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PEACOCK AND SMITH

CAPITOL PARK, BARNSELY

GREAT CRESTED NEWT EDNA SURVEY REPORT

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PEACOCK AND SMITH

CAPITOL PARK, BARNESLEY

GREAT CRESTED NEWT EDNA SURVEY REPORT

MAY 2019

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Appendix 1 eDNA Analysis Report

DRAWINGS	TITLE	SCALE
SH11957-001	Extended Phase 1 Habitat Plan	1:2,500

EXECUTIVE SUMMARY

Wardell Armstrong LLP (WA) was commissioned by Peacock and Smith to undertake Environmental DNA (eDNA) testing for great crested newt *Triturus cristatus* at a proposed development scheme on land adjacent to Capitol Park, near Barnsley.

The site comprises agricultural land bounded on all sides by fencing or stone walls, with the M1 corridor immediately beyond the north-eastern site boundary and Higham Lane to the south and west. The Capitol Park industrial estate is located on land to the south east. The town of Barnsley lies immediate beyond the Motorway corridor to the north and east and the village settlement of Dodworth lies 0.5 km to the south with open arable and pastoral farmland dominating the wider landscape particularly to the west and south.

The survey involved eDNA sampling of three ponds within 500m of the site all of which are located well beyond the site boundary but within 250m. All three ponds returned negative results for GCN eDNA i.e. no GCN eDNA was recorded in the collected water samples. However, the laboratory recorded deterioration of the markers and as such the possibility of GCN eDNA being similarly deteriorated cannot be ruled out. Deterioration of the markers is considered to be due to the high levels of sediment and likely contamination present. Currently, the ponds are considered to be unsuitable for GCN due to very low water levels and likely contamination.

In conclusion, there are no further surveys required due to the unsuitability of the ponds to support GCN.

1 INTRODUCTION

1.1 Terms of Reference

- 1.1.1 Wardell Armstrong LLP (WA) was commissioned by Peacock and Smith to undertake Environmental DNA (eDNA) testing for great crested newt (GCN) at a proposed development scheme (hereafter referred to as the 'site'), located on land adjacent to Capitol Park, near Barnsley, central Ordnance Survey (OS) grid reference: SE 315 063.
- 1.1.2 The site comprises agricultural land covering approximately 5.7 ha. The site is bounded on all sides by fencing or stone walls, with the M1 corridor immediately beyond the north-eastern site boundary and Higham Lane to the south and west. The Capitol Park industrial estate is located on land to the south east. The town of Barnsley lies immediate beyond the Motorway corridor to the north and east, and the village settlement of Dodworth lies 0.5 km to the south with open arable and pastoral farmland dominating the wider landscape particularly to the west and south.
- 1.1.3 Surveys followed recommendations from an updated Preliminary Ecological Appraisal (PEA) undertaken by WA in 2017.

1.2 Legislative Framework

- 1.2.1 All native amphibians receive legal protection in Great Britain arising from the following legislation:
- Wildlife and Countryside Act 1981 (as amended) (in Great Britain).
 - Nature Conservation (Scotland) Act 2004.
 - Conservation of Habitats and Species Regulations 2010 (as amended).
- 1.2.2 In England and Wales all amphibians are listed on schedule 5 of the 1981 Act and the more threatened species (great crested newt, natterjack toad *Epidalea calamita* and pool frog *Pelophylax lessonae*) are also listed on Schedule 2 of the Conservation of Habitats and Species Regulations (2010 as amended).
- 1.2.3 It is an offence to deliberately capture, possess, disturb, kill, injure, or trade in great crested newts. In addition, it is an offence to damage or destroy the places they use for breeding or resting.
- 1.2.4 Other amphibian species, including smooth newt *Lissotriton vulgaris*, palmate newt *Lissotriton helveticus*, common frog *Rana temporaria* and common toad *Bufo bufo* are

protected against unlicensed trade. The legislation applies to all life stages of these animals.

2 SURVEY METHODOLOGY

2.1 Desk Study

2.1.1 A desk study was carried out prior to the survey to identify suitable habitats for great crested newts, such as additional water features within the site and within 500m of the site boundary. This included a review of OS maps, aerial photographs and the Multi-Agency Geographical Information for the Countryside (MAGIC) website.

2.1.2 Sheffield Biological Records Centre (SBRC) were contacted to ascertain whether there were any known records of great crested newts within the last 10 years within a 2km radius of the central grid reference of the site. Any records exceeding a 10-year period are omitted from reference in the report.

2.2 Field Survey

2.2.1 The eDNA testing of the three ponds was carried out on the 17th April 2019. Methodologies were undertaken in strict accordance with the relevant DEFRA guidelines¹ (Biggs et al., 2014).

2.2.2 The following field sampling protocols were followed when taking water samples:

- Twenty sub-samples at each pond were taken and evenly spaced around the pond margin and where possible, targeted areas where there was vegetation which could be used by great crested newts for egg laying.
- Using gloves, the surveyor opened the sterile Whirl-Pak bag plastic strip and collected 20 samples of 30mL each of pond water from around the margins of the pond. The samples were emptied into the Whirl-Pak bag and closed securely and shaken for 10 seconds.
- With a fresh pair of gloves on the surveyor used the clear plastic pipette provided and take 15ml of water from the Whirl-Pak bag into a sterile tube containing 35ml of ethanol to preserve the eDNA samples. The tubes were closed and shaken for 10 seconds to mix the samples and the preservatives.
- The above process was repeated to obtain seven conical tubes for each pond.
- The remaining water in the Whirl-Pak bag was emptied into the pond.

¹ Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). *Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067*. Freshwater Habitats Trust: Oxford

- The above process was carried out for each pond surveyed.

2.2.3 All samples were labelled with the relevant eDNA testing kit reference and pond number.

2.2.4 The eDNA samples were returned to FERA on 23rd April 2019.

2.3 Constraints

2.3.1 Sample kits contain a DNA marker, if marker levels are reduced on analysis this indicates that any DNA present, may have undergone some degradation. This is potentially due to presence of enzymes (nucleases) or compounds (e.g. phenolics) which can degrade DNA. In this case the markers did show inhibition, however this is likely due to the presence of sediments and potentially contamination within the water column, and the very shallow nature of the ponds. Hence the ponds are currently considered to be unsuitable for GCN.

3 RESULTS AND DISCUSSION

3.1 Desk Study

3.1.1 SBRC provided one record of great crested newt located 2km north of the site.

3.2 Field Survey

3.2.1 None of the three ponds surveyed for great crested newt eDNA returned a positive result for the presence of eDNA. The surveyors noted at the time of collecting the water samples that the ponds were almost dry, being a maximum of 100mm deep with a total absence of aquatic/marginal vegetation. All three ponds were turbid, with a whitish sediment noted².

3.2.2 The eDNA sampling was undertaken as a precaution, however the surveyors suspected that marker inhibition may result in an inconclusive analysis once the samples were returned to the lab.

3.2.3 The following information is relevant to the consideration of potential GCN presence of site and consequently whether additional surveys are required:

- the unsuitability of the ponds for GCN. All three ponds are very shallow with no aquatic vegetation and high turbidity levels, with the possible presence of contaminated sediment.
- The application site lies in excess of 200m from the ponds and is separated by the presence of Higham Lane (a trunk road measuring c.70m in width).
- The paucity of suitable habitats for GCN within the application site – the majority of which is dominated by intensively farmed arable land.
- Studies by Cresswell and Whitworth (2004) indicate that terrestrial foraging by GCN occurs mainly within 50 metres of their breeding pond and that few individuals are encountered at greater distances. GCN are also known to display directional bias during migration from their breeding ponds towards the most favourable terrestrial habitat (Jehle, 2000; Jehle and Arntzen, 2000; Malmgren, 2002). GCNs are, therefore more likely to stay within the woodlands which surround the ponds or migrate towards boundary hedgerows, field margins and marginal habitats to the north of Higham Lane rather than into the proposed construction footprint.
- The proposed development does not present any barriers to GCN dispersal or result in fragmentation of habitats.

² The whitish sediment is a potential source of the contamination which caused marker inhibition.

3.2.4 Given the above considerations. Additional surveys of the ponds are not considered to be necessary. The following precautionary mitigation is recommended as a further safeguard against incidental impacts:

- Toolbox talks to inform all staff engaged in site clearance works at the site about GCN and protected species.
- In the very unlikely event that amphibian are discovered during the course of the development works, all works in the immediate vicinity should immediately cease and a suitably qualified ecologist be contacted for advice.

4 REFERENCES

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). *Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067*. Freshwater Habitats Trust: Oxford.

Jehle, R. (2000). The terrestrial summer habitat of radio-tracked great crested newts (*Triturus cristatus*) and marbled newts (*T. marmoratus*). *Herpetological Journal* 10 pp. 137-142.

Jehle R & Arntzen JW (2000). Post breeding migrations of newts (*Triturus cristatus* and *T. marmoratus*) with contrasting ecological requirements. *Journal of Zoology* 241 (3) 297-306

Malmgren, J.C. (2002). How does a newt find its way from a pond? Migration patterns after breeding and metamorphosis in great crested newts (*Triturus cristatus*) and smooth newts (*T. vulgaris*). *Herpetological Journal* 12 pp. 29-35.

APPENDICES

Appendix 1 – DNA Analysis Report

DNA Analysis Report - Commercial in Confidence



Customer: Wardell Armstrong LLP
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Contact: Michael Moores
Email: m.moores@wardell-armstrong.com
Tel: 01204227227
Report date: 01-May-2019
Order Number: GCN19-1021
Samples: Pond Water
Analysis requested: Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:

The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

- Positive:** DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence



CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
P1	S19-015964	Inconclusive	0	No	YES
P3	S19-015968	Inconclusive	0	No	YES
P2	S19-015952	Inconclusive	0	No	YES

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. However, with all the samples we detected degradation of the internal control. Therefore, due to the risk of any eDNA also being degraded resulting in a false negative, we have issued an inconclusive result for each sample. We did note a substantial amount of white sediment in these samples which may have contributed to this result.

Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

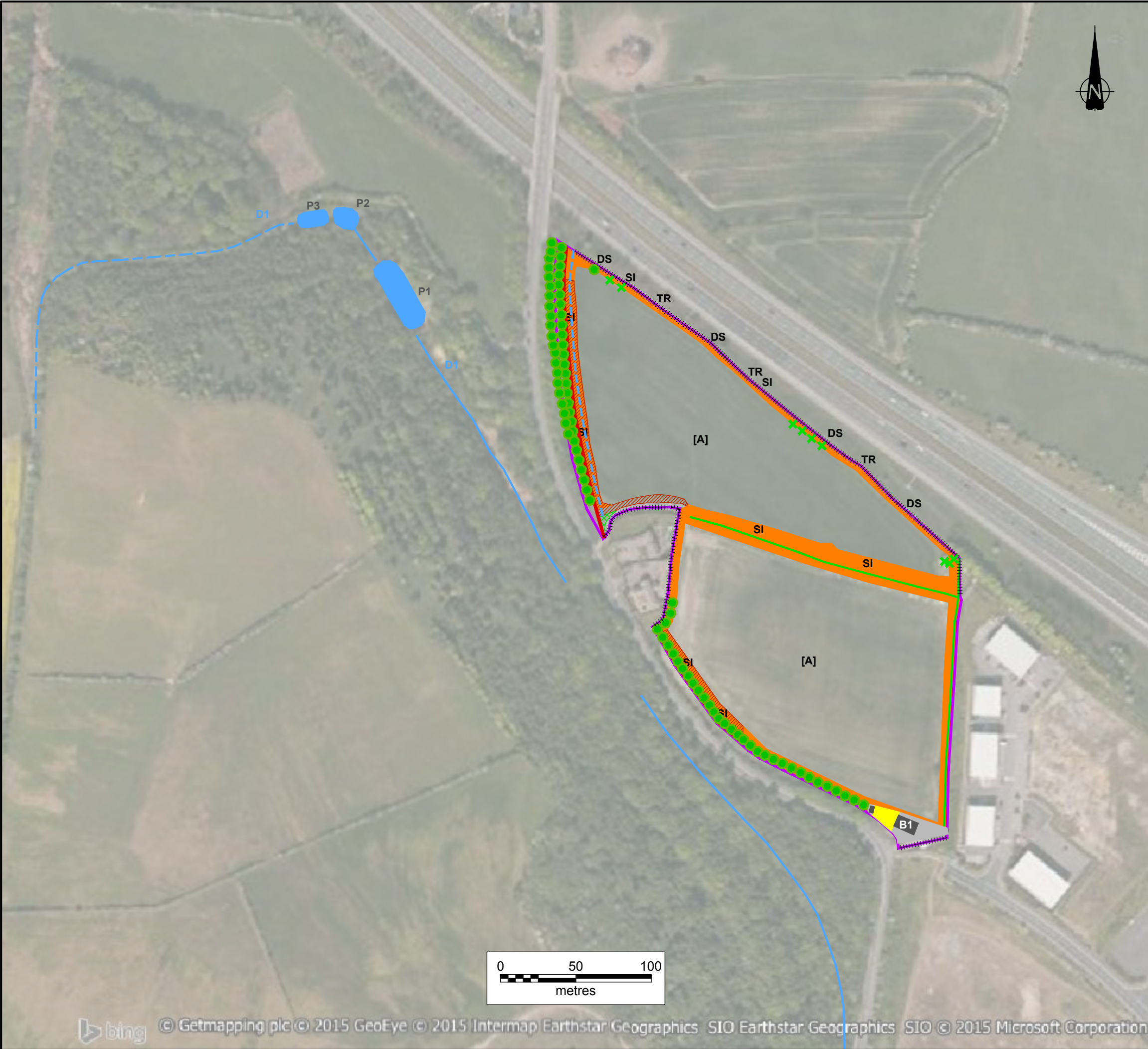
This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce

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DRAWINGS



DO NOT SCALE FROM THIS DRAWING

KEY

- Survey Area
- Amenity Grassland
- A Arable
- Buildings
- SI Semi Improved Neutral Grassland
- Dense Scrub
- Dry Drain
- Fence
- Hardstanding
- Pond
- Scattered Scrub
- Species Rich Hedge
- Stone Wall
- Tall Ruderal
- Tree
- Wet/damp drain
- TR Tall Ruderals
- DS Dense Scrub

REVISION	DETAILS	DATE	DRAWN	CHK'D	APP'D
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CLIENT	Sterling Capitol				
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PROJECT	Capitol Park Barnsley				
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DRAWING TITLE	Extended Phase 1 Habitat				
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DRG No.	SH11957-001	SCALE	1:2500 @ A3	DATE	April 2017
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