Non-Technical Summary

This report summarises work undertaken to support the development of surveillance monitoring for the Great Crested Newt. The work had two components: Part A was an evaluation of the use of environmental DNA (eDNA) to detect the presence and abundance of Great Crested Newts, particularly when used by volunteers. Part B comprised complementary work to develop statistically valid sampling strategies for detecting trends in pond occupancy by Great Crested Newts, the quality of their pond habitat and the numbers of ponds at national and Great Britain level.

Part A

Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin, hair and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7-21 days, depending on the conditions. Recent research has shown that the DNA of a range of aquatic organisms can be detected in water samples at very low concentrations using qPCR (quantitative Polymerase Chain Reaction) methods.

In this project we first developed and tested a primer – a length of artificial DNA which specifically binds to and amplifies the DNA of the target organism – which was able to detect Great Crested Newt eDNA successfully in water samples. We then undertook an intensive study at 35 ponds to compare the ability of eDNA and traditional survey methods (torch counting, bottle trapping and egg searches) to detect newts in the breeding season, from late April to late June. Volunteer surveyors also collected single eDNA samples from 239 ponds known to be used by Great Crested Newts across England, Wales and Scotland. We used the volunteer data to assess whether eDNA detection was affected by variations in pond physical and chemical environmental factors, and also to assess the practicality of the technique for use by volunteers. eDNA samples were collected at sites where newts were known to be present in order to determine how often false negatives occur i.e. when the survey method failed to detect animals that we knew to be present. At further sets of sites we also tested for false positives i.e. we wanted to check that the technique would not report that animals were present when they were not. For this analysis we visited 30 sites *outside* the known range of the Great Crested Newt in Great Britain and 30 sites *within the range* where we had good evidence that newts were absent.

In the detailed methodological study, eDNA detected Great Crested Newts 99.3% of the time i.e. out of 140 samples from ponds where we knew newts were present, eDNA detected newts 139 times. Of the traditional survey methods, bottle trapping and torching were similar in effectiveness, followed by egg searches, with the individual methods detecting newts respectively 76%, 75% and 44% of the

time over the full survey period from April to June. When torch counting and bottle trapping were combined, as is normal practice in amphibian surveying, the traditional method was only slightly less effective than eDNA. At the volunteer survey sites, newts were successfully detected 91.2% of the time (218 out of 239 sites). There were no false positives: eDNA did not record newts where we believed them to be absent. We found that newt abundance was weakly correlated with the eDNA 'score': sites with low eDNA scores always had low newt counts but sites with higher eDNA scores did not always have more newts.

Overall, collecting eDNA appears to be a highly effective method for determining whether Great Crested Newts are present or absent during the breeding season. We do not know how effective the method is outside this period and, at the moment, eDNA provides only limited information on newt abundance. eDNA also seems to offer more certainty about zero values: traditionally it has been difficult to say that there are no Great Crested Newts at a pond because surveys might just have missed them. eDNA is also substantially quicker than traditional methods with a sample taking about 2 person/hours in the field (including travel to site) compared to about 48 person/hours for a four visit / multiple methods traditional survey to confirm absence with a similar level of certainty. Overall, the cost of an eDNA survey in England, with 50% of samples collected by volunteers, would be just over £400,000. In Wales and Scotland the cost would be about half this amount. Costs of analysing and reporting the results would be about £50,000.

Part B

A monitoring strategy for Great Crested Newts should provide information on stock (e.g. the total number of ponds supporting newts) and change in variables which are important for the conservation of the species. The UK statutory agencies have decided that for the Great Crested Newt monitoring should focus are pond numbers, Habitat Suitability Index, an indicator of pond habitat quality for newts, and the number of ponds where newts are found (pond occupancy).

There are already several schemes underway which collect data at Great Britain level on the number and condition of ponds and their biota, including the Countryside Survey, the National Amphibian and Reptile Recording Scheme and PondNet. However, the effectiveness of these schemes in generating statistically robust data to assess change for Great Crested Newts is uncertain. None of these surveys was designed to report solely on Great Crested Newt, and all have different approaches to survey design. In addition, Great Crested Newts, in particular, are difficult to monitor because they have a scattered distribution, can be hard to detect and fluctuate in population abundance and pond occupancy between years. As a result, real changes can be hidden in this noise if the number of sites being monitored is too small.

Power analysis can be used to determine the sample size required to detect changes between separate survey years. For example, power analysis can be used to decide whether it is better to assess pond occupancy using a survey conducted in 1996 followed by a second conducted in 2006; or by surveying annually over that time (e.g. 1996, 1997, 1998, 1999 and annually until 2006) to detect change. In both cases the output of power analysis is dependent on a good understanding of the variability of data within and between years. At present such data are surprisingly sparse for Great Crested Newts, despite the amount of information collected about the species, so that there is still considerable uncertainty in estimates of variability and whether this is different for the different regions.

We undertook a wide range of power analyses investigating potential scenarios for the design of Great Crested Newt surveys. Overall, results indicate that, in England, to monitor change in the number of ponds occupied by Great Crested Newts per 1 km grid square, about 550 1 km squares need to be visited, which is roughly 15 per county. This number of squares would also provide sufficient data for pond number assessments and could detect a c10% change in HSI score. The survey strategy requires all ponds in the 1 km square to be surveyed for newts. As the national average pond density is about 2 ponds / km^2 this indicates that, in England, data on Great Crested Newts would need to be collected at c1100 ponds, most likely by the collection of an eDNA sample. In Wales a total of about 300 1 km squares would need to be visited to assess the number of ponds with newts, about 30 per county within the Great Crested Newt's range. Again this is sufficient to estimate change in pond numbers and large changes in HSI score. Newt data would be needed from about 600 individual ponds. In Scotland a total of 290 1 km grid squares would need to be visited to assess change in the number of occupied newt ponds. This would be sufficient to detect at least 20% change in the number of ponds within the newt's range and large changes in HSI scores. Newt data would be needed from about 380 individual ponds. These numbers of squares and ponds would need to be surveyed each time the survey was undertaken.

Estimates for trend analysis were more speculative because of the lack of existing data on which to base models. Stand-alone surveys provide an immediate assessment of change, whereas trend analysis can only confidently report on whether change is occurring at the end of the survey period.

We think that it would be difficult to recruit sufficient volunteers to undertake a 4 visit / multiple method survey using traditional survey methods, or to fund a professional survey using this approach. However, an eDNA survey appears much more feasible, and could probably be undertaken by volunteers assisted by professional staff. However, it is also important to recognise that, for many volunteers, seeing newts is often an important factor motivating them to participate and they may be less interested in an eDNA survey which simply involves collecting water. For this reason, we also discuss the potential for a 'mixed method' survey which combines traditional survey methods at sites where newts can be recorded quickly and easily on a single visit (e.g. by egg searches) with an eDNA sample taken if newts are not immediately and quickly found on the first survey visit.