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eDNA-based detection of *Astacus astacus*, *Aphanomyces astaci* and invasive alien crayfish species in Estonian water bodies

Michael Aluma, Katrin Kaldre, Lilian Pukk
Estonian University of Life Sciences

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Veterinærinstituttet
Norwegian Veterinary Institute

Eesti Maaülikool
Estonian University of Life Sciences
Chair of Aquaculture

Background

- *Astacus astacus* is the only native freshwater crayfish species in Estonia
- It is threatened by the introduction of invasive non-indigenous crayfish species (NICS) and crayfish plague
- The NICS of North American origin are latent carriers of the crayfish plague pathogen *Aphanomyces astaci*
- The crayfish species native to Europe are highly susceptible to crayfish plague
- Between 2008 and 2017, three NICS were detected in Estonia through trapping
  I. *P. leniusculus* in 2008
  II. *F. limosus* and in 2017
  III. *Procambarus virginalis* in 2017

Background cont.

- When using traps for monitoring of NICS and noble crayfish, early detection remains a challenge at low densities
- Early detection of NICS is crucial for conservation-based management of indigenous and endangered species
- Studies show that the environmental DNA (eDNA) method allows early detection of native and invasive species at low population density
- eDNA method uses traces of DNA left in the environment by cells shed from multicellular organisms in the form of mucus, body fluids, scraped off epithelial cells etc.
- eDNA are filtered from a water sample followed by extraction and analysis
- Our study aimed to find the possibility of applying the eDNA method to study the spread of invasive NICS and crayfish plague pathogen *A. astaci* in Estonia

Material and methods

Study area

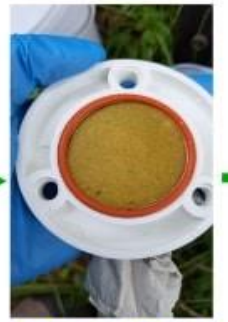
- We collected eDNA water samples from **16** water bodies across Estonia
- Waterbodies were grouped as **lotic** (flowing) or **lentic** (still waters)
- A total of **37** sampling points (1 up to 4 per location) were selected
- Sampling period

Round I	Aug-Sept	**2022**
Round II	Aug-Sept	**2023**

eDNA water sampling and qPCR detection

Filtration of water samples using Peristaltic pump, tubing, and the sampling vessel

Between 2-5L of water filtered through glass fibre filter



47mm (2µm pore size) used filter

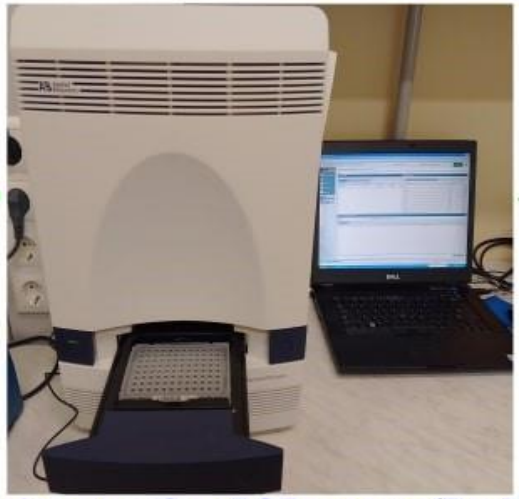
Filter folded and inserted into 15ml falcon tube with ATL buffer

Samples collected are transported to the lab

eDNA extraction following Qiagen DNeasy Blood & Tissue Spin-column Protocol

MACHEREY-NAGEL Nucleo Spin Filters Midi and Plant II Midi Columns used

PCR inhibitor removal using Zymo Research kit



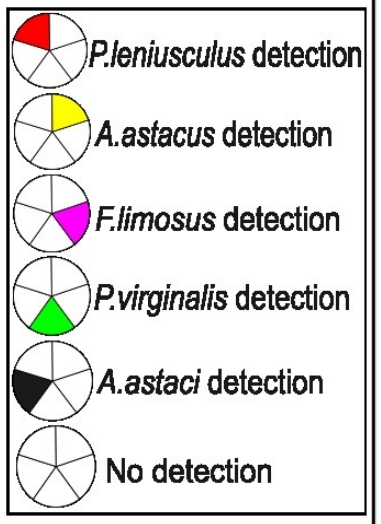
Species specific qPCR assays (TaqMan) screening for target species eDNA and determination of LOD and LOQ

Results and analysis:
We obtained trapping data (CPUE-catch per unit effort) from an annual monitoring of crayfish for comparison and verification of our eDNA results

RESULTS

Results

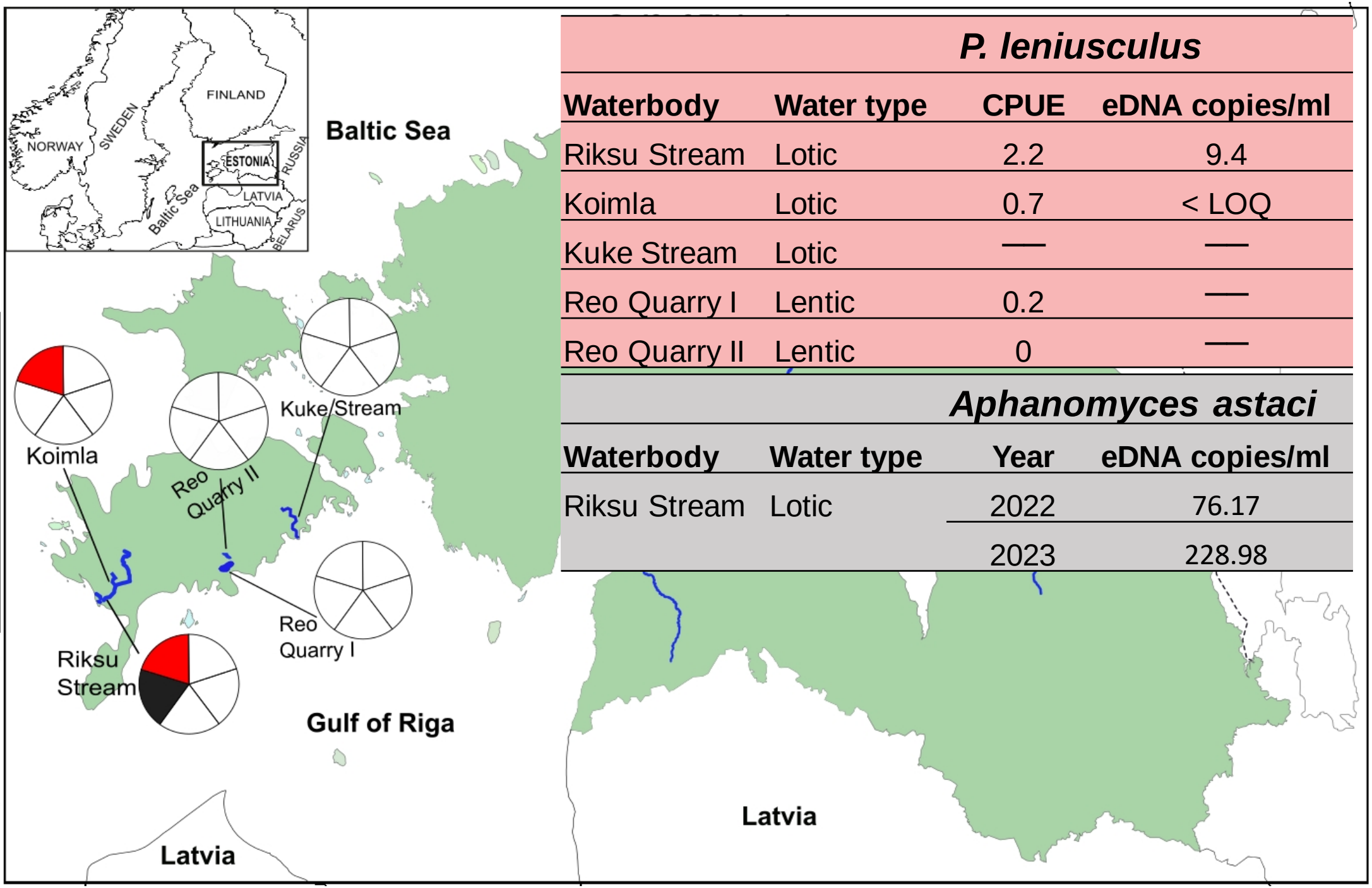
Results



CPUE < 1 (Low density)

CPUE = 1- 4 (moderate)

CPUE > 4 (High density)



P. leniusculus

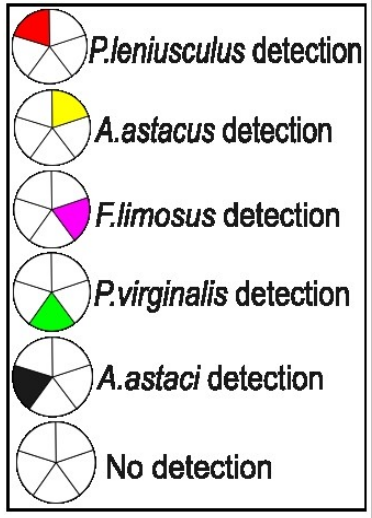
Waterbody	Water type	CPUE	eDNA copies/ml
Riksu Stream	Lotic	2.2	9.4
Koimla	Lotic	0.7	< LOQ
Kuke Stream	Lotic	—	—
Reo Quarry I	Lentic	0.2	—
Reo Quarry II	Lentic	0	—

Aphanomyces astaci

Waterbody	Water type	Year	eDNA copies/ml
Riksu Stream	Lotic	2022	76.17
		2023	228.98

Results

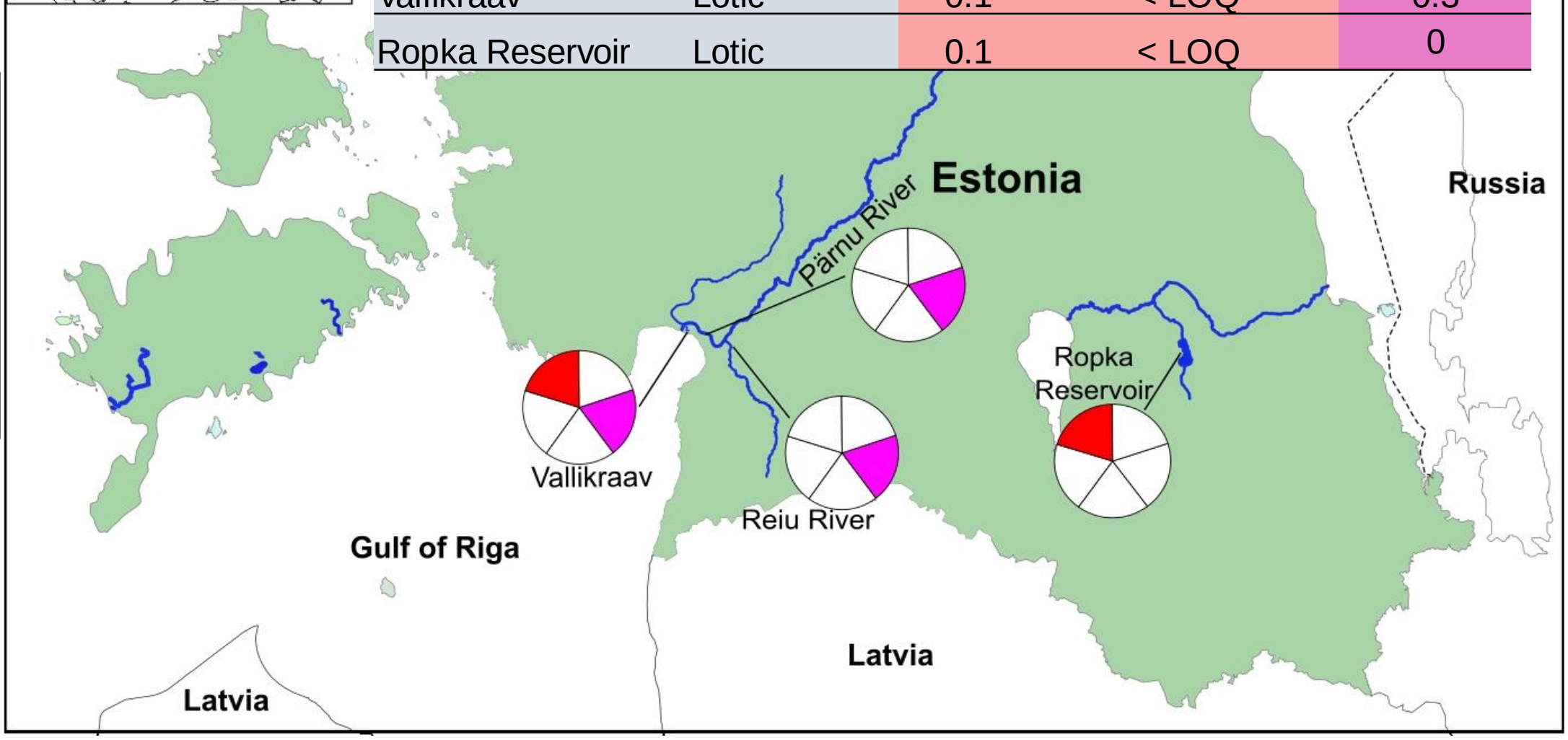
		P.leniusculus		*F.limosus*
Waterbody	**Water type**	**CPUE**	**eDNA copies/ml**	**CPUE**
Reiu River	Lotic	0	—	0.3
Pärnu River	Lotic	0	—	1.7
Vallikraav	Lotic	0.1	< LOQ	0.3
Ropka Reservoir	Lotic	0.1	< LOQ	0



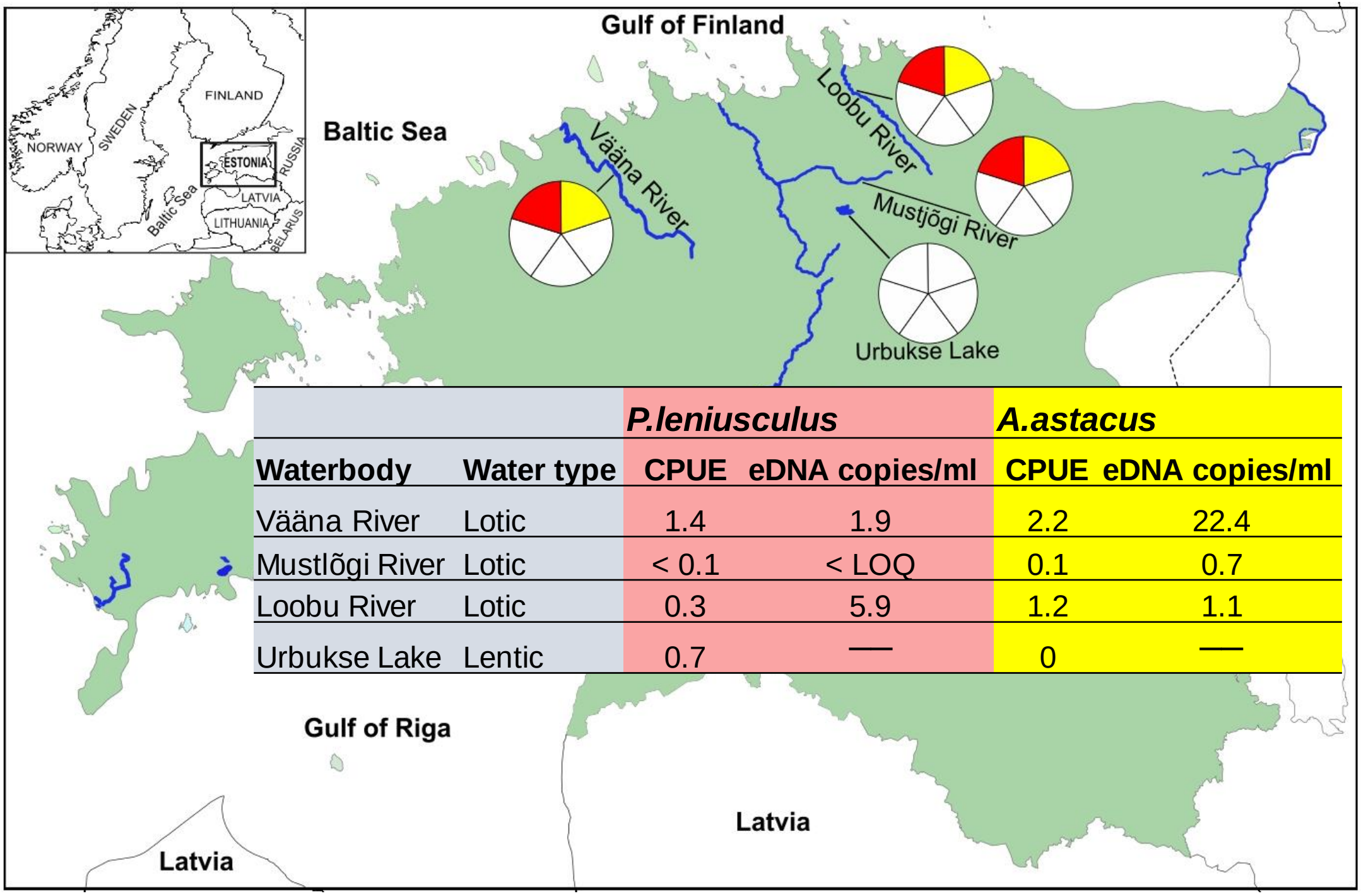
CPUE < 1 (Low density)

CPUE = 1- 4 (moderate)

CPUE > 4 (High density)



Results



P.leniusculus detection

A.astacus detection

F.limosus detection

P.virginalis detection

A.astaci detection

No detection

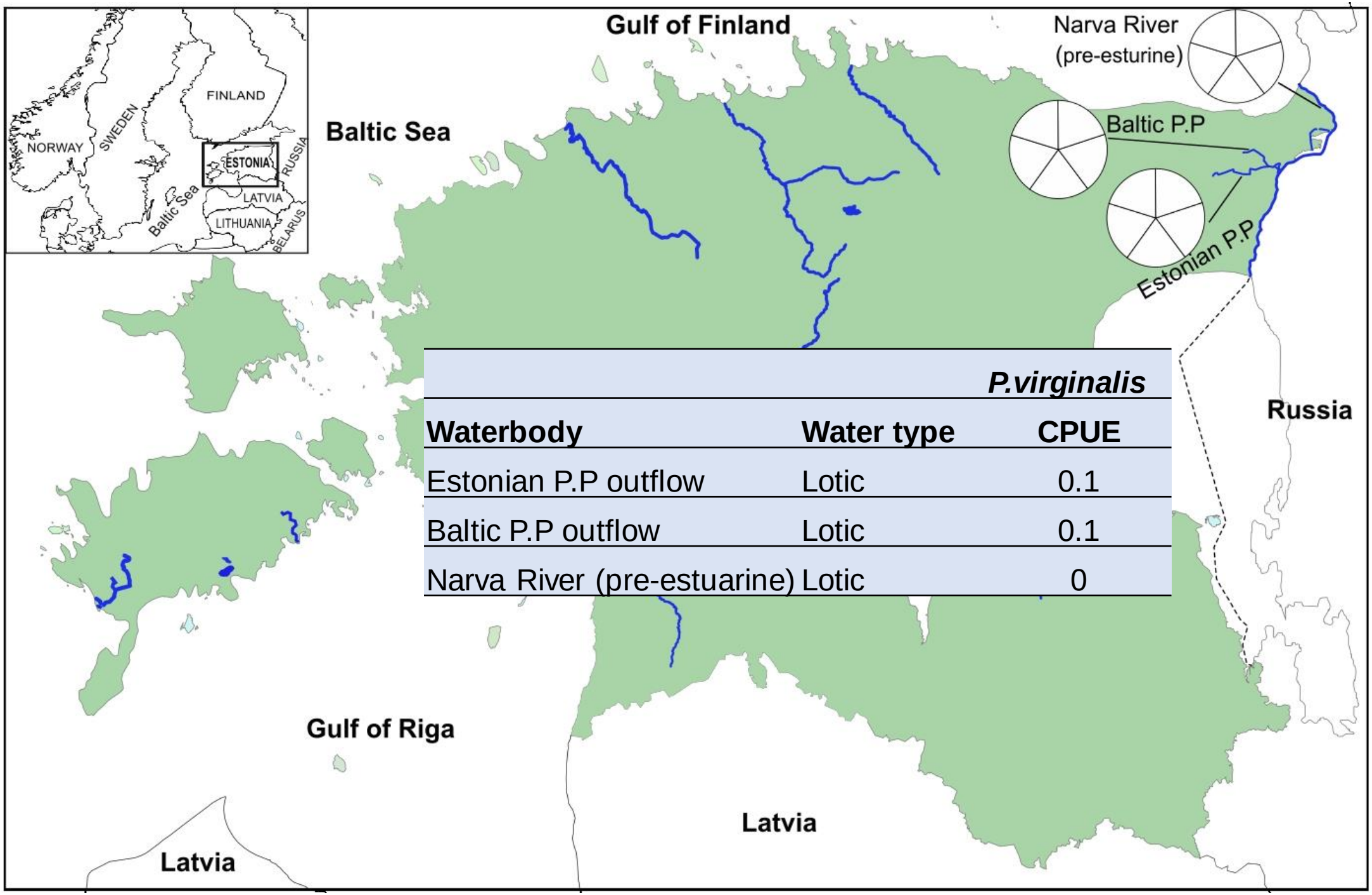
CPUE < 1 (Low density)

CPUE = 1- 4 (moderate)

CPUE > 4 (High density)

		P.leniusculus		*A.astacus*	
Waterbody	**Water type**	**CPUE**	**eDNA copies/ml**	**CPUE**	**eDNA copies/ml**
Vääna River	Lotic	1.4	1.9	2.2	22.4
Mustlõgi River	Lotic	< 0.1	< LOQ	0.1	0.7
Loobu River	Lotic	0.3	5.9	1.2	1.1
Urbukse Lake	Lentic	0.7	—	0	—

Results



		P.virginalis
Waterbody	**Water type**	**CPUE**
Estonian P.P outflow	Lotic	0.1
Baltic P.P outflow	Lotic	0.1
Narva River (pre-estuarine)	Lotic	0

Conclusions

- In lotic waters with either low or moderate crayfish population density, the eDNA method reliably detected the presence of *A.astacus and P.leniusculus*
- eDNA from the invasive NICS or *A.astacus* was not detected in lentic water bodies with low crayfish population density
- *A.astaci* eDNA was detected in only one water body (one sampling point)
- eDNA from *P. virginalis* was not detected in their distribution areas
- *F. limosus* eDNA detection was positive and consistent with the CPUE data despite the assay showing some cross amplification with *P. virginalis*
- With optimization and development, the eDNA method can be employed to enhance the use of traps in the detection, monitoring, and control of invasive NICS

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