

Great Crested Newt and Water Vole Survey
Boston West Golf Course
Hubbert's Bridge
Lincolnshire



Issued to:

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Head Office
The County-hall
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GREAT CRESTED NEWT AND WATER VOLE SURVEY BOSTON WEST GOLF COURSE, HUBBERT'S BRIDGE, LINCOLNSHIRE

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1 INTRODUCTION

Inspired Ecology Ltd has been commissioned by Smart Move Boston Ltd. to undertake a great crested newt *Triturus cristatus* environmental DNA (eDNA) and water vole survey of ponds and drains at Boston West Golf Course, Hubbert's Bridge, Boston, Lincolnshire. The survey is required in connection with plans to construct holiday lodges on the former golf course, as well as a hub building and sales area at the south-east of the site.

Inspired Ecology Ltd initially surveyed half of the site in October 2019, with the other half surveyed in February 2020, and the results reported in McNally (2020). Nine ponds and three drains were surveyed and assessed using the habitat suitability index (HSI), and were determined to have suitability to support great crested newt ranging from below average to good suitability. The data search did not return any results of great crested newt within 2km of the survey site. The report recommended that the two ponds determined to have good suitability to support breeding great crested newt (Pond 8 and Pond 9) should be subject to eDNA testing. After discussions with the local planning authority (LPA) and their ecologist, it was determined that it was necessary to conduct eDNA surveys of all of the ponds and drains on Boston West Golf Course, in order to confirm the presence or absence of this species from the survey site. The drains on site were considered to have either low or low-moderate potential to support water vole. The LPA advised that water vole surveys should be undertaken on these drains in order to determine whether water vole are present or absent from the survey site.

The eDNA and water vole surveys were carried out on the 22nd April 2020 by Ian Nixon (registered to use Natural England Class Licences WML-CL08 to survey great crested newts, WML-CL19 and WML-CL20 to survey bats and WML-CL29 to survey barn owls; registration numbers 2015-16823-CLS-CLS, 2015-12336-CLS-CLS, 2015-12338-CLS-CLS and CL29/00110 respectively), and Rachel McNally.

This report details the methods used, describes the species found on the site, discusses the results and makes recommendations for further work.

2 SITE DESCRIPTION

The survey site comprises nine ponds and three drains located at Boston West Golf Course, Hubbert's Bridge, Boston, Lincolnshire – central grid reference TF264440. The locations of the ponds and drains surveyed are shown in Figure 1 below. Representative photographs can be found in the initial report (McNally, 2020) and also in Appendix 1 of this report.



Figure 1: Locations of the nine ponds and three drains surveyed

Pond 1 is the westernmost pond on the former golf course and is choked by common reed *Phragmites australis* and bulrush *Typha latifolia*. The original survey determined that this pond was considered to have below average suitability to support breeding great crested newts.

Pond 2 has locally dominant common reed. Other marginal and emergent plants include yellow iris *Iris pseudacorus*, bulrush, false fox-sedge *Carex otrubae*, great willowherb *Epilobium hirsutum*, clustered dock *Rumex conglomeratus* and grey club-rush *Schoenoplectus tabernaemontani*. Water plants include a water-lily species *Nymphaea* sp. and mare's-tail *Hippuris vulgaris*. The pond is spanned by a bridge. The original survey determined that this pond was considered to have below average suitability to support breeding great crested newts.

Pond 3 is the southernmost pond on site. There are some areas of open water amongst extensive and dense swamp vegetation, with common reed and bulrush dominant. There is also bittersweet *Solanum dulcamara*, great willowherb, lesser bulrush *Typha angustifolia*, clustered dock and false fox-sedge. Grey willow *Salix cinerea*, goat willow *Salix caprea*, white willow *Salix alba* and crack-willow *Salix fragilis* are located at the edge of the pond, as well as guelder-rose

Viburnum opulus and ornamental dogwood *Cornus sp.* Plants in the open water are common duckweed *Lemna minor*, water-cress *Nasturtium officinale*, mare's-tail, gypsywort *Lycopus europaeus*, and a water-speedwell species *Veronica sp.* The original survey determined that this pond was considered to have below average suitability to support breeding great crested newts.

Pond 4 is located to the north of Pond 2 and has a waterfall. Species present include bulrush, weeping willow *Salix x sepulcralis*, water-lily species and common reed. The original survey determined that this pond was considered to have average suitability to support breeding great crested newts. The waterfall was not running at the time of survey on 22nd April 2020.

Pond 5 is a small pond towards the north of the survey site. Species present here include bulrush, rosebay willowherb *Chamerion angustifolium*, dogwood *Cornus sanguinea*, hazel *Corylus avellana* and common knapweed *Centaurea nigra*. The original survey determined that this pond was considered to have below average suitability to support breeding great crested newts. Pond 5 was dry on 22nd April 2020, thus it was not possible to conduct an eDNA test on this pond.

Pond 6 is a very small pond adjacent to the woodland at the north of the site. It is dominated by bulrush with rosebay willowherb, dogwood, hazel and common knapweed. The original survey determined that this pond was considered to have below average suitability to support breeding great crested newts. Pond 6 was dry on 22nd April 2020, thus it was not possible to conduct an eDNA test on this pond.

Pond 7 is a long, sinuous pond which is spanned by a footbridge. Along the banks of the pond there is vegetation such as alder *Alnus glutinosa* and willow species *Salix sp.* saplings, as well as ornamental dogwood bushes. There are patches of common reed, bulrush and lesser bulrush, with other marginal plants present such as willowherb species *Epilobium sp.*, false fox-sedge, hard rush *Juncus inflexus*, yellow iris and the wetland moss *Calliergon cuspidatum*. Exposed pond liner was noted in various places around the pond. The original survey determined that this pond was considered to have average suitability to support breeding great crested newts.

Pond 8 is a C-shaped pond with a short, grassy culvert towards the northern end. Alder saplings, a mature willow species and several sub-mature elder *Sambucus nigra* trees were noted along the banks of the pond. Common reed and bulrush patches are present around the pond, with other marginal vegetation including false fox-sedge, hard rush, soft-rush *Juncus effusus*, clustered dock, great willowherb and purple-loosestrife *Lythrum salicaria*. Water-lily species, water-cress and celery-leaved buttercup *Ranunculus sceleratus* are present as submerged

plants, with much water-speedwell species in the pond to the north of the culvert. The original survey determined that this pond was considered to have good suitability to support breeding great crested newts.

Pond 9 is a small waterbody with a fountain at the southern end, along with some ornamental brickwork with slabs. Around the margins of the pond, there are numerous large pebbles/cobbles for decoration. Marginal vegetation comprises patches of bulrush, yellow iris and greater pond-sedge *Carex riparia*, along with jointed rush *Juncus articulatus*, water-lily species, great willowherb and spiked water-milfoil *Myriophyllum spicatum*. The original survey determined that this pond was considered to have good suitability to support breeding great crested newts.

Drain 1 is located towards the west of the active golf course. The banks of the drain are steep, with vegetation present including dogwood, common reed, dog-rose *Rosa canina*, great willowherb, alder, blackthorn *Prunus spinosa*, dead-nettle species *Lamium sp.*, common nettle *Urtica dioica*, bristly oxtongue *Helminthotheca echinoides*, spear thistle *Cirsium vulgare*, creeping thistle *Cirsium arvense*, daisy *Bellis perennis* and ribwort plantain *Plantago lanceolata*. Within the channel, there is vegetation including bulrush and common reed, with submerged celery-leaved buttercup. The drain is culverted at the east and west, and continues to the west near the site boundary; here there is a very dense patch of common reed within the drain channel. The eastern side of the drain is culverted underneath the adjacent fairway, with the easternmost end of the drain after the culvert having low water levels and being dry in places. The original survey determined that this drain was considered to have average suitability to support breeding great crested newts and low-moderate potential to support water vole.

Drain 2 is a short length of wet ditch along the western site boundary. The ditch has steep banks of rough grassland, with the channel being very narrow. Species including great willowherb, bulrush and alder are present here. The original survey determined that this drain was considered to have average suitability to support breeding great crested newts and low potential to support water vole. On 22nd April 2020, Drain 2 was largely dry, particularly towards the north of the drain, thus eDNA samples could only be taken from the southern end of the drain which had between 75mm and 120mm of water present at the time of survey.

Drain 3 is a further small wet ditch, located to the east of the survey site. The ditch is crossed by the tarmac access road to site, and joins a roadside ditch at the eastern end. The banks are steep with rough grassland vegetation. Common reed lines the water-filled channel, with great willowherb and duckweed species *Lemna sp.* also present. The original survey determined that this drain was considered to have below average suitability to support breeding great crested newts and low-moderate potential to support water vole.

3 METHODS

3.1 Great crested newt eDNA tests

This test involves collecting water samples from each of the ponds and drains on site in accordance with a strict protocol provided by Natural England. The samples are then sent to a Natural England approved laboratory and analysed for traces of environmental DNA produced by great crested newts. The test was undertaken in accordance with the protocol specified by Natural England by Ian Nixon and Rachel McNally on 22nd April 2020 and the analysis was undertaken by NatureMetrics Ltd, who are an approved laboratory.

3.2 Water vole

The drain channels were assessed for their potential to support water vole *Arvicola amphibius* and a search was made of the banks (where access allowed) for signs of use by water voles including feeding stations, burrows, latrine sites, runs through the vegetation and cropped grass around burrow entrances. In accordance with the latest survey guidelines (Dean *et al.*, 2016), the drains were checked from the channel and April is an appropriate time of year to confirm water vole presence.

4 RESULTS AND DISCUSSION

4.1 Great crested newt eDNA tests

4.1.1 Results

The eDNA tests for Ponds 1, 2, 4, 7, 8 and 9, and of Drain 2 were returned as negative. The test results for Pond 3 and Drains 1 and 3 were inconclusive. It was not possible to test Pond 5 or Pond 6 as these ponds were dry at the time of survey. The results are provided as Appendix 2.

4.1.2 Discussion

The results of the eDNA surveys of the waterbodies within Boston West Golf Course suggest that great crested newt are not present on the survey site. Although the results for Pond 3 were inconclusive, large numbers of fowl were noted on this pond. This included greylag goose *Anser anser*, mallard *Anas platyrhynchos*, moorhen *Gallinula chloropus* and coot *Fulica atra*, all of which were nesting on this pond. Similarly, fish were noted within Pond 3, which would make it unfavourable habitat for breeding great crested newt. As such, it is considered highly unlikely that great crested newt would be present within this pond. Fowl and fish were also noted within several other ponds on the survey site.

Drains 1 and 3 had low water levels at the time of survey and there was lots of sediment within the water, which may explain why the results of the tests for these drains were inconclusive. Taken together with the absence of great crested newt from other waterbodies on site however, it is considered that great crested newt are also likely to be absent these drains.

Similarly, Ponds 5 and 6 could not be tested as they were dry at the time of survey; although rare drying of ponds can be conducive to great crested newt occupation of a pond, on the basis that great crested newt were absent from the rest of the survey site, it is considered highly unlikely that this species would be present within these ponds.

The results of this eDNA survey indicate that great crested newt are **absent** from Boston West Golf Course. No further survey work or mitigation is required with respect to this species.

4.2 Water vole

4.2.1 Results

No signs of use by water vole were noted within any of the drains on site.

4.2.2 Discussion

The easternmost section of Drain 1 was not considered to offer potential for water vole due to the lack of water and shallower banks, however the central and western sections had been considered to offer low-moderate potential to support water vole. The results of this survey suggest that water vole are not present on this drain, however. Similarly, Drain 3 had been considered to offer low-moderate potential to support water vole, but no signs were noted during this survey.

The original report considered Drain 2 to offer low potential to support water vole. During this survey however, the drain was mostly dry, with just 75mm-120mm of water present within the southern end of the drain. The lack of water likely makes this drain unsuitable for use by water vole, with no signs of use by water vole noted within this drain either.

Water vole are considered to be **absent** from the drains on the survey site. The work is highly unlikely to impact upon the water vole population in the local area. As the drains will not be impacted by the proposed works, it is not considered necessary to conduct further surveys or undertake any mitigation work for this species on site.

5 REFERENCES AND BIBLIOGRAPHY

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**APPENDIX 1
Photographs**



Photograph 1: Pond 2 (October 2019)



Photograph 2: Pond 3 (October 2019)



Photograph 3: Pond 4 (October 2019)



Photograph 4: Pond 5 (October 2019)



Photograph 5: Pond 5 (22nd April 2020)



Photograph 6: Pond 6 (October 2019)



Photograph 7: Pond 6 (22nd April 2020)



Photograph 8: Pond 7 (February 2020)



Photograph 9: Pond 8 (February 2020)



Photograph 10: Pond 9 (February 2020)



Photograph 11: Drain 1 (February 2020)



Photograph 12: Drain 2 (February 2020)



Photograph 13: Drain 2 (22nd April 2020)



Photograph 14: Drain 3 (February 2020)

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**APPENDIX 2
eDNA test results**

Report: 101200-1

Order number: 101200

Great Crested Newt eDNA Results

Company: Inspired Ecology Ltd

Project code | Task code: Boston West

Date of Report: 1 May 2020

Number of samples: 10

Thank you for sending your sample(s) for analysis by NatureMetrics. Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

DNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen Blood and Tissue extraction kits.

qPCR amplification was carried out in 12 replicates per sample, using the primers and probe described by Biggs et al. (2014), in the presence of both positive and negative controls.

Results indicate GCN absence in 'Drain 2', 'Pond 1', 'Pond 2', 'Pond 4', 'Pond 7', 'Pond 8' and 'Pond 9'. All controls performed as expected and so the results are conclusive. Inhibition was detected in 'Drain 1', 'Drain 3' and 'Pond 3', which was not resolved with multiple rounds of DNA dilution as prescribed by Biggs et al. 2014, we therefore return these results as inconclusive.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of Great Crested Newts at a level below the limits of detection.

Sample	Pond ID	Arrived	Inhibition	Degradation	Score	GCN status
183	'Drain 1'	24-Apr	Yes	No	0	Inconclusive
182	'Drain 2'	24-Apr	No	No	0	Negative
180	'Drain 3'	24-Apr	Yes	No	0	Inconclusive
174	'Pond 1'	24-Apr	No	No	0	Negative
175	'Pond 2'	24-Apr	No	No	0	Negative
177	'Pond 3'	24-Apr	Yes	No	0	Inconclusive
176	'Pond 4'	24-Apr	No	No	0	Negative
185	'Pond 7'	24-Apr	No	No	0	Negative
184	'Pond 8'	24-Apr	No	No	0	Negative



181	'Pond 9'	24-Apr	No	No	0	Negative
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End of report

Report issued by: Dr. Narin Kirikyali

Contact: gcn@naturemetrics.co.uk | 01491 829042



Understanding your results

Positive GCN DNA has been detected in this sample, meaning that at least one of the 12 replicates has been amplified. Remember that this is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative No GCN DNA has been detected in this sample, and the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by the degradation of the DNA (when the DNA marker contained in the ethanol in the kits fails to amplify) or by inhibition of the reaction (when the marker added in the lab fails to amplify) caused by certain chemicals or organic compounds that may be present in the water sample.

inhibitors Naturally-occurring chemicals/compounds that cause DNA amplification to fail, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

negative control Used to determine if PCR reactions are contaminated.

positive control Used to determine whether the assay is working correctly.

primers Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR.

probe A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

qPCR Stands for 'quantitative PCR', sometimes also known as 'real-time PCR'. A PCR reaction incorporating a coloured dye that fluoresces during amplification, allowing a machine to track the progress of the reaction. Often used with species-specific Primers where detection of amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

