People, Ponds and Water

PondNet:
A national citizen science-based monitoring programme for Great Crested Newt in England
2015 – 2017

Version 1.1

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Executive summary

Overview

This report summarises the findings of a three-year national monitoring programme for Great Crested Newts in England; part of Freshwater Habitat Trust's PondNet project.

Under the Habitats Directive (Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora) an Article 17 report has to be submitted for Great Crested Newts on a member state level with data from all four countries of the UK (or in this case in the three countries of GB since Ireland is not included in the GCN range). The current report includes data that will form part of the English submission to JNCC and will be used in conjunction with data from the other countries.

It provides data on the status of Great Crested Newts in England for three key parameters:

- Pond occupancy by Great Crested Newt.
- Habitat suitability for Great Crested Newt, principally through use of the 'Habitat Suitability Index'.
- Pond turnover (i.e. balance of losses and gains in the number of ponds).

To assess pond occupancy, we initially intended to combine eDNA (environmental DNA) and traditional surveys (a minimum of four visits using torch surveys to establish absence), to report the following:

- Change in the number of occupied 1 km grid squares in England
- Change in the number of occupied ponds in England
- Change in the number of occupied ponds per 1 km grid squares

We also evaluated Habitat Suitability Index (HSI) values and pond turnover over the three years of the survey.

In practice, it was only possible to obtain robust data using eDNA surveys because traditional surveys were insufficiently comprehensive to provide a robust data source.

PondNet surveys from 2015 to 2017 were coordinated nationally and delivered by a team of four regional project officers who facilitated volunteer recruitment, undertook volunteer training and provided on-going volunteer support in partnership with national and local recording schemes and the Association of Local Environmental Record Centres (LERCs).

Stock and change results

Change over time in occupancy was analysed in 'R' using Generalized Estimating Equations with post-hoc Wald chi-square test; and 'Longpower' was then used to undertake power sample size calculations. Differences between Habitat Suitability Index scores for ponds with and without Great Crested Newts were assessed using chi-squared test with post-hoc Cramer's V. Estimates for the proportion of occupied 1 km squares in England were calculated using the sample proportion (95% CI). We then used these data to calculate the minimum sample size needed to reduce the margin of error for the estimate.

In total, eDNA surveys were undertaken in 131 1 km grid squares (380 ponds) every year for three years 2015-2017. We describe this as the 'complete data set' which can be used for analysis. Although we aimed to survey 100 further ponds using traditional methods, volunteers either failed to visit all the ponds in each square, or were only able to visit the ponds for one year out of the three. This gave a partial dataset of 69 1 km grid squares where we only have one years-worth of data, or an incomplete result. These data are not robust enough to contribute to trend analysis.

In terms of 1 km square occupancy we estimate that 24.6% (± 8.5%) of 1 km grid squares with ponds in England were occupied by Great Crested Newts in 2015, increasing to 25.9%

(± 8.5%) in 2016 and returned to 24.4% (± 8.5%) in 2017 (95% CI). Changes were not statistically significant over the survey period.

Power analysis indicated that with relatively low sample sizes (i.e. 100-150 sites) power to detect changes of less than 10% in square occupancy would be low with only three years of data: in the region of 50%, and therefore less than would be regarded as statistically robust. Extending the trend analysis over 5-6 years would substantially increase power to detect changes of 10% in 1 km grid square occupancy to between 94% and 98%.

The original experiment was designed to detect change in pond occupancy using the minimum number of squares possible. It was not deigned to make a national estimate of square occupancy at a single point in time, however, this information is useful for reporting purposes and we have therefore made this calculation with the caveat that there will be a large margin of error. Taking the average occupancy over the three years, we estimate that between 16-33% of 1 km grid squares (95% CI) known to support ponds, in England are occupied by Great Crested Newts. This equates to 10-20% of all 1 km grid squares in England. So the estimate for square occupancy by Great Crested Newts is c.13,470 – 26,940 1 km grid squares based on the 2015 - 2017 results.

To obtain more precise estimates (±5%, ±2.5%) of the true number of occupied 1 km grid squares, requires substantially larger samples ranging from around 300 to over 2000 squares depending on the level of precision regarded as acceptable. For example, to reduce the margin of error to within ±2.5% of the estimate at 95% confidence would require c.1,500 1 km grid squares, which is probably prohibitive.

In terms of estimates of pond occupancy, we estimate that 13.3% (± 7.7%) of ponds in England were occupied by Great Crested Newts in 2015 (95% CI), this was similar to results for 2016 (13.2% ± 7.6%) and 2017 (13.3% ± 7.8%) in 2017 (95% CI). The change was not statistically significant. Overall, we estimate that between 6-21% of ponds in England are occupied by Great Crested Newts (95% CI).

The result for c.13% pond occupancy seen in this survey, is comparable with previous estimates of 12% pond occupancy (Wilkinson and Arnell 2013), and 11% pond occupancy (Swan and Oldham 1993). Both the previous estimates were GB level, which would explain lower estimates of occupancy overall, and the authors highlight the lack of returns for Great Crested Newts presence/absence in Wales which may bias the results. The slightly higher estimate in this survey may also be due to improved detection with eDNA.

The current survey was only able to achieve 48% power to detect <10% change in pond occupancy. Therefore, there is a risk that smaller changes may have been missed between 2015 and 2017. If this trend analysis were repeated over a 5-6 period (i.e. for 2 or 3 further years) we would achieve 86%-92% power to detect a change of less than 10% change in pond occupancy.

To investigate occupancy versus the availability of pond habitat per square, we categorised the number of squares in different pond occupancy categories as follows: unoccupied squares, satellite squares which were sometimes occupied and sometimes unoccupied, and core squares which were occupied by Great Crested Newts in every year of the survey. We also divided occupancy per square in the known Great Crested Newts squares as follows: 1-25% of ponds occupied, 26-50% of ponds occupied, 51-75% of ponds occupied, and 76-100% in any year of the survey.

The eDNA results suggested that Great Crested Newt occupancy and the likelihood of consistent occupancy between years was related to the number of ponds per square. The average number of ponds per square in unoccupied squares was low (2 ponds/square). Permanently occupied (core) 1 km grid squares had an average of 4 ponds per square; whilst occasionally occupied (satellite) squares fell in-between, with an average of 3 ponds per square.

When Great Crested Newts were present in the square, the number of occupied ponds per square was low, regardless of the number of ponds available. Only 13 squares, out of 70 which had positive records in any year, had 100% pond occupancy every year. In other words, in these squares, all of the available habitat was occupied by Great Crested Newts every year. In many squares where Great Crested Newts were recorded, they were only able to occupy a small proportion of the ponds available to them. In the majority of squares (63% of squares) known for Great Crested Newts, between 2015-2017, less than 50% of the available ponds were occupied in some years.

We then looked at turnover in pond occupancy between years; excluding squares which were never occupied by Great Crested Newts. As with other metrics there was a degree of turnover in pond occupancy per square per year. 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. However, average pond occupancy per grid square remained steady throughout the survey period, so whilst the identity of the occupied ponds changed, the number of occupied ponds per square remained the same; 2015: 1.95 ± 0.42 occupied ponds per square, 2016: 1.96 ± 0.50 occupied ponds per square, 2017: 1.96 ± 0.45 occupied ponds per square (95% CI). There was no significant change in the number of occupied ponds per square per year.

As predicted, the number of occupied ponds per 1 km grid square was able to provide a more statistically robust measure of change (73% power, to detect changes of <10%, 90% CI), than binary data. There is a tendency for over dispersion in binary data (the variation not accounted for by the model), which may not be overcome without a large number of samples (ponds in this instance) or increasing the number of sample years (Ferrari and Comelli, 2016). Power to detect <10% change in pond occupancy would increase to between 80-90% with if we were able to extend the survey for 4-6 years.

Habitat Suitability Index scores were positively correlated with Great Crested Newt occupancy; 40% of ponds positive for Great Crested Newts had an excellent HSI score. They were less 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.

HSI scores did not change significantly over the course of the survey (2015-2017). In 2016 and 2017, volunteer surveyors were given the Habitat Suitability Index scores from the first year and asked to confirm whether anything had changed. Most responded no, without repeating the full survey from scratch. This may have led to subtle changes being overlooked. Full HSI surveys will be conducted in future years.

Recommendation for continuation of PondNet approach

Given the success and limitations of a three year survey window, we recommend that PondNet 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. 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, but the small sample size and requirement to detect small levels of change has resulted in low levels of power – an increased risk that significant changes may be missed (Type II errors). Analysis predicts that 4-6 year surveys would substantially increase power across all metrics (up to c.90% power to detect <10% change with 6 years of survey).

As an additional analysis, we used the time series to estimate square occupancy by Great Crested Newts in England; 24% ± 8.5% 1 km squares occupied (95% CI). This is a snap-shot of occupancy at a single point in time, and because this was not part of the original experimental design, the sample size is smaller than required to provide a precise estimate. To increase precision, for example to within ± 5% of the real value, would require a large sample with c.400 1 km squares. This is more costly, needing c.700 kits plus extra co-ordination time.

There is a trade-off between monitoring change and estimating square occupancy. With enough resources, the sample size would be large enough to estimate occupancy with a small margin of error, and could also be repeated annually to monitor change over time whilst overcoming annual variability in pond occupancy. If resources are limited, annual surveys, with a smaller number of samples per year, can be used to detect change. Alternatively, periodic surveys for example once every six years, could be used to estimate square occupancy, but this design may not be sufficient to detect change over time because of inter-annual variation.

It may be possible to pool data from different surveys to look at occupancy modelling. However, as was noted in the original design of PondNet (Biggs et al. 2013), few if any surveys exist where all the ponds in each 1 km grid square are surveyed to establish absence.

Summary of issues and opportunities

- A break in the continuity of the project is likely to cause problems as we will lose contact with the landowners. Re-establishing the network at a later date is likely to incur similar costs as the initial set-up.
- All volunteers who completed the eDNA survey would be willing to take part again, these trained individuals represent a significant cost saving to a national monitoring network for Great Crested Newts.
- We found that even although volunteers become independent to some extent, able to undertake the survey with minimal input from the regional coordinator, they still needed someone to administer the eDNA kits, coordinate volunteer activities to ensure that kits were used within the survey window, and to ensure that results were submitted. We recommend a project management structure using a national FTE officer employed for 5 months to administer and coordinate the survey.
- Volunteer distribution hubs for eDNA kits has streamlined the delivery and collection of survey equipment. All the hubs have expressed a willingness to be involved if the survey was to be repeated.

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.

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1. Introduction

This report summarises the findings of a three-year national monitoring programme for Great Crested Newts in England; part of Freshwater Habitat Trust's PondNet project. The work has been funded by Heritage Lottery Fund, Defra, and Natural England; with additional elements supported by Beryl Thomas Animal Welfare Trust, BGL Group, Garfield Weston Foundation, New Forest Catchment Partnership, Thames Water, ToE2, ValPak and Yorkshire County Council.

1.1 Background

1.1.1 Legislative background

Great Crested Newts *Triturus cristatus* are strictly protected under EU and domestic legislation (Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora). Under Annex IV of the Habitats Directive, EU Member States are required to report every six years on the status of Great Crested Newts (Article 17 reporting). The current report includes data that will form part of the English submission to JNCC and will be used in conjunction with data from the other countries in Great Britain (excluding Ireland since this is outside of the Great Crested Newt's range).

A sampling strategy for Great Crested Newts to meet these monitoring requirements must be proportionate. A monitoring programme must make the best use of resources, and balance surveillance needs against conservation action for Great Crested Newts; and to balance the resources required for Great Crested Newts against action required for other species and habitats.

1.1.2 Survey history

Historically, information on the status of Great Crested Newts for nature conservation purposes has been derived from four main sources: opportunistic 'natural history' sightings (Jehle *et al*. 2011), questionnaire surveys of herpetologists to assess trends in populations (e.g. Beebee 1975), and co-ordinated regional (e.g. Hollinshead *et al.* 2008) and national surveys (see below).

Large scale, co-ordinated, national surveys for Great Crested Newts in the UK, and amphibians generally, began with the National Amphibian Survey of the 1990s (Swan and Oldham 1993), and were more formally implemented with the National Amphibian and Reptile Recording Scheme (NARRS), organised by Amphibian and Reptile Conservation, which was started in 2007 (Wilkinson and Arnell 2013).

NARRS uses volunteer-based efforts to monitor and report on the status of amphibians and reptiles, including the five widespread native amphibian species: Common Frog (*Rana temporaria*), Common Toad (*Bufo bufo*), Smooth Newt (*Lissotriton vulgaris*) Palmate Newt (*L. helveticus*) and Great Crested Newt (*Triturus cristatus*).

Reporting on the first six years of NARRS, Wilkinson and Arnell (2013) provided estimates of current pond occupancy by Great Crested Newts in Great Britain over 5 years 2007- 2012, indicating that 12% of ponds were occupied by Great Crested Newts. However, the natural variability of Great Crested Newt populations and the relatively small pool of surveyors available for survey work, presented a substantial challenge to obtaining robust national surveillance data on Great Crested Newts. Thus Wilkinson and Arnell (2013) commented that "Current (NARRS) survey sample sizes will not detect useful levels of change in occupancy rate [of Great Crested Newts] at anything other than low power".

1.1.3 A new approach

The statutory conservation agencies agreed (Defra, 2012) that in order to be able to report on the UK status of Great Crested Newt, data were still required at a national level (on an ongoing rather than one off basis) for three key parameters:

1. Pond occupancy by Great Crested Newt.
2. Habitat suitability for Great Crested Newt, principally through use of the 'Habitat Suitability Index'.
3. Pond turnover (i.e. balance of losses and gains in the number of ponds). Note that pond turnover is not discussed further in this report.

The need for a new approach to fill in the gaps in the monitoring network for Great Crested Newts coincided with the development of a structured surveillance monitoring network for pond habitats; the PondNet project.

1.2 PondNet

1.2.1 Development of 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*', funded by Defra, with additional elements supported by Natural England. 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 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 (launching the project initially in England and Wales, with a view to extending the network in the future to Scotland and Northern Ireland).
- 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.

The project comprised three project elements: PondNet, Clean Water for Wildlife and Flagship Ponds (Figure1).

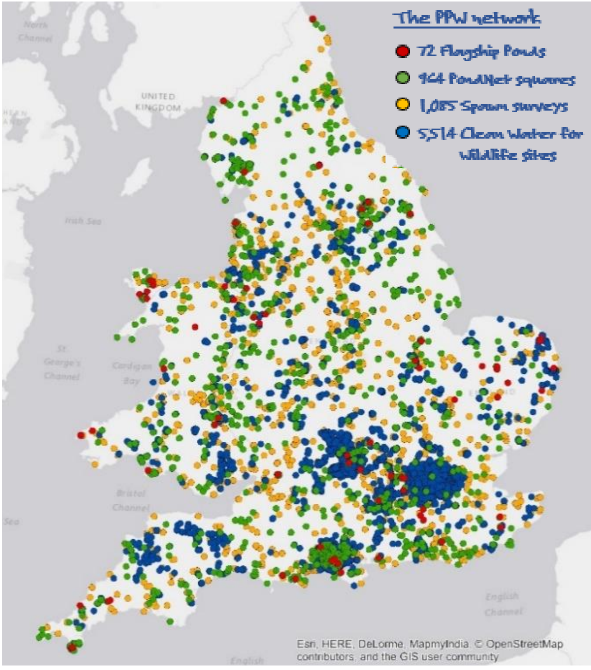


Figure 1: Map of the People, Ponds and Water network

1.2.2 PondNet principles

PondNet is a volunteer survey network that aims to collect statistically robust data to identify trends in pond quality and pond species, including uncommon plants and animals. Survey methodologies for pond species are standardized and compatible with national recording schemes. Environmental data are also recorded, giving information that will help to explain the reasons for changes in biological quality and, ultimately, help to guide the direction of freshwater policy.

PondNet comprises several overlapping sub-networks:

- **Amphibian network:** based on recording the number of occupied ponds per randomly selected 1 km grid square (Common Toad / Great Crested Newt).
- **Pond quality network:** based on a standardised survey (PSYM surveys – the Predictive System of Multimetrics) of priority pond habitats and a randomly selected wider-countryside pond network.
- **Uncommon plants and invertebrates:** monitoring populations of c.30 S41/ S42 plant and invertebrate species at specific known ponds using a standardised methodology specific to that species.
- **Added value sites:** Volunteer selected sites.

Once in place, the aim was that PondNet would provide a long-term monitoring network for a critical part of the freshwater environment (ponds) and contribute to national biodiversity reporting for England and Wales.

1.3 Overview of the work

1.3.1 Great Crested Newt Monitoring Network

We aimed to design a sampling programme which would provide statistically meaningful data to monitor change in Great Crested Newts in the UK. In England, occupancy would be determined through a combination of eDNA (environmental DNA) and traditional surveys (a minimum of four visits using torch surveys to establish absence), to report the following:

- Change in the number of occupied 1 km grid squares
- Change in the number of occupied ponds
- Change in the number of occupied ponds per 1 km grid squares

A-priori power analysis (Biggs, *et al*. 2013) indicated that a design based on repeat surveys of the same ponds would reduce the sample size needed to detect change with a reasonable level of certainty (80% power). But, to overcome the spatial variation in Great Crested Newt occupancy, the sample size needed to detect change (30% change) between time A and time B would still require a survey of more than 300 1 km grid squares (c.900 ponds).

The sample size required could be reduced further if trend data were collected (Gibbons et al. (2010), visiting the same ponds in successive years. However, the sample size required was uncertain because of the lack of existing data on which to base power analysis. Estimates suggested that between 100-200 1 km grid squares could have 80% power to detect 30% change over 3-6 years.

1.3.2 Survey timetable

Establishment of the PondNet volunteer monitoring network was timetabled to roll out over three years, 2015-2017; agreeing landowner permissions on behalf of the volunteers and organising volunteer recruitment and training for a third of the network in year 1, a third in year 2 and final establishment in year 3. Using this approach, year 3 would become the first year of complete survey, with years 4, 5, 6, etc. providing data for trend analysis.

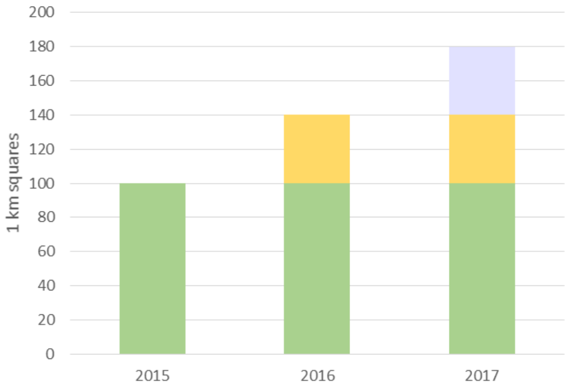


Figure 2: Target number of 1 km squares. Site selection 'fast-tracked' in year 1 to maximise the number of squares for trend analysis. Green: the planned core network for years 1, 2, and 3; Yellow: squares added in year 2 and surveyed again in year 3; Blue squares: added in year 3 to complete the 180 square network.

However, additional funding from Defra made it possible to 'fast-tack' the PondNet network for Great Crested Newts, agreeing landowner permissions for more than half of the 1 km grid squares in year 1 (2015) to increase the number of sites (c.100 1km grid squares) where we would have three years' worth of data by the end of the 2017 for trend analysis.

1.4 Aims and objectives

- **Implement survey design:** To identify randomly stratified network of 1 km grid squares and secure landowner permissions.
- **Collect data on pond occupancy:** To recruit and train volunteers to survey a minimum of 100 1 km in 2015, 2016 and 2017. Recruit additional volunteers in 2016 and 2017 to build the network towards 200 1 km grid squares.
- **Report on change in Great Crested Newt occupancy in England:** To analyse any changes in Great Crested Newt occupancy (number of occupied 1 km grid squares, number of occupied ponds, and number of occupied ponds per square) over the three years of survey.
- **Investigate other outputs:** Determine whether these data could also provide an estimate of Great Crested Newt occupancy in England; and report on additional data collected during the survey e.g. habitat quality for Great Crested Newts.
- **Undertake power analysis:** To report on the validity and robustness of the survey results over the three years of survey. To understand the difference in power between short and long term trend surveys.
- **Understand the limitations of the survey:** To understand the limitations of the data - what doesn't the data tell us, what are the greatest gaps in the data, and what are the factors that contribute to these limitations.
- **Make recommendations for future monitoring approaches:** Use the results from the survey to inform a future monitoring strategy for Great Crested Newts.

2. Methods

2.1 Delivery structure

Over the period 2015-17 PondNet was coordinated nationally in England and Wales and delivered by a team of four regional project officers who facilitated volunteer recruitment, undertook volunteer training and provided on-going volunteer support in partnership with national and local recording schemes and the Association of Local Environmental Record Centres (LERCs).

Volunteers from the National Amphibian and Reptile Recording Scheme (NARRS), Amphibian and Reptile Groups of the UK (ARG-UK) and a wider network of specialist and non-specialist volunteers were recruited to undertake surveys for Great Crested Newts.

2.2 Monitoring metrics

2.2.1 Pond occupancy

There are a number of ways to monitor change in Great Crested Newt occupancy at a national level, with PondNet and NARRS using complementary methods (Figure 3), and providing outputs that can be combined.

- **The number of occupied 1 km grid squares:** because we aim to monitor all the ponds in each 1km grid square (PondNet).
- **The number of occupied ponds:** based on a survey of the pond nearest the south-west corner of the square (PondNet and NARRS).
- **The number of occupied ponds per 1 km square:** to detect change in the strength of populations over time (PondNet).

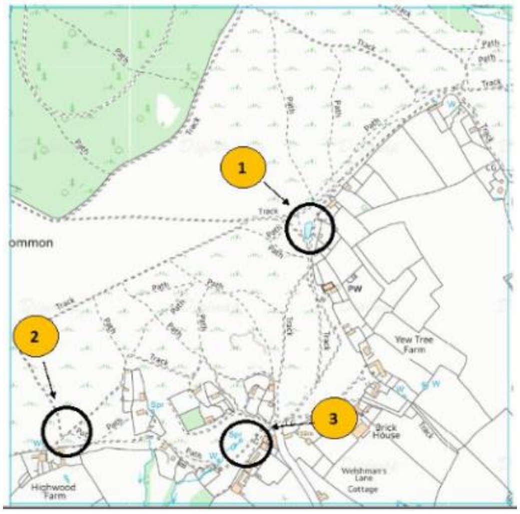


Figure 3: A PondNet monitoring square: pond 2 is used in the analysis of the number of occupied ponds (NARRS equivalent pond)

2.2.2 Habitat suitability for Great Crested Newt

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
SI_5 Shade
SI_6 Waterfowl
SI_7 Fish
SI_8 Pond count
SI_9 Terrestrial habitat
SI_{10} Macrophytes

By monitoring the same ponds each year it is possible to use HSI data collected over the same period to understand the reasons for any change recorded.

PondNet also asked volunteers to record additional information on pond habitats and surrounding land use, which could be used to assess pond quality for other species (S41/S42) and biological metrics (PSYM) [1].

2.2.3 Pond turnover

Estimates of pond turnover are dependent on the quality of the datasets used to estimate change. Previous estimates made from walkovers (e.g. early Countryside Survey data from the early 1990s) have often underestimated the number of ponds; overlooking ponds in woodland, man-made ponds, temporary ponds, ponds within other wetland habitats, or through lack of surveyor skill in identifying pond features.

Remote sensing methods may also underestimate the number of ponds due to poor resolution in identifying temporary ponds and those under tree cover or they may overestimate the number of ponds by failing to identify pond features which have since been lost.

PondNet aims to survey all the ponds in each 1 km grid square, every year, and therefore it is possible to report on pond turnover over time.

2.3 Monitoring network

In 2013, work to develop improved statistical designs for sampling methodologies for Great Crested Newts (Biggs, *et al*. 2013) concluded that repeat surveys of approx. 200 1 km grid squares over time (trend analysis) could potentially have 80% power to detect 30% change in Great Crested Newt pond occupancy in England and Wales.

The total PondNet network for Great Crested Newts would comprise a minimum network of 180 1 km grid squares in England and 20 1 km grid squares in Wales, with additional squares selected to account for losses (e.g. volunteer drop-off or loss of landowner permission between years). At an average of 3 ponds per square km, PondNet volunteers would survey approximately 600 ponds per year.

Grid squares known to support ponds, 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.

The initial analysis assumed assessment between two points in time, year t_1 compared with year t_2, but the report hypothesised that it may be possible to reduce the number of sites and/or detect smaller changes in the population if trend analyses were used from repeated visits to the same squares annually. PondNet aimed to visit 100 1 km grid squares annually over the three years 2015-2017.

2.4 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

[1] https://freshwaterhabitats.org.uk/wp-content/uploads/2015/03/3-eDNA-GREAT-CRESTED-NEWT-RECORDING-FORM-FINAL.pdf

by LERCs to identify any overlap with existing local projects (where this occurred the LERCs were able to contact the landowners on our behalf).

Landowners were left with a letter (Appendix 1) 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).

2.5 Survey methods

NARRS primarily uses trained volunteer surveyors who carry out presence-absence surveys using a standard protocol, proposed by Sewell *et al* (2010), comprising a ‘four visit’/four method’ protocol 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. The proposed protocol is a compromise between rigour and simplicity, necessary for volunteer-based field work. PondNet adopted the same survey protocol to undertake traditional surveys of ponds in the network (Appendix 1).

Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment (Levy-Booth et al. 2007). 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 is detectable for 7–21 days, depending on environmental conditions (Alvarez et al. 1996; Deagle et al. 2006; Matsui et al. 2001; Romanowski et al. 1992). Thomsen et al. (2012) 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 (Appendix 2).

Natural England and Defra agreed to fund eDNA surveys of the ponds in 100 1 km grid squares (England only) in the network, and PondNet volunteers aimed to undertake eDNA surveys (funded from other sources) and traditional surveys on the remainder.

eDNA kits and sample analysis was conducted by the SpyGen laboratory, France. The survey protocol and eDNA analysis followed the protocol as outlined in Biggs *et al.* (2014).

2.6 Data analysis

2.6.1 Trend analysis

We used Generalized Estimating Equations (GEE) in SPSS to analyse change over time in pond occupancy. Generalised Estimating Equations (Liang and Zeger 1986; Zeger and Liang 1986) are a type of longitudinal data analysis which extends the generalized linear model to allow for analysis of repeated measurements or other correlated observations. The response of the subjects, in our case occupancy of ponds, can be binary (present/absent) or count data (number of occupied ponds). Post-hoc Wald chi-square test of the model effect was used to look for the significance of change over time.

2.6.2 Power analysis

We used Package 'longpower' in 'R' to undertake sample size calculations for longitudinal data. The lmmpower function (based on the formulae of Diggle et al. 2002; Liu and Liang 1997) translates model parameters from GEE into marginal model parameters, so that the formula can be applied to predict sample size, percentage change, or power.

2.6.3 Analysis of other survey variables

Habitat suitability index scores were calculated using the standard formula of the geometric mean of 10 suitability indices:

$$\mathbf{HSI = (SI_1 \times SI_2 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10})^{1/10}}$$

Differences between HSI scores for ponds with and without Great Crested Newts were assessed using chi-squared test with post-hoc Cramer's V.

2.6.4 Number of occupied 1 km squares

In addition to change analysis we were asked to determine whether the sample design could also be used to estimate the proportion of occupied 1 km squares in England.

The sample proportion was used to estimate the proportion of occupied 1 km grid squares at a national level with standard errors at 95% confidence levels:

$$\hat{p} \pm z_{\alpha/2}\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

Where, $\hat{p}$ is the sample proportion, $Z_{\alpha/2}$ is the critical value of the normal distribution and n is the sample size.

We then used these data to calculate the minimum sample size needed to reduce the margin of error for the estimate at 95% certainty.

$$n = N^* x / (x + N - 1)$$

where

$$x = (Z_{\alpha/2})^2 * \hat{p} * (1 - \hat{p}) / MOE^2$$

$Z_{\alpha/2}$ is the critical value of the normal distribution, MOE is the margin of error, $\hat{p}$ is the sample proportion and N is the population size (c.135,000 1km grid squares in England).

3. Results

3.1 Results summary

The PondNet Great Crested Newt volunteer monitoring network in England surveyed 200 randomly selected 1 km grid squares, encompassing 558 ponds, between 2015 and 2017.

Network development occurred over 3 years 2015-2017:

- Grid squares established in 2015 were surveyed in 2015, 2016 and 2017 using eDNA (Target A 100 1 km grid squares).
- Grid squares established in 2016 and 2017 were surveyed using either eDNA or traditional methods (Target B 100 1km grid squares).

We exceeded Target A and established a significant proportion of the network in 2015. As a result, 131 1 km grid squares, 380 ponds, were surveyed every year for three years 2015-2017 using eDNA (Figure 4). We describe this as the 'complete data set' which can be used for analysis.

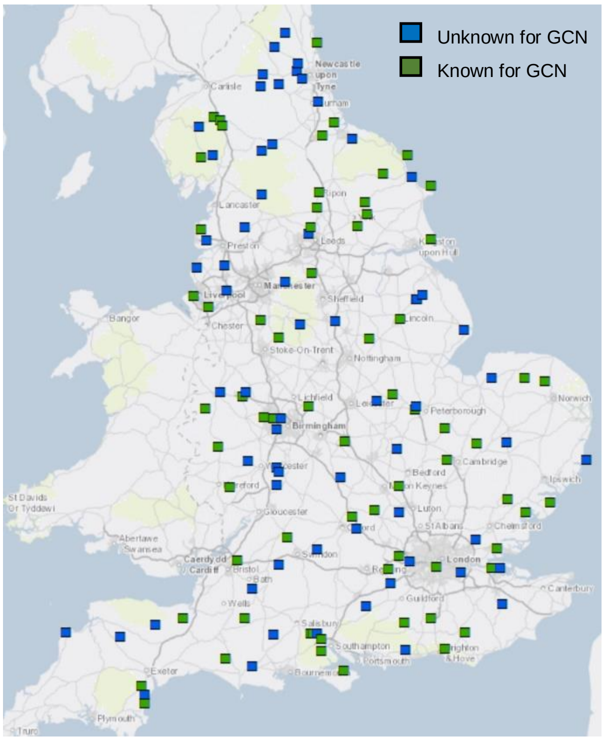


Figure 4: Great Crested Newt volunteer monitoring network in England - map showing the location of 131, 1 km grid squares surveyed annually 2015-2017

We were unable to establish another 100 1 km grid squares (Target B) using traditional techniques. Volunteers either failed to visit all the ponds in each square, or were only able to visit the ponds for one year out of the three. The partial dataset describes the remaining 69 1 km grid squares where we only have one years worth of data, or an incomplete result. The partial dataset can provide data on the presence of Great Crested Newts for atlas purposes but is not robust enough to be analysed here.

3.2 1 km grid square occupancy

Volunteers surveyed all the ponds[2] within 131 1 km grid squares using eDNA 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 been surveyed at some point in the last 30 years and returned a null result, or they had not been surveyed.

3.2.1 Estimating change in the number of occupied 1 km grid squares

To estimate the number of occupied 1 km grid squares in England each year (2015-2017), we recorded the number of occupied 'known squares' and the number of occupied 'unknown squares'. All squares in the design were known to contain ponds. Because we had allowed for proportional representation in the design, and including 1 km grid squares which do not

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

currently contain ponds, we were then able to estimate the number of 1 km squares occupied by Great Crested Newts in England (Table 1).

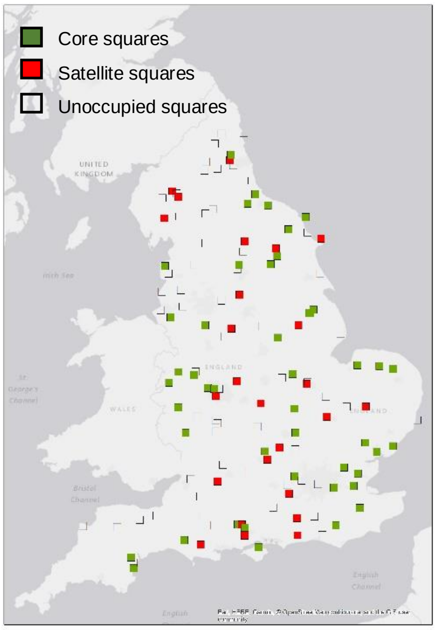
Within the survey design, we estimate that 24.6% (± 8.5%) of 1 km grid squares with ponds in England were occupied by Great Crested Newts in 2015, this increased to 25.9% (± 8.5%) in 2016 and returned to 24.4% (± 8.5%) in 2017 (95% CI). There was no significant change in the number of occupied 1 km grid squares over the survey period (Generalised estimating equation assessed using Wald test statistic =1.918, p=0.383) (Figure 5).

Table 1. Number of occupied squares revealed by eDNA surveys of all the ponds in each 1 km grid square in each of the PondNet survey years.

	Number of occupied 1 km squares with ponds (and as a % of the squares)		
	2015	2016	2017
1 km squares 'known' for GCN in the survey design	46 (69%)	40 (63%)	42 (66%)
1 km squares 'unknown' for GCN in the survey design	15 (23%)	16 (25%)	15 (23%)
Estimate of the percentage of occupied 1 km grid squares with ponds in England (± margin of error, CI 95%)	**24.6% (± 8.5%)**	**25.9% (± 8.5%)**	**24.4% (± 8.5%)**

- Ten 1 km squares which supported Great Crested Newts in 2015, were null records in 2016. Only 5 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.

Figure 5: Estimated number of 1 km grid squares occupied by Great Crested Newts in England (± 8.5% margin of error at 95% confidence). Change over time: y=-0.07x + 25.07 (not significant)



Great Crested Newt occurrence in 1 km squares was variable between years. Core squares were identified where newts were found to be present every year. Other squares appeared to be part of a satellite network which were only occupied in some years (Figure 6). Change from occupied squares to 'no Great Crested Newts recorded' was often the loss or gain of a single pond, either because the square only had a single pond or more often because Great Crested Newts were only recorded from a single pond in a multiple pond square. There was no geographical clustering to either core or satellite squares (Figure 6).

Figure 6: Distribution of core squares (occupied by GCN every year), and satellite squares (only occupied in some years).

3.2.2 Power to detect change in 1 km grid square occupancy

The amount of spatial variation in occupancy of 1 km grid squares was expected, but the level of variability between years was new information. Variability is the key driver of Type II errors; failure to reject the null hypothesis because of insufficient power to overcome natural variability and observe the effect of the parameter of interest. Power can be increased with sufficient sample size to overcome this natural variability.

Package 'longpower' in 'R' was used to determine the level of power achieved in the current survey; when sample size n=131, repeated samples over three years, alternative two-sided response. The current survey was only able to achieve 51% power to detect changes of <10% (90% confidence interval). Therefore, there is a risk that a significant change may have occurred in the number of occupied squares between 2015 and 2017, but we were unable to detect it because the sample size was not large enough.

A priori analysis had indicated that we would be unlikely to achieve sufficient power with low levels of change (<10% change) over short time periods (3 years) if the sample size was low (n=131). If we were able to extend the survey for longer the power to detect change would increase (Table 2).

Table 2. Power to detect change (<10% change) in 1km square occupancy where the number of sample squares n=131, over 3, 4, 5, and 6 years (90% confidence level).

Number of years of consecutive survey	% power
3 years	51%
4 years	81%
5 years	94%
6 years	98%

We would recommend a minimum of a further 2-3 years survey to report on change in 1 km grid square occupancy in the short term with a high level of confidence and power. The survey protocol could be repeated on a six year cycle to report on long term changes.

3.2.3 Estimating the total number of occupied 1 km grid squares

Taking the average occupancy over the three years, we estimate that between 16-33% of 1 km grid squares (95% CI) known to support ponds, in England are occupied by Great Crested Newts. This equates to 10-20% of all 1 km grid squares in England. So the estimate for square occupancy by Great Crested Newts is c.13,470 – 26,940 1 km grid squares based on the 2015 - 2017 results.

Table 3. Table showing an estimate of 1 km grid square occupancy (± margin of error) at difference levels of confidence based on the sample size (n=131) of the current survey.

	Level of confidence		
	90%	95%	99%
Estimate of the number of occupied 1 km grid squares in England (%)	11-19%	10-20%	8-22%
Margin of error (± %)	± 7.1%	± 8.5%	± 11.2%

Table 4 Table showing the sample size needed to increase the level of certainty and reduce the margin of error to provide more accurate estimates of the number of occupied 1 km grid squares in England in future surveys.

	Level of confidence		
	90%	95%	99%
Sample size required to reduce the margin of error to within ± 5% of the estimate	n=269	n=382	n=657
Sample size required to reduce the margin of error to within ± 2.5% of the estimate	n=1,067	**n=1,508**	n=2,577

We could repeat the survey and include a greater number of 1km grid squares in the survey sample to reduce the margin of error or increase the level of certainty (Table 4). However, to reduce the margin of error to within ±2.5% of the estimate at 95% confidence would require a survey network of c.1,500 1 km grid squares, which is likely to be prohibitive.

3.3 Pond occupancy

3.3.1 Estimating change in the number of occupied ponds

Pond occupancy was calculated by reference to the pond in the SW corner of each 1 km grid square, to maintain independence between each sample pond. Allowing for proportional representation in the design, we produced an estimate for the number of ponds occupied by Great Crested Newts in England.

We estimate that 13.3% (± 7.7%) of ponds in England were occupied by Great Crested Newts in 2015; 13.2% ± 7.6% in 2016, and 13.3% (± 7.8%) in 2017 (95% confidence). There was no significant change in the number of occupied ponds over the survey period (Generalised estimating equation assessed using Wald test statistic = 2.118, p=0.347) (Figure 7). This result is comparable with previous estimates of 12% pond occupancy (Wilkinson and Arnell 2013), and 11% pond occupancy (Swan and Oldham 1993). The slightly higher estimates may be due to improved detection with eDNA.

- Five ponds which supported Great Crested Newts in 2015, were null records in 2016. Only 2 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.

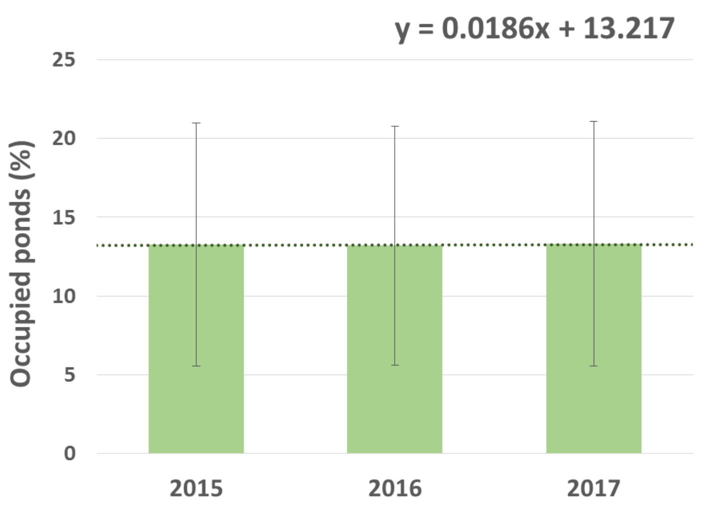
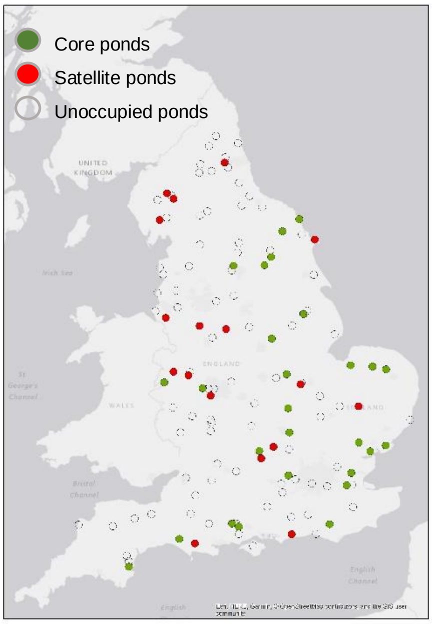


Figure 7: Estimated number of ponds occupied by Great Crested Newts in England (± c.8% margin of error at 95% confidence)
Change over time y=0.0186x + 13.2 (not significant)



Taking the pond in the SW corner across known and unknown Great Crested Newt squares meant the overall most ponds were unoccupied (c.87% of ponds in the data set unoccupied).

There was also high turnover in Great Crested Newt occupancy of these SW corner ponds between years. Core ponds were occupied by Great Crested Newts every year, and satellite ponds were only occupied in occasional years. As with 1 km grid square occupancy, there was no geographical clustering to either core or satellite ponds (Figure 8).

Figure 8: Distribution of core ponds (occupied by GCN every year), and satellite ponds (only occupied in some years).Based on analysis of the pond in the SW corner of the square.

3.3.2 Power to detect change in pond occupancy

The unbalanced nature of the design for pond occupancy based on analysis of the pond in the SW corner of the square (within known and unknown 1 km grid squares) resulted in a large margin of error within years. In the first year of survey only 27% of the sample ponds had a positive record for Great Crested Newts. This coupled with the temporal variation in pond occupancy between years (as newts moved between satellite ponds) resulted in very low power to detect change over three years of survey.

Package ‘longpower’ in ‘R’ was used to determine the level of power achieved in the current survey to detect changes of less than 10%; when sample size n=131, repeated samples over three years, alternative two-sided response. The current survey was only able to achieve 48% power. Therefore, there is a risk that a significant change may have occurred in the number of occupied ponds between 2015 and 2017, but we had not sampled enough ponds to effectively detect the change.

The level of power to detect small changes (<10% change) improved with longer survey periods (Table 5). A four year, rather than three year repeat survey, increased the level of power by 25%. Repeat surveys over a 6 year period, would increase the level of power to detect less than 10% change, to 93% power.

Table 5. Power to detect change (<10% change) in pond occupancy where the number of samples n=131, over 3, 4, 5, and 6 years (90% confidence level).

Number of years of consecutive survey	% power
3 years	48%
4 years	73%
5 years	86%
6 years	92%

3.3.3 Estimating the total number of occupied 1 km grid squares

Taking the average of occupancy over the three years, we estimate that between 6-20% of ponds in England are occupied by Great Crested Newts. The 7.7% margin of error given to this estimate at 95% certainty is large, due to the variability in pond occupancy between years and the large number of unoccupied ponds in any given year, even in occupied ponds.

Table 6. Table showing an estimate of pond occupancy (± margin of error) at difference levels of confidence based on the sample size (n=131) of the current survey.

	Level of confidence		
	90%	95%	99%
Estimate of the number of occupied ponds in England (%)	7-20%	6-21%	3-23%
Margin of error (± %)	± 6.5%	± 7.7%	± 10.1%

The analysis suggest that the PondNet survey design may not be optimal for reporting on pond occupancy in England, although with repeat surveys it can report on change in pond occupancy. Pond occupancy models may be better served by large sample sizes, such as collation of large datasets to compare data over time and space.

3.4 Pond occupancy per km square

PondNet aimed to survey at least 70% of the ponds in each 1 km grid square, to report on the number of occupied ponds per square. The aim of this metric was to act as a proxy for population stability, as clusters of ponds could provide opportunities for metapopulations to develop (Marsh and Trenham, 2008).

The eDNA results suggested that Great Crested Newt occupancy and the likelihood of consistent occupancy between years was related to the number of ponds per square. The average number of ponds per square in absent squares, i.e. 1 km grid squares where Great Crested Newts were not recorded in any year was low (2 ponds/square). Permanently occupied (core) 1 km grid squares had an average of 4 ponds per square; whilst occasionally occupied (satellite) squares fell in-between, with an average of 3 ponds per square (Table 7).

Table 7. Table showing the average number of ponds per square in a) unoccupied (no Great Crested Newts recorded in any ponds over the three years of survey), b) core squares (Great Crested Newts recorded in one or more ponds every year) and c) satellite squares (Great Crested Newts recorded in one or more ponds, in one or more years, but not every year)..

	Square Type		
	Unoccupied	Core	Satellite
Average number of ponds per square	2.4	3.4	3.8
Standard deviation	1.6	2.4	2.8

However, analysis of the number of squares with 1, 2, 3, 4, or 5 or more ponds (Figure 9), showed no significant difference ($\chi^2 = 14.8$, df. 6, P>0.05) between square types: unoccupied squares, core occupied squares, or satellite squares.

Even when Great Crested Newts were present in the square, the number of occupied ponds per square was low, regardless of the number of ponds available.

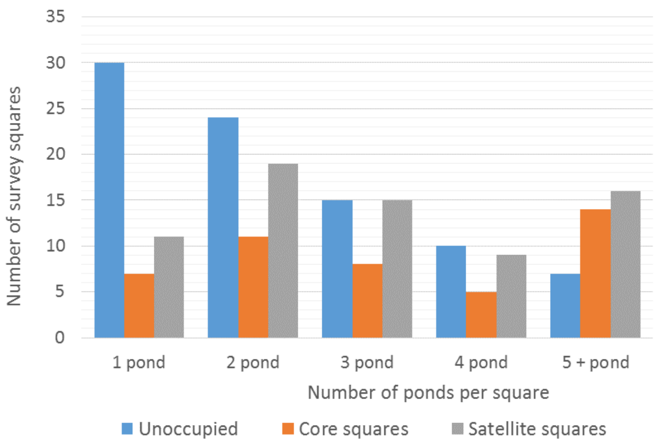


Figure 9: Number of survey squares with 1, 2, 3, 4, or 5+ ponds per square; in each square type - unoccupied by Great Crested Newts in any year, core squares (always occupied), and satellite squares (sometimes occupied).

Only 13 squares, out of 70 which had positive records in any year, had 100% pond occupancy every year. In other words, in these squares all of the available habitat was occupied by Great Crested Newts every year. Of these, seven squares only contained 1 pond, three squares contained 2 ponds, two squares contained 3 ponds; and only one square contained 5 ponds, all of which were occupied every year.

It was interesting to note, that in many squares where Great Crested Newts were recorded, they were only able to occupy a small proportion of the ponds available to them (Figure 10). In the majority of squares (19+44 = 63% of squares), known for Great Crested Newts, between 2015-2017, less than 50% of the available ponds were occupied in some years.

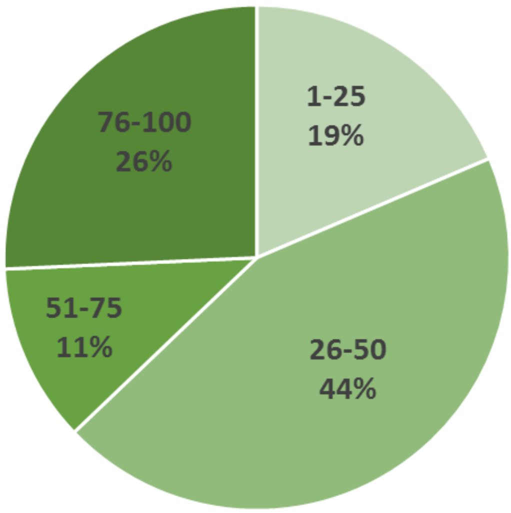


Figure 10: Percentage of squares within pond per square occupancy categories; 1-25%; 26-50%; 51-75%; and 76-100% of ponds in each square occupied.

3.4.1 Change in the number of occupied ponds per year

In total, across the survey network, 117 ponds were positive in 2015, 110 ponds were positive in 2016, and 110 ponds were positive in 2017.

Between 2015 and 2017, 61 (47%) sample squares had zero occupancy every year.

The average number of occupied ponds per square remained consistent between years, 2 ponds per square, within Great Crested Newt occupied squares (Figure 11). As such, there was no significant change in the number of occupied ponds per square over the survey period (Generalised estimating equation assessed using Wald test statistic =1.408, p=0.495).

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.

3.4.2 Power to detect change in the number of occupied ponds per 1 km grid square

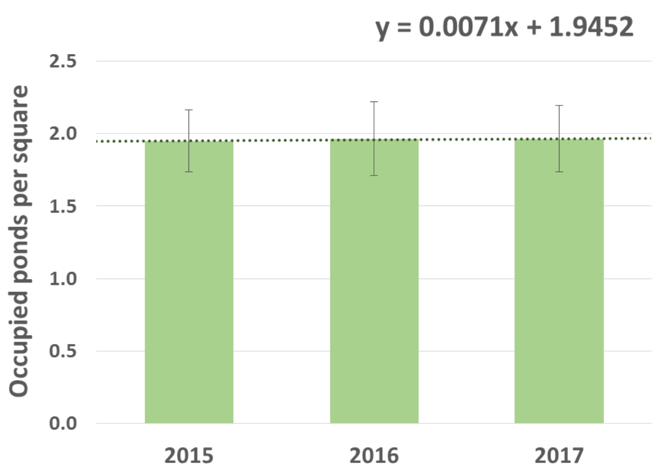


Figure 11: Change in the average number of occupied ponds per square (± 0.2 SE Mean) Change over time y=-0.0071x + 1.9452 (not significant)

Package 'longpower' in 'R' was used to determine the level of power achieved in the current survey; when sample size n=131, repeated samples over three years, alternative two-sided response. The current survey was able to achieve 73% power to detect changes of <10% (90% confidence interval).

As predicted, the number of occupied ponds per 1 km grid square was able to provide a statistically robust measure of change following three years of survey because count data can provide more robust models than binary data with greater levels of power. There is a tendency for over dispersion in binary data (the variation not accounted for by the model), which may not be overcome without a large number of samples (ponds in this instance) or increasing the number of sample years (Ferrari and Comelli, 2016).

Table 8. Power to detect change (<10% change) in ponds per square occupancy, where the number of sample squares n=131, over 3, 4, 5, and 6 years (90% confidence level).

Number of years of consecutive survey	% power
3 years	73%
4 years	84%
5 years	88%
6 years	**89%**

Power to detect <10% change in pond occupancy would increase to between 80-90% with if we were able to extend the survey for 4-6 years. But, at 90% confidence and 73% power, the experimental design may still fail to detect change, because the sample size may not be sufficiently large to overcome random variation in the data. If we were able to extend the survey for longer the power to detect change would increase (Table 8).

3.5 Habitat suitability index

We categorised the number of ponds (n=380) with a positive or negative record for Great Crested Newts, into Habitat Suitability Index (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 (Figure 10).

The results show that Great Crested Newt occupancy was positively correlated with Habitat Suitability Index scores (y = 6.9828x - 0.9483 $R^2 = 0.8381$).

There was a significant difference between the HSI scores for occupied and unoccupied ponds (Pearson Chi-square = 12.61, p=0.013). Only 8% of ponds that were positive for Great Crested Newts had a poor HSI score; whilst 40% of ponds positive for Great Crested Newts had an excellent HSI score.

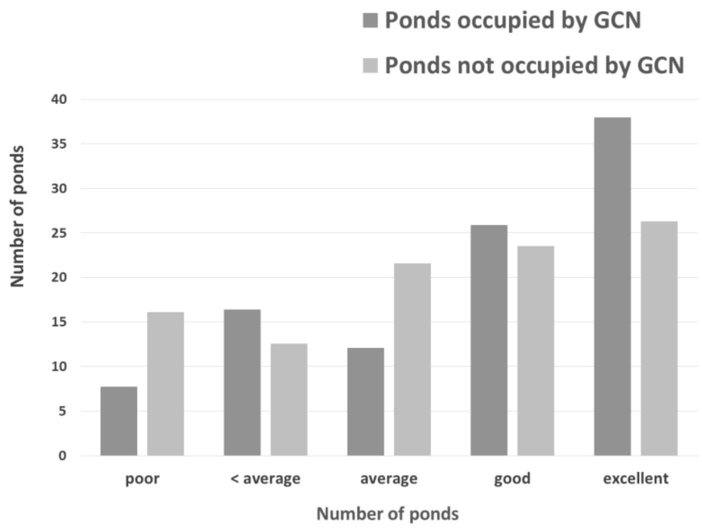


Figure 12: Number of ponds occupied and unoccupied by Great Crested Newts and classified by Habitat Suitability Index category.

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.

The results may also indicate a slight bias in the ponds selected for survey as there were more ponds in the excellent HSI category compared with the poor HSI category even in ponds with no records for Great Crested Newts. Ponds were selected at random during network development, but the need to reselect sites when access permission was refused may have caused some bias.

Habitat Suitability Index scores did not change over the course of the survey. Volunteers were asked to complete an HSI survey in 2015 and update it in subsequent years. This may have led to under-recording as most volunteers reported ‘no change’, rather than submitting a new form.

4. Discussion

4.1 Developing the PondNet programme

4.1.1 Change in pond occupancy

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. 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 random, rather than repeated measures, approach is adopted. Analysis predicts that a 4-6 year surveys would substantially increase power across all metrics (up to c.90% power to detect <10% change with 6 years of survey).

This option would cost c.55k per year for eDNA kits and co-ordination of volunteer surveyors.

4.1.2 1 km grid square occupancy

Trend analysis looks at change over time in occupancy but does not try to estimate the true level of 1 km square occupancy. The advantage of trend analysis to detect change, over a total population occupancy estimate, is that substantially smaller sample sizes are needed to determine whether there is a trend. The trade-off is that there is less precision in the estimate of true 1 km square occupancy. Estimates of occupancy at a national level require a different design to ensure that the sample size is large enough to have certainty that this is a true estimate. It’s important to note that as a result of collecting trend analysis data for the first three years, we now have a dataset that enables us to assess statistically how large a sample we really need to assess the number of occupied 1km squares with a small margin of error and high level of certainty.

At present we have 131 1 km squares with which we can assess occupancy and this allows us to say that we have 1 km square occupancy of 25.0% +/- 7.1% (with 90% certainty) relatively large errors around the estimated occupancy. If plus or minus 7% is an acceptable level of certainly (i.e. actual occupancy could be anything between 17-32% 1 km occupancy, assuming 90% certainty) we could stick with this number of sites. To achieve confidence limits of +/- 5% with 95% certainty would require a larger sample with c.400 1 km squares.

The 400 site option is a quite a lot more costly, needing c.700 kits plus extra co-ordination time so in the order of £150k. Although this is more costly than trend analysis it would not be needed every year (say one year in six would be reasonable) and in the grand scale of Great Crested Newt conservation is still perhaps less than 0.5% of total expenditure on Great Created Newt conservation work.

More detailed exploration of the need for a wider conservation monitoring programme for Great Crested Newts has been submitted as a separate report.

4.2 Application of the eDNA method (issues and opportunities)

4.2.1 Site permissions

As expected, site permissions took a long time to secure. Staff made considerable efforts to ensure that survey squares were representative, and not biased towards semi-natural landuse, where it would have been much easier to get permission. Each of the four regional project officers, spent 14 weeks on landowner permissions in total to establish the PondNet network; 10 of which were allocated to year 1. We have now built a relationship with these landowners over the delivery of this project and all have said that they would be willing to continue allowing access to their land.

Four landowners refused permission on the basis that they did not want the data made public. We decided to make an exception for these sites and keep the records private in the interest of maintaining the un-biased nature of the network.

4.2.2 Volunteer engagement

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 in eDNA because it's simple, and because it's a novel technique. There was also a strong sense amongst volunteers that they were taking part in 'real' science.

Initially our aim was to complete the eDNA surveys each year between late April and late June, which is generally considered the optimal survey window (Biggs et al. 2013).

In 2015, the majority of eDNA kits (79%) were used during this window. But at some sites we had minor delays agreeing access permissions and at others, volunteers were relaxed about adhering to the deadline. This meant that 16% of samples were collected in the first two weeks in July, and the remaining 5% of samples were collected late; between 17th July and 8th August.

In light of this, we revised the methodology to a fixed survey window of May for 2016 and 2017. Some volunteers were still unable to keep to the deadline because, for example, they initially signed up for the survey, but found they were unable to go out. However, we now had more time to resolve the issues. In 2016, 97% of kits were collected before the end of June, and in 2017 100% of samples were collected in May or June.

4.2.3 Kit distribution

eDNA has revolutionised the approach to Great Crested Newt surveys in the UK. But, for volunteer engagement, these is still an issue around postage of kits because they contain pure ethanol which cannot be sent through the Royal Mail without incurring prohibitive costs. This has meant finding other ways to distribute kits to volunteers.

In 2015, kits were mostly distributed to volunteers directly by the regional project officers and collected in the same way. This was time consuming for the project officers and incurred greater travel costs, but was advantageous because it gave project officers the opportunity to demonstrate the methodology and answer any queries.

In 2016/2017, three distribution methods worked particularly well: i) several volunteers met at an agreed location with the project officer and collected / dropped off their kits, ii) individual volunteers offered to act as distribution hubs, e.g. the regional officer dropped off and collected kits from the volunteer distributors, then volunteers from the region collected their kits from their appointed kit coordinator, and iii) Local Environmental Record Centres (e.g. NE England) and Universities (e.g. Ambleside) offered to act as distribution centres for volunteers.

Of the eDNA kits distributed over the three years, only six were lost (c.1%). One, because the volunteer arrived at the pond and on finding it dry, filled in the details on the box and returned it as if completed. Five kits were distributed to volunteers, but for personal/ family reasons they did not complete the survey and were unable to find the time to return the kits.

4.2.4 Use of traditional surveys versus eDNA

Funding from Natural England and Defra allowed us to purchase eDNA kits to cover half of the PondNet network i.e. 100 1 km grid squares. Additional funding from HLF and others allowed us to purchase extra kits for other project elements in the People, Ponds and Water project, and these were made available to PondNet volunteers if they were struggling to complete their survey using traditional methods.

All of the 131 1 km grid squares, 380 ponds, surveyed every year for three years 2015-2017 were completed using eDNA. There were no sites where volunteers managed to complete four visits to all the ponds in the square, over three years.

Volunteers using traditional methods did complete 4 visits to all the ponds in 64 1 km grid squares, 139 ponds but only completed the survey in one or two years of the three year survey. Five squares, 39 ponds had to be removed from the analysis because volunteers did not complete the four visits to establish Great Crested Newt absence.

4.2.5 Summary of issues and opportunities

- A break in the continuity of the project is likely to cause problems as we will lose contact with the landowners. Re-establishing the network at a later date is likely to incur similar costs as the initial set-up.
- All volunteers who completed the eDNA survey would be willing to take part again, these trained individuals represent a significant cost saving to a national monitoring network for Great Crested Newts.
- We found that even although volunteers become independent to some extent, able to undertake the survey with minimal input from the regional coordinator, they still needed someone to administer the eDNA kits, coordinate volunteer activities to ensure that kits were used within the survey window, and to ensure that results were submitted. We recommend a project management structure using a national FTE officer employed for 5 months to administer and coordinate the survey.
- Volunteer distribution hubs for eDNA kits has streamlined the delivery and collection of survey equipment. All the hubs have expressed a willingness to be involved if the survey was to be repeated.
- 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.

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Appendix 1. Information provided to landowners

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Information for landowners about PondNet 2015-2020

Who are we?

Freshwater Habitats Trust is a national charity that works to understand and conserve all freshwater habitats (ponds, lakes, rivers, streams and ditches).

Ponds are a really important habitat. Our work has shown that they can be the most important freshwater habitat in the landscape, supporting more freshwater species than any other.

The problem is that we don't have enough information. If we can show how important they are on a national level we can raise their profile, and in some cases secure funding for others to conserve the most important sites.

What is PondNet?

PondNet is a volunteer survey network that aims to collect information about *trends* in pond quality and pond species in England and Wales. Environmental data is also recorded: providing information that will help explain the *reasons* for any change.

The PondNet network is made up of *randomly* selected one-kilometre grid squares – 250 squares in total in England. Within these grid squares our trained volunteers carry out survey visits to one or more ponds to record wetland plants, aquatic insects or amphibians.

A wide range of partner organisations are also involved in PondNet, including: British Dragonfly Society, Botanical Society of the British Isles, British Trust for Ornithology, Amphibian and Reptile Groups of the UK and the Freshwater Biological Association. The project is supported by funding from Heritage Lottery Fund and Natural England.

Why have we contacted you?

The enclosed 1 km grid square has been chosen as part of the random selection process. We would like to survey the ponds in this square and have contacted you for permission because we believe you are the land owner/ land manager for at least one of the ponds indicated on the map.

We would be very grateful if you would grant permission for our volunteers to carry out pond surveys on your land. Details of what the surveys involve are provided overleaf.

Why take part?

PondNet surveys will not damage your property and we have full liability insurance. The results will be analysed at a national level and used to benefit nature conservation. You can use the results in your own site management plans, as part of a farm assessment or include them in agri-environment scheme applications.

Data collected by volunteers will be entered into a database. You will also be able to log on to the project website and see results (www.freshwaterhabitats.org.uk/projects/pondnet).

What does a PondNet survey involve?

PondNet is based on annual surveys; to give us an idea of change over time. All surveys will be carried out by volunteers who have received appropriate training from us and agreed to our code of conduct.

There are different types of survey depending on how the square was allocated. We aim to survey all the ponds on the enclosed map for (**delete as appropriate**):

Amphibians - a visit to your pond on four evenings between the beginning of April and the end of May. Two volunteers will walk around the pond using a torch to detect newts, frogs and toads in the water.

Frog and toad surveys – one visit to your pond, during the day, in March or April. We are looking for frog or toad spawn and any adults which are hanging out in the pond.

eDNA – one visit, during the day, in May. This is an exciting new technique which makes it possible to look for newts by taking a sample of water to look for their DNA. This is much easier than the traditional survey which involves four night time visits.

Environmental variables – one visit, during the day, between June and August. We will undertake a survey of the pond habitat, including information such as its size, the amount of shade, and whether it is a permanent pond or one that dries out periodically.

One pond (the focal pond which is highlighted on the map) will also be surveyed for plants and invertebrates:

Wetland plants – one visit between June and August to record all the wetland plants within the pond.

Invertebrates – one visit between May and July. We will use a net to collect a sample of invertebrates and identify what is living in the pond.

Dragonflies – five visits between May and September. All the dragonflies seen flying over or around the pond are recorded. This is an optional survey if volunteers and the landowner are interested in having the pond surveyed for them.

Specific surveys for plants or invertebrate species – this pond has been selected because it is a known location for .... (**insert common and scientific name here**). We are monitoring this species because little is known about change in the national population. A volunteer will visit the pond once or twice during the summer to record the number of individuals occurring at this pond.

In summary, each pond on the attached map will be visited a maximum 1, 3 or 5 times (**change as appropriate**). One pond will be visited a further 2 times to survey plants and invertebrates (**delete if not a focal pond**).

What will happen next?

Visits to your ponds will be made in accordance with your wishes. If there are ponds which you don't want volunteers to access we will exclude them from the survey – but don't worry about the pond's condition because we want to survey all types of pond, even the ones that don't look like they have anything in them!

If you want volunteers to contact you before they come out to do a survey we will give them your contact details, and ask them to call or email depending on your preference. If you provide us with your contact details, we will store these separately on a secure server, where they will only be used to provide volunteers with the contact information they need. We will not pass your details to anyone else.

If you have any queries please contact me (details below). We will confirm via email or telephone that you are happy with these arrangements.

e.g. Francesca Dunn

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Freshwater Habitats Trust

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