Ecological survey:
Bat activity and Great crested newt eDNA survey.

(To be read in conjunction with 'Protected species and Habitats survey', JP ecology, 7th Nov 2022).

Replacement Garage,
Meadowside,
3 Upper Street,
Oakley,
Diss,
IP21 4AX.

Final report: 3rd June 2023.

Author: John Parden
Natural England Bats (All species) Licence No. 2015-14697-CLS-CLS
Natural England Great Crested Newt Licence No. 2021-53785-CLS-CLS (GCN)

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1.0: Summary:

The site was subject to a bat activity survey and the pond was subject to e-DNA analysis for Great crested newts.

The bat activity survey did not identify bats roosting within the structure.
The e-DNA survey returned a Negative result for the presence of Great crested newts.

The Mitigation and Enhancement proposals described in the Protected Species and Habitats Survey, JP ecology, of 7th Nov 2022 as amended below, are recommended.

Mitigation.
General mitigation – all species.

- The contact details of a suitably licenced ecologist should be made available to the development contractors.
- Advice must be sought from an ecologist if any protected species are inadvertently disturbed.

Obligatory mitigation.

- Nesting birds –
  - Nesting birds should not be disturbed during the nesting season typically 1st March to 31st August (species dependant).
  - Should it be necessary to strip the site during the nesting season, specifically the demolition of any parts of the garage, the site should be searched by a suitably qualified ecologist for nests and any active nests protected until the young have fledge.

Precautionary mitigation.

- Site clearance.
  - Amphibians. The specifics of the clearance of the site with regard to Amphibians are as follows:
    - Any debris piles should be dismantled by hand and the materials kept in skips until moved off site or disposed of.
    - Any debris and materials arising from the proposed construction should be stored in skips and/or on pallets to prevent creating refuge sites for reptiles or amphibians.
    - The clearance of ruderals and vegetation > 300mm in height should be done during spring / summer (Feb to October) when amphibians and reptiles are active, all vegetation should be cut down to 150mm above ground level and left for at least an hour before final clearance to allow any reptiles or amphibians that may be present to disperse or to be carefully relocated to hedgerows in the local vicinity. Once cleared the land should be maintained as bare ground or short mown grassland throughout the development process.
    - If a great crested newt is discovered at any stage of the development, work should cease immediately, and an ecologist should be contacted for further advice.
  - Small mammals including hedgehogs.
    - Any debris and materials arising from the proposed construction should be stored in skips and/or on pallets to prevent creating refuge sites for reptiles or amphibians.
    - Clearance of any debris or waste should be done sensitively with consideration to disturbance of hedgehogs.
    - Vegetation above 300mm above ground level should not be cleared until temperatures are above 6C for at least 6 consecutive days to avoid disturbance of hibernating hedgehogs.
    - Any fences that might be erected should include a gap of 150mm long by 100mm high at some point in the base of each run of fencing to enable terrestrial vertebrates, including hedgehogs, to move through the plot and prevent entrapment.
- To avoid the risk of causing injury or harm to small mammals, amphibians and reptiles during the construction process the generic method statement attached in appendix 1 should be made available to all contractors.
- Should the Local Planning Authority be minded to grant planning permission then it is advised that the site be maintained as bare ground or close mown grassland until the development works start. Reason, to prevent the establishment of any features of ecological interest becoming established on the site prior to the commencement of works.

Ecological Enhancement.

- Birds. - 2 x bird box (house sparrow terrace) to be mounted under the eaves on the north or west facing aspect of the proposed building.
- 1 x built in bat box (Schweglar 2FE or similar) to be mounted as high as practically possible on the south facing gable of the proposed building.

Clients responsibility towards protected species.

The site owner has a responsibility to ensure that protected species or their resting places are not killed, injured or disturbed as a consequence of their actions.
Whilst the results of the survey are considered to be conclusive at the time that the survey was conducted, there is always a possibility that protected species might occupy the site between the period of the survey and the commencement of any works on the site. If any protected species are discovered during any construction works a qualified ecologist should be contacted for advice or assistance.

Contents:

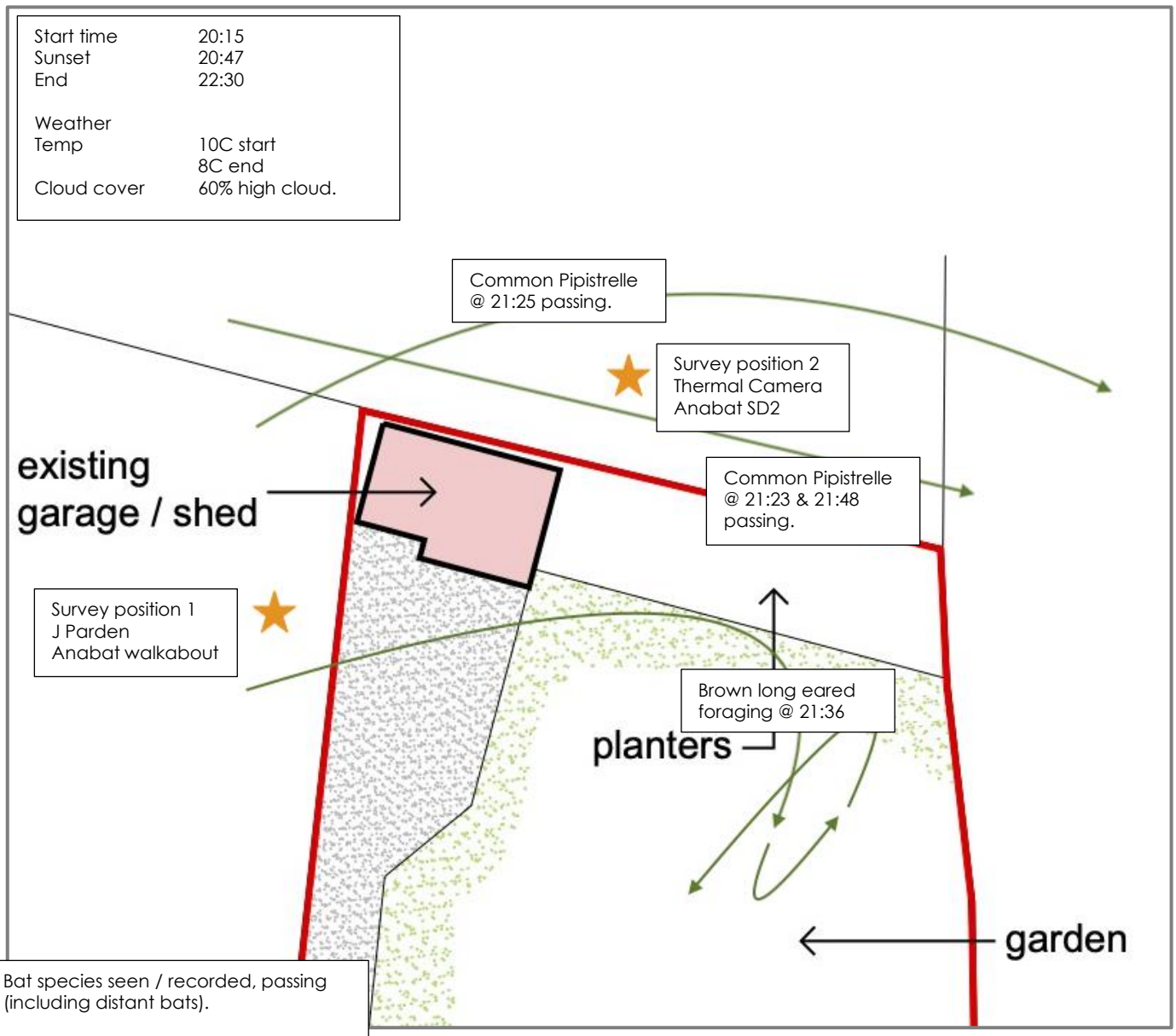
2.0: Bat Activity Survey

2.1 Methodologies

- Bat Survey – the bat activity survey was conducted in accordance with the guidance described in 'Bat Survey Good Practice Guidelines 3rd edition 2016'.
- The Scoping survey classified the buildings as having 'Low' favourability for roosting bats, consequently in accordance with the survey guidelines a single bat activity survey was required to give confidence to the results.
- The site was surveyed by John Parden of JP ecology on 17th May 2023. Given the building is a simple structure, a small garage, a single surveyor and a thermal camera was considered to be sufficient to cover all aspects of the building.
- Equipment used.
    - Anabat SD2
    - Anabat Walkabout
    - Hikmicro Lynx Pro LE15 Thermal Camera

2.2 Results.

No bats were observed emerging from the building.



3.0 Great crested newt e-DNA survey.

3.1 Methodology

The pond was sampled by John Parden on 23rd May 2023.

The DNA analysis was conducted by Surescreen Scientific, the sampling was conducted in accordance with their prescribed methodology (See attached Surescreen Scientific report).

Note: the pond has been subject to recent extensive maintenance, including felling and thinning of surrounding trees, clearing / dredging sediment, and reprofiling / extending the extent of the pond.

Whilst the maintenance work will have long term ecological benefits, it is likely to have compromised the ponds suitability for breeding Great crested newts in the immediate and short term.

Image of pond on 17th May 2023 following recent clearance.

3.2 Results.

The e-DNA analysis returned a 'Negative' result for the presence of Great crested newts.

SureScreen Scientifics

Folio No: E17651
Report No: 1
Purchase Order: OAKLEY 230524
Client: JP Ecology
Contact: John Parden

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: 25/05/2023
Date Reported: 01/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2364	Oakley 03	TM 1568 7672	Pass	Pass	Pass	Negative	0

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

Reported by: Gabriela Danickova **Approved by:** Jennifer Higginbottom

Forensic Scientists and Consultant Engineers
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Company Registration No. 08950940

SureScreen Scientifics

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.

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