北海道大学
HOKKAIDO UNIVERSITY

Environmental DNA for Fish Monitoring in the Wild

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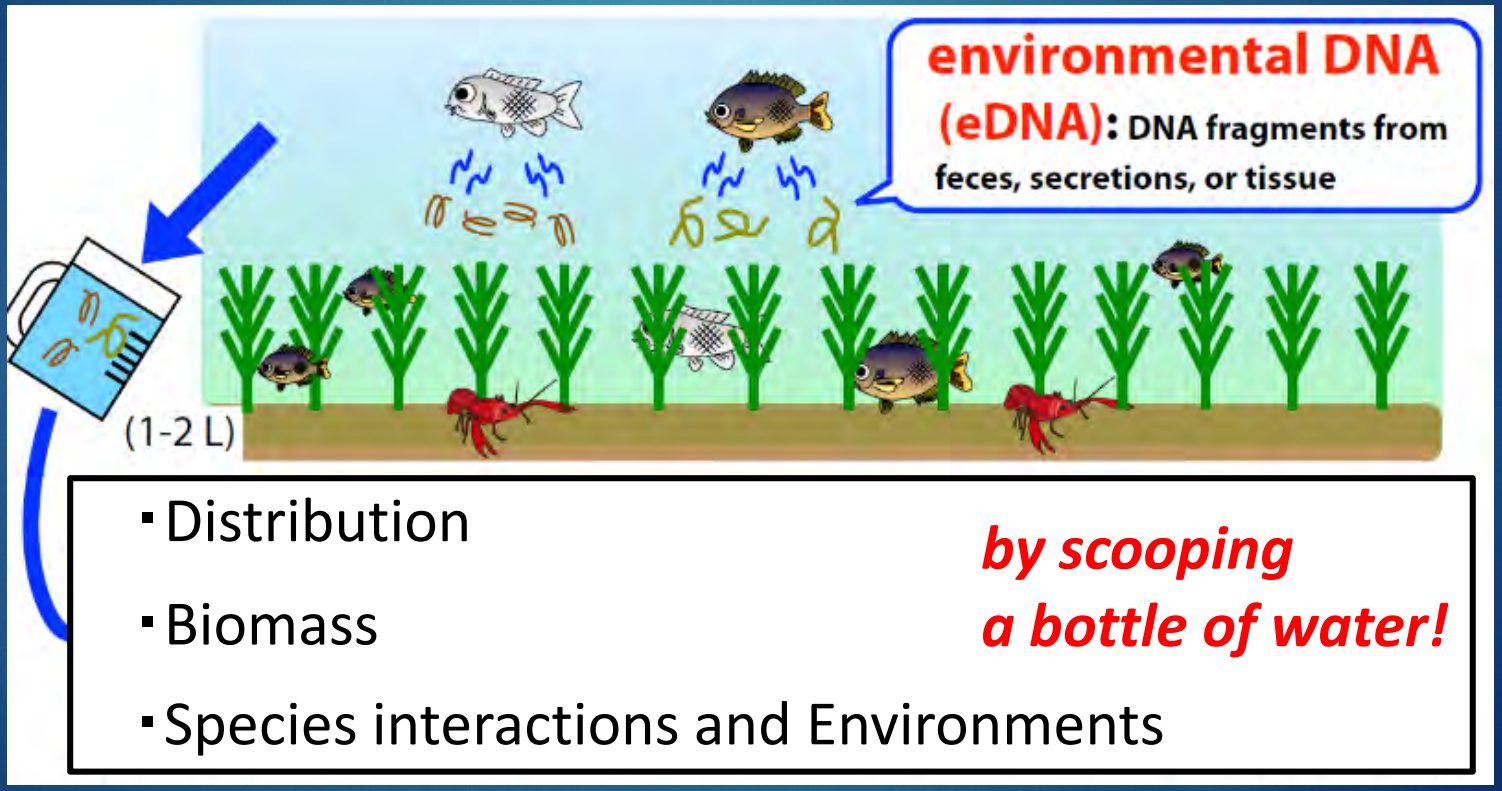
What is environmental DNA?

eDNA (environmental DNA):

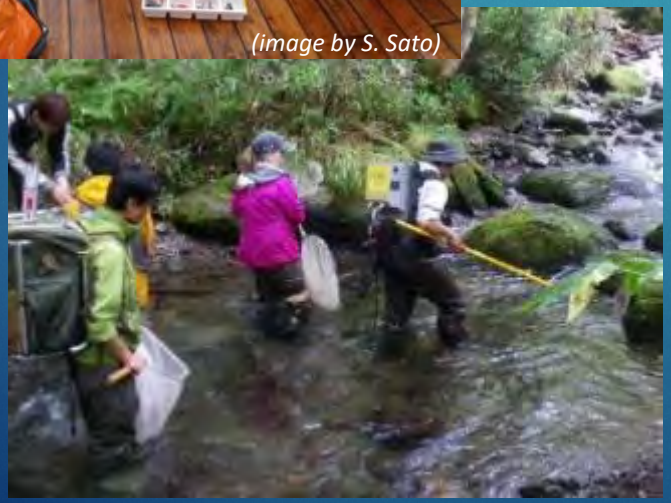
DNA released from living organisms to their environmental media



(image by T. Takahara)

Aquatic biodiversity monitoring

In the past:
a lot of work & cost

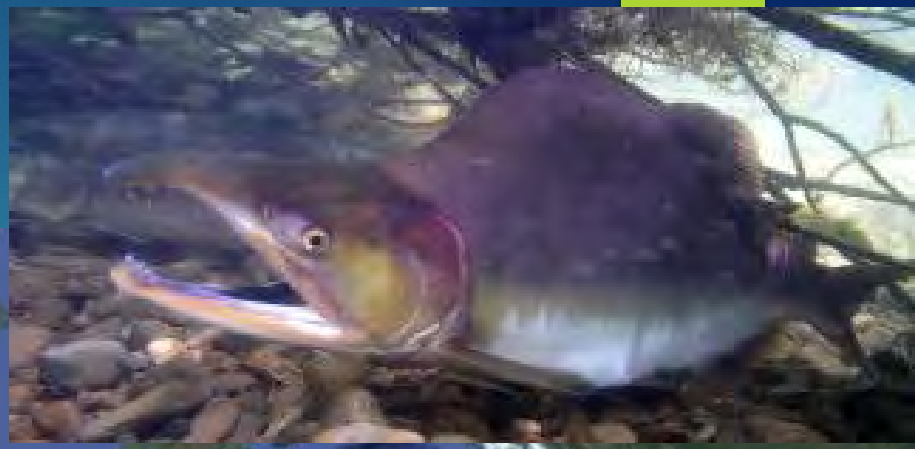


Today:
water sampling & eDNA analysis

- Easy
- Efficient
- Objective
- No taxonomic skills required

Hokkaido, JAPAN

Hokkaido, JAPAN



(images by K. Morita, M. Yamamoto et al.)

eDNA-based monitoring

1. Species-specific approach (q-PCR)

2. Taxon-wide approach (NGS metabarcoding)

eDNA-based monitoring

1. Species-specific approach (q-PCR)

Q1. Is "my target species" there?

Q2. If yes, how many?

Sakhalin taimen
(Parahucho perryi)

Chum salmon
(Oncorhynchus keta)

Shishamo smelt
(*Spirinchus lanceolatus*)

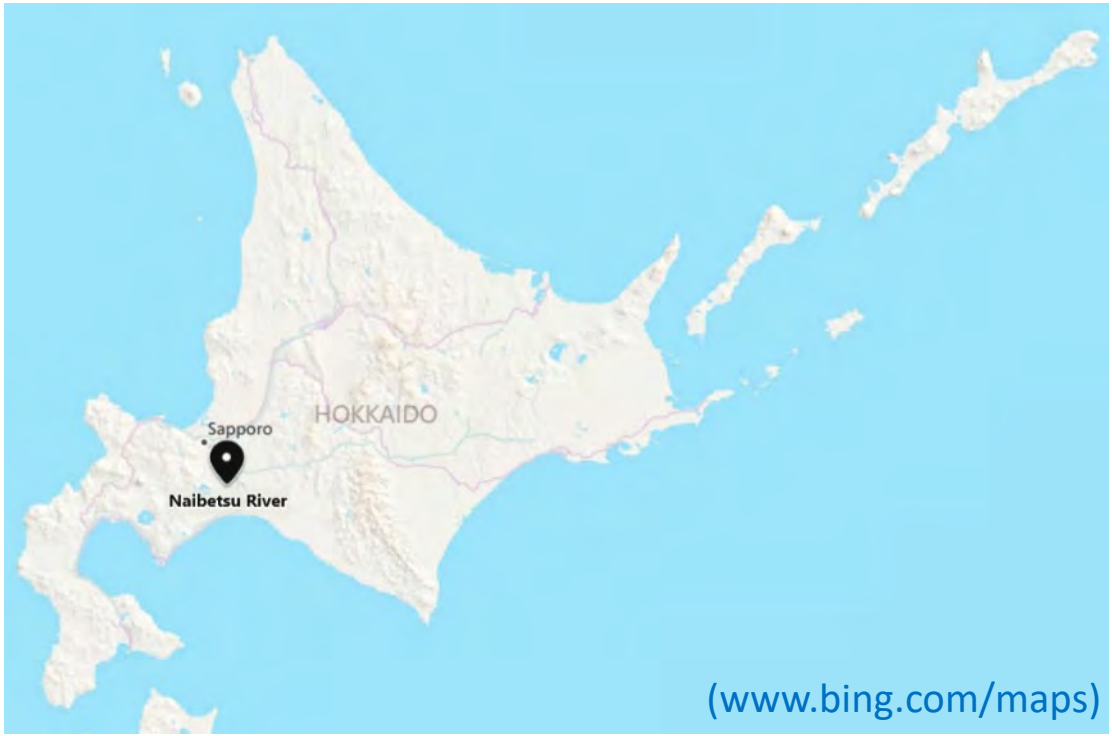
1. Species-specific approach

Chum salmon *(Oncorhynchus keta)*

1. Species-specific approach

O. keta

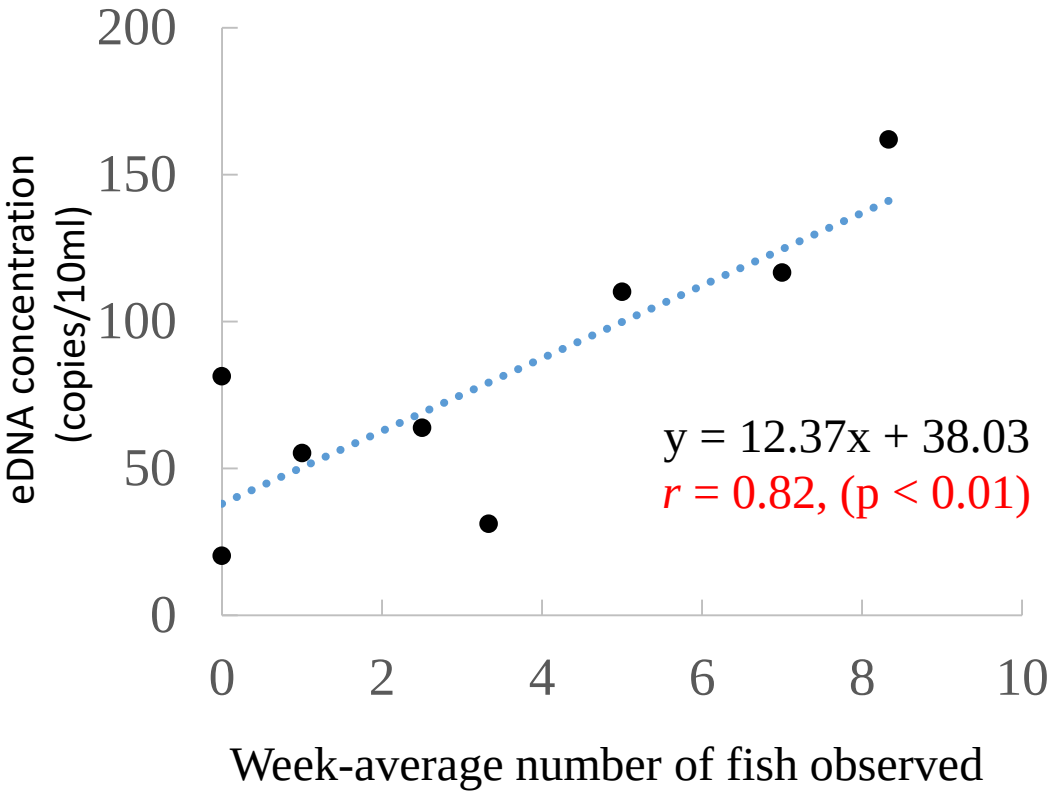
Visual observation vs. eDNA in Naibetsu R.



1. Species-specific approach

O. keta

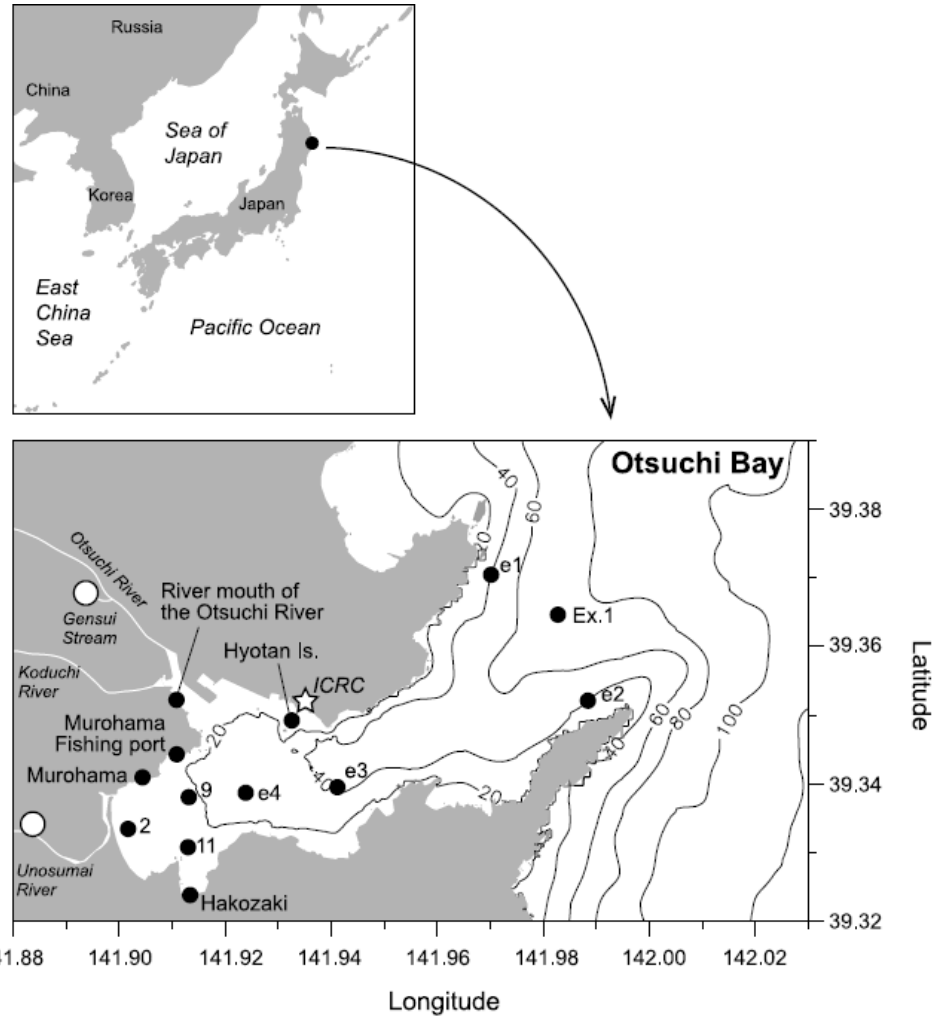
Visual observation vs. eDNA in Naibetsu R.

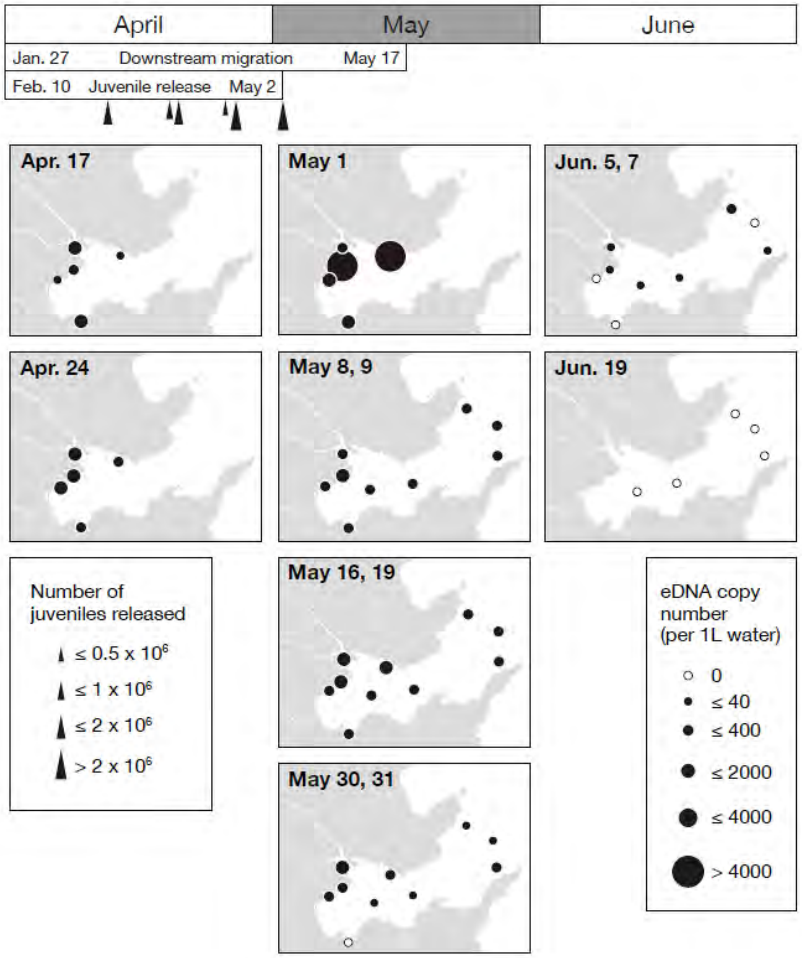


1. Species-specific approach

O. keta

Chum salmon juvenile out-migration monitoring





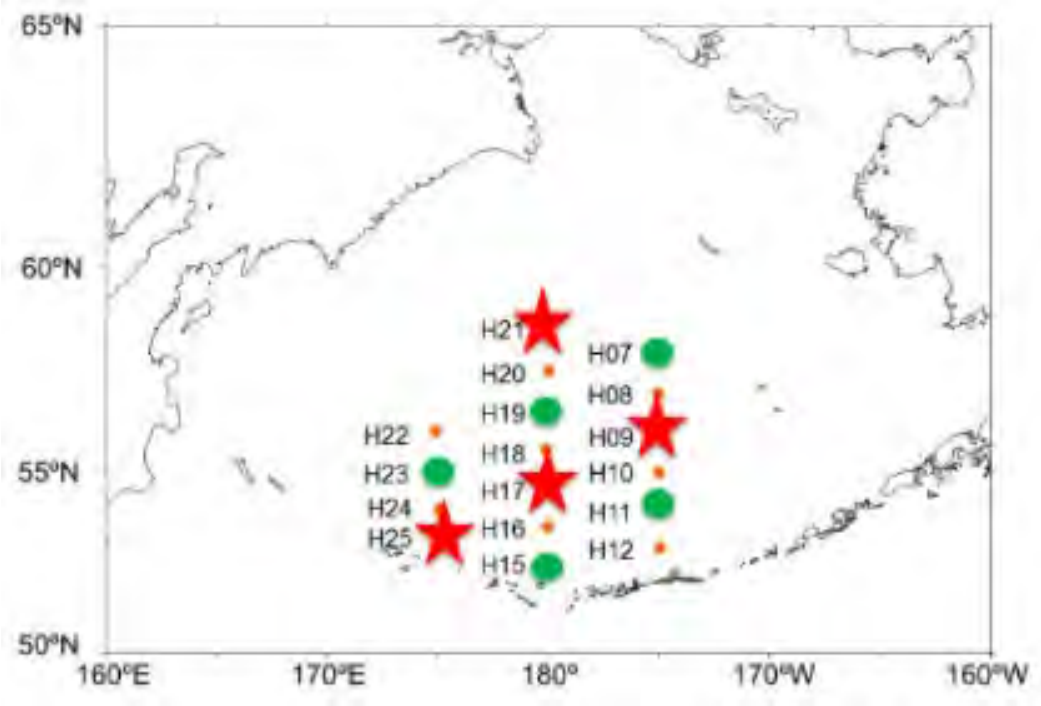
（Minegishi et al. 2019）

1. Species-specific approach

O. keta

Chum salmon migration monitoring

In Bering Sea? → *to be continued*



1. Species-specific approach

Sakhalin taimen (*Parahucho perryi*)

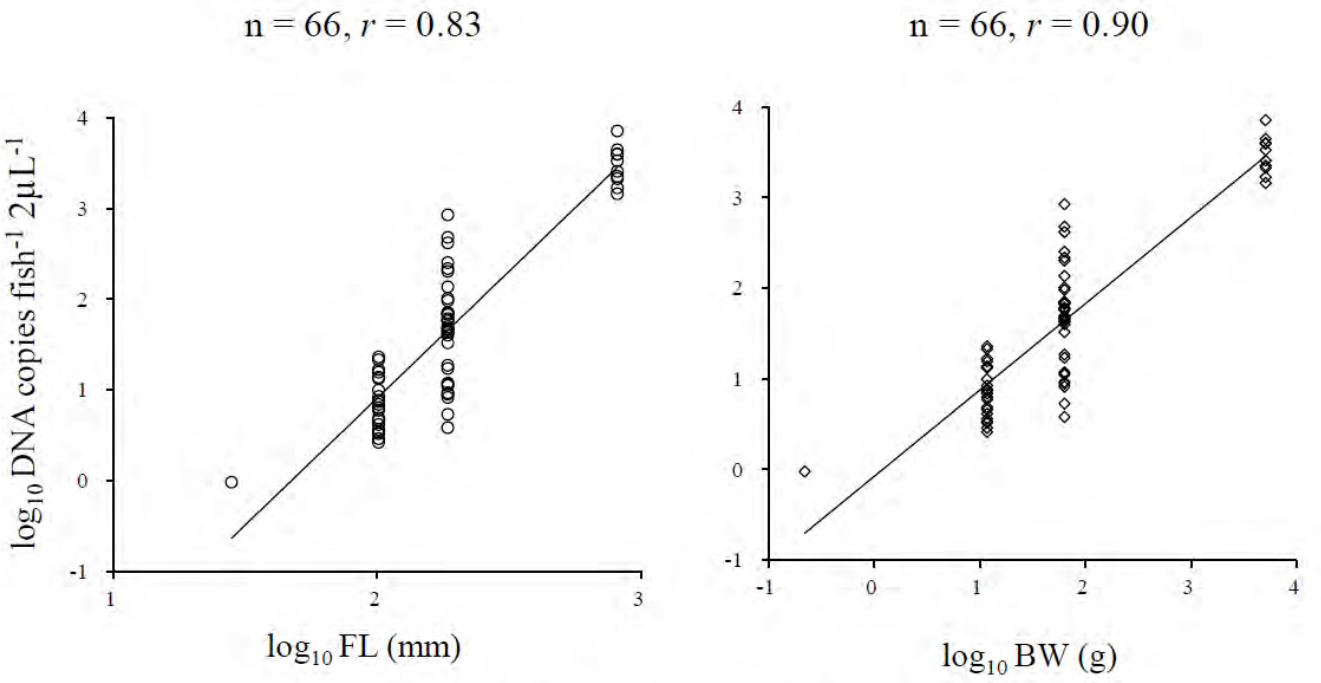
- Largest freshwater fish in Japan (up to 1.5m)
- Anadromous, Long-lived (>20 years)
- Critically endangered (CR) in IUCN Red List

1. Species-specific approach

Sakhalin taimen (*P. perryi*)

Aquarium experiment for eDNA concentration

Q1. Is eDNA conc. correlated with fish size (FL & BW)? *A. YES*



Regression analyses: All p <0.001

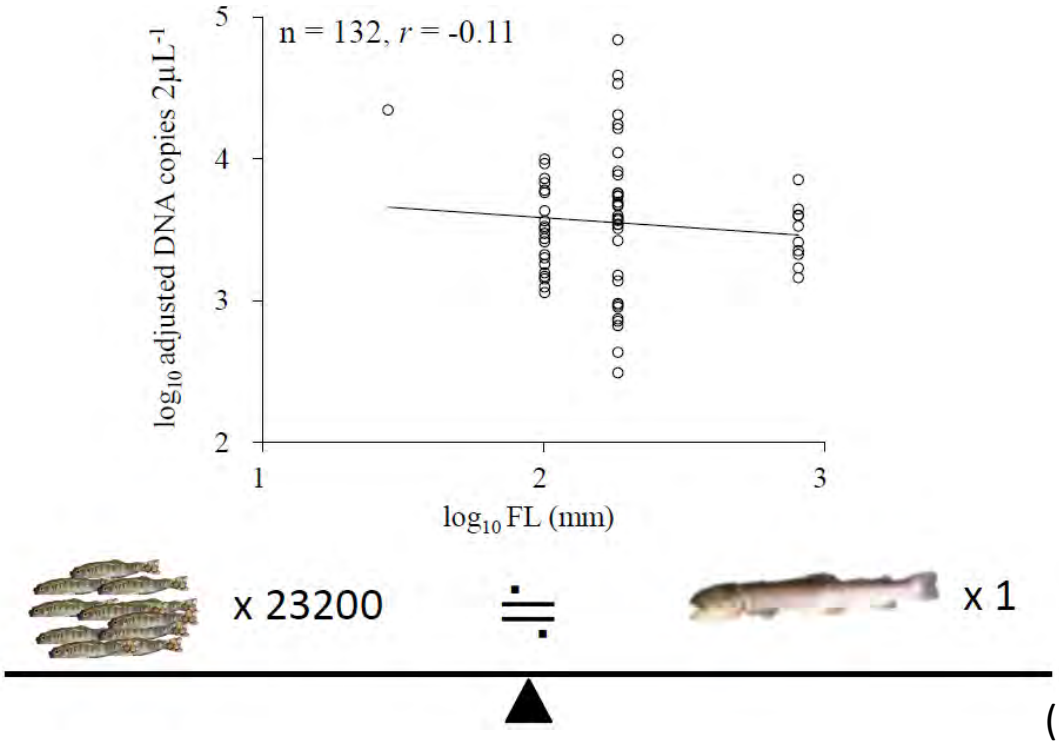
(Mizumoto et al. 2018)

1. Species-specific approach

Sakhalin taimen (*P. perryi*)

Aquarium experiment for eDNA concentration

Q2. Does eDNA conc. reflect biomass of fish nearby? *A. YES*



(Mizumoto et al. 2018)

1. Species-specific approach

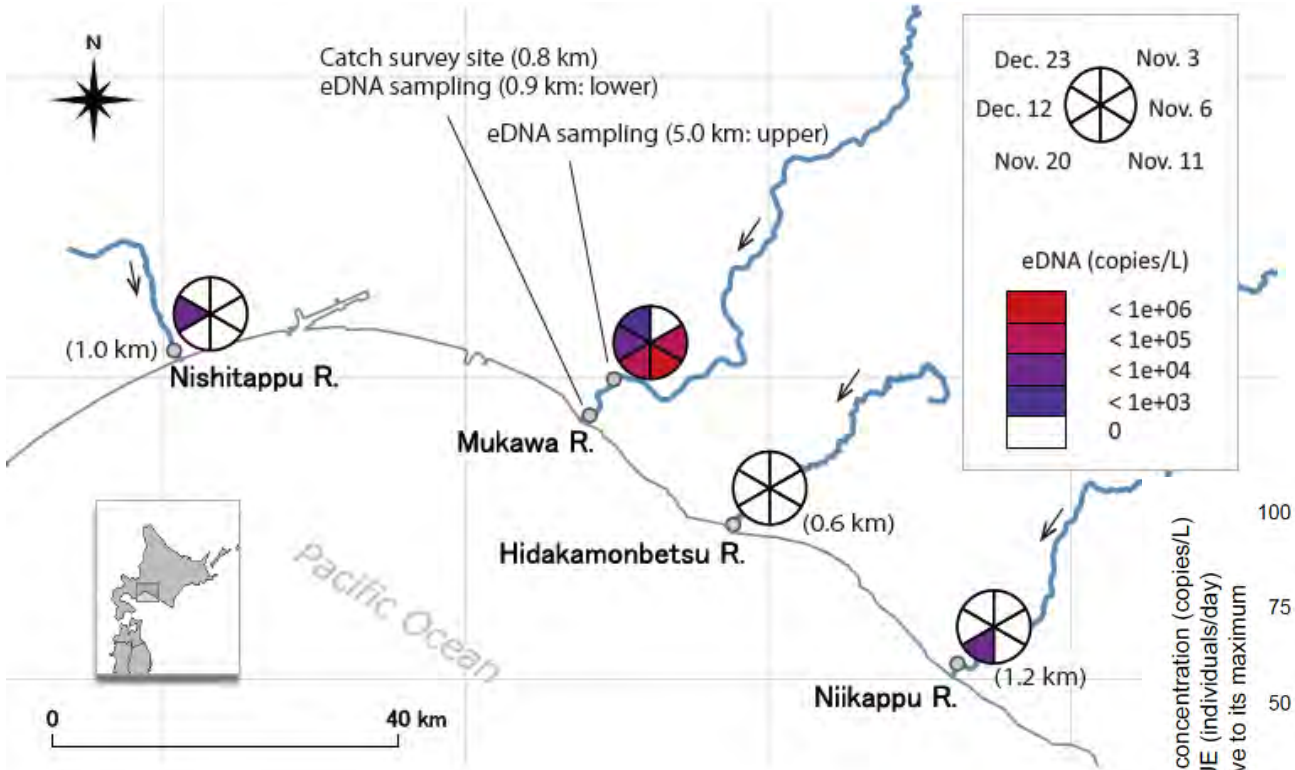
Shishamo smelt (*Spirinchus lanceolatus*)

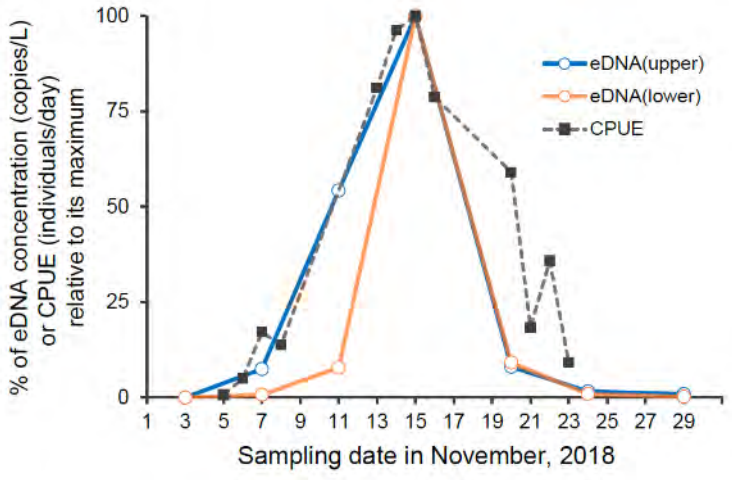
- Endemic to Japan (found only in Hokkaido)
- Anadromous, Short-term freshwater migration
- Local populations threatened

1. Species-specific approach

Shishamo smelt
(*Spirinchus lanceolatus*)

→ Short-term spawning migration captured by eDNA





(Yatsuyanagi et al., *just accepted!*)

eDNA-based monitoring

2. Taxon-wide approach (NGS metabarcoding)

Q1. Who is there?

Q2. Any between-species interaction?

eDNA metabarcoding

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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. Fukunaga[4], T. Sado[1,2], J. Y. Poulsen[1,2,5], K. Sato[6], 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]

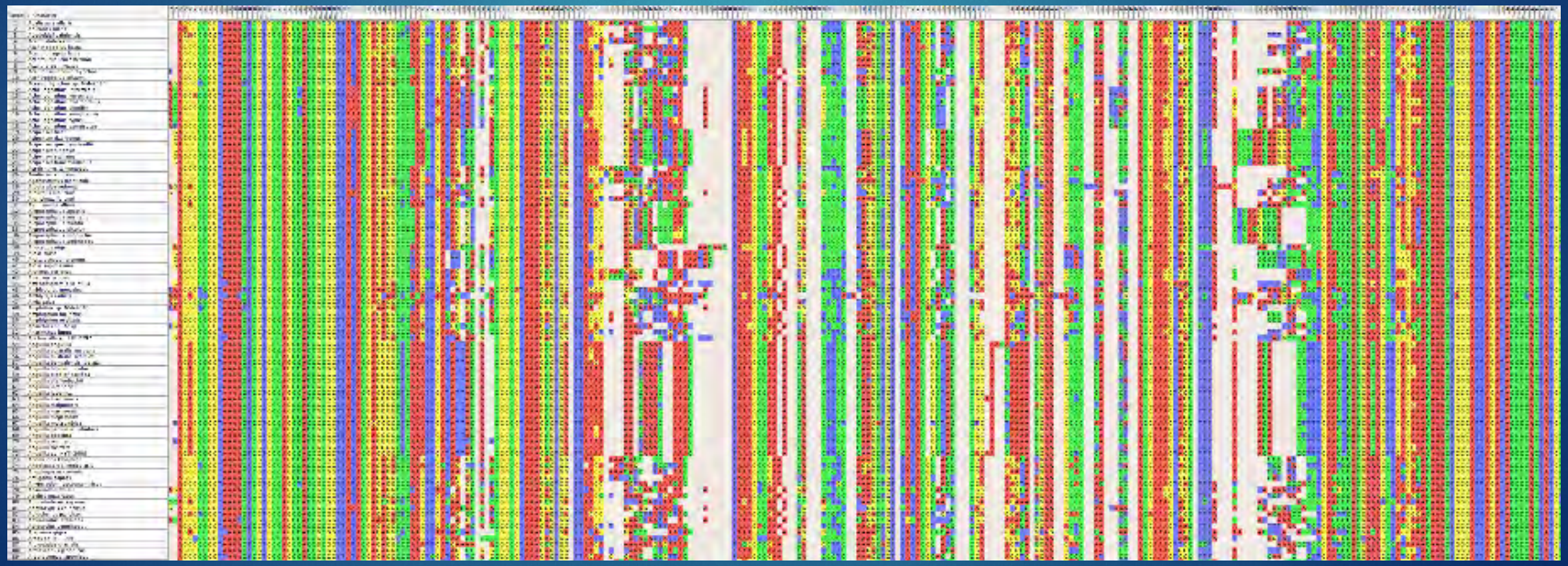
(Miya et al. 2015)

eDNA metabarcoding

Fish universal primers for eDNA metabarcoding (MiFish)

- Originally based upon the MitoFish database
- Super-variable region between highly conserved regions in 12S
- Reference DNA sequences over 7000+ fish species

MiFish F-Primer ← Species specific region → MiFish R-Primer



(Miya et al. 2015)

eDNA metabarcoding

Test at the Churaumi Aquarium in Okinawa

(Miya et al. 2015)

eDNA metabarcoding with MiFish

Aquarium test ・・・ 93.3% of species identified (168/180 sp.)

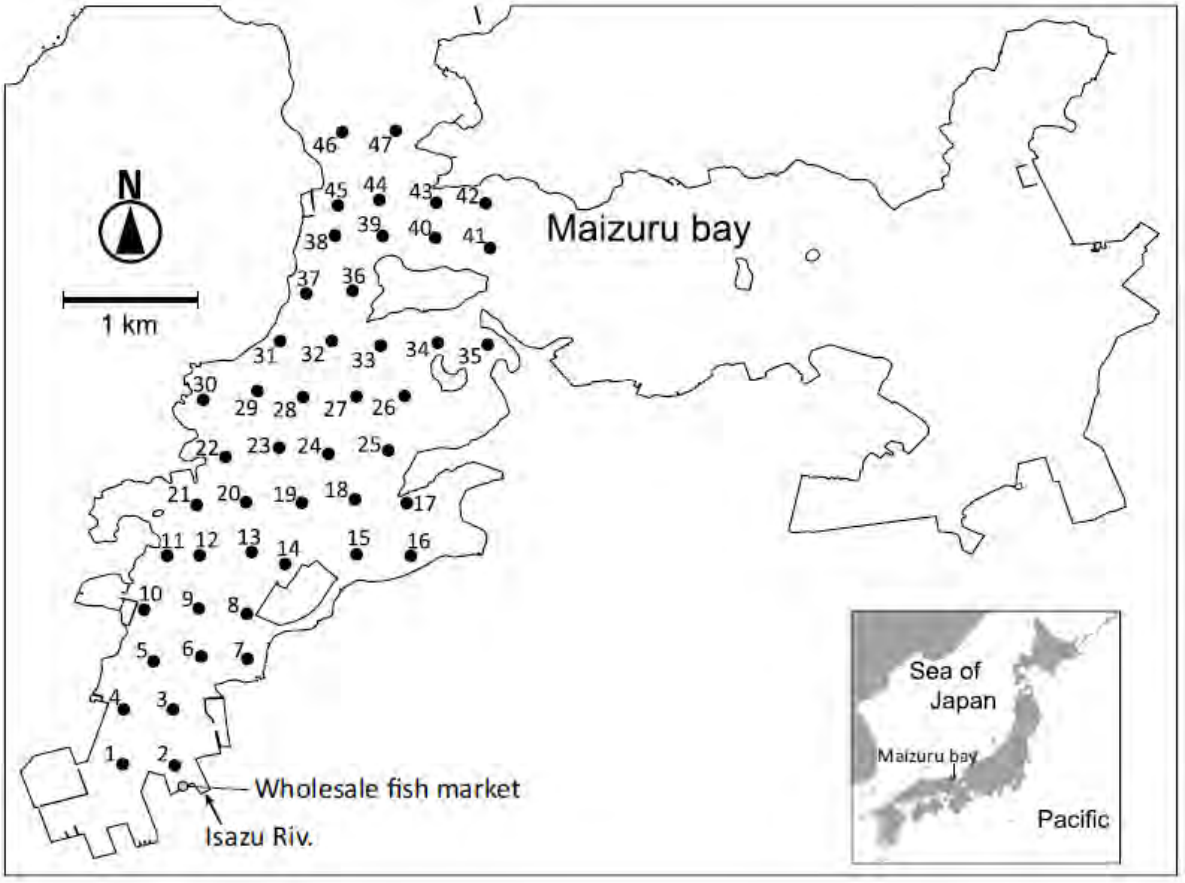
tank	Volume (m^3)	Species No. (with reference)	Total DNA reads	Species detected (%)
Tank-1	7,500	63	2,568,008	61 (96.8%)
Tank-2	700	105	1,301,723	95 (90.5%)
Tank-3	230	15	240,508	13 (100%)
Tank-4	35.6	8	212,643	8 (100%)

(Miya et al. 2015)

eDNA metabarcoding with MiFish

R. Masuda
(Kyoto U.)

eDNA survey in Maizuru Bay, Kyoto



(Yamamoto et al. 2017)

eDNA metabarcoding with MiFish

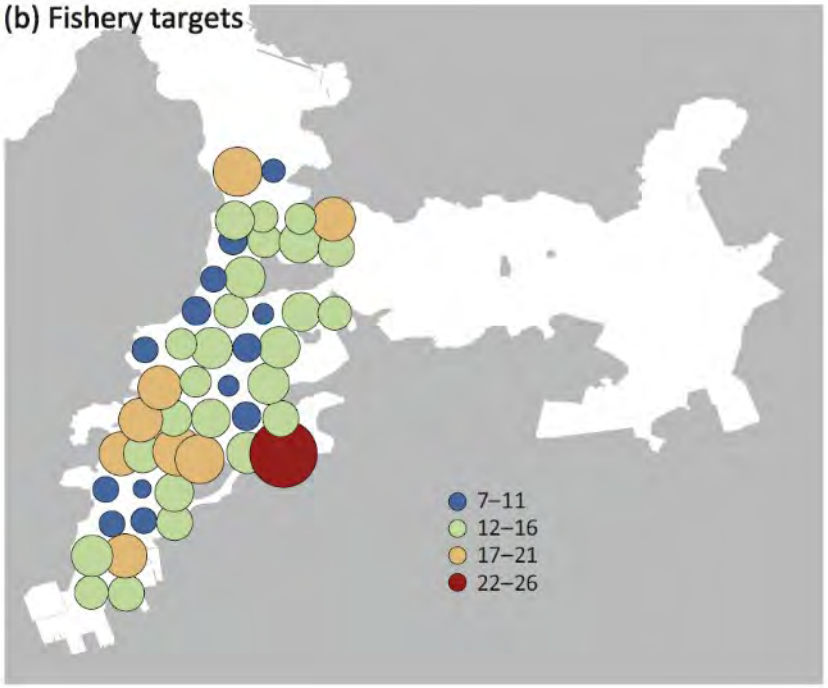
eDNA survey in Maizuru Bay, Kyoto

6hr water collection → 128 species

14yr visual censuses → 80 species

x 1.6

R. Masuda
(Kyoto U.)



(Yamamoto et al. 2017)

2. Taxon-wide approach

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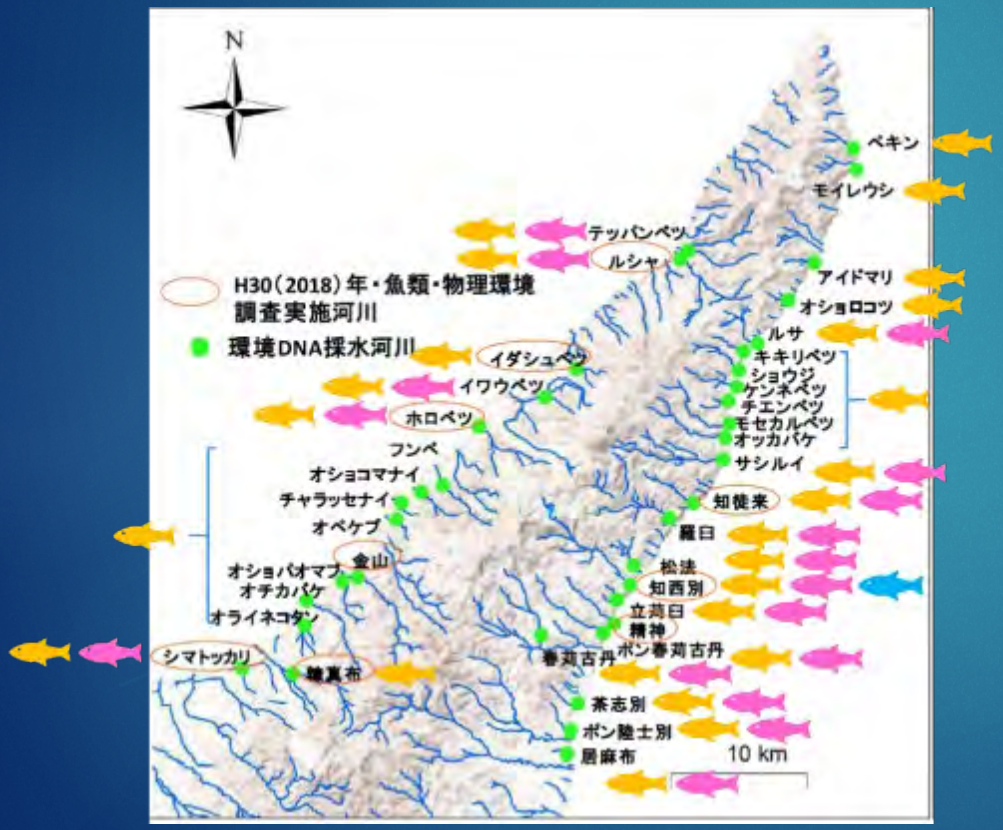
38 Shiretoko rivers
(UNESCO world natural heritage)



Dolly Varden
(*Salvelinus malma*)

Masu Salmon
(*Oncorhynchus masou*)

Rainbow trout
(*Oncorhynchus mykiss*)

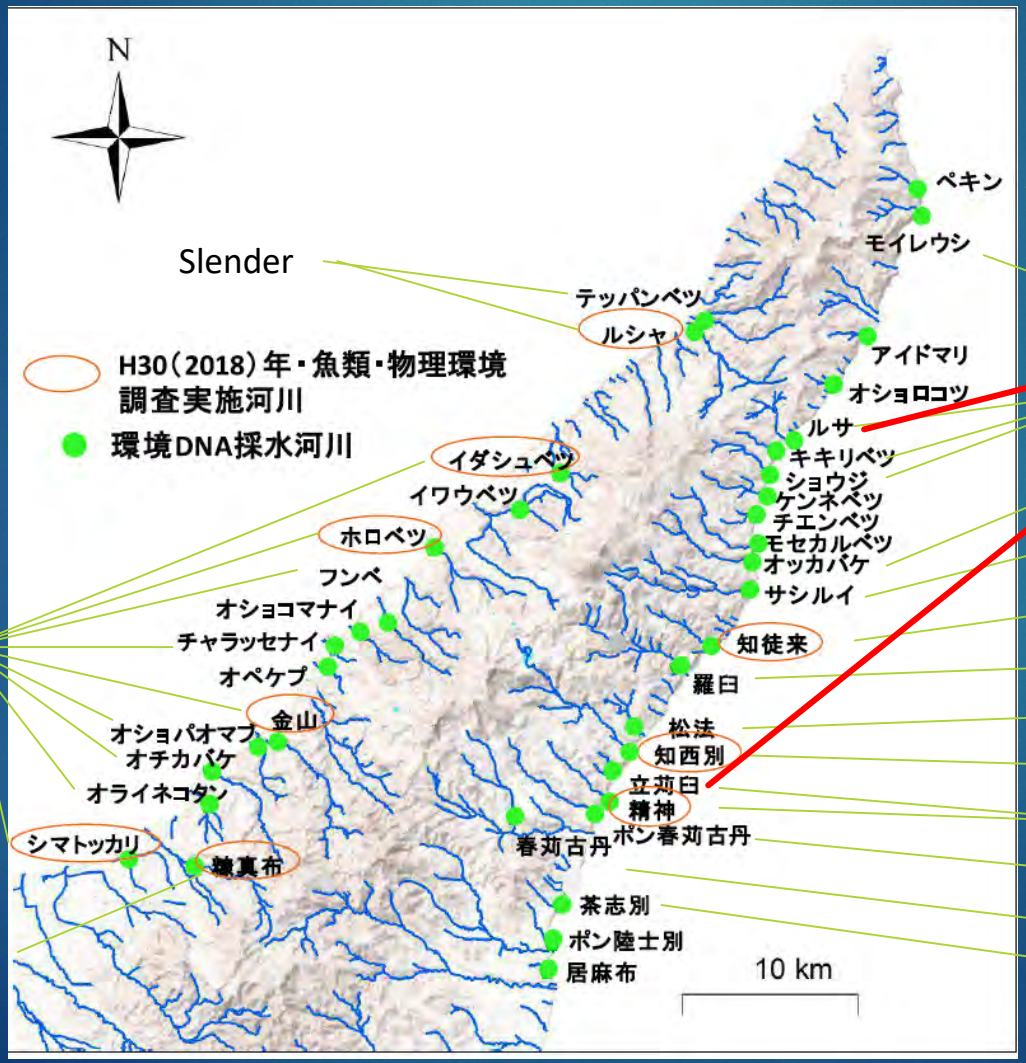
(Araki et al. *in prep*.)

2. Taxon-wide approach

Long-clawed shrew
(*Sorex unguiculatus*)

Slender shrew
(*Sorex gracillimus*)

American mink
(*Neovison vison*)



Slender

Long-clawed

both

Long-claw

both

Slender

Long-clawed

both

Long-clawed

both

Long-clawed

both

Long-clawed

Slender

Long-clawed

Slender

(Araki et al. *in prep.*)

Take-home messages

eDNA technology provides:

- an efficient tool for estimating distribution of target species
- moderately good estimation of biomass (in BW)
- opportunity for taxon-wide estimate of fish fauna

Current & Future directions

Challenges:

- High risk of contamination (in each step)
- Detection threshold (time & space)
- Influencing factors (environment restrictions?)

Goals:

- Whole life history monitoring of migratory species
- Wider range of taxonomic groups
- Local stakeholder monitoring on “their habitat”
- Intra-specific variation with eDNA?

eDNA-based biodiversity monitoring

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- K. Morita and all SWSP members

eDNA-salmon symposium in Hokkaido U. (2014)

Thank you for listening!