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Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea☆

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ABSTRACT

Biomass assesment of fish stocks is a difficult task that often involves costly fisheries trawl surveys. Trawl surveys rarely return replicate samples, as trawling attempts are expensive and difficult to reproduce. Furthermore, traditional benthic trawling is often detrimental to habitats and the organisms associated with the sea bottom. The rapidly developing field of environmental DNA (eDNA) analysis offers a new approach to non-invasive monitoring of fish. In the present study, we develop and test species-specific primers and probes for qPCR detection of eDNA from Atlantic herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*), European flounder (*Platichthys flesus*), European plaice (*Pleuronectes platessa*), and Atlantic mackerel (*Scomber scombrus*) in the Baltic Sea. A recently published qPCR system was applied for European eel (*Anguilla anguilla*). Filtered water samples were collected during a stratified benthic trawl survey, enabling parallel comparisons of eDNA concentrations with biomass caught by trawling. No significant correlation was found between eDNA concentrations and the biomass of fish caught by the trawl, although an association was observed between the measured concentrations of eDNA and the known distributions and main abundances of cod, herring, plaice and flounder. This indicates that while eDNA concentrations may not be directly comparable to results from existing methods – likely because aquatic eDNA concentrations are not controlled by the same combinations of factors as e.g. trawl biomass catch – eDNA analysis could prove a useful supplement for monitoring fish stocks in the future.

1. Introduction

Estimation of fish stock biomass is typically conducted using bottom trawling, which is both invasive and costly (Baldwin et al., 1996; Heessen et al., 2015). Not all commercially important fish species are evenly and commonly detected in traditional trawl surveys, and correct taxonomic identification can be difficult for many species (Daan, 2001). All types of fishing gear can be considered as being selective. Thus the catchability differs between species and between size classes of conspecifics, seriously influencing stock size estimates (Fraser et al., 2007). As a consequence, conventional surveys often target a limited number of commercially important species at a time. In addition, bottom trawling is banned in some countries (e.g. Belize and Qatar) and in thousands of Marine Protected Areas globally, preventing qualified stock assessments. Trawl surveys are also very costly due to the many man-hours needed, as well as expensive equipment and bunker fuel for

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Table 1
Life history traits, stock size in the Baltic Sea and catch data for the six species in the present study. Data from ICES (http://ices.dk/marine-data/tools/Pages/stock-assessment-graphs.aspx) and the Danish Minestery for Food and Environment (http://fd-statweb.fd.dk/landingsrapport/landingsrapport_front_matter).

Species	Migrations	Habitat	Baltic Sea Landings plus discard 2014, tonnes	Baltic Sea stock biomass 2014, tonnes	Caught in bottom trawls
Anguilla anguilla	Migratory	Benthic[a]	283	No data[d]	No[c]
Clupea harengus	Resident[e]	Pelagic	897771	1259603	Yes
Gadus morhua	Resident	Bentho-pelagic	49987	No data[d]	Yes
Platichthys flesus	Resident	Benthic	28278	No data[d]	Yes
Pleuronectes platessa	Resident	Benthic	4892	14593	Yes
Scomber scombrus	Migratory[b]	Pelagic	0	No data[d]	No[c]

[a] May swim pelagically during spawning migration.
[b] Most of the population migrates out of the Baltic Sea in autumn, but some stay.
[c] A few might be caught occasionally.
[d] Not calculated for the eastern Baltic.
[e] Spawning is often local, but migration is common inside the Baltic Sea.

survey vessels – costs that in many cases will be an obstacle for frequent sampling. Also, the level of exploitation of fish stocks needs to be regarded as a fairly unknown parameter (Worm et al., 2009; Zeller et al., 2011). Even though fisheries surveys aim at fishing at randomized catch positions evenly distributed across depth, seabed strata and economically important regions, it is not always possible to complete a haul if the seabed is too rocky, too muddy or too shallow. As a result, fish stock estimates are generally based on where it is possible to fish, and not necessarily on a broad and random representation of all the different habitats where the fish stocks can be expected to occur. Still, fisheries surveys are currently among the most widely used approaches for obtaining estimates of commercially important fish stocks and of the distribution of species at depths deeper than > 20 m, which is of great importance for giving advice for managers on allowable catch. Although fisheries surveys today provide valuable estimates of fish stocks and distributions for commercially important fish species, there is a need for the development of non-invasive methods that are capable of monitoring marine resources and biodiversity more broadly (Murphy and Jenkins, 2010).

Environmental DNA (eDNA) provides such an alternative tool for determining the presence of species in aquatic environments (Taberlet et al., 2012; Evans et al., 2016), and could potentially assist marine fisheries surveys with estimation of fish stock biomasses of commercially important species (Thomsen et al., 2012a; Miya et al., 2015; Thomsen et al., 2016). Biological monitoring based on eDNA does not rely on the taxonomic identification skills of the researcher once the primers and databases are in place. Because of this the eDNA approach may be advantageous when the taxonomic expertise required to discriminate e.g. closely related species or different life stages of the same species is unavailable (Thomsen and Willerslev, 2015).

Detection of eDNA from macroorganisms in water samples have shown promising results (e.g. Ficotela et al., 2008; Port et al., 2016). Although the release rate of eDNA is likely to be different among species, ages, and growth rates (Dejean et al., 2011; Thomsen et al., 2012b; Klymus et al., 2015), and will depend on biomass (Takahara et al., 2012), life stages (Jerde et al., 2011) and the time of year (Ushio et al., 2017), multiple studies have found a positive correlation between catch per unit effort (CPUE) data with levels of eDNA detected in water samples (e.g. Thomsen et al., 2016; Yamamoto et al., 2016). This includes studies that compare eDNA with seining catch of tidal gobies (Schmelzle and Kinziger, 2016), biomass of a freshwater smelt estimated by snorkelling (Doi et al., 2017), and biomass estimates from echo sounding (Yamamoto et al., 2016). Still, studies that compares detection of eDNA from water samples with traditional fish stock assessments from simultaneous bottom trawl fisheries surveys have only been done once, in Greenland, to the best of our knowledge (Thomsen et al., 2016).

The two most common approaches for detecting eDNA in water samples are based on 1) single-species detection by quantitative real-time polymerase chain reaction (qPCR) (Heid et al., 1996) with species-specific primers and probes (e.g. Thomsen et al., 2012b; Wilcox et al., 2013; Sigsgaard et al., 2015) and 2) multispecies detection by Next-Generation Sequencing (NGS) after PCR amplification of eDNA using universal primers that target short mitochondrial DNA barcoding regions – *eDNA metabarcoding* (e.g. Valentini et al., 2015; Sigsgaard et al., 2016).

The aim of the present study was to develop qPCR-based species-specific primer-probe systems targeting eDNA from six of the most important commercial fish species in the Eastern North Atlantic: Atlantic herring (*Clupea harengus* Linnaeus, 1758), Atlantic cod (*Gadus morhua* Linnaeus, 1758), Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758), European eel (*Anguilla Anguilla* Linnaeus, 1758), European flounder (*Platichthys flesus* (Linnaeus, 1758) and European plaice (*Pleuronectes platessa* Linnaeus, 1758). The system for eel was recently published in a study of mesopelagic predation of eel in the Sargasso Sea (Jensen et al., 2018). These species are among the most common and abundant species of marine fishes in the Baltic Sea (Dickey-Collas et al., 2015; Ellis and Heessen, 2015; Goldsmith et al., 2015; Heessen et al., 2015; Hislop et al., 2015), and play an important ecological role in the North Atlantic Ocean. The six species all have different life histories and different migration patterns across the Baltic and North Sea, and their habitats vary from mainly benthic to exclusively pelagic (Table 1).

We applied the eDNA qPCR detection systems on Baltic Sea seawater samples to investigate whether there were correlations between eDNA concentrations; and traditional benthic trawl catches, temperature, salinity, trawling depth, as well as average length of species caught and targeted with eDNA assays. Based on the results, we discuss whether species-specific eDNA detection can serve as an efficient way of supporting and expanding current surveys in order to obtain a better understanding of the distribution of fish stocks.

2. Materials and methods

2.1. Field sites and sample collection

Water samples were collected onboard R/V *Dana* from October 28th to November 5th, 2014, during a fisheries survey in the southeastern and central part of the Baltic Sea. The survey was carried out in connection with the Danish contribution to the Baltic International Trawl Survey (BITS) program. Occurrence of commercial species and their catch weight were noted for each of the 50 trawled positions. All trawling was carried out during daytime. Water samples were collected with a rosette water collector (SBE 32) containing 12 Niskin 5 L bottles mounted on a Conductivity Temperature Depth (CTD) SeaBird probe. The CTD probe also measured salinity and temperature down through the water column (data in Suppl. File S1.4), and stopped 1–3 m above the bottom. The 5 L water samples were collected at 17 of the 50 trawled stations, as close to the planned trawling depth (between 37.8

Table 2
Positions for trawling and hydrographical stations. Trawling was done in late 2014. Trawling and hydrographical stations were placed as close as possible to the same position. The 'Ex54' station marks an extra hydrographical station not included in the BITS trawling survey. Depths are where the trawling was done. The Conductivity Temperature Depth (CTD) Seabird probe was stopped 1–3 m above this depth, and the water sample for eDNA analysis was thus collected at a depth that is 1–3 m above the trawling depth.

Hydrographical and trawling station reference number	Catch position	Depth (m)	Trawl date	Haul time (hours)
12	55° 38 ′N; 14° 42 ′E	61.7	29-Oct	0.506
14	55° 42 ′N; 14° 41 ′E	54.7	29-Oct	0.501
16	55° 38 ′N; 15° 04 ′E	85.7	29-Oct	0.501
33	55° 44 ′N; 15° 34 ′E	62.4	30-Oct	0.501
35	55° 48 ′N; 15° 48 ′E	56.2	30-Oct	0.504
37	55° 50 ′N; 15° 57 ′E	56.8	30-Oct	0.496
39	55° 52 ′N; 16° 26 ′E	53.0	30-Oct	0.502
54	55° 48 ′N; 17° 41 ′E	57.8	31-Oct	0.501
Ex54	56° 06 ′N; 18° 02 ′E	56.7	31-Oct	0.502
60	56° 08 ′N; 18° 19 ′E	77.8	01-Nov	0.503
69	55° 31 ′N; 18° 08 ′E	75.7	01-Nov	0.503
73	55° 26 ′N; 18° 56 ′E	92.0	02-Nov	0.503
75	55° 30 ′N; 18° 33 ′E	87.6	02-Nov	0.501
91	55° 36 ′N; 18° 25 ′E	94.3	02-Nov	0.501
93	55° 44 ′N; 17° 57 ′E	62.9	02-Nov	0.309
95	55° 37 ′N; 17° 36 ′E	40.2	03-Nov	0.502
97	55° 30 ′N; 17° 56 ′E	68.3	03-Nov	0.502

and 93.8 m) as possible, and before trawling was initiated to avoid contamination of the water samples (Table 2). The water sample for subsequent eDNA analysis was collected 1–3 m above the bottom to avoid sampling suspended sediment and thereby ensure that (as far as possible) only recently sloughed eDNA was collected (Turner et al., 2015). The trawling depth listed (Table 2) is therefore 1-3 m deeper than the water sampling depth. Trawl hauls were performed with a standard towing speed of 3 knots, measured as the speed over the ground, for around 30 minutes (Table 2). Trawl hauls were mainly targeting *G. morhua*, *P. flesus* and *P. platessa* (Anon, 2011) whereas *A. anguilla*, *C. harengus, S. scombrus* and other pelagic species were classified as bycatch. In every haul, subsamples of each species were counted and measured, allowing for estimation of the average length and the variance in length distribution for each species (Supplementary Table S3.1).

2.2. Filtration of water samples and extraction of DNA

From 17 of the trawled stations, 1.5 L of water was filtered using a Sterivex filter (VGPL10RC Polyethersulfone Sterivex-GP Radio-Sterilized Syringe Filter Units with 0.22 µm pore size) in line with filter pore size recommendations put forward by Turner et al. (2014), and with previous studies that have filtered water with the aim of optimizing the detection of eDNA from aquatic organisms (Deiner et al., 2015; Spens et al., 2017). A person that did not do any sorting of the catch performed the filtering immediately after retrieval of the CTD probe. Filtering was done in a room not associated with handling of the caught fish, by using a 60 mL sterile syringe to push a total of 1.5 L through the Sterivex filter, and then forcing out residual water in the filter, prior to immediate storage at −20 °C.

Extractions of DNA were done using the Qiagen DNeasy Blood & Tissue kit. We used tissue samples from various North East Atlantic species of bony fishes that are sympatric with the target species (Table 3, S3.2-S3.7, Suppl. Text S1.1, setup 01) for tests of primer- and probe specificity (Table 4). The filtered water samples were extracted using a modified version of the protocol presented by Sigsgaard et al. (2017) (Suppl. Text S1.1, setup 05).

2.3. Design of species-specific primers and probes

Primers and probes were designed by aligning sequences of mitochondrial (mtDNA) cytochrome *b* (*cytb*) and NADH dehydrogenase subunit 4 (*nd4*) in the software Geneious v.R7.1.7 (Kearse et al., 2012) and identifying gene regions differing between the target species and other sympatric nontarget species. As eDNA fragments excreted from an organism to the environment rapidly degrade; often within days or weeks (e.g. Thomsen et al., 2012a, 2012b), we set out to develop species-specific primers and probes for a short mitochondrial gene fragment (around 100 bp), targeting gene regions that vary among sympatric species (Riaz et al., 2011). Differing gene regions of 90–150 bp were identified by eye in Geneious R7. Suggestions for primers and probes were found using the built-in Primer3 v.2.3.4 (Untergasser et al., 2012) in Geneious, with annealing temperature set to 60 °C and 72 °C for primers and probes, respectively, and length of oligos set to 20–22 bp and 25–27 bp, for primers and probes, respectively. Potential primers and probes were then tested with Primer3 v.0.4.0 (Koressaar and Remm, 2007) to verify the match between primers and the target region. Lastly, primers were matched against the National Center for Biotechnology Information (NCBI) GenBank database using Primer-BLAST (Ye et al., 2012), to ascertain the level of specificity. Any matches to non-marine species and species not found in the North Atlantic were disregarded. We used Geneious R7 to produce distance matrices of nucleotide differences between the targeted species and closely related nontarget species for the full fragment, and for the primers and probes (Tables 3–4 and S3.2-S3.7), to check if primers and probes might amplify eDNA from nontarget species. The specificity of the primers was also tested using OBITools (Boyer et al., 2016) and ecoPCR (Bellemain et al., 2010; Ficetola et al., 2010) at a generic level, with data analysis in R v.3.2.4 (R Core Team, 2016) to produce sequence logo plots for the primers (Fig. 1) and primer mismatch plots (Fig. 2 and S2.1) (Suppl. Text S1.2).

2.4. Laboratorial tests of species-specific and quantitative PCR on water samples

To make sure that each species-specific system would not be affected in the PCR detection by competition from eDNA of sympatric nontarget species, we tested each primer set on DNA extracted from target- and nontarget species (Suppl. Text S1.1, setup 01) to ascertain specificity (Table 3, S3.2-S3.7). We then optimized the reaction concentrations of primers and probes (Suppl. Text S1.1, setup 02). To determine the concentration of target DNA copies per L of seawater, we prepared a standard dilution series (Suppl. Text S1.1, setup 03–04). This approach is similar to the testing described by Agersnap et al. (2017). The concentrations of eDNA target copies from the 17 trawling stations were measured in a qPCR setup (Suppl. Text S1.1, setup 05),

Table 3
Cross reactivity for each species-specific assay. The '*in vitro*'-column lists whether the amplification result on DNA extracted from tissue tested in a qPCR tests were (P) ositive or (N)egative. The closest relative occurring in the North Sea and the Baltic Sea was identified for *A. anguilla* among Anguilliformes (Bauchot, 1986), *C. harengus* among Clupeiformes (Whitehead, 1986), *G. morhua* among Gadiformes (Møller et al., 2002; Svetovidov, 1986; Teletchea et al., 2006), *P. platessa* and *P. flesus* among Pleuronectiformes (Nielsen, 1986; Pardo et al., 2005; Azevedo et al., 2008), and for *S. scomber* among Scombriformes (Collette, 1986). All samples are from Zoological Museum at the University of Copenhagen (ZMUC). 'Concentration assay test' is the concentration of the DNA extracted from tissue used for the qPCR in vitro test. Abbreviations for occurrence is Atlantic (A), Baltic Sea (Ba), Black Sea (Bl), Mediterranean (M), North Sea (NS), New Foundland (NF), Pacific (Pa) and Red Sea (RS).

Assay	Species assay was tested *in vitro* on	Author	Museum catalog number	sequence accession number	*In vitro* test	Occurence	Concentration assay test (ng/mL)
AngangND4_02 assay targeting eDNA from *Anguilla anguilla*. Also see table S3.6 and Jensen et al. (2018).							
	Nemichthys scolopaceus	Richardson, 1848	P313229	NC_013620	N	A	130
	Serrivomer beanii	Gill & Ryder, 1883	P313606	NC_013627	N	A	125
	Anguilla anguilla	(Linnaeus, 1758)	Angsp9_09	NC_006531	P	A	7360
	Anguilla rostrata	(Lesueur, 1817)	Angsp9_10	NC_006547	P	A	8480
CluharCYB_02 assay targeting eDNA from *Clupea harengus*. Also see table S3.2.							
	Alosa alosa	(Linnaeus, 1758)	P18783	NC_009575	N	NS	1260
	Engraulis encrasicolus	(Linnaeus, 1758)	P183796	NC_009581	N	NS	5800
	Sardina pilchardus	(Walbaum, 1792)		NC_009592	N	NS	123
	Sprattus sprattus	(Linnaeus, 1758)	P183862	NC_009593	N	NS	284
	Clupea harengus	Linnaeus, 1758	P183827	NC_009577	P	NS	128
GadmorCYB_02 assay targeting eDNA from *Gadus morhua*. Also see table S3.3.							
	Lota lota	(Linnaeus, 1758)	P375475	KC844053	N	A, Ba, NS	7960
	Merlangius merlangus	(Linnaeus, 1758)	P375951	NC_007395	N	A, Bl, M, NS	127
	Micromesistius poutassou	(Risso, 1827)	P375894	FR751401	N	A, M, NS	532
	Molva molva	(Linnaeus, 1758)	P375732	EU492133	N	A, M, NS	1230
	Pollachius pollachius	(Linnaeus, 1758)	P375896	FR751400	N	A, NS	218
	Pollachius virens	(Linnaeus, 1758)	P375934	FR751399	N	A, NF, NS	129
	Gadus morhua	Linnaeus, 1758	P375874	HG514359	P	A, Ba, NS	50
PlafleCYB_02 assay targeting eDNA from *Platicthys flesus*. Also see table S3.4.							
	Hippoglossoides platessoides	(Fabricius, 1780)		EU492110	N	NS	1680
	Limanda limanda	(Linnaeus, 1758)	P856245	EU492119	N	NS	20000
	Pleuronectes platessa	Linnaeus, 1758	P86427	AY164472	N	NS	1710
	Platichthys flesus	(Linnaeus, 1758)	P855954	AB125334	P	NS	2090
PleplaCYB03 assay targeting eDNA from: *Pleuronectes platessa*. Also see table S3.5.							
	Hippoglossoides platessoides	(Fabricius, 1780)		EU492110	N	NS	1680
	Hippoglossus hippoglossus	(Linnaeus, 1758)		AM749122	N	NS	126
	Limanda limanda	(Linnaeus, 1758)	P856245	EU492119	N	NS	20000
	Platichthys flesus	(Linnaeus, 1758)	P855954	AB125334	N	NS	2090
	Pleuronectes platessa	Linnaeus, 1758	P86427	AY164472	P	NS	1710
ScoscoCYB_03 assay targeting eDNA from *Scomber scombrus*. Also see table S3.7.							
	Euthynnus alletteratus	(Rafinesque, 1810)		NC_004530	N	A	130
	Sarda sarda	(Bloch, 1793)	P74232	EU036483	N	A	1740
	Scomber japonicus	Houttuyn, 1782	10145	NC_021470	N	A, NS, M, Pa, RS	1020
	Scomber scombrus	Linnaeus, 1758	P74240	NC_006398	P	A, M, NF, NS	722

one setup for each species of fish. To make it possible to use the qPCR-amplicons for cloning (Suppl. Text S1.1, setup 06–08), all qPCR reactions were immediately stored at −20 °C. Confirmation of the identity of qPCR amplicons were made by cloning and sequencing on an Illumina MiSeq platform with separation of amplicons using Tapestation 2200 (Agilent) performed by DNA Sense ApS (Aalborg, Denmark). The qPCR amplicons obtained with the assays for *C. harengus*, *P. flesus*, *P. platessa*and *S. scombrus* (CluharCYB_02-, PlafleCYB_02-, PleplaCYB_03- and ScoscoCYB_03-assay, respectively) were sequenced and matched with their assay primers and probe, and reads were counted and matched against the NCBI GenBank database using BLAST. The amplicons from the assays for *A. anguilla* and *G. morhua* (AngangND4_02- and the GadmorCYB_02-assay, respectively) were checked by cloning (suppl. Text S1.1, setup 08, Jensen et al., 2018).

2.5. Estimation of target copy numbers in seawater and data analysis

The eDNA copy number was estimated for the original seawater samples (Suppl. Text S1.1, setup 05). Trawl catches obtained from each haul were standardized as CPUE (as mass of fish caught per time unit of trawling) (Table 5) for each haul at each trawling station for each of the four species caught (i.e. *C. harengus*, *G. morhua*, *P. flesus* and *P. platichthys*). The CPUE was calculated as the weight of the target species of fish caught in each haul divided by fishing time for the given haul. With a standard towing speed of 3 knots the fishing time spent on each haul will be a direct reflection of the length of seabed covered by the haul. The CPUE values reported in this study are all given as kg of fish per hour of fishing (kg/hour).

For the four species of marine fishes (*C. harengus*, *G. morhua*, *P. flesus* and *P. platichthys*) where CPUE and eDNA could be estimated, we plotted eDNA levels (copies per L seawater), and catch data as CPUE (kg/hour) on bathymetric maps of the southeastern part of the Baltic Sea (Figs. 3–4). As there were no catches of *A. anguilla* and *S. scombrus,* we only plotted the eDNA levels for these two species. All plots and maps were prepared using R v.3.2.4 (R Core Team, 2016) (suppl. Text S1.2).

To be able to compare the concentration of target eDNA (copies/L seawater) in the filtered water samples with the CPUE (kg/hour) in a regression analysis, we applied logarithmic transformation of both eDNA concentrations and CPUE measurements (Table 5). Transformation returned distributions that were less skewed for both response variables (Fig. S2.3). Furthermore, the log-transformed data returned normally distributed residuals when regression analysis was performed (Fig. S2.4-S2.7).

The regression analyses performed for each of the four species caught (i.e. *C. harengus*, *G. morhua*, *P. flesus* and *P. platichthys*) were tested for whether there was a significant correlation between the concentration of eDNA and CPUE measurements, and how much of the variation would be explained in the eDNA concentrations as expected from the CPUE measurements as indicated by the coefficient of

Table 4
Primer and probe qPCR detection systems for six marine fishes. Optimal concentrations for primers and probes are given as final concentrations in the qPCR reaction. Probes are 5′-end modified with a FAM-flourescent dye, and equipped with a BHQ1-modification at the 3′-end.

Target species (assay name)	Primer (F and R) and probe (P) name	Sequence, primer and probe 5′- > 3′ direction, with FAM and BHQ1 modifications	Optimal primer − /probe concentration (nM) per individual qPCR well	Molecular weight of dsDNA target fragment [Da]	Target fragment length (bp)
Clupea harengus	Cluhar_CYBF14928	CCCATTTGTGATTGCAGGGG	200	53017.5	86
(CluharCYB_02)	Cluhar_CYBR15013	CTGAGTTAAGTCCTGCCGGG	1000		
See Table S3.2	Cluhar_CYBP14949	FAM-TACTATTCTCCACCTTCTGTTCCTC-BHQ1	200		
Gadus morhua	Gadmor_F15076	TTCGCACCTAATTTACTCGGAG	400	49307.1	80
(GadmorCYB_02)	Gadmor_R15155	TCGGGCTTAACATGAGGTGG	800		
See Table S3.3	Gadmor_P15102	FAM-AGATAATTTCACCCCTGCTAACCCCATC-BHQ1	300		
Platichthys flesus	Plafle_F15107	TAGGCTTTGCAGTTCTCCTT	200	54254.2	88
(PlafleCYB_02)	Plafle_R15194	GCAGGCGTAAAGTTGTCCG	800		
See Table S3.4	Plafle_P15131	FAM-CACTGGCTTCGCTCGCCCTATTTTC-BHQ1	300		
Pleuronectes platessa	Plepla_F15107	TAGGCTTCGCAGTCCTCCTC	1000	55489.0	90
(PleplaCYB_03)	Plepla_R15196	TTGCAGGCGTGAAGTTGTCT	200		
See Table S3.5	Plepla_P15169	FAM-CTAAAAGATTTGGGGAAAATAGGGCGAGT-BHQ1	300		
Anguilla anguilla	Angang_F10571	ATCTAGCAACGGACCCCTTA	800	65364.7	106
(AngangND4_02)	Angang_R10676b	TTGGTTGGTTCTAGCCGCA	1200		
See Table S3.6	Angang_P10595	FAM-ACACCACTACTAGTTTTATCTTGCT-BHQ1	300		
Scomber scombrus	Scosco_CYBF14517	TTCCCTGCTTGGTCTCTGTT	400	61667.1	100
(ScoscoCYB_03)	Scosco_CYBR14597	GGCGACTGAGTTGAATGCTG	800		
See Table S3.7	Scosco_CYBP14541	FAM-TTCCCAAATCCTCACAGGACTATTC-BHQ1	200		

Target fragments for each assay:

Clupea harengus	CCCATTTGTGATTGCAGGGGCTACTATTCTCCACCTTCTGTTCCTCCACGAAACGGGGTCAAACAACCCGGCAGGACTTAACTCAG
Gadus morhua	TTCGCACCTAATTTACTCGGAGATCCAGATAATTTCACCCCTGCTAACCCCATCGTTACCCCACCTCATGTTAAGCCCGA
Platichthys flesus	TAGGCTTTGCAGTTCTCCTTACTGCACTGGCTTCGCTCGCCCTATTTTCCCCCAATCTCTTAGGAGACCCGGACAACTTTACGCCTGC
Pleuronectes platessa	TAGGCTTCGCAGTCCTCCTCACTGCACTGGCTTCACTCGCCCTATTTTCCCCAAATCTTTTAGGAGACCCAGACAACTTCACGCCTGCAA
Anguilla anguilla	ATCTAGCAACGGACCCCTTATCAACACCACTACTAGTTTTATCTTGCTGACTTCTACCATTAATAATTTTAGCGAGCCAAAACCACATGCGGCTAGAACCAACCAA
Scomber scombrus	TTCCCTGCTTGGTCTCTGTTTAGCTTCCCAAATCCTCACAGGACTATTCCTTGCAATGCACTACACGCCCGACGTCGAATCAGCATTCAACTCAGTCGCC

determination in a model comparing eDNA concentrations with CPUE measurements (Table S3.8, Figs. 5–6, S2.2 and S2.8). For the three species with more than three trawling stations above the limit of quantification (LOQ) (see Suppl. Text S1.1, set up 05), (i.e. *C. harengus*, *G. morhua* and *P. platichthys*), a generalized linear model test was performed on one or more of the measured variables (salinity, temperature, fish length, latitude, longitude and trawling depth) to check if any two or three of these in combination could be regarded as explanatory variables for a correlation between eDNA and CPUE levels (Table S3.9 and Fig. 7).

The concentration of eDNA (copy/L seawater) and CPUE (kg/hour) were plotted in double logarithmic plots (Fig. 5 and S2.2, S2.8) for each species, and a linear regression analysis was performed on the logarithmic transformed data. This was done for i) average values of eDNA levels per filter for eDNA levels above the LOQ, ii) averages of eDNA levels per filter including eDNA values below the LOQ with zero (i.e. no qPCR signal) omitted and iii) averages of eDNA levels per filter including measurements below the LOQ and adding 1 to all eDNA levels (i.e. qPCRs showing no amplification were set equal to 1), which would ensure that eDNA levels of zero would be included after logarithmic transformation of data. Exclusion of negative samples (i.e. below the LOQ) was preferred as these would bias linear regression (Ellison et al., 2006). Technical qPCR replicates were averaged for each filter (Table S3.8-S3.9, Figs. 5–6). An average eDNA level was only calculated for filter samples if at least one of four technical qPCR replicates returned eDNA levels above the LOQ. A value of 1 was added to all the CPUE levels so that zero catch values would be included after logarithmic transformation of data.

3. Results

3.1. Species-specific primers and probe systems designed and tested

The primers and probes were found to amplify only mtDNA-*cytb* and mtDNA-*nd4* of the intended target species.

In the tests on DNA extracted from target and nontarget species, no unspecific amplification was observed for the assays: CluharCYB_02, GadmorCYB_02, PlafleCYB_02, PleplaCYB_03 and ScoscoCYB_03 (Table 3 and S3.2-S3.5 and S3.7, S1.1 set up 08). The AngangND4_02-assay was found to be specific for *Anguilla* (Table 3 and S3.6, Jensen et al., 2018). Cloned amplicons (S1.1 set up 08) checked by sequencing of a subsequent PCR were all found to be unique matches to the mtDNA-*cytb* fragment for *G. morhua*. Illumina sequencing resulted in 3518, 2145, 11325 and 11704 reads for the CluharCYB_02-, PlafleCYB_02-, PleplaCYB_03- and the ScoscoCYB_03-assay, respectively (with 48%, 79%, 1%, and 67% of the reads matching the target fragment, respectively). The reads that could not be matched to the primers, the probe or the target fragment, did not match any sequences on NCBI GenBank when 0–3 mismatches were allowed.

The qPCR assays were specific for the target species, with the exception of the assay for *G. morhua* and *A. anguilla* (Table 3 and S3.2-S3.7). Comparison of mtDNA-*cytb* sequences showed that the GadmorCYB_02-assay does not allow for distinction between *G. morhua* and various other species of *Gadus* spp., which is important if eDNA from *G. morhua* needs to be detected in subarctic regions. From the genus *Gadus* only *G. morhua* occurs in the North Sea and the Baltic Sea, making the GadmorCYB_02-assay species-specific in this geographical area. The AngangND4_02-assay is unable to distinguish between *A. anguilla* and the closely related *A. rostrata* (Lehmann et al., 2000; Jensen et al., 2018) (Table 3 and S3.6). As all eels found in northern European seas are *A. anguilla* (Walker and Ellis, 2015), positive amplification should only arise from *A. anguilla* eDNA, when usage of this assay is restricted to the northeastern part of the Atlantic. The PlafleCYB_02-assay is specific to *P. flesus* in the Atlantic Ocean (Table 3 and S3.4). In summary, the six assays are species-specific for eDNA for their intended target species as long as usage of these six assays is restricted to the North Sea and the Baltic Sea.

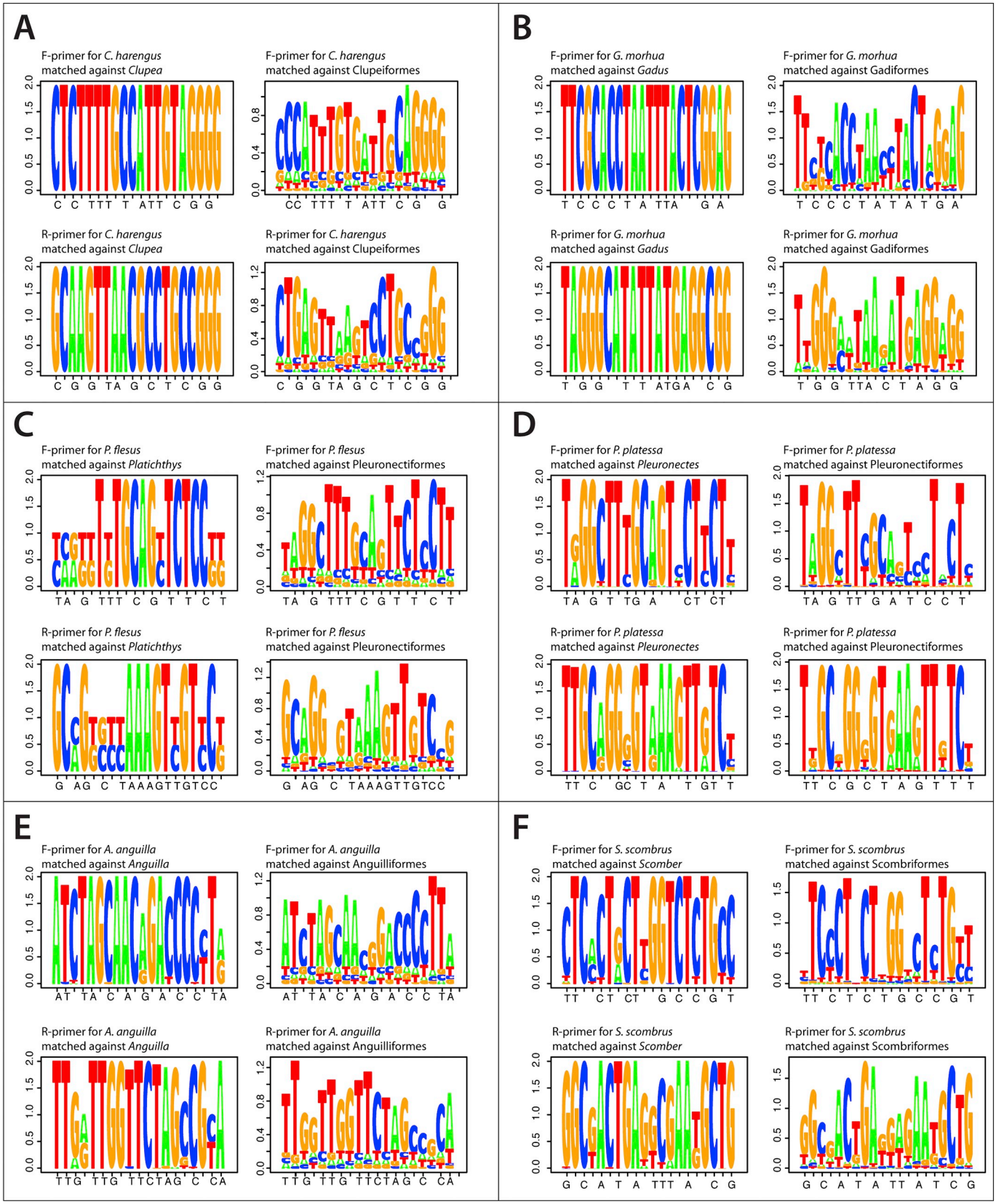

Fig. 1. Sequence logo plots for forward and reverse primers for the species-specific assays targeting *Clupea harengus* (A), *Gadus morhua* (B), *Platichthys flesus* (C), *Pleuronectes platessa* (D), *Anguilla anguilla* (E) and *Scomber scombrus* (F). Primer sequences are also listed in Table 4.

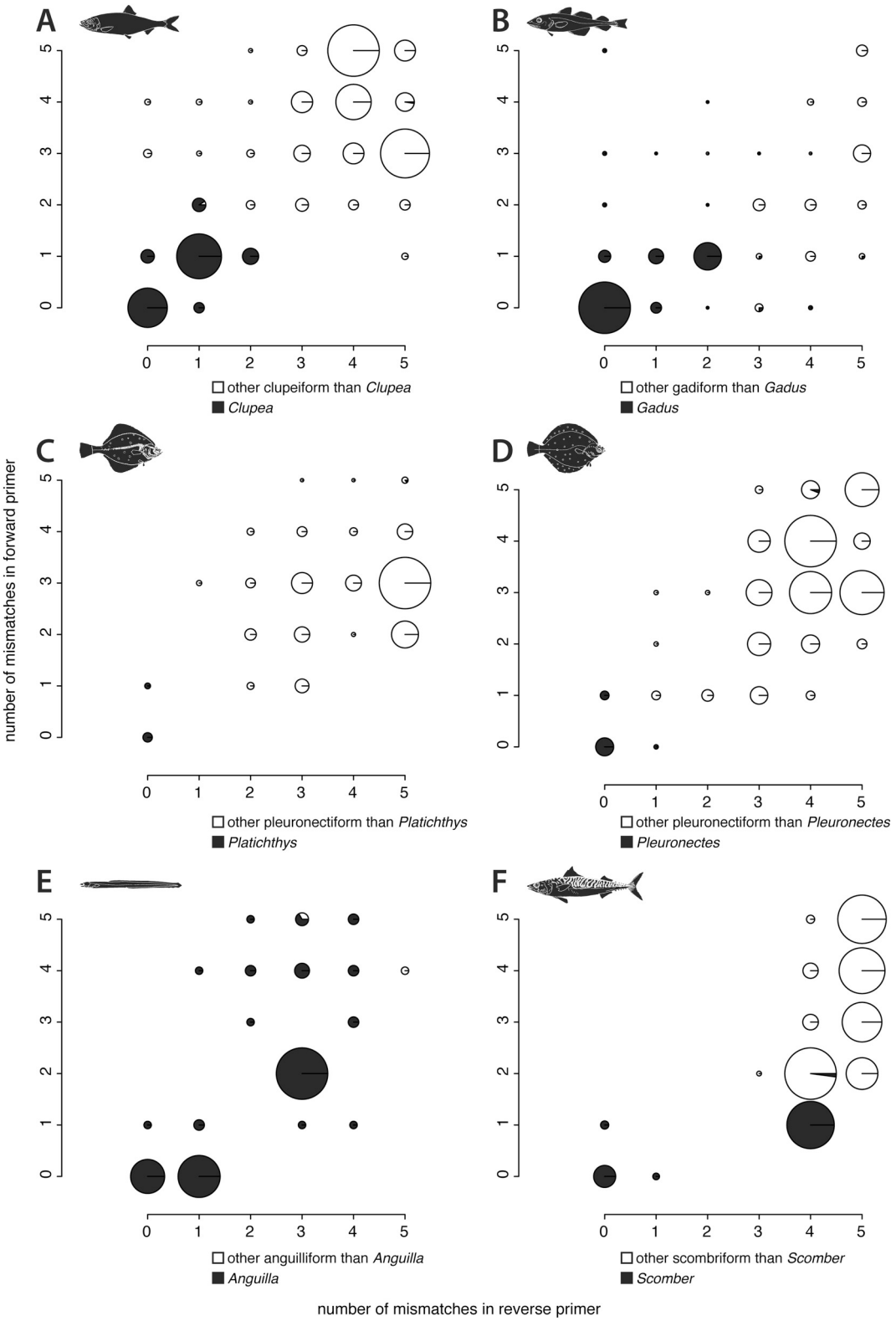

Fig. 2. Mismatch plots for forward and reverse primers to show potential cross reactivity with nontarget species, for *Clupea harengus* (A), *Gadus morhua* (B), *Platichthys flesus* (C), *Pleuronectes platessa* (D), *Anguilla anguilla* (E) and *Scomber scombrus* (F). The abscissa and ordinate indicate the number of nucleotide differences in the primer sequence required before the primer will anneal to another species within the same order instead of annealing to the target genus. The size of the circles reflects the number of sequence matches in the European Molecular Biology Laboratory (EMBL) database. Drawings by SWK.

Table 5
Average of measured eDNA (copies/L seawater) in seawater samples from 17 stations are shown together with catch (kg), levels of eDNA (copies/L filtered water) and catch per unit effort (kg/hour). *Anguilla anguilla* and *Scomber scombrus* were not caught in any trawls. All water samples were analysed with quantitative PCR in four replicates. The number of replicates with positive detection of eDNA, (eDNA level above the LOD) is indicated for each sample (columns denoted by 'pos. repl.'). If one or more replicates were above the level of quantification, LOQ = 667 copies of eDNA/L, an average was calculated using all four measurements, including values below LOQ. Hence, the average calculated may be lower than LOQ. Presence is denoted by 'p' – i.e. when eDNA response levels are below the LOQ but above the LOD. No detection of eDNA (below the LOD) are indicated by 'n'. If the eDNA content was not analysed this is denoted by 'na'. Catch positions for trawling stations are listed in Table 2.

Latin	*Clupea harengus*				*Gadus morhua*				*Platichthys flesus*				*Pleuronectes platessa*				*Anguilla anguilla*		*Scomber scombrus*	
English	Atlantic herring				Atlantic cod				European flounder				European plaice				European eel		Atlantic mackerel	
Station no.	pos. repl.	Catch (kg)	eDNA (copies/L)	CPUE (kg/h)	pos. repl.	Catch (kg)	eDNA (copies/L)	CPUE (kg/h)	pos. repl.	Catch (kg)	eDNA (copies/L)	CPUE (kg/h)	pos. repl.	Catch (kg)	eDNA (copies/L)	CPUE (kg/h)	pos. repl.	eDNA (copies/L)	pos. repl.	eDNA (copies/L)
12	4	87.9	689	173.7	4	20.4	474	40.3	3	0.2	p	0.3	1	0.9	2343	1.9	na	–	2	p
14	4	148.6	p	296.4	3	104.1	p	207.6	4	54.9	p	109.6	2	4.5	3863	9.0	na	–	4	p
16	4	11.6	579	23.1	1	9.2	p	18.4	4	0.6	p	1.2	2	0.0	1189	0.0	4	1948	2	p
33	4	293.3	1034	585.3	4	243.5	549	485.9	4	1.0	p	2.1	1	0.5	p	1.0	na	–	2	p
35	4	1332.7	528	2644.8	4	254.9	p	505.9	4	2.8	p	5.5	2	0.2	1669	0.4	na	–	0	n
37	4	367.4	2416	741.4	4	129.0	594	260.3	4	1.9	p	3.8	0	0.0	n	0.0	na	–	0	n
39	4	146.7	p	292.4	4	259.1	674	516.5	2	5.9	p	11.7	0	1.1	n	2.1	na	–	0	n
54	4	219.5	p	438.5	3	154.9	539	309.5	4	1.1	p	2.2	1	0.6	2009	1.2	na	–	1	p
Ex54	4	0.0	583	0.0	4	0.0	p	0.0	4	0.0	p	0.0	2	0.0	2256	0.0	na	–	1	p
60	4	29.7	933	59.1	2	0.6	483	1.2	4	0.2	p	0.5	1	0.0	1592	0.0	na	–	2	p
69	4	61.8	p	122.9	4	202.9	p	403.6	4	0.0	p	0.0	1	0.0	3146	0.0	4	1897	0	n
73	4	7.8	955	15.5	4	248.5	1540	494.3	4	0.5	p	1.1	na	0.0	–	0.0	4	999	2	p
75	4	17.8	p	35.5	4	515.1	10415	1027.9	3	0.5	p	1.0	na	0.0	–	0.0	na	–	1	p
91	4	2.6	p	5.1	4	0.0	1094	0.0	4	0.0	5029	0.0	na	0.0	–	0.0	na	–	3	p
93	4	148.7	p	481.0	4	1026.1	1495	3318.9	4	0.8	2936	2.5	na	0.0	–	0.0	na	–	2	p
95	4	27.3	p	54.4	4	686.4	2926	1367.5	4	19.2	p	38.2	na	0.0	–	0.0	na	–	4	571
97	4	42.2	p	84.1	4	371.6	3970	740.3	4	0.0	p	0.0	na	0.0	–	0.0	na	–	0	n

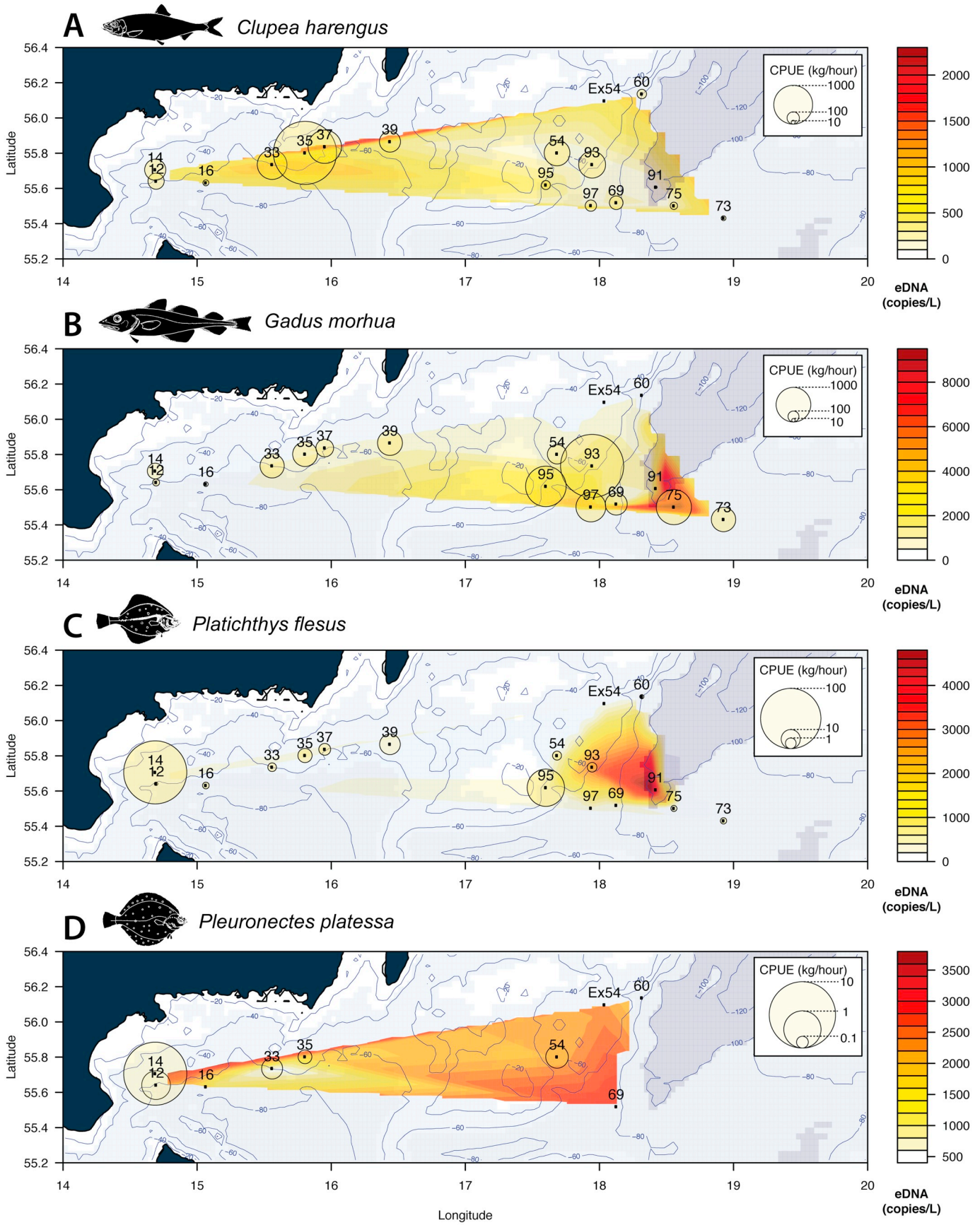

Fig. 3. Catch per unit effort (CPUE, kg per hour) and average eDNA concentrations (copies per L seawater) quantified with qPCR across 17 trawling stations in the Baltic Sea. Concentrations of eDNA are represented by heat map colouring, and the CPUE as circles. A) *Clupea harengus*. B) *Gadus morhua*. C) *Platichthys flesus*. D) *Pleuronectes platessa*. Bathymetry is indicated by underlying isobars and shading. Numbers underneath each catch circle refer to trawling stations as listed in Table 2. Note that *A. anguilla* and *S. scombrus* were not caught in any hauls, and therefore not included on these maps. Drawings by SWK.

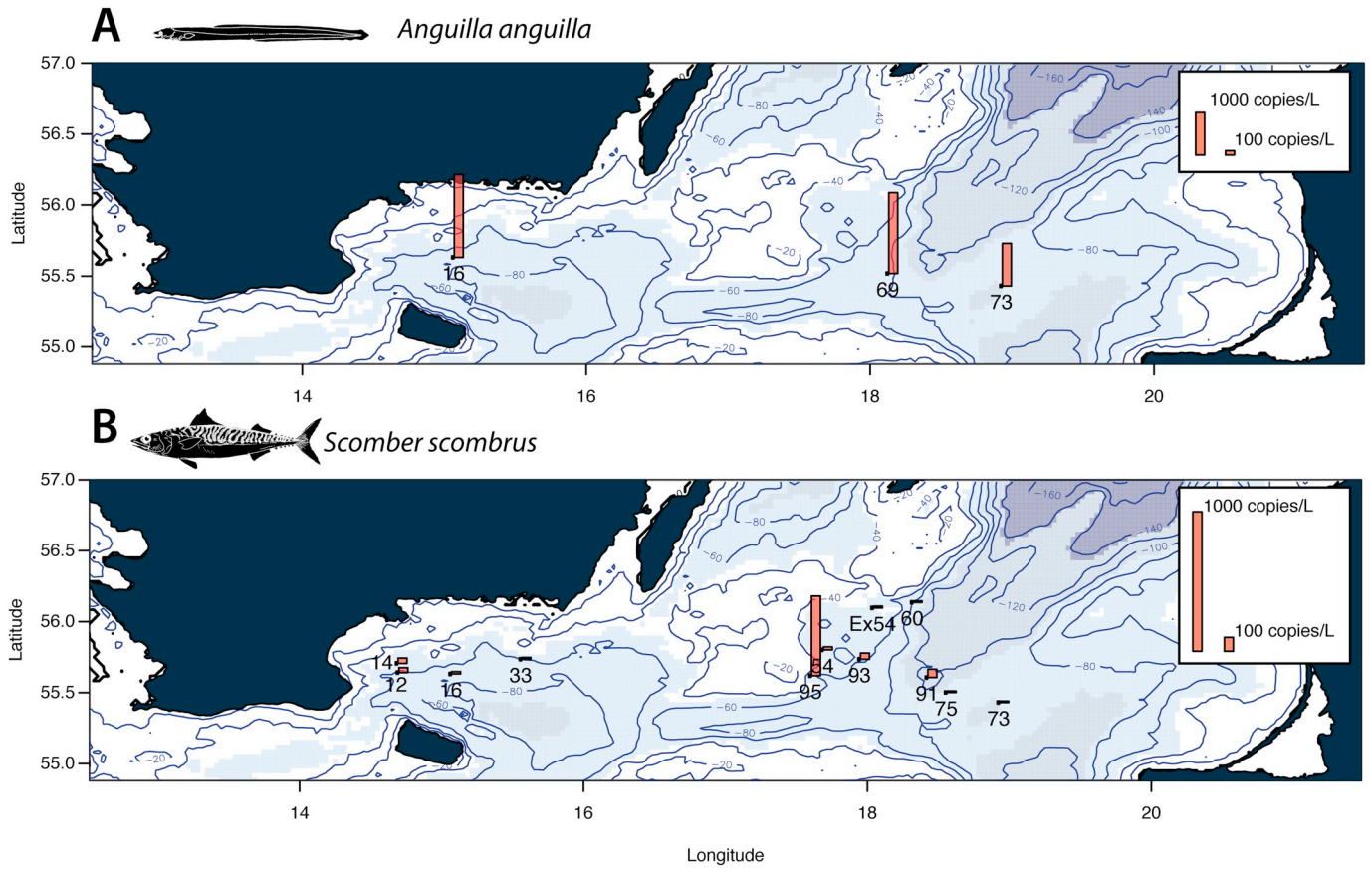

Fig. 4. Average levels of eDNA (copies per L seawater) for *Anguilla anguilla* and *Scomber scombrus*, for the 17 trawling stations (Table 2). The eDNA levels are presented as bars. *Anguilla anguilla* and *S. scombrus* were not caught in any of the hauls, and only eDNA levels are therefore available. Bathymetry is indicated by underlying isobars and shading. Drawings by SWK.

3.2. Quantitative PCR estimated target eDNA copies in water samples and catch data

The limit of detection (LOD) is defined as 1 target copy per qPCR reaction, as inferred from the standard dilution series. The LOD per L of filtered seawater therefore equals 66 copies/L (Suppl. Text S1.1 set up 05).

In the following, we compare the distribution of eDNA copy numbers between the hydrographic stations and relate the eDNA levels to the catch size for the four species caught in trawl hauls, i.e. *C. harengus, G. morhua*, *P. flesus* and *P. platessa* (Figs. 3–4).

Considering only eDNA levels above the LOQ across the 17 trawled stations (Fig. 3), the highest average levels of target eDNA filtered from water samples were 1948, 2416, 10415, 5029 and 3863 copies/L for *A. anguilla*, *C. harengus*, *G. morhua*, *P. flesus* and *P. platesssa*, respectively (Table 5). For *S. scombrus*, all eDNA measurements were below the LOQ (the highest eDNA level was 571 copies/L), and therefore only the presence of eDNA from *S. scombrus* could be confirmed (Table 5). The maximum catch per species for a trawled position was 1333, 1026, 55 and 6 kg for *C. harengus*, *G. morhua*, *P. flesus* and *P. platessa*, respectively.

The highest amount of eDNA for *C. harengus* and *P. platessa* were found in the northwestern part of the surveyed area (Fig. 3A and D). For *C. harengus* the northwestern part also returned the highest CPUE levels (Table 5), while the western part of the surveyed area had the highest CPUE for *P. platessa*. The largest catch and the highest levels of eDNA from *G. morhua* were found in the southeastern part of the surveyed area (Fig. 3B), at trawling station 75 (Table 5). For *P. flesus* and for *S. scombrus*, the highest levels of eDNA were found in the southeastern part of the surveyed area at trawling station 91 (Figs. 3C and 4B). For *P. flesus* the highest CPUE levels were found in the western part of the surveyed area, furthest away from the highest eDNA levels in the east (Fig. 3C). *Anguilla anguilla* and *S. scombrus* were not caught at any of the 17 trawling stations tested for eDNA (Table 5; Fig. 4). For *A. anguilla*, the highest levels of eDNA were found in the southeastern part of the surveyed area (Fig. 4A trawling station 69), and a relatively high eDNA level was found at trawling station 16 (Fig. 4A; Table 5). *Platichthys platessa* was caught in very small quantities (in total 8 kg when summed across all 17 hauls) compared to *G. morhua* and *C. harengus*, which were caught in quantities of several tons each.

3.3. Comparing three variables in a generalized linear model

Three explanatory variables (latitude, trawling depth and concentration levels of eDNA from other fish species) provided the lowest AIC score to best explain the eDNA levels (Table S3.9). The generalized linear model test (Table S3.9) supported that other eDNA levels (eDNA from *A. anguilla* and *P. platessa*) and CPUE for *C. harengus* as explanatory variables provided the best correlation with the eDNA levels for *C. harengus*. For *G. morhua* the latitude, the eDNA levels for *P. platessa* and the CPUE for *G. morhua* provided the best correlation with the eDNA levels for *G. morhua*. The average depth, the eDNA levels for *G. morhua* and CPUE for *P. platessa* provided the best correlation with the eDNA levels for *P. platessa* (Table S3.9). The plots from the eDNA concentration as response based on two explanatory variables (Fig. 7), showed that latitude and eDNA levels from other species together with CPUE levels could be used as explanatory variables. The AIC score (Akaike, 1974) for the models tested, all favoured three explanatory variables for the eDNA levels as a response for *C. harengus*, *G. morhua* and *P. platessa* (Table S3.9). Only two fishing localities returned eDNA

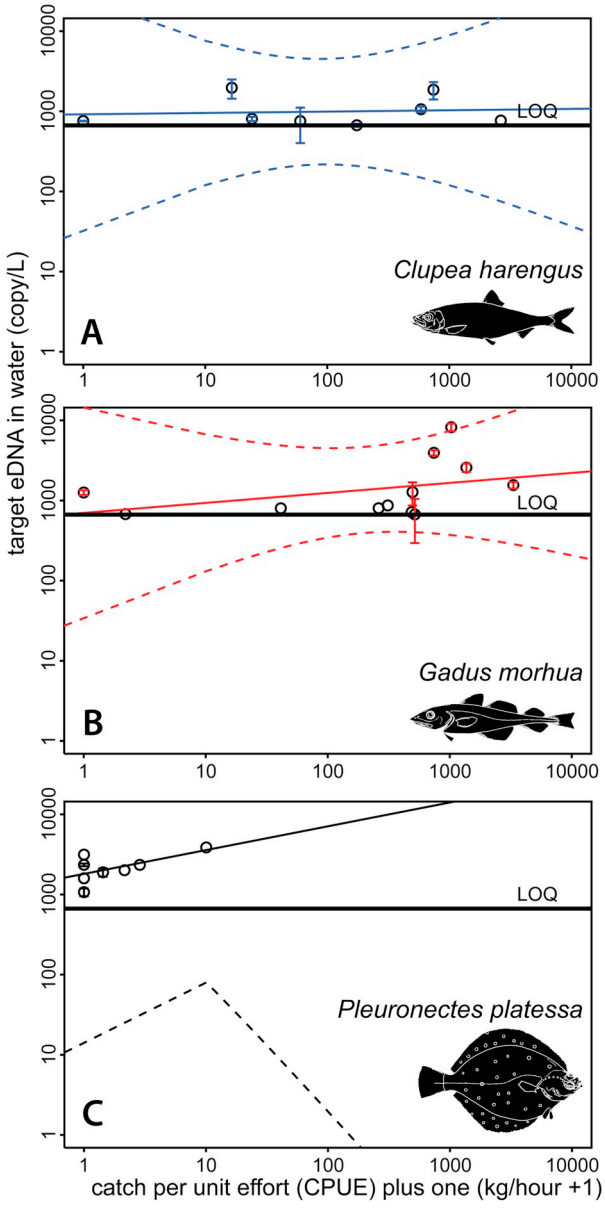

Fig. 5. Logarithmic plots comparing catch per unit effort (CPUE) in kg per hour of fishing time plus 1, with average concentrations of eDNA per filter (copies per L seawater) for four marine fish species A) *Clupea harengus*, B) *Gadus morhua* and C) *Pleuronectes platessa* in the Baltic Sea. The thick horizontal line indicates the limit of quantification (LOQ, i.e. 667 copies per L filtered water) as estimated by the standard curve for each assay. The stipled curved lines indicate 95% confidence interval. Only qPCR replicates with minimum one out of the four replicates returning eDNA concentrations higher than the LOQ were used to calculate the average concentration of eDNA per filter and the standard error. Drawings by SWK.

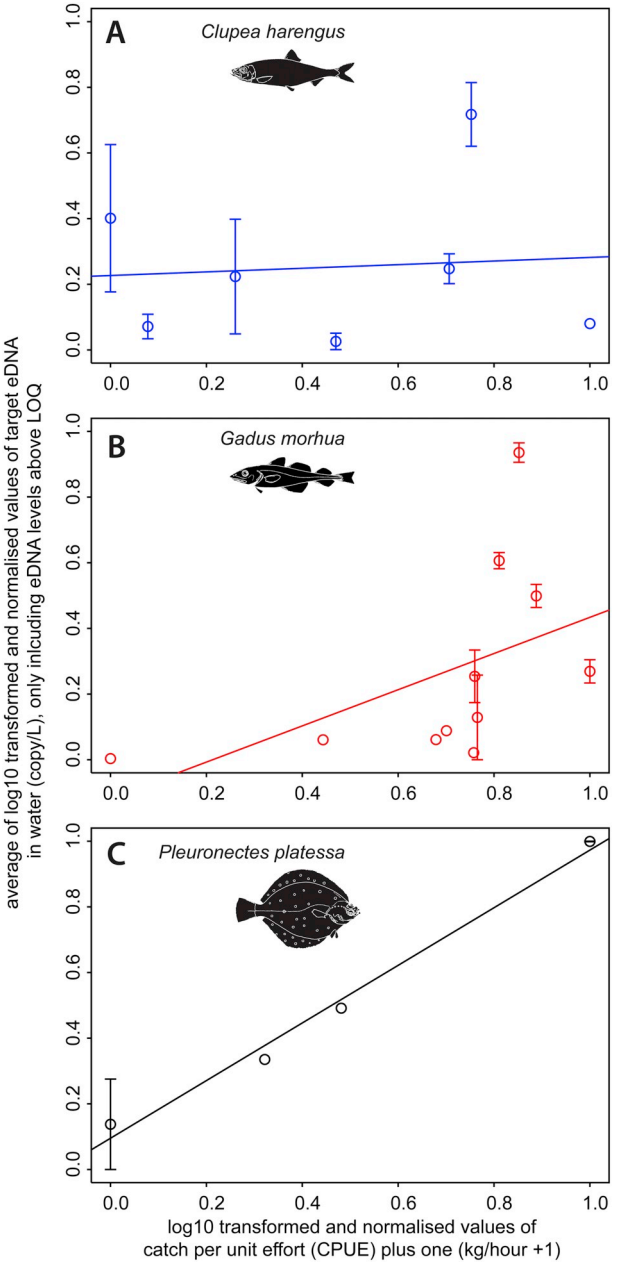

Fig. 6. Plots of normalized log10-transformed catch per unit effort (CPUE) in kg per hour of fishing time plus 1, against normalized log10-transformed average concentrations of eDNA per filter (copies per L seawater) for three marine fish species: A) *Clupea harengus*, B) *Gadus morhua* and C) *Pleuronectes platessa*, in the Baltic Sea. Only eDNA levels above the limit of quantification (LOQ, i.e. 667 copies per L filtered water) is included. Drawings by SWK.

levels for *P. flesus* above the LOQ, and two data points is insufficient to perform a regression analysis. The eDNA levels for *P. flesus* were therefore not considered for the plots based on two explanatory variables (Fig. 7). For *C. harengus*, *G. morhua* and *P. flesus*, the eDNA levels were compared with CPUE and a third variable was determined by removal of covariate variables and identification of variables improving the squared distance among residuals, and comparison of AIC scores for models obtained in a generalized linear model test (see Table S3.9). For *C. harengus* (Fig. 7A) and for *G. morhua* (Fig. 7B) an improved squared distance of residuals was obtained when eDNA levels and CPUE levels were included in the model together with eDNA levels for *P. platessa* and latitude, respectively. For *P. platessa* (Fig. 7C) an improved squared distance of residuals was obtained when eDNA levels and CPUE levels were included in the model together with eDNA levels for *G. morhua*.

4. Discussion

4.1. Specificity in the developed primers and probes

All the designed qPCR assays were shown to be species-specific in the North Sea-Baltic Sea region (Table 3 and S3.2-S3.7, Jensen et al., 2018). It is possible that the primers could amplify other related species, if used in other geographical areas. The NCBI GenBank database cannot be regarded as providing complete coverage of nucleotide

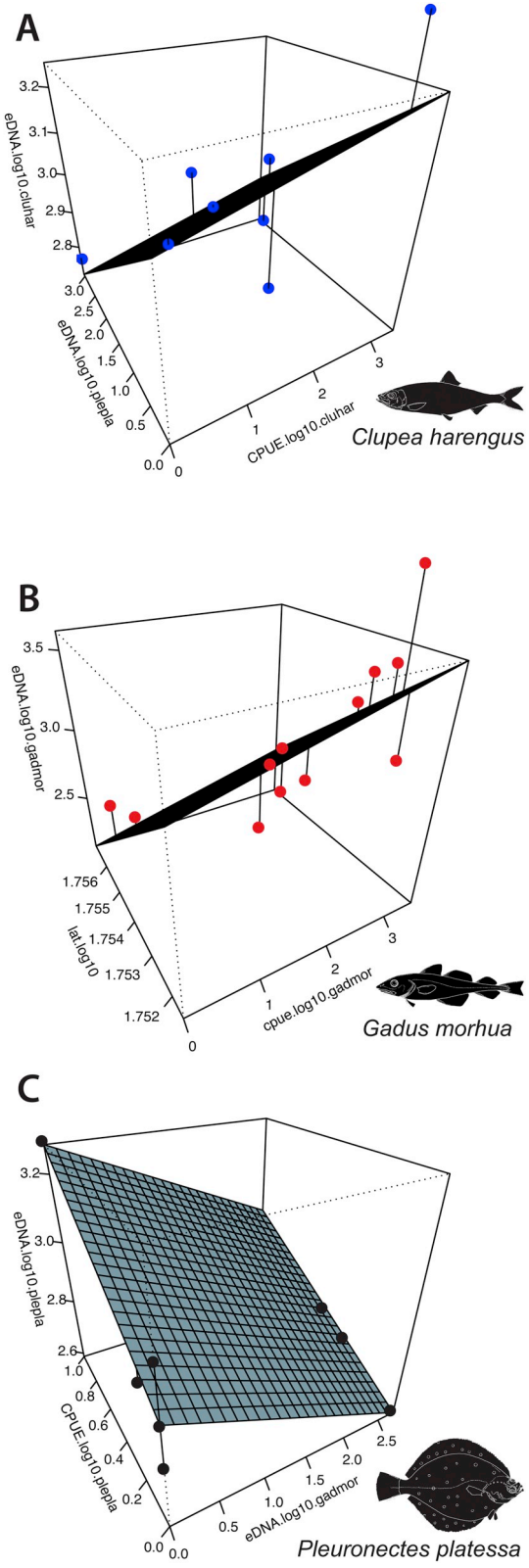

Fig. 7. Logarithmic plots comparing an extra variable with the target species levels of eDNA (copies per L seawater) and the catch per unit effort (CPUE) in kg per hour fishing time for three of the marine fish species: A) *Clupea harengus*, B) *Gadus morhua* and C) *Pleuronectes platessa*. For each species the extra explanatory variable was identified in a generalized linear model test. All axes are on a logarithmic scale (log10). Only eDNA levels above the limit of quantification (LOQ, i.e. 667 copies per L filtered water) are included. Drawings by SWK.

diversity for all organisms found in the Baltic Sea (Nilsson et al., 2006; Ojaveer et al., 2010). However, the Baltic Sea is a well-studied area in regards to diversity of marine fishes (Kontula and Haldin, 2012).

4.2. Distribution of eDNA from target species in relation to catch, migration, life histories and historical data

In the following sections, each species and the eDNA levels measured for them are discussed in relation to their known distributions and ecology as well as to past and contemporaneous catch rates – i.e. the CPUE levels recorded in past studies and the CPUE levels measured in the present study (Table 5).

Clupea harengus was found to have the highest CPUE levels along the southeastern coast of Sweden (Fig. 3A), which historically (1977–2013) is known to provide relatively high catch rates of *C. harengus* (Dickey-Collas et al., 2015). The migratory behaviour of *C. harengus* (Bekkevold et al., 2005; Jørgensen et al., 2005; Limborg et al., 2012) results in a low degree of genetic differentiation (André et al., 2011; Guo et al., 2016), which makes it likely that the CluharCYB_02-assay will perform well if applied across the distribution of *C. harengus*. Time of spawning and salinity is correlated with population structure in *C. harengus* as the genetic variation reflects the salinity levels found in the North Atlantic, the North Sea and the Baltic Sea (Limborg et al., 2012). Future sampling of water with the aim of monitoring spawning in *C. harengus* could be made to coincide with peaks in spawning, to check if eDNA levels are able to reflect the distribution of spawning *C. harengus* and if eDNA levels correlate with salinity and dispersal of eggs and sperm. The regression analysis performed on eDNA levels and CPUE (Figs. 4–6, Table S3.8) did not reveal any significant correlation, which most likely is due to discrepancy between the pelagic behaviour exhibited by *C. harengus* and the benthic trawl which is more efficient at catching fish that are associated with the bottom.

Gadus morhua in the Baltic Sea have the highest catch rates reported along the southern coast of Sweden (Hislop et al., 2015). The eDNA levels measured here from *G. morhua* were found to reflect the catch rates inferred from the trawling and the CPUE levels for the species, and not the recent historical (1972–2013) abundance of *G. morhua* in the Baltic Sea. This indicates that eDNA monitoring provides a reflection of the current species distribution, and not a reflection of the past or surrounding distribution as could occur due to eDNA being swept in by currents from outside the surveyed area, or because of old eDNA being accidentally sampled from the sediment raised from the seabed by currents. *Gadus morhua* has a benthic associated behaviour, and is therefore more likely to be caught in the benthic trawl than *C. harengus*. However, the species also makes frequent vertical feeding migrations to the pelagic zone. The eDNA levels detected are therefore likely a combination of eDNA shed from individuals in the pelagic zone and from individuals closer to the seabed, while trawling only includes individuals in the benthic zone.

Platichthys flesus is found throughout the Baltic Sea, with high catch rates reported from 1977 to 2013 in the eastern part close to Latvia and Lithuania (Goldsmith et al., 2015). The eDNA levels for *P. flesus* is high in the eastern part of the surveyed area (Fig. 3C), and thus appear to match the catch rates recorded in the eastern parts of the Baltic from 1977 to 2013 (Goldsmith et al., 2015). As the populations of *P. flesus* are slightly different genetically (Hemmer-Hansen et al., 2007a) it cannot be said with certainty whether the PlafleCYB_02-assay could return false negative detections in the western part of the North Sea. The life history of *P. flesus* likely influences the level of eDNA detected, as adults do not move around much, but do venture off to spawning areas and feeding grounds. Juveniles are relatively inactive but use currents to disperse to nursery grounds (Hemmer-Hansen et al., 2007b). The eDNA levels in this study are most likely a result of the eDNA sloughed from the local adult in the western part of the Baltic. Opposed to *G. morhua* which is associated with both the pelagic and benthic environments, *P. flesus* is exclusively associated with the bottom. Water

sampling carried out a few meters above the bottom might therefore not yield sufficiently high levels of eDNA from *P. flesus*.

Pleuronectes platessa are less abundant in the southern part of the Baltic Sea, with increasing catch rates westward through the Kattegat Sea and into the North Sea (Goldsmith et al., 2015). A drop in salinity east off the island of Bornholm acts as a boundary for the distribution, and the highest densities are found off the southeastern coast of Sweden (Goldsmith et al., 2015). This distribution is supported by the eDNA levels and the CPUE levels from our study (Fig. 3D). The PlafleCYB_02-assay should also be tested for affinity to the recently described new species of flounder *Platichthys solemdali* (Momigliano et al., 2018), which apparently is endemic to the eastern Baltic Sea, as this potentially could lead to false positive detection of *P. flesus*. *Pleuronectes platessa* displays a complicated image of partially overlapping populations (Hoarau et al., 2005; Ulrich et al., 2017), and populations in the Baltic are to a large extent dependent on the nursery grounds along the western coast of Sweden (Wennhage and Stål, 2007). Juveniles settle in shallow waters in the spring where they stay until autumn. They then migrate to deeper waters and return again to shallow waters the following spring (Wennhage and Stål, 2007). The high CPUE levels and eDNA levels obtained along the southeastern coast of Sweden correspond well with the high level of landings from this area (Ulrich et al., 2013).

Anguilla anguilla have been reported to have a more dense occurrence in the southwestern part of Baltic Sea (Walker and Ellis, 2015), and a tendency towards a similar pattern was found in the eDNA levels (Fig. 4A; Table 5), albeit that eDNA from *A. anguilla* was only recorded at three haul positions. The migration from the Sargasso Sea undertaken by *A. anguilla* and the lack of genetic variation in the mitochondrial genome (Daemen et al., 2001) suggests that it is possible to utilize this species-specific assay across the distribution in the northeastern Atlantic. The current benthic trawl is unlikely to catch *A. anguilla*, as they usually escape from the trawl through the holes in the mesh, but the species-specific assay developed in this study can easily be used in both marine and freshwater environments for specific detection of eel. The low eDNA levels from the Baltic are not surprising as *A. anguilla* is more abundant in shallow fjords, bays and closer to the coastal areas compared to open waters such as the central Baltic Sea. Furthermore, the stock has declined dramatically in recent decades (Walker and Ellis, 2015).

Scomber scombrus decreases in abundance from the saline North Sea towards the more brackish Kattegat and the Baltic Sea (Ellis and Heessen, 2015). In the Baltic Sea, *S. scombrus* is mostly caught around Bornholm (Ellis and Heessen, 2015). Since the type of trawling performed during the survey in this study targets benthic species and rarely performs well catching fast pelagic species, the absence of *S. scombrus* in the catches is not a surprise. The population of *S. scombrus* in the North Sea does not appear to have any genetic structuring (Nesbø et al., 2000) suggesting that our species-specific assay can be used to detect eDNA from all seas surrounding northern Europe. However, as *S. scombrus* is capable of traversing large distances by fast swimming dispersal, future studies will have to take into consideration that eDNA detected from *S. scombrus* might not stem from the individuals in the immediate vicinity of where fishing is carried out.

4.3. Environmental DNA versus biomass of marine species of fish

All six developed qPCR assays were proven to be species-specific and can be a valuable additional tool for future surveys investigating commercial fish species in the Baltic Sea. Here we have demonstrated that eDNA analysis can be used for detection of species such as *S. scombrus* and *A. anguilla,* which normally go undetected in benthic trawling surveys.

Although the quantitative application of qPCR-based eDNA detection can be questioned (Iversen et al., 2015), our data indicated that the largest amounts of eDNA came from the dominant species (i.e. *C. harengus* and *G. morhua*), and that lower eDNA concentrations were found for less common species (*A. anguilla, P. flesus, P. platessa* and *S. scombrus*), and that there appears to be a correlation between the concentrations of eDNA and CPUE from three of the investigated species (Table S3.9 and Fig. 7).

Overall, the 17 stations sampled showed some indication of eDNA correlating with CPUE for *C. harengus*, *G. morhua* and *P. platessa* (Figs. 3–7 and S2.1, S2.7, Table S3.8). However, qPCR assays vary in efficiency because of different target sequence lengths and differences in affinity between primer and target sequence. Hence, quantitative interpretations should mainly focus on variations between different samples for the same target species, rather than on different target species in the same sample. Even without correlation, the findings reported here underline that monitoring with eDNA could provide an effective supplement to traditional fisheries surveys. The high variation in the estimated levels of eDNA concentrations, point to the need for both an increased number of biological sample replicates and also a high number of technical replicates in the qPCR analysis, especially if the eDNA levels are to be evaluated quantitatively.

Individual trawl hauls are nearly impossible to replicate, so a statistically significant correlation with eDNA levels would likely require an intense sampling effort and a high number of hauls. It is unlikely that direct comparison is possible. Strong correlations between eDNA concentrations and trawl catches are not necessarily to be expected as the eDNA concentrations in seawater (here measured as copies per L) and CPUE (here measured as kg per hour) are two completely different measures, and are obtained in different ways and have different kinds of origin. Trawling represents a real-time picture of the fish distribution, while eDNA will represent a picture of shedding during the past days/weeks, with a successively diluted signal. This could particularly skew results in the oceans, where eDNA can be transported over greater distances. Several factors potentially obscure the relationship between fish density and corresponding species-specific eDNA concentration: eDNA shedding rates, eDNA degradation rates specific to the environment, abiotic factors such as temperature, currents, salinity and pH, as well as water sampling method, DNA extraction method, primer chemistry, stochasticity in PCR reactions et cetera. Nevertheless, there are studies where quantitative levels of eDNA in water samples have correlated with catch size of fish caught with traditional fishing methods in marine environments (e.g. Yamamoto et al., 2016; Thomsen et al., 2016; Ushio et al., 2017).

Although the correlations in the current study were not significantly different from zero, eDNA and catch correlated better for *G. morhua* than for *C. harengus* (Figs. 5–6 and S2.7). Trawling in the Baltic Sea mainly targets benthic species such as *G. morhua*, *P. platessa*, and *P. flesus.* Hence, a better correlation was expected between eDNA concentration and catch for these three species as compared to the pelagic *C. harengus*. Fast-swimming fish like *S. scombrus* are able to escape the trawl, and can therefore be expected to be detected more efficiently by eDNA than by trawling. This difference in trawl selectivity may be the cause of the low correlation observed between eDNA and catch for *C. harengus* (Table S3.8).

Biomass and stock size estimates based on bottom trawling are difficult to obtain for fish stocks closer to the shore, since rocky coasts or shallow seas make trawling difficult, if not impossible. Especially for these coastal areas, eDNA monitoring could potentially contribute greatly to existing monitoring programs of fish stocks.

The ecological part of this study could have been improved with more comprehensive sampling. A single 1.5 L water sample per haul was sufficient to demonstrate an increase in eDNA levels by an increase in CPUE for *C. harengus* and *G. morhua*, but not for the less abundant species. Future studies that wish to estimate eDNA concentrations in marine water samples and relate this to catch, should probably aim at sampling a minimum of three filters per fishing locality (Schmelzle and Kinziger, 2016), and perform 12 qPCR replicates, or eight replicates as a minimum (Ficotela et al., 2015).

4.4. Conclusions

eDNA is a rapidly evolving research field and the "optimal experimental design" changes between research fields and funds invested. The current project is a medium-sized project targeting commercial Baltic fish species and with the main focus of producing species-specific detection systems. While eDNA concentrations may not always be directly comparable to results from existing methods - likely because aquatic eDNA concentrations are not controlled by the same combinations of factors as e.g. trawl biomass catch, measurement of additional factors might help coupling eDNA concentrations to catch size. Some of these factors were addressed in a generalized linear model, showing that latitude and concentration levels of eDNA from other fish species also could be helpful for evaluating eDNA levels and CPUE. Although, no significant correlations were found between eDNA concentrations and the biomass of fish caught by the trawl, associations were observed between concentrations of eDNA and the known distributions and main abundances of *G. morhua*, *C. harengus*, *P. platessa* and *P. flesus*, and eDNA levels of co-occuring species. Thus, our study demonstrates that eDNA analysis can be a useful supplement for monitoring fish stocks in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2018.09.004.

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