NORTH FALLS

Offshore Wind Farm

ENVIRONMENTAL STATEMENT

Appendix 23.2 Great Crested Newt eDNA Survey Report

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NORTH FALLS
Offshore Wind Farm

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ECOLOGY
RESOURCES

eDNA Great Crested Newt Results Report

North Falls Offshore Wind Farm Ltd

August 2022

ECOLOGY | ARBORICULTURE

Status	Name	Date
Draft	Jo Dent BSc (Hons) PGDip QCIEEM	22/07/2022
Rev 1	Gavin Mullan (BA) Hons MCIEEM	19/08/2022
Rev 2	Gavin Mullan (BA) Hons MCIEEM	06/09/2022

EXECUTIVE SUMMARY

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of North Falls Offshore Wind Farm Limited, to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling of suitable standing water bodies within the onshore project area plus a 250m buffer.

In total 123 standing water bodies were identified as having potential for great crested newts, three of which were found incidentally during site walkovers.

A total of 95 HSI assessments and eDNA sampling surveys were completed great crested newt survey area, 13 of which returned positive eDNA results confirming great crested newt presence. The outstanding 28 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions or were unsuitable at time of survey.

Contents

1. INTRODUCTION

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of North Falls Offshore Wind Farm Limited , to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling of suitable standing water bodies within the onshore project area plus a 250m buffer (herein 'the great crested newt survey area').

1.1 Project Background

North Falls Offshore Wind Farm (herein North Falls or 'the project') is a proposed extension to the operational Greater Gabbard Offshore Wind Farm (GGOW), which is located off the east coast of England in the Southern North Sea and was opened in 2013. North Falls is located to the west of the existing GGOW and at its closest point is approximately 22km offshore. The wind farm is being developed by North Falls Offshore Wind Farm Limited , a joint venture between SSE Renewables and RWE.

North Falls is currently awaiting a formal grid connection offer from National Grid. Whilst this process is ongoing, in order to ensure that adequate baseline data is collected to inform the Environmental Impact Assessment (EIA), North Falls has progressed with site selection of the project's onshore infrastructure (landfall location, onshore cable route and onshore substation location) at risk. The outputs of North Falls site selection process have then been used to generate a study area for the purposes of undertaking a suite of ecological surveys during 2021 and 2022 so that baseline data for the project can be gathered. This is referred to herein as the 'onshore project area'.

An Extended Phase 1 Habitat Survey of the onshore project area was undertaken between April and October 2021, the findings of which were used to inform the scope of further 'Phase 2' ecology surveys required in 2022 to inform the project's Ecological Impact Assessment (EcIA) in support of its Development Consent Order (DCO) application.

This report details the scope, methodology and findings of a great crested newt HSI and eDNA survey, which forms part of this suite of Phase 2 surveys.

1.2 Legislation

Great crested newts are a European protected species, as such they are afforded a high level of protection. The animals and their eggs, breeding sites and resting places are protected by law. Great crested newts are fully protected under the following UK legislation / international agreements: Bern Convention 1979: Appendix II Strictly Protected Fauna Species, Wildlife & Countryside Act (as amended) 1981, The Conservation of Habitats and Species Regulations 2017 (The Conservation of Habitats and Species Regulations 2017 transposes into UK law the EU Habitats Directive Council Directive 92/43/EEC) and Countryside Rights of Way Act 2000 (CRoW 2000). Protection under these laws makes it an offence to: -

- intentionally kill, injure, or capture, or take great crested newts
- deliberately take or destroy eggs of great crested newts
- possess or control alive or dead great crested newt or any part or thing derived from them,
- intentionally or recklessly
- damage, destroy, or obstruct access to, any structure or place which great crested newts use for shelter or protection, intentionally or recklessly
- disturb great crested newts while occupying a structure or place which it uses for that purpose

- sell, offer, or expose for sale, or possess or transport for the purpose of sale, any live or dead great crested newt or any part or thing derived from them. It is also an offence to publish or cause to be published any advertisement likely to be understood as conveying that great crested newts, or parts or derived things of them are bought, sold, or are intended to be. This applies to all stages in their life cycle
- damage or destroy a breeding site or resting place
- keep, or transport, or exchange great crested newts or any part or thing derived from them.

2.0 METHODOLOGY

The great crested newt eDNA survey was completed in accordance with Natural England's *Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA* (Biggs et al., 2014). Each of the suitable standing water bodies were visited once and eDNA samples taken following the field protocol set out in Biggs et al. (2014).

In total 123 standing water bodies were identified as having potential for great crested newts, three of which were found incidentally during site walkovers (i.e. not part of the original scope). A total of 95 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area.

2.1 Habitat Suitability Index

The HSI for great crested newts is a measure of habitat suitability, where water bodies are assessed and scored on the following ten key variables which are known to influence breeding populations of great crested newts, in accordance with standard methods (ARG UK, 2010):

- geographic location
- water body area
- water body permanence
- water quality
- water body shading
- presence/impact of waterfowl
- fish stocks
- number of water bodies within 1km
- terrestrial habitat around the water body; and
- macrophyte cover of the water body

Table 1: Water body suitability category based on HSI score

HSI Score	Water body Suitability
<0.5	Poor
0.5 – 0.59	Below Average
0.6 – 0.69	Average
0.7- 0.79	Good

2.2 eDNA Water Sampling

The eDNA sampling collection was by led Johnnie Johnson, Great Crested Newt Level 1 licence holder (2018-33728-CLS-CLS). Each of the suitable water bodies was visited once, and all standing water bodies were subject to eDNA analysis irrespective of their HSI score. The samples were taken following the field protocol set out in Biggs et al. (2014). Visits were made between late-April and mid-June, during the newt breeding season (survey dates provided in Table 3 page 8.)

Samples underwent laboratory analysis by Surescreen and followed the laboratory protocol detailed in Biggs et al. (2014). The laboratory testing provided a 'presence / likely absence' result for each standing water body. The laboratory testing provided a 'presence / likely absence' result for each standing water body, shown in Appendix B.

2.3 Survey Limitations

Most of the standing water bodies were surveyed and assessed without any limitations to survey, however 28 standing water bodies were not surveyed due to restricted land access (11 water bodies) and water body unsuitability i.e., dry at time of survey (17 water bodies). Table 2 page 7 identifies the reasons water bodies were not surveyed.

Table 2: Standing Water Bodies not surveyed

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO32	Photograph unavailable	Unable to reach pond due to dense vegetation	163; spins.instance.influence
PO146		Unsuitable – Pond was mostly dry at time of survey	703, 707; tummy.kickbacks.newsreel
PO33		Unsuitable – Ditch was dry at time of survey and dense vegetation	176; landed.moth.hound

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO70		Unsuitable – Pond was dry at time of survey	93; larger.tested.plants
PO71		Unsuitable – Pond was dry at time of survey	93; rugs.became.aura

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO72		Unsuitable – Pond was dry at time of survey	93; commented.relate.rang
PO73		Unsuitable – Pond was dry at time of survey	93; divide.fund.clear

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO77		Unsuitable – Pond was dry at time of survey	90; composed.patrolled.gold
PO80	Photograph unavailable	Restricted access - Locked gate, with caravans present on site preventing access.	80; lyricism.cake.releasing

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO97		Unsuitable – Pond was dry at time of survey	332; menu.excuse.newlyweds
PO110		Unsuitable – Pond was dry at time of survey	235, 246; landscape.zealous.stubbed

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO121		Unsuitable– swimming pool	205; begins.shopper.amplified
PO122		Unsuitable – Pond was dry at time of survey	1112; objective.fizzle.belonging
PO126	Photograph unavailable	Unsuitable – Pond was dry at time of survey	985; marathons.discount.attends

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO133		Unsuitable – Pond was dry at time of survey	1265; cadet.prayers.amends
PO144		Unsuitable – Ditch dry present at time of survey	784; author.strapped.scribbled

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
PO148		Unsuitable – Pond was dry at time of survey	611; agency.shelter.aviators
PO60		Large waterbody, only around 40% of edge accessed	93; value.still.same

Water body ID	Photograph	Constraint	Land Parcel Number and What3Words
			
PO75	Photograph unavailable	. Four attempts made at gaining landowner access permission, but this was unsuccessful.	110; culling.marketing.rungs
PO90	Photograph unavailable	Five attempts made at gaining landowner access permission, but this was unsuccessful.	351; majoring.sits.shook
PO91	Photograph unavailable	Five attempts made at landowner access permission, but this was unsuccessful.	1367; prices.saddens.offstage
PO95	Photograph unavailable	Four attempts made at gaining landowner access permission, but this was unsuccessful.	337; coast.beauty.cashew
PO123	Photograph unavailable	Six attempts made at gaining landowner access permission, but this was unsuccessful.	1013; care.greed.dominate
PO136	Photograph unavailable	Four attempts made at gaining landowner access permission, but this was unsuccessful.	818; layers.skillet.probing
PO140	Photograph unavailable	Two attempts made at gaining landowner access permission, but this was unsuccessful.	682; comedy.date.slim
PO179	Photograph unavailable	Seven attempts made at gaining landowner access permission, but this was unsuccessful.	1254; shredder.transmits.pizzeria
PO196	Photograph unavailable	Two attempts made at gaining landowner access permission, but this was unsuccessful.	392; cuff.funded.ambushes
INC03	Photograph unavailable	Three attempts made at gaining landowner access permission, but this was unsuccessful.	356; crew.gains.smuggled

3.0 RESULTS

In total 123 standing water bodies were identified within the great crested newt survey area, three of which were identified as incidental finds during site walkovers.

Twenty-eight of the standing water bodies were either unsuitable or inaccessible for reasons identified in Table 2: Water Bodies not surveyed, Page 7. As a result, these were not subject to HSI assessments and eDNA sampling.

A total of 95 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 13 of which returned positive eDNA results confirming great crested newt presence. Those water bodies which returned positive eDNA results are detailed in Table 3. The full eDNA results are shown in Appendix A.

Table 3: Water body HSI and Positive eDNA Results

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO60		TM 20262 19254	0.5	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO65		TM 20193 19342	0.58	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
PO67		TM 20121 19408	0.57	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022

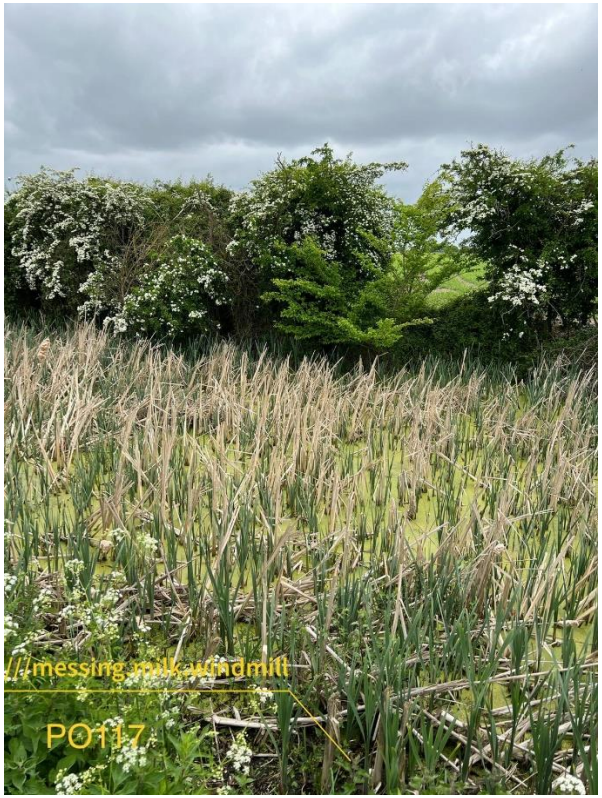
Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO68		TM 20182 19480	0.49	Poor	Positive	HSI – 20/09/2022 eDNA - 08/06/2022

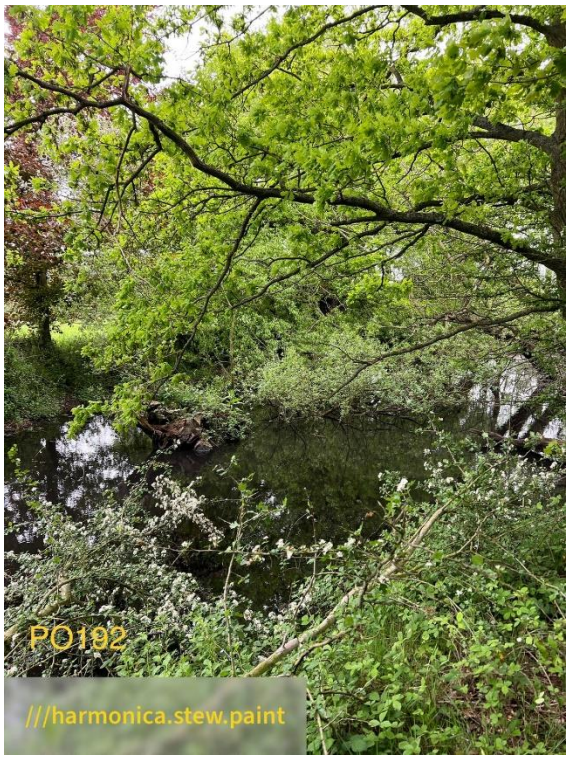
Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO69		TM 20202 19503	0.53	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
PO82	HSI completed by Royal HaskoningDHV prior to the eDNA surveys (Royal HaskoningDHV, 2022) – no photograph available	TM 19080 22018	0.70	Good	Positive	09/06/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO84		TM 19728 22526	0.72	Good	Positive	HSI – 13/10/22 eDNA - 10/05/2022
PO99		TM 16475 23393	0.81	Excellent	Positive	HSI – 23/09/2022 eDNA - 12/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 101		TM 16161 23542	0.63	Average	Positive	HSI – 12/40/21 eDNA - 11/05/2022
PO 102		TM 16082 23551	0.80	Excellent	Positive	HSI – 24/09/2021 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 103		TM 16098 23862	0.72	Good	Positive	11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 117		TM 15438 25475	0.65	Average	Positive	10/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 192		TM 15211 26033	0.78	Good	Positive	11/05/2022

4.0 CONCLUSION

In total 123 standing water bodies were identified as having potential for great crested newts, three of which were found incidentally during site walkovers.

A total of 95 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 13 of which returned positive eDNA results confirming great crested newt presence. The outstanding 28 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions (11 waterbodies) or were unsuitable (17 waterbodies) at time of survey.

5.0 REFERENCES

ARG UK Advice Note 5, (2010)

Biggs et al., (2014). Natural England's Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA

Oldham, Keeble, Swan, & Jeffcote, (2000). Evaluating the suitability of habitat for the great crested newt (Triturus cristatus) British Herpetological Society, pp.143-155

Royal HaskoningDHV, (2022). North Falls Extended Phase 1 Habitat Survey Report

LEGEND

- eDNA Negative; GCN Absent
- eDNA Positive; GCN Present
- No Access Granted
- eDNA Not Complete
- Onshore plus 50m
- 250m Buffer

GREAT CRESTED NEWT WATERBODIES FIGURE 1

PROJECT TITLE:
North Falls Offshore Wind Farm

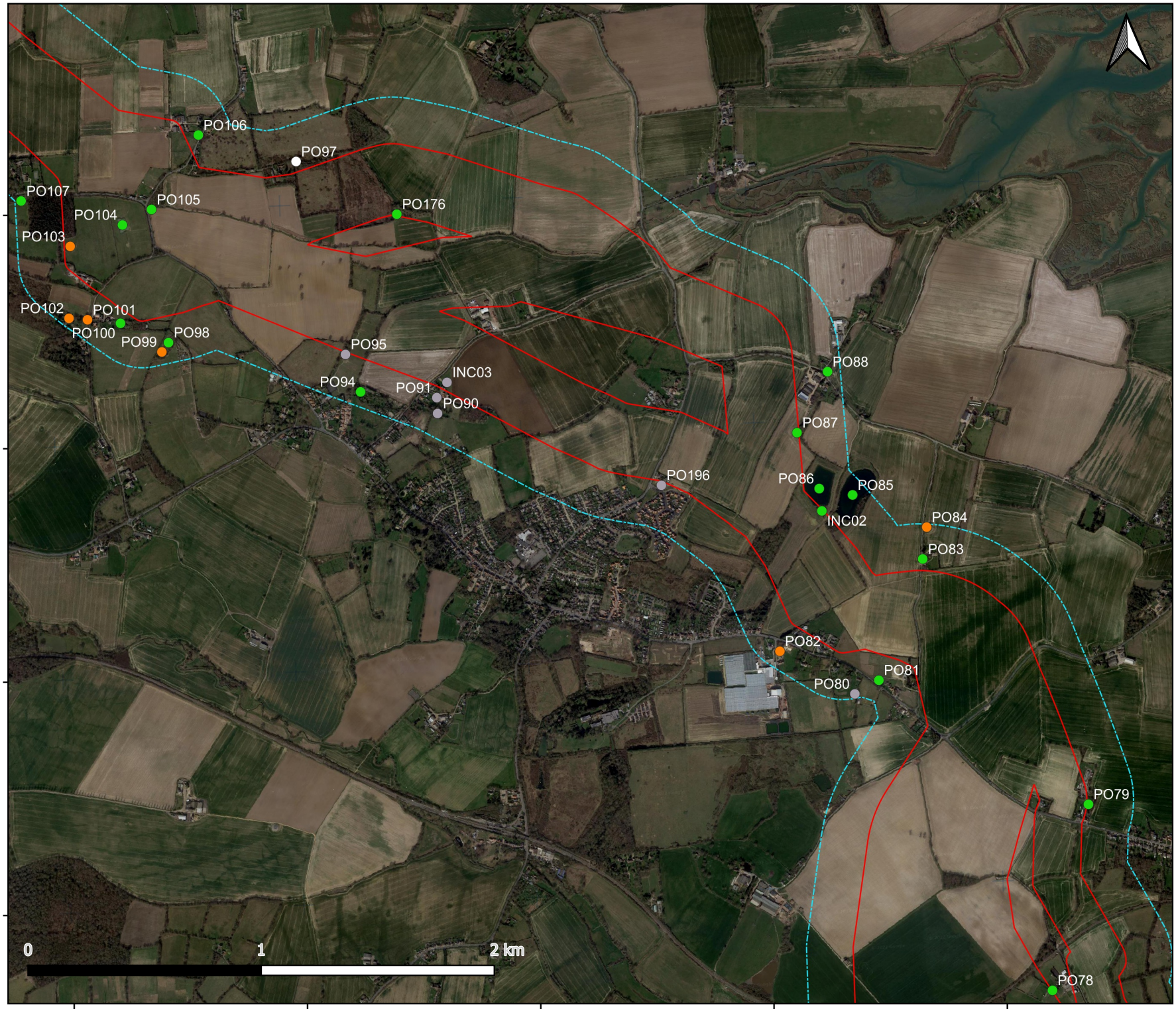
CLIENT:
Royal HaskoningDHV

DATE:
14.09.2022

PRODUCED BY:
J. McMahon

REF: 22042

ECOLOGY RESOURCES



LEGEND

- eDNA Negative; GCN Absent
- eDNA Positive; GCN Present
- No Access Granted
- eDNA Not Complete
- Onshore plus 50m
- 250m Buffer

GREAT CRESTED NEWT WATERBODIES
FIGURE 2

PROJECT TITLE:
North Falls Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
14.09.2022

PRODUCED BY:
J. McMahon

REF: 22042

ECOLOGY RESOURCES

LEGEND

- eDNA Negative; GCN Absent
- eDNA Positive; GCN Present
- No Access Granted
- eDNA Not Complete
- Onshore plus 50m
- 250m Buffer

GREAT CRESTED NEWT WATERBODIES
FIGURE 3

PROJECT TITLE:
North Falls Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
14.09.2022

PRODUCED BY:
J. McMahon

REF: 22042

ECOLOGY RESOURCES

LEGEND

- eDNA Negative; GCN Absent
- No Access Granted
- eDNA Not Complete
- Onshore plus 50m
- 250m Buffer

GREAT CRESTED NEWT WATERBODIES
FIGURE 4

PROJECT TITLE:
North Falls Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
14.09.2022

PRODUCED BY:
J. McMahon

REF: 22042

ECOLOGY RESOURCES

APPENDIX A: All Standing Water Body Results

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO01		TM 23238 18578	1	1	0.67	1	1	1	0.65	0.67	0.8	1	0.86	Excellent	Negative	17/05/222
PO02		TM 23060 18351	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/222

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO03		TM 23063 18339	1	0.9154	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/22
PO04	No photograph available	TM 23029 18319	1	0.9154	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/10/21 17/05/22
PO05	No photograph available	TM 23022 18306	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22
PO06	No photograph available	TM 23034 18306	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22
PO07	No photograph available	TM 23027 18300	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22
PO08	No photograph available	TM 22935 18244	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22
PO09	No photograph available	TM 22920 18228	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22
PO10	No photograph available	TM 15652 24379	1	0.9154	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO11		TM 22872 18209	1	0.2	0.5	0.67	1	1	0.67	0.9	0.67	0.9	0.68	Average	Negative	17/05/22
PO12		TM 22873 18193	1	0.1	1	0.67	1	1	0.67	0.65	0.33	0.9	0.62	Average	Negative	17/05/22
PO13	No photograph available	TM 22869 18185	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/22
PO14	No photograph available	TM 61544 22507	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22
PO15	No photograph available	TM 62286 21818	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22
PO16	No photograph available	TM 22859 18183	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22
PO17	No photograph available	TM 22864 18176	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	174/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO18		TM 22798 18127	1	0.7	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.76	Good	Negative	17/05/22
PO19		TM 22718 18053	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO20		TM 22708 18046	1	1	0.5	0.67	1	1	0.67	0.7	0.67	1	0.79	Excellent	Negative	17/05/22
PO21		TM 22622 17966	1	0.2	1	0.67	1	1	0.67	0.7	0.67	1	0.72	Good	Negative	17/05/22
PO22		TM 22589 17929	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/22
PO23		TM 22575 17920	1	0.3	0.5	0.67	1	0.67	0.67	0.8	0.67	0.9	0.68	Average	Negative	17/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO24		TM 22557 17918	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/22
PO25		TM 22558 17910	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/22
PO26	No photograph available	TM 22334 17693	1	0.8001	1	0.67	1	0.67	0.67	1	0.67	0.9	0.82	Excellent	Negative	18/05/22
PO27		TM 21924 17653	1	0.1	0.1	0.33	1	0.67	1	0.7	1	0.95	0.52	Below average	Negative	20/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO28		TM 21916 17582	1	0.6	1	0.67	1	0.67	0.67	0.7	0.67	0.5	0.72	Good	Negative	20/05/22
PO29	No photograph available	TM 21768 17568	1	0	1	0.67	1	0.67	0.67	0.95	0.67	0.3	0.72	Good	Negative	18/05/22
PO34	No photograph available	TM 21136 18788	1	0.2	1	0.67	0.6	0.67	0.67	0.65	0.67	0.3	0.58	Below average	Negative	16/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO35		TM 21174 18656	1	0.2	0.9	1	1	0.01	0.01	0.9	1	0.6	0.31	Poor	Negative	06/06/22
PO36	No photograph available	TM 20916 18505	1	0.877	0.9	0.67	1	0.67	0.67	0.72	0.67	0.4	0.73	Good	Negative	16/05/22
PO37		TM 20452 18437	1	0.8001	0.9	0.67	1	0.67	0.67	0.69	0.67	0.35	0.71	Good	Negative	16/05/22

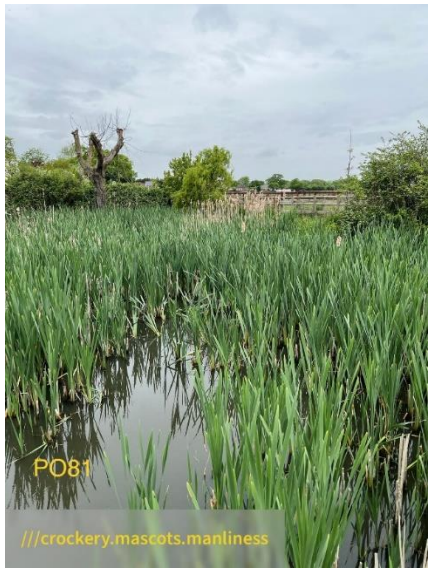
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO59		TM 20329 19152	1	0.05	0.90	0.67	1.00	0.67	0.67	1	1.00	0.50	0.74	Average	Negative	16/05/22
PO60		TM 20262 19254	1	0.00	0.90	0.33	0.70	0.01	0.67	1	1.00	0.35	0.50	Below Average	Positive	16/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO64		TM 20225 19307	1	1.00	0.10	0.33	1.00	0.67	1.00	1	1.00	0.40	0.62	Average	Negative	16/05/22
PO65		TM 20193 19342	1	0.21	0.90	0.33	0.50	0.67	0.67	1	1.00	0.30	0.58	Below Average	Positive	16/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO66		TM 20149 19368	1	0.4	1	1	0.7	1	1	0.95	1	0.9	0.86	Excellent	Negative	17/05/22
PO67		TM 20121 19408	1	0.50	0.10	0.33	1.00	0.67	1.00	1	1.00	0.35	0.57	Below Average	Positive	16/05/22

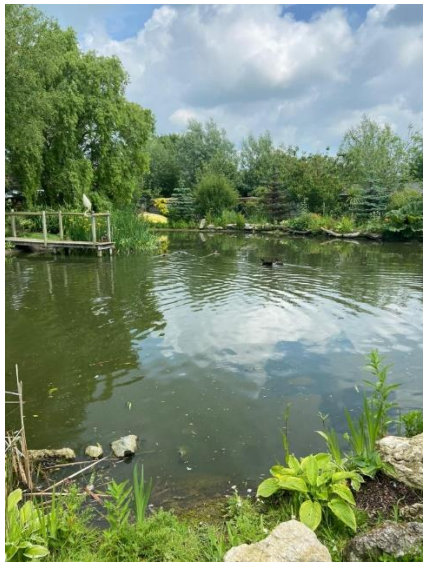
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO68		TM 20182 19480	1	0.1	0.1	0.33	1	1	1	0.65	0.33	1	0.49	Poor	Positive	08/06/22
PO69		TM 20202 19503	1	0.21	0.10	0.33	0.60	0.67	0.67	1	1.00	0.90	0.53	Below Average	Positive	16/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO76		TM 20614 20151	1	0.1	0.9	0.67	1	1	0.67	0.65	0.67	0.35	0.60	Average	Negative	29/06/22
PO78	No photograph available	TM 20198 20524	1	0.2	0.9	1	1	0.01	0.01	0.95	0.67	0.6	0.30	Poor	Negative	07/06/22
PO79		TM 20381 21316	1	0.5	0.9	0.67	1	0.67	0.67	0.66	0.67	0.4	0.68	Average	Negative	06/06/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO81		TM 19500 21879	1	0.3	0.5	0.67	1	1	1	0.9	0.67	0.5	0.70	Good	Negative	10/05/22
PO82	No photograph available	TM 19080 22018	1	0.3	1	0.67	1	1	0.67	0.8	0.67	0.45	0.70	Good	Positive	09/06/22
PO83		TM 19706 22392	1	0.8001	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.69	Average	Negative	10/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO84		TM 19728 22526	1	0.80	0.90	0.33	0.60	0.67	0.67	0.85	0.67	1.00	0.72	Good	Positive	10/05/22
PO85		TM 19415 22677	1	0.00	0.90	0.67	1.00	0.67	0.67	0.9	1.00	0.35	0.80	Excellent	Negative	10/05/22
PO86		TM 19273 22709	1	0.00	0.90	1.00	1.00	0.67	0.67	0.93	1.00	0.40	0.84	Excellent	Negative	10/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO87		TM 19189 22953	1	0.90	0.90	0.33	1.00	0.67	0.67	0.85	0.67	0.30	0.68	Average	Negative	10/05/22
PO88		TM 19326 23209	1	0.70	0.90	0.33	1.00	0.67	0.67	0.83	0.67	0.35	0.67	N/A	Negative	10/05/22

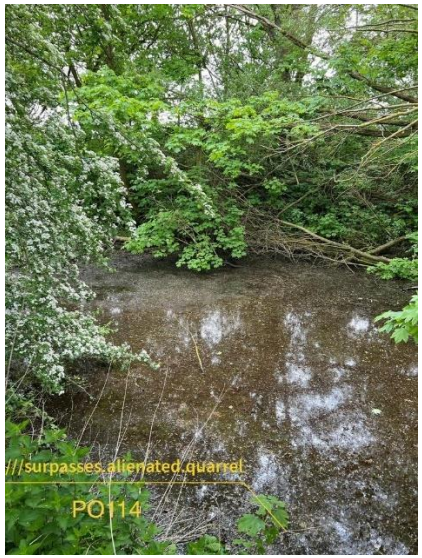
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO94		TM 17317 23195	1	0.3	0.9	1	1	0.01	0.01	0.9	0.67	0.7	0.32	Poor	Negative	08/06/22
PO98		TM 16505 23432	1	0.98	0.90	0.67	1.00	0.01	0.67	0.96	1.00	0.50	0.53	Below Average	Negative	12/05/22

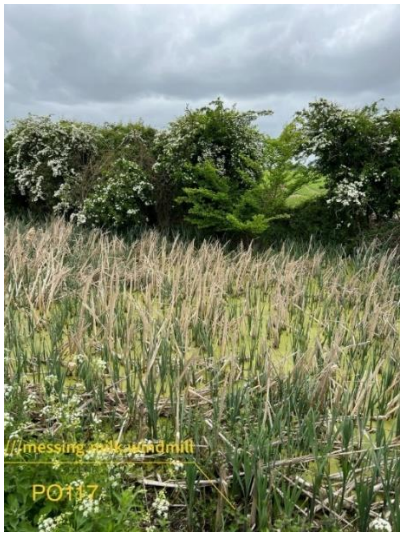
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO99		TM 16475 23393	1	1.00	0.90	0.67	0.60	0.67	0.67	0.98	1.00	0.80	0.81	Excellent	Positive	12/05/22
PO 100		TM 16303 23523	1	1.10	0.90	0.33	1.00	0.67	0.33	0.93	1.00	0.45	0.70	Good	Negative	11/05/22
PO 101		TM 16161 23542	1	0.21	0.90	0.67	0.60	0.67	0.67	0.9	1.00	0.30	0.63	Average	Positive	11/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 102		TM 16082 23551	1	0.98	0.90	0.67	1.00	0.67	0.67	0.96	1.00	0.40	0.80	Excellent	Positive	24/09/21 11/05/22
PO 103		TM 16098 23862	1	0.3	0.5	0.33	1	0.67	1	0.92	0.67	0.35	0.61	Average	Positive	11/05/22
PO 104		TM 16325 23943	1	0.83	0.90	0.33	1.00	0.67	1.00	0.93	1.00	0.30	0.73	Good	Negative	12/10/21 16/06/22

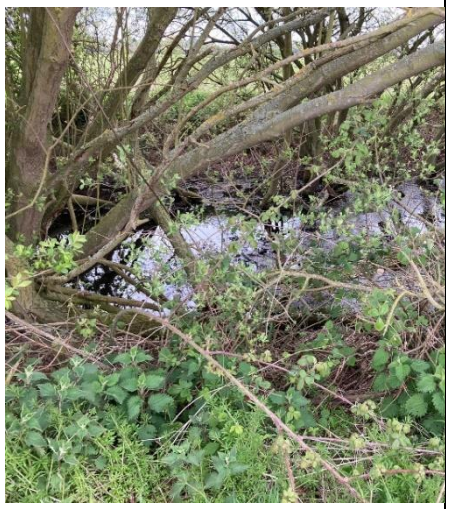
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 105	No photograph available	TM 16452 24005	1	0.31	0.90	0.33	0.20	0.67	0.67	0.76	1.00	0.35	0.54	Average	Negative	27/09/21 26/04/22
PO 106	No photograph available	TM 16664 24317	1	0.60	0.90	0.33	1.00	0.67	0.67	0.69	0.67	0.30	0.64	Average	Negative	27/09/21 06/05/22
PO 107		TM 15894 24061	1	0.1	0.5	0.67	1	1	1	0.55	1	1	0.67	Average	Negative	26/04/22
PO 112	No photograph available	TM 15709 25239	1	0	0.9	1	1	0.67	0.67	0.65	1	0.3	0.75	Good	Negative	26/04/22

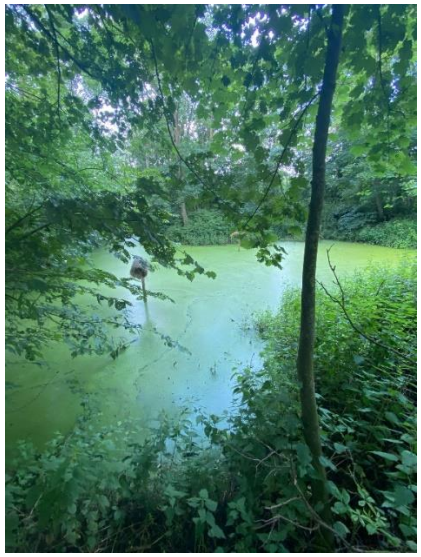
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 113		TM 15557 25153	1	0.88 47	1	0.33	0.6	0.67	0.67	0.7	1	0.4	0.68	Average	Negative	12/07/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 114		TM 15442 25077	1	0.95 39	0.5	0.33	0.6	1	1	0.65	0.67	0.35	0.65	Average	Negative	10/05/22
PO 115	No photograph available	TM 15030 24861	1	0.2	0.5	0.33	1	0.67	1	0.65	1	0.95	0.65	Average	Negative	26/04/22
PO 116		TM 14887 24994	1	0	0.9	0.67	1	0.67	0.67	0.75	0.67	0.4	0.72	Good	Negative	26/04/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 117		TM 15438 25475	1	0.2	0.5	0.33	1	1	1	0.65	0.67	0.9	0.65	Average	Positive	10/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 118		TM 14691 25342	1	0.00	0.90	0.67	1.00	0.67	0.01	0.85	1.00	0.30	0.54	Below Average	Negative	26/04/22
PO 120		TM 15022 25938	1	N/A	0.90	0.67	1.00	0.67	0.33	0.85	0.33	0.50	0.70	Good	Negative	26/04/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 124		TM 14102 26511	1	0.92	0.50	0.33	0.20	0.67	0.67	0.9	0.33	0.50	0.54	Below Average	Negative	26/04/22
PO 125		TM 14217 26683	1	0.2	0.9	0.67	1	0.67	1	0.69	0.67	0.35	0.64	Average	Negative	12/05/22
PO 127	No photograph available	TM 13093 27091	1	0.60	0.90	0.67	1.00	0.67	0.67	0.58	1.00	0.35	0.71	Good	Negative	28/04/22
PO 128	No photograph available	TM 12555 28100	1	0.31	0.90	0.33	1.00	0.67	0.67	0.58	0.33	0.40	0.56	Below Average	Negative	28/04/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 129	No photograph available	TM 13255 28265	1	0.31	0.90	0.33	0.20	0.67	0.67	0.76	0.67	0.30	0.51	Below Average	Negative	28/04/22
PO 130	No photograph available	TM 13137 29295	1	0	0.9	1	1	0.67	0.33	0.65	0.67	0.4	0.69	Average	Negative	28/04/22
PO 131	No photograph available	TM 13205 29298	1	0.5	1	1	0.4	0.67	1	0.65	0.33	0.45	0.65	Average	Negative	28/04/22
PO 132	No photograph available	TM 13265 29325	1	0.00	0.90	0.67	1.00	0.67	0.67	0.69	0.67	0.30	0.74	Poor	Negative	28/09/21 28/04/22
PO 134		TM 12275 29994	1	1	0.9	0.67	0.4	0.67	0.67	0.65	1	0.7	0.74	Good	Negative	09/06/22

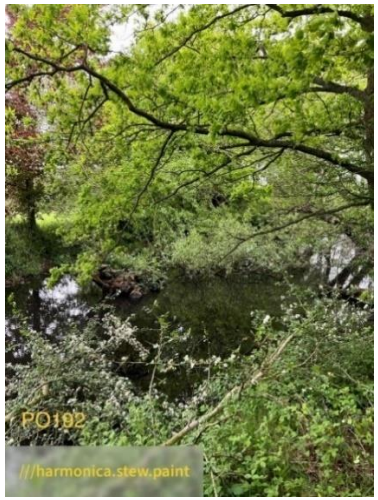
Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 135		TM 11336 29737	1	0.2	0.9	1	1	0.01	0.01	0.7	1	0.6	0.30	Poor	Negative	06/09/22
PO 138		TM 10198 30183	1	0.1	1	0.67	0.8	1	1	0.43	0.33	0.35	0.55	Below average	Negative	27/04/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 141		TM 10858 28469	1	0	0.9	1	1	0.67	0.67	0.65	0.67	0.4	N/A	N/A	Negative	11/05/22
PO 142		TM 11851 27529	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.6	0.75	Good	Negative	11/05/22
PO 143	No photograph available	TM 11123 27625	1	1.10	0.90	0.67	1.00	0.67	0.67	0.66	1.00	0.70	0.82	Excellent	Negative	08/10/21 28/04/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 147		TM 09673 27216	1	0.1	0.1	0.33	1	1	1	0.47	0.67	0.35	0.45	Poor	Negative	18/05/22
PO 174	No photograph available	TM 22864 18176	1	0.8001	0.9	0.67	1	0.67	0.67	1	0.67	0.5	0.76	Good	Negative	14/06/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 176		TM 17503 23948	1	0.50	0.90	0.33	1.00	0.67	0.67	0.96	1.00	0.90	0.75	Good	Negative	07/06/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 178		TM 11446 29778	1	0.3	0.9	1	1	0.67	0.01	0.8	0.67	0.4	0.45	Poor	Negative	06/06/22
PO 183		TM 11680 27882	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.71	Good	Negative	11/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 192		TM 15211 26033	1	0.8	1	0.67	1	0.67	0.67	0.89	1	0.4	0.78	Good	Positive	11/05/22
PO 193		TM 15175 26030	1	0.1	0.1	0.33	1	0.67	1	0.88	1	0.7	0.51	Below average	Negative	12/05/22

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO 195		TM 11643 28069	1	0.8154	1	0.67	1	0.67	0.67	0.7	0.67	0.35	0.72	Good	Negative	11/05/22
Inc01	No photograph available	TM 61498 22485	1	0.3	1	0.67	1	0.67	0.33	0.75	1	0.6	0.68	Average	Negative	26/04/22
Inc02	No photograph available	TM 61928, 22261	1	0.9	0.9	0.67	1	1	0.67	0.84	0.67	0.4	0.778583	Good	Negative	01/06/22

APPENDIX B: Surescreen Scientifics – eDNA Results

SureScreen Scientifics

Folio No: E13559
Report No: 1
Purchase Order: 173EM1204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 13/05/2022
Date Reported: 23/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1737	PO83 North Falls	TM 19706 22392	Pass	Pass	Pass	Negative	0
1738	PO84 North Falls	TM 19728 22526	Pass	Pass	Pass	Positive	1
1739	PO86 North Falls	TM 19273 22709	Pass	Pass	Pass	Negative	0
1740	PO85 North Falls	TM 19415 22677	Pass	Pass	Pass	Negative	0
1741	PO87 North Falls	TM 19189 22953	Pass	Pass	Pass	Negative	0
1742	PO88 North Falls	TM 19326 23209	Pass	Pass	Pass	Negative	0
1744	PO143 North Falls	TM 11123 27625	Pass	Pass	Pass	Negative	0

1747	PO124 North Falls	TM 14102 26511	Pass	Pass	Pass	Negative	0
1749	PO129 North Falls	TM 13255 28265	Pass	Pass	Pass	Negative	0
1751	PO128 North Falls	TM 12555 28100	Pass	Pass	Pass	Negative	0
1752	PO120 North Falls	TM 15022 25938	Pass	Pass	Pass	Negative	0
1753	PO118 North Falls	TM 14691 25342	Pass	Pass	Pass	Negative	0
1754	PO115 North Falls	TM 15030 24861	Pass	Pass	Pass	Negative	0
1756	PO105 North Falls	TM 16452 24005	Pass	Pass	Pass	Negative	0
1757	POIncol North Falls	614980 224854	Pass	Pass	Pass	Negative	0
1758	PO116 North Falls	TM 14887 24994	Pass	Pass	Pass	Negative	0
1759	PO107 North Falls	TM 15894 24061	Pass	Pass	Pass	Negative	0
1760	PO112 North Falls	TM 15709 25239	Pass	Pass	Pass	Negative	0
1857	PO106 North Falls	TM 16664 24317	Pass	Pass	Pass	Negative	0
1859	PO132 North Falls	TM 13265 29325	Pass	Pass	Pass	Negative	0
1860	PO138 North Falls	TM 10198 30183	Pass	Pass	Pass	Negative	0
1861	PO131 North Falls	TM 13205 29298	Pass	Pass	Pass	Negative	0
1862	PO130 North Falls	TM 13137 29295	Pass	Pass	Pass	Negative	0
1863	PO127 North Falls	TM 13093 27091	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Esther Strafford **Approved by:** Gabriela Danickova

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

SureScreen Scientifics

Folio No: E13850
Report No: 1
Purchase Order: 173EM1204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 27/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1745	PO98 North Falls	TM 16505 23432	Pass	Pass	Pass	Negative	0
1746	PO141 North Falls	TM 10858 28469	Pass	Pass	Pass	Negative	0
1761	PO101 North Falls	TM 16161 23542	Pass	Pass	Pass	Positive	4
1762	PO103 North Falls	TM 16098 23862	Pass	Pass	Pass	Positive	3
1764	PO102 North Falls	TM 16082 23551	Pass	Pass	Pass	Positive	10
1765	PO142 North Falls	TM 11851 27529	Pass	Pass	Pass	Negative	0
1766	PO183 North Falls	TM 11680 27882	Pass	Pass	Pass	Negative	0

1767	PO195 North Falls	TM 11643 28069	Pass	Pass	Pass	Negative	0
1768	PO100 North Falls	TM 16303 23523	Pass	Pass	Pass	Negative	0
1769	PO114 North Falls	TM 15442 25077	Pass	Pass	Pass	Negative	0
1770	PO113 North Falls	TM 15557 25153	Pass	Pass	Pass	Negative	0
1772	PO99 North Falls	TM 16475 23393	Pass	Pass	Pass	Positive	12
1774	PO193 North Falls	TM 15175 26030	Pass	Pass	Pass	Negative	0
1775	PO124 North Falls	TM 14102 26511	Pass	Pass	Pass	Negative	0
1781	PO117 North Falls	TM 15438 25475	Pass	Pass	Pass	Positive	1
1782	PO81 North Falls	TM 19500 21879	Pass	Pass	Pass	Negative	0
1812	PO18 North Falls	TM 22798 18127	Pass	Pass	Pass	Negative	0
1816	PO03 North Falls	TM 23063 18339	Pass	Pass	Pass	Negative	0
1817	PO11 North Falls	TM 22872 18209	Pass	Pass	Pass	Negative	0
1819	PO02 North Falls	TM 23060 18351	Pass	Pass	Pass	Negative	0
1822	PO01 North Falls	TM 23238 18578	Pass	Pass	Pass	Negative	0
1823	PO13-17 North Falls	TM 22869 18185	Pass	Pass	Pass	Negative	0
1824	PO12 North Falls	TM 22873 18193	Pass	Pass	Pass	Negative	0
1858	PO125 North Falls	TM 14217 26683	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Esther Strafford **Approved by:** Chris Troth

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

SureScreen Scientifics

Folio No: E13885
Report No: 1
Purchase Order: 173EM1204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 30/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1743	PO147 North Falls	TM 09673 27216	Pass	Pass	Pass	Negative	0
1771	PO192 North Falls	TM 15211 26033	Pass	Pass	Pass	Positive	1
1777	PO27 North Falls	TM 21924 17653	Pass	Pass	Pass	Negative	0
1783	PO28 North Falls	TM 21916 17582	Pass	Pass	Pass	Negative	0
1801	PO26 North Falls	TM 22334 17693	Pass	Pass	Pass	Negative	0
1806	PO29 North Falls	TM 21768 17568	Pass	Pass	Pass	Negative	0
1809	PO25 North Falls	TM 22558 17910	Pass	Pass	Pass	Negative	0

1810	PO19 North Falls	TM 22718 18053	Pass	Pass	Pass	Negative	0
1811	PO20 North Falls	TM 22708 18046	Pass	Pass	Pass	Negative	0
1813	PO22 North Falls	TM 22589 17929	Pass	Pass	Pass	Negative	0
1814	PO24 North Falls	TM 22557 17918	Pass	Pass	Pass	Negative	0
1815	PO23 North Falls	TM 22575 17920	Pass	Pass	Pass	Negative	0
1818	PO21 North Falls	TM 22622 17966	Pass	Pass	Pass	Negative	0
1820	PO37 North Falls	TM 20452 18437	Pass	Pass	Pass	Negative	0
1821	PO64 North Falls	TM 20225 19307	Pass	Pass	Pass	Negative	0
1825	PO69 North Falls	TM 20202 19503	Pass	Pass	Pass	Positive	12
1826	PO59 North Falls	TM 20329 19152	Pass	Pass	Pass	Negative	0
1827	PO67 North Falls	TM 20121 19408	Pass	Pass	Pass	Positive	9
1828	PO65 North Falls	TM 20193 19342	Pass	Pass	Pass	Positive	2
1829	PO36 North Falls	TM 20916 18505	Pass	Pass	Pass	Negative	0
1830	PO34 North Falls	TM 21136 18788	Pass	Pass	Pass	Negative	0
1831	PO60 North Falls	TM 20262 19254	Pass	Pass	Pass	Positive	7
1832	PO66 North Falls	TM 20149 19368	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Esther Strafford **Approved by:** Chris Troth

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

SureScreen Scientifics

Folio No: E14177
Report No: 1
Purchase Order: 173EM1204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 15/06/2022
Date Reported: 23/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1763	PO 068 North Falls	TM 20182 19480	Pass	Pass	Pass	Positive	1
1773	PO 176 North Falls	TM 17503 23948	Pass	Pass	Pass	Negative	0
1793	PO 078 North Falls	TM 20198 20524	Pass	Pass	Pass	Negative	0
1794	PO82 North Falls	TM 19080 22018	Pass	Pass	Pass	Positive	4
1796	PO 134 North Falls	TM 12275 29994	Pass	Pass	Pass	Negative	0

1797	PO135 North Falls	TM 11336 29737	Pass	Pass	Pass	Negative	0
1798	PO178 North Falls	TM 11446 29778	Pass	Pass	Pass	Negative	0
1799	PO94 North Falls	TM 17317 23195	Pass	Pass	Pass	Negative	0
1800	PO35 North Falls	TM 21174 18656	Pass	Pass	Pass	Negative	0
1803	PO79 North Falls	TM 20381 21316	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Esther Strafford **Approved by:** Chelsea Warner

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

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INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

SureScreen Scientifics

Folio No: E14893
Report No: 1
Purchase Order: 173EM/204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 05/07/2022
Date Reported: 19/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1787	PO 174	TM 22864 18176	Pass	Pass	Pass	Negative	0
1788	PO 104	TM 16325 23943	Pass	Pass	Pass	Negative	0
1791	PO 76	TM 20614 20151	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chelsea Warner **Approved by:** Chelsea Warner

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

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A joint venture company owned equally by SSE Renewables and RWE.

To contact please email contact@northfallsoffshore.com

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