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Great crested newt surveys for proposed new prison, bowling club, and boiler house on land adjacent to HMP Garth and HMP Wymott, Leyland

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Christchurch

9th September 2021

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Volume code: GHX0000 Project wide

Project: Garth Wymott 2

Document number: 608623-0000-CGO-GHX0000-XX-RP-X-0007

Issue number: P01

Suitability code: S3 Suitable for Review & Comment

Date of issue: 09/09/2021

Classification: Official

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Project: MoJ NPP Garth Wymott 2

Deliverable: Great crested newt surveys

Our reference: ALRZ GCN

Version: 1

Date: 9th September 2021

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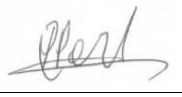
Checked by:	Rebecca Perl BA MA	
Approved by:	Dr Chris Gleed-Owen BSc (Hons) PhD MCIEEM	

Issued to: Mace Ltd. Anticipated circulation includes Mace internal use, Ministry of Justice, other appointed consultancies, Chorley Council, Natural England, and other relevant stakeholders.

Version control:

Version	Date	Summary of changes
1	09/09/2021	n/a

Non-technical summary

Introduction

CGO Ecology Ltd (CGO) was instructed by Mace Ltd, on behalf of the Ministry of Justice (MoJ), to conduct presence-absence and population surveys for great crested newt (GCN) at HMPs Garth and Wymott, Leyland, Lancashire. The Ministry of Justice proposes a development as part of its New Prisons Programme on land centred on (SD 502 205). The Local Planning Authority (LPA) is Chorley Council.

Methodology

Haycock and Jay Associates Ltd (HJA) undertook the surveys as subconsultants for CGO. All ponds on MoJ land and some ponds on third-party land were surveyed, but permission was not forthcoming from some landowners. Licensed surveyors conducted Habitat Suitability Index (HSI) assessments on 28 ponds. GCN presence-absence surveys were conducted on 16 ponds, following standard guidance, and led by licensed surveyors. GCN presence at one pond on site led to two additional population-assessment surveys. These surveys were conducted between 16th March and 24th May 2021, with at least half the visits during the mid-April to mid-May optimal period. GCN eDNA sampling was conducted on four off-site ponds, three on-site ditches, and one on-site pond, again by licensed surveyors.

Results

A small population of GCN is present in pond 39 (maximum count 12 GCN), around 90m south of the proposed bowling green location. Low GCN presence was also identified by eDNA in a ditch between the prisons, around 290m south of the proposed boiler house, and in two ponds among fishing lakes to the north of HMP Garth.

Conclusions, mitigation and enhancement recommendations

The GCN in pond 39 are likely to migrate west to the nearest woodland, rather than north; and are therefore unlikely to be impacted by the bowling green. The numbers of GCN likely to be affected by the boiler house or new prison development are likely to be very low (fewer than five individuals) or none at all. The fishing lakes are too far away, and isolated by woodland, to be affected by the new prison. Access to additional off-site ponds would not be likely to affect these conclusions.

Given that low numbers of GCN may be disturbed and/or harmed in the absence of mitigation, it must be considered whether avoidance measures, traditional licensed mitigation, or District Level Licence (DLL) scheme offsetting would be the most appropriate response.

The Natural England rapid risk assessment tool gave an ‘amber’ result, suggesting that an offence is likely in the absence of mitigation. However, the tool does not differentiate between large and small populations such as the case here.

On balance, avoidance measures could be used to mitigate the risk of harm to GCN, and prevent offences under the Habitats Regulations 2017 (as amended). Woodland, scrub, ditch, and pond clearance work would need to be done under licensed ecologist supervision. The bowling green footprint must be kept mown short, to minimise suitability for GCN. Bowling green construction could also use seasonal avoidance to prevent the need for fencing to protect low numbers of migrating newts.

Should a licensed mitigation approach be needed, the options are traditional methods (lengthy Natural England application process, drift fencing, bucket traps, bottle-traps, 30 days of capture, destructive search) or DLL route (scope entry into Natural England-led scheme, offset payment, no mitigation required).

The creation of six new ponds and grassland enhancement will yield a net gain in GCN breeding habitat.

Contents

1. Introduction

1.1. Background

CGO Ecology Ltd (CGO) was instructed by Mace Ltd, on behalf of the Ministry of Justice (MoJ), to conduct presence-absence and population surveys for great crested newt (GCN, *Triturus cristatus*) at HMPs Garth and Wymott, Leyland, Lancashire. The Ministry of Justice proposes a development as part of its New Prisons Programme on land centred on (SD 502 205). The Local Planning Authority (LPA) is Chorley Council.

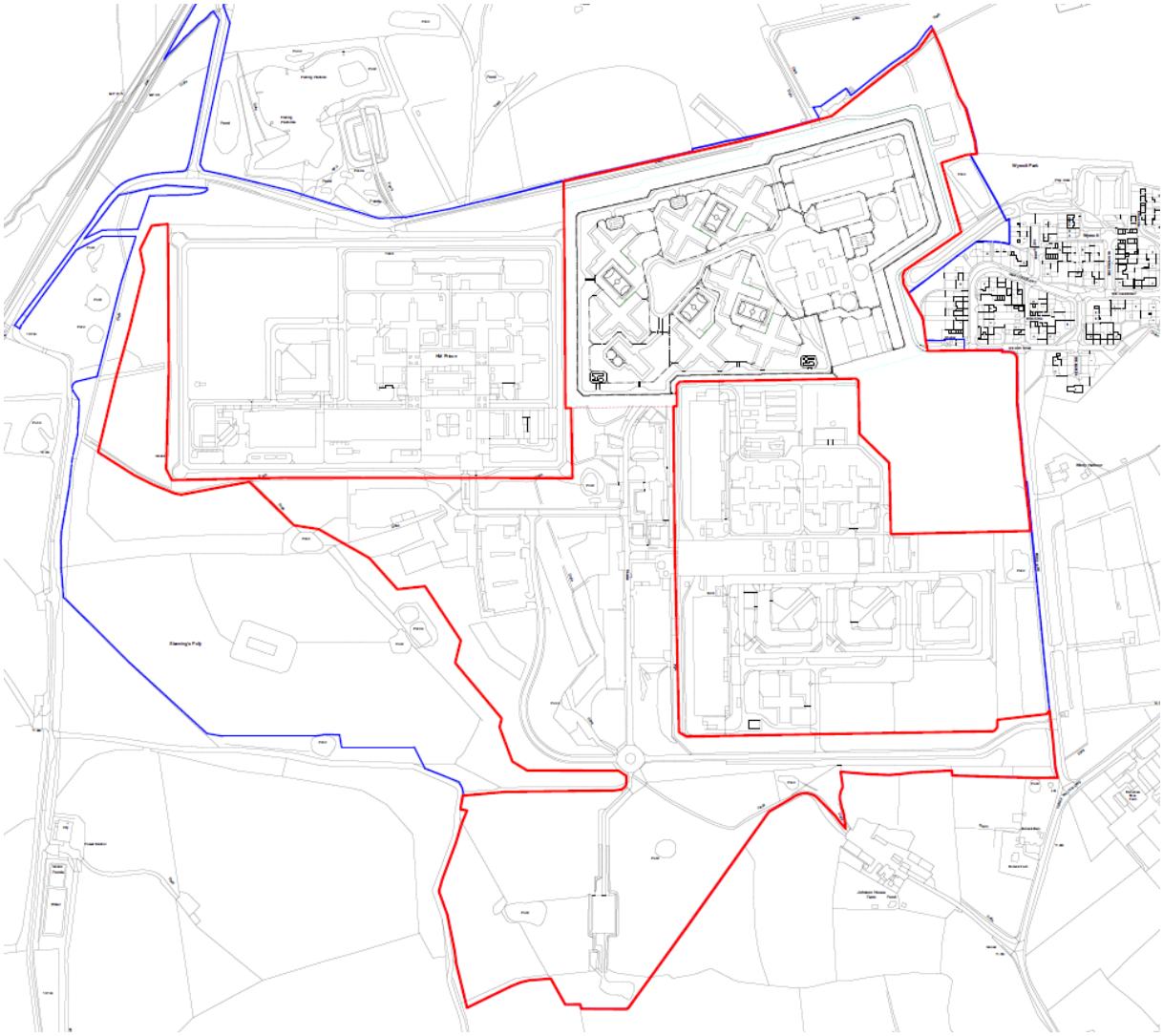


Figure 1 – Development site boundary (red line) and MoJ ownership boundary (blue line).

GCN is strictly protected by the Habitats Regulations 2017 (as amended) and Schedule 5 of the Wildlife and Countryside Act 1981 (as amended). It is also a Lancashire Key Species.

A Preliminary Ecological Appraisal (PEA) conducted by Ramboll (Molesworth, 2020). Additional areas to the north and east were subjected to a PEA by CGO (Gleed-Owen, 2021a). An Ecological Impact Assessment (EcIA) was conducted by CGO (Gleed-Owen, 2021b).

Haycock and Jay Associates Ltd (HJA) was commissioned to carry out the GCN surveys as subconsultant to CGO.

Dr Chris Gleed-Owen MCIEEM is Director and Principal Ecologist of CGO, and project lead for the Garth Wymott 2 GCN surveys and other phase 2 ecological surveys.

This report aims to follow CIEEM (2017) guidance and provide sufficient information to enable an EcIA conforming to CIEEM (2018) guidance.

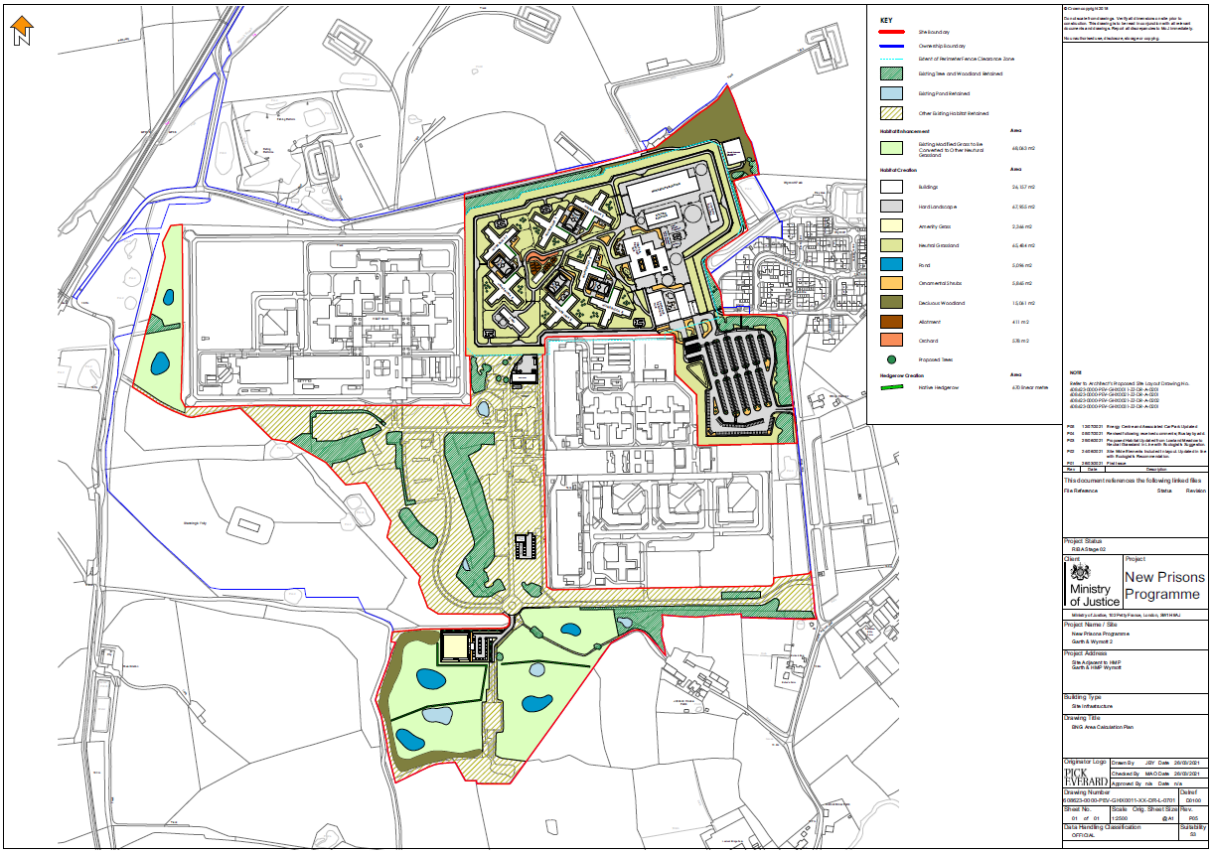


Figure 2 – Proposed development and landscaping plan, with habitat areas for BNG purposes, produced by Pick Everard.

1.2. Site context

The development site comprises predominantly land north of HMP Wymott, currently used as a sheep (*Ovis aries*) farm, stables, bowling club, boiler house, and utility buildings. The part within HMP Wymott is a sports field and disused assault course. The new boiler house will be between the existing prisons. The new bowling club will be on farmland to the south. Some woodland will be lost for the new prison development. Larger areas of woodland will remain.

The surrounding area is intensively farmed for a mixture of livestock and arable crops, but there are significant areas of woodland and other land uses. A large area of woodland lies to the southwest of the site, extending around the west and north of HMP Garth. There are major urban areas to the northeast (Leyland and Preston), and a network of minor roads, railway lines, villages, hamlets, and farms in all directions.

1.3. Proposed works

The proposed development is a hybrid planning application seeking: Outline planning permission (with all matters reserved except for access, parking and landscaping) for a new prison (up to 74,531.71m^2) within a secure perimeter fence following demolition of existing buildings and structures and together with associated engineering works; Outline planning permission for a replacement boiler house (with all matters reserved except for access); and Full planning permission for a replacement bowling green and club house.

2. Methodology

2.1. Desk study

The Ramboll PEA (Molesworth, 2020) included a 2km data search by Lancashire Environment Record Network (LERN). A GCN mitigation licence search of the Defra MAGIC Application (https://magic.defra.gov.uk/MagicMap.aspx) was also conducted.

Greater Manchester Ecology Unit (GMEU), which advises Chorley Council on ecology matters, was consulted. Mace Ltd liaised with MoJ to seek landowner permissions for survey access.

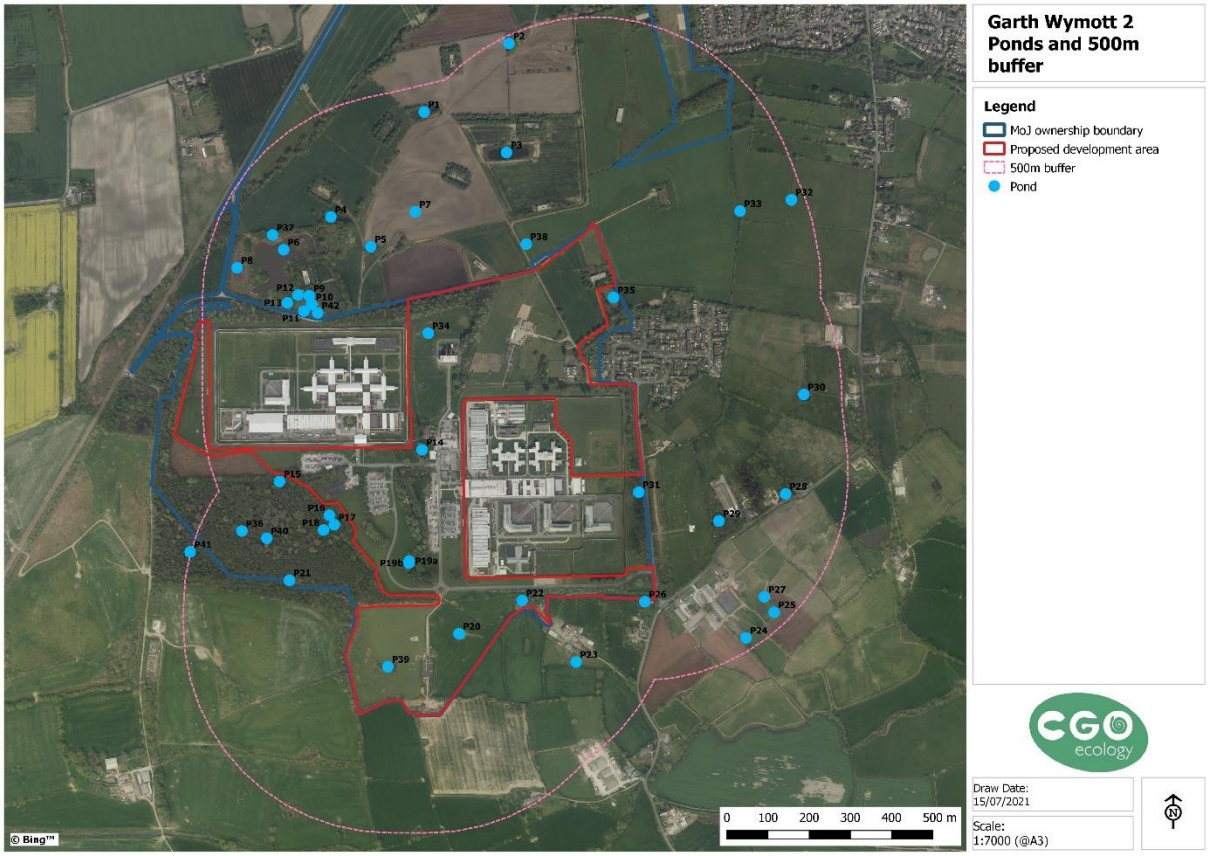


Figure 3 – Ponds within a 500m radius of the three proposed development areas within the red line application boundary.

2.2. Habitat Suitability Index

HJA and CGO conducted scoping surveys of the whole site in early February 2021. HJA conducted GCN Habitat Suitability Index (HSI) surveys of 28 ponds on MoJ land, four ponds on third-party land on 1-4th March 2021, and four third-party ponds on 6th May 2021, following standard guidance (ARGUK, 2010). MoJ sought third-party permissions for access to all off-site ponds, but this was not forthcoming for many. HSI assessment was therefore only possible on a few off-site ponds to the southeast of HMP Wymott, and four Prince Albert Angling Society (PAAS) ponds to the north of HMP Garth (part of the Ulnes Walton Biological Heritage Site). The surveyors were Will Steele (CL08 licensed), Rachel Whitaker (CL08 licensed), and Chris Gleed-Owen of CGO (CL09 licensed).

2.3. Presence-absence surveys

GCN presence-absence surveys were then conducted on all accessible ponds with HSI scores in the 'average', 'good', or 'excellent' Brady categories (cf. ARGUK, 2010). Following English Nature (2001) survey methodologies, four nocturnal visits using three techniques (typically torch, bottle-trap, egg-search) were conducted at 16 ponds. The surveys were conducted between 16th March and 24th May 2021, in all cases with at least half the visits taking place in the mid-April to mid-May optimal period (cf. English Nature, 2001).

Surveys were conducted in suitable weather conditions, times of day and night, and following accepted guidance (English Nature, 2001). All GCN and other amphibians were recorded, sexed where possible, their lifestages recorded, and other observations noted. All trapped animals were released immediately at the location of capture.

The surveyors were Will Steele (CL08 licensed), Rachel Whitaker (CL08 licensed), Clare Cashon (CL08 licensed), Richard Else, and Emma Sutton. The full results and survey information are provided in Appendices 1 and 2.

Evenings			Mornings	
Date	**Times**	**Weather**	**Date**	**Times**
16/03/2021	1800-2220	calm, dry, 8C	17/03/2021	0630-0830
17/03/2021	1730-2245	calm, dry, 8C	18/03/2021	0630-0945
18/03/2021	1710-2230	calm, dry, 9C	19/03/2021	0630-0830
19/03/2021	1745-2300	calm, dry, 9C	20/03/2021	0630-1000
08/04/2021	1800-2225	light wind, some rain, 8C	09/04/2021	0600-0930
19/04/2021	1800-0030	calm, dry, 8C	20/04/2021	0600-0800
20/04/2021	1830-2325	calm, dry, 9C	21/04/2021	0600-0800
26/04/2021	1750-2215	calm, dry, 11C	27/04/2021	0600-0850
27/04/2021	1740-2210	calm, dry, 8C	28/04/2021	0600-0820
28/04/2021	2130-2315	calm, dry, 8C		
04/05/2021	2119-2325	calm, rain before survey, 4C		
06/05/2021	1830-2200	light wind, some rain, 6C	07/05/2021	0630-0830
11/05/2021	1925-2310	calm, dry, 8C	12/05/2021	0600-0805
12/05/2021	1900-2215	calm, dry, 7C	13/05/2021	0600-0730
17/05/2021	1800-2235	calm, rain before survey, 11C	18/05/2021	0630-0730
24/05/2021	1900-1125	calm, slight rain, 8C	25/05/2021	0630-0730

Table 1 – Survey visits information.

Pond	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Notes
P14	35	30	35	35			
P15	35	30	30	30			
P16	25						Bottle trapping discontinued after Water shrew caught on first survey.
P17	25						Bottle trapping discontinued after Water shrew caught in adjacent P16.
P18	10						Bottle trapping discontinued after Water shrew caught in adjacent P16.
P19a	20	25	20	20			
P19b	10	15	10	10			
P21	20	20					Bottle trapping discontinued after Water shrew caught on second survey.
P26	30		30	30			Too cold to bottle trap on second visit.
P28	20	25	25	15			
P29	25	25	25	25			
P31	35	35		35			Too cold to bottle trap on third visit.
P34	35	20	20	20			
P36	25	20	10				Water level dropped a lot by third visit. Too cold to bottle trap on fourth visit.
P39	40	35	35	40	40	40	
P40	25	25	15				Too cold to bottle trap on fourth visit.

Table 2 – Number of bottle-traps used per pond.

2.4. Population size class surveys

As per guidance, GCN presence led to population size class assessment (an additional two survey visits) of one pond (P39). The additional surveys were conducted on 17th and 24th May 2021. When considered alongside the four presence-absence visits to P39, at least half the visits took place during the mid-April to mid-May optimal period.

Methodology followed the same standard techniques and timings as above (cf. English Nature, 2001). The surveyors were Will Steele (CL08 licensed), Rachel Whitaker (CL08 licensed), Clare Cashon (CL08 licensed), Richard Else, and Emma Sutton. The full results and survey information are provided in Appendices 2 and 3.

2.5. Environmental DNA

GCN environmental DNA (eDNA) sampling was conducted at four off-site ponds at the PAAS fishing lakes where permission was only granted for a single visit. (Permission for full presence-absence and population size class surveys was not forthcoming for these ponds).

Three ditches (D1-3) on MoJ land were surveyed for GCN eDNA, where other survey techniques were not practical due to lack dense emergent vegetation and lack of open water.

One pond on MoJ land (P22) was surveyed for eDNA as a control to check the validity of the presence-absence surveys conducted on it.

The surveyors were Rachel Whitaker (CL08 licensed) and Chris Gleed-Owen (CL09 licensed). The samples were processed by Cellmark in Abingdon. The resulting reports are attached in Appendices 3 and 4.

2.6. Incidental observations

Other notable wildlife, including mammals, birds, fish, and invertebrates observed during the GCN surveys, was also recorded.

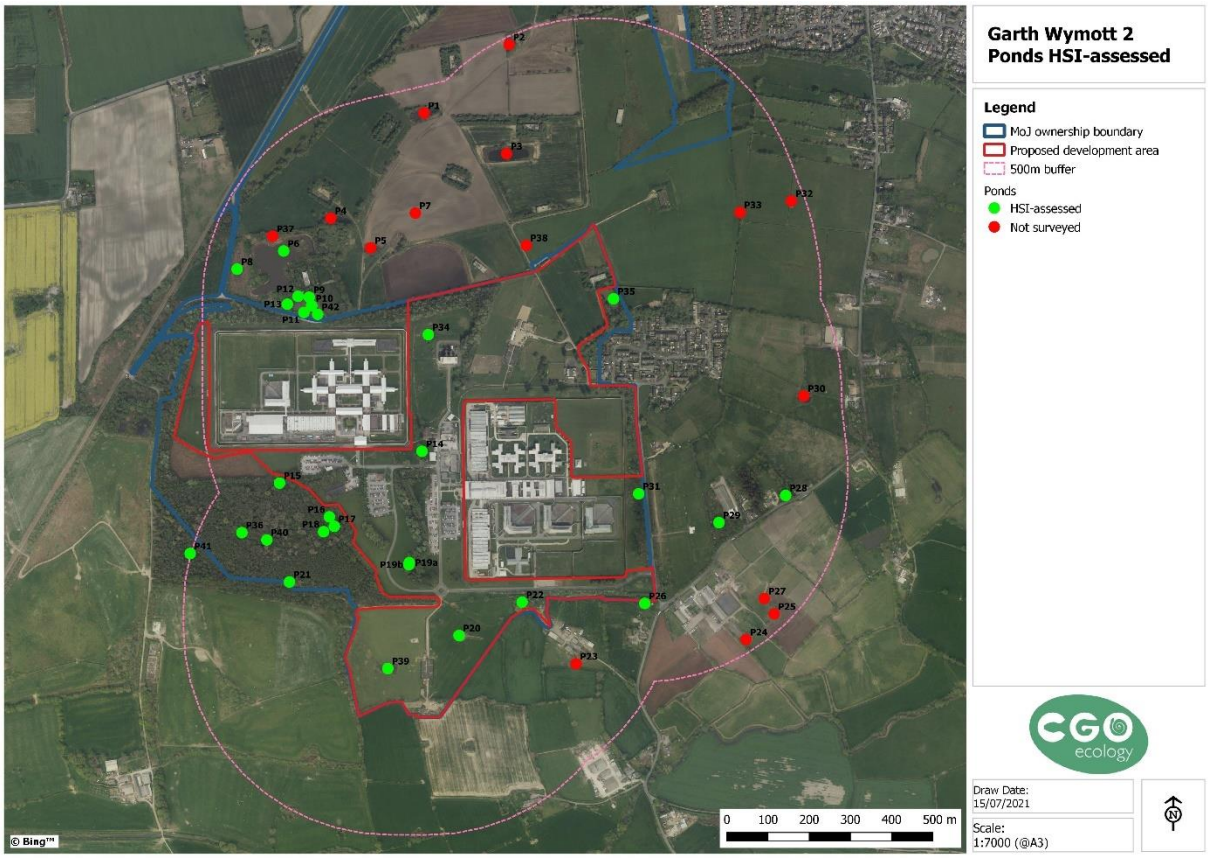


Figure 4 – Ponds with access permission to conduct an HSI assessment (21 of 42 ponds within 500m).

2.7. Limitations

The survey sought access to all ponds within 500m of the development, but access permission was not forthcoming for many ponds on third-party land. This amounts to 21 of 42 ponds within

500m, or 50% of the total. However, within 250m of the development areas, access permission was lacking for only 3 out of 23 ponds, which corresponds to only 13% of them. Of these three ponds, two of them are separated by a woodland ecological barrier from the proposed new prison development, and only one of them (P38) is within the Zone of Influence (ZoI) of the development. Pond 38 is around 70m north of the proposed new prison, adjacent to a ditch and hedge line. The nearest ponds to it are 250-300m away. On balance, the chances of an isolated population of GCN existing in this pond, when nearly all other surveyed ponds are negative, are slim.

Air and water temperatures were too cold for bottle-trapping on the evenings/nights of 28th April and 4th May 2021. However, this did not affect the overall presence-absence results for the ponds in question.

Incidental trapping and death of water shrew (*Neomys fodiens*) occurred on two occasions, preventing further use of bottle-traps at ponds P16 and adjacent ponds P17-18 after visit 1, and P21 after visit 2. This is not considered a significant impact on the results.

P22 was not accessible due to dense surrounding vegetation and poor water visibility, and therefore was not subjected to presence-absence surveys. GCN is unlikely to inhabit this pond in any case, despite an 'average' HSI score.

3. Results

3.1. Desk study

The Ramboll PEA (Molesworth, 2020) described the data obtained from LERN and MAGIC. Both sources were revisited during this desk study, and the MAGIC data was updated.

MAGIC shows that Natural England has issued eight EPS mitigation licences within 5km for GCN, three of them within 2km. The nearest is 1.3km east of development activity; the others are 1.4km southwest and 2.6km west of the nearest development areas. MAGIC also shows nine GCN occurrence records from surveys. The nearest is 1.4km east. Another is 1.9km north. The others are 3-5km away. There are also two recent GCN records from surveys at 1.4km east and 2.1kkm north.

Figure 5 – Defra MAGIC map showing GCN records (red dots) from Natural England's mitigation licence database and targeted surveys within 2km of development application boundary.

The LERN data includes 60 records of GCN within 2km. These include 18 records from 2006 at two ponds in woodland immediately outside the west edge of the red line boundary, to the west of HMP Garth. The LERN dataset describes them as part of “Ulnes Walton Landfill Site”, presumably mitigation/receptor ponds. They are 500m to 800m from the proposed development areas, separated by significant ecological barriers (HMP Garth and large woodland blocks). The ponds are densely wooded now, and could not support GCN; but they could have been the source for GCN in the PAAS fishing lakes around 200m to the northeast. Other records from the same landfill site are spread across a triangle of land extending around 1km to the south.

The Leyland Waste Water Treatment Works 1.3km to the east produced 18 GCN records in 2007, again presumably mitigation-related records.

None of the LERN GCN records are within the ZoI of the proposed development areas.

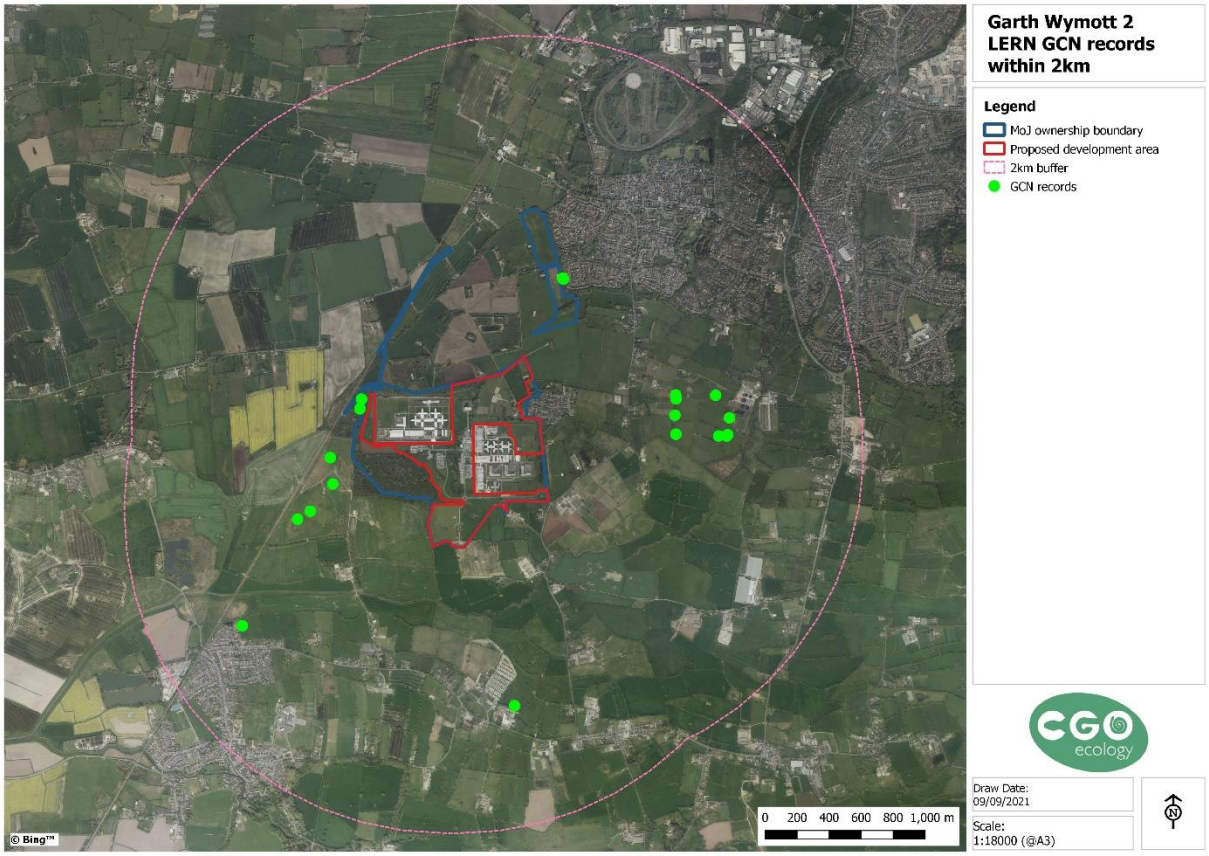


Figure 6 – GCN records within 2km from LERN’s database.

3.2. Habitat Suitability Index

Of the 28 ponds assessed for HSI, the following Brady classes (ARGUK, 2010) were allocated: two ponds were ‘poor’, three were ‘below average’, 13 were ‘average’, six were ‘good’, and four were ‘excellent’.

All ponds with access permission and a Brady class of ‘average’ or above (HSI score of 0.60 or above) were put forward for GCN presence-absence surveys.

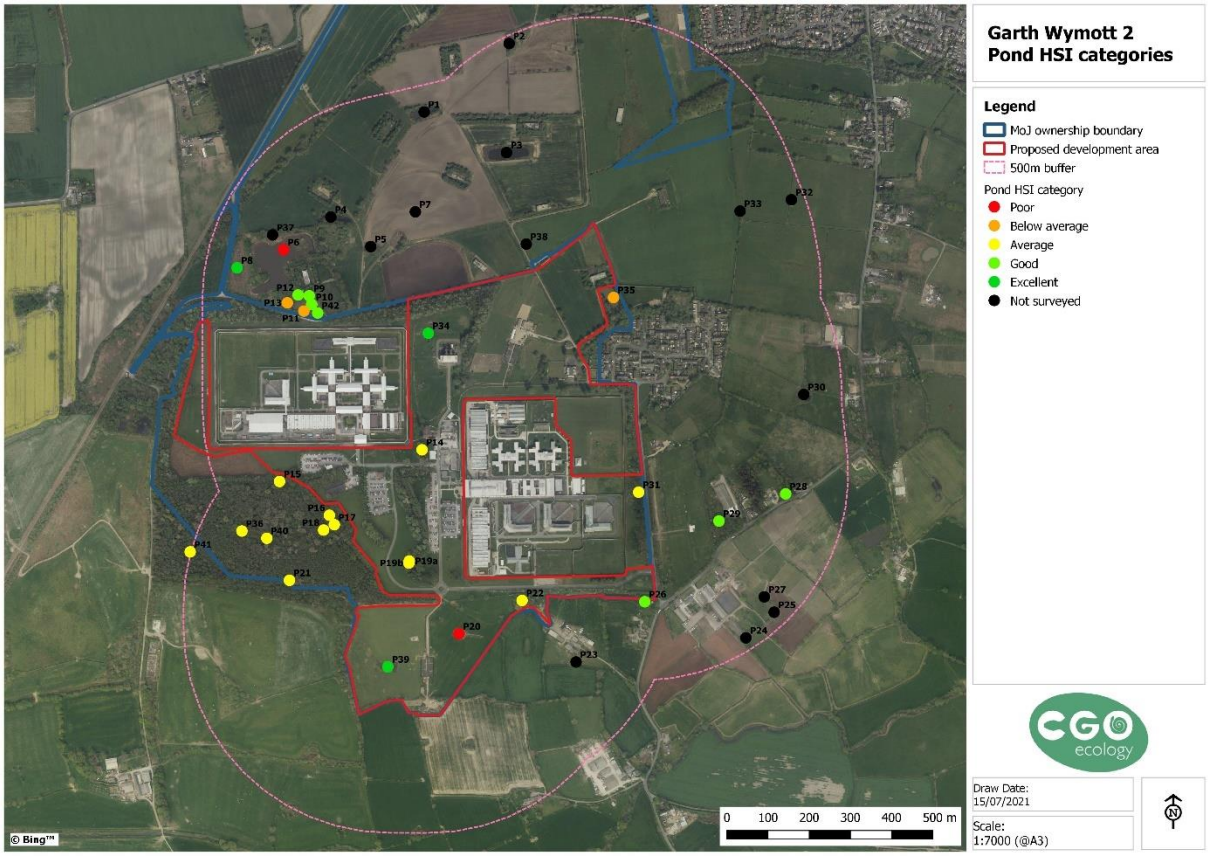


Figure 7 – Schematic plan of GCN HSI results.

Pond	Grid reference	Location	Ownership	HSI score	HSI Brady	eDNA survey	Pres-abs surveys
1	SD 50307 21409	Large pond within farmland.	Unknown third party			No access	No access
2	SD 50504 21572	Within farmland. Appears dry from distance.	Unknown third party			No access	No access
3	SD 50498 21312	Large pond within farmland.	Unknown third party			No access	No access
4	SD 50081 21148	Farmland, might be associated with fishing lake	Unknown third party			No access	No access
5	SD 50170 21079	Small pond within farmland. Appears dry.	Unknown third party			No access	No access
6	SD 49957 21071	Fishing lake	PAAS	0.34	Poor	No access	No access
7	SD 50276 21162	Located within farmland	Unknown third party			No access	No access
8	SD 49844 21027	Fishing lakes. Appears dry.	PAAS	0.86	Excellent	Yes	No access
9	SD 50019 20959	Small pond among fishing lakes.	PAAS	0.78	Good	Yes	No access

10	SD 50025 20938	Small pond among fishing lakes.	PAAS	0.79	Good	Yes	No access
11	SD 50006 20922	Fishing lakes	PAAS	0.54	Below average	No access	No access
12	SD 49992 20961	Small pond among fishing lakes.	PAAS	0.7	Good	No access	No access
13	SD 49966 20942	Small pond among fishing lakes.	PAAS	0.56	Below average	No access	No access
14	SD 50293 20585	Ornamental pond between prisons.	MoJ	0.65	Average		Yes
15	SD 49947 20508	Within woodland	MoJ	0.66	Average	Yes	Yes
16	SD 50068 20426	Within woodland	MoJ	0.68	Average		Yes
17	SD 50080 20403	Within woodland	MoJ	0.65	Average		Yes
18	SD 50054 20390	Within woodland	MoJ	0.66	Average		Yes
19a	SD 50262 20315	Within ditch, in woodland belt	MoJ	0.63	Average		Yes
19b	SD 50262 20315	Within ditch, in woodland belt	MoJ	0.61	Average		Yes
20	SD 50383 20138	Farmland	MoJ	0.49	Poor	No access	No access
21	SD 49971 20268	Within woodland	MoJ	0.67	Average		Yes
22	SD 50536 20219		?	0.66	Average	No access	No access
23	SD 50659 20073	Within paddock	Unknown third party			No access	No access
24	SD 51083 20130	Farmland	Unknown third party			No access	No access
25	SD 51149 20191	Farmland	Unknown third party			No access	No access
26	SD 50834 20216	Within woodland	Unknown third party	0.79	Good		Yes
27	SD 51126 20231	Farmland	Unknown third party			No access	No access
28	SD 51176 20478	Within woodland	Unknown third party	0.74	Good		Yes
29	SD 51014 20412	Within woodland	Unknown third party	0.72	Good		Yes
30	SD 51219 20718	Farmland	Unknown third party			No access	No access
31	SD 50819 20482	Roadside	MoJ	0.69	Average		Yes
32	SD 51191 21191	Farmland	Unknown third party			No access	No access
33	SD 51069 21165	Farmland	Unknown third party			No access	No access
34	SD 50308 20868	Pasture, within proposed prison area	MoJ	0.85	Excellent		Yes

35	SD 50758 20955	Wymott fishing lake	MoJ	0.51	Below average		
36	SD 49856 20404	Within woodland	MoJ	0.7	Average		Yes
37	SD 49935 21105	Fishing lakes.	Unknown third party			No access	No access
38	SD 50554 21083	Farmland	Unknown third party			No access	No access
39	SD 50210 20058	Farmland	MoJ	0.83	Excellent		Yes
40	SD 49916 20370	Within woodland	MoJ	0.68	Average		Yes
41	SD 49730 20337	Flooded woodland, poss over 2ha	MoJ	0.64	Average		
42	SD 50039 20919	Fishing lakes. Separated from P11.	PAAS	0.72	Excellent	Yes	No access
D1	SD50272062 to SD50332091	Ditch network in pasture, within proposed prison area	MoJ	n/a	n/a	Yes	
D2	SD50372071 to SD50572077	Ditch network in pasture, within proposed prison area	MoJ	n/a	n/a	Yes	
D3	SD50252030 to SD50302035	Section of ditch within woodland, connected to P19a/b	MoJ	n/a	n/a	Yes	

Table 3 – Pond details, survey methods applied and presence-absence results (GCN positive green, negative red).

3.3. Presence-absence and population size class

A small population of GCN is present in pond 39 (P39) at SD 50210 20058. The maximum count was 12 GCN on 17th May 2021 (two female, five male, five unsexed), obtained from torchlight survey. Bottle-trap counts were higher than torchlight survey on some visits. P39 is located around 90m south of the proposed bowling club, 500m south of the proposed boiler house, and 600m south of the proposed new prison.

15 other ponds returned no GCN presence. Low numbers of common toad (*Bufo bufo*), common frog (*Rana temporaria*), and smooth newt (*Lissotriton vulgaris*) were variously recorded in some ponds. Stickleback (*Gasterosteus aculeatus*) was recorded in several ponds.

Note that four presence-absence surveys of pond 19 yielded no GCN presence, but eDNA of the ditch system to which it is connected yielded a positive result (see 3.4. below).

3.4. Environmental DNA

GCN presence was identified by eDNA survey in a ditch (D3), about 290m south of the proposed boiler house, and 340m south of the new prison development. Ponds P19a and P19b are part of this ditch in the sinuous plantation woodland belt between the car parks for HMP Wymott to the east and HMP Garth to the west. Four nocturnal surveys failed to identify GCN presence in P19, and the Cellmark assay detected GCN eDNA in only four out of 12 replicates.

This suggests that only a few GCN are present in this ditch, and could be scattered anywhere along its length. The standard eDNA sampling technique involves taking 20 samples from around the shore of a pond, and in this case, the 20 samples were taken from along its length.

GCN presence was also detected by eDNA in the PAAS fishing ponds north of HMP Garth (part of the Ulnes Walton BHS local site). Four ponds were deemed GCN-worthy by HSI (P8-10, P42), of which two (P8, P42) proved positive for GCN. The Cellmark analyses only found GCN DNA in two out of 12 replicates for these ponds, which suggests a low population density.

Two ditches within the proposed new prison area (D2, D3) were negative for GCN eDNA. Pond P15, which was used as a control, was also negative for GCN eDNA.

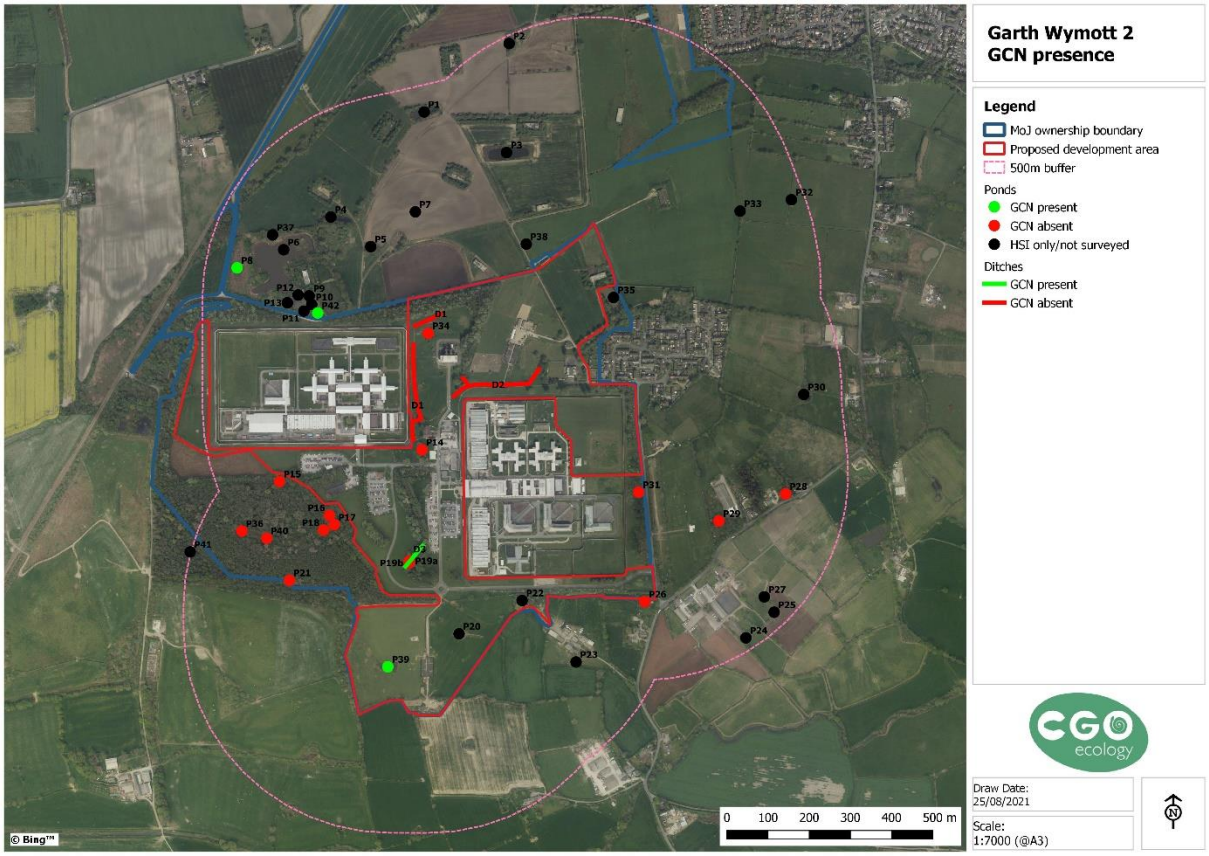


Figure 8 – GCN presence-absence in ponds (dots) and ditches (lines) surveyed by nocturnal surveys and/or eDNA sampling.

4. Baseline Ecological Conditions

A small population of GCN (peak count 12) is present in pond 39 within the red line boundary, around 90m south of the proposed bowling club. This is the only one out of 16 ponds surveyed for GCN presence-absence that produced a positive result. It is within the ZoI of the proposed bowling club.

A small but undefined number of GCN are also present in a ditch between the proposed bowling club and boiler house developments. It is likely to be outside the ZoI of both, due to distance, intervening ecological barriers and woodland areas providing favourable habitat.

A small but undefined number of GCN are also present in the PAAS fishing lakes site to the north of HMP Garth. Its distance and separation by a band of woodland from the proposed new prison make it unlikely to be within the ZoI.

There is no evidence from MAGIC and LERN datasets of additional GCN presence within the ZoI. On balance, the number of GCN within the ZoI is likely to be fewer than five.

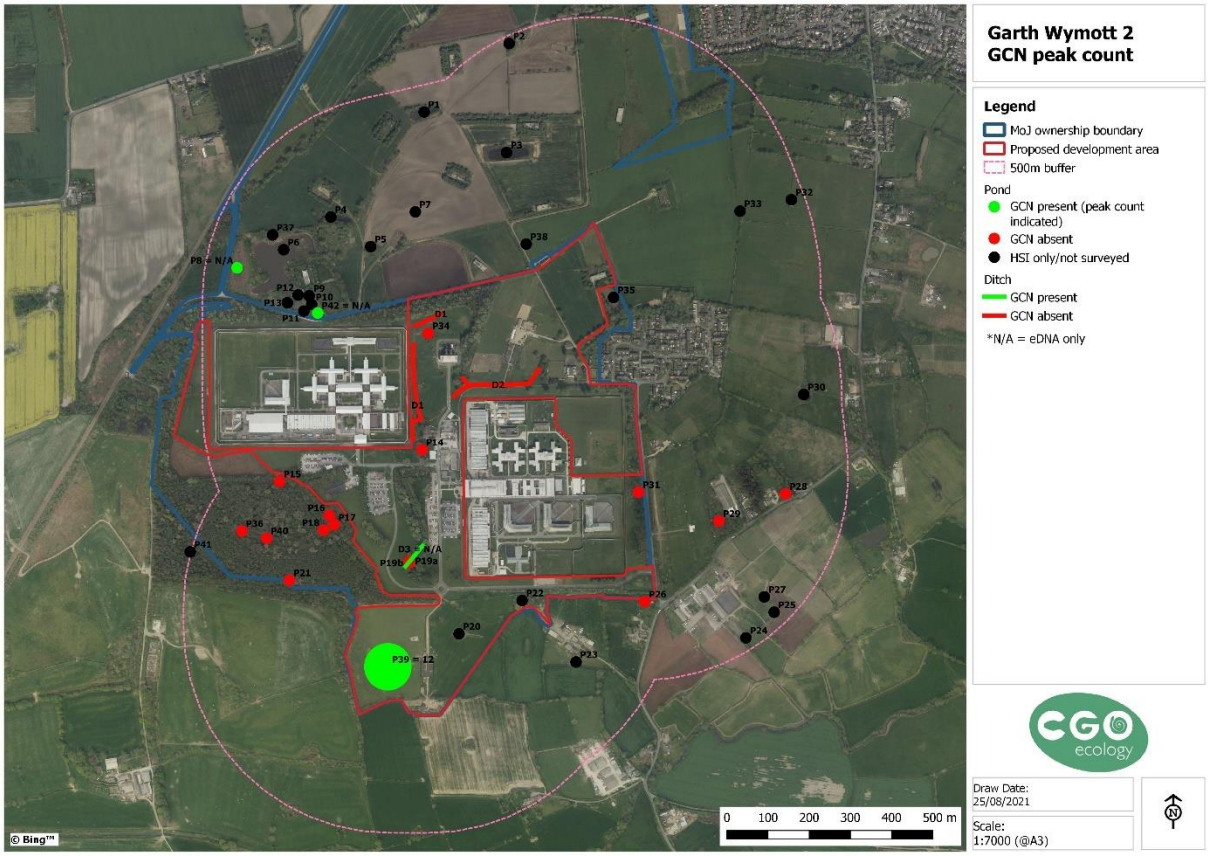


Figure 9 – Schematic plan of GCN presence-absence and peak count.

5. Impact Assessment

The combination of field survey in 2021, and previous data from MAGIC and LERN datasets, suggest that the number of GCN within the ZoI is likely to be fewer than five.

The GCN in pond 39 are likely to migrate west to the nearest woodland, rather than north; and are therefore unlikely to be impacted by the bowling green. P39 is 90m south of bowling club, and the Natural England rapid risk assessment tool considers this to be at risk of an offence under the Habitats Regulations 2017 (as amended). However, given the small size of the P39 population, the tool's prediction can be downgraded to a low likelihood of an offence.

Any GCN in ditch 3 are unlikely to be affected by the boiler house or new prison development. Their numbers are undetectably small by standard methods (zero presence detected by nocturnal surveys), and only a minor eDNA presence was detected (2 out of 12 replicates). The boiler house is over 250m away, and the prison even further.

The PAAS fishing lakes are too far away, and isolated by woodland, from the proposed new prison. The distance between the PAAS lakes and prison is over 250m, and the intervening woodland is likely to be an ecological barrier. Any GCN leaving the PAAS lakes in their terrestrial phase would encounter woodland in which to forage and hibernate within 50m, and newts are unlikely to travel 250m east through woodland to the prison site.

Access to additional off-site ponds would not be likely to affect these conclusions. Only pond 38 on third-party land to the north of the proposed new prison site is of interest. However, its location and relative isolation suggests that it would not hold GCN, or would only hold a small population. It presents a low risk of GCN being impacted by the proposed new prison.

It is likely that larger GCN populations exist in surrounding areas within 5km of the site, possibly within 2km. The general picture is of a patchy, relict distribution of GCN in the local landscape.

6. Mitigation

Given that low numbers of GCN (fewer than five) may be disturbed and/or harmed in the absence of mitigation, it must be considered whether avoidance measures, traditional licensed mitigation, or District Level Licence (DLL) scheme offsetting would be the most appropriate response.

The Natural England rapid risk assessment tool in the GCN licence method statement template gave an 'amber' result, suggesting that an offence is likely in the absence of mitigation. However, the tool does not differentiate between large and small populations such as the case here.

On balance, avoidance measures could be used to mitigate the risk of harm to GCN, and prevent offences under the Habitats Regulations 2017 (as amended). Woodland, scrub, ditch, and pond clearance work would need to be done under licensed ecologist supervision. The bowling green footprint must be kept mown short, to minimise suitability for GCN. Bowling green construction could also use seasonal avoidance to prevent the need for fencing to protect low numbers of migrating newts.

Should a licensed mitigation approach be needed, the options are traditional methods (lengthy Natural England application process, drift fencing, bucket traps, bottle-traps, 30 days of capture, destructive search) or DLL route (scope entry into Natural England-led scheme, offset payment, no mitigation required).

7. Residual effects and enhancements

Once the agreed mitigation route is implemented, no residual effects on GCN are anticipated. The proposed creation of six new ponds within the wider enhancement areas of the application area, replacing loss of one non-GCN pond and two non-GCN ditches, will bring a significant net gain of GCN breeding and terrestrial habitat.

The lost pond (P34) and ditches (D1-2) are within the proposed new prison area, and have no GCN presence within 250m. They are ecologically isolated from other GCN breeding habitat. Whereas, four of the new ponds will be in the area around pond 39 which already has a GCN presence, thus creating a network of GCN breeding ponds and intervening enhanced neutral grassland.

Two other new ponds will be to the west of HMP Garth, adjacent to where the Ulnes Walton Landfill Site population existed in 2006. It is possible that emigrants from that population colonised newly-created ponds at the PAAS fishing lakes. Survivors in the woodland to the west of HMP Garth could potentially colonise the two proposed ponds.

The creation of six new ponds could allow connectivity between currently-disjunct populations. The new ponds will be fish-free, and will be managed to benefit GCN. The existing ponds on the MoJ estate could also be treated to eradicate the stickleback presence that was recorded in most of them.

8. References

CIEEM (2017) *Guidelines for Ecological Report Writing.* Chartered Institute of Ecology and Environmental Management, Winchester.

CIEEM (2018) *Guidelines for Ecological Impact Assessment in the UK and Ireland: Terrestrial, Freshwater, Coastal and Marine. Version 1.1 (Sept 2019).* Chartered Institute of Ecology and Environmental Management, Winchester.

English Nature (2001) *Great Crested Newt Mitigation Guidelines.* English Nature, Peterborough.

Gleed-Owen, C. (2021a) *Preliminary Ecological Appraisal of additional areas for proposed new prison, bowling club, and boiler house on land adjacent to HMP Garth and HMP Wymott, Leyland.* CGO Ecology Ltd, Christchurch.

Gleed-Owen, C. (2021b) *Ecological Impact Assessment for proposed new prison, bowling club, and boiler house on land adjacent to HMP Garth and HMP Wymott, Leyland.* CGO Ecology Ltd, Christchurch.

Molesworth, J. (2020) *Albatross & Razorbill. Preliminary Ecological Appraisal.* Ramboll, Exeter.

9. Appendices

Appendix 1 – GCN Habitat Suitability Index results

Appendix 2 – GCN presence-absence and population size class survey results

Appendix 3 – eDNA results for ditches 1, 2, 3

Appendix 4 – eDNA results for ponds 8, 9, 10, 42

Appendix 5 – eDNA results for pond 15

Appendix 1 – GCN Habitat Suitability Index assessment results

Pond number	P6		P8		P9		P10		P11		P12		P13	
Grid reference	SD4995721071		SD4984421027		SD5001920959		SD5002520938		SD5000620922		SD4999220961		SD4996620942	
SI1 location	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1
SI2 pond area	>2000		2000	0.8	100	0.2	100	0.2	800	0.99	100	0.2	100	0.2
SI3 pond drying	Never drie	0.9	Rarely drie	1	Rarely drie	1	Rarely drie	1	Never drie	0.9	Sometimes	0.5	Sometime	0.5
SI4 water quality	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Good	1	Moderate	0.67	Poor	0.33
SI5 shade	0.1	1	0.05	1	0.3	1	0	1	0.2	1	0.6	1	1	0.2
SI6 fowl	Major	0.01	Minor	0.67	Absent	1	Absent	1	Minor	0.67	Minor	0.67	Absent	1
SI7 fish	Major	0.01	Possible	0.67	Possible	0.67	Possible	0.67	Major	0.01	Possible	0.67	Absent	1
SI8 ponds	>12	1	>12	1	12	0.98	>12	1	>12	1	12	0.98	>12	1
SI9 terrestrial habitat	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI10 macrophytes	0.65	0.95	0.9	0.9	0.9	0.9	0.8	1	0.05	0.35	0.7	1	0.2	0.5
Product		0.00		0.22		0.08		0.09		0.00		0.03		0.00
HSI (10th root)		0.34		0.86		0.78		0.79		0.54		0.70		0.56
Brady category		Poor		Excellent		Good		Good		Below average		Good		Below ave

Pond number	P14		P15		P16		P17		P18		P19a		P19b	
Grid reference	SD5029320585		SD4994720508		SD5006820426		SD5008020403		SD5005420390		SD5026220315		SD5026120310	
SI1 location	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1
SI2 pond area	400	0.8	700	1	1200	0.93	1600	0.87	300	0.6	200	0.4	150	0.3
SI3 pond drying	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9
SI4 water quality	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67
SI5 shade	0.95	0.3	1	0.2	1	0.2	1	0.2	1	0.2	1	0.2	1	0.2
SI6 fowl	Minor	0.67	Minor	0.67	Absent	1	Minor	0.67	Absent	1	Absent	1	Absent	1
SI7 fish	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67
SI8 ponds	>12	1	>12	1	>12	1	>12	1	>12	1	>12	1	>12	1
SI9 terrestrial habitat	Moderate	0.67	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI10 macrophytes	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
Product		0.01		0.02		0.02		0.01		0.01		0.01		0.01
HSI (10th root)		0.65		0.66		0.68		0.65		0.66		0.63		0.61
Brady category		Average		Average		Average		Average		Average		Average		Average

Pond number	P20		P21		P22		P26		P28		P29		P31	
Grid reference	SD5038320138		SD4997120268		SD5053620219		SD5083420216		SD5117620478		SD5101420412		SD5081920482	
SI1 location	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1
SI2 pond area	700	1	2000	0.8	600	1	200	0.4	350	0.7	300	0.6	1100	0.94
SI3 pond drying	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9	Rarely drie	1	Never drie	0.9
SI4 water quality	Moderate	0.67	Moderate	0.67	Moderate	0.67	Good	1	Moderate	0.67	Good	1	Moderate	0.67
SI5 shade	0	1	1	0.2	1	0.2	0.8	0.6	0.5	1	0.95	0.3	0.85	0.5
SI6 fowl	Major	0.01	Absent	1	Absent	1	Minor	0.67	Minor	0.67	Minor	0.67	Minor	0.67
SI7 fish	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67	Minor	0.33	Possible	0.67	Possible	0.67
SI8 ponds	>12	1	>12	1	>12	1	>12	1	>12	1	12	0.98	>12	1
SI9 terrestrial habitat	Moderate	0.67	Good	1	Moderate	0.67	Good	1	Good	1	Good	1	Moderate	0.67
SI10 macrophytes	0	0.3	0	0.3	0	0.3	0.7	1	0.2	0.5	0.2	0.5	0	0.3
Product		0.00		0.02		0.02		0.10		0.05		0.04		0.03
HSI (10th root)		0.49		0.67		0.66		0.79		0.74		0.72		0.69
Brady category		Poor		Average		Average		Good		Good		Good		Average

Pond number	P34		P35		P36		P39		P40		P41		P42	
Grid reference	SD5030820868		SD5075820955		SD4988220357		SD5021020058		SD4991620370		SD4973020337		SD5003920919	
SI1 location	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1	Zone A	1
SI2 pond area	500	1	1600	0.87	700	1	600	1	400	0.8	>2000		100	0.2
SI3 pond drying	Never drie	0.9	Never drie	0.9	Rarely drie	1	Never drie	0.9	Never drie	0.9	Never drie	0.9	Never drie	0.9
SI4 water quality	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Good	1
SI5 shade	0.1	1	0.05	1	1	0.2	0	1	1	0.2	1	0.2	0.05	1
SI6 fowl	Minor	0.67	Minor	0.67	Absent	1	Minor	0.67	Absent	1	Minor	0.67	Minor	0.67
SI7 fish	Possible	0.67	Major	0.01	Possible	0.67	Possible	0.67	Possible	0.67	Possible	0.67	Minor	0.33
SI8 ponds	>12	1	>12	1	>12	1	>12	1	>12	1	>12	1	>12	1
SI9 terrestrial habitat	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI10 macrophytes	0.4	0.7	0.05	0.35	0	0.3	0.3	0.6	0.05	0.35	0.05	0.35	0.6	0.9
Product		0.19		0.00		0.03		0.16		0.02		0.02		0.04
HSI (10th root)		0.85		0.51		0.70		0.83		0.68		0.64		0.72
Brady category		Excellent		Below average		Average		Excellent		Average		Average		Good

Appendix 2 – GCN presence-absence/population size class survey results

Count is given for each species, prefixed with method (B = bottle, T = torch).

Pond 14	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt		B1				
Common toad						
Common frog	Y					

Pond 15	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt	B1			B/T1		
Smooth/palmate newt (females)			T2			
Common toad						
Common frog		Y	Y			

Pond 16	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog						

Pond 17	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Smooth/palmate newt (females)	T1					
Common toad						
Common frog	Y					

Pond 18	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog						

Pond 19a	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt	B1					
Common toad	Y					
Common frog	Y	Y				

Pond 19b	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog	Y		Y			

Pond 21	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog						

Pond 26	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt	B6	T2	T4	B2		
Smooth/palmate newt (females)		T3	T4	T3		
Common toad	Y					
Common frog		Y	Y	Y		

Pond 28	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Smooth/palmate newt (females)	T1					
Common toad	Y	Y	Y			
Common frog	Y	Y	Y	Y		

Pond 29	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt	B6	T11	T5	T6		
Smooth/palmate newt (females)	T8	T17	T7	T5		
Common toad						
Common frog			Y	Y		

Pond 31	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Smooth/palmate newt (females)	T1					
Common toad		Y	Y	Y		
Common frog	Y	Y		Y		

Pond 34	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt	B1	T1				
Common toad	Y	Y		Y		
Common frog	Y	Y	Y	Y		

Pond 36	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog	Y					

Pond 39	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt	B/T1	B2	B4	B6	T12	T5
Smooth newt	B4		B2	B7	B4	
Smooth/palmate newt (females)	T1	T1	T1			T2
Common toad	Y			Y	Y	Y
Common frog	Y	Y		Y	Y	Y

Pond 40	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Great crested newt						
Smooth newt						
Common toad						
Common frog						

eDNA Technical Report

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Report Reference	R0000063
Report Date	02 Jun 2021
Reported By	cbutton

Site Name	D2/SD50391 20734						
Site Location	Ulnes walton						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000959	26/05/2021	18/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

Site Name	D1/SD50279 20706						
Site Location	Ulnes walton						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000971	26/05/2021	18/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

Site Name	D3/ SD50275 20333						
Site Location	Ulnes Walton						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000961	26/05/2021	18/05/2021	PASS	PASS	PASS	POSITIVE	4 out of 12

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eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

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The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

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eDNA Technical Report

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Report Reference	R0000050
Report Date	13 May 2021
Reported By	akarlsson

Site Name	P42/SD 5003920919 Ulnes Walton						
Site Location	Ulnes Walton						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000723	10/05/2021	06/05/2021	PASS	PASS	PASS	POSITIVE	2 out of 12

Site Name	P9/SDS002220957 Ulnes Walten						
Site Location	Ulnes Walten						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000701	10/05/2021	06/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

Site Name	P10/SD5002120938 Ulnes Walten						
Site Location	Ulnes Walten						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000708	10/05/2021	06/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

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eDNA Technical Report

Site Name	P8/SD4986620997 Ulnes Walten						
Site Location	Ulnes Walten						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000692	10/05/2021	06/05/2021	PASS	PASS	PASS	POSITIVE	2 out of 12

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eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

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The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

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eDNA Technical Report

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Report Reference	R0000087
Report Date	25 Jun 2021
Reported By	hbelcher

Site Name	Garth South						
Site Location	Pond W of car park						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000968	22/06/2021	22/06/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

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