



JUNE e-DNA UPDATE / OTHER SERVICES / R&D RELIEF

The Great Crested Newt, *Triturus cristatus*, or GCN, is an ideal subject for eDNA surveying methods. Rather than surveying the presence or absence of the organism itself, environmental DNA or eDNA can be detected from the organism's cellular material via their saliva, urine, faeces, skin cells etc. This eDNA may persist for several weeks. Thomsen showed in 2012 that GCN eDNA in water degrades in the pond in about 20 days, so a positive result shows the species has been present recently.

In our earlier bulletins we explained the process of sample collection and some of the theory behind eDNA. Here, we touch on some of the quality issues behind the lab testing and 'behind the scenes' work that we undertake to ensure you receive accurate result in a timely manner. We're hands-on forensic scientists, and we know how difficult it can be to sample and how demanding clients can be.

LAB TESTING

The laboratory testing is conducted in three phases, and each phase is zoned in the laboratory to prevent airborne contamination. DNA analysis is extremely sensitive so to avoid any risk of contamination the area is confined to authorized staff and samples are segregated. Each kit is booked into the laboratory system on receipt so the analysts have no knowledge of the client or location of the sample. (Our Laboratory also analyses urine and blood from famous sportspeople but only the Laboratory Director knows their identity).

Samples remain refrigerated throughout their journey in the Laboratory, and even the ultracentrifuges we use are refrigerated to 4 degrees Celsius. The kit first goes through an extraction process where all 6 tubes are centrifuged and the eDNA is extracted and pooled together to acquire as much as possible from the pond. The pooled sample is then reacted with an enzyme and fluorescence labelling system which finds, reacts with and labels the GCN eDNA. This process is lengthy which is why even our 'fast track' system takes a few days.

eDNA BENEFITS	
• Evidence of presence valid for previous 20 days	
• Sample any time of day	
• More accurate than surveys	
• Less Intrusive and safer	
• Perfect for 'murky water'	
• Accurate for 'evidence of presence'	
• Negatives give good guidance	
• One sample makes it easy	
• qPCR technique may determine population densities	
• Future data possibilities.	

Now comes the clever bit. An aliquot from this reacted sample is tested via real time PCR (or q-PCR). This process not only amplifies select part of DNA allowing it to be detected and measured, it continuously tracks the increase in target DNA and plots the result against known controls and any other calibration DNA we have introduced for quality control purposes. Each kit we receive is sampled and replicated in the instrument 12 times to ensure results are accurate. All samples are randomly distributed in the array of samples which include negative and positive controls and calibrators. These non-sample controls and calibrators are necessary whether we have one sample or six samples to run, making it very expensive to test a single sample. That's probably why other labs have a slow turn-round of results, they probably wait for more samples to arrive before conducting a run, but while we lose money on small batches we believe it's the speed of service that is critical to the success of the eDNA service.

The q-PCR works in cycles, which are made up of different stages. The DNA is heated to break the bonds and make 2 separate complementary single strands of DNA. Primers that are specific to a section of the target DNA are then attached at a lower temperature. Once attached these primers act as a starting point for the polymerase enzyme, this completes the missing DNA creating two exact copies of the target sequence. This cycle is repeated so that any samples that have the target DNA sequence

are amplified exponentially. At the end of every cycle the amount of DNA is measured giving a real time picture of the amplification process. The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified. If one or more of the 12 replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

WHEN CAN WE TEST?

Currently, for GCN presence/absence surveys, Natural England dictate that eDNA can only be sampled between 15 April and 30 June. However, samples taken outside this period can be used to show presence (say, if larval newts are in a pond) but negative results cannot be used to prove absence. Even so, a positive result can be helpful in large planning exercises where the developer might consider an alternative scheme to avoid inhabited ponds.

WHAT OTHER SERVICES ARE ON OFFER?

We have a fully equipped forensic laboratory that serves many industries. We handle water quality, contamination, planning issues, and construction materials as well as component failures and analysis. We're used by many well known companies as their centre of excellence. Here are a few examples of how we can serve you better and impress your clients:

- Microscopy, including sharp, high definition images of fecal matter, frass, debris, contamination etc up to x10,000 magnification.
- Analysis of debris, particles, contaminants etc.
- 'Source of origin' or 'who pollutes?' on unknown samples.
- Drone surveys with report-ready stills or videos.
- Bat species eDNA for bat droppings including photomicrographs
- Crayfish, Water Vole, Badger, Otter eDNA; others in test phase
- Research partnerships using Scientifics as your lab resource

RESEARCH AND DEVELOPMENT TAX REBATE

Did you know that ecological advice for clients may sometimes qualify for the R&D tax rebate, making your fees very tax-efficient?

For Small and Medium Enterprises, the Research and Development Expenditure Credit (RDEC) Scheme runs alongside the Large Company enhanced-deduction scheme which it will replace in April 2016. Tax relief is given on eligible projects as an 11% taxable credit on the amount of qualifying R&D expenditure. If your company is small or medium-sized, you may be able to claim R&D relief under the SME Scheme for one project and the Large Company Scheme for another.

For larger companies, the tax relief on allowable R&D costs is 130% - that is, for each £100 spent, your client could have the income on which Corporation Tax is paid reduced by an additional £30 on top of the £100 spent. If instead there's an allowable trading loss for the period, this can be increased by 30% of the qualifying R&D costs - £30 for each £100 spent. This loss can be carried forwards or back in the normal way that deferred losses work.

This means that where surveys are done as part of research or development, especially when supported by eDNA results, your fees will be rebated by the R&D tax allowance. Government introduced this rebate to oil the wheels of commerce, industry and improve the environment so everyone wins.

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