A preliminary report on the implementation of eDNA-based techniques to biodiversity monitoring of fish from the Far East of Russia

Sergei V. Turanov[1,2], Olesia A. Rutenko[1]

sturcoal@mail.ru; **sturcoal.github.io**

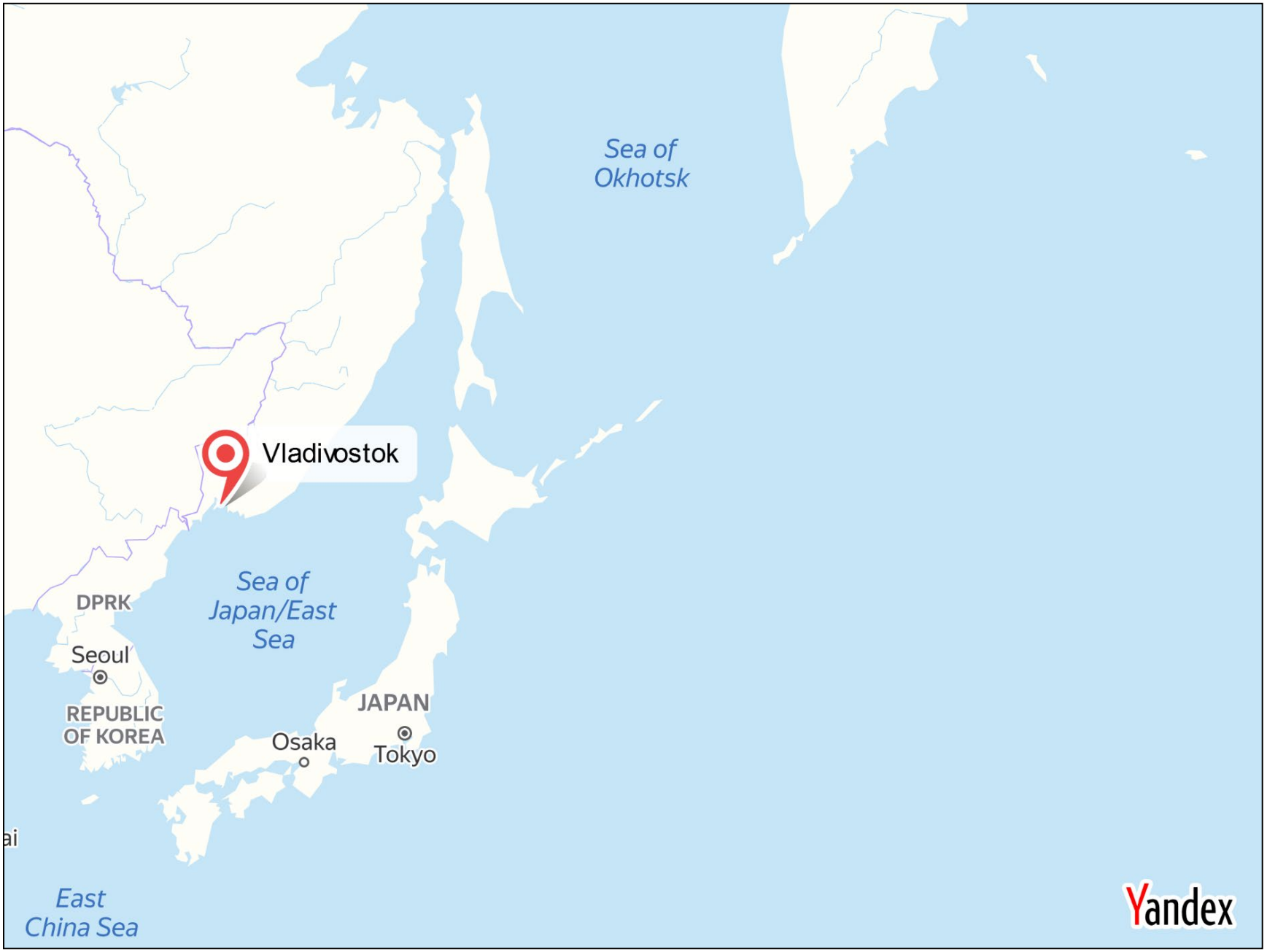
Laboratory of Molecular Systematics

[1]A.V. Zhirmunski National Scientific Center of Marine Biology, Russian Academy of Sciences,
[2]Far Eastern State Technical Fisheries University,
Vladivostok, Russia

A.V. Zhirminski National Scientific Center of Marine Biology (Vladivostok, Russia)

Primorsky Aquarium

Map of where we are



Monitoring the water biological resources (mostly fish)

What about eDNA?

Specially protected natural areas

Far Eastern Marine Reserve

Endangered and threatened species

Fish

- Sakhalin sturgeon
Acipenser mikadoi (Hilgendorf, 1892)

- Sakhalin taimen
Parahucho perryi (Brevoort, 1856)

Crayfish

Cambaroides wladiwostokensis
(Winogradow, 1934)

Cambaroides schrenckii ?

Why do we need to use aquatic eDNA for it?

1. All we need is water.
2. There is no need to physically locate and/or capture individuals to determine whether it is present in the area.
3. The cost of surveys is relatively low.

[The picture is stolen from https://fishbio.com/field-notes/conservation/traces-left-behind]

Fish eDNA studies on the **Far East of Russia** are **now** concentrated on:

1. Building the DNA barcode reference library based on the *COI* and *12S* rRNA mitochondrial markers. There are about 1200 fish species documented to date in Russia (including 35 endangered species). **Reference database is still half-completed**.

2. **Express-monitoring** of **specially protected natural areas** (Far Eastern Marine Reserve) including detection of invasive species.

3. **Non-invasive monitoring** of endangered and threatened species:

   - Sakhalin sturgeon
   (Acipenser mikadoi Hilgendorf, 1892)

The techniques we are using

1. Collecting eDNA.


Gloves
Filter, combi-stoppers, tube with Longmaier's buffer
Sample label
A "Small" syringe (up to 3 ml)
A "Big" syringe (160 ml)

How do we use it

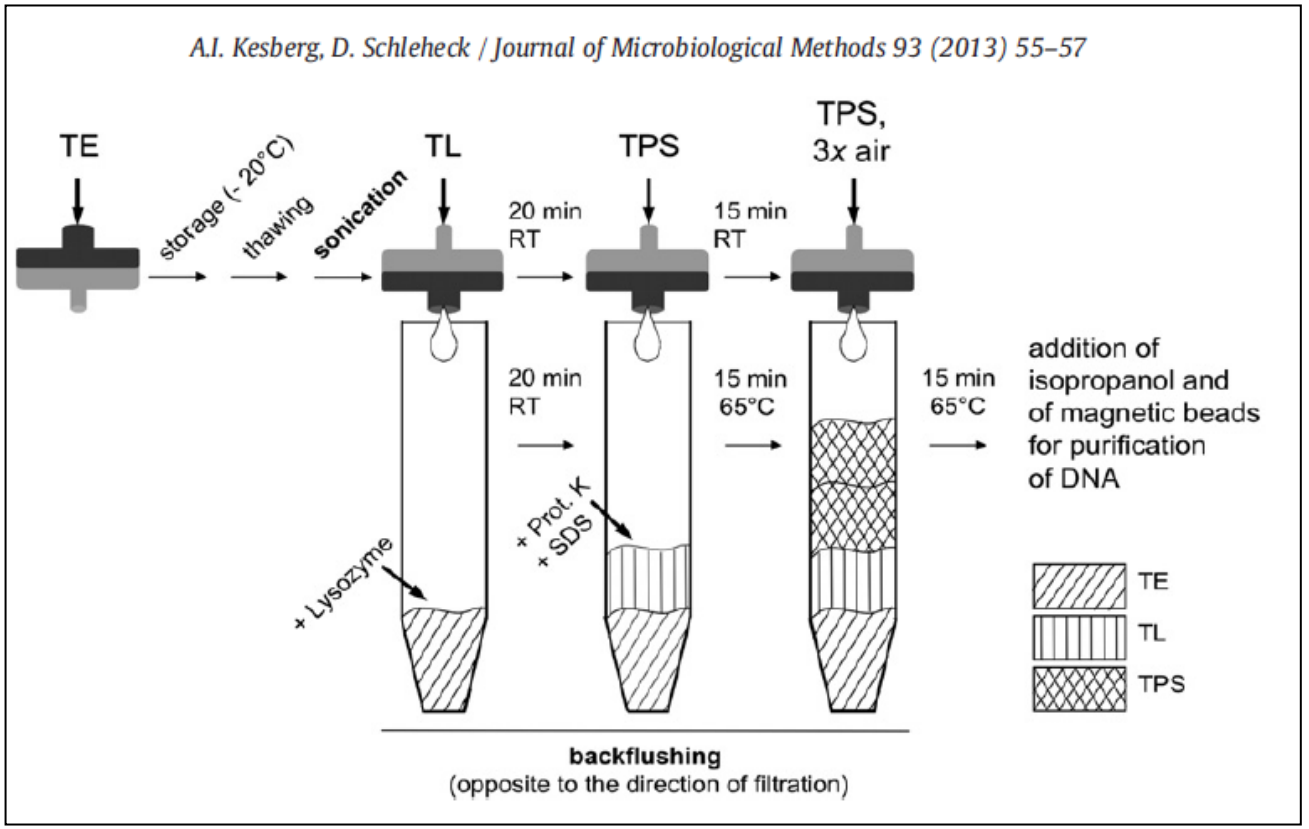





A modified sealant gun :)

The techniques we are using

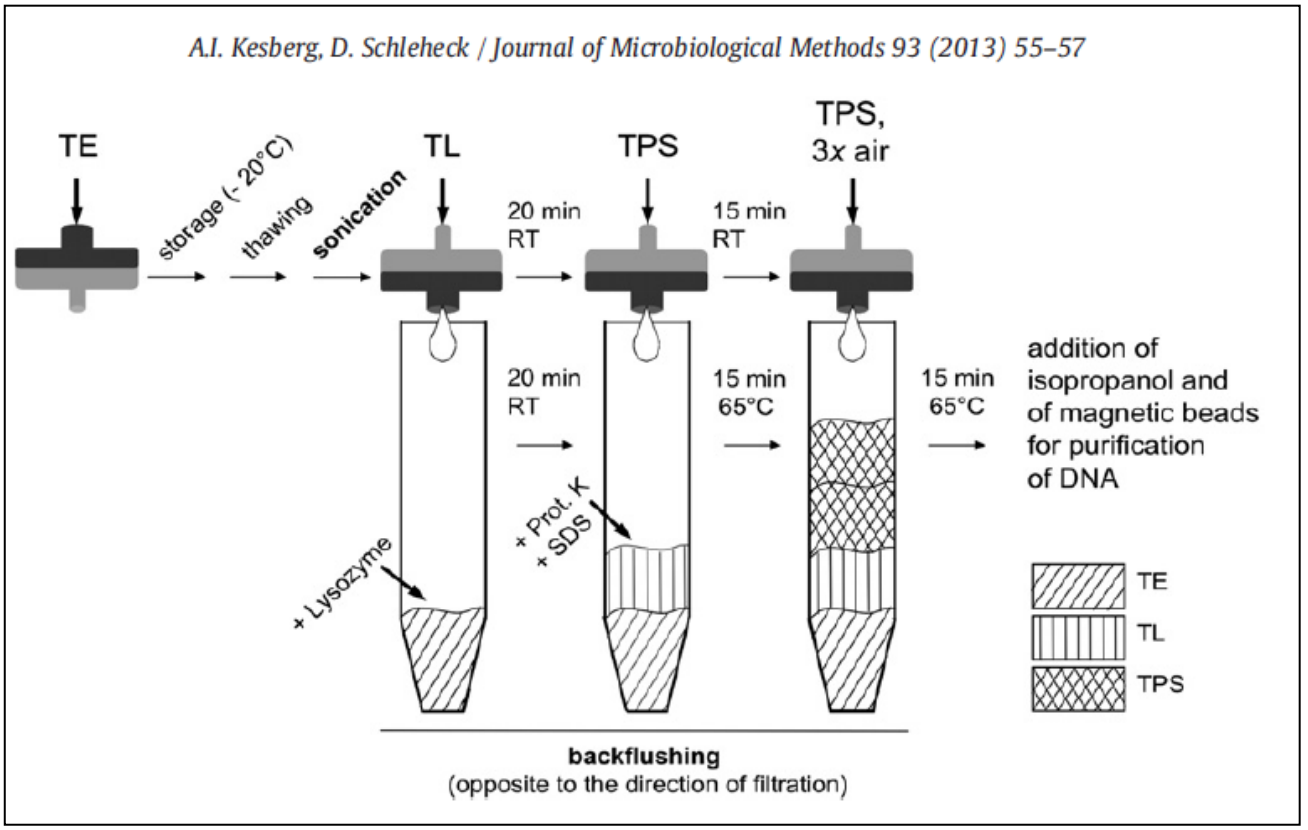
2. eDNA isolation.



Modified backflushing technique (Kesberg, Schleheck, 2013)

The techniques we are using

2. eDNA isolation.

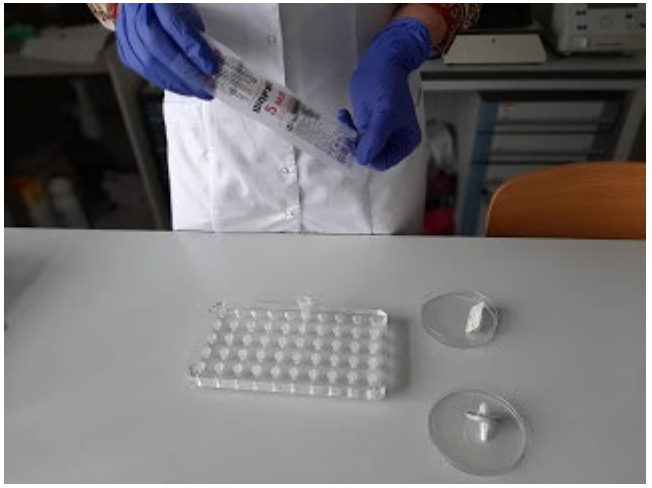


Modified backflushing technique (Kesberg, Schleheck, 2013)

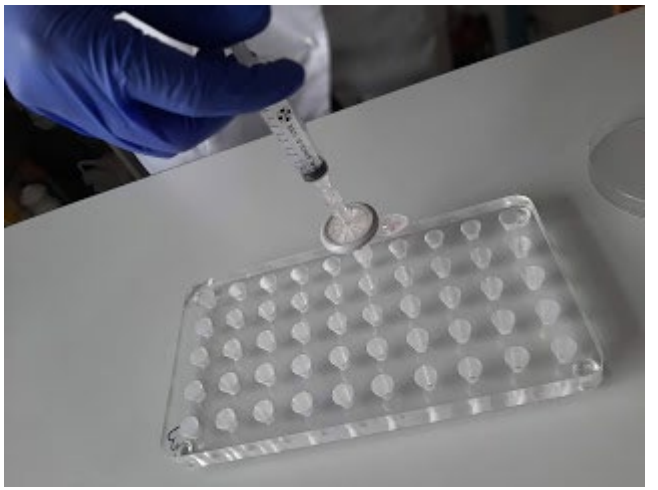
Commercial kit whish is based on magnetic beads. Sintol Co., Russia.

The techniques we are using

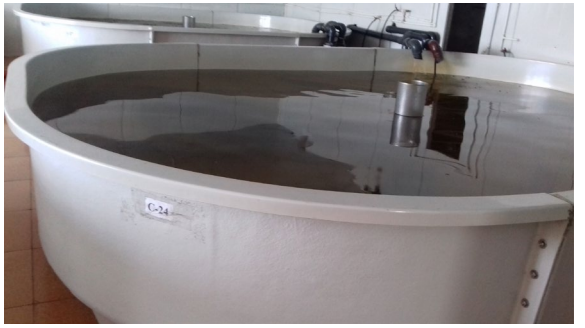
2. eDNA isolation.

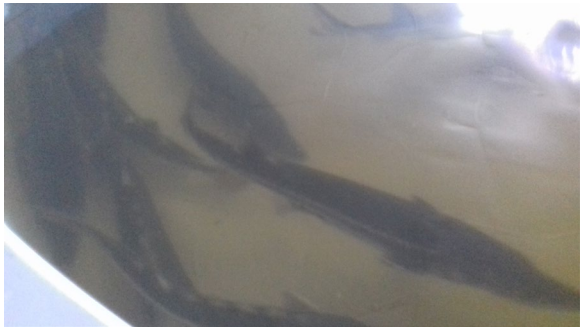





What have we done for today
1. Sakhalin sturgeon eDNA.





Sampling site:
Anyuiskiy fish hatchery

Water tank of 1000 liters with
10 sturgeon specimens from 2008 year
generation.

In September of 2019 we had filtered 450 ml water
from these tanks (0.45 μm syringe-filter) with 3
replicates. DNA is already isolated. We are now developing the
species-specific primers and probes.

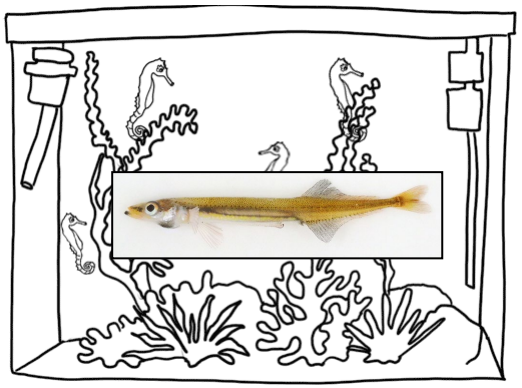
What have we done for today

2. The development of express-monitoring techniques for fish species using eDNA.

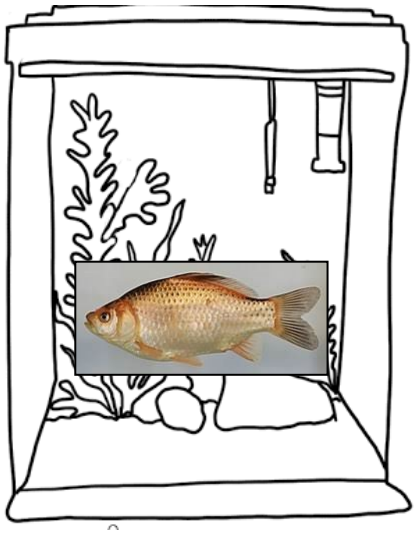
Primorsky Aquarium

What have we done for today

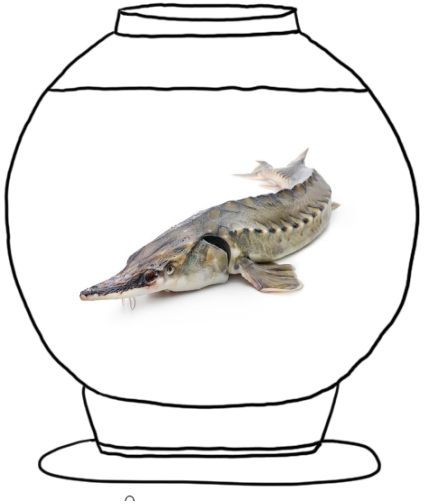
2. The development of express-monitoring techniques for fish species using eDNA.



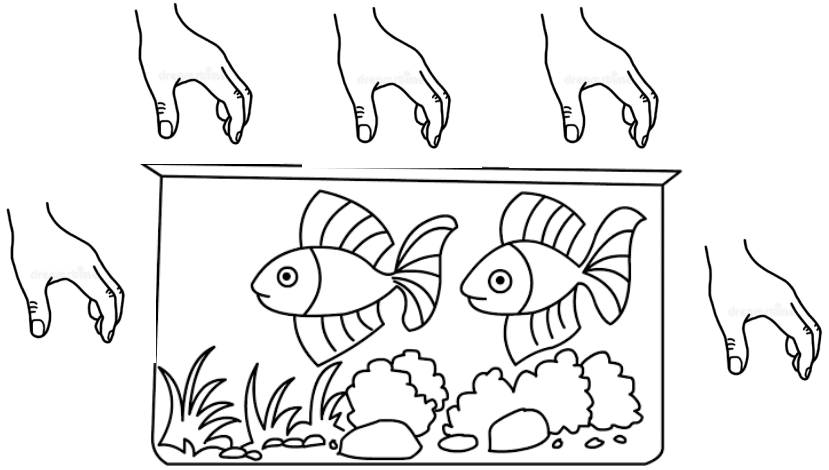
Marine fishes (Sea of Japan/East Sea)
TF13, **13 species.**



Freshwater fishes (Lake Khanka)
TF5, **16 species**



Sturgeons (has no Sakhalin sturgeon)
T1, **4 species**

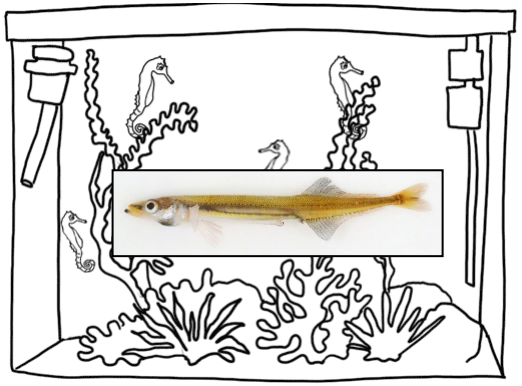


Touch pool (fishes and invertebrates)
T3, **11 species**

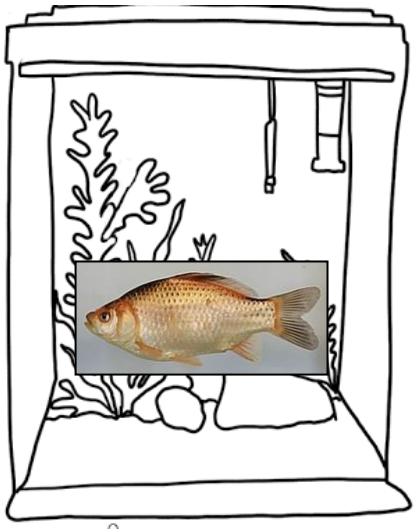
What have we done for today

2. The development of express-monitoring techniques for fish species using eDNA.

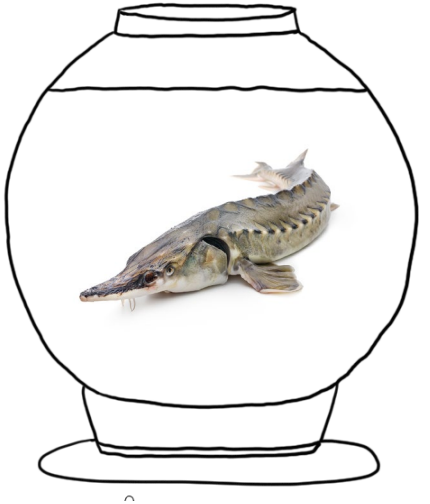
- collecting and isolating eDNA
- implementing metabarcoding techniques with two sets of *twin-tagged* primers – Leray **COI** and **12S rRNA** MiFish
- using individual tag pair for each sample (replicate) for PCR
- normalizing and construct the common pool based on both markers
- inspecting the results, dereplicating, filtering, clustering and assigning OTUs to known taxa



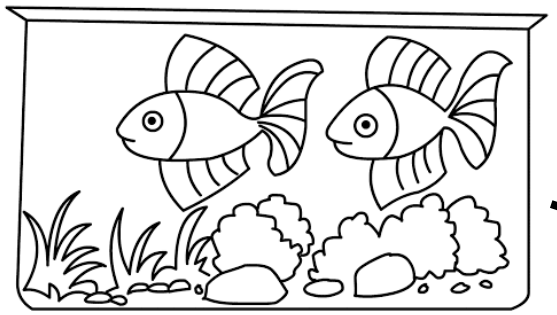
Marine fishes (Sea of Japan/East Sea)
TF13, 1 replicate



Freshwater fishes (Lake Khanka)
TF5, 3 replicates



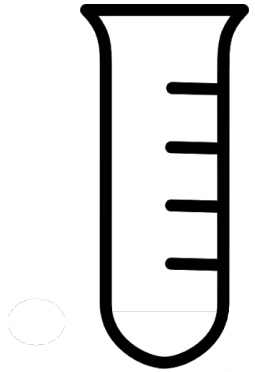
Sturgeons (has no Sakhalin sturgeon)
T1, 3 replicates



Touch pool (fishes and invertebrates)
T3, 2replicates

Joint Pool of normalized amplicons from **COI** and **12S rRNA** markers

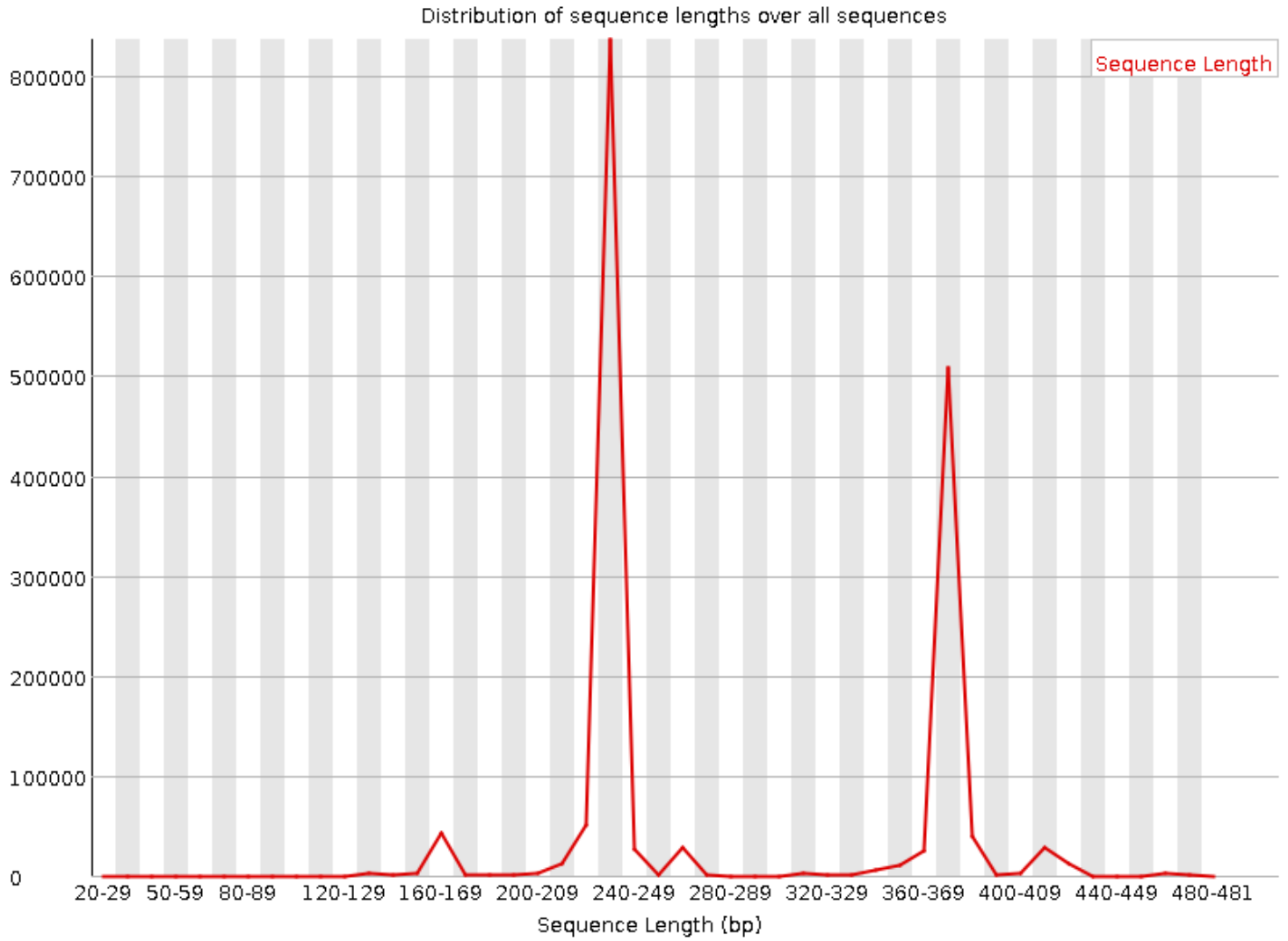
Joint Pool of normalized amplicons from **COI** and **12S rRNA** markers



Sending for sequencing to Novogen company

What have we done for today

2. The development of express-monitoring techniques for fish species using eDNA.

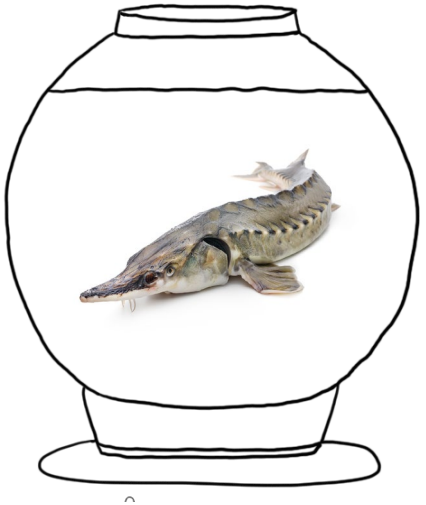


What have we done for today

2. The development of express-monitoring techniques for fish species using eDNA.

Metabarcoding data processing was done based on **Begum** pipeline (Zepeda-Mendoza et al., 2016; Yang et al., 2020):

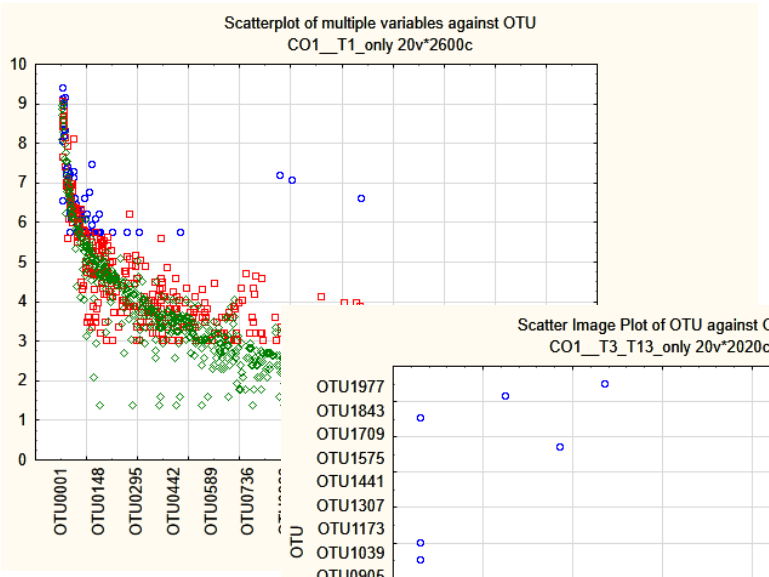
COI	**vs**	**12S rRNA**
Dereplication		Dereplication
Clustering (sumaclust)		Clustering (sumaclust)
blasting (blastn)		blasting (blastn)
Building OUT table based on MEGAN output		Building OUT table based on MEGAN output
~~lulu r package OTUs correction~~		lulu r package OTUs correction
Visualization		Visualization

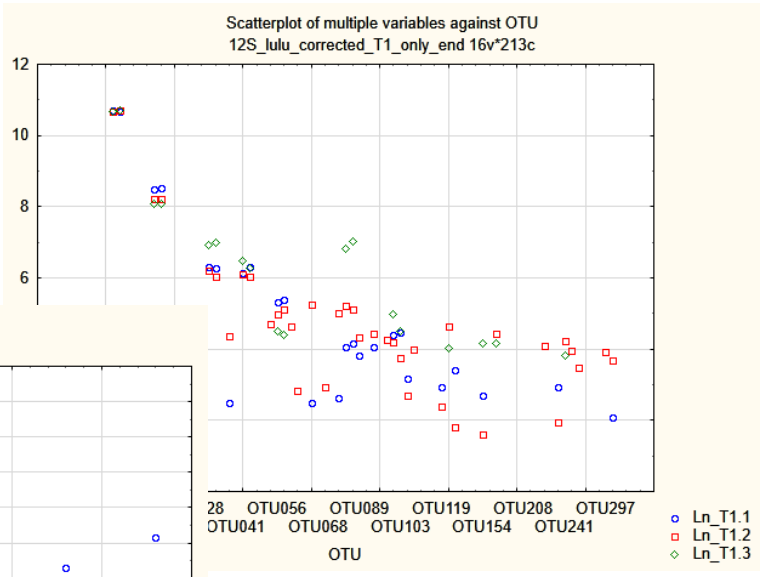


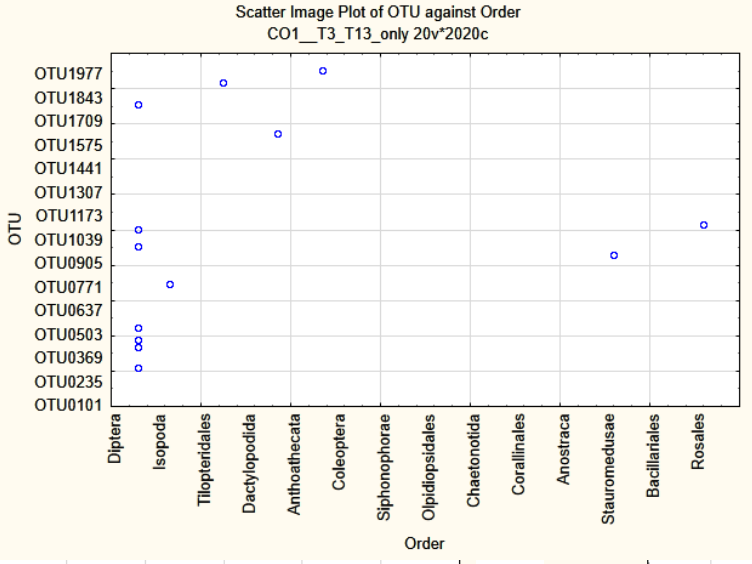
Sturgeons
T1, **4 species**

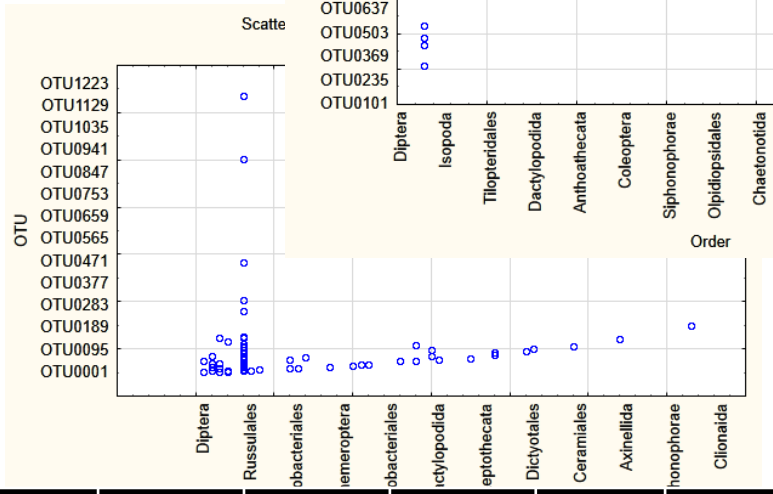
COI

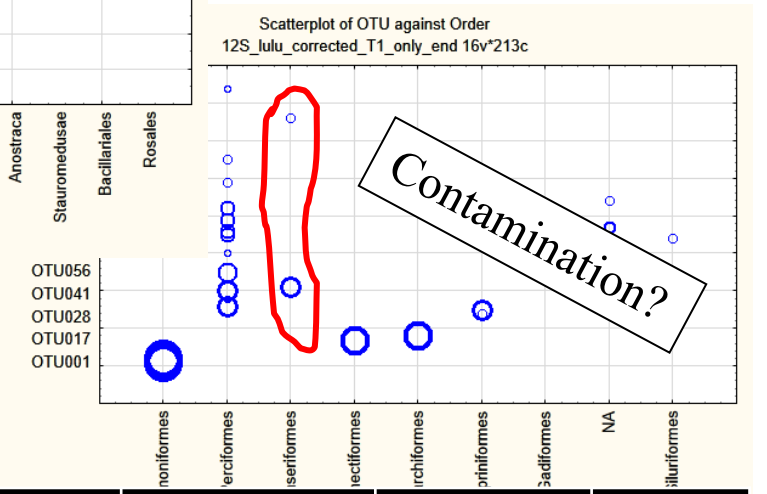
12S rRNA



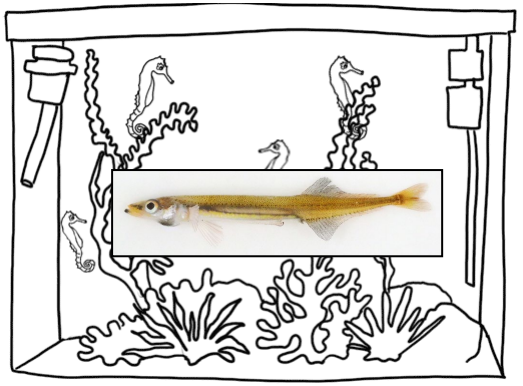






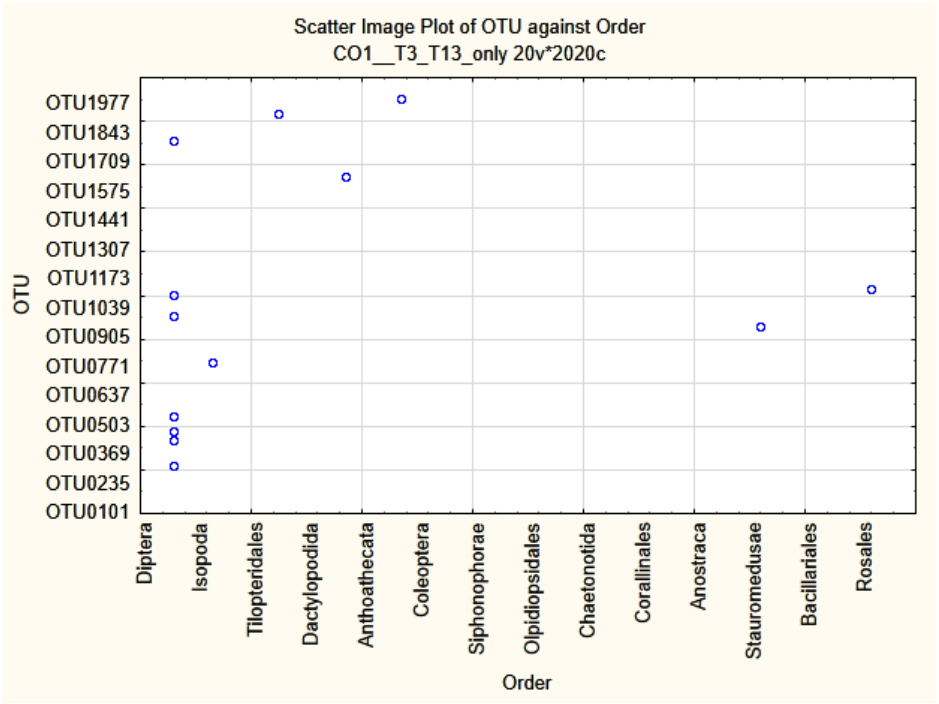


	T1_1.1	T1_1.2	T1_1.3	TF5_1.1	TF5_1.2	TF5_1.3	T3_1.1	T3_1.2	TF13_1.2	Order	Family	Genus
OTU0051	765	571	431	0	0	0	0	0	0	Acipenseriformes	Acipenseridae	NA
OTU0118	1786	252	163	0	0	0	0	0	0	Acipenseriformes	Acipenseridae	Acipenser
OTU0172	0	33	146	0	0	0	0	0	0	Acipenseriformes	Acipenseridae	Acipenser
OTU0243	0	58	79	0	0	0	0	0	0	Acipenseriformes	Acipenseridae	Acipenser

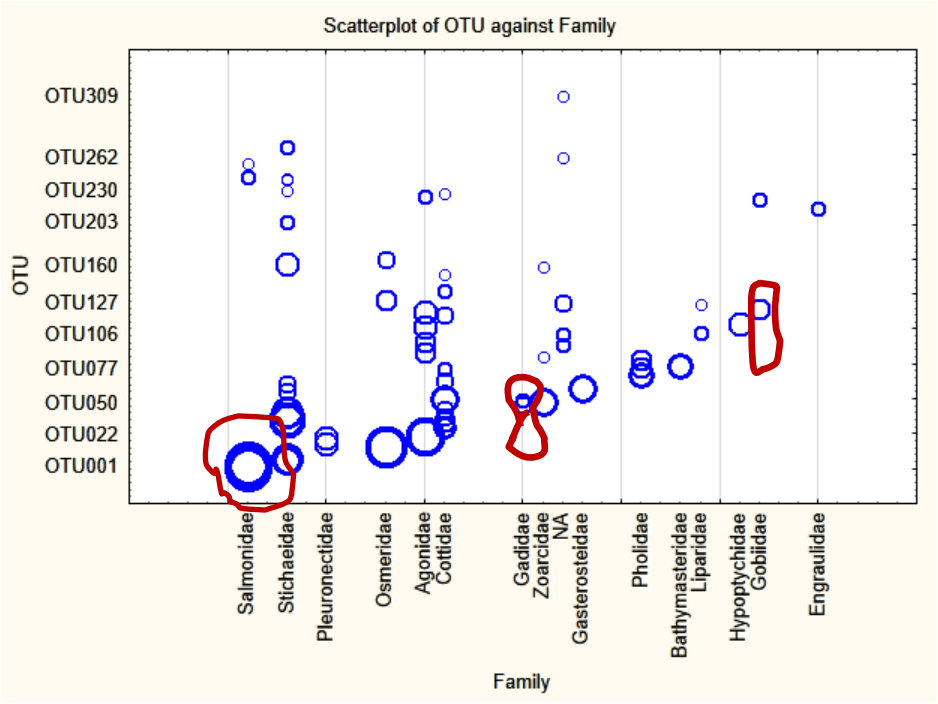


Marine fishes (Sea of Japan/East Sea)
TF13, **13 species.**

COI



12S rRNA



Possible sources of contamination:

- Water intake (we didn't take a sample from it)
- DNA isolation (we didn't sequence a control from it)
- ?

In addition:

- Salmonidae were everywhere

Acknowledgements

Instructors of **Yunnan Metabarcoding School 2019 (China)**
Special thanks to Prof. **Douglas Yu**

Primorsky Aquarium staff

Anyuiskiy fish hatchery staff

Thank you!