



PondNet

Using eDNA to implement a national citizen science-based monitoring programme for great crested newt (*Triturus cristatus*)



Volunteer GCN monitoring using eDNA

What is eDNA?

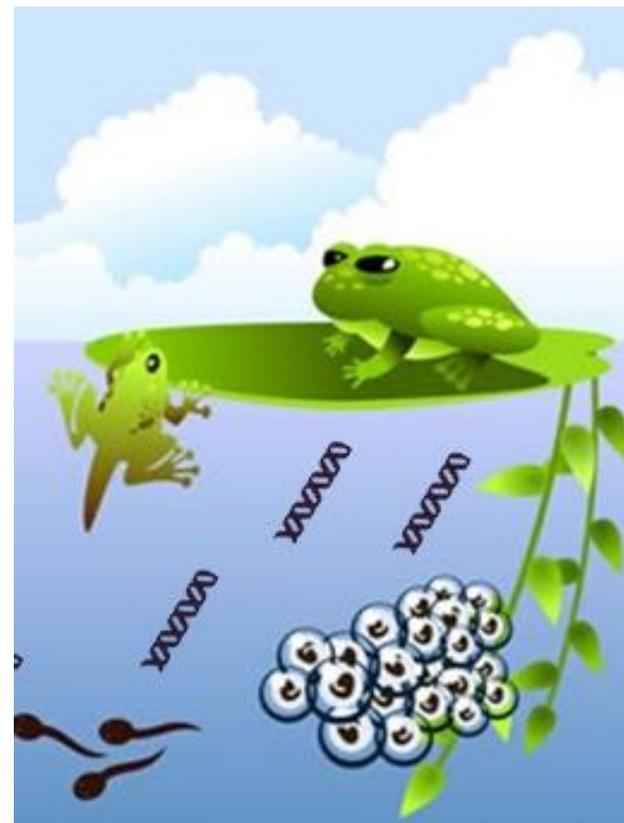
Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment.

Sources of eDNA include secreted faeces, mucous, and gametes, shed skin and hair, and carcasses.

In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, depending on environmental conditions.

Recent research has shown that the DNA of a range of aquatic organisms can be detected in water samples at very low concentrations using qPCR (quantitative Polymerase Chain Reaction) methods.

Collecting eDNA appears to be a highly effective method for determining whether Great Crested Newts are present or absent at a pond, although at the moment, eDNA provides only limited information on newt abundance.



eDNA shed into the environment from amphibians may be especially abundant during the breeding season

Volunteer GCN monitoring using eDNA

Background

At how many sites do we need to survey GCN to see if they are declining?

Statistical analysis says that we need annual surveys of approx. 200 1 km grid squares to have 80% power to detect 30% change in GCN pond occupancy in England and Wales.

This is based on the random selection of squares, from within two categories - 50% of squares are known to have previous records of GCN and 50% are unknown.

At an average of 3 ponds per square, we would need to survey around 600 ponds per year.

We can reduce the number of sites and, or detect smaller changes in the population if we use *trend analysis* from repeated visits to the same squares.

Natural England agreed to fund eDNA surveys of the ponds in 100 1 km grid squares (England only) in the network, and PondNet volunteers went out to collect the samples.



GCN eDNA monitoring network –
England

Volunteer GCN monitoring using eDNA

Overview

Traditional effective monitoring techniques for GCN are time consuming and require skilled and licenced surveyors.

Traditional methods:

- **Bottle trapping**
- **Torching at night**
- **Egg searching**
- **Netting**

Number of survey visits needed to know whether GCN are present /absent from a pond: (using all techniques)

- **3 visits (80% certainty)**
- **4 visits (90% certainty)**
- **5 visits (95% certainty)**

(Sewell et al. 2010)

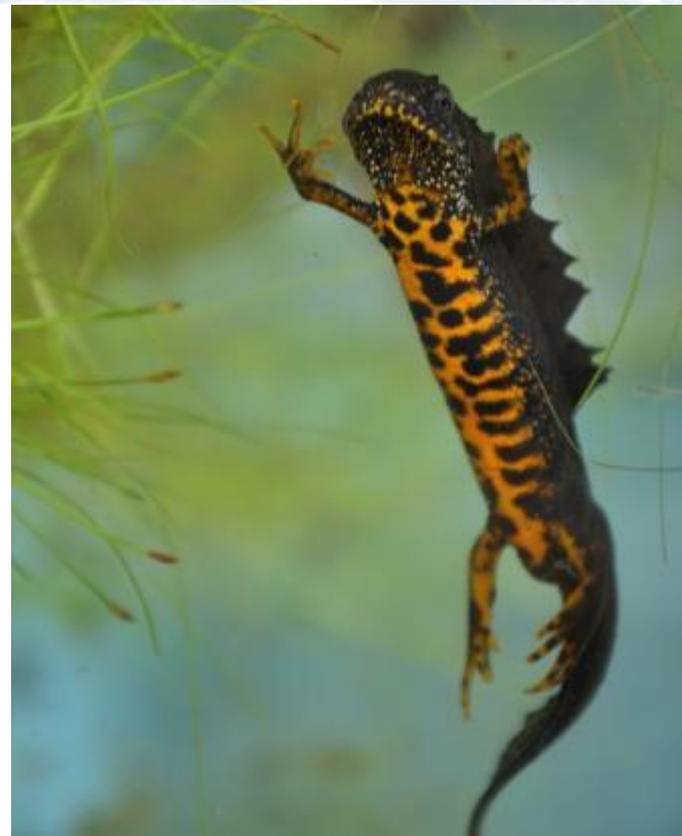


Photo credit: Chris Dresh ARC

Great crested newt (*Triturus cristatus*) a protected species listed in Annexes II and IV of the EC Habitats Directive (EC 1992).

Volunteer GCN monitoring using eDNA

Other issues with traditional techniques

Undertaking traditional surveys is difficult at some site and has a low success rate when undertaken by volunteers because it is so demanding in terms of time and effort.



Volunteer GCN monitoring using eDNA

2014 test of the eDNA method

Professional comparison of eDNA with traditional methods (bottle trapping, torching, egg searching) on 4 visits (at 35 ponds, 140 survey visits)

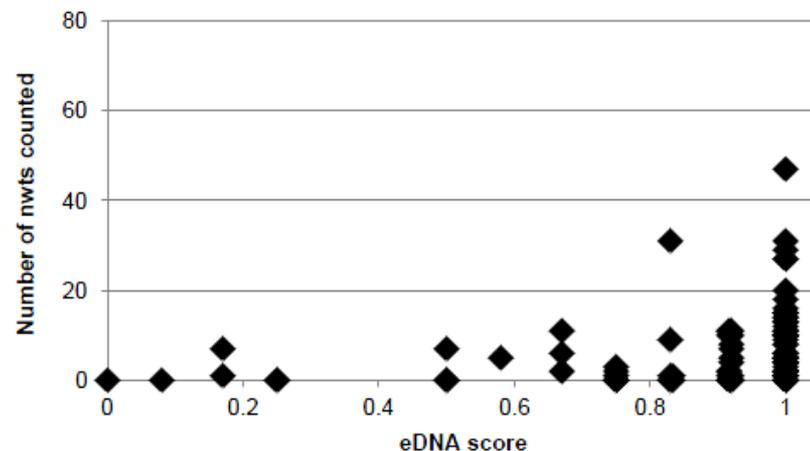
Result: eDNA significantly ($p < 0.0005$, McNemar's) more effective than traditional techniques on a single visit 99.3% detection rate using eDNA*

Volunteer evaluation of eDNA technique for (86 volunteers, 239 ponds across the UK)

Result: eDNA positive at 91.3% of sites known to support GCN

Volunteer evaluation of false positives using eDNA (60 ponds within and outside of GCN range where GCN were known to be absent)

No false positives using eDNA at ponds with no GCN



eDNA was great for identifying whether GCN were present – but not for estimating their abundance:

Relationship between eDNA score and great crested newt abundance (bottle trapping). eDNA scores range from 0 = 0/12 positive qPCR replicates to 1 = 12/12 positive qPCR replicates. eDNA was not a consistent predictor of GCN abundance.

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Monitoring measures

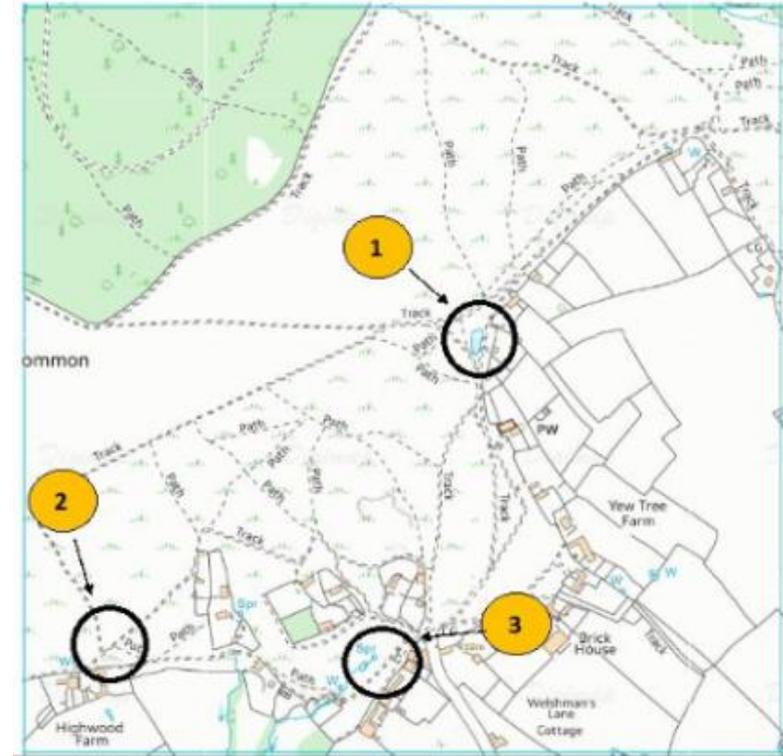
There are a number of ways of to monitor change in GCN at national level. PondNet and NARRS use complimentary methods, and their data can be combined

Only PondNet: The number of *occupied 1km grid squares* - because we aim to monitor all the ponds in each 1km grid square

Both PondNet and NARRS: The number of *occupied ponds* - based on a survey of the pond nearest the south-west corner of the square

Only PondNet: The number of *occupied ponds per square* – to detect change in the strength of populations over time

Only NARRS: The *number of newts per pond*, estimated using traditional methods. NB PondNet doesn't use this measure, even using traditional surveys because it is concerned with presence/absence only.



A PondNet square (only pond 2 would be surveyed as part of NARRS)

Volunteer GCN monitoring using eDNA

2015 PondNet Kit collection

PondNet project officers secured landowner permissions for 342 ponds in 100 1km grid squares in 2 months.

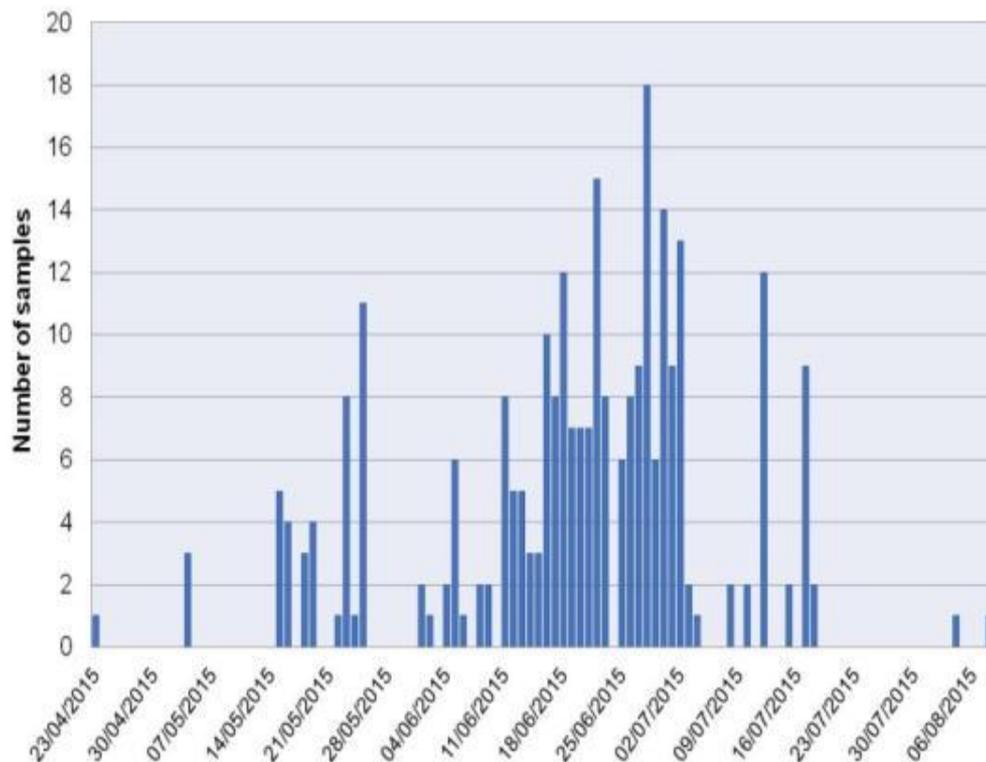
112 volunteers and staff collected the eDNA samples and completed pond habitat surveys.

Most eDNA kits (79%) were collected between late April and late June, the optimal survey window (Biggs et al. 2013).

At some sites we had minor delays agreeing access + matching up volunteers which meant that 16% of kits were collected in the first two weeks in July.

The remaining 5% of kits were collected late; between 17th July and 8th August.

eDNA samples were couriered to France for analysis by SpyGen . The results were all received by November 2015.



Date on which eDNA samples were collected in 2015

Volunteer GCN monitoring using eDNA

2015 Results: square occupancy in England

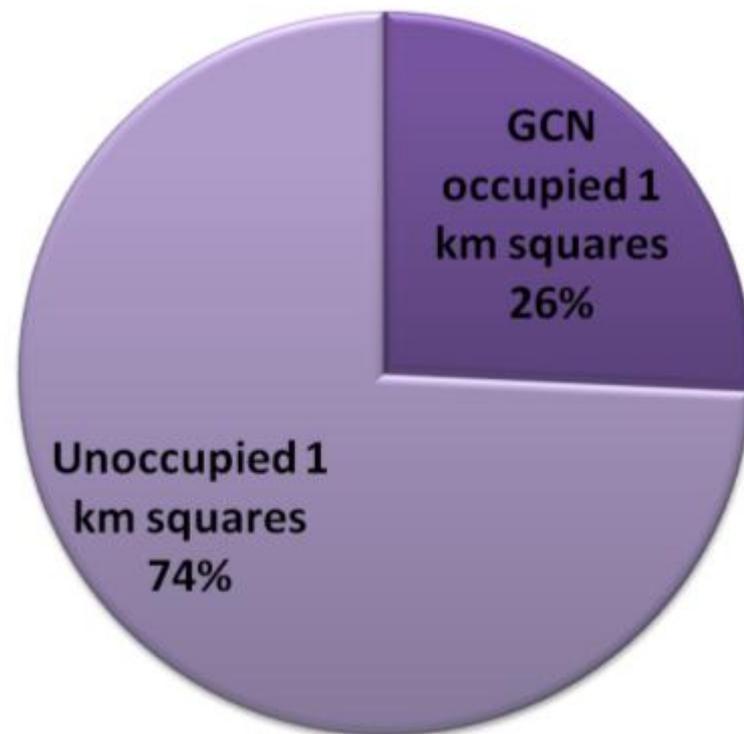
In the original database which listed where GCN had been previously recorded in England - only 4% of 1 km squares had records of GCN

Volunteers surveyed 50 of the 1 km squares that had previous GCN records using eDNA, 34 returned a positive result

In the original database 96% of 1km squares had no previous records of GCN

Volunteers used eDNA to survey 50 squares with no previous record of GCN, 12 returned a positive result (24%)

Overall, this suggests that around 26% of 1 km grid squares in England are occupied by GCN



Volunteer's eDNA results: Occupied versus unoccupied GCN squares in England

Volunteer GCN monitoring using eDNA

Results: pond occupancy in England

To make our results comparable with previous NARRS surveys we selected the pond in the SW corner of the square and tallied the results at a national level

Of the 50 known GCN squares surveyed using eDNA, only 15 ponds in the SW corner were positive (34 squares were positive)

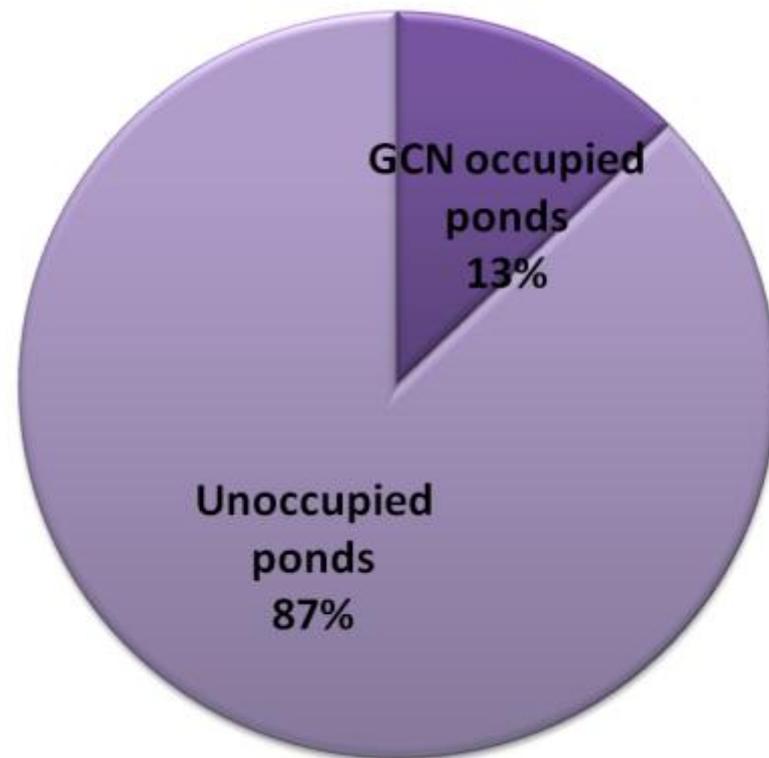
Of the 50 unknown GCN squares surveyed using eDNA, only 15 ponds in the SW corner were positive (12 squares were positive)

Overall, this suggests that around 13% of ponds in England are occupied by GCN

Previous estimates:

Swan + Oldham (1993) = 11%

Wilkinson + Arnell (2013) = 12%



Occupied versus unoccupied GCN ponds

Volunteer GCN monitoring using eDNA

Results: pond occupancy per 1km square

The eDNA results tell us, for the first time, that Great Crested Newt occupied ponds are quite scattered - even in squares where GCN are known to occur:

There were only 9 squares (out of 46 with positive records) where *all* ponds were occupied (20%)

In 18 squares (39%) less than half of the available ponds were occupied

On average , only around half (55%) of the ponds in a square with GCN were actually occupied



Alderford Common – Great Crested Newt SSSI

Volunteer GCN monitoring using eDNA

Results: Summary

The eDNA monitoring network has identified new records for Great Crested Newts, indeed around a quarter of survey squares with no previous records, did show the presence of GCN

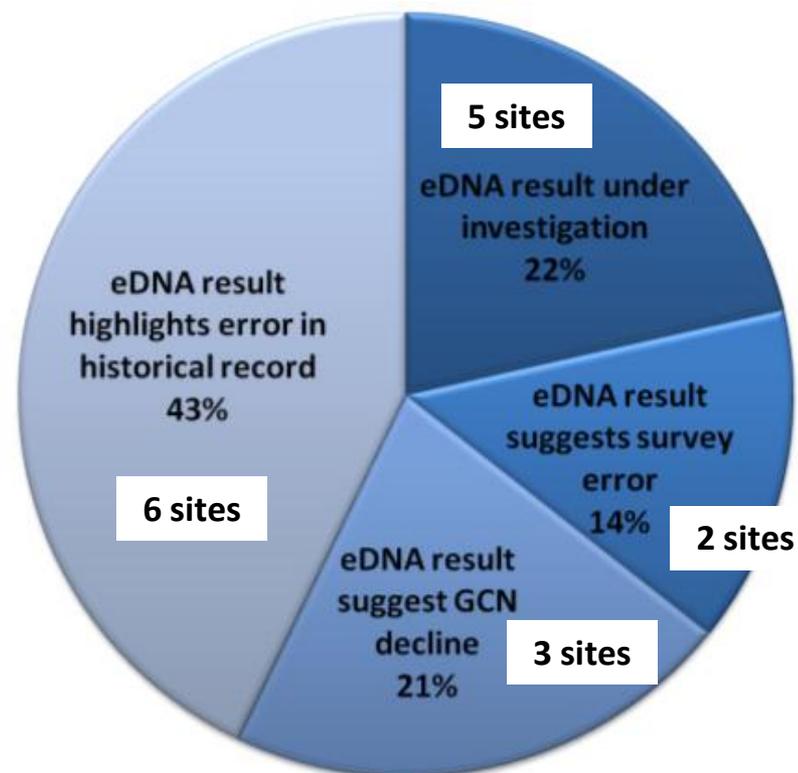
In some ponds 'local knowledge' suggests that newts were known to be present, but not officially recorded

Only 1 of the 16 results that were different to expected has been confirmed as a false negative i.e. GCN were known to be present but was not picked up by eDNA - this may have been because the pond was heavily used by geese and very turbid

The eDNA monitoring network showed that were present at sites where traditional surveys would be very difficult e.g. ponds with heavy scrub or steep sites

The survey has provided useful information for site managers on the status of Great Crested Newts where their current status was uncertain

eDNA monitoring indicates that Great Crested Newts may have been lost from some ponds and squares with previous records



Possible explanation for differences in the results of known GCN squares

Volunteer GCN monitoring using eDNA

Conclusions and next steps

eDNA is proving to be a really useful monitoring tool. Surveys undertaken by volunteers are already providing unique and reliable information about the occurrence of GCN in England.

The 2015 survey provided a baseline – continuing the survey in future years has the potential to provide both short and longer term information on the status of Great Crested Newts at local and national levels.

Lessons learnt from this year's survey:

- We will restrict the survey window to May only.
- We will identify distribution hubs, either training sessions, individual volunteers or Local Environmental Record Centres who can act as a point of pick up and drop off within the region.
- As a minimum we need to repeat the survey in 2016 and 2017 to begin to detect trends, and confirm presence/ absence at sites which may have fluctuating populations.



..... A HUGE THANKS to all the wonderful volunteers who helped with this year's survey!!