

Hampshire Biodiversity Information Centre

HBIC Ref: 9344

Non-statutory designated sites (Not Wader and Brent Goose) within the of Land North of Fareham, Hampshire (SU55900814):

Legend

- 5x5km Search Area
- SINC (Labelled pink)
- Surrounding SINC
- RVEI (Labelled purple)
- Surrounding RVEI

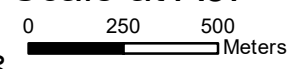
Ancient Woodland Inventory

- Semi-Natural
- Replanted

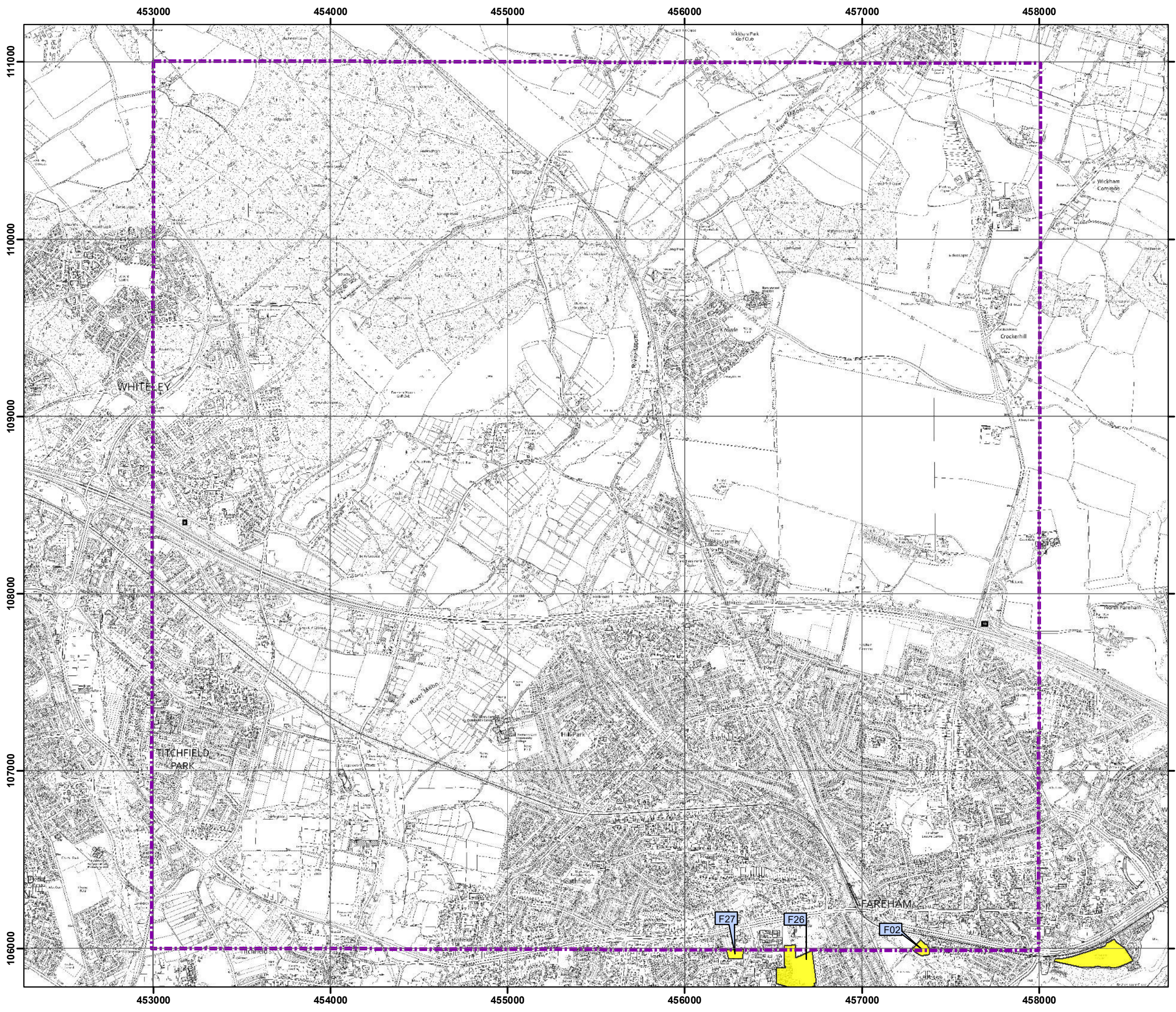
* Ancient Woodland boundaries are provided by Natural England and have been derived from the 1996 provisional Hampshire Ancient Woodland Inventory. These boundaries are indicative not definitive and tend not to include ancient woodlands below 2ha in size.

Your licence to re-use expires on 17/09/2021

Created: 17/09/2020
Scale at A3: 1:20,000



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Hampshire Biodiversity
Information Centre

HBIC Ref: 9344

**Non-statutory designated
sites (Wader and Brent
Goose only) within
the Land North of
Fareham, Hampshire
(SU55900814):**

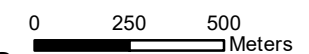
Legend

-  5x5km Search Area
- Solent Waders and Brent Goose Strategy**
- Classification**
-  Core Area
-  Primary Support Area
-  Secondary Support Area
-  SPA site
-  Low use
-  Candidate
-  Uncertain
-  Sites with only negative records
-  Brent Goose Sites 2002 without records

Your licence to re-use expires on 17/09/2021

Created: 17/09/2020

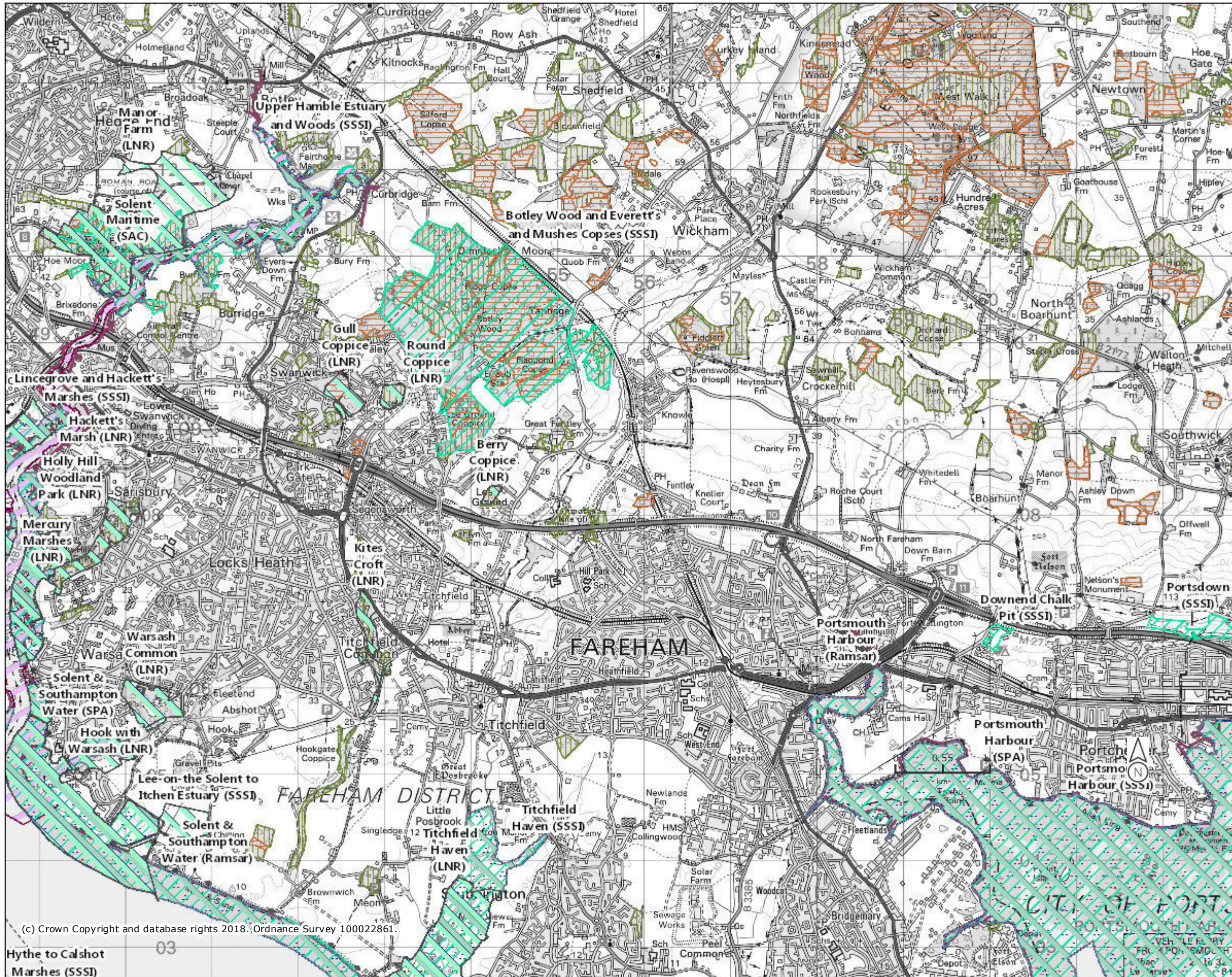
Scale at A3: 1:20,000



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APPENDIX 2


Information downloaded from MAGIC



Legend

-  Local Nature Reserves (England)
-  Ramsar Sites (England)
-  Sites of Special Scientific Interest (England)
-  Special Areas of Conservation (England)
-  Special Protection Areas (England)
- Ancient Woodland (England)**
-  Ancient and Semi-Natural Woodland
-  Ancient Replanted Woodland

Projection = OSGB36
 xmin = 448200
 ymin = 102800
 xmax = 463300
 ymax = 113700



Map produced by MAGIC on 22 January, 2018.
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APPENDIX 3

2017 Bat survey results

1.1. Bats

Bat Activity Surveys

- 1.1.1. Three bat activity surveys were undertaken at the application site, in line with the methodology outlined in Section 2 above. Table 1 below outlines the weather conditions during each survey visit.

Date	Weather Conditions
31.08.2017	17°C, 80% cloud cover, dry, light breeze
27.09.2017	15°C, 100% cloud cover, light rain, light breeze
19.10.2017	16°C, 100% cloud cover, persistent rain late on, moderate breeze

Table 1: Weather conditions during bat activity surveys

Activity Survey 30/08/2017

- 1.1.2. The activity survey on 30th August recorded low numbers of bat registrations, the majority of which were Common Pipistrelle *Pipistrellus pipistrellus*.
- 1.1.3. In total 111 registrations were recorded, of which 78 were Common Pipistrelle, with the first recorded at 20:17, the last at 21:23, and activity mostly located in the north of the application site. Soprano Pipistrelle *Pipistrellus pygmaeus*, was recorded 20 times between 20:19 and 21:53, with activity evenly distributed throughout the application site. An unidentified *Myotis* species was recorded seven times between 20:34 and 21:51. Brown Long-eared Bat *Plecotus auritus*, was recorded twice, at 20:31 and 21:53. Noctule *Nyctalus noctula*, was recorded twice at 20:46 and 21:02. Serotine was recorded twice at 20:35.
- 1.1.4. No groups of bats were noted during the surveys. All direct observations of bats were of individuals, either commuting or foraging along hedgerows.

Automated Surveys 30/08/2017 – 03/09/2017

- 1.1.5. Automated detectors were deployed at the northern (**D1**), central (**D2**) and southern (**D3**) locations (see Plan ECO3) and set to record for five consecutive nights from 30th August to 3rd September. The results from each night are detailed below for all three detector locations.

30/08/2017

D1 – Northern location

- 1.1.6. In total 84 registrations were recorded over the active period. Soprano Pipistrelle was recorded 60 times from 20:17 to 20:47. Common Pipistrelle was recorded 22 times from 20:16 to 20:54. A single Brown Long-eared Bat was recorded at 23:18. A single unidentified *Myotis* Bat was recorded at 02:09.

D2 – Central location

- 1.1.7. In total 117 registrations were recorded over the active period. Common Pipistrelle was recorded 67 times from 20:05 to 00:09. Soprano Pipistrelle was recorded 38 times from 20:08 to 20:26. A single Nathusius' Pipistrelle *Pipistrellus nathusii* was recorded at 22:20. A single Serotine *Eptesicus serotinus* was recorded at 20:34.

D3 – Southern location

- 1.1.8. In total 81 registrations were recorded over the active period. Common Pipistrelle was recorded 60 times from 20:06 to 22:17. Soprano Pipistrelle was recorded 21 times from 20:05 to 05:57.

31/08/2017

D1 – Northern location

- 1.1.9. In total 88 registrations were recorded over the active period. Soprano Pipistrelle was recorded 64 times from 20:18 to 22:34. Common Pipistrelle was recorded 23 times from 20:17 to 21:44. A single Noctule was recorded at 22:18.

D2 – Central location

- 1.1.10. In total 126 registrations were recorded over the active period. Common Pipistrelle was recorded 81 times from 20:13 to 22:46. Soprano Pipistrelle was recorded 43 times from 20:07 to 05:47. A single Brown Long-eared Bat was recorded at 21:32. A single unidentified *Myotis* Bat was recorded at 02:28.

D3 – Southern location

- 1.1.11. In total 124 registrations were recorded over the active period. Common Pipistrelle was recorded 104 times from 20:08 to 05:48. Soprano Pipistrelle was recorded 14 times from 19:59 to 05:47. An unidentified *Myotis* species was recorded six times from 20:36 to 00:25.

01/09/2017

D1 – Northern location

- 1.1.12. In total 129 registrations were recorded over the active period. Soprano Pipistrelle was recorded 92 times from 20:12 to 22:56. Common Pipistrelle was recorded 31 times from 20:21 to 03:40. Brown Long-eared Bat was recorded four times from 23:52 to 04:05. An unidentified *Myotis* species was recorded twice, at 22:14 and 03:34.

D2 – Central location

- 1.1.13. In total 188 registrations were recorded over the active period. Common Pipistrelle was recorded 128 times from 20:23 to 01:52. Soprano Pipistrelle was recorded 58 times from 20:15 to 23:51. Noctule was recorded twice, at 20:46 and 23:39.

D3 – Southern location

- 1.1.14. In total 189 registrations were recorded over the active period. Common Pipistrelle was recorded 163 times from 20:01 to 05:38. Soprano Pipistrelle was recorded 25 times from 20:39 to 05:42. A single unidentified *Myotis* Bat was recorded at 01:02.

02/09/2017

D1 – Northern location

- 1.1.15. In total 113 registrations were recorded over the active period. Soprano Pipistrelle was recorded 76 times from 20:06 to 22:45. Common Pipistrelle was recorded 34 times from 20:13 to 23:28. Brown Long-eared Bat was recorded twice, at 03:12 to 05:06. A single Noctule was recorded at 21:08.

D2 – Central location

- 1.1.16. In total 136 registrations were recorded over the active period. Common Pipistrelle was recorded 81 times from 20:01 to 22:47. Soprano Pipistrelle was recorded 45 times from 20:11 to 05:48. Noctule was recorded six times from 21:38 to 00:46. An unidentified *Myotis* species was recorded four times from 20:47 to 03:13.

D3 – Southern location

- 1.1.17. In total 133 registrations were recorded over the active period. Common Pipistrelle was recorded 86 times from 20:19 to 06:01. Soprano Pipistrelle was recorded 45 times from 20:04 to 06:05. A single unidentified *Myotis* Bat was recorded at 21:29. A single Noctule was recorded at 20:09.

03/09/2017

D1 – Northern location

- 1.1.18. In total 51 registrations were recorded over the active period. Soprano Pipistrelle was recorded 27 times from 20:04 to 05:46. Common Pipistrelle was recorded 24 times from 20:05 to 02:16.

D2 – Central location

- 1.1.19. In total 48 registrations were recorded over the active period. Soprano Pipistrelle was recorded 30 times from 20:07 to 04:46. Common Pipistrelle was recorded 17 times from 20:06 to 02:44. A single Noctule was recorded at 20:07.

D3 – Southern location

- 1.1.20. In total 16 registrations were recorded over the active period. Common Pipistrelle was recorded 11 times from 20:02 to 02:52. Soprano Pipistrelle was recorded four times from 19:59 to 05:24. A single Noctule was recorded at 22:52.

Activity Survey 27/09/2017

- 1.1.21. The activity survey on September 27th recorded very low numbers of bat registrations, the majority of which were Soprano Pipistrelle and Common Pipistrelle.
- 1.1.22. In total 26 registrations were recorded, of which 13 were Soprano Pipistrelle, with the first recorded at 19:23 and the last at 20:08. Common Pipistrelle was recorded 12 times from 19:20 to 20:25. A single Brown Long-eared Bat was recorded at 21:47. All activity was recorded in the northern half of the application site. Technical issues meant recording ceased partway through the second transect, comprising the southern half of the application site. Although part of one of the transects was not fully completed within the southern areas, it is considered that sufficient data was collected to allow for an effective analysis of bat activity across the application site, noting that additional data was obtained on this transect during the automated survey work.
- 1.1.23. No groups of bats were noted during the transects. The only direct observation of a bat was of an individual commuting along a hedgerow.

Automated Surveys 27/09/2017 – 02/10/2017

- 1.1.24. Automated detectors were deployed at locations **D1** and **D3** locations and set to record for six consecutive nights from 27th September to 2nd October. The results from each night are detailed below for both detector locations.

27/09/2017

D1 – Northern location

- 1.1.25. Only one registration was recorded over the active period. A single Soprano Pipistrelle was recorded at 19:59.

D3 – Southern location

- 1.1.26. In total 893 registrations were recorded over the active period. Common Pipistrelle was recorded 866 times from 19:25 to 06:30. Soprano Pipistrelle was recorded 27 times from 19:20 to 06:10.

28/09/2017

D1 – Northern location

- 1.1.27. In total 49 registrations were recorded over the active period. Soprano Pipistrelle was recorded 45 times from 19:16 to 22:25. Common Pipistrelle was recorded four times from 20:23 to 05:25.

D3 – Southern location

- 1.1.28. In total 1073 registrations were recorded over the active period. Common Pipistrelle was recorded 1046 times from 19:11 to 06:40. Soprano Pipistrelle was recorded 16 times from 19:12 to 06:25. An unidentified *Myotis* species was recorded six times from 19:35 to 05:31. Noctule was recorded five times from 19:00 to 01:18.

29/09/2017

D1 – Northern location

- 1.1.29. In total 28 registrations were recorded over the active period. Soprano Pipistrelle was recorded 27 times from 19:19 to 06:42. A single Common Pipistrelle was recorded at 05:57.

D3 – Southern location

- 1.1.30. In total 603 registrations were recorded over the active period. Common Pipistrelle was recorded 548 times from 19:08 to 05:03. Soprano Pipistrelle was recorded 35 times from 19:12 to 05:49. An unidentified *Myotis* species was recorded 18 times from 23:18 to 05:53. Noctule was recorded twice, at 19:58 to 21:58.

30/09/2017

D1 – Northern location

- 1.1.31. In total 13 registrations were recorded over the active period. Soprano Pipistrelle was recorded ten times from 19:06 to 05:21. Common Pipistrelle was recorded three times from 19:16 to 04:48.

D3 – Southern location

- 1.1.32. In total 373 registrations were recorded over the active period. Common Pipistrelle was recorded 357 times from 19:24 to 07:06. Soprano Pipistrelle was recorded 11 times from 19:10 to 06:45. An unidentified *Myotis* species was recorded five times from 04:22 to 04:29.

01/10/2017

D1 – Northern location

- 1.1.33. In total 14 registrations were recorded over the active period. Soprano Pipistrelle was recorded eight times from 19:13 to 22:22. Common Pipistrelle was recorded six times from 19:44 to 22:52.

D3 – Southern location

- 1.1.34. In total 792 registrations were recorded over the active period. Common Pipistrelle was recorded 742 times from 19:03 to 05:57. Soprano Pipistrelle was recorded 41 times from 19:03 to 06:43. An unidentified *Myotis* species was recorded nine times from 03:18 to 03:26.

02/10/2017

D1 – Northern location

- 1.1.35. In total 10 registrations were recorded over the active period. Soprano Pipistrelle was recorded five times from 19:11 to 19:44. Common Pipistrelle was recorded five times from 19:12 to 20:08.

D3 – Southern location

- 1.1.36. In total 12 registrations were recorded over the active period. Common Pipistrelle was recorded seven times from 19:00 to 22:31. Soprano Pipistrelle was recorded five times from 19:00 to 06:28.

Activity Survey 19/10/2017

- 1.1.37. The activity survey on 19th October recorded relatively high numbers of bat registrations, all of which were Soprano Pipistrelle and Common Pipistrelle. The activity survey was terminated after approximately one hour due to the onset of heavy rain. However, additional automated survey effort was employed for this month.
- 1.1.38. In total 205 registrations were recorded, of which 116 were Soprano Pipistrelle, with the first recorded at 18:18 and the last at 18:43. Common Pipistrelle was recorded 89 times from 18:21 to 18:45. Most activity occurred along the western boundary of the application site.
- 1.1.39. During the survey multiple groups of bats were noted. In all cases these groups were a maximum of two pipistrelles, which typically circled during foraging bouts.

Automated Surveys 19/10/2017 – 29/10/2017

- 1.1.40. Automated detectors were deployed at locations **D1**, **D2** and **D3** and set to record for ten consecutive nights from 19th October to 29th October. However, the detector at **D1** ceased to record data on 21st October. An additional detector was deployed at location **D3** for 20 consecutive nights from 19th October to 7th November. The results from each night are detailed below for both detector locations.

19/10/2017

D1 – Northern location

- 1.1.41. In total 63 registrations were recorded over the active period. Soprano Pipistrelle was recorded 52 times from 18:20 to 23:16. Common Pipistrelle was recorded nine times from 18:35 to 18:46. Noctule was recorded twice at 18:03.

D2 – Central location

In total 42 registrations were recorded over the active period, all relating to Soprano Pipistrelle. The registrations were recorded from 18:34 to 23:43.

D3 – Southern location

- 1.1.42. In total 107 registrations were recorded over the active period. Common Pipistrelle was recorded 86 times from 18:19 to 18:47. Soprano Pipistrelle was recorded 21 times from 18:18 to 23:00.

20/10/2017

D1 – Northern location

- 1.1.43. In total 197 registrations were recorded over the active period. Soprano Pipistrelle was recorded 192 times from 18:30 to 04:30. Common Pipistrelle was recorded four times from 19:12 to 19:56. A single Noctule was recorded at 18:42.

D2 – Central location

- 1.1.44. In total 20 registrations were recorded over the active period. Common Pipistrelle was recorded 16 times from 21:06 to 21:08. Soprano Pipistrelle was recorded four times from 18:34 to 18:44.

D3 – Southern location

- 1.1.45. In total 133 registrations were recorded over the active period. Common Pipistrelle was recorded 117 times from 18:37 to 23:46. Soprano Pipistrelle was recorded 11 times from 18:45 to 05:01. An unidentified Myotis species was recorded five times from 23:00 to 04:19.

21/10/2017

D1 – Northern location

- 1.1.46. Two registrations were recorded over the active period, both relating to Common Pipistrelle. They were recorded at 03:41 and 06:19.

D3 – Southern location

- 1.1.47. Seven registrations were recorded over the active period. Common Pipistrelle was recorded five times from 21:22 to 05:07. Soprano Pipistrelle was recorded twice, at 06:52 and 06:57.

22/10/2017

D1 – Northern location

- 1.1.48. In total 43 registrations were recorded over the active period. Common Pipistrelle was recorded 22 times from 18:25 to 05:13. Soprano Pipistrelle was recorded 19 times from 18:19 to 06:45. A single Brown Long-eared Bat was recorded at 22:12. A single Nathusius' Pipistrelle was recorded at 01:43.

D3 – Southern location

- 1.1.49. In total 128 registrations were recorded over the active period. Common Pipistrelle was recorded 115 times from 18:18 to 06:57. Soprano Pipistrelle was recorded ten times from 18:28 to 06:49. An unidentified Myotis species was recorded three times, at 19:11, 02:35 and 04:31.

23/10/2017

D1 – Northern location

- 1.1.50. In total 138 registrations were recorded over the active period. Soprano Pipistrelle was recorded 118 times from 18:09 to 19:29. Common Pipistrelle

was recorded 18 times from 18:21 to 00:37. Noctule was recorded twice, at 18:51 and 23:05.

D3 – Southern location

- 1.1.51. In total 50 registrations were recorded over the active period. Common Pipistrelle was recorded 46 times from 18:13 to 04:32. An unidentified Myotis species was recorded three times, at 19:08, 00:15 and 02:41. A single Soprano Pipistrelle was recorded at 18:41.

24/10/2017

D1 – Northern location

- 1.1.52. Three registrations were recorded over the active period, all relating to Common Pipistrelle. They were recorded at 23:52, 01:14 and 04:12.

D3 – Southern location

- 1.1.53. Ten registrations were recorded over the active period. Common Pipistrelle was recorded five times from 18:35 to 22:56. Soprano Pipistrelle was recorded five times from 18:20 to 06:29.

25/10/2017

D1 – Northern location

In total 212 registrations were recorded over the active period. Soprano Pipistrelle was recorded 145 times from 18:13 to 07:21. Common Pipistrelle was recorded 66 times from 18:20 to 22:33. A single unidentified Myotis Bat was recorded at 21:50.

D3 – Southern location

- 1.1.54. In total 478 registrations were recorded over the active period. Common Pipistrelle was recorded 468 times from 18:12 to 07:10. Soprano Pipistrelle was recorded nine times from 18:19 to 18:49. A single unidentified Myotis Bat was recorded at 03:12.

26/10/2017

D1 – Northern location

- 1.1.55. In total 375 registrations were recorded over the active period. Soprano Pipistrelle was recorded 160 times from 18:08 to 06:52. Common Pipistrelle was recorded 115 times from 18:09 to 03:55. An unidentified Myotis species was recorded 99 times from 18:32 to 19:08. A single Nathusius' Pipistrelle was recorded at 02:19.

D3 – Southern location

- 1.1.56. In total 302 registrations were recorded over the active period. Common Pipistrelle was recorded 259 times from 18:11 to 06:12. An unidentified Myotis species was recorded 35 times from 19:50 to 02:31. Soprano Pipistrelle was recorded eight times from 18:08 to 07:05.

27/10/2017

D1 – Northern location

- 1.1.57. In total 93 registrations were recorded over the active period. Soprano Pipistrelle was recorded 89 times from 18:13 to 06:51. Common Pipistrelle was recorded four times from 18:52 to 22:12.

D3 – Southern location

- 1.1.58. In total 88 registrations were recorded over the active period. Common Pipistrelle was recorded 81 times from 18:17 to 23:44. Soprano Pipistrelle was recorded five times from 18:20 to 18:37. An unidentified *Myotis* species was recorded twice, at 19:06 and 20:49.

28/10/2017

D1 – Northern location

- 1.1.59. In total 53 registrations were recorded over the active period. Soprano Pipistrelle was recorded 30 times from 18:07 to 06:51. Common Pipistrelle was recorded 23 times from 18:19 to 05:31.

D3 – Southern location

- 1.1.60. In total 95 registrations were recorded over the active period. Common Pipistrelle was recorded 83 times from 18:03 to 06:41. Soprano Pipistrelle was recorded 12 times from 18:25 to 07:09.

29/10/2017

D1 – Northern location

- 1.1.61. Three registrations were recorded over the active period, all relating to Common Pipistrelle. They were recorded at 18:41, 19:23 and 19:47.

D3 – Southern location

- 1.1.62. In total 78 registrations were recorded over the active period. An unidentified *Myotis* species was recorded 38 times from 18:51 to 06:49. Soprano Pipistrelle was recorded 22 times from 18:16 to 18:53. Common Pipistrelle was recorded 17 times from 18:13 to 19:18.

30/10/2017

D3 – Southern location

- 1.1.63. In total 32 registrations were recorded over the active period. Common Pipistrelle was recorded 22 times from 18:20 to 21:34. An unidentified *Myotis* species was recorded nine times from 18:51 to 05:51. A single Soprano Pipistrelle was recorded at 18:21.

31/10/2017

D3 – Southern location

- 1.1.64. In total 62 registrations were recorded over the active period. Common Pipistrelle was recorded 34 times from 18:08 to 03:01. An unidentified Myotis species was recorded 15 times from 19:33 to 06:59. Soprano Pipistrelle was recorded 13 times from at 17:52 to 22:33.

01/11/2017

D3 – Southern location

- 1.1.65. In total 128 registrations were recorded over the active period. Common Pipistrelle was recorded 116 times from 18:01 to 19:19. Soprano Pipistrelle was recorded 11 times from at 17:57 to 19:00. A single unidentified Myotis Bat was recorded at 19:00.

02/11/2017

D3 – Southern location

- 1.1.66. In total 24 registrations were recorded over the active period. An unidentified Myotis species was recorded 15 times from 18:27 to 19:25. Common Pipistrelle was recorded seven times from 18:11 to 18:40. Soprano Pipistrelle was recorded twice at 18:38.

03/11/2017

D3 – Southern location

- 1.1.67. In total 240 registrations were recorded over the active period. Common Pipistrelle was recorded 177 times from 18:01 to 07:24. An unidentified Myotis species was recorded 40 times from 18:30 to 21:28. Soprano Pipistrelle was recorded 23 times from 17:52 to 07:43.

04/11/2017

D3 – Southern location

- 1.1.68. In total 23 registrations were recorded over the active period. An unidentified Myotis species was recorded 19 times from 18:09 to 06:14. Common Pipistrelle was recorded twice, at 18:18 and 18:45. Soprano Pipistrelle was recorded twice, at 17:58 and 18:08.

05/11/2017

D3 – Southern location

- 1.1.69. Six registrations were recorded over the active period, all of which related to An unidentified Myotis species. These were recorded from 18:12 to 18:18.

06/11/2017

D3 – Southern location

- 1.1.70. No bats were recorded over the active period.

07/11/2017

D3 – Southern location

1.1.71. No bats were recorded over the active period.

APPENDIX 4

Great Crested Newt eDNA results

Technical Report
Confidential

Folio No: D2116
Report No: 1
Client: Ecology Solutions
Order No: TS7065
Attn: Tom Smith

Date: 18th July 2016

TECHNICAL REPORT

EXAMINATION OF ENVIRONMENTAL DNA

IN POND WATER FOR THE DETECTION OF

GREAT CRESTED NEWTS

T. Wood



SUMMARY

When Great Crested Newts (GCN) inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (e-DNA) traces to confirm GCN inhabitation, or prove absence of presence.

The following samples were submitted for e-DNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

Date received at Laboratory: 11th July 2016
 Date DNA extracted started: 12th July 2016
 Date of analysis: 14th July 2016
 Matters Affecting Results: None

RESULTS

Laboratory Reference	Sample Detail	Co-Ordinates	Inhibition Check	Sample integrity	Result / Score
eDNA23497	Pond – Roebuck	-	Acceptable	Acceptable	Negative 0/12
eDNA23498	Drain 1 Roebuck	-	Acceptable	Acceptable	Negative 0/12

METHODS AND INFORMATION.

In the above table, **NEGATIVE** means that GCN DNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence.

POSITIVE means that GCN DNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed.

Inhibition check refers to a laboratory evaluation of the sample tested by adding a known amount of an artificial gene to the sample and running the qPCR analysis in duplicate. Variation in the point at which qPCR amplification starts indicates inhibition, which requires the sample to be diluted and retested.

Sample integrity refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. PCR inhibitors can cause false results. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will



cause an indeterminate sample and must be sampled again. In that case the result will be reported as inconclusive.

To generate the results all of the tubes from each pond are combined to produce one e-DNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. It may be assumed that small fractions of positive analyses suggest low level presence but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

A positive result may be a true positive but there is also a possibility that it is due to contamination of the sample from another pond, or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA from one site to another. The optimum accuracy of the method depends on careful sampling methodology alongside our forensic standards of laboratory analysis.

We provide training sheets and can offer formalised training in collection, even though certified training of collectors is not currently a Natural England requirement when sampling for GCN.

Kits are manufactured by Scientifics to strict quality procedures adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit.

LABORATORY ANALYSIS

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time'.

qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.



The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 as follows:

Primer	Sequence	Fragment	Gene
TCCBL	CGTAAACTACGGCTGACTAGTACGAA	81	Cyt-b
TCCBR	CCGATGTGTATGTAGATGCAAACA	81	Cyt-b
Probe			
TCCB	CATCCACGCTAACGGAGCCTCGC	81	Cyt-b

Using this protocol, there should be no detection of closely related species. In the case of the great crested newt in the UK, the risk is mainly of detecting the Italian crested newt (*Triturus carnifex*) which is present at a few locations. The primers and probe were also tested on tissue samples of marbled newt (*Triturus marmoratus*). None of these samples were amplified, confirming the suitability of the primer pair and probe for the great crested newt. The primers and probe also did not bind with the DNA of other UK native newts (smooth and palmate newt) which are in the genus *Lissotriton*.

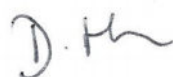
Reported By: **Arthur Green**



Analysed By: **Thomas Wood**



Approved by: **Derry Hickman**



APPENDIX 5

Nitrogen Budget Calculations

Nitrogen Budget Calculation

Planning Application Reference No.	
Site Name:	Land south of Funtley Road, Funtley (FS2)
Additional Information:	
Date:	08.07.20

Stage 1	Calculate total Nitrogen in kg per year derived from the development that would exit the Wastewater Treatment Works (WwTW) into Solent catchments after treatment	
Step 1	Calculate additional population	
	Enter the number of units proposed	125
	Net population increase per housing unit	2.40
	Total net population increase generated by the development	300.00
Step 2	Calculate wastewater volume generated by the development	
	Water use in litres per person per day	110
	Total wastewater volume generated by the development (litres per day)	33,000
Step 3	Confirm receiving WwTW and permit limit	
	Select the wastewater treatment works the development will connect to	Peel Common
	Wastewater treatment works' permit limit (mg per litre)	9.0
	Wastewater treatment works' discharge level (mg per litre)	8.1
Step 4	Calculate total nitrogen in kg per year discharged by the WWTW	
	Deduct acceptable Nitrogen loading in wastewater (mg per litre)	6.1
	Total Nitrogen discharged by WwTW (mg per day)	201,300.0
	Total Nitrogen discharged by WwTW (kg per day)	0.2013
	Total Nitrogen discharged by WwTW (kg per year)	73.5

Stage 2	Calculate existing (pre-development) nitrogen from current land use of the development site	
Step 1	Total area of development site	
	Enter the total area of the development site (hectares)	6.23
Step 2	Identify current land uses of the development site	
	Enter area currently used for urban development (hectares)	0.87
	Enter area currently used for open space / greenfield (hectares)	0.00
	Enter area currently used for woodland (hectares)	0.00
	Enter area currently used for community food growing / catchment average (hectares)	0.00
	Enter area currently used for cereals (hectares)	0.00
	Enter area currently used for dairy (hectares)	0.00
	Enter area currently used for general cropping (hectares)	0.00
	Enter area currently used for horticulture (hectares)	0.00
	Enter area currently used for pig farming (hectares)	0.00
	Enter area currently used for lowland grazing (hectares)	5.36
	Enter area currently used for mixed farming (hectares)	0.00
	Enter area currently used for poultry farming (hectares)	0.00
	Check to help ensure that sum total of land uses in Step 2 equals site area in Step 1	6.2
Step 3	Calculate nitrogen load from current land usage	
	Total Nitrogen load from current land usage (kg per year)	82.1

Nitrogen Budget Calculation

Stage 3		Calculate nitrogen load for the non-built land uses proposed for the development site		
Step 1	Identify proposed land uses of the development site			
	Enter the total urban area to be created (hectares)		3.61	
	Enter the total designated open space / SANG area to be created (hectares)		2.62	
	Enter the total nature reserve area to be created (hectares)		0.00	
	Enter the total woodland area to be created (hectares)		0.00	
	Enter the total community orchard area to be created (hectares)		0.00	
	Enter the total community food growing / allotment area to be created (hectares)		0.00	
	<i>Check to help ensure that sum total of proposed land uses equals site area in Stage 2</i>			6.23
Step 2	Calculate total Nitrogen load from proposed land uses			
	<i>Total Nitrogen load from future land uses (kg per year)</i>			64.72

Stage 4		Calculate the net change in Nitrogen load from the proposed development		
Step 1	Identify Nitrogen load from wastewater (Stage 1)			
	<i>Nitrogen leaving wastewater treatment works (kg per year)</i>			73.47
Step 2	Calculate net change in Nitrogen load from land use changes			
	<i>Total Nitrogen load from future land use (kg per year)</i>			-17.40
Step 3	Calculate total Nitrogen budget for the development site			
	<i>Nitrogen budget for the site (kg per year)</i>			56.08
Step 4	Calculate precautionary buffer if Nitrogen budget exceeds zero			
	<i>Precautionary Nitrogen buffer (kg per year)</i>			11.22

Total Nitrogen budget for the proposed development (kg per year)		67.3
Development will generate additional Nitrogen - Mitigation is required Please liaise with your Local Planning Authority for advice on next steps		

APPENDIX 6


Map showing Ancient Woodland, Priority Habitat and
National Forest Inventory boundaries



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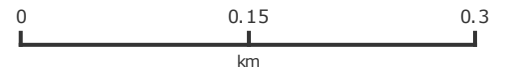
Legend

Ancient Woodland (England)

 Ancient and Semi-Natural Woodland

 Ancient Replanted Woodland

 Priority Habitat Inventory - Deciduous Woodland (England)



Projection = OSGB36

xmin = 455100

ymin = 107600

xmax = 456800

ymax = 108800

Map produced by MAGiC on 22 January, 2018.
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APPENDIX 7

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boundary