APPENDIX 6-3

AQUATIC BASELINE REPORT

Aquatic baseline report for Borrisbeg wind farm, Co. Tipperary

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 Borrisbeg wind farm project. The following report provides a baseline assessment of the aquatic ecology including fisheries and biological water quality, as well as protected aquatic species and habitats in the vicinity of the proposed Borrisbeg wind farm, located approximately 3km north-east of Templemore, Co. Tipperary.

Undertaken on a catchment-wide scale, the baseline surveys focused on aquatic habitats and species of high conservation value. This included surveys for white-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussel (*Margaritifera margaritifera*) (eDNA only), macro-invertebrates (biological water quality), macrophytes & aquatic bryophytes, aquatic invasive species, fish of conservation value and Annex I Habitats that could utilise the watercourses in the vicinity of the proposed project (**Figure 2.1**). Aquatic surveys were undertaken in September 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 project were considered as part of the current baseline. A total of *n*=13 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 Shanakill River (EPA code: 16S34), River Suir (16S02), Eastwood River (16E17), Farranacahill Stream (16F69) and unnamed tributary, Clonmore Stream (16C11) and the Adamstown River (16A69) (**Table 2.1**). The *n*=13 aquatic survey sites were located within the Suir_SC_010 river sub-catchment. The proposed wind farm and associated infrastructure were not located within a European site.

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

2.2 Aquatic site surveys

Aquatic surveys of the watercourses within the vicinity of the proposed wind farm project were conducted on 28th and 29th September 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*=3 strategically chosen riverine locations within the vicinity of the project. This holistic approach informed the overall aquatic ecological evaluation of each site in context of the proposed project and ensured that any habitats and species of high conservation value would be detected to best inform mitigation for the wind farm project.

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

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

2.3 Fish stock assessment (electro-fishing)

A single anode Smith-Root LR24 backpack (12V DC input; 300V, 100W DC output) was used to electro-fish sites on watercourses in the vicinity of the proposed Borrisbeg wind farm in September 2022, following notification to Inland Fisheries Ireland, under the conditions of a Department of the Environment, Climate and Communications (DECC) licence. Electro-fishing was undertaken at all wetted riverine survey sites. Therefore, a total of *n*=12 sites were surveyed via electro-fishing given that site C2 was dry at the time of survey (**Table 2.1, Figure 2.1; Appendix A**). The survey was undertaken in accordance with best practice (CEN, 2003; CFB, 2008) and Section 14 licencing requirements.

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

Table 2.1 Location of *n*=13 aquatic survey sites in the vicinity of Borrisbeg wind farm, Co. Tipperary (* denotes eDNA sampling)

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Shanakill River	16S34	Skehanagh	614869	678187
A2	Shanakill River	16S34	Skehanagh	613890	676753
B1	River Suir	16S02	Knockanroe Bridge	613482	677642
B2	River Suir	16S02	Knockanroe	613504	676677
B3*	River Suir	16S02	Ballycahill	613751	674344
B4	River Suir	16S02	Knocknageragh Bridge (R433)	613043	672550
B5*	River Suir	16S02	Loughmore Bridge	611761	667377
C1	Eastwood River	16E17	College Bridge, N62	612058	675462
C2	Unnamed stream	n/a	Knockanroe	612603	675689
C3	Farranacahill Stream	16F69	Knockanroe	612942	675703
C4*	Eastwood River	16E17	Ballycahill	612749	674284
D1	Clonmore Stream	16C11	L7039 road crossing, Clonmore	614585	674055
E1	Adamstown River	16A69	L3230 road crossing, Gortacurra	609871	674499

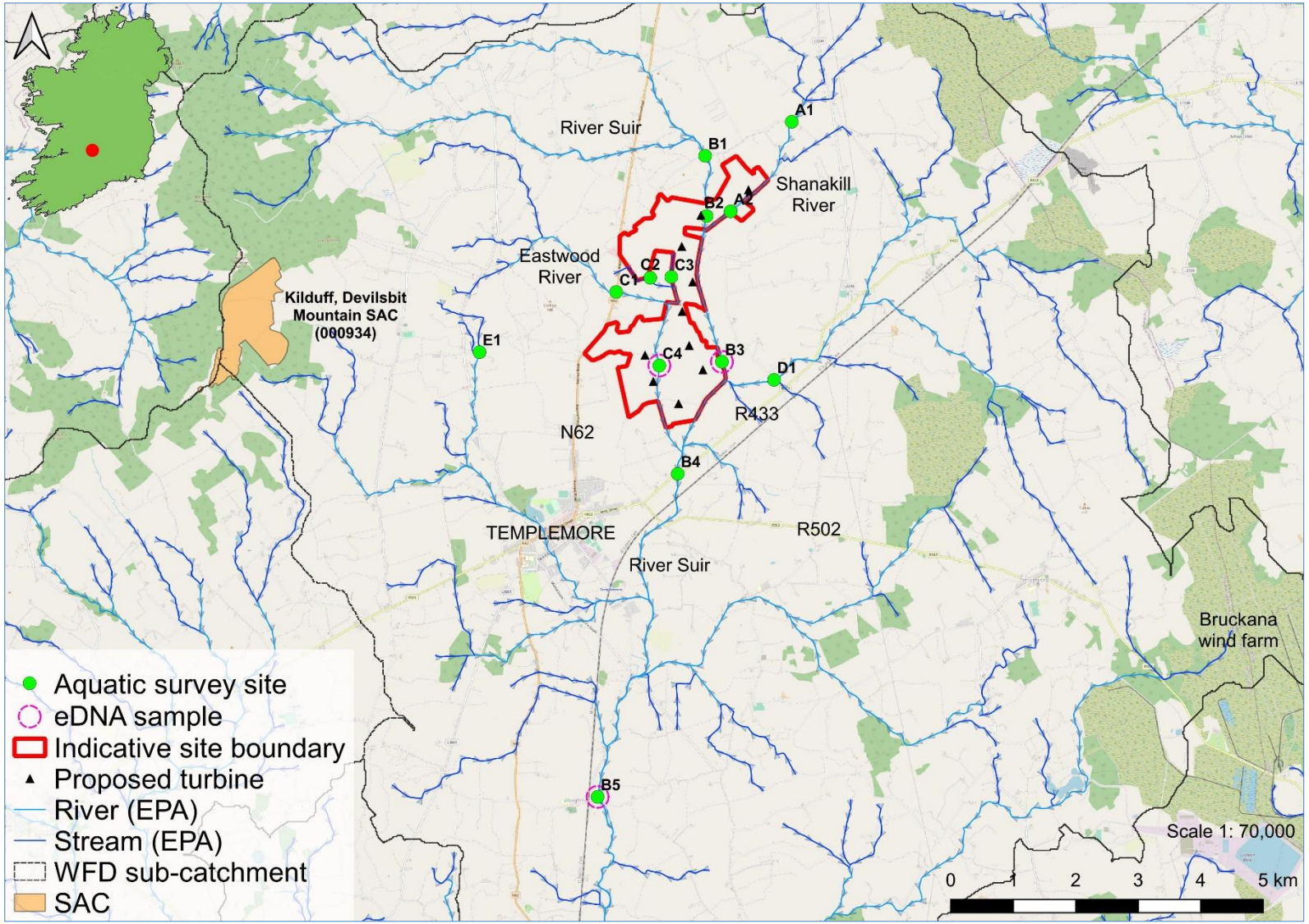

Figure 2.1 Overview of the *n*=13 aquatic survey site locations in the vicinity of Borrisbeg wind farm, Co. Tipperary

2.4 White-clawed crayfish survey

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

2.5 Otter signs

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

2.6 eDNA analysis (including freshwater pearl mussel)

To validate habitat suitability appraisal and to detect potentially cryptically low populations of freshwater pearl mussel within the study area, *n*=3 composite water samples were collected from the River Suir and Eastwood River and analysed for freshwater pearl mussel eDNA (**Figure 2.1**). This would help validate presence and or absence of the species given that no data was available on the status of pearl mussel in these rivers. Samples were also analysed for white-clawed crayfish and crayfish plague. The water samples were collected on 29th September 2022, with the sites strategically chosen to maximise longitudinal (instream) coverage within the catchment (i.e. facilitating a greater likelihood of species detection).

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

2.7 Biological water quality (Q-sampling)

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

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

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

2.8 Macrophytes and aquatic bryophytes

Surveys of the macrophyte and aquatic bryophyte community were conducted by instream wading at each of the *n*=13 riverine sites, with specimens collected (by hand, sweep nets or via grapnel) for on-site identification. An assessment of the aquatic vegetation community helped to identify any rare macrophyte species (Flora Protection Order or Wyse-Jackson et al., 2016) or habitats corresponding to the Annex I habitats, e.g., 'Water courses of plain to montane levels, with submerged or floating vegetation of the *Ranunculion fluitantis* and *Callitricho-Batrachion* (low water level during summer) or aquatic mosses [3260]' (more commonly referred to as 'floating river vegetation').

2.9 Aquatic ecological evaluation

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

2.10 Biosecurity

A strict biosecurity protocol following IFI (2010) and the Check-Clean-Dry approach was adhered to during surveys for all equipment and PPE used. Disinfection of all equipment and PPE before and after use with Virkon™ was conducted to prevent the transfer of pathogens or invasive propagules between

survey sites. Surveys were undertaken at sites in a downstream order to minimise the risk of upstream propagule mobilisation. Particular cognisance was given towards preventing the spread or introduction of crayfish plague given the known distribution of white-clawed crayfish in the wider survey area and previous outbreaks of crayfish plague in the Suir catchment. 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. Receiving environment

3.1 Borrisbeg wind farm catchment and survey area description

The proposed Borrisbeg wind farm project is located in a lowland area within the townlands of Borrisbeg, Ballycahill, Graffin, Knockanroe, Eastwood and Skehenagh, approximately 3km north-east of Templemore, Co. Tipperary (**Figure 2.1**). The proposed wind farm site is within the South-eastern River Basin District and within hydrometric area 16 (Suir) within the Suir_SC_010 river sub-catchment. The proposed wind farm site is drained by the Shankill River (16S34), River Suir (6S02), Eastwood River (16E17), Farranacahill Stream (16F69) and unnamed tributary and the Adamstown River (16A69) (**Table 2.1**), all of which flow in a largely north to south direction. The survey area also overlapped with the Suir *Margaritifera* sensitive area (a catchment with previous records of *Margaritifera*, but current status unknown). However, the only known extant population in the wider Suir catchment is located in the Clodiagh River (Ross, 2006), a sub-catchment with no hydrological connectivity to the proposed wind farm.

The watercourses and aquatic surveys sites in the vicinity of Borrisbeg wind farm are typically small, historically modified lowland depositing channels (FW2; Fossitt, 2000). Predominantly, the watercourses flow over areas of Tournaisian limestone (Geological Survey of Ireland data). Land use practices in the wider survey area is exclusively pasture (CORINE 231).

3.2 Fisheries asset of the survey area

The River Suir (16S02) rises in Devilsbit Mountain some 8km north-west of Templemore and flows in a southerly direction through Templemore, Thurles, Cahir, Clonmel and Carrick-on Suir before joining the River Nore near Cheekpoint, Co. Waterford. The Suir has the largest quantity of accessible fluvial salmonid habitat in the country (8.8 million m^2) or 7.8% of the national total (McGinnity et al., 2003). The Suir is one of Ireland's premier recreational angling venues for both Atlantic salmon (*Salmo salar*) (Grilli et al., 2021) and brown trout (*Salmo trutta*) (O'Reilly, 2009). The Suir was not meeting its conservation limit for Atlantic salmon in 2022 (Gargan et al., 2022). The growth of trout in the Suir has been assessed as 'fast' according to the classification scheme of Kennedy and Fitzmaurice (1971) (Kelly et al., 2011).

At Knocknageragh Bridge (survey site B4), the river supports brown trout, Atlantic salmon, European eel (*Anguilla anguilla*), pike (*Esox lucius*), stone loach (*Barbatula barbatula*), gudgeon (*Gobio gobio*), invasive roach (*Rutilus rutilus*), three-spined stickleback (*Gasterosteus aculeatus*) and lamprey (*Lampetra* sp.) (Kelly et al., 2011, 2010; O'Connor, 2007; IFI data[1]). Further downstream, the middle and lower reaches of the river are also known to support minnow (*Phoxinus phoxinus*) and invasive dace (*Leuciscus leuciscus*) (Matson et al., 2019; Kelly et al., 2011).

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

[1] Inland Fisheries Ireland data for Water Framework Directive Fish Ecological Status 2008-2021. Available at https://opendata-ifigis.hub.arcgis.com/datasets/IFIgis::water-framework-directive-fish-ecological-status-2008-2021/

3.3 Protected aquatic species

A comprehensive desktop review of available data (NPWS, NBDC & BSBI data) for 10km grid squares containing and adjoining the project (i.e. S05, S07, S15, S17 & S18) identified records for a low number of rare and or protected aquatic species within the vicinity of the proposed wind farm.

A large number of records for white-clawed crayfish were available for the S05, S15 and S16 grid squares (>70 records; NPWS data). These ranged from 1986 to 2008 with records for a number of rivers including the Black River, Rossestown River, Drish River, Clodiagh River and River Suir (**Figure 3.1**). However, no records were available in the vicinity of the proposed wind farm (most records in the vicinity of Thurles), with the nearest record present at Penane Bridge located between survey sites B4 and B5.

A low number of otter (*Lutra lutra*) records were available for the 10km grid squares, including records on the River Suir at Knocknageragh Bridge (survey site B4) (from 2010; NPWS data). With the exception of this location, no records were available in vicinity if the proposed wind farm (**Figure 3.1**).

A single historical record for river lamprey (*Lampetra fluviatilis*) was available for the Aughrim River (grid square S17), with historical records for brook lamprey (*Lampetra planeri*) available for the Ollatrim River (S07). Neither of these rivers have hydrological connectivity to the proposed wind farm.

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 project. Only recent water quality is summarised below. There was no contemporary EPA biological monitoring data available for a number of the surveyed watercourses, namely the Shanakill River (16S34), Eastwood River (16E17), Farranacahill Stream (16F69) and unnamed tributary, and the Adamstown River (16A69). Please note that biological water quality analysis (Q-sampling) was undertaken as part of this survey, with the results presented in the **section 4** and **Appendix A** of this report.

3.4.1 River Suir

A number of contemporary EPA biological monitoring stations were located on the River Suir (16S02) in the vicinity of the proposed wind farm. Upstream of the site boundary, the river achieved **Q3-4 (moderate status)** at Knockanroe Bridge (station RS16S020100, survey site B1) in 2020. Downstream of the site boundary, the river also achieved **Q3-4 (moderate status)** at Knocknageragh Bridge (station RS16S020200, survey site B4) and Penane Bridge (Station RS16S020300) in 2020. Whilst the status was elevated to **Q4 (good status)** at Rossestown Bridge (station RS16S020500) in 2020, downstream of Thurles the river consistently achieved Q3-4 (moderate status) at numerous locations in 2020 (EPA data).

In the vicinity of the proposed wind farm, the Suir_020 and Suir_030 river waterbodies achieved poor status and moderate status in the 2016-2021 period and were considered 'not at risk' (due to WWTP upgrades) and 'at risk' of achieving target good status water quality (WFD Risk 3rd cycle), respectively. Agriculture is considered a major pressure in the Suir_020 river waterbody (EPA, 2019).

3.4.2 Clonmore Stream

As single contemporary EPA biological monitoring station was located on the Clonmore Stream (16C11). The river achieved **Q3-4 (moderate status)** at station RS16C111000 (survey site D1) in 2020.

The Clonmore Stream (Suir)_010 river waterbody (containing the Clonmore Stream) achieved moderate status in the 2016-2021 period and was considered 'at risk' of not achieving target good status water quality (WFD Risk 3rd cycle). Peat extraction is the major water quality threat on this watercourse (EPA, 2019).

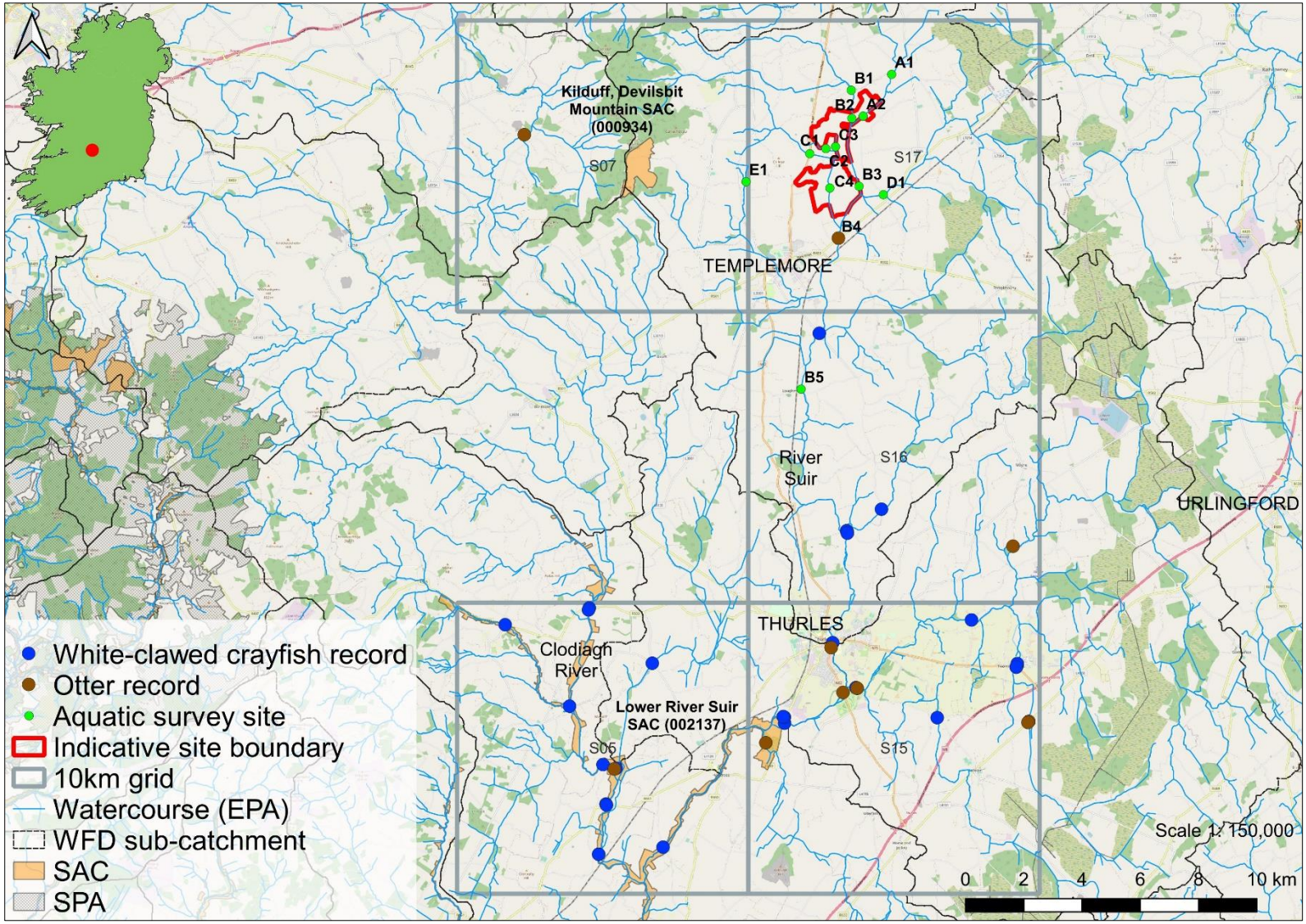

Figure 3.1 White-clawed crayfish (> year 2000) and otter records (>2010) in the vicinity of the proposed Borrisbeg wind farm (source: NPWS & NBDC data)

4. Results of aquatic surveys

The following section summarises each of the *n*=13 survey sites in terms of aquatic habitats, physical characteristics and overall value for fish, white-clawed crayfish and macrophyte/aquatic bryophyte communities. Biological water quality (Q-sample) results are also summarised for each riverine sampling site (*n*=12) and in **Appendix B**. Habitat codes are according to Fossitt (2000). Scientific names are provided at first mention only. Sites were surveyed in September 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 – Shanakill River, Skehanagh

Site A1 was located on the Shanakill River (EPA code: 16S34) at a local road crossing c.1km upstream of the proposed site boundary. The small lowland depositing watercourse (FW2) had been extensively straightened and deepened historically, with resulting poor hydromorphology. The river suffered from low seasonal flows at the time of survey. The river averaged 2m wide and 0.2-0.4m deep in a trapezoidal channel with bankfull heights of up to 2m. The profile comprised slow-flowing depositional glide with occasional small pool and only very localised riffle. A heavily silted, rendered bridge apron was also present. The substrata were dominated by boulder and cobble with localised beds of mixed gravels. However, these were exposed to very heavy levels of siltation, with abundant deep organic-rich silt deposits throughout. Given very high riparian shading, macrophyte growth was limited to localised fool's watercress (*Apium nodiflorum*), watercress (*Nasturtium officinale*) and water mint (*Mentha aquatica*) in open areas with common duckweed (*Lemna minor*) throughout. Branched bur-reed (*Sparganium erectum*) and brooklime (*Veronica beccabunga*) were present but rare overall. Aquatic bryophytes to limited to very occasional *Rhynchostegium riparioides* on larger boulder in riffle zones. Large woody debris (LWD) was abundant throughout. Aside from a short section adjoining the bridge, the river was very heavily tunnelled with dense hedgerows/treelines of ash (*Fraxinus excelsior*), hawthorn (*Crataegus monogyna*), sycamore (*Acer psuedoplatanus*), grey willow (*Salix cinerea*), elder (*Sambucus nigra*) and abundant bramble scrub (*Rubus fruticosus* agg.). The site was bordered by improved pasture (GA1) with narrow riparian buffers and abundant livestock poaching.

Three-spined stickleback (*Gasterosteus aculeatus*) and lamprey (*Lampetra* sp.) were the only fish species recorded via electro-fishing at site A1 (**Appendix A**). The site was of poor value for salmonids given gross siltation, historical modifications and poor flows. No salmonids were recorded which reflected the very poor spawning and poor nursery habitat present. Some holding habitat for adult salmonids was present. Whilst soft sediment accumulations were abundant, these were typically either flocculent or clay-dominated, and supported a very low density of *Lampetra* sp. ammocoetes. Lamprey spawning habitat was present but highly localised and significantly compromised by siltation. Despite some low suitability for European eel and white-clawed crayfish, none were recorded. A regular otter spraint site was recorded on the northern bridge ledge (ITM 614866, 678191) (this contained fish remains only).

The biological water quality (Q sample) was calculated as of **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 presence of *Lampetra* sp. lamprey and otter, the aquatic ecological evaluation of site A1 was of **local importance (higher value) (Table 4.4).**

Plate 4.1 Representative image of site A1 on the Shanakill River upstream of the site boundary, September 2022

4.1.2 Site A2 – Shanakill River, Skehanagh

Site A2 was located on the Shanakill River (16S34) at a ford crossing approx. 1.8km downstream of site A1. As per upstream, the small lowland depositing watercourse (FW2) had been extensively straightened and deepened historically, with resulting very poor hydromorphology. The river suffered from low seasonal flows at the time of survey with only a slight flow observed in shallow glide habitat. Riffle areas were absent. The river averaged 2-2.5m wide and 0.2-0.3m deep in a trapezoidal channel with bankfull heights of up to 2m. The substrata in vicinity of the ford crossing were dominated by compacted cobble and small boulder with localised interstitial mixed gravels. Soft sediment accumulations were occasional along channel margins. The channel was heavily silted elsewhere. The site was very heavily tunnelled and vegetated with abundant water mint and fool's watercress instream. Brooklime was rare. Reed canary grass (*Phalaris arundinacea*) was abundant along the margins and often encroached instream. Aquatic bryophytes were not recorded. Filamentous algae were observed throughout (>5% cover), indicating enrichment pressures. With the exception of the ford crossing, the small channel was very heavily tunnelled (95% covered) throughout with abundant hawthorn, great willowherb (*Epilobium hirsutum*), dog rose (*Rosa canina*) and bramble scrub, in addition to scattered willow and elder. The site was bordered by arable crops (BC1) and improved pasture (GA1).

Brown trout (*Salmo trutta*) and three-spined stickleback (*Gasterosteus aculeatus*) were the only fish species recorded via electro-fishing at site A2 (**Appendix A**). The site was of poor value for salmonids given heavy siltation, historical modifications and poor flows. However, the site supported a very low density of brown trout, with a single juvenile and small adult recorded via electro-fishing. Three-spined stickleback were also present in low densities. The site provided moderate quality (at best) salmonid nursery and spawning habitat in the upstream vicinity of the ford crossing. Soft sediment accumulations were of poor suitability for larval lamprey given the flocculent nature and poor flows. The river at this location was also of poor suitability for European eel and white-clawed crayfish (i.e. poor bed conditions and hydromorphology) and neither species was recorded. No otter signs were recorded in vicinity of the site which likely relates to the small size of the channel and poor foraging opportunities.

The biological water quality (Q sample) was calculated as of **Q3 (poor status)** (**Appendix B**). However, it should be noted that this was a tentative rating given poor flows and an absence of suitable riffle areas for sampling (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 presence of a brown trout population, the aquatic ecological evaluation of site A2 was of **local importance (higher value) (Table 4.4).**

Plate 4.2 Representative image of site A2 on the Shanakill River, September 2022

4.1.3 Site B1 – River Suir, Knockanroe Bridge

Site B1 was located on the upper reaches of the River Suir (16S02) at a local road crossing, approx. 0.8km upstream of the proposed wind farm boundary. The swift-flowing lowland depositing river (FW2) had been straightened locally and deepened throughout, with steep (near vertical) trapezoidal banks of up to 2.5m in height. The river averaged 3-4m wide and 0.2-0.4m deep with deeper glide and pool predominating upstream of the bridge (to 1m in depth). The profile was dominated by glide

habitat with occasional riffle and pool. The substrata comprised compacted cobble and boulder with localised small interstitial beds of finer gravels and sand. Beds of coarser gravels were present but highly localised. Soft sediment accumulations were shallow and flocculent, where present along channel margins. Siltation was moderate overall (plumes underfoot). Macrophyte coverage was low with only occasional fool's watercress and watercress along channel margins and on occasional exposed cobble bars. Branched bur-reed was present but rare. Aquatic bryophyte coverage was also low with localised *Leptodictyum riparium* and rare *Cinclidotus fontinaloides*. Floc[2] and filamentous algae were present (2% cover), indicating enrichment. The river channel was shaded by mature treelines of ash, grey willow, crack willow (*Salix fragilis*), dog rose and hawthorn that had become invasive of the banks due to historical channel and riparian clearance. Bramble scrub was locally abundant with localised tunnelling downstream of the bridge. The survey site was bordered by improved pasture (GA1) (some of which had been re-seeded).

Atlantic salmon (*Salmo salar*), brown trout and stone loach (*Barbatula barbatula*) were recorded via electro-fishing at site B1 (**Appendix A**). The site was of high value for salmonids, supporting healthy populations of mixed cohort Atlantic salmon and brown trout. The site was of most value as a salmonid nursery and holding habitat. Good quality salmonid and lamprey spawning habitat was present in the River Suir at Knockanroe Bridge albeit localised and compromised by compaction of substrata with excessive siltation. Whilst some soft sediment accumulations were present, these were superficial/flocculent in nature and did not support lamprey ammocoetes. Despite some good suitability for both European eel and white-clawed crayfish, none were recorded present. An otter old otter spraint site was recorded on the bridge's mammal underpass (containing fish remains) (ITM 613480, 677639).

The biological water quality (Q sample) was calculated as of **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) and high-quality salmonid habitat, in addition to utilisation by otter, the aquatic ecological evaluation of site B1 was of **local importance (higher value) (Table 4.4).**

[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)

Plate 4.3 Representative image of site B1 on the River Suir at Knockanroe, September 2022 (facing downstream from bridge illustrating tunnelling of the channel)

4.1.4 Site B2 – River Suir, Knockanroe

Site B2 was located on the River Suir (16S02) at a farm access bridge crossing, approx. 1km downstream of site B1 (i.e. within the proposed site boundary). The river had been straightened and deepened throughout, with steep (near vertical) trapezoidal banks of up to 2.5m in height. The river averaged 4-6m wide and 0.1-0.3m deep with deeper glide and pool (to 1m in depth) present locally. The profile featured shallow glide and riffle habitat with very localised (rare) pool. The substrata were dominated by mixed gravels with abundant beds of sand and soft sediment accumulations. Cobble (mostly small) dominated in faster-flowing areas. Boulder was rare overall. Siltation was moderate (plumes underfoot). The macrophyte community was dominated by fool's watercress which was frequent on exposed bars and soft sediment areas. Water crowfoot (*Ranunculus* sp.) was frequent (small beds) with occasional watercress, water mint and rare brooklime. Given finer, more mobile substrata, aquatic bryophyte coverage was low with occasional *Leptodictyum riparium*. *Pellia* sp. liverwort was locally frequent on exposed muddy areas of bank and the inundation zones of the channel margins supported narrow fringes of reed canary grass. The riparian zone and steep banks supported abundant bramble scrub (WS1) with mature, dense hedgerows/treelines of hawthorn, ash, grey willow, dog rose and elder. The River Suir at survey site B2 was bordered by arable crops (BC1) and improved pasture (GA1).

A total of five fish species were recorded at site B2 via electro-fishing, namely Atlantic salmon, brown trout, *Lampetra* sp., three-spined stickleback and stone loach (**Appendix A**). The site was of high value for salmonids, supporting a healthy population of mixed-cohort brown trout in addition to low numbers of Atlantic salmon. The site was of high value as a spawning area, with abundant mixed gravels and cobbles providing suitable spawning habitat for brown trout and, to a lesser degree, Atlantic salmon. The site provided good quality salmonid nursery habitat although the shallow nature

of the site and paucity of instream refugia reduced the value overall. Holding habitat for adult salmonids was rare but of good quality where present (e.g. small pools associated with overhanging vegetation/bank scours and LWD). The site was of greatest importance as a lamprey habitat, with a high density of *Lampetra* sp. ammocoetes recorded via targeted electro-fishing in the abundant soft sediment and sand deposits (mean density of 23.25 per m^2). The site was also of excellent value as a lamprey spawning habitat given the predominance of finer gravel substrata. Despite some suitability for European eel and white-clawed crayfish, none were recorded. No otter signs were recorded in vicinity of the site (although marking opportunities were rare). A kingfisher (*Alcedo atthis*)[3] was observed in flight during the survey.

The biological water quality (Q sample) was calculated as of **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 presence of salmonids (including Atlantic salmon) and *Lampetra* sp., in addition to the high-quality salmonid and lamprey habitat, the aquatic ecological evaluation of site B2 was of **local importance (higher value) (Table 4.4).**

Plate 4.4 Representative image of site B2 on the River Suir, September 2022

[3] Kingfishers are protected under Annex I of the EU Birds Directive (79/409/EEC as amended 2009/147/EC) and are Amber-listed (medium conservation concern) in Ireland according to the Birds of Conservation Concern of Ireland (BoCCI; Gilbert et al., 2022)

4.1.5 Site B3 – River Suir, Ballycahill

Site B3 was located on the River Suir (16S02) at a farm access crossing, approx. 2.4km downstream of site B2, adjoining the proposed site boundary. As per upstream, the lowland depositing watercourse (FW2) had been historically straightened and deepened throughout, with resulting poor hydromorphology and steep (often near vertical) trapezoidal banks of up to 2.5m in height. The profile was dominated by slow-flowing glide with occasional small pool and riffle areas. The substrata were dominated by sands and soft sediment with a high clay fraction (compacted). Boulder, cobble and superficial mixed gravels were present but confined to faster-flowing areas (including underneath the bridge) and heavily bedded in silt where present. Siltation was very high overall with excessive livestock poaching observed both upstream and downstream of the bridge (**Plate 4.5**). An adjoining drainage channel had recently been excavated and was contributing sediment to the river. Macrophyte growth was limited with only occasional fool's watercress, brooklime and water mint along channel margins. Curled pondweed (*Potamogeton crispus*) and water crowfoot (*Ranunculus* sp.) were present but rare. Narrow fringes of reed canary grass were scattered throughout. Aquatic bryophytes were limited to occasional *Leptodictyum riparium* on larger substrata. The liverwort *Conocephalum conicum* was locally frequent on exposed loamy/muddy banks. Filamentous algae were present (2% cover), indicating enrichment. The riparian zones supported abundant bramble scrub with overlying treelines of ash, elder, hawthorn and willow species. The site was bordered by improved pasture (GA1).

Atlantic salmon, brown trout, *Lampetra* sp., minnow (*Phoxinus phoxinus*) and stone loach were recorded via electro-fishing at site B3 (**Appendix A**). The site was of good value to salmonids, supporting a moderate density of mixed-cohort brown trout and low numbers of Atlantic salmon parr. However, the aquatic value of the site was reduced by hydromorphological changes to the channel (i.e. historical drainage and cattle poaching) with associated siltation that compromised the spawning and nursery habitat for salmonids. Localised pool and overhanging vegetation provided some good holding habitat for adult salmonids. However, while these areas supported the majority of the fish populations recorded by electro-fishing, these mesohabitats were rare. The River Suir at Ballycahill at the survey site was considered of high value as a lamprey nursery. Lamprey (*Lampetra* sp.) ammocoetes were recorded in moderate to locally high densities of >10 per m^2. As per upstream, the majority of ammocoetes represented larger size classes - this reflected the clay-dominated soft sediment. Despite some suitability for European eel and white-clawed crayfish, none were recorded. No otter signs were recorded in vicinity of the survey site which maybe as a consequence of the poor availability of marking substrata (i.e. no exposed boulders, tree limbs and suitable marking outposts) or inherently low utilisation of the channel by otter.

The biological water quality (Q sample) was calculated as of **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) and *Lampetra* sp., in addition to high-quality lamprey nursery habitat, the aquatic ecological evaluation of site B3 was of **local importance (higher value) (Table 4.4).**

Plate 4.5 Representative image of site B3 on the River Suir, September 2022 (facing downstream from bridge)

4.1.6 Site B4 – River Suir, Knocknageragh Bridge

Site B4 was located on the upper reaches of the River Suir (16S02) at Knocknageragh Bridge (R433 road crossing), approx. 1km downstream of the proposed site boundary. As per upstream, the river had been extensively straightened and deepened historically with a trapezoidal profile and bankfull heights of up to 3m. The river averaged 5-6m wide and 0.4-0.8m deep, with locally deeper glide and pool to 1.2m. The flow profile comprised deep slow-flowing glide with occasional pool. Riffle areas were absent. The substrata were dominated by compacted mixed gravels and sand with frequent boulder and occasional cobble. Sand deposits with a moderate silt fraction were abundant in association with macrophyte beds and pool slacks. Macrophyte cover was high with frequent unbranched bur-reed (*Sparganium emersum*) and occasional fool's watercress and common duckweed in marginal areas. Water starwort (*Callitriche* sp.) was present but rare. Aquatic bryophytes were limited to occasional *Fontinalis antipyretica* and *Leptodictyum riparium*. The littorals supported abundant reed canary grass which often formed overhanging stands. The steeply sloping banks supported a typical nitrophilous community comprised of reed canary grass in addition to nettle (*Urtica dioica*), hedge bindweed (*Calystegia sepium*), great willowherb and occasional bramble. A mature treeline of sycamore and beech (*Fagus sylvatica*) lined the south bank downstream of the bridge. The site was bordered by residential properties (with GA2) and improved pasture (GA1) with narrow riparian buffers.

A total of six fish species were recorded at site B4 via electro-fishing, namely Atlantic salmon, brown trout, roach (*Rutilus rutilus*), minnow, three-spined stickleback and stone loach (**Appendix A**). Site B4 was of high value for salmonids, supporting a high density of mixed-cohort brown trout. Only a single Atlantic salmon parr was recorded. The site was of highest value as a holding habitat for adult salmonids with abundant deep glide and associated overhanging cover via reed canary grass stands.

The site provided good quality salmonid nursery habitat with frequent instream macrophyte beds and occasional boulder offering valuable refugia. The site was of limited value as a spawning habitat for salmonids or lamprey given the compacted nature of the substrata, in addition to siltation pressures. Whilst soft sediment accumulations were frequent, no lamprey ammocoetes were recorded via targeted electro-fishing (however, *Lampetra* sp. are known from the site; O'Connor, 2007). This was taken to reflect the typically flocculent and or compacted nature of the soft sediment. Despite some good suitability, no European eel or white-clawed crayfish were recorded. No otter signs were recorded in vicinity of the bridge, although suitability was high as a foraging habitat.

The biological water quality (Q sample) was calculated as of **Q3-4 (moderate status)** (**Appendix B**). However, it should be noted that this was a tentative rating given an absence of suitable riffle areas for sampling (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 presence of salmonids (including Atlantic salmon) and *Lampetra* sp., the aquatic ecological evaluation of site B4 was of **local importance (higher value) (Table 4.4).**

Plate 4.6 Representative image of site B4 on the River Suir at Knocknageragh Bridge, September 2022

4.1.7 Site B5 – River Suir, Loughmore Bridge

Site B5 was located on the River Suir (16S02) at Loughmore Bridge, approx. 5.8km downstream of site B4. The swift-flowing lowland depositing watercourse (FW2) had been historically modified in the vicinity of the bridge with local straightening, bank revetment works with a mill race channel and bypass channel construction. However, instream the river retained a good degree of naturalness despite historical modifications. The river was 6-7m wide and 0.3-0.6m deep, with locally deeper pool to 1.6m (under the bridge arch). The profile comprised glide and riffle with localised pool. The substrata were dominated by cobble and boulder with frequent mixed gravels. These were often

partially compacted although more mobile substrata (cobble and gravels) were present upstream of the bridge (in riffle areas). Abundant soft sediment accumulations were present under the bridge arch (east bank). Siltation was moderate overall. Macrophyte cover was low with occasional fool's watercress, (submerged) blue water speedwell (*Veronica anagallis-aquatica*) and water mint along the river margins. Amphibious bistort (*Persicaria amphibia*) was present but rare and present more often in its terrestrial form along the banks. The moss *Fontinalis antipyretica* was frequent on larger cobble and boulder, with *Cinclidotus fontinaloides* also locally frequent. *Leptodictyum riparium* was rare. Filamentous algae were frequent (5% cover), indicating enrichment. The narrow riparian zones supported abundant reed canary grass and great willowherb and bittersweet (*Solanum dulcamara*) with scattered willow. The site was bordered by residential properties, an amenity area (BL3, BC4, GA2) and improved pasture (GA1).

A total of seven fish species were recorded at site B5 via electro-fishing, namely Atlantic salmon, brown trout, *Lampetra* sp., European eel (*Anguilla anguilla*), three-spined stickleback, minnow and stone loach (**Appendix A**). This was the highest species diversity of any site surveyed. Site B5 was of high value for salmonids, supporting moderate densities of juvenile Atlantic salmon and primarily adult brown trout. The site was an excellent quality salmonid nursery, especially for Atlantic salmon, given an abundance of boulder and cobble refugia in deep glide. Whilst localised, the site provided excellent quality holding areas for adult salmonids by way of deep pool under the bridge and undercut banks downstream of the bridge. Good quality spawning habitat for both salmonids and lamprey was present locally. Excellent quality lamprey ammocoete habitat was present underneath the bridge arch and supported a moderate density of c.6 larvae per m^2. The site was also of high value for European eel with excellent quality refugia and a low density of fish present. A regular (ITM 611755, 667373) otter spraint and older otter spraint site (ITM 611754, 667397) were present upstream of the bridge and under the bridge, respectively. Fresh otter prints were also identified in littoral mud under the bridge arch.

The biological water quality (Q sample) was calculated as of **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), *Lampetra* sp. and Red-listed European eel, in addition to utilisation by otter, the aquatic ecological evaluation of site B5 was of **local importance (higher value) (Table 4.4).**

Plate 4.7 Representative image of site B5 on the River Suir at Loughmore Bridge, September 2022 (downstream of bridge)

4.1.8 Site C1 – Eastwood River, College Bridge

Site C1 was located on the upper reaches of the Eastwood River (16E17) at College Bridge (N62 road crossing). The small upland eroding watercourse (FW1) had been historically straightened and deepened in vicinity of the twin-arch bridge but demonstrated some good instream recovery. The river averaged 2-2.5m wide and 0.1-0.2m deep at the time of survey, with localised deeper areas to 0.3m. The profile comprised swift-flowing glide with occasional riffle and rare pool in a deep U-shaped channel. The substrata were dominated by cobble and boulder that were heavily calcified, with abundant cyanobacterial bacterial crusts on the bed. Mixed gravels were present locally and interstitially but these were both compacted and exposed to siltation pressures (moderate overall). Soft sediment accumulations were flocculent, where present (<0.01m deep). The site was heavily vegetated with abundant fool's watercress and frequent water mint, often occupying up to 50% of the channel width. Brooklime was occasional along the margins. The aquatic bryophyte community was dominated by abundant submerged *Pellia endiviifolia*, with rare *Rhynchostegium riparioides* on larger substrata. The channel was lined by a mature treeline on the north bank supporting beech, hazel (*Corylus avellana*), sycamore, holly (Ilex aquifolium) and hawthorn with scattered bramble scrub. A narrow strip of amenity grassland (GA2) adjoined the channel on the south bank along a residential access track. The site was bordered by improved pasture (GA1).

Atlantic salmon, brown trout and lamprey (*Lampetra* sp.) were recorded via electro-fishing at site C1 (**Appendix A**). The site was of moderate value for salmonids, supporting a very low density of Atlantic salmon parr and mixed-cohort brown trout. This was considered to reflect the absence of good quality spawning and holding habitat given high rates of bed calcification and the shallow nature of the site. However, the site provided some locally good quality nursery habitat given the abundance of swift-flowing glide and instream macrophyte refugia. The site was of poor value for lamprey given a paucity

of suitable nursery habitat (flocculent silt deposits) and calcified spawning substrata. However, a single *Lampetra* sp. ammocoete was recorded, indicating the channel was of some value for the species. Suitability for European eel was poor given a paucity of refugia and the shallow depths and none were recorded present. Despite some good physical habitat suitability for white-clawed crayfish, the paucity of refugia, calcification of the bed and compacted banks reduced the suitability for the species significantly. No crayfish were recorded during the survey. A single otter spraint, containing fish remains, was recorded on a marginal boulder at the bridge (ITM 612036, 675454).

The biological water quality (Q sample) was calculated as of **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) and *Lampetra* sp., in addition to utilisation by otter, the aquatic ecological evaluation of site C1 was of **local importance (higher value) (Table 4.4).**

Plate 4.8 Representative image of site C1 on the Eastwood River at College Bridge, September 2022

4.1.9 Site C2 – unnamed stream, Knockanroe

Site C2 was located on an unnamed stream, approx. 0.3km upstream of the Farranacahill Stream confluence. The modified stream had been realigned, straightened and deepened historically along a farm access track and was located 50-140m north of the course mapped by the EPA. The small stream averaged 1-1.5m wide and 0.2-0.3m deep and suffered from low seasonal flows at the time of survey. The profile was of very slow-flowing glide and near stagnant pool. The substrata comprised exclusively deep anoxic silt (up to 0.3m in depth). Livestock poaching was contributing to the siltation of the site. The channel was very heavily vegetated throughout with abundant fool's watercress and watercress instream (>75% cover). Branched bur-reed was also locally abundant. Riparian shading was high with abundant reed canary grass and herbaceous vegetation such as great willowherb heavily encroaching

the channel. Localised sections were also heavily tunnelled with bramble, hawthorn and willow scrub. The site was bordered by improved pasture (GA1).

Three-spined stickleback was the only fish species recorded via electro-fishing at site C2 (**Appendix A**). With the exception of low densities of this species, the site was not of fisheries value given historical modifications, poor flows, heavy siltation and poor connectivity with downstream habitats. There was no suitability for white-clawed crayfish and no crayfish were recorded during the survey. The channel had poorer suitability for otter given it was heavily choked with vegetation making otter passage difficult and no signs were recorded.

The biological water quality (Q sample) was calculated as of **Q2-3 (poor status)** (**Appendix B**). However, it should be noted that this was a tentative rating given poor flows and an absence of suitable riffle areas for sampling (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 habitats or species of high conservation value, the aquatic ecological evaluation of site C2 was of **local importance (lower value) (Table 4.4).**

Plate 4.9 Representative image of site C2 on an unnamed Farranacahill Stream tributary, September 2022

4.1.10 Site C3 – Farranacahill Stream, Knockanroe

Site C3 was located on the Farranacahill Stream (16F69) at a farm access crossing. The lowland depositing watercourse (FW2) had been extensively straightened and deepened historically, with resulting very poor hydromorphology. The stream suffered from low seasonal water levels at the time of survey, with an imperceptible flow and standing water. The bridge abutments represented a significant barrier to fish passage. The stream, which represented a drainage channel habitat, averaged a homogenous 2.5m wide and 0.2-0.3m deep, with occasional areas of up to 0.5m. The flow

profile was of stagnant pool and glide in a trapezoidal channel with bankfull heights of up to 2m. The substrata comprised exclusively deep silt, with deposits of up to 0.3m deep on the channel bed. Boulders and superficial mixed gravels were present at the track crossing but these were heavily bedded in silt. The site was heavily vegetated throughout with abundant fool's watercress covering >75% of the channel. Common duckweed was frequent with occasional water mint, branched bur-reed and water starwort (*Callitriche* sp.). Aquatic bryophytes were not recorded. The channel was heavily shaded by abundant bramble scrub with scattered elder, blackthorn (*Prunus spinosa*), hawthorn and ash. The site was bordered by improved agricultural grassland (GA1).

Three-spined stickleback was the only fish species recorded via electro-fishing at site C3 (**Appendix A**). Therefore, the site was not considered of fisheries value given no species of high conservation value were present. The historical modifications, poor flows, gross siltation and poor connectivity with downstream habitats supported the observed poor fisheries value of the watercourse. There was no suitability for white-clawed crayfish the species was not recorded present. Foraging opportunities were limited for otter and the heavy overgrowth in the channel reduced suitability further. No otter signs were recorded in vicinity of the site.

The biological water quality (Q sample) was calculated as of **Q2-3 (poor status)** (**Appendix B**). However, it should be noted that this was a tentative rating given poor flows and an absence of suitable riffle areas for sampling (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 habitats or species of high conservation value, the aquatic ecological evaluation of site C3 was of **local importance (lower value) (Table 4.4).**

Plate 4.10 Representative image of site C3 the Farranacahill Stream, September 2022

4.1.11 Site C4 – Eastwood River, Ballycahill

Site C4 was located on the Eastwood River (16E17) at a livestock access point approx. 1.6km downstream of site C1. The lowland depositing watercourse (FW2) had been extensively straightened and deepened throughout with resulting poor hydromorphology. The river suffered from low seasonal flows at the time of survey with only a very slight flow observed in the trapezoidal channel. The river averaged 3m wide (locally up to 5m) and 0.2-0.5m deep, with locally deeper glide to 0.7m. Slow-flowing depositional glide predominated with only very localised pool and no riffle areas. The river at this location was heavily silted with clay-dominated soft sediment deposits covering >95% of bed of up to 0.3m in depth. Any localised boulder or cobble present was heavily bedded in silt. Given the very high shading (tunnelling), macrophyte growth was sparse with only occasional fool's watercress, brooklime and water mint present in localised open areas of channel. Aquatic bryophytes were not recorded. Apart from the livestock access point (excessive livestock poaching), the river was heavily tunnelled with dense hawthorn, blackthorn, grey willow, hedge bindweed, dog rose and abundant bramble scrub on steep banks. The site was bordered by improved pasture (GA1) and semi-improved, species-poor wet grassland (GS4).

Brown trout, lamprey (*Lampetra* sp.), minnow and three-spined stickleback were recorded via electro-fishing at site C4 (**Appendix A**). The site was of poor value for salmonids given gross siltation pressures and poor flows. However, a very low density of mixed-cohort brown trout were recorded via electro-fishing. Spawning habitat for both salmonids and lamprey was absent given the dominance of deep soft sediment deposits, with nursery habitat of poor quality given poor flows and poor habitat heterogeneity. Some limited, moderate quality holding habitat for adult salmonids was present, typically in association with instream large woody debris. Whilst the site was dominated by soft sediment accumulations, these were of relatively poor value for lamprey ammocoetes given the dominance of clay particles. However, a low number of larger size class larvae (and transformers) were recorded. Suitability for European eel and white-clawed crayfish was poor and none were recorded. 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 was a tentative rating given poor flows and an absence of suitable riffle areas for sampling (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 presence of salmonids and *Lampetra* sp., the aquatic ecological evaluation of site C4 was of **local importance (higher value) (Table 4.4).**

Plate 4.11 Representative image of site C4 the Farranacahill Stream, September 2022

4.1.12 Site D1 – Clonmore Stream, Clonmore

Site D1 was located on the Clonmore Stream (16C11) at the L7039 road crossing, approx. 0.8km upstream of the River Suir confluence. The lowland depositing river (FW2) had been extensively straightened and deepened historically with resulting poor hydromorphology. Furthermore, upstream of the bridge the south bank of the river had been recently cleared (see **Plate 4.13**). The river averaged 4-5m wide and 0.2-0.3m deep in a deep trapezoidal channel with bankfull heights of up to 3m. Water levels were seasonally low at the time of survey with some peat-staining evident. The homogenous flow profile comprised slow flowing depositional glide with very localised riffle areas. Some pool habitat was present under the bridge. The substrata were dominated by compacted cobble and mixed gravels which were heavily bedded in silt. Boulder was present in vicinity of the bridge but limited elsewhere. Siltation was high with abundant flocculent deposits (mostly peat-derived). Given the very high shading present at the survey site macrophyte growth was limited to localised fool's watercress upstream of the bridge. Aquatic mosses were not recorded present. The liverwort *Pellia* sp. was locally frequent on muddy/peaty banks. The Clonmore Stream at the survey location was heavily shaded by mature treelines of sycamore, hawthorn, holly, hazel, grey willow with abundant ivy and bramble scrub. The survey site was bordered by a residential property (BL3) near the bridge and also improved pasture (GA1).

Small numbers of Atlantic salmon, brown trout, European eel and lamprey (*Lampetra* sp.) were the recorded via electro-fishing at site D1 (**Appendix A**). The survey area was considered of poor value for salmonids, supporting only a low density of Atlantic salmon parr and mixed-cohort brown trout. This was consequential of the significant siltation at the site in addition to poor observed hydromorphology. However, some moderate quality nursery and holding habitat was nonetheless present. Spawning substrata for both salmonids and lamprey was not recorded present but make occur upstream. Whilst the site was heavily silted, the generally flocculent nature of the shallow soft

sediment deposits rendered the bed substrata unsuitable for ammocoetes, with only a single *Lampetra* sp. transformer recorded via electro-fishing. Whilst the bridge area provided some good European eel habitat given the presence of boulder refugia, suitability was poor elsewhere. There was poor suitability for white-clawed crayfish (paucity of refugia, compacted substrata & banks) and none were recorded. No otter signs were recorded in vicinity of the site.

The biological water quality (Q sample) was calculated as of **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 presence of salmonids (including Atlantic salmon), *Lampetra* sp. and Red-listed European eel, the aquatic ecological evaluation of site D1 was of **local importance (higher value) (Table 4.4).**

Plate 4.12 Representative image of site D1 on the Clonmore Stream, September 2022 (downstream of the bridge)

Plate 4.13 The historically straightened Clonmore Stream had been recently cleared of instream and riparian vegetation upstream of the survey site

4.1.13 Site E1 – Adamstown River, Gortacurra

Site E1 was located on the Adamstown River (16A69) at a local road crossing, c.17km west of the proposed site boundary. The small lowland depositing watercourse (FW2) flowed under the local road via two 600m pipe culverts. The river had been historically straightened and deepened, with more recent modifications (bank clearance, instream excavations) were evident upstream of the culvert. The river averaged 1-1.5m wide and 0.1m deep, with only very localised deeper areas to 0.25m present. The river suffered from low seasonal water levels at the time of survey, with shallow, slow-flowing glide and riffle predominating. Pool was occasional (often caused by instream debris). The substrata comprised mixed gravels, sands and cobble with localised areas of small boulder. However, the substrata were heavily silted throughout with significant, persistent silt plumes underfoot. Soft sediment deposits (with high clay fractions) of up to 0.1m deep were present downstream of the culvert. Given the very high shading present, macrophyte growth was sparse with only occasional fool's watercress and water mint along channel margins. Common duckweed and watercress were present but rare. Aquatic bryophytes were limited to rare *Leptodictyum riparium* on occasional larger substrata. Terrestrial encroachment of the channel was high with abundant great willowherb and bramble scrub (WS1). The narrow channel was heavily tunnelled by mature treelines of ash, alder (*Alnus glutinosa*), elder and hawthorn. The site was bordered by improved pasture (GA1).

Three-spined stickleback and lamprey (*Lampetra* sp.) were the only fish species recorded via electro-fishing at site E1 (**Appendix A**). The site was of poor value for salmonids given heavy siltation, low seasonal flows, historical modifications, shallow depths and the location in the upper reaches of the catchment. No salmonids were recorded via electro-fishing. However, the site did support a low density of *Lampetra* sp. ammocoetes. Whilst soft sediment accumulations were abundant, these were typically dominated by sand and clay particles and therefore sub-optimal for the species. Some

moderate quality lamprey spawning habitat was present but highly localised and compromised by siltation. Suitability for European eel and white-clawed crayfish was poor and none were recorded. No otter signs were recorded in the vicinity of the site.

The biological water quality (Q sample) was calculated as of **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 *Lampetra* sp., the aquatic ecological evaluation of site E1 was of **local importance (higher value) (Table 4.4).**

Plate 4.14 Representative image of site E1 on the Adamstown River, illustrating heavy siltation

4.2 White-clawed crayfish

No white-clawed crayfish were recorded via hand-searching or sweep netting of instream refugia during the survey and no crayfish remains were identified in otter spraints recorded during the survey. These results supported the absence of available records for the species within the survey area with exception of the River Suir (historical records only) considering recent plague outbreaks in the river. The eDNA assessment also failed to detect the species in the Eastwood River and River Suir via eDNA analysis (refer to section 4.3 below).

4.3 eDNA analysis

Composite water samples collected from the River Suir at sites B3 and B5 and the Eastwood River (site C4) returned a negative result for freshwater pearl mussel and white-clawed crayfish, i.e. eDNA not present or was present below the limit of detection in a series of 12 qPCR replicates (0 positive replicates out of 12, respectively) (**Table 4.1; Appendix C**). These results were considered as evidence of the species' absence at and or upstream of the sampling locations.

Whilst not detected at sites B3 or C4, crayfish plague was present at site B5 on the River Suir at Loughmore Bridge (12 positive replicates out of 12) (**Table 4.1**).

Table 4.1 eDNA results in the vicinity of the proposed Borrisbeg wind farm, Co. Tipperary (positive qPCR replicates out of 12 in parentheses)

Sample	Watercourse	Freshwater pearl mussel	White-clawed crayfish	Crayfish plague
FK901	River Suir (site B3)	Negative (0/12)	Negative (0/12)	Negative (0/12)
FK900	River Suir (site B5, Loughmore Bridge)	Negative (0/12)	Negative (0/12)	**Positive** (12/12)
FK902	Eastwood River (site C4)	Negative (0/12)	Negative (0/12)	Negative (0/12)

4.4 Otter signs

Despite some good suitability at numerous survey locations, otter signs (*n*=6) were only recorded at a total of 4 no. locations during the aquatic surveys undertaken in September 2022. A regular spraint site (containing fish remains only) was recorded at site A1 on the Shanakill River. An old spraint site (greyed out) was recorded on the mammal underpass at Knockanroe Bridge on the River Suir at site B1. Both a regular (holding mixed age spraint) and an old spraint site were recorded in the vicinity of Loughmore Bridge on the River Suir at site B5. Fresh prints were also recorded under Loughmore Bridge in littoral mud. A single otter spraint, containing fish remains, was recorded on a marginal boulder at College Bridge on the Eastwood River at site E1. No breeding (holts) or resting (couch) areas were identified in the vicinity of the survey sites in September 2022.

4.5 Invasive aquatic species

Roach (*Rutilus rutilus*) were recorded from the River Suir at site B4 (Knocknageragh Bridge). The species was previously known from this site (Kelly et al., 2011, 2010). This non-native cyprinid is listed on the Third Schedule of the European Communities (Birds and Natural Habitats) Regulations 2011-2021 (S.I. 477/2011) and is considered a medium impact invasive species in Ireland (O' Flynn et al., 2014).

No other aquatic invasive species were recorded during the survey of a total of *n*=13 riverine sites in September 2022.

4.6 Biological water quality (macro-invertebrates)

No rare or protected macro-invertebrate species (according to national red lists) were recorded in the biological water quality samples taken from *n*=13 riverine sites in September 2022 (**Appendix B**).

None of the survey sites achieved target good status (≥Q4) requirements of the European Union Environmental Objectives (Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (**Figure 4.1** below).

Six sites on the River Suir (B1, B3, B4 & B5), Eastwood River (C1) and Adamstown River (E1) achieved **Q3-4 (moderate status)** water quality. This was given the low numbers (<5%) of group A species, such as the mayfly *Ecdyonurus dispar* and low numbers of group B species such as the cased caddis *Sericostoma personatum* and the stonefly *Leuctra hippopus* (**Appendix B**).

The remaining 7 no. sites on the Shanakill River (A1 & A2), River Suir (B2), unnamed stream (C2), Farranacahill Stream (C3), Eastwood River (C4) and the Clonmore Stream (D1) achieved **Q2-3 or Q3 (poor status)** water quality. This was given the absence of group A species, a paucity of group B species and dominance of pollution-tolerant group C species such as the mayfly *Baetis rhodani*, the caseless caddis *Hydropsyche instabilis*, and *Serratella ignita*, New Zealand mud snail (*Potamopyrgus antipodarum*), freshwater shrimp (*Gammarus duebeni*), riffle beetles *Elmis aenea* and *Limnius volckmari* and blackfly (Simuliidae) larvae (**Appendix B**).

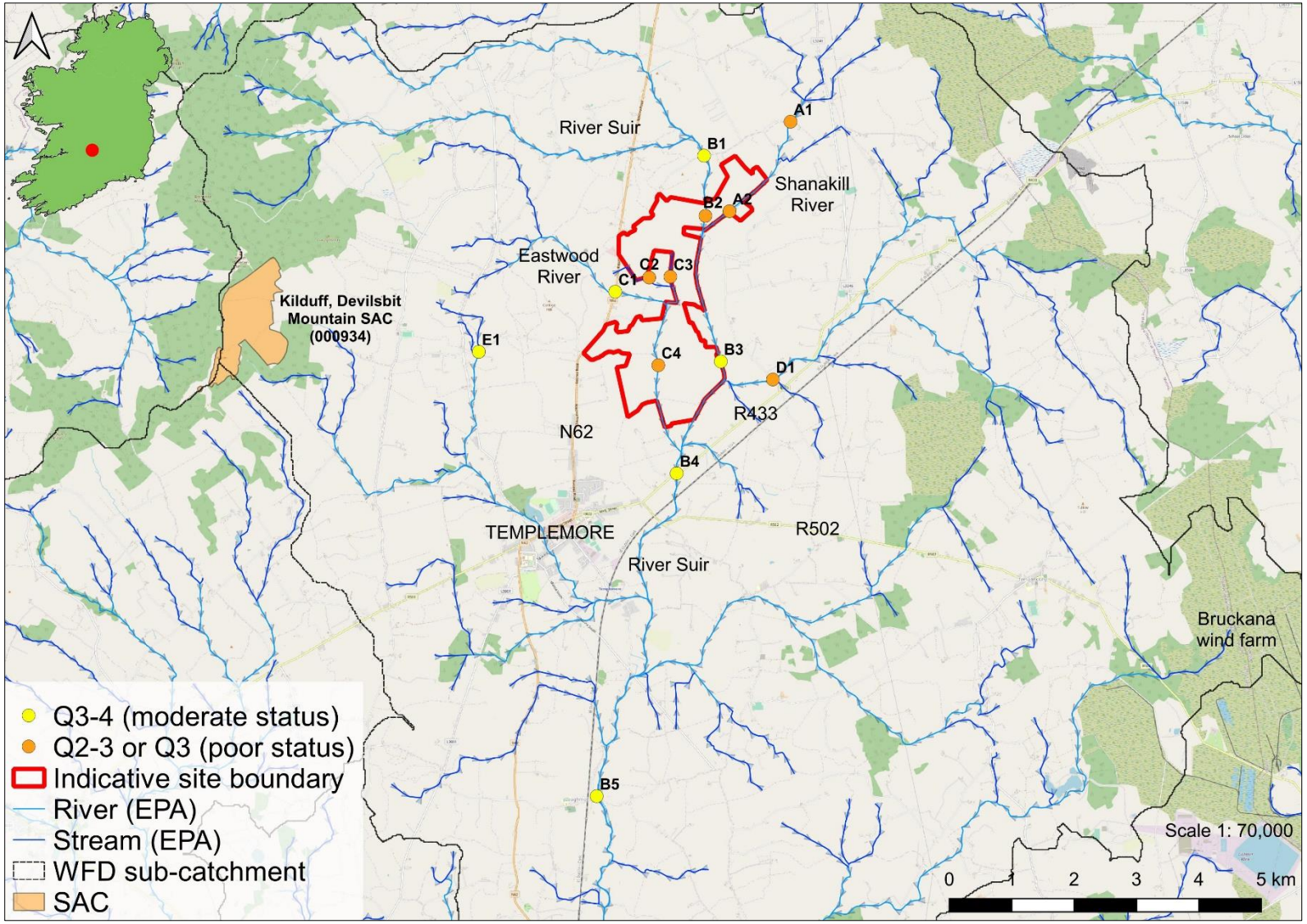

Figure 4.1 Overview of the biological water quality status in the vicinity of the proposed Borrisbeg wind farm project, Co. Tipperary, September 2022

4.7 Macrophytes and aquatic bryophytes

No rare or protected macrophytes or aquatic bryophytes were recorded at the *n*=13 survey sites in September 2022. Similarly, no examples of the Annex I habitat 'Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation or aquatic mosses [3260]' (aka floating river vegetation) were recorded during the surveys. The absence of floating river vegetation primarily relates to the significant hydromorphological pressures in the study area, i.e. extensive straightening and deepening of the river channels. Such conditions exacerbate siltation pressure and creates conditions inimical to support floating river vegetation habitat (i.e. mixed flow profiles with a stable bed, good floodplain connectivity and associated low siltation).

4.8 Aquatic ecological evaluation

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

Apart from two sites on the Farranacahill Stream and unnamed tributary (sites C2 and C3 respectively) that were of **local importance (lower value)**, the remaining 11 no. survey sites were evaluated as **local importance (higher value)** in terms of their aquatic ecological evaluation. Primarily this higher evaluation was due to the presence of salmonids, lamprey (*Lampetra* sp.) and or otter. None of the 13 no. aquatic survey sites were evaluated as greater than **local importance (higher value)**. The fisheries attributes and other important aquatic ecological attributes are summarised in **Tables 4.2 and 4.3** respectively. A full aquatic ecological evaluation is provided in **Table 4.4** below.

Table 4.2 Summary of fish species of higher conservation value recorded via electro-fishing per survey site in the vicinity of the proposed Borrisbeg wind farm, September 2022

Site	Watercourse	Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Shanakill River			✓		Three-spined stickleback
A2	Shanakill River		✓			Three-spined stickleback
B1	River Suir	✓	✓			Stone loach
B2	River Suir	✓	✓	✓		Three-spined stickleback, stone loach
B3	River Suir	✓	✓	✓		Minnow, stone loach
B4	River Suir	✓	✓			Roach, minnow, stone loach, three-spined stickleback
B5	River Suir	✓	✓	✓	✓	Minnow, stone loach, three-spined stickleback
C1	Eastwood River	✓	✓	✓		
C2	Unnamed stream					Three-spined stickleback
C3	Farranacahill Stream					Three-spined stickleback
C4	Eastwood River		✓	✓		Three-spined stickleback, minnow
D1	Clonmore Stream	✓	✓	✓	✓	
E1	Adamstown River			✓		Three-spined stickleback

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**) and habitats of higher conservation value recorded in the vicinity of the proposed Borrisbeg 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	Shanakill River	None recorded		**Spraint site**	Not present	None recorded	None recorded	None recorded
A2	Shanakill River	None recorded			Not present	None recorded	None recorded	None recorded
B1	River Suir	None recorded		**Spraint site**	Not present	None recorded	None recorded	None recorded
B2	River Suir	None recorded			Not present	None recorded	None recorded	None recorded
B3	River Suir	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment		Not present	None recorded	None recorded	None recorded
B4	River Suir	None recorded			Not present	None recorded	None recorded	None recorded
B5	River Suir	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment	**2 no. spraint sites & prints**	Not present	None recorded	None recorded	None recorded
C1	Eastwood River	None recorded			Not present	None recorded	None recorded	None recorded
C2	Unnamed stream	None recorded			Not present	None recorded	None recorded	None recorded
C3	Farranacahill Stream	None recorded			Not present	None recorded	None recorded	None recorded
C4	Eastwood River	None recorded; negative eDNA result at site	Negative eDNA result at site, no records in catchment		Not present	None recorded	None recorded	None recorded
D1	Clonmore Stream	None recorded			Not present	None recorded	None recorded	None recorded
E1	Adamstown River	None recorded		**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 Borrisbeg wind farm survey sites according to NRA (2009) criteria

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary
A1	Shanakill River	16S34	**Local importance (higher value)**	Small, shallow, heavily silted, lowland depositing watercourse with low fisheries value; low density of *Lampetra* sp. recorded via electro-fishing; otter spraint site recorded under bridge; **Q3** (poor status) water quality
A2	Shanakill River	16S34	**Local importance (higher value)**	Heavily modified, silted lowland depositing watercourse with poor flows and low fisheries value; low density of brown trout recorded via electro-fishing; **Q3** (poor status) water quality (tentative)
B1	River Suir	16S02	**Local importance (higher value)**	Upper reaches of swift-flowing lowland depositing river with high value for salmonids & an excellent salmonid nursery & holding habitat; healthy mixed populations of Atlantic salmon & brown trout recorded via electro-fishing; otter spraint site recorded under bridge; **Q3-4** (moderate status) water quality
B2	River Suir	16S02	**Local importance (higher value)**	Upper reaches of swift-flowing, modified lowland depositing river with high value for salmonids & a very good salmonid spawning & nursery habitat; healthy mixed populations of Atlantic salmon & brown trout recorded via electro-fishing with high density of *Lampetra* sp. ammocoetes present (excellent quality nursery habitat); **Q3** (poor status) water quality
B3	River Suir	16S02	**Local importance (higher value)**	Modified, heavily silted lowland depositing river with high value for salmonids & good salmonid holding & nursery habitat; moderate densities of Atlantic salmon & brown trout recorded via electro-fishing with locally high densities of *Lampetra* sp. ammocoetes present (very good quality nursery habitat); **Q3-4** (moderate status) water quality
B4	River Suir	16S02	**Local importance (higher value)**	Heavily modified, heavily silted, slow-flowing lowland depositing river with high value for salmonids & good salmonid spawning & nursery habitat; high densities of mixed-cohort brown trout recorded via electro-fishing with a single Atlantic salmon parr; **Q3-4** (moderate status) water quality (tentative)
B5	River Suir	16S02	**Local importance (higher value)**	Large, modified, swift flowing lowland depositing watercourse with very high value for salmonids & excellent salmonid nursery & holding; also of high value for European eel and *Lampetra* sp.; highest fish species diversity of any survey site (7); moderate densities of Atlantic salmon & brown trout recorded via electro-fishing with low numbers of European eel & Lampetra

Site no.	Watercourse	EPA code	Evaluation of importance	Rationale summary
				sp.; two otter spraint sites and prints recorded; **Q3-4** (moderate status) water quality
C1	Eastwood River	16E17	**Local importance (higher value)**	Small, semi-natural, calcareous upland eroding watercourse with moderate fisheries value; low densities of Atlantic salmon, brown trout & *Lampetra* sp. recorded via electro-fishing; **Q3-4** (moderate status) water quality
C2	Unnamed stream	n/a	Local importance (lower value)	Heavily modified, heavily silted lowland depositing stream with poor flows & poor connectivity resembling drainage channel with poor fisheries value; only of value for three-spined stickleback; **Q2-3** (poor status) water quality (tentative); no aquatic habitats or species of high conservation value
C3	Farranacahill Stream	16F69	Local importance (lower value)	Heavily modified, very heavily silted lowland depositing stream with poor flows & poor connectivity with poor fisheries value; only of value for three-spined stickleback; **Q2-3** (poor status) water quality (tentative); no aquatic habitats or species of high conservation value
C4	Eastwood River	16E17	**Local importance (higher value)**	Heavily modified, heavily silted lowland depositing river with poor flows and relatively poor fisheries value; very low density of brown trout and *Lampetra* sp. recorded via electro-fishing; **Q2-3** (poor status) water quality (tentative)
D1	Clonmore Stream	16C11	**Local importance (higher value)**	Heavily modified, heavily silted lowland depositing Suir tributary with poor hydromorphology but of moderate fisheries value with some moderate quality salmonid & lamprey nursery habitat; low densities of Atlantic salmon, brown trout, European eel & *Lampetra* sp. recorded via electro-fishing; **Q3** (poor status) water quality
E1	Adamstown River	16A69	**Local importance (higher value)**	Heavily modified, heavily silted swift flowing lowland depositing stream with low fisheries value; low density of *Lampetra* sp. recorded via electro-fishing (sub-optimal habitat); **Q3-4** (moderate status) water quality

Conservation value: Atlantic salmon (*Salmo salar*), *Lampetra* spp. and otter (*Lutra lutra*) are all listed under Annex II of the Habitats Directive [92/42/EEC]. Furthermore, Atlantic salmon, *Lampetra* spp. are also listed under Annex V of the Habitats Directive [92/42/EEC] while otter are also listed on under Annex IV of the Habitats Directive [92/42/EEC]. Otters (along with their breeding and resting places) are also protected under provisions of the Irish Wildlife Acts 1976 to 2021. European eel are 'critically endangered' according to most recent ICUN red list (Pike et al., 2020) and listed as 'critically engendered' in Ireland (King et al., 2011). With the exception of the 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

None of the 13 no. aquatic survey sites in the vicinity of the proposed Borrisbeg wind farm project were evaluated as of greater than **local importance (higher value)** in terms of their aquatic ecology. Poor hydromorphology due to drainage pressures (deepening and straightening) had impacted the flow profiles and exacerbated sedimentation. These pressures evidently reduced the fisheries value of the riverine sites and also created conditions inimical to support Annex I floating river vegetation that was not recorded during the surveys. Apart from two sites on the Farranacahill Stream and unnamed tributary (see below) that achieved **local importance (lower value)**, the remaining 11 survey sites were evaluated as **local importance (higher value)** in terms of their aquatic ecology. Primarily this evaluation was due to the presence of salmonids (*n*=9 sites), lamprey (*Lampetra* sp.) (*n*=8 sites) and or otter (*n*=4 sites). Sites B5 on the River Suir and D1 on the Clonmore Stream also supported other aquatic species of high conservation value, such as Red-listed European eel.

5.1.1 Fish species of high conservation value

With the exception of sites A1 on the Shanakill Stream and E1 on the Adamstown River, the remaining nine **local importance (higher value)** sites supported salmonids (**Table 4.2**), despite considerable, widespread hydromorphological and siltation pressures. All 5 no. sites on the River Suir supported Atlantic salmon in addition to brown trout, with salmon also present at Suir tributary sites on the Eastwood River (C1) and Clonmore Stream (D1) (**Table 4.2**). Site B1 in the upper reaches of the River Suir supported the highest densities of both Atlantic salmon and brown trout (**Appendix A**).

Whilst the high rates of siltation observed across the study area reduced the quality of lamprey spawning habitat, soft sediment accretions often provided high value nursery areas (**Appendix A**). *Lampetra* sp. are known to be widespread in the upper Suir catchment (O'Connor, 2007). Lamprey ammocoetes (*Lampetra* sp.) were recorded from 8 no. sites (**Table 4.2**), with particularly high densities present at sites B2 (23.25 per m^2) and B3 (14.3 per m^2) on the upper reaches of the River Suir. Low numbers of early-stage transformers (no speciation possible) were also recorded from sites on the River Suir (B2, B3, B5), Eastwood River (C4) and Clonmore Stream (D1).

Despite widespread suitability, European eel were only recorded in low densities from sites B5 on the River Suir and D1 on the Clonmore Stream (**Table 4.2; Appendix A**). European eel are Red-listed in Ireland (King et al., 2011) and are classed as 'critically endangered' on a global scale (Pike et al., 2020). Eel were recorded in low numbers via electro-fishing. As eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019), the patchy distribution of eel observed in the upper Suir catchment – including the upper River Suir - could be explained by the distance between the survey area and marine habitats (Chadwick et al., 2007) (>150km instream distance).

5.1.2 Annex II otter

Despite some good suitability at numerous survey locations, otter signs were only recorded on the Shanakill River (site A1), River Suir (B1 & B5) and Adamstown River (E1) (i.e. four locations with a total of n=6 signs). In light of the often-suitable prey resources present, this paucity of signs may reflect the

low number of observed marking opportunities in vicinity of the survey sites (Sittenthaler et al., 2020) and or local otter population demographics. The smaller size and shallower nature of the channels makes foraging more difficult for otter and otter sign distribution regularity increases significantly with increasing stream order (pers. obs.). No breeding (holts) or resting (couch) areas were identified in the vicinity of the survey sites in September 2022.

5.1.3 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*=13 riverine sites in September 2022 (**Appendix B**).

None of the survey sites achieved target good status (≥Q4) water quality requirements of the European Union Environmental Objectives (Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (**Figure 4.1**). The majority (7 no.) of the riverine sites achieved poor status water (**Q2-3** or **Q3**) with the remaining six sites achieving (**Q3-4**) moderate status. Siltation (peat extraction, agriculture) and urban wastewater, in addition to alterations to hydromorphology, are known to be the major pressures within the survey area (EPA, 2019) and this was supported by observations made during the aquatic surveys.

5.2 eDNA analysis

No freshwater pearl mussel eDNA was detected in the River Suir (sites B3 & B5) or Eastwood River (C4) samples collected in September 2022 (0 positive qPCR replicates out of 12, respectively) (**Table 4.1**; **Appendix C**). Suitability was poor or absent throughout the survey sites (heavy siltation, enrichment, historical modifications, compaction of substrata etc.) and these results were in keeping with the known distribution of this species within the wider Suir catchment, i.e. the only extant population is located on the Clodiagh River (Ross, 2006).

Similarly, no white-clawed crayfish eDNA was detected within the survey area, supporting the absence of available records within much of the Suir_010 river sub-catchment. However, a single historical record (2005, EPA data) for crayfish was available for the River Suir at Penane Bridge (located between survey sites B4 and B5) (**Figure 3.1**). This record was prior to the first outbreaks of crayfish plague on the River Suir which occurred in May 2017 (NPWS, 2017) and resulted in mass mortalities of up to 400,000 crayfish (Swords et al., 2020) among the highest numbers observed nationally. Environmental DNA monitoring (aside from this report) has continued to detect and confirm the spread of crayfish plague in the Suir catchment since (Swords et al., 2021). Crayfish plague is listed at one of the world's 100 worst invasive species (GISD, 2022; Lowe et al., 2000) and is becoming widespread across Ireland.

Despite an apparent absence of crayfish from the survey area, crayfish plague was also detected via eDNA (12 of 12 positive replicates) in this study at Loughmore Bridge on the River Suir (downstream of the proposed wind farm, site B5) (**Table 4.1**). Generally, *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). Thus, the detection of crayfish plague in a sub-catchment putatively devoid of crayfish in indicative of the ongoing spread of the pathogen across Irish catchments (pers. obs.), including the Suir, likely by a multitude of anthropogenic and natural vectors (Svoboda et al., 2020, 2016). However, it should be noted that the patchy distribution and often low abundances of white-clawed crayfish (especially since crayfish plague outbreaks) in a given catchment

may also strongly influence detection probability via eDNA (Sint et al., 2022) and, therefore, remnant, hitherto undocumented crayfish populations may still be present in tributaries of the upper Suir catchment.

5.3 Aquatic ecology summary

In summary, the majority of watercourses in the vicinity of the proposed Borrisbeg wind farm were of **local importance (higher value)** in terms of their aquatic ecology. However, historical drainage pressures (hydromorphology) and siltation have significantly reduced the quality of aquatic habitats on most watercourses in the vicinity of the proposed project. Nevertheless, most surveyed watercourses were found to support salmonid populations, lamprey (*Lampetra* sp.) and or otter. The River Suir was the highest value watercourse within vicinity of the project supporting Atlantic salmon at all survey sites and typically high to moderate densities of *Lampetra* sp.

None of the 13 no. sites sampled achieved target good status (≥Q4) biological water quality requirements of the European Union Environmental Objectives (Surface Waters) (Amendment) Regulations 2019 and the Water Framework Directive (2000/60/EC) (i.e. all sites **≤Q3-4 (moderate status)**. Primarily, this was considered to reflect the widespread hydromorphological and or more intensive agricultural pressures within the catchment adjoining the proposed project.

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7. Appendix A – fisheries assessment report

Please see accompanying fisheries assessment report

Fisheries assessment of Borrisbeg wind farm, Co. Tipperary

Prepared by Triturus Environmental Ltd. for MKO

December 2022

Please cite as:

Triturus (2022). Fisheries assessment of Borrisbeg wind farm, Co. Tipperary. Report prepared by Triturus Environmental Ltd. for MKO. December 2022.

Table of contents

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 Borrisbeg wind farm, located approximately 3km north-east of Templemore, Co. Tipperary (**Figure 2.1**).

The survey was undertaken to establish baseline fisheries data used in the preparation of the EIAR for the proposed project. 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*=13 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 (*Petromyzon marinus* and *Lampetra* spp.) and European eel (*Anguilla anguilla*). Other species of lower conservation value were also recorded. The presence and or absence of fish populations and or associated supporting habitat would help inform impact assessment and any subsequent mitigation for the project.

Triturus Environmental Ltd. made an application under Section 14 of the Fisheries (Consolidation) Act, 1959 as substituted by Section 4 of the Fisheries (Amendment) Act, 1962, to undertake a catchment-wide electro-fishing survey in the vicinity of the proposed Borrisbeg wind farm. Permission was granted on the 22nd September 2022 and the survey was undertaken on the 28th and 29th September 2022.

1.2 Fisheries asset of the survey area

The *n*=13 aquatic survey sites were located within the Suir_SC_010 river sub-catchment. The proposed wind farm was not located within a European site. Fisheries survey sites were present on the Shanakill River (EPA code: 16S34), River Suir (16S02), Eastwood River (16E17), Farranacahill Stream (16F69) and unnamed tributary, Clonmore Stream (16C11) and the Adamstown River (16A69) (**Table 2.1**).

The River Suir (16S02) rises in Devilsbit Mountain some 8km north-west of Templemore and flows in a southerly direction through Templemore, Thurles, Cahir, Clonmel and Carrick-on Suir before joining the River Nore near Cheekpoint, Co. Waterford. The Suir has the largest quantity of accessible fluvial salmonid habitat in the country (8.8 million m^2) or 7.8% of the national total (McGinnity et al., 2003). The Suir is one of Ireland's premier recreational angling venues for both Atlantic salmon (*Salmo salar*) (Grilli et al., 2021) and brown trout (*Salmo trutta*) (O'Reilly, 2009). The Suir was not meeting its conservation limit for Atlantic salmon in 2022 (Gargan et al., 2022). The growth of trout in the Suir has been assessed as 'fast' according to the classification scheme of Kennedy and Fitzmaurice (1971) (Kelly et al., 2011).

At Knocknageragh Bridge (survey site B4), the river supports brown trout, Atlantic salmon, European eel (*Anguilla anguilla*), pike (*Esox lucius*), stone loach (*Barbatula barbatula*), gudgeon (*Gobio gobio*), invasive roach (*Rutilus rutilus*), three-spined stickleback (*Gasterosteus aculeatus*) and lamprey

(*Lampetra* sp.) (Kelly et al., 2011, 2010; O'Connor, 2007; IFI data[1]). Further downstream, the middle and lower reaches of the river are also known to support minnow (*Phoxinus phoxinus*) and invasive dace (*Leuciscus leuciscus*) (Matson et al., 2019; Kelly et al., 2011).

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

[1] Inland Fisheries Ireland data for Water Framework Directive Fish Ecological Status 2008-2021. Available at https://opendata-ifigis.hub.arcgis.com/datasets/IFIgis::water-framework-directive-fish-ecological-status-2008-2021/

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 Borrisbeg wind farm on the 28th and 29th September 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*=13 sites (see **Table 2.1, Figure 2.1**).

2.1.1 Salmonids and European eel

For salmonid species and European eel, as well as all other incidental species, electro-fishing was carried out in an upstream direction for a 10-minute CPUE, an increasingly common standard approach for wadable streams (Matson et al., 2018). A total of approx. 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 high conductivity waters of the sites (draining limestone geologies) a voltage of 200-230v, 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 using targeted box quadrat-based electro-fishing (as per Harvey & Cowx, 2003) in objectively suitable areas of sand/silt, where encountered. As lamprey take longer to emerge from silts and require a more persistent approach, they were targeted at a lower frequency (30Hz) burst DC pulse setting which also allowed detection of European eel in sediment, if present. Settings for lamprey followed those recommended and used by Harvey & Cowx (2003), APEM (2004) and Niven & McAuley (2013). Using this approach, the anode was placed under the water's surface, approx. 10-15cm 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 (Environment Agency, 2003) and Fishery Assessment Methodology (O'Grady, 2006) to broadly characterise the riverine sites (i.e., channel profiles, substrata etc.).

2.3 Biosecurity

A strict biosecurity protocol following IFI (2010) and the Check-Clean-Dry approach was adhered to during surveys for all equipment and PPE used. Disinfection of all equipment and PPE before and after use with Virkon™ was conducted to prevent the transfer of pathogens or invasive propagules between survey sites. Surveys were undertaken at sites in a downstream order to minimise the risk of upstream propagule mobilisation. Particular cognisance was given towards preventing the spread or introduction of crayfish plague given the known distribution of white-clawed crayfish in the wider survey area and previous outbreaks of crayfish plague in the Suir catchment. 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*=13 aquatic survey sites in the vicinity of Borrisbeg wind farm, Co. Tipperary

Site no.	Watercourse	EPA code	Location	X (ITM)	Y (ITM)
A1	Shanakill River	16S34	Skehanagh	614869	678187
A2	Shanakill River	16S34	Skehanagh	613890	676753
B1	River Suir	16S02	Knockanroe Bridge	613482	677642
B2	River Suir	16S02	Knockanroe	613504	676677
B3	River Suir	16S02	Ballycahill	613751	674344
B4	River Suir	16S02	Knocknageragh Bridge (R433)	613043	672550
B5	River Suir	16S02	Loughmore Bridge	611761	667377
C1	Eastwood River	16E17	College Bridge, N62	612058	675462
C2	Unnamed stream	n/a	Knockanroe	612603	675689
C3	Farranacahill Stream	16F69	Knockanroe	612942	675703
C4	Eastwood River	16E17	Ballycahill	612749	674284
D1	Clonmore Stream	16C11	L7039 road crossing, Clonmore	614585	674055
E1	Adamstown River	16A69	L3230 road crossing, Gortacurra	609871	674499

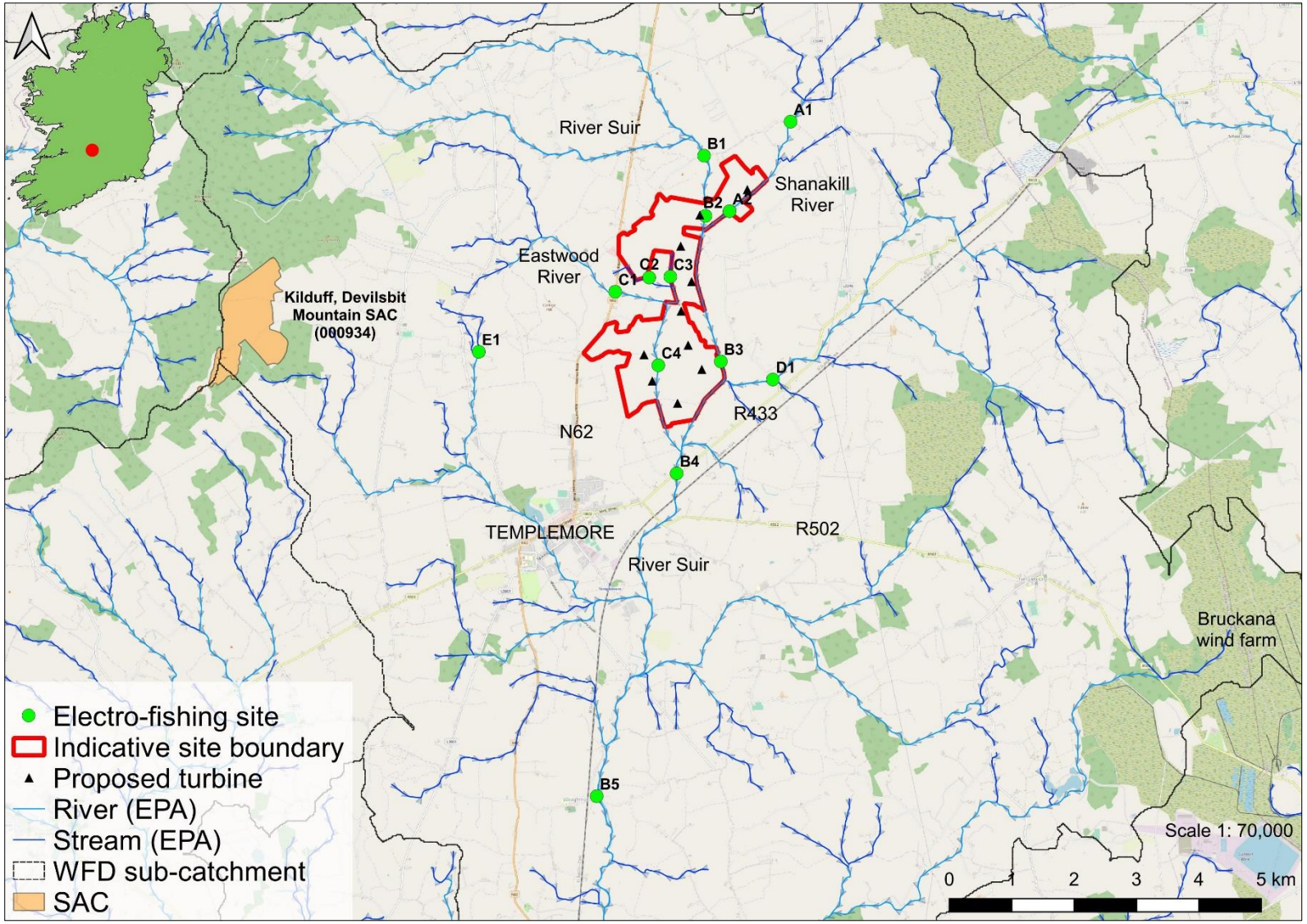

Figure 2.1 Overview of the *n*=13 electro-fishing survey site locations for Borrisbeg wind farm, Co. Tipperary

3. Results

A catchment-wide electro-fishing survey of *n*=13 riverine sites in the vicinity of the proposed Borrisbeg wind farm was conducted on the 28th and 29th September 2022 following notification to Inland Fisheries Ireland. The results of the survey are discussed below in terms of fish population structure, population size and the suitability and value of the surveyed areas as nursery and spawning habitat for salmonids, European eel and lamprey species. Scientific names are provided at first mention only.

3.1 Fish stock assessment (electro-fishing)

3.1.1 Site A1 – Shanakill River, Skehanagh

Three-spined stickleback (*Gasterosteus aculeatus*) (*n*=13) and lamprey (*Lampetra* sp.) (*n*=2) were the only fish species recorded via electro-fishing at site A1 (**Figure 3.1**).

The site was of poor value for salmonids given gross siltation, historical modifications and poor flows. No salmonids were recorded which reflected the very poor spawning and poor nursery habitat present. Some holding habitat for adult salmonids was present. Whilst soft sediment accumulations were abundant, these were typically either flocculent or clay-dominated, and supported a very low density of *Lampetra* sp. ammocoetes (1 ammocoete per m^2 of targeted habitat). Lamprey spawning habitat was present but highly localised and significantly compromised by siltation. Despite some low suitability for European eel, none were recorded.

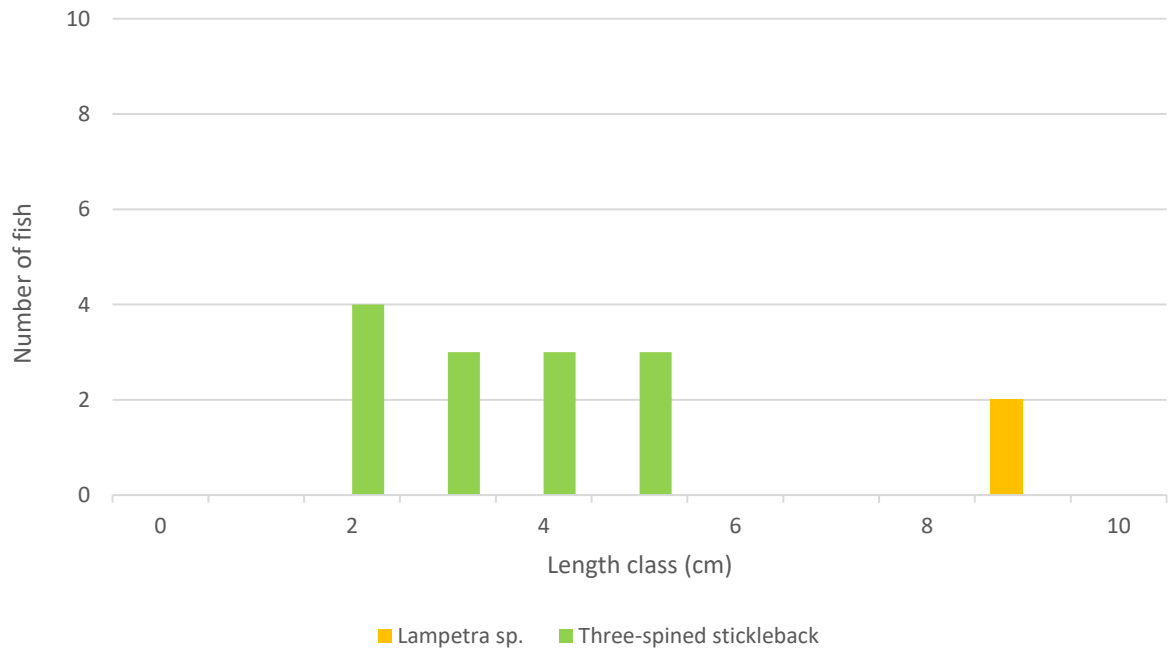

Figure 3.1 Length frequency distribution recorded via electro-fishing at site A1 on the Shanakill River, September 2022

Plate 3.1 Three-spined stickleback and *Lampetra* sp. recorded at site A1 on the Shanakill River, September 2022

3.1.2 Site A2 – Shanakill River, Skehanagh

Brown trout (*Salmo trutta*) (*n*=2) and three-spined stickleback (*n*=10) were the only fish species recorded via electro-fishing at site A2 (**Figure 3.2**).

The site was of poor value for salmonids given heavy siltation, historical modifications and poor flows. However, the site supported a very low density of brown trout, with a single juvenile and small adult recorded via electro-fishing. Three-spined stickleback were also present in low densities. The site provided moderate quality (at best) salmonid nursery and spawning habitat in the upstream vicinity of the ford crossing. Soft sediment accumulations were of poor suitability for larval lamprey given the flocculent nature and poor flows. The river at this location was of very poor suitability for European eel and none were recorded.

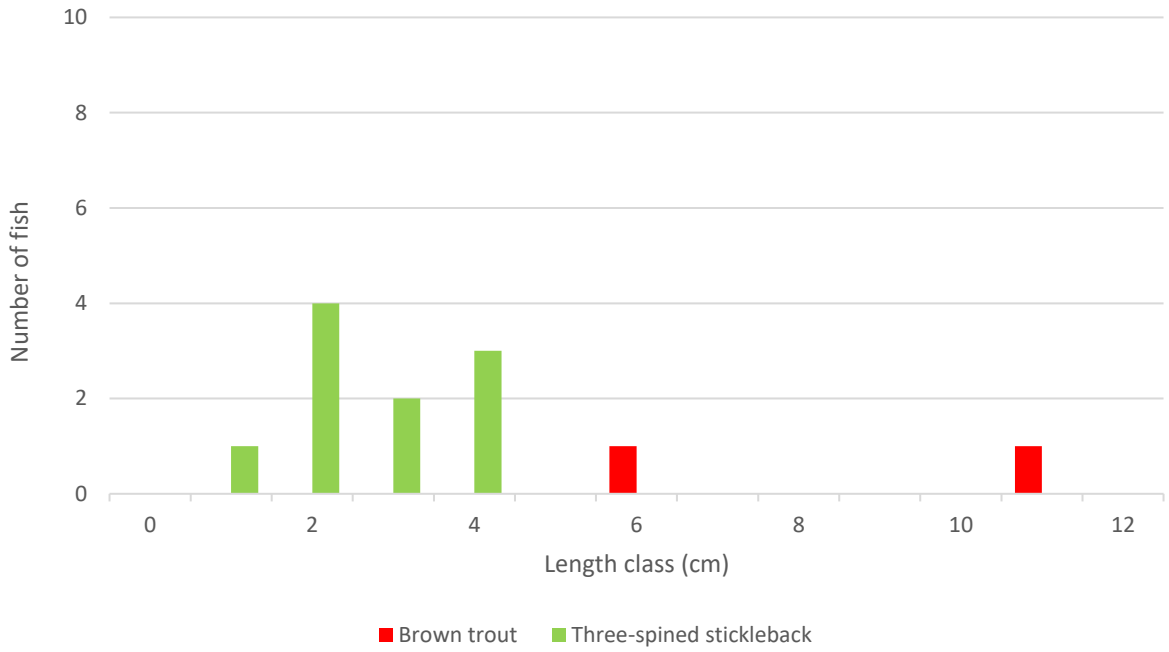

Figure 3.2 Length frequency distribution recorded via electro-fishing at site A2 on the Shanakill River, September 2022

Plate 3.2 Mixed-cohort brown trout recorded at site A2 on the Shanakill River, September 2022

3.1.3 Site B1 – River Suir, Knockanroe Bridge

Atlantic salmon (*Salmo salar*) (*n*=14), brown trout (*n*=56) and stone loach (*Barbatula barbatula*) (n=4) were recorded via electro-fishing at site B1 (**Figure 3.3**).

The site was of high value for salmonids, supporting healthy populations of mixed cohort Atlantic salmon and brown trout. The site was of most value as a salmonid nursery and holding habitat. Good quality salmonid and lamprey spawning habitat was present albeit localised and compromised by siltation and compaction of substrata. Whilst some soft sediment accumulations were present, these were superficial/flocculent in nature and did not support lamprey ammocoetes. Despite some good suitability for European eel, none were recorded.

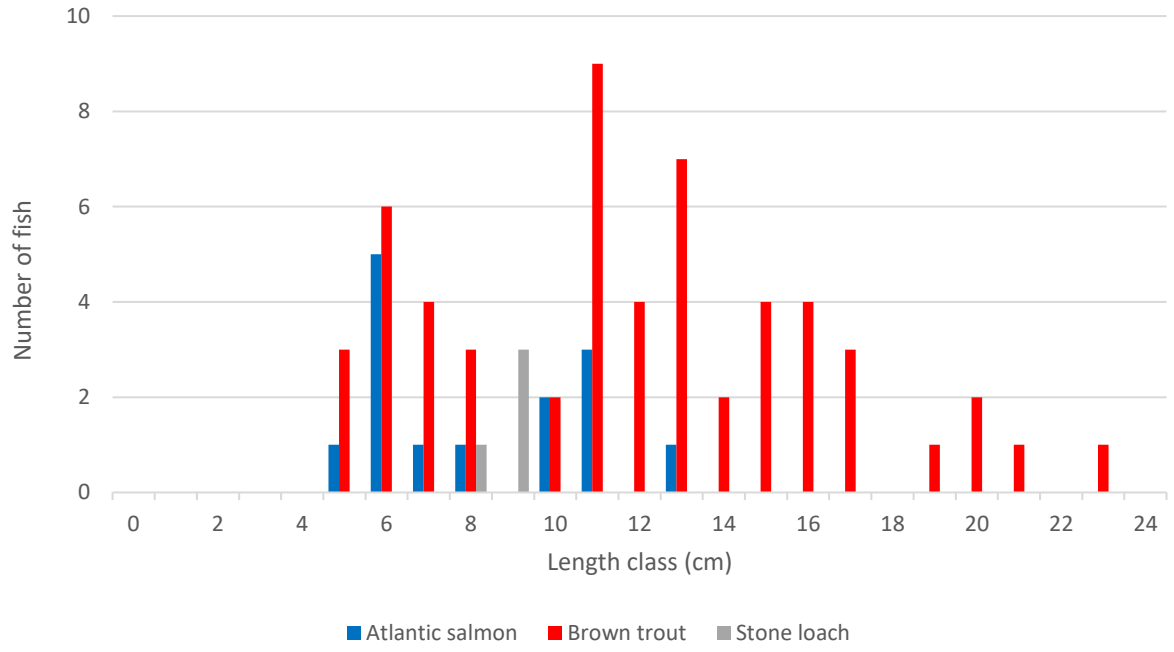

Figure 3.3 Length frequency distribution recorded via electro-fishing at site B1 on the River Suir, September 2022

Plate 3.3 Mixed-cohort Atlantic salmon recorded at site B1 on the River Suir, September 2022

3.1.4 Site B2 – River Suir, Knockanroe

A total of five fish species were recorded at site B2 via electro-fishing, namely Atlantic salmon (*n*=2), brown trout (*n*=29), *Lampetra* sp. (*n*=93), three-spined stickleback (*n*=22) and stone loach (*n*=3) (**Figure 3.4**).

The site was of high value for salmonids, supporting a healthy population of mixed-cohort brown trout in addition to low numbers of Atlantic salmon. The site was of high value as a spawning area, with abundant mixed gravels and cobbles providing good quality spawning habitat for brown trout and, to a lesser degree, Atlantic salmon. The site provided good quality salmonid nursery habitat although the shallow nature of the site and paucity of instream refugia reduced the value overall. Holding habitat for adult salmonids was rare but of good quality where present (e.g. small pools associated with overhanging vegetation/bank scours and LWD). The site was of greatest importance as a lamprey habitat, with a high density of *Lampetra* sp. ammocoetes recorded via targeted electro-fishing in the abundant soft sediment and sand deposits (mean density of 23.25 per m^2). The site was also of excellent value as a lamprey spawning habitat given the predominance of finer gravel substrata. Despite some suitability for European eel, none were recorded.

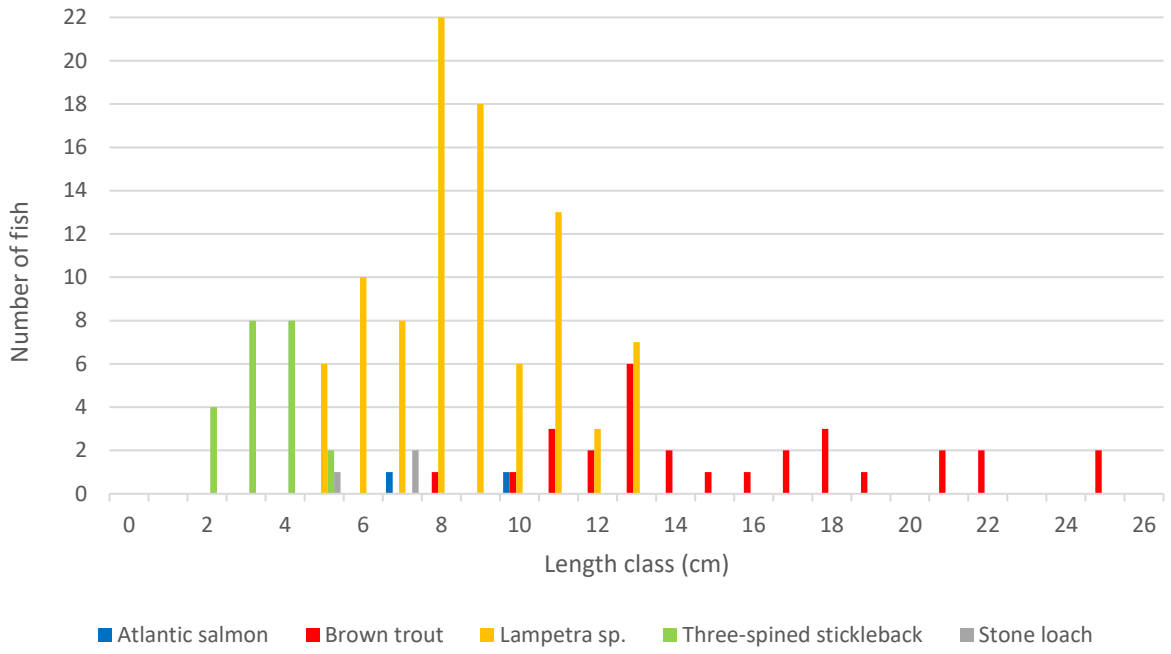

Figure 3.4 Length frequency distribution recorded via electro-fishing at site B2 on the River Suir, September 2022

Plate 3.4 Early-stage *Lampetra* sp. transformer recorded at site B2 on the River Suir, September 2022

3.1.5 Site B3 – River Suir, Ballycahill

Atlantic salmon (*n*=4), brown trout (*n*=24), *Lampetra* sp. (*n*=43), minnow (*Phoxinus phoxinus*) (*n*=3) and stone loach (*n*=8) were recorded via electro-fishing at site B3 (**Figure 3.5**).

The site was of good value to salmonids, supporting a moderate density of mixed-cohort brown trout and low numbers of Atlantic salmon parr. However, the value of the site was significantly reduced by gross siltation, which compromised the spawning and nursery habitat for salmonids. Localised pool and overhanging vegetation provided some good holding habitat for adult salmonids but such areas were highly localised (and, as a result, so were most of the fish recorded). The site was of high value as a lamprey nursery with *Lampetra* sp. ammocoetes recorded in moderate to locally high densities of >10 per m^2. As per upstream, the majority of ammocoetes were represented by larger size classes that reflected the clay-dominated soft sediment. Despite some suitability for European eel, none were recorded.

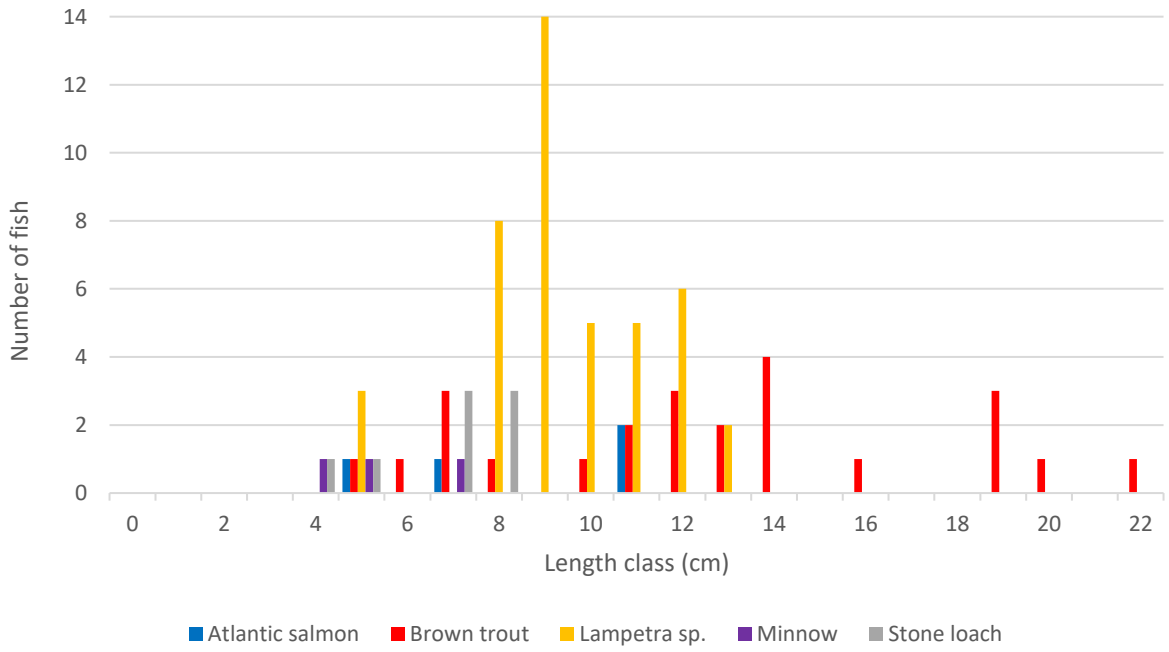

Figure 3.5 Length frequency distribution recorded via electro-fishing at site B3 on the River Suir, September 2022

Plate 3.5 Mixed cohort brown trout recorded at site B3 on the River Suir, September 2022

3.1.6 Site B4 – River Suir, Knocknageragh Bridge

A total of six fish species were recorded at site B4 via electro-fishing, namely Atlantic salmon (*n*=1), brown trout (*n*=47), roach (*Rutilus rutilus*) (*n*=2), minnow (*n*=6), three-spined stickleback (*n*=37) and stone loach (*n*=6) (**Figure 3.6**).

Site B4 was of high value for salmonids, supporting a high density of mixed-cohort brown trout. Only a single Atlantic salmon parr was recorded. The site was of highest value as a holding habitat for adult salmonids with abundant deep glide and associated overhanging cover via reed canary grass stands. The site provided good quality salmonid nursery habitat with frequent instream macrophyte beds and occasional boulder offering valuable refugia. The site was of limited value as a spawning habitat for salmonids or lamprey given the compacted nature of the substrata, in addition to siltation pressures. Whilst soft sediment accumulations were frequent, no lamprey ammocoetes were recorded via targeted electro-fishing (however, *Lampetra* sp. are known from the site; O'Connor, 2007). Despite some good suitability, no European eel were recorded. Roach, an invasive fish species was recorded at low density with two adults recorded (**Figure 3.6**).

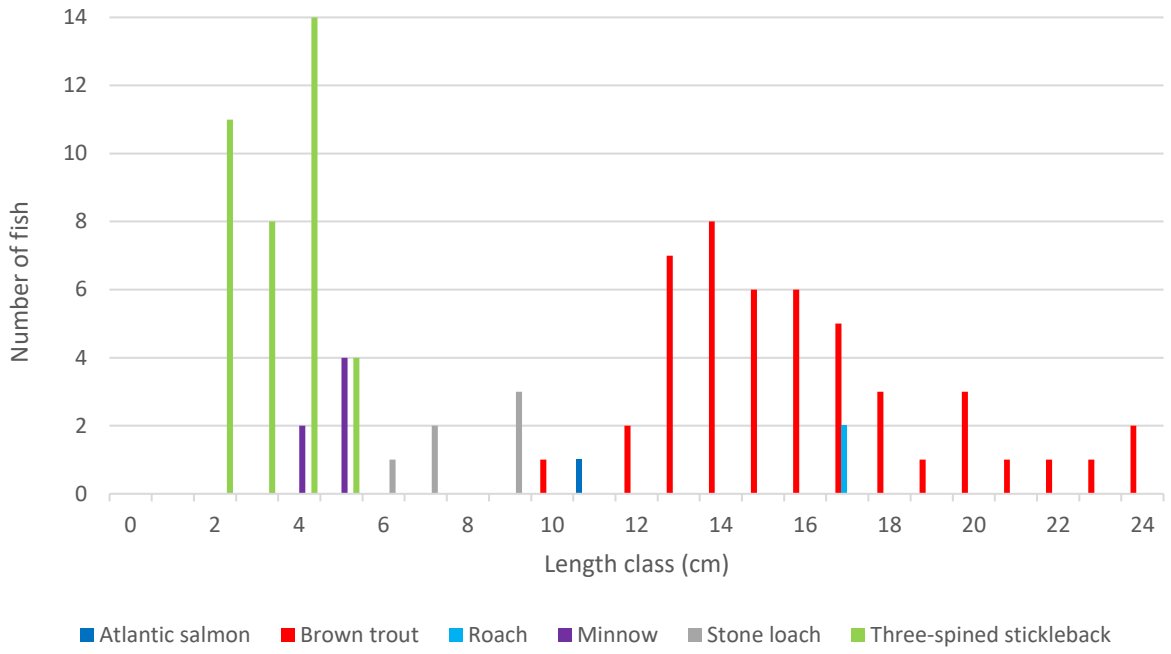

Figure 3.6 Length frequency distribution recorded via electro-fishing at site B4 on the River Suir at Knocknageragh Bridge, September 2022

Plate 3.6 Roach and brown trout recorded at site B4 on the River Suir, September 2022

3.1.7 Site B5 – River Suir, Loughmore Bridge

A total of seven fish species were recorded at site B5 via electro-fishing, namely Atlantic salmon (*n*=25), brown trout (*n*=15), *Lampetra* sp. (*n*=12), European eel (*Anguilla anguilla*) (*n*=7), three-spined stickleback (*n*=7), minnow (*n*=10) and stone loach (*n*=20) (**Figure 3.7**). This was the highest species diversity of any site surveyed.

Site B5 was of high value for salmonids, supporting moderate densities of juvenile Atlantic salmon and primarily adult brown trout. The site was an excellent quality salmonid nursery, especially for Atlantic salmon, given an abundance of boulder and cobble refugia in deep glide. Whilst localised, the site provided excellent quality holding areas for adult salmonids by way of deep pool under the bridge and undercut/scoured banks downstream of the bridge. Good quality spawning habitat for both salmonids and lamprey was present locally. Excellent quality lamprey ammocoete habitat was present underneath the bridge arch and supported a moderate density of c.6 larvae per m^2. The site was also of high value for European eel with excellent quality refugia and a low density of fish present.

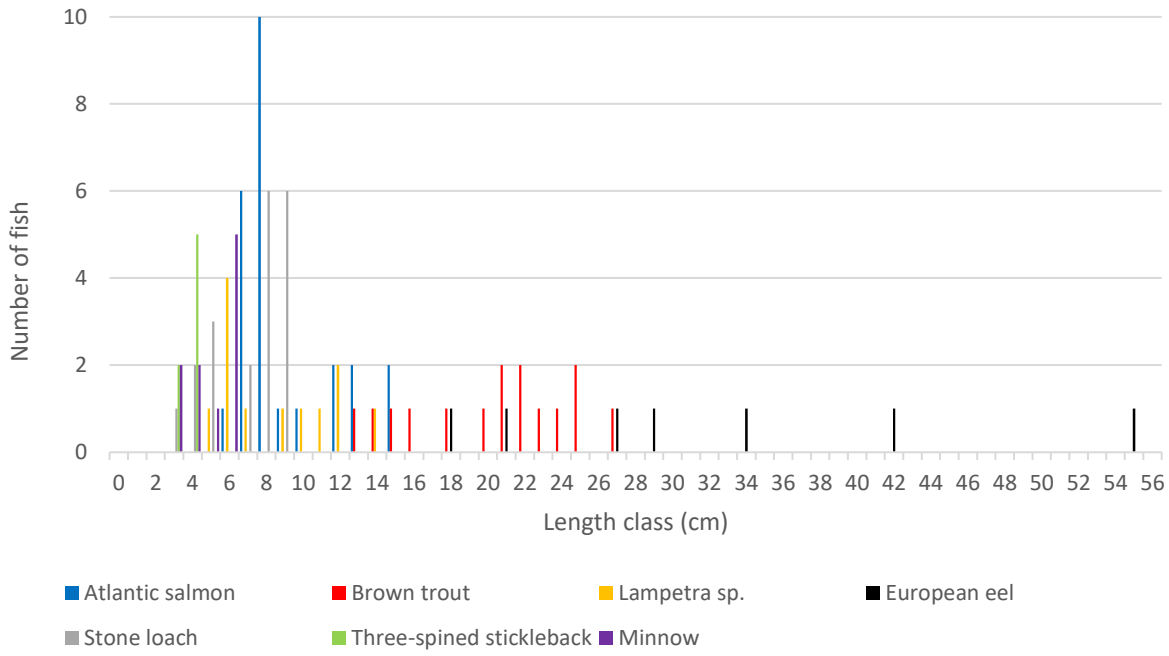

Figure 3.7 Length frequency distribution recorded via electro-fishing at site B5 on the River Suir at Loughmore Bridge, September 2022

Plate 3.7 Large adult European eel recorded at site B5 on the River Suir at Loughmore Bridge, September 2022

3.1.8 Site C1 – Eastwood River, College Bridge

Atlantic salmon (*n*=2), brown trout (*n*=4) and lamprey (*Lampetra* sp.) (*n*=1) were recorded via electro-fishing at site C1 (**Figure 3.8**).

The site was of moderate value for salmonids, supporting a very low density of Atlantic salmon parr and mixed-cohort brown trout. This was considered to reflect the absence of good quality spawning and holding habitat given high rates of bed calcification and the shallow nature of the site. However, the site provided some locally good quality nursery habitat given the abundance of swift-flowing glide and instream macrophyte refugia. The site was of poor value for lamprey given a paucity of suitable nursery habitat (flocculent silt deposits) and calcified spawning substrata. However, a single *Lampetra* sp. ammocoete was recorded, indicating the channel was of some value for the species. Suitability for European eel was poor given a paucity of refugia and the shallow depths - none were recorded.

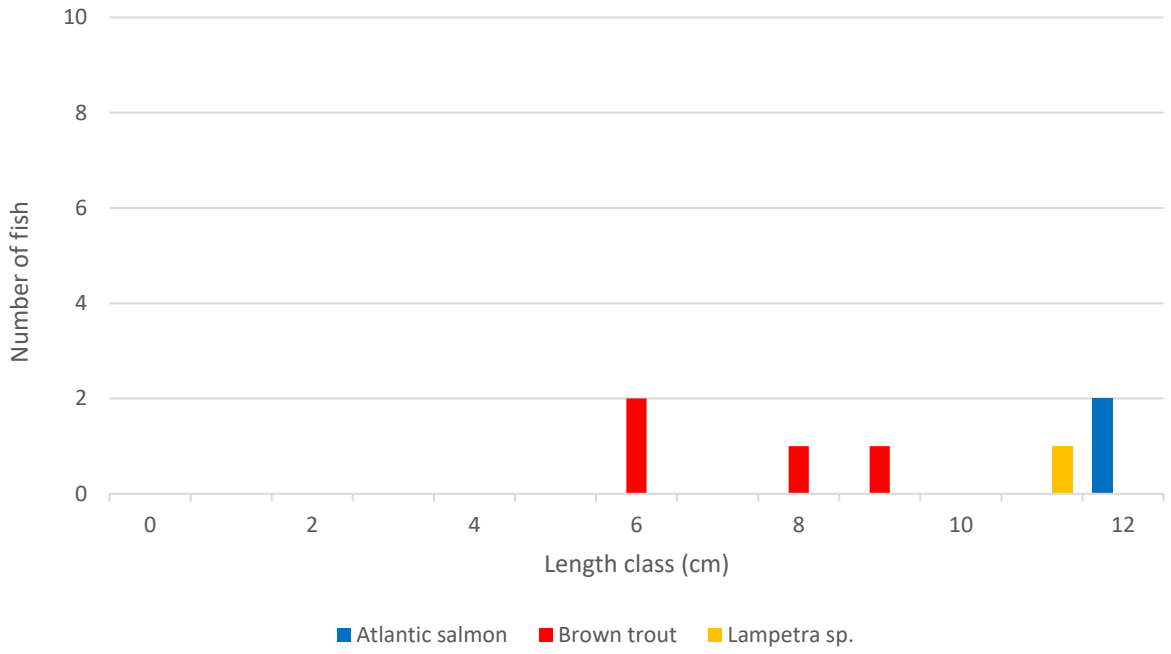

Figure 3.8 Length frequency distribution recorded via electro-fishing at site C1 on the Eastwood River, September 2022

Plate 3.8 Juvenile brown trout and *Lampetra* sp. ammocoete recorded at site C1 on the Eastwood River, September 2022

3.1.9 Site C2 – unnamed stream, Knockanroe

Three-spined stickleback (*n*=7) was the only fish species recorded via electro-fishing at site C2 (**Figure 3.9**). With the exception of low densities of this species, the site was not of fisheries value given historical modifications, poor flows, heavy siltation and poor connectivity with downstream habitats.

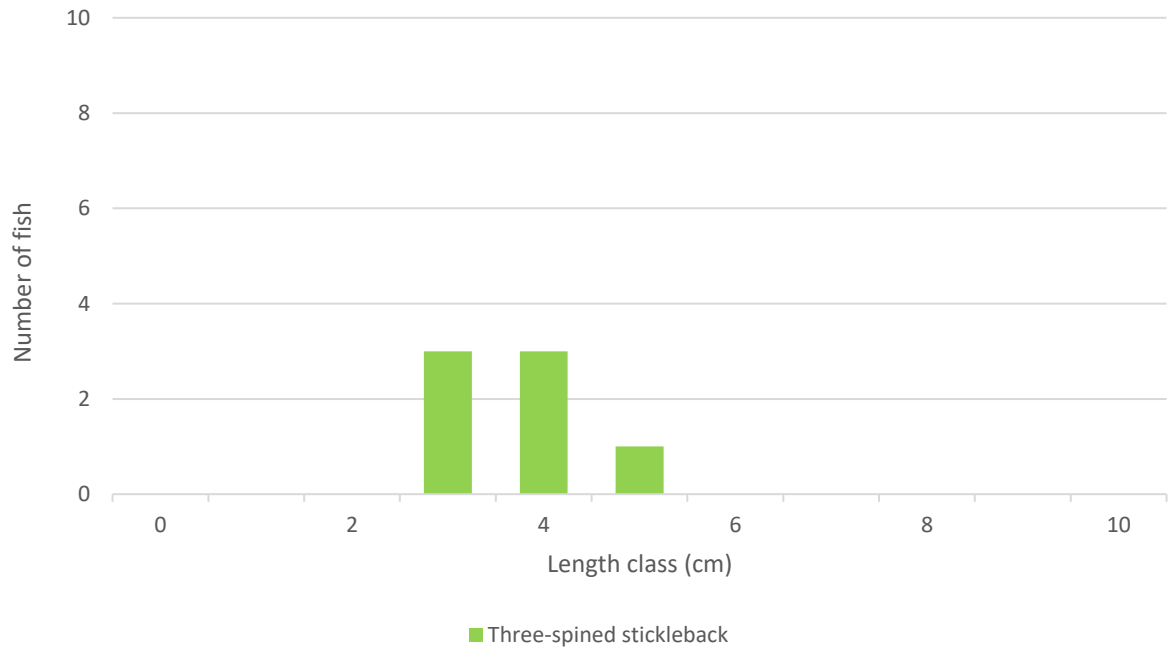

Figure 3.9 Length frequency distribution recorded via electro-fishing at site C2 on an unnamed Farranacahill Stream tributary, September 2022

Plate 3.9 Representative image of site C2 on an unnamed Farranacahill Stream tributary, September 2022

3.1.10 Site C3 – Farranacahill Stream, Knockanroe

Three-spined stickleback (*n*=7) was the only fish species recorded via electro-fishing at site C2 (**Figure 3.10**). With the exception of low densities of this species, the site was not of fisheries value given historical modifications, poor flows, heavy siltation and poor connectivity with downstream habitats.

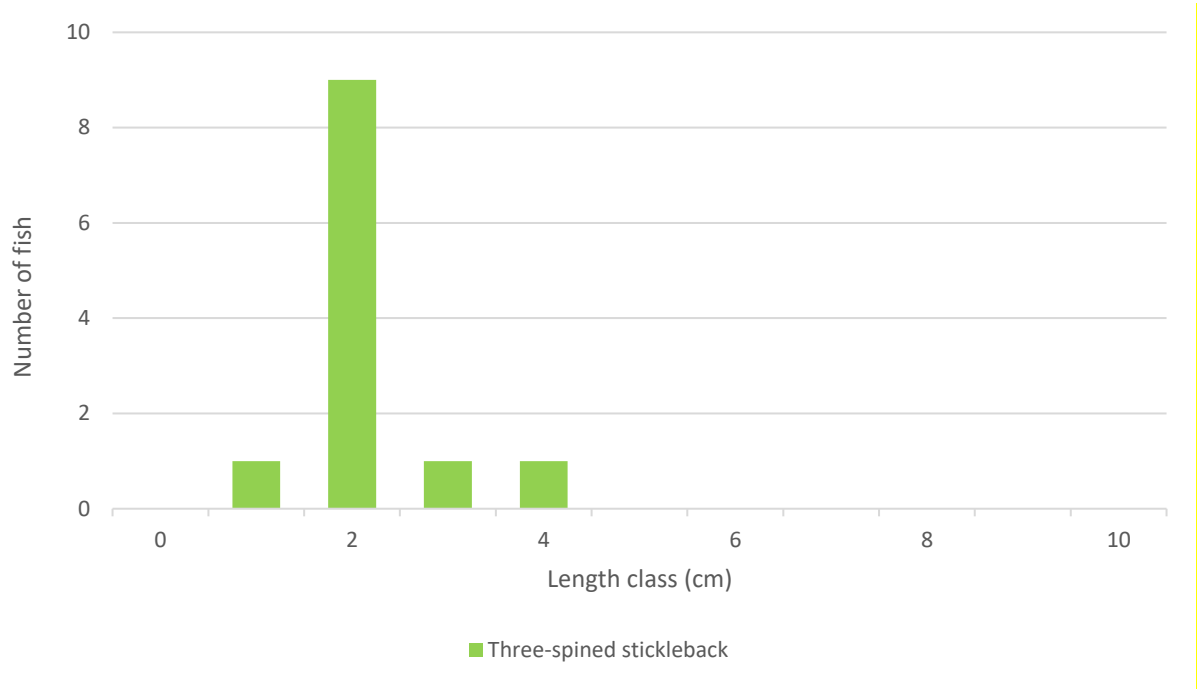

Figure 3.10 Length frequency distribution recorded via electro-fishing at site C3 on the Farranacahill Stream, September 2022

Plate 3.10 Representative image of site C3 the Farranacahill Stream, September 2022

3.1.11 Site C4 – Eastwood River, Ballycahill

Brown trout (*n*=3), lamprey (*Lampetra* sp.) (*n*=2), minnow (*n*=17) and three-spined stickleback (*n*=12) were recorded via electro-fishing at site C4 (**Figure 3.11**).

The site was of poor value for salmonids given gross siltation pressures and poor flows. However, a very low density of mixed-cohort brown trout were recorded via electro-fishing. Spawning habitat for both salmonids and lamprey was absent given the dominance of deep soft sediment deposits, with nursery habitat of poor quality given poor flows and poor habitat heterogeneity. Some limited, moderate quality holding habitat for adult salmonids was present, typically in association with instream large woody debris. Whilst the site was dominated by soft sediment accumulations, these were of relatively poor value for lamprey ammocoetes given the dominance of clay particles. However, a low number of larger size class larvae (and transformers) were recorded. Suitability for European eel and was poor and none were recorded.

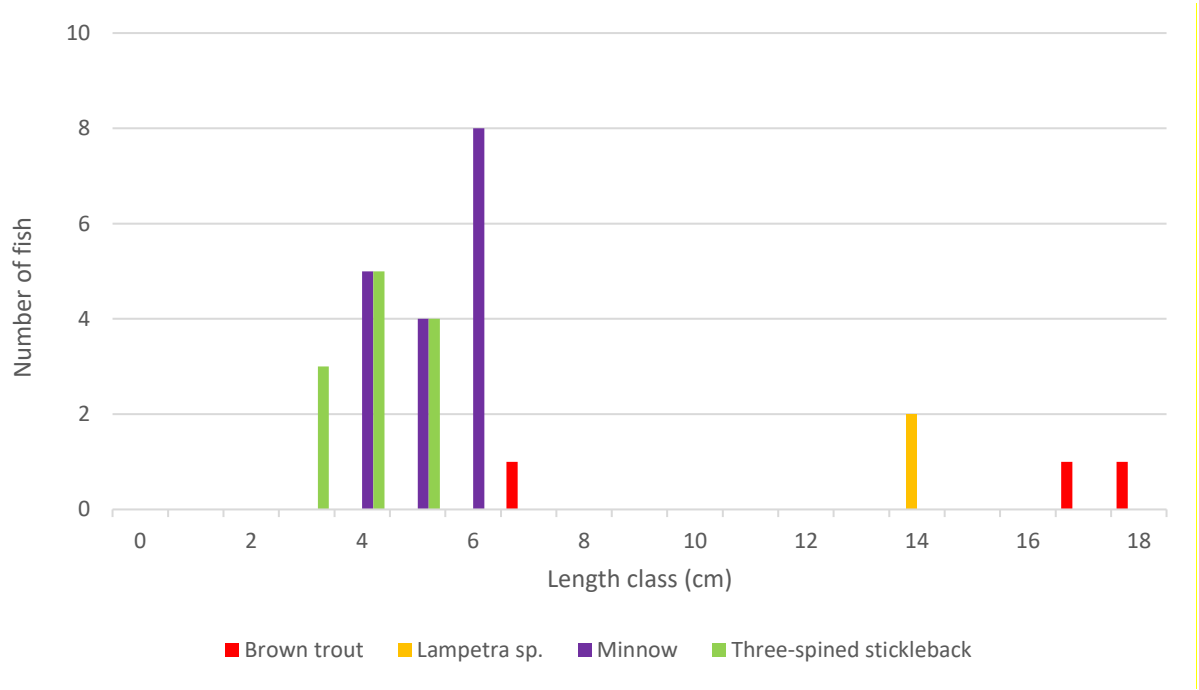

Figure 3.11 Length frequency distribution recorded via electro-fishing at site C4 on the Eastwood River, September 2022

Plate 3.11 Mixed-cohort brown trout and minnow recorded at site C4 on the Eastwood River, September 2022

3.1.12 Site D1 – Clonmore Stream, Clonmore

Atlantic salmon (*n*=2), brown trout (*n*=12), European eel (*n*=2) and lamprey (*Lampetra* sp.) (*n*=1) were the fish species recorded via electro-fishing at site D1 (**Figure 3.12**).

Site D1 was of relatively poor value for salmonids, supporting only a low density of Atlantic salmon parr and mixed-cohort brown trout. This reflected the significant siltation at the site in addition to poor hydromorphology. However, some moderate quality nursery and holding habitat was nonetheless present. Spawning substrata for both salmonids and lamprey was not present. Whilst the site was heavily silted, the generally flocculent nature of the shallow soft sediment deposits rendered them unsuitable for ammocoetes, with only a single *Lampetra* sp. transformer recorded via electro-fishing. Whilst the bridge area provided some good European eel habitat given the presence of boulder refugia, suitability was poor elsewhere.

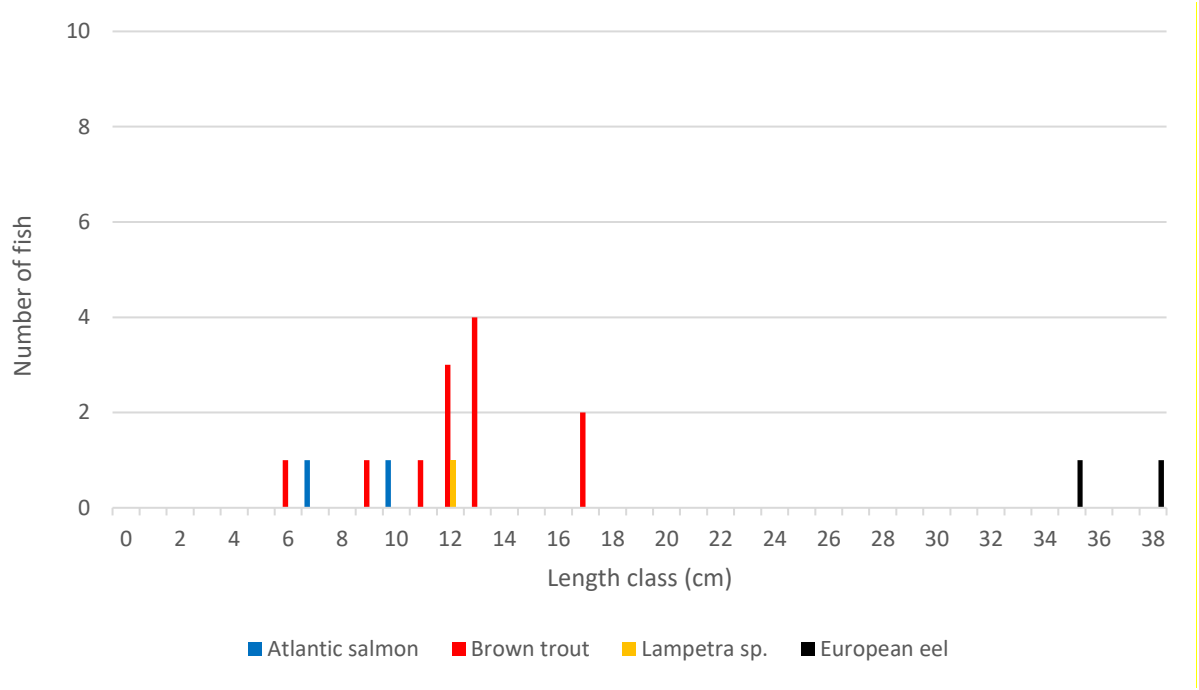

Figure 3.12 Length frequency distribution recorded via electro-fishing at site D1 on the Clonmore River, September 2022

Plate 3.12 Brown trout and Atlantic salmon parr recorded at site D1 on the Clonmore River, September 2022

3.1.13 Site E1 – Adamstown River, Ballycahill

Three-spined stickleback (*n*=16) and lamprey (*Lampetra* sp.) (*n*=3) were the only fish species recorded via electro-fishing at site E1 (**Figure 3.13**).

The site was of poor value for salmonids given heavy siltation, low seasonal flows, historical modifications, shallow depths and the location in the upper reaches of the catchment. No salmonids were recorded via electro-fishing. However, the site did support a low density of *Lampetra* sp. ammocoetes. Whilst soft sediment accumulations were abundant, these were typically dominated by sand and clay particles and therefore sub-optimal for the species. Some moderate quality lamprey spawning habitat was present but highly localised and compromised by siltation. Suitability for European eel was poor and none were recorded

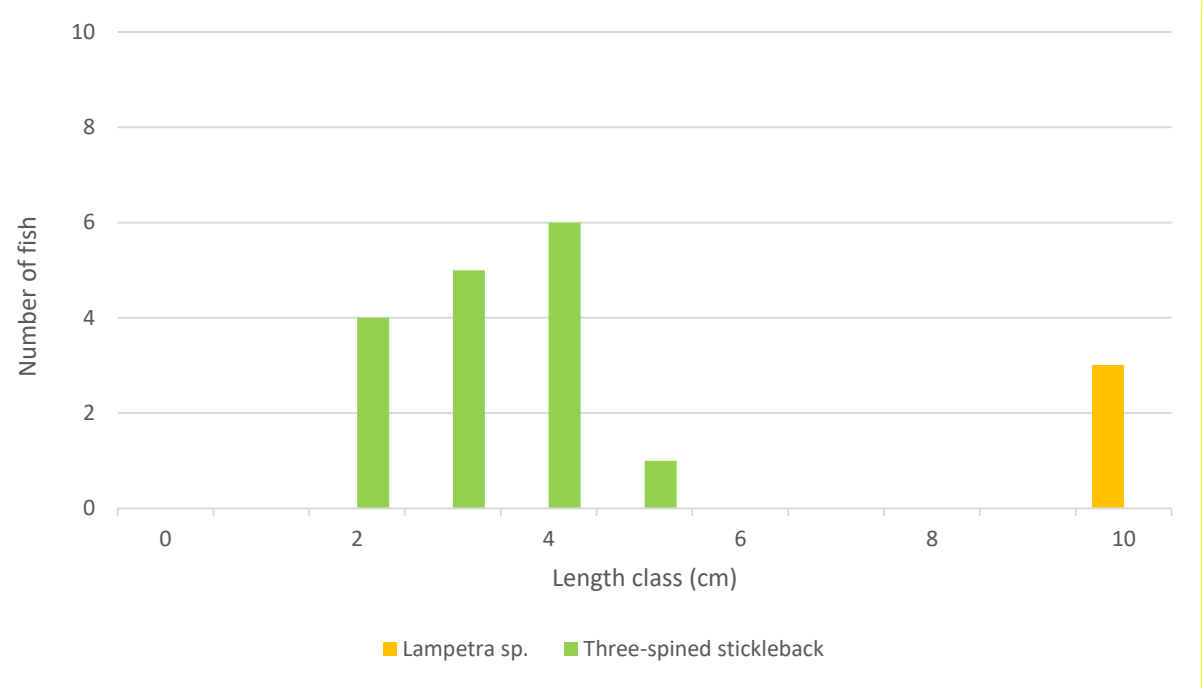

Figure 3.13 Length frequency distribution recorded via electro-fishing at site E1 on the Adamstown River, September 2022

Plate 3.13 *Lampetra* sp. ammocoetes recorded at site E1 on the Adamstown River, September 2022

Table 3.1 Fish species densities per m^2 recorded at sites in the vicinity of the proposed Borrisbeg wind farm via electro-fishing in September 2022 (values in bold represent the highest densities recorded for each species, respectively)

Site	Watercourse	CPUE (elapsed time)	Approx. area fished (m^2)	Fish density (number fish per m^2)							
				Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Stone loach	Three-spined stickleback	Minnow	Roach
A1	Shanakill River	5	25	0.000	0.000	1 per m2	0.000	0.000	0.144	0.000	0.000
A2	Shanakill River	5	67.5	0.000	0.050	0.000	0.000	0.000	0.250	0.000	0.000
B1	River Suir	10	320	**0.117**	**0.467**	0.000	0.000	0.033	0.000	0.000	0.000
B2	River Suir	10	350	0.007	0.097	**23.25 per m^2**	0.000	0.010	0.073	0.000	0.000
B3	River Suir	10	380	0.020	0.120	14.3 per m^2	0.000	0.040	0.000	0.015	0.000
B4	River Suir	5	150	0.004	0.188	0.000	0.000	0.024	0.148	0.024	**0.008**
B5	River Suir	5	50	0.083	0.050	6 per m^2	**0.023**	**0.067**	0.023	0.033	0.000
C1	Eastwood River	5	20	0.013	0.025	0.5 per m^2	0.000	0.000	0.000	0.000	0.000
C2	Unnamed stream			n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
C3	Farranacahill Stream			0.000	0.000	0.000	0.000	0.000	0.160	0.000	0.000
C4	Eastwood River	10	300	0.000	0.017	0.5 per m^2	0.000	0.000	0.067	**0.094**	0.000
D1	Clonmore Stream	10	400	0.007	0.040	0.5 per m^2	0.007	0.000	0.000	0.000	0.000
E1	Adamstown River	10	380	0.000	0.000	3 per m^2	0.000	0.000	**0.356**	0.000	0.000

Table 3.2 Summary of fish species of higher conservation value recorded via electro-fishing per survey site in the vicinity of the proposed Borrisbeg wind farm, September 2022.

Site	Watercourse	Atlantic salmon	Brown trout	*Lampetra* sp.	European eel	Other species
A1	Shanakill River			✓		Three-spined stickleback
A2	Shanakill River		✓			Three-spined stickleback
B1	River Suir	✓	✓			Stone loach
B2	River Suir	✓	✓	✓		Three-spined stickleback, stone loach
B3	River Suir	✓	✓	✓		Minnow, stone loach
B4	River Suir	✓	✓			**Roach**[2], minnow, stone loach, three-spined stickleback
B5	River Suir	✓	✓	✓	✓	Minnow, stone loach, three-spined stickleback
C1	Eastwood River	✓	✓	✓		
C2	Unnamed stream					Three-spined stickleback
C3	Farranacahill Stream					Three-spined stickleback
C4	Eastwood River		✓	✓		Three-spined stickleback, minnow
D1	Clonmore Stream	✓	✓	✓	✓	
E1	Adamstown River			✓		Three-spined stickleback

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

[2] Roach are an invasive fish species listed on the 3rd Schedule of S.I. No. 477/ 2011 - the European Communities (Birds and Natural Habitats) Regulations.

4. Discussion

The watercourses in the vicinity of the proposed Borrisbeg wind farm were typically small, modified, lowland depositing channels. Historical drainage pressures (straightening & deepening) and siltation have significantly reduced the quality and heterogeneity of aquatic habitats in the vicinity of the proposed project. Nevertheless, most surveyed watercourses were found to support salmonid populations and or lamprey (*Lampetra* sp.). The River Suir was the highest value watercourse within vicinity of the project supporting Atlantic salmon at all survey sites and typically moderate to high densities of lamprey ammocoetes.

All 13 no. survey sites supported fish at the time of survey. With the exception of sites A1 (Shanakill River), C2 (unnamed stream), A3 (Farranacahill Stream) and E1 (Adamstown River), all sites supported brown trout (9 no. in total). Atlantic salmon were recorded from all survey sites (5) on the River Suir (B1, B2, B3, B4 & B5), in addition to sites E1 on the Eastwood River and D1 on the Clonmore Stream (both Suir tributaries). Sites B1 and B5 supported the greatest abundances of Atlantic salmon parr. As might be expected given it is the most significant watercourse in vicinity of the project, the River Suir provided the best quality salmonid habitat and supported the highest densities of both Atlantic salmon and brown trout (**Table 3.1**).

In lowland rivers, Atlantic salmon density is known to be positively correlated with instream vegetation (especially *Ranunculus* sp.) and numbers of nearby upstream spawning areas (redds), whilst brown trout density is typically dependant on flow velocity heterogeneity (Marsh et al., 2020). Historical straightening and deepening of watercourses removes habitat and hydromorphological heterogeneity, encourages sediment deposition and invariably results in an irreparable reduction in fisheries potential, particularly for salmonids (O'Grady et al., 2017, O'Grady, 2006). Diffuse siltation is one of the greatest threats to salmonid populations, particularly in agricultural catchments such as that of the proposed Borrisbeg wind farm. 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). Sedimentation of salmonid habitat is a particular problem in Irish rivers flowing through agricultural catchments (Evans et al., 2006).

Lamprey ammocoetes (*Lampetra* sp., likely *L. planeri* given the location in the upper catchment) were recorded from a total of 8 no. sites on the Shanakill River (site A1), River Suir (B2, B3, B5), Eastwood River (C1 & C4), Clonmore Stream (D1) and Adamstown River (E1) (**Table 3.1, 3.2**). Early-stage *Lampetra* sp. transformers were also recorded from sites on the River Suir (B2, B3, B5), Eastwood River (C4) and Clonmore Stream (D1) but speciation in the field was not possible due to the early stage of development (Gardiner, 2003). *Lampetra* sp. are known to be widespread in the upper Suir catchment (O'Connor, 2007). Particularly high densities of ammocoetes were recorded at sites B2 (23.25 per m^2) and B3 (14.3 per m^2) on the upper reaches of the River Suir. These sites featured abundant deposition of fine, organic-rich sediment ≥5cm in depth; areas considered optimal for larval *Lampetra* spp. (Aronsuu & Virkkala, 2014; Goodwin et al., 2008; Gardiner, 2003). Lower ammocoete densities (0.5 to 6 per m^2) were recorded at the other aforementioned sites. Despite widespread siltation at these sites, the generally clay-dominated composition of the soft sediment reduced the suitability for larval

lamprey (e.g. site B3). Such conditions typically support lower ammocoete densities and are normally better suited to larger size classes (pers. obs.). *Lampetra* spp. generally fine, clean gravels required for spawning (Dawson et al., 2015; Rooney et al., 2013; Lasne et al., 2010). The quality of lamprey spawning habitat was compromised by siltation throughout the survey area (also for salmonids). Larval lamprey distribution and settlement is passive and entirely regulated by local, dynamic hydrographical (flow) regimes (Kelly & King, 2001; Potter, 1980; Hardisty & Potter 1971). Thus, a paucity of suitable spawning sites (i.e. sources of larvae) can often counteract the presence of even widespread ammocoete burial habitat (i.e. soft sediment) and impact the demographics and establishment of local populations unless suitable spawning upstream and outside of the observable area exists. The paucity of spawning habitat was exemplified at several survey sites where mean densities of ≤3 larvae per m^2 were recorded (e.g. sites A1, C1, D1).

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). European eel were only recorded in low densities from sites B5 on the River Suir and D1 on the Clonmore Stream (**Table 3.1, 3.2**). This restricted distribution largely reflected the poor hydromorphology and instream habitat heterogeneity resulting from historical modifications, providing a low frequency of suitable refugia (e.g. boulders, pools) required by the species (Laffaille et al., 2003). Furthermore, as eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019), the low numbers of eel and patchy distribution recorded during the electro-fishing survey could be further explained by the considerable distance between the survey area and marine habitats (>150km instream distance) (Matondo et al., 2021; Chadwick et al., 2007).

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

Table 8.1 Macro-invertebrate Q-sampling results for sites in the vicinity of the proposed Borrisbeg wind farm, September 2022

Group	Family	Species	A1	A2	B1	B2	B3	B4	B5	C1	C2	C3	C4	D1	E1	EPA class
Ephemeroptera	Heptageniidae	*Ecdyonurus dispar*			8		5			4					1	A
Ephemeroptera	Heptageniidae	*Heptagenia* sp.							1							A
Plecoptera	Nemouridae	*Nemoura cinerea*						3								A
Ephemeroptera	Baetidae	*Alainites muticus*					2								1	B
Plecoptera	Leuctridae	*Leuctra hippopus*								4					4	B
Trichoptera	Cased caddis pupa	sp. indet.							2							B
Trichoptera	Leptoceridae	*Athripsodes* sp.							22							B
Trichoptera	Limnephilidae	*Limnephilus lunatus*		3									4			B
Trichoptera	Limnephilidae	sp. indet. (early instar)		1												B
Trichoptera	Odontoceridae	*Odontocerum albicorne*			1											B
Trichoptera	Rhyacophilidae	*Rhyacophila dorsalis*			2											B
Trichoptera	Glossosomatidae	sp. indet.												10		B
Trichoptera	Sericostomatidae	*Sericostoma personatum*	3		1	24	1							14		B
Odonata	Calopterygidae	*Calopteryx* sp.						2								B
Ephemeroptera	Ephemerellidae	*Serratella ignita*			10				1							C
Ephemeroptera	Baetidae	*Baetis rhodani*		5	49	45	4	29	32	86		1		5	21	C
Ephemeroptera	Caenidae	*Caenis luctuosa*					2	2	1							C
Ephemeroptera	Caenidae	*Caenis rivulorum*							2							C
Trichoptera	Hydropsychidae	*Hydropsyche instabilis*			47	31	3			14				31	2	C
Trichoptera	Hydropsychidae	*Hydropsyche siltalai*			10									5		C
Trichoptera	Polycentropodidae	*Holocentropus dubius*		1												C
Trichoptera	Polycentropodidae	*Plectrocnemia conspersa*													3	C
Trichoptera	Polycentropodidae	*Polycentropus kingi*			1		2	4						1		C
Trichoptera	Caseless caddis pupa	sp. indet.			1			1								C
Crustacea	Gammaridae	*Gammarus duebeni*	107	14	71	45	43	26	166	34	21	36	22	47	36	C

Group	Family	Species	A1	A2	B1	B2	B3	B4	B5	C1	C2	C3	C4	D1	E1	EPA class
Mollusca	Lymnaeidae	*Lymnaea stagnalis*						1	2		4					C
Mollusca	Lymnaeidae	*Stagnicola fuscus*										1				C
Mollusca	Planorbidae	*Ancylus fluviatilis*	1	10	14	7								1		C
Mollusca	Planorbidae	*Anisus leucostoma*					1									C
Mollusca	Planorbidae	*Bathyomphalus contortus*		2							2	8				C
Mollusca	Planorbidae	*Gyraulus albus*						20								C
Mollusca	Tateidae	*Potamopyrgus antipodarum*	1				24	25	10				51	56		C
Mollusca	Valvatidae	*Valvata piscinalis*					9	4								C
Coleoptera	Dytiscidae	*Dystiscus marginalis*									2					C
Coleoptera	Dytiscidae	Dytiscidae larva						3								C
Coleoptera	Dytiscidae	*Hydroporus tessellatus*						2				1				C
Coleoptera	Dytiscidae	*Nebrioporus depressus*			6			1								C
Coleoptera	Elmidae	*Elmis aenea*	4	7	31	23	21	5	36	8		1		2	4	C
Coleoptera	Elmidae	*Limnius volckmari*	19		15	29	7	17	23	12				5	19	C
Coleoptera	Halipliidae	*Brychius elevatus*					2	2		1						C
Coleoptera	Haliplíidae	*Haliplus lineatocollis*						1					3			C
Coleoptera	Halipliidae	*Haliplus ruficollis* group						1								C
Coleoptera	Hydrophilidae	*Helophorus brevipalpis*									1	1				C
Coleoptera	Hydrophilidae	Hydrophilidae larva		1												C
Coleoptera	Gyrinidae	*Gyrinus* sp.									6		3		4	C
Diptera	Ceratopogonidae	sp. indet.													1	C
Diptera	Chironomidae	non-*Chironomus* spp.		1			1			1	2	3			9	C
Diptera	Culicidae	sp. indet.					2			1						C
Diptera	Dixidae	sp. indet.								9		1				C
Diptera	Muscidae	*Limnophora* sp.							1							C
Diptera	Pediciidae	*Dicranota* sp.	1		9	2			3	1						C
Diptera	Scirtidae	sp. indet.								1						C

Group	Family	Species	A1	A2	B1	B2	B3	B4	B5	C1	C2	C3	C4	D1	E1	EPA class
Diptera	Simuliidae	sp. indet.	11		43	10		20		47						C
Diptera	Tipuliidae	*Tipula* sp.	1			8	1		25						1	C
Hemiptera	Corixidae	*Hesperocorixa linnaei*						4					1			C
Hemiptera	Gerridae	Gerris sp.			1			1								C
Hemiptera	Notonectidae	*Notonecta marmorea viridis*						4								C
Hydracarina	Hydrachnidiae	sp. indet.		1				8				1		1		C
Crustacea	Asellidae	*Asellus aquaticus*	22	12			5	1		15	127	159	15	25	26	D
Mollusca	Lymnaeidae	*Ampullacaena balthica*					1		17				31			D
Mollusca	Physidae	*Physa fontinalis*										1				D
Mollusca	Sphaeriidae	sp. indet.					14		1		3	22				D
Hirudinidae	Glossiphoniidae	sp. indet.			1		2		2					9		D
Hirudinidae	Erpobdellidae	sp. indet.												15		D
Diptera	Chironomidae	*Chironomus* spp.				8	6			1	4	3	5		3	E
Annelida	Lumbricidae	*Eiseniella* sp.				2			5							n/a
Oligochaeta	Oligochaeta	sp. indet.			2				11	2		1		4	10	n/a
Nematomorpha	Gordiidae	sp. indet.													1	n/a
Abundance			**170**	**58**	**323**	**234**	**158**	**187**	**363**	**241**	**172**	**240**	**135**	**231**	**146**	
Q-rating			**Q3**	**Q3***	**Q3-4**	**Q3**	**Q3-4**	**Q3-4***	**Q3-4**	**Q3-4**	**Q2-3***	**Q2-3***	**Q2-3***	**Q3**	**Q3-4**	
WFD status			Poor	Poor	Mod	Poor	Mod	Mod	Mod	Mod	Poor	Poor	Poor	Poor	Mod	

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

9. Appendix C – eDNA analysis lab report

SureScreen Scientifics

Folio No: E15685
Report No: 1
Client: Triturus Environmental LTD
Contact: Bill Brazier

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: 10/10/2022
Date results reported: 14/10/2022
Matters affecting result: None

TARGET SPECIES: Crayfish plague *(Aphanomyces astaci)*

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK900	B5 - River Suir, Borrisbeg WF	ITM 611761 667377	Pass	Pass	Pass	Positive	12/12
FK901	B3 - River Suir, Borrisbeg WF	ITM 613751 674344	Pass	Pass	Pass	Negative	0/12
FK902	C4 - Eastwood River, Borrisbeg WF	ITM 612749 674284	Pass	Pass	Pass	Negative	0/12

Forensic Scientists and Consultant Engineers
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Company Registration No. 08950940

SureScreen Scientifics

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

Lab ID	Site Name	OS Reference	SIC	DC	IC	Result	Positive Replicates
FK900	B5 - River Suir, Borrisbeg WF	ITM 611761 667377	Pass	Pass	Pass	Negative	0/12
FK901	B3 - River Suir, Borrisbeg WF	ITM 613751 674344	Pass	Pass	Pass	Negative	0/12
FK902	C4 - Eastwood River, Borrisbeg WF	ITM 612749 674284	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
FK900	B5 - River Suir, Borrisbeg WF	ITM 611761 667377	Pass	Pass	Pass	Negative	0/12
FK901	B3 - River Suir, Borrisbeg WF	ITM 613751 674344	Pass	Pass	Pass	Negative	0/12
FK902	C4 - Eastwood River, Borrisbeg WF	ITM 612749 674284	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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