

Balcombe Estate
Estate Office Stone Hall
Haywards Heath Road
Balcombe
West Sussex
RH17 6QN

Via Email Only

Wednesday 5th May 2021

Re: Land at Townhouse Farm, Church Lane, Ardingly, East Sussex – GCN eDNA Assessment

Dear Sir / Madam,

I am writing this letter following our recent correspondence regarding the environmental DNA (eDNA) assessment of waterbody P1 situated immediately adjacent the application site at Townhouse Farm, Church Lane, Ardingly, East Sussex.

A preliminary ecological appraisal (PEA) was undertaken of the application site in 2021 (PJC Consultancy, 2021). The PEA also included a great crested newt (GCN) *Triturus cristatus* habitat suitability index (HSI) assessment of waterbody P1.

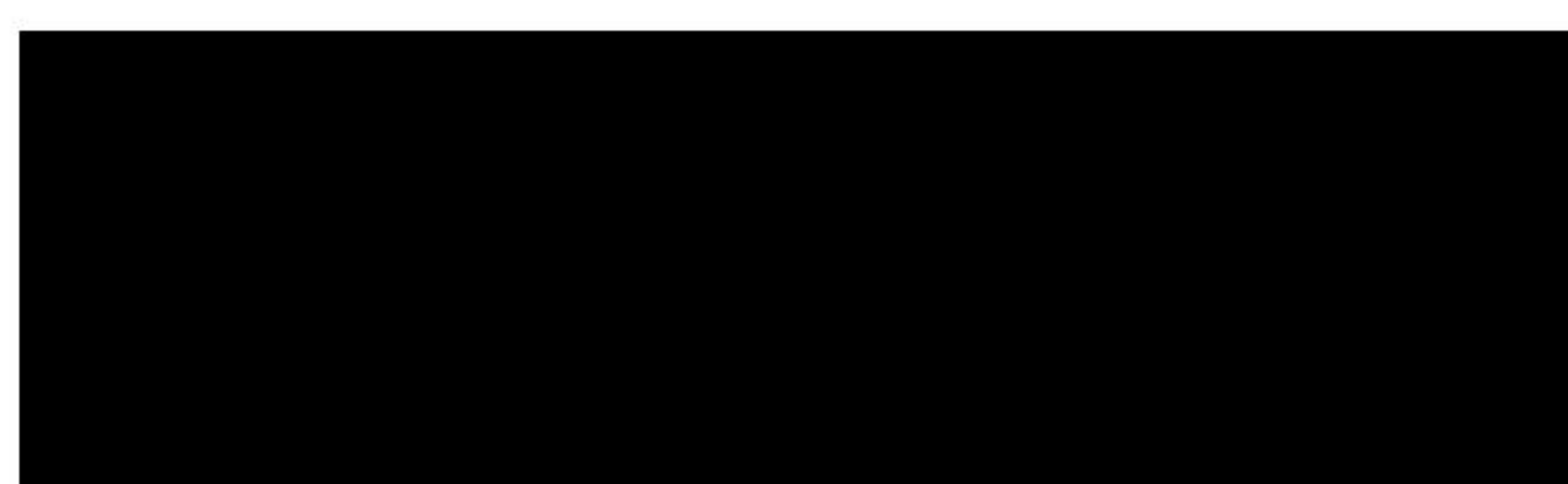
The results of this HSI assessment determined waterbody P1 as having 'good' suitability to support breeding GCN.

On this basis and in accordance with recognized survey guidelines, an eDNA assessment was subsequently undertaken to establish the presence/likely absence of GCN within waterbody P1.

When GCN inhabit a pond, cells containing their DNA are continually shed into the water via their saliva, urine, faeces, skin cells and dead individuals etc. This eDNA may persist for several weeks and can be collected through water sampling, and analysed in a controlled laboratory environment to assess the presence/likely absence of GCN within a waterbody.

An eDNA survey was carried out on waterbody P1 on 23rd April 2021 during daylight hours and when newts are most likely to be present (mid-April to late-June). Samples of water were taken from the pond by Thomas Knight BSc(Hons) MSc MCIEEM (Natural England GCN class 1 licence holder).

The eDNA collection was undertaken in accordance with best practice survey standards (Biggs et al., 2014). Samples were taken from 20 suitable sampling sites around the perimeter of the pond. The 20 samples were combined, and a pipette used to transfer 15ml of the water in to preserve filled sample tubes, where they were combined with the preservative. These water samples were stored and transported in accordance with best practice protocols for subsequent laboratory analysis.



The result of this analysis was “Negative” (see Appendix I for eDNA Technical Report), meaning that GCN eDNA was not detected and GCN are therefore considered likely absent from waterbody P1.

It should be noted that the PEA report specifies a further four further waterbodies (P2, P3, P4 & P5) within 250m of the Site, located approximately 110m north, 160m north, 180m east and 150m south-east of the application site respectively.

However, given the distance between the Site and these four waterbodies and given the nature of the proposed development which is anticipated to result in an approximate loss of 0.4ha of sub-optimal GCN terrestrial habitat, assuming that GCN are present within these two waterbodies, the proposed development is considered highly unlikely to result in the death or injury, or disturbance to GCN or result in the damage or destruction of a GCN breeding site or resting place.

This is supported by the rapid risk assessment tool within the GCN licence application form (WML-A14-2), which was used to assess the risk of the proposed development on GCN. Based on the current proposals that indicate the permanent loss 0.1ha of sub-optimal GCN terrestrial habitat, the tool indicates a ‘green’ risk meaning the risk of an offence being committed is considered to be highly unlikely and that a European Protected Species (EPS) licence is not required.

Nevertheless, as a precaution, it is recommended that construction works (including habitat clearance works) be undertaken in accordance with a Precautionary Non-Licensed Method Statement (see Appendix II).

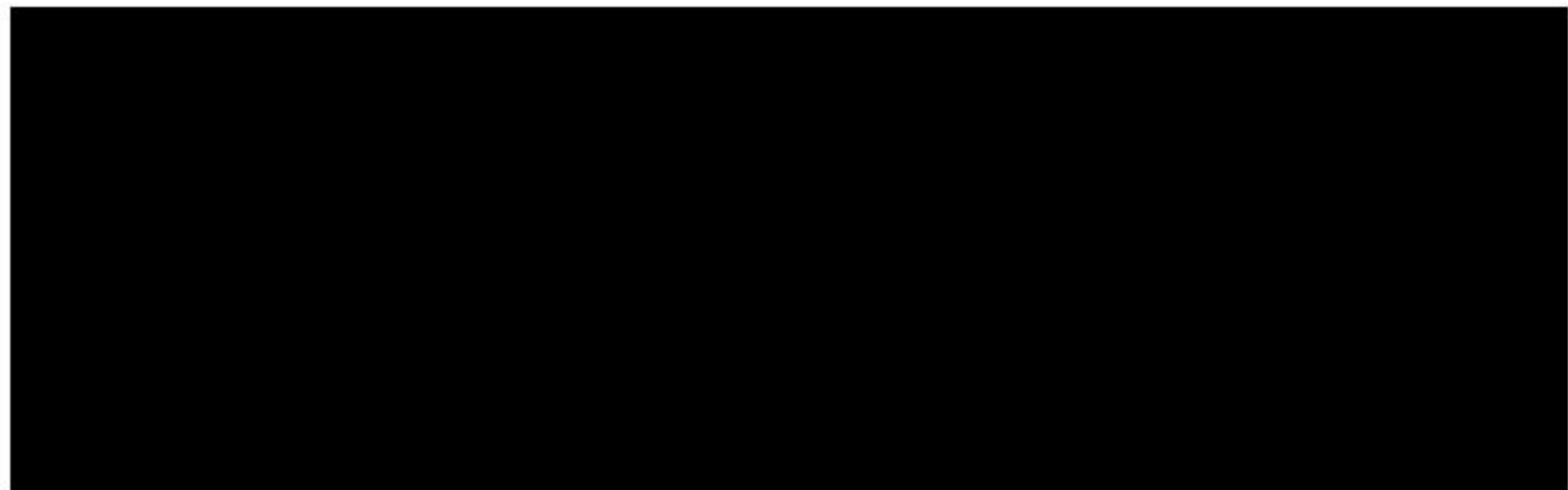
The purpose of the Precautionary Non-Licensed Method Statement is to detail all reasonable avoidance and mitigation measures to be implemented to ensure that there are no detrimental impacts on the favourable conservation status of GCN and no risk to individual GCN potentially present within the Site as a result of construction activities.

Avoidance and mitigation measures detailed within the Precautionary Non-Licensed Method Statement include but are not limited to the following:

- Ensuring that construction works are undertaken during the spring/summer season (April to mid-June) when GCN enter into/are around aquatic habitat to breed, and outside the hibernation period (avoiding November to February, inclusive).
- Ensuring that construction works (including habitat clearance and excavations) are undertaken under the direction and supervision of a suitably qualified and licensed ecologist.

Providing the avoidance and mitigation measures detailed within the Precautionary Non-Licensed Method Statement are implemented in full, the proposed works are considered highly unlikely to adversely affect the favourable conservation status of GCN, or breach relevant nature conservation legislation pertaining to GCN. On this basis, further GCN presence/likely absence surveys and/or a EPSL is not required prior to proposed works commencing.

Appendix I: eDNA Technical Report



Rocks Yard, Victoria Rd
Herstmonceux, Hailsham
East Sussex, BN27 3AB

Folio No: E9755
Report No: 1
Purchase Order: 4531E
Client: PJC CONSULTANCY
Contact: Thomas Knight

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: 28/04/2021
Date Reported: 30/04/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1740	TOWNHOUSE FARM	TQ 337 293	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth

Approved by: Chris Troth



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.



Appendix II: GCN Precautionary Non–Licensed Method Statement

MITIGATION MEASURES

The mitigation measures detailed below are provided to ensure that in the unlikely event of a GCN being present within the Site, they are protected from death, injury or disturbance, and that their resting places are also protected from damage, destruction, or obstruction of access.

PRIOR TO CONSTRUCTION WORKS

All site workers undertaking any habitat clearance works will receive an ecological Tool Box Talk (TBT) with a focus on GCN from a suitably qualified ecologist prior to undertaking habitat clearance works on Site.

DURING CONSTRUCTION WORKS

Access and egress routes for people and plant must be kept to existing areas of hardstanding, bare earth and grassland (providing the grassland sward has been maintained below 150mm in height).

Habitat clearance must be conducted using a two–stage directional approach, first reducing the vegetation to approximately 150mm above ground, then the second cut reducing it to ground level. This technique will allow any protected species potentially present within the Site to naturally disperse to other areas of suitable semi–natural habitat within the surroundings.

An ecologist must hand search the cleared areas either before the first cut, or immediately after, depending on the height of the vegetation and visibility.

Potential refuge features, such as brash piles and exposed tree roots potentially used by hibernating GCN must not be moved or affected during the hibernation season (November to March). All features must be deconstructed sensitively, whereby removing half of the feature (top–down) and removing the second half after 24 hours.

All suitable GCN habitat removal must be supervised at all times by a suitably qualified ecologist.

All arisings generated from habitat clearance works must be removed from Site and should not be stored on Site for any longer than a 24–hour period. In the event that this is not possible, all arisings must be stored within a skip or a minimum of 10m away from retained suitable GCN habitat, and only on existing hardstanding or bare earth.

All excavations should be excavated individually and back filled immediately where possible. Where this is not possible, excavations must be covered to prevent GCN (and other animals) becoming trapped within the excavation. If this is also not possible, one or both sides of the excavation must be sloped in order to allow egress from the excavation. All machinery, equipment and materials must be stored on areas of hardstanding or bare earth or on other areas identified by an ecologist as being unsuitable for GCN, for example, grassland of a short sward.

All site workers, particularly those involved in habitat clearance works, must remain vigilant at all times during construction works if at any point during construction works any

protected species or signs of protected species, including GCN are identified, the following instructions must be adhered to:

- Stop works immediately and leave the area;
- Inform an ecologist immediately who will then provide further guidance/instructions;
- Do not try to handle a GCN; and
- Do not resume construction works until advised it is safe to do so by a suitably qualified ecologist.