Appendix 7.4

Great Crested Newt e-DNA & Presence/Absence Surveys 2015 of the Proposed Douglas West & Dalquhandy DP Renewable Energy Project, South Lanarkshire

Dunnock Environmental Services

Final Report

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1	07/05/2015	Interim draft	
2	26/05/2015	Updated interim draft	incorporating additional data from 22/05/2015
3	27/05/2015	Final interim report	Incorporating client comments received 27/05/2015

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1. Introduction

A planning application is being drawn up by 3R Energy for a 15-turbine wind farm and associated wood drying facility at Douglas West and Dalquhandy DP, located to the north-west of the village of Douglas in South Lanarkshire (central OS grid reference: NS 820 325).

In addition to the turbines and wood drying facility the proposed development would contain associated infrastructure, such as substation/control building, hardstandings, crane pads, access tracks, etc. It is anticipated that the grid connection would be laid largely underground along the former Dalquhandy access road which leaves the north-eastern corner of the site and runs northwards past the Dewars bonded warehouses towards the M74 motorway.

The turbines would be sited largely on land disturbed by the former Dalquhandy Opencast Coal Site and to the north-east of the operational Hagshaw Hill Wind Farm.

As part of this planning application, a suite of ecological and ornithological surveys is being carried out to feed into the Environmental Impact Assessment process. This report describes the methods and results of an e-DNA assessment and presence/absence surveys. It follows on from a Great Crested Newt Habitat Suitability Assessment which was carried out by DES on 12th and 19th October 2014 at the site (DES, 2014).

2. Site Description

The revised site boundary is 245 ha in extent and consists of two distinctive sections: a northern section and a southern section, which are separated by the former coal haul road, now a tarmac road that crosses the site in an east-west direction.

The northern half of the site consists of previously worked opencast coal land which was restored in the mid-1990s and which has reverted predominantly to a rough grassland consisting of a mixture of Soft-rush (*Juncus effusus*) and Tufted Hair-grass (*Deschampsia cespitosa*) with patches of more open and improved grassland scattered in between. A number of small waterbodies, including former settlement lagoons, and running streams are scattered across the site. The concrete hardstanding of the former dispatch point (DP) in the north-east corner of the site and the tarmac road are remnants of the previous opencast coal infrastructure.

The southern section of the site consists of unworked land that is more semi-natural in character, although has been drained in the past, and consists of a mixture of Purple Moor-grass (*Molinia caerulea*) dominated wet heath, marshy and acidic grassland. There is also a band of young mixed woodland plantation along the southwestern site boundary.

The Poniel Water corridor, deeply incised in the west, runs along the northern boundary of the site (in a diverted channel), while dense Sitka Spruce plantation borders the western boundary and a mixture of broadleaved woodland, coniferous woodland and a disused railway flanked by broadleaved trees border the eastern boundary. The access track to the Hagshaw Hill Wind Farm

forms the southern boundary of the site beyond which the rough grassland of the southern section continues south-westwards for some distance.

The entire site is grazed by sheep and there is a low level of informal recreational use of the site, primarily along the former coal haul road in the centre of the site.

3. Legislation

The following paragraphs provide a summary of the protection afforded to Great Crested Newts. The summary is not comprehensive and is included here for illustrative purposes only. For a definite list of offences, the reader is referred to the original legislative texts[1].

The Conservation (Natural Habitats, &c.) Regulations 1994 (as amended) (the 'Habitats Regulations') provide protection to European Protected Species (EPS) of animals listed in Annex II of the Council Directive 92/43/EEC on the Conservation of Natural Habitats of Wild Fauna and Flora (the 'Habitats Directive'). Great Crested Newts are listed in Schedule 2 of the Habitats Regulations.

The following provides a summary of relevant offences included in the Habitats Regulations in relation to Great Crested Newts.

It is an offence, amongst others, to deliberately or recklessly:

- capture, injure or kill a wild animal of this species
- disturb such an animal whilst it is using any structure or place for shelter or protection (e.g. a breeding pond, a hibernation site)
- obstruct access to a breeding site or resting place of such an animal or otherwise deny the animal use of that site
- disturb such an animal in a manner that is, or in circumstances which are, likely to significantly affect the local distribution or abundance of the species
- disturb such an animal in a manner that is, or in circumstances which are, likely to impair its ability to survive, breed or reproduce, or rear or otherwise care for its young.

It is also an offence to:

- damage or destroy a breeding site or resting place of such an animal even when this is not occupied. This does not need to be deliberate or reckless to constitute an offence.

[1] Further details are provided at http://www.snh.gov.uk/protecting-scotlands-nature/protected-species/which-and-how/amphibians-reptiles/.

4. Survey Limitations

Surveys were carried out within the recommended survey periods and in suitable weather conditions. There were therefore no survey limitations.

5. Methodology

The approach adopted consisted of a desk study and a field survey, as described in sections 5.1 and 5.2 below.

5.1 Desk Study

A desk study had been carried out for the Habitat Suitability Assessment (DES, 2014). The NBN gateway (https://data.nbn.org.uk/Reports/Sites/NS83/Groups) was consulted again on 15th April 2015 for any records that may have become available in the meantime. The SNHi web site (http://www.snh.gov.uk/publications-data-and-research/snhi-information-service/map/) was also consulted but was not available at the time of writing.

5.2 Field Survey

The habitat suitability assessment (DES, 2014) looked at 26 ponds located at the proposed development site and within an approximately 500 m buffer of the then site boundary[2]. The outcome is summarized in Table 1 below.

Table 1 - Number of ponds per habitat suitability category within the development site and a surrounding buffer zone of around 500 m, as assessed in 2014

Habitat suitability category	Number of ponds
Good	3
Average	4
Below average	2
Poor	3
Unsuitable	7

The habitat suitability assessment methodology of the Amphibian and Reptile Groups of the United Kingdom (ARG-UK, 2010) does not make any recommendations about what habitat suitability categories should be considered for further assessment (e.g. presence/absence surveys, population size class assessments), although mentions that data from Oldham *et al*. (2000), upon which the methodology is based, showed that a total of 55% of ponds assessed as of average suitability supported Great Crested Newts, while that proportion dropped to 20% for the below average category of ponds, implying that the cut-off should be between the average category (to be scoped in) and the below average category (to be scoped out). However, Sellars (2010) found a higher proportion (29%) of ponds in the below average category containing Great Crested Newts

[2] The site boundary has since been reduced and is illustrated in Figure 1.

than Oldham *et al*. (2000) did and implied that the cut-off for presence/absence surveys should be made between the below average and poor habitat suitability categories.

In line with the latter, more conservative, approach, all ponds of at least below average habitat suitability were considered for further assessment. These included ponds 6, 8 and 12 (good), ponds 9, 10, 11 and 13 (average) and pond 15 (below average). Pond 2 had a score of 0.50, putting it marginally into the below average category. However, it was very shallow, ephemeral and did not support any submerged plants for egg laying. For these reasons pond 2 was not considered for further assessment. Moreover, an inspection on 20th April 2015 revealed that agricultural soil mounds had been deposited into this pond. The ponds included for further assessment (ponds 6, 8 to 13 and 15) are shown in Figure 1.

Two methods were employed for further assessment: e-DNA sampling and presence/absence surveys.

E-DNA survey

E-DNA sampling has been approved by Natural England (NE) for the determination of the presence/absence of Great Crested Newts in ponds in England since 2014 (Natural England, 2014) following publication of the results of a study by the Freshwater Habitats Trust (Biggs *et al*., 2014a) which showed that the e-DNA technique detected Great Crested Newts where they were known to be present in over 99% of cases, compared to 76%, 75% and 44%, respectively, for the conventional methods of bottle trapping, torching and egg searches or 95% for a combination of these techniques.

NE make use of the e-DNA technique subject to a number of conditions, which include the following:

- that the technical advice note accompanying the report (Biggs *et al*., 2014b) is strictly followed
- that licensed GCN surveyors take the samples, should a licence application be necessary at a later stage
- that samples are only collected following the onset of suitable weather conditions for surveying Great Crested Newts, which is defined by NE as the period 15th April to 30th June, bearing in mind geographical location and conditions early in the year
- that the laboratory undertaking the analyses can achieve a satisfactory level of performance, similar to that of the high specification laboratory used by the Freshwater Habitats Trust.

Confirmation that the e-DNA method is acceptable in Scotland was obtained from SNH (e-mail dated 27th March 2015 from John McKinnell, SNH Policy and Advice Officer - Mammals, Reptiles and Amphibians, to Alison Hannah, DES), subject to the DEFRA/NE protocol (detailed in Biggs *et al*., 2014b) being followed.

Eight e-DNA sampling kits were received by courier on 13th March 2015 from Fera, a high specification lab that adheres to the NE protocol, and were stored at room temperature.

The e-DNA survey was carried out between 16:30 and 20:00 on 17th April 2015 jointly by Findlay Ecology Surveys and Dunnock Environmental Services under the licence of Melanie Findlay (licence number: 14199). The other surveyors (RI, JWG and AT) were agents/assistants under that licence and were familiar with or had been trained in the e-DNA methodology. Prior to the survey all the surveyors familiarised themselves with the Biggs *et al.* (2014b) technical advice note and the guidance notes issued by the lab (Fera, 2014) as well as watching a commercially available video showing how to collect the e-DNA samples in the field. On the day of survey, surveyors were again briefed on the e-DNA sampling technique by one of the surveyors with prior experience of the method (RI). All surveyors followed the amphibian disease precautions (ARG-UK, 2008).

Weather conditions during the survey were blustery and cold (*ca*. 10ºC) with no precipitation. An inspection of a pond in Midlothian known to hold Great Crested Newts was carried out prior to the survey on 14th April 2015 under colder and windier conditions and revealed the presence of good numbers of Great Crested Newts, thereby confirming that Great Crested Newts had migrated to ponds and that it was an appropriate time for carrying out surveys in Scotland.

The surveyors split up into two teams, with the first team (RI and JWG) sampling ponds 6 and 8 to 10 and the second team (MF and AT) sampling ponds 11 to 13 and 15. The surveyors followed the field water sample collection protocol described in Biggs *et al*. (2014b), which is reproduced in Appendix 1 to this report.

In short, 20 locations were identified spaced as evenly around the pond as possible and targeting vegetated areas (used for egg laying) and open water areas (used for displaying). A 30 ml sample of pond water was collected by one of the surveyors wearing sterile gloves from each of these locations with a sterile ladle, stirring the water column gently to close to the pond bottom but taking care to avoid disturbing sediment from the bottom (which can contain old e-DNA from past years) and emptied into a plastic bag. No samples were taken in shallow water areas less than 5-10 cm deep. Once all 20 samples had been taken, the second surveyor shook the plastic bag vigorously for approximately 10 seconds to mix any e-DNA across the water sample. Using a new pair of sterile gloves, the first surveyor then took a 15 ml sample of pond water from the bag with a sterile pipette and decanted it into a sterile centrifuge tube containing 35 ml of preservative (absolute ethanol (200 proof), molecular biology grade, Fisher BioReagents (Product Code: 10644795), sodium acetate and other markers). The tube was then sealed, marked with the pond number and date and shaken vigorously for approximately 10 seconds to mix the sample and preservative. This step was repeated for five more tubes, stirring the water in the bag before each pipette draw to prevent e-DNA sinking to the bottom of the bag. Following this procedure, the tubes were placed into a cardboard box in which they had been delivered by the lab and the pond number and date marked on the box. The surplus water was returned to the pond and the box with the six tubes put in a cool box. Following completion of the other surveys (see below), the water samples were driven to the home of one of the surveyors (AT) and stored in a fridge at a temperature of 2-4ºC for 2.5 days before being picked up by courier and sent to the Fera lab in Sand Hutton, Yorkshire, on 20th April 2015 where they were delivered the following morning.

The Fera lab analysed the samples using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA following the Biggs *et al*. (2014b) protocol, as specified by NE. For details of the lab procedure the reader is referred to section 4 in Biggs *et al*. (2014b). A lab report with the results of the analyses was e-mailed to DES on 29th April 2015.

Due to a somewhat ambiguous way of marking the test tubes, which had the 50 ml mark at the back of the tubes with a standard 45 ml scale at the front, the first team of surveyors misinterpreted the scale and the four samples from ponds 6 and 8 to 10, respectively, were only filled to the 45 ml instead of the 50 ml mark. Under such circumstances under-sampling and the risk of a false negative cannot be ruled out.

As a result four additional e-DNA kits were ordered and received by Findlay Ecology Services on 7th May 2015. The first team of surveyors re-sampled ponds 6 to 8 and 10 the same day between 18:15 and 20:35 following the same methods as described above. Weather conditions during the survey were clear skies and cold (*ca*. 10ºC) with no precipitation.

The e-DNA kits were picked up by courier on 11th May 2015 but transferred to the wrong destination before reaching the Fera lab on 15th May 2015. Fera analysed the samples according to the Biggs *et al*. (2014b) protocol and e-mailed a lab report with the results to Findlay Ecology Services on 22nd May 2015.

Presence/absence surveys

Due to the time windows of the e-DNA surveys (mid-April to end June) and those of the conventional survey techniques (mid-March to mid-June, with at least two visits between mid-April and mid-May or at least three visits between mid-April and mid-May where relative population size class assessments are required) and the time between e-DNA sample collection and return of lab results (about two weeks), it is prudent to carry out some conventional survey technique visits prior to the lab results becoming available, should the latter confirm the presence of Great Crested Newts and three conventional survey visits are then required during the period mid-April to mid-May.

Therefore, in addition to the e-DNA survey, egg searches, natural refugia searches and torching were carried out by the same surveyors on 17th April 2015 following standard guidance (Langton *et. al*., 2001; Natural England, nd.). Bottle trapping was not carried out on this visit for two reasons: i) the licence under which the surveys were carried out specified that bottle trapping should only be employed when torching is not practical and ii) temperatures were too low (< 5ºC) during the night. Instead placement of artificial refugia to be checked on subsequent visits was undertaken as an additional survey technique.

Following the e-DNA survey, both surveyors in each team searched each pond for newt eggs, did occasional natural refugia searches and put out ten 0.5 m x 0.5 m felt mats in suitable habitat around each pond, apart from pond 9, which is much larger than the other ponds and where 30 felt mats were put out.

Following the e-DNA, egg and refugia surveys, the two teams of surveyors carried out torch surveys after dark between 21:30 on 17th April 2015 and 01:00 on 18th April 2015 at the same ponds they had covered during the afternoon surveys. Surveyors in each team walked around opposite shorelines of each pond where these were accessible and made a count of all amphibians seen. The counts from both surveyors were then added up to give the totals per pond.

Weather conditions had considerably calmed down in the evening for the torch surveys with only a light breeze, a cold temperature (down to -1ºC) and occasional surface mist from the ponds. The latter was not considered to be a significant impediment to carrying out the torch surveys in an efficient manner. Torches used were Viking head torches with Cree T6 LEDs giving 800 lumens. These torches had been trialled in parallel to Clulite torches at the Midlothian site on 14th April 2015 and considered suitable for torching survey purposes.

After the e-DNA re-sampling of ponds 6 and 8 to 10 on 7th May 2015 the artificial refugia that had been laid out on 17th April 2015 were checked for the presence of newts. This was followed by a torch survey carried out between 21:30 and 23:30 under clear skies and a temperature of 5ºC.

6. Results

This section presents the results of the desk study and the field survey elements.

6.1. Desk Study

The desk study revealed no additional records to those that had been found for the Habitat Suitability Assessment (DES, 2014). In summary, Smooth Newts (*Lissotriton vulgaris*) and Palmate Newts (*Lissotriton helveticus*) were recorded in the 10 km square in which the development site is located and at 6 km to the south-east during the period 1960-1985, while unspecified newt (*Triturus* sp.) records were recorded at the site in 1991. There were no positive records of Great Crested Newts at the site and in the wider surrounding area.

6.2. Field Survey

E-DNA survey

The results of the e-DNA assessment are summarized in Table 2 below. A copy of the lab report of 29th April 2015 is included in Appendix 2 and that of the lab report of 22nd May 2015 in Appendix 3.

Table 2 - e-DNA sample results

Pond	Sampling date	FERA sample reference	GCN DNA present/absent	Inhibition	Degradation
6	17/04/2015	S15-049720	inconclusive	No	No
	07/05/2015	S15-050233	absent	No	No
8	17/04/2015	S15-049722	inconclusive	No	No
	07/05/2015	S15-050234	absent	No	No
9	17/04/2015	S15-049723	inconclusive	No	No

Pond	Sampling date	FERA sample reference	GCN DNA present/absent	Inhibition	Degradation
	07/05/2015	S15-050235	absent	No	No
10	17/04/2015	S15-049724	inconclusive	No	No
	07/05/2015	S15-050236	absent	No	No
11	17/04/2015	S15-049725	absent	No	No
12	17/04/2015	S15-049718	absent	No	No
13	17/04/2015	S15-049719	absent	No	No
15	17/04/2015	S15-049721	absent	No	No

According to the lab report of 29th April 2015 the results indicate that Great Crested Newt DNA was not detected in any of the eight samples submitted. However, for the four samples (from ponds 6 and 8 to 10, respectively) that had insufficient water under-sampling and the risk of a false negative cannot be ruled out. Consequently, inconclusive results were issued for these ponds. Following analyses of the re-samples collected on 7th May for these for ponds, negative results were also issued for these four ponds.

Analysis was conducted in the presence of the following controls: 1.) extraction blank and 2.) appropriate positive and negative PCT controls for each of the TaqMAn assays (GCN, Inhibition and Degradation). All controls performed as expected, meaning that the samples were not inhibited or degraded.

Presence/absence surveys

The results of the presence/absence surveys of 17th April 2015 are summarised in Table 3 below.

Table 3 - Results of egg searches, natural refugia searches and torching

Pond	% of pond perimeter accessible	Eggs	Refugia search	Torching				Notes
				Small Newts*	Frogs	Toads	Unidentified Frog/Toad	
6	60	-	-	2	-	-	-	
8	80	-	-	6	-	-	-	
9	90	-	-	2	-	-	-	
10	50	-	-	2			-	
11	80	-	-	-	1	10	1	Swampy with no standing water at one end. One area of frog spawn. Hydrologically linked to pond 12.
12	50	-	-	5	-	12	-	Deep swampy sedge bed around shoreline. Hydrologically linked to pond 11.
13	60	-	-	2	9	73	-	Lots of dried frog spawn on muddy margin where water level had dropped. High turbidity in area around main inflow.
15	100	of small newts	-	69	2	59	-	

" Small newts refer to either Palmate or Smoot Newts

Torching revealed the presence of small newts (Palmate/Smooth Newts) in all ponds, except pond 11. Numbers were generally small (up to six), except for pond 15 (see photo on title page), where a

much larger number of small newts were recorded (69). The latter was also the only pond where small newt eggs were recorded. The searches of natural and artificial refugia did not reveal any newts.

With regards to other amphibians, small numbers of Common Toads (10-12) were recorded at ponds 11 and 12, with much larger numbers recorded at pond 15 (59) and pond 13 (73). Small numbers of Common Frogs (1 to 9) were also recorded at ponds 11, 13 and 15.

The torch survey of 7th May 2015 revealed the presence of a single small newt in pond 8 but none in ponds 6, 9 and 10.

A small number of the artificial refugia that were put out on 17th April 2015 were checked incidentally during other survey work on 20th, 22nd and 23rd April 2015. No newts were recorded under any of the mats checked. A full check of all the refugia on 7th May 2015 did not record any newts either.

7. Discussion

Neither the desk study nor the e-DNA sampling, egg, natural and artificial refugia searches and torch survey revealed any positive evidence of Great Crested Newts but Smooth/Palmate Newts were present in all but pond 11.

On the basis of these surveys, especially the e-DNA assessment, the presence of Great Crested Newts in the eight ponds assessed can be ruled out at the time of survey.

Pond 15 appears to provide the highest diversity of amphibians with 69 small newts and 59 Common Toads recorded. It is separated from the development site by the Poniel Water but a land bridge into the site exists within ca. 200 m to the east of the pond (and at ca. 700 m to the west). Pond 15 differs from the other ponds in its more sheltered location within the Poniel Water valley; it also appears to be less acidic than most of the other ponds, though this was not tested in the field.

Pond 13 had the highest number of Common Toads (73). In addition there was lots of dried out Common Frog spawn on its muddy shoreline where the water level had dropped. This pond dried out further, unlike any of the other ponds, to the point where only about half of its surface remained by 1st May before somewhat filling up again by mid-May 2015. Its water level thus appears to fluctuate significantly, which may make it less suitable for amphibians.

8. Recommendations

Based on the desk study and field survey, the presence of Great Crested Newts at the Douglas West & Dalquhandy DP site is unlikely and colonization from the surrounding area also looks unlikely. No further survey is therefore proposed.

Given the presence of other amphibians - Smooth/Palmate Newts, Common Frogs and Common Toads -, all reasonable measures should be taken to avoid causing harm to these species during construction activities.

An opportunity for biodiversity enhancement exists in the form of additional pond creation for amphibians within 250 m of pond 15 to the north of the Poniel Water, outside the site boundary.

9. References

ARG-UK (2008) *Amphibian Disease Precautions: A Guide for UK Fieldworkers*. Amphibian and Reptile Groups of the United Kingdom Advice Note 4.

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DES (2014) *Great Crested Newt Habitat Suitability Assessment 2014 of the Proposed Douglas West & Dalquhandy DP Renewable Energy Project, South Lanarkshire*. Unpublished report by DES to 3R Energy.

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Sellars, C. (2010) Habitat Suitability Index Scores as an Indicator of the Presence of Great Crested Newts. *In Practice*, **69** (September), 22-24.

Appendix 1 - Field water sample collection protocol (from Biggs *et al.*, 2014b)

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.

Appendix 2 - Fera lab DNA analysis report of 29th April 2015

DNA ANALYSIS REPORT Commercial in Confidence

Customer:	Findlay Ecology Services
Address:	2 Blakelaw Farm Cottages Kelso TD5 8PB United Kingdom
Contact:	**Melanie Findlay**
Email:	melanie.ingledew@btconnect.com
Tel:	07909524202
Report date:	22 May 2015
Order Number:	GCN089
Samples:	Pond Water
Analysis Requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., *et al*, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:
Positive: DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA ANALYSIS REPORT
Commercial in Confidence

Results:

Customer Reference	Fera Sample Ref.	GCN Detection	GCN Score	Inhibition	Degradation
Pond 6 Douglas West Site	S15-050233	Negative	0	No	No
Pond 9 Douglas West Site	S15-050234	Negative	0	No	No
Pond 8 Douglas West Site	S15-050235	Negative	0	No	No
Pond 10 Douglas West Site	S15-050236	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the four samples submitted. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs, and was performed under the conditions of licensing arrangements with Applied Biosystems and patent rights owned by F. Hoffman-La Roche Ltd.

Issuing officer: Steven Bryce
Tel: 01904 462 324
Email: e-dna@fera.co.uk

Appendix 3 - Fera lab DNA analysis report of 22nd May 2015

DNA ANALYSIS REPORT Commercial in Confidence

Customer:	3R Energy Solutions Ltd
Address:	Lanark Auction Market Hyndford Road Lanark ML11 9AX
Contact:	**Alison Hannah** **Dunnock Environmental Services**
Email:	alisonhannah@live.co.uk
Tel:	01382 330 486
Report date:	29 April 2015
Order Number:	GCN012
Samples:	Pond Water
Analysis Requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., *et al*, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:
Positive: DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

Results:

Customer Reference	Fera Sample Ref.	GCN Detection	GCN Score	Inhibition	Degradation
DW12 17/04/2015 AT	S15-049718	Negative	0	No	No
DW13 17/04/15 AT	S15-049719	Negative	0	No	No
-	S15-049720	Inconclusive	0	No	No
DW # 15 17/04/15 AT	S15-049721	Negative	0	No	No
-	S15-049722	Inconclusive	0	No	No
-	S15-049723	Inconclusive	0	No	No
-	S15-049724	Inconclusive	0	No	No
DW 11 AT 17/04/2015	S15-049725	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the eight samples submitted. However, four samples (S15-049720, S15-049722, S15-049723 and S15-049724) were received at the lab with the tubes filled to the 45ml mark. Under such circumstances we cannot rule out under-sampling and the risk of a false negative result that this carries. Therefore, we have issued an inconclusive result for these four samples. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs, and was performed under the conditions of licensing arrangements with Applied Biosystems and patent rights owned by F. Hoffman-La Roche Ltd.

Issuing officer: Steven Bryce
Tel: 01904 462 324
Email: e-dna@fera.co.uk

279000 280000 281000 282000 283000 284000 285000

635000 634000 633000 632000 631000

Opencast Workings

Workings (disused)

Coalburn

Bellfield

Netherfield

Nethertown

Muttown

Crowhill

Happendon Wood

Happendon

Douglas West

Douglas

Long Plantation

Common Hill

Henry's Hill

Wind Farm

17 18 19 20 21 16 14 15 13 12 11 10 9 25 26 1 2 3 4 5 7 8 6 24 23 22

Legend

- Site Boundary
- 1 Existing Pond
- 4 Pond No Longer Exists
- Pond surveyed for e-DNA and presence/absence of Great Crested Newts

3R Energy
Wind • Biomass

Lanark Auction Market, Hyndford Road,
Lanark, ML11 9AX
T : 01555 660244
W : www.3renergy.co.uk

PROJECT:	Douglas West and Dalquhandy DP Renewable Energy Project.
TITLE:	Figure 1 - Great Crested Newt e-DNA and presence/absence surveys
SITE CO-ORD:	Centre of Site: E282346 N632612

DRAWN BY: SP	SCALE: 1:17,500	DATE: 07/05/2015
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