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**Preliminary Ecological Assessment (PEA) and Phase 1 of land at Bryn Morfa,
Bodelwyddan, Denbighshire**

On behalf of

Cadnant Planning

29 July 2019

ECO 399

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CONTENTS	Page no.
SUMMARY:	1
1. INTRODUCTION	2
1.1 Description of Brief	2
2. METHOD	3
2.1 Desk study	3
2.2 Field survey	3
3. RESULTS	4
3.1 Desk study	4
3.2 Site Description	5
4. CONCLUSION	9
4.1 Summary of results	9
4.2 Risk assessment	9
4.3 Conclusion	10
5. RECOMMENDATIONS	12
5.1 Mitigation Strategy	12
5.2 Avoidance	12
5.3 Protection	12
5.4 Enhancement & Mitigation	13
6. REVISED RISK ASSESSMENT	16
APPENDIX 1 PHASE 1 HABITAT MAPPING	I
APPENDIX 2 SPECIES LIST	III
APPENDIX 3 SUMMARY OF LEGISLATION	V
APPENDIX 4 EDNA RESULTS	VII

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TABLES & FIGURES:

Figure 1 Target Note Images	5
Figure 2 Ponds in the grounds of Glan Clwyd Hospital	7
Figure 3 Evidence of water vole in ponds within 300m	7
Figure 4 Barrier fencing	13
Figure 5 Bat mitigation	13
Figure 6 Bird boxes	14
Figure 7 Phase 1 Map and Key	i
Table 1 Personnel	3
Table 2 Protected sites and Qualifying Features within 5km	4
Table 3 Significant species within +/-1000m	4
Table 4 Species recorded on site 18th June	5
Table 5 Risk assessment (without mitigation)	10
Table 6 Hierarchy of impacts	11
Table 7 Risk assessment (after mitigation)	16
Table 8. Target Notes	ii
Table 9 Species list for field using the ACFOR scale	iii
Table 10 Woody species recorded in hedges	iii

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SUMMARY:

Ecoscope undertook a Preliminary Ecological Assessment (inc. Phase 1 extended survey) of land at Bryn Morfa, Bodelwyddan, in advance of planning for +16 residential developments (including access).

The survey also included sampling and eDNA analysis from ponds within 320m of the site.

The results indicate that the site has a population of resident nesting birds (including three which are amber or red-listed), potential to support roosting bats in perimeter trees, a resident population of common frog and Great Crested Newt within 500m, necessitating EPSL in advance of works.

Ponds within 320m were also found to contain a population of Water Vole, and the development site is bounded by a wet ditch, which though sub-optimal, may contain water vole.

The report includes a Phase 1 map and assessment of potential impacts on protected species.

A development licence will be required in respect of Great Crested Newts. Further survey work is recommended before planning submission to rule out the presence of Water Voles, reptiles and bats.

Document Issue Date: Monday, 29 July 2019

Approved by: Mr. Stuart Kato M.Sc., MCIEEM

Prepared by: Dr Richard Birch CEcol

1. INTRODUCTION

1.1 Description of Brief

1.1.1 Ecoscope was commissioned to undertake a Preliminary Ecological Assessment (PEA) and Phase 1 of land at Bryn Morfa, Bodelwyddan, Denbighshire LL18 5TR (SH 99687 75985).

1.1.2 Proposals are presented to develop the land for+16 residential properties and associated facilities.

2. METHOD

2.1 Desk study

2.1.1 As part of reporting, Ecoscope undertake a desk study of existing information from sources including:

- Magic map (<https://magic.defra.gov.uk/MagicMap.aspx>);
- The Local Environmental Records Centre (LERC) Cofnod.

2.1.2 Protected habitats and sites within 5 km are included in the Desk Study.

2.2 Field survey

2.2.1 A site visit was undertaken on 18th June 2019 by the following personnel:

Table 1 Personnel

PERSONNEL	EXPERIENCE
Dr Richard Birch CEcol	Qualified horticulturalist and 1 st class degree in Botany. 20 years practising ecologist. Licences for bats & newts in England & Wales. Chartered since 2016
Miss Katie Williams	Undergraduate studying a BSc Honours degree in Environmental Science with an interest in a career in Ecology. Experience in bat surveys.

2.2.2 The site visit included a Phase 1 survey and assessment of ponds within 500m with the potential to support a population of great crested newt.

2.2.3 eDNA samples from three ponds within the grounds of Glan Clwyd Hospital were taken on 26th June 2019.

3. RESULTS

3.1 Desk study

3.1.1 Protected sites within 5km are given in Table 2:

Table 2 Protected sites and Qualifying Features within 5km

NAME	CLASSIFICATION	SUMMARY OF QUALIFYING FEATURES	AREA (Ha)	DISTANCE (Km)
COED Y GOPA	SSSI	<ul style="list-style-type: none"> Limestone plants Lesser Horseshoe Bat Hibernacula 	36.3 Ha	5.1 Km
COEDYDD AC OGOFAU ELWY A MEIRCHION	SSSI	<ul style="list-style-type: none"> Limestone plants Geology Hibernating bats 	82.4 Ha	4.4. Km
MOEL HIRADDUG A BRYN GOP	SSSI	<ul style="list-style-type: none"> Vegetated shingle 	50.6 Ha	6.6 Km
TRAETH PENSARN	SSSI	<ul style="list-style-type: none"> Limestone vegetation Geology 	51.4	5.7 Km

3.1.2 A summary of the data provided from the LERC Cofnod is given in Table 3:

Table 3 Significant species within +/-1000m

COMMON NAME	LATIN NAME	TYPE	DISTANCE	NOTES
Herring gull	<i>Larus argentatus</i>	Bird – red list	496m	x3 reported nesting
Kestrel	<i>Falco tinnunculus</i>	Bird – amber list	645m	
Skylark	<i>Alauda arvensis</i>	Bird – red list	962m	
Curlew	<i>Numenius arquata</i>	Bird – red list	962m	
Grey partridge	<i>Perdix perdix</i>	Bird – red list	960m	
Lapwing	<i>Vanellus vanellus</i>	Bird – red list	960 m	
Marsh tit	<i>Poecile palustris</i>	Bird – red list	843m	
Dunnock	<i>Prunella modularis</i>	Bird – amber list	843m	Male holding territory during site visit
Starling	<i>Sturnus vulgaris</i>	Bird – red list	843m	
Song thrush	<i>Turdus philomelos</i>	Bird – red list	843m	2 singing males recorded during site visit
House sparrow	<i>Passer domesticus</i>	Bird – red list	707m	Nesting within 20m of site
Bullfinch	<i>Pyrrhula pyrrhula</i>	Bird – amber list	286m	Territorial bird heard during site visit
Reed bunting	<i>Emberiza schoeniclus</i>	Bird – amber list	949m	
Lesser redpoll	<i>Acanthis cabaret</i>	Bird – red list	1.1km	
Tree sparrow	<i>Passer montanus</i>	Bird – red list	1.1km	
Woodcock	<i>Scolopax rusticola</i>	Bird – red list	1 km	
Mute swan	<i>Cygnus olor</i>	Bird – amber list	852m	
Great black-backed gull	<i>Larus marinus</i>	Bird - amber list	852m	
Mallard	<i>Anas platyrhynchos</i>	Bird – amber list	278m	
Willow warbler	<i>Phylloscopus trochilus</i>	Bird - amber list	278m	
Shelduck	<i>Tadorna tadorna</i>	Bird – amber list	278m	
Swift	<i>Apus apus</i>	Bird – amber list	900m	

COMMON NAME	LATIN NAME	TYPE	DISTANCE	NOTES
Common lizard	<i>Zootoca vivipara</i>	Reptile	300m	Recorded during site clearance for hospital
Great Crested Newt	<i>Triturus cristatus</i>	Amphibian	700m	TBC
Grass snake	<i>Natrix natrix</i>	Reptile	300m	Recorded during site clearance for hospital

3.1.3 In addition, the following species were recorded as resident during the Phase 1 survey on 18th June:

Table 4 Species recorded on site 18th June

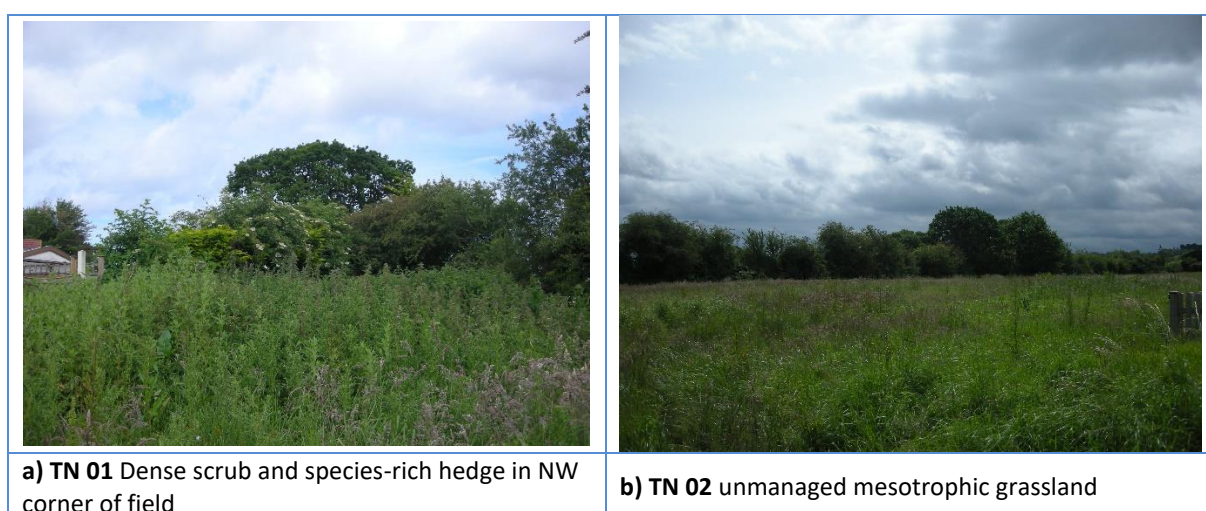
COMMON NAME	SCIENTIFIC NAME	COMMON NAME	SCIENTIFIC NAME
Common Frog	<i>Rana temporaria</i>	Blackbird	<i>Turdus merula</i>
Whitethroat	<i>Sylvia communis</i>	Wren	<i>Troglodytes troglodytes</i>
Blackcap	<i>Sylvia atricapilla</i>	House sparrow	<i>Passer domesticus</i>
Song thrush	<i>Turdus philomelos</i>	Goldfinch	<i>Carduelis carduelis</i>



3.2 Site Description

3.2.1 The site boundary and habitats therein are shown in the Phase 1 map in Figure 7 Phase 1 Map and Key in APPENDIX 1, p. i

3.2.2 the site is a field consisting of semi-improved unmanaged grassland appropriating to a National Vegetation Classification (NVC) of Neutral (Mesotrophic) Grassland 1 (MG 1), reverting to W22 scrub incursion (dominated by Blackthorn and Bramble) around the margins (Figure 1b).

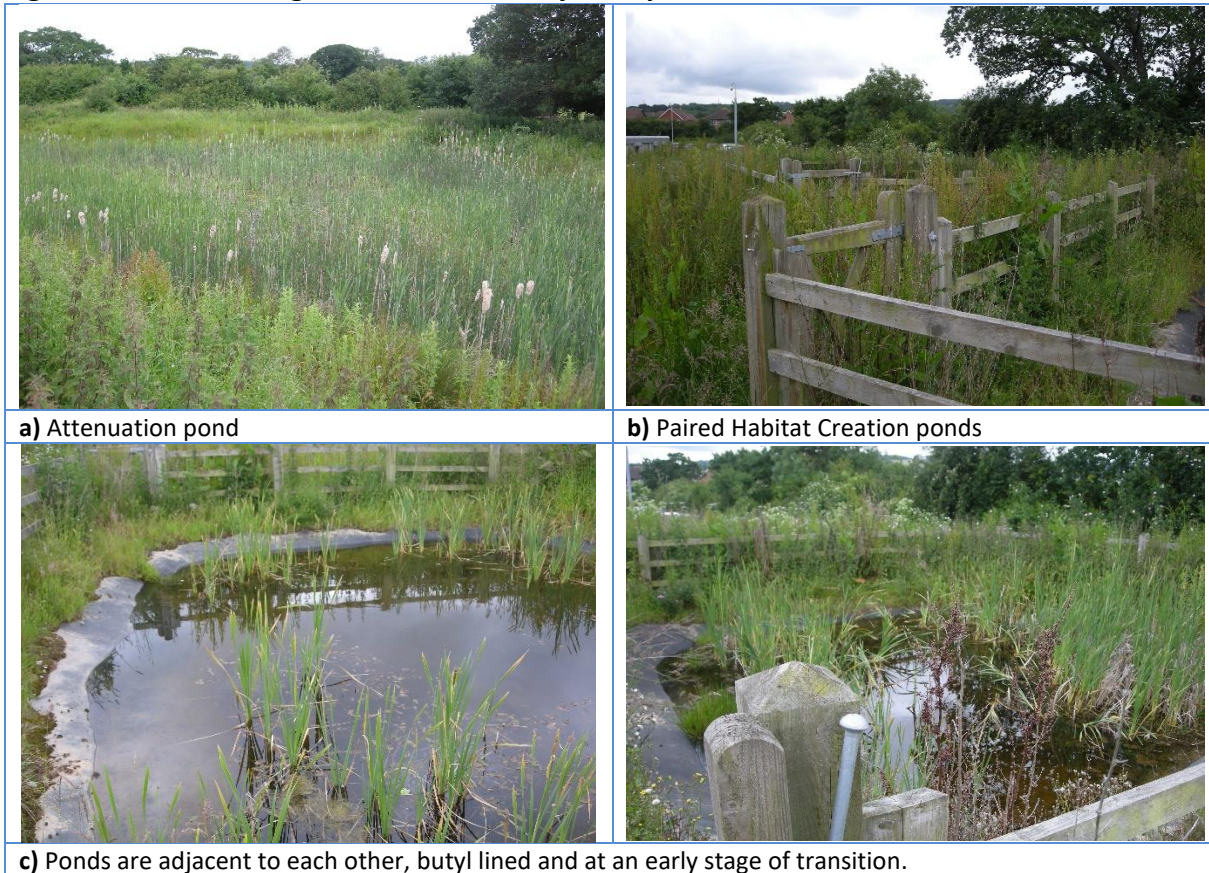
Figure 1 Target Note Images



	
<p>c) TN 03 Semi-mature sessile oak with nesting birds and some potential for bats</p>	<p>d) TN 07 One of two semi-mature oaks in field boundary. No potential for bats.</p>

- 3.2.3 While not especially species-rich, the species list for the field is included in Table 9, and attendant invertebrates included large skipper butterfly, meadow brown and speckled wood close to the boundary hedges.
- 3.2.4 The field was bordered on all sides by fences and unmanaged boundary hedges. Individually some of these were species-poor, but collectively the hedges are regarded as species-rich. The woody species are recorded in Table 10, APPENDIX 2.
- 3.2.5 The site is partially bordered on its eastern boundary by a slow-flowing ditch. During the Phase 1 mapping, four Common frog (*Rana temporaria*) were disturbed in the field. Given that Common frog do not wander far from their spawning areas, it must be concluded that a hitherto unidentified amphibian spawning site must be reasonably local.
- 3.2.6 There are four ponds within 300m of the site boundary, three of which were within the grounds of Glan Clwyd Hospital and accessible to surveyors (Figure 2a-d).

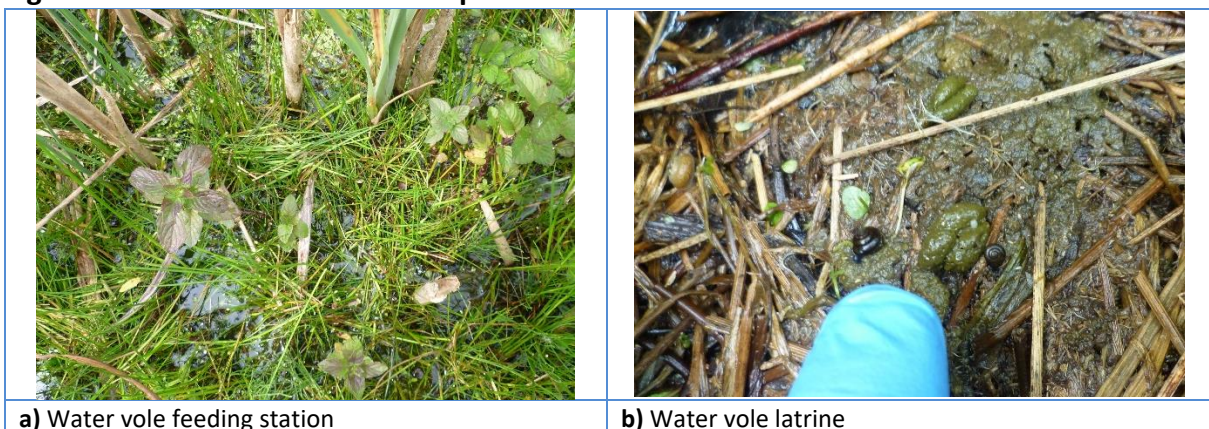
Figure 2 Ponds in the grounds of Glan Clwyd Hospital



3.2.7 eDNA sampling and analysis indicated that great crested newt (*Triturus cristata*) is present in both ponds featured in Figure 2; a distance of 322m from the proposed development (see APPENDIX 4, p. vii)

3.2.8 Pond sampling also indicated a hitherto unrecorded population of water vole (*Arvicola terrestris*) concentrated around the attenuation pond in the grounds of (Figure 3):

Figure 3 Evidence of water vole in ponds within 300m



3.2.9 Although this is 320m from site, the presence of a boundary ditch – although sub-optimal – means it cannot be ruled out that water vole may be present.

4. CONCLUSION

4.1 Summary of results

4.1.1 Analysis of biodiversity data, eDNA sampling and Phase 1 survey concludes that the location has the following features of biodiversity interest:

- The boundary hedgerow is regarded as species-rich and is bordered by both agricultural and common land, and therefore falls within the criterion of the Hedgerow Regulations 1997;
- The hedgerow contains one semi-mature oak which has the potential to support bats and nesting birds;
- The hedgerow has suitable nesting habitat for **Amber** and **Red**-listed bird species recorded during survey, including Song thrush, House sparrow, Bullfinch and Dunnock;
- While the neutral grassland in the field is not especially diverse, it does provide terrestrial habitat for Common frog;
- Although none are recorded locally, the site is suitable for reptiles, and is adjacent allotments which are potentially good places for reptiles;
- The proposed development is within 300m of ponds in which great crested newt was confirmed by eDNA evidence.
- Water vole occur within 300m of the proposals.

4.1.2 Further survey is required to confirm the presence of water vole, bats and reptiles.

4.1.3 The legislation pertaining to water vole, great crested newt and breeding birds is included in APPENDIX 3.

4.2 Risk assessment

4.2.1 Having identified the biodiversity interest, it is assessed against the impacts of the proposed development (without mitigation) in Table 5:

Table 5 Risk assessment (without mitigation)

IMPACT	FEATURE AFFECTED	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Water vole	5	5	25
Removal of hedges & trees		5	4	20
Construction		5	2	10
Permanent loss of habitat		5	5	25
 				
Site clearance	Nesting birds	5	4	20
Removal of hedges & trees		4	5	20
Construction		5	4	20
Permanent loss of habitat		5	5	25
 				
IMPACT	FEATURE AFFECTED	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Amphibians (inc. Great crested newt)	5	5	25
Removal of hedges & trees		5	3	15
Construction		5	5	25
Permanent loss of habitat		5	5	25
 				
IMPACT	FEATURE AFFECTED	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Tree-roosting bats	5	5	25
Removal of hedges & trees		5	5	25
Construction		3	3	9
Permanent loss of habitat		5	2	10

Key

RISK		SEVERITY		RISK × SEVERITY	
1	Negligible	1	Negligible	1-9	Cumulative effect of likelihood × severity = minor negative (potentially positive) impact
2	Slight risk	2	Low level of impact		
3	Moderate risk	3	Moderate impact	10-16	Cumulative effect of likelihood × severity = moderate negative impact
4	Event likely to occur	4	Major impact		
5	High risk of event occurring	5	Severe impact	17 - 25	Cumulative effect of likelihood × severity = major negative impact

4.3 Conclusion

4.3.1 The potential impacts of development – without any form of mitigation being implemented – are considered overall as being **Moderate** to **Severe** at a **National** to **Regional** level (see Table 6).

Table 6 Hierarchy of impacts

HIERARCHY OF IMPACT	DEFINITION
International	Having an impact on the population size or habitat area on a Worldwide scale
National	Having an impact on a habitat or species distributed throughout the British Isles
Regional	Having an impact on a habitat or species distribution in any of the individual countries making up the British Isles
Local	Having an impact on a habitat or species that may be significant at a local level (Borough or Parish)

4.3.2 the PEA is sufficient to identify the potential for all species included in the Risk Assessment in Table 5, but further survey is required for confirmation.

5. RECOMMENDATIONS

5.1 Mitigation Strategy

4.1.1 The level of mitigation is determined by evidence of presence, which is beyond the scope of the current preliminary assessment. The mitigation strategy makes assumptions based on the proximity of species and the likelihood of the habitat to support protected species. Further survey is required in order to determine the level of mitigation needed, which may entail more or less than is specified in the PEA. To comply with National Planning policy all developments need to show a biodiversity gain wherever possible.

4.1.2 Risk is reduced by implementation of a hierarchy of strategies:

- Avoidance
- Protection
- Reduction
- Enhancement
- Mitigation

4.1.3 Not all levels are appropriate in all cases.

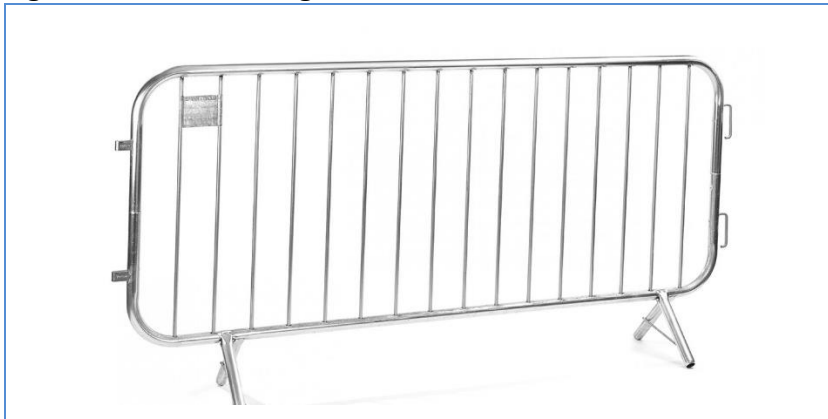
5.2 Avoidance

5.2.1 Site clearance (including hedgerows and trees) must be undertaken outside the bird nesting season (March to August respectively).

5.3 Protection

5.3.1 Features identified as of significant biodiversity value within the construction perimeter (including trees, hedgerows and water bodies) must be protected by suitable demarcation barriers.

Figure 4 Barrier fencing



5.3.2 A 5m margin between the perimeter of the site and the boundary ditch must be demarcated by the use of barrier fencing (see Figure 4) to prevent incursion near the ditch by Plant (this may be accommodated in the site boundary).

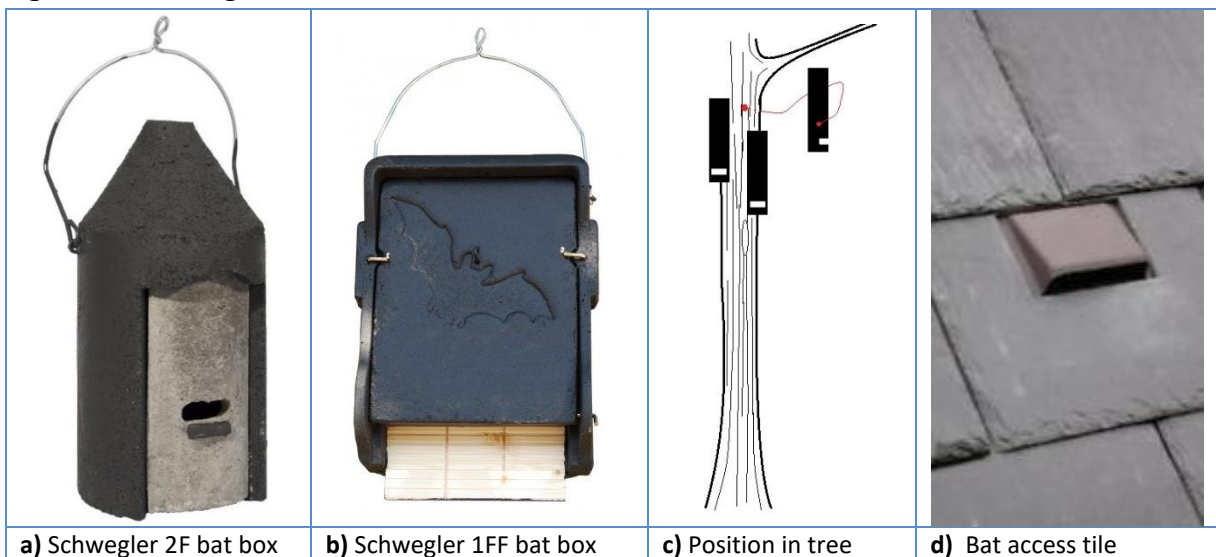
5.4 Enhancement & Mitigation

Bats

5.4.1 Further survey of the semi-mature Oak featured as TN 03 will be required.

5.4.2 The potential of the environment to maintain a population of bats is increased by fitting paired Schwegler© 2F bat boxes and a single Schwegler© 1FF bat box on a suitable tree at a height of no less than 4 metres (see Figure 5a-c).

Figure 5 Bat mitigation



5.4.3 To enhance the potential of new build to provide suitable places for bats, each property must be fitted with a bat tile, one per property (see Figure 5d) on alternate east or west aspects.

5.4.4 A lighting scheme must be provided detailing the position of external light sources and the type of lighting required to limit impact on bats.

Birds

5.4.5 Provision of a design drawing that shows all trees to be removed and like-for-like replacement planting.

5.4.6 Two each of:

- Schwegler© 1B hole-type bird boxes, and;
- Schwegler© 2H open-fronted nestboxes

In positions advised by an appointed ecologist (see Figure 6a/b).

Figure 6 Bird boxes



5.4.7 Swift can be accommodated in new build by the inclusion of 2× Swift bricks integral to the design of properties facing public open spaces or parks (see Figure 6c).

Amphibians & reptiles

- 5.4.8 Great crested newt were confirmed within 320 metres of the scheme. The presence of reptiles can only be confirmed by additional survey. Mitigation considers both as being present.
- 5.4.9 The confirmed presence of great crested newt within 500m may require a European Protected Species Licence prior to works, to include details of:
- One-way herpetological fencing around the site;
 - A 30-day trapping and exclusion procedure prior to works, with translocation site identified;

Water voles

- 5.4.10 Additional survey is required to determine the presence of water voles. If present a licence may be required from NRW to enable the proposal.
- 5.4.11 Avoidance strategies to avoid negative impacts to water courses will need to be developed and implemented.
- 5.4.12 Retention of wetland to provide suitable habitat for water voles, including landscape planting and potential food plants, will be required if presence is confirmed.

6. REVISED RISK ASSESSMENT

6.1.1 Compliance with the strategies outlined in Section 5 RECOMMENDATIONS will require a review of the risk assessment post-mitigation (Table 7).

Table 7 Risk assessment (after mitigation)

IMPACT	FEATURE AFFECTED	MITIGATION STRATEGY	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Water voles	<ul style="list-style-type: none"> • Barriers protecting watercourses; • 5m margin to ditch; • Habitat improvements 	2	5	10
Removal of hedges & trees					
Construction					
Permanent loss of habitat					
IMPACT	FEATURE AFFECTED	MITIGATION STRATEGY	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Breeding birds	<ul style="list-style-type: none"> • 2× 1B nest boxes; • 2× 2H nest boxes; • 2× swift brick • Design drawing • Habitat enhancement 	2	4	8
Removal of hedges & trees					
Construction					
Permanent loss of habitat					
IMPACT	FEATURE AFFECTED	MITIGATION STRATEGY	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Amphibians & reptiles	<ul style="list-style-type: none"> • EPS licence; • Exclusion barrier; • Trapping & relocation 	2	4	8
Removal of hedges & trees					
Construction					
Permanent loss of habitat					
IMPACT	FEATURE AFFECTED	MITIGATION STRATEGY	RISK	SEVERITY	RISK × SEVERITY
Site clearance	Bats – all species	<ul style="list-style-type: none"> • Lighting diagram; • 2× 2F bat boxes; • 1× 1FF bat box; • 1× bat tile per property 	2	4	8
Removal of hedges & trees					
Construction					
Permanent loss of habitat					

Key

RISK		SEVERITY		RISK × SEVERITY	
1	Negligible	1	Negligible	1-9	Cumulative effect of likelihood × severity = minor negative (potentially positive) impact
2	Slight risk	2	Low level of impact		
3	Moderate risk	3	Moderate impact		

RISK		SEVERITY		RISK × SEVERITY	
4	Event likely to occur	4	Major impact		Cumulative effect of likelihood × severity = moderate negative impact
5	High risk of event occurring	5	Severe impact	17 - 25	Cumulative effect of likelihood × severity = major negative impact

6.1.2 Note that the residual risk to water voles remains **amber** because of proximity to habitation and the threat posed by domestic pets (particularly cats).

6.1.3 Notwithstanding the residual impact on water voles, compliance with the mitigation as proposed, reduces the impacts to **Negligible** at a **Regional** & **National** level.

APPENDIX 1 PHASE 1 HABITAT MAPPING

Figure 7 Phase 1 Map and Key

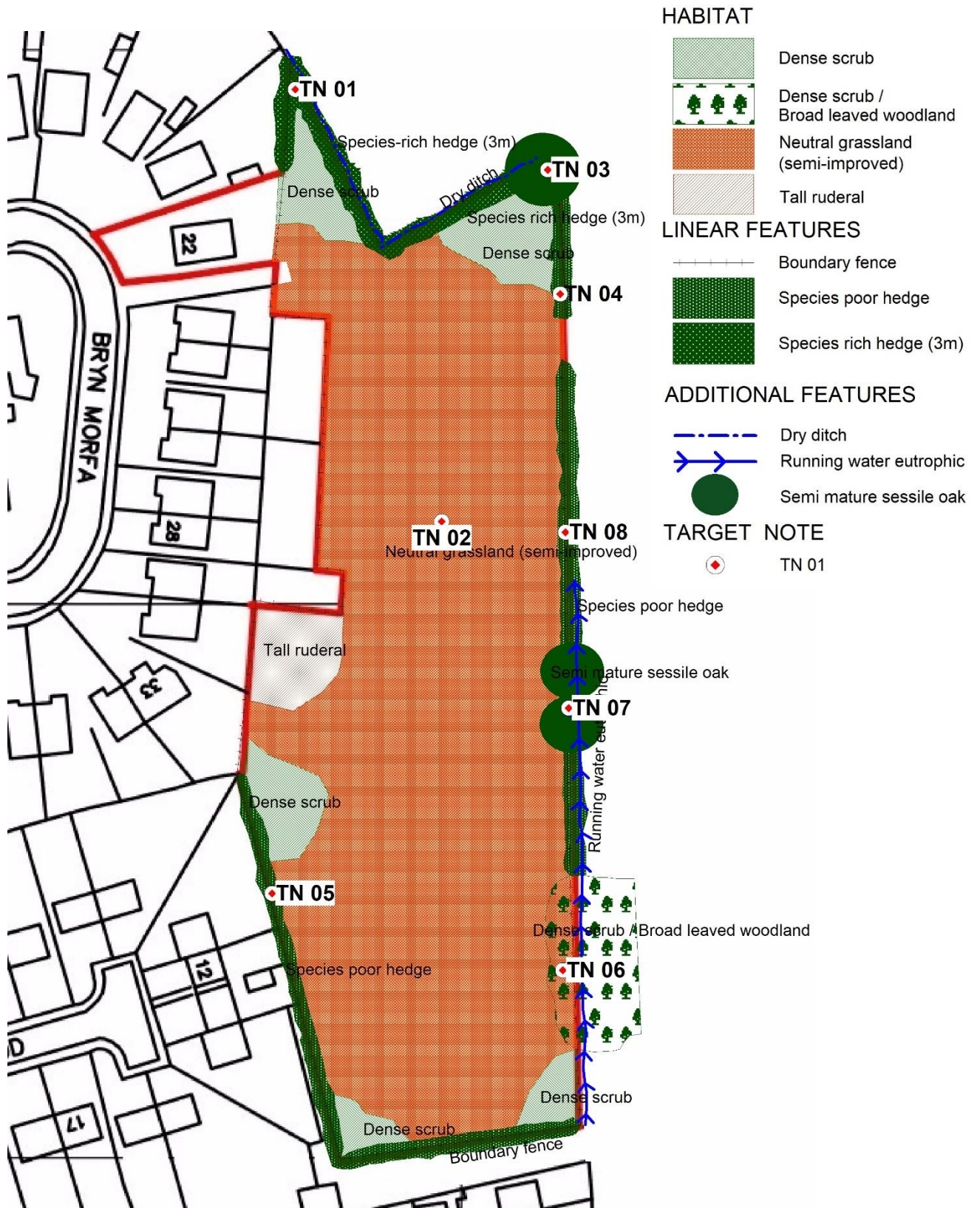


Table 8. Target Notes

TN	NOTES	PHOTOGRAPH No.
1	Species-rich hedge up to 5m, with species listed in ____. Resident Blackcap & Dunnock.	
2	Semi-improved neutral grassland (MG1) grading to scrub (W22) along the boundaries.	
3	semi-mature Sessile oak (<i>Quercus petraea</i>) with resident Blue tit and some potential for roosting bats.	
4	Fence and managed hedge (to 2m) of Blackthorn and Hawthorn, forming allotment boundary.	
5	Boundary hedge with dead Wych elm (<i>Ulmus glabra</i>) and suckers extending into field.	
6	Boundary hedge to 5m, with dense scrub / woodland extending into neighbouring land. Singing Whitethroat.	
7	Two semi-mature Sessile oak. Not suitable for roosting bats.	

APPENDIX 2 SPECIES LIST

Table 9 Species list for field using the ACFOR scale¹

COMMON NAME	SCIENTIFIC NAME	ACFOR	COMMON NAME	SCIENTIFIC NAME	ACFOR
American willow herb	<i>Epilobium ciliatum</i>	O	Hard rush	<i>Juncus inflexus</i>	O
Black bindweed	<i>Fallopia convolvulus</i>	A	Hedge woundwort	<i>Stachys sylvatica</i>	O
Black knapweed	<i>Centaurea nigra</i>	F	herb Robert	<i>Geranium robertianum</i>	O
Bramble	<i>Rubus fruticosus</i> agg.	A	Hogweed	<i>Heracleum</i>	O
Broad leaved dock	<i>Rumex obtusifolius</i>	F	Lesser stitchwort	<i>Stellaria graminea</i>	O
Caration sedge	<i>Carex panicea</i>	R	Marsh ragwort	<i>Jacobaea aquatica</i>	R
Cocksfoot	<i>Dactylis glomerata</i>	A	Meadow buttercup	<i>Ranunculus acris</i>	O
Common sorrel	<i>Rumex acetosa</i>	F	Meadow foxtail	<i>Alopecurus pratensis</i>	O
Common sow-thistle	<i>Sonchus oleraceus</i>	R	Meadow vetchling	<i>Lathyrus pratensis</i>	O
Common vetch	<i>Vicia sativa</i>	A	Nettle	<i>Urtica dioica</i>	F
Common vetch	<i>Vicia sativa</i>	A	Oval sedge	<i>Carex leporina</i>	R
Creeping bent	<i>Agrostis stolonifera</i>	A	Prickly sedge	<i>Carex echinata</i>	R
Creeping cinquefoil	<i>Potentilla reptans</i>	O	Prickly sow thistle	<i>Sonchus asper</i>	O
Creeping thistle	<i>Cirsium arvense</i>	O	Red clover	<i>Trifolium pratense</i>	O
Cuckoo pint	<i>Arum maculatum</i>	R	Red fescue	<i>Festuca rubra</i>	A
Curled dock	<i>Rumex crispus</i>	F	Rough meadow-grass	<i>Poa trivialis</i>	A
Cut-leaved Crane's bill	<i>Geranium dissectum</i>	O	Silverweed	<i>Argentina anserina</i>	O
False Fox-sedge	<i>Carex otrubae</i>	A	Smooth meadow-grass	<i>Poa pratensis</i>	F
False Oat Grass	<i>Arrhenatherum elatius</i>	F	Sweet vernal grass	<i>Anthoxanthum odoratum</i>	O
Fleabane	<i>Erigeron</i>	O	Upright hedge-parsley	<i>Torilis japonica</i>	LF
Geat willow herb	<i>Epilobium hirsutum</i>	O	Wood dock	<i>Rumex sanguineus</i>	O
Giant fescue	<i>Festuca gigantea</i>	R	Wood false-brome	<i>Brachypodium sylvaticum</i>	O
Greater bird's foot	<i>Lotus pedunculatus</i>	O	Yorkshire Fog	<i>Holcus lanatus</i>	A
Hairy tare	<i>Vicia hirsuta</i>	LA	Tutsan	<i>Hypericum androsaemum</i>	R

Table 10 Woody species recorded in hedges

COMMON NAME	SCIENTIFIC NAME	COMMON NAME	SCIENTIFIC NAME
Blackthorn	<i>Prunus spinosa</i>	Honeysuckle	<i>Lonicera periclymenum</i>

¹ ACFOR: Abundant, Common, Frequent, Occasional Rare

COMMON NAME	SCIENTIFIC NAME	COMMON NAME	SCIENTIFIC NAME
Bramble	<i>Rubus fruticosus agg.</i>	Ivy	<i>Hedera helix</i>
Bryony	<i>Dioscorea communis</i>	Northern-white cedar	<i>Thuja occidentalis</i>
Creeping thistle	<i>Cirsium arvense</i>	Oak	<i>Quercus petraea</i>
Dog rose	<i>Rosa canina</i>	Privet	<i>Ligustrum ovalifolium</i>
Elder	<i>Sambucus nigra</i>	Wych elm	<i>Ulmus glabra</i>
Hawthorn	<i>Crataegus monogyna</i>		

APPENDIX 3 SUMMARY OF LEGISLATION

Water vole

Water vole is protected under **Section 9** of the Wildlife and Countryside Act 1981(as amended) (WCA) and it is illegal to:

- Intentionally kill, injure or take any wild water vole. 9(1)
- Possess or control any live or dead wild water vole or any part of, or anything derived from, such an animal. 9(2)
- Intentionally or recklessly damage or destroy, any structure or place which any wild water vole uses for shelter or protection. 9(4)(a)
- Intentionally or recklessly disturb any such animal while it is occupying a structure or place which it uses for that purpose. 9(4)(b)
- Intentionally or recklessly obstruct access to any structure or place which any wild water vole uses for shelter or protection. 9(4)(c)
- Sell, offer or expose for sale, or have in possession or transports for the purpose of sale, any live or dead wild water vole, or any part of, or anything derived from, such an animal. 9(5)(a)
- Publishes or causes to be published any advertisement likely to be understood as conveying that you buy or sell, or intend to buy or sell, any of those things 9(5)(b)

Great crested newt

Great crested newt is protected under the **Conservation of Habitats and Species Regulations**

2017, known as the '**Habitats Regulations**', where it is an offence to:

- Deliberately capture, injure or kill any wild animal of an EPS,
- Deliberately disturb wild animals of any such species,
- Deliberately take or destroy the eggs of such an animal, or
- Damage or destroy a breeding site or resting place of such an animal

Great crested newt is also protected under the WCA, where it is illegal to:-

- Intentionally or recklessly disturb any great crested newt while it is occupying a structure or place which it uses for shelter or protection, 9(4)(b)

- Intentionally or recklessly obstructs access to any structure or place used by a great crested newt for shelter or protection, 9(4)(c)
- Sell, offer or expose for sale any great crested newt, 9(5)

Breeding birds

All birds, their nests and eggs are protected by law and it is thus an offence, with certain exceptions to

- Intentionally kill, injure or take any wild bird.
- Intentionally take, damage or destroy the nest of any wild bird while it is in use or being built.
- Intentionally take or destroy the egg of any wild bird.
- Have in one's possession or control any wild bird, dead or alive, or any part of a wild bird.
- Have in one's possession or control any egg or part of an egg.
- Use traps or similar items to kill, injure or take wild birds.
- Have in one's possession or control any bird of a species occurring on Schedule 4 of the Act unless registered, and ringed, in accordance with the Secretary of State's regulations.
- Recklessly disturb any wild bird listed on Schedule 1 while it is nest building, or at a nest containing eggs or young, or disturb the dependent young of such a bird.

APPENDIX 4 EDNA RESULTS



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ADAS
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172 Chester Road
Helsby
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Tel: 01159 516747
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www.adas.uk

Sample ID: 2019-1668 Condition on Receipt: Good Volume: Passed
Client Identifier: Mitigation Ponds Description: pond water samples in preservative
A and B
Date of Receipt: 27/06/2019 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	11/07/2019
Degradation Control [§]	Within Limits	Real Time PCR	11/07/2019
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	11/07/2019
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 12/07/2019 Date of issue: 12/07/2019

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.



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Sample ID: 2019-1670 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: Attenuation Pond Description: pond water samples in preservative
Date of Receipt: 27/06/2019 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	11/07/2019
Degradation Control [§]	Within Limits	Real Time PCR	11/07/2019
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	11/07/2019
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)