DOGGER BANK D WIND FARM

Preliminary Environmental Information Report

Volume 2
Appendix 23.3 Great Crested Newt Technical Advice Note

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Prepared For:	**Dogger Bank D Offshore Wind Farm**

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Great Crested Newt Technical Advice Note
Dogger Bank D Wind Farm

April 2025

Ecus Ltd

Report to:	Royal HaskoningDHV UK Ltd	
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4.0	**Fourth issue**	**24/04/2025**

Contents

1. Introduction

1.1.1 The Applicant commissioned Ecus Limited (Ecus Ltd) to produce a 'Great Crested Newt *Triturus cristatus* (GCN) Technical Advice Note' for the Onshore Development Area of Dogger Bank D Offshore Wind Farm (hereafter known as the Project or DBD).

1.1.2 The Onshore Development Area includes a cable corridor up to 55 km long, running through a predominantly agricultural landscape with occasional rural settlements within East Riding of Yorkshire. The Onshore Development Area can be seen in Figures 1 and 2 (presented at the end of the report).

1.1.3 Habitats in the Onshore Development Area to be impacted by the Project predominantly comprises arable farmland containing various cereal and non-cereal crops and occasional woodland, with field boundary features such as ditches, hedgerows and grassland field margins.

1.1.4 This report addresses comments received by the Applicant from Natural England on 31 March 2023 for the infrastructure which was described in the 2023 Dogger Bank D Scoping Report (LF000016-CST-DOG-REP-0001), where appropriate.

1.1.5 Comments provided through the Natural England Discretionary Advice Service (Charged Advice), reference DAS/426550 (provided at Appendix 1), state that:

> *"Natural England (NE) expects Great Crested Newt (GCN) surveys, which may inform a future GCN licence application, to include visual inspections of all ponds up to 250 m (or 500 m from development sites). Factors such as scale of the development, habitat connectivity, barriers to dispersal, etc. should be considered when determining the survey area. These factors can also be considered when excluding specific ponds from a survey (e.g. significant barriers to dispersal between a pond and the development site). If ponds are excluded from the survey effort and/or if only ponds within 250 m of the development are surveyed, NE recommend the ecologist retains evidence of their justification for their own records. If there is clear habitat connectivity between ponds within 250 m to 500 m and the development site, it may be necessary to extend the survey area. In general, surveys of ponds greater than 250 m from developments are normally appropriate only when all of the following conditions are met:*
>
> - *Maps, aerial photos, walk-over surveys or other data indicate that the pond(s) has potential to support a large great crested newt population;*
> - *The footprint contains particularly favourable habitat, especially if it constitutes the*

majority available locally;

- *The development would have a substantial negative effect on that habitat; and,*
- *There is an absence of dispersal barriers".*

1.1.6 Therefore, based on advice from Natural England, the purpose of this GCN Technical Advice Note is to evidence the rationale to undertake GCN environmental DNA (eDNA) surveys and GCN Habitat Suitability Index (HSI) assessments only at waterbodies within 250m of the Onshore Development Area that are identified as extant and relevant.

1.2 Site Description and Project Scope

1.2.1 The Onshore Development Area is in the East Riding of Yorkshire. A 250 m buffer surrounding the Onshore Development Area was surveyed for GCN. The Onshore Development Area and the 250 m buffer can be seen in Figures 1 and 2.

1.2.2 The onshore elements of the Project forming the Onshore Development Area will include landfall, onshore export cables within the onshore export cable corridor (ECC) and the Onshore Converter Station (OCS) and Energy Storage and Balancing Infrastructure (ESBI). A full description of the Project is provided in **Volume 1, Chapter 4 Project Description**.

1.2.3 Works will involve varying levels of vegetation clearance and light and noise disturbance during the construction phase. These will range from total clearance and high disturbance to minimal clearance and low disturbance. Within this report, the highest level of clearance and disturbance have been assumed and assessments made accordingly.

2. Waterbody Location: Desk Based Review

2.1.1 The English Nature Great Crested Newt Mitigation Guidelines (2001) state:

"A survey for great crested newts may be indicated when background information on distribution suggests that they may be present. More detailed indicators are:

- *Any historical records for great crested newts on the site, or in the general area;*
- *A pond on or near the site (within around 500 m), even if it holds water only seasonally. Note that muddy, cattle-poached, heavily vegetated or shady ponds, ditches and temporary, flooded hollows can be used by great crested newts; and,*
- *Sites with refuges (such as piles of logs or rubble), grassland, scrub, woodland or hedgerows within 500 m of a pond".*

2.1.2 Current Natural England GCN Mitigation Licence Application Survey Guidance (2023) states:

"In keeping with a proportionate and risk-based approach, surveys need reasonable boundaries. The great crested newt mitigation guidelines explain that surveys of ponds up to around 500 m from the development might need to be surveyed. The decision on whether to survey depends primarily on how likely it is that the development would affect newts using those ponds. For developments resulting in permanent or temporary habitat loss at distances over 250 m from the nearest pond, carefully consider whether a survey is appropriate. Surveys of land at this distance from ponds are normally appropriate when all of the following conditions are met: (a) maps, aerial photos, walk-over surveys or other data indicate that the pond(s) has potential to support a large great crested newt population; (b) the footprint contains particularly favourable habitat, especially if it constitutes the majority available locally; (c) the development would have a substantial negative effect on that habitat; and, (d) there is an absence of dispersal barriers".

2.1.3 The Onshore Development Area, waterbody locations, and ditch networks, including a 250 m and 500 m buffer zone, are shown on Figures 1 and 2.

2.1.4 A total of 180 ponds were identified within 500 m of the Onshore Development Area. Of these, 111 ponds were located within 250 m, including 20 ponds situated within the Onshore Development Area itself.

2.1.5 Given proximity and habitat connectivity to the Onshore Development Area all the waterbodies within 250 m were subject to a GCN HSI assessment in 2024 to indicate GCN presence/likely

absence, as discussed and agreed with Natural England.

2.1.6 A complex network of interlinked ditches was identified, via a desk-based Ordnance Survey and aerial imaging review, as potentially extant and relevant to the survey effort within 500 m of the Onshore Development Area.

2.1.7 Based on the desk study information, it is anticipated that only limited sections of the ditch network within 250 m of the Onshore Development Area are likely to offer suitable aquatic habitat for GCN breeding. Given a connection to the North Sea, it is highly likely that the ditch networks towards the east supports brackish waters and is, therefore, unsuitable for GCN. The south westerly ditch networks are likely more suitable. The status of the ditch network should be established, in advance of detailed ecological impact assessment, so any suitable sections of the ditches can be included in the GCN eDNA survey and further HSI assessment.

2.1.8 Fast flowing ditches and other watercourses, such as streams and rivers, have been excluded from the assessment as fast flowing water is unsuitable for GCN.

2.1.9 The survey data obtained from the waterbodies and any sections of the ditch network and provided within this technical note and as part of further surveys will inform any requirement for further GCN survey, including GCN population size class surveys. Collectively these data would also inform a submission for a Natural England GCN mitigation licence application in respect of the development, should one be required.

2.1.10 A total of 180 of the waterbodies and extensive sections of the ditch network have been identified, via a desk-based Ordnance Survey and aerial imaging review, as potentially extant at distances of up to 500 m from the Onshore Development Area, including 20 waterbodies within the Onshore Development Area.

2.1.11 A review of the available habitat survey data, undertaken to inform the production of this GCN Technical Advice Note, has established that the Onshore Development Area comprised predominantly intensively managed arable farmland and does not offer particularly favourable or extensive terrestrial GCN habitat.

2.1.12 The Natural England GCN Risk Zone dataset (Figure 3, presented at the end of the report) was used to give an understanding of the potential presence of GCN in the local area and, therefore the likelihood of the species being associated with the identified waterbodies and/or the ditch network. This dataset identifies areas where the distribution of GCN has been categorised by Natural England into distinct zones relating to GCN occurrence and the level of impact development

is likely to have on this species. These zones are split into Red, Amber, Green, and White and are described as follows:

- Red zone – contains key populations of GCN, which are important on a regional, national, or international scale and include designated Sites of Special Scientific Interest for GCN;
- Amber zone – contains main population centres for GCN and comprise important connecting habitat that aids natural dispersal;
- Green zone – contains sparsely distributed GCN and are less likely to contain important pathways of connecting habitat for this species; and,
- White zone – contains no GCN. However, as most of England forms the natural range of GCN, white zones are rare and will only be used when it is certain that there are no GCN.

2.1.13 The review of the GCN Risk Zone dataset has established that the majority of the waterbodies and ditches within the Onshore Development Area are located in Orange Zones, and some within Green Zones, as shown on Figure 3. Therefore, connecting habitat and dispersal of GCN could be impacted, although most identified waterbodies are outside of the Onshore Development Area.

3. Habitat Suitability Index Methodology

3.1.1 Only ponds identified within the Onshore Development Area and within the 250 m buffer were visited between the 5 August 2024 to 8 October 2024, where accessible. The surveyor teams comprised of at least one suitably experienced ecologist. The surveyors are outlined in Table 1 below.

Table 1: Lead and assistant ecologists conducting HSI surveys.

Lead ecologists	**Assistant ecologists**
RJJ - Principal Ecologist BSc (Hons) MSc; RNJ - Senior Ecologist BSc (Hons); JA - Senior Ecologist BSc (Hons) MCIEEM; HC - Senior Ecologist BSc (Hons) MSc; EH - Consultant Ecologist BSc (Hons) MSc; SA - Consultant Ecologist BSc (Hons) MSc; and LS - Consultant Ecologist BSc (Hons) MSc.	LS - Consultant Ecologist BSc (Hons) MSc; GT - Assistant Ecologist BSc (Hons) MSc; ZC - Assistant Ecologist BSc (Hons); EP - Assistant Ecologist BSc (Hons) MSc; DH - Graduate Ecologist BSc (Hons); RL - Graduate Ecologist BSc (Hons); BH - Graduate Ecologist BSc (Hons); AB - Graduate Ecologist BSc (Hons); and, JB - Graduate Ecologist BSc (Hons) MSc.

3.1.2 The GCN status in a given waterbody is influenced by the existence of 10 suitability indices, all of which are factors known to affect the species (e.g. fish, heavy shading) and/or the absence of others (e.g. suitable terrestrial habitat within 500 m). The HSI score is determined using the Amphibian and Reptile Group guidance for HSI surveys[1].

3.1.3 The HSI provides a numerical value (ranging from 0 to 1) based on measurable parameters that indicates the suitability of a waterbody for GCN. The higher the HSI score, the more suitable the waterbody is for GCN.

3.1.4 It should be noted that a low score is not sufficient on its own to rule out the presence of GCN but provides a predicted presence of GCN from survey results[1].

[1] *Oldham, R.S; Keeble, J; Swan, M.J.S. and Jeffcote, M. (2000). Evaluating the suitability of habitat for the great crested newt (Triturus cristatus). Herpetological Journal. 10: 143-155.*

4. HSI Limitations

4.1.1 A total of 43 ponds were not surveyed due to varying access restrictions. This is a significant limitation as suitability of these waterbodies for GCN has not been assessed. To address this, it is recommended that access is re-attempted in 2025 to complete the HSI assessments on ponds not visited – this could be completed at the same time as eDNA surveys.

4.1.2 Netting of water was not possible and therefore water quality was assessed from what could be seen from the bank. This is not a significant limitation because it does not determine if GCN are present or not. It is recommended that GCN eDNA surveys are undertaken to determine presence / likely absence of GCN within the waterbodies.

4.1.3 At least five waterbodies were not visible from all sides or were assessed through vegetation. This is not a significant limitation because it was still possible to get an understanding of the habitat present and the suitability of factors for GCN.

4.1.4 Some refinements to the Onshore Development Area have been made after the HSI surveys were undertaken. This resulted in seven ponds being added into the 250 m buffer (145, 187, 205, 211, 229, 234, and 235), which have not had HSI surveys undertaken on them. This is a limitation because their suitability for GCN has not been assessed at this stage. To address this limitation, surveys are scheduled for April - June 2025 to complete HSI assessments on these additional ponds and the results will be presented at the ES stage.

4.1.5 None of the ditches or ditch networks were subject to HSI surveys. This is a limitation as their suitability for GCN has not been assessed at this stage. To address this limitation, these surveys will be completed in 2025 at the same time as the eDNA surveys.

5. HSI Survey Results

5.1.1 Of the 111 ponds within 250 m, 66 ponds were visited in total, as presented on Figure 4 at the end of this report. Photographs of ponds can be seen in Appendix 2 and the full HSI results of 42 waterbodies are detailed in Appendix 3.

5.1.2 A total of 16 ponds no longer existed or were not actual ponds, excluding dry ponds. Additionally, seven ponds were dry at the time of the survey, but HSI surveys were still carried out on six of these ponds based on the presence of water. It was not possible to complete a HSI assessment on one of the dry ponds due to limited access, although it was likely to be dry.

5.1.3 A total of 42 ponds were successfully surveyed, including six dry ponds. The survey identified that Ponds 67 and 68 formed a single, connected pond. The HSI calculations showed that two ponds were of 'excellent' suitability, seven were of 'good' suitability, three were of 'average' suitability, 17 were of 'below average' suitability, and 13 were of 'poor' suitability. This includes the seven ponds that were dry at the time of the survey.

5.1.4 A total of 45 ponds could not be accessed, either due to a lack of landowner permission or because dense vegetation prevented surveyors from reaching the ponds. These ponds will be surveyed in April – June 2025, subject to accessibility.

5.1.5 A total of seven new ponds were added after the boundary of the Onshore Development Area was revised following the survey. As a result, these ponds were not included in the survey but will be surveyed in April – June 2025.

6. Impact Assessment

6.1.1 GCN is a European Protected Species (EPS) and as such receives protection under The Conservation of Habitats and Species Regulations 2017 (as amended) and the Wildlife and Countryside Act (WCA) 1981 (as amended).

6.1.2 GCN is also a Species of Principal Importance under Section 41 of the Natural Environment and Rural Communities Act 2006.

6.1.3 It is illegal to kill, injure, capture, handle, or disturb GCN and the habitats they use for breeding, resting, shelter, and protection are also legally protected from damage or destruction.

6.1.4 The potential predicted effects and scale of impacts on GCN arising from the development are highlighted (grey) in Table 2 below (adapted from the English Nature 'Great Crested Newt Mitigation Guidelines' (2001)).

6.1.5 It has been assumed that all waterbodies within the Onshore Development Area will be destroyed as part of the works. This is a total of 20 ponds. When full plans are available, the assessment will need to be reviewed.

6.1.6 It is currently unknown whether GCN are present in the ponds within the Onshore Development Area, as eDNA surveys to confirm their presence have not yet been completed. As a result, the assessment assumes the worst-case scenario, where all ponds are considered to have GCN present and breeding. This assessment will be reviewed once the eDNA survey results are available.

Table 2. Predicted Effect and Scale of Impacts to GCN

Habitat Feature	Development Effect	Scale of Impact		
		Low	Medium	High
Confirmed GCN breeding pond/water body (on and off site)	Destruction			✓
	Isolation caused by fragmentation			✓
	Partial destruction; modification		✓	
	Temporary disturbance	✓		
	Post-development interference	✓		

Habitat Feature	Development Effect	Scale of Impact		
		Low	Medium	High
Other pond or water body	**Destruction**		✓	
	Isolation caused by fragmentation		✓	
	Partial destruction; modification	✓		
	Temporary disturbance	✓		
	Post-development interference	✓		
Immediate Terrestrial Habitat (less than 50 m from a GCN/breeding pond or other waterbody identified to be or potentially used by the species)	Destruction			✓
	Isolation caused by fragmentation			✓
	Partial destruction		✓	
	Modified management, resurfacing etc.		✓	
	Temporary disturbance	✓		
	Post-development interference		✓	
	Temporary destruction & reinstatement	✓		
Intermediate Terrestrial Habitat (at 50 m up to 250 m from a GCN/breeding pond or other waterbody identified to be or potentially used by the species)	Destruction		✓	
	Isolation caused by fragmentation		✓	
	Partial destruction	✓		
	Modified management, resurfacing, etc.	✓		
	Temporary disturbance	✓		
	Post-development interference	✓		
	Temporary destruction & reinstatement	✓		
Distant Terrestrial Habitat (more than 250 m from a GCN/breeding pond or other water body potentially used by the	Destruction	✓		
	Isolation caused by fragmentation	✓		
	Partial destruction	✓		
	Modified management, resurfacing etc.	✓		
	Temporary disturbance	✓		
	Post-development interference	✓		

Habitat Feature	Development Effect	Scale of Impact		
		Low	Medium	High
species)	Temporary destruction & reinstatement	✓		

6.1.7 The predicted impacts of the development on waterbodies and ditches are destruction, partial destruction and modification, isolation caused by fragmentation, and temporary disturbance.

6.1.8 The predicted impacts of the development on potential 'Immediate' and 'Intermediate' GCN terrestrial habitats (habitats located within 250 m of the Onshore Development Area) have been assessed as: partial destruction, temporary disturbance, modified management, resurfacing etc., and temporary destruction and reinstatement.

6.1.9 The predicted impacts of the development on potential distant GCN terrestrial habitat associated with the waterbodies and ditches located between 250 m and up to 500 m from the Onshore Development Area have been assessed as partial destruction, temporary disturbance, modified management, resurfacing etc., and temporary destruction and reinstatement.

6.1.10 A total of 111 waterbodies and a complex network of interlinked ditches were identified as potentially extant within 250 m of the Onshore Development Area through ecologist-led desk-based searches. Following the 2024 field surveys, 23 of these waterbodies were confirmed to either be non-existent or dry. As a result, up to 88 ponds are likely to be extant within 250 m of the Onshore Development Area, subject to confirmation through further survey in 2025. Given their proximity and habitat connectivity to the Project, there is a moderate level risk of encountering and negatively impacting on dispersing GCN (from these waterbodies and ditches), if present.

6.1.11 Therefore, given the predicted level of negative impact/s as detailed in Table 2 above, it is recommended that waterbodies located within 250 m of the Onshore Development Area should be subject to eDNA surveys. The survey methodology is outlined below in Section 4. It is important to note that any sections of the ditch network potentially offering GCN with suitable aquatic breeding habitat should be identified and included within the eDNA surveys.

6.1.12 The survey will indicate GCN presence/likely absence at each waterbody (or ditch section) and will inform the requirement for further GCN survey (population size class surveys) or any possible required application for a Natural England GCN mitigation licence of the Project.

6.1.13 There is only a relatively low-level risk of encountering and negatively impacting GCN from the

further 69 waterbodies and ditch networks situated between 250 m and 500 m from the Onshore Development Area, given their distance from the Onshore Development Area and the presence of extensive similar habitats in the wider area. Consequently, waterbodies and ditches located at distances further than 250 m from the proposals have been excluded.

7. GCN eDNA Survey Methodology

7.1.1 The complete survey methodology is outlined in Appendix 4. Surveys must be carried out by an ecologist who is suitably trained and experienced, as detailed in Appendix 4.

7.1.2 It is advised that the eDNA survey waterbody sampling is undertaken by the ecologist immediately prior to the HSI assessment at each target waterbody. This approach will enable the collection of the required evenly spaced water samples from twenty suitable locations around the waterbody margins, in accord with the approved sampling techniques for eDNA surveys (refer to Appendix 4: WC1067). Additionally, this approach will also negate the risk of the hand netting, to be undertaken as part of the HSI assessment, disturbing silt and mud and clouding/contaminating the water column which could adversely impact on the laboratory analysis of the collected water samples (refer to Appendix 5: GCN HSI Advice Note 5).

7.1.3 If the best practice methodology is updated in the interim, then this would supersede any guidance or recommendations within this technical note.

8. Conclusion

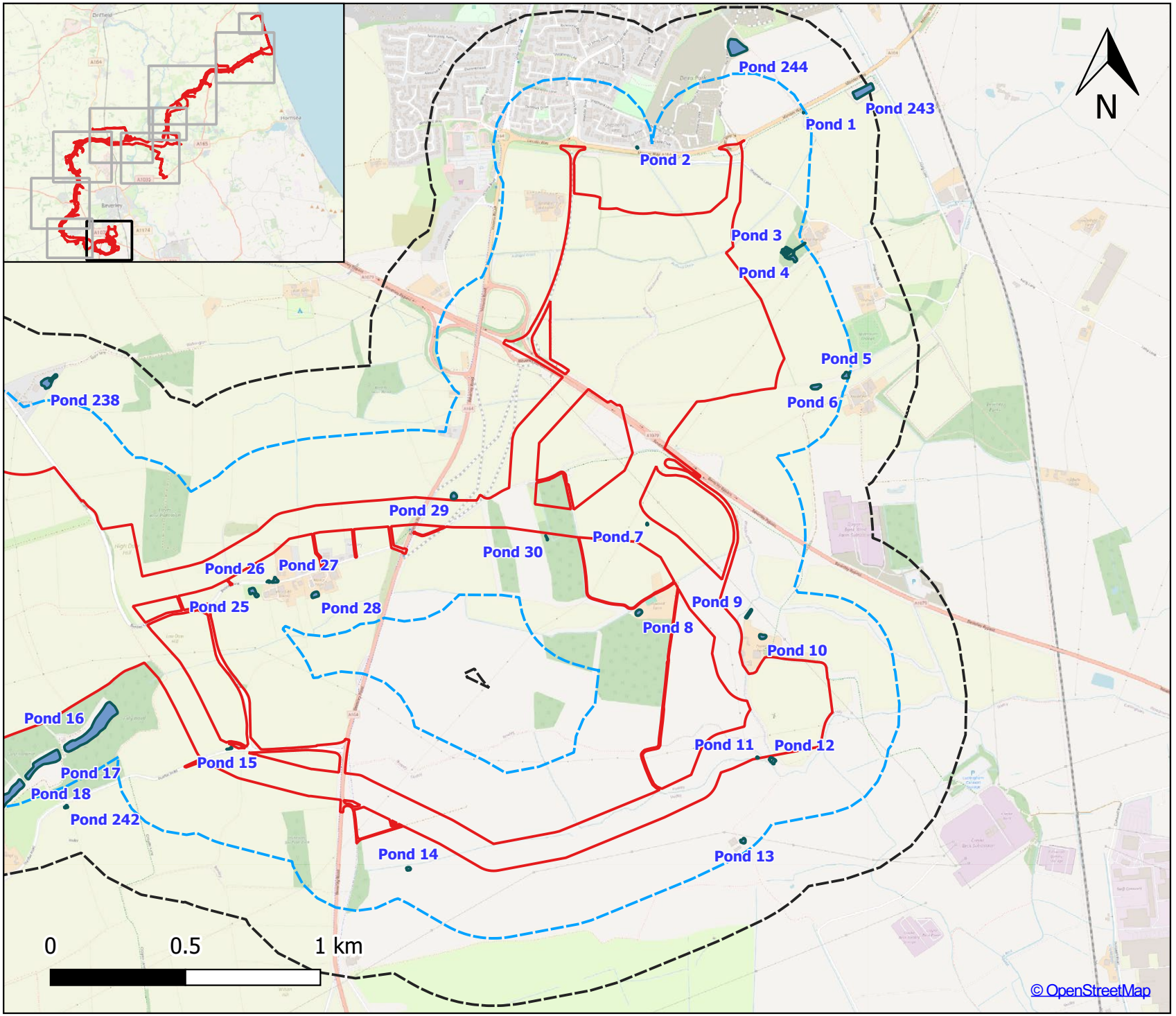
8.1.1 This GCN Technical Advice Note demonstrates that two out of the four conditions, as referenced by Natural England in their previous discretionary advice response, have not been met. All four conditions must be met to justify conducting detailed GCN surveys on waterbodies located between 250 m and 500 m from the Onshore Development Area. The habitats within the Onshore Development Area do not offer particularly favourable or extensive GCN habitat and the predicted impacts of the Project on terrestrial habitats inside the project footprint have been assessed as 'Low'.

8.1.2 This GCN Technical Advice Note details and justifies the rationale for undertaking GCN environmental DNA (eDNA) surveys and GCN Habitat Suitability Index (HSI) assessments only at those waterbodies identified as extant and relevant within 250 m of the Onshore Development Area.

8.1.3 Based on the findings detailed in this GCN Technical Advice Note, the previous Natural England discretionary advice response, and the English Nature Great Crested Newt Mitigation Guidelines (2001), it is recommended that further GCN surveys are undertaken. These surveys should include additional HSI surveys and eDNA surveys at the waterbodies and ditches located 250 m from the Onshore Development Area. The results of these surveys will determine whether GCN population class assessments are required and if an EPS licence application may be needed.

8.1.4 Given GCN terrestrial range, the limited and localised potential terrestrial habitat, and presence of extensive similar habitats in the wider area, it is concluded that any GCN populations located between 250 m and up to 500 m from the Onshore Development Area would not be negatively impacted by the development proposals and therefore further surveys are not required.

Figure 1. Waterbodies Location Plan



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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Waterbodies

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Dogger Bank D
GCN Technical Note

Figure 1
Waterbody map

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A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
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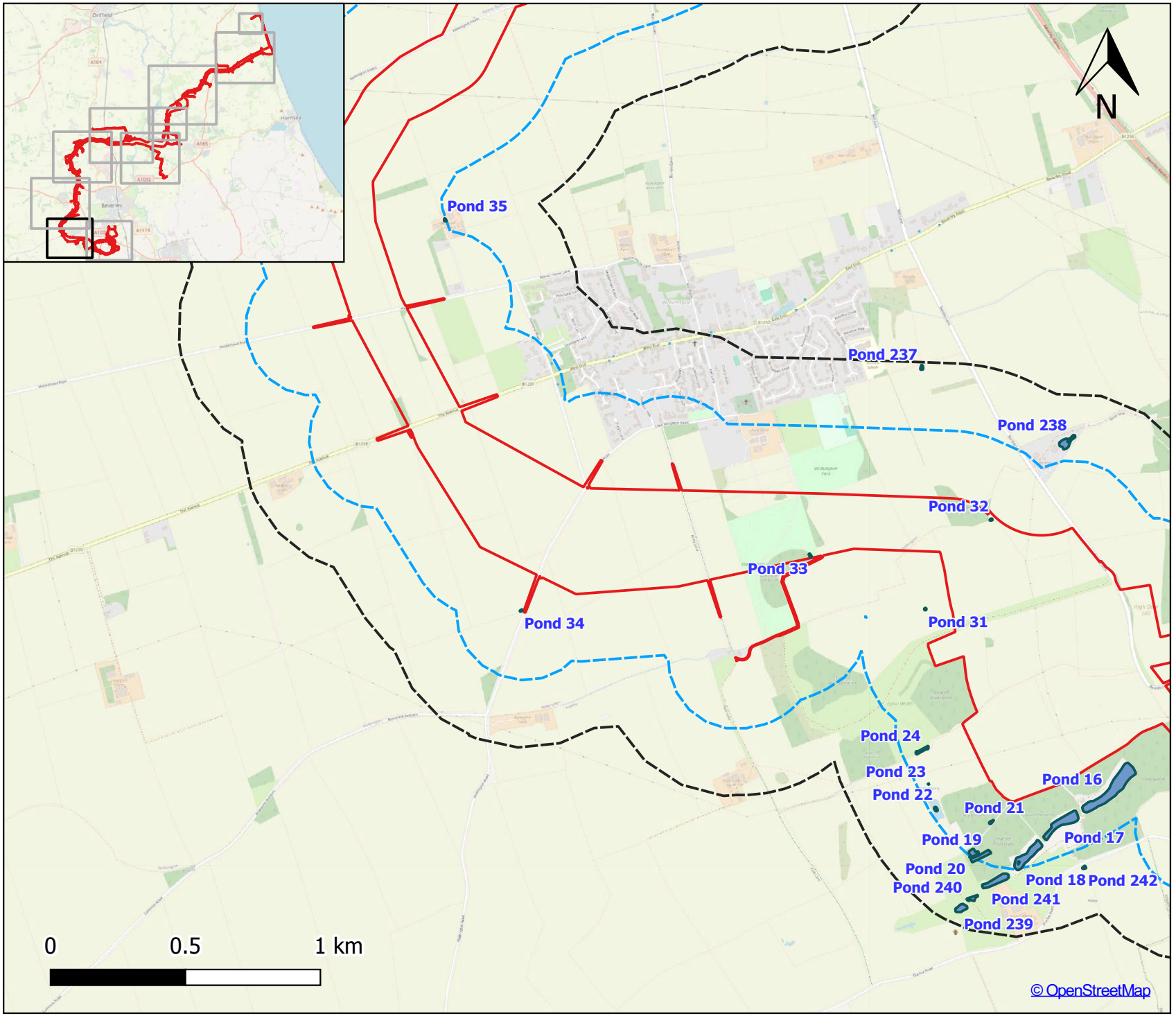

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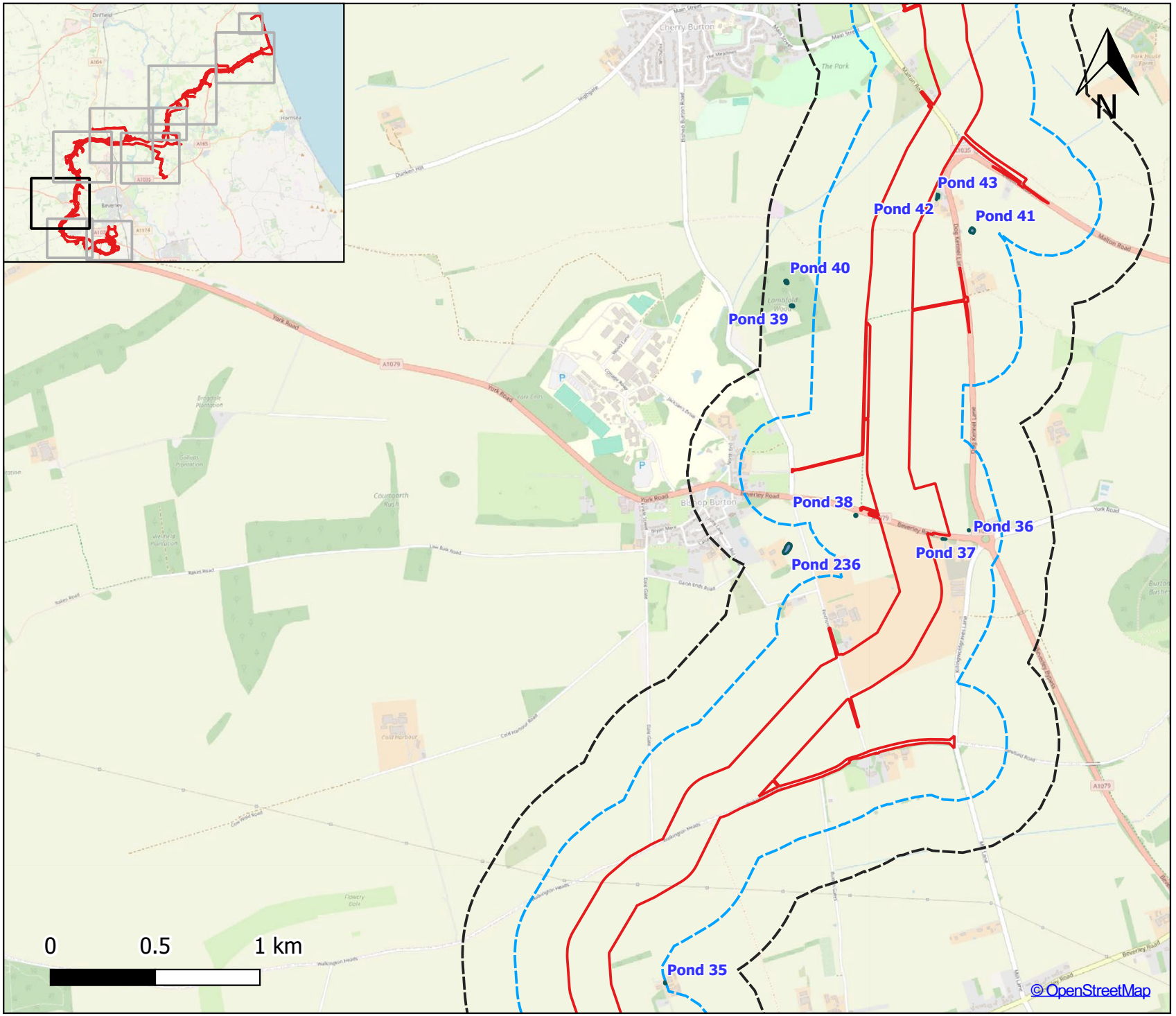

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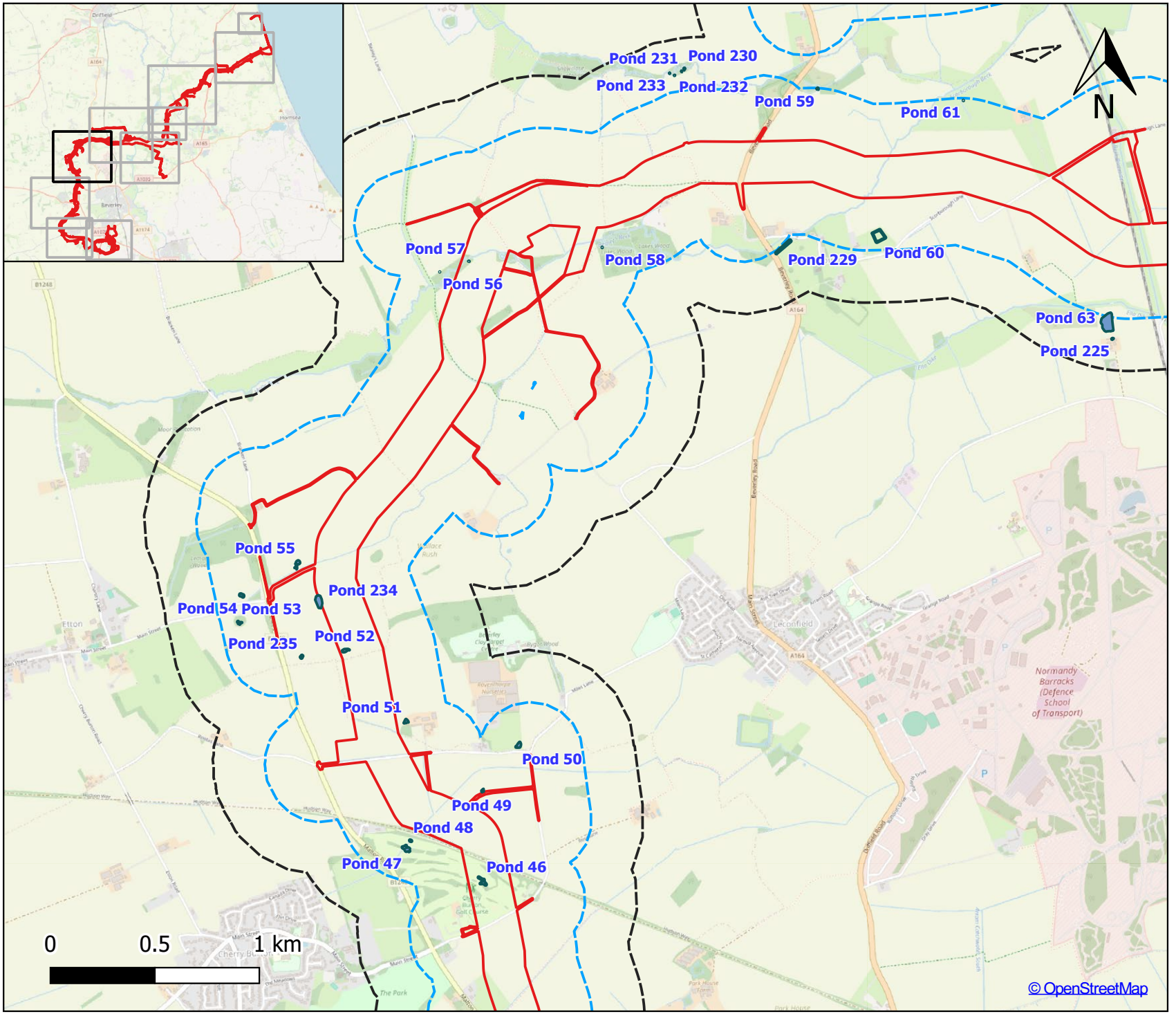

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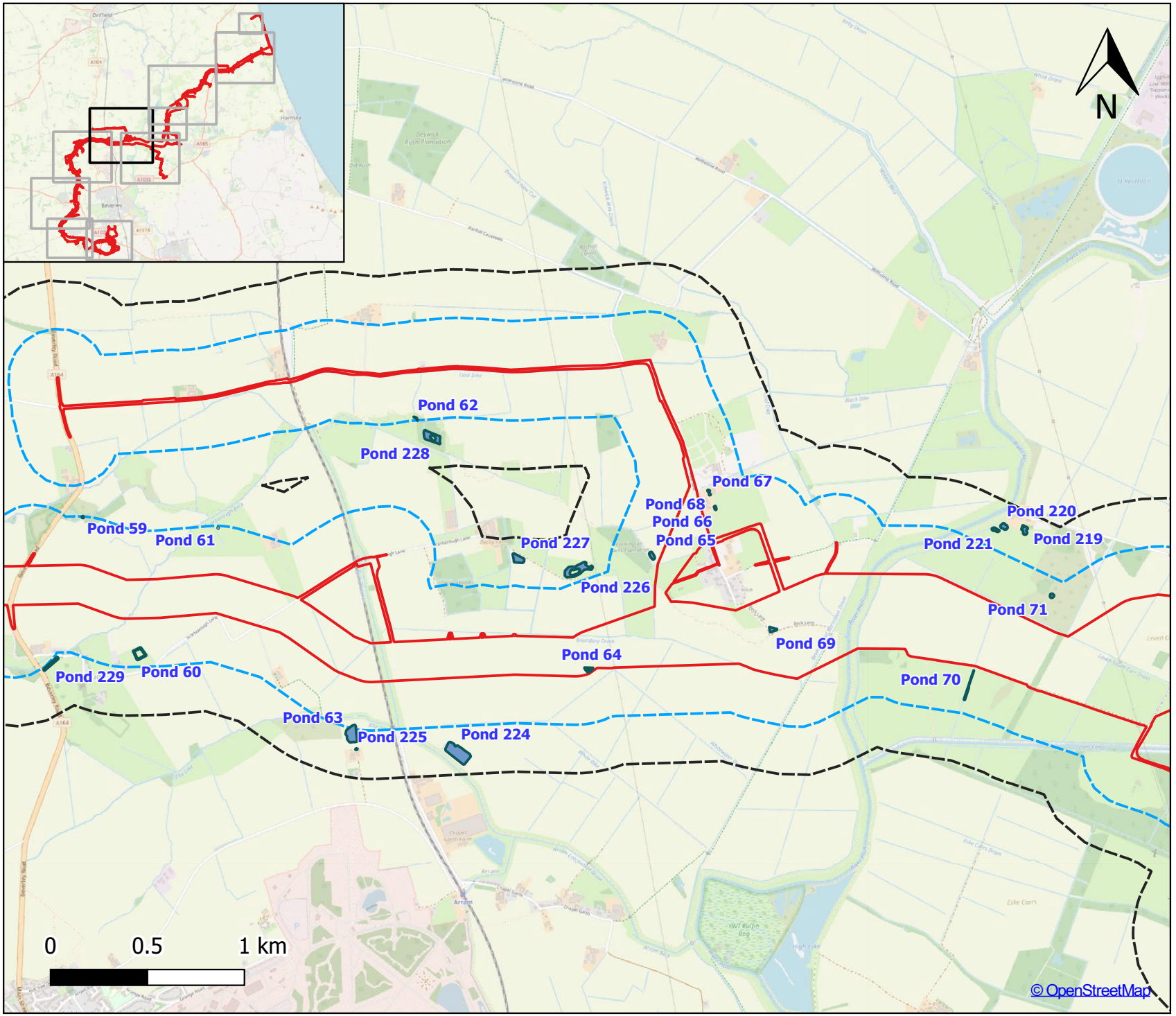

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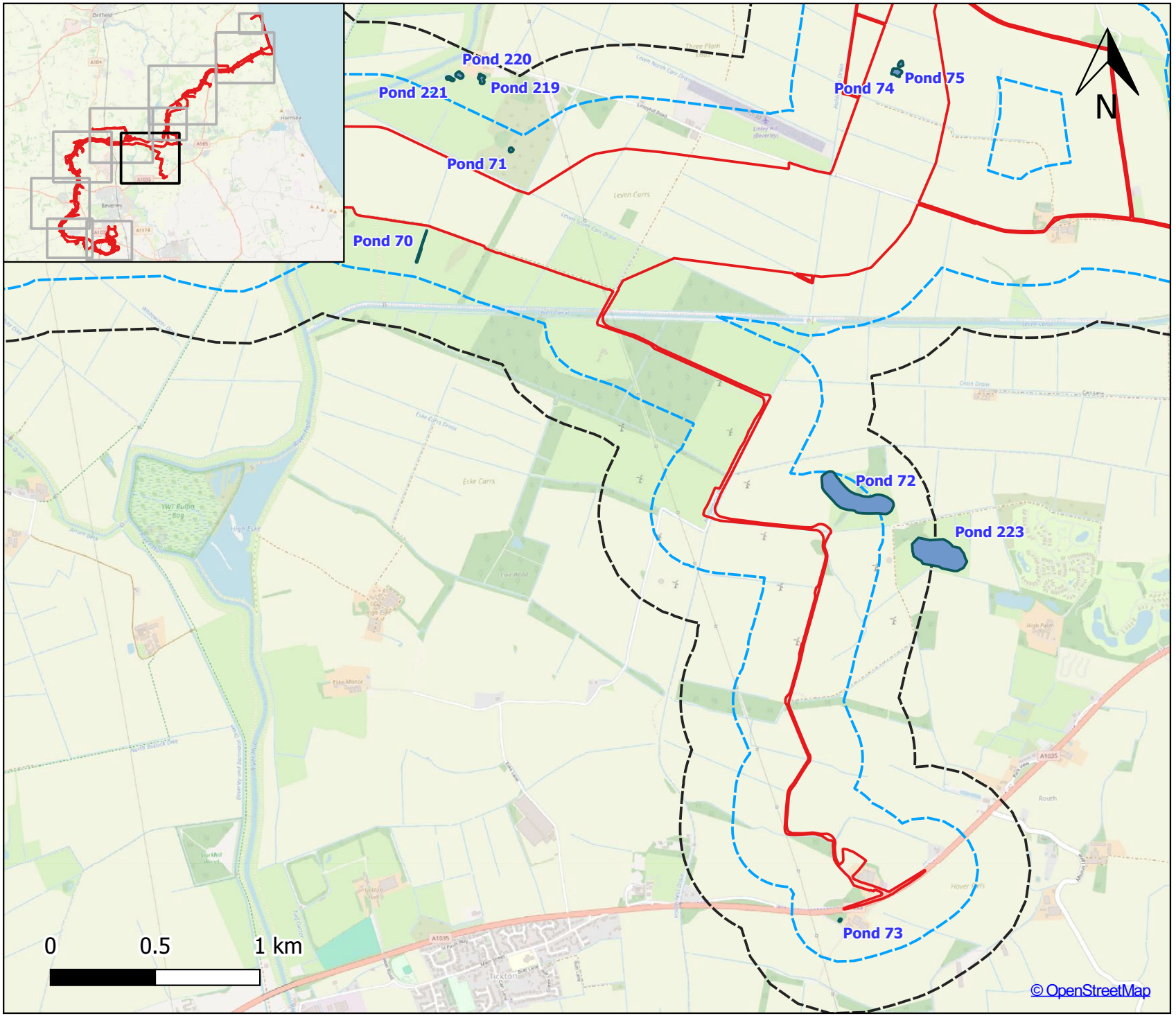

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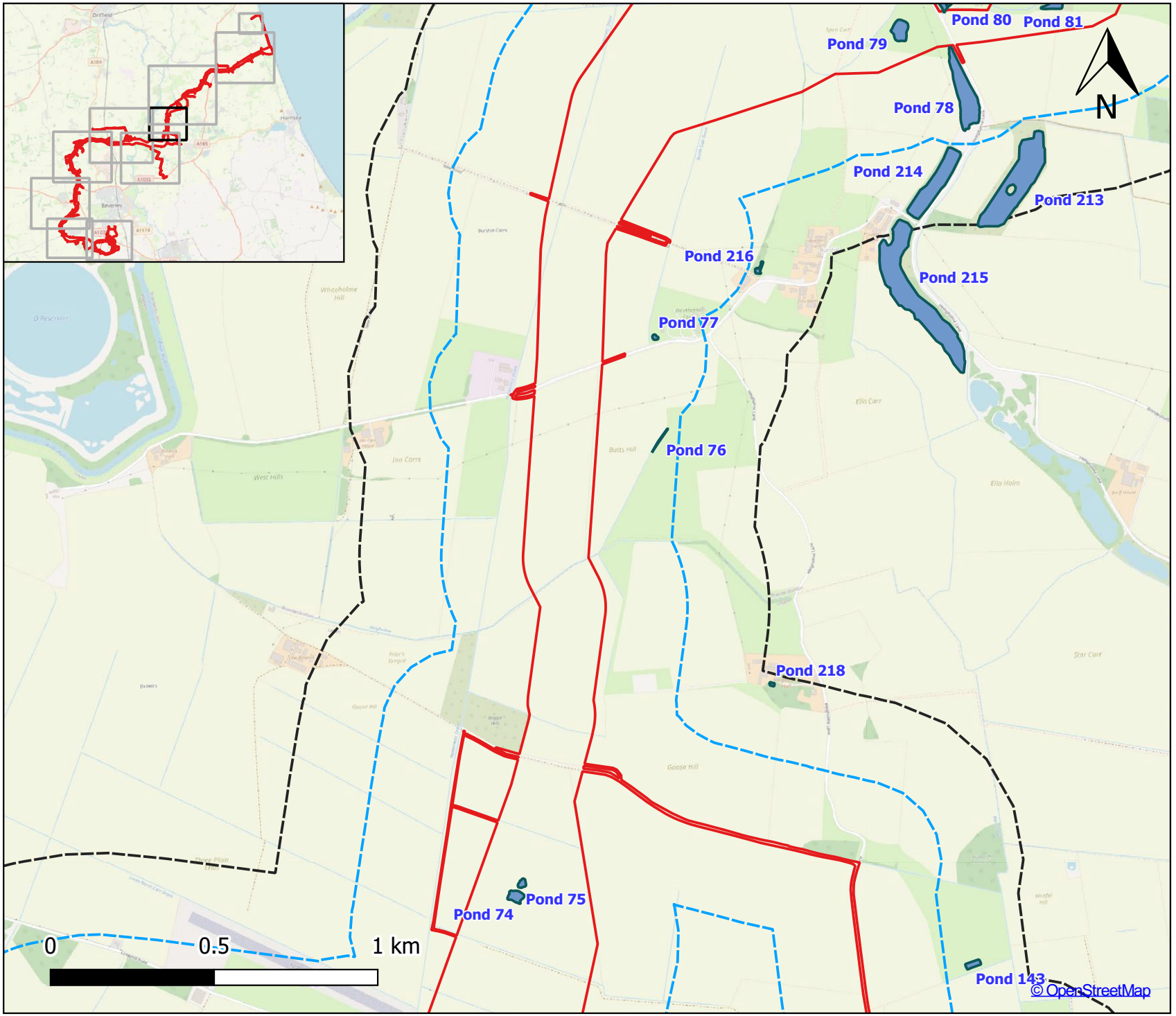

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Waterbodies

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

Figure 1
Waterbody map

Rev	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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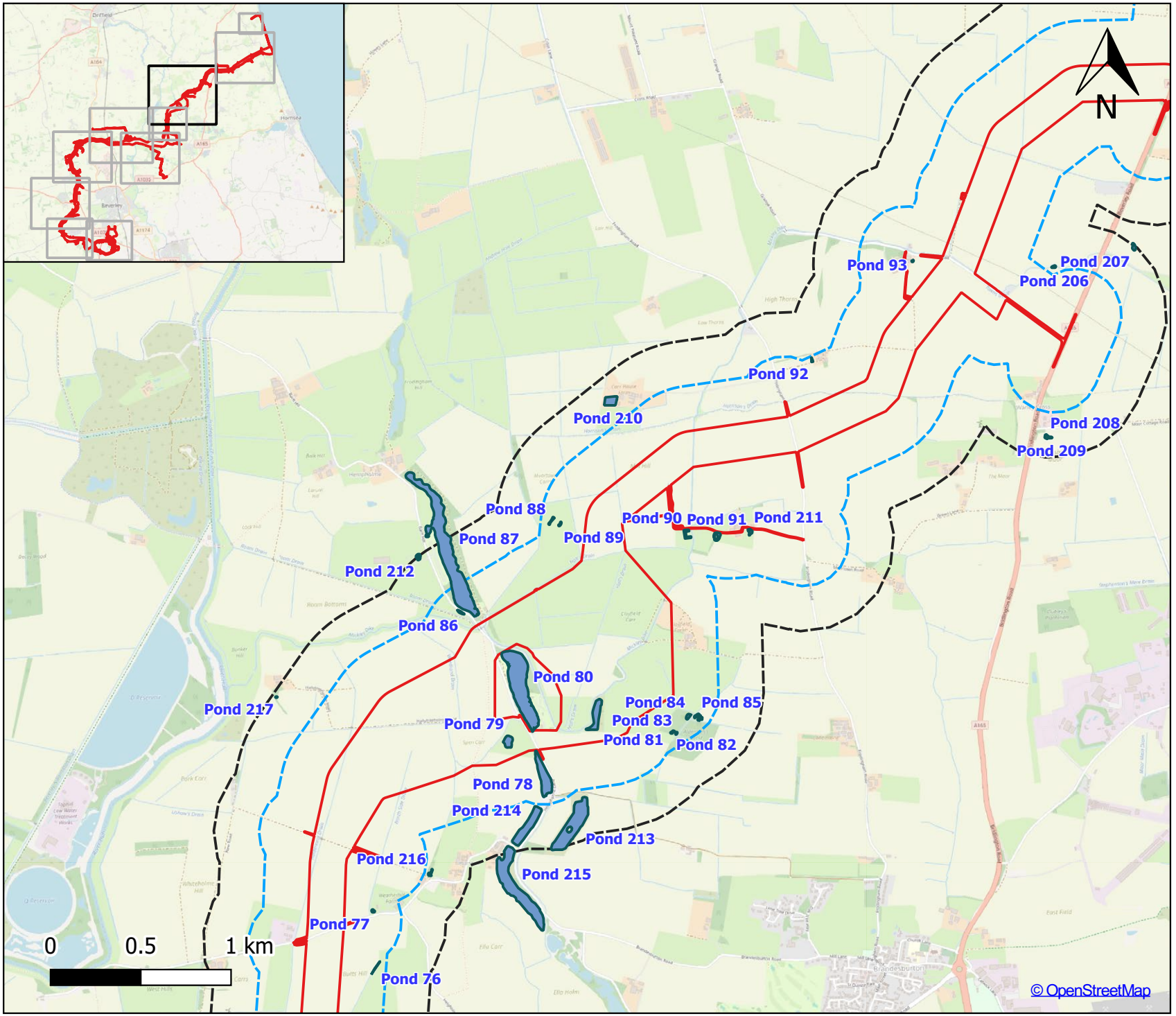

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Waterbodies

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

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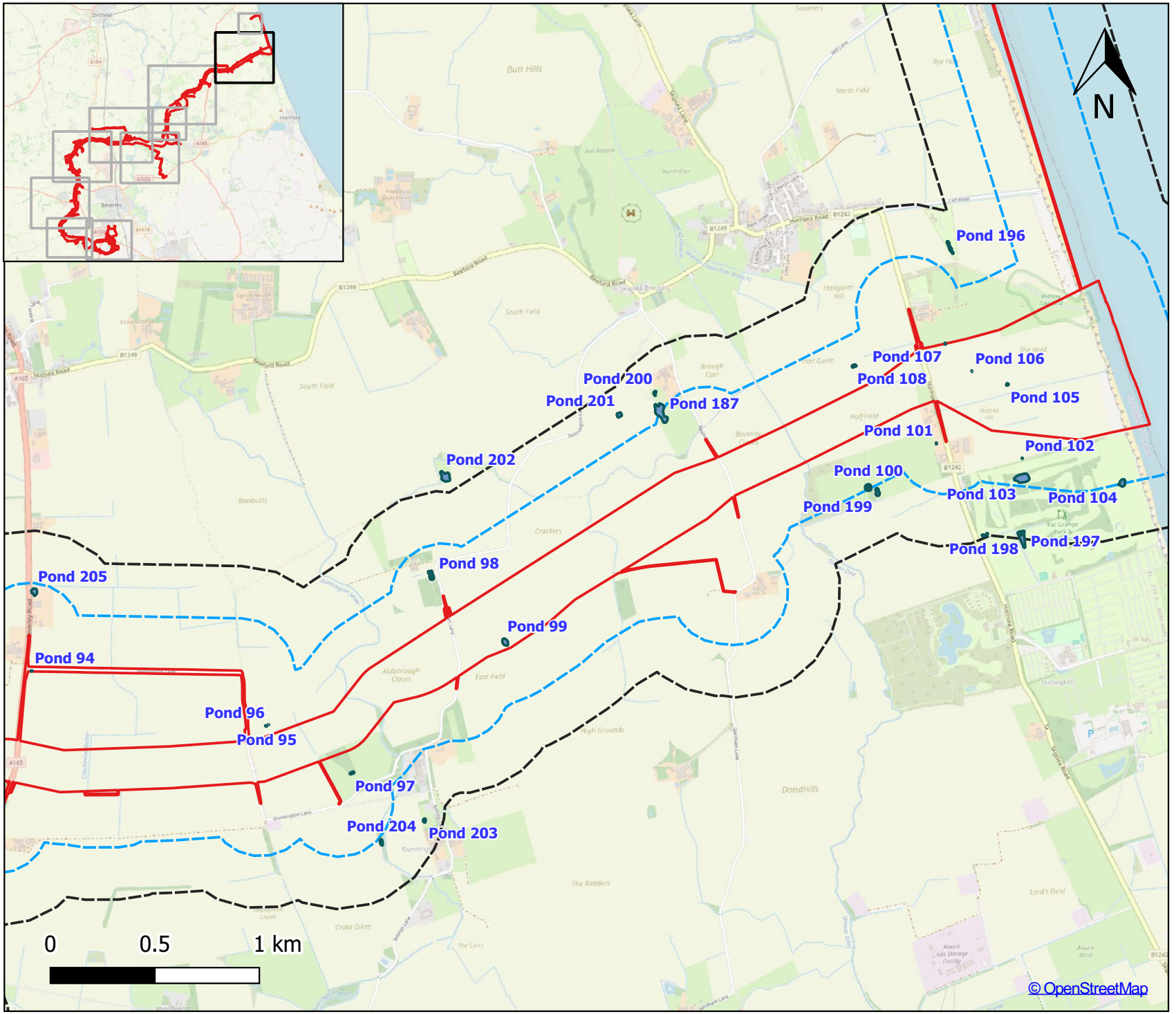

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Waterbodies

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

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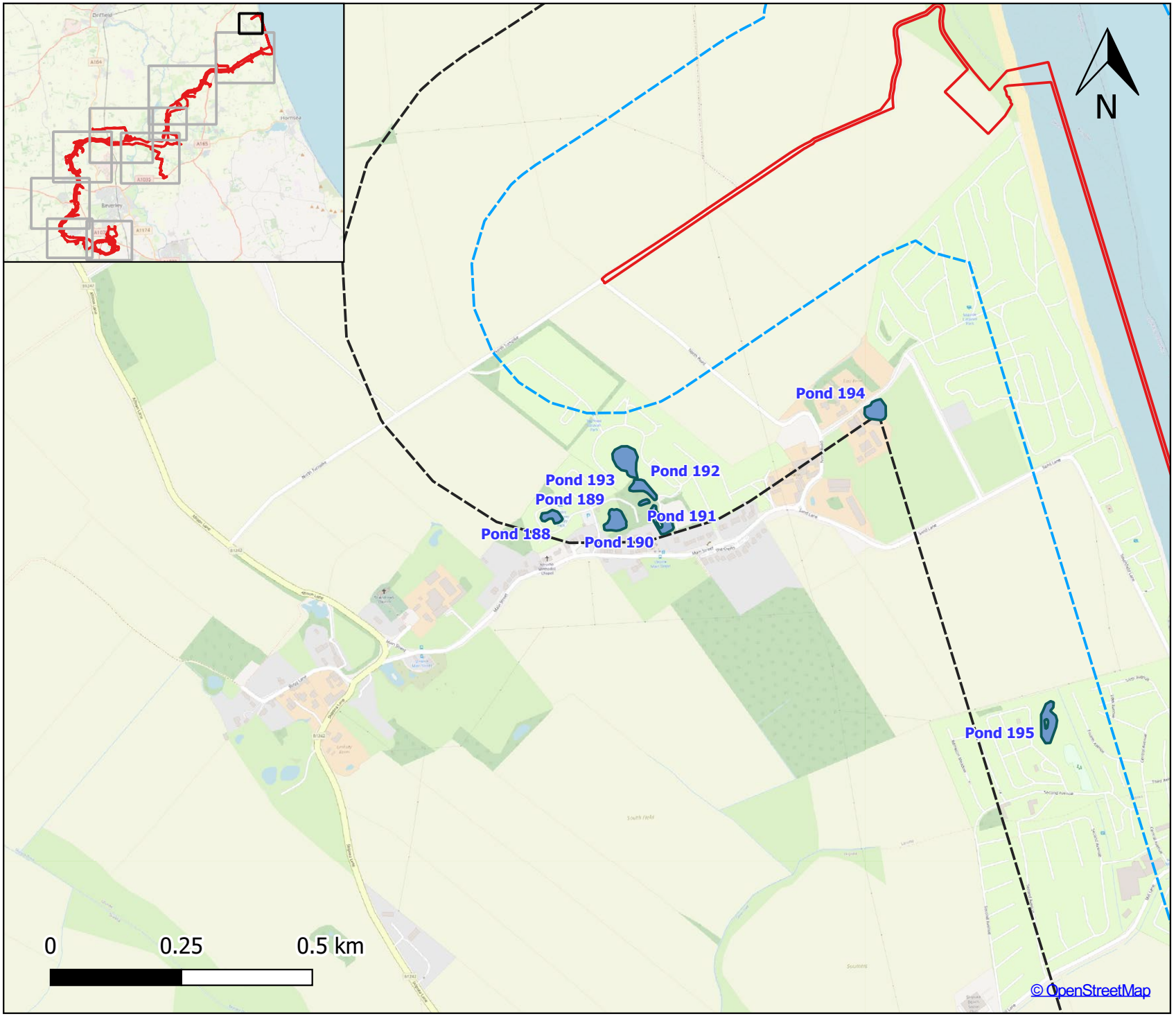

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Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

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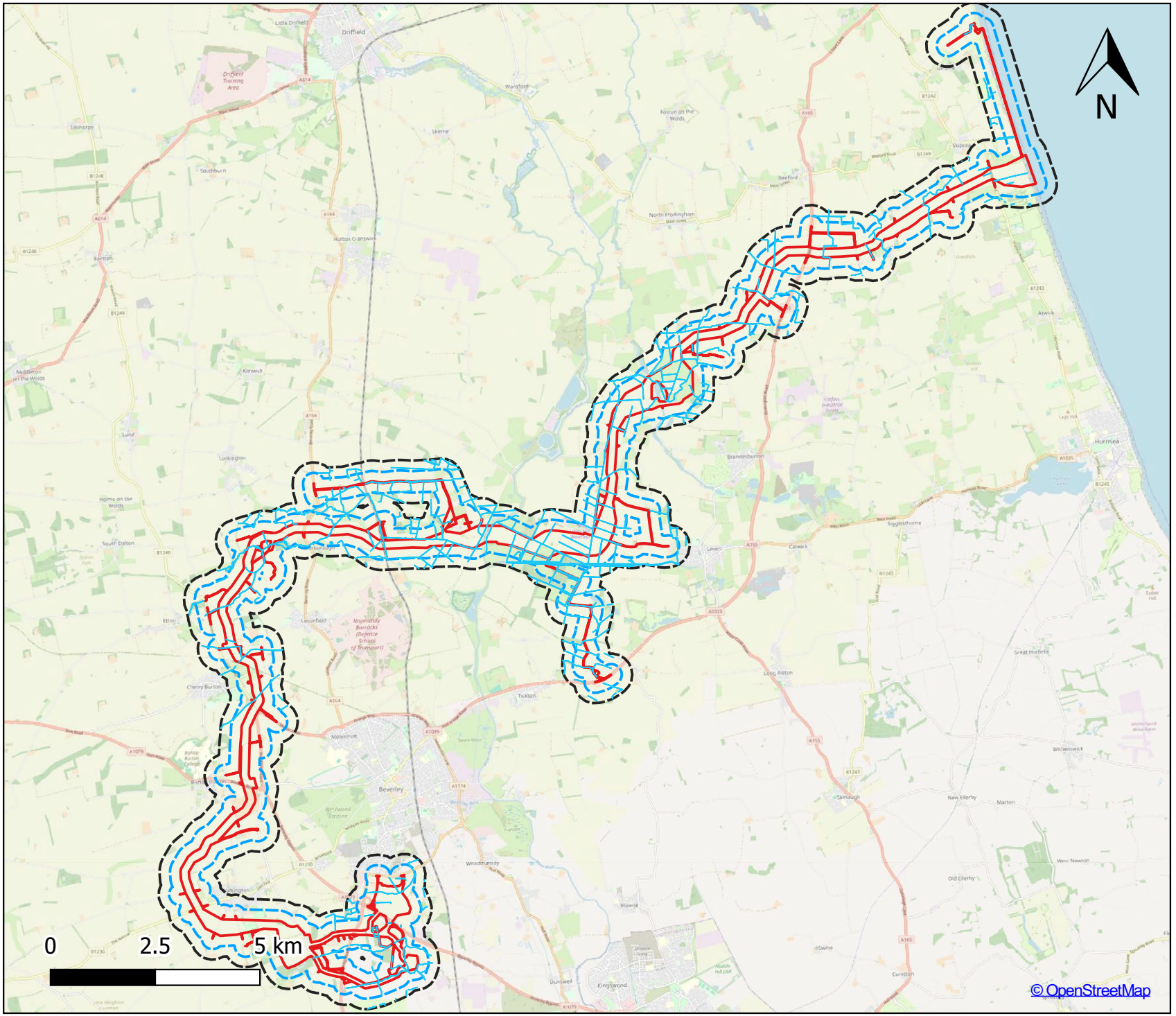
Figure 2. Watercourses and Ditch Networks Location Plan



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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Watercourses

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

Rev	Date	Drawn by	Checked by
A	15/10/2024	JB	RJJ
B	10/12/2024	LS	SS
C	02/04/2025	SW	RJJ

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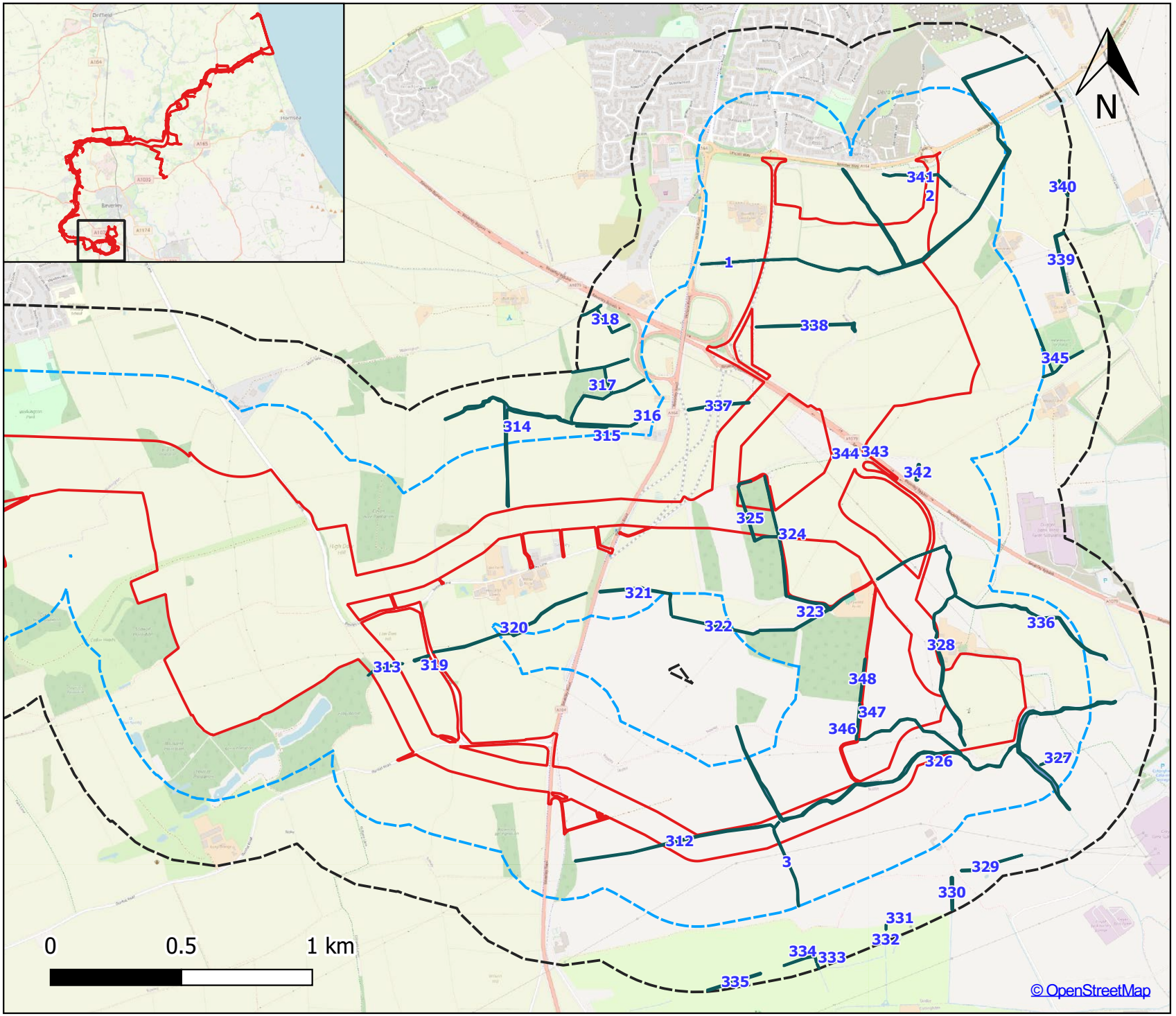

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
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Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

Rev	Date	Drawn by	Checked by
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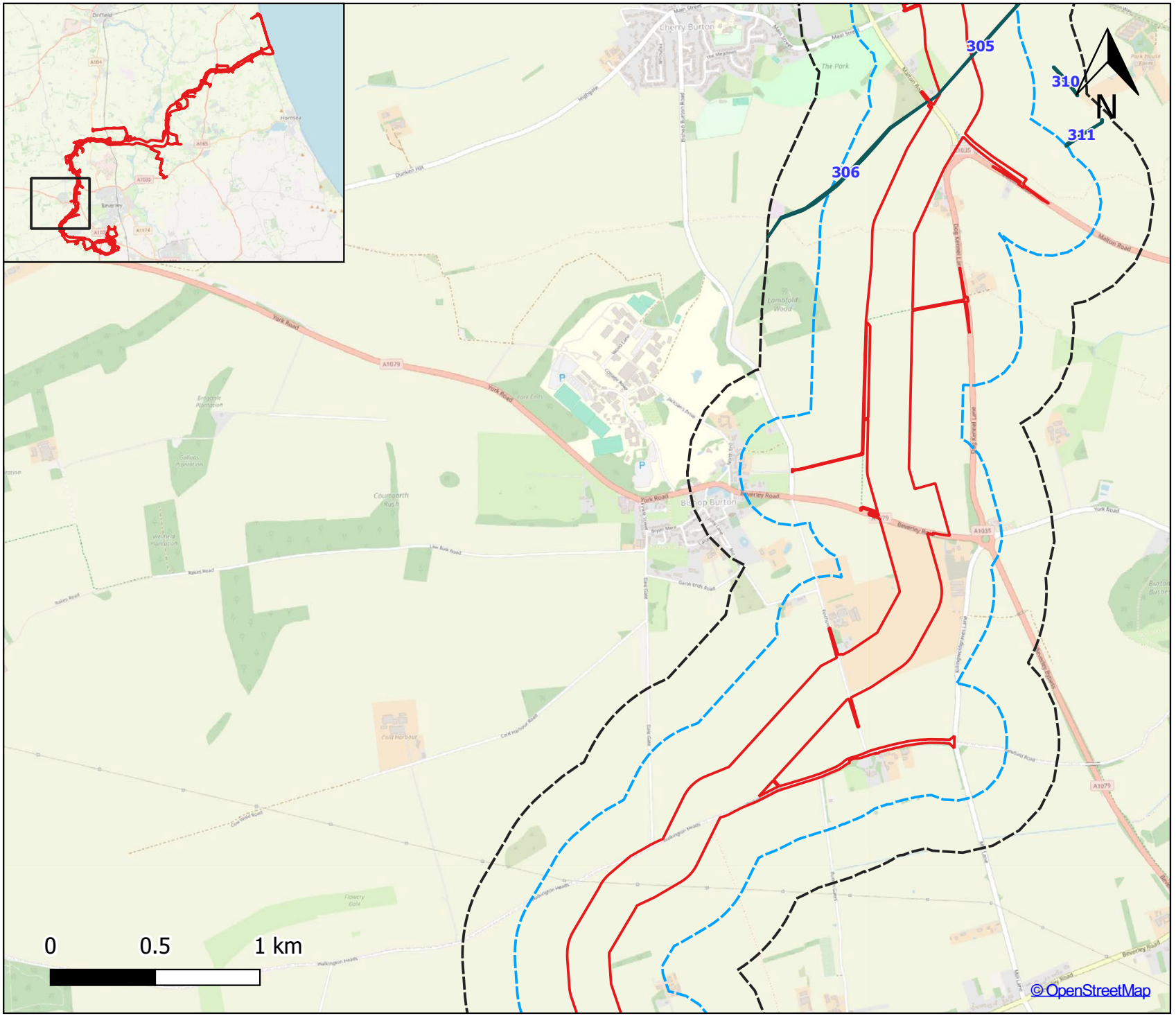

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Legend

- Onshore Development Area
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- 500 m buffer
- Watercourses

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Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

Rev	Date	Drawn by	Checked by
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C	02/04/2025	SW	RJJ

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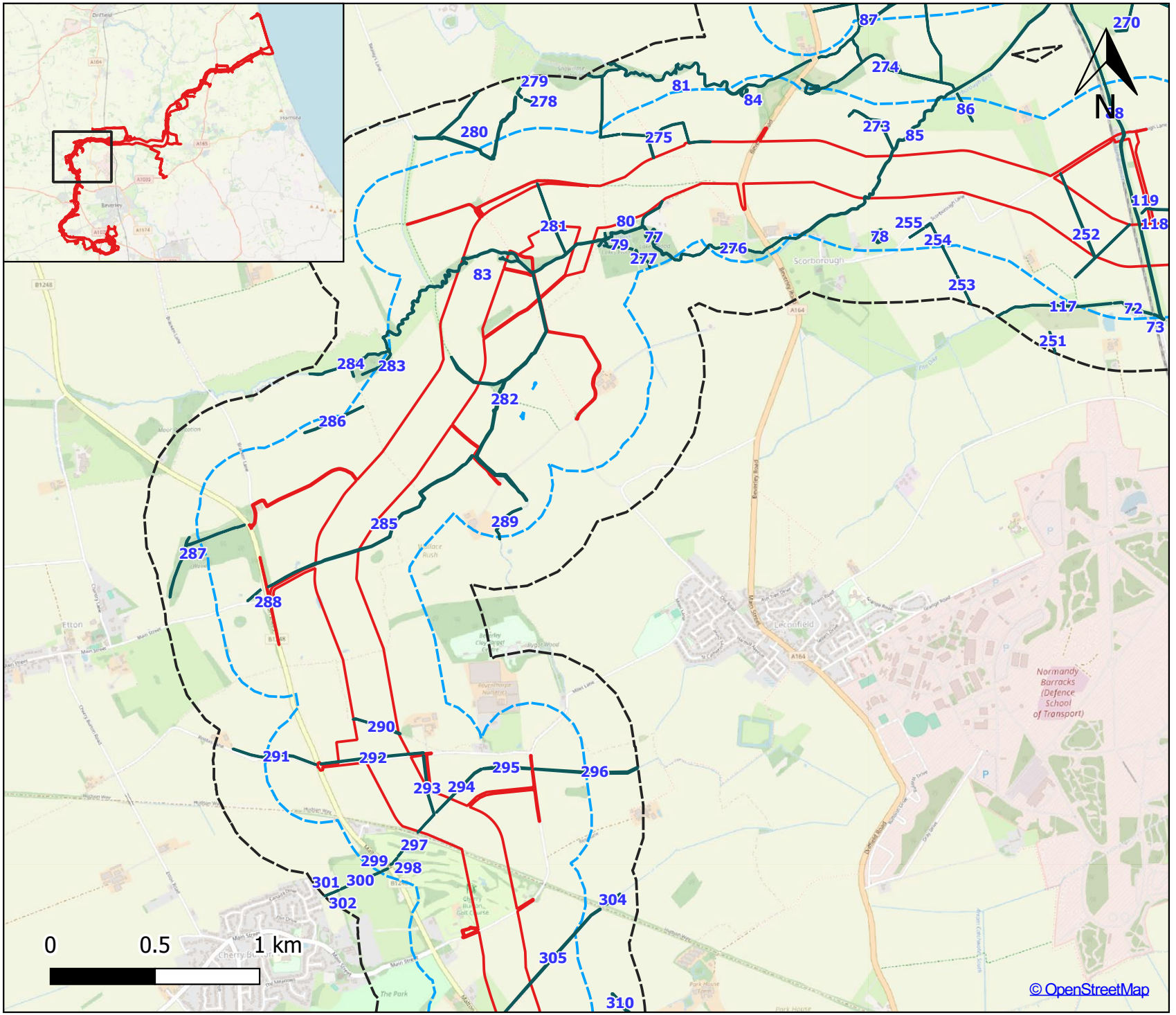

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Watercourses

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

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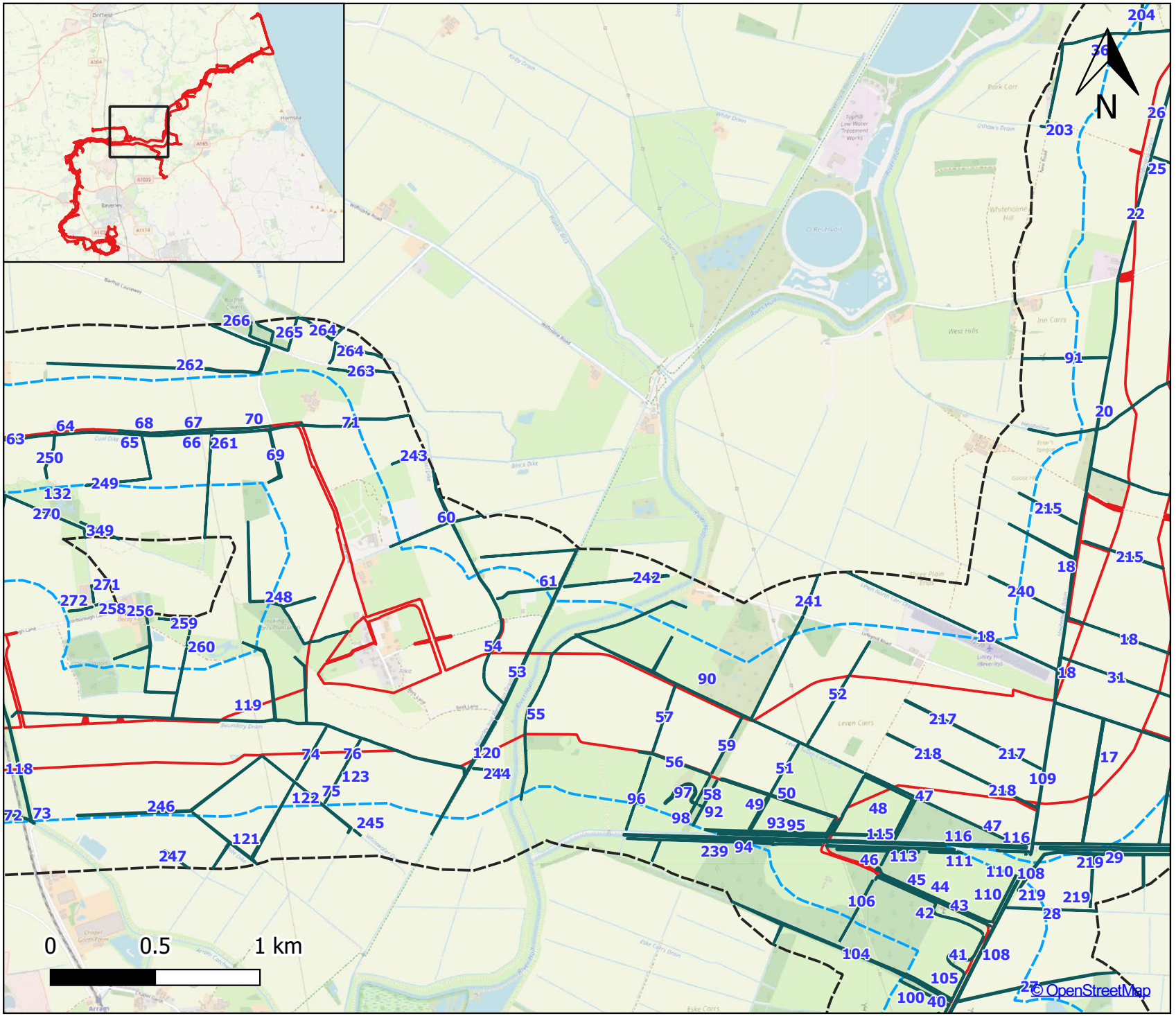

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Dogger Bank D
GCN Technical Note

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C	02/04/2025	SW	RJJ

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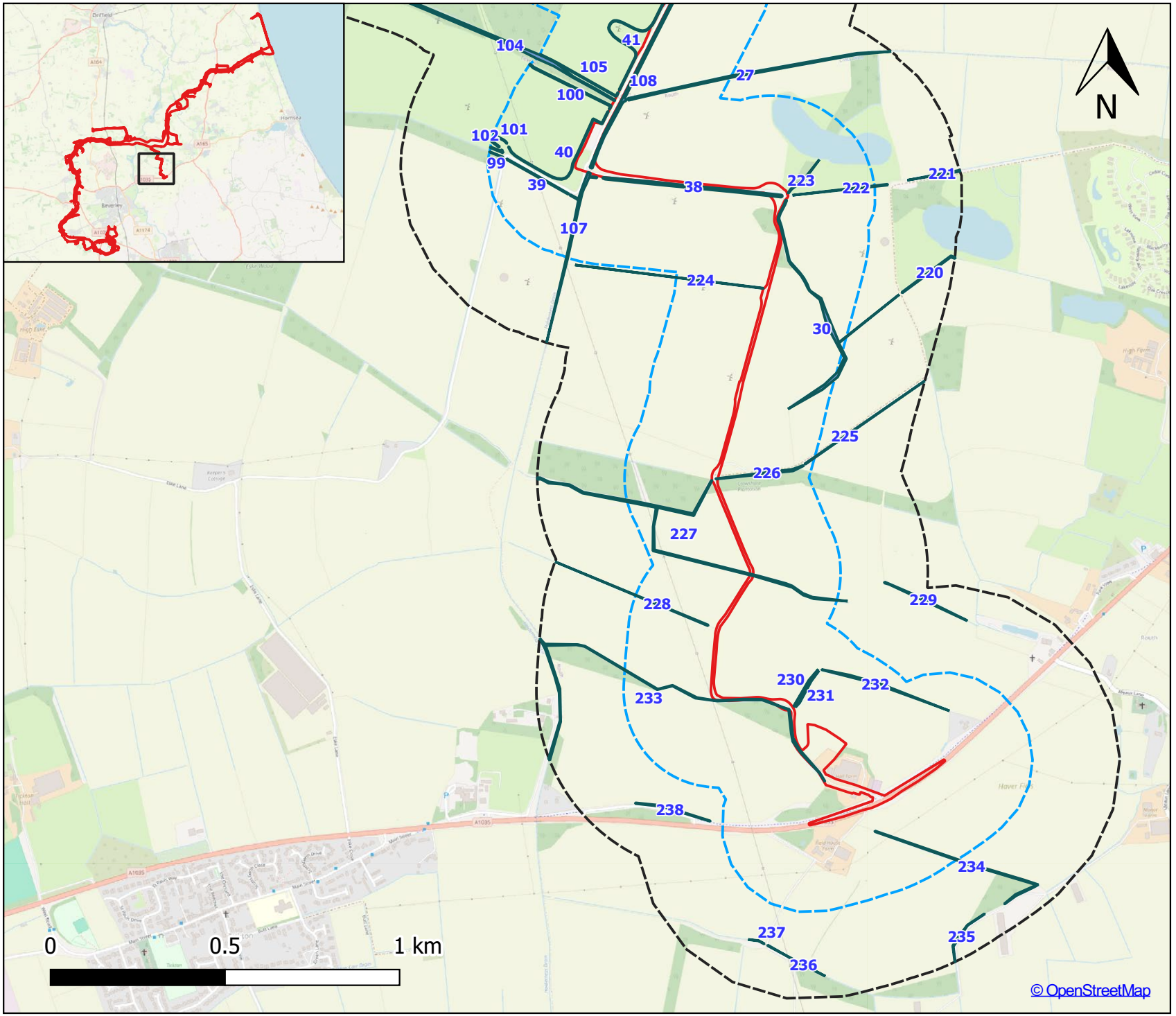

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Watercourses

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Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

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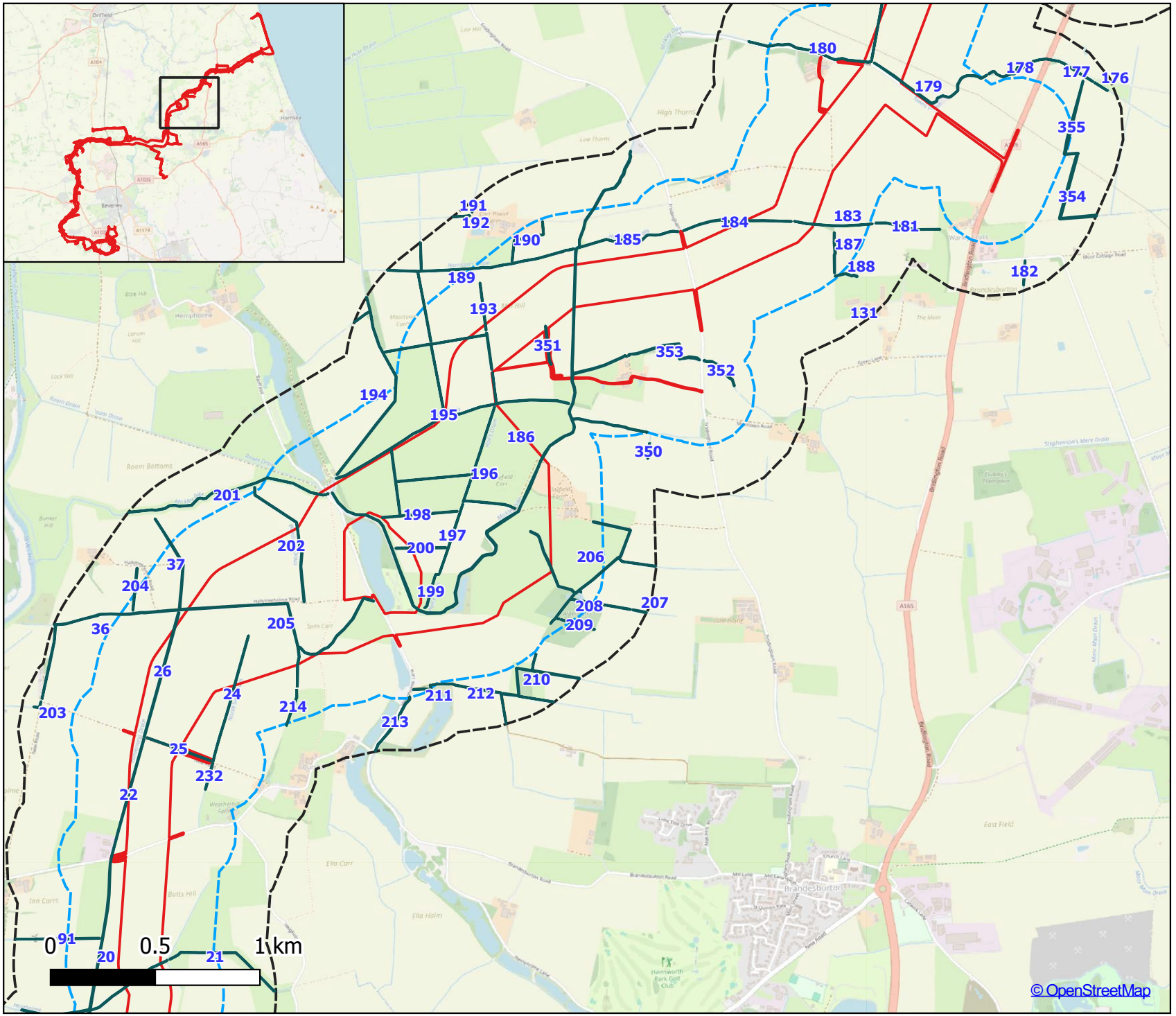

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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Watercourses

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

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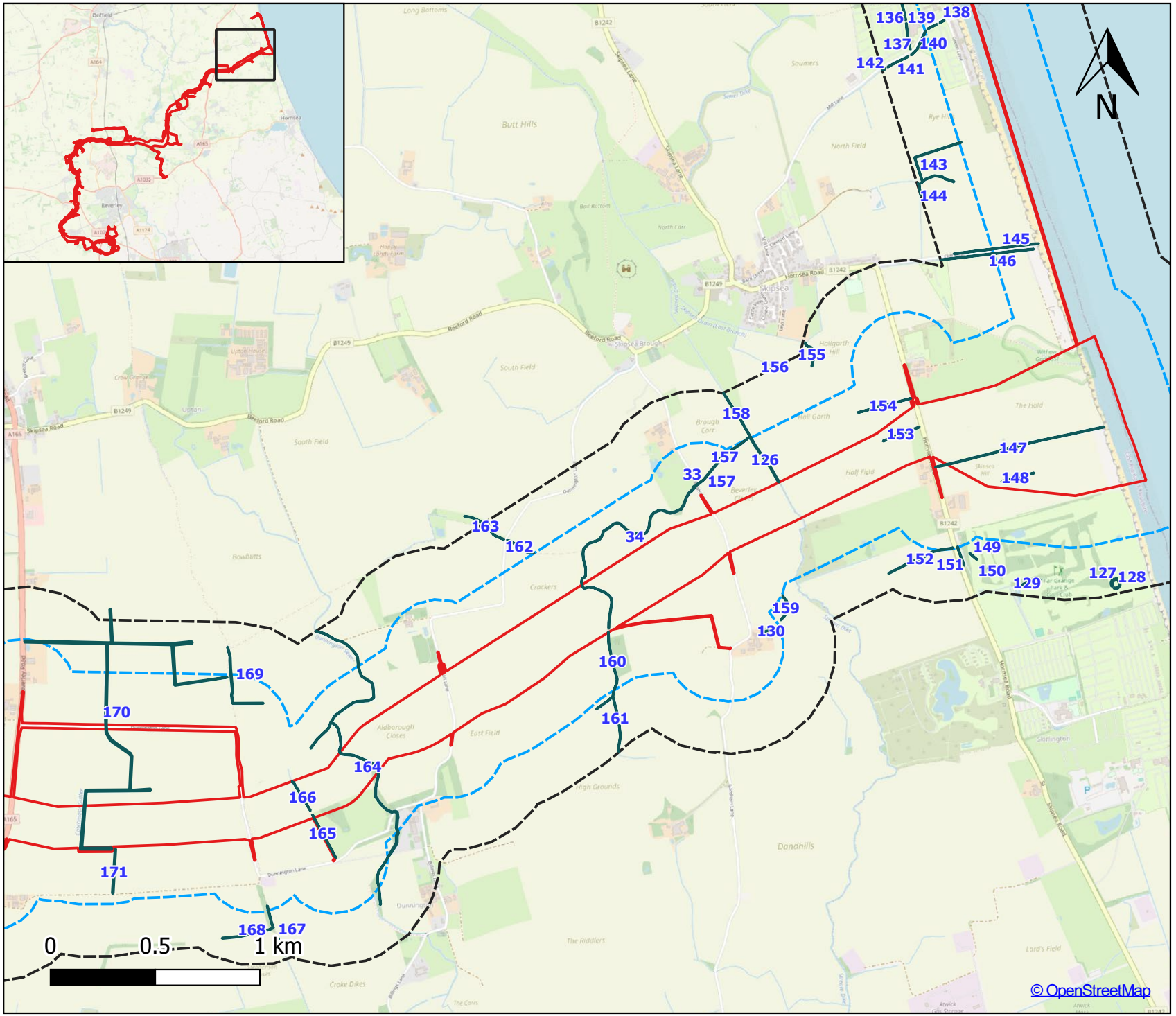

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Legend

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- 500 m buffer
- Watercourses

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Dogger Bank D
GCN Technical Note

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Watercourse and ditch network

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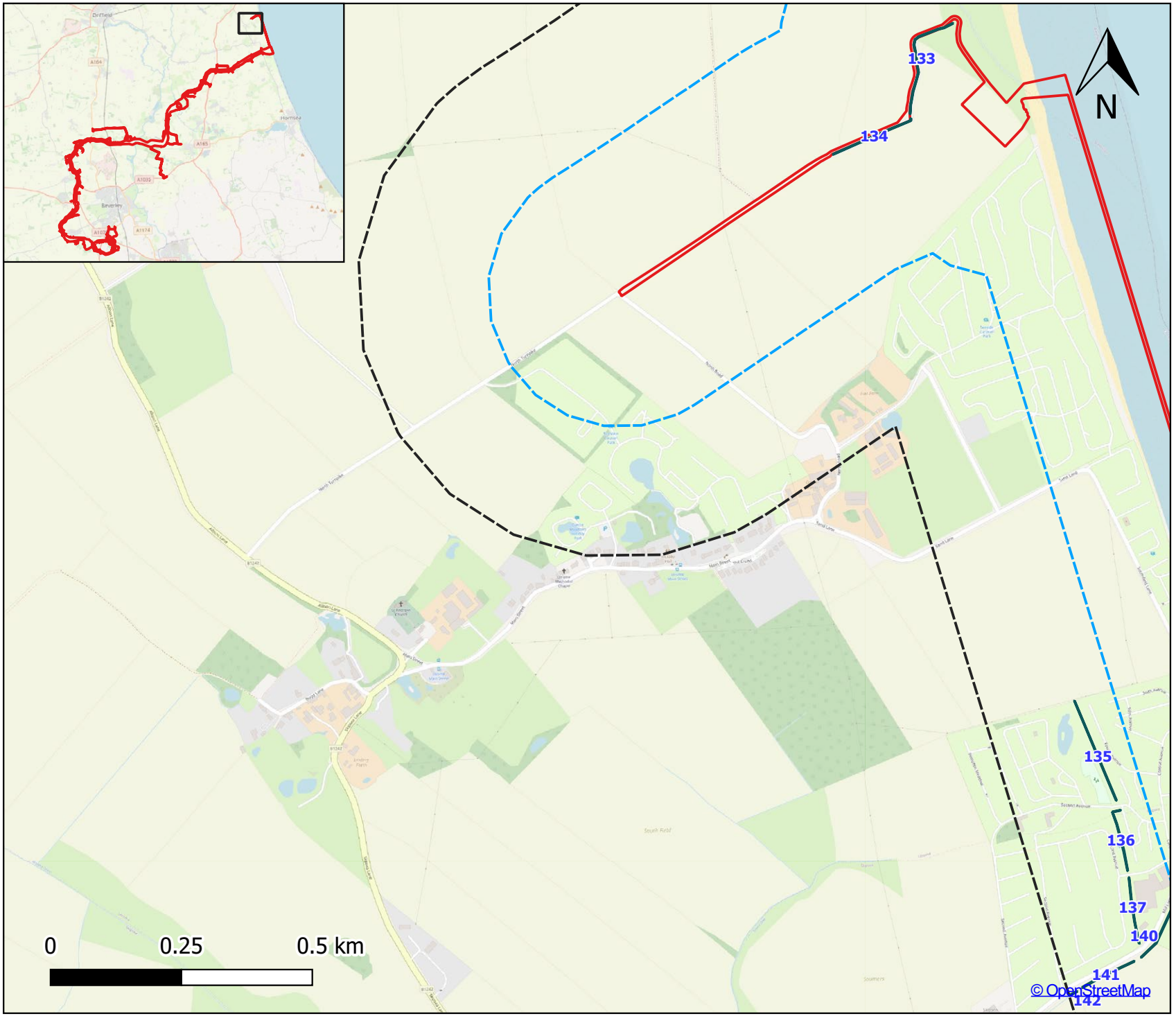

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Legend

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- 250 m buffer
- 500 m buffer
- Watercourses

Royal HaskoningDHV

Dogger Bank D
GCN Technical Note

Figure 2
Watercourse and ditch network

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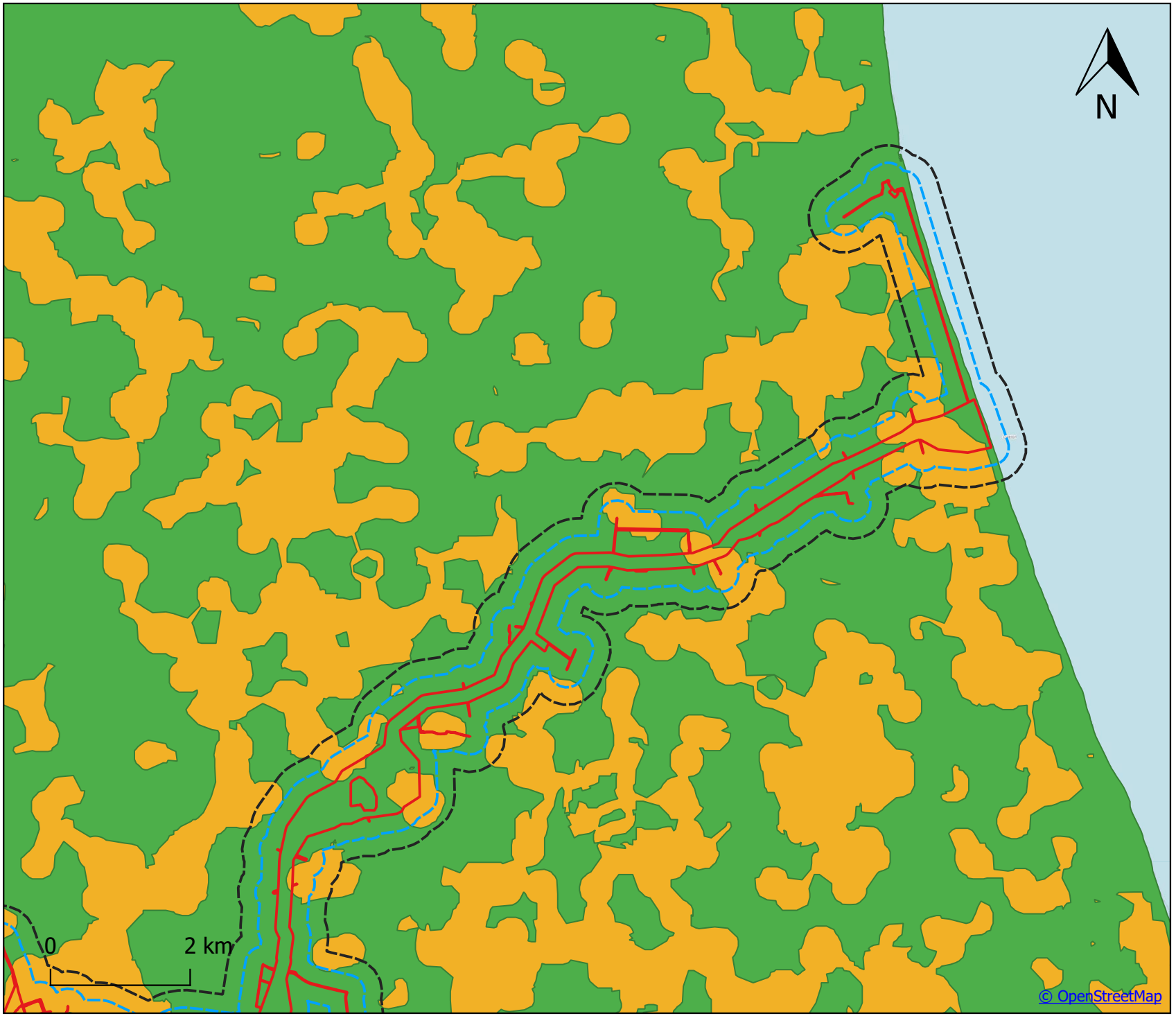
Figure 3. Natural England GCN Risk Zones



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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Amber Zone
- Green Zone

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23696 Dogger Bank D
GCN Technical Note

Figure 3
GCN Risk Zones

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	02/04/2025	SW	RJJ

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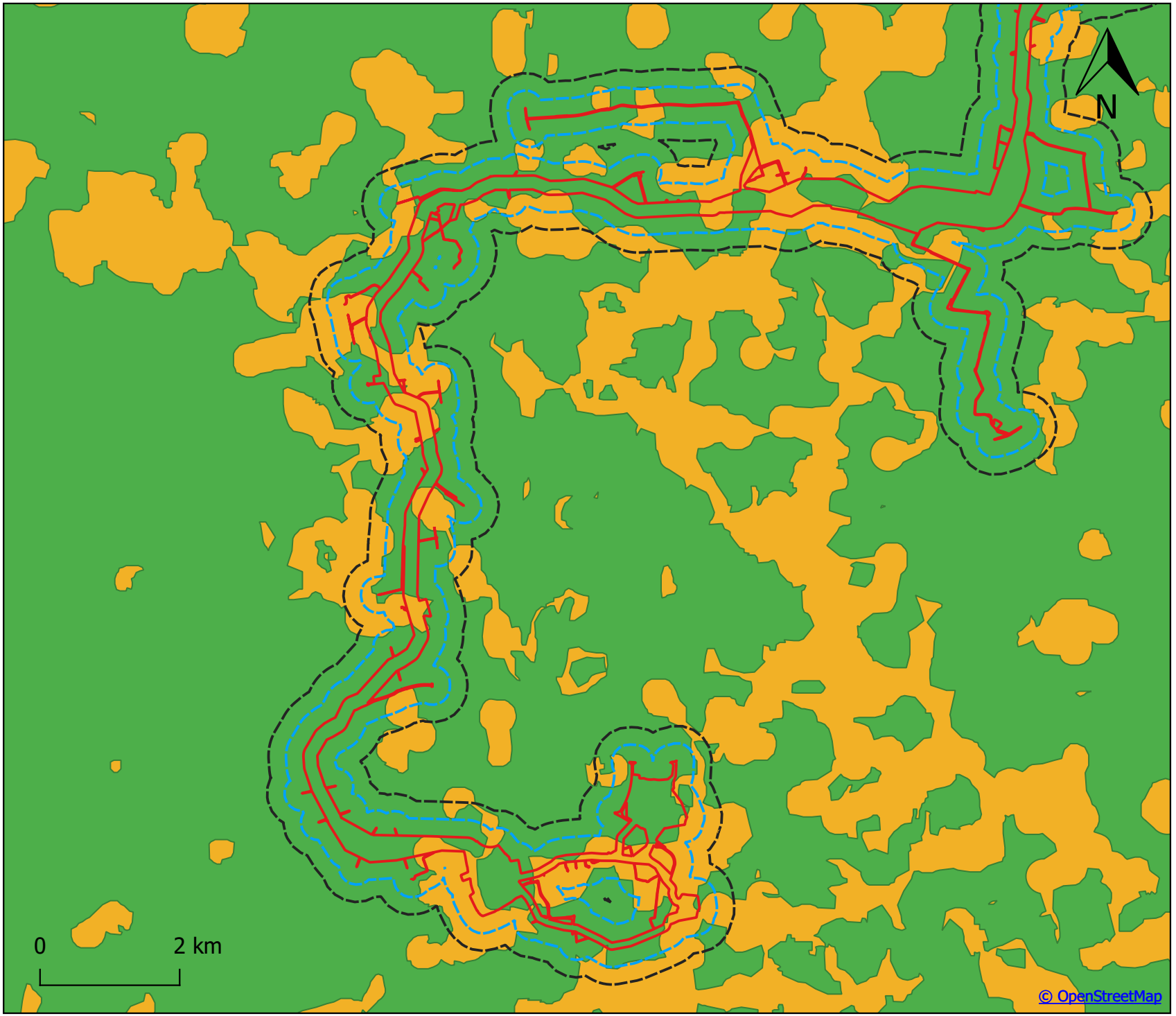
Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:75,000 @A4 | Drg.Ref: 23696.006-ECO-3-C



ecus
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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Amber Zone
- Green Zone

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23696 Dogger Bank D
GCN Technical Note

Figure 3
GCN Risk Zones

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	02/04/2025	SW	RJJ

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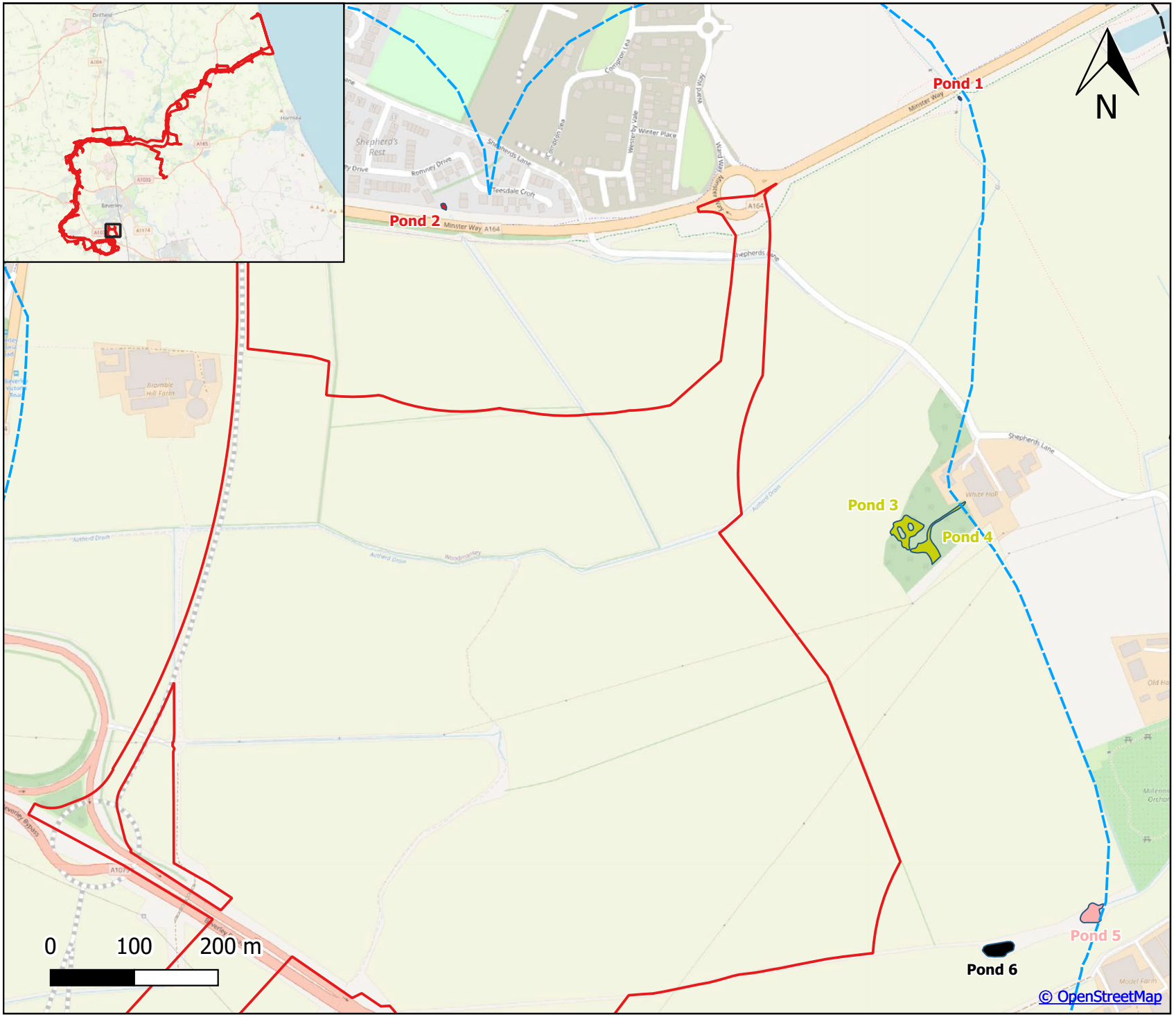
April 2025 | Scale:1:75,000 @A4 | Drg.Ref: 23696.006-ECO-3-C

Figure 4. HSI Results



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Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Good
- Below average
- Poor
- No pond

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
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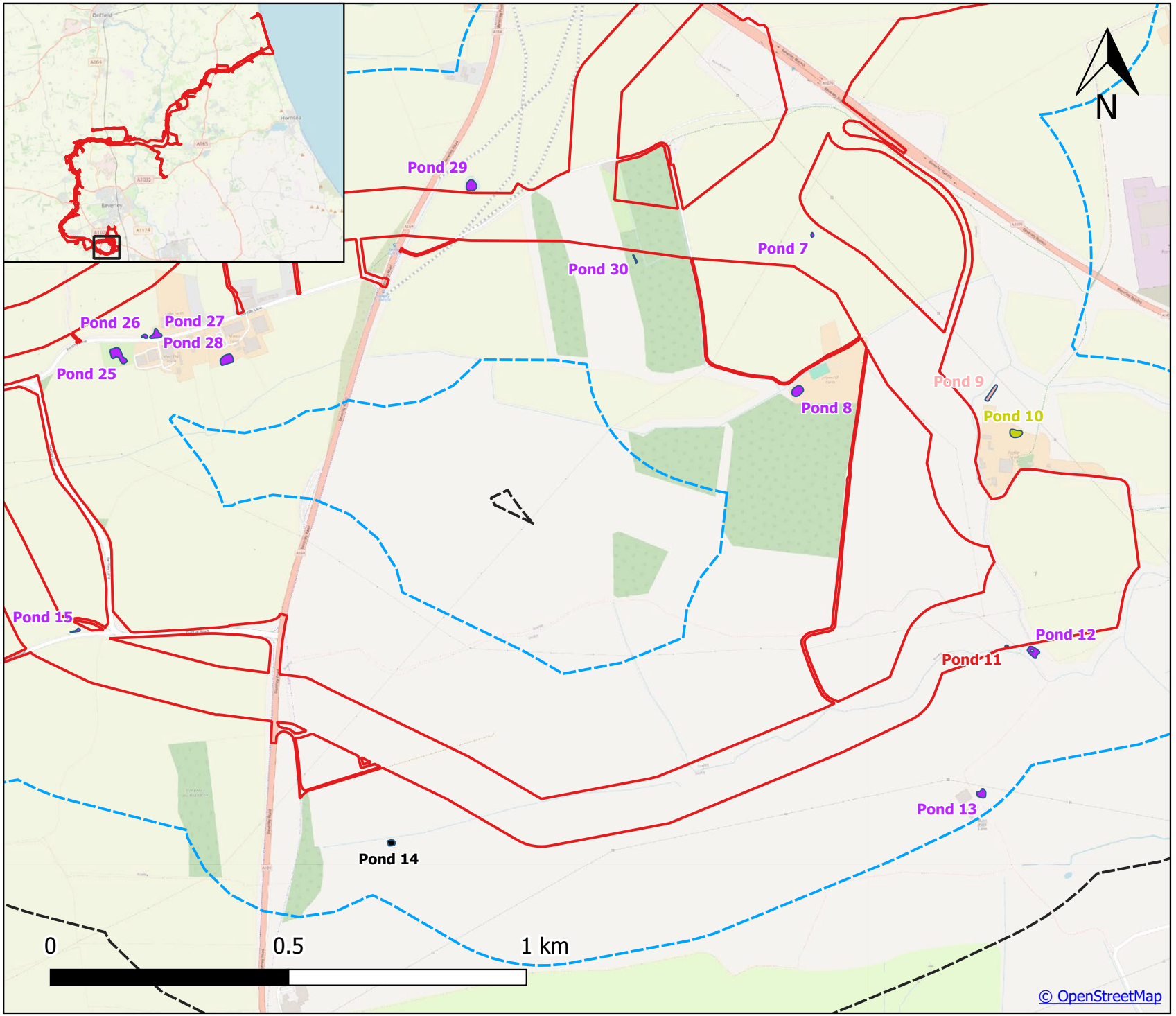

ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Good
- Below average
- Poor
- No pond
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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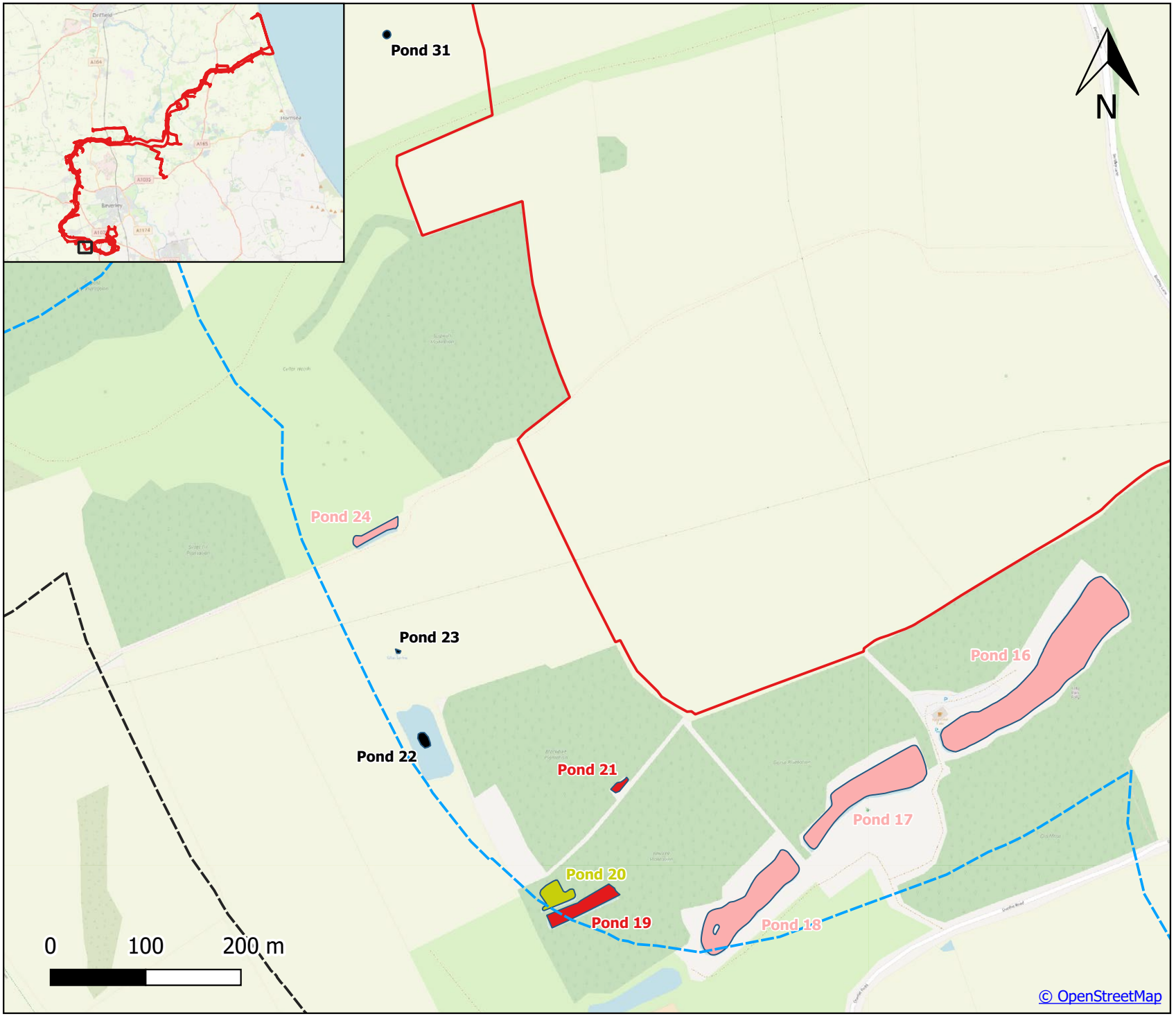
April 2025 | Scale:1:11,000 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Good
- Below average
- Poor
- No pond

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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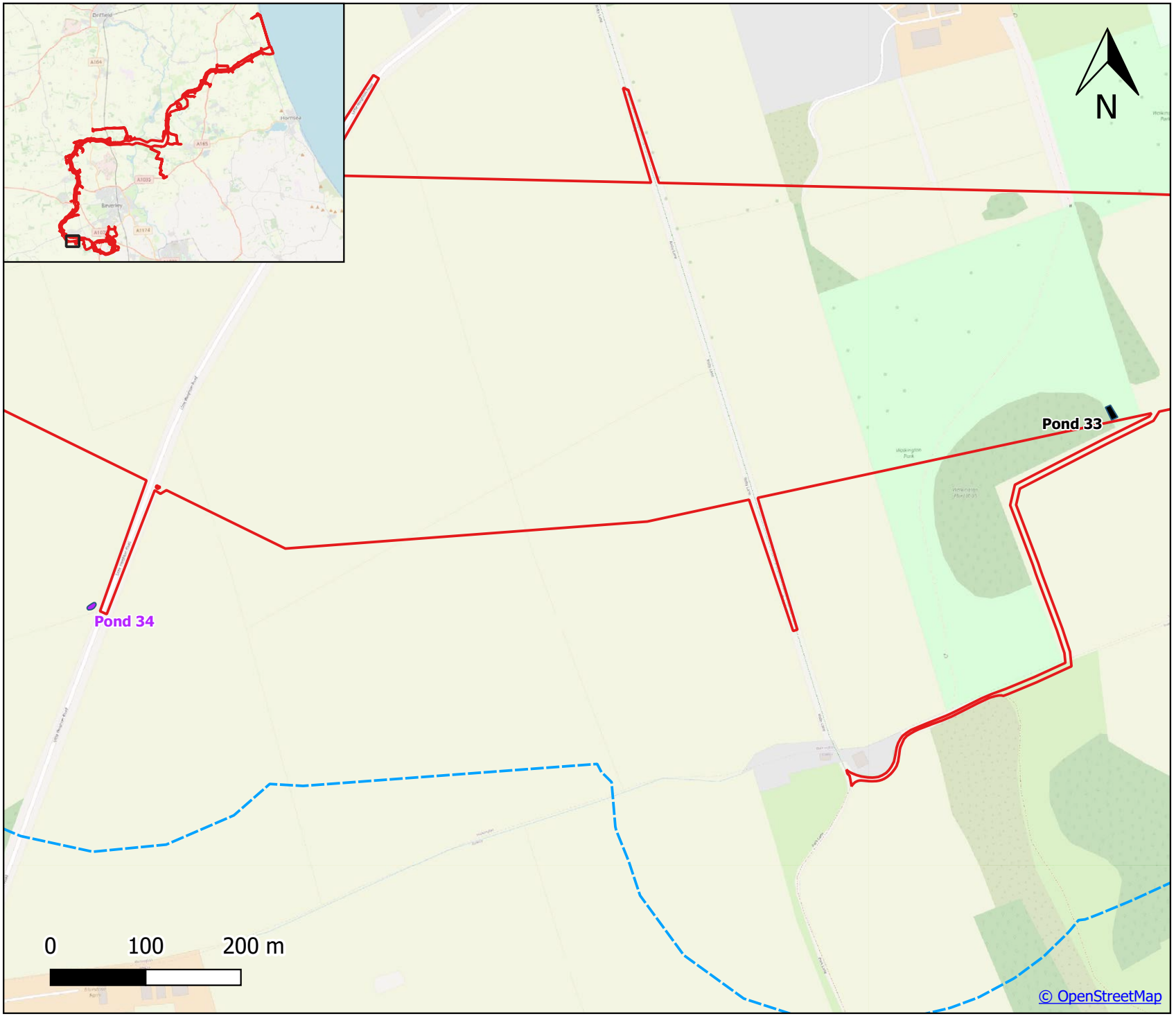

ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- No pond
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
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B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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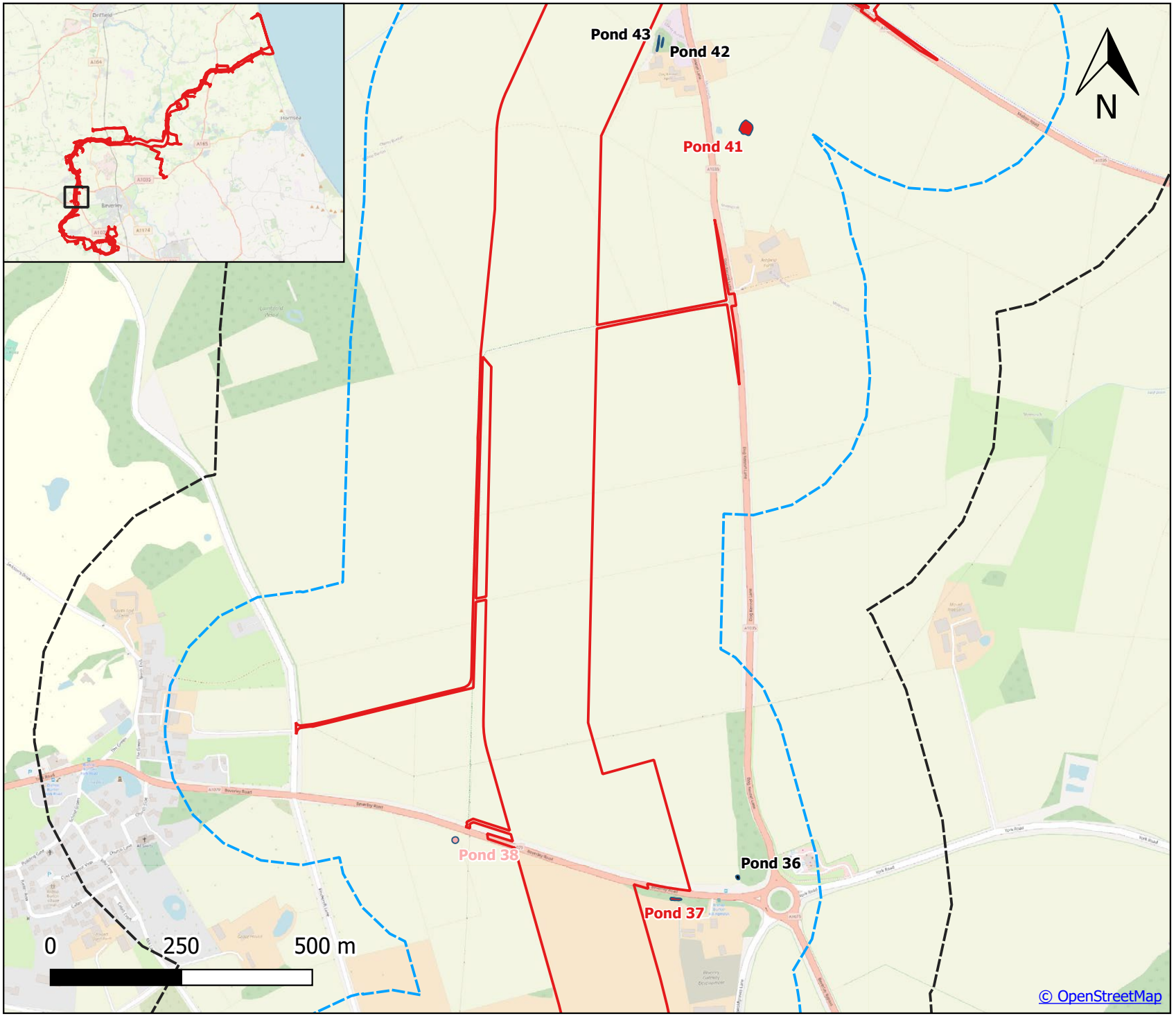

ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Below average
- Poor
- No pond

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
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C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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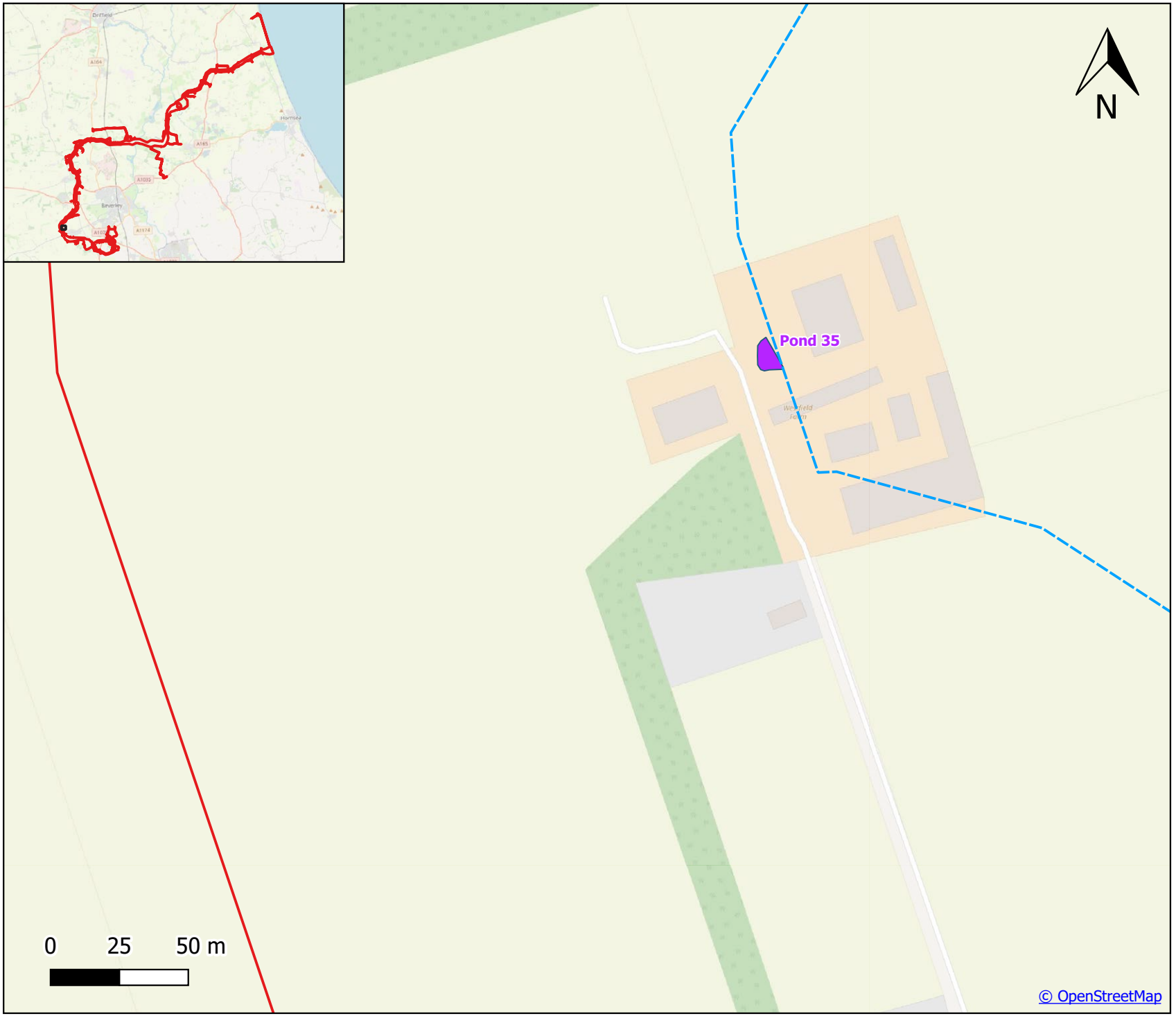
April 2025 | Scale:1:10,000@A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
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C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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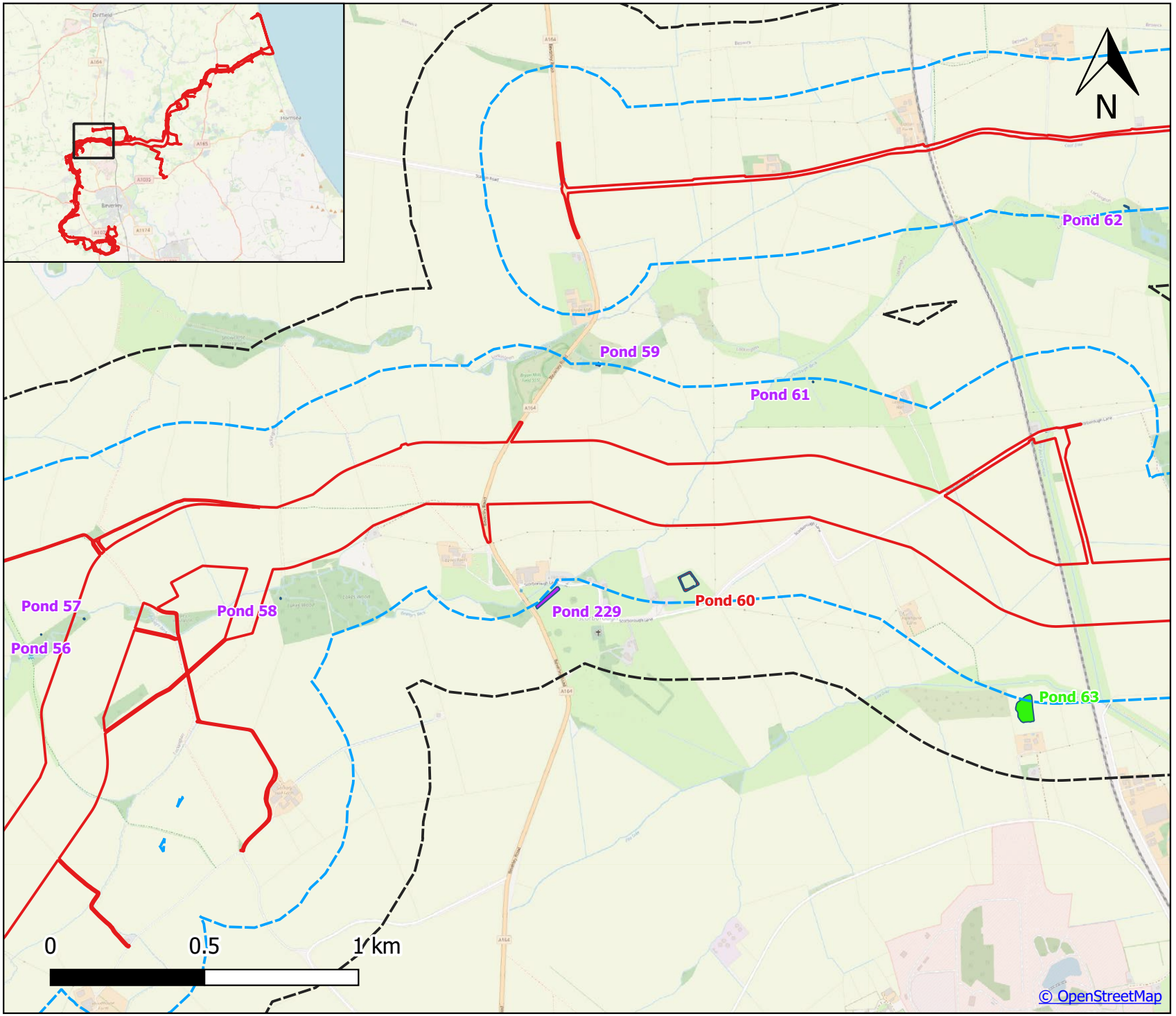
April 2025 | Scale:1:1,900 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Excellent
- Poor
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note
Figure 4
HSI results

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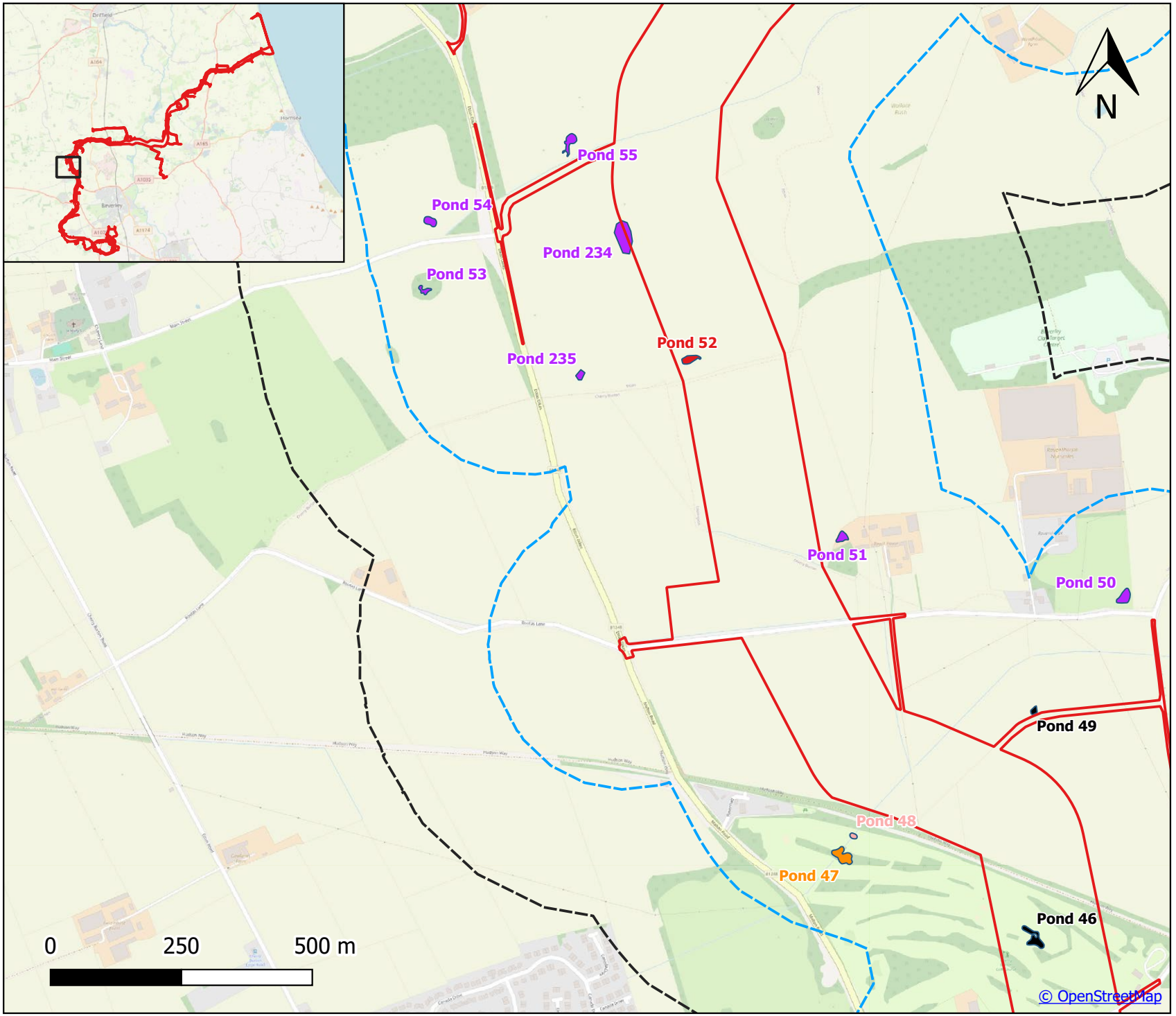

ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Average
- Below average
- Poor
- No pond
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note
Figure 4
HSI results

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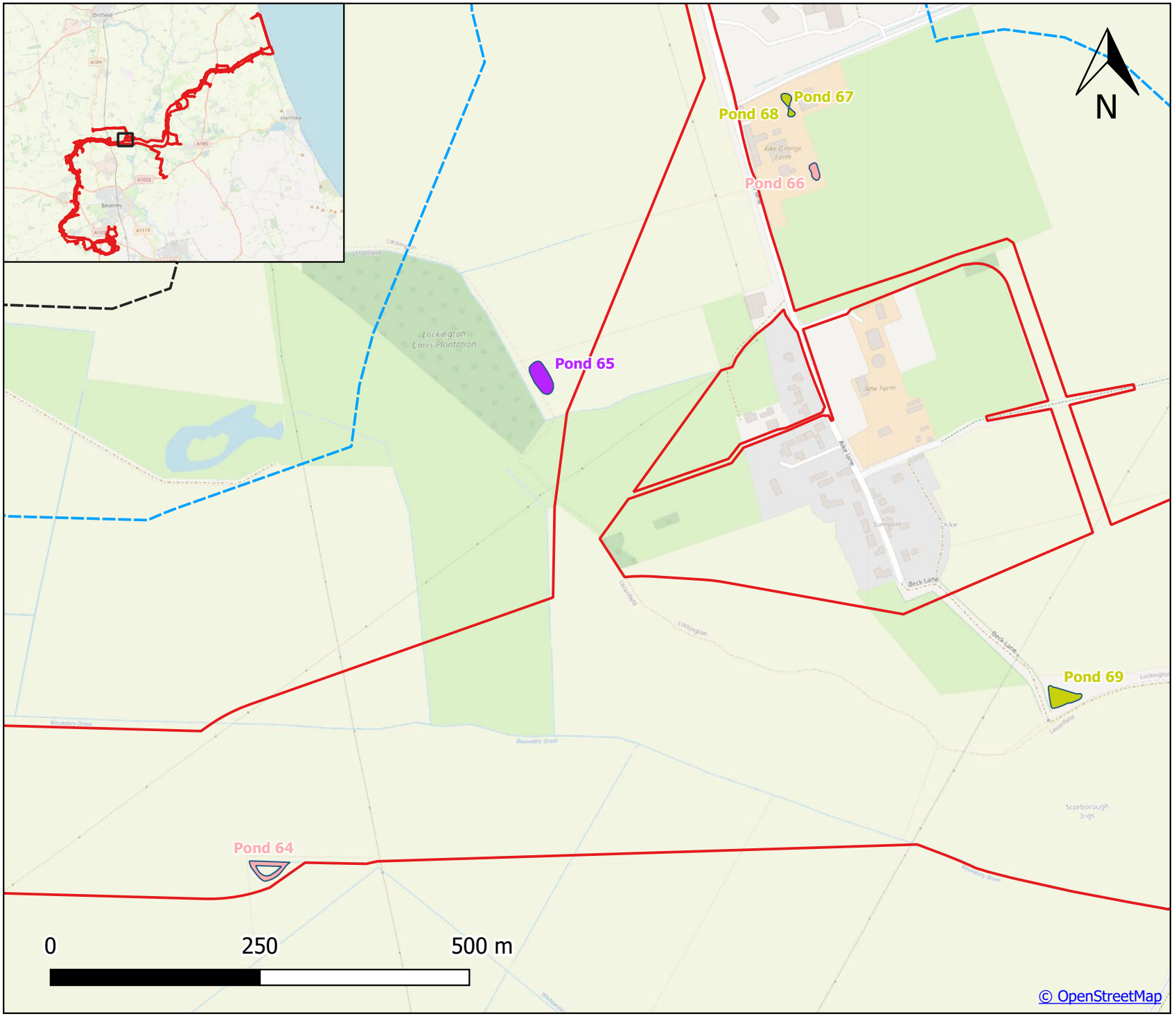
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

Building 350 ▪ The Crescent ▪ Bristol Business Park
Bristol ▪ BS16 1EJ
tel: 0117 4039050 ▪ www.ecusltd.co.uk

Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:10,000@A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Good
- Below average
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

Building 350 ▪ The Crescent ▪ Bristol Business Park
Bristol ▪ BS16 1EJ
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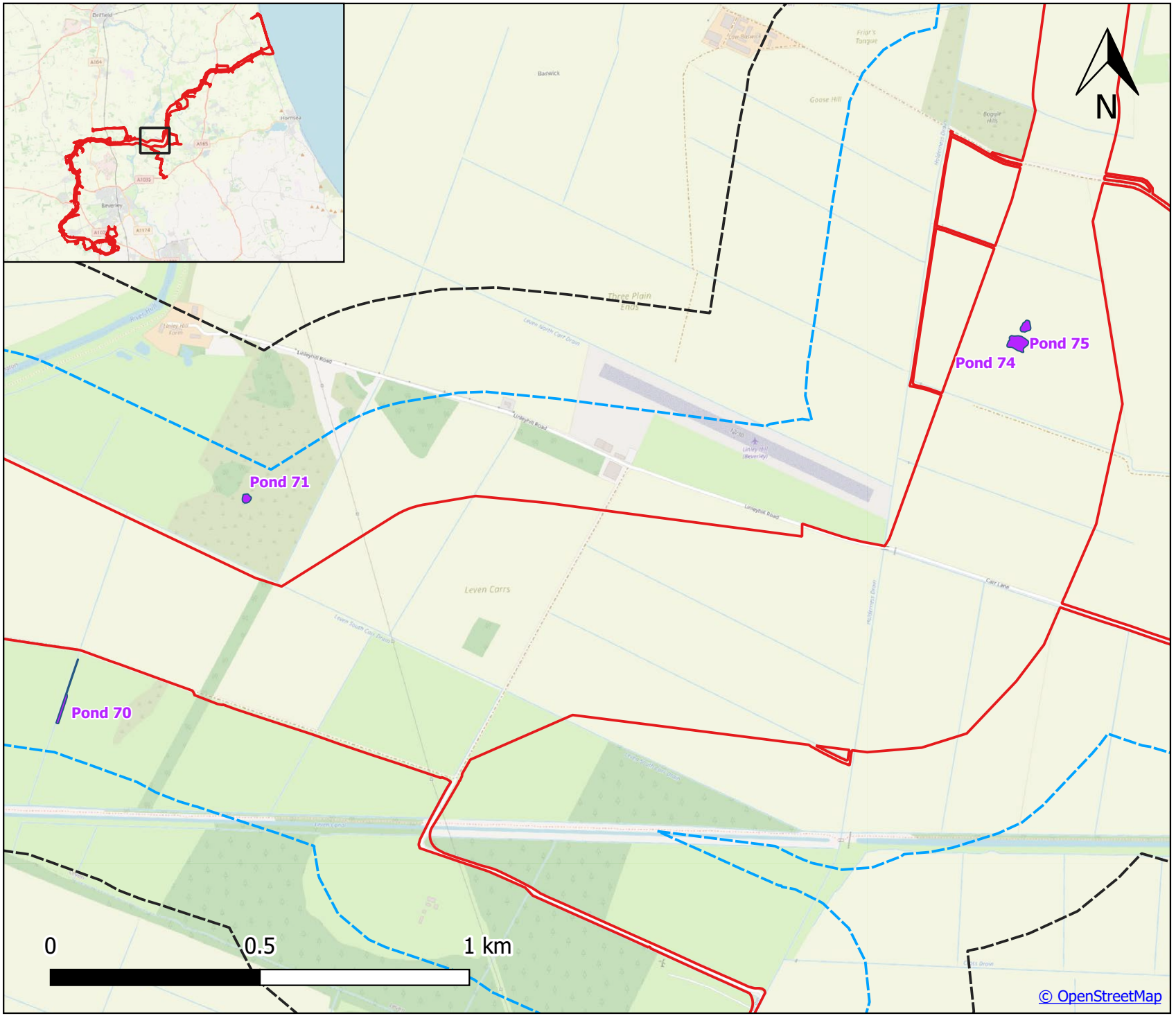
Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:6,250 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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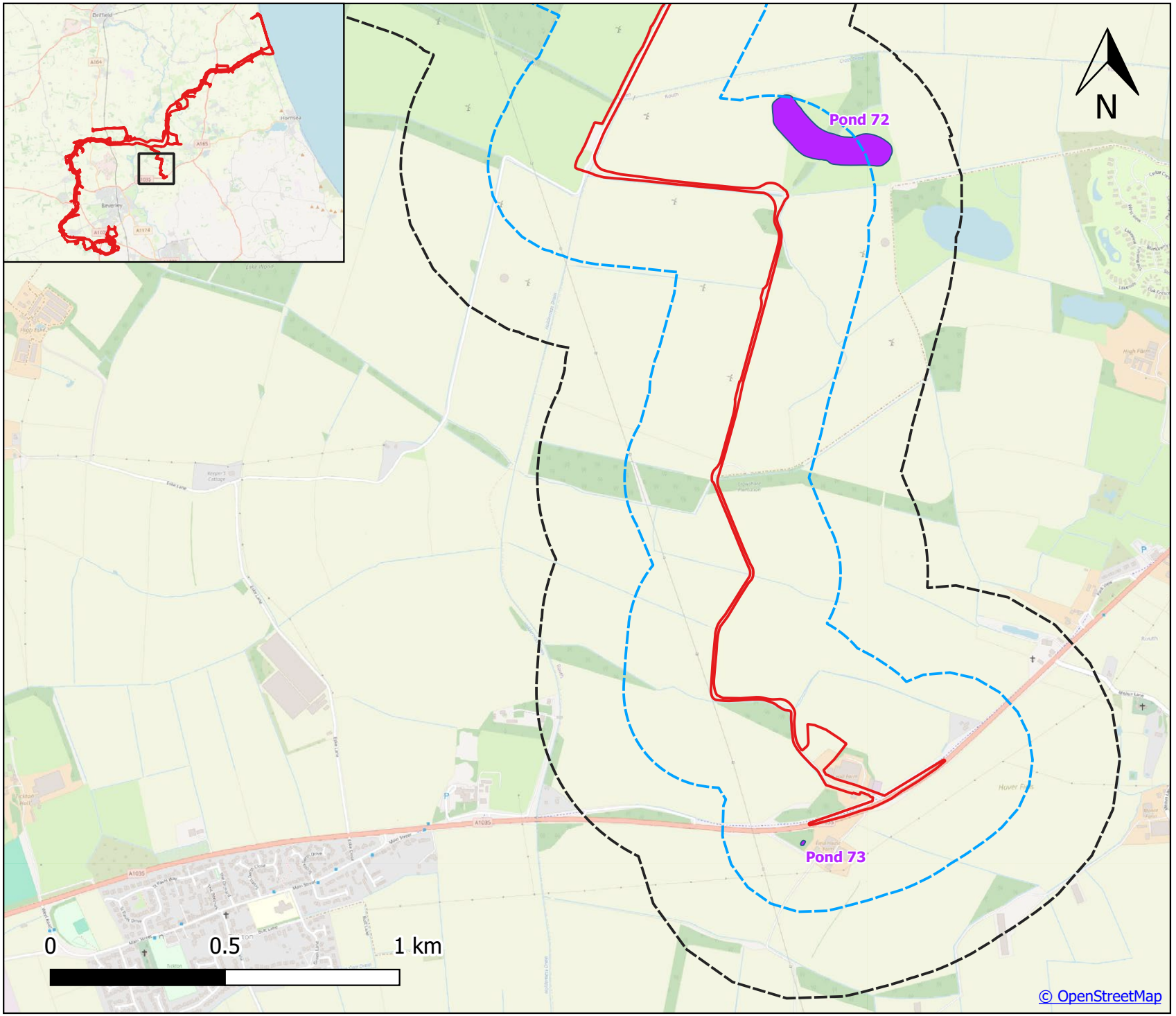
Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:12,500 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

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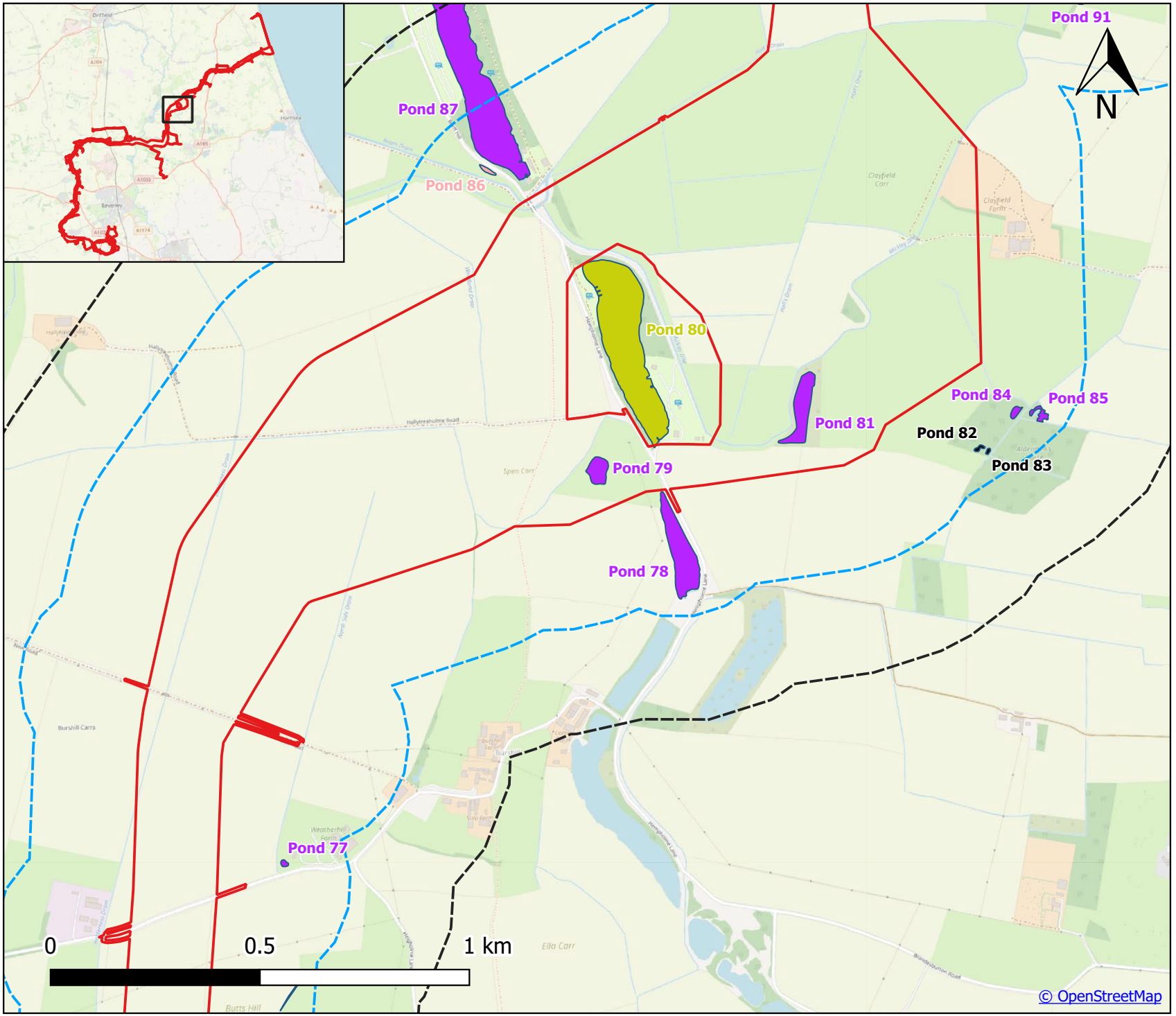
Bristol ▪ BS16 1EJ
tel: 0117 4039050 ▪ www.ecusltd.co.uk

Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:15,000 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Good
- Below average
- Poor
- No pond
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

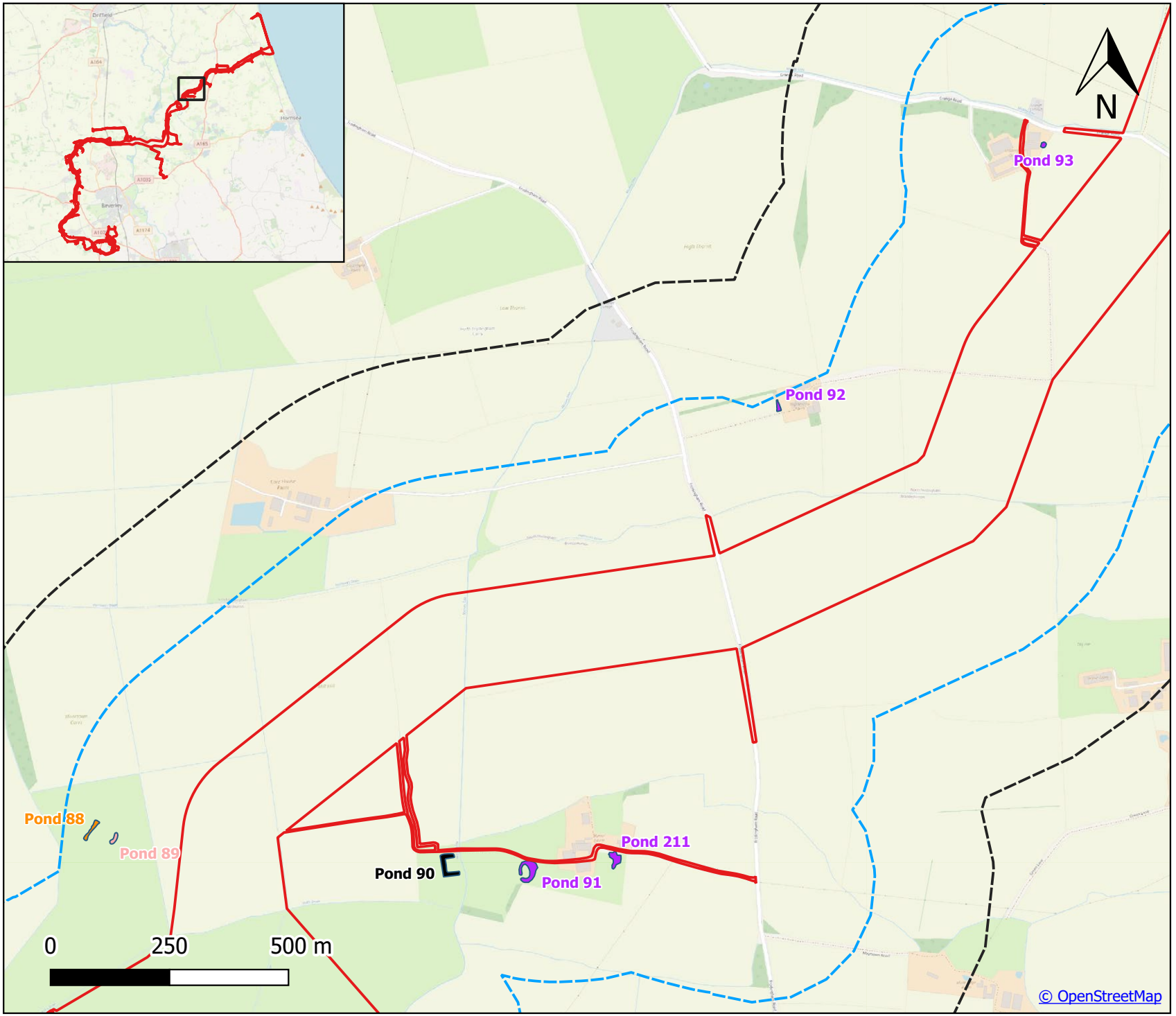
Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

Building 350 ▪ The Crescent ▪ Bristol Business Park
Bristol ▪ BS16 1EJ
tel: 0117 4039050 ▪ www.ecusltd.co.uk

Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:12,500 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Average
- Below average
- No pond
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

Building 350 ▪ The Crescent ▪ Bristol Business Park
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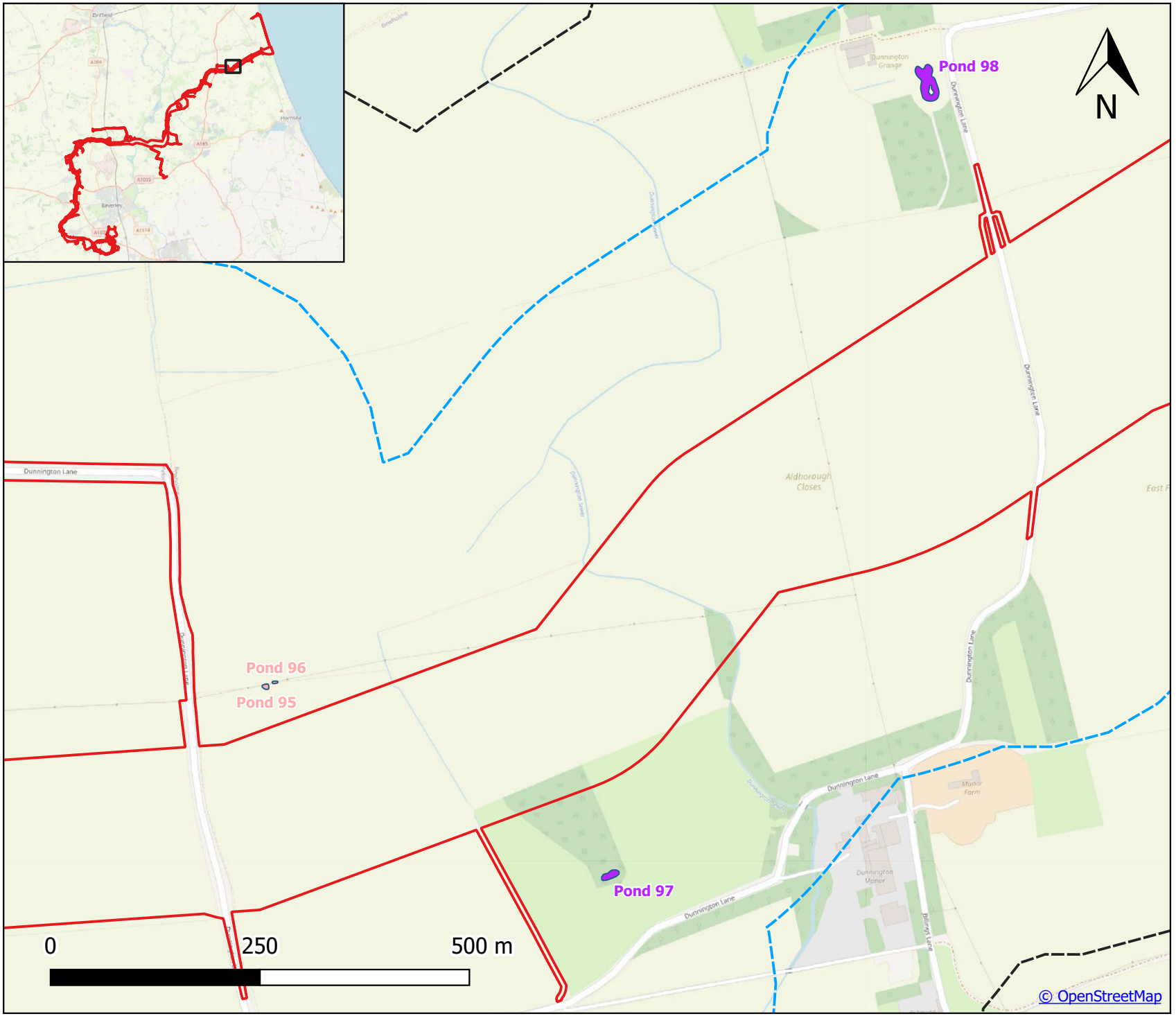
Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:11,000 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Below average
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

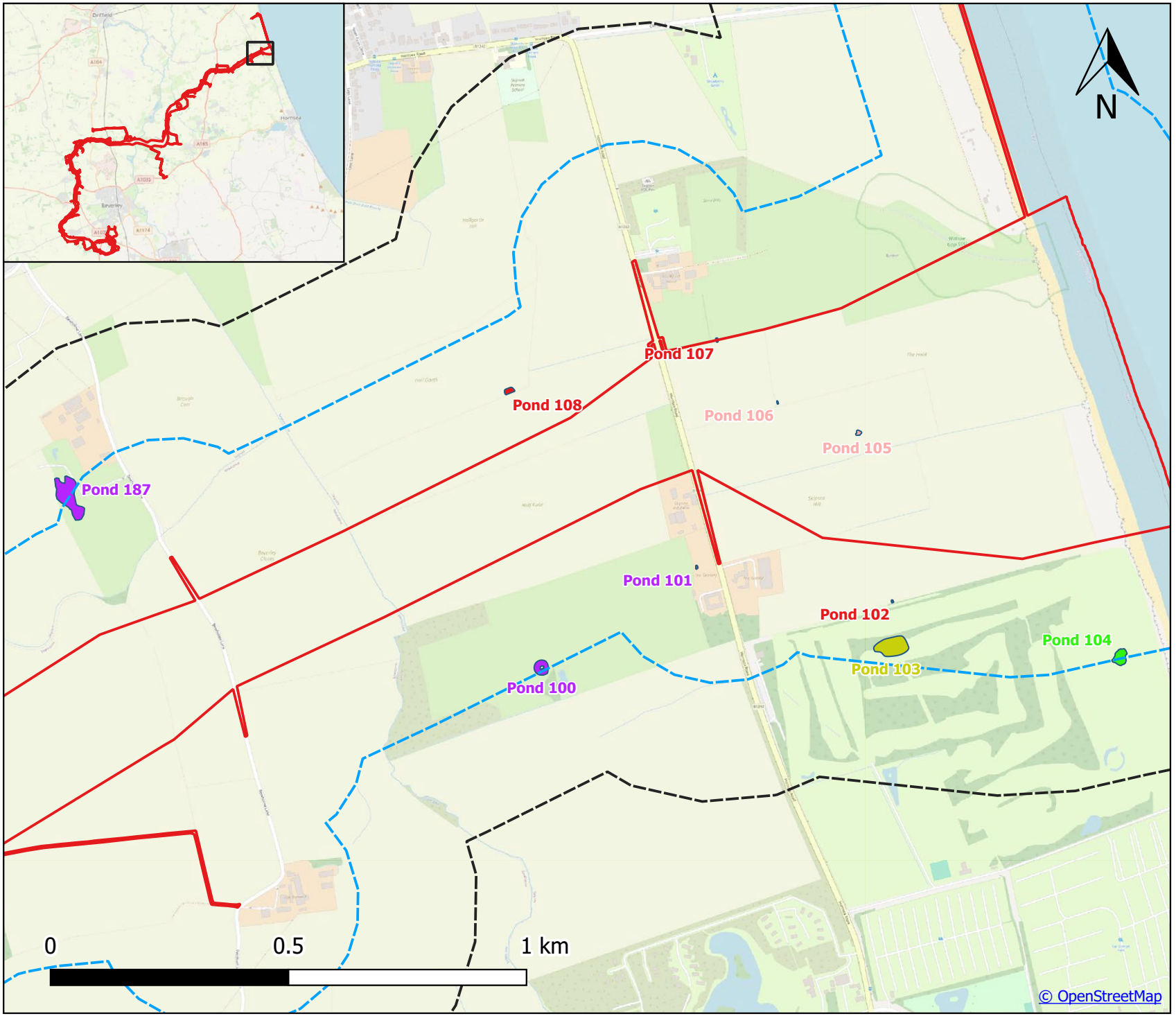
Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
D	02/04/2025	SW	RJJ

Building 350 ▪ The Crescent ▪ Bristol Business Park
Bristol ▪ BS16 1EJ
tel: 0117 4039050 ▪ www.ecusltd.co.uk

Sources: OpenStreetMap, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

April 2025 | Scale:1:6,250 @A4 | Drg.Ref: 23696.006-ECO-4-D



ecus
part of CuraTerrae

Legend

- Onshore Development Area
- 250 m buffer
- 500 m buffer
- Excellent
- Good
- Below average
- Poor
- Not surveyed

Royal HaskoningDHV

23696 Dogger Bank D
GCN Technical Note

Figure 4
HSI results

Revision	Date	Drawn by	Checked by
A	08/11/2024	LS	RJJ
B	09/12/2024	LS	SS
C	16/12/2024	LS	RJJ
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April 2025 | Scale:1:11,000 @A4 | Drg.Ref: 23696.006-ECO-4-D

Appendix 1. Natural England DAS Response

Date: 31 March 2023
Our ref: DAS/426550
Your ref: N/A

Natural England
4th Floor Foss House
Kings Pool
1-2 Peasholme Green
York
YO1 7PX

BY EMAIL ONLY

Dear [redacted]

Discretionary Advice Service (Charged Advice)
UDS A006626
Development proposal and location: Dogger Bank D Offshore Wind Farm – North Sea

Thank you for your consultation on the above dated 28 February 2023, which was received on 28 February 2023.

This advice is being provided as part of Natural England's Discretionary Advice Service in accordance with the Quotation and Agreement dated 30th September 2022. Dogger Bank D offshore wind farm (SSE Renewables Services (UK) Limited. has asked Natural England to provide advice upon:

- **DBD Preliminary Ecological Appraisal Scope (002)**

Natural England welcomes the Preliminary Ecological Appraisal Scope and the opportunity to provide feedback on the proposed approach. We have provided our comments below.

General comments

- The desk based study (data consolidation) should be undertaken prior to the field survey. Combining the desk study results with the habitat assessment will help best identify where field surveys should be undertaken.

- For a few species Natural England do not consider the 50m buffer to be a large enough area of search and we would like to see this extended (please see below for species this applies to).

- In some instances Natural England would recommend that the survey season window is restricted further to allow the best assessment to be made e.g. during breeding season, or avoiding certain weather events.

Species specific comments

Great Crested Newts:

Survey area - Natural England (NE) expects Great Crested Newt (GCN) surveys, which may inform a future GCN licence application, to include visual inspections of all ponds up to 250m (or 500m from development sites). Factors such as scale of the development, habitat connectivity, barriers to dispersal, etc. should be considered when determining the survey area. These factors can also be considered when excluding specific ponds from a survey (e.g. significant barriers to dispersal between a pond and the development site). If ponds are excluded from the survey effort and/or if only ponds within 250m of the development are surveyed, NE recommend the ecologist retains evidence of their justification for their own records. If there is clear habitat connectivity between ponds within 250m to 500m and the development site, it may be necessary to extend the survey area.
In general, surveys of ponds greater than 250m from developments are normally appropriate only when all of the following conditions are met:

- maps, aerial photos, walk-over surveys or other data indicate that the pond(s) has potential to support a large great crested newt population
- the footprint contains particularly favourable habitat, especially if it constitutes the majority available locally
- the development would have a substantial negative effect on that habitat
- there is an absence of dispersal barriers

HSI assessment/eDNA - It is understood that Habitat Suitability Index (HSI) assessments and eDNA surveys will be undertaken on all waterbodies within 250m of the project at some stage pre development (where criteria met above). As explained above, assessments of waterbodies within 500m of the development may be necessary. Furthermore, HSI scores can be used as an indication of pond suitability for GCN, which can in turn help determine which ponds to survey. Ponds should not be excluded from surveys solely based on HSI scores (unless it can be demonstrated that they are totally unsuitable) as GCN are regularly recorded in ponds with poor HSI scores. HSI assessment findings should be used in combination with historical survey data, habitat connectivity information, etc. when determining which waterbodies should be subject to further survey.

Age of survey data - To best inform any licensing decisions, it is recommended that surveys are undertaken as close as possible to when works will commence. The required age of the survey data also depends on the predicted impacts of the development. Specific requirements regarding the age of survey data can be found on the Instructions tab of the GCN Method Statement template[1]. Consideration should therefore also be given to when population size class surveys should be undertaken.

Water voles

An initial habitat survey should be carried out to help identify suitable water vole habitat. NE would suggest the initial habitat survey is undertaken during the breeding season to best inform any surveying decisions. Further to this multiple habitat surveys may be required over the course of the breeding season as the habitat suitability may change over time e.g., variations in water levels, changes to habitat management techniques, etc. which may in turn impact the suitability of the habitat for water voles. Overall, habitat initially ruled out as unsuitable for water voles could change during the year to become suitable water vole habitat and this needs to be considered to accurately determine water vole presence across the development and surrounding habitat.

Otters

Otter surveys can be carried out at any time of year but should avoid periods following prolonged heavy rainfall and/or high water when spraints and other signs of otter may have been washed away. Heavy frost or recent snow can also make finding spraints difficult.

[1] Great crested newts: apply for a mitigation licence (A14) - GOV.UK (www.gov.uk)

All suitable otter habitat within 200m of the proposed works should be surveyed. The survey should be undertaken by an experienced otter surveyor, and should include a systematic search for spraints, paw prints, otter paths, slides, food remains, holts and places used for shelter.

For clarification of any points in this letter, please contact [redacted]

Senior adviser to QA letter and check box below

☒ The advice provided in this letter has been through Natural England's Quality Assurance process

The advice provided within the Discretionary Advice Service is the professional advice of the Natural England adviser named below. It is the best advice that can be given based on the information provided so far. Its quality and detail is dependent upon the quality and depth of the information which has been provided. It does not constitute a statutory response or decision, which will be made by Natural England acting corporately in its role as statutory consultee to the competent authority after an application has been submitted. The advice given is therefore not binding in any way and is provided without prejudice to the consideration of any statutory consultation response or decision which may be made by Natural England in due course. The final judgement on any proposals by Natural England is reserved until an application is made and will be made on the information then available, including any modifications to the proposal made after receipt of discretionary advice. All pre-application advice is subject to review and revision in the light of changes in relevant considerations, including changes in relation to the facts, scientific knowledge/evidence, policy, guidance or law. Natural England will not accept any liability for the accuracy, adequacy or completeness of, nor will any express or implied warranty be given for, the advice. This exclusion does not extend to any fraudulent misrepresentation made by or on behalf of Natural England.

Yours sincerely,

[redacted]
Yorkshire and North Lincolnshire area team

Appendix 2. Site photographs

1 Pond 1

2 Pond 2

3 Pond 3

4 Pond 4

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

December 2024
Drg. Ref: LS/23696/A1

5 Pond 5

6 Pond 6

7 Pond 9

8 Pond 10

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

9	Pond 11
10	Pond 12
11	Pond 14
12	Pond 16

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

December 2024
Drg. Ref: LS/23696/A1

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

13 Pond 17

14 Pond 18

15 Pond 19

16 Pond 20

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

December 2024
Drg. Ref: LS/23696/A1

17	Pond 21
18	Pond 22
19	Pond 23
20	Pond 24

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

21	Pond 31
22	Pond 33
23	Pond 34
24	Pond 36

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

December 2024
Drg. Ref: LS/23696/A1

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

25	Pond 37
26	Pond 38
27	Pond 41
28	Pond 42

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

29	Pond 43
30	Pond 46
31	Pond 47
32	Pond 48

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

33	Pond 49
34	Pond 52
35	Pond 60
36	Pond 63

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

37	Pond 64
38	Pond 66
39	Pond 67/68
40	Pond 67/68

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

December 2024
Drg. Ref: LS/23696/A1

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

41	Pond 69
42	Pond 76
43	Pond 80
44	Pond 86

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

45	Pond 88
46	Pond 89
47	Pond 90
48	Pond 94

Royal HaskoningDHV

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

December 2024

Drg. Ref: LS/23696/A1

49	Pond 95
50	Pond 96
51	Pond 99
52	Pond 100

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

December 2024
Drg. Ref: LS/23696/A1

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

53 Pond 102

54 Pond 103

55 Pond 104

56 Pond 106

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

December 2024
Drg. Ref: LS/23696/A1

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

57	Pond 107
58	Pond 108
59	Pond 82 / 83
60	Pond 82 / 83

December 2024

Drg. Ref: LS/23696/A1

Royal HaskoningDHV

23696 Dogger Bank D
Great Crested Newt Technical Note

Appendix 1: Site Photographs

Ecus Ltd.
Building 350, Bristol Business Park, The Crescent, Stoke Gifford, BS16 1EJ

Appendix 3. HSI survey results

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
1	TA 03831 37670	1	0.05	0.1	0.33	0.5	1	1	0.65	0.33	0.5	0.39	Poor	eDNA	0 – 250 m
2	TA 03216 37542	1	0.05	0.1	0.33	1	1	1	0.75	0.67	0.5	0.46	Poor	eDNA	0 – 250 m
3	TA 03769 37159	1	1	0.9	0.33	1	0.67	0.7	0.9	0.67	0.4	0.71	Good	eDNA	0 – 250 m
4	TA 03794 37132	1	1	0.9	0.67	0.7	0.67	0.7	0.9	0.67	0.4	0.73	Good	eDNA	0 – 250 m & 250 – 500 m
5	TA 03990 36696	1	0.9	0.1	0.33	0.4	1	1	1	0.67	0.5	0.58	Below Average	eDNA	0 – 250 m & 250 – 500 m

2 SI^1 = Geographical location, SI^2 = Pond area, SI^3 = Pond permanence, SI^4 = Water quality, SI^5 = Shade, SI^6 = Water fowl effect, SI^7 = Fish presence, SI^8 = Pond density, SI^9 = Terrestrial habitat, SI^{10} = Macrophyte cover.

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
6	TA 03876 36657	HSI not completed (pond does not exist)												None	0 – 250 m
7	TA 03252 36147	HSI not completed (no land access)												HSI and eDNA	Onshore Development Area
8	TA 03220 35817	HSI not completed (land parcel inaccessible due to road closures)												HSI and eDNA	0 – 250 m
9	TA 03627 35818	1	0.1	1	1	0.33	0.2	1	1	1	0.8	0.59	Below Average	eDNA	0 – 250 m
10	TA 03680 35732	1	0.7	0.9	0.9	0.67	1	0.67	0.9	0.67	0.4	0.73	Good	eDNA	0 – 250 m
11	TA 03660 35284	1	0.05	0.1	0.1	0.33	0.4	0.67	0.75	0.67	0.4	0.40	Poor	eDNA	Onshore Development

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
															Area
12	TA 03717 35271	HSI not completed (pond inaccessible)												HSI and eDNA	0 – 250 m
13	TA 03607 34972	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
14	TA 02360 34869	HSI not completed (pond does not exist)												None	0 – 250 m
15	TA 01707 35315	HSI not completed (no land access)												HSI and eDNA	Onshore Development Area
16	TA 01189 35398	1	0.8	0.9	0.67	1	0.67	0.01	1	1	0.4	0.51	Below Average	eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
17	TA 01012 35257	1	0.955	0.9	0.67	1	0.67	0.01	1	1	0.4	0.52	Below Average	eDNA	0 – 250 m
18	TA 00891 35146	1	0.955	0.9	0.67	1	0.67	0.01	1	1	0.4	0.52	Below Average	eDNA	0 – 250 m and 250 – 500 m
19	TA 00716 35142	1	0.955	0.9	0.33	1	0.01	0.7	0.85	1	0.4	0.48	Poor	eDNA	0 – 250 m
20	TA 00689 35154	1	1	0.9	0.67	1	0.67	0.3	0.85	1	0.4	0.73	Good	eDNA	0 – 250 m and 250 – 500 m
21	TA 00756 35272	1	0.2	0.1	0.67	0.3	0.67	1	0.75	1	0.4	0.49	Poor	eDNA	0 – 250 m
22	TA 00550 35319	HSI not completed (pond does not exist)												None	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
23	TA 00521 35411	HSI not completed (pond does not exist)												None	0 – 250 m
24	TA 00497 35536	1	0.8	0.5	0.33	0.2	1	1	1	0.33	0.4	0.57	Below Average	eDNA	0 – 250 m
25	TA 01793 35896	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
26	TA 01850 35934	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
27	TA 01874 35935	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
28	TA 02021 35885	HSI not completed (no land access)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
29	TA 02536 36250	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
30	TA 02880 36095	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
31	TA 00510 36059	HSI not completed (pond does not exist)												None	0 – 250 m
32	TA 00753 36391	HSI not completed (pond does not exist)												None	Onshore Development Area
33	TA 00084 36257	HSI not completed (pond does not exist)												None	Onshore Development Area
34	SE 99011	HSI not completed (pond inaccessible)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
	36051														
35	SE 98732 37497	HSI not completed (no land access)												HSI and eDNA	0 – 250 m and 250 – 500 m
36	TA 00181 39663	HSI not completed (pond does not exist)												None	0 – 250 m
37	TA 00062 39619	1	0.1	0.1	0.33	0.2	1	1	0.85	0.67	0.4	0.41	Poor	eDNA	0 – 250 m
38	SE 99644 39736	1	0.25	0.1	0.33	1	1	1	0.975	0.67	0.6	0.56	Below Average	eDNA	0 – 250 m
41 (dry)	TA 00196 41091	1	1	0.1	0.33	1	0.67	1	0.1	0.33	0.4	0.44	Poor	None, as dry	0 – 250 m
42	TA 00036	HSI not completed (pond does not exist)												None	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
	41256														
43	TA 00030 41256	HSI not completed (pond does not exist)												None	0 – 250 m
46	SE 99966 42409	HSI not completed (pond does not exist)												None	Onshore Development Area
47 (dry)	SE 99602 42563	1	1	0.1	0.33	1	1	1	0.89	0.67	0.4	0.62	Average	None, as dry	0 – 250 m
48 (dry)	SE 99625 42600	1	0.25	0.1	0.33	1	1	1	0.89	0.67	0.4	0.54	Below Average	None, as dry	0 – 250 m
49	SE 99969 42841	HSI not completed (pond does not exist)												None	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
50	TA 00142 43053	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
51	SE 99605 43172	HSI not completed (pond does not exist)												None	0 – 250 m
52 (dry)	SE 99305 43511	1	0.05	0.1	0.33	0.3	1	1	0.6	0.67	0.8	0.42	Poor	None, as dry	Onshore Development Area
53	SE 98804 43639	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
54	SE 98816 43773	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
55	SE 99078 43921	HSI not completed (no land access)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
56	SE 99763 45319	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
57	SE 99903 45370	HSI not completed (no land access)												HSI and eDNA	Onshore Development Area
58	TA 00541 45437	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
59	TA 01572 46196	HSI not completed (no land access)												HSI and eDNA	0 – 250 m and 250 – 500 m
60	TA 01861 45501	1	0.91	1	0.33	1	0.01	1	0.6	0.33	0.4	0.43	Poor	eDNA	0 – 250 m
61	TA 02269 46140	HSI not completed due to health and safety reasons												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
62	TA 03290 46707	HSI not completed (no land access)												HSI and eDNA	0 – 250 m and 250 – 500 m
63	TA 02959 45093	1	0.8	0.9	1	1	0.67	0.67	0.89	1	0.8	0.86	Excellent	eDNA	0 – 250 m and 250 – 500 m
64 (dry)	TA 04180 45411	1	0.05	0.5	0.33	0.4	1	1	0.84	0.67	0.8	0.52	Below Average	None, as dry	Onshore Development Area
65	TA 04507 45997	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
66	TA 04833 46245	1	0.1	0.9	0.67	0.9	0.67	0.67	0.45	0.67	0.4	0.56	Below Average	eDNA	0 – 250 m
67/68	TA 04801 46325	1	1	0.9	0.67	0.9	0.67	0.67	0.84	0.67	0.7	0.79	Good	eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
69	TA 05133 45619	1	1	0.9	0.67	0.9	0.67	0.67	0.84	0.67	0.7	0.79	Good	eDNA	Onshore Development Area
70	TA 06138 45322	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
71	TA 06569 45792	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
72	TA 08195 44118	HSI not completed (no land access)												HSI and eDNA	0 – 250 m and 250 – 500 m
73	TA 08140 42112	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
74	TA 08407 46160	HSI not completed (no land access)												HSI and eDNA	Onshore Development

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
															Area
75	TA 08428 46200	HSI not completed (no land access)												HSI and eDNA	Onshore Development Area
76	TA 08850 47558	1	1	0.1	0.33	1	0.67	1	0.85	0.01	0.4	0.39	Poor	eDNA	0 – 250 m
77	TA 08837 47869	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
78	TA 09782 48622	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
79	TA 09581 48806	HSI not completed (no land access)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
80	TA 09651 49089	1	0.8	0.9	0.67	1	0.67	0.33	1	0.67	0.5	0.72	Good	eDNA	Onshore Development Area
81	TA 10075 48962	HSI not completed (no land access)												HSI and eDNA	Onshore Development Area
82	TA 10479 48821	HSI not completed (pond does not exist)												None	0 – 250 m
83	TA 10498 48810	HSI not completed (pond does not exist)												None	0 – 250 m
84	TA 10583 48956	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
85	TA 10651	HSI not completed (no land access)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
	48933														
86	TA 09322 49528	1	0.65	0.1	0.33	0.4	1	1	0.9	0.67	0.4	0.54	Below Average	eDNA	0 – 250 m
87	TA 09243 49868	HSI not completed (no land access)												HSI and eDNA	0 – 250 m and 250 – 500 m
88	TA 09824 50032	1	0.6	0.1	0.33	1	1	1	1	1	0.4	0.62	Average	eDNA	0 – 250 m
89 (dry)	TA 09870 50018	1	0.4	0.1	0.33	1	1	1	1	1	0.4	0.59	Below Average	None, as dry	0 – 250 m
90	TA 10578 49959	HSI not completed (pond does not exist)												None	0 – 250 m
91	TA 10749	HSI not completed (no land access)												HSI and eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
	49950														
92	TA 11268 50920	HSI not completed, due to landowner refusal												HSI and eDNA	0 – 250 m
93	TA 11824 51470	HSI not completed, due to dense scrub making it inaccessible												HSI and eDNA	0 – 250 m
94	TA 13335 52891	HSI not completed (inaccessible)												HSI and eDNA	Onshore Development Area
95	TA 14455 52627	1	0.05	1	0.67	1	0.67	0.67	0.45	0.67	0.4	0.53	Below Average	eDNA	0 – 250 m
96	TA 14467 52630	1	0.05	0.5	0.33	1	1	1	0.72	0.67	0.8	0.56	Below Average	eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
97 (dry)	TA 14867 52400	HSI not completed (inaccessible, but looked dry)												None, as dry	0 – 250 m
98	TA 15246 53349	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
99	TA 15600 53027	1	0.99	0.1	0.33	1	1	1	0.72	0.33	0.4	0.56	Below Average	eDNA	Onshore Development Area
100	TA 17336 53767	HSI not completed as it was inaccessible												HSI and eDNA	0 – 250 m and 250 – 500 m
101	TA 17664 53975	HSI not completed (no land access)												HSI and eDNA	0 – 250 m
102	TA 18075 53906	1	0.05	0.1	0.33	0.3	1	1	0.75	0.67	0.4	0.40	Poor	eDNA	0 – 250 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
103	TA 18072 53813	1	0.8	1	0.33	1	0.67	0.7	0.75	0.67	0.9	0.75	Good	eDNA	0 – 250 m
104	TA 18551 53789	1	1	0.9	1	1	0.67	0.7	0.75	0.67	1	0.85	Excellent	eDNA	0 – 250 m and 250 – 500 m
105	TA 18003 54261	1	0.1	0.5	0.33	1	0.67	1	0.75	0.33	0.6	0.53	Below Average	eDNA	Onshore Development Area
106	TA 17833 54323	1	0.05	1	0.33	1	0.67	1	0.75	0.67	0.4	0.54	Below Average	eDNA	Onshore Development Area
107	TA 17707 54454	1	0.05	1	0.67	1	0.67	0.7	0.1	0.67	0.4	0.46	Poor	eDNA	Onshore Development Area

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
108	TA 17270 54347	1	0.4	1	0.33	1	0.67	1	0.75	0.67	0.4	0.67	Average	eDNA	0 – 250 m
145	TA 10081 45260	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	0 – 250 m
187	TA 16344 54123	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	0 – 250 m and 250 – 500 m
205	TA 13347 53265	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	0 – 250 m
211	TA 10924 49969	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	0 – 250 m
229	TA 01409 45439	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	250 – 500 m

ID number	Grid reference	SI Values[2]										HSI score	Pond suitability	Further survey required	Location
		1	2	3	4	5	6	7	8	9	10				
234	SE 99187 43743	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	Onshore Development Area
235	SE 99102 43478	HSI not completed (new pond, was not within 250 m before the boundary change)												HSI and eDNA	0 – 250 m

Appendix 4. WC1067: Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA

Analytical and methodological development for improved surveillance of the Great Crested Newt

WC1067

Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA

This report should be cited as:
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. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

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Contents

1. Scope of document

Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, and gametes; shed skin and hair; and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, according to the detection limits of qPCR approaches and associated fragment sizes, and depending on environmental conditions (Biggs et al. 2014). Recent research has shown that the DNA from a range of aquatic organisms can be detected in water samples at very low concentrations using qPCR (quantitative Polymerase Chain Reaction) methods.

This document provides technical advice for laboratories and field staff collecting and analysing samples for qPCR analysis of great crested newt (*Triturus cristatus*) environmental DNA. The document:

- Sets out the standards required
- Sets out field and laboratory approaches for screening the presence/absence of the great crested newts
- Is designed to deliver a consistent approach, and hence comparable data, between laboratories for use in decision making.

Deviations from this protocol will need to demonstrate equivalence.

This document is based mainly on research undertaken during Defra project WC1067 "Analytical and methodological development for improved surveillance of the great crested newt" (Biggs et al. 2014). We advise that this report is used as a reference document for those carrying out great crested newt surveys using the methods described here.

2. Quality assurance and quality control

The methods described in this technical advice note are designed to reduce as far as possible the risk of field or laboratory generated false positive and false negative results. Quality control measures must be extended to sample collection, preservation and handling, as well as laboratory protocols, since assurances of sample quality will prove critically important to the avoidance of false negatives.

The field of aquatic eDNA is developing rapidly and it is likely that, as methods evolve, appropriate updates will need to be made to the processes detailed in this technical advice note.

2.1 Laboratory standard

Laboratories undertaking eDNA analysis should be able to demonstrate adequate quality assurance standards. Typically these will comprise a documented quality management system which would usually follow, or be equivalent to, the outline of ISO/IEC 17025 standard.

Ultimately it may be necessary to develop a proficiency testing scheme for eDNA analysis to enable the identification of laboratories certified as achieving the appropriate level of proficiency with the eDNA methods. At present a proficiency testing scheme for eDNA is not available because an appropriate proficiency testing methodology has not been established. Further research and development work will be needed to establish such a scheme.

In the meantime, agencies and organisations may wish to include samples from ponds known to support great crested newt and samples from sites known to be free from great crested newts to validate sampling programmes.

2.2 Sample acceptance

The laboratory analysing eDNA water samples should have a standard and documented sample acceptance procedure in place. This should include:

- Date and time of sample receipt
- Sample condition (i.e. has the sample container been damaged in any way)
- A visual verification of the sample volume (to detect any leakages)
- A note to confirm appropriate handling in transit (e.g. courier packaging intact).

The receiving laboratory should transfer the sample number to the sample acceptance record at this point.

2.3 Stability of field sampling kits

The stability of field sampling kits should be assessed through the use of an appropriate artificial DNA marker to check for unexpected decay of DNA between sampling and sample analysis. Details of the marker used, expected rates of decay and actual decay rates should be published alongside eDNA results for the target species. The marker can be chosen by the laboratory or the marker used in WC1067 can be purchased from Spygen.

2.4 Outcome required

Biggs et al. (2014) achieved a Limit of Quantification of $3 * 10^{-3}$ ng/L: at present there is no evidence that great crested newt eDNA can be quantified with precision and accuracy below this level. Failure to achieve detection at this limit will lead to increased risk of false negative results for sites where great crested newt occur at low density. 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 of 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*.

2.5 Identification of risks of false positives and false negatives

There are risks of both false positives and false negatives in eDNA analysis (Darling and Mahon, 2011). Errors can occur in both field and laboratory stages of the work. Given the test's sensitivity it appears that the main risk from contamination will be from false positives.

The main risks, and their mitigation for great crested newt eDNA work, are:

(i) Molecular assay design: mitigated in research and development phase of primer and probe design. Salt free primers should be used. The quality of the primer and of the PCR mix is assured by the standards.

(ii) Laboratory quality control: mitigated by laboratory design and process control.

(iii) Sampling design: mitigated by site selection procedures in field monitoring programmes.

(iv) Uncertainty in the relationship between presence of target DNA and presence of viable target organisms: mitigated partially by research so far undertaken, and by future research increasing knowledge of great crested newt eDNA.

Table 1 summarises information on situations which may have an increased risk of generating false negatives and false positives, and potential ways to mitigate these risks. For the field sampling protocol, the risk of contamination may be greater for specialist contractors undertaking large numbers of great crested newt surveys compared to volunteers making infrequent visits to a small number of sites.

Table 1. Risk, and mitigation, of false positives and false negatives

Risk factor	Mitigation
Field-based false positives	
Cross contamination between sites (due to equipment, clothing etc.).	Ensure that there is no contact between contaminated material and the water being preserved in the sampling process.
Inflows, bringing eDNA from sites with newts into unoccupied ponds. Note that there is so far little evidence that this is a significant problem but it is a theoretical possibility.	This risk cannot be eliminated at present and its extent is not understood. Where ponds have inflows, survey teams will have to make judgements about the likely impact of any inflow. However, the majority of great crested newt ponds lack substantial inflows. The presence/absence of inflows, and whether they are wet or dry at the time of survey should be recorded in field notes.
Aquatic animals (e.g. herons, water voles) transferring newt DNA between sites (e.g. in faeces, in water trapped in fur)	This risk cannot be eliminated and the extent to which it occurs is currently unknown. Further research will be required to assess whether this is a significant risk, although at present it seems likely to be small.
Field-based false negatives	
Low numbers of newts	This risk is minimised by following good field protocol. Note that at present the minimum number of newts that can be detected in different waterbodies is not known. However, ponds with torch counts of zero animals in the breeding season, where newts were known to be present, have provided positive eDNA results in the breeding season.
Very wide, shallow drawdown zones may increase the likelihood of collecting water samples in areas where there has been no newt activity even though the pond is currently occupied.	To access deeper water areas it is possible that the water sampler could be added to a long pole. It is important not to enter the water as sediments will be disturbed which may contain historical great crested newt DNA. Further research data on sediment DNA is likely to be available within 6-12 months to refine understanding of this issue. In all water depths it is necessary to gently stir the water throughout its depth, without disturbing sediments, as eDNA is believed to sink. It is advisable to avoid sampling very shallow water (less than 5-10 cm deep) as it may be difficult to avoid stirring up sediment in these areas.
There is evidence that DNA is less likely to be detected in water taken from densely packed mats of vegetation; either because of a lack of newt activity or because of the difficulty of sample collection in these areas.	Avoid sampling in these areas: sample from water in areas where vegetation is suitable for egg-laying and open water areas suitable for displaying.
There is evidence that eDNA is less likely to be detected if the whole pond perimeter is not sampled.	Every effort should be made to access 20 sites around the pond for sampling. Sites where 80-90% of pond margins were accessed achieved 99.3% detection rates. Attaching the sampling ladle to an extension pole may be an option for reaching a wider range of areas. Effective cleaning of the extension pole between sites is essential. The pole must be kept separate from any equipment that is in contact with newts.

Table 1 (cont). Risk, and mitigation, of false positives and false negatives

Risk factor	Mitigation
Laboratory false positives	
Contamination of eDNA sampling kits.	Mitigation is largely ensured by good laboratory design, set-up and processes, particularly separation of the sample preparation room from all other stages of the process.
Contamination during DNA amplification.	Mitigation is largely ensured by physical separation of the different stages of the PCR process, use of dedicated equipment and lab coats for each stage and a uni-directional work flow from clean to DNA contaminated rooms.
The risk of contamination in the laboratory is likely to be greatest when larger numbers of samples (>20) and multiple batches of samples are handled.	Mitigation is largely ensured by good laboratory design, set-up and processes. It is to be expected that handing of smaller batches of samples (i.e. <20 samples), in single trials, will be easier than larger throughput operations.
Laboratory false negatives	
Very low eDNA concentrations.	Samples with DNA amounts below the Limit of Detection will generate false negatives. It is not currently possible to mitigate this risk.

2.6 Laboratory specifications

2.6.1How the laboratory should be set up

The set-up of an eDNA laboratory should broadly follow the outline below. Note that this is not a detailed specification for building a laboratory: rather it provides guidance on the standard which is needed.

Successful eDNA work has so far been undertaken both in laboratories designed to standards established over the last 20 years for ancient DNA (aDNA) work (Knapp et al., 2012), and in more conventional DNA labs. There is as yet no evidence available to evaluate whether these different set-ups produce different results.

The main principles of the laboratory set-up should be (PHE, 2013):

- **Physical separation of pre and post-PCR work:** to prevent amplified DNA from contaminating samples there should be physical separation of pre and post PCR stages of the work. This should include separation of the area where sampling kits are prepared.
- **Unidirectional workflow**: The arrangement of activities in the rooms should be unidirectional to reduce potential for contamination. This can be achieved by physically having one room leading to another or by set working practices.

Two potential layouts of facilities based on existing constructed systems are exemplified below (Figure 1). The simpler design has some recognised limitations which are noted in the figure. Good results are known to have been produced in higher specification laboratories. The main features of the designs are:

- **Reagent preparation clean room:** a clean DNA free room is needed for the preparation of field sampling kits. Samples containing DNA should never be brought into this room and no DNA extractions or PCRs are performed in this room[1].
- **Nucleic acid extraction room**: the only area where DNA extractions are performed, and an area where PCR products and stocks of cloned material have not been handled.
- **Amplification room**: this is the area where PCR machines are housed.

The schematic designs shown in Figure 1 fulfil these criteria.

2.6.2Appropriate precautions to avoid laboratory contamination

As PCR products are ubiquitous in post-PCR laboratories it is important to make sure that no consumables or equipment for the DNA facility have been sourced from laboratories which undertake post-PCR amplification analysis.

Full body suits have been adopted by some eDNA laboratories for work in the sample kit preparation room and the eDNA sample preparation room, including full body suit, face masks, face shields and hairnets. In other rooms disposable laboratory coats are sufficient. Dedicated clean room shoes are useful to reduce carry-over contamination. Wearing two pairs of gloves will prevent exposure of skin when changing gloves. However, not all laboratory managers consider 'suiting-up' necessary, preferring separation of staff as the contamination control method (i.e. staff do not move between pre- and post-PCR laboratories). Those working with full body suits regard this approach as good practice for rare DNA work which generally reduces the amount of DNA present in the rooms to very low levels. Face masks reduce the breathing out of DNA which has been inhaled outside the clean rooms.

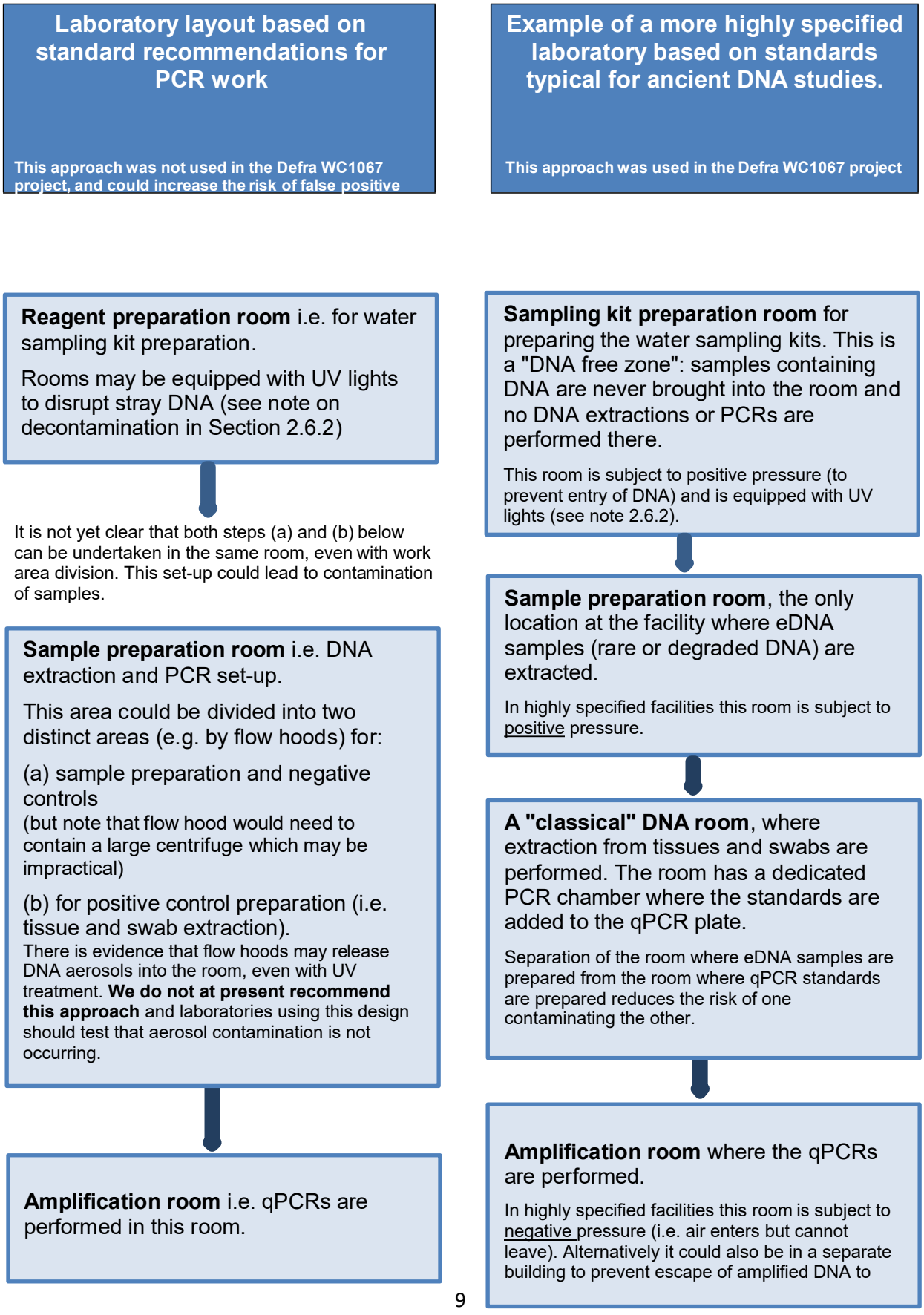
To reduce the risk of DNA contamination regular bleaching of the laboratory should be undertaken weekly. qPCR work should be undertaken inside a cabinet with UV light and in a room which is also lit by UV light outside the cabinet; to control aerosol DNA. Although UV lights are widely recommended for decontamination they need to be high power and close enough to the surface for decontamination to be effective and only then for low level contamination - cleaning and liquid decontamination is more effective (for detailed discussion see Champlot et al., 2010). They are also used in some laboratories to keep levels of environmental DNA low, including UV irradiating the facility when it is not in use.

Dedicated laminar flow hoods and fume hoods for DNA extraction and manipulation can reduce the risk of contamination still further. However, note that laminar flow hoods and fume hoods can under some circumstances make contamination worse by circulating contaminating aerosols around the laboratory. Most PCR hoods either do not have air flow, or are used switched off, providing a dedicated work station that is contained and can be easily decontaminated.

Further useful features are a positive pressure system and HEPA-filtered air conditioning. Some teams regard positive and negative pressure as desirable features to control contamination effectively. However, others suggest that procedural aspects are more important. At present it is not possible to tell which of these positions is correct. The more stringent standards of ancient DNA workers normally include positive / negative pressure and several successful laboratories working with eDNA have used this set-up. However, other groups have produced published results (e.g. Pilliod et al., 2013) without such systems. A highly specified laboratory (e.g. a forensic laboratory) may also have dedicated staff for each area because people are a major source of contamination. Vestibules with shoe/coat changing are effective techniques to prevent transfer of DNA in a highly specified laboratory, but can be adopted less expensively in less well specified laboratories by having dedicated shoes and coats for each laboratory.

[1] It is possible that a Class II cabinet in a non-DNA free room could be used for this step. If this approach is used it would be advisable for laboratories to demonstrate that this process did not lead to contamination of sample test kits. Cabinets are at risk of contamination from DNA aerosols which can be present in DNA laboratories even with UV lighting.

Figure 1. Examples of laboratory specifications proposed or in use for eDNA work.



earlier preparation stages.

3. Field protocol

Field sampling should be undertaken by a suitably trained and experienced great crested newt surveyor (trained volunteer or professional). At present it is believed that eDNA water sampling does not disturb newts enough to justify the procedure being licensed by the national regulatory authority.

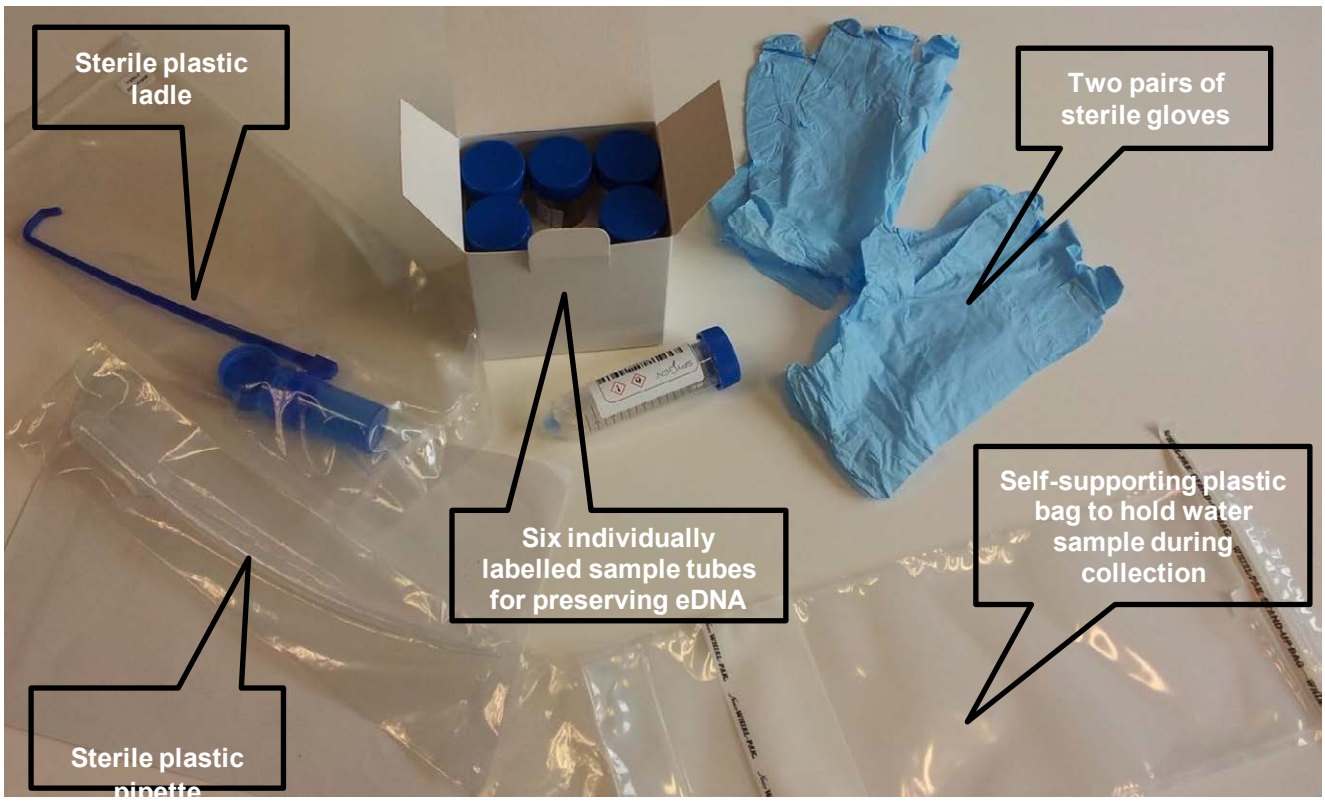
A single visit to the target pond should be made between mid-April and June, during the newt breeding season. eDNA samples can be collected at any time of day and in any reasonable weather conditions, including light rain. It may be best to avoid heavy rain as this makes sampling more difficult and might increase the risk of cross contamination (e.g. splashing of mud which could contain great crested newt DNA from wet ground). There is evidence that unpreserved amphibian eDNA decays slightly more quickly in full sun than shaded conditions, becoming undetectable after 8 and 11 days respectively (Pilliod et al., 2014), but as long as samples are preserved the impact on detection should be slight.

3.1 Sampling equipment

The field sampling equipment used by Biggs et al. (2014) has five components (Figure 2):

- A sterile 30 mL ladle
- A sterile self-supporting Whirl-Pak plastic bag with 1 L capacity
- A sterile 10 mL pipette to resample the pond water
- Six sterile 50 mL centrifuge tubes containing preservative (Absolute Ethanol (200 Proof), Molecular Biology Grade, Fisher BioReagents (Product Code: 10644795), sodium acetate and other markers)
- Two pairs of sterile gloves.

Figure 2 Sampling equipment used for eDNA water samples by Biggs et al. (2014)



Kits can be stored at room temperature before use in an appropriate solvent store, consistent with Home Office regulations, and should be used within about two weeks of receipt. The time between kit receipt and use should be noted (see Section 5.1). Use one kit per pond up to an area of 1 ha. Beyond this, use an additional kit per hectare. However, note that as yet there is no practical experience of the effectiveness of kits used on ponds greater than 1 ha in area. Note that sampling techniques are still developing rapidly in this field and alternative preservatives to ethanol are currently being sought.

3.2 Field water sample collection protocol

The field sampling protocol should follow the steps outlined below. Gloves should be worn at all times during the sampling process, replacing the gloves between sample collection from the pond and pipetting into the sterile sub-sample tubes. Samples should be collected without entering the water, i.e. the surveyor stands only on the pond bank or muddy pond edges. This prevents disturbance of the substrate and may limit cross-contamination.

Stages of field sampling protocol

Step 1 Identify where 20 samples will be taken from the pond. The location of sub-samples should be spaced as evenly as possible around the pond margin, and if possible targeted to areas where there is vegetation which may be being used as egg laying substrate and open water areas which newts may be using for displaying.

Step 2 Open the sterile Whirl-Pak bag by tearing off the clear plastic strip c 1cm from the top (along the perforated line), then pulling the tabs. The bag will stand-up by itself.

Step 3 Collect 20 samples of 30 mL of pond water from around the pond (see 1 above) using the ladle (fill the ladle), and empty each sample into the Whirl-Pak bag. At the end the Whirl-Pak bag should be just under half full (600 mL).

NOTE: Before each ladle sample is taken, the pond water column should be mixed by gently using the ladle to stir the water from the surface to close to the pond bottom without disturbing the sediment on the bed of the pond. It is advisable not to sample very shallow water (less than 5-10 cm deep).

Step 4 Once 20 samples have been taken, close the bag securely using the top tabs and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.

Step 5 Put on a new pair of gloves to keep the next stage as uncontaminated as possible.

Step 6 Using the clear plastic pipette provided take c15 mL of water from the Whirl-Pak bag and pipette into a sterile tube containing 35 mL of ethanol to preserve the eDNA sample (i.e. fill tube to the 50 mL mark). Close the tube ensuring the cap is tight.

Step 7 Shake the tube vigorously for 10 seconds to mix the sample and preservative. This is essential to prevent DNA degradation. Repeat for each of the 6 conical tubes in the kit. Before taking each sample, stir the water in the bag to homogenize the sample - this is because the DNA will constantly sink to the bottom.

Step 8 Empty the remaining water from the Whirl-Pack bag back into the pond.

Step 9 The box of preserved sub-samples is then returned at ambient temperature immediately for analysis. If batches of samples are collected and stored prior to analysis they should be refrigerated at 2-4° C. Kits can be stored for up to one month in a refrigerator before analysis. It is not necessary to freeze samples. Freezing may damage storage bottles, which can lead to leaking during transit, and also unnecessarily increases costs by requiring refrigerated transport. The length of time eDNA samples are stored in a refrigerator prior to analysis should be recorded and passed on to the analysing laboratory. Use an appropriate labelling system to ensure that the kits are supplied with a unique reference number.

4. Laboratory protocol

4.1 Introduction

This section describes the laboratory protocol for analysis of eDNA samples. It is assumed that laboratory staff are familiar with the techniques for using the proprietary products specified.

It is important that the analysing laboratory has no prior knowledge of whether sites being tested do or do not have newts. Samples should be identified only by a unique reference number which contains no site identifying information.

4.2 Analytical methods

Primers and probes

Great crested newt (*Triturus cristatus*) DNA should be amplified using the primers and probes listed in Appendix 2. They amplify a fragment of the mitochrondrial cytochrome oxidase I gene (cytb). It may be desirable for laboratories undertaking analyses to demonstrate that these primers and probes have been tested *in vitro* against real great crested newt tissue (which can be collected by external swabbing), and *in situ* from real ponds with great crested newts (unless they have already undertaken eDNA work with great crested newts). There are a number of amphibian biologists around the UK who have licenses to swab newts and they can be contracted to do this work. An alternative approach to standardisation is to purchase synthetic DNA.

Water

Water used in eDNA analysis should be ultrapure water for molecular biology grade, which can either be purchased or made in the laboratory, using proprietary equipment.

1. DNA extraction

DNA should be extracted using the DNA Blood and Tissue kit (Qiagen®) following the manufacturer's instructions.

Step 1 For each sample from a site, the six subsamples per site should be centrifuged at 14000 x g[1], for 30 minutes, at 6 °C and the supernatant discarded.

Step 2 360 µL of ATL Buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen®) is added to the first tube, the tube is vortexed for several minutes (time depends on degree of film accumulation on tubes) and the supernatant poured into the second tube. This operation is repeated for all the six tubes, resulting in the 6th tube containing the ATL buffer that has been vortexed sequentially in each of the six sample tubes. Vortexing is needed to remove films of DNA which become attached to the tubes at high centrifuge speeds. Flicking the tube or pipetting have not been found sufficiently vigorous to remove these films. Other kits may be suitable for this step but would need to be evaluated, perhaps as part of a proficiency testing process.

Step 3 The supernatant in the sixth tube, containing the DNA concentrated from all 6 sub-samples, is transferred to a 2 mL tube and the DNA extraction performed following the manufacturer's instructions. The DNA extraction should be performed in the room or laboratory area dedicated for degraded DNA samples.

Step 4 An extraction control should be performed at the same time to monitor possible

[1]The centrifugation speed suggested originally by Ficetola et al. (2008) was 5500 x g. Internal tests made by Spygen indicated that better results were found with the highest centrifugation speed, which led to the adoption of 14,000 x g for the Great Crested Newt DNA extraction.

laboratory contamination. The extraction control is undertaken using an 11th tube containing buffers alone and no sample (i.e. no alcohol mix and no pond water). Note that the quality of the alcohol (i.e. absence of DNA contamination) is assessed with the negative controls in the field. These can be either out of range sites where great created newts are definitely absent or sites within the newt's range where there is high certainty that newts are absent. If no negative field sites are available in a study, a different approach may be needed. In the analytical process the extraction control sample is, from Step 4 onwards, processed as a normal sample.

Additional control samples may be added to the process depending on where it is believed contamination may be originating.

2. qPCR

Step 5 DNA inhibition should be tested by adding a known amount of an artificial gene tothe sample and running qPCR in duplicate. If a different than expected Ct[2] value is observed in a least one replicate, the sample should be considered inhibited. In this instance dilute the sample twice before amplification with great crested newt primer and probes.

Step 6 qPCR analysis. Each sample should be run in 12 replicates. A dilution series of *T. cristatus* DNA, ranging from 10^{-1} ng μL^{-1} to 10^{-4} ng μL^{-1} (increments 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) and measured using a Nanodrop ND-1000 or equivalent, should be used as a qPCR standard. The qPCR standards are made using DNA extracted from great crested newt tissue samples, and the quantification made on extracted DNA before the dilution. Samples should be run on a BIO-RAD® CFX96 Touch real time PCR detection system or equivalent.

Note that the standards are the positive controls for qPCR in this approach (i.e. assuring that the method successfully detects DNA when present). Negative controls are provided by one extraction blank, which is run with 12 replicates, as a normal sample, and with four qPCR negative controls which also run during the qPCR step, using ultrapure water for molecular biology grade.

Step 7 The quantitative PCR is performed in a final volume of 25 µL made up from:

- 3 µL of template DNA
- 12.5 µL of TaqMan® Environmental Master Mix 2.0 (Life Technologies ®)
- 6.5 µL of ddH_2O
- 2 µL of primer (1 µL each of primer 10 µM TCCBL and TCCBR)
- 1 µL of probe (2.5 µM TCCB Probe)

Step 8 The PCR is performed under thermal cycling at 56.3 °C for 5 minutes and 95 °C for 10 minutes, followed by 55 cycles of 95 °C for 30 seconds and 52°C for one minute.

[2](C_t = C_t threshold value, the number of PCR cycles after which amplification becomes exponential)

5. Data recording and reporting

Accurate detailed records of the sites surveyed should be kept by the commissioning ecologists for reporting, reference and auditing purposes.

5.1 Sampling information

Sampling kits should be identified by a unique identifying code when provided to field ecologists. All site information should be associated with this unique number. Laboratory staff do not need further site reference information.

The commissioning ecologists should maintain records which include:

- Site name
- Nearest settlement (provides double check against grid reference errors)
- County (provides double check against grid reference errors)
- Time between receipt of sampling kit and date of sampling
- Date of sampling
- Personnel collecting sample
- Ordnance Survey grid reference, ideally to 1 m (i.e. a 12 figure grid reference)
- Site maps showing locations of sites
- Percentage of pond perimeter that is accessible for survey
- Data on inflows, and whether these were wet or dry at the time of survey
- If available, data on presence and number of great crested newt recorded during eDNA collection to help with further assessment / refinement of this technique
- Information on any difficulties experienced during sample collection.

5.2 Laboratory data

The laboratory should maintain records which include:

- Personnel involved identified
- Date of kit preparation
- Duration of storage of samples once returned from the field
- Dates of analysis
- Details on type and any degradation of the marker DNA in sample kits
- A record of any modifications to standard operation procedures of laboratory equipment.

Standard laboratory data should be maintained by the laboratory.

Information on sample inhibition should be reported with the reporting of positive or negative DNA results.

At present there is no intention to archive eDNA samples although this may become necessary in the future.

6. References

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Appendix 1. Frequently asked questions about eDNA sampling from volunteer and non-specialist surveyors

What is eDNA?
eDNA in the case of larger organisms, is DNA that is collected from the environment in which an organism lives, rather than directly from the plants or animals themselves. In aquatic environments, animals including amphibians and fish, shed cellular material into the water via their saliva, urine, faeces, skin cells etc. This DNA may persist for several weeks, and can be collected through a water sample, and analysed to determine if target species of interest have been present in the waterbody.

Why must surveyors remain out of the pond?
There is a considerable risk of contaminating your pond sample by bringing in Great Crested Newt DNA in mud and water from other areas on your boots and equipment. This is a real risk: DNA can remain on surfaces even after they have been dried, and can persist in soil for many years. There are recorded examples of eDNA cross-contaminating pond water samples from surveyor's boots.

Why are sampling points spread around the pond?
Existing data shows that eDNA can be very patchy depending on where the animals have been. By sampling in many areas you considerably increase your chance of collecting their DNA successfully.

Why is the water column mixed before sampling?
DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. However, it is important not to collect sediment because it is currently thought that DNA may persist in the sediment for substantially longer than in the water column. If you collect sediment, there is a risk your sample might show a false positive indicating great crested newts were present recently, when in fact this was a long time in the past.

Why is such a large volume of water collected?
In this methodology we collect a larger volume of water than previous methods have recommended (e.g. Thomsen et al. 2012). Our experience indicates that collecting a larger volume of water than was taken by Thomsen et al. (2012) is important to the success of the method.

Does it matter if I get things like duckweed, algae or zooplankton in my sample?
No, small amounts don't matter. However try not to collect bottom sediment in the sample, because the DNA can be absorbed by sediment and may give false positive results (see above).

What happens if I spill the preservative - or the sample tube itself
If you spill some of the preservative from one of the tubes, just add proportionally less water from your pond sample. The samples from all six tubes are later combined for the laboratory analysis, so it's not disastrous if some sample is lost.

Will samples degrade in the post?
The preservative (alcohol) in the sample bottle will slow, but not eliminate, degradation of any DNA. Keeping the samples refrigerated also slows this process. The rate of decay during posting at ambient temperatures will be faster, but it will not be sufficient to degrade the sample completely.

What evidence is there to support the use of this technique?
Defra project WC1067 has demonstrated the effectiveness of environmental DNA in the detection of Great Crested Newts. In detailed field studies eDNA detected Great Crested Newts 99.3% of the time in ponds where they were known to occur. When used by volunteers surveyors, eDNA detected Great Crested Newts at 91% of ponds where they were known to be present. No false positives were recorded from sites either outside or within the known range of the newt.

Appendix 2 Details of primers and probes

Primers are salt free and HPLC-purified.

Primer	Sequence (5'-'3)	Fragment	Gene
TCCBL	CGTAAACTACGGCTGACTAGTACGAA	81	Cyt-b
TCCBR	CCGATGTGTATGTAGATGCAAACA	81	Cyt-b
Probe			
TCCB	CATCCACGCTAACGGAGCCTCGC	81	Cyt-b

Degradation control

A length of artificial DNA is added to the samples to assess post-sampling degradation. This DNA does not have an analogue in the natural world so it can clearly be separated from all DNAs that can be sampled in the field. The structure of the molecule is commercially confidential to Spygen so is not reproduced in this guide. Laboratories may either design their own synthetic DNA or purchase material from Spygen.

Acknowledgements

We would like to thank all those who helped with Defra project WC1067 including the landowners who facilitated access to their sites, and particularly the many people and groups who volunteered time and resources to collect eDNA samples. This includes NARRS and PondNet volunteers, the team in Wales co-ordinated by Matt Ellis of Natural Resources Wales, who generated an excellent dataset for the detailed methodological component of the project, and Tom Langton who not only collected many eDNA samples, but provided access to a dataset from Suffolk extending over 20 years which has helped us to better interpret the relationship between eDNA and Great Crested Newt abundance. The project lead was Natasha Chick (Defra) and the Steering Group was Matt Ashton (Defra), Pete Brotherton (Natural England), Paul Edgar (Natural England), John McKinnel (Scottish Natural Heritage), Katharine Woods (Natural England) and Anna Robinson (JNCC). Our thanks also to Barbara Zweifel for delivering eDNA samples to France for analysis.

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Appendix 5. HSI Advice Note 5

Amphibian and Reptile Groups of the United Kingdom
ARG UK Advice Note 5
www.arguk.org

Great Crested Newt Habitat Suitability Index

May 2010

Background

The Habitat Suitability Index (HSI) for the great crested newt was developed by Oldham *et al.* (2000). HSI scoring systems were originally developed by the US Fish and Wildlife Service as a means of evaluating habitat quality and quantity. An HSI is a numerical index, between 0 and 1. Values close to 0 indicate unsuitable habitat, 1 represents optimal habitat. The HSI for the great crested newt incorporates ten suitability indices, all of which are factors known to affect this species. These ten suitability indices are retained in this current Advice Note.

In the HSI system proposed by Oldham *et al.* (2000) one of the suitability indices (SI_9, terrestrial) involves more lengthy measurement and calculation than the others. In using the HSI system with volunteer surveyors in Kent, Lee Brady has substituted a simpler evaluation of terrestrial habitat quality (a four-point scale), for ease of use.

Several other, local, surveys have utilised the HSI, but incorporating their own variations on the original system. In 2007 a workshop was held at the Herpetofauna Workers' Meeting to evaluate the use of the HSI for the great crested newt, with the aims of:

- Identifying components of the system that may need clarification or refinement
- Agreeing on a standard that can readily be used by volunteers and professionals alike.

The outputs of the workshop and subsequent consultation have been used to formulate the current Advice Note. As far as possible a conservative approach has been adopted in modifying the use of the original HSI suitability indices. However, a major departure is the adoption of Lee Brady's four-point evaluation of terrestrial habitat. This differs from the original HSI in that it has been developed with respect to newt presence/absence at a pond, rather than estimating population size.

Use and limitations of the HSI

The HSI for great crested newts is a measure of habitat suitability. **It is not a substitute for newt surveys**. In general, ponds with high HSI scores are more likely to support great crested newts than those with low scores. However, the system is not sufficiently precise to conclude that any particular pond with a high score will support newts, or that any pond with a low score will not do so.

There is a positive correlation between HSI scores and the numbers of great crested newts observed. In general, high HSI scores are likely to be associated with greater numbers of great crested newts. The relationship is not sufficiently strong, however, to allow estimations of the numbers of newts in any particular pond.

HSI scoring can be useful in:

- Evaluating the general suitability of a pond, or ponds, for great crested newts
- Comparing general suitability of ponds across different areas
- Evaluating the suitability of receptor ponds in a proposed mitigation scheme
- Identifying habitat management priorities.

How to collect data and calculate the HSI

The HSI is a geometric mean of ten suitability indices:

$$HSI = (SI_1 \times SI_2 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10})^{1/10}$$

- Ten factors are scored for a pond, in the field and from map work (field scores).
- The ten field scores are converted to SI scores, on a scale from 0.01 to 1 (0.01 is used as the lower end of the scale in stead of 0, because multiplying by 0 reduces all other SI scores to 0).
- The ten SI scores are multiplied together.
- The tenth root of this number is calculated $(x)^{1/10}$ i.e. x to the power of 0.1.

The calculated HSI for a pond should score between 1 and close to 0 (the calculations above do not allow the HSI to be exactly 0).

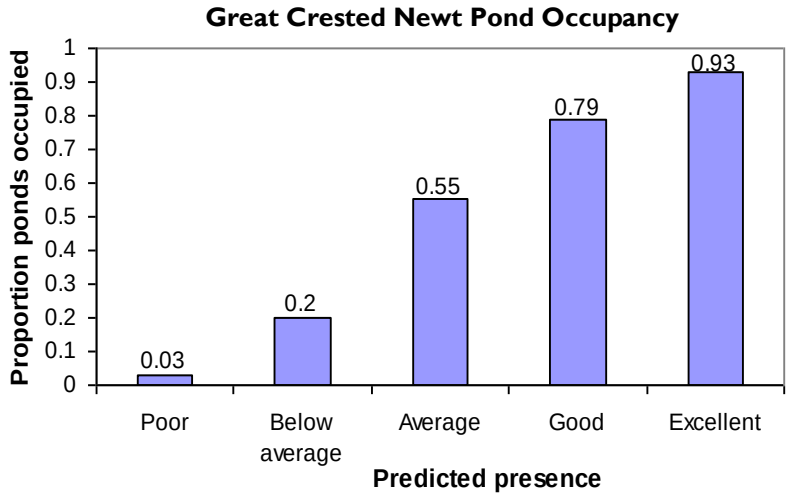
Some of the field scores are categorical, some are numerical. The numerical field scores are converted to SI scores by reading off the values from graphs produced by Oldham *et al.* (2000) reproduced in this Advice Note.

Full details of the scoring system, including descriptions of the criteria used in the categorical scores are given in *Details of suitability indices and definitions of categories* (below). Scores for two of the factors (SI_1 and SI_8) can be gained as desktop/map exercises and so do not have to be completed in the field. The remaining factors should be recorded as field scores, and later converted to suitability indices, in some cases reading SI scores from the graphs provided. A summary of data to collect is given in the appendix *Summary of scoring system*.

Categorisation of HSI scores

Lee Brady has developed a system for using HSI scores to define pond suitability for great crested newts on a categorical scale:

HSI		Pond suitability
< 0.5	=	poor
0.5-0.59	=	below average
0.6-0.69	=	average
0.7-0.79	=	good
> 0.8	=	excellent



The graph shows occupancy of ponds by great crested newts in south-east England. 248 ponds were surveyed on three to six occasions, using egg-searching, torching and bottle-trapping. As pond suitability increases from 'poor' to 'excellent', so does the proportion of ponds occupied by great crested newts.

Details of suitability indices and definitions of categories

Factor 1. Geographic location (SI_1)

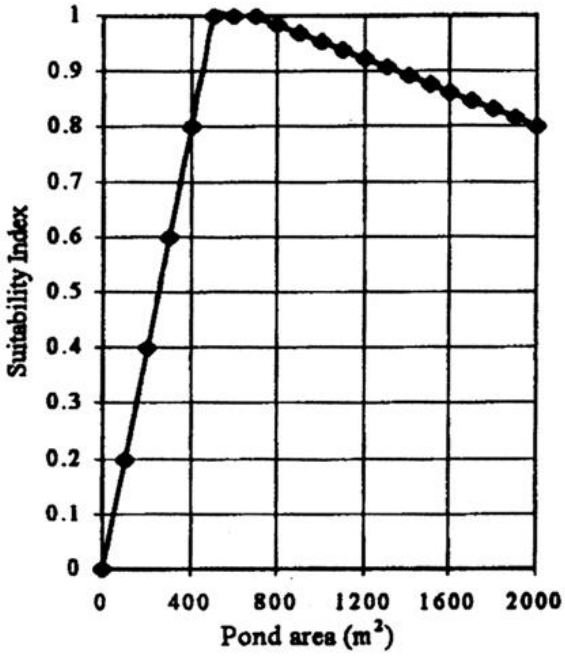
Sites should be scored according to the zone in which they occur. This scoring can be carried out either in the field, or as part of a desktop exercise.

Zone A, location is optimal, SI = 1
Zone B, location is marginal, SI = 0.5
Zone C, location is unsuitable, SI = 0.01.

Some sites will fall on boundary lines between zones. In such cases, select medium-value scores i.e. Zone B.

Factor 2. Pond area

Pond area is the surface area of the pond when water is at its highest level (excluding flooding events). This is usually in the spring. If the pond is being measured at another time of year, the spring time area should still be evident from vegetation types and evidence of a draw down zone around the pond.



Pond area should be measured as accurately as possible. There are several ways of doing this, for example by measuring axes of regularly shaped ponds, either by pacing out in the field, or taking measurements from a map. Irregularly shaped ponds may have to be treated as a series of geometric shapes, calculating the area for each and adding together.

Since it can be difficult reading off SI scores from the graph, pond area should be rounded to the nearest 50 m^2.

It can be particularly difficult to read off SI scores for very small ponds. For ponds smaller than 50 m^2 use a score of 0.05.

For ponds larger than 2000 m^2 omit this factor from the HSI calculation (as there are no data for such large ponds). i.e. $HSI = (SI_1 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10})^{1/9}$.

Factor 3. Permanence

Pond permanence should be deduced from local knowledge and personal judgement. A landowner may know how often a pond dries. However, if not, the surveyor should make a judgement based on water level at the time of the survey, and taking seasonality into consideration. For example, a pond that is already dry by late spring is likely to dry out every year, etc.

Category	SI	Criteria
Never dries	0.9	Never dries.
Rarely dries	1.0	Dries no more than two years in ten or only in drought.
Sometimes dries	0.5	Dries between three years in ten to most years.
Dries annually	0.1	Dries annually.

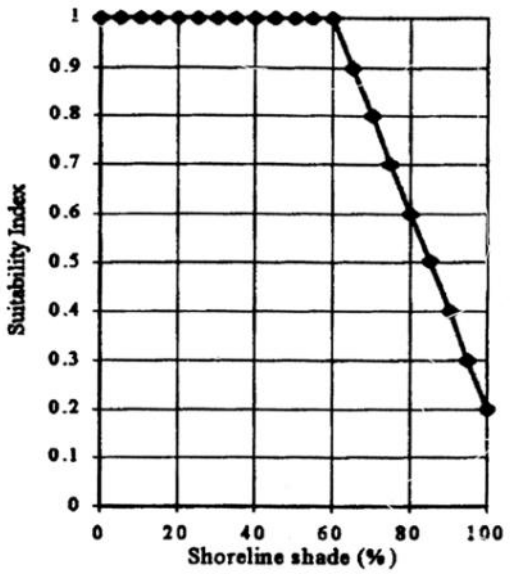
Factor 4. Water quality

The assessment of water quality is subjective and should be based on invertebrate diversity, the presence of submerged water plants and knowledge of the water sources feeding the pond. Water quality should not be confused with water clarity. Sometimes clear water can be devoid of invertebrates, and turbid ponds can support a wealth of invertebrates. There is no quick and simple invertebrate index of water quality. However, some species are indicators of water quality.

Category	SI	Criteria
Good	1.0	Water supports an abundant and diverse invertebrate community. Netting reveals handfuls of diverse invertebrates, including groups such as mayfly larvae and water shrimps.
Moderate	0.67	Moderate invertebrate diversity
Poor	0.33	Low invertebrate diversity (e.g. species such as midge and mosquito larvae). Few submerged plants.
Bad	0.01	Clearly polluted, only pollution-tolerant invertebrates (such as rat-tailed maggots), no submerged plants.

Other cues may also provide information about water quality. For example, ponds subject to agricultural inputs are likely to have poor water quality.

Factor 5. Shade



Estimate percentage pond perimeter shaded, to at least 1m from the shore. Shading is usually from trees, but can include buildings. Shading should not include emergent pond vegetation. The estimate should be made during the period from May to the end of September.

Factor 6. Waterfowl

This factor is concerned with the impact of waterfowl upon the pond and newts. At high densities, as created when waterfowl are encouraged to use a pond by provision of food, the birds can remove all aquatic vegetation, pollute water and persistently stir sediments. Some waterfowl may also actively hunt adult newts and their larvae. Score as one of three categories.

Category	SI	Criteria
Absent	1	No evidence of waterfowl impact (moorhens may be present).
Minor	0.67	Waterfowl present, but little indication of impact on pond vegetation. Pond still supports submerged plants and banks are not denuded of vegetation.
Major	0.01	Severe impact of waterfowl. Little or no evidence of submerged plants, water turbid, pond banks showing patches where vegetation removed, evidence of provisioning waterfowl.

'Waterfowl' includes most water birds, such as ducks, geese and swans. Moorhens should be excluded because almost every pond has at least one or two.

Factor 7. Fish

Information on fish should be gleaned from local knowledge and the surveyor's own observations. Pond owners will usually be aware of stocking with fish for commercial or aesthetic reasons. However, stickleback (which can be significant predators of great crested newt larvae, when present in large numbers) are unlikely to be deliberately introduced to a pond, but may arrive through other means. Netting is useful in detecting smaller fish, such as sticklebacks, or the fry of larger species.

Category	SI	Criteria
Absent	1	No records of fish stocking and no fish revealed by netting or observed by torchlight.
Possible	0.67	No evidence of fish, but local conditions suggest that they may be present.
Minor	0.33	Small numbers of crucian carp, goldfish or stickleback known to be present.
Major	0.01	Dense populations of fish known to be present.

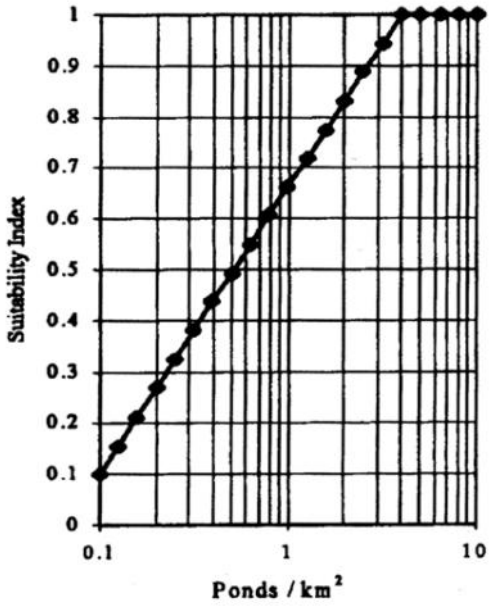
Factor 8. Pond count

This is the number of ponds occurring within 1 km of survey pond. Do not count the survey pond itself. Ponds on the far side of major barriers, such as main roads, should not be counted. Use 1:25,000 scale O.S. data, such as Explorer maps, GIS or web-based mapping sources, such as:

Getamap	www.ordnancesurvey.co.uk/oswebsite/getamap/
Magic	www.magic.gov.uk/site_map.html
Digimap	edina.ac.uk/digimap/

Pond counts can be carried out a by a survey coordinator and so do not necessarily have to be performed by surveyors.

Divide the number of ponds by π (3.14) to calculate the density of ponds per km^2 and read off the SI value from graph.



Factor 9. Terrestrial habitat

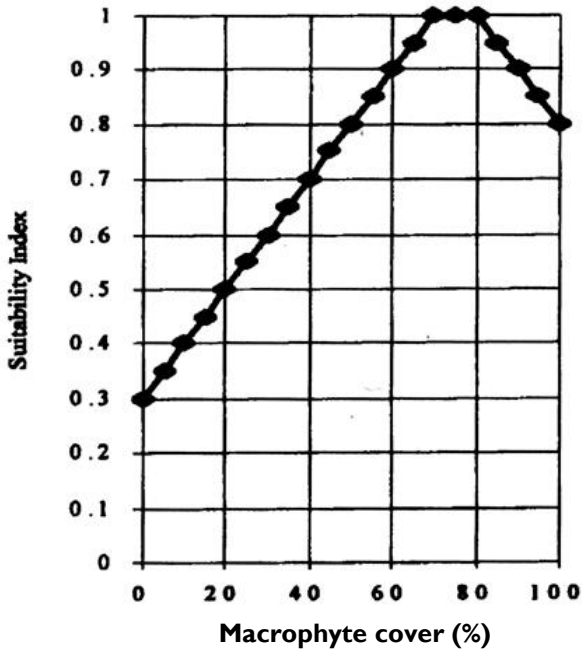
Scoring terrestrial habitat depends on the surveyor's understanding of newt habitat quality. Good terrestrial habitat offers cover and foraging opportunities and includes meadow, rough grassland with tall sward height, scrub, woodland or mature gardens. Terrestrial habitat should be considered within approximately 250 m from the pond, but only on the near side of any major barriers to dispersal (e.g. main roads or large expanses of bare habitat).

Category	SI	Criteria
Good	1	Habitat that offers good opportunities for foraging and shelter (e.g. most semi-natural environments, such as rough grassland, scrub or woodland, also brownfield sites and low intensity farmland) covers more than 75% of available area.
Moderate	0.67	Habitat offers opportunities for foraging and shelter but may not be extensive (25-75%) of available area.
Poor	0.33	Habitat with poor structure (e.g. amenity grassland, improved pasture and arable) that offers limited opportunities (less than 25% of available area) for foraging and shelter.
None	0.01	No suitable habitat around pond (e.g. centre of arable field or large expanse of bare habitat).

Great crested newts do not have specific terrestrial habitat requirements. However, good quality terrestrial habitat has structure. The presence of hedges, ditches, stone walls, old farm buildings, piles of loose stone or rock, rabbit burrows and small mammal holes all contribute towards 'good' terrestrial habitat. Note that it is rare to encounter a pond falling within the terrestrial habitat category of 'none'.

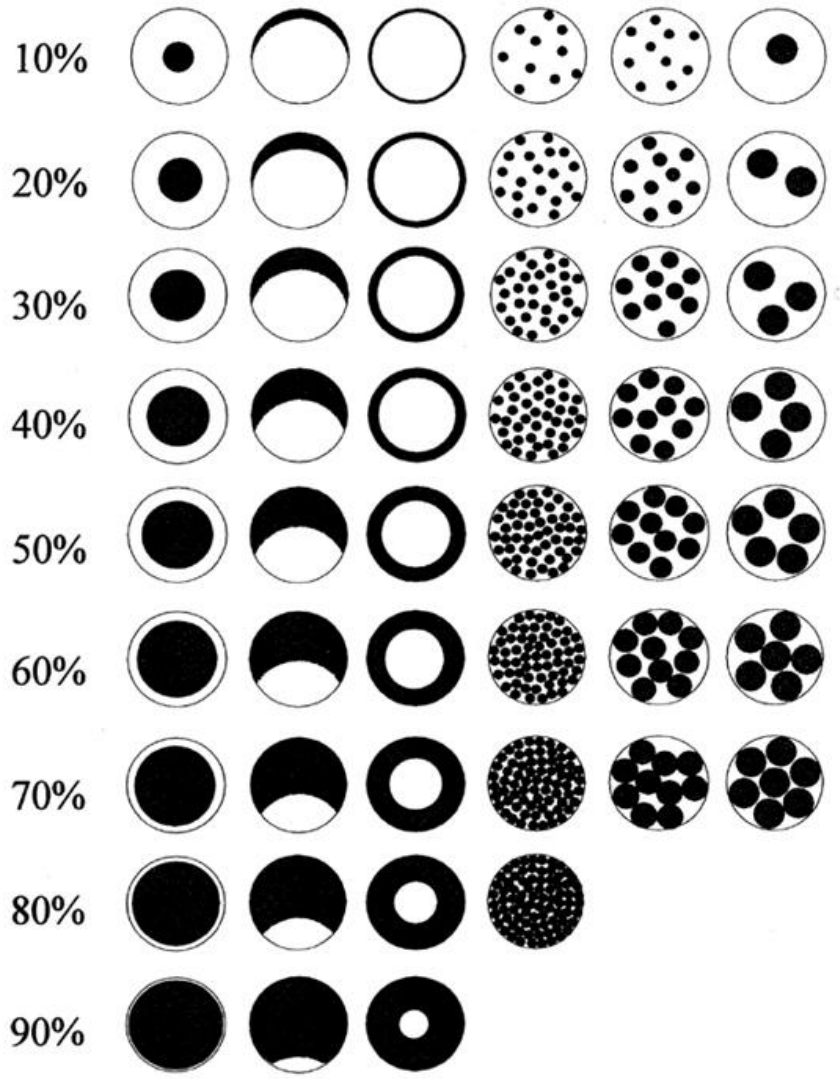
Factor 10. Macrophytes

Estimate the percentage of the pond surface area occupied by macrophyte cover. This includes emergents, floating plants (excluding duckweed) and submerged plants reaching the surface. Make an estimate between March and the end of September. Read off the SI value from graph.



Guide for assessment of macrophyte cover in a pond

The areas of dark shading simulate a variety of vegetation dispersion patterns.



Reference

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Publication date: May 2010.

ARG UK is the network of volunteer conservation groups concerned with the native amphibians and reptiles of the UK.

Summary of scoring system

SI_1 Location

Field score	SI
A (optimal)	1
B (marginal)	0.5
C (unsuitable)	0.01

SI_2 Pond area

Field score	SI
Measure pond surface area (m^2) and round to nearest 50 m^2	Read off graph.

SI_3 Pond drying

Field score	SI	Criteria
Never	0.9	Never dries
Rarely	1.0	Dries no more than two years in ten or only in drought.
Sometimes	0.5	Dries between three years in ten to most years
Annually	0.1	Dries annually

SI_4 Water quality

Field score	SI	Criteria
Good	1.0	Abundant and diverse invertebrate community.
Moderate	0.67	Moderate invertebrate diversity
Poor	0.33	Low invertebrate diversity, few submerged plants
Bad	0.01	Clearly polluted, only pollution-tolerant invertebrates, no submerged plants.

SI_5 Shade

Field score	SI
Estimate percentage perimeter shaded to a least 1 m from shore.	Read off graph.

SI_6 Fowl

Field score	SI	Criteria
Absent	1	No evidence of water fowl (although moorhen may be present)
Minor	0.67	Waterfowl present, but little sign of impacts
Major	0.01	Severe impact of waterfowl

SI_7 Fish

Category	SI	Criteria
Absent	1	No records of fish stocking and no fish revealed during survey.
Possible	0.67	No evidence of fish, but local conditions suggest that they may be present.
Minor	0.33	Small numbers of crucian carp, goldfish or stickleback known to be present.
Major	0.01	Dense populations of fish known to be present.

SI_8 Pond count

Field score	SI
Count the number of ponds within 1 km of the survey pond (not separated by major barriers) and divide by 3.14. This can be done from maps rather than in the field.	Read off graph.

SI_9 Terrestrial habitat

Category	SI
Good	1
Moderate	0.67
Poor	0.33
None	0.01

SI_{10} Macrophytes

Field score	SI
Estimate the percentage of the pond surface area occupied by macrophyte cover (between May and the end of September)	Read off graph.

Appendix 6. Biosecurity Advice Note 4

ARG UK
amphibian and reptile conservation
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Amphibian and Reptile Groups of the United Kingdom

www.arc-trust.org **Advice Note 4** www.arguk.org

Amphibian Disease Precautions: A Guide for UK Fieldworkers

Version 2, revised March 2017

Background

Amphibians are one of the most rapidly declining groups of animals globally, and infectious disease is a major cause of these declines in some areas. Fieldworkers have a key role to play in combating this; they can help detect disease, and through good practice they can reduce the risk of introducing and spreading disease.

This note advises field workers and others who may come into contact with amphibians (through ecological survey and monitoring, training, research or education activities) on simple procedures to substantially reduce the risk of introducing and spreading amphibian pathogens. The advice is based on the latest available evidence relating to fungal and viral pathogens, but this should also be effective for many other types of pathogen and may also help reduce the spread of invasive animals and plants.

Three key findings from research underpin the advice in this note: (1) amphibian diseases are frequently found to be spread by human activity, and amphibian fieldworkers therefore have a particular responsibility; (2) amphibian disease emergence is commonly associated with the introduction of non-native species; and (3) signs of infection are not necessarily evident on visual inspection.

Given the alarming situation regarding amphibian diseases overseas, and the poor understanding of disease impacts in the UK, this advice note advocates a precautionary approach to minimise the chance of introducing and spreading amphibian pathogens. Controlling disease outbreaks in the wild is practically impossible except under very particular circumstances, and so prevention is the best strategy. Alongside prevention, this note highlights the importance of reporting signs of infection, so that we can learn more about diseases and their effects and develop an early warning system for detection of novel threats.

Growing numbers of amphibian diseases have been described in recent years. The most notable of these are chytridiomycosis and ranavirus. Chytridiomycosis is caused by two species of microscopic fungi *Batrachochytrium dendrobatidis* ("Bd") and *B. salamandrivorans* ("Bsal"), which are often simply referred to as "chytrid". Chytrid infection has been responsible for mass mortalities of amphibians with declines and extinctions in some species on six continents, including Europe. Ranavirus (ranaviral disease), caused by infection with one of many types of ranavirus, also has a wide global distribution, though it appears to cause population declines less frequently than chytridiomycosis. Other infectious agents, about which much less is known, include herpesvirus and the parasitic infections *Amphibiocystidium* and *Ribeiroia*. It is likely that additional, currently undescribed diseases will emerge in the future.

In the UK, a number of pathogens and infected amphibians have been detected, yet the implications for conservation remain largely unclear. Perhaps the most obvious concern relates to ranavirus. This is now commonly reported from parts of England, and infection can lead to local population declines of 80% in common frogs. Whilst Bd is now known to be widespread in Great Britain, it does not appear to have caused the types of mass die-offs reported overseas. However, the lack of evidence for mass mortalities should not lead to complacency; such events can be difficult to detect even when they are occurring, and in any case it can take many years for the full effects of disease introduction to manifest. Whilst Bsal is known to be present in captive amphibians in the UK, it has not yet been detected in the wild. However, it is believed to be a major potential

threat to newt health should it become established, particularly the great crested newt which is known to be highly susceptible.

General guidance is given here, followed by recommendations for specific activities. The advice may be revised in future in the light of further research findings. Presently there is no evidence to suggest that amphibian diseases found in the UK present a hazard to human health.

General guidance

- Handle amphibians only when necessary.
- If handling amphibians, or if contact with pond water is necessary, wear powder-free disposable vinyl gloves which you rinse before contact with the amphibians. Use vinyl rather than latex or nitrile because the latter two may be harmful to amphibians.
- Use a fresh pair of gloves for each site[1] visited. For higher risk activities, it may be appropriate to change gloves between handling individual amphibians, even within the same site (see below).
- Disinfect survey equipment or containers used to hold amphibians between each site[1] visited (see disinfection procedures below).
- If entering the water, footwear should be washed and disinfected (see disinfection procedures below) immediately after the site visit. If you do not enter the water, there is no need to disinfect footwear unless visiting a high risk site (see guidance on specific activities below).
- Wash all clothing on a 40°C cycle with biological detergent, after exposure to amphibians or pond water. If visiting several sites bring a change of clothes. Use a lower temperature wash if care instructions indicate this may be harmful to your clothing, and take additional precautions such as washing twice or spraying with disinfectant before next use.
- Do not release amphibians anywhere except at the place of capture.
- If travelling by vehicle, park on hard standing (rather than vegetated areas) and walk to the pond.
- Treat dead or sick amphibians as a high infection risk and do not handle unless necessary.

Guidance on specific activities

In addition to the general guidance given above, the following additional precautions are recommended for specific activities, which may carry a higher risk of introducing or spreading disease:

Activity	Additional precautions
Monitoring an amphibian population.	At a site where amphibians are monitored from one year to the next: • Ensure that all surveyors are aware of disease issues and precautions. • Use survey equipment and footwear dedicated solely to the target site. • Store field equipment on site where possible. • Some sites may already have risk assessments in place so it is important to check for these before commencing field work.
Amphibian survey work at several sites. This is most common among ecological consultants surveying sites to inform the planning process.	• Ensure that all field workers are aware of disease issues and precautions. • Disinfect field equipment between sites. • Consider allocating a set of field equipment and footwear to each site within a season, rather than using the same equipment at different sites. • Consider having two sets of field gear, so that one can be in the disinfection and drying process while the other is in use.

[1] Ponds within 1km of each other and not separated by major barriers to dispersal can be considered as a single site. This working guide is equivalent to the distance over which amphibians may disperse and spread pathogens in a single generation.

Translocating (moving) amphibians. This should be avoided where possible, but occasionally it is desirable for conservation, research or mitigation purposes.	In general translocation of amphibians should be avoided. It may be acceptable if: • There is a strong case for the benefits of the translocation; and • There is no satisfactory alternative; and • Rigorous efforts to analyse and minimise disease risks are taken and independently assessed, and any residual risk is outweighed by the benefit of translocation.
Training courses or educational dip-netting for amphibians.	Training and educational work is important and disease risks should not prevent these activities occurring if simple precautionary measures are taken. The following points should be observed: • For training courses, ensure that all participants are aware of disease issues and precautions. • Ensure that all participants thoroughly scrub and disinfect footwear before going on site. • Use equipment (nets, trays and tanks) dedicated to the site only. • Where training or educational work is undertaken at several sites within a season, and the equipment cannot be assigned to a specific site, ensure all equipment is thoroughly disinfected and allowed to dry between sites. • Disposable gloves should be worn by the instructor. • Allow trainees to view amphibians but try to minimise handling as far as possible (realistically, it is impossible to prevent all handling). • Ensure that all participants wash hands thoroughly with soap or hand sanitising gel and water after the visit.[2] Use the event to discourage movements of all life stages of amphibians including spawn, whilst still giving positive messages (e.g. the value of garden ponds).
Fieldwork at infected sites.	Restrict fieldwork to essential activities only (e.g. research to track the progress of infection or to assess amphibian population status). • Use field equipment dedicated to the target site only. • Store field equipment on site where possible. • Keep the number of survey visits to the minimum necessary. • Minimise the number of field workers, and visit no more than one site per day. • Disinfect equipment between individual ponds within the site. • Use a fresh pair of gloves for each amphibian handled (or if that is not feasible, at least every 2-5 individuals), to minimise the chance of amplifying infection levels.
Fieldwork at other high risk sites. These include sites near to an area where disease has been detected or where non-native amphibian species are present.	• Use field equipment dedicated to the target site only. • Store field equipment on site where possible. • Keep the number of survey visits to the minimum necessary. • Minimise the number of field workers, and visit no more than one site per day.

[2] This is also good practice to prevent infection with disease agents that are pathogenic to humans including Weil's disease and tetanus

Fieldwork by persons who keep non-native amphibian species in captivity.	• Implement rigorous barrier methods (gloves, minimal handling, disinfection, change of clothes and footwear, etc.) to minimise the risk of transmitting pathogens from captive stock to wild sites. • Regularly screen captive stock to detect infection. • Do not bring native amphibians into captivity, or release animals that have been in captivity back into the field. • Consider curtailing fieldwork that involves handling amphibians at sites supporting important native populations. • Do not use any equipment in the field that has been previously used in captive facilities, even if it has been cleaned. • If the above points are not feasible, then such persons should consider refraining from undertaking fieldwork at amphibian sites.

Disinfection procedures

Disinfect all field equipment that has come into contact with amphibians or pond water. This includes footwear (boots or waders), pond nets and aquatic trapping equipment such as bottle-traps and canes or the box section of Dewsbury traps. Note: water may be drawn up into canes, and so we recommend that the whole cane is soaked in a disinfectant solution.

To disinfect equipment in the field the following will be required:

- a bucket or washing up bowl
- a brush
- disinfectant (bleach[3] or Virkon[4])
- disposable or washing up gloves (to wear while disinfecting)
- a source, or container, of clean water
- bin bags for waste and wrapping field equipment.

Disinfection solutions

Bleach (diluted with water to produce a 4% solution).
Virkon (10mg/ml, as per supplier's instructions).

Virkon-S (licensed for veterinary/animal livestock applications but not human use) can also be purchased in tablet form. One 5mg tablet can be dissolved in 500ml of water.

Pond water can be used to make up solutions so long as it contains little or no organic matter (as this reduces disinfectant efficacy).

1. Use brush to scrub off any debris, plant fragments, mud etc.
2. Rinse with water (pond water will suffice).
3. Soak in bleach solution for at least 5 minutes, or Virkon for at least one minute (5 minutes where Bsal is suspected).
4. Rinse with clean water.
5. If possible, allow to dry for before next use.
6. Keep field equipment inside plastic bags during transit and storage (after thorough drying) to reduce the chance of transmitting pathogens.

Dispose of disinfectant solutions following the supplier's instructions. Unless otherwise stated, it is recommended that used disinfectant solutions should be poured directly into a drain connected to the sewerage system[5] and flushed with clean water (note that not all drains are connected to sewerage systems), or disposed of as hazardous waste. Used gloves should be disposed of as domestic rubbish. Fabrics including clothes worn while

[3] Sodium hypochlorite is the active ingredient in household bleach, and concentrations vary between brands typically from 8-15%. It is important you check the concentration of the brand you are using, and adjust your dilution rate to arrive at 4%.

[4] Virkon is a disinfectant sold as a powder or in tablet form and used in large animal husbandry (readily available online and at farm supply stores or outlets aimed at horse owners).

[5] Surface water drains, including road and car park drains, often discharge into rivers or the sea without treatment, so only dispose of disinfectant in this way if you are sure the drain is connected to the foul sewer. The sewerage undertaker – usually the water company – maintains a map of public sewers.

doing amphibian fieldwork can be washed on a 40°C cycle with biological detergent (see comments above regarding clothing requiring colder washes).

Investigating dead amphibians

The *Garden Wildlife Health* project has been established to investigate disease in a range of native wildlife species in Great Britain, and in some cases the project vets, based at the Wildlife Epidemiology Unit at the Institute of Zoology, may be able to carry out post mortem examinations on dead amphibians.

Reports of sick and dead amphibians are valuable to help gather information about the diseases that affect native species and to monitor their impact. Photographs of affected animals and the site in which they are found can be very helpful, along with information on the potential disease symptoms observed. Reports of amphibian ill health from all sites are welcome, and are not limited to gardens. Please report sightings via the project web portal www.gardenwildlifehealth.org.

When freshly dead amphibian carcasses in a good state of preservation are available, it may be possible to test them for disease. Where appropriate, arrangements for submission are made with the GWH veterinary surgeons and all costs covered by the project. If you are concerned about a sick or injured amphibian, please contact your local veterinary surgeon, or experienced wildlife rescue centre for guidance. Disease factsheets on the common conditions affecting British amphibians are available at www.gardenwildlifehealth.org.

Further information

Amphibian Ark www.amphibianark.org/the-crisis/chytrid-fungus.

Amphibian and Reptile Conservation, www.arc-trust.org/habitat-management-handbooks

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