THE LANES

PENWORTHAM

Appendix 7.11
Great Crested Newt Survey Report

August 2019

Taylor Wimpey

Homes England

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Prepared for	Taylor Wimpey North West/Homes England
Prepared by	TEP - Warrington
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Author	Lizi Pimlott
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Checked	Sofie Swindlehurst
Approved	Sofie Swindlehurst

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Version	Date	Modified by	Check / Approved by	Reason(s) issue	Status
2.0	26/11/18	LP	SS	Amended red line site boundary	Superceded
3.0	01/03/19	LP	SS	Client amendments	Superceded
4.0	06/08/19	LP	SS	Updated eDNA surveys and site description	Issued

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G6900.014 - Pond Location Plan

1.0 Introduction

1.1 Taylor Wimpey and Homes England are seeking to obtain planning permission for residential-led mixed-use development on land to the east of Penwortham Way known as 'The Lanes, Penwortham' (hereafter referred to as 'the site').

1.2 TEP was commissioned, in June 2017, to determine the presence or likely absence of great crested newts *Triturus cristatus* in ponds on and within 500m of the site. An updated great crested newt survey was then commissioned in June 2019.

Description of Survey Area

1.3 The central grid reference of the site is SD 53329 25884 and the location of the site is shown in Figure 1 below. Hatched areas within the red line site boundary in Figure 1 do not fall within the scope of the current planning application and these were not subject to surveys.

1.4 The site is irregular in shape and occupies approximately 53ha on land to the east of Penwortham Way to the south of the settlement of Penwortham.

1.5 The site is bound by Penwortham Way to the west, existing residential development south of Kingsfold Drive to the north, the West Coast mainline railway to the east and agricultural fields to the south.

1.6 The site comprises a mix of land uses including:

- Agricultural land separated into a number of fields by fences, hedgerows and trees;
- Pylon accommodation land;
- Pylon corridor; and
- Roads.

1.7 The site surrounds a number of residential dwellings and light industrial buildings which do not lie within the application boundary.

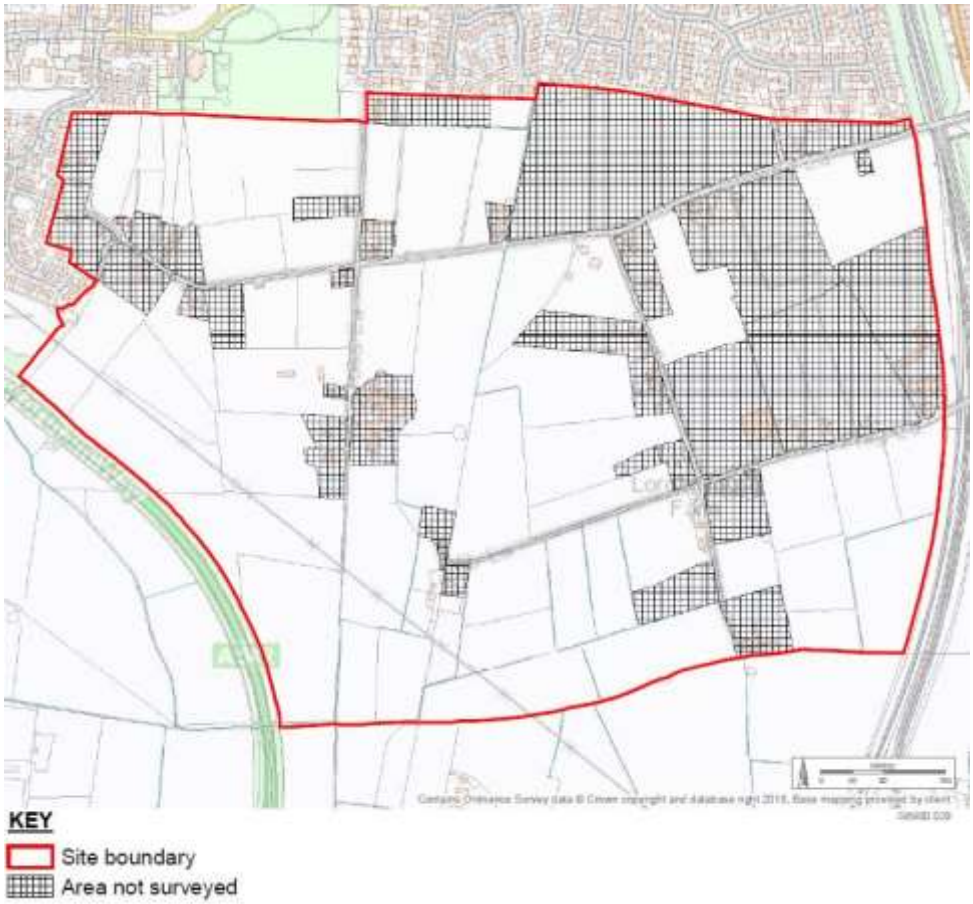

Figure 1: Site Plan. Contains Ordnance Survey data © Crown copyright and database right 2018

Suitability for Great Crested Newts

1.8 Ecology surveys were completed by TEP in 2014 and 2015 in connection with former site proposals. A review of the survey information identified 14 ponds within 500m of the site, with habitat connectivity. This includes six ponds within the site itself.

1.9 The hedgerows and tree roots within the site provide some opportunities for sheltering amphibians, including great crested newts, with some connectivity to nearby aquatic habitat. The remaining land is arable or closely grazed grassland which is too exposed to be used by these species.

2.0 Methods

2.1 Great crested newt environmental DNA (eDNA) surveys of the ponds were carried out by licensed surveyors Lee Moat (licence ref: WML-CL08 2015-16327-CLS-CLS) and Andrew Crone (licence ref: WML-CL08 2015-18548-CLS-CLS) on the 28th June 2017. The updated eDNA surveys of the ponds were carried out by licensed surveyor Anthony Carr (licence ref: WML-CL08 2015-19056-CLS-CLS) on 11th June 2019.

2.2 On 28th March 2014, DEFRA published a report (Biggs et al. 2014[1]) into the effectiveness of environmental DNA testing to detect great crested newt presence from samples of pond water. Shortly after publication of this report, Natural England European protected species (EPS) licensing department confirmed that they would accept quantitative Polymerase Chain Reaction (qPCR) analysis of eDNA from water samples as proof of presence or absence of great crested newts in a pond. Natural England also stated that sampling must take place between the 15th April and the 30th June and be undertaken by a licensed great crested newt surveyor.

2.3 The eDNA analysis technique does not provide a great crested newt population estimate and if great crested newts are present in a waterbody, and site proposals necessitate that a population estimate is required for Natural England licensing purposes, then six visits using traditional survey methods are required.

2.4 Further information on the reliability of this technique and the methods used can be found in Appendix A.

Limitations

2.5 Access was not granted to P3 within the site which is under third party ownership. It is understood that amphibian surveys have been completed of the pond in connection with other planning applications but these are not publicly available at the time of writing this report.

[1] Biggs et al (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

3.0 Results

3.1 No records of great crested newts were found within 1km of the site during the desk based assessment completed by TEP in June 2018 (Report Ref: 6900.007).

3.2 The results of the eDNA surveys are shown in Table 1 below and in Drawing G6900.014.

3.3 Of the 14 ponds surveyed eight were found to be dry or no longer present (P1, P4, P5, P7, P10, P12, P13 and P14). Access was not granted to one pond (P3).

3.4 All five ponds subject to eDNA surveys (P2, P6, P8, P9 and P11) were found to be negative which confirmed the likely absence of great crested newts.

Table 1: eDNA survey results

Pond Ref	Photograph/Description	eDNA result
1	Pond no longer present	
2		Negative (2017 and 2019)
3	No access permission from landowner	
4	Dry	
5	Pond no longer present	
6		Negative (2017 and 2019)

Pond Ref	Photograph/Description	eDNA result
7	Pond no longer present	
8		Negative (2017 and 2019)
9		Negative (2017 and 2019)
10	Pond no longer present	
11	Pond within field margins at the end of a boundary ditch. No photo available.	Negative (2017 and 2019)
12	Pond no longer present	
13	Pond no longer present	
14	Dry	

4.0 Conclusions

4.1 No records of great crested newt were returned within 1km of the site during the desk based assessment.

4.2 Likely absence of great crested newts was confirmed in five ponds within the site and surrounding 500m through eDNA testing, with a further eight ponds being confirmed as dried out or not present.

4.3 One pond (P3) was not accessible to survey and therefore likely absence of great crested newts cannot be confirmed in this waterbody. Given the likely absence of great crested newts from other ponds within the site, and the lack of records locally, it is considered highly unlikely that this species would be present in Pond P3.

4.4 Therefore no impacts to great crested newts are anticipated as a result of the proposals.

APPENDIX A: eDNA History and Method

ENVIRONMENTAL DNA ANALYSIS

A METHOD TO DETERMINE GREAT CRESTED NEWT PRESENCE OR ABSENCE IN PONDS

Background

On 28th March 2014, DEFRA published a report[1] into the effectiveness of Environmental DNA testing to detect great crested newt (GCN) presence from samples of pond water. Shortly after Natural England European protected species (EPS) licensing department confirmed that they would accept quantitative Polymerase Chain Reaction (qPCR) analysis of eDNA from water samples as proof of presence or absence of GCN in a pond. Natural England also stated that for the 2014 survey season, sampling must take place between the 15th April and the 30th June and be undertaken by a licensed surveyor.

This eDNA technique does not provide a population estimate and if GCN are present in a waterbody and site proposals necessitate that a population estimate[2] is required for Natural England licensing purposes then a six visits using traditional survey methods are required.

Sources of environmental DNA (eDNA) include shed skin cells, mucous, faeces and gametes. These sources are diluted and distributed within the aquatic environments and persist within the water column for 7-21 days.

The study was a collaborative effort led by the Freshwater Habitats Trust (formerly known as Pond Conservation) included an in-house study of 35 ponds to test the efficiency of the eDNA method against traditional survey methods. The study was then expanded to volunteers (including consultants and members of wildlife organisations) to provide a wider sample set, to test the practicality of the technique for use by volunteers and to determine reliability was affected by pond characteristics.

To determine the risk of obtaining a false negative result, the study included sampling known GCN breeding ponds. To determine the risk of obtaining a false positive result, the study also included sampling ponds outside the known range of GCN and ponds within the known range of GCN but where confidence of GCN absence was high. A subsection of the samples within the volunteer survey were resurveyed by professionals to assess if surveyor experience influenced reliability. In total over 270 ponds were included in the study.

No false positive results were obtained from the eDNA technique. A small number of false negative results were obtained from the eDNA technique and these were associated with difficulties collecting water samples from areas of the pond used by GCN, difficulties accessing around the entire perimeter of the pond, and very small GCN populations. The study concludes that false positives were most likely when more than one of these constraints occurred.

The study concluded that the eDNA technique was accurate 99.3% (91.2% in the wider volunteer survey) compared with 76% for bottle trapping 75% for torching and 44% for egg searching across the full survey period (April to June). However, when the results of bottle trapping and torching were combined, traditional methods were nearly as efficient as the eDNA technique. Professional surveyors obtained the same result as volunteers on 92% of occasions.

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[1] Biggs et al 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

[2] Calculations of the type and extent of temporary and permanent habitat impacts of proposals are required to determine if presence/absence or population estimates are required for licensing purposes. Up to date guidance can be found on Natural England's GCN licence method statement template.

Statistical analysis of the various pond characteristics only found correlation between GCN detection (with the eDNA technique) and HSI and the study concludes that GCN presence is the major factor determining in the ability to detect GCN eDNA.

The eDNA analysis results in a score of zero to twelve. Zero is a negative result, anything from one to 12 is a positive result. The study found that newt abundance was weakly correlated with the eDNA score, so far as a low score was always associated with a low count using traditional methods. However, the same correlation was not true of high scores.

A small study (provided at Appendix 4 of the main report) has also been undertaken to determine the effectiveness of the eDNA technique outside the breeding season. This study (although of a very limited sample size) concluded that eDNA sampling outside the breeding season could not be relied upon to determine presence / absence.

Method

The an extract of the field sampling protocol outlined in the published Defra funded study (Appendix 5 of the study) is provided at the end of this document. TEP followed this protocol in undertaking their sampling.

Training & Qualifications

Principal TEP Ecologist Elizabeth Seal underwent training with Dr Jeremy Biggs of the Freshwater Habitats Trust (FHT) on the eDNA sampling method on 11th April 2014. A copy of the certificate of this training can be provided on request. Elizabeth has worked with GCN as a consultant ecologist since 2004 and has held a Natural England licence since late 2005.

Before commencing eDNA sampling GCN licensed TEP ecologists were personally trained by Elizabeth Seal on the eDNA sampling method, additional biosecurity measures and record keeping procedures. A record of this training can be provided for named surveyors on request.

Equipment

All equipment for the collection of water samples was as detailed in the published protocol. The equipment was purchased from SpyGen, the laboratory in France who developed the test, supplied the equipment and who undertook all the analysis for the Defra funded study.

Chain of Custody and Storage

All sterilised sampling equipment was received in sealed bags. A check to confirm all seals were intact was undertaken prior to issuing to surveyors. All sample preserving tubes were received in sealed boxes with a unique bar code. On receipt, all seals were checked and the kits were registered on a central database using the unique bar code.

Sample preserving tubes were issued to surveyors with unique individual Sample Forms. The unique bar code was used on each Sample Form to identify each sample. The following information was recorded on the Sample Form (and the central database) at the point of issuing the surveyor:

- Unique bar code
- Site name
- Date of issue

Once in the field and at the ponds, the surveyor confirmed the appropriate field survey sheet was being completed, by checking the bar code on the box and double checking the corresponding bar codes on the sample tubes. The surveyor then filled in the date of survey and the pond ID number (as well as other information relating to survey conditions) on the Sample Form.

On returning to the office the Sample Forms were signed to confirm for each sample:

- The member of staff who received the samples back into the office and stored them in the fridge
- The date the fridge temperature was last checked and the temperature at that time of checking

The pond IDs were checked against a site map confirming which ponds had been sampled and this map was stored with the Sample Forms. All this information was also recorded on the central database.

A blank Sample Form is provided at the end of this document.

The sample preserving tubes were stored in a fridge until the morning of collection by the courier. The Sample Forms and the central database were updated to confirm the date of collection by the courier.

The unique bar codes were used by SpyGen to report results. All results were recorded in the central database by one member of staff and cross checked by a second member of staff. The results were then immediately issued to the lead survey for each site who then checked if the results were as expected (based on historic knowledge, on traditional survey methods if these were being utilised in parallel and based on HSI results). This process was to allow any unexpected results to be investigated further if necessary and to ensure full amphibian surveys were undertaken where necessary.

Sampling Method

The sampling protocol issued with the published Defra study was adhered to. In summary:

- 20 samples were taken from around the entire perimeter of the waterbody.
- The surveyor stayed out of the water while taking the samples (extension poles were used in situations where open/sufficiently deep water was at a distance from the dry banks.
- Survey locations were distributed around the pond perimeter but micro-siting was used to select location most likely to be used by GCN.
- At each sample location the water column was stirred prior to taking the sample but care was taken to avoid disturbing the sediment on the base of the pond.
- Once all 20 samples were taken, 15ml of the total sample were pipetted into each of the 6 sampling tubes ensuring the water in the sample bag was mixed before taking each 15ml sample and that only one sample tube was opened at any one time.
- At all times the surveyor ensured the sampling equipment avoided risk of contamination by not placing the ladle or pipet on the ground or otherwise contaminated surfaces and by changes gloves between the initial sampling and the pipetting stages of the method.

Lab Analysis

All samples were sent to the Spygen laboratory in France. SpyGen developed the qPCR GCN eDNA test and the associated laboratory protocol and undertook all the analysis for the Defra funded study.

Sample Kit ID:			
Received @ TEP (date):		**Signed into Store by:**	
Signed out of Store by:		**Date:**	

Site Name:			
Job Number:			
Have previous pond surveys been undertaken this year?	**Yes/No** **Date:**	**If yes, has evidence of anti-contamination protocol been provided?**	**Yes/No**

DOUBLE CHECK SAMPLING KIT ID

Pond Number:	

Date of Survey:		**Time of Survey:**	
Lead Surveyor Name:			
Surveyor GCN Licensed: (surveyor must be licensed)	**Yes/No**	**Surveyor eDNA trained:** (surveyor must be trained)	**Yes/No**
Number of Samples Taken at Pond: (20 samples required)		**% of Pond Sampled:**	
Is the Pond <1ha?	**Yes/No**		
Notes of any Constraints to Sampling: (circle relevant item and add description)	*Access difficulties, surveyor entered water, potential contamination of sampling equipment, loss of ethanol, sediment disturbed, shallow water, cattle disturbance of sediment, noticeable disturbance of sediment by waterfowl, other.*		

Returned to Fridge (date & time):		**Signed into Fridge by:**	
Fridge Temperature checked (date & temp):		**Fridge Temperature checked (by TEP staff):**	
Collected by Courier (date):		**Collected by Courier (TEP staff responsible):**	
eDNA sampling results:	**Positive/negative**		
Other amphibian survey results:	Visit #of#/Date/Method/Results		

3. Field protocol

Field sampling should be undertaken by a suitably trained and experienced great crested newt surveyor (trained volunteer or professional). At present it is believed that eDNA water sampling does not disturb newts enough to justify the procedure being licensed by the national regulatory authority.

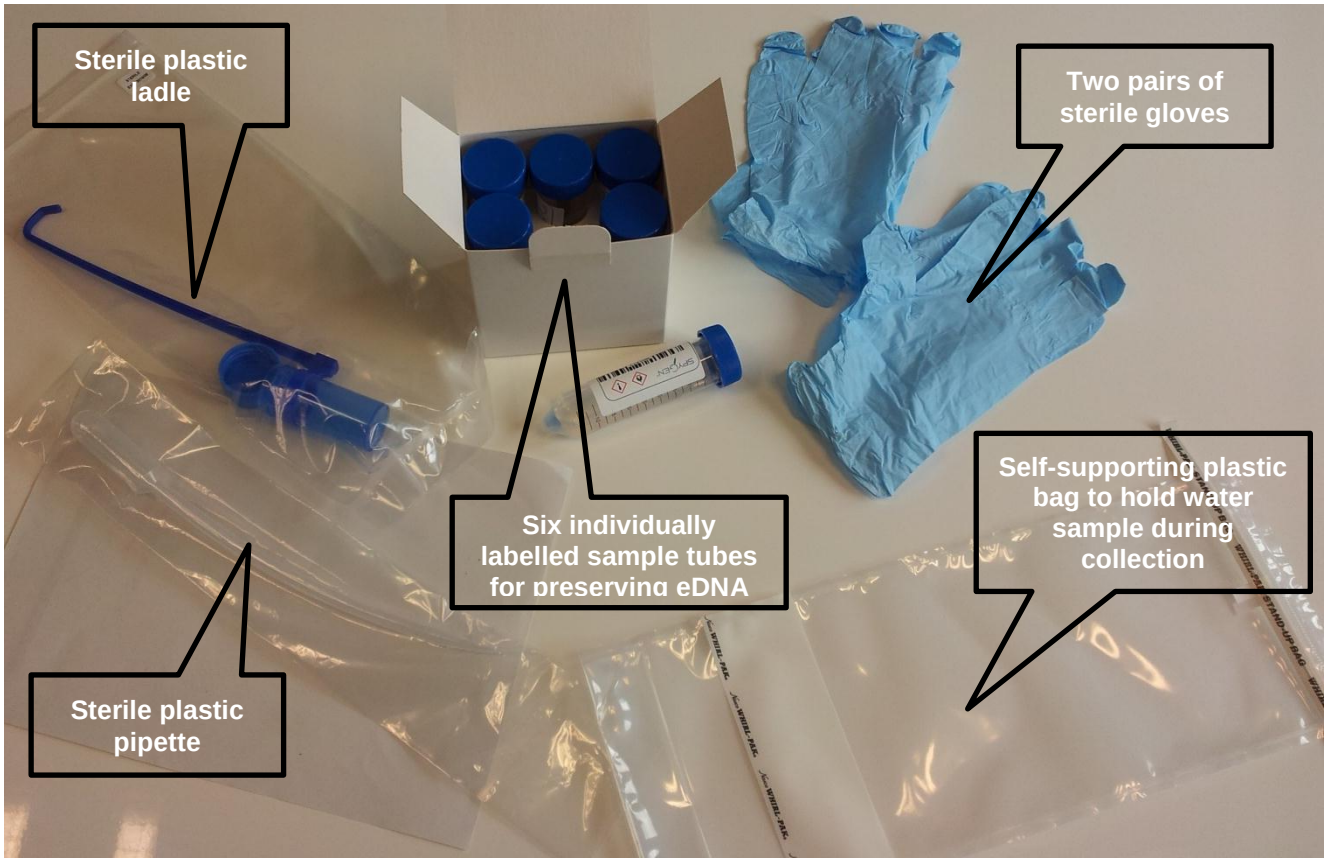
A single visit to the target pond should be made between mid-April and June, during the newt breeding season. eDNA samples can be collected at any time of day and in any reasonable weather conditions, including light rain. It may be best to avoid heavy rain as this makes sampling more difficult and might increase the risk of cross contamination (e.g. splashing of mud which could contain great crested newt DNA from wet ground). There is evidence that unpreserved amphibian eDNA decays slightly more quickly in full sun than shaded conditions, becoming undetectable after 8 and 11 days respectively (Pilliod et al., 2014), but as long as samples are preserved the impact on detection should be slight.

3.1 Sampling equipment

The field sampling equipment used by Biggs et al. (2014) has five components (Figure 2):

- A sterile 30 mL ladle
- A sterile self-supporting Whirl-Pak plastic bag with 1 L capacity
- A sterile 10 mL pipette to resample the pond water
- Six sterile 50 mL centrifuge tubes containing preservative (Absolute Ethanol (200 Proof), Molecular Biology Grade, Fisher BioReagents (Product Code: 10644795), sodium acetate and other markers)
- Two pairs of sterile gloves.

Figure 2 Sampling equipment used for eDNA water samples by Biggs et al. (2014)



Kits can be stored at room temperature before use in an appropriate solvent store, consistent with Home Office regulations, and should be used within about two weeks of receipt. The time between kit receipt and use should be noted (see Section 5.1). Use one kit per pond up to an area of 1 ha. Beyond this, use an additional kit per hectare. However, note that as yet there is no practical experience of the effectiveness of kits used on ponds greater than 1 ha in area. Note that sampling techniques are still developing rapidly in this field and alternative preservatives to ethanol are currently being sought.

3.2 Field water sample collection protocol

The field sampling protocol should follow the steps outlined below. Gloves should be worn at all times during the sampling process, replacing the gloves between sample collection from the pond and pipetting into the sterile sub-sample tubes. Samples should be collected without entering the water, i.e. the surveyor stands only on the pond bank or muddy pond edges. This prevents disturbance of the substrate and may limit cross-contamination.

Stages of field sampling protocol

Step 1 Identify where 20 samples will be taken from the pond. The location of sub-samples should be spaced as evenly as possible around the pond margin, and if possible targeted to areas where there is vegetation which may be being used as egg laying substrate and open water areas which newts may be using for displaying.

Step 2 Open the sterile Whirl-Pak bag by tearing off the clear plastic strip c 1cm from the top (along the perforated line), then pulling the tabs. The bag will stand-up by itself.

Step 3 Collect 20 samples of 30 mL of pond water from around the pond (see 1 above) using the ladle (fill the ladle), and empty each sample into the Whirl-Pak bag. At the end the Whirl-Pak bag should be just under half full (600 mL).

NOTE: Before each ladle sample is taken, the pond water column should be mixed by gently using the ladle to stir the water from the surface to close to the pond bottom without disturbing the sediment on the bed of the pond. It is advisable not to sample very shallow water (less than 5-10 cm deep).

Step 4 Once 20 samples have been taken, close the bag securely using the top tabs and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.

Step 5 Put on a new pair of gloves to keep the next stage as uncontaminated as possible.

Step 6 Using the clear plastic pipette provided take c15 mL of water from the Whirl-Pak bag and pipette into a sterile tube containing 35 mL of ethanol to preserve the eDNA sample (i.e. fill tube to the 50 mL mark). Close the tube ensuring the cap is tight.

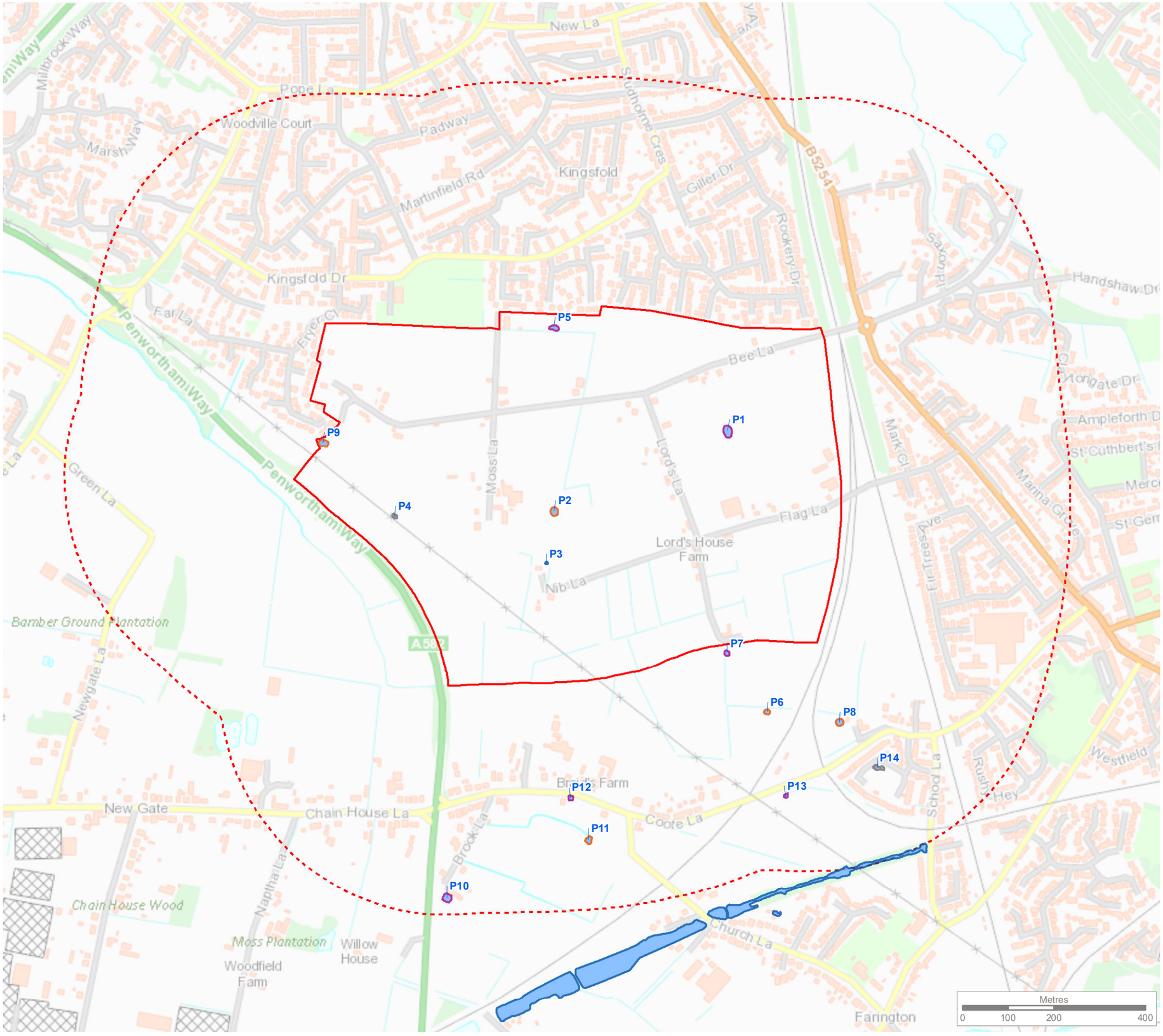
Step 7 Shake the tube vigorously for 10 seconds to mix the sample and preservative. This is essential to prevent DNA degradation. Repeat for each of the 6 conical tubes in the kit. Before taking each sample, stir the water in the bag to homogenize the sample - this is because the DNA will constantly sink to the bottom.

Step 8 Empty the remaining water from the Whirl-Pack bag back into the pond.

Step 9 The box of preserved sub-samples is then returned at ambient temperature immediately for analysis. If batches of samples are collected and stored prior to analysis they should be refrigerated at 2-4° C. Kits can be stored for up to one month in a refrigerator before analysis. It is not necessary to freeze samples. Freezing may damage storage bottles, which can lead to leaking during transit, and also unnecessarily increases costs by requiring refrigerated transport. The length of time eDNA samples are stored in a refrigerator prior to analysis should be recorded and passed on to the analysing laboratory. Use an appropriate labelling system to ensure that the kits are supplied with a unique reference number.

Drawing

G6900.014 - Pond Location Plan

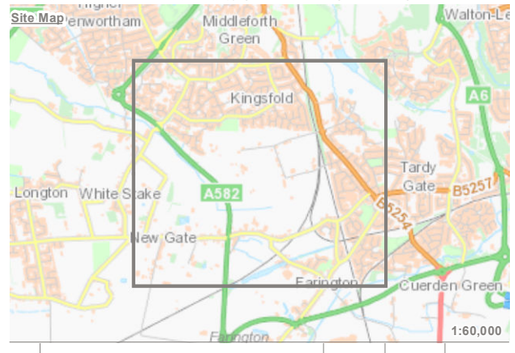


KEY

- Site boundary
- Site boundary - 500m offset

Pond data

- Dry pond
- Pond not surveyed
- Pond eDNA negative
- Pond was not evident during survey



Rev	Description	Drawn	Approved	Date
A	Changes in ponds IDs.	MK	LP	10/12/2018

THE ENVIRONMENT PARTNERSHIP
Genesis Centre, Birchwood Science Park, Warrington WA3 7BH
Tel 01925 844004 e-mail tep@tep.uk.com www.tep.uk.com

Project
The Lanes, Penwortham

Title
Pond Location Plan

Drawing Number
G6900.014A

Drawn	Checked	Approved	Scale	Date
JO	JS	LP	1:8,500 @ A3	10/12/2018

THE ENVIRONMENT PARTNERSHIP

HEAD OFFICE

Genesis Centre,
Birchwood Science Park,
Warrington
WA3 7BH

Tel: 01925 844004
E-mail: tep@tep.uk.com

MARKET HARBOROUGH

Harborough Innovation Centre,
Airfield Business Park,
Leicester Road,
Market Harborough
Leicestershire
LE16 7WB

Tel: 01858 383120
E-mail: mh@tep.uk.com

GATESHEAD

Office 26, Gateshead International Business Centre,
Mulgrave Terrace,
Gateshead
NE8 1AN

Tel: 0191 605 3340
E-mail: gateshead@tep.uk.com

LONDON

8 Trinity Street,
London,
SE1 1DB

Tel: 020 3096 6050
E-mail: london@tep.uk.com

CORNWALL

4 Park Noweth,
Churchtown,
Cury,
Helston
Cornwall
TR12 7BW

Tel: 01326 240081
E-mail: cornwall@tep.uk.com