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### Appendix A – Great Crested Newt eDNA Survey Methodology

# Great Crested Newt eDNA

Technical White Paper





## Great Crested Newt

Great crested newts (*Triturus cristatus*) are a widespread amphibian species in England and Wales with some localized populations in Scotland. They perform important ecosystem services such as the cycling of nutrients from water to land, which contributes to soil fertility. With declining populations due to changes in farming practices, habitat fragmentation and the destruction of ponds for building developments, great crested newts are now protected under the Wildlife and Countryside Act 1981. They are also a priority Species under the UK Post-2010 Biodiversity Framework and are listed as a European Protected Species under Annex IV of the European Habitats Directive.

## Traditional Survey Methods

All standard survey methods require a great crested newt survey licence. In order to obtain a licence from Natural England, the surveyor must demonstrate both knowledge and experience: applications, training, and references are also required.

#### Egg searching



Great crested newts lay a single egg on a leaf. They then fold the leaf over and the egg adheres to the leaf, giving protection from predation. Egg searching is a relatively effective method of detecting newt presence used by ecologists during April and June. The surveyor must unfold the leaves from plants within the pond to check for eggs, however once unfolded, eggs will not re-adhere therefore leaving them exposed and at risk.

#### Torching



Torching should be done from mid-March to mid-June, between dusk and midnight. It is not recommended whilst raining (due to poor visibility in the water), during cold conditions (newts are more likely to be inactive at low temperatures), or in ponds with a high density of vegetation. Surveyors need to walk slowly around the perimeter of the pond shining the torch back and forth with attention to vegetation and the bottom of the pond in order to get a visual sighting of any newts that are present.

#### Netting



Netting can be done from March to June for adult newts and in August for larvae. Fifteen minutes of netting per 50 metres of pond shoreline is recommended, moving the net in a figure of eight motion. This technique is not as efficient as egg searching, torching or bottle trapping, and care must be taken as netting can cause damage and disturbance to ponds as well as risk the transfer of invasive non-native species between ponds.

#### **Bottle trapping**



An effective yet highly invasive technique, bottle trapping can prove difficult and the risk of harm to newts and small aquatic mammals is relatively high. A large plastic bottle with an inverted neck attached to a stick is placed with the opening submerged, at an angle and secured into the sediment. It is crucial that enough air is in the part of the bottle that is out of the water or there is a risk of newts and aquatic mammals drowning. Traps are set in the evening and checked and removed early the following morning. This survey method should only be undertaken after thorough training, due to the high risk of mortality if incorrectly executed.

Following the latest Natural England guidelines, these surveys should be undertaken between mid-March and mid-June. Three techniques must be used per visit and the site must be surveyed on four separate occasions throughout the survey season, unless the presence of great crested newts is detected on an earlier visit. As bottle trapping must be carried out overnight, each survey requires two days to complete, making these traditional survey techniques highly time consuming and expensive for ecologists.

# eDNA Survey Methods

The environmental DNA survey method for great crested newts has been approved by Natural England since 2014. Organisms release DNA into the environment constantly in the form of urine, faeces, gametes, shedding skin or hair etc., remaining present in aquatic environments for up to three weeks. This DNA can be extracted from water samples and analysed to determine the presence or likely absence of great crested newts. Detailed guidance for accepted sample collection and laboratory analysis can be found in the DEFRA Technical Report WC1067 Appendix 5.

Unlike traditional survey methods, eDNA analysis of a water sample is a quicker, cheaper and more reliable method to establish the presence/absence of great crested newts within a pond. In addition, this non-invasive method does not cause any harm, as opposed to the traditional methods which may cause stress, increased exposure to predation or even accidental death to the newts or developing young.

## eDNA at SureScreen

SureScreen have carried out great crested newt eDNA analysis since the technique was first approved in 2014, strictly following the methods set out in WC1067 and actively participating in the annual Natural England eDNA proficiency testing scheme.





# Sample Collection

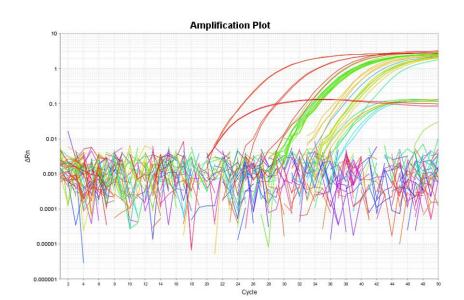
Natural England will accept results from samples collected between 15th April and 30th June, during the great crested newt breeding season, as the concentration of DNA in ponds should be relatively high during this time. We have developed our kits following WC1067 with input from our end-users and ecologists, ensuring they contain everything needed for easy collection of samples, including an ethanol preservative which prevents the degradation of DNA, and detailed sample collection instructions. Our kits have a long use-by date of three months (lasting for the entire eDNA sampling season) and contain a 'spike DNA' degradation marker which can inform at the point of analysis if any inhibitors are present within the sample or if the sample has degraded post sample collection. Once collected, any sample which will not be immediately returned to SureScreen Scientifics can be stored in a refrigerator for up to 4 weeks before analysis.

## Sample Analysis

All of our great crested newt eDNA sample analysis steps follow strict guidelines outlined in Report WC1067, as approved for use by Natural England. At all stages along the process we make use of internal laboratory positive and negative controls in order to assure sample results are always accurate, reliable and free of contamination or inconsistencies. We utilize separate laboratories for each stage in the analytical pipeline to ensure sample integrity is maintained throughout.

Once the 6 subsample tubes arrive into the laboratory they are concentrated, and the DNA is then extracted and isolated to form a single sample for qPCR (quantitative polymerase chain reaction) analysis. This makes use of an enzyme and molecular markers which are designed to amplify the DNA of great crested newts to a great enough extent that it can be detected using fluorescent imaging.

Each sample is analysed with 12 replicates, allowing the methodology to have a high sensitivity and detect populations of great crested newts which are very small in abundance. If any one or more of these 12 replicates results in the positive detection of great crested newt eDNA then it is considered as positive for great crested newt presence. Negative results are assessed for the presence of spike DNA which if also negative or at lower concentrations indicates sample inhibition or degradation.



Typical output from qPCR analysis. Upper red curves represent known positive control sample, lower red curves represent spike DNA control, upper multi-coloured curves represent positive detection in eDNA samples, lower multi-coloured curves represent positive detection of spike DNA, 'noise' lines across center of graph represent negative samples and negative control samples.

eDNA@surescreen.com

(+44) 0 1332 292003

surescreenscientifics.com





#### Appendix B – Great Crested Newt eDNA Survey Result

 Folio No:
 1932-2024

 Purchase Order:
 PO-24-652-00876

 Contact:
 Sweco Services UK Ltd

 Issue Date:
 24.06.2024

# GCN Report

Technical Report



Folio No: Purchase Order: Contact: Issue Date: 1932-2024 PO-24-652-00876 Sweco Services UK Ltd 24.06.2024



# GCN eDNA Analysis

## 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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

### Results

Lab ID	Site Name	OS Reference	erence Degradation Inhibition Check Check		Result	Positive Replicates
4193	Corriemoillie, Pond 7	NH344641	Pass	Pass	Negative	0/12
6064	Corriemoillie, Pond 1	NH349639	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

Folio No: Purchase Order: Contact: Issue Date: 1932-2024 PO-24-652-00876 Sweco Services UK Ltd 24.06.2024



### Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

## Interpretation of Results

Sample Integrity Check:	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. Any samples which fail this test are rejected and eliminated before analysis.
Degradation Check:	<b>Pass/Fail</b> . 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.
Inhibition Check:	<b>Pass/Fail.</b> The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
Result:	<ul> <li>Presence of GCN eDNA (Positive/Negative/Inconclusive)</li> <li>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.</li> <li>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 the WC1067 Natural England protocol, even a score of 1/12 is declared positive. O/12 indicates negative GCN presence.</li> <li>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.</li> <li>Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.</li> </ul>



### Appendix C – National Vegetation Classification Results

#### Habitat area number: 1

NVC classification: M15

Common nome	Scientific name	"Old" scientific names	Cover (DOMIN scale)			Fraguanay	Abundance Range			
Common name	Scientific fiame	matching Rodwell	Q1	Q2	Q3	Frequency	Low -	High	V	1
Heather	Calluna vulgaris	Calluna vulgaris	6	8	8	V	6	8	IV	
Purple moor grass	Mollinea cerulea	Mollinea cerulea	8	5	6	V	5	8	Ш	
Red-stemmed feather-mo	Pleurozium schreberi	Pleurozium schreberi	4	4	4	V	4	4	Ш	
Marsh thistle	Cirsium palustre	Cirsium palustre	4	2	4	V	2	4	1	
Heath bedstraw	Galium saxatile	Galium saxatile	4	2	4	V	2	4		-
Common haircap	Polytrichum commune	Polytrichum commune	2	5	2	V	2	5	Domin	Т
Cross-leaved heath	Erica tetralix	Erica tetralix	2	4	3	V	2	4	10	T
Compact rush	Juncus conglomeratus	Juncus conglomeratus	4	2	1	V	1	4	9	
Lustrous bog-moss	Sphagnum subnitens	Sphagnum subnitens	2	2	3	V	2	3	8	
Tormentil	Potentilla erecta	Potentilla erecta	2	3	1	V	1	3	7	
Deer fern	Blechnum spicant	Blechnum spicant	1	3	2	V	1	3	6	
	Dryopteris sp.	Dryopteris sp.	1	1	1	V	1	1	5	
Red fescue	Festuca rubra	Festuca rubra	4	3	0	IV	0	4	4	
Soft rush	Juncus effusus	Juncus effusus	2	2	0	IV	0	2	3	
Marsh violet	Viola palustris	Viola palustris	2	0	2	IV	0	2	2	
Blunt-leaved bog-moss	Sphagnum palustre	Sphagnum palustre	0	3	3	IV	0	3	х	
Foxglove	Digitalis purpurea	Digitalis purpurea	1	2	0	IV	0	2	<b>-</b>	
Slender St-John's wort	Hypericum pulchrum	Hypericum pulchrum	0	1	1	IV	0	1		
Velvet bent	Agrostis canina	Agrostis canina	0	0	5	IV	0	5		
Common tamarisk moss	Thuidium tamariscinum	Thuidium tamariscinum	0	4	0	IV	0	4		
Common bent	Agrostis capillaris	Agrostis capillaris	0	4	0	IV	0	4		
Creeping bent	Agrostis stolonifera	Agrostis stolonifera	3	0	0	IV	0	3		
Flat-topped bog moss	Sphagnum fallax	Sphagnum recurvum	3	2	0	IV	0	3		
Mat grass	Nardus stricta	Nardus stricta	0	0	2	1	0	2		
Willowherb	Epilobium sp.	Epilobium sp.	1	0	0	1	0	1		
Rowan	Sorbus acuparia	Sorbus acuparia	1	0	0	1	0	1		
Heath speedwell	Veronica officinalis	Veronica officinalis	1	0	0	1	0	1		
Common yellow-sedge	Carex demissa	Carex demissa	0	0	1	1	0	1		
Glittering wood-moss	Hylocomium splendens	Hylocomium splendens	1	0	0	1	0	1		
Neat feather-moss	Pseusoscleropodium purum	Pseusoscleropodium purum	1	0	0	1	0	1		
	Number of species	3	30	30	30					

Frequency					
V	Constant				
IV	Constant				
Ш	Frequent				
Ш	Occasional				
1	Scarce				
Domin	% cover				
10	91-100				
9	76-90				
8	51-75				
7	34-50				
6	26-33				
5	11-25				

5	11-25
4	4-10
3	<4, many
2	<4, several
х	Outside guadrat but nearby

#### Habitat area number: 2

NVC classification: M15

Common name	Scientific name	Species ("Old" latin names	•	Cover (DOMIN scale)				nce Range	
Common name	Sciencinc name	matching Rodwell)	Q1	Q2	Q3	Frequency	Low	- High	
Heather	Calluna vulgaris	Calluna vulgaris	4	5	6	V	4	6	
Compact rush	Juncus conglomeratus	Juncus conglomeratus	2	5	5	V	2	5	
Common haircap	Polytrichum commune	Polytrichum commune	4	3	5	V	3	5	
Blunt-leaved bog-moss	Sphagnum palustre	Sphagnum palustre	6	3	2	V	2	6	
Red-stemmed feather-mo	Pleurozium schreberi	Pleurozium schreberi	5	2	4	V	2	5	
Tormentil	Potentilla erecta	Potentilla erecta	3	3	4	V	3	4	D
Puple moor-grass	Mollinea cerulea	Mollinea cerulea	2	4	4	V	2	4	
Cross-leaved heath	Erica tetralix	Erica tetralix	1	4	4	V	1	4	
Star sedge	Carex echinata	Carex echinata	3	3	3	V	3	3	
Mat grass	Nardus stricta	Nardus stricta	3	2	4	V	2	4	
Soft rush	Juncus effusus	Juncus effusus	4	1	2	V	1	4	1
Heath rush	Juncus squarrosus	Juncus squarrosus	2	2	1	V	1	2	1
Heath bedstraw	Galium saxatile	Galium saxatile	4	0	6	IV	0	6	1
Common tamarisk moss	Thuidium tamariscinum	Thuidium tamariscinum	0	1	5	IV	0	5	
Deer fern	Blechnum spicant	Blechnum spicant	2	0	3	IV	0	3	
Bulbous rush	Juncus bulbosus	Juncus bulbosus	3	2		IV	2	3	
Marsh violet	Viola palustris	Viola palustris	3	0	1	IV	0	3	
Red fescue	Festuca rubra	Festuca rubra	3	1		IV	1	3	
Common yellow-sedge	Carex demissa	Carex demissa	0	3	1	IV	0	3	1
Bell heather	Erica cinerea	Erica cinerea	0	1	2	IV	0	2	1
Slender St-John's wort	Hypericum pulchrum	Hypericum pulchrum	1	0	1	IV	0	1	1
Yorkshire fog	Holcus lanatus	Holcus lanatus	1	0	1	IV	0	1	1
Marsh thistle	Cirsium pallustre	Cirsium pallustre	0	1	1	IV	0	1	1
Lustrous bog-moss	Sphagnum subnitens	Sphagnum subnitens	0	5	0	I	0	5	1
Common bent	Agrostis capillaris	Agrostis capillaris	4	0	0	I	0	4	1
Carnation sedge	Carex panicea	Carex panicea	0	0	4	I	0	4	1
Velvet bent	Agrostis canina	Agrostis canina	0	3	0	I	0	3	1
Foxglove	Digitalis purpurea	Digitalis purpurea	1	0	0	I	0	1	1
Devil's bit scabious	Succisa pratensis	Succisa pratensis	1	0	0	1	0	1	1
Sweet vernal grass	Anthoxanthem odoratum	Anthoxanthem odoratum	1	0	0	1	0	1	
Heath speedwell	Veronica officinalis	Veronica officinalis	0	1	0	1	0	1	1
Sitka spruce	Pisea sichensis	Pisea sichensis	0	0	1	1	0	1	
Common wintergreen	Lysimachia europaea	Lysimachia europaea	0	0	1	I	0	1	l
0	Sphagnum inundatum	Sphagnum auriculatum	0	0	1	I	0	1	1
Heath milkwort	Polygala serpyllifolia	Polygala serpyllifolia	0	0	1	I	0	1	l
Glittering wood-moss	Hylocomium splendens	Hylocomium splendens	0	1	0	I	0	1	1
	Pseudoscleropodium purum	Pseudoscleropodium purum	0	1	0	1	0	1	i i
Neat feather-moss	Pseudoscieropoulum purum	r seddoseleropodiain param	0	-	, , , , , , , , , , , , , , , , , , ,	· ·	0	1	1

	Frequency					
V	Constant					
IV	Constant					
Ш	Frequent					
Ш	Occasional					
1	Scarce					
Domin	% cover					
10	91-100					
9	76-90					
8	51-75					

34-50 26-33 11-25 4-10 <4, many <4, several <4 few Outside quadrat but nearby

### Habitat area number:3NVC classification:M15

C	Scientific name	"Old" scientific names	"Old" scientific names Cover (DOMIN scale)			Frequency	Abundar	nce Range		
Common name	Scientific name	matching Rodwell)	Q1	Q2	Q3	Frequency	Low -	High		V
Heather	Calluna vulgaris	Calluna vulgaris	9	9	9	V	9	9		IV
Red-stemmed feather-mo	Pleurozium schreberi	Pleurozium schreberi	5	8	7	V	5	8		111
Purple moor-grass	Mollinea cerulea	Mollinea cerulea	6	5	5	V	5	6		Ш
Lustrous bog-moss	Sphagnum subnitens	Sphagnum subnitens	7	4	5	V	4	7		1
Deergrass	Trichophorum germanicum	Scirpus cespitosum	2	5	5	V	2	5		
Cross-leaved heath	Erica tetralix	Erica tetralix	3	0	2	IV	0	3		Domi
Cladonia lichen	Cladonia sp. 2	Cladonia sp. 2	3	1	0	IV	0	3		10
Cladonia lichen	Cladonia sp. 1	Cladonia sp. 1	3	0	0	I	0	3		9
Tormentil	Potentilla erecta	Potentilla erecta	2	0	0	1	0	2		8
Bell heather	Erica cinerea	Erica cinerea	1	0	0	1	0	1		7
Neat feather-moss	Pseudoscleropodium purum	Pseudoscleropodium purum	1	0	0	1	0	1		6
Marsh fern	Thelypteris palustris	Thelypteris palustris	1	0	0	I	0	1		5
Bluntleaved bog-moss	Sphagnum palustre	Sphagnum palustre	0	0	1	1	0	1		4
Glittering wood-moss	Hylocomium splendens	Hylocomium splendens	0	0	1	I	0	1		3
Cypress-leaved plait-moss	Hypnum cupressiforme	Hypnum cupressiforme	1	0	0	I	0	1		2
Neat feather-moss	Pseudoscleropodium purum	Pseudoscleropodium purum	0	0	1	I	0	1		1
									1	Х
	Number of species		16	16	16		•			

Frequency					
Constant					
Constant					
Frequent					
Occasional					
Scarce					
% cover					
91-100					
76-90					
76-90 51-75					
51-75					
51-75 34-50					
51-75 34-50 26-33					
51-75 34-50 26-33 11-25					

<4 few Outside quadrat but nearby

Number of species

#### Habitat area number: Diffuse drainage lines

NVC classification: M15a

C	"Old" scientific names (show		(	Frequency	Abundance Range			
Common name	scientific name	in Rodwell)	Q1	Q2	Q3	Frequency	Low -	High
Bulbous Rush	Juncus bulbosus	Juncus bulbosus	7	7	5	V	5	7
Star sedge	Carex echinata	Carex echinata	4	4	4	V	4	4
Papillose bog-moss	Sphagnum papillosum	Sphagnum papillosum	1	4	7	V	1	7
Lustrous bog-moss	Sphagnum subnitens	Sphagnum subnitens	1	1	9	V	1	9
Cross-leaved heath	Erica tetralix	Erica tetralix	4	3	3	V	3	4
Round-leaved sundew	Drosera rotundiflora	Drosera rotundiflora	1	4	3	V	1	4
Common yellow-sedge	Carex demissa	Carex demissa	3	2	1	V	1	3
Soft rush	Juncus effusus	Juncus effusus	2	2	2	V	2	2
Tormentil	Potentilla erecta	Potentilla erecta	2	2	2	V	2	2
Purple moor grass	Mollinea cerulea	Mollinea cerulea	1	2	3	V	1	3
Heather	Calluna vulgaris	Calluna vulgaris	1	1	1	V	1	1
Bog pondweed	Potamogeton polygonifolius	Potamogeton polygonifolius	0	4	7	IV	0	7
Pale glaucous thread-moss	Pohlia walenbergii	Pohlia walenbergii	3	1	0	IV	0	3
Common cottongrass	Eriophorum angustifolium	Eriophorum angustifolium	3	0	0	I	0	3
Willowherb	Epilobium sp.	Epilobium sp.	1	0	0	I	0	1
Marsh violet	Viola palustris	Viola palustris	1	0	0	I	0	1
New Zealand willowherb	Epilobium brunnescens	Epilobium brunnescens	1	0	0	I	0	1
Yorkshire fog	Holcus lanatus	Holcus lanatus	1	0	0	I	0	1
Foxglove	Digitalis purpurea	Digitalis purpurea	1	0	0	I	0	1
Marsh thistle	Cirsium palustre	Cirsium palustre	1	0	0	I.	0	1
Common liverwort	Marchantia polymorpha	Marchantia polymorpha	1	0	0	I	0	1
Golden head-moss	Breutolia chrysocoma	Breutolia chrysocoma	1	0	0	I	0	1
Common tamarisc-moss	Thuidium tamariscinum	Thuidium tamariscinum	1	0	0	I	0	1
Heath rush	Juncus squarrosus	Juncus squarrosus	0	3	0	I	0	3
Mat grass	Nardus stricta	Nardus stricta	0	3	0	I	0	3
Sheep's fescue	Festuca ovina	Festuca ovina	0	0	1	I	0	1
Carnation sedge	Carex panicea	Carex panicea	2	0	0	1	0	2
	N. school of the		27	27	27			
	Number of species	1	27	27	27	1		

Frequency							
V	Constant						
IV	Constant						
Ш	Frequent						
Ш	Occasional						
1	Scarce						
Domin	% cover						
10	91-100						
9	76-90						
8	51-75						

34-50

26-33

11-25

4-10

<4, many

<4 few

Outside quadrat but nearby

7

6

5

4

3

1

Х



	Score 1 if present			
Habitat Suitability Feature	1	2	3	4
Well-developed (>60%) bankside and emergent vegetation to provide cover.	1	0	0	1
Year-round availability of food sources.		0	0	1
Suitable refuge areas above extremes in water levels.		1	1	1
Steep banks suitable for burrowing.		1	1	0
Permanent open water.		1	1	1
Presence of berm (ledge at water level).		1	1	0
Lack of disturbance through poaching, grazing and/or recent management.		1	1	1
Nest building opportunities in vegetation above water level.		1	1	1
TOTAL	6	6	6	6

#### Appendix D – Water Vole Habitat Suitability Results



#### Appendix E – Ecological Impact Assessment Summary and Delivering Positive Effects

Actions Considered	Mitigation: Measures included as mitigation to avoid and minimise impacts	Enhancement: Measures included to enhance biodiversity (or explanation for not applying)
Protection and enhancement of existing habitats on or adjacent to the site	<ul> <li>All upland birchwood and Scots pine woodland, both of which are priority habitats, will be retained.</li> <li>Approximately 0.2ha of wet heath is being retained.</li> <li>Approximately 0.16ha of acid grassland is being retained.</li> <li>Approximately 0.1km of ditch will be retained.</li> <li>Landscaping will incorporate recreation of wet heathland, acid grassland, using topsoils displaced from onsite so that species present may persist.</li> <li>Measures to protect retained habitats during construction and operation will be detailed in the CEMP.</li> </ul>	<ul> <li>As compensation for loss of the heathland and acid grassland habitats on site, 2.9ha of felled coniferous woodland to the north of the site will be restored to wet heathland.</li> <li>The proposed development will result in a 24.33% increase in linear watercourse habitat on site.</li> <li>A bioswale will increase the wetland habitat on site.</li> </ul>
Creation of new habitat on the site	Areas of priority habitat that are being retained such as the wet heathland in the south of the site, will not be used for tree planting when landscaping the site.	<ul> <li>Three new permanently wet SuDS ponds will be created and planted with native aquatic and wetland species, providing new vegetated standing water habitat on site.</li> <li>The proposed landscaping post development includes parcels of acid grassland. The management of this grassland will follow NatureScot's guidance [38] employing methods such as reduced mowing regimes and avoidance of</li> </ul>

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Actions Considered	Mitigation: Measures included as mitigation to avoid and minimise impacts	Enhancement: Measures included to enhance biodiversity (or explanation for not applying)
		pesticides, this will be detailed in the Landscape and Habitat Management Plan.
Protection and enhancement of connectivity throughout the site and with its surroundings	<ul> <li>A sensitive lighting strategy will be prepared to avoid light spill. Details of this will be provided in the CEMP.</li> </ul>	• The landscaping on site and habitat creation in the biodiversity enhancement areas will enhance the habitat connectivity in the areas surrounding the site.
Protections and enhancement of existing species on or adjacent to the site	<ul> <li>A sensitive lighting strategy will be prepared to minimise the potential effects during construction of the proposed development on species using habitat adjacent to the site.</li> <li>Vegetation clearance to be undertaken outside of breeding bird season (March-August inclusive) or if this is not possible a pre-construction check of vegetation by an Ecological Clerk of Works will be carried out.</li> <li>Vegetation clearance will be undertaken following precautionary methods with respect to reptiles (these methods will be detailed in the CEMP).</li> <li>Excavations will be covered overnight during the construction phase of the development to avoid the entrapment of badger and other wildlife.</li> <li>A pollution protection plan will be in place (detailed in the CEMP) during the construction phase.</li> </ul>	<ul> <li>The creation of additional log piles from felled trees and vegetation will provide food and shelter for a variety of invertebrate species as well as small mammals, birds and amphibians.</li> </ul>
Enhancement for new species		<ul> <li>The creation of three permanently wet SuDS ponds which will be planted with native species will introduce the new ecologically valuable habitat of</li> </ul>



Actions Considered	Mitigation: Measures included as mitigation to avoid and minimise impacts	Enhancement: Measures included to enhance biodiversity (or explanation for not applying)
		<ul> <li>permanent standing water to the site. This will be of value to invertebrates, amphibians and species that prey upon these groups such as birds, bats and otter.</li> <li>Bat boxes will be installed to offer roosting opportunities that were not available preconstruction due to the lack of mature/veteran trees.</li> <li>Bird boxes to be installed on site will offer cavity nesting opportunities that were not available preconstruction.</li> </ul>
Avoidance, control and removal of invasive species from the site	Rhododendron will be removed from the site.	<ul> <li>All planted species on site will be non-invasive. Species have been chosen to prioritise benefits to wildlife, including pollinators. No invasive species listed in Appendix B of NatureScot's NPF4 guidance [39] have been included.</li> </ul>
Protecting wildlife from negative interactions with people and / or infrastructure	<ul> <li>The protected species surveys undertaken identified species likely present on site and this EcIA has assessed the potential effects and provided mitigation to protect the wildlife present from the development.</li> <li>Pre-construction and surveys will be carried out where appropriate</li> </ul>	<ul> <li>Operational lighting has been designed to minimise light spill into surrounding natural habitats through the use of low-level lighting, where possible, directed away from any unlit areas off site.</li> <li>The maintenance of the bird and bat boxes on site will be detailed in the Habitat Management and Monitoring Plan.</li> </ul>
Promoting awareness and encouraging further actions for nature	N/A	<ul> <li>The restoration of a large area of wet heathland from felled coniferous woodland will set a positive precedent regarding opportunities to improve land use and restore valuable habitats.</li> </ul>

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Actions Considered	Mitigation: Measures included as mitigation to avoid and minimise impacts	Enhancement: Measures included to enhance biodiversity (or explanation for not applying)
SUMMARY: Positive effects that will be delivered	<ul> <li>A CEMP will be prepared prior to construction to include details on pollution prevention. The correct implementation of this will reduce any future impacts of the development.</li> <li>The proposed mitigation will ensure that the species confirmed/potentially present on site are protected during construction by following the CEMP to minimise disturbance and/or potential mortality.</li> <li>Minimising any potential disturbance to the surrounding habitat will ensure connectivity is not reduced and proposed planting will further benefit species using these areas.</li> </ul>	<ul> <li>A greater area of wetland than is being lost will be restored in the biodiversity enhancement area.</li> <li>Biodiversity enhancements have been included in the design including permanently wet SuDS ponds, bird boxes, bat boxes and log piles.</li> <li>Construction, monitoring and maintenance of all habitat creation and biodiversity enhancements will be detailed in a Habitat Management and Monitoring Plan for the site.</li> </ul>

