ORIGINAL ARTICLE

A comparison of European eel *Anguilla anguilla* eDNA concentrations to fyke net catches in five Irish lakes

Laura Weldon[1,2] | Ciara O'Leary[3] | Mark Steer[1] | Lyn Newton[1] | Heather Macdonald[1] | Stephanie L. Sargeant[1]

[1]Centre for Research in Biosciences, University of the West of England, Bristol, UK

[2]Wildfowl & Wetlands Trust, Slimbridge, Gloucestershire, UK

[3]Inland Fisheries Ireland, Dublin, Ireland

Correspondence
Stephanie L. Sargeant, Centre for Research in Biosciences, University of the West of England, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK.
Email: stephanie.sargeant@uwe.ac.uk

Funding information
University of the West of England

Abstract

The European eel, *Anguilla anguilla*, is classified as critically endangered by the IUCN. To protect what remains of the European eel population, accurate monitoring methods for this species are important. Environmental DNA (eDNA) techniques are gaining popularity for ecological monitoring of aquatic organisms because they are sensitive and noninvasive. This study directly compared catch data from a standardized fyke-net fishing survey with a single species *A. anguilla* eDNA survey in five freshwater lakes in Ireland. The eDNA was recovered by the filtration of water samples and amplified by quantitative real-time polymerase chain reaction (qPCR). European eel eDNA was reliably determined in 83% (70/84) of surface water samples collected from lakes classified as having high, medium, and low eel populations. In addition, there was a positive association between the eDNA concentrations recovered and the eel population classification with lower eDNA concentrations in lakes classified as low eel population lakes. Similar amounts of *A. anguilla* eDNA were detected in water samples collected from open water and shore-side, suggesting shore sampling is an adequate method for eel detection. Together, the results demonstrate that eDNA sampling is more sensitive for detecting eel presence in low eel population environments than standard survey methods and may be a useful noninvasive tool for monitoring *A. anguilla* species distribution.

KEYWORDS
Anguillidae, environmental DNA, Lakes, population monitoring, quantitative real-time polymerase chain reaction

1 | INTRODUCTION

The critically endangered European eel *Anguilla anguilla* (Jacoby & Gollock, 2014) inhabits inland and coastal waters from northern Norway throughout the Mediterranean as far south as the north-western coast of Africa (Dekker, 2000, 2003). Reports of drastic population decline across the whole region are well documented, with abundance currently estimated at 5% of that observed in the 1980s (Dekker, 2004, 2016; Henderson, Plenty, Newton, & Bird, 2012; MacNamara, McCarthy, & Barry, 2016; Stone, 2003). In parts of Europe and East Asia, eels are still considered to be a rare and luxury food item. European and other eel species cannot yet be captive bred to maturity (Butts, Sørensen, Politis, & Tomkiewicz, 2016), so consequently fishing that removes

individuals directly depletes the remaining breeding populations. Wild-caught glass eels (juvenile) and elvers (pigmented juvenile) are in demand to stock commercial eel farms that "grow on" eels to supply the market (Sustainable Eel Group, 2018). This, coupled with their scarcity, has ensured that they maintain a high monetary value and these fish are among the most trafficked critically endangered fish species in Europe. The UK based Sustainable Eel Group estimates that 100 tonnes of glass eels are exported from Europe to Asia annually; the final eel fillets produced from these glass eels will have a value of €2.27 billion at the point of consumption (Stein, 2018).

Legislation exists to enhance the recovery of *A. anguilla* populations (European Union Eel Regulation, 2007) which is interpreted and implemented in each EU country through Eel Management Plans. It is recommended that, in order to conserve *A. anguilla* numbers, at least 40% of mature eels should leave European waters to migrate to spawning sites. To meet the goals of the Eel Management Plans and ensure these levels are reached, all life stages of *A. anguilla* residing in European waters, need to be accurately monitored.

Nearly, all watercourses in Ireland have a population of *A. anguilla* with the large lake-wetted areas that are available being the preferred habitat for eel growth (Moriarty, 2003). Once a thriving eel fishery, fishing for *A. anguilla* was suspended in Ireland in 2009 (Conservation of eel fishing, bye-law number C.S. 303) through its Eel Management Plan (Inland Fisheries Division, 2008). Consequently, the only fishing for *A. anguilla* is now exclusively for monitoring purposes. The Irish agency, Inland Fisheries Ireland (IFI), has a dedicated eel monitoring team and during the summer months (June–September) conducts fyke-net surveys in freshwater lakes to measure eel abundance and health.

Fyke nets are a standard research method for capturing benthic fish species such as eels and have been used in Ireland to study eel populations since 1965 (Moriarty, 1975). They have a large capture capacity and reduced mortality rate compared with other netting techniques (Krueger, Hubert, & Price, 1998). However, despite their widespread use they are difficult to set correctly, require considerable handling of the eels, and in low eel populations may underestimate the total number of eels (Jellyman & Graynoth, 2005). A more sensitive noninvasive detection method is required, particularly for declining eel populations when eels are scarce.

Fish and other organisms leave behind DNA (through skin cells, scales, feces, and mucus) as they move through their environment, which if sampled before it degrades or settles into the sediments can be a useful indication of their recent presence. This shed DNA is more commonly referred to as environmental DNA (eDNA). Methods that detect eDNA from aquatic environments are frequently found to be more sensitive than traditional presence/absence methods of detection (Sengupta et al., 2019; Smart, Tingley, Weeks, Van Rooyen, & Mccarthy, 2015) and can be powerful tools for the ecological monitoring of aquatic organisms. eDNA has already been used to detect single species and methods have been described for many aquatic organisms (Agersnap et al., 2017; Harper et al., 2019; Rees, Bishop, et al., 2014; Rees, Maddison, Middleditch, Patmore, & Gough, 2014;

Sigsgaard, Carl, Møller, & Thomsen, 2015). To advance eDNA for routine and reliable monitoring, methods need to undergo robust validation and demonstrate their ease of use. They should also reduce labor requirements and increase sensitivity over traditional survey methods (Goldberg et al., 2016).

Recent eDNA studies have detected *A. anguilla* through eDNA markers through both single species methods and metabarcoding (Cardeñosa, Gollock, & Chapman, 2019; Knudsen et al., 2019; Seymour et al., 2018; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). However, to monitor *A. anguilla* in freshwater lakes this study designed and validated a qPCR method in order to directly compare eDNA monitoring to the results of fyke net fishing for *A. anguilla*. Other comparative eDNA studies have been found to be more sensitive than fyke net fishing when detecting the presence or absence of common carp (Cyprinus carpio), red fin perch (Perca fluviatilis) and Oriental weatherloach (*Misgurnus anguillicaudatus*; Hinlo, Furlan, Suitor, & Gleeson, 2017) and crucian carp (*Carassius carassius*; Harper et al., 2019). A recent study that compared eDNA monitoring with electrofishing for Japanese eels *Anguilla japonica* in rivers, also found eDNA methods to be more sensitive at detecting riverine distribution of eels (Itakura et al., 2019).

This study focussed on *A. anguilla* in Irish lakes. The study compared a single species eDNA method for the detection of *A. anguilla* with paired fyke net surveys conducted by the IFI eel monitoring team during summer 2018. The method was developed with compliance to the minimum information for publication of quantitative real-time PCR experiments, MIQE guidelines (Bustin et al., 2009). The field validation of this eDNA method is described using filtered surface water samples collected from five freshwater lakes across Ireland. These lakes are classed as having high to low eel populations based on historic fyke net data. The potential application of monitoring eel populations in lakes using eDNA is discussed.

2 | METHODS

2.1 | Study species

The European eel, *A. anguilla*, is a migratory fish that spawns in the Sargasso sea (van Ginneken & Maes, 2005). The larval eels (leptocephali) are thought to drift on ocean currents toward European waters, a journey that can take between a year and maybe more than eighteen months (Bonhommeau, Castonguay, Rivot, Sabatié, & Le Pape, 2010; Friedland, Miller, & Knights, 2007; Lecomte-Finiger, 1994; Zenimoto, Sasai, Sasaki, & Kimura, 2011). Here, environmental triggers initiate the transformation of the larvae into glass eels (up to 5 cm long and transparent), which move into coastal estuaries and migrate upstream into freshwater becoming pigmented juveniles (elvers) and developing into the yellow eel (growth stage; van Ginneken & Maes, 2005). The pigmented yellow eels (growth stage) may spend 6–22 years here in a benthic sedentary stage, feeding, and growing until they start to reach sexual maturity (Arai, Kotake, & McCarthy, 2006). Once mature, the adult silver eels (reproductive stage) will move back downstream

TABLE 1 Site descriptions and water samples collected for eDNA analysis and results

Lake	Latitude	Longitude	Surface area (km²)	Maximum lake depth (mean depth) (m)	Water samples collected (% positive detection)				Trapping effort (fyke net days)	Eel population (IFI description prior to 2018)
					Shore[a]	Open lake[a]	Pre[a]	Post[a]		
Lough Corrib	53.4906	9.2913	176	42 (>4)[b]	17 (77)	2 (50)	16 (75)	8 (100)	16	High
White Lough	54.1092	−6.9875	0.54	6 (>4)[c]	3 (50)	2 (50)	3 (100)	X	3	Medium
Lough Derg	52.9056	−8.4050	130	36 (7.6)[d]	2 (100)	2 (100)	4 (100)	4 (75)	4	Medium
Lough Owel	53.5707	−7.3910	10.31	22 (>4)[e]	4 (75)	2 (100)	4 (100)	4 (100)	4	Low
Muckanagh Lough	52.9826	−8.9368	0.96	19 (3)[f]	2 (100)	2 (100)	2 (100)	2 (50)	2	Low

Note: Water sample collection was conducted concurrently with fyke net fishing surveys.

[a]Shore, open lake, pre- and post-refers to type of sample collection, shore, and open lake are non-net associated, pre and post are collected at the fyke net locations.

[b]Kelly et al. (2012a).

[c]Kelly et al. (2013a).

[d]Kelly et al. (2017).

[e]Kelly et al. (2012b).

[f]Kelly et al. (2013b).

into coastal waters and commence the lengthy migration back to the North Atlantic spawning sites. These migratory events are seasonal, with glass eels arriving in Ireland in significant numbers between January and May and the mature silver eels leaving between August and December (Russell Poole & Reynolds, 1998).

2.2 | Location of study

A number of lakes were scheduled for monitoring in 2018 as part of the larger IFI eel monitoring program and out of these lakes, six were chosen for this eDNA analysis. The lakes sampled for the study were categorized as high, medium, and low/absent eel populations. Unfortunately, Lower Corrib, which would have paired with White Lough (a medium population lake), was canceled due to a bereavement in the local fishing crew leaving just five lakes for the analysis (Table 1). The eel population designation is simple due to the variation in eel catches over time and the size of lake. Simple categories have been used in other eDNA detection studies (Ficetola, Miaud, Pompanon, & Tab erlet, 2008). Historically, Lough Derg (Shannon catchment) and Lough Corrib (Corrib catchment) supported a large commercial eel fishery and together with survey data indicated a large eel population. Lough Owel (Shannon catchment) was also the site of a commercial fishery but small in scale, and recent fyke surveys already indicated a declining population. Lough Muckanagh (Fergus catchment) and White Lough (Erne catchment) have been surveyed by IFI over the last 10 years and their classification is based on the results of those surveys. Sustained fish stocking has only been carried out in two systems in Ireland the Shannon (Lough Derg) and the Erne system (not included in this study). The Electricity Supply Board (ESB) carries out a program to assist the migration of eels by trapping elvers at the base of the stations and transporting them upstream. This program has been ongoing since the 1950s. Lough Derg and White Lough are located above these hydrostations and therefore do not have natural recruitment.

Four of the five lakes selected are within the largest three catchments in Ireland; Shannon, Erne, and Corrib. Loughs Derg and Corrib are in the lower reaches of the catchments, Lough Owel and White Lough are in the upper reaches. The location of the lake within a catchment does impact on the distribution of eels (Degerman, Tamario, Watz, Nilsson, & Calles, 2019) but the focus of this study was designed to test the eDNA method with specific levels of eel abundance (high, medium, and low). Within all lakes, the net depths were set <20 m, with the majority set <5 m (Table 1). Analysis of eDNA was undertaken in the lowest population lakes (Lough Owel and Lough Muckanagh) to determine whether eDNA sampling for *A. anguilla* is more sensitive than fyke netting.

2.3 | Field sampling

Sampling and filtration took place on fifteen days between June and September during 2018. Water samples were collected using a

boat on twelve days, at fyke-net locations before and after fishing and at independent open water lake locations not associated with fishing sites. An additional three days of water sampling was conducted from lake shores. In total, including the filtration controls, 110 water samples were filtered for eDNA analysis. Across all five lakes, 1 L samples were collected from 21 fyke net locations. Water samples were collected before nets were deployed (referred to as presamples) and again above the fyke nets 18–24 hr later (n = 42) before the nets were lifted (referred to as postsamples). At each lake, water samples were also taken at shore-side and open water locations not directly associated with fyke net trapping locations. To compare the eDNA recovery from 1 to 2 L water samples, 2 L samples (n = 27) were collected from Upper Corrib. Sampling took place over four days. On day one, 1 L presamples were collected at each of the eight fyke net locations and from open water and shore-side (non-net associated). On day two, 1 L postsamples were collected and the eel catch returned to shore. Then, later the same day an additional set of 2 L presamples were collected at new net locations along with open water and shore-side (non-net associated) samples. Poor weather precluded boat-based sampling on days 3 and 4, so 2 L

water samples were collected on foot from accessible points along the shore-side at the same locations on both days. Sample locations can be found in Figure 1. Temporal autocorrelation is highly unlikely to be a significant factor in the data as eDNA concentrations are not expected to change significantly within the water column over a 24 hr time horizon.

Sample collection was standardized for both one- and two-liter water samples. Wearing clean disposable nitrile gloves, the sample bottle was briefly rinsed using water from the lake. The water bottle was immersed at the water surface until full. The bottle was labeled with the time of collection and the sample location recorded using GPS. Where water samples were collected from the shore-side specific care was taken to avoid disturbing lake sediments.

Filtering of water samples was conducted at temporary field laboratories within eight hours of collection. A Masterflex[tm] peristaltic pump head (Cole-Parmer) was operated using a generic cordless drill. This drew a single water sample via silicone tubing through a closed in-line filter holder (Sarstedt) containing a 49 mm cellulose nitrate 3 μm pore filter (Whatman[®]). Wearing clean gloves and using bleach-treated forceps, each filter was transferred into a 2 ml screw
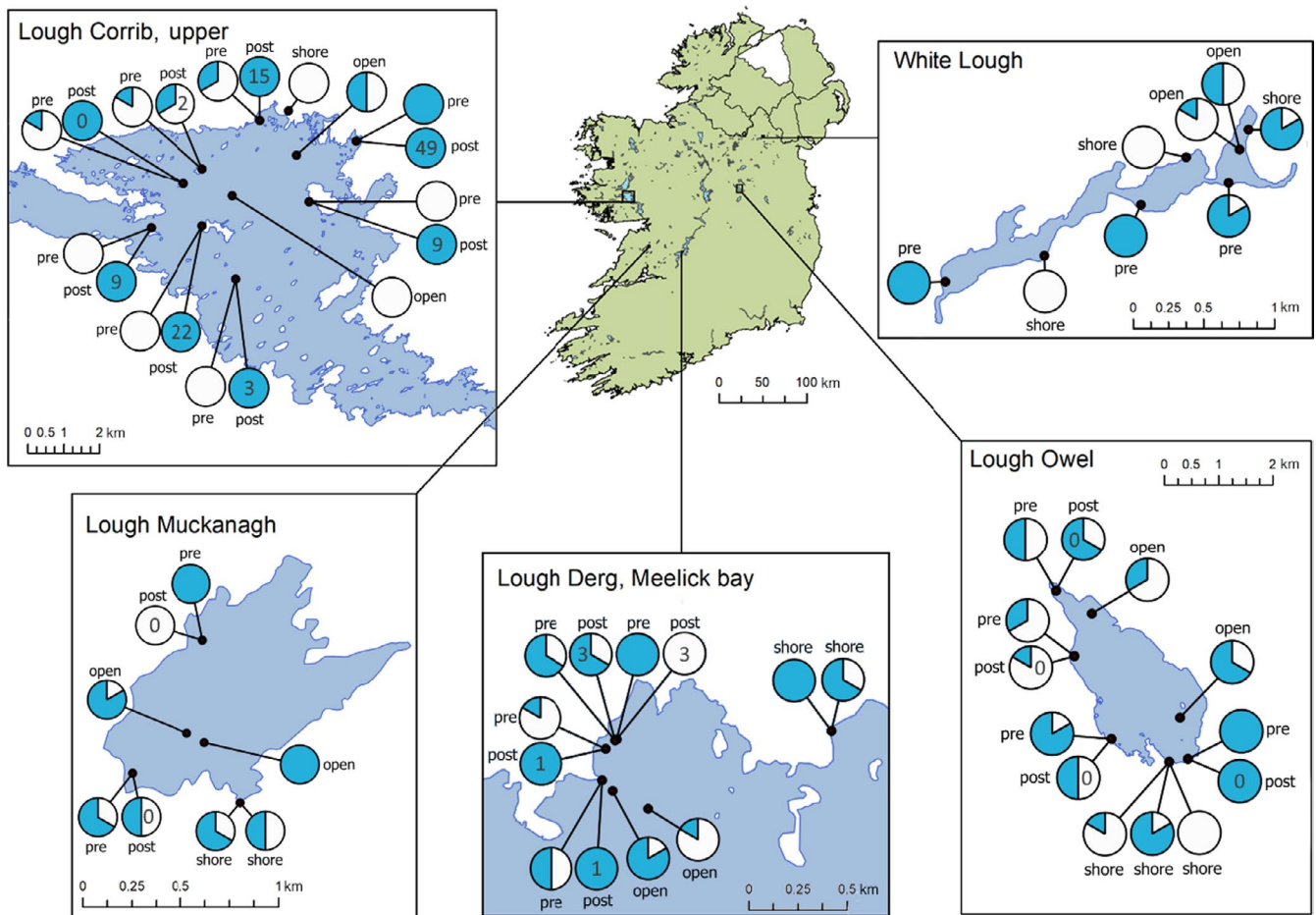
FIGURE 1 Map of Ireland with the locations of the five freshwater lakes sampled. Within each lake, the pie charts indicate the location of 1 L water sample locations. Positive *Anguilla anguilla* eDNA PCR replicates (n = 6) are in blue and negative are white for each water sample. The labels, shore, open, pre and post describe the type of sample collection, shore, and open lake are non-net associated, pre and post are collected at the fyke net locations before the nets are set and the following day before the nets are lifted. Numbers on the postnet samples indicate the number of eels caught

cap sample vial that contained 800 µl of Longmire's storage buffer (Williams, Huyvaert, & Piaggio, 2016). Occasionally suspended solids blocked the filter membrane, when this occurred it was replaced with a second filter and the remainder of the water sample filtered. The two filter papers were rolled together and placed into a single vial thus all filtered samples were stored in the same standard volume of buffer. On no occasion were more than two filters required. After each filtration procedure, the filter housing and tubing were completely submerged in a 10% bleach (sodium hypochlorite) solution for 20 min, then transferred and completely submerged in cold tap water to remove the bleach. Each filtering batch included a filtration control of 1 L tap water (filtration negative control). The labeled vials with the filter paper and Longmire's buffer were stored at ambient temperature and transferred to the laboratories at the University of the West of England, Bristol (UWE) for analysis.

2.4 | eDNA extraction

Total DNA was recovered from the filter and buffer using a phenol:chloroform:isoamyl alcohol-based extraction (Renshaw, Olds, Jerde, Mcveigh, & Lodge, 2015; Sambrook & Russell, 2001). Proteinase K 400 µg/ml (New England Biolabs) was added to each tube before proceeding with an overnight incubation at 55°C. Then, 800 µl phenol:chloroform:isoamyl alcohol (24:24:1, Sigma-Aldrich now Merck) was added to each tube and incubated at room temperature for 30 min with occasional vortex mixing, the tubes were centrifuged for 5 min at 15,000 g, 4°C. The supernatant (600 µl) was transferred to a clean DNA-free 1.5 ml Eppendorf tube and an equivalent volume of propan-2-ol (Fisher Scientific) added. The tubes were gently mixed to precipitate the DNA then centrifuged for 5 min at 15,000 g, 4°C. The supernatant was removed and the pellet washed using 100% ethanol followed by 70% ethanol (both Fisher Scientific). The DNA pellets were then allowed to dry at 45°C for 30 min. Each batch of DNA extractions included a blank extraction of 800 µl of Longmire storage buffer (extraction negative control) to indicate any DNA carryover. The dried DNA pellets were rehydrated in 100 µl TE buffer (10 mM Tris-HCl containing 1 mM EDTA, Sigma-Aldrich now Merck) and stored at −20°C until qPCR amplification.

2.5 | Design of species-specific qPCR primers and probes

Anguilla anguilla mitochondrial cytochrome b sequences were obtained from NCBI Genbank and aligned using MEGA version 10.0.4 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Species-specific primers and probe were identified using the NCBI primer-BLAST feature (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and optimized visually for qPCR. Specificity of the primers was determined in silico using the primer-BLAST tool. This showed no risk of cross-amplification with nontarget species and closely related Anguillid species. The primers' specificity was next challenged in vitro by amplification

with genomic DNA extracted from UK and Irish fish species likely to be encountered during sampling and DNA from the closely related freshwater eel species *Anguilla rostrata* (Table S1). The third specificity check was a confirmation sequencing step of environmental-derived positive PCR products to establish that the correct *A. anguilla* cytochrome b fragment was being amplified.

2.6 | Quantitative real-time PCR

All of the DNA extractions were assayed for *A. anguilla* eDNA in a qPCR, using specific primers and a hydrolysis probe on a StepOne Plus™ Real-Time PCR System (Thermo Fisher Scientific). Plates were prepared in a clean laboratory using a dedicated PCR workstation previously decontaminated using UV light for 30 min. The limit of detection (LOD) for the qPCR primer-probe set was established using a standard method previously described by Broeders et al. (2014). Briefly, a serial dilution series was prepared of a 998 base pair synthetic gene copy of the *A. anguilla* cytochrome b gene (Integrated DNA Technologies) from which the exact copy number of qPCR amplicons was determined. Using this, two forms of LOD were calculated. The LOD_6, which is determined by identifying the last dilution in a series of sixfold dilutions where all six PCR replicates give rise to specific and positive amplification. The second the $LOD_{95\%}$ determines the specific sensitivity of the primer set with a 95% confidence level. This was obtained by amplification of 60 replicates of the lowest serial dilutions around the LOD_6. For this primer-probe set, both LOD_6 and $LOD_{95\%}$ was twelve haploid genome equivalents per reaction.

During sample analysis each reaction contained, 2× qPCRBIO probe mix (PCRBiosystems), 200 nM forward primer Aangcytb1F 5′–3′ TTGCCCTATTCTACCCGAACC, and 200 nM reverse primer Aangcytb1R 5′–3′ ACAAGGCTAATACCCCGCC, 100 nM fluorescently labeled probe Aangcytb1P, 5′–3′ TTGGAGACCCAGACAACTTCACCCCGGCA (Integrated DNA Technologies), 6 µg/µl bovine serum albumin and DMSO 1.25% v/v (both Sigma-Aldrich now Merck). The reactions were made up to 13.5 µl with sterile distilled water and the qPCR plate moved to the DNA bench for the addition of 1.5 µl DNA sample to each well. Six replicates were amplified for each sample and every plate included three, no template controls of 1.5 µl sterile distilled water. The reactions were run on a fast presence/absence test using the following cycling parameters; 2 min denaturation at 95°C, followed by 40 cycling steps of 5 s at 95°C and 20 s at 59°C. Each plate included a prepared serial dilution of standard of *A. anguilla* genomic DNA (extracted from *A. anguilla* muscle tissue using the protocol described earlier), in triplicate. The concentration of the standard was confirmed using a Qubit 4.0 immediately prior to qPCR. The standards, typically seven per plate, provide a regression line from which the unknown quantities of the DNA extracts can be estimated. A positive result was recorded for each sample if amplification reached the C_t threshold in one or more of the six replicates. Positive qPCR products representative of environmental water samples were retained;

these were cleaned using a Qiaquick PCR purification kit (Qiagen, N.V.) quantified using a Qubit 4.0 (Thermo Fisher Scientific) and sent to Aberystwyth University for Sanger sequencing. The amplicons were derived from samples collected during the first weeks at Lake Corrib and Lough Owel. A range of PCR concentrations (0.001–1.016 ng/μl) were included from samples of which between one and six of the replicates (*n* = 6) had amplified. The sequence data were visualized, checked for miscalls and aligned using MEGA version 10.0.4 (Kumer et al., 2018). Sequences were then compared to *A. anguilla* sequence on the National Centre for Biotechnology database using the basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequenced amplicons represented 14% of the positive field samples and all confirmed the correct *A. anguilla* target had been amplified (Table S2).

2.7 | Fyke net survey

IFI conducts an annual program of fyke net surveys for *A. anguilla* to monitor the eel population, determine the health status and collect morphometric data. The surveys take place in freshwater lakes between June and September, using open motorized fishing boats to set the Dutch fyke nets. The sampling effort is standardized by IFI and involves setting fyke nets linked by the cod end to form chains of three or five nets. The nets are left in place for 18–24 hr.

During this study, the total catch from each net was returned to shore where the fish were weighed and counted before being returned to the lake (Table 2). All eels caught were yellow eels (growth stage) specimens. A proportion of the catch were randomly selected by IFI to collect morphometric data. This included total length (to nearest mm), weight (to nearest g), horizontal eye diameter (mm), vertical eye diameter (mm), pectoral fin length (mm), diameter of head and pigmentation (data not presented). These eels were then released back to the lake.

2.8 | Data analysis

Statistical relationships were analyzed using Gaussian generalized linear mixed models with identity links. During analysis, residuals from each model were visually inspected to ensure normality, homogeneity of variance and linearity. The relationship between eel biomass in individual fyke nets and mean eDNA concentration in the paired post-net samples was modeled using biomass as a covariate and lake as a random factor. To determine whether eDNA concentrations predict overall biomass within individual lakes, a critical question for fisheries management, the mean eel biomass was calculated from all fyke nets for each lake. This was included as a covariate nested within lake, with lake being included as a random factor. Variation between the concentrations obtained from individual PCR replicates from each water sample was controlled for by including sample as a further random factor. This model also included sampling location (shore-side or open water) as a fixed factor. The influence of the amount of water filtered

TABLE 2 Summary table of *Anguilla anguilla* catch data from 2018 season, for the five lakes included in the eDNA survey

Lake	Total number of eels 2018	Number of eels caught during eDNA survey	Total weight (kg)	Mean length (cm)	Min. length (cm)	Max. length (cm)	Mean weight (kg)	Min. weight (kg)	Max. weight (kg)
Lough Corrib Upper	226	51	52.6	50.3	32.3	86.9	0.23	0.07	1.35
Lough Derg	511	8	100.6	47.2	26.5	75.6	0.2	0.06	1.04
White Lough	24	24	7.1	54.74	45.5	67	0.29	0.12	0.59
Lough Owel	0	0	NA	NA	NA	NA	NA	NA	NA
Lough Muckanagh	1	0	0.20	49.4	49.4	49.4	0.20	0.20	0.20

Note: Data is the combined data from two fyke net surveys conducted during 2018.

WELDON ET AL.

Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences
Open Access
WILEY 7

(1 vs 2 L samples) on concentrations of DNA was modeled separately using volume filtered as a fixed factor and sample as a random factor. All analysis was carried out using the lme4 package (Bates, Mächler, Bolker, & Walker, 2015) in R v.3.5.2 (R Core Team, 2019). Marginal R^2 values are reported for mixed models. Figures were prepared using SPSS 24.0 (IBM corp.) and ArcMap 10.4 (ESRI).

3 | RESULTS

3.1 | eDNA sampling in freshwater lakes identifies the presence of European eels

The data demonstrate that *A. anguilla* can be detected in Irish lakes using this single species eDNA method. *A. anguilla* eDNA was detected in 83% (70/84) of all surface lake water samples, indicated by successful amplification in one or more of the six PCR replicates for each sample (Figure 1). All lakes and sample types, regardless of eel population sizes produced both positive and negative qPCR reactions in all sample types collected pre, post netting, shore and open water.

In the laboratory analysis, all extraction and PCR negative controls tested negative with no evidence of contamination. The PCR efficiency ranged from 94.04% to 99.67%, the slope ranged from −3.33 to −3.53 and the y-intercept from 22.36 to 27.36. The R^2 values ranged from 0.98 to 0.99. Samples that gave rise to a negative result were tested by re-amplification with 1 µl 0.03 ng/ml *A. anguilla* genomic DNA. This indicated that failure to amplify was not due to environmental inhibition, and that these samples were likely to be either negative or below the detection limits (LOD_6 and $LOD_{95\%}$) of the qPCR for field samples of *A. anguilla* eDNA.

3.2 | The eDNA concentrations at net locations reflected the eel biomass recovered in fyke nets

The abundance of *A. anguilla* was established by eel catches in the fyke nets deployed, 67% (14/ 21) of nets deployed caught eels, all growth stage specimens. All eel catches were from lakes historically described as having high (Upper Corrib and Derg) and medium (White Lough) eel populations. No eels were caught during the paired surveys at lakes described as having low eel populations (Lough Owel and Lough Muckanagh). In addition, two nets in the high eel net locations (one at Lough Corrib and one in Lough Derg) did not catch eels.

There was a significant positive relationship between eel biomass in individual fyke nets and the mean eDNA concentration (calculated from $n = 6$ amplifications) in the paired postnet samples ($F_{1,3} = 321.51$, $p < .001$, $r^2 = .950$). While the relationship is highly influenced by a single large eel catch (Figure 2), the significant positive relationship between biomass and eDNA concentration remains when the same model is run excluding this data point ($F_{1,3} = 10.78$, $p = .046$).

3.3 | Lake eDNA concentrations predict average biomass

The influence of the *A. anguilla* biomass and sample location (shore-side vs open water) were analyzed. The eDNA concentrations predicted the average biomass retrieved from each lake ($F_{1,3} = 20.60$, $p = .020$, $r^2 = .425$; Figure 3). Furthermore, the eDNA did not differ significantly between samples collected shore-side and from open water ($F_{1,3} = 1.60$, $p = .295$; Figure 4).

FIGURE 2 *Anguilla anguilla* eDNA concentration in 1 L postnet samples and the biomass (kg) of eels subsequently recovered by fyke net at those net locations ($n = 18$). White Lough postnet samples and catch data were removed from the dataset due to detection of eDNA in the filtration control. Note the log scale on the y-axis and two data points of low eel catches with no eDNA detected. IFI categorizations of eel population size in each lake are given in brackets
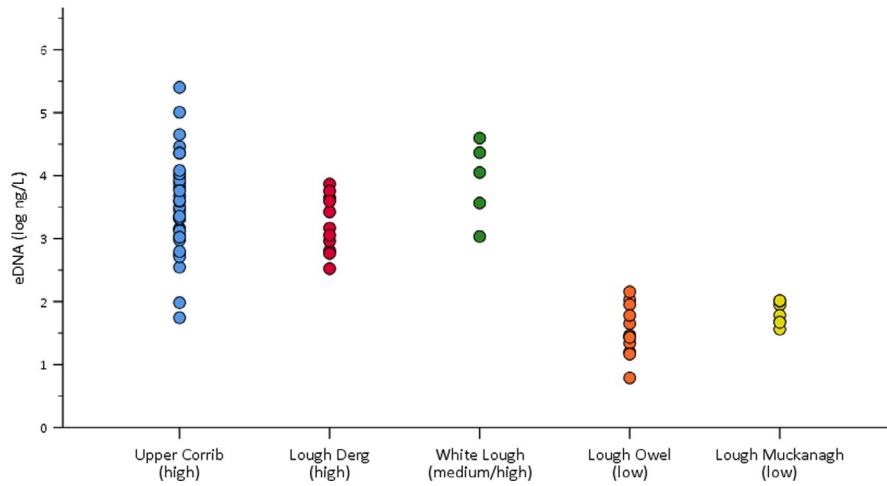
FIGURE 3 Quantity of eDNA recovered by location for each lake. Each data point represents a water sample from which *Anguilla anguilla* eDNA was recovered. IFI categorizations of eel population size in each lake are given in brackets
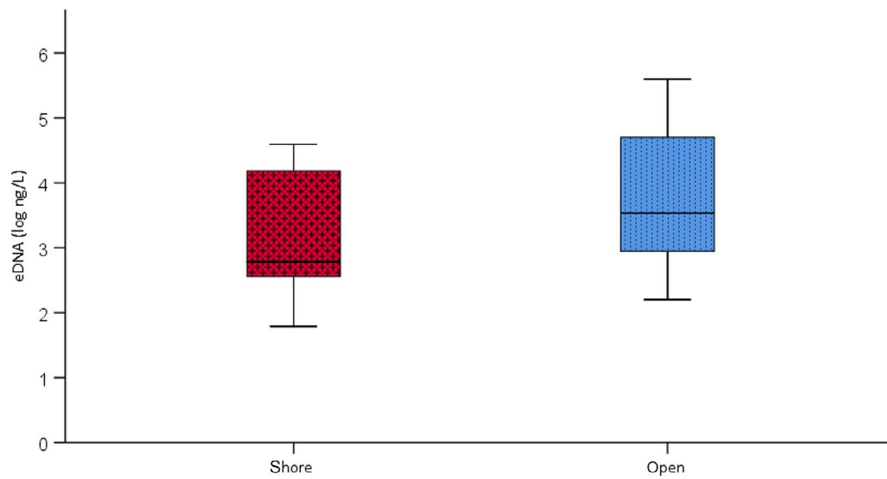

FIGURE 4 Comparison of eDNA quantities (log-transformed) in 1 L water samples (*n* = 40) collected from shore-side (*n* = 11) and open water (non-net associated and prenet, *n* = 29) from all five lakes. The eDNA quantities are log-transformed for visual clarity, the box plot represents quartile range of the data
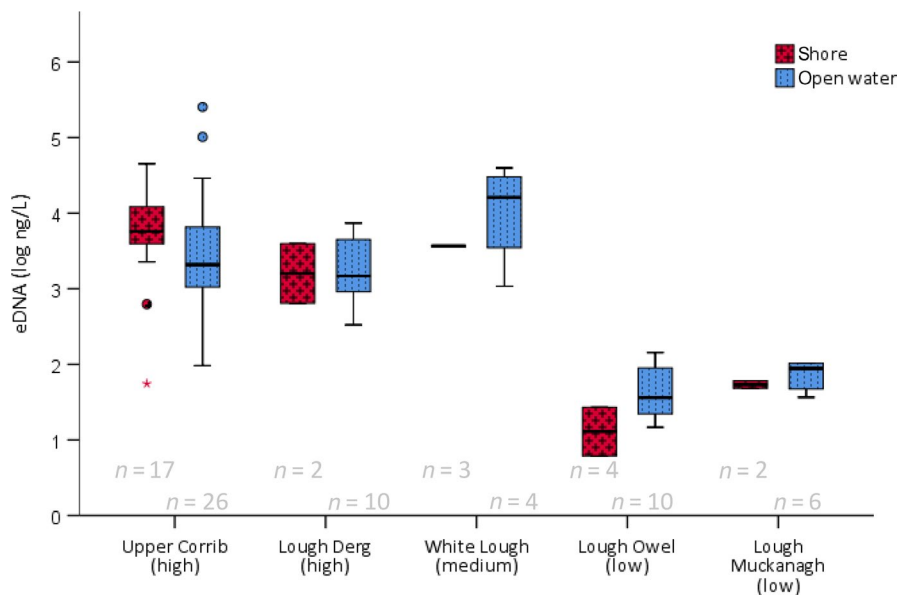
FIGURE 5 Quantity of eDNA in 1 L samples from shore-side and open water samples shown at all five lakes. The box plots describe log-transformed data represented as quartile ranges. Here, * is more than three times the interquartile range (extreme outlier), and ° is more than 1.5 times the interquartile range (outlier). IFI categorizations of eel population size in each lake are given in brackets

At two lakes, Lough Owel and Lough Muckanagh, no eels were caught using fyke nets during this monitoring period (Table 2). However, *A. anguilla* eDNA was detected in 91% (*n* = 23) of all the 1 L water samples (including shore-side and open water, pre- and postnetting) collected from these lakes (Figures 1 and 3). The eDNA quantities recovered in these 1 L water samples from low eel

populations, was much lower than in the other lakes (Figure 5). Fyke netting at Lough Muckanagh subsequent to this study confirmed eel presence within the lake.

3.4 | Sample volume

We tested whether the volume of water filtered influenced the concentrations of DNA using 1 and 2 L samples taken at Upper Corrib. While there was more eDNA in the 2 L samples than the 1 L samples where eDNA detection occurred there was no significant difference in the quantity of eDNA recovered per L between 1 and 2 L samples ($F_{1,25}$ = 0.65, p = .428, r^2 = 0.013; Figure S1).

3.5 | Contamination control

A potential challenge with eDNA sampling is cross-contamination of the samples. Extensive contamination controls were employed at each step of the eDNA analysis which were negative for all laboratory steps. All of the sample controls (filtration; DNA extraction; PCR amplification) from the low population lakes were negative. Field contamination occurred on one occasion (White Lough, discussed further below). All samples associated with this filtration batch were removed from the dataset.

4 | DISCUSSION

4.1 | The detection of *Anguilla anguilla* eDNA in freshwater lakes

The primers and probe designed for this study were able to detect eDNA of *A. anguilla* origin in all five freshwater Irish lakes. Although the detection of *A. anguilla* eDNA has been previously reported in single species and metabarcoding detection studies (Seymour et al., 2018; Takeuchi et al., 2019; Thomsen, Kielgast, Iversen, Møller, et al., 2012), it was not applied for field monitoring. There is significant homology between the mtDNA of *A. anguilla* and *A. rostrata*—its North American counterpart. In an eDNA study to detect marine fish, Knundsen et al. (2019) were unable to distinguish eDNA of *A. anguilla* from *A. rostrata*. This study targeted a conserved region of the cytochrome b gene where heterology could be found between the two species. By concentrating on the specificity of the primers, which Wilcox et al., (2013) reported to be more important than the specificity of the probe, in silico analysis indicated these primers as unique for *A. anguilla*. During specificity tests in the laboratory samples of freshwater fish collected from UK and Irish locations and *A. rostrata* sourced from N. America confirmed the specificity of these primers and did not amplify using the primer-probe set (Table S1). However, two additional samples from eels identified as *A. rostrata* caught in a European lake (most likely derived from a restocking event) did weakly amplify using these primers and probe.

For this study, we relied in part, upon the natural geographical distribution of these two species, with the chance of encountering naturally occurring *A. rostrata* in the Irish lakes sampled being highly. Globally, the movement of anguillid species is a concern and there are reports of *A. rostrata* in Europe (Frankowski et al., 2009). It is suspected that *A. rostrata* located in Europe are introductions and a result of imported eels for stocking and aquaculture. Ireland has never imported glass eels as part of a stocking program. Large *A. anguilla* stocking programs have only been carried out in two catchments in Ireland, the Shannon, and the Erne system. All stocking that was carried out in these rivers was the movement of locally sourced glass eels from below the hydrostations, supplemented with catches from neighboring catchments but only in the River Shannon. Therefore, the risk of *A. rostrata* being present in Ireland is very low.

4.2 | Using eDNA to monitor *Anguilla anguilla* in freshwater lakes

In this study, five freshwater lakes in Ireland were simultaneously monitored for European eels using fyke nets and a single species eDNA technique. The sampling took place between June and September 2018, in freshwater lakes categorized by IFI into high, medium, and low eel populations based on historical catch data. This study shows that *A. anguilla* eDNA can be successfully recovered and amplified from freshwater lakes (Figure 1). There was a clear relationship between the overall quantity of eDNA recovered and eel biomass in the lakes. The eDNA concentrations recovered from the five lakes compared well to their high and low eel population descriptions.

Simple sampling from surface waters is another positive aspect for applying eDNA methods for monitoring. Taking samples at the surface are logistically an easier method than taking samples at depth. Collecting samples at depth requires specialist equipment and disturbing the lake sediments must be avoided which can be difficult to control for from a small boat. Hinlo, Furlan, et al. (2017) looked at the DNA copy number of benthic weatherloach and found no effects sampling water at the surface or subsurface. Furthermore, other studies of benthic species have collected samples taken at the water surface (Forsström & Vasemägi, 2016; Wilson et al., 2014) and successfully detected the target species. Notably, in order to standardize eDNA sampling during this study water samples were always collected at the surface and the eDNA results obtained compared favorably with the eel population fyke net survey descriptions.

During summer months in deep lakes, stratification can occur, resulting in minimal mixing between the surface waters and the layers below. Lawson Handley et al. (2019) found a greater spatial structure to the distribution of fish eDNA sampled in Lake Windemere during the summer months than in Winter. In addition, yellow eels (growth-phase) are demersal fish, and generally found in the lowest benthic region of rivers or lakes (Capoccioni, Lin, Iizuka, Tzeng, & Ciccotti, 2014). Taken together, it would be reasonable to suggest that eDNA sampling for *A. anguilla* should

be from lower lake depths. Unless that is, the lakes are shallow and mixing of the water column is occurring. In this study, the majority of nets were set in shallow water (<5 m) in Lough Derg, Lough Muckanagh, Lough Owel, and White Lough. The eDNA comparisons with the nets set in these shallow lakes are good, it may be because there are unlikely to be strong lake thermoclines at these depths. The nets in Lough Corrib were a mix of depths but all less than 20 m. By sampling DNA at the surface in the summer, our results should not necessarily link to the net depths at 20 m (especially as it was in the summer). The fact they do potentially demonstrates that a seasonal thermocline was not limiting for this surface eDNA surveys for demersal species, including eels. With the nets set at different depths (by the nature of the lake floor) both across a lake and between lakes, standardizing the eDNA sampling at the water surface also reduced the variation between samples.

In the first week of sampling, both 1 and 2 L water samples were collected for method validation. Sample collection was constrained by the fishing requirements (i.e., limited by time and space on the boat). Collecting 2 L samples in particular was difficult in open water while leaning out from the boat. These samples took longer to collect, longer to filter and the additional weight in the boat made collection more hazardous. With early analysis demonstrating that while more eDNA was recovered in the 2 L samples, the eDNA quantities per L were not significantly different (Figure S1), for the remainder of the study 1 L samples were collected.

At White Lough, historically a medium eel population lake, eDNA concentrations were higher than anticipated, with values similar to those at Upper Corrib and Derg (historically classified as high populations). Possible reasons for this could include; a cross-contamination event between lakes, different sizes of the lakes influencing eDNA concentrations or fyke net sampling providing unrepresentative estimates of the true population density. A system of controls both in the field and laboratory was used to detect cross contamination. Field contamination occurred on only one occasion when a single positive amplification (*n* = 6) was detected in the filtration batch controls at White Lough on day two. All samples associated with this batch were removed from the dataset. Therefore, we are confident that cross-contamination was not the cause of this result. It may be possible that the concentrations of eDNA measured at White Lough were influenced by the size of the lake, as Derg and Upper Corrib are considered to be large lakes (11,519 and 11,650 ha respectively) compared with White Lough which is a much smaller lake (54 ha). However, Lacoursière-Roussel, Côté, Leclerc, and Bernatchez (2016) sampled twelve lakes varying in size from 44 to 6702 ha and found no correlation between eDNA concentration of lake trout *Salvelinus namaycush* and lake size; therefore, it seems unlikely that lake size has played an important role in our study. It is possible that fyke net catches do not represent the true population density of a lake and/or are highly impacted by environmental stochasticity, particularly when sample sizes are low. A high number of eels (450 eels caught over three nights) were caught in fyke nets in an earlier

fishing survey in June, whereas following prolonged periods of dry and hot weather only eight eels were caught in fyke nets in August with a similar level of trapping effort. If our eDNA, findings are accurate it is possible that the eDNA survey is representative of the higher eel numbers observed in the June catch and the later fyke survey is an under representative estimate. Further sampling by fyke nets and by eDNA is needed to confirm that White Lough has changed from a medium to high eel population lake in 2018.

In the two low eel population lakes, 91% of the water samples collected were positive for *A. anguilla* eDNA. These lakes were included in this study specifically to determine if eDNA sampling may be more sensitive than fyke netting especially if catches are low and infrequent. Traditionally, both lakes had been commercially fished for *A. anguilla* but long-term monitoring by IFI over the past 10 years has indicated that eel populations are declining in these small upstream lakes, with very low numbers of eels recorded in recent surveys (Table 1). In 2002, Ibbotson, Smith, Scarlett, and Aprhamian (2002) predicted that the decline in recruitment of *A. anguilla* would lead to a decrease in the proportion of eels moving into these freshwater habitats. Although fyke net sampling failed to catch any eels in these lakes (during the eDNA survey), both the pre- and post-eDNA samples indicated the recent presence of eels at multiple locations around the lakes. Furthermore, the positive *A. anguilla* eDNA results were confirmed at Lough Muckanagh in a repeat fyke net survey. We recovered eDNA from these lakes with a high degree of repeatability suggesting that eDNA can be more sensitive for assessing species presence than fyke net sampling when populations are low. Other freshwater eDNA studies comparing eDNA sampling to established sampling methods have also reported an increased sensitivity of detection, including for electrofishing of *A. japonica* in rivers (Itakura et al., 2019) and for other fish species in both lakes and rivers (Hinlo, Gleeson, Lintermans, & Furlan, 2017; Lacoursière-Roussel et al., 2016; Ogburn et al., 2018; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012).

It was a difficult undertaking to conduct the eDNA sampling alongside fyke net fishing and a testament to the careful adherence to collection methods that cross contamination occurred only once during the study. The eDNA methods are highly sensitive, in the laboratory under optimal LOD conditions the primer and probe set are able to amplify as few as twelve haploid genome equivalent copies of target mitochondrial DNA.

4.3 | Variation in eDNA concentrations between lakes

Although the mean eDNA quantities differed between the lakes, within each lake the eDNA quantities did not significantly differ between samples collected either at the shore-side or from the open water locations (Figure 5). As well, the collective relative eDNA concentrations for each lake compared well to the fyke net categories (Figures 3 and 5). This raises two points concerning the distribution of eels within each lake and category (high, medium, and low). If the

eel density across the lakes is evenly distributed, then simple shore side sampling is sufficient to inform the lakes general population, potentially a key finding. However, if eel density is not evenly distributed then this survey strategy was not reflective of local differences in the numbers of eels.

Exactly, how eels behave in lakes is still poorly understood, different sampling methods conclude differing distributions. Using a baited longline, Yokouchi, Aoyama, Miller, McCarthy, & Tsukamoto, 2009, found the eel density (CPUE) in a lake in Ireland was highest in the deepest water (open water) than shallow water (shore-side). Conversely, at Upper Corrib during this (unbaited) fyke net survey the highest number and biomass of eels were caught in the shallowest nets set at 0.5 m, and no eels were caught in the deepest nets set at 16 m (Figure S2). To understand the distribution of eels in lakes through an eDNA survey, it is likely that a completely different sampling strategy is required. The data hint that it may be possible to reflect local eel abundance within each lake. Figure 2 shows the eDNA concentrations from samples collected above nets with eels and suggests eDNA concentrations do reflect the number of eels contained below. However, samples collected at net locations before the nets were set did not always predict subsequent eel catch (Figure 1). Most likely other factors are having an effect that were not measured during this study and an intensive standardized sampling effort would make a useful future study—again paired with comprehensive capture methods. Determining a link between eDNA concentrations and eel abundance in the field will ultimately allow a more accurate assessment of eel presence and abundance.

The aim for this study was to confirm the presence or absence of eels in freshwater lakes. In addition, the data confirmed the comparative population status (determined through fyke net surveys) of the lakes through relative eDNA concentrations in the water samples. Both survey methods (fyke nets and eDNA) provide valuable and complementary information and together can provide a robust sampling regime. This is particularly important as the distribution and recruitment of *A. anguilla* is so low (Dekker, 2004; Henderson et al., 2012).

4.4 | Method validation to inform management practise

For eel management programs, using fyke net catch data alone can be problematic. The fyke net is a biased sampling method, the size of the eels caught and retained is determined by the mesh size of the cod end (usually eels larger than 25 cm). It is only the eels caught that are over 30 cm where 100% of the catch is retained (Bevacqua, De Leo, Gatto, & Melià, 2009). In this study, the fyke cod-end mesh size was 12 μm and the eels captured ranged from 26.5 to 86.9 cm (Table 2). If eel numbers are low and netting is infrequent the survey may fail to catch any eels, leading to a false zero (Martin et al., 2005) or when the distribution of eels is patchy within a lake this can result in highly variable catches (Rose & Kulka, 1999).

In low eel population lakes, where fyke net surveys no longer catch eels, switching to eDNA methods has the potential to confirm local extinction or to detect an upturn and re-establishment of a population. This will be particularly useful for habitat remediation works and barrier mitigation where assessment of recruitment of this critically endangered species without using invasive capture measures will be valuable.

In conclusion, this study compared single species *A. anguilla* eDNA survey method to a fyke net survey. The eDNA method is quick and easy to perform in the field and samples can be collected from the shore as well as from a boat by staff with minimal training. Sampling could be conducted shore-side and was more sensitive than fyke nets in detecting low eel populations. The increased sensitivity of eDNA methods will be a useful additional tool to guide survey design and effort. Furthermore, repeated use of eDNA surveys over seasons would build a clearer picture of changing eel abundance to compliment fyke net catch data. While the eDNA method cannot replace the fyke net surveys, this method is a more sensitive indicator of eel presence and particularly useful for monitoring population advances or retreats within freshwater systems and for enabling rapid assessments of populations across more locations than is possible using live-capture techniques.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

LW, CO, MS, HM, LN, and SS conceived the study and designed the methodology; LW and CO collected the data; LW, MS, and LN analyzed the data; LW led the writing of the article. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

The sequence data generated for this study are available in the supplementary data. The eDNA dataset analyzed during the current study is available from the University of the West of England data repository http://researchdata.uwe.ac.uk/571.

ORCID

Laura Weldon https://orcid.org/0000-0002-0761-4414

Ciara O'Leary https://orcid.org/0000-0001-8740-9455

Mark Steer https://orcid.org/0000-0002-0528-2874

Lyn Newton https://orcid.org/0000-0001-5132-4185

Heather Macdonald https://orcid.org/0000-0001-6227-6684

Stephanie L. Sargeant https://orcid.org/0000-0002-9007-7583

REFERENCES

Agersnap, S., Larsen, W. B., Knudsen, S. W., Strand, D., Thomsen, P. F., Hesselsøe, M., … Møller, P. R. (2017). Monitoring of noble, signal and narrow-clawed crayfish using environmental DNA from freshwater samples. *PLoS ONE*, *12*, e0179261. https://doi.org/10.1371/journal.pone.0179261

Arai, T., Kotake, A., & McCarthy, T. K. (2006). Habitat use by the European eel *Anguilla anguilla* in Irish waters. *Estuarine, Coastal and Shelf Science*, *67*(4), 569–578. https://doi.org/10.1016/j.ecss.2006.01.001

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bevacqua, D., De Leo, G. A., Gatto, M., & Melià, P. (2009). Size selectivity of fyke nets for European eel *Anguilla anguilla*. *Journal of Fish Biology*, *74*(9), 2178–2186. https://doi.org/10.1111/j.1095-8649.2009.02243.x

Bonhommeau, S., Castonguay, M., Rivot, E., Sabatié, R., & Le Pape, O. (2010). The duration of migration of Atlantic Anguilla larvae. *Fish and Fisheries*, *11*(3), 289–306. https://doi.org/10.1111/j.1467-2979.2010.00362.x

Broeders, S., Huber, I., Grohmann, L., Berben, G., Taverniers, I., Mazzara, M., … Morisset, D. (2014). Guidelines for validation of qualitative real-time PCR methods. *Trends in Food Science and Technology*, *37*(2), 115–126. https://doi.org/10.1016/j.tifs.2014.03.008

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., … Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Butts, I. A. E., Sørensen, S. R., Politis, S. N., & Tomkiewicz, J. (2016). First-feeding by European eel larvae: A step towards closing the life cycle in captivity. *Aquaculture*, *464*, 451–458. https://doi.org/10.1016/j.aquaculture.2016.07.028

Capoccioni, F., Lin, D.-Y., Iizuka, Y., Tzeng, W.-N., & Ciccotti, E. (2014). Phenotypic plasticity in habitat use and growth of the European eel (*Anguilla anguilla*) in transitional waters in the Mediterranean area. *Ecology of Freshwater Fish*, *23*, 65–76. https://doi.org/10.1111/eff.12049

Cardeñosa, D., Gollock, M. J., & Chapman, D. D. (2019). Development and application of a novel real-time polymerase chain reaction assay to detect illegal trade of the European eel (*Anguilla anguilla*). *Conservation Science and Practice*, *1*(5), e39. https://doi.org/10.1111/csp2.39

Degerman, E., Tamario, C., Watz, J., Nilsson, P. A., & Calles, O. (2019). Occurrence and habitat use of European eel (*Anguilla anguilla*) in running waters: Lessons for improved monitoring, habitat restoration and stocking. *Aquatic Ecology*, *53*(4), 639–650. https://doi.org/10.1007/s10452-019-09714-3

Dekker, W. (2000). The fractal geometry of the European eel stock. *ICES Journal of Marine Science*, *57*(1), 109–121. https://doi.org/10.1006/jmsc.1999.0562

Dekker, W. (2003). On the distribution of the European eel and its fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*, 787–799.

Dekker, W. (2004). *Slipping through our hands Population dynamics of the European eel*. PhD thesis. Amsterdam: University of Amsterdam. Retrieved from http://www.diadfish.org/doc/these_2004/dekker_thesis_eel.pdf.

Dekker, W. (2016). Management of the eel is slipping through our hands! Distribute control and orchestrate national protection. *ICES Journal of Marine Science: Journal Du Conseil*, *73*(10), 2442–2452. https://doi.org/10.1093/icesjms/fsw094

European Union Eel Regulation (2007). Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. *Official Journal of the European Union*, *248*(1100/2007), 17–23.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*, 423–425. https://doi.org/10.1098/rsbl.2008.0118

Forsström, T., & Vasemägi, A. (2016). Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea? *Marine Pollution Bulletin*, *109*(1), 350–355. https://doi.org/10.1016/j.marpolbul.2016.05.054

Frankowski, J., Jennerich, S., Schaarschmidt, T., Ubl, C., Jürss, K., & Bastrop, R. (2009). Validation of the occurrence of the American eel Anguilla rostrata (Le Sueur, 1817) in free-draining European inland waters. *Biological Invasions*, *11*(6), 1301–1309. https://doi.org/10.1007/s10530-008-9337-8

Friedland, K. D., Miller, M. J., & Knights, B. (2007). Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. *ICES Journal of Marine Science*, *64*(3), 519–530. https://doi.org/10.1093/icesjms/fsm022

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., … Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Harper, L. R., Griffiths, N. P., Lawson Handley, L., Sayer, C. D., Read, D. S., Harper, K. J., … Hänfling, B. (2019). Development and application of environmental DNA surveillance for the threatened crucian carp (*Carassius carassius*). *Freshwater Biology*, *64*(1), 93–107. https://doi.org/10.1111/fwb.13197

Henderson, P. A., Plenty, S. J., Newton, L. C., & Bird, D. J. (2012). Evidence for a population collapse of European eel (*Anguilla anguilla*) in the Bristol Channel. *Journal of the Marine Biological Association of the United Kingdom*, *92*, 843–851. https://doi.org/10.1017/S002531541100124X

Hinlo, R., Furlan, E., Suitor, L., & Gleeson, D. (2017). Environmental DNA monitoring and management of invasive fish: Comparison of eDNA and fyke netting. *Management of Biological Invasions*, *8*(1), 89–100. https://doi.org/10.3391/mbi.2017.8.1.09

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE*, *12*, e0179251. https://doi.org/10.1371/journal.pone.0179251

Ibbotson, A., Smith, J., Scarlett, P., & Aprhamian, M. (2002). Colonisation of freshwater habitats by the European eel *Anguilla anguilla*. *Freshwater Biology*, *47*(9), 1696–1706. https://doi.org/10.1046/j.1365-2427.2002.00930.x

Inland Fisheries Division (2008). *National Report for Ireland on Eel Stock Recovery Plan Including River Basin District Eel Management Plans National Report for Ireland on Eel Stock Recovery Plan Including River Basin District Eel Management Plans*, (December).

Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T., & Minamoto, T. (2019). Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*, 361–373. https://doi.org/10.1002/aqc.3058.

Jacoby, D., & Gollock, M. (2014). *Anguilla anguilla*, *European eel*. The IUCN Red List of Threatened Species, 8235. Retrieved from https://www.iucnredlist.org/species/60344/45833138.

Jellyman, D. J., & Graynoth, E. (2005). The use of fyke nets as a quantitative capture technique for freshwater eels (*Anguilla* spp.) in rivers. *Fisheries Management and Ecology*, 12(4), 237–247. https://doi.org/10.1111/j.1365-2400.2005.00445.x

Kelly, F. L., Connor, L., Morrissey, E., McLoone, P., Kelly, K., Delanty, K., & Buckley, S. (2017). *Fish stock survey of lough derg, June 2016.* Retrieved from http://wfdfish.ie/wp-content/uploads/2017/10/Derg_2016.pdf.

Kelly, F. L., Connor, L., Morrissey, E., Wogerbauer, C., Matson, R., Feeney, R., & Rocks, K. (2012a). *Water framework directive fish stock survey of Lough Corrib, August 2011.*

Kelly, F. L., Connor, L., Morrissey, E., Wogerbauer, C., Matson, R., Feeney, R., & Rocks, K. (2012b). *Water framework directive fish stock survey of Lough Owel, July 2011.*

Kelly, F. L., Connor, L., Morrissey, E., Wogerbauer, C., Matson, R., Feeney, R., & Rocks, K. (2013a). *Water framework directive fish stock survey of Muckanagh Lough, September 2012.*

Kelly, F. L., Connor, L., Morrissey, E., Wogerbauer, C., Matson, R., Feeney, R., & Rocks, K. (2013b). *Water framework directive fish stock survey of white Lough, August 2012.*

Knudsen, S. W., Ebert, R. B., Hesselsøe, M., Kuntke, F., Hassingboe, J., Mortensen, P. B., … Møller, P. R. (2019). Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, 510, 31–45. https://doi.org/10.1016/j.jembe.2018.09.004

Krueger, K. L., Hubert, W. A., & Price, R. M. (1998). Tandem-set fyke nets for sampling benthic fishes in lakes. *North American Journal of Fisheries Management*, 18(1), 154–160. https://doi.org/10.1577/1548-8675(1998)018<0154:tsfnfs>2.0.co;2

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *Journal of Applied Ecology*, 53(4), 1148–1157. https://doi.org/10.1111/1365-2664.12598

Lawson Handley, L., Read, D. S., Winfield, I. J., Kimbell, H., Johnson, H., Li, J., … Hänfling, B. (2019). Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. *Environmental DNA*, 1(1), 26–39. https://doi.org/10.1002/edn3.5

Lecomte-Finiger, R. (1994). The early life of the European eel [7]. *Nature*, 370, 424. https://doi.org/10.1038/370424a0

MacNamara, R., McCarthy, T. K., & Barry, J. (2016). Estimation of production and long-term changes in the population of European eel *Anguilla anguilla* (L.) in an Irish lake. *Ecology of Freshwater Fish*, 26, 602–608. https://doi.org/10.1111/eff.12304

Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., … Possingham, H. P. (2005). Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. *Ecology Letters*, 8(11), 1235–1246. https://doi.org/10.1111/j.1461-0248.2005.00826.x

Moriarty, C. (1975). The small fyke net as a sampling instrument in eel research. In *Symposium on the methodology for the survey, monitoring and appraisal of fishery resources in lakes and large rivers*. EIFAC Technical Paper – EIFAC/T23Suppl.1. Retrieved from http://www.fao.org/3/AA043B/AA043B06.htm#ch6.

Moriarty, C. (2003). The yellow eel. In K. Aida, K. Tsukamoto, & K. Yamauchi (Eds.), *Eel biology* (pp. 89–105). Tokyo, Japan: Springer. https://doi.org/10.1007/978-4-431-65907-5_7

Ogburn, M. B., Richie, K. D., Marafino, G. A., Plough, L. V., Fitzgerald, C. L., & Geranio, R. (2018). Environmental DNA analysis of river herring in Chesapeake Bay: A powerful tool for monitoring threatened keystone species. *PLoS ONE*, 13, e0205578. https://doi.org/10.1371/journal.pone.0205578

R Core Team (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/index.html

Rees, H. C., Bishop, K., Middleditch, D. J., Patmore, J. R. M. M., Maddison, B. C., & Gough, K. C. (2014). The application of eDNA for monitoring of the Great Crested Newt in the UK. *Ecology and Evolution*, 4(21), 4023–4032. https://doi.org/10.1002/ece3.1272

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA – A review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51, 1450–1459. https://doi.org/10.1111/1365-2664.12306

Renshaw, M. A., Olds, B. P., Jerde, C. L., Mcveigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, 15(1), 168–176. https://doi.org/10.1111/1755-0998.12281

Rose, G. A., & Kulka, D. W. (1999). Hyperaggregation of fish and fisheries: How catch-per-unit-effort increased as the northern cod (*Gadus morhua*) declined. *Canadian Journal of Fisheries and Aquatic Sciences*, 56(S1), 118–127.

Russell Poole, W., & Reynolds, J. D. (1998). Variability in growth rate in European eel *Anguilla anguilla* (L.) in a western Irish catchment. *Biology and Environment*, 98(3), 141–145.

Sambrook, D., & Russell, D. W. (2001). *Molecular cloning: A laboratory manual* (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sengupta, M. E., Hellström, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., … Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proceedings of the National Academy of Sciences of the United States of America*, 116(18), 8931–8940. https://doi.org/10.1073/pnas.1815046116

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., … Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications Biology*, 1(1), 4. https://doi.org/10.1038/s42003-017-0005-3

Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, 183, 46–52. https://doi.org/10.1016/j.biocon.2014.11.023

Smart, A. S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., Mccarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecological Applications*, 25, 1944–1952.

Stein, F. (2018). *Europe's largest wildlife crime; The illegal trade of the European eel*. Retrieved from http://www.illegalwildlifetrade.net/2018/07/27/europes-largest-wildlife-crime-illegal-trade-of-the-european-eel/.

Stone, R. (2003). Freshwater eels are slip-sliding away. *Science*, 302(5643), 221–222.

Sustainable Eel Group (2018). *Quantifying the illegal trade in European glass eels (*Anguilla anguilla*): Evidences and Indicator*s. Sustainable Eel Group. SEG-Report, 1–V1(January), 1–5.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLoS ONE*, 7, e35868. https://doi.org/10.1371/journal.pone.0035868

Takeuchi, A., Sado, T., Gotoh, R. O., Watanabe, S., Tsukamoto, K., & Miya, M. (2019). New PCR primers for metabarcoding environmental DNA from freshwater eels, genus Anguilla. *Scientific Reports*, 9(1), 7977. https://doi.org/10.1038/s41598-019-44402-0

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using

14 | **WILEY**—Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences
Open Access

WELDON ET AL.

environmental DNA from seawater samples. *PLoS ONE*, *7*(8), 1–9. https://doi.org/10.1371/journal.pone.0041732

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, *21*(11), 2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

van Ginneken, V. J. T., & Maes, G. E. (2005). The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: A literature review. *Reviews in Fish Biology and Fisheries*, *15*(4), 367–398. https://doi.org/10.1007/s11160-006-0005-8

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environmental DNA: The importance of primer specificity. *PLoS ONE*, *8*(3), e59520. https://doi.org/10.1371/journal.pone.0059520

Williams, K. E., Huyvaert, K. P., & Piaggio, A. J. (2016). No filters, no fridges: A method for preservation of water samples for eDNA analysis. *BMC Research Notes*, *9*(1), 298. https://doi.org/10.1186/s13104-016-2104-5

Wilson, C., Wright, E., Bronnenhuber, J., Macdonald, F., Belore, M., & Locke, B. (2014). Tracking ghosts: Combined electrofishing and environmental DNA surveillance efforts for Asian carps in Ontario waters of Lake Erie. *Management of Biological Invasions*, *5*(3), 225–231. https://doi.org/10.3391/mbi.2014.5.3.05

Yokouchi, K., Aoyama, J., Miller, M. J., McCarthy, T. K., & Tsukamoto, K. (2009). Depth distribution and biological characteristics of the European eel *Anguilla anguilla* in Lough Ennell, Ireland. *Journal of Fish Biology*, *74*(4), 857–871. https://doi.org/10.1111/j.1095-8649.2008.02167.x

Zenimoto, K., Sasai, Y., Sasaki, H., & Kimura, S. (2011). Estimation of larval duration in *Anguilla* spp., based on cohort analysis, otolith microstructure, and Lagrangian simulations. *Marine Ecology Progress Series*, *438*, 219–228. https://doi.org/10.3354/meps09255

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Weldon L, O'Leary C, Steer M, Newton L, Macdonald H, Sargeant SL. A comparison of European eel *Anguilla anguilla* eDNA concentrations to fyke net catches in five Irish lakes. *Environmental DNA*. 2020;00: 1–14. https://doi.org/10.1002/edn3.91