

# **Great Crested Newt Survey Report**

Development of National Significance Pre-Application Consultation

# Alaw Môn Solar Farm

Land west of the B5112, 415m south of Llyn Alaw, 500m east of Llantrisant and 1.5km west of Llannerch-y-Medd, Anglesey

October 2023





# **Alaw Môn Solar Farm Ecology**

Great Crested Newt Survey Report

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# 1 Introduction

# **Background**

1.1 BSG Ecology was commissioned by Wylfa Green to carry out survey work and report on the status of great crested newt *Triturus cristatus* at the proposed Alaw Môn Solar Farm (the 'Site'). The Site is located in Anglesey: to the west of the B5112, to the south of Llyn Alaw, 500m to the east of the small hamlet of Llantrisant, and 1.5km to the west of the village of Llannerch-y-medd. The Site is centred at Ordnance Survey (OS) grid reference SH 38304 83896. The Site location is presented in Figure 1.

# **Purpose of this Document**

- 1.2 This document forms an appendix to the Alaw Môn Solar Farm Environmental Statement (Chapter 8 Biodiversity (BSG Ecology, 2023)). The aim of this document is to present the results of survey work undertaken in 2020, 2021 and 2023 to assess the status of great crested newt at the Site.
- 1.3 The objectives of this document are to:
  - Provide details of the survey methods, timings, and any limitations;
  - · Present the results of the fieldwork and laboratory analysis; and
  - Provide interpretation of the results.



# 2 Methods

- 2.1 The survey approach has involved a combination of desk-based assessment and field survey techniques:
  - A review of aerial photographs (Google Earth), online maps (OS 1:25,000 maps) and the extended Phase 1 habitat survey was undertaken to identify ponds. From the initial review in April 2020, 33 ponds were identified within a Study area, which included the Site<sup>1</sup> and a 250m radius around its boundary.
  - Given the number of ponds within 250m, ponds beyond this distance were scoped out of the assessment. When the Site boundary was subsequently revised as the project design was refined, five ponds (9, 15, 18, 22, 25) that were included in the initial assessment fell outside of the 250m survey area, and were scoped out of the assessment. An additional pond (Pond 34) was located in 2023, in an area that was not previously accessible; this was included in the survey work in 2023.
  - Wherever access was possible all ponds were subject to an initial Habitat Suitability Index (HSI) assessment for great crested newt. For some ponds, HSI assessment was only possible at a later stage, when access was agreed.
  - Following the HSI assessment, all accessible ponds within 250m of the Site and found to be holding water, and therefore capable of being sampled, were subsequently subject to eDNA survey for great crested newt.
  - Pond 7 which returned a positive eDNA result in 2020 was subject to a population class assessment survey in 2021. Other ponds (Ponds 3 and 8) which returned a positive eDNA result in 2023 were also subject to further assessment.
  - A data search for any existing great crested newt records was also undertaken for a 2km search radius around the Site. The data was obtained through Cofnod, the North Wales Environmental Information Service. The data were provided in May 2020 and renewed in April 2021, and again in July 2023.

# Habitat Suitability Index (HSI) Assessment

- The HSI for great crested newts is a measure of habitat suitability. The HSI score is calculated by allocating scores to ten suitability indices (SI) which include features such as size of the waterbody, water quality, quality of surrounding habitat, and presence of fish or wildfowl (see Oldham *et al.*, 2000). These scores are then used to calculate the overall HSI for each waterbody as a number between 0 and 1. In general, ponds with high HSI scores are more likely to support great crested newts than those with low scores. The system is not sufficiently precise to conclude that any particular pond with a high score will support newts, or that any pond with a low score will not.
- 2.3 Based on the HSI score, each waterbody can be broadly categorised in terms of suitability for great crested newt (ARG UK, 2010):

HSI score	Pond suitability for great crested newt		
>0.8	excellent		
0.7 - 0.79	good		
0.6 – 0.69	average		

<sup>&</sup>lt;sup>1</sup> NB For the purposes of this report, the 'Site' relates to the proposed solar farm area and excludes the grid connection cable route; this be provided by underground cabling located within the adopted highway and will not affect vegetated areas.

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0.5 - 0.59	below average
<0.5	poor

2.4 The HSI assessment was carried out on 6/7 May 2020 by Consultant Ecologist Richard Birch CEcol. Further confirmatory assessment was carried out on 4 May 2021 by Senior Ecologist Emily McVean ACIEEM.

### **Great Crested Newt - eDNA Survey**

- 2.5 Great crested newt DNA is released into aquatic environments through shed skin cells, urine, faeces and saliva. It can persist in water for several weeks and can be collected in water samples. A test has been developed for detecting the environmental DNA (eDNA) which is an effective way to determine presence or absence of great crested newt.
- eDNA survey involves collecting water samples following a set survey protocol, collecting samples between 15 April and 30 June (see Biggs *et al.*, 2014). The samples are sent off for analysis to determine the presence of great crested newt eDNA.
- 2.7 In total, water samples for 22 ponds were collected and analysed for great crested newt eDNA: 4 samples were collected in 2020; 11 further ponds were sampled in 2021. Subsequently, 20 ponds were sampled in 2023. The numbers of ponds sampled varied each year, depending on two factors: access being agreed, and sufficient water being present to collect a sample.

# **eDNA Survey 2020 and 2021**

- 2.8 Consultant Ecologist Richard Birch CEcol undertook the first phase of eDNA sample collection on 3 June 2020. The lab analysis was undertaken by Surescreen Ltd. The results were reported back to BSG Ecology on 19 June 2020. During this phase of survey, samples were taken from all accessible ponds with HSI scores of 0.5 and above.
- 2.9 The second phase of eDNA sample collection was undertaken on 13 and 14 May 2021. This was carried out by Senior Ecologist Emily McVean ACIEEM. The lab analysis was undertaken by Surescreen Ltd. The results were reported back to BSG Ecology on 28 May 2021. During this phase of survey, samples were taken from a) all ponds not sampled in 2020, regardless of HSI score, and b) any off-site ponds which was inaccessible in 2020 but for which access had subsequently been secured.
- 2.10 A summary of the ponds sampled is shown in Table 4. The testing methods and lab results are provided in Annex B.
- 2.11 The survey approach is in line with the guidance published on Natural Resources Wales (NRW) website when the survey work was carried out: <a href="https://naturalresources.wales/media/3509/guidance-on-use-of-dna-sampling-of-great-crested-newts.pdf">https://naturalresources.wales/media/3509/guidance-on-use-of-dna-sampling-of-great-crested-newts.pdf</a>
- 2.12 This guidance states that NRW will accept eDNA test results as evidence of presence or absence of great crested newt. It also states that eDNA survey can have a better rate of great crested newt detection (99%) than a combination of conventional survey techniques (95%); that the visit must be targeted when great crested newt is likely to be present in water bodies in the area (which may change on a yearly basis depending on local / regional conditions); and that NRW will accept samples taken between 15 April to 30 June.

### eDNA Survey 2023

2.13 The survey work described above was repeated in 2023 to update the survey previous survey results, and sample additional ponds to which access had not previously been agreed (see Table 5); water samples were taken from 20 ponds.

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eDNA sample collection was undertaken on 11 and 12 May 2023. This was carried out by Senior Ecologist Gemma Watkinson ACIEEM. The lab analysis was undertaken by Surescreen Ltd. The results were reported back to BSG Ecology on 24 May 2023. The testing methods and results are provided in Annex B.

# Great Crested Newt - Population size class assessment survey 2021

- 2.15 This survey method was carried on any ponds with a positive eDNA survey result:
  - a positive eDNA survey result was returned for one pond (Pond 7) in 2020; all other ponds sampled were negative.
  - no positive eDNA survey results were returned from the survey in 2021.
- 2.16 Pond 7 was therefore subject to a population class assessment survey in spring 2021; this involved six visits between April and June, using three survey techniques: bottle-trapping, torch-light survey, and egg searching. The survey methods were carried out with reference to standard industry guidance (English Nature, 2001) and in suitable weather conditions.
- 2.17 The survey visits were undertaken by consultant ecologist Stuart Thomas (who holds a Natural Resources Wales survey licence). The survey data are presented in Annex C of this report and the dates of the survey are summarised in Table 1 below.

Table 1: Population size class assessment survey dates 2021

Visit	1	2	3	4	5	6
Dates	07/04/2021	13/04/2021	02/05/2021	21/05/2021	09/06/2021	16/06/2021

# Great Crested Newt - Population size class assessment survey 2023

2.18 The population class assessment survey on Pond 7 was repeated in spring 2023 to update the previous survey results. This survey was carried out by Enfys Ecology Ltd (led by Consultant Ecologist Tim Yardley, ACIEEM, who holds a Natural Resources Wales survey licence) on behalf of BSG Ecology. The survey was carried out with reference to standard industry guidance in suitable weather conditions.

Table 2: Population size class assessment survey dates 2023

Visit	1	2	3	4	5	6
Dates	25/04/2023	02/05/2023	15/05/2023	23/05/2023	30/05/2023	07/06/2023

- 2.19 In 2023, bottle-trapping was not used during the first two visits as due to the density of vegetation and lack of suitable substrate for securing traps, meant that the pond could not be safely trapped; torching and egg searching was possible. Floating bottle traps were used for the remaining visits to allow traps to be safely employed.
- 2.20 Positive eDNA results were obtained from two additional ponds (Ponds 3 and 8) part-way through the population size class assessment survey of Pond 7. Previously, Pond 3 had been too dry to sample; a negative eDNA result had been returned for Pond 8 in 2021. These two ponds were subsequently included in the remaining 2023 population size class assessment survey (Visits 5 and 6), as described in Table 2 above.

# **Consideration of limitations**

2.21 <u>Access</u>: Access was not granted to all of the off-site ponds: ponds are on the far (west) side of Corsy-bol Local Wildlife Site (LWS) were not included in the survey; it is therefore not possible to rule out or confirm the presence of great crested newt in these ponds.

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- 2.22 The closest (Pond 21) is 114m from the Site; others (Ponds 22, 23, 24, 25) are more distant (332m, 236m, 227m and 373m respectively; and the two furthest are scoped out on distance). It is also noted that they are separated from the Site by a minor watercourse, Cors-y-bol, which may provide a partial barrier for dispersal, were great crested newt to be present in these ponds. The ponds are also close to extensive areas of terrestrial habitat (wetland) within Cors-y-bol LWS. It is considered likely that if great crested newts were using these ponds they are more likely to stay in nearby terrestrial habitat during the terrestrial phase, rather than moving into more distant suboptimal habitat (i.e. habitats within the Site). Given that the majority of ponds within the study area have been sampled, the significance of this constraint is considered to be minor; it is not considered to significantly alter the conclusions of the report, or the ability to assess impacts on this species from the development.
- 2.23 <u>Dry ponds</u>: Some ponds were found to be dry or have water levels too shallow to take water samples. Those that were dry in spring 2020 were revisited in 2021 after a cooler, wetter spring, and water samples for eDNA survey were taken where possible to do so. This approach was repeated in 2023. Some ponds that were dry on all visits and were not sampled. These are identified in Tables 4 and 5. This is not considered to be a significant constraint on the findings of this report.
- 2.24 Scope: Ponds that were within 250m of the Site but, based on the HSI assessment, were assessed as having low suitability for great crested newt, were not included of the first phase of eDNA survey in 2020. Survey effort was therefore initially targeted to the more suitable ponds (those with HSI scores of 0.5 and above). In the second phase of eDNA survey, all ponds on-site ponds were incorporated into the eDNA survey work in 2021. This approach was devised to provide additional data and to ensure the increase confidence in the assessment.
- 2.25 Population class assessment: The two additional ponds that returned positive eDNA results in 2023 were subject to additional survey (torching) and assessment once the eDNA results were confirmed. The survey effort (number of visits) was less than that for Pond 7. Given the character of these two ponds, and the survey previous results for both (Pond 7 dry on two previous visits, and dry again by late May 2023; and Pond 8, poor HSI score and a previous negative eDNA result), neither are considered likely to be breeding ponds and the survey approach is not considered to be a significant constraint or significantly alter the findings of this report or the assessment. This is discussed in further detail below.
- 2.26 <u>Conclusion</u>: Overall, the survey approach, which has involved a variety of techniques and repeat visits over three seasons and has included all accessible ponds, is considered to be sufficient to allow an assessment of impacts on great crested newts from the proposed development.

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# 3 Results and Evaluation

# **Desk study**

- 3.1 Three great crested newt records, dating from 1992, were returned for a location 61 m to the east of the northern part of the Site (at grid reference SH396847; this grid reference is close to Pond 7 and is considered to relate to this pond). A further two records, dating from 2017, were returned for a location 642m west of the Site (at grid reference SH37168515); this is from a pond surrounded by vegetation and scrub, close to a farm, on the opposite side of the valley formed by Cors y Bol LWS.
- 3.2 It is noted that the north-west part of the Site corresponds with part of a wider area identified for potential pond creation for great crested newt with the aim of improving favourable conservation status of the species (Russell *et al.*, 2017). This has been modelled by Natural Resources Wales (NRW) based on existing populations and flood maps, with the aim of identifying areas where dispersal corridors would be created.

# Field survey

- 3.3 A summary of survey results is presented below in Table 4 (for the 2020/21 survey results) and Table 5 (for the 2023 survey results); A map of the ponds is presented in Figure 2.
- 3.4 The HSI assessments for each pond are provided in Annex A.

# Habitat Suitability Index Assessment

Ponds within the Site

- There are three ponds within the Site: Ponds 4,11, and 16. A fourth pond (Pond 27) is shown on the OS 1:25,000 map, however, it is not actually a pond. It is an area of marshy grassland with no depression or standing water.
  - Pond 4 (Below Average suitability; dry in 2020 and 2023)
  - Pond 11 (Poor Suitability)
  - Pond 16 (Below Average suitability; dry in 2020 and 2021)

# Off-site ponds

3.6 The following off-site ponds were visited:

Excellent suitability: Pond 7

Good suitability: Ponds 6, 17, 20

Average suitability: Pond 3<sup>2</sup>

Below Average suitability: Ponds 12, 14, 16, 19

Poor suitability:
 Ponds 1, 5, 8, 9, 12, 13, 18, 26, 28, 31, 32, 33

Dry or not present: Ponds 2, 3, 10, 29, 30.

# eDNA analysis 2020/21

- 3.7 Positive results were returned for one pond (Pond 7).
- 3.8 All other ponds surveyed tested negative; in these, great crested newt DNA could not be detected, and it was concluded that great crested newt is likely to be absent from these ponds.
- 3.9 The eDNA analysis results are presented in Annex B. There were no limitations to the eDNA analyses.

<sup>&</sup>lt;sup>2</sup> This HSI score is when Pond 3 holds water (in wet conditions only), however, it was dry in 2020 and 2021, and in 2023 was dry by early June, suggesting that its typical condition is dry.



### eDNA analysis 2023

- 3.10 Positive results were returned from three ponds:
  - Pond 7 (as previously)
  - Pond 3 (not sampled previously as it was dry in 2020/21. This is a small rush filled depression immediately to the west of the Site boundary).
  - Pond 8 (previously sampled; negative result). This is an open, unvegetated pond which is used by wildfowl just (c. 50m) to the north-west of the northern part of the Site).
- 3.11 The eDNA analysis results are presented in Annex B. There were no limitations to the eDNA analyses.

# Population class assessment survey

3.12 Pond 7 (2021): Six survey visits were conducted between April and June 2021. One female great crested newt was observed on the penultimate visit (9 June 2021). Great crested newt was not recorded on any other visit. A single palmate newt *Lissotriton helveticus* was also observed on the 9 June 2021.

Table 2: Population size class assessment survey results 2021

Visit	1	2	3	4	5	6
Dates	07/04/2021	13/04/2021	02/05/2021	21/05/2021	09/06/2021	16/06/2021
Great crested newt (male)	-	-	-	-	-	-
Great crested newt (female)	-	-	-	-	1	-
Great crested newt egg	-	-	-	-	-	-

3.13 Pond 7 (2023): Six survey visits were conducted between April and June 2023. A maximum of three great crested newts were recorded (2 males and 1 female), as summarised in Table 3, below (Further information is provided in Annex C):

Table 3: Population size class assessment survey results 2023

Visit	1	2	3	4	5	6
Dates	25/04/2023	02/05/2023	15/05/2023	23/05/2023	30/05/2023	07/06/2023
Great crested newt (male)	2	1	1	2	1	-
Great crested newt (female)	-	1	1	1	-	-
Great crested newt egg	-	1	-	-	-	-

- 3.14 Small numbers of palmate newt (up to two) and smooth newt *Lissotriton vulgaris* (peak of 10 on 25 May 2023, but typically just one or two individuals) were also recorded from Pond 7.
- 3.15 The survey results from both years indicates that a small great crested newt population is present in Pond 7<sup>3</sup>. The presence of an egg, located in 2023, indicated that breeding is occurring in Pond 7.
- 3.16 No great crested newts were recorded during the survey to Pond 3 or Pond 8.

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<sup>&</sup>lt;sup>3</sup> For the purposes of assessment, a 'small' population is one that is not likely to have a maximum observable count greater than 10 newts (English Nature, 2001).



- 3.17 By 30 May 2023, Pond 3, which is a rush-filled depression in a pasture field, held barely any water and could not be surveyed using bottle traps; by the 7 June 2023 this pond was completely dry. It is not assessed to provide suitable habitat for breeding great crested newt.
- 3.18 Pond 8 has a rocky base and pond does not support vegetation; it is used by wildfowl. It could be fully surveyed throughout by torching; no evidence of great crested newt was identified. Given the HSI assessment (poor), the previous negative eDNA result, the lack of vegetation (required for egglaying), and lack of any other evidence of use, this pond is considered unlikely to be used as a breeding pond. The eDNA result is considered most likely to indicate a temporary presence when moving through the local landscape.

### Habitat assessment

- 3.19 The 2023 survey results are considered to confirm the previous assessment that this species present in the local landscape, but occurs at a very low density, and given the presence of eggs, and both male and female newts, breeding occurs in Pond 7, where a small population is present.
- 3.20 Suitable habitat for great crested newt exists on site in the form of scrub, hedgerows and drystone walls, and patches of marshy grassland. The most suitable habitats typically form boundary features. The improved grassland within the fields is typically close-grazed and does not offer structure, cover or places of shelter, and is assessed to be of limited suitability for great crested newt.
- 3.21 Habitats within 250m of a breeding pond are likely to be used most frequently by great crested newt. Where suitable habitat is present, great crested newts typically occur at highest densities close to breeding ponds (i.e. within 50m); very few occur at greater distances (Cresswell & Whitworth, 2004).

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Table 4: Survey Results Summary Table 2020/21

Pond	х	Υ	HSI Score	HSI category	eDNA Status	Distance to Site (m)
Pond 1	238236	384957	0.47	Poor	Negative (19/05/2021)	193
Pond 2	237600	384485		Dry	n/a	On-site
Pond 3	237635	384522		Dry	n/a	On-site
Pond 4	237747	384257	0.47	Poor	Negative (19/05/2021)	On-site
Pond 5	238691	383988	0.34	Poor	Negative (19/05/2021)	5
Pond 6	238637	383903	0.71	Good	Negative (19/05/2021)	10
Pond 7	239386	384686	0.81	Excellent	Positive (19/06/2020)	32
Pond 8	239133	385381	0.48	Poor	Negative (19/05/2021)	42
Pond 9	238900	385367	0.46	Poor	Scoped out (distance >250m) <sup>4</sup>	255
Pond 10	239806	384918		Dry	n/a	179
Pond 11	238695	383682	0.37	Poor	Negative (19/05/2021)	On-site
Pond 12	239108	383296	0.32	Poor	No access	22
Pond 13	239434	383400	No access		No access	135
Pond 14	239527	383420	No access		No access	92
Pond 15	240270	383557	No access		No access and scoped out (distance >250m)	380
Pond 16	238558	382697		Dry	n/a	On-Site
Pond 17	238851	382519	0.72	Good	Negative (19/05/2021)	190
Pond 18	238182	382307	0.30	Poor	Scoped out (distance >250m)	260
Pond 19	237985	382784	0.52	Below average	Negative (19/05/2021)	110
Pond 20	237270	383444	0.7	Good	Negative (19/06/2020)	105
Pond 21	236810	383635	No access		No access	114
Pond 22	237205	384426	No access		No access and scoped out (distance >250m)	332
Pond 23	237406	384576	No access		No access	236
Pond 24	237537	384695	No access		No access	227
Pond 25	237464	384791	No access		No access and scoped out (distance >250m)	373
Pond 26	239522	383545	0.43	Poor	Negative (19/05/2021)	6
Pond 27	238102	382733		Dry	n/a	On-site
Pond 28	238618	384696	0.43	Poor	Negative (19/06/2020)	5
Pond 29	238417	383833		Dry	n/a	17
Pond 30	237688	384524		Dry	n/a	On-site
Pond 31	238389	383845	0.31	Poor	Negative (19/05/2021)	50
Pond 32	238364	383842	0.44	Poor	Negative (19/05/2021)	25
Pond 33	238745	383978	0.34	Poor	Negative (19/06/2020)	5

 $<sup>^4</sup>$  Due to changes in the Site boundary, which increased the distance of Ponds 9, 15, 18, 22 and 25 from the Site, these were scoped out not sampled for eDNA.



Table 5: Survey Results Summary Table 2023

Pond	x	Y	HSI Score⁵	HSI category <sup>6</sup>	eDNA Status	Distance to Site (m)
Pond 1	238236	384957			Negative	193
Pond 2	237600	384485	0.74	Good	Negative	On-site
Pond 3	237635	384522	0.64	Average (but dry by June)	<u>Positive</u>	On-site
Pond 4	237747	384257		Dry	n/a	On-site
Pond 5	238691	383988			Negative	5
Pond 6	238637	383903			Negative	10
Pond 7	239386	384686			<u>Positive</u>	32
Pond 8	239133	385381			Positive	42
Pond 9	238900	385367			Scoped out (distance >250m)	255
Pond 10	239806	384918		Dry	n/a	179
Pond 11	238695	383682			Negative	On-site
Pond 12	239108	383296			Negative	22
Pond 13	239434	383400	0.49	Poor	Negative	135
Pond 14	239527	383420	0.53	Below average	Negative	92
Pond 15	240270	383557	No Access		n/s and scoped out (distance >250m)	380
Pond 16	238558	382697	0.56	Below average	Negative	On-Site
Pond 17	238851	382519			Negative	190
Pond 18	238182	382307			Scoped out (distance >250m)	260
Pond 19	237985	382784			Negative	110
Pond 20	237270	383444		Dry	n/a	105
Pond 21	236810	383635	No access		n/a	114
Pond 22	237205	384426	No access		n/a and scoped out (distance >250m)	332
Pond 23	237406	384576	No access		n/a	236
Pond 24	237537	384695	No access		n/a	227
Pond 25	237464	384791	No access		n/a and scoped out (distance >250m)	373
Pond 26	239522	383545			Negative	6
Pond 27	238102	382733		Dry	n/a	On-site
Pond 28	238618	384696			Negative	5
Pond 29	238417	383833		Dry	n/a	17
Pond 30	237688	384524		Dry	n/a	On-site
Pond 31	238389	383845			Negative	50
Pond 32	238364	383842			Negative	25
Pond 33	238745	383978			Negative	5
Pond 34 <sup>7</sup>	239520	383338	0.68	Average	Negative	200

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 <sup>&</sup>lt;sup>5</sup> HSI scores provided in Table 5 only where not obtained during the previous phases of work.
 <sup>6</sup> HSI assessment provided where not obtained during the previous phases of work.
 <sup>7</sup> This pond was located during the survey in 2023, in an area that was not previously accessible.



# 4 References

ARG UK (Amphibian and Reptile Groups of the United Kingdom) (2010) ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. May 2010.

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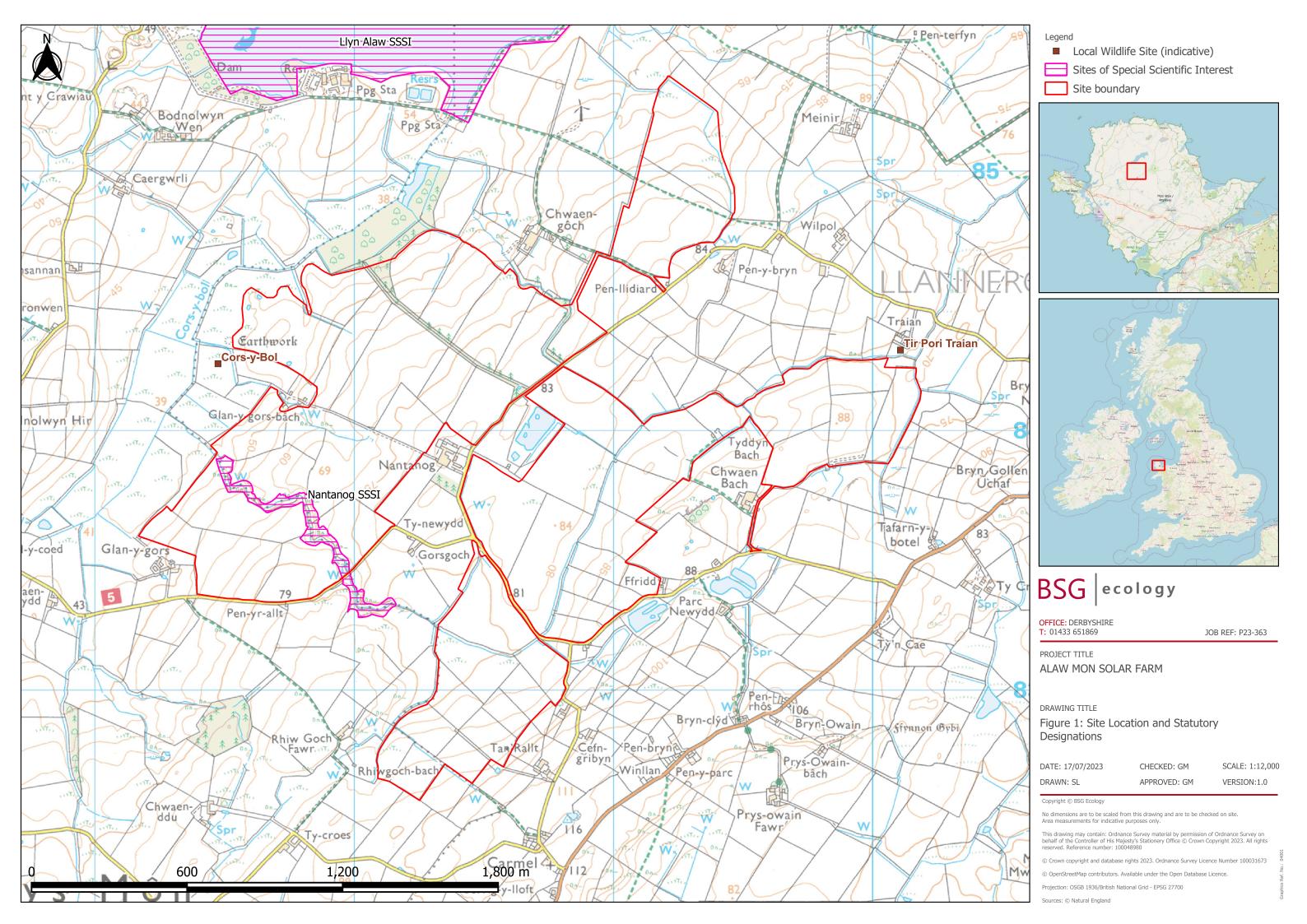
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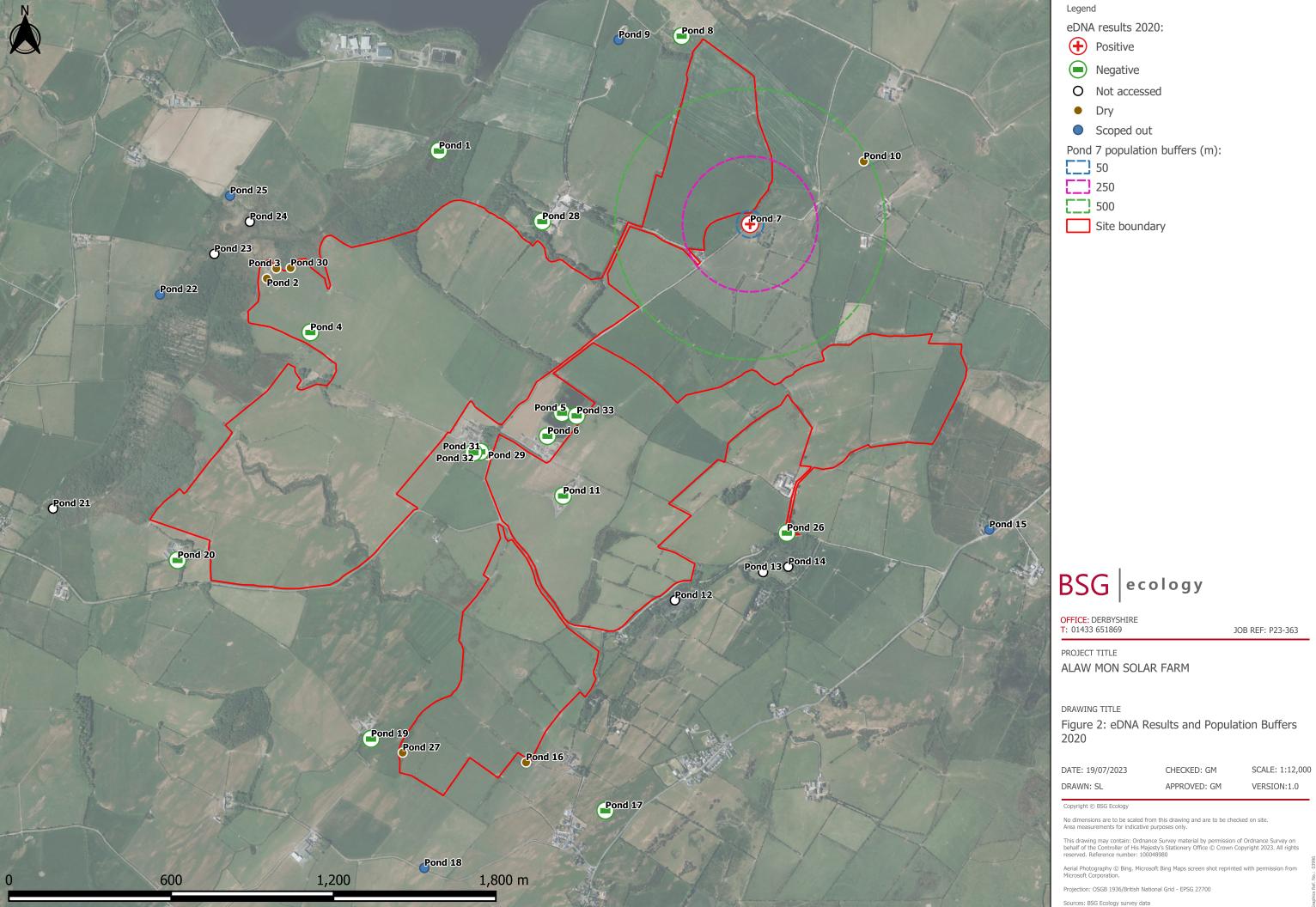
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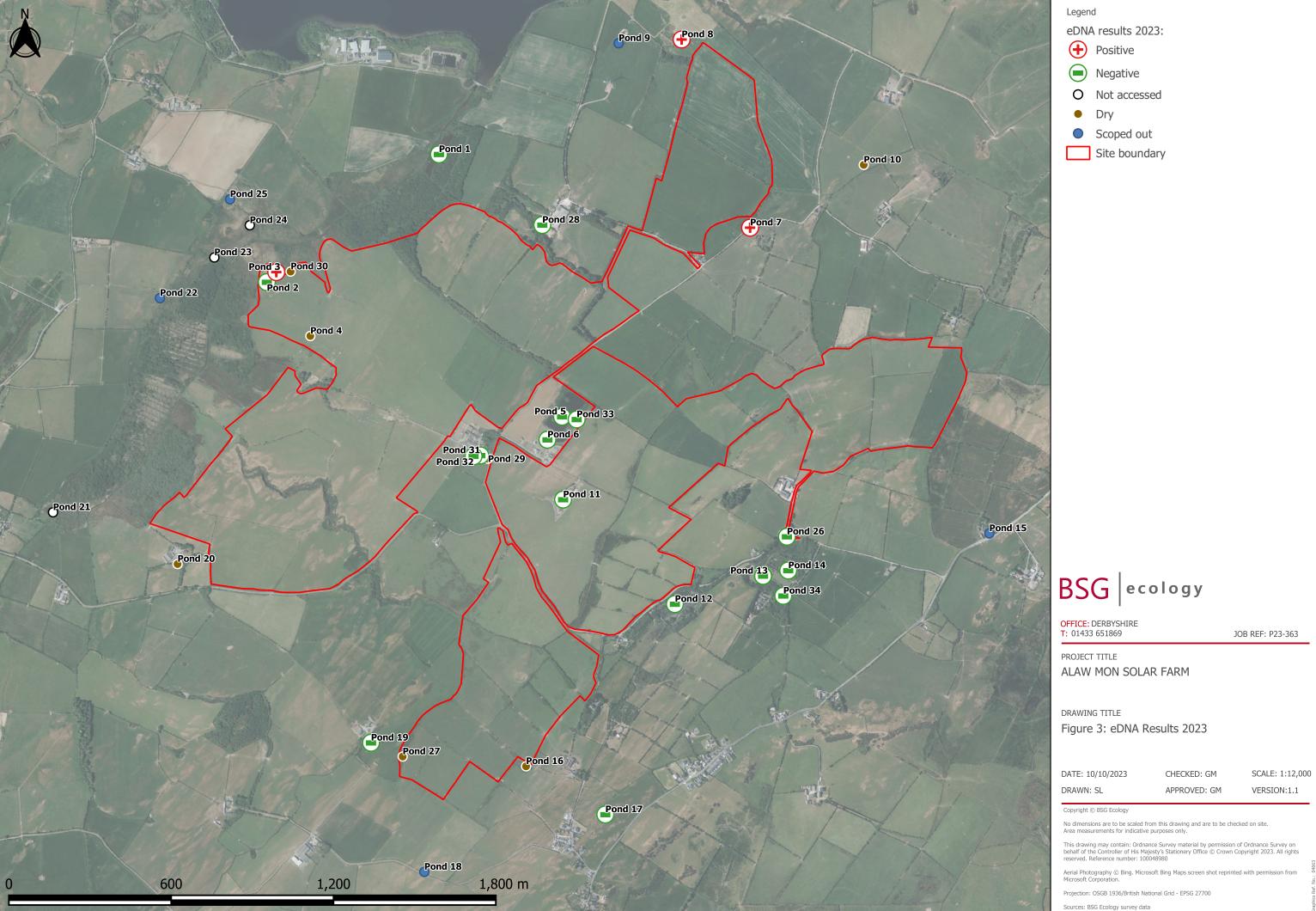
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# 5 Figures

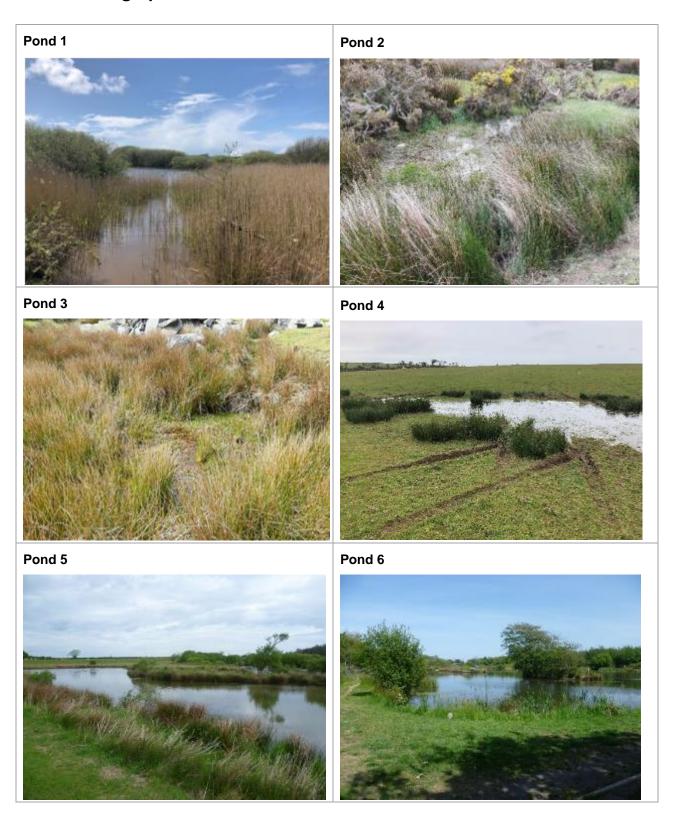








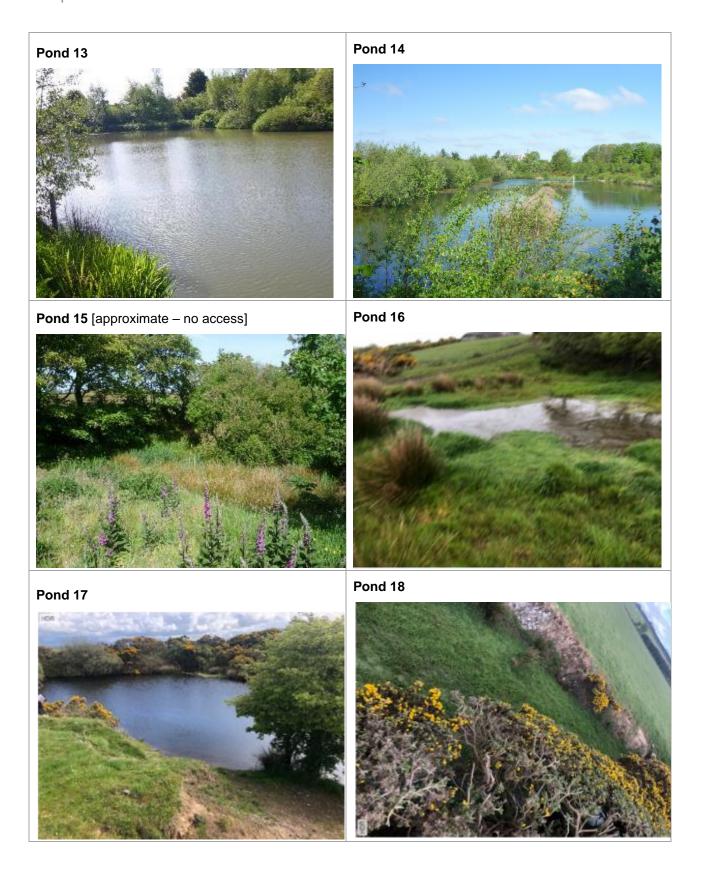
# 6 Photographs













# Pond 19 Pond 21 [Not accessed] Pond 25 [Not accessed]



Pond 24
[Not accessed]

Pond 22

[Not accessed]













# 7 Annexes



# Annex A: GCN Habitat Suitability Index (HSI) results

	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5		Pond 6	
HSI criteria	HSI Score		HSI Score		HSI Score		HSI Score		HSI Score		HSI Score	
Location	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5
Pond area (m²)	5700	0.8	217	0.4	260	0.5	40	0.05	4500	0.9	2500	0.8
Pond drying	Never	0.9	Sometimes	0.5	Frequently	0.1	Frequently	0.1	Never	0.9	Never	0.9
Water quality	Moderate	0.67	Good	1	Good	1	Good	1	Good	1	Good	1
Shade	60%	1	10%	1	0%	1	0%	1	0-60%	1	10	1
Waterfowl	Major	0.01	Absent	1	Absent	1	Absent	1	Major	0.01	Minor	0.67
Fish	Possible	0.67	Absent	1	Absent	1	Absent	1	Major	0.01	Minor	0.33
Pond density	8	1	9	0.9	9	0.9	10	1	4	1	6	1
Terrestrial habitat	Good	1	Moderate	0.67	Moderate	0.67	Moderate	0.67	Good	1	Good	1
Macrophyte	50%	0.36	100%	0.8	100%	0.8	0%	0.31	40%	0.7	20%	0.51
Overall HSI score	0.47	7	0.7	<b>'</b> 4	0.6	64	0.47		0.3	4	0.71	
GCN suitability	Poo	r	God	od	Aver	age	Poo	or	Poo	or	Goo	d



	Pond 7		Pond 8		Pond 9		Pond 11		Pond 12		Pond 13	
HSI criteria	HSI Score	Э	HSI Score		HSI Score		HSI Score		HSI Score		HSI Score	
Location	Zone B	0.5	Zone B	0.5	Zone B	0.5						
Pond area (m²)	360	0.6	800	0.99	800	0.98	4800	0.8	10000	0.8	5900	0.8
Pond drying	Never	0.9	Sometimes	0.5	Sometimes	0.5	Sometimes	0.5	Never	0.9	Never	0.9
Water quality	Good	1	Moderate	0.67	Moderate	0.67	Poor	0.33	Poor	0.33	Good	1
Shade	40%	1	5%	1	0%	1	10%	1	100%	1	10%	1
Waterfowl	Minor	0.67	Major	0.01	Major	0.01	Major	0.01	Major	0.01	Minor	0.67
Fish	Possible	0.67	Absent	1	Absent	1	Absent	1	Major	0.01	Major	0.01
Pond density	3	1	4	1	4	1	4	1	8	1	15	1
Terrestrial habitat	Good	1	Good	1	Good	1	Poor	0.33	Good	1	Good	1
Macrophyte	80%	1	0%	0.3	0%	0.3	95%	0.3195	90%	0.9	5%	0.36
Overall HSI score	0.8	1	0.4	5	0.46		0.3	37	0.3	32	0.49	
GCN suitability	Excel	lent	Pod	or	Poor		Poor		Poor		Poor	



	Pond 14		Pond 16		Pond 17		Pond 18		Pond 19	
HSI criteria	HSI Score		HSI Score		HSI Score		HSI Score		HSI Score	
Location	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5
Pond area (m²)	2850	0.8	180	0.35	960	0.97	6	0.05	24	0.05
Pond drying	never	0.9	frequently	0.1	never	0.9	frequently	0.1	sometimes	0.5
Water quality	good	1	poor	0.33	moderate	0.67	good	1	moderate	0.67
Shade	10%	1	20%	1	60	1	5	1	40	1
Waterfowl	minor	0.67	absent	1	minor	0.67	major	0.01	minor	0.67
Fish	major	0.01	absent	1	possible	0.67	absent	1	possible	0.67
Pond density	15	1	16	1	5	1	7	1	8	1
Terrestrial habitat	good	1	moderate	0.67	good	1	good	1	good	1
Macrophyte	40%	0.71	50%	0.81	5	0.36	0	0.31	10	0.41
Overall HSI score	0.53		0.5	6	0.72	2	0.3		0.52	
GCN suitability	Below Av	erage	Below A	verage	Goo	d	Pod	or	Below Av	erage



	Pond 20		Pond 26		Pond 28		Pond 31		Pond 32		Pond 33		Pond 34	
HSI criteria	HSI Score		HSI Score		HSI Score		HSI Score	)	HSI Score		HSI Score		HSI Score	
Location	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5	Zone B	0.5
Pond area (m²)	40	0.2	72 m²	1	280 m <sup>2</sup>	1	6	0.05	225	0.4	30	0.05	140	0.25
Pond drying	Sometimes	0.5	Frequently	0.1	Never	0.9	Rarely	1	Sometimes	0.5	Sometimes	0.5	Sometimes	0.5
Water quality	Good	1	Bad	0.01	Good	1	Bad	0.01	Bad	0.01	Good	1	Good	1
Shade	70%	0.8	0-60%	1	15%	1	5	1	0	1	60	1	30	1
Waterfowl	Absent	1	Absent	1	Major	0.01	Minor	0.67	Minor	0.67	Major	0.01	Minor	0.67
Fish	Absent	1	Absent	1	Minor	0.33	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67
Pond density	2	1	3	1	3	1	7	1	7	0.85	4	0.72	15	1
Terrestrial habitat	Good	1	Moderate	0.67	Moderate	0.67	Good	1	Good	1	Good	1	Good	1
Macrophyte	70%	0.9	60%	0.9	0%	0.3	0	0.31	40	0.71	0	0.31	40%	0.71
Overall HSI score	0.7		0.43		0.43		0.3	1	0.44		0.34		0.68	
GCN suitability	Good		Poor		Poor		Poo	r	Poor		Poor		Avera	ge



# Annex B: eDNA Laboratory results



Folio No: E7676 Report No: 1

Purchase Order: ECO 486 Client: ECO-SCOPE Contact: Richard Birch

# 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:09/06/2020Date Reported:19/06/2020Matters Affecting Results:None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC		Result	Positive Replicates
2132	Pond 20, Anglesey Solar BSG	SH 37284 83436	Pass	Pass	Pass		Negative	0
2133	Pond 28, BSG Solar	SH 38624 84690	Pass	Pass	Pass		Negative	0
2134	Pond 7, Solar BSG	SH 39371 84663	Pass	Pass	Pass		Positive	10
2135	Pond 29, Solar BSG	SH 38745 33978	Pass	Pass	Pass	Ī	Negative	0

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

Reported by: Sarah Evans

Approved by: Chris Troth





Folio No: E10424 Report No: 1

Purchase Order: P20-947

Client: BSG ECOLOGY LTD Contact: Emily McVean

# 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:19/05/2021Date Reported:28/05/2021Matters Affecting Results:None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5089	Alaw Mon Pond 11	SH386628362 5	Pass	Pass	Pass	Negative	0
5091	Alaw Mon Pond 31	SH383888385 4	Pass	Pass	Pass	Negative	0
5092	Alaw Mon Pond 6	SH389748391 6	Pass	Pass	Pass	Negative	0
5095	Alaw Mon Pond 5	SH386548393 5	Pass	Pass	Pass	Negative	0
5096	Alaw Mon Pond 32	SH383768384 0	Pass	Pass	Pass	Negative	0
5097	Alaw Mon Pond 4	SH377698428 9	Pass	Pass	Pass	Negative	0
5098	Alaw Mon Pond 26	SH395108353 8	Pass	Pass	Pass	Negative	0





5099	Alaw Mon Pond 1	SH382368483 4	Pass	Pass	Pass	Negative	0	
5100	Alaw Mon Pond 8	SH391128536 6	Pass	Pass	Pass	Negative	0	
5101	Alaw Mon Pond 17	SH388418252 8	Pass	Pass	Pass	Negative	0	
5102	Alaw Mon Pond 19	SH380178279 4	Pass	Pass	Pass	Negative	0	

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

Reported by: Chris Troth

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: E17412 Report No: 1

Purchase Order: P23-363

Client: BSG ECOLOGY LTD Contact: Gemma Watkinson

# 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:15/05/2023Date Reported:24/05/2023Matters Affecting Results:None

Lab Sample No.	Site Name	O/S Reference		SIC	DC		IC		Result	itive icates
2246	Alaw Mon - Pond 26	-		Pass	Pass	1	Pass		Negative	0
3454	Alaw Mon - Pond 32	-		Pass	Pass		Pass		Negative	0
3455	Alaw Mon - Pond 3	-		Pass	Pass		Pass	-	Positive	1
3456	Alaw Mon - Pond 19	-		Pass	Pass		Pass	-	Negative	0
3457	Alaw Mon - Pond 11	-		Pass	Pass		Pass		Negative	0
3458	Alaw Mon - Pond 33	-		Pass	Pass		Pass	-	Negative	0
3459	Alaw Mon - Pond 6	-	Ī	Pass	Pass		Pass	Ι	Negative	0





3460	Alaw Mon - Pond 5	-	Pass	Pass	Pass	Negative	0
3461	Alaw Mon - Pond 31	-	Pass	Pass	Pass	Negative	0
3462	Alaw Mon - Pond 7	-	Pass	Pass	Pass	Positive	12
3463	Alaw Mon - Pond 12	-	Pass	Pass	Pass	Negative	0
3464	Alaw Mon - Pond 13	-	Pass	Pass	Pass	Negative	0
3465	Alaw Mon - Pond 34	-	Pass	Pass	Pass	Negative	0
3466	Alaw Mon - Pond 16	-	Pass	Pass	Pass	Negative	0
3467	Alaw Mon - Pond 14	-	Pass	Pass	Pass	Negative	0
3468	Alaw Mon - Pond 17	-	Pass	Pass	Pass	Negative	0
3469	Alaw Mon - Pond 2	-	Pass	Pass	Pass	Negative	0
3480	Alaw Mon - Pond 8	-	Pass	Pass	Pass	Positive	4
3483	Alaw Mon - Pond 28	-	Pass	Pass	Pass	Negative	0
3485	Alaw Mon - Pond 1	-	Pass	Pass	Pass	Negative	0

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

**Reported by:** Jennifer Higginbottom

Approved by: Jackson Young

# **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.





# **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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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.

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# **Result:** Presence of GCN eDNA [Positive/Negative/Inconclusive]

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**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.





# **Annex C: Population Class Assessment Survey Results**

Pond reference: Pond 7	Torch			Bottle-trap	)		Egg search
	Male	Female	lmm.	Male	Female	lmm.	
(1) Date: 07/04/21							
(2) Date: 13/04/21							
(3) Date: 02/05/21							
(4) Date: 21/05/21							
(5) Date: 09/06/21					1		
(6) Date: 16/06/21							

Peak adult count for this pond in any one visit (by torch, trap or net):

<sup>1</sup> female great crested newt

Pond reference: Pond 7	Torch			Bottle-trap	)		Egg search
	Male	Female	lmm.	Male	Female	lmm.	
(1) Date: 25/04/23	2						(1, possible, but could not be closely examined)
(2) Date: 02/05/23	1	1					1
(3) Date: 15/05/23	1	1					
(4) Date: 23/05/23	1			2	1		
(5) Date: 30/05/23				1			
(6) Date: 07/06/22							

Peak adult count for this pond in any one visit (by torch, trap or net):

3 (2 male; 1 female)

Pond reference: Pond 3	Torch			Bottle-trap	)		Egg search
	Male	Female	lmm.	Male	Female	lmm.	
(5) Date: 30/05/23							
(6) Date: 07/06/22							

Peak adult count for this pond in any one visit (by torch, trap or net):

0

Pond reference: Pond 8	Torch			Bottle-trap			Egg search
	Male	Female	lmm.	Male	Female	lmm.	
(5) Date: 30/05/23							
(6) Date: 07/06/22							

Peak adult count for this pond in any one visit (by torch, trap or net):

0