

# Detection of fish sedimentary DNA in aquatic systems: A review of methodological challenges and future opportunities

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**Abstract**

Environmental DNA studies have proliferated over the last decade, with promising data describing the diversity of organisms inhabiting aquatic and terrestrial ecosystems. The recovery of DNA present in the sediment of aquatic systems (sedDNA) has provided short- and long-term data on a wide range of biological groups (e.g., photosynthetic organisms, zooplankton species) and has advanced our understanding of how environmental changes have affected aquatic communities. However, substantial challenges remain for recovering the genetic material of macro-organisms (e.g., fish) from sediments, preventing complete reconstructions of past aquatic ecosystems, and limiting our understanding of historic, higher trophic level interactions. In this review, we outline the biotic and abiotic factors affecting the production, persistence, and transport of fish DNA from the water column to the sediments, and address questions regarding the preservation of fish DNA in sediment. We identify sources of uncertainties around the recovery of fish sedDNA arising during the sedDNA workflow. This includes methodological issues related to experimental design, DNA extraction procedures, and the selected molecular method (quantitative PCR, digital PCR, metabarcoding, metagenomics). By evaluating previous efforts (published and unpublished works) to recover fish sedDNA signals, we provide suggestions for future research and propose troubleshooting workflows for the effective detection and quantification of fish sedDNA. With further research, the use of sedDNA has the potential to be a powerful tool for inferring fish presence over time and reconstructing their population and community dynamics.

**KEYWORDS**

environmental DNA, fish monitoring, lake sediment, marine sediment, paleolimnology, sedimentary DNA

**1 | INTRODUCTION**

Aquatic ecosystems are experiencing a rapid decline in fish biodiversity and fisheries stocks due to escalating anthropogenic pressures, such as habitat degradation, invasive species, overfishing, and climate change (Lotze et al., 2019; Reid et al., 2019; Su et al., 2021). As secondary consumers and top predators, fishes are among the most ecologically important taxa in aquatic ecosystems, exerting strong influences on the population dynamics of both their predators and prey. Accurate and timely assessments of fish diversity and population dynamics are therefore essential to support databases needed for conservation and management decisions (Pereira et al., 2013). Over the past two decades, environmental DNA (eDNA) monitoring approaches have expanded rapidly and are now frequently used worldwide to study fish populations (Deiner et al., 2017; Rourke et al., 2022). Some studies have shown that eDNA-derived fish biodiversity profiles are comparable to those reported through conventional sampling techniques based on observational and historical records (Cantera et al., 2022; Fediajevaite et al., 2021; Keck, Blackman, et al., 2022; Keck, Couton, & Altermatt, 2022). Increasingly, evidence suggests that less intrusive eDNA methods

will complement more invasive methods (e.g., mark and recapture) for analyzing the diversity and composition of fish communities.

Although most fish eDNA studies are focused on water column sampling, analyses of sedimentary DNA (sedDNA) – environmental DNA present in the sediment of aquatic or non-aquatic ecosystems – are also gaining momentum (Capo, Giguet-Covex, et al., 2021; Crump, 2021; Cuenca-Cambronero et al., 2022). Both surface and core sediment layers constitute natural archives of biological and environmental changes that can be useful in reconstructing short- and long-term dynamics of aquatic communities prior to the beginning of observational records, which are frequently limited to the most recent decades (Barouillet et al., 2023; Kuwae et al., 2020; Monchamp et al., 2018). Many paleolimnological approaches rely on the preservation and subsequent identification of microfossils from the sediment archive, such as diatom remains, algae cysts, or pollen (Smol, 2008). Unfortunately, many soft-bodied species, such as rotifers and microbial eukaryotes, do not leave behind diagnostic morphological features. Fish can leave remains like otoliths, scales, teeth, and bones that might be identifiable to broad groups (Sibert & Rubin, 2021), but these are often found in sparse quantities and have therefore

been largely excluded from many reconstructive analyses used to infer past ecosystem changes (Gregory-Eaves et al., 2023). Indirect proxies, such as stable isotope analyses (Finney et al., 2000) or zooplankton-based transfer functions (Jeppesen et al., 1996, 2001), have been used to reconstruct past fish abundances, but often fail to provide definite information on species identification and community composition. As an untapped proxy, sedDNA analyses have the potential to fill this gap and allow for better characterizations of short- and long-term trends in fish assemblages. SedDNA analyses involve the recovery and study of genetic material preserved in the sediment archive to identify fish taxa, whether from DNA bound to sediment particles or DNA still contained in cells buried in the sediment (Capo, Giguët-Covex, et al., 2021; Cuenca-Cambronero et al., 2022). The detection of fish sedDNA would (i) allow for more complete reconstructions of past ecosystems, (ii) enable new research approaches to tackle fundamental ecological questions related to community interactions (e.g., predation, competition, parasitism) and food web dynamics over longer time scales, and (iii) strengthen ecosystem-wide conservation and management practices. However, current challenges with reliably detecting and characterizing fish communities from sediments limit our ability to fully understand their long-term spatiotemporal variations in aquatic ecosystems (Ficetola & Taberlet, 2023).

Presently, several challenges constrain the successful recovery of fish DNA from sediments. As a result, fish have not been as widely represented in sedDNA studies as other aquatic taxa, though there have been notable exceptions (Table 1). Matisoo-Smith et al. (2008) first detected fish sedDNA matching the native common bully (*Gobiomorphus cotidianus*) in an 1800-year-old sediment core from a New Zealand Lake. Fish sedDNA has since been used to estimate colonization histories (Olajos et al., 2018; Stager et al., 2015), confirm species introductions (Nelson-Chorney et al., 2019), and reconstruct quantitative time series (Kuwae et al., 2020; Sakata et al., 2022) in multiple ecosystems.

In parallel, progress has been made to optimize the recovery of fish DNA from contemporary surface sediments. By testing a range of sediment quantities and extraction methods, Thomson-Laing et al. (2022) developed an optimized protocol to detect fish DNA from freshwater surface sediments. Similarly, Sakata et al. (2021) established an effective sampling technique for contemporary fish sedDNA metabarcoding. Other attempts to analyze aquatic sediments using universal fish primers for DNA metabarcoding have also been performed (Cheang et al., 2020; Naro-Maciel et al., 2022; Sakata et al., 2020; Sales et al., 2021; Shiragaki et al., 2021) with varying degrees of success, and many unsuccessful attempts remain unreported or unpublished (Table 1). Promising results have also been obtained by metagenomic analyses (e.g., shotgun sequencing) of sedDNA in freshwater (Hebda et al., 2022; Pedersen et al., 2016) and marine systems (Armbrecht, 2020).

Despite these advances, many uncertainties remain, and additional research is needed to elucidate the various factors affecting fish DNA transport, deposition, and preservation in sediments.

Biotic factors (e.g., fish biomass and ecology) impact the amount of eDNA released into the water column (Yao et al., 2022). In addition, the process of preservation and degradation (i.e., taphonomy) of fish eDNA in water and sediments is understudied, and the effects of sedimentation rate, water residence time, and other abiotic factors (e.g., water chemistry) on DNA deposition are still poorly understood. For instance, sediment geochemistry may impact sedDNA accumulation and preservation (Kanbar et al., 2020). Armbrecht et al. (2022) found that marine eukaryote sedDNA damage was associated with organic matter decomposition indicators (e.g., ammonia, phosphate, alkalinity). Picard et al. (2023) also suggested that sediment geochemistry may impact the preservation of fish sedDNA, with indications of poor preservation in sediments of low density and high-organic content. However, the extent to which geochemical properties and diagenetic processes affect sedDNA preservation, recovery, and amplification remains unclear.

Given that the study of fish sedDNA is an emerging field, standardized protocols need to be developed. In this review, we summarize knowledge gaps and discuss the main factors and methodological uncertainties that limit the detection of fish sedDNA. We begin by providing common terminologies being used in the field (Table 2) and examine the factors affecting fish sedDNA dynamics in aquatic systems, from the release of DNA into the water column (aqueous eDNA) to its preservation in sedimentary archives (sedDNA). We then discuss the various methodological considerations that can potentially affect fish sedDNA detection and provide an extensive list of successful and unsuccessful fish sedDNA studies globally. At last, we propose a comprehensive workflow and troubleshooting guide for the effective detection and quantification of fish DNA from sediments. The outcomes of the present review will help guide future research and advance the use of sedDNA as a powerful tool for detecting fish and reconstructing their population and community dynamics for fundamental environmental research and the conservation of aquatic systems.

## 2 | FISH SEDDNA DYNAMICS: FROM FISH TO SEDIMENTARY ARCHIVES

Aqueous eDNA concentrations are affected by multiple biotic and abiotic factors at the production, transport, and persistence steps, all of which ultimately impact the DNA signals in sediments (Figure 1). Therefore, understanding (i) how fish eDNA is released into the water column, (ii) deposited in sediments, and (iii) preserved in sedimentary archives is essential in understanding the dynamics of fish sedDNA.

### 2.1 | Production and persistence of fish environmental DNA in the water column

The amount of eDNA shed by fish is influenced by various factors such as fish size, diet, life events (e.g., spawning), and ecology, and

TABLE 1 Summary of successful and unsuccessful fish sedimentary DNA studies in various aquatic systems.

Reference	Location	Type of aquatic system	Sediment type	Sediment age (oldest estimate)	Sediment used per sample
Matisoo-Smith et al. (2008)	Round Lake, New Zealand	Lentic	Sediment core	1.8 kya	N/A
Stager et al. (2015)	St. Regis Lake, New York, USA	Lentic	Sediment core	4 kya	0.25 g packed sediments
Pedersen et al. (2016)	Charlie Lake and Spring Lake in the Peace River drainage, Canada	Lentic	Sediment core	15 kya	2 g
Ficetola et al. (2018)	La Poule Lake, Kerguelene Island	Lentic	Sediment core	1 kya	12 g
Olajos et al. (2018)	Hotagen and Stora Lögdsjön Lake, Sweden	Lentic	Sediment core	12 kya	0.25 g/extraction * 4 extractions = 1 g
Nelson-Chorney et al. (2019)	Mystic and Marvel Lake, Alberta, Canada	Lentic	Sediment core	100 ya	0.1–0.2 g
Ambrecht et al. (2022)	Scotia Sea, West Antarctica	Marine	Sediment core	1 mya	0.25 g
Turner et al. (2015)	Experimental ponds (Kansas, USA) and natural rivers: the Wabash River (Indiana, USA), the Kansas River (Kansas, USA), and the Wakarusa River (Kansas, USA)	Lentic (experimental) and lotic (natural)	Surface sediment	NA	5 mL wet sediment (5.5 to 10.9 g)
Kuwae et al. (2020)	Beppu Bay, Japan	Marine	Sediment core	400 ya	3 g
Cheang et al., (2020)	Western waters, Hong Kong	Marine	Surface sediment	N/A	0.25 g/extraction * 5 extractions = 1.25 g
Sakata et al., (2020)	Lake Iba, Japan	Lentic	Surface sediment	N/A	3 g/extraction * 9 extractions = 27 g
Thomson-Laing et al., 2020	Lake Rotoiti, Maitai River, Tasman Valley Stream, New Zealand	Lotic Lentic	Surface sediment	N/A	0.25 g

Extraction method description	Purification method description	Molecular approach for detection	Target fish species	Outcome
Modified FastDNA Spin Kit for soil (Qbiogene)	Humic acid wash (solution containing GuSCN)	PCR	<i>Gobiomorphus cotidianus</i>	Successful detection of native fish species
N/A	Power Soil DNA purification kit (MoBio Laboratories)	PCR	<i>Perca flavescens</i>	Confirmed native status of target fish species
Modified version of the organic extraction protocol (Wales et al., 2014)	Mobio C2 and C3 buffers (Mobio Laboratories)	Metagenomics	N/A	Complete biodiversity reconstruction including a few fish species
NucleoSpin Soil Kit (Macherey-Nagel)	N/A	Metagenomics	N/A	Mammal DNA detected using mammal primers, while no fish DNA was detected from the same sediments
Power Soil DNA isolation kit (MoBio Laboratories)	OneStep inhibitor removal kit (Zymo Research)	PCR	<i>Coregonus lavaretus</i>	Estimated species colonization dates of target fish species
Power Soil DNA isolation kit (MoBio Laboratories)	Agencourt AMPure XP beads (Beckman Coulter; Brea, CA)	Metabarcoding	<i>Oncorhynchus clarkii bouvieri</i> and <i>Oncorhynchus clarkii lewisi</i>	Confirmed historical records of non-native introductions
"Combined": EDTA + bead-beating + liquid silica in QG Buffer (1) MinElute Reaction Cleanup Kit (Qiagen); (2) "reverse AxyPrep" DNA purification post-library preparation; (3) AxyBeads purification	"Combined": EDTA + bead-beating + liquid silica in QG Buffer (1) MinElute Reaction Cleanup Kit (Qiagen); (2) "reverse AxyPrep" DNA purification post-library preparation; (3) AxyBeads purification	Metagenomics	N/A	Presented a 1-million-year-old record of marine eukaryote ancient DNA from Antarctic marine sediments
CTAB extraction (modification of Coyne et al. 2005, 2006, 2001)	OneStep Inhibitor Removal Kit (Zymo Research)	qPCR	<i>Hypophthalmichthys spp.</i>	SedDNA was detected up to 132 days (versus 25 days for aqueous eDNA) after the presence of target species. Target species eDNA was 8-1846 times more concentrated in sediments (per g) than in water (per mL)
Power Soil DNA isolation kit (MoBio Laboratories)	N/A	qPCR	<i>Engraulis japonicus</i> , <i>Sardinella melanostictus</i> , and <i>Tranchurus japonicus</i>	Reconstructed decadal-centennial dynamics of fish abundance in marine waters. DNA signatures were consistent with landing records
Modified DNeasy PowerSoil Kit (Qiagen)	N/A	Metabarcoding	N/A	Successfully sequenced 2/3 of the studied sediment samples. Identified 22 fish species, which is an underestimate of the fish diversity on record
Combined alkaline DNA extraction (Kouduka et al., 2012) with ethanol precipitation and the PowerSoil DNA Isolation Kit (MoBio Laboratories)	N/A	Metabarcoding	<i>Hemigrammocypris rasborella</i> , <i>Cyprinus carpio</i> , <i>Lepomis macrochirus</i> , and <i>Micropterus salmoides</i>	Species composition obtained by metabarcoding was not significantly different between sediment and water
DNeasy PowerSoil Isolation Kit (Qiagen)	N/A	dPCR	<i>Anguilla australis</i> , <i>Anguilla dieffenbachii</i>	Detected both target species but with variability between site replicates (varied 40%- 100% detection rate depending on site)

(Continues)

TABLE 1 (Continued)

Reference	Location	Type of aquatic system	Sediment type	Sediment age (oldest estimate)	Sediment used per sample
Sales et al., (2021)	Jequitinhonha River catchment, Brazil	Lotic	Surface sediment	N/A	15 mL
Shiragaki et al., (2021)	Otsuchi Bay, Japan	Marine	Surface sediment	N/A	0.25 g/extraction * 8 extractions = 2 g
Sakata et al., (2021)	Koide River, Japan	Lotic	Surface sediment	N/A	9 g
Huang et al., (2021)	13 sub-alpine and alpine lakes, Jämtland, western Sweden	Lentic	Surface sediment	N/A	0.5 g/extraction * 3 extractions = 1.5 g (wet)
Hebda et al., (2022)	Little Woss Lake, British Columbia, Canada	Lentic	Sediment core	16 kya	2 g
Sakata et al., (2022)	Lake Biwa, Japan	Lentic	Sediment core	100 ya	10 g
Naro-Maciel et al., (2022)	Bronx River Estuary, New York City, USA	Lotic	Surface sediment	N/A	0.25 g
Thomson-Laing et al., (2022)	Richmond Reservoir, Lake Ngakeketo, Lake Hurimoana, Lake Pounui, New Zealand	Lentic	Surface sediment	N/A	~10 g
Picard et al., (2023)	Lake Pounui, Tomarata, and Waitawa, New Zealand	Lentic	Surface sediment	N/A	~3 g
Thomson-Laing et al., (Under review)	Lake Pounui, New Zealand	Lentic	Sediment core	800 ya	~10g
Lopez et al., (Under review)	Cowpar Lake, Alberta, Canada	Lentic	Sediment core	100 ya	2 g
Wood et al., in prep <sup>a</sup>	Lakes Wiritoa, Alice, Westmere, Waipu, Karere and Oporoa New Zealand	Lentic	Sediment cores	1.8 kya	~3 g
Duxbury et al., in prep <sup>b</sup>	Lashmars Lagoon, Kangaroo Island, Australia	Lentic	Sediment Core	7 kya	0.25 g
Kurte et al., in prep <sup>c</sup>	Gippsland Lake, Australia	Estuary	Sediment core	300 ya	0.25 g

Extraction method description	Purification method description	Molecular approach for detection	Target fish species	Outcome
DNeasy PowerMax Soil Kit (Qiagen)	N/A	Metabarcoding	N/A	Sediment samples provided a different overview of species richness and $\beta$ -diversity than water samples
DNeasy PowerSoil Kit (Qiagen)	PowerClean Cleanup Kit (Qiagen)	Metabarcoding	17 different fish species	Temporal changes in fish species composition reconstructed using sedDNA were consistent with visual censuses
Combined alkaline DNA extraction (Kouduka et al., 2012) with ethanol precipitation and the PowerSoil DNA Isolation Kit (Qiagen)	N/A	Metabarcoding	Fish communities	Fish sedDNA is heterogeneously distributed in the environment
C1 solution from the DNeasy PowerSoil Kit (Qiagen) vs. three homemade buffers	PowerClean Cleanup Kit (Qiagen)	PCR and dPCR	<i>Salvelinus alpinus</i> and <i>Salmo trutta</i>	Unsuccessful detection (confirmed by sequencing). Digital PCR performed better than conventional PCR
Digestion in CTAB, followed by chloroform-isoamyl alcohol extraction, and purification in Qiagen Minielute column	Mobio C2 and C3 buffers (Mobio Laboratories)	Metagenomics	<i>Oncorhynchus tshawytscha</i>	Reconstructed late Pleistocene palaeoenvironments in Vancouver Island
Combined alkaline DNA extraction (Kouduka et al., 2012) with ethanol precipitation and the PowerSoil DNA Isolation Kit (Qiagen)	N/A	qPCR	<i>Plecoglossus altivelis</i> and <i>Gymnogobius isaza</i>	Reconstructed past fish fauna of studied lake system
PowerSoil kit (Qiagen, USA)	Calibrated Ampure XP beads (Agencourt Bioscience)	Metabarcoding	N/A	Failed to detect fish undergoing restoration but common fish were found
Lakes ABPS protocol (Described in this paper)	N/A	dPCR	<i>Perca fluviatilis</i> , <i>Anguilla australis</i> , <i>Anguilla dieffenbachii</i>	Presented an optimized extraction protocol for dPCR detection of target fish species in freshwater surface sediments
Lakes ABPS	N/A	dPCR	<i>Perca fluviatilis</i> , <i>Scardinius erythrophthalmus</i>	Fish sedDNA was found throughout the lakes with some patchy detections. In two of the three study lakes, sediment was better than water in detecting fish
Lakes ABPS	N/A	dPCR and Metabarcoding	<i>Perca fluviatilis</i> , <i>Anguilla australis</i> , <i>Anguilla dieffenbachii</i> , <i>Salmo trutta</i> , <i>Oncorhynchus mykiss</i>	Successful but variable target fish detection using dPCR. Detection varied between nearshore and depocenter locations. Variable metabarcoding results depending on sites
Norgen Soil DNA Isolation Maxi Kit	OneStep PCR Inhibitor Removal Kit (Zymogen)	qPCR	<i>Esox lucius</i> and <i>Coregonus artedii</i>	Confirmed indigenous knowledge on fish baseline records
Lakes ABPS	N/A	Metabarcoding	Wide range of native and non-native fish	Showed spatial variability in detection between lakes and with core depth (age). Worked well in some lakes but not others
DNeasy PowerLyzer PowerSoil Kit	MinElute Reaction Cleanup Kit (Qiagen) and AxyBeads purification	Metagenomics	N/A	Complete biodiversity reconstruction focusing on <i>Viridiplantae</i> . A few fish taxa detected at low read counts in some samples
Laboratory extraction methods described in Weyrich et al., 2017	AxyPrep (Axygen, Bioscience)	Metagenomics	Fish biodiversity described in Gippsland Lake	Database of fish (Metagenome RefSeq, NCBI and Mitofish) were insufficient to obtain conclusive results

(Continues)

TABLE 1 (Continued)

Reference	Location	Type of aquatic system	Sediment type	Sediment age (oldest estimate)	Sediment used per sample
Myler et al., unpublished (2023)	Marden Creek, Ontario, Canada	Lotic	Surface sediment and suspended sediment	N/A	0.25 g
King et al., unpublished <sup>d</sup>	Utah Lake, Utah, USA	Lentic	Sediment core	275 ya	1 mL
Huston et al., unpublished (2023)	Walker Pond, Maine, USA	Lentic	Surface sediment and sediment core	NA	10 g

Note: When available, sediment age is listed as reported in its respective publication.

Abbreviations: dPCR, digital PCR; qPCR, quantitative PCR.

Citation notes:

<sup>a</sup>Wood, S. A., Vandergoes, M. J., Pearman, J. K., Waters, S., Thomson-Laing, G., Thompson, L., ... Howarth, J. D. (2023). Analysis of sediment cores from 16 lakes in the Manawatū-Whanganui region – implication for lake management. Cawthron Report XXX. In preparation.

<sup>b</sup>Duxbury, L. C., Pérez Godoy, V., Cadd, H., Tyler, J. J., Francke, A., Law, W. B., & Armbricht, L. (in prep). Lake sedimentary ancient DNA reveals ecosystem response to fire and climate on Kangaroo Island (Karti), Australia.

<sup>c</sup>Kurte, L., Pérez, V., Quezada-Romegialli, C., Yichen, L., Wei, W. W., Kessler, A., ... Sintern, A. (in prep). Ancient DNA analysis from Gippsland Lakes sediment cores: impact on publish database biases.

<sup>d</sup>King, L., Brothers, S., & Brahney, J. Fish sedDNA from Goshen Bay, Utah Lake. Unpublished.

is expected to correlate with the biomass and number of individuals present in an environment (Harrison et al., 2019; Stewart, 2019). Even though species abundance and biomass have a direct mechanistic effect on and statistical relationship with the amount of eDNA in the water column (Rourke et al., 2022), shedding rates of fish DNA vary between species and may confound the ability to estimate their comparative abundances based on the amount of eDNA collected (Yates et al., 2021). Regarding the major sources of fish eDNA, most are likely derived from mucosal epithelial cells, excreted products (e.g., urine and feces), and decomposing tissues (Harrison et al., 2019). Moreover, when fish spawn or are exposed to stress, eDNA production can increase up to 100-fold (Sassoubre et al., 2016; Wu et al., 2022).

The persistence of fish DNA in the water column depends on whether the eDNA is intra- or extracellular, as well as the ecosystem's biotic and abiotic factors, such as water temperature, sunlight, pH, and microbial activity (Barnes et al., 2014; Barnes & Turner, 2016; Rourke et al., 2022). Environmental DNA can be found in a variety of states (such as extra-organismal [dissolved or particle adsorbed], intracellular, and even held within an organelle), with environmental factors differentially affecting the recovery of each state (Brandão-Dias et al., 2023; Kirtane et al., 2023; Mauvisseau et al., 2022; Nagler et al., 2022). Depending on the state of eDNA, it is anticipated that microbial activity and abundance, as well as temperature, pH, and oxygen concentrations, are crucial factors in the degradation of eDNA in the water column (Hofreiter et al., 2001; Seymour et al., 2018).

Higher temperatures and oxygen concentrations enhance microbial activity, which in turn results in faster DNA degradation. For instance, bacteria use extracellular enzymes and ectoenzymes to break down and assimilate DNA in aquatic ecosystems (Tsuji et al., 2017).

The rate of eDNA decay in the water column likely differs between aquatic environments, potentially impacting DNA transfer to sediments. Water residence time is an overarching variable that can influence eDNA decay rates (Harrison et al., 2019). In addition, ion concentrations have a substantial influence on DNA degradation in the water column. Hypersaline lakes rich in chaotropic salts (salts that can increase proteins solubility) can effectively preserve DNA, while hard water lakes also offer good preservation due to higher sedimentation rates promoted by calcite production (Capo et al., 2017; Hallsworth et al., 2007). Environmental DNA persistence in the water column is also affected by irradiance and water temperature in different ways (Andruszkiewicz et al., 2017). Although UV light damages DNA directly, studies on freshwater systems indicate that UV radiation has minimal effects on eDNA degradation in temperate regions (Mächler et al., 2018; Merkes et al., 2014; Yu et al., 2022). Instead, higher water temperatures, linked to irradiance, have more significant effects on eDNA degradation (Mächler et al., 2018; Yu et al., 2022). Environmental DNA decay may be faster in marine vs. freshwater environments, potentially due to differences in metabolic processes, though the effects of marine currents and climate impacts serve as the primary driving factors (Andruszkiewicz et al., 2017; Sassoubre et al., 2016).

Extraction method description	Purification method description	Molecular approach for detection	Target fish species	Outcome
PowerSoil Pro Kit (Qiagen)	None	Metabarcoding	Fish communities	Poor DNA yields, low concentration libraries, did not pass QC for sequencing
DNeasy PowerSoil HTP 96 Kit	N/A	Metabarcoding	Fish communities ( <i>Chasmistes liorus</i> and <i>Cyprinus carpio</i> of particular interest)	Poor fish DNA yields. Dominant taxa identified by sedDNA not present in the lake. Phytoplankton and higher plant sedDNA was successful
Lake ABPS with increased DNA template during qPCR	NA	qPCR	Alewife ( <i>Alosa pseudoharengus</i> )	Found fish sedDNA concentrations to vary spatially across the lake. Higher DNA concentrations in water samples than in sediments

## 2.2 | Transfer of fish DNA from the water column to sediments

Understanding DNA taphonomy and sedimentological processes (e.g., depositional environment and sedimentation rates) can assist in the accurate interpretation of eDNA signals from aquatic sediments (Figure 1). DNA can be transferred from the water column to sediments within carcasses or via binding to particulate organic matter (known as sinking particles or marine/lacustrine snow) (Turner et al., 2015). Fish DNA can be transferred quickly to sediments, as shown in the work of Nevers et al. (2020) detecting round goby sedDNA on the first day of their mesocosm experiment. This trend may be similar in littoral zones and shallow lakes, especially if high fish DNA input is combined with a longer water residence time. While whole fish carcasses may release locally high concentrations of DNA into the environment of experimental settings (Merkes et al., 2014; Tillotson et al., 2018), scavenging likely influences fish sedDNA deposition in natural settings. For instance, scavenger fish and invertebrates (e.g., catfish and crayfish) feed directly on dead fish, potentially reducing the concentration and altering the distribution of sedDNA from the source. A study on a small lake in Michigan (USA) reported that scavengers consumed up to 82% of dead fish biomass found on the lake bottom, leaving only 18% to decompose naturally (Schneider, 1998).

Rapid burial may be crucial in the preservation of eDNA transported to sediments because DNA degradation through enzymatic and abiotic processes is more likely to occur in the more

metabolically active surface sediment layer. This is especially true for extracellular DNA, though intracellular DNA will also degrade quickly once cells stop active repair after the death of the organism (Ellegaard et al., 2020). The sediment accumulation rate may also affect sedDNA detections. Sediment may accumulate due to an external source (e.g., clastic material brought by erosion or currents) or an internal source (e.g., organogenic sediments, chemical precipitates). Both sediment typologies are known to preserve and yield sedDNA, but may influence the communities detected using sedDNA. For instance, studies analyzing the sedDNA of plant communities in lakes receiving limited sedimentary input from terrestrial sediments found relatively high amounts of DNA of aquatic and semi-aquatic plants (Alsos et al., 2018). Conversely, in lakes receiving strong inputs from erosion, plant sedDNA represents the communities of terrestrial plants inhabiting the surrounding drainage, while aquatic plants are more scarce (Giguet-Covex et al., 2019). These observations suggest that high-erosion rates in surrounding landscapes might bias the reconstruction of ecosystem changes through time, making it more difficult to detect aquatic organisms, including fish species (Giguet-Covex et al., 2019).

## 2.3 | Persistence and preservation of DNA in sediments

The physical and geochemical composition of aquatic sediments, as well as the form of DNA (intra- or extracellular), play a key role in

TABLE 2 Definition of terms used in the review.

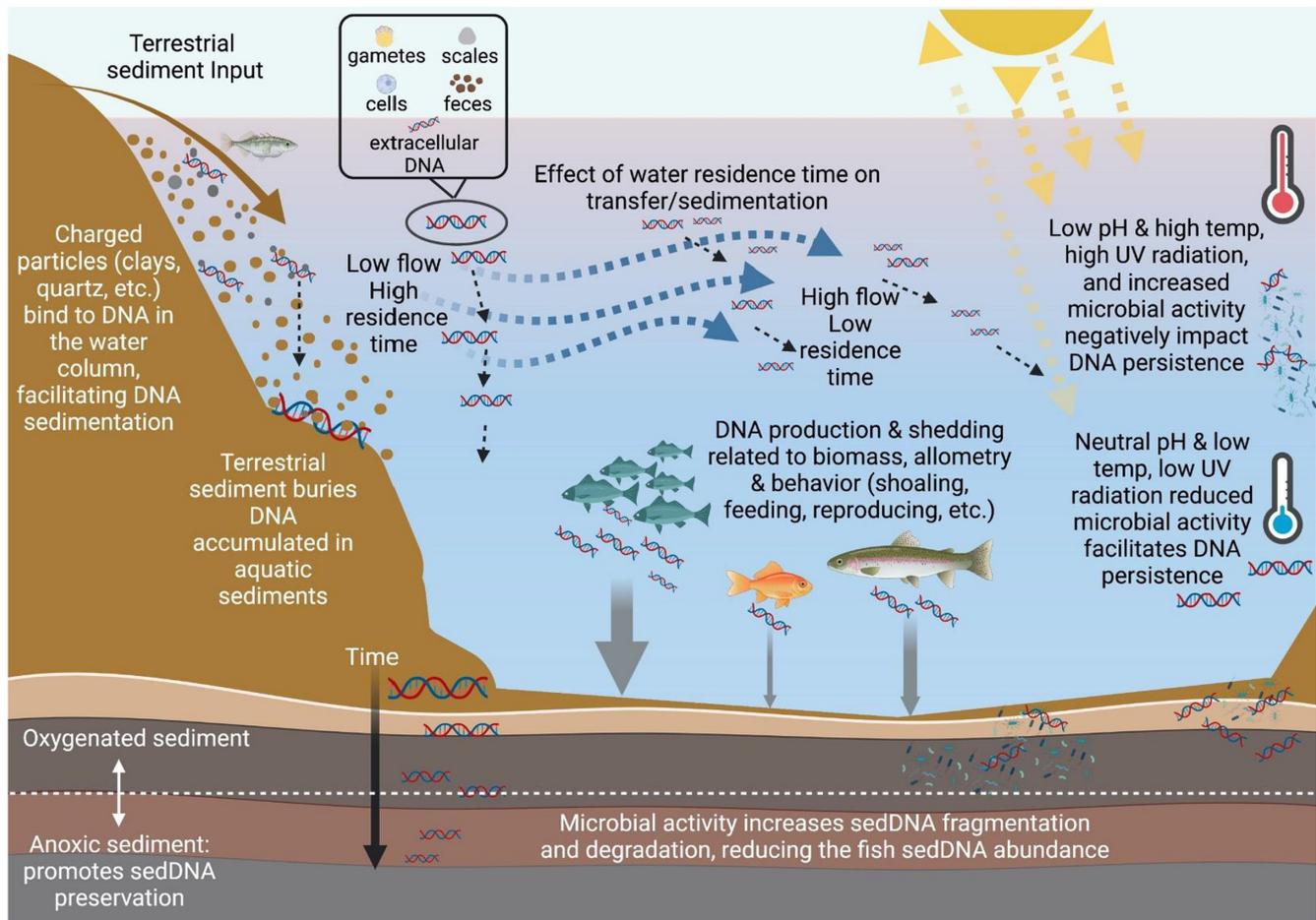
Terms	Definition
Amplicon Sequence Variants (ASVs)	Also called exact sequence variants (ESVs). 'True' DNA sequences (reads) are identified and separated from false reads (chimeras) by an algorithm during the bioinformatic pipeline of a sequencing run. As opposed to Operational Taxonomic Units (OTUs), ASVs are not study-specific groupings, and a clustering algorithm based on sequence similarity is not employed. If the same bioinformatics parameters are used, ASVs can be compared and merged across sequencing runs, studies, and databases
Digital PCR (dPCR)	Method of amplifying and quantifying DNA concentration. Unlike qPCR, in dPCR the PCR reaction is split into thousands of individual reactions prior to amplification, allowing for more precise quantification. Quantification of the PCR product is measured at the end of the reaction (end-point PCR)
Environmental DNA (eDNA)	Genetic material (from skin, excrement, etc.) is shed by an organism into its environment (e.g., soil, water, etc.)
High-Throughput Sequencing (HTS)	Also called next-generation sequencing (NGS) – the term is used to describe technologies that sequence large numbers of DNA fragments in parallel, encompassing both second and third-generation sequencing to target short and long-read lengths
Metabarcoding	Large-scale taxonomic detection of multiple species from an environmental sample via high-throughput sequencing of a targeted gene fragment (amplicon).
Metagenomics	The analysis of all nucleotide sequences detected in a bulk environmental sample using shotgun sequencing, as opposed to targeting specific species or gene regions
PCR Inhibitors	Organic and inorganic compounds (e.g., humic acids, tannic acids, ethanol, etc.) that prevent the amplification of nucleic acids during PCR
Primer	Short single-stranded synthetic DNA sequence that is used in the PCR process to hybridize with the DNA template and identify the region to be amplified
Probe	Single-stranded DNA or RNA sequence matching the DNA template and enhancing PCR specificity. Used in quantitative PCR, probes have a tag that emits fluorescence upon amplification, making it possible to visualize and quantify the amplicons it has bound to
Quantitative PCR (qPCR, also quantitative real-time qPCR – rt-qPCR)	Amplification of DNA product whereby the accumulation of the amplification product is measured as the reaction progresses (in real-time), thus quantifying the amount of DNA amplified after each cycle
RPA/CRISPR	Short for Recombinase polymerase amplification (RPA) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). Recombinase is an isothermal alternative to the polymerase used in standard PCR (using cycling machines), useful to detect and amplify low levels of DNA, but with a possibility of non-specific amplification. CRISPR combined with an associated Cas protein, for example, Cas12a, performs a double-stranded DNA cleavage which is very specific. Combining RPA and CRISPR allows for fast, specific amplification at a constant temperature
Sedimentary DNA (sedDNA)	Environmental DNA is preserved in the sediment, e.g., of aquatic systems, caves, and terrestrial soils
Universal Primers	Primer sets that amplify conserved DNA regions of specific taxonomic groups are used for detecting broader sets of species

controlling the preservation of fish DNA after its deposition into sediments. Buried fish sedDNA is most likely to consist of predominantly extracellular DNA. Unlike other organisms with recalcitrant structural elements, such as resting stages (e.g., cyanobacteria akinetes), dormant eggs (e.g., contained in cladoceran ephippia), propagules (e.g., protist cysts), frustules (e.g., diatoms), and lignin (e.g., terrestrial plants), the majority of fish biomass is composed of unprotected cells and is, therefore, more susceptible to degradation. SedDNA preservation is also regulated by the adsorption and desorption of DNA to mineral particles; thus, it is influenced by the mineralogical composition, pore-water pH, and the valence and concentrations of cations in the sediments (Kanbar et al., 2020; Torti et al., 2015).

Previous studies suggest that DNA degrades over time while it is adsorbed onto sediment particles. For example, sedDNA can be degraded or damaged rapidly during the first years of burial (early diagenesis) by microbes using extracellular DNA as energy sources

(Dell'Anno & Danovaro, 2005) or by environmentally induced strand breakage (Dabney et al., 2013). In the coastal marine sediments, DNA damage in eukaryotes increased in the first ~30 cm to remain relatively stable (at ~25% damage) in deeper (1–3 m) sediments (Armbricht et al., 2021). This differential damage pattern may be related to differences in compaction between upper and deeper sediments, as well as oxygen levels and microbial activity. On the other hand, experimental evidence from lake sediments suggests that there are moderate to limited effects of early diagenesis on the DNA signal of protist communities (Capo et al., 2017). Nonetheless, significant knowledge gaps remain regarding the importance of each of these factors in contributing to fish sedDNA degradation, and how the species-specific DNA signal might be modified with sediment depth.

The time scales of DNA preservation in aquatic sediments are not yet fully determined. Presently, the oldest fish sedDNA detection occurred between 16.1–16.2 kya (Table 1; Hebda et al., 2022). In



**FIGURE 1** Schematic diagram showing the main abiotic and biotic factors that can affect the production, shedding, vertical transfer, sedimentation, preservation, and persistence of fish sedDNA. Differences in marine versus freshwater as well as spatial heterogeneity of fish in water bodies are not explicitly covered to simplify the diagram.

comparison, decay of chloroplast DNA has been reported based on analyses of Bering Sea sediments within 100–200 kya after deposition (Kirkpatrick et al., 2016), and DNA from planktonic organisms has been detected in 270kya sediment samples from Lake Van (Randlett et al., 2014), 1 Mya in the Scotia Sea (Armbrecht et al., 2022), and up to 1.4Mya in the Bering Sea (Kirkpatrick et al., 2016). Most recently, the DNA of several terrestrial organisms was detected in 2 Mya-old sediment samples from Northern Greenland (Kjær et al., 2022). Due to natural degradation, it is predicted that eukaryote sedDNA cannot survive for much longer than 2Mya, even under conditions favorable to DNA preservation (e.g., cold, hypoxic, no UV radiation) (Kjær et al., 2022). Moreover, after this time (~1 Mya), the ancient DNA signal may be strongly obscured by DNA from living organisms (e.g., fungi, bacteria) (Armbrecht et al., 2022; Kjær et al., 2022).

Other key factors influencing the preservation of DNA in lake sediments include the chemical properties of lake water, specifically salinity, and pH. SedDNA preservation may be facilitated by intermediate water conductivities ( $100\text{--}500\mu\text{Scm}^{-1}$ ) and neutral to slightly alkaline water pH (7–9) (Jia et al., 2021). Increases in water temperature promote fish eDNA decay rates (Eichmiller et al., 2016). Therefore, excellent sedDNA repositories may be produced in dark

benthic habitats experiencing conditions of anoxia, little bioturbation, and low temperatures (Ellegaard et al., 2020).

In sedDNA studies focusing specifically on fish, the quantification of fish DNA copies in younger core sections compared to older sections may provide a useful measure of the degradation of fish sedDNA through time (Sakata et al., 2022; Thomson-Laing et al. (under review); Lopez et al. (under review)). However, this method may only be valid if the population size remains constant over time and should still be validated with independent catch data.

## 2.4 | Composition of the DNA pool in aquatic sediments

The proportion of fish DNA relative to total sedDNA remains a key uncertainty and is likely to vary across ecosystems. Bacterial and archaeal DNA compose the majority of the sedDNA pool in both surface and deep sediment layers. This is due to their relatively high densities (compared with eukaryotic organisms) in both the water column and sediments, the activity of some prokaryotes in surface

sediments, and the survival of other prokaryotes in low-energy deep sediment layers (Capo et al., 2022). In comparison, eukaryotic DNA and thus fish DNA only account for a small portion of the sedDNA pool. For example, based on the analyses of Swedish lake sediments dated between 14.5 and 9.5 kya, bacterial DNA sequences were dominant (78%–99%), with a relatively low proportion of archaeal (1%–21%) and eukaryotic (1%–22%) DNA sequences (Parducci et al., 2019). Analyses of more recent sediments from three eastern Canadian lakes yielded a dominance of bacterioplankton, microbial eukaryotes, and viruses in sediment metagenomes (Garner et al., 2020). From a sediment sample dated back to the pre-industrial period, less than 1% of DNA sequences could be assigned to eukaryotes, with only 0.0145% of total reads assigned to fish (Monchamp, unpublished data). In a high-altitude tropical lake in Mexico, Moguel et al. (2021) reported a higher proportion of DNA from bacteria (81%), followed by archaea (15%) and eukaryotes (3%) in 12 metagenomes from a 12 kya-long sediment record. Finally, in an analysis of Antarctic deep ocean sediments, Armbrrecht et al. (2022) assigned ~3% of all eukaryotic sequences to Chordata, with ~0.29% being assigned to Actinopterygii (ray-finned fishes). However, when considering the total sedDNA pool, these contributions decreased to ~0.24% and ~0.02% for Chordata and Actinopterygii, respectively (Armbrrecht, personal communication). In coastal marine sediments from eastern Australia, the fish sedDNA fraction was only slightly higher (~1%–2%; Armbrrecht, 2020; Armbrrecht et al., 2021).

Collectively, these recent findings demonstrate that fish sedDNA may only occur in trace amounts in sediment records. However, there are techniques to target and amplify the fish sedDNA signal, such as PCR-based and hybridization capture (or target capture) analyses that show promise for more detailed investigations into fish and other macro-eukaryotes (see Section 3.3).

### 3 | METHODOLOGICAL CONSIDERATIONS FOR FISH SEDDNA DETECTION

#### 3.1 | Sampling design: Effect of sampling location and effort

A summary of the main steps and associated methodological considerations for successful fish sedDNA detection is shown in Figure 2. The type of sediment, water depth, biomass, and biology of target fish taxa are crucial considerations for sampling design. In paleolimnology, sediment cores are typically taken from the deepest part of a lake to ensure that the effects of wave action and other physical disturbances of the sediment record are minimized. An offshore location also provides an integrated portrait of the basin as littoral contributions are redistributed during mixing events (supported by the detection of subfossils of organisms from the littoral zone). This has been shown in microbial diversity studies where a single sediment core at the depositional center of the lake can capture dominant microbial communities (Weisbrod et al., 2020).

However, larger subfossils derived from macrophytes and fish vary substantially in their abundance across study basins (Šolcová et al., 2018; Zhao et al., 2006). Sakata et al. (2021) suggested fish sedDNA was heterogeneously distributed, with samples collected 5 m apart representing different fish communities. Spatial variation in fish sedDNA signals could be related to species-specific habitat preference due to thermal stratification, where cooler areas may be ideal for detecting cold-water fish species, such as trout or whitefish (Klobucar et al., 2017; Lawson Handley et al., 2019; Littlefair et al., 2021). For warm water fishes, sampling in the littoral zone may be ideal, especially to target high concentrations of juveniles that prefer these shallow, more productive zones (Lawson Handley et al., 2019; Valdez-Moreno et al., 2019; Yao et al., 2022). Thomson-Laing et al. (under review) compared the detection of short-finned eel (*Anguilla australis*), and European perch (*Perca fluviatilis*) in sediment cores collected from the depositional center and nearshore in a small lake in New Zealand. Their analysis showed significantly higher detections in samples from the nearshore compared to the depositional center core, which they attribute to the habitat of the target species. However, for time-series studies using sediment cores, littoral zones may not represent high-quality and datable archives of lake dynamics as they are subject to increased terrestrial sedimentation, higher microbial degradation, and potential sediment resuspension through bioturbation or wind-driven mixing (Dearing, 1997). Instead, sampling at intermediate-depth depositional basins may be more suitable to detect these warm water and littoral fish species, though further research is needed to determine this effect. Regardless of sample location, it is still encouraged to collect sediment cores on a settling basin versus slopes, to get accurate interpretations of chronological sequences.

Sediment type will influence the choice of equipment, sample sizes, and number of replicates needed. Coarse sediments on hard substrates are more challenging to sample for sedDNA, whereas soft sediments are easier to sample and yield relatively high amounts of total DNA (Pawlowski et al., 2022). The population biomass of the target taxa determines the ideal size for a sediment sample. While small amounts of sediment (0.25–0.5 g) are adequate for describing microbial diversity (Capo, Giguët-Covex, et al., 2021; Xie et al., 2017), larger amounts are often required to accurately recover the fish sedDNA signal (~10 g, Thomson-Laing et al., 2022), because fish represent smaller total biomass and can have a more heterogeneous distribution. The choice of sampling equipment to collect sediment samples must also consider the type of sediment, the depth of the water column, and the time period targeted. While different coring equipment can be used to preserve the vertical sediment profile, Ekman or Van Veen grabs can be used to collect larger volumes of surface sediments from shallow aquatic bodies (Stoeck et al., 2018). Subsampling of sediment cores or surface sediments makes it possible to obtain more replicates, which is necessary to improve the precision of sedDNA analysis. Based on benthic community studies, between 10 and 20 replicate samples were needed for a reliable assessment of the species to detect 50% macrofauna richness (Lins et al., 2021). Similarly, Hestetun et al. (2021) performed an eDNA

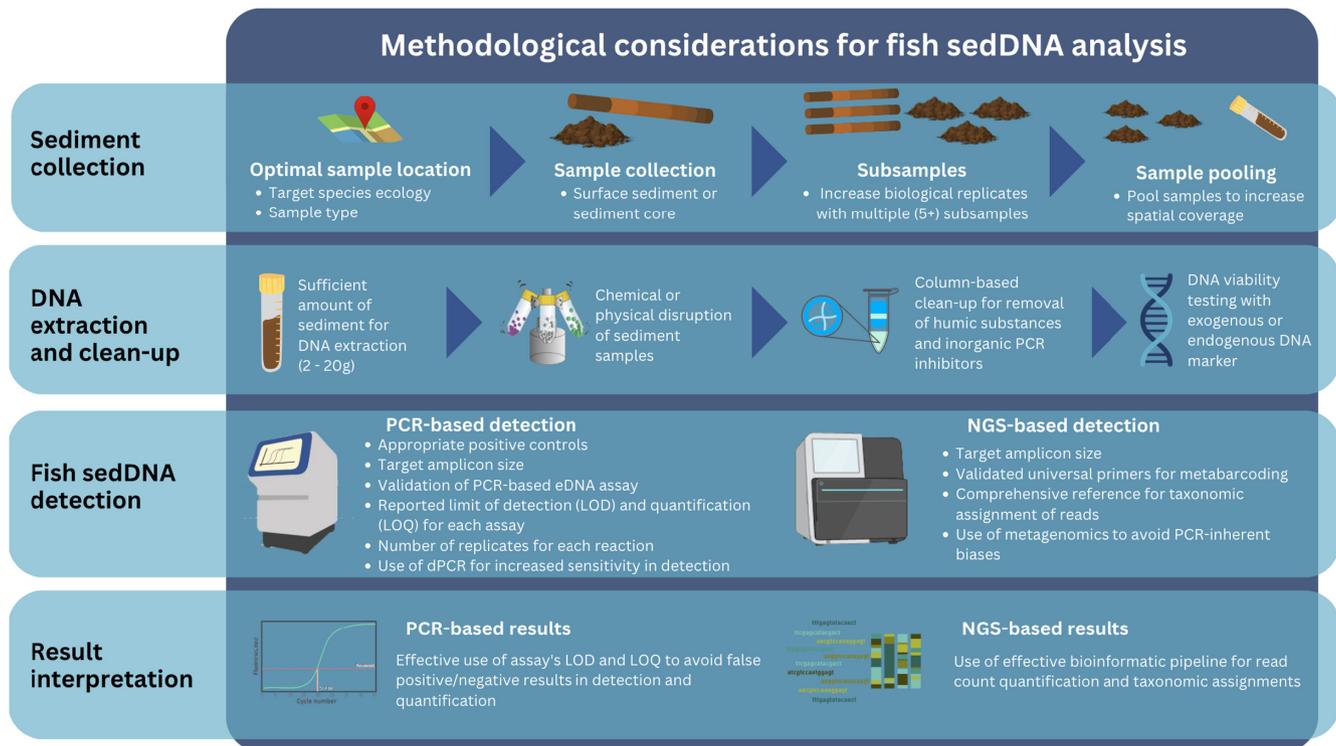


FIGURE 2 Consolidated methodological workflow for fish sedDNA analysis. LOD, limit of detection; LOQ, limit of quantification.

metabarcoding analysis of surface sediments and revealed that five physical subsamples from a Van Veen grab, with five sedDNA extraction replicates from each subsample, roughly doubled the total eukaryotic richness detected. Picard et al. (2023) used occupancy modeling to show that for perch and rudd sedDNA in shallow small lakes, at least six sites and five replicates per site were needed to reliably detect fish sedDNA. To create a small-volume sample that is representative of a larger area and considers fine-scale heterogeneity, the subsamples can be combined and homogenized before being resampled (Hestetun et al., 2021). However, there is an implicit trade-off with this method as precise compositional data analysis methods may require losses in fine-scale spatiotemporal resolution. For successful fish sedDNA detection, high-spatial heterogeneity of targets may require many sediment core replicates.

### 3.2 | DNA extraction, concentration, and purification

Different DNA extraction techniques can be considered for targeting different states of sedDNA (i.e., intra- vs. extracellular; Capo, Giguet-Covex, et al., 2021; Pearman et al., 2021) because chemistry affects how much particle-bound DNA is released into solution. For efficient DNA extraction from sediment particles that are not easily permeated by chemical cell disruptors, Hestetun et al. (2021) showed that moderate, rather than rigorous, physical disruption (e.g., use of beads for homogenization) more efficiently increased the completeness of the microbial community recovered in individual DNA extractions, though it is not clear how this would

relate to fish sedDNA. To increase the likelihood of fish DNA detection, one of the key factors in the DNA extraction procedure is the volume of sediment that needs to be analyzed. Thomson-Laing et al. (2022) found that fish DNA was more likely to be detected in higher-volume sediment samples up to 20g. However, extracellular DNA recovery methods employing commercial kits can only hold ~10g of sediment, and the greater elution volume (2mL) requirement of such kits results in diluted DNA samples as a trade-off. Thus, concentrating DNA with ethanol precipitation (Sakata et al., 2020; Thomson-Laing et al., 2022), paramagnetic beads (e.g., AMPure XP), concentrating columns, or vacuum concentration/freeze-drying should be considered.

In addition, the DNA extraction method must involve the removal of organic and metal compounds (e.g., polyphenolic compounds, humic acids, fulvic acids, tannins, melanin, and traces of heavy metals), known as potential PCR inhibitors, from aquatic sediments (Hermans et al., 2018; Lloyd et al., 2010). Commercial kits for DNA extraction from soils and sediments usually include inhibitor removal for humic substances, among other organic and inorganic materials (such as the PowerSoil DNA isolation kit from Qiagen or the Soil DNA Isolation Maxi Kit from Norgen). Alternatively, column-based clean-up kits (e.g., Zymo Research OneStep PCR Inhibitor Removal Kit) are also available for the efficient removal of contaminants that can inhibit downstream enzymatic reactions.

Endogenous or exogenous DNA spiking can be used to test for the successful extraction and amplification of DNA from sediments, proving that DNA is neither lost nor destroyed in the extraction process. False-negative results can be reduced by using IntegriE-DNA™, which evaluates DNA samples for their capacity to support

amplification from ubiquitous endogenous plant chloroplast DNA as a measure of sample integrity that includes identifying inhibited and/or degraded samples (Hobbs et al., 2019; Veldhoen et al., 2016). As an internal positive control to investigate inhibition, the sedDNA samples can also be spiked with exogenous DNA, such as lambda phage DNA (Sakata et al., 2022; Xu et al., 2009). These steps are considered crucial because older DNA is preserved in the sediment matrix with a high likelihood of PCR inhibitor co-precipitation.

### 3.3 | Comparison of fish sedDNA detection approaches

The following molecular methods have been used to detect fish sedDNA.

#### 3.3.1 | Quantitative PCR (qPCR)

Quantifying gene copies of target fish species from sedDNA has proven to be successful with qPCR-based approaches (Sakata et al., 2020, 2022). Currently, qPCR is the most widely available, cost-effective approach that is also amenable to the development of standards (Langlois et al., 2021; Tsuji et al., 2018). However, there are still challenges with qPCR assays that affect the specificity, sensitivity, and reliability of this approach. Compared to digital PCR, this technology is highly sensitive to inhibitors that are found at high concentrations in sediments. If not removed or mitigated (e.g., diluting DNA template, using column-based clean-up kits, Bovine Serum Albumin, or PCR master mixes that are resilient to inhibition), the presence of inhibitors can lead to false negatives (Albers et al., 2013; Savichtcheva et al., 2011). Since qPCR is deemed less sensitive than more advanced quantitative digital PCR, running samples in multiple replicates is needed to improve and validate fish sedDNA detection (Gagné et al., 2021; Langlois et al., 2021; Lopez et al., *under review*; Matthias et al., 2021). Furthermore, a reliable qPCR-based test should follow the core guidelines of qPCR best practices by reporting performance characteristics like the limit of detection (LOD) and limit of quantification (LOQ) (Abbott et al., 2021; Gagné et al., 2021; Langlois et al., 2021). The LOD measures an assay's capacity to detect the target sequence at low levels. The LOQ, on the other hand, measures the assay's ability to quantify copy numbers within a predefined variance (Klymus et al., 2019; Lesperance et al., 2021). For low-concentration fish eDNA in sediments, reporting the LOD and LOQ enables a more informed interpretation of assay results (Hocking et al., 2022). This is particularly useful for detecting DNA in older sediment samples that are expected to be more degraded and fragmented in form (Golenberg et al., 1996).

#### 3.3.2 | Digital PCR (dPCR)

With dPCR, the PCR reaction is divided into 8000–20,000 separate amplification units (droplets or plate partitions) before amplification,

reducing the normalization and calibration concerns of qPCR (Hindson et al., 2011). Due to its binary nature, dPCR can detect and provide absolute quantification of target markers that are present at low levels, while simultaneously reducing the potential of inhibitors on PCR amplifications (Capo et al., 2019; Capo, Spong, et al., 2021). Comparative standard curves are not needed and are thus not affected by differential amplification efficiencies between samples and standards. Additionally, dPCR is less affected by many of the variabilities associated with qPCR (e.g., Cq values interpolations, amplification delays), allowing for increased precision and consistency between samples (Cao et al., 2016). Digital PCR has shown better performance than qPCR in quantifying fish abundance in waters (Doi et al., 2015), and studies using sediment samples to compare qPCR, dPCR, and/or metabarcoding found that dPCR significantly increased the sensitivity and detection of target genes at low concentrations (Mejbel et al., 2021; Singh et al., 2017; Thomson-Laing et al., 2020).

#### 3.3.3 | DNA metabarcoding (amplicon sequencing)

DNA metabarcoding can provide an inventory of taxa/species present in an environmental sample using a set of universal primers and can be used to evaluate species interactions. This approach has been successful in detecting a broad range of biological groups from environmental samples (Pawlowski et al., 2018; Sakata et al., 2020; Zinger et al., 2019). Most recently, Thomson-Laing et al. (*under review*) successfully detected five fish species in sediment core samples up to 500 years in age. However, other applications to detect fish DNA in surface and subsurface sediments have rarely been successful.

There are many different metabarcoding primer pair sets for fish, yet the amplified taxonomic ranges and the make-up of the fish communities vary significantly among them. Higher fish diversity was typically detected using primers that amplify a portion of the 12S rRNA gene rather than primers for other gene segments such as 16S rRNA, COI, and cytochrome b genes. Different primer sets can have qualitative and quantitative effects on the biodiversity that is identified (i.e., primer bias), and these effects should be taken into account when designing experiments and interpreting the results (Zhang et al., 2020). PCR inhibitors also reduce the likelihood of detecting fish (Capo, Spong, et al., 2021). To minimize this effect, the presence of inhibitors should be quantified and at least one clean-up step should be included. Alternatively, DNA templates can be diluted to reduce the PCR inhibitor concentration (Wang et al., 2017). Moreover, lowering the annealing temperature during the hybridization steps of the PCR reactions can help enhance primer binding (Yang et al., 2021). However, this approach increases non-specific target amplification. For example, DNA metabarcoding of sedDNA using universal fish primers (e.g., tele01, Valentini et al., 2016) and an annealing temperature of 56°C reported dominance of non-target sequences from plants and bacteria (Huang et al., 2021). Overall, DNA metabarcoding can provide data on the taxonomic composition of a target community. However, metabarcoding of sedDNA can be

sensitive to PCR-related errors, pseudogene contamination, primer bias, and universality issues (Lopez et al., 2021). Last, the lack of a comprehensive and accurate reference database needed for taxonomic assignment also limits the data derived from this approach (Keck, Blackman, et al., 2022; Keck, Couton, & Altermatt, 2022).

### 3.3.4 | Metagenomics (shotgun sequencing)

Metagenomics is a promising method for detecting fish sedDNA, particularly when an enrichment step is employed (e.g., Armbrrecht et al., 2021). Metagenomics has three advantages over DNA metabarcoding: (i) DNA fragments found in environmental samples are randomly sequenced, which avoids many of the above-mentioned PCR amplification biases; (ii) ancient DNA originating from fish can be verified by quantifying post-mortem DNA damage (as in Seerholm et al., 2016); (iii) if enough DNA is sequenced, entire mitochondrial genomes can be reconstructed, which substantially increases confidence in recovered fish DNA compared with metabarcoding (Deiner et al., 2017). Another benefit of reconstructed mitochondrial genomes from sedDNA is the ability to design more robust species-targeted qPCR tools (Allison et al., 2023; Chua et al., 2021). Despite its advantages over DNA metabarcoding, the use of metagenomics is currently limited by high costs and by the complexity of bioinformatic pipelines. Furthermore, taxonomic identification should use data across the genome. However, the availability of genomic information outside barcode regions is so far limited. Ongoing broad-scale sequencing efforts, such as the European Genome Atlas or the Vertebrates Genome Project have the potential to fill these gaps, enhancing metagenomic databases and boosting the possibility of genomic approaches in the next future (Theissinger et al., 2023).

Hybridization capture is a technique in metagenomics that relies on the creation of *in silico* RNA baits – short oligonucleotides designed to be complementary to nuclear, mitochondrial, or chloroplast genomes or gene regions of any target species. When mixed with eDNA extracts, baits hybridize with matching DNA fragments, and the resulting complexes are purified, enriched, and sequenced to generate more targeted biological insights. Recent sedDNA investigations have successfully used this approach to retrieve DNA from low-abundant targets such as mammals and plants (Murchie et al., 2021) as well as marine phytoplankton and zooplankton (Armbrrecht et al., 2021). To our knowledge, hybridization capture has never been utilized to identify fish sedDNA; nevertheless, this method is promising for future studies.

## 3.4 | Detection of fish DNA from the water column, surface sediment, and sediment core samples

Based on copy numbers of a specific marker as an indicator of fish DNA concentrations, studies have found fish eDNA to be more concentrated and to persist for longer periods in sediment than in water, with fish, sedDNA detection reported 3–12 months after the removal

of organisms from experimental ponds and tanks (Ogata et al., 2021; Turner et al., 2015). When comparing aqueous eDNA and sedDNA, Sakata et al. (2020) found that surface sediments contained a higher concentration of fish DNA (12.5–1456.9 times) and a lower decay rate (56.45 times) than water samples. Metabarcoding data of fish species reported by Sakata et al. (2020) showed no noticeable variations in species composition between surface sediment and water samples. Brandt et al. (2021) however, displayed contrasting results when comparing community data between water and surface sediment samples, as illustrated by the small number of metazoan taxa (3%–8% of ASVs) found in common between the two types of environmental samples. In preliminary results from lakes in Maine (USA) and Canada, higher concentrations of fish DNA were observed in the water column 1 m above the water–sediment interface, with little to no fish DNA detected in the surface sediment (Huston et al., 2023, unpublished; Myler, 2023). These differences might be related to variations in sediment input, DNA inhibitors, and fish phenology across lakes. Comparisons between the genetic signals from water and sediment core samples showed the reliability of sedDNA to detect many, but not all, planktonic organisms (Capo et al., 2015; Gauthier et al., 2021; Nwosu et al., 2021; Zhu et al., 2023). For instance, *Planktothrix* – filamentous cyanobacteria – was not well represented in sediment traps and surface sediments despite being present in high proportions at 10 m water depth (Nwosu et al., 2021).

Currently, there is a lack of data comparing the detection of fish DNA present in surface sediments with DNA from deeper core samples, which is often degraded into shorter fragments as a result of abiotic degradation processes (i.e., ancient DNA). The topmost section of the core (generally <50 cm depth and ~<200 years old) tend to contain more fish DNA copies than older sediment sections based on sediment cores analyzed to date (Sakata et al., 2022; Thomson-Laing et al., under review). Surface sediments likely contain more intact DNA molecules, which would facilitate a higher likelihood of fish DNA detection in these sediments. The higher likelihood of fish sedDNA detection may also be addressed by collecting more surface sediment samples within any one lake. However, the full potential of sedDNA is unlocked by incorporating the temporal component of down-core sediments to reconstruct changes in past aquatic fauna.

## 3.5 | Inferring fish abundance from sedDNA

The relationship between eDNA concentration and fish species abundance is anticipated to differ greatly between taxa and ecological context, making it challenging to demonstrate a link between fish sedDNA and abundance (Rourke et al., 2022; Spear et al., 2021). To advance our understanding of the relationship between sedDNA signals and fish abundance, it is necessary to take into consideration extrinsic factors affecting eDNA production in the water column and preservation in sedimentary archives, including climatic and hydrologic conditions. Accounting for the biotic degradation of sedDNA in surface sediments, and the abiotic degradation over time, may improve estimates of sedDNA concentration and its potential use to evaluate the past

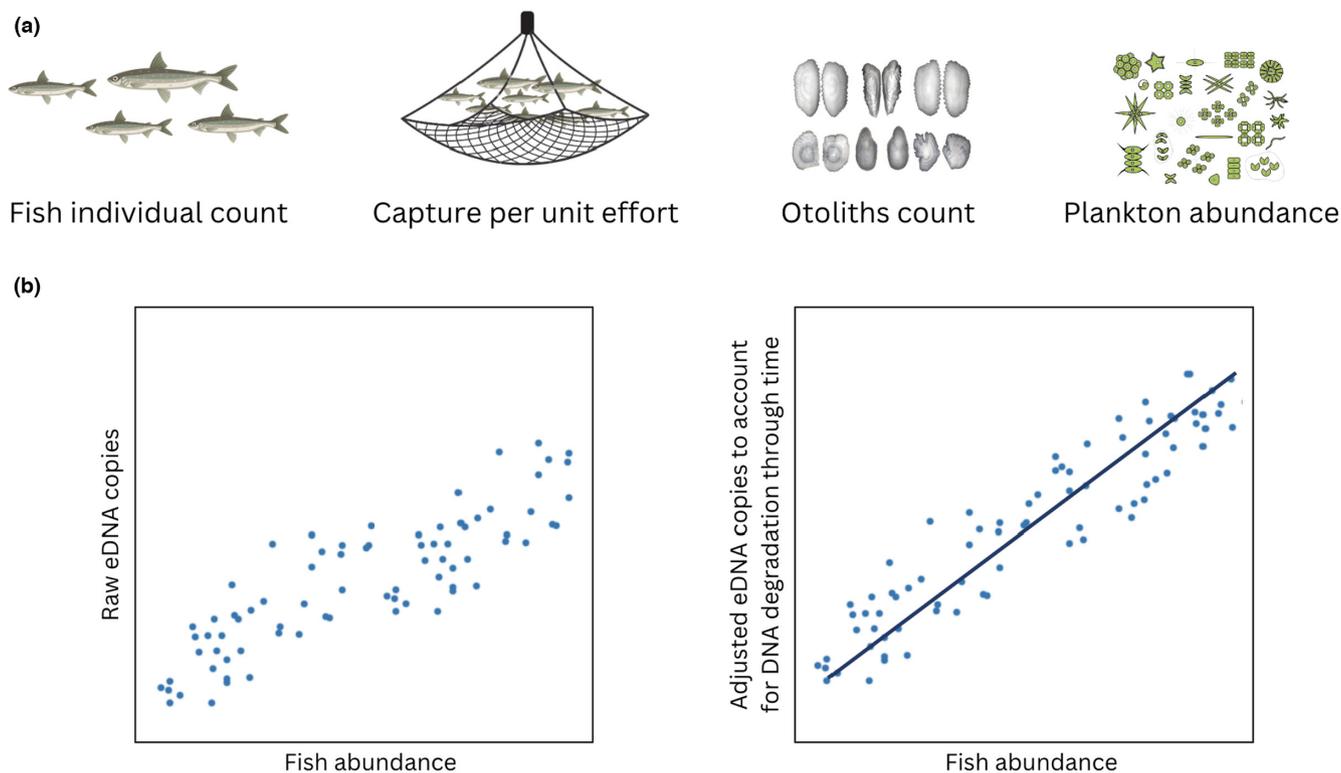
abundance of specific taxa. For instance, with the use of first-order kinetics for plant pigment degradation, it was possible to estimate the rate of degradation of total chlorophyll-*a* and its derivatives before and after burial in sediment (Tsugeki et al., 2017). These degradation rate values can be used as a “normalization coefficient” to account for biotic and abiotic factors affecting sedDNA concentration (e.g., quantitative PCR approaches) and percentage of reads (e.g., metabarcoding) (Sakata et al., 2022; Tsugeki et al., 2022). Ideally, available historical abundance data would facilitate correctly relating fish sedDNA concentrations and biomass, as catch data may not accurately reflect ecosystem abundances. Sakata et al. (2022) found a significant correlation between the capture per unit effort statistics of their target fish species in Lake Biwa and adjusted sedDNA concentration (Figure 3, accounting for DNA degradation). The use of otolith counts present in sediments as a proxy for abundance, as done by Lin et al. (2019), could also be investigated to confirm the connection with quantifiable copies of fish DNA in sedimentary environments. Likewise, one could normalize the fish sedDNA copies using estimated sedimentation rates (measured as mm/year).

#### 4 | TROUBLESHOOTING FISH SEDDNA WORKFLOWS

In terms of basic workflow, extensive effort must be exerted to validate different sampling locations, sample sizes, and necessary

replication levels to improve the ecological relevance of generated data, while also considering the temporal and spatial variations of target fish taxa (Pawlowski et al., 2022). A consolidated troubleshooting workflow for no and/or low yields of fish sedDNA is shown in Figure 4. Collecting multiple sediment cores, across different water depths and habitat types may increase the probability of detecting fish sedDNA. Similarly, larger extraction volumes of up to 20g of wet sediment can increase detection likelihood (Thomson-Laing et al., 2022). When using larger volumes, alternative or additional extractions methods (i.e., non-commercial alkaline extractions), are recommended to help unbind DNA from sediments or remove PCR inhibitors (Thomson-Laing et al., 2022). DNA concentration post-extraction should also be considered, especially when higher elution volumes are required (e.g., DNeasy PowerMax Soil Kit, Qiagen). A consolidated list of fish sedimentary DNA extraction, concentration, and cleanup protocols can be accessed through the links listed in Table S1. This list was generated from the successful and unsuccessful studies reported in Table 1, with each protocol explicitly stating whether it was successful in extracting fish sedDNA at sufficient concentrations.

Increasing the amount of extracted/concentrated DNA template used in each PCR reaction can help improve fish sedDNA detectability. In addition, the use of internal positive controls (e.g., exogenous spiked DNA) to detect inhibitors, or endogenous DNA (e.g., amplification of endogenous plant chloroplast DNA with IntegritE-DNA™) to detect inhibitors and/or sample degradation, is recommended to identify potential false negatives. If needed, a clean-up step should



**FIGURE 3** Correlating fish abundance and fish sedimentary DNA (sedDNA) concentration: (a) fish abundance and other proxy data; and (b) correlating fish abundance with raw environmental DNA (eDNA) copies and adjusted values after normalizing with coefficient to consider biotic and abiotic factors affecting DNA degradation through time.

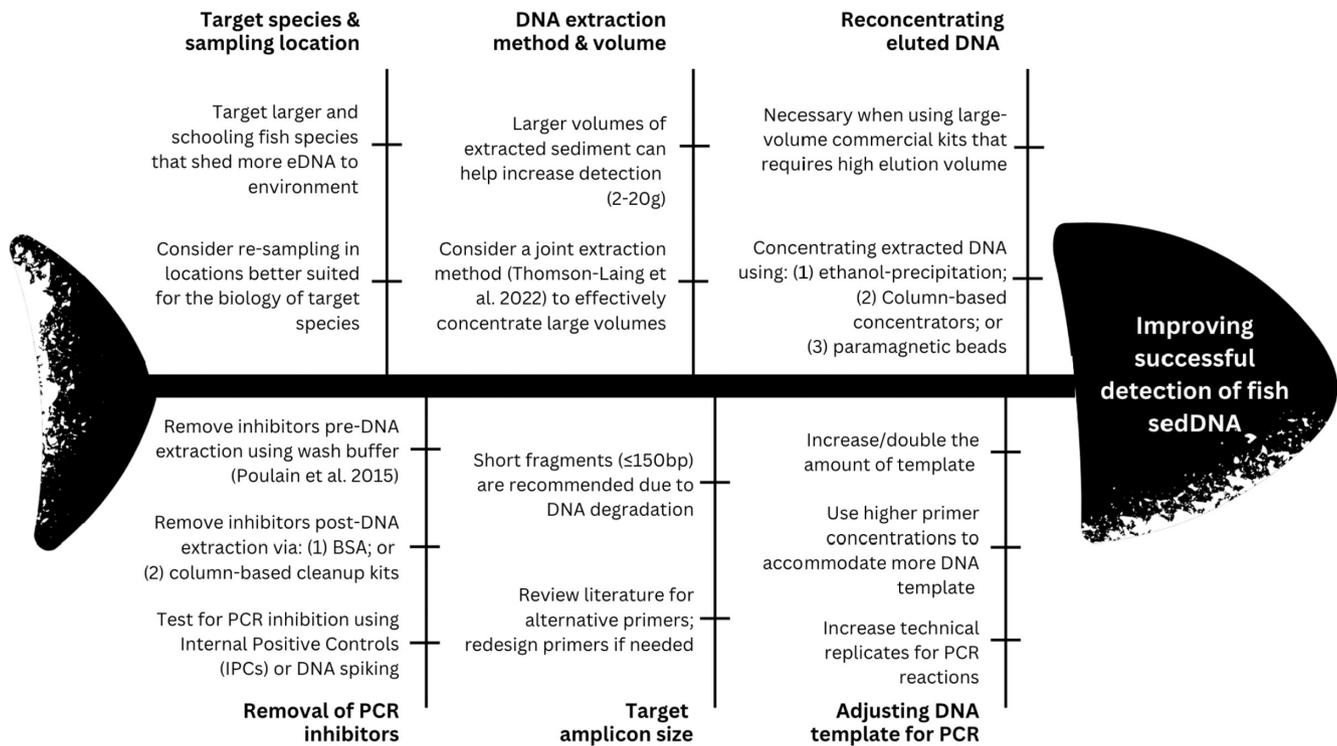


FIGURE 4 Troubleshooting steps to enhance fish sedimentary DNA yield and detection.

be included in the workflow to remove humic substances and other inorganic PCR inhibitors, followed by re-testing for sample integrity.

For detection, primer and probe design may be improved by targeting shorter amplicon sizes, due to the fragmented nature of preserved DNA in older sediment sections. This, however, can result in reduced taxonomic resolution; therefore, it is important to carefully design PCR-based eDNA assays to achieve high specificity with target taxa, e.g., use of entire mitochondrial regions to identify unique sequences (Allison et al., 2023), and should be accompanied by well-characterized sensitivity (LOD and LOQ) values (Klymus et al., 2019; Lesperance et al., 2021). Even with such cautions, testing of known negative site controls may be especially useful in confirming specificity beyond initial *in silico* and lab testing. Given the relative rarity of fish sedDNA, employing multiple redundant qPCR or dPCR assays targeting high-copy gene regions (e.g., mitochondrial) could further enhance detection and quantification.

DNA metabarcoding can provide high-resolution data on the taxonomic composition of the target community, however, it can be highly susceptible to PCR-related errors, pseudogene contamination, and non-target amplification issues. PCR-inherent biases can be addressed to some extent by increasing sequencing depth, using multiple markers in combination, and using appropriate bioinformatic pipelines for accurate taxonomic assignment to avoid false positive detection. Metagenomics also appears to be a good alternative for detecting trace amounts of fish DNA in sedimentary archives and should continue to be explored. It is strongly recommended to use advanced next-generation sequencing methods that enable better sequencing coverage (such as Illumina NextSeq or NovaSeq) to produce informative data about fish community structure in sedDNA

samples (Tringe & Rubin, 2005). Moreover, employing third-generation sequencing technologies (e.g., PacBio and Nanopore sequencing) can produce longer sequence reads, which can help ease the difficulties of assembling sequences (Marx, 2021). Combining short- and long-strand sequencing of environmental metagenomes may also be an alternative, allowing thorough investigation of fragmented and thus poorly preserved DNA from the sediments, but can be costly and time-consuming (Pedersen et al., 2016). Importantly, all these methods are dependent upon adequate DNA sequence resources and reference databases (Monchamp et al., 2023). Overall, further research is required to meet ecological and methodological challenges, but in the meantime, it is essential to consolidate best practices from existing workflows to enable higher success in detecting fish DNA from sediment samples.

## 5 | FUTURE DIRECTIONS

In the coming years, we expect major improvements in various aspects of fish sedDNA sampling design, extraction and purification techniques, detection methods, and data analysis and interpretation. Specifically, we anticipate the following advancements in the field of fish sedDNA: (1) development in sequencing platforms and emerging detection approaches (e.g., hybridization-capture, RPA-CRISPR; Williams et al., 2023; Williams et al., 2021; Williams et al., 2019) to reduce costs while improving limits of detection; (2) development of controlled experiments to validate, better understand, and calibrate the taphonomic processes behind fish sedDNA; (3) collaborations with geochemists and other researchers to better characterize the

binding properties of DNA with complex and variable sediment matrices; (4) improvement of methods to concentrate and detect rare or degraded fish sedDNA without co-concentrating PCR-inhibitors; (5) expansion of efforts to improve the accuracy and augment fish DNA reference sequence database resources; (6) integration of fish sedDNA studies with indigenous and local ecological knowledge; (7) expansion of fish sedDNA studies to more diverse regions and communities to help identify the best methods for particular settings and contexts; (8) development of analytical approaches to better statistically account for incomplete detection and time scale biases; and (9) standardization of sampling, metadata processing, and reporting to increase the confidence and repeatability of fish sedDNA-inferred biological data.

Knowledge of fish sedDNA dynamics in natural ecosystems is in its infancy. The present review lays the groundwork for the successful integration of fish sedDNA in ecological and conservation studies by providing an overview of the critical ecological and methodological factors affecting fish sedDNA analysis. By consolidating both the successful and unsuccessful experiences of experts, we identified current challenges that can be addressed in future fish sedDNA studies. In this review, we highlight troubleshooting steps that can be used in confronting difficulties that can be encountered within the fish sedDNA workflow. In doing so, we hope to advance the use of sedDNA as a powerful tool in providing baseline data in environmental monitoring programs and in reconstructing past and current dynamics of fish fauna in aquatic systems.

#### AUTHOR CONTRIBUTIONS

GPH and MLL contributed equally as the co-first authors of this work. GPH, MLL, and EC are responsible for model concepts, data acquisition and analysis, and manuscript preparation. GPH, MLL, YC, LK, and LCD compiled and generated fish sedDNA protocols, and YC generated Table 1. GPH, MLL, and EC wrote the first draft of the manuscript and all the other authors contributed to the drafting and editing of the manuscript and gave their final approval for publication.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

#### DATA AVAILABILITY STATEMENT

Raw data are available upon request to the corresponding author.

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