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

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Caribbean Coral Reef food web
249 species, 3,313 interactions
(Opitz 1996)
We need a lot of data to understand ecological systems.
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.
Methods

Conduct surface snorkel surveys parallel to shore along a 75 m transect at high tide. Have two snorkelers in the water and a shore-based observer. The water depth and distance from shore may vary with the site—target 3 m and 10 m from shore for deep sites, 1.5 m water depth for shallower sites. These are good ranges for juvenile Chinook salmon. Smaller juvenile chum and pink salmon may be in shallower water.

Record observations of fish species, number (approximate if over 20), length range (2.5 cm increments), water column position (surface, mid-water, bottom), and feeding behavior.

Swim slowly and consistently, scanning the water column with a focus near the water's surface where juvenile salmon are likely to be (tilt your head sideways for this). Pause to record data as appropriate.

Data can be written on either an underwater writing tablet or clipboard with datasheet printed on waterproof paper. Use the transect tape to measure the transect length, water depth, and underwater visibility (horizontal distance that you can see the writing tablet underwater—needs to be at least 2.5 m).

May is a good month to target the peak outmigration of juvenile chum and pink salmon, June and July are good peak months for Chinook.

Data to record in the field: Date, time, site name, transect length, water depth, distance from shore, underwater visibility, fish data. An underwater digital camera can help document fish presence.

Processing

Enter the field data into computer spreadsheets. Fish counts are standardized by numbers/m² as: fish number/(transect length x underwater visibility).

Materials

- Snorkel gear—drysuit or wetsuit, mask, snorkel, fins, ankle weights
- 50 m or longer transect tape
- Underwater writing tablet, or clipboard with datasheet printed on waterproof paper

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.
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

1. Collect an environmental sample
2. DNA extraction from environmental sample
3. Amplify DNA markers
4. High-throughput sequencing
5. Bioinformatic processing
6. Species identification

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 m³)
- 10L of water sampled from each tank and analysed by eDNA metabarcoding
93.3% of the fish species (168 species of 59 family, 123 genus) detected only from a “bucket of water”
Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea

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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 km² revealed the fish community structure at a species resolution. The eDNA metabarcoding based on a 6-l 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 potentially become a standard tool for surveying fish communities.

- 2L water sampling from 47 stations covering 10km² 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

Figure 1. Location of the research site (a). The arrow indicates our research site. A floating pier in the Maizuru Fishery Research Station of Kyoto University, Maizuru, Kyoto, Japan, where the weekly water sampling was performed (b). Photo taken in winter season by R. Masuda.
Time-series of eDNA of 10 dominant species in Maizuru Bay

- **Takifugu** sp1 (Fugu)
- **Engraulis japonicus** (Anchovy)
- **Takifugu** sp2 (Fugu)
- **Acanthopagrus schlegelii** (Black porgy)
- **Dictyosoma burgeri**
- **Parablennius yatabei** (Combtooth blenny)
- **Trachurus japonicus** (Jack mackerel)
- **Pagrus major** (Red seabream)
- **Konosirus punctatus** (Dotted gizzard shad)
- **Sphyraena pinguis** (Barracuda)

Ushio et al. (2017)
Figure 5. Dynamics of the total fish eDNA (a), Japanese anchovy (*Engraulis japonicus*; b) and Japanese jack mackerel (*Trachurus japonicus*; c) quantified by qMiSeq and qPCR. Solid and dashed lines indicate the number of eDNA copies quantified by qMiSeq and qPCR, respectively. Note that the copy numbers of total fish eDNA were normalised to have zero mean and unit variance.

Quantification of eDNA from only one fish species in a single experiment, this method is much more efficient compared with qPCR. In addition, this method can take effects of PCR inhibition into account. Although it should be mentioned that fish eDNA copy numbers are still only a rough index of fish biomass/abundance (or population size) and this problem should be addressed in a future study (e.g. estimating taxon-specific correction factors is a promising direction; see Krehenwinkel et al. 2017), these results show that eDNA metabarcoding with the inclusion of internal standard DNAs can be a promising tool to monitor fish biodiversity. This method will improve the efficiency of obtaining data and may contribute to more effective resource management and ecosystem 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

- Largescale Blackfish  
  *Girella punctata*  
  285 sites

- Japanese black porgy  
  *Acanthopagrus schlegelii*  
  284 sites

- Japanese anchovy  
  *Engraulis japonicus*  
  279 sites

- Japanese rock fish  
  *Sebastes spp.*  
  279 sites

- Grass puffer  
  *Takifugu niphobles*  
  276 sites

- Spotbelly Greenling  
  *Hexagrammos agrammus*  
  232 sites

- Snake blenny  
  *Enneapterygius etheostomus*  
  229 sites

- Surfperch  
  *Ditrema temmincki temmincki*  
  191 sites

- Motleystripe rainbowfish  
  *Halichoeres tenuispinis*  
  184 sites

- Blenny  
  *Parablennius yatabei*  
  179 sites

- 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.