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# The effect of temperature on environmental DNA degradation of Japanese eel

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## Abstract

The environmental DNA (eDNA) technique is a convenient and powerful tool to detect rare species. Knowledge of the degradation rate of eDNA in water is important for understanding how degradation influences the presence and/or estimate biomass of aquatic animals. We developed a new set of species-specific primers and probe to detect eDNA of Japanese eel *Anguilla japonica*, which is a commercially important and endangered species, and then conducted a laboratory experiment to quantify the temperature-dependent degradation of emitted eDNA. Eels were held in tanks at five different temperature levels from 10 to 30 °C and water from each tank was sampled and kept in bottles at each temperature over 6 days. The concentration of eDNA was measured every day and the results showed that temperature ($T$) had a significant and positive effect on the degradation rate ($k$) as $k = 0.02T + 0.18$. Improved understanding of the effect of temperature on degradation rates would help data interpretations and adjustments would increase the reliability of eDNA analysis in future studies.

**Keywords** Environmental DNA · Degradation rate · Temperature · Primer · Japanese eel

## Introduction

The Japanese eel *Anguilla japonica* is a catadromous fish species that spawns in waters west of the Mariana Islands (Tsukamoto 2006). Their eggs and larvae are transported to coastal areas in East Asian countries by the North Equatorial Current and the subsequent Kuroshio Current (Kimura et al. 1994). Once they recruit to coastal areas, juveniles migrate to rivers and lakes, although some juveniles may remain in saline habitats (Tsukamoto et al. 1998). The Japanese eel is a commercially important species, but the catch of eels has declined drastically since the 1970s (Tsukamoto et al. 2009). As the biomass of Japanese eel has reached historically critical levels, it was classified as an endangered species (EN) in Japan by the Ministry of the Environment in 2013 (Announcement of the 4th version of the Japanese Red Lists (Fresh and brackish water fishes) https://www.env.go.jp/press/16264.html/ Accessed 25 Oct 2019), and an EN based on the International Union for Conservation of Nature Red List criteria in 2014 (IUCN Red List of Threatened Species, p 1. https://www.iucnredlist.org/ja/species/166184/1117791/ Accessed 25 Oct 2019). Precise estimates of abundance and distribution are essential for managing and protecting endangered species. However, the precise distribution of Japanese

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eel has not yet been identified because it migrates across an extremely wide area from ocean to upland headwaters (Wakiya et al. 2016).

Conventionally, backpack electrofishers are often used for monitoring eels in freshwater areas, but it requires advanced skills to collect eels from turbid waters. Eels tend to hide in refuges such as holes and crevices or burrow in the mud during the day (Tesch 2003; Aoyama et al. 2005), which further contributes to the difficulty in capturing them. Furthermore, electrofishers are ineffective in deep areas and are disabled in saline water. Consequently, accurate data on the spatial and temporal distribution of Japanese eel is lacking. Alternative methods may prove fruitful for monitoring their precise distribution and abundance.

Recent developments and investigations using environmental DNA (eDNA) have demonstrated that eDNA is an effective monitoring tool for organisms in aquatic environments (Dejean et al. 2012; Lodge et al. 2012; Pilliod et al. 2013; Rees et al. 2014), as it can be used in any water depth or any salinity range. eDNA is DNA released from organisms into their environments such as water, air or soil. eDNA is effective for identifying the presence of species that are difficult to find or catch and it is highly useful for detecting endangered species, as the presence and abundance of a target species can be estimated by detecting eDNA from water samples without capturing or damaging individuals. Itakura et al. (2019) conducted surveys of Japanese eels in several small rivers using both electrofishing and eDNA analysis. In a comparison between the methods, they showed that eDNA analysis was more sensitive for detecting the presence of eels than electrofishing. This suggests that eDNA may be more powerful than conventional methods for monitoring the spatial distribution of eels. The eDNA analysis was not only used for riverine environments, but also for detecting eels in the ocean. Takeuchi et al. (2019) successfully detected eDNA in the southern West Mariana Ridge that is one of the most plausible spawning areas of Japanese eel. Although eDNA analyses have been shown to be highly effective for the detection of target species, there are still some uncertainties regarding eDNA ecology that could affect interpretation of eDNA quantification results. For example, it has been reported that eDNA degrades rapidly and does not persist over time after being released from organisms into the water (Thomsen et al. 2012; Maruyama et al. 2014). In addition, degradation rates may differ among species and water conditions (Eichmiller et al. 2016). Understanding the factors affecting degradation is required for achieving better quantitative estimates and improving sampling strategies for eDNA monitoring.

Differences in eDNA degradation rates can be caused by variation in local environmental conditions, such as water temperature, salinity, and light intensity. Exposure to high levels of ultraviolet radiation can photochemically damage DNA (Ravanat et al. 2001; Häder et al. 2003). Naturally occurring levels of solar radiation can have variable effects on exonuclease activity, and thus eDNA degradation, although the extent of the effects would depend on the bacterial communities. However, in laboratory experiments, Strickler et al. (2015) showed that temperature exhibited a stronger influence on the degradation of eDNA than UV-B radiation exposure and bacterial species. Tsuji et al. (2017) conducted experiments in which the concentration of eDNA of common carp *Cyprinus carpio*, temperature, and bacterial abundance were measured in time series. The results showed that eDNA degradation was accelerated at higher water temperatures, but bacterial abundance did not have a significant effect on eDNA degradation, although bacterial abundance and water temperature would be expected to be correlated. Taken together, these studies indicate that temperature may be most important for the degradation of eDNA, among various environmental factors.

To apply the eDNA analysis in natural waters, *A. japonica* should be strictly distinguished from other species of the same genus that may exist in the target region. Besides *A. japonica*, the giant mottled eel *Anguilla marmorata* is also distributed in the natural waters of Japan (Masuda et al. 1992). In addition, some foreign species, mainly the European eel *Anguilla anguilla* have been imported and cultured in Japan (Kishikawa 1997). The presence of *A. anguilla* in the natural waters of Japan was discovered by DNA analyses: they had escaped from cultured ponds (Zhang et al. 1999). Although a primer set for Japanese eel was used in the previous eDNA studies (Itakura et al. 2019; Takeuchi et al. 2019), its species-specificity had not been checked among the *Anguilla* species potentially inhabiting in the inland and coastal areas of Japan. Therefore, we originally designed a primer set for the Japanese eel, A. japonica, to specifically distinguish this species from other species in the same genus.

It is expected that the effect of temperature on the degradation rate of eDNA would vary with species. However, the degradation rates of eDNA for Japanese eel have not been estimated yet. Thus, using the species-specific primer, we conducted laboratory experiments to determine the temperature-dependent degradation rate of eDNA for Japanese eel.

## Materials and methods

### Species-specific assay development

To design the species-specific primer set for the Japanese eel, *A. japonica*, the sequences of the D-loop region of the mitochondrial DNA of this species as well as 14 other species and subspecies in the same genus were collected from GenBank (https://www.ncbi.nlm.nih.gov/genbank/

Accessed 25 Oct 2019) and RefSeq (https://www.ncbi.nlm.nih.gov/refseq/ Accessed 25 Oct 2019). Accession numbers of sequences collected for each species are listed in Online Resource, Table S1. All sequences were aligned and species-specific sites for *A. japonica* were searched by eye (for details of the specificity, see next Section). Finally, the forward primer, Aja-Dlp-F2: 5'-TACATTTAATGG AAAACAAGCATAAGCC-3' and the reverse primer, Aja-Dlp-R3: 5'-CGTTAACATTACTCTGTCAACTTACCTG-3', were designed as a pair. The expected amplified length was 138 bp. A TaqMan™ probe was designed against the amplified region between the forward and reverse primers. Probe candidates were generated using Primer Express 3.0 (Thermo Fisher Scientific, Waltham, MA, USA) and the one with high species-specificity to the target species was selected: Aja-Dlp-Pr-FAMMGB: 5'-FAM-ACCCAT AAACTGATAAATAG-MGB-3'. To adjust the annealing temperature of the probe to work under a general thermal condition for real-time PCR, the probe adopted MGB quencher on its 3' end.

## Specificity check of the assay

Consensus sequences of each species listed in Online Resource Table S1 were aligned and are shown in Online Resource Figure S1. For the forward primer, the number of nucleotide mismatches between *A. japonica* and the other non-target species were at least 9 out of 28 bp and at least 1 out of the 5 nucleotides on the 3' end. For the reverse primer, the number of nucleotide mismatches between *A. japonica* and the other non-target species were at least 12 out of 28 bp and at least 3 out of the 5 nucleotides on the 3' end. For the probe, the number of nucleotide mismatches between *A. japonica* and the other non-target species were at least 10 out of 20 bp.

To confirm the coverage of intra-specific sequence variation in the target species by the primer and probe set, additional sequence data was downloaded from Genbank (857 sequences in total including the sequences of *A. japonica* listed in Online Resource, Table S2; accession numbers are not shown) and aligned (Online Resource, Figure S2). The average of consensus percentage (%) in each nucleotide position among the 5 nucleotides on the 3' end of the forward primer was 98.5. As for the reverse primers, it was 98.3. In the probe sequence, the average was 99.3 across the entire probe sequence. Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/ Accessed 25 Oct 2019) was used to evaluate the specificity of the primer set with default settings.

As a second specificity check for the primer and probe set, a real-time PCR test was conducted with a total reaction volume of 15 μl which included 900 nM of each primer, 125 nM of probe, 7.5 μl of TaqMan™ Gene Expression Master Mix 2.0 (Thermo Fisher Scientific), and 1 μl of DNA template in a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific). Genomic DNA at a concentration of 0.1 ng/μl of the target and each of 4 non-target related species (*A. anguilla anguilla, A. bicolor bicolor, A. marmorata, and A. rostrata*), which potentially inhabit Japanese inland waters as native or introduced species, were used as the DNA templates. Thermal cycle settings for PCR were: 2 min at 50 °C, 10 min at 95 °C and 55 cycles of 15 s at 95 °C and 60 s at 60 °C. PCR was performed with 3 replications for each species. Non-template negative controls (NTC) with 3 replications were included in the PCR test in which ultrapure water was added in place of DNA template.

## Determination of limit of detection and quantification of the assay

Real-time PCR was performed to determine the limit of detection (LOD) and the limit of quantification (LOQ) of the assay with StepOne™ Real-Time PCR System (Thermo Fisher Scientific). Commercially synthesized cloned DNA including the target sequence of *A. japonica* was used as a DNA template (hereafter standard DNA; see Online Resource Table S2 for the product details). Total reaction volume of PCR was 15 μl which included 900 nM of each primer, 125 nM of probe, 7.5 μl of TaqMan™ Environmental Expression Master Mix 2.0 (Life Technologies), 0.075 μl of AmpErase Uracil N-Glycosylase (Thermo Fisher Scientific) and 2 μl template of standard DNA with known copies of the target sequence. Each concentration of the standard DNA at 30,000, 3000, 300, 30, and 20 copies per reaction were tested in triplicate, and the other concentrations, 10, 3, and 1 copy per reaction were tested with 6 replications. A triplicate of NTC was also included which contained 2 μl of ultrapure water in place of the standard DNA.

## Experimental design

We conducted an experiment to test whether the degradation rate of eel eDNA varies depending on water temperature. The experimental set-up consisted of five 20-l glass tanks and three Japanese eels were kept in each 20-l glass tank under a constant temperature for 3–7 days prior to beginning the experiment. Using a chiller/heater and aeration, the temperature in each tank was kept at one of five temperature levels: 10 °C, 15 °C, 20 °C, 25 °C and 30 °C. The temperature levels were selected to represent the range of temperatures found in rivers and streams that Japanese eels inhabit. Japanese eels were unfed during the experiments. Two liters of rearing water from each tank were transferred to four polyethylene bottles. The bottled water was kept in a dark environment for 6 days under the

same tank temperature for each treatment level. We added a negative control bottle filled with 500 ml of distilled water to monitor potential contamination among treatments for each temperature treatment. The negative control bottles had never been exposed to Japanese eels but were otherwise treated the same as the experimental bottles. All the equipment for the tank experiment and water sampling, including the tanks, chiller/heater, air stones and sampling bottles were decontaminated prior to use by soaking in a 10% bleach solution for 10 min and were subsequently rinsed with a large amount of tap water and distilled water.

## Sample collection and DNA collection

Water samples (60 ml) were collected from each bottle, including a negative control bottle, at the beginning of the degradation experiment (day 0), and every day for 6 days (days 1–6). All water samples were filtered through a 47-mm-diameter glass microfiber filter GF/F (nominal pore size 0.67 m; GE Healthcare Life Sciences, Piscataway, NJ, USA). Each filter was folded inward and kept in aluminum foil, then placed in a plastic bag with a zipper to avoid contamination and stored at − 30 °C until DNA extractions. Filtering devices (i.e., funnels, beakers, tweezers and cylinders used for water sampling) were bleached after every use, using 0.1% sodium hypochlorite solution for at least 5 min, washed with running tap water, and then rinsed with distilled water.

DNA was extracted from filters as follows, referring to Uchii et al. (2016) with minor modifications. Each half folded frozen filter was rolled into a cylindrical shape and placed in the upper part of the spin column with collection tubes (Salivette, SARSTEDT AG&Co., Nümbrecht, Germany). The cotton in the Salivette tubes was removed before use. The Salivette tubes were centrifuged for 2 min at $3000 \times g$ to remove water contained in the filter. A mixture containing 200 µl of Milli-Q water, 100 µl of Buffer AL, and 10 µl of proteinase K was added onto the filter in each tube and then incubated for 30 min at 56 °C. After incubation, the Salivette tubes were centrifuged for 2 min at $3000 \times g$ to collect DNA in the lower part of the spin column. Tris–EDTA buffer (200 µl, pH 8.0) was added to each filter and incubated for 1 min at room temperature before being centrifuged for 2 min at $3000 \times g$. The upper part of the Salivette was then discarded. The eluted mixture was added to new column provided with DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) after the addition of 100 µl of Buffer AL and 300 µl of 99% ethanol for each tube, and then the tubes were centrifuged for 1 min at $6000 \times g$ following the manufacturer's instructions. Due to the large volume of each mixture, this step was repeated 3 times to catch all the DNA on the membrane. The silica-gel membrane was washed twice with the washing buffers Buffer AW1 and Buffer AW2, and DNA was eluted from the column with 100 µl of Buffer AE. Buffer AL, AW1, AW2 and proteinase K were the regents provided with the DNeasy Blood and Tissue Kit. The eluted DNA samples were stored at − 20 °C until the real-time qPCR step.

## PCR set-up

Real-time quantitative PCR targeting on Japanese eel was conducted in 15-µl reaction volumes which included 900 nM of forward and reverse primers (Aja-Dlp-F2 and Aja-Dlp-R3, respectively), 125 nM of a probe (Aja-Dlp-Pr-FAMMGB), 7.5 µl of TaqMan™ Environmental Expression Master Mix 2.0 (Thermo Fisher Scientific), 0.075 µl of AmpErase Uracil N-Glycosylase (Thermo Fisher Scientific) and 2 µl of template eDNA sample. A dilution series of a standard DNA was included in each PCR test as a quantification standard with the copy numbers diluted to 30,000, 3000, 300, and 30 copies per reaction. All samples and standard DNA were run in triplicate. All PCR runs included triplicates of NTCs which included 2 µl of ultrapure water instead of template DNA. Thermal cycle settings for PCR were 2 min at 50 °C, 10 min at 95 °C and 55 cycles of 15 s at 95 °C and 60 s at 60 °C.

## Decay model and statistical analysis

The degradation rate was estimated by the overall change in eDNA concentration detected in each bottle over the duration of the experiment. As the degradation of eDNA is expected to be proportional to eDNA concentration, the temporal change in the eDNA concentration is expressed by

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -kC$$

where $C$ is the DNA concentration at time $t$ and $k$ is the decay rate. This equation can be solved as an exponential decay model.

$$C = C_0 \mathrm{e}^{-kt} \tag{1}$$

where $C_0$ is the initial concentration of eDNA. Because the starting concentration of eDNA varied among bottles, $C$ is transformed to $\hat{C}$, which is the eDNA concentration relative to the initial value.

It is considered that the degradation of eDNA is caused by bacteria in the water (Minegishi et al. 2019). Higher temperature leads to the higher activity of bacteria. It is therefore supposed that the degradation rate is higher in higher water temperatures (Tsuji et al. 2017). According to the previous studies, a linear model was used to test the effect of temperature on the degradation rate.

R version 3.5.1 (R Core team 2019) was used to perform the statistical analyses.

## Results

### Assay development

Primer BLAST in silico test on the newly developed assay estimated potential amplification of 886 *A. japonica* sequences without any other candidates from different species, suggesting a strict specificity to the target species as well as good coverage for the intra-specific genetic variation of the target. Moreover, in vitro real-time PCR specificity testing using DNA samples of the target and the other four non-target species in the genus *Anguilla* confirmed species-specific amplification of *A. japonica* without nonspecific amplification in the non-target species. No unintended amplification was observed in NTCs. In the LOD and LOQ check, all standard DNA with 30,000, 3000, 300, 30, 20, and 10 copies per reaction were amplified across all technical replications. In the standard DNA with 3 copies per reaction, successful amplifications were observed in 5 out of 6 replications. In the standard DNA with 1 copy per reaction, 3 out of 6 replications were amplified. No unintended amplification was observed in NTCs. Based on the result, LOD and LOQ of the assay were defined as 1 copy per reaction and 3 copies per reaction, respectively.

### Degradation experiment

The $R^2$ values of the standard curve for the real-time PCR experiments on the tank samples ranged from 0.980 to 0.999 and PCR efficiencies ranged from 80.306 to 97.137%. All negative control samples from the negative control bottles tested negative. All the NTCs in PCR tests were also negative.

The concentration of eel eDNA in all bottles declined rapidly, confirming the exponential decay model (Eq. 1) is applicable (Fig. 1; Table 1, $R^2$ values ranged from 0.81 to 0.99). Half of the eDNA decayed within the first 2 days. Decay was highest in 30 °C treatment, as eel eDNA was below the detection level on the 6th day. *k* values at each temperature are listed in Table 1. The degradation rate was positively correlated with temperature (*T*), as $k = 0.02T + 0.18$ ($p < 0.01$, Fig. 2).



**Fig. 1** Temporal change in eDNA concentration after being released from Japanese eel. *Different symbols* in **a**–**e** indicate different bottles, and *different symbols* in **f** indicate different temperatures. *Lines* in **f** represent the best fit exponential regression curves for each temperature experiment
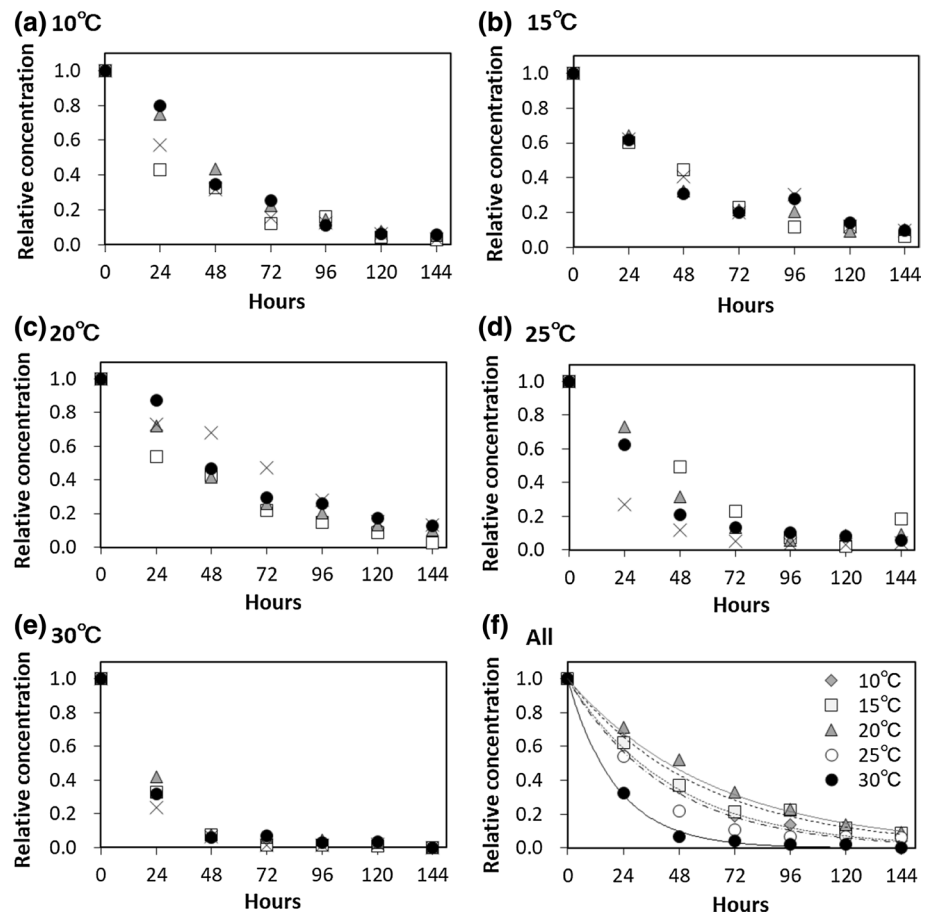
**Table 1** Degradation rates (mean ± standard error), coefficients of determination ($R^2$) and half decay time ($T_{50}$) for Japanese eel eDNA at five temperatures

| Temperature (°C) | $k$ (h$^{-1}$) | $R^2$ | $T_{50}$ (h) |
|---|---|---|---|
| 10 | 0.022 ± 0.002 | 0.99 | 31.1 |
| 15 | 0.018 ± 0.001 | 0.96 | 39.1 |
| 20 | 0.017 ± 0.004 | 0.99 | 40.2 |
| 25 | 0.023 ± 0.004 | 0.85 | 29.8 |
| 30 | 0.040 ± 0.005 | 0.81 | 17.3 |
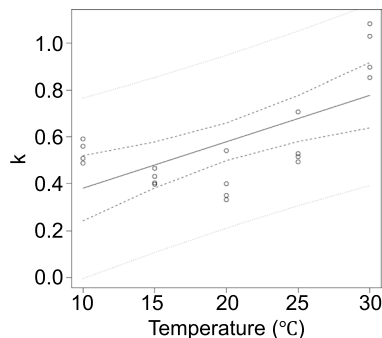


**Fig. 2** Relation between degradation rates ($k$) and temperature ($T$). *Dashed* and *dotted lines* show 95% confidence and prediction intervals, respectively

## Discussion

The newly developed assay for *A. japonica* in this study has exact species-specificity and wide coverage of the intraspecific nucleotide variation in the target amplification region of the target sequence. As for the sensitivity and quantification ability of the assay, LOD and LOQ were low enough to be acceptable for eDNA field surveys for the relatively rare species with low population density.

Our degradation experiment showed that the eDNA of Japanese eels in sampled water degraded rapidly with time. The results indicate that water samples should be filtered as soon as possible to avoid degradation, preferably just after sampling in the field. Alternatively, the degradation of eDNA in the sample may be suppressed by adding stabilizing agents such as benzalkonium chloride (Yamanaka et al. 2017).

Our estimated degradation rates (0.017–0.040) were smaller than those reported for freshwater fishes. The rates were reported as 0.051–0.159 for bluegill sunfish *Lepomis macrochirus* (Maruyama et al. 2014) and 0.105 for common carp *Cyprinus carpio* (Barnes et al. 2014). These values indicate that the half-decay times for bluegill sunfish and common carp are 4.4–13.6 h and 6.6 h, which

are considerably smaller than our results for Japanese eel (17.3–40.2 h, Table 1). The identification of eDNA is difficult because eDNA exists as a heterogeneous mixture of genetic materials freely floating or adsorbed onto other particles within the aquatic environment (Barnes et al. 2014). However, a portion of eDNA is released from the body surface. In eels, the body surface is protected by a viscous mucus layer. Thus, eel DNA that is released from the body surface may be protected by viscous mucus and may therefore be less susceptible to degradation compared to other fishes.

The degradation rate was significantly affected by water temperature in this study (Fig. 2). Environmental DNA degraded faster at higher water temperatures as $k = 0.02T + 0.18$. This temperature-dependent tendency is consistent with previous studies. Eichmiller et al. (2016) reported the degradation constant $k$ for common carp eDNA as 0.015, 0.078, 0.10, and 0.10/h at 5 °C, 15 °C, 25 °C and 35 °C, respectively. Tsuji et al. (2017) reported the degradation rates for Ayu sweetfish *Plecoglossus altivelis altivelis* as 0.035, 0.142 and 0.248/h at 10 °C, 20 °C and 30 °C, respectively, and for common carp *C. carpio* as 0.034, 0.141 and 0.249/h at 10 °C, 20 °C and 30 °C, respectively. Higher temperatures promote degradation of eDNA by denaturing DNA molecules and by increasing enzyme kinetics and microbial metabolism (Barnes et al. 2014).

The temperature-dependency of eDNA degradation implies that eDNA concentrations in the field should vary depending on seasons; therefore, interpretation of eel biomass estimates when using eDNA may be more accurate when temperature is taken into account. Less degradation at low temperatures indicates that the eDNA can reflect a wider area and a longer time in winter than in summer. As the water flows from upstream to downstream in rivers where eels live, detected eDNA would reflect a greater upstream area in the winter season.

Salinity would be another water parameter that affects eDNA degradation. This is particularly important for eel eDNA, as eels encounter a wide range of salinity in their habitats, ranging from coastal waters to upland headwaters. The present study showed that the eDNA degradation rate is strongly influenced by water temperature. Further clarification of the relationship between eDNA degradation and other parameters will increase the reliability of biomass estimations based on eDNA analysis.

# References

Aoyama J, Shinoda A, Sasai S, Miller MJ, Tsukamoto K (2005) First observations of the burrows of *Anguilla japonica*. J Fish Biol 67:1534–1543

Barnes M, Turner CR, Jerde CL, Renshaw MA, Chadderton WL, Lodge DM (2014) Environmental conditions influence eDNA persistence in aquatic systems. Environ Sci Technol 48:1819–1827

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C (2012) Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. J Appl Ecol 49:953–959

Eichmiller J, Best SE, Sorensen PW (2016) Effects of temperature and trophic state on degradation of environmental DNA in lake water. Environ Sci Technol 50:1859–1867

Häder DP, Kumar HD, Smith RC, Worrest RC (2003) Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochem Photobiol Sci 2:39–50

Itakura H, Wakiya R, Yamamoto S, Kaifu K, Sato T, Minamoto T (2019) Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. Aquat Conserv Mar Freshw Ecosyst. https://doi.org/10.1002/aqc.3058

Kimura S, Tsukamoto K, Sugimoto T (1994) A model for the larval migration of the Japanese eel—roles of the trade winds and salinity front. Mar Biol 119:185–190

Kishikawa T (1997) The poor catch of glass eels and survival strategy of the eel farm. Yoshoku 34:52–68 **(in Japanese)**

Lodge DM, Turner CR, Jerde CL, Barnes MA, Chadderton L, Egan SP, Feder J, Mahon AR, Pfrender ME (2012) Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. Mol Ecol 21:2555–2558

Maruyama A, Nakamura K, Yamanaka H, Kondoh M, Minamoto T (2014) The release rate of environmental DNA from juvenile and adult fish. PLoS ONE 9:e114639

Minegishi Y, Wong MK, Kanbe T, Araki H, Kashiwabara T, Ijichi M, Kogure K, Hyodo S (2019) Spatiotemporal distribution of juvenile chum salmon in Otsuchi Bay, Iwate, Japan, inferred from environmental DNA. PLoS ONE 14:e222052

Masuda H, Amaoka K, Araga C, Uyeno T, Yoshino T (1992) The fishes of the Japanese Archipelago, 3rd edn. Tokai University Press, Tokyo

Pilliod DS, Goldberg CS, Arkle RS, Waits LP (2013) Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can J Fish Aquat Sci 70:1123–1130

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Ravanat JL, Douki T, Cadet J (2001) Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B Biol 63:88–102

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC (2014) The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. J Appl Ecol 51:1450–1459

Strickler KM, Fremier AK, Goldberg CS (2015) Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol Conserv 183:85–92

Takeuchi A, Watanabe S, Yamamoto S, Miller MJ, Fukuba T, Miwa T, Okino T, Minamoto T, Tsukamoto K (2019) First use of oceanic environmental DNA to study the spawning ecology of the Japanese eel *Anguilla japonica*. Mar Ecol Prog Ser 609:187–196

Tesch FW (2003) The eel biology and management of anguillid eels. Blackwell Science, London

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol 21:2565–2573

Tsuji S, Ushio M, Sho S, Minamoto T, Yamanaka H (2017) Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. PLoS ONE 12:e176608

Tsukamoto K (2006) Oceanic biology: spawning of eels near a seamount. Nature 439:929

Tsukamoto K, Aoyama J, Miller MJ (2009) The present status of the Japanese eel: resources and recent research. In: Casselman J, Cairns D (eds) Eels at the edge. Bethesda, Maryland, pp 21–35

Tsukamoto K, Nakai I, Tesch WV (1998) Do all freshwater eels migrate? Nature 396:635–636

Uchii K, Doi H, Minamoto T (2016) A novel environmental DNA approach to quantify the cryptic invasion of non-native genotypes. Mol Ecol Res 16:415–422

Wakiya R, Kaifu K, Mochioka N (2016) Growth conditions after recruitment determine residence—emigration tactics of female Japanese eels *Anguilla japonica*. Fish Sci 82:729–736

Yamanaka H, Minamoto T, Matsuura J, Sakurai S, Tsuji S, Motozawa H, Hongo M, Sogo Y, Naoki K, Iori T, Sugita M, Baba M, Kondo A (2017) A simple method for preserving environmental DNA in water samples at ambient temperature by addition of cationic surfactant. Limnology 18:233–241

Zhang H, Mikawa N, Yamada Y, Horie N, Okamura A, Utoh T, Tanaka S, Motonobu T (1999) Foreign eel species in the natural waters of Japan detected by polymerase chain reaction of mitochondrial cytochrome b region. Fish Sci 65:684–686

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