

RE: eDNA Survey at P. 2048.24 2048.24 Halewood LUF – Grace Park

Introduction

A Preliminary Ecological Appraisal (PEA) of the site at Halewood LUF – Grace Park was undertaken in August 2023. One pond (P1) was identified within 500m of the proposed development works that may provide suitable breeding habitat for Great Crested Newt (GCN).

Legislation

The great crested newt (*Triturus cristatus*) is fully protected under the Wildlife and Countryside Act, 1981 (as amended) and the Habitats Regulations, 2017. It is also a Species of Principal Importance. The legislation makes it an offence to:

- Deliberately (or intentionally) kill, injure or capture (or take) a great crested newt, or great crested newt egg or eft;
- Deliberately (intentionally) damage or destroy any breeding site or resting place (i.e. pond, refuge, hibernaculum);
- Deliberately or recklessly obstruct access to any breeding site or resting place;
- Deliberately, intentionally or recklessly disturb a great crested newt, in particular disturbance which is likely to:
- impair the ability of the great crested newt to survive, breed, reproduce, or to rear or nurture young;
- impair the ability of the great crested newt to hibernate or migrate; or significantly affect the local distribution or abundance of great crested newts.

Methodology

The site was visited on the 16th April 2025 by Alex Rose (Natural England Great Crested Newt Licence 2022-10517-CL08-GCN) and Jack Kay, when eDNA samples were taken from pond P1. The eDNA sampling followed the methods detailed in WC1067 Appendix 5 (Biggs et al, 2014). In summary, the sampling protocol was as follows:

- 1) The water samples were taken on the 16th April 2025, which falls within the advised sampling period (between mid-April and 30th June). The samples were taken during dry conditions between 10:00 and 11:00 and were shielded from the sun to prevent degradation of DNA;
- 2) Sampling equipment was provided by SureScreen Scientifics and included:
 - A sterile 30ml ladle;
 - A sterile Whirl-Pak plastic bag with 1l capacity;
 - A sterile 10ml pipette to resample the pond water from the Whirl-pak;
 - Six sterile 50ml centrifuge tubes containing preservative, sodium acetate and other markers;and
 - A pair of sterile gloves;
- 3) The kits were stored at room temperature and used within 2 weeks of receipt. One kit was used on one pond within the site.
- 4) Gloves were worn at all times during the sampling process and replaced between sample collection from the pond and pipetting into the sterile subsample tubes. Samples were collected without entering the water.
- 5) Twenty sampling location points were identified around the margin of the pond. The location of sub-samples was spaced as evenly as possible around the pond margins, targeted to areas of vegetation with egg laying potential and open water areas which newts may be using for displaying.
- 6) Wearing the first pair of sterile gloves, the sterile Whirl-Pak bag was opened and 20 samples of 30ml of pond water was taken from the sampling points identified in 5 above and emptied into the Whirl-pak bag. Prior to sampling, the pond water column was gently mixed (avoiding the disturbance of the sediment) using the ladle at each sampling point. This was to mix DNA as it has a tendency to sink.
- 7) The Whirl-Pak was closed once filled with 20 samples (approximately 600ml) and shaken for 10 seconds to mix the DNA across the water sample.

- 8) A second sterile pair of gloves was put on and approximately 15ml of water was pipetted from the Whirl-Pak bag into a sterile tube containing 35ml of eDNA preservative, to make the tube up to the 50ml mark. The tube was closed ensuring the cap was tight.
- 9) The tube was shaken vigorously for 10 seconds to mix the sample and preservative and prevent DNA degradation.
- 10) Steps 8 and 9 were repeated for each of the 6 conical tubes in the kit, with the water in the Whirl-Pak stirred to homogenise prior to taking each sample.
- 11) The remaining water from the Whirl-Pak bag was emptied back into the pond.
- 12) The box of preserved sub-samples was returned to the office and stored at room temperature (as advised by SureScreen Scientifics for samples returned within 1 month of collection).

Survey results and evaluation

The eDNA samples returned a **negative** (0 out of 12 replicates) result for GCN at pond P1. The analysis technical report is enclosed. (GCN eDNA Analysis, appendix 2).

Conclusion

Pond (P1) is considered to not be a suitable breeding pond for GCN and GCN are considered to not be using ponds P1 -P4 for breeding and therefore further population size class surveys are not required to support a planning application.

Yours Sincerely,

Liz Kenyon
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