

ENVIRONMENTAL DNA FOR IDENTIFYING “RED ZONE” OF ENDANGERED/INVASIVE SPECIES

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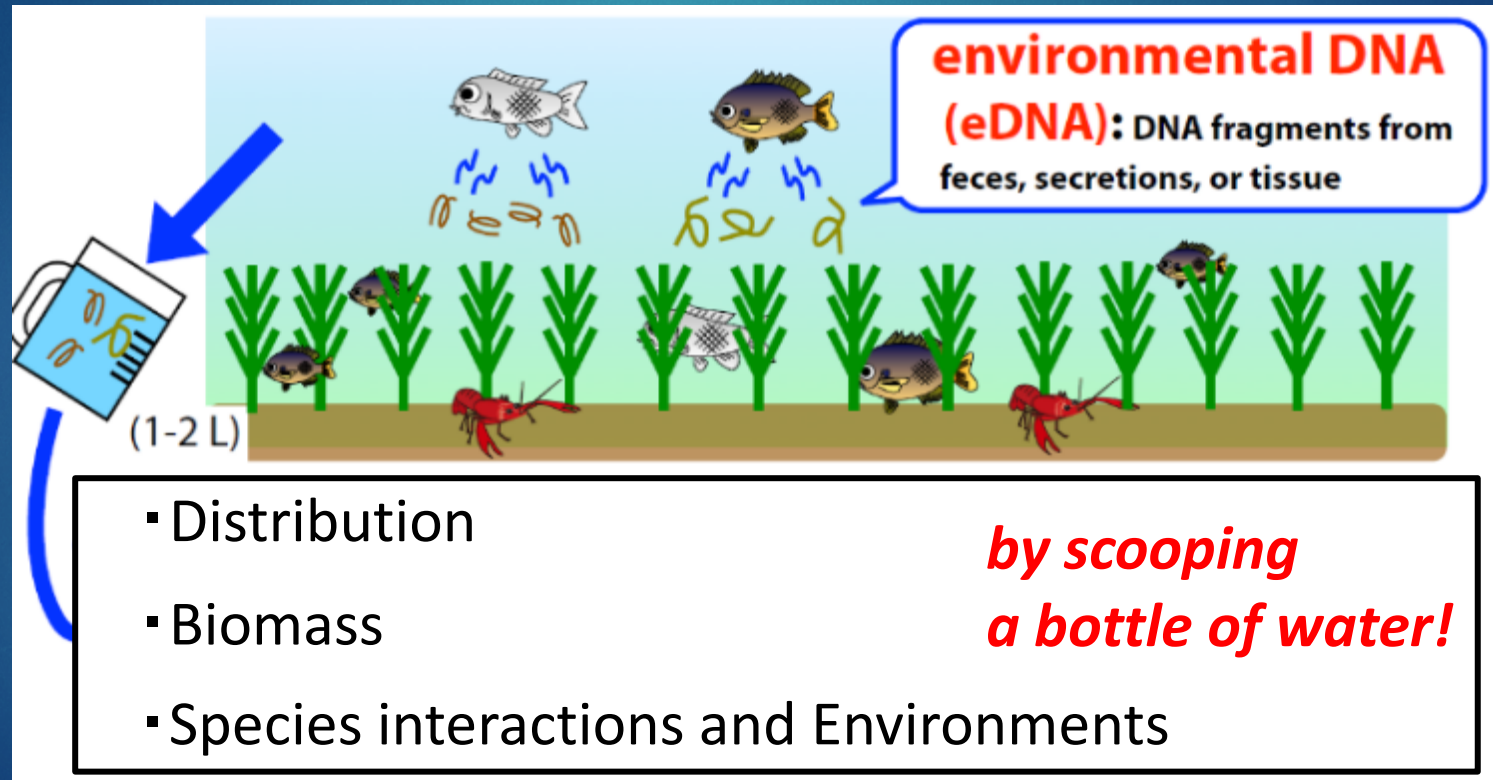
Today's menu

1. What is eDNA?
2. Species-specific approach
3. Taxon-wide approach
4. Challenges & Future studies

1. What is eDNA?

eDNA (environmental DNA):

DNA released from living organisms to their environmental media



Aquatic biodiversity monitoring

In the past:
a lot of work & cost



Today:
water sampling & eDNA analysis



- Non-invasive
- Easy & efficient
- Objective
- No taxonomic skills required

eDNA Sampling

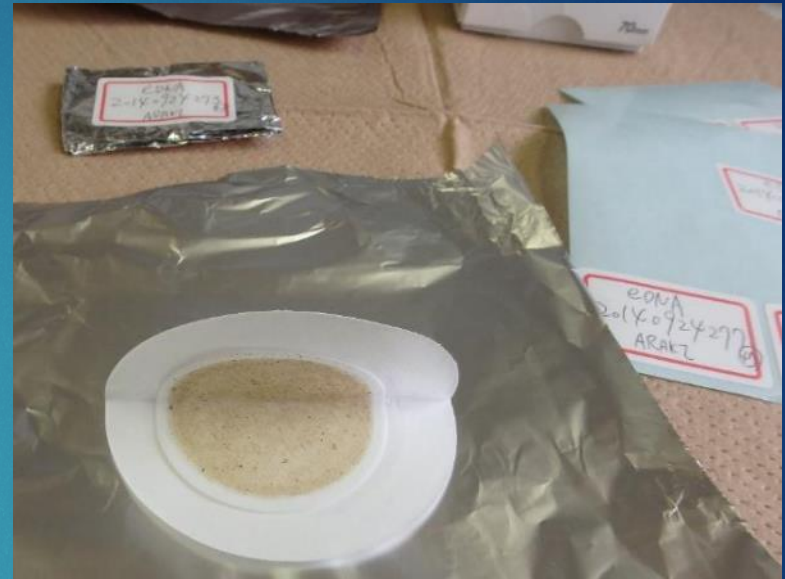


eDNA Sampling



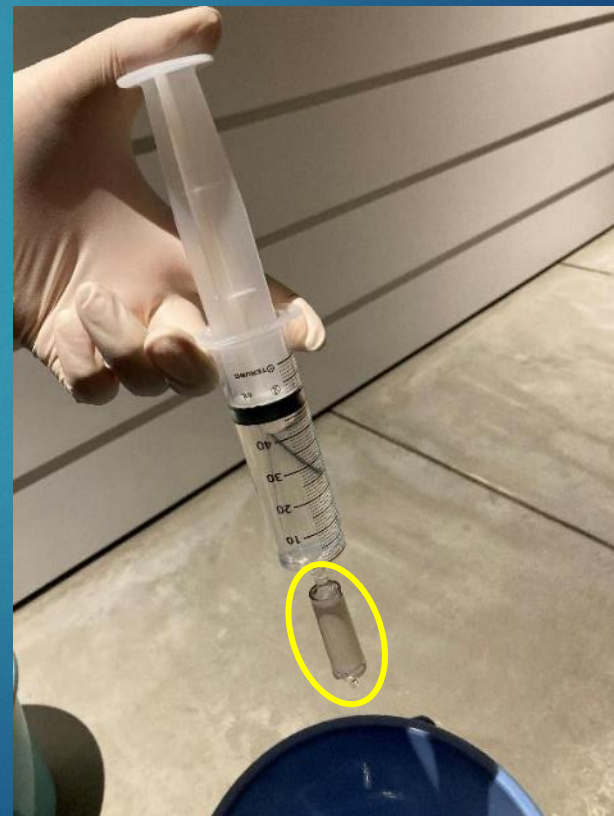
eDNA Sampling

Filtering (Whatman GF/F)



eDNA Sampling

Filtering (Sterivex)



eDNA Extraction, Purification & Analysis

DNA extraction



www.qiagen.com

Template DNA
(100~200 μ l)



q-PCR applications



www.thermofisher.com
www.agilent.com

NGS applications



<https://jp.illumina.com>



“species list” through data analysis
pipeline (e.g., Sato et al. 2018)

2. Species-specific approach

eDNA for answering questions such as:

Is there “my target species” nearby?

Where are they? And how many?

q-PCR applications



2-1. Species-specific approach



Species-specific primer + q-PCR

Sakhalin taimen (*Parahucho perryi*)

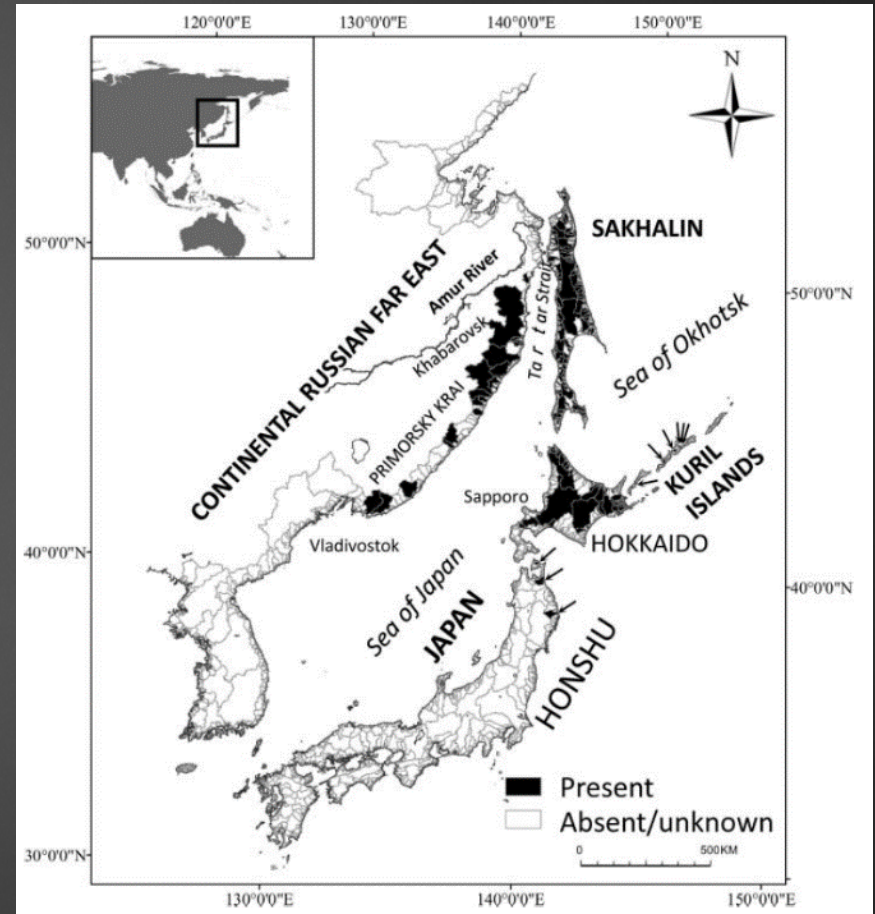


Largest freshwater fish in Japan

Used to be distributed widely in Far East

IUCN Red List:

Critically Endangered (CR)



(Fukushima et al. 2011)

2-1. Species-specific approach

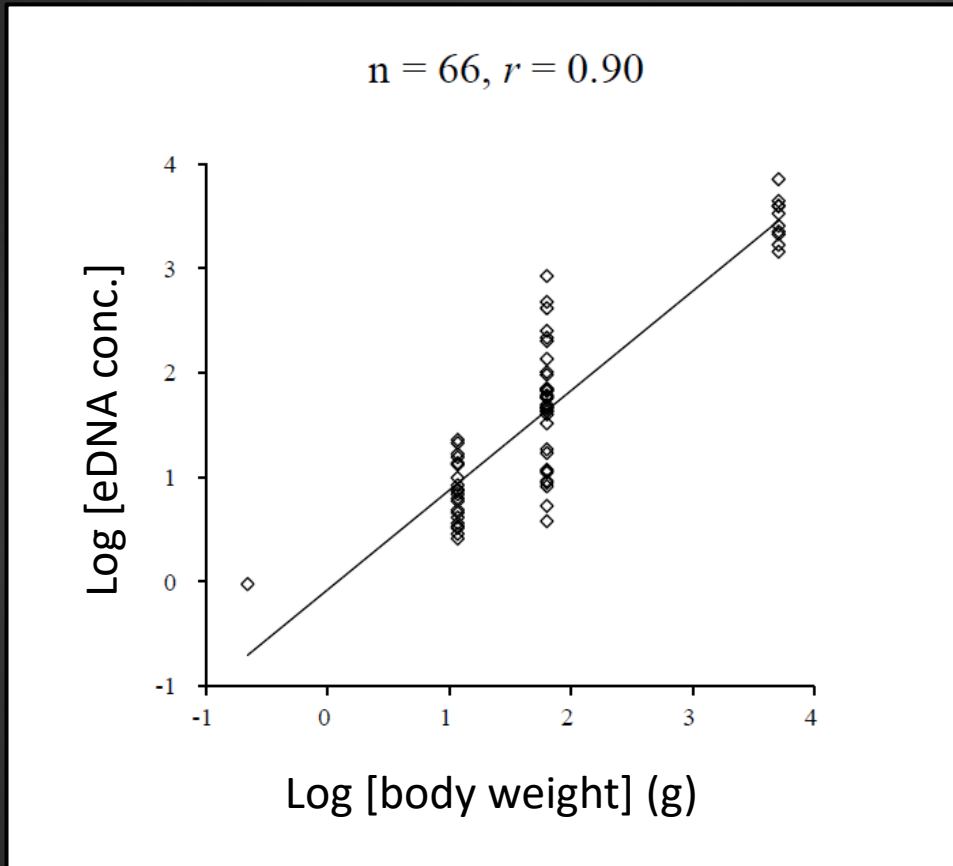
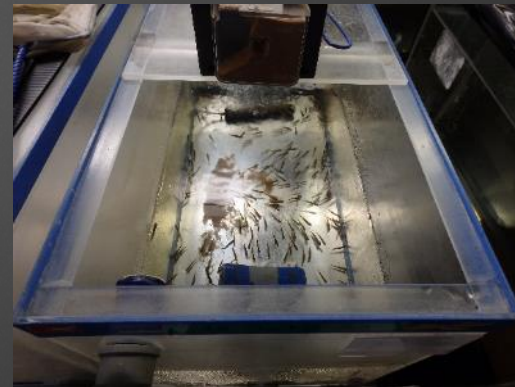
Species-specific primer + q-PCR



Sakhalin taimen (*P. perryi*)



H. Mizumoto
(Hokkaido U.)



➔ *More fish = More eDNA*

(Mizumoto et al. 2018 *Limnology*)

2-1. Species-specific approach

Species-specific primer + q-PCR

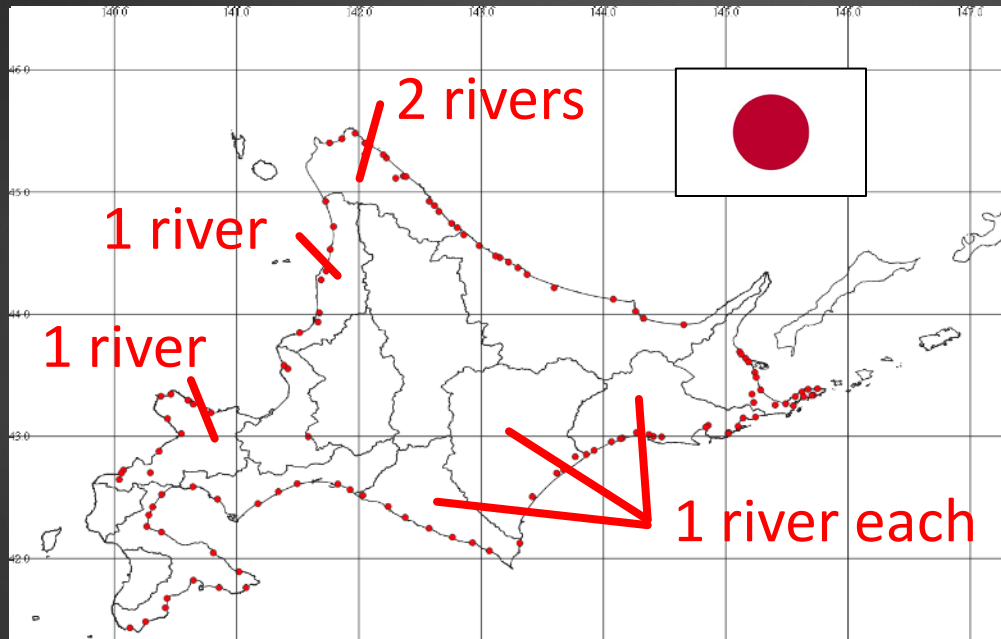
Application to 120 rivers in Hokkaido



Sakhalin taimen (*P. perryi*)



H. Mizumoto



- 7 rivers with positive eDNA detection
- >2000 Sakhalin taimen in Hokkaido?
- 3 rivers for >95% total biomass?

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 06 November 2020
doi: 10.3389/fevo.2020.589425

An Environmental DNA Survey on
Distribution of an Endangered
Salmonid Species, *Parahucho perryi*,
in Hokkaido, Japan

Hiroki Mizumoto¹, Takashi Mitsuzuka² and Hitoshi Araki^{1*}

(Mizumoto et al. 2020 *In press*)

2-2. Species-specific approach

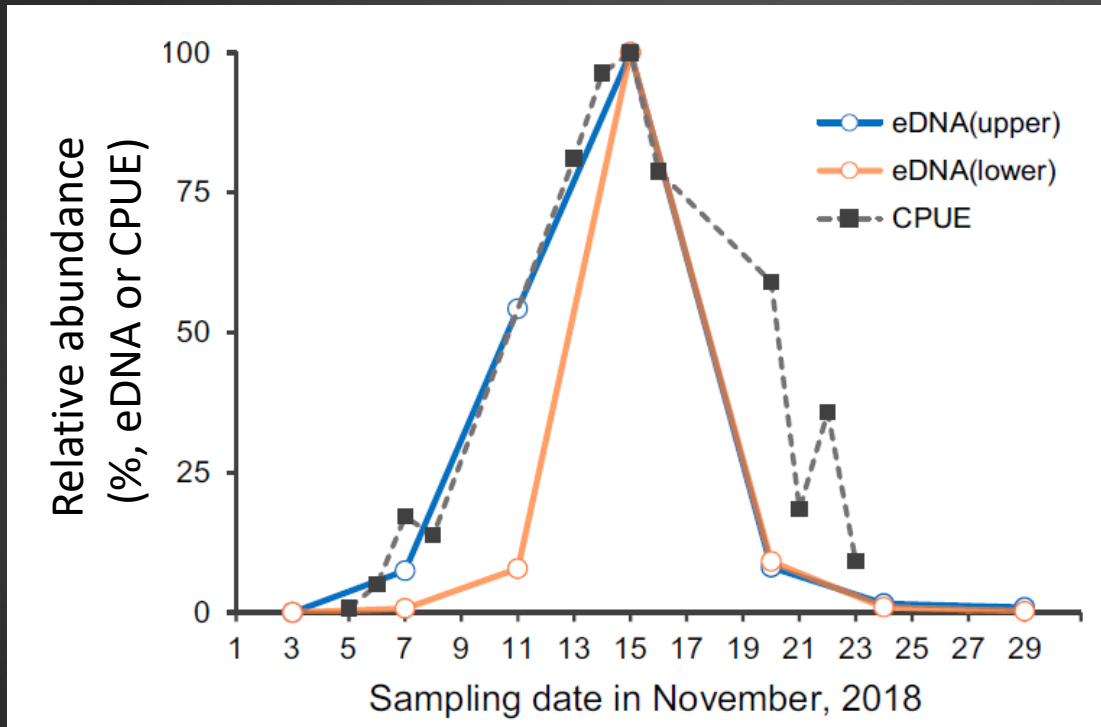
Species-specific primer + q-PCR



Shishamo smelt
(*Spirinchus lanceolatus*)



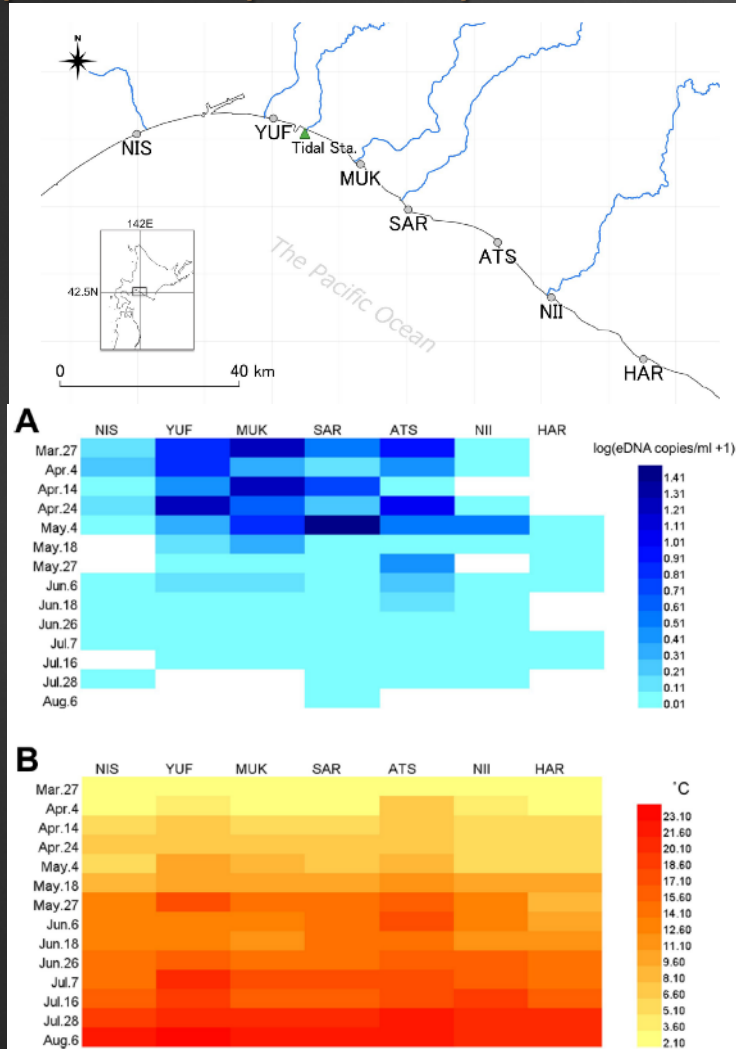
T. Yatsuyanagi
(Hokkaido U.)



➔ eDNA identifying a sharp peak of the fish spawning migration

2-2. Species-specific approach

Species-specific primer + q-PCR



Shishamo smelt
(*Spirinchus lanceolatus*)

PLOS ONE

RESEARCH ARTICLE

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

Tetsu Yatsuyanagi¹, Hitoshi Araki^{2*}

→ Seasonal off-shore migration triggered by surface water temp?

(Yatsuyanagi & Araki 2020, *PLoS One*)

2-3. Species-specific approach

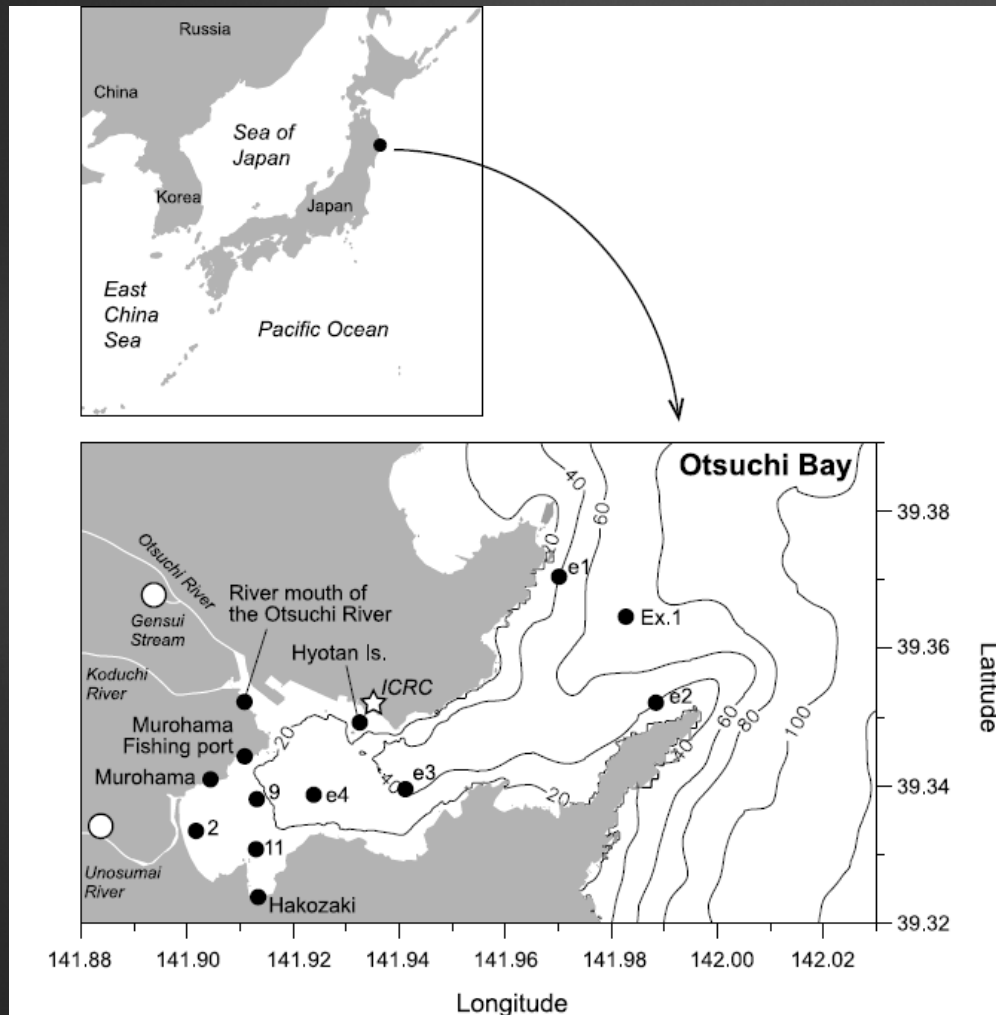
Species-specific primer + q-PCR



Chum salmon (*Oncorhynchus keta*)



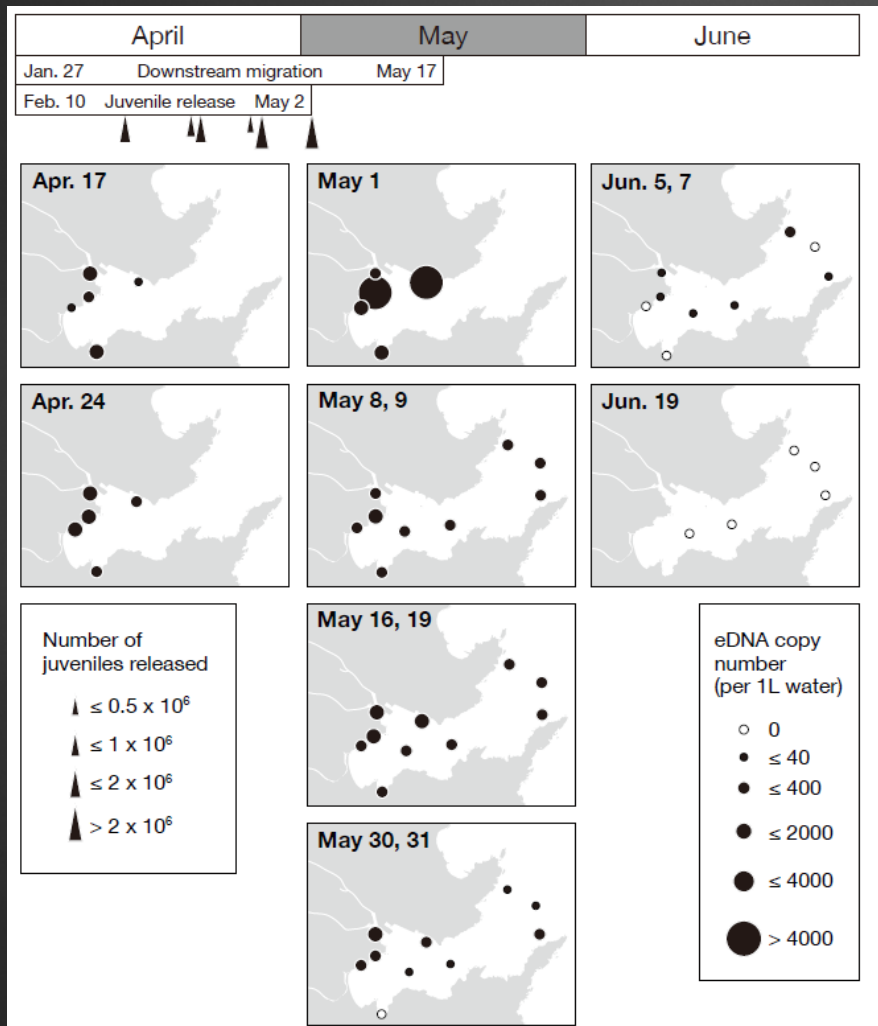
Y. Minegishi
(U. Tokyo)



(Minegishi et al. 2019 *PLoS One*)

2-3. Species-specific approach

Species-specific primer + q-PCR



Chum salmon (*Oncorhynchus keta*)

PLOS ONE

RESEARCH ARTICLE

Spatiotemporal distribution of juvenile chum salmon in Otsuchi Bay, Iwate, Japan, inferred from environmental DNA

Yuki Minegishi^{1*}, Marty Kwok-Shing Wong², Takashi Kanbe³, Hitoshi Araki³, Tomomi Kashiwabara², Minoru Ijichi², Kazuhiro Kogure², Susumu Hyodo²

➔ *Salmon juvenile migration toward open-sea*

(Minegishi et al. 2019 *PLoS One*)

3. Taxon-wide approach

eDNA for answering questions such as:

Who are around?

Any relationship among species?

NGS applications



3-1. Taxon-wide approach

NGS applications



MiFish, fish universal primers for eDNA metabarcoding

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OPEN SCIENCE

rsos.royalsocietypublishing.org

Research



Cite this article: Miya M *et al.* 2015 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.* 2: 150088. <http://dx.doi.org/10.1098/rsos.150088>

Received: 26 February 2015
Accepted: 25 June 2015

MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species

M. Miya^{1,2}, Y. Sato^{2,3}, T. Fukunaga⁴, T. Sado^{1,2},
J. Y. Poulsen^{1,2,5}, K. Sato⁶, T. Minamoto^{2,7},
S. Yamamoto^{2,7}, H. Yamanaka^{2,8}, H. Araki^{2,9},
M. Kondoh^{2,8} and W. Iwasaki^{2,4,10}



M. Miya
(Nat. Hist. Museum, Chiba)

(Miya et al. 2015, *RSOS*)

3-1. Taxon-wide approach



MiFish, fish universal primers for eDNA metabarcoding

- Originally based upon the MitoFish database (<http://mitofish.aori.u-tokyo.ac.jp/>)
- Super-variable region between highly conserved regions in 12S (mt-genome)
- Reference DNA sequences over 7000+ fish species

MiFish F-Primer

← Species specific region →

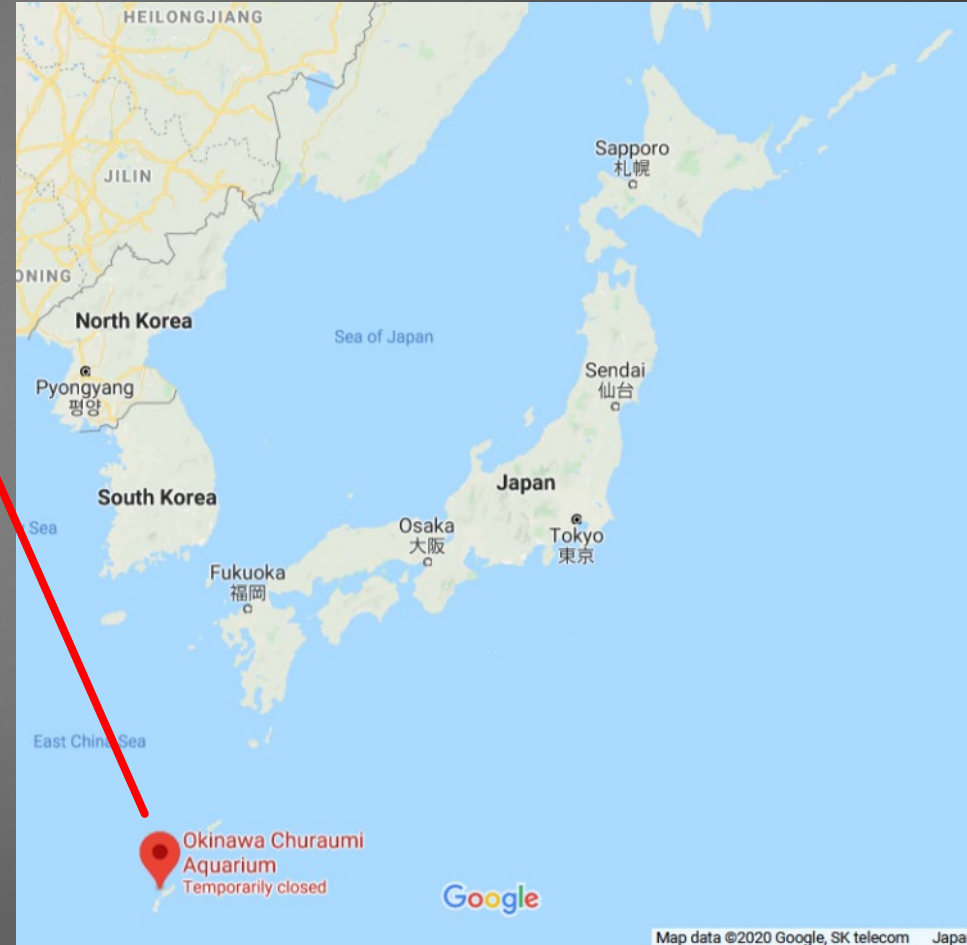
MiFish R-Primer



3-1. Taxon-wide approach



Empirical test at Okinawa Aquarium



3-1. Taxon-wide approach



Empirical test at Okinawa Aquarium

tank	Volume (m ³)	Species No. (with reference)	Total DNA reads	Species detected (%)
Tank-1	7,500	63	2,568,008	61 (96.8%)
Tank-2	700	105	1,301,723	95 (90.5%)
Tank-3	230	13	240,508	13 (100%)
Tank-4	35.6	8	212,643	8 (100%)



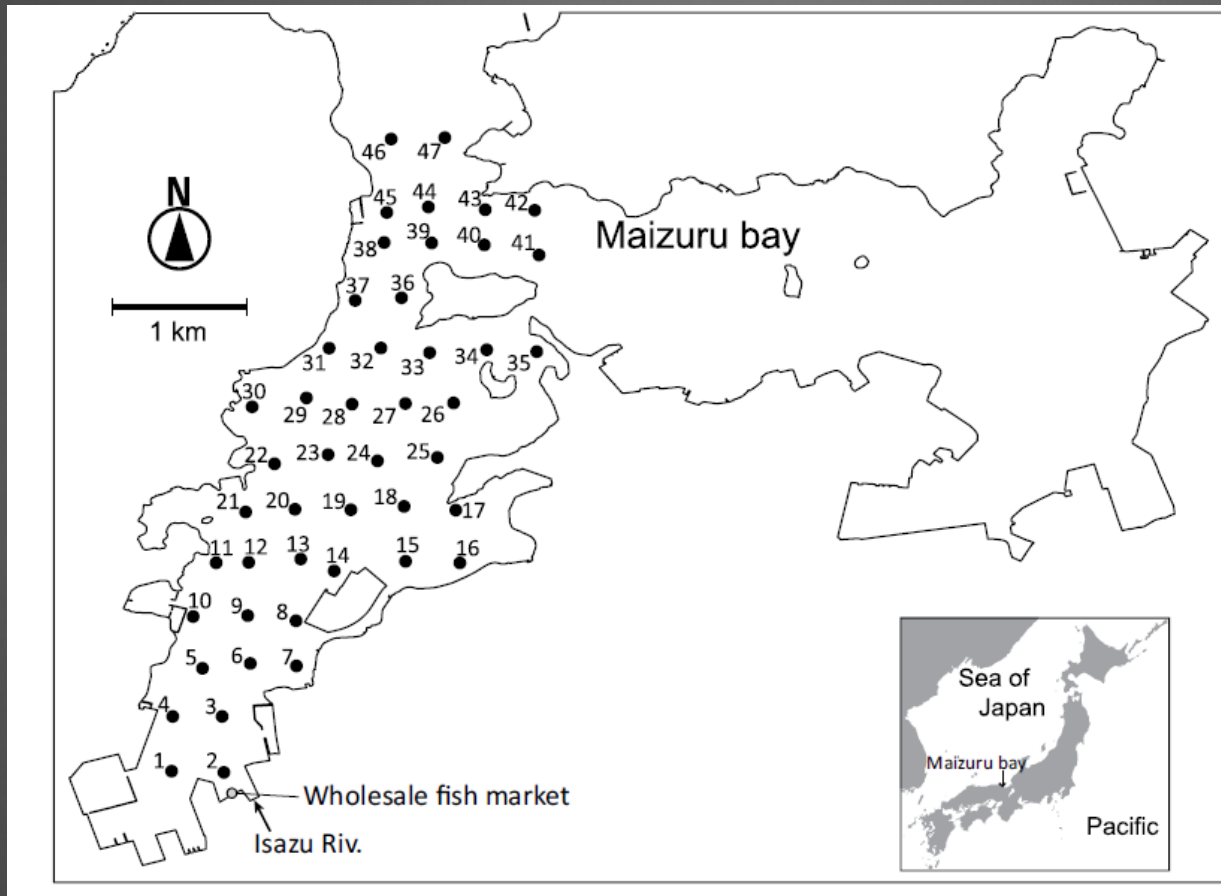
→ 93.3% of species identified (168/180 sp.)

(Miya et al. 2015, *RSOS*)

3-2. Taxon-wide approach



eDNA metabarcoding in Maizuru Bay, Kyoto



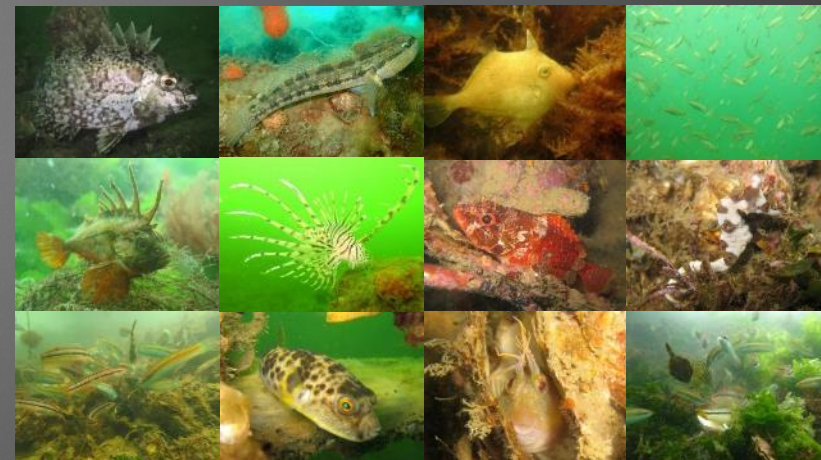
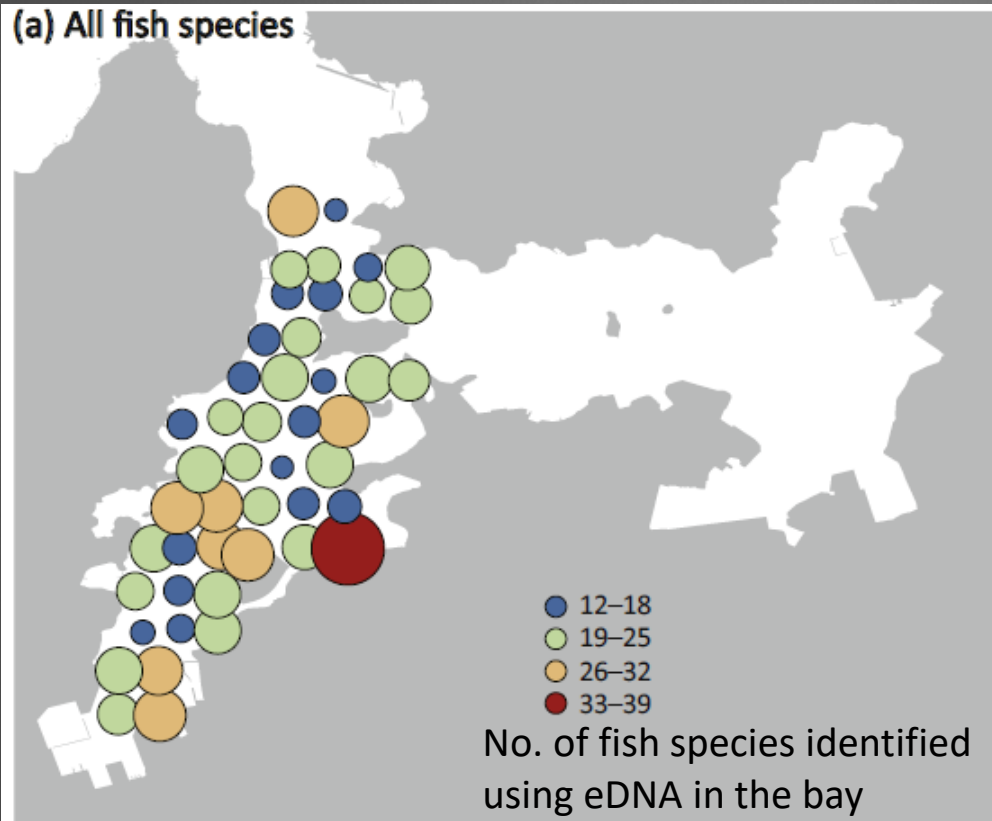
3-2. Taxon-wide approach



eDNA metabarcoding in Maizuru Bay, Kyoto

6-hour water collection → 128 species

14-year visual censuses → 80 species



(Photo by R. Masuda)

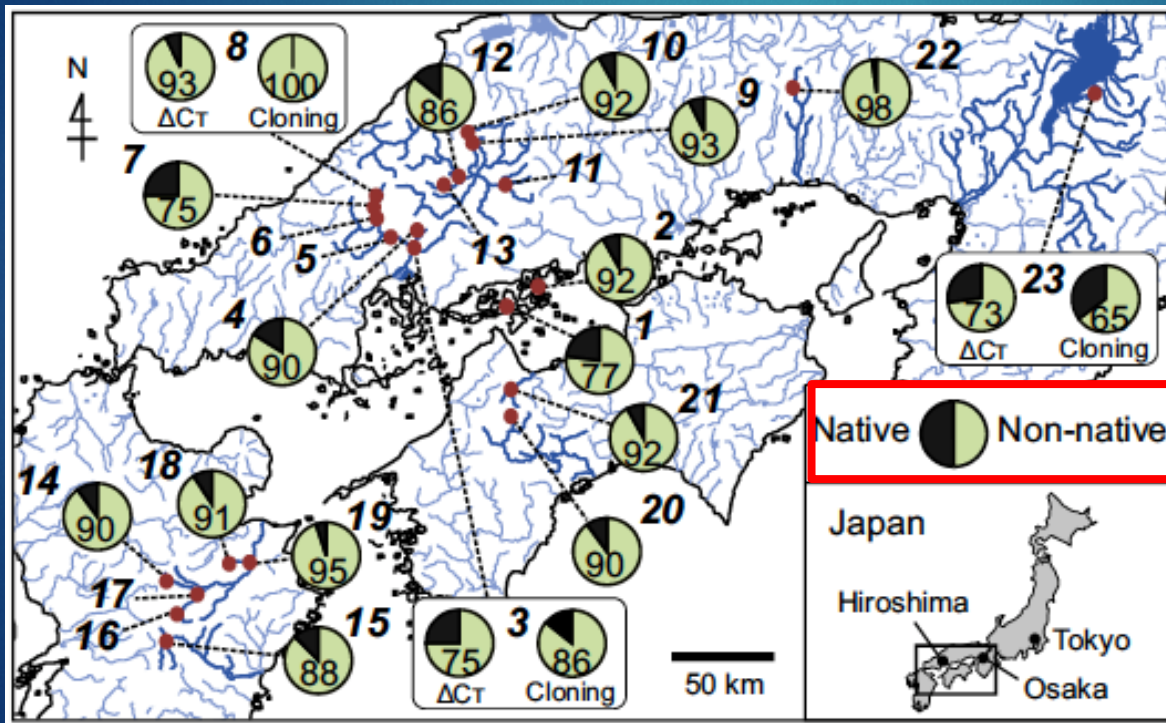
4-1. Challenges & Future studies

eDNA for detecting cryptic invasions

Intra-specific Mt-genome variation



Common carp
(*Cyprinus carpio*)



K. Uchii
(Osaka Ohtani U.)

(Uchii et al. 2016, *Mol. Ecol. Res.*)

4-2. Challenges & Future studies

eDNA from sediment core samples

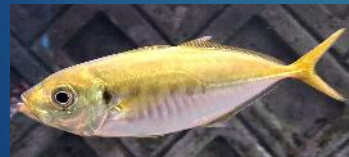
Beppu Bay, Japan



Anchovy (*E. japonicus*)



Sardine (*S. melanostictus*)



Jack mackerel
(*T. japonicus*)

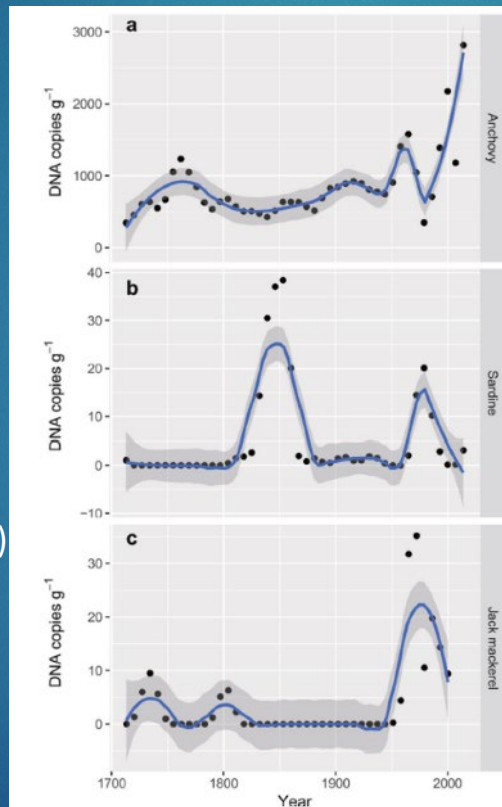


Fig. 2 The results of general additive models (GAM) from inter-core, seven-year averaged sedDNA concentrations. **a** *Engraulis japonicus* (Japanese anchovy); **b** *Sardinops melanostictus* (Japanese sardine); **c** *Trachurus japonicus* (jack mackerel). Blue line denotes a regression line of GAM with the 95% confidence interval (gray zone).



~100cm sediment core in the bay
covering 300 years

→ Abundance change of 3 dominant
fish species in 300 years

ARTICLE

<https://doi.org/10.1038/s42003-020-01282-9>

OPEN



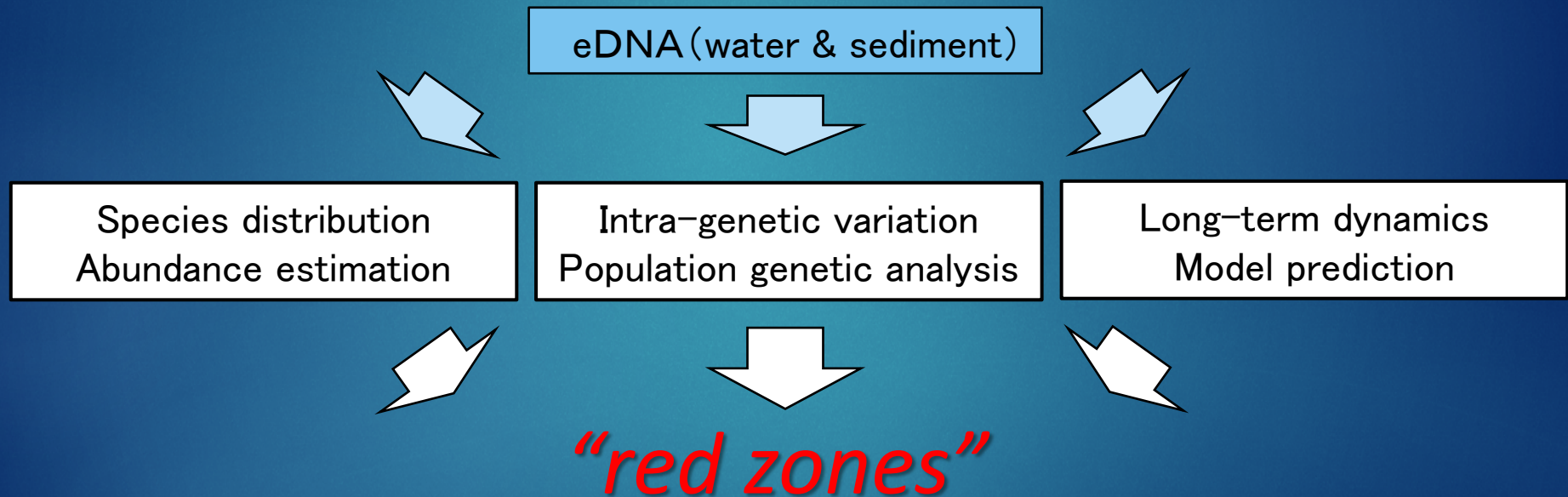
Sedimentary DNA tracks decadal-centennial changes in fish abundance

Michinobu Kuwae^{1,5}, Hiromichi Tamai¹, Hideyuki Doi^{2,5}, Masayuki K. Sakata³, Toshifumi Minamoto³ & Yoshiaki Suzuki⁴

(Kuwae et al. 2020 *Comm. Biol.*)

4-3. Challenges & Future studies

Environment Research and Technology Development Project (2020-2022)
*Development and Application of Environmental DNA Techniques for
Evaluating Distribution and Population Status of Rare/Invasive Species*



"red zones"

Endangered species



Photo: H. Mizumoto

Species
conservation
priority

Population
management
priority

Invasive species



Photo: J. Tsuboi



The eDNA Society

The eDNA Society, a general incorporated association, aims at fostering and developing eDNA Science as a discipline that contributes to the human well-being, such as sustainable use of ecosystems and environmental conservation.

About the Society

The eDNA Society, a general incorporated association, aims at fostering and developing eDNA Science as a discipline that contributes to the human well-being, such as sustainable use of ecosystems and environmental conservation.

[What is eDNA?](#)

[The eDNA Society](#)

[Environmental DNA Sampling and Experiment Manual](#)

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第1回環境DNA学会東京大会

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The eDNA Society, a general incorporated association, aims at fostering and developing eDNA Science as a discipline that contributes to the human well-being, such as sustainable use of ecosystems and environmental conservation.

Joint Annual Meeting of The eDNA Soci...

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The Joint Meeting of The eDNA Society & The Society of Population Ecology

Ecology in the Age of Big Data & Open Data

14-16 November, 2020

online

The eDNA Society, a general incorporated association, aims at fostering and developing eDNA Science as a discipline that contributes to the sustainable use of ecosystems and environmental conservation.

Environmental DNA Sampling and Experiment Manual

The eDNA Society published the standardized manual for eDNA analysis, "Environmental DNA Sampling and Experiment Manual". We wish this manual will contribute for solving the global issues relating to the biodiversity loss and for promoting the conservation.

- [Environmental DNA Sampling and Experiment Manual \(ver. 2.2\) \(in Jpn\)](#) (Published on April 3, 2020)
- [Environmental DNA Sampling and Experiment Manual \(ver. 2.1\) \(in Eng\)](#) (Published on April 25, 2019)

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Topics

COMMENTARY

Environmental DNA
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

An illustrated manual for environmental DNA research: Water sampling guidelines and experimental protocols

Toshifumi Minamoto¹  | Masaki Miya²  | Tetsuya Sado² | Satoquo Seino³ | Hideyuki Doi⁴  | Michio Kondoh⁵ | Keigo Nakamura⁶  | Teruhiko Takahara⁷ | Satoshi Yamamoto⁸  | Hiroki Yamanaka⁹  | Hitoshi Araki¹⁰  | Wataru Iwasaki¹¹  | Akihide Kasai¹² | Reiji Masuda¹³ | Kimiko Uchii¹⁴ 

(Minamoto et al. 2020, *Environmental DNA*)



TIME FOR YOUR QUESTIONS!

(PICES VS3, Oct. 28th, 2020)