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RESEARCH ARTICLE OPEN ACCESS

Allometric Scaling in Environmental DNA Concentration of Japanese Eel *Anguilla japonica* Confirmed Under Laboratory and Natural Conditions

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Received: 20 June 2025 | **Accepted:** 6 October 2025

Editor-in-Chief: Binbin Li | **Handling Editor:** Eric Ameca

Funding: This study was supported by River Fund of the River Foundation and Grant-in-Aid for JSPS Research Fellows (JP16J01523, JP22J00439, JP22KJ3043).

Keywords: abundance | allometric scaling | conservation | environmental DNA (eDNA) | Japanese eel (*Anguilla japonica*) | stock assessment

ABSTRACT

Environmental DNA (eDNA) analysis offers a noninvasive, efficient, and sensitive approach for biomonitoring in aquatic ecosystems compared with traditional capture-based methods. However, accurately estimating species abundance using eDNA analysis remains challenging due to uncertainty in the correlative relationship between eDNA concentration and organism abundance in natural environments. This uncertainty is partly attributed to variation in body mass distribution within and among populations, which may be addressed by integrating body size allometry into eDNA production models. In this study, we focused on the Japanese eel (*Anguilla japonica*), an endangered species of anguillid eel that is globally recognized as a significant food and cultural resource, to investigate whether eDNA production scales allometrically with body size. We reanalyzed existing datasets describing the relationships between *A. japonica* eDNA concentration and abundance under both laboratory and riverine conditions. Our results show that eDNA concentrations were scaled allometrically with body mass ($0 < b < 1$), with scaling coefficients closely matching those previously estimated for *A. japonica* oxygen consumption rate. The effect of allometric scaling, however, appeared weaker in riverine environments than under laboratory conditions, and integrating body size allometry did not substantially strengthen the relationship between eDNA concentration and abundance. These findings underscore the importance of body size structure in eDNA production for anguillid eels and highlight the potential of allometric scaling to refine eDNA-based abundance estimates. Further studies on the mechanisms of eDNA production and the influence of environmental parameters are needed to enhance the accuracy and applicability of eDNA analytical procedures for population assessments in the wild.

1 | Introduction

Environmental DNA (eDNA) analysis has recently revolutionized biomonitoring in aquatic and terrestrial environments, offering great potential for optimizing ecosystem and resource management (Deiner et al. 2017; Evans and Lamberti 2018; Sales et al. 2020; Yao et al. 2022). Organisms release DNA into the environment through epidermal cells, mucus, feces, and gametes (i.e., eDNA), and species distribution and composition can be inferred from eDNA collected from water, soil, or air without capturing or directly observing individuals (Jo et al. 2022a). The noninvasive, cost-effective, and highly sensitive

Summary

Environmental DNA (eDNA) analysis provides a sensitive, noninvasive way to monitor aquatic life, offering an alternative to traditional methods that rely on capturing organisms. However, accurately estimating population abundance from eDNA remains difficult because the link between eDNA concentration and the actual biomass in nature is not yet fully understood. One reason is that differences in body size across populations can influence how much eDNA organisms release into the environment. Incorporating body size allometry—the way biological traits scale with body size—into eDNA studies may help improve these estimates. This study focused on the Japanese eel (*Anguilla japonica*), an endangered species of major cultural and economic value, to test whether eDNA production follows an allometric relationship with body size. By reanalyzing existing laboratory and river datasets, we found that eDNA concentration scales allometrically with body mass (scaling exponent $0 < b < 1$), with scaling coefficients similar to those previously reported for oxygen consumption rates in this species. This suggests that eDNA production may be linked to metabolic activity. However, including body size allometry did not greatly enhance the accuracy of abundance predictions from eDNA data. Overall, the findings highlight the role of body size structure in eDNA production and the potential for allometric approaches to improve eDNA-based monitoring. Further research is needed to clarify how environmental factors affect eDNA release and to refine analytical methods for more reliable field applications.

- **Practitioner Points**
  - Given the allometric relationship between Japanese eel eDNA concentration and its biomass, ignoring body size information in field sites may result in under- or overestimation of biomass.
  - Obtaining approximate data on the body size structure of target populations through limited capture surveys can help calibrate interpretations of eDNA quantification.
  - Further research is needed to clarify the mechanisms of eDNA production in relation to body size and allometry.

nature of eDNA-based biomonitoring has been demonstrated across diverse taxa, including fish, amphibians, mammals, and macroinvertebrates (Thomsen et al. 2012; Valentin et al. 2018; Leempoel et al. 2020; Li et al. 2021). Moreover, eDNA analysis can be applied to estimate population abundance, based on the general assumption that eDNA concentration positively correlates with population abundance (Jo et al. 2022b; Rourke et al. 2022). Here, "abundance" refers to both the number of individuals and total biomass within a population, with "biomass" defined as the sum of individual body masses in the population.

In natural environments, the eDNA-abundance relationship tends to be less precise than under laboratory conditions, and abundance estimation using eDNA analysis is currently in its infancy (Yates et al. 2019). Laboratory studies typically employ individuals of a similar age and body size to generate multiple density or biomass treatments, whereas in nature, populations often exhibit substantial variation in body size and structure (Yates et al. 2021a). Previous studies have shown that larger fish tend to have lower mass-specific eDNA production rates (i.e., eDNA production per unit body mass; e.g., copies/hour/gram) (Maruyama et al. 2014; Takeuchi et al. 2019a) (Table 1). Spear et al. (2021) also demonstrated that the relationship between walleye (*Sander vitreus*) eDNA concentration and its abundance (both biomass and number of individuals estimated through mark–recapture sampling) improved when mean walleye body size was included in the model, with a negative coefficient. These findings suggest that both organism abundance and body size impact observed eDNA concentrations, and that the weaker eDNA–abundance relationship observed in natural environments may be partly attributed to variations in size structure.

Recently, this concept has been formalized through the integration of allometric scaling in eDNA production models (Yates et al. 2021b) (Table 1). Major processes contributing to eDNA production—such as metabolic excretion (e.g., urine and fecal matters) and shedding from the body surface (e.g., epidermis and mucus) (Lacoursière-Roussel et al. 2016; Stewart 2019)—are known to scale nonlinearly with individual mass and body size (i.e., allometric scaling; Brown et al. 2004). Metabolic activity underpins diverse biological processes (e.g., excretion, secretion, and cellular turnover) that govern the release of organic matter into the environment (Brown et al. 2004), and thus the rate of eDNA production is expected to follow the same principle. Accordingly, differences in eDNA production due to size structure variation can be expressed as the sum of individual masses raised to the power of a scaling coefficient b (i.e., allometrically scaled mass [ASM]; Yates et al. 2021b). This is expressed as follows:

$$eDNA\ production\ rate = f(abundance) = f\left(\sum(individual\ mass^{b})\right)$$

where eDNA production rate (e.g., copies/hour) is considered to scale allometrically with individual mass when $0 < b < 1$, while individuals exhibit similar eDNA production rates regardless of their size (i.e., eDNA production rates are explained by the individual density but not biomass density) when $b = 0$, and there is a perfect linear relationship between eDNA production and biomass when $b = 1$ (Yates et al. 2021a). This hypothesis was first tested for brook trout (*Salvelinus fontinalis*) populations (Yates et al. 2021b, 2021c), followed by other fish species (Yates et al. 2023) and amphibians (Breton et al. 2022), showing allometric scaling effects on population-level eDNA production. To strengthen the generality and applicability of the allometric scaling hypothesis, further research across species with different physiologies and morphologies—as well as tests at the individual level under controlled laboratory conditions—is essential. Such efforts would further improve the reliability and precision of quantitative biomonitoring using eDNA.

The genus *Anguilla* has a catadromous life history, reproducing in the open ocean and growing in continental waters, with 16 species distributed across more than 150 countries (Tsukamoto and Arai 2001; International Union for the Conservation of Nature IUCN. 2023). Anguillid eels are globally recognized as important food and cultural resources (Kuroki et al. 2014). They are also ecologically significant, serving as

TABLE 1 | Previous studies supporting the allometric scaling of eDNA production (excerpted).

Study	Target species	Environment	Major finding
Maruyama et al. (2014)	Bluegill sunfish *Lepomis macrochirus*	Aquarium	The rate of eDNA release per fish body weight was higher in the juvenile than in the adult group.
Takeuchi et al. (2019a)	Japanese eel *Anguilla japonica*	Aquarium	eDNA concentration per wet weight decreased as the developmental stages progressed.
Spear et al. (2021)	Walleye *Sander vitreus*	Lake	The relationship between eDNA concentration and abundance metrics (both biomass and the number of individuals) was strengthened by considering the mean walleye size in each lake.
Yates et al. (2021b)	Brook trout *Salvelinus fontinalis*	Lake	ASM (allometrically scaled mass) explained more variation in eDNA concentration than individual and biomass densities ($b = 0.73$).
Yates et al. (2021c)	Brook trout *S. fontinalis*	River	ASM explained more variation in eDNA concentration than biomass density ($b = 0.36$) but had comparable explanatory power to individual density.
Breton et al. (2022)	Northern leopard frog *Rana pipiens* Wood frog *R. sylvatica*	Mesocosm	ASM explained more variation in *R. pipiens* eDNA concentration than individual and biomass densities ($b = 0.72$) but had comparable explanatory power to individual density for *R. sylvatica* eDNA concentration ($b = 0.79$).
Nakagawa et al. (2022)	Bullhead *Cottus pollux* Chinese minnow *Rhynchocypris oxycephalus*	River	Integrating mean body mass improved the relationship between *C. pollux* and *R. oxycephalus* eDNA concentration and fish abundance (but not significantly for other fishes).
Yates et al. (2023)	Northwestern Atlantic bony fishes	Sea coast	Integrating allometry improved correlations between bony fish abundance and metabarcoding read count relative to individual and biomass densities ($b = 0.77$).
Berger et al. (2024)	Atlantic salmon *Salmo salar*	River	The strongest relationship was observed between eDNA concentration and ASM, relative to fish abundance, biomass, and body surface area ($b = 0.73$).

Note. *b* values are noted as used in calculating the ASM in each study.

umbrella, indicator, and flagship species for the conservation of freshwater biodiversity (Itakura et al. 2020). However, stocks of several anguillid eel species have drastically declined in recent decades due to multiple factors, including climate change, pollution, overexploitation, and habitat loss or modification (Itakura et al. 2015; Jacoby et al. 2015; Drouineau et al. 2018; Yokouchi et al. 2022). Consequently, some species are listed on the IUCN Red List of Threatened Species (e.g., *A. anguilla* as critically endangered [CR], and *A. japonica*, *A. rostrata*, and *A. dieffenbachii* as endangered [EN]) (Pike et al. 2019, 2020a, 2020b, 2023).

Precise monitoring of anguillid eel distribution and abundance is therefore an urgent priority for their effective conservation. However, traditional capture-based survey methods (e.g., set nets, bait traps, and electrofishing) are often limited in efficiency and accuracy due to the species' cryptic hiding behavior in refuges and complex migratory life cycle across broad geographic ranges (Aoyama et al. 2005; Itakura et al. 2019). In contrast, several studies have shown that eDNA analysis offers higher monitoring efficiency and sensitivity for detecting anguillid eels' distribution, abundance, and spatial dynamics in rivers, lakes, coastal zones (Itakura et al. 2019; Burgoa Cardás et al. 2020; Kasai et al. 2021; George et al. 2023; Halvorsen et al. 2023), and even open oceans (Takeuchi et al. 2019b, 2022). Moreover, because some eel species initially recruit to freshwater tidal limit areas and subsequently disperse both upstream and downstream, their density and body size often vary substantially with distance from the river mouth (Kaifu et al. 2010; Itakura and Wakiya 2020; Wakiya et al. 2020). Therefore, if body size allometry influences eDNA production in anguillid eel species, eDNA quantification results should be interpreted cautiously to avoid over- or underestimating abundance depending on population body size structure. Integrating body size allometry scaling into eDNA analysis may thus improve the reliability of field-based abundance assessments for anguillid eels.

In this study, we reanalyzed datasets obtained from two previous studies to test allometric scaling in eDNA production of Japanese eel (*A. japonica*) under laboratory and riverine conditions in Japan. The first data set (individual-level data set; Takeuchi et al. 2019a) examined eDNA concentrations of single eels across life stages (larval, juvenile, and adult stages) in laboratory settings. The second data set (population-level data set; Itakura et al. 2019) involved basin-scale river surveys combining eDNA analysis and electrofishing, which showed a

positive relationship between eDNA concentration and both individual and biomass densities. Together, these individual- and population-level datasets offer complementary perspectives for understanding allometry in eDNA release by Japanese eels. Specifically, we tested allometry relationships between eDNA concentration and body size using the following approaches: (i) mass-specific eDNA concentration in relation to individual body size, (ii) the slope of the logarithmic relationship between eDNA concentration and body mass at the individual level, and (iii) optimal scaling coefficient *b* relating eDNA concentration to allometrically scaled mass (ASM) (see below for details).

2 | Materials and Methods

2.1 | Data Compilation

Takeuchi et al. (2019a) (hereafter referred to as the laboratory study) conducted a laboratory experiment at the IRAGO Institute (Aichi, Japan) to compare *A. japonica* eDNA concentrations across different life stages (leptocephalus, glass eel, elver, yellow eel, and silver eel). A single individual from each stage was reared in 30-L flow-through tanks containing saline groundwater (N = 3 for each stage). During the experiment, water temperature and flow rate ranged from 24.3°C to 25.1°C and 0.7 to 1.03 L/min, respectively. Total length (TL) and wet weight ranged from 32 to 770 mm and 0.1– 500 g, respectively (Table S1). Although *A. japonica* eggs and preleptocephali (early larval stage) were also used in the experiment, their eDNA was barely detectable from the tanks and thus excluded from the analysis in our study. After a 24-h acclimation period, 500 mL of seawater was collected from the outlet of each tank using disposable plastic bags and filtered through filter cartridges (0.45 μm pore size; Merck Millipore, Germany). Total eDNA on each filter was extracted using DNeasy Blood and Tissue Kit (Qiagen, Germany) in a final elution volume of 100 μL and stored at −20°C. *A. japonica* eDNA concentrations were quantified using a Light Cycler Nano (Roche, Switzerland) with species-specific primers and a TaqMan probe targeting a 154 base pair (bp) amplicon within the mitochondrial 16S ribosomal RNA gene (Watanabe et al. 2004; Takeuchi et al. 2019b). Plasmid DNA at known concentrations (6×10^{0} to 10^{5} copies per reaction) was simultaneously run for eDNA quantification.

Itakura et al. (2019) (hereafter referred to as the field study) conducted field surveys in ten small rivers (< 20 km in length; < 100 km^2 in basin area) located in the Fukui, Kagoshima, and Shizuoka prefectures of Japan between August 2015 to November 2016. Seven to 31 sites were selected along each river, from downstream to upstream reaches (a total of 125 sites). At each site, depth (cm), width (m), and flow velocity (cm per sec) were measured. Water depth, river width, and current velocity ranged from 7.7 to 72.0 cm, 1.4 to 56.0 m, and 4.6 to 119.5 cm/s, respectively (Table S2). One liter of surface water was collected using a bottle at each site, and 1 mL of benzalkonium chloride (BAC) solution was immediately added to each water sample to prevent eDNA degradation (Jo et al. 2021a). Eels were collected using battery-powered backpack electrofishing units operating at 200 V DC (LR-20B; Smith-Root Inc., US), sampling from downstream to upstream reaches. Abundance and biomass densities at each site were calculated as the number and total mass of captured eels divided by the surveyed area of each site (29.5–330.0 m^2). All captured eels were confirmed to be yellow eels based on body color and pectoral fin morphology in accordance with the silvering index (Okamura et al. 2007). Water samples were filtered within 1 week of collection using 47 mm glass microfiber filters (GF/F, nominal pore size 0.7 μm; GE Healthcare Life Science, UK). Total eDNA on the filter was extracted using the same Qiagen kit as in the laboratory study (final elution volume = 100 μL) and stored at −20°C. *A. japonica* eDNA concentrations were quantified using a StepOnePlus Real-Time PCR system (Applied Biosystems, US), with the same primers–probe set as described above. A dilution series of genomic DNA extracted from the target species (1×10^{0} to 10^{3} pg per reaction) was simultaneously run for eDNA quantification.

Of the surveyed rivers, both target eDNA and individuals were scarcely detected in four, which were therefore excluded from the analysis in our study. Sites lacking target eDNA detection or eel captures were also removed, resulting in 55 sites retained for further analyses. In the remaining six rivers, the number of individuals per site ranged from 1 to 18, biomass from 5 to 1424 g, and TLs from 145.5 to 708.0 mm (Table S2). Full experimental details are provided in the original publications.

Additionally, we reanalyzed data on estimated oxygen consumption rates of Japanese eels from Egusa (1958), who measured metabolic rates of pond-cultured eels (5–270 g) under resting conditions using a flowing-water respirometry system at approximately 25°C. Following Yates et al. (2021b), oxygen consumption was used as a proxy for metabolic rate, given the absence of alternative data (e.g., excretion rates) for this species. The water temperature range in Egusa (1958) overlapped with those in the laboratory study (24.3–25.1°C) and field study (15.1–30.1°C). Although laboratory experiments may not fully reflect natural metabolic and excretion processes (Vanni and McIntyre 2016), such data provide valuable validation for testing allometric scaling relationships in eDNA production (Yates et al. 2021b). Egusa (1958) found that oxygen consumption per individual followed a power-law relationship with body mass, with an exponent of 0.71, which we adopted as the reference scaling coefficient.

2.2 | Statistical Analyses

All the statistical analyses were performed using R version 4.2.2 (R Core Team 2023). The target eDNA concentrations per PCR reaction (copies per μL template DNA for the laboratory study and pg per 2 μL template DNA for the field study) were converted to concentrations per water sample (copies per 500 mL water for the laboratory study and pg per L water for the field study). To account for the dilutive effect of flow discharge on observed eDNA concentrations in lotic systems, all the eDNA concentrations in the field study were further converted to eDNA flux (pg per sec; the amount of eDNA transported downstream per unit time). This was calculated by multiplying eDNA concentrations per water sample by flow discharge (L per sec; approximated as the product of river depth, width, and velocity at each site) (Levi et al. 2019; Yates et al. 2021a).

The following subsections describe how the allometric relationships between Japanese eel eDNA concentration and biomass were tested multifacetedly.

2.2.1 | Mass-Specific eDNA Concentration With Relation to Individual-Level Body Size

If eDNA concentration scales linearly to body size ($b = 1$), mass-specific eDNA concentration should be independent of body size. In contrast, if eDNA concentration scales allometrically ($0 < b < 1$), mass-specific eDNA concentration should decline with increasing body size. We therefore examined the relationship between mass-specific Japanese eel eDNA concentration and individual-level body mass using the datasets to verify whether the slopes in their relationships were significantly negative. For the laboratory study, a linear model was run for the target eDNA concentration per water sample (natural log [ln] -transformed), in which individual body mass (gram; ln-transformed) was included as an explanatory variable. Individual body mass (ln-transformed) was also included as an offset term to statistically adjust the eDNA concentration (Sólymos et al. 2013; Nervo et al. 2014). Similarly, for the field study, a linear mixed-effects model (LMM) was run using the *lmerTest* package (Kuznetsova et al. 2017). The target eDNA flux (pg per sec; ln-transformed) was included as an objective variable, the biomass in each site (gram; ln-transformed) was included as a fixed effect, and river name was included as a random effect. The biomass in each site (ln-transformed) was also included as an offset term. In the latter model, both eDNA flux and biomass are proportional to the number of individuals for each site; however, the number of individuals is consequently canceled out in the equation, and thus eDNA flux and biomass per site were used.

2.2.2 | Logarithmic Relationship Between eDNA Concentration and Individual-Level Body Mass

We assumed that the individual-level target eDNA concentration is explained by the power function of the individual mass as follows:

$$C = C_0 * M^b$$

where C is eDNA concentration, C_0 is a normalization constant, M is body mass, and b is the allometric scaling coefficient (Yates et al. 2021b). This equation can be transformed by taking the logarithm of both sides as follows:

$$log(C) = log(C_0) + b * log(M)$$

where the slope in the linear relationship between ln-transformed eDNA concentration and ln-transformed mass is equal to the scaling coefficient. We therefore related the Japanese eel eDNA concentration to their biomass at the individual level using the datasets to verify whether the slopes in their relationships (and their 95% confidence intervals [CIs]) fell between 0 and 1. For the laboratory study, a linear model was fitted with eDNA concentration per water sample (ln-transformed) as the response variable and individual body mass in each tank (gram; ln-transformed) as the explanatory variable. For the field study, a linear mixed-effect model (LMM) was fitted using individual-level eDNA flux (i.e., site-level eDNA flux divided by the number of individuals captured in each site; ln-transformed) as the response variable, individual-level biomass (i.e., site-level biomass divided by the number of individuals captured in each site; ln-transformed) as the fixed effect, and river name as a random effect.

2.2.3 | Optimal Scaling Coefficient Relating eDNA Concentration to ASM

Following previous studies (Yates et al. 2021b, 2021c; Breton et al. 2022), the relationship between eDNA concentration and ASM was modeled iteratively by using various scaling coefficients ($b = 0$–1, at 0.05 intervals). Although the optimal scaling coefficient can be estimated within a single Bayesian model, this study adopted an alternative, more intuitive iterative approach to minimize the risk of model convergence failure caused by relatively small sample sizes (Yates et al. 2023, 2025). For the laboratory study, in which a single Japanese eel individual was reared per tank, ASM was calculated as the individual body mass raised to the power of the scaling coefficient b. Because $b = 0$ cannot be expressed, we substituted $b = 0.01$. Linear models were then sequentially fitted with ln-transformed eDNA concentration per water sample as the response variable and ASMs calculated at different b variables as the explanatory variable. For the field study, ASM was calculated according to the following formula:

$$\text{ASM per } 100\ m^2 = \frac{\sum_{i=1}^{N} M_i^b}{\text{area}\quad (m^2)} * 100$$

where N is the number of individuals and M_i is the body mass of each individual captured at a site. Because linear mixed-effect models (LMMs) did not converge, generalized linear mixed-effect models (GLMMs) with a Gamma distribution were fitted instead using the *lmerTest* package. In each model, eDNA flux was included as the response variable, ASM (computed at different b values) as a fixed effect, and river name as a random effect. Model performance was evaluated using corrected Akaike information criterion (AICc) for the laboratory study and AIC for the field study. AIC(c) values were plotted against b (ranging from 0.01 to 1 for the laboratory data set and 0 to 1 for the field data set). If eDNA production follows a power-law allometric relationship, AIC(c) values across models with different b should form a downward parabolic distribution, with the minimum AIC(c) corresponding to the optimal allometric scaling coefficient (Yates et al. 2021b, 2021c).

3 | Results

For the laboratory study, the linear model incorporating individual body mass as an offset term showed that body mass had a significant negative effect on Japanese eel eDNA concentration ($t = -4.32$; $p < 0.001$). This indicates that mass-specific eDNA concentration decreased with increasing individual body size (Figure 1a; Table S3), consistent with the qualitative findings

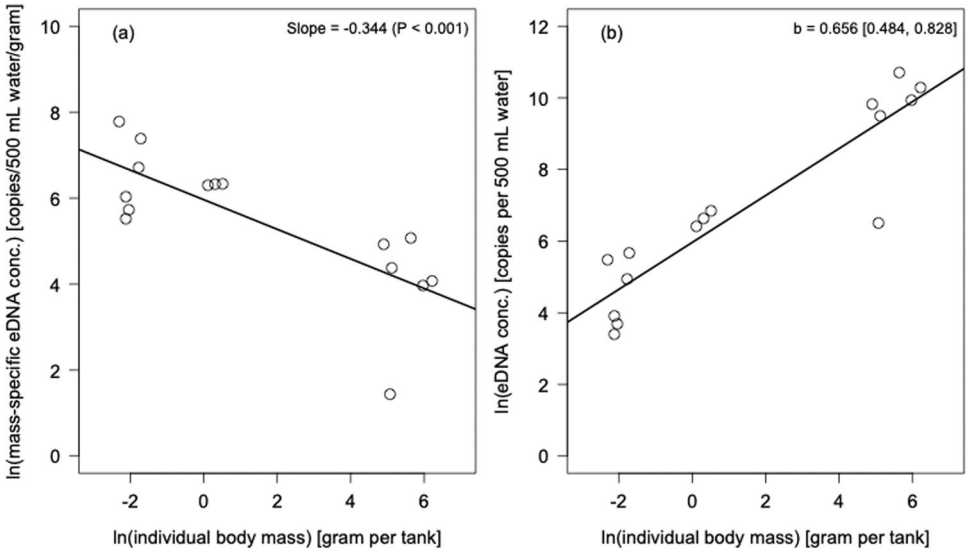

FIGURE 1 | Relationships (a) between mass-specific Japanese eel eDNA concentration (natural log [ln] -transformed; copies per gram of fish weight) and individual mass (ln-transformed; gram), and (b) between Japanese eel eDNA concentration (ln-transformed; copies per 500 mL water sample) and individual mass (ln-transformed; gram). In (a), the slope of the linear regression and its statistical significance are shown in the top right. In (b), the slope (i.e., the scaling coefficient *b*) of the linear regression and its 95% CI are shown in the top right.

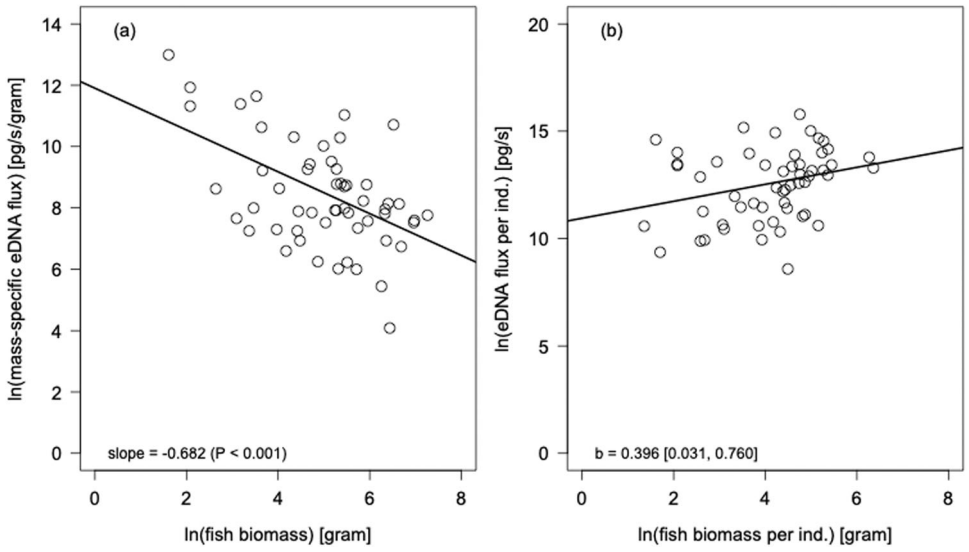

FIGURE 2 | Relationships (a) between mass-specific Japanese eel eDNA flux (ln-transformed; pg per second per gram of fish weight) and fish biomass (ln-transformed; gram), and (b) between Japanese eel eDNA flux per individual (ln-transformed; copies per second) and fish biomass per individual (ln-transformed; gram). In (a), the slope of the linear regression and its statistical significance are shown in the bottom left. In (b), the slope (i.e., the scaling coefficient *b*) of the linear regression and its 95% CI are shown in the bottom left.

reported in the original study (Takeuchi et al. 2019a). The slope of the logarithmic relationship between Japanese eel eDNA concentration and individual body mass was significantly positive (slope: 0.656, 95% CI: 0.484 to 0.828) (Figure 1b; Table S3). The 95% confidence interval (CI) included the reference scaling coefficient ($b = 0.71$) estimated from the data in Egusa (1958). Sequential linear regressions further showed that the AICc for the relationship between eDNA concentration and ASM was lowest at $b = 0.6$ (Figure S1). In particular, when b ranged from 0 to 0.2, ΔAICc (difference in AICc relative to the model with the smallest AICc) exceeded 2.

For the field study, the LMM with biomass as an offset term revealed that fish biomass had a significant negative effect on eDNA flux ($t = -4.45$; $p < 0.001$). (Figure 2a; Table S4). The slope of the logarithmic relationship between individual-level eel eDNA flux and mean biomass was also significantly positive (slope = 0.396, 95% CI: 0.031–0.760), and the 95% CI encompassed the reference scaling coefficient from Egusa (1958) (Figure 2b; Table S4). Sequential LMMs showed that AIC values for the relationship between eDNA flux and ASM were lowest at $b = 0.65$, although ΔAIC values remained below 2 across the full range of tested b values (Figure S2).

4 | Discussion

Our study demonstrated that Japanese eel eDNA concentration scaled allometrically with biomass in both laboratory and riverine environments. Allometry in eDNA production has been reported mainly for salmon and trout species (Salmonidae) (Yates et al. 2021b, 2021c; Berger et al. 2024), which have distinct morphological and ecological traits compared with anguillid eel species (Anguillidae) examined in our study. For example, salmonids have streamlined bodies covered with circular or oval scales, whereas anguillid eels possess elongated, snake-like bodies with minute scales embedded beneath the skin and are covered by a thick mucus layer (Tasumi et al. 2002). In addition, salmonids have an adipose fin located between the dorsal and caudal fins that helps them sense river currents (Aiello et al. 2016), whereas anguillid eels have a single continuous fin extending from the dorsal to the caudal region (Mehta et al. 2010). Despite these interspecific differences, our findings support the physiological link between organism body size and eDNA production across a wide range of aquatic animals. These results highlight the potential of allometric scaling to strengthen the relationship between eDNA concentration and organism abundance and, conversely, underscore the risk of over- or underestimating abundance from eDNA concentrations when body size information is ignored. Acquiring approximate data on the body size structure of a target population through a supplementary but less labor-intensive capture survey could help calibrate eDNA quantification results and improve biomass and individual density estimations from ASM-based models (Maruyama et al. 2014; Yates et al. 2021b).

Nonlinear relationships between eDNA concentration and biomass have also been reported for other anguillid species in riverine environments. Chin et al. (2021) found that mass-specific eDNA concentration of American eels (*A. rostrata*) negatively correlated with average body mass at study sites, consistent with our field-based findings, suggesting that allometrically scaled eDNA production may be common among *Anguilla* species. However, American eel eDNA concentration in that study was more strongly correlated with individual density than with biomass density, and the lowest AICc value in the model linking American eel eDNA concentration with ASM occurred at $b = 0.07$. Chin et al. (2021) did not account for variation in river discharge among sites, which may have biased the eDNA–biomass relationship and obscured the extent of allometry in eDNA production. Because high discharge can dilute eDNA concentrations and weaken the eDNA–abundance correlations in lotic environments (Curtis et al. 2021; Yates et al. 2021a), incorporating discharge measurements is essential. This issue can be addressed by quantifying eDNA flux (eDNA concentration × discharge), as done in our study and others (Yates et al. 2021a, 2021c).

As expected, the scaling coefficients estimated from the slopes of the logarithmic relationships largely overlapped between laboratory conditions, where single individuals were reared (95% CI: 0.484–0.828), and riverine environments, where the number of individuals and biomass varied among sites (95% CI: 0.031–0.760). Both results included the reference scaling coefficient estimated from oxygen consumption rates (Egusa 1958). The scaling coefficients optimal for relating eDNA concentration to ASM were also similar between laboratory ($b = 0.6$) and riverine ($b = 0.65$) conditions, and both fell within the range of the scaling coefficients derived from the logarithmic relationships. Although some aspects of eDNA capture and detection (e.g., filter types, real-time PCR platform, and materials used for quantification standards) differed between datasets, this study is the first to demonstrate that the body-size allometry in Japanese eel eDNA production is consistent across both individual and population levels, as well as between laboratory and natural environments. These integrative findings strengthen the reliability and robustness of the allometric scaling hypothesis in eDNA production and reinforce its applicability for improving eDNA–abundance relationships in natural settings.

Interestingly, the optimal scaling coefficients for relating eDNA concentration to ASM were closer to 2/3 (the theoretical surface-area scaling exponent) than to 3/4 (the theoretical metabolic scaling exponent) for both datasets. This pattern may reflect physiological and ecological traits unique to Japanese eels, and likely to anguillid eels more generally, compared with active-swimming species such as salmonids (e.g., Yates et al. 2021b; Berger et al. 2024). Eels exhibit highly developed mucus secretion and cutaneous respiration (Smith et al. 1983; Tasumi et al. 2002), suggesting that eDNA production from the body surface through direct water contact may contribute more substantially than metabolic-dependent secretion. For some taxa, allometry in eDNA production may have to be considered from both metabolic and surface-area perspectives (e.g., Berger et al. 2024; Ledger et al. 2024). A more integrated understanding of eDNA production mechanisms that accounts for physiological diversity and species-specific lifestyles is therefore required.

It should be acknowledged, however, that the allometric scaling effect appeared weaker in Japanese eels, with ASM explaining eDNA-abundance relationships similarly to biomass. Several confounding factors may underlie this outcome. In lotic systems, much of the eDNA released upstream can remain detectable in sampling sites a few kilometers downstream (Jo and Yamanaka 2022b). Although neither eel abundance nor biomass at upstream sites significantly affected the eDNA concentrations in the field study (Itakura et al. 2019), the relatively short distances between sampling sites (typically < 100 m) may have obscured fine-scale allometric patterns. In earlier studies on brook trout eDNA concentrations and their abundances, the magnitude of allometry was also smaller in streams (Yates et al. 2021c) than in the lakes (Yates et al. 2021b), supporting the idea that hydrological processes can weaken eDNA–biomass relationships. Additionally, in the field study, water samples were filtered several days after collection. Although BAC solution was added to them immediately to inhibit degradation, this delay could have impacted target eDNA yields. Moreover, eDNA sampling and electrofishing were conducted at different times in some rivers in the field study. While resident large yellow eels generally display strong site fidelity and limited seasonal distribution (Itakura et al. 2018), smaller nonresident individuals (TL < 240 mm) are more mobile (Wakiya et al. 2016; Itakura and Wakiya 2020), which may have introduced temporal mismatches and added uncertainty in the eDNA-abundance relationship. In other words, clearer allometric scaling patterns in anguillid eel eDNA concentration might have been achieved under optimized conditions for eDNA collection, preservation, and synchronization with abundance surveys.

The allometric scaling effect on Japanese eel eDNA concentration was also weaker for the field study than the laboratory study. The slope of the logarithmic relationship under natural conditions was smaller, and its 95% CI was broader than in the laboratory setting. This difference is likely attributable to the nature of the datasets: in the laboratory study, eDNA concentration and wet weight were measured for each individual, whereas in the field study, eDNA flux and biomass per individual (biomass divided by the number of individuals captured at each site) were used as proxies for individual-level eDNA flux and mass. Additionally, saline groundwater used in the laboratory is expected to have lower bacterial loads, more stable temperatures, and higher salinity than riverine water, all of which could promote eDNA persistence in tank water relative to field conditions (Collins et al. 2018; Jo and Minamoto 2021). Such factors may have affected both the quantification range and the sensitivity of eDNA detection in response to changes in organism abundance (Jo and Yamanaka 2022a; Ogonowski et al. 2023).

Our study did not account for biotic and abiotic parameters—other than river discharge—that could influence the relationship between Japanese eel eDNA concentration and abundance. Various environmental parameters (e.g., temperature, pH, salinity, turbidity, and substrate type) may have affected eDNA production and degradation, thereby shaping the strength of the eDNA–abundance relationship (Stewart 2019; Yates et al. 2021a). Temperature, in particular, is a major determinant of eDNA degradation (Collins et al. 2018; Jo et al. 2020) and likely affects eDNA production through its influence on organism physiology and metabolism (Lacoursière-Roussel et al. 2016; Jo et al. 2020). Although higher temperatures can accelerate eDNA degradation, eDNA detectability tends to increase in warmer seasons or periods that coincide with a species' active phase (De Souza et al. 2016; Jo et al. 2021b). Nakagawa et al. (2022) also found that water temperature significantly influenced models linking eDNA concentrations with fish abundance, while Ogonowski et al. (2023) reported steeper regression slopes in the eDNA–abundance relationships under warmer conditions. Furthermore, depending on species ecology, the presence of sperm-derived eDNA during reproduction can disrupt the relationship between eDNA concentration and population abundance, as well as their allometric relationships. For Anguillid eels, however, this is not an issue in rivers since reproduction occurs exclusively in the open ocean (Takeuchi et al. 2019b, 2022). Future studies should examine and incorporate these environmental drivers to ensure that eDNA signals more accurately reflect true organism abundance and to further improve the quantitative precision of eDNA-based monitoring (Yates et al. 2021a, 2025).

5 | Conclusions and Perspectives

Our study provides evidence for allometric scaling in Japanese eel eDNA concentration using individual-level (laboratory) and population-level (riverine) datasets. In both cases, mass-specific eDNA concentrations were negatively correlated with body mass, and the estimated scaling coefficients fell between 0 and 1, with their CIs supporting allometric effects. These consistent results highlight the importance of organism physiology and body-size structure for Japanese eel eDNA production, as well as the potential errors or biases from neglecting body size or developmental stage when inferring organism abundance from eDNA quantification. However, incorporating allometric scaling did not markedly strengthen the relationship between Japanese eel eDNA concentration and abundance—particularly in riverine environments. Whether this reflects eel-specific traits or confounding factors such as environmental parameters and methodological variation remains an open question for future research.

Previous studies identifying allometric scaling effects in eDNA production have primarily concentrated on field observations, leaving gaps in our understanding of how eDNA production mechanisms vary with developmental stage and individual body size (Table 1). Although laboratory experiments cannot fully reproduce natural physiological or ecological conditions (Yates et al. 2021b), testing the allometric scaling hypothesis in controlled environments can help validate its relevance under field conditions. It also remains necessary to clarify under what circumstances the allometric scaling effect meaningfully improves eDNA-based abundance estimation. For example, using ASM may offer little advantage unless populations include a sufficient number of individuals spanning a wide size range (Yates et al. 2021b; Ogonowski et al. 2023). A recent study developed mathematical frameworks to jointly estimate the number of individuals and their biomass using population size structure data and allometric scaling coefficients, demonstrating that models assuming eDNA production allometrically scaled with biomass ($0 < b < 1$) did not always outperform those assuming eDNA production linearly scaled with biomass ($b = 1$) (Yates et al. 2025). According to this finding, assuming a linear relationship between eDNA concentration and biomass might be a sufficient approximation in some systems. Altogether, future research should address these remaining challenges by linking organism physiology to eDNA production, thereby refining the eDNA–abundance relationship and enhancing the accuracy and applicability of eDNA-based abundance estimation across species and ecosystems.

Author Contributions

Toshiaki S. Jo: conceptualization, data curation, formal analysis, funding acquisition, methodology, validation, visualization, writing – original draft, and writing – review and editing. **Aya Takeuchi:** investigation, resources, and writing – review and editing. **Hikaru Itakura:** investigation, resources, and writing – review and editing.

Acknowledgements

We are deeply grateful to K. Kenzo and R. Wakiya (Chuo University, Japan) for their help in the fieldwork for Itakura et al. (2019). The proofreading of the manuscript was partly supported by Grammarly (https://app.grammarly.com). Anonymous reviewers provided helpful and constructive comments to improve our manuscript. This work was supported by the Grant-in-Aid for JSPS Research Fellows (Grant Number: JP22J00439 and JP22KJ3043 to T.S.J. and JP16J01523 to H.I.) and the River Fund of the River Foundation, Japan (to H.I.).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article. All the data is available from the Supplemental Information and the original studies.

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Supporting Information

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

Figure S1: AICc values for GLMs relating Japanese eel eDNA concentration and ASM with different scaling coefficients b ranging 0.01 to 1. A vertical dashed line indicates the reference scaling coefficient ($b = 0.71$; Egusa, 1958). **Figure S2:** AIC values for LMMs relating Japanese eel eDNA concentration and ASM with different scaling coefficients b ranging 0 to 1. A vertical dashed line indicates the reference scaling coefficient ($b = 0.71$; Egusa, 1958). **Table S1:** Dataset from Takeuchi et al. (2019b). **Table S2:** Dataset from Itakura et al. (2019). **Table S3:** Summary of linear models using the dataset from Takeuchi et al. (2019b). **Table S4:** Summary of LMMs using the dataset of Itakura et al. (2019).