H2Teesside Project

Environmental Statement

Volume III – Appendices

Appendix 12B: Great Crested Newt Survey Report

Document Reference: 6.4.19

The Infrastructure Planning (Environmental Impact Assessment) Regulations 2017 (as amended)

The Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009 - Regulation 5(2)(a) and 5(2)(l)

Rev: 0

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TABLES

PLATES

12B.0 GREAT CRESTED NEWT SURVEY REPORT

12B.1 Introduction

Background

12B.1.1 This report details the approach and findings of the Great Crested Newt (*Triturus cristatus*) (GCN) surveys undertaken on land within and up to 500 m from the Proposed Development Site. This report has been prepared by AECOM on behalf of H2Teesside Ltd (hereafter referred to as the Applicant). Baseline data presented in this Appendix has been used to inform the assessment within Chapter 12: Ecology and Nature Conservation (ES Volume I, EN070009/APP/6.2).

12B.1.2 The aim of the Proposed Development is to deliver up to 30% of the UK's 2030 target for hydrogen production as a part of the current clean energy goals.

12B.1.3 This technical appendix is supported by the following figures (Annex 1):

- Figure 12-B-1: the Proposed Development Site;
- Figure 12-B-2: Waterbodies and watercourses and positive eDNA results; and
- Figure 12-B-3: Desk Study Results.

12B.1.4 This report refers to the relevant wildlife legislation and planning policy, summarised in Annex 2 and is consistent with the requirements of British Standard 42020:2013 Biodiversity Code of Practice for Planning and Development.

The Proposed Development

12B.1.5 The Applicant proposes the construction, operation (including maintenance where relevant) and decommissioning of an approximately 1.2-Gigawatt Thermal (GWth) Lower Heating Value (LHV) Carbon, Capture and Storage (CCS) enabled Hydrogen Production Facility (the 'Production Facility' hereby referred to as 'The Main Site') located in Teesside, along with the pipeline infrastructure required to supply hydrogen (H_2) to offtakers (customers) and the necessary utility connections. A description of the Proposed Development and Terms of Reference are provided in Chapter 4 (ES Volume I, EN070009/APP/6.2).

12B.5.1 The Proposed Development's purpose is the conversion of methane, from the North Sea storage sites, into hydrogen (with carbon dioxide byproduct captured and stored), for the increased provision of hydrogen supplies as the UK aims to expand its hydrogen energy targets and decarbonise heavy industry and transport.

Scope of the Report

12B.1.6 The aims of the survey work undertaken, and the subsequent report presented are to:

- Outline the legislation, planning policy and guidance relevant to GCN;
- Determine suitability of habitats within the Proposed Development Site to support GCN; and

- Report on the presence / likely absence of GCN within 250 m of the Proposed Development Site and 500 m of the Main Site.

12B.2 Method

12B.2.1 An evaluation of the potential constraints associated with GCN, incorporating both desk-based and field-based data collection, was conducted in accordance with established guidelines for best practices (Natural England, 2022; Froglife, 2001; and Gent and Gibson, 1998). A compilation of desk study records took place in August 2022, followed by field surveys undertaken in June 2023 with environmental DNA (eDNA) analysis completed by the end of June (ADAS, 2023).

Desk Study

12B.2.2 A desk study was completed as part of the Extended Phase 1 Habitat Survey to identify records of protected or notable species (including GCN) within 2 km of the Proposed Development Site. The sources accessed during the desk study are listed in Table 12B-1 below.

Table 12B-1: Desk Study Data Requests

DATA SOURCE	DATE ACCESSED	RECORDS REQUESTED
Environmental Records Information Centre for the Northeast (ERIC NE)	August 2022	GCN records within 2 km of the Proposed Development within the last decade
Multi-Agency Geographic Information for the Countryside (MAGIC) website	November/December 2022	European Protected Species Licences for GCN within 2 km of the Proposed Development
Industry Nature Conservation Association (INCA)	July 2022	Records of notable species including GCN within 2 km of the Proposed Development
Net Zero Teesside (NZT) GCN report (bp, 2021)	December 2022	Reviewed for relevant GCN data and findings

Extended Phase 1 Habitat Survey (and screening of waterbodies)

12B.2.3 Waterbodies within 500 m of the Main Site and 250 m of the Connection Corridors were identified through a review of Ordnance Survey (OS) maps and aerial photography. Habitats within the Proposed Development Site were appraised for their suitability to support GCN during the Extended Phase 1 Habitat Survey, and additional survey visits were completed where waterbodies were present within the 250 or 500 m buffers.

12B.2.4 The habitat suitability assessment was guided by the Herpetofauna Worker's Manual (Gent and Gibson, 2003) and the Great Crested Newt Conservation Handbook (Langton, Beckett and Foster, 2001). Habitat Suitability Index (HSI) scores were calculated for accessible waterbodies within 250 m of the Proposed

Development Site and 500 m of the Main Site, using the methodology outlined by Oldham et al. (2000).

12B.2.5 Extended Phase 1 Habitat Surveys initially covered a larger area than the current Proposed Development boundary, identifying potential waterbodies indicated by numbering in Annex 1, Figure 12-B-2. Breaks in numbering occur where waterbodies are no longer relevant to the Proposed Development Site and have been removed from the assessment.

12B.2.6 OS Mastermap data supplemented the Extended Phase 1 Habitat Surveys to identify waterbodies within 250 m of the Proposed Development Site and 500 m of the Main Site. This distance aligns with GCN movement limits for 250 m (Langton et al, 2001), extended to 500 m for the Main Site due to permanent land take (Natural England, 2020).

12B.2.7 GCN, preferring freshwater habitats, face challenges in brackish waters. Beebee and Griffiths (2000) and Sparreboom *et al*. (2008) emphasise GCN freshwater preference, crucial for breeding and Laravel development.

12B.2.8 Waterbodies were excluded based on salinity, confirmed by aquatic ecology surveys (refer to Appendix 12G: Aquatic Ecology) and OS Mastermap data indicating connections to tidal areas such as Greatham Creek or the River Tees Estuary. Aquatic ecology surveys identified high salinity levels (>1500 SPC (specific conductance), within the following watercourses and connected waterbodies making them unsuitable for GCN:

- The Fleet at NGR NZ 57347 24488;
- Knitting Wife Beck at NGR NZ 54876 22708;
- A ditch on Phillips Tank Farm LWS at NGR NZ 51182 26912;
- A ditch on the Brine fields at NGR NZ 49376 24076;
- Holme Fleet at NGR NZ 49387 23931;
- A ditch on Greenabella Marsh LWS at NGR NZ 51453 25943;
- The Tees Estuary at NGR NZ 51976 25996;
- A ditch on the Brine fields at NGR NZ 51111 24820; and
- A ditch on Greenabella Marsh LWS at NGR NZ 51531 26137.

12B.2.9 Specific conductance is a measure of the ability of water to pass an electrical current, which increases with the amount of dissolved iconic solids (i.e., salts) that the water contains, and therefore provides a measure of total dissolved solids.

12B.2.10 To refine the assessment of screening, additional efforts were given to narrowing down the number of waterbodies requiring further consideration to GCN. This process involved the identification and exclusion of waterbodies based on specific criteria:

- Waterbody no longer present: no waterbody presence through analysis of aerial imagery in Google Earth, spanning the time series from 2018 to 2023.

Additionally, temporary features, such as those retaining water after winter rainfall but drying up in spring and summer, were discounted;

- Site Inspections: waterbodies lacking conditions suitable for GCN (for example man-made settlement lagoons), as determined through on-site inspections, were discounted from further survey;
- Running Water: waterbodies identified as running water, including rivers, streams and drains with evident flow, were excluded from assessment, as they are not typical habitats for GCN;
- Brackish Water Connectivity: waterbodies displaying evident connectivity to brackish waters were also excluded, acknowledging the unsuitability of such habitats for GCN; and
- Other grounds: additional scoping criteria.

12B.2.11 The following 'other grounds' were considered a reasonable basis for scoping out potentially suitable waterbodies from further consideration:

- Waterbodies isolated by major barriers (roads, industrial development) and standalone artificial waterbodies (e.g., chemical works tanks surrounded by hard standing) were excluded due to a lack of habitat connectivity with the Proposed Development Site;
- Waterbodies within 250 m of the Proposed Development Site, where existing infrastructure will be used, were excluded. The utilisation of current roads and pipe racking infrastructure eliminates the need for significant construction or disturbances to potentially suitable semi-natural habitats for GCN; and
- Waterbodies linked to chemical works and gas processing infrastructure, like concrete tanks, are excluded due to ongoing operational use, minimising the likelihood of GCN presence. These structures have unstable water levels and suboptimal water quality.

12B.2.12 A summary table (Table 12-B-6) of the screening evidence for all potential waterbodies, and the data sources used to inform the scoping for suitability for GCN is provided in Annex 4.

Field Surveys

Habitat Suitability Index Assessment for Great Crested Newts

12B.2.13 The assessment of habitat suitability for GCN within the Study Area involved the application of the HSI. This method aims to quantitatively evaluate the potential of a given waterbody to support the species based on various environmental parameters.

12B.2.14 The parameters used within the HSI are:

- geographical location;
- pond area;
- permanence;

- water quality;
- shading;
- waterfowl presence;
- fish presence;
- number of ponds within 1 km;
- terrestrial habitat; and
- macrophyte cover.

12B.2.15 The collected data was used to calculate the scores for each surveyed waterbody. HSI scores were determined using established guidelines outlined in the Great Crested Newt Habitat Suitability Index Note 5 (Oldham *et al.*, 2000). These scores provided a numerical representation of the overall suitability of each habitat for GCN (HSI results are presented in Annex 3).

Great Crested Newt eDNA

12B.2.16 eDNA surveys were conducted following protocols established in technical advice note 2 for field and laboratory sampling of GCN (Biggs *et al*., 2014) to detect the presence of GCN genetic material in water samples. Rigorous sampling procedures were employed, involving the collection of water samples from suitable waterbodies within 250 m of the Proposed Development Site and within 500 m of the Main Site.

Table 12B-2: Surveyed Waterbodies

WATERBODY ID	DATE SURVEYED
2 and 4	28/06/2023
12 and 145	12/06/2023
14	13/06/2023
34	14/06/2023
44	13/06/2023
23	13/06/2023
88, 90,184, 186, 187, 188 and 189	07/06/2023
94 and 96	09/06/2023
176 and 180	08/06/2023
312, 313, 315 and 316	05/06/2023

12B.2.17 All surveyors undertaking eDNA were all licenced.

12B.3 Limitations

12B.3.1 The unusually warm start to summer of June 2023 introduced noticeable challenges to the eDNA surveys as the elevated temperatures and lack of rainfall led to significant reductions in water levels across numerous waterbodies and watercourses within the survey areas and thus many waterbodies were dry in 2023, see Table 12-B-6.

12B.3.2 Some of the waterbody eDNA samples produced inconclusive results (waterbodies 12, 14, 44, 88, 90, 94, 96, 145, 184, 186, 187, 188 and 189) and therefore were unable to confirm GCN presence/absence. However, it is likely that the indeterminate results were due to water salinity, influenced by specific habitats such as coastal sand dunes and coastal and floodplain grazing marsh, as well as the underlying geology and connectivity to Greatham Creek and therefore this is not a significant limitation.

12B.4 Results

Desk Study

12B.4.1 GCN records within the Study Area are restricted to Cowpen Bewley Woodland Park which is within 250 m of the Proposed Development and Phillips Tank Farm Grassland Local Wildlife Site (LWS) which is more than 250 m away from the Proposed Development. Notably, both locations have held European Protected Species (EPS) Licences specifically for GCN from 2015 to 2017 as illustrated by MAGIC, 2022. INCA provided no records of GCN within 2 km of the Proposed Development Site.

12B.4.2 NZT eDNA results confirmed absence of GCN at waterbody 1 (which was dry in 2023), waterbody 2, waterbody 3 (which is no longer present after site clearance), and waterbody 4. Further assessment was required at waterbodies 96 and 337 (which was dry in 2023).

12B.4.3 The Local Biodiversity Action Plan (see Annex 2) indicates that there are no records of GCN within the South Tees areas (Tees Valley Nature Partnership, 2012). The NZT surveys found two potentially suitable waterbodies (95 and 105) and performed eDNA testing. The results were inconclusive, and therefore it was considered that GCN may be present.

12B.4.4 There are no current, confirmed records from Middlesbrough and few from around the lower Tees Estuary. As much of the land in the lower Tees Estuary is reclaimed, it is possible that great crested newts were never present in these areas.

12B.4.5 The desk study results are shown in Annex 1, Figure 12-B-3.

Extended Phase 1 Habitat Survey (screening of waterbodies)

12B.4.6 Initial investigations revealed that the OS Mastermap data used in Extended Phase 1 Habitat Surveys was unreliable for identifying waterbodies suitable for GCN within 250 m of the Proposed Development and within 500 m of the Main Site. This was because some waterbodies that were present on OS Mastermap data did not exist when site visits were undertaken.

12B.4.7 The initial 571 potential waterbodies identified (using OS Mastermap data) as being within 250 m of the Proposed Development Site and within 500 m of the Main Site was revised to 409 based on updates to the Proposed Development Site.

12B.4.8 The suitability of the 409 potential waterbodies was identified by the screening process. Unsuitable waterbodies were then excluded from further assessment. Excluded waterbodies included:

- 152 dry waterbodies;
- 38 waterbodies within 250 m of the Proposed Development Site, where the use of existing infrastructure prevents potential for impact;
- 153 waterbodies identified as running water and associated ditches;
- 13 operational industrial waterbodies;
- 23 waterbodies identified as being unsuitable because they were part of the estuary/port, connected to or were saline water, so are not suitable for GCN; and
- 8 waterbodies identified as part of the RSPB reserve and were dry and therefore unsuitable for GCN.

12B.4.9 A total of 22 waterbodies were sampled for GCN eDNA. The locations of waterbodies and watercourses are shown in Annex 1, Figure 12-B-2. The figure shows all the initial waterbodies identified including those within the 250 m buffer of the Proposed Development Site and within the 500 m buffer of the Main Site.

Field surveys

Great Crested Newt eDNA

12B.4.10 The eDNA results were positive for GCN at waterbodies 315, 316 and 176. Two of these waterbodies (Annex 1, Figure 12-B-2) (Annex 6, Plates 12B-1 and 12B-2) can be found in the broad-leaved plantation woodland that forms part of Cowpen Bewley Woodland Park and the third waterbody is located at Greenabella Marsh LWS.

12B.4.11 The Greenabella Marsh LWS waterbody 176 sits within an area of semi-improved neutral grassland with areas of swamp habitat to the east and west as part of an open mosaic habitat on previously developed land.

12B.4.12 Waterbodies 12, 14, 44, 88, 90, 94, 96, 145, 184, 186, 187, 188 and 189 resulted in indeterminate results because calcium ions present in the water samples, and this reacted with the preservatives used in the eDNA water sampling. These indeterminate waterbodies are discussed within the conclusion section of this appendix.

12B.4.13 The eDNA results for all waterbodies surveyed are provided in Annex 5.

12B.4.14 Due to the large number of waterbodies, a photograph for each has not been included and photographs representative of ponds which support GCN have been included within Annex 6.

12B.5 Conclusions

12B.5.1 GCN were confirmed present within three waterbodies within 250 m of the Proposed Development Site (waterbodies 315, 316 and 176). In relation to indeterminate results, within an evidence review for GCN eDNA monitoring protocols (Natural England, 2023), Natural England indicate that where local geology leads to high levels of dissolved calcium in the water, the calcium ions can react with the preservatives used in eDNA water sampling, creating a white precipitate (which is often only visible a few hours after sampling). Through correspondence received from ADAS post-analyses, they suggested the indeterminate results could be due to the salinity of these habitats and therefore, GCN is assumed absent from these areas.

12B.5.2 Brackish waters are characterised by a moderate salinity level that falls between freshwater and seawater and is generally considered unsuitable for GCN (Beebee and Griffiths, 2000; Sparreboom et al., 2008).

12B.5.3 GCN are primarily freshwater species and exhibit high sensitivity to changes in water quality, particularly salinity levels. GCN predominantly breed and inhabit freshwater bodies, such as ponds, where they lay their eggs and undergo various life stages (Griffiths, 1996). Brackish water, with its elevated salinity content, poses significant challenges to the delicate physiological balance of GCN, particularly in areas within the Proposed Development Site that are known for their brackish conditions within areas such as the floodplain grazing marshes and the Brine fields.

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12B.7 ANNEX 1: Figures

Figure 12-B-1: The Proposed Development Site and Survey Areas

Figure 12-B-2: Desk Study Results

Figure 12-B-3: Positive eDNA Results

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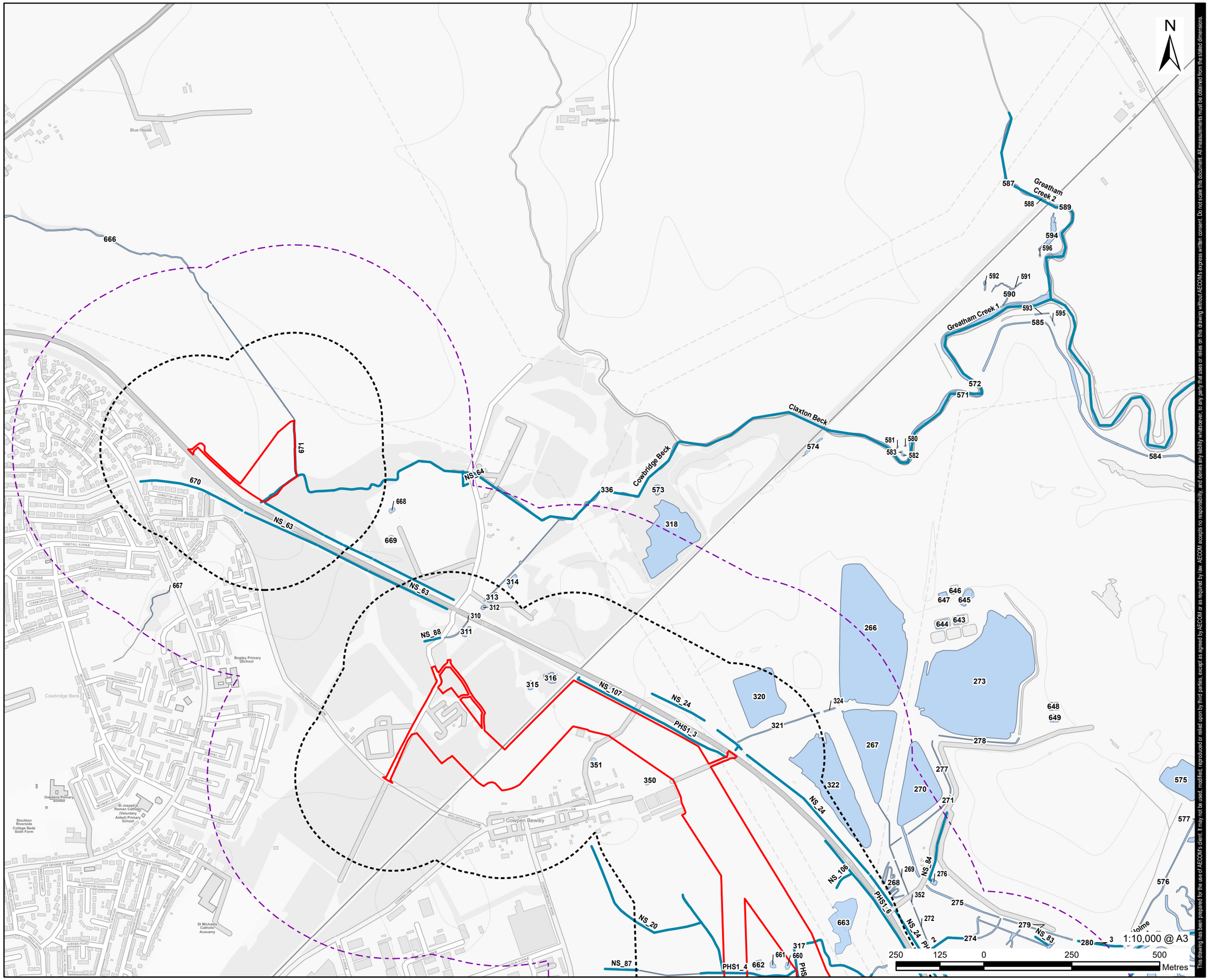

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LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Waterbody
- Waterbody Area

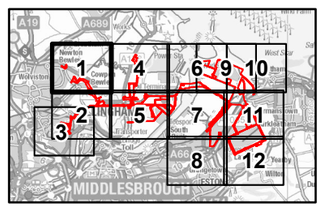

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FIGURE TITLE

The Proposed Development Site and Survey Areas

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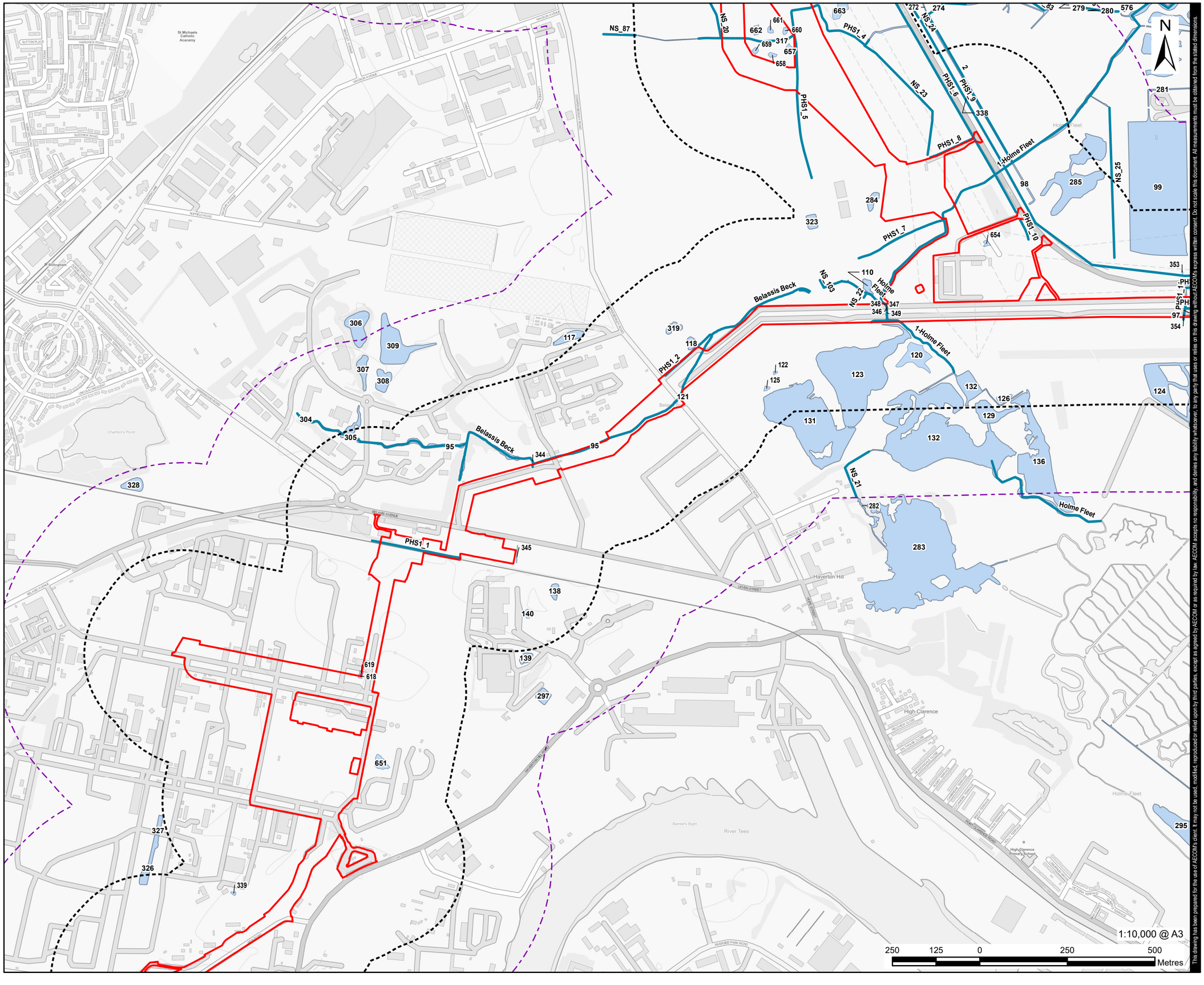

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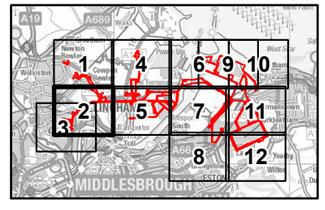

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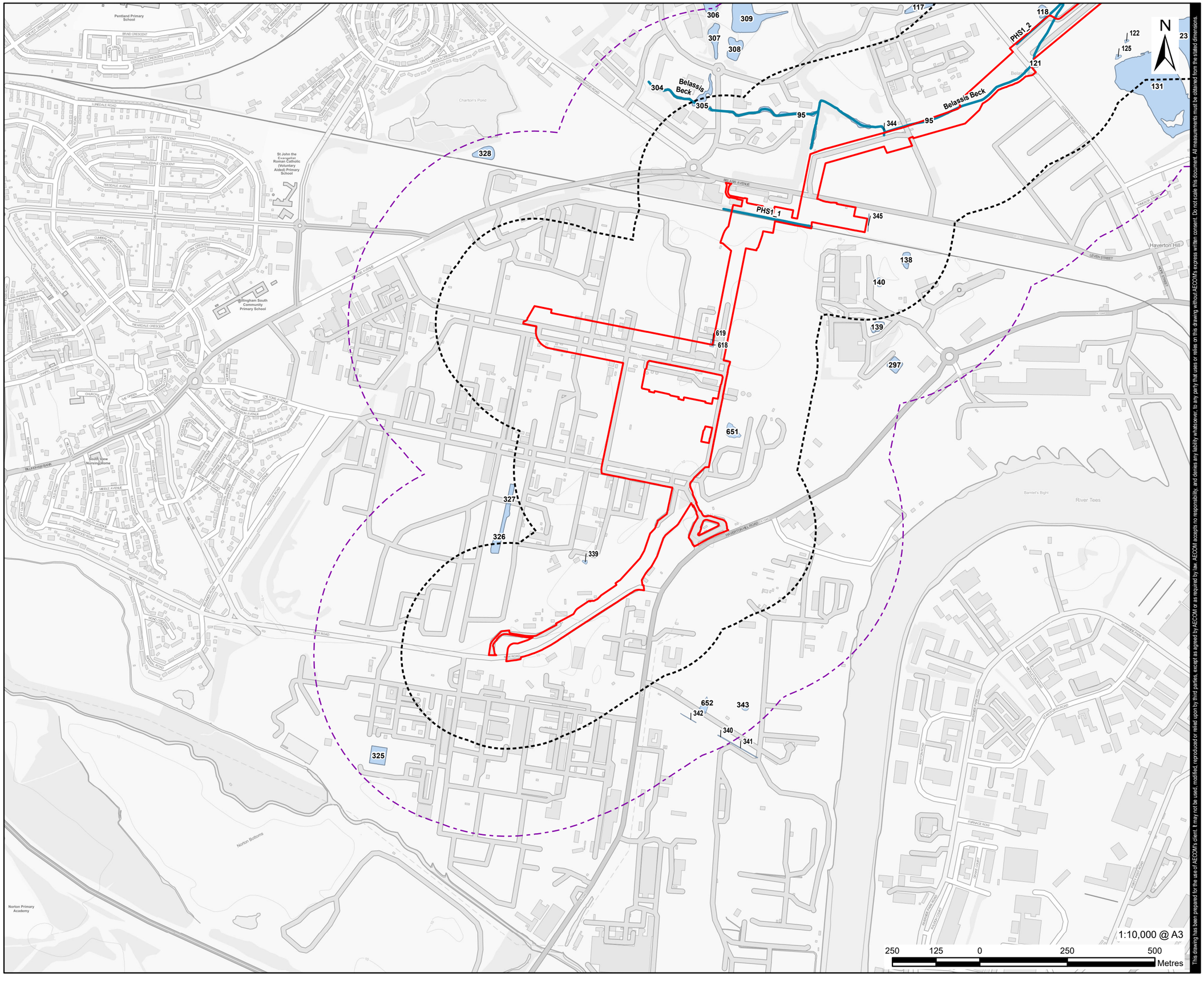

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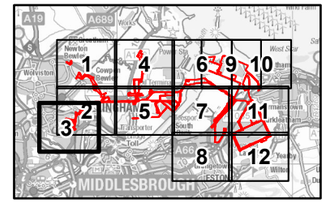

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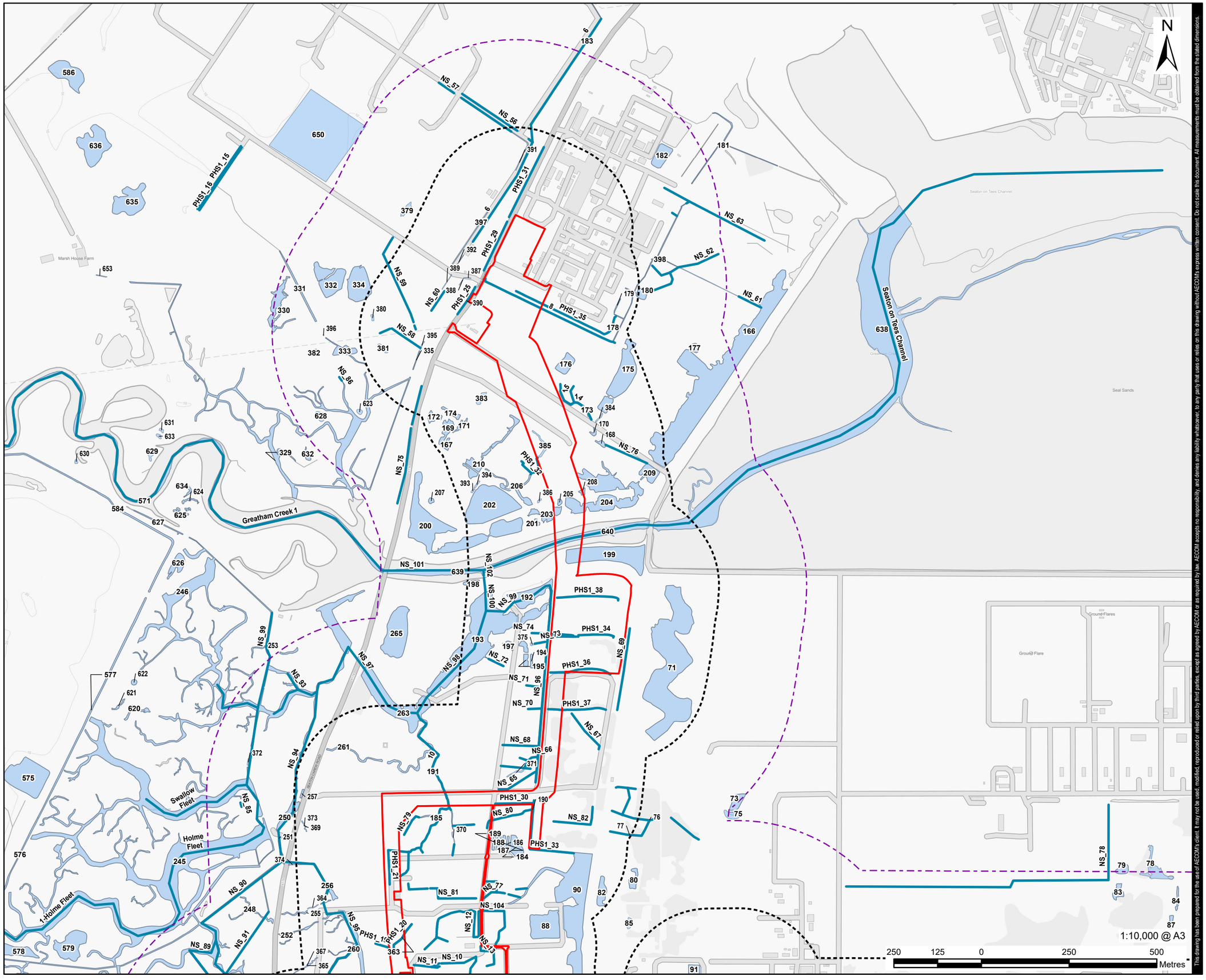

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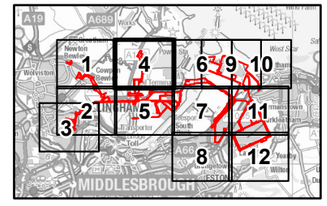
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 4 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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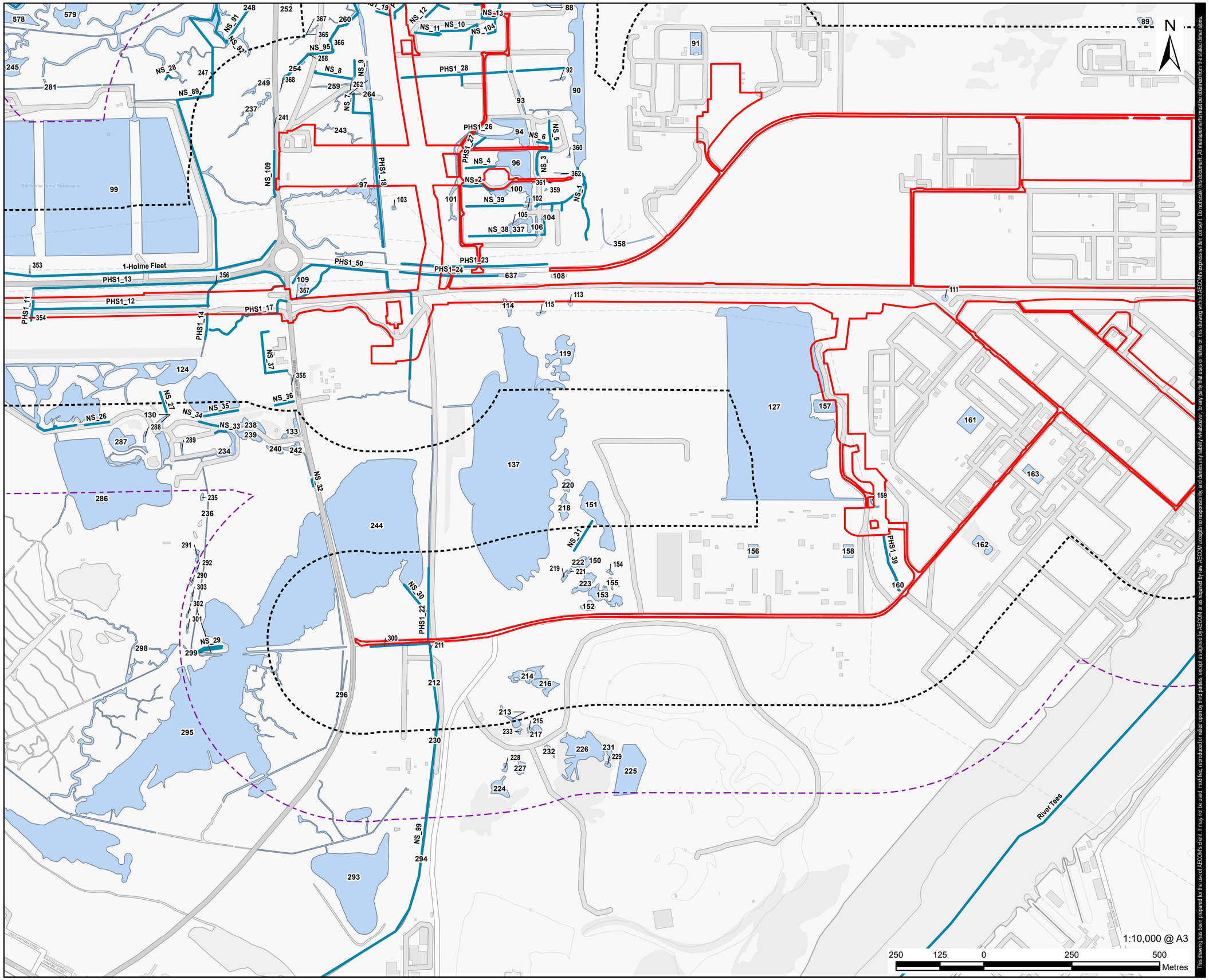

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AECOM

PROJECT

H2Teesside DCO

APPLICANT

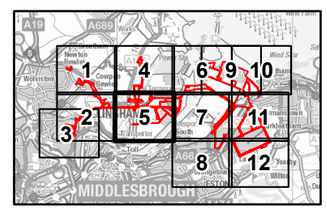
H2 Teesside Limited

CONSULTANT

AECOM Limited
100 Embankment,
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 5 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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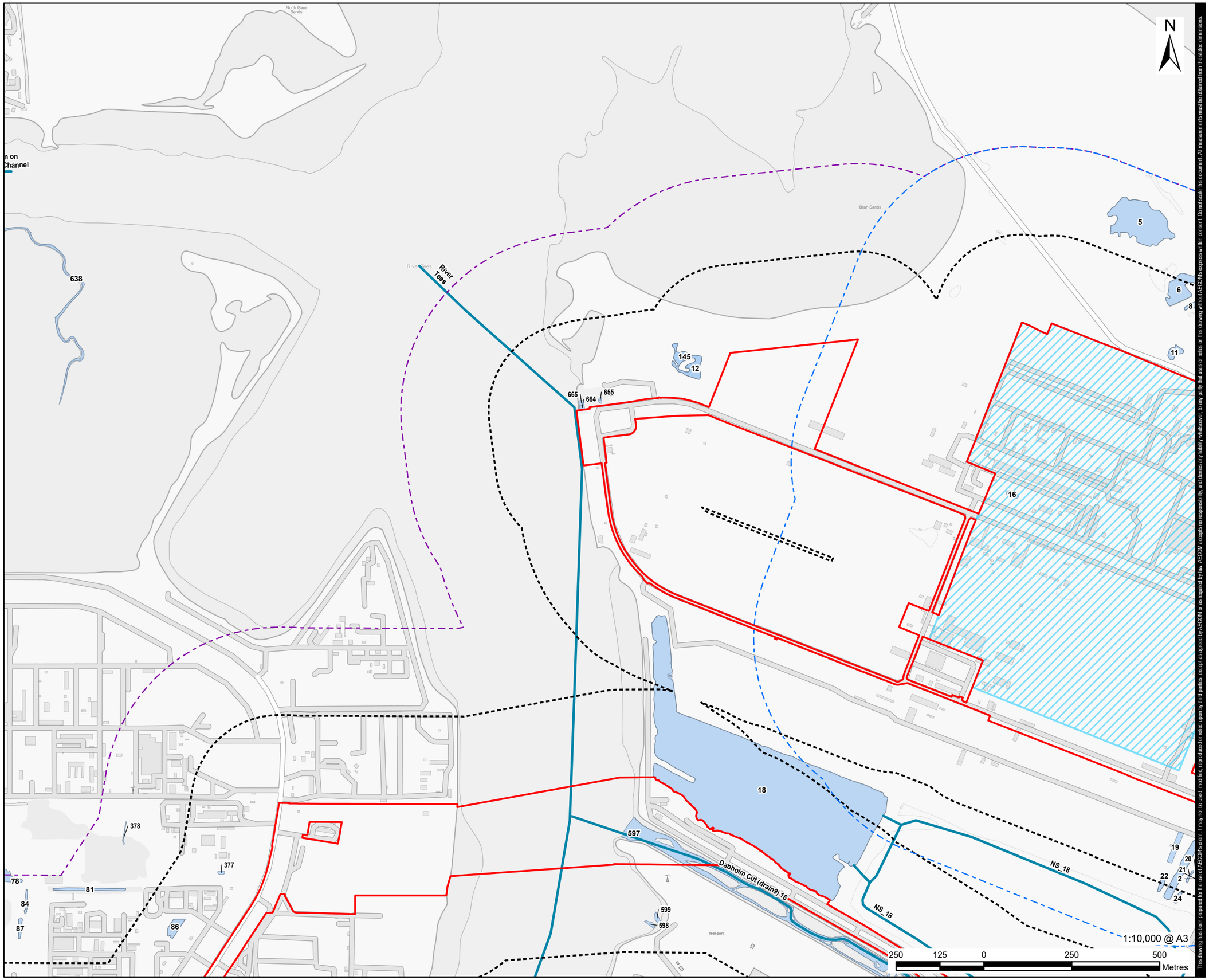

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PROJECT

H2Teesside DCO

APPLICANT

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CONSULTANT

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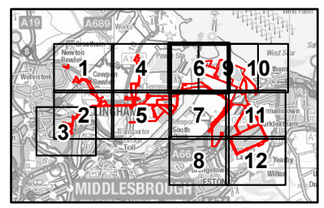
LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 6 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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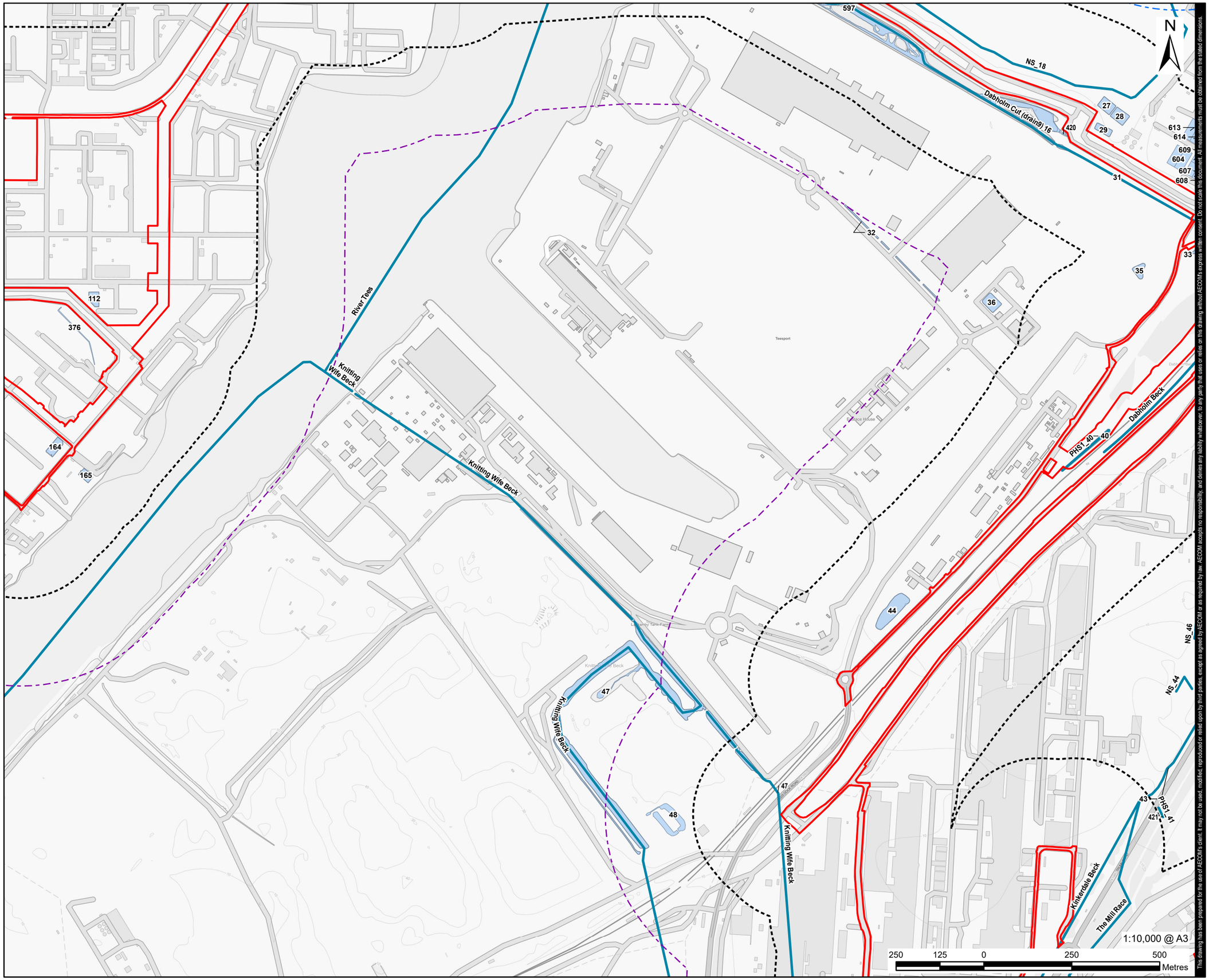

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PROJECT

H2Teesside DCO

APPLICANT

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CONSULTANT

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100 Embankment,
Cathedral Approach,
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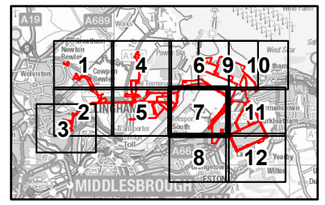
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 7 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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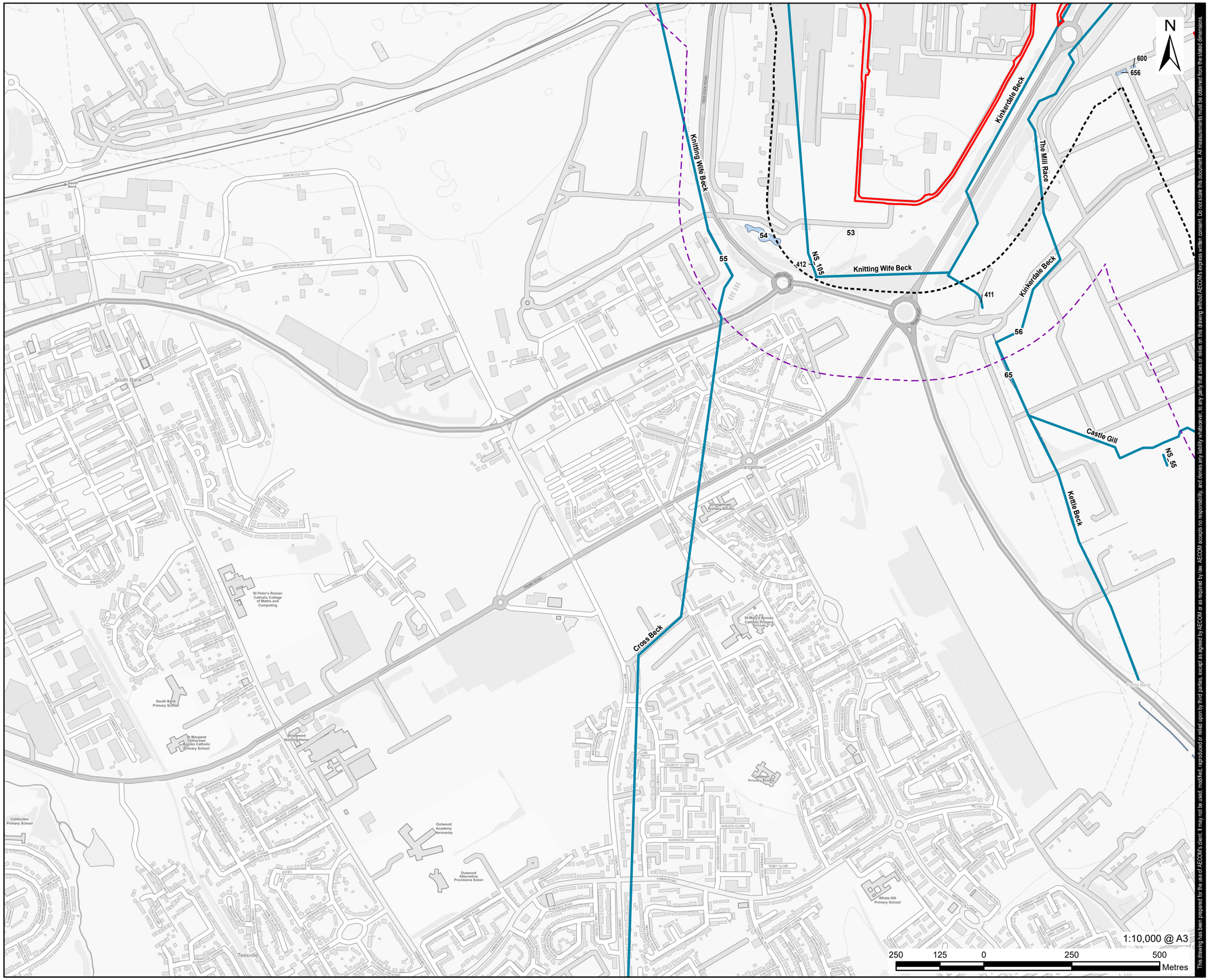

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APPLICANT

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Cathedral Approach,
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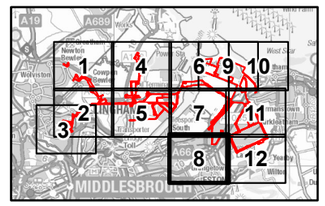
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 8 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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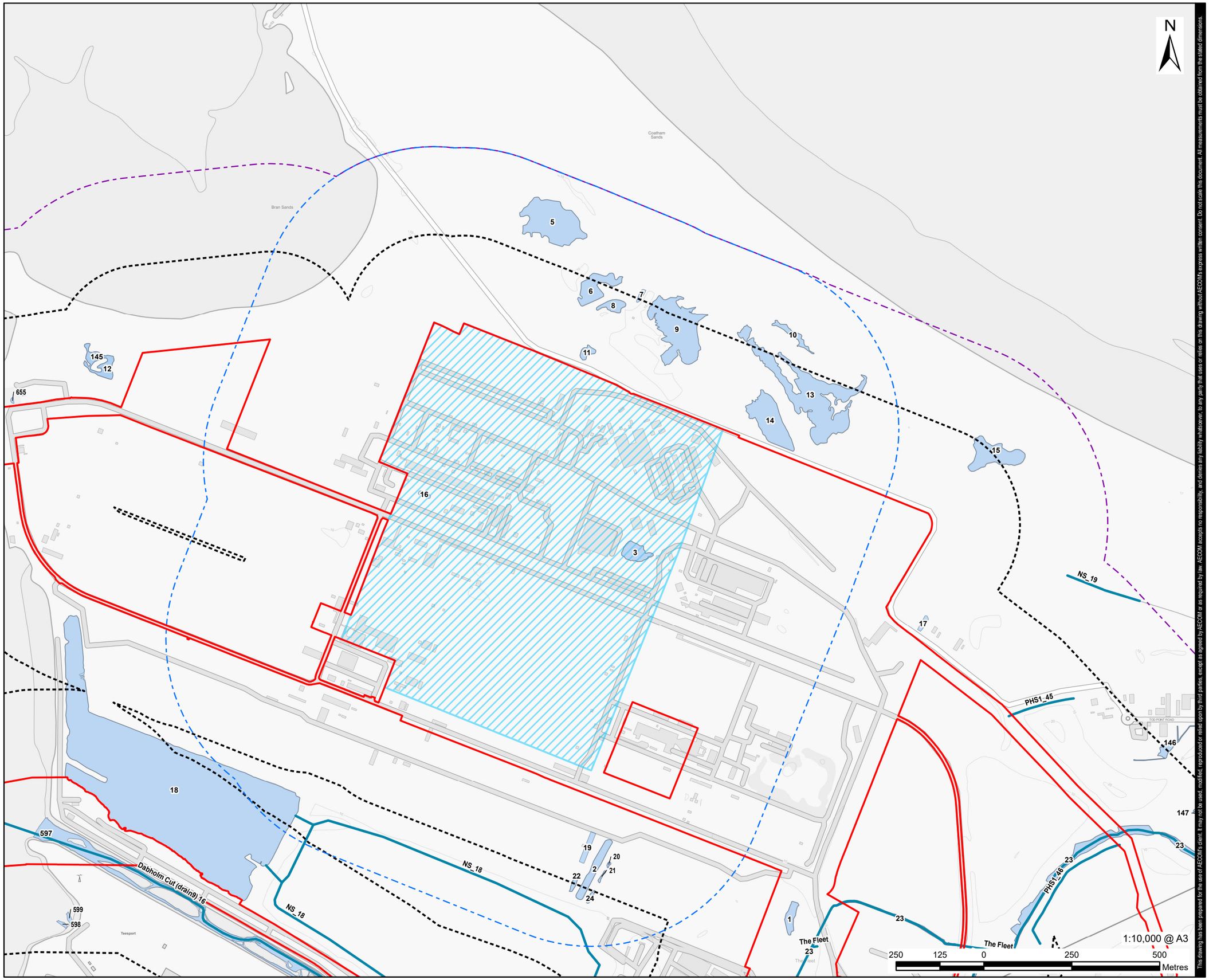

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CONSULTANT

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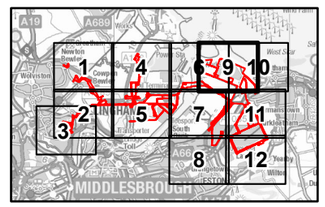
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 9 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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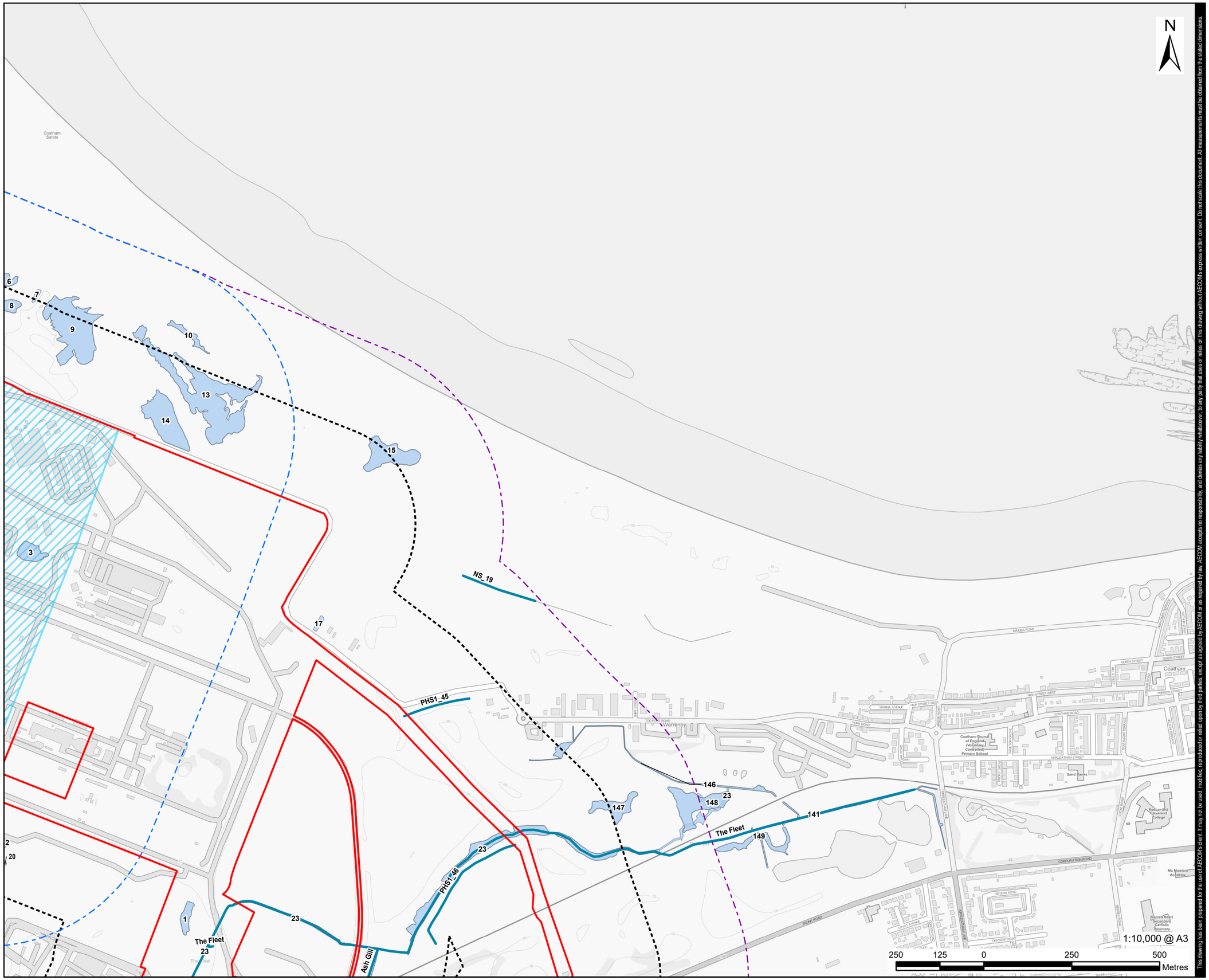

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APPLICANT

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CONSULTANT

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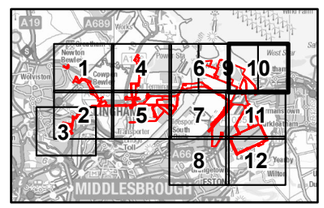
100 Embankment,
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 10 of 12)

Revision: 0 Drawn: MAB Checked: LL Approved: JW Date: 2024-03-13

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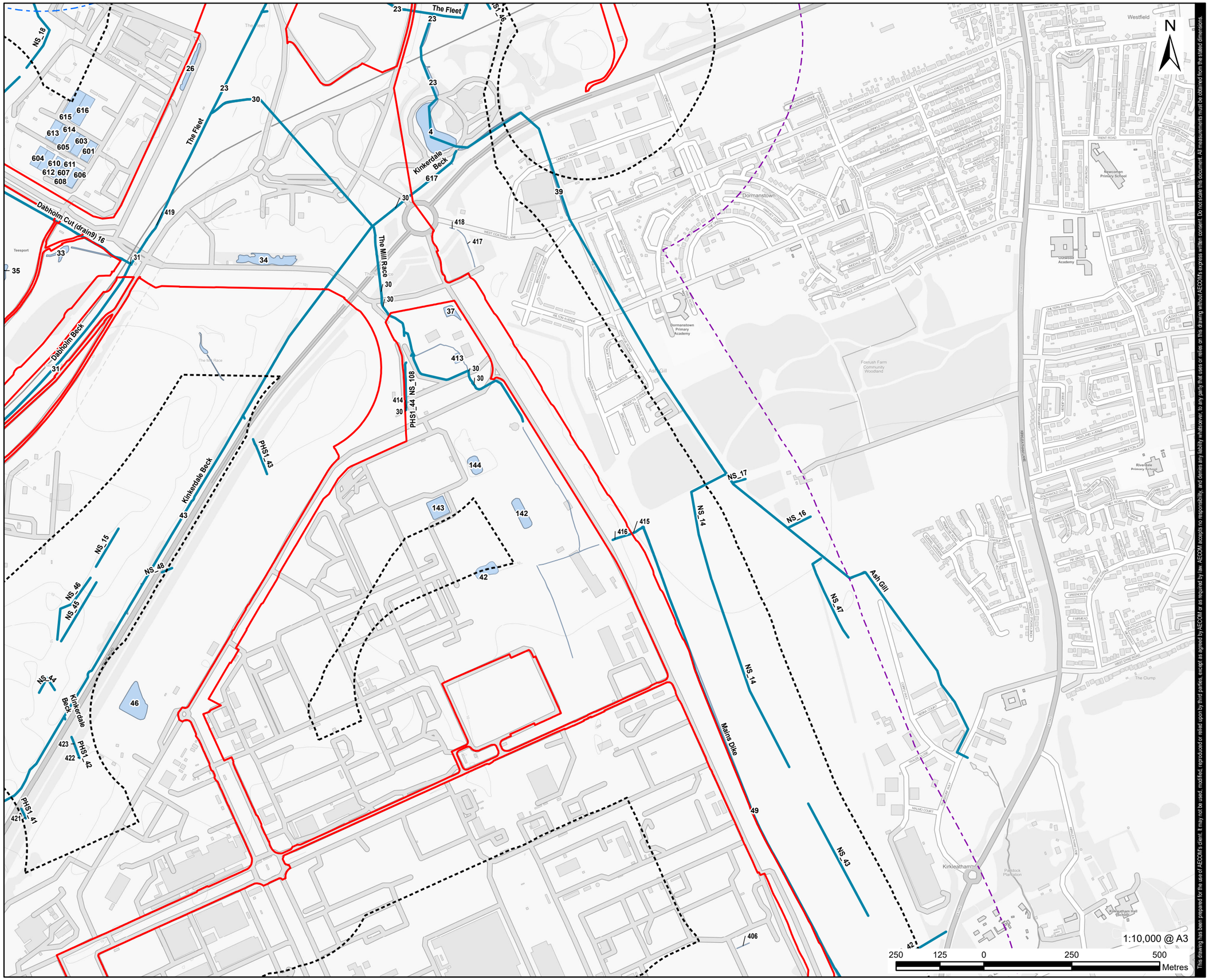

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H2Teesside DCO

APPLICANT

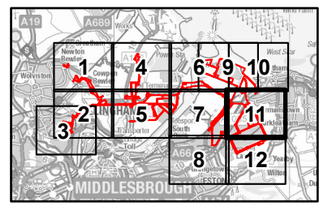
H2 Teesside Limited

CONSULTANT

AECOM Limited
100 Embankment,
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 11 of 12)

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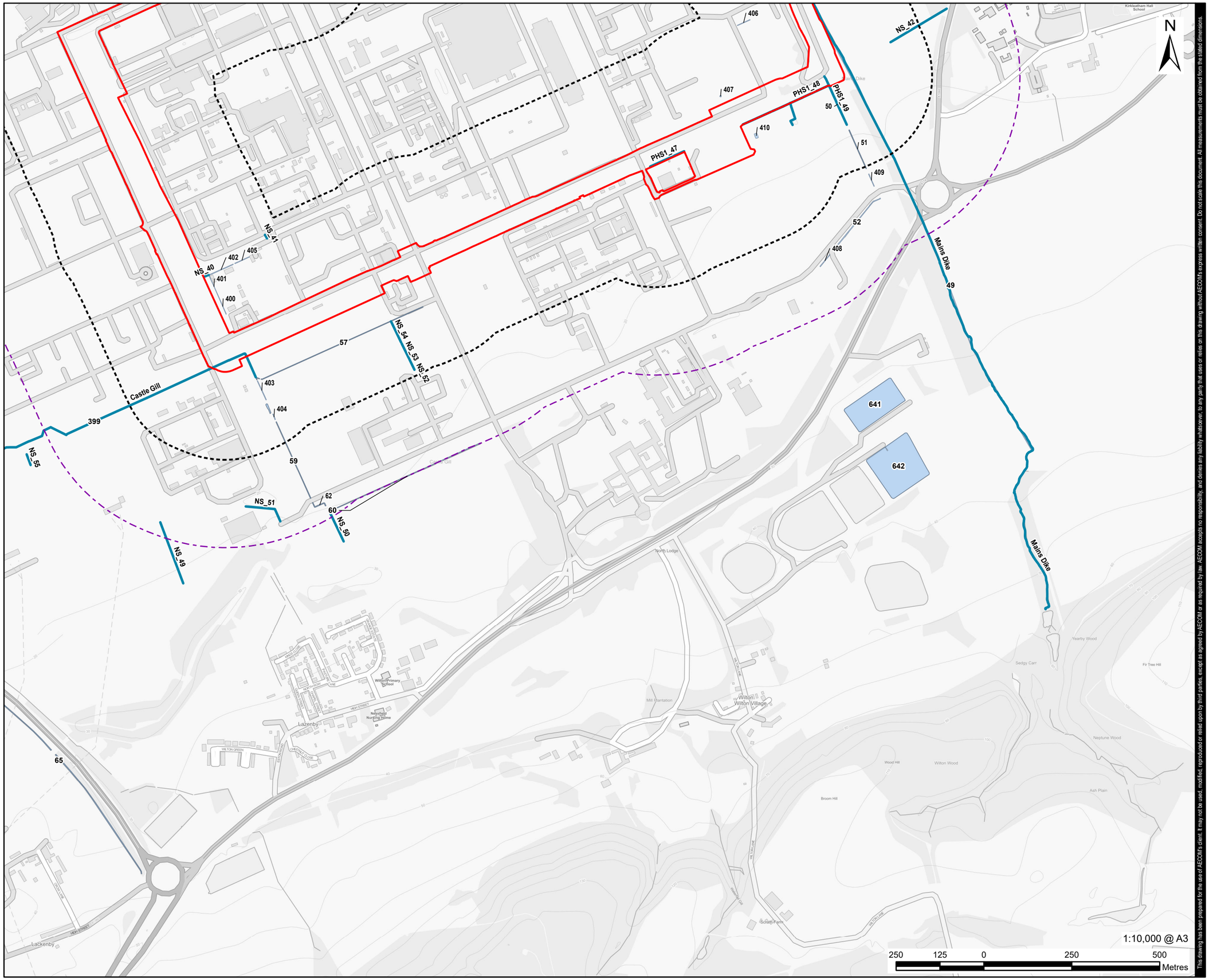

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PROJECT

H2Teesside DCO

APPLICANT

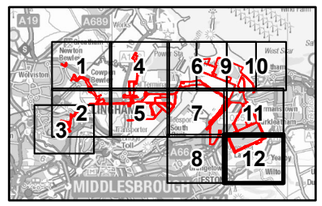
H2 Teesside Limited

CONSULTANT

AECOM Limited
100 Embankment,
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Waterbody
- Waterbody Area



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

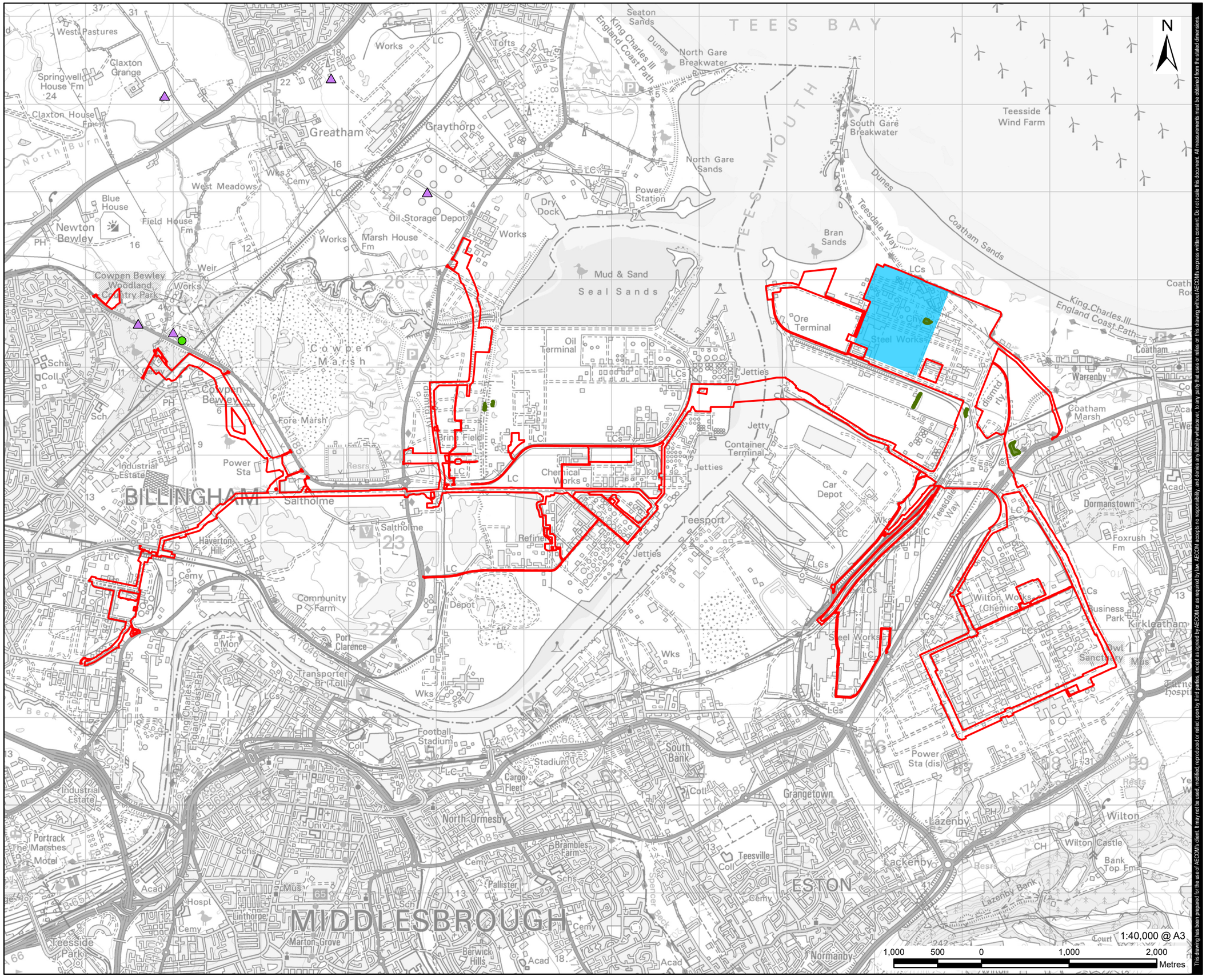
60689030

FIGURE TITLE

The Proposed Development Site and Survey Areas

FIGURE NUMBER

Figure 12-B-1 (Page 12 of 12)



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PROJECT

H2Teesside DCO

APPLICANT

H2 Teesside Limited

CONSULTANT

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Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Main Site
- Great Crested Newt Class Survey Licence Return

Great Crested Newt (GCN) Record

- Environmental Records Information Centre North East (From 2012 to 2021) - GCN Present

Net Zero Teesside

- Waterbody Area with Negative eDNA - GCN Confirmed Absent

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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Desk Study Results

FIGURE NUMBER

Figure 12-B-2

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Revision: 0 Drawn: MAB Checked: VC Approved: JW Date: 2024-03-13

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PROJECT

H2Teesside DCO

APPLICANT

H2 Teesside Limited

CONSULTANT

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Cathedral Approach,
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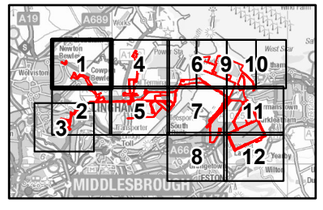
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- P - Present
- A - Absent
- NS - Not Surveyed
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 1 of 12)

Revision: 0 Drawn: MAB Checked: VC Approved: JW Date: 2024-03-13

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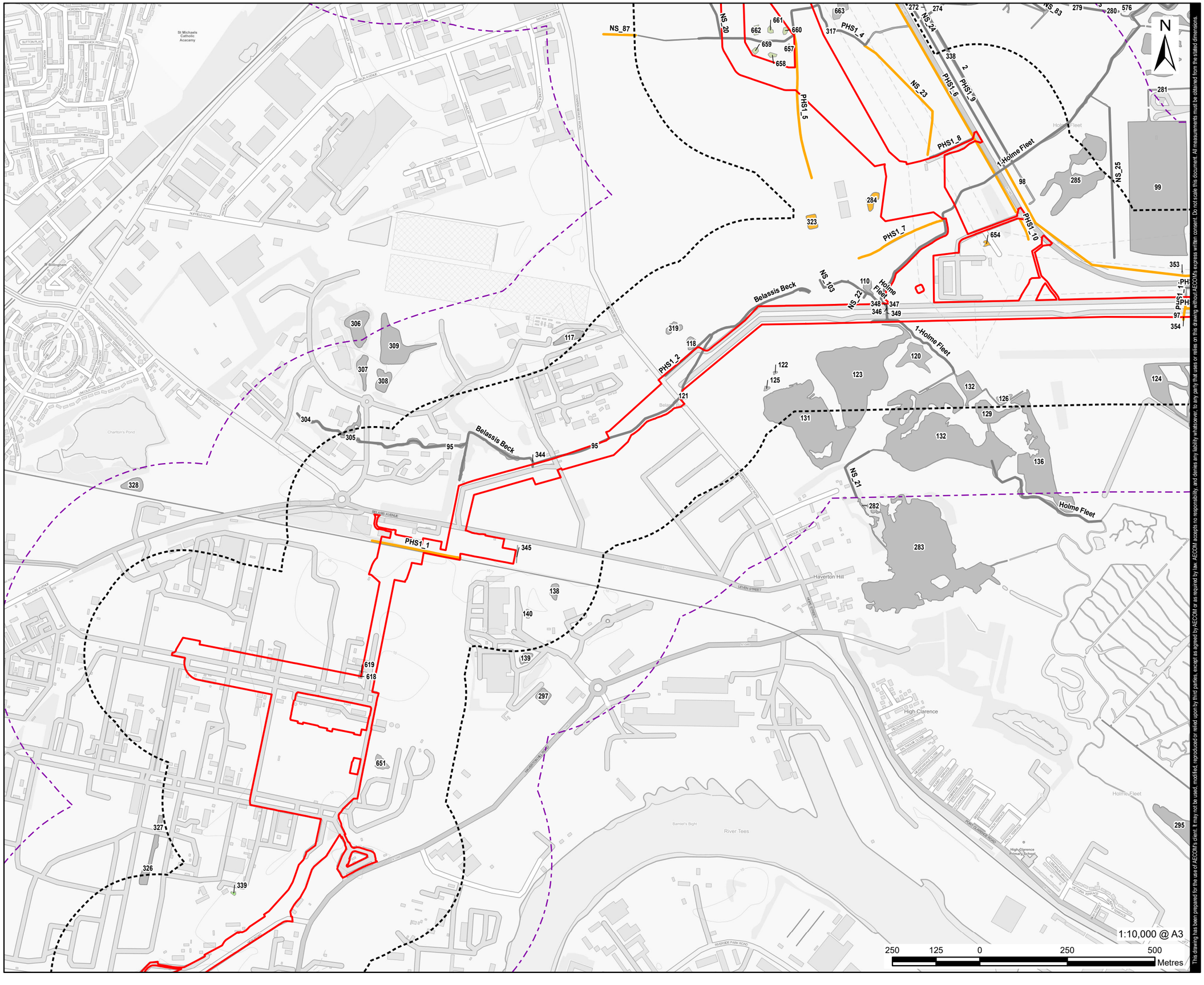

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APPLICANT

H2 Teesside Limited

CONSULTANT

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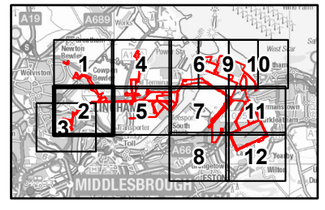
100 Embankment,
Cathedral Approach,
Manchester, M3 7FB
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 2 of 12)

Revision: 0 Drawn: MAB Checked: VC Approved: JW Date: 2024-03-13

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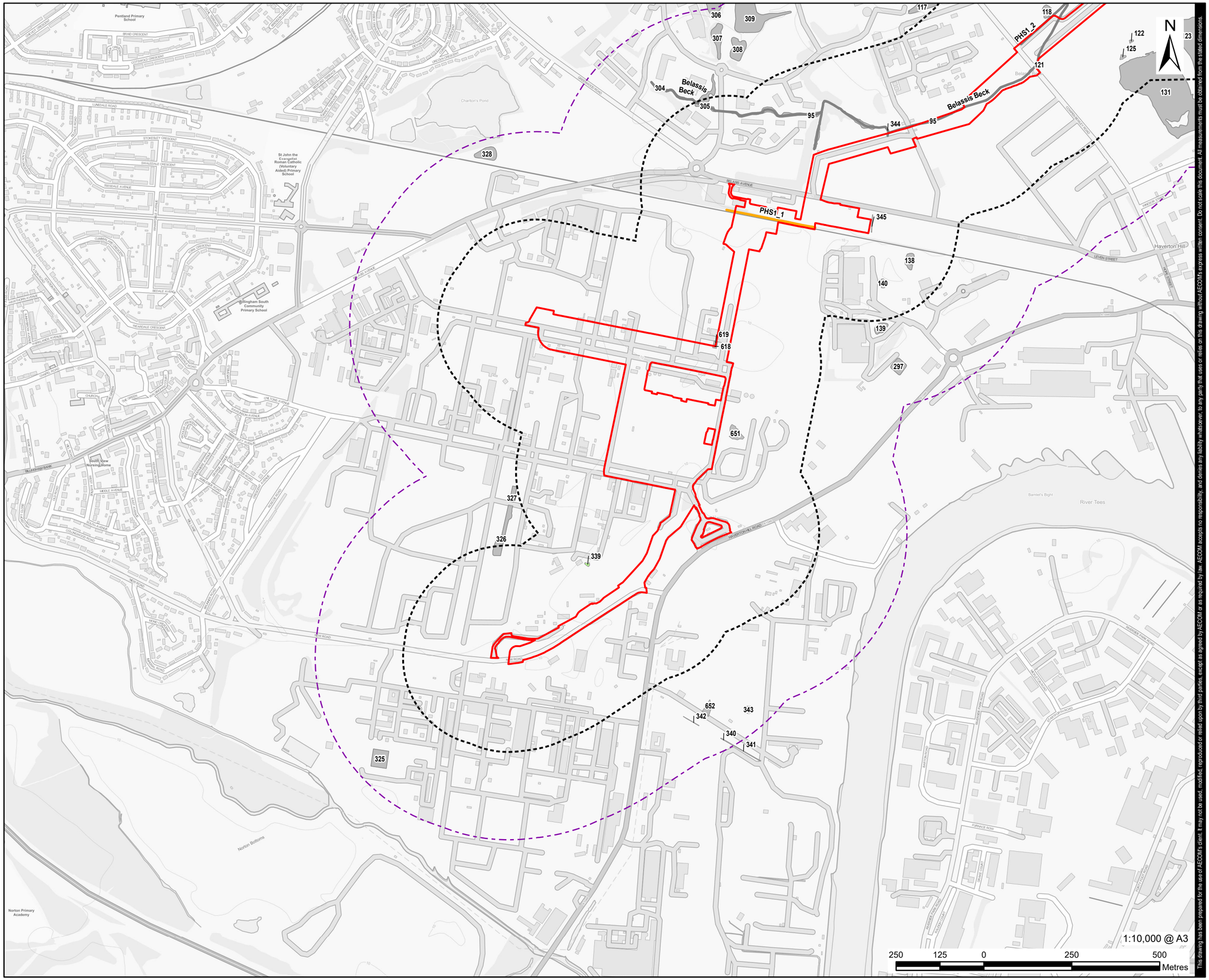

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APPLICANT

H2 Teesside Limited

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Cathedral Approach,
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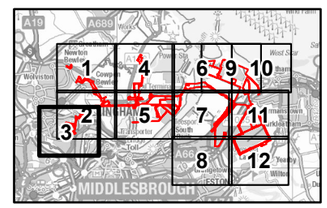
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 3 of 12)

Revision: 0 Drawn: MAB Checked: VC Approved: JW Date: 2024-03-13

Filename: \\na.aecomnet.com\tls\EMEA\Bristol-UKBRI2\Legacy\UKBRI2FP001\VOL1\BE\Projects\Environmental\General\GIS\Projects\H2 Teesside\02_Maps\Ecology\H2Teesside_ES_EcologyGCN.aprx

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PROJECT

H2Teesside DCO

APPLICANT

H2 Teesside Limited

CONSULTANT

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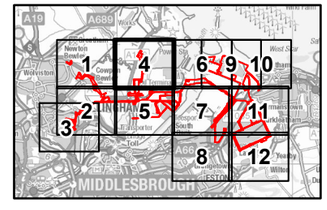
www.aecom.com

LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- NS - Not Surveyed (Saline)
- Scoped Out
- P - Present
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- NS - Not Surveyed (Saline)
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 4 of 12)

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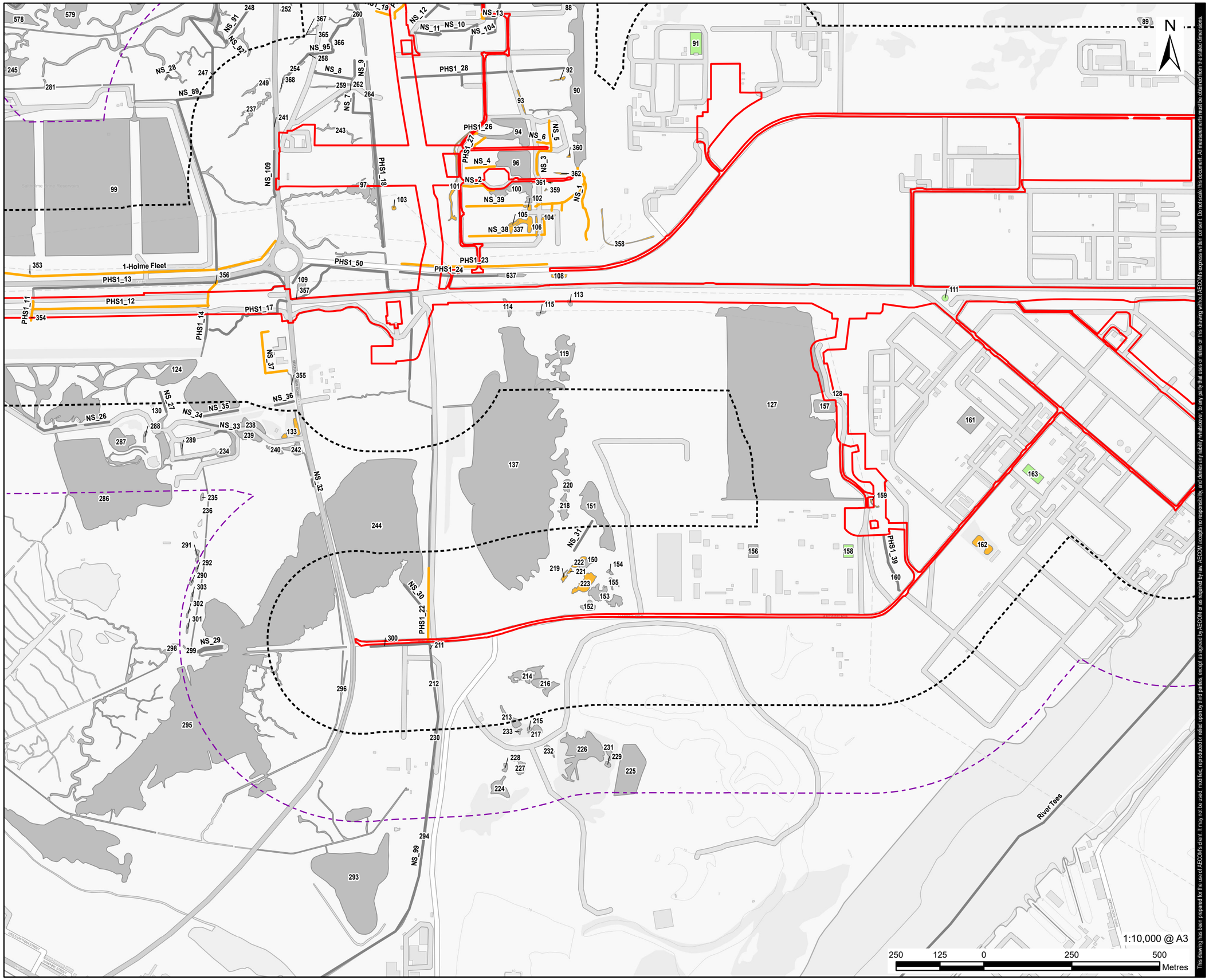

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PROJECT

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APPLICANT

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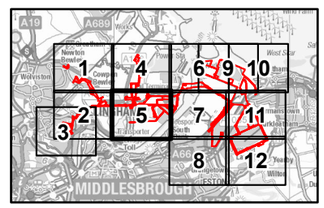
LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out



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Environmental Statement

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FIGURE TITLE

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FIGURE NUMBER

Figure 12-B-3 (Page 5 of 12)

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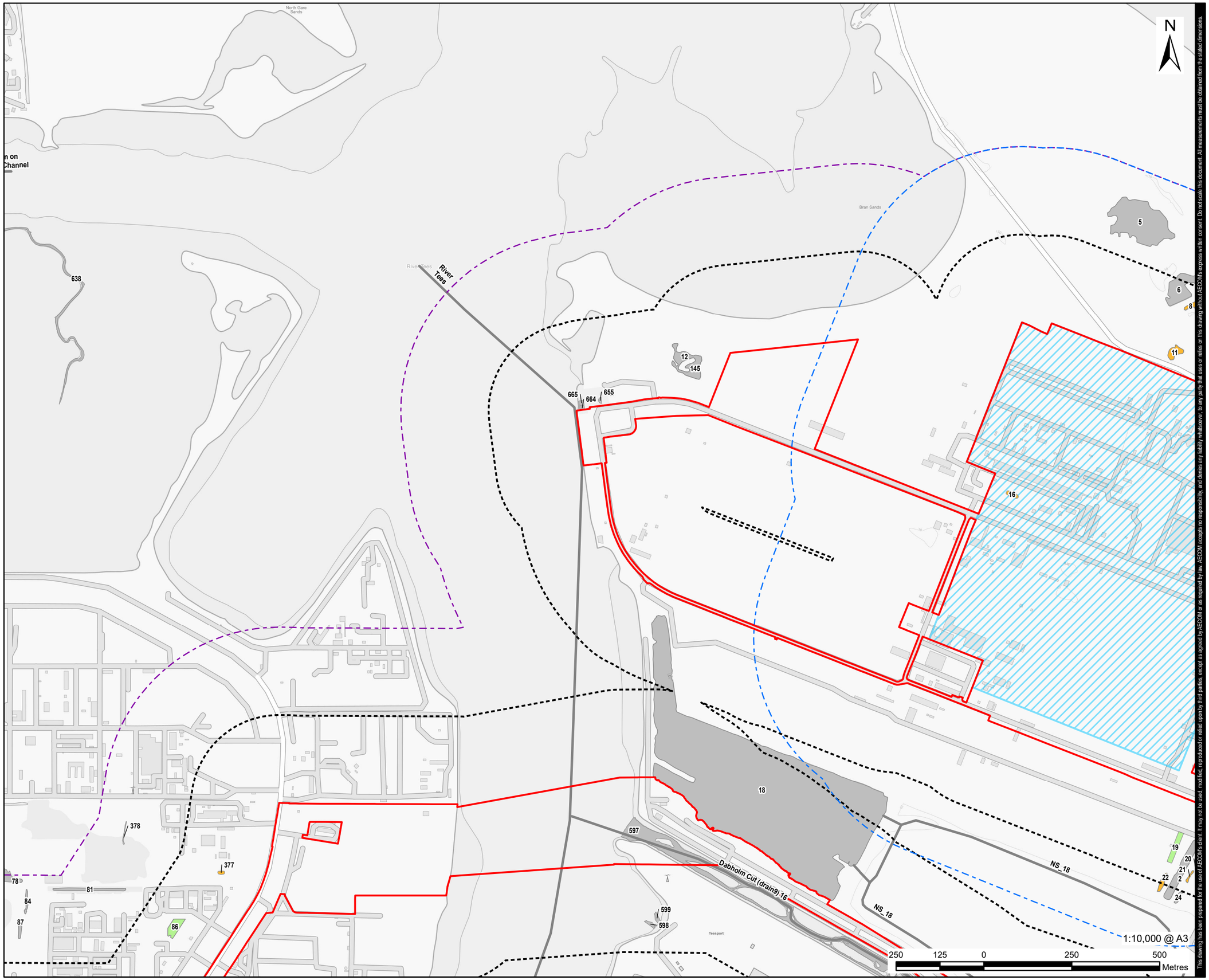

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LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer

GCN Results

- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out

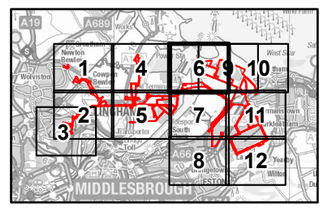

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FIGURE TITLE

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FIGURE NUMBER

Figure 12-B-3 (Page 6 of 12)

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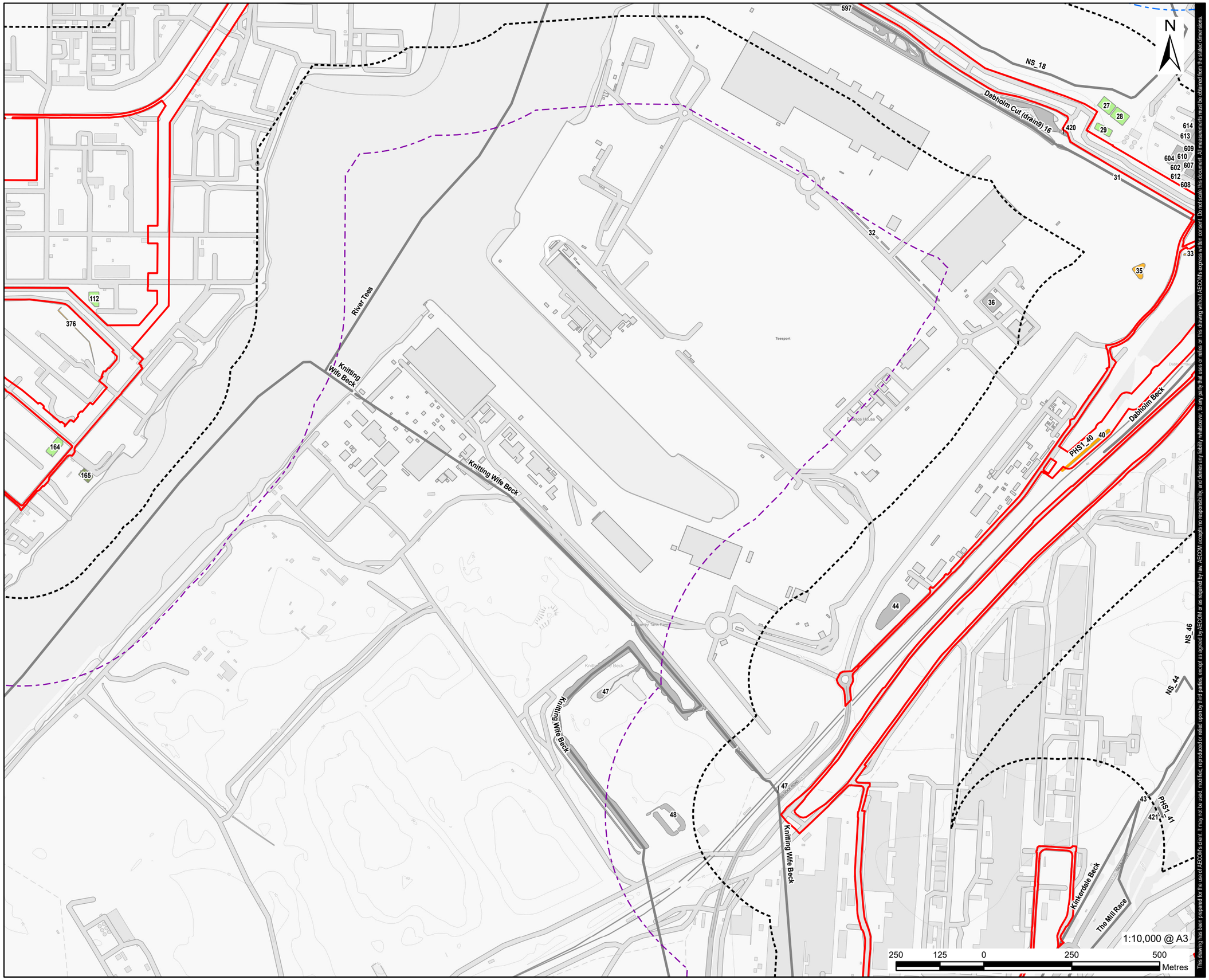

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LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- NS - Not Surveyed (Saline)
- Scoped Out

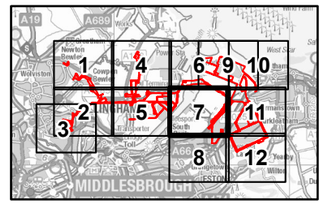

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FIGURE NUMBER

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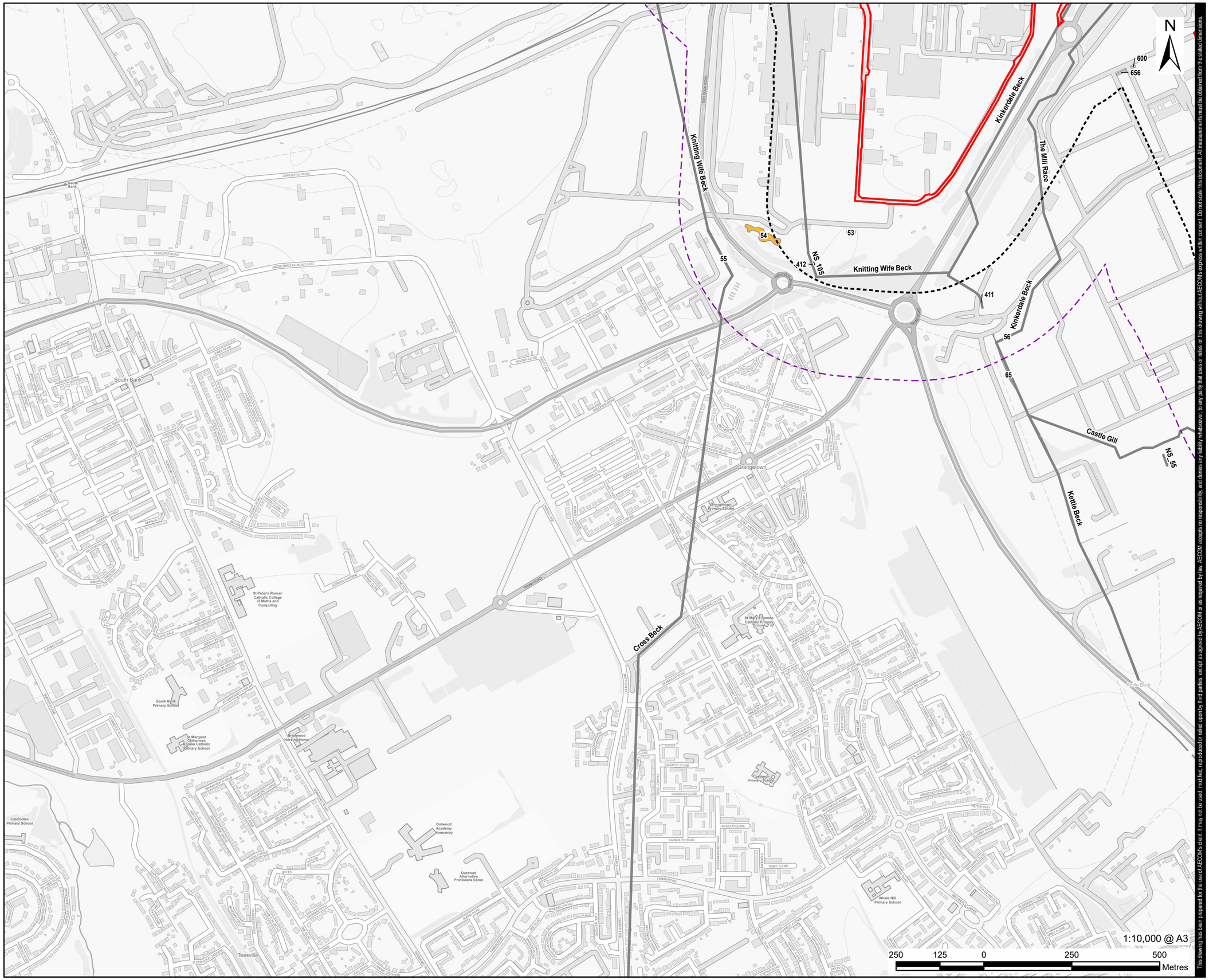

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LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- Scoped Out
- A - Absent
- Scoped Out

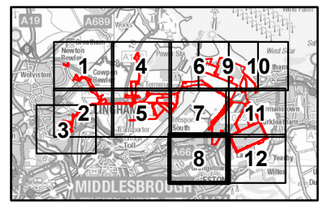

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FIGURE NUMBER

Figure 12-B-3 (Page 8 of 12)

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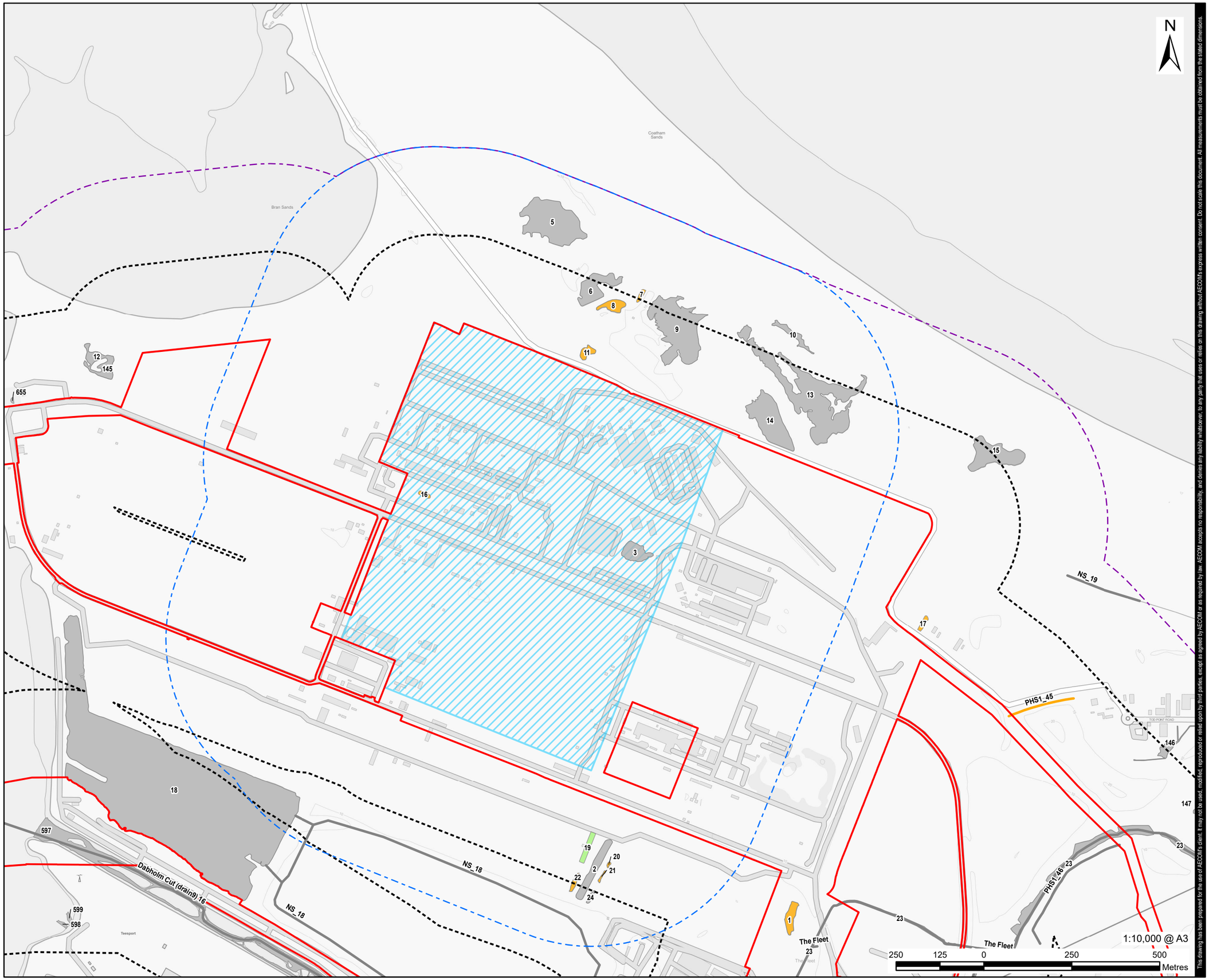

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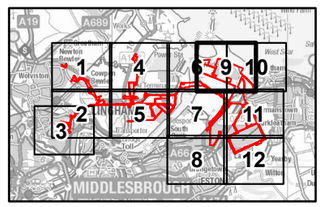
LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out



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Environmental Statement

PROJECT NUMBER

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FIGURE NUMBER

Figure 12-B-3 (Page 9 of 12)

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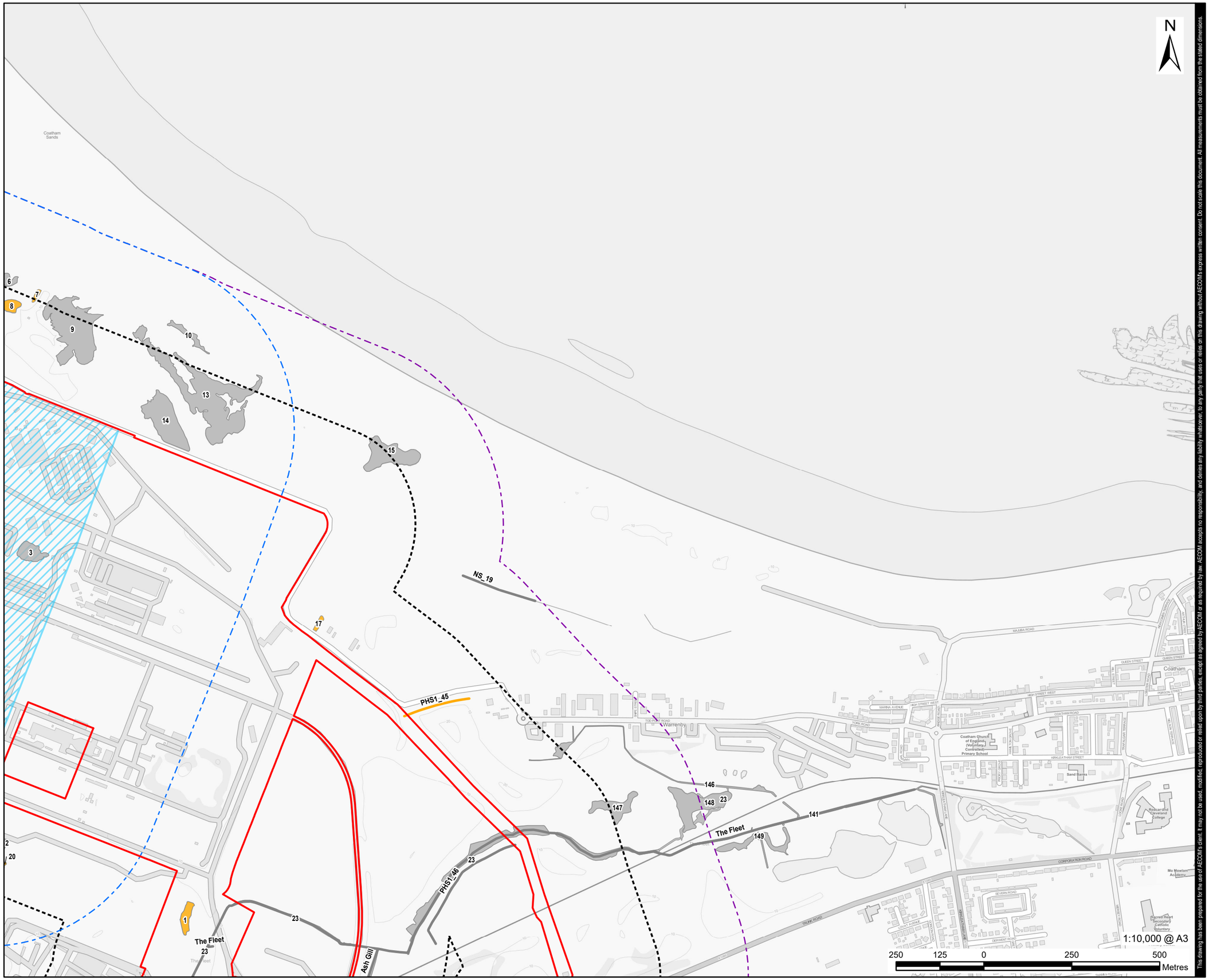

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LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site
- Main Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- Scoped Out

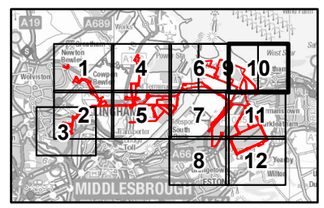

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FIGURE TITLE

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FIGURE NUMBER

Figure 12-B-3 (Page 10 of 12)

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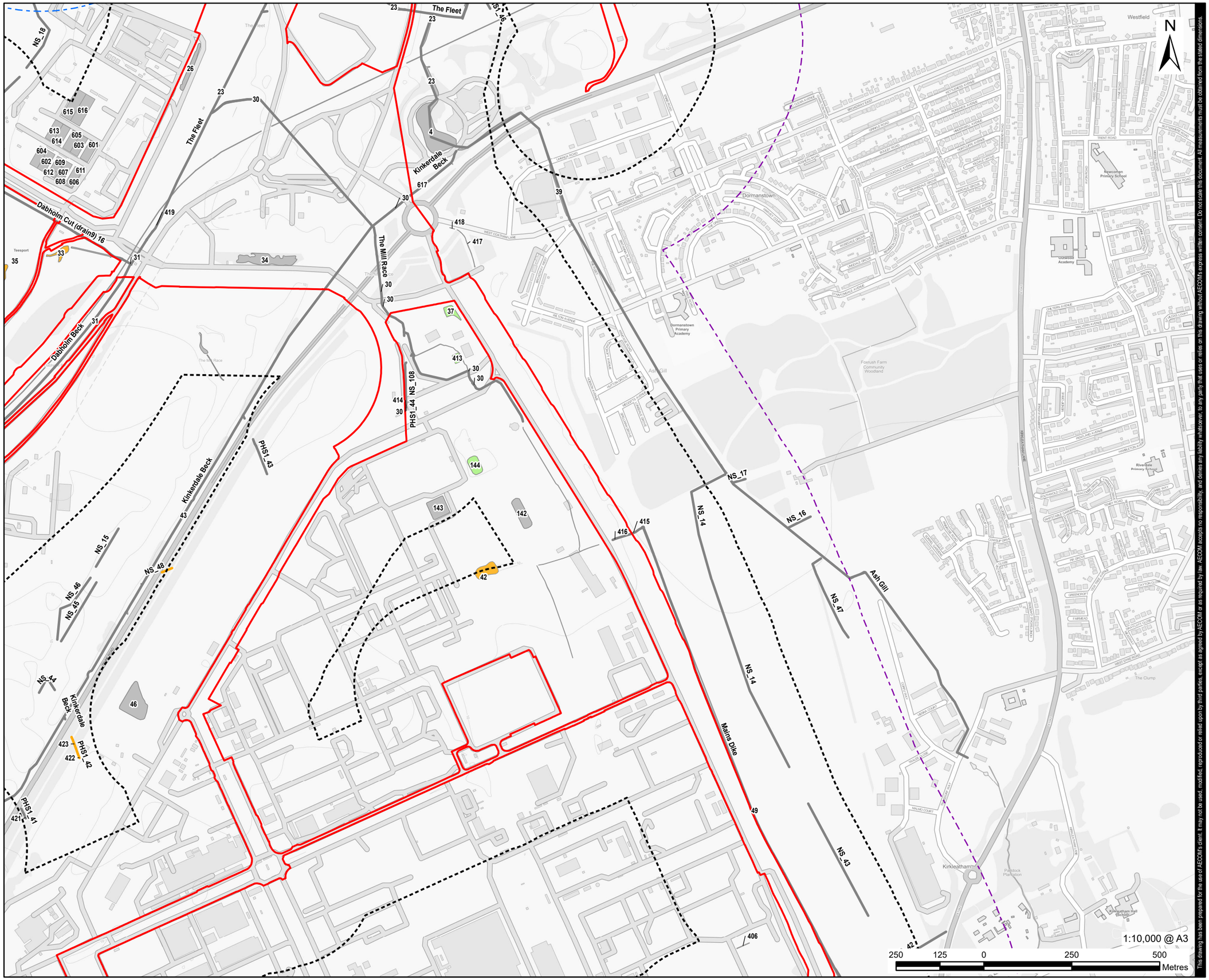

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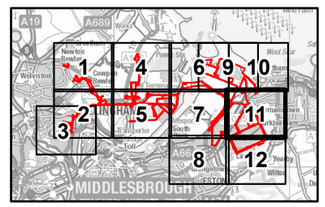
LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer
- Main Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- NS - Not Surveyed (Industrial Waterbody)
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

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60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 11 of 12)

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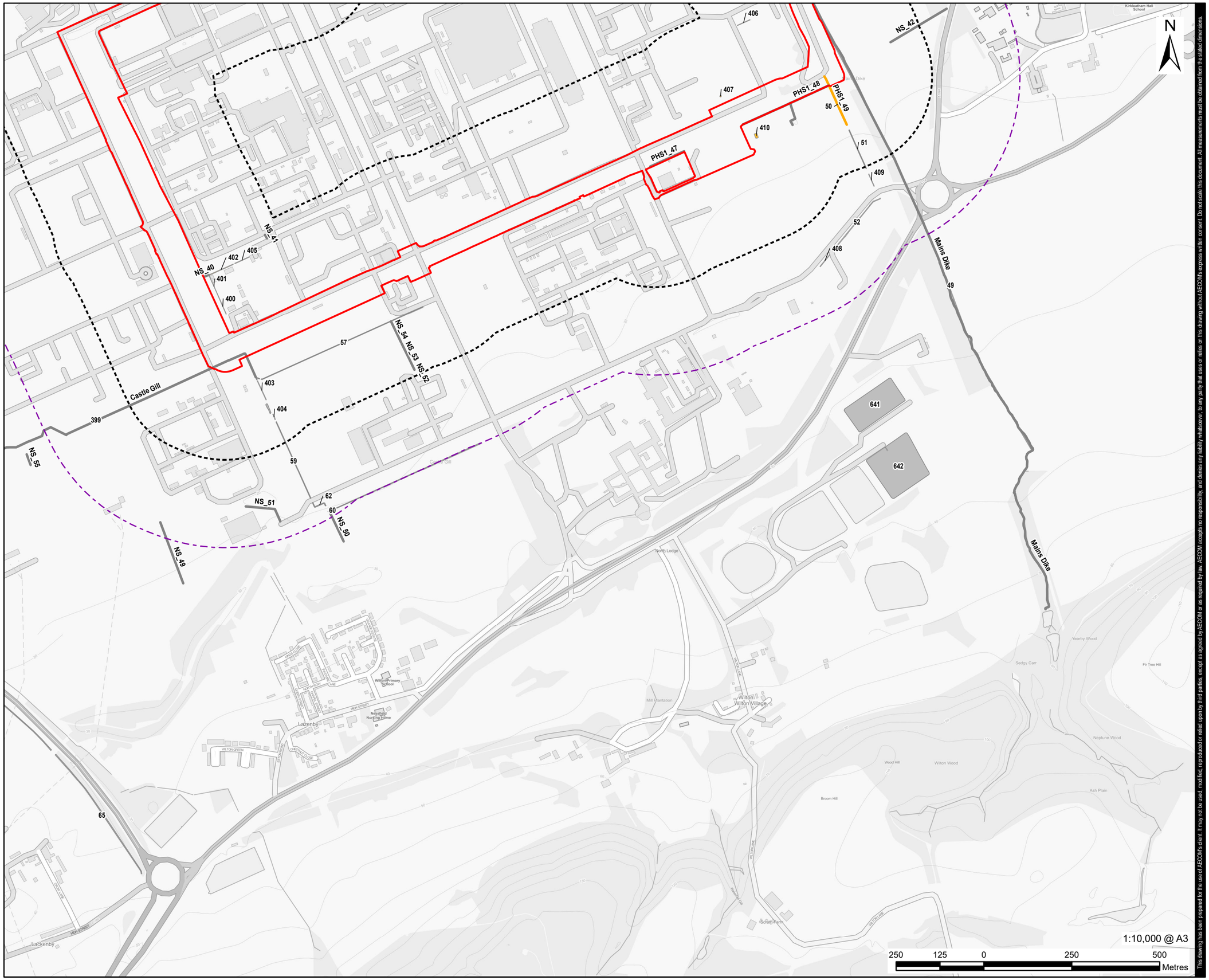

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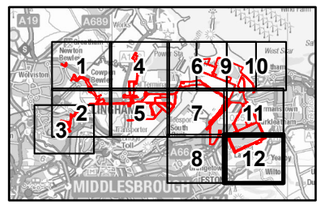
LEGEND

- Proposed Development Site
- Proposed Development Site - 250 m Buffer
- Proposed Development Site - 500 m Buffer

GCN Results

- A - Absent
- Scoped Out
- A - Absent
- Scoped Out



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ISSUE PURPOSE

Environmental Statement

PROJECT NUMBER

60689030

FIGURE TITLE

Positive eDNA Results

FIGURE NUMBER

Figure 12-B-3 (Page 12 of 12)

12B.8 ANNEX 2: Legislation and Policy

Relevant Legislation, Planning Policy and Guidance

12B.8.1 A summary of the relevant international, national, and local legislation, planning policy, and guidance is set out below.

Legislation

12B.8.2 The following legislation is relevant to this report:

- Wildlife and Countryside Act 1981 (as amended (WCA);
- The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019;
- European Council Directive 92/43/EEC (Annex II and IV); and,
- Bern Convention (Appendix II).

12B.8.3 GCN are protected in England under The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 (The Habitat Regulations) and are included on Schedules 5 of the Wildlife and Countryside Act 1981 (as amended). The above collectively prohibits the following:

- Deliberately or recklessly capturing, injuring, taking or killing of a GCN;
- Deliberately or recklessly harassing a GCN;
- Intentionally or recklessly disturbing of a GCN in its place of rest, or which is used for protection or rearing young;
- Deliberately or recklessly damaging, destroying or obstructing access to any resting place or breeding area used by GCN;
- Deliberately or recklessly disturbing a GCN in any way which is likely to significantly affect the local populations of the species, either through affecting their distribution or abundance, or affect any individual's ability to survive, reproduce or rear young; and,
- Possession or advertisement/sale/exchange of a GCN (dead or alive) or any part of a GCN.

12B.8.4 GCN are included as a European Protected Species under the Annex II and Annex IV of Council Directive 92/43/EEC.

12B.8.5 GCN are also strictly protected under Appendix II of the Bern Convention. This Appendix prohibits the capture, injuring/killing, disturbance and trade of GCN.

12B.8.6 Natural England has also published guidance for Local Planning Authorities (LPAs) in determining planning decisions, for proposals which have the potential to affect GCN alongside other protected species. The guidance sets out responsibilities and minimum requirements for GCN surveys and potential mitigation (Natural England, 2022).

Biodiversity Action Plans

12B.8.7 The UK Biodiversity Action Plan (BAP) was withdrawn in March 2011 with the lists of Priority Species and Habitats being superseded by those within Section 41 of the NERC Act (2006).

12B.8.8 Local Biodiversity Action Plans (LBAPs) are no longer used as a formal expression of delivery of biodiversity targets but identify sub-regional priorities for nature conservation and propose agreed actions to conserve, maintain, enhance and increase local Priority Species and Habitats.

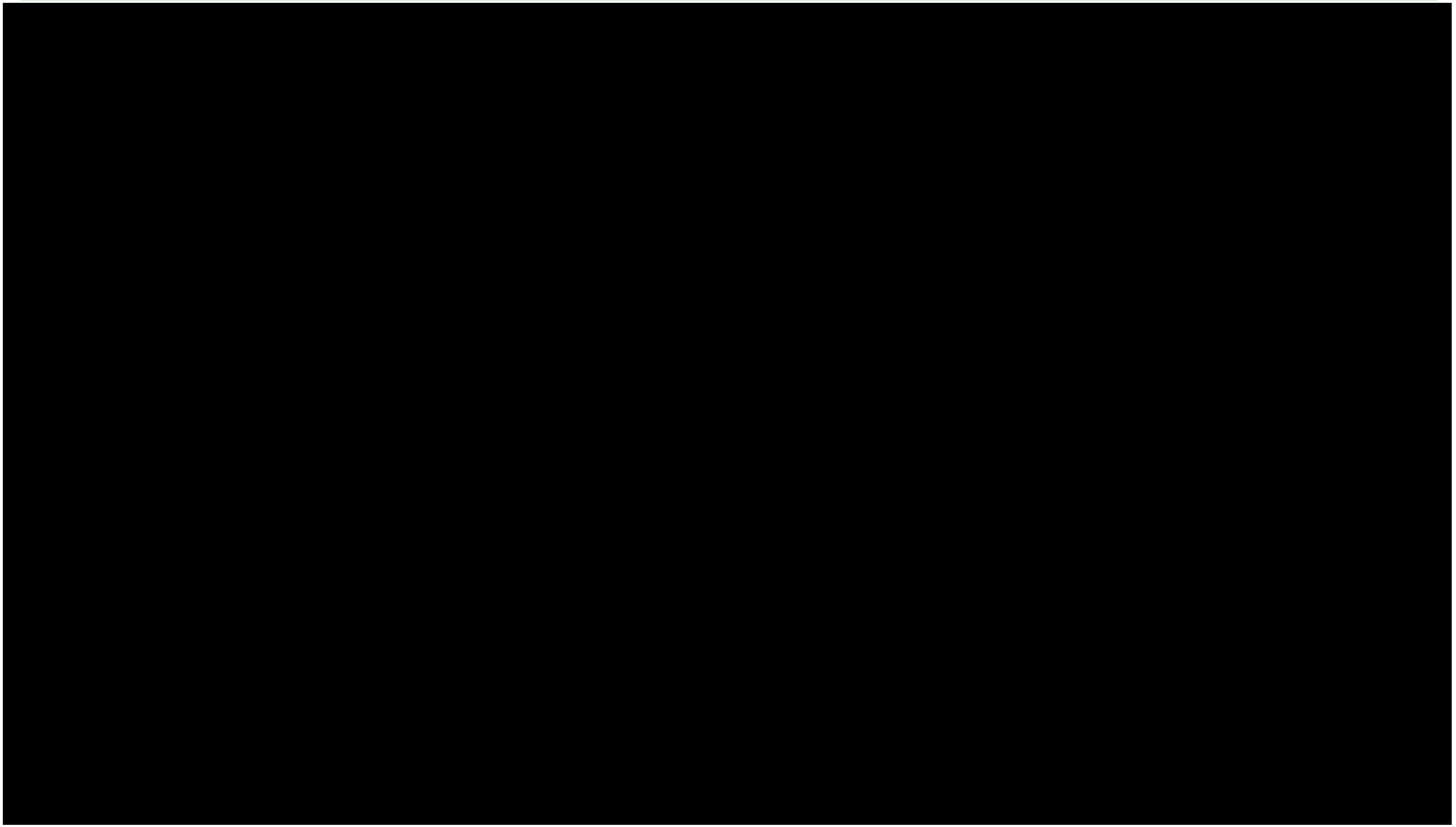
12B.8.9 The Tees Valley Biodiversity Action Plan (Tees Valley Nature Partnership, 2012) is the relevant LBAP for the defined Study Area and was updated in in 2012. The LBAP outlines biodiversity conservation objectives within the region and identifies priorities for action for priority habitats, species, locally important wildlife, and sites.

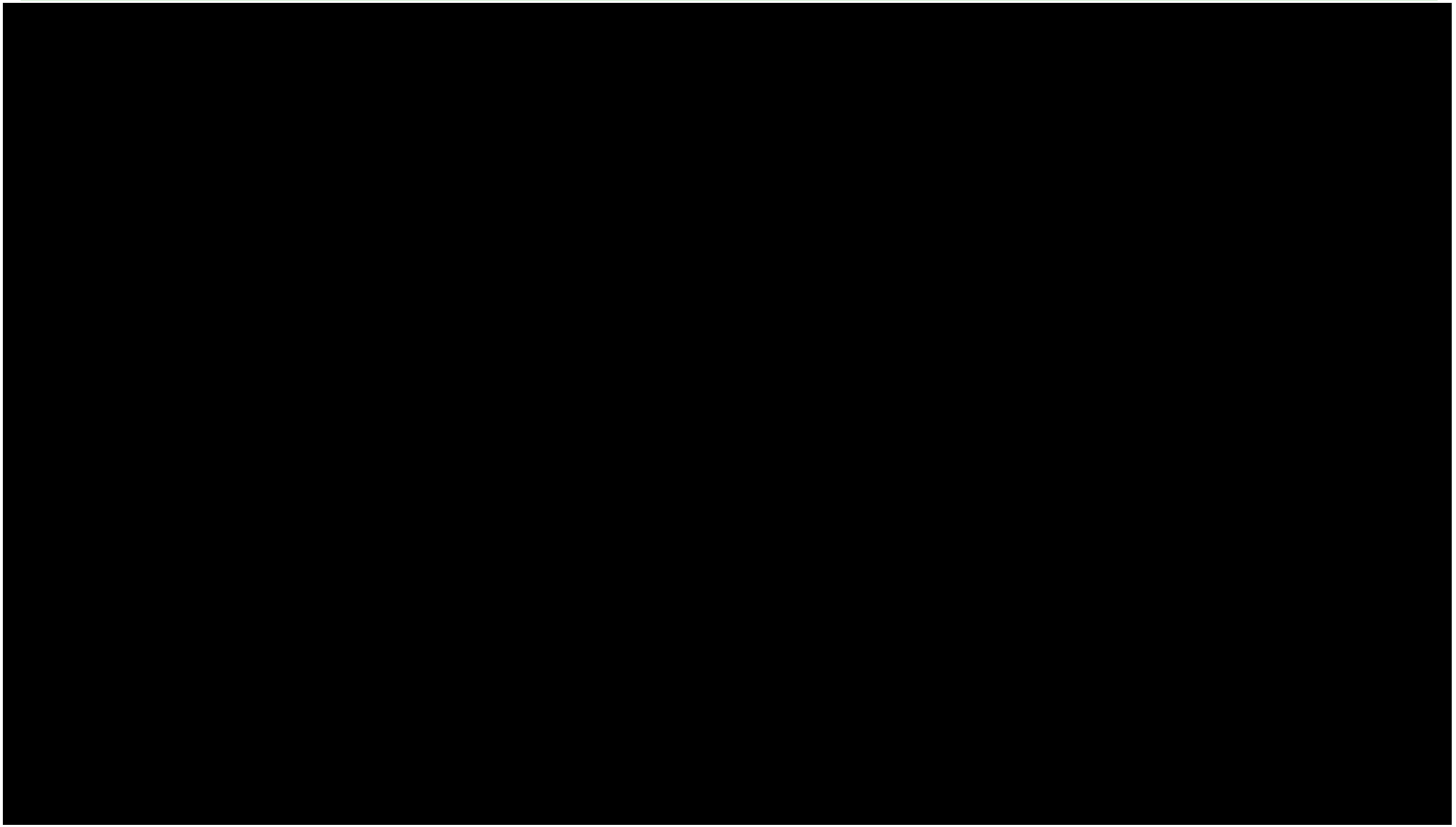
12B.8.10 The LBAP states “Britain is an important stronghold for the species. It is widely distributed in England but with a decline in range and abundance in recent years. The main factor for their decline is loss of suitable breeding ponds and loss and fragmentation of terrestrial habitats. They are widely distributed across most of the Tees Valley but there are no current, confirmed records from Middlesbrough and few from around the lower Tees Estuary. As much of the land in the lower Tees Estuary is reclaimed, it is possible that great crested newts were never present in these areas. There are healthy populations around the Eaglescliffe and Cowpen Bewley area but otherwise populations seem to be small and fragmented.”

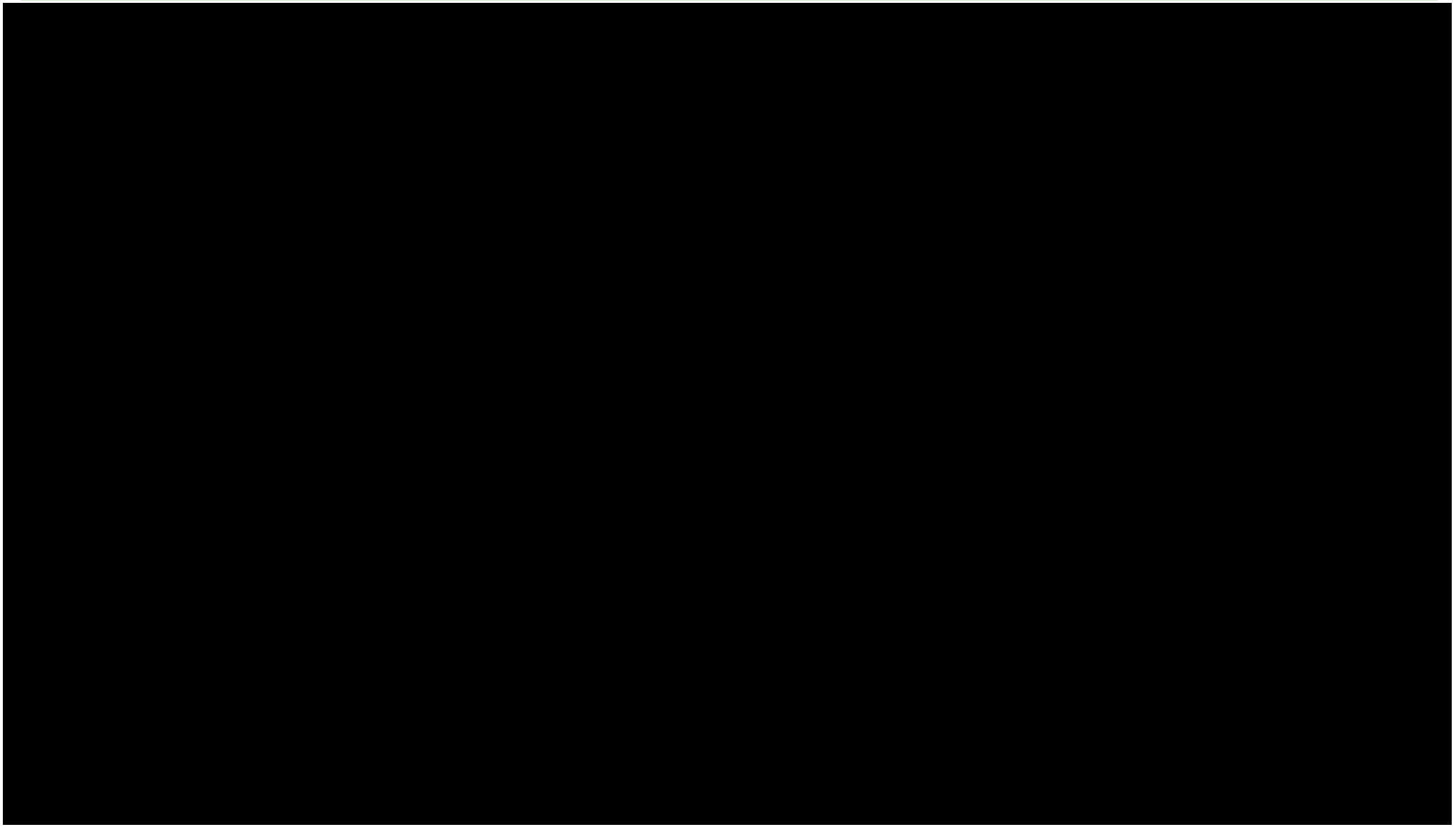
12B.9 ANNEX 3: HSI Data

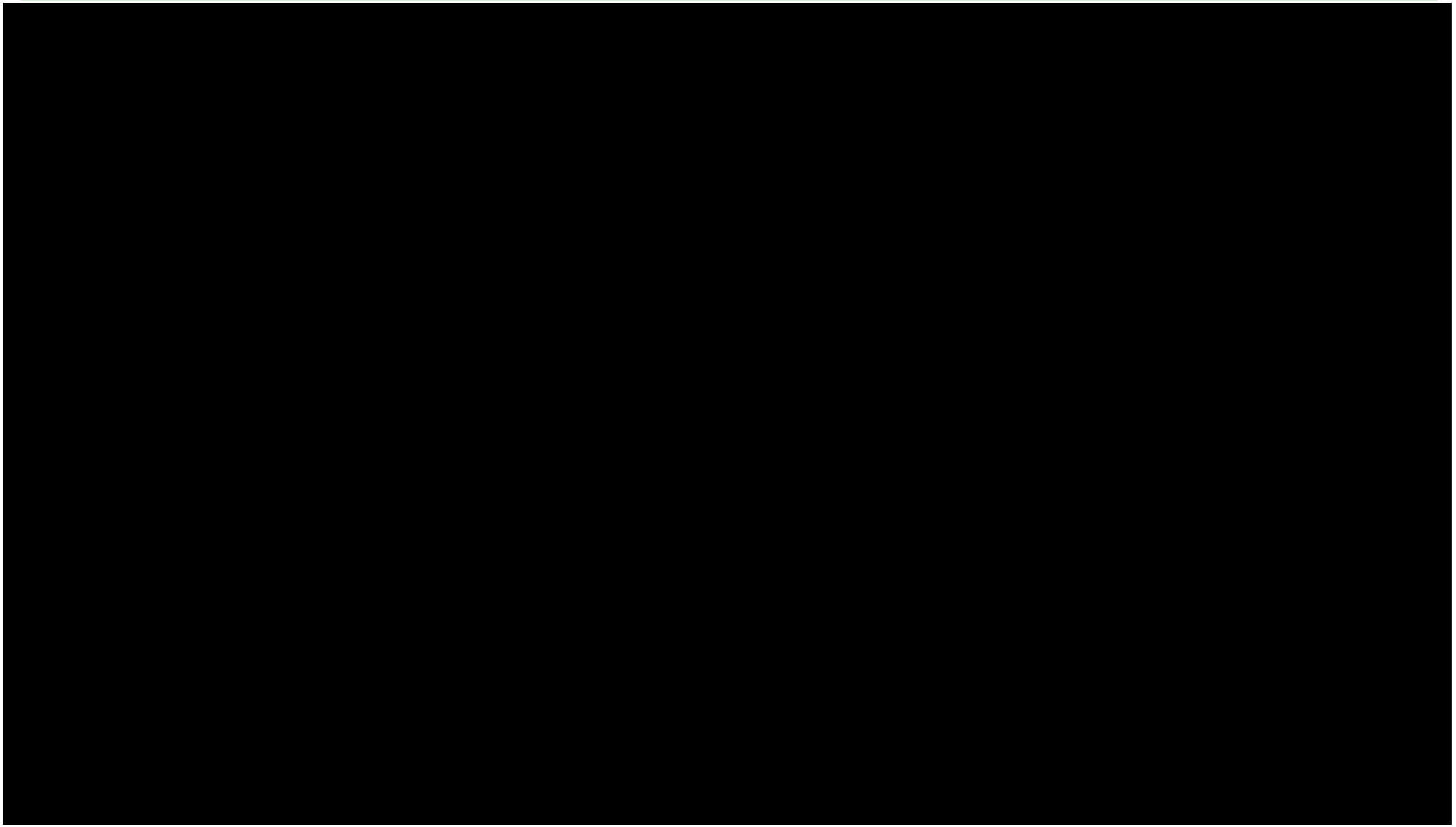
WATERBODY ID	DATE	DESCRIPTION	SITE LOCATION	POND AREA	WATER QUALITY	% OF POND PERIMETER SHADED	WATERFOWL	FISH EVIDENCE/ POPULATION	PERMANENCE	TERRESTRIAL HABITAT FOR FORAGING/SHELTER	NUMBER OF PONDS WITHIN 1KM OF SURVEY POND	MACROPHYTES	HS SCORE	HS RATING
312	04/06/2023	Country park pond	1	0.05	0.67	1	1	1	1	1	0.96	0.3	0.68	Average
313	04/06/2023	Large woodland pond	1	0.2	1	1	1	0.67	0.9	1	0.96	0.55	0.77	Good
315	04/06/2023	Pond within a woodland clearing	1	0.2	1	1	1	1	1	1	0.96	0.3	0.75	Good
316	04/06/2023	Large waterbody within woodland	1	0.4	1	0.3	1	1	0.5	1	0.96	0.3	0.67	Average
NO ID	06/09/2023	Small pond (drying out)	1	0.1	0.67	1	1	1	0.1	1	0.75	0.35	0.67	Average
34	14/06/2023	Eco Pond at Wilton	1	0.4	1	1	1	0.67	0.9	1	0.75	0.45	0.78	Good
90	06/07/2023	Large lake on the Brine fields	1	0	1	1	0.01	0.67	0.9	0.67	0.87	0.35	0.5	Below average
184	06/07/2023	Brine fields waterbody	1	0.6	0.67	1	1	1	0.9	0.67	0.93	0.35	0.77	Good
186	06/07/2023	Linear manmade waterbody on the Brine fields	1	0.2	0.67	1	1	1	0.5	0.67	0.93	0.6	0.69	Average
187	06/07/2023	Linear manmade waterbody on the Brine fields	1	0.2	0.67	1	1	1	0.5	0.67	0.93	0.6	0.69	Average
188	06/07/2023	Linear manmade waterbody on the Brine fields	1	0.2	0.67	1	1	1	0.5	0.67	0.93	0.6	0.69	Average
88	06/07/2023	Large manmade pond on Brine fields	1	0.87	1	1	0.01	0.67	0.9	0.67	0.87	0.35	0.5	Below average
180	06/08/2023	Pond with common reed throughout	1	1	1	1	1	1	0.1	1	0.87	0.35	0.71	Good
96	06/09/2023	Large pond with central island on Brine fields	1	0.98	1	1	0.67	1	0.9	0.67	0.93	0.3	0.8	Excellent
94	06/09/2023	Small waterbody on Brine fields (drying out)	1	0.6	0.67	1	1	1	1	0.67	0.93	0.5	0.81	Excellent

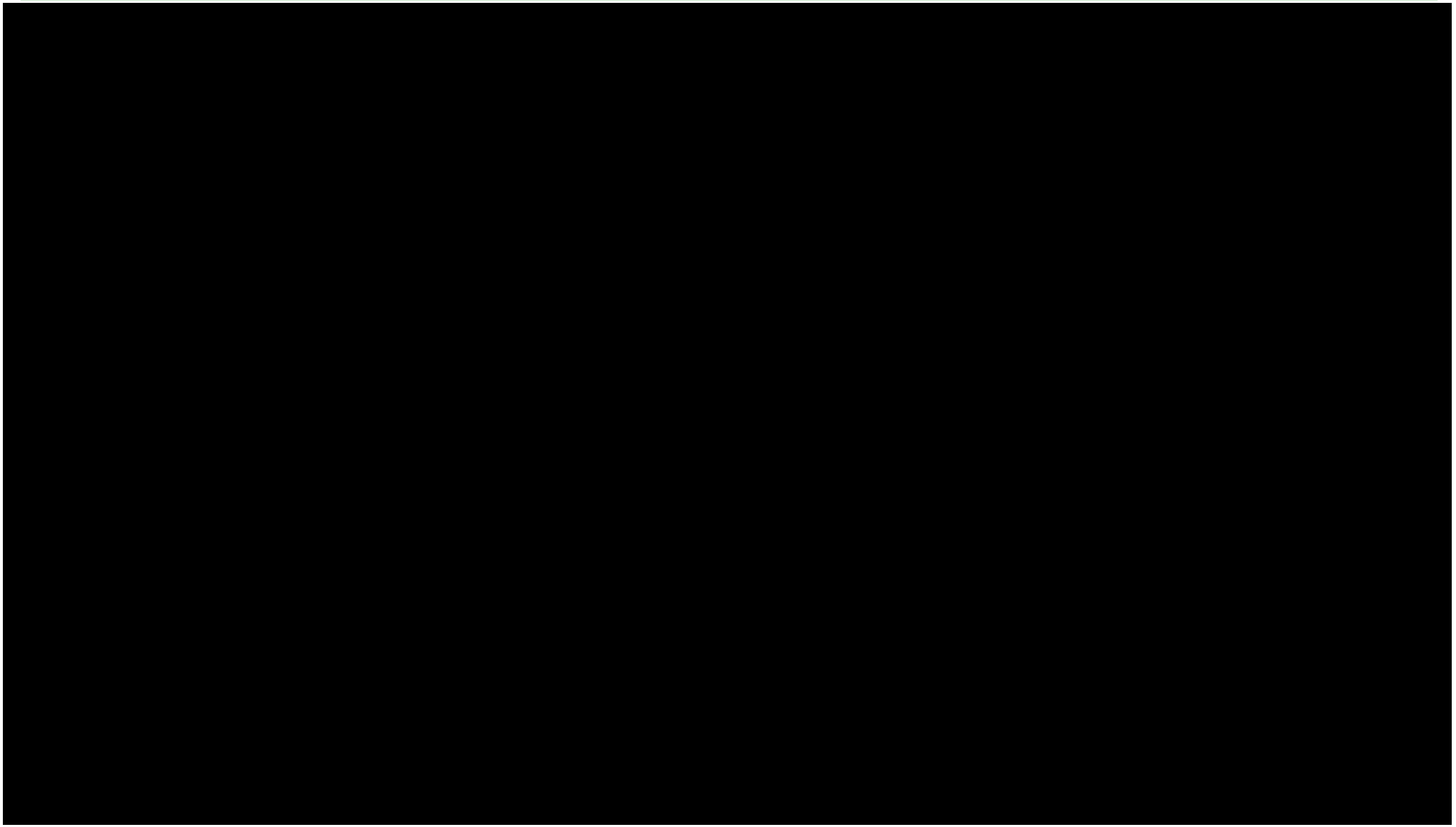
WATERBODY ID	DATE	DESCRIPTION	SITE LOCATION	POND AREA	WATER QUALITY	% OF POND PERIMETER SHADED	WATERFOWL	FISH EVIDENCE/ POPULATION	PERMANENCE	TERRESTRIAL HABITAT FOR FORAGING/SHELTER	NUMBER OF PONDS WITHIN 1KM OF SURVEY POND	MACROPHYTES	HS SCORE	HS RATING
264	06/09/2023	Large ditch with dense common reed on eastern side	1	1	1	1	0.67	1	0.9	0.67	0.93	0.85	0.9	Excellent
NO ID	06/09/2023	Flooded depression in field	1	0.1	0.67	1	1	1	0.1	1	0.75	0.35	0.9	Excellent
12 / 145	06/12/2023	Swamp with a foot of water	1	1	1	1	0.67	1	0.5	1	1	0.35	0.8	Excellent
2	27/06/2023	Artificial pond	1	0.98	0.67	1	1	0.67	0.9	0.67	0.75	0.35	0.76	Good
4	28/06/2023	Large pond adjacent to abandoned building	1	0.87	1	1	0.67	0.67	0.9	0.67	0.75	0.4	0.77	Good
44	13/06/2023	Large pond with island	1	0.98	1	1	1	0.67	0.9	0.67	0.75	0.45	0.82	Excellent
14	13/06/2023	Large pond within dunes	1	0.98	1	1	0.67	1	0.5	0.67	0.75	0.3	0.74	Good
23	13/06/2023	Large waterbody start of The Fleet	1	0	1	1	0.01	0.33	0.9	1	0.75	0.3	0.47	Poor
193	13/06/2023	Saline lagoon	1	0.6	1	1	0.01	0.33	0.9	0.33	1	0.4	0.47	Poor

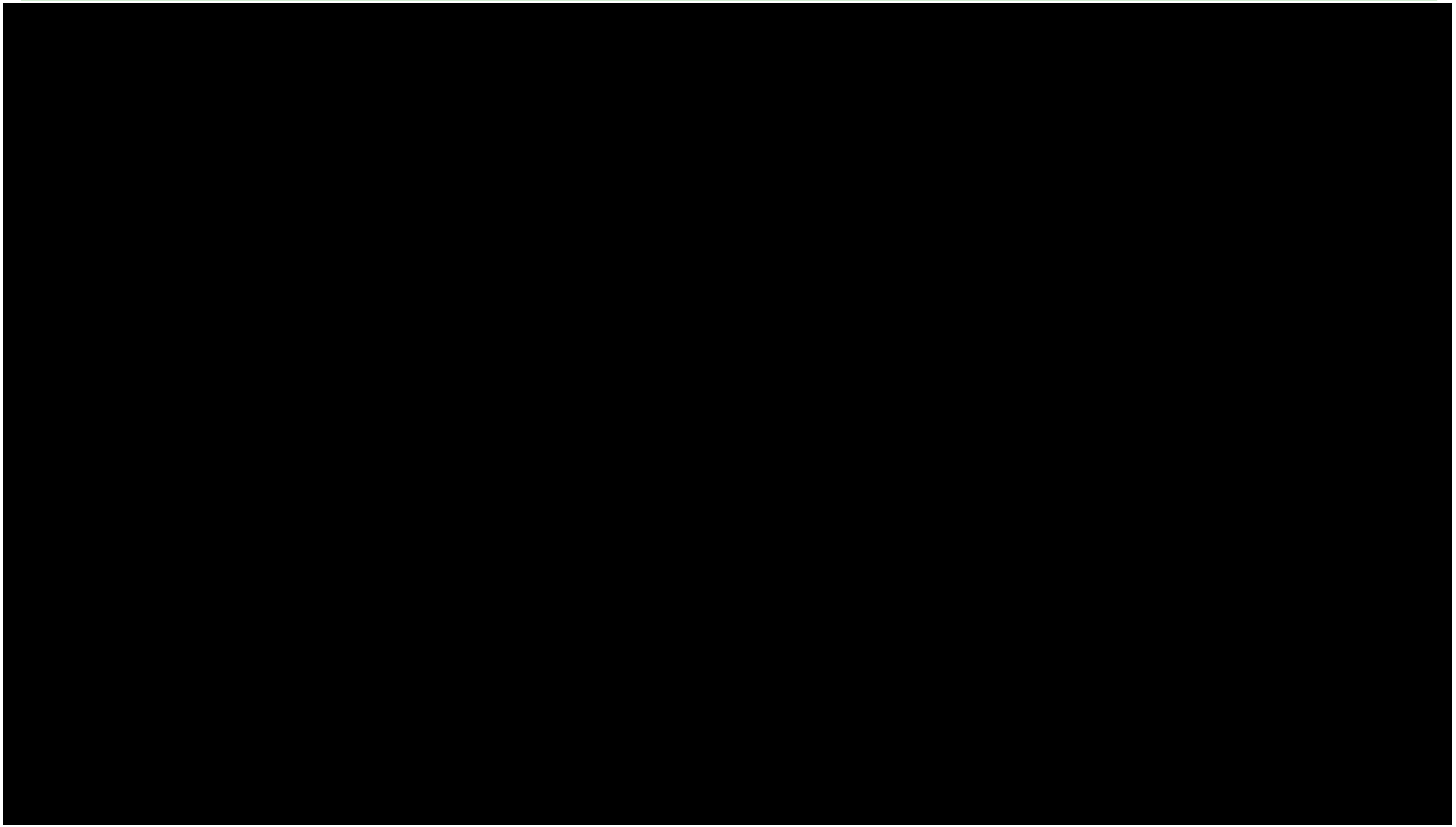


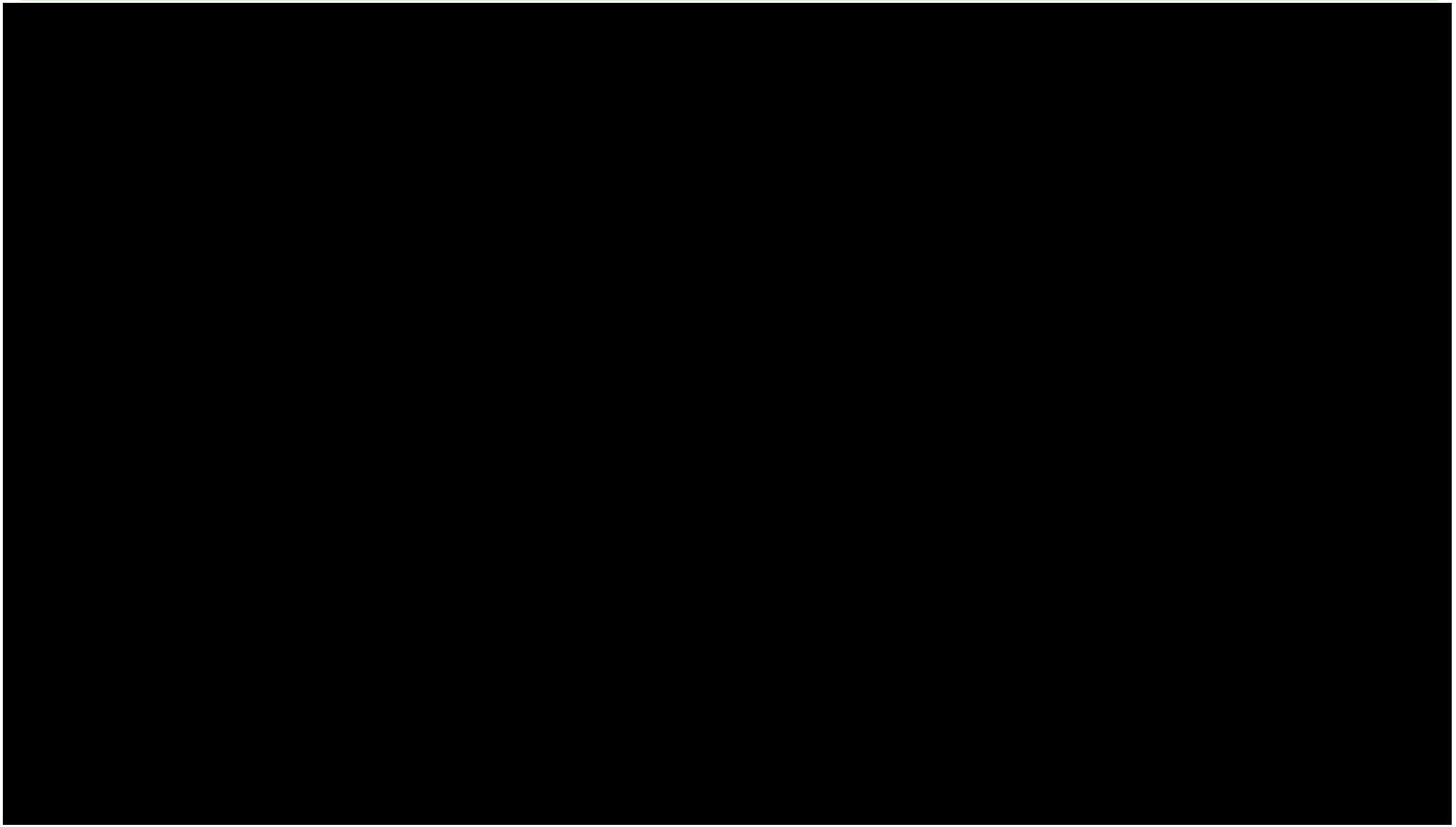


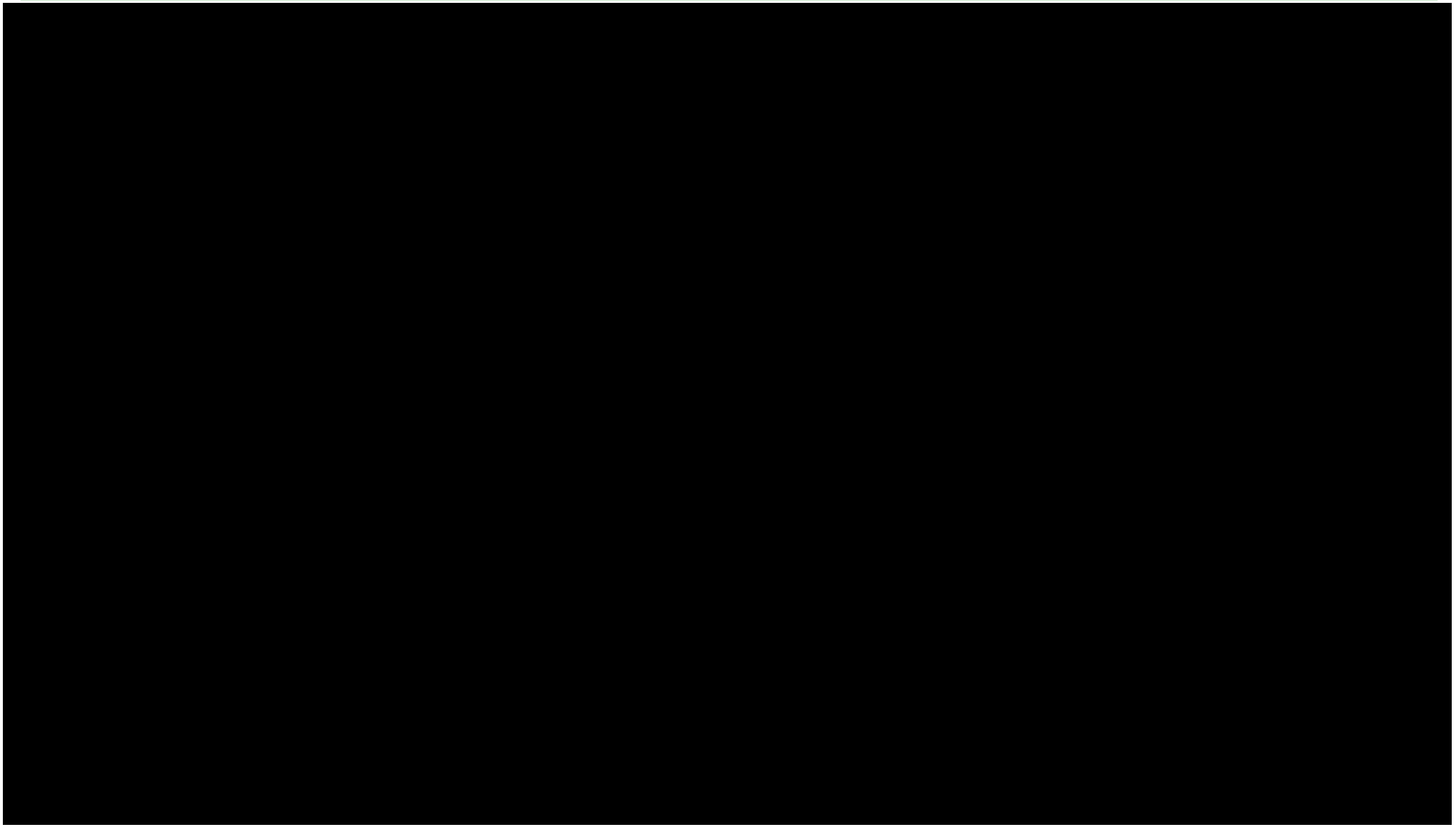


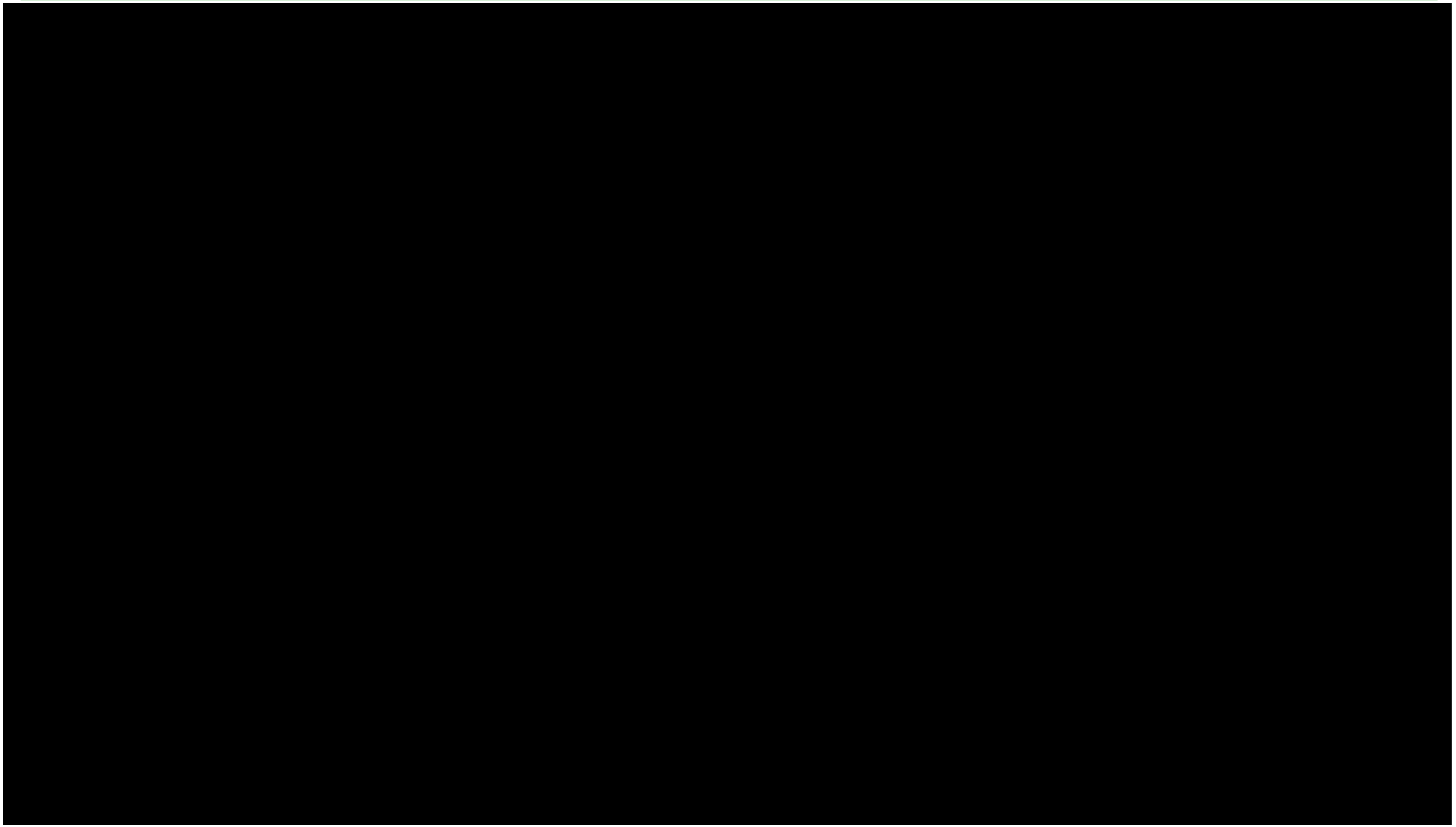


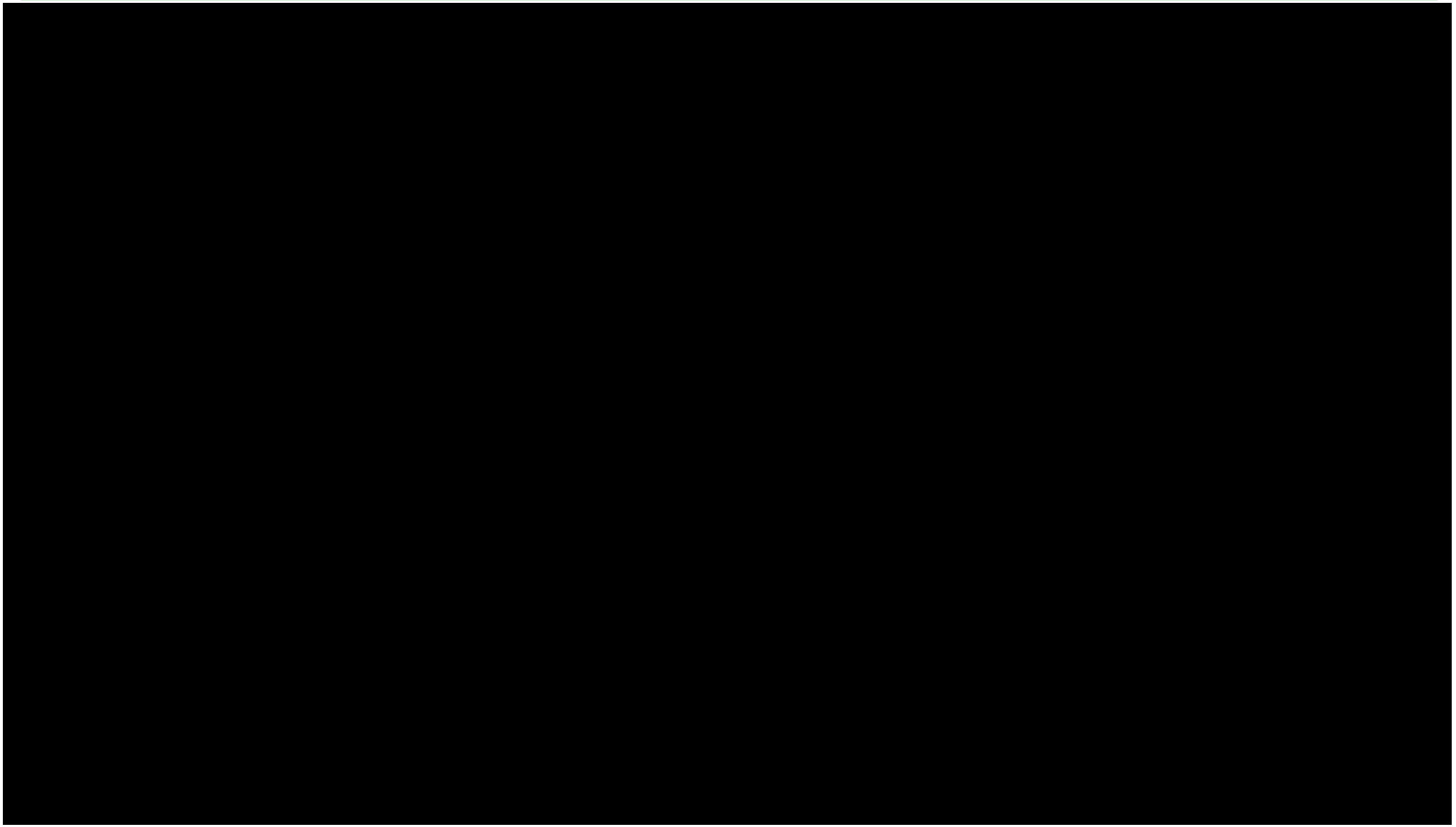


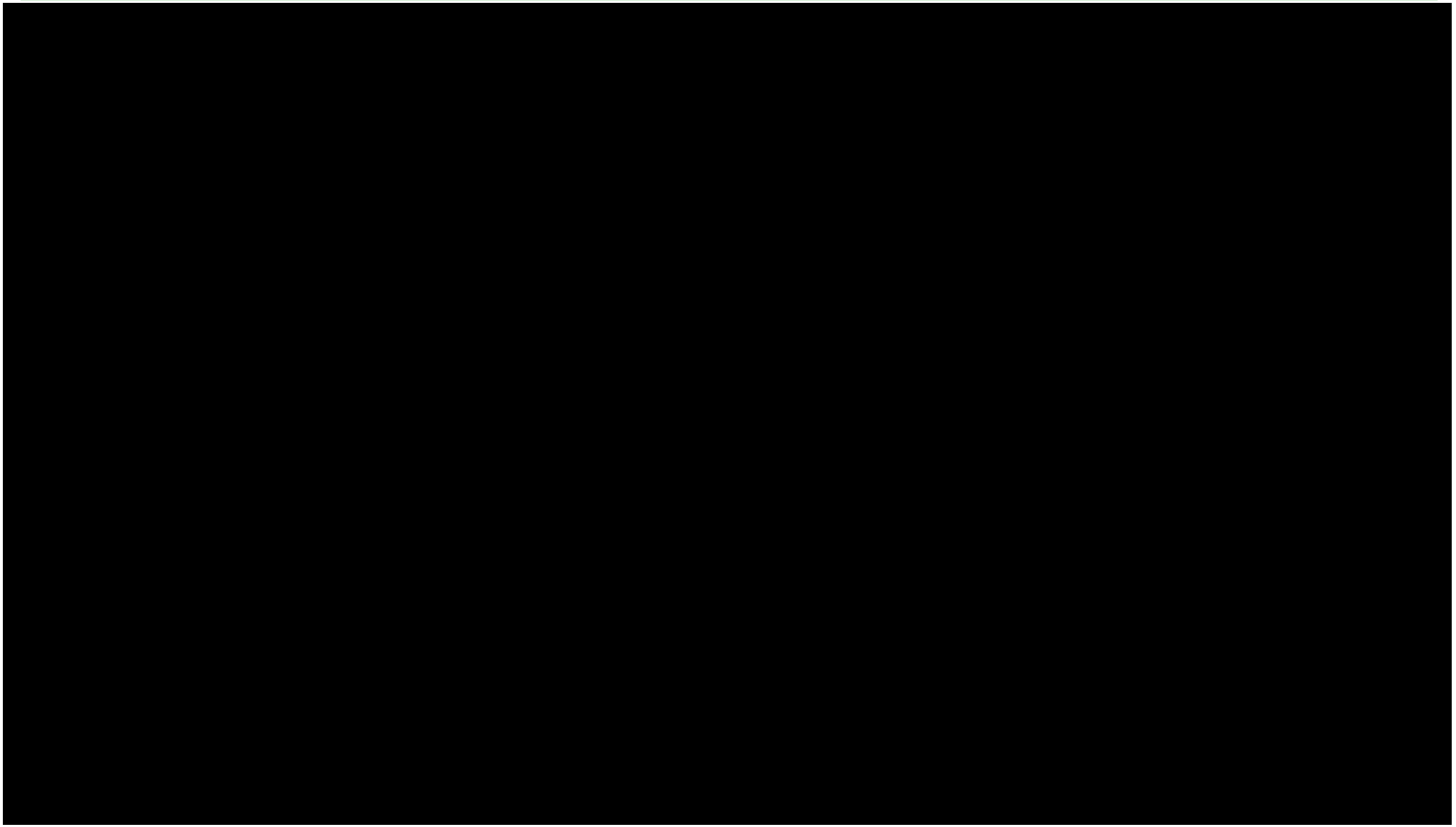


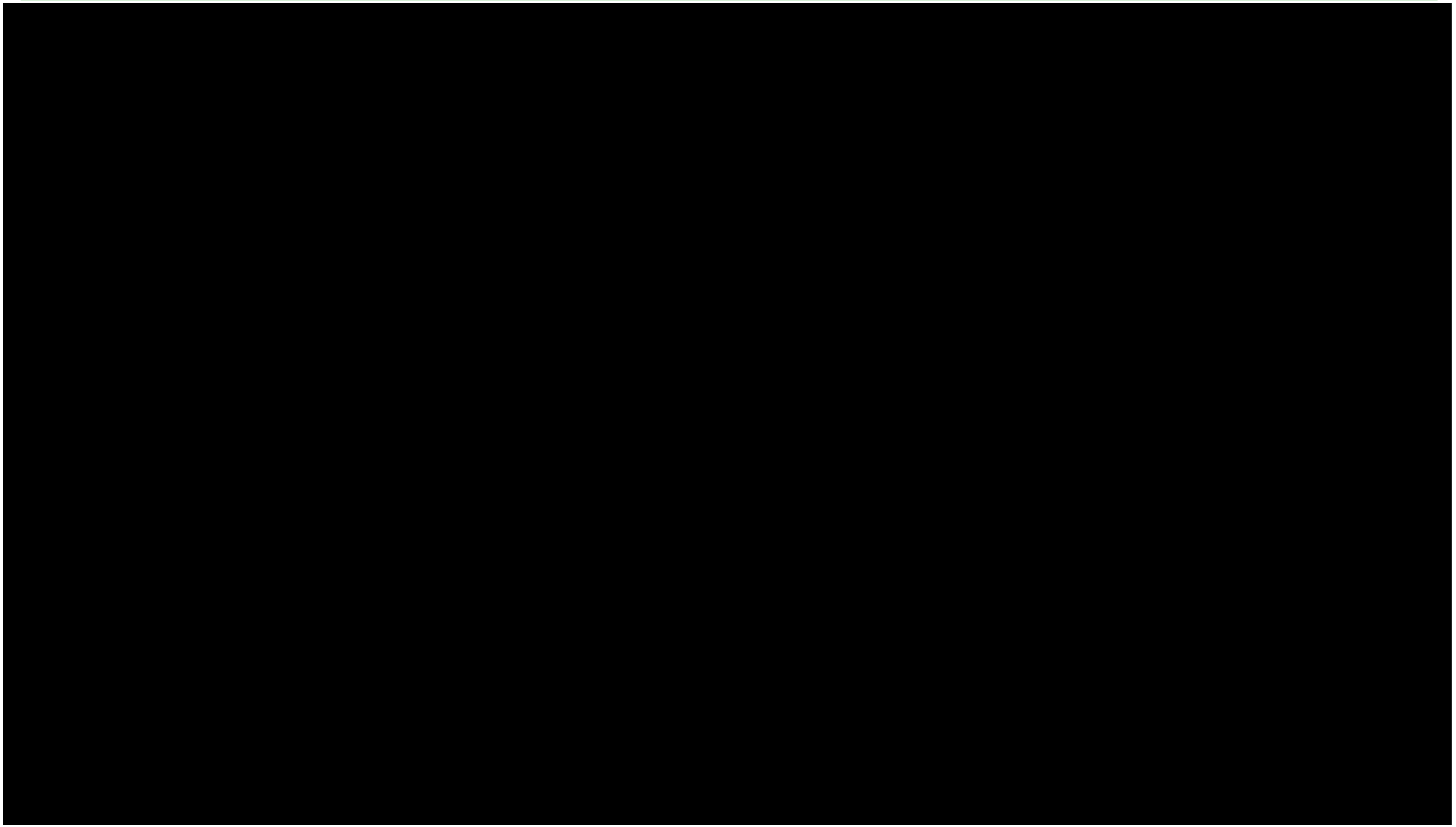


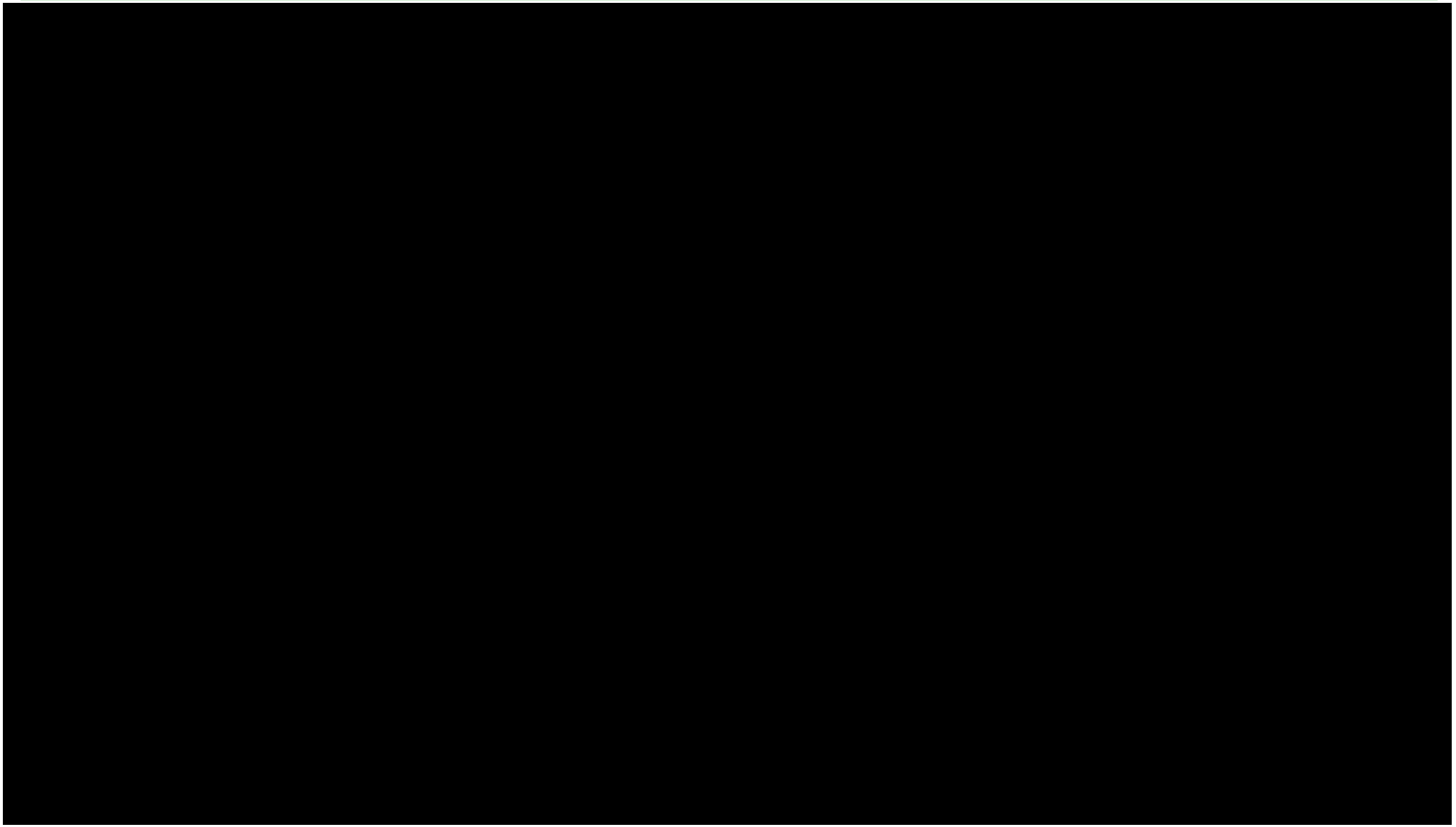


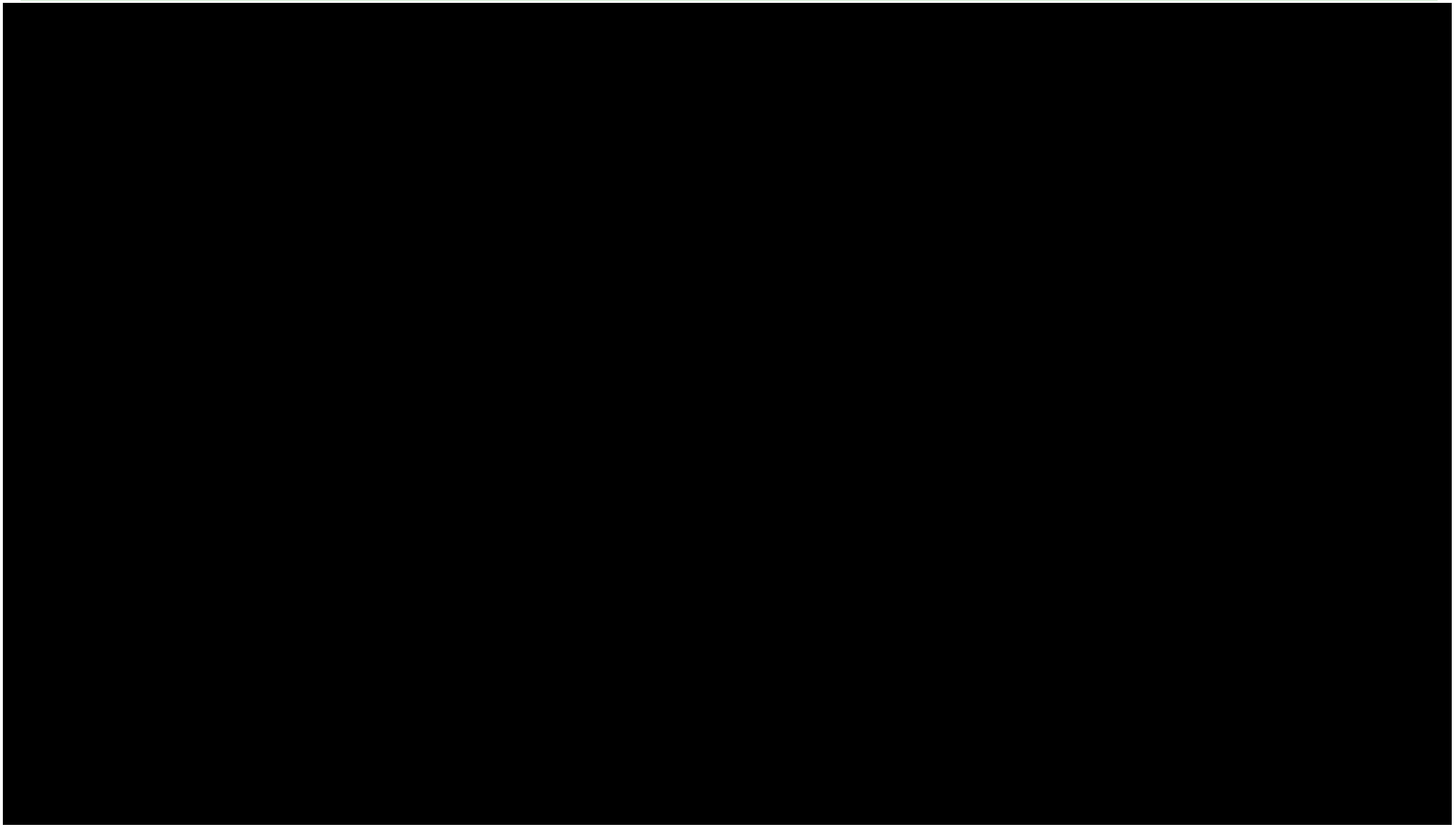


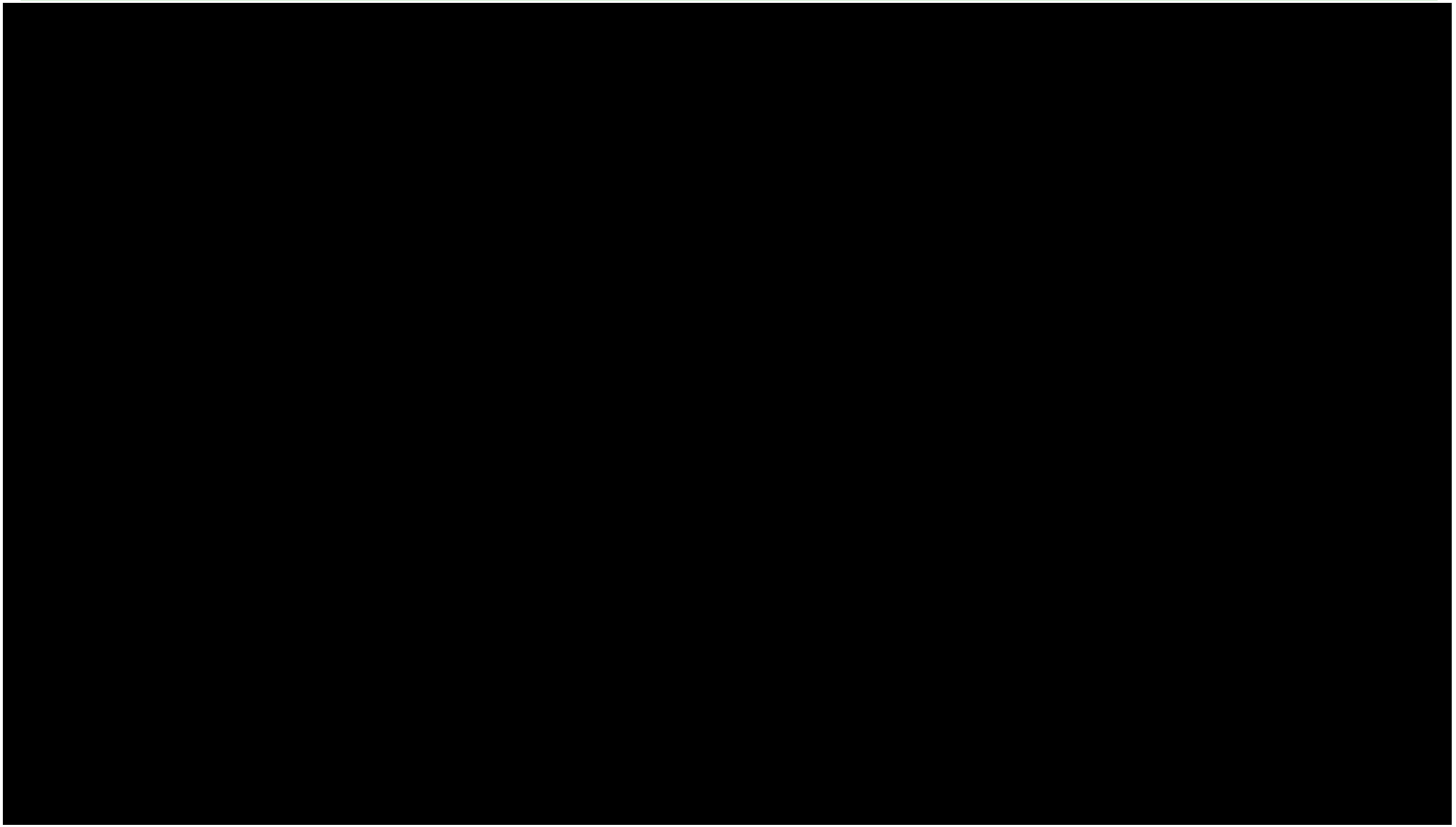


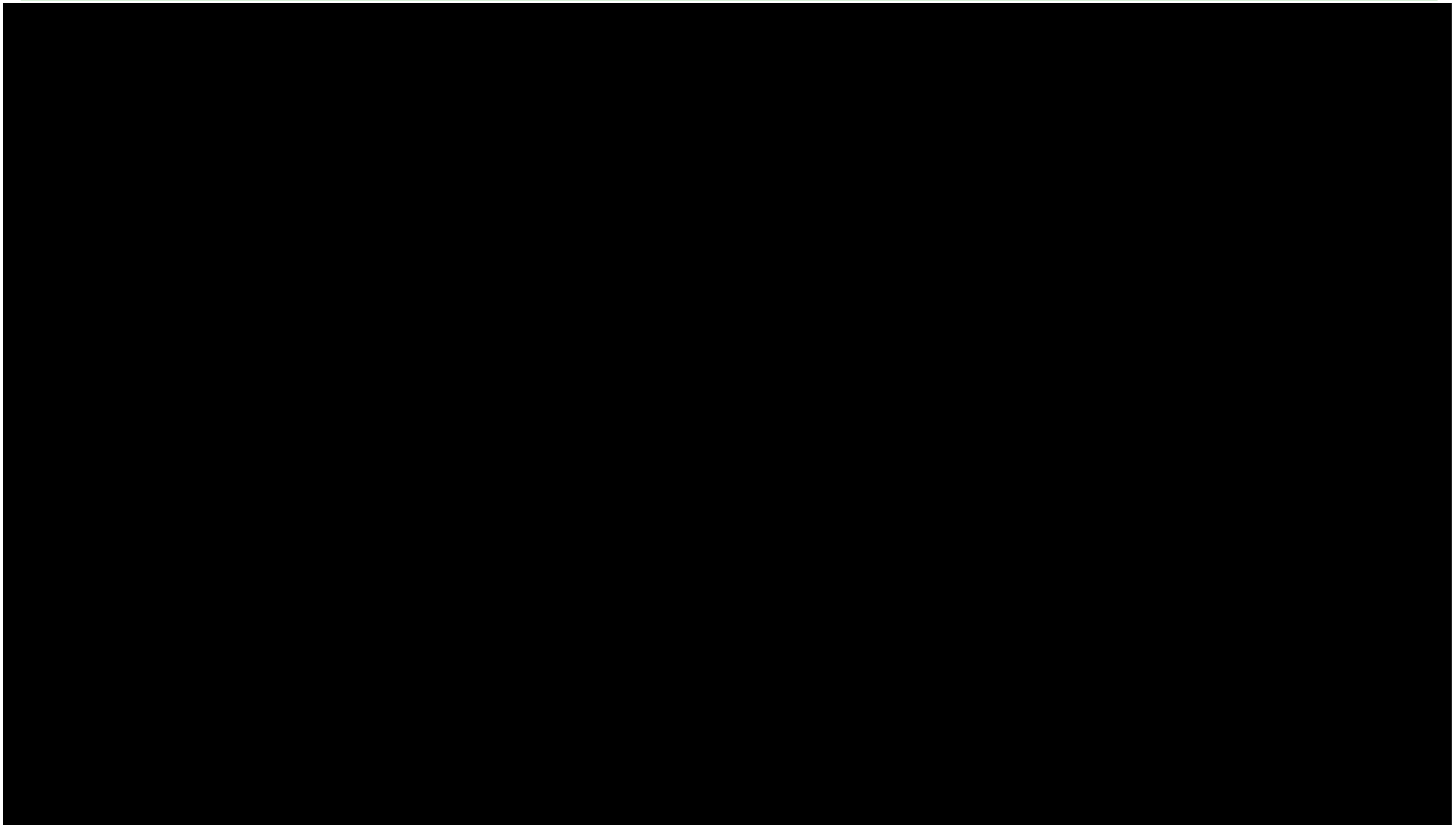


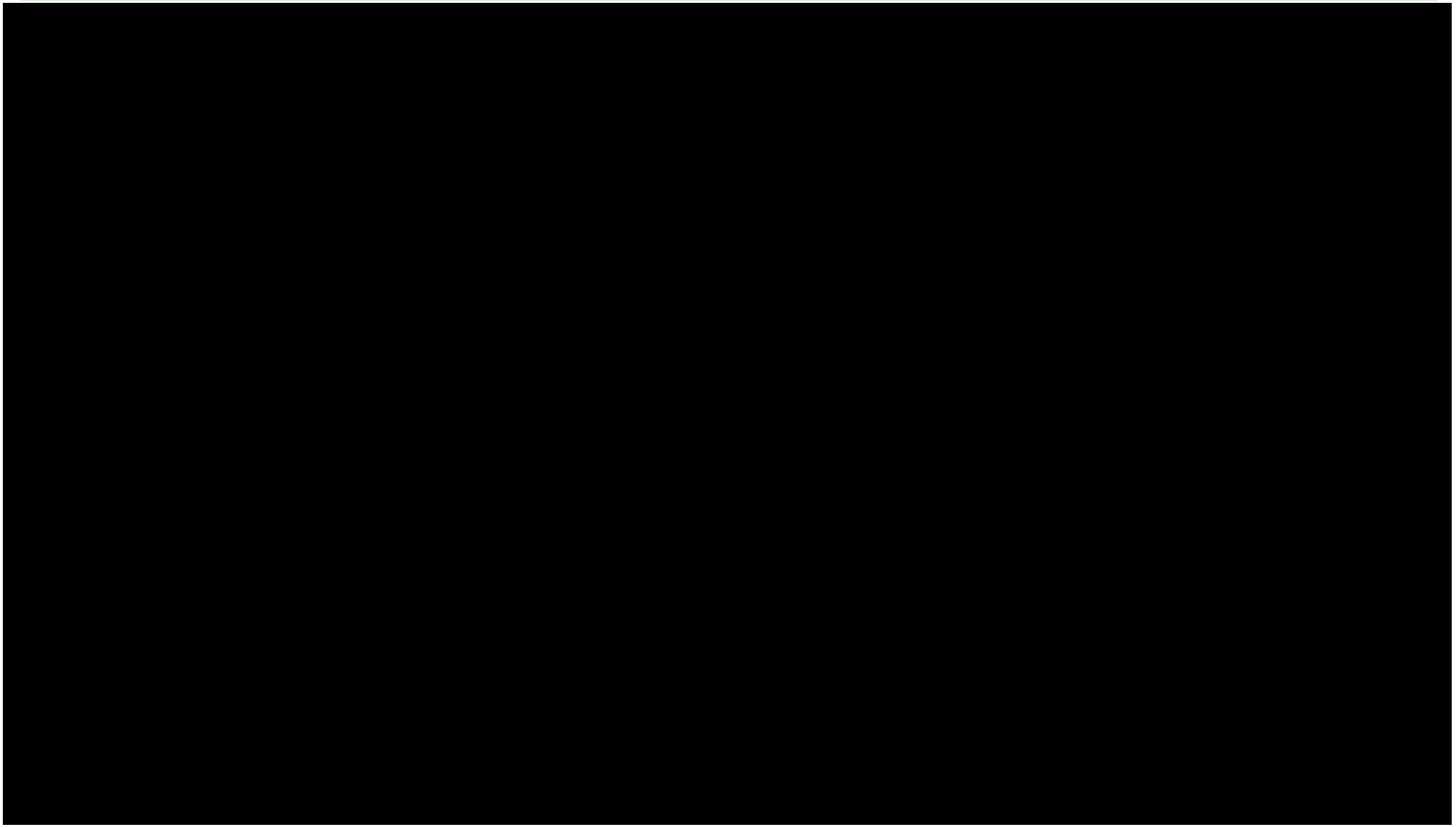


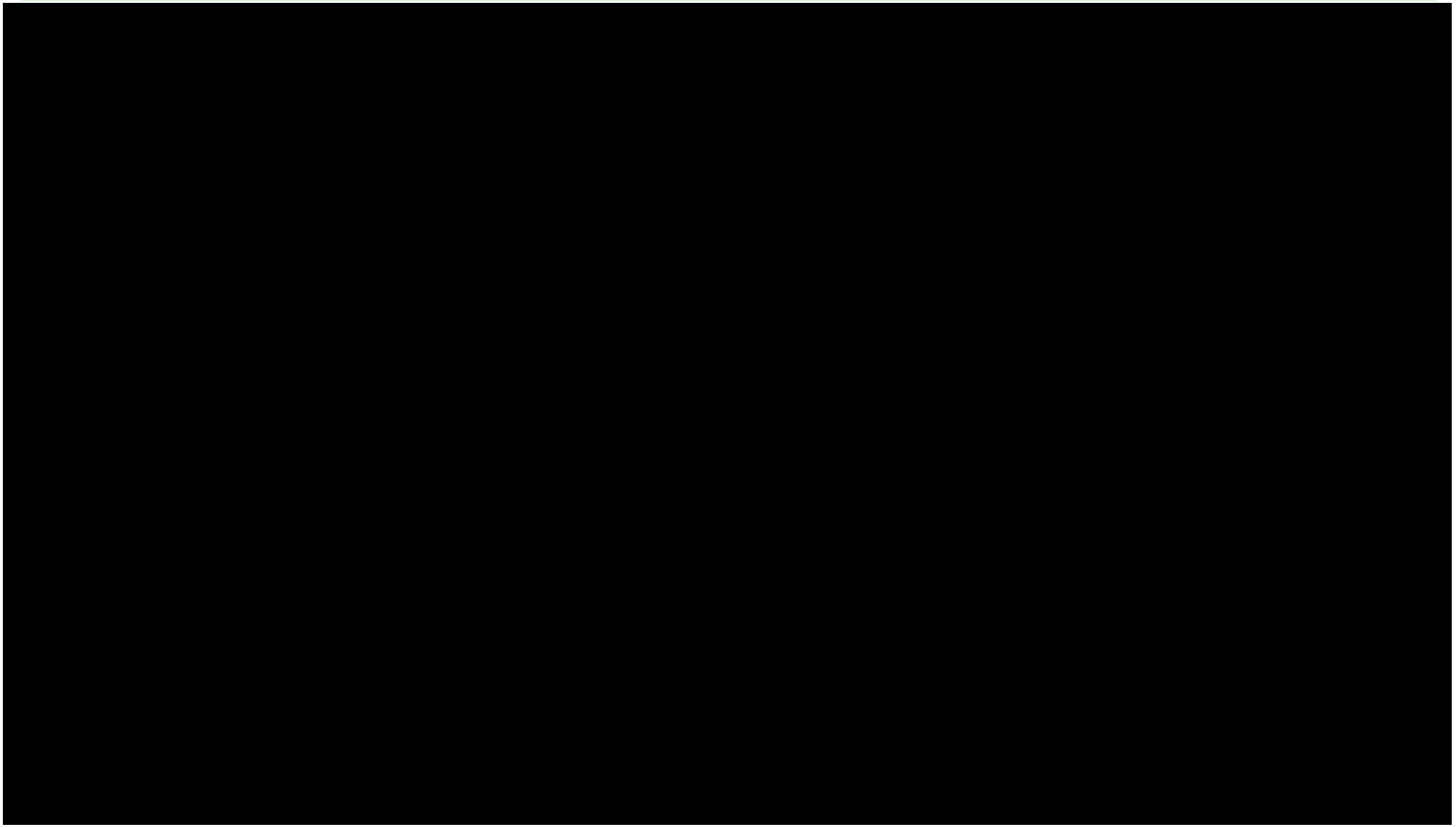


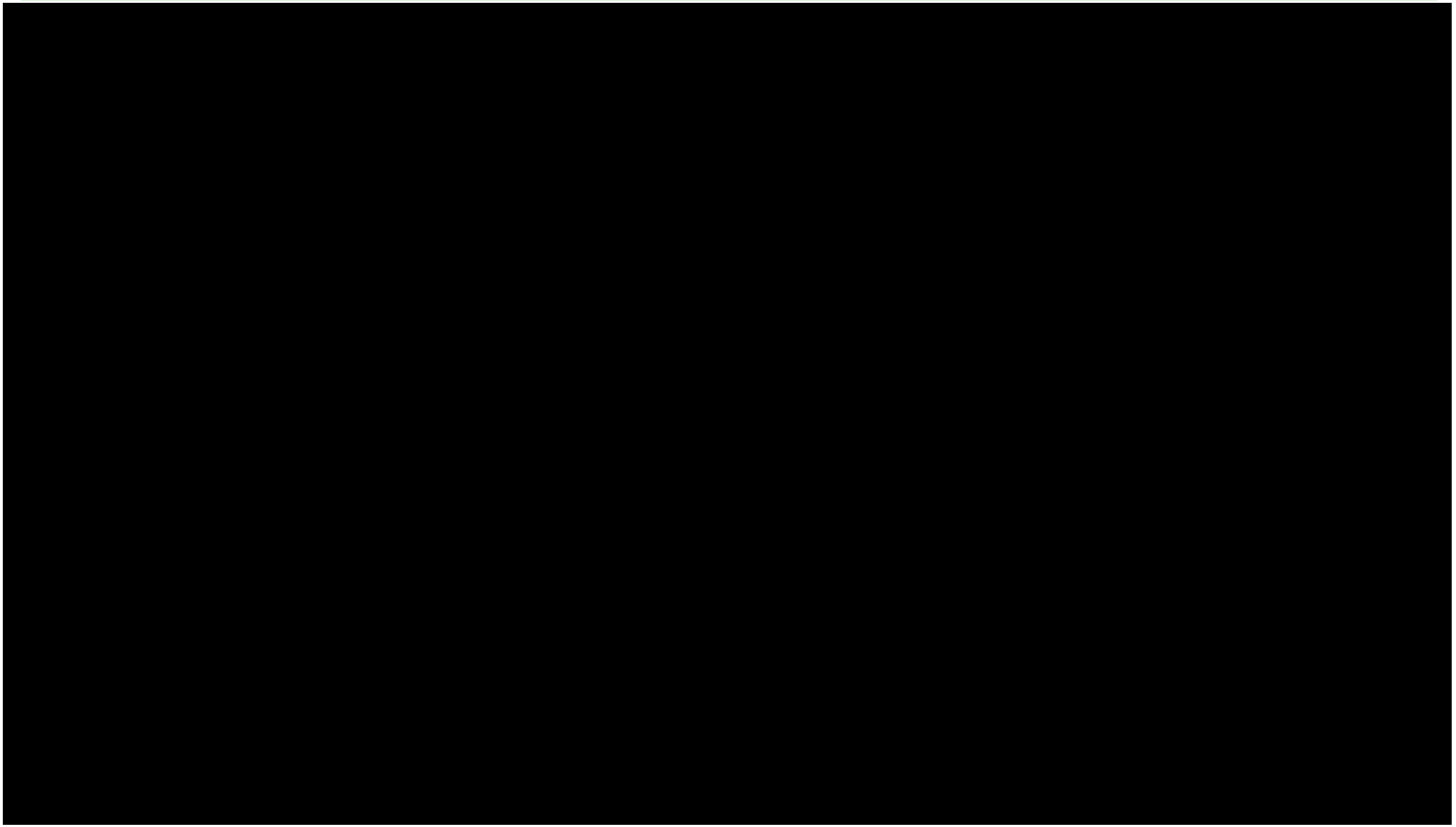


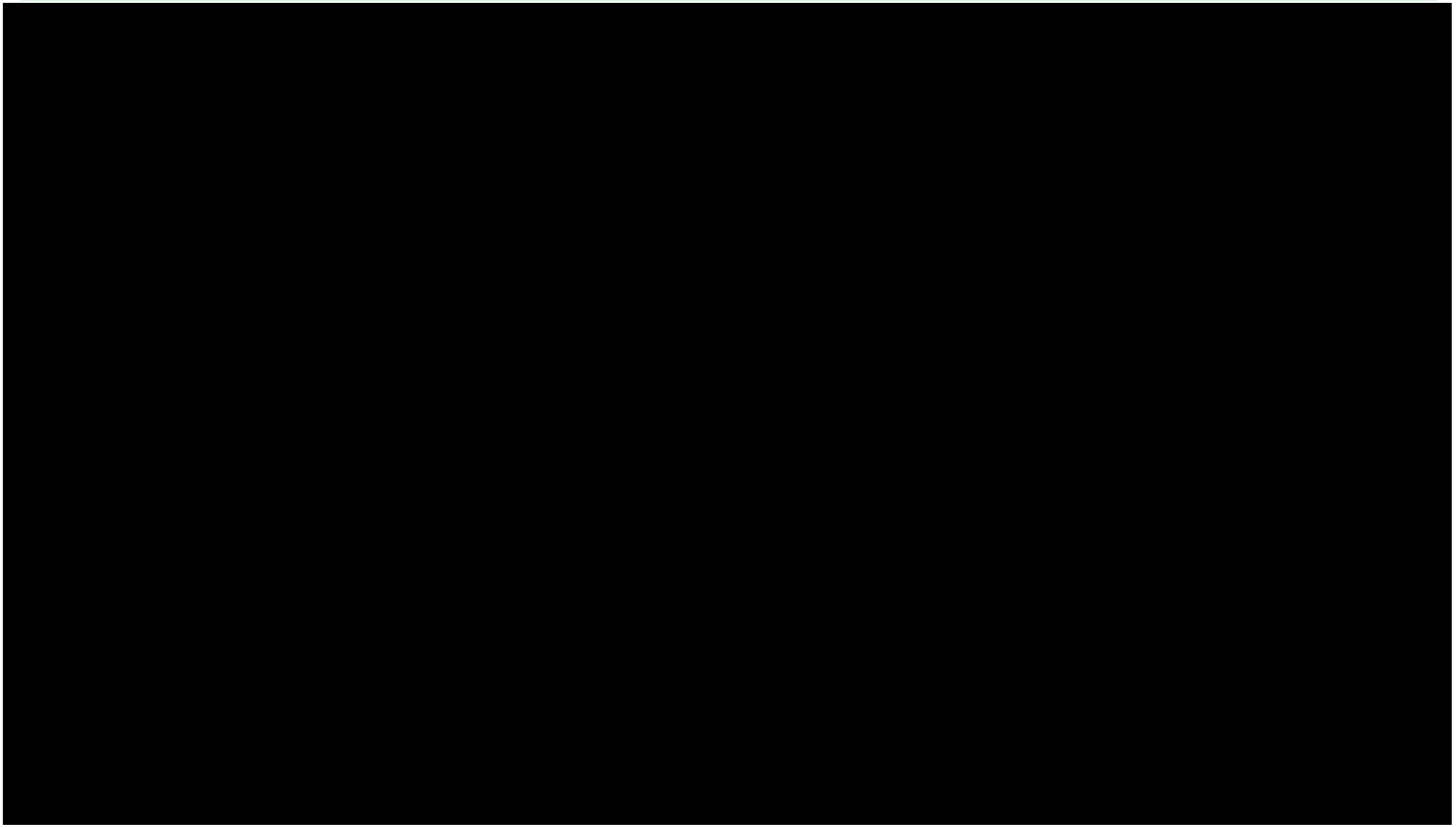


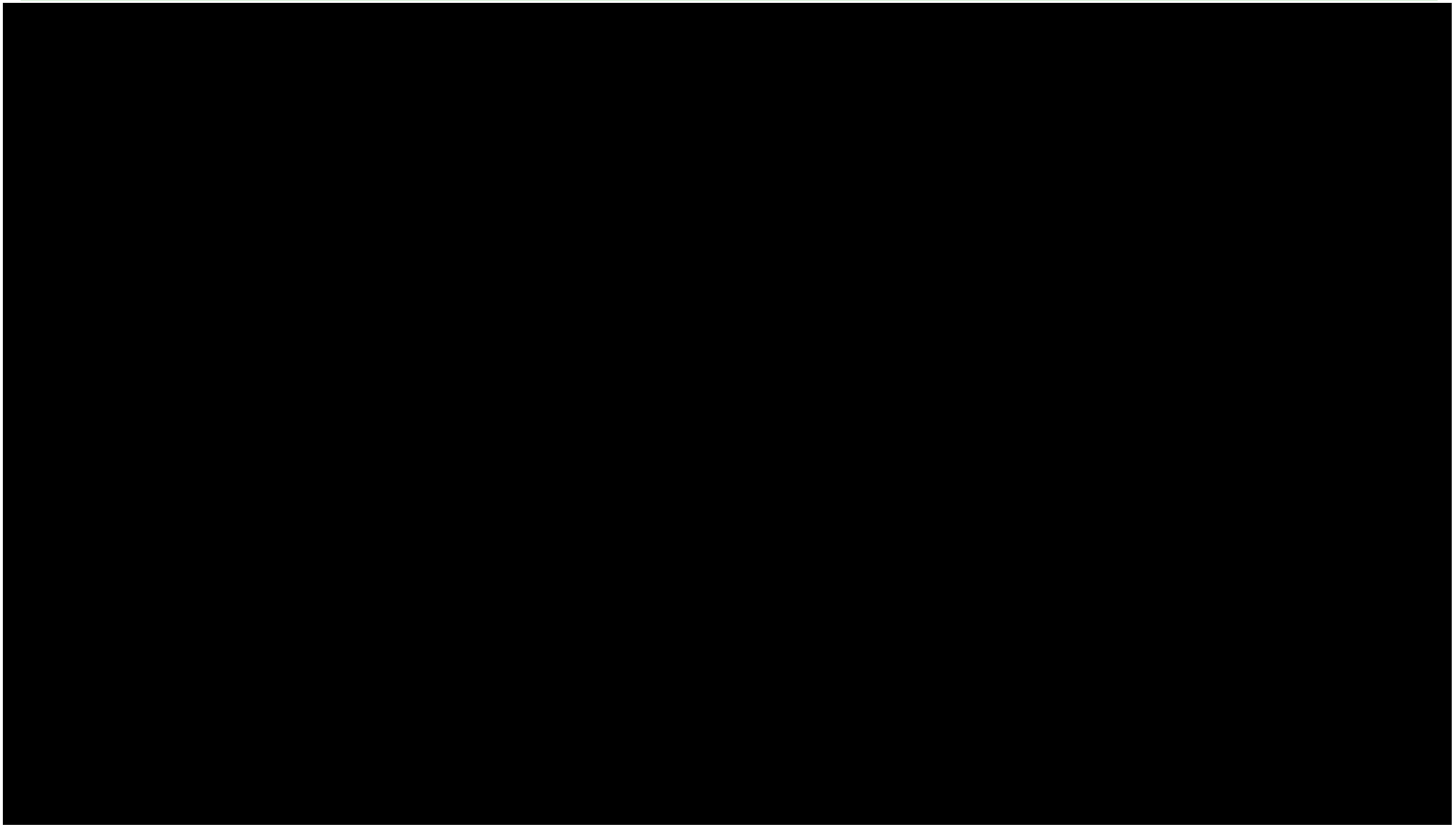


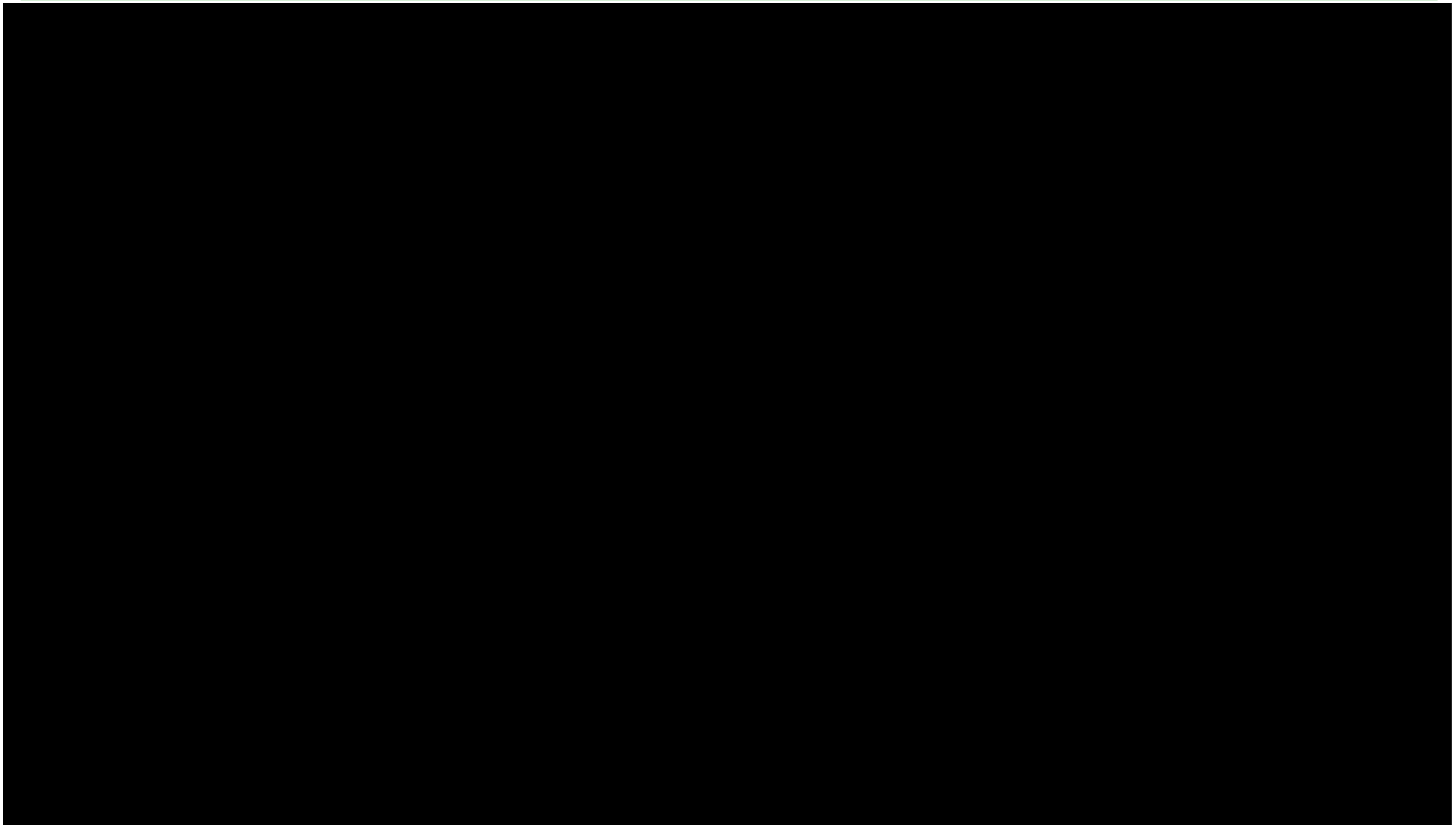


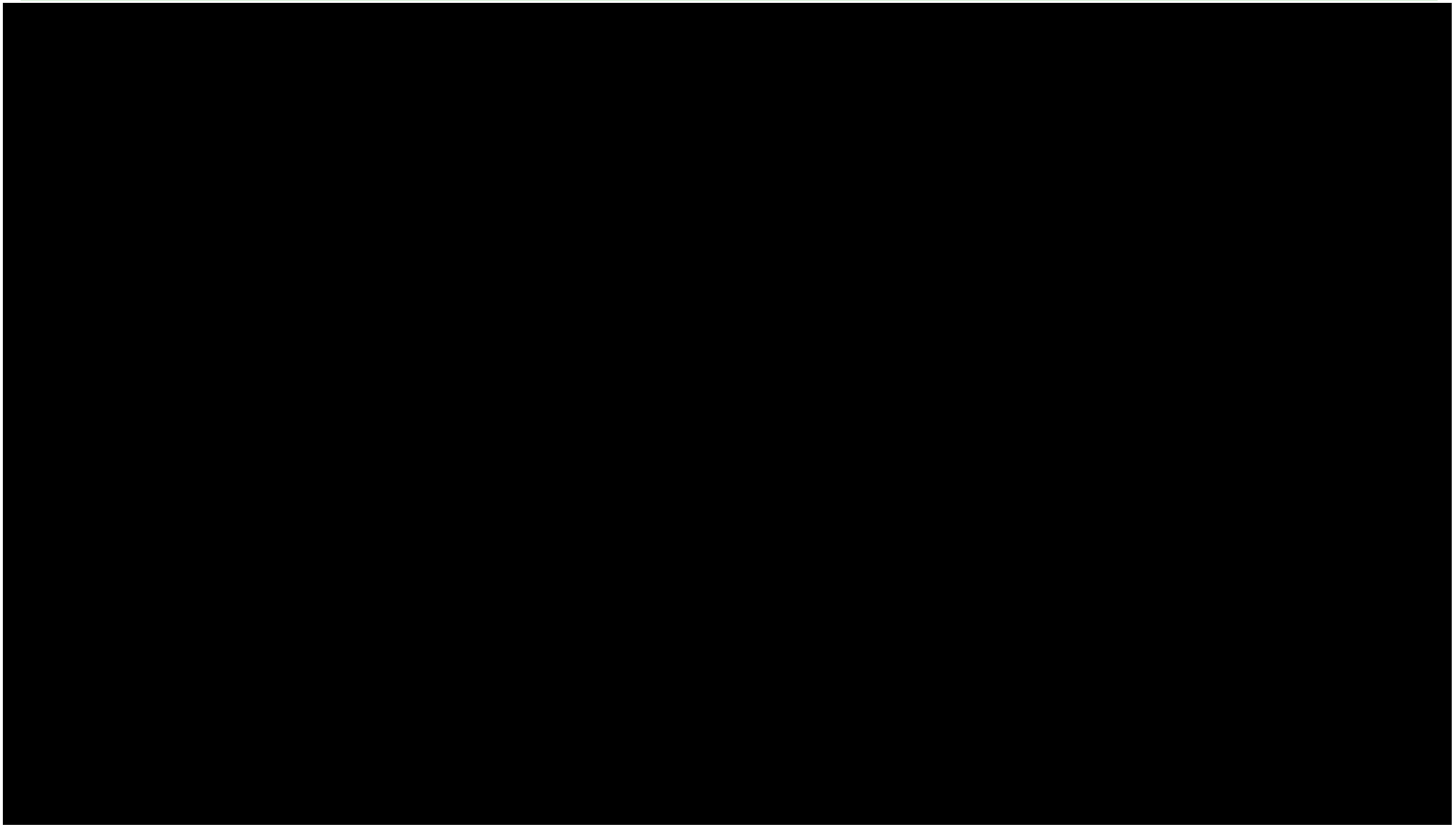


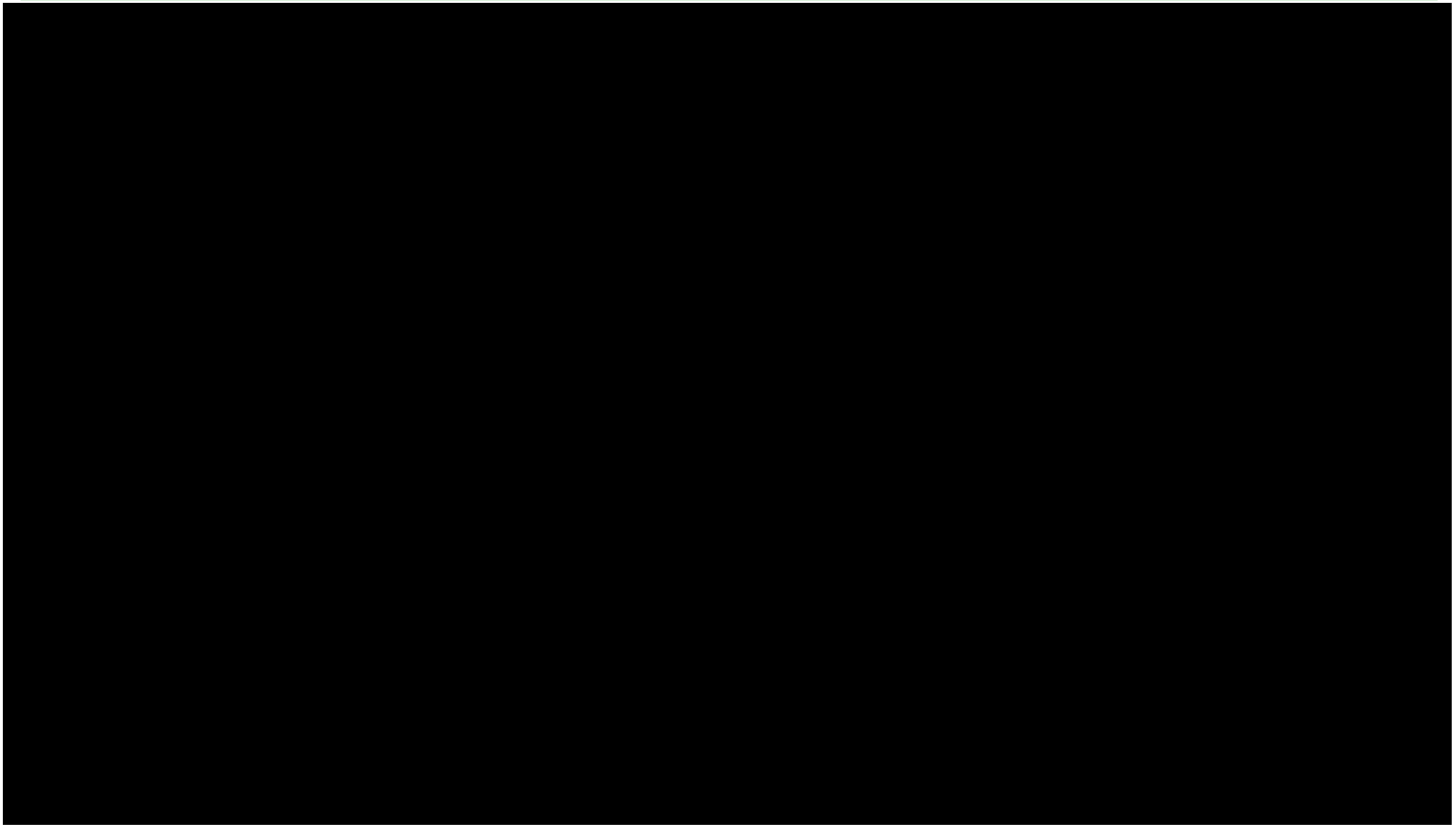


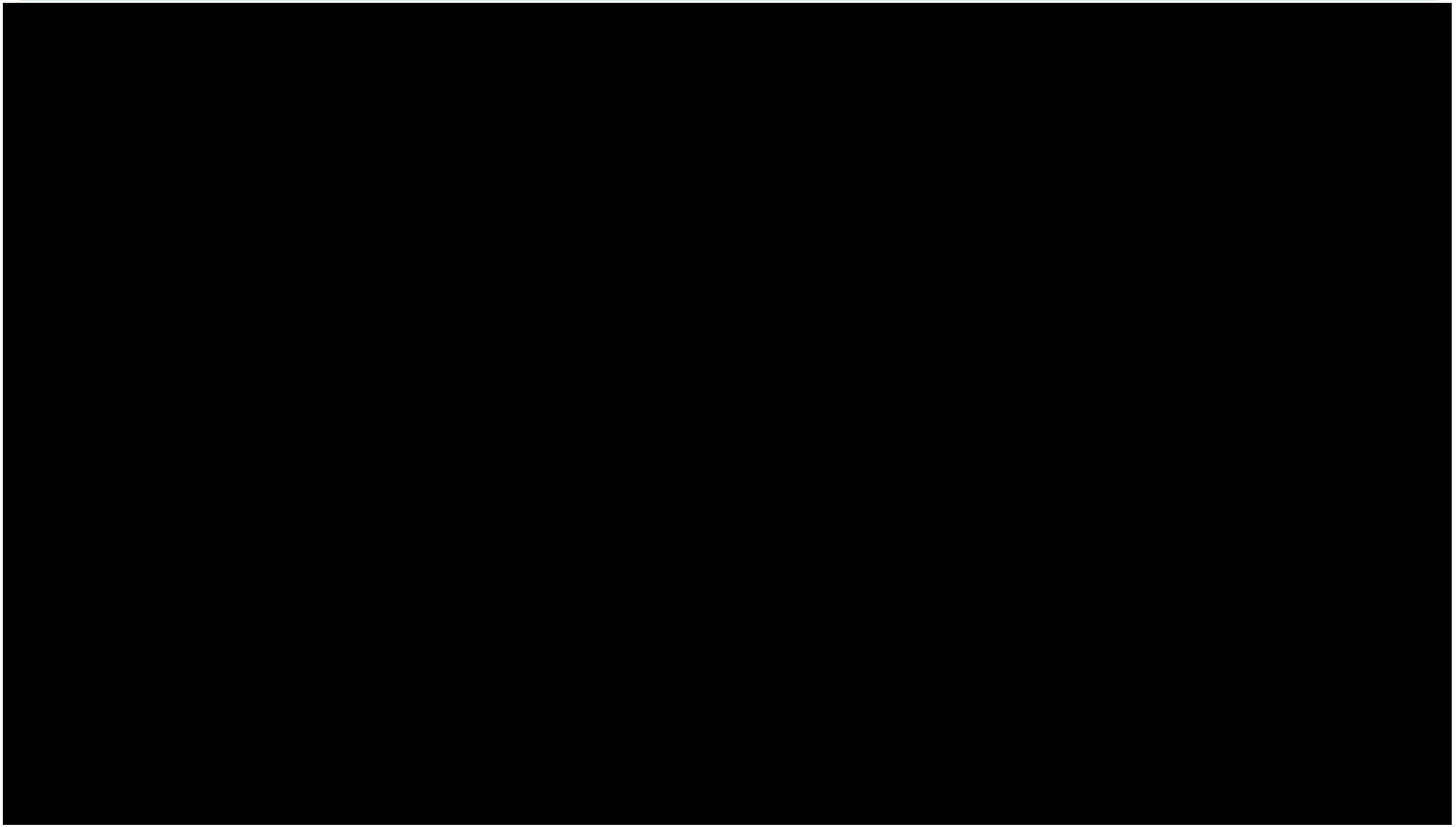


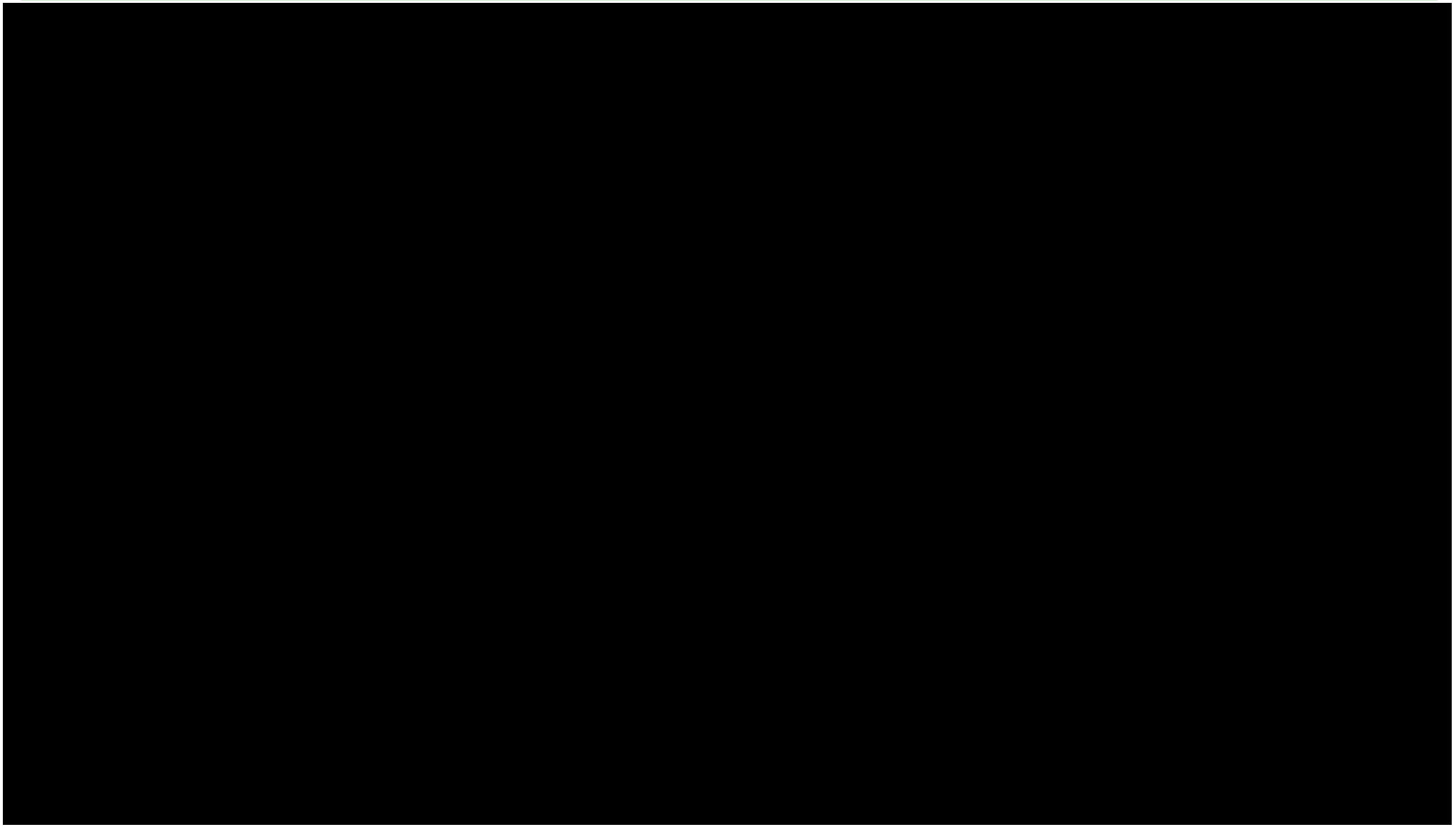


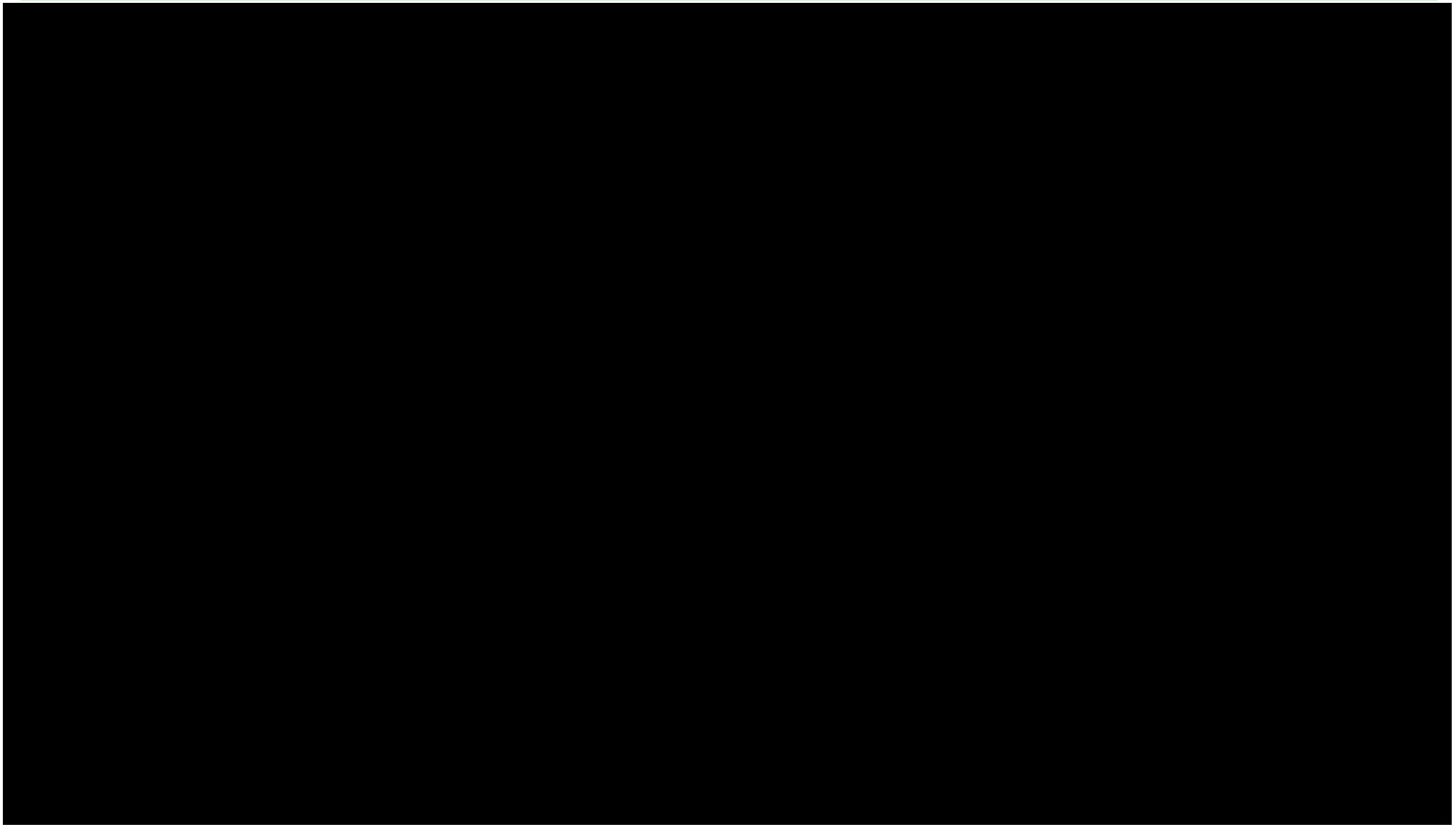


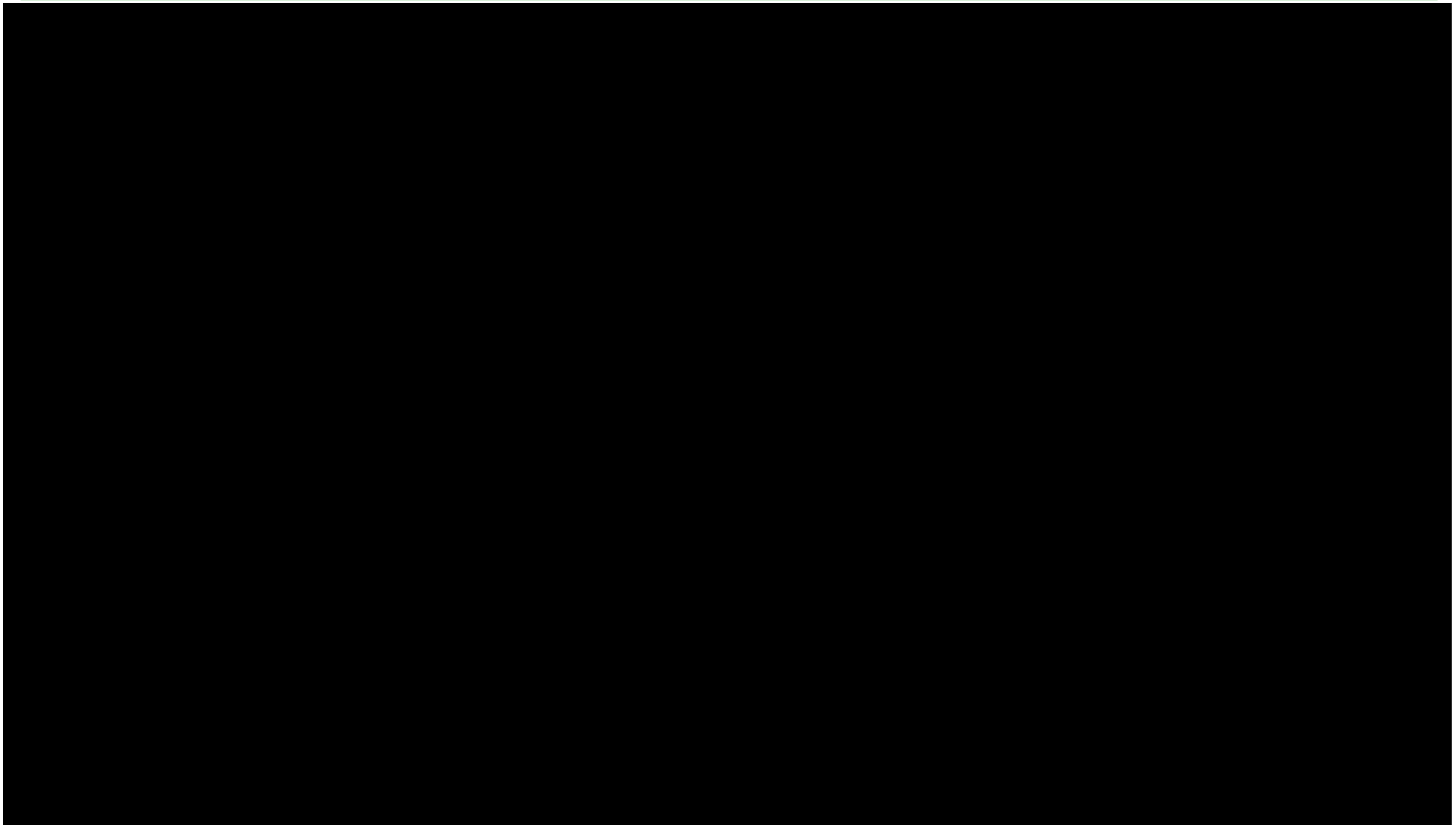


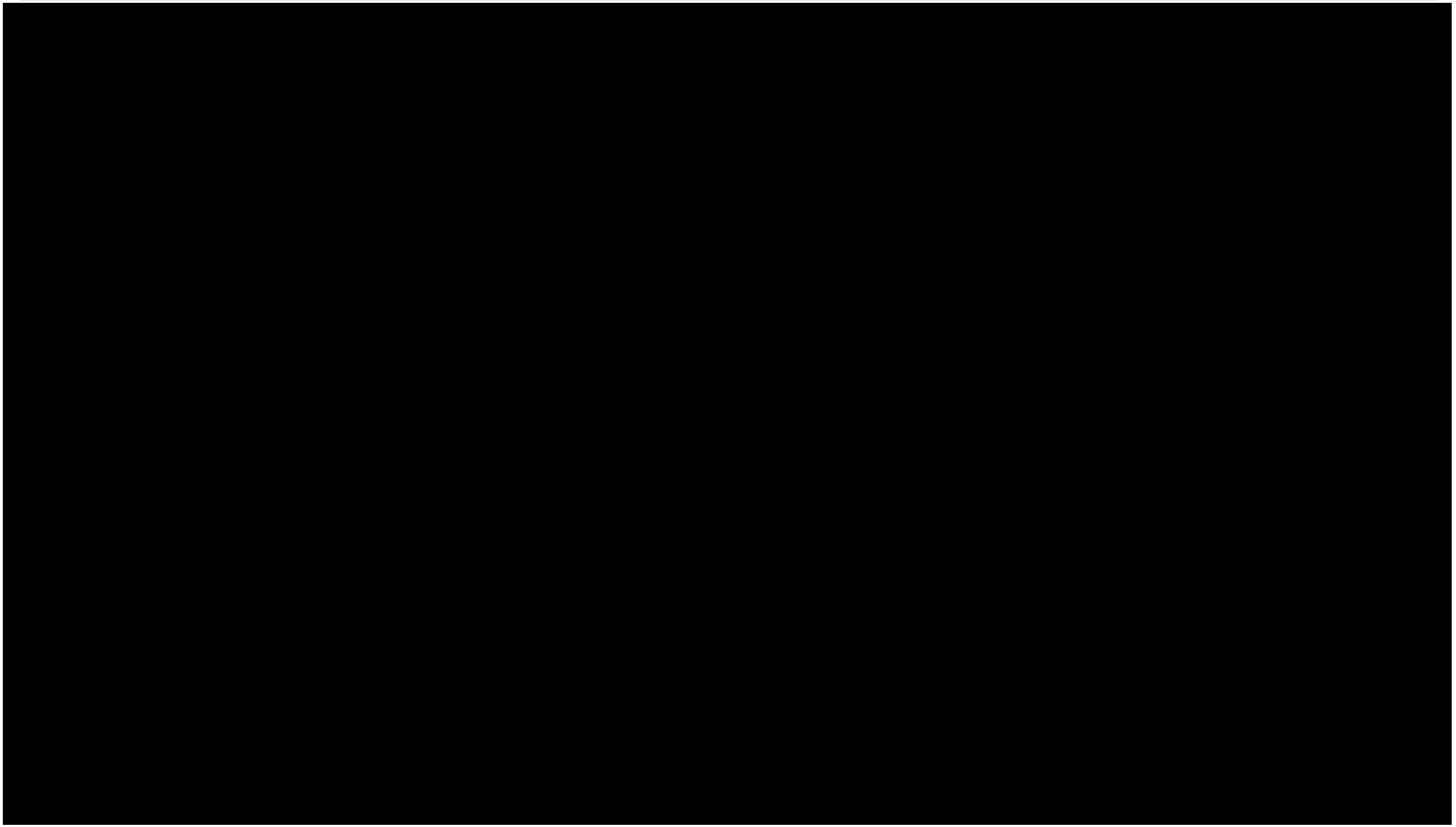


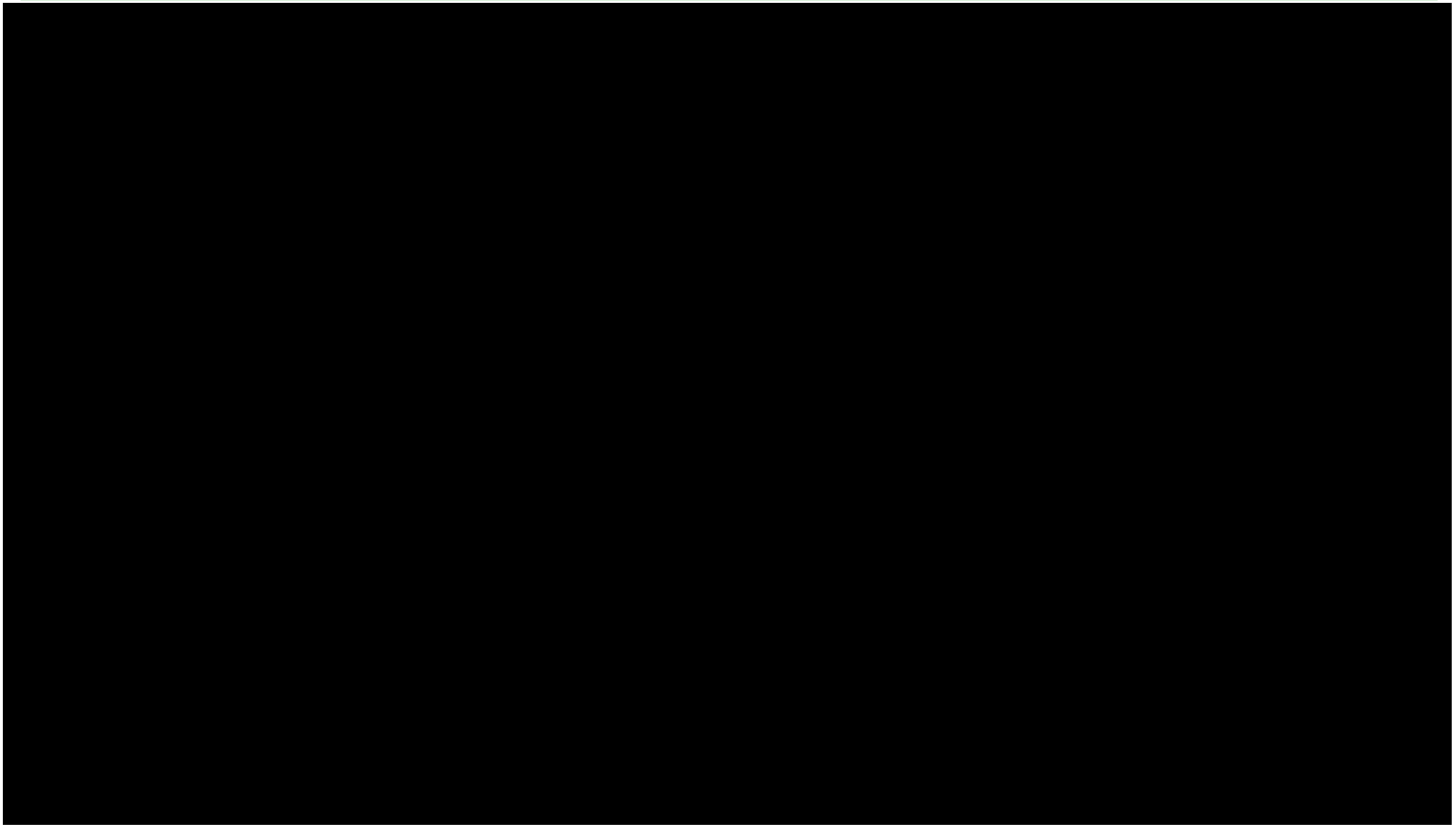


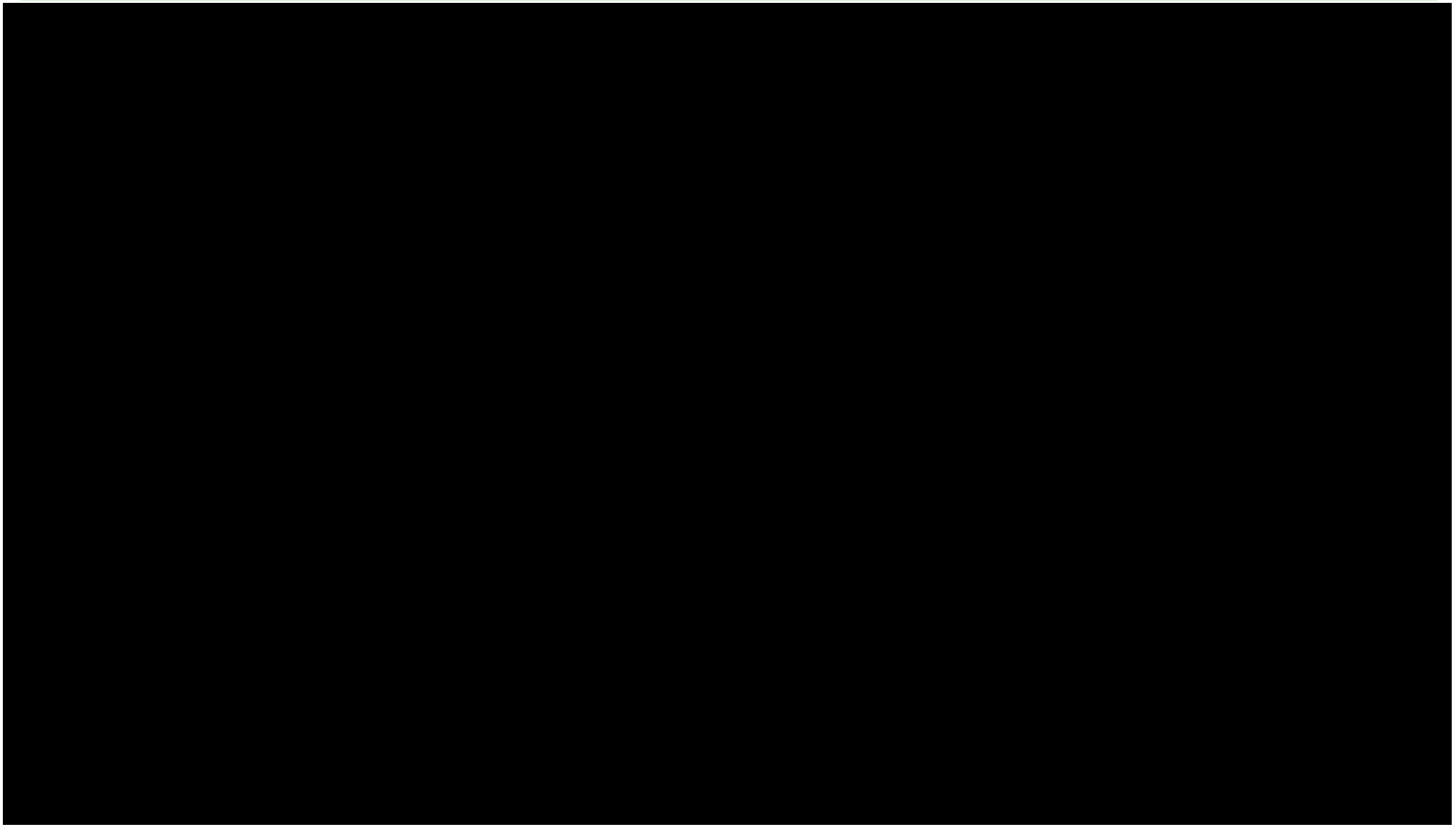


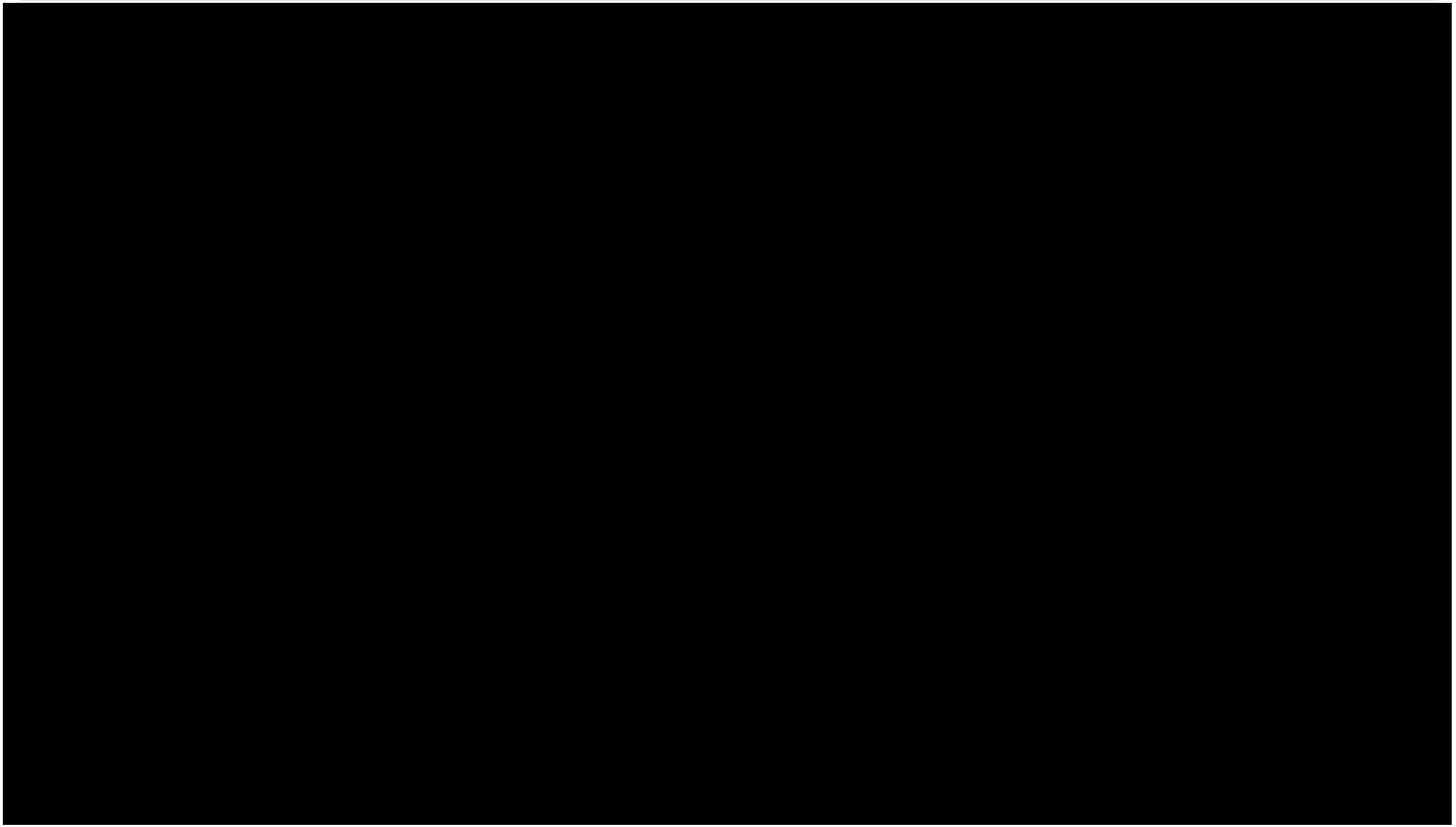


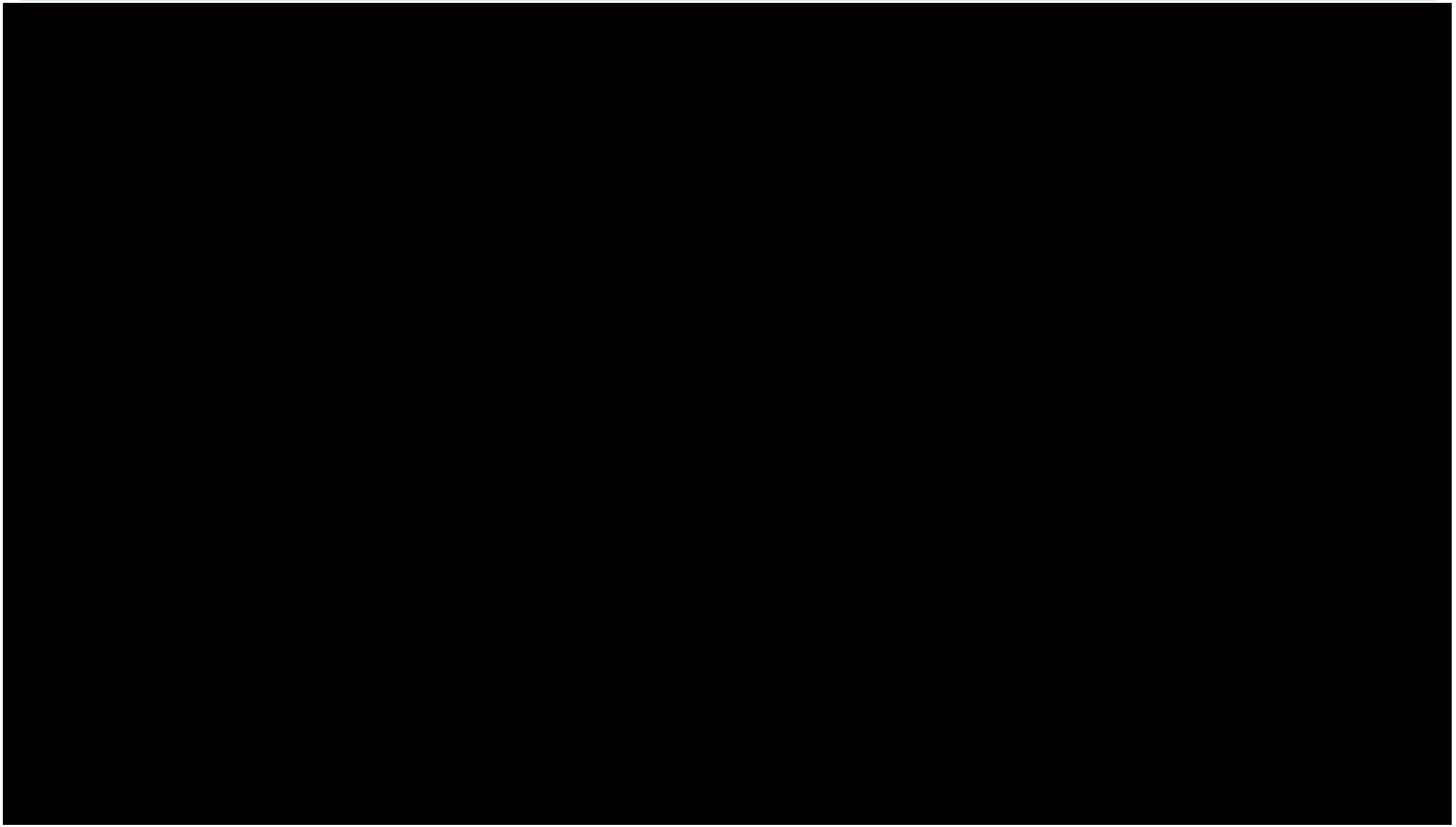


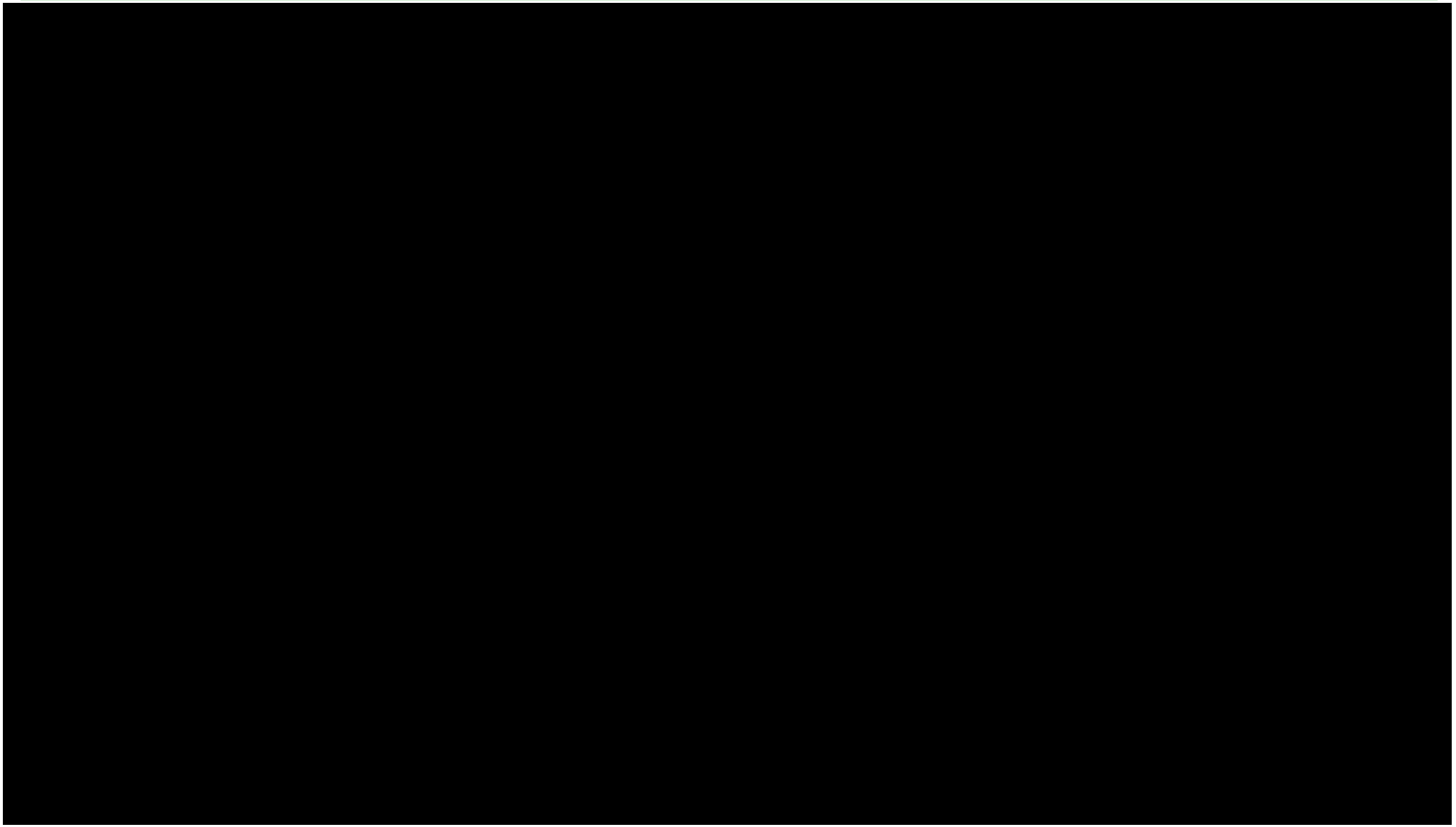


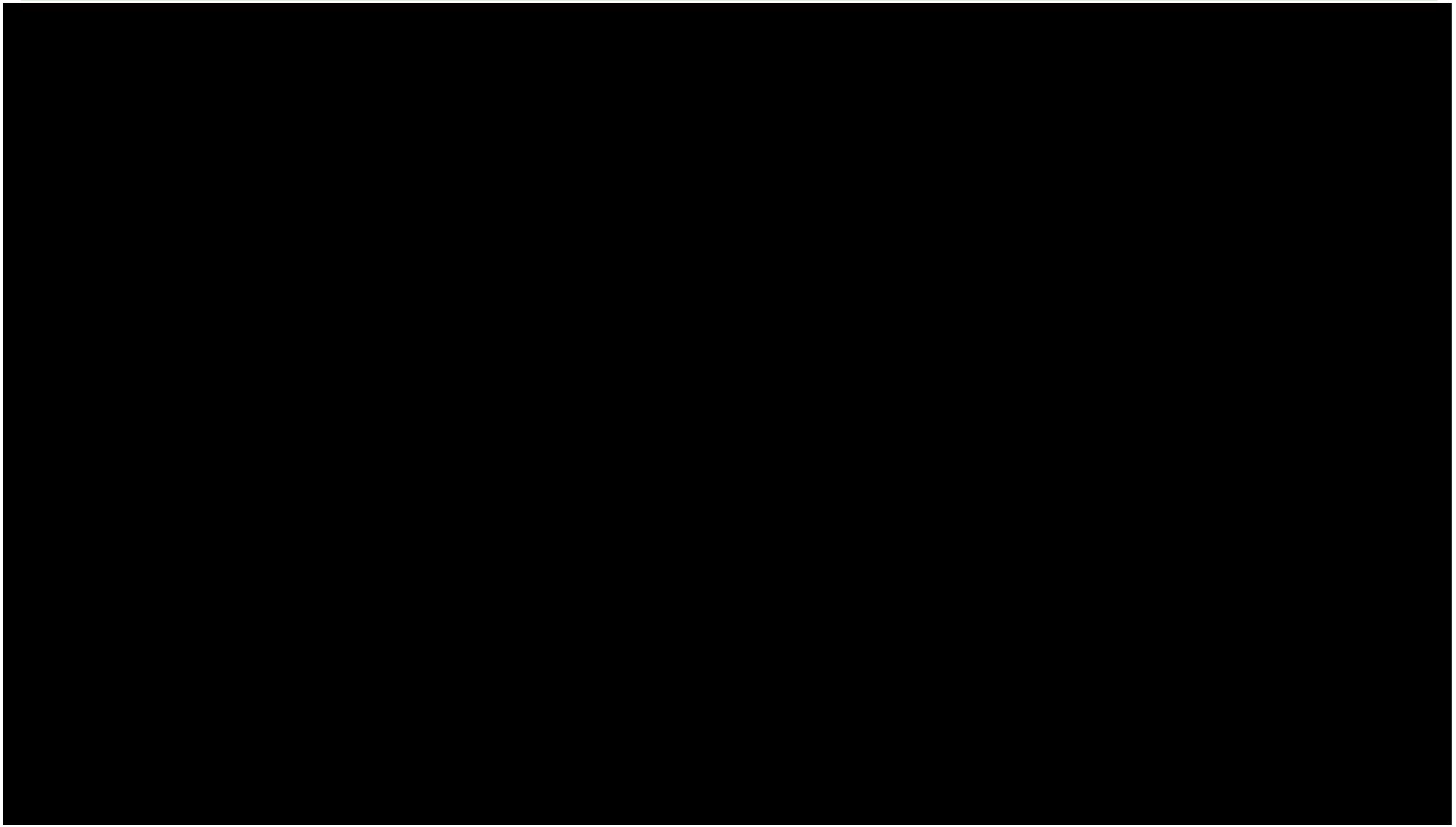


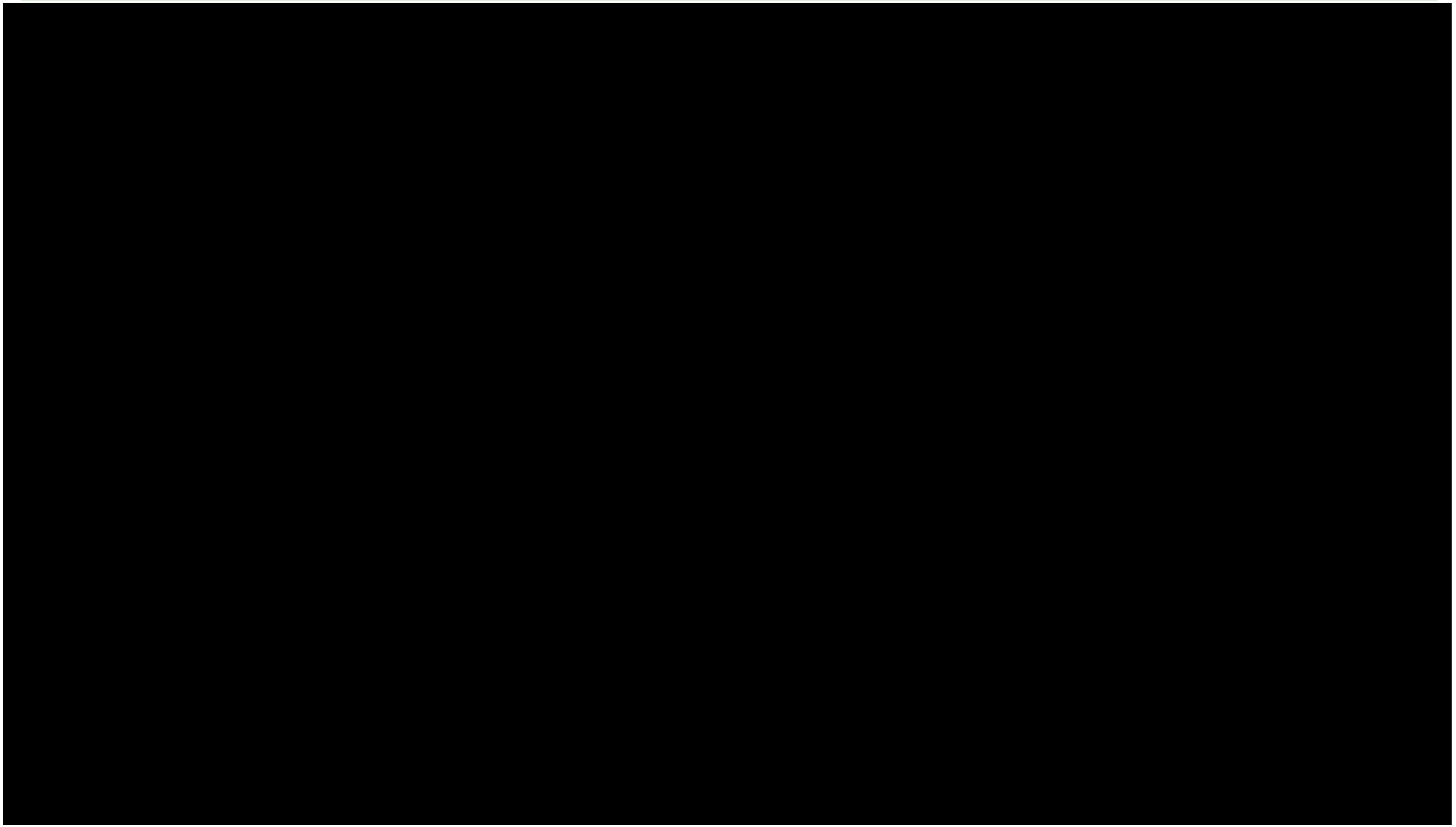


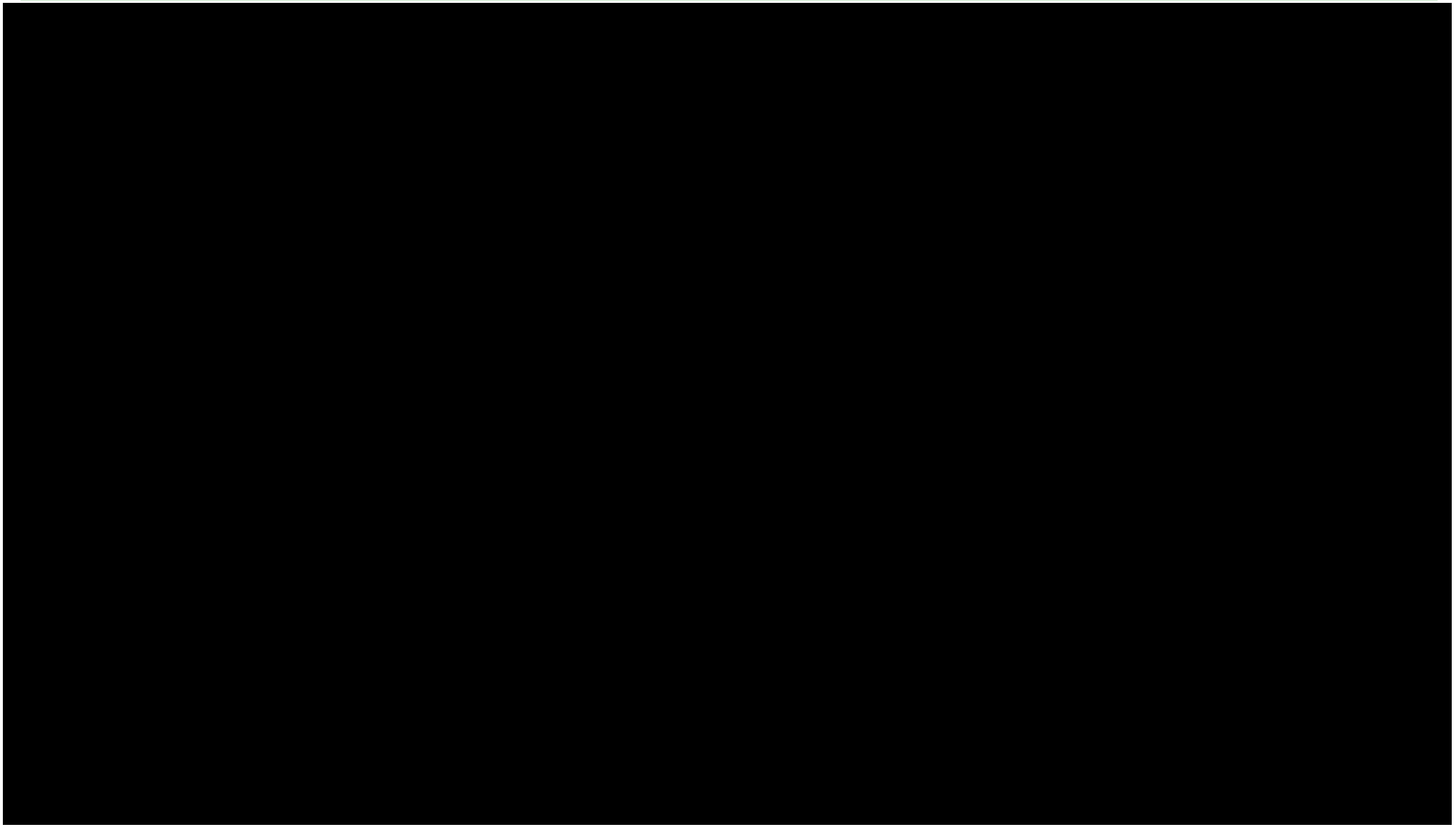


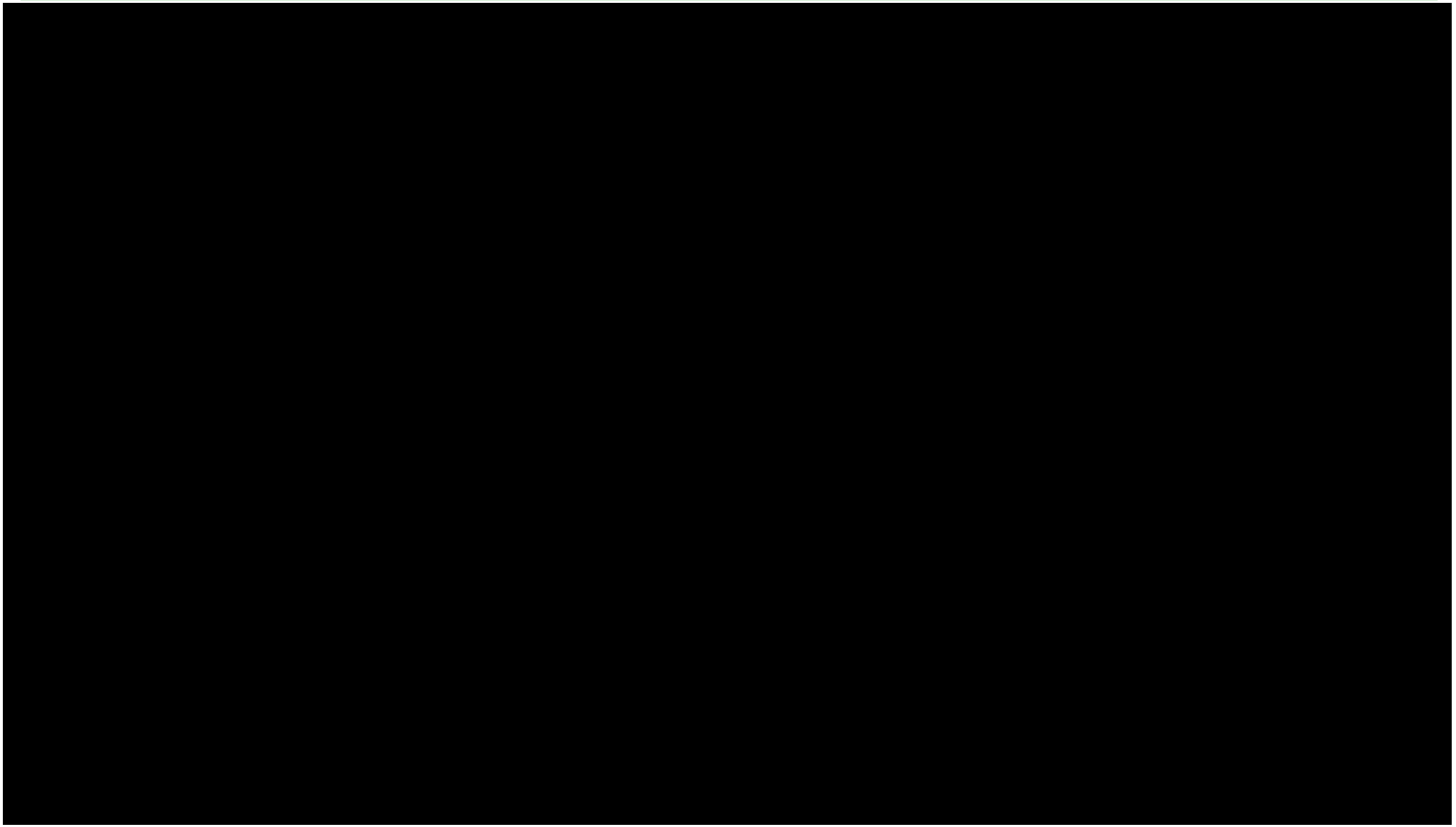


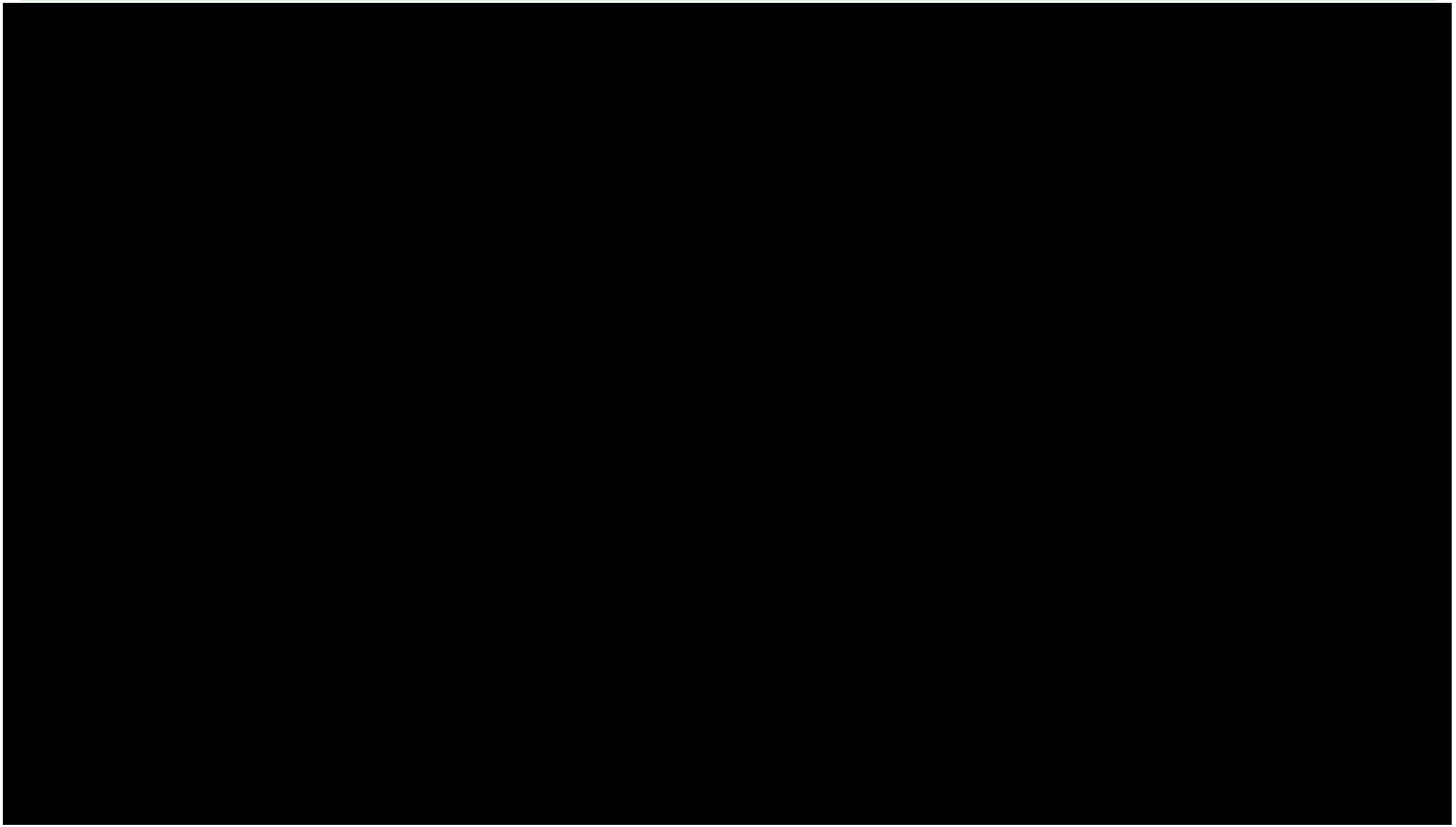


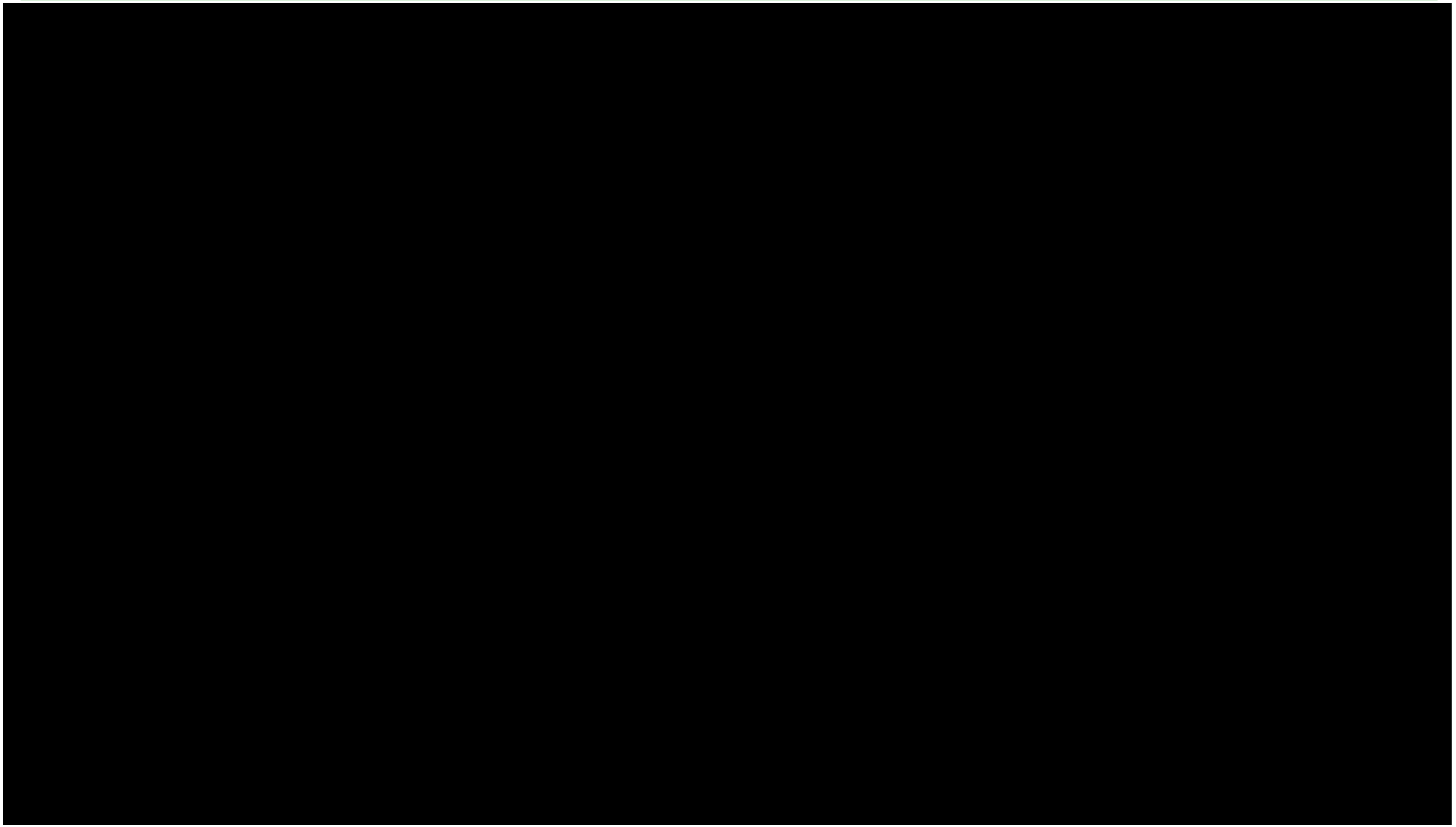


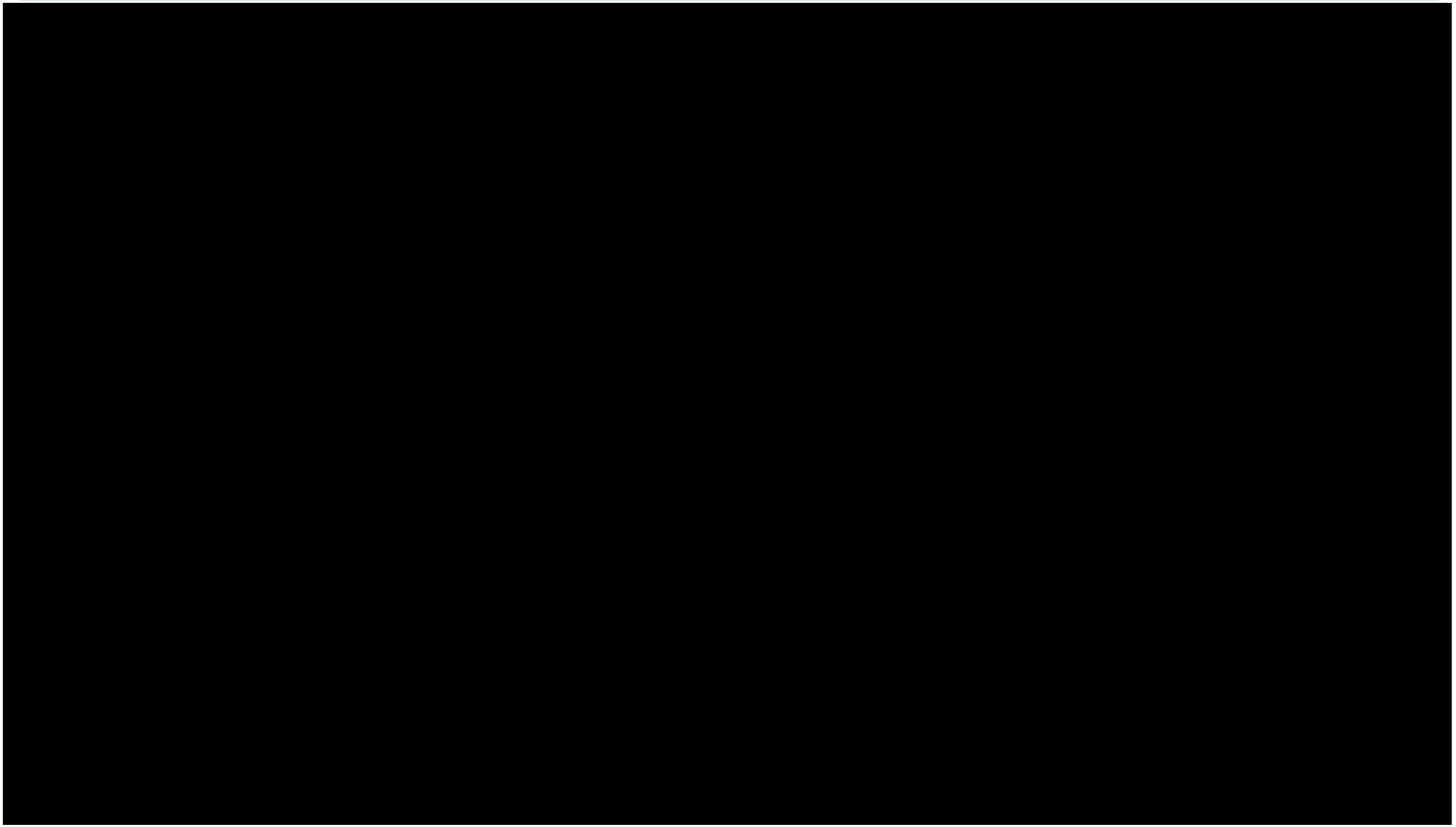


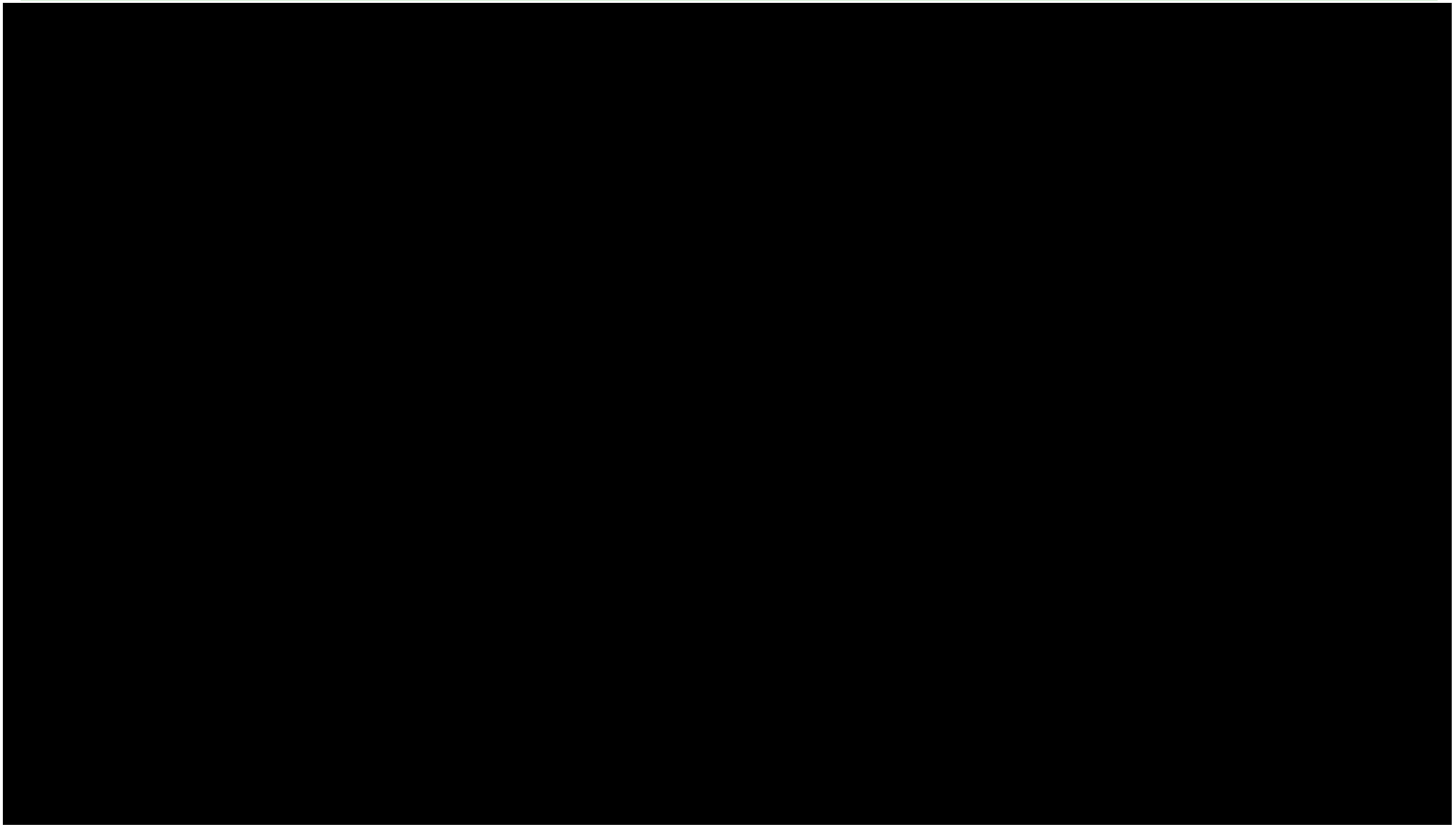


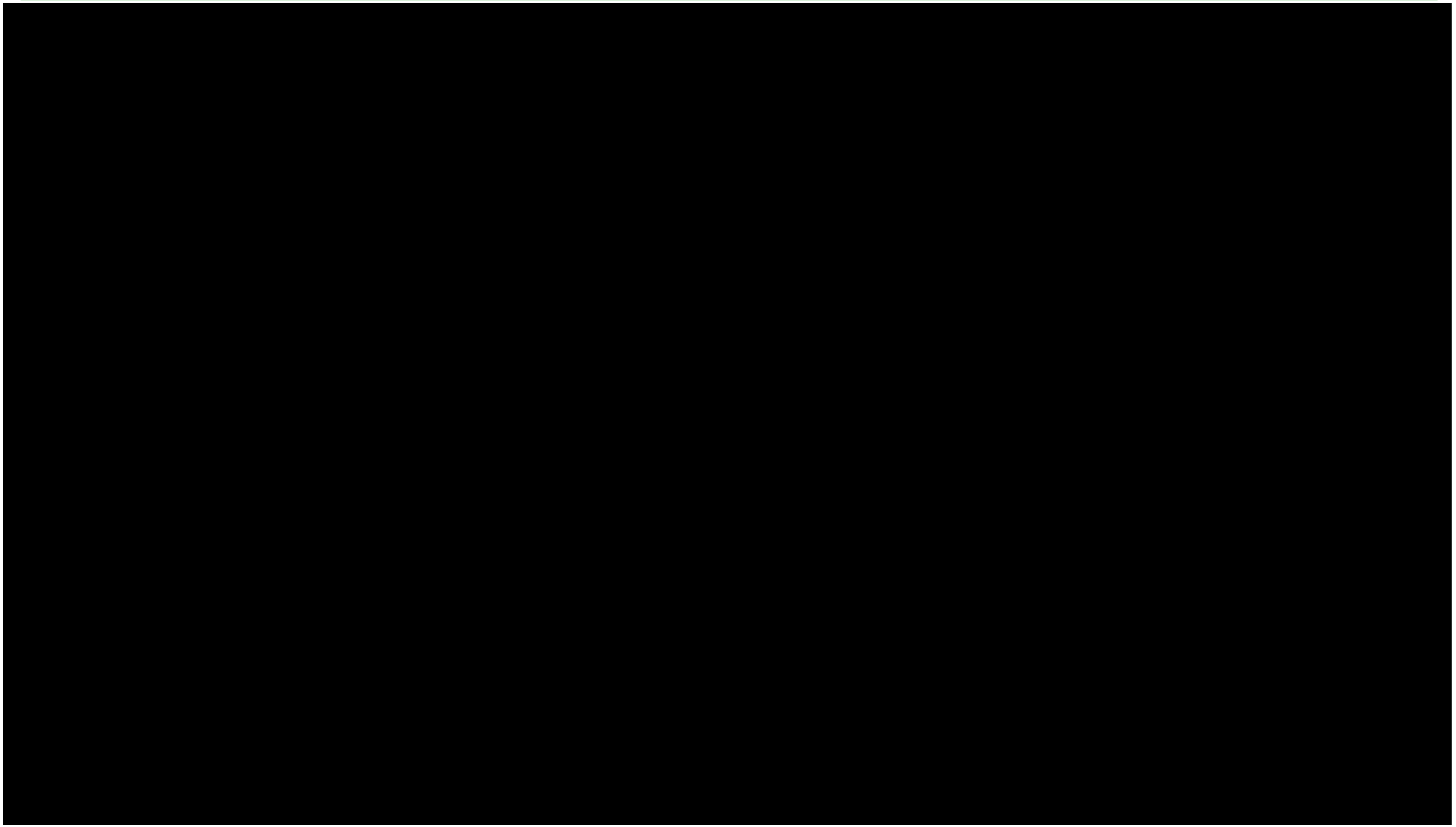


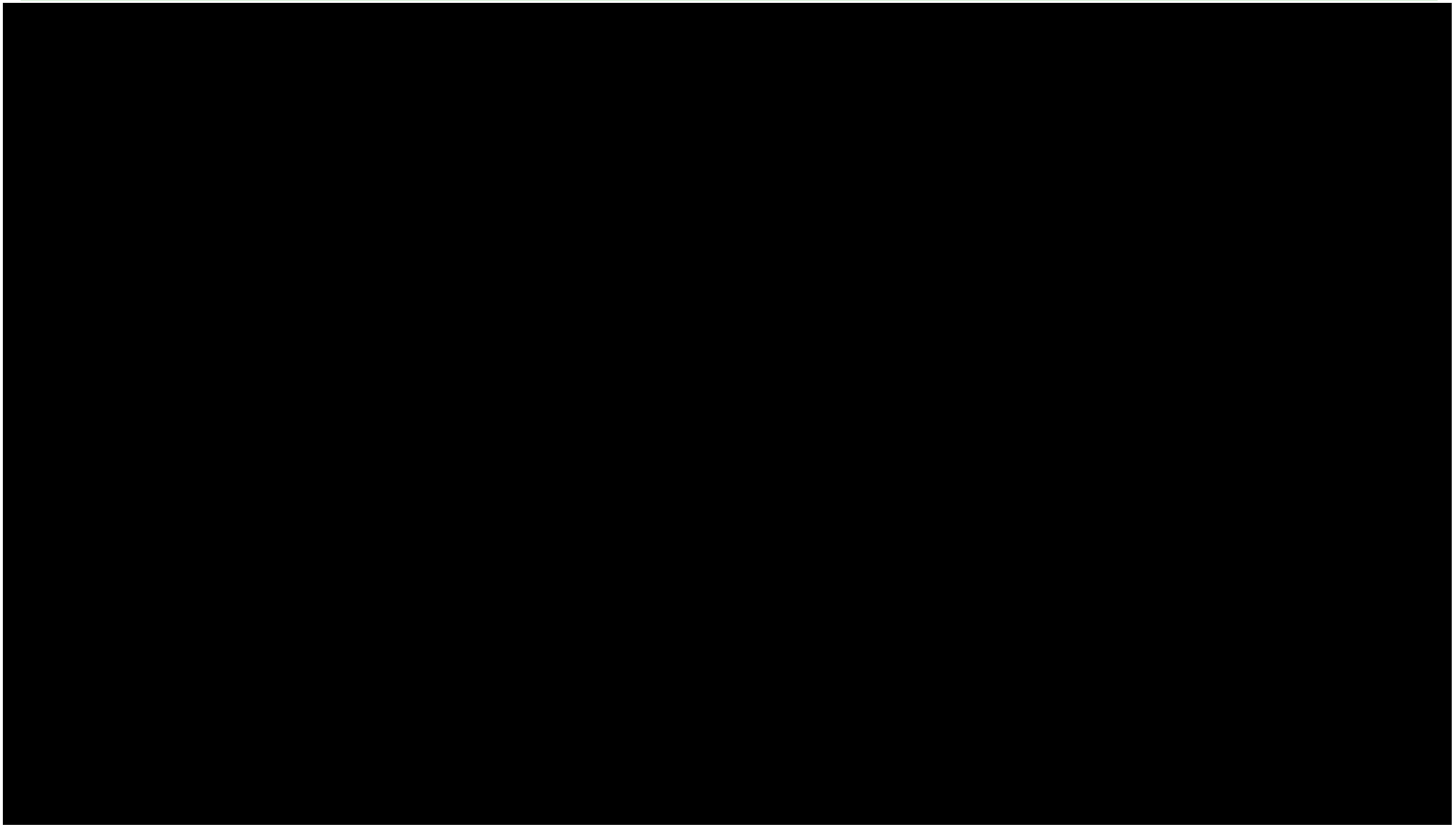


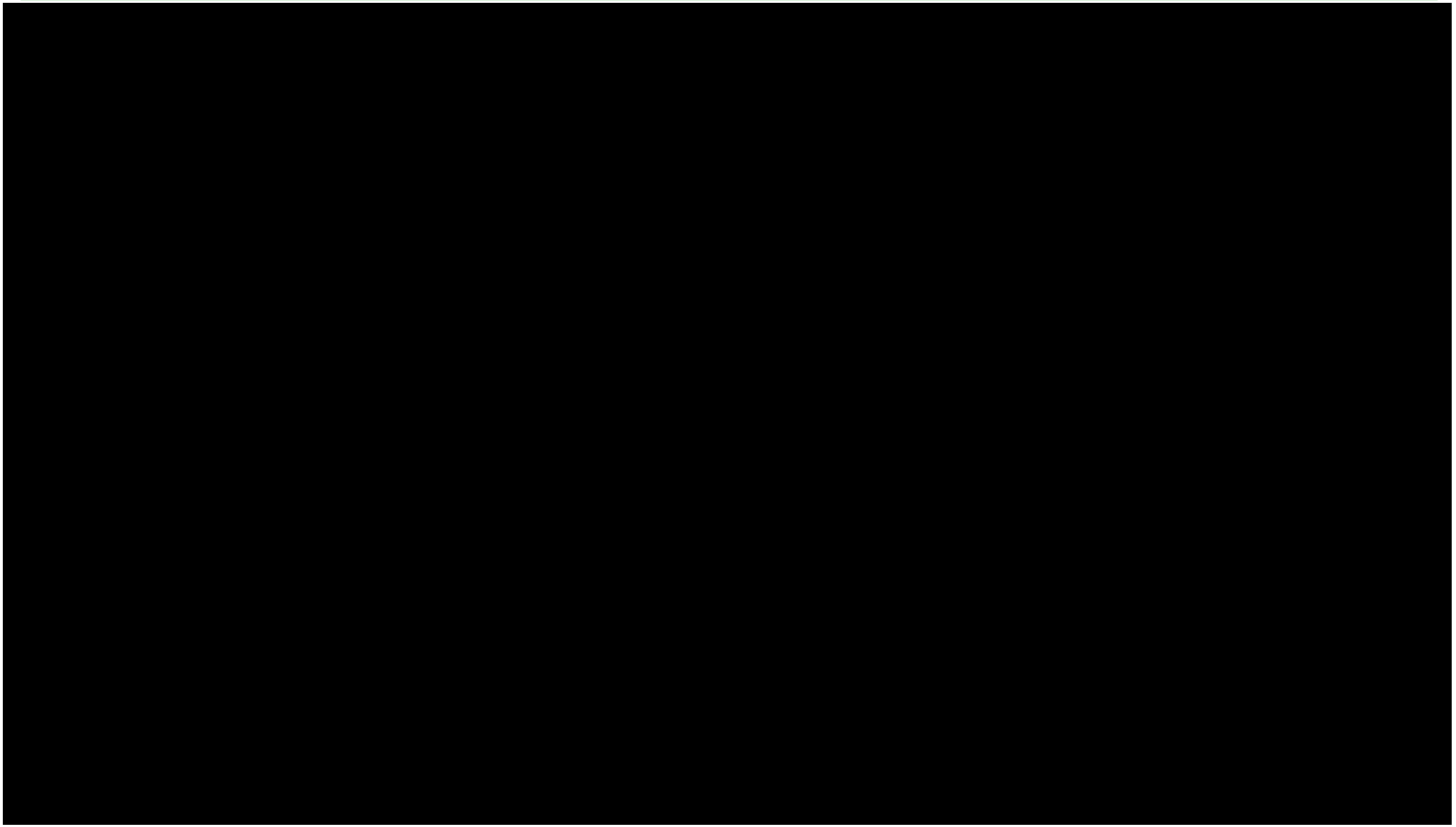


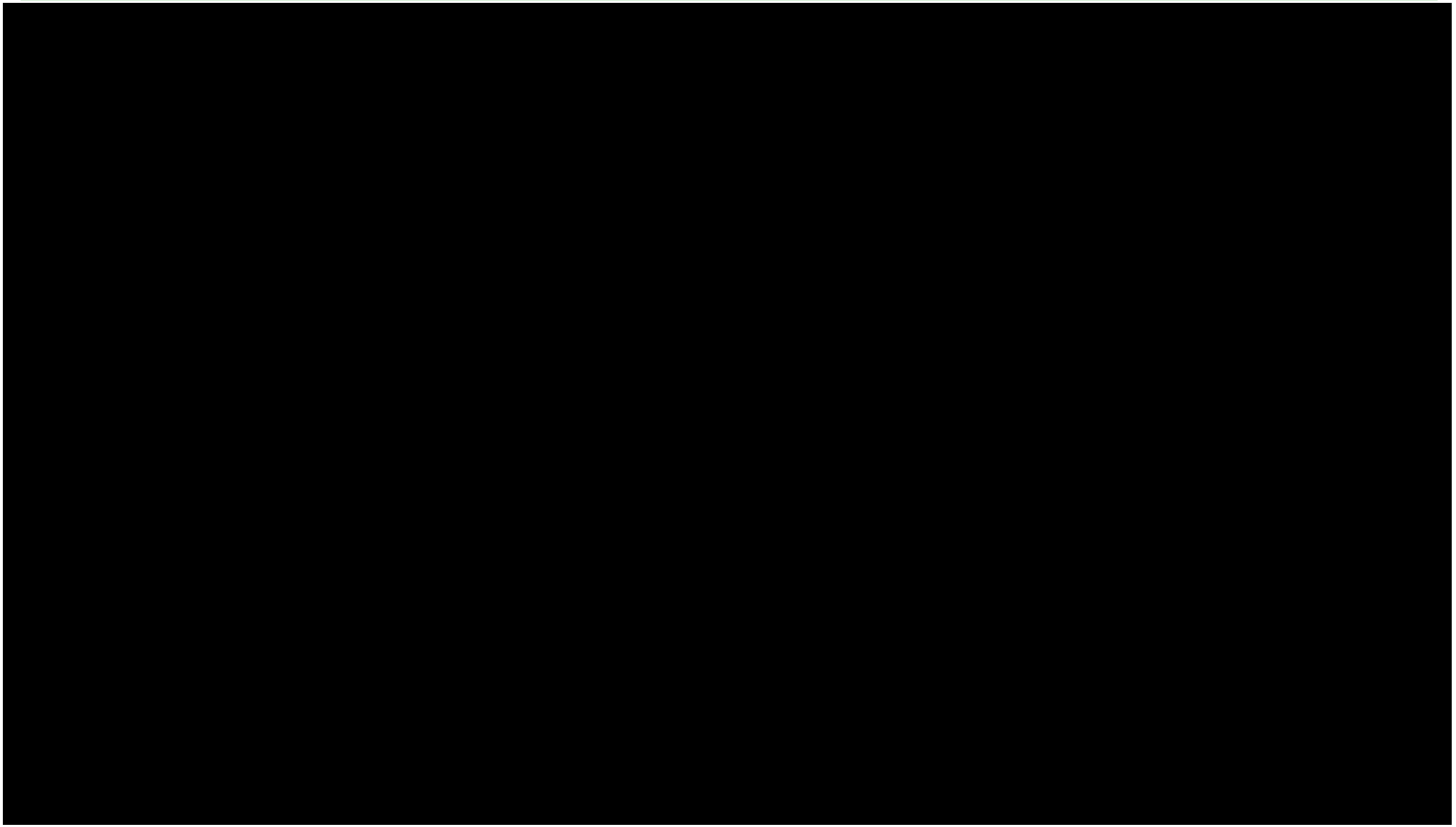


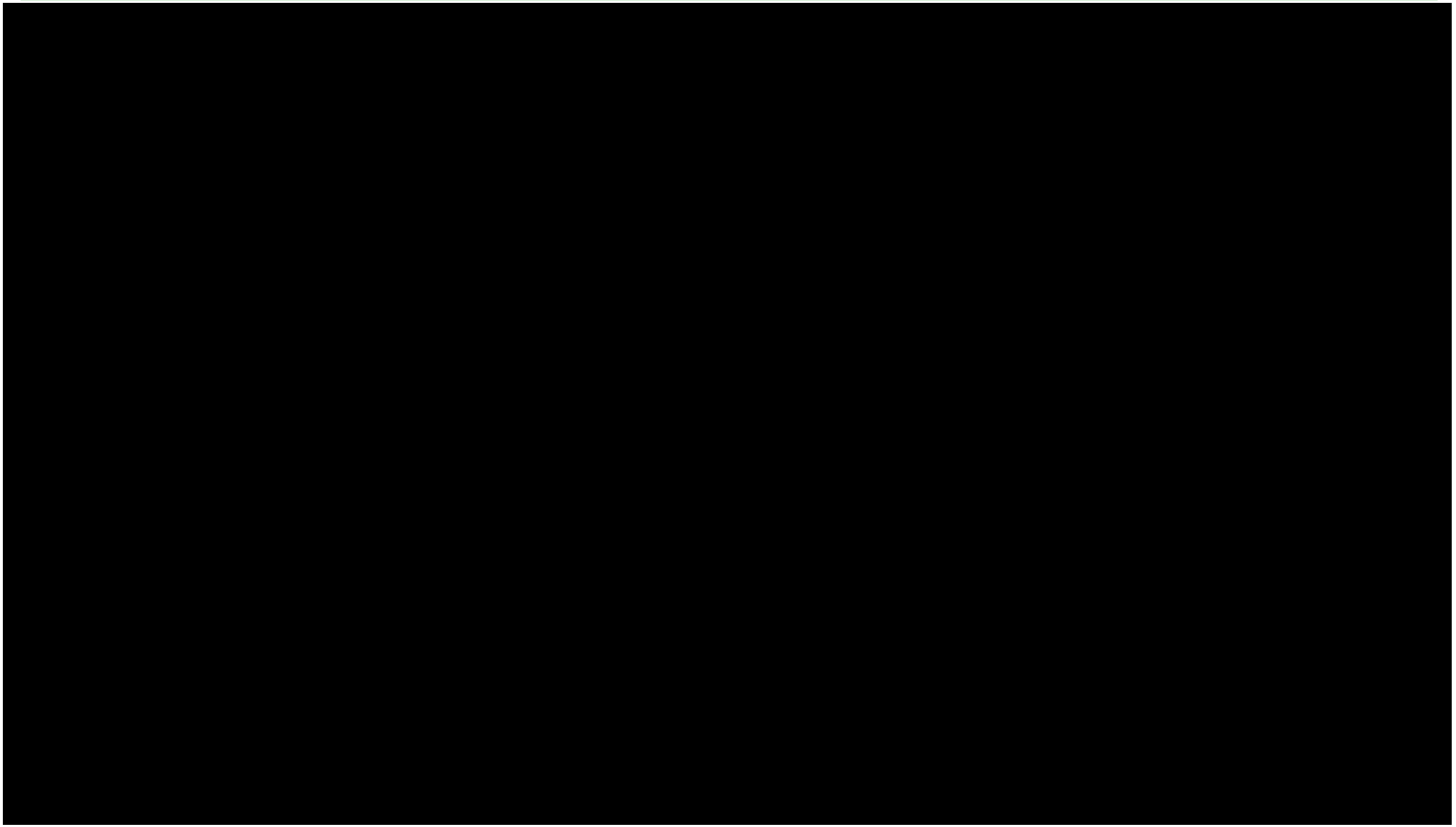


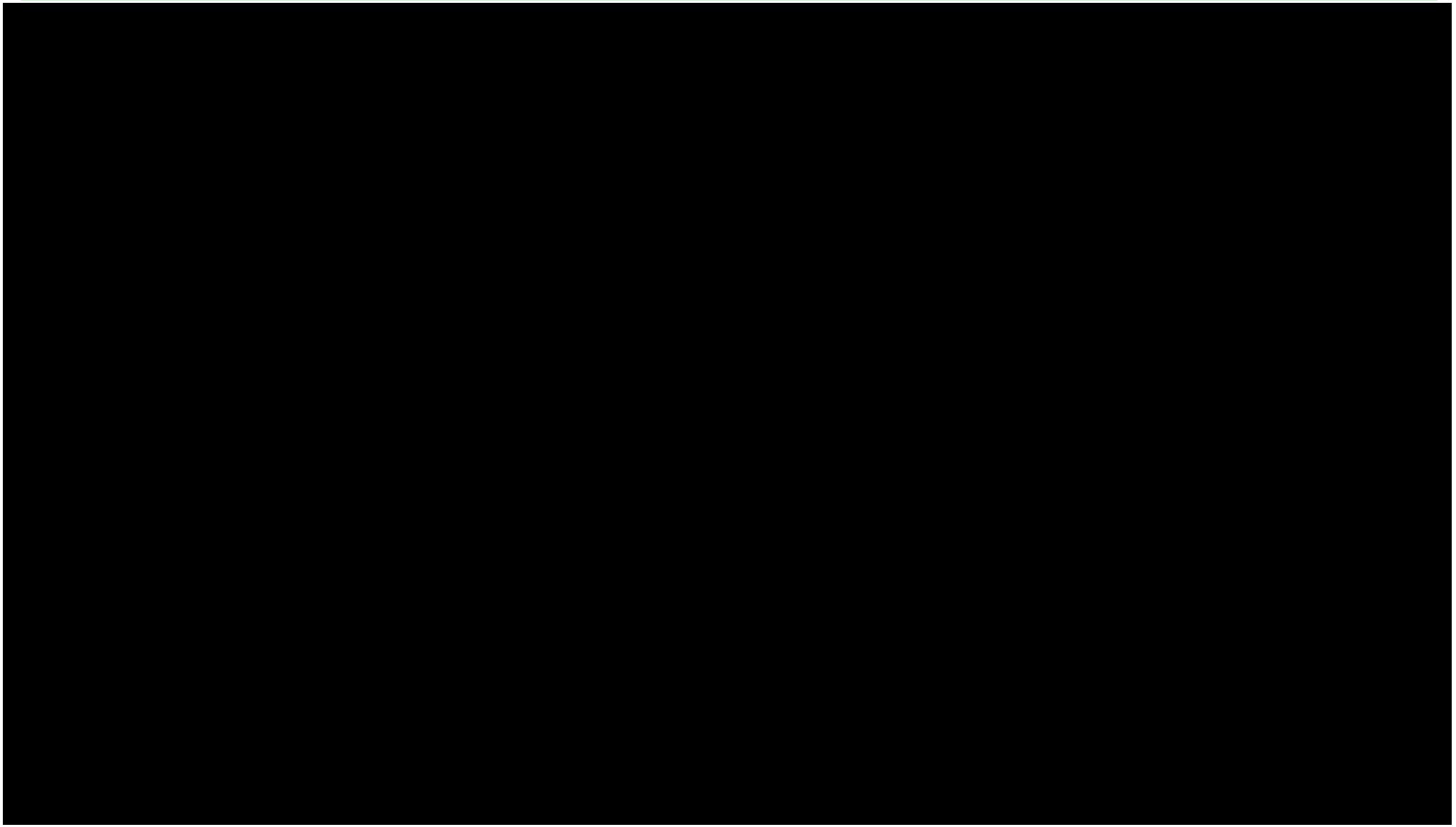


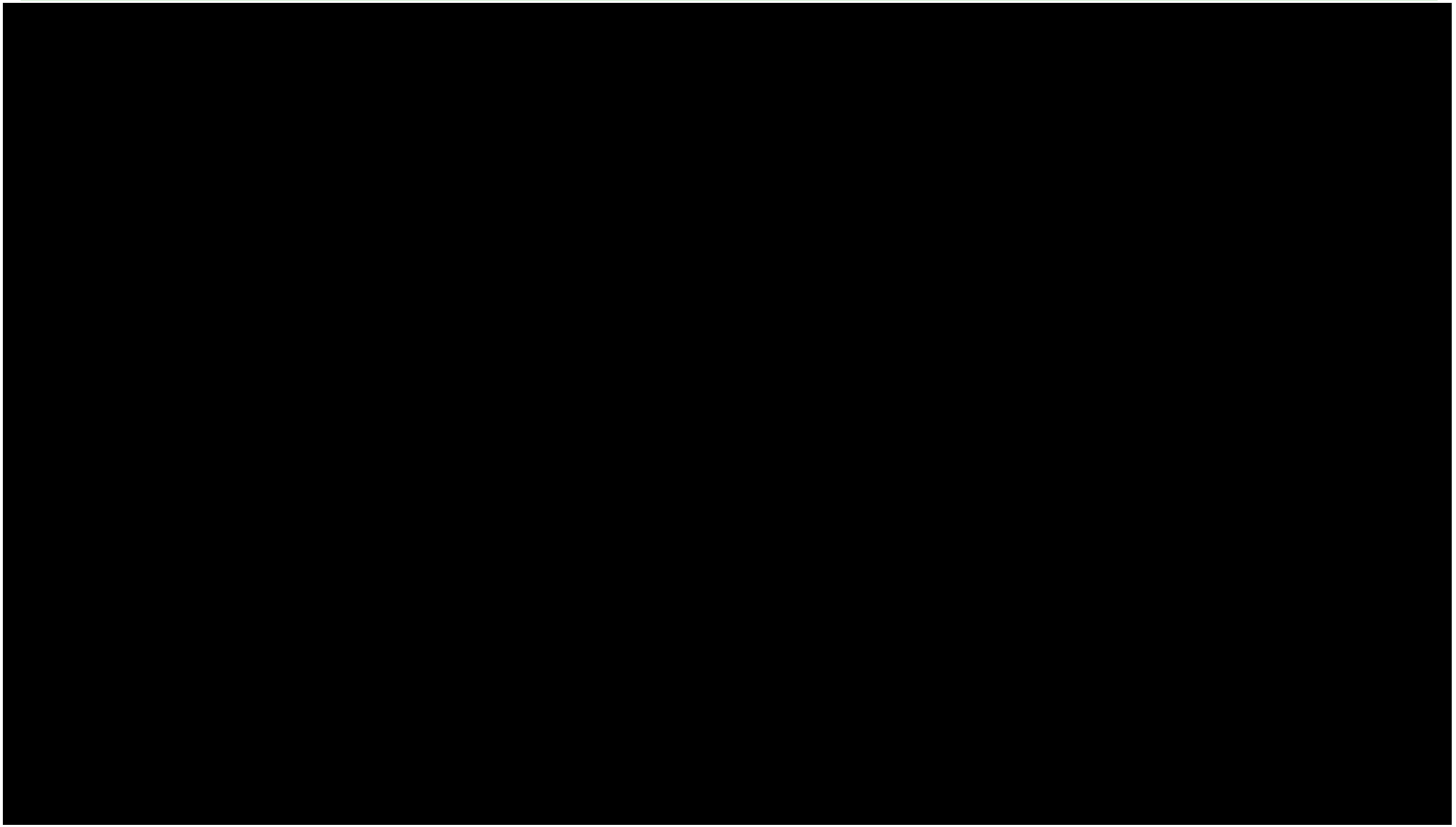


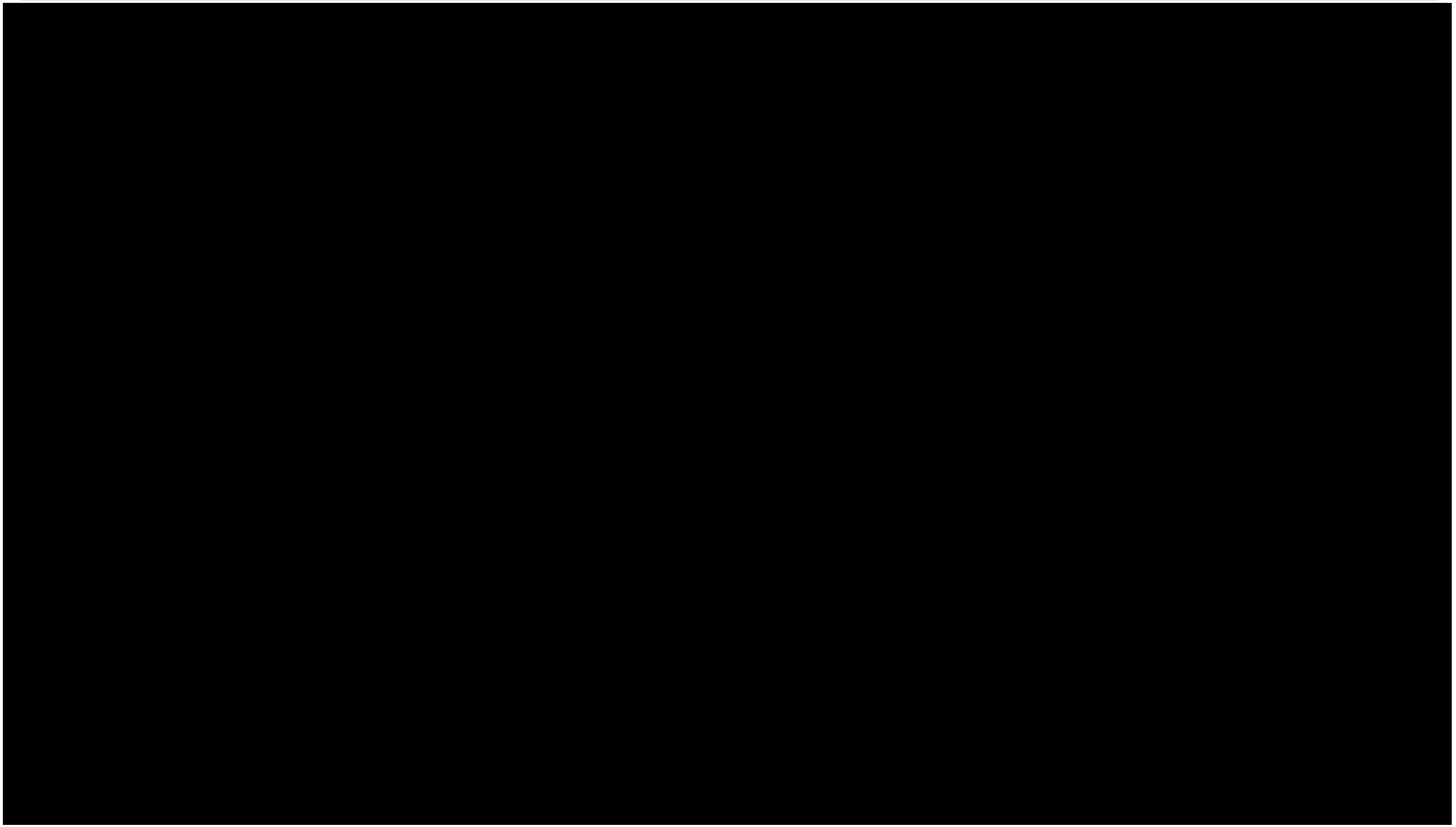


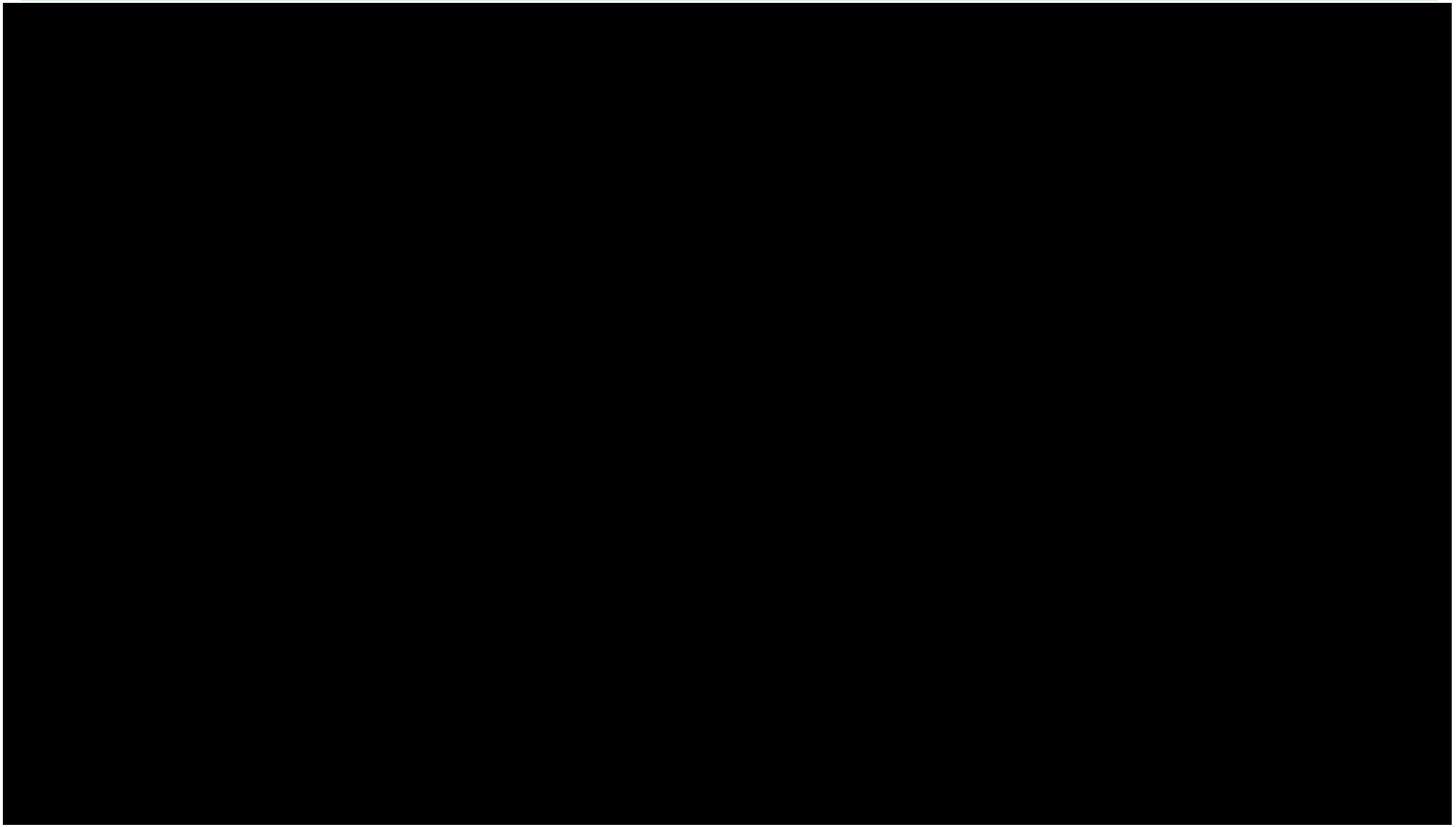


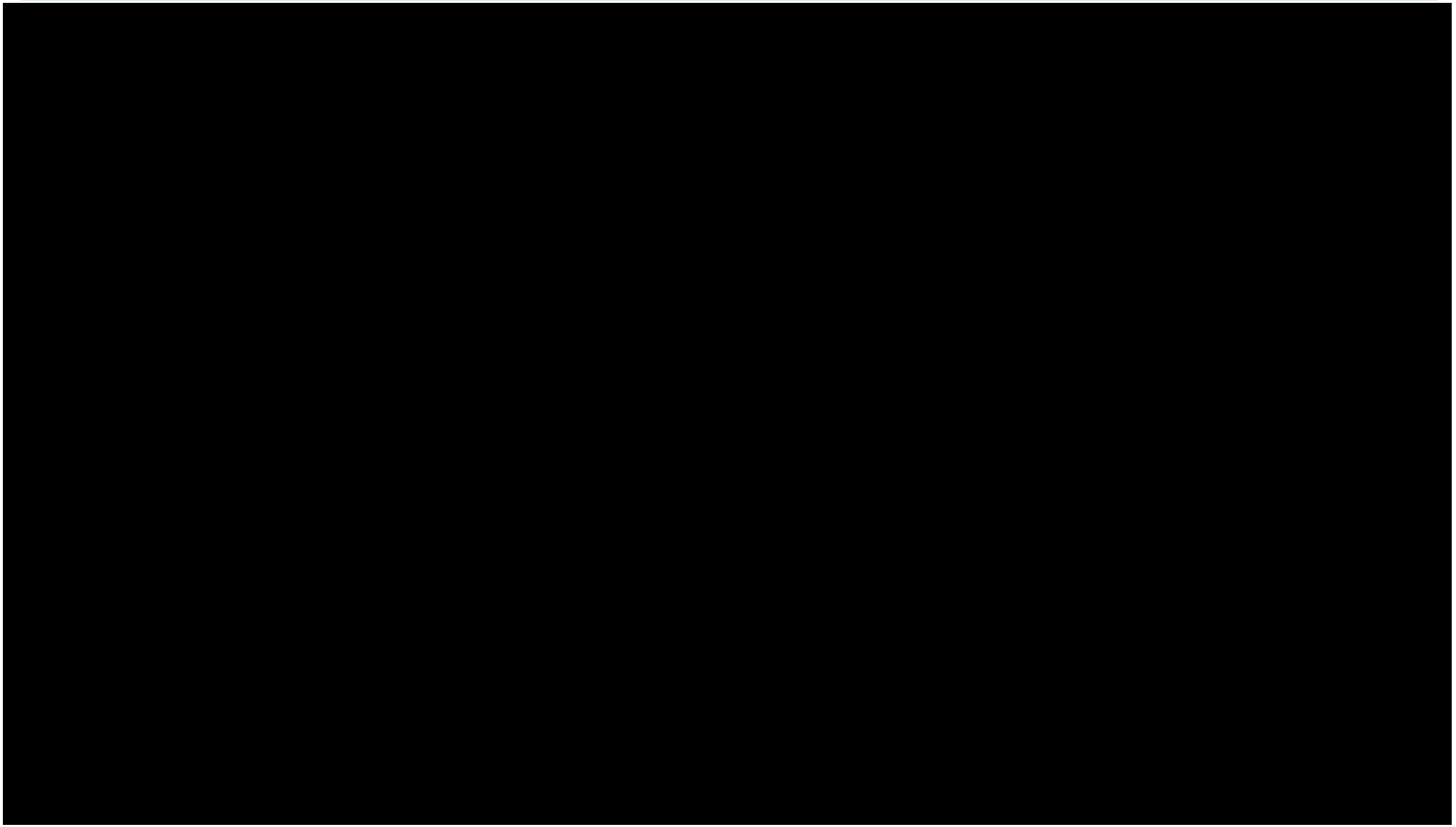


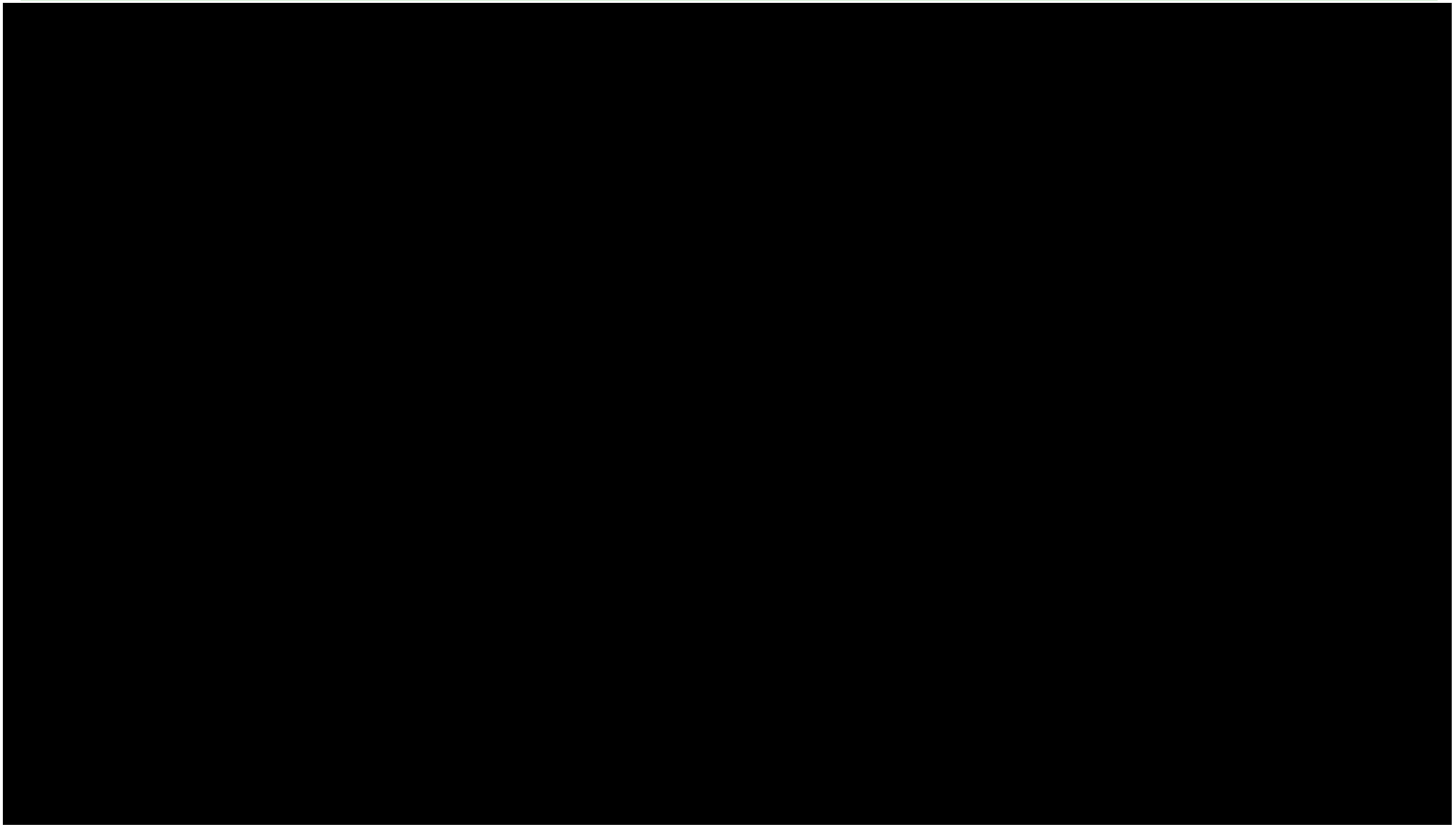


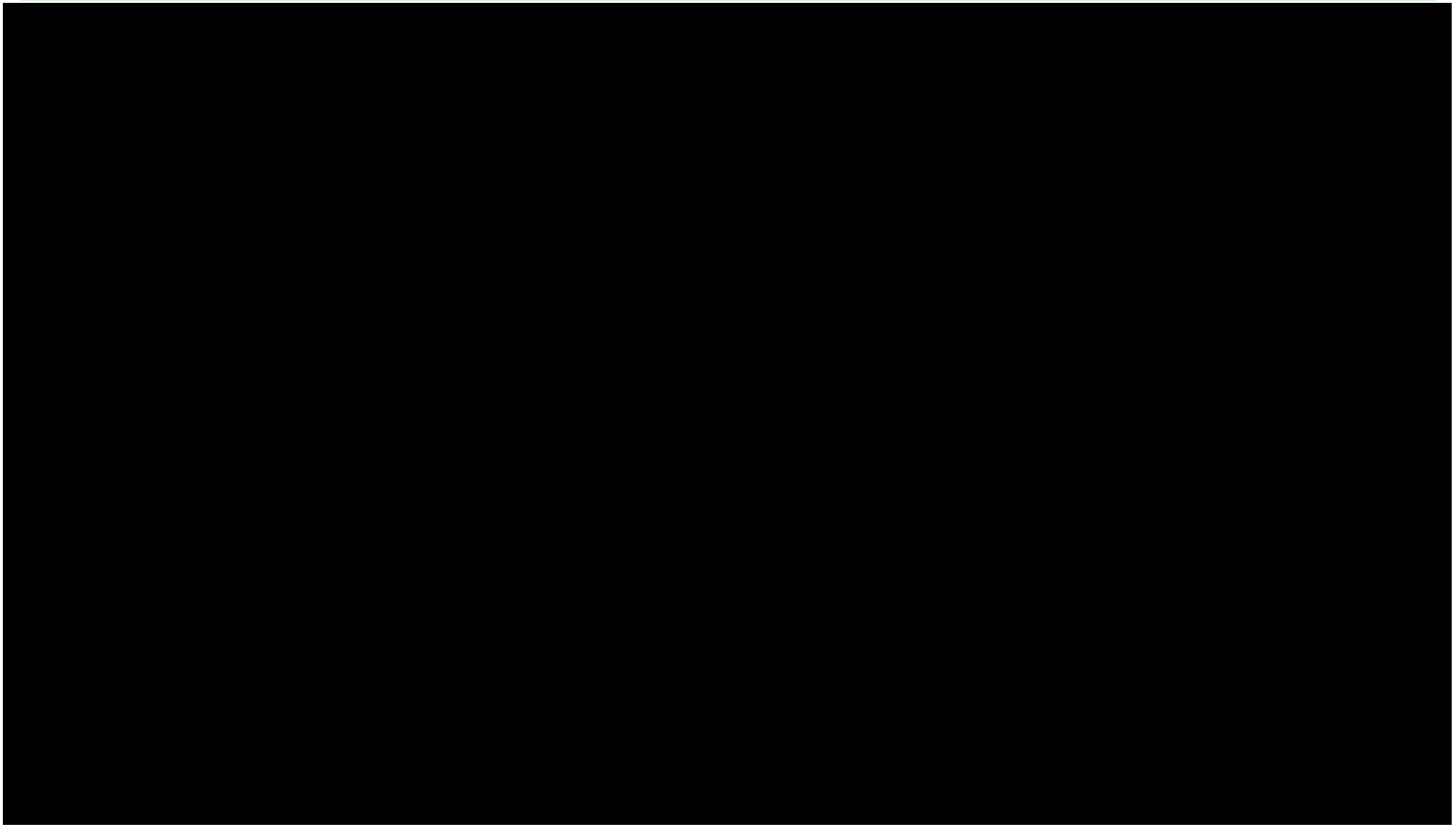


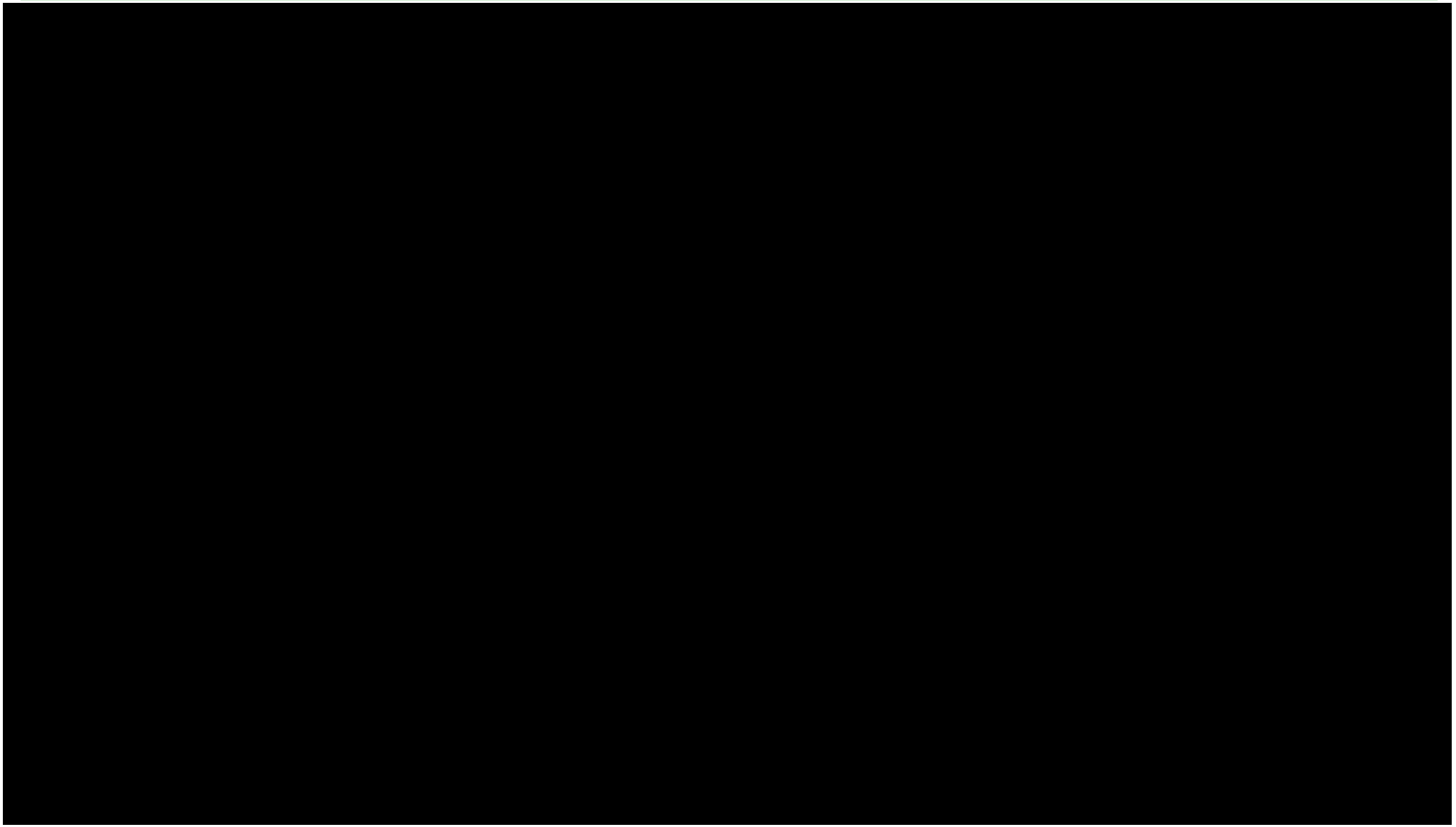


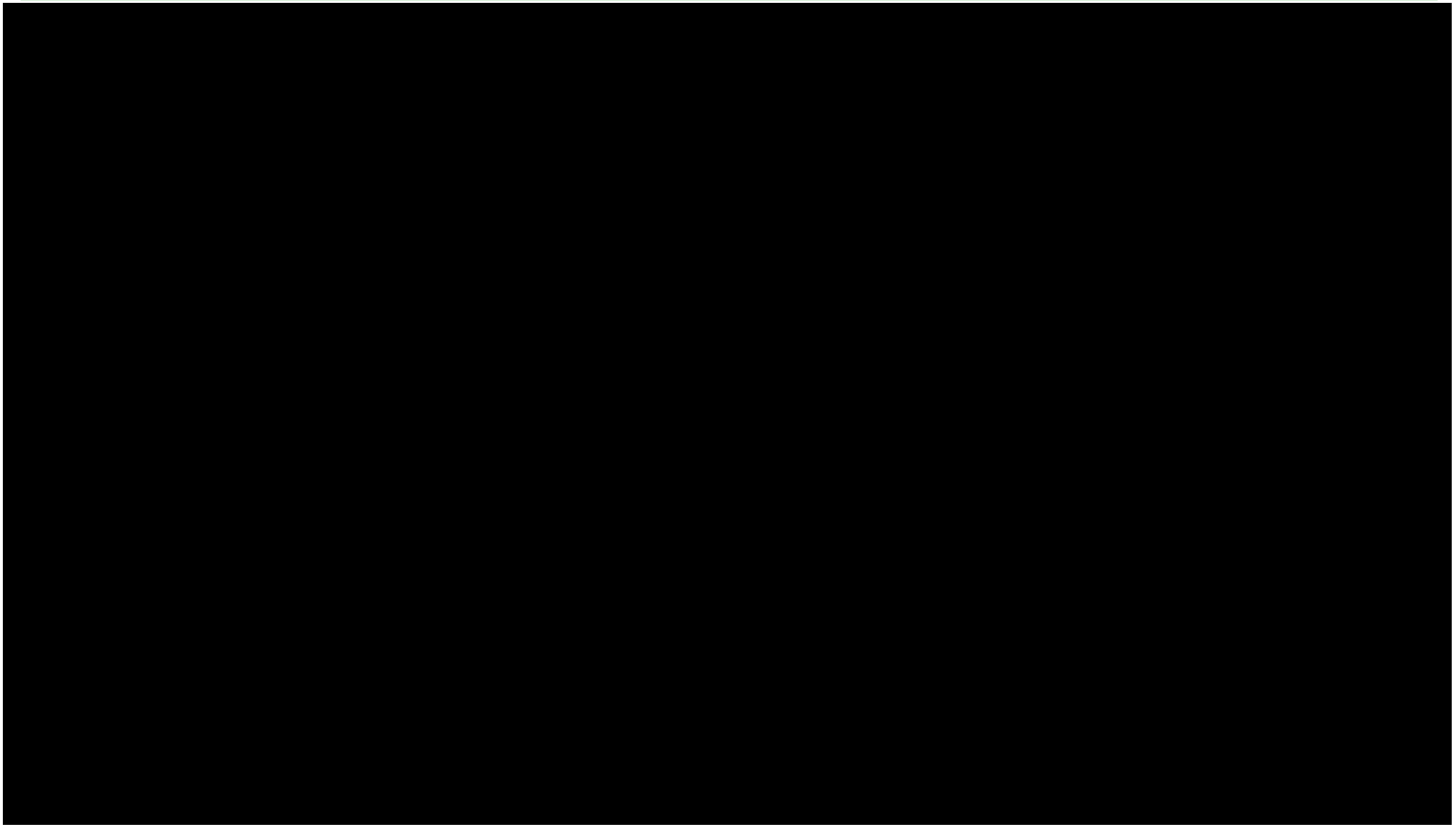












12B.11 ANNEX 5: Great Crested Newt eDNA Results

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
[redacted]

Sample ID: ADAS-1474	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Cowpen three	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1476	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Cowpen one	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1478	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Cowpen four	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1479	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Cowpen two	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2156	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: Highfield two	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	0 of 2	Real Time PCR	15/06/2023
Degradation Control§	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

ADAS eDNA Results Sheet: 1040055-Highfield (01) Page | 1 Edition: 01

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2157	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: Highfield one	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	0 of 2	Real Time PCR	15/06/2023
Degradation Control§	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1475	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Six	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Evidence of degradation	Real Time PCR	14/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1477	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Five	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Evidence of degradation	Real Time PCR	14/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1480	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Three	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Evidence of degradation	Real Time PCR	14/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1481	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC One	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Evidence of degradation	Real Time PCR	14/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1482	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Two	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	0 of 2	Real Time PCR	15/06/2023
Degradation Control§	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1483	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Four	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	0 of 2	Real Time PCR	15/06/2023
Degradation Control§	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
[redacted]

Sample ID: ADAS-1485	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: SABIC Seven	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	0 of 2	Real Time PCR	15/06/2023
Degradation Control§	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2023
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
[redacted]

Sample ID: ADAS-2166	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: SABIC Eight	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
[redacted]

Sample ID: ADAS-2167	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: SABIC Nine	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2173	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Substation one	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	14/06/2023
Degradation Control§	Within Limits	Real Time PCR	14/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

ADAS eDNA Results Sheet: 1040055-Substation (01) Page | 1 Edition: 01

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2160	Condition on Receipt: High Sediment	Volume: Passed
Client Identifier: Venator two	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	15/06/2023
Degradation Control§	Within Limits	Real Time PCR	15/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

ADAS eDNA Results Sheet: 1040055-Highfield (01) Page | 1 Edition: 01

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2161	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Venator one	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	15/06/2023
Degradation Control§	Within Limits	Real Time PCR	15/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggott,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-2162	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Venator three	Description: pond water samples in preservative	
Date of Receipt: 13/06/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	15/06/2023
Degradation Control§	Within Limits	Real Time PCR	15/06/2023
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	15/06/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/06/2023	Date of issue:	16/06/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggot,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1467	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: Beech one	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	18/07/2023
Degradation Control§	Evidence of degradation	Real Time PCR	18/07/2023
Great Crested Newt*	Indeterminate	Real Time PCR	18/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggot,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

[redacted]

Sample ID: ADAS-1470	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: Coatham two	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	18/07/2023
Degradation Control§	Evidence of degradation	Real Time PCR	18/07/2023
Great Crested Newt*	Indeterminate	Real Time PCR	18/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Joshua Waggot,
Aecom

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
[redacted]

Sample ID: ADAS-1472	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Coatham one	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	19/07/2023
Degradation Control§	Within Limits	Real Time PCR	19/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Client: Joshua Waggot,
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[redacted]

Sample ID: ADAS-2170	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: PD one	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	19/07/2023
Degradation Control§	Evidence of degradation	Real Time PCR	19/07/2023
Great Crested Newt*	Indeterminate	Real Time PCR	19/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

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

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

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

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

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[redacted]

Sample ID: ADAS-2169	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: RBT one	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	19/07/2023
Degradation Control§	Evidence of degradation	Real Time PCR	19/07/2023
Great Crested Newt*	Indeterminate	Real Time PCR	19/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
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Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

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

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[redacted]

Sample ID: ADAS-1469	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Senbcorp one	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	19/07/2023
Degradation Control§	Within Limits	Real Time PCR	19/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

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

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

Client: Joshua Waggot,
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172 Chester Road
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[redacted]

Sample ID: ADAS-2172	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 3 Teesworks	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	19/07/2023
Degradation Control§	Within Limits	Real Time PCR	19/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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

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

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

#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

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

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

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

Client: Joshua Waggot,
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172 Chester Road
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[redacted]

Sample ID: ADAS-1468	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond two Teesworks	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	18/07/2023
Degradation Control§	Within Limits	Real Time PCR	18/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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

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#Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

ADAS eDNA Results Sheet: 1040055-Teesworks (01) Page | 1 Edition: 01

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[redacted]

Sample ID: ADAS-2164	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: Pond one Teesworks	Description: pond water samples in preservative	
Date of Receipt: 10/07/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	18/07/2023
Degradation Control§	Within Limits	Real Time PCR	18/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/07/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/07/2023	Date of issue:	19/07/2023

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Appendix 1: Interpretation of results

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1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
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12B.12 ANNEX 6: Plates

Plate 12B-1: Waterbody 315 (Positive for GCN eDNA)

Plate 12B-2: Waterbody 316 (Positive for GCN eDNA)