European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

16th Annual Workshop of the National Reference Laboratories for Crustacean Diseases
28th May 2025

Technical Presentations


DTU

Niccoló Vendramin and Thomas Weise

Survey and diagnosis of crustacean diseases in Europe 2024

Disease situation in EU for crustaceans

All NRLs were asked to answer the following questions for their country:

- 1) Report the number of farms belonging to each health status category according to COMMISSION DELEGATED REGULATION (EU) 2020/689.

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2020/689
Approved disease free
Eradication/control program
Farm under surveillance but not in eradication program
Not approved disease free and not under eradication/control program

Disease situation in EU for crustaceans

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- 3) Report the number of samples tested for WOAH listed crustacean diseases and how many of these gave a positive result.

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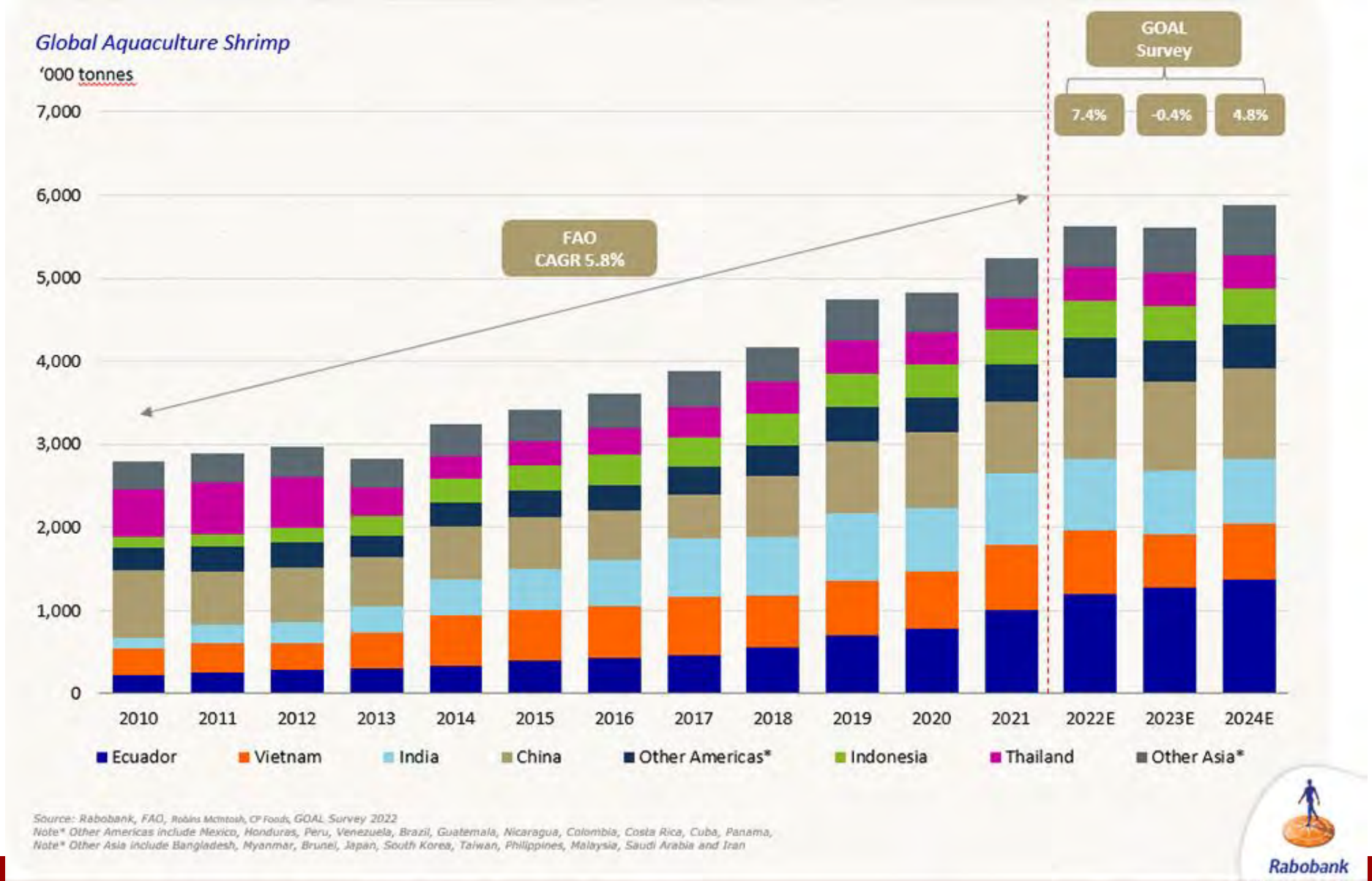
- 3) Report the number of samples tested for WOAH listed crustacean diseases and how many of these gave a positive result.
- 4) Describe the current status of crustacean aquaculture in the country, as well as the strategy used for surveillance of crustacean diseases.

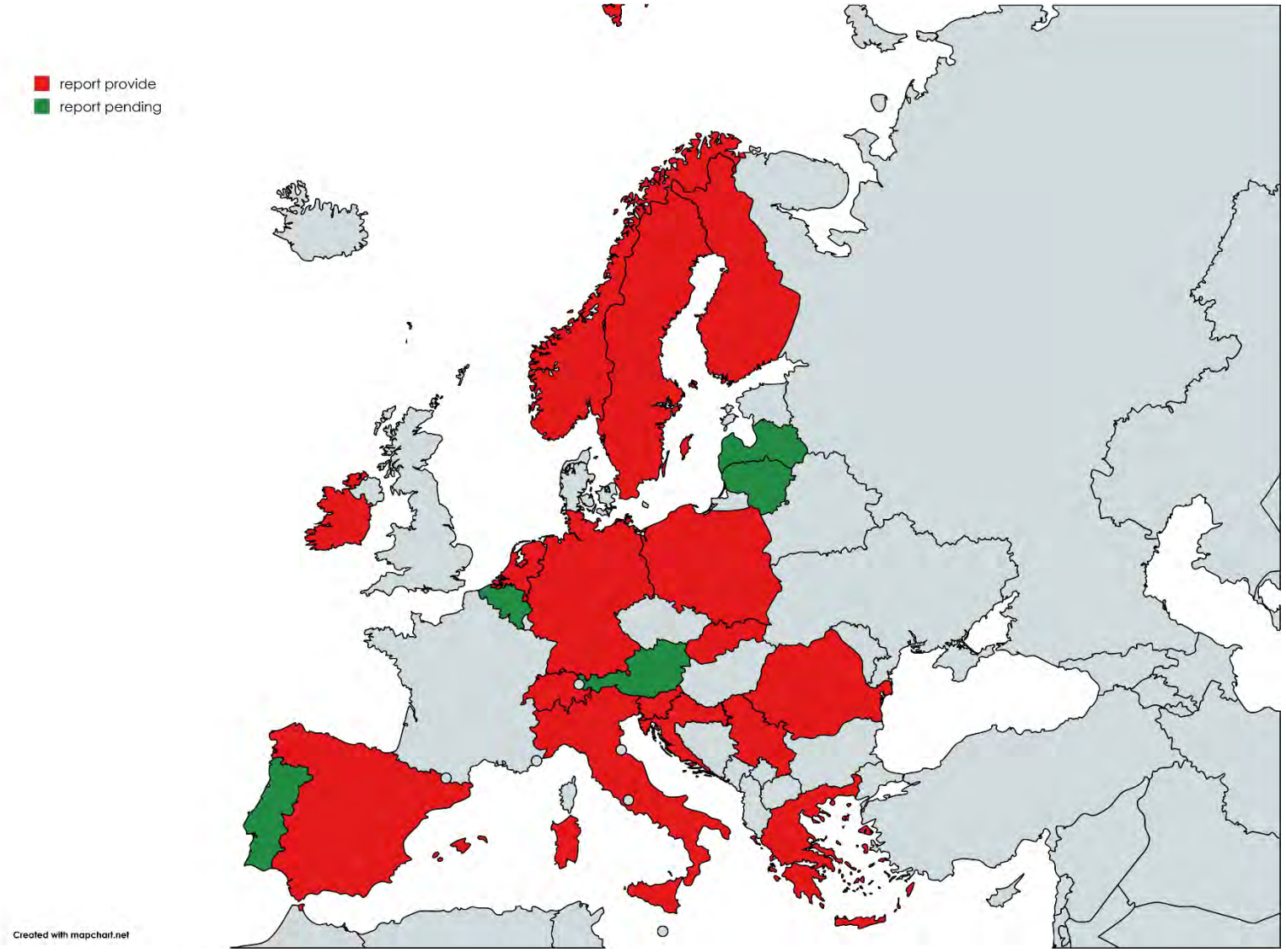
Thanks to all NRLs that answered the survey!

Shrimp production worldwide

Survey results indicate that the world's production of farmed shrimp in 2023 will likely be slightly lower (down 0.4 percent) at around 5.6 million metric tons (MMT) than in 2022, but that it is expected to grow by about 4.8 percent in 2024 to close to 5.88 MMT

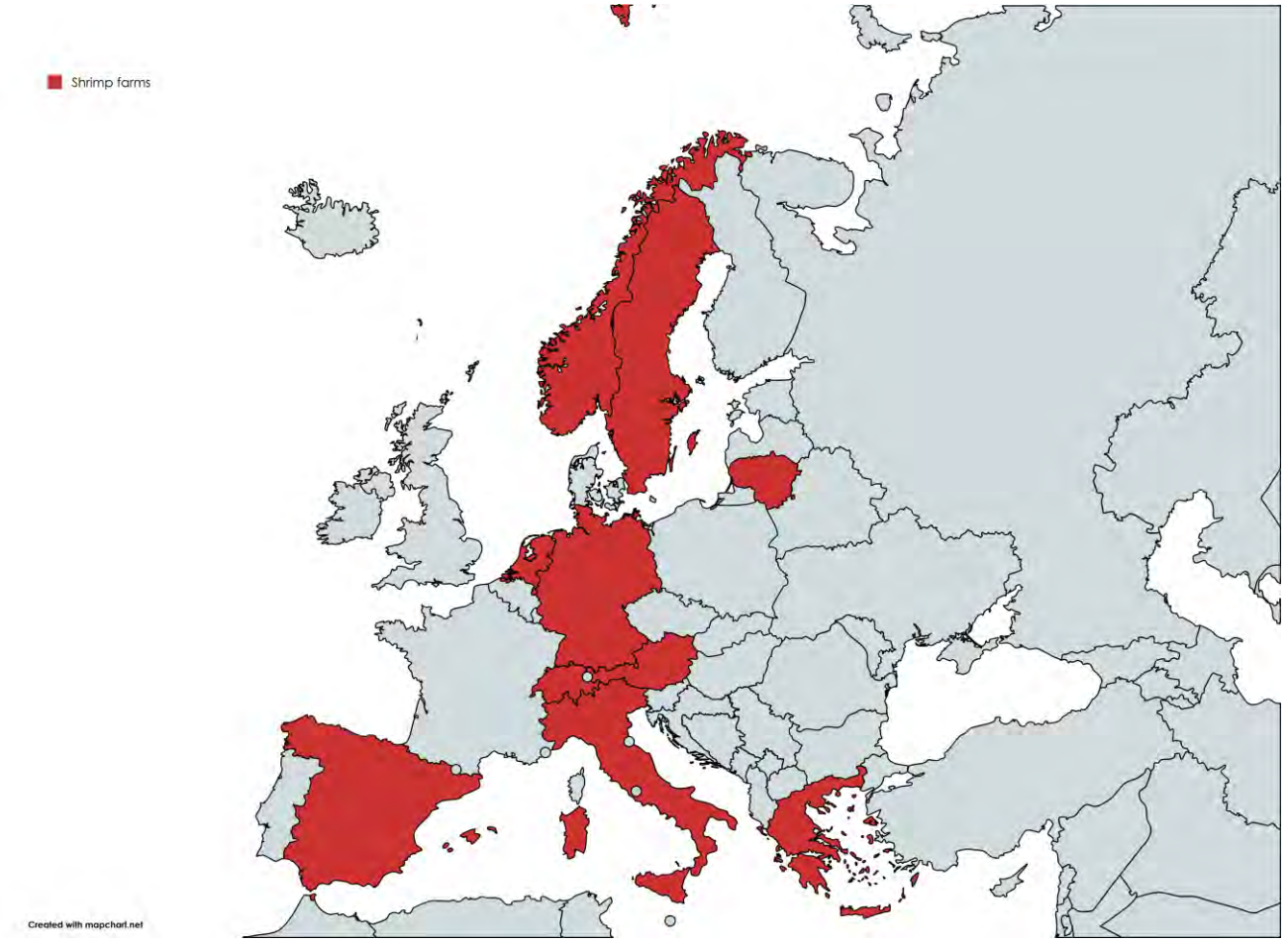


16 countries have currently answered

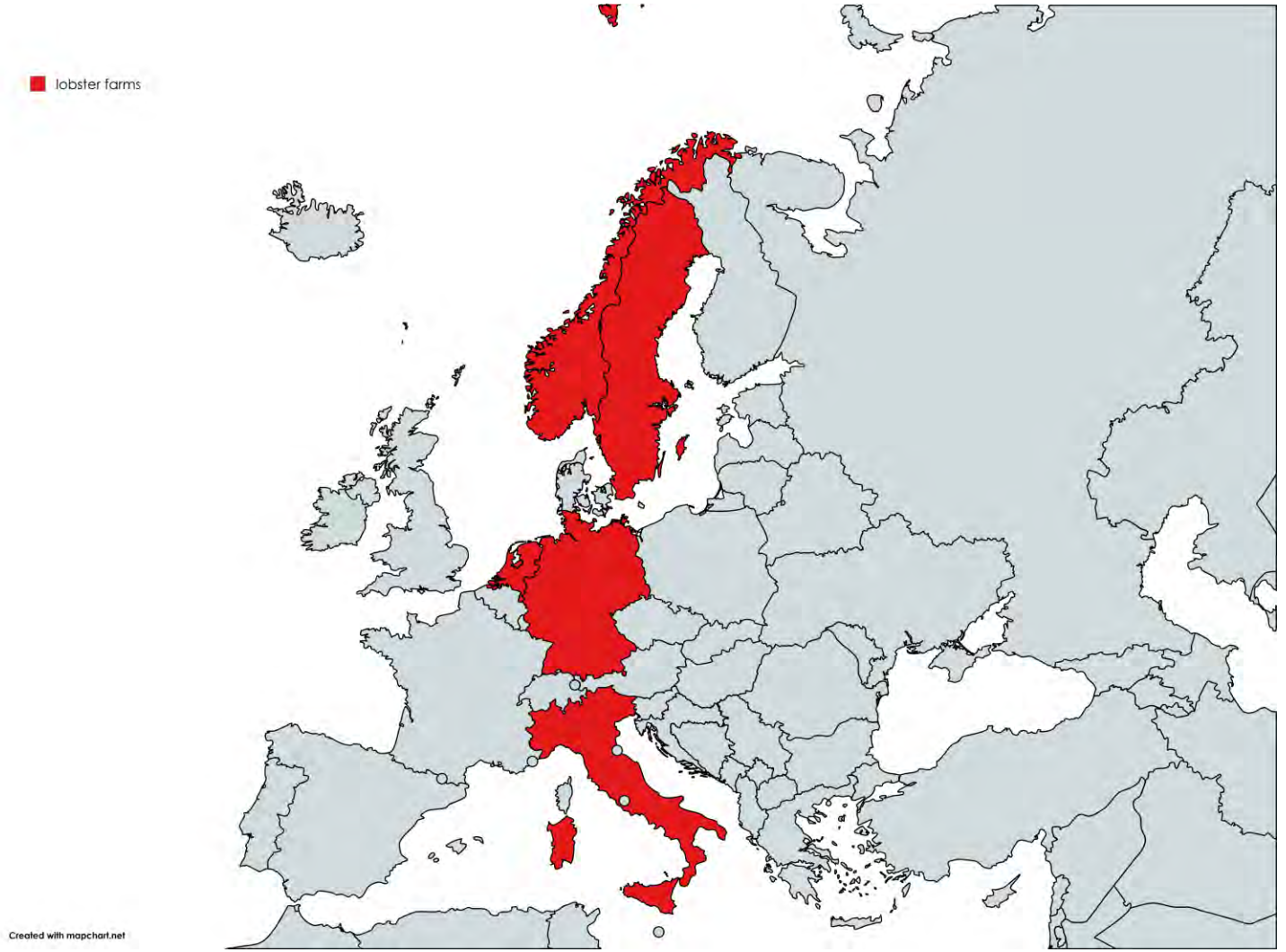


10 countries with shrimp farms. Total: 31 farms

Country	Shrimp					
	2024	2023	2022	2021	2020	2019
Spain	1	2		1	9	30-36
Germany	6	8	8	6	11	8
Greece	1	1	1			
France	-	-	10	8		
Belgium	-	1	2	2	2	
Switzerland	4	4	4	4	3	3
Italy	5	5	5	5	8	11
Lithuania	Report Pending	2	2	2	2	2
Norway	3	2	2	2	1	
Netherlands	1	1	1	1	1	1
Poland	0	2				
Sweden	4	4				
Austria	Report Pending	4				



5 countries with lobster farms (mostly repopulation/aquaria). Total: 22 farms



11 countries with crayfish farms. Total: 105 farms

Country	Crayfish					
	2024	2023	2022	2021	2020	2019
Spain	2	2	0	2		4
Germany	21	43	40	38	<42	33
Belgium	-	5	2	2	3	
Switzerland	1	1	1	1		
Italy	19	19	16	15	15	23
Lithuania	report pending	1	1	1	1	
Norway	2	2	2	2	1	
Poland	<10	16	9	10	10	10
Finland	7	11	8	5	5	5 – 28
Serbia	1	1				
Sweden	33	1	≥11			
Austria	report pending	18	1			
Denmark	-	7	≥3	≥3	≥3	≥3

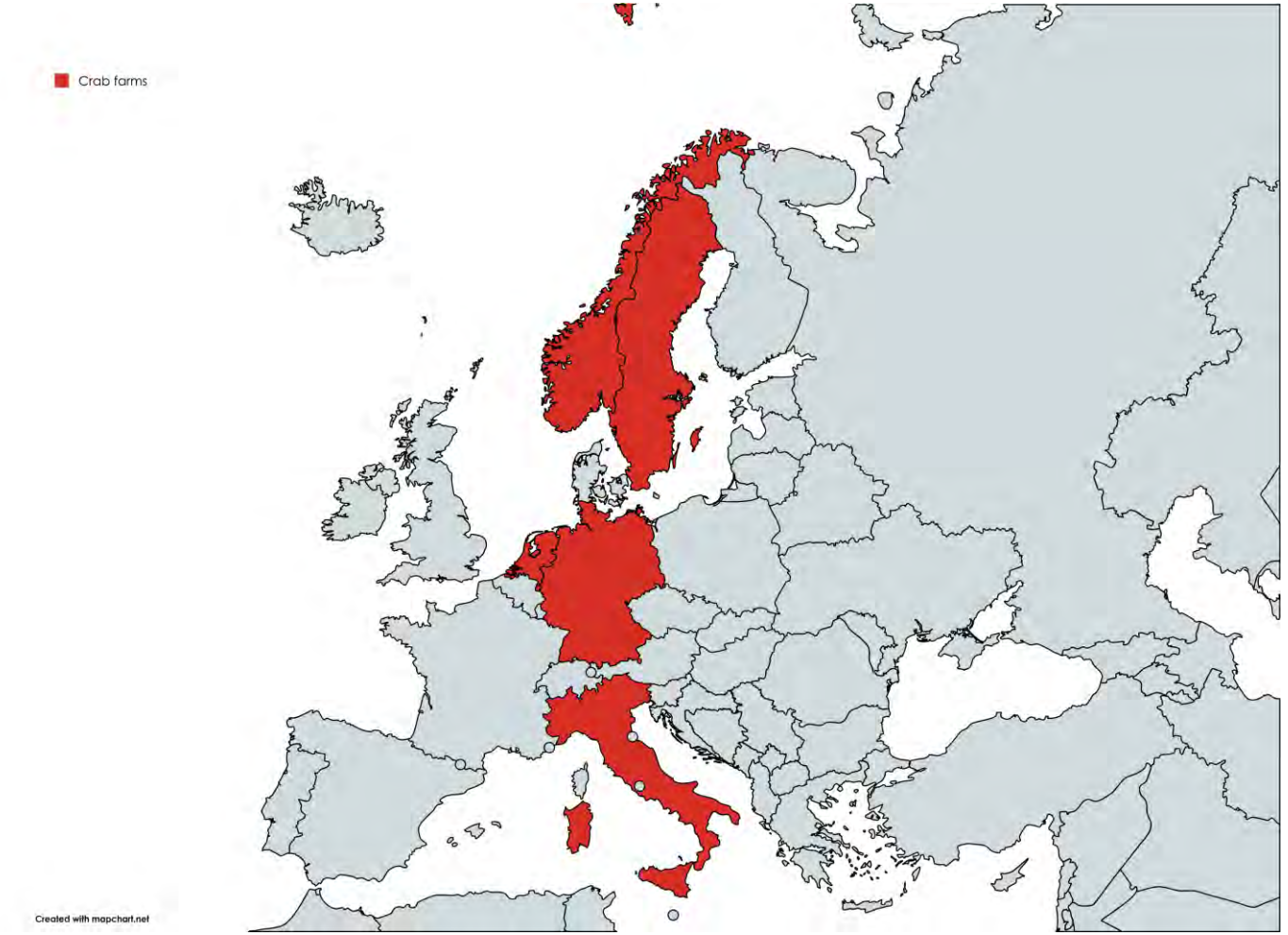
Crustacean farms in Europe

(Some farms may not currently be active, many lobster and crayfish farms produce animals for restocking of wild populations)

*from 2020 only active farms are included; **2021 data from some regions pending; ***data from many regions pending

Country	Shrimp						Crayfish						Lobsters					
	2024	2023	2022	2021	2020	2019	2024	2023	2022	2021	2020	2019	2024	2023	2022	2021	2020	2019
Spain	1	2	0***	1**	9*	30-36	2	2	0***	2		4			0***			
Germany	6		8	6**	11?	8	21		40	38	<42	33	4		4	5	1	4
France			10	8														
Belgium		1	2	2	2			5	2	2	3							
Switzerland	4	4	4	4	3	3	1	1	1	1								
Italy	5	5	5	5	8	11	19	19	16	15	15	23	1	1	1	1	1	
Lithuania		2	2	2	2	2		1	1	1	1							
Norway	3	2	2	2	1		2	2	2	2	1		1	1	1	1	1	
Netherlands	1	1	1	1	1	1							1	1				
Greece	1	1	1															
Poland		2						16	9	10	10	10						
Finland							7		8	5	5	5 – 28						
Denmark									≥3	≥3	≥3	≥3						
Sweden	4	4					33	1	≥11				15		2			
Austria		4						18	1									

4 countries with crab farms. Total: 10 farms.



What are then disease problems observed in crustacean in Europe?

Country	Crustacean species	Diseases Symptoms	Crustacean species	Diseases Symptoms	Crustacean species	Diseases Symptoms
Austria	Astacus astacus and other indigenous crayfish species	crayfish plague				
Finland	Astacus astacus	Aphanomyces astaci	Pacifastacus leniusculus	Aphanomyces astaci carriers		
Germany						
Italy	Austropotamobius pallipes complex (Austropotamobius italicus)	Astathelohania contejeani /Nosema austropotamobii chronic infections in wild populations	Austropotamobius pallipes complex (Austropotamobius italicus)	Aphanomyces astaci	Callinectes sapidus	Hematodinium sp.; Lagenidium callinectes
Sweden	Noble crayfish, Astacus astacus	Crayfish plague				
Norway	Noble Crayfish (Wild populations)	Crayfish plague				
Switzerland	different species of native and non-native crayfish	Crayfish plague				
Ireland	Austropotamobius pallipes	Crayfish Plague				
The netherladns	lobsters		mortalities in the Oosterschelde area (unresolved			

Crayfish plague

Countries with surveillance and diagnostics in 2024

Country	Diagnostics	Diagnostic Samples	Positive tests
Croatia	Aphanomyces	12	0
Finland	WSSV Aphanomyces	5 12	0 8
Germany	WSSV Aphanomyces	19 201	0 29
Ireland	WSSV Aphanomyces	9 9	0 2
Italy	WSSV Aphanomyces cuticle /swabs/eDNA filter Thelohania contejeani Hematodinium	26 27 /380/19 19 117	0 9 15 16
Norway	WSSV Aphanomyces	1 12	0 3
Netherlands	WSSV	10	0
Serbia	Aphanomyces	2	0
Slovakia	Aphanomyces	1	0
Switzerland	Aphanomyces	46	19
Sweden	WSSV TSV YSV Aphanomyces	113 1 1 147	0 0 0 10

No testing performed:

Austria

Bulgaria

Denmark

Greece

Latvia

Lithuania

Poland

Romania

Slovakia

Slovenia

Spain

Detection of WSSV in Austria in 2024

- EURL contacted from Austrian NRL during AW 2024.
  - Increased mortality in wild stock of *Orconectes limosus*
  - The crayfish tested positive for Crayfish plague and tested also weakly positive for WSSV
  - First batch of purified DNA shipped to DTU which tested positive at Austrian NRL
  - Along with purified DNA (+ve for WSSV) a set of Crayfish from the same sampling in isopropanolol

First testing:

DNA samples: 7/10 WSSV +ve by qPCR

2/10 WSSV +ve end point PCR and sequencing

(sequencing provide no epidemiological information as is a very conserved region)

Crayfish in isopropanol, pleopods and telson tested: 3/10 +ve qPCR

Sampling and testing - 2

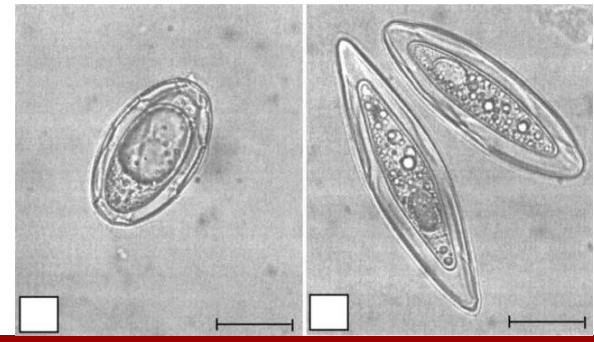
Additional sampling of crayfish ongoing mortality event performed

5 crayfish send to EURL for further confirmation

- Tested gills, pleopods and muscle by qPCR for WSSV -> -ve

Crayfish fixed in Davidson send to histopath. At IZSVe

Histopathology investigation revealed massive infestation with in 5 out of 6 specimens presence of the parasite *Psorospermium haeckeli* in the connective tissues and in 3/6 specimens presence of intranuclear bacilliform virus (putative Nudivirus) in the hepatopancreas.



Callinectes sapidus – Blue Crab

- Firstly observed in Adriatic sea in 1949
- Likely introduced with ballast water
- Potentially in relation to climate change steep increase in population
- High impact on bivalves production – predation

- Projects on health status running at IZSVe
- Projects on biological control (using Octopus or eels)

Callinectes sapidus – Blue Crab

Vol. 113: 163–167, 2015
doi: 10.3354/dao02829

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 9

NOTE

Blue crabs *Callinectes sapidus* as potential biological reservoirs for white spot syndrome virus (WSSV)

James W. B. Powell[1], Craig L. Browdy[2,4], Erin J. Burge[3,*]

Official Journal
of the European Union

EN
L series

2024/216

12.1.2024

COMMISSION IMPLEMENTING REGULATION (EU) 2024/216

of 11 January 2024

amending the Annex to Implementing Regulation (EU) 2018/1882 concerning listed diseases of aquatic animals and the list of species and groups of species posing a considerable risk for the spread of those listed diseases

(Text with EEA relevance)

Likely to be amended by WOAH !

Name of listed disease	Category of listed disease	Listed species	
		vannamei	Vector species
nfection with white spot syndrome virus	C+D+E	All decapod crustaceans (order Decapoda)	

Conclusions

- Production of farmed crustaceans in Europe is still very low
- Number of shrimp farms relatively stable
- One German and one Greek farm “Approved disease-free” all others in “Not approved disease free and not under eradication/control program”
- Apart from one detection of WSSV and crayfish , disease incidents are very low

Questions or comments?

Shrimp aquaculture in Europe: status, prospects, and the role of networking

28/05/2024

16th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Lyngby, Denmark

Author:
Paolo Gamberoni
Mirko Bögner

Shrimp aquaculture worldwide

European shrimp aquaculture

- Production: *Penaeus vannamei* leads with 6.8 million tons/year
- Why *P. vannamei* so successful? Palatability, disease resistance, fast growth
- Asian countries (China) main supplier, mostly using ponds
- 555k tons (377k Penaeid shrimps) imported in EU in 2024 (+5% from 2023), > 6 billion euros

Species or species group	2018	2019	2020	2021	2022	Share of species in species group, 2022 (%)
	(thousand tonnes, live weight equivalent)					
Crustacean	**9 501**	**10 422**	**11 108**	**11 948**	**12 751**	**100**
Penaeid shrimps	6 056	6 504	6 881	7 405	7 934	62.2
Red swamp crayfish	1 714	2 168	2 469	2 710	2 967	23.3
Chinese mitten crab	757	779	776	808	815	6.4
River prawns	533	536	553	590	600	4.7
Swimming crabs	419	404	399	396	395	3.1
Other crustaceans	22	31	30	39	40	0.3

Source: FAO, 2024

Source: DELOS Aqua

Why production in Europe?

European shrimp aquaculture

- Local sustainable production

- Unknown imports:
  1. Illicit products used
  2. Poor working conditions
  3. Habitat impact

- Technical optimized production systems:
  1. Health and biosecurity
  2. Improvement of breeding
  3. Low water consumption and waste
  4. Adjustable parameters

Slavery still a problem in Thai shrimp industry despite scrutiny

AP finds grueling conditions in Indian shrimp industry that report calls 'dangerous and abusive'

Gel-injected shrimp a growing problem in China

Indian company sold contaminated shrimp to U.S. grocery stores, 'whistleblower' says

Source: NBC NEWS, APNEWS, CBC, Seafood Sources

Source: NBC NEWS

Current situation

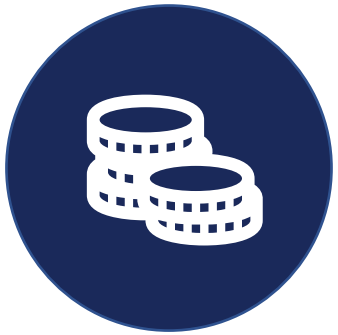
European shrimp aquaculture

Between 20-30 farms:

- Small to medium size (3–100 t), mostly startups
- Estimated annual production: 418 t

Sale size:

- 12–30 g
- With/out head, peeled/not peeled



Sale prices:

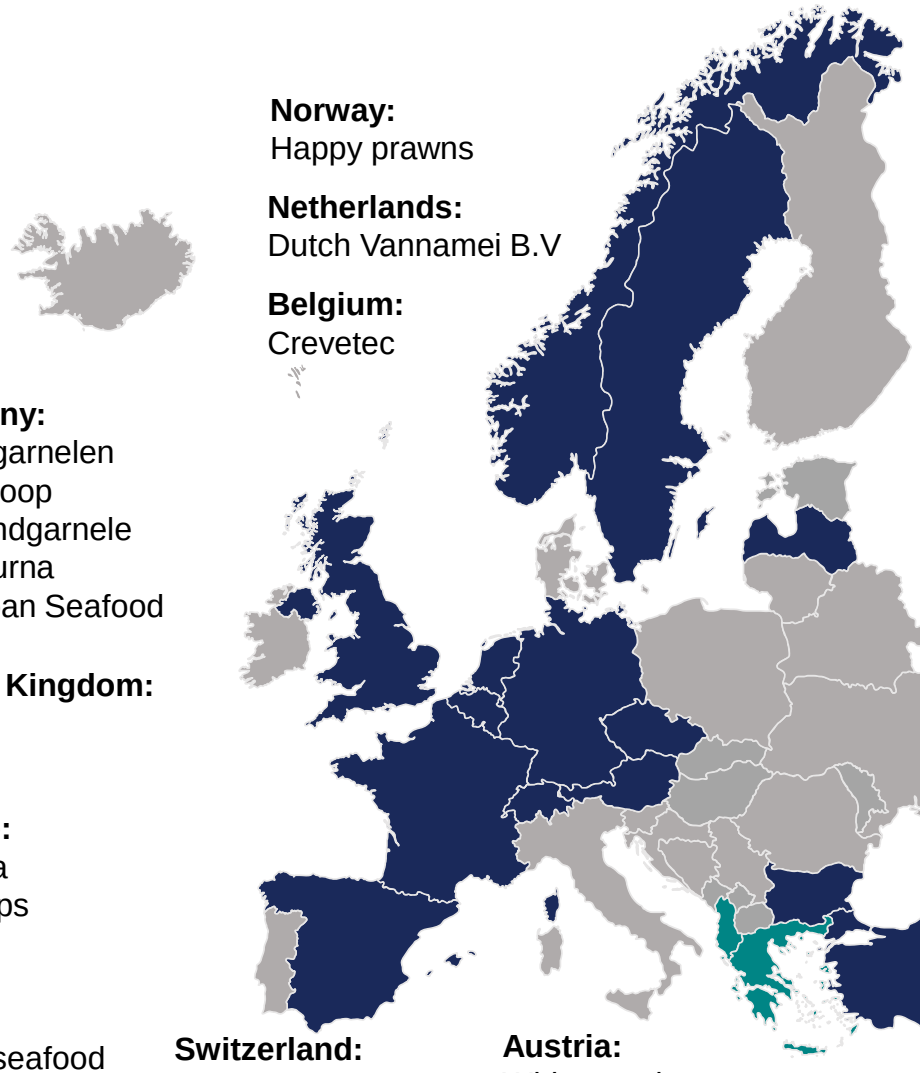
- Spain: 25 €/kg
- Germany: 60-100 €/kg
- Austria: 70 €/kg

Market concepts:

- Online shop
- Farm sale
- Retailers (supermarkets)

Current situation

European shrimp aquaculture



Norway:
Happy prawns

Netherlands:
Dutch Vannamei B.V

Belgium:
Crevetec

Germany:
Hansegarnelen
Oceanloop
Die Landgarnele
Aquapurna
Suburban Seafood

United Kingdom:
FloGro

France:
Lisaqua
Agriloops

Spain:
Noray seafood

Switzerland:
SwissShrimp GmbH
Mayer Shrimps
Lucky shrimp AG

Austria:
White panther
Kremstal Garnelen
Alpengarnelen

Albania:
Narta farm

Greece:
Europrawn Hellas

Sweden:
Hava

Latvia:
Mere Shrimp farm

Czech Republic:
Pure shrimp

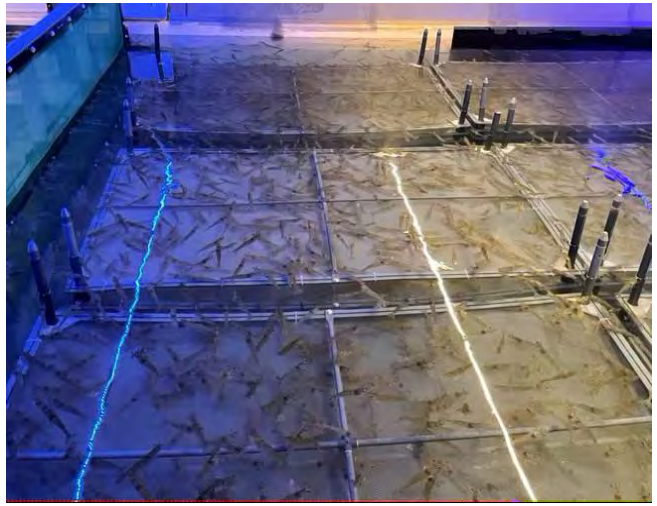
Bulgaria:
Bilyana Ltd

Turkey:
Mem's Karides

- *P. vannamei*
- Other shrimp species

Farm designs

Indoor systems:



Source: The Fish Site

RAS

Source: B. Andlauer

Biofloc

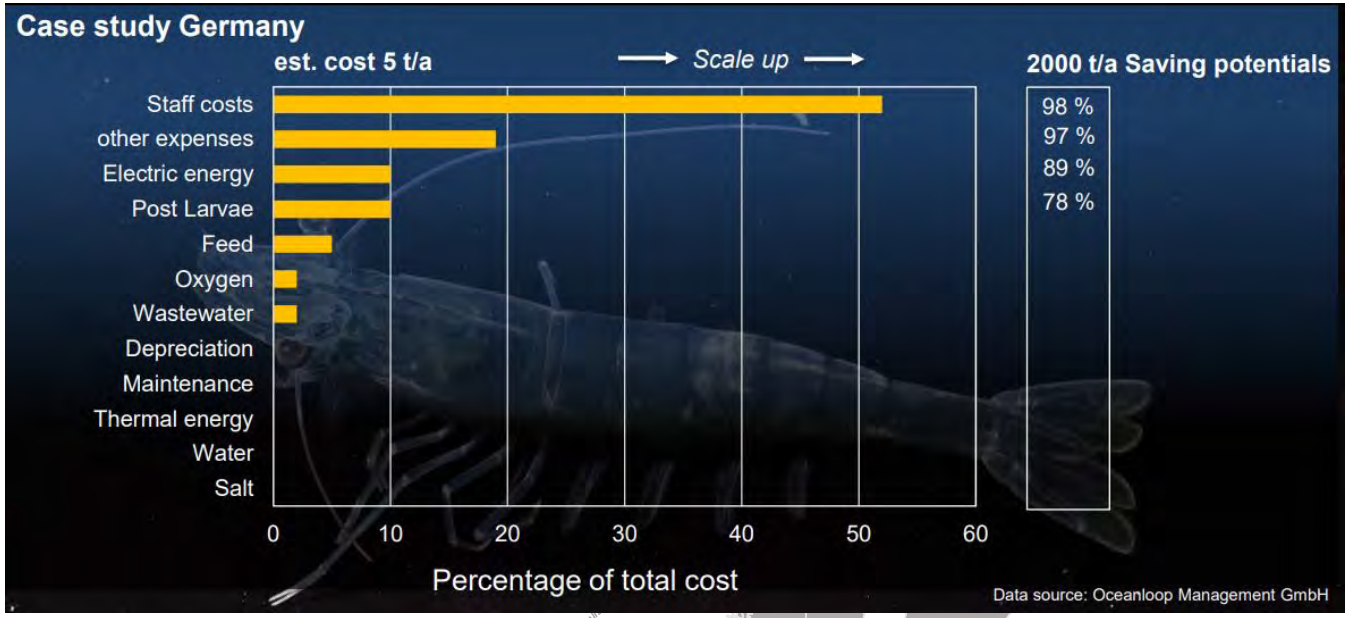
Source: MyFishPlant
Feed and Additive

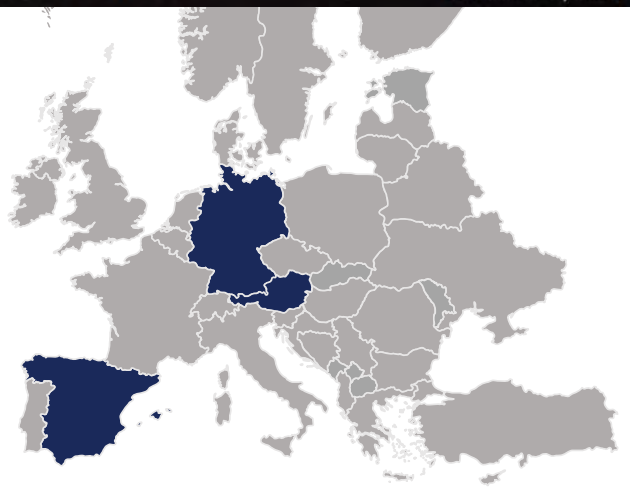
Hybrid systems (Bio-RAS)

Main challenges

European shrimp aquaculture

- Seed availability and quality
- High production costs
- Waste water/solids treatment
- Up-scaling
- Market coordination and bureaucratic regulations



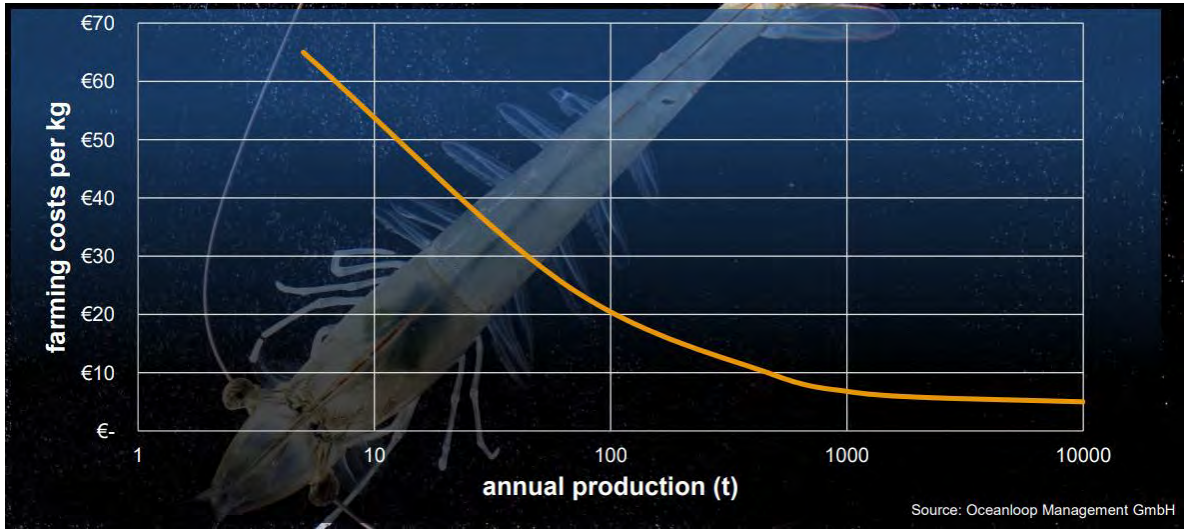


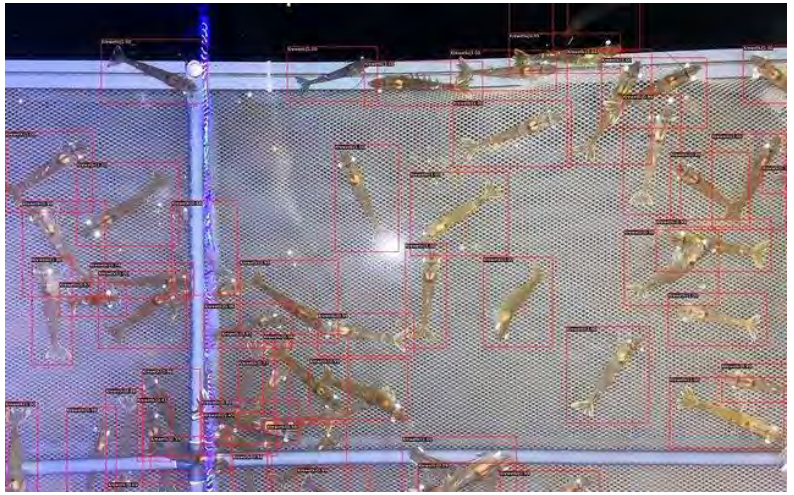
Shrimp hatcheries in Europe

Future perspectives

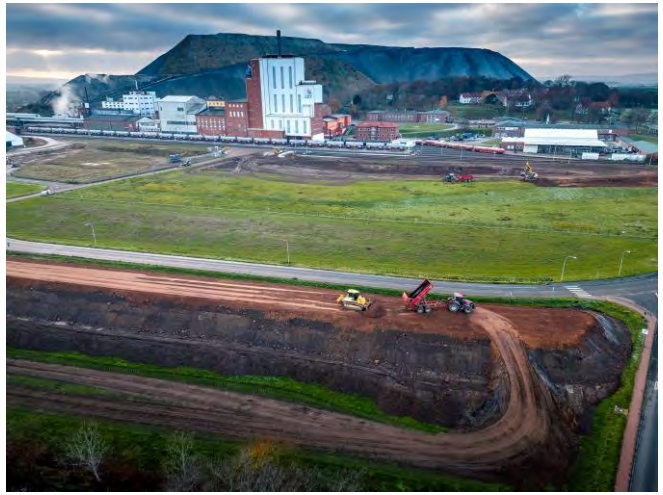
European shrimp aquaculture

- High quality seeds available
- Technical improvements: AI monitoring, IMTA systems
- Up-scaling (new farms) to established companies + reduction of production costs
- European shrimp still sold as premium product





Source: Hatchery Feed Management



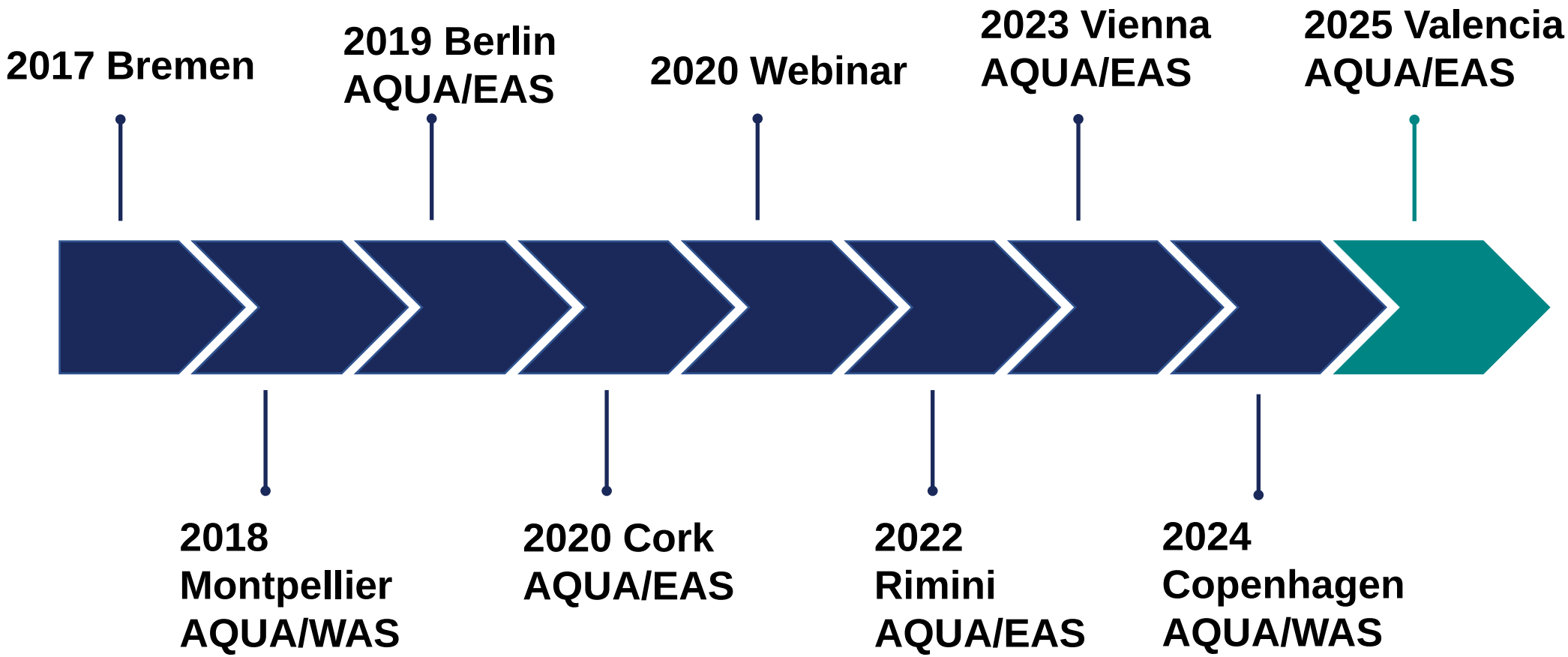
Source: Billund Aquaculture

EUROSHRIMP

- 287 members (2023) EU and not EU members
- Why? Expanding shrimp production in Europe
- Who? Enthusiastic producers, scientists, networkers
- What? Connection point for European shrimp community:

“The Euroshrimp is a place where we can identify areas needing development, bottlenecks, challenges, that can be addressed in the future”

Annual Euroshrimp Forum meetings

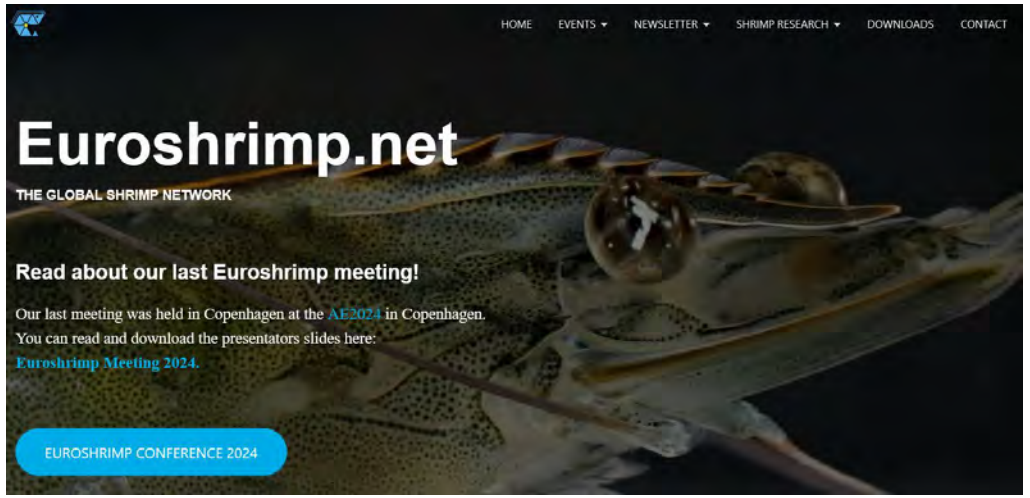


https://www.euroshrimp.net/events/

Euroshrimp website and newsletter

- All information summarized
- Past and future events and activities
- Links and downloads of presentations
- Surveys, research updates
- **Newsletter: Free registration**

https://www.euroshrimp.net/newsletter



Areas of interest

- Fields of research: applied research with companies, supported by third-party funding

Technical innovations

AI application

Nutritional trials

Biofloc trials

Animal welfare

Centre of aquaculture research (ZAF)

Euroshrimp

- Closed RAS (tanks from 250 to 2500L) + rack systems with smaller tanks for marine and freshwater organisms

Source: H. Mueller-Elsner

- Glasshouse for algae and aquaponic systems, culture rooms for microalgae

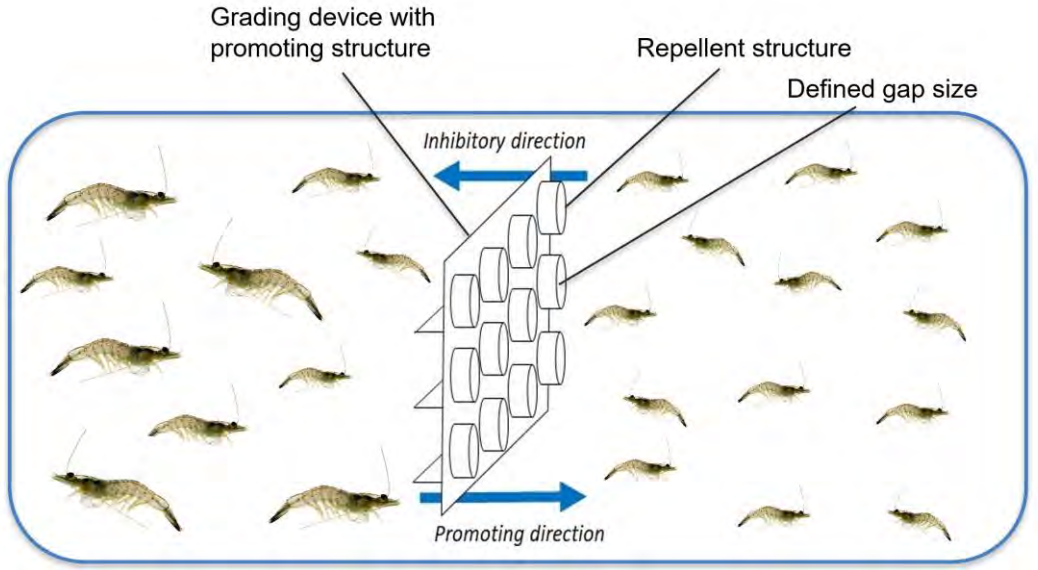
Source: H. Mueller-Elsner

- Customization with automatic feeders, additional technology

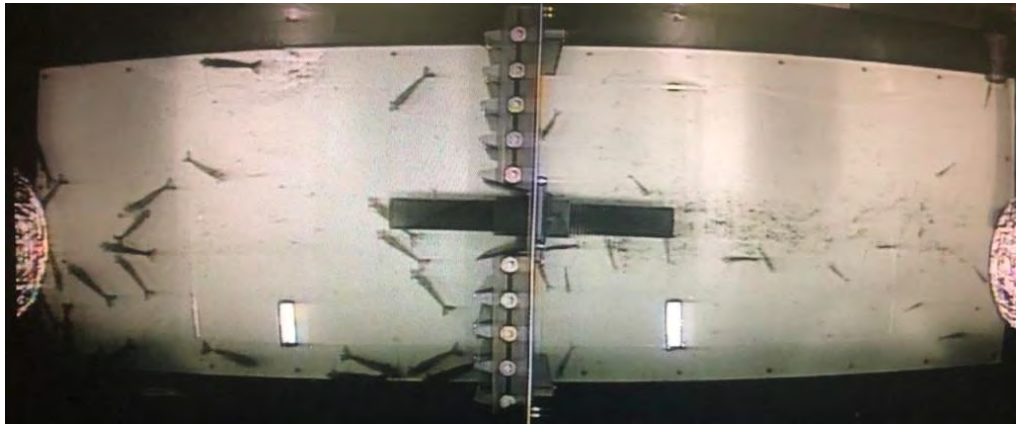
Technical innovations: Sorted

Euroshrimp

- **Problem**: no viable mechanisms to grade (cannibalism, reduced harvest) without stress and too resource-demanding
- **Target**: shrimp Sorting Technology to improve welfare and commercial production efficiency
- **Solution**: exploits natural behaviour, avoids stress, limits manpower. Wall covered with structures difficult for the shrimp to return back
- **MarbleGrade**: transfer Sorted technology to marbled crayfish (*Procambarus virginalis)*



Source: M. Bögner



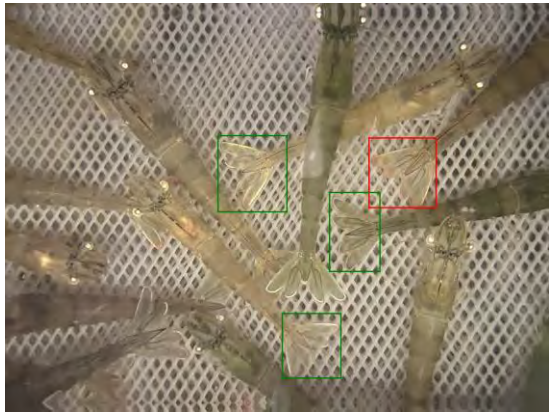
Source: M .Bögner

AI application: Monitor shrimp and ShrimpWiz

Euroshrimp

- **Problem**: exact biomass is unknown and feed mismanagement can impact yields, animal welfare or health
- **Target**: Release a market-ready software for counting shrimp and early detection of visual stress indicators.
- **Solution**: online tool allowing a counting shrimp accuracy of about 90%, with an automated length and animal welfare detection (detection of red tails, as early stress response)

Source: Oceanloop



Source: B. Wecker

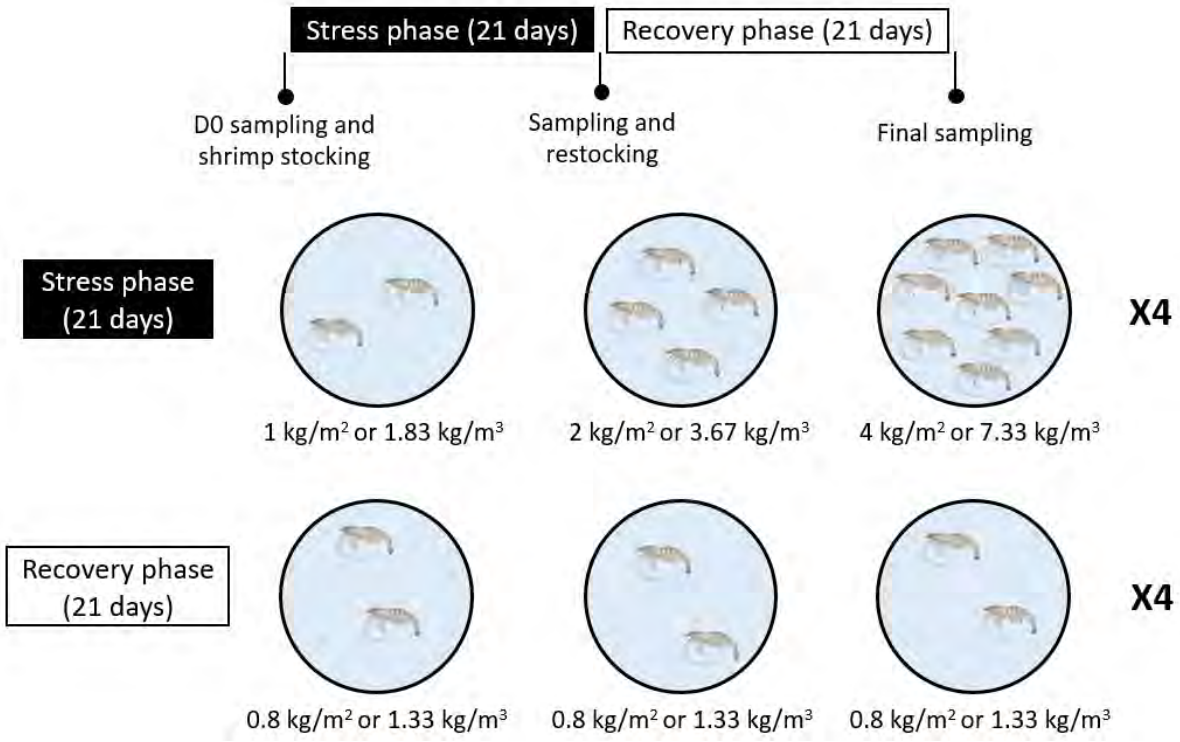
Animal welfare: Crustawohl

- **Problem**: Shrimp welfare is an emerging topic that remains largely unexplored in research
- **Target**: Development and validation of an assessment system for improved animal welfare and animal health in shrimp farming (index)
- **Solution**: Work closely with farmers (surveys, workshops, index testing). Better understanding of chronic stress effects on animal welfare, through application of multidisciplinary methodologies

Source: M. Bögner

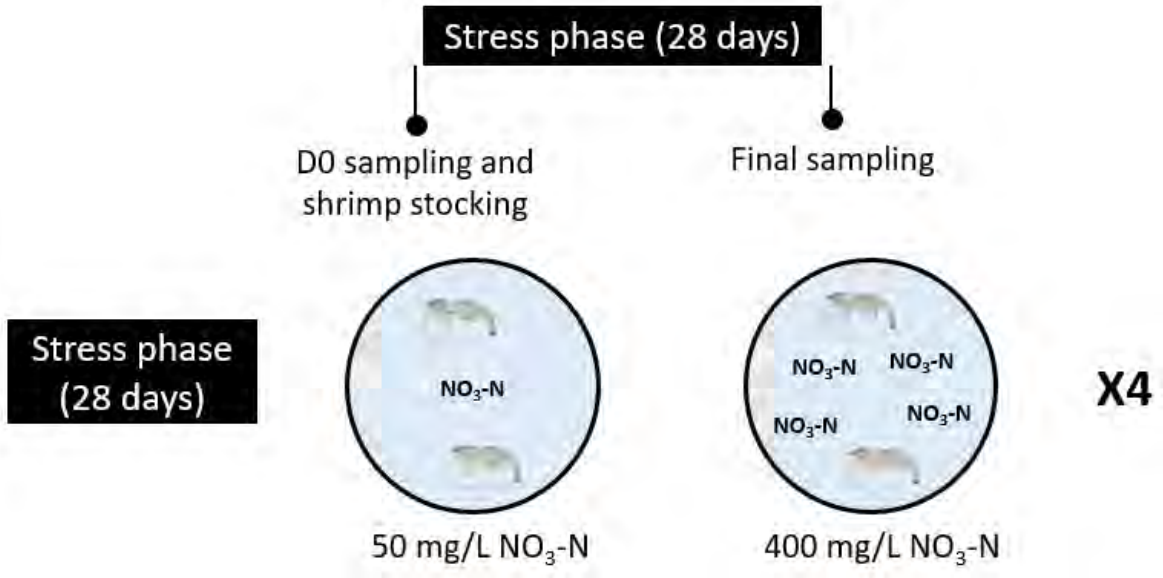
Source: M. Bögner

Crustawohl: Crowding stress



- Survival, growth performance negatively affected by crowding, both re-established during recovery phase
- Stress phase impacts on antennae and uropods quality, with quick recovery
- Stress phase increases frequency of abnormal swimming and loss of balance, drop in recovery
- Gpx and HSP70 expression increase at high density, lower after recovery phase

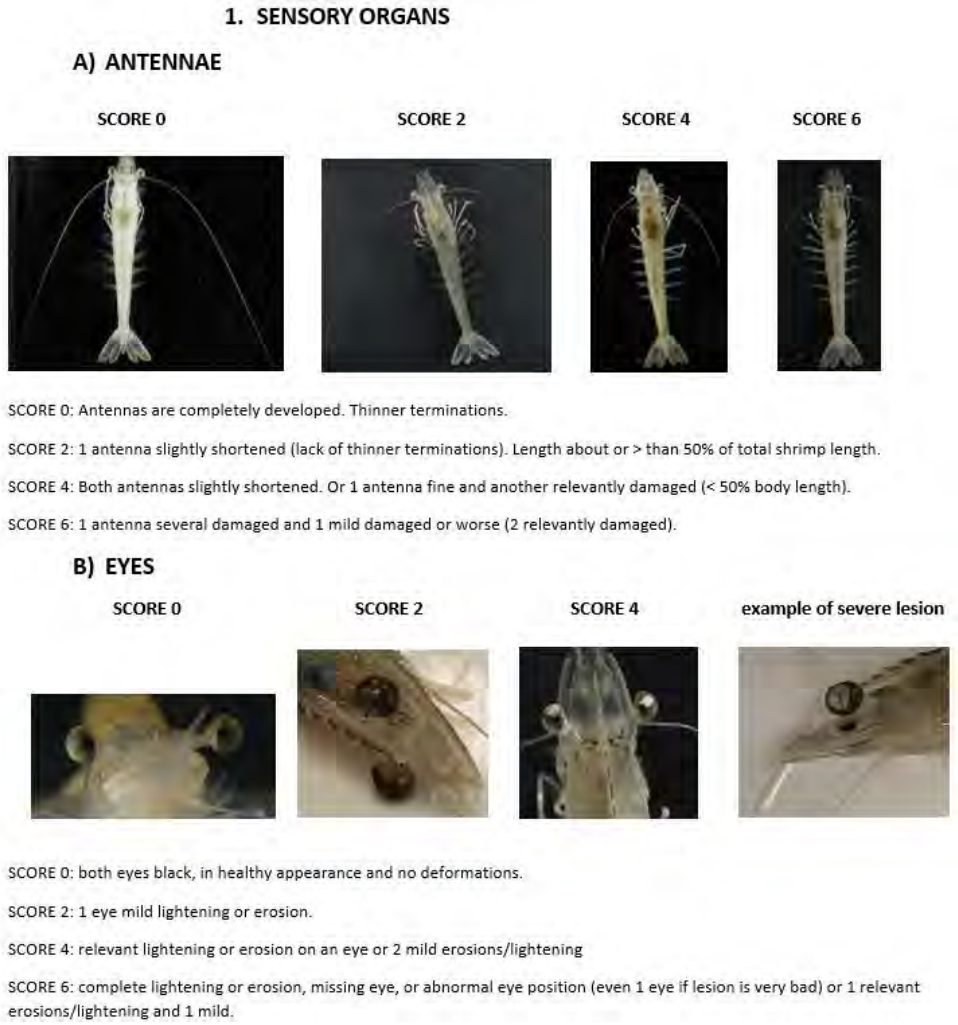
Crustawohl: Chronic nitrate stress



- Survival not significantly affected
- High nitrate load impacts on shrimp growth performance
- No significant variation in physiological parameters
- hepatopancreas quality deterioration (colour and consistence)

Crustawohl: Next steps

- **Minerals management**: different water Magnesium: Calcium ratios on shrimp health and performance
- **Feeding management**: different feeding strategies to test shrimp health, performance and bacterial community in the intestine
- **Index testing**: testing and calibration at farms. Data collection on different sites

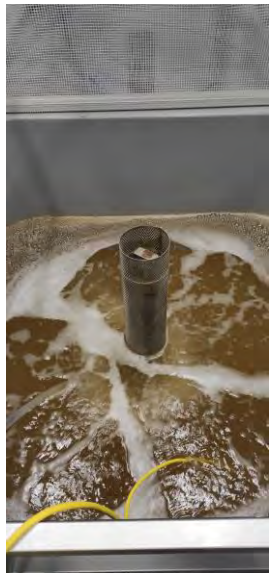


Biofloc and nutritional trials

Euroshrimp

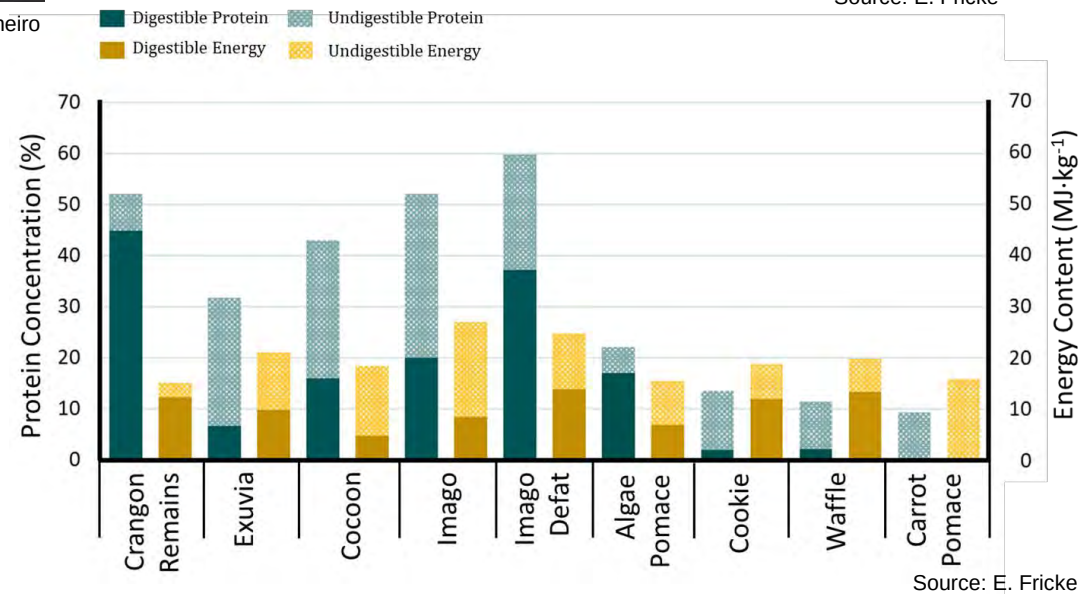
- **Biofloc trials:**
  1. Brewery waste as carbon source
  2. Oxygen depletion, crowding stress

- **Nutritional trials:**
  1. Bioavailability of by-products have to serve as protein, energy and functional ingredient
  2. Effects on shrimp performance



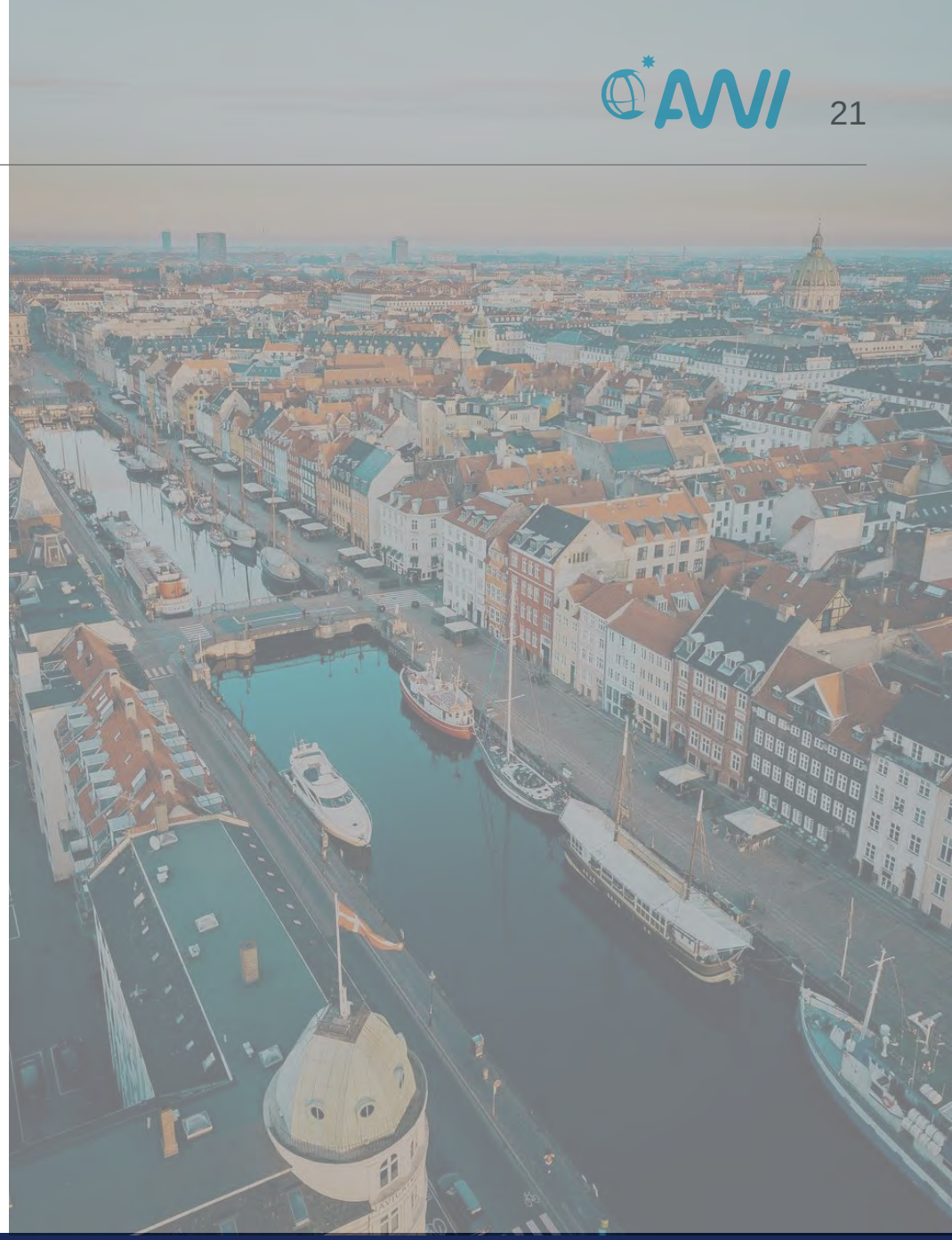
Source: I. Pinheiro

Source: E. Fricke



Source: E. Fricke

Thank you!




DTU

16th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Advanced computing and camera technology-based AI-powered probe for the real-time detection of shrimp diseases: *A proof-of-concept*

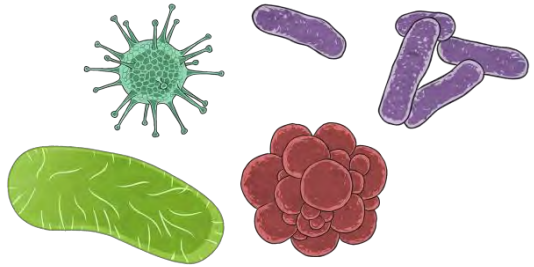
Shyam K Uthaman[1], Niccolò Vendramin[1], Casper Stæhr[2], Gustav Stæhr[2], Fridi Mellemgaard[2] and Britt Bang Jensen[1]

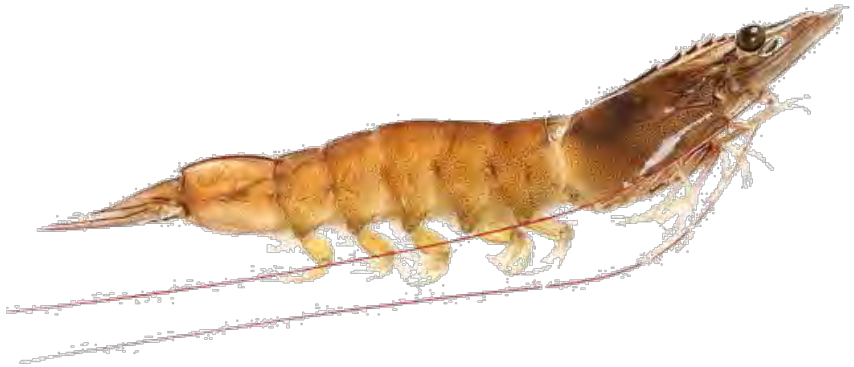
[1]DTU Aqua – National Institute of Aquatic Resources

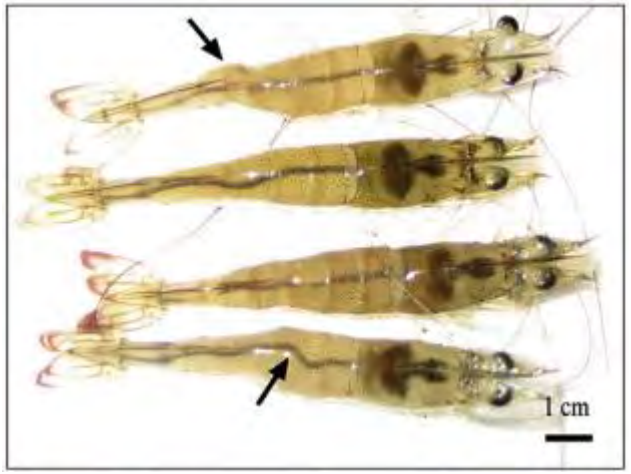
[2]Sincere Aqua, Denmark

Background

Pathogen



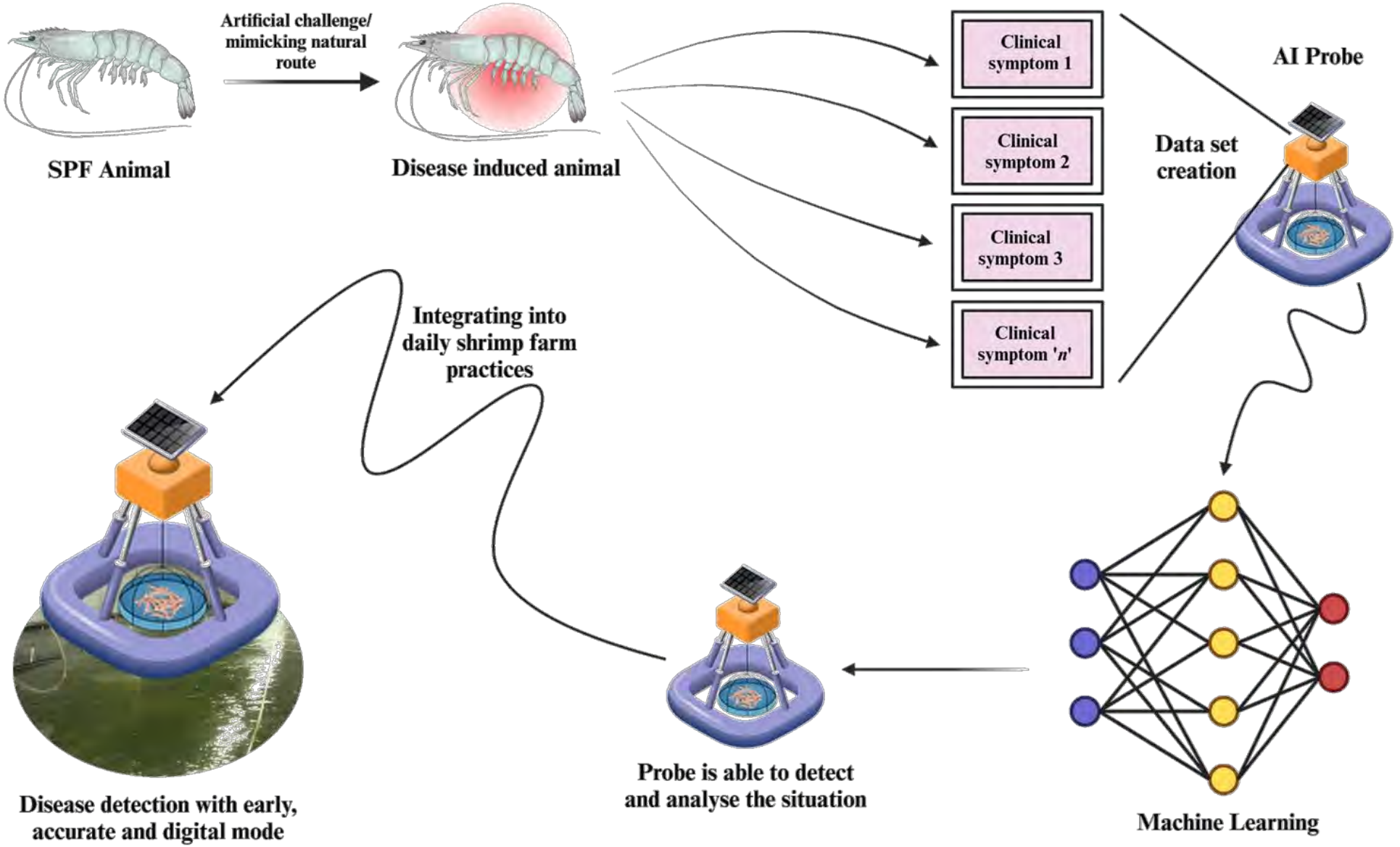




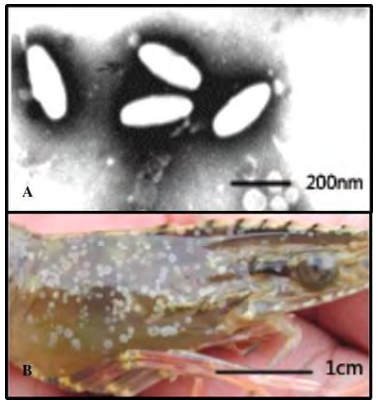
Diseases

- **Unhealthy shrimp** → Expense on feeding, Loss of biomass, Unappealing, Ecological consequences
- **AHPND** in shrimp → 45 million USD Loss in Asia
- **If diseased, prevention/treatment** → causes unfocused and misuse of drugs → drug residue, AMR
- **Options** → manual inspection, Early detection (POCT), Laboratory Confirmation
- **Idea** → some means detection the pathogen early and accurate, easily integrate into daily farm operations

Methodology



Experimental Trials and Tasks



We will establish Infection models for EU-listed crustacean pathogens

1. **White spot syndrome virus (WSSV)**
2. **Taura syndrome virus (TSV)**
3. **Yellowhead virus genotype 1 (YHV1)**

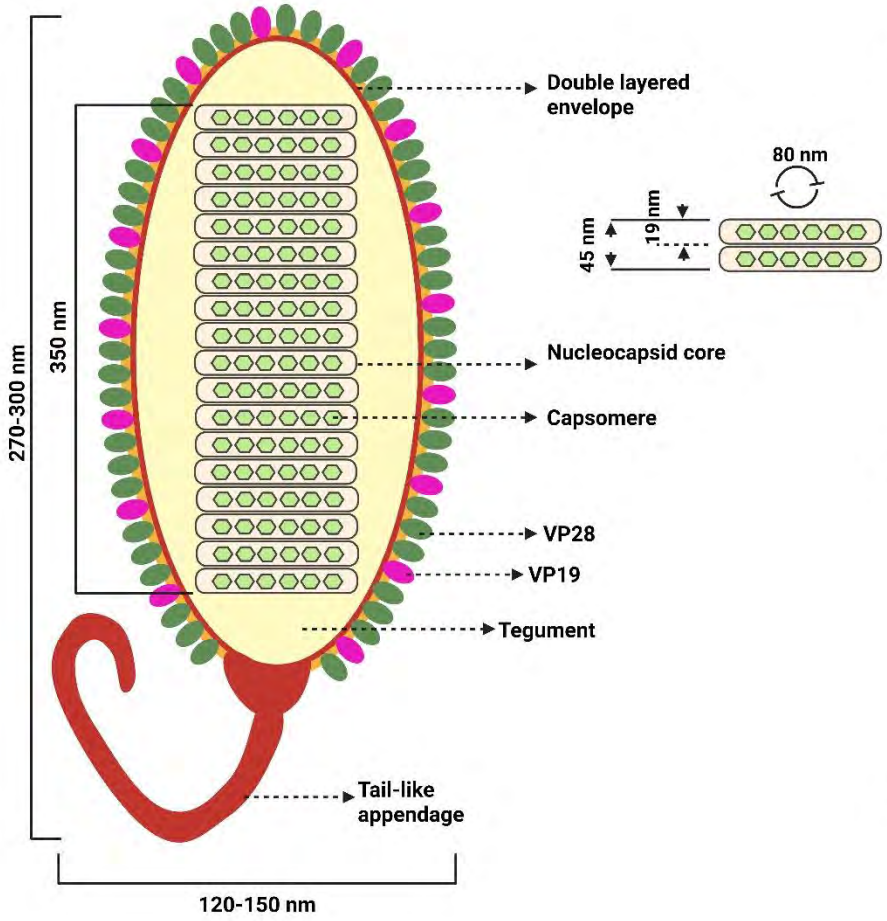
We just finished experiments for the WSSV

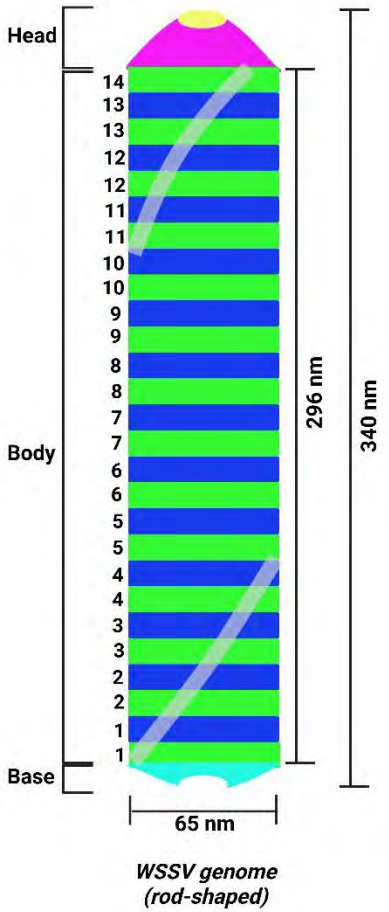
We will start the experiments for the TSV in Early July

- We create clinical data set for the probe
- Analyze disease progression and detection in lab for the probe comparison
- Provide Specialized scientific insights

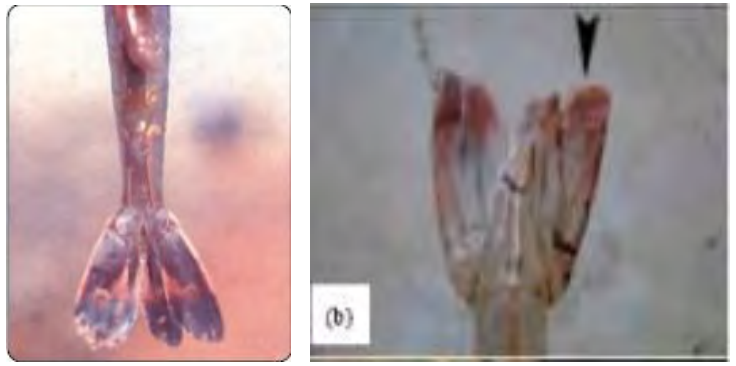
- **LD50 Estimation**
- **Validating the clinics**
- **Probe-installed experiments**
- **Additional experiments on probe-installed natural route of infection challenge**

WSSV





WSSV genome (rod-shaped)

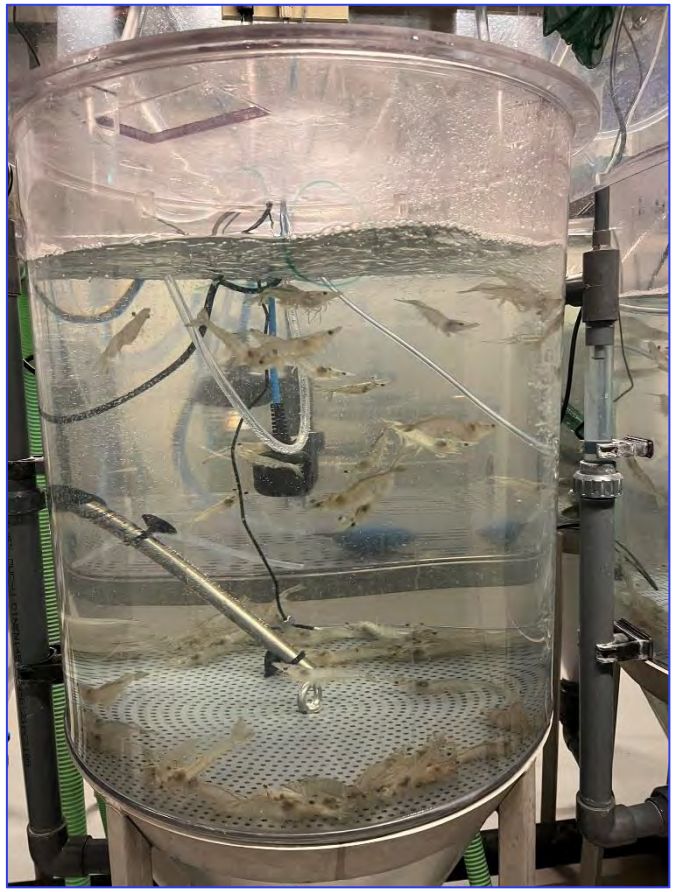


Reddish discoloration of the body and appendages

Infected shrimp gather near pond edge

White spots

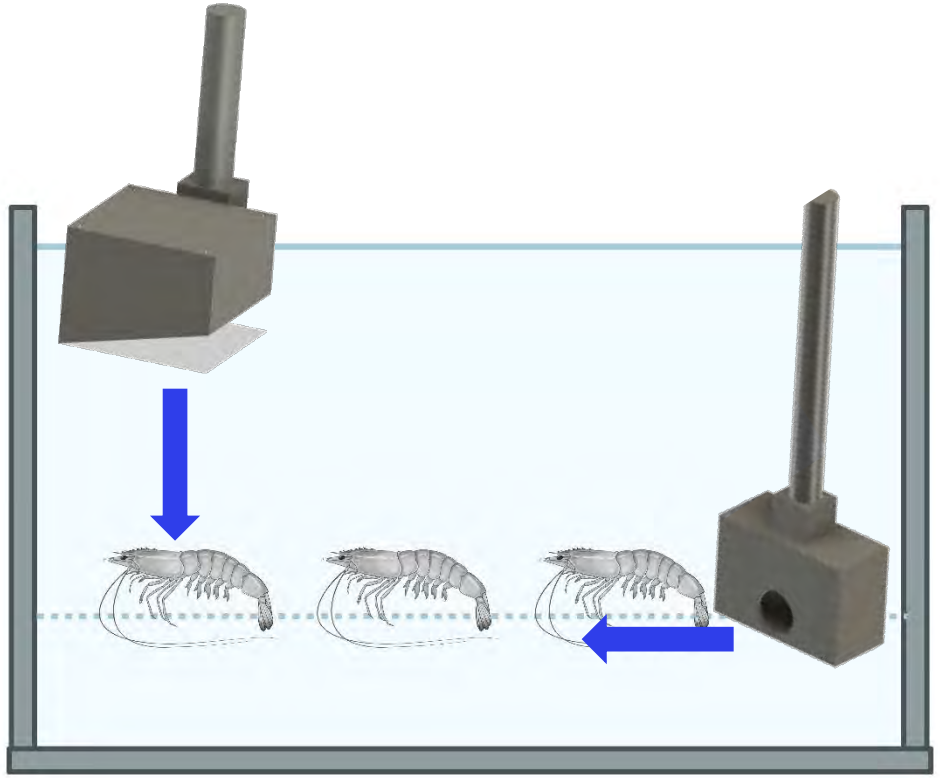
Experimental Animal



- We procured animals from AquaPurna, GmBH, Germany
- Juvenile stages
- 5-8 g Body weight sized animals
- Acclimatized for 2 weeks in the facility
- We used 60 L Grey tanks for the experimental challenge trails
- 180-200 L tanks for keeping the shrimp for acclimatization
- All the tanks were equipped with biological filters, aerators, thermostat with temperature controller, water level detector in 180L tank
- We fed the shrimp with BioMar Inicio Focus pellet feed at 2-3% body weight while acclimatizing
- Animals were starved for 24 h before the challenge
- Animals were not fed during the trial
- Used artificial seawater prepared in-house, salinity @25 ppt, temperature @25°C
- 50% water exchange was done every other day or whenever it is necessary

High-speed Camera

- 3D Image
- Slanting underwater phase – avoid bubble trapping
- Water-tight resin coating



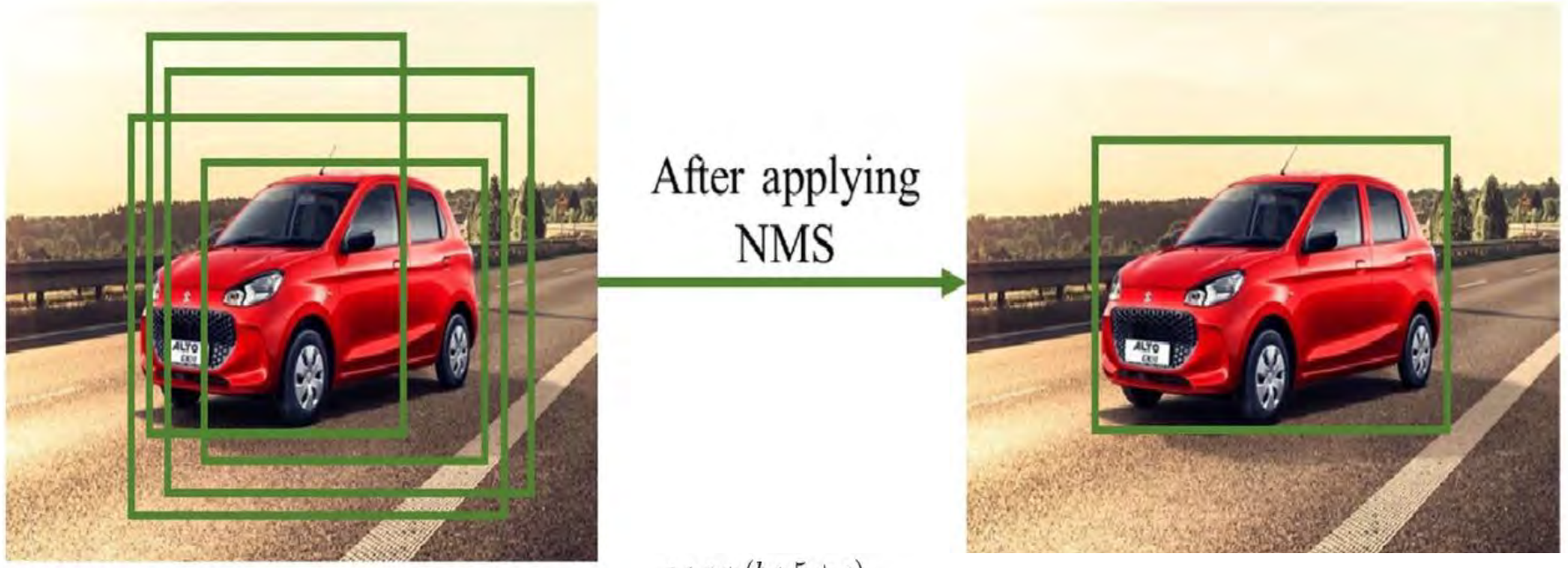
Camera 1: Submersible

- 3D Image
- Additional weight bars attached to make it sink, stay at the bottom
- Water-tight resin coating

Camera 2: Submerged

Analysis and teaching the AL

- We use a **central object detection model** in real-time environments with impressive accuracy and speed, CNN (Convolutional Neural Network) model and examines it on the **PASCAL VOC** detection dataset
- One-shot detection approach – speed
- Dividing the entire image into a small grid and making a predictions directly within every grid cell



Non-Maximal Suppression

$$s * s * (b * 5 + c) \quad (6)$$

The model is evaluated on the Pascal VOC dataset, with parameters b=2, s=8, and c=20. By applying this value in Eq.2

Partnership

Sincere Aqua
We bring biomass insights

https://www.sincereaqua.com/

Accurate pond stocking

Our shrimp counter uses advanced technology to accurately count shrimp, eliminating the need for manual counting, which can be time-consuming and error-prone. This ensures optimal stocking densities for your shrimp farm.

Real-time biomass monitoring

Our shrimp biomass monitoring probe provides instant data on the shrimp in your ponds, allowing you to make informed decisions about feeding rates, harvest times, and overall farm management.

Improved feed management

With precise shrimp population and biomass data, you can optimize your feed distribution, minimizing waste and improving feed conversion ratios. This results in healthier shrimp, higher yields, and increased profits

Reduced labor costs

By automating shrimp counting and biomass monitoring, our products help you save time and reduce labor expenses, allowing you to focus on other essential aspects of your shrimp farming business.

Enhanced disease prevention

Early detection of changes in shrimp biomass can indicate potential health issues or disease outbreaks. By monitoring your shrimp population closely, our products enable you to take preventative measures and mitigate risks, ensuring the well-being of your shrimp and safeguarding your investment.

Data-driven decision

Our shrimp counter and monitoring probe provide valuable data that can be easily integrated into your farm management software, enabling you to make informed, data-driven decisions to enhance productivity and optimize your shrimp farming operation.

Shrimp Counter

Precision Shrimp farming

Automatic Shrimp Monitoring Probe

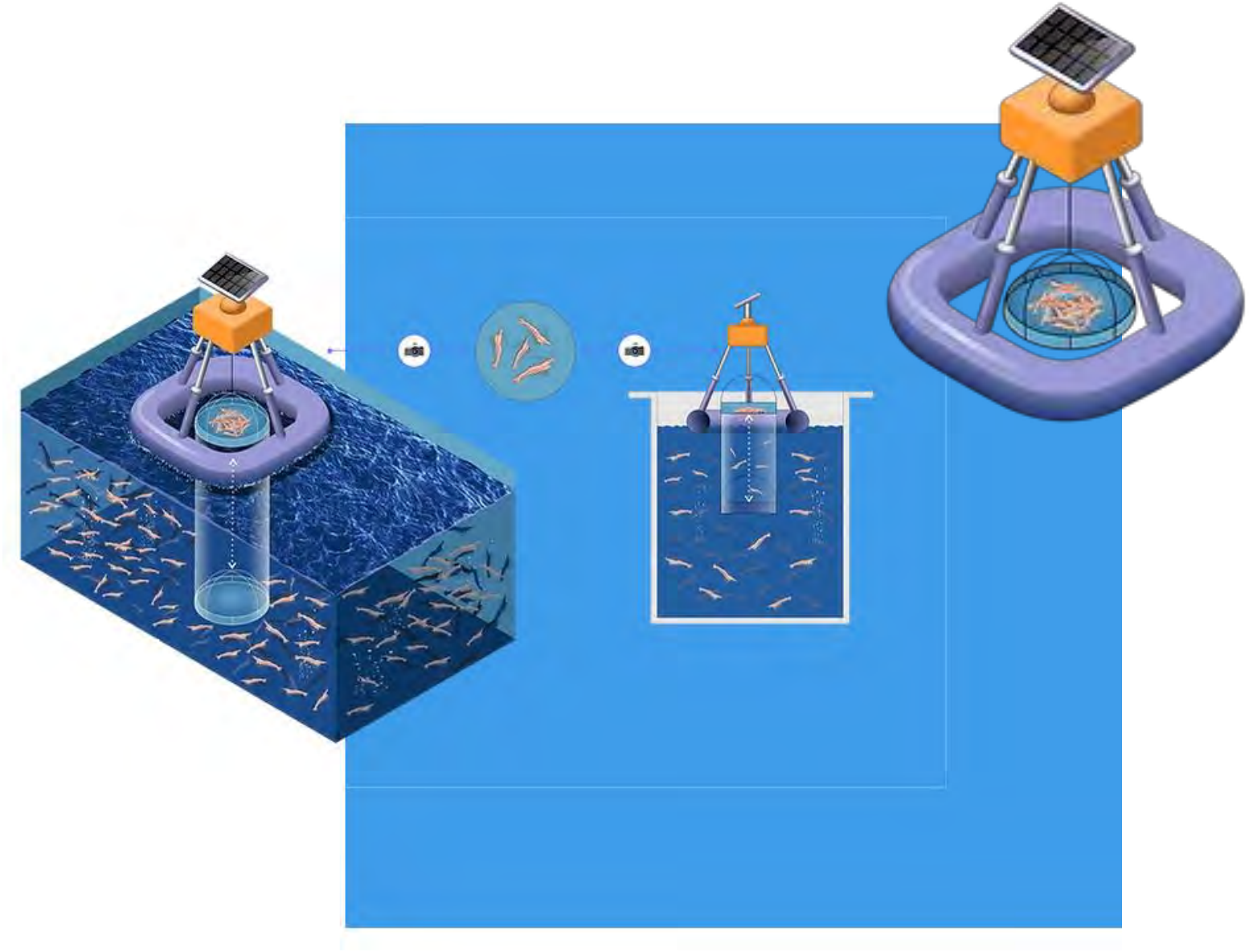
AI Probe

Automatic Shrimp Monitoring Probe

Monitor Biomass (Coming later)

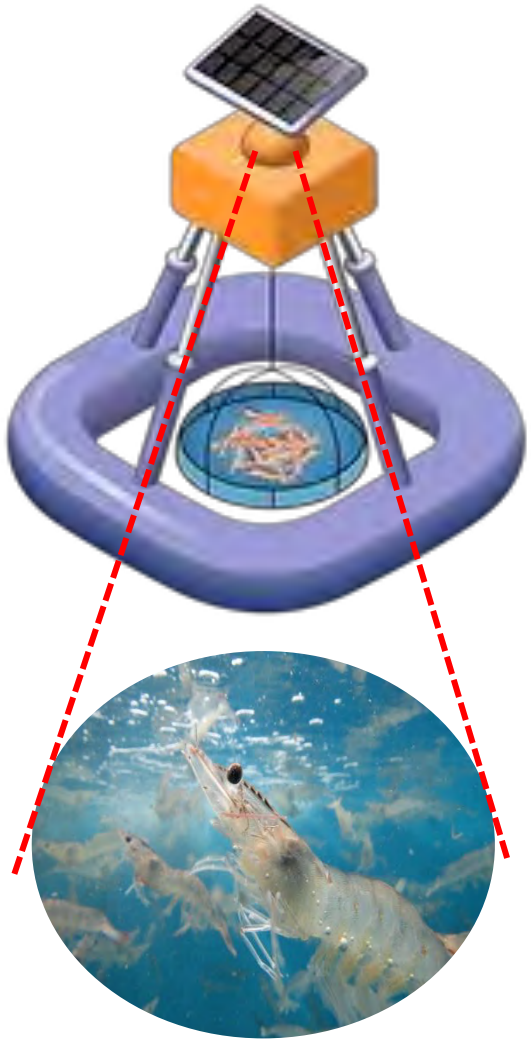
Monitor the shrimp daily using our Sincere Probe. See the growth rates and better predict when it's time to harvest a pond or adjust feeding schedules. Get alerts if any health concerns are found through our alarm system.

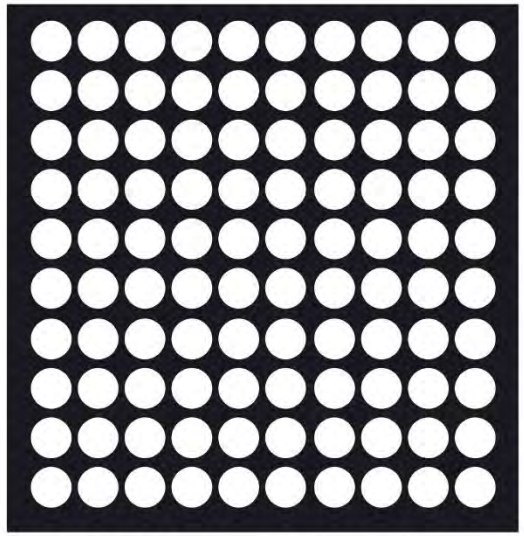
Keep track of the biomass in every pond of your farm forever. Go back and revisit old cycles and compare them to the current cycle. Check out every individual record, and look at differences in shrimp from past cycles to current.



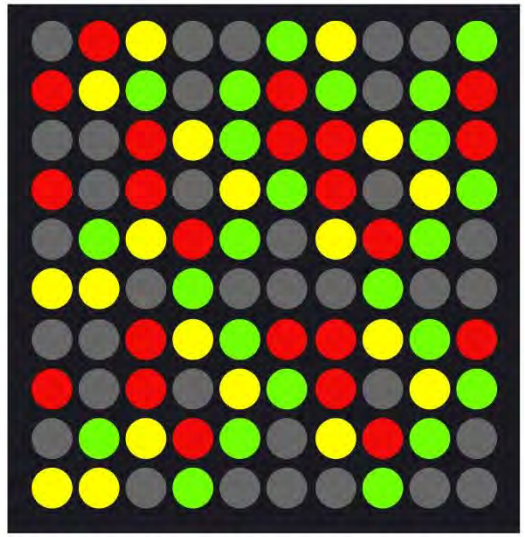
Automatic Shrimp Monitoring Probe

- Image-based disease detection system





Probe Captures high quality images of the shrimp in tank



AI algorithm identify, and analyze the clinical signs from the images according the ML database

- No clinical signs
- Clinical sign A
- Clinical sign B
- Clinical sign C

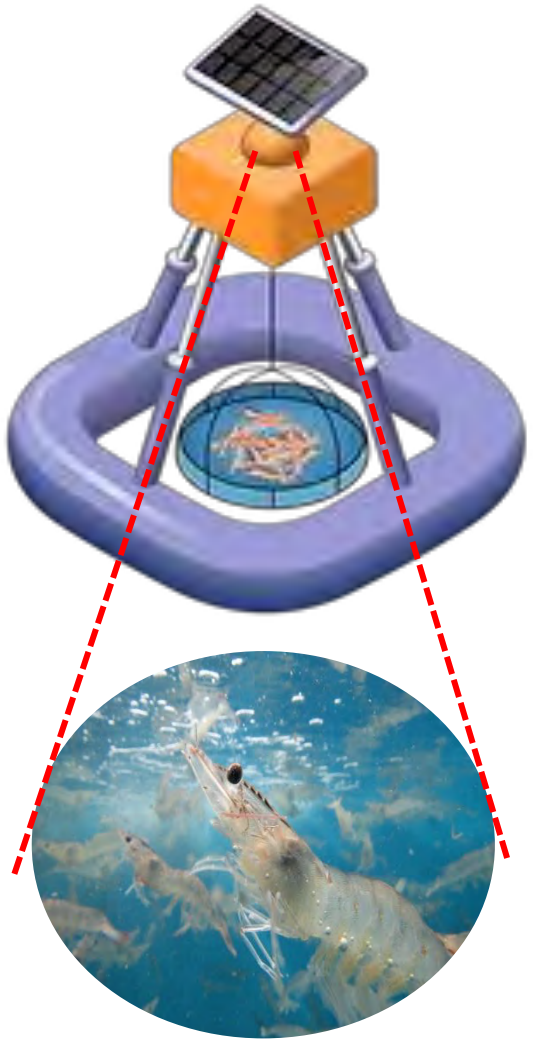
Outcome/Benefit

- Data can be tracked
- Online monitoring
- Individual records
- Easy comparison
- Digital storage

- Growth rates - Biomass
- Predict about the time of harvest
- Feeding behaviour - Feeding adjustments

Alert the predicted health concerns through alarm system

Outcome



Final output of the project

- Image-based disease detection system – use of digital technology in shrimp farming
- Precise and accurate, individual animal-based health management strategy
- Daily routine integrated health monitoring and record keeping
- Promising data which encourage to use AI-driven technologies in farming practices (both for finfish and shellfish)

Prospects of the project

- Teaching the AI probe to be more pathogen-specific (WSSV or TSV) and shrimp life-stage specific (PL, Juvenile and adult)
- Integrating with other sensing technologies – single, multi-sensor camera probes, robotic probes (shrimp miniature forms) etc.

The Team

DTU Aqua

Shyam K Uthaman
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Argelia Cuenca
Charlotte Bjørner Larsen
Jacob Günther Schmidt
Britt Bang Jensen

SincereAqua

Casper Stæhr
Bergur Clementsen
Gustav Stæhr
Fridi Mellemgaard

Thank you

Tak

Any Questions/Suggestions/Comments?

FINNISH FOOD AUTHORITY
Ruokavirasto • Livsmedelsverket

Studies on crayfish plaque *Aphanomyces astaci* genotype D in northern noble crayfish

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EURL Annual Workshop for Crustacean Diseases

28 May 2025

Background

- *Aphanomyces astaci* is a pathogenic oomycete, causing devastating crayfish plague, which can be fatal especially to indigenous European crayfish populations
- North American crayfish are usually a carrier species of *A. astaci*
- Five different genotypes, which alter in their virulence and susceptible species:

- Genotype A: Astacus

- Genotype B: Pacifastacus, Astacus, Austropotamobius

- Genotype C: Pacifastacus

- Genotype D: Procambarus

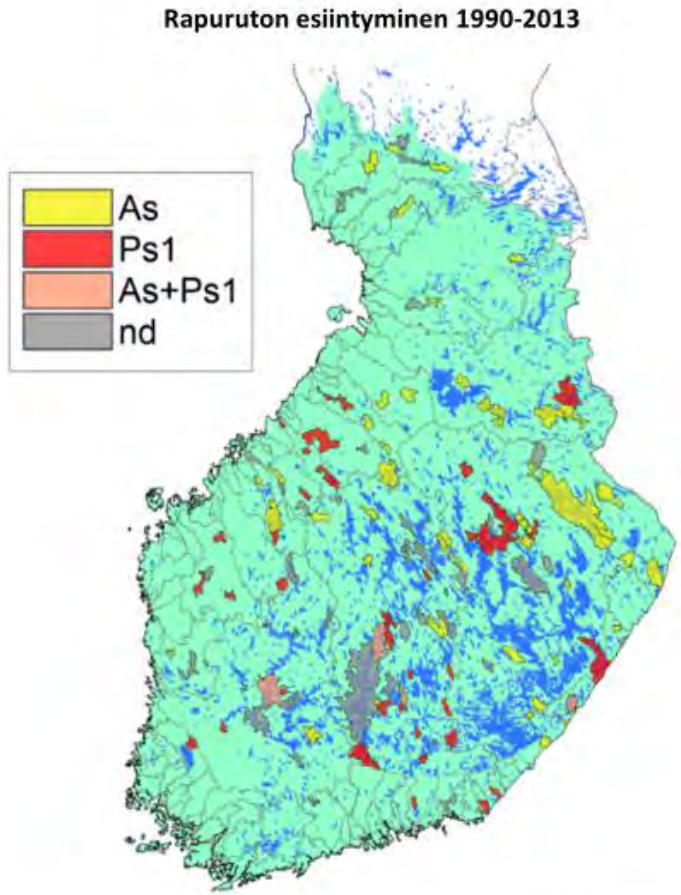
- Genotype E: Faxonius

(Huang et al. 1994, Dieguez-Uribeondo et al. 1995, Kozubikova et al. 2011)

Heavy infection of crayfish plaque in signal crayfish
Photo: S. Viljamaa-Dirks

Crayfish plaque in Finland

- First crayfish plague in Finland was detected in 1893, and was presumably *A. astaci* genotype A (As), which is still present in noble crayfish populations.
- In 1960s signal crayfish (*Pacifastacus leniusculus*) was introduced in Finnish lakes, a carrier specie of *A. astaci* genotype B (Ps1), causing mass mortalities for noble crayfish (*Astacus astacus*) populations.
- In the last decades, the crayfish plague epizooties in Finland were caused by the genotypes A or B.
- Genotype A was only connected with noble crayfish. Genotype B caused acute disease episodes in noble crayfish but was also found regularly from the signal crayfish populations.



Crayfish plague genotypes in Finland 1990- 2013. As = genotype A, Ps1 = genotype B, nd = not detected. (Pursiainen & Viljamaa-Dirks, 2014)

First detection of *A. astaci* genotype D in Finland

- Noble crayfish populations in Kemijoki river in the Northern Finland has suffered mortality event in the productive populations of noble crayfish in the years 2010-2011 due to the crayfish plague.
- In Kemijoki river estuary a small population had survived until 2020, which 6 moribund individuals were studied and diagnosed for *A. astaci* genotype D. This was first isolation of *A. astaci* genotype D in noble crayfish in Finland. (Viljamaa-Dirks et al. 2020)

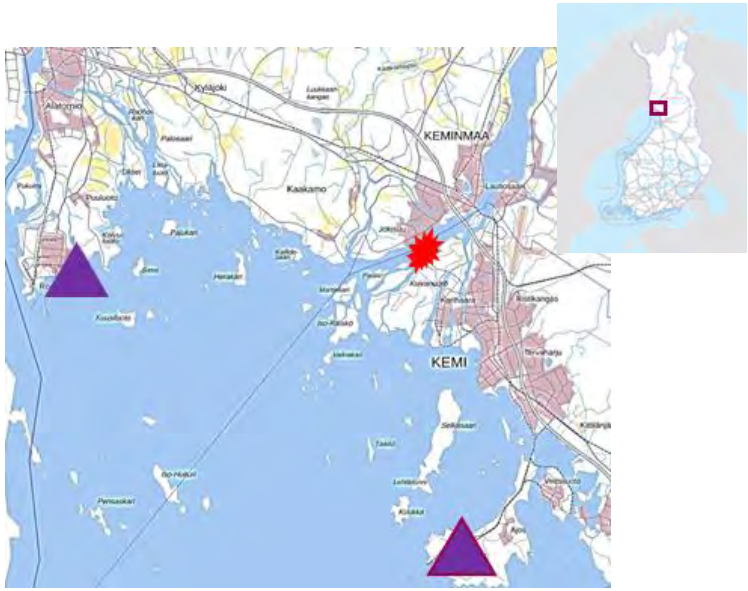
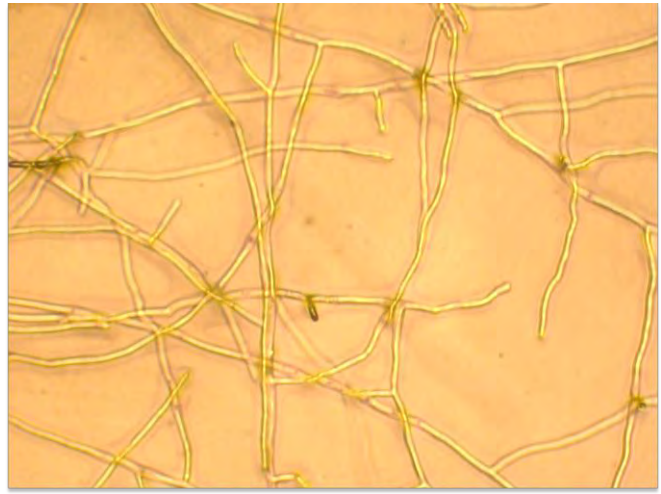


Fig. Bay of Bothnia and Kemijoki estuary. Red star: crayfish plague. Purple triangle: seaport. Map © CC MLL

Studies of *A. astaci* genotype D Kemijoki strain

- *A. astaci* was diagnosed by microscopic observation of cuticulum and cultivation from moribund noble crayfish and for one isolation genotype was determined by RAPD-PCR (Huang et al. 1994). The culture from the other 5 crayfish were tested with the specific PCR for genotype D (Minardi et al. 2019) with positive results.
- Genotype D is considered a warm-water- type strain and usually isolated from red swamp crayfish (*Procambarus clarkia)*.
- Epizootic in noble crayfish suggested that strain can also cause devastating disease in colder climate, so isolated strain was studied further in laboratory



Hyphae of A. astaci on PG-1 Agar. 10x magnification. Photo: S. Viljamaa-Dirks

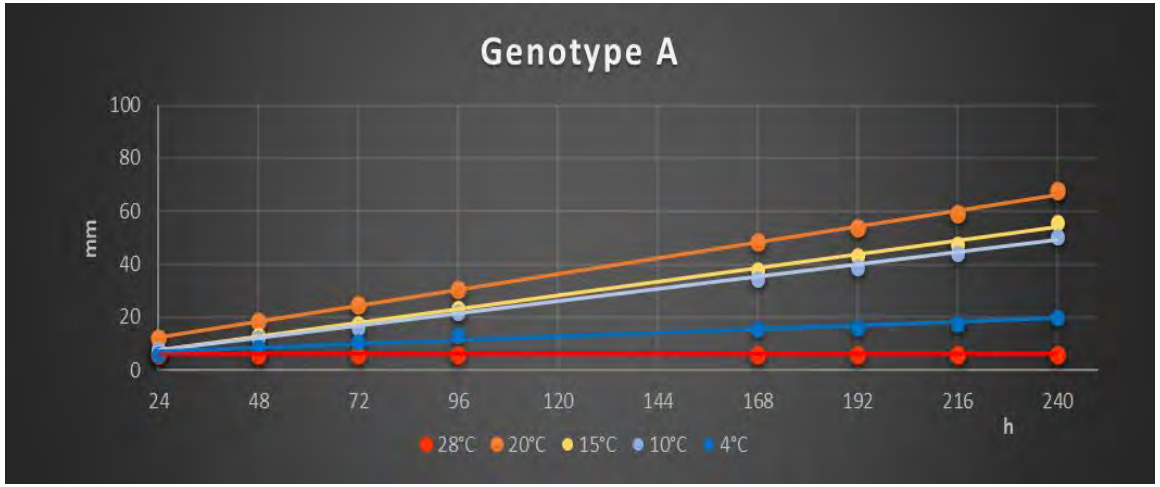
Objective and methods

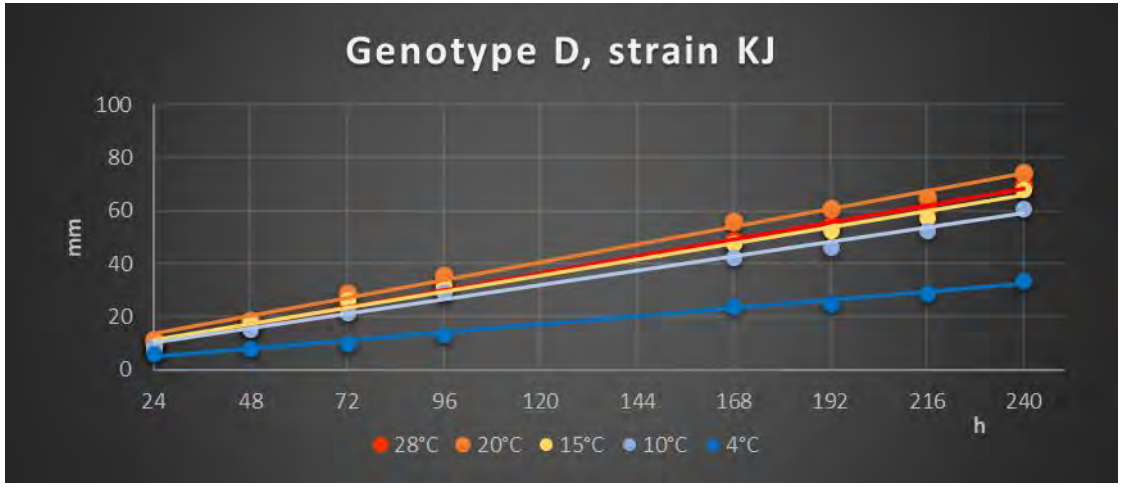
- Does *A. astaci* genotype D thrive also in cold water temperatures?
  - *A. astaci* genotypes A, B, C, D, E and genotype D Kemijoki strain were cultivated on PG-1 agar as triplicate plates
  - Growth rate of genotypes were studied by measuring colony diameter in different temperatures (4 °C, 10 °C, 15 °C, 20 °C, 28 °C) for 11 days.

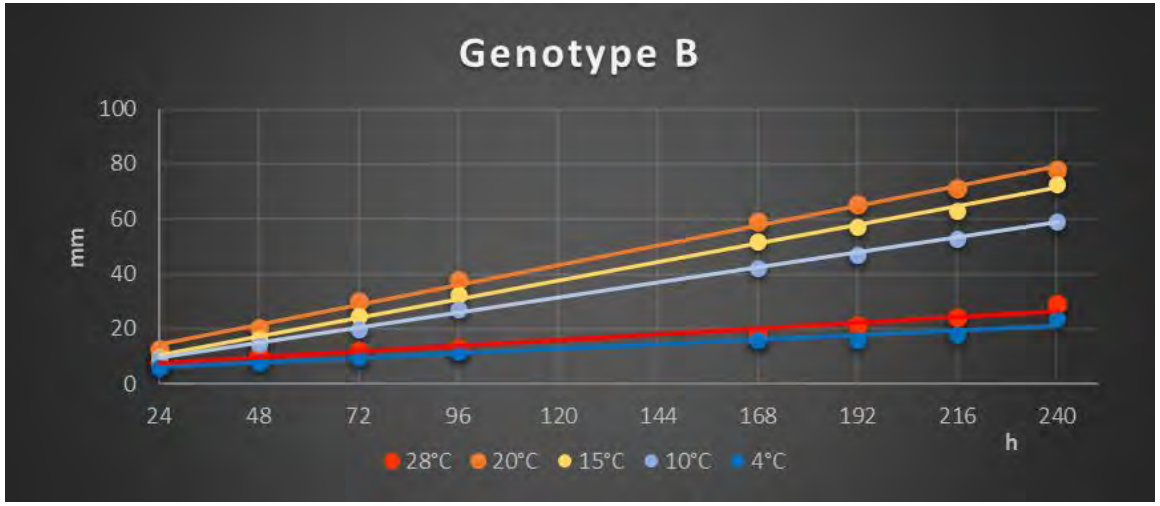
A. astaci strain	Genotype	Host	From
RR242	A	Noble crayfish	Venesjärvi, Finland
RR257	B	Signal crayfish	Kitee, Finland
RR160	C	Noble crayfish	Pitt lake, Canada
RR196	D	Red swamp crayfish	Spain
RR143	E	Spinycheek crayfish	Smecno, Czech Republic
RR 267, Kemijoki (KJ)	D	Noble crayfish	Kemijoki, Finland

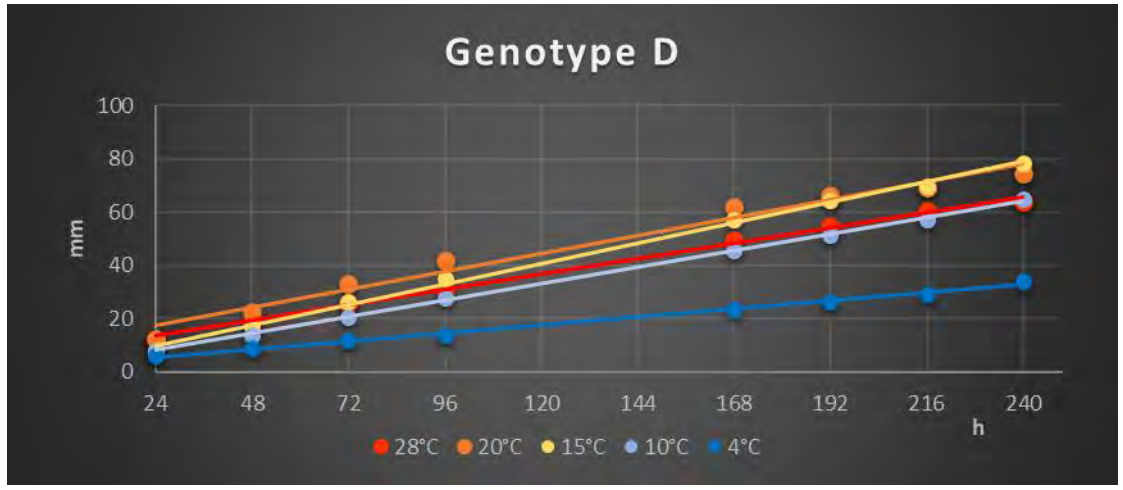
Table. Strains, genotype, host and place of *A. astaci* isolated and used in growth experiment

Growth rate of *A. astaci* genotypes in different temperatures









Growth rate of *A. astaci genotypes* in different temperatures

- For all genotypes optimum growth temperature for hyphan was 15 - 20 °C
- In cold temperature 4°C growth was less than in 15-20 C in all strains.
- Genotypes D had good growth in 28 C, while for genotype A and B growth was compromised in this temperature
- Both genotype D strains had higher growth rate in 4°C than other genotypes and this was significantly different compared to genotype A growth rate (p=0.018) or genotype B growth rate (p=0.018).

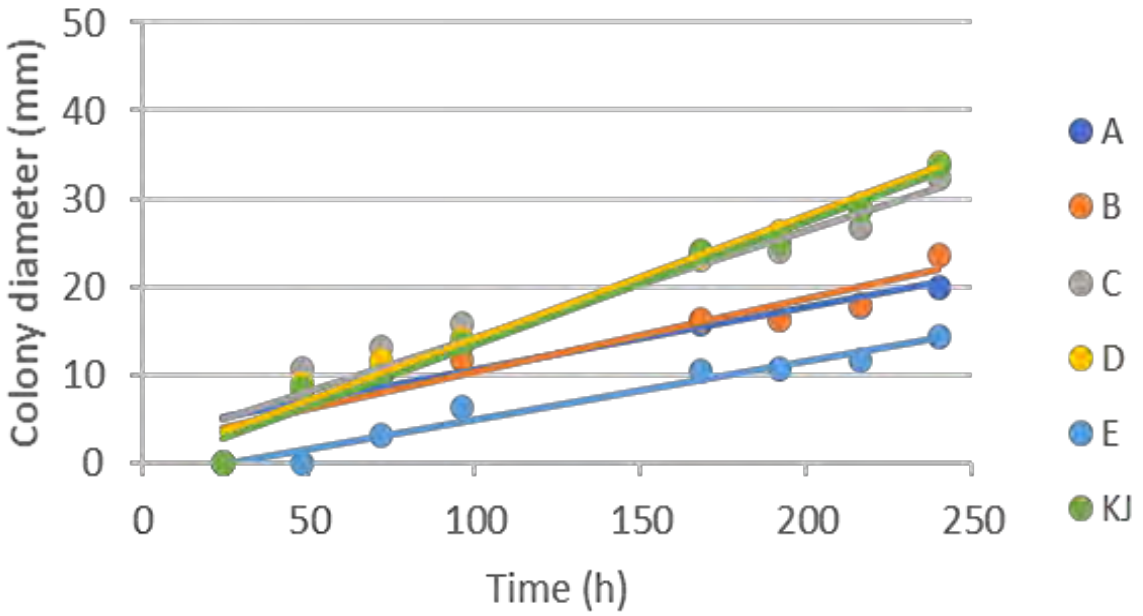


Fig. Colony diameters of different genotypes of *A. astaci* at 4 °C. Genotype D and the Kemijoki-strain KJ are the fastest growers

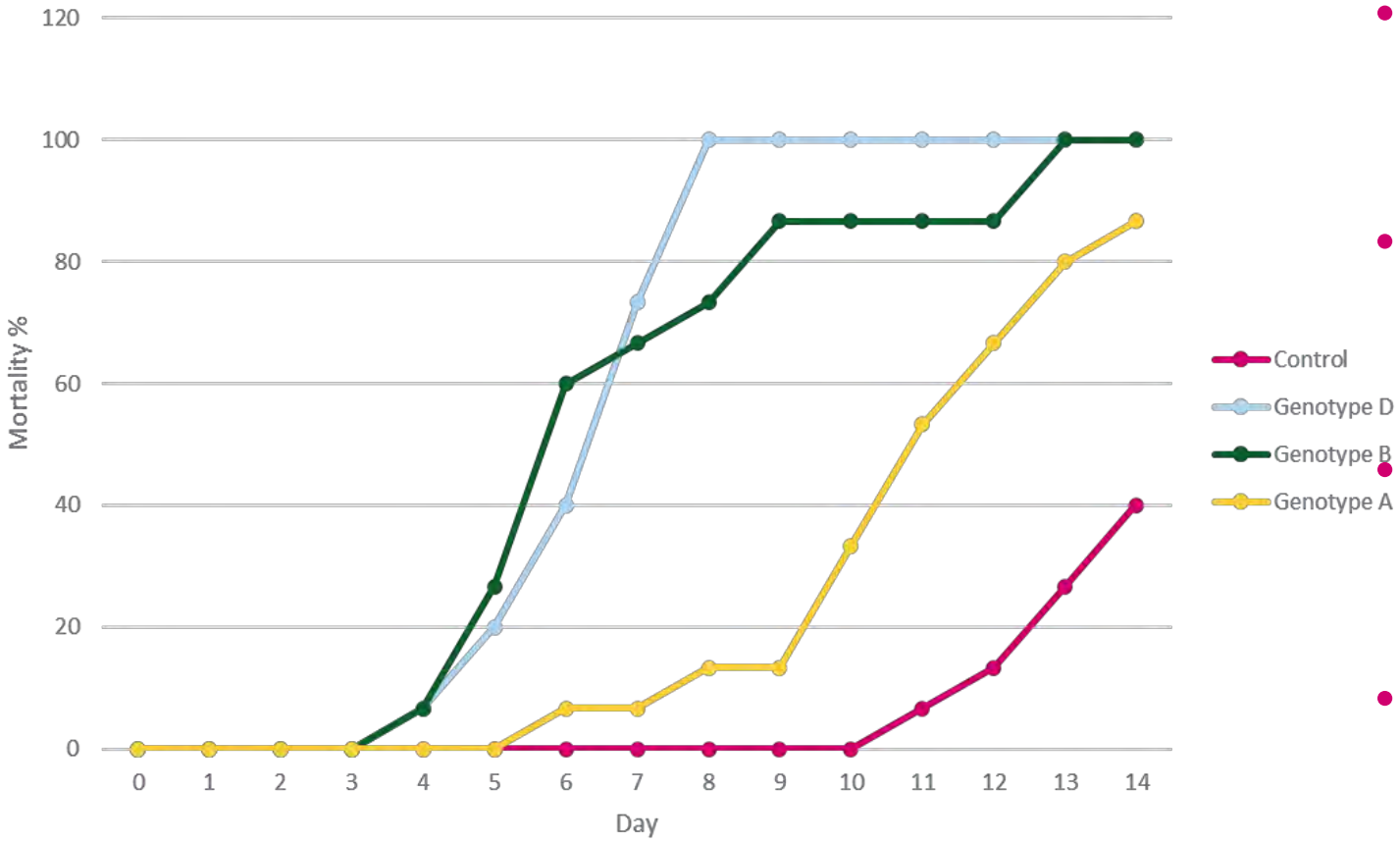
Objective and methods

- How susceptible noble crayfish is for *A. astaci* genotype D Kemijoki strain?
  - Noble crayfish were received from *A. astaci* free crayfish farm.
  - Five Crayfish were included in standing water with aeration in tanks
  - 15 crayfish (three triplicate tanks) were exposed to 1000 spores/ml of one *A. astaci* genotype (A, B, or D Kemijoki strain, see table)
  - Water temperature in tanks were around 17 °C

A. Astaci strain	Genotype	Host	From
RR267, Kemijoki	D	Noble crayfish	Kemijoki, Finland
RR263	B	Noble crayfish	Hyrynjärvi, Finland
RR262	A	Noble crayfish	Luvanjärvi, Finland

Table. Strains, genotype, host and place of *A. astaci* isolated and used in exposure experiment

Cumulative mortality in exposure experiment



- Similar virulence was detected with *A. astaci* genotypes D Kemijoki strain and B exposure: first mortality was recorded 4 days post-infection in both genotypes.
- 100 % mortality was detected already 7 days post-infection with genotype D Kemijoki strain exposure and 13 days post-infection with genotype B.
- Genotypes B and D Kemijoki had significantly higher cumulative mortality than in control group (p=0,004 and p=0,002, respectively)
- 40 % mortality in control group was detetected on day 14. No *A. astaci* was detected in control group crayfish, tested with qPCR

- **Conclusions**
  - *A. astaci* genotype D strain isolated from Kemijoki noble crayfish population showed
    - high growth rate also in cold temperatures
    - high virulence for northern noble crayfish
  - *A. astaci* genotype D pose great risk for native Noble crayfish populations also in Northern Europe
  - *A. astaci* genotype D has been associated with Louisiana red swamp crayfish (*Procambarus clarkii*) and in southern European countries with wild populations of red swamp crayfish. Another reservoir of genotype D is the colorful *Procambarus* species which are common in the aquarium trade.
  - The affected population in Kemijoki estuary were catched between two seaports (appr. 15 and 20 km distance), possible infection route is alien crayfish species arriving with ship ballast , or less probably aquarium trade species escapes.

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Thank you for your attention!

FINNISH FOOD AUTHORITY

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World Organisation for Animal Health

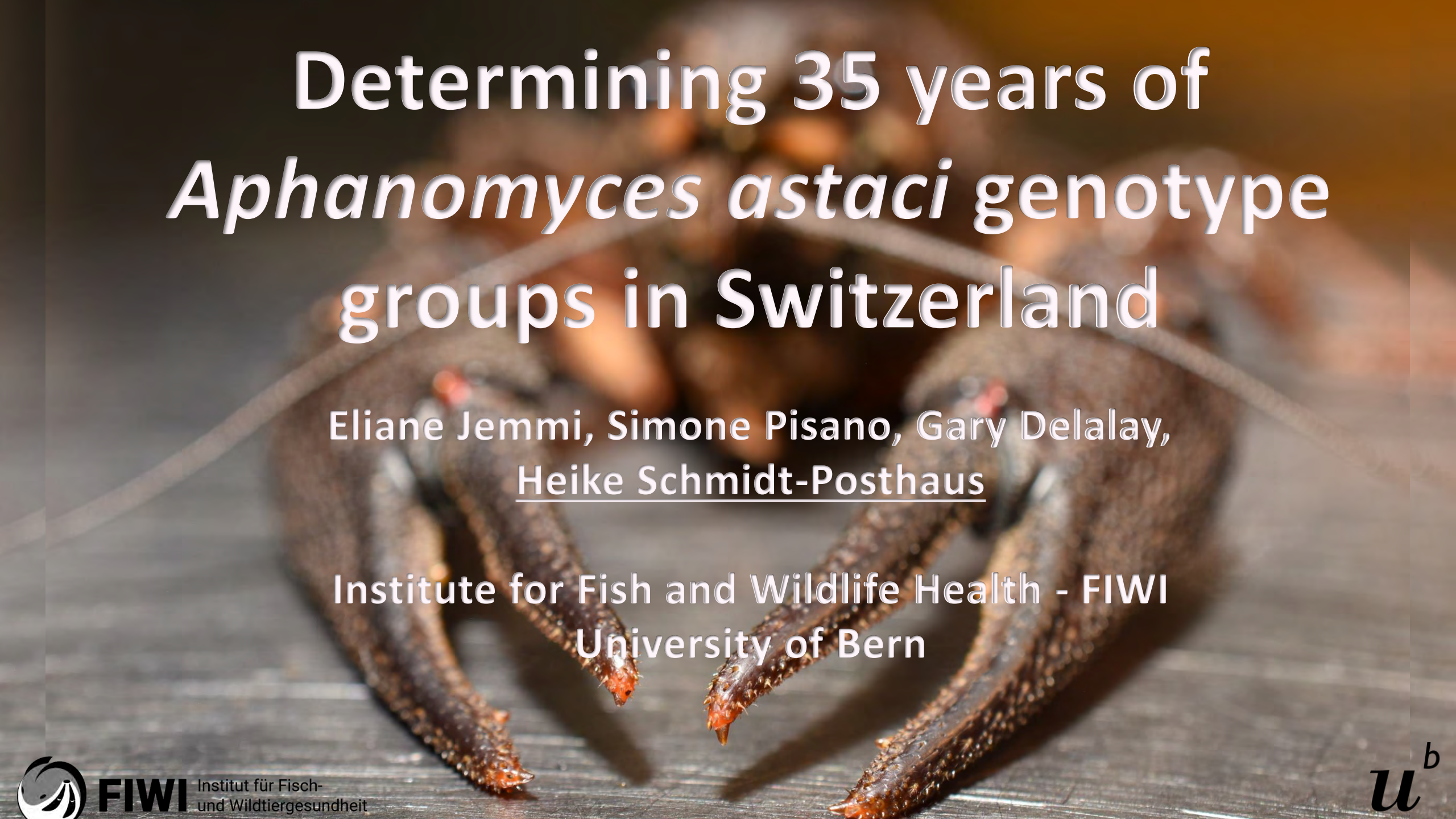
Founded as OIE

CRAYFISH PLAGUE

Determining 35 years of *Aphanomyces astaci* genotype groups in Switzerland

Eliane Jemmi, Simone Pisano, Gary Delalay,
Heike Schmidt-Posthaus

Institute for Fish and Wildlife Health - FIWI
University of Bern



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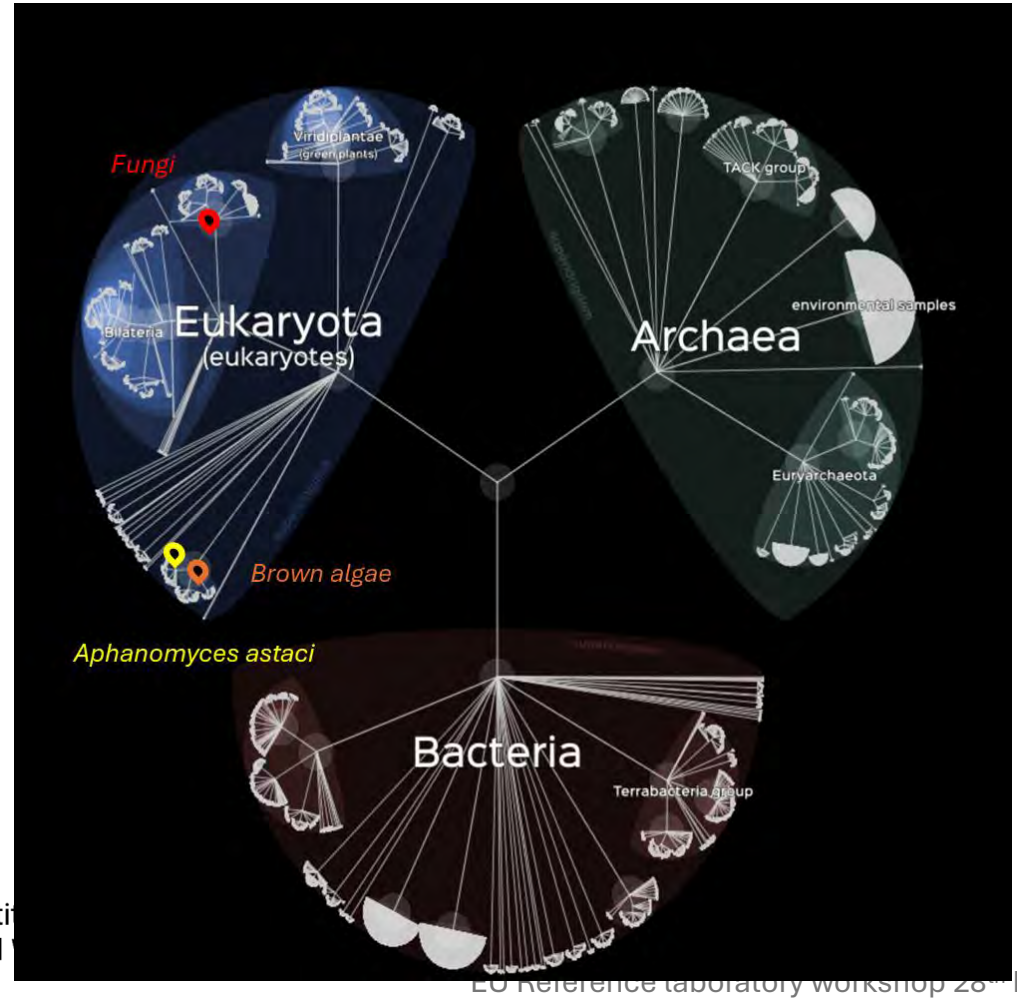
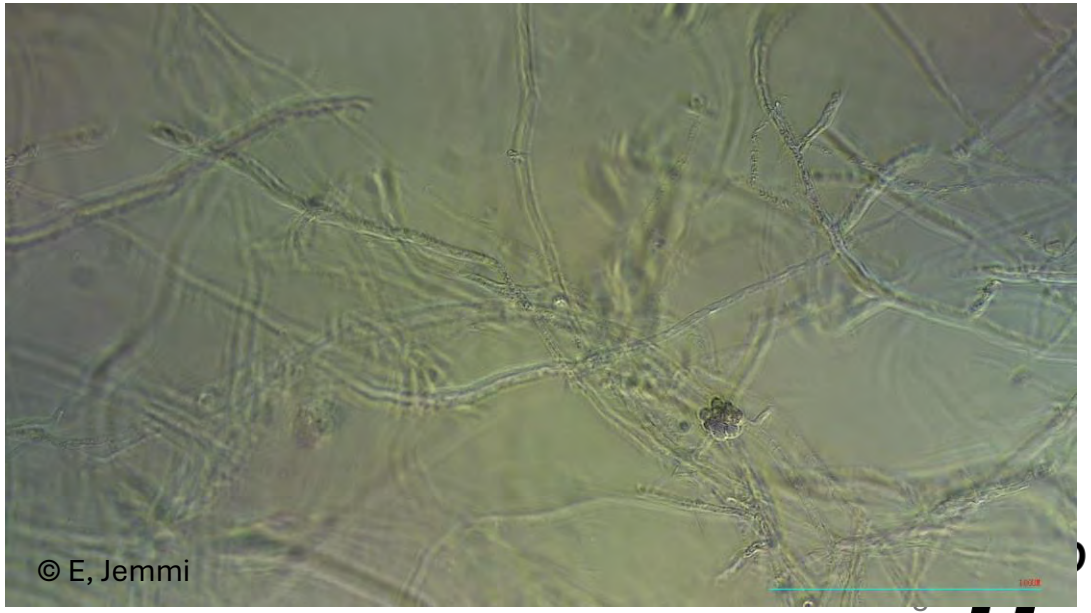
u b

What is crayfish plague?

- Caused by the oomycete *Aphanomyces astaci*
- Fungus-like eukaryotic microorganism

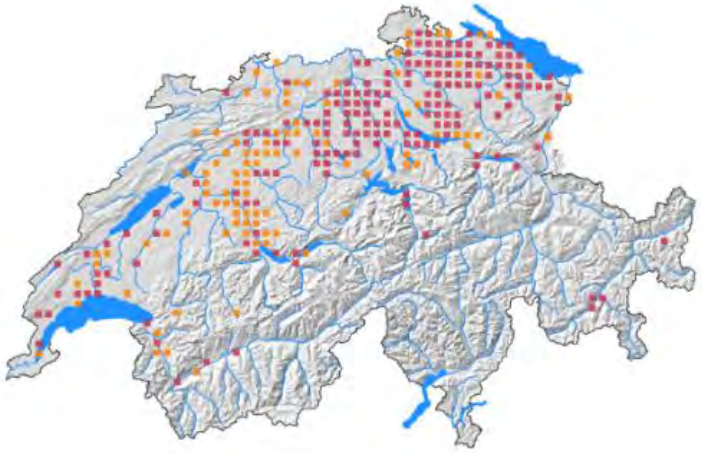




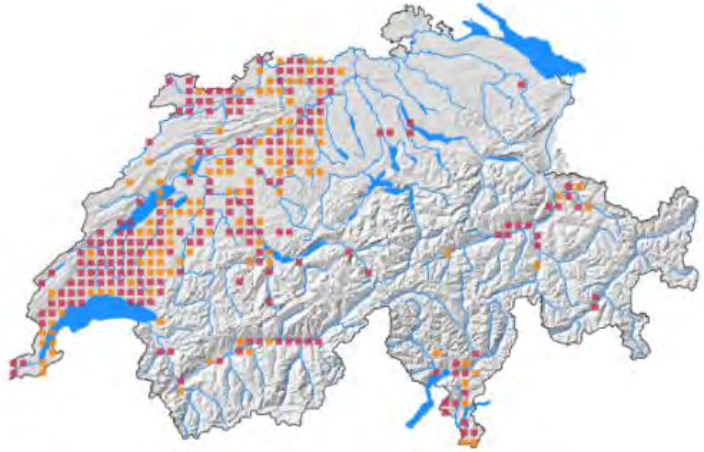
What is crayfish plague?

- Listed among the "100 of the World's Worst Invasive Alien Species (IUCN)"
- Acute disease
- Decline and local extinction of **European crayfish**
- All CH native crayfish species are either **endangered** or **highly endangered**

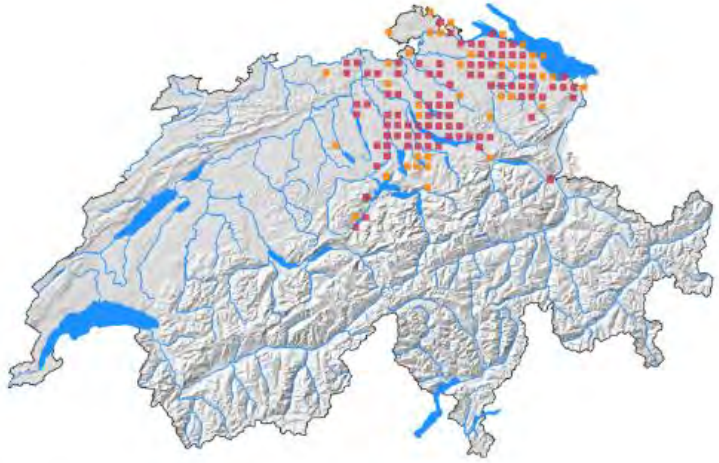
noble crayfish
Astacus astacus



white-clawed crayfish
Austropotamobius pallipes

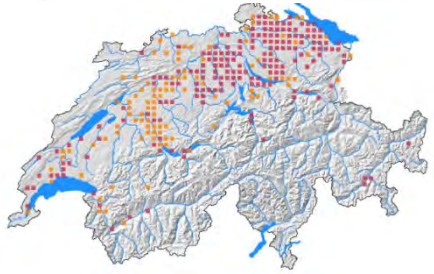


stone crayfish
Austropotamobius torrentium

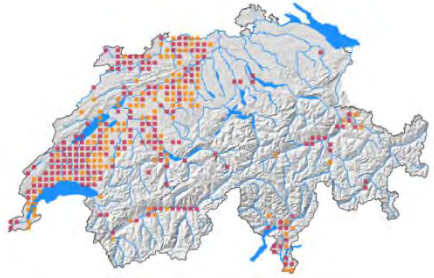


native crayfish species

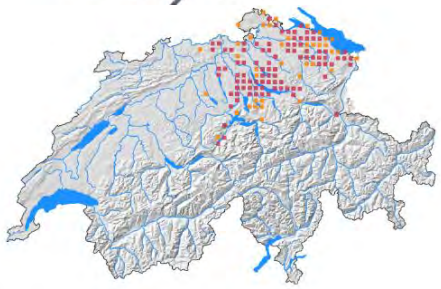
noble crayfish



white-clawed crayfish



stone crayfish



native crayfish species

narrow-clawed crayfish

east Europe

signal crayfish

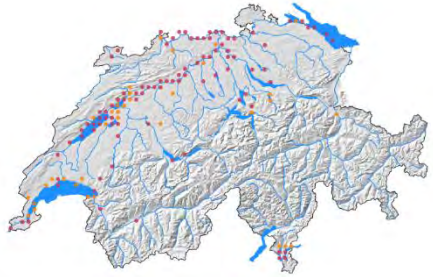
1959 in EU

red swamp crayfish

1957 in EU

spiny cheek crayfish

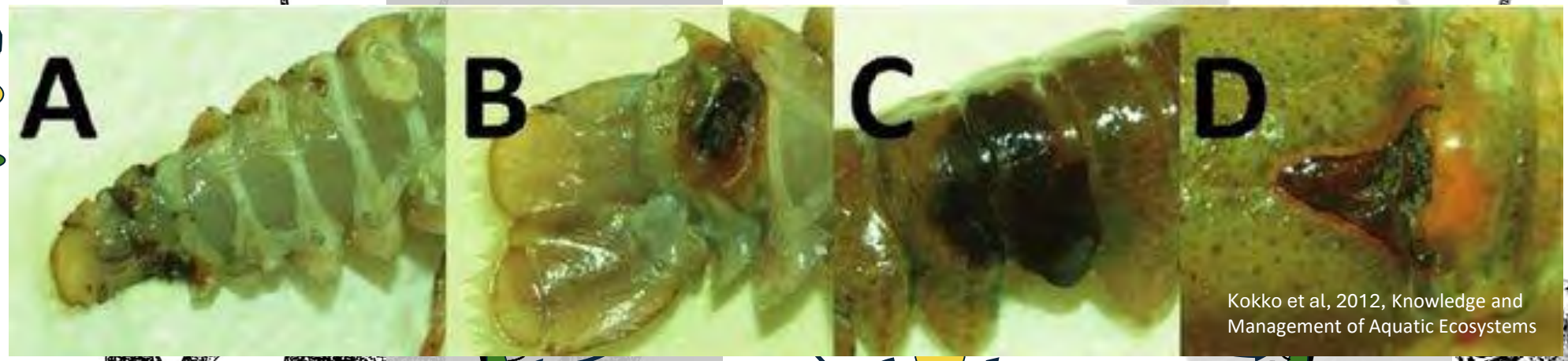
1890 in EU



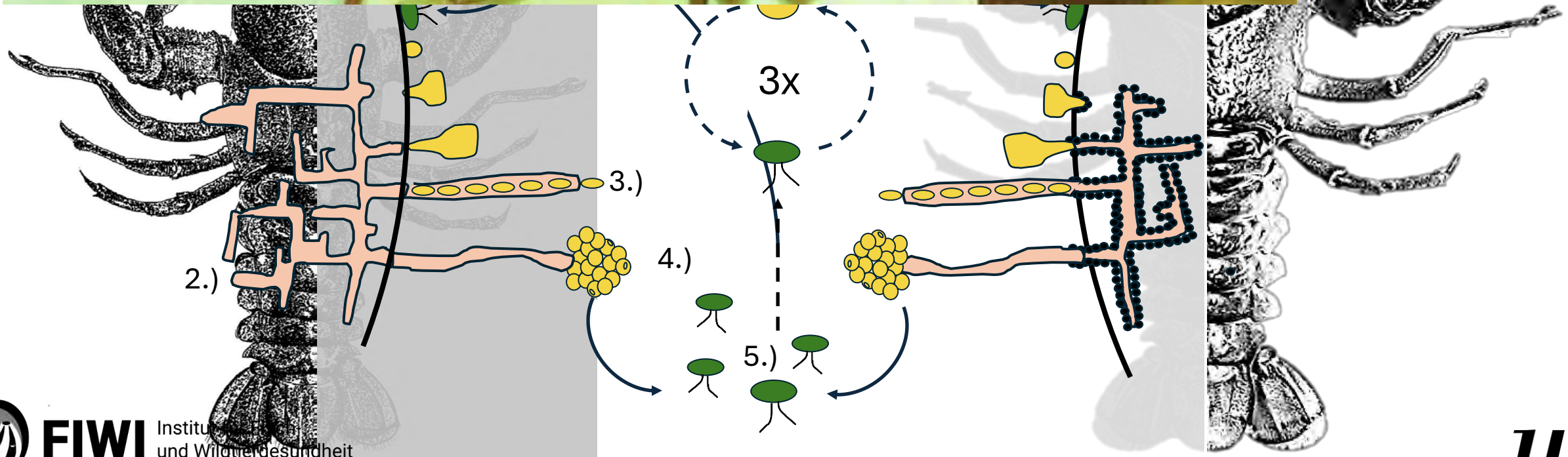
invasive North American crayfish species

endemic crayfish

invasive crayfish

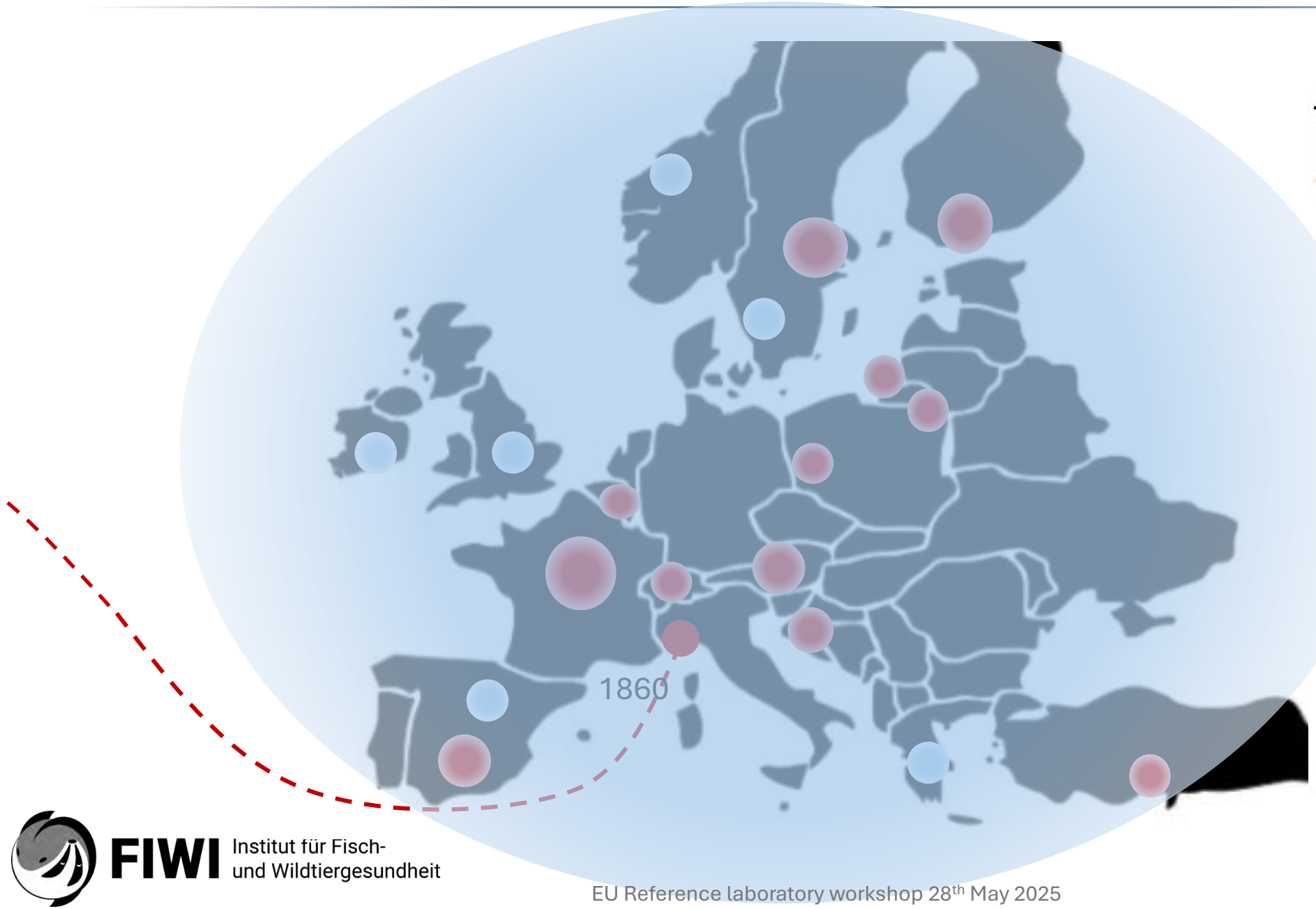


Kokko et al, 2012, Knowledge and Management of Aquatic Ecosystems



How did it spread in Europe?

How did it spread in Europe?



Genetic diversity of *Aphanomyces astaci* in Europe

defined by their distinct RAPD patterns (Huang et al, 1994)

A

American host still unknown

B

associated with **signal crayfish**

Sweden
1959

C

only in Sweden (invasive species) and Ireland (native species), in **Canada**

D

associated with **red swamp crayfish**

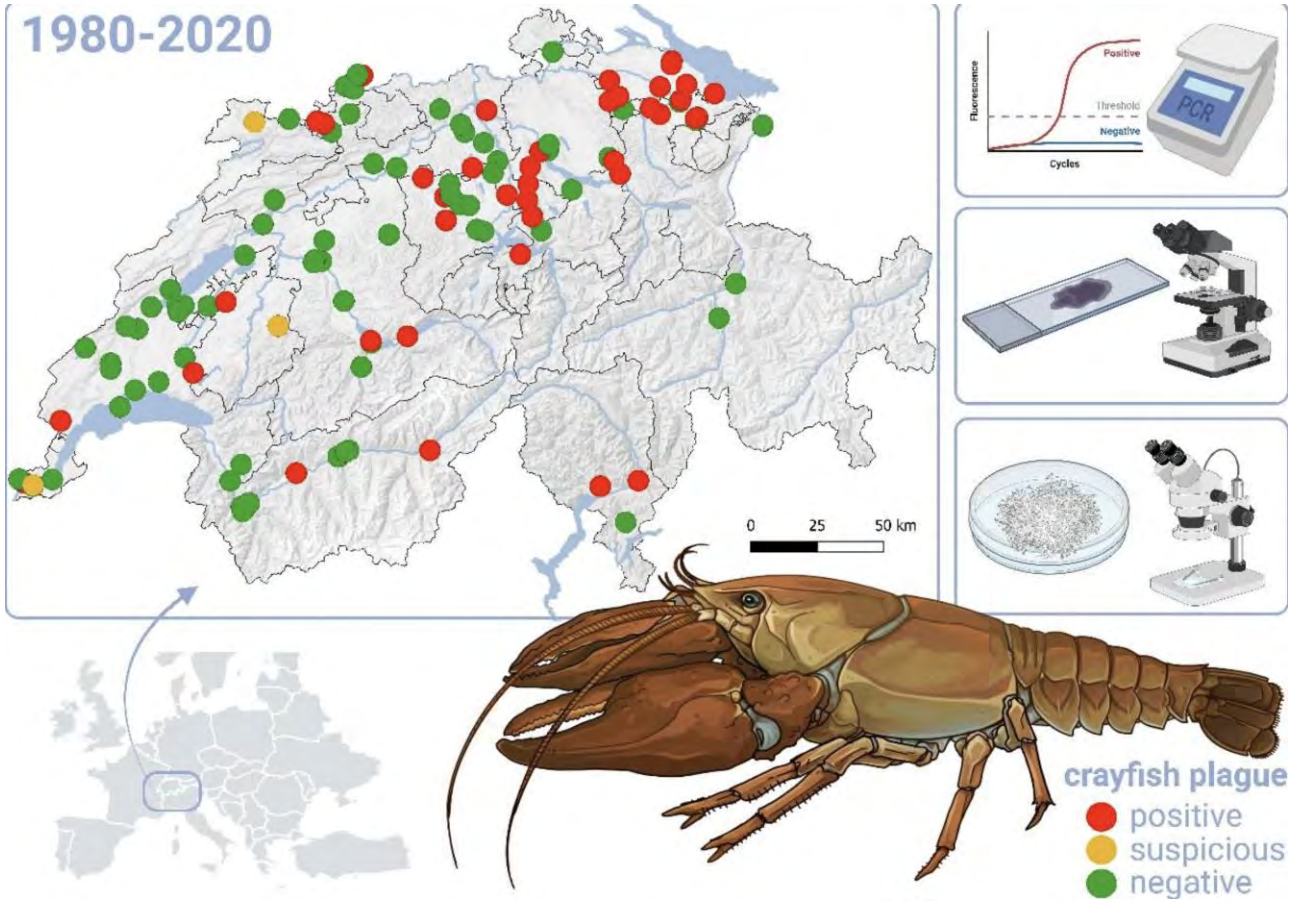
Spain
1973

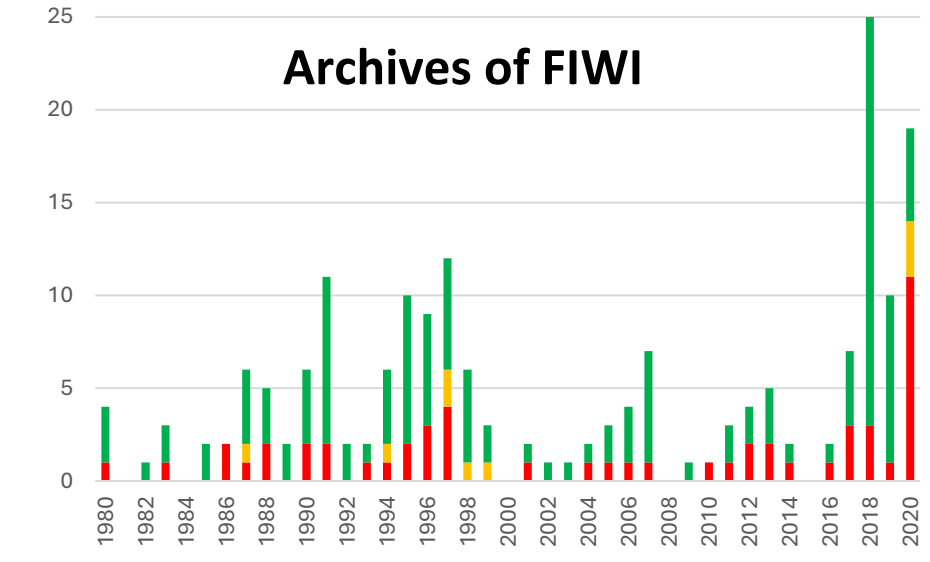
E

associated with **spiny cheek crayfish**

Poland
1890

Genotyping *Aphanomyces astaci* in Switzerland





Culture

Histology

PCR Oidtmann et al. 2006

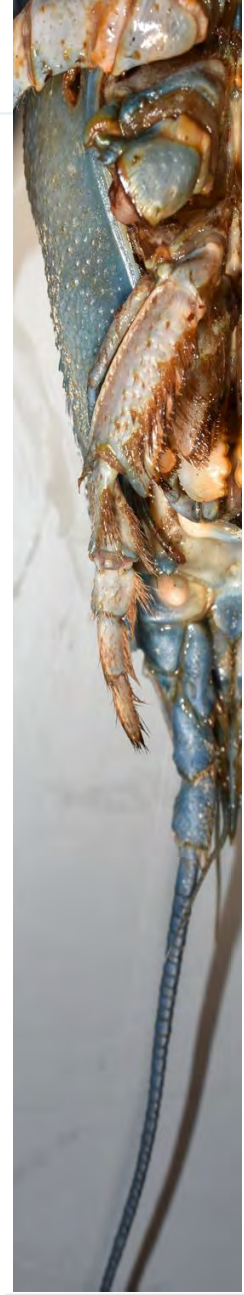
Archived FFPE

Pisano R.R.P. et al., 2024, Journal of Invertebrate Pathology

Journal of Invertebrate Pathology
Volume 206, September 2024, 108159

An old unknown: 40years of crayfish plague monitoring in Switzerland, the water tower of Europe

Simone Roberto Rolando Pisano [a], Jonas Steiner [a], Elodie Cristina [a], Zoé Delefortrie [a], Gary Delalay [a], Raphael Krieg [b], Armin Zenker [b], Heike Schmidt-Posthaus [a]



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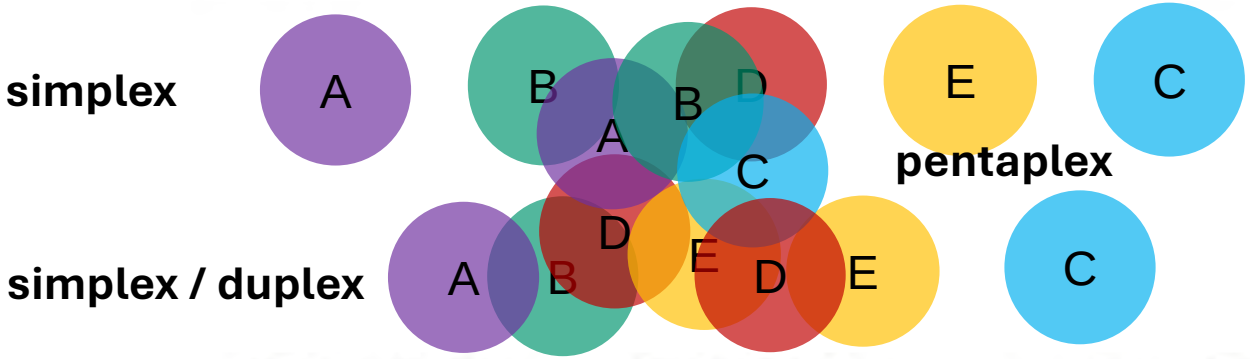
Genetic diversity of *Aphanomyces astaci* in Europe

defined by their distinct RAPD patterns

frontiers in Ecology and Evolution

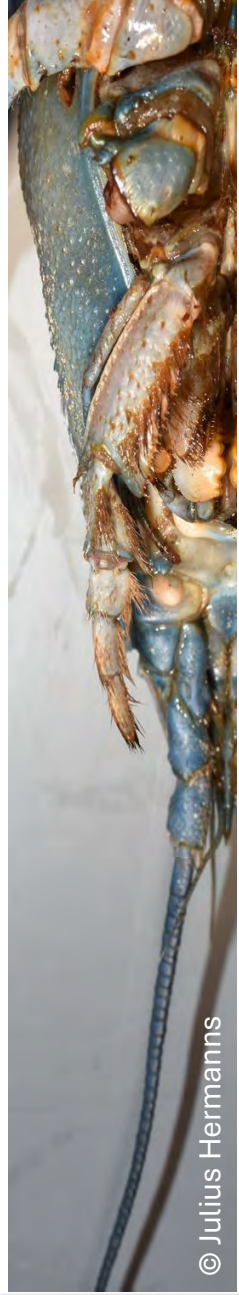
METHODS
published: 28 June 2021
doi: 10.3389/fevo.2021.597585



Real-Time PCR Assays for Rapid Identification of Common *Aphanomyces astaci* Genotypes

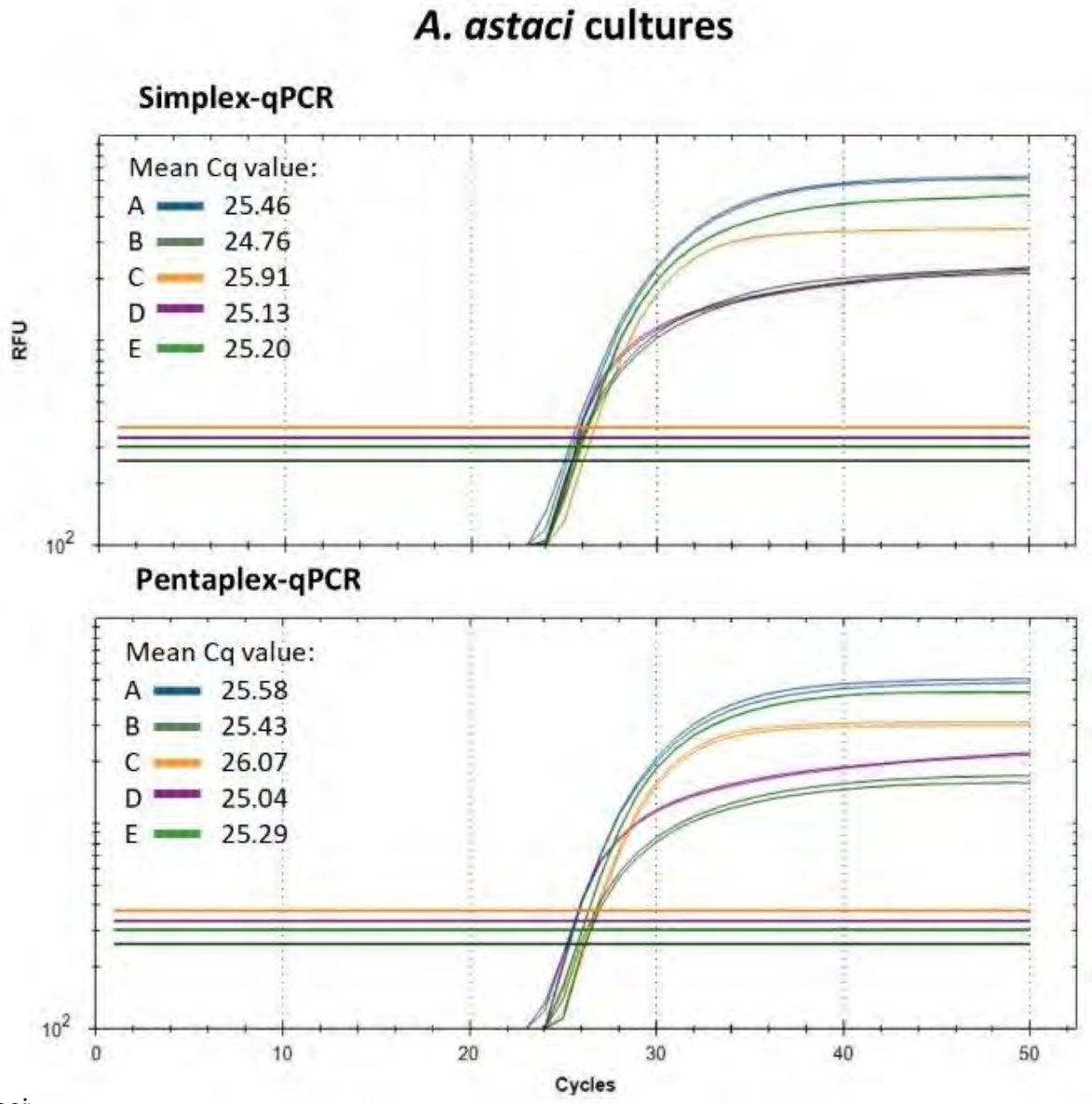
Marco Di Domenico[1], Valentina Curini[1], Riccardo Caprioli[1,2], Carla Giansante[1], Agata Mrugała[3,4,5,6], Michaela Mojžišová[3], Cesare Cammà[1] and Adam Petrusek[3]*

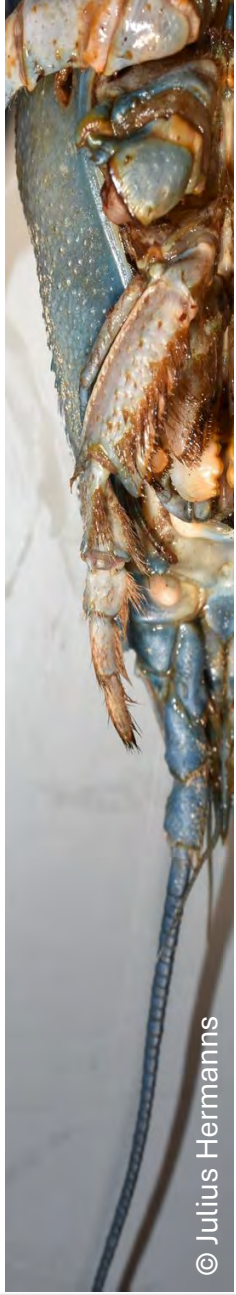
[1] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Teramo, Italy, [2] Agenzia Regionale Protezione Ambientale del Lazio, Rome, Italy, [3] Department of Ecology, Faculty of Science, Charles University, Prague, Czechia, [4] Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany, [5] Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany, [6] Berlin-Brandenburg Institute of Advanced Biodiversity Research, Berlin, Germany



Genetic diversity of *Aphanomyces astaci* in Europe

Multiplexing of all 5 genotype group primers (Di Domenico et al, 2021)

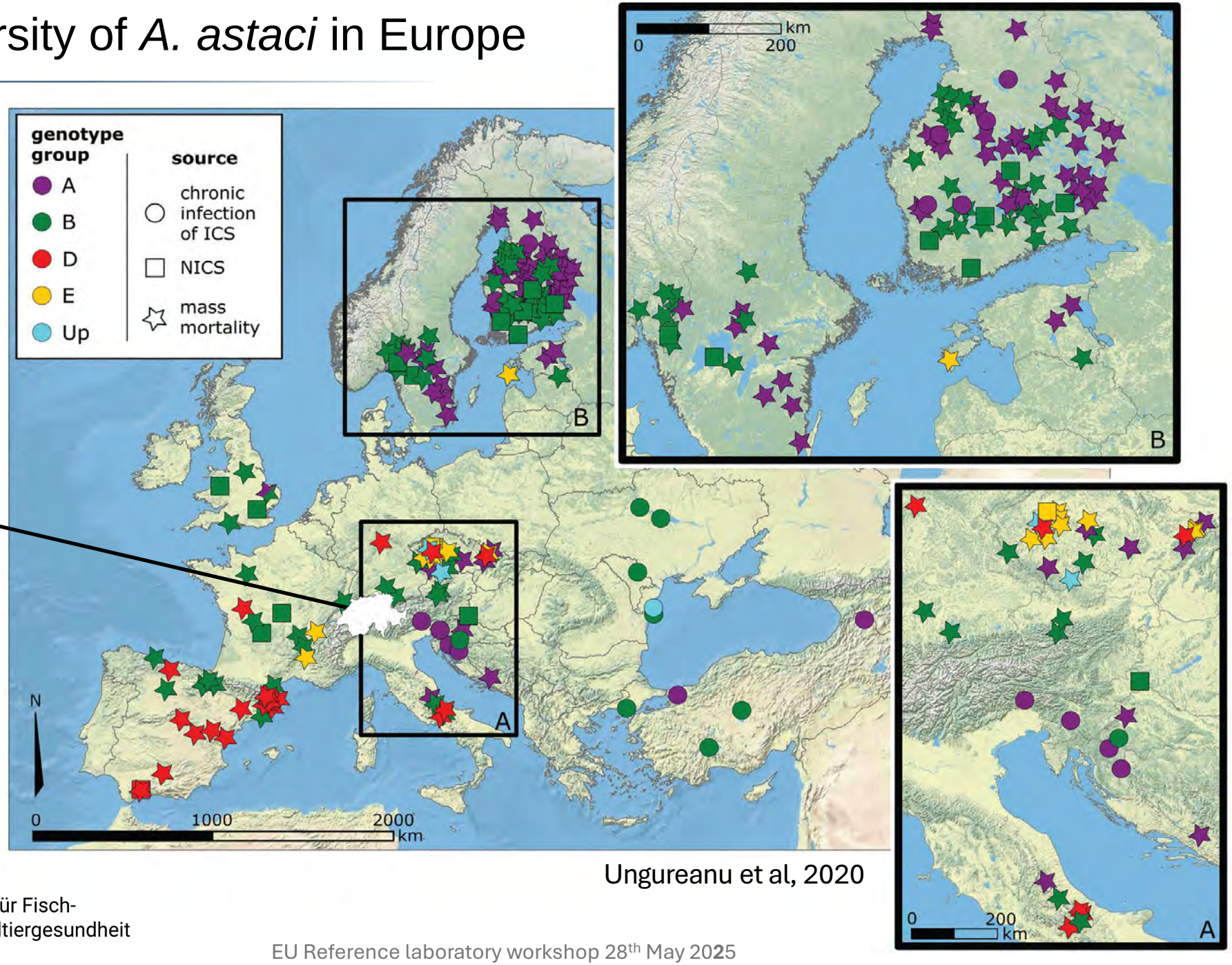




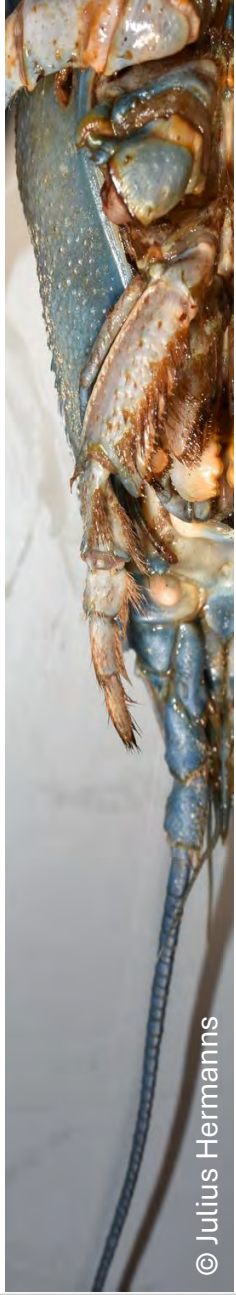
Genetic diversity of *A. astaci* in Europe

based on microsatellites and the large and small ribosomal subunit rnnL & rnnS

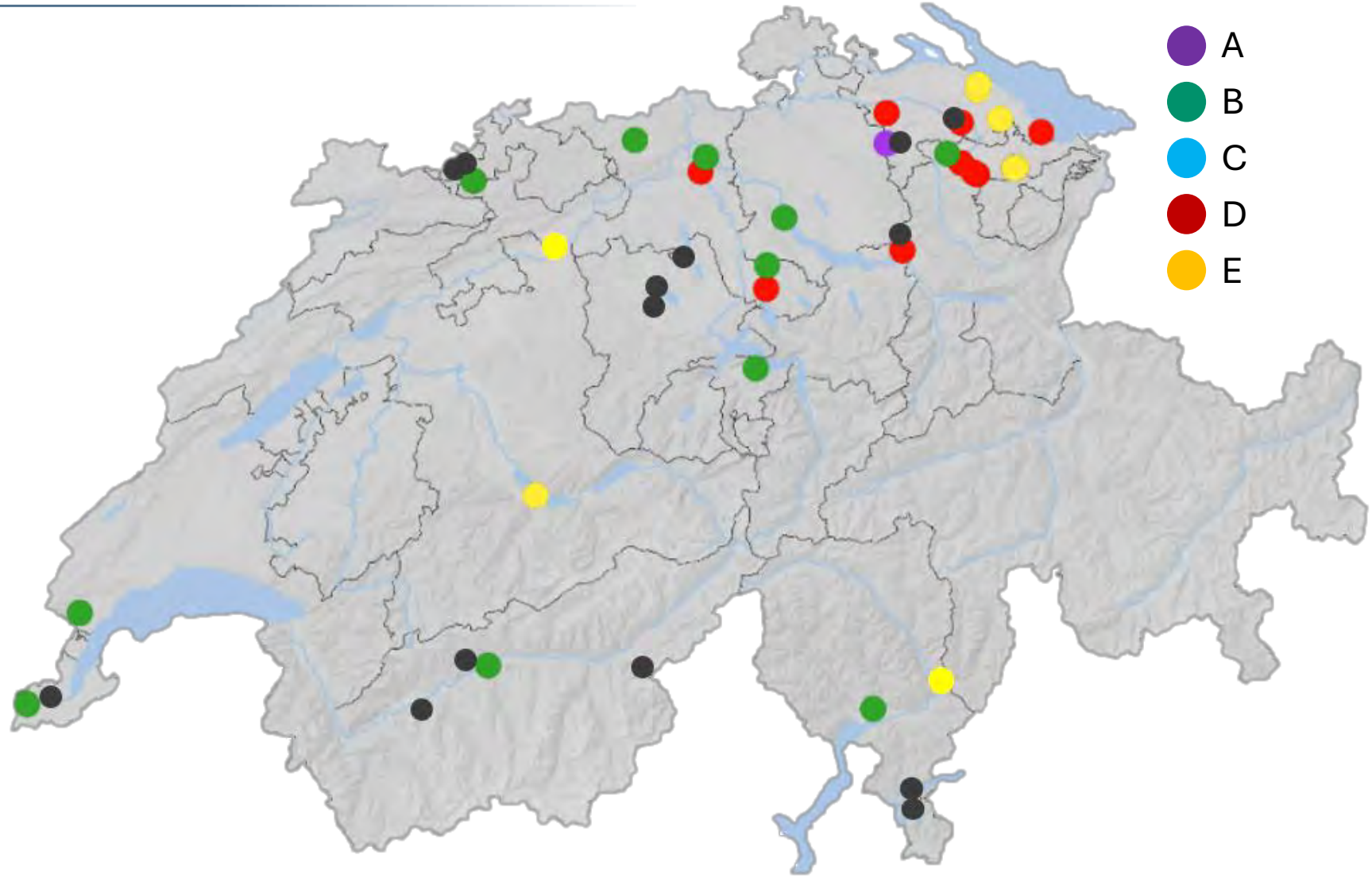
?

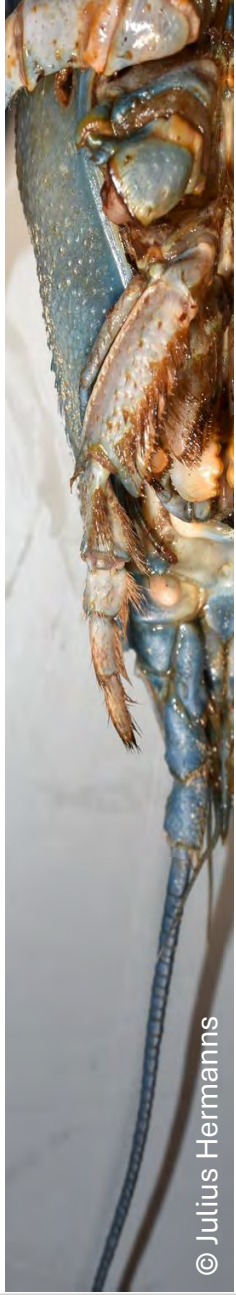


Ungureanu et al, 2020



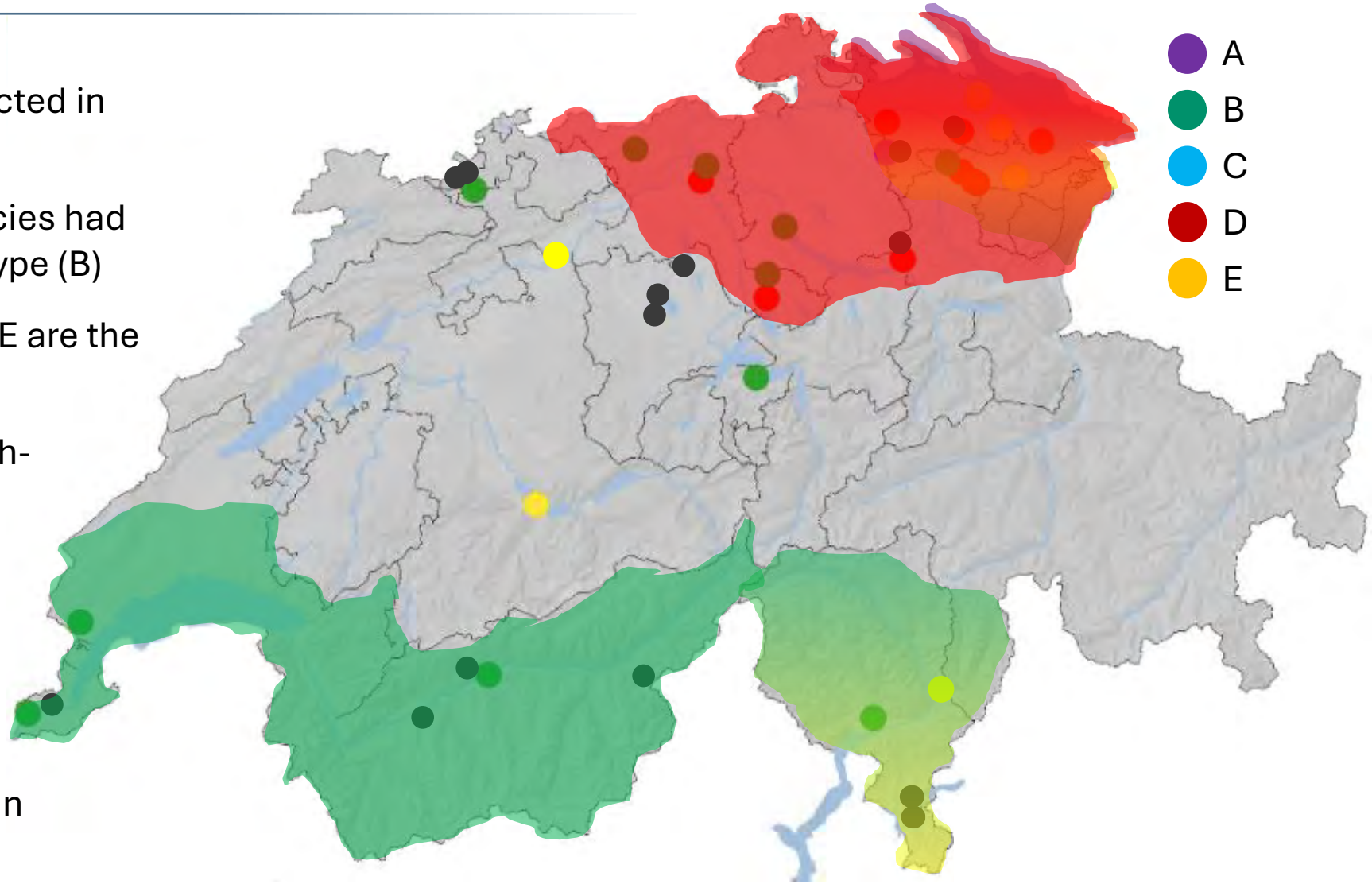
Genetic diversity of *A. astaci* in Switzerland (1991-2023)

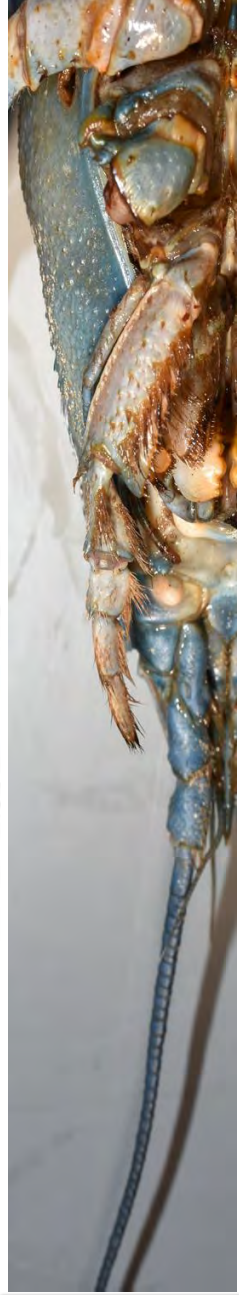




Genetic diversity of *A. astaci* in Switzerland (1991-2023)

- A, B, D, and E were detected in native crayfish
  - only 2 invasive species had a detectable genotype (B)
- Genotype groups B and E are the most prevalent
- Highest diversity in North-Eastern Switzerland
- In Rhine subbasin only genotype group B
- In Ticino subbasin genotype group B and E
- Genotype group D only in the North of Switzerland





Genetic diversity of *A. astaci* in Switzerland

A 2017 in the Rhone basin

- ❖ detected only once in a population with low mortality

B 1996 in the Limmat subbasin

- ❖ potentially underrepresented in dataset
- ❖ highest percentage of all genotype groups in Switzerland

C not detected

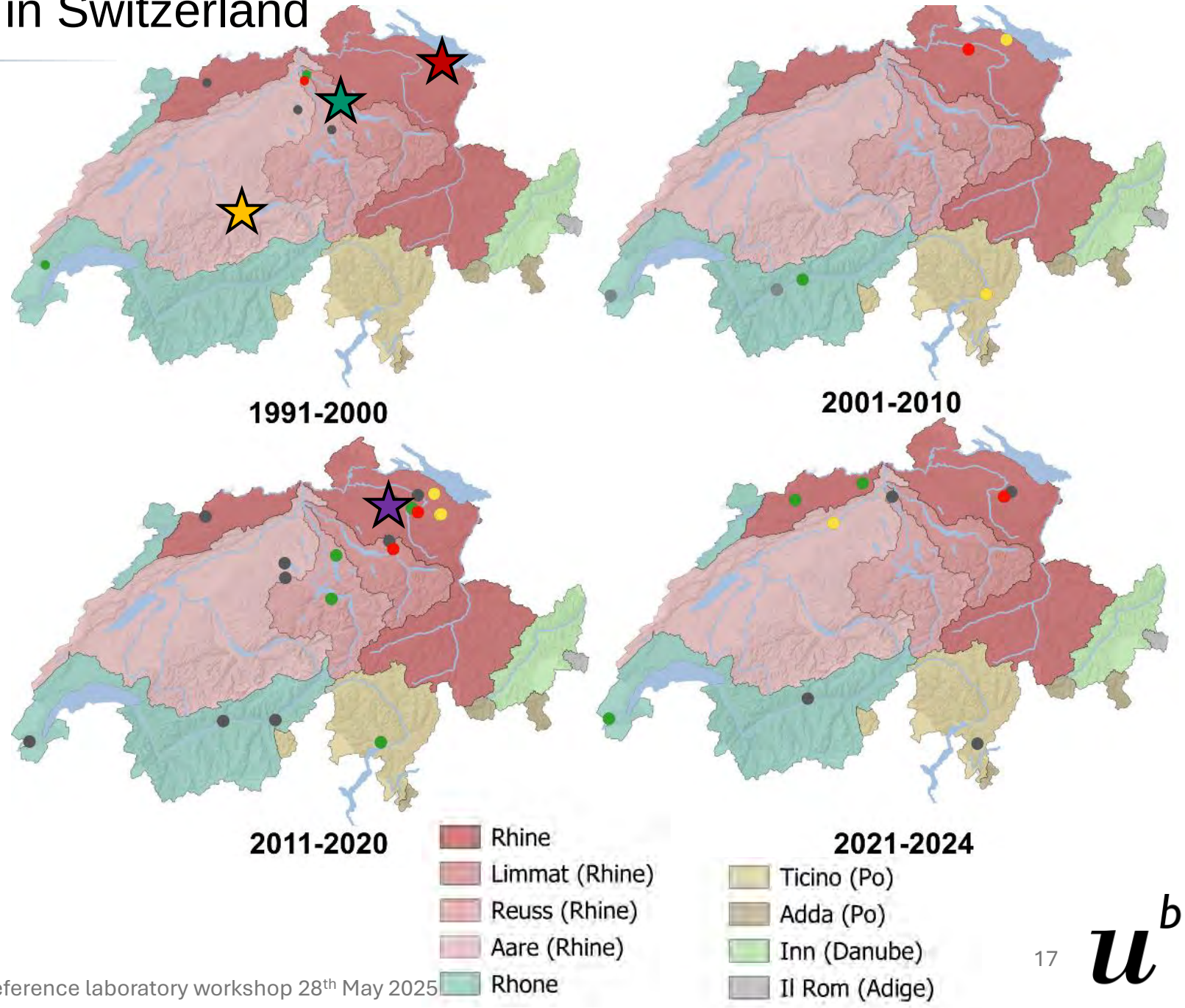
- ❖ similar to other European countries

D 1991 in the Rhine subbasin

- ❖ detected before the presence of its American host

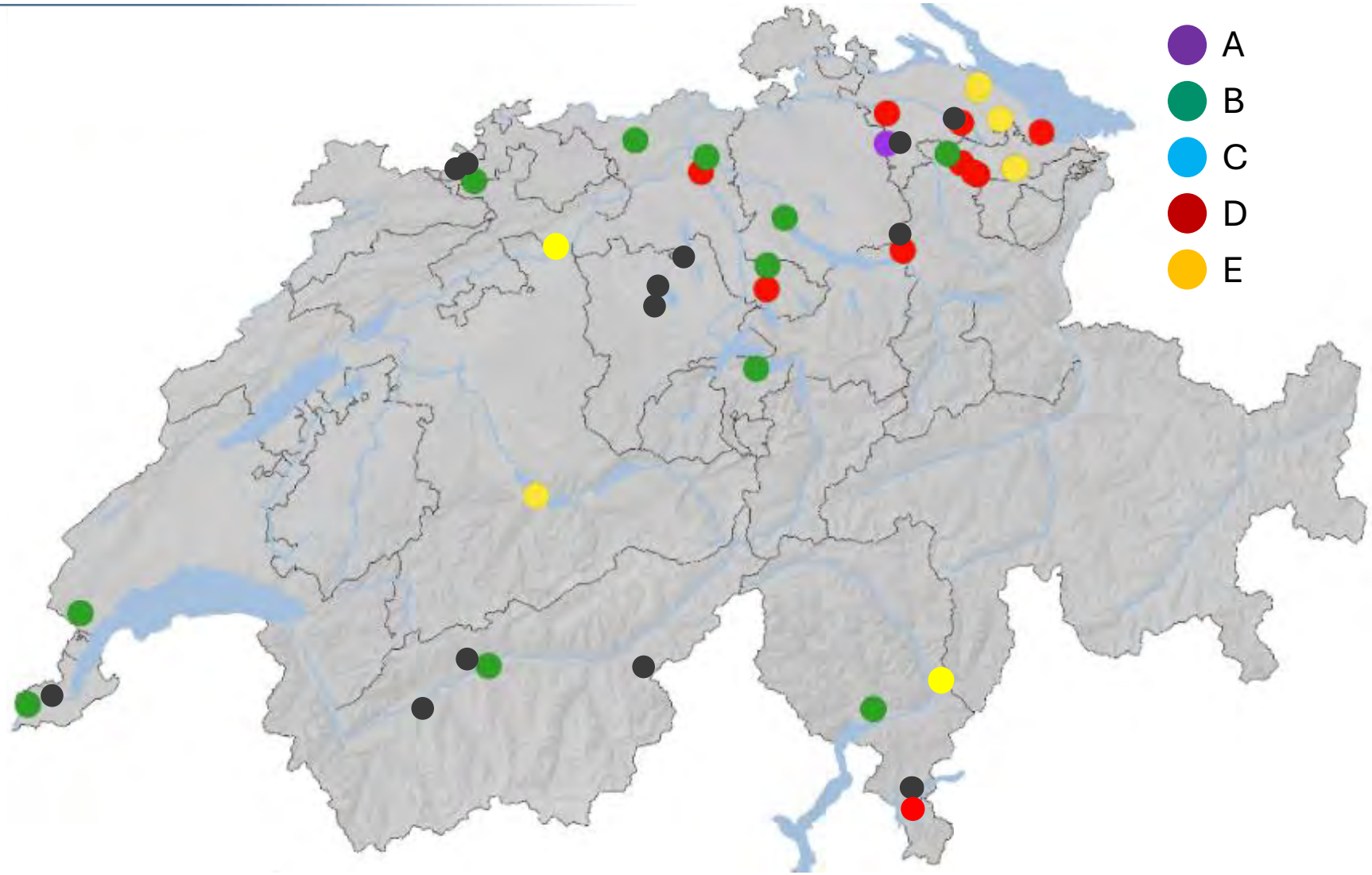
E 1994 in the Aare subbasin

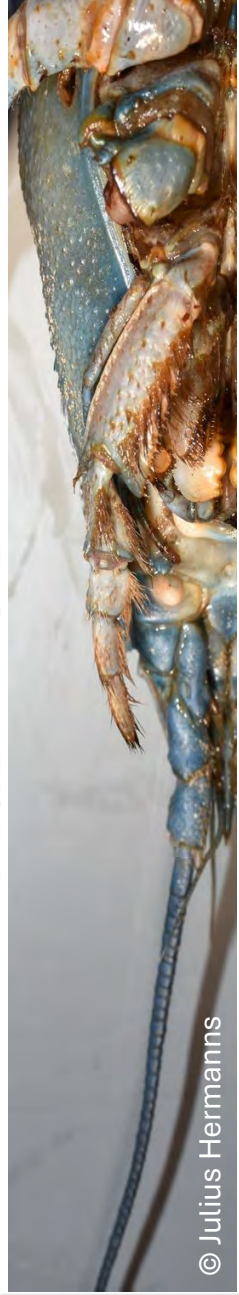
- ❖ might assume that only few carrier individuals are needed to cause high mortality
- ❖ detected once in Ticino subbasin and thereafter only in the North of Switzerland



Genetic diversity of *A. astaci* in Switzerland (1991-2023)

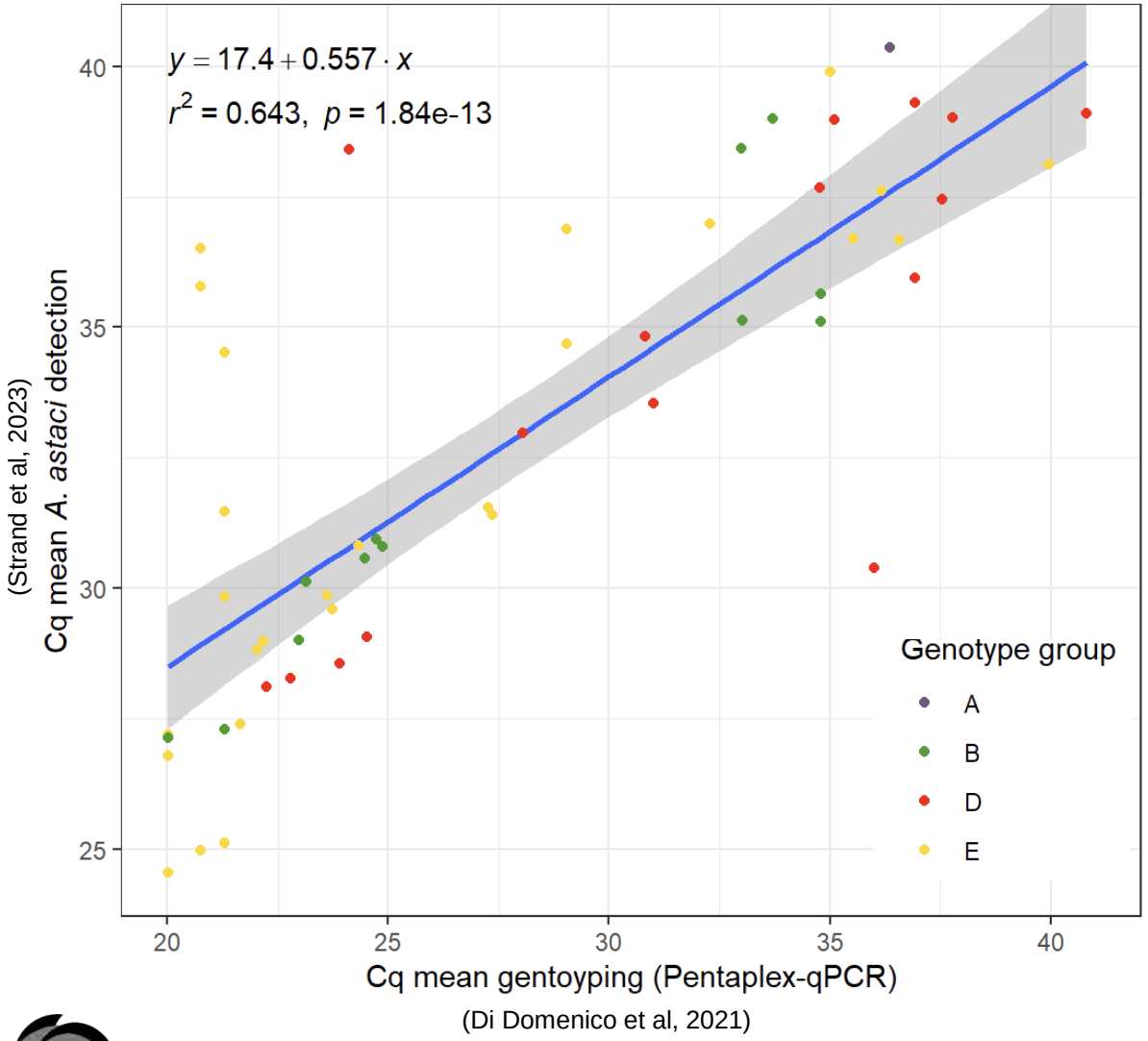
40 % of analysed populations could not be genotyped

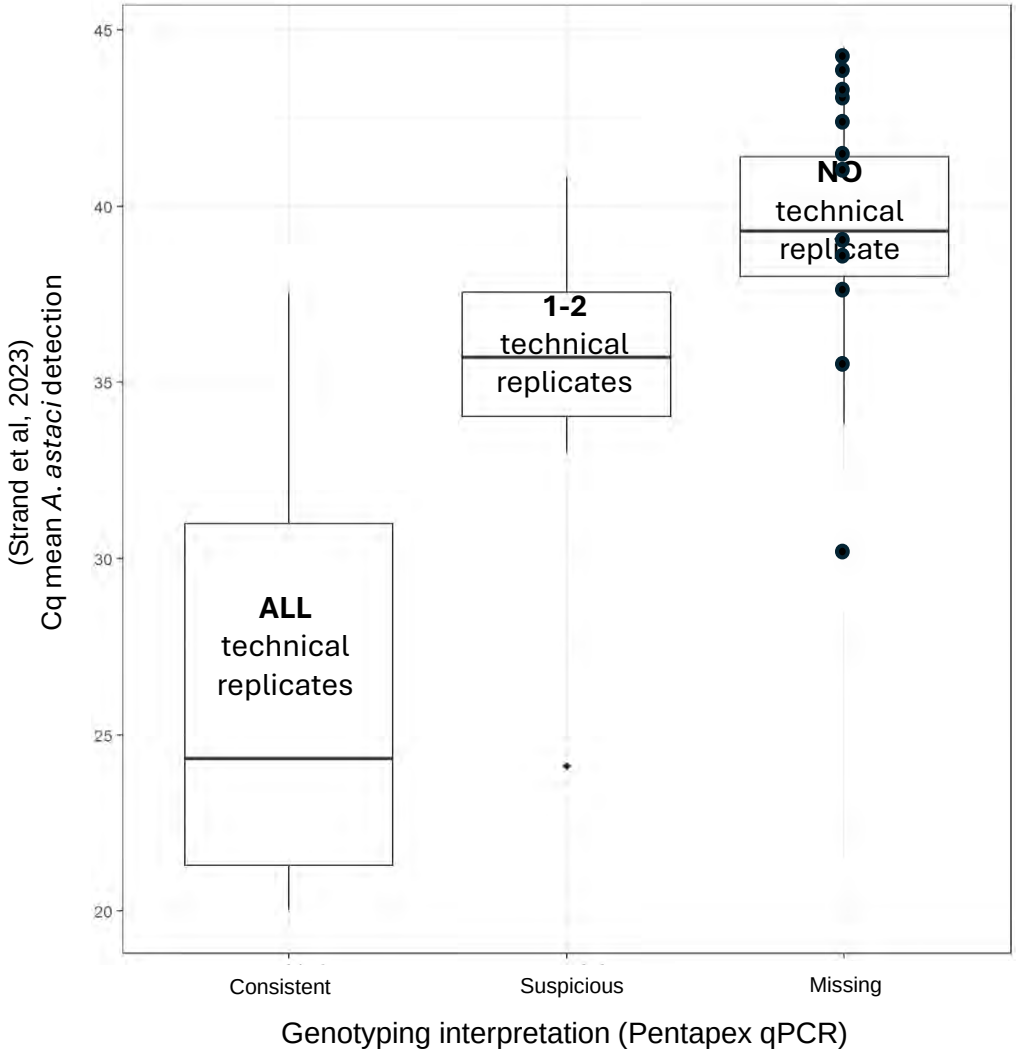


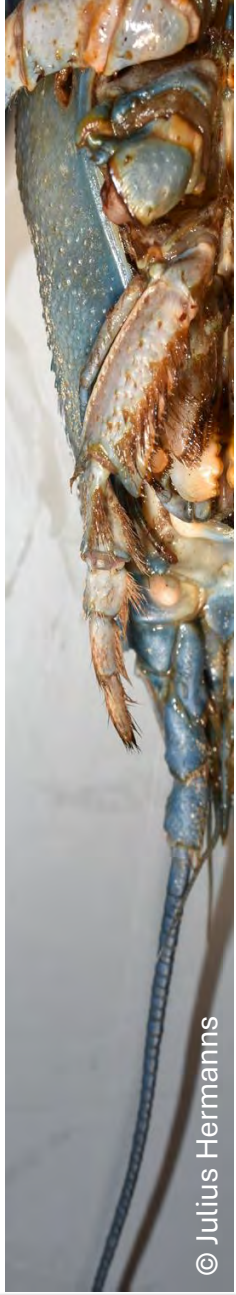


Genetic diversity of *Aphanomyces astaci* in Europe

Prediction of genotype groups (Di Domenico et al, 2021) based on Strand et al. qPCR (Strand et al, 2023)







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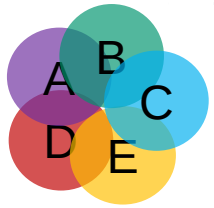
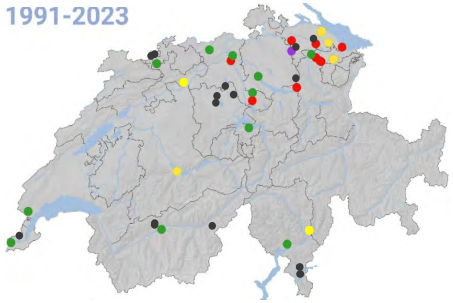
Summary



Pentaplex-qPCR shows comparable performance to Simplex-qPCR



Genotype groups A, B, D and E could be detected in Switzerland

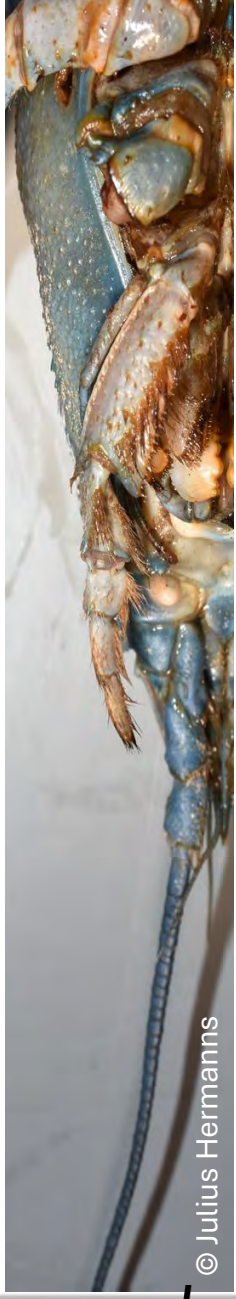
Archived samples can be used to get an understanding of the spatiotemporal pattern of the plague

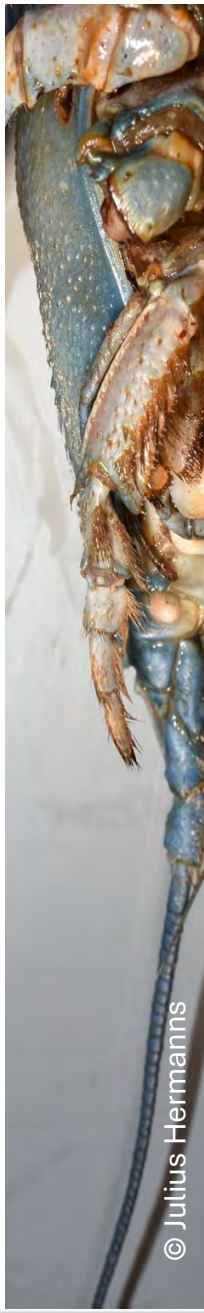
Our data is representative for the genotype groups **associated to outbreaks** of crayfish plague

Many NA's. potentially new genotypes?

Pentaplex assay is a quick tool for a first screening

- Microsatellites and ribosomal subunits of Genotype B will be further analysed





Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Bundesamt für Umwelt BAFU

Schweizerischer Nationalfonds

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Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Bundesamt für Lebensmittelsicherheit
und Veterinärwesen

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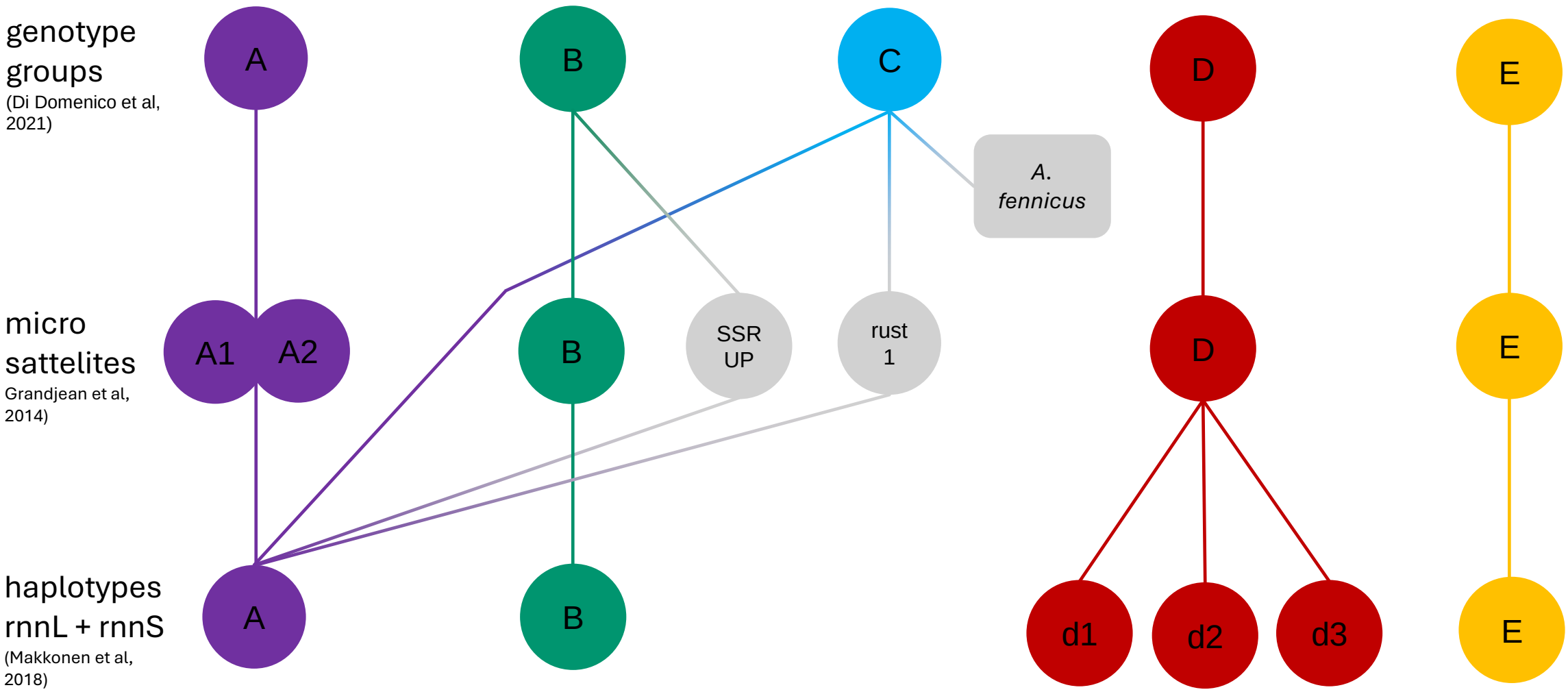
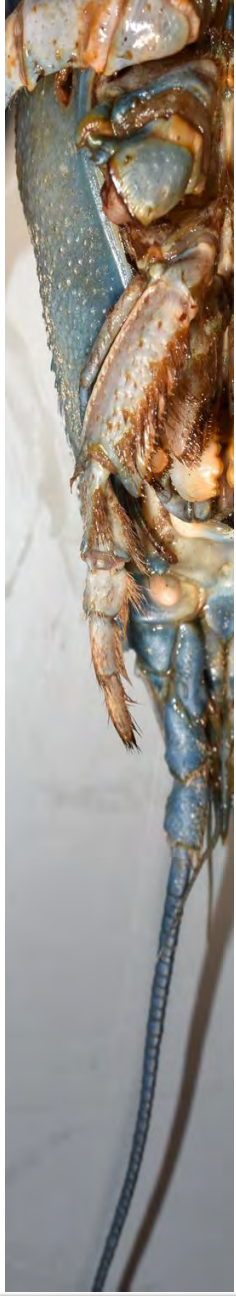
Questions?

Genetic diversity of *A. astaci*

different detection methods






DTU

European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Crustacean Inter-Laboratory Proficiency Testing (ILPT) 2025

RESULT ANNOUCEMENT

Shyam K Uthaman*, Niccolò Vendramin, Argelia Cuenca, Charlotte Bjørner Larsen, Thomas Weise

EURL, DTU Aqua-National Institute of Aquatic Resources, Lyngby, Denmark

Crustacean Inter-Laboratory Proficiency Testing (ILPT) 2025

Sample Preparation & Quality Check

FTA card Samples in C-ILPT25

FTA Card Sl. No	Code
1	S01
2	S02
3	S03
4	S04
5	S05
6	S06
7	S07
8	S08
9	S09

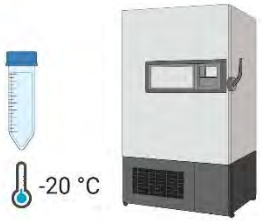
- We sent 9 FTA cards adsorbed with sampled (designated in the table) to each labs participated
- We included samples of EU listed pathogens of crustaceans and negative samples
-
- Derogations are possible

Viral inoculum preparation

Infected shrimp

Ground 2 g carcass in 8 mL sterile cell culture media and sand

Centrifuged at 3000 x *g* for 30 min, 4°C



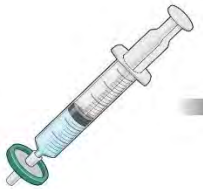
Supernatant stored at -20°C

WSSV - from recent challenge experiment

TSV - from ILPT 2024 stock

YHV1- from previous experimental stock

Supernatant

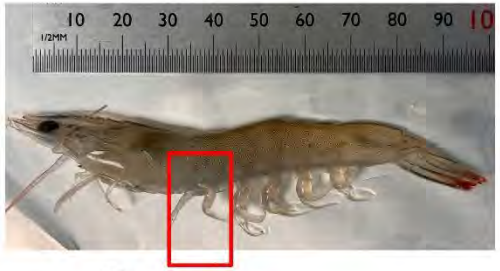


Diluted and filtered

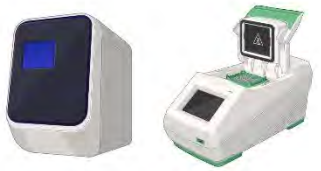
Live animals injected with 100 µL diluted extract

Dead and moribund shrimp collected and sampled

Pathogen confirmation & FTA card preparation



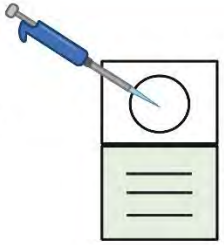
Total nucleic acid extraction using IndiMag48s



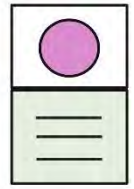
PCRTesting and confirmation of the presence of virus

1st pair of pleopod Collected and tested in EURL

WSSV - qPCR (Durand & Lightner, 2002)
TSV - qPCR (Tang et al., 2004)
YHV1 - RT-PCR (Mohr et al., 2015) + RT-qPCR (Moody et al., 2023)



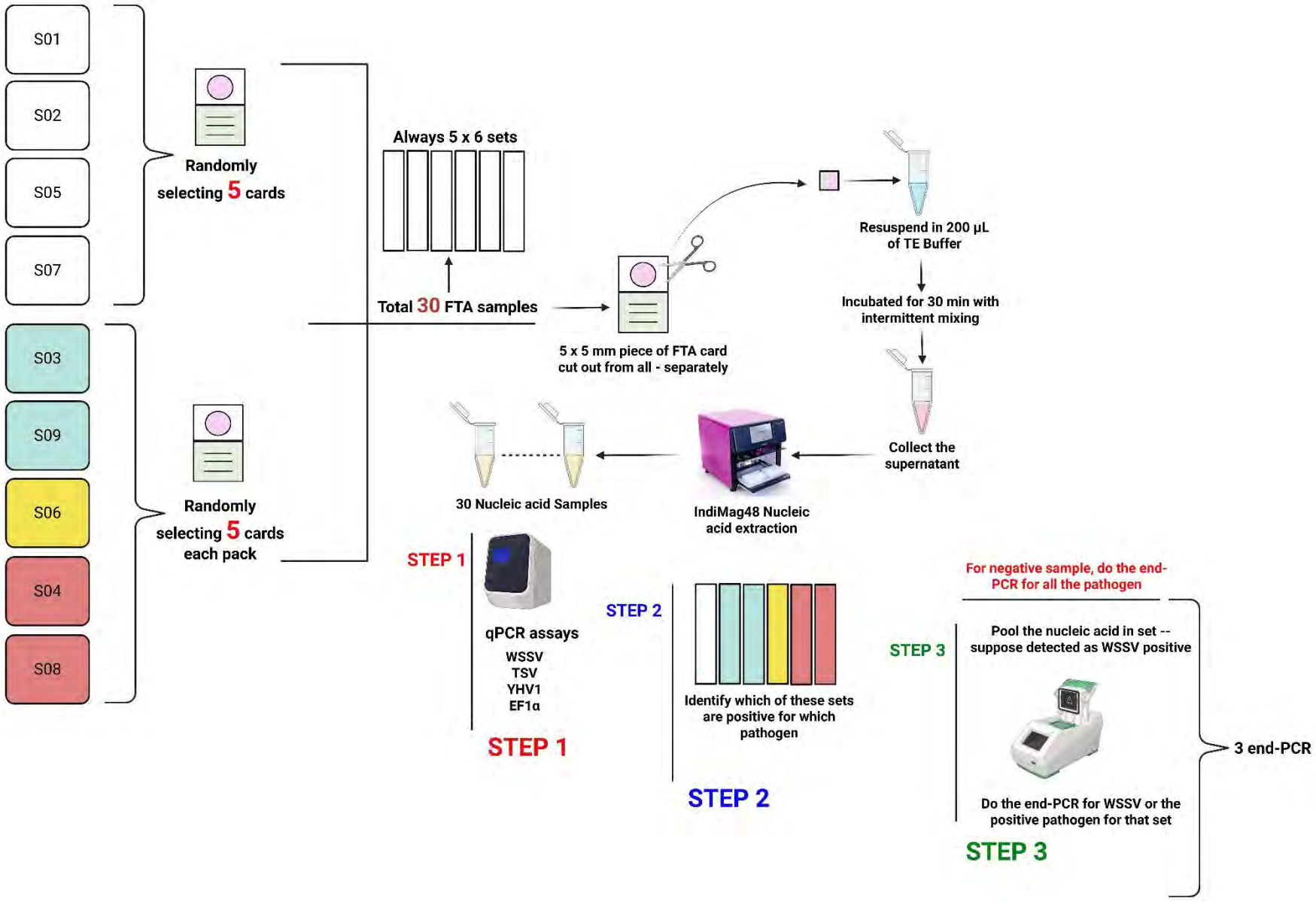
65 µL of the extract was added and dried on to the cards



Prepared cards were stored at 4°C for 2 weeks before testing

- Protects nucleic acid
- can be shipped at ambient temperature
- we sent them at refrigerated condition and requested to store at 4°C

FTA card – Quality Check



Crustacean Inter-Laboratory Proficiency Testing (ILPT) 2025

Results and Analysis

Crustacean Inter-laboratory Proficiency Test 2025

Participants

19 EU National Reference Laboratories (NRLs)

07 Non-EU Countries

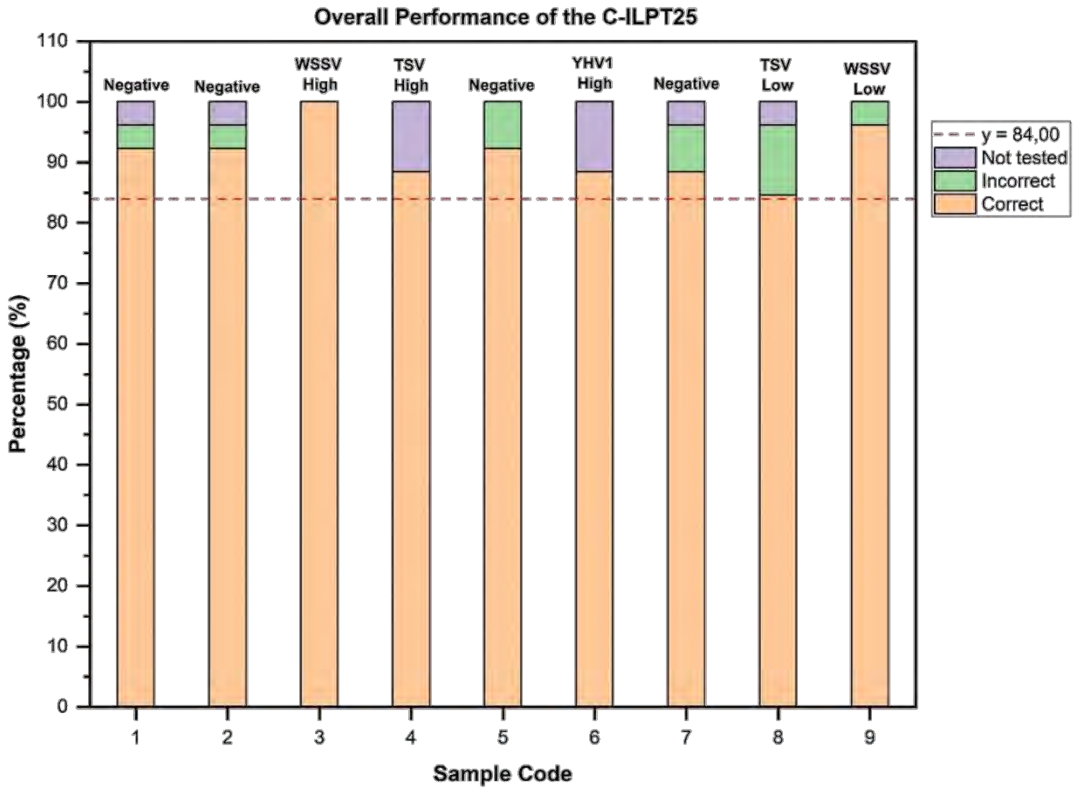
Sample Designation in C-ILPT25

FTA Card Sl. No	Code	Content	Dilution	Description
1	S01	Neg	1:20	SPF shrimp homogenate
2	S02	Blank	-	Cell Culture Media
3	S03	WSSV	1:10	High
4	S04	TSV	1:10	High
5	S05	Neg	1:20	SPF shrimp homogenate
6	S06	YHV1	1:10	High
7	S07	Neg	1:20	SPF shrimp homogenate
8	S08	TSV	1:200	Low
9	S09	WSSV	1:100	Low

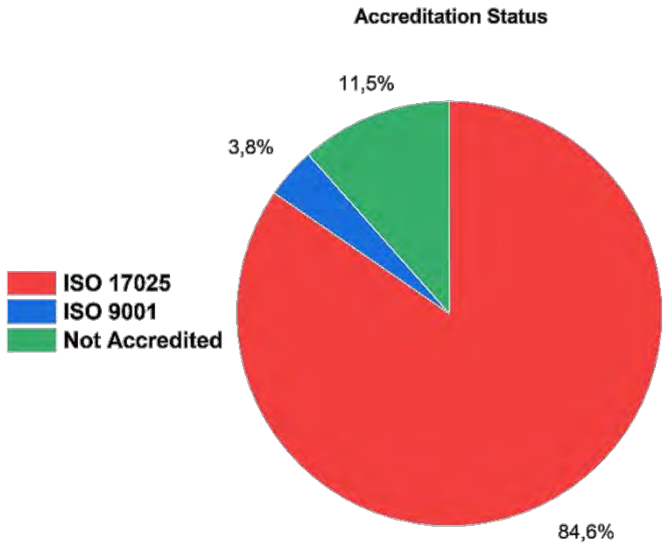
- We sent 9 FTA cards adsorbed with sampled (designated in the table) to each labs participated
- Of which, 4 FTA cards were –Blank or SPF homogenate (Negative)
- 2 FTA cards – for WSSV
- 2 FTA cards - for TSV
- 1 FTA card for - YHV1
- 27 laboratories/NRLs were participated
- 26 labs were submitted back the results
- One right detection get 1 score – total evaluation on 9 scores if the lab tested for all pathogen
- If any labs tested for only WSSV, then evaluated out of 6

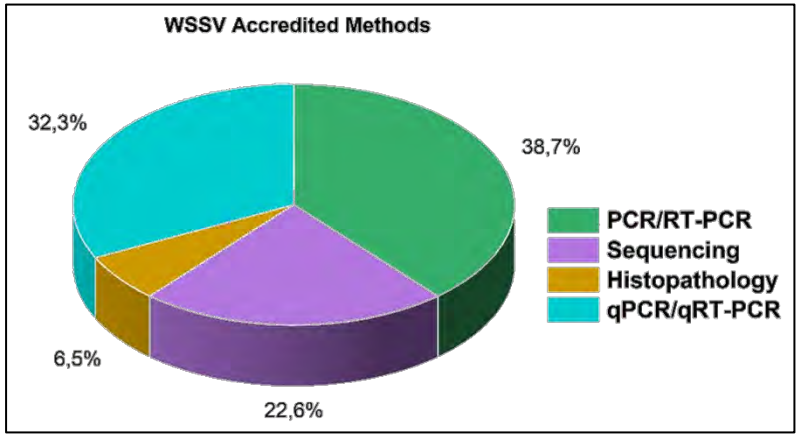
Overall Performance of the Participants across samples

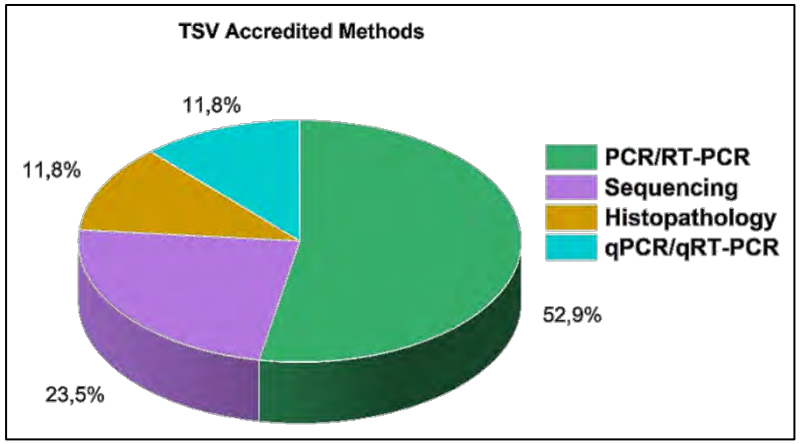
Sample code	S01	S02	S03	S04	S05	S06	S07	S08	S09
Sample Description	Negative	Negative	WSSV High	TSV High	Negative	YHV1 High	Negative	TSV Low	WSSV Low
Total No of Participants	**27**								
Total Results received	**26 (*Overall* 96,30%)**								
Correct Detection	24	24	26	23	24	23	23	22	25
	92,31%	**92,31%**	**100%**	**88,46%**	**92,31%**	**88,46%**	**88,46%**	**84,62%**	**96,15%**
Incorrect Detection	1	1	0	0	2	0	2	3	1
	3,85%	**3,85%**	**0**	**0**	**7,69%**	**0**	**7,69%**	**11,54%**	**3,85%**
Not Tested	1	1	0	3	0	3	1	1	0
	3,85%	**3,85%**	**0**	**11,54%**	**0**	**11,54%**	**3,85%**	**3,85%**	**0**
Result not submitted	**1**								
	3,70%								

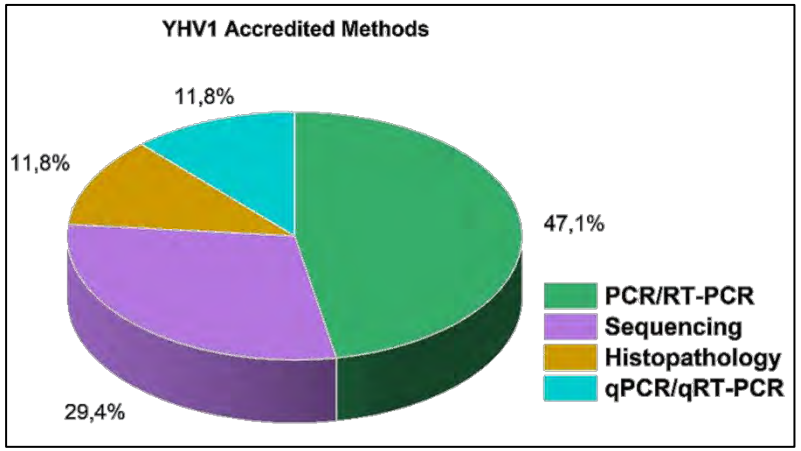


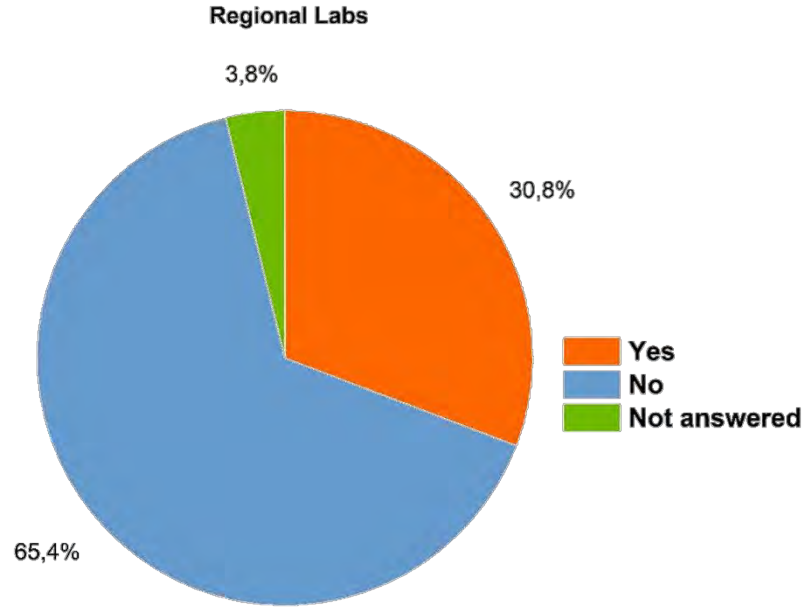
C-ILPT25 CORRECT DETCETION →	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	SCORE	
	Negative	Blank	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV		
	Blank	Blank	High	High	Blank	High	Blank	Low	Low		
Laboratory code	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Out of 9	%
1	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
2	Negative	TSV	WSSV	TSV	Dual detection*	YHV1	YHV1	Negative	WSSV	5/9	55,56
3	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
4	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
5	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
6	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
7	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
8	0	0	WSSV	TSV	0	YHV1	0	TSV	WSSV	9/9	100
9	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
10	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
11	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
12	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
13	negative	negative	WSSV	TSV	negative	YHV1	negative	TSV	WSSV	9/9	100
14	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
15	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	Negative	8/9	88,89
16	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
17	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
18	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
19	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
20	Negative	Negative	WSSV	TSV	Negative	YHV1	YHV1	TSV	WSSV	8/9	88,89
21	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
22	YHV1	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	8/9	88,89
23	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
24	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
25	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
26	0	0	0	0	0	0	0	0	0	NR	-
27	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100

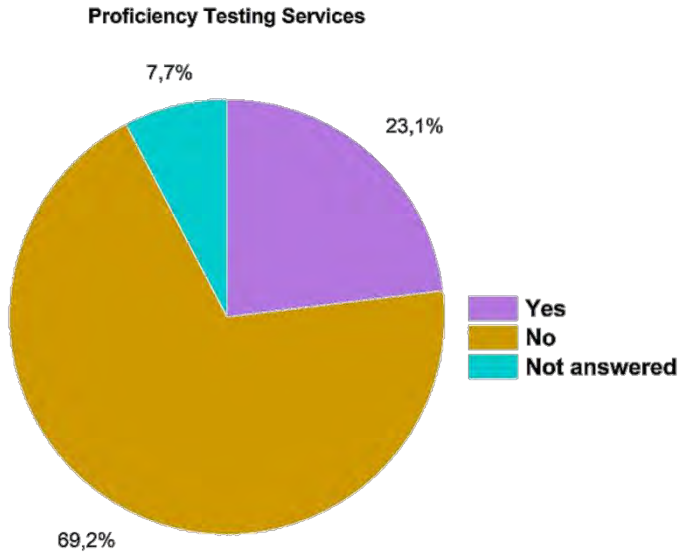

Accreditation Status
11,5%
3,8%
84,6%
ISO 17025
ISO 9001
Not Accredited

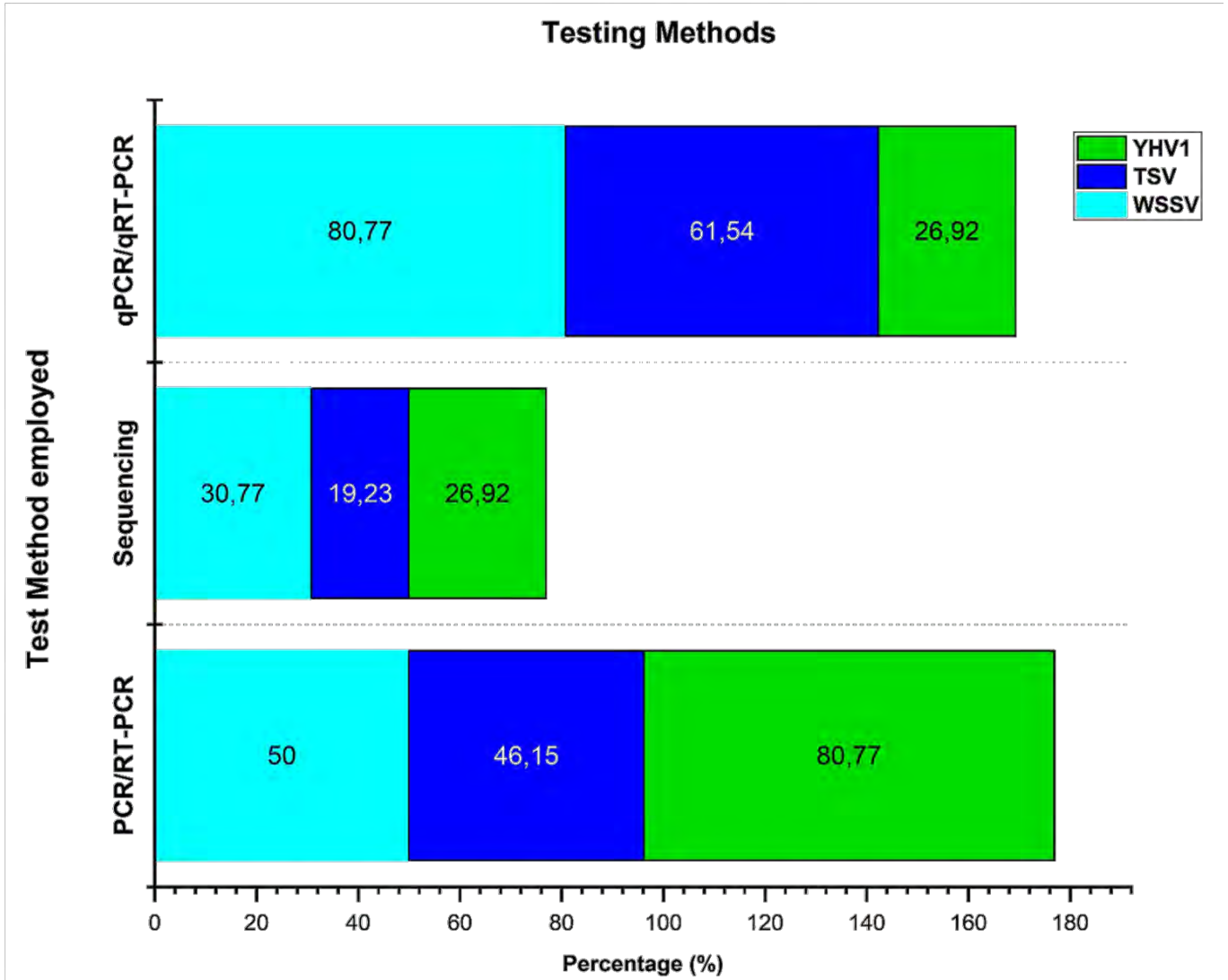

WSSV Accredited Methods
32,3%
38,7%
6,5%
22,6%
PCR/RT-PCR
Sequencing
Histopathology
qPCR/qRT-PCR

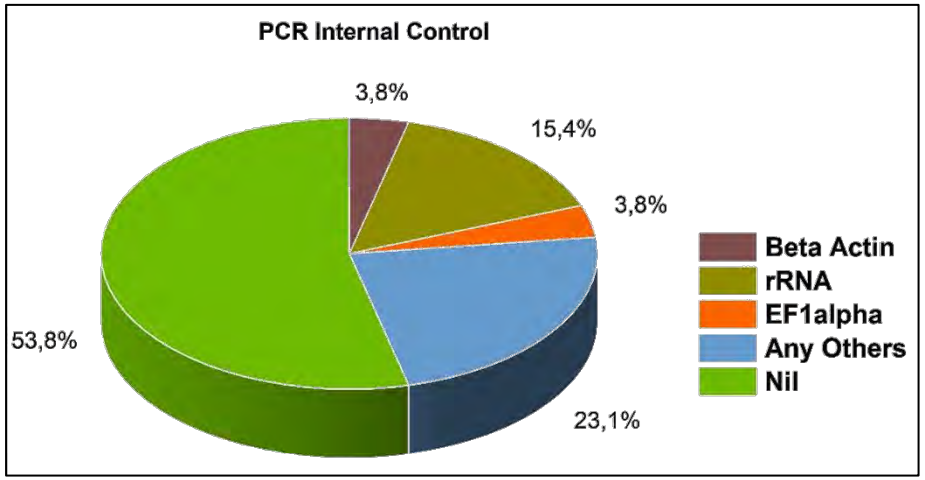

TSV Accredited Methods
11,8%
11,8%
52,9%
23,5%
PCR/RT-PCR
Sequencing
Histopathology
qPCR/qRT-PCR

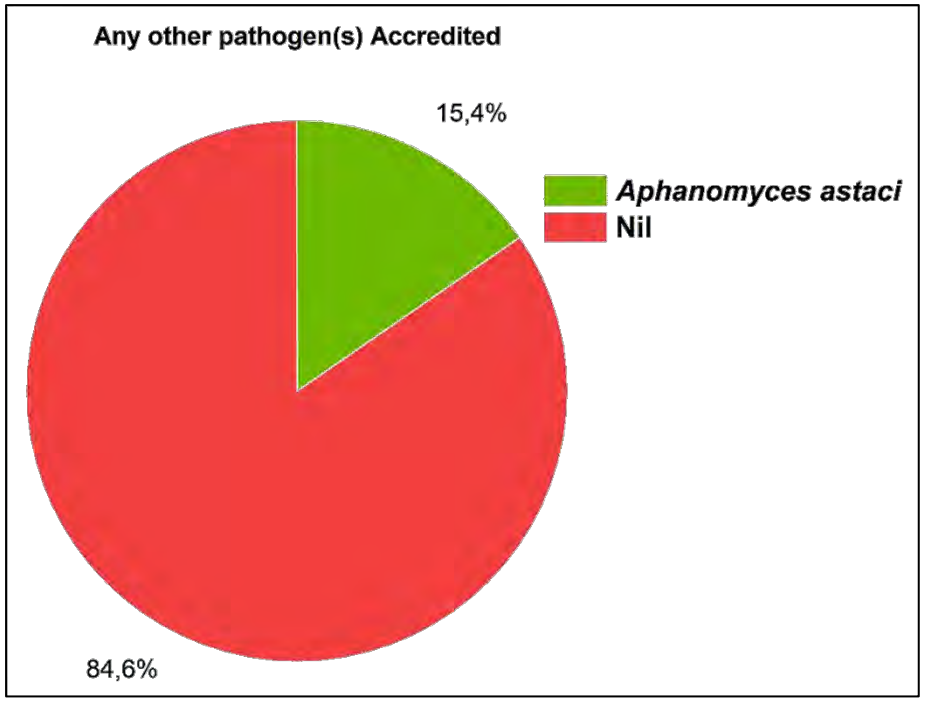

YHV1 Accredited Methods
11,8%
11,8%
47,1%
29,4%
PCR/RT-PCR
Sequencing
Histopathology
qPCR/qRT-PCR


Regional Labs
3,8%
30,8%
65,4%
Yes
No
Not answered


Proficiency Testing Services
7,7%
23,1%
69,2%
Yes
No
Not answered


Testing Methods
Test Method employed
qPCR/qRT-PCR
Sequencing
PCR/RT-PCR
80,77
61,54
26,92
30,77
19,23
26,92
50
46,15
80,77
YHV1
TSV
WSSV
0
20
40
60
80
100
120
140
160
180
Percentage (%)

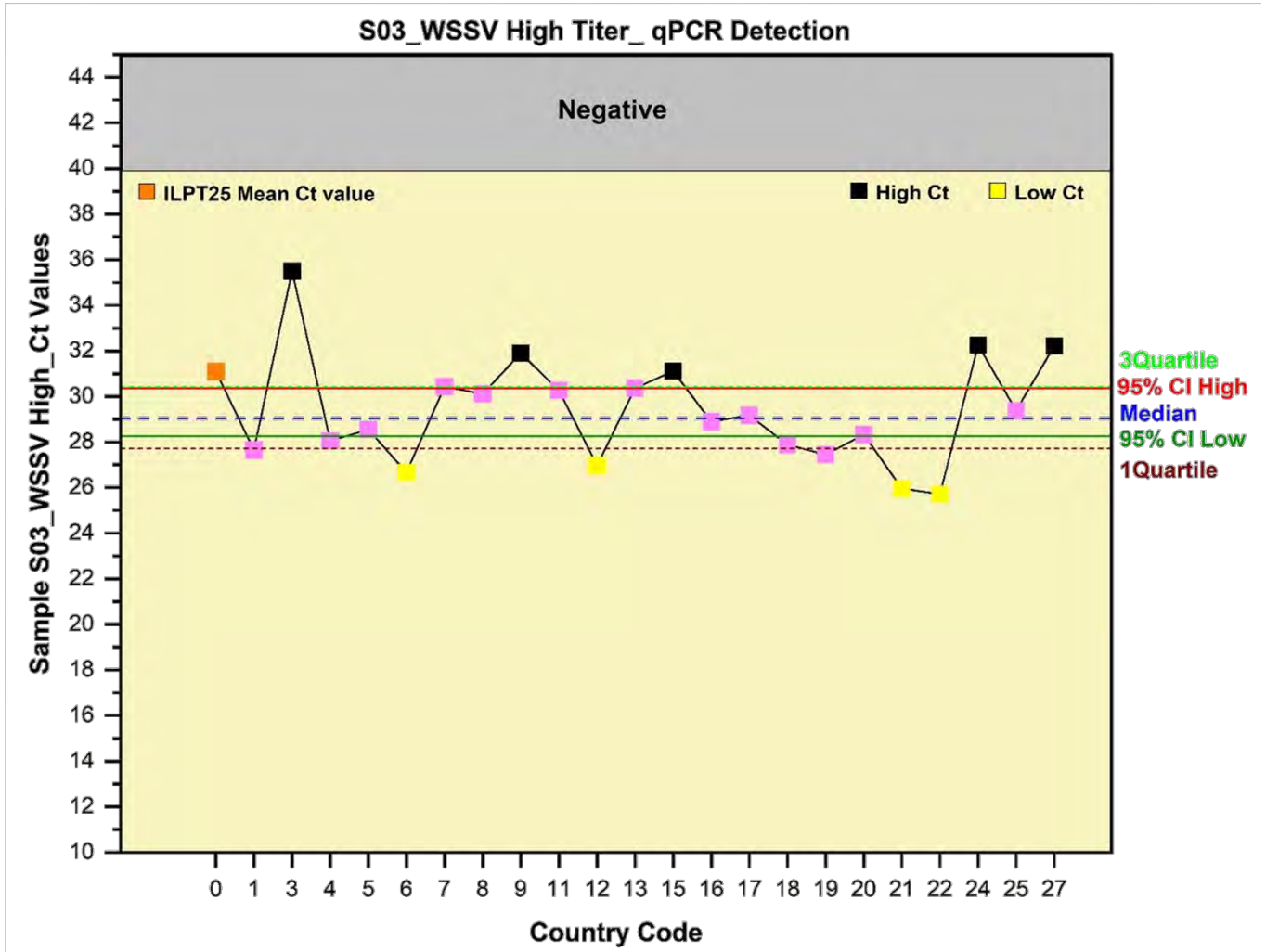

PCR Internal Control
3,8%
15,4%
3,8%
53,8%
23,1%
Beta Actin
rRNA
EF1alpha
Any Others
Nil


Any other pathogen(s) Accredited
15,4%
84,6%
Aphanomyces astaci
Nil

Result Analysis – Negative FTA Card Samples

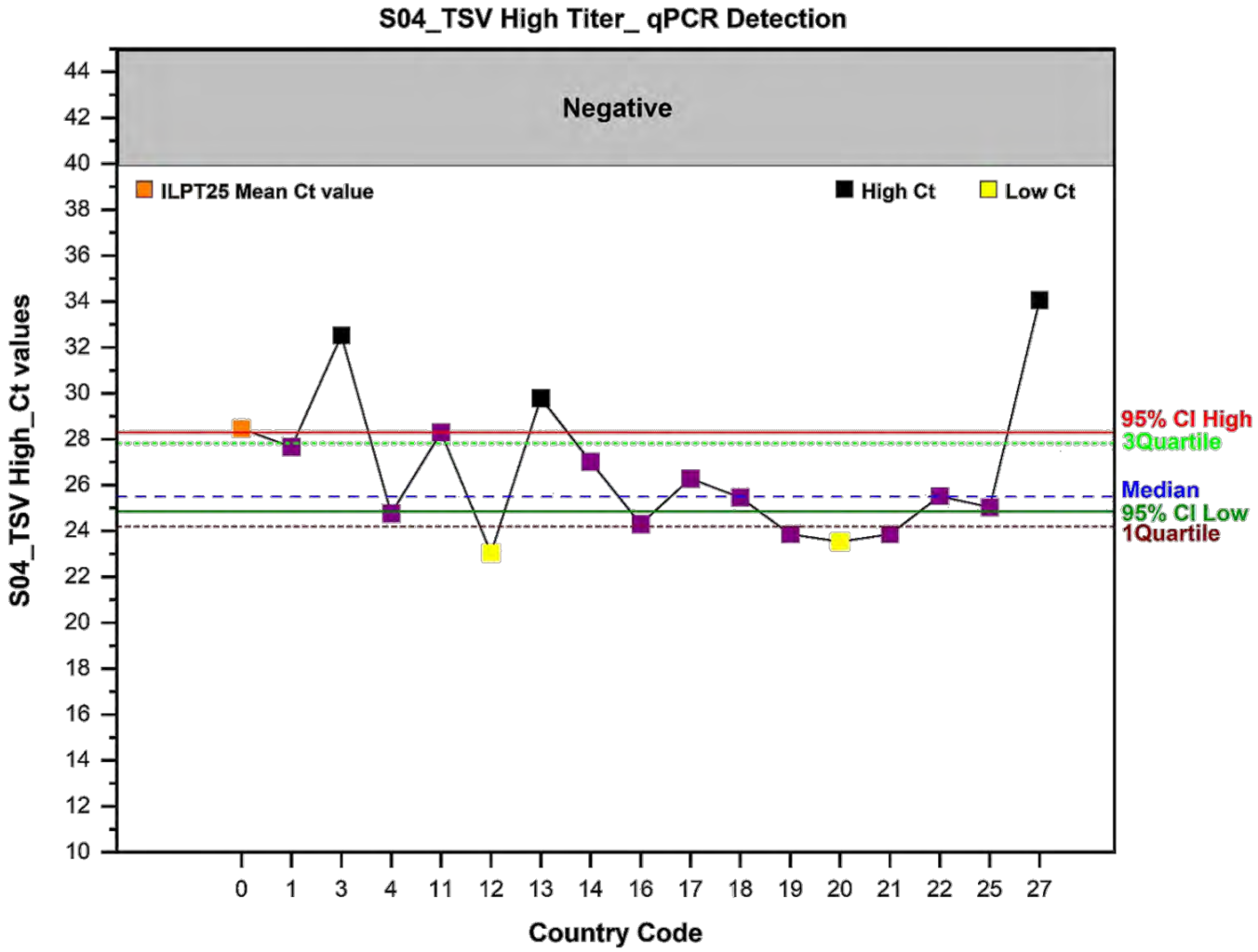
Sample Code	Sample Description	Total Number of Results	Correct Detection	Incorrect Detection	Not Tested
S01	SPF Shrimp Homogenate Diluted 1:20 Negative	26	**24 (92.31%)**	**1 (3.85%)**	**1**
S02	Sterile Culture Media Blank	26	**24 (92.31%)**	**1 (3.85%)**	**1**
S05	SPF Shrimp Homogenate Diluted 1:20 Negative	26	**24 (92.31%)**	**1 (3.85%)**	**1**
S07	SPF Shrimp Homogenate Diluted 1:20 Negative	26	**23 (88.46%)**	**2 (7.69%)**	**1**

Sample S03 – WSSV High Titer



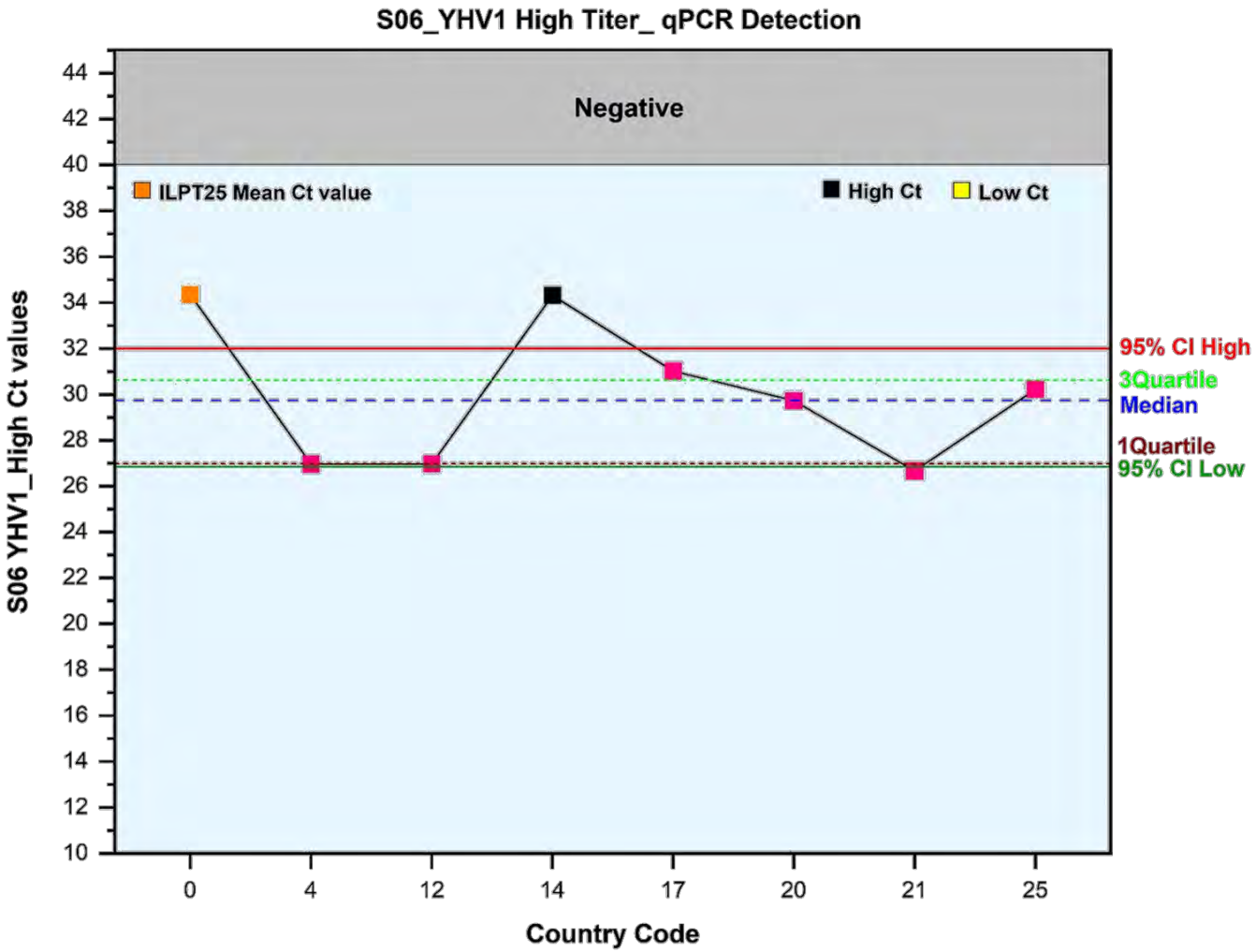
- 22 Labs were performed probe-based qPCR for the detection of WSSV
- 13 Labs have Ct values within the range (MAGENTA)
- 5 Labs have high Ct value – Code 3, 9, 21, 22, 24, 27 (BLACK)
- 4 Labs have low Ct value (YELLOW)
- Median – 29,035
- 95% CI High – 30,36
- 95% CI Low – 28,26
- 25% Quartile – 27,71
- 75% Quartile – 30,41

Sample S04 – TSV High Titer



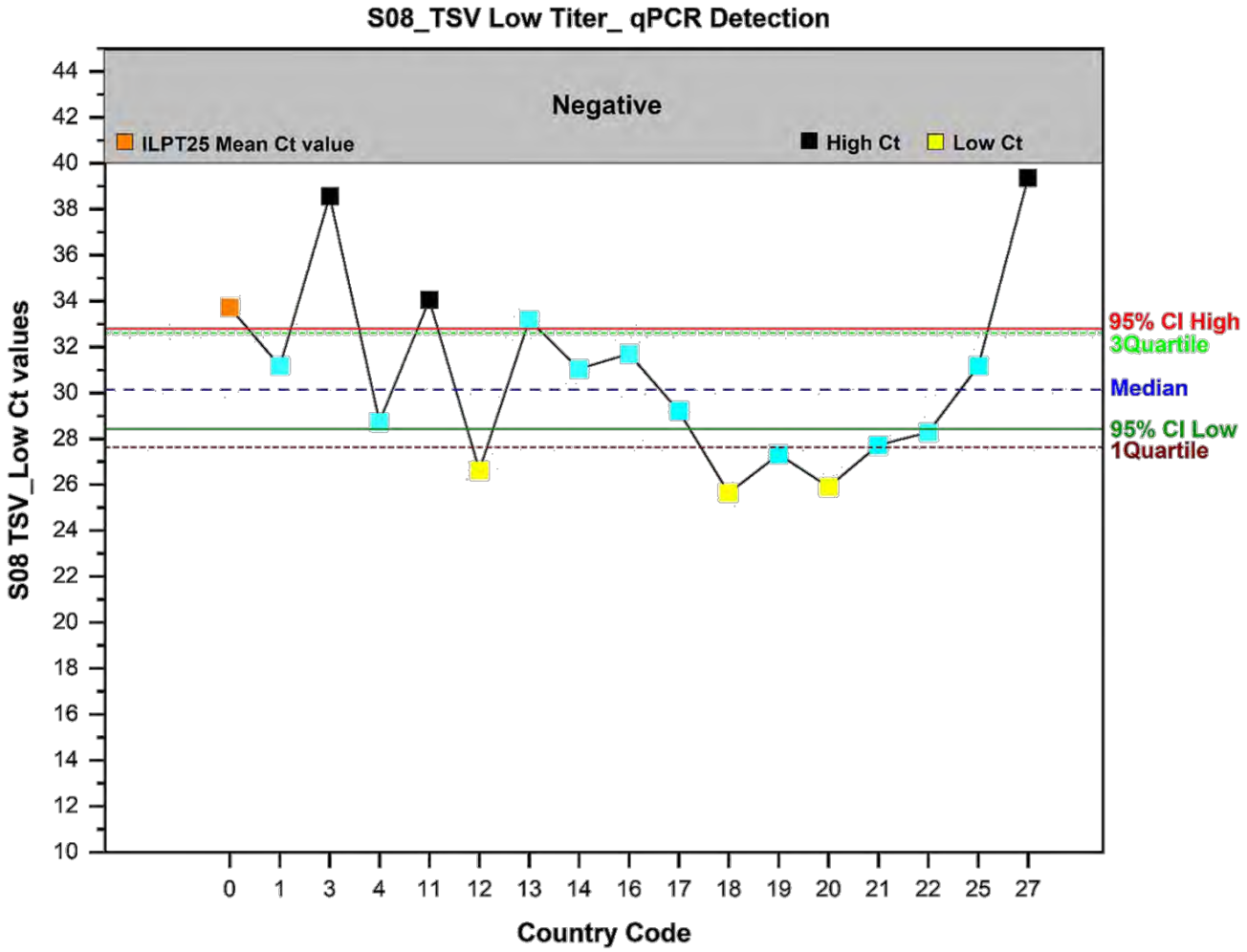
- 16 Labs were performed probe-based qPCR for the detection of TSV
- 11 Labs were performed well (VIOLET)
- 3 Labs were underperformed – Code 3, 13, 27 (BLACK)
- 2 Labs with less efficiency (YELLOW)
- Median – 25,49
- 95% CI High – 28,28
- 95% CI Low – 24,85
- 25% Quartile – 24,19
- 75% Quartile – 27,82

Sample S06 – YHV1 High Titer



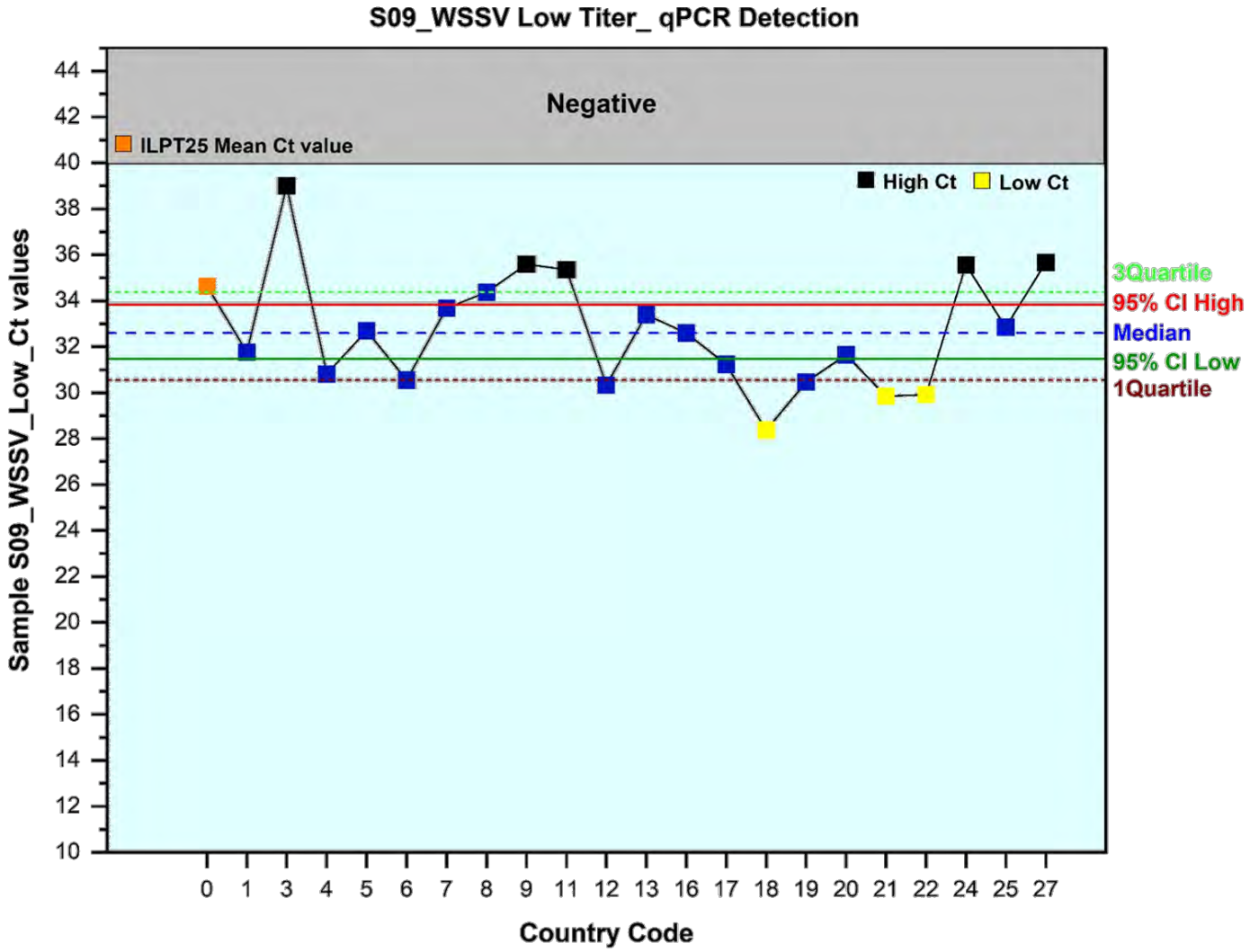
- 7 Labs were performed probe-based qPCR for the detection of YHV1
- 6 Labs have Ct values within the range (MAGENTA)
- 1 Lab has high Ct value – Code 14 (BLACK)
- Median – 29,73
- 95% CI High – 32,80
- 95% CI Low – 28,42
- 25% Quartile – 26,98
- 75% Quartile – 30,62

Sample S08 – TSV Low Titer



- 16 Labs were performed probe-based qPCR for the detection of TSV
- 10 Labs have Ct values within the range (CYAN)
- 3 Labs have high Ct values – Code 3, 11, 27 (BLACK)
- 3 Labs have low Ct values (YELLOW)
- Median – 30,125
- 95% CI High – 32,80
- 95% CI Low – 28,42
- 25% Quartile – 27,63
- 75% Quartile – 32,07

Sample S09 – WSSV Low Titer



- 22 Labs were performed probe-based qPCR for the detection of WSSV
- 1 Lab has incorrect detection, as NEGATIVE
- 13 Labs have Ct value within the range (BLUE)
- 5 Labs have high Ct value – Code 3, 9, 11 24, 27 (BLACK)
- 3 Labs have low Ct value (YELLOW)
- Median – 32,60
- 95% CI High – 33,83
- 95% CI Low – 31,48
- 25% Quartile – 30,55
- 75% Quartile – 34,37

Conclusion

- Participants were asked to identify 9 FTA card contents
- For each answer accounted for 1 point, for a total of 9 points.
- Some are testing only one virus (WSSV), as they have derogated their testing for TSV and YHV1
- In that case maximum score that can be obtained is 6 points
- 27 Labs were totally participated
- Results were received from 26 participating laboratories
- 22 Laboratories correctly diagnosed all samples (100%),
- 1 Lab had 4 underperformances (5/9, 55.56%)
- 3 Labs had 1 underperformance (8/9, 88.89%)
- 3 out of 26 labs tested only for WSSV
- 1 Lab does not respond to the ILPT25
- 1 Lab had a dual detection for S05 (Negative Sample) – Code 2
- 1 Lab could not detect the WSSV low titer even though they correctly detect High titer – Code 15

Probable causes of the variation in Ct values outside the range

- Less efficient total nucleic acid extraction procedure
- Error (equipment & manual) during the downstream processing of the FTA cards
- Improper storage and handling of the FTA cards upon arrival
- Incorrect setting of Ct Threshold line in analysis
- Cross-contamination from the positive control used
- Reagent issues

We kindly encourage the laboratories that showed underperformance in some samples to analyze the remaining sample they have available.

If they do not have sufficient sample material, the EURL is happy to provide additional FTA cards upon request

Crustacean Inter-Laboratory Proficiency Testing (ILPT) 2025

New Result Report Form

Spreadsheet – Result Reporting Form

Instructions to fill out the Crustacean Inter-Laboratory Proficiency Test (ILPT) 2025 Report sheet

1. The report form in MS Excel contains three worksheet. All NRLs are requested to fill out first two worksheets (**Accrediation status & PT_Crustacia_Sample1-9 skabelon**) as mandatory, and third one (**Sequencing Data**) if any sequencing has been done.

2. Drop-down menus have been provided wherever necessary. NRLs are advised to fill out the form by selecting the appropriate option relevant to the result. If you do not a value to input in a cell, please select '**0**'. By default, all the blank cells have the value '**0**'

3. For detailed response beyond the selected value in the cell, please write in the '**Comment**' section of sheet 3. NRLs can also express their concerns about their provided results in the '**Comment**' box.

4. Wherever the values '**0**' and '**1**' are provided in the drop-down menu, they indicate the answer '**NO**' and '**YES**', respectively. Therefore, if you haveperformed a PCR for a pathogen included, please select '**1**' from the drop-down menu; otherwise, select '**0**'.

5. For all columns requesting '**Ct values of the qPCR**', please write the actual numerical value of the average Ct value obtained, rounded to two decimal places (Eg. Ct value 26,325 should be written as '26,33'). It is recommended to run samples at least in duplicatewhen performing the qPCR assay.

6. NRLs can select the '**Concentration**' in Column C of worksheet 3 as either '**LOW**' or '**HIGH**' depending on the Ct values/PCR band intensity they obtained. Providing a response is '**OPTIONAL**' but encouraged.

7. If the lab detects any sample with dual pathogens ('**Dual detection**'), please indicate in the '**Concluding Results**' by selecting '**Dual Detection***' from the drop-down menu. Additionally, the details of the results must be filled out in the '**Comment**' section provided at the end of sheet.

8. If the NRLs performed any sequencing for any of the sample under ILPT25, please answer '**YES**' under the '**Sequencing**' heading and then provide best NCBI hit sequence data **ACCESSION NUMBER** in the '**Other, Specify**' column. Please refer to the '**Example**' provided.

9. Should you require any assistance or have any questions, please conatct the concerned person at EURL

INSTRUCTION TO FILL OUT THE REPORT

1

Instructions to fill | Accreditation status | PT_Crustacea_Sample1-9 skabelon | Sequencing Data

Accreditation Status			
Country:			
Name of the National Reference Laboratory:			
Do you have regional laboratories in your country/region, if yes how many?			
Do you organize inter-laboratory proficiency test annually for the regional laboratories in your country/region?			
The accreditation situation in your laboratory:			
Are you accredited?			
Which system (e.g. ISO EN 17025)?			
Which diagnostic techniques for identification of NON-EXOTIC DISEASES are accredited in your lab			
		Yes	**No**
WSSV	**Histopathology**		
	ELISA		
	Conventional PCR (one step/two step)		
	Sequencing		
	Real-time PCR (Probe/SYBR)		
	Other - specify:		
YHV1	**Histopathology**		
	ELISA		
	Conventional (RT-) PCR (one step/two-step)		
	Sequencing		
	Real-time (RT-) PCR		
	Other - specify:		
TSV	**Histopathology**		
	ELISA		
	Conventional (RT-) PCR (one step/two-step)		
	Sequencing		
	Real-time (RT-) PCR		
	Other - specify:		
Diagnostic procedures of other crustacean diseases/pathogens accredited in your laboratory (e.g. AHPND, EHP)			
Disease/pathogen	***Method***		

REPORTING ACCREDITATION STATUS OF NRL

2

Instructions to fill | Accreditation status | PT_Crustacea_Sample1-9 skabelon | Sequencing Data

Mandatory to fill

Mandatory to fill

RESULT REPORT

Annual Interlaboratory Proficeincy Test 2025

Country:	0
Name of the National Reference Laboratory:	0
Date in which the ampoules arrived the laboratory:	00-00-2025

Instructions

Please have a look in the full description of the instructions given in worksheet 1. Please select which pathogen you identified under Concluding Results:

Select your results from the drop-down menu, see example below. Please write Ct values if real-time assays ahve been used.

EXAMPLE

EXAMPLE	Pathogen	Concentration	PCR	Real-time PCR	Ct-value (Real-time PCR)	Internal Control (Real-time PCR)	RT-PCR	Real-time RT-PCR	Ct value (Real-time RT-PCR)	Internal Control (Real-time RT-PCR)	Sequencing: Fill in the information on the sheet regarding "Sequencing Results"	Other-Specify:	Concluding Results
S01	WSSV	Low	0	1	22,06	EF1-α	0	0	0	0	0	0	WSSV
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S02	WSSV	0	0	0	0	0	0	0	0	0	0	0	TSV
	YHV1	0	0	0	0	0	0	0	0	β-actin	0	0	
	TSV	0	0	0	0	0	1	0	0	0	1	MT877008,	
	Negative	0	0	0	0	0	0	0	0	0	0	0	

Sample N	Pathogen	Concentration (Low/High)	PCR	Real-time PCR	Ct-value (Real-time PCR)	Internal Control (Real-time PCR)	RT-PCR	Real-time RT-PCR	Ct value (Real-time RT-PCR)	Internal Control (Real-time RT-PCR)	Sequencing: Fill in the information on the sheet regarding "Sequencing Results"	Other-Specify:	Concluding Results
S01	WSSV	0	0	0	0	0	0	0	0	0	0	0	0
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S02	WSSV	0	0	0	0	0	0	0	0	0	0	0	
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S03	WSSV	0	0	0	0	0	0	0	0	0	0	0	
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S04	WSSV	0	0	0	0	0	0	0	0	0	0	0	0
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S05	WSSV	0	0	0	0	0	0	0	0	0	0	0	0
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S06	WSSV	0	0	0	0	0	0	0	0	0	0	0	0
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
S07	WSSV	0	0	0	0	0	0	0	0	0	0	0	0
	YHV1	0	0	0	0	0	0	0	0	0	0	0	
	TSV	0	0	0	0	0	0	0	0	0	0	0	
	Negative	0	0	0	0	0	0	0	0	0	0	0	
	WSSV	0	0	0	0	0	0	0	0	0	0	0	

Mandatory to fill

Drop-down menu

Infromation
For the '*' responses required deatiled description in the comment section below

Instructions to fill | Accreditation status | PT_Crustacea_Sample1-9 skabelon | Sequencing Data

3

SEQUENCING DATA REPORT

Annual Interlaboratory Proficeincy Test 2025

Country:	0
Name of the National Reference Laboratory:	0
Date in which the ampoules arrived the laboratory:	00-00-2025

Instructions

Please write which pathogen you identified under Concluding Results of sequencing

Data regarding sequencing is optional to add but encouraged to input

Sequencing Parameter	Value
Sample Code	S01
Concluding Results	0
Sequenced Result: (*Pathogen*)	
Sequenced done in:	
Sequencing primers	
% Sequence identity (BLAST results)	
Top 1 hit (Accession Number)	
Top 2 hit (Accession Number)	
Sequencing Deviations, if any	
Remarks/Comments, if any	

Sequencing Parameter	Value
Sample Code	S02
Concluding Results	0
Sequenced Result: (*Pathogen*)	
Sequenced done in:	
Sequencing primers	
% Sequence identity (BLAST results)	
Top 1 hit (Accession Number)	
Top 2 hit (Accession Number)	
Sequencing Deviations, if any	
Remarks/Comments, if any	

Sequencing Parameter	Value
Sample Code	S03
Concluding Results	0
Sequenced Result: (*Pathogen*)	
Sequenced done in:	
Sequencing primers	
% Sequence identity (BLAST results)	
Top 1 hit (Accession Number)	
Top 2 hit (Accession Number)	
Sequencing Deviations, if any	
Remarks/Comments, if any	

Sequencing Parameter	Value
Sample Code	S04

Automatically fill & locked

Instructions to fill | Accreditation status | PT_Crustacea_Sample1-9 skabelon | Sequencing Data

4

I appreciate your time.

If you have any questions, I'd be glad to answer them now.

****

The Sending Team

Shyam K Uthaman
Crustacean Co-ordinator

Niccoló Vendramin
EURL Director

Argelia Cuenca
Senior Researcher

Charlotte Bjorner Larsen
Lab Technician

Thomas Weise
Lab Technician

Teena Vendel Blade
Lab Technician

European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL-Crustacean work done in 2024,2025 plans for 2026

Shyam Kokkattunivarthil Uthaman, Argelia Cuenca, Teena V. Klinge, Charlotte Larsen, Niccoló Vendramin

Shyam

EURL for crustacean diseases

Anna Alencar

postdoc

EURL director Niccoló Vendramin

Britt Bang Jensen

Section leader

Senior researcher Argelia Cuenca

Senior researcher Lone Madsen

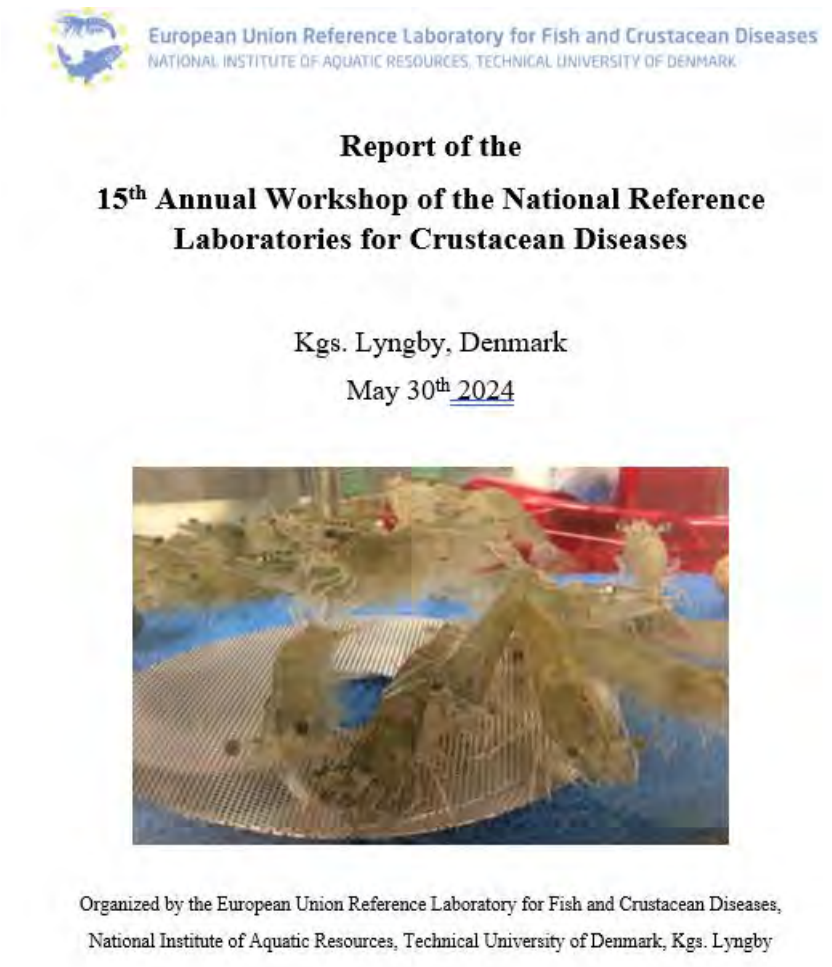
Lab technician Teena Klinge

Lab technician Charlotte Larsen

Lab technician Thomas Weise

1-1 Organise and prepare for the 15th Annual Workshop

The 15th Annual Workshop of the National Reference Laboratories for Crustacean Diseases was held online on 30th of May 2024. There were 68 participants attending the workshop in person, representing 34 countries.


European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

15th Annual Workshop of the National Reference Laboratories for Crustacean Diseases

Kgs. Lyngby, Denmark

May 30th 2024

Organized by the European Union Reference Laboratory for Fish and Crustacean Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby

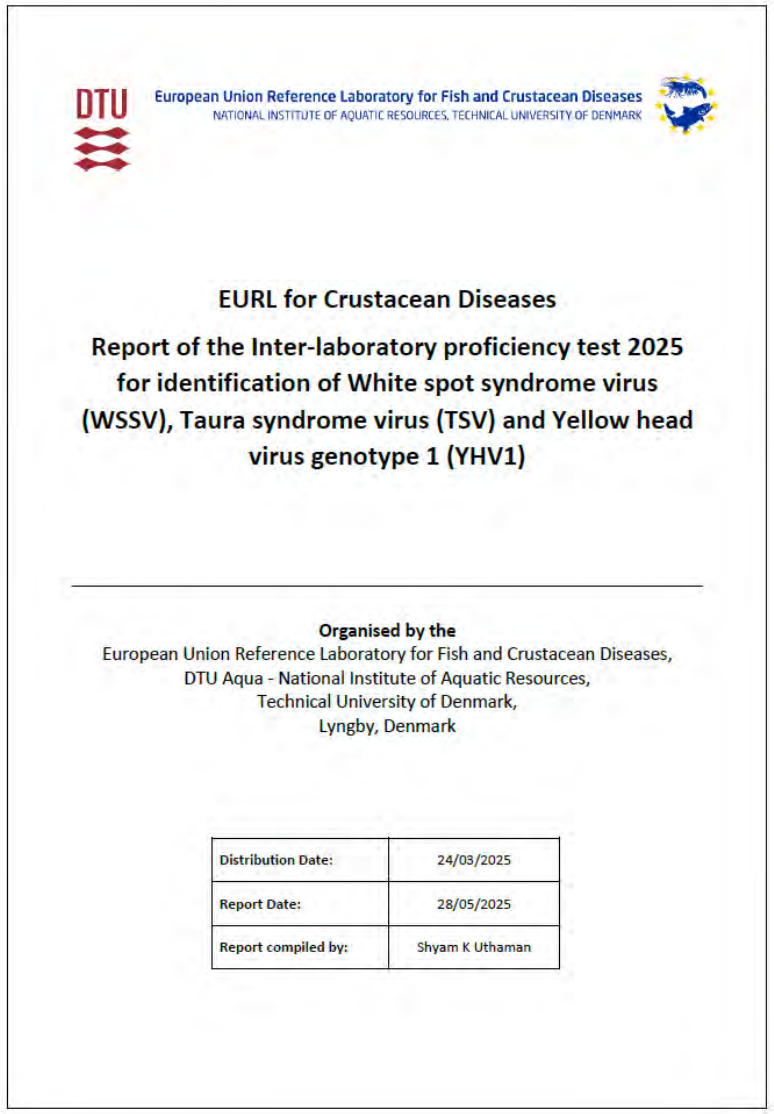
1-2 Organise scientific working group meetings

Working group on Diagnostic manuals for TSV and YHV1

Participants:

- EURL: (Morten Schiøtt, Argelia Cuenca and Niels Jørgen Olesen)
- IZSVe (Tobia Pretto)
- CEFAS (Kelly Bateman and Grant Stentiford)
- AAHL, Geelong ACDP (Nick Moody and Peter Mohr)

1-3 Organise Proficiency tests



European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL for Crustacean Diseases

Report of the Inter-laboratory proficiency test 2025 for identification of White spot syndrome virus (WSSV), Taura syndrome virus (TSV) and Yellow head virus genotype 1 (YHV1)

Organised by the
European Union Reference Laboratory for Fish and Crustacean Diseases,
DTU Aqua - National Institute of Aquatic Resources,
Technical University of Denmark,
Lyngby, Denmark

Distribution Date:	24/03/2025
Report Date:	28/05/2025
Report compiled by:	Shyam K Uthaman

Results were received from all 26 participating laboratories.

- 22 laboratories correctly diagnosed all samples, 9/9 (100 %) or 6/6 (100 %).
- 3 laboratories correctly diagnosed Eight samples, 8/9 (88,89 %).
- 1 laboratory correctly diagnosed Five samples, 5/9 (55,56%)

27 laboratories including 16 NRLs of EU Member States accepted the invitation to participate and send in their test results for diagnostic assays not derogated to other laboratories.

2-1 Training:

Facilitate and provide training in laboratory diagnosis

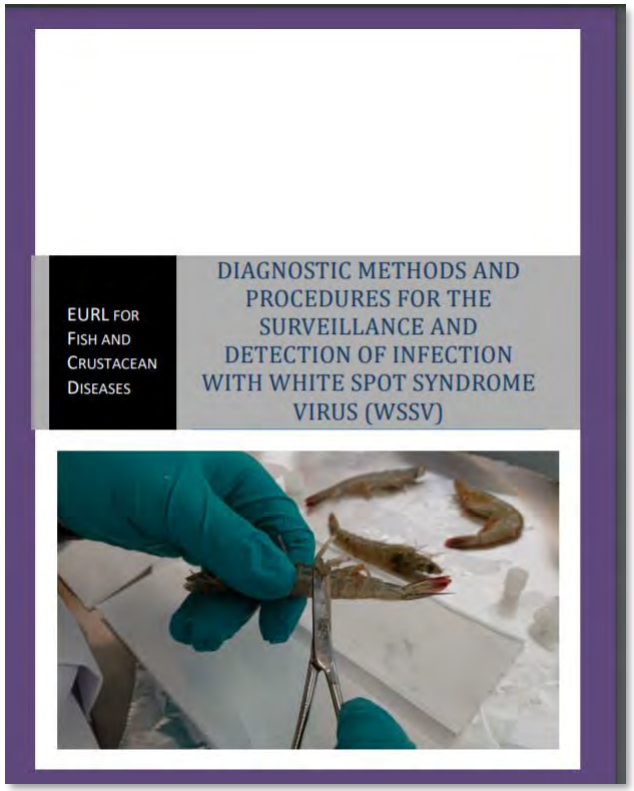
A 3 days training course on **Introduction to validation of Diagnostic methods for Aquatic animal diseases**

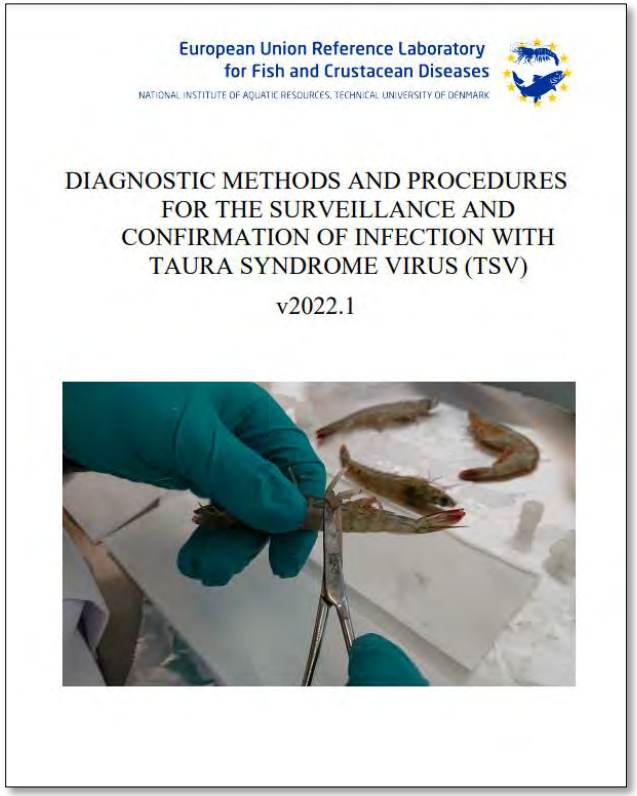
With 16 trainees was held in week 41

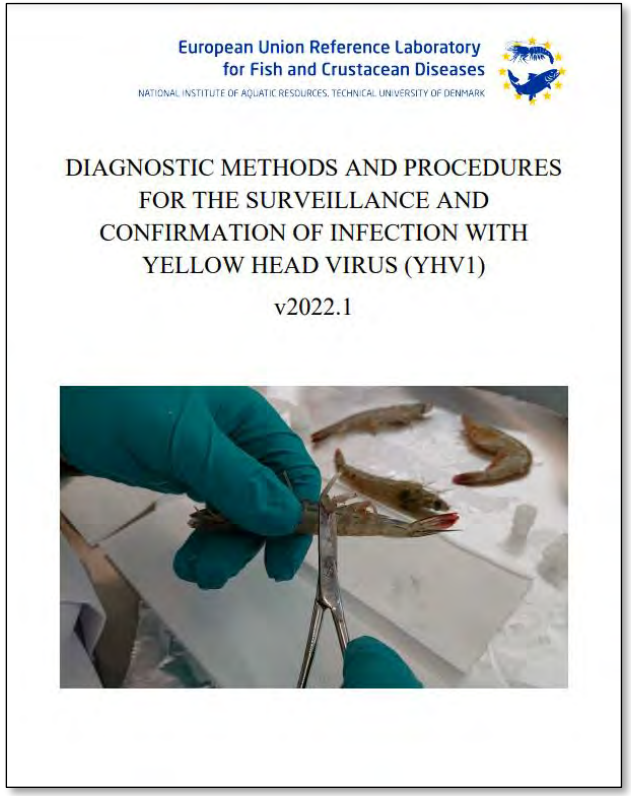
3. TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

3.1. Diagnostic manuals.

To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs on the EURL website www.eurl-fish-crustacean.eu.







Diagnostic methods based on diagnostic qPCR introduced in the lab. Through interlaboratory proficiency test provided by CSIRO

Infectious hypodermal and hematopoietic necrosis virus (IHHNV)

Infectious myonecrosis virus (IMNV)

V. Parahaemolyticus PirA and PirB positive strains (pVA1^{+}) causing Acute Hepatopancreatic Necrosis Disease (AHPND)

- We will also try to validate internally the diagnostic methods other pathogens in crustaceans relevant to the routine diagnosis services, such as
- Bacteria → NHP - *Hepatobacter penaei* (hepatopancreatitis necrotizante)
- We have the PCR method which is not validated yet.

3.2. Survey and diagnosis. "collate and *forward information on exotic and endemic diseases, that are potentially emerging in* **Community"**

Surveillance and diagnostics of crustacean diseases in Europe

Niccoló Vendramin and Thomas Weise

Sub-activity 3.3 *Confirmatory diagnosis*

For the EURL to be able to identify and characterize isolates of listed viral fish and crustacean pathogens on request from the Member State NRLs

Corroborating finding of WSSV in Crayfish in Austria

Establishment and characterization of tissue sample bank of shrimp infected with WSSV, YHV-1, TSV

5.1 Scientific advice in relation to aquatic animal health legislation

The experts of the EURL have since September 2022 been part of a working group established by the European Food Safety Authority (EFSA) to produce guidelines for defining vector species of listed aquatic animal diseases. These guidelines will be used in commissioned work to search the scientific literature for evidence of aquatic animal species working as vector species for the relevant diseases. The work has been finalized and published in 2024.

EURL Workplan 2023-2024

Non-accomplished tasks

1. Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR and provision of guidelines on how to pool.

2. Establishment of facility for maintaining permanent stock of shrimp.

This work is now initiated in 2025

Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR and provision of guidelines on how to pool.

ESTABLISHED collaboration with CSIRO

Establishment of facility for maintaining permanent stock of shrimp.

Designed and prepared customized tanks for conducting shrimp trials

EURL Workplan 2025-2027

3-year program

1. PTs same business as usual, yearly delivery. Possibilities to include *A.astaci* (?)
2. Annual Workshop 2025 accomplished. AW 2026: online – back to back to AW for fish diseases.
3. Training courses 2026: surveillance of listed diseases in EU week 41
4. Quality assurance: provide help for implementing in NRL´s (SOPs, visits etc.), accreditation of ILPT
5. Diagnostic methods: Implementation of additional diagnostic methods of crustacean diseases
6. Scientific assessment of the effect of pooling samples for surveillance and diagnostics by PCR and provision of guidelines on how to pool.
7. Upgrade facility for maintaining permanent stock of shrimp
8. Further update of Diagnostic manuals
9. Proposals for topics are VERY welcome!

End of 16th Annual workshop of NRL´s for Crustacean Diseases 2025

Important! Please give us feed back as soon as possible by filling the evaluation scheme send to all of you.

Upon request we will send you a signed certificate of participation.

Thank you for all the excellent presentations, valuable questions and contributions and for participating in this workshop

We are looking forward to seeing you in 2026


Volume Four in
THE HITCHHIKER'S GUIDE TO THE GALAXY
37
SO LONG, AND THANKS FOR ALL THE FISH
AND CRUSTACEANS
Foreword by Neil Gaiman

1-4 Novel molecular methods

For the EURL to have molecular diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs.

In 2021 the following new diagnostic PCR methods were introduced in the laboratory:

RT-qPCR for IMNV

qPCR for IHHNV

qPCR for AHPND

16th Annual workshop 2025

28th + 29th of May (Wednesday and Thursday) ONLY virtual.

Three workshops back to back, on fish and crustacean diseases, respectively and a closed session for the NRLs in EU and EFTA.