Update on environmental DNA (eDNA) monitoring of *Aphanomyces astaci* and freshwater crayfish

Trude Vrålstad

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10th AW of the NRL for Crustacean Diseases – May 29th 2019

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Fish health
Aquatic animal health
Disease
Understanding - Prevention - Surveillance - Control

The Fish Health Research Group

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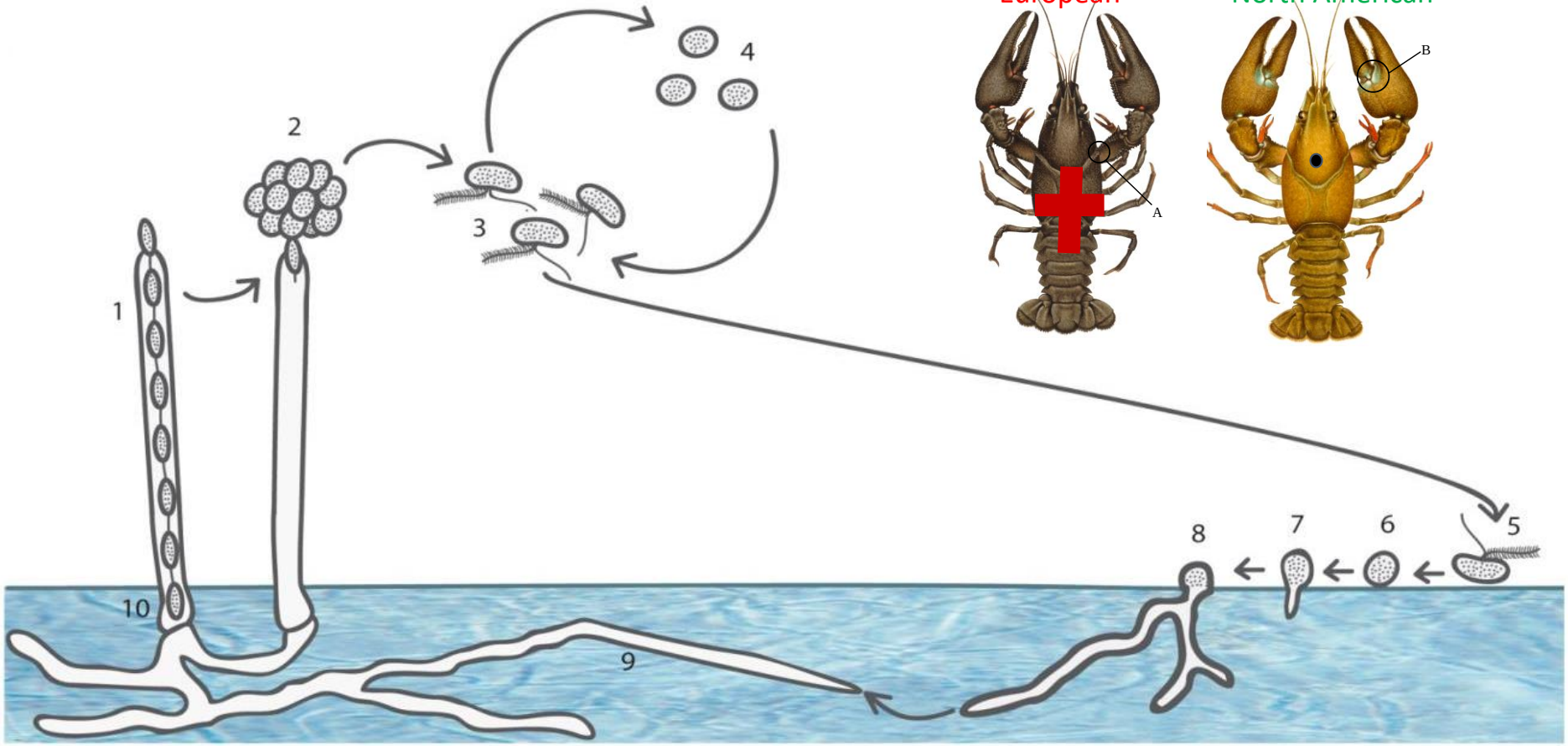
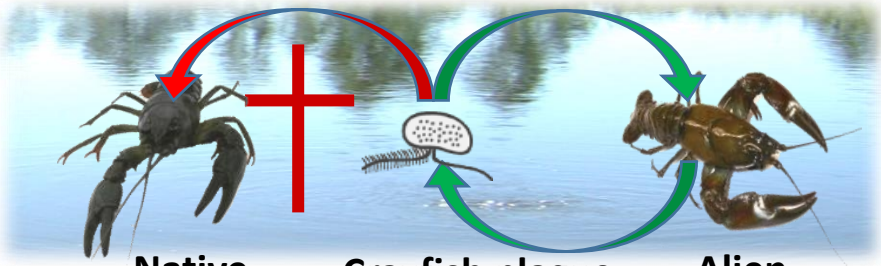
Crayfish plague – *Aphanomyces astaci*

(Oomycetes » Saprolegniales » Leptolegniaceae)

- OIE listed, lethal to European crayfish
- **Norway: List 3 (national) disease**
- 100 worlds worst invasive species
- American crayfish healthy carriers



qPCR crayfish plague
From diagnostic method to eDNA monitoring tool



Native Noble crayfish | Crayfish plague (*Aphanomyces astaci*) | Alien Signalkreps

Strand et al. 2019

2016: Implementation in National surveillance

2015: Validation during an outbreak

2014: detect in water Nature – large systems

Strand et al. 2014

2012: detect in water Aquarium conditions

Strand et al. 2012

2011: detect in water Laoratory conditions

Strand et al. 2011

2012: OIE method

2009: qPCR diagnostic assay tissue samples

Vrålstad et al. 2009

Detection & quantification of host-pathogen eDNA in water

50 YEARS WITH IMPACT
Journal of Applied Ecology
British Ecological Society
Journal of Applied Ecology 2014, **51**, 544–553 doi: 10.1111/1365-2664.12218

Detection of crayfish plague spores in large freshwater systems

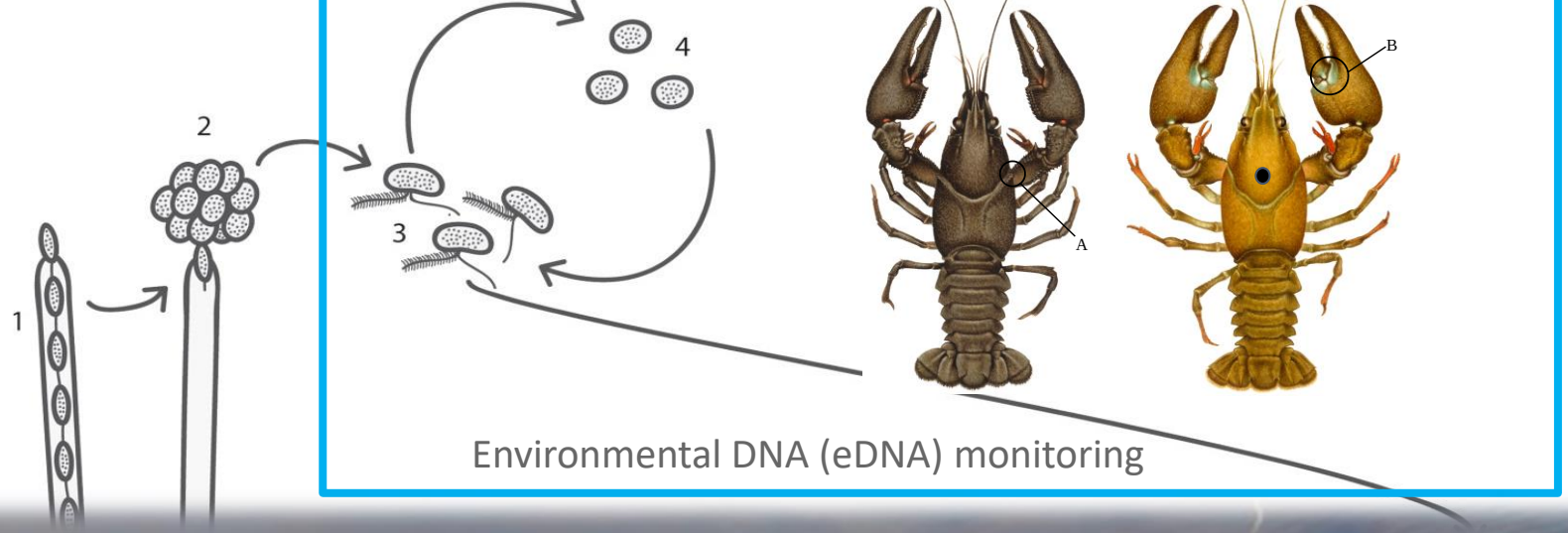
David A. Strand[1,2], Japo Jussila[3], Stein I. Johnsen[4], Satu Viljamaa-Dirks[5], Lennart Edsman[6], Jannicke Wiik-Nielsen[1], Hildegunn Viljugrein[1], Frederik Engdahl[6] and Trude Vrålstad[1,2]*

PLOS ONE

RESEARCH ARTICLE

Monitoring of noble, signal and narrow-clawed crayfish using environmental DNA from freshwater samples

Sune Agersnap[1☯*], William Brenner Larsen[1☯], Steen Wilhelm Knudsen[1], David Strand[2], Philip Francis Thomsen[3], Martin Hesselsøe[4], Peter Bondgaard Mortensen[5], Trude Vrålstad[2], Peter Rask Møller[1]



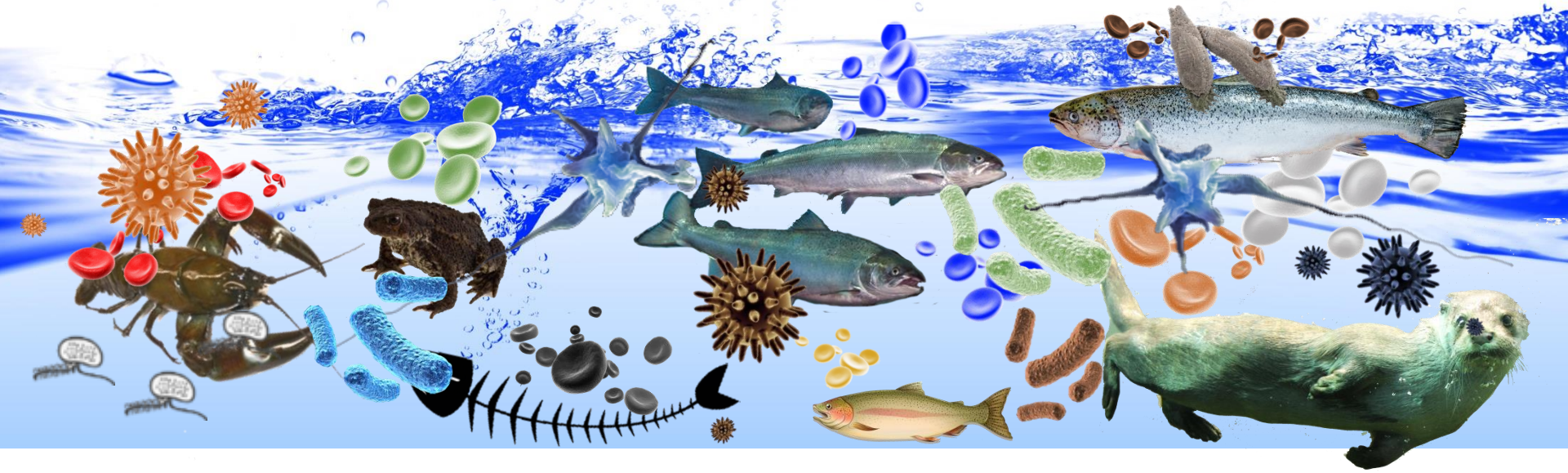
Environmental DNA (eDNA) monitoring

eDNA sources

- Aquatic animals shed cells with DNA to the environment through feces, skin, urine, sperm, eggs, mucus, blood, decomposing individuals, etc.

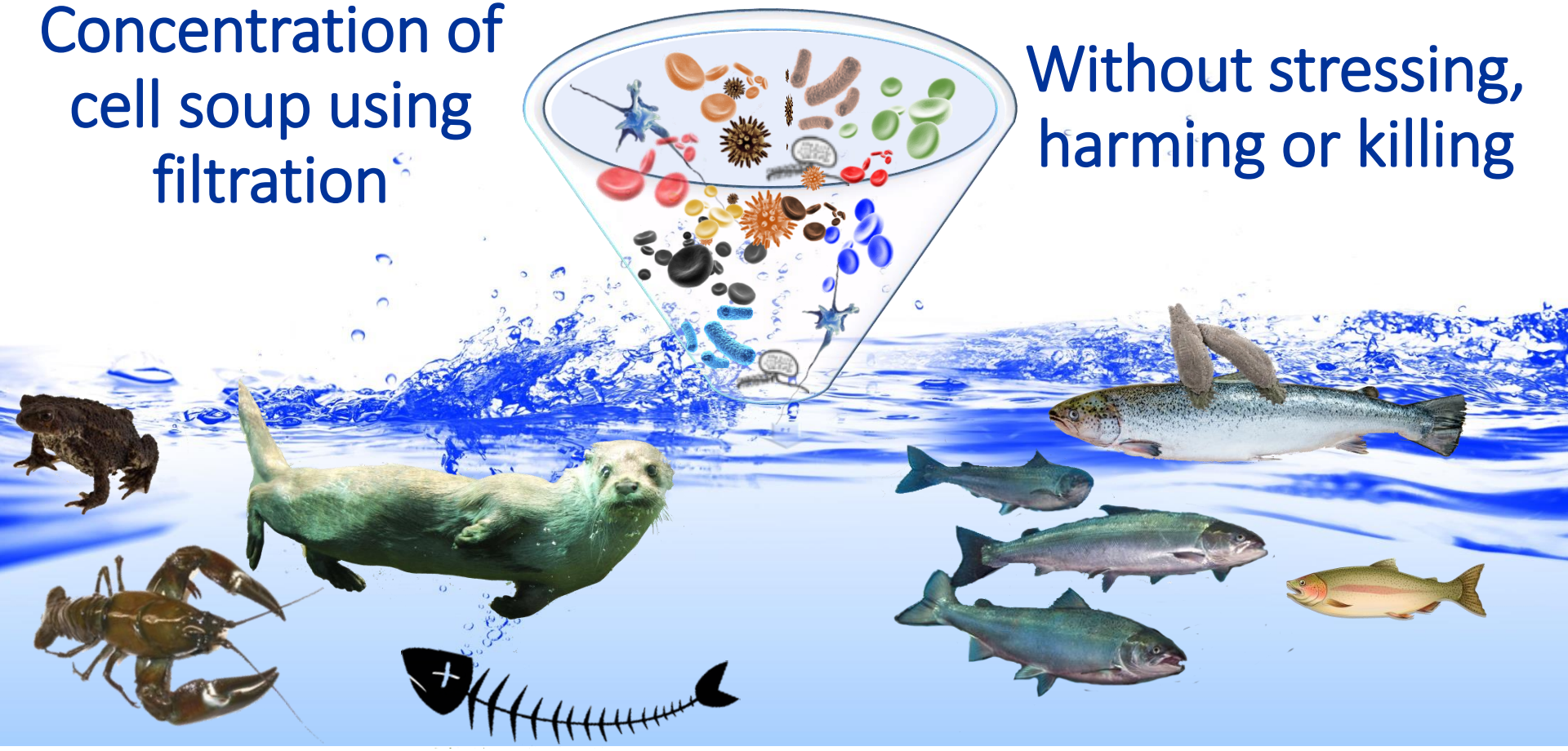
eDNA sources

- Microorganisms including pathogens lives in the environment, or are emitted from infected organisms



Concentration of cell soup using filtration

Without stressing, harming or killing



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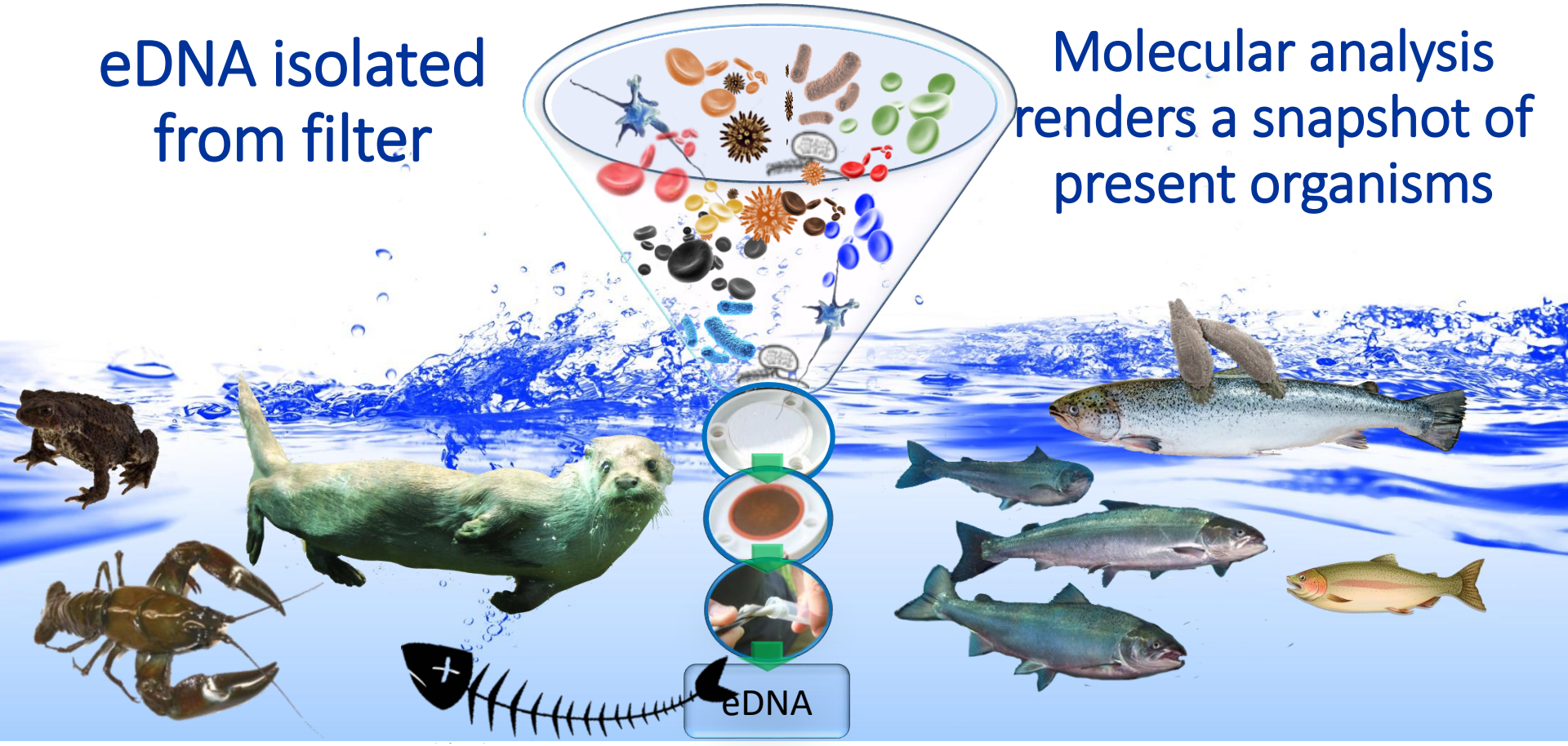
eDNA isolated from filter

Molecular analysis renders a snapshot of present organisms



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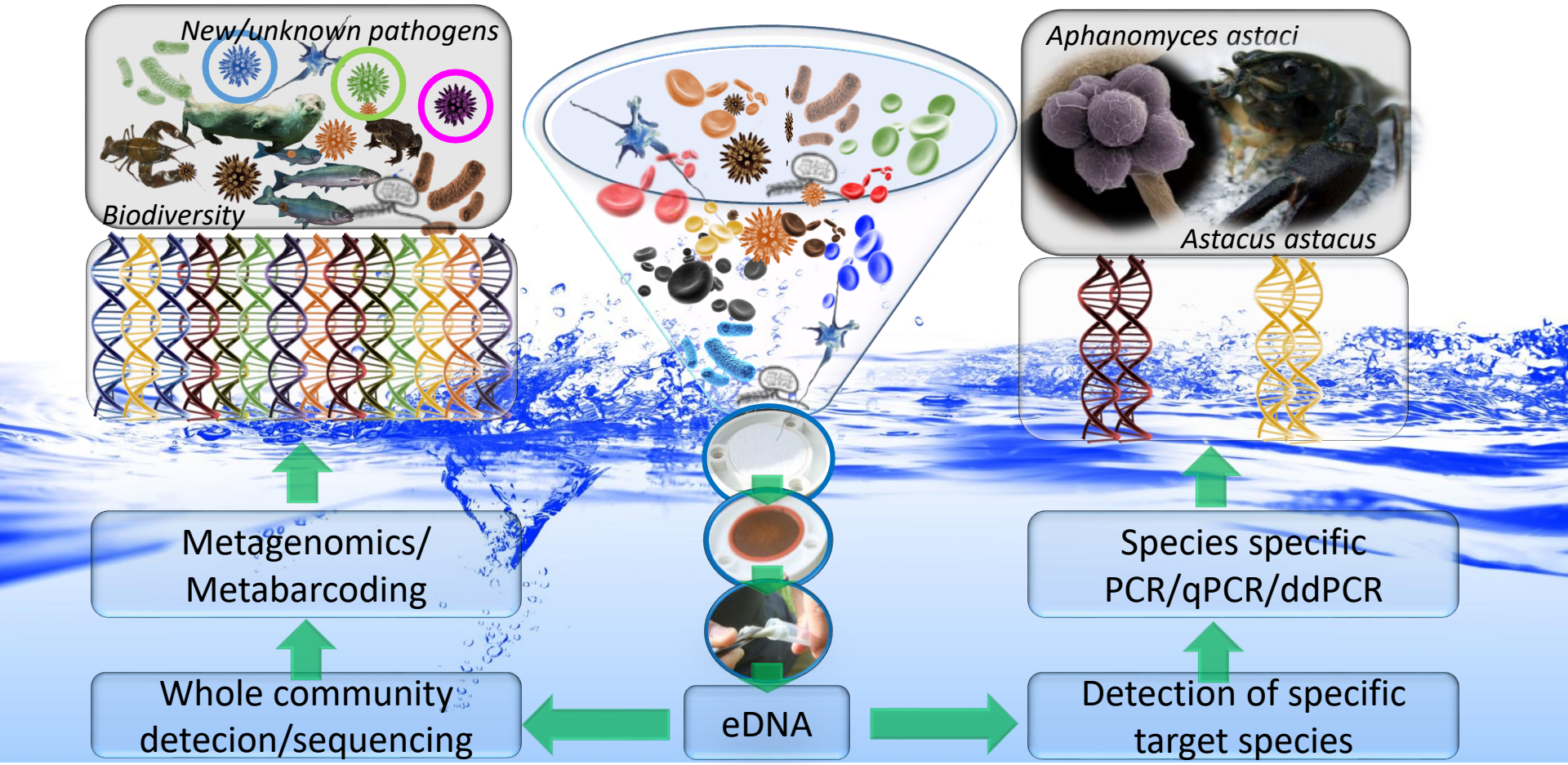

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eDNA sampling protocols

Collecting vessel with lead & tube

Pump, power & filtration system

Alternative

Battery driven easy portable water sampler (all in one box system, expensive)

Canister meassue filtered water volume (~5 L)

Alternative

Alternative

Disposable filter cup

Drill-powered pump (cheap)

eDNA sampling protocols and downstream analyses

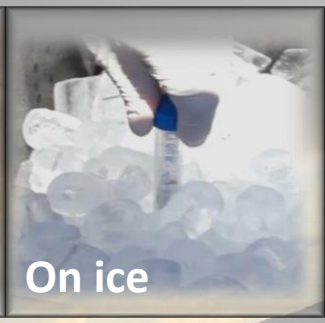

Clean filter


Filter holder

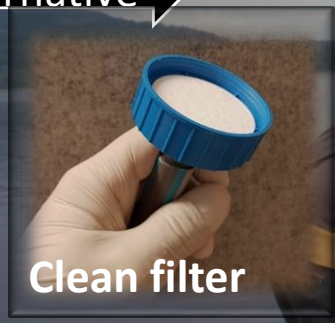

5 L later

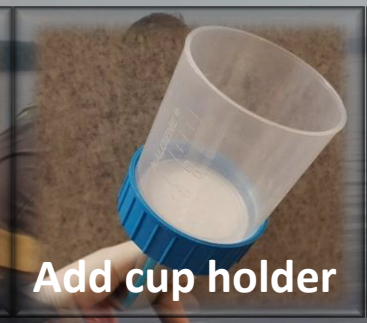

Remove

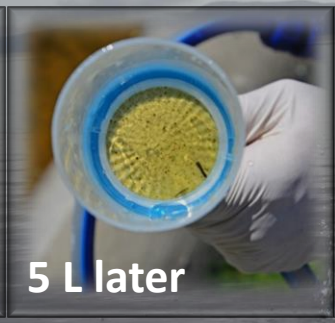

Store


On ice

Alternative


Clean filter


Add cup holder

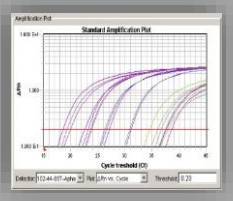

5 L later


Remove


Store in silica

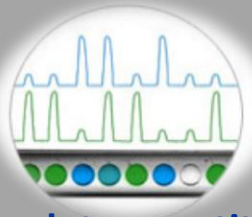
Large volume DNA extraction

qPCR



Relative quantity

ddPCR



Absolute quantity

eDNA monitoring of A. astaci and freshwater crayfish in a real-life situation

- Ørje locks closed since 2005
  - Stop the spread of crayfish plague and signal crayfish
- Signal crayfish moved illegally upstream Ørje locks
  - Discovered during noble crayfish surveillance in 2014
  - **The onset of a crayfish plague outbreak unavoidable**

Received: 29 November 2018 | Accepted: 10 March 2019
DOI: 10.1111/1365-2664.13404

RESEARCH ARTICLE

Journal of Applied Ecology | BRITISH ECOLOGICAL SOCIETY

Monitoring a Norwegian freshwater crayfish tragedy: eDNA snapshots of invasion, infection and extinction

David A. Strand[1,2] | Stein Ivar Johnsen[3] | Johannes C. Rusch[1,4] | Sune Agersnap[5,6] | William Brenner Larsen[5] | Steen Wilhelm Knudsen[5] | Peter Rask Møller[5] | Trude Vrålstad[1]

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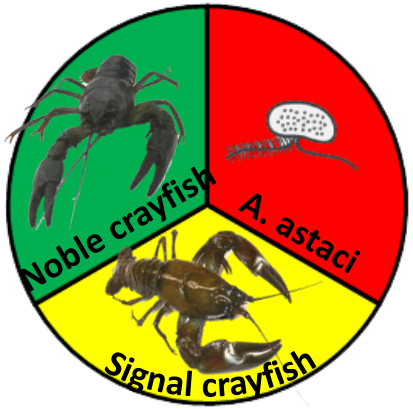
Comparing methods during an outbreak

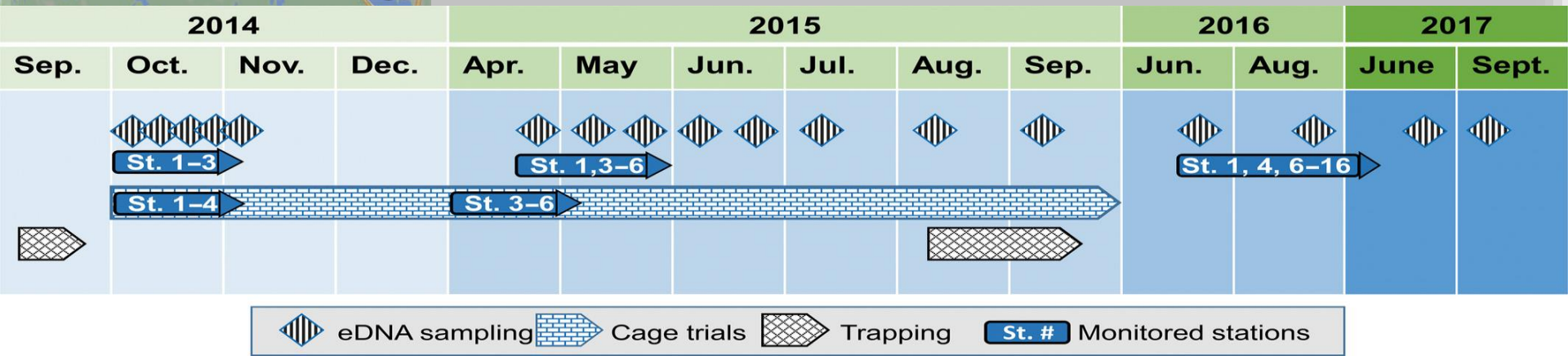
- Compare conventional surveillance with eDNA monitoring
- National surveillance noble crayfish = trapping
- National surveillance crayfish plague = cage trials



eDNA monitoring

Cage trials

Trapping (CPUE)



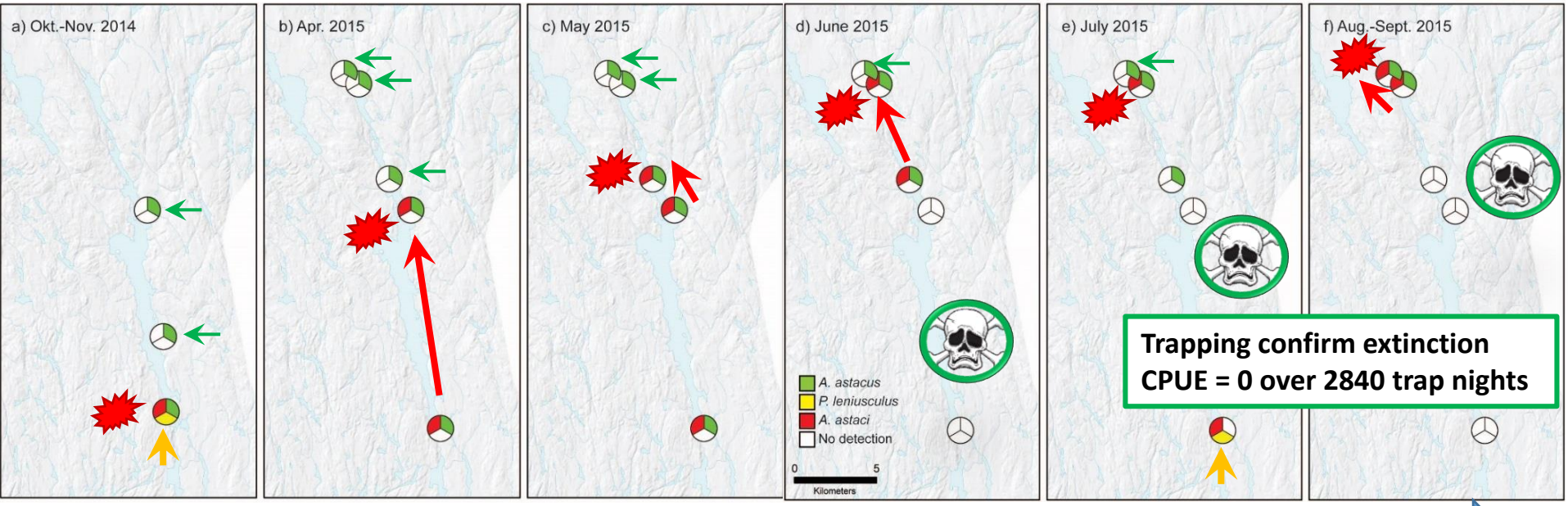
eDNA monitoring


Cage trials

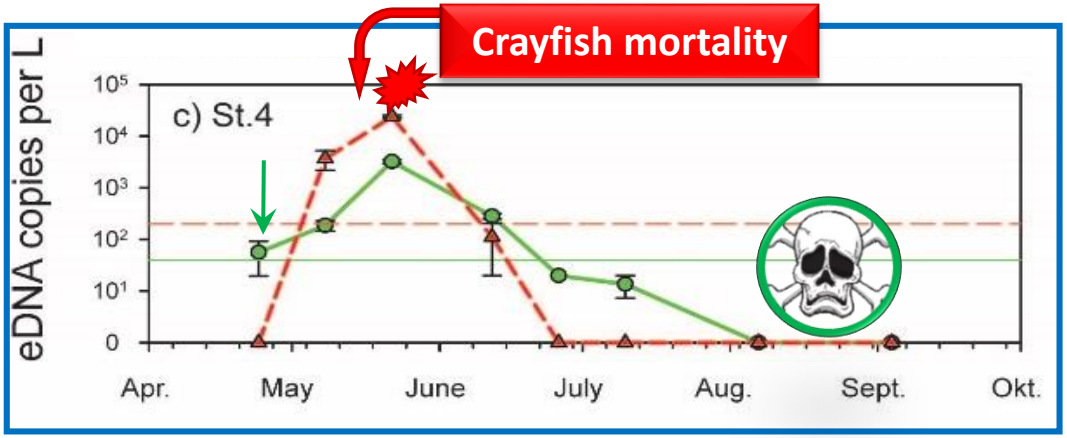

Trapping (CPUE)

Comparing methods during an outbreak

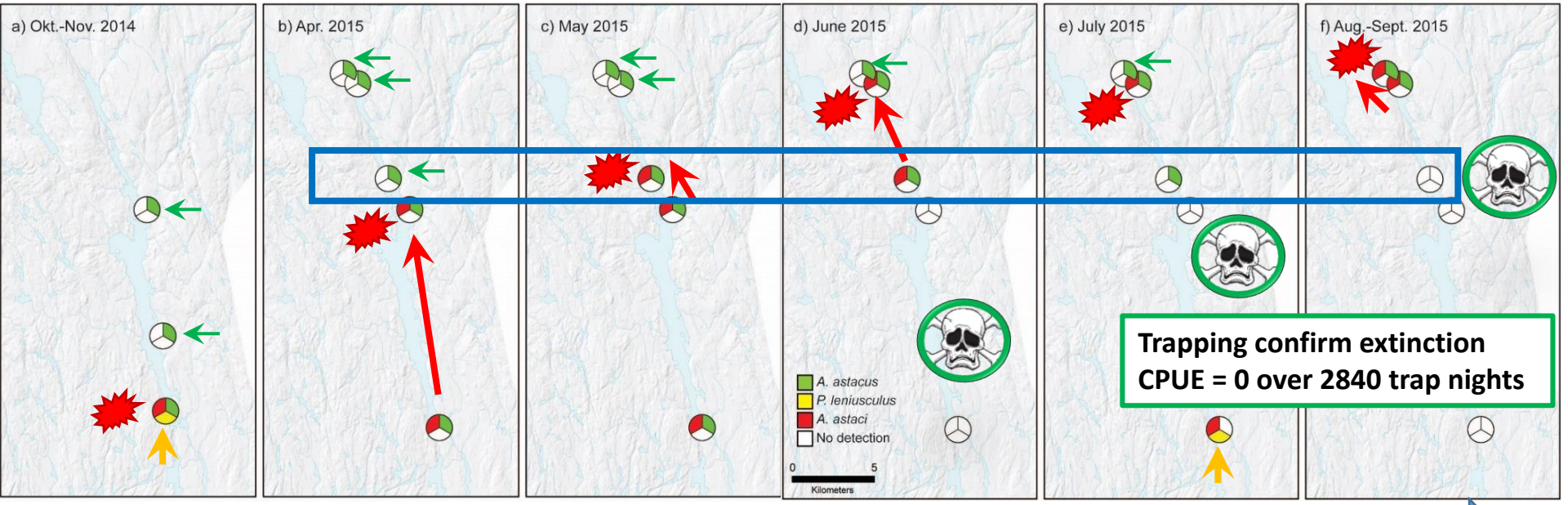
- The crayfish plague outbreak started late in 2014.
- *A. astaci* eDNA detected 3 weeks before mortality of crayfish observed in the cages
- Upstream spread also during winter
- After one year, 23 km spread
- Noble crayfish died in the cages and lake – and eDNA of both targets vanished
- A huge trapping effort with 0 noble crayfish confirm local extinction
- Detect signal crayfish at low densities (~0.12 CPUE) – but not every time



eDNA fluctuations - from infection to extinction



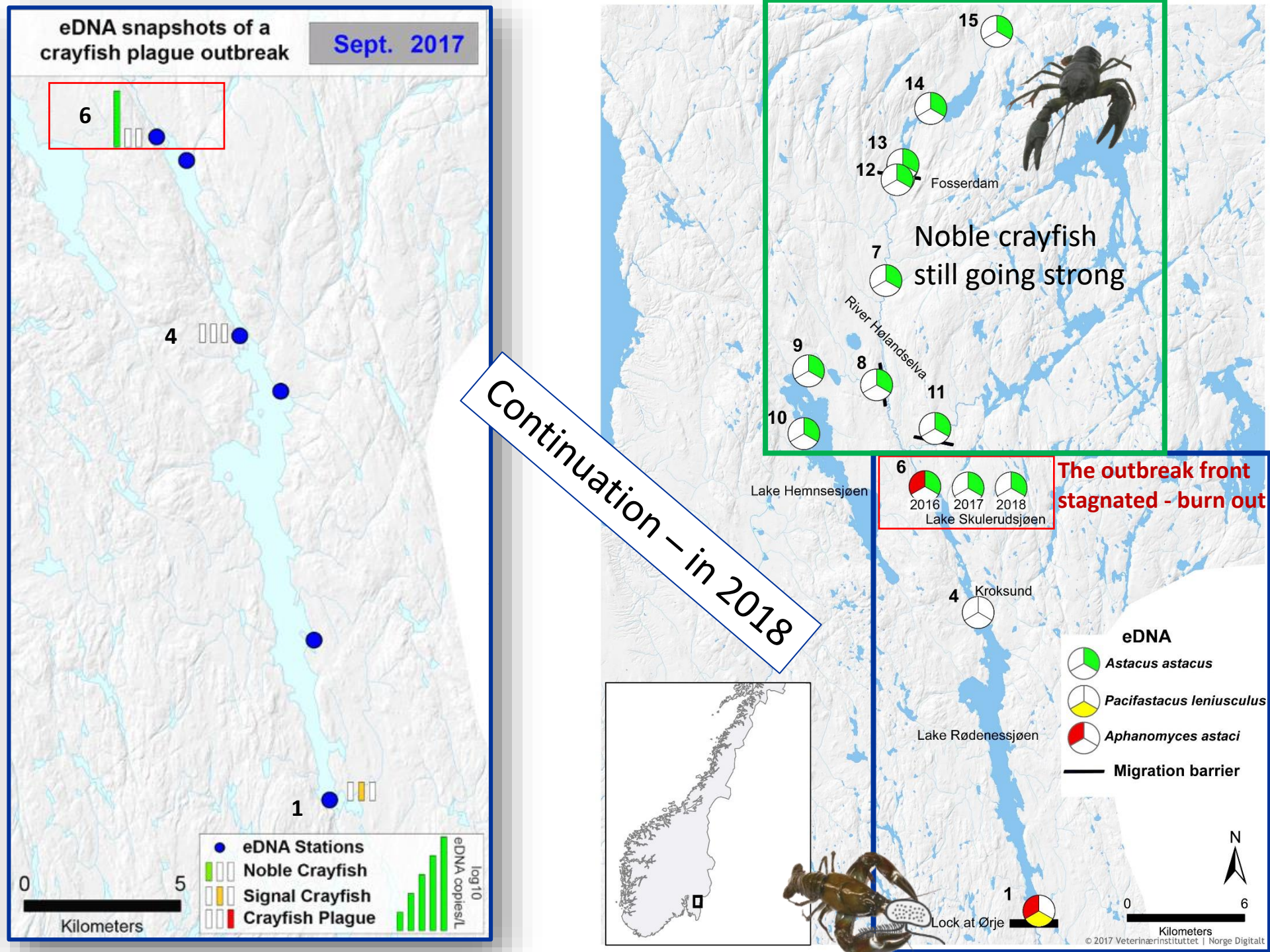
- First only eDNA of noble crayfish.
- Then *A. astaci* detectable at low levels – then increased
- Parallel trend for noble crayfish eDNA
- Then eDNA of both targets disappeared, reflecting the local extinction



Oktober April Mai Juni Juli August

eDNA snapshots of a crayfish plague outbreak

- 2014
  - eDNA of all targets at the water locks
  - Upstream spread under the ice
- 2015
  - Outbreak spreads steadily
  - eDNA increase during outbreak – then disappear
- 2016
  - The outbreak front stagnated and burn out
- 2017
  - *A. astaci* only seen in the signal crayfish location
  - Noble crayfish eDNA remain where the outbreak burnt out
- See the time-lapse here: https://appliedecologistsblog.com/2019/06/07/edna-snapshots-of-invasion/


eDNA snapshots of a crayfish plague outbreak
Sept. 2017
6
4
1
eDNA Stations
Noble Crayfish
Signal Crayfish
Crayfish Plague
log10 eDNA copies/L
0
5
Kilometers
Continuation – in 2018
15
14
13
12
Fosserdam
Noble crayfish still going strong
7
River Høylandselva
9
8
11
10
Lake Hemnsesjøen
6
2016 2017 2018
Lake Skulerudsjøen
The outbreak front stagnated - burn out
4
Kroksund
eDNA
Astacus astacus
Pacifastacus leniusculus
Aphanomyces astaci
Migration barrier
Lake Rødenessjøen
1
Lock at Ørje
N
0
6
Kilometers
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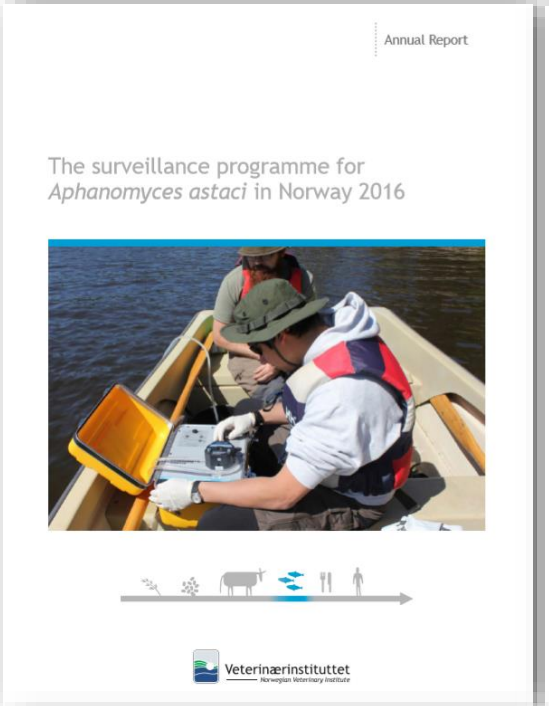

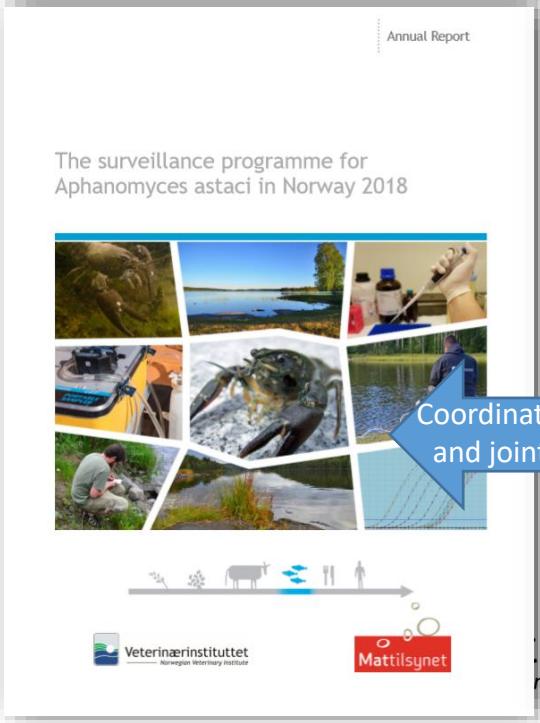

Coordination work and joint results

eDNA implemented in Norwegian surveillance

- 2016: eDNA supplement cage experiments in *A. astaci* surveillance
- 2017: eDNA replace cage experiments in *A. astaci surveillance*
- 2018: Joint use of eDNA results *A. astaci* and crayfish surveillance programs
- Continues in 2019…

eDNA study in Czech Republic (2017-2019)

Rusch et al. *in prep*

Evaluate the presence of *A. astaci* and crayfish in waterbodies within Czech Republic
Collaboration with Prof. Adam Petrusek, Charles University Prague

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Mobile technology and on-site detection of crayfish plague

https://www.youtube.com/watch?v=flvIH_Zj9aM&feature=youtu.be

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Summary - eDNA monitoring

- An efficient and rapid monitoring tool – but still only a snapshot
- Fluctuations of eDNA amount can reflect stages of infection – outbreak - extinction
- Detect the infectious spores in the water earlier than caged crayfish
- Simultaneous detection of crayfish eDNA – extra confirmation for little extra cost
- Well suited for
  - Early warning of invasion or infection
  - Risk assessments / Habitat evaluation / Surveillance of pathogen/host
- Non-invasive and animal welfare friendly
- Has replaced the cage trials with live crayfish (“canaries in a coalmine”)
- What is next?  Automated continuous eDNA monitoring?

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- National surveillance program Noble crayfish
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  - Tom Andersen, UiO
  - Stein Ivar Johnsen, NINA
  - Peter Rask Møller, Sune Agersnap, Steen Knudsen, UoC
  - Adam Petrusek, CUNI