PondNet: Great Crested Newt surveys 2015-2017

PondNet is a national citizen science-based monitoring programme for pond habitats and the species they support. Between 2015 and 2017 the PondNet team in England, including more than 400 volunteers and regional staff collected data on the presence of Great Crested Newts from more than 230 1 km grid squares, and over 670 ponds.

Thanks to funding from Defra, Natural England and our Heritage Lottery Funded People Ponds and Water project and support from Amphibian and Reptile Conservation and the Amphibian and Reptile Groups, we rolled out the first ever national eDNA survey WORLDWIDE!

- **c.1,1400 results** from **380 ponds** over the three years of survey will contribute to the PondNet national monitoring network for Great Crested Newts.
- **c.126 results** from **113 ponds** will help us to understand the distribution of Great Crested Newts on our Flagship Pond Sites – some of the best pond sites in England.
- **c.120 ponds** were also surveyed in 2016 by Thames Water for Wildlife volunteers on local sites across the Thames Valley.

We can now share with you some of the exciting information which the PondNet surveys have revealed about the status and change of Great Crested Newts in England.

eDNA has allowed volunteers to smash the record from the largest number of ponds surveyed for Great Crested Newts in a single year

Background to PondNet

In 2012, Freshwater Habitats Trust commenced a two-year pilot project '*Biodiversity of ponds: developing and testing new approaches to biodiversity data collection in the voluntary sector*'. The project aimed to investigate whether it was possible to establish a new volunteer-based biodiversity surveillance network to provide statistically valid distribution, stock and change data for target species and habitats. The network's approach was novel in that surveillance was habitat-based (ponds), rather than species focused, seeking to record a number of species groups (plants, invertebrates, amphibians, including protected (Article 17 and S41) species etc.) and environmental data, at the same site.

The pilot was successful and, in 2015, PondNet became one of the core projects for delivery of Freshwater Habitat Trust's three-year HLF funded People, Ponds and Water project.

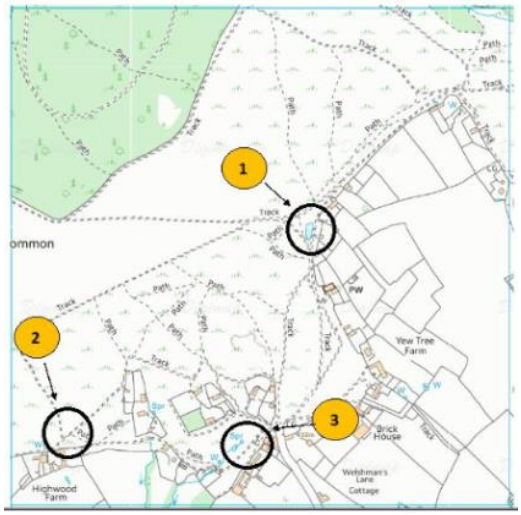
In **People, Ponds and Water** we aimed to achieve two overarching outcomes:

- To engage many thousands of people with activities that helped them to learn about, participate in, and enjoy their freshwater heritage, and
- To make a nationally significant difference to the protection of freshwater biodiversity in the UK – increasing understanding by gathering evidence on the status of pond habitats in England and Wales, and undertaking practical action on the ground to secure the future of some of the most important pond sites in the UK.

PondNet's Great Crested Newt Monitoring Network

We aimed to design a sampling programme which would provide data to monitor change in Great Crested Newts in England. We did some number crunching before we began the survey and discovered that the best design would be based on a repeat surveys of the same ponds over a minimum of three years (2015-2017). Occupancy would be determined through a combination of eDNA and traditional surveys, to report on the following metrics:

- **Change in the number of occupied 1 km grid squares**: by monitoring all the ponds in each 1 km grid square.
- **Change in the number of occupied ponds:** based on a survey of the pond nearest the south-west corner of each square (this is compatible with the National Amphibian and Reptile Scheme (NARRS) survey approach).
- **Change in the number of occupied ponds per 1 km grid squares:** to detect change in the strength of populations over time by providing counts of the number of ponds occupied.



A PondNet monitoring square

We also asked volunteers to complete a pond habitat survey for each pond which included data to calculate a Habitat Suitability Index score for Great Crested Newts. This would allow us to assess the quality of ponds for Great Crested Newts in England and understand the reasons why any changes that we saw may have occurred.

Grid squares would be chosen at random based on a stratified sample within two categories - 50% of squares known to have previous records of Great Crested Newt and 50% in which it was unknown whether Great Crested Newts were present or absent. The stratified sampling strategy targets sample squares to those within the Great Crested Newt's range and reduces the number of squares with zero values by selecting 50% as supporting newts. At the same time, having 50% of the squares as 'unknown' (and most likely 'non-newt' squares) still allows new sites to be detected and for range expansion because a proportion of the survey squares will always be of unknown status.

Landowner permission

Landowner permissions to access ponds were arranged by the regional project officers, using a combination of email, phone, postal and door-to-door cold calling, following a review by Local Environmental Record Centres to identify any overlap with existing local projects. Landowners were left with a letter explaining the project which included a clause stating that data collected on their land would be shared via the National Biodiversity Network (NBN) without restriction, i.e. were Open Data.

We were aware that we might not be able to access all ponds in each 1 km grid square if landowner permission was refused or it was not possible to access a pond for other reasons (e.g. access unsafe for volunteers). For this reason, for a 1 km grid square to remain eligible for survey we stipulated that we should have access to at least 70% of the ponds in the square. If a square failed to "pass", an alternative square was selected at random from the same category (i.e. Great Crested Newts present or Great Crested Newt status unknown).

Survey methods

The majority of sites were surveyed using the new Environmental DNA (eDNA) technique. 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. 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. Biggs *et al.* (2013) were able to show that collecting eDNA was a highly effective method for determining whether Great Crested Newts were present or absent at a pond. PondNet volunteers were trained in eDNA techniques following the standard protocol of one eDNA sample per pond per year. eDNA kits and sample analysis was conducted by the SpyGen laboratory, France.

Volunteer surveyors were also trained to carry out presence-absence surveys using a standard protocol, proposed by Sewell *et al* (2010), comprising a 'four visit'/four method' of visual searching, torch counting, netting and (for licenced individuals) bottle trapping in order to achieve reliable assessments of the presence or absence of species. This protocol is based on an occupancy modelling approach that goes some way to resolving issues concerned with variation in detectability that can lead to 'false absences' in presence-absence surveys.

Results summary

The PondNet Great Crested Newt volunteer monitoring network in England surveyed 131 randomly selected 1 km grid squares, encompassing 380 ponds, between 2015 and 2017.

Volunteers collected data from another 69 1 km grid squares but, to date, these squares have only been visited once or twice, which means we can't do trend analysis with them at the moment.

In a few squares we weren't able to visit all the ponds in the square, and sometimes when traditional methods were used we didn't have a full set of four visits to establish absence. The partial dataset can still provide useful data on the presence of Great Crested Newts for atlas purposes and to add to analyses where repeat data are not required, but we haven't analysed them in the short term trend dataset discuss below.

One of the advantages of eDNA technique has been the ease with which we can survey large numbers of sites with just one visit.

Great Crested Newt volunteer monitoring network in England - map showing the location of 131, 1 km grid squares surveyed annually 2015-2017

Stock and change in the number of occupied 1 km grid sqaures

Volunteers surveyed all the ponds[1] within 131 1 km grid squares over three years. In the survey design we began with the following proportion of 'known' and 'unknown' squares in 2015:

- 67 1 km squares (51%) had previous records for Great Crested Newts (known squares) from one or more ponds in the square in the last 30 years.
- 64 1 km squares (49%) were unknown for Great Crested Newts (unknown squares) and had no records for Great Crested Newts. They had either been surveyed at some point in the last 30 years and returned a null result, or they had not been surveyed.

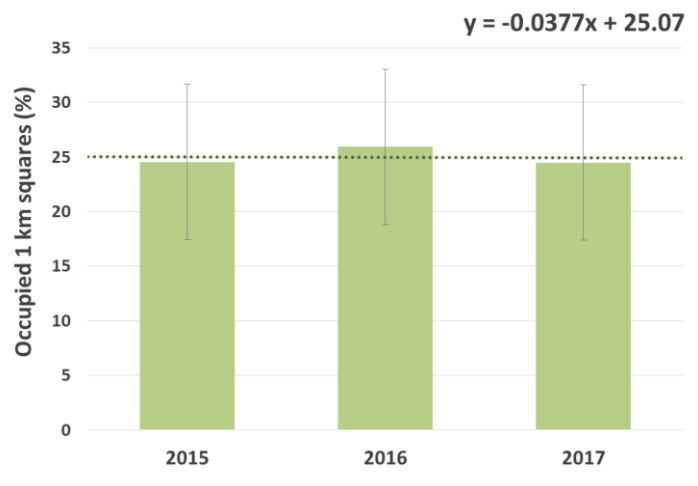
Because we had allowed for proportional representation in the design we were then able to estimate the number of 1 km squares occupied by Great Crested Newts in England.

We estimate that 24.6% of 1 km grid squares in England were occupied by Great Crested Newts in 2015, this increased to 29.5% in 2016 and returned to 24.5% in 2017.

Great Crested Newt occurrence in 1 km squares was variable between years.

- Ten 1 km squares which supported Great Crested Newts in 2015, were null records in 2016. Only five of these squares recovered newts in 2017.
- Five 1 km squares which had no Great Crested Newts in 2015, gained them in 2016. Three of these squares lost them again in 2017.
- Four 1 km squares had no Great Crested Newts in 2015 or 2016 and gained them in 2017.

Statistically these are small changes and there was no significant change in the number of occupied 1 km grid squares over the survey period.



Estimated number of 1 km grid squares occupied by Great Crested Newts in England (± 7% margin of error)
Change over time y=-0.0377x + 25.07 (not significant)

Overall, we estimate that between 18-32% of 1 km grid squares in England are occupied by Great Crested Newts, and in the short term this isn't changing, but we need to collecct data over a longer time period to be certain.

[1] At least 70% of ponds within each square were surveyed.

Stock and change in the number of occupied ponds

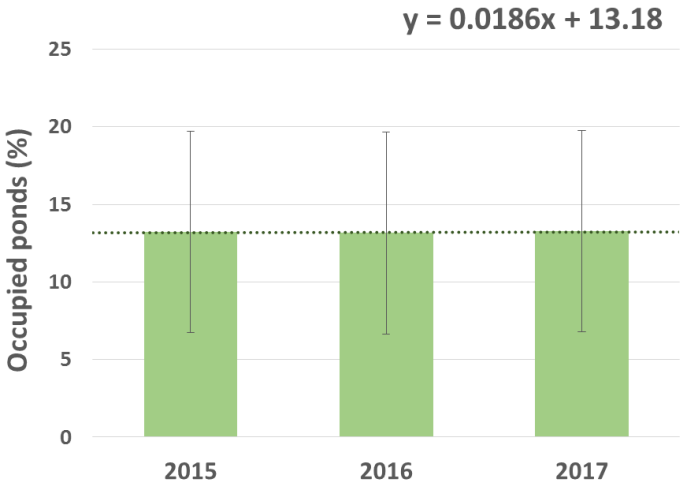
To estimate the total number of ponds occupied at a national level we looked at the pond in the south-west corner of each of our survey squares. This prevents any correlation between sites, because you might expect ponds which are close together to be more or less likely to be occupied.

Using the same technique to allow for proportional representation as above we were able to estimate the number of ponds occupied by Great Crested Newts in England.

We estimate that 13.2% of ponds in England were occupied by Great Crested Newts in 2015 and 2016, and this increased fractionally to 13.3% in 2017.

Great Crested Newt occurrence in ponds was also variable between years.

- Five ponds which supported Great Crested Newts in 2015, were null records in 2016. Only two of these ponds recovered newts in 2017.
- Four ponds which supported Great Crested Newts in 2015 and 2016, were null records in 2017.
- Three ponds which had no Great Crested Newts in 2015, gained them in 2016. Five ponds which had no Great Crested Newts in 2015 or 2016, gained them in 2017.



Estimated number of ponds occupied by Great Crested Newts in England (± 6.5% margin of error)
Change over time y=0.0186x + 13.18 (not significant)

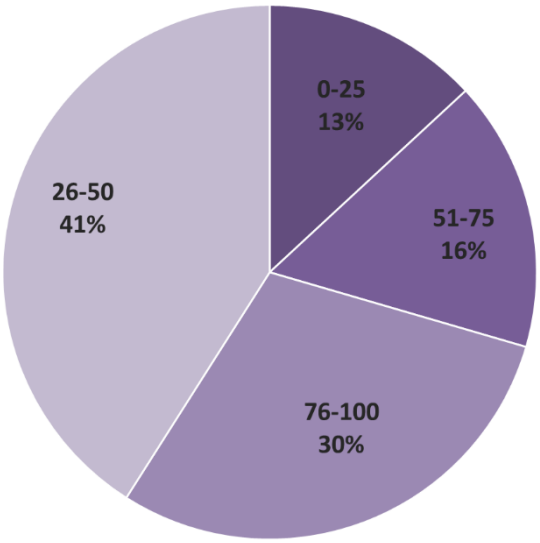
Statistically these are small changes and there was no significant change in the number of occupied 1 km grid squares over the survey period.

Overall, we estimate that between 7-20% of pond in England are occupied by Great Crested Newts in any one year, and in the short term this isn't changing, but because the margin of error in this result was large compared with the number of ponds surveyed we can't say for certain that no change was occurring in the number of individual ponds occupied.

Stock and change in the number of occupied ponds per square

To investigate the number of occupied ponds per square we categorised the number of squares in different pond occupancy categories. We were surprised by the results. Even in squares with lots of ponds, Great Crested Newts are often only found in a small number of the available ponds.

- Between 2015 and 2016, 61 (47%) sample squares had zero occupancy every year.
- Only 18 squares (30%) known for Great Crested Newts, had full occupancy i.e. all the ponds were positive for Great Crested Newts. Half of these, nine squares, only had one pond and only three fully occupied squares had four or more ponds.
- More than half of the squares (54%) known for Great Crested Newts, had below average occupancy. In other words, less than half of the ponds in the square were occupied. Of these 12 squares only had two ponds, so it was 50:50 occupancy. But, 15 poorly occupied squares had more than four ponds and less than 50% occupancy.
- Most squares (41%) known for Great Crested Newts had between 26-50% of the available ponds occupied.

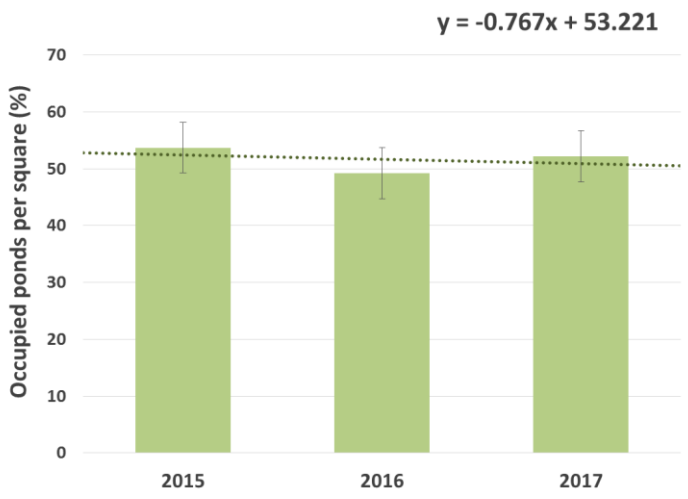


Proportion of ponds within pond per square occupancy categories; 0-25% of ponds in each square occupied, 26-50% of ponds in each square occupied; 51-75% of ponds in each square occupied; and 76-100% of ponds in each square occupied.

Average pond occupancy per grid square in the occupied squares remained steady throughout the survey period; 53.7% ponds per square occupied in 2015, 49.2% ponds per square occupied in 2016, and 52.2% ponds per square occupied (4.5% *SE Mean*).

As with other metrics there was a degree of turnover in pond occupancy per square per year:

- Pond occupancy per square per year remained the same in 24% of squares.
- In 34% of squares, pond occupancy either increased or decreased in 2016 from 2015, but returned to 2015 occupancy levels in 2017.
- Pond occupancy increased over the period of survey in 19% of squares, and decreased over the period of the survey in 23% of squares.



Change in the average number of occupied ponds per square (± 4% SE Mean)
Change over time y=-0.0767x + 53.221 (not significant)

Overall, there were no significant short term changes in pond occupancy, but we'd like to collect more data over longer time scales, ideally for another 2-3 years (6 years in total) to be sure.

Pond quality for Great Crsted Newts

Habitat quality for Great Crested Newts in PondNet and NARRS is primarily based on Habitat Suitability Index (HSI) scores, recording ten critical environmental metrics for each pond surveyed (Oldham et al. 2000):

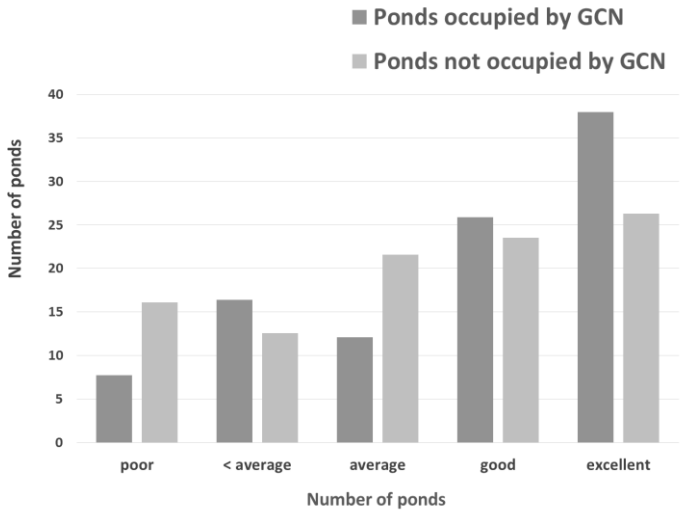
SI_1 Location
SI_2 Pond area
SI_3 Pond drying
SI_4 Water quality
$SISI_5$ Shade
$SISI_6$ Waterfowl
$SISI_7$ Fish
$SISI_8$ Pond count
$SISI_9$ Terrestrial habitat
$SISI_{10}$ Macrophytes

We categorised all the ponds volunteers had surveyed into four HSI categories: < 0.5 = poor, 0.5-0.59 = below average, 0.6-0.69 = average, 0.7-0.79 = good, and > 0.8 = excellent.

The results show that Great Crested Newt occupancy was positively correlated with Habitat Suitability Index scores, and there was a significant difference between the HSI scores for occupied and unoccupied ponds.

Ponds which were occupied by Great Crested Newts were more likely to have excellent Habitat Suitability Index Scores.

For example: 40% of ponds occupied by Great Crested Newts had an excellent HSI score; whilst only 8% of ponds that were positive for Great Crested Newts had a poor HSI score.



Number of ponds occupied and unoccupied by Great Crested Newts -classified by Habitat Suitability Index category.

Habitat Suitability Index scores were not useful for predicting the absence of Great Crested Newts; 26% of ponds with no record for Great Crested Newts were categorised as excellent; whilst 16% of ponds with no record for Great Crested Newts were categorised as poor. This means that not every pond that has an excellent HSI score will support Great Crested Newts, but the likelihood is that their quality could support other species of interest and may have important plant and invertebrate communities.

Habitat Suitability Index scores did not change significantly over the course of the survey, which may explain why, in the short term, we did not see a change in any of the occupancy metrics we were monitoring.

What next for Great Crested Newt monitoring?

There has been no significant change in square occupancy, pond occupancy or the number of occupied ponds per square over the first three years of the trend analysis period, and no change in the number of ponds per Habitat Suitability Index category over the same time period. The amount of variability in pond occupancy both spatially and temporally requires very large sample sizes if a short term, rather than a long term approach is adopted.

Given the success and limitations of a three year survey window, we recommend that eDNA work is continued for 2-3 further years (we originally proposed a 6 year series of data for trend analysis) giving a trend analysis covering 2015 - 2020.

We've learnt a lot of new information about the extent of Great Crested Newt occupancy in England, and about how this changes between years on lots of sites, so that whilst the overall picture remains the same in the short term individual sites may lose and gain newts regularly over time. It's therefore really important that we keep and create ponds of good quality near to one another in the landscape so that newts can move from one site to another as they need.

More than 450 volunteers used a combination of eDNA and traditional methods to complete the surveys. The time contribution from volunteers was c.11,500 hours, equivalent to £245,000. Volunteer feedback has been overwhelmingly positive. Most enjoyed taking part and there was also a strong sense amongst volunteers that they were taking part in 'real' science. The volume of information we had been able to use from this work has clearly reflected the value of citizen science to collect statistically robust information.

In conclusion, eDNA made this volunteer monitoring network possible and it is unlikely that the spatial and temporal scale of this survey would have been possible without this technique.

Acknowledgements

We would like to thank all those who have helped with this project including the landowners who facilitated access to their sites, and particularly the many people and groups who volunteered time and resources to collect eDNA samples. This includes NARRS and PondNet volunteers.

Development of the Great Crested Newt national pond monitoring in England was completed as part of Freshwater Habitats Trust's People, Ponds and Water Project. The network concept was developed in partnership with Amphibian and Reptile Conservation, Amphibian and Reptile Groups of the UK, the Association of Local Environmental Record Centres, Defra, Durrell Institute of Conservation and Ecology, JNCC, Natural England and the SpyGen laboratory.

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