council Directive 92/43/EEC, on the conservation of natural habitats and of wild fauna and flora (EC Habitats Directive), into national law. They also transpose elements of the EU Wild Birds Directive in England and Wales. The Regulations provide for the designation and protection of 'European Sites', the protection of 'European protected species', and the adaptation of planning and other controls for the protection of European Sites ('Natura 2000 Sites termed Special Areas of Conservation SACs or Special Protection Areas (SPAs)). The Regulations designate these sites as being important for either habitats or species (listed in Annexes I and II of the Habitats Directive respectively).

Wildlife and Countryside Act 1981

National legislation for the special protection of selected species is provided in the Wildlife and Countryside Act 1981, as amended (WCA) and the Habitats Regulations.

Under Section 1(1) and 1(2) of the WCA, all British bird species, their nests and eggs (excluding some pest and game species) are protected from intentional killing, injury or damage. Under Sections 1(4) and 1(5), special penalties are applied to bird species included in Schedule 1 of the WCA and protection is extended for these species to disturbance to birds whilst building, in or near a nest and disturbance to dependant young. Schedule 5 provides special protection to selected animal species other than birds, through Section 9(4) of the WCA, against damage to "any structure or place which any [wild animal included in the schedule] uses for shelter and protection" and against disturbance whilst in such places.

Non-native invasive plants are listed in Schedule 9 of the WCA which makes it an offence to spread or enable them to be spread in the wild. The list includes species such as Japanese knotweed *Reynoutria japonica* and Himalayan balsam *Impatiens glandulifera*.

A number of animals, known as European protected species (EPS), are provided full protection through inclusion in Schedule 2 of The Habitats Regulations. The Habitats Regulations provide protection against deliberate disturbance to those animals wherever they are present and provides tests against which the permission for a development (that may have an effect on a Schedule 2 protected species) must be assessed before permission can be given.

In addition to species protection, the WCA and Habitats Regulations also set out requirements/procedures for the notification, designation and protection of a range of statutory site designations in order to preserve important nature conservation resources.

All public authorities have a requirement to pay due regard to the conservation and enhancement of habitats and species through Section 41 of the Natural Environment and Rural Communities Act 2006 (NERC). Section 41 states, "Every public authority must, in exercising its functions, have regard, so far as is consistent with the proper exercise of those functions, to the purpose of conserving biodiversity". To this end, Section 41 of the NERC provides for the establishment of a list of habitat and species that are considered to be of "principal importance for the conservation of biological diversity in England".

Great Crested Newt

Great crested newt (GCN) *Triturus cristatus* are afforded full statutory protection as a European protected species listed on Schedule 2 of The Conservation (Natural Habitats, &c.) Regulations 2017 (1994, as amended), which transpose into British law the European Community's Habitats Directive (92/43/EEC).

Under the terms of Regulation 39(1), with certain exceptions, a person commits an offence if he/she:

“(a) deliberately captures, injures or kills any [a great crested newt];

(b) deliberately disturbs wild [great crested newts].

(1A) For the purposes of paragraph (1)(b), disturbance of animals includes in particular any disturbance which is likely —

(a) to impair their ability —

(i) to survive, to breed or reproduce, or rear or nurture their young; or

(ii) in the case of animals of a hibernating or migratory species, to hibernate or migrate; or

(b) to affect significantly the local distribution or abundance of [great crested newts];

(c) deliberately takes or destroys the eggs of [a great crested newt]; or

(d) damages or destroys a breeding site or resting place of [a great crested newt].”

It is also an offence under Regulation 39 to keep, transport, sell or exchange, or offer for sale or exchange, any live or dead wild Great crested newt, or any part of, or anything derived from one.

All of the above protections apply regardless of the stage of the life of the animal in question.

Protection of great crested newts' is also provided for in the Wildlife and Countryside Act 1981, as amended. The great crested newt is listed on Schedule 5 of the Act, and is afforded partial protection under the terms of section 9(4)(b) and (c) and (5). This makes it an offence if any person:

9(4) *“... intentionally or recklessly ... (b) ... disturbs any [great crested newt] while it is occupying a structure or place which it uses for shelter or protection; or (c) ... obstructs access to any structure or place which any [great crested newt] uses for shelter or protection.”*

9(5) *“... (a) sells, offers or exposes for sale, or has in his possession or transports for the purpose of sale, any live or dead [great crested newt], or any part of, or anything derived from, such an animal; or (b) publishes or causes to be published any advertisement likely to be understood as conveying that he buys or sells, or intends to buy or sell, any of those things”.*

There are provisions in the above legislation for the licensing of activities to facilitate development that would otherwise constitute an offence. However, the statutory agency Natural England (NE) advises that the requirement for licensing should be carefully considered by developers, on a site/activity-specific basis, and recommends consideration of non-licensed mitigation such as reasonable avoidance measures relating to timing of works and sensitive site clearance to minimise potential for adverse impacts to this species where this can be achieved.

3. Methodology

The relevant 1:25,000 scale Ordnance Survey (OS) sheet and aerial photography were searched to check for waterbodies within 500m of the Assessment Boundary, as recommended in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

3.1 Habitat Suitability Index (HSI)

Waterbodies within a 500, radius of the Assessment Boundary were identified using several methods including ordnance survey maps, aerial photographs and walkover surveys. Identified waterbodies were then assessed for their potential to support GCN using the standardised Habitat Suitability Index (HSI) methodology (Oldham *et al.*, 2000).

The HSI assessment follows a standardised assessment criteria using habitat components such as water quality, fish/waterfowl presence and surrounding terrestrial habitat quality to derive a suitability score, or 'index'. Waterbodies with high scores are considered more likely to support GCNs compared to those with lower scores. HSI scores and the inferred suitability of the ponds assessed to support GCN are described within Table 9-4-1 below.

Table 9 - 4 - 1 Pond Suitability to Support Great Crested Newts

HSI Score	Habitat Suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.80	Excellent

During eDNA sampling a number of ponds were noted as being dry. However, it is possible that these ponds fill with water during wetter years therefore these were still subject to HSI assessments.

3.2 eDNA Survey

GCN eDNA surveys were carried out on all suitable waterbodies within 500m of the Assessment Boundary on the 15th May 2023, 15th – 16th April 2024 and 12th June 2024; which is within the recognised survey period (15th April until 30th June, inclusive). Some waterbodies were not sampled due to lack of land owner permission, with other ponds noted to be dry or non-existent, these are presented on Figure 9-6. The waterbodies were surveyed by two ecologists using the standard methodology, which has been tested by DEFRA research and approved for use by the nature conservation agencies. The laboratory testing was undertaken by SureScreen Scientifics using the following methodology.

The laboratory testing was conducted in two phases; first the sample goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested through real time Polymerase Chain Reaction (q-PCR). This process amplifies selected parts of DNA allowing it to be detected and measured.

QPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signal during the exponential phase of the reaction is measured for fast and objective data analysis.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified. Samples are tested in a clean laboratory and the different phases

of testing are kept separate to reduce any risk of cross contamination. Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates test positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

3.3 GCN Population Size Class Assessment Surveys

GCN presence was confirmed within a single pond, namely P1, through eDNA surveys, therefore further surveys were undertaken on this pond to establish the population size.

This was undertaken in order to provide a population class estimate, as the determination of population size in accordance with the English Nature 'Great Crested Newt Mitigation Guidelines', recommends that a minimum of six survey visits to each pond are conducted; with at least half of these survey visits completed between mid-April and mid-May.

Table 9 - 4 - 2 Survey dates and weather conditions

Survey	Date	Temperature (°C)	Rainfall	Wind (Beaufort Scale)	Cloud Cover
1	11/04/24 – 12/04/24	13-14	Light rain most of the evening survey that stopped at approximately 20:30 prior to torching. Dry on the morning visit.	3	50%
2	09/05/24 – 10/05/24	12-19	Dry	2	0%
3	16/05/24 – 17/05/24	13-14	Light rain for up to 1hr after arriving, stopping around 20:00 before torching. Dry on the morning visit.	1	50%
4	20/05/24 – 21/05/24	11-15	Dry	5	50%
5	03/06/24 – 04/06/24	13-15	Dry	3	100%
6	12/06/24 – 13/06/24	7-12	Dry	3	0%

The six surveys used at least three survey methods per visit, with methodology discussed further below.

3.3.1 Torchlight surveys

This method involves searching for newts at night by shining a torch into the waterbodies being surveyed. It should be undertaken only when the sky is fully dark (time after sunset may vary depending on cloud cover and nearby trees).

One million candlepower torches (e.g., CluLights) must be used to standardise the detecting of newts present. The margins of the pond are often the best areas to search for newts. The entire margin of the pond was walked once (where safe to do so), slowly checking for great crested newts

Torch survey results are subject to high variation due to weather conditions and are only be carried out under the following conditions: night-time air temperature above 5°C, no/little wind, no rain. A note was made of the percentage of the waterbody margin covered.

3.3.2 Bottle trapping

Specially designed bottle traps (1.5 to 2.0 litre inverted plastic bottles) were submerged in the waterbody on stakes at intervals of one trap per 2m length of shoreline. The bottle traps are designed

and installed in such a way that air pockets are trapped and will provide ample oxygen for at least 12 hours for captured newts. Bottle traps are placed in the water at dusk.

After night fall active newts enter the traps. The traps are designed on the same principle as lobster pots and the animals cannot escape easily. Traps were not left in situ for more than 12 hours. Surveyors returned in the morning between the hours of 06:00 and 11:00 to record the species, gender and number of newts captured. Newts are then returned to the water. Handling of newts is avoided as much as possible or, where necessary, carried out wearing vinyl gloves (not latex or nitrile, which can cause harm) and limited to as short a time as possible.

3.3.3 Netting

GCN can be captured, using a long-handled dip-net, by sampling the area around the pond edge. Netting can be conducted by day or night, but better results may be obtained at night when adult newts are more likely to be in open water. Handling of newts is avoided as much as possible or, where necessary, carried out wearing vinyl gloves (not latex or nitrile, which can cause harm) and limited to as short a time as possible.

3.3.4 Population size

Following the completion of the surveys, the peak count of GCN is calculated. This is the maximum number of GCN adults observed via one survey method (to avoid double-counting of individuals) per pond. This leads to the population size being assigned as follows:

- 'Small' population – peak count of between 1 and 10 adults;
- 'Medium' population – peak count of between 11 and 100 adults; and
- 'Large' population – peak count of 100 + adults.

Ponds with GCN populations within 250m of each other with no significant barrier to prevent dispersal are considered to be a meta-population. However, there was only one pond with GCN present.

3.3.5 Competence of surveyors

During the identification of broad features of potential value to GCN, suitably experienced ecologists were used, with an understanding of the ecological dependencies of the species in the wider environment.

Where field surveys were completed, an appropriately licensed ecologist, holding a Natural England (NE) great crested newt survey licence (licence number: 2023-11134-CL08-GCN) which permits the use of hand, nets, torches and aquatic funnel traps (including bottle traps) were used. All surveyors worked under supervision of the license holder.

Biosecurity

During all interactions with waterbodies, biosecurity practices were implemented to prevent the spread of diseases including chytridiomycosis and invasive plant species such as floating pennywort *Hydrocotyle ranunculoides*, following guidance within ARG UK Advice Note 4 (2017) and the Invasive Non-Native Secretariat's Check, Clean, Dry guidance. Equipment and footwear were disinfected between non-adjacent waterbodies using Virkon or a similar non-persistent and water-safe product.

3.4 Limitations

3.4.1 HSI and eDNA

The eDNA surveys were carried out within the recommended timeframe (mid-April to end of June), although P7 was inaccessible due to the steep pond embankment during 2023 yet this pond was able to surveyed during 2024. The following ponds did not undergo eDNA surveys, or HSI assessments, in 2024 because access permission was not granted: P18, P23, P24, P26, P27, P28, P29, P32, P33, P34, P38, P39, P40, P41, P42, P43, P44 and P53. Furthermore P47 was inaccessible due to surrounding watercourses and P45 was not accessible at the time of survey because of cows restricting access. On closer inspection of P30 there was no pond present with this area being a waterlogged reed patch; thus no HSI or eDNA survey was carried out.

In 2023, both P11 and P14 were dry at the time of eDNA surveying, with both P52 and P56 dry in 2024.

Surveys found no ponds where P15, P17, P19 and P46 were located on OS maps and aerial imagery, therefore no HSI or eDNA surveys were completed and these ponds were marked non-existent on Figure 9-6.

The late changes to the Assessment Boundary are not considered a limitation as an initial 500m buffer was used when designing survey scope, and therefore all ponds that were accessible within the Assessment Boundary have been surveyed.

3.4.2 GCN Population surveys

Torch survey results are subject to high variation due to weather conditions and are only to be carried out under the following conditions: night-time air temperature above 5°C, no/little wind and no rain. Whilst there was light rainfall during surveys 1 and 3 rain had ceased prior to torching, thus the impact of rain hitting the pond surface distorting and hiding any newt activity was not a factor here. Stronger wind speeds noted during survey 4 resulted in torching being an unsuitable survey method because wind distorts the water, making it harder to detect newts under the surface. However a terrestrial refuge search could not be completed due to the lack of refuges such as logs, bark and rocks surrounding P1. Therefore torching was substituted by netting as an additional method. Air temperature remained above 5°C throughout all surveys, allowing bottle traps to be deployed and left out overnight.

4. Results

4.1 HSI Results

Following completing the HSI, the waterbodies were scored with results found in Appendix B.

4.2 eDNA results

The full 2023 and 2024 technical reports from SureScreen Scientifics can be viewed in Appendix C. A figure of the results is available in Figure 9-6.

4.2.1 2023 Surveys

Following laboratory analysis of the samples, one pond (P1) returned positive for GCN presence with all other ponds sampled returning negative results.

At the time of surveying during 2023, two palmate newts were observed within the water in P7. This pond was very low set and inaccessible for testing at this time however was accessible in 2024, with eDNA results being negative for GCN. Ponds P11 and P14 were dry at the time of eDNA surveying.

4.2.2 2024 Surveys

A total of twenty three ponds were sampled during 2024, with no GCN DNA found in any of these ponds. Ponds P52 and P56 were dry at the time of eDNA surveying, so no samples could be taken.

4.3 Population Size Class Assessment Survey Results

Tables 3-5 below detail the results from the six surveys undertaken on P1 across April to June 2024. Overall no adult GCN were captured within bottle traps or observed during torching, however GCN eggs were found indicating the presence of a small breeding population.

Table 9 - 4 - 3 Torchlight survey results

Date	GCN Male	GCN Female	Palmate Female	Palmate Male	Smooth Male	Smooth Female	Smooth/Palmate	Juvenile
11/04/24 – 12/04/24	0	0	1	1	0	0	29	0
09/05/24 – 10/05/24	0	0	1	7	0	0	24	0
16/05/24 – 17/05/24	0	0	1	6	0	2	33	1 Smooth
20/05/24 – 21/05/24	0	0	0	2	0	0	14	0
03/06/24 – 04/06/24	0	0	0	2	0	0	10	0
12/06/24 – 13/06/24	0	0	0	0	0	0	0	0

Table 9 - 4 - 4 Bottle trapping survey results

Date	GCN Male	GCN Female	Palmate Female	Palmate Male	Smooth Male	Smooth Female	Juvenile
11/04/24 – 12/04/24	0	0	5	3	2	0	0
09/05/24 – 10/05/24	0	0	1	4	3	1	1 Smooth

Date	GCN Male	GCN Female	Palmate Female	Palmate Male	Smooth Male	Smooth Female	Juvenile
16/05/24 – 17/05/24	0	0	4	2	1	3	0
20/05/24 – 21/05/24	0	0	8	5	2	6	0
03/06/24 – 04/06/24	0	0	1	0	4	1	0
12/06/24 – 13/06/24	0	0	0	0	0	0	0

Table 9 - 4 - 5 Egg search survey results

Date	GCN	Smooth / Palmate
11/04/24 – 12/04/24	0	0
09/05/24 – 10/05/24	0	0
16/05/24 – 17/05/24	1	6
20/05/24 – 21/05/24	N/A	N/A
03/06/24 – 04/06/24	N/A	N/A
12/06/24 – 13/06/24	N/A	N/A

5. Summary

5.1 GCN Presence

Sixty four waterbodies were identified within 500m of the Assessment Boundary from OS mapping and aerial imagery, however five (P15, P17, P19, P46 and P30) of these were deemed non-existent. eDNA surveys were completed on all ponds listed within Table 6 below, with other ponds not sampled because of access limitations and being dry at the time of survey.

Table 9 - 4 - 6 eDNA survey results

Pond	Year	Result
P1	2023	Positive
P2	2023	Negative
P3	2023	Negative
P4	2023	Negative
P5	2023	Negative
P6	2023	Negative
P7	2024	Negative
P8	2023	Negative
P9	2023	Negative
P10	2023	Negative
P12	2023	Negative
P13	2023	Negative
P16	2023	Negative
P20	2024	Negative – P20 and P22 are connected, so were sampled as one pond
P21	2024	Negative
P22	2024	Negative – P20 and P22 are connected, so were sampled as one pond
P25	2024	Negative
P31	2024	Negative
P35	2024	Negative
P36	2024	Negative
P37	2024	Negative
P48	2024	Negative
P49	2024	Negative
P50	2024	Negative
P51	2024	Negative
P55	2024	Negative
P57	2024	Negative
P58	2024	Negative
P59	2024	Negative
P60	2024	Negative
P61	2024	Negative
P62	2024	Negative
P63	2024	Negative
P64	2024	Negative

Further surveys were undertaken on P1 to establish GCN population size, however no GCN were seen during the six surveys. Although one GCN egg was found on the third survey, suggesting a small breeding population does utilise P1.

5.2 Licencing

Although the law provides strict protection to GCN, it also allows this protection to be set aside (derogated) under Regulation 53 of the Conservation of Habitats and Species Regulations 2017 (the Habitats Regulations) through the issuing of European Protected Species Mitigation Licences (EPSML) for the purpose of preserving public health, public safety, other imperative reasons of overriding public interest, including those of a social or economic nature and beneficial consequences of primary importance for the environment. However, in accordance with the requirements of the Habitats Regulations a licence can only be issued where the following requirements are satisfied that:

- There is no satisfactory alternative; and
- The action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range.

In England, EPSML applications are currently determined by Natural England.

5.2.1 District Level Licencing

Given that only a small population of GCN are present in a single pond, with all other ponds sampled returning negative eDNA results, District Level Licensing (DLL) may provide a suitable alternative to the traditional licencing approach for this Assessment Boundary. DLL is available within the Greater Manchester area as well as in Lancashire.

DLL is a relatively new approach to authorising developments affecting GCN that focuses conservation effort where it will create maximum benefit whilst reducing delays, costs, risks and uncertainty for developers. It shifts investment from site-based assessment and mitigation into strategic habitat improvements which can be integrated with Local Plans and biodiversity strategies.

In appropriate cases, instead of requiring harm to GCN to be avoided or mitigated at a development site, the species is benefited elsewhere in the area at the developer's expense. This safeguards the conservation status of GCN in the area concerned and also represents a significant streamlining of the GCN licensing element of the consenting process.

The contribution required from developers will be designed to cover all the costs of implementing the scheme, including pond creation and restoration, maintenance, monitoring, reviewing and updating the strategy, administering the licence, authorisation of developments and any other overheads. The costs will depend on the scale of habitat creation/restoration required to mitigate the expected development in any given local authority area, so that will be calculated during the development of the strategy.

Depending on the location of the work, the application for the licence and the ultimate habitat creation, will be facilitated by Natural England or NatureSpace but ultimately, Natural England remains the regulator. The developer only pays for the habitat creation to take place and is not required to include it in their scheme.

6. References

ARG UK (2017). *ARG UK Advice Note 4: Amphibian Disease Precautions: A Guide for UK Fieldworkers*. Amphibian and Reptile Groups of the United Kingdom.

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Appendices



Appendix A. Waterbodies Surveyed

Table 9 - 4 - 7 Waterbodies within 500m

Pond number	Grid Reference	Image
P1	SD 86647 16046	
P2	SD 86570 16484	
P3	SD 86827 16081	

Pond number	Grid Reference	Image
P4	SD 87080 16143	
P5	SD 87066 16372	
P6	SD 86982 16438	

Pond number	Grid Reference	Image
P7	SD 86744 16504	
P8	SD 86259 17144	
P9	SD 86445 17051	

Pond number	Grid Reference	Image
P10	SD 86686 17257	
P11	SD 86022 18264	
P13	SD 85974 18189	

Pond number	Grid Reference	Image
P16	SD 86101 17541	
P20	SD 83833 20124	
P21	SD 84550 19586	

Pond number	Grid Reference	Image
P22		
P25	SD 86031 20749	
P31	SD 87848 19383	

Pond number	Grid Reference	Image
P35	SD 86509 18988	
P36	SD 87288 18527	
P37	SD 86668 17467	

Pond number	Grid Reference	Image
P48	SD 86021 20583	
P49	SD 86063 20850	
P50	SD 85292 20235	

Pond number	Grid Reference	Image
P51	SD 87398 19786	
P54	SD 85980 18769	
P55	SD 85468 18585n	

Pond number	Grid Reference	Image
P57	SD 86101 20748	 A photograph showing a pond with brown, silty water. The water surface is calm, reflecting the sky. The bank is covered in dry, brown grass and some small shrubs.
P58	SD 85418 19587	 A photograph of a pond surrounded by tall, dry grasses. The water is dark and still, reflecting the surrounding vegetation. The grasses are a mix of green and brown.

Pond number	Grid Reference	Image
P59	SD 86452 17044	
P60	SD 86136 18254	

Pond number	Grid Reference	Image
P61	SD 86097 20793	
P62	SD 86141 20802	
P63	SD 86809 16407	

Pond number	Grid Reference	Image
P64	SD 86746 17297	

Table 9 - 4 - 8 HSI Matrix 2023 Results

Pond	1	2	3	4	5	6	7	8	9	10	11	12
Geographic location	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pond area	1	0.8	0.2	1	1	1	0.1	0.7	0.8	1	0.1	0.8
Pond permanence	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.5	1	0.1	0.9
Water quality	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Shade	1	1	1	1	1	1	1	1	1	1	1	1
Water fowl effect	0.67	1	0.67	1	0.67	1	1	1	1	1	1	1
Fish presence	1	1	1	1	1	1	1	1	1	1	1	1
Pond Density	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Terrestrial habitat	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Macrophyte cover	0.6	0.5	0.85	1	0.6	0.6	0.7	0.7	0.8	1	0.8	0.6
HSI Score	0.76	0.76	0.67	0.83	0.76	0.79	0.64	0.78	0.75	0.84	0.52	0.77
Pond Suitability	Good	Good	Average	Excellent	Good	Good	Average	Good	Good	Excellent	Below Average	Good

Table 9 - 4 - 9 HSI Matrix 2024 Results

Pond	7	20/22	21	25	31	32	35	36	37	48	49	50	51	54
Geographic location	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pond area	0.1	0.05	0.89	1	0.1	0.1	0.05	0.85	0.2	0.4	0.05	0.1	0.1	0.05
Pond permanence	0.9	1	0.9	1	0.5	0.1	0.1	0.9	0.5	0.5	0.5	0.5	0.5	1
Water quality	0.67	0.33	0.01	0.33	0.67	0.01	0.67	0.67	0.67	0.33	0.67	0.33	0.67	0.67
Shade	1	1	1	1	0.8	1	1	1	1	1	1	1	1	1
Water fowl effect	1	1	1	0.01	1	1	1	1	1	0.67	0.67	0.67	1	1
Fish presence	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Great Crested Newt and Amphibians

Pond Density	0.8	0.5	0.35	0.8	0.7	0.8	0.5	0.1	0.1	0.8	0.9	0.6	0.7	0.3
Terrestrial habitat	0.67	0.67	0.33	0.67	1	0.33	0.67	0.67	0.67	0.67	0.67	1	1	0.67
Macrophyte cover	0.7	0.4	0.4	0.4	0.4	0.4	1	0.5	0.6	0.6	0.9	0.5	1	0.4
HSI Score	0.64	0.51	0.42	0.45	0.57	0.3	0.47	0.62	0.52	0.61	0.54	0.53	0.64	0.52
Pond Suitability	Average	Below Average	Poor	Poor	Below Average	Poor	Poor	Average	Below Average	Average	Below Average	Below Average	Average	Below Average

Table continued below

Pond	55	57	58	59	60	61	62	63	64
Geographic location	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pond area	0.05	0.6	0.3	0.2	0.05	0.8	0.9	0.05	0.05
Pond permanence	0.5	1	0.5	1	0.5	1	1	0.5	0.1
Water quality	0.33	0.33	0.33	0.01	0.67	0.33	0.33	0.67	0.67
Shade	0.6	1	1	1	1	1	1	1	1
Water fowl effect	1	0.01	1	1	1	0.67	0.67	1	1
Fish presence	1	1	1	1	1	1	1	1	1
Pond Density	0.1	0.8	0.4	0.5	1	0.75	0.5	1	1
Terrestrial habitat	1	0.67	1	0.67	1	0.67	0.67	1	1
Macrophyte cover	1	0.6	0.6	0.4	1	0.9	0.8	0.5	0.9
HSI Score	0.44	0.45	0.6	0.41	0.62	0.72	0.7	0.58	0.52
Pond Suitability	Poor	Poor	Average	Poor	Average	Good	Good	Below Average	Below Average

Appendix C. Surescreen eDNA Results

Results appended to the report.

Folio No: E17588
Report No: 1
Purchase Order: 4790
Client: ATMOS CONSULTING LTD
Contact: Thomas Bamber

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: 23/05/2023
Date Reported: 01/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3892	Scout Moor 2 Pond 4	-	Pass	Pass	Pass	Negative	0
3894	Scout Moor 2 Pond 5	-	Pass	Pass	Pass	Negative	0
3896	Scout Moor 2 Pond 6	-	Pass	Pass	Pass	Negative	0
3898	Scout Moor 2 Pond 1 (Part 2)	-	Pass	Pass	Pass	Positive	1
3899	Scout Moor 2 Pond 9	-	Pass	Pass	Pass	Negative	0
3901	Scout Moor 2 Pond 10	-	Pass	Pass	Pass	Negative	0
3902	Scout Moor 2 Pond 2	-	Pass	Pass	Pass	Negative	0



3903	Scout Moor 2 Pond 1 (Part 1)	-	Pass	Pass	Pass	Negative	0
3904	Scout Moor 2 Pond 12	-	Pass	Pass	Pass	Negative	0
3906	Scout Moor 2 Pond 13	-	Pass	Pass	Pass	Negative	0
3907	Scout Moor 2 Pond 8	-	Pass	Pass	Pass	Negative	0
3908	Scout Moor 2 Pond 3	-	Pass	Pass	Pass	Negative	0
3909	Scout Moor 2 Pond 16	-	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Gabriela Danickova

Approved by: Jackson Young

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: 382-2024
Purchase Order: PO 6874
Contact: Atmos Consulting Ltd
Issue Date: 25.04.2024

GCN Report

Technical Report



SureScreen Scientifics

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
0403	Scout Moor 2, P57	SD8610120748	Pass	Pass	Negative	0/12
0404	Scout Moor 2, P21		Pass	Pass	Negative	0/12
0405	Scout Moor 2, P25	SD8603120749	Pass	Pass	Negative	0/12
0406	Scout Moor 2, P85	SD861207	Pass	Pass	Negative	0/12
0407	Scout Moor 2, P58	SD8541819587	Pass	Pass	Negative	0/12
0409	Scout Moor 2, P59		Pass	Pass	Negative	0/12
0410	Scout Moor 2, P7		Pass	Pass	Negative	0/12
0411	Scout Moor 2, P22		Pass	Pass	Negative	0/12
0412	Scout Moor 2, P62	SD8614120802	Pass	Pass	Negative	0/12
0416	Scout Moor 2, P80		Pass	Pass	Negative	0/12



Folio No: 382-2024
Purchase Order: PO 6874
Contact: Atmos Consulting Ltd
Issue Date: 25.04.2024

GCN0417	Scout Moor 2, P51	SD8738319792	Pass	Pass	Negative	0/12
0418	Scout Moor 2, P48	SD8602120583	Pass	Pass	Negative	0/12
0421	Scout Moor 2, P54		Pass	Pass	Negative	0/12
0424	Scout Moor 2, P37		Pass	Pass	Negative	0/12
0425	Scout Moor 2, P35		Pass	Pass	Negative	0/12
0426	Scout Moor 2, P81		Pass	Pass	Negative	0/12
0440	Scout Moor 2, P60		Pass	Pass	Negative	0/12
GCN0826	Scout Moor 2, P49	SD860208	Pass	Pass	Negative	0/12
GCN0827	Scout Moor 2, P50	SD8529220235	Pass	Pass	Negative	0/12
GCN0830	Scout Moor 2, P55		Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Chelsea Warner



Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

- Sample Integrity Check:** 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. Any samples which fail this test are rejected and eliminated before analysis.
- 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.
- Inhibition Check:** **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 2034-2024
Purchase Order: 7388
Contact: Atmos Consulting Ltd
Issue Date: 26.06.2024

GCN Report

Technical Report



SureScreen Scientifics

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN0852	Scout Moor, Pond 31	SD8784819383	Pass	Pass	Negative	0/12
GCN0861	Scout Moor, Pond 36	SD8728818527	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes



Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

- Sample Integrity Check:** 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. Any samples which fail this test are rejected and eliminated before analysis.
- 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.
- Inhibition Check:** **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.

