Great Crested Newt eDNA

Technical White Paper

SureScreen Scientifics

Great Crested Newt

Great crested newts (*Triturus cristatus*) are a widespread amphibian species in England and Wales with some localized populations in Scotland. They perform important ecosystem services such as the cycling of nutrients from water to land, which contributes to soil fertility. With declining populations due to changes in farming practices, habitat fragmentation and the destruction of ponds for building developments, great crested newts are now protected under the Wildlife and Countryside Act 1981. They are also a priority Species under the UK Post-2010 Biodiversity Framework and are listed as a European Protected Species under Annex IV of the European Habitats Directive.

Traditional Survey Methods

All standard survey methods require a great crested newt survey licence. In order to obtain a licence from Natural England, the surveyor must demonstrate both knowledge and experience: applications, training, and references are also required.

Egg searching

Great crested newts lay a single egg on a leaf. They then fold the leaf over and the egg adheres to the leaf, giving protection from predation. Egg searching is a relatively effective method of detecting newt presence used by ecologists during April and June. The surveyor must unfold the leaves from plants within the pond to check for eggs, however once unfolded, eggs will not re-adhere therefore leaving them exposed and at risk.

Torching

Torching should be done from mid-March to mid-June, between dusk and midnight. It is not recommended whilst raining (due to poor visibility in the water), during cold conditions (newts are more likely to be inactive at low temperatures), or in ponds with a high density of vegetation. Surveyors need to walk slowly around the perimeter of the pond shining the torch back and forth with attention to vegetation and the bottom of the pond in order to get a visual sighting of any newts that are present.

Netting

Netting can be done from March to June for adult newts and in August for larvae. Fifteen minutes of netting per 50 metres of pond shoreline is recommended, moving the net in a figure of eight motion. This technique is not as efficient as egg searching, torching or bottle trapping, and care must be taken as netting can cause damage and disturbance to ponds as well as risk the transfer of invasive non-native species between ponds.

Bottle trapping

An effective yet highly invasive technique, bottle trapping can prove difficult and the risk of harm to newts and small aquatic mammals is relatively high. A large plastic bottle with an inverted neck attached to a stick is placed with the opening submerged, at an angle and secured into the sediment. It is crucial that enough air is in the part of the bottle that is out of the water or there is a risk of newts and aquatic mammals drowning. Traps are set in the evening and checked and removed early the following morning. This survey method should only be undertaken after thorough training, due to the high risk of mortality if incorrectly executed.

Following the latest Natural England guidelines, these surveys should be undertaken between mid-March and mid-June. Three techniques must be used per visit and the site must be surveyed on four separate occasions throughout the survey season, unless the presence of great crested newts is detected on an earlier visit. As bottle trapping must be carried out overnight, each survey requires two days to complete, making these traditional survey techniques highly time consuming and expensive for ecologists.

eDNA Survey Methods

The environmental DNA survey method for great crested newts has been approved by Natural England since 2014. Organisms release DNA into the environment constantly in the form of urine, faeces, gametes, shedding skin or hair etc., remaining present in aquatic environments for up to three weeks. This DNA can be extracted from water samples and analysed to determine the presence or likely absence of great crested newts. Detailed guidance for accepted sample collection and laboratory analysis can be found in the DEFRA Technical Report WC1067 Appendix 5.

Unlike traditional survey methods, eDNA analysis of a water sample is a quicker, cheaper and more reliable method to establish the presence/absence of great crested newts within a pond. In addition, this non-invasive method does not cause any harm, as opposed to the traditional methods which may cause stress, increased exposure to predation or even accidental death to the newts or developing young.

eDNA at SureScreen

SureScreen have carried out great crested newt eDNA analysis since the technique was first approved in 2014, strictly following the methods set out in WC1067 and actively participating in the annual Natural England eDNA proficiency testing scheme.

Sample Collection

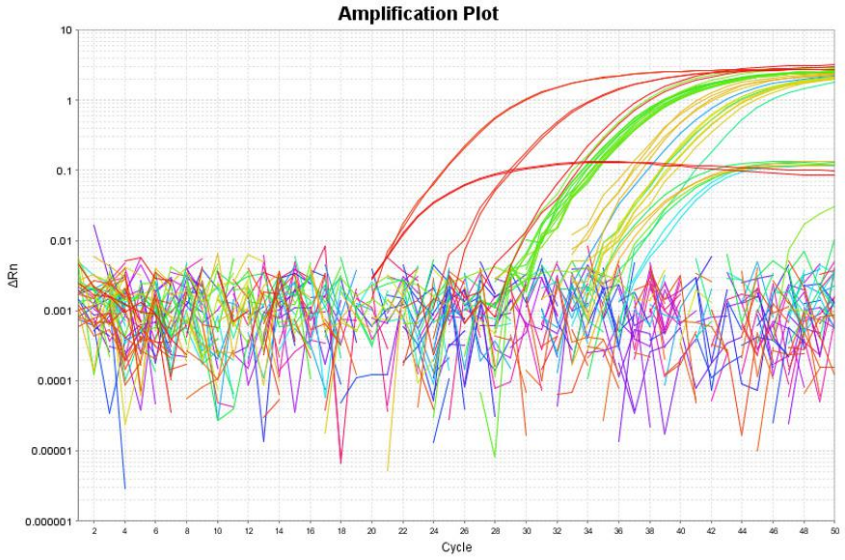
Natural England will accept results from samples collected between 15th April and 30th June, during the great crested newt breeding season, as the concentration of DNA in ponds should be relatively high during this time. We have developed our kits following WC1067 with input from our end-users and ecologists, ensuring they contain everything needed for easy collection of samples, including an ethanol preservative which prevents the degradation of DNA, and detailed sample collection instructions. Our kits have a long use-by date of three months (lasting for the entire eDNA sampling season) and contain a 'spike DNA' degradation marker which can inform at the point of analysis if any inhibitors are present within the sample or if the sample has degraded post sample collection. Once collected, any sample which will not be immediately returned to SureScreen Scientifics can be stored in a refrigerator for up to 4 weeks before analysis.

Sample Analysis

All of our great crested newt eDNA sample analysis steps follow strict guidelines outlined in Report WC1067, as approved for use by Natural England. At all stages along the process we make use of internal laboratory positive and negative controls in order to assure sample results are always accurate, reliable and free of contamination or inconsistencies. We utilize separate laboratories for each stage in the analytical pipeline to ensure sample integrity is maintained throughout.

Once the 6 subsample tubes arrive into the laboratory they are concentrated, and the DNA is then extracted and isolated to form a single sample for qPCR (quantitative polymerase chain reaction) analysis. This makes use of an enzyme and molecular markers which are designed to amplify the DNA of great crested newts to a great enough extent that it can be detected using fluorescent imaging.

Each sample is analysed with 12 replicates, allowing the methodology to have a high sensitivity and detect populations of great crested newts which are very small in abundance. If any one or more of these 12 replicates results in the positive detection of great crested newt eDNA then it is considered as positive for great crested newt presence. Negative results are assessed for the presence of spike DNA which if also negative or at lower concentrations indicates sample inhibition or degradation.



Typical output from qPCR analysis. Upper red curves represent known positive control sample, lower red curves represent spike DNA control, upper multi-coloured curves represent positive detection in eDNA samples, lower multi-coloured curves represent positive detection of spike DNA, 'noise' lines across center of graph represent negative samples and negative control samples.

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