Longitudinal and Seasonal Profiles of Concentration of Environmental DNA for Stream Invertebrates in freshwater

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Background

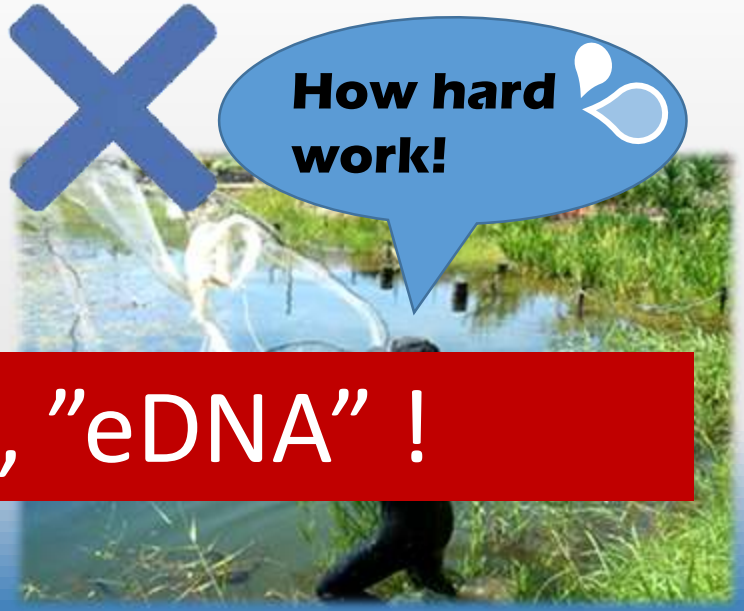
- Needs of biological information for conservation
  - -Are there **Endangered** species ?
  - -Where **Invasive** species inhabit ?
  - -**How many** rare species ?
  - -How are **faunal compositions** balanced ?

etc…

→ Constant & broader are Monitoring is needed

▲**Common problems of biological survey**

- ✓ Effort and time-consuming
- ✓ Hardly avoidable to hurt animals
- ✓ Highly risk of inter-observer errors



Using new method, "eDNA" !

http://www.odakyu.jp/csr/topics/2010/0223.html

What is “eDNA”?

- DNA extracted from animal body to environment
  water ----pond, sea, lake, river, etc.
- Originates from metabolite of animals
  (e.g. skin cells, saliva, urine, feces)

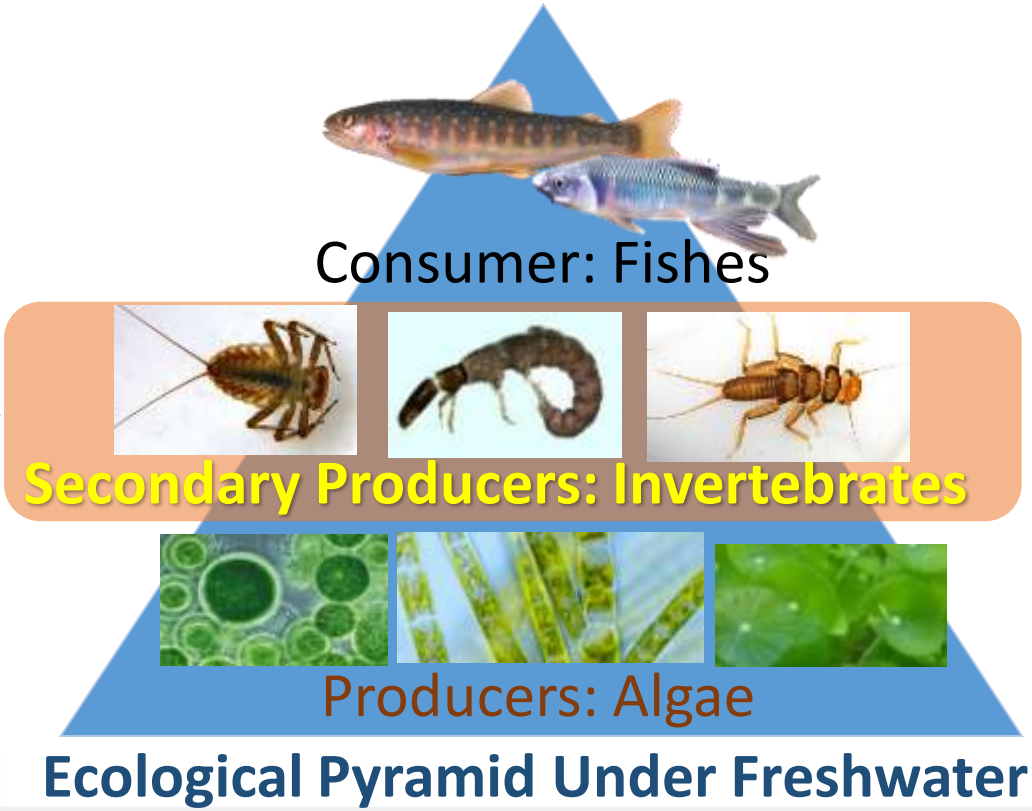
●Expected results of eDNA

- Detect endangered species (Fukumoto *et al.*,2014,Goldberg *et al.*,2013,etc.)
- invasive species (James *et al.*,2015, Dejean *et al.*,2012,etc.)
- Estimate biomass (Minamoto,2012)
- species composition (Miya *et al.*,2015; Thomsen *et al.*,2012)

Background_2
Previous Studies about environmental DNA

● **Target species of eDNA study**

- ✓ Invertebrate species are just 16% of total literature
- ✓ Very few study focused on **Aquatic Insects**



Ecological Pyramid Under Freshwater

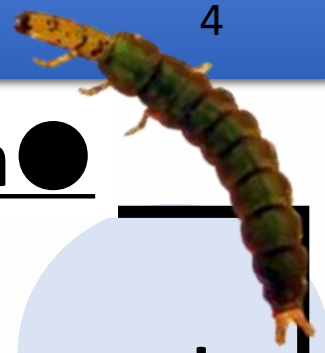
● **They are..**

- ✓ Core fauna for maintaining Freshwater ecosystem
- ✓ used as **a indicator of environmental change**

(e.g. climate changes, modifications by artificial structures, deterioration of water quality, etc.)

● Monitoring their biomass and species composition is fruitful to assess the condition of river ecosystem

Objectives & Methodology

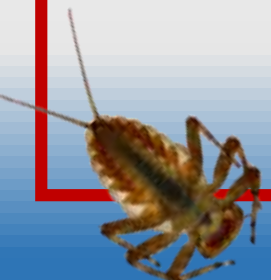


●Objectives●

1. Applying eDNA method to aquatic invertebrates

2. Revealing spacial & Seasonal profiles

3. Knowing characteristics Of the eDNA



●Approach●

Step 1.
Develop a experimental protocol

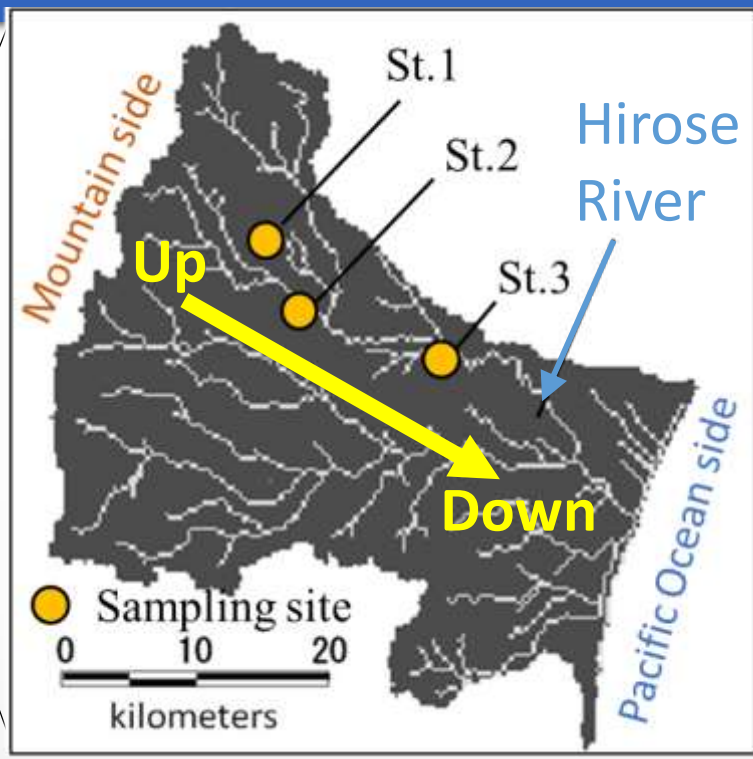
Step 2:
Conduct longitudinal and seasonal field survey

Step 3:
Comparing eDNA and indicators

Field Survey

① Sampling sites & term

- *Natori* River Basin, Northeast Japan
  - Catchment Area: 315.9 km^2
  - The length of river: 45.2 km



- **3 sampling sites**
  - Upper, Middle, Lower
- August to December 2015, per a month
- Sampling 2L of water per a site

- Capture aquatic insects using 30x30cm quadrat server net
- Environmental survey (e.g. water temperature, pH, turbidity, V, TP)

DNA analysis

② Filtration

・1L / a filter (GF /F, φ0.7μm）

③ DNA extraction

・extract DNA from the filter :DNA extraction Kit (QIAGEN)

・Quantify the concentration using Absorption meter

・All Mixed- DNA solution → **Total DNA (ng/L)**

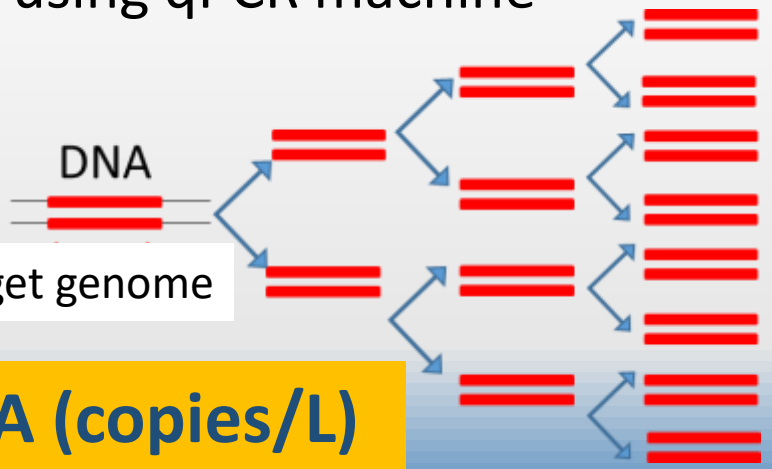
④ amplify the target DNA (qPCR)

・Amplify the target DNA fragments using qPCR machine

・reagent for detection:
specific for most of invertebrates

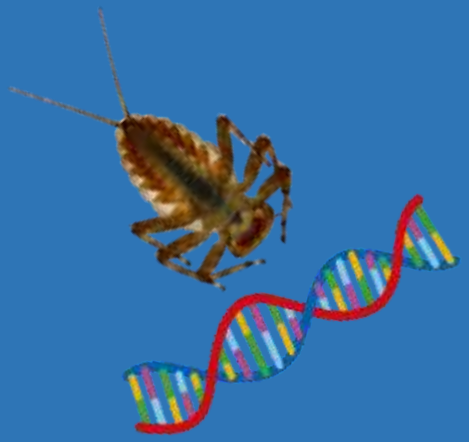
→ Detecting & Quantifying
invertebrates' DNA

→ **Only invertebrates' DNA (copies/L)**



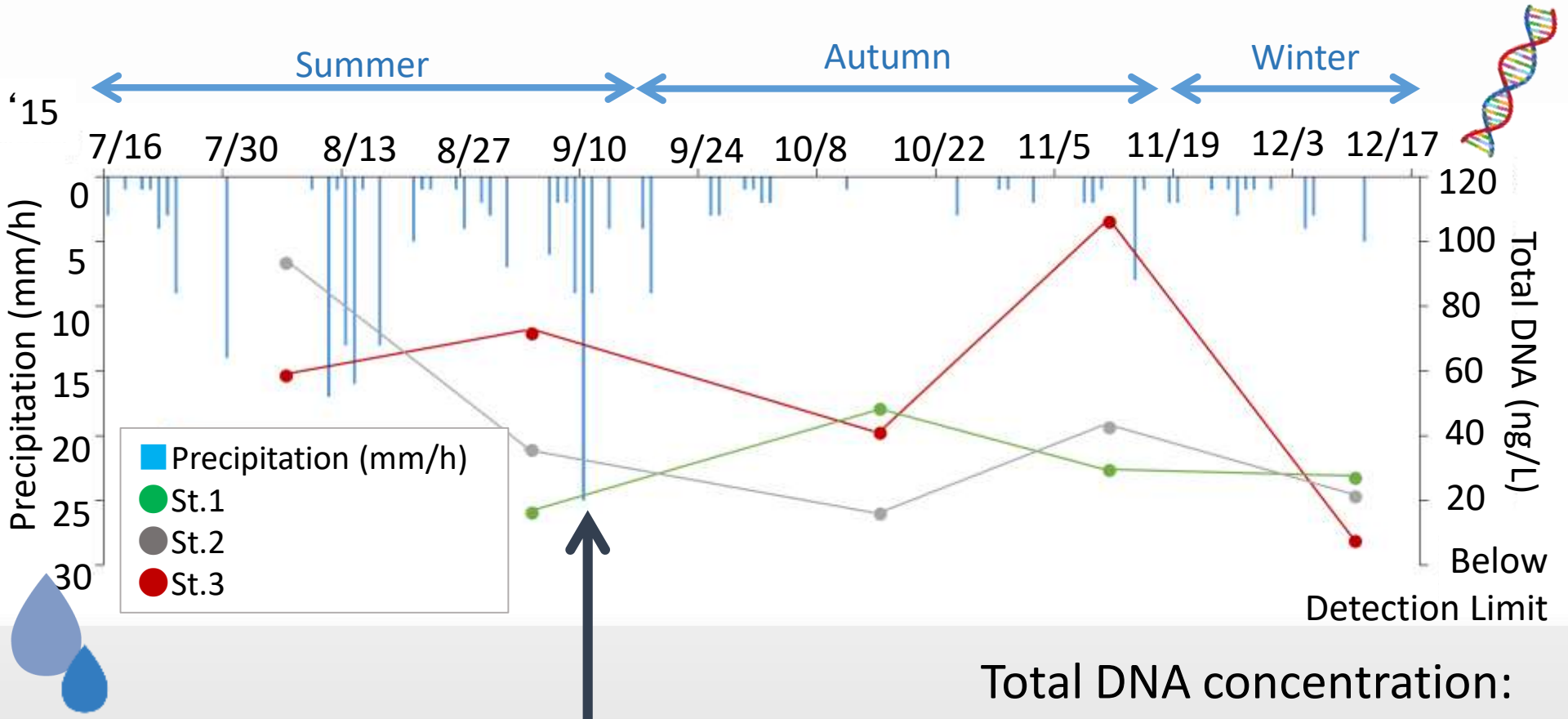
Result 1:

Detection of invertebrate DNA from river water



Result 1: Detection invertebrates eDNA

✓ Successfully measured total DNA (except Aug. St.1)



Flood on 10th Sept. caused serious disturbance of habitat

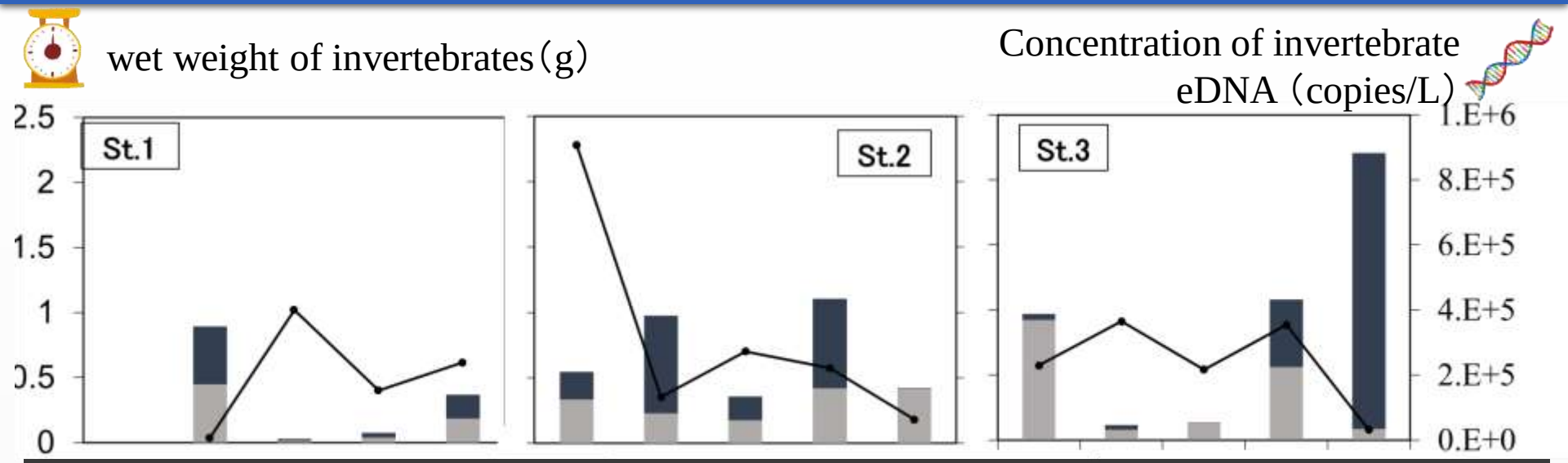
Total DNA concentration:

- min: 17.8 ng/L
- Max: 264.6 ng/L
- Mean: 109.0 ng/L

Result 2:

Comparing invertebrate eDNA and wet weight

Result 2: eDNA vs wet weight



From eDNA detection,

Even after serious disturbance, it seems potential mass of aquatic invertebrates will be maintained

✓It seemed there is no positive correlation; because..

- eDNA came from upper stream
- originated from not only the insects we collected

✓After September:

the weight decreased,

but eDNA was not so much decreased, but stable

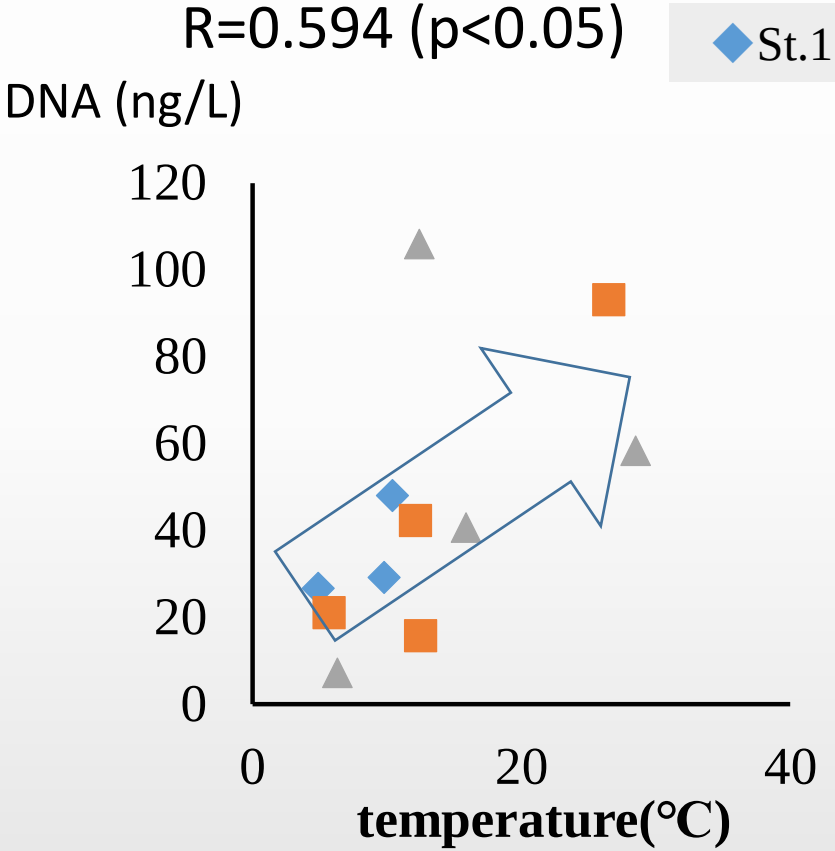
Result 3:

Relationship between
eDNA and environmental variables

eDNA vs environmental factors 1/3

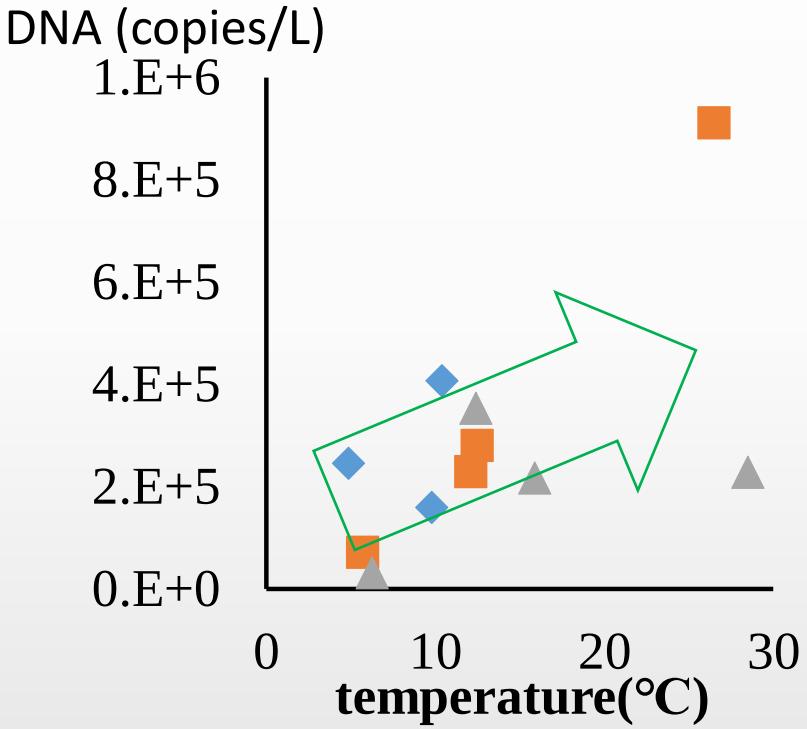
Total DNA

R=0.594 (p<0.05)



Only Invertebrates DNA

R=0.602 (p<0.05)



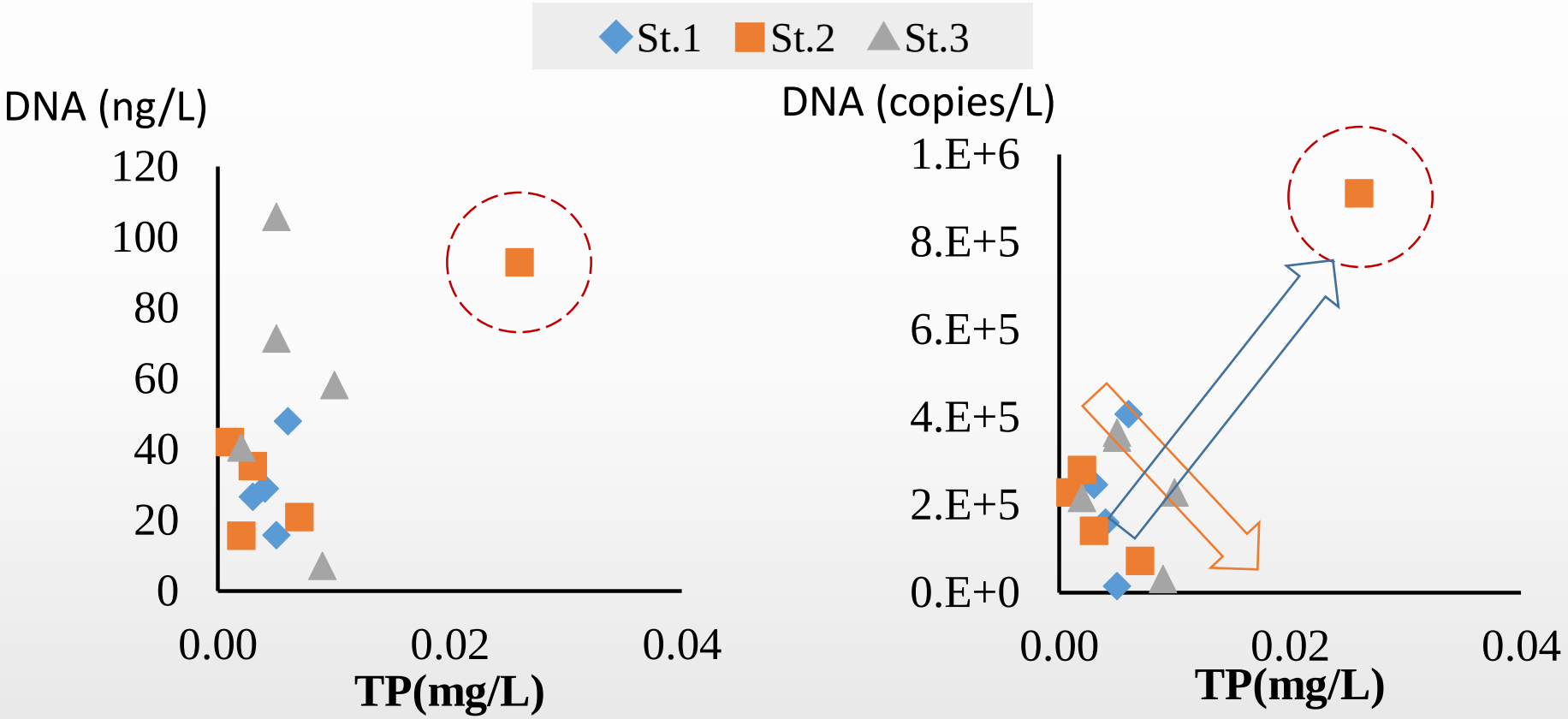
✓ Metabolite activity is promoted by higher water temperature

⇒ There is positive correlation

eDNA vs environmental factors 2/3

Total DNA

Only Invertebrates DNA



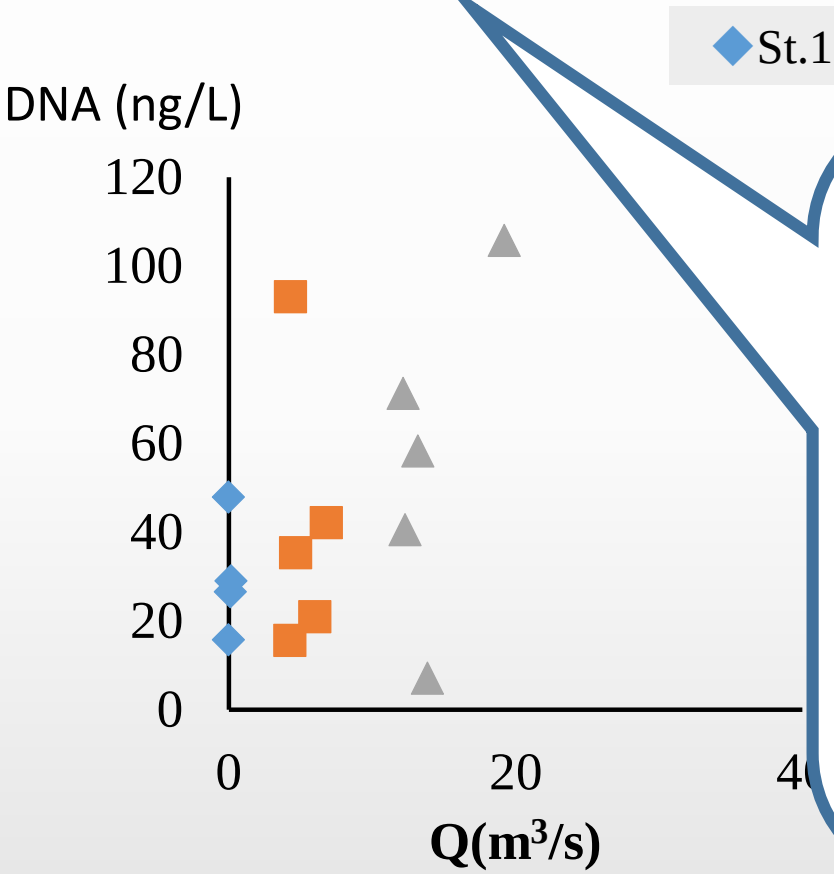
✓ One high TP value leads positive correlation

⇒ It needs more sample to define the tendency

eDNA vs environmental factors 3/3

Total DNA

Only Invertebrates DNA



R=-0.184 ($p<0.05$)

ANCOVA
(analysis of covariance)

- ✓ Studied sites gave the differences of Total DNA ($p<0.05$)
- ✓ Characteristic of the site possibly made it

(e.g. land-use, soil properties)

- ✓ Invertebrate DNA has negative correlation with discharge
- ✓ Land characteristics may affect concentration of total DNA.

Summary

●RESULTS●

1. Applied eDNA method to aquatic invertebrates
2. Result from spacial & Seasonal profiles, eDNA showed potential biomass or existence even after severe disturbance
3. eDNA has positive correlation with Water Temp.

●FUTURE VIEW●

1. Laboratory experiments
2. Combing DNA sequence analysis to know the composition

eDNA can be utilized to make biomonitoring easier!