







# Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys

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## Abstract

Environmental DNA (eDNA) has revolutionized our ability to identify the presence and distributions of terrestrial and aquatic organisms. Recent evidence suggests the concentration of eDNA could also provide a rapid, cost-effective indicator of abundance and/or biomass for fisheries stock assessments. Globally, fisheries resources are under immense pressure, and their sustainable harvest requires accurate information on the sizes of fished stocks. However, in many cases the required information remains elusive because of a reliance on imprecise or costly fishery-dependent and independent data. Here, we review the literature describing relationships between eDNA concentrations and fish abundance and/or biomass, as well as key influencing factors, as a precursor to determining the broader utility of eDNA for monitoring fish populations. We reviewed 63 studies published between 2012 and 2020 and found 90% identified positive relationships between eDNA concentrations and the abundance and/or biomass of focal species. Key influencing biotic factors included the taxon examined as well as their body size, distribution, reproduction, and migration. Key abiotic factors mostly comprised hydrological processes affecting the dispersal and persistence of eDNA, especially water flow and temperature, although eDNA collection methods were also influential. The cumulative influence of these different factors likely explains the substantial variability observed in eDNA concentrations, both within and among studies. Nevertheless, there is considerable evidence to support using eDNA as an ancillary tool for assessing fish population abundance and/or biomass across discrete spatio-temporal scales, following preliminary investigations to determine species- and context-specific factors influencing the eDNA abundance/biomass relationship. Advantages of eDNA monitoring relative to other approaches include reduced costs, increased efficiencies, and nonlethal sampling.

## KEYWORDS

abundance, biomass, environmental DNA, fishery-independent survey, stock assessment

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## 1 | INTRODUCTION

The global harvest from wild marine and inland fisheries peaked at ~90 million tonnes during the mid-1990s and has fluctuated around this value to the present day (FAO, 2018). While there remains conjecture over the accuracy of these estimates (Branch et al., 2011; Pauly & Zeller, 2017), it is clear that within the same period (and prior to the early 1970s) the proportion of sustainably harvested stocks declined. The most recent estimate suggests only 67% of stocks are sustainably harvested (FAO, 2018). In addition to concerns over the future sustainability of the world's harvested stocks are the impacts to populations of bycatch species, particularly endangered, threatened, and protected (ETP) species (Gray & Kennelly, 2018; Lewison et al., 2004).

Sustainable harvesting requires accurate information on population size, exploitation rates, population connectivity, dynamics, and size structures. However, much of the intrinsic information can be challenging to obtain for many species. Estimating the size (usually biomass) of fished populations, or proxies of size that allow relative temporal trends to be examined, is usually achieved by collating *in situ* fishery-dependent or fishery-independent data as a basis for stock assessments (Beverton & Holt, 1957; Hilborn & Walters, 1992).

Fishery-dependent methods typically involve collecting catch data from vessel logbooks and/or scientific sampling onboard or during landings (Doubleday & Rivard, 1983). While cost-effective and broad scale, such data are subject to numerous biases, including variable fishing effort or efficiency and/or hyperstability (where catch per unit of effort remains stable while the population declines; Dennis et al., 2015). Commercial catches are also a diminishing data source for assessment in some developed nations, owing to declining yields due to fleet consolidations and increased pressure from recreational fisheries that are often poorly assessed themselves (Gray & Kennelly, 2017). Other assessment methods (mostly among developed nations) utilize fishery-independent surveys by deploying similar gears as those fished commercially (Dennis et al., 2015) that are sometimes supplemented by larval sampling (e.g., egg counts for fish, or puerulus surveys for rock lobsters) (Chittleborough & Phillips, 1975; Ospina-Álvarez et al., 2013). These methods can provide high-quality representative data for assessments, but are often costly and logistically demanding. For ETP species, conventional survey methods for stock assessment are generally too invasive and often conflict with animal-welfare legislation. Cost-effective data sources that are free from fisheries operational biases and do not impact populations of declining species will be invaluable for supporting sustainable fisheries worldwide (Gray & Kennelly, 2018).

One alternative approach to conventional survey methods involves genetic analyses through sampling environmental DNA (eDNA) (Kamoroff & Goldberg, 2018; Shelton et al., 2019). All organisms release mucus, feces, urine, gametes, and skin cells which, for aquatic species, result in intra- and extracellular DNA being suspended in the water column and detectable for up to ~60 days (Dejean et al., 2012; Strickler et al., 2015; Thomsen et al., 2012). This

eDNA can be collected and used to identify the recent presence and distribution of a species; often at lower costs than traditional survey methods (Jerde, 2019; Lugg et al., 2018; Yamamoto et al., 2016). Alternatively, eDNA metabarcoding using universal primers can allow multiple species to be rapidly and concurrently identified (Berry et al., 2019; McInerney & Rees, 2018; Shaw et al., 2016; Stein et al., 2014; Thomsen et al., 2016; Yamamoto et al., 2017).

Most of the early research on eDNA was constrained to detecting species presence (Cristescu & Hebert, 2018; Deiner et al., 2017; Evans & Lamberti, 2018; Rees et al., 2014; Thomsen & Willerslev, 2015). More recent investigations have examined whether eDNA concentrations correlate with the abundance and/or biomass of aquatic taxa (e.g., fish—Takahara et al. (2012); amphibians—Thomsen et al. (2012); Pilliod et al. (2013); and molluscs—Goldberg et al. (2013)). For some fish species, these relationships have been confirmed in controlled environment (aquaria/mesocosm) studies (Doi et al., 2015; Klymus et al., 2015), but are less clear in natural environments (Spear et al., 2015; Yamamoto et al., 2016; Yates et al., 2019)—reflecting at least two considerations. First, most studies have compared eDNA concentrations to abundance and/or biomass estimated via traditional survey methods, which have their own inherent biases (Lyon et al., 2014). Second, and perhaps more importantly, a plethora of uncontrolled species-specific biotic and abiotic factors may affect eDNA concentrations, and in turn abundance and/or biomass estimates (Deiner et al., 2017; Hansen et al., 2018; Stewart, 2019; Yates et al., 2019). Nevertheless, multiple studies showing correlations between abundance and/or biomass and eDNA concentrations for various taxa justify not only ongoing efforts to improve accuracy and precision, but also a synthesis of existing information to help refine future efforts (Mace et al., 2000; Shelton et al., 2016). Ultimately, consolidation of efforts in this area is required to inform fisheries management and conservation efforts globally (Shelton et al., 2016).

Based on the information above, our primary objective in this synthesis paper was to evaluate the broader utility and limitations of eDNA for quantifying the abundance and/or biomass of fish through a systematic review of the available literature. Specifically, we aimed to (a) collate published peer-reviewed studies and describe biotic and abiotic factors affecting the relationship between eDNA concentrations and abundance and/or biomass, and then (b) use this information to propose future research efforts in this developing field.

## 2 | METHODS

A search was conducted on the Web of Science for published, peer-reviewed literature (January 2000 to October 2020) with pre-determined stem keywords (and variants) including 'eDNA or environmental DNA', 'abundance, or biomass, or quanti\*', 'marine or aquatic or freshwater or river\*' or estuar\*'. This initial search identified 631 publications, which were further filtered using 'fish'. All papers referencing fish were manually filtered and only retained if their titles or abstracts were consistent with the stated objective above.

**TABLE 1** Alphabetical list of family, scientific and common names, and taxonomic authority and target region of species-specific primers for fish mentioned in the review, with superscripts describing the most recent International Union for the Conservation of Nature (IUCN) Red List global classification: NE, not evaluated; DD, data deficient; LC, least concern; NT, near threatened; VU, vulnerable; and EN, endangered. The number of reviewed studies assessing each species (species-specific markers only) was either one, or as marked in parentheses. †The specificity of the oligonucleotides may differ depending on co-occurring species

Family	Accepted Latin name	Taxonomic authority	Common name	Target region and reference for oligonucleotides†
Acipenseridae	<i>Acipenser fulvescens</i> <sup>LC</sup>	Rafinesque, 1817	Lake sturgeon	COI and CytB (Yusishen et al., 2020)
Anguillidae	<i>Anguilla japonica</i> <sup>EN (2)</sup>	Temminck & Schlegel, 1846	Japanese eel	16S rRNA (Watanabe et al., 2005)
	<i>Anguilla marmorata</i> <sup>LC</sup>	Quoy & Gaimard, 1824	Giant mottled eel	16S rRNA (Itakura et al., 2020)
Carangidae	<i>Trachurus japonicus</i> <sup>NT (5)</sup>	(Temminck & Schlegel, 1844)	Japanese jack mackerel	CytB (Fukaya et al., 2020; Jo et al., 2017; Takahashi et al., 2020; Yamamoto et al., 2016)
Centrarchidae	<i>Micropterus salmoides</i> <sup>LC</sup>	(Lacepède, 1802)	Largemouth bass	COI and ND4 (Perez et al., 2017)
Cichlidae	<i>Lepomis macrochirus</i> <sup>LC</sup>	Rafinesque, 1819	Bluegill sunfish	CytB (Maruyama et al., 2014)
	<i>Hemichromis letourneuxi</i> <sup>LC</sup>	Sauvage, 1880	African jewelfish	COI (Diaz-Ferguson et al., 2014)
Clupeidae	<i>Alosa aestivalis</i> <sup>VU</sup>	(Mitchill, 1814)	Blueback herring	COI (Plough et al., 2018)
	<i>Alosa pseudoharengus</i> <sup>LC</sup>	(Wilson, 1811)	Alewife	COI (Plough et al., 2018)
Cobitidae	<i>Clupea harengus</i> <sup>LC</sup>	Linnaeus, 1758	Atlantic herring	CytB and ND4 (Knudsen et al., 2019)
	<i>Dorosoma cepedianum</i> <sup>LC</sup>	(Lesueur, 1818)	Gizzard chad	ND3 and ND5 (Perez et al., 2017)
Cyprinidae	<i>Sardinops sagax</i> <sup>LC</sup>	(Jenyns, 1842)	Pacific sardine	mtD-loop (Sassoubre et al., 2016)
	<i>Misgurnus anguillicaudatus</i> <sup>LC (2)</sup>	(Cantor, 1842)	Oriental weatherloach	12S rRNA (Furlan et al., 2016)
Cyprinidae	<i>Cyprinus carpio</i> <sup>VU (6)</sup>	Linnaeus, 1758	Common carp	CytB (Eichmiller et al., 2014; Takahara et al., 2012); 12S rRNA (Furlan & Gleeson, 2016b)
	<i>Hypophthalmichthys molitrix</i> <sup>NT (3)</sup>	(Valenciennes, 1844)	Silver carp	mtD-loop (Coulter et al., 2013); mtDNA (Farrington et al., 2015)
Engraulidae	<i>Hypophthalmichthys nobilis</i> <sup>DD (2)</sup>	(Richardson, 1845)	Bighead carp	mtD-loop (Coulter et al., 2013; Erickson et al., 2016)
	<i>Opsariichthys uncirostris</i> <sup>NE</sup>	(Temminck & Schlegel, 1846)	Three-lips	mtD-loop (Yamanaka et al., 2018)
Engraulidae	<i>Rhinichthys cobitis</i> <sup>VU</sup>	(Girard, 1856)	Loach minnow	CytB (Dysthe et al., 2016)
	<i>Engraulis mordax</i> <sup>LC</sup>	Girard, 1854	Northern anchovy	mtD-loop (Sassoubre et al., 2016)
Gadidae	<i>Engraulis japonicus</i> <sup>LC</sup>	Temminck & Schlegel, 1846	Japanese anchovy	CytB (Ushio et al., 2018)
	<i>Gadus morhua</i> <sup>VU</sup>	Linnaeus, 1758	Atlantic cod	CytB and ND4 (Knudsen et al., 2019)
Gobiidae	<i>Eucyclogobius newberryi</i> <sup>NT</sup>	(Girard, 1856)	Tidewater goby	CytB (Schmelzle & Kinziger, 2016)
	<i>Neogobius melanostomus</i> <sup>LC</sup>	(Pallas, 1814)	Round goby	COI (Nathan et al., 2015)
Labridae	<i>Halichoeres tenuispinis</i> <sup>LC</sup>	(Günther, 1862)	Wrasse	CytB (Takahashi et al., 2020)
	<i>Thalichthys pacificus</i> <sup>LC</sup>	(Richardson, 1836)	Eulachon	COI (Pochardt et al., 2019)
Oplegnathidae	<i>Oplegnathus fasciatus</i> <sup>NE</sup>	(Temminck and Schlegel, 1844)	Striped knifejaw	CytB (Takahashi et al., 2020)

(Continues)

TABLE 1 (Continued)

Family	Accepted Latin name	Taxonomic authority	Common name	Target region and reference for oligonucleotides <sup>‡</sup>
Percichthyidae	<i>Macquaria australasica</i> <sup>EN</sup>	Cuvier, 1830	Macquarie perch	12S rRNA and 18S rRNA (Piggott, 2016)
Percidae	<i>Perca fluviatilis</i> <sup>LC</sup>	Linnaeus, 1758	Redfin perch	12S rRNA (Furlan & Gleeson, 2016a)
	<i>Etheostoma flabellare</i> <sup>LC</sup>	Rafinesque, 1819	Fantail darter	CytB (Guivas & Brammell, 2020)
Petromyzontidae	<i>Petromyzon marinus</i> <sup>LC</sup> (2)	Linnaeus, 1758	Sea lamprey	COI (Gingera et al., 2016; Gustavson et al., 2015); CytB, ND1 and ND4 (Schlosser et al., 2018)
Plecoglossidae	<i>Plecoglossus altivelis</i> <sup>DD</sup>	(Temminck & Schlegel, 1846)	Ayu	CytB (Yamanaka & Minamoto, 2016)
	<i>Plecoglossus altivelis ryukyuensis</i> <sup>EN</sup>	(Temminck & Schlegel, 1846)	Ryukyu ayu	ND5 and COI (Akamatsu et al., 2020)
Pleuronectidae	<i>Platichthys flesus</i> <sup>LC</sup>	(Linnaeus, 1758)	European flounder	CytB and ND4 (Knudsen et al., 2019)
	<i>Pleuronectes platessa</i> <sup>LC</sup>	Linnaeus, 1758	European plaice	CytB and ND4 (Knudsen et al., 2019)
Salmonidae	<i>Oncorhynchus kisutch</i> <sup>NE</sup>	(Walbaum, 1792)	Coho salmon	COI (Rasmussen Hellberg et al., 2010)
	<i>Oncorhynchus nerka</i> <sup>LC</sup> (2)	(Walbaum, 1792)	Sockeye salmon	COX3 (Tillotson et al., 2018); COI (Rasmussen Hellberg et al., 2010)
	<i>Oncorhynchus tshawytscha</i> <sup>NE</sup>	(Walbaum, 1792)	Chinook salmon	CO3/ND3 (Shelton et al., 2019)
	<i>Parahucho perryi</i> <sup>NE</sup>	(Brevoort, 1856)	Sakhalin taimen	ND2 (Mizumoto et al., 2018)
	<i>Salmo salar</i> <sup>VU</sup>	Linnaeus, 1758	Atlantic salmon	NAD5 and COI (Wood et al., 2020)
	<i>Salmo trutta</i> <sup>LC</sup> (2)	Linnaeus, 1758	Brown trout	COI (Deutschmann et al., 2019); CytB (Capo et al., 2020)
	<i>Salvelinus alpinus</i> <sup>LC</sup> (2)	(Linnaeus, 1758)	Arctic char	CytB (Capo et al., 2020; Rodgers et al., 2018)
	<i>Salvelinus fontinalis</i> <sup>NE</sup> (5)	(Mitchill, 1814)	Brook trout/brook charr	CytB (Jane et al., 2015; Wilcox et al., 2013)
	<i>Salvelinus namaycush</i> <sup>NE</sup>	(Walbaum, 1792)	Lake trout	COI (Lacoursière-Roussel et al., 2016a)
Scombridae	<i>Scomber scombrus</i> <sup>LC</sup>	Linnaeus, 1758	Atlantic mackerel	CytB and Nd4 (Knudsen et al., 2019)
	<i>Scomber japonicus</i> <sup>LC</sup>	Houttuyn, 1782	Pacific chub mackerel	COI (Sassoubre et al., 2016)
Sparidae	<i>Acanthopagrus schlegelii</i> <sup>LC</sup>	(Bleeker, 1854)	Blackhead seabream	CytB (Takahashi et al., 2020)

The collected literature was also assessed for any additional relevant references within each publication. The key methods, outcomes, and/or limitations of each paper were tabulated, and the most common themes were used to partition topics for this review. The focus was to identify studies that tested the relationship between eDNA concentrations and fish abundance and/or biomass. We identified a relationship as being positive (or negative) when it was explicitly stated by the authors of the publications and objectively supported by hypothesis testing with  $p = 0.05$ .

### 3 | RESULTS AND DISCUSSION

In total, 63 papers assessing both freshwater and saltwater fish species satisfied the review criteria, with an almost linear increase in publishing between the first article in 2012 and the most recent in 2020 (Tables 1 and 2; Figure 1). Of note, we failed to find any references to species-specific or metabarcoding approaches based on eDNA analyses in aquatic environments until 2008 (amphibians) and 2012 (amphibians, invertebrates, fish, and mammals), respectively (Ficetola et al., 2008; Thomsen et al., 2012). Most studies were completed in the United States of America (USA; 53%) and Japan (25%) and were biased toward experiments done in the field (rivers, lakes, estuaries, and the sea; 60%) followed by those in controlled environments (aquaria or artificial ponds; 25%). The remaining studies (14%) were conducted across a combination of the field and controlled environments, but all within developed nations (Table 1, Figure 2).

Salmonidae (includes salmon, trouts, chars, freshwater whitefishes, and graylings) was the most assessed family with 16 papers using species-specific detection methods, followed by Cyprinidae (13 papers; includes carps, the true minnows and their relatives) (Table 1). Only three species-specific assessments involved globally listed ETP species (Table 1). Most studies used species-specific detection methods (51 or 81% across 46 species; Tables 1 and 2) rather than metabarcoding methods (12 or 19% across > 200 species; Table 2) to evaluate eDNA concentrations, with the latter restricted to estimating only relative abundance and/or biomass.

Irrespective of the experimental location, only six (10%) of the 63 studies accepted the null hypothesis of no relationship between eDNA and the abundance and/or biomass of focal species (Capo et al., 2019; Deutschmann et al., 2019; Fraija-Fernandez et al., 2020; Hinlo et al., 2018; Knudsen et al., 2019; Perez et al., 2017) (Table 2). While this general outcome is compelling, it is important to acknowledge the possibility of biases whereby researchers are less likely to publish their null or negative results. Nevertheless, among the published studies there was consistent support for the utility of the approach with 46 (90%) and 11 (92%) studies incorporating species-specific or metabarcoding methods, respectively, reporting positive correlations between eDNA concentrations or read counts and abundance and/or biomass (Table 2).

Notwithstanding the clear trend identified, most studies noted at least some uncertainty around absolute (versus relative) abundance

or biomass estimates. Such uncertainty was due to the processes affecting the production, degradation, and transport of DNA in the environment (Hansen et al., 2018; Klymus et al., 2015; Wood et al., 2020), as well as the choice of eDNA capture, extraction methods, and primer amplification biases (Eichmiller et al., 2016; Kelly et al., 2014). In some cases, this uncertainty might be considered acceptable (e.g., generating a relative abundance index for temporal comparisons), but in other cases could produce unacceptable biases (e.g., attempting to understand the population size of an ETP species). The biotic and abiotic factors most commonly identified across studies are critically discussed below and used to consider the future utility of eDNA concentrations for estimating abundances and/or biomasses of fish populations.

#### 3.1 | Biotic factors affecting eDNA concentrations

Considerable research has been completed to examine what is perhaps the key biological consideration for estimating abundance/biomass: How the quantity of eDNA in the water is affected by biotic factors directly related to eDNA production by a focal organism (Table 2). Various biotic factors affect eDNA concentrations, with interactive effects frequently identified among both biotic and abiotic influences. Such an outcome is somewhat implicit considering the source for eDNA is the biological organism itself, and so the movements of these molecules reflect intrinsic processes, as well as external influences on those intrinsic processes. The influence of each of these variables is discussed separately below, although the interrelated effects of intrinsic factors on eDNA production mean that isolating individual effects is challenging. Despite this, knowledge of combined effects may be sufficient to correct estimates of abundance or biomass derived from measures of eDNA concentration.

##### 3.1.1 | Intraspecific variation in DNA production

Intraspecific variation in DNA production and shedding rates among individuals experiencing the same environment and exhibiting the same behavior could potentially impact estimates of abundance and/or biomass (Kelly et al., 2014). Several aquaria studies reported large variations in DNA shedding rates, despite using similar-sized individuals of the same species and after eliminating some other confounding influences (Horiuchi et al., 2019; Klymus et al., 2015; Minamoto et al., 2017; Wilcox et al., 2016). For example, Klymus et al. (2015) observed up to a 100-fold variation in day-to-day DNA shedding (interpolated from eDNA in the water) from the same fish under controlled conditions—variability that the authors postulated was due to different sources of the DNA, including tissues, cells, and fecal debris that were unlikely to have been evenly dispersed in the water column. In another example, the greatest DNA shedding rates during experimental trials using tanks housing round gobies (*Neogobius melanostomus*) came from a tank with a single fish versus

**TABLE 2** Spatio-temporal summary of peer-reviewed literature published between January 2000 and October 2020 describing methods for assessing null hypotheses associated with the utility of eDNA, and key limiting factors, for quantifying the abundance and/or biomass of fish. C, Controlled environment; L, lake; R, river; E, estuary, S, sea; Sp, species-specific; Met, metabarcoding; and significant (+) positive or (-) negative effect if null hypothesis was rejected (continuous variables only),  $p < 0.05$ . Refer to Table 1 for the Latin names. \*Key null hypothesis was interpreted where not clearly stated

Country	Species	Key null hypothesis ( $H_0$ ) <sup>*</sup>	Water volume collected/filtered and DNA extraction method
Japan <sup>C,L</sup>	Common carp <sup>Sp</sup>	Difference between eDNA concentrations and water temperature and known biomass	0.02 L centrifuged into pellet, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit (aquaria exp). 2.0 L through a 3.0 or 0.8- $\mu$ m polycarbonate filter, with DNA extracted as above (mesocosm exp)
USA <sup>C,R</sup>	African jewelfish <sup>Sp</sup>	Difference between eDNA concentrations and known abundance	1.0 L through a 0.45- $\mu$ m cellulose nitrate filter, with DNA extracted using the Rapid Water DNA Isolation Kit
USA <sup>C,L</sup>	Common carp <sup>Sp</sup>	Difference between eDNA concentrations and mark-recapture and telemetry for estimating distribution	0.2 L through 1.5-mm glass microfiber filters, with DNA extracted using the QIAamp DNA Stool Mini Kit
USA <sup>C</sup>	Confined assemblage of 12 marine species <sup>Met</sup>	Difference between eDNA metabarcoding reads and known species abundance	1.0 L through 0.22- $\mu$ m membrane filters, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
USA <sup>C</sup>	Round goby <sup>Sp</sup>	Difference between eDNA concentrations derived via PCR, qPCR and ddPCR for known abundance/biomass	2.0 L through 1.5- $\mu$ m glass filter papers, with DNA extracted using a MoBio PowerWater DNA Isolation Kit
Japan <sup>C</sup>	Bluegill sunfish <sup>Sp</sup>	Difference between eDNA concentrations and life stage and known abundance	0.015 L centrifuged into pellet, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
Japan <sup>C</sup>	Common carp <sup>Sp</sup>	Difference between eDNA concentrations derived via qPCR and ddPCR and known abundance/biomass.	0.015 L centrifuged into pellet, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Brook trout <sup>Sp</sup>	Difference between eDNA concentrations and sampling distance, water flow and known biomass	6.0 L through a 1.5- $\mu$ m glass fiber filter, with DNA extracted using the MoBio PowerWater DNA Isolation Kit
USA <sup>C</sup>	Silver carp and bighead carp <sup>Sp</sup>	Difference between eDNA concentrations and temperature, diet, and known biomass	0.05 L centrifuged into pellet, with DNA extracted using a phenol-chloroform method
USA <sup>C</sup>	Common carp <sup>Sp</sup>	Difference between eDNA concentration and capture methods (precipitation, centrifugation, and filtration) and six commercially available DNA extraction kits.	1.0 L with eDNA captured via precipitation, centrifugation or filtration, with DNA extracted using the QIAamp DNA Stool Mini Kit. 1.0 L through 0.6- $\mu$ m polycarbonate membrane filters, with DNA extracted by one of six commercial kits.
USA <sup>R</sup>	Silver carp and bighead carp <sup>Sp</sup>	Difference between eDNA concentrations and fish movement, water discharge and egg densities	0.05 L centrifuged into pellet, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
USA <sup>C</sup>	Eight fish and one amphibian species <sup>Met</sup>	Difference between eDNA metabarcoding reads and known abundance/biomass	0.25 L through 1.2- $\mu$ m polycarbonate membrane filters, DNA extracted using a CTAB method
UK <sup>L</sup>	Freshwater fish assemblages in three lakes <sup>Met</sup>	Difference between eDNA metabarcoding reads and gill-netting surveys for estimating abundance	2.0 L through a 0.4- $\mu$ m cellulose nitrate membrane filter, with DNA extracted using the PowerWater DNA Isolation Kit
Canada <sup>L</sup>	Lake trout <sup>Sp</sup>	Difference between eDNA concentrations and gillnet surveys for estimating abundance/biomass	1.0 L through a 1.2- $\mu$ m glass microfiber filter, with DNA extracted using QIAshredder and Qiagen DNeasy Blood and Tissue Kit

Assay and target region	eDNA quantification method	Biotic/abiotic factors assessed	Reference
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	eDNA capture method, water temperature in aquaria (no effect), water temperature in the lake (+) and biomass (+)	Takahara et al. (2012)
qPCR TaqMan assay of the COI gene	Standard curves based on serial dilutions of DNA and Ct values used as a proxy for DNA	Abundance (+)	Diaz-Ferguson et al. (2014)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	High-use versus low-use areas versus concentration of eDNA (+) and sampling depth	Eichmiller et al. (2014)
Metabarcoding of the 12S rRNA gene, Illumina MiSeq platform	eDNA sequence read abundance used as a proxy for relative fish abundance	Rank biomass abundance of bony fish versus rank DNA sequence abundance (+)	Kelly et al. (2014)
PCR, qPCR and dPCR TaqMan assays of the COI gene	Standard curves (qPCR), and QuantaSoft software (ddPCR)	qPCR/ddPCR accuracy, analysis time/cost and biomass (+)	Nathan et al. (2014)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	eDNA shedding rate with life stage (adult/juvenile) and abundance (+)	Maruyama et al. (2014)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA (qPCR), Bio-Rad QuantaSoft software used to quantify DNA (ddPCR)	Biomass (+), abundance (+) and qPCR/ddPCR accuracy	Doi et al. (2015)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	eDNA transport distance (+/-), flow (+/-), biomass (+) and PCR inhibition	Jane et al. (2015)
qPCR TaqMan assay of the mtD-loop region	Standard curves based on serial dilutions of DNA	Biomass (+), diet (+), water temperature (no effect) and eDNA shedding rates	Klymus et al. (2015)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	eDNA capture method, type of extraction kit and biomass (+)	Eichmiller et al. (2016)
qPCR Taqman assay of the mtD-loop region	Standard curves based on serial dilutions of DNA	Telemetry counts (+), river discharge (+), egg densities (no effect) and relative abundance (+)	Erickson et al. (2016)
Metabarcoding of the CytB, 12S and 16S genes, Illumina MiSeq platform	eDNA sequence read abundance used as a proxy for relative fish abundance/biomass	Species detection rate (+), eDNA read abundance and species abundance and biomass (+)	Evans et al. (2016)
Metabarcoding of the 12S rRNA and CytB genes, Illumina MiSeq platform	eDNA sequence read abundance used as a proxy for relative fish abundance	Species detection rates (+), eDNA read count and site occupancy versus rank abundance or biomass (+)	Hänfling et al. (2016)
qPCR Taqman assay of the COI gene	Standard curves based on serial dilutions of DNA	Water temperature, dissolved oxygen, pH and turbidity (no effect), abundance (+) and biomass (+)	Lacoursière-Roussel et al. (2016a)

(Continues)

TABLE 2 (Continued)

Country	Species	Key null hypothesis ( $H_0$ ) <sup>*</sup>	Water volume collected/filtered and DNA extraction method
Canada <sup>C</sup>	Brook char <sup>Sp</sup>	Difference between eDNA concentrations and water temperature, capture method, and known abundance/biomass	1.0 L through one of five filters (0.2 and 0.45- $\mu$ m mixed cellulose ester, and 0.7, 1.2 and 3.0- $\mu$ m glass microfiber), with DNA extracted using salt extraction method
Australia <sup>C</sup>	Macquarie perch <sup>Sp</sup>	Difference between eDNA concentrations and sampling methods, extraction methods, marker choice, amplicon size and dilutions of DNA as a proxy for biomass	0.25 L though (1) 0.45- $\mu$ m cellulose nitrate filter and DNeasy Blood and Tissue Kit, (2) 0.45- $\mu$ m cellulose nitrate filter and Phenol-Chloroform Isoamyl or (3) sodium acetate and ethanol precipitation and the Qiagen DNeasy Blood Tissue Kit
USA <sup>C</sup>	Northern anchovy, Pacific sardine and, Pacific chub mackerel <sup>Sp</sup>	Difference in eDNA shedding and decay rates	0.25–0.50 L through 0.2- $\mu$ m polycarbonate filters, with DNA extracted using the Qiagen DNeasy Blood Tissue Kit
USA <sup>E</sup>	Tidewater goby <sup>Sp</sup>	Difference between eDNA concentrations and seining for species detection and estimating abundance	2.0 L through a 3.0 $\mu$ m polycarbonate, track-etched filter, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit and QIAshredder
Greenland <sup>S</sup>	Marine fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding reads and bottom-trawl survey methods for estimating abundance	1.5 L through a 0.45- $\mu$ m nylon filter, with DNA extracted using bead beating and the Qiagen DNeasy Blood and Tissue Kit
USA <sup>C, R</sup>	Brook trout <sup>Sp</sup>	Difference between eDNA concentrations and electrofishing for estimating detection probabilities/abundance. Difference in rates of eDNA shedding and persistence	1.0–6.0 L through 1.5- $\mu$ m pore glass filters, with DNA extracted using QIAshredder and Qiagen Blood and Tissue DNeasy Kit
Japan <sup>S</sup>	Japanese jack mackerel <sup>Sp</sup>	Difference between eDNA concentrations and echo-sounder surveys for estimating biomass	1.0 L through 0.7- $\mu$ m pore glass filters and DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Brook trout <sup>Sp</sup>	Difference between eDNA concentrations and electrofishing surveys for estimating abundance	Up to 6.0 L through 1.5- $\mu$ m glass filters and several grams of sediment stored in plastic bags, with DNA was extracted using MoBio PowerWater and PowerSoil DNA Isolation Kits, respectively
Japan <sup>R</sup>	Ayu <sup>Sp</sup>	Difference between eDNA concentrations and snorkeling surveys for estimating abundance/biomass	1.0 L filtered through 0.7- $\mu$ m glass filters, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
Australia <sup>R</sup>	Common carp, redfin perch and oriental weatherloach <sup>Sp</sup>	Difference between eDNA concentrations and fyke netting for estimating abundance	2.0 L through 1.2- $\mu$ m glass fiber filters, with DNA extracted using the MoBio PowerWater DNA Isolation Kit
Japan <sup>C, S</sup>	Japanese jack mackerel <sup>Sp</sup>	Difference between the decay rates of long and short eDNA fragments. Difference between eDNA concentration and acoustic surveys for estimating biomass	1.0 L filtered through 0.7- $\mu$ m pore glass filters, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
USA <sup>L</sup>	Arctic char <sup>Sp</sup>	Difference between eDNA concentrations and mark-recapture for estimations of abundance/biomass. Difference between seasons and sampling depth	5.0 L through 10- $\mu$ m polyamide filters, with DNA extracted using Qiagen DNeasy Blood and Tissue Kit
USA <sup>L</sup>	Largemouth bass and gizzard chad <sup>Sp</sup>	Difference between eDNA concentrations and electrofishing/gill netting for estimating species presence, relative abundance and biomass	0.015 L centrifuged into pellet, with DNA extracted using the IBI gMAX Mini Genomic DNA Kit



Assay and target region	eDNA quantification method	Biotic/abiotic factors assessed	Reference
qPCR Taqman assay of the CytB gene	Standard curves based on serial dilutions of DNA	Water temperature (+), eDNA capture method and biomass (+)	Lacoursière-Roussel et al. (2016b)
PCR and qPCR SYBR Green assay of the 12S rRNA and 18S rRNA genes	Standard curves based on serial dilutions of DNA	Sampling and extraction methods, PCR strategy, amplicon size, marker choice and biomass (+)	Piggott (2016)
qPCR Taqman assay of the mtD-loop and COI genes	Standard curves based on serial dilutions of DNA	eDNA shedding (+) and decay rates (+), eDNA size fractionation and abundance (+)	Sassoubre et al. (2016)
qPCR TaqMan assay for the CytB gene	Standard curves based on serial dilutions of DNA	Species detection rates (+), abundance (+) and salinity (-)	Schmelzle and Kinziger (2016)
Metabarcoding of the 12S rRNA gene, Illumina MiSeq platform	eDNA sequence read abundance used as a proxy for relative fish abundance	Species detection rates, relative eDNA read abundance versus relative abundance (+) and relative biomass (+) and depth	Thomsen et al. (2016)
qPCR Taqman assay of the CytB gene	Standard curves based on serial dilutions of DNA	Rate of eDNA production, eDNA transport distance, detection probability (+) and abundance (+)	Wilcox et al. (2016)
qPCR Taqman assay of the CytB gene	Standard curves based on serial dilutions of DNA	Sampling location (surface/bottom), species distribution and biomass (+)	Yamamoto et al. (2016)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Species detection rate (+), abundance (+), biomass (+) and eDNA substrate (water/sediment)	Baldigo et al. (2017)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Abundance (+), biomass (+) and season	Doi et al. (2017)
qPCR TaqMan assay of the 12S rRNA gene	Standard curves based on serial dilutions of DNA	Species detection rate (+), season, sampling location (surface/subsurface), abundance of carp (no effect), abundance of redfin perch and oriental weatherloach (+)	Hinlo et al. (2017a)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	eDNA decay rate, fragment length (+) and biomass (long fragment +)	Jo et al. (2017)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Abundance (+), biomass (+), depth ( $\pm$ ) and season	Klobucar et al. (2017)
qPCR TaqMan assay of the COI, ND4, ND3 and ND5 genes	Standard curves based on serial dilutions of DNA	Season, relative abundance (no effect) and biomass (no effect)	Perez et al. (2017)

(Continues)

TABLE 2 (Continued)

Country	Species	Key null hypothesis ( $H_0$ ) <sup>a</sup>	Water volume collected/filtered and DNA extraction method
USA <sup>E</sup>	Estuarine fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding reads and traditional surveys for estimating abundance	1.0 L through a 0.45- $\mu$ m polyamide filter, with DNA extracted using MoBio Powersoil Kit
USA <sup>C, R</sup>	Common carp <sup>Sp</sup>	Difference in statistical models accounting for factors influencing biomass estimates from eDNA concentration data	0.015 L centrifuged into pellet, with DNA extracted from pellet using the Qiagen DNeasy Blood and Tissue Kit
Australia <sup>R</sup>	Oriental weatherloach <sup>Sp</sup>	Difference between eDNA concentrations and fyke netting/electrofishing for estimating abundance	2.0 L through 1.2- $\mu$ m glass fiber filter, with DNA extracted using a modified Qiagen DNeasy Kit
USA <sup>L</sup>	Brook trout <sup>Sp</sup>	Difference in eDNA concentrations between where fish had been eradicated versus where fish remained, using gillnets for estimating abundance/biomass	0.25 L through 1.2- $\mu$ m polycarbonate filter membrane, with DNA extracted using a QIAshredder and Qiagen DNeasy Blood and Tissue Kit
UK <sup>C</sup>	Freshwater fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding reads and known species presence and abundance/biomass	0.3 L through one of six filtration types, with DNA extracted using the MoBio PowerWater DNA Isolation Kit
Japan <sup>R</sup>	Three-lips <sup>Sp</sup>	Difference between eDNA concentrations and visual sampling for estimating abundance	0.5 L through a 0.7- $\mu$ m glass filter membrane, with DNA extracted using a Qiagen DNeasy Blood and Tissue DNA Kit
Japan <sup>C</sup>	Sakhalin taimen <sup>Sp</sup>	Difference between eDNA concentration and known biomass	1.0 L through 0.7- $\mu$ m glass-membrane filters, with DNA extracted using a slightly modified Qiagen DNeasy Blood and Tissue Kit.
USA <sup>C, L</sup>	Round goby <sup>Sp</sup>	Difference between eDNA concentrations and seining/traps/mark-recapture for estimating abundance. Difference in rates of eDNA shedding and decay	0.05–1 L through either 1.5- or 0.22- $\mu$ m nitrocellulose filters, with DNA extracted using a slightly modified MoBio PowerWater Kit
USA <sup>R</sup>	Alewife and blueback herring <sup>Sp</sup>	Difference between eDNA concentration and visual surveys/ichthyoplankton sampling for presence and estimating abundance	~0.8 L through a 1.0- $\mu$ m cellulose nitrate filter, with DNA extracted using a slightly modified Omega Biotek EZNA Water Kit
France/ Switzerland <sup>R</sup>	Freshwater fish assemblage <sup>Met</sup>	Difference between eDNA concentrations and electrofishing for estimating relative abundances	30.0 L through a 0.45- $\mu$ m cross-flow filtration capsule, with DNA extracted using a combination of the Qiagen DNeasy Blood and Tissue Extraction Kit and the NucleoSpin Soil Kit
USA <sup>C</sup>	Sea lamprey <sup>Sp</sup>	Relationship between eDNA (concentration and detection probability) and known abundances/biomass	1.0 L through a 1.5- $\mu$ m glass microfiber filter, with DNA extracted using the gMax Mini Genomic Extraction Kit
USA <sup>R</sup>	Sockeye salmon <sup>Sp</sup>	Difference between eDNA concentrations and visual counts for estimating abundances	1.0 L through a 0.45- $\mu$ m cellulose nitrate filter, with DNA extracted using Qiagen DNeasy Blood and Tissue Kit
Ireland <sup>R</sup>	Sea lamprey <sup>Sp</sup>	Difference between eDNA concentrations and visual surveys of individuals/nests for estimating abundance	2.0 L through a 0.45- $\mu$ m cellulose nitrate filter, with DNA extracted using a Chelex Chelating resin
Sweden <sup>L</sup>	Brown trout and Arctic char <sup>Sp</sup>	Difference between eDNA concentrations and standardized gillnet surveys and relationships with environmental parameters	0.75–1 L through 1.2 $\mu$ m glass fiber and 0.45 $\mu$ m mixed cellulose ester filters, with DNA extracted using the DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Silver carp <sup>Sp</sup>	Difference between eDNA concentrations and hydroacoustic and electrofishing/gillnet surveys for estimating abundance/biomass	2.0 L through a 1.5-mm glass microfiber filter, with DNA extracted using a FastDNA Spin Kit

Assay and target region	eDNA quantification method	Biotic/abiotic factors assessed	Reference
Metabarcoding of the 12S rRNA gene, Illumina MiSeq platform	eDNA sequence read abundance used as a proxy for relative abundance estimates	Seasonal abundance (+), eDNA read abundance versus relative abundance (+), and habitat preference	Stoeckle et al. (2017)
ddPCR TaqMan assay of the CytB gene	Bio-Rad QuantaSoft software used to quantify DNA	Statistical model choice and biomass (+)	Chambert et al. (2018)
qPCR TaqMan assay for the 12S rRNA gene	Standard curves based on serial dilutions of DNA	Abundance (no effect)	Hinlo et al. (2018)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Abundance (+) and biomass (+)	Kamoroff and Goldberg (2018)
Metabarcoding of the 12S rRNA, Illumina MiSeq platform	Species detection rates based on the relative read counts as a proxy for abundance estimates	eDNA capture method, species detection rates, relative abundance (+) and relative biomass (+)	Li et al. (2018)
qPCR TaqMan assay of the mtD-loop region	Standard curves based on serial dilutions of DNA	Abundance (+), eDNA transport distance (no effect) and seasonal changes (+)	Maruyama et al. (2018)
qPCR TaqMan assay for the ND2 gene	Standard curves based on serial dilutions of DNA	Aquarium size, fish age, body size and biomass (+)	Mizumoto et al. (2018)
qPCR TaqMan assay of the COI gene	Standard curves based on serial dilutions of DNA	Species detection rate (+), abundance (+) and DNA shedding and decay rate	Nevers et al. (2018)
qPCR TaqMan assay for the COI gene	Standard curves based on serial dilutions of DNA	Species detection rate (+) and abundance (+)	Plough et al. (2018)
Metabarcoding of the 12S rRNA gene, Illumina HiSeq platform	Species detection rates based on the relative read counts as a proxy for abundance estimates	Species detection rate and relative abundance (+)	Pont et al. (2018)
qPCR TaqMan assay of the COI, CytB, ND1 and ND4 genes	Standard curves based on serial dilutions of DNA	Fish life stage, abundance (+) and biomass (+)	Schloesser et al. (2018)
qPCR TaqMan assay of the COX3 gene	Standard curves based on serial dilutions of DNA	eDNA transport distance (+), water temperature (minor +) and abundance (+)	Tillotson et al. (2018)
qPCR TaqMan assay of the COI gene	Standard curves based on serial dilutions of DNA	Seasonal movement, spawning and abundance (+)	Bracken et al. (2019)
ddPCR assay of the CytB gene	Divided the mean number of positive droplets by the volume used in ddPCR and multiplied by the total volume of the DNA extract	Biomass, abundance, dissolve organic carbon and temperature (no effect)	Capo et al. (2019)
qPCR TaqMan assay of mitochondrial genes	Standard curves based on serial dilutions of DNA	Abundance (+) and biomass (+)	Coulter et al. (2019)

(Continues)

TABLE 2 (Continued)

Country	Species	Key null hypothesis ( $H_0$ ) <sup>*</sup>	Water volume collected/filtered and DNA extraction method
Germany <sup>C, R</sup>	Brown trout <sup>SP</sup>	Difference between eDNA concentrations and known biomass. Difference between eDNA concentrations and distance	1.0 L through 0.45- $\mu$ m nitrocellulose filters, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
Japan <sup>C</sup>	Japanese jack mackerel <sup>SP</sup>	Difference between eDNA concentration and known biomass	1.0 L through a 0.7- $\mu$ m glass filter, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
Japan <sup>R</sup>	Japanese eel <sup>SP</sup>	Difference between eDNA concentrations and electrofishing for estimating species presence and abundance/biomass	1 L through a 0.7- $\mu$ m glass filter, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
Denmark <sup>S</sup>	Atlantic herring, Atlantic cod, European flounder, European plaice and Atlantic mackerel <sup>SP</sup>	Difference between eDNA concentrations and benthic trawls for estimating abundance/biomass	1.5 L through a 0.22- $\mu$ m Sterivex filter, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Sockeye salmon and coho salmon <sup>SP</sup>	Difference between eDNA concentrations and daily human count data for estimating abundances	1.0 L through a 0.45- $\mu$ m cellulose nitrate filter, with DNA extracted using a Qiagen DNeasy Blood and Tissue Kit
UK <sup>L</sup>	Freshwater assemblages in 14 lakes <sup>Met</sup>	Difference between eDNA metabarcoding reads and historical information for estimating relative abundances	2.0 L through a 0.45- $\mu$ m mixed cellulose acetate and nitrate filter, with DNA extracted using a Qiagen DNeasy PowerWater Kit
USA <sup>E</sup>	Eulachon <sup>SP</sup>	Difference between eDNA concentrations and mark-recapture for estimating abundance	1.0 L through a 0.45- $\mu$ m cellulose nitrate filter, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Loach minnow <sup>SP</sup>	Difference between eDNA concentrations and electrofishing/seining for estimating species presence and biomass	5.0 L through a 1.5- $\mu$ m pore-size glass fiber filter, with DNA extracted using a slightly modified Qiagen DNeasy Blood and Tissue Kit
Faroe Islands <sup>S</sup>	Atlantic cod <sup>SP</sup>	Difference between eDNA concentrations and benthic trawls for estimating biomass	1.5 L through a 0.2- $\mu$ m Sterivex filter, with DNA extracted using a modified protocol of the Qiagen DNeasy Blood and Tissue Kit
USA <sup>L</sup>	Freshwater assemblages in eight lakes <sup>Met</sup>	Difference between eDNA metabarcoding reads and traditional methods for estimating species presence and relative abundances	1.0 L through a 0.45 $\mu$ m nitrocellulose filter, DNA was extracted using a Qiagen QIAshredder and the Qiagen DNeasy Blood and Tissue Kit
USA <sup>E</sup>	Chinook salmon <sup>SP</sup>	Difference between eDNA concentrations and beach seining for estimating abundance/biomass	1.0 L through a 0.45- $\mu$ m cellulose acetate membrane, with DNA extracted using a chloroform:isoamyl alcohol method
Japan <sup>R</sup>	Ryukyu ayu <sup>SP</sup>	Difference between eDNA concentration and snorkeling surveys for estimating abundance	1.0 L through a 0.7- $\mu$ m glass fiber filter, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit
UK <sup>C</sup>	Freshwater fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding reads and known species presence and abundance/biomass and differences between filter types	1.0 L through 0.45- $\mu$ m mixed cellulose ester filters or 0.35 L through 0.45- $\mu$ m Sterivex filter, with DNA extracted using the Mu-DNA protocol for water samples
Spain <sup>S</sup>	Marine fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding reads and biomass estimated from trawling	5 L through Sterivex 0.45- $\mu$ m enclosed filters, DNA extracted using the DNeasy Blood and Tissue Kit
Japan <sup>S</sup>	Japanese jack mackerel <sup>SP</sup>	Difference between eDNA concentration and echo-sounder abundance	1.0 L through 0.7- $\mu$ m glass microfiber filter, with DNA extracted using the DNeasy Blood and Tissue Kit
France <sup>R</sup>	Freshwater fish assemblage <sup>Met</sup>	Difference between eDNA metabarcoding read count and electrofishing relative abundance	30 L through 0.45- $\mu$ m VigiDNA cross-flow filtration capsule, with DNA extracted using the DNeasy Blood and Tissue Kit
USA <sup>C</sup>	Fantail darter <sup>SP</sup>	Difference between eDNA concentration and known biomass and difference between single versus. multiple (3) filters	1.2 L through 0.7- $\mu$ m glass fiber filter, with DNA extracted using the DNeasy Blood and Tissue kit

Assay and target region	eDNA quantification method	Biotic/abiotic factors assessed	Reference
qPCR TaqMan assay of the COI gene	Standard curves based on serial dilutions of DNA	eDNA transport distance and biomass (no effect)	Deutschmann et al. (2019)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Biomass (+)	Horiuchi et al. (2019)
qPCR TaqMan assay of the 16S rRNA gene	Standard curves based on serial dilutions of DNA	Species detection rate (+), biomass (+) and abundance (+)	Itakura et al. (2019)
qPCR TaqMan assay of the CytB and Nd4 genes	Standard curves based on serial dilutions of DNA	Species distribution (+) and biomass (no effect)	Knudsen et al. (2019)
qPCR TaqMan assay of the COI gene	Standard curves based on serial dilutions of DNA	Flow, water temperature, fish life stage and abundance (+)	Levi et al. (2019)
Metabarcoding of the 12S rRNA and CytB gene, Illumina MiSeq platform	Site occupancy based on the relative read counts as a proxy for abundance estimates	Species distribution (+) and relative abundance (+)	Li et al. (2019)
ddPCR assay of the COI gene	Bio-Rad QuantaSoft software used to quantify DNA	Flow and biomass (+)	Pochardt et al. (2019)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Species detection rate (+), eDNA persistence rate and biomass (+)	Robinson et al. (2019)
qPCR TaqMan assay of the mtD-loop region	Standard curves based on serial dilutions of DNA	Abundance (+) and biomass (+)	Salter et al. (2019)
Metabarcoding of the 12S rRNA and 16S rRNA genes, Illumina MiSeq platform	Species detection rates based on the relative read counts as a proxy for abundance estimates	Sampling effort, species detection rate (+) and relative abundance (+)	Sard et al. (2019)
qPCR TaqMan assay of the CO3/ND3 gene	Standard curves based on serial dilutions of DNA	Spatial scale (+), biomass (+) and abundance (+)	Shelton et al. (2019)
qPCR TaqMan assay of the ND4 gene	Standard curves based on serial dilutions of DNA	Species detection rate (+) and biomass (+)	Akamatsu et al. (2020)
Metabarcoding of the 12S rRNA and 16S rRNA genes, Illumina MiSeq platform	Spearman's rank correlation coefficient to calculate correlation between biomass/abundance and average read counts and site occupancy	Biomass (+), abundance (+) and filter types and biomass/abundance (no effect)	Di Muri et al. (2020)
Metabarcoding of the 12S rRNA gene, Illumina MiSeq platform	Pearson correlation coefficient to calculate correlation between the number of reads and biomass	Biomass (no effect)	Frajia-Fernandez et al. (2020)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Abundance (+)	Fukaya et al. (2020)
Metabarcoding of the 12S rRNA gene, Illumina HiSeq platform	Spearman's rank order correlation to calculate correlation between electrofishing relative abundance and relative number of reads per species	Relative abundance (+)	Goutte et al. (2020)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Biomass (+) and number of filters (+)	Guivas and Brammell (2020)

(Continues)

TABLE 2 (Continued)

Country	Species	Key null hypothesis ( $H_0$ ) <sup>a</sup>	Water volume collected/filtered and DNA extraction method
Japan and Taiwan <sup>R</sup>	Giant mottled eel and Japanese eel <sup>Sp</sup>	Difference between eDNA concentration and electrofishing biomass and abundance	1.0 L through 0.7- $\mu$ m glass fiber filters, with DNA extracted using the DNeasy Blood and Tissue Kit
Japan <sup>S</sup>	Blackhead seabream, Japanese anchovy, wrasse, stiped knifejaw and Japanese jack mackerel <sup>Sp</sup>	Difference between eDNA concentrations and visual (SCUBA) biomass estimates and differences between filter types	1.0 L through 0.45- $\mu$ m Sterivex filter and 1 L through 0.7- $\mu$ m glass fiber filters, with DNA extracted using the DNeasy Blood and Tissue Kit
USA <sup>R</sup>	Atlantic salmon <sup>Sp</sup>	Difference between eDNA concentrations/detection rates and downstream distance	1.0 L through a 1.5- $\mu$ m glass fiber filter, with DNA extracted using the Qiagen DNeasy Spin Column Kit
Canada <sup>C, R</sup>	Lake sturgeon <sup>Sp</sup>	Difference between eDNA concentration and relative abundance/species presence	1.0–2 L through 1.5- $\mu$ m glass fiber filter, with DNA extracted using the Qiagen DNeasy Blood and Tissue Kit

those containing three fish, although fish sexes were not determined and could have affected shedding rates (Nevers et al., 2018). Additional considerations here are possible confounding effects of animal stress, size and/or basal metabolism.

### 3.1.2 | Stress

Stress may account for considerable intraspecific variation in DNA shedding, especially in aquaria. In some studies, eDNA concentrations have been greatest immediately after fish were first introduced into tanks. The elevated eDNA resulted from increased activity of individuals is presumably associated with physiological stress during the acclimation period, with a subsequent decline in concentrations recorded as individuals became accustomed to confinement (Maruyama et al., 2014; Nevers et al., 2018; Sassoubre et al., 2016; Takahara et al., 2012). To minimize the confounding effects of fish stress on the outcomes of future aquaria experiments, pilot work should always be undertaken to determine when eDNA concentrations plateau following fish introduction into experimental tanks.

### 3.1.3 | Metabolism and size

The metabolic rate describes the energy expenditure of an organism while it is at rest and varies with body size and temperature (Gillooly et al., 2001). Given that temperature variably affects many physiological, ecological, and biological processes via complex interactions among fish (discussed below), metabolic rate likely affects eDNA shedding by species or individuals, potentially influencing subsequent interpretations of eDNA quantification. For example, Lacoursière-Roussel et al. (2016b) hypothesized that increased eDNA concentrations at higher temperatures were due to an increased metabolic rate leading to shedding of more epidermal cells and other secretions (feces

and urine). Thus, to avoid overestimating fish abundance and/or biomass from eDNA concentrations, temperature must be considered.

In many teleost fishes, their metabolic rate varies with life stage and is therefore directly linked to body size (Post & Lee, 1996). Consequently, different-sized individuals of the same species can exhibit different DNA shedding rates (Klymus et al., 2015; Maruyama et al., 2014; Wilcox et al., 2016), potentially influencing the interpretation of eDNA quantification for some species. Small individuals have a relatively greater metabolic rate per unit bodyweight versus larger individuals (Chabot et al., 2016), and therefore may shed relatively more DNA compared to a single large fish. For example, during tank trials, Maruyama et al. (2014) observed that the DNA release rates by individual bluegill sunfish (*Lepomis macrochirus*) were three or four times greater in adults than juveniles, but when scaled to body size, juvenile release rates were four times greater owing to the ontogenetic decrease in metabolic activity. Consequently, attempting to estimate a population's abundance or biomass from eDNA concentrations, without knowing size distributions, could potentially give misleading estimates if size-based variation in DNA shedding rates was substantial. In such situations, concomitant knowledge of the size and age structures of the focal species within a sampling area, derived via ancillary fishery-dependent or fishery-independent techniques, is therefore essential (Hansen et al., 2018). An implicit requirement here is also to determine the magnitude of differences (if any) in DNA shedding rates according to the size or age of the fish species.

### 3.1.4 | Distribution and density

The generally patchy distributions of fish will result in eDNA concentrations that vary across space and time (Eichmiller et al., 2014; Itakura et al., 2019; Takahara et al., 2012). Such variation may affect the accuracy of subsequent abundance or biomass estimates, particularly if sampling is not done across appropriate spatio-temporal

Assay and target region	eDNA quantification method	Biotic/abiotic factors assessed	Reference
qPCR TaqMan assay of the 16S rRNA gene	Standard curves based on serial dilutions of DNA	Abundance (+), biomass (+), spatial distribution of eDNA concentration (Japanese eel only) (-)	Itakura et al. (2020)
qPCR TaqMan assay of the CytB gene	Standard curves based on serial dilutions of DNA	Biomass (+) and filter types (no effect)	Takahashi et al. (2020)
qPCR TaqMan assay of the ND5 and COI genes	Standard curves based on serial dilutions of DNA	eDNA transport distance, optimal sampling distance, assay type and biomass (+)	Wood et al., 2020 (Wood et al., 2020)
qPCR TaqMan assay of the COI and CytB gene	Ct values used as a proxy DNA concentration	Biomass (+)	(Yusishen et al., 2020)

scales and/or insufficient water is collected to enable replication (Furlan et al., 2016; Moyer et al., 2014). For example, when common carp (*Cyprinus carpio*) were heterogeneously distributed across lakes in the upper Mississippi River basin, their eDNA was occasionally detectable and only loosely associated with moderately dense groups of fish (Ghosal et al., 2018). However, when fish were attracted to a single site (using bait), a doubling of fish density resulted in a 500-fold increase in eDNA concentrations.

In another study in a temperate freshwater lake in the northern USA, Eichmiller et al. (2014) noted that common carp eDNA concentrations were over seven times greater in areas with a high frequency of carp habitation than areas with a low habitation frequency. Specifically, eDNA was detected in 100% of water samples from areas of high use by common carp compared with only 63% of samples from the low-use areas (Eichmiller et al., 2014). Given the strength of this latter relationship and the known patchy distribution of eDNA in the environment, the authors concluded that any sampling methods and data interpretation should be primarily informed by existing fish distributions because the probability of detecting a target organism may drastically decline within tens of meters.

There was not always a consistent linear relationship between fish density and eDNA concentration (Coulter et al., 2019; Doi et al., 2017; Ghosal et al., 2018). As one example, both the numerical and biomass densities of silver carp (*Hypophthalmichthys molitrix*) in a river system were positively correlated with eDNA concentrations and detection rates at low densities, but plateaued at greater densities (Coulter et al., 2019). Consequently, reliable population estimates from eDNA concentrations for this species are currently restricted to areas with low densities of fish.

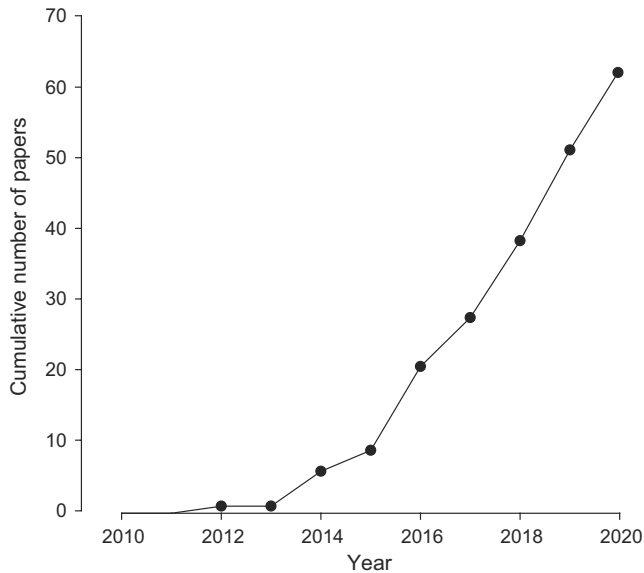
### 3.1.5 | Feeding and diet

Feeding and diet can significantly affect DNA shedding rates. Ghosal et al. (2018) demonstrated that actively feeding common carp in a

lake produced substantially more eDNA than less actively feeding conspecifics. Similarly, among aquaria-housed bighead carp (*Hypophthalmichthys nobilis*) the amount of DNA shed was up to tenfold greater in fed than nonfed individuals (Klymus et al., 2015). Klymus et al. (2015) also hypothesized that a diet of textured crustacean food would slough more epithelial cells from the gut and thus increase DNA shedding rates versus a diet consisting of soft textured algae; however, fish fed algal diets had greater shedding rates, which the authors attributed to a higher feeding rate. This latter study implies that both diet composition and the frequency of feeding may influence DNA shedding and thus eDNA concentrations in the water.

### 3.1.6 | Reproduction and migration

The reviewed studies also show that eDNA concentrations in aquatic systems depend on the life stages of the fish inhabiting that body of water and any spawning activity and/or movements/migration (Table 2). Spawning and associated migrations, which are typically seasonal, evoke fluctuations in eDNA concentrations due to the communal release of gametes, increased activity and congregation of often large numbers of adults and the presence of juvenile fish (Bracken et al., 2019; Bylemans et al., 2017; Kamoroff & Goldberg, 2018; Maruyama et al., 2018; Nevers et al., 2018; Plough et al., 2018; Schloesser et al., 2018; Spear et al., 2015). For example, Bracken et al. (2019) observed the concentration of eDNA in river water increased during the spawning season of sea lamprey (*Petromyzon marinus*), simply attributed to more adult fish (including the carcasses of those that died after spawning) and gametes. Another study demonstrated the ratio of nuclear-to-mitochondrial eDNA from Macquarie perch (*Macquaria australasica*) was greatest during their spawning season (Bylemans et al., 2017). One simple way of limiting the impact that spawning can have on baseline levels of eDNA is to sample outside spawning seasons.



**FIGURE 1** Cumulative number of articles published in the primary literature from 2012 to October 2020 assessing the utility of environmental DNA for estimating the abundance and/or biomass of fish across all environments (i.e., controlled and wild)

### 3.2 | Abiotic factors affecting eDNA concentrations

Six key abiotic factors were commonly identified as affecting estimates of abundance or biomass from eDNA concentrations (Table 2). Further, nearly all abiotic factors have complex interactive or confounding effects that encapsulate influences on the general ecology and biology of species, which then affect DNA shedding rates and/or volume (as discussed above). Here, we limit our discussion to the direct effects of abiotic factors on eDNA concentrations.

#### 3.2.1 | Water flow

One of the most important abiotic factors affecting eDNA concentrations is regional water flow (Harrison et al., 2019). By dispersing eDNA away from its source, water flow influences the spatial scale over which abundance and/or biomass can be meaningfully estimated, and affects numerous other factors that influence eDNA production, degradation, dilution, and deposition (Fukaya et al., 2020; Hansen et al., 2018; Itakura et al., 2020; Pont et al., 2018; Thomsen et al., 2016). Due to the challenges involved, few studies have directly examined the effects of water flow on eDNA concentrations, but those that have support complex system-specific responses that require further investigation (Hinlo et al., 2018; Laporte et al., 2020; Robinson et al., 2019; Wilcox et al., 2016). For example, using cage introductions of brook trout (*Salvelinus fontinalis*) in lotic systems, Jane et al. (2015) noted the relationship between water flow and eDNA copy number varied depending on system flow rates. Specifically, the eDNA copy number was highest immediately downstream of the caged trout in low flow scenarios, but relatively constant at the most upstream and downstream locations during high flow scenarios (Jane et al., 2015).

The potential for nonlinear relationships between flow and eDNA concentrations with distance from a point source is also apparent, with a peak in concentration observed 70 m downstream of caged Atlantic salmon (*Salmo salar*) (Wood et al., 2020) and 50 to 70 m downstream of Japanese eel (*Anguilla japonica*) capture sites (Itakura et al., 2020). The recognized influence of flow on eDNA concentrations led to the recent development of an “eDNA rate” for Pacific salmon (*Oncorhynchus* spp.) in lotic systems—a correction metric that combines eDNA concentrations with flow velocities to improve abundance estimates (Levi et al., 2019). Systems influenced by tidal flow or influxes from tide-dominated estuaries also pose challenges to estimate abundance or biomass. As one solution, Pochardt et al. (2020) used a flow-corrected eDNA rate to predict the abundance of eulachon (*Thaleichthys pacificus*) at the mouth of a river adjacent to an estuary as determined through mark-recapture population estimates. The eDNA concentration, combined with sampling at low tide to minimize the effects of dilution or intrusion of eDNA from the tidal flow, resulted in the authors successfully predicting eulachon abundance (Pochardt et al., 2020).

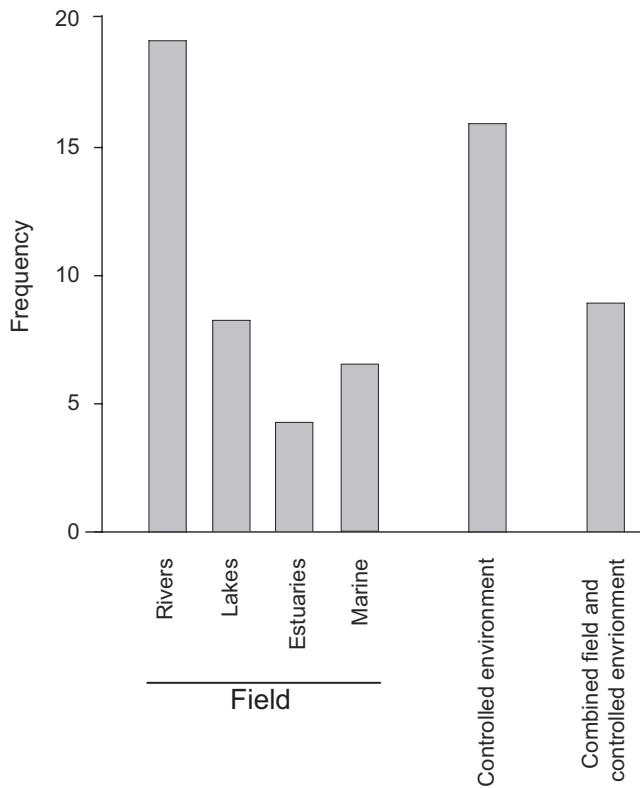
Despite the potential for broad and complex dispersal patterns of eDNA, the reviewed studies imply effective spatial scales of abundance and/or biomass estimation for fish may be considerably smaller than previously considered (Table 2). In lotic systems, studies either identified a relatively rapid decline of eDNA concentrations beyond their source (Deutschmann et al., 2019; Shogren et al., 2017; Wood et al., 2020), or found no evidence of downstream eDNA accumulation (Maruyama et al., 2018; Tillotson et al., 2018). The latter might be expected if production and transport exceeded degradation and deposition (Tillotson et al., 2018).

In coastal marine systems, slower and multi-directional flows may further narrow the effective spatial scale of abundance and/or biomass estimation. For example, the best-fit model between acoustic signal intensity and eDNA concentrations of Japanese jack mackerel (*Trachurus japonicus*) in the Sea of Japan was within 10 to 150 m of the eDNA collection point, implying eDNA concentrations likely reflected biomass within 150 m (Yamamoto et al., 2016) (Table 2). Similarly, most detections of striped jack (*Pseudocaranx dentex*) were within 30 m of holding cages at the same study location, with no effect of flow direction on eDNA detections, although eDNA concentrations did decrease with distance from the cages (Murakami et al., 2019) (Table 2).

#### 3.2.2 | Water temperature

Water temperature affects many aspects of the ecology and biology of aquatic species (Person-Le Ruyet et al., 2004; Takahara et al., 2011), and consequently has interactive and/or divergent effects on eDNA concentrations (mostly through shedding, but also decay), which makes drawing conclusions beyond broad trends difficult in the absence of ancillary data on species-specific physiological requirements (Lacoursière-Roussel et al., 2016b). As one example, Takahara et al. (2012) found no





**FIGURE 2** The percentage of environmental DNA studies completed between 2012 and October 2020 assessing the abundance and/or biomass of fish in each study environment

effect of three different temperatures (7, 15 and 25°C) on common carp eDNA concentrations in aquaria. In contrast, another aquaria-based study using five densities of brook charr (*Salvelinus fontinalis*) exposed to two temperatures (7 and 14°C) reported a significant effect of temperature on eDNA concentration, with more DNA being released at higher temperatures and a better relationship between abundance and biomass at the higher temperature (Lacoursière-Roussel et al., 2016b). Such divergent effects at least partially reflect species-specific tolerance ranges and metabolic rates affecting shedding, and also any subsequent eDNA decay once shed from the fish.

Field studies similarly report contrasting effects of temperature on eDNA concentrations. In one relevant study, water sampling from a freshwater lagoon in winter revealed a strong correlation between water temperature and the concentration of common carp eDNA (Takahara et al., 2012). Warmer areas of the lagoon had greater concentrations of eDNA potentially because the fish were seeking optimal water temperatures to maintain their metabolism. In contrast, another study of 12 Canadian freshwater lakes found that lake trout (*Salvelinus namaycush*) eDNA concentrations did not vary with temperature; however, the temperatures were all low (<15°C), and it was hypothesized conditions were conducive to fish being variably distributed throughout the water column and thus randomly shedding DNA (Lacoursière-Roussel et al., 2016a).

Further, it is important to note that any effects of water temperature likely reflect broader seasonal influences, which can affect both the detection probability and the concentration of eDNA

present in the environment due to the activity levels of the fish (Furlan et al., 2016). For example, Doi et al. (2017) collected water samples from Japanese rivers in spring, summer, and autumn and measured the eDNA concentration of ayu (*Plecoglossus altivelis*). The relationships between eDNA concentrations and estimated biomass of ayu were similar across seasons, likely because the fish did not migrate and were highly active. These studies serve to illustrate potential issues with extrapolating the effects of temperature between species (even those occupying similar habitats) and underscore the need to adequately understand the biology of the focal species and the factors affecting their likely distributions.

### 3.2.3 | Water depth

Although not assessed at the same level of detail as horizontal flow or temperature, the eDNA sampling depth can clearly bias estimates of fish abundance or biomass, reflecting not only species-specific vertical distributions and habitat preferences, but also the absolute depth of the system (Diaz-Ferguson et al., 2014; Eichmiller et al., 2014; Hinlo et al., 2017a; Klobucar et al., 2017; Moyer et al., 2014). For example, Hinlo et al. (2017a) compared eDNA concentrations from Oriental weatherloach (*Misgurnus anguillicaudatus*) between the surface and subsurface of an Australian river and found few differences, although they attributed the homogeneity to the shallow depths of the surveyed river and the strong effects of vertical mixing. In contrast, findings from lakes have been more variable, with eDNA concentrations throughout the water column correlated to stratification and/or preferred depth distributions for each species (Eichmiller et al., 2014; Klobucar et al., 2017; Moyer et al., 2014). Such effects were illustrated by Hänfling et al. (2016) who observed that eDNA detection of multiple fish species in UK freshwater lakes reflected species-specific depth preferences, with deep-dwelling species like Arctic charr (*Salvelinus alpinus*) only recorded at the deepest sampling points (Hänfling et al., 2016).

A few investigations within the marine environment have also identified depth-specific variations in fish eDNA concentrations (Murakami et al., 2019; Yamamoto et al., 2016). In particular, despite a strong relationship between surface eDNA concentrations and acoustic intensity for Japanese jack mackerel, Yamamoto et al. (2016) noted surface and bottom water eDNA concentrations were not correlated, and bottom samples were frequently at the lower bound of eDNA concentration detectability. Considering the observed variation with horizontal flow, greater focus should be directed toward assessing the contributing effects of depth on eDNA concentration.

### 3.2.4 | Environmental DNA decay

Among the reviewed papers, it was clear that the decay of eDNA variably impacts estimates of abundance and biomass (Coulter et al., 2019; Jo et al., 2017; Nevers et al., 2018; Sassoubre et al., 2016). Decay may be beneficial to some extent by restricting

eDNA concentrations over ecologically relevant scales (Nevers et al., 2018; Sassoubre et al., 2016), or detrimental, in cases where low production and/or rapid decay could potentially prevent establishing a relationship with abundance or biomass (Perez et al., 2017).

Decay rate depends on whether the eDNA is intra- or extracellular and is potentially influenced by several biotic and abiotic factors, including salinity, water temperature, sunlight, pH, microbial activity, and enzymatic digestion (Andruszkiewicz et al., 2017; Barnes & Turner, 2016; Collins et al., 2018; Dejean et al., 2012; Hansen et al., 2018; Sassoubre et al., 2016; Schmelzle & Kinziger, 2016). The rate of eDNA decay may be faster in marine than freshwater environments (Sassoubre et al., 2016). Indeed, Schmelzle and Kinziger (2016) reported that for every unit increase in salinity within the marine environment, eDNA concentrations reduced by 0.07 standard deviations from the mean, thus affecting abundance estimates of tidewater goby (*Eucyclogobius newberryi*).

As stated above, water temperature variably affects eDNA decay, which appears to depend on the species and the environment. At a broader level, there are likely to be seasonal effects, simply because ultraviolet radiation directly degrades DNA (Lindahl, 1993). Nevertheless, studies in aquatic systems suggest that sunlight has a limited influence, probably because other confounding effects such as depth or pH play much larger roles (Andruszkiewicz et al., 2017; Merkes et al., 2014; Strickler et al., 2015). More research under controlled conditions is warranted to assess the influence of sunlight on eDNA decay.

### 3.2.5 | Methods of capturing, extracting, and amplifying eDNA

Irrespective of the various factors affecting the concentration of eDNA in an aquatic system, subsequently estimating a population's abundance or biomass is contingent on the repeatability of the eDNA concentration estimate. This estimate is the product of the compounding effects of many processes including the substrate used (water/sediment) and method of eDNA capture, extraction, storage, eDNA primer design and validation, and PCR amplification (Baldigo et al., 2017; Eichmiller et al., 2016; Furlan et al., 2016; Hinlo et al., 2017b; Jane et al., 2015; Knudsen et al., 2019; Kumar et al., 2020; Nathan et al., 2014; Thalinger et al., 2020; Thomsen et al., 2016; Yusishen et al., 2020). In the reviewed papers, most studies used a filtration method (e.g., glass fiber filters, mixed cellulose ester filters) to capture eDNA. Only a few studies (predominantly in controlled environments) used alternative methods such as centrifugation and precipitation, most likely because the latter methods utilize substantially smaller volumes of water and thus may be more suited to controlled environments where eDNA concentrations can be relatively high (Eichmiller et al., 2016; Erickson et al., 2016; Klymus et al., 2015; Maruyama et al., 2014; Piggott, 2016).

Only a few studies have directly compared different eDNA capture and extraction methods and their effects on abundance or biomass estimates. Lacoursière-Roussel et al. (2016b) found that five

different filters combined with water temperature could affect the strength of the relationship between eDNA concentration and abundance or biomass estimates of brook charr in aquaria. In another aquaria experiment, Eichmiller et al. (2016) evaluated the ability of three eDNA capture methods and six commercial DNA extraction kits to detect and quantify common carp biomass. Their results indicated the choice of capture and extraction methods could affect results. In contrast, in a study of Macquarie perch in an artificial pond, Piggott (2016) tested the effect of three eDNA capture - (filtration and precipitation) - and extraction methods - (Qiagen DNeasy Kit and phenol-chloroform-isoamyl) - on simulated biomass estimates (dam water spiked into the bore water that supplied the dam). All methods produced a strong positive correlation between eDNA concentration and biomass. Similarly, Takahashi et al. (2020) observed a strong correlation between eDNA concentration quantified from open filtration (glass filter) to enclosed filtration (Sterivex) and both of these concentrations were correlated with visually estimated biomasses of five marine fish—although different extraction methods were not assessed. While several studies have assessed the effects of filter type on eDNA concentrations, only one study has investigated the impact of dividing a sample among multiple filters (Guivas & Brammell, 2020). In that study, using either a single versus three filters produced positive correlations with biomass, although multiple filters resulted in a stronger relationship.

For species-specific studies, the vast majority have used qPCR to estimate eDNA concentrations with only three studies utilizing ddPCR (Capo et al., 2019; Doi et al., 2015; Nathan et al., 2014), possibly due to the higher relative cost of the latter instrument. Nathan et al. (2014) conducted mesocosm experiments to compare the performance of three PCR platforms (PCR, qPCR, and ddPCR) for estimating the concentration of round goby eDNA in the water. There was no statistical difference in the estimates of eDNA concentration between qPCR and ddPCR. In contrast, mesocosm experiments with common carp revealed eDNA concentrations determined by ddPCR better estimated absolute abundance and biomass than those determined by qPCR (Doi et al., 2015). While there are few studies evaluating fish eDNA concentrations using different PCR platforms, the available data suggest ddPCR has an advantage over qPCR. Three reasons for this preference are that it (a) does not require a standard curve (which can be compromised by pipetting error); (b) is more accurate for quantifying DNA at low concentrations; and (c) is becoming more cost-effective and thus may be more routinely used in the future (Doi et al., 2015; Nathan et al., 2014).

The success of PCR can be affected by the presence of PCR inhibitors in the sample that persist through the eDNA extraction stage (Akamatsu et al., 2020; Sassoubre et al., 2016). As one relevant example, in an experiment quantifying eDNA concentrations downstream of caged brook trout in a flowing stream in Massachusetts, USA, from midsummer to late autumn, eDNA was consistently detected (Jane et al., 2015). However, the eDNA signal became negligible to absent during autumn (which coincided with an increase in leaf litter) unless an inhibitor-reducing extraction method was used (Jane et al., 2015). This result implies inhibition can potentially mask

high eDNA concentrations, and profoundly affect the detectability and interpretation of abundance and/or biomass estimates (Jane et al., 2015).

Of the 63 reviewed papers, only 12 used metabarcoding to estimate relative abundance and/or biomass. Among these, four were carried out in controlled environments (Di Muri et al., 2020; Evans et al., 2016; Kelly et al., 2014; Li et al., 2018) and the remainder in natural environments (Fraija-Fernandez et al., 2020; Goutte et al., 2020; Hanfling et al., 2016; Li et al., 2019; Pont et al., 2018; Sard et al., 2019; Stoeckle et al., 2017; Thomsen et al., 2016). Nearly all metabarcoding studies (except Fraija-Fernandez et al., 2020) have reported positive correlations between eDNA read counts and relative abundance and/or biomass, supporting its potential utility as a cost-effective and accurate relative quantification method beyond simply identifying species presence.

The few assessed metabarcoding approaches also appear less affected by the filter types or extraction methods. In one study of artificial ponds, Li et al. (2018) reported comparable correlations between eDNA read counts and biomass across six different combinations of filter types (mixed cellulose ester and Sterivex-HV PVDF units), pore sizes and extraction methods (DNeasy Blood & Tissue Kit, PowerWater Kit and PowerWater Sterivex Kit). A more recent metabarcoding study of artificial ponds with known data on abundance and biomass also had consistent read counts from samples filtered using mixed cellulose ester and Sterivex filters (Di Muri et al., 2020). Nevertheless, an important consideration with metabarcoding is the additional primer-specificity biases that it introduces (Kelly et al., 2014).

### 3.2.6 | Spatial and temporal scales represented by eDNA sampling

It is clear from this review that eDNA concentrations in an aquatic environment vary considerably in space and time, which needs to be considered when designing a sampling regime to ensure accurate estimates of abundance or biomass (Hanfling et al., 2016; Klobucar et al., 2017; Salter et al., 2019; Shelton et al., 2019). This caveat was illustrated by Takahara et al. (2012) during aquaria trials with common carp which demonstrated eDNA concentrations peaked on the third day after fish were added to the tank before reaching equilibrium on the sixth day. Ideally, aquaria trials seeking to compare eDNA concentrations to abundance or biomass should first determine the most appropriate time for sampling after fish have been introduced into a tank. In natural populations, eDNA distributions can be heterogeneous and abiotic mechanisms that remove eDNA, such as decay, sedimentation or water flow, can be rapid (Eichmiller et al., 2014; Jane et al., 2015; Wilcox et al., 2016).

While there is considerable spatio-temporal variability in the movement or dispersal of eDNA (and therefore concentrations) within experiments, an important point here is that fishing gear-based methods of estimating fish abundance or biomass are constrained by single points in space and time (i.e., where and when

the gear is deployed). The detectability of eDNA across space and time might therefore provide relatively more information. In support of this statement, Shelton et al. (2019) showed that while eDNA and seine nets provided the same quantitative information for chinook salmon (*Oncorhynchus tshawytscha*) in terms of abundance indices reflecting seasonal migrations, the methods were less correlated when compared across sites, suggesting each approach provides different information about a heterogeneously distributed species. Indeed, seine nets only catch fish if they are present at the target site, whereas DNA is shed into the surrounding water where it is mixed and can persist for some time after the fish have moved on, but in some cases, may not be correlated with abundance. This effect can result in a smoother distribution (in time and space) of eDNA versus the presence of the fish themselves (Shelton et al., 2019).

## 4 | CONCLUSIONS AND FUTURE DIRECTIONS

It is well established that eDNA can provide valuable information on the spatio-temporal distributions of marine and freshwater fish and can be used to detect the presence of ETP or invasive species, but its utility to quantify the abundance and/or biomass of animals in the field has remained equivocal. Clarifying the efficacy of a quantitative eDNA approach and understanding its utility as an ancillary tool within conventional stock assessments (incorporating data on biology, mortality, recruitment, etc. Quetglas et al., 2017) would be an important step toward developing a cost-effective means for monitoring regional fisheries of both sessile and migratory species.

In this review, we have critically evaluated whether eDNA concentrations (i.e., number of DNA sequences) or read counts as quantified by qPCR, ddPCR or metabarcoding were correlated with the absolute or relative abundance or biomass of fish. We accomplished this evaluation by sourcing studies from the available literature that met select criteria and identified points of congruence. We acknowledge the potential for publication bias, whereby some null results are less likely to be presented. Nevertheless, we note the overwhelmingly positive evidence in support of the development of this quantitative approach, with 90% of the sourced studies demonstrating positive correlations between detectable DNA in the environment and abundance/biomass of the focal species. Variation around this positive correlation was attributed to key biotic (taxon, life history, diet, metabolism, and behavior) and abiotic (water flow, temperature, and capture method) factors. Species-specific assays were more frequently used than metabarcoding assays for quantifying eDNA, and recent research suggests that the former may be more effective in this regard for fish (Bylemans et al., 2019).

Considering the reviewed information, in addition to initial laboratory considerations that include *in silico* primer development and validation using source-voucher tissues, we suggest focusing on understanding the influences of the biotic and abiotic factors listed above on eDNA concentrations within and among key

species to guide the refinement of future efforts. Indeed, to control for the heterogeneous dispersal of eDNA in the environment, we suggest study designs that include sampling replicates across multiple spatio-temporal scales. It is especially imperative that future studies investigate any potential relationships between eDNA concentrations and abundance or biomass in the field to verify the technique is appropriate for species of interest.

To account for differences in life history and metabolism, complementary knowledge of the size and age structures of the focal species within a sampling area is important, unless the goal is to explicitly detect variation in eDNA concentrations through time as a proxy for spawning activity or seasonal migrations. However, studies should not be initiated without *a priori* ecological information, unless there is a strong incentive to do so (e.g., EPT species). The diet and behavior of the focal species may also bias eDNA concentrations in the field, but temporally standardized sampling could help to mitigate this problem. Moreover, the effects of abiotic factors such as water flow, depth, and temperature can be partially controlled for by collecting detailed knowledge of the hydrodynamics of the sampling site, vertical stratification of the focal species and historical temperature ranges, in addition to standardizing the time of sampling to account for tides and seasonal changes. Hierarchical sampling designs will be essential for identifying and partitioning spatio-temporal variation in eDNA concentrations, and ensuring such variability is accounted for when estimating abundance or biomass (Hanfling et al., 2016). Newer modeling-based approaches developed in aquatic settings may also compliment such initiatives (Chambert et al., 2018).

Irrespective of the approach, the collection method (i.e., type and pore size of filter, volume of water processed, and preservation method) should be standardized to facilitate comparisons between sampling sites and time points, and where possible, between studies. Based on the accumulated evidence, we therefore recommend developing eDNA as a complimentary (nonlethal) tool for assessing population abundance and/or biomass across different species of fish in both freshwater and marine environments, provided key biotic and abiotic factors are identified, considered, and, where possible, controlled for. With further development, eDNA may provide an effective fishery-independent method for assessing targeted stocks, while avoiding many of the potential biases associated with traditional fishery-dependent methods.

Data Archiving Statement: Data sharing is not applicable to this article because no data were created or analyzed outside of those summarized in the paper.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

MLR, AMF, JMH, MKB, JDB, and SF conceived and designed the research. MLR, AMF, JMH, MKB, JDB, SF, and JWW performed the literature review. MKB produced the figures. MLR, AMF, JMH, MKB, JDB, SF, JWW, and EMF wrote and edited the review.

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