Understanding seasonal migration of Shishamo smelt in coastal regions using environmental DNA

Tetsu Yatsuyanagi¹, Hitoshi Araki²*

¹ Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan, ² Research Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan

* arakih@res.agr.hokudai.ac.jp

Abstract

Migratory organisms have their own life histories that efficiently link multiple ecosystems. Therefore, comprehensive understanding of migration ecologies of these organisms is essential for both species conservation and ecosystem management. However, monitoring migration at fine spatiotemporal scales, especially in open marine systems, often requires huge costs and effort. Recently, environmental DNA (eDNA) techniques that utilize DNA released from living organisms into their environment became available for monitoring wild animals without direct handling. In this study, we conducted an eDNA survey for understanding marine migration of an endemic fish species, Shishamo smelt (Spirinchus lanceolatus). We examined 1) seasonal habitat changes in coastal regions and 2) environmental factors potentially driving the migration of this species. The eDNA concentrations along a 100 km-long coastline exhibited spatiotemporal variation, suggesting that this species shifts their habitat away from nearshore areas between spring and summer. We also found a significantly negative association between the eDNA concentration and sea surface temperature. That finding suggests that the offshore migration of this species is associated with increased sea surface temperature. This study reveals new aspects of S. lanceolatus life history in coastal regions. Together with our previous eDNA study on the freshwater migration of S. lanceolatus, this study illustrates the potential of eDNA techniques for understanding the whole life history of this migratory species.

Introduction

Migratory organisms move across heterogeneous ecosystems. Their life-history strategies not only determine the fate of individuals but also influence structures and dynamics of populations, communities, and ecosystems [1]. For example, anadromous fishes migrate between saltwater and freshwater to complete their life cycles. These large-scale migrations sustain ecosystem structures and functions through food webs and by transporting marine-derived nutrients to riparian zones [2–4]. Because such migrations can be the key to maintaining community dynamics and ecosystem functions, proper monitoring of fish migration is essential for both species conservation and ecosystem management.
Shishamo smelt (Spirinchus lanceolatus), in the family Osmeridae, is an anadromous forage fish endemic to the Pacific regions of Hokkaido, Japan [5]. This forage fish is expected to contribute to energy supplies for seabirds, marine mammals, terrestrial animals, and predatory fish species. *S. lanceolatus* also has a significant economic value for commercial fisheries and is known as a local specialty product, despite the species listing as threatened on the Japanese Red List due to its limited distribution and resource degradation [6]. In early winter, *S. lanceolatus* migrates into rivers for reproduction at one or two years of age. Previous studies investigated their reproductive migration by examining the spatial distribution and environmental conditions of the spawning grounds [7] and temporal migration dynamics [8]. However, two important questions about marine migration of this species are unsolved to date: (i) how do *S. lanceolatus* move through seawater habitats? (ii) What environmental factors (if any) trigger their habitat changes? While commercial fisheries target this species in coastal regions around October, distribution trends through the other seasons are almost entirely unknown.

Investigating migration at fine spatial and temporal scales is often very challenging due to huge effort and cost requirements, especially in open marine systems (e.g., catch-based survey on a research vessel). While bio-mechanical methods such as biologging, biotelemetry, or GPS techniques may enabled us to track species’ movements in detail [9, 10], the recovery rates of tracking instruments are often very low, and these surveys require certain fish sizes and durability to fit those instruments. Moreover, in the case of migration ecology studies, it is often necessary to visualize movement at the population level; therefore, individual movement data often have limited use [11].

Environmental DNA (eDNA) analysis, to trace organismal DNA found in the environment, can be an alternative way to study fish migration [12]. By detecting and quantifying eDNA, researchers can estimate the presence, absence, and abundance of target species’ populations in aquatic systems [13–17]. Detections of eDNA may also provide useful information about seasonal movements or habitat changes of target species in various environments, such as rivers [18–20], lakes [21, 22], and estuaries [23]. Even in marine environments, eDNA surveys have displayed their abilities to estimate spatial abundance and biomass [24–26], and the community composition of various species [27–32]. These labor-saving sampling processes enable us to carry out large-scale surveys in fine spatiotemporal scales and can be applied to quantitative analysis of species migration.

In this study, we conducted an eDNA survey from March to August along the Pacific coast of Hokkaido to gain an understanding of the seasonal habitat changes of *S. lanceolatus* at sea. Firstly, we investigated spatial and temporal eDNA concentrations at fixed sampling points nearshore using the species-specific eDNA detection system established in our previous study [8]. Next, we evaluated the relationships between eDNA concentrations and environmental factors. Since the shallow coastal environment has unique properties such as strong seasonal variation in temperature [33] and high productivity by phytoplankton [34], we tested a hypothesis that one or a few of these environmental factors are associated with the seasonal habitat changes of *S. lanceolatus* in coastal regions.

**Materials & methods**

**Ethical statement**

We declare no ethical statement. There was no need to obtain any permission for conducting this study including field samplings and lab experiments because water sample collection in our study sites is not prohibited, and no animals were captured or killed in our experiments.
eDNA sample collection

Seven locations along the western Pacific coast of Hokkaido were selected as eDNA sampling sites (Nishikioka, Yufutsu, Mukawa, Saru, Atsuga, Niikappu, and Harutachi from the west to east; S1 Table, Fig 1). Within the study region, several spawning rivers for *S. lanceolatus* were identified according to previous records [7, 8]. Sampling for eDNA was conducted from March to August in 2019, 14 times in total.

Seawater samples were collected at coastal sites with plastic dippers cleaned by soaking in a 10% sodium hypochlorite solution for 20 min before every use. Samples were moved into sterile plastic bags and 500 ml were immediately filtered using Sterivex™-HV filter cartridges with a pore size of 0.45 μm (Merck Millipore, Inc., Darmstadt, Germany) and a sterile 50 ml syringe (TERUMO, Inc., Tokyo, Japan) following protocols outlined in the Environmental DNA Sampling and Experiment Manual version 2.1 by The eDNA Society (available from: http://ednasociety.org/eDNA_manual_Eng_v2_1_3b.pdf). Two filter cartridge samples were collected as field replicates per site, and negative control samples were prepared by filtration of 500 ml of ultrapure water at the end of every field sampling. All the filter samples were stored at -80˚C until eDNA could be extracted.

Environmental data collection

Four environmental factors; seawater temperature (˚C), salinity (parts per thousand; ppt), daily mean Chlorophyll-a (Chl-a) concentration (mg/m³), and tidal height (cm) were...
collected. The seawater temperature and salinity were measured with a YSI Pro30 water quality sensor (Xylem, Inc., New York, USA) at each sampling site right after seawater sampling. The datasets of Chl-a concentration, an indicator of phytoplankton productivity, were collected from the P-Tree System database (https://www.eorc.jaxa.jp/ptree/index_j.html) supplied by the satellite Himawari-8, Japan Aerospace Exploration Agency (JAXA) [35, 36]. The daily mean values measured at the closest point to each eDNA sampling site were used. The tidal height datasets were collected from the database of oceanographic observatories maintained by the Japan Meteorological Agency (https://www.jma.go.jp/jma/indexe.html). The location of the station used is illustrated in Fig 1.

**DNA extraction and quantification**

DNA extraction from the Sterivex™-HV filter cartridge was performed largely following Yatsuyanagi et al. [8] with a slight modification of the vacuuming step. Firstly, RNAlater put into the filter cartridge was vacuumed from the outlet port using a QIAvac 24 Plus manifold (QIagen, Inc., Hilden, Germany) and circulating aspirator (ADVANTEC, Inc., Tokyo, Japan). To remove the RNAlater, 1,000 μl ultrapure water was injected into the cartridges and vacuumed again (repeated twice). The eluted DNA solution was purified using a DNeasy Blood and Tissue Kit (Qiagen Inc.). The final volume of the extracted DNA was 100 μl.

DNA quantification was performed using a species-specific qPCR assay established in Yatsuyanagi et al. [8]. Details for PCR components, thermal cycling conditions, and standard curve preparation can also be found in Yatsuyanagi et al. [8]. In brief, quantitative PCR was run with a quantification standard in triplicate 20-μl reactions which included 800 nM of each primer, 400 nM probe, 0.1 μl Bovine Serum Albumin solution (20 mg/ml, New England Biolabs, Inc., Massachusetts, USA), and 2 μl of the DNA template in a Brilliant III Ultra-Fast qPCR Master Mix with Low ROX (Agilent Technology, Inc., California, USA). To test for cross-contamination, ultrapure water was applied as a PCR negative control, instead of the DNA template. In total, six replicates were analyzed per site, and the number of DNA copies per 2 μl template was calculated by averaging over them. The standard curve efficiency ranged from 0.910 to 1.000 and R² ranged from 0.996 to 1.000 (intercept: 38.28–42.18). No DNA was detected in any of the negative controls. Based on the number of detected DNA copies, eDNA concentration per 1 ml of collected water was estimated following this formula:

\[
eDNA \text{ concentration} = \frac{\text{DNA number (copies)} \times \frac{\text{DNA extract volume (ml)}}{\text{DNA template volume (ml)}} \times 1}{\text{filtered volume (ml)}}
\]

which means that DNA copy number was divided by ten.

**Statistical analysis**

To confirm the seasonal variability in temporal eDNA concentrations of *S. lanceolatus*, we compared the eDNA concentrations between spring (March-May, n = 49) and summer (June-August, n = 49) using Wilcoxon signed-rank test with R package "exactRankTests".

We evaluated the relationships between eDNA concentrations and environmental factors using generalized linear mixed models (GLMMs) with negative binomial distribution. The eDNA concentration was used as a response variable, and the seawater temperature, salinity, Chl-a concentration, and tidal height datasets were used as explanatory variables. Sampling sites were treated as random effects. The GLMMs were conducted using the Automatic Differentiation Model Builder [37] with R package “glmmADMB”. Prior to GLMM analyses, we
calculated variation inflation factors (VIFs) with R package “fmsb” to confirm the colinearity between the environmental factors. The VIFs of less than 3.000 indicate that the colinearity among the factors does not significantly influence the GLMMs [38]. All the statistical analyses were performed using R ver. 3-4-2 [39]. Heatmaps for data visualization were generated with HemI software [40].

Results

Spatial and temporal variation in eDNA concentrations

The eDNA shed by *S. lanceolatus* was detected in 73 out of the 98 seawater samples (Fig 2A). The minimum eDNA concentration (0.005 copies/ml) was obtained from Atsuga (ATS) on April 14th, and the maximum eDNA concentration (24.91 copies/ml) was from Saru (SAR) on May 4th. Of the 73 detections, 33 were below 1 copy per 2 μl of PCR template, which was categorized as below-the-detection level in S1 Fig (but not in Fig 2A). Given zero detections of *S. lanceolatus* eDNA from both field and PCR negative controls, those 33 samples were included in the following analyses as positive samples.

In general, high eDNA concentrations were detected in the early sampling periods. In fact, the eDNA concentrations in Yufutsu (YUF), Mukawa (MUK), Saru (SAR), and Atsuga (ATS) showed significant differences between spring (March-May) and summer (June-August) (Wilcoxon signed-rank test; *P* < 0.05; Fig 3). Although results from the other sites (NIS, NII, and HAR) showed no significant differences in eDNA concentrations between the two seasons, an overall comparison pooling the data among sites suggested a significant difference between the seasons (*P* < 0.001, Fig 3).

According to catch reports filed by the Hokkaido Government [41], numbers of *S. lanceolatus* caught by fisheries between October and November in 2018 were larger in the central areas (around YUF: 23 tons, MUK: 45 tons, ATS: 48 tons) in comparison to the margins (around NIS: 5 tons, NII: less than 1 ton, HAR: 1 ton). The median eDNA concentrations detected in spring represented a significantly positive correlation with the regional fishery catches (*R*² = 0.742, *P* < 0.05; Fig 4).

Spatial and temporal variations in environmental factors

Variations in examined environmental factors are shown in Fig 2B (seawater temperature) and S2 Fig (others), and the VIFs among them are shown in S2 Table. The seawater temperature ranged from 2.1˚C (in Mukawa on March 27th) to 23.2˚C (in Yufutsu on August 6th). The salinity ranged from 19.8 ppt (in Yufutsu on July 7th) to 29.7 ppt (in Harutachi on April 4th). The Chl-a concentrations ranged from 35 g/m³ (in Saru on April 14th) to 1,275 g/m³ (in Nishikioka on June 26th). The tidal height ranged from 3 cm (in Atsuga at 12 noon on June 6th) to 129 cm (in Harutachi at 5 p.m. on June 18th). All the VIF values were less than 2, indicating that colinearity among the environmental factors did not significantly influence the GLMMs.

Evaluating relationships between eDNA concentration and environmental factors

GLMM and linear regression analyses suggested a significantly negative correlation between eDNA concentration and seawater temperature, indicating that eDNA concentrations declined gradually with increase of the seawater temperature (*R*² = 0.273, *P* < 0.001; Table 1; Fig 5), whereas the salinity, Chl-a concentration, and tidal height were not significantly associated with the eDNA concentration.
In this study, a time-series eDNA survey for *S. lanceolatus* demonstrates the ability to trace the species’ coastal distribution and its spatiotemporal changes between spring and summer (Figs 2 and 3). Among eDNA detections, the highest eDNA concentration was detected in spring (March-May) around the central study sites, which was consistent with regional biomass inferred by fishery catch data from late fall (Fig 4). In summer (June-August), the eDNA concentrations decreased near shore, which, if the correlation between eDNA concentrations and...
fisheries catch data holds true, indicates *S. lanceolatus* habitat changes at that time. Currently, fish migration studies require a great amount of labor to monitor populations in a wide geographical range. Here, intensive eDNA sampling along a 100 km-long coastline enabled us to monitor the unseen migration trends of *S. lanceolatus*.

Indirect surveys using the eDNA approach do not provide detailed information (e.g., distance of sampling point to target organism) especially in open marine systems. However, recent eDNA studies have enhanced understandings about eDNA dispersion in coastal marine systems. Yamamoto et al. [25] compared eDNA concentrations with echo intensities in Japanese jack mackerel (*Trachurus japonicus*) and suggested that eDNA reflected relative abundance of those fish sources within 150 m in Maizuru Bay, the Sea of Japan. Using caged fishes in the Maizuru Bay, Murakami et al. [42] indicated that eDNA was detectable mostly within 30 m from the source. In the present study, *S. lanceolatus* likely stayed close to the shore in spring, at least around the central study sites according to the high eDNA concentrations detected.

The eDNA concentration of *S. lanceolatus* was significantly negatively correlated with the seawater temperature (GLMM, *P* < 0.001; Table 1; Fig 5). Previous studies reported that eDNA decays rapidly in warmer water [43–47]. For instance, Tsuji et al. [47] showed that eDNA in water at 25°C decayed 2.3 times faster than that at 15°C (−0.088/h at 15°C, −0.200/h
at 25˚C). Jo et al. [45] indicated that at higher water temperatures and with larger fish biomass, both eDNA shedding and eDNA decay rates increased. Among the study sites, however, the seawater temperature should not have been high enough to significantly decompose eDNA in June (11.0–16.8˚C), suggesting that the decrease of eDNA concentrations was an ecological consequence of *S. lanceolatus* migration.

![Fig 4. Relationship between eDNA concentration and regional fishery catch.](https://doi.org/10.1371/journal.pone.0239912.g004)

The blue regression line represents a significantly positive correlation between median eDNA concentrations in seawater and fishery catch numbers in 2018 for six regions ($R^2 = 0.742, P < 0.05$). Shaded area shows 95% confidence intervals for the regression model. The fishery catch data was provided by Hokkaido government under the CC BY 4.0 license [41].

**Table 1. Summary of statistical analyses on GLMMs showing the variables and outputs.**

| Response variable | Explanatory variable | Estimate | SD        | z-value | P-value |
|-------------------|----------------------|----------|-----------|---------|---------|
| eDNA concentrations | Temperature         | -2.75 x 10^{-1} | 3.82 x 10^{-2} | -7.23       | <2e-16 |
|                   | Salinity             | -1.68 x 10^{-1} | 9.85 x 10^{-2} | -1.71       | 0.088   |
|                   | Chl-a conc.          | 1.28 x 10^{-3}  | 8.90 x 10^{-4} | 1.44       | 0.151   |
|                   | Tidal height         | 5.51 x 10^{-3}  | 7.62 x 10^{-3} | 0.72       | 0.470   |

The eDNA concentration was used as a response variable and the four environmental factors were used as the explanatory variables. Point estimates, standard deviations (SDs), z-values, and P-values were provided for each model.
Water temperature in shallow coastal environments tends to be higher than that in deeper offshore spaces. In fact, temperatures at 50 m depth in the western Pacific Ocean off Hokkaido in June ranged from about 5.0˚C to 7.0˚C according to the Japan Meteorological Agency (http://www.jma.go.jp/jma/indexe.html). Together with the eDNA concentrations we estimated in the current study, we believe that S. lanceolatus migrates from nearshore/surface areas to relatively deeper layers in summer due to the increase in sea surface temperature. For a more precise understanding of habitat use in summer, however, further investigations with multi-depth water sampling will be needed.

The state of eDNA changes depending on various factors besides temperature, such as pH [48], ultraviolet radiation [49], biochemical oxygen demand, and Chl-a concentration [50]. Some previous studies suggested that tidal amplitude has little effect on temporal eDNA concentration in nearshore environment [42, 51]. In this study, tested environmental factors such as salinity, Chl-a concentration, and tidal height had little effect on the eDNA concentration of S. lanceolatus (Table 1). However, in order to interpret the eDNA data observed in open marine systems and improve the accuracy of biomass estimation, one needs to seek more
detailed information on the relationships between eDNA dynamics and the environmental factors in seawater.

Smelt fishes, in the family Osmeridae, display diverse life-history characteristics with marine, anadromous, and freshwater forms. While the family has a widespread distribution, most Osmerid species have more limited ranges, generally along single coastlines of the North Pacific and North Atlantic. Notable exceptions include the Holarctic Osmerids, Capelin (Mallotus villosus) and Rainbow smelt (Osmerus dentex) [52]. Longfin smelt (Spirinchus thalaeichthys) is closely related to S. lanceolatus and inhabits lakes, coastal river estuaries, and nearshore marine environments from Alaska to central California. S. thalaeichthys juveniles aggregate in low-salinity estuaries from winter through spring and then outmigrate seaward during summer [53], showing the same seasonal movement pattern as S. lanceolatus. Our findings suggest the similarities in their phenology between the two closely related species for the first time.

This study uncovered new aspects of S. lanceolatus life history in the coastal regions. The outmigration of this species in summer reveals likely survival strategies for the species such as metabolic optimization or escape from predators. Osmerids are important forage fishes acting as energetic pathways between zooplankton and higher-trophic-level predators, which are keys for the maintenance of aquatic food webs and ecosystem functions [2, 54]. Therefore, continuous evaluation of the interaction between population dynamics of S. lanceolatus and environmental and community changes will be required for long-term management of this species and the surrounding ecosystems. Together with our previous eDNA study on the freshwater migration of S. lanceolatus [8], this study illustrates the usefulness of eDNA techniques for understanding the whole life history of this valuable endemic species.

Supporting information
S1 Table. Geographical information of seven eDNA sampling sites and sampling dates. (DOCX)
S2 Table. Variance inflation factors (VIFs) among environmental factors. (DOCX)
S3 Table All the raw data for quantitative PCR. (XLSX)
S1 Fig. Heatmap illustrating the spatiotemporal variation of eDNA concentrations (except for samples below 1 copy per 2 μl PCR template) among sampling sites from March 27th to August 6th, 2019. (TIF)
S2 Fig. Heatmaps illustrating the spatiotemporal variation of environmental factors. A) salinity (ppt), B) Chlorophyll-a concentration \(\log_{10}(g/m^3)\), and C) tidal height (cm) among sampling sites from March 27th to August 6th. Blank (white) columns in the heatmap of Chl-a concentrations mean that the data were not available due to weather disturbances. (TIF)

Acknowledgments
We thank Takashi Kanbe, Hiroki Mizumoto, and all members of the Animal Ecology Lab in the Research Faculty of Agriculture, Hokkaido University, for technical support and suggestions and Theodore E. Squires for English corrections. We are also grateful to Ryotaro Ishida, of the Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization,
and Hisaya Nii, Hokkaido Aquaculture Promotion Corporation, for meaningful comments and cooperation. We further appreciate the contributions of a former lab member Theodore Squires for edits to the manuscript.

Author Contributions

Conceptualization: Tetsu Yatsuyanagi, Hitoshi Araki.
Data curation: Tetsu Yatsuyanagi.
Formal analysis: Tetsu Yatsuyanagi.
Funding acquisition: Hitoshi Araki.
Project administration: Hitoshi Araki.
Supervision: Hitoshi Araki.
Writing – original draft: Tetsu Yatsuyanagi.
Writing – review & editing: Tetsu Yatsuyanagi, Hitoshi Araki.

References

1. Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, et al. A movement ecology paradigm for unifying organismal movement research. PNAS. 2008; 105(49):19052–19059. https://doi.org/10.1073/pnas.0800375105 PMID: 19060196
2. Hall CJ, Jordaan A, Frisk MG. Centuries of anadromous forage fish loss: consequences for ecosystem connectivity and productivity. BioScience 2012; 62:723–731. https://doi.org/10.1525/bio.2012.62.8.5
3. Hellfield JM, Naiman RJ. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology. 2001; 82(9):2403–2409. https://doi.org/10.1002/1051-1511.3
4. Schmitz OJ, Hawlena D, Trussell GC. Predator control of ecosystem nutrient dynamics. Ecol. Lett. 2010; 13(10):1199–1209. https://doi.org/10.1111/j.1461-0248.2010.01511.x PMID: 20602626
5. Goto A, Nakano S. Distribution and ecology of freshwater fishes in Hokkaido. In: Higashi S, Osawa A, Kanagawa K. Biodiversity and ecology in the northernmost Japan; 1993. pp. 113–126.
6. Ministry of the Environment Government of Japan. Red List 2019—Brackish and Freshwater Fishes. 2019. Available from: https://www.env.go.jp/press/files/jp/109278.pdf.
7. Ni H, Murakami K, Yoneda T, Ueda H. The relationship between spawning ground and physical river environmental conditions of shishamo smelt *Spirinchus lanceolatus*. Nippon Suisan Gakkaishi. 2006; 72(3):390–400
8. Yatsuyanagi T, Ishida R, Sakata MK, Kanbe T, Mizumoto H, Kobayashi Y, et al. Environmental DNA monitoring for short-term reproductive migration of endemic anadromous species, Shishamo smelt (*Spirinchus lanceolatus*). Environmental DNA. 2020; 2:130–139. https://doi.org/10.1002/edn3.50
9. Rutz C, Hays GC. New frontiers in biologging science. Biol. Lett. 2009; 5(3):289–292. https://doi.org/10.1098/rsb.2009.0089 PMID: 19324624
10. Tomkiewicz SM, Fuller MR, Kie JG, Bates KK. Global positioning system and associated technologies in animal behaviour and ecological research. Philos. Trans. R. Soc. B. 2010; 365(1550):2163–2176. https://doi.org/10.1098/rstb.2010.0090
11. Dingle H, Drake VA. What is migration? BioScience. 2007; 57(2):113–121. https://doi.org/10.1641/B570206
12. Thomsen PF, Willerslev E. Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 2015; 183:4–18. http://dx.doi.org/10.1016/j.biocon.2014.11.019
13. Doi H, Inui R, Akamatsu Y, Kanno K, Yamanaka H, Takahara T, et al. Environmental DNA analysis for estimating the abundance and biomass of stream fish. Freshw. Biol. 2017; 62(1):30–39. https://doi.org/10.1111/fwb.12846
14. Ficetola GF, Miaud C, Pompanon F, Taberlet P. Species detection using environmental DNA from water samples. Biol. Lett. 2008; 4(4):423–425. https://doi.org/10.1098/rsbl.2008.0118 PMID: 18400683
15. Lacoursière-Roussel A, Côté G, Leclerc V, Bernatchez L. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J. Appl. Ecol. 2016; 53(4):1148–1157. https://doi.org/10.1111/1365-2664.12598
Mizumoto H, Urabe H, Kanbe T, Fukushima M, Araki H. Establishing an environmental DNA method to detect and estimate the biomass of Sakhalin taimen, a critically endangered Asian salmonid. Limnology. 2018; 19(2):219–227. https://doi.org/10.1007/s10201-017-0535-x

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z. Estimation of Fish Biomass Using Environmental DNA. PLoS ONE. 2012; 7(4):e35868. https://doi.org/10.1371/journal.pone.0035868 PMID: 22563411

Laramie MB, Pilliod DS, Goldberg CS. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol. Conserv. 2015; 183:29–37. https://doi.org/10.1016/j.bioccon.2014.11.025

Maruyama A, Sugata ni K, Watanabe K, Yamana ka H, Imamura A. Environmental DNA analysis as an innovative quantitative tool for reproductive migration of a threatened endemic fish in rivers. Ecol. Evol. 2018; 8(23):11964–11974. https://doi.org/10.1002/ece3.4653 PMID: 30596791

Yamanaka H, Minamoto T. The use of environmental DNA of fishes as an efficient method of determining habitat connectivity. Ecol. Indic. 2016; 62:147–153. https://doi.org/10.1016/j.ecolind.2015.11.022

Handley LL, Read DS, Winfield IJ, Kimbell H, Johnson H, Li J, et al. Temporal and spatial variation in distribution of fish environmental DNA in England’s largest lake. Environmental DNA. 2019; 1(1):26–39. https://doi.org/10.1002/edn3.5

Wu Q, Kawano K, Ishikawa T, Sakata MK, Nakao R, Hiraiwa MK, et al. Habitat selection and migration of the common shrimp, *Palaemon paucidentes* in Lake Biwa, Japan—An eDNA-based study. Environmental DNA. 2019; 1(1):54–63. https://doi.org/10.1002/edn3.6

Stoeckle MY, Soboleva L, Charlop-Powers Z. Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. PLoS ONE. 2017; 12(4):e0175186. https://doi.org/10.1371/journal.pone.0175186 PMID: 28403183

Minamoto T, Fukuda M, Katsumara KR, Fujiwara A, Hidaka Y, Yamamoto S, et al. Environmental DNA reflects spatial and temporal jellyfish distribution. PLoS ONE. 2017; 12(2):e0173073. https://doi.org/10.1371/journal.pone.0173073 PMID: 28245277

Yamamoto S, Minami K, Fukaya K, Takahashi K, Sawada H, Murakami H, et al. Environmental DNA as a ‘snapshot’ of fish distribution: a case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. PLoS ONE. 2016; 11:e0149786. https://doi.org/10.1371/journal.pone.0149786 PMID: 26933889

Salter I, Joensen M, Kristiansen R, Steingrund P, Vestergaard P. Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. Commun. Biol. 2019; 2(461):1–9. https://doi.org/10.1038/s42003-019-0696-8

Andruszkiewicz EA, Starks HA, Chavez FP, Sassoubre LM, Block BA, Boehm AB. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS ONE. 2017; 12(4):e0176343. https://doi.org/10.1371/journal.pone.0176343 PMID: 28441466

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, et al. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. R. Soc. Open Sci. 2015; 2(7):150088. https://doi.org/10.1098/rsos.150088 PMID: 26587265

O’Donnell JL, Kelly RP, Shelton AO, Samhouri JF, Lowell NC, Williams GD. Spatial distribution of environmental DNA in a nearshore marine habitat. PeerJ. 2017; (2):1–24. https://doi.org/10.7717/peerj.3044

Sigsgaard EE, Nielsen IB, Carl H, Krag MA, Knudsén SW, Xing Y, et al. Seawater environmental DNA reflects seasonality of a coastal fish community. Mar. Biol. 2017; 164(6):1–15. https://doi.org/10.1007/s00227-017-3147-4

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE. 2012; 7(8):e41732. https://doi.org/10.1371/journal.pone.0041732 PMID: 22952584

Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, et al. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. Sci. Rep. 2017; 7(1):1–12. https://doi.org/10.1038/s41598-016-0028-x PMID: 28127051

Bigelow HB. Studies of the waters on the continental shelf, Cape Cod to Chesapeake Bay. Papers in Physical Oceanography and Meteorology. 1933; 2(4). https://doi.org/10.1057/palaeo.1935-1912-1144

Ryther JH. Photosynthesis and fish production in the sea. Science. 1969; 166(3901):72–76. https://doi.org/10.1126/science.166.3901.72 PMID: 5817762

Bessho K, Date K, Hayashi M, Ikeda A, Imai A, Inoue H, et al. An introduction to Himawari-8/9- Japan’s new-generation geostationary meteorological satellites. J. Meteorol. Soc. Jpn. 2016; 94(2):151–183. https://doi.org/10.2151/jmsj.2016-009

Murakami H. Ocean color estimation by Himawari-8/AHI. Proc. SPIE 9878. 2016. https://doi.org/10.1117/12.2225422.
37. Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, et al. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim. Method Softw. 2012; 27(2):233–249. https://doi.org/10.1080/10556788.2011.597854

38. Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 2010; 1(1):3–14. https://doi.org/10.1111/j.2041-2100.2009.00001.x

39. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria; 2017. Available from: http://www.R-project.org.

40. Deng W, Wang Y, Liu Z, Cheng H, Xue Y. HemI: A toolkit for illustrating heatmaps. PLoS ONE. 2014; 9(11):e111988. https://doi.org/10.1371/journal.pone.0111988 PMID: 25372567

41. Hokkaido Government. Catches and production of fisheries in Hokkaido. 2019. Available from: http://www.pref.hokkaido.lg.jp/sr/sum/03kanrig/suitoukei/suitoukei.htm

42. Murakami H, Yoon S, Kasai A, Minamoto T, Yamamoto S, Sakata MK, et al. Dispersion and degradation of environmental DNA from caged fish in a marine environment. Fish. Sci. 2019; 85(2):327–337. https://doi.org/10.1007/s12562-018-1282-6

43. Collin RA, Wangensteen OS, O’Gorman EJ, Mariani S, Sims DW, Genner MJ. Persistence of environmental DNA in marine systems. Commun. Biol. 2018; 1(1):1–11. http://doi.org/10.1038/s42003-018-0192-6

44. Eichmiller JJ, Best SE, Sorensen PW. Effects of temperature and trophic state on degradation of environmental DNA in lake water. Environ. Sci. Technol. 2016; 50(4):1859–1867. https://doi.org/10.1021/acs.est.5b05672 PMID: 26771292

45. Jo T, Murakami H, Yamamoto S, Masuda R, Minamoto T. Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. Ecol. Evol. 2019; 9(3):1135–1146. https://doi.org/10.1002/ece3.4802 PMID: 30805147

46. Sassoubre LM, Yamaha KM, Gardner LD, Block BA, Boehm AB. Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. Environ. Sci. Technol. 2016; 50(19):10456–10464. https://doi.org/10.1021/acs.est.6b03114 PMID: 27580258

47. Tsuji S, Uschio M, Sakurai S, Minamoto T, Yamanaka H. Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. PLoS ONE. 2017; 12(4):e0176608. https://doi.org/10.1371/journal.pone.0176608 PMID: 28448613

48. Tsuji S, Yamanaka H, Minamoto T. Effects of water pH and proteinase K treatment on the yield of environmental DNA from water samples. Limnology. 2017; 18(1):1–7. https://doi.org/10.1007/s10201-016-0483-x

49. Pilliod DS, Goldberg CS, Arkle RS, Waits LP. Factors influencing detection of eDNA from a stream-dwelling amphibian. Mol. Ecol. Res. 2014; 14(1):109–116. https://doi.org/10.1111/1755-0998.12159

50. Barnes MA, Tumer CR, Jerde CL, Renshaw MA, Chadderton WL, Lodge DM. Environmental conditions influence eDNA persistence in aquatic systems. Environ. Sci. Technol. 2014; 48(3):1819–1827. https://doi.org/10.1021/es404734p PMID: 24422450

51. Kelly RP, Gallego R, Jacobs-Palme E. The effect of tides on nearshore environmental DNA. PeerJ. 2018;(3):2018–2018. https://doi.org/10.7717/peerj.4521

52. Ilves KL, Taylor EB. Molecular resolution of the systematics of a problematic group of fishes (Teleostei: Osmeridae) and evidence for morphological homoplasy. Mol. Phylogenetics Evol. 2009; 50(1):163–178. https://doi.org/10.1016/j.ympev.2008.10.021

53. Rosenfield JA, Baxter RD. Population dynamics and distribution patterns of Longfin Smelt in the San Francisco Estuary. Trans. Am. Fish. Soc. 2007; 136:1577–1592. https://doi.org/10.1577/T06-148.1

54. Pikitch EK, Rountos KJ, Essington TE, Santora C, Pauly D, Watson R, et al. The global contribution of forage fish to marine fisheries and ecosystems. Fish and Fisheries. 2014; 15(1):43–64. https://doi.org/10.1111/faf.12004