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Innovative approaches to monitoring and assessing marine environment and nature values in Estonian sea area

Task 2.4 eDNA based methodology for early detection of alien species

Task 2.7. Development of DNA-based approach for estimation of effective spawner abundance

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Task 2.4 eDNA based methodology for early detection of alien species

Aim of the activity

- To develop a detailed protocol with the description of the sampling, sample storage and eDNA extraction
- To develop species-specific approach (quantitative PCR) for early detection of alien species in the Baltic Sea environment
- To develop the High Throughput Sequncing (HTS) based metabarcoding approach to detect alien species in marine coastal communities
- To carry out a pilot monitoring survey in selected sea areas

Timeframe

- **Fieldworks**: May 2019 – April 2020
- **DNA extractions**: February – May 2020
- **qPCR optimisation**: April – June 2020
- **High Throughput Sequencing**: June – September 2020
- **Data analysis**: September – October 2020
- **Reporting**: November – Detsember 2020

Before eDNA approaches can be used for monitoring for alien species, several technical and analytical issues must be tested and resolved

- sampling strategy
- selection of the species
- testing various filters
- sample storage method
- testing DNA extraction protocols
- qPCR (primer and probe design)

- selection of marker genes for metabarcoding (COI, rRNA genes)
- optimizing the bioinformatic pipeline

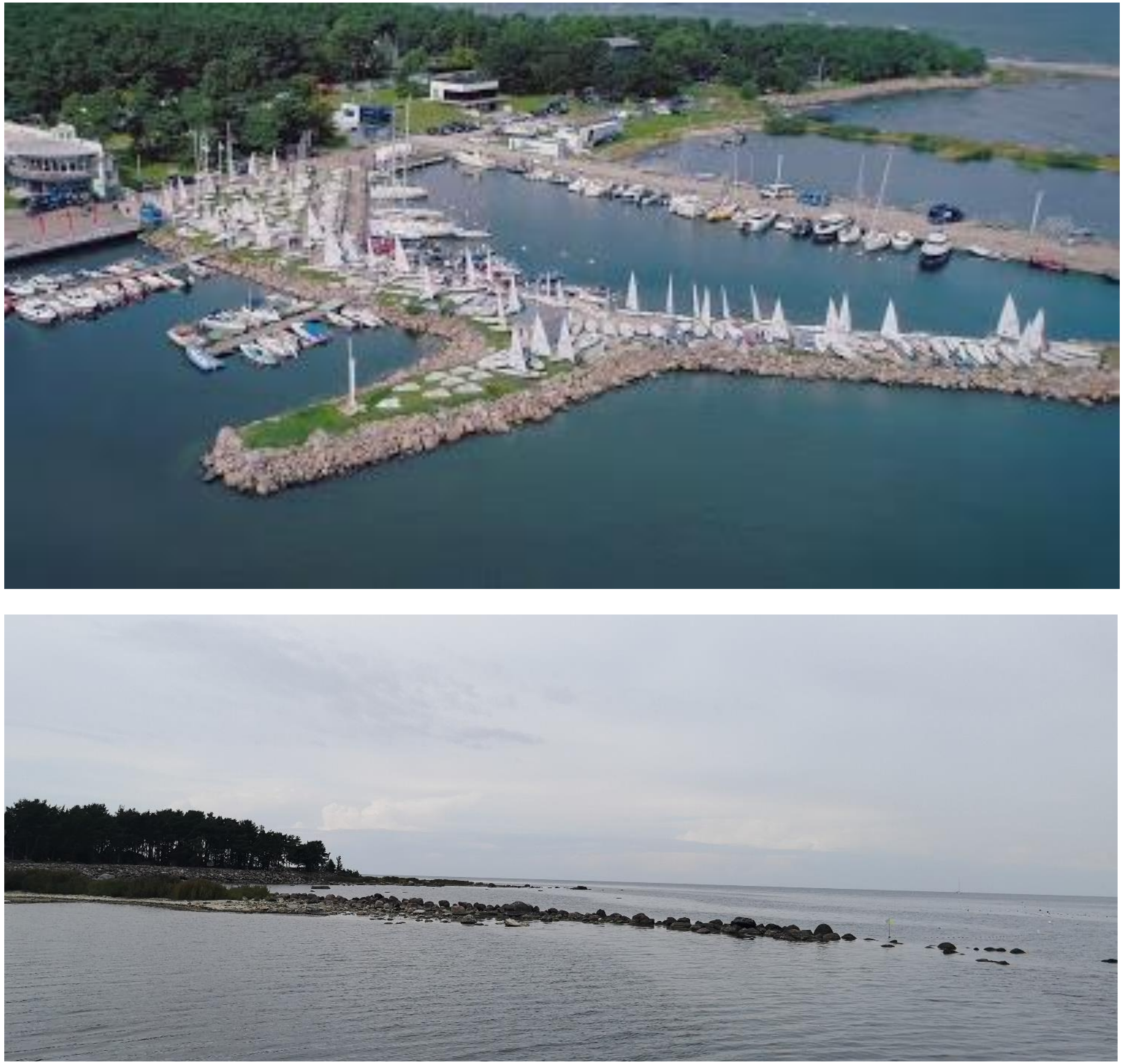
Sampling strategy, selection of pilot and reference areas

Muuga Harbour - the main cargo harbour for Port of Tallinn

Lohusalu Harbour - small harbour

Muuga harbour

Lohusalu harbour



Known selected species for pilot study

- *Laonome xeprovala*
- *Gammarus tigrinus*
- *Dreissena polymorpha*
- *Cercopagis pengoi*
- *Rhithropanopeus harrisii*
- *Marenzelleria neglecta*
- *Neogobius melanostomus*
- *Palaemon elegans*

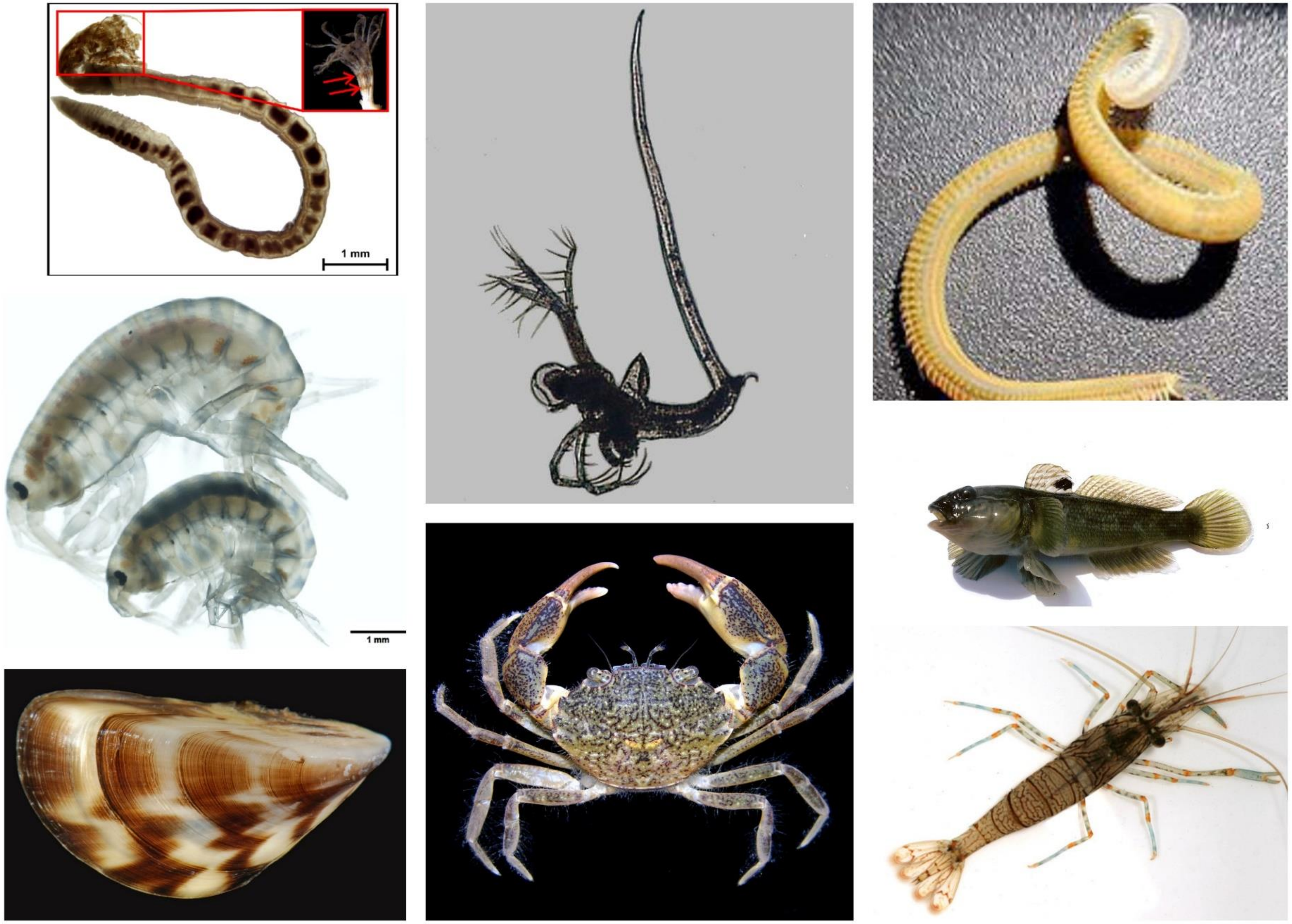
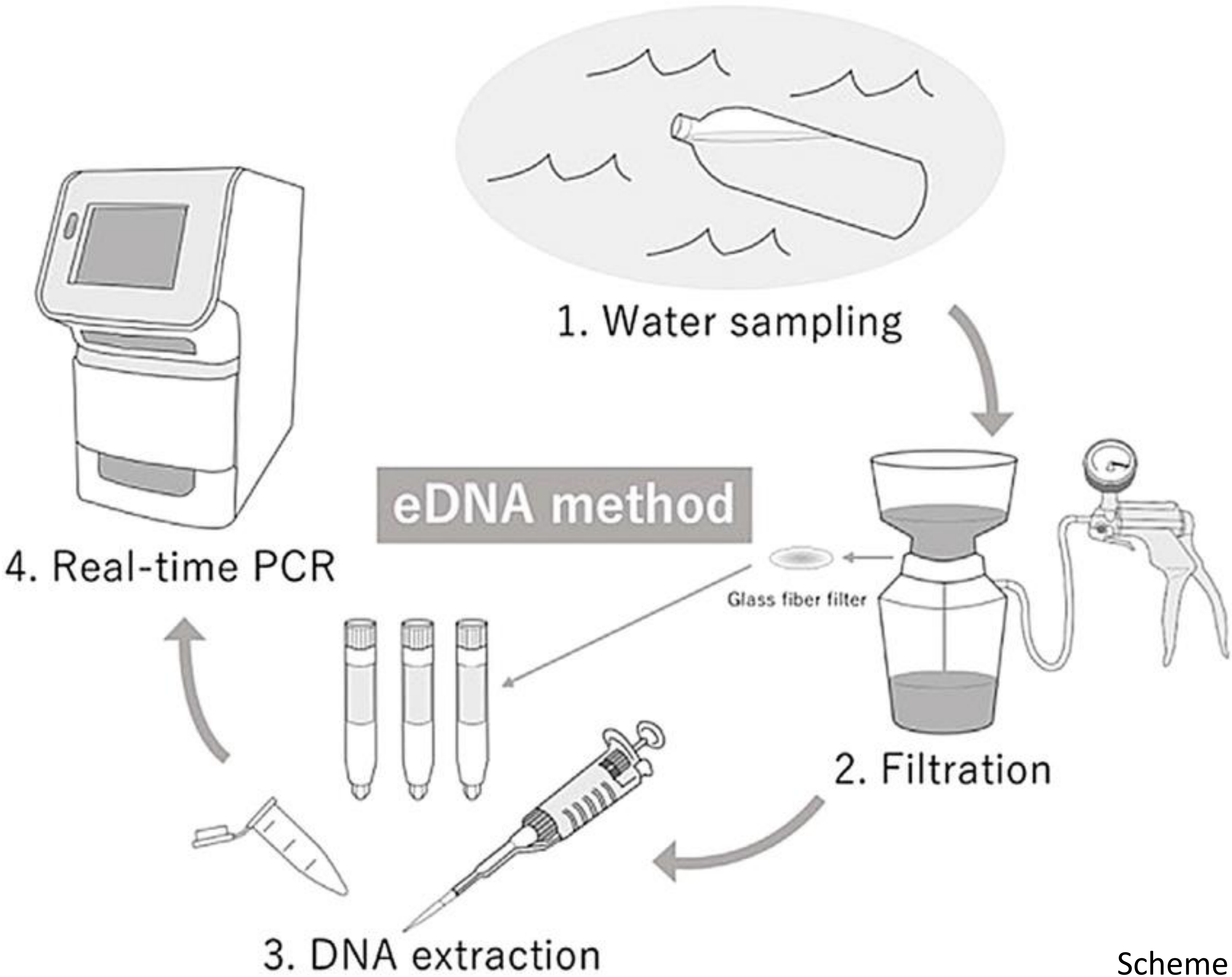


Photo: J. Kotta; A. Bick; R. Neideman; M. Storey; P. Sluijs;
Smithsonian Environmental Research Center; TÜ Mereinstituut



Scheme: Marcello Otake Sato

Filters, filter storage, negative controls

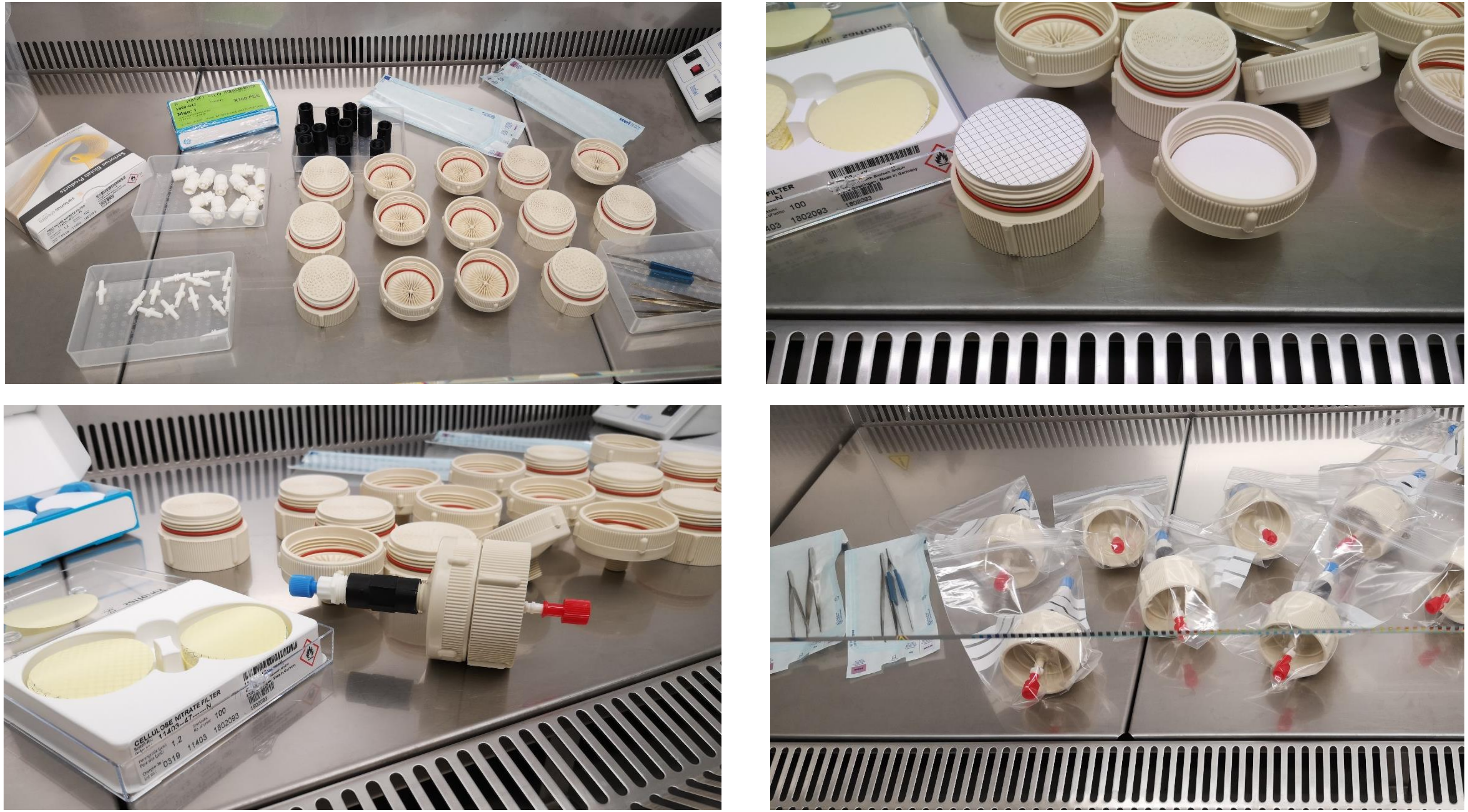
Sterivex

NatureMetrics

Self-constructed filter unit

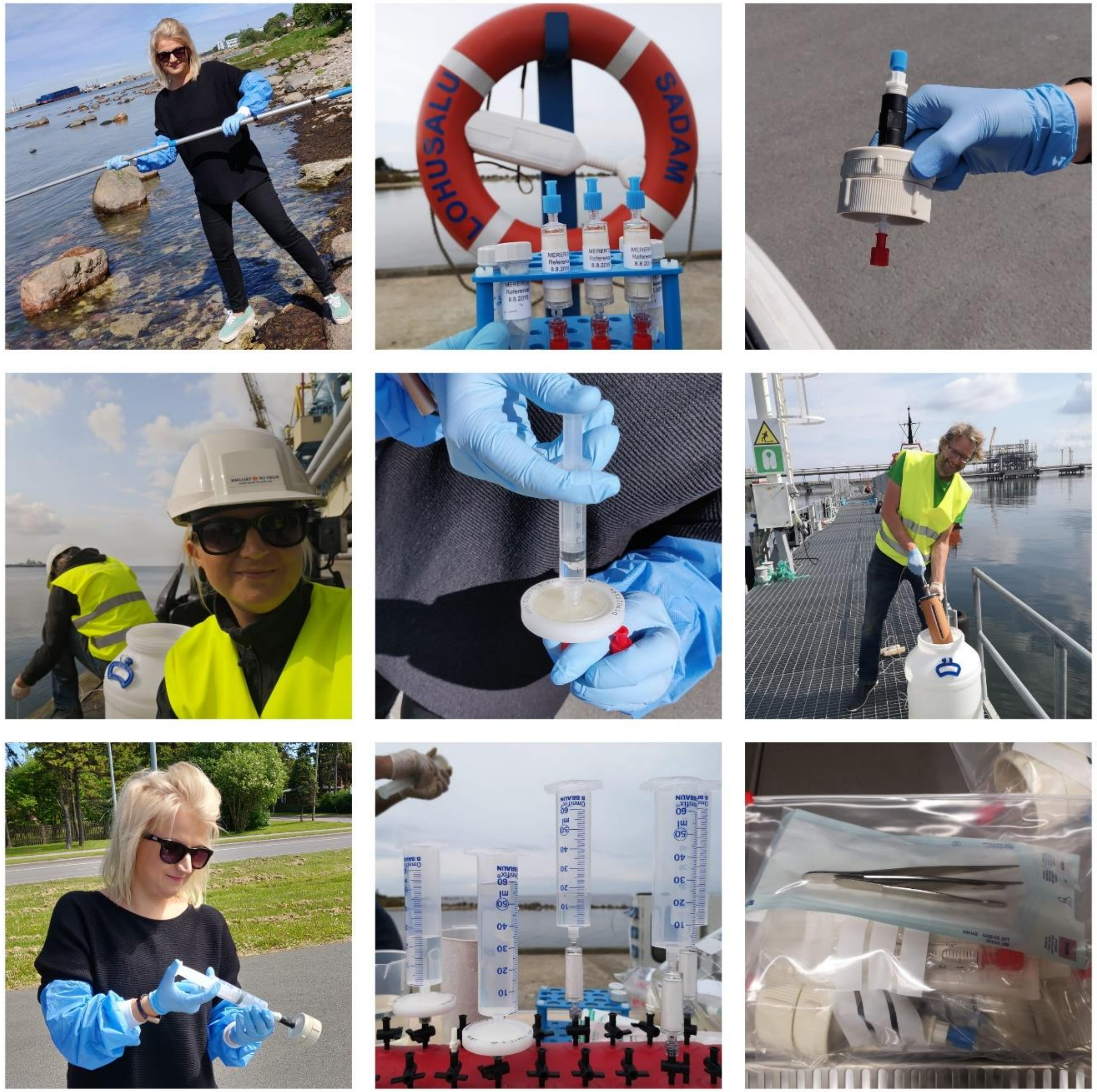
Smith-Root eDNA filter

Time vs Money



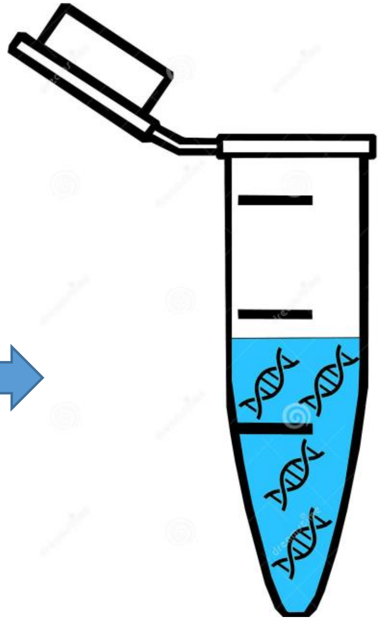
Testing the system in the field

- Same sampling points with traditional monitoring
- Depth integrated water
- Pooled samples



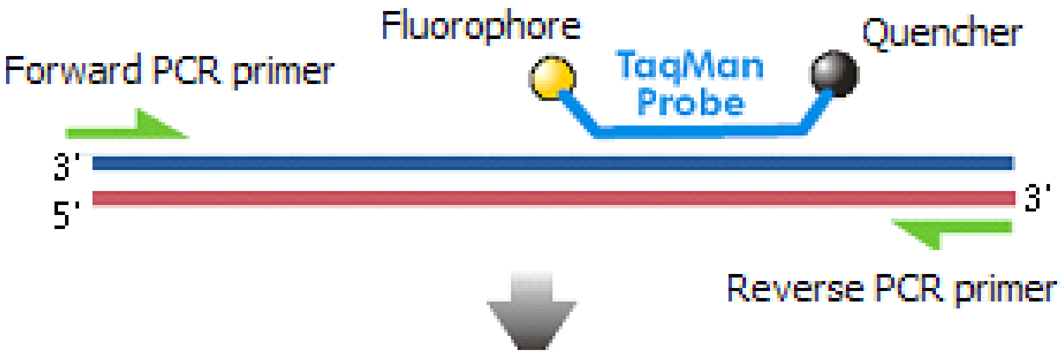
eDNA extraction – commerial kits

- Qiagen DNeasy PowerWater Sterivex Kit
- Qiagen DNeasy PowerWater Kit
- Qiagen Blood and Tissue Kit



Design of species-specific Real-Time PCR primers and probes

- primers and probes for all the previously shown species
- region of the COI gene - most commonly used barcode region for animals
- primer specificity test with Primer-BLAST



Task 2.7. Development of DNA-based approach for estimation of effective spawner abundance

- To develop and test the performance of DNA-based approach for estimation of effective spawner abundance focusing on Northern pike (*Esox lucius*) in the coastal Baltic Sea areas.
- In 2019 juvenile samples collected from 5 locations (n=111); Adults collected from 6 locations (n=92)
- DNA extractions – finished
- Development of multiplex panel of 11 highly variable microsatellite markers
- Microsatellite genotyping for population structure – ongoing
- Additional sampling of juveniles in summer 2020
- The effective number of breeders and the total number of breeders will be estimated using the sibship method implemented in the most recent version of COLONY (Jones & Wang, 2010).

Tänan kuulamast!

Uuringut rahastab Euroopa Regionaalarengu Fond Eesti Teadusagentuuri ja Keskkonnaministeeriumi tellimusel programmi RITA raames.

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