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Environmental DNA analysis reveals the spatial distribution, abundance and biomass of Japanese eels at the river basin scale

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Abstract

1. There is growing international concern about declines in populations of anguillid eels, resulting in their inclusion in the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. However, monitoring the population dynamics of these species is often challenging due to their broad distributions and complex, catadromous life histories.
2. Whether environmental DNA (eDNA) analysis could be used to monitor the spatial distribution of anguillid eels in rivers was investigated by conducting basin-scale surveys of Japanese eels *Anguilla japonica* in 10 rivers in Japan and comparing the results obtained using eDNA analysis and the electrofishing method. Moreover, the relationship between the eDNA concentration and the abundance and biomass of Japanese eels was examined.
3. The eDNA of Japanese eels was detected at 56 (91.8%) of the 61 study sites from which individuals were collected by electrofishing and at an additional 35 sites where individuals were not directly collected. This indicates that eDNA analysis has greater sensitivity for detecting the presence of eels, making it a powerful tool for monitoring the spatial distribution of anguillid eels in rivers.
4. A significant, but weak, positive relationship between the eDNA concentration and the abundance and biomass of Japanese eels was also found, suggesting that eDNA analysis may be useful for estimating the abundance and biomass of anguillid eels in rivers.

5. This is the first study to demonstrate the potential usefulness of eDNA analysis for estimating the spatial distribution, abundance and biomass of Japanese eels in rivers. eDNA analysis will allow anguillid eel populations to be monitored over large spatial and temporal scales using a consistent protocol with reduced time and effort compared with conventional techniques, providing invaluable information for managing populations of these endangered species.

Keywords: abundance, biomass, *Anguilla japonica*, anguillid eel, conservation, endangered species, eDNA, Japanese eel spatial distribution

1 Introduction

Reductions in the quality and quantity of coastal, estuarine and freshwater habitats and the resulting loss of biodiversity have become a global concern (Butchart et al., 2010; Davidson, 2014; Dudgeon et al., 2006; Lotze et al., 2006). This situation is particularly critical in freshwater environments, where nearly one-third of species have been classified as endangered (Collen et al., 2014).

The genus *Anguilla* includes 19 species and sub-species of catadromous eels that spawn in the open ocean and grow in continental waters. Anguillid eel populations are distributed across more than 150 countries (IUCN, 2017) but have experienced remarkable declines in recent decades, likely as a result of both oceanic and continental factors, including habitat loss/modification, migration barriers, pollution, parasitism, overexploitation and oceanic conditions (Jacoby et al., 2015). This has led to half of all

anguillid eel species now being listed as Vulnerable (VU), Endangered (EN) or Critically Endangered (CR) in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2017), and the American eel *Anguilla rostrata*, European eel *A. anguilla* and Japanese eel *A. japonica*, which are distributed in developed, temperate regions of the Northern Hemisphere, being classified as EN or CR (Jacoby & Gollock, 2014a, b; Jacoby, Casselman, DeLucia, & Gollock, 2017).

Conservation efforts to protect biodiversity require precise data on species distributions and population sizes, which are generally obtained through biological monitoring. The dynamics of the target population should ideally be monitored quantitatively and continuously throughout its distribution range using a consistent protocol to enable the direct comparison of results obtained from different regions or studies. However, quantitative monitoring requires extensive fieldwork and great effort, as well as different sampling protocols in different environments, making it difficult to achieve consistency.

Anguillid eels inhabit a wide range of habitats within a river, from brackish estuaries to upland headwaters (Moriarty, 2003; Wakiya, Kaifu, & Mochioka, 2016), exhibit hiding behaviours in refuges (Aoyama, Shinoda, Sasai, Miller, & Tsukamoto, 2005) and have complex life histories and broad geographic ranges as a result of their migration between saline and freshwater environments, all of which represent challenges for monitoring them continuously using a standardised capture-based method throughout their range (McDowall, 1992). For instance, although backpack electrofishers are frequently used to collect eels in rivers, they often cannot be used in

areas with deep or salt water. Consequently, data on the spatial and temporal variation in anguillid eel population dynamics are often sparse, patchy or imbalanced (Jacoby et al., 2015), making it imperative to find a novel method for monitoring their distributions and abundances.

Environmental DNA (eDNA) analysis is rapidly increasing in popularity as a monitoring tool for studying and managing organisms in aquatic ecosystems (Lodge et al., 2012; Rees, Maddison, Middleditch, Patmore, & Gough, 2014) as it can be used in any water depth or habitat type (fresh or salt water). Indeed, it has been effectively used to determine the presence of aquatic species inhabiting lakes and ponds (Dougherty et al., 2016; Ficetola, Miaud, Pompanon, & Taberlet, 2008; Takahara, Minamoto, & Doi, 2013), rivers (Deiner, Fronhofer, Mächler, Walser & Altermatt, 2016; Doi et al., 2017; Fukumoto, Ushimaru, & Minamoto, 2015; Minamoto, Yamanaka, Takahara, Honjo, & Kawabata, 2012; Wilcox et al., 2016) and marine habitats (Minamoto, Fukuda, Katsuhara, & Fujiwara, 2017; Stoeckle, Soboleva, & Charlop-Powers, 2017; Thomsen et al., 2012a; Yamamoto et al., 2016, 2017). Moreover, this method may be more sensitive for detecting the presence or absence of fish than conventional capture-based sampling methods (Doi et al., 2017; Jerde et al., 2013; Sakata, Maki, Sugiyama, & Minamoto, 2017; Takahara et al., 2013; Wilcox et al., 2016) and can also be used to estimate their abundance and biomass in both freshwater and marine habitats (Doi et al., 2017; Dougherty et al., 2016; Minamoto et al., 2017; Pilliod, Goldberg, Arkle, Waits, & Richardson, 2013; Wilcox et al., 2016; Yamamoto et al., 2016); however, it has been demonstrated that the estimation of abundance and biomass is more difficult in running

waters (i.e. rivers and streams) compared with standing waters (i.e. lakes and ponds) (Rice, Larson & Taylor 2018; Stoeckle, Kuehn & Geist 2015). Having the ability to estimate the spatial distribution of anguillid eels in rivers as well as their abundance and biomass using eDNA analysis would allow investigators to undertake large-scale eDNA surveys throughout their distribution range using a consistent method.

Although eDNA analysis has proven to be highly sensitive in standing waters, it remains challenging in running waters (Rice et al., 2018; Stoeckle et al., 2015). For example, according to Thomsen et al. (2012b), the detection rate for aquatic animals in streams is less than half of that in ponds. The detection of eDNA and its concentration are influenced by the transport distance from the source organisms, which will be affected by DNA degradation and the environmental conditions, including river discharge, velocity, depth and stream morphology (Minshall et al., 2000; Wilcox et al., 2016), all of which can vary greatly among reaches of the same river and between rivers. Therefore, an assessment of the efficacy of eDNA analysis for estimating abundance and biomass of species from the downstream to upstream reaches of multiple rivers and a comparison of its performance with other survey methods is required. However, to the best of our knowledge, there has been no such multiple basin-scale survey (i.e. from the downstream to upstream reaches of rivers) of any aquatic species to date.

In this study, basin-scale surveys of Japanese eels were conducted across 10 rivers in Japan which are located in four different regions that were expected to have varying eel abundances, and the results of eDNA analysis were compared with the electrofishing method by estimating the presence or absence of eels. Then, the

relationship between the eDNA concentration and the abundance and biomass of eels was examined.

2 Methods

2.1 Study species

Japanese eels spawn in waters west of the Mariana Islands (Tsukamoto et al., 2011), from where their leaf-like leptocephalus larvae drift westwards to growth habitats in East Asia, including Taiwan, eastern China, Korea and Japan. After metamorphosing into glass eels, they migrate into brackish and freshwater habitats where they remain as growth-phase yellow eels. Although some eels appear to remain in saline habitats throughout this stage (Tsukamoto, Nakai, & Tesch, 1998), others grow in rivers, lakes and estuaries, with some individuals switching between different types of habitats (Kaifu, Tamura, Aoyama, & Tsukamoto, 2010; Yokouchi et al., 2012). Yellow eels are generally nocturnal, tending to hide in refuges such as holes and crevices, or burrowing into mud during the day (Aoyama et al., 2005; Itakura, Miyake, Kitagawa, & Kimura, 2018) and have a small home range (<l km) within a particular river (Itakura et al., 2018). After approximately 10 years' growth, the yellow eels metamorphose into reproductive-stage silver eels (Yokouchi, Sudo, Kaifu, Aoyama, & Tsukamoto, 2009), following which they migrate from the rivers and estuaries to their spawning areas (Tsukamoto, 2009).

When undertaking an eDNA survey, it is important to consider the phenology and life cycle events of the target animal. In this study, we focused on yellow eels, as they exhibit relatively sedentary behaviour compared with recruiting glass eels and downstream-migrating silver eels. Consequently, nearly all surveys were conducted during summer (August to November; Table 1) to avoid sampling the eDNA of glass eels or silver eels during their upstream or downstream migrations, which mostly occur during winter and autumn, respectively (Sudo, Okamura, Fukuda, Miller, & Tsukamoto, 2017).

2.2 Study sites

The eDNA sampling and conventional capture-based sampling of Japanese eels were conducted in 10 small rivers in the Fukui, Kagoshima and Shizuoka Prefectures of Japan (Table 1, Fig. 1), each of which has a length of <20 km and a basin area of <100 km^2. These rivers are located in four different regions: the Pacific side of Honshu, which is the central main island of Japan (the Hatauchi, Tomoe and Aono Rivers); Kyushu, which is the southern main island of Japan (the Kaizoko, Atsumari and Mawatari Rivers); the Sea of Japan side of Honshu (the Sanbongi River); and Amami-Oshima, which is a subtropical island (the Kawauchi, Sumiyo and Yakugachi Rivers). It was expected that the abundance of eels would be higher in the first two

regions and lower in the remaining regions because the recruitments are low in catchments adjacent to the Sea of Japan side of Honshu (Kaifu et al., 2014) and that Japanese eels may not be well adapted to living on small islands where a tropical anguillid eel resides. The Tomoe River flows through residential areas, while all of the other rivers flow through agricultural and forest lands. A total of 125 study sites were selected from the downstream to upstream reaches of these rivers (7–31 sites per river), all of which were in the freshwater area but some of which were influenced by the tide. The depth and velocity were measured at the centre of the downstream, middle and upstream rivers at each study site. At each study site, water sampling was conducted for the eDNA analysis and eels were collected by electrofishing.

2.3 eDNA analysis

2.3.1 Field sampling

Surface water (1 L) was collected by submerging a bottle by *c*. 10 cm from the downstream side of the centre of the river in each study site just before collecting eels by electrofishing. Benzalkonium chloride solution (1 mL) was immediately added to each water sample to prevent eDNA degradation, following Yamanaka et al. (2017). Each water sample was vacuum-filtered through a 47 mm GF/F glass filter (pore size *c*. 0.7 μm; GE Healthcare Life Science, Whatman) within an average of 3 days (maximum 1 week) from collection. The filters were then immediately wrapped in commercial aluminium foil and stored at −20 °C until eDNA extraction. The bottles that were used to collect the samples were bleached using 0.1% sodium hypochlorite and washed two

or more times with surface river water from each sampling site immediately prior to water collection, and the filtering devices (i.e. filter funnels and measuring cups used for filtration) were decontaminated using the same method as described by Fukumoto et al. (2015).

2.3.2 eDNA extraction

eDNA was extracted from the filters following the method described by Yamamoto et al. (2016). Total eDNA was extracted from each filter using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) with a minor modification to adjust for eDNA extraction. Briefly, the sample filter was placed in the suspended insert within a Salivette® tube (Sarstedt, Nümbrecht, Germany) and 420 µL of a solution consisting of 20 µL Proteinase K, 200 µL AL buffer and 200 µL water was poured onto the filter. The tube was then incubated at 56 °C for 30 min, following which the liquid held in the filter was collected by centrifugation. To increase the yield of eDNA, 200 µL TE buffer was poured onto the filter, and the liquid was again collected by centrifugation. Then, 200 µL AL buffer and 600 µL ethanol were added to the collected liquid, the mixture was transferred to a spin column and the final volume of eDNA was eluted in 100 µL AE buffer, following the manufacturer's protocol. To check for cross-contamination during the eDNA extraction procedures, eDNA was simultaneously extracted from DNA-free distilled water (extraction negative control) as one sample for every extraction procedure (i.e. there was one negative control for every 7–23 river water samples).

2.3.3 Real-time quantitative polymerase chain reaction (qPCR)

The eDNA samples were quantified by real-time TaqMan® qPCR using a StepOnePlus Real-Time PCR system (Life Technologies, Foster City, USA). The mitochondrial 16S ribosomal RNA (rRNA) gene fragments were amplified and quantified using the following primers and probe from Watanabe, Minegishi, Yoshinaga, Aoyama, & Tsukamoto (2005): forward primer, 5′-AATCAGTAATAAGAGGGCCCAAGC-3′; reverse primer, 5′-TGTTGGGTTAACGGTTTGTGGTA-3′; probe, 5′-FAM-CACATGTGTAAGTCAGAACGGACCGACC-TAMRA-3′. These primers specifically amplify a 153 bp fragment of the Japanese eel's 16S rRNA gene. Each 20 μL TaqMan reaction contained 2 μL extracted eDNA solution, a final concentration of 900 nM forward and reverse primers and 125 nM TaqMan probe in 1×PCR Master Mix (TaqMan Gene Expression Master Mix). qPCR was performed in triplicate for each eDNA sample under the following conditions: 2 min at 50 °C, 10 min at 95 °C and 55 cycles of 15 s at 95 °C and 1 min at 60 °C.

To estimate the relative eDNA concentration in each sample, a dilution series of genomic DNA extracted from Japanese eel tissue was simultaneously analysed in triplicate in each round of qPCR. The dilution series consisted of 1 ng, 100 pg, 10 pg and 1 pg of genomic DNA, which was made by repeated tenfold dilution of a single extracted DNA sample. Pure water (2 μL) was also analysed in triplicate in all rounds of qPCR as a negative control. It was found that the calibration curves from all rounds of

qPCR had R^2 values of 0.986–0.998, slopes of −3.821 to −3.424 and intercept values of 39.545–41.850. That some of the amplified samples contained 16S rRNA gene sequences were confirmed by Sanger sequencing and subsequent Basic Local Alignment Search Tool searches using the National Center for Biotechnology Information nucleotide database.

2.4 Eel collection

Eels were collected from the downstream to upstream reach of each study site using a battery-powered backpack electrofishing units operating at 200-V DC (LR-20B; Smith-Root, Inc., Vancouver, WA, USA) following the collection of water for eDNA analysis. The length of the study sites ranged from 12.0 to 40.0 m with a mean ± standard deviation (SD) of 25.0 ± 8.9 m, and the width of the study sites ranged from 1.4 to 56.0 m with a mean ± SD of 8.9 ± 8.0 m. In most study sites (94 of 125 sites, 75.2 %), where the river width was 5.7 ± 2.5 m (mean ± SD), electrofishing was conducted in the entire area within each study site, whereas in the remaining sites, where the river width was 19.2 ± 10.1 m, electrofishing was conducted in some areas within each study site (4.7 ± 1.8 m in the offshore direction from either right or left banks). Finally, the area of study sites, where electrofishing was conducted, ranged from 30 to 330 m^2 with a mean ± SD of 131 ± 62 m^2 (Table 1). The growth stage of each captured eel was confirmed based on the colour of its body and pectoral fins in accordance with the silvering index (Okamura et al., 2007), which indicated that all of

the eels collected were yellow eels. The body weight of each eel was measured to the nearest 0.1 g. In addition, the observed abundance and biomass densities of eels at each site were calculated by dividing the number or total mass of captured eels, respectively, by the area of the study site (m^2).

The observed abundance and biomass densities of eels in the Aono, Kawauchi, Sumiyo, Tomoe and Yakugachi Rivers were measured in 2015 as part of a separate investigation on the effects of habitat loss on eel distribution, whereas the water sampling for eDNA analysis was carried out in 2016 (Table 1). However, since yellow eels show strong site fidelity (Itakura et al., 2018), we did not expect their distribution in these rivers to have changed considerably over the course of a year. In all other rivers, the water samplings for eDNA analysis and eel collections were carried out in the same year.

2.5 Statistical analysis

To examine the relationship between the eDNA concentration and the abundance and biomass of Japanese eels in the study rivers, a linear mixed-effects (LME) model (*lmer* in the package *lme4* for R) was used. This model included the eDNA concentration as the dependent variable, the abundance and biomass at each sampling site and its adjacent upstream site as fixed effects and the river as a random effect. The abundance and biomass at the adjacent upstream site was included in the initial model to examine whether the drift of eDNA from upstream to downstream sites affects the eDNA concentrations at the sampling sites. Model selection was performed using the *lmerTest*

package for R, which allows for automatic model selection using the *step* function. This function eliminates non-significant random effects before eliminating non-significant fixed effects using backwards selection to yield the optimal model (Kuznetsova,Christensen, Bavay, & Brockhoff, 2014).

It has previously been reported that the abundance of Japanese eels decreases with increasing distance from the river mouth (Kaifu et al., 2010; Yokouchi, Aoyama, Oka, & Tsukamoto, 2008). Therefore, the spatial distribution of eel eDNA concentration was also investigated in those rivers in which a relatively large number of eels was captured (Aono, Atsumari, Hatauchi, Kaizoko, Mawatari and Tomoe rivers) using an LME model. In this model, the eDNA concentration was included as the dependent variable, the distance from the river mouth as a fixed effect and the river as a random effect. All statistical analyses were performed with R statistical package 3.3.2.

3 Results

3.1 Comparison of the spatial distribution of Japanese eels using eDNA analysis and electrofishing

The findings of the field survey and eDNA analysis are summarised in Table 2 and Figs 2–3. Japanese eels were collected by electrofishing from 61 of the 125 study sites, whereas the eDNA of Japanese eels was detected at 91 of the study sites. Among these, eDNA was detected at 56 (91.8%) of the 61 sites where eels were collected as well as at 35 sites where the species was not directly collected.

A relatively large number of Japanese eels were collected from a high proportion of sites in the Aono, Atsumari, Hatauchi, Kaizoko, Mawatari and Tomoe Rivers located on the central and southern main islands of Japan, ranging from 11 to 70 eels and 55% to 80% of sites (Table 2). The eDNA of Japanese eels was also detected at all sites in these six rivers except the most upstream site in the Hatauchi River where eels were also not collected (Fig. 3a, b, d, e). The eDNA concentration was higher at the downstream sites (generally sites 1 to 3) in each river than at the middle and upstream sites and generally decreased with increasing distance from the river mouth (LME: coefficient ± SE = −1.92 ± 0.86, t = −2.22, P = 0.035; Fig. 4), although this relationship was less clear in the Aono and Tomoe Rivers, where water sampling and eel collection were conducted in different years.

By contrast, only a few or no eels were captured from a small proportion of sites in the Kawauchi, Sanbongi, Sumiyo and Yakugachi Rivers located in the Sea of Japan and on the subtropical island, ranging from 0 to 5 eels and 0% to 44% of sites. Similarly, no eDNA of Japanese eel was detected in the majority of sites in these rivers (range = 0%–30%) (Fig. 3c, f).

3.2 Relationships between eDNA concentration and abundance and biomass

The optimal LME model revealed that the eDNA concentration of Japanese eels was significantly positively related to both the abundance and biomass of eels at a particular sampling site (abundance: coefficient ± SE = 187.64 ± 74.84, t = 2.51, P = 0.014, pseudo R^2 = 0.34; biomass: coefficient ± SE = 3.52 ± 1.14, t = 3.10, P = 0.002, pseudo

$R^2 = 0.32$). Neither eel abundance nor biomass at the adjacent upstream site to each sampling site significantly affected the eDNA concentration (abundance: $F = 0.02$, df = 1, $P = 0.88$; biomass: $F = 0.0038$, df = 1, $P = 0.95$), and so these variables were removed during the backwards selection process.

There was a relatively clear relationship between the eDNA concentration and the abundance and biomass of eels found in the Hatauchi, Kaizoko, Atsumari and Mawatari Rivers (Fig. 5), although some high eDNA values were detected at sites where eel densities were relatively low. However, this relationship was less clear in the Aono and Tomoe Rivers, where water sampling and eel collection were conducted in different years (Fig. 6).

4 Discussion

4.1 Effectiveness of eDNA analysis for surveying the distribution of anguillid eels

In this study, basin-scale surveys of Japanese eels were conducted from near the river mouths to the upstream reaches of 10 rivers in Japan and the results obtained from eDNA analysis and direct collection of fish by electrofishing were compared. The eDNA of Japanese eels was detected from nearly all of the study sites where the species was collected by electrofishing (56 of 61 sites, 91.8%), which were mainly located on the Pacific side of Honshu and Kyushu where the species was expected to be present at a high abundance. In contrast, eels were rarely detected through eDNA analysis or electrofishing in the Sea of Japan side or on Amami-Oshima, indicating that there may be a very low abundance of this species in these regions. However, eel eDNA was also

detected at an additional 35 study sites where the species was not directly collected. Most of these sites were located in the upper or middle reaches of the rivers, where eel densities are generally low (Tzeng, Cheng, & Lin, 1995; Yokouchi et al., 2008; Fig. 3). Therefore, eDNA analysis appears to have greater sensitivity for detecting the presence of eels than conventional survey techniques, as previously reported for other fishes (Doi et al., 2017; Jerde et al., 2013; Sakata et al., 2017; Takahara et al., 2013; Wilcox et al., 2016) and so is likely to be a powerful tool for monitoring the spatial distribution of anguillid eels in rivers.

It was also found that significant positive relationship between the eDNA concentration and the abundance and biomass of Japanese eels, suggesting that eDNA analysis may be useful for estimating the abundance and biomass of this species in rivers–although it should be noted that only a relatively small proportion of the variation in eel eDNA concentration was explained by their abundance (pseudo $R^2 = 0.34$) or biomass (pseudo $R^2 = 0.32$). Interestingly, this relationship as well as the relationship between eDNA concentration and distance from the river mouth were less clear in the Aono and Tomoe Rivers located on the Pacific side of Honshu, however, where fish sampling and water collection for eDNA analysis were carried out in different years, indicating that, contrary to expectation, the distribution of eels in rivers may change over the course of a year, and the eDNA analysis can detect such annual variation. Therefore, eDNA analysis would be effective for estimating the abundance and biomass of Japanese eels within a particular year.

Although only 1 L of surface water was collected from a single location (the centre of the river) at each site for eDNA analysis, the eDNA of Japanese eels was detected at nearly every site, even when no individuals were directly collected (Fig. 3; Table 2). Erickson et al. (2016) previously reported that eDNA concentrations do not vary across sampling transects within rivers (i.e. there is little difference between the centre and edges of rivers). In addition, the river scale of the present study may have been sufficiently small to allow mixing of the river waters (Table 1). Thus, the sampling method used here for eDNA analysis may be appropriate for detecting the presence or absence of eels. However, some high eDNA values were detected at sites where eel densities were low. This was likely due to relatively large tissue fragments of eels having incidentally entered the water samples. For example, Turner, Uy, & Everhart, (2015) found that fish eDNA is more concentrated in sediments than in the water and can persist here for a long time. Therefore, it is possible that the resuspension of sediments resulted in the observed outliers. To avoid this issue, it may be better to collect more than one water sample for eDNA analysis from different locations at each site [e.g. three replicate water samples, as recommended by Stoeckle et al. (2015)]. Another possibility is that hiding eels were overlooked during the electrofishing. Yellow eels are generally nocturnal and tend to hide in refuges during the day (Aoyama et al., 2005; Itakura et al., 2018), making them easier to detect by eDNA analysis than by electrofishing.

It was found that neither the abundance nor the biomass of Japanese eels at the upstream site adjacent to each sampling site was included in the final model, suggesting

that the drifting of eel eDNA from upstream sites made little contribution to the eDNA concentration at the sampling sites. The 25th, 50 th and 75 th percentiles of the distance of sites used in this study were 200.0, 400.0 and 1025.0 m, respectively (mean ± SD = 776.5 ± 934.4 m; range = 50.0–5100.0 m) and similarly, Wilcox et al. (2016) reported that the transport of fish eDNA occurs over distances of <1 km. The transport distance of fine particulate organic matter is influenced by a large number of environmental factors, including river discharge, velocity, depth and stream morphology (Minshall et al., 2000), and thus the transport distance of eDNA may be influenced by similar factors as well as DNA degradation rates (Wilcox et al., 2016), all of which make it difficult to monitor the distribution, abundance and biomass of target species in running waters (Rice et al., 2018; Stoeckle et al., 2015).

eDNA analysis may be superior to conventional capture-based methods when conducting large-scale surveys both in terms of the time and human resources required. In this study, the electrofishing survey took three or more people at least 3 days to conduct per river. In contrast, water samples for eDNA analysis were collected by two people within half a day maximum per river, and water filtering, eDNA extraction and qPCR were conducted by one person within 1.5 days, highlighting the simplicity of eDNA analysis and its applicability for surveying an entire river, as previously undertaken for other species (Eva et al., 2016; Fukumoto et al., 2015; Sakata et al., 2017).

4.2 Spatial distribution of Japanese eel eDNA

It has previously been shown that the abundance of freshwater eels decreases with increasing distance from the river mouth at different scales and across a range of species, including the Japanese eel (Kaifu et al., 2010; Yokouchi et al., 2008), American eel (Goodwin & Angermeier, 2003; Smogor, Angermeier, & Gaylord, 1995), European eel (Laffaille et al., 2003; Lasne & Laffaille, 2008), shortfinned eel *A. australis* (Glova, Jellyman, & Bonnett, 1998) and giant mottled eel *A. marmorata* (Itakura et al., unpublished data). Similarly, in this study, it was found that the eDNA concentration decreased with increasing distance from the river mouth, indicating that eDNA analysis can be used to reveal the general ecological characteristics of anguillid species.

4.3 Contributions of eDNA analysis to eel conservation

The findings of the present study suggest that eDNA analysis could be used to monitor not only the spatial distribution of anguillid eels but also potentially their abundance and biomass from the downstream to upstream reaches of rivers. eDNA analysis requires less time and effort than more conventional approaches, enabling eel populations to be monitored over large spatial and temporal scales using a consistent protocol. Moreover, since this is a non-lethal method, it would be suitable for monitoring populations of endangered anguillid eels in the same way as it has been applied to other endangered species, including fishes (Boothroyd, Mandrak, Fox, & Wilson, 2016; Eva et al., 2016; Laramie, Pilliod, & Goldberg, 2015; Pfleger, Rider, Johnston, & Janosik, 2016), bivalves (Currier, Morris, Wilson, & Freeland, 2018) and amphibians (Fukumoto et al., 2015). This method could also be used to monitor species of invasive eels, as it has for

other invasive species (Clusa and García-Vázquez, 2018; Dougherty et al., 2016; Hinlo, Furlan, Suitor, & Gleeson, 2017). Non-native eel species (e.g. the European eel) have been reported in Japanese waters (Aoyama et al., 2000; Arai et al., 2017) but cannot be discriminated from the native Japanese eel based on appearance alone. Therefore, eDNA analysis will make it easier to detect these eels, contributing to the conservation of not only the native eels but the entire ecosystem.

eDNA analysis could also be used to investigate the effects of environmental factors on the distribution of eels. For example, changes in the eDNA concentration could be used to detect the negative effects of cross-river structures (i.e. migration barriers), such as weirs and barrages, which are known to impact on Japanese eel abundance (Ministry of Environment, 2016) and have been identified as a major contributing factor to the reduction in anguillid eel stock (Chen, Huang, & Han, 2014; Feunteun, 2002; Laffaille, Acou, Legault, & Guilloue, 2005).

In the present study, water samples were collected from relatively shallow, freshwater areas in the rivers. However, some anguillid eels also inhabit deep parts of rivers and lakes and estuarine waters (Tsukamoto et al., 1998; Yokouchi, Aoyama, Miller, McCarthy, & Tsukamoto, 2009), none of which can be sampled by electrofishing. Therefore, results from eDNA analysis and conventional capture methods should also be compared in such areas to further understand the effectiveness of this technique for monitoring the spatial distribution, abundance and biomass of anguillid eels across all habitat types.

Author contributions

HI, SY and TM conceived the ideas and designed the methodology; HI, RW and KK collected the data; HI, SY and TM analysed the eDNA samples; HI and TS analysed the data; HI led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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TABLE 1 Characteristics of the study rivers and the sampling sites that were used for the environmental DNA (eDNA) analysis and electrofishing of Japanese eels *Anguilla japonica*.

River	Prefecture	Length (km)	Basin area (km^2)	No. of study sites	Length of study sites (m)	Width (m)	Area (m^2)	Depth (cm)	Velocity ($cm\ s^{-1}$)	Collection year	
										Eels	eDNA water samples
Hatauchi	Shizuoka	4.3	8	10	40	4.9 ± 1.5 (3.1–8.6)	200 ± 43 (163–286)	29 ± 8 (21–44)	51 ± 14 (32–69)	Sep. 2016	Sep. 2016
Tomoe	Shizuoka	17.98	94.02	20	23.5 ± 3.3 (16–30)	10.8 ± 8.8 (2.2–26)	86 ± 31 (48–169)	28 ± 14 (11–55)	22 ± 15 (5–54)	Aug. 2015	Sep. 2016
Aono	Shizuoka	17.2	72	31	18.5 ± 2.4 (12–20)	8.4 ± 9.8 (1.4–56)	99 ± 34 (30–222)	36 ± 15 (8–71)	32 ± 26 (6–119)	Sep.–Oct. 2015	Oct. 2016
Kaizoko	Kagoshima	3	-	10	40	5.1 ± 1.7 (3–8.1)	203 ± 61 (137–330)	31 ± 12 (13–45)	22 ± 11 (5–43)	Aug. 2016	Aug. 2016
Atsumari	Kagoshima	5.9	-	10	21.4 ± 4.4 (20–34)	5.5 ± 1.4 (3.3–8.1)	119 ± 35 (80–196)	41 ± 15 (22–72)	42 ± 23 (11–69)	Sep. 2016	Sep. 2016
Mawatari	Kagoshima	11.5	-	7	18.3 ± 3 (13–20)	5.6 ± 2.1 (3–8)	105 ± 43 (45–168)	53 ± 13 (35–67)	57 ± 17 (24–79)	Sep. 2016	Sep. 2016
Yakugachi	Kagoshima	15.1	45.1	9	19.6 ± 1.3 (16–20)	14.4 ± 10 (5.4–36)	155 ± 70 (83–291)	60 ± 60 (21–215)	45 ± 24 (7–79)	Aug.–Sep. 2015	Aug. 2016
Sumiyo	Kagoshima	16.8	48.5	9	20	6.6 ± 10.2 (3.7–37.7)	129 ± 52 (87–245)	38 ± 11 (21–55)	17 ± 8 (6–34)	Aug.–Sep. 2015	Nov. 2016
Kawauchi	Kagoshima	11.6	41.7	9	20	8.7 ± 5 (2.7–18)	128 ± 46 (67–217)	46 ± 18 (26–73)	30 ± 16 (5–54)	Aug.–Sep. 2015	Aug. 2016
Sanbongi	Fukui	5.8	-	10	40	5.1 ± 2 (2.5–7.7)	197 ± 68 (106–309)	53 ± 36 (28–152)	42 ± 12 (24–61)	Sep. 2016	Sep. 2016

TABLE 2 Summary of the results of the environmental DNA (eDNA) analysis and the electrofishing surveys of Japanese eels *Anguilla japonica*.

River	No. of captured eels	Total length (mm)	Eel capture sites (%)	eDNA detection sites (%)	eDNA detection sites where eels were captured (%)	eDNA detection sites where no eels were captured (%)
Hatauchi	11	361 ± 108 (201–544)	6/10 (60)	9/10 (90)	6/6 (100)	3/4 (75)
Tomoe	39	299 ± 168 (95–679)	11/20 (55)	20/20 (100)	11/11 (100)	9/9 (100)
Aono	70	339 ± 140 (91–599)	21/31 (68)	31/31 (100)	21/21 (100)	10/10 (100)
Kaizoko	48	301 ± 119 (110–609)	8/10 (80)	10/10 (100)	8/8 (100)	2/2 (100)
Atsumari	24	422 ± 158 (154–780)	6/10 (60)	10/10 (100)	6/6 (100)	4/4 (100)
Mawatari	22	233 ± 102 (19–470)	4/7 (57)	7/7 (100)	4/4 (100)	3/3 (100)
Yakugachi	5	318 ± 83 (176–379)	4/9 (44)	0/9 (0)	0/4 (0)	0/5 (0)
Sumiyo	0	-	0/9 (0)	1/9 (11)	-	1/9 (11)
Kawauchi	0	-	0/9 (0)	0/9 (0)	-	0/9 (0)
Sanbongi	1	745	1/10 (10)	3/10 (30)	0/1 (0)	3/9 (33)
Total	220	324 ± 147 (19–780)	61/125 (49)	91/125 (73)	56/61 (92)	35/64 (55)

Total length is indicated as mean ± standard deviation (range).

Figure legends

FIGURE 1 Locations of the study rivers used for the environmental DNA (eDNA) analysis and electrofishing of Japanese eels *Anguilla japonica*.

FIGURE 2 Presence and absence of Japanese eels *Anguilla japonica* in the study rivers based on electrofishing and the eDNA analysis. The charts show the number of sites where (a) eels were (or were not) collected; (b) eDNA was (or was not) detected among those sites where eels were collected and (c) eDNA was (or was not) detected among those sites where eels were not collected.

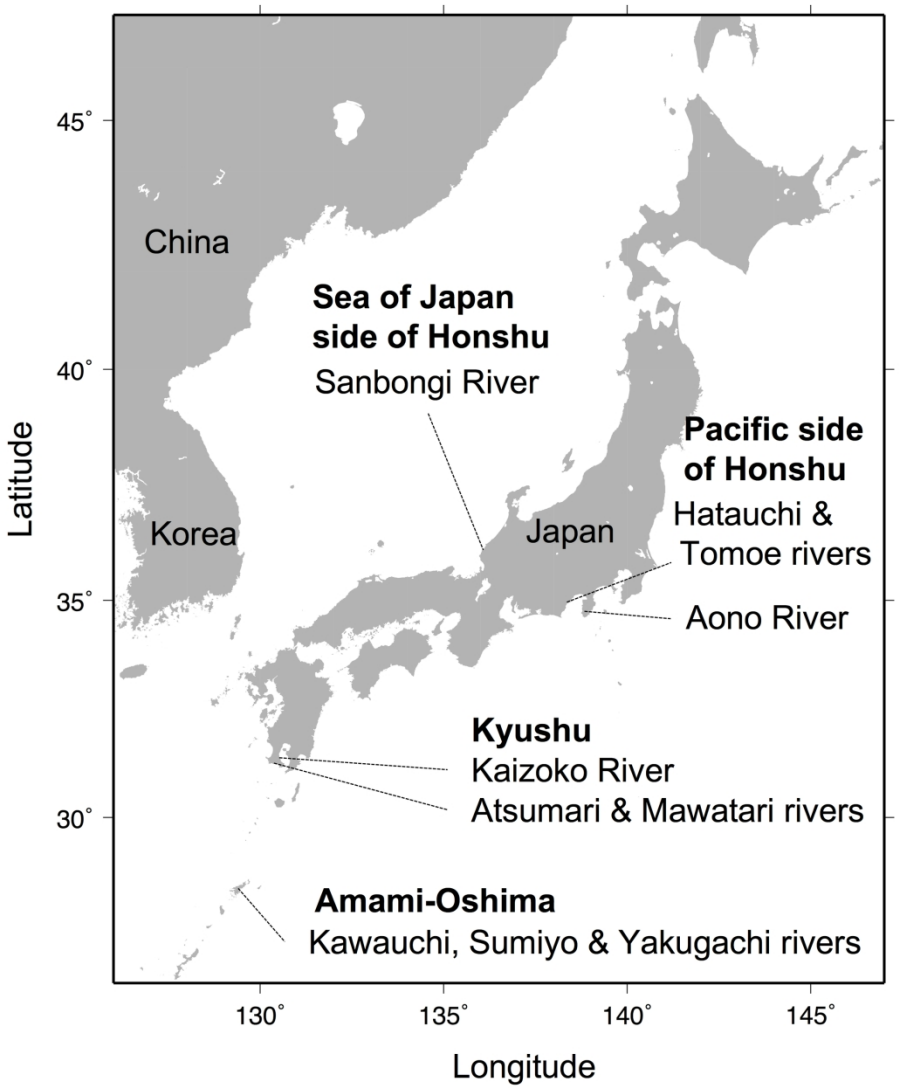
FIGURE 3 Maps showing the locations of the study sites in each river and the environmental DNA (eDNA) concentrations for Japanese eels *Anguilla japonica*. The eDNA concentrations are shown as different coloured circles, the numbers above which indicate the study site within each river. (a) Atsumari and Mawatari rivers; (b) Kaizoko River; (c) Sanbongi River; (d) Hatauchi and Tomoe rivers; (e) Aono River and (f) Kawauchi, Sumiyo and Yakugachi rivers. These rivers are divided into two figures and shown according to the size of the drainage areas. The dashed lines indicate the presence of one or more cross-river structure (e.g. weirs or dams), while the solid lines indicate waterfalls.

FIGURE 4 Relationships between the environmental DNA (eDNA) concentrations for Japanese eels *Anguilla japonica* in the surface waters of the Aono, Hatauchi, Tomoe,

Atsumari, Mawatari and Kaizoko rivers and distance from the river mouth. The grey lines and shaded areas indicate the regression lines and 95% confidential intervals, respectively. The x- and y-axis scales differ among rivers.

FIGURE 5 Relationships between the environmental DNA (eDNA) concentrations for Japanese eels *Anguilla japonica* in the surface waters of the Hatauchi, Atsumari, Mawatari and Kaizoko rivers and their abundance and biomass. Different colours represent different study sites within each river. The grey lines and shaded areas indicate the regression lines and 95% confidential intervals, respectively. The x- and y-axis scales differ among rivers.

FIGURE 6 Relationships between the environmental DNA (eDNA) concentrations for Japanese eels *Anguilla japonica* in the surface waters of the Aono and Tomoe rivers and their abundance and biomass. Different colours and shapes represent different study sites within each river. The grey lines and shaded areas indicate the regression lines and 95% confidential intervals, respectively. The x- and y-axis scales differ among rivers.



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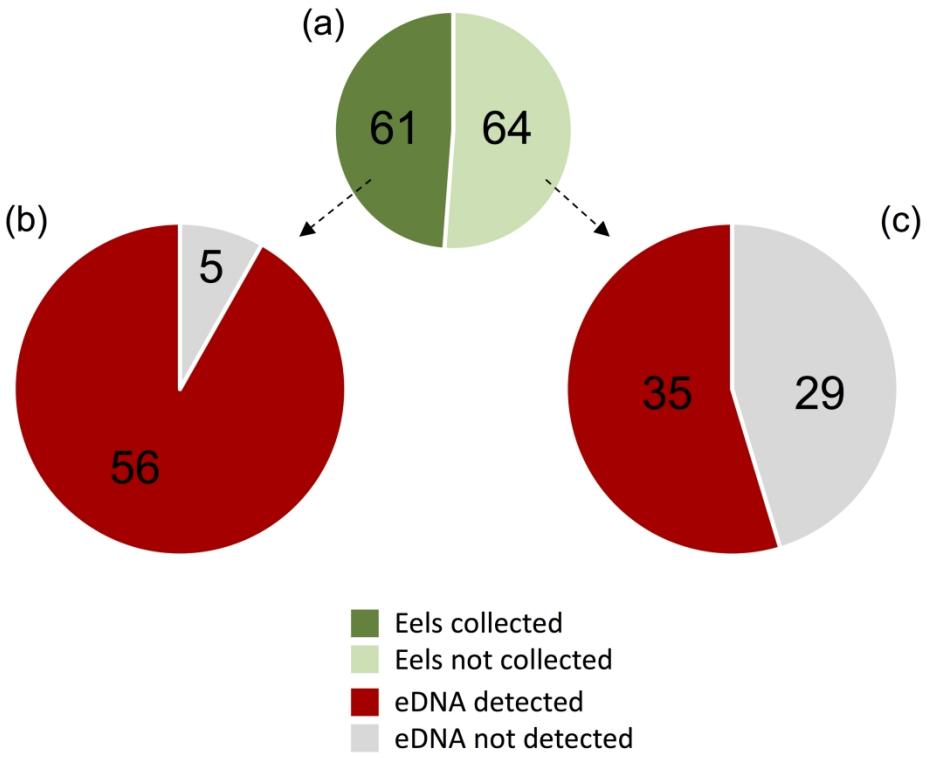

137x116mm (600 x 600 DPI)

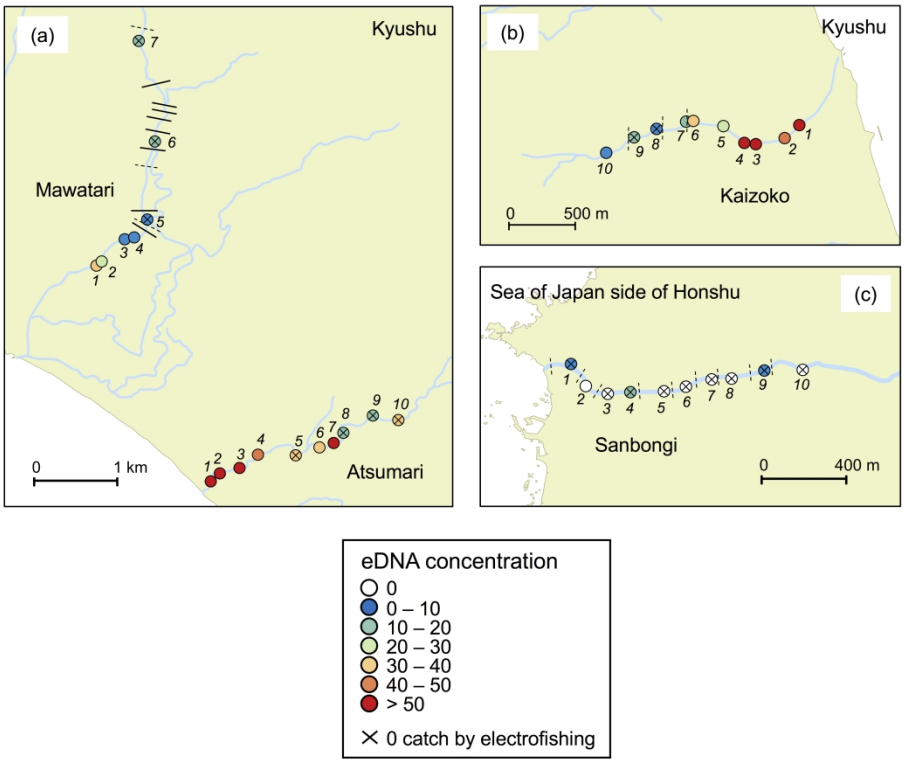

Figure 3

190x157mm (600 x 600 DPI)

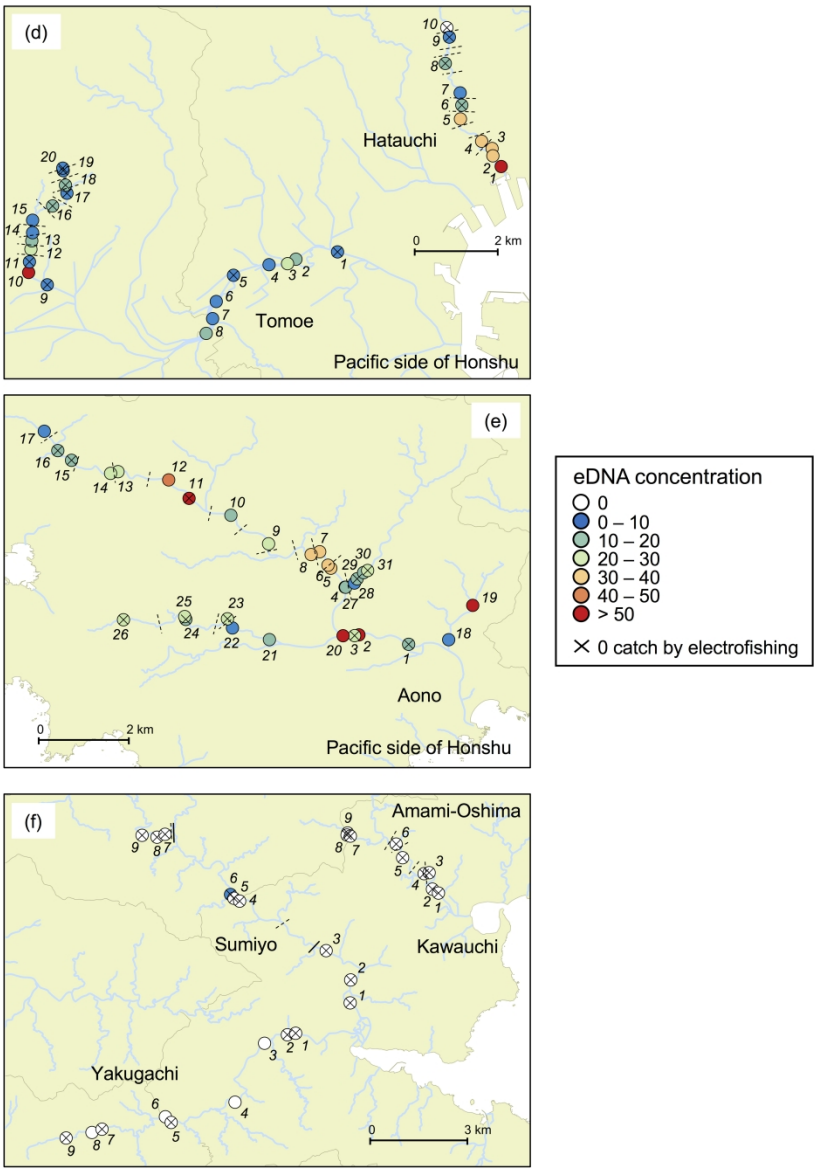

Figure 3 continued

166x237mm (600 x 600 DPI)

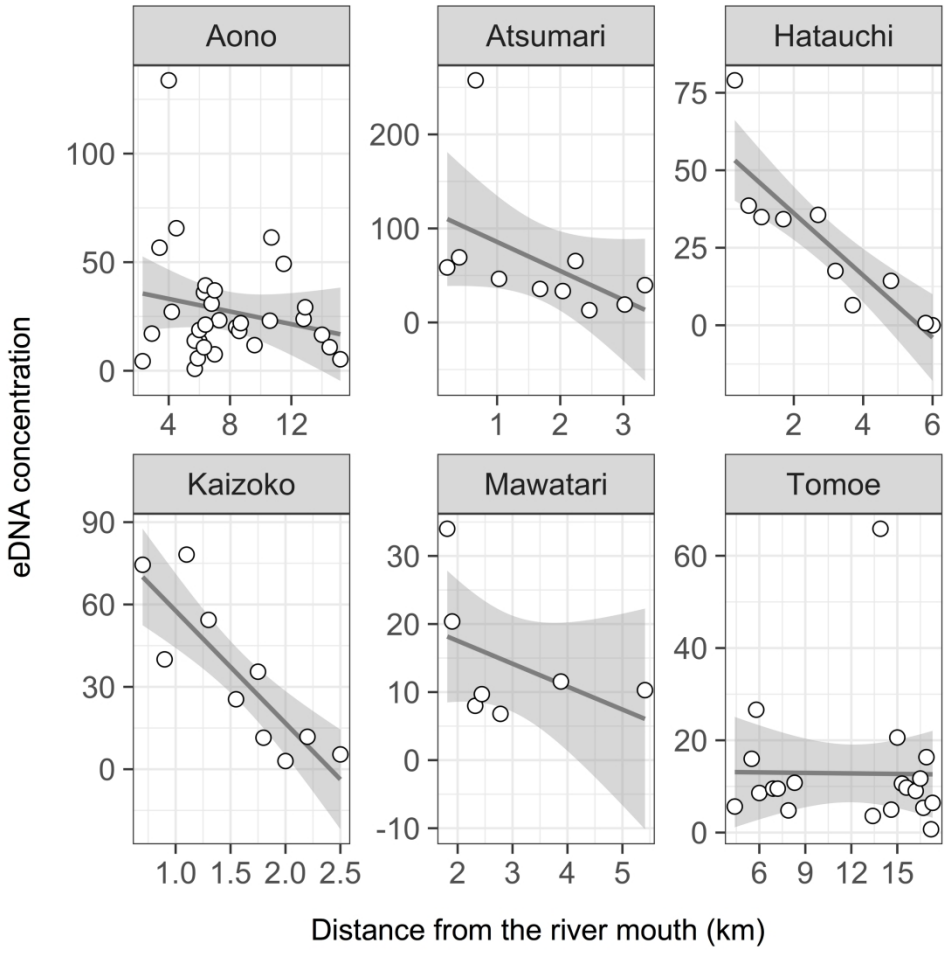

Figure 4

143x143mm (600 x 600 DPI)

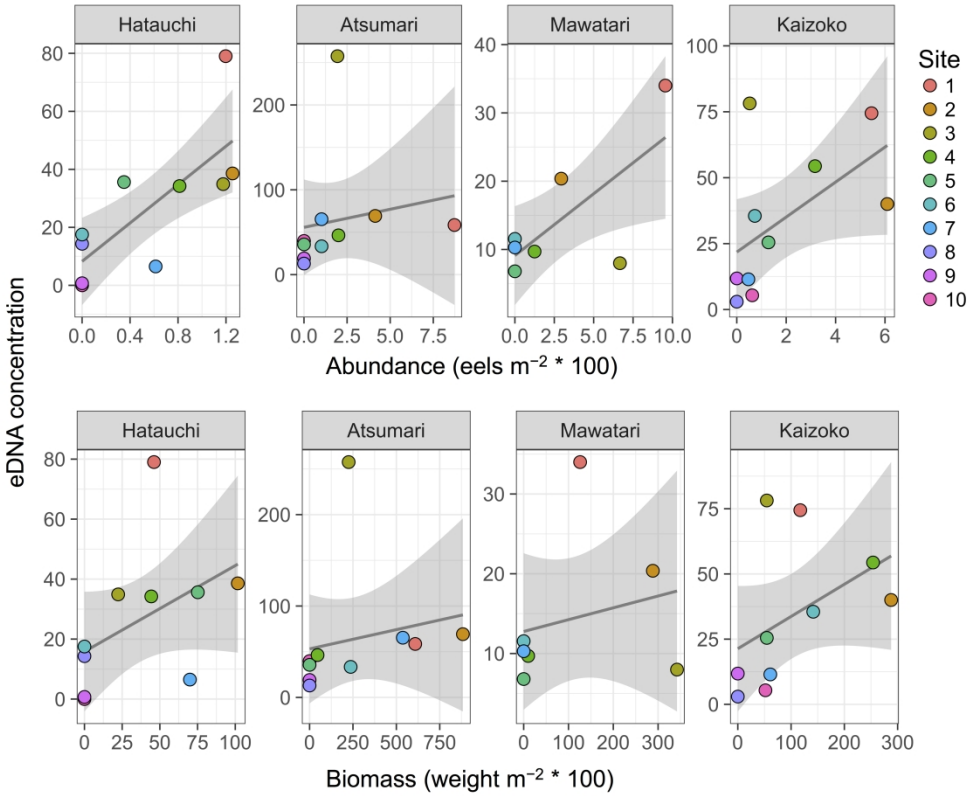

Figure 5

178x151mm (600 x 600 DPI)

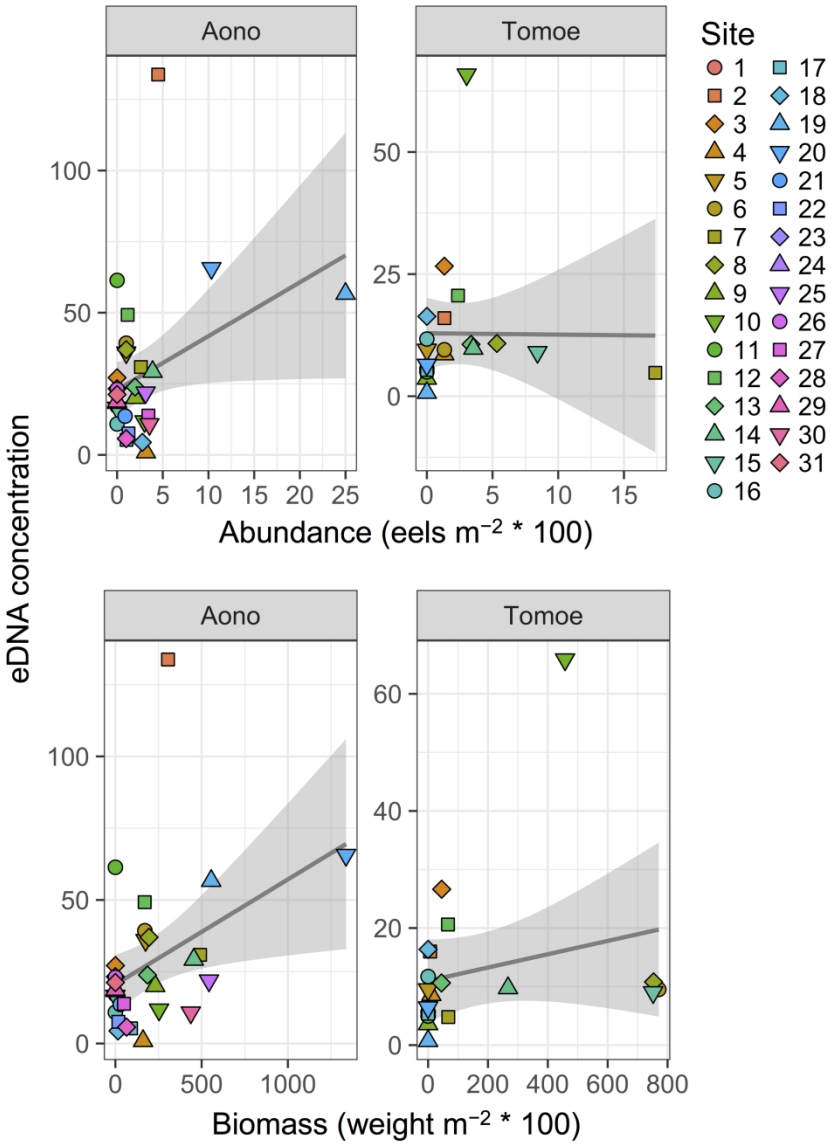

Figure 6

124x166mm (600 x 600 DPI)