# Estimation accuracy of species abundance based on environmental DNA with relation to its production source, state, and assay methodology suggested by meta-analyses

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## Abstract

Environmental DNA (eDNA) analysis is a promising tool for non-disruptive and cost-efficient estimation of species abundance. However, its practical applicability in natural environments is limited because it is unclear whether eDNA concentrations actually represent species abundance in the field. Although the importance of accounting for eDNA dynamics, such as transport and degradation, has been discussed, the influences of eDNA characteristics, including production source and state, and methodology, including collection and quantification strategy and abundance metrics, on the accuracy of eDNA-based abundance estimation were entirely overlooked. We conducted a meta-analysis using 56 previous eDNA literature and investigated the relationships between the accuracy (R2) of eDNA-based abundance estimation and eDNA characteristics and methodology. Our meta-regression analysis found that R2 values were significantly lower for crustaceans than fish, suggesting that less frequent eDNA production owing to their external morphology and physiology may impede accurate estimation of their abundance via eDNA. Moreover, R2 values were positively associated with filter pore size, indicating that selective collection of larger-sized eDNA, which is typically fresher, could improve the estimation accuracy of species abundance. Furthermore, R2 values were significantly lower for natural than laboratory conditions, while there was no difference in the estimation accuracy among natural environments. Our findings shed a new light on the importance of what characteristics of eDNA should be targeted for more accurate estimation of species abundance. Further empirical studies are required to validate our findings and fully elucidate the relationship between eDNA characteristics and eDNA-based abundance estimation.

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## Abstract (< 250 words)

Environmental DNA (eDNA) analysis is a promising tool for non-disruptive and cost-efficient estimation of species abundance. However, its practical applicability in natural environments is limited because it is unclear whether eDNA concentrations actually represent species abundance in the field. Although the importance of accounting for eDNA dynamics, such as transport and degradation, has been discussed, the influences of eDNA characteristics, including production source and state, and methodology, including collection and quantification strategy and abundance metrics, on the accuracy of eDNA-based abundance estimation were entirely overlooked. We conducted a meta-analysis using 56 previous eDNA literature and investigated the relationships between the accuracy  $(R^2)$  of eDNA-based abundance estimation and eDNA characteristics and methodology. Our meta-regression analysis found that  $\mathbb{R}^2$  values were significantly lower for crustaceans than fish, suggesting that less frequent eDNA production owing to their external morphology and physiology may impede accurate estimation of their abundance via eDNA. Moreover,  $R^2$  values were positively associated with filter pore size, indicating that selective collection of larger-sized eDNA, which is typically fresher, could improve the estimation accuracy of species abundance. Furthermore,  $\mathbf{R}^2$  values were significantly lower for natural than laboratory conditions, while there was no difference in the estimation accuracy among natural environments. Our findings shed a new light on the importance of what characteristics of eDNA should be targeted for more accurate estimation of species abundance. Further empirical studies are required to validate our findings and fully elucidate the relationship between eDNA characteristics and eDNA-based abundance estimation.

## Introduction

In the past decades, environmental DNA (eDNA) analysis has been developed and successfully applied in multiple fields in ecology, fisheries, and environmental science (Ficetola et al., 2008; Bálint et al., 2018; Ruppert et al., 2019; Spear et al., 2021). Environmental DNA is defined as a total pool of DNA isolated from environmental samples such as water and sediment (Pawlowski et al., 2020); in a narrower sense, it is generally defined as extra-organismal DNA released from macro-organisms in the form of feces, skin, mucus, and gamete (Barnes & Turner, 2016; Rodriguez-Ezpeleta et al., 2021). Contrary to traditional methods, PCR-based detection of target eDNA does not require capturing nor observing individuals, and thus eDNA analysis is a feasible approach for non-disruptive, highly-sensitive, and cost-effective biomonitoring (Takahara et al., 2013; Yamanaka & Minamoto, 2016; Deiner et al., 2017; Djurhuus et al., 2020). Therefore, eDNA analysis has potential to improve the monitoring of biodiversity and ecosystems, allowing for more effective conservation and management of biodiversity and resources.

In addition to species presence/absence, eDNA analysis can be used to estimate species abundance from target eDNA quantity. Several studies have reported positive relationships between eDNA concentrations and species abundance for various taxa and environments (e.g., Takahara et al., 2012; Pilliod et al., 2013; Klymus et al., 2015; Salter et al., 2019). However, a recent meta-analysis demonstrated that the relationships between eDNA concentration and species abundance was weaker in natural environments than in controlled laboratory conditions (i.e., aquaria, tanks, or mesocosms) (Yates et al., 2019). According to the study, the mean R<sup>2</sup> values were 0.81 and 0.57 in laboratory conditions and natural environments, respectively. This finding is

intuitively unsurprising given that abundance can be precisely set in laboratory experiments, but we cannot know 'true' species abundance in natural environments where some individuals are not analyzable depending on their developmental stage and the survey method (Yates et al., 2019). In addition, the effects of diffusion and degradation on eDNA detection/quantification would be more substantial in natural environments due to compounding and complicated environmental conditions, including temperature, water chemistry, flow rate, and substrate (Strickler et al., 2015; Jane et al., 2015; Shogren et al., 2018; Jo et al., 2019a). Such factors could hamper the practical application of eDNA-based abundance estimation in natural environments (Hansen et al., 2018). Therefore, toward effective conservation management of biodiversity and precise stock assessment *via* eDNA analysis, it is critical to assess the factors affecting such variabilities concerning the estimation accuracy and improve the accuracy of eDNA-based abundance estimation.

Given that the amount of eDNA is determined by a function of its production, transport, and degradation (Strickler et al., 2015; Barnes & Turner, 2016; Jo & Minamoto, 2021), the relationships between eDNA quantity and species abundance may also be affected by target eDNA characteristics, including its production source and cellular/molecular state. For example, eDNA production sources and processes may differ among taxa, which could accordingly influence the estimation accuracy of species abundance *via* eDNA analysis, as well as detection sensitivity of target eDNA. Andruszkiewicz et al. (2021) estimated eDNA shedding rates (pg/hour) of multiple taxa under similar experimental conditions and found that crustaceans (*Palaeomenes* spp.) had lower shedding rates than fish (*Fundulus heteroclitus*) and scyphomedusae (*Aurelia aurita* and *Chrysaora* spp.). These findings suggest that external morphology and/or physiology could explain the difference in eDNA production sources and processes among taxa.

Cellular and molecular states of eDNA can also be closely associated with its transport and degradation processes, consequently influencing the spatiotemporal range of target eDNA signals (Barnes & Turner, 2016) and even eDNA-based estimation accuracy of species abundance. Although studies linking eDNA state to its spatiotemporal dynamics are scarce, it has been reported that intra-cellular eDNA collected from larger size fraction (i.e., larger eDNA particles) contained longer DNA fragments more frequently (Jo et al., 2020a), and eDNA decay rates could be determined by eDNA states, such as target gene (mitochondrial/nuclear) and particle size, as well as abiotic factors, including temperature and water chemistry (Strickler et al., 2015; Jo & Minamoto, 2021). In the context of abundance estimation, given the rapid degradation of longer eDNA fragments (Jo et al., 2017) and persistence of smaller-sized eDNA particles (i.e., eDNA from smaller size fractions) in water due to the inflow of degraded eDNA from larger to smaller fractions (Jo et al., 2019b), biological signals from longer eDNA fragments and larger eDNA particles (i.e., eDNA from larger size fractions) could be fresher and spatiotemporally finer in the field, which may consequently improve the accuracy of eDNA-based abundance estimation. Nevertheless, aside from Stewart (2019), who reviewed how biotic factors, such as developmental stage, life history, and species interaction might influence eDNA production and eDNA-based abundance estimation performance, exploration of the effects of eDNA production sources and states on estimation accuracy has been limited.

Furthermore, the estimation accuracy of species abundance can rely on some technical aspects in eDNA analysis. First, although the earlier studies collected eDNA in water samples by centrifugation and precipitation (e.g., Ficetola et al., 2008; Takahara et al., 2012), filtration of water samples is now the most common method for collecting aqueous eDNA (Kumar et al., 2020). Between these collection methods, the volume of water samples (typically 15 mL in the former while hundreds to thousands of milliliters in the latter) and the state of eDNA collected (both membranous and dissolved DNA in the former while only membranous DNA in the latter) could be different. Second, compared to the eDNA concentration estimated by real-time and digital PCR, the eDNA read number estimated by metabarcoding is expected to reflect species abundance less precisely, given the biases introduced during PCR, sequencing, and bioinformatics steps required high-throughput sequencing (Lamb et al., 2019). Nevertheless, some studies have reported a positive relationship between the relative abundance of eDNA read detected *via* high-throughput sequencing and species abundance (e.g., Evans et al., 2016; Li et al., 2021). Third, it is unclear which metrics of species abundance, biomass or number of individuals, exhibit a stronger relationship with eDNA quantity. Although a precedent meta-analytic study found no evidence that there was no significant difference in these metrics

for the relationship with eDNA quantity (Yates et al., 2019), the study also acknowledged the need of accumulating future research to specify the difference. As well as the production source and state of eDNA, these technical aspects in eDNA analysis have potentials affecting the estimation accuracy of species abundance *via* eDNA, whereas these points had not been assessed so far.

As far as we know, there is no study to directly assess the importance of eDNA production source and state, as well as technical aspects in eDNA analysis, for the accuracy of eDNA-based abundance estimation. However, meta-analyses, synthesizing previous findings and statistically re-analyzing them, may shed light on the relationship between eDNA-based estimation of species abundance and eDNA characteristics and methodology. In this study, we investigated how different eDNA production sources, states, and methodology influenced eDNA-based species abundance estimation accuracy by performing meta-analyses of eDNA studies targeting macro-organisms. We conducted a literature search and extracted data on factors influencing eDNA characteristics and methodology. Moreover, since it is unclear how the relationship between species abundance and eDNA concentration differs among various natural environments (e.g., freshwater/marine, lentic/lotic), we also assessed the effect of target environments on eDNA-based abundance estimation accuracy. Integrating and collating previous findings *via*meta-analysis will enable us to find generalizable patterns in these relationships and to elucidate the hitherto unknown findings in the estimation accuracy of species abundance *via* eDNA.

#### Materials and methods

#### $Literature \ search$

We conducted a literature search according to The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021) (Figure 1). First, we compiled all the studies described in the literature list of Jo et al. (2021) (N = 728), which screened the literature regarding macrobial eDNA published until 2020 on the following criteria; (i) targeted eDNA from macro-organisms (not from microbes such as bacteria and fungi, or viruses), (ii) published in international journals, (iii) peer-reviewed (not preprints), and (iv) not review articles, news, views, introductions, opinions, responses, or perspectives. Additionally, using Google Scholar (https://scholar.google.co.jp/), we searched for the latest eDNA literature, where the search included the terms "eDNA" or "environmental DNA" in the title and/or text (the final date of the literature search was 15 September 2021) and screening strategy was the same as above (N = 195). We then assessed the abstracts of the remaining 923 literature and excluded 805 literature that were out of the scope of our meta-analysis (i.e., not describing the relationship between eDNA quantity and species abundance).

We further assessed the full-texts of the remaining 118 literature and excluded 62 literature that did not present  $\mathbb{R}^2$  values and Pearson's correlation coefficients of the relationship between species abundance and eDNA quantity, and the values of eDNA quantification were not reliable (< limit of quantification). Besides, a part of dataset in Akamatsu et al. (2020) was excluded because the sample size was too small to estimate variance under a Fisher's z-transformation (see below). 56 literature were finally included in our meta-analysis, assessing the relationships between eDNA quantity in water samples and species abundance. Quantity of eDNA was categorized into (a) eDNA concentration measured by real-time PCR or digital PCR (species-specific assay) or (b) eDNA read number measured by high-throughput sequencing (metabarcoding assay). Species abundance metrics were categorized into (a) biomass or (b) number of individuals. Most studies reported positive relationships with statistical significance, while some results were not significant (Table S1).

## Data compilation

We used  $R^2$  values from linear fitting as indices of species abundance estimation accuracy based on eDNA quantity because the values were calculated in most studies collected in our meta-analysis. If the manuscripts only reported Pearson's correlation coefficients, we squared the coefficients and substituted them for  $R^2$ values. We then extracted the information on collection strategy (filtration or centrifugation), quantification strategy (species-specific or metabarcoding assay), target taxa, filter pore sizes used for water filtration

( $\mu$ m), volume of water sample (mL), PCR amplicon sizes (base pair; bp), study environments, and abundance metrics (biomass or individuals) from the studies. As all the metabarcoding studies in our meta-analysis collected aqueous eDNA by filtration, collection and quantification strategy were combined as the factor 'assay strategy', which was classified as 'species-specific/filtration', 'species-specific/precipitation', and 'metabarcoding'. Taxa were classified as fish, herptiles (i.e., reptiles and amphibians), crustaceans, mollusks, and coral and seastars. In studies involving aqueous eDNA collection *via* centrifugation, the filter pore size was regarded as 0  $\mu$ m. Study environments were classified as laboratory (i.e., tank, aquarium, or mesocosm experiments), lentic freshwater, lotic freshwater, and marine environments. Multiple R<sup>2</sup> values based on different experimental conditions within the same study (e.g., species, filter type, and amplicon size) were treated individually. Moreover, we calculated the sample size (the number of water samples or sampling sites) required for calculating R<sup>2</sup> values or coefficients based on figures and/or text in the corresponding literature.

#### Statistical analyses

All the statistical analyses were performed by using R version 4.0.4 (R Core Team, 2021). First, we estimated the effect sizes and their variances of each collected dataset using the *metacor* function in the package 'meta' (Balduzzi et al., 2019), where  $R^2$  values were Fisher's z-transformed to meet normality (Fisher, 1921). We ran the random-effect model taking multiple effect sizes derived from the same study into account by the *rma.mv* function in the package 'metafor' (Viechtbauer, 2010). This assumed that the variances of the effect sizes in all the collected studies included the heterogeneity across the studies and datasets, as our collected dataset is not functionally identical (i.e., some  $R^2$  values could be derived from the same study). More strictly, the weight (inverse variance) for averaging  $R^2$  values comprised both intra- and inter-study variances of effect size (Borenstein et al., 2010).

We then performed a meta-regression analysis to assess the effects of each factor on eDNA-based estimation accuracy of species abundance accounting for the variances of the effect sizes. We used a generalized linear mixed model (GLMM) assuming Gaussian distribution with the Bayesian Markov chain Monte Carlo (MCMC) algorithm in the package 'MCMCglmm' (Hadfield, 2010). R<sup>2</sup> values (Fisher's z-transformed) were included as the objective variable, assay strategy, target taxa, filter pore size, amplicon size, study environment, and abundance metrics were included as the fixed effects, and study groups (i.e., each literature) were included as the random effects. We did not include the volume of water samples because of its significant correlation with filter pore size (Pearson's coefficient = 0.360; P < 0.001). The variances of Fisher's z-transformed R<sup>2</sup> values were included in our model as the measurement error variances for each dataset. The prior distribution of all the fixed and random effects were set as default settings. 13,000 iterations of the MCMC algorithm were run and burn-in was set at 3,000 to discard the initial transient region of the chain to obtain precise parameter estimates. Prior to model fitting, we excluded all the dataset from Salter et al. (2019) (N = 2) because they used a commercially available assay kit whose amplicon size was unknown.

We further assessed the heterogeneity across studies/datasets by performing Cochran's Q test and calculating  $I^2$  values (Nakagawa & Santos, 2012), which were performed using the *metacor* function.  $I^2$  values have recently been used to quantify heterogeneity in the data;  $I^2 = 25$ , 50, and 75% are considered as low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). In addition, we assessed the publication bias, occasionally termed as the 'file drawer problem' (Haddaw ay et al., 2020), by visualizing a funnel plot (Fisher's z values plotted against their variances) and conducting a modified Egger's regression (Egger et al., 1998) using the *regtest* function. Moreover, to test the effects of outliers in our modeling, we excluded the dataset whose filter pore size was 10  $\mu$ m (N = 1) and amplicon size was approximately 900 bp (N = 2) (Table S1) and performed the similar meta-regression analysis as described above.

#### Results

We compiled 204  $\mathbb{R}^2$  values in total from the collected 56 literature (Tables 1 and S1). Most studies reported the relationships between eDNA quantity and species abundance by the species-specific assay (47 studies) and targeting fish species (40 studies). Contrary, such relationships were reported less frequently for

metabarcoding assay (9 studies) and other taxa (7, 4, 3, and 3 studies for herptiles, crustaceans, mollusks, and coral and seastars). The filter pore size and amplicon size ranged from 0 to 10  $\mu$ m and 52 to 719 bp, respectively. Almost all the studies used filters with less than 3  $\mu$ m pore size and amplified less than 400 bp target DNA fragments. The number of studies conducted in laboratory, lentic freshwater, lotic freshwater, and marine environments were 18 17, 18, and 6, respectively.

A GLMM with MCMC algorithm showed the significant effects of target taxa, filter pore size, and study environments (Table 2). Relative to fish, estimation accuracy (Fisher's z-transformed  $\mathbb{R}^2$ values) was significantly lower for crustaceans (posterior mean = -0.544 [95% CI: -1.074, -0.030]) and marginally higher for coral and seastars (0.500 [-0.116, 1.063]). Among the target taxa, the relationship between crustacean eDNA quantity and abundance was the weakest and the estimated  $\mathbb{R}^2$  values for herptiles and crustaceans were more variable than other taxa (Figure 2a). Laboratory experiments produced higher estimation accuracy than natural environments, whereas there seemed to be no substantial differences in the estimation accuracy among natural environment types (Figure 2b). Filter pore size had a significantly positive effect on the estimation accuracy (0.094 [0,011, 0.180]; Figure 3). In contrast, we did not confirm any statistically significant effects of assay strategy, amplicon size, and abundance metrics (Figures S1 and S2). These statistical trends were not different even if the outliers were excluded (Table S2). We confirmed the convergences of MCMC algorithm for all the run and no substantial multi-collinearity among the factors (adjusted GVIFs: 1.23 to 1.68).

The heterogeneity across studies/datasets was relatively high ( $I^2 = 77.4 \%$  for the overall dataset; Table 3). Although the values were re-calculated by dividing the dataset into each categorical factor (assay strategy, target taxa, study environment, and abundance metrics), the heterogeneities were generally high (> 70 %) except for some categories. In addition, Egger's regression and funnel plot showed the significant publication bias in our dataset (P < 0.01; Figure 4).

#### Discussion

#### Estimation accuracy and eDNA production source

We found that eDNA-based estimation accuracy of species abundance was significantly lower for crustaceans than fish. A previous study hypothesized that species morphology and/or behavior might affect eDNA production, reporting lower eDNA shedding rates in grass shrimp than in fish and jellyfish (Andruszkiewicz et al., 2021). Fish and jellyfish are likely to constantly produce eDNA as epidermis and/or muco-substances (Merkes et al., 2014; Sassoubre et al., 2016), while crustaceans are characterized by their hard exoskeletons and segmented bodies plans (Hadley, 1986) and are thus unlikely to shed large amounts of eDNA from their body surfaces unless they are molting. Consequently, crustaceans infrequently and irregularly shed eDNA, which may impede sufficient eDNA detection in the field and prevent accurate abundance estimation *via* eDNA analysis (Dougherty et al., 2016; Machler et al., 2016; Johnsen et al., 2020). In contrast, estimation accuracy of species abundance was marginally higher for coral and seastars than fish. These species are sessile or move relatively slower than other marine animals. Accordingly, their eDNA after released into environments might reflect species presence abundance more precisely. In particular, under the recent climate change and anthropogenic disturbances, rapid and sensitive monitoring of tropical coral reefs *via* eDNA would be helpful for the effective conservation of biodiversity and ecosystem functioning on reefs (Nichols & Marko, 2019).

The mean  $\mathbb{R}^2$  value between herptile eDNA quantity and abundance was similar to that of fish, which is reasonable given amphibians likely shed eDNA constantly from their epidermis and/or mucus. However, the variation (95 % CI) was much larger for herptiles relative to fish. This point could simply be explained by biases derived from the smaller number of corresponding studies or different experimental conditions. Most of the relationships between herptile eDNA quantity and abundance were studied in natural environments and depended on visual counts for abundance estimation (e.g., Thomsen et al., 2012; Kakuda et al., 2019), which might make the relationships less certain than other capture-based survey. On the other hand, Everts et al. (2021) assessed the relationships between eDNA concentration and abundance of American bullfrog (*Lithobates catesbeianus*) tadpoles and juveniles using mesocosm experiments, and reported relatively high  $R^2$  values (0.64 to 0.99). Accumulating studies targeting various taxa in laboratory conditions and natural environments could help us understand the effects of ecological characteristics (morphology, physiology, and ethology) on the process of eDNA production, and may provide us with keys for improving eDNA-based abundance estimation.

#### Estimation accuracy and eDNA state

We showed that the use of larger pore size filters could improve the accuracy of eDNA-based abundance estimation. According to previous studies, the cellular and molecular structure of larger eDNA particles derived from intra-cellular DNA, such as cells and tissues, is degraded into smaller eDNA particles over time (Jo et al., 2019b). Apparent persistence of such smaller-sized eDNA could thus be longer than that of larger-sized eDNA, and larger eDNA particles collected using larger pore size filters are more likely to be recently released and less degraded than smaller eDNA particles. Such larger-sized 'fresher' eDNA is expected to reflect species presence and abundance at a spatiotemporally finer scale, consequently improving the accuracy of eDNA-based abundance estimation. Alternatively, given that filter pore size was positively correlated with the volume of water samples (see Materials and Methods), larger volume of water samples might allow to diminish the heterogeneity of target eDNA quantity across sampling replicates and improve the estimation accuracy of species abundance. Although individual eDNA studies could not clearly infer the effects of filter pore size and water volume on the estimation accuracy (Takahara et al., 2012; Eichmiller et al., 2016), further empirical studies will be required which factors substantially contribute to the relationship between eDNA quantity and species abundance. In any case, our meta-analysis supports the applicability of larger pore size filters for improved abundance estimation *via* eDNA analysis for the first time.

In contrast, some datasets reported high  $R^2$  values using smaller pore size filters (Figure 3), which can collect both larger-sized fresher eDNA and smaller-sized older eDNA. Thus, these studies may have collected a higher proportion of larger-sized eDNA while using smaller pore size filters; in particular, laboratory experiments with high abundances (e.g., Takahara et al., 2012; Doi et al., 2015) may have collected large quantities of large-sized fresh eDNA. Although studies using larger pore size filters (especially >3  $\mu$ m) were limited in our dataset, further empirical studies targeting larger-sized eDNA particles would conceivably contribute to the robustness of our results and potentially provide a new approach to improve the accuracy of eDNA-based abundance estimation in the field.

PCR amplicon size (i.e., DNA fragment length of target eDNA) was not significantly correlated with  $R^2$  values. However, almost all the studies in our meta-analysis targeted eDNA fragments (<500 bp) much shorter than overall genome and thus the effect of PCR amplicon size on the accuracy of eDNA-based abundance estimation may be underestimated. Owing to higher decay rates, detection of longer eDNA fragments may mitigate the effect of degraded eDNA and improve species abundance estimation accuracy (Jo et al., 2017). Nonetheless, Jo et al. (2017) was conducted in a situation where false-positive inferences of target individuals could be obvious (i.e., the effect of fish markets and dead individuals); thus, the performance of longer eDNA fragments for species abundance estimation in more ordinary situations is unknown. In addition, some studies reported that there were no significant differences in eDNA decay rate or persistence time depending on amplicon size (e.g., Ma et al., 2016; Bylemans et al., 2018). Future empirical studies targeting longer eDNA fragments are needed to elucidate the importance of PCR amplicon size on eDNA-based abundance estimation.

## Estimation accuracy and eDNA assay strategy

In addition to eDNA production source and state, we investigated the effects of abundance metrics and eDNA assay strategy, including collection and quantification strategies, on the estimation accuracy of species abundance *via* eDNA. First, there was no significant difference in the estimation accuracy between biomass and number of individuals. This is consistent with the finding in Yates et al. (2019) although our study meta-analyzed a larger number of studies. However, given that wild populations are often composed of individuals with different age classes and body sizes, selecting either metrics itself may ultimately hamper

the accurate estimation abundance to select either of abundance metrics. A few studies recently applied allometric scaling coefficients to the relationship between eDNA quantity and species abundance, showing higher  $R^2$  values for allometrically scaled mass (ASM) than biomass and individuals (Chin et al., 2021; Yates et al., 2021a; 2021b).

Second, the estimation accuracy of species abundance was not affected by the assay strategy (filtration/centrifugation and species-specific/metabarcoding). In particular, although the variation of  $\mathbb{R}^2$  values were higher for metabarcoding than species-specific assay, it is surprising that metabarcoding assay had similar abundance estimation accuracy to species-specific assay (real-time or digital PCR). However, the result must be tempered with an acknowledgement that (i) the proportion of studies conducting laboratory experiments were higher for metabarcoding assay, (ii) there was no result of metabarcoding assay targeting crustaceans eDNA, and (iii) the number of literatures performing metabarcoding assay was much fewer (9 out of 56) than that of species-specific assay (Tables 1 and S1). Theoretically, as the number of eDNA reads is estimated from the PCR final product, metabarcoding assay should not be suitable for the accurate estimation of species abundance, except for the application of internal standard DNAs (i.e., known amounts of short artificial DNA fragments; Ushio et al., 2018). In addition, regardless of the substantial difference in the volume of water sample, there was no difference in the estimation accuracy between filtration and centrifugation. This implies that water sample volume does not have a substantial effect on the estimation accuracy, partially contradicting the above hypothesis that larger volume of water samples might allow to improve the estimation accuracy of species abundance. Further studies are required how the assay strategy and sampling water volume relate to the accuracy of eDNA-based species abundance estimation.

## Estimation accuracy and environments

Higher R<sup>2</sup> values were reported for laboratory conditions than natural environments, which is consistent with previous findings (Yates et al., 2019) and supports the methodological validity of our statistical analyses. As discussed in Yates et al. (2019), myriad and complex dynamics of eDNA could hamper the prediction of species abundance based on eDNA concentration in natural relative to controlled environments. However, we observed little difference in estimation accuracy among natural environments. Relative to lentic freshwater environments, such as ponds and lakes, eDNA diffusion and degradation would be substantial in riverine, coastal, and marine environments due to flow and tidal effects. Such factors can transport eDNA very long distances (Deiner & Altermatt, 2014; Andruszkiewicz et al., 2019) while also resulting in rapid dilution (Baker et al., 2018). In contrast, although eDNA may diffuse less in lentic environments (Fremier et al., 2019; Curtis et al., 2020), residual eDNA may cause false-positive inferences of species presence and inaccurate estimation of species abundance. It is noted that our results must be cautiously interpreted given the bias in the number of studies among natural environment types, but eDNA-based abundance estimation accuracy might not necessarily be worsened for lotic freshwater and marine environments relative to lentic environments.

#### Conclusions, limitations, and perspectives

To our knowledge, the present meta-analysis is the first to report variations in eDNA-based estimation accuracy of species abundance among different target taxa and filter pore sizes (reflecting eDNA particle size distribution). The significance is that the relationship between eDNA quantity and species abundance can be driven not only by eDNA transport and degradation processes but also by the characteristics of eDNA (its production source and state). Some recent studies have suggested the possibility of improving the accuracy of eDNA-based abundance estimation by statistically accounting for the processes of eDNA production, transport, and degradation (Carraro et al., 2018; Cerco et al., 2018; Fukaya et al., 2021). In contrast, our meta-analyses shed a new light on the importance of what characteristics of eDNA should be targeted for more accurate estimation of species abundance. In particular, our findings on the effects of eDNA state imply that 'more recently released' eDNA, existing as larger eDNA particles and potentially longer eDNA fragments, more precisely reflect species abundance in the field. This knowledge will complement abundance estimation approaches that consider eDNA spatiotemporal dynamics; that is, understanding eDNA characteristics, including production source, particle size, and fragment length, as well as eDNA production, transport, and degradation processes, will enable us to further enhance the potential of eDNA analysis as a non-disruptive and cost-efficient tool for species abundance estimation. Therefore, accumulating knowledge of eDNA states and their interactions with the spatiotemporal dynamics (e.g., the processes of production, transport, and degradation) is crucial (Jo & Minamoto, 2021).

It should be noted that there are some potential biases and limitations in our meta-analyses. First, regardless of accounting for random effects and multiple factors, we observed the high degree of heterogeneity across studies and datasets in our meta-analysis. This infers that there remain a number of variables that our study could not consider. For example, environmental parameter such as water qualities and temperature has not considered here, although this point was barely considered as the categorical factor (laboratory/natural, lentic/lotic, freshwater/marine). Degradation of eDNA, which is accelerated by higher temperature (Strickler et al., 2015; Jo et al., 2019a), could affect its persistence time in water and possibly the goodness of relationships between eDNA quantity and species abundance. In addition, the difference in other technical steps, including eDNA storage and extraction, in the analysis should be considered. Some studies filtered water samples on site and transferred the filter samples to the laboratory, while others transported water samples and filtered them in the laboratory (Kumar et al., 2020). There are also substantial variations of DNA extraction protocols across studies (commercial kits or in-house formulations; Kumar et al., 2020). These methodologies might accordingly influence the estimation accuracy of species abundance *via* eDNA, as well as eDNA detectability and quantification.

Second, we also acknowledge the publication bias in this study. The asymmetry funnel plot infers that there may be some 'hidden' studies that both the estimation accuracy and its variance are small. This may partly be attributed to the studies that were not included in this meta-analysis because the indices of abundance estimation accuracy (Pearson's correlation coefficients or  $\mathbb{R}^2$  values) were not estimated or presented in the manuscript. Besides, our collected dataset was concentrated toward studies targeting fish species, which might cause biased and over-dispersed estimation for other taxa. Accumulating additional empirical studies for various taxa, assay strategy, and environmental conditions is necessary to validate the findings of our meta-analyses and further elucidate the influence of eDNA characteristics on eDNA-based estimation of species abundances.

Furthermore, although not considered in the present study, nuclear eDNA, particularly targeting multiple copies of ribosomal RNA genes, may be applicable for more accurate eDNA-based species abundance estimations. Relative to mitochondrial eDNA, targeting multi-copy nuclear eDNA can improve detectability and yield (Minamoto et al., 2017; Jo et al., 2020b) and nuclear eDNA may degrade more rapidly due to potential differences in membrane and DNA structures (Bylemans et al., 2018; Jo et al., 2020b). In addition, nuclear eDNA production may also be less biased by individual growth and developmental stages, whereas mitochondrial eDNA production is expected to be suppressed with maturity and aging (Jo et al., 2020b). Understanding both the characteristics and dynamics of eDNA will fill a gap between eDNA concentration and species abundance in the field, and update current eDNA analysis as a more refined tool for biodiversity and ecosystem monitoring and stock assessment.

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#### Data accessibility

Detailed information on published studies reporting the relationship between eDNA quantity and species abundance can be found in the Supporting Information.

#### Author contributions

T.J. and H.Y. conceived the study. T.J. performed the literature search, analyzed the data, and wrote the

first draft of the manuscript. Both authors edited and provided feedback on the manuscript. There is no conflict of interest to declare.

## **Supporting Information**

Table S1. A detailed list of all eDNA literature selected and analyzed in this study.

Table S2. Summary of a GLMM with MCMC algorithm assessing the effects of multiple factors on  $\mathbb{R}^2$  values (Fisher's z-transformed) without the outliers.

Figure S1. Comparison of  $\mathbb{R}^2$  values among the assay strategy (a) and abundance metrics (b).

Figure S2.  $\mathbb{R}^2$  values (a) and Fisher's z values (b) with relation to filter pore sizes used for water filtration.

Figure S3. Forest plots for integrating individual  $R^2$  values for the assay strategy (a), target taxa (b), environments (c), and abundance metrics (d).

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## Tables

Table 1. Published literature on the relationship between eDNA quantity and species abundance analyzed in this study.

| Study                                   | $\# \mathbf{R}^2$ values | Assay<br>strategy         | Target taxa           | Amplicon<br>size [bp] | Filter pore<br>size [µm] | Environment                     | Metrics               | Sample  |
|---|--------------------------|---------------------------|-----------------------|-----------------------|--------------------------|---------------------------------|-----------------------|---------|
| Takahara<br>et al. 2012                 | 3                        | Filtration<br>Centrifugat | Fish<br>ion           | 78                    | 0 to 3                   | Laboratory<br>Fresh /<br>Lentic | Biomass               | 6 to 12 |
| Thomsen<br>et al.<br>2012               | 2                        | Centrifugat               | ionHerptile           | 72 to 81              | 0                        | Fresh /<br>Lentic               | Individual            | 9 to 10 |
| Pilliod et<br>al. 2013                  | 4                        | Filtration                | Herptile              | 78 to 85              | 0.45                     | Fresh /<br>Lotic                | Individual<br>Biomass | 14 to 1 |
| Doi et al.<br>2015                      | 4                        | Centrifugat               | ionFish               | 78                    | 0                        | Laboratory                      | Individual<br>Biomass | 36      |
| Klymus<br>et al.<br>2015                | 2                        | Centrifugat               | ionFish               | 108 to<br>190         | 0                        | Laboratory                      | Biomass               | 24      |
| Dougherty<br>et al.<br>2016             | 1                        | Filtration                | Crustacean            | 128                   | 1.2                      | Fresh /<br>Lentic               | Individual            | 12      |
| Eichmiller<br>et al.                    | 4                        | Filtration                | Fish                  | 149                   | 0.2 to $5$               | Laboratory                      | Biomass               | 9       |
| Erickson<br>et al.<br>2016              | 1                        | Centrifugat               | ionFish               | 108                   | 0                        | Fresh /<br>Lotic                | Individual            | 12      |
| Evans et al. 2016                       | 53                       | Metabarcod                | lingFish<br>Herptile  | 202 to $413$          | 1.2                      | Laboratory                      | Biomass               | 12      |
| Lacoursière-<br>Roussel et<br>al. 2016a | 20                       | Filtration                | Fish                  | 139                   | 0.2 to 3                 | Laboratory                      | Individual<br>Biomass | 15      |
| Lacoursière-<br>Roussel et<br>al. 2016b | 2                        | Filtration                | $\operatorname{Fish}$ | 66                    | 1.2                      | Fresh /<br>Lentic               | Individual<br>Biomass | 12      |
| Schmelzle<br>&<br>Kinziger<br>2016      | 1                        | Filtration                | Fish                  | 119                   | 3                        | Fresh /<br>Lentic               | Individual            | 20      |
| Thomsen et<br>al. 2016                  | 2                        | Metabarcod                | lingFish              | 100                   | 0.45                     | Marine                          | Biomass<br>Individual | 54      |
| Wilcox<br>et al.<br>2016                | 1                        | Filtration                | $\operatorname{Fish}$ | 90                    | 1.5                      | Fresh /<br>Lotic                | Individual            | 46      |

| Study                          | $\# \mathbf{R}^2$ values | Assay<br>strategy | Target taxa          | Amplicon<br>size [bp] | Filter pore<br>size [µm] | Environment          | Metrics               | Sample  |
|--------------------------------|--------------------------|-------------------|----------------------|-----------------------|--------------------------|----------------------|-----------------------|---------|
| Yamamoto<br>et al.             | 1                        | Filtration        | Fish                 | 127                   | 0.7                      | Marine               | Biomass               | 94      |
| 2016<br>Baldigo et<br>al. 2017 | 2                        | Filtration        | Fish                 | 140                   | 1.5                      | Fresh /<br>Lotic     | Individual<br>Biomass | 27      |
| Doi et al.<br>2017             | 6                        | Filtration        | Fish                 | 131                   | 0.7                      | Fresh /<br>Lotic     | Individual<br>Biomass | 7       |
| Dunn et<br>al. 2017            | 1                        | Centrifugation    | nCrustacean          | 88                    | 0                        | Laboratory           | Biomass               | 30      |
| Klobucar<br>et al.<br>2017     | 1                        | Filtration        | Fish                 | 145                   | 10                       | Fresh /<br>Lentic    | Individual            | 5       |
| Larson<br>et al.<br>2017       | 2                        | Filtration        | Crustacean           | 184                   | 1.2                      | Fresh /<br>Lentic    | Individual            | 9       |
| Mauvisseau<br>et al.<br>2017   | 1                        | Filtration        | Mollusk              | 204                   | 0.2                      | Laboratory           | Biomass               | 9       |
| Currier<br>et al.              | 3                        | Filtration        | Mollusk              | 99 to<br>129          | 1.2                      | Fresh /<br>Lotic     | Individual            | 216     |
| Kamoroff<br>& Goldberg<br>2018 | 2                        | Filtration        | Fish                 | 140                   | 1.2                      | Fresh /<br>Lentic    | Individual<br>Biomass | 18      |
| Maruyama<br>et al.<br>2018     | 1                        | Filtration        | Fish                 | 129                   | 0.7                      | Fresh /<br>Lotic     | Individual            | 32      |
| Mizumoto<br>et al. 2018        | 5                        | Filtration        | Fish                 | 124                   | 0.7                      | Laboratory           | Individual<br>Biomass | 10 to 6 |
| Nevers<br>et al.<br>2018       | 1                        | Filtration        | Fish                 | 147                   | 0.22                     | Fresh /<br>Lentic    | Individual            | 5       |
| Shinzato<br>et al.<br>2018     | 2                        | Metabarcodin      | ngCoral &<br>Seastar | 900                   | 0.8                      | Laboratory           | Biomass               | 9       |
| Uthicke et<br>al. 2018         | 4                        | Filtration        | Coral &<br>Seastar   | 126                   | 0.22 to $1$              | Laboratory<br>Marine | Biomass<br>Individual | 6 to 14 |
| Wu et<br>al. 2018              | 1                        | Filtration        | Crustacean           | 166                   | 0.7                      | Fresh /<br>Lentic    | Individual            | 10      |
| Bracken<br>et al.<br>2019      | 1                        | Filtration        | Fish                 | 72                    | 0.45                     | Fresh /<br>Lotic     | Individual            | 10      |
| Horiuchi<br>et al.<br>2019     | 1                        | Filtration        | Fish                 | 127                   | 0.7                      | Laboratory           | Biomass               | 22      |
| Itakura et<br>al. 2019         | 2                        | Filtration        | Fish                 | 153                   | 0.7                      | Fresh /<br>Lotic     | Individual<br>Biomass | 31      |
| Iwai et al.<br>2019            | 2                        | Filtration        | Herptile             | 96                    | 0.7                      | Fresh /<br>Lotic     | Individual<br>Biomass | 53      |

| Study                            | $\# \mathbb{R}^2$ values | Assay<br>strategy | Target taxa           | Amplicon<br>size [bp] | Filter pore<br>size [µm] | Environment       | Metrics               | Sample  |
|----------------------------------|--------------------------|-------------------|-----------------------|-----------------------|--------------------------|-------------------|-----------------------|---------|
| Kakuda<br>et al.                 | 1                        | Filtration        | Herptile              | 153                   | 0.7                      | Fresh /<br>Lentic | Individual            | 20      |
| 2019<br>Minegishi<br>et al.      | 2                        | Filtration        | Fish                  | 163                   | 0.7                      | Laboratory        | Individual            | 10      |
| 2019<br>Nichols<br>&<br>Marko    | 4                        | Metabarcod        | ingCoral &<br>Seastar | 120 to<br>400         | 0.22                     | Marine            | Biomass               | 12      |
| 2019<br>Salter et al.<br>2019    | 2                        | Filtration        | Fish                  | NA                    | 0.2                      | Marine            | Biomass<br>Individual | 8       |
| Takeuchi<br>et al.               | 1                        | Filtration        | Fish                  | 107                   | 0.45                     | Laboratory        | Biomass               | 18      |
| Akamatsu<br>et al.               | 2                        | Filtration        | Fish                  | 124                   | 0.7                      | Fresh /<br>Lotic  | Individual            | 4 to 9  |
| Guivas<br>&<br>Bram-<br>mell     | 2                        | Filtration        | Fish                  | 118                   | 0.7                      | Laboratory        | Biomass               | 14      |
| 2020<br>Itakura et<br>al. 2020   | 2                        | Filtration        | Fish                  | 171                   | 0.7                      | Fresh /<br>Lotic  | Individual<br>Biomass | 25      |
| Stoeckle<br>et al.<br>2020       | 4                        | Metabarcod        | ingFish               | 106                   | 0.45                     | Marine            | Biomass               | 17 to 5 |
| Weldon<br>et al.<br>2020         | 2                        | Filtration        | Fish                  | 159                   | 3                        | Fresh /<br>Lentic | Biomass               | 18 to 8 |
| Boivin-<br>Delisle et<br>al 2021 | 6                        | Metabarcod        | ingFish               | 135                   | 1.2                      | Fresh /<br>Lotic  | Individual<br>Biomass | 14 to 1 |
| Brys et<br>al. 2021              | 1                        | Filtration        | Fish                  | 119                   | 0.45                     | Laboratory        | Biomass               | 6       |
| Capo et al.<br>2021              | 4                        | Filtration        | Fish                  | 134                   | 0.2                      | Fresh /<br>Lentic | Individual<br>Biomass | 14 to 1 |
| Chin et al.<br>2021              | 2                        | Filtration        | Fish                  | 151                   | 0.45                     | Fresh /<br>Lotic  | Individual<br>Biomass | 7       |
| Cornman<br>et al.<br>2021        | 1                        | Metabarcod        | ingFish               | 106                   | 0.8                      | Fresh /<br>Lotic  | Individual            | 30      |
| Everts et<br>al 2021             | 4                        | Filtration        | Herptile              | 84                    | 0.8                      | Laboratory        | Individual<br>Biomass | 7 to 8  |
| Li et al.<br>2021                | 7                        | Metabarcod        | ingHerptile           | 52                    | 1.5                      | Fresh /<br>Lentic | Individual            | 71      |
| Ponce et<br>al. 2021             | 1                        | Filtration        | Mollusk               | 93                    | 5                        | Fresh /<br>Lotic  | Individual            | 5       |

| Study                       | $\# R^2$ values | Assay<br>strategy | Target taxa           | Amplicon<br>size [bp] | Filter pore<br>size [µm] | Environment       | Metrics               | Sample |
|-----------------------------|-----------------|-------------------|-----------------------|-----------------------|--------------------------|-------------------|-----------------------|--------|
| Sepulveda<br>et al.<br>2021 | 3               | Filtration        | Fish                  | 88 to<br>172          | 1.5                      | Fresh /<br>Lotic  | Biomass               | 65     |
| Shu et al.<br>2021          | 8               | Metabarcod        | ingFish               | 218 to 321            | 0.45                     | Laboratory        | Biomass<br>Individual | 72     |
| Spear et al.<br>2021        | 2               | Filtration        | Fish                  | 175                   | 0.7                      | Fresh /<br>Lentic | Individual<br>Biomass | 22     |
| Yates et al.<br>2021a       | 2               | Filtration        | Fish                  | 90                    | 1.5                      | Fresh /<br>Lotic  | Individual<br>Biomass | 27     |
| Yates et al.<br>2021b       | 2               | Filtration        | $\operatorname{Fish}$ | 90                    | 0.7                      | Fresh /<br>Lentic | Individual<br>Biomass | 9      |

Note. All the studies collecting eDNA by filtration or centrifugation measured eDNA quantity by real-time or digital PCR. All the metabarcoding studies collected aqueous eDNA by filtration. Amplicon size was not reported in Salter et al. (2019) because they used a commercial kit.

Table 2. Summary of a GLMM with MCMC algorithm assessing the effects of multiple factors on  $\mathbb{R}^2$  values (Fisher's z-transformed).

| Variable                     | Df | Posterior mean [95 $\%$ CI]    | Effective sample size | P value in MCMC | P value in MO |
|------------------------------|----|--------------------------------|-----------------------|-----------------|---------------|
| Intercept                    |    | 1.013 [0.717, 1.280]           | 1000.0                | < 0.001         | ***           |
| Assay (Centrifugation)       | 2  | 0.270 [-0.126, 0.667]          | 1000.0                | 0.156           |               |
| Assay (Metabarcoding)        |    | -0.018 $[-0.335, 0.328]$       | 1000.0                | 0.908           |               |
| Taxa (Herptile)              | 4  | -0.150 $[-0.397, 0.063]$       | 1000.0                | 0.184           |               |
| Taxa (Crustacean)            |    | -0.544 $[-1.074, -0.030]$      | 1207.0                | 0.046           | *             |
| Taxa (Mollusk)               |    | -0.348 $[-0.882, 0.288]$       | 882.1                 | 0.256           |               |
| Taxa (Coral & Seastar)       |    | 0.500 [-0.116, 1.063]          | 1000.0                | 0.088           |               |
| Amplicon size                | 1  | 0.000 [-0.001, 0.000]          | 729.3                 | 0.316           |               |
| Filter pore size             | 1  | 0.094 $[0.011, 0.180]$         | 1030.0                | 0.028           | *             |
| Environment (Fresh / Lentic) | 3  | -0.457 [ $-0.775$ , $-0.106$ ] | 1866.2                | 0.004           | **            |
| Environment (Fresh / Lotic)  |    | -0.388 [-0.692, -0.106]        | 1000.0                | 0.016           | *             |
| Environment (Marine)         |    | -0.591 [-1.004, -0.124]        | 1000.0                | 0.006           | **            |
| Metrics (Individual)         | 1  | $0.077 \ [-0.025, \ 0.182]$    | 1000.0                | 0.140           |               |

Note: The abbreviation 'Df' means degrees of freedom. Asterisks and dot indicate statistical (\*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05) and marginal (P < 0.1) significances of the fixed effects. The posterior means in the fixed effect 'Assay' were estimated against the studies collecting eDNA by filtration. The posterior means in the fixed effect 'Taxa were estimated against the studies targeting fish species. The posterior means in the fixed effect 'Environment were estimated against the studies performing in laboratory conditions. The posterior means in the fixed effect 'Metrics were estimated against the studies using species biomass as an abundance metrics.

Table 3. Summary of heterogeneity in the meta-analysis.

| Category        | Category        | I <sup>2</sup> [95 % CI] (%) | Q value | P value | P value | P value |
|-----------------|-----------------|------------------------------|---------|---------|---------|---------|
| Overall dataset | Overall dataset | 77.4 [74.2, 80.2]            | 897.38  | 897.38  | < 0.001 | ***     |
| Assay strategy  | Filtration      | 72.4 [66.5, 77.2]            | 380.9   | 380.9   | < 0.001 | ***     |

| Category    | Category        | $I^2 [95 \% CI] (\%)$ | Q value | P value | P value | P value |
|-------------|-----------------|-----------------------|---------|---------|---------|---------|
|             | Centrifugation  | 78.3 [61.5, 87.7]     | 46.0    | 46.0    | < 0.001 | ***     |
|             | Metabarcoding   | 78.1 [73.3, 82.1]     | 392.7   | 392.7   | < 0.001 | ***     |
| Target taxa | Fish            | 76.2 72.4, 79.6       | 661.1   | 661.1   | < 0.001 | ***     |
| 0           | Herptile        | 58.1 [35.3, 72.9]     | 59.7    | 59.7    | < 0.001 | ***     |
|             | Crustacean      | $0.0 \ [0.0, \ 12.5]$ | 1.0     | 1.0     | 0.917   |         |
|             | Mollusk         | 0.0 $[0.0, 59.8]$     | 2.1     | 2.1     | 0.723   |         |
|             | Coral & Seastar | 64.7 [30.7, 82.1]     | 25.5    | 25.5    | 0.002   | **      |
| Environment | Laboratory      | 70.7 [64.6, 75.7]     | 385.0   | 385.0   | < 0.001 | ***     |
|             | Fresh / Lentic  | 32.9 [0.0, 56.2]      | 47.7    | 47.7    | 0.037   | *       |
|             | Fresh / Lotic   | 80.3 [74.0, 85.1]     | 208.2   | 208.2   | < 0.001 | ***     |
| Metrics     | Marine          | 71.5[52.1, 83.1]      | 49.2    | 49.2    | < 0.001 | ***     |
|             | Biomass         | 74.2 [69.4, 78.3]     | 492.5   | 492.5   | < 0.001 | ***     |
|             | Individual      | $79.7 \ [74.9, 83.5]$ | 368.7   | 368.7   | < 0.001 | ***     |

Note. Q values were used to test the heterogeneity across studies/datasets in Cochran's Q test (corresponding P values were shown in the right). Asterisks indicate the significant heterogeneity (\*\*\* P < 0.001; \*\* P < 0.01; \*P < 0.01; \*P < 0.05).

## Figure legends

Figure 1. Summary of our literature search in the meta-analysis according to the PRISMA statement. We compiled all the eDNA studies collected in the previous review article (Jo et al., 2021) (N = 728) and additionally searched for the eDNA studies published in this year (N = 195). By reading the abstracts of them, we retained 118 literature describing the relationship between eDNA quantity and species abundance. We further carefully read the full-texts of the remaining literature and finally included 56 literature in the meta-analysis.

Figure 2. Comparison of  $\mathbb{R}^2$  values among the target taxa (a) and study environments (b). Circles and error bars represent the mean  $\mathbb{R}^2$  values and their 95 % CIs estimated by the forest plots (Figure S3). The gray dotted line shows  $\mathbb{R}^2 = 0$ . Numerals in parentheses mean the number of individual  $\mathbb{R}^2$  values required for each plot.

Figure 3.  $R^2$  values (a) and Fisher's z values (b) with relation to filter pore sizes used for water filtration. The size of each plot represents the sample size required to calculate original  $R^2$  values in each individual study. The regression line is based on the result of the meta-regression analysis.

Figure 4. The asymmetry funnel plot showing the publication bias in this study.

## Figures

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## Figure 1.

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Figure 2.

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Figure 3.

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production-source-state-and-assay-methodology-suggested-by-meta-analyses
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Figure 4.