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ORIGINAL ARTICLE

Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA

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Abstract

Aqueous environmental DNA (eDNA) analysis has been applied to the monitoring of various ecosystems and taxa, and the characteristics of aqueous eDNA have been previously studied. In contrast, although sedimentary eDNA has been used to restore past information, the characteristics of sedimentary eDNA are not well understood. In this study, we compared the properties of sedimentary and aqueous eDNA of macro-organisms. First, to clarify the preservation ability of sediments, we compared the difference in decay rates between aqueous and sedimentary eDNA using samples collected from a biotope (an artificial pond prepared with concrete). Next, to clarify the biological information retained in sedimentary eDNA both qualitatively and quantitatively, we compared eDNA concentrations between sediment and water samples collected simultaneously from a lake, and the fish species detected by eDNA metabarcoding were also compared. The results demonstrated the following: (a) the decay rate (decreased eDNA copy number divided by the initial eDNA copy number per unit time) of sedimentary eDNA (0.00033 ± 0.000049 [mean ± SE]/hr) was lower than that of aqueous eDNA (0.01863 ± 0.0011/hr); (b) sedimentary eDNA concentration of the mitochondrial marker of three fish species was higher than aqueous eDNA concentration for the same sample weight (12.5–1,456.9 times); and (c) the species composition obtained by metabarcoding was not significantly different between sediment and water; however, considering the lower decay rate of sedimentary eDNA, using both sample types may provide more comprehensive information of species distribution. Thus, sedimentary eDNA analysis will expand future biomonitoring and ecological studies by providing a difference in timescale.

KEYWORDS
decay rate, environmental DNA, MiFish metabarcoding, real-time PCR, sediment, sedimentary eDNA

1 | INTRODUCTION

In recent years, environmental DNA (eDNA) analysis of macro-organisms has been applied to monitoring of various ecosystems and taxa because it enables large-area or multipoint surveys to be conducted inexpensively and noninvasively (Ficetola, Miaud, Pompanon, & Taberlet, 2008; Jerde, Mahon, Chadderton, & Lodge, 2011; Minamoto, Yamanaka, Takahara, Honjo, & Kawabata, 2012). In eDNA analysis, DNA molecules released into the surrounding environment from organisms are collected and analyzed. In addition to estimating presence or absence of target species, successful applications for estimating biomass or abundance have been reported, although most of them are performed in controlled environments using real-time PCR or droplet digital PCR (Doi et al., 2015; Goldberg, Sepulveda, Ray, Baumgardt, & Waits, 2013; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). Furthermore, eDNA metabarcoding assays that comprehensively detect organisms belonging to a certain taxonomic group, such as fishes, by high throughput sequencing (HTS) with universal primers have been developed (e.g., Miya et al., 2015), with various HTS pipelines being used to analyze metabarcoding data (see, e.g., Bálint et al., 2016; Taberlet, Bonin, Coissac, & Zinger, 2018). This eDNA metabarcoding technique has already been applied in various environments, for example, marine (Port et al., 2016; Yamamoto et al., 2017), lotic (Nakagawa et al., 2018; Shaw et al., 2016), and lentic freshwater environments (Hänfling et al., 2016; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). Furthermore, basic properties of eDNA, such as release and decay rates and transportation distance in natural water bodies, have also been investigated (Barnes & Turner, 2016; Jane et al., 2015; Jo et al., 2017; Murakami et al., 2019).

Most eDNA studies on macro-organisms analyzed eDNA in water samples (Goldberg, Strickler, & Fremier, 2018; Ishige et al., 2017; Minamoto et al., 2012; Miya et al., 2015; Ushio et al., 2018; Valentini et al., 2016); however, several recent studies have targeted eDNA in underwater sediments (Buxton, Groombridge, & Griffiths, 2018; Shaw et al., 2016; Turner, Uy, & Everhart, 2015; Wei, Nakajima, & Tobino, 2018). To date, comparison of the basic properties between sample types (i.e., water/sediment) has been performed only for fish eDNA. According to Turner et al. (2015), fish eDNA derived from sediment samples contains a higher copy number per unit weight than that derived from water samples in both experimental ponds and a natural river. For micro-organisms, underwater sediments accumulate information of the surrounding ecosystem. It is considered that some of aqueous eDNA-bearing particles, such as feces, are too large to remain suspended in water (Maggi, 2013) and consequently precipitate onto the sediments (Turner et al., 2014). Such precipitation of aqueous eDNA-bearing particles forms a part of the mechanism of sedimentary eDNA accumulation. It has been reported that animal feces contain viable epithelial cells (10^{-1}–10^{6} cells/g) and large amounts of mtDNA (10^{-1}–10^{7} copies/g) (reviewed in Caldwell, Payment, & Villemur, 2011) and that feces from aquatic macrofauna rapidly sinks (Robison & Bailey, 1981; Wotton & Malmqvist, 2001). Additionally, several studies have reported that sediments reduce biologically driven DNA decay by adsorbing both DNases and DNA

molecules and in low-oxygen environment such as deeper sediment (Levy-Booth et al., 2007; Pietramellara et al., 2009). This delay in DNA decay caused by the binding of DNA and DNases to sediment particles would also be observed in shallow sediments (Shogren et al., 2017). Furthermore, in aquatic sediments, it was reported that the chemical DNA decay rate appeared to be low (Corinaldesi, Barucca, Luna, & Dell'anno, 2011), and Ogram, Mathot, Harsh, Boyle, and Pettigrew (1994) reported that the decay of DNA absorbed to soil particles was minimal. In fact, Turner et al. (2015) discovered that the detectable period of sedimentary eDNA was longer than that of aqueous eDNA. Moreover, it has been reported that virus DNA infecting common carp showed higher concentration in sediment than in the water column (Honjo, Minamoto, & Kawabata, 2012), and that fish eDNA did not rise to the surface in the water column (Kamoroff & Goldberg, 2018). Another study reported that the concentration of bigheaded carp eDNA was higher per g of sediment than per ml of water (8- to 1846-fold) (Turner et al., 2015). Therefore, it is expected that sedimentary eDNA concentration will be higher than aqueous eDNA concentration, and it can be hypothesized that the difference in concentration is caused by differences in decay rates. However, a direct comparison of decay rates between sedimentary and aqueous eDNA in controlled experimental conditions and the subsequent comparison of information contained in sedimentary and aqueous eDNA in a natural lentic environment have not been reported. Although it has been reported that eDNA in biofilms (part of the surface sedimentation) decays to nondetectable levels within 2 days (Seymour et al., 2018), knowledge on the persistence of sedimentary eDNA is limited. If the decay rate varies between sedimentary eDNA and aqueous eDNA, it may follow that information on the biota obtained from both types of samples would be qualitatively different.

In a previous study that compared the detected fish species between sedimentary and aqueous eDNA, eDNA metabarcoding for fish was performed using 1 L water samples and 0.25 g sedimentary samples from rivers (Shaw et al., 2016). The species detected from sedimentary eDNA were fully encompassed within those from aqueous eDNA. In contrast, a different study reported that the fish species detected in 10 g of sediment only partially overlapped with those from 0.9 L water samples (Siegenthaler et al., 2018). Therefore, in simple comparisons between sediment and water, such as the present study, it may be necessary to consider the weight of the sediment sample in order to test the qualitative difference between sediment and 1 L of water. In addition, it is reported that eDNA retention is influenced by sediment substrate (Shogren et al., 2017) and bacterial abundance (Tsuji, Ushio, Sakurai, Minamoto, & Yamanaka, 2017). Shogren et al. (2017) showed that the dynamics of aqueous eDNA, such as transport, retention, and resuspension, were influenced by sediment substrates (in particular, particle size). Therefore, the dynamics of sedimentary eDNA may be influenced by sediment substrates because it is assumed that DNA adsorption is affected by changes in surface area with particle size.

In recent years, studies that use ancient DNA from sediment cores have increased (Parducci et al., 2017). Much of that research has been targeted to species for which the body remains in the sample,

such as bacteria (Domaizon et al., 2013), other microbes (Hou et al., 2014), and plants (Pansu et al., 2015), or species for which part of the body, such as bones, are preserved (Wooller, Gaglioti, Fulton, Lopez, & Shapiro, 2015). More recently, studies have been developed to detect macro-organisms using extracellular DNA remaining in sediments (i.e., sedimentary eDNA), for example, for fish (Nelson-Chorney et al., 2019; Stager, Sporn, Johnson, & Regalado, 2015). These studies showed that fish sedimentary eDNA can be detected in sediments from ~140 years ago. As such, revealing past information by analyzing the extracellular DNA of macro-organisms in sediments has been realized. The abovementioned studies on fish are important not only for ecology but also for the fishery industry because they could enable predictions of future trends by observing the past. However, although it is known that DNA molecules remain for a long time in low-oxygen environments, such as deeper sediments as mentioned above, there is little information on the decay rate of fish sedimentary eDNA on the surface before deposition in the anoxic deep layer. As basic information of sedimentary eDNA is lacking, we measured the decay rate of sedimentary eDNA and compared with that of aqueous eDNA. Our findings regarding decay rates will provide a part of mechanism on holding long time of sedimentary eDNA.

In this study, to elucidate the characteristics of sedimentary eDNA compared with aqueous eDNA, we first compared the decay rates of sedimentary and aqueous eDNA under the condition of a random sediment substrate type. Next, we collected paired sediment and water samples from a natural lake and compared fish eDNA concentration between sample types. Finally, the fish species detected by eDNA metabarcoding were compared. Our study partially clarified the characteristics of sedimentary eDNA. Specifically, we clarified quantitatively that detection was possible over a longer period of time when the decay rate of sedimentary eDNA was slower and found that sedimentary eDNA works in a complimentary manner with aqueous eDNA in biomonitoring.

2 | MATERIALS AND METHODS

2.1 | Comparison of decay rates between sediment and aqueous eDNA

2.1.1 | eDNA sampling

Sediments and water were sampled from a biotope (an artificial pond made of concrete with a volume of approximately 4,000 L) in the Tsurukabuto Second Campus of Kobe University, Japan (34.734°N, 135.234°E), to compare the decay rates of eDNA in the water and sediment samples. Two fish species (*Hemigrammocypris rasborella* and *Oryzias latipes*) inhabited the biotope, and half of the water area in the biotope was covered with emergent plant species. The sediment in biotope mainly consists of organic matter and mud. Nine bulk sedimentary samples of approximately 45 g each were collected from surface sediments in 50-ml tubes (includes random particle size), and after thoroughly stirring, 3 g

was transferred into each of nine 15-ml tubes per one bulk sample (81 sediment samples in total; Figure 1). Nine water samples were collected using 5-L plastic tanks (sample series ID: 1–9; Figure 1) and mixed well, and then, 250 ml of the sample was transferred into each of nine 250-ml bottles per one bulk sample (81 water samples in total; Figure 1). In addition, reverse osmosis membrane water (5 L) was divided into nine subsamples to serve as negative controls (NCs). To test for decay and avoid any large temperature fluctuations, all samples and controls were retained for different time periods (0.5 day (12 hr), 1 day (24 hr), 2 days (48 hr), 3 days (72 hr), 7 days (168 hr), 14 days (336 hr), 21 days (504 hr), and 28 days (672 hr)) in boxes prior to filtration for a maximum of 28 days (672 hr). Furthermore, to monitor only the water temperature fluctuation, a 250-ml bottle with 250 ml of water containing a temperature logger (HOBO pendant logger, HOBO) was placed in each box. Temperature range was 15–19.6°C except for the first 48 hr before stabilizing the water temperature (Figure S1). One of the nine water samples per single bulk sample (i.e., 250 ml of water) was filtered with a glass fiber filter with nominal pore size of 0.7 μm (GF/F; GE Healthcare Life Science) immediately after the sampling, and this was defined as the Time 0 sample. Subsequently, filtration was performed at 12, 24, 48, 72, 168, 336, 504, and 672 hr. Filtration of the control series was performed at the same times to evaluate any possible DNA contamination during filtration. Sediment samples were stored frozen at −25°C at the same timing as filtration. To prevent cross-contamination among samples, all tools used were decontaminated with chlorine bleach (0.1% effective chlorine concentration).

2.1.2 | eDNA extraction

eDNA on the filters (i.e., eDNA from water samples) was extracted using the Salivette (Sarstedt) and DNeasy Blood & Tissue Kit (Qiagen,) methods and stored at −25°C according to the methods described by Minamoto, Hayami, Sakata, and Imamura (2019). DNA was extracted from sediment samples by combining alkaline DNA extraction (Kouduka et al., 2012) with ethanol precipitation and a fecal-soil DNA extraction kit (PowerSoil DNA Isolation Kit, MO Bio Laboratories). Briefly, 6 ml of 0.33 M sodium hydroxide solution and 3 ml of Tris-EDTA buffer (pH 6.7) were added to the sediment sample, and it was thoroughly mixed by vortex and then incubated at 94°C for 50 min. The sample was cooled down to room temperature and centrifuged at $5,000 \times g$ for 30 s, and then, 7.5 ml of supernatant was transferred to a new 50-ml tube and neutralized with the same volume of Tris-HCl (1 M, pH 6.7). Next, 1.5 ml of 3 M sodium acetate solution (pH 5.2) and 30 ml of absolute ethanol were added to the mixture and placed in a freezer (−25°C) for more than 1 hr. Cooled samples were centrifuged at $5,350 \, g$ for 20 min, and the supernatant was discarded. The pellet was transferred to a Power Bead Tube (PowerSoil DNA Isolation Kit). To retrieve any residual DNA, the remaining precipitate in the 50-ml tube was dissolved with 100 μl of ultrapure water and
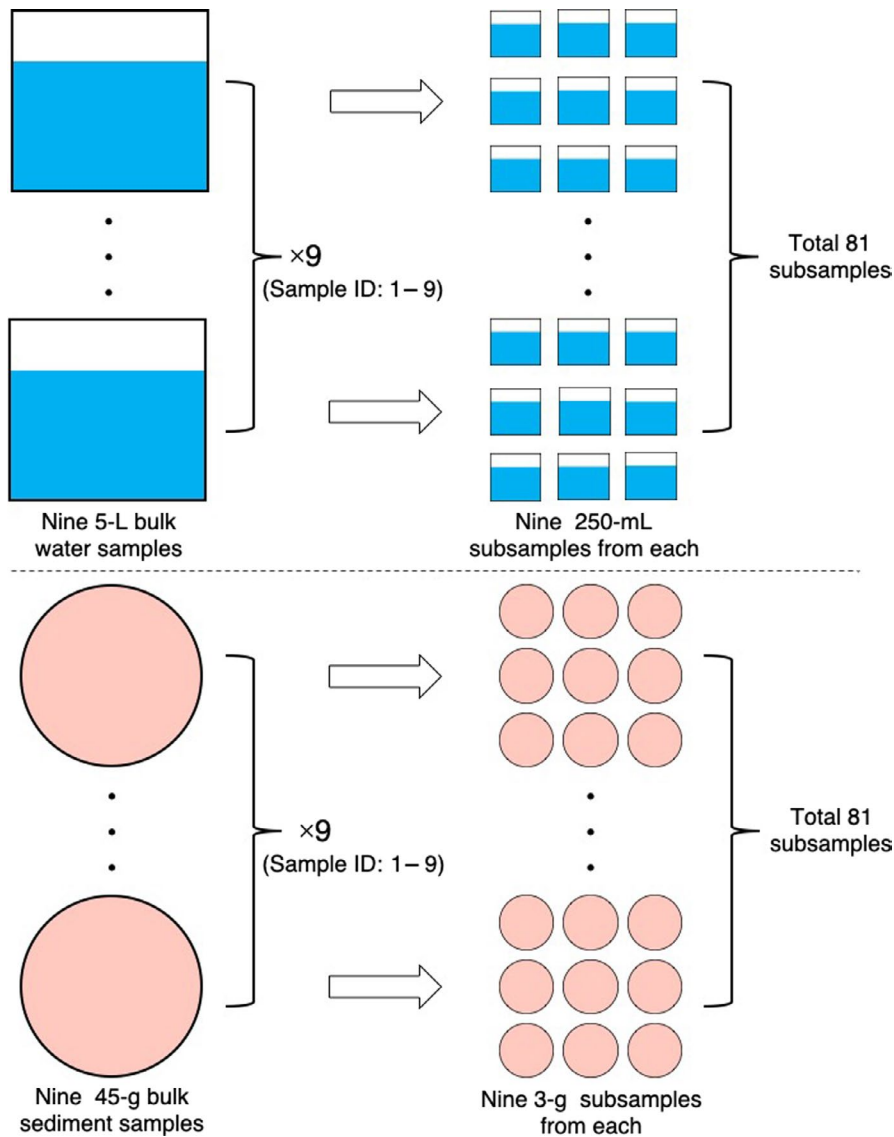
FIGURE 1 Workflow of sampling and subsampling

transferred to the same Power Bead Tube. Subsequently, DNA extraction was performed according to the "Experienced User Protocol 3 to 22" of the PowerSoil DNA Isolation Kit. eDNA extraction was performed in a separate room from PCR operations to prevent contamination.

2.1.3 | Real-time quantitative PCR (qPCR)

The amount of eDNA of *H. rasborella* was quantified by TaqMan real-time quantitative PCR (qPCR) targeting the cytochrome *b* region of *H. rasborella* using previously developed primers and a probe (Fukuoka, Takahara, Matsumoto, Ushimaru, & Minamoto, 2016; Table 1), and the specificity of the primers/probe set was confirmed by Fukuoka et al. (2016) through specificity tests using DNA of closely related species. Real-time qPCRs were carried out in triplicate using extracted eDNA from each sample as template. Each reaction (20 µl final volume) contained 900 nM primers and 125 nM TaqMan probe in 1× TaqMan Gene Expression Master Mix

(Life Technologies) and 2 µl eDNA. The real-time PCR conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 55 cycles of 15 s at 95°C and 60 s at 60°C. To obtain calibration curves, a dilution series of standards (3×10^1–3×10^4 copies in each reaction) were simultaneously quantified: The standard was linearized plasmids that contained synthesized artificial DNA fragments of the target cytochrome *b* gene sequence of *H. rasborella*. Ultrapure water was used instead of DNA in three reaction mixtures as nontemplate negative controls.

2.2 | Field surveys

2.2.1 | eDNA sampling and extraction

On 15 July 2015, we sampled water and sediments at four points at the Lake Iba shore (Figure 2), one of the lakes adjacent to Lake Biwa, Shiga Prefecture, Japan (surface: 0.5 km², average depth: 1.5 m; Figure 2; Table S1). Approximately 45 g of sediment, scooped

TABLE 1 Primers and probes used in qPCR experiments

Target species	Name of primer/ probe	Sequences (5′–>3′)	Reference
Hemigrammocypris rasborella	Hra-CyB-F	CACCCCAGCAAACCCCTTA	Fukuoka et al. (2016)
	Hra-CyB-R	ACTAGAATAGAGAACAGTAACGCGAGAA	Fukuoka et al. (2016)
	Hra-CyB-P	FAM - CCTGTTCGCTTACGCCATTCTACGATCA - TAMRA	Fukuoka et al. (2016)
Cyprinus carpio	CpCyB_496F	GGTGGGTTCTCAGTAGACAATGC	Takahara et al. (2012)
	CpCyB_573R	GGCGGCAATAACAAATGGTAGT	Takahara et al. (2012)
	CpCyB_550p probe	FAM - CACTAACACGATTCTTCGCATTCCACTTCC - TAMRA	Takahara et al. (2012)
Lepomis macrochirus	Bluegill_CytB_F	GCCTAGCAACCCAGATTTTAACA	Takahara, Minamoto, and Doi (2013)
	Bluegill_CytB_R	ACGTCCCGGCAGATGTGT	Takahara et al. (2013)
	Bluegill_CytB_probe	FAM - CGACATCGCAACTGCCTTCTCTTCAGT - TAMRA	Takahara et al. (2013)
Micropterus salmoides	LMB-F	GCCCACATTTGTCGTGATGTAA	Yamanaka et al. (2016)
	LMB-R	AGCCCCGGCCGATATG	Yamanaka et al. (2016)
	LMB-P	FAM - CTAACGGTGCATCCTTCTTTTTCATCTGCA - TAMRA	Yamanaka et al. (2016)

from the surface of the lake bottom, and 1 L of surface water were collected at these points (lake shore). Sediment and water samples were transported to the laboratory on ice, and water samples were immediately filtered at the laboratory. We used two glass fiber filters with nominal pore size of 0.7 μm (GF/F) to filter a 1-L water sample because filters sometimes clogged, and it was impossible to process 1 L through a single filter. Two filters were pooled into a single tube and preserved at −25°C. Sediment samples were stored frozen at −25°C until DNA extraction. eDNA was extracted by the methods described in "eDNA extraction" section and stored at −25°C. All tools used were decontaminated with chlorine bleach (> 0.1% effective chlorine concentration).

2.3 | Comparison of eDNA concentration between sediment and water samples by qPCR

To compare eDNA concentration between sediment and water samples, qPCRs were performed targeting the cytochrome *b* region of the common carp (*Cyprinus carpio*), bluegill sunfish (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*), using previously developed primers and probes (Table 1). All qPCRs were performed under the conditions described above ("Real-time quantitative PCR" section) except for primers and probes. Further, to test PCR inhibition, each qPCR solution was spiked with 2,000 copies of lambda phage DNA as an internal positive control (IPC). This test was carried out using Lambda-7184F (5′-TTCTCTGTGGAGGAGTCCATGAC-3′) and Lambda-7267R (5′-GCTGACATCACGGTTCAGTTGT-3′) primers, and a TaqMan probe lambda-7210P (5′-FAM-AGATGAACTGATTGCCCGTCTCCGCT-TAMRA-3′) (Honjo et al., 2010). The results of this inhibition test showed no inhibition for any sample (ΔCt (Ct sample − Ct positive control) < 0.93; corresponding to a difference of <1.91 times between estimated eDNA copy numbers) because ΔCt values larger than 3 are considered as

inhibition because a shift of 3.3 cycles is equivalent to one log10 difference in general (Hartman, Coyne, & Norwood, 2005).

2.4 | Detection of fish species by eDNA metabarcoding

To compare the fish-faunal information retained in eDNA between the sediment and water samples, eDNA metabarcoding was performed using a universal primer set for fish species (MiFish-U; Miya et al., 2015). The first-round PCR (1st PCR) was performed in a total reaction volume of 12 μl, containing 6.0 μl 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems), 3.6 pmol each of MiFish-U primers, 1 μl eDNA template, and ultrapure water. The thermal cycle profile was 95°C for 3 min; 40 cycles at 98°C for 20 s, 65°C for 15 s, and 72°C for 15 s; and 72°C for 5 min. Eight technical replicates were amplified per sample. Ultrapure water was used instead of eDNA in eight reaction mixtures (nontemplate negative controls). As positive controls, eight replicates using a breeding water sample of *Trachurus japonicus*, which is a marine fish and never occurs in our study field, were used instead of eDNA to examine possible contamination.

After eight technical replicates were pooled into a single tube, we removed unreacted reagents and primer dimers from the 1st PCR products with the SPRIselect Reagent Kit (Beckman Coulter) according to manufacturer's instructions. An equal volume of SPRIselect was added to the 1st PCR products (96 μl) for each sample. Then, the DNA concentration of each sample was quantified using a Qubit dsDNA HS assay kit and a Qubit fluorometer 3.0 (Thermo Fisher Scientific). After the measurement, we calculated the required dilution ratio to give 0.1 ng/μl for each sample other than the negative control. All samples were diluted using an average dilution ratio, and the diluted samples were used as DNA template for the second-round PCR (2nd PCR).

The 2nd PCR was performed to add MiSeq adaptor sequences and 8-bp index sequences to both ends of the amplicons. The total
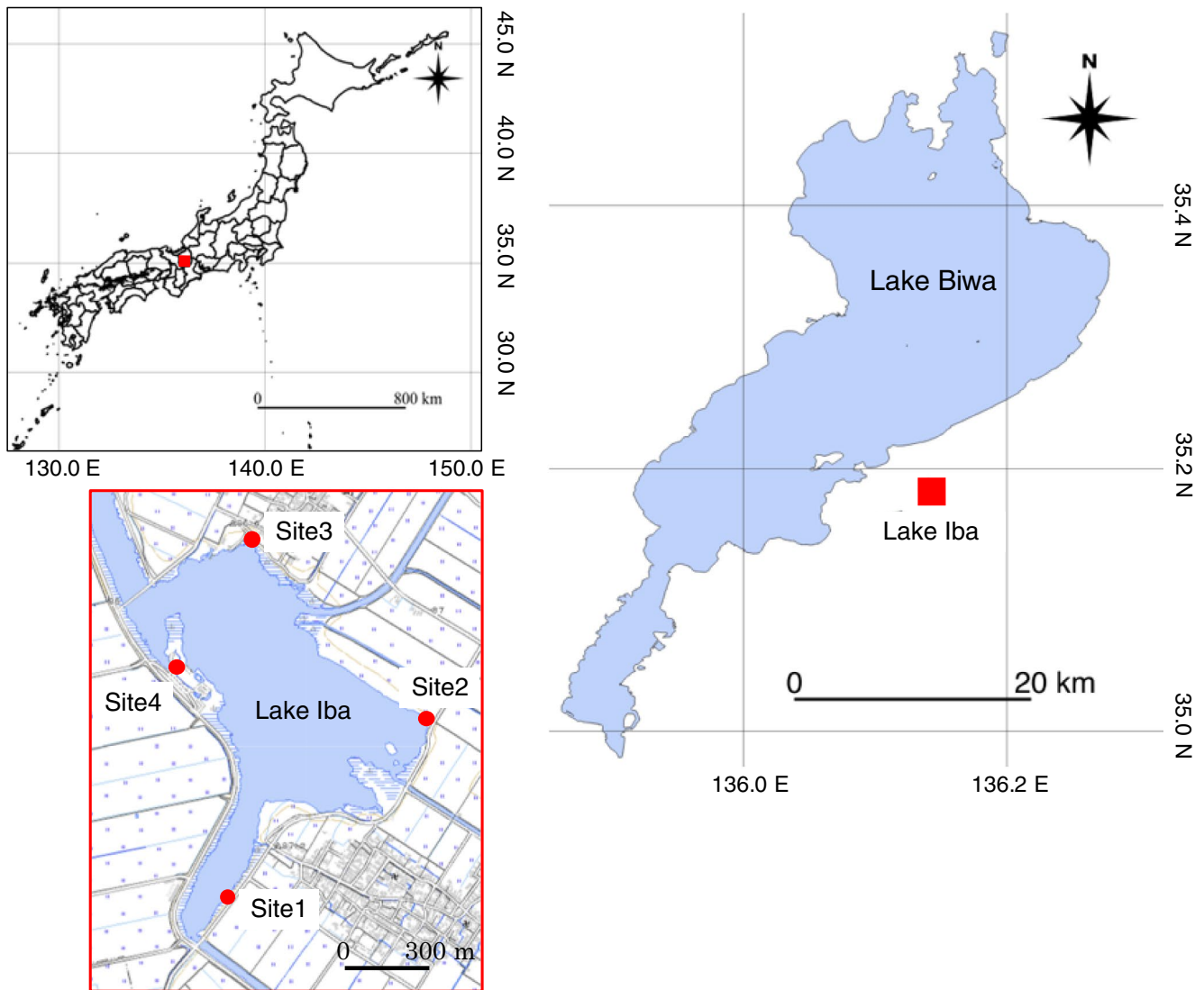
FIGURE 2   Maps of study sites for field survey in Lake Iba. Maps are based on the Digital Map (Basic Geospatial Information) published by the Geospatial Information Authority of Japan (http://maps.gsi.go.jp/)

reaction volume of the 2nd PCR was also 12 µl, containing 6.0 µl of 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems), 3.6 pmol each of forward and reverse primers, 1 µl template, and ultrapure water. The thermal cycle profile for the 2nd PCR was 95°C for 3 min; 12 cycles of 98°C for 20 s and 72°C for 30 s; and 72°C for 5 min.

The 2nd PCR products were pooled in equal volumes into a single 1.5-ml tube and then diluted five times with TE buffer (pH 8.0). We obtained a 200 µl library sample of target size amplicons by electrophoresis using E-Gel® SizeSelect 2% (Thermo Fisher Scientific) with the E-Gel Precast Agarose Electrophoresis System (Thermo Fisher Scientific). Subsequently, we confirmed whether only DNA of the target length (around 370 bp) was isolated using Agilent 2100 Bioanalyzer (Agilent Technologies). The concentration of the DNA library was adjusted to 4 nM. Finally, the library was sequenced using an Illumina MiSeq v2 Reagent kit for 2× 150 bp PE (Illumina).

2.5 | Bioinformatics

We performed data preprocessing and analysis of MiSeq raw reads using USEARCH v10.0.240 (Edgar, 2010) according to the following steps. (a) Paired-end reads (reads 1 and 2) were merged using the command "fastq_mergepairs" with a default setting. During this process, low-quality tail reads with a cutoff threshold set at a quality (Phred) score of 2, too short reads (<100 bp) after tail trimming, and those paired reads with too many differences (>5 positions) in the aligned region (ca. 65 bp) were discarded; (b) Primer sequences were removed from those merged reads using the command "fastx_truncate"; (c). Quality filtering using the "fastq_filter" command was performed to remove low-quality reads with an expected error rate (Edgar & Flyvbjerg, 2015) of >1% and too short reads of <100 bp; (d) The preprocessed reads were dereplicated using the "fastx_uniques" command, and all singletons, doubletons, and tripletons were removed from the subsequent analysis following the recommendation of the author of the

program. (e) The dereplicated reads were denoised using the "unoise3" command to generate amplicon sequence variants (ASVs). We removed ASVs with all putatively chimeric, erroneous sequences (Edgar, 2016), and those with less than 10 reads; 6) Finally ASVs were subjected to taxonomic assignments to species names using the "usearch_global" command with a sequence identity of >98.5% (two nucleotide differences allowed) with the reference sequences and a query coverage of ≥90%. Finally, species reads that were detected in both sample and positive control were regarded as possible contamination if the number of reads in the sample was less than that in the positive control, and these species reads were discarded.

2.6 | Statistical analysis

In the qPCR results, the DNA concentration was calculated as the average of the three replicates. When a negative detection was obtained in any of the replicates, the DNA concentration of that replicate was assigned as zero (Ellison, English, Burns, & Keer, 2006). To compare eDNA concentration between sediments and water, eDNA concentrations were converted to copy numbers per unit weight and then log-transformed. These concentration comparisons implicitly assume the equivalence of 1 ml and 1 g of water. To analyze the decay rates of both sample types, we fit a linear mixed model using the function LMER in R package LME4 (Bates, Mächler, Bolker, & Walker, 2015). In this model, eDNA copy numbers served as a response variable. The time point, the sample type (water/sediment), and their interaction were set as explanatory variables, and the sample series ID was set as a random effect to consider differences in decay rates between sample series. Additionally, the decay rate was calculated based on the untransformed data assuming exponential decay. To compare sedimentary and aqueous eDNA concentrations for field samples, linear mixed modeling was performed. In this model, eDNA copy numbers served as a response variable. The sample type (water/sediment) was set as an explanatory variable, and the fish species was set as a random effect. To compare the number of detected species between sample types, a paired t test was performed. In addition, to compare fish species composition between sampling types, nonmetric multidimensional scaling (NMDS) was performed with "Jaccard methods" and 10,000 permutations, and the PERMANOVA analysis was performed with "Jaccard methods" and 10,000 permutations using the "adonis" function. In this analysis, abundance information was not included. All analyses were performed using the software R ver. 3.5.1 (R Core Team, 2018).

3 | RESULTS

3.1 | Comparison of decay rates of eDNA for biotope samples

The R^2 values of calibration curves were >0.976 in all runs. The slopes ranged between −3.428 and −4.077, the intercepts ranged

between 42.648 and 45.455, and the PCR efficiency ranged between 75.908% and 95.741%.

The slopes of the regression lines based on all eDNA concentrations at each time point differed significantly between sample types ($p < .05$; Figure 3; Table 2). The decay rates (decreased eDNA copies divided by the initial eDNA copies per unit time) calculated for each sample type were 0.00033 ± 0.000049 [mean ± SE]/hr and 0.019 ± 0.0011/hr for sedimentary and aqueous eDNA, respectively. In this experiment, there was no organism that could be a source of target eDNA. In other words, aqueous eDNA showed a clear decline, but sedimentary eDNA showed little decline over the timescale of this experiment. All PCR NC and water NC showed no amplification.

3.2 | Comparison of eDNA concentration between sediment and water samples

The R^2 values of calibration curves were >0.980 in all runs. The values of slopes, intercepts, and PCR efficiency were, respectively, −3.901%, 45.151%, and 80.439% for common carp, −3.633%, 40.477%, and 88.469% for bluegill sunfish, and −3.341%, 44.396%, and 99.194% for largemouth bass.

For all four sites, the eDNA concentration of common carp and bluegill sunfish was higher per weight (g) of sediment than per volume (ml) of water (sediment: 163.7–1,450.0 copies/g; water: 0.1–31.2 copies/ml for common carp, and sediment: 15.4–72.8 copies/g; water: 0.3–3.8 copies/ml for bluegill sunfish; Figure 4). For three of the four sites, the eDNA concentration of largemouth bass was higher per g of sediment than per ml of water (sediment: 84.9–570.1 copies/g; water 0.5–12.7 copies/ml; Figure 4); however, sedimentary eDNA was not detected at one of the four sites. The results of the LMM analysis revealed that sedimentary eDNA concentration was significantly higher than aqueous eDNA one ($p < .05$; Table 3, Figure 4).
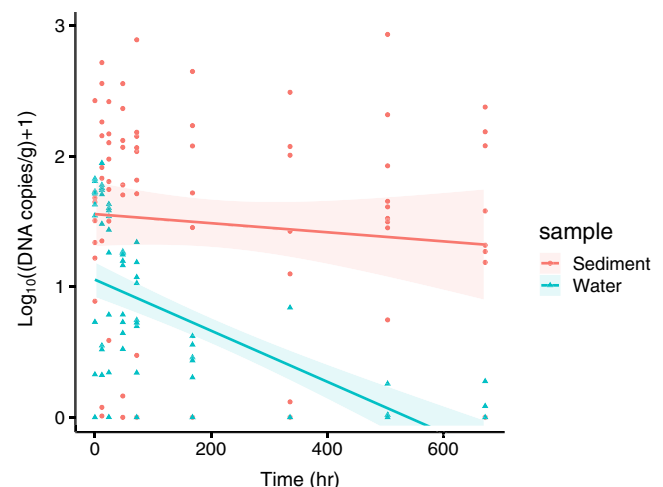

FIGURE 3 The difference of decay trends of *Hemigrammocypris rasborella* eDNA in water and sediments. The red circles and blue triangles show sediment and water samples, respectively. Shaded areas show 95% CI

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TABLE 2 Summary results of linear mixed models for biotope experiments

Coefficients	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	1.557	0.1379	18.36	11.291	<0.001***
Time	−0.0003479	0.0003098	150	−1.123	0.2633
Sample type	−0.5022	0.135	150	−3.719	<0.001***
Time:sample type	−0.001607	0.0004281	150	−3.668	<0.001***

Note: In this model, eDNA copy numbers served as a response variable. The time point, the sample type (sediment and water are included as 0 and 1, respectively), and their interaction were set as explanatory variables. Asterisks show the significant effects of each parameter (***$p < .001$).

TABLE 3 Summary results of linear mixed models for field samples

Coefficients	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	309.257	111.869	3.548	2.764	0.0579
Sample type	−304.105	112.638	20	−2.7	0.0138*

Note: In this model, eDNA copy number served as the response variable and the sample type (sediment and water are included as 0 and 1, respectively) was set as the explanatory variable. Asterisks show the significant effects of each parameter (*$p < .05$).

3.3 | Detection of fish species by eDNA metabarcoding

We obtained 1,244,636 MiSeq reads in total (113,149 ± 67,455 [mean ± *SD*]), of which 1,214,725 passed the merging processes (110,430 ± 65,979), and 1,194,390 reads passed the quality control processes (108,581 ± 64,887). Of these reads, 3.03% (36,135 reads) were singletons and the other 1,158,255 reads clustered into 15,843 unique sequences. Subsequently, 999,859 reads (231 ASVs) passed the denoizing step, and the sequences judged as "not chimera" formed 166 clusters. Finally, 105 clusters were assigned to fish taxa (>98.5% identity) and the other clusters were assigned to mammals, reptiles, and bacteria. After possible contaminant sequences were removed, the number of clusters subjected to the following analyses was reduced to 90. Only *T. japonicus* sequences were detected in the positive controls.

A total of 22 fish species were identified by eDNA metabarcoding (Table 4). The detected number of species was saturated for the number of reads in all samples (Figure S4). Fourteen fish species were detected from sediment samples, and 20 fish species were detected from water samples. In the paired *t* test, the number of detected species was higher in that water sample than in the sediment sample (Figure 5; $p < .01$). On the contrary, the fish species composition detected by eDNA metabarcoding was not statistically different between the sediment and water samples (PERMANOVA: $p = .59$; Figure S5). However, although there was no statistical significance, some fish species were detected only in sediment or water samples (Figure 5; Table 4; Figure S5).

4 | DISCUSSION

The present study revealed the decay rate of sedimentary eDNA in surface sediments compared with aqueous eDNA. Long-term retention in low-oxygen environments, such as deeper sediment, is well

known, but our finding that the decay rate in surface sediment is also very slow reveals part of the mechanism underlying the long holding time of sedimentary eDNA. The sedimentary eDNA concentration was higher than the aqueous eDNA concentration both in an artificial biotope and a natural lake, as reported in a previous study (Turner et al., 2015). Further, the fish species identified from eDNA metabarcoding were different between sediment and water samples. These results
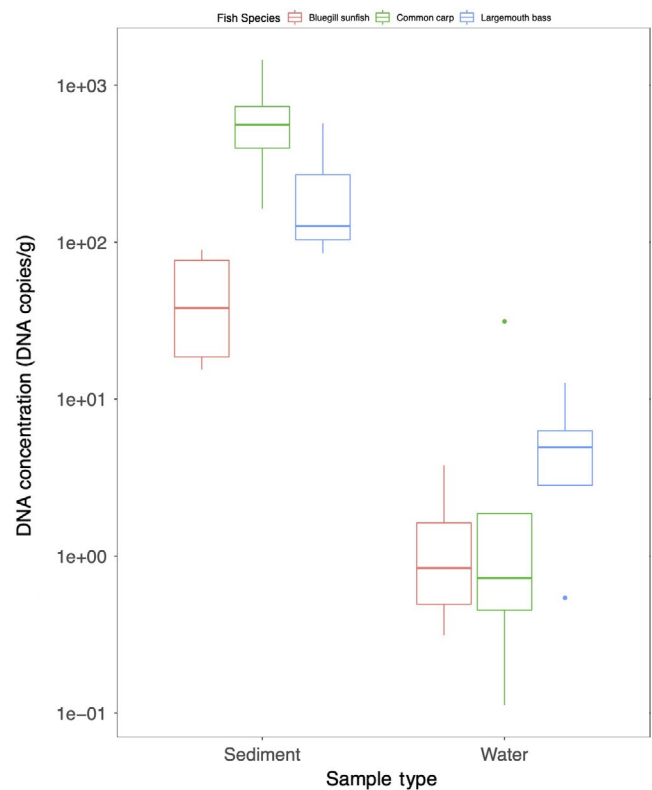

FIGURE 4 Comparison of eDNA concentrations for three species between sediment and water samples in a natural lake. The LMM result shows that DNA concentration is higher in sediment sample than water one ($p < .05$)

indicate that sedimentary eDNA has a slower decay rate than aqueous eDNA (approximately 1/57; Figure 3) and suggest that sedimentary eDNA may complement aqueous eDNA for revealing previously undetectable species (Figure 5). For example, fish that migrate seasonally may not be detected in water samples when they are not present, but they may be detected in sediment samples.

The decay rate of eDNA in sediment samples was much lower than that in water (sedimentary eDNA = 0.00033/hr, and aqueous eDNA = 0.019/hr; Figure 3). In the present study, we clarified the decay rate of sedimentary eDNA, which was rarely reported in previous studies. This decay rate (0.019/hr) of aqueous eDNA is comparable with that of natural environments reported in previous studies (0.0097–0.101: Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016; Sansom & Sassoubre, 2017). Therefore, although our experiments were conducted in a controlled environment, the decay rate of sedimentary eDNA can be assumed to be within the range of rates found in the natural environment. However, previous studies reported that aqueous eDNA was degraded rapidly by the effects of water state, temperature, sunlight (UV), and pH (Andruszkiewicz, Sassoubre, & Boehm, 2017; Eichmiller, Best, & Sorensen, 2016; Strickler, Fremier, & Goldberg, 2015; Tsuji, Yamanaka, & Minamoto, 2017). Sedimentary eDNA might be protected from such decay by

adsorption to soil particles, and this may explain its slow-decay rate compared with aquatic eDNA. For example, a previous study indicated that the DNA adsorbed to soil particles was protected from decay by nucleases (Ogram et al., 1994). Our finding that the decay of sedimentary eDNA was slower than that of aqueous eDNA supports the findings of previous studies on the dynamics of DNA molecules in sediments (Corinaldesi et al., 2011; Levy-Booth et al., 2007; Pietramellara et al., 2009). A previous study found that carp eDNA remained detectable up to 132 days in sediment after removing carp fish (Turner et al., 2015), whereas the detectable time of aqueous eDNA was reported to be several days to several weeks (Barnes et al., 2014; Dejean et al., 2011; Goldberg et al., 2013; Pilliod, Goldberg, Arkle, & Waits, 2014; Thomsen, Kielgast, Iversen, Møller, et al., 2012). Therefore, the sedimentary eDNA would persist longer than aqueous eDNA, and deriving historical information on macro-organisms by collecting eDNA from sediment core samples is possible as reported previously (Bálint et al., 2018; Parducci, Suyama, Lascoux, & Bennett, 2005; Stager et al., 2015). Moreover, combining the decay rate of sedimentary eDNA as shown in our study and the initial concentration of sedimentary eDNA in sediment cores will be useful for revealing past information. For example, our results (decay rate and initial concentration in the controlled experiments) revealed that fish sedimentary

TABLE 4 Fish species detected by eDNA metabarcoding

Species name	Site1 Sediment	Site1 Water	Site2 Sediment	Site2 Water	Site3 Sediment	Site3 Water	Site4 Sediment	Site4 Water
Acheilognathus rhombeus	0	1,647	3,940	2,259	0	584	0	1,623
Biwia zezera	0	205	0	0	0	0	0	0
Carassius cuvieri	7,061	0	0	6,157	0	0	0	450
Carassius spp.	0	0	64,897	44,818	8,801	1,437	36,953	8,188
Channa argus	0	1,281	11,586	0	14,148	26,796	0	2,675
Cyprinus carpio	57,528	51,732	0	1,348	6,998	3,231	5,030	3,946
Lepomis macrochirus	49,862	21,212	0	4,396	744	4,932	12,661	26,056
Micropterus salmoides	30,341	21,879	0	6,443	0	8,137	0	22,899
Misgurnus anguillicaudatus	0	0	0	383	4,992	42,860	0	0
Nipponocypris sieboldii	0	0	4,316	0	0	0	0	0
Nipponocypris temminckii	0	0	6,278	0	0	432	0	0
Odontobutis obscura	0	0	0	1,102	0	0	0	0
Oreochromis niloticus	0	0	17,413	0	0	0	0	0
Opsariichthys platypus	0	182	0	381	5,950	0	3,429	0
Pseudogobio esocinus	0	0	0	350	0	0	0	0
Pungtungia herzi	0	0	0	323	0	0	0	0
Rhinogobius flumineus	0	866	0	0	0	0	0	0
Rhinogobius spp.	0	0	7,587	6,594	0	0	11,328	1,757
Rhynchocypris lagowskii steindachneri	0	0	0	165	0	0	0	0
Silurus asotus	0	0	0	0	0	31	0	280
Squalidus spp.	0	244	0	0	0	0	0	0
Tridentiger obscurus	16,642	4,493	0	0	0	0	0	754

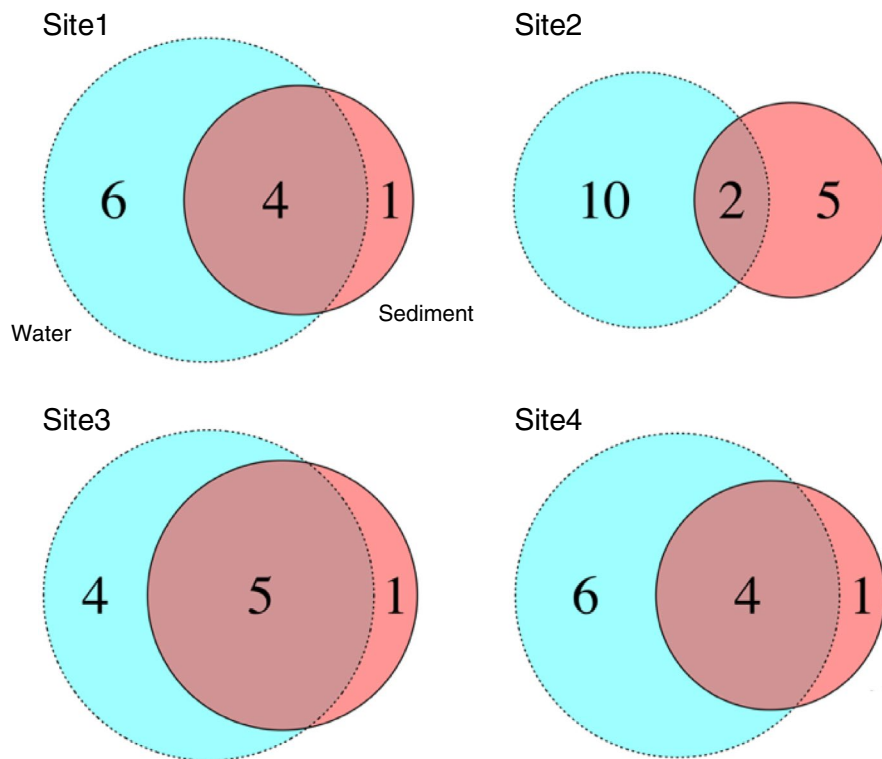*Note:* The number shows obtained reads. "0" means no detection.

FIGURE 5 Number of fish species detected by eDNA metabarcoding. Blue and red show results from water and sediment samples, respectively

eDNA might be detected up to 537 days in surface sediment. This low decay rate in the surface sediment may enable DNA retention until reaching a low-oxygen environment and may be a part of the mechanism of DNA maintenance for a long time in low-oxygen environments, such as deeper sediment. The microenvironment of the sedimentary eDNA on the surface may become anoxic through the accumulation of sediments before the eDNA becomes undetectable owing to decay. However, in our experiments, sediment substrate types were not controlled or measured. Therefore, it would be worth investigating the effect of a longer period on the decay rate of sedimentary eDNA in various substrate types in various environments.

The qPCR results of field samples showed that sedimentary eDNA concentration was higher than that of aqueous eDNA for the same sample weight (Figure 4). This result matches that of a previous study (Turner et al., 2015), and the concentration of eDNA in sediment would be generally higher than that in water.

The number of fish species detected by eDNA metabarcoding was significantly higher in water sample than in sediment sample (paired t test: $p < .01$; Figure 5). This may be because of aqueous eDNA, indicating a wider spatial scale, but in this comparison, the volume of sample was not considered (sediment: 3 g, water: 1 L). Therefore, water sample may be advantageous because a large amount of sample can be used. On the contrary, the fish species composition detected by eDNA metabarcoding was not statistically different between sediment and water samples (Figure S5). However, some species were detected only in sediment or water samples (Figure 5; Table 4). For example, very abundant fish in the sampling area, such as *Carassius* spp., were detected in both sample types. However, rare species with small populations, such as *Biwia*

zezera and *Oreochromis niloticus*, were detected either in sediment or water samples. Although the number of detected species from sedimentary eDNA was lower than that from aqueous eDNA, information from water and sediment samples could be complementary because sedimentary eDNA reflects a longer timescale, whereas aqueous eDNA reflects a wider spatial scale. This result was consistent with a previous study that used 10 g sediment sample (Siegenthaler et al., 2018). In another previous study, which used low sample weight (0.25 g), the species detected from aqueous eDNA subsumed those from sedimentary eDNA (Shaw et al., 2016). This inconsistency could be caused by differences in the eDNA timescale reflected, or differences in spatial scale, in addition to differences in decay rate between sediment and water samples. For example, aqueous eDNA would be expected to reflect a wide spatial scale because it diffuses in the water (Dunker et al., 2016), whereas it would be more difficult for sedimentary eDNA to diffuse. Overall, our result suggested that information on fish species composition obtained from 3 g of sediment samples and 1 L of water samples were comparable, and this result is similar to that of a previous study (10 g and 2 L; Siegenthaler et al., 2018). Sediment samples of 3–10 g are preferred over less samples (0.25 g; Shaw et al., 2016), because greater sample sizes may further improve the results of sedimentary eDNA analysis. Meanwhile, in metabarcoding assays, the estimation of abundance was prevented by PCR bias (Bista et al., 2018). Therefore, the estimation of abundance by using eDNA metabarcoding needs careful interpretation.

Wei et al. (2018) observed two phases in the sedimentary eDNA decay process: a fast-decay phase (until 72 hr after removal of individuals), followed by a slow-decay phase (until 480 hr). However, we

assumed that we observed only a single decay phase in the present study (Figures S2 and S3). It appears that the fast-decay phase did not occur in our study. The slow-decay speed may have been due to the low temperature early in the experiment (until approximately 72 hr; Fig. S1) (Eichmiller et al., 2016; Strickler et al., 2015).

5 | CONCLUSION

In this study, we demonstrated sedimentary eDNA characteristics in comparison with aqueous eDNA under a controlled and natural lentic condition. The decay rate of sedimentary eDNA was low even for surface sediment, and the sedimentary eDNA concentration was higher than that of aqueous eDNA for the same sample weight. Additionally, the composition obtained by metabarcoding was not statistically different between sediment or water samples. However, using both sample types may provide complementary species information. Therefore, sedimentary eDNA could complement aqueous eDNA in eDNA biomonitoring for species composition because the sedimentary eDNA should reflect a longer timescale, as shown in this study, and a narrower spatial scale, owing to its physical properties, than aqueous eDNA. In addition, although the analysis of eDNA from sediment cores can reconstruct information on past biological communities by targeting various taxa (e.g., plants: Pansu et al., 2015; eukaryotes: Kouduka et al., 2017; rabbits: Ficetola et al., 2018), knowledge of the characteristics of extracellular sedimentary eDNA of macro-organisms and detection methods remains limited, especially for sediment core samples. Therefore, further research related to detection methods and analysis is needed to achieve more sensitive detection and accurate estimation of bioinformation using ancient eDNA from sediment cores.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION
M.K.S., S.Y., and T.M. conceived and designed the research. M.K.S. and T.M. collected samples. M.K.S., R.O.G., M.M., and H.Y. performed the experiments, bioinformatics, and statistical analysis of the data. M.K.S. and T.M. wrote the first draft of the manuscript, and all authors edited the manuscript. All authors discussed the results and contributed to development of the manuscript. We thank Dr. A. S. Tanabe (Ryukoku University) for assistance with metabarcoding analysis.

ETHICAL STATEMENT
No animal experiments were performed in this study. All experiments were performed according to the current law of Japan.

DATA AVAILABILITY STATEMENT
The raw data were deposited to Dryad (https://doi.org/10.5061/dryad.mgqnk98wd).

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SUPPORTING INFORMATION

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