APPENDIX 1

AQUATIC BASELINE REPORT

Aquatic ecology baseline report for Seskin Wind Farm, Co. Carlow

Triturus

Prepared by Triturus Environmental Ltd. for MKO

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1. Introduction

1.1 Background

Triturus Environmental Ltd. were commissioned by MKO to conduct baseline aquatic surveys to inform EIAR preparation for the proposed Seskin Wind Farm, Co. Carlow. 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 Wind Farm, located approx. 6km north-west of Leighlinbridge, Co. Carlow.

Undertaken on a catchment-wide scale, the baseline surveys focused on the detection of freshwater habitats and species of high conservation value. These included surveys for white-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussel (*Margaritifera margaritifera*) (eDNA only), macro-invertebrates (biological water quality) and fish of high conservation value, inclusive of supporting nursery and spawning habitat. The surveys also documented macrophyte and aquatic bryophyte communities including Annex I habitat associations in the vicinity of the Proposed Wind Farm (**Figure 2.1**). Aquatic surveys were undertaken during August 2022.

1.2 Project description

A full description of the Proposed Project 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 Wind Farm (excluding the Proposed Grid Connection Route[1]) were considered as part of the current baseline. A total of *n*=20 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 Seskinrea Stream (EPA code: 15S14) and tributaries, Knocknabranagh (Knockbaun) River (15K25), Agharue Stream (15A14), Dinin River (15D08), Seskin Upper Stream (14S28), Rathornan River (14R43), River Barrow (14B01), Parknakyle Stream (14P10) and the Oldleighlin Stream (14O02) (**Table 2.1**). The aquatic survey sites were located within the Dinin[South]_SC_010 and Barrow_SC_110 river sub-catchments. The Proposed Wind Farm site is not located within a European site. However, there was potential downstream hydrological connectivity between the Proposed Project and River Barrow and River Nore SAC (002162), a site designated for numerous aquatic qualifying interests (NPWS, 2011).

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 Wind Farm were conducted on Tuesday 2nd to Friday 5th 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*=5 strategically chosen riverine locations within the vicinity of the Proposed Project. These water samples were also analysed for white-clawed crayfish and crayfish plague (*Aphanomyces astaci*). This holistic approach informed the overall aquatic ecological evaluation of each site in context of the Proposed Wind Farm 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

[1] Proposed Grid Connection Route did not form part of this aquatic survey, please refer to Chapter 6 of the EIAR for summary of the multidisciplinary surveys undertaken along the Proposed Grid Connection Route

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

Table 2.1 Location of *n*=20 aquatic survey sites in the vicinity of the Proposed Wind Farm

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Unnamed stream	n/a	Ridge	662900	668302
A2	Unnamed stream	n/a	Seskinrea	663485	668787
A3	Unnamed stream	n/a	Seskinrea	662751	668934
A4	Unnamed river	n/a	L30372 road crossing, Seskinrea	662266	669109
A5	Seskinrea Stream	15S14	Seskinrea	663451	669455
A6	Seskinrea Stream	15S14	L3037 road crossing, Seskinrea	662134	669191
A7	Knocknabranagh (Knockbaun) River	15K25	Philips Bridge	661971	669091
A8	Agharue Stream	15A14	L3037 road crossing, Agharue	662027	669464
A9	Dinin River	15D08	Black Bridge, L3037	661736	670133
A10*	Dinin River	15D08	Coolcullen	659426	670287
A11	Dinin River	15D08	Uskerty	655788	669139
A12*	Dinin River	15D08	Dysart Bridge, N78	653022	669867
B1	Seskin Upper Stream	14S28	Seskin Upper	665192	669420
B2	Rathornan River	14R43	Coolnakisha	668010	667714
B3*	Rathornan River	14R43	River Barrow confluence	669665	666911
B4	River Barrow	14B01	Downstream of Rathvinden Weir	669526	666580
C1	Parknakyle Stream	14P10	Parknakyle	664839	666839
C2	Parknakyle Stream	14P10	Coolnakeeran	666849	666034
C3*	Oldleighlin Stream	14O02	Madlin Bridge, R448	668668	664605
C4*	River Barrow	14B01	Downstream of Rathellin Weir	669102	664554

* eDNA sampling for freshwater pearl mussel, white-clawed crayfish & crayfish plague

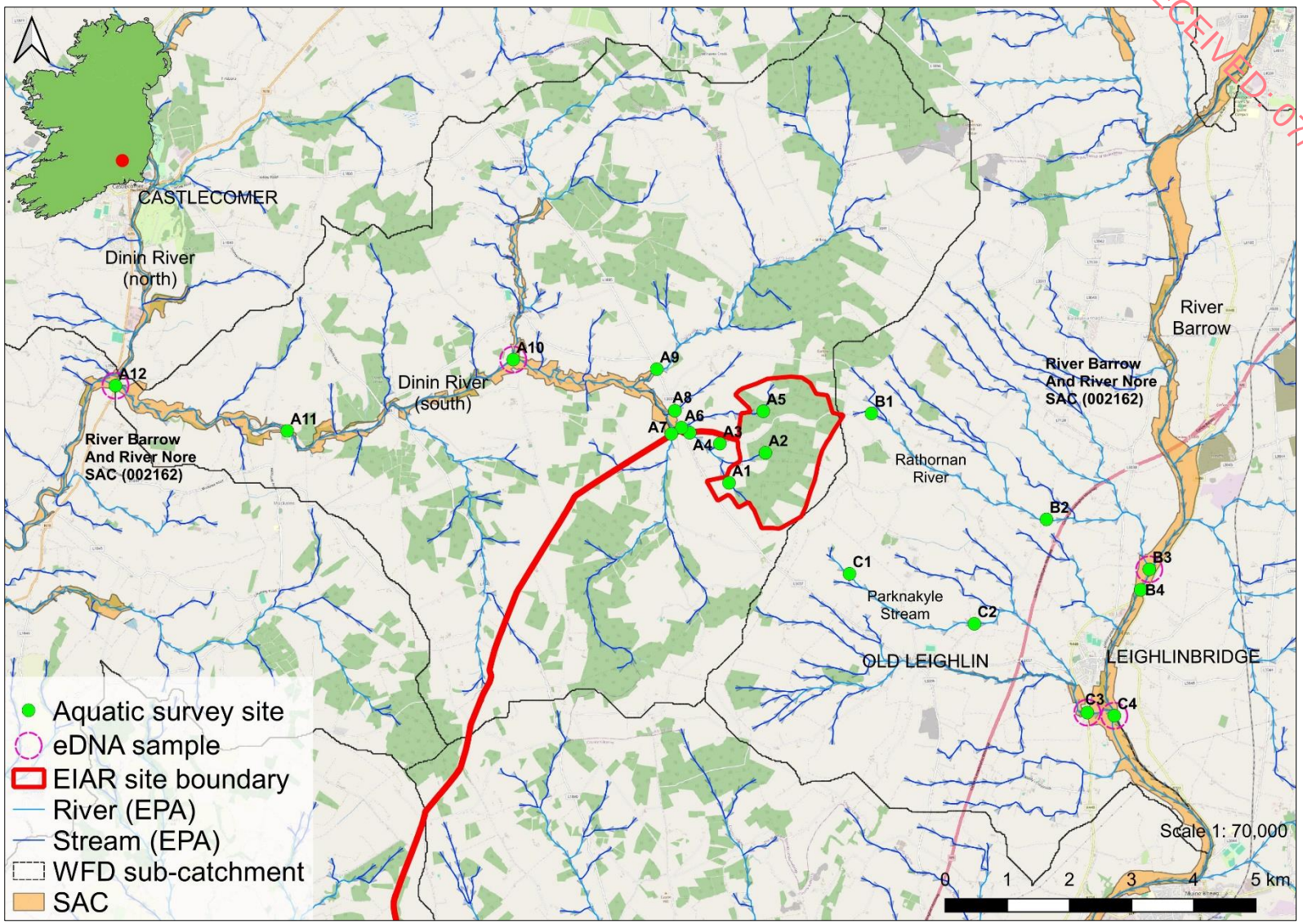

Figure 2.1 Overview of the *n*=20 aquatic survey site locations for the Proposed Wind Farm, August 2022

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 Wind Farm in August 2022 (**Table 2.1, Figure 2.1; Appendix A**). The surveys were undertaken following notification to Inland Fisheries Ireland, under the conditions of a Department of the Environment, Climate and Communications (DECC) section 14 license. The survey was also undertaken in accordance with best practice for electro-fishing of wadable riverine sites (CFB, 2008; CEN, 2003).

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 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 Proposed Wind Farm catchment/sub-catchments in the survey area to minimise the risk of transferring 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.

2.5 eDNA analysis (including freshwater pearl mussel)

To validate site surveys and to detect potentially cryptically low populations of freshwater pearl mussel and white-clawed crayfish within the study area, *n*=2 composite water samples were collected from the Dinin River (sites A10 & A12), Rathornan River (B3), Oldleighlin Stream (C3) and the River Barrow (C4) and analysed for freshwater pearl mussel eDNA (**Figure 2.1**). This would help validate the species' presence and or absence given that no data was available on the status of pearl mussel in these rivers (apart from the River Barrow). Samples were also analysed for white-clawed crayfish and crayfish plague. The water samples were collected on 5th 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 within 48 hours of collection. A total of *n*=12 qPCR replicates were analysed

for each 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.

2.6 Biological water quality (Q-sampling)

The 20 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

2.7 Macrophytes and aquatic bryophytes

Surveys of the macrophyte and aquatic bryophyte community were conducted by instream wading at each of the *n*=20 riverine survey 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').

2.8 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.).

2.9 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. Cognisance was given towards preventing the spread or introduction of crayfish plague given the known historical distribution of white-clawed crayfish and previous outbreaks of crayfish plague in the wider survey area. Furthermore, staff did not undertake any work in a known crayfish plague catchment for a period of <72hrs in advance of the survey. 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. Desktop review

3.1 Proposed Wind Farm catchment and survey area description

The Proposed Wind Farm site boundary is situated in an upland area within the townlands Ridge, Seskinrea and Agharue, near Gallows Hill approximately 6km north-west of Leighlinbridge, Co. Carlow (**Figure 2.1**). The Proposed Wind Farm site is within the South-eastern River Basin District and within hydrometric area 15 (Nore). The aquatic survey sites were located within the Dinin[South]_SC_010 and Barrow_SC_110 river sub-catchments. The Proposed Wind Farm site is drained by the Seskinrea Stream (EPA code: 15S14) and two unnamed tributaries, with downstream connectivity to the Dinin River 15D08), a major tributary of the River Nore (**Figure 2.1**).

The watercourses and aquatic surveys sites in the vicinity of the Proposed Wind Farm are typically small, upland eroding channels (FW1; Fossitt, 2000). Predominantly, the watercourses flow over areas of Westphalian shale, sandstone, siltstone & coal in upland areas with Namurian shale, sandstone, siltstone & coal in the adjoining lowlands (Geological Survey of Ireland data). The River Barrow, to the east, flows over Visean limestone & calcareous shale. Land use practices in the wider survey area are dominated by pastures (CORINE 231) with localised coniferous forest plantations (CORINE 312).

3.2 Fisheries

In proximity to the Proposed Wind Farm site boundary, the Knocknabranagh & Knockbaun River (also known as the Coolcullen River) is a tributary of the Dinin River known to support Atlantic salmon, brown trout and stone loach at Philip's Bridge (survey site A7) (Matson et al., 2018). The site supported the highest density of juvenile Atlantic salmon recorded in nationwide Water Framework Directive (WFD) surveys undertaken by Inland Fisheries Ireland (IFI) in 2021 (Corcoran et al., 2022).

The Dinin River is a major tributary of the River Nore and much of the river (downstream of Black Bridge) forms part of the River Barrow and River Nore SAC (002162). The Dinin is known to support Atlantic salmon, brown trout, minnow (*Phoxinus phoxinus*) and stone loach (Gordon et al., 2021a; Matson et al., 2018). In the lower reaches (Dinin Bridge), European eel (*Anguilla anguilla*), three-spined stickleback (*Gasterosteus aculeatus*) and lamprey (*Lampetra* sp.) have also been recorded (Kelly et al., 2017, 2013).

To the east of the Proposed Wind Farm, the Rathornan River, a tributary of the River Barrow, is known to support Atlantic salmon, brown trout and stone loach (Gordon et al., 2021b) (at same location as survey site B2).

At Madlin Bridge (survey site C3), the Oldleighlin Stream (also known as the Madlin River) is known to support brown trout, minnow, stone loach, three-spined stickleback and invasive dace (*Leuciscus leuciscus*) (Gordon et al., 2021b). The river is noted as an important brown trout spawning habitat in the wider Barrow catchment (Delanty et al., 2017).

The River Barrow is Ireland's second-longest river, flowing for some 192km and draining an area of approx. 3010km^2 (Delanty et al., 2017). In the vicinity of the Leighlinbridge, downstream of the Proposed Project, the river is known to support Atlantic salmon, brown trout, European eel, dace,

minnow, three-spined stickleback, roach (*Rutilus rutilus*), pike (*Esox lucius*), gudgeon (*Gobio gobio*) and perch (*Perca fluviatilis*) (Kelly et al., 2013).

Fisheries data for the other watercourses surveyed was not available at the time of survey.

3.3 Protected aquatic species

A comprehensive desktop review of available data (NPWS, NBDC, BSBI & other data) for 10km grid squares adjoining the Proposed Wind Farm (i.e. S46, S56, S57, S65, S66 & S67) identified a number of records for a low number of rare and or protected aquatic species within the vicinity of the Proposed Wind Farm.

Records for otter (*Lutra lutra*) were widespread throughout the respective grid squares, with approximately half of records (46 no. total) historical only (i.e. pre-1989). The more contemporary records were available for several watercourses including the Dinin River at Black Bridge (survey site A9) and near survey site A12, in addition to numerous locations on the River Barrow

A low number of records for white-clawed crayfish (*Austropotamobius pallipes*) were available for the wider survey area although the majority were historical only (1987 to 1998). Most contemporary records were available for the River Barrow, with a low number of records for the Dinin River and the Oldleighlin Stream at survey site C3 (**Figure 3.1**).

Two records (from 1991 and 2007) were available for freshwater pearl mussel (M*argaritifera margaritifera*) for the River Nore in grid square S46. Both records were located upstream of the Dinin River confluence.

3.4 EPA water quality data (existing data)

The following outlines the available water quality data for the watercourses in context of the Proposed Wind Farm. Only recent water quality is summarised below. There was no contemporary EPA biological monitoring data available for several of the surveyed watercourses, namely the Seskinrea Stream and unnamed tributaries, Knocknabranagh & Knockbaun River, Agharue Stream, Seskin Upper Stream, Rathornan River and the Parknakyle Stream.

Please note that biological water quality analysis was undertaken as part of this study, with the results presented in the **section 4** and **Appendix B** of this report.

3.4.1 Dinin River

There were 2 no. contemporary EPA biological monitoring stations located on the Dinin River (15D08) in the vicinity of the Proposed Wind Farm. At Black Bridge (station RS15D080450, survey site A9), the river achieved **Q4 (good status)** in 2019. Some 11km downstream, at Dysart Bridge (station RS15D080600, survey site A12) the river also achieved **Q4 (good status)** in 2019.

Between Black Bridge and Dysart Bridge, the Dinin River (Dinin (south)_010 river waterbody) achieved good status in the 2016-2021 period and was considered 'not at risk' of failing to achieve good ecological status (WFD Risk 3rd cycle). However, moving downstream, the Dinin (Main channel)_010 and Dinin (Main channel)_020 waterbodies fell to moderate status in the same period and were both

considered 'at risk' of not achieving good ecological status (WFD Risk 3rd cycle). Abstraction and agricultural eutrophication are the primary threat to water quality in these river waterbodies (EPA, 2018).

3.4.2 Oldleighlin Stream

There were 2 no. contemporary EPA biological monitoring stations located on the Oldleighlin Stream (Madlin River) (14O02). The river achieved **Q4-5 (high status)** at station RS14O020500 (upstream of hydrological pathway with the Proposed Wind Farm) in 2020. However, in the lower reaches, at Madlin Bridge (station RS14O020700, survey site C3), the river achieved **Q3-4 (moderate status)** in 2020.

The Old Leighlin Stream_020 river waterbody, containing the Parknakyle Stream and lower reaches of the Oldleighlin Stream achieved moderate status in the 2016-2021 period and was considered 'at risk' of not achieving good ecological status (WFD Risk 3rd cycle). Agriculture and wastewater treatment are the significant pressures for this waterbody (EPA, 2019).

3.4.3 River Barrow

There was a single contemporary EPA biological monitoring station located on the River Barrow (14B01) in the downstream vicinity of the Proposed Project. At Cardinal Moran Bridge (station RS14B012680, downstream of survey site B4), the river achieved **Q3-4 (moderate status)** in 2020.

This section of the River Barrow (Barrrow_180 river waterbody) was of moderate status in the 2016-2021 period and was considered 'at risk' of not achieving good ecological status (WFD Risk 3rd cycle). Agriculture and urban wastewater are the significant pressures for this waterbody (EPA, 2019).

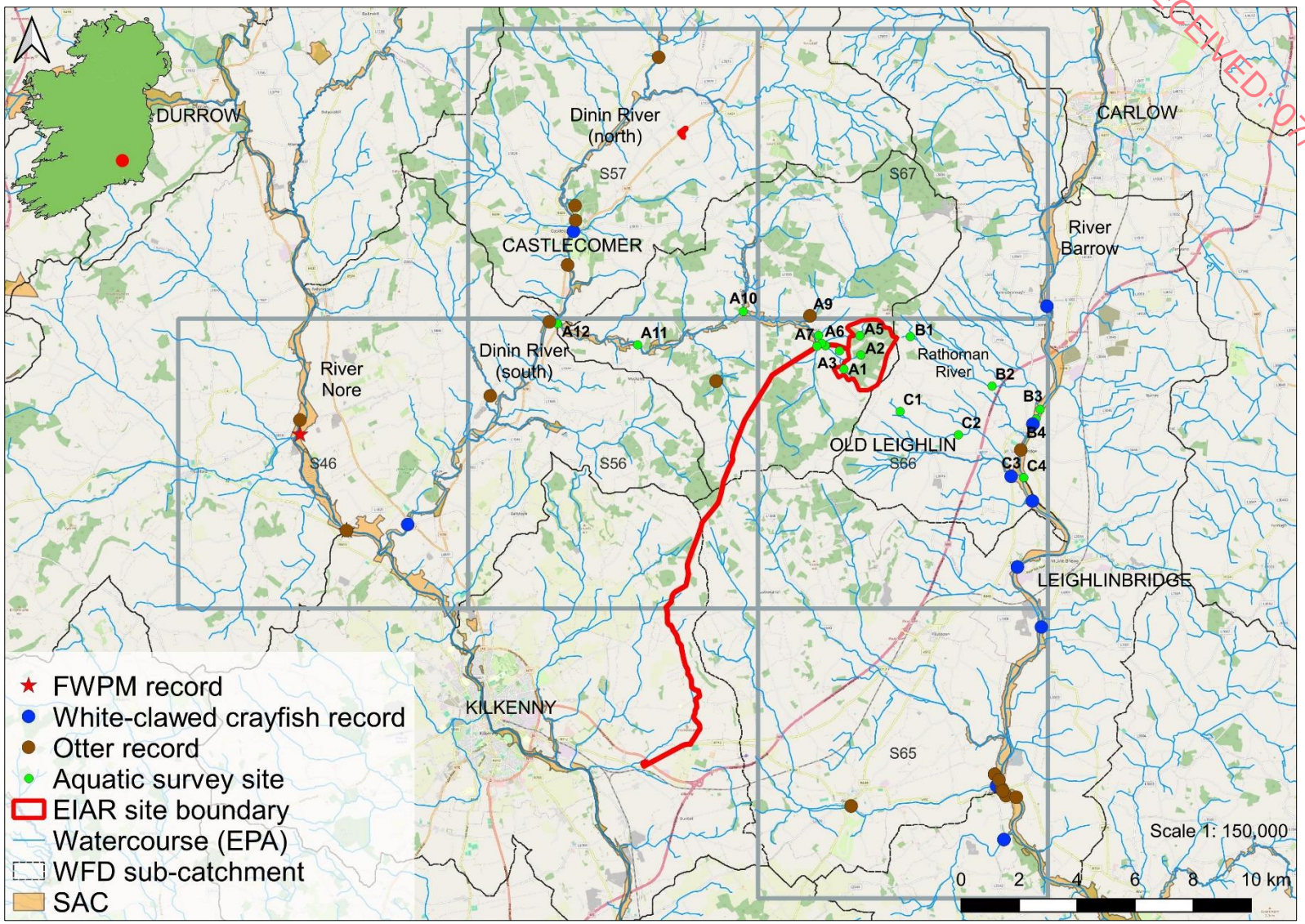

Figure 3.1 Selected protected aquatic species records in the vicinity of the Proposed Wind Farm (source: NPWS & NBDC data, 2000-2018)

4. Results of aquatic surveys

The following section summarises each of the *n*=20 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 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 – unnamed stream, Ridge

Site A1 was located on an unnamed Seskinrea Stream tributary at a farm access track crossing. The small upland eroding stream (FW1) had been straightened and over-deepened historically, resulting in a deep U-shaped channel and poor hydromorphology. The stream averaged 2m wide and <0.05 m deep and suffered from low summer flows at the time of survey with a semi-dry channel. The likely shallow stream featured steep banks of 2.5-3m and the substrata comprised of compacted angular cobble and coarse gravels. Siltation was moderate and exacerbated by low summer flows. Given low flows and very high shading (tunnelling), no macrophytes or aquatic bryophytes were recorded. The stream was heavily tunnelled by scrub dominated by bramble (*Rubus fruticosus* agg.). The site was bordered by clear-fell (WS5) and improved pasture (GA1) to the east and degraded blanket bog (PB4) to the west. Coniferous plantations (WD4) were present upstream.

No fish were recorded via electro-fishing at site A1 (**Appendix A**). The site was not of fisheries value given the very shallow nature and location in the uppermost reaches of the catchment with evident siltation pressures. There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey site.

Biological water quality, based on Q-sampling, was calculated as **Q3 (poor status)** (**Appendix B**). However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). 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 hydromorphology and poor status water quality, the aquatic ecological evaluation of site A1 was of **local importance (lower value) (Table 4.4).**

Plate 4.1 Representative image of site A1 on an unnamed Seskinrea Stream tributary, August 2022

4.1.2 Site A2 – unnamed stream, Seskinrea

Site A2 was located at the headwaters of an unnamed Seskinrea Stream tributary at a forestry access track crossing. The small upland eroding stream (FW1) had been straightened and deepened historically, resulting in a U-shaped channel and poor flows. The stream averaged 1m wide and 0.05-0.1m deep and suffered from very low summer flows at the time of survey. The profile was of riffle and shallow glide with localised shallow pool areas. The substrata comprised angular boulder and cobble which were heavily silted (exacerbated by low flows). Macrophytes were limited to bog pondweed (*Potamogeton polygonifolius*), with occasional water mint (*Mentha aquatica*) along the margins. Aquatic bryophytes were not recorded. The immediate riparian areas supported scrub (WS1) and wet grassland (GS4). The site was bordered by clear-fell (WS5) and coniferous plantations (WD4).

No fish were recorded via electro-fishing at site A2 (**Appendix A**). The site was not of fisheries value given the very shallow nature, location in the headwaters of the stream and evident siltation pressures. There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey site.

Biological water quality, based on Q-sampling, was calculated as **Q2-3 (poor status)** (**Appendix B**). However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). 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 A2 was of **local importance (lower value) (Table 4.4).**

Plate 4.2 Representative image of site A2 on an unnamed Seskinrea Stream tributary, August 2022

4.1.3 Site A3 – unnamed stream, Seskinrea

Site A3 was located on an unnamed Seskinrea Stream tributary. The small upland eroding stream (FW1) had been heavily modified historically (straightened and deepened) and was dry at the time of survey. The U-shaped channel was 0.5m wide with steep 3m-high trapezoidal banks. The dry mud base and absence of aquatic vegetation indicated only occasional water flows. The dry channel was heavily shaded by mature treelines of ash (*Fraxinus excelsior*), hawthorn (*Crataegus monogyna*) and grey willow (*Salix cinerea*) with bramble and bracken (*Pteridium aquilinum*) in the understories. The site was bordered by heavily improved pasture (GA1).

Site A3 was not of fisheries value given its dry, ephemeral nature and absence of aquatic habitats. No otter signs were recorded in the vicinity of the site. Given the dry nature of the site, it was not possible to collected a biological water quality sample at the time of survey.

Given the absence of aquatic habitats in the ephemeral channel, the aquatic ecological evaluation of site A3 was of **local importance (lower value) (Table 4.4).**

Plate 4.3 Representative image of site A3 on an unnamed stream, August 2022 (dry channel)

4.1.4 Site A4 - unnamed river, Seskinrea

Site A4 was located on an unnamed Seskinrea Stream tributary at the L30372 local road crossing, approx. 1.1km downstream of site A1 and <150m upstream of the Seskinrea River confluence. The small upland eroding river (FW1) had been straightened and deepened historically, resulting in a deep trapezoidal channel (2-3m) and poor hydromorphology. The river averaged 2-3m deep and suffered from low summer flows at the time of survey with a profile of slow-flowing glide (0.05m depth). The substrata were dominated by exposed bedrock slabs with boulder and cobble that were heavily silted (exacerbated by low flows). Macrophytes were not recorded although the moss *Brachythecium rivulare* was present locally on boulder tops. The riparian zones supported dense scrub vegetation, with bramble, grey willow and gorse (*Ulex europaeus*). The site was bordered by heavily improved pasture (GA1).

No fish were recorded via electro-fishing at site A4 (**Appendix A**). The site was not of fisheries value at the time of survey given the very shallow nature and evident siltation pressures. There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey site.

Biological water quality, based on Q-sampling, was calculated as **Q3-4 (moderate status)** (**Appendix B**). However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). 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 moderate status water quality, the aquatic ecological evaluation of site A4 was of **local importance (lower value) (Table 4.4).**

Plate 4.4 Representative image of site A4 on an unnamed Seskinrea River tributary, August 2022

4.1.5 Site A5 – Seskinrea Stream, Seskinrea

Site A5 was located on the uppermost reaches of the Seskinrea Stream (15S14), at a forestry access track crossing within the Proposed Wind Farm site boundary. The upland eroding stream (FW1) had been heavily modified historically in vicinity of coniferous plantations and suffered from low summer flows at the time of survey (near imperceptible flows). The stream averaged 0.5m wide and <0.05m deep in a U-shaped channel. The profile was of shallow riffle and glide with an absence of pool. The substrata comprised angular boulder and cobble with sand and high rates of siltation. Given high shading and poor flows, macrophytes and aquatic bryophytes were absent. The riparian areas supported dense scrub (WS1) with bramble, gorse and rosebay willowherb (*Chamaenerion angustifolium*), with grey willow causing heavy tunnelling of the stream, locally. The site was surrounded by semi-mature sitka spruce plantations (WD4).

No fish were recorded via electro-fishing at site A5 (**Appendix A**). The site was not of fisheries value given poor flows, the very shallow nature of the site and evident siltation pressures. There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey 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 A5 was of **local importance (lower value) (Table 4.4).**

Plate 4.5 Representative image of site A5 on the upper reaches of the Seskinrea Stream, August 2022

4.1.6 Site A6 – Seskinrea Stream, Seskinrea

Site A6 was located on the Seskinrea Stream (15S14) at the L3037 local road crossing, approx. 1.4km downstream of site A5. The upland eroding stream (FW1) had been historically straightened and averaged 2-4m wide and 0.1-0.2m deep. The stream suffered from low summer flows at the time of the survey. The profile was of boulder cascade with frequent pool. The substrata comprised angular boulder, cobble and mixed gravels that were heavily bedded and silted. Much of the large boulder and cobble were exposed due to the low summer flows. The site did not support macrophytes due to the high energy of the channel. The aquatic moss *Brachythecium rivulare* was present locally on boulder tops. The riparian areas supported mature treelines (WL2) of ash, elder (*Sambucus nigra*), hawthorn and ivy (*Hedera* sp.). The site was bordered by improved pasture (GA1) and residential properties (BL3).

Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) were the only fish species recorded via electro-fishing at site A6 (**Appendix A**). The site was considered a moderate quality salmonid nursery, supporting a low density of juveniles but its nursery value was reduced due to siltation. Spawning habitat was of moderate quality due to the higher energy and dominance of coarse substrata with heavy siltation. Holding areas for adult salmonids were sparse but present, nonetheless. The site was considered a moderate quality European eel habitat given the presence of pools and coarse substrata refugia. The upland site was unsuitable for lamprey and had low potential for white-clawed crayfish (none recorded). There was no suitability for freshwater pearl mussel. No otter signs were recorded in vicinity of the survey 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 (including Atlantic salmon), the aquatic ecological evaluation of site A6 was of **local importance (higher value) (Table 4.4).**

Plate 4.6 Representative image of site A6 on the Seskinrea Stream, August 2022

4.1.7 Site A7 – Knocknabranagh & Knockbaun River, Philip's Bridge

Site A7 was located on the unusually named Knocknabranagh & Knockbaun River (15K25) at Philip's Bridge. Also known as the Coolcullen River, the upland eroding river (FW1) was semi-natural with a meandering profile. The river averaged 2-4m wide and 0.1-0.5m depth water with moderate flows at the time of the survey. The profile was dominated by riffle and glide with localised pool. The substrata comprised boulder, cobble and mixed gravels that were moderately bedded and silted. Up to half of the channel width had exposed large boulder and cobble beds due to the low summer flows. The cover of filamentous algae was c.30%, indicating enrichment. Macrophytes were limited to localised water mint and water starwort (*Callitriche* sp.). Aquatic bryophytes were not recorded. The riparian areas supported mature alder (*Alnus glutinosa*), grey willow, sitka spruce (*Picea sitchensis*), ash and hawthorn. The banks were however, mostly open and graded into heavily improved pasture (GA1).

Atlantic salmon and brown trout were the only fish species recorded via electro-fishing at site A7 (**Appendix A**). The stream was a very good quality salmonid nursery due to abundant shallow glide and riffle with boulder refugia and supported a relatively high number of juveniles. Spawning habitat was of moderate quality due to the dominance of boulder and cobble but improved locally where more expansive gravels were present. The site was a poor holding habitat due to the paucity of pool areas. The site was considered a good quality European eel habitat given the presence of abundant refugia (none recorded). The upland site was unsuitable for lamprey and had low potential for white-clawed crayfish (none recorded). There was no suitability for freshwater pearl mussel. Despite some foraging suitability, no otter signs were recorded in vicinity of the survey 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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site A7 was of **international importance (Table 4.4).** The site also supported salmonids (including Atlantic salmon) and contributes to the SAC population.

Plate 4.7 Representative image of site A7 on the Knocknabranagh & Knockbaun River at Philip's Bridge, August 2022

4.1.8 Site A8 – Agharue Stream, Agharue

Site A8 was located on the lower reaches of the Agharue Stream (15A14) at the L3037 local road crossing, approx. 150m upstream of the Knocknabranagh & Knockbaun River confluence. The small upland eroding stream (FW1) was present in a semi-natural V-shaped valley with a channel averaging 1.5m wide. The stream was dry at the time of survey although the bed of small angular boulder, cobble and mixed gravels indicated occasional water flows. Macrophytes and aquatic bryophytes were not recorded. The ephemeral stream was situated in a narrow band of conifer woodland (WD4) with mature Japanese larch (*Larix kaempferi*), Norway spruce (*Picea abies*) with a dense bramble and ivy understory. The site was bordered by improved pasture (GA1).

Site A8 was not of fisheries value given its dry, ephemeral nature and absence of aquatic habitats. However, some migration of fish from the downstream-connecting river may occur periodically at higher water levels with fish dropping back downstream during dry summer flows. No otter signs were recorded in the vicinity of the site.

Given the dry nature of the site at the time of the survey, it was not possible to collected a biological water quality sample at the time of survey.

Given the absence of aquatic habitats in the ephemeral channel, the aquatic ecological evaluation of site A8 was of **local importance (lower value) (Table 4.4).**

Plate 4.8 Representative image of site A8 on the Agharue Stream, August 2022 (dry channel)

4.1.9 Site A9 – Dinin River, Black Bridge

Site A9 was located on the Dinin River (15D08) at Black Bridge, approx. 0.5km upstream of the Knocknabranagh & Knockbaun River confluence (i.e. an upstream control). The large upland eroding river (FW1) was of variable width, between 10-12m wide, and averaged 0.2-0.5m deep. The river cascaded over a moderate gradient in a steep, incised valley with banks of 2-3m in height. The natural profile of the river was characteristic of a very high energy site, with cascading riffle, glide and pool sequences over bedrock steps. In addition to frequent bedrock, the substrata comprised exposed boulder with frequent cobble and occasional patches of coarse gravel. The substrata were mobile and had light siltation due to the high energy of the site. Given the high energy of the channel, macrophytes were not present. However, the exposed bedrock adjoining the cascading sections supported *Brachythecium rivulare* and *Cinclidotus fontinaloides*. The riparian areas supported mature sycamore and ash with bluebell (*Hyacinthoides non-scripta*), bracken, bramble, hogweed (*Heracleum sphondylium*) and nettle in the understories. The site was bordered by dry grassy meadows (GS2) and improved pasture (GA1).

Atlantic salmon, brown trout, minnow (*Phoxinus phoxinus*) and stone loach (*Barbatula barbatula*) were recorded via electro-fishing at site A9 (**Appendix A**), in keeping with previous surveys of the site (Gordon et al., 2021a; Matson et al., 2018). The site was a good quality salmonid nursery habitat given the presence of abundant broken oxygenated water with cascading riffle, glide and pool sequences. The nursery value was only reduced due to the high energy and steep gradient of the channel. Nonetheless, the river still supported healthy mixed cohorts of Atlantic salmon and brown trout. The site was of moderate spawning value only due to the dominance of bedrock and boulder, with suitable spawning areas restricted to small patches of cobble and gravels in pool tailings. Holding habitat was of moderate quality due to the shallow nature of the cascade pool areas. The site was a low to moderate quality European eel habitat (reduced due to high energy) and none were recorded. The upland site was unsuitable for lamprey or white-clawed crayfish and none were recorded). There was no suitability for freshwater pearl mussel (bedrock with mobile substrata). Despite some foraging suitability, no otter signs were recorded in vicinity of the survey 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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site A9 was of **international importance (Table 4.4).** The site also supported qualifying interest Atlantic salmon.

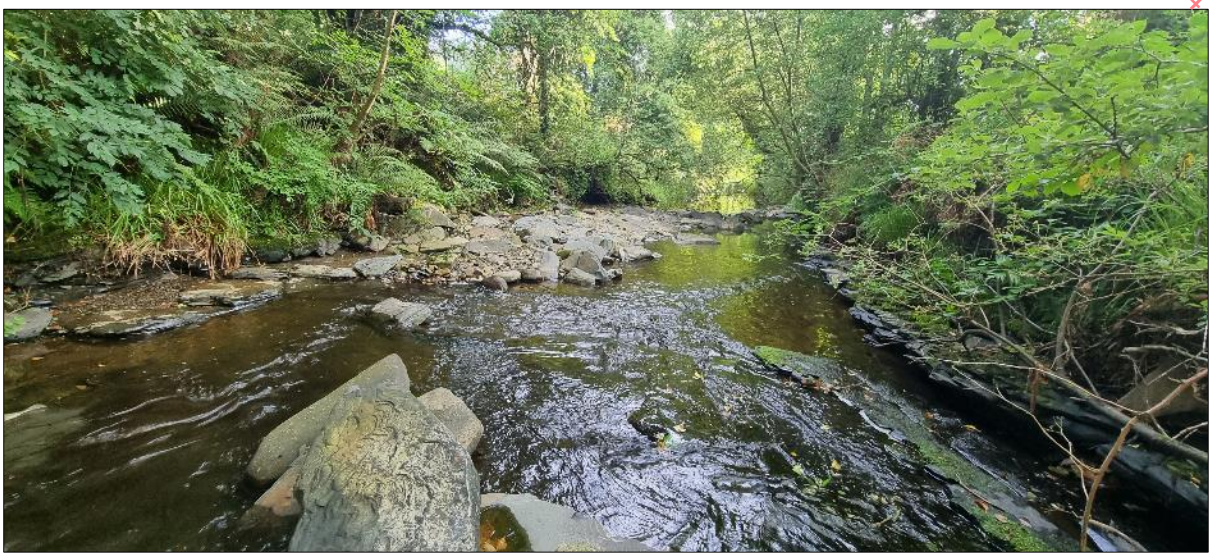

Plate 4.9 Representative image of site A9 on the Dinin River at Black Bridge, August 2022

4.1.10 Site A10 – Dinin River, Coolcullen

Site A10 was located on the Dinin River (15D08) at a local road crossing, approx. 2.9km downstream of site A9. The large, semi-natural upland eroding river (FW1) varied between 6-15m wide and averaged 0.2-0.6m deep, with localised glide and pool to 1.5m. The profile was of cascading riffle, glide and shallow pool sequences. A large, stepped barrier of concrete was present in excess of 5m high downstream of the bridge. A small Larinier fish pass was present on the south bank downstream of the bridge but was dry at the time of the survey. The substrata comprised boulder cobble and mixed gravels. These were uncompacted and had light siltation only due to the high energy of the channel. However, floc[2] was abundant in slacks with filamentous algae present locally (indicating enrichment). Macrophytes were limited to localised blue water speedwell (*Veronica anagallis-aquatica*) and narrow fruited watercress (*Nasturtium microphyllum*) on the margins of seasonally exposed cobble and gravel bars. The areas also supported great willowherb (*Epilobium hirsutum*), creeping yellowcress (*Rorippa sylvestris*), redshank (*Persicaria maculosa*), coltsfoot (*Tussilago farfara*) and invasive Himalayan balsam (*Impatiens glandulifera*). The riparian areas supported mature woodland (WD1) of sycamore, ash and grey willow. The site was bordered by dry grassy meadows (GS2).

[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)

Atlantic salmon, brown trout, minnow and stone loach were recorded via electro-fishing at site A10 (**Appendix A**). The site was an excellent quality salmonid nursery habitat, supporting a high abundance of Atlantic salmon parr (mostly 0+). Nursery habitat was of especially good quality upstream of the deep pool near the bridge where riffle and glide sequences were present. Good quality spawning and holding habitat was also present in this area. The site was considered a good quality European eel habitat due to the abundant cobble and boulder refugia. However, the weir downstream of the bridge may partially restrict eel passage. The high energy site was unsuitable for lamprey and white-clawed crayfish, with none recorded. However, an eDNA sample detected white-clawed crayfish at and or upstream of this site (see section 4.3 below). There was no suitability for freshwater pearl mussel and no pearl mussel eDNA was detected in the sample. The site provided good foraging opportunities for otter and a single regular sprainting site was recorded downstream of the bridge on a marginal boulder (ITM 659412, 670288). This did not contain crayfish remains.

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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site A10 was of **international importance (Table 4.4).** The site also supported qualifying interest Atlantic salmon.

Plate 4.10 Representative image of site A10 on the Dinin River, August 2022

4.1.11 Site A11 – Dinin River, Uskerty

Site A11 was located on the Dinin River (15D08) at a local road crossing, approx. 4.4km downstream of site A10. The upland eroding spate river (FW1) varied from 4m to 12m wide and averaged 0.2-0.5m deep although suffered from low summer flows at the time of survey with frequent exposed areas of bed. The channel had 1.5m high banks and a semi-natural profile of cascading riffle, glide and shallow pool sequences. A water impoundment area at the bridge resulted in a run of deeper glide and pool upstream. V-notch fish passes had been installed with chambers to facilitate fish passage at the downstream side of the bridge. The substrata were dominated by rounded boulder and cobble with

coarse interstitial gravels in. The bed was uncompacted with light siltation only due to the high energy of the site. However, floc was abundant in slower-flowing areas and pool. Filamentous algae were not present due to high shading. Macrophytes were not present given the high energy of the channel and the absence of smooth angular cobble and boulder precluded the presence of aquatic bryophytes. The exposed boulder and cobble adjoining the low flow summer channel supported great willowherb creeping yellowcress, redshank, coltsfoot and invasive Himalayan balsam. The riparian areas downstream of the bridge supported mature hawthorn, ash, alder, hazel (*Corylus avellana*) and grey willow, with historically cleared banks upstream adjoining improved pasture (GA1).

Atlantic salmon, brown trout, European eel (*Anguilla anguilla*), minnow and stone loach were recorded via electro-fishing at site A11 (**Appendix A**). The site was a good quality salmonid nursery downstream of the weir, supporting a low density of juveniles. The site was of moderate value as a spawning habitat with suitable areas limited to localised coarse gravels between boulders. Holding habitat was of moderate quality overall due to a paucity of pool. The site was of good value as a European eel habitat with abundant refugia. The channel was of too high energy for lamprey species. The high energy conditions precluded the presence of lamprey and white-clawed crayfish. There was no suitability for freshwater pearl mussel. The site provided moderate foraging opportunities for otter and a single spraint was recorded downstream near the bridge (ITM 655775, 669148). This did not contain crayfish remains (fish only).

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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site A11 was of **international importance (Table 4.4).** The site also supported salmonids (including Atlantic salmon) and Red-listed European eel.

Plate 4.12 Representative image of site A11 on the Dinin River, August 2022

4.1.12 Site A12 – Dinin River, Dysart Bridge

Site A12 was located on the Dinin River (15D08) at Dysart Bridge, approx. 3.6km downstream of site A11. The large, semi-natural upland eroding river (FW1) was 6-12m wide and averaged 0.2-0.6m deep with 2m high banks. The profile was of riffle, glide and localised pool with only localised historical drainage works evident that were limited to bank reinforcement works. The spate channel had a bed dominated by rounded boulder and cobble with mixed interstitial gravels. The substrata were uncompacted and had light siltation only due to the high energy of the channel. Filamentous green algae dominated by *Cladophora glomerata* covered 70% surface area of slow-moving deep glide and pool, indicating significant enrichment. Macrophytes were limited to water mint which was locally frequent downstream of the bridge on exposed boulder and cobble. Aquatic bryophytes were not recorded. The riparian areas were more open and supported treelines and hedgerows of scattered crack willow (*Salix fragilis*), hawthorn, sycamore, ash with scattered bramble and gorse. These patches of hedgerow and treeline graded into the adjoining improved pasture (GA1).

Atlantic salmon, brown trout, European eel, minnow and stone loach were recorded via electro-fishing at site A12 (**Appendix A**). The site was a good quality nursery habitat (abundant refugia), supporting a medium density of juveniles. Spawning and holding habitat was of moderate quality. The site was of good value as a European eel habitat with abundant refugia, with a low density recorded. The high energy conditions precluded the presence of lamprey and white-clawed crayfish. There was no suitability for freshwater pearl mussel. An eDNA sample collected at the site did not detect white-clawed crayfish or freshwater pearl mussel although crayfish plague was present (see section 4.3 below). The site provided moderate foraging opportunities for otter and a single spraint was recorded on the bridge abutment (ITM 653022, 669884). This did not contain crayfish remains (salmonid fish only).

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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site A12 was of **international importance (Table 4.4).** The site had good hydromorphology overall in addition to supporting qualifying interest Atlantic salmon and Red-listed European eel.

Plate 4.12 Representative image of site A12 on the Dinin River, August 2022 (upstream of bridge)

4.1.13 Site B1 – Seskin Upper Stream, Seskin Upper

Site B1 was located on the Seskin Upper Stream (14S28) at the L71231 local road crossing, approx. 0.3km upstream of the Rathornan River confluence. The small upland eroding spate channel (FW1) suffered from low summer flows at the time of survey and averaged 1m wide and 0.05m deep. The stream meandered over a steep gradient through the small incised wooded valley. The profile was of boulder cascade and pool formations. The substrata comprised angular boulder, cobble, mixed gravels and sand. The bed was compacted given the high energy of the channel and had moderate to heavy siltation. Given low flows and the very shallow nature of the site, macrophytes were not recorded. Exposed boulders supported *Hygroamblystegium sp*. moss. The stream flowed through hazel woodland (WN1) with steep valley escarpments supporting wood anemone (*Anemone nemorosa*), wood sorrel (*Oxalis acetosella*), herb Robert (*Geranium robertianum*), ivy, honeysuckle (*Lonicera periclymenum)* and opposite-leaved golden saxifrage (*Chrysosplenium oppositifolium*). The site was bordered by wet grassland (GS4) and improved pasture (GA1).

No fish were recorded via electro-fishing at site B1 (**Appendix A**). The site was not of fisheries value given the very shallow nature, low summer flows and evident siltation pressures despite being situated in a natural valley form. There was no suitability for white-clawed crayfish or freshwater pearl mussel due to the steep gradient, small size of the stream and low summer flows. No otter signs were recorded in vicinity of the survey 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 absence of aquatic species or habitats of higher conservation value, in addition to poor status water quality, the aquatic ecological evaluation of site A4 was of **local importance (lower value) (Table 4.4).**

Plate 4.13 Representative image of site B1 on the Seskin Upper Stream, August 2022

4.1.14 Site B2 – Rathornan River, Coolnakisha

Site B2 was located on the middle reaches of the Rathornan River (14R43) at a local road crossing, approx. 2.5km upstream of the River Barrow confluence. The upland eroding stream (FW1) had been historically straightened and deepened and suffered from low summer flows at the time of survey. The river averaged 1m wide in a 3m wide deep U-shaped channel and 0.05-0.1m deep. The profile was of very slow-flowing glide and riffle with an absence of pool areas. The substrata comprised small boulder and cobble which were bedded and exposed to moderate levels of siltation (exacerbated by low flows). Much of the bed was exposed due to low summer flows. Cover of filamentous algae and floc was high (20%), indicating enrichment. Macrophytes were not present given the shallow, compacted bed. Aquatic bryophytes were not recorded. Abundant great willowherb (*Epilobium hirsutum*) grew on the exposed boulder and cobble bed. Scattered mature oak (*Quercus* sp.), alder, hazel and grey willow were present on the banks. The site was bordered by heavily improved pasture (GA1) and tillage (BC3).

Three-spined stickleback was the only fish species recorded via electro-fishing at site B2 (**Appendix A**). Apart from stickleback (present in low densities), the site was not of fisheries value at the time of survey given very poor flows and shallow water. However, the site is known to support Atlantic salmon, brown trout and stone loach (Gordon et al., 2021b), presumably at higher water levels. Under such conditions, there would be some moderate suitability for salmonids and European eel. The upland characteristics of the site presented conditions inimical to lamprey and none were recorded. There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey 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 absence of aquatic species or habitats of higher conservation value, in addition to moderate status water quality, the aquatic ecological evaluation of site B2 was of **local importance (lower value) (Table 4.4).** However, this value may improve under higher flow conditions.

Plate 4.14 Representative image of site B2 on the Rathornan River, August 2022

4.1.15 Site B3 – Rathornan River, River Barrow confluence

Site B3 was located on the lowermost reaches of the Rathornan River (14R43) immediately upstream of the River Barrow confluence, 2.5km downstream of site B2. The lowland depositing river (FW2) had been heavily modified historically (straightened and deepened), resulting in a deep U-shaped channel throughout except for a small area immediately upstream of the River Barrow confluence where the channel braided. The stream suffered from low summer water levels at the time of survey and averaged 1-2m wide and 0.2-0.6m deep, with a flow profile of very slow moving glide and pool habitat. The substrata were dominated by silted cobble and mixed gravels, with much of the bed exposed due to low summer flows. The site supported abundant common duckweed (*Lemna minor*) in the margins with occasional blue water speedwell, fool's watercress (*Apium nodiflorum*), brooklime (*Veronica beccabunga*) and watercress (*Nasturtium officinale*). Invasive Nuttall's pondweed (*Elodea nuttallii*) was also present in pool pockets with water starwort (*Callitriche* sp.). The exposed gravel banks supported great willowherb, wavy bittercress (*Cardamine flexuosa*), redshank and fat hen (*Atriplex prostrata*). The river became heavily tunnelled moving upstream with very high shading. The riparian areas supported mature ash, hawthorn and grey willow with bramble in the understories. The more open areas of bank near the River Barrow confluence supported abundant great willowherb and reed sweet grass (*Glyceria maxima*). The site was bordered by improved pasture (GA1).

Three-spined stickleback, stone loach and pike (*Esox lucius*) were the only fish species recorded via electro-fishing at site B3 (**Appendix A**). The site had limited fisheries value at the time of survey due to the stagnant water, heavy siltation and historical drainage pressures. The fisheries value would improve under higher water levels. It had some low value as a European eel nursery in its lower reaches due to the shading, deep pools and connection with the River Barrow (superior fisheries habitat). The intermittent flows and paucity of soft sediment presented conditions inimical to lamprey

(none recorded). The site had limited suitability for white-clawed crayfish given the stagnant nature and heavy siltation of the channel and none were recorded. There was no suitability for freshwater pearl mussel. An eDNA sample collected at the site did not detect white-clawed crayfish or freshwater pearl mussel (see section 4.3 below). Despite some low foraging suitability, no otter signs were recorded in the vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q2-3 (poor status) (Appendix B)**. However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site B3 was of **international importance (Table 4.4).**

Plate 4.15 Representative image of site B3 on the Rathornan River, August 2022, immediately upstream of the River Barrow confluence

4.1.16 Site B4 – River Barrow, downstream of Rathvinden Weir

Site B4 was located on the River Barrow (14B01) immediately downstream of Rathvinden Weir, approx. 0.3km downstream of the Rathornan River confluence. The large lowland depositing river (FW2) had been historically modified for navigation, with a weir and associated navigation lock along the west bank. Nonetheless the weir areas remain some of the most important nursery habitats for fish in the River Barrow with deep pool and glide upstream of the weir and fast riffle-glide habitat downstream of the weir. The river averaged 30-35m wide and c.2-2.5m deep. Soft sediment accumulations were present in depositing areas but cobble and mixed gravels dominated in faster-flowing areas adjoining the weir. The open water areas supported abundant arrowhead (*Sagittaria sagittifolia*) in deep glide and pool with occasional perfoliate pondweed (*Potamogeton perfoliatus*). The pondweed hybrid *Potamogeton* x *angustifolius* was also present near the weir crest in deep water. The invasive pondweed Nuttall's pondweed was present locally in deep pool and adjoining the reeded littorals. The reeded littorals supported abundant common clubrush (*Schoenoplectus lacustris*), occasional branched bur-reed (*Sparganium erectum*) and frequent reed canary grass (*Phalaris*

arundinacea) and reed sweet grass with hedge bindweed (*Calystegia sepium*), meadowsweet (*Filipendula ulmaria*), nettle (*Urtica dioica*) and great willowherb in the drier areas of the riverbank. The riparian areas on the bank tops supported trees characteristic of the River Barrow including white willow (*Salix alba*), grey willow, crack willow, sycamore, black poplar (*Populus nigra*), alder and ash. The small island between the navigation channel at the canal lock and the River Barrow was heavily scrubbed over with butterbur (*Petasites hybridus*) and invasive Himalayan balsam (*Impatiens glandulifera*). The site was bordered by improved pasture (GA1) in the adjoining fields.

Electro-fishing was not undertaken at site B4 given prohibitive depths of >1.5m. As observed throughout the River Barrow, the weir area provided good quality nursery habitat for juvenile salmonids given ample broken water (riffle and glide) with cobble and boulder refugia. Atlantic salmon are common below weirs in the River Barrow with more limited brown trout populations (pers. obs.). Site B4 also provided some salmonid and lamprey spawning habitat that was restricted to gravel pockets in the fast water downstream of the weir. The site was an excellent quality holding habitat for adult salmonids given abundant deep glide and pool. Depositing sand and silt in pool slacks and deep glide below the weir face offered good lamprey ammocoete burial habitat. The site was also of high value as a European eel habitat (abundant refugia) and coarse fish habitat for a range of species. The survey area historically supported white-clawed crayfish but despite searching cobble and boulder refugia below the weir no crayfish were recorded present. The site had good foraging potential for otter and a single spraint site was recorded on boulders between Rathvinden Lock and Cardinal Moran Bridge (R448) (ITM 669420, 666424).

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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site B4 was of **international importance (Table 4.4).**

Plate 4.16 Representative image of site B4 on the River Barrow at Rathvinden Weir, August 2022

4.1.17 Site C1 – Parknakyle Stream, Parknakyle

Site C1 was located on the upper reaches of the Parknakyle Stream (14P10) at the L30375 road crossing. The small upland eroding stream (FW1) had been straightened historically and was present in a semi-natural V-shaped channel averaging 1.5-2m wide. The stream suffered from very low summer flows at the time of survey and was semi-dry with an imperceptible flow and localised ponding of water. Pools to a maximum depth of 0.15m were present. Despite the low summer flows, the site characteristics were of a small spate channel. The substrata comprised small angular boulder, cobble and mixed gravels, with natural bank scouring contributing to the siltation load. Macrophytes and aquatic bryophytes were not recorded. The likely ephemeral site was heavily shaded by holly (*Ilex aquifolium*) and Japanese larch with a dense bramble, bracken and ivy understory. The site was bordered by improved pasture (GA1).

No fish were recorded via electro-fishing at site C1 (**Appendix A**). The site was not of fisheries value given the low summer flows, very shallow nature and ephemeral character of downstream-connecting habitats (see 4.1.18 below). There was no suitability for white-clawed crayfish or freshwater pearl mussel. No otter signs were recorded in vicinity of the survey site.

Biological water quality, based on Q-sampling, was calculated as **Q3-4 (moderate status)** (**Appendix B**). However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). 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 moderate status water quality, the aquatic ecological evaluation of site C1 was of **local importance (lower value) (Table 4.4).**

Plate 4.17 Representative image of site C1 on the uppermost reaches of the Parknakyle River, August 2022 (semi-dry channel)

4.1.18 Site C2 – Parknakyle Stream, Coolnakeeran

Site C2 was located on the Parknakyle Stream (14P10) at the L7125 local road crossing, approx. 2.3km downstream of site C1. The small upland eroding stream (FW1) had been extensively straightened and deepened historically, resulting in a homogenous U-shaped channel that averaged 1.5m wide. Whereas the stream supported remnant pools of water upstream (site C1), the stream at this location was dry at the time of survey. The presence of an exposed, dry cobble-dominated bed and very high coverage of macrophyte vegetation (fool's watercress and watercress) indicated intermittent water flows (i.e. an ephemeral channel). The banks supported scattered mature hazel, wych elm (*Ulmus glabra*), hawthorn, ash and black poplar with great willowherb, bramble, nettle and thistles (*Cirsium* spp.) in the understories. The site was bordered by improved pasture (GA1) and tillage (BC3).

Site C2 was not of fisheries value given its dry, ephemeral nature and absence of aquatic habitats. No otter signs were recorded in the vicinity of the site.

Given the dry nature of the site, it was not possible to collected a biological water quality sample at the time of survey.

Given the absence of aquatic habitats in the ephemeral channel, the aquatic ecological evaluation of site C2 was of **local importance (lower value) (Table 4.4).**

Plate 4.18 Representative image of site C2 on the uppermost reaches of the Parknakyle River, August 2022 (dry channel)

4.1.19 Site C3 – Oldleighlin Stream, Madlin Bridge

Site C3 was located on the lower reaches of the Oldleighlin Stream (14O02) at Madlin Bridge (R448), approx. 0.4km upstream of the River Barrow confluence. The lowland depositing watercourse (FW2), also known as the Madlin River, had been heavily modified historically (straightened and deepened). The stream suffered from low summer flows at the time of survey and averaged 4-5m wide and 0.2-0.5m deep in a U-shaped channel. The flow was near imperceptible with a profile of very slow-flowing glide and occasional pool. The substrata comprised mixed gravels which were heavily compacted and

silted (exacerbated by low flows). Soft sediment accumulations were present locally. Watercress and fool's watercress were present along channel margins. Aquatic bryophytes were not present. Cover of filamentous algae was high (70%), indicating significant enrichment. The banks supported scattered mature alder with great willowherb, hedge bindweed and gorse in the understories. Mixed broad-leaved woodland (WD1) was present on the north bank. The site was bordered to the south by heavily improved pasture (GA1).

A total of 8 no. fish species were recorded via electro-fishing at site C3, namely brown trout, European eel, lamprey (*Lampetra* sp.), minnow, stone loach, three-spined stickleback, roach (*Rutilus rutilus*) and dace (*Leuciscus leuciscus*) (**Appendix A**). This was the highest fish diversity recorded during the survey although abundances of most species were low. The site was a poor quality salmonid habitat at the time of survey given poor flows, historical modifications, and siltation pressures (i.e. a single brown trout recorded). Spawning, nursery and holding habitat were of poor quality. However, the fisheries value of the site is known to be significantly higher during higher flow periods (Gordon et al., 2021b; Delanty et al., 2017). Soft sediment areas were sub-optimal for larval lamprey due to poor flows but supported a low density of ammocetes. The site was of good value for European eel although only a single fish was recorded. The stream had low potential for white-clawed crayfish given the very low flows, heavy siltation and eutrophication. There was no potential for freshwater pearl mussel due to poor flows and enrichment pressures. An eDNA sample collected at the site did not detect white-clawed crayfish or freshwater pearl mussel (see section 4.3 below). There was good foraging potential for otter but no signs were recorded in vicinity of the site.

Biological water quality, based on Q-sampling, was calculated as **Q3 (poor status)** (**Appendix B**). However, it should be noted that this is a tentative rating given poor flows and lack of suitable riffle areas for sampling (as per Toner et al., 2005). No macro-invertebrate species of conservation value greater than 'least concern', according to national red lists, were recorded via Q-sampling.

Given the location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site C3 was of **international importance (Table 4.4).**

Plate 4.19 Representative image of site C3 on the lower reaches of the Oldleighlin Stream (Madlin River) at Madlin Bridge, August 2022

4.1.20 Site C4 – River Barrow, downstream of Rathellin Weir

Site B4 was located on the River Barrow (14B01) immediately downstream of Rathellin Weir, immediately downstream of the Oldleighlin Stream confluence. The large lowland depositing river (FW2) adjoining the upstream extent of Rathellin Lock that adjoined the Barrow at the weir crest. The river had been historically modified for navigation but retained a semi-natural character downstream of the weir with riffle, pool and glide sequences bordered by lush reed swamp (FS1). The river averaged 25-30m wide and >1.5m deep. Soft sediment accumulations were present in depositing areas but compacted cobble, sand and gravels dominated in faster-flowing areas adjoining the weir (mostly compacted by argillaceous clay). Common clubrush and branched bur-reed were frequent along the channel margins with abundant arrowhead and occasional perfoliate pondweed and invasive Nuttall's pondweed upstream of the weir. The coarser substrata supported scattered patches of the moss species *Leptodictyum riparium* and *Rhynchostegium riparoides*. The aquatic vegetation community was not diverse enough to be representative of the Annex I floating river vegetation habitat [3260]. The riparian areas supported the inundation vegetation fringe characteristic of the River Barrow with reed canary grass, reed sweet grass, hedge bindweed, great willowherb, meadowsweet, purple loosestrife (*Lythrum salicaria*), and scattered grey willow, osier, crack willow and white willow. The site was bordered by improved grassland (GA1).

Electro-fishing was not undertaken at site C4 given prohibitive depths of >1.5m. The weir area, inclusive of broken riffle and glide downstream, provided good quality nursery habitat for juvenile salmonids. Juvenile (1+) Atlantic salmon were visible with larger brown trout also observed in fast glides. Moderate quality spawning habitat as present locally below the weir but compaction of substrata was evident, with moderate siltation. The site was an excellent quality holding habitat for adult salmonids given abundant deep glide and pool. Depositing sand and silt in pool slacks and deep glide below the weir face offered good lamprey ammocoete burial habitat with nearby spawning in mixed gravels but this habitat was more localised. The site was also of high value as a European eel habitat (abundant refugia) and coarse fish habitat for a range of species. The survey area historically supported white-clawed crayfish but despite searching cobble and boulder refugia below the weir no crayfish were recorded present. There was no potential for freshwater pearl mussel. An eDNA sample collected at the site did not detect white-clawed crayfish or freshwater pearl mussel (see section 4.3 below). The site had good foraging potential for otter and a single spraint site was recorded under a crack willow tree on the east bank with salmonid and roach remains (ITM 669099, 664520).

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 location of the site within the River Barrow and River Nore SAC (002162), the aquatic ecological evaluation of site C4 was of **international importance (Table 4.4).**

Plate 4.20 Representative image of site C4 on the River Barrow downstream of Rathellin Weir, August 2022

4.2 White-clawed crayfish survey

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 spraint sites recorded during the survey.

However, white-clawed crayfish eDNA was detected in a water sample collected from the Dinin River at site A10 (see section 4.3 below). No crayfish eDNA was detected at sites A12 (Dinin River), B3 (Rathornan River), C3 (Oldleighlin Stream) or B4 (River Barrow).

4.3 eDNA analysis

White-clawed eDNA was detected at site A10 on the Dinin River (1 positive qPCR replicates out of 12, respectively) (**Table 4.1; Appendix C**). However, no crayfish eDNA was detected in composite water samples collected from the other sites, namely the Dinin River (A12), Rathornan River (B3), Oldleighlin Stream (C3) and River Barrow (B4) (0 positive qPCR 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 (see Discussion).

Site A12 on the Dinin River tested positive for crayfish plague (*Aphanomyces astaci*) (10 positive qPCR replicates out of 12, respectively) (**Table 4.1**).

No freshwater pearl mussel eDNA was detected in the 5 no. samples (0 positive qPCR replicates out of 12, respectively) (**Table 4.1; Appendix C**). These results were considered as evidence of the species' absence within the survey area, in keeping with the known distribution (absence) of the species in the wider survey area.

Table 4.2 eDNA results in the vicinity of the Proposed Wind Farm (positive qPCR replicates out of 12 in parentheses)

Sample	Watercourse	Freshwater pearl mussel	White-clawed crayfish	Crayfish plague
FK622	Dinin River (site A10)	Negative (0/12)	**Positive (1/12)**	Negative (0/12)
FK627	Dinin River (site A12)	Negative (0/12)	Negative (0/12)	**Positive (10/12)**
FK618	Rathornan River (site B3)	Negative (0/12)	Negative (0/12)	Negative (0/12)
FK623	Oldleighlin Stream (site C3)	Negative (0/12)	Negative (0/12)	Negative (0/12)
FK601	River Barrow (site B4)	Negative (0/12)	Negative (0/12)	Negative (0/12)

4.4 Otter signs

A total of 5 no. otter signs were recorded across 20 no. survey sites during the course of aquatic surveys undertaken in August 2022.

On the Dinin River, a regular spraint site was recorded at site A10 (ITM 659412, 670288), with a single spraint downstream of the road bridge at site A11 (ITM 655775, 669148) and on the bridge abutment at site A12 (ITM 653022, 669884). Spraint sites were also recorded on the River Barrow downstream of site B4 (ITM 669420, 666424) and site C4 (ITM 669099, 664520). None of the identified spraint sites contained white-clawed crayfish remains.

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

4.5 Invasive aquatic species

The invasive macrophyte Nuttall's pondweed (*Elodea nuttallii*) was recorded at site B3 on the lowermost reaches of the Rathornan River and on the River Barrow at site B4. The species is relatively widespread in Ireland and is listed on the Third Schedule of the European Communities (Birds and Natural Habitats) Regulations 2011-2021 (S.I. 477/2011). It is considered a high-risk invasive species in Irish waters (O' Flynn et al., 2014).

Dace (*Leuciscus leuciscus*) is an invasive cyprinid species in Ireland and has been present in the River Barrow since 1992 (Caffrey et al., 2007). The species is now firmly established throughout the River Barrow catchment and was recorded (via electro-fishing) at site C3 on the lower reaches of the Oldleighlin Stream. Another invasive cyprinid, roach (*Rutilus rutilus*), was also recorded at site C3. Roach have been present in the River Barrow catchment since the 1980s (Brazier, 2018). Both species are subject to restrictions under Regulations 49 and 50 of the Third Schedule of the European Communities (Birds and Natural Habitats) Regulations 2011-2021 (S.I. 477/2011).

Environmental DNA analysis detected the non-native pathogen crayfish plague (*Aphanomyces astaci*) in the Dinin River (**Table 4.1**; see section 4.3 above).

Incidentally, the invasive terrestrial plant Himalayan balsam (*Impatiens glandulifera*), typically associated with riparian corridors, was abundant on the island between Rathvinden Weir and the navigation lock at site B4 on the River Barrow. It was also present at sites A10 and A11 the Dinin River. This species is listed on the Third Schedule of the European Communities (Birds and Natural Habitats) Regulations 2011-2021 (S.I. 477/2011) and the Invasive Alien Species of Union Concern ('Union list') (European Commission, 2022). In Ireland it is considered a high-risk invasive species (O' Flynn et al., 2014).

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*=17 wetted sites in August 2022 (**Appendix B**).

Site A11 on the Dinin River achieved **Q4 (good status)** water quality and thus met the 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**). This was given the presence of one group A taxa (in this case the stonefly *Perla bipunctata*) in fair numbers (5% of total abundance) (**Appendix B**).

A total of 8 no. sites on an unnamed stream (A4), Seskinrea Stream (A6), Knocknabranagh & Knockbaun River (A7), Dinin River (A10 & A12), Seskin Upper Stream (B1), Rathornan River (B2) and Parknakyle Stream (C1) achieved **Q3-4 (moderate status)** water quality (**Figure 4.1**). This was given the low numbers (<5%) of group A species, typically the mayfly *Ecdyonurus dispar*; low to moderate abundances of group B species such as the mayfly *Alainites muticus* and stonefly *Leuctra hippopus,* and a dominance of group C species such as the mayflies *Baetis rhodani* and *Seratella ignita* and biting midge larvae (non-*Chironomus* spp.) (**Appendix B**).

The remaining 8 no. samples on unnamed Seskinrea Stream tributaries (A1 & A2), Seskinrea Stream (A5), Dinin River (A9), Rathornan River (B3), Oldleighlin Stream (C3) and the River Barrow (C4 & B4) achieved **Q2-3** (A2, B3) **or Q3 (poor status)** based on an absence of group A species; low numbers or absence of group B species and a dominance of group C species, particularly *Baetis rhodani* and *Gammarus duebeni* (**Appendix B**). Sites A2 (unnamed stream) and B3 (Rathornan River) supported higher proportions of group D and E taxa and thus were reduced to **Q2-3 (poor status).**

It should be noted that the ratings for sites A1, A2, A3, B3 and C3 were tentative due to low summer flows and or a lack of suitable riffle areas for sampling (Toner et al., 2005).

Sites on an unnamed Seskinrea Stream tributary (A3), Agharue Stream (A8) and Parknakyle Stream (C2) were dry at the time of survey (August 2022) and thus no biological water quality samples could be collected.

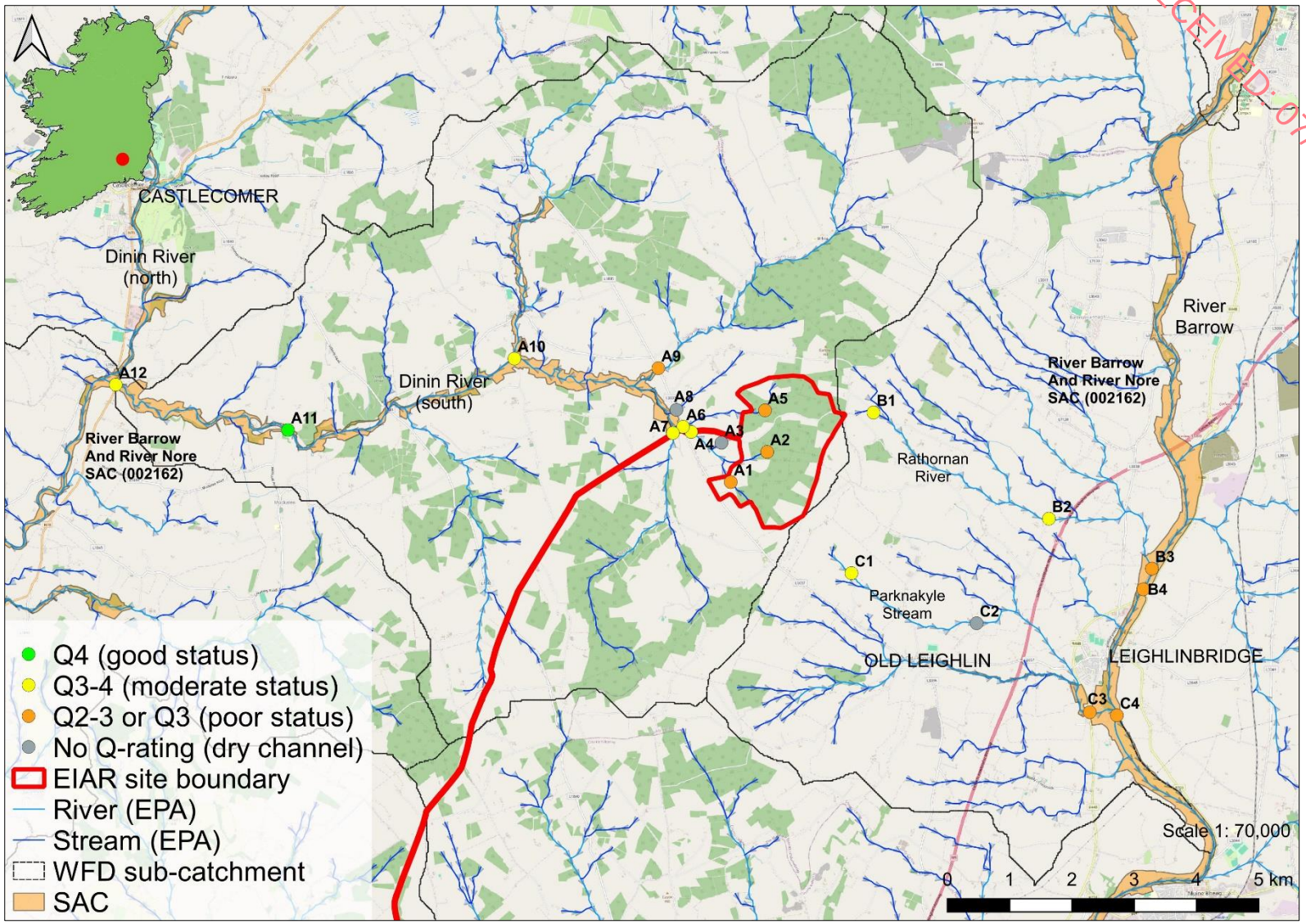

Figure 4.1 Overview of the biological water quality status in the vicinity of the Proposed Wind Farm, Co. Carlow, August 2022

4.7 Macrophytes and aquatic bryophytes

No rare or protected macrophytes or aquatic bryophytes were recorded at the *n*=20 survey sites in August 2022. Similarly, no examples of Annex I aquatic vegetation habitats were recorded during the surveys.

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 species of high conservation value), fisheries assessments and 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**).

A total of 9 no. sites on the Knocknabranagh & Knockbaun River (A7), Dinin River (A9, 10, A11 & A12), Rathornan River (B3), Oldleighlin Stream (C3) and River Barrow (B4 & C4) were evaluated as **international importance** given their location within the River Barrow and River Nore SAC (002162).

Site A6 on the Seskinrea Stream was evaluated as **local importance (higher value)** due to the presence of salmonids (including Atlantic salmon) (**Table 4.4**).

The remaining 10 no. sites on unnamed streams (A1, A2, A3 & A4), Seskinrea Stream (A5), Agharue Stream (A8), Seskin Upper Stream (B1), Rathornan River (B2), Parknakyle Stream (C1 & C2) were evaluated as **local importance (lower value)** in terms of their aquatic ecology given an absence of aquatic species or habitats of high conservation value and or less than Q4 (good status) water quality (**Table 4.4**).

Table 4.2 Summary of fish species of higher conservation value and relative abundances (low, medium, high & very high) recorded via **electro-fishing** per survey site in the vicinity of the Proposed Wind Farm, August 2022

Site	Watercourse	Relative abundance				
		Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Unnamed stream	No fish recorded				
A2	Unnamed stream	No fish recorded				
A3	Unnamed stream	No fish recorded				
A4	Unnamed river	No fish recorded				
A5	Seskinrea Stream	No fish recorded				
A6	Seskinrea Stream	Low	Medium	Not recorded		
A7	Knocknabranagh & Knockbaun River	Medium	Medium	Not recorded		
A8	Agharue Stream	No fish recorded				
A9	Dinin River	High	Medium	Not recorded		Stone loach, minnow
A10	Dinin River	Very high	Low	Not recorded		Stone loach, minnow
A11	Dinin River	Medium	Low	Not recorded	Low	Stone loach, minnow
A12	Dinin River	Medium	Medium	Not recorded	Low	Stone loach, minnow
B1	Seskin Upper Stream	No fish recorded				
B2	Rathornan River			Not recorded		Three-spined stickleback
B3	Rathornan River			Not recorded		Three-spined stickleback, stone loach, pike
B4	River Barrow	n/a – too deep for electro-fishing (fisheries appraisal only)				
C1	Parknakyle Stream	No fish recorded				
C2	Parknakyle Stream	No fish recorded				
C3	Oldleighlin Stream		Low	Low	Low	Dace, minnow, roach, stone loach, three-spined stickleback
C4	River Barrow	n/a – too deep for electro-fishing (fisheries appraisal only)				

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 Inland Fisheries Acts 1959 to 2017, brown trout and coarse fish species have no legal protection in Ireland.

Table 4.3 Summary of aquatic species (**excluding fish**) & habitats of higher conservation value recorded in the vicinity of the Proposed Wind Farm

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	Unnamed stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A2	Unnamed stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A3	Unnamed stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A4	Unnamed river	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A5	Seskinrea Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A6	Seskinrea Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A7	Knocknabranagh & Knockbaun River	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A8	Agharue Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A9	Dinin River	None recorded		No signs	Not present	None recorded	None recorded	None recorded
A10	Dinin River	**None recorded; positive eDNA result at site**	Negative eDNA result at site, no records in catchment	**Regular spraint site**	Not present	None recorded	None recorded	None recorded
A11	Dinin River	None recorded		**Spraint site**	Not present	None recorded	None recorded	None recorded
A12	Dinin River	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment	**Spraint site**	Not present	None recorded	None recorded	None recorded
B1	Seskin Upper Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
B2	Rathornan River	None recorded		No signs	Not present	None recorded	None recorded	None recorded
B3	Rathornan River	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment	No signs	Not present	None recorded	None recorded	None recorded
B4	River Barrow	None recorded		**Regular spraint site**	Not present	None recorded	None recorded	None recorded

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
C1	Parknakyle Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
C2	Parknakyle Stream	None recorded		No signs	Not present	None recorded	None recorded	None recorded
C3	Oldleighlin Stream	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment	No signs	Not present	None recorded	None recorded	None recorded
C4	River Barrow	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment	**Regular 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.

[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	Unnamed stream	n/a	Local importance (lower value)	Small, shallow, heavily modified upland eroding stream with ephemeral characteristics & siltation pressures; no fish recorded via electro-fishing & not of fisheries value; **Q3** (poor status) water quality (tentative rating); no aquatic species or habitats of high conservation value
A2	Unnamed stream	n/a	Local importance (lower value)	Headwaters of small, heavily modified upland eroding stream with low summer flows & siltation pressures; no fish recorded via electro-fishing & not of fisheries value; **Q3** (poor status) water quality (tentative rating); no aquatic species or habitats of high conservation value
A3	Unnamed stream	n/a	Local importance (lower value)	Small, shallow, heavily modified upland ephemeral eroding stream that was dry at the time of survey with an absence of aquatic species or habitats
A4	Unnamed river	n/a	Local importance (lower value)	Shallow, heavily modified upland eroding stream with low summer flows & poor hydromorphology; no fish recorded via electro-fishing & not of fisheries value; **Q3-4** (poor status) water quality (tentative rating); no aquatic species or habitats of high conservation value
A5	Seskinrea Stream	15S14	Local importance (lower value)	Uppermost reaches of small, shallow, heavily modified, upland eroding stream with low summer flows & siltation pressures; no fish recorded via electro-fishing & not of fisheries value; **Q3** (poor status) water quality; no aquatic species or habitats of high conservation value
A6	Seskinrea Stream	15S14	**Local importance (higher value)**	Small, historically straightened upland eroding stream with low summer flows & siltation pressures; Atlantic salmon & brown trout recorded via electro-fishing (low densities); **Q3-4** (moderate status) water quality
A7	Knocknabranagh & Knockbaun River	15K25	**International importance**	Located within the River Barrow & River Nore SAC (002162); semi-natural, meandering upland eroding river with low summer flows but high value as a salmonid nursery; Atlantic salmon & brown trout recorded via electro-fishing; **Q3-4** (moderate status) water quality
A8	Agharue Stream	15A14	Local importance (lower value)	Small, shallow, semi-natural, ephemeral upland eroding stream that was dry at the time of survey with an absence of aquatic species or habitats
A9	Dinin River	15D08	**International importance**	Located within the River Barrow & River Nore SAC (002162); medium sized, high energy, natural upland eroding river of good value for salmonids but with enrichment pressures; Atlantic salmon, brown trout, minnow & stone loach recorded via electro-fishing; **Q3-4** (moderate status) water quality
A10	Dinin River	15D08	**International importance**	Located within the River Barrow & River Nore SAC (002162); medium sized, high energy, semi-natural upland eroding river with enrichment pressures but of very high value as a salmonid nursery; high abundance of juvenile Atlantic salmon with brown trout, minnow & stone loach recorded via electro-fishing; regular otter spraint site recorded; **Q3-4** (moderate status) water quality

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary
A11	Dinin River	15D08	**International importance**	Located within the River Barrow & River Nore SAC (002162); medium sized, semi-natural upland eroding river with enrichment pressures but of good value for salmonids; Atlantic salmon, brown trout, European eel, minnow & stone loach recorded via electro-fishing; otter spraint site recorded; **Q4** (good status) water quality
A12	Dinin River	15D08	**International importance**	Located within the River Barrow & River Nore SAC (002162); medium sized, semi-natural upland eroding river with enrichment pressures but of good value for salmonids; Atlantic salmon, brown trout, European eel, minnow & stone loach recorded via electro-fishing; otter spraint site recorded; **Q3-4** (moderate status) water quality
B1	Seskin Upper Stream	14S28	Local importance (lower value)	Shallow, shallow, high gradient upland eroding spate stream with low summer flows & siltation pressures; no fish recorded via electro-fishing & not of fisheries value; **Q3-4** (moderate status) water quality (tentative rating); no aquatic species or habitats of high conservation value
B2	Rathornan River	14R43	Local importance (lower value)	Shallow, heavily modified upland eroding stream with low summer flows, poor hydromorphology & enrichment pressures; three-spined stickleback recorded via electro-fishing; **Q3-4** (poor status) water quality (tentative rating); no aquatic species or habitats of high conservation value
B3	Rathornan River	14R43	**International importance**	Located within the River Barrow & River Nore SAC (002162); lowermost reaches of heavily modified lowland depositing river with low summer flows; pike, stone loach & three-spined stickleback recorded via electro-fishing; **Q2-3** (poor status) water quality
B4	River Barrow	14B01	**International importance**	Located within the River Barrow & River Nore SAC (002162); large, semi-natural lowland river with high value for salmonids, lamprey & coarse fish species; too deep for electro-fishing survey; otter spraint site recorded in vicinity of site; **Q3** (poor status) water quality
C1	Parknakyle Stream	14P10	Local importance (lower value)	Small, shallow, historically modified, semi-dry upland eroding stream with ephemeral characteristics; no fish recorded via electro-fishing & not of fisheries value; **Q3-4** (moderate status) water quality (tentative rating); no aquatic species or habitats of high conservation value
C2	Parknakyle Stream	14P10	Local importance (lower value)	Shallow, heavily modified upland ephemeral eroding stream that was dry at the time of survey with an absence of aquatic species or habitats
C3	Oldleighlin Stream	14O02	**International importance**	Located within the River Barrow & River Nore SAC (002162); heavily modified lowland stream with low summer flows plus siltation & enrichment pressures;; brown trout, European eel, lamprey (*Lampetra* sp.), minnow, stone loach, three-spined stickleback, roach & dace recorded via electro-fishing; **Q3** (poor status) water quality (tentative rating)

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary
C4	River Barrow	14B01	**International importance**	Located within the River Barrow & River Nore SAC (002162); large, semi-natural lowland river with high value for salmonids, lamprey & coarse fish species; too deep for electro-fishing survey; otter spraint site recorded in vicinity of site; **Q3** (poor 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). Apart from the Inland Fisheries Acts 1959 to 2017, brown trout and coarse fish species have no legal protection in Ireland.

5. Discussion

5.1 Most valuable areas for aquatic ecology

A total of 9 no. sites in the vicinity of the Proposed Wind Farm were evaluated as of **international importance** given their location within the River Barrow and River Nore SAC (002162). These were on the Knocknabranagh & Knockbaun River (A7), Dinin River (A9, 10, A11 & A12), Rathornan River (B3), Oldleighlin Stream (C3) and River Barrow (B4 & C4).

Apart from site A6 on the Seskinrea Stream that was of **local importance (higher value**), the remaining aquatic survey sites on unnamed streams (A1, A2, A3 & A4), Seskinrea Stream (A5), Agharue Stream (A8), Seskin Upper Stream (B1), Rathornan River (B2) and Parknakyle Stream (C1 & C2) were evaluated as **local importance (lower value)** in terms of their aquatic ecology. This was given an absence of aquatic species or habitats of high conservation value and or less than Q4 (good status) water quality (**Table 4.4**).

5.1.1 Fish species of high conservation value

Salmonids were present at 7 no. sites in total, with Atlantic salmon present at six of these (i.e. A6, A7, A9, A01, A11 & A12). All sites supporting Atlantic salmon were located in the Nore catchment (Dinin[South]_SC_010 sub-catchment), to the west of the Proposed Wind Farm. Atlantic salmon parr were recorded at all four survey sites on the Dinin River and these sites also supported the highest densities of the species, indicating the importance of the river for anadromous salmonids (**Appendix A**). The Dinin, along with the Knocknabranagh & Knockbaun River (A7) and River Barrow, can be considered overall the most important salmonid habitats in the survey area. Sites A7[3] and A10 were particularly valuable as salmonid nurseries. It should be noted that whilst no salmonids were recorded from 2 no. sites on the Rathornan River (Barrow tributary, east of the Proposed Wind Farm), the watercourse is known to support Atlantic salmon and brown trout (Gordon et al., 2021b), including at the same location as survey site B2. Their absence during this survey reflected the low summer flows observed and resulting influences on fish distribution. Although the Oldleighlin Stream (Madlin River) suffered from low summer flows and supported only a low density of brown trout during the current survey, the stream is noted as an important trout spawning habitat in context of the wider Barrow catchment (Delanty et al., 2017) (at least under higher flows).

Lamprey ammocoetes (*Lampetra* sp.) were only recorded from a single site during targeted electro-fishing across the 19 no. survey sites in the vicinity of the Proposed Wind Farm (**Table 4.2; Appendix A**). A low density of ammocoetes was present at site C3 on the lowermost reaches of the Oldleighlin Stream near the River Barrow confluence. This highly restricted distribution reflected the upland, higher-energy nature of many of the survey watercourses, in addition to low summer flows and siltation pressures, which present conditions inimical to lamprey population establishment and persistence (**Appendix A**).

Despite widespread suitability, European eel were only recorded in low densities from sites A11 & A12 on the Dinin River and C3 on the Oldleighlin Stream (**Table 4.2; Appendix A**). European eel are Red-

[3] This site supported the highest density of juvenile Atlantic salmon recorded in nationwide Water Framework Directive (WFD) surveys undertaken by Inland Fisheries Ireland (IFI) in 2021 (Corcoran et al., 2022)

listed in Ireland (King et al., 2011) and are classed as 'critically endangered' on a global scale (Pike et al., 2020). As eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019), the paucity of eel observed in the Dinin[South]_SC_010 and Barrow_SC_110 river sub-catchments can be partly explained by the distance between the survey area and marine habitats (Chadwick et al., 2007) (c.80km nearest instream distance). The absence of eel from many physically suitable sites also likely reflects the high number of barriers to fish passage present in the Nore and Barrow catchments, as well as widespread very low summer flow conditions the reduce the suitability of smaller rivers and streams for the species during these conditions (**Appendix A**).

5.1.2 Otter

Despite some suitability at numerous survey locations, otter signs were only recorded at a total of 5 no. sites on the Dinin River (A10, A11 & A12) and River Barrow (B4 & C4). This paucity of signs was considered to mainly reflect the influence of low (summer) water levels and flows on the health and distribution of fish populations, the key prey resource of otter (Krawczyk et al., 2016; Ruiz-Olmo & Jiménez, 2009). Otters are food-limited and prey availability is a crucial factor in determining mortality, breeding success and the status of local populations (Sittenhaler et al., 2019; Ruiz-Olmo et al., 2002). Numerous survey sites were largely unsuitable for otter given their location on smaller, higher-energy upland eroding channels. These would typically provide more restricted, stochastic prey resources and reduced foraging opportunities (Sittenthaler et al., 2019; Scorpio et al., 2016; Remonti et al., 2009). No breeding (holt) or couch (resting) areas were identified in the vicinity of the survey sites in August 2022.

5.1.3 Freshwater pearl mussel

No freshwater pearl mussel eDNA was detected in samples collected in August 2022 from the Dinin River (A10 & A12), Rathornan River (B3), Oldleighlin Stream (C3) or the River Barrow (C4) (0 positive qPCR replicates out of 12, respectively) (**Table 4.1**; **Appendix C**). Suitability was typically absent throughout the survey sites given the considerable siltation, eutrophication and historical drainage pressures in addition to low summer flows and the small size of many of the surveyed watercourses. These results were in keeping with the known absence of this species within the wider survey area (NPWS data). Extant freshwater pearl mussel populations are confined to the Mountain and Ballymurphy sub-catchments in the Barrow catchment, and the River Nore in the Nore catchment[4].

5.1.4 White-clawed crayfish & crayfish plague

No white-clawed crayfish were detected via hand searching (*n*=17 wetted sites) or field examination of otter spraint (*n*=5 sites). In validation of site observations, no white-clawed crayfish eDNA was detected in samples collected in August 2022 from sites A12 (Dinin River), B3 (Rathornan River), C3 (Oldleighlin Stream) or B4 (River Barrow) (0 positive qPCR replicates out of 12, respectively) (**Table 4.1**; **Appendix C**).

However, white-clawed crayfish eDNA was detected from the Dinin River at site A10 (1 positive qPCR replicate out of 12, respectively) (**Table 4.1**; **Appendix C**). Whilst highly sensitive and often detectable

[4] see Freshwater Pearl Mussel Plans 2009-2015 at: https://www.catchments.ie/download/freshwater-pearl-mussel-plans-2009-2015/

over long distances instream (including in crayfish; Chucholl et al., 2021), the detection of environmental DNA from an upstream (riverine) population depends on downstream transport of genetic material. The low summer flows present on the Dinin River at the time of survey may have limited the flow of eDNA and thus influenced detection rates of crayfish (i.e. DNA may have temporarily settled out of suspension; Buxton et al., 2018). The patchy distribution and often low abundances of white-clawed crayfish in a given river system may also strongly influence eDNA detection probability (Sint et al., 2022). Nevertheless, the weak eDNA signature at site A10, coupled with the failure to record live crayfish elsewhere on the river (surveys & eDNA) and an absence of crayfish remains in otter spraint, would suggest the presence of a small, cryptic crayfish population within the Dinin River and or its tributaries. Our results highlight the importance of a multifaceted approach to crayfish surveying, using a combination of crayfish surveys, inspection of otter spraint and eDNA to improve detection rates.

Regrettably, crayfish plague was also detected on the Dinin River, at site A12 (10 positive qPCR replicates out of 12, respectively) (**Table 4.1**). Crayfish plague is listed at one of the world's 100 worst invasive species (GISD, 2022; Lowe et al., 2000) and is becoming highly prevalent across Ireland. *Aphanomyces astaci* is considered an obligate crayfish parasite not capable of surviving for a long period outside a crayfish host (Strand et al., 2011; Söderhall & Cerenius, 1999). The pathogen has been known in the wider Nore (and Barrow) catchments since 2017, resulting in widespread mortality (NPWS, 2017). Environmental DNA monitoring (aside from this report) has continued to detect and confirm the spread of crayfish plague in these wider catchments since (National Crayfish Plague Surveillance Programme; Swords et al., 2021). The detection of crayfish plague in the River Dinin is also likely to jeopardise any such remaining populations within the sub-catchment.

5.1.5 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*=17 wetted riverine sites in August 2022 (**Appendix B**).

Site A11 on the Dinin River achieved **Q4 (good status)** water quality and thus met the target good status (≥Q4) requirements of the European Union Environmental Objectives (Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (**Appendix B**). The remaining 16 no. sites achieved **Q3-4 (moderate status)** or **Q2-3 or Q3 (poor status)**.

The biological water quality of the survey area was generally poor, with the majority of the watercourses in the study area significantly impacted via eutrophication, siltation and or historical modifications (hydromorphology). The widespread low summer flows and water volumes further reduced the water quality within the survey area in August 2022. Abstraction and agricultural eutrophication are among the primary threats to water quality within the survey area (EPA, 2019, 2018) and this was observed during the site surveys.

5.2 Aquatic ecology summary

With the exception of the Dinin River (a larger semi-natural upland river) and the River Barrow (large lowland river), the watercourses in the vicinity of the Proposed Wind Farm were typically small, modified channels which suffered from reduced summer flows in August 2022. These characteristics resulted in in reduced habitat and water quality, often poor fluvial connectivity, habitat fragmentation

and fish passage issues. Low summer flows are a common occurrence in the wider survey area and, in addition to considerable agricultural (eutrophication, siltation) pressures, is a significant threat to aquatic ecology in the vicinity of the Proposed Wind Farm. Approximately half of the survey sites were of **international importance** by virtue of their location within the River Barrow and River Nore SAC (002162) but these were not always of inherently high aquatic value (e.g. site C3).

Salmonids were relatively widespread within the Dinin[South]_SC_010 river sub-catchment, with European eel and *Lampetra* sp. showing a much more restricted distraction in the wider survey area. Atlantic salmon were not recorded in the surveyed watercourses to the east of the Proposed Wind Farm (i.e. Barrow_SC_010 sub-catchment). The invasive fish species dace and roach were confined to the Barrow_SC_010 sub-catchment. Freshwater pearl mussel were not recorded during the surveys, in keeping with the known distribution of these species in the wider survey area (i.e. absent). Both white-clawed crayfish and crayfish plague were recorded via eDNA analysis on the Dinin River, whilst neither were recorded in the Barrow_SC_010 sub-catchment. A low number of otter signs were recorded in vicinity of the Proposed Wind Farm (no holts or couches). No rare or protected macro-invertebrates, macrophytes or aquatic bryophytes were recorded and no examples of Annex I floating river vegetation habitat [3260] were present. Biological water quality was less than good status (<Q4) at all sites with the exception of site A11 on the Dinin River. Broadly speaking, the highest value watercourses within vicinity of the Proposed Wind Farm were the Dinin River and its tributary the Knocknabranagh & Knockbaun River (east) and, to the west of the Proposed Wind Farm, the River Barrow.

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7. Appendix A – fisheries assessment report

Please see accompanying fisheries assessment report

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1. Introduction

1.1 Background

Triturus Environmental Ltd. were commissioned by MKO to undertake a baseline fisheries assessment of numerous watercourses in the vicinity of the proposed Seskin wind farm ('Proposed Wind Farm'), located approx. 6km north-west of Leighlinbridge, Co. Carlow (**Figure 2.1**).

The survey was undertaken to establish baseline fisheries data used in the preparation of the EIAR for the Proposed Project (excluding the grid cable route, Proposed Grid Connection Route[1]). In order to gain an accurate overview of the existing and potential fisheries value of the riverine watercourses within the vicinity of the Proposed Project, a catchment-wide electro-fishing survey across *n*=20 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 (*Lampetra* sp.) and European eel (*Anguilla anguilla*). Other species of lower conservation value were also recorded. The presence and or absence of fish populations of high conservation value and or associated supporting habitats would help inform impact assessment and any subsequent mitigation for the Proposed 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 Project. Permission was granted on the 27th June 2022 and the survey was undertaken on Tuesday 2nd to Thursday 4th August 2022.

1.2 Fisheries asset of the survey area

In proximity to the EIAR site boundary, the Knocknabranagh & Knockbaun River (also known as the Coolcullen River) is a tributary of the Dinin River known to support Atlantic salmon, brown trout and stone loach at Philip's Bridge (survey site A7) (Matson et al., 2018a). The site supported the highest density of juvenile Atlantic salmon recorded in nationwide Water Framework Directive (WFD) surveys undertaken by Inland Fisheries Ireland (IFI) in 2021 (Corcoran et al., 2022).

The Dinin River is a major tributary of the River Nore and much of the river (downstream of Black Bridge) forms part of the River Barrow and River Nore SAC (002162). The Dinin is known to support Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), minnow (*Phoxinus phoxinus*) and stone loach (*Barbatula barbatula*) (Gordon et al., 2021a; Matson et al., 2018a). In the lower reaches (Dinin Bridge), European eel (*Anguilla anguilla*), three-spined stickleback (*Gasterosteus aculeatus*) and lamprey (*Lampetra* sp.) have also been recorded (Kelly et al., 2017, 2013).

To the east of the proposed project, the Rathornan River, a tributary of the River Barrow, is known to support Atlantic salmon, brown trout and stone loach (Gordon et al., 2021b). This survey area was also surveyed in the current fisheries report (i.e. site B2).

At Madlin Bridge (survey site C3), the Oldleighlin Stream (also known as the Madlin River) is known to support brown trout, minnow, stone loach, three-spined stickleback and invasive dace (*Leuciscus*

[1] Proposed Grid Connection Route did not form part of this aquatic survey, please refer to Chapter 6 of the EIAR for summary of the multidisciplinary surveys undertaken along the Proposed Grid Connection Route

leuciscus) (Gordon et al., 2021b). The river is noted as an important brown trout spawning habitat in the wider Barrow catchment (Delanty et al., 2017).

The River Barrow is Ireland's second-longest river, flowing for some 192km and draining an area of approx. 3010km^2 (Delanty et al., 2017). In the vicinity of the Leighlinbridge, downstream of the proposed project, the river is known to support Atlantic salmon, brown trout, European eel, dace, minnow, three-spined stickleback, roach (*Rutilus rutilus*), pike (*Esox lucius*), gudgeon (*Gobio gobio*) and perch (*Perca fluviatilis*) (Kelly et al., 2013). Fisheries data for the other watercourses surveyed was not available at the time of survey.

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 Wind Farm on Tuesday 2nd to Friday 5th 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*=20 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., 2018b). A total of approx. 50-100m 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 moderate to high conductivity waters of the sites (draining both sandstone & limestone) a voltage of 220-280v, frequency of 35-40Hz 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 by using targeted electro-fishing in objectively suitable areas of sand/silt, where encountered and estimating surface area for density estimates (as per Harvey & Cowx, 2003). 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. 10-15cm 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 (EA, 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. Cognisance was given towards preventing the spread or introduction of crayfish plague given the known historical distribution of white-clawed crayfish and previous outbreaks of crayfish plague in the wider survey area. Furthermore, staff did not undertake any work in a known crayfish plague catchment for a period of <72hrs in advance of the survey. 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*=20 electro-fishing survey sites in the vicinity of Proposed Wind Farm, Co. Carlow (* indicates fisheries appraisal only due to prohibitive depths)

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Unnamed stream	n/a	Ridge	662900	668302
A2	Unnamed stream	n/a	Seskinrea	663485	668737
A3	Unnamed stream	n/a	Seskinrea	662751	668934
A4	Unnamed river	n/a	L30372 road crossing, Seskinrea	662266	669109
A5	Seskinrea Stream	15S14	Seskinrea	663451	669455
A6	Seskinrea Stream	15S14	L3037 road crossing, Seskinrea	662134	669191
A7	Knocknabranagh & Knockbaun River	15K25	Philips Bridge	661971	669091
A8	Agharue Stream	15A14	L3037 road crossing, Agharue	662027	669464
A9	Dinin River	15D08	Black Bridge, L3037	661736	670133
A10	Dinin River	15D08	Coolcullen	659426	670287
A11	Dinin River	15D08	Uskerty	655788	669139
A12	Dinin River	15D08	Dysart Bridge, N78	653022	669867
B1	Seskin Upper Stream	14S28	Seskin Upper	665192	669420
B2	Rathornan River	14R43	Coolnakisha	668010	667714
B3	Rathornan River	14R43	River Barrow confluence	669665	666911
B4*	River Barrow	14B01	Downstream of Rathvinden Weir	669526	666580
C1	Parknakyle Stream	14P10	Parknakyle	664839	666839
C2	Parknakyle Stream	14P10	Coolnakeeran	666849	666034
C3	Oldleighlin Stream	14O02	Madlin Bridge, R448	668668	664605
C4*	River Barrow	14B01	Downstream of Rathellin Weir	669102	664554

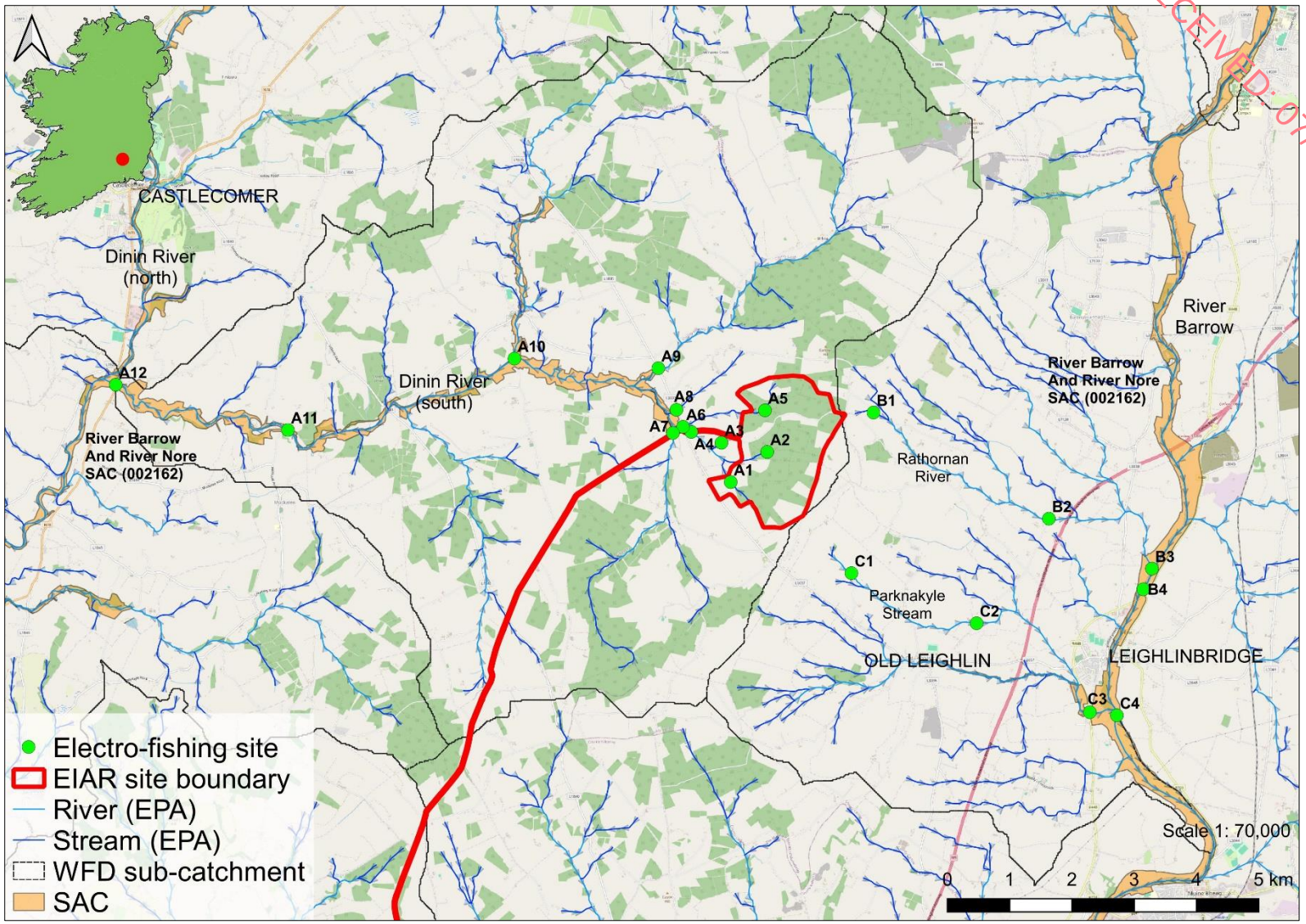

Figure 2.1 Overview of the *n*=20 electro-fishing & fisheries appraisal survey site locations for the Proposed Wind Farm, Co. Carlow

3. Results

A catchment-wide electro-fishing survey of *n*=20 riverine sites in the vicinity of the proposed Old Leighlin wind farm was conducted on Tuesday 2nd to Friday 5th 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 Fisheries assessment & appraisal

3.1.1 Site A1 – unnamed stream, Ridge

No fish were recorded via electro-fishing at site A1 on an unnamed poorly accessible Seskinrea Stream tributary. The shallow, heavily modified upland eroding site was not of fisheries value given the very shallow nature of the stream with trickles of water and shallow pools. The channel was likely ephemeral in nature and its location in the uppermost reaches of the catchment and evident siltation pressures precluded it from having any fisheries value.

Plate 3.1 Representative image of site A1 on an unnamed Seskinrea Stream tributary, August 2022

3.1.2 Site A2 – unnamed stream, Seskinrea

No fish were recorded via electro-fishing at site A2 in the headwaters of an unnamed Seskinrea Stream tributary. The diminutive upland eroding site was not of fisheries value given the low summer flows, very shallow nature, location in the headwaters of the stream and evident siltation pressures.

Plate 3.2 Representative image of site A2 on an unnamed Seskinrea Stream tributary, August 2022

3.1.3 Site A3 – unnamed stream, Seskinrea

Site A3 on an unnamed Seskinrea Stream tributary was not of fisheries value given its dry, ephemeral nature and absence of aquatic habitats at the time of survey.

Plate 3.3 Representative image of site A3 on an unnamed stream, August 2022 (dry channel)

3.1.4 Site A4 - unnamed river, Seskinrea

No fish were recorded via electro-fishing at site A4 on an unnamed Seskinrea Stream tributary. The heavily modified upland eroding site was not of fisheries value at the time of survey given the low summer flows, very shallow nature and evident siltation pressures.

Plate 3.4 Representative image of site A4 on an unnamed Seskinrea River tributary, August 2022

3.1.5 Site A5 – Seskinrea Stream, Seskinrea

No fish were recorded via electro-fishing at site A5 on the uppermost reaches of the Seskinrea Stream. The diminutive upland eroding site was not of fisheries value given poor summer flows, the very shallow nature of the site, evident siltation pressures and the location in the headwaters of the stream.

Plate 3.5 Representative image of site A5 on the upper reaches of the Seskinrea Stream, August 2022

3.1.6 Site A6 – Seskinrea Stream, Seskinrea

Atlantic salmon (*Salmo salar*) (*n*=4) and brown trout (*Salmo trutta*) (*n*=12) were the only fish species recorded via electro-fishing at site A6 on the Seskinrea Stream (**Figure 3.1**).

The upland eroding site was considered a moderate quality salmonid nursery, supporting a low density of juveniles. The value was reduced due to siltation. Spawning habitat was of moderate quality due to

the higher energy and dominance of coarse substrata with heavy siltation. Holding areas for adult salmonids were sparse but present nonetheless. The site was considered a moderate quality European eel habitat given the presence of pools and coarse substrata refugia but the species was not recorded. The upland site was unsuitable for lamprey and no lamprey were recorded during the survey.

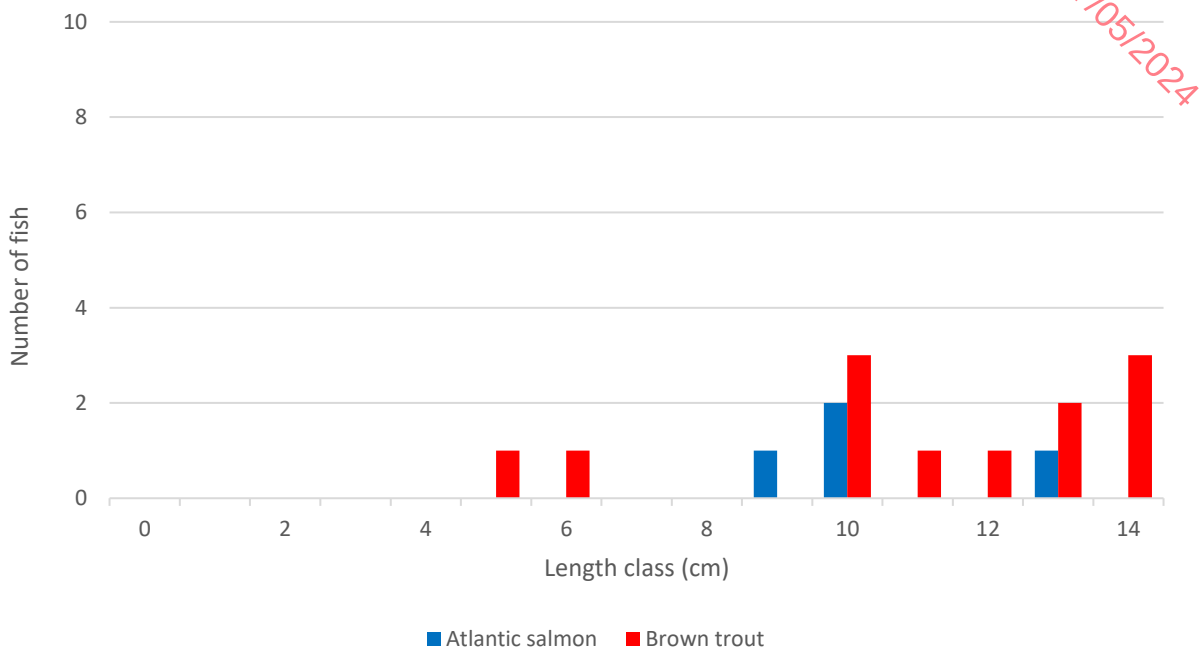

Figure 3.1 Length frequency distribution recorded via electro-fishing at site A6 on the Seskinrea Stream, August 2022

Plate 4.5 Representative image of site A6 on the Seskinrea Stream, August 2022

3.1.7 Site A7 – Knocknabranagh & Knockbaun River, Philip's Bridge

Atlantic salmon (*n*=16) and brown trout (*n*=28) were the only fish species recorded via electro-fishing at site A7 on the Knocknabranagh & Knockbaun River (**Figure 3.2**).

The site was a very good quality salmonid nursery due to abundant shallow glide and riffle with boulder refugia and supported a relatively high number of juveniles. Spawning habitat was of moderate quality due to the dominance of boulder and cobble but improved locally. The site was a poor holding habitat due to the paucity of pool areas. The site was considered a moderate quality European eel habitat given the presence of abundant refugia albeit no eel were recorded during the survey. The upland spate river channel was of too high energy for lamprey.

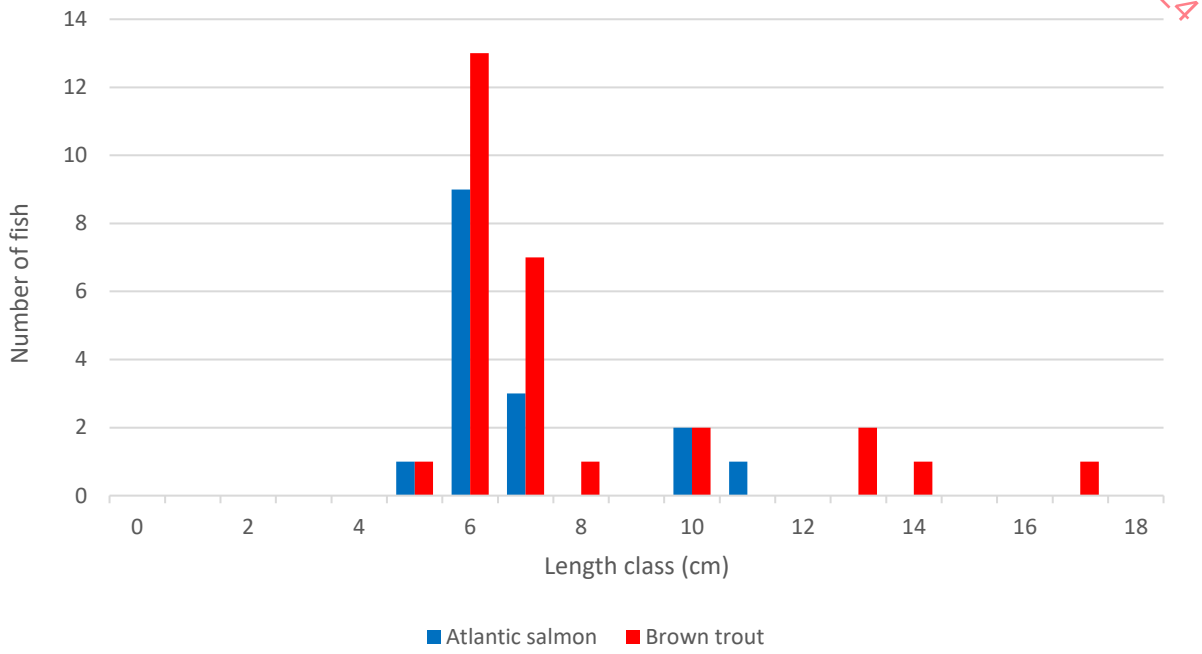

Figure 3.2 Length frequency distribution recorded via electro-fishing at site A7 on the Knocknabranagh & Knockbaun River, August 2022

Plate 3.7 Representative image of site A7 on the Knocknabranagh & Knockbaun River, August 2022

3.1.8 Site A8 – Agharue Stream, Agharue

Site A8 on the Agharue Stream was not of fisheries value given its dry, ephemeral nature and absence of water at the time of survey.

Plate 3.8 Representative image of site A8 on the Agharue Stream, August 2022 (dry channel)

3.1.9 Site A9 – Dinin River, Black Bridge

Atlantic salmon (*n*=32), brown trout (*n*=19), minnow (*Phoxinus phoxinus*) (*n*=1) and stone loach (*Barbatula barbatula*) (*n*=8) were recorded via electro-fishing at site A9 (**Figure 3.3**), in keeping with previous surveys of the site (Gordon et al., 2021a; Matson et al., 2018a).

The site was a good quality salmonid nursery habitat in light of abundant broken oxygenated water with cascading riffle, glide and pool sequences. The nursery value was only reduced due to the high energy and steep gradient of the channel. Nonetheless, the river still supported healthy mixed cohorts of Atlantic salmon and brown trout. The site was of moderate spawning value, with suitable areas restricted to small patches of cobble and gravels in pool tailings. Holding habitat was of moderate quality due to the shallow nature of the cascade pool areas. The site was a moderate quality European eel habitat (reduced due to high energy) but none were recorded. The upland site was unsuitable for lamprey (none recorded).

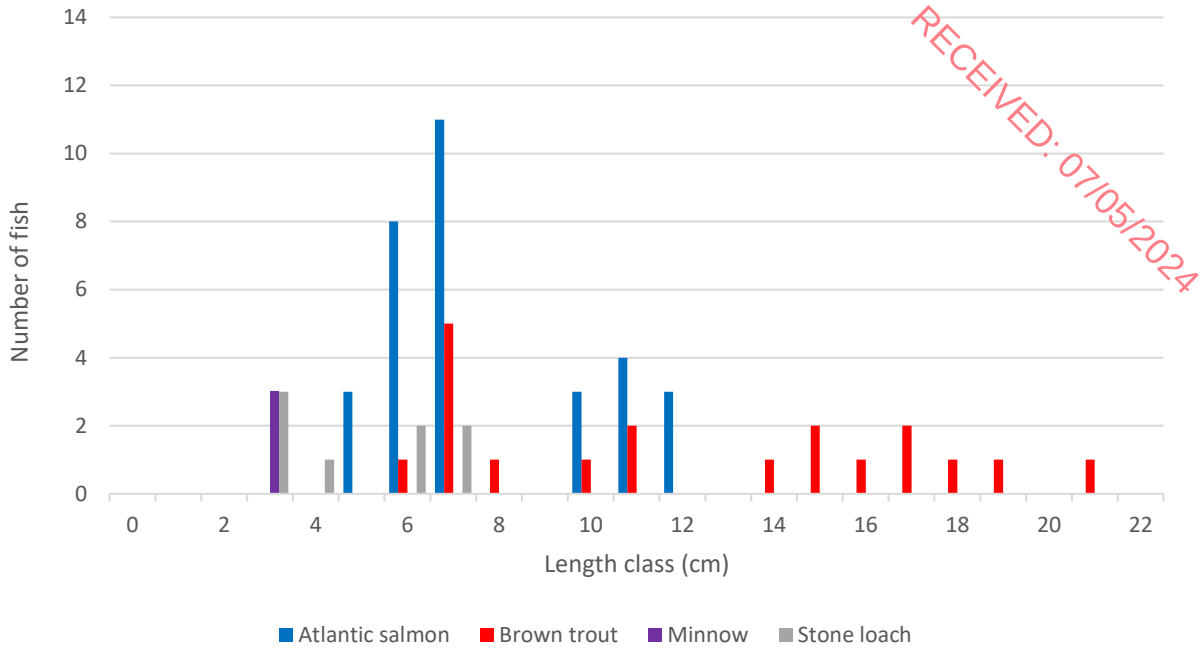

Figure 3.3 Length frequency distribution recorded via electro-fishing at site A9 on the Dinin River, August 2022

Plate 3.9 Brown trout (top) and Atlantic salmon (bottom) recorded at site A9 on the Dinin River at Black Bridge, August 2022

3.1.10 Site A10 – Dinin River, Coolcullen

Atlantic salmon (*n*=117), brown trout (*n*=4), minnow (*n*=1) and stone loach (*n*=1) were recorded via electro-fishing at site A10 on the Dinin River (**Figure 3.4**).

The site was an excellent quality salmonid nursery habitat, supporting a high abundance of Atlantic salmon parr (mostly 0+). Nursery habitat was of especially good quality upstream of the deep pool near the bridge. Good quality spawning and holding habitat was also present. The site was considered a good quality European eel habitat due to the abundant cobble and boulder refugia but the species

was not recorded present. The weir downstream of the bridge may partially restrict eel passage. The high energy site was unsuitable for lamprey, with none recorded.

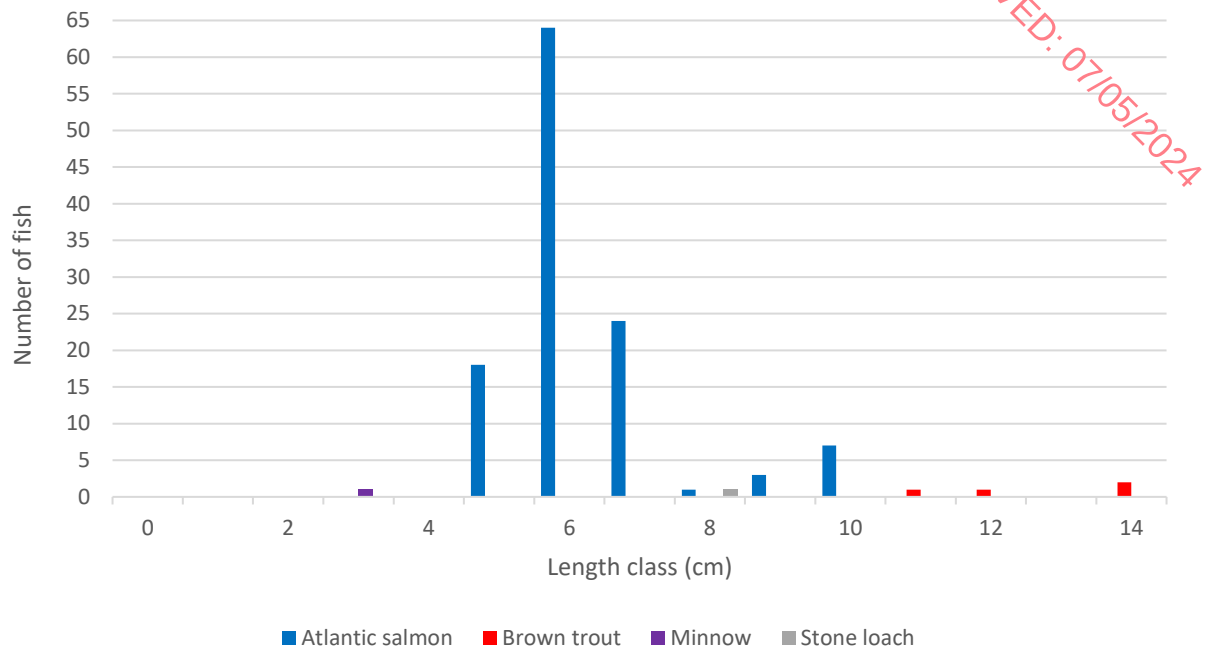

Figure 3.4 Length frequency distribution recorded via electro-fishing at site A10 on the Dinin River, August 2022

Plate 3.10 Representative image of site A10 on the Dinin River, August 2022

3.1.11 Site A11 – Dinin River, Uskerty

Atlantic salmon (*n*=15), brown trout (*n*=7), European eel (*Anguilla anguilla*) (*n*=1), minnow (*n*=17) and stone loach (*n*=13) were recorded via electro-fishing at site A11 on the Dinin River (**Figure 3.5**).

The site was a good quality salmonid nursery, supporting a low density of juveniles. The site was of moderate value as a spawning habitat with suitable areas limited to localised coarse gravels between

boulders. Holding habitat was of moderate quality overall due to a paucity of pool. The site was of good value as a European eel habitat with abundant refugia. The channel was of too high energy for lamprey species and none were recorded.

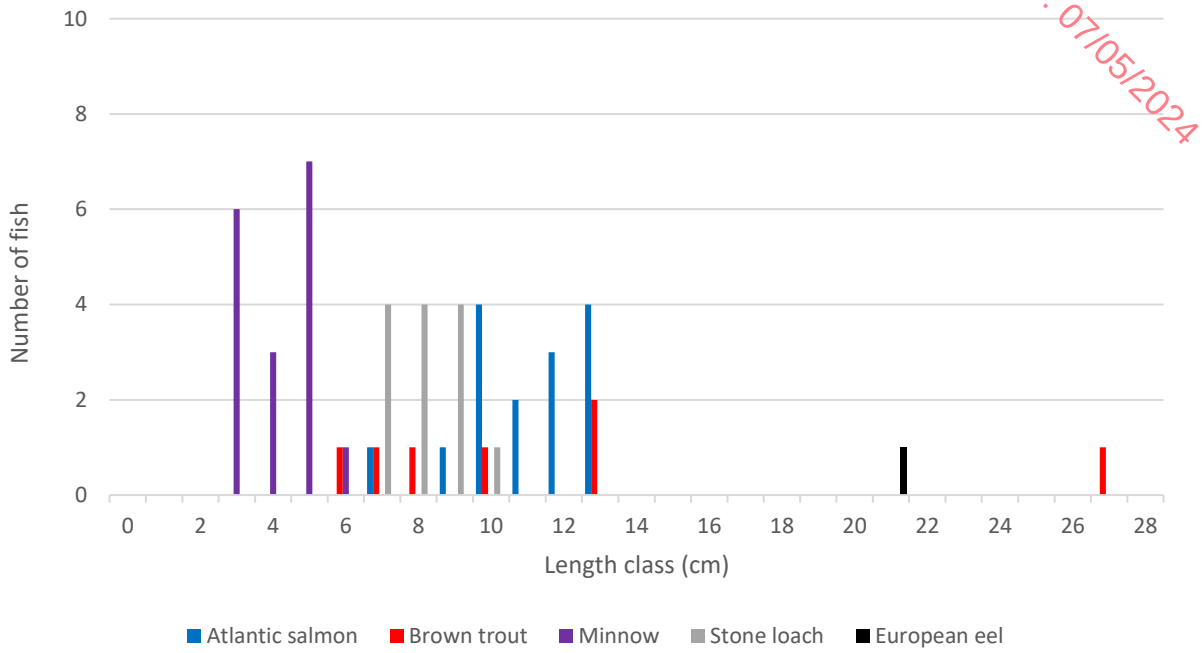

Figure 3.5 Length frequency distribution recorded via electro-fishing at site A11 on the Dinin River, August 2022

Plate 3.12 Stone loach recorded at site A11 on the Dinin River, August 2022

3.1.12 Site A12 – Dinin River, Dysart Bridge

Atlantic salmon (*n*=27), brown trout (*n*=12), European eel (*n*=1), minnow (*n*=7) and stone loach (*n*=12) were recorded via electro-fishing at site A12 on the Dinin River (**Figure 3.6**).

The swift-flowing site was a good quality salmonid nursery habitat (abundant refugia), supporting a medium density of juveniles. Spawning and holding habitat was of moderate quality. The site was of good value as a European eel habitat with abundant refugia, with a low density recorded. The high energy conditions precluded the presence of lamprey.

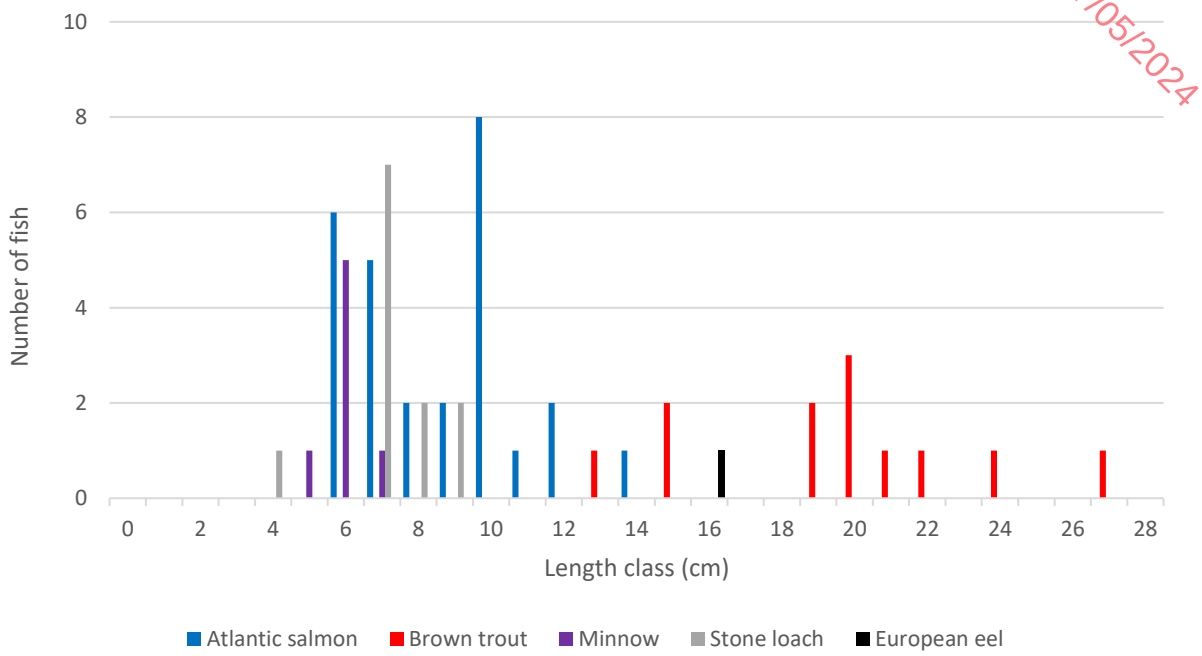

Figure 3.6 Length frequency distribution recorded via electro-fishing at site A12 on the Dinin River, August 2022

Plate 3.12 Representative image of site A12 on the Dinin River, August 2022 (upstream of bridge)

3.1.13 Site B1 – Seskin Upper Stream, Seskin Upper

No fish were recorded via electro-fishing at site B1 on the Seskin Upper Stream. The upland site was not of fisheries value given the very shallow nature, low summer flows and evident siltation pressures.

Plate 3.13 Representative image of site B1 on the Seskin Upper Stream, August 2022

3.1.14 Site B2 – Rathornan River, Coolnakisha

Three-spined stickleback (*n*=18) was the only fish species recorded via electro-fishing at site B2 the Rathornan River (**Figure 3.7**).

With the exception of stickleback (present in low densities), the site was not of fisheries value at the time of survey given very poor flows and shallow water. However, the site is known to support Atlantic salmon, brown trout and stone loach (Gordon et al., 2021b), presumably at higher water levels. Under such conditions, there would be some moderate suitability for salmonids and European eel. The upland characteristics of the site presented conditions inimical for lamprey establishment and none were recorded.

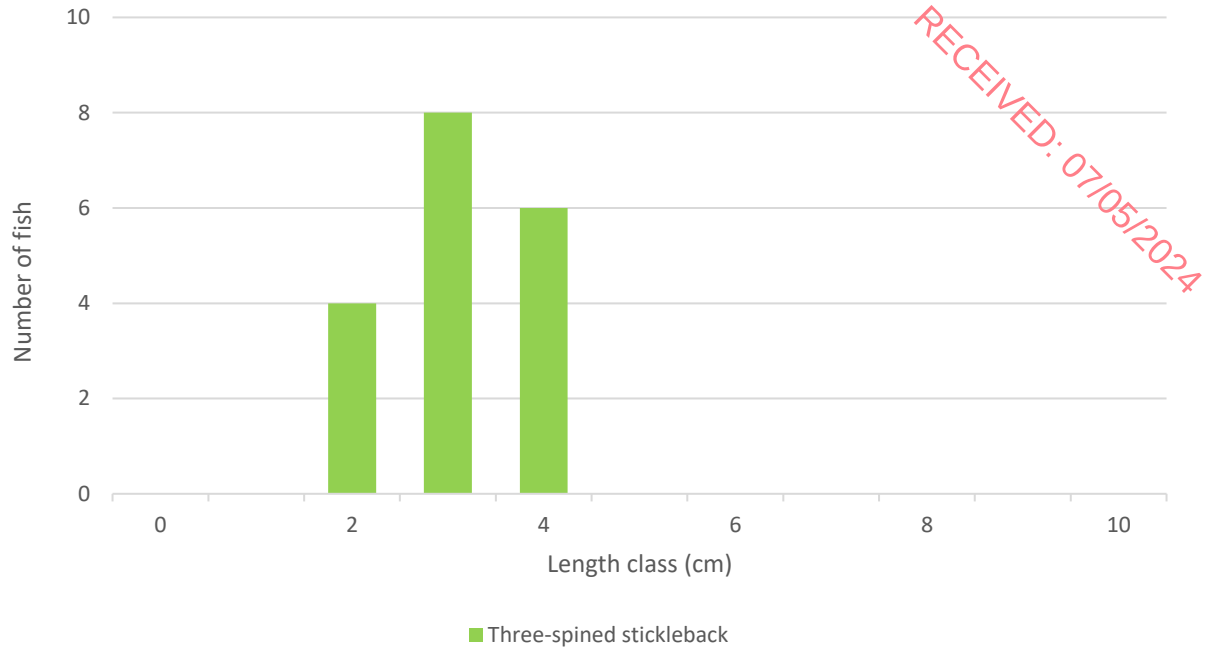

Figure 3.7 Length frequency distribution recorded via electro-fishing at site B2 on the Rathornan River, August 2022

Plate 3.14 Representative image of site B2 on the Rathornan River, August 2022

3.1.15 Site B3 – Rathornan River, River Barrow confluence

Three-spined stickleback (*n*=6), stone loach (*n*=4) and pike (*Esox lucius*) (*n*=1) were the only fish species recorded via electro-fishing at site B3 on the lowermost reaches of the Rathornan River (**Figure 3.8**).

The lowland depositing site had limited fisheries value at the time of survey due to the stagnant water, heavy siltation and historical drainage pressures. As per upstream (site B2), the fisheries value would improve under higher water levels. The site had some low value as a European eel nursery in its lower reaches due to the shading, deep pools and connection with the River Barrow (which supported far

superior fisheries habitat). The intermittent flows and paucity of soft sediment presented conditions inimical to lamprey (none recorded).

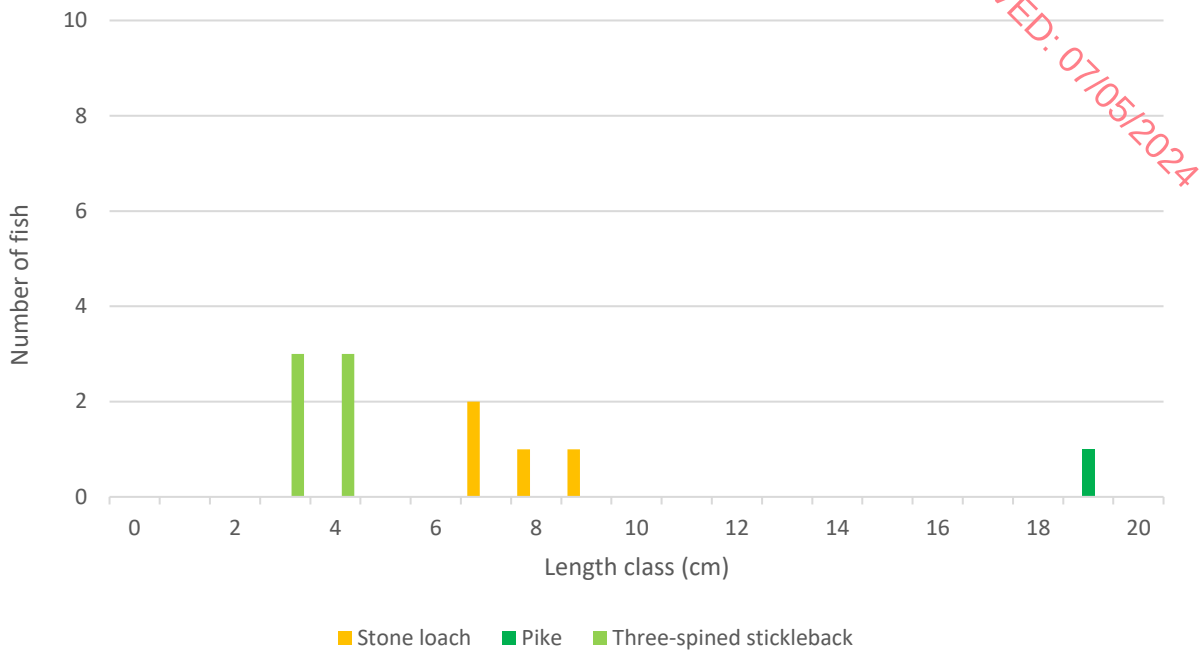

Figure 3.8 Length frequency distribution recorded via electro-fishing at site B3 on the Rathornan River, August 2022

Plate 3.15 Juvenile pike recorded at site B3 on the Rathornan River, August 2022, immediately upstream of the River Barrow confluence

3.1.16 Site B4 – River Barrow, downstream of Rathvinden Weir

Electro-fishing was not undertaken at site B4 on the River Barrow given prohibitive depths of >1.5m. As observed throughout the River Barrow, the weir area provided good quality nursery habitat for juvenile salmonids given ample broken water (riffle and glide) with cobble and boulder refugia. This area also provided some limited salmonid and lamprey spawning habitat. The site was an excellent

quality holding habitat for adult salmonids given abundant deep glide and pool. Depositing sand and silt in pool slacks and deep glide below the weir face offered good lamprey ammocoete burial habitat. The site was also of high value as a European eel habitat (abundant refugia) and coarse fish habitat for a range of species.

Plate 3.16 Representative image of site B4 on the River Barrow at Rathvinden Weir, August 2022

3.1.17 Site C1 – Parknakyle Stream, Parknakyle

No fish were recorded via electro-fishing at site C1 on the upper reaches of the Parknakyle Stream. The site was not of fisheries value given the low summer flows, very shallow nature and ephemeral character of downstream-connecting habitats (see 4.1.18 below).

Plate 3.17 Representative image of site C1 on the uppermost reaches of the Parknakyle River, August 2022 (semi-dry channel)

3.1.18 Site C2 – Parknakyle Stream, Coolnakeeran

Site C2 on the Parknakyle Stream was not of fisheries value given its dry, ephemeral nature and absence of aquatic habitats.

Plate 3.18 Representative image of site C2 on the uppermost reaches of the Parknakyle River, August 2022 (dry channel)

3.1.19 Site C3 – Oldleighlin Stream, Madlin Bridge

A total of 8 no. fish species were recorded via electro-fishing at site C3 on the lower reaches of the Oldleighlin Stream, namely brown trout (*n*=1), European eel (*n*=1), lamprey (*Lampetra* sp.) (*n*=3), minnow (*n*=11), stone loach (*n*=37), three-spined stickleback (*n*=5), roach (*Rutilus rutilus*) (*n*=1) and dace (*Leuciscus leuciscus*) (*n*=1) (**Figure 3.9**). This was the highest fish diversity recorded during the survey although abundances of most species were low.

The site was a poor quality salmonid habitat at the time of survey given poor flows, historical modifications and siltation pressures (single brown trout recorded). Spawning, nursery and holding habitat were all of poor quality. However, the fisheries value of the site is known to be significantly higher during higher flow periods (Gordon et al., 2021b; Delanty et al., 2017). Soft sediment areas were sub-optimal for larval lamprey due to poor flows but supported a low density of ammocetes. The site was of good value for European eel although only a single fish was recorded.

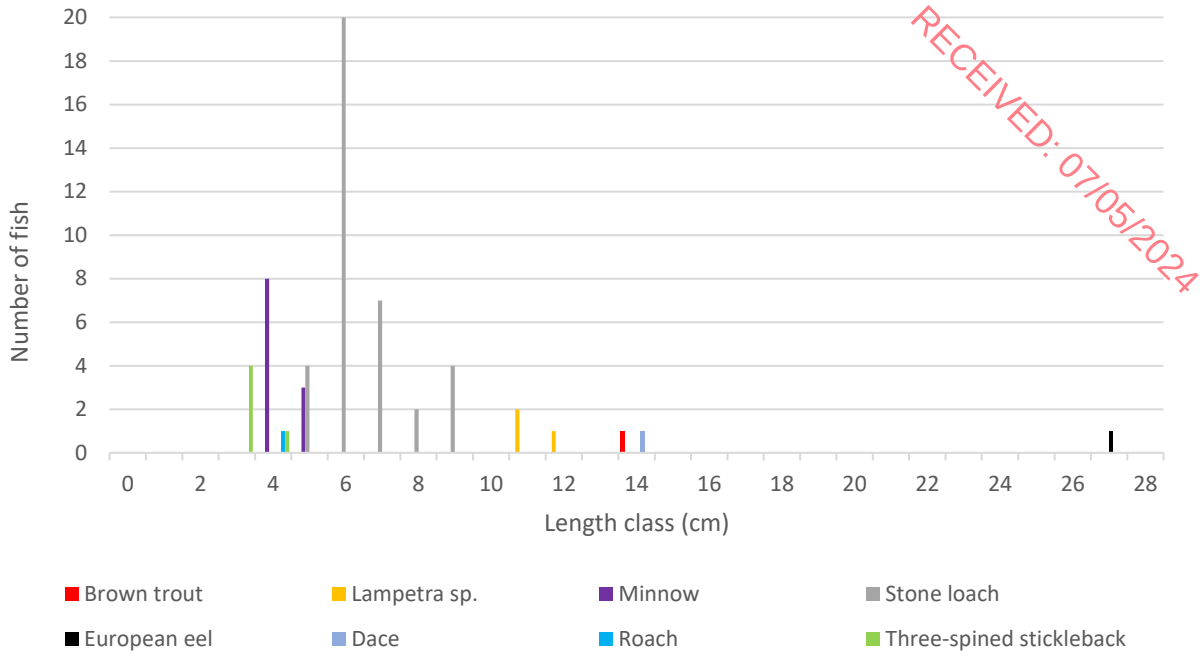

Figure 3.9 Length frequency distribution recorded via electro-fishing at site C3 on the Oldleighlin Stream, August 2022

Plate 3.19 Dace and brown trout recorded at site C3 on the lower reaches of the Oldleighlin Stream (Madlin River) at Madlin Bridge, August 2022

3.1.20 Site C4 – River Barrow, downstream of Rathellin Weir

Electro-fishing was not undertaken at site C4 on the River Barrow given prohibitive depths of >1.5m. The weir area, inclusive of broken riffle and glide downstream, provided good quality nursery habitat for juvenile salmonids. Juvenile (1+) Atlantic salmon were visible with larger brown trout also observed in fast glides. Moderate quality spawning habitat as present locally below the weir but compaction of substrata was evident, with moderate siltation. The site was an excellent quality holding habitat for adult salmonids given abundant deep glide and pool. Depositing sand and silt in pool slacks and deep glide below the weir face offered good lamprey ammocoete burial habitat with nearby spawning in

mixed gravels but this habitat was more localised. The site was also of high value as a European eel habitat (abundant refugia) and coarse fish habitat for a range of species.

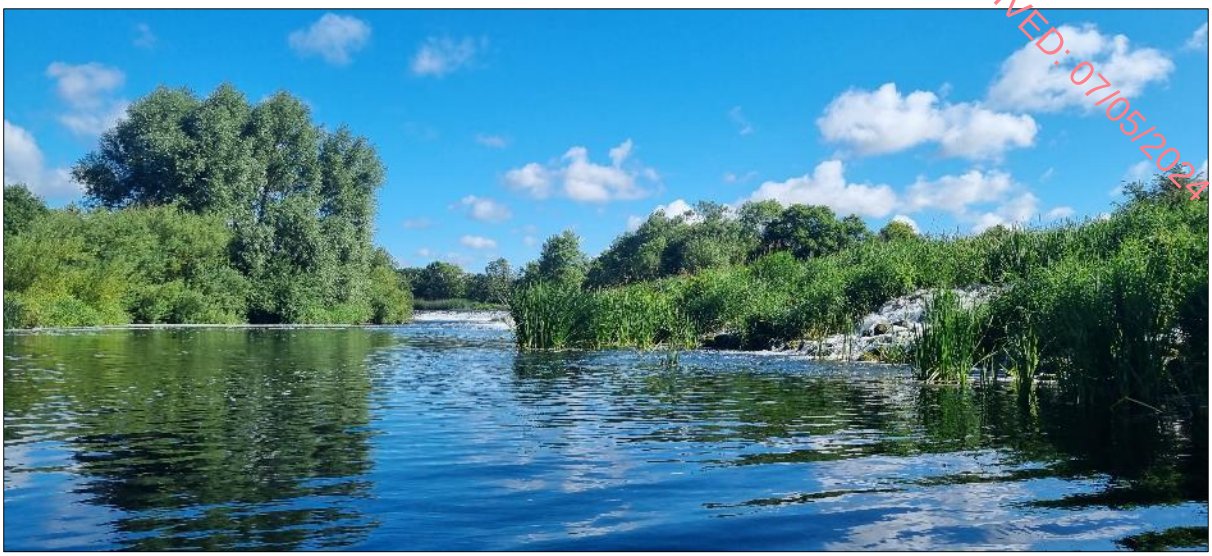

Plate 3.20 Representative image of site C4 on the River Barrow downstream of Rathellin Weir, August 2022

Table 3.1 Fish species densities per m^2 recorded at sites in the vicinity of the Proposed Wind Farm 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 (per m^2)									
				Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Three-spined stickleback	Stone loach	Minnow	Pike	Roach	Dace
A1	Unnamed stream	5	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A2	Unnamed stream	5	25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A3	Unnamed stream	n/a - dry channel		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
A4	Unnamed river	5	40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A5	Seskinrea Stream	5	35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A6	Seskinrea Stream	10	150	0.027	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A7	Knocknabranagh & Knockbaun River	10	300	0.053	**0.093**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A8	Agharue Stream	n/a - dry channel		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
A9	Dinin River	10	375	0.085	0.051	0.000	0.000	0.000	0.021	0.003	0.000	0.000	0.000
A10	Dinin River	10	300	**0.390**	0.013	0.000	0.000	0.000	0.003	0.003	0.000	0.000	0.000
A11	Dinin River	10	275	0.055	0.025	0.000	**0.004**	0.000	0.047	**0.062**	0.000	0.000	0.000
A12	Dinin River	10	280	0.096	0.043	0.000	**0.004**	0.000	0.043	0.025	0.000	0.000	0.000
B1	Seskin Upper Stream	5	80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B2	Rathornan River	5	100	0.000	0.000	0.000	0.000	**0.180**	0.000	0.000	0.000	0.000	0.000
B3	Rathornan River	5	110	0.000	0.000	0.000	0.000	0.055	0.036	0.000	**0.009**	0.000	0.000
B4	River Barrow	n/a - too deep for electro-fishing		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Site	Watercourse	CPUE (elapsed time)	Approx. area fished (m^2)	Fish density (per m^2)									
				Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Three-spined stickleback	Stone loach	Minnow	Pike	Roach	Dace
C1	Parknakyle Stream	n/a - dry channel		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
C2	Parknakyle Stream	n/a - dry channel		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
C3	Oldleighlin Stream	10	350	0.000	0.003	**2 per m^2**	0.003	0.014	**0.106**	0.031	0.000	**0.003**	**0.003**
C4	River Barrow	n/a - too deep for electro-fishing		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 3.2 Summary of fish species of higher conservation value and relative abundances (low, medium, high & very high) recorded via **electro-fishing** per survey site in the vicinity of the Proposed Wind Farm, August 2022

Site	Watercourse	Relative abundance				
		Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Unnamed stream	No fish recorded				
A2	Unnamed stream	No fish recorded				
A3	Unnamed stream	No fish recorded				
A4	Unnamed river	No fish recorded				
A5	Seskinrea Stream	No fish recorded				
A6	Seskinrea Stream	Low	Medium			
A7	Knocknabranagh & Knockbaun River	Medium	Medium			
A8	Agharue Stream	No fish recorded				
A9	Dinin River	High	Medium			Stone loach, minnow
A10	Dinin River	Very high	Low			Stone loach, minnow
A11	Dinin River	Medium	Low		Low	Stone loach, minnow
A12	Dinin River	Medium	Medium		Low	Stone loach, minnow
B1	Seskin Upper Stream	No fish recorded				
B2	Rathornan River					Three-spined stickleback
B3	Rathornan River					Three-spined stickleback, stone loach, pike
B4	River Barrow	n/a – too deep for electro-fishing (fisheries appraisal only)				
C1	Parknakyle Stream	No fish recorded				
C2	Parknakyle Stream	No fish recorded				
C3	Oldleighlin Stream		Low	Low	Low	Dace, minnow, roach, stone loach, three-spined stickleback
C4	River Barrow	n/a – too deep for electro-fishing (fisheries appraisal only)				

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 Inland Fisheries Acts 1959 to 2017, brown trout and coarse fish species have no legal protection in Ireland.

4. Discussion

With the exception of the Dinin River (a larger semi-natural upland river) and the River Barrow (large lowland river), the watercourses in the vicinity of the Proposed Wind Farm were typically small, modified channels which suffered from reduced summer flows. Many were higher gradient, upland eroding channels which are invariably unproductive and or unsuitable habitats in terms of fish (Wood & Budy, 2009; O'Grady, 2006; Amiro, 1993; Richardson, 1993). Historical drainage pressures (straightening & deepening), eutrophication and siltation have significantly reduced the quality and heterogeneity of aquatic habitats in the vicinity of the Proposed Project. Low summer water levels and ephemeral conditions are a characteristic of the Nore and Barrow sub-catchments (pers. obs.) and were evidently an issue for fish populations. Intermittent flows resulted in degraded fisheries habitat, particularly due to high thermal stress and agricultural (siltation and eutrophication) pressures. Poor hydromorphology due to historical drainage pressures including riparian tree removal evidently exacerbated the pressures of low summer flows. Diffuse siltation is another significant threat to salmonid populations, particularly in agricultural catchments (Evans et al., 2006) such as that of the landscape in the catchments of the study area. Sediment not only blocks interstitial spaces in substrata (colmation) and limits oxygen supply to salmonid eggs (required for healthy embryonic development & successful hatching) but can also smother substrata, thus reducing available spawning habitat and impact macro-invertebrate communities on which salmonids feed (Kelly-Quinn et al., 2020; Davis et al., 2018; Conroy et al., 2016; Cocchiglia et al., 2012; Louhi et al., 2008, 2011; Walling et al., 2003; Soulsby et al., 2001).

Low water levels also exacerbated known instream barriers within the wider survey area (**Plate 4.1; Figure 4.1**). Approximately half of the survey sites did not support fish at the time of survey (i.e. dry or semi-dry channels). Nevertheless, a total of ten fish species were recorded during the survey, namely Atlantic salmon, brown trout, *Lampetra* sp., European eel, stone loach, minnow, three-spined stickleback, pike, roach and dace (the latter two being invasive species).

Salmonids were present at 7 no sites in total, with Atlantic salmon present at six of these (i.e. sites A6, A7, A9, A01, A11 & A12). All sites supporting Atlantic salmon were situated in the Nore catchment (Dinin[South]_SC_010 sub-catchment), to the west of the Proposed Project. Atlantic salmon parr were recorded at all four survey sites on the Dinin River and these sites also supported the highest densities of the species. (**Table 3.1**). The Dinin, along with its tributary the Knocknabranagh & Knockbaun River (A7) and River Barrow, can be considered the most important salmonid habitats in the survey area. Sites A7[2] and A10 were particularly valuable salmonid nurseries. It should be noted that whilst no salmonids were recorded from 2 no. sites on the Rathornan River (Barrow tributary, east of the project), the watercourse is known to support Atlantic salmon and brown trout (Gordon et al., 2021b), including at the same location as survey site B2 in the current study. Their absence during this survey reflected the low summer flows observed and resulting influences on fish distribution. Although the Oldleighlin Stream (Madlin River) suffered from low summer flows and supported only a low density of brown trout during the current survey, the stream is noted as an important trout spawning habitat in context of the wider Barrow catchment (Delanty et al., 2017).

[2] This site supported the highest density of juvenile Atlantic salmon recorded in nationwide Water Framework Directive (WFD) surveys undertaken by Inland Fisheries Ireland (IFI) in 2021 (Corcoran et al., 2022)

Lamprey ammocoetes (*Lampetra* sp.) were only recorded from a single site during targeted electro-fishing across the 19 no. survey sites in the vicinity of the Proposed Wind Farm (**Table 3.1, 3.2**). A low density of ammocoetes (2 per m^2 of targeted habitat) was present at site C3 on the lowermost reaches of the Oldleighlin Stream near the River Barrow confluence. This highly restricted distribution reflected the upland, higher-energy nature of most of the surveyed sites which reduce 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). The patchy distribution of lamprey within the survey area has been previously noted, particularly in the Barrow catchment (Delanty et al., 2017; King, 2006). Furthermore, low summer flows and siltation pressures contributed to a reduction in the quality of habitat for larval (nursery) and adult lamprey (spawning), respectively. Larval lamprey dispersal and settlement is passive and entirely regulated by local, dynamic hydrographical (flow) regimes (Kelly & King, 2001; Malmqvist, 1983; Potter, 1980; Hardisty & Potter 1971) and distribution is often sporadic in watercourses which suffer from low summer flows and poor fluvial connectivity (such as several in the vicinity of the proposed project).

Despite widespread suitability, European eel were only recorded in low densities from sites A11 & A12 on the Dinin River and C3 on the Oldleighlin Stream (**Table 3.1, 3.2**). 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). As eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019), the paucity of eel observed in the Dinin[South]_SC_010 and Barrow_SC_110 river sub-catchments can be partly explained by the distance between the survey area and marine habitats (Chadwick et al., 2007) (c.80km nearest instream distance). The absence of eel from many physically suitable sites also likely reflects the high number of barriers to fish passage present in the Nore and Barrow catchments (**Figure 4.1**), as well as widespread low summer flow conditions which influence and restrict fish distribution.

Plate 4.1 Several significant instream barriers were present in the survey area, such as the weir at site A10 on the Dinin River (passable to salmonids under higher flows but likely impassable to *Lampetra* sp.)

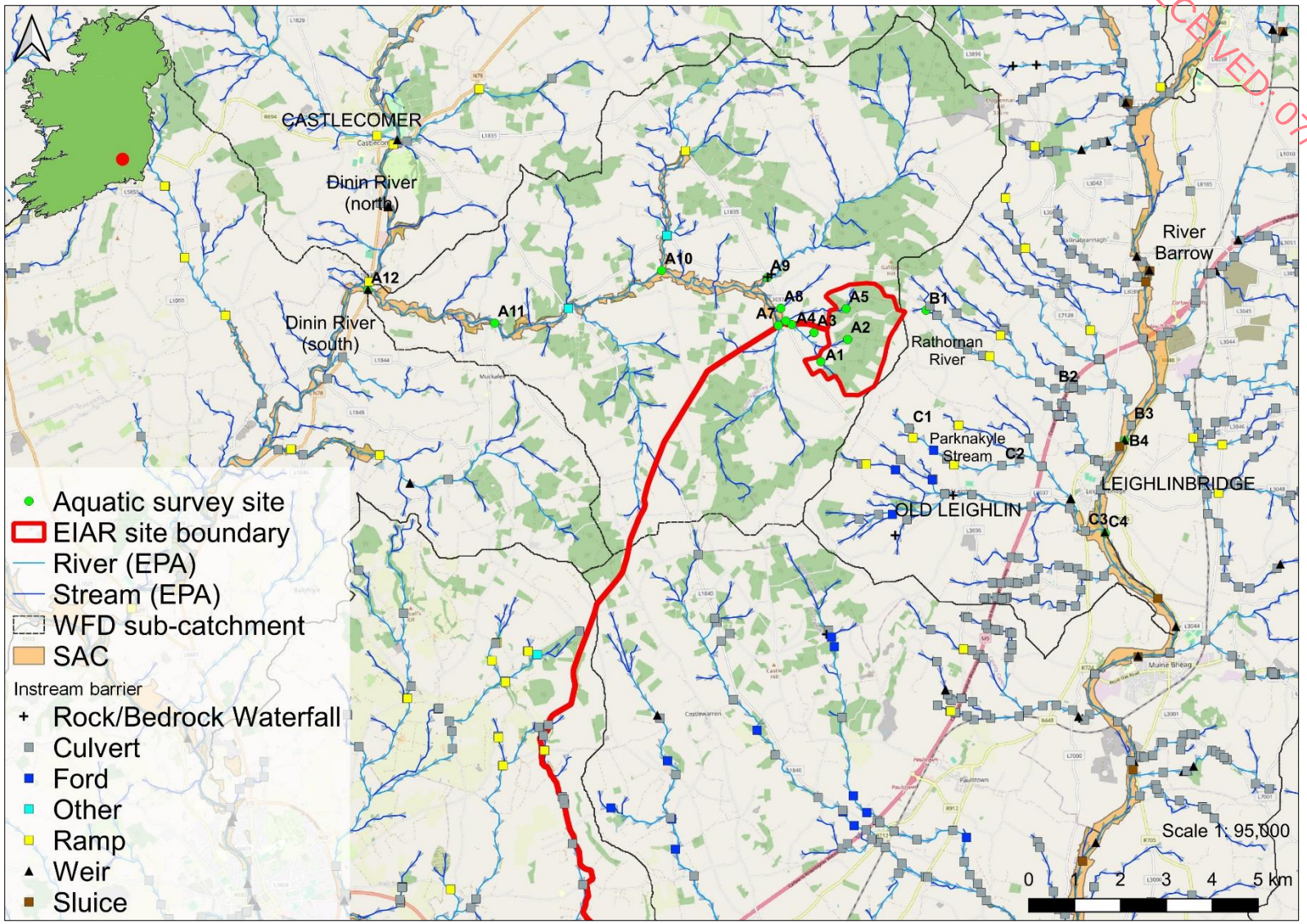

Figure 4.1 Overview of the known instream barriers in the vicinity of Proposed Wind Farm, Co. Carlow (source: AMBER & IFI's National Barriers Programme)

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8. Appendix B – Q-sample results (biological water quality)

Table 8.1 Macro-invertebrate Q-sampling results for sites A1, A2, A4, A5, A6, A7, A9, A10, A11 & A12, August 2022

Group	Family	Species	A1	A2	A4	A5	A6	A7	A9	A10	A11	A12	EPA class
Ephemeroptera	Heptageniidae	*Ecdyonurus dispar*			2		3	20		1	4	5	A
Plecoptera	Perlidae	*Perla bipunctata*									10		A
Ephemeroptera	Baetidae	*Alainites muticus*		2		1	4	57		1	3		B
Plecoptera	Leuctridae	*Leuctra hippopus*					2	3	2	6	11	13	B
Trichoptera	Cased caddis pupa	sp. indet.				2		1					B
Trichoptera	Limnephilidae	*Potamophylax cingulatus*				2							B
Trichoptera	Sericostomatidae	*Sericostoma personatum*									1		B
Odonata	Coenagrionidae	*Coenagrion* sp.		4									B
Ephemeroptera	Baetidae	*Baetis rhodani*	78	125	52		2	262	30	80	78	30	C
Ephemeroptera	Caenidae	*Caenis luctuosa*										1	C
Ephemeroptera	Ephemerellidae	*Serratella ignita*			1		4	13		2	7	13	C
Trichoptera	Hydropsychidae	*Hydropsyche instabilis*					2			1	3	21	C
Trichoptera	Hydropsychidae	*Hydropsyche siltalai*							1			5	C
Trichoptera	Philopotamidae	*Wormaldia occipitalis*				2					1		C
Trichoptera	Polycentropodidae	*Plectrocnemia conspersa*	1	4			1			1		1	C
Trichoptera	Polycentropodidae	*Polycentropus kingi*	1		2		13	4	7	6	9	23	C
Trichoptera	Rhyacophilidae	*Rhyacophila dorsalis*					3					3	C
Gastropoda	Planorbidae	*Ancylus fluviatilis*			1	6	2			1		5	C
Gastropoda	Tateidae	*Potamopyrgus antipodarum*									45	4	C
Crustacea	Gammaridae	*Gammarus duebeni*							8				C
Coleoptera	Dytiscidae	Dytiscidae larva	1	3			1		1	1			C
Coleoptera	Dytiscidae	*Dytiscus dimidiatus*		1									C
Coleoptera	Dytiscidae	*Ilybius fuliginosus*	3	1									C
Coleoptera	Dytiscidae	*Oreodytes sanmarkii*					7	2	3	4	1		C
Coleoptera	Dytiscidae	*Stictotarsus duodecimpustulatus*							1				C

Group	Family	Species	A1	A2	A4	A5	A6	A7	A9	A10	A11	A12	EPA class
Coleoptera	Elmidae	*Elmis aenea*	3	1	1	1	2	1		11	3	6	C
Coleoptera	Elmidae	*Esolus parallelepipedus*					1					1	C
Coleoptera	Elmidae	*Limnius volckmari*					2	2	1	2	3	4	C
Coleoptera	Halipliidae	*Haliplus ruficollis* group		2									C
Coleoptera	Hydraenidae	*Hydraena gracilis*									1		C
Coleoptera	Hydrophilidae	*Helophorus brevipalpis*		6									C
Coleoptera	Scirtidae	sp. indet.				1							C
Diptera	Ceratopogonidae	sp. indet.			1								C
Diptera	Chironomidae	Non-*Chironomus* spp.	5	10	2	7	6	58	3	5	1	1	C
Diptera	Culicidae	sp. indet.		1			2						C
Diptera	Pediciidae	*Dicranota* sp.				1				1			C
Diptera	Simuliidae	sp. indet.		6	13		4	243			1		C
Diptera	Tipuliidae	*Tipula* sp.									4		C
Hemiptera	Corixidae	Corixidae nymph	8	3						2			C
Hemiptera	Gerridae	Gerridae nymph	2				4	1					C
Hemiptera	Gerridae	*Gerris* sp.	5										C
Hemiptera	Hydrometridae	*Hydrometra stagnorum*					1						C
Hemiptera	Veliidae	*Velia caprai*			2								C
Hemiptera	Veliidae	Veliidae nymph		2	2				1				C
Arachnida	Hydrachnidiae	sp. indet.		2			2	1	3	5	5	7	C
Crustacea	Asellidae	*Asellus aquaticus*										1	D
Gastropoda	Lymnaeidae	*Ampullacaena balthica*										39	D
Diptera	Chironomidae	*Chironomus* spp.	15	53	1	2	4		5		1	8	E
Annelidae	Oligochaeta	sp. indet.										1	n/a
Abundance			**122**	**226**	**80**	**25**	**72**	**668**	**66**	**130**	**192**	**192**	
Q-rating			**Q3***	**Q2-3***	**Q3-4***	**Q3**	**Q3-4**	**Q3-4**	**Q3**	**Q3-4**	**Q4**	**Q3-4**	
WFD status			Poor	Poor	Mod	Poor	Mod	Mod	Poor	Mod	Good	Mod	

Table 8.2 Macro-invertebrate Q-sampling results for sites B1, B2, B3, B4, C1, C3 & C4, August 2022

Group	Family	Species	B1	B2	B3	B4	C1	C3	C4	EPA class
Ephemeroptera	Heptageniidae	*Ecdyonurus dispar*		2						A
Ephemeroptera	Heptageniidae	*Electrogena lateralis*					1			A
Ephemeroptera	Heptageniidae	*Heptagenia sulphurea*	2							A
Ephemeroptera	Baetidae	*Alainites muticus*	9	5			3			B
Ephemeroptera	Baetidae	*Cloeon simile*			5					B
Plecoptera	Leuctridae	*Leuctra hippopus*	7	14			3	1		B
Trichoptera	Glossosomatidae	*Agapetus fuscipes*		5						B
Trichoptera	Leptoceridae	*Athripsodes aterrimus*							5	B
Trichoptera	Leptoceridae	*Mystacides* sp.			1					B
Trichoptera	Leptoceridae	*Triaenodes bicolor*							1	B
Trichoptera	Limnephilidae	*Potamophylax cingulatus*	2							B
Trichoptera	Sericostomatidae	*Sericostoma personatum*	1	2			5		2	B
Odonata	Calopterygidae	*Calopteryx splendens*							5	B
Odonata	Coenagrionidae	*Coenagrion* sp.			7					B
Ephemeroptera	Baetidae	*Baetis rhodani*	6	77	114	16	6		11	C
Ephemeroptera	Caenidae	*Caenis rivulorum*		4						C
Ephemeroptera	Ephemerellidae	*Serratella ignita*		25		16			22	C
Trichoptera	Hydropsychidae	*Hydropsyche instabilis*							6	C
Trichoptera	Philopotamidae	*Philopotamus montanus*	2							C
Trichoptera	Polycentropodidae	*Plectrocnemia conspersa*	8	1			4			C
Trichoptera	Polycentropodidae	*Polycentropus kingi*		2		4				C
Trichoptera	Rhyacophilidae	*Rhyacophila dorsalis*							3	C
Gastropoda	Bithnyiidae	*Bithynia tentaculata*				7			39	C
Gastropoda	Lymnaeidae	*Lymnaea stagnalis*			2					C
Gastropoda	Physidae	*Physa fontinalis*			2	2		25	1	C

Group	Family	Species	B1	B2	B3	B4	C1	C3	C4	EPA class
Gastropoda	Planorbidae	*Planorbis planorbis*				2			4	C
Gastropoda	Tateidae	*Potamopyrgus antipodarum*			3			3		C
Crustacea	Gammaridae	*Gammarus duebeni*	41	1		117	64	1	6	C
Coleoptera	Dytiscidae	Dytiscidae larva			7			7		C
Coleoptera	Dytiscidae	*Nebrioporus depressus*			1			56		C
Coleoptera	Dytiscidae	*Oreodytes sanmarkii*		13						C
Coleoptera	Dytiscidae	*Stictotarsus duodecimpustulatus*		1						C
Coleoptera	Elmidae	*Brychius elevatus*				1		8		C
Coleoptera	Elmidae	*Elmis aenea*		5				4		C
Coleoptera	Elmidae	*Esolus parallelepipedus*		3						C
Coleoptera	Elmidae	*Limnius volckmari*		5	2			1		C
Coleoptera	Gyrinidae	*Gyrinus substriatus*			1					C
Coleoptera	Halipliidae	Halipliidae larva						1		C
Coleoptera	Halipliidae	*Haliplus ruficollis* group		1	4			2	1	C
Coleoptera	Hydraenidae	*Hydraena gracilis*		1						C
Coleoptera	Hydrophilidae	*Helophorus brevipalpis*						1		C
Diptera	Chironomidae	Non-*Chironomus* spp.	2	30	1		1	19		C
Diptera	Limoniidae	*Antocha sp.*	2						2	C
Diptera	Pediciidae	*Dicranota* sp.		6			16			C
Diptera	Tipuliidae	*Tipula* sp.						5		C
Hemiptera	Corixidae	Corixidae nymph			44	39		5	24	C
Hemiptera	Corixidae	*Siagara* sp.				3			26	C
Hemiptera	Gerridae	Gerridae nymph			9					C
Hemiptera	Gerridae	*Gerris* sp.			1			4	1	C
Hemiptera	Hydrometridae	*Hydrometra stagnorum*			7			2		C
Hemiptera	Notonectidae	Notonectidae nymph			2					C
Hemiptera	Veliidae	*Velia caprai*	1							C

Group	Family	Species	B1	B2	B3	B4	C1	C3	C4	EPA class
Hemiptera	Veliidae	Veliidae nymph	2				2			C
Arachnida	Hydrachnidiae	sp. indet.		2	3			1		C
Crustacea	Asellidae	*Asellus aquaticus*		1	46	64		22	32	D
Gastropoda	Lymnaeidae	*Ampullacaena balthica*		1	25			1	2	D
Gastropoda	Sphaeriidae	sp. indet.							7	D
Megaloptera	Sialidae	*Sialis lutaria*			25	1		1		D
Hirudinidae	Glossiphoniidae	sp. indet.		1	1					D
Diptera	Chironomidae	*Chironomus* spp.	2		237	1	2	18	1	E
Annelidae	Oligochaeta	sp. indet.	1	2					1	n/a
Abundance			**88**	**210**	**550**	**273**	**107**	**188**	**202**	
Q-rating			**Q3-4**	**Q3-4**	**Q2-3***	**Q3**	**Q3-4**	**Q3***	**Q3**	
WFD status			Mod	Mod	Poor	Poor	Mod	Poor	Poor	

*tentative Q-rating due to poor flows and or lack of suitable riffle areas for sampling (Toner et al., 2005)

9. Appendix C – eDNA analysis lab report

SureScreen Scientifics

RECEIVED: 07/05/2024

Folio No: E15233
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: 08/08/2022
Date results reported: 16/08/2022
Matters affecting result: None

TARGET SPECIES: Crayfish plague *(Aphanomyces astaci)*

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK601	C4 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK618	B3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK622	A10 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK623	C3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK627	A12 Old Leighlin	-	Pass	Pass	Pass	Positive	10/12

Forensic Scientists and Consultant Engineers
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Company Registration No. 08950940

SureScreen Scientifics

RECEIVED: 07/05/2024

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

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK601	C4 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK618	B3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK622	A10 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK623	C3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK627	A12 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12

TARGET SPECIES: White-clawed crayfish *(Austropotamobius pallipes)*

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK601	C4 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK618	B3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK622	A10 Old Leighlin	-	Pass	Pass	Pass	Positive	1/12
FK623	C3 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12
FK627	A12 Old Leighlin	-	Pass	Pass	Pass	Negative	0/12

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

Reported by: **Chelsea Warner** Approved by: **Gabriela Danickova**

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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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