Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses

Toshiaki Jo[1] and Toshifumi Minamoto[1]

[1]Kobe University

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Abstract

Understanding the processes of environmental DNA (eDNA) persistence and degradation is essential to determine the spatiotemporal scale of eDNA signals and accurately estimate species distribution. The effects of environmental factors on eDNA persistence have previously been examined; however, the influence of the physiochemical and molecular states of eDNA on its persistence is not completely understood. Here, we performed meta-analyses including 26 previously published papers on the estimation of first-order eDNA decay rate constants, and assessed the effects of filter pore size, DNA fragment size, target gene, and environmental conditions on eDNA decay rates. Almost all supported models included the interactions between the filter pore size and water temperature, between the target gene and water temperature, and between the target gene and water source, implying the influence of complex interactions between the eDNA state and environmental conditions on eDNA persistence. These findings were generally consistent with the results of a re-analysis of a previous tank experiment which measured the time-series changes in marine fish eDNA concentrations in multiple size fractions after fish removal. Our results suggest that the mechanism of eDNA persistence and degradation cannot be fully understood without knowing not only environmental factors but also cellular and molecular states of eDNA in water. Further verification of the relationship between eDNA state and persistence is required by obtaining more information on eDNA persistence in various experimental and environmental conditions, which will enhance our knowledge on eDNA persistence and support our findings.

Introduction

Organisms release their DNA molecules into their surroundings, which are termed as environmental DNA (eDNA) (Levy-Booth et al., 2007; Nielsen et al., 2007; Taberlet et al., 2012). The analysis of eDNA has recently been applied to monitor the abundance and composition of macro-organisms, such as fish and amphibians (Ficetola et al., 2008; Minamoto et al., 2012; Bohmann et al., 2014; Deiner et al., 2017; Jo et al., 2020a). Detection of eDNA in water samples does not involve any damage to the target species and their habitats, thus enabling non-invasive and cost-effective monitoring of species in aquatic environments, contrary to traditional monitoring methods such as capturing and observing (Darling & Mahon, 2011). However, the characteristics and dynamics of eDNA are not yet completely understood, and thus, the spatiotemporal scale of eDNA signals at a given sampling time and location is not certain, which can result in false-positive or false-negative detection of eDNA in natural environments (Darling & Mahon, 2011; Hansen et al., 2018; Beng & Corlett, 2020).

To determine the spatiotemporal scale of eDNA signals and accurately estimate species presence/absence and abundance in the environment, understanding the processes of eDNA persistence and degradation is important. Aqueous eDNA is detectable from days to weeks (Barnes & Turner, 2016; Collins et al., 2018), depending on various environmental factors. For example, moderately high temperature (Strickler et al., 2015; Eichmiler et al., 2016; Lance et al., 2017; Jo et al., 2020b) and low pH (Strickler et al., 2015; Lance

et al, 2017; Seymour et al., 2018) accelerate eDNA degradation. In addition, eDNA decay rates are higher in environments with higher species biomass density (Bylemans et al., 2018; Jo et al., 2019a). These abiotic and biotic factors contribute to the increase in microbial activities and abundance in water, thus indirectly affecting eDNA degradation (Strickler et al., 2015). Moreover, eDNA decay rates were found to be different between the trophic states of studied lakes, and were negatively correlated with the dissolved organic carbon (DOC) concentrations (Eichmiller et al., 2016). This may be attributed to the binding of DNA molecules to humic substances, protecting eDNA from enzymatic degradation.

However, apart from the effects of such environmental conditions, little is known about the influence of the physiochemical and molecular states of eDNA on its persistence and degradation. Fish eDNA has been detected at various size fractions (<0.2 μm to >180 μm in diameter; Turner et al., 2014; Jo et al., 2019b) in water, suggesting that eDNA is present as various states and cellular structures, from larger-sized and intra-cellular DNA (e.g., cell and tissue fragments) to smaller-sized and extra-cellular DNA (e.g., organelles and dissolved DNA). Enzymatic and chemical degradation of DNA molecules in the environment depends on the presence of cellular membranes around the DNA molecules, and thus, the persistence of eDNA is likely to be linked to its state. In addition, eDNA persistence may be different depending on the target genetic regions. Recent studies have suggested that eDNA decay rates may vary between mitochondrial and nuclear DNA (Bylemans et al., 2018; Moushomi et al., 2019; Jo et al., 2020b). Moreover, studies comparing eDNA degradation between different target DNA fragment lengths (i.e. PCR amplification length) have yielded inconsistent conclusions; Jo et al. (2017) and Wei et al. (2018) reported higher eDNA decay rates for longer DNA fragments, whereas Bylemans et al. (2018) did not observe any difference in the eDNA decay rates of different DNA fragment sizes. Notably, Jo et al. (2020c) reported that selective collection of larger-sized eDNA using a larger pore size filter increased the ratio of long to short eDNA concentrations and altered the ratio of nuclear to mitochondrial eDNA concentrations; however, such reports linking eDNA state to its persistence are scarce.

Although our understanding of the relationship between eDNA state and persistence is currently limited, this relationship can be inferred by integrating previous findings of eDNA persistence and degradation. Here, we used meta-analyses to examine the relationship between eDNA states and persistence. We extracted data on filter pore size, DNA fragment size, target gene, and environmental parameters from previous studies estimating first-order eDNA decay rate constants, and investigated the influence of these factors on eDNA degradation. By assembling and integrating the results of previous eDNA studies, our meta-analyses revealed the hitherto unknown relationships between eDNA state and persistence, which could not have been observed in the individual studies. Furthermore, we assessed the validity of the findings of the meta-analyses by re-analysing the dataset from a previous tank experiment (Jo et al., 2019b).

Materials and methods

1. Literature search and data extraction

We searched for literature relating to eDNA persistence and degradation, published during 2008 to 2020 (final date for the literature search was 20 Jun 2020), using Google Scholar (*https://scholar.google.co.jp/*). The terms "eDNA" or "environmental DNA", included in the title and/or text, were used for the literature search. We then filtered and selected papers that (i) targeted eDNA from macro-organisms (i.e. not from microbes, fungi, plankton, virus, and bacteria), (ii) were written in English, (iii) were peer-reviewed (i.e. not preprints), and (iv) described aqueous eDNA decay rate constants using a first-order exponential decay model ($C_t = C_0 e^{-kt}$, where C_t is the eDNA concentration at time t, C_0 is the initial eDNA concentration, and k is the first-order decay rate constant). The eDNA decay rate constants estimated using multi-phasic exponential decay models (e.g. biphasic or Weibull models) (Eichmiller et al., 2016; Bylemans et al., 2018; Wei et al., 2018) were not included in our meta-analyses, because of the limited number of such studies and difficulty in directly comparing the constants between first-order and multi-phasic models.

From the filtered eDNA studies, we then extracted data on the eDNA decay rate constant (per hour), filter pore size used for water filtration (μm), target DNA fragment size (base pair; bp), and target gene

(mitochondrial or nuclear). The decay rate constant was converted to "per hour" if it was originally described as "per day". Different eDNA decay rate constants based on different experimental conditions within the same study (e.g. species, temperature, pH, and biomass density) were treated separately. The filter pore size in studies involving aqueous eDNA collection *via* ethanol precipitation or centrifugation was regarded as 0 μm. In addition, we extracted information on the water temperature (°C), water source used for experiments, and target species and taxa. Although other biotic and abiotic factors are known to affect eDNA degradation, we extracted only temperature and water source data, because of their consistent and informative descriptions in all selected papers (i.e. other water physicochemical parameters such as pH, conductivity, and dissolved oxygen were sometimes not specified in the paper). If necessary, we used the mean temperature obtained by averaging the maximum and minimum temperatures during the experimental period. Water source was classified as 'artificial', including tap water and distilled water (DW); 'freshwater', including wells, ponds, lakes, and river water; and 'seawater', including harbour, inshore, and offshore seawaters. Because Moushomi et al. (2019) had estimated decay rates of *Daphnia magna*eDNA at each size fraction, we calculated total eDNA concentrations collected by a 0.2 μm pore size filter and ethanol precipitation, and re-estimated the eDNA decay rates (Appendix S1).

2. Statistical analyses

All statistical analyses were performed in R version 3.6.1 (R Core Team, 2019). We first performed a generalized linear model (GLM) with Gaussian distribution to assess the relationship between eDNA persistence, eDNA state, and environmental conditions. The eDNA decay rate constants (per hour) were treated as the dependent variable, and the filter pore size (μm), DNA fragment size (bp), target gene (mitochondrial or nuclear), water temperature (°C), water source (artificial, freshwater, or seawater), and their primary interactions were included as the explanatory variables. We first confirmed that the multi-collinearity among the variables was negligible (1.028 to 1.096), by calculating the generalized variance inflation factors (GVIF). We then selected models based on Akaike's Information Criterion (AIC), using the *dredge*function in the 'MuMIn' package in R (Bartoń, 2019). We adopted the model with the smallest AIC value, and all models with [?]AIC (i.e. difference in the AIC value) less than two were selected as the supported models (Burnham & Anderson, 2002).

We performed an additional meta-analysis to examine the relationship between the DNA fragment size and eDNA decay rate constant. Most eDNA studies conducted to date have targeted short DNA fragments (<200 bp), and only three papers have reported eDNA decay rates targeting longer DNA fragments (>200 bp); however, they yielded inconsistent conclusions (Tables 1 & S1). Taking this into consideration and targeting eDNA decay rate constants derived from <200 bp DNA fragments, we performed a linear regression to assess the effect of DNA fragment size on eDNA degradation.

3. Re-analysis of the time-series changes in eDNA particle size distribution

To assess the validity of the findings of the meta-analyses, we re-analysed the dataset from a previous study investigating the particle size distribution of eDNA derived from the mitochondria and nuclei of Japanese jack mackerel (*Trachurus japonicus*) and the time-series changes therein, after fish removal from tanks (Jo et al., 2019b). In the aforementioned study, mitochondrial and nuclear eDNA degradation was examined under multiple size fractions, and both degradations tended to be suppressed at smaller size fractions. We estimated the eDNA decay rate constants at different size fractions using the dataset from the said study, and assessed the variation in eDNA decay rates depending on the eDNA particle size, target gene, and water temperature. Detailed information on the experimental design, water sampling, and molecular analyses can be found in Jo et al. (2019b).

We included all eDNA samples that could pass through sequential filters with 10, 3, 0.8, and 0.2 μm pore sizes at 0, 6, 12, and 18 hours, which yielded four eDNA size fractions, i.e. >10, 3-10, 0.8-3, and 0.2-0.8 μm. Linear regressions were performed between eDNA concentrations (original concentration + 1 followed by log-transformation) and sampling time points for each size fraction, target gene (mitochondrial or nuclear), and temperature level (13, 18, 23, or 28 °C), to estimate the slope (i.e. eDNA decay rate constant) and

the corresponding 95% CI, using *lm* and *confint* functions in R, respectively. Here, the two fish biomass levels (Small and Large; see Jo et al. (2019b)) were pooled to increase the sample size. We then performed ANOVA to assess the relationship between eDNA degradation, particle size, target gene, and temperature. We included the median of the slope (eDNA decay rate) as the dependent variable, and the filter pore size, target gene, water temperature, and their primary interactions as the explanatory factors.

Results

1. Literature review

We selected 26 published papers in total, including 106 eDNA decay rate constants, ranging from 0.0005 to 0.6969 (per hour) (Tables 1 & S1). The filter pore size, DNA fragment size, and water temperature ranged from 0 to 3 μm, 70 to 719 bp, and -1.0 to 36.0°C, respectively. The number of eDNA decay rate constants derived from mitochondrial and nuclear genes were 89 and 17, respectively, and those derived from artificial water, freshwater, and seawater sources were 31, 15, and 60, respectively. Most studies reported eDNA decay rates targeting freshwater and marine fishes, whereas only few papers reported decay rates targeting amphibians and other invertebrates.

2. Model selection

In the full model, interactions between filter pore size and water temperature and between target gene and water temperature were statistically significant (both $P < 0.05$), and effects of the filter pore size and interaction between fragment size and water source were marginally significant (both $P < 0.1$) (Table 2). All supported models resulting from model selection included the effects of filter pore size, target gene, and water source, whereas the effects of fragment size and temperature were uncertain, owing to their small coefficient and large SE. However, we focused on the effects of the interactions among variables; all supported models included interactions between filter pore size and temperature (Figure 1) and between target gene and temperature (Figure 2). In addition, 11 of the 13 models included the interaction between target gene and water source (Figure 3), and four models included the interaction between filter pore size and water source (Figure S1). Other interactions were included in less than three supported models, and the uncertainties of the corresponding coefficients were relatively large.

Although DNA fragment size was included in most supported models, its effect was relatively small due to its high variability (Table 2). Considering the smaller number of eDNA decay rate constants targeting longer DNA fragments as mentioned previously, we instead assessed the relationship between the eDNA decay rate and shorter DNA fragment size (<200 bp). Consequently, the fragment size was found to have a significantly positive effect on the decay rate ($P < 0.01$; Figure S2).

3. Re-analysis of the time-series changes in eDNA particle size distribution

The ANOVA test showed that all factors significantly affected the eDNA decay rate constants (all $P < 0.001$, Table 3). Decay rate constants tended to be lower in smaller size fractions and at lower temperature levels, and were higher for nuclear than for mitochondrial genes (Figure 4). In addition, the interaction between filter pore size and temperature was a significant factor affecting the decay rate constant ($P < 0.01$), and interaction between target gene and temperature was marginally significant ($P = 0.0902$). Decay rates of eDNA were smaller for smaller size fractions, and there was a greater tendency to decay at higher temperature levels than at lower levels.

Discussion

Most studies conducted in the past decade have focused on the relation of eDNA persistence with environmental conditions, and little attention has been paid to the relationship between the persistence of eDNA and its cellular states and molecular structures. In the present study, we integrated the findings of previous reports on eDNA and provided new insights into the relationship between the persistence and state of eDNA. Our findings indicated significant influences of the complex interactions between eDNA states and environmental factors on eDNA persistence.

1. Meta-analyses of eDNA literature

Our meta-analyses showed that filter pore size, water temperature, target gene, and water source could influence eDNA degradation, not as individual parameters but in conjunction. We focused on three substantial interactions that were included in almost all supported models. Firstly, the interaction between filter pore size and water temperature influenced eDNA decay rates (Figure 1). Considering that a larger pore size filter can selectively collect eDNA particles in larger size fractions, our result implied that higher water temperature could accelerate the degradation of eDNA in larger size fractions by a greater degree than that in smaller size fractions. However, it is unlikely that smaller-sized eDNA itself is less affected by higher temperature-mediated degradation, and its apparent persistence can be increased by the inflow of eDNA from larger to smaller size fractions, as described in Jo et al. (2019b). Organic matter in water, including eDNA, is degraded by microbes and extra-cellular enzymes in the environment for uptake, and their activities are promoted by moderately high temperatures (less than 50°C) (Price & Sowers, 2004; Nielsen et al., 2007; Arnosti, 2014; Strickler et al., 2015). During the degradation processes, aqueous eDNA in larger size fractions, such as intra-cellular DNA, is believed to flow into smaller size fractions, such as extra-cellular DNA. This suggests that water temperature does not uniformly influence the apparent degradation of eDNA among the different size fractions, and the effect of temperature on eDNA degradation might be buffered in smaller-sized eDNA particles. Thus, the effect of temperature on eDNA degradation would be smaller when using a smaller pore size filter and collecting eDNA particles at various size fractions.

Secondly, the interaction between the target gene (nuclear or mitochondrial) and water temperature influenced the eDNA decay rates; higher water temperature could accelerate the degradation of nuclear eDNA by a greater extent when compared with mitochondrial DNA (Figure 2). This may be attributed to the difference in the protection conferred to the DNA molecules against the attack of extra-cellular enzymes in the environment by the outer nuclear and mitochondrial membranes. In contrast to mitochondrial DNA, which is surrounded by a non-porous outer membrane (Ernster & Schatz, 1981), nuclear DNA is enclosed in a porous membrane (45-50 nm in diameter; Fahrenkrog & Aebi, 2003), rendering it more susceptible to environmental extra-cellular enzymes, and thus, more likely be degraded by a greater degree at higher temperatures (Price & Sowers, 2004; Strickler et al., 2015). However, these results should be interpreted with caution, because the number of nuclear eDNA decay rate constants (n = 17) included was considerably lower than that of mitochondrial eDNA decay rate constants (n = 89). It is necessary to estimate nuclear eDNA decay rates in various environmental and experimental conditions in the future, which would enable a more robust comparison of eDNA degradation between nuclear and mitochondria DNA.

Thirdly, the interaction between the target gene and water source influenced the eDNA decay rates (Figure 3). Although the effects of water source on eDNA degradation differed between nuclear and mitochondrial DNA, it was evident that eDNA degradation was suppressed in artificial waters, such as tap water and DW, when compared to that in natural waters. Eichmiller et al. (2016) compared the degradation of common carp (*Cyprinus carpio*) eDNA in natural waters with different trophic states, and found that eDNA decay rates in well water were lower than those in eutrophic and oligotrophic waters, which could be attributed to the lower microbial activity in the former. Our results were generally consistent with those of Eichmiller et al. (2016). Using tap water and DW as water sources can lead to underestimation of eDNA persistence in the natural environment. Moreover, no significant difference could be observed in the eDNA decay rates between freshwater and seawater. The difference in eDNA persistence between freshwater and seawater has previously been reported; some studies indicated faster eDNA degradation in seawater than in freshwater (Thomsen et al., 2012; Sassoubre et al., 2016), whereas Collins et al. (2018) showed that eDNA degradation was higher in terrestrially-influenced inshore waters than in ocean-influenced offshore environments. Marine systems are generally characterized by higher salinity and ionic content, higher pH, and more stable temperatures when compared with freshwater systems, which can promote DNA preservation in water (Okabe & Shimazu, 2007; Schulz & Childers, 2011; Collins et al., 2018). However, the direct effects of microbial abundance and composition and other physicochemical parameters of water were not included in our meta-analyses. Thus, greater variations in eDNA decay rates in seawater when compared with artificial water and freshwater observed in our meta-analyses might partly be explained by such microbial and physicochemical conditions.

The effects of various nutrient salts and microbial activities on eDNA persistence and differences in the eDNA degradation processes between freshwater and seawater systems require further investigation.

The interaction between filter pore size and water source influenced the eDNA decay rates in some supported models; however, its effect was relatively smaller when compared with those of the interactions discussed above (Table 2). The water source might affect the apparently longer persistence of smaller-sized eDNA described previously. Although no linear regressions were statistically significant (Figure S1; $P > 0.05$), the increase in eDNA decay rates with larger filter pore sizes appeared to be greater in seawater than in artificial water, which might also be attributed to the differences in microbial activities among the different water sources.

Contrary to these four factors, model selection in the present study did not strongly support the effects of DNA fragment size and its interactions with other variables on the eDNA decay rate (Table 2), which may be due to the potential bias of DNA fragment sizes in the eDNA studies included in the meta-analysis. Only three studies have previously estimated eDNA decay rates in water targeting longer DNA fragments (>200 bp) (aqueous eDNA; Jo et al. 2017; Weltz et al., 2017; Bylemans et al., 2018), and there was no consensus on the relationship between eDNA degradation and DNA fragment size among these studies. Although our additional meta-analysis, which targeted only shorter DNA fragments (70 to 190 bp), supported rapid eDNA degradation in longer DNA fragments, as suggested by Jo et al. (2017) and Wei et al. (2018), the analysis might be considered slightly arbitrary, and thus, the validity of the result would need to be tested in the future. Interactions between DNA fragment size and other factors may become evident when more information is available on eDNA persistence and degradation at different fragment sizes.

2. Re-analysis of the time-series changes in eDNA particle size distribution

Our meta-analyses provided new insights into the relationship between eDNA persistence and its state. We then re-analysed the dataset from a previous tank experiment (Jo et al., 2019b) to estimate mitochondrial and nuclear eDNA decay rates at multiple size fractions and water temperature levels. The results of the re-analysis appeared to be generally consistent with those of the meta-analyses; as indicated by the meta-analyses, eDNA persistence depended on the interactions between its size fraction, type of the target gene, and water temperature (Table 3; Figure 4). In particular, a significant interaction between filter pore size and temperature indicated that inflow of the degraded, larger-sized eDNA into smaller size fractions could buffer the effect of temperature on eDNA degradation in these smaller size fractions, as described in previous sections. The dependence of eDNA degradation on water temperature would likely be smaller when targeting smaller-sized eDNA or using a smaller pore size filter.

Some recent studies attempted to estimate species biomass and abundance by integrating quantitative eDNA analysis and hydrodynamic modelling, allowing the consideration of eDNA dynamics, such as its production, transport, and degradation (Carraro et al., 2018; Tillotson et al., 2018; Fukaya et al., 2020). For a more accurate estimation, environmental parameters affecting these eDNA dynamics may be included in the statistical modelling framework. The effect of temperature on eDNA degradation can be minimized during statistical modelling by considering eDNA particles at smaller fraction sizes, which will allow simplification of the modelling procedure while retaining its accuracy and reliability. However, considering the apparent suppression of eDNA degradation in smaller size fractions, owing to the inflow of the degraded larger-sized eDNA, it is possible that such smaller-sized eDNA yield 'older and less fresh' biological signals than the larger-sized eDNA. Such non-fresh eDNA signals can result in false-positives during eDNA detection (Yamamoto et al, 2016; Jo et al., 2017), in which case the use of eDNA particles in the smaller size fractions would be disadvantageous for eDNA-based biomass or abundance estimation. The applicability of smaller-sized eDNA for such estimations can be verified by comparing the correlation between eDNA quantification and species biomass and abundance, and the availability of longer eDNA fragments among the filter pore sizes or eDNA particle sizes, for which meta-analyses such as the present study may be suitable.

3. Limitations and perspectives

We noted some potential biases and limitations of the dataset used in our meta-analyses. Firstly, studies

estimating the decay rates of nuclear eDNA were substantially fewer when compared with those on mitochondrial eDNA, particularly in freshwater systems (Figure 3), which might limit our ability to infer the effect of water source on eDNA degradation between the target genes. In addition, eDNA decay rates targeting longer DNA fragments (>200 bp) and taxa other than fish were relatively scarce. Moreover, estimation of eDNA decay rates using a 0.7 μm pore size filter appeared to be relatively more common, which suggests greater knowledge of eDNA persistence in this filter pore size, and a potential bias in our meta-analyses. It is expected that eDNA analysis will be applied to ecological monitoring of more varied taxa and environments in the future, and will have to be developed accordingly to determine the spatiotemporal scale of eDNA signals and to maximize the biological information obtained from eDNA samples. More information on eDNA persistence and degradation should therefore be collected, by targeting different taxa and environments and using various collection and analysis methods.

Although our findings and their implications require further verification, this study is the first to propose that the persistence of eDNA from macro-organisms can be determined by the state of the eDNA and its complex interactions with environmental conditions, i.e. the mechanism of eDNA persistence and degradation cannot be fully understood without knowing not only the environmental biotic and abiotic factors involved in eDNA degradation but also the cellular and molecular states of eDNA occurring in water. If our findings are correct, the spatiotemporal scale and intensity of eDNA signals would be different depending on the eDNA particle size and state. The fact that Weibull or biphasic exponential decay models fit better to eDNA degradation implies the differences in eDNA persistence depending its state (e.g., intra- or extra-cellular, living or dead cells, particulate or dissolved) (Eichmiller et al., 2016; Bylemans et al., 2018), which support our results linking eDNA persistence to its state. In addition, the study by Jo et al. (2020c), where it was reported that the genomic information obtained from eDNA samples can differ depending on the filter pore size, can further support the link between eDNA state and persistence. Experimental verification of our findings and implications will highlight the importance of clarifying the characteristics and dynamics of aqueous eDNA, and will contribute substantially to the development of eDNA analysis in the future.

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Supplemental Information

Appendix S1. Re-estimation of eDNA decay rates in Moushomi et al. (2019).

Table S1. Detailed information on published studies estimating first-order eDNA decay rate constants.

Figure S1. The effects of water source and filter pore size on eDNA decay rate constant.

Figure S2. The effects of DNA fragment size on eDNA decay rate constant.

Data accessibility

Detailed information on published studies estimating first-order eDNA decay rate constants can be found in Supplemental Information.

Author contribution

Both authors designed the study. T.J. performed a literature search, analysed the data, and wrote the first draft of the manuscript. Both authors edited and provided feedback for the manuscript.

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Tables

Table 1. Published literature on the estimation of first-order eDNA decay rate constants included in the present study.

Study	# Decay rate constant	Filter pore size [µm]	Fragment size [bp]	Target gene	Temperature [°C]	Water source	Target taxa
Thomsen et al. (2012)	2	0.45	101 to 104	mt	15	Seawater	Fish
Barnes et al. (2014)	1	1.2	146	mt	25	Freshwater	Fish
Maruyama et al. (2014)	1	0	100	mt	20	Artificial	Fish
Strickler et al. (2015)	3	0.45	84	mt	5 to 35	Artificial	Amphibian
Eichmiller et al. (2016)	4	0.2	149	mt	5 to 35	Freshwater	Fish
Forsström & Vasemägi (2016)	1	0	75	mt	17	Artificial	Crustacean
Sassoubre et al. (2016)	5	0.2	107 to 133	mt	19 to 22	Seawater	Fish
Andruszkiewicz et al. (2017)	2	0.22	107	mt	17	Seawater	Fish
Jo et al. (2017)	2	0.7	127 to 719	mt	26	Seawater	Fish

Study	# Decay rate constant	Filter pore size [μm]	Fragment size [bp]	Target gene	Temperature [°C]	Water source	Target taxa
Lance et al. (2017)	4	0.22	190	mt	4 to 30	Artificial	Fish
Minamoto et al. (2017)	1	0.7	151	mt	19	Seawater	Invertebrate
Sansom & Sassoubre (2017)	6	0.4	147	mt	22	Artificial	Invertebrate
Sigsgaard et al. (2017)	2	0.22	105	mt	35 to 36	Seawater	Fish
Tsuji et al. (2017)	6	0.7	78 to 131	mt	10 to 30	Freshwater	Fish
Weltz et al. (2017)	2	0.45	331	mt	4	Seawater	Fish
Bylemans et al. (2018)	12	1.2	95 to 515	mt & nu	20	Artificial	Fish
Collins et al. (2018)	8	0.22	132 to 153	mt	10 to 15	Seawater	Fish & Crustacean
Cowart et al. (2018)	1	0.45	70	mt	-1	Seawater	Fish
Nevers et al. (2018)	2	1.5	150	mt	12 to 19	Seawater	Fish
Nukazawa et al. (2018)	2	0.7	149	mt	21 to 22	Freshwater	Fish
Jo et al. (2019)	12	0.7	127	mt	13 to 28	Seawater	Fish
Moushomi et al. (2019)	4	0 to 0.2	101 to 128	mt & nu	20	Artificial	Invertebrate
Sengupta et al. (2019)	1	0	86	mt	23	Artificial	Invertebrate
Jo et al. (2020)	12	0.7	164	nu	13 to 28	Seawater	Fish
Kasai et al. (2020)	5	0.7	138	mt	10 to 30	Seawater	Fish
Sakata et al. (2020)	1	0.7	132	mt	17	Freshwater	Fish
Wood et al. (2020)	4	3	90 to 150	mt	19	Seawater	Invertebrate

Note: Abbreviations 'mt' and 'nu' indicate mitochondrial and nuclear DNA, respectively. Filter pore size in studies collecting eDNA*via* ethanol precipitation or centrifugation was regarded as 0 μm.

Table 2. Results of model selection for the effects of filter pore size, DNA fragment size, target gene,

temperature, and water source on the first-order eDNA decay rates.

Variable	GVIF	Full model	Full model	Full model	Model_1	Model_1	Model_2	Mod
		Coeff.	SE	P value	Coeff.	SE	Coeff.	SE
Intercept		**0.0506**	0.0975	0.6050	**0.0358**	0.0552	**0.0506**	0.05
Filter pore size	1.0308	**-0.2269**	0.1341	0.0942	**-0.2058**	0.0993	**-0.2933**	0.10
Fragment size	1.0440	**0.0004**	0.0005	0.3889	**-0.0002**	0.0001		
Gene (nu)	1.0472	**-0.3073**	2.5630	0.9048	**-0.3591**	0.1010	**-0.3268**	0.10
Temperature	1.0281	**-0.0043**	0.0038	0.2612	**-0.0008**	0.0026	**-0.0012**	0.00
Water source (fre)	1.0955	**0.1909**	0.1567	0.2266	**0.0571**	0.0272	**0.0525**	0.05
Water source (sea)		**0.0308**	0.0791	0.6982	**0.0858**	0.0207	**0.0452**	0.02
Filter pore size: Fragment size		**-0.0004**	0.0004	0.2547				
Filter pore size: Gene (nu)		**0.0034**	0.5853	0.9953				
Filter pore size: Temperature		**0.0138**	0.0056	0.0151	**0.0130**	0.0052	**0.0142**	0.00
Filter pore size: Water source (fre)		**-0.0164**	0.0948	0.8632			**0.0238**	0.08
Filter pore size: Water source (sea)		**0.0709**	0.0466	0.1318			**0.0783**	0.03
Fragment size: Gene (nu)		**-0.0001**	0.0196	0.9969				
Fragment size: Temperature		**0.0000**	0.0000	0.7966				
Fragment size: Water source (fre)		**-0.0015**	0.0009	0.0796				
Fragment size: Water source (sea)		**-0.0004**	0.0003	0.1526				
Gene (nu): Temperature		**0.0149**	0.0047	0.0023	**0.0162**	0.0046	**0.0158**	0.00
Gene (nu): Water source (fre)		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Gene (nu): Water source (sea)		**0.3064**	1.0600	0.7731	**0.3239**	0.0491	**0.2966**	0.04
Temperature: Water source (fre)		**0.0041**	0.0039	0.2964				
Temperature: Water source (sea)		**0.0036**	0.0033	0.2786				
AIC		-208.16			-217.38		-217.08	
AIC		9.22			0.00		0.30	

(Table 2 continued)

Variable	Model_4	Model_4	Model_5	Model_5	Model_6	Model_6	Model_7	Model_7
	Coeff.	SE	Coeff.	SE	Coeff.	SE	Coeff.	SE
Intercept	**0.0647**	0.0585	**-0.0048**	0.0838	**0.0388**	0.0553	**0.0387**	0.0553
Filter pore size	**-0.3266**	0.1109	**-0.2579**	0.1257	**-0.2103**	0.0996	**-0.2103**	0.0996
Fragment size	**0.0000**	0.0001	**0.0005**	0.0004	**-0.0002**	0.0001	**-0.0002**	0.0001
Gene (nu)	**-0.3173**	0.1013	**-0.3193**	0.1011	**-0.3116**	0.1145	**-0.5802**	0.2718
Temperature	**-0.0019**	0.0026	**-0.0014**	0.0026	**-0.0010**	0.0026	**-0.0010**	0.0026
Water source (fre)	**0.2557**	0.1359	**0.2711**	0.1363	**0.0567**	0.0272	**0.0567**	0.0272
Water source (sea)	**0.0725**	0.0379	**0.0982**	0.0439	**0.0852**	0.0207	**0.0852**	0.0207
Filter pore size: Fragment size			**-0.0004**	0.0004				
Filter pore size: Gene (nu)					**-0.0615**	0.0697		
Filter pore size: Temperature	**0.0159**	0.0053	**0.0152**	0.0053	**0.0134**	0.0052	**0.0134**	0.0052
Filter pore size: Water source (fre)	**-0.0238**	0.0894	**-0.0164**	0.0895				
Filter pore size: Water source (sea)	**0.0789**	0.0388	**0.0738**	0.0390				
Fragment size: Gene (nu)							**0.0021**	0.0023
Fragment size: Temperature								
Fragment size: Water source (fre)	**-0.0014**	0.0008	**-0.0015**	0.0009				
Fragment size: Water source (sea)	**-0.0002**	0.0002	**-0.0004**	0.0002				
Gene (nu): Temperature	**0.0153**	0.0046	**0.0153**	0.0046	**0.0161**	0.0046	**0.0161**	0.0046
Gene (nu): Water source (fre)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Variable	Model_4	Model_4	Model_5	Model_5	Model_6	Model_6	Model_7	Model_7
Gene (nu): Water source (sea)	**0.2998**	0.0500	**0.3029**	0.0500	**0.3213**	0.0493	**0.2106**	0.1383
Temperature: Water source (fre)								
Temperature: Water source (sea)								
AIC	-216.71		-216.25		-216.25		-216.23	?¿?
AIC	0.67		1.13		1.13		1.15	

(Table 2 continued)

Variable	Model_8	Model_8	Model_9	Model_9	Model_10	Model_10	Model_11	Model
	Coeff.	SE	Coeff.	SE	Coeff.	SE	Coeff.	SE
Intercept	**0.0387**	0.0553	**0.0142**	0.0636	**0.0383**	0.0557	**0.0932**	0.0685
Filter pore size	**-0.2103**	0.0996	**-0.1781**	0.1074	**-0.2174**	0.1001	**-0.1777**	0.1013
Fragment size	**-0.0002**	0.0001	**0.0000**	0.0002	**-0.0002**	0.0001	**-0.0002**	0.0001
Gene (nu)	**-1.0870**	0.1886	**-0.3626**	0.1014	**-0.9222**	0.1542	**-0.3484**	0.1026
Temperature	**-0.0010**	0.0026	**-0.0006**	0.0026	**-0.0012**	0.0026	**-0.0036**	0.0033
Water source (fre)	**0.0568**	0.0272	**0.0535**	0.0278	**0.0594**	0.0274	**-0.0314**	0.0825
Water source (sea)	**0.0853**	0.0207	**0.0818**	0.0215	**0.0892**	0.0207	**0.0001**	0.0660
Filter pore size: Fragment size			**-0.0002**	0.0003				
Filter pore size: Gene (nu)	**0.1140**	0.0762						
Filter pore size: Temperature	**0.0134**	0.0052	**0.0128**	0.0052	**0.0139**	0.0052	**0.0115**	0.0053
Filter pore size: Water source (fre)								
Filter pore size: Water source (sea)								
Fragment size: Gene (nu)	**0.0059**	0.0009			**0.0054**	0.0008		
Fragment size: Temperature								
Fragment size: Water source (fre)								
Fragment size: Water source (sea)								
Gene (nu): Temperature	**0.0161**	0.0046	**0.0161**	0.0046	**0.0160**	0.0047	**0.0157**	0.0047
Gene (nu): Water source (fre)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Gene (nu): Water source (sea)			**0.3276**	0.0496			**0.3213**	0.0492
Temperature: Water source (fre)							**0.0044**	0.0039
Temperature: Water source (sea)							**0.0044**	0.0032
AIC	-216.14		-215.91		-215.68		-215.61	?¿?
AIC	1.24		1.47		1.70		1.77	

(Table 2 continued)

Variable	Model_12	Model_12	Model_13	Model_13
	Coeff.	SE	Coeff.	SE
Intercept	**-0.0051**	0.0514	**-0.0558**	0.0809
Filter pore size	**-0.1851**	0.1000	**-0.1340**	0.1117
Fragment size			**0.0005**	0.0004
Gene (nu)	**-0.3504**	0.1022	**-0.3527**	0.1010
Temperature	**-0.0001**	0.0026	**-0.0002**	0.0026
Water source (fre)	**0.0670**	0.0270	**0.2148**	0.1109
Water source (sea)	**0.0932**	0.0205	**0.1282**	0.0418
Filter pore size: Fragment size			**-0.0005**	0.0004
Filter pore size: Gene (nu)				

Variable	Model_12	Model_12	Model_13	Model_13
Filter pore size: Temperature	**0.0117**	0.0052	**0.0125**	0.0052
Filter pore size: Water source (fre)				
Filter pore size: Water source (sea)				
Fragment size: Gene (nu)				
Fragment size: Temperature				
Fragment size: Water source (fre)			**-0.0012**	0.0008
Fragment size: Water source (sea)			**-0.0003**	0.0002
Gene (nu): Temperature	**0.0164**	0.0047	**0.0160**	0.0046
Gene (nu): Water source (fre)	n.a.	n.a.	n.a.	n.a.
Gene (nu): Water source (sea)	**0.3069**	0.0490	**0.3224**	0.0494
Temperature: Water source (fre)				
Temperature: Water source (sea)				
AIC	-215.50		-215.39	?¿?
AIC	1.88		1.99	

Note: Abbreviation 'Coeff.' indicates the coefficient of each variable in GLM. Positive values for the coefficient of the variable 'Gene (nu)' indicate higher eDNA decay rate constant for nuclear than mitochondrial DNA. Positive values for the coefficient of the variable 'Water source (fre/sea)' indicate higher eDNA decay rate constant for freshwater or seawater than artificial water samples. The coefficient of the interaction 'Gene (nu): Water source (fre)' was not analysed because no study described eDNA decay rate constants using a nuclear DNA marker and freshwater samples. P values of each parameter are not shown in the model, except for the full model. Coefficients of each parameter are shown in bold.

Table 3. The result of the ANOVA test for the effects of eDNA particle size, target gene, and water temperature on eDNA decay rate constants.

Response	Factor	F value	P value
Decay rate constant	Filter pore size	39.2770	***
	Gene	45.8534	***
	Temperature	27.3524	***
	Filter pore size: Gene	0.2535	0.8570
	Filter pore size: Temperature	5.9051	**
	Gene: Temperature	2.9600	0.0902

Note: Asterisks indicate the statistical significance of the factor (**,$P < 0.01$; ***, $P < 0.001$).

Figures

Figure 1. The effects of water temperature and filter pore size on eDNA decay rate constants. Left, middle, and right graphs show the linear relationships between decay rate constants and temperature targeting all filter pore sizes (circle), <0.45 μm pore sizes (square), and >0.7 μm pore sizes (triangle), respectively. Bold and dotted lines indicate the regression line and the corresponding 95% confidence intervals (CI) estimated by *lm* and *confint*functions in R, respectively. R^2 values of the linear regressions are shown in the top-left corner of each figure, and the asterisks indicate the statistical significance of the linear regressions (**, $P <$ 0.01).

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persistence-suggested-by-meta-analyses

Figure 2. The effects of water temperature and target gene on eDNA decay rate constants. Left, middle, and right graphs show the linear relationships between decay rate constants and temperature targeting all genes (circle), mitochondrial DNA (square), and nuclear DNA (triangle), respectively. Bold and dotted lines indicate the regression line and the corresponding 95% CI estimated by *lm* and *confint*functions in R, respectively. R^2 values of the linear regressions are shown in the top-left corner of each figure, and the asterisks indicate the statistical significance of the linear regressions (*, $P < 0.05$; **, $P < 0.01$).

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Figure 3. The effects of water source and target gene on eDNA decay rate constants. Left, middle, and right graphs show the boxplots of eDNA decay rate constants targeting all genes, mitochondrial DNA, and nuclear DNA, respectively. In each graph, decay rate constants derived from artificial water, freshwater, and seawater are shown in white, bright grey, and dark grey, respectively. Note that no study described eDNA decay rate constants using a nuclear DNA marker and freshwater samples.

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Figure 4. The effects of eDNA particle size, water temperature, and target gene on eDNA decay rate constants. Upper and lower graphs show the results for mitochondrial (bright grey) and nuclear (dark grey) eDNA, respectively. Medians and 95% CI of eDNA decay rate constants are indicated by circles and bars, respectively. Each filter pore size (10, 3, 0.8, and 0.2 μm) corresponded to a size fraction (>10, 3-10, 0.8-3, and 0.2-0.8 μm).

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