A Model and Simulation of the Influence of Temperature and Amplicon Length on Environmental DNA Degradation Rates: A Meta-Analysis Approach

Tatsuya Saito* and Hideyuki Doi

Graduate School of Simulation Studies, University of Hyogo, Kobe, Japan

Environmental DNA (eDNA) analysis can detect aquatic organisms, including rare and endangered species, in a variety of habitats. Degradation can influence eDNA persistence, impacting eDNA-based species distribution and occurrence results. Previous studies have investigated degradation rates and associated contributing factors. It is important to integrate data from across these studies to better understand and synthesize eDNA degradation in various environments. We complied the eDNA degradation rates and related factors, especially water temperature and amplicon lengths of the measured DNA from 28 studies, and subjected the data to a meta-analysis. In agreement with previous studies, our results suggest that water temperature and amplicon length are significantly related to the eDNA degradation rate. From the 95% quantile model simulation, we predicted the maximum eDNA degradation rate in various combinations of water temperature and amplicon length. Predicting eDNA degradation could be important for evaluating species distribution and inducing innovation (e.g., sampling, extraction, and analysis) of eDNA methods, especially for rare and endangered species with small population size.

Keywords: environmental DNA, polymerase chain reaction, degradation rate, quantile model, meta-analysis

INTRODUCTION

Environmental DNA (eDNA) methods are innovative methods developed for monitoring macroorganisms, especially aquatic species (Ficetola et al., 2008; Minamoto et al., 2012; Taberlet et al., 2012; Takahara et al., 2012; Ushio et al., 2018; Kakuda et al., 2019; Tsuji et al., 2019). The eDNA method is used to investigate species distribution. It is less invasive to organisms, and is especially useful for rare and endangered species, which generally have low tolerance to sampling disturbance and may be difficult to detect. Consequently, eDNA methods have been used to detect rare and endangered species in various taxa, such as fish, salamander, and aquatic insects (Fukumoto et al., 2015; Sigsgaard et al., 2015; Pfleger et al., 2016; Doi et al., 2017; Sakata et al., 2017).

Environmental DNA, which is compromised of DNA fragments released by organisms into environments such as water or soil, is thought to be derived from mixtures of feces (Martellini et al., 2005), skin cells (Ficetola et al., 2008), mucus (Merkes et al., 2014), and secretions (Bylemans et al., 2018) of organisms. Previous studies have suggested that eDNA is mainly derived from fractions of cells or cellular organs (i.e., mitochondria and nuclei), but it can also be derived from fragmented DNA (degraded DNA) in the water (Turner et al., 2014; Minamoto et al., 2016).

Many points regarding the general behavior of eDNA in water (reviewed in Barnes and Turner, 2016) are still unclear, especially the state (fragment length) and degradation of eDNA (Turner et al., 2015; reviewed in Barnes and Turner, 2016). Understanding eDNA states and degradation is essential for the effective sampling and storage of eDNA, and may provide pertinent information to better interpret the results of species distribution and abundance and biomass estimations. This may be especially problematic for rare and endangered species, which are thought to have small populations and small amounts (or concentrations) of DNA (Fukumoto et al., 2015; Sigsgaard et al., 2015; Pfleger et al., 2016; Doi et al., 2017; Sakata et al., 2017). Both factors can influence eDNA persistence, potentially inducing false negatives which impact accuracy in occurrence and distribution data.

Many experiments have been conducted to reveal the detailed states and degradation rates of eDNA under various conditions (Thomsen et al., 2012; Barnes et al., 2014; Maruyama et al., 2014; Tsuji et al., 2017; Jo et al., 2019). In most cases, the eDNA degradation curves declined exponentially and quickly, often in less than a week (Thomsen et al., 2012; Barnes et al., 2014). Earlier meta-analyses for eDNA degradation (Collins et al., 2018) found that water conditions, such as salinity (Collins et al., 2018), water temperature (Tsuji et al., 2017; Jo et al., 2019), and pH (Barnes et al., 2014; Tsuji et al., 2017), influenced the eDNA degradation rate. In addition, the characteristics of DNA itself, such as its measured amplicon length, affected the eDNA degradation rate (Bylemans et al., 2018; Jo et al., 2019). From the data so far (temperature and amplification length), it seems possible to predict the approximate degradation rate and estimate the state of eDNA. Therefore, we conducted a novel meta-analysis to model the effects of water conditions and DNA amplicon length on the eDNA degradation rate using data generated in previous eDNA degradation studies. The previous meta-analysis (Collins et al., 2018) used the half-life of the degradation curve as an index of degradation. Although half-life has the advantage of being more intuitively meaningful, we instead used here the degradation rate constants "k" because our model uses the degradation rate, not half-life.

Using this approach, we aimed to evaluate the effects of water conditions (i.e., ecosystem, source, temperature, and pH), and target DNA region on eDNA degradation in previously published data. Also, we tested the relationship between DNA amplicon length and eDNA degradation because degradation may differ with amplicon length. Specifically, we conducted a simulation to predict the maximum degradation rate using quantile regression modeling with temperature and DNA amplicon length.

MATERIALS AND METHODS

Search Strategy
A Google Scholar search on September 9, 2020, using the search terms "eDNA" OR "environmental DNA" AND "degradation" OR "decay" OR "decomposition," returned 11,300 hits. The initial filtering of the articles was based on their title; any articles that obviously had no relevance to eDNA degradation were discarded. After title screening, 1,000 articles remained. After abstract screening, 42 articles remained. We manually inspected these remaining articles and selected papers describing the degradation rate of eDNA using experiments or field settings (**Supplementary Table 1**). Upon completion of the screening process, we obtained relevant eDNA data from 28 articles (**Table 1** and **Supplementary Table 1**) for the meta-analysis.

Data Extraction
From the selected publications, we assembled a list of factors for eDNA degradation (**Supplementary Table 1**). We collected the following factors and categories: "Ecosystem" was divided into marine and freshwater. "Source" was categorized into water sources (Freshwater: river, lake, well water, pond, tap water, and deionized water; Marine: marine and artificial seawater). "Temperature" and "pH" refer to the water temperature and pH of the water sample for each experiment, respectively. "Region" and "Amplicon length" refer to the amplified DNA region used for quantitative PCR (qPCR) and the number of amplified-DNA bases targeted by the qPCR reaction (bp). "Region" was divided into mtDNA (COI, CytB, 16s, 18s, D-loop, NADH, ND2, ND4), nuDNA (ITS), and RNA. "DNA type" was divided into spike (i.e., the DNA contained in the environment water) and organism. "Experiment type" was divided into "in tank" and "in field."

We extracted the simple exponential slope (hereafter referred to as "degradation rate") from the article contents and/or plots according to the simple exponential equation (Motulsky and Christopoulos, 2003) in each experiment:

$$C = C_o E^{kt}$$

where C_0 is the eDNA concentration at time 0 (i.e., the initial eDNA concentration), and k is the degradation slope (rate) constant per hour. We used the standardized degradation rate per hour. The degradation rate by day was divided by 24 to calculate the degradation rate per hour.

Statistical Analysis and Simulation
We performed the statistical analysis and graphics using R ver. 4.0.2 (R Core Team, 2020). We tested the differences in the eDNA degradation rate in measured DNA regions and water resources using a linear mixed-effect model (LMM) using "lme4" ver. 1.1.23 package with "lmerTest" ver. 3.1.2 package in R. We excluded data points without temperature information in the statistical analyses. We set each study as a random effect. Jo et al. (2020) compared the degradation of mtDNA and nuDNA and found the difference. However, we could not analyze mtDNA and nuDNA due to the limited data.

We performed quantile models (QM) for 0.1, 0.5, and 0.95 quantiles for the regression. By performing 0.95 and 0.1 quantiles for the regression, we evaluated the maximum and minimum degradation rate. The 0.5-quantile used median for the regression, so almost similar to simple linear regression. We employed the Bayesian mixed-effect quantile model using the "lqmm" function of "lqmm" package ver. 1.5.5 in R. In the QM, we set water temperature and amplicon length as explanatory effects and each study as the random effect. We performed the Nelder–Mead algorithm using 10000 MCMC permutations with

TABLE 1 | The organisms, ecosystem types (Ecosystem), water source (Source), and PCR-amplified DNA regions by quantitative PCR (Region) for all papers analyzed in this meta-analysis.

Organism	Ecosystem	Source	Region	References	Year	Study
Gasterosteus aculeatus	Marine	Marine	CytB	Thomsen et al.	2012	1
Platichthys flesus	Marine	Marine	CytB	Thomsen et al.	2012	1
Lepomis macrochirus	Freshwater	Tap	CytB	Maruyama et al.	2014	2
Cyprinus carpio	Freshwater	Well	CytB	Barnes et al.	2014	3
Lithobates catesbeianus	Freshwater	Tap	CytB	Strickler et al.	2015	4
Cyprinus carpio	Freshwater	Well	CytB	Eichmiller et al.	2016	5
Cyprinus carpio	Freshwater	Lake	CytB	Eichmiller et al.	2016	5
Engraulis mordax	Marine	Marine	D-loop	Sassoubre et al.	2016	6
Sardinops sagax	Marine	Marine	D-loop	Sassoubre et al.	2016	6
Scomber japonicus	Marine	Marine	COI	Sassoubre et al.	2016	6
Scomber japonicus	Marine	Marine	COI	Andruszkiewicz et al.	2017	7
Zearaja maugeana	Marine	Marine	ND4	Weltz et al.	2017	8
Chrysaora pacifica	Marine	Marine	COI	Minamoto et al.	2017	9
Trachurus japonicus	Marine	Marine	CytB	Jo et al.	2017	10
Plecoglossus altivelis	Freshwater	River	CytB	Tsuji et al.	2017	11
Cyprinus carpio	Freshwater	River	CytB	Tsuji et al.	2017	11
Margaritifera margaritifera	Freshwater	River	NADH	Sansom and Sassoubre	2017	12
Carcinus maenas	Marine	Marine	COI	Collins et al.	2018	13
Lipophrys pholis	Marine	Marine	COI	Collins et al.	2018	13
Hypophthalmichthys nobilis	Freshwater	Deionized	D-loop	Lance et al.	2017	14
Chionodraco rastrospinosus	Marine	Marine	ND2	Cowart et al.	2018	15
Carassius auratus	Freshwater	Tap	ITS	Bylemans et al.	2018	16
Neogobius melanostomus	Freshwater	Lake	COI	Nevers et al.	2018	17
Cyprinus carpio	Freshwater	River	CytB	Nukazawa et al.	2018	18
Grandidierella japonica	Marine	Artificial seawater	COI	Wei et al.	2018	19
Trachurus japonicus	Marine	Marine	CytB	Jo et al.	2019	20
Daphnia magna	Freshwater	Tap	COI	Moushomi et al.	2019	21
Daphnia magna	Freshwater	Tap	18S	Moushomi et al.	2019	21
cyanobacterial	Freshwater	Lake	16S	Zulkefli et al.	2019	22
Schistosoma mansoni	Freshwater	Tap	COI	Sengupta et al.	2019	23
Trachurus japonicus	Marine	Marine	CytB	Jo et al.	2020	24
Trachurus japonicus	Marine	Marine	ITS	Jo et al.	2020	24
Styela clava	Marine	Marine	COI	Wood et al.	2020	25
Spirographis spallanzani	Marine	Marine	COI	Wood et al.	2020	25
Styela clava	Marine	Marine	RNA	Wood et al.	2020	25
Spirographis spallanzani	Marine	Marine	RNA	Wood et al.	2020	25
Anguilla japonica	Freshwater	Tap	D-loop	Kasai et al.	2020	26
Rhinella marina	Freshwater	Tap	16S	Villacorta-Rath et al.	2020	27
Trachurus japonicus	Marine	Marine	CytB	Saito and Doi	2020	28
Cyprinus carpio	Freshwater	Pond	CytB	Saito and Doi	2020	28

the Gauss–Hermite quadrature approach. We set the statistical alpha as 0.05 for parameter evaluation. We did not find a significant interaction ($p > 0.1$) between water temperature and amplicon length, so we used the model excluding the interaction, i.e., eDNA degradation rate = water temperature + amplicon length. We evaluated the QM models using the Akaike information criteria (AIC), in which the best QM is identified by having the lowest AIC.
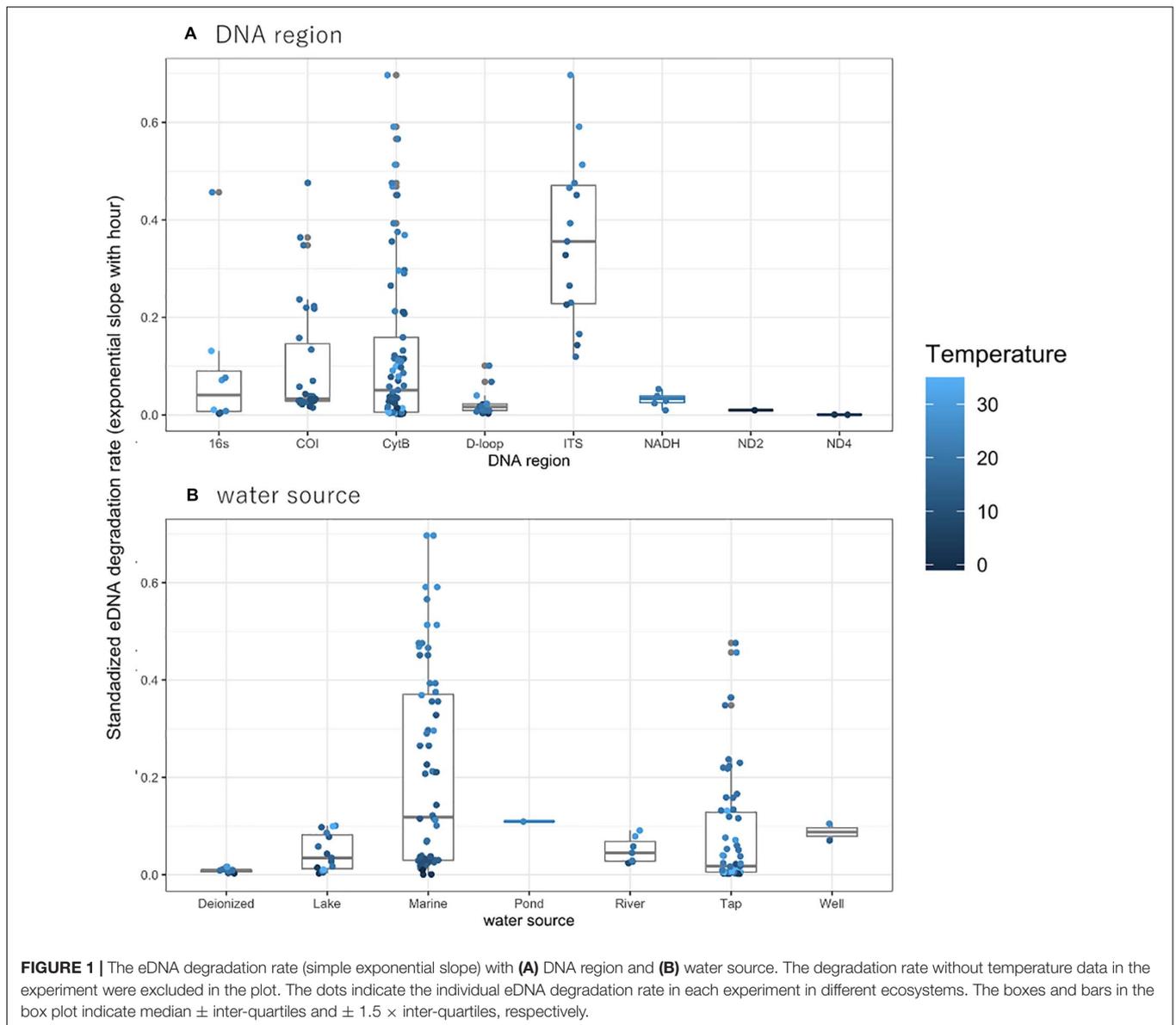
We simulated the combined effects of water temperature and amplicon length, using the obtained 0.95-quantile QM. We generated 100,000 random values for the combination of water temperature (ranging in published values from −1 to 35 °C; see the results) and amplicon length used for the experiments (ranging in published values from 70 to 719) using "runif" function in R, which generates a random number from the

Mersenne-Twister method. We used 100,000 random values to predict the eDNA degradation rate from the 0.95-quantile QM (see results).

RESULTS

Degradation Rate Experiments From Literature

The number of obtained time points for the eDNA degradation data ranged from 3 to 25 (mean: 8.3, median: 8.0, **Supplementary Table 1**). Details of the sites are listed as water sources (**Table 1**). In total there were 21 marine sites, 1 artificial marine site, and 19 freshwater sites. Within the freshwater sites, there were 9 experiments that used tap or deionized water, 4 river sites, 3 lake

FIGURE 1 | The eDNA degradation rate (simple exponential slope) with **(A)** DNA region and **(B)** water source. The degradation rate without temperature data in the experiment were excluded in the plot. The dots indicate the individual eDNA degradation rate in each experiment in different ecosystems. The boxes and bars in the box plot indicate median ± inter-quartiles and ± 1.5 × inter-quartiles, respectively.

sites, 2 well water sites, and 1 pond site. The temperature for the experiments ranged from −1 to 35 °C (mean: 19, median: 20, **Supplementary Table 1**). The amplicon length used for the experiments ranged from 70 to 719 bp (mean: 150, median: 131, **Supplementary Table 1**), and the DNA fragment regions used were mainly Cyt B or COI regions in mtDNA (**Table 1**). Degradation experiments of nuDNA and RNA were very few data compared to mtDNA.
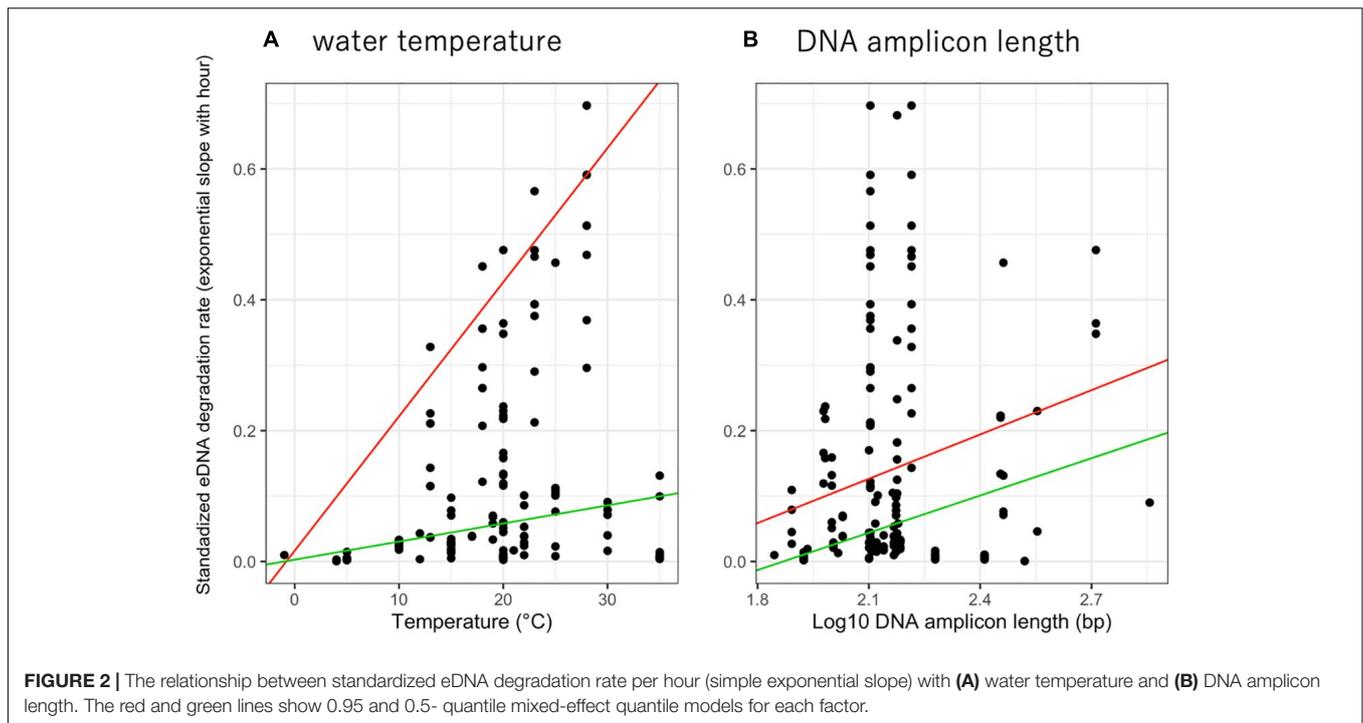
Degradation Rate

The observed degradation rate for the previously published eDNA data ranged from 0.0005 to 0.7010 (mean: 0.1317, median: 0.0440, **Supplementary Table 1**). Differences in PCR regions did not affect the rate of DNA degradation, nor did differences in water sources (**Figures 1A,B**). Although the degradation rates for Tap and Marine water sources appear

much higher than that observed for other sources, there were no significant differences among water sources, nor among taxa or PCR regions (LMM, $t < 1.859$, $p > 0.07$, **Figure 1** and **Supplementary Figure 1**, respectively). With the limited data excluded, such as ND2, ND4 for PCR region and pond for water source, there were no significant differences among water sources (LMM, $t < 1.965$, $p > 0.06$, **Supplementary Figure 2**, respectively), but significant differences among PCR region (LMM, $t = -3.414$, $p = 0.002538$, **Supplementary Figure 2**).

Quantile Model for Temperature and Amplicon Length

The relationship between eDNA degradation rate and water temperature was significant in 0.95- quantile and showed that higher water temperatures accelerated eDNA degradation

FIGURE 2 | The relationship between standardized eDNA degradation rate per hour (simple exponential slope) with **(A)** water temperature and **(B)** DNA amplicon length. The red and green lines show 0.95 and 0.5- quantile mixed-effect quantile models for each factor.

(**Figure 2A**, p = 0.02004 and 0.5761 for 0.95- and 0.5- quantiles, respectively). Upon comparing the QM of 0. 1-, 0. 5-, and 0.95- quantiles, the QM with 0.95-quantile was observed to have the lowest AIC value (0.1-quantile: 41.82, 0.5-quantile: −120.78, and 0.95-quantile: −161.26), indicating that the best model for the relationship. Therefore, we simulated these data using the QM with a 0.95-quantile with a positive slope (slope = 0.020, **Figure 2A**). The relationship between eDNA degradation rate and amplicon length suggests that longer amplicon length undergo greater eDNA degradation (**Figure 2B**). For amplicon length, as for water temperature, the QM with 0.95-quantile had the lowest AIC value (0.1-quantile: 155.1, 0.5-quantile: −110.2, and 0.95-quantile: −145.6). Therefore, we simulated and discussed these data using the QM with a 0.95-quantile with a positive slope (slope = 0.225). We also showed the categories of water temperature range (divided into four levels: −1, 0–10, 11–20, and > 21 °C) and amplicon length (divided into three levels: 0–100, 101–200, and > 201 bp) with eDNA degradation rate (**Supplementary Figures 3, 4** respectively) with similar trends of **Figure 2**.
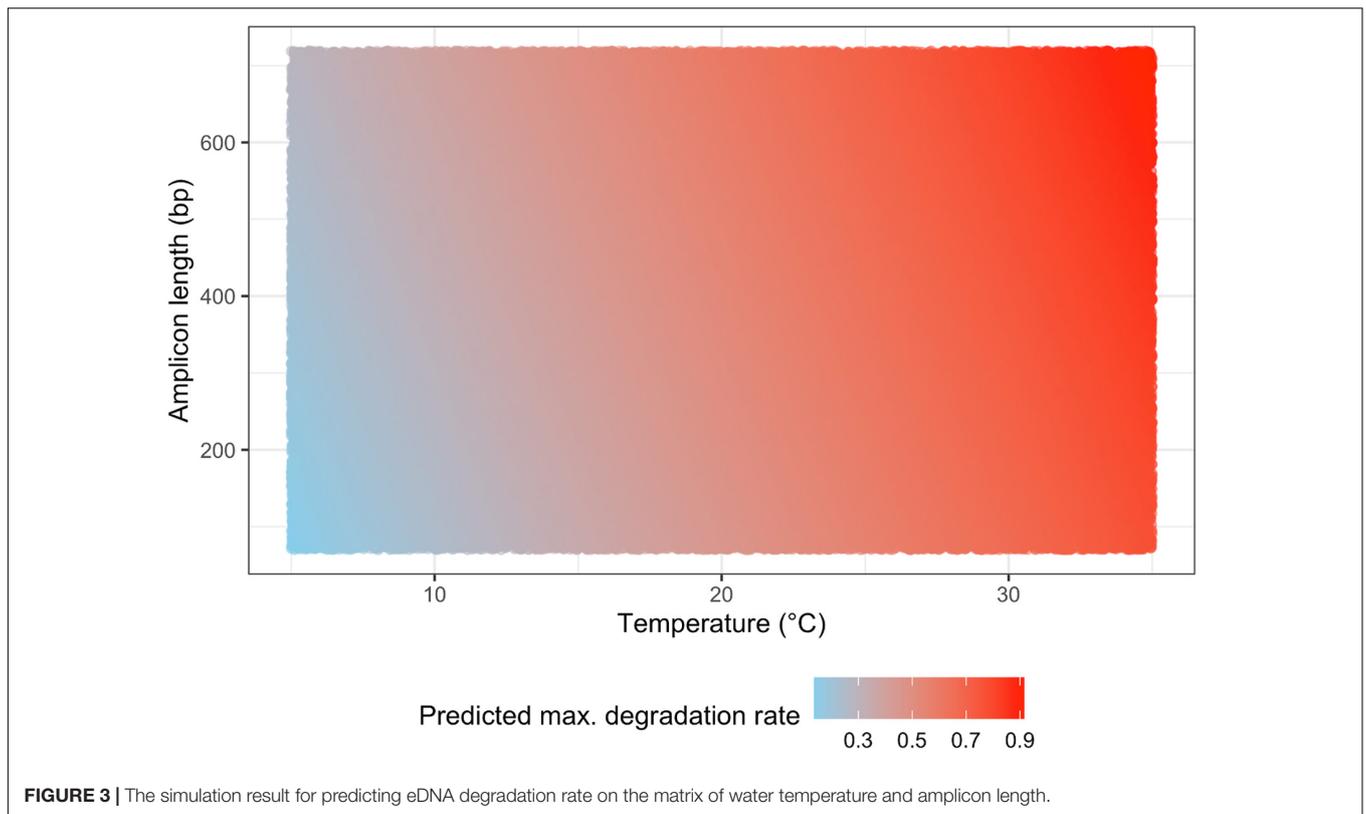
eDNA Degradation Simulation

Our QM simulation lead to plotting the eDNA degradation on a matrix of water temperature and amplicon length (**Figure 3**), which showed that the water temperature had a great influence on the eDNA degradation rate. At lower (e.g., −1 to 5 °C) and higher (e.g., 15 to 35 °C) water temperatures, our model predicted that amplicon length would have a smaller effect on the eDNA degradation rate, while at moderate (e.g., 5 to 15 °C) water temperatures, our prediction more clearly showed that the longer amplicon length would have a faster degradation rate. Thus, at

moderate water temperatures, the amplicon length should also be considered in evaluating eDNA degradation.

DISCUSSION

Our meta-analysis results showed that eDNA degradation was accelerated in higher water temperatures and in longer amplicon length. These generally supported the effect of water temperature on the eDNA degradation rate in previous hypotheses for each condition and species (e. g., Strickler et al., 2015; Eichmiller et al., 2016; Lance et al., 2017; Tsuji et al., 2017; Jo et al., 2019; Kasai et al., 2020). Previous studies have assumed that water temperature does not directly affect eDNA degradation, but indirectly affects it through enzymatic hydrolysis by microbes and extracellular nucleases (reviewed in Barnes and Turner, 2016). At high temperatures, with increasing activity of microorganisms and extracellular enzymes, the eDNA in water would degrade more quickly (reviewed in Barnes and Turner, 2016). Our meta-analysis results showed that there were no significant differences between laboratory water (purified or tap water) and environmental water (seawater or freshwater). This may indicate the enzymes and bacteria possessed by experimental organisms affected the eDNA degradation. In fact, the degradation experiment, which intracellular DNA and fragmented DNA were added to purified water, showed that intracellular and fragments DNA were not degraded in the water for a week (Saito and Doi, 2020).

Evidence from previous studies suggested that, in eDNA samples, long amplicon length are less likely to be detected than short amplicon (Jo et al., 2017). Our meta-analysis supports these previous results. A possible explanation is provided by Jo et al. (2017), in which it was suggested that the DNA degradation rate

FIGURE 3 | The simulation result for predicting eDNA degradation rate on the matrix of water temperature and amplicon length.

was higher in longer amplicon length (719 bp) than in shorter amplicon (127 bp). Our simulation by QM indicated that shorter amplicon lengths were more likely to be detected when eDNA degradation was less affected by water temperature. When the eDNA degradation rates were very fast or very slow due to water temperature (e.g., 15 to 35 °C or 0 to 5°C, respectively), the amplicon length had a smaller effect on eDNA degradation than at other water temperature ranges. In higher temperatures, microbial activity that breaks down DNA is occurring fast on both large and short DNA fragments, such that both classes of fragments are not detectable by either a large or small fragment amplicon assay at a similar rate. Whereas in colder temperatures, both fragment classes are degraded at lower rates, and thus it is possible that the longer fragments are able to last longer than under warmer conditions, thus remaining detectable for longer (suggesting a slower decay rate).

In our meta-analysis, we evaluated amplicon lengths ranging from 70 to 719 bp, but there were no experiments in which longer amplicon were measured. Recently, however, long range PCR was used to amplify full mitogenomes from eDNA samples (Deiner et al., 2017a,b). Additional investigation is needed to better understand retention of such extremely long DNA (> 16,000 bps), and the role of degradation in these cases.

CONCLUSION

In conclusion, our meta-analysis results showed that eDNA degradation was accelerated in higher water temperatures and

in longer DNA amplicon. We predicted the combined effects of water temperature and amplicon length on the maximum eDNA degradation rate. Our meta-analysis and simulation provided new insights for future eDNA studies. We should note the limitations: The number of papers used for our meta-analysis was limited to 28 studies, and the data was limited especially for other environmental factors, such as UV, pH, and salinity, which are important factors for eDNA degradation (Barnes et al., 2014; Lance et al., 2017; Tsuji et al., 2017; Collins et al., 2018; Mächler et al., 2018). When data such as UV, pH, and salinity are obtained in addition to water temperature, more complex phenomena can be evaluated to determine the eDNA degradation rate in water. A greater understanding and accumulation of eDNA degradation data would improve future eDNA methods.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TS and HD designed the study, analyzed the data, interpreted the results, and wrote the manuscript. TS collected the data. Both authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.623831/full#supplementary-material

Supplementary Figure 1 | The eDNA degradation rate (simple exponential slope) with the targeted taxon group. The dots indicate the individual eDNA degradation rate in each experiment. The boxes and bars in the box plot indicate median ± inter-quartiles and ± 1.5 × inter-quartiles, respectively.

Supplementary Figure 2 | The eDNA degradation rate (simple exponential slope) with (A) DNA region and (B) water source. In the plot, the limited data were excluded; NADH, D-loop, ND2, and ND4 for PCR region and pond, well for water source. The dots indicate the individual eDNA degradation rate in each experiment in different ecosystems. The boxes and bars in the box plot indicate median ± inter-quartiles and ± 1.5 × inter-quartiles, respectively.

Supplementary Figure 3 | The eDNA degradation rate (simple exponential slope) with temperature category. The temperature categories are divided into four levels: −1, 0–10, 11–20, and > 21 °C. The dots indicate the individual eDNA degradation rate in each experiment in different ecosystems. The boxes and bars in the box plot indicate median ± inter-quartiles and ± 1.5 × inter-quartiles, respectively.

Supplementary Figure 4 | The eDNA degradation rate (simple exponential slope) with amplicon length category. The amplicon length categories are divided into three levels: 0–100, 101–200, and > 201. The dots indicate the individual eDNA degradation rate in each experiment in different ecosystems. The boxes and bars in the box plot indicate median ± inter-quartiles and ± 1.5 × inter-quartiles, respectively.

Supplementary Table 1 | All data analyzed in this meta-analysis.

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