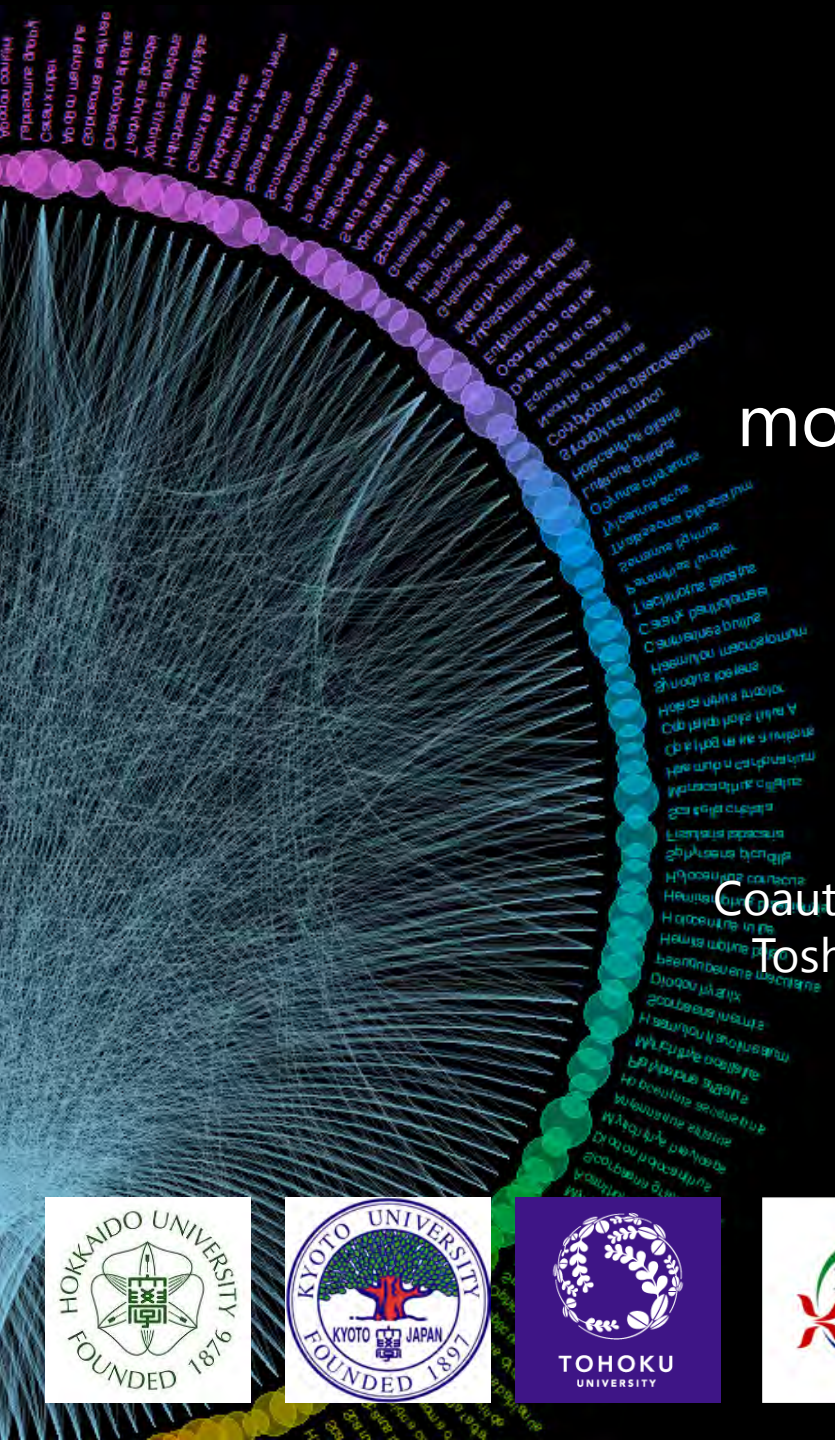

Multi-site, high-frequency monitoring of marine ecosystem using environmental DNA

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千葉県立中央博物館
NATURAL HISTORY MUSEUM AND INSTITUTE, CHIBA

HOKKAIDO UNIVERSITY FOUNDED 1876

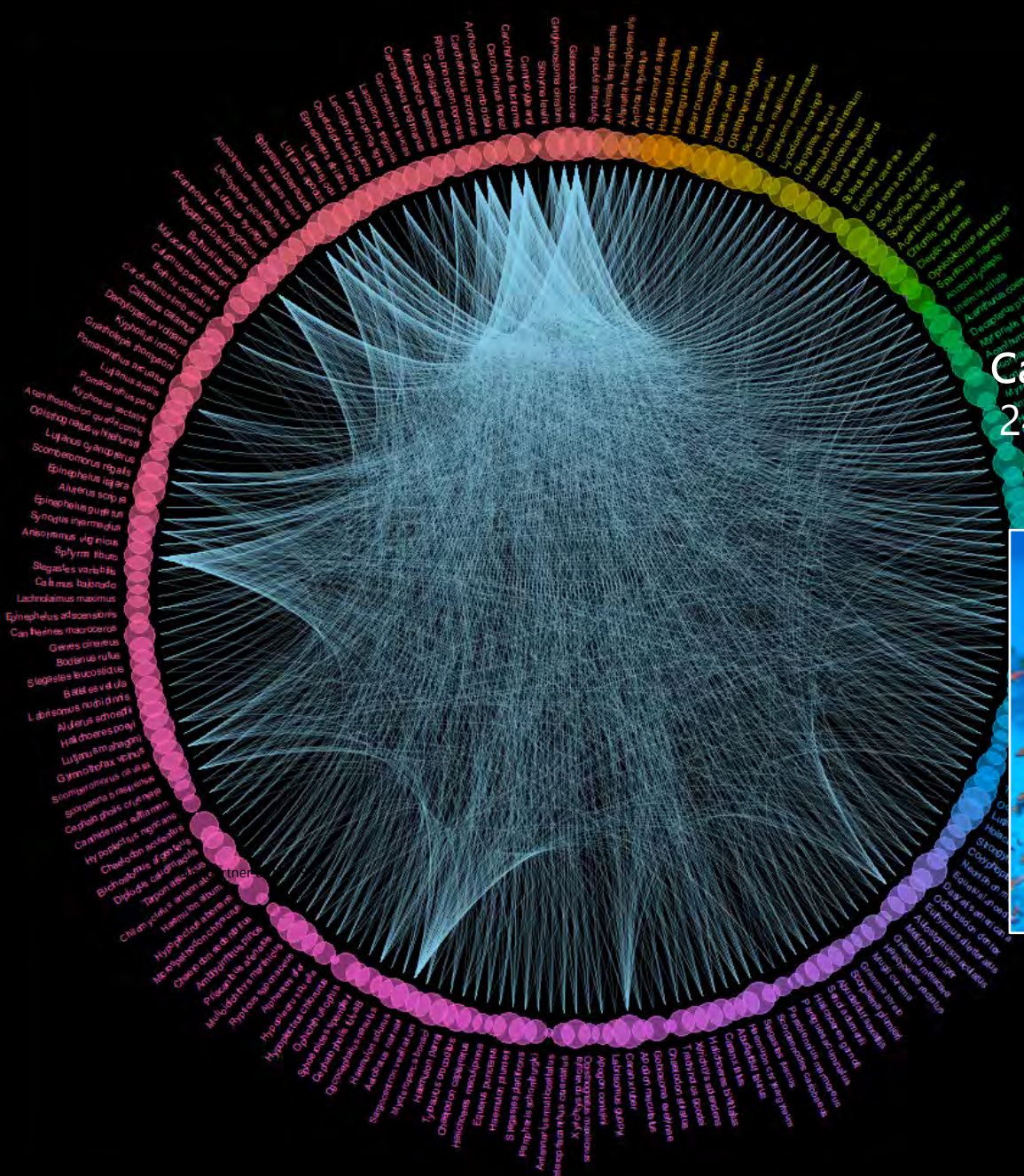
KYOTO UNIVERSITY KYOTO JAPAN FOUNDED 1897

TOHOKU UNIVERSITY

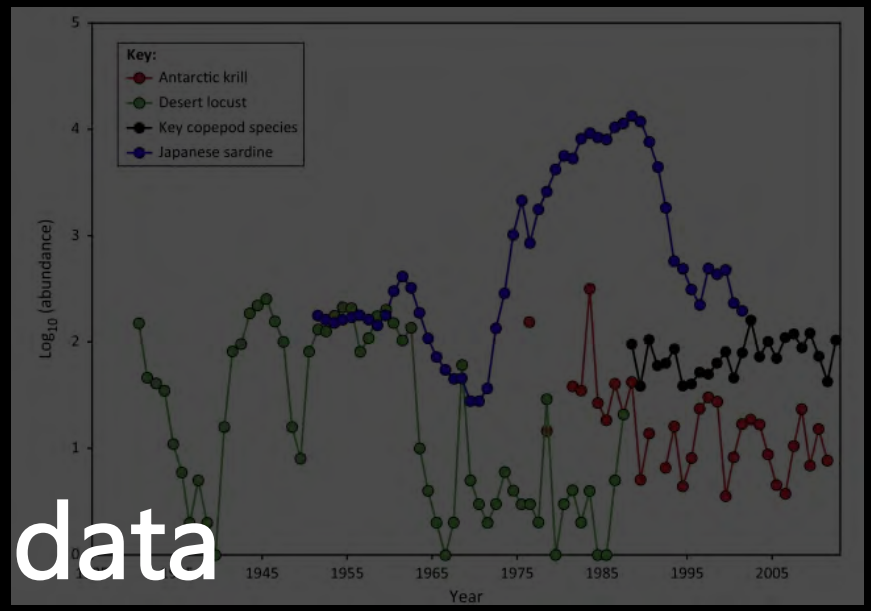
KOBE UNIVERSITY

KYUSHU UNIVERSITY

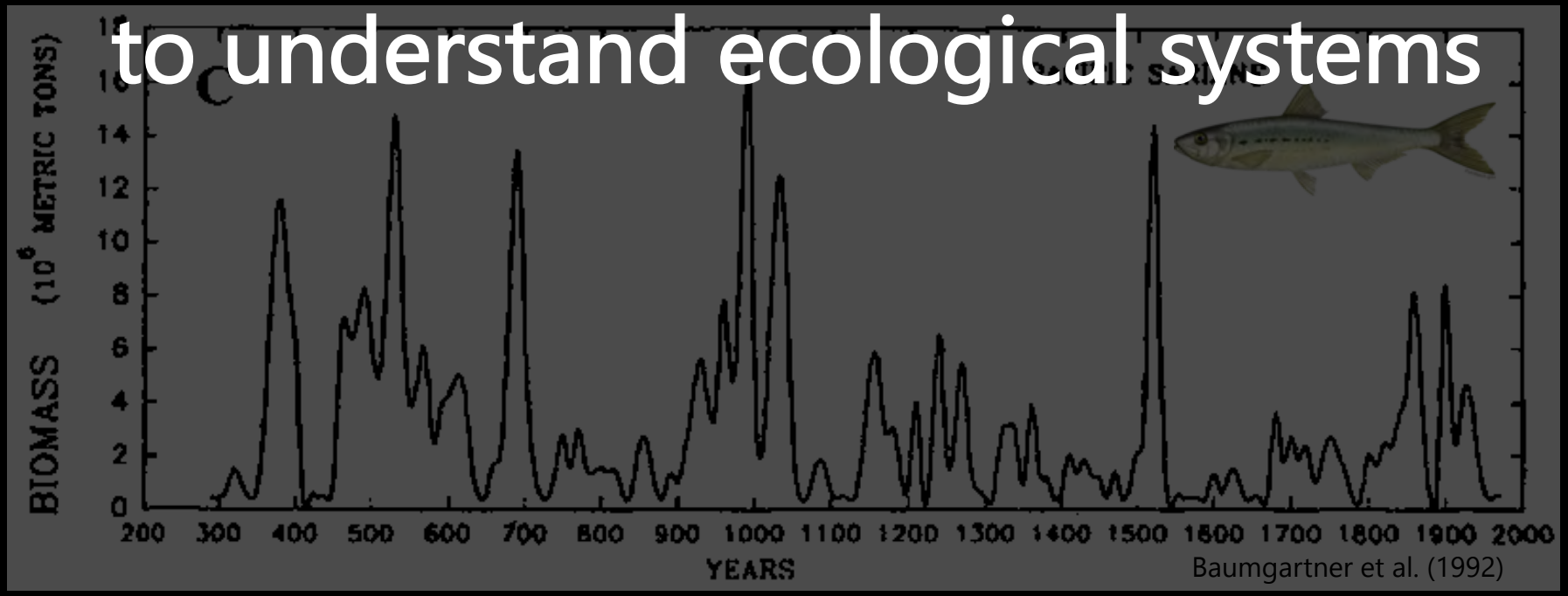
JST CREST



Caribbean Coral Reef food web
249 species, 3,313 interactions
(Opitz 1996)



We need a lot of data to understand ecological systems



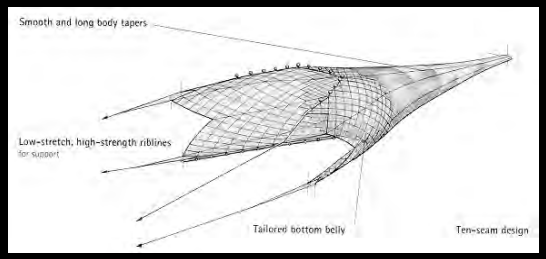
Baumgartner et al. (1992)

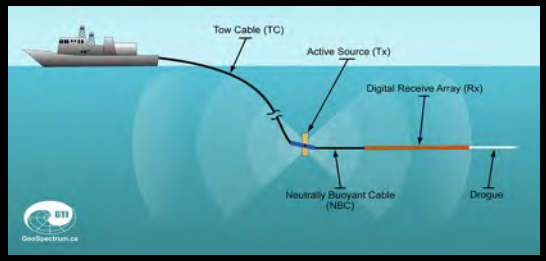
Understanding complex systems requires large amount of data ("Big Data"), which allow determining the parameters that characterize the complex system (high dimensionality, nonlinearity)

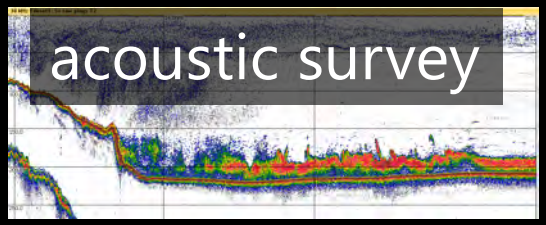
"Big Data" and sophisticated analysis enables better understanding:

- Knowing the present status
- Forecasting the future dynamics
  (Ye et al. 2015, McGowan et al. 2017)
- Understand the mechanism behind the dynamics
  (Deyle et al. 2016, Ushio et al. 2018)

Ecosystem monitoring is the key process to understand the ecological complexity

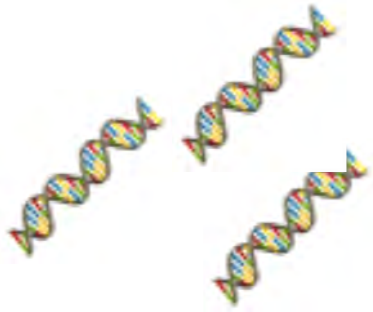






Not easy to get “Big Data”, as contemporary methods are costly and destructive and/or less effective to identify species or cover a large area

living organisms
(fish, mammals, birds…)

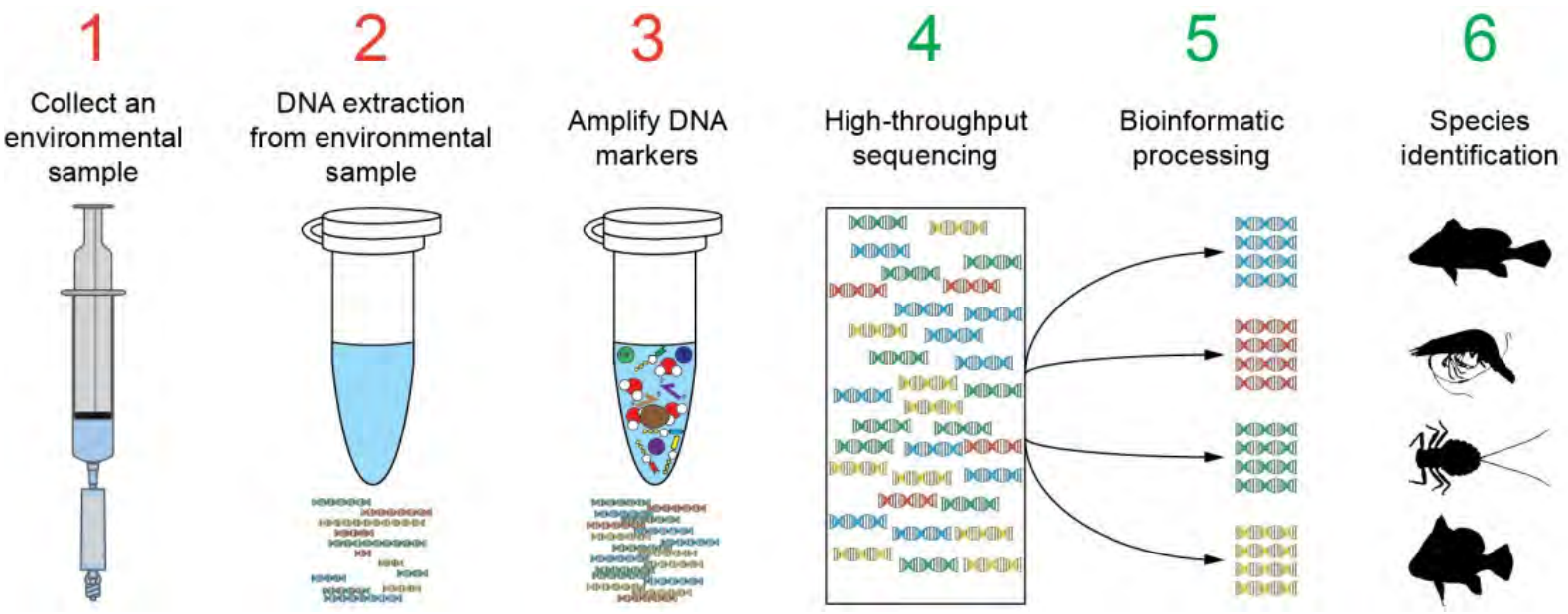


DNA fragments
(feaces, urine, gametes, mucus…)

degradation
(bacteria, UV radiation…)

- Environmental DNA (eDNA) is DNA that can be extracted from environmental samples, such as water, soil and air.
- eDNA originates from various sources such as feaces, urine, muscus, gametes, etc.
- eDNA in the water are decomposed within a week and can be used to detect organisms or determine the species composition
- eDNA allows non-invasive, species-resolved monitoring of biodiversity.

eDNA metabarcoding – how to know the fish using water samples



Credit: Nature Metrics

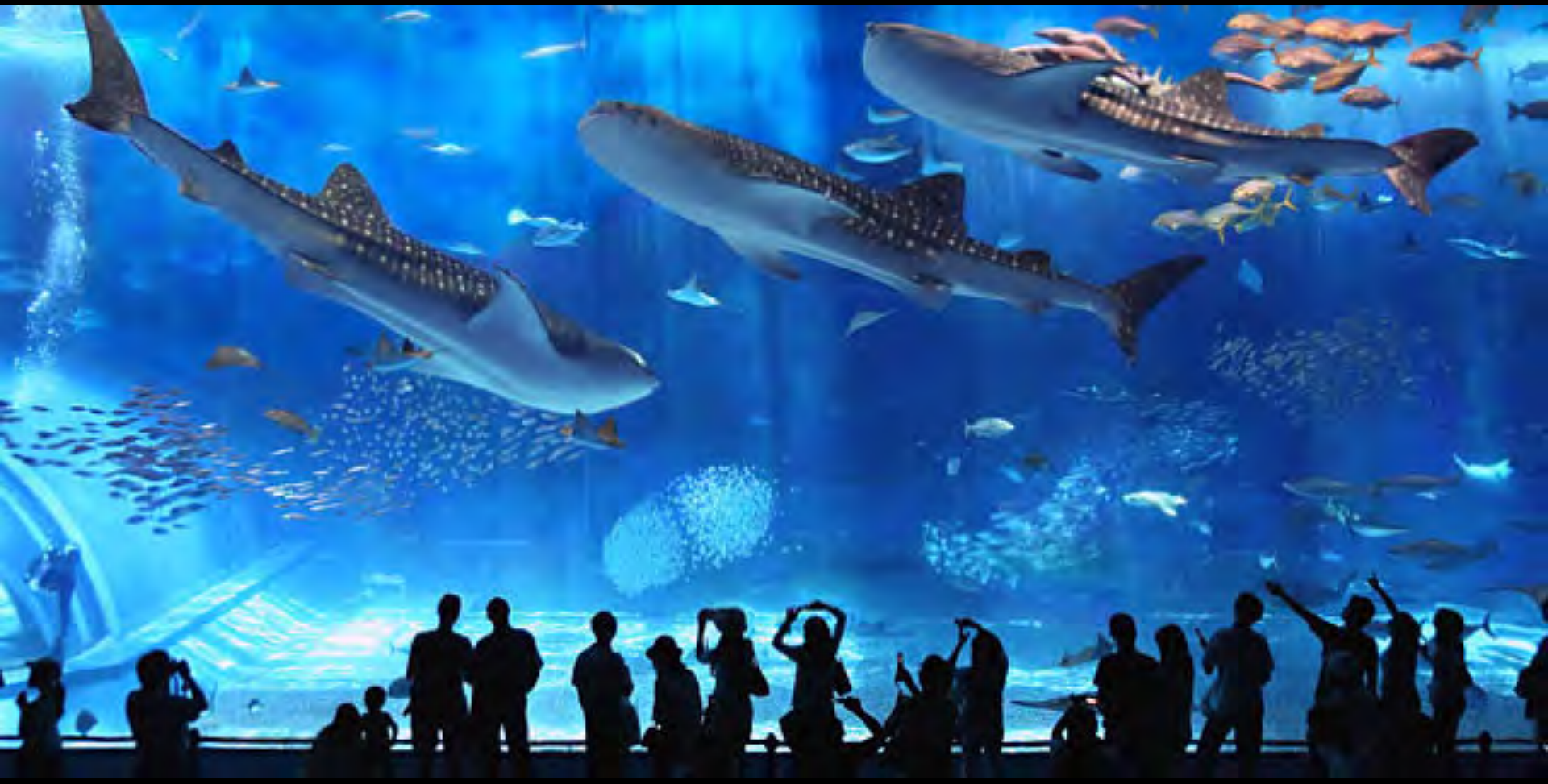
blood test for ecosystem

MiFish Pipeline

Analyzing Fish eDNA amplified by MiFish primers.

Get Started Now →

MiFish is a set of universal PCR primers for metabarcoding environmental DNA (eDNA) that are shed into waters from fishes. MiFish primers target a hypervariable region of fish mitochondrial 12S rRNA gene (approximately 160-190 bp), which contains information to identify fishes to taxonomic family, genus and species except for some closely related congeners. After amplification by MiFish primers, MiFish pipeline accepts your sequence data in FASTQ (paired-end or single file) or FASTA format and returns a



- Okinawa Churaumi Aquarium (one of the world's largest)
- 249 fish species in the four large tanks (36~7500 m^3)
- 10L of water sampled from each tank and analysed by eDNA metabarcoding

number of reads[a]	total	Kuroshio	tropical fish	deep-sea	mangrove
more than or equal to 97% identity with reference sequences (number of libraries)	4 322 882 (14)	2 568 008 (5)	1 299 788 (4)	259 191 (3)	212 643 (2)
tank fish	4 053 184 (93.4%)	2 375 892 (92.5%)	1 237 546 (95.2%)	245 201 (94.6%)	194 545 (91.5%)
non-tank fish	286 446 (6.6%)	192 116 (7.5%)	62 242 (4.8%)	13 990 (5.4%)	18 098 (8.5%)
number of tank species	249	75	159	15	8
number of tank species with reference sequences	180	63	105	13	8
number of tank species detected in MiSeq analysis	168 (93.3%)	61 (96.8%)	95 (90.5%)	13 (100%)	8 (100%)
water volumes of tank (m^3)	8465	7500	700	230	35.6

- 93.3% of the fish species (168 species of 59 family, 123 genus) detected only from a “bucket of water”

Miya et al. (2015)

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SCIENTIFIC REPORTS

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Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea

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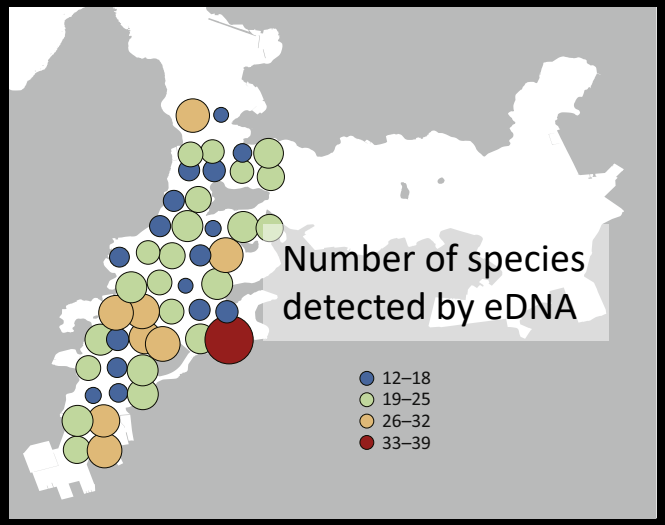
Accepted: 06 December 2016
Published: 12 January 2017

Satoshi Yamamoto[1], Reiji Masuda[2], Yukuto Sato[3], Tetsuya Sado[4], Hitoshi Araki[5], Michio Kondoh[6], Toshifumi Minamoto[1] & Masaki Miya[4]

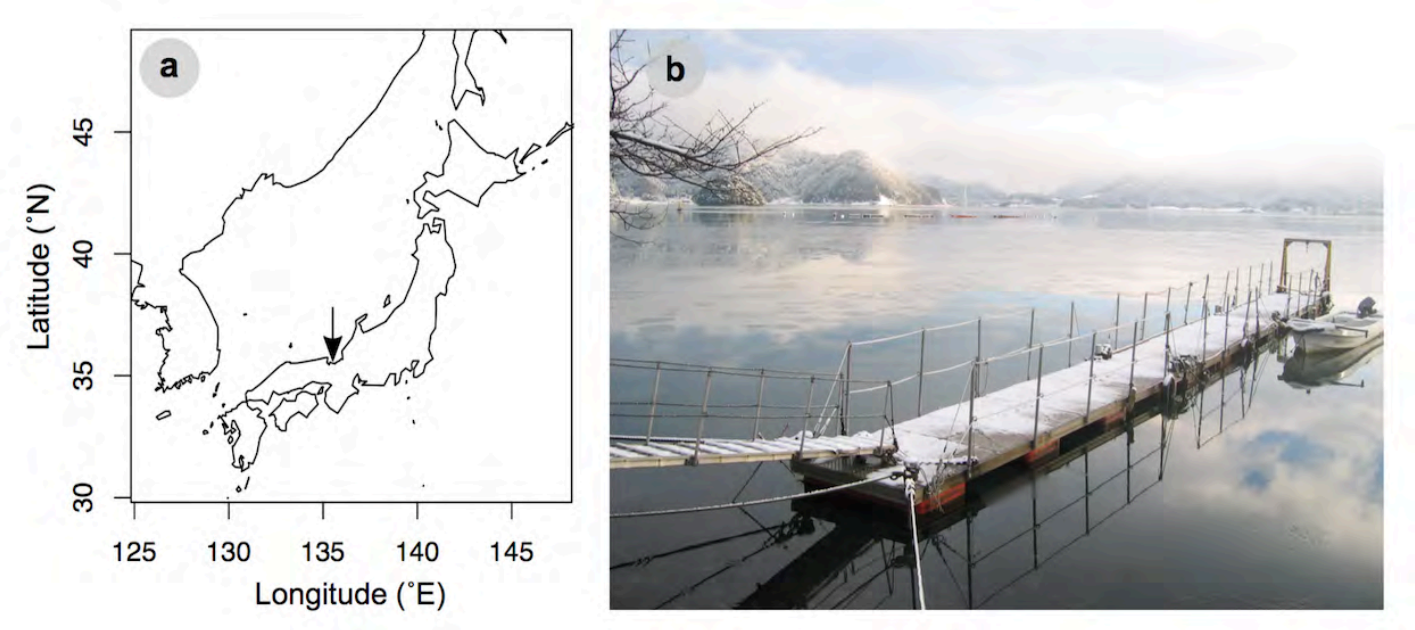
Environmental DNA (eDNA) metabarcoding has emerged as a potentially powerful tool to assess aquatic community structures. However, the method has hitherto lacked field tests that evaluate its effectiveness and practical properties as a biodiversity monitoring tool. Here, we evaluated the ability of eDNA metabarcoding to reveal fish community structures in species-rich coastal waters. High-performance fish-universal primers and systematic spatial water sampling at 47 stations covering ~11 km^2 revealed the fish community structure at a species resolution. The eDNA metabarcoding based on a 6-h collection of water samples detected 128 fish species, of which 62.5% (40 species) were also observed by underwater visual censuses conducted over a 14-year period. This method also detected other local fishes (≥23 species) that were not observed by the visual censuses. These eDNA metabarcoding features will enhance marine ecosystem-related research, and the method will



- 2L water sampling from 47 stations covering 10km^2 of Maizuru-Nishi Bay
- Detected from the 6H survey were 128 local fish species, which include >60% of the 80 species observed in the past 140 diving surveys

How to maximize the potential merit

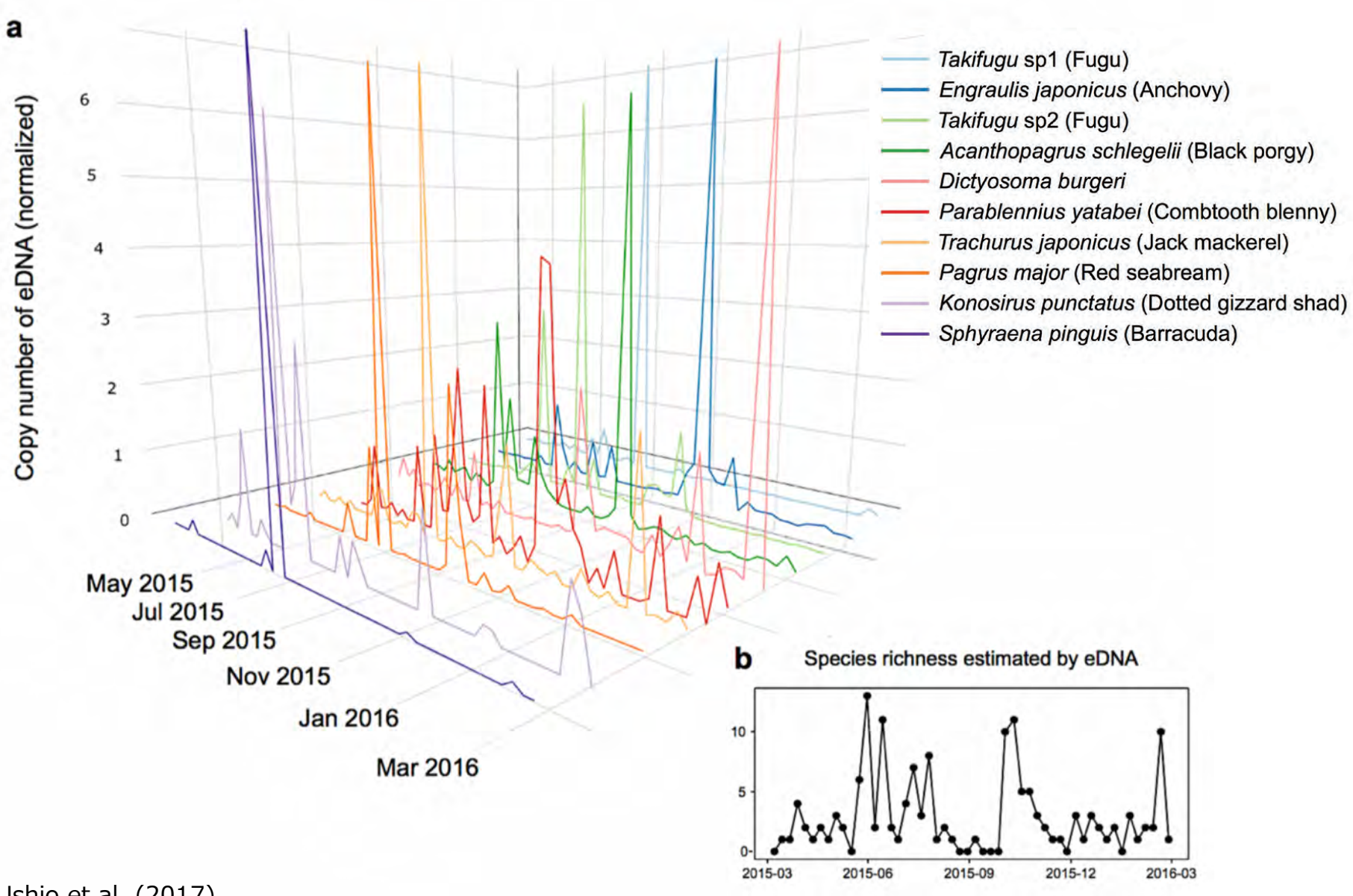
1. High-frequency eDNA Monitoring



Weekly monitoring with eDNA metabarcoding for >3 years

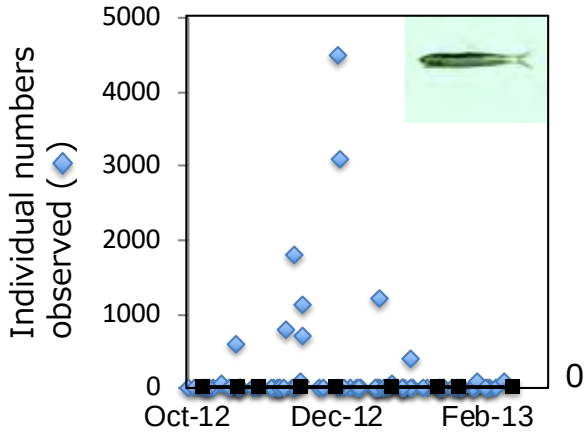
The amount of eDNA quantified for all detected fish species

Time-series of eDNA of 10 dominant species in Maizuru Bay

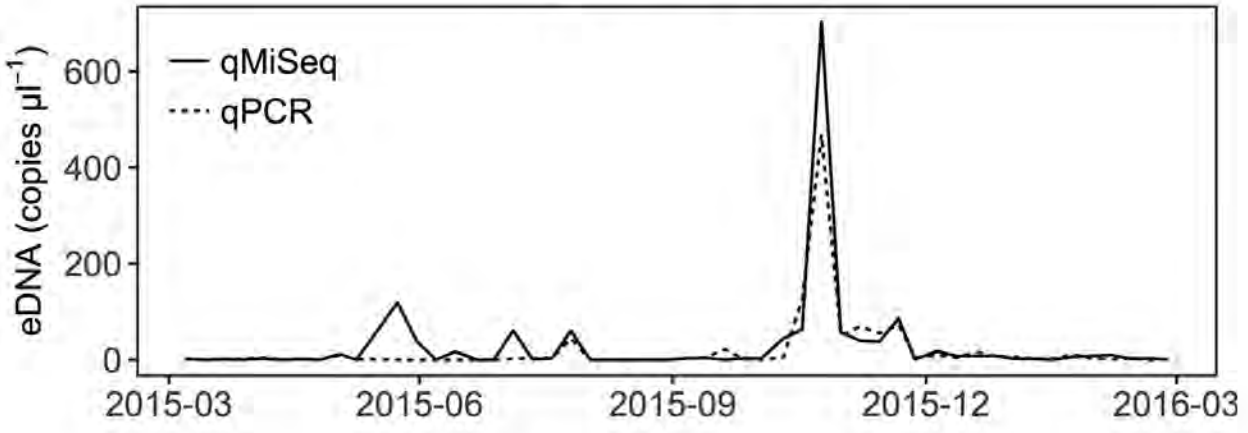


Ushio et al. (2017)

visual observation



eDNA monitoring



How to maximize the potential merit

2. Multi-site eDNA Monitoring

eDNA Monitoring of 567 coastal sites in 3 months

Date Started：5 June, 2017

Date Completed：30 August, 2017

Monitoring Sites：**567 sites**

Northernmost: Soya Misaki (lat. 45.52°N)

Southernmost: Minami-Io Island (lat. 24.22°N)

Westernmost: Nosappu Misaki (long. 145.82°E)

Easternmost: Yonaguni Island (long. 122.68°E)

Number of people Joined：**114 (accumulated)**

The top 10 species most frequently detected from the 567 sites


285 sites

Largescale Blackfish
メジナ
Girella punctata


284 sites

Japanese black porgy
クロダイ
Acanthopagrus schlegelii


279 sites

Japanese anchovy
カタクチイワシ
Engraulis japonicus


279 sites

Japanese rock fish
メバル属
Sebastes spp.


276 sites

Grass puffer
クサフグ
Takifugu niphobles


232 sites

Spotbelly Greenling
クジメ
Hexagrammos agrammus


229 sites

Snake blenny
ヘビギンポ
Enneapterygius etheostomus


191 sites

Surfperch
ウミタナゴ
Ditrema temmincki temmincki


184 sites

Motleystripe rainbowfish
ホンベラ
Halichoeres tenuispinis


179 sites

Blenny
イソギンポ
Parablennius yatabei

- 1,218 fish species of 136 family detected
- This is 43.5% of all Japanese “coastal” species (2,800 species)

- eDNA provide a non-destructive tool for species-resolved biodiversity monitoring of fish, enabling high-frequent or multi-site biodiversity monitoring
- eDNA monitoring provides “Big Data”, which may provide more information than the contemporary monitoring methods
- We believe that eDNA monitoring will open a new era of data-driven marine ecology
- How the eDNA data should be analyzed is an open question, given some weakness of eDNA monitoring (contamination, dead/alive not distinguishable, unclear whether eDNA amount reflects fish amount, spatial/temporal scale of monitoring unclear)
- eDNA is a “young” method and more study is required to confirm its utility

The eDNA Society

- ✓ The eDNA Society was founded on 27th April 2018 to promote the eDNA science and its social implementation with an aim to realize the sustainable use of ecosystem service.
- ✓ The 1st Annual Meeting was held in Tokyo on 29th-30th September 2018 with 309 participants.