FEHILY TIMONEY

CONSULTANTS IN ENGINEERING,
ENVIRONMENTAL SCIENCE
& PLANNING

APPENDIX 5.4

Aquatic Ecology Report

Aquatic baseline report for Barnadivane Wind Farm & Substation, Co. Cork

Prepared by Triturus Environmental Ltd. for Fehily Timoney & Company Ltd.

November 2022

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Table of contents

1. Introduction

1.1 Background

Triturus Environmental Ltd. were commissioned by Fehily Timoney & Company Ltd. to conduct baseline aquatic surveys to inform EIAR preparation for the Proposed Wind Farm & Substation Project. The following report provides a baseline assessment of the aquatic ecology including fisheries and biological water quality, as well as protected aquatic species and habitats in the vicinity of the Proposed Development, located approx. 5km southwest of Kilmurry, Co. Cork.

Undertaken on a catchment-wide scale, the baseline surveys focused on aquatic habitats and species of high conservation value. This included surveys for white-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussel (*Margaritifera margaritifera*) (eDNA only), macro-invertebrates (biological water quality), macrophytes & aquatic bryophytes, aquatic invasive species and fish of conservation value which may use the watercourses in the vicinity of the Proposed Project (**Figure 2.1**). Aquatic surveys were undertaken in August 2022.

1.2 Project description

A full description of the Proposed Development is provided in the accompanying Environmental Impact Assessment Report (EIAR).

2. Methodology

2.1 Selection of watercourses for assessment

All freshwater watercourses which could be affected directly or indirectly by the Proposed Development (the Proposed Wind Farm and Substation) were considered as part of the current baseline. A total of *n*=11 riverine sites were selected for detailed aquatic assessment (see **Table 2.1, Figure 2.1** below). The nomenclature for the watercourses surveyed is as per the Environmental Protection Agency (EPA). Aquatic survey sites were present on the Cummer River (EPA code: 19C02), Clearagh Stream (19C64), River Bride [Cork] (19B04), Moneygaff East Stream (19F09) and Barnadivane Stream (19B22) (**Table 2.1**). The *n*=11 aquatic survey sites were located within the Lee[Cork]_SC_030 and Lee[Cork]_SC_050 river sub-catchments. The Proposed Wind Farm and associated infrastructure were not located within a European site.

Please note this aquatic report should be read in conjunction with the final Environmental Impact Assessment Report (EIAR) prepared for the Proposed Project. More specific aquatic methodology is outlined below and in the appendices of this report.

2.2 Aquatic site surveys

Aquatic surveys of the watercourses within the vicinity of the Proposed Development were conducted on 17th-18th August 2022. Survey effort focused on both instream and riparian habitats at each aquatic sampling location (**Figure 2.1**). Surveys at each of these sites included a fisheries assessment (electro-fishing and or fisheries habitat appraisal), white-clawed crayfish survey, macrophyte and aquatic bryophyte survey and (where suitable) biological water quality sampling (Q-sampling) (**Figure 2.1**).

Suitability for freshwater pearl mussel was assessed at each survey site with environmental DNA (eDNA) sampling undertaken for the species at *n*=2 strategically chosen riverine locations within the vicinity of the Proposed Project. This holistic approach informed the overall aquatic ecological evaluation of each site in context of the Proposed Project and ensured that any habitats and species of high conservation value would be detected to best inform mitigation for the Proposed Project.

In addition to the ecological characteristics of the site, a broad aquatic and riparian habitat assessment was conducted utilising elements of the methodology given in the Environment Agency's 'River Habitat Survey in Britain and Ireland Field Survey Guidance Manual 2003' (EA, 2003) and the Irish Heritage Council's 'A Guide to Habitats in Ireland' (Fossitt, 2000). This broad characterisation helped define the watercourses' conformity or departure from naturalness. All sites were assessed in terms of:

- Physical watercourse/waterbody characteristics (i.e. width, depth etc.) including associated evidence of historical drainage
- Substrate type, listing substrate fractions in order of dominance (i.e. bedrock, boulder, cobble, gravel, sand, silt etc.)
- Flow type by proportion of riffle, glide and pool in the sampling area
- An appraisal of the macrophyte and aquatic bryophyte community at each site
- Riparian vegetation composition

2.3 Fish stock assessment (electro-fishing)

A single anode Smith-Root LR24 backpack (12V DC input; 300V, 100W DC output) was used to electro-fish sites on watercourses in the vicinity of the Proposed Development in August 2022, following notification to Inland Fisheries Ireland, under the conditions of a Department of the Environment, Climate and Communications (DECC) licence. Electro-fishing was undertaken at all riverine survey sites. Therefore, a total of *n*=11 sites were surveyed via electro-fishing (**Table 2.1, Figure 2.1; Appendix A**). The survey was undertaken in accordance with best practice (CEN, 2003; CFB, 2008) and Section 14 licencing requirements.

Furthermore, a fisheries habitat appraisal of the aquatic survey sites (**Figure 2.1**) was undertaken to establish their importance for salmonid, lamprey, European eel and other fish species. The baseline assessment also considered the quality of spawning, nursery and holding habitat for salmonids and lamprey within the vicinity of the survey sites. For detailed survey methodology, please refer to accompanying fisheries assessment report in **Appendix A.**

2.4 White-clawed crayfish survey

White-clawed crayfish (*Austropotamobius pallipes*) surveys were undertaken at the aquatic survey sites in August 2022 under a National Parks and Wildlife (NPWS) open licence (no. C31/2022), as prescribed by Sections 9, 23 and 34 of the Wildlife Act (1976-2021), to capture and release crayfish to their site of capture, under condition no. 6 of the licence. As per Inland Fisheries Ireland recommendations, the crayfish sampling started at the uppermost site(s) of the wind farm catchment/sub-catchments in the survey area to minimise the risk of transfer invasive propagules (including crayfish plague) in an upstream direction.

Hand-searching of instream refugia and sweep netting was undertaken according to Reynolds et al. (2010). An appraisal of white-clawed crayfish habitat at each site was conducted based on physical channel attributes, water chemistry and incidental records in mustelid spraint. Additionally, a desktop review of crayfish records within the wider Proposed Wind Farm survey area was completed.

Table 2.1 Location of *n*=11 aquatic survey sites in the vicinity of the Proposed Wind Farm, Co. Cork (* denotes eDNA sampling)

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Cummer River	19C02	Lackareagh	534472	564764
A2	Clearagh Stream	19C64	Lackareagh	535506	564531
A3	Cummer River	19C02	Greenville	535311	565896
A4	Cummer River	19C02	Teereeven	535346	567245
A5*	Cummer River	19C02	Ballymichael Bridge	538941	567084
B1	River Bride [Cork]	19B04	Moneygaff East	533173	562259
B2	Moneygaff East Stream	19F09	Barnadivane	533455	562476
B3	Barnadivane Stream	19B22	Barnadivane	533994	562217
B4	River Bride [Cork]	19B04	Garranereagh	534607	561454
B5	River Bride [Cork]	19B04	Hornhill Bridge	538342	562962
B6*	River Bride [Cork]	19B04	Currabeha	541813	564722

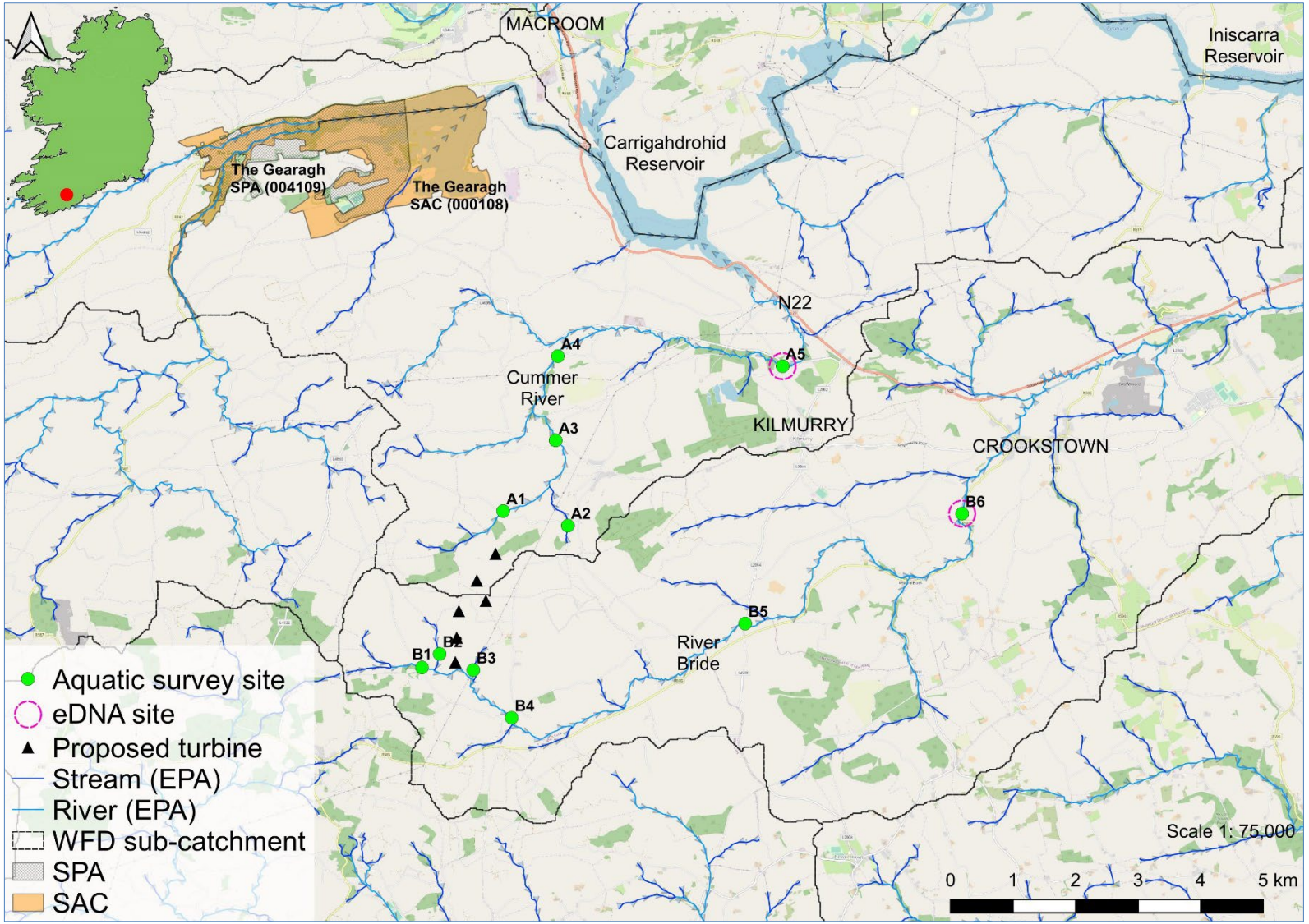

Figure 2.1 Overview of the *n*=11 aquatic survey site locations in the vicinity of the Proposed Wind Farm, Co. Cork

2.5 Otter signs

The presence of otter (*Lutra lutra*) at each aquatic survey site was determined through the recording of otter signs within 150m of each survey site. Notes on the age and location (ITM coordinates) were made for each otter sign recorded, in addition to the quantity and visible constituents of spraint (i.e. remains of fish, crustaceans, molluscs etc.).

2.6 eDNA analysis (including freshwater pearl mussel)

To validate habitat suitability appraisal and to detect potentially cryptically low populations of freshwater pearl mussel within the Study Area, *n*=2 composite water samples were collected from the River Bride and Cummer River and analysed for freshwater pearl mussel eDNA (**Figure 2.1**) given the absence of known records for pearl mussel in these catchments. This would help validate presence and or absence given that no data was available on the status of pearl mussel in these rivers. The water samples were collected on 18th August 2022, with the sites strategically chosen to maximise longitudinal (instream) coverage within the catchment (i.e. facilitating a greater likelihood of species detection).

In accordance with best practice, a composite (500ml) water sample was collected from the sampling point, maximising the geographic spread at the site (20 x 25ml samples at each site), thus increasing the chance of detecting the target species' DNA. The composite sample was filtered on site using a sterile proprietary eDNA sampling kit. The fixed sample was stored at room temperature and sent to the laboratory for analysis with 48 hours of collection. A total of *n*=12 qPCR replicates were analysed for the site. Given the high sensitivity of eDNA analysis, a single positive qPCR replicate is considered as proof of the species' presence (termed qPCR No Threshold, or qPCR NT). Whilst an eDNA approach is not currently quantitative, the detection of the target species' DNA indicates the presence of the species at and or upstream of the sampling point. Please refer to **Appendix C** for full eDNA laboratory analysis methodology.

2.7 Biological water quality (Q-sampling)

The 11 no. riverine survey sites were assessed for biological water quality through Q-sampling in August 2022 (**Figure 2.1).** All samples were taken with a standard kick sampling hand net (250mm width, 500µm mesh size) from areas of riffle/glide utilising a 2-minute kick sample, as per Environmental Protection Authority (EPA) methodology (Feeley et al., 2020). Large cobble was also washed at each site for 1-minute (where present) to collect attached macro-invertebrates (as per Feeley et al., 2020). Samples were elutriated and fixed in 70% ethanol for subsequent laboratory identification. Samples were converted to Q-ratings as per Toner et al. (2005) and assigned to WFD status classes. Any rare invertebrate species were identified from the NPWS Red List publications for beetles (Foster et al., 2009), mayflies (Kelly-Quinn & Regan, 2012), stoneflies (Feeley et al., 2020) and other relevant taxa (i.e. Byrne et al., 2009; Nelson et al., 2011).

Table 2.2 Reference categories for EPA Q-ratings (Q1 to Q5)

Q Value	WFD status	Pollution status	Condition
Q5 or Q4-5	High status	Unpolluted	Satisfactory
Q4	Good status	Unpolluted	Satisfactory
Q3-4	Moderate status	Slightly polluted	Unsatisfactory
Q3 or Q2-3	Poor status	Moderately polluted	Unsatisfactory
Q2, Q1-2 or Q1	Bad status	Seriously polluted	Unsatisfactory

2.8 Macrophytes and aquatic bryophytes

Surveys of the macrophyte and aquatic bryophyte community were conducted by instream wading at each of the *n*=11 riverine sites, with specimens collected (by hand, sweep nets or via grapnel) for on-site identification. An assessment of the aquatic vegetation community helped to identify any rare macrophyte species (Flora Protection Order or Wyse-Jackson et al., 2016) or habitats corresponding to the Annex I habitats, e.g., 'Water courses of plain to montane levels, with submerged or floating vegetation of the *Ranunculion fluitantis* and *Callitricho-Batrachion* (low water level during summer) or aquatic mosses [3260]' (more commonly referred to as 'floating river vegetation').

2.9 Aquatic ecological evaluation

The evaluation of aquatic ecological receptors contained within this report uses the geographic scale and criteria defined in the 'Guidelines for Assessment of Ecological Impacts of National Road Schemes' (NRA, 2009).

2.10 Biosecurity

A strict biosecurity protocol following IFI (2010) and the Check-Clean-Dry approach was adhered to during surveys for all equipment and PPE used. Disinfection of all equipment and PPE before and after use with Virkon™ was conducted to prevent the transfer of pathogens or invasive propagules between survey sites. Surveys were undertaken at sites in a downstream order to minimise the risk of upstream propagule mobilisation. Where feasible, equipment was also thoroughly dried (through UV exposure) between survey areas. Any aquatic invasive species or pathogens recorded within or adjoining the survey areas were geo-referenced. All Triturus staff are certified in 'Good fieldwork practice: slowing the spread of invasive non-native species' by the University of Leeds.

3. Receiving environment

3.1 Barnadivane wind farm catchment and survey area description

The Proposed Development (Barnadivane wind farm and substation) is located in an upland area within the townlands of Capeen East, Moneygaff East and Lactanashinnagh, approximately 5km southwest of Kilmurry, Co. Cork (**Figure 2.1**). The Proposed Wind Farm site is within the Southwestern River Basin District and within hydrometric area 19 (Lee, Cork Harbour and Youghal Bay) within the Lee[Cork]_SC_030 and Lee[Cork]_SC_050 river sub-catchments. The Proposed Development is drained by the Cummer River (EPA code: 19C02) to the north and the Moneygaff East Stream (19F09), Barnadivane Stream (19B22) and River Bride (EPA code: 19B04) to the south.

The watercourses and aquatic surveys sites in the vicinity of the Proposed Development are typically small, upland eroding channels (FW1; Fossitt, 2000). Predominantly, the watercourses flow over areas of Devonian old red sandstone, sandstone, conglomerate and mudstone (Geological Survey of Ireland data). Land use practices in the wider survey area comprise pastures (CORINE 231), with localised coniferous forests (CORINE 312) and transitional woodland scrub (CORINE 324).

3.2 Fisheries asset of the survey area

The River Bride (19B04) rises 1.5km upstream of the Proposed Development (near Coppeen) and meanders for approx. 35km before it joins the River Lee (19L03) at Inniscarra Graveyard near Ballincollig. It is a productive river and contains a good population of brown trout (*Salmo trutta*) and, in the lower reaches, Atlantic salmon (*Salmo salar*) (O'Reilly, 2009). Lamprey (*Lampetra* sp.) are also known from the River Bride (see section 3.3).

Fisheries data for the other watercourses within the survey area was not available at the time of survey although many are locally known to support brown trout populations.

3.3 Protected aquatic species[1]

A comprehensive desktop review of available data (NPWS, NBDC & BSBI data) for 10km grid squares containing and adjoining the project (i.e. W36, W46, W56) identified a low number of records for rare and or protected aquatic species within the vicinity of the Proposed Wind Farm.

With the exception of a historical (1903) record for the River Lee (upstream of the Proposed Project), there are no known freshwater pearl mussel (*Margaritifera margaritifera*) records in the Lee[Cork]_SC_030 and Lee[Cork]_SC_050 river sub-catchments. This was based on an extensive literature review and also examination of NPWS sensitive species data. A low number of records for freshwater pearl mussel were available for the River Lee (upstream of The Gearagh) in 10km grid square W36 (NPWS data). However, these were located upstream of the Proposed Development (i.e. no hydrological connectivity) (**Figure 3.1**). Pearl mussel are also known from the Sullane River near Macroom (NPWS data not shown) but these populations are also located upstream of and in a separate sub-catchment (Sullane_SC_020) to the Proposed Wind Farm. Whilst part of the project is

[1] This report may not be made available to the public without redaction given the inclusion of sensitive species data

located within the Lee Lower *Margaritifera* sensitive area, this is designated for the aforementioned River Lee populations.

Records for Annex II otter (*Lutra lutra*) were available throughout the W36, W46 & W56 10km grid squares, including records on the Cummer River and River Bride (NPWS & NBDC data; **Figure 3.1**) A record was located in the upper reaches of the Cummer River in close proximity to the Proposed Development (i.e. <0.7km).

A single record for brook lamprey (*Lampetra planeri*) was available for the River Bride upstream of Béal na Bláth, located between survey sites B5 and B6 (Figure 3.1). However, this record was from 1993.

3.4 EPA water quality data (existing data)

The following outlines the available water quality data for the watercourses in context of the Proposed Development. Only recent water quality is summarised below. There was no contemporary EPA biological monitoring data available for a number of the surveyed watercourses, namely the Moneygaff East Stream (19F09), the Barnadivane Stream (19B22) and the Clearagh Stream (19C64). Please note that biological water quality analysis (Q-sampling) was undertaken as part of this survey, with the results presented in the **section 4** and **Appendix A** of this report.

3.4.1 River Bride [Cork]

A total of four contemporary EPA biological monitoring stations were located on the River Bride (19B04). The river achieved **Q4 (good status)** at Hornhill Bridge (station RS19B040400, survey site B5) in 2020. The river achieved **Q3-4 (moderate status)** at station RS19B040600 near Crookstown in 2020, approx. 1.6km downstream of survey site B6. The water quality improved to **Q4 (good status)** at Coolmucky Bridge (station RS19B040900) in 2020 but declined to **Q3-4 (moderate status)** at Kilcrea Bridge (station RS19B041300) in 2020.

The upper reaches of the Bride (Bride (Lee)_010 river waterbody) achieved high status in the 2013-2018 period and were considered 'not at risk' of achieving target good status water quality (WFD Risk 3rd cycle). The Bride (Lee)_020, Bride (Lee)_030 and Bride (Lee)_050 also achieved good status in the same period. The Bride (Lee)_030 and Bride (Lee)_040 river waterbodies were considered 'at risk' of not achieving target good status water quality (WFD Risk 3rd cycle). The primary risk to water quality within these river waterbodies is wastewater discharge (EPA, 2019).

3.4.2 Cummer River

Three contemporary EPA biological monitoring stations were located on the River Cummer (19C02). The river achieved **Q4 (good status)** at station RS19C020200 (survey site A3) in 2020. The river achieved **Q4-5 (high status)** at station (RS19C020500) approx. 0.6km downstream of survey site A4. In the lower reaches, at Athsollis Bridge (station RS19C020800), the river achieved **Q4 (good status)** in 2020.

The Cummer River in Cummer_010 and _020 river waterbodies achieved good status in the 2013-2018 period and were considered 'not at risk' of achieving target good status water quality (WFD Risk 3rd cycle).

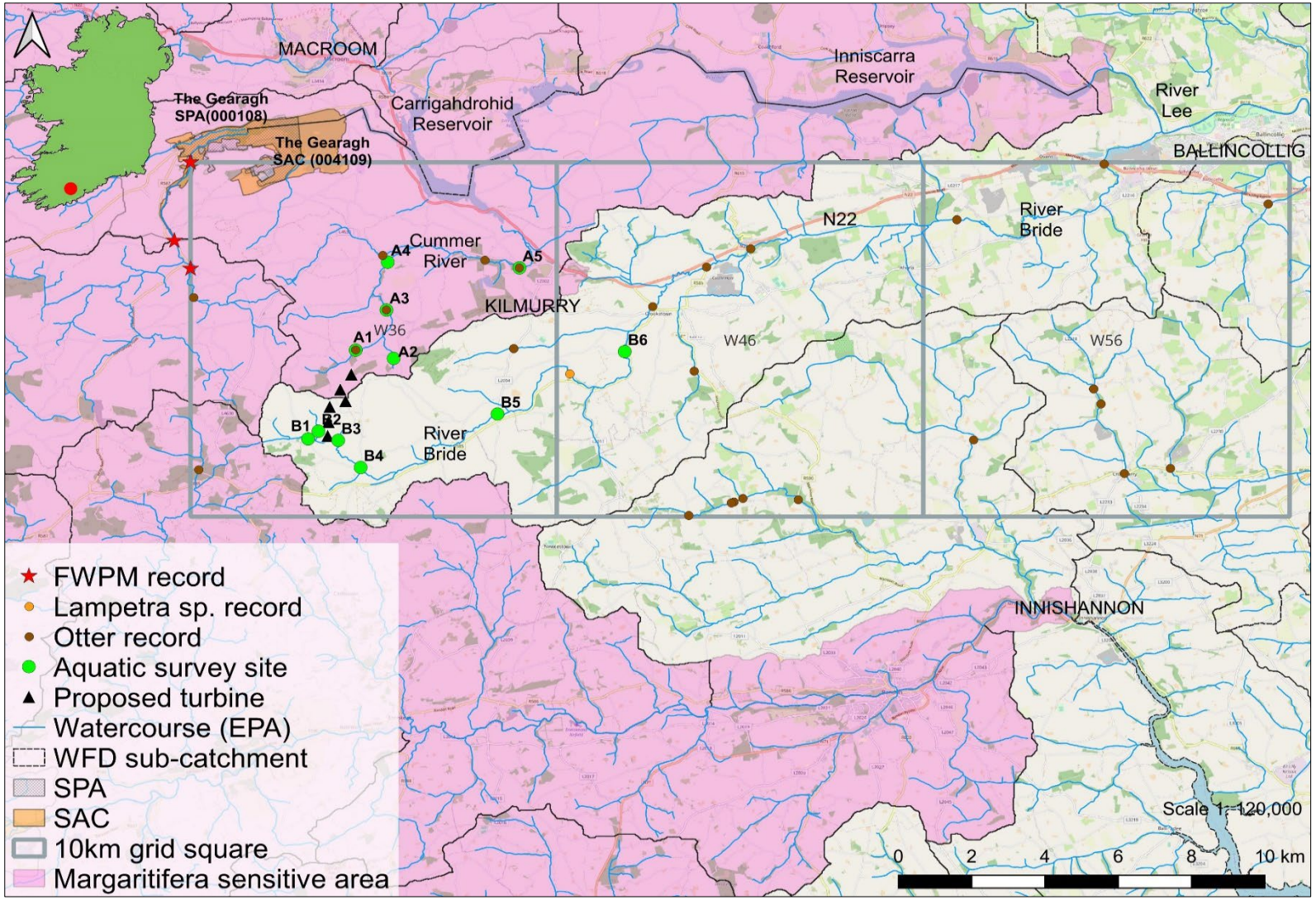

Figure 3.1 Freshwater pearl mussel, otter & *Lampetra* sp. records in the vicinity of the Proposed Wind Farm and Substation (source: NPWS & NBDC data)

4. Results of aquatic surveys

The following section summarises each of the *n*=11 survey sites in terms of aquatic habitats, physical characteristics and overall value for fish, white-clawed crayfish and macrophyte/aquatic bryophyte communities. Biological water quality (Q-sample) results are also summarised for each riverine sampling site (*n*=11) and in **Appendix B**. Habitat codes are according to Fossitt (2000). Scientific names are provided at first mention only. Sites were surveyed in August 2022. Please refer to **Appendix A** (fisheries assessment report) for more detailed fisheries results. A summary of the fish species recorded at each survey site is provided in **Table 4.2**. A summary of the aquatic species and habitats of high conservation concern recorded during the surveys is provided in **Table 4.3**. An evaluation of the aquatic ecological importance of each survey site based on these aquatic surveys is provided and summarised in **Table 4.4**.

4.1 Aquatic survey site results

4.1.1 Site A1 – Cummer River, Lackereagh

Site A1 was located on the uppermost reaches of the Cummer River (19C02) at a local road crossing. The low gradient upland eroding watercourse (FW1) averaged 1-2m wide and 0.05-0.15m deep with 1m high banks grading into an area of valley flat. The river at this location had a semi-natural meandering profile but had been straightened historically along the local road. It was dominated by glide and riffle with localised deeper pool. The substrata were dominated by small boulder and cobble with coarse gravels between boulder and cobble with light siltation only and with uncompacted substrata. Siltation was moderate but the substrata were largely uncompacted. The site supported localised water mint (*Mentha aquatica*) and water starwort (*Callitriche* sp.). In terms of aquatic bryophytes, the moss species *Brachythecium rivulare* was present on boulder tops in addition to *Fontinalis antipyretica* on cobble. The riparian zone supported scattered mature grey willow (*Salix cinerea*) and ash (*Fraxinus excelsior*) with frequent great willowherb (*Epilobium hirsutum*), meadowsweet (*Filipendula ulmaria*), wild angelica (*Angelica sylvestris*) and marsh woundwort (*Stachys palustris*) along the boundaries. The site was bordered by improved pasture (GA1) and dry meadows (GS2) with wet grassland locally (GS4).

Brown trout (*Salmo trutta*) was the only fish species recorded via electro-fishing at site A1 (**Appendix A**). Site A1 was considered a moderate quality brown trout nursery, with a low density recorded. The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile salmonids. Spawning was of moderate to good quality given the presence of mixed gravels in pool and gravel tailings. Moderate quality holding habitat as present locally in deeper pool areas. The site was considered a lower quality European eel habitat given the high gradient and none were recorded. The upland eroding site was unsuitable for lamprey. There was no suitability for freshwater pearl mussel. white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids, in addition to good status water quality, the aquatic ecological evaluation of site A1 was of **local importance (higher value) (Table 4.4).**

Plate 4.1 Representative image of site A1 on the upper reaches of the Cummer River, August 2022

4.1.2 Site A2 – Clearagh Stream, Lackereagh

Site A2 was located on the uppermost reaches of the Clearagh Stream (19C64), a Cummer River tributary, at a local road crossing. The upland eroding watercourse (FW1) averaged 2-3m wide and 0.05-0.15m deep and flowed over a steep gradient with 0.5m high banks grading into a gently sloping valley. The stream had a semi-natural meandering profile with riffle, glide and localised pool at gradient drops. The riverbed was dominated by small boulder and cobble with frequent mixed gravels in pool and glide pockets. Siltation was moderate but the substrata were largely uncompacted. Given the high energy, macrophytes were not present. However, the moss species *Brachythecium rivulare* was present on boulder tops. The liverwort *Pellia endiviifolia* was also present locally in shaded areas. The riparian zone supported scattered mature grey willow, ash and hawthorn (*Crataegus monogyna*), with bramble (*Rubus fruticosus* agg.) and gorse (*Ulex europaeus*) in the understories. The site was bordered by improved pasture (GA1).

Brown trout was the only fish species recorded via electro-fishing at site A2 (**Appendix A**). The site was considered a moderate quality brown trout nursery, with a low density recorded. The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well oxygenated water with refugia for juveniles. Spawning was of moderate to good quality given the presence of mixed gravels in pool and gravel tailings. Moderate quality holding habitat as present locally in deeper pool areas. The site was considered a lower quality European eel habitat given the high gradient and none were recorded. The upland eroding site was unsuitable for lamprey. There was no suitability for freshwater pearl mussel. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q3-4 (moderate status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids, the aquatic ecological evaluation of site A2 was of **local importance (higher value) (Table 4.4).**

Plate 4.2 Representative image of site A2 on the Clearagh Stream, August 2022

4.1.3 Site A3 – Cummer River, Greenville

Site A3 was located on the Cummer River (19C02) at a local road crossing, approx. 1.8km downstream of site A1. The low gradient upland eroding watercourse (FW1) averaged 4-6m wide and varied between 0.1m and 0.6m deep with 0.6m high banks. The river had a semi-natural meandering profile with equal proportions of riffle, glide and pool. The substrata were dominated by cobble but abundant mixed gravels were present in the tailings of pool and deeper glide. Scattered boulder was also present. Siltation was moderate but the substrata were largely uncompacted. The bed featured a low deposition of floc[2] (approx. 5%) with a low cover of filamentous algae (<5%) indicating enrichment. The site supported occasional hemlock water dropwort (*Oenanthe crocata*), fool's watercress (*Apium nodiflorum*), narrow-fruited watercress (*Nasturtium microphyllum*) and brooklime (*Veronica beccabunga*) locally on exposed cobble islands or at depositing margins of meanders. Water crowfoot (*Ranunculus* sp.) was occasional in glide areas adjoining pool where light permitted growth, water starwort (*Callitriche* sp.) recorded locally. The liverwort *Chiloscyphus polyanthos* was recorded as rare with *Fontinalis antipyretica* occasionally present on boulders. The macrophyte and aquatic bryophyte community shared links with the Annex I habitat floating river vegetation (3260) (EC, 2013; Weekes et al., 2018). The riparian zone supported scattered mature alder (*Alnus glutinosa*) that shaded the small river channel. The river was bordered by large expanses of open improved pasture (GA1).

Brown trout was the only fish species recorded via electro-fishing at site A3 (**Appendix A**). The site was considered an excellent brown trout nursery, supporting high density of juveniles and a low number of adults. The presence of broken riffle and glide with an abundance of mixed cobble and gravels provided well oxygenated water with high quality refugia. A sinuous river profile, shading and

[2] floc is defined as an aggregation of (mostly dead) organic material, mainly from algae and diatoms, but also with potential origins from decaying macrophytes and associated decomposers (bacteria and fungi). The floc can form a layer at the surface of the substrate, or infiltrate the substrate, generally where there is insufficient flow to keep the material in suspension (Moorkens & Killeen, 2020)

macrophyte cover (e.g. water crowfoot) further increased the nursery value. Spawning was of high quality given the presence of mixed gravels in deeper pool habitat being only diminished slightly due to moderate siltation. The holding value was good with ample deep glide and pool for adult brown trout. Site A3 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. The upland eroding site was unsuitable for lamprey. There was no suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids and high-quality salmonid habitat, in addition to Annex I floating river vegetation (3260) and good status water quality, the aquatic ecological evaluation of site A3 was of **local importance (higher value) (Table 4.4).**

Plate 4.3 Representative image of site A3 on the Cummer River, August 2022

4.1.4 Site A4 – Cummer River, Teereeven

Site A4 was located on the Cummer River (19C02) at a local road crossing, approx. 1.7km downstream of site A3. The upland eroding spate watercourse (FW1) averaged 8-9m wide and 0.1-0.3m deep with locally deeper pool to 1.1m with bank heights of 1.2m in height. The river had been historically deepened but retained a well-defined meandering thalweg with equal proportions of riffle, glide and pool. The substrata comprised boulder, cobble and mixed gravels. There were no depositing silt areas given the higher energy of the channel. Although the gravels were exposed to moderate siltation, they were largely uncompacted. The site featured a deposition of floc (approx. 20%) with a low cover of filamentous algae (<5%) indicating enrichment. The site supported frequent hemlock water dropwort on exposed cobble and gravel bars. The liverwort *Chiloscyphus polyanthos* was occasional with *Brachythecium rivulare* present locally on boulder tops. *Hygroamblystegium* sp. moss was recorded as rare. The riparian zone supported mature treelines of alder with scattered hawthorn and scrubby

understories with nettle (*Urtica dioica*), bramble, hogweed (*Heracleum sphondylium*), hedge bindweed (*Calystegia sepium*) and ivy (*Hedera hibernica*). The survey site was bordered by dry meadows (GS2) and improved pasture (GA1).

Brown trout was the only fish species recorded via electro-fishing at site A4 (**Appendix A**). The site was considered a good quality salmonid nursery (for brown trout). The presence of numbers of 0+ fish indicated more optimal nursery conditions (i.e. ample broken water good shading and abundant refugia in gravels). The spawning attributes were considered locally good in the tailing of deep glide and pool where abundant clean mixed gravels were present. The holding value was good with ample deep glide and pool for adult brown trout. Site A4 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. The upland eroding nature of the survey site was unsuitable for lamprey. There was no suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. A regular otter latrine site was recorded under the eastern arch of the bridge (ITM 535352, 567251).

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids, high-quality salmonid habitat and good status water quality, in addition to utilisation by Annex II otter, the aquatic ecological evaluation of site A4 was of **local importance (higher value) (Table 4.4).**

Plate 4.4 Representative image of site A4 on the Cummer River, August 2022

4.1.5 Site A5 – Cummer River, Ballymichael Bridge

Site A5 was located on the lower reaches of the Cummer River (19C02) at Ballymichael Bridge, approximately 4.1km downstream of site A4 and 2.3km upstream of the Carrigadrohid Reservoir confluence with the river. The low gradient upland eroding watercourse (FW1) averaged 8-10m wide and 0.1-0.4m deep, with locally deeper pool to 0.9m and banks of 1m in height. The river at this location had a semi-natural meandering profile with equal proportions of riffle, glide and pool. The substrata were dominated by small cobble and mixed gravels with more localised boulder. Small pockets of shallow silt were also present in depositing margins of the channel adjoining exposed bars of cobble and mixed gravel. Siltation was light overall and the substrata were uncompacted. The site supported frequent hemlock water dropwort on exposed cobble and gravel bars with occasional water crowfoot (*Ranunculus* sp.) and very localised starwort (*Callitriche* sp.). Common duckweed (*Lemna minor*) was occasional in floating patches with occasional fool's watercress in the margins. The liverwort *Chiloscyphus polyanthos* was abundant on the riverbed with occasional *Fontinalis antipyretica and Leptodictyum riparium*[3]. The macrophyte community corresponded to Annex I floating river vegetation (3260) given the presence of three or more indicator species (EC, 2013; Weekes et al., 2018). Filamentous algae were not present due to higher riparian shading. The riparian areas supported mature buffers of mixed broad-leaved woodland (WD1) dominated by sycamore (*Acer pseudoplatanus*) with ash, alder and cherry laurel (*Prunus laurocerasus*) with an understory of ferns, nettle, opposite leaved golden saxifrage (*Chrysosplenium oppositifolium*), wood avens (*Geum urbanum*) and bramble. The site was bordered by improved pasture (GA1).

Brown trout, *Lampetra* sp. and three-spined stickleback (*Gasterosteus aculeatus*) were recorded via electro-fishing at site A5 (**Appendix A**). The site was considered a good quality salmonid nursery (for brown trout). The presence of mixed cohorts supported this observation and good numbers of 0+ fish indicated more optimal nursery conditions (i.e. ample broken water good shading and abundant refugia in gravels). The spawning attributes were considered locally good in the tailing of deep glide and pool where abundant clean mixed gravels were present. The holding value was good with ample deep glide and pool for adult brown trout. Site A5 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. Some moderate suitability for *Lampetra* sp. existed in localised areas of shallow organic rich silt. However, given these areas were typically <5cm deep and localised in the survey reach, they were not capable of supporting high densities of *Lampetra* sp. ammocoetes. There was limited suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. A recent otter spraint was recorded on boulders adjoining the north abutment of the bridge on the upstream (west) side (ITM 538940, 567092).

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

[3] *Leptodictyum riparium* is an indicator of enrichment (Weekes et al., 2021)

Given the presence of salmonids, high-quality salmonid habitat and good status water quality, in addition to Annex II *Lampetra* sp., Annex I floating river vegetation (3260) and utilisation by Annex II otter, the aquatic ecological evaluation of site A5 was of **local importance (higher value) (Table 4.4).**

Plate 4.5 Representative image of site A5 on the Cummer River at Ballymichael Bridge, August 2022

4.1.6 Site B1 – River Bride, Moneygaff East

Site B1 was located on the uppermost reaches of the River Bride (19B04) at a local road crossing. The small upland eroding watercourse (FW1) averaged 2m wide and 0.05-0.15m deep with 1.5m high banks. The river had been straightened and deepened historically but retained a semi-natural, meandering profile. The profile was dominated by glide and riffle with very localised pool. The substrata comprised small boulder and cobble with coarse interstitial gravels. The bed was moderately compacted with moderate siltation evident (silt plumes underfoot). Given high shading, macrophytes were limited to very localised water cress (*Nasturtium officinale*) and blue water speedwell (*Veronica anagallis-aquatica*) in open areas downstream of the bridge. The site also supported the moss species *Fontinalis antipyretica* occasionally on small boulder. The instream cobble and boulder were heavily strewn with the liverwort *Chiloscyphus polyanthos*. The riparian zone supported abundant grey willow with dense bramble, hedge bindweed and bracken (*Pteridium aquilinum*) scrub. The site was bordered by scrub (WS1) and improved pasture (GA1).

Brown trout was the only fish species recorded via electro-fishing at site B1 (**Appendix A**). The site was considered a moderate quality salmonid nursery with a low density of juveniles recorded (no adults). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile trout. Spawning was of poor quality given siltation pressures, bedding of substrata and dominance of coarser substrata. Localised deeper pool provided some moderate quality holding habitat. Site B1 was considered a moderate quality European eel habitat given the presence of boulder and cobble refugia although none were recorded. The upland eroding site was unsuitable for lamprey. There was limited suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. none were recorded in the eDNA sample. No white-

clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids, in addition to good status water quality, the aquatic ecological evaluation of site B1 was of **local importance (higher value) (Table 4.4).**

Plate 4.6 Representative image of site B1 on the upper reaches of the River Bride August 2022

4.1.7 Site B2 – Moneygaff East Stream, Barnadivane

Site B2 was located on the Moneygaff East Stream (19M09) approx. 0.3km upstream of the River Bride confluence. The small upland eroding watercourse (FW1) averaged 1m wide and 0.05-0.15m deep with 1.2m high banks. The channel had a semi-natural profile meandering through dense scrub. The profile was dominated by glide and riffle with localised and limited pool habitat. The substrata were dominated by small boulder and cobble with limited coarse gravels. The bed was moderately compacted and had moderate siltation with silt plumes underfoot. Given high shading, macrophyte growth was not present. However, the moss species *Fontinalis antipyretica* was occasional on small boulders. Instream cobble and boulders were heavily strewn with the liverwort *Chiloscyphus polyanthos*, a species that favours shaded channels (Atherton et al., 2010). The riparian zone supported dense bramble, gorse, hedge bindweed and bracken scrub (WS1) with scattered grey willow. The site was bordered by semi-improved pasture (GA1).

Brown trout was the only fish species recorded via electro-fishing at site B2 (**Appendix A**). The site was considered a moderate quality salmonid nursery, supporting a low density of juveniles (no adults). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile trout. Spawning was of poor quality given siltation pressures, bedding of substrata and dominance of coarser substrata. Localised deeper pool provided

some moderate quality holding habitat. Site B1 was considered a moderate quality European eel habitat given the presence of boulder and cobble refugia although none were recorded. The upland eroding site was unsuitable for lamprey. There was no suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids, in addition to good status water quality, the aquatic ecological evaluation of site B2 was of **local importance (higher value) (Table 4.4).**

Plate 4.7 Representative image of site B2 on the Moneygaff East Stream, August 2022

4.1.8 Site B3 – Barnadivane Stream, Barnadivane

Site B3 was located on the Barnadivane Stream (19B22) approx. 0.2km upstream of the River Bride confluence. The stream at this location was a very narrow, high-gradient upland eroding stream (FW1) that averaged <1m wide and 0.05m deep. The stream had been straightened and deepened historically (along a field boundary) but retained a semi-natural profile with 1m high banks. The profile comprised very shallow glide and riffle with no pool. The substrata were dominated by small boulder and cobble with mixed interstitial gravels. The bed was moderately compacted and had moderate siltation with silt plumes underfoot. Given high shading, macrophyte growth was not present. However, instream boulders supported occasional *Brachythecium* sp. moss. The site was bordered by improved pasture (GA1) outside the scrubbed riparian areas (WS1).

No fish were recorded via electro-fishing at site B3 (**Appendix A**) and the site was not of fisheries value given its diminutive size and location in the upper reaches of the catchment. There was no suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed

crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q3 (poor status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the absence of aquatic species or habitats of higher conservation value, in addition to poor status water quality, the aquatic ecological evaluation of site B3 was of **local importance (lower value) (Table 4.4).**

Plate 4.8 Representative image of site B3 on the Barnadivane Stream at Barnadivane, August 2022

4.1.9 Site B4 – River Bride, Garranereagh

Site B4 was located on the River Bride (19B04) at a local road crossing approx. 2km downstream of site B1. The upland eroding watercourse (FW1) had been straightened and deepened historically but retained a semi-natural profile and demonstrated some good instream recovery. The river averaged 3-6m wide and 0.1-0.4m deep with locally deeper glide and pool to 0.7m. The banks were up to 1.6m in height. The profile featured equal proportions of riffle, glide and pool. The substrata were dominated by cobble but abundant mixed gravels were present at the tailings of pool and deeper glide. Boulder was scattered throughout. The site suffered from moderate siltation but the substrata were largely uncompacted. The site supported frequent hemlock water dropwort, occasional fool's watercress, broadleaved pondweed (*Potamogeton natans*), water crowfoot (*Ranunculus* sp.) and water starwort (*Callitriche* sp.) The liverwort *Chiloscyphus polyanthos* and *Leptodictyum riparium* were locally frequent. The aquatic vegetation community shared links with Annex I floating river vegetation (3260) habitat (EC, 2013; Weekes et al., 2018). The riparian zone supported scattered mature grey willow, beech (*Fagus sylvatic*a) and ash but was dominated by scrub (WS1) comprising bracken, cleavers (*Galium aparine*), nettle, bramble and wild angelica.

Atlantic salmon (*Salmo salar*), brown trout, European eel (*Anguilla anguilla*) and stone loach (*Barbatula barbatula*) were recorded via electro-fishing at site B2 (**Appendix A**). The site was considered a good quality salmonid nursery supporting moderate densities of brown trout and low densities of Atlantic salmon. The presence of abundant broken riffle and glide with an abundance of mixed cobble and gravels provided well-oxygenated water with high quality refugia. Shading and macrophyte plant cover (e.g. water crowfoot) further increased the nursery value. Spawning was of good quality given the presence of mixed gravels in deeper pool and glide. The holding value was good with ample deep glide and pool for adult salmonids. Site B4 was considered a good quality European eel habitat given the presence of boulder and cobble refugia although only a single adult was recorded. The upland eroding site was unsuitable for lamprey. There was limited suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. No otter signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids (including Annex II Atlantic salmon), high-quality salmonid habitat and good status water quality, in addition to Annex I floating river vegetation (3260), the aquatic ecological evaluation of site B4 was of **local importance (higher value) (Table 4.4).**

Plate 4.9 Representative image of site B4 on the River Bride, August 2022

4.1.10 Site B5 – River Bride, Hornhill Bridge

Site B5 was located on the River Bride (19B04) at Hornhill Bridge approx. 4.8km downstream of site B4. The upland eroding watercourse (FW1) averaged 5-10m wide (up to 15m) and 0.1-0.5m deep, with frequent braiding present upstream of the bridge. The banks were typically 1.5m in height. The river at this location featured a natural channel form with boulder cascade sequences over exposed sandstone bedrock. The bed of the high energy spate channel was dominated by bedrock, small boulder and cobble with patches of mixed gravels in pool and deeper glide pockets between exposed

bedrock. Siltation was light due to the high energy nature of the site. The site supported abundant hemlock water dropwort in the margins with frequent water crowfoot (*Ranunculus* sp.) and water starwort (*Callitriche* sp.) species were rare. The liverwort *Chiloscyphus polyanthos* was occasional on boulders with the moss species *Fontinalis antipyretica*, *Fontinalis squamosa* and *Rhynchostegium riparioides* on wet bedrock and boulders. The moss *Racomitrium aciculare* was also present but rare. The aquatic vegetation community shared links with the Annex I floating river vegetation [3260] habitat (EC, 2013; Weekes et al., 2018). The riparian zone supported frequent alder, grey willow and ash with bramble and nettle in the understories. The site was bordered by scrub (WS1) and improved pasture (GA1).

Atlantic salmon, brown trout and European eel were recorded via electro-fishing at site B5 (**Appendix A**). The site was considered a good quality salmonid nursery supporting moderate densities of brown trout and Atlantic salmon. The presence of abundant broken riffle and glide with an abundance of mixed cobble and gravels provided well-oxygenated water with high quality refugia. Shading and macrophyte plant cover (e.g. water crowfoot) further increased the nursery value. Spawning was of good quality given the presence of mixed boulder, coble and gravels in the boulder-cascade sequences. The holding value was good with ample deep glide and pool for adult salmonids. Site B5 was considered a good quality European eel habitat given the presence of boulder and cobble refugia although only a single juvenile was recorded. The upland eroding site was unsuitable for lamprey. There was limited suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. A regular otter spraint site was recorded under the southern arch of the masonry bridge, containing salmonid remains (ITM 538341, 562955).

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids (including Annex II Atlantic salmon), high-quality salmonid habitat and good status water quality, in addition to Annex I floating river vegetation (3260) and utilisation by Annex II otter, the aquatic ecological evaluation of site B5 was of **local importance (higher value) (Table 4.4).**

Plate 4.10 Representative image of site B5 on the River Bride at Hornhill Bridge, August 2022

4.1.11 Site B6 – River Bride, Currabeha

Site B6 was located on the River Bride (19B04) adjacent to the R585 road, approx. 5.6km downstream of site B5. The upland eroding watercourse (FW1) averaged 15-18m wide and 0.2-0.6m deep, with locally deeper areas to 1.4m. The banks were typically 1.5m in height and graded into a natural V-shaped valley. The river at this location featured a natural channel form with boulder cascade sequences over exposed sandstone bedrock. The bed of the high energy spate channel was dominated by bedrock, small boulder and cobble with patches of mixed gravels in pool and deeper glide pockets between exposed bedrock. Siltation was light due to the high energy nature of the site. The site supported occasional hemlock water dropwort in the margins with no other macrophytes due to the very high energy. The moss species *Brachythecium rivulare* and *Hyocomium* sp. were present on large bedrock and boulders. The riparian zone supported mixed broad-leaved woodland (WD1) with alder, ash and sycamore on the north bank and improved pasture (GA1) adjoining the south bank.

Atlantic salmon and brown trout were the only two fish species recorded via electro-fishing at site B6 (**Appendix A**). The site was considered an excellent salmonid nursery for both brown trout and Atlantic salmon, with high numbers present. The presence of abundant broken riffle and glide associated with boulder cascade sequences adjoining deep pool and glide provided well-oxygenated water with high quality refugia. Shading from the adjoining mature trees and woodland protected the river from thermal stress and excessive light, further increasing the nursery value. Spawning was of very good quality given the presence of mixed boulder, coble and gravels in the boulder-cascade sequences. The holding value was very good with ample deep glide and pool for adult salmonids. Site B6 was considered a good quality European eel habitat given the presence of boulder and cobble refugia but none were recorded. The upland eroding site was unsuitable for lamprey. There was limited suitability for freshwater pearl mussel and no signatures were recorded in the eDNA sample. No white-clawed crayfish were recorded and the sandstone geology was considered unsuitable for the species. An otter spraint site was recorded on an instream boulder (ITM 541819, 564737) and the site offered excellent foraging potential for the species.

Biological water quality, based on Q-sampling, was calculated as **Q4 (good status)** (**Appendix B**). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the presence of salmonids (including Annex II Atlantic salmon), high-quality salmonid habitat and good status water quality, in addition to utilisation by Annex II otter, the aquatic ecological evaluation of site B6 was of **local importance (higher value) (Table 4.4).**

Plate 4.11 Representative image of site B6 on the River Bride, August 2022

4.2 White-clawed crayfish

No white-clawed crayfish were recorded via hand-searching or sweep netting of instream refugia during the survey and no crayfish remains were identified in otter spraints recorded during the survey. These results supported the absence of available records for the species within the survey area, reflecting unsuitable (sandstone) geologies (Demers et al., 2005; Lucey & McGarrigle, 1987).

4.3 eDNA analysis

Composite water samples collected from the Cummer River at site A5 (FK593) and the River Bride at site B6 (FK598) returned a negative result for freshwater pearl mussel, i.e. freshwater pearl mussel eDNA not present or was present below the limit of detection in a series of 12 qPCR replicates (0 positive replicates out of 12, respectively) (**Table 4.1; Appendix C**). These results were considered as evidence of the species' absence at and or upstream of the sampling locations.

Table 4.1 eDNA results in the vicinity of the Proposed Development at Barnadivane, Co. Cork (positive qPCR replicates out of 12 in parentheses)

Sample	Watercourse	Freshwater pearl mussel
FK593	Cummer River, Ballymichael Bridge (site A5)	Negative (0/12)
FK598	River Bride (site B6)	Negative (0/12)

4.4 Otter signs

Despite some good suitability at numerous survey locations, otter signs were only recorded at a total of *n*=4 locations during the course of aquatic surveys undertaken in August 2022. A latrine site and spraint site were recorded at sites A4 and A5 on the Cummer River, respectively. Regular spraint site was recorded on the River Bride at site B5 (Hornhill Bridge), with a single spraint recorded on the River Bride at site B6.

No breeding (holts) or resting (couch) areas were identified in the vicinity of the survey sites in August 2022.

4.5 Invasive aquatic species

No aquatic invasive species were recorded during the survey of a total of *n*=11 riverine sites in August 2022.

4.6 Biological water quality (macro-invertebrates)

No rare or protected macro-invertebrate species (according to national red lists) were recorded in the biological water quality samples taken from *n*=11 riverine sites in August 2022 (**Appendix B**).

With the exception of sites A2 on the Clearagh Stream and B3 on the Barnadivane Stream, all survey sites achieved target good status (Q4) requirements of the European Union Environmental Objectives (Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (**Figure 4.1**).

Sites on the Cummer River (sites A1, A3, A4 & A5), Moneygaff East Stream (B2) and River Bride (B1, B4, B5 & B6) achieved **Q4 (good status)** water quality. This was given the presence of fair numbers (5-10%) of group A species, particularly the flattened mayfly *Ecdyonurus dispar*. These sites also supported a low number of group B species such as *Alainites muticus* and *Leuctra hippopus*, and a variety of group C species such as the mayflies *Baetis rhodani* and *Seratella ignita*, the riffle beetle Elmis aenea and freshwater shrimp (*Gammarus duebeni*) (**Appendix B**). Site A4 was the only site to support the group A stonefly *Amphinemura sulcicollis*, a species typically found in fast flowing stony streams (Feeley et al., 2020).

Site A2 on the Clearagh Stream achieved **Q3-4 (moderate status)** based on low abundance of group A species (single example of *Ecdyonurus dispar*), low numbers of group B species *Alainites muticus* and *Leuctra hippopus* and a dominance of group C species, particularly *Baetis rhodani* and Simuliidae larvae (**Appendix B**).

Site B3 on the Barnadivane Stream achieved **Q3 (poor status)**. This was given an absence of group A and B species and a dominance of low-abundance pollution tolerant group C species such as the non-native New Zealand mud snail (*Potamopyrgus antipodarum*) and crane fly larvae (*Dicranota* sp.).

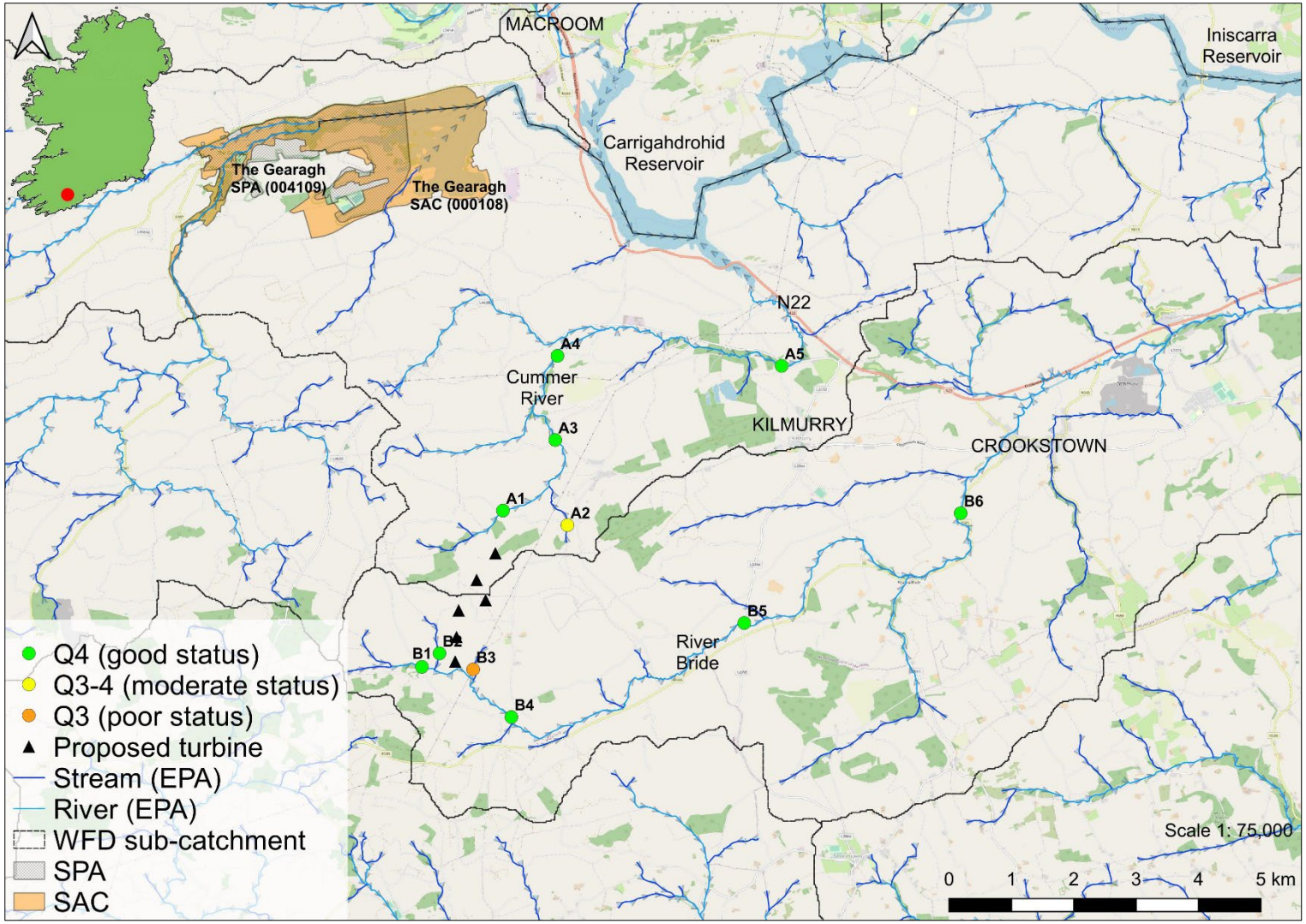

Figure 4.1 Overview of the biological water quality status in the vicinity of the Proposed Wind Farm and Substation, Co. Cork, August 2022

4.7 Macrophytes and aquatic bryophytes

No rare or protected macrophytes or aquatic bryophytes were recorded at the *n*=11 survey sites in August 2022.

However, examples of the Annex I habitat 'Water courses of plain to montane levels, with submerged or floating vegetation of the *Ranunculion fluitantis* and *Callitricho-Batrachion* (low water level during summer) or aquatic mosses [3260]' (more commonly referred to as 'floating river vegetation') were recorded at sites on the Cummer River (A3 & A5) and River Bride (B4 & B5). This was given the presence of several indicator species for the habitat (EC, 2013; Weekes et al., 2018), including water crowfoot (Ranunculus sp.) and the moss *Fontinalis antipyretica*.

4.8 Aquatic ecological evaluation

An aquatic ecological evaluation of each survey site was based on the results of desktop review (i.e., presence of fish of conservation value), fisheries habitat assessments, the presence of protected or rare invertebrates (e.g. white-clawed crayfish, freshwater pearl mussel), environmental DNA analysis, the presence of rare macrophytes and aquatic bryophytes and or associated representations of Annex I habitats. Furthermore, biological water quality status also informed the aquatic evaluation (**Table 4.4**).

None of the 11 no. aquatic survey sites were evaluated as greater than **local importance (higher value)**. With the exception of site B3 on the Barnadivane Stream, all survey sites were of **local importance (higher value)**. Primarily this evaluation was due to the presence of salmonids but some sites also supported other aquatic species or habitats of conservation value, such as Red-listed European eel, Annex II and IV otter or Annex I aquatic habitats.

Site B3 on the Barnadivane Stream was evaluated as **local importance (lower value)** in terms of its aquatic ecology given an absence of aquatic species or habitats of high conservation value.

Table 4.2 Summary of fish species of higher conservation value recorded via electro-fishing per survey site in the vicinity of the Proposed Development, August 2022

Site	Watercourse	Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Cummer River		✓			
A2	Clearagh Stream		✓			
A3	Cummer River		✓			
A4	Cummer River		✓			
A5	Cummer River		✓	✓		Three-spined stickleback
B1	River Bride		✓			
B2	Moneygaff East Stream		✓			
B3	Barnadivane Stream	No fish recorded				
B4	River Bride	✓	✓		✓	Stone loach
B5	River Bride	✓	✓		✓	
B6	River Bride	✓	✓			

Conservation value: Atlantic salmon (*Salmo salar*), brook lamprey (L*ampetra planeri*) and river lamprey (*Lampetra fluviatilis*) are listed under Annex II of the Habitats Directive [92/42/EEC]. Atlantic salmon and river lamprey are also listed under Annex V of the Habitats Directive [92/42/EEC]. European eel are 'critically endangered' according to most recent ICUN red list (Pike et al., 2020) and listed as 'critically engendered' in Ireland (King et al., 2011). With the exception of the Fisheries Acts 1959 to 2019, brown trout have no legal protection in Ireland.

Table 4.3 Summary of aquatic species (excluding fish) and habitats of higher conservation value recorded in the vicinity of the Proposed Development, August 2022

Site	Watercourse	White-clawed crayfish	Freshwater pearl mussel (eDNA)	Otter signs[4]	Annex I aquatic habitats	Rare or protected macrophytes/ aquatic bryophytes	Rare or protected macro-invertebrates	Other species/habitats of high conservation value
A1	Cummer River	None recorded			Not present	None recorded	None recorded	None recorded
A2	Clearagh Stream	None recorded			Not present	None recorded	None recorded	None recorded
A3	Cummer River	None recorded			**Floating river vegetation [3260]**	None recorded	None recorded	None recorded
A4	Cummer River	None recorded		**Latrine site**	Not present	None recorded	None recorded	None recorded
A5	Cummer River	None recorded	Negative eDNA result at site, no records in catchment	**Spraint site**	**Floating river vegetation [3260]**	None recorded	None recorded	None recorded
B1	River Bride	None recorded			Not present	None recorded	None recorded	None recorded
B2	Moneygaff East Stream	None recorded			Not present	None recorded	None recorded	None recorded
B3	Barnadivane Stream	None recorded			Not present	None recorded	None recorded	None recorded
B4	River Bride	None recorded			**Floating river vegetation [3260]**	None recorded	None recorded	None recorded
B5	River Bride	None recorded		**Spraint site**	**Floating river vegetation [3260]**	None recorded	None recorded	None recorded
B6	River Bride	None recorded	Negative eDNA result at site, no records in catchment	**Spraint site**	Not present	None recorded	None recorded	None recorded

*** Conservation value:** White-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussel (*Margaritifera margaritifera*) and Eurasian otter (*Lutra lutra*) are listed under Annex II and Annex V of the Directive on the Conservation of Natural Habitats of Wild Fauna and Flora (92/43/EEC) ('EU Habitats Directive'). and all are protected under the Irish Wildlife Acts 1976-2021. White-clawed crayfish (Füreder et al., 2010) and freshwater pearl mussel (Moorkens et al., 2017) are also both listed as 'Endangered' according to the IUCN Red List. The European Union (Invasive Alien Species) (Freshwater Crayfish) Regulations 2018 (SI 354/2018) affords further protection to native white-clawed crayfish by prohibiting the introduction and spread of five no. invasive 'Union concern' crayfish species listed under EU Regulation 1143/2014. Common frog (*Rana temporaria*) and smooth newt (*Lissotriton vulgaris*) are protected under the Irish Wildlife Acts 1976-2021. Common frog are also afforded protection under Annex V of the Habitats Directive [92/42/EEC]. Floating river vegetation habitat [326] is protected under Annex I of the Directive on the Conservation of Natural Habitats of Wild Fauna and Flora (92/43/EEC) ('EU Habitats Directive'). [4] Otter signs within 150m of the survey site

Table 4.4 Aquatic ecological evaluation summary of the Proposed Wind Farm survey sites according to NRA (2009) criteria

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary*
A1	Cummer River	19C02	**Local importance (higher value)**	Upper reaches of small, shallow upland eroding watercourse with moderate fisheries value; low density of brown trout recorded via electro-fishing; **Q4** (good status) water quality
A2	Clearagh Stream	19C64	**Local importance (higher value)**	Upper reaches of small, shallow upland eroding watercourse with moderate fisheries value; low density of brown trout recorded via electro-fishing; **Q3-4** (moderate status) water quality
A3	Cummer River	19C02	**Local importance (higher value)**	Semi-natural, medium-sized upland eroding watercourse with high value for salmonids & an excellent salmonid nursery; high density of brown trout recorded via electro-fishing; Annex I habitat floating river vegetation (3260) present; **Q4** (good status) water quality
A4	Cummer River	19C02	**Local importance (higher value)**	Large, semi-natural upland eroding watercourse with high value for salmonids; high density of mixed-cohort brown trout recorded via electro-fishing; otter latrine identified; **Q4** (good status) water quality
A5	Cummer River	19C02	**Local importance (higher value)**	Large, semi-natural upland eroding watercourse of lower gradient with good value for salmonids; moderate numbers of mixed-cohort brown trout recorded via electro-fishing in addition to *Lampetra* sp. & three-spined stickleback; Annex I habitat floating river vegetation (3260) present; **Q4** (good status) water quality
B1	River Bride	19B04	**Local importance (higher value)**	Uppermost reaches of small, shallow, semi-natural upland eroding watercourse with moderate fisheries value; low numbers of brown trout recorded via electro-fishing; **Q4** (good status) water quality
B2	Moneygaff East Stream	19F09	**Local importance (higher value)**	Small, high gradient upland eroding watercourse with moderate fisheries value; low numbers of brown trout recorded via electro-fishing; **Q4** (good status) water quality
B3	Barnadivane Stream	19B22	Local importance (lower value)	Upper reaches of small, semi-natural upland eroding watercourse with low fisheries value; no fish recorded via electro-fishing; **Q3** (poor status) water quality; no aquatic species or habitats of high conservation value
B4	River Bride	19B04	**Local importance (higher value)**	Medium-sized, semi-natural upland watercourse of lower gradient with good value for salmonids with good quality salmonid spawning & nursery habitat; high numbers of brown trout and low numbers of

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary*
				Atlantic salmon recorded via electro-fishing; Annex I habitat floating river vegetation (3260) present; **Q4** (good status) water quality
B5	River Bride	19B04	**Local importance (higher value)**	Large, natural, high energy upland eroding watercourse of good value for salmonids; brown trout, Atlantic salmon & Red-listed European eel recorded via electro-fishing; a regular otter spraint site was recorded; Annex I habitat floating river vegetation (3260) present; **Q4** (good status) water quality
B6	River Bride	19B04	**Local importance (higher value)**	Large, natural, high energy upland eroding watercourse of high value for salmonids & an excellent salmonid nursery; moderate numbers of brown trout and high numbers of Atlantic salmon recorded via electro-fishing; regular otter spraint site recorded; **Q4** (good status) water quality

***Conservation value:** Atlantic salmon (*Salmo salar*), *Lampetra* spp. and otter (*Lutra lutra*) are all listed under Annex II of the Habitats Directive [92/42/EEC]. Furthermore, Atlantic salmon, *Lampetra* spp. are also listed under Annex V of the Habitats Directive [92/42/EEC] while otter are also listed on under Annex IV of the Habitats Directive [92/42/EEC]. Otters (along with their breeding and resting places) are also protected under provisions of the Irish Wildlife Acts 1976 to 2021. European eel are 'critically endangered' according to most recent ICUN red list (Pike et al., 2020) and listed as 'critically engendered' in Ireland (King et al., 2011). With the exception of the Fisheries Acts 1959 to 2019, brown trout have no legal protection in Ireland.

5. Discussion

5.1 Most valuable areas for aquatic ecology

None of the 11 no. aquatic survey sites in the vicinity of the Proposed Development were evaluated as of greater than **local importance (higher value)** in terms of their aquatic ecology.

Sites on the Cummer River (A1, A3, A4, & A5), Clearagh Stream (A2), River Bride (B1, B4, B5 & B6) and Moneygaff East Stream (B2) (i.e. all sites except B3) were evaluated as **local importance (higher value)**. Primarily this evaluation was due to the presence of salmonids at these 10 no. sites. A proportion of these sites also supported other aquatic species or habitats of conservation value, such as Red-listed European eel (sites B4 & B5), Annex II and IV otter (A4, A5, B5 & B6) and or the Annex I aquatic habitat 'floating river vegetation [3260]' (A3, A5, B4 & B5). Ten of the eleven **local importance (higher value)** sites had **Q4 (good status)** water quality, with only site A2 on the Clearagh Stream having lesser **Q3-4 (moderate status)** water quality.

5.1.1 Fish species of high conservation value

All of the eleven **local importance (higher value)** sites supported salmonids. However, these were typically brown trout populations, with Annex II Atlantic salmon only recorded from sites B4, B5 and B6 on the River Bride (**Table 4.2**). The absence of Atlantic salmon from the Cummer River and the Clearagh Stream (within the Lee[Cork]_SC_030 river sub-catchment) reflects the presence of significant downstream barriers rather than unsuitable habitat. These watercourses are located upstream of both Inniscarra and Carrigadrohid hydro-electric dams, resulting in poor fluvial connectivity. Historically the upper Lee system supported large runs of Atlantic salmon although salmon runs above Inniscarra Dam on the lower River Lee are now negligible (O'Donovan, 2018) with the Lee salmon population classified as being 'non-self-sustaining' (McGinnity et al., 2003). Whilst small numbers of salmon are still known from the River Sullane catchment upstream of Carrigadrohid Reservoir (Kelly et al., 2015), the confluence of the Cummer River with the reservoir (downstream of Athsollis Bridge) in a small, shallow bay with fluctuating, hydro-regulated water levels that is culverted under the N22 road is an evident barrier to salmonid passage within the system. Furthermore, as would be expected for higher-gradient, spate systems (O'Grady, 2006; Amiro, 1993), higher fish biomass and better-quality salmonid habitat was largely confined to the lower gradient reaches of the larger surveyed watercourses. These sites also supported higher salmonid densities (**Appendix A**).

Despite widespread suitability, European eel were only recorded in low densities from sites B4 and B5 on the River Bride (**Table 4.2**). European eel are Red-listed in Ireland (King et al., 2011) and are classed as 'critically endangered' on a global scale (Pike et al., 2020). Eel were typically recorded in very low numbers via electro-fishing (**Appendix A**), a pattern routinely observed in the upper reaches of watercourses given increasingly sub-optimal habitat (Matondo et al., 2021; Chadwick et al., 2007). Additionally, as per Atlantic salmon above, the absence of eel from the Cummer River and Clearagh Stream survey sites can be explained largely due to the presence of significant downstream migration barriers. However, the species is known upstream of Carrigadrohid Dam in both the Sullane River (Triturus, 2021) and Carrigadrohid Reservoir, albeit in very low numbers (pers. obs.).

The distribution of Annex II lamprey (*Lampetra* sp.) in the vicinity of the Proposed Wind Farm was highly restricted, reflecting the upland, higher-energy nature of most survey watercourses which present conditions inimical to lamprey population establishment and persistence (**Appendix A**). The species was only recorded at very low densities (2.5 per m^2) from the Cummer River at site A5. A single record for brook lamprey (*Lampetra planeri*) was available for the River Bride upstream of Béal na Bláth, located between survey sites B5 and B6 from the desktop review (**Figure 3.1**). There was no suitability for lamprey at the River Bride sites surveyed in August 2022, primarily due to higher energy. The species is however likely to occur in the lower River Bride downstream of the areas surveyed during the current survey where lower energy conditions and improved spawning and or depositional areas for ammocoetes are present (pers. obs.).

5.1.2 Annex II otter

Despite some good suitability at numerous survey locations, otter signs were only recorded at a total of *n*=4 locations on the Cummer River (sites A4 & A5) and River Bride (B5 & B6). This paucity of signs was considered to reflect the upland, higher-gradient, higher-energy nature of the survey watercourses which generally provide more restricted, stochastic prey resources and reduced foraging opportunities for otter when compared with the lower reaches of watercourses (Sittenthaler et al., 2019; Scorpio et al., 2016; Remonti et al., 2009).

5.1.3 Macrophytes & aquatic bryophytes and Annex I habitats

Whilst no rare or protected macrophytes or aquatic bryophytes were recorded during the survey, the Annex I habitat 'Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation or aquatic mosses [3260]' (also known as 'floating river vegetation') was recorded at sites on the Cummer River (A3 & A5) and River Bride (B4 & B5). Site B5 supported the best example of this habitat given a higher diversity of indicator species (EC, 2013) including the aquatic mosses *Fontinalis antipyretica*, *Fontinalis squamosa* and *Rhynchostegium riparioides* and the aquatic liverwort *Chiloscyphus polyanthos*, in addition to water crowfoot (*Ranunculus* sp.). Numerous studies have shown that *Ranunculus*-dominated areas provide highly valuable habitat for juvenile salmonids (e.g. Marsh et al., 2020). Indeed, these sites also supported the highest recorded numbers of brown trout (A3, A5 and B4) and two of the three sites supporting Atlantic salmon parr (B4 and B5).

According to the most recent round of Article 17 reporting for Ireland (NPWS, 2019), the overall conservation status of floating river vegetation [3260] is 'inadequate and deteriorating', remaining unchanged since the previous (2013) assessment.

5.1.4 Macro-invertebrates & biological water quality

No rare or protected macro-invertebrate species (according to national red lists) were recorded in the biological water quality samples taken from *n*=11 riverine sites in August 2022 (**Appendix B**). With the exception of sites A2 on the Clearagh Stream (**Q3-4**) and B3 on the Barnadivane Stream (**Q3**), all survey sites achieved target good status (**Q4**) requirements of the European Union Environmental Objectives

(Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (**Figure 4.1**).

5.2 eDNA analysis

No freshwater pearl mussel eDNA was detected in the Cummer River (site A5) or River Bride (B6) samples collected in August 2022 (0 positive qPCR replicates out of 12, respectively) (**Table 4.1**; **Appendix C**). Suitability was poor throughout the survey sites (siltation, historical modifications, compaction of substrata etc.) and these results were in keeping with the known distribution of this species within the Lee[Cork]_SC_030 and Lee[Cork]_SC_050 river sub-catchments (**Figure 3.1**).

5.3 Aquatic ecology summary

The surveyed watercourses in the vicinity of the Proposed Development were typically small, higher energy spate channels draining areas of afforestation in the lower order riverine sites and pasture in the higher order lower gradient areas. The watercourses generally supported salmonids, a healthy diversity of macro-invertebrate species and biological water quality of **Q4 (good status)**.

With the exception of site B3 (**local importance (lower value)**), all survey sites were evaluated as **local importance (higher value)** given they supported salmonids, Red-listed European eel, otter and or **≥Q4 (good status)** water quality. Sites A3 and A5 on the Cummer River and sites B4 and B5 on the River Bride also supported an aquatic vegetation community with links to the Annex I habitat 'Water courses of plain to montane levels, with submerged or floating vegetation of the *Ranunculion fluitantis* and *Callitricho-Batrachion* (low water level during summer) or aquatic mosses [3260]'.

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7. Appendix A – fisheries assessment report

Fisheries assessment for Barnadivane wind farm & Substation, Co. Cork

Prepared by Triturus Environmental Ltd. for Fehily Timoney & Company Ltd.

November 2022

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Table of contents

1. Introduction

1.1 Background

Triturus Environmental Ltd. were commissioned by Fehily Timoney & Company Ltd. to undertake a baseline fisheries assessment of numerous watercourses in the vicinity of the Proposed Development (Barnadivane Wind Farm and Substation), located approx. 5km southwest of Kilmurry, Co. Cork (**Figure 2.1**).

The survey was undertaken to establish baseline fisheries data used in the preparation of the EIAR for the Proposed Development. In order to gain an accurate overview of the existing and potential fisheries value of the riverine watercourses within the vicinity of the Proposed Development, a catchment-wide electro-fishing survey across *n*=11 riverine sites was undertaken (**Table 2.1; Figure 2.1**). Electro-fishing helped to identify the importance of the watercourses as nurseries and habitats for salmonids, lamprey and European eel (*Anguilla anguilla*), as well as other species, and helped to further inform impact assessment and any subsequent mitigation for the project.

Triturus Environmental Ltd. made an application under Section 14 of the Fisheries (Consolidation) Act, 1959 as substituted by Section 4 of the Fisheries (Amendment) Act, 1962, to undertake a catchment-wide electro-fishing survey in the vicinity of the proposed Barnadivane Wind Farm and Substation. Permission was granted on 4th August 2022 and the survey was undertaken on 17th and 18th August 2022.

1.2 Fisheries asset of the survey area

The survey sites were located within the Lee [Cork]_SC_030 and Lee [Cork]_SC_050 river sub-catchments. The Proposed Development was not located within a European site. Fisheries survey sites were present on the Cummer River (EPA code: 19C02), Clearagh Stream (19C64), River Bride (19B04), Moneygaff East Stream (19F09) and Barnadivane Stream (19B22) (**Table 2.1**).

The River Bride (19B04) rises 1.5km upstream of the Proposed Development (near Coppeen) and meanders for approx. 35km before it joins the River Lee (19L03) at Inniscarra Graveyard near Ballincollig. It is a productive river and contains a good population of brown trout (*Salmo trutta*) and, in the lower reaches, Atlantic salmon (*Salmo salar*) (O'Reilly, 2009). Lamprey (*Lampetra* sp.) are also known from the River Bride (NPWS data).

Fisheries data for the other watercourses within the survey area was not available at the time of survey although many are locally known to support brown trout populations.

2. Methodology

2.1 Fish stock assessment (electro-fishing)

A single anode Smith-Root LR24 backpack (12V DC input; 300V, 100W DC output) was used to electro-fish sites on watercourses in the vicinity of the Proposed Development on the 17th and 18th August 2022 following notification to Inland Fisheries Ireland and under the conditions of a Department of the Environment, Climate and Communications (DECC) licence. Both river and holding tank water temperature was monitored continually throughout the survey to ensure temperatures of 20°C were not exceeded, thus minimising stress to the captured fish due to low dissolved oxygen levels. A portable battery-powered aerator was also used to further reduce stress to any captured fish contained in the holding tank.

Salmonids, European eel and other captured fish species were transferred to a holding container with oxygenated fresh river water following capture. To reduce fish stress levels, anaesthesia was not applied to captured fish. All fish were measured to the nearest millimetre and released in-situ following a suitable recovery period.

As three primary species groups were targeted during the survey, i.e., salmonids, lamprey, and eel, the electro-fishing settings were tailored for each species. By undertaking electro-fishing using the rapid electro-fishing technique (see methodology below), the broad characterisation of the fish community at each sampling reach could be determined as a longer representative length of channel can be surveyed. Electro-fishing methodology followed accepted European standards (CEN, 2003) and adhered to best practice (e.g., CFB, 2008).

The catchment-wide electro-fishing (CWEF) survey was undertaken across *n*=11 sites (see **Table 2.1, Figure 2.1**).

2.1.1 Salmonids and European eel

For salmonid species and European eel, as well as all other incidental species, electro-fishing was carried out in an upstream direction for a 10-minute CPUE, an increasingly common standard approach for wadable streams (Matson et al., 2018). A total of approx. 40-100m channel length was surveyed at each site, where feasible, in order to gain a better representation of fish stock assemblages. At certain, more minor watercourse sites or sites with limited access, it was more feasible to undertake electro-fishing for a 5-minute CPUE. Discrepancies in fishing effort (CPUE) between sites are accounted for in the subsequent results section (**Table 3.1**).

Relative conductivity of the water at each site was checked in-situ with a conductivity meter and the electro-fishing backpack was energised with the appropriate voltage and frequency to provide enough draw to attract salmonids and European eel to the anode without harm. For the low-moderate conductivity waters of the sites (draining sandstone geologies) a voltage of 220-250v, frequency of 35-45Hz and pulse duration of 3.5-4ms was utilised to draw fish to the anode without causing physical damage.

2.1.2 Lamprey

Electro-fishing for lamprey ammocoetes was conducted using targeted box quadrat-based electro-fishing (as per Harvey & Cowx, 2003) in objectively suitable areas of sand/silt, where encountered. As lamprey take longer to emerge from silts and require a more persistent approach, they were targeted at a lower frequency (30Hz) burst DC pulse setting which also allowed detection of European eel in sediment, if present. Settings for lamprey followed those recommended and used by Harvey & Cowx (2003), APEM (2004) and Niven & McAuley (2013). Using this approach, the anode was placed under the water's surface, approx. 10-15 cm above the sediment, to prevent immobilising lamprey ammocoetes within the sediment. The anode was energised with 100V of pulsed DC for 15-20 seconds and then turned off for approximately five seconds to allow ammocoetes to emerge from their burrows. The anode was switched on and off in this way for approximately two minutes. Immobilised ammocoetes were collected by a second operator using a fine-mesh hand net as they emerged.

Lamprey species were identified to species level, where possible, with the assistance of a hand lens, through external pigmentation patterns and trunk myomere counts as described by Potter & Osborne (1975) and Gardiner (2003).

2.2 Fisheries habitat

A broad appraisal / overview of the upstream and downstream habitat at each site was also undertaken to evaluate the wider contribution to salmonid and lamprey spawning and general fisheries habitat. River habitat surveys and fisheries assessments were also carried out utilising elements of the approaches in the River Habitat Survey Methodology (Environment Agency, 2003) and Fishery Assessment Methodology (O'Grady, 2006) to broadly characterise the riverine sites (i.e., channel profiles, substrata etc.).

2.3 Biosecurity

A strict biosecurity protocol following IFI (2010) and the Check-Clean-Dry approach was adhered to during surveys for all equipment and PPE used. Disinfection of all equipment and PPE before and after use with Virkon™ was conducted to prevent the transfer of pathogens or invasive propagules between survey sites. Surveys were undertaken at sites in a downstream order to minimise the risk of upstream propagule mobilisation. Where feasible, equipment was also thoroughly dried (through UV exposure) between survey areas. Any aquatic invasive species or pathogens recorded within or adjoining the survey areas were geo-referenced. All Triturus staff are certified in 'Good fieldwork practice: slowing the spread of invasive non-native species' by the University of Leeds.

Table 2.1 Location of *n*=11 electro-fishing survey sites in the vicinity of Barnadivane wind farm and Substation, Co. Cork

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Cummer River	19C02	Lackareagh	534472	564764
A2	Clearagh Stream	19C64	Lackareagh	535506	564531
A3	Cummer River	19C02	Greenville	535311	565896
A4	Cummer River	19C02	Teereeven	535346	567245
A5	Cummer River	19C02	Ballymichael Bridge	538941	567084
B1	River Bride [Cork]	19B04	Moneygaff East	533173	562259
B2	Moneygaff East Stream	19F09	Barnadivane	533455	562476
B3	Barnadivane Stream	19B22	Barnadivane	533994	562217
B4	River Bride [Cork]	19B04	Garranereagh	534607	561454
B5	River Bride [Cork]	19B04	Hornhill Bridge	538342	562962
B6	River Bride [Cork]	19B04	Currabeha	541813	564722

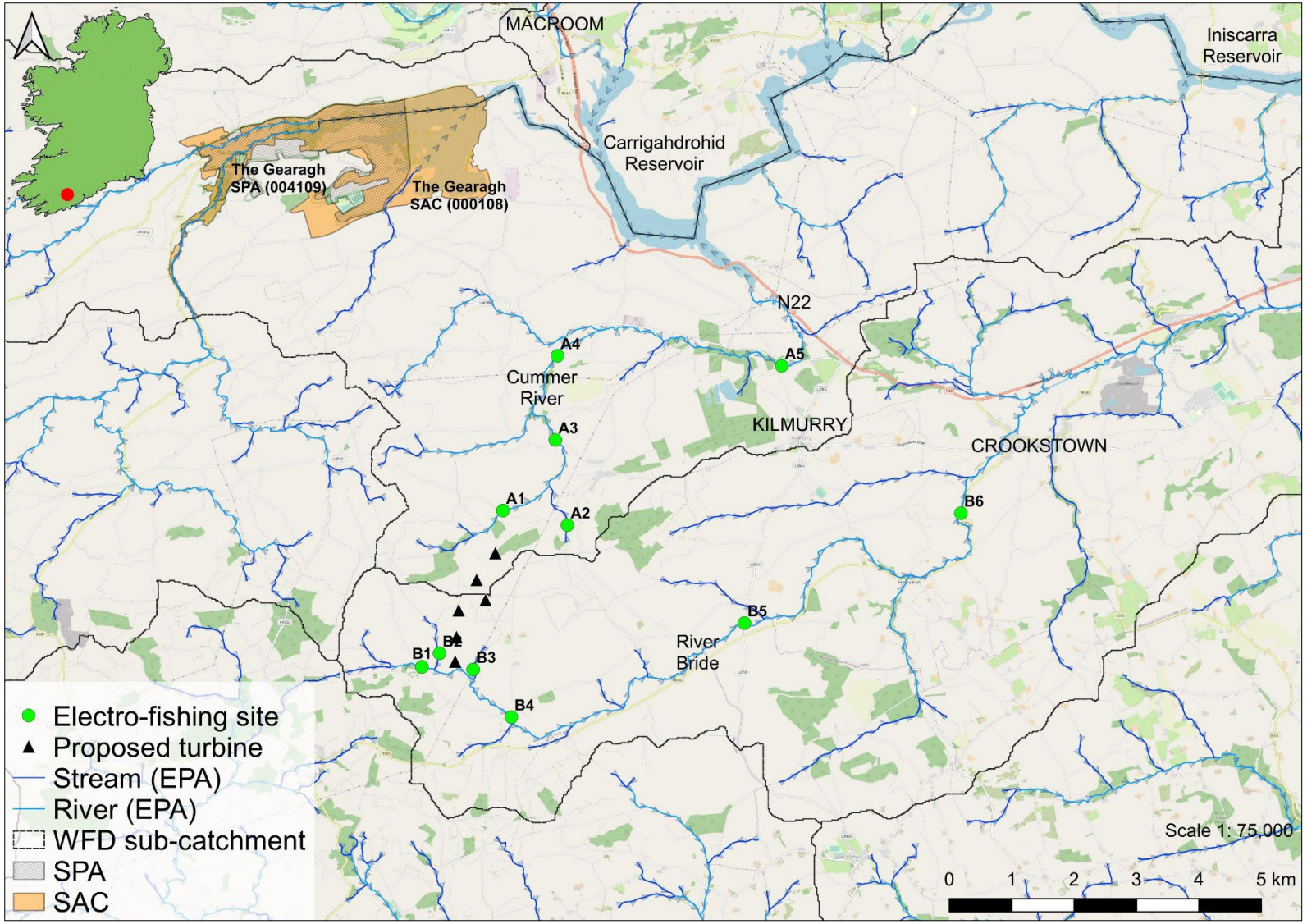

Figure 2.1 Overview of the *n*=11 electro-fishing survey site locations for Barnadivane wind farm and Substation, Co. Cork

3. Results

A catchment-wide electro-fishing survey of *n*=11 riverine sites in the vicinity of the Proposed Development was conducted on the 17th and 18th August 2022 following notification to Inland Fisheries Ireland. The results of the survey are discussed below in terms of fish population structure, population size and the suitability and value of the surveyed areas as nursery and spawning habitat for salmonids, European eel and lamprey species. Scientific names are provided at first mention only.

3.1 Fish stock assessment (electro-fishing)

3.1.1 Site A1 – Cummer River, Lackareagh

Brown trout (*Salmo trutta*) was the only fish species recorded via electro-fishing at site A1 (**Figure 3.1**).

Site A1 was considered a moderate quality brown trout nursery, with a low density recorded (*n*=4). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile salmonids. Spawning was of moderate to good quality given the presence of mixed gravels in pool and gravel tailings. Moderate quality holding habitat as present locally in deeper pool areas. The site was considered a lower quality European eel habitat given the high gradient and none were recorded. The upland eroding site was unsuitable for lamprey.

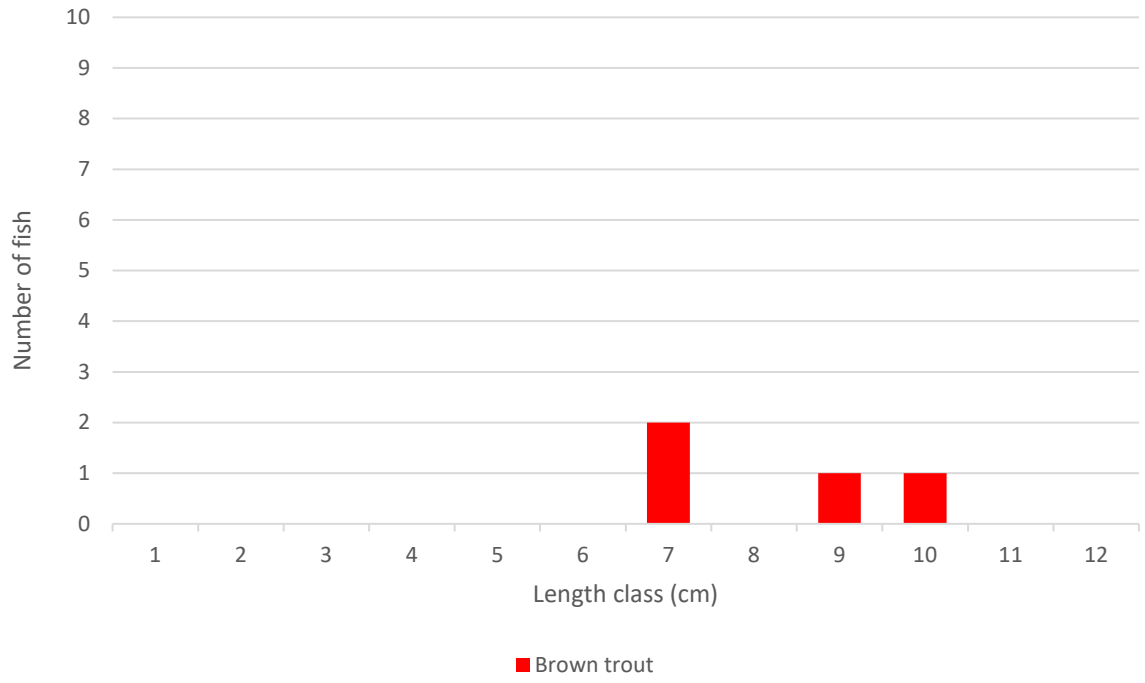

Figure 3.1 Length frequency distribution recorded via electro-fishing at site A1 on the Cummer River, August 2022

Plate 3.1 Juvenile brown trout recorded at site A1 on the Cummer River, August 2022

3.1.2 Site A2 – Clearagh Stream, Lackareagh

Brown trout was the only fish species recorded via electro-fishing at site A2 (**Figure 3.2**).

The site was considered a moderate quality brown trout nursery, with a low density recorded (*n*=5, all juveniles). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well oxygenated water with refugia for juveniles. Spawning was of moderate to good quality given the presence of mixed gravels in pool and gravel tailings. Moderate quality holding habitat as present locally in deeper pool areas. The site was considered a lower quality European eel habitat given the high gradient and none were recorded. The upland eroding site was unsuitable for lamprey.

Plate 3.2 Representative image of site A2 on the Clearagh Stream, August 2022

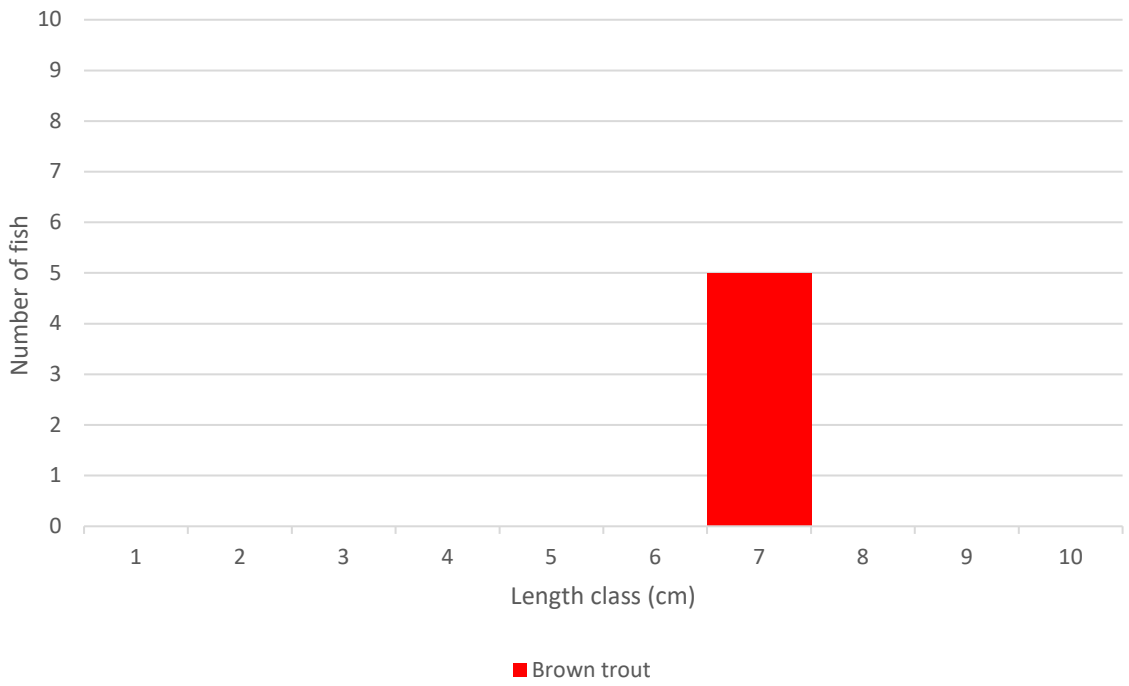

Figure 3.2 Length frequency distribution recorded via electro-fishing at site A2 on the Clearagh Stream, August 2022

3.1.3 Site A3 – Cummer River, Greenville

Brown trout was the only fish species recorded via electro-fishing at site A3 (**Figure 3.3**).

The site was considered an excellent brown trout nursery, supporting high density of juveniles and a low number of adults (total of *n*=63). This was the highest density of trout recorded during the survey (**Table 3.1**). The presence of broken riffle and glide with an abundance of mixed cobble and gravels provided well oxygenated water with high quality refugia. A sinuous river profile, shading and macrophyte cover (e.g. *Ranunculus* sp.) further increased the nursery value. Spawning was of high quality given the presence of mixed gravels in deeper pool habitat being only diminished slightly due to moderate siltation. The holding value was good with ample deep glide and pool for adult brown trout. Site A3 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. The upland eroding site was unsuitable for lamprey.

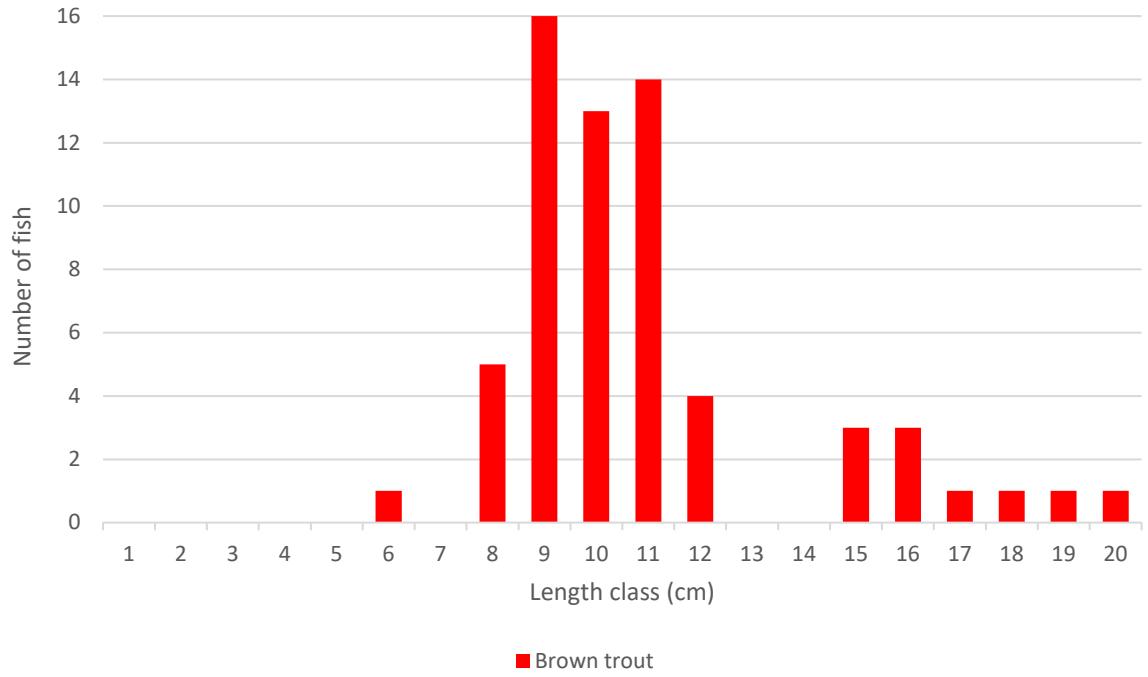

Figure 3.3 Length frequency distribution recorded via electro-fishing at site A3 on the Cummer River, August 2022

Plate 3.3 Mixed-cohort brown trout recorded at site A3 on the Cummer River, August 2022

3.1.4 Site A4 – Cummer River, Teereeven

Brown trout was the only fish species recorded via electro-fishing at site A4 (**Figure 3.4**).

The site was considered a good quality salmonid nursery (for brown trout). The presence of mixed cohorts (total of *n*=48) supported this observation and good numbers of 0+ fish indicated more optimal nursery conditions (i.e. ample broken water good shading and abundant refugia in gravels). The spawning attributes were considered locally good in the tailing of deep glide and pool where

abundant clean mixed gravels were present. The holding value was good with ample deep glide and pool for adult brown trout. Site A4 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. The upland eroding site was unsuitable for lamprey.

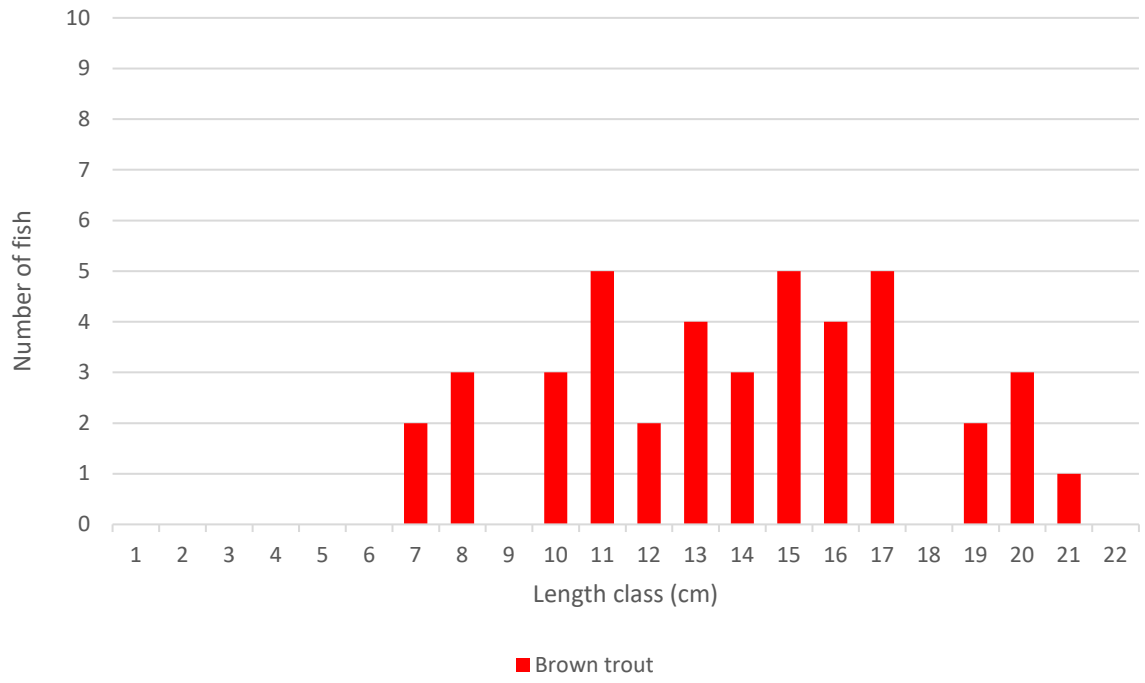

Figure 3.4 Length frequency distribution recorded via electro-fishing at site A4 on the Cummer River, August 2022

Plate 3.4 Mixed-cohort brown trout recorded at site A4 on the Cummer River, August 2022

3.1.5 Site A5 – Cummer River, Ballymichael Bridge

Brown trout (*n*=33), *Lampetra* sp. (*n*=5) and three-spined stickleback (*Gasterosteus aculeatus*) (*n*=2) were recorded via electro-fishing at site A5 (**Figure 3.5**).

The site was considered a good quality salmonid nursery (for brown trout). The presence of mixed cohorts supported this observation and good numbers of 0+ fish indicated more optimal nursery conditions (i.e. ample broken water good shading and abundant refugia in gravels). The spawning attributes were considered locally good in the tailing of deep glide and pool where abundant clean mixed gravels were present. The holding value was good with ample deep glide and pool for adult brown trout. Site A5 was considered a good quality European eel habitat given the presence of suitable boulder and cobble refugia although none were recorded. Some moderate suitability for *Lampetra* sp. existed in localised areas of shallow organic rich silt. However, given these areas were typically <5cm deep and localised in the survey reach, they supported only low densities of *Lampetra* sp. ammocoetes (2.5 per m^2).

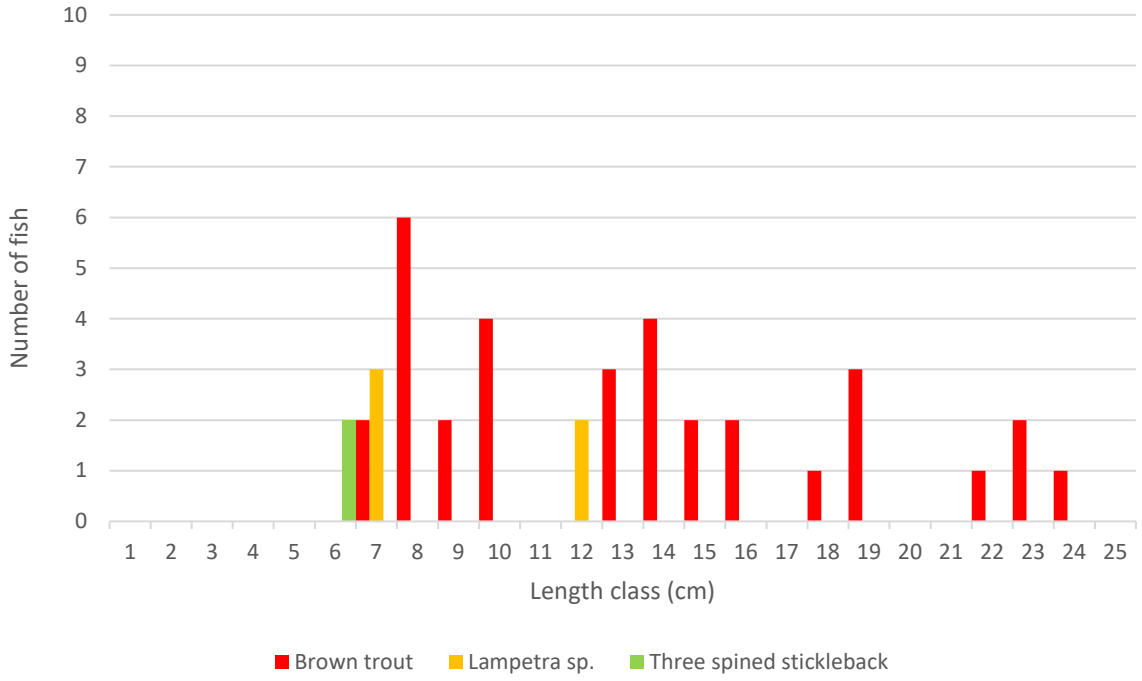

Figure 3.5 Length frequency distribution recorded via electro-fishing at site A5 on the Cummer River, August 2022

Plate 3.5 *Lampetra* sp. ammocoete recorded at site A5 on the lower reaches of the Cummer River, August 2022

3.1.6 Site B1 – Bride River, Moneygaff East

Brown trout was the only fish species recorded via electro-fishing at site B1 (**Figure 3.6**).

The site was considered a moderate quality salmonid nursery with a low density of juveniles only (*n*=10). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile trout. Spawning was of poor quality given siltation pressures, bedding of substrata and dominance of coarser substrata. Localised deeper pool provided some moderate quality holding habitat. Site B1 was considered a moderate quality European eel habitat given the presence of boulder and cobble refugia although none were recorded. The upland eroding nature of the site was unsuitable for lamprey.

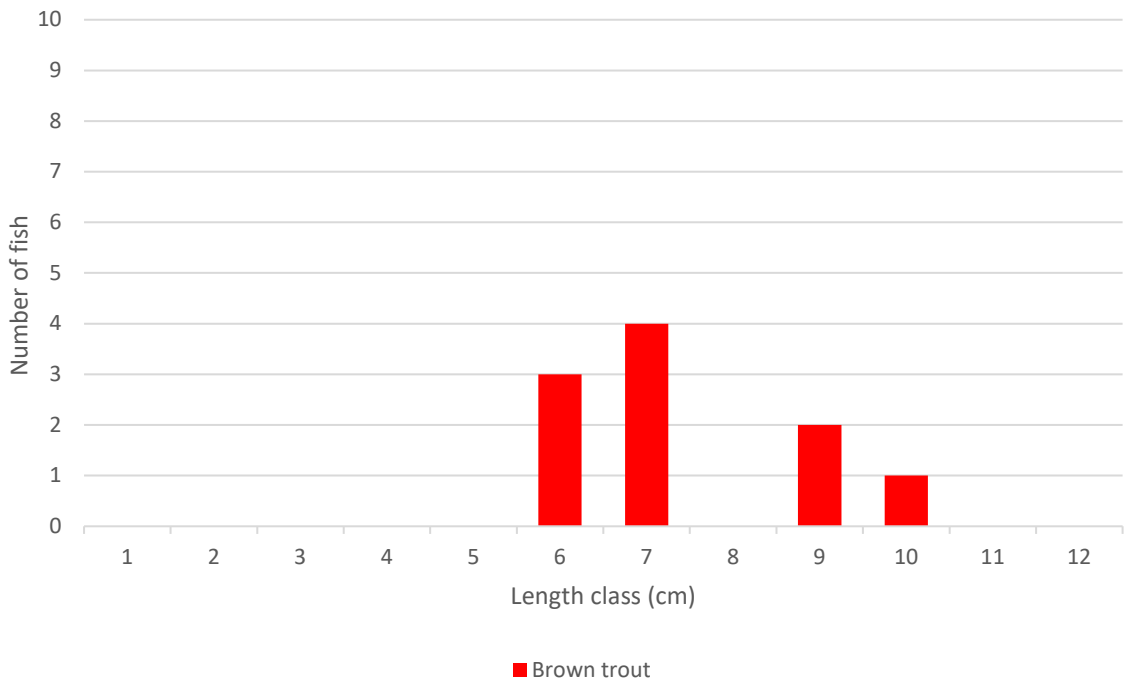

Figure 3.6 Length frequency distribution recorded via electro-fishing at site B1 on the Bride River, August 2022

Plate 3.6 Representative image of site B1 on the upper reaches of the River Bride August 2022

3.1.7 Site B2 – Moneygaff East Stream, Barnadivane

Brown trout was the only fish species recorded via electro-fishing at site B2 (**Figure 3.7**).

The site was considered a moderate quality salmonid nursery, supporting a low density of juveniles only (*n*=5). The presence of broken riffle and glide with an abundance of boulder, cobble and gravel provided well-oxygenated water with refugia for juvenile trout. Spawning was of poor quality given siltation pressures, bedding of substrata and dominance of coarser substrata. Localised deeper pool provided some moderate quality holding habitat. Site B1 was considered a moderate quality European eel habitat given the presence of boulder and cobble refugia although none were recorded. The upland eroding nature of the site was unsuitable for lamprey.

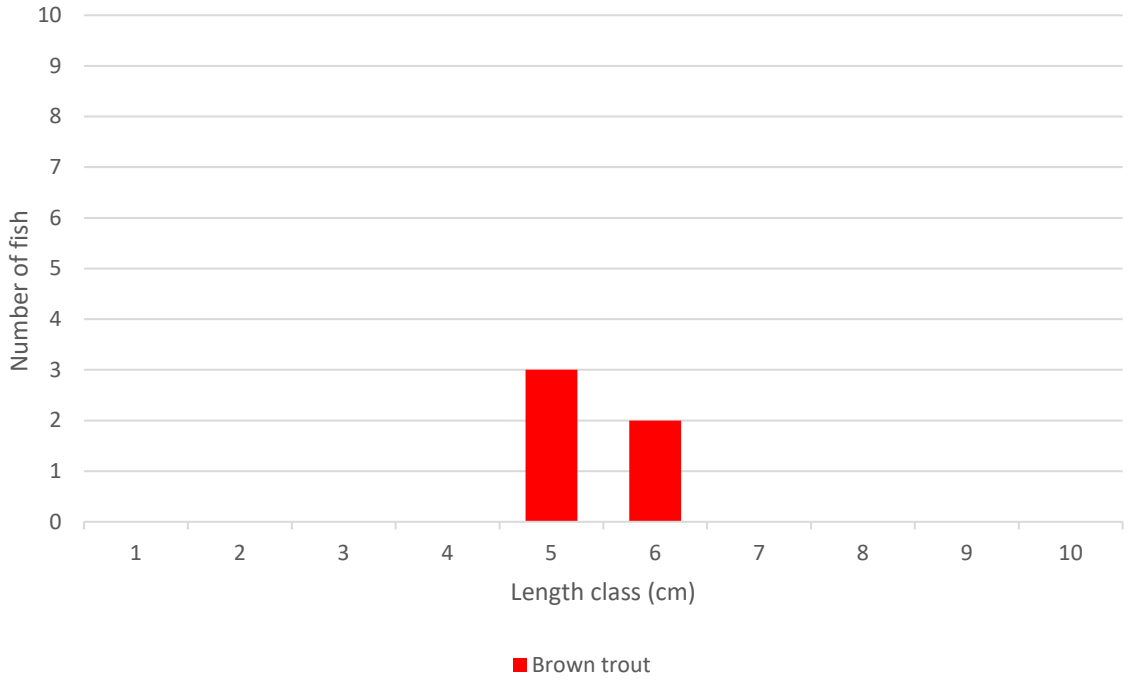

Figure 3.7 Length frequency distribution recorded via electro-fishing at site B2 on the Moneygaff East Stream, August 2022

Plate 3.7 Representative image of site B2 on the Moneygaff East Stream, August 2022

3.1.8 Site B3 – Barnadivane Stream, Barnadivane

No fish were recorded via electro-fishing at site B3. The stream at this location was not of fisheries value given its diminutive size (<1m wide, <0.1m deep) and location in the upper reaches of the catchment (**Plate 3.8**).

Plate 3.8 Representative image of site B3 on the Barnadivane Stream at Barnadivane, August 2022

3.1.9 Site B4 – Bride River, Garranereagh

Atlantic salmon (*Salmo salar*), brown trout, European eel (*Anguilla anguilla*) and stone loach (*Barbatula barbatula*) were recorded via electro-fishing at site B4 (**Figure 3.8**). The site was considered a good quality salmonid nursery supporting moderate densities of brown trout (*n*=40) and low densities of Atlantic salmon parr (*n*=7). The presence of abundant broken riffle and glide with an

abundance of mixed cobble and gravels provided well-oxygenated water with high quality refugia. Shading and macrophyte plant cover (e.g. water crowfoot) further increased the nursery value. Spawning was of good quality given the presence of mixed gravels in deeper pool and glide. The holding value was good with ample deep glide and pool for adult salmonids. Site B4 was considered a good quality European eel habitat given the presence of boulder and cobble refugia although only a single adult was recorded. The upland eroding nature of the site was unsuitable for lamprey.

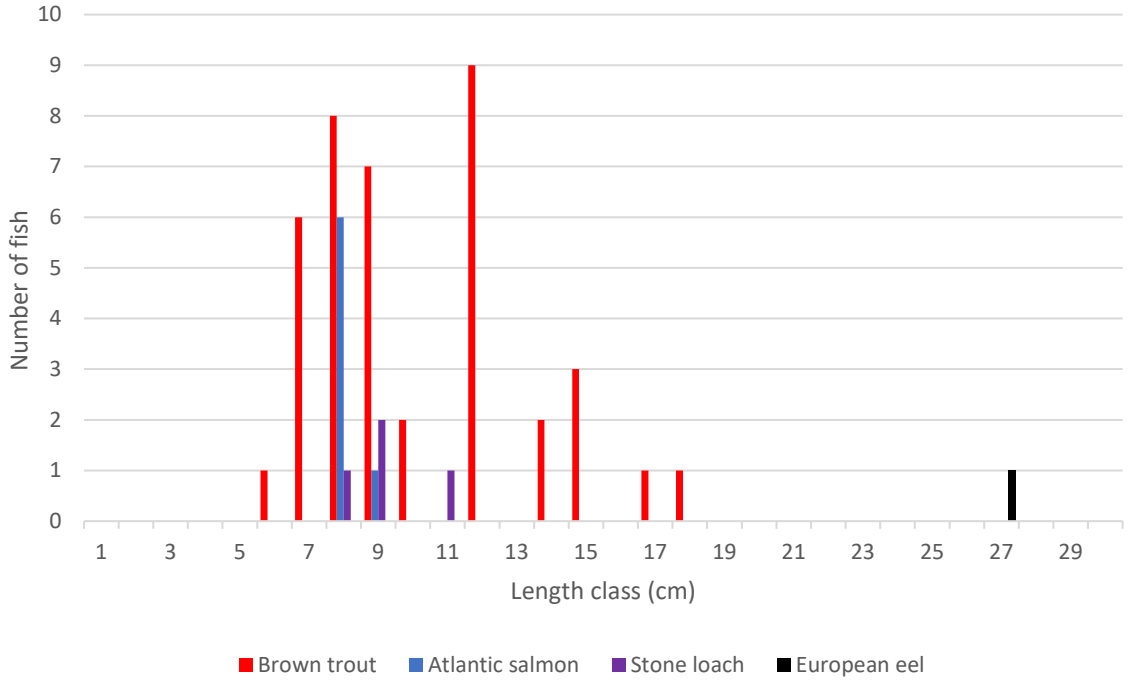

Figure 3.8 Length frequency distribution recorded via electro-fishing at site B4 on the Bride River, August 2022

Plate 3.9 Juvenile brown trout (top) and Atlantic salmon (bottom) recorded at site B4 on the River Bride, August 2022

3.1.10 Site B5 – Bride River, Hornhill Bridge

Atlantic salmon, brown trout and European eel were recorded via electro-fishing at site B5 (**Figure 3.9**).

The site was considered a good quality salmonid nursery supporting moderate densities of brown trout (*n*=17) and Atlantic salmon (*n*=28). The presence of abundant broken riffle and glide with an abundance of mixed cobble and gravels provided well-oxygenated water with high quality refugia. Shading and macrophyte plant cover (e.g. *Ranunculus* sp.) further increased the nursery value. Spawning was of good quality given the presence of mixed boulder, coble and gravels in the boulder-cascade sequences. The holding value was good with ample deep glide and pool for adult salmonids. Site B5 was considered a good quality European eel habitat given the presence of boulder and cobble refugia although only a single juvenile was recorded (**Plate 3.10**). The upland eroding nature of the site was unsuitable for lamprey.

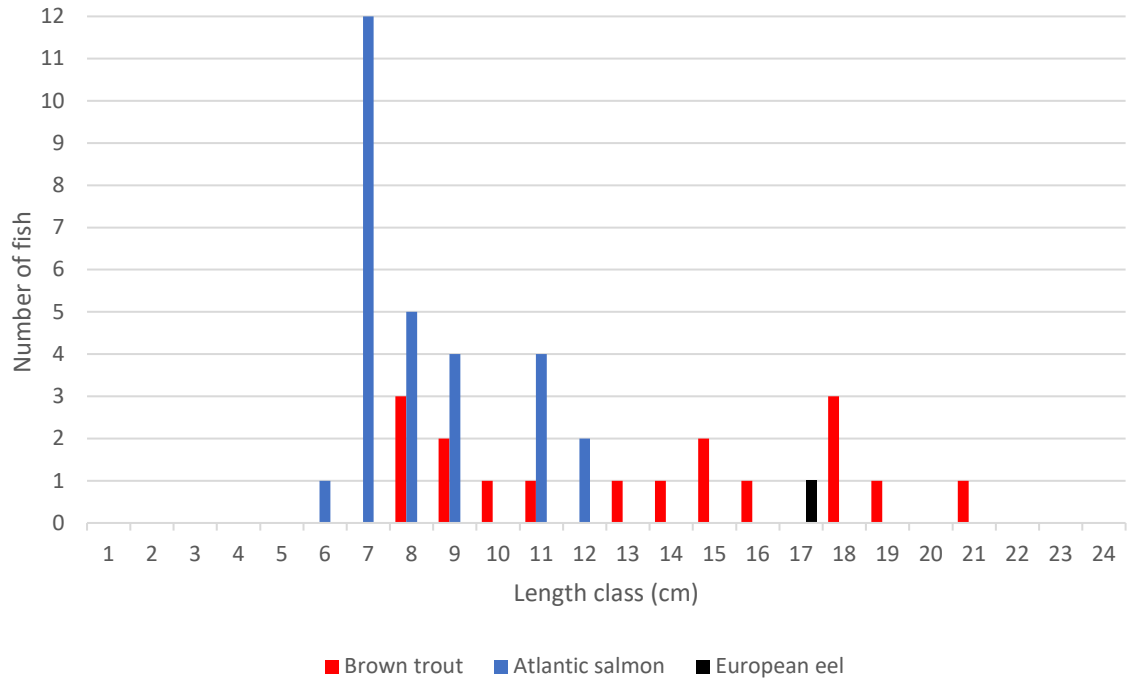

Figure 3.9 Length frequency distribution recorded via electro-fishing at site B5 on the River Bride, August 2022

Plate 3.10 European eel elver recorded at site B5 on the River Bride, August 2022

3.1.11 Site B6 –Bride River, Currabeha

Atlantic salmon and brown trout were the only two fish species recorded via electro-fishing at site B6 (**Figure 3.10**).

The site was considered an excellent salmonid nursery for both brown trout and Atlantic salmon, with high numbers present (*n*=27 & *n*=53, respectively). This was the highest density of Atlantic salmon recorded during the survey (**Table 3.1**). The presence of abundant broken riffle and glide associated with boulder cascade sequences adjoining deep pool and glide provided well-oxygenated water with high quality refugia. Shading from the adjoining mature trees and woodland protected the river from thermal stress and excessive light, further increasing the nursery value. Spawning was of very good quality given the presence of mixed boulder, coble and gravels in the boulder-cascade sequences. The holding value was very good with ample deep glide and pool for adult salmonids. Site B6 was considered a good quality European eel habitat given the presence of boulder and cobble refugia but none were recorded. The upland eroding nature of the site was unsuitable for lamprey.

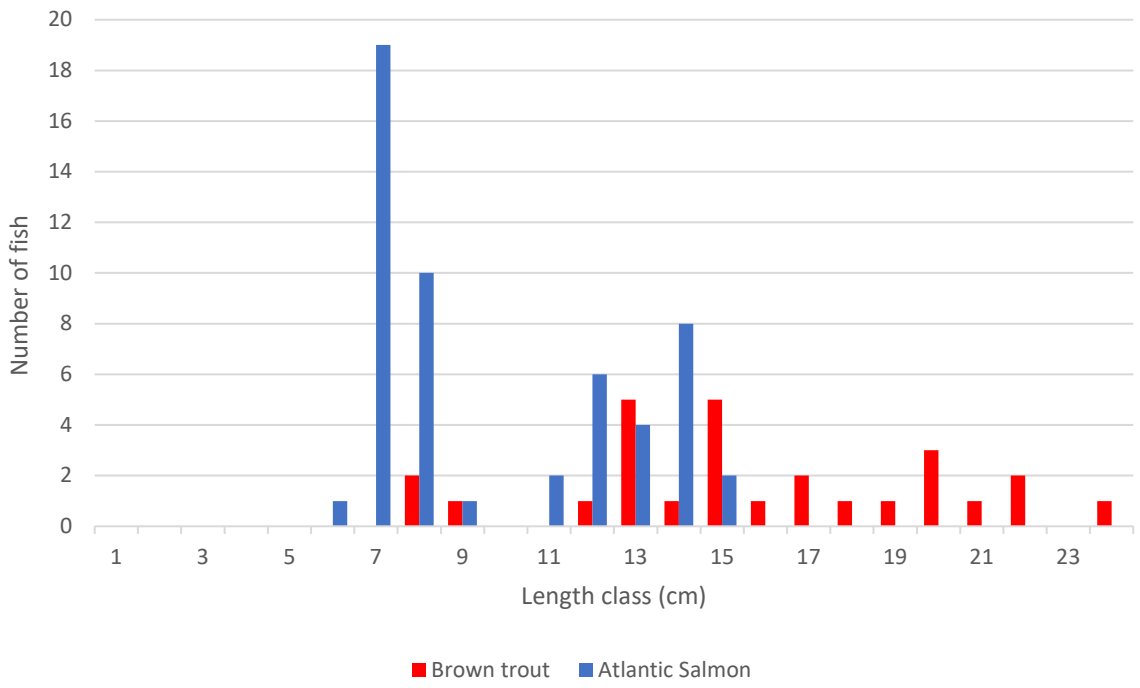

Figure 3.10 Length frequency distribution recorded via electro-fishing at site B6 on the Bride River, August 2022

Plate 3.11 Adult brown trout recorded at site B6 on the River Bride, August 2022

Table 3.1 Fish species densities per m^2 recorded at sites in the vicinity of the Proposed Development via electro-fishing in August 2022 (values in bold represent the highest densities recorded for each species, respectively)

Site	Watercourse	CPUE (elapsed time)	Approx. area fished (m^2)	Fish density (number fish per m^2)					
				Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Three spined stickleback	Stone loach
A1	Cummer River	5	25	0.000	0.160	0.000	0.000	0.000	0.000
A2	Clearagh Stream	5	67.5	0.000	0.074	0.000	0.000	0.000	0.000
A3	Cummer River	10	320	0.000	**0.196**	0.000	0.000	0.000	0.000
A4	Cummer River	10	350	0.000	0.137	0.000	0.000	0.000	0.000
A5	Cummer River	10	380	0.000	0.086	**2.5 per m^2**	0.000	**0.005**	0.000
B1	Bride River	5	150	0.000	0.066	0.000	0.000	0.000	0.000
B2	Moneygaff East Stream	5	50	0.000	0.100	0.000	0.000	0.000	0.000
B3	Barnadivane Stream	5	20	0.000	0.000	0.000	0.000	0.000	0.000
B4	Bride River	10	300	0.023	0.133	0.000	0.003	0.000	**0.013**
B5	Bride River	10	400	0.069	0.042	0.000	0.003	0.000	0.000
B6	Bride River	10	380	**0.139**	0.071	0.000	0.000	0.000	0.000

Table 3.2 Summary of fish species of higher conservation value recorded via electro-fishing per survey site in the vicinity of the proposed Barnadivane wind farm and Substation, August 2022

Site	Watercourse	Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Cummer River		✓			
A2	Clearagh Stream		✓			
A3	Cummer River		✓			
A4	Cummer River		✓			
A5	Cummer River		✓	✓		Three-spined stickleback
B1	River Bride		✓			
B2	Moneygaff East Stream		✓			
B3	Barnadivane Stream	No fish recorded				
B4	River Bride	✓	✓		✓	Stone loach
B5	River Bride	✓	✓		✓	
B6	River Bride	✓	✓			

Conservation value: Atlantic salmon (*Salmo salar*), brook lamprey (L*ampetra planeri*) and river lamprey (*Lampetra fluviatilis*) are listed under Annex II of the Habitats Directive [92/42/EEC]. Atlantic salmon and river lamprey are also listed under Annex V of the Habitats Directive [92/42/EEC]. European eel are 'critically endangered' according to most recent ICUN red list (Pike et al., 2020) and listed as 'critically engendered' in Ireland (King et al., 2011). With the exception of the Fisheries Acts 1959 to 2019, brown trout have no legal protection in Ireland.

4. Discussion

The watercourses in the vicinity of the proposed Barnadivane wind farm and Substation were typically small, higher energy upland eroding channels. Lower natural gradients allowed for the accumulation of suitable substrata (i.e., boulder, cobble, gravels), within broken riffle, glide and pool sequences, which provided moderate to good salmonid spawning, nursery and holding habitats at the majority of sites.

A total of 10 no. sites supported brown trout at the time of survey, with only site B3 on the Barnadivane Stream not supporting fish due to its diminutive nature, high gradients, shallow water and insufficient flows. Annex II Atlantic salmon were only recorded from sites B4, B5 and B6 on the River Bride and their absence from the Cummer River and Clearagh Stream, despite suitability, is reflective of downstream migration barriers (refer to discussion of accompanying baseline aquatic report). As would be expected for higher energy spate systems (O'Grady, 2006; Richardson, 1993; Amiro, 1993), better quality salmonid habitat and higher fish densities were present in the lower gradient survey reaches of the Cummer River (A4, A5) and River Bride (B4, B5, B6). These sites contained abundant broken riffle and glide with an abundance of mixed cobble and gravels, providing well-oxygenated water with high-quality refugia. The salmonid nursery value was further increased by shading and macrophyte cover (e.g. *Ranunculus* sp.) which protected the river from thermal stress and excessive light with macrophytes providing good cover and foraging opportunities for juvenile salmonids. The presence of deeper glide and pool provided good holding habitat for adult salmonids with ample mixed gravel areas providing good quality spawning areas.

On both a global and Irish scale, the European eel is listed as 'critically endangered' (Pike et al., 2020; King et al., 2011). A number of sites (i.e., A3, A4, A5, B4, B5, B6) exhibited good habitat suitability for European eel, containing coarse substrata (boulder, cobble) which are typically favoured by larger eels as diurnal refugia (Laffaille et al., 2003). However, only 2 no. sites were found to contain eel during the electro-fishing survey (i.e. sites B4 and B5 on the River Bride) despite good habitat suitability and suitable prey resources elsewhere. As eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019), the low numbers of eel recorded during the electro-fishing survey could be explained by the distance between the survey area and marine habitats (minimum 30km instream distance) (Matondo et al., 2021; Chadwick et al., 2007). Additionally, as per Atlantic salmon, the absence of catadromous eel from the Cummer River and Clearagh Stream survey sites can be explained largely due to the presence of significant downstream migration barriers, i.e. Carrigadrohid and Inniscarra hydro-electric dams. Less considerable barriers exist in the River Bride catchment (AMBER Consortium, 2020).

Low numbers lamprey were recorded during the electro-fishing survey and habitat suitability was poor or absent throughout. This reflected the upland, higher-energy, spate nature of the survey watercourses which reduces the extent of fine gravels required for spawning (Dawson et al., 2015; Rooney et al., 2013; Lasne et al., 2010) and discourages the deposition of fine, organic-rich sediment ≥5cm in depth generally required by larval *Lampetra* spp. (Aronsuu & Virkkala, 2014; Goodwin et al., 2008; Gardiner, 2003). Lamprey ammocoetes were only recorded at site 5 on the Cummer River during the survey, in low densities (2.5 per m^2; **Table 3.1**). Site A5 featured a number of habitat characteristics

conducive to lamprey ammocoete occupation i.e., pockets of shallow silt in depositing margins, adjoining exposed bars of uncompacted cobble and mixed gravel.

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8. Appendix B – Macro-invertebrates (biological water quality)

Table 8.1 Macro-invertebrate Q-sampling results for sites in the vicinity of the Proposed Wind Farm and Substation, August 2022

Group	Family	Species	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	EPA class
Ephemeroptera	Heptageniidae	*Ecdyonurus dispar*	3	1	14	14	8	15	18		15	3	6	A
Ephemeroptera	Heptageniidae	*Rhithrogena semicolorata*	2		1	1	12							A
Ephemeroptera	Heptageniidae	*Heptagenia* sp.	1											A
Plecoptera	Chloroperlidae	*Siphonoperla torrentium*	2								1			A
Plecoptera	Nemouridae	*Amphinemura sulcicollis*				5								A
Plecoptera	Nemouridae	*Protonemura meyeri*					10	5			1			A
Plecoptera	Perlidae	*Perla bipunctata*				2	1							A
Ephemeroptera	Baetidae	*Alainites muticus*	3	16	4	3	12				11			B
Plecoptera	Leuctridae	*Leuctra hippopus*	8	2	10	25	42	1	9		26		10	B
Trichoptera	Cased caddis pupa	sp. indet.					1							B
Trichoptera	Limnephilidae	*Halesus radiatus*									1			B
Trichoptera	Limnephilidae	*Potamophylax cingulatus*									1			B
Trichoptera	Odontoceridae	*Odontocerum albicorne*									1			B
Trichoptera	Sericostomatidae	*Sericostoma personatum*				1						1		B
Ephemeroptera	Baetidae	*Baetis rhodani*	37	31	22	5	2	2	14	1	5	8	58	C
Ephemeroptera	Ephemerellidae	*Serratella ignita*	15	1	27	9	1	2	9		6	2	4	C
Trichoptera	Caseless caddis pupa	sp. indet.			1									C
Trichoptera	Hydropsychidae	*Hydropsyche instabilis*				2	16		4		11	3	2	C
Trichoptera	Hydropsychidae	*Hydropsyche siltalai*					12					4		C
Trichoptera	Philopotamidae	*Wormaldia occipitalis*			1									C
Trichoptera	Polycentropodidae	*Holocentropus dubius*							2					C
Trichoptera	Polycentropodidae	*Plectrocnemia conspersa*	2	4						1				C
Trichoptera	Polycentropodidae	*Polycentropus kingi*				5			1				2	C
Trichoptera	Rhyacophilidae	*Rhyacophila dorsalis*		1		3	3		3			1	2	C

Group	Family	Species	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	EPA class
Trichoptera	Rhyacophilidae	*Rhyacophila munda*										1		C
Crustacea	Gammaridae	*Gammarus duebeni*	4	3	3	1	11		2		1	1	6	C
Mollusca	Planorbidae	*Ancylus fluviatilis*			1	5						2		C
Mollusca	Tateidae	*Potamopyrgus antipodarum*								5				C
Coleoptera	Dytiscidae	*Dytiscidae larva*			2					1				C
Coleoptera	Dytiscidae	*Oreodytes sanmarkii*	4			3			2					C
Coleoptera	Elmidae	*Elmis aenea*	3		2	6	24	8	1	2	7	11	1	C
Coleoptera	Elmidae	*Esolus parallelepipedus*				1								C
Coleoptera	Elmidae	*Limnius volckmari*	1			8	2	1				1	1	C
Coleoptera	Halipliidae	*Brychius elevatus*							1			2		C
Coleoptera	Hydraenidae	*Hydraena gracilis*	1			3	3	1	1		1			C
Diptera	Chironomidae	non-*Chironomus* spp.		3	5			1	5	1		1	13	C
Diptera	Culicidae	sp. indet.			1							1		C
Diptera	Dixidae	sp. indet.		1							1			C
Diptera	Pediciidae	*Dicranota* sp.		1			1	1	1	1	9			C
Diptera	Simuliidae	sp. indet.	1	9	77			4			5	3	2	C
Diptera	Tipuliidae	sp. indet.							1					C
Hemiptera	Gerridae	*Gerridae nymph*										1		C
Hemiptera	Gerridae	*Gerris* sp.										1		C
Hemiptera	Veliidae	Veliidae nymph			1									C
Arachnida	Hydrachnidiae	sp. indet.			2									C
Crustacea	Asellidae	*Asellus aquaticus*									1	2	1	D
Hirudinidae	Glossiphoniidae	sp. indet.										1		D
Mollusca	Lymnaeidae	*Ampullacaena balthica*										2		D
Diptera	Chironomidae	*Chironomus* spp.	1	1		1					1		1	E
Annelidae	Oligochaeta	sp. indet.		1	3	1								n/a

Group	Family	Species	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	EPA class
Nematomorpha	Gordiidae	sp. indet.		2										n/a
Abundance			**88**	**77**	**177**	**104**	**161**	**41**	**74**	**12**	**105**	**52**	**109**	
Q-rating			Q4	Q3-4	Q4	Q4	Q4	Q4	Q4	Q3	Q4	Q4	Q4	
WFD status			Good	Mod	Good	Good	Good	Good	Good	Poor	Good	Good	Good	

9. Appendix C – eDNA analysis lab report

SureScreen Scientifics

Folio No: E15442
Report No: 1
Client: Triturus Environmental Ltd
Contact: Ross Macklin

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN WATER FOR AQUATIC SPECIES DETECTION

SUMMARY

When aquatic organisms inhabit a waterbody such as a pond, lake or river they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm the presence or absence of the target species within the waterbody.

RESULTS

Date sample received in laboratory: 20/09/2022
Date results reported: 21/09/2022
Matters affecting result: None

TARGET SPECIES: Freshwater pearl mussel *(Margaritifera margaritifera)*

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK593	A5 - Cummer River	-	Pass	Pass	Pass	Negative	0/12
FK598	B6 - River Bride	-	Pass	Pass	Pass	Negative	0/12

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: **Gabriela Danickova** Approved by: Jennifer Higginbottom

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SureScreen Scientifics

METHODOLOGY

The samples detailed above have been analysed for the presence of target species eDNA following scientifically published eDNA assays and protocols which have been thoroughly tested, developed and verified for use by SureScreen Scientifics.

The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample. The extracted sample is then tested via real time PCR (also called q-PCR) for each of the selected target species. This process uses species-specific molecular markers (known as primers) to amplify a select part of the DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this reaction is measured for fast and objective data analysis. The primers used in this process are specific to a part of mitochondrial DNA only found in each individual species. Separate primers are used for each of the species, ensuring no DNA from any other species present in the water is amplified.

If target species DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If target species DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

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INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample, between the date it was made to the date of analysis. Degradation of the spiked DNA marker may indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of eDNA** [Positive/Negative/Inconclusive]

Positive: DNA was identified within the sample, indicative of species presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for species presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. Even a score as low as 1/12 is declared positive. 0/12 indicates negative species presence.

Negative: eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of species absence, however, does not exclude the potential for species presence below the limit of detection.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for species presence or absence.

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