

A drop in the ocean: Monitoring fish communities in spawning areas using environmental DNA

Frances C. Ratcliffe  | Tamsyn M. Uren Webster  | Carlos Garcia de Leaniz  |
Sofia Consuegra 

Centre for Sustainable Aquatic Research (CSAR), Swansea University, Swansea, UK

Correspondence

Sofia Consuegra, Swansea University, Singleton Park, Swansea SA2 8PP, UK
Email: s.consuegra@swansea.ac.uk

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Abstract

Early life stages of aquatic organisms are particularly vulnerable to climatic stressors; however, they are difficult to monitor due to challenges in sampling and morphological identification. Environmental DNA (eDNA) from water samples represents an opportunity for rapid, nondestructive monitoring of aquatic community composition as well as single species monitoring. eDNA can also detect spawning events, although has not been yet tested in offshore spawning grounds. Here, we used metabarcoding of water samples to detect the presence of key fish taxa in spawning areas that are difficult to monitor using traditional means. We analyzed DNA from water samples and fish larvae samples at 14 offshore sites, using 12S mitochondrial metabarcoding and compared taxa detections, diversity, and community structure estimated by both sample types. Species richness and diversity did not differ between water and larvae samples. Both sample types detected a core of 12 taxa across the survey, with an average agreement in detections of 75% at sampling site level. Water samples detected two of the three most abundant taxa, the sandeel, *Ammodytes marinus*, and clupeids, *Clupea harengus/Sprattus sprattus*, at 31% and 38% more sites than larvae samples respectively, while *Callionymus* sp. was more prevalent in larvae samples. Mackerel (*Scomber scombrus*) and blue whiting (*Micromestius poutassou*) were only detected in water samples despite sampling taking place at peak spawning times for these species. Our results demonstrate that eDNA metabarcoding provides a rapid and feasible monitoring method for the management of key taxa, such as sandeel, that cannot be easily monitored using traditional capture surveys.

KEYWORDS

12S, larvae monitoring, metabarcoding, sandeel

1 | INTRODUCTION

As climate stressors increase globally (Masson-Delmotte et al., 2018), spatial and temporal monitoring of biodiversity is required to detect changes in both species composition (Poloczanska et al., 2013)

and geographic range (Burrows et al., 2011). This information is critical to informing policy decisions and assessing the efficacy of conservation/management interventions (Douve & Ehler, 2011; Geijzenborffer et al., 2016). In the marine environment, monitoring is sometimes complicated by inaccessibility (Bicknell, Godley, Sheehan,

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Votier, & Witt, 2016) or limited resources (Costello et al., 2010). In addition, the velocity of climate change and seasonal shifts in timing of temperature changes is, in some cases, greater in the ocean than on land (Burrows et al., 2011); therefore, development of rapid (and feasible) monitoring methods is crucial to detecting the magnitude of these changes (Costello et al., 2010; Thomsen et al., 2012).

Monitoring spawning grounds and fish recruitment is essential in the face of these global pressures as larval and juvenile developmental stages of fishes are highly sensitive to environmental stressors (Pitois, Lynam, Jansen, Halliday, & Edwards, 2012). Temperature changes (Lee, Nash, & Danilowicz, 2005), prey availability (Régner, Gibb, & Wright, 2017), and offshore construction (Cordes et al., 2016) may all impact recruitment. Globally, spawning areas are often protected by policy measures (Pastoors, Rijnsdorp, & Van Beek, 2000), such as MPAs (Christie et al., 2010) or restrictions in offshore development such as pile driving or oil drilling (La Védrine, 2014). However, current data or time series information on larval distribution and spawning aggregations within these sensitive areas tends to be limited (Greve, Prinage, Zidowitz, Nast, & Reiners, 2005; Kimmerling et al., 2018). Traditional larvae monitoring methods involve deploying a plankton net from a research vessel and morphologically identifying individual larvae (Habtes, Muller-Karger, Roffer, Lamkin, & Muhling, 2014). Morphological identification is challenging and time consuming (Brechon, Coombs, Sims, & Griffiths, 2013) and, in some cases, only accurate to family level (Ellis, Milligan, Readdy, Taylor, & Brown, 2012). Monitoring economic and ecological costs can be, therefore, high (Koslow & Wright, 2016) and as a consequence data on spawning distributions of is globally sparse (Ellis et al., 2012; Kimmerling et al., 2018; Maggia et al., 2017). Furthermore, information on key taxa that are difficult to capture and identify using traditional trawls (e.g., sandeel) is currently lacking, even in areas that are surveyed more regularly (Ellis et al., 2012; Lynam et al., 2013).

Environmental DNA (eDNA) extracted from water samples is potentially a rapid, cost effective tool for monitoring species distributions (Lodge et al., 2012), thus reducing the need for destructive sampling (Bylemans et al., 2017). Metabarcoding analysis of eDNA can be useful for whole community/broad range assessment (Bohmann et al., 2014; Lacoursière-Roussel et al., 2018; Thomsen et al., 2016) and also perform well for target species monitoring (Harper et al., 2018), offering a potential alternative to traditional monitoring of spawning grounds. The use of eDNA has shown potential for detecting pulses of spawning in freshwater systems where mass release of gametes results in sudden increases of concentration of mitochondrial DNA in the water column (Bylemans et al., 2017). In some cases, increases in eDNA concentration reveal the movement of adults toward a spawning area, rather than the release of gametes (Erickson et al., 2016), but its usefulness to detect spawning in the marine environment remains uncertain.

Although the nondestructive nature of eDNA would make it ideal for use in sensitive environments (Stat et al., 2019), or for the monitoring of rare or threatened species (Bylemans et al., 2017), understanding how eDNA sampling reflects or differs from traditional

monitoring techniques can be challenging (Hansen, Bekkevold, Clausen, & Nielsen, 2018). For example, eDNA cannot distinguish among year classes, which in turn can be specifically targeted by physical sampling (Maruyama, Nakamura, Yamanaka, Kondoh, & Minamoto, 2014). In addition, eDNA may show differing dispersal patterns in the open ocean to those physically exhibited by the fish larvae themselves (Goldberg et al., 2016). In fact, comparisons of simultaneous water and visual/physical sampling in the marine environment have found variable level of agreement in detections between eDNA analyses and morphological taxonomy (Leduc et al., 2019; Stat et al., 2019; Thomsen et al., 2016). In order to address these discrepancies, we assessed both eDNA (water samples) and ichthyoplankton physical samples (larvae) using metabarcoding and an identical bioinformatics pipeline. We compared species detection levels, relative abundance, and community composition in both sample types, taken simultaneously at the same locations from the Celtic and Irish seas.

2 | METHODOLOGY

2.1 | Field sampling

Water and larvae samples were taken onboard RV Celtic Voyager in the Irish and Celtic seas at 14 sites in known spawning areas for Ammodytidae, Clupeidae, Gadidae, Scombridae, and Pleuronectidae (Ellis et al., 2012) (Figure 1, Table S1). Sampling was carried out in May (17th to 26th, 2018), during or shortly after the spawning season for many of the fish species in the sampling area (Table S2). Filtration of water samples was carried out in an area physically separated from the processing of larvae samples. Gloves were changed between samples and surfaces cleaned using a 10% bleach solution, before and after each sampling event. Sea surface water samples

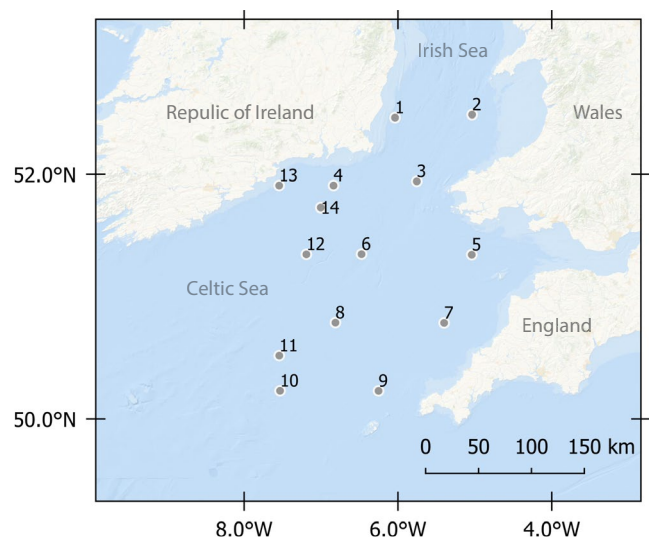


FIGURE 1 Map of the sampling locations (14 sites) across the Irish and Celtic sea region. For coordinates of each sampling location, see Table S3

were taken by rope and bucket (bleach sterilized and swilled in sea water from the same site). At each of the 14 sites, 400 ml from three replicate buckets of sea water (true biological replicates) and one control (de-ionized water) were filtered using a syringe (Terumo, 50 ml), through polycarbonate filter holders, (25 mm, Cole-Parmer) containing a 0.22 μm hydrophilic polyethersulfone filter (Merck Millipore). Filters were left in the holders to minimize contamination, and holders were then filled with ethanol and stored at -20°C until extraction. After water samples had been taken, a larvae haul was conducted starting from the same coordinates, using MultiNet plankton sampler (Hydro-Bios) that continuously filtered water from the surface to 50 m depth and back to the surface (for volumes filtered, see Table S1). Ichthyoplankton were separated from other zooplankton species and preserved in RNA later at room temperature for 24 hr, then refrigerated at 4°C until laboratory processing. Temperature, salinity, and density were recorded at each sampling site (Table S1).

2.2 | Laboratory processing—DNA extraction and library preparation

All water samples were extracted using the QIAGEN PowerSoil kit, using a homogenization step (a Precellys 24 tissue homogenizer, Bertin Instruments). Extracted DNA was stored at -80°C until library preparation. Extraction blanks (where no filter was added) were carried through all steps of the library preparation and bioinformatic analysis.

Pooled homogenates of all fish larvae present in the MultiNet haul corresponding to each sampling site were extracted in bulk, by taking a 5 mg (± 3 mg) of tissue anterior to the tail (or the complete larva for smaller specimens) from all individuals in a haul (Ratcliffe et al., 2020).

Environmental DNA libraries were prepared using 12S V5 primers (Riaz et al., 2011) which amplify a 106 bp fragment of the 12S mitochondrial gene. A nested PCR approach was optimized, using Platinum™ Hot Start PCR Master Mix (2x) (Thermo Fisher Scientific). Initially, all samples were amplified in triplicate, in 12 μl PCR reactions, with 2 μl of template and 0.5 μl of 12S primers, for 25 cycles, with an annealing temperature of 52°C . Subsequently, 5 μl of this first reaction was used as a template for a second reaction, using the 12S primers with the addition of overhang adaptors for subsequent Nextera indexing, using identical PCR conditions, for 10 cycles. PCR triplicates were then pooled, using 10 μl of each triplicate from the nested PCR reaction and purified using Agencourt AMPure XP beads (Beckman Coulter). Subsequently, amplicons were indexed using the Nextera XT Index Kit v2 (Illumina, Inc.), and DNA concentration of each reaction was quantified via Qubit dsDNA HS Assay (Invitrogen, Thermo Fisher Scientific) and pooled in equal molar concentrations. Filtration blanks for each site, extraction blanks for each round of extractions, and PCR blanks were carried through all steps of library preparation, including triplication in the first PCR, and bioinformatics processing.

Larvae samples (and associated extraction and PCR blanks) were directly amplified using the 12S primers with Nextera overhang adapters attached, using Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific) as described in Ratcliffe et al. (2020).

The effect (if any) of the different DNA polymerases and the nested and non-nested PCR protocols was assessed using mock communities, constructed from known molar concentrations of DNA extracted individually from larvae in the survey. Community 1 contained 12.5% of the following taxa: *Ammodytes marinus*, *Callionymus* sp., *Ciliata mustela*, *Lepidorhombus* sp., *Merlangius merlangius*, *Merluccius merluccius*, *Sprattus sprattus*, and *Trisopterus minutus*. Community 2 contained *A. marinus* (1.83%), *Callionymus* sp., (23.77%), *C. mustela* (2.64%), *Lepidorhombus* sp. (18.78%), *M. merlangius* (26.92%), *M. merluccius* (22.10%), *S. sprattus* (1.66%), and *T. minutus* (2.30%). Water and larvae/mock community libraries were prepared separately to avoid contamination and were pooled after indexing and sequenced in the same Illumina Miseq run to avoid any sequencing bias. A tag jumping/sequencing control, where no sample was added, was used to monitor any effect of concurrent sequencing of water and larvae samples.

2.3 | Bioinformatic analyses

Water, larvae, and mock community and all blanks were subjected to the same bioinformatics pipeline and processed simultaneously. The amplicon sequence variant (ASV) approach was used because it enables detection of single nucleotide differences (Callahan, McMurdie, & Holmes, 2017) and therefore provides a higher resolution than a traditional OTU approach.

Qiime2 (version 2019.1, Bolyen et al., 2019) was used to process de-multiplexed paired end sequences. DADA2 (Callahan et al., 2016) within Qiime2 was used for de-noising steps. Based on read quality scores, the first 10 bp of each sequence was trimmed and all sequences truncated to 100 bp in length. Subsequently, sequencing errors were corrected where possible, and chimeric sequences removed, paired end reads were joined and sequences de-replicated using the default DADA2 settings in Qiime2.

Taxonomic assignment was conducted using custom databases (Ratcliffe et al., 2020). Initially, reads were classified against a full database that included all taxa available on NCBI amplified in silico with the 12S V5 primers, using the KNN method in mothur (Schloss et al., 2009). A second screening was then carried out using a smaller database that included all available sequences of fish encountered (native and non-native) in the British Isles (Fish Base: accessed 31/3/2019) as well as outgroups known to be present from the classification against the full database, again using the KNN method, and the parameter “numwanted = 1” (Findley et al., 2013). Due to the potential for false positive assignments using this parameter, these assignments were verified using NCBI megablast (Morgulis et al., 2008), where the top 10 hits were screened on a case by case basis, for the highest match of a UK fish taxon. Assignments below

98% identity were assigned to genus or family level using the lowest common ancestor algorithm (Huson, Auch, Qi, & Schuster, 2007) in MEGAN (6.15.1).

Once taxonomic assignment was complete, non-fish ASVs were removed from downstream analysis. To remove contaminant ASVs (false positives) from the data, filtration blank read counts (subjected to the same workflow at all steps of the process as field samples) were subtracted from each field replicate in the corresponding site, (Grey et al., 2018). This was carried out before any downstream data analysis (Andruszkiewicz et al., 2017).

2.4 | Statistical analysis

All statistical analysis was performed using R (version 3.5.3, R Core Team, 2017). For comparison purposes, as only one larvae haul was sampled per site, the reads from the three water biological replicates and three larvae technical replicates were pooled for each site. Reads were then converted to relative abundance data (proportion) to account for unequal sequencing depths between samples. Subsequently, to remove any reads present due to tag jumping (Schnell, Bohmann, & Gilbert, 2015) taxa that accounted for <0.05% (set using the tag jumping blank) of the relative abundance of a sample were removed from that site for downstream analysis.

Mock communities were analyzed using a chi-square (goodness of fit) test to ascertain if there was any difference between relative abundance of genomic DNA in the sample (expected) and relative abundance of reads after sequence processing (observed). Species richness was calculated for each sample using "specnumber" in R, and Shannon Wiener diversity was calculated using the "diversity" function with "method" = "Shannon." Wilcoxon signed rank tests (paired samples) were used to ascertain if medians differed between water and larvae samples and variances within each treatment were calculated as median absolute deviation (MAD), using r function "mad." After checking the residuals for normality using "skewness" (Moments package), an F test was used to check for significant differences in variance between the sample types. Log-likelihood ratio (G-test) test of independence with Williams' correction was used to test the effect of sample site and taxon on detections between the two methods.

To assess differences in community composition between the two methods, a dummy number of species of 1 was added to all samples in order compute Brae-Curtis dissimilarity for sites where no larvae were captured (Clarke, Somerfield, & Chapman, 2006). Reads (relative abundance) were square-root transformed, a Bray Curtis dissimilarity matrix was generated and PERMDISP (to test for homogeneity of dispersion) and subsequent PERMANOVA analysis was used (Anderson, 2014) (Figure S1). SIMPER analysis was then carried out on untransformed relative abundance data to ascertain which species were driving the differences observed between the two sampling methods (Clarke, 1993).

TABLE 1 Number of reads remaining and removed at each step of the denoising process. Denoising was carried out using DADA2 (Callahan et al., 2016) within Qiime2 (version 2019.1, Bolyen et al., 2019). Removal of nonvertebrate and non-fish reads was conducted after taxonomy had been assigned

	Reads remaining	Reads removed
Raw reads	13,096,645	
Filtered	7,961,080	5,135,565
Denosed	7,961,080	0
Merged	7,653,885	307,195
Nonchimeric	7,371,118	282,767
Vertebrate	6,030,566	1,340,552
Fish	6,010,983	19,583

3 | RESULTS

A total of 42 water biological replicate samples from 14 sites (three per site) were collected in the survey. At nine of the sites, the larvae hauls contained multiple larvae suitable for metabarcoding; however, at two sites, the larvae hauls contained only one individual and three sites the hauls did not contain larvae.

A total of 13,149,751 raw paired end reads were generated from the water and larvae samples. After DADA denoising, 7,379,309 reads remained for downstream analysis (Table 1). A total of 209 ASVs were generated across all samples in the study. Of these, 95 matched to fishes, 71 had no vertebrate match, 36 matched to family Hominidae, three to Delphinidae, two to Felidae, and one to Laridae and Phasianidae respectively. The primary contaminant observed in filtration blanks was *Homo sapiens*; however, a proportionally small amount (3.5% of blank reads) mapped to fish (*Salmo salar*, not found in any field replicates, *A. marinus*, *Clupea harengus*/*S. sprattus* and *Pollacchius* sp./*M. merlangus*) and were used to set a cutoff below which reads were subtracted from each field replicate in the corresponding site. Filtration blank read removal resulted in the removal of 0.36% of water reads across the study. After pooling site replicates for analysis, the mean number of water sample reads per site was 223,745. Site 11 contained only 108 reads (no larvae were physically captured at this location) and was therefore discarded from downstream analysis. The mean number of larvae reads per site (nine sites) was 279,667.

Each of the eight species added to both mock communities was detected using both Phusion (Thermo Fisher) and Platinum (Thermo Fisher) Taq polymerases. Only reads assigned to the input DNA taxa were observed in mock community reads, except for 23 and 19 reads in mock community 1 assigned to *Trisopterus esmarkii* when amplifying with Phusion and Platinum, respectively. For mock community 1, which contained equal concentrations of DNA from each of the eight taxa, the relative quantity of DNA inputted (expected) and relative proportion of reads (observed) differed significantly (Phusion, Chi-square: $X^2 = 14.59$, $df = 7$, $p = .041$, Platinum $X^2 = 18.26$, $df = 7$, $p = .011$), mainly due to an excess and deficit of *A. marinus* reads

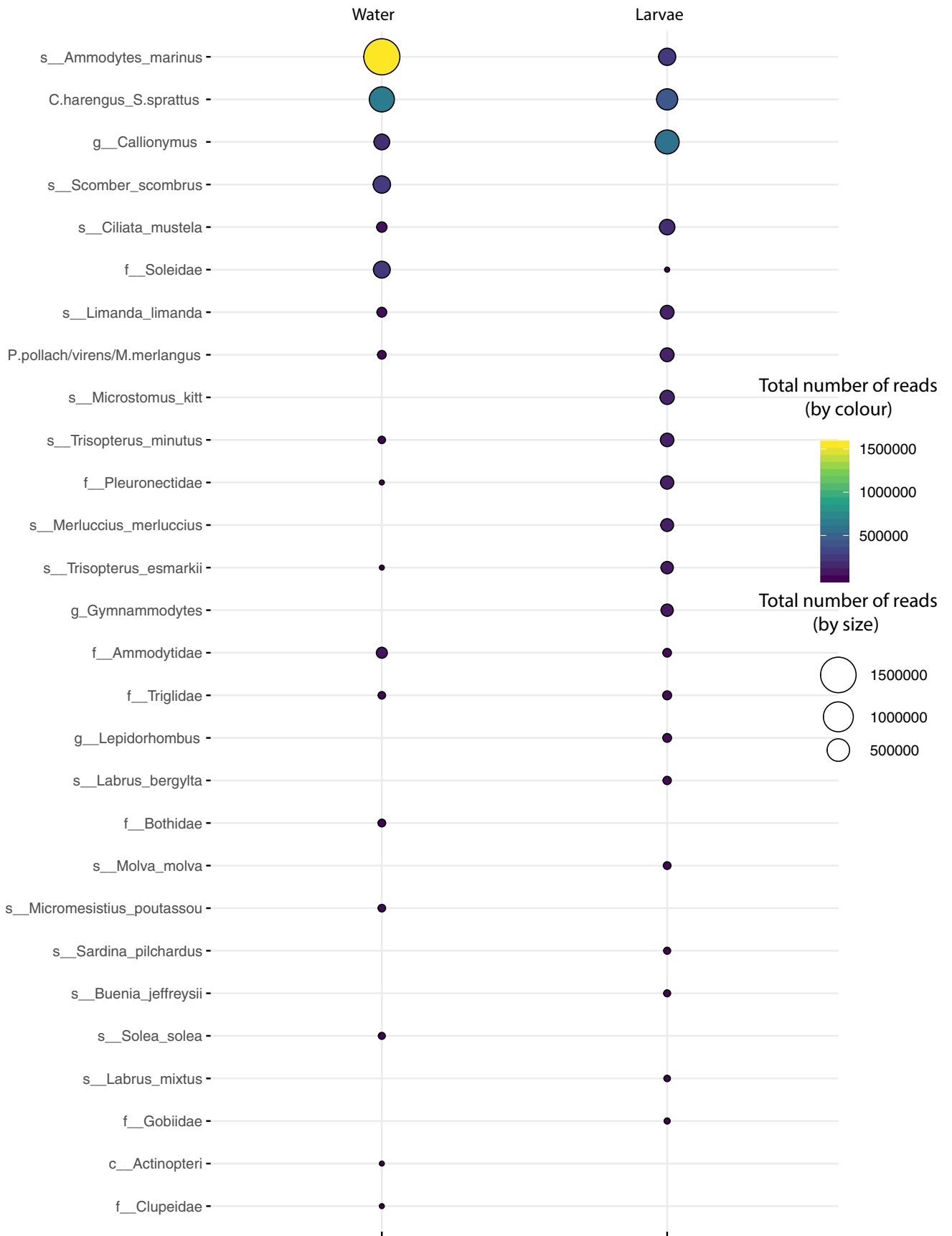


FIGURE 2 Overview of the total number of raw reads per taxon in the two sample types: water samples = Water and larvae samples = Larvae. Taxa are identified to lowest possible taxonomic level. s__ = species level, g__ = genus level, f__ = family level

and *S. sprattus*, respectively. However, in Mock 2, where input molar concentrations varied, there was no difference observed between the relative input of DNA and the observed proportion of reads (Phusion, $X^2 = 11.39$, $df = 7$, $p = .123$, Platinum $X^2 = 8.11$, $df = 7$, $p = .323$) (Figure S2).

In both water and larvae samples, we successfully detected 12 of the same taxa (Figure 2) and a similar number of taxa (19 taxa in water, 20 taxa in larvae) were detected overall. At a site level, there was an average of 75% agreement in taxa detection between the sample types (Table 2; Figure 3). The number of taxa detected in the two sample types depended on the sampling site ($G = 31.43$, $df = 12$, $p = .002$) and the taxon considered ($G = 42.80$, $df = 27$, $p = .027$, Figure 3, Table 2). In general, the more abundant taxa were detected by both sampling methods while less abundant taxa exhibited much greater variance, with some taxa being detected in one sample type. Of these, 10 taxa were detected at only one site, 60% of which were observed in larvae samples (Figure 3, Table 2).

Patterns of relative abundance broadly followed those observed in the number of detections. *C. harengus/S. sprattus*, *A. marinus* were detected in higher relative abundance in water than in larvae samples (Figure 2, *C. harengus/S. sprattus*: $W = 37.5$, $p = .016$. *A. marinus*: $W = 32$, $p = .008$). In contrast, the third most abundant taxa, *Callionymus* sp., were more frequently detected in the larvae samples; however, there was no difference in relative abundance between sample types ($W = 113$, $p = .110$). Comparisons of relative abundance estimates between the sample types were only significantly correlated for *Limanda limanda* ($S = 76.44$, $p < .001$, $\rho = 0.79$) (Table S4).

Community composition differed between water and larvae samples (PERMANOVA $df: 1,24$, $ps-F = 4.107$, $R^2 = 0.146$, $P(\text{perm}) = .001$, permutations: 999). These differences were driven by the pattern of higher abundances of *A. marinus*, *C. harengus/S. sprattus*, and *S. scombrus* in water samples, in contrast to higher abundances of *L. limanda* and *Callionymus* sp. in larvae samples, together contributing to

Taxa	Both	Neither	Larvae	Water	% Agreement
<i>Clupea harengus/Sprattus sprattus</i>	7	0	8	12	53.8
<i>Ammodytes marinus</i>	8	0	8	13	61.5
<i>Callionymus</i> sp.	1	3	7	4	30.8
<i>Limanda limanda</i>	5	7	6	5	92.3
Triglidae	1	7	5	2	61.5
<i>Scomber scombrus</i>	0	6	0	7	46.2
<i>Ciliata mustela</i>	2	7	3	5	69.2
<i>P. pollachius/virens/M. merlangus</i>	2	4	7	4	46.2
<i>Trisopterus minutus</i>	1	6	6	2	53.8
<i>Microstomus kitt</i>	0	8	5	0	61.5
Pleuronectidae	1	7	5	2	61.5
<i>Trisopterus esmarkii</i>	1	9	4	1	76.9
<i>Merluccius merluccius</i>	0	11	1	1	84.6
<i>Gymnammodytes</i> sp.	0	11	2	0	84.6
Soleidae	0	9	0	4	69.2
Bothidae	0	11	0	2	84.6
<i>Ammodytidae</i>	1	7	2	4	61.5
<i>Solea solea</i>	0	12	0	1	92.3
<i>Lepidorhombus</i> sp.	0	11	2	0	84.6
<i>Micromesistius poutassou</i>	0	12	0	1	92.3
<i>Labrus bergylta</i>	0	12	1	0	92.3
<i>Molva molva</i>	0	12	1	0	92.3
<i>Sardina pilchardus</i>	0	12	1	0	92.3
<i>Buenia jeffreysii</i>	0	12	1	0	92.3
Gobiidae	0	12	1	0	92.3
<i>Labrus mixtus</i>	0	12	1	0	92.3
Clupeidae	0	12	0	1	92.3
Actinopteri	0	12	0	1	92.3

TABLE 2 Agreement in detections between larvae and water samples at 13 sites. Both = number of sites where the taxon was detected using both sample types. Larvae = number of sites where taxon was detected in larvae samples. Water = number of sites where taxon was detected in water samples. Neither = number of sites where a taxon was not detected by either sample type. % Agreement = Sum of "Both" and "Neither"/total sites $\times 100$

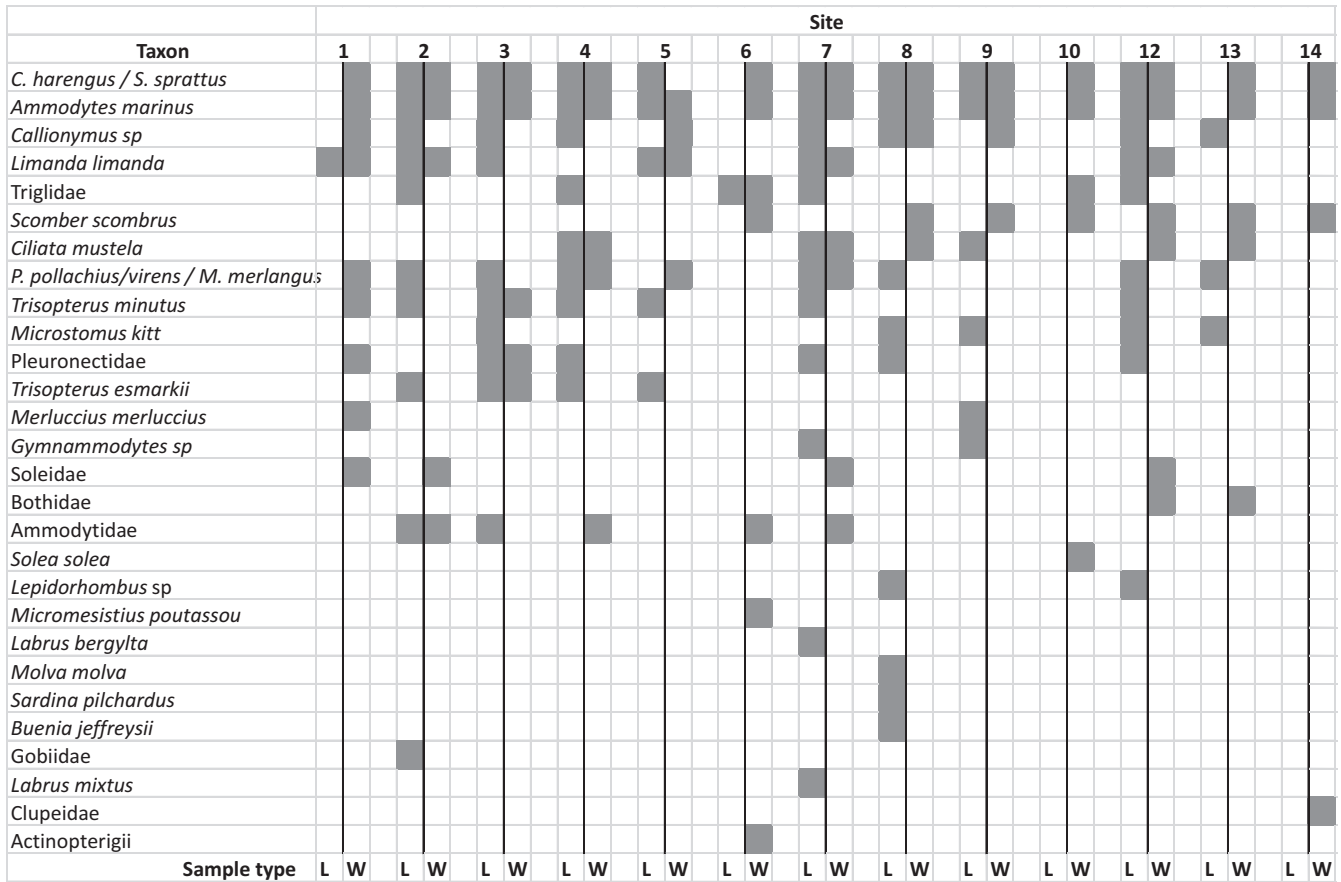
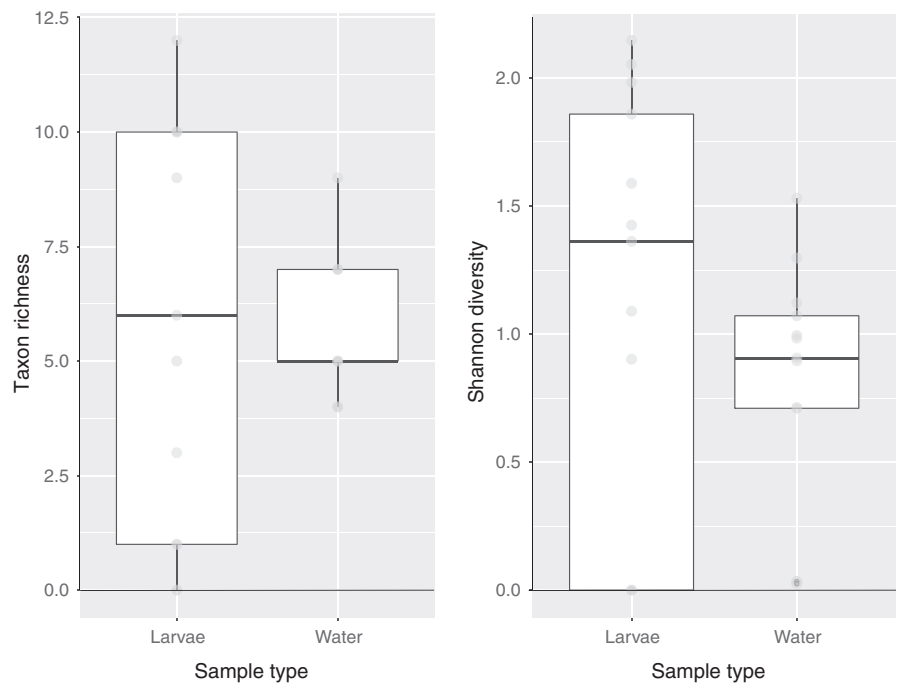


FIGURE 3 Site by site detections (presence/absence) between the two different sampling methods, larvae sampling “L” and water sampling, “W.” Taxa are identified to lowest taxonomic level possible and ordered by overall abundance in the survey

FIGURE 4 Median taxon richness and diversity (lowest possible taxonomic level) for eDNA and bulk samples across 14 sites in the survey. The median is represented by the horizontal line within each box, boxes define the 25th and 75th percentiles and the most extreme data point which is no more than 1.5 times the interquartile range from the corresponding box



70.64% of the differences observed between sample types (SIMPER analysis, Table S5). Taxon richness and alpha diversity did not differ between the two sampling methods (richness: $V = 42, p = .83,$

Shannon–Wiener Diversity: $V = 29, p = .27.$ Larvae samples did, however, exhibit greater variance than water samples in terms of species richness (richness Larvae MAD = 4, Water MAD = 1, F

test: $df = 12$, $F = 8.85$, $p < .001$. Shannon–Wiener Diversity Bulk MAD = 0.62, water MAD = 0.2, F test: $df = 12$, $F = 3.04$, $p = .065$ (Figure 4).

4 | DISCUSSION

We applied water eDNA metabarcoding to detect fish in marine spawning areas and demonstrated that water samples not only broadly reflect larvae samples, with 75% average agreement on site by site basis but can also be more sensitive in the case of particular taxa, such as sandeels and herring/sprat. While rare species, those detected at one site only, were more likely to be found in bulk samples, some taxa expected to be part of the spawning assemblage (e.g., mackerel *S. scombrus* and blue whiting, *Micromestius poutassou*) were only detected in water samples, highlighting the potential of this tool to complement traditional sampling.

Previous studies that compare water sampling to visual or capture surveys have found varying levels of agreement between the taxa detected (Cilleros et al., 2019; Stat et al., 2019; Thomsen et al., 2016). Thomsen et al., (2016) found correlations between capture biomass and numbers of water sample reads, while Stat et al., (2019) found fish assemblages differed between visual (BRUV) and water samples. Differences in detection between traditional methods and eDNA are influenced by eDNA dispersal range, year class, and detection sensitivity of the particular methods compared. Importantly, all monitoring techniques are subject to biases, for example trawl types may also differ in the species captured due to gear selectivity biases (Hansen et al., 2018). Hence, while water samples may not always perfectly reflect capture/visual samples, they represent a rapid and feasible and nondestructive way to efficiently assess fish community assemblages (Cilleros et al., 2019; Thomsen et al., 2016).

Here, we compared surface water samples to larvae sampled between the surface and 50 m depth. Fish eggs and larvae are most abundant in depths shallower than 50 m (Conway, Coombs, & Smith, 1997; Sabatés, 2004). In the Irish Sea, densities of fish eggs increase with decreasing depth and peak at the surface, while larval density increases toward a peak at of 10–15 m, with little difference observed between species, including the families most abundant in this study (Clupeidae, Ammodytidae, Callionymidae) (Conway et al., 1997). For some taxa, however, this general pattern may not apply; for instance, hake larvae (*M. merluccius*) have been shown aggregate at maximum density at depths of 60–80 m (Sabatés, 2004). This taxon was not detected in water samples, despite being detected in one larvae haul; therefore, while sampling depth is unlikely to have affected the majority of taxa, it may be a reason for some of the discrepancies in the detection of rare species. Thus, as eDNA exhibits sensitivity to vertical zonation in stratified water (Jeunen et al., 2020), multiple sampling depths may be advisable, depending on the taxa and life stages of interest.

For sensitive taxa such as sandeels that are hard to monitor using traditional means (Ellis et al., 2012), this survey demonstrates the potential of eDNA metabarcoding as a monitoring tool.

Sandeels are a key prey species, consumed by fish, seabirds, and marine mammals; however, due to their short life cycle, and the reliance of their stocks on larval recruitment, these taxa are difficult to sample and the stocks are therefore difficult to manage (Lynam et al., 2013). Neither otter nor beam trawls are effective methods for assessing their abundance, particularly at early life stages (Ellis et al., 2012). In addition, morphological identification is often unreliable (Thiel & Kneib, 2016), limiting the assessment to family level only (Ellis et al., 2012). Our survey encountered the lesser sandeel, *A. marinus*, a taxon of the genus *Gymnammodytes* and a further taxon identified to family level, distributed in areas where sandeels are known to spawn in the Irish Sea and the Bristol channel (Ellis et al., 2012; Lynam et al., 2013). *A. marinus* was always detected in water samples where the larvae were also encountered. Therefore, while water sample metabarcoding alone cannot determine the age class of organisms encountered, it can give a picture of the distribution of these taxa during spawning events, to a higher taxonomic resolution than is often available through traditional means (Ellis et al., 2012).

Herring/sprat (*C. harengus/S. sprattus*) were also frequently encountered using both sample types at the same sites, with water samples displaying higher sensitivity. Most *C. harengus* spawning in the Irish Sea occurs in September/October, and some can spawn as late as March (Brophy & Danilowicz, 2002). However, due to difficulties in morphologically separating these two species, spring surveys tend to assume that larvae caught in this period are *S. sprattus* (Fox, Dickey-Collas, & Winpenny, 1997), which spawn from March to August (de Silva, 1973). While the primers used in this study cannot separate *S. sprattus* and *C. harengus*, a qPCR approach could be used in the samples where the presence of one or both species is identified by metabarcoding, to rapidly separate these species in water samples (e.g., Brechon et al., 2013). *S. sprattus* is relatively under-assessed within the Celtic Seas ecoregion and is considered data limited (Moore et al., 2019); therefore, information obtained from a water sampling approach has the potential to add to traditional methods of assessment.

Relative abundances were correlated for *L. limanda* between the two sample types. However, relative abundances estimated from metabarcoding should be treated with caution (Lamb et al., 2019; Thomas, Deagle, Eveson, Harsch, & Trites, 2016) due to amplification bias. In fact, the sequencing of mock community 1 indicated that, with the primers used in this study, the relative abundance of *A. marinus* reads could be on average 2 times higher than the relative abundance of input material, while *Lepidorhombus* sp. reads were 0.6 times as abundant as their known inputs. In addition, DNA shedding may differ between organisms (Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016) and sizes of fish (Maruyama et al., 2014), and can further complicate abundance signals (Hansen et al., 2018). However, using a presence/absence approach can also overestimate the importance of rare taxa and relative abundance estimates may provide useful information (Deagle et al., 2019; Lamb et al., 2019) for comparative studies. In this case, *C. harengus/S. sprattus* and *A. marinus* were more frequently detected, and had a higher relative

abundance, in water samples, while *Callionymus* sp. was more frequently detected in bulk samples, but no difference in relative abundance was observed between sample types. This indicates that, in some contexts, measures of relative abundance can provide useful information.

For rarer species, detections by both methods were more sporadic. For example, taxa such as mackerel and blue whiting, which are known to undergo peak spawning in May (Ellis et al., 2012), were not encountered in larvae samples, but were detected in water samples. While it is not possible to know whether spawning had occurred and was missed by the larvae sampling, or whether only adults were present, this indicates that taxa potentially missed using traditional means can still be detected in water samples.

eDNA metabarcoding detections are sensitive to computational filtering thresholds (Evans et al., 2017). In this case, blank filtering (Grey et al., 2018) and discarding of taxa with low reads to account for index hopping (Schnell et al., 2015) resulted in less water than larvae sample detections for some of the less common/abundant taxa for example *M. merluccius*, and *L. limanda* (Figure 3). This reflects the trade-off between stringency and uncertainty when applying thresholds of detection to metabarcoding data, as while more stringent filtering can underestimate taxa richness, it also reduces the risk of false positives due to tag jumping (Schnell et al., 2015) or cross contamination. Potential solutions include using combinations of markers (Evans et al., 2017) or specific qPCR assays (Harper et al., 2018; Schneider et al., 2016) in conjunction with metabarcoding (Deiner et al., 2017).

While water and larvae samples did not differ overall in richness and alpha diversity measures, larvae samples exhibited greater variance in species richness, community composition, and detection of rare taxa differed between sample types, demonstrating how these two sampling strategies may complement each other. When considering how to interpret eDNA data, the ecology of the eDNA molecules should be considered (Barnes & Turner, 2016). eDNA transport in offshore areas has not been studied extensively (Collins et al., 2018); however, in freshwater systems, eDNA signals may travel up to 10 km (Deiner & Altermatt, 2014). In the marine environment, tidal currents, seasonal stratification, pH, and temperature (Lacoursière-Roussel et al., 2018) may all influence the distribution of eDNA in the marine environment (Hansen et al., 2018). In coastal environments, with a small tidal amplitude, eDNA has been shown to have a limited dispersion area, <1,000 m, and may only remain detectable for as little as an hour after the source has been removed, providing a snapshot of the organisms present (Murakami et al., 2019). In offshore environments however, eDNA may degrade more slowly than in coastal areas (Collins et al., 2018). Environmental factors can, therefore, lead to widely variable dispersal of eDNA particles, dependent on oceanographic, biological and chemical parameters (Hansen et al., 2018).

5 | CONCLUSIONS

Water and larval sampling both coincided in the detection of the most common taxa, which constituted 63% and 60% of the taxa in

the water and larvae samples, respectively. On a site by site basis, there was a 75% agreement in detection between sample types. Sandeels were detected more frequently in water samples, which improves upon traditional methods that often are unable to capture or identify this family. Herring/sprat were also more frequently detected in water samples, indicating that metabarcoding combined with a targeted approach such as qPCR could also provide higher sensitivity distributions for these taxa. While eDNA still requires an extensive sampling effort, its noninvasive and rapid nature renders it particularly suitable for use in spawning and protected areas and for fisheries management applications.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

SC, FR, and CGL conceived the idea. FR carried out the sampling. FR and TUW carried out the laboratory work. FR performed the bioinformatic analyses with TUW. FR led the writing of the manuscript with SC and all authors contributed critically to the drafts and final version.

ETHICAL APPROVAL

Sampling has been conducted following Home Office regulations and approved by Swansea University Ethics Committees under approval No. 181019/1996.

DATA AVAILABILITY STATEMENT

Metabarcoding sequences from larvae samples (BioProject PRJNA576002) and water samples (PRJNA596623) have been deposited in the NCBI.

ORCID

Frances C. Ratcliffe  <https://orcid.org/0000-0001-5185-9200>

Tamsyn M. Uren Webster  <https://orcid.org/0000-0002-0072-9745>

[org/0000-0002-0072-9745](https://orcid.org/0000-0002-0072-9745)

Carlos Garcia de Leaniz  <https://orcid.org/0000-0003-1650-2729>

Sofia Consuegra  <https://orcid.org/0000-0003-4403-2509>

REFERENCES

- Anderson, M. J. (2014). Permutational multivariate analysis of variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online*, 1–15. <https://doi.org/10.1002/9781118445112.stat07841>
- Andruszkiewicz, E. A., Starks, H. A., Chavez, F. P., Sassoubre, L. M., Block, B. A., & Boehm, A. B. (2017). Biomonitoring of marine vertebrates in

- Monterey Bay using eDNA metabarcoding. *PLoS ONE*, 12(4), 1–20. <https://doi.org/10.1371/journal.pone.0176343>
- Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, 17(1), 1–17. <https://doi.org/10.1007/s10592-015-0775-4>
- Bicknell, A. W. J., Godley, B. J., Sheehan, E. V., Votier, S. C., & Witt, M. J. (2016). Camera technology for monitoring marine biodiversity and human impact. *Frontiers in Ecology and the Environment*, 14(8), 424–432. <https://doi.org/10.1002/fee.1322>
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., ... de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, 29(6), 358–367. <https://doi.org/10.1016/j.tree.2014.04.003>
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Brechon, A. L., Coombs, S. H., Sims, D. W., & Griffiths, A. M. (2013). Development of a rapid genetic technique for the identification of clupeid larvae in the Western English Channel and investigation of mislabelling in processed fish products. *ICES Journal of Marine Science*, 70(2), 399–407. <https://doi.org/10.1093/icesjms/fss178>
- Brophy, D., & Danilowicz, B. S. (2002). Tracing populations of Atlantic herring (*Clupea harengus* L.) in the Irish and Celtic Seas using otolith microstructure. *ICES Journal of Marine Science*, 59(6), 1305–1313. <https://doi.org/10.1006/jmsc.2002.1304>
- Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E. S., Brander, K. M., ... Richardson, A. J. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science*, 334(6056), 652–655. <https://doi.org/10.1126/science.1210288>
- Bylemans, J., Furlan, E. M., Hardy, C. M., McGuffie, P., Lintermans, M., & Gleeson, D. M. (2017). An environmental DNA-based method for monitoring spawning activity: A case study, using the endangered Macquarie perch (*Macquaria australasica*). *Methods in Ecology and Evolution*, 8(5), 646–655. <https://doi.org/10.1111/2041-210X.12709>
- Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, 11(12), 2639–2643. <https://doi.org/10.1038/ismej.2017.119>
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>
- Christie, M. R., Tissot, B. N., Albins, M. A., Beets, J. P., Jia, Y., Ortiz, D. M., ... Hixon, M. A. (2010). Larval connectivity in an effective network of marine protected areas. *PLoS ONE*, 5(12), 1–10. <https://doi.org/10.1371/journal.pone.0015715>
- Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., ... Brosse, S. (2019). Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Molecular Ecology Resources*, 19(1), 27–46. <https://doi.org/10.1111/1755-0998.12900>
- Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18(1), 117–143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- Clarke, K. R., Somerfield, P. J., & Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, 330(1), 55–80. <https://doi.org/10.1016/j.jembe.2005.12.017>
- Collins, R. A., Wangenstein, O. S., O’Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology*, 1(1), 185. <https://doi.org/10.1038/s42003-018-0192-6>
- Conway, D. V. P., Coombs, S. H., & Smith, C. (1997). Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. *ICES Journal of Marine Science*, 54(1), 136–147. <https://doi.org/10.1006/jmsc.1996.0176>
- Cordes, E. E., Jones, D. O., Schlacher, T., Amon, D. J., Bernardino, A. F., Brooke, S., ... Witte, U. (2016). Environmental impacts of the deep-water oil and gas industry: A review to guide management strategies. *Frontiers in Environmental Science*, 4, 1–54. <https://doi.org/10.3389/fenvs.2016.00058>
- Costello, M. J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H., & Miloslavich, P. (2010). A census of marine biodiversity knowledge, resources, and future challenges. *PLoS ONE*, 5(8), e12110. <https://doi.org/10.1371/journal.pone.0012110>
- de Silva, S. S. (1973). Aspects of the reproductive biology of the sprat, *Sprattus sprattus* (L.) in inshore waters of the west coast of Scotland. *Journal of Fish Biology*, 5(6), 689–705. <https://doi.org/10.1111/j.1095-8649.1973.tb04505.x>
- Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., ... Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, 28(2), 391–406. <https://doi.org/10.1111/mec.14734>
- Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE*, 9(2), e88786. <https://doi.org/10.1371/journal.pone.0088786>
- Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26(21), 5872–5895. <https://doi.org/10.1111/mec.14350>
- Douvere, F., & Ehler, C. N. (2011). The importance of monitoring and evaluation in adaptive maritime spatial planning. *Journal of Coastal Conservation*, 15(2), 305–311. <https://doi.org/10.1007/s11852-010-0100-9>
- Ellis, J. R., Milligan, S. P., Readdy, L., Taylor, N., & Brown, M. J. (2012). Spawning and nursery grounds of selected fish species in UK waters. Retrieved from <https://www.cefas.co.uk/publications/techrep/TechRep147.pdf>
- Erickson, R. A., Rees, C. B., Coulter, A. A., Merkes, C. M., McCalla, S. G., Touzinsky, K. F., ... Amberg, J. J. (2016). Detecting the movement and spawning activity of bigheaded carps with environmental DNA. *Molecular Ecology Resources*, 16(4), 957–965. <https://doi.org/10.1111/1755-0998.12533>
- Evans, N. T., Li, Y., Renshaw, M. A., Olds, B. P., Deiner, K., Turner, C. R., ... Pfrender, M. E. (2017). Fish community assessment with eDNA metabarcoding: Effects of sampling design and bioinformatic filtering. *Canadian Journal of Fisheries and Aquatic Sciences*, 74(9), 1362–1374. <https://doi.org/10.1139/cjfas-2016-0306>
- Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J. A., ... Becker, J. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 498(7454), 367. <https://doi.org/10.1038/nature12171>
- Fox, C. J., Dickey-Collas, M., & Winpenny, A. J. (1997). *Spring plankton surveys of the Irish Sea in 1995: The distribution of fish eggs and larvae*. Fisheries Research Technical Report – UK Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, 104(104). [https://doi.org/10.1016/S0967-0653\(98\)80494-8](https://doi.org/10.1016/S0967-0653(98)80494-8)
- Geijzendorffer, I. R., Regan, E. C., Pereira, H. M., Brotons, L., Brummitt, N., Gavish, Y., ... Walters, M. (2016). Bridging the gap between biodiversity data and policy reporting needs: An essential biodiversity variables perspective. *Journal of Applied Ecology*, 53(5), 1341–1350. <https://doi.org/10.1111/1365-2664.12417>
- 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>
- Greve, W., Prinage, S., Zidowitz, H., Nast, J., & Reiners, F. (2005). On the phenology of North Sea ichthyoplankton. *ICES Journal of Marine Science*, 62(7), 1216–1223. <https://doi.org/10.1016/j.icesjms.2005.03.011>
- Grey, E. K., Bernatchez, L., Cassey, P., Deiner, K., Deveney, M., Howland, K. L., ... Lodge, D. M. (2018). Effects of sampling effort on biodiversity patterns estimated from environmental DNA metabarcoding surveys. *Scientific Reports*, 8(1), 2–11. <https://doi.org/10.1038/s41598-018-27048-2>
- Habtes, S., Muller-Karger, F. E., Roffer, M. A., Lamkin, J. T., & Muhling, B. A. (2014). A comparison of sampling methods for larvae of medium and large epipelagic fish species during spring SEAMAP ichthyoplankton surveys in the Gulf of Mexico. *Limnology and Oceanography: Methods*, 12(2), 86–101. <https://doi.org/10.4319/lom.2014.12.86>
- Hansen, B. K., Bekkevold, D., Clausen, L. W., & Nielsen, E. E. (2018). The sceptical optimist: Challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish and Fisheries*, 19(5), 751–768. <https://doi.org/10.1111/faf.12286>
- Harper, L. R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H. C., Gough, K. C., ... Hänfling, B. (2018). Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (*Triturus cristatus*). *Ecology and Evolution*, 8(12), 6330–6341. <https://doi.org/10.1002/ece3.4013>
- Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, 17(3), 377–386. <https://doi.org/10.1101/gr.5969107>
- Jeunen, G. J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., ... Gemmell, N. J. (2020). Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environmental DNA*, 2(1), 99–111. <https://doi.org/10.1002/edn3.49>
- Kimmerling, N., Zuqert, O., Amitai, G., Gurevich, T., Armoza-Zvuloni, R., Kolesnikov, I., ... Sorek, R. (2018). Quantitative species-level ecology of reef fish larvae via metabarcoding. *Nature Ecology & Evolution*, 2(2), 306–316. <https://doi.org/10.1038/s41559-017-0413-2>
- Koslow, J. A., & Wright, M. (2016). Ichthyoplankton sampling design to monitor marine fish populations and communities. *Marine Policy*, 68, 55–64. <https://doi.org/10.1016/j.marpol.2016.02.011>
- La Védrine, M. (2014). *Oil and gas fisheries risk assessment advice, updated cefas recommendations for spawning finfish – English & Welsh Blocks*. <https://doi.org/10.13140/RG.2.1.3543.3126>
- Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E. K., Archambault, P., Deiner, K., ... Bernatchez, L. (2018). eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. *Ecology and Evolution*, 8(16), 7763–7777. <https://doi.org/10.1002/ece3.4213>
- Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology*, 28(2), 420–430. <https://doi.org/10.1111/mec.14920>
- Leduc, N., Lacoursière-Roussel, A., Howland, K. L., Archambault, P., Sevellec, M., Normandeau, E., ... Bernatchez, L. (2019). Comparing eDNA metabarcoding and species collection for documenting Arctic metazoan biodiversity. *Environmental DNA*, 1(4), 342–358. <https://doi.org/10.1002/edn3.35>
- Lee, O., Nash, R. D. M., & Danilowicz, B. S. (2005). Small-scale spatio-temporal variability in ichthyoplankton and zooplankton distribution in relation to a tidal-mixing front in the Irish Sea. *ICES Journal of Marine Science*, 62(6), 1021–1036. <https://doi.org/10.1016/j.icesjms.2005.04.016>
- Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., ... Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, 21(11), 2555–2558. <https://doi.org/10.1111/j.1365-294X.2012.05600.x>
- Lynam, C. P., Halliday, N. C., Höffle, H., Wright, P. J., Van Damme, C. J. G., Edwards, M., & Pitois, S. G. (2013). Spatial patterns and trends in abundance of larval sandeels in the North Sea: 1950–2005. *ICES Journal of Marine Science*, 70(3), 540–553. <https://doi.org/10.1093/icesjms/fst006>
- Maggia, M. E., Vigouroux, Y., Renno, J. F., Duponchelle, F., Desmarais, E., Nunez, J., ... Mariac, C. (2017). DNA metabarcoding of amazonian ichthyoplankton swarms. *PLoS ONE*, 12(1), 1–14. <https://doi.org/10.1371/journal.pone.0170009>
- Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PLoS ONE*, 9(12), e114639. <https://doi.org/10.1371/journal.pone.0114639>
- Masson-Delmotte, V., Zhai, P., Pörtner, H.O., Roberts, D., Skea, J., Shukla, P.R., ... Connors, S. (2018). Global Warming of 1.5 C: An IPCC Special Report on the Impacts of Global Warming of 1.5 C above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change. https://www.ipcc.ch/site/assets/uploads/sites/2/2018/07.SR15_SPM_version_stand_alone_LR.pdf
- Moore, C., Lynch, D., Clarke, M., Officer, R., Mills, J., Louis-Defour, J., & Brophy, D. (2019). Age verification of north Atlantic sprat. *Fisheries Research*, 213, 144–150. <https://doi.org/10.1016/j.fishres.2019.01.018>
- Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schäffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics*, 24(16), 1757–1764. <https://doi.org/10.1093/bioinformatics/btn322>
- Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M. K., ... Masuda, R. (2019). Dispersion and degradation of environmental DNA from caged fish in a marine environment. *Fisheries Science*, 85(2), 327–337. <https://doi.org/10.1007/s12562-018-1282-6>
- Pastors, M., Rijnsdorp, A. D., & Van Beek, F. A. (2000). Effects of a partially closed area in the North Sea (“plaice box”) on stock development of plaice. *ICES Journal of Marine Science*, 57(4), 1014–1022. <https://doi.org/10.1006/jmsc.2000.0586>
- Pitois, S. G., Lynam, C. P., Jansen, T., Halliday, N., & Edwards, M. (2012). Bottom-up effects of climate on fish populations: Data from the continuous plankton recorder. *Marine Ecology Progress Series*, 456, 169–186. <https://doi.org/10.3354/meps09710>
- Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., ... Richardson, A. J. (2013). Global imprint of climate change on marine life. *Nature Climate Change*, 3(10), 919–925. <https://doi.org/10.1038/nclimate1958>
- R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Ratcliffe, F. C., Uren Webster, T. M., Rodriguez-Barreto, D., O'Rourke, R., Garcia de Leaniz, G., & Consuegra, S. (2020). Rapid quantitative assessment of fish larvae community composition using metabarcoding. *BioRxiv*. <https://doi.org/10.1101/2020.01.01.884544>
- Régnier, T., Gibb, F. M., & Wright, P. J. (2017). Importance of trophic mismatch in a winterhatching species: Evidence from lesser sandeel. *Marine Ecology Progress Series*, 567, 185–197. <https://doi.org/10.3354/meps12061>
- Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). ecoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, 39(21), e145. <https://doi.org/10.1093/nar/gkr732>
- Sabatés, A. (2004). Diel vertical distribution of fish larvae during the winter-mixing period in the Northwestern Mediterranean. *ICES Journal of Marine Science*, 61(8), 1243–1252. <https://doi.org/10.1016/j.icesjms.2004.07.022>

- Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science and Technology*, 50(19), 10456–10464. <https://doi.org/10.1021/acs.est.6b03114>
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., ... Sahl, J. W. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–7541. <https://doi.org/10.1128/AEM.01541-09>
- Schneider, J., Valentini, A., Dejean, T., Montarsi, F., Taberlet, P., Glaizot, O., & Fumagalli, L. (2016). Detection of invasive mosquito vectors using environmental DNA (eDNA) from water samples. *PLoS ONE*, 11(9), 1–18. <https://doi.org/10.1371/journal.pone.0162493>
- Schnell, I. B., Bohmann, K., & Gilbert, M. T. P. (2015). Tag jumps illuminated - reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, 15(6), 1289–1303. <https://doi.org/10.1111/1755-0998.12402>
- Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., & Harvey, E. S. (2019). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology*, 33(1), 196–205. <https://doi.org/10.1111/cobi.13183>
- Thiel, R., & Kneibelsberger, T. (2016). How reliably can northeast Atlantic sand lances of the genera *Ammodytes* and *Hyperoplus* be distinguished? A comparative application of morphological and molecular methods. *ZooKeys*, 2016(617), 139–164. <https://doi.org/10.3897/zookeys.617.8866>
- Thomas, A. C., Deagle, B. E., Eveson, J. P., Harsch, C. H., & Trites, A. W. (2016). Quantitative DNA metabarcoding: Improved estimates of species proportional biomass using correction factors derived from control material. *Molecular Ecology Resources*, 16(3), 714–726. <https://doi.org/10.1111/1755-0998.12490>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE*, 7(8), 1–9. <https://doi.org/10.1371/journal.pone.0041732>
- Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., & Willerslev, E. (2016). Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLoS ONE*, 11(11), e0165252. <https://doi.org/10.1371/journal.pone.0165252>

SUPPORTING INFORMATION

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

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