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

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21 **Running head:**

22 Meta-analyses of eDNA persistence and state

23

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26 water chemistry

27

28 **Abstract**

29 Understanding the processes of environmental DNA (eDNA) persistence and degradation is

30 essential to determine the spatiotemporal scale of eDNA signals and accurately estimate

31 species distribution. The effects of environmental factors on eDNA persistence have

32 previously been examined; however, the influence of the physiochemical and molecular states

33 of eDNA on its persistence is not completely understood. Here, we performed meta-analyses

34 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; Bohmann et al., 2014; Deiner et al., 2017). 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

67 factors. For example, moderately high temperature (Strickler et al., 2015; Eichmiler et al.,
68 2016; Lance et al., 2017; Jo et al., 2020b) and low pH (Strickler et al., 2015; Lance et al,
69 2017; Seymour et al., 2018) accelerate eDNA degradation, whereas the effect of UV-B
70 radiation was contradictory among studies (Strickler et al., 2015; Mächler et al., 2018). In
71 addition, eDNA decay rates are higher in environments with higher species biomass density
72 (Bylemans et al., 2018; Jo et al., 2019a). These abiotic and biotic factors contribute to the
73 increase in microbial activities and abundance in water, thus indirectly affecting eDNA
74 degradation (Strickler et al., 2015). Moreover, eDNA decay rates were found to be different
75 between the trophic states of studied lakes, and were negatively correlated with the dissolved
76 organic carbon (DOC) concentrations (Eichmiller et al., 2016). This may be attributed to the
77 binding of DNA molecules to humic substances, protecting eDNA from enzymatic
78 degradation.

79 However, apart from the effects of such environmental conditions, little is known
80 about the influence of the physiochemical and molecular states of eDNA on its persistence
81 and degradation. Fish eDNA has been detected at various size fractions (<0.2 μm to >180 μm
82 in diameter; Turner et al., 2014; Jo et al., 2019b) in water, suggesting that eDNA is present as
83 various states and cellular structures, from larger-sized and intra-cellular DNA (e.g., cell and

84 tissue fragments) to smaller-sized and extra-cellular DNA (e.g., organelles and dissolved
85 DNA). Enzymatic and chemical degradation of DNA molecules in the environment depends
86 on the presence of cellular membranes around the DNA molecules, and thus, the persistence
87 of eDNA is likely to be linked to its state. In addition, eDNA persistence may be different
88 depending on the target genetic regions. Recent studies have suggested that eDNA decay rates
89 may vary between mitochondrial and nuclear DNA (Bylemans et al., 2018; Moushomi et al.,
90 2019; Jo et al., 2020b). Moreover, studies comparing eDNA degradation between different
91 target DNA fragment lengths (i.e. PCR amplification length) have yielded inconsistent
92 conclusions; Jo et al. (2017) and Wei et al. (2018) reported higher eDNA decay rates for
93 longer DNA fragments, whereas Bylemans et al. (2018) did not observe any difference in the
94 eDNA decay rates of different DNA fragment sizes. Notably, Jo et al. (2020c) reported that
95 selective collection of larger-sized eDNA using a larger pore size filter increased the ratio of
96 long to short eDNA concentrations and altered the ratio of nuclear to mitochondrial eDNA
97 concentrations; however, such reports linking eDNA state to its persistence are scarce.
98 Although our understanding of the relationship between eDNA state and persistence
99 is currently limited, this relationship can be inferred by integrating previous findings of eDNA
100 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)

118 described aqueous eDNA decay rate constants using a first-order exponential decay model
119 ($C_t = C_0 e^{-kt}$, where C_t is the eDNA concentration at time t, C_0 is the initial eDNA
120 concentration, and k is the first-order decay rate constant). The eDNA decay rate constants
121 estimated using multi-phasic exponential decay models (e.g. biphasic or Weibull models)
122 (Eichmiller et al., 2016; Bylemans et al., 2018; Wei et al., 2018) were not included in our
123 meta-analyses, because of the limited number of such studies and difficulty in directly
124 comparing the constants between first-order and multi-phasic models.
125 From the filtered eDNA studies, we then extracted data on the eDNA decay rate
126 constant (per hour), filter pore size used for water filtration (μm), target DNA fragment size
127 (base pair; bp), and target gene (mitochondrial or nuclear). The decay rate constant was
128 converted to "per hour" if it was originally described as "per day". Different eDNA decay rate
129 constants based on different experimental conditions within the same study (e.g. species,
130 temperature, pH, and biomass density) were treated separately. The filter pore size in studies
131 involving aqueous eDNA collection *via* ethanol precipitation or centrifugation was regarded
132 as 0 μm. In addition, we extracted information on the water temperature (°C), water source
133 used for experiments, and target species and taxa. Although other biotic and abiotic factors are
134 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

152 temperature (°C), water source (artificial, freshwater, or seawater), and their primary
153 interactions were included as the explanatory variables. We first confirmed that the multi-
154 collinearity among the variables was negligible (1.028 to 1.096), by calculating the
155 generalized variance inflation factors (GVIF). We then selected models based on Akaike's
156 Information Criterion (AIC), using the *dredge* function in the 'MuMIn' package in R (Bartoń,
157 2019). We adopted the model with the smallest AIC value, and all models with ⊿AIC (i.e.
158 difference in the AIC value) less than two were selected as the supported models (Burnham &
159 Anderson, 2002). Moreover, we performed model averaging targeting all the supported
160 models (⊿AIC < 2) to estimate the parameters averaged among the supported models (Rice et
161 al., 2018).
162 We performed an additional meta-analysis to examine the relationship between the
163 DNA fragment size and eDNA decay rate constant. Most eDNA studies conducted to date
164 have targeted short DNA fragments (<200 bp), and only three papers have reported eDNA
165 decay rates targeting longer DNA fragments (>200 bp); however, they yielded inconsistent
166 conclusions (Tables 1 & S1). Taking this into consideration and targeting eDNA decay rate
167 constants derived from <200 bp DNA fragments, we performed a linear regression to assess
168 the effect of DNA fragment size on eDNA degradation.

169

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

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

181 We included all eDNA samples that could pass through sequential filters with 10, 3,
182 0.8, and 0.2 μm pore sizes at 0, 6, 12, and 18 hours, which yielded four eDNA size fractions,
183 i.e. >10, 3-10, 0.8-3, and 0.2-0.8 μm. Linear regressions were performed between eDNA
184 concentrations (original concentration + 1 followed by log-transformation) and sampling time
185 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). Other interactions were included in less than four supported models, and the uncertainties of the corresponding coefficients were relatively large. Moreover, model averaging also showed that the interactions between filter pore size

and temperature ($P < 0.05$), target gene and temperature ($P < 0.001$), and target gene and water source ($P < 0.001$) were statistically significant (Table S2).

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 S1).

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 interactions that were included in almost all supported models and were significant in the averaged model. Firstly, the interaction between filter pore size and water

251 temperature influenced eDNA decay rates (Figure 1). Considering that a larger pore size filter
252 can selectively collect eDNA particles in larger size fractions, our result implied that higher
253 water temperature could accelerate the degradation of eDNA in larger size fractions by a
254 greater degree than that in smaller size fractions. However, it is unlikely that smaller-sized
255 eDNA itself is less affected by higher temperature-mediated degradation, and its apparent
256 persistence can be increased by the inflow of eDNA from larger to smaller size fractions, as
257 described in Jo et al. (2019b). Organic matter in water, including eDNA, is degraded by
258 microbes and extra-cellular enzymes in the environment for uptake, and their activities are
259 promoted by moderately high temperatures (less than 50°C) (Price & Sowers, 2004; Nielsen
260 et al., 2007; Arnosti, 2014; Strickler et al., 2015). During the degradation processes, aqueous
261 eDNA in larger size fractions, such as intra-cellular DNA, is believed to flow into smaller size
262 fractions, such as extra-cellular DNA. This suggests that water temperature does not
263 uniformly influence the apparent degradation of eDNA among the different size fractions, and
264 the effect of temperature on eDNA degradation might be buffered in smaller-sized eDNA
265 particles. Thus, the effect of temperature on eDNA degradation would be smaller when using
266 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.

283 Thirdly, the interaction between the target gene and water source influenced the
284 eDNA decay rates (Figure 3). Although the effects of water source on eDNA degradation
285 differed between nuclear and mitochondrial DNA, it was evident that eDNA degradation was
286 suppressed in artificial waters, such as tap water and DW, when compared to that in natural
287 waters. Eichmiller et al. (2016) compared the degradation of common carp (*Cyprinus carpio*)
288 eDNA in natural waters with different trophic states, and found that eDNA decay rates in well
289 water were lower than those in eutrophic and oligotrophic waters, which could be attributed to
290 the lower microbial activity in the former. Our results were generally consistent with those of
291 Eichmiller et al. (2016). Using tap water and DW as water sources can lead to
292 underestimation of eDNA persistence in the natural environment. Moreover, no significant
293 difference could be observed in the eDNA decay rates between freshwater and seawater. The
294 difference in eDNA persistence between freshwater and seawater has previously been
295 reported; some studies indicated faster eDNA degradation in seawater than in freshwater
296 (Thomsen et al., 2012; Sassoubre et al., 2016), whereas Collins et al. (2018) showed that
297 eDNA degradation was higher in terrestrially-influenced inshore waters than in ocean-
298 influenced offshore environments. Marine systems are generally characterized by higher
299 salinity and ionic content, higher pH, and more stable temperatures when compared with

300 freshwater systems, which can promote DNA preservation in water (Okabe & Shimazu, 2007;
301 Schulz & Childers, 2011; Collins et al., 2018). However, the direct effects of microbial
302 abundance and composition and other physicochemical parameters of water were not included
303 in our meta-analyses. Thus, greater variations in eDNA decay rates in seawater when
304 compared with artificial water and freshwater observed in our meta-analyses might partly be
305 explained by such microbial and physicochemical conditions. The effects of various nutrient
306 salts and microbial activities on eDNA persistence and differences in the eDNA degradation
307 processes between freshwater and seawater systems require further investigation.
308 Contrary to these factors, model selection in the present study did not strongly
309 support the effects of DNA fragment size and its interactions with other variables on the
310 eDNA decay rate (Table 2), which may be due to the potential bias of DNA fragment sizes in
311 the eDNA studies included in the meta-analysis. Only three studies have previously estimated
312 eDNA decay rates in water targeting longer DNA fragments (>200 bp) (aqueous eDNA; Jo et
313 al. 2017; Weltz et al., 2017; Bylemans et al., 2018), and there was no consensus on the
314 relationship between eDNA degradation and DNA fragment size among these studies.
315 Although our additional meta-analysis, which targeted only shorter DNA fragments (70 to
316 190 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 legacy 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. Therefore, more information on eDNA degradation should be collected by targeting different taxa and environments. 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. Accumulating eDNA decay rates based on more varied filter pore sizes and size fractions could contribute to our findings in the meta-analysis. In addition, this knowledge might in the future contribute to revealing the relationships between

366 eDNA degradation and its cellular and molecular states (e.g., intra-/extra-cellular, genetic
367 region, DNA fragment size).
368 Although our findings and their implications require further verification, this study
369 is the first to propose that the persistence of eDNA from macro-organisms can be determined
370 by the state of the eDNA and its complex interactions with environmental conditions. That is,
371 the mechanism of eDNA persistence and degradation cannot be fully understood without
372 knowing not only the environmental biotic and abiotic factors involved in eDNA degradation
373 but also the cellular and molecular states of eDNA occurring in water. If our findings are
374 correct, the spatiotemporal scale and intensity of eDNA signals would be different depending
375 on the eDNA particle size and state. The fact that Weibull or biphasic exponential decay
376 models fit better to eDNA degradation implies the differences in eDNA persistence depending
377 its state (e.g., intra- or extra-cellular, living or dead cells, particulate or dissolved) (Eichmiller
378 et al., 2016; Bylemans et al., 2018), which support our results linking eDNA persistence to its
379 state. In natural environments, the persistence of nuclear and larger-sized eDNA could also be
380 more sensitive to environmental conditions including temperature and water chemistry, than
381 to mitochondrial and smaller-sized eDNA. Despite the potential to use nuclear and larger-
382 sized eDNA in the estimation of population-level inferences and the improvement of the

detection sensitivity in the field (Minamoto et al., 2017; Jo et al., 2020b; Sigsgaard et al., 2020), understanding the effect of biotic/abiotic factors on their degradation will be further required particularly in the case of the estimation of species abundance/biomass based on nuclear and larger-sized eDNA. Experimental verification of our findings and implications will be necessary, which could clarify the characteristics and dynamics of aqueous eDNA and 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.

Table S2. The result of model averaging targeting all the supported models.

Figure S1. 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.

409

410 **Author contribution**

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

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588 **Tables**

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

590

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
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.

595 Table 2. Results of model selection for the effects of filter pore size, DNA fragment size, target gene, temperature, and water source on the
596 first-order eDNA decay rates.
597

Variable	GVIF	Full model			Model_1		Model_2		Model_3	
		Coeff.	SE	P value	Coeff.	SE	Coeff.	SE	Coeff.	SE
Intercept		**0.0506**	0.0975	0.6050	**0.0358**	0.0552	**0.0506**	0.0563	**0.0709**	0.0582
Filter pore size	1.0308	**-0.2269**	0.1341	0.0942	**-0.2058**	0.0993	**-0.2933**	0.1099	**-0.2911**	0.1095
Fragment size	1.0440	**0.0004**	0.0005	0.3889	**-0.0002**	0.0001			**-0.0001**	0.0001
Gene (nu)	1.0472	**-0.3073**	2.5630	0.9048	**-0.3591**	0.1010	**-0.3268**	0.1012	**-0.3365**	0.1011
Temperature	1.0281	**-0.0043**	0.0038	0.2612	**-0.0008**	0.0026	**-0.0012**	0.0026	**-0.0016**	0.0026
Water source (fre)	1.0955	**0.1909**	0.1567	0.2266	**0.0571**	0.0272	**0.0525**	0.0555	**0.0573**	0.0554
Water source (sea)		**0.0308**	0.0791	0.6982	**0.0858**	0.0207	**0.0452**	0.0295	**0.0491**	0.0295
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**	0.0053	**0.0149**	0.0053
Filter pore size: Water source (fre)		**-0.0164**	0.0948	0.8632			**0.0238**	0.0841	**0.0031**	0.0853
Filter pore size: Water source (sea)		**0.0709**	0.0466	0.1318			**0.0783**	0.0351	**0.0631**	0.0368
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**	0.0046	**0.0156**	0.0046
Gene (nu): Water source (fre)		n.a.	n.a.	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**	0.0484	**0.3110**	0.0495
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		-217.00	
⊿AIC		9.22			0.00		0.30		0.38	

598 (Table 2 continued)

599

Variable	Model_4		Model_5		Model_6		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.
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	

600

(Table 2 continued)

Variable	Model_8		Model_9		Model_10		Model_11	
	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	

604 (Table 2 continued)

605

Variable	Model_12		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)				
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	

606

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.

614 Table 3. The result of the ANOVA test for the effects of eDNA particle size, target gene, and
615 water temperature on eDNA decay rate constants.
616

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

617
618 Note: Asterisks indicate the statistical significance of the factor (**, $P < 0.01$; ***, $P <$
619 0.001).
620

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).

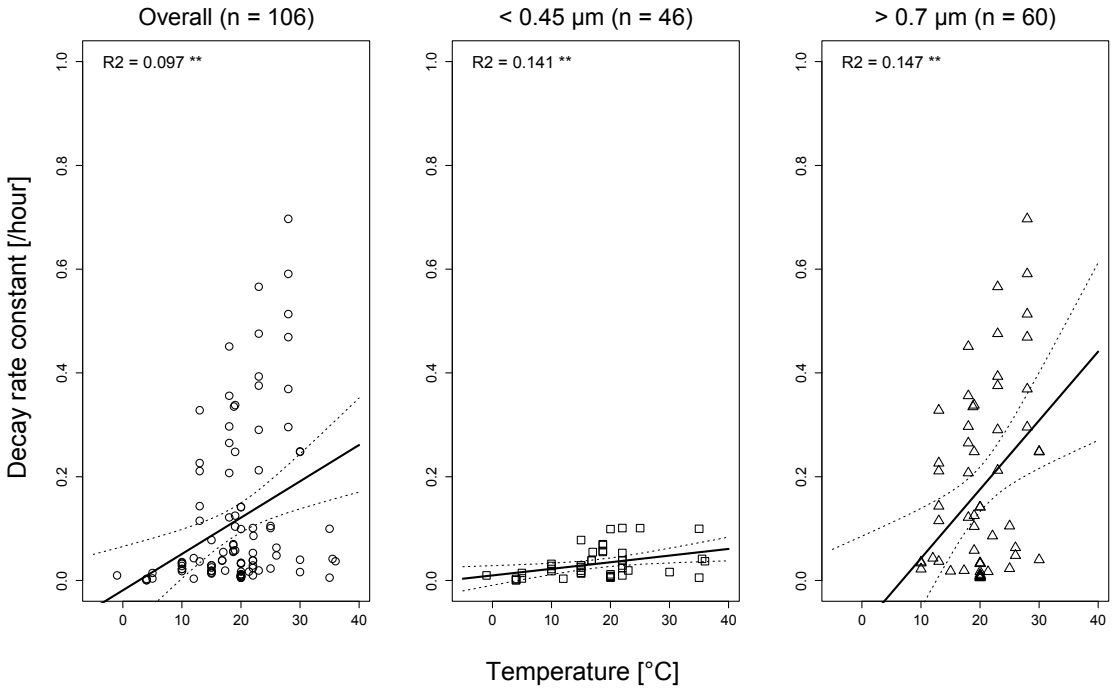


Figure 2. The effects of water temperature and target gene on eDNA decay rate constants. Left, middle, and right graphs show the linear relationships between decay rate constants and temperature targeting all genes (circle), mitochondrial DNA (square), and nuclear DNA (triangle), respectively. Bold and 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 (*, $P < 0.05$; **, $P < 0.01$).

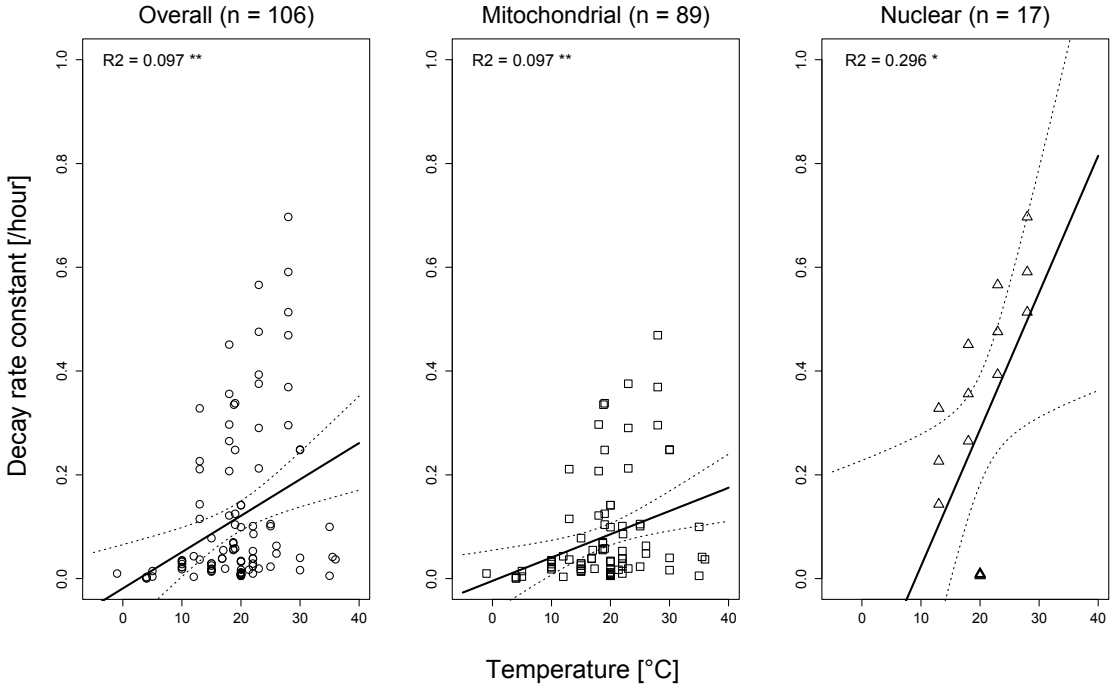
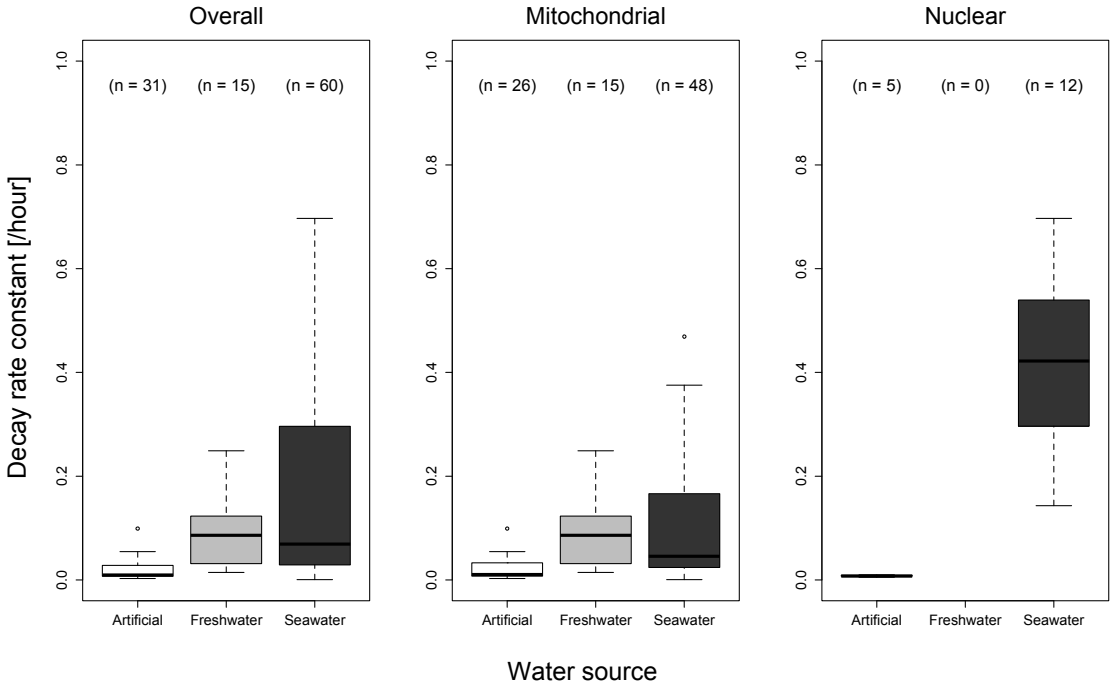
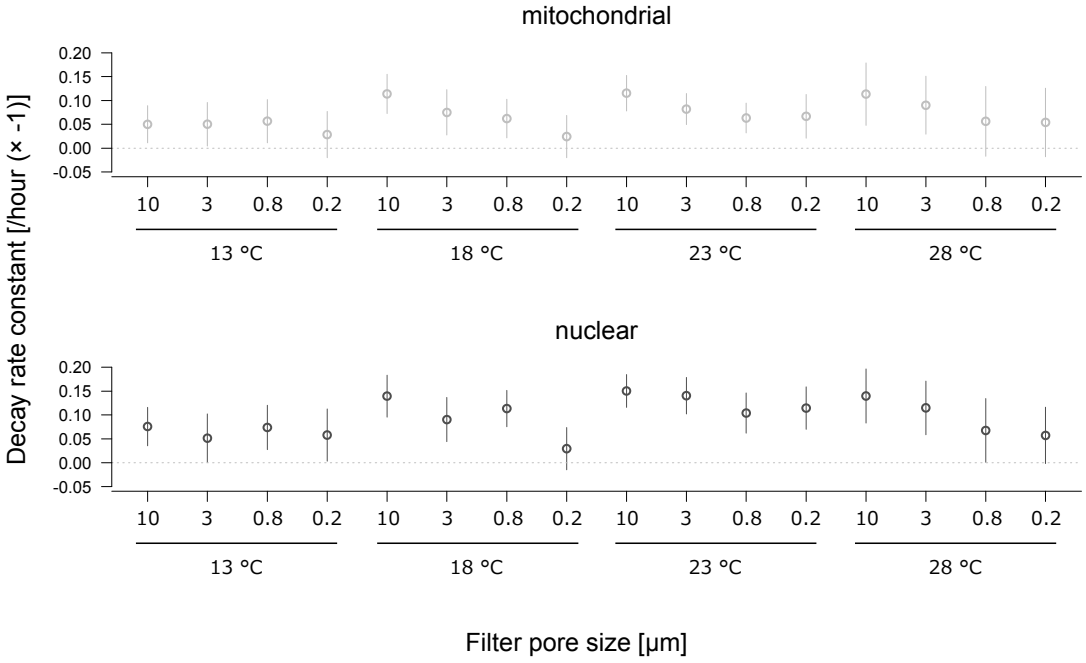


Figure 3. The effects of water source and target gene on eDNA decay rate constants. Left, middle, and right graphs show the boxplots of eDNA decay rate constants targeting all genes, mitochondrial DNA, and nuclear DNA, respectively. In each graph, decay rate constants derived from artificial water, freshwater, 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.



649 Figure 4. The effects of eDNA particle size, water temperature, and target gene on eDNA
650 decay rate constants. Upper and lower graphs show the results for mitochondrial (bright grey)
651 and nuclear (dark grey) eDNA, respectively. Medians and 95% CI of eDNA decay rate
652 constants are indicated by circles and bars, respectively. Each filter pore size (10, 3, 0.8, and
653 0.2 μm) corresponded to a size fraction (>10, 3-10, 0.8-3, and 0.2-0.8 μm).
654



655