



FIVE
ESTUARIES
OFFSHORE WIND FARM

FIVE ESTUARIES
OFFSHORE WIND FARM
PRELIMINARY ENVIRONMENTAL
INFORMATION REPORT

VOLUME 5, ANNEX 4.5: GREAT CRESTED
NEWT SURVEY REPORT: SOUTH OF A120

Document Reference 004685545-01
Revision A
Date August 2022



Project	Five Estuaries Offshore Wind Farm
Sub-Project or Package	Preliminary Environmental Information Report
Document Title	Volume 5, Annex 4.5: Great Crested Newt Survey Report: South of A120
Document Reference	004685545-01
Revision	A

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Revision	Date	Status/Reason for Issue	Originator	Checked	Approved
A	Aug-22	Final for PEIR	Ecology	GoBe	VE OWFL

In general, field survey data used to inform the Five Estuaries Offshore Wind Farm PEIR were gathered specifically for the Project. However, in instances where the North Falls Offshore Wind Farm Project held pertinent survey data and reports, these have been provided to the Five Estuaries Offshore Wind Farm Project for use in the PEIR.

This annex is an example of information that has been provided by the North Falls Offshore Wind Farm Project for use by the Five Estuaries Offshore Wind Farm Project. It should be noted that all relevant technical information is included in the Five Estuaries Offshore Wind Farm Project PEIR, regardless of initial source.



ECOLOGY
RESOURCES

**eDNA
Great Crested
Newt
Results Report**

**5 Estuaries
Offshore Wind
Farm Ltd**

August 2022



Status	Name	Date
Draft	Francesca Austin BSc (Hons) QCIEEM	20/10/2022
Rev 1	Gavin Mullan BA (Hons) MCIEEM	01/11/22
Rev 2	Gavin Mullan BA (Hons) MCIEEM	17/11/22
Rev 3		

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EXECUTIVE SUMMARY

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), 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 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions or were unsuitable at time of survey.

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1. INTRODUCTION

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling for great crested newt *Triturus cristatus* for suitable standing water bodies within the onshore project area plus a 250m buffer (herein ‘the great crested newt survey area’).

1.1 Project Background

Five Estuaries Offshore Windfarm (VE) is a proposed extension to the operational Galloper Offshore Wind Farm (OWF) which consists of 56 wind turbine generators (WTGs). The VE will comprise up to 79 WTGs situated within two array areas to the east of the operational Galloper OWF. The array areas will be located approximately 30km off the coast of Suffolk, England.

Cables will connect the turbines to the offshore substation platforms and then export the power generated to shore. It is expected that there will be a number of inter-array cables, up to four export cables and up to two offshore substations platforms.

A landfall area has been identified between Holland-on Sea and Frinton-on-Sea on the Essex coast. The landfall point is yet to be determined but will be located within this area of coastline. A new VE onshore substation will be needed and will be constructed in an area to the north of the A120.

The VE cables will be installed underground between the landfall and the grid connection point north of the A120. A preferred corridor has not yet been determined with several corridors still under consideration at the time of writing. Potential substation land parcels and associated corridor options north of the A120 also remain under review at the time of writing.

A more detailed description of the project, several elements of which have yet to be finalised at this time, will be provided in the PEIR and ES in due course.

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 breeding, 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;
- 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 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers (i.e. not part of the original scope). A total of 84 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 13.)



Samples underwent laboratory analysis by Surescreen Scientifics Ltd. 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 22 standing water bodies were not surveyed due to restricted land access (10 water bodies) and water body unsuitability i.e., dry at time of survey (12 water bodies). Table 2 page 7 identifies the reasons water bodies were not surveyed.



Table 2: Standing Water Bodies not Surveyed

Ponds labelled with PO preceding the number are also ponds within the North Falls survey area

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
1	Photograph unavailable	No pond present, does not exist	LPN 144; TM2113217604
12		No pond was present, waterbody was dry	LPN 93; TM2019219517
14		No pond was present, waterbody was dry	LPN 93; TM2020519520
21	Photograph unavailable	No access granted	LPN 46; TM1924321450
31	Photograph unavailable	No access granted	LPN 351; TM1764823088
32	Photograph unavailable	No access granted	LPN 352; TM1764723156

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
PO32	Photograph unavailable	No pond was present, waterbody was dry	LPN 163; TM2170117823
33	Photograph unavailable	No access granted	LPN 356; TM1769323220
PO33		No pond was present, waterbody was dry	LPN 176; TM2234318446
35	Photograph unavailable	No access granted	LPN 337; TM1726223355
58	Photograph unavailable	No access granted	LPN 1273; TM15481 4343
59	Photograph unavailable	No access granted	LPN 1273; TM1535224415

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
67		No pond was present, waterbody was dry	LPN 1112; TM1433026076
68	Photograph unavailable	No access granted	LPN 1013; TM1368026232
PO70		Waterbody is dry	LPN 93; TM2023219499

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
71	Photograph unavailable	Waterbody is dry	LPN 985; TM1339326865
PO77		No pond was present, waterbody was dry	LPN 90; TM1991920198
PO80	Photograph unavailable	No access granted	No LPN; TM1939521824
PO110		No pond was present, waterbody was dry	LPN 235; TM1565224379

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
IP0192		Temporary pond but the waterbody was dry	No LPN; TM1528326070
PO196	Photograph unavailable	No access granted	No LPN; TM1859723220


3.0 RESULTS



In total 106 standing water bodies were identified within the great crested newt survey area, two of which were identified as incidental finds during site walkovers, a location plan of all the water bodies that were assessed within the great crested newt survey can be found in Appendix A; Figures 1a – 1e. The ponds identified in table 1 that were dry at the time of survey are excluded from these figures.



Twenty two 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 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 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 for all waterbodies are shown in Appendix B.

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
6		TM 20262 19255	0.5	Below Average	Positive	eDNA – 16/05/2022



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

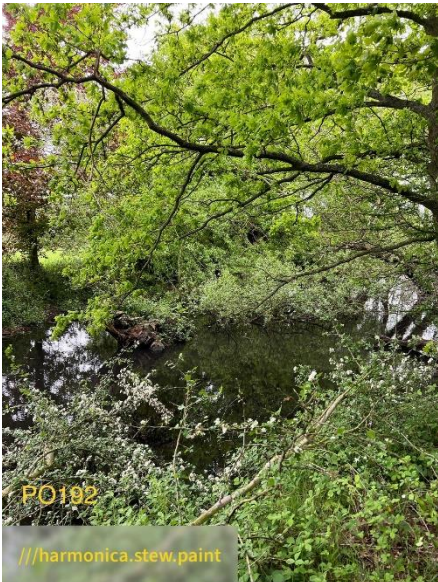
Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
11		TM 20182 19480	0.49	Poor	Positive	HSI – 08/06/2022 eDNA – 08/06/2022
13		TM 20202 19503	0.53	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
24	*Image not available for this pond*	TM 19080 22018	0.7	Good	Positive	HSI – 09/06/2022 eDNA – 09/06/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
25		TM 19728 22526	0.72	Good	Positive	HSI – 13/10/22 eDNA - 10/05/2022
50		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
53		TM 16161 23542	0.63	Average	Positive	HSI – 12/40/21 eDNA - 11/05/2022
54		TM 16098 23859	0.61	Average	Positive	HSI – 11/05/2022 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
57		TM 15739 24129	0.66	Average	Positive	HSI - 01/06/2022 eDNA - 01/06/2022
65		TM 15438 25475	0.65	Average	Positive	HSI – 10/05/2022 eDNA – 10/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
72		TM 12979 26811	0.72	Good	Positive	HSI – 01/06/2022 eDNA – 01/06/2022
PO102		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
PO192		TM 15211 26033	0.78	Good	Positive	HSI – 11/05/2022 eDNA – 11/05/2022

4.0 CONCLUSION

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions (10 waterbodies) or were unsuitable (12 waterbodies) at time of survey.

5.0 REFERENCES

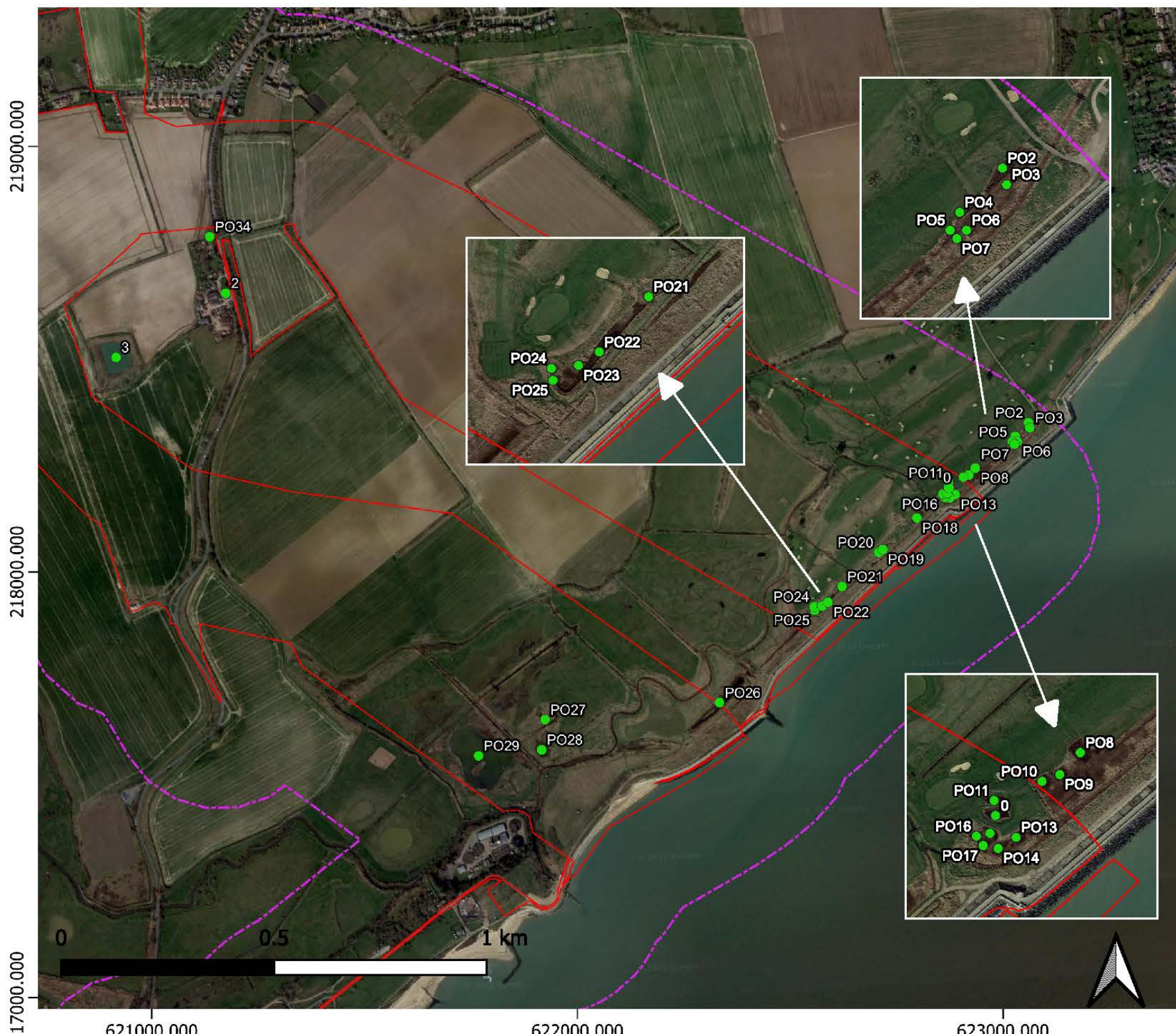
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Oldham R.S, Keeble J., Swan M.J.S., & Jeffcote M., (2000). Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*) British Herpetological Society, pp.143-155

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

APPENDIX A: Standing Water Body Location Plan



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1a**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1b**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1c**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

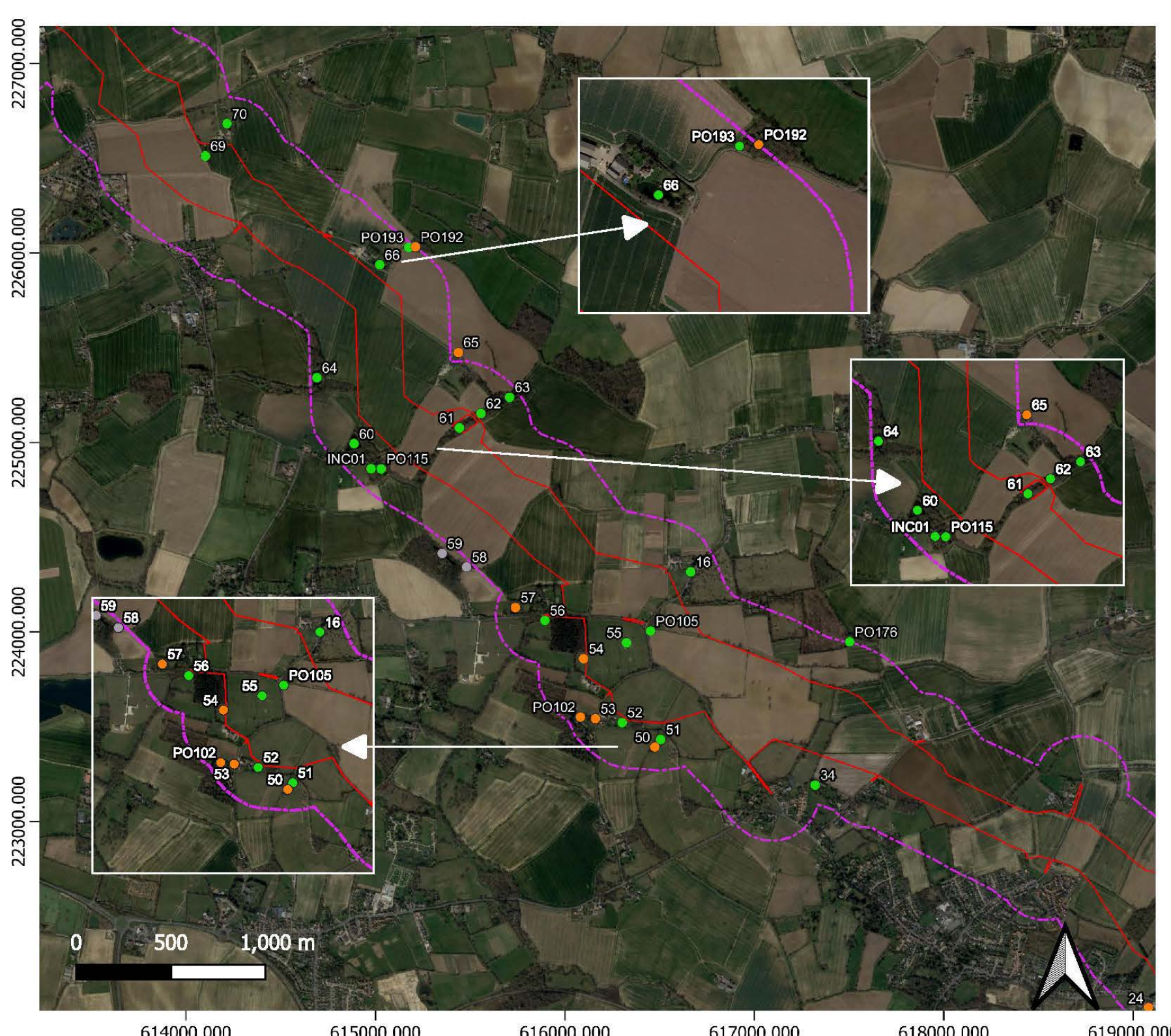
DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1d**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1e**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022


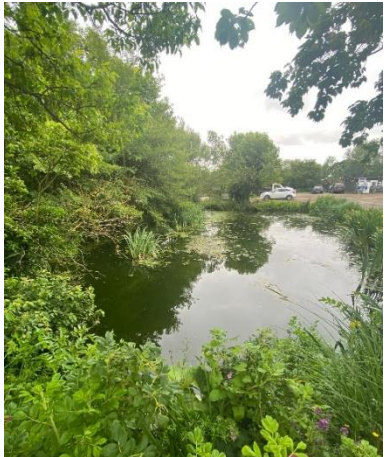
PRODUCED BY:
F. Austin



REF: 22043







ECOLOGY
RESOURCES



APPENDIX B: 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
0		TM 22873 18198	1	0.1	1	0.67	1	1	0.67	0.65	0.33	0.9	0.62	Average	Negative	17/05/2022
2		TM 21174 18655	1	0.2	0.9	1	1	0.01	0.01	0.9	1	0.6	0.31	Poor	Negative	06/06/2022



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
P02		TM 23060 18351	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
3	No photograph available	TM 20916 18504	1	0.87 7	0.9	0.67	1	0.67	0.67	0.72	0.67	0.4	0.73	Good	Negative	16/05/2022
P03		TM 23063 18339	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022



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
4		TM 20451 18437	1	0.80 01	0.9	0.67	1	0.67	0.67	0.69	0.67	0.35	0.71	Good	Negative	16/05/2022
P04	No photograph available	TM 23029 18319	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
5		TM 20329 19152	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	16/05/2022
P05	No photograph available	TM 23022 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022


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
6		TM 20262 19255	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
P06	No photograph available	TM 23034 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
7		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
P07	No photograph available	TM 23027 18300	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022



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
8		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
P08	No photograph available	TM 22935 18244	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
9		TM 20149 19368	1	0.4	1	1	0.7	1	1	0.95	1	0.9	0.86	Excellent	Negative	16/05/2022 17/05/2022



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
P09	No photograph available	TM 22920 18228	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
10		TM 20121 19407	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
P010		TM 22907 18223	1	0.91 54	1	0.67	1	1	0.67	0.90	0.67	0.90	0.86	Excellent	Negative	17/05/2022

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
11		TM 20182 19480	1	0.1	0.1	0.33	1	1	1	0.65	0.33	1	0.49	Poor	Positive	08/06/2022
P011		TM 22872 18209	1	0.2	0.5	0.67	1	1	0.67	0.9	0.67	0.9	0.68	Average	Negative	17/05/2022

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
13		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	16/05/2022
P013	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
14		TM 20205 19520	1	0.5	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	N/A	07/06/2022
P014	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




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
15		TM 20228 19525	1	0.5	0.1	0.01	1	1	1	1	0.1	0.3	0.2	Poor	N/A	07/06/2022
P015	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
16	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
P016	No photograph available	TM 22859 18183	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
17	No photograph available	TM 20629 19731	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.5	0.76	Good	Negative	14/06/2022
P017	No photograph available	TM 22864 18176	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022



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
P018		TM 22798 18127	1	0.7	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.76	Good	Negative	17/05/2022
P019		TM 22718 18053	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
20	No photograph available	TM 20198 20524	1	0.2	0.9	1	1	0.01	0.01	0.95	0.67	0.6	0.3	Poor	Negative	14/06/2022




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
P020		TM 22708 18046	1	1	0.5	0.67	1	1	0.67	0.7	0.67	1	0.79	Excellent	Negative	17/05/2022
P021		TM 22622 17966	1	0.2	1	0.67	1	1	0.67	0.7	0.67	1	0.72	Good	Negative	17/05/2022


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
22		TM 20380 21316	1	0.5	0.9	0.67	1	10.6 7	0.67	0.66	0.67	0.4	0.68	Average	Negative	06/05/2022
P022		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	17/05/2022



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
23		TM 19500 21879	1	0.3	0.5	0.67	1	1	1	0.9	0.67	0.5	0.7	Good	Negative	10/05/2022
P023		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	17/05/2022
24	No photograph available	TM 19080 22018	1	0.3	1	0.67	1	1	0.67	0.8	0.67	0.45	0.7	Good	Positive	09/06/2022

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
P024		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	17/05/2022
25		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
P025		TM 22558 17910	1	03	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022




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
26	No photograph available	TM 19281 22613	1	0.9	0.9	0.67	1	1	0.67	0.84	0.67	0.4	0.77	Good	Negative	01/06/2022
P026	No photograph available	TM 22334 17693	1	0.80 01	1	0.67	1	0.67	0.67	1	0.67	0.9	0.82	Excellent	Negative	18/05/2022
27		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	10/05/2022
P027		TM 21924 17653	1	0.1	0.1	0.33	1	0.67	1	0.7	1	0.95	0.52	Below Average	Negative	20/05/2022



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
28		TM 19273 22709	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	10/05/2022
P028		TM 21916 17582	1	0.6	1	0.67	1	1	0.67	0.7	0.67	0.7	0.5	Good	Negative	20/05/2022
29		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



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
P029		TM 21768 17568	1	0	1	0.67	1	0.67	0.67	0.95	0.67	0.3	0.72	Good	Negative	18/05/2022
30	*Image not available for this pond*	TM 19408 23333	1	0.3	1	0.67	1	1	1	0.68	0.67	0.35	0.7	Good	Negative	01/06/2022

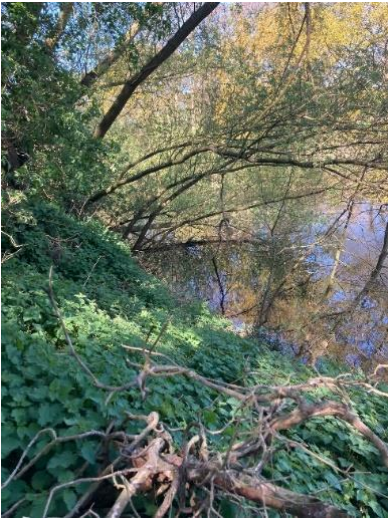

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
34		TM 17321 23192	1	0.3	0.9	1	1	0.01	0.01	0.9	0.67	0.7	0.32	Poor	Negative	08/06/2022
P034	No photograph available	TM 21136 18788	1	0.2	1	0.67	0.6	0.67	0.67	0.65	0.67	0.3	0.58	Below Average	Negative	16/05/2022
50		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	12/05/2022

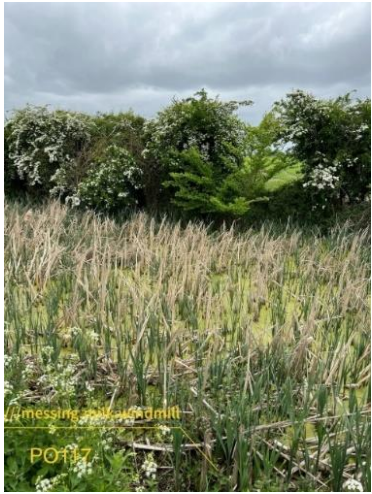

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
51		TM 16505 23432	1	0.98	0.90	0.67	1.00	0.01	0.67	0.96	1.00	0.50	0.53	Below Average	Negative	12/05/2022
52		TM 16302 23522	1	1.10	0.90	0.33	1.00	0.67	0.33	0.93	1.00	0.45	0.70	Good	Negative	11/05/2022
53		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	11/05/2022



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
54		TM 16098 23859	1	0.3	0.5	0.33	1	0.67	1	0.92	0.67	0.35	0.61	Average	Positive	11/05/2022
55		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
56		TM 15894 24061	1	0.1	0.5	0.67	1	1	1	0.55	1	1	0.67	Average	Negative	26/04/2022



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
57		TM 15739 24129	1	0.1	1	0.67	1	1	1	0.7	1	0.35	0.66	Average	Positive	01/06/2022
60		TM 14887 24994	1	0	0.9	0.67	1	0.67	0.67	0.75	0.67	0.4	0.72	Good	Negative	26/04/2022



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
61		TM 15443 25077	1	0.95 39	0.5	0.33	0.6	1	1	0.65	0.67	0.35	0.65	Average	Negative	10/05/2022
62		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	11/05/2022 12/07/2022



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
63		TM 15708 25239	1	0	0.9	1	1	0.67	0.67	0.65	1	0.3	0.75	Good	Negative	26/04/2022
64		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	26/04/2022



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
65		TM 15438 25475	1	0.2	0.5	0.33	1	1	1	0.65	0.67	0.9	0.65	Average	Positive	10/05/2022
66		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	26/04/2022

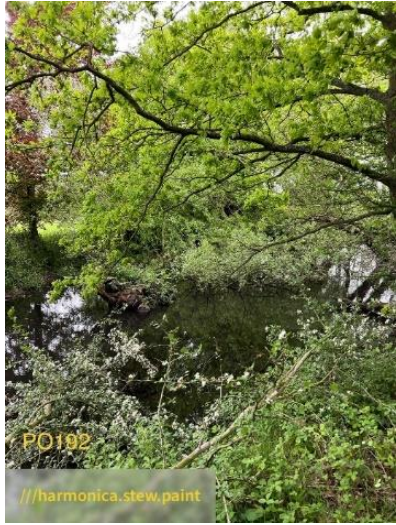

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
69		TM 14102 26512	1	0.92	0.50	0.33	0.20	0.67	0.67	0.9	0.33	0.50	0.54	Below Average	Negative	26/04/2022
70		TM 14216 26683	1	0.2	0.9	0.67	1	0.67	1	0.69	0.67	0.35	0.64	Average	Negative	12/05/2022



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
P070		TM 20232 19499	1	0.05	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	Negative	07/06/2022
72		TM 12979 26811	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.65	0.72	Good	Positive	01/06/2022
73	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	28/04/2022

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
74	No photograph available	TM 12555 28104	1	0.31	0.90	0.33	1.00	0.67	0.67	0.58	0.33	0.40	0.56	Below Average	Negative	28/04/2022
P083		TM 19706 22392	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.69	Average	Negative	10/05/2022
P0102		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	11/05/2022

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
P0105		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
P0115	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	Excellent	26/04/2022
P0142		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	11/05/2022

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
P0176		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
P0183		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	11/05/2022

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
P0192		TM 15211 26033	1	0.8	1	0.67	1	0.67	0.67	0.89	1	0.4	0.78	Good	Positive	11/05/2022
IP0192		TM 15283 26070	1	0.1	0.1	0.67	1	1	1	0.6	0.67	1	0.55	Below Average	N/A	11/05/2022

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
P0193		TM 15175 26030	1	0.1	0.1	0.33	1	0.67	1	0.88	1	0.7	0.51	Below Average	Negative	12/05/2022
P0195		TM 11643 28069	1	0.81 54	1	0.67	1	0.67	0.67	0.7	0.67	0.35	0.72	Good	Negative	11/05/2022
INC01	No photograph available	TM 14977 24862	1	0.3	1	0.67	1	0.67	0.33	0.75	1	0.6	0.68	Average	Negative	26/04/2022

APPENDIX C: Surescreen Scientifics – eDNA Results

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	TM 19706 22392	Pass	Pass	Pass	Negative	0
1738	PO84	TM 19728 22526	Pass	Pass	Pass	Positive	1
1739	PO86	TM 19273 22709	Pass	Pass	Pass	Negative	0
1740	PO85	TM 19415 22677	Pass	Pass	Pass	Negative	0
1741	PO87	TM 19189 22953	Pass	Pass	Pass	Negative	0
1742	PO88	TM 19326 23209	Pass	Pass	Pass	Negative	0
1744	PO143	TM 11123 27625	Pass	Pass	Pass	Negative	0



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



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	TM 16505 23432	Pass	Pass	Pass	Negative	0
1746	PO141	TM 10858 28469	Pass	Pass	Pass	Negative	0
1761	PO101	TM 16161 23542	Pass	Pass	Pass	Positive	4
1762	PO103	TM 16098 23862	Pass	Pass	Pass	Positive	3
1764	PO102	TM 16082 23551	Pass	Pass	Pass	Positive	10
1765	PO142	TM 11851 27529	Pass	Pass	Pass	Negative	0
1766	PO183	TM 11680 27882	Pass	Pass	Pass	Negative	0



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



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	TM 09673 27216	Pass	Pass	Pass	Negative	0
1771	PO192	TM 15211 26033	Pass	Pass	Pass	Positive	1
1777	PO27	TM 21924 17653	Pass	Pass	Pass	Negative	0
1783	PO28	TM 21916 17582	Pass	Pass	Pass	Negative	0
1801	PO26	TM 22334 17693	Pass	Pass	Pass	Negative	0
1806	PO29	TM 21768 17568	Pass	Pass	Pass	Negative	0
1809	PO25	TM 22558 17910	Pass	Pass	Pass	Negative	0



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



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	TM 20182 19480	Pass	Pass	Pass	Positive	1
1773	PO 176	TM 17503 23948	Pass	Pass	Pass	Negative	0
1776	ID391-LP49 5E	TM 192 226	Pass	Pass	Pass	Negative	0
1780	ID222-LP239 5 Estuaries	TM 157 241	Pass	Pass	Pass	Positive	12
1794	PO82	TM 19080 22018	Pass	Pass	Pass	Positive	4
1796	PO 134	TM 12275 29994	Pass	Pass	Pass	Negative	0



1797	PO135	TM 11336 29737	Pass	Pass	Pass	Negative	0
1798	PO178	TM 11446 29778	Pass	Pass	Pass	Negative	0
1799	PO94	TM 17317 23195	Pass	Pass	Pass	Negative	0
1800	PO35	TM 21174 18656	Pass	Pass	Pass	Negative	0
1803	PO79	TM 20381 21316	Pass	Pass	Pass	Negative	0
1807	10260 - LP996 5E	TM 130 268	Pass	Pass	Pass	Positive	1
1808	ID145 - LP75 5 Estuaries	TM 193 233	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.

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.



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.

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.





F I V 
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