Executive Summary

Abbreviations of project partner names and surveys widely used in this report are:

ARC = Amphibian and Reptile Conservation

DICE = Durrell Institute of Conservation and Ecology

FHT = Freshwater Habitats Trust, formerly Pond Conservation

NARRS = National Amphibian and Reptile Recording Scheme.

Background

This report summarises work to support the development of a surveillance programme for the Great Crested Newt in Great Britain. It has two main components:

- an evaluation of the use of environmental DNA (eDNA) in detecting the presence of Great Crested Newts in ponds, particularly its use by volunteer surveyors, and its ability to detect newt abundance (Part A); and
- complementary statistical work to establish reliable survey strategies for detecting change in pond occupancy by Great Crested Newts, Habitat Suitability Index scores and pond numbers (Part B).

Methods

Part A: eDNA methods

We developed and tested a primer for the Great Crested Newt using a three stage protocol: *in silico*, *in vivo* and *in situ*. Markers were first tested *in silico* using ecoPCR software, followed by an *in vivo* check of primer specificity using tissue samples collected by swab sampling from 16 Great Crested Newts from south Hampshire, north-west England and north-east England. Finally we tested the primer *in situ* at three out-of-range ponds in Shetland, and six in-range locations in south Hampshire with known low/medium or high density populations. Out of range sites were all negative and in-range sites all positive. The quantities of eDNA detected were broadly, but not exactly, correlated with the low/medium and high densities.

To test the practical utility of the eDNA method we collected five main datasets.

(i) Out-of-range sites to test for false positive eDNA responses: eDNA samples were collected from a set of sites (n=30) just beyond the edge of the Great Crested Newt's known range, in Cornwall.

(ii) Sites for detailed comparison with 'traditional' survey methods: we surveyed 35 sites (20 in south Hampshire, 15 in north-east Wales) on four occasions between mid-April and late June, collecting eDNA on each sampling visit and at the same time recording newt occurrence and abundance by torch counting, bottle trapping, daytime visual searching and egg searching. In south Hampshire, surveys were undertaken by a professional survey team; in north-east Wales the work was conducted by a volunteer team of approximately 50 people, organised by Natural Resources Wales. All sites in the detailed methods study were known to support Great Crested Newts at varying densities, with peak torch counts varying from 1 to 47 individuals.

(iii) Volunteer survey sites: in order to assess the potential for volunteers to use the eDNA method, eDNA samples were collected on one occasion from 239 ponds across England, Wales and Scotland. Volunteers were either part of the PondNet[1] project, or had been involved in amphibian surveys previously, for example through the NARRS network. All sites were intended to be known Great Crested Newt ponds with evidence from the 2013 breeding season that newts were present. Just over 80 volunteers, plus six members of the project team, were involved in collecting the samples. Most volunteers (55%) collected samples from 1 or 2 sites; 1 volunteer, a highly experienced herpetologist with a special interest in the project, collected samples from 30 sites.

(iv) Test for within-range false positives: during the sampling programme we added a further subset of sites (n=30), which were not part of the originally planned work. These were ponds *within* the core range of the Great Crested Newt (in south Hampshire, Kent and London) where we were had good reason to believe Great Crested Newts were *absent* as assessed by local expert knowledge.

(v) Volunteer sampling quality assurance: professional members of the project team resurveyed 11% of sites (n=26) previously surveyed by volunteers to quality assure volunteer sampling.

The volunteer sites surveyed in the present project were representative of the sites occupied by Great Crested Newts across Great Britain in terms of their range, altitude, pond size, geology and associated land-use. However, it should be noted that the sites were not strictly statistically representative i.e. they were not a random stratified sample. Rather, the objective of the study was simply to collect eDNA samples from a good range of sites.

Part B: Statistical design of surveillance surveys for Great Crested Newt

We undertook a wide range of analyses, and associated tests of power, to determine the optimum sampling designs to detect change in three parameters: pond numbers, Habitat Suitability Index (HSI) and Great Crested Newt pond occupancy.

Unlike some other taxonomic groups, particularly birds and butterflies, stock and change data for Great Crested Newts and HSI score is poor, although data are available for pond numbers. This places some limits on the statistical design of survey strategies because we do not have reliable estimates of variability and it is not possible to fully validate the models against real data.

[1]PondNet is a Natural England/Defra funded project which is investigating whether it is possible to establish a new volunteer-based biodiversity surveillance network that will provide statistically valid stock and change data for target species and habitats. The network, called PondNet, uses a habitat-centred monitoring approach with ponds used as the pilot habitat. The project is working with volunteers in three regions: south Hampshire, Cheshire and north-east Yorkshire.

(i) Pond numbers: We used existing data on pond numbers from the Countryside Survey and Ordnance Survey MasterMap to explore sampling strategies and sample sizes needed to assess change in pond numbers.

Countryside Survey field data were originally collected as part of a Great Britain level survey comprising a stratified random sample of 1 km squares, undertaken by professional survey teams. Surveys were undertaken in 1998 and again in 2007 and data were available from 544 1 km squares. Field surveys are currently the only practical way of accurately estimating pond numbers and, in particular, overcoming problems associated with detecting small or temporary ponds, ponds beneath trees, ponds in generally wet environments, and determining which ponds no longer exist. Remotely surveyed data are unlikely to deal with such problems effectively (Biggs *et al.* 1996).

Ordnance Survey data are based on a mixture of ground survey and remote sensing. They are collected over a number of years and have several short-comings including variable dates of survey, irregular updating, inconsistent recording of waterbodies, an ill-defined lower size limit and uncertainties in recording of temporary ponds. Initial ground-truthing by Freshwater Habitats Trust staff of the difference between actually existing ponds and those shown on OS maps indicates that up to 30% of ponds that exist on the ground are not shown on OS maps. However, the Ordnance Survey data provide a much larger pool of samples on which to base pond number estimates than Countryside Survey, effectively all 243,000 1 km square that make up Great Britain, compared to the 544 1 km squares available from Countryside Survey. Thus, despite the inaccuracies in Ordnance Survey data, the very large sample size provides better estimates of variability than the relatively small number of Countryside Survey samples.

(ii) HSI: We used data from the regional surveys undertaken by DICE / FHT in Kent and Wales, Countryside Survey and NARRS to provide data on HSI scores.

Recording HSI scores is important to both understand pond condition for Great Crested Newts at individual sites and also for Favourable Conservation Status (FSC) reporting at national and European levels. However, until recently HSI score has not been included in most monitoring schemes, with the exception of NARRS, so that data on levels of change and variability in the index between years is uncertain.

We re-surveyed 23 ponds in Kent and 25 ponds in Wales which had been surveyed previously by DICE in 2007 to better understand change over time. These data and existing results from analysis of Countryside Survey data (77 ponds) suggest that HSI scores may be increasing (i.e. pond quality is increasing for Great Crested Newts), but that rates of change are slow e.g. a 3% increase in HSI scores over 10 years. A much higher rate of change in is suggested by HSI scores collected during NARRS surveys (372 ponds).

Because of this difference in assessment of change we evaluated the different sampling strategies' abilities to detect an ecologically meaningful value (10%) in HSI scores with 95% confidence and 80% power, using the existing surveys to provide information about variability in HSI scores within and between years.

(iii) Great Crested Newt occupancy: We used two principal datasets to analyse the power of alternative sampling strategies for Great Crested Newts: (a) distribution data from the National Biodiversity Network Gateway (NBN), and other sources, for the period 1988 to 2012 localised by a grid reference but not associated with a specific waterbody and (b) National Amphibian and Reptile Recording Scheme data (NARRS) collected between 2007 and 2012 and based on surveys of ponds nearest to the south-west corner of the sampling square in 410 randomly selected 1 km squares, and so associated with a known waterbody.

We cleaned the NBN data and developed a simulated pond occupancy dataset by overlaying the NBN data on the MasterMap pond layer. We identified all ponds within 1 km of a newt record as potentially suitable for Great Crested Newts, giving a dataset of just over 57,000 ponds and a simulated pond occupancy of 10% in Great Britain, which is consistent with field data. This dataset was then used to explore a sampling strategy based on recording occupancy of *all* ponds in a 1 km square. This approach has a number of advantages both statistically and practically and is the approach currently being tested in the Defra and Natural England supported PondNet project. We evaluated the power of various sampling strategies based on this overall approach, testing different levels of power and different levels of change between sampling years.

We used the NARRS data to evaluate sampling strategies based on the pond occupancy with independence of sample units maintained through survey of only one pond in a 1 km grid square. An alternative strategy would be to survey all the ponds in a square to provide data on the number of occupied ponds per square. This strategy is being used in PondNet, but this network is still in a pilot phase and has not yet generated sufficient data for power analysis. Therefore, variability between squares was modelled using NBN data. Both strategies are valid and have benefits and drawbacks: importantly we are seeking ways to integrate the results to make the best use of all available data.