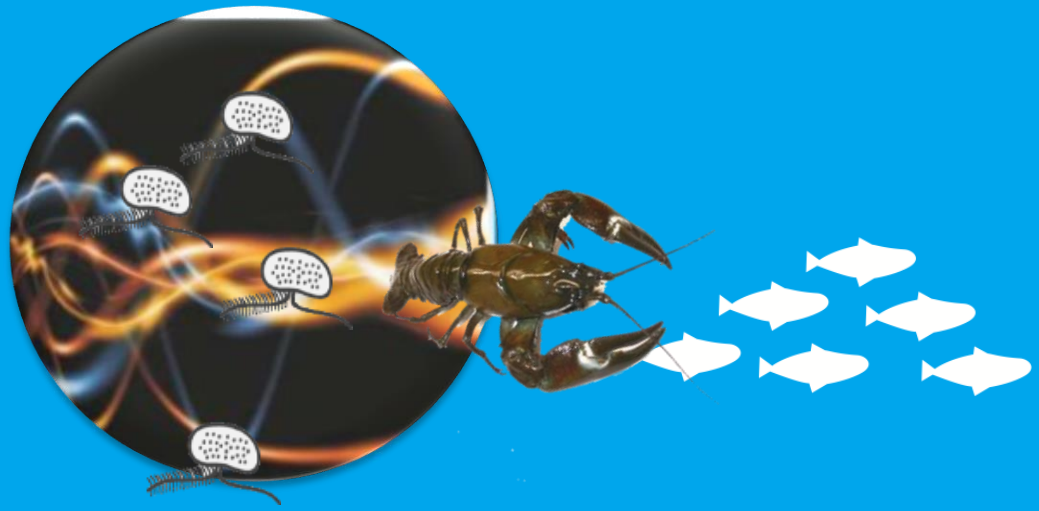
Veterinærinstituttet
Norwegian Veterinary Institute

Testing ultrasonic treatment against crayfish plague

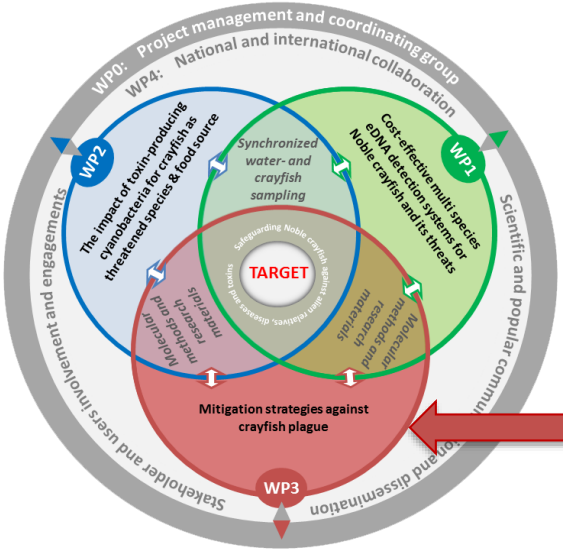
Trude Vrålstad
Head of Fish Health Research Section



The Research Council of Norway

People involved:

David Strand, Lennart Edsman, Johannes Rusch, Lisa Brand, Lars Haavi, Elin Rolén, Trude Vrålstad



TARGET project WP3 Overall objective

Explore the potential of ultrasonic technology to eliminate the crayfish plague agent in the water and in carrier crayfish

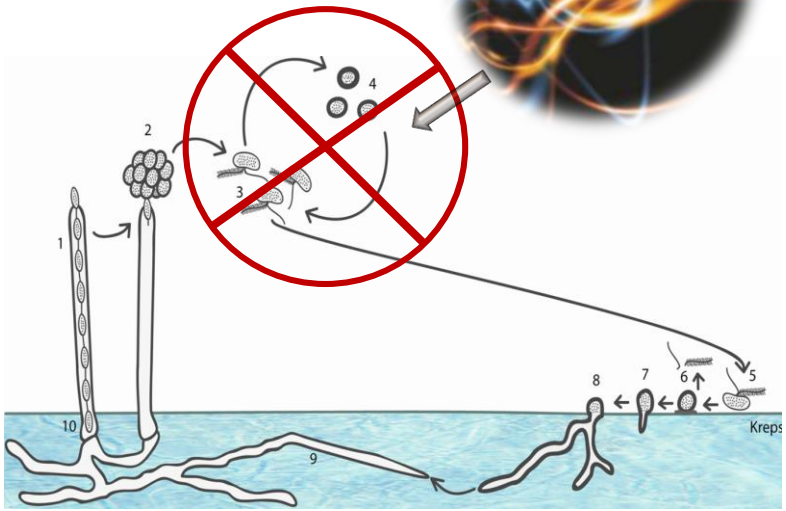
LG SOUND Leading in ultrasonic algae control

SLU

Veterinærinstituttet
Norwegian Veterinary Institute

Background:

- Currently no treatment/cure for crayfish plague infection
- Ultrasound: environmental friendly control of cyanobacteria & green algae
  - Eliminate by rupture of gas vesicles, rupture of cell structures etc
  - Target specific groups (vary frequency, amplitude, waveform, signal duration)
- **Can this be transferred to oomycetes/the crayfish plague pathogen?**



Objectives and experiments

1. **Identify ultrasonic (US) wave lengths that eliminate *A. astaci***
   - ***Spore-US treatment trial – failed** (missing methods for live-dead spore separation)*
   - ***Co-habitation experiment signal crayfish-noble crayfish – US treatment***
   - ***Challenge experiments noble crayfish - A. astaci – US treatment***
2. **Test if ultrasound prevents re-infection of *A. astaci* after moults**
   - ***Molting experiments signal crayfish – US-treatment***

Co-habitation experiments

- Signal crayfish originating from Lake Båven
- In-lake prevalence: 80% *A. astaci infected crayfish*
- Aquaria facility at SLU Aqua, Institute of freshwater research
- Utrasound devices and recommendations from LG-SOUND
- Testing 3 different ultrasound programs (hereafter US treatments)

Experimental design – co-habitation

- Duration: November 2016 – march 2017 (5 months)
- Fed once a week with corn, temp varied from 8 – 18 °C

US3

- Noble crayfish N = ~5
- Signal crayfish N = ~5
- Ultrasound US3

US8

- Noble crayfish N = ~5
- Signal crayfish N = ~5
- Ultrasound US8

US5

- Noble crayfish N = ~5
- Signal crayfish N = ~5
- Ultrasound US5

Control

- Noble Crayfish N = ~5
- No ultrasound

Control

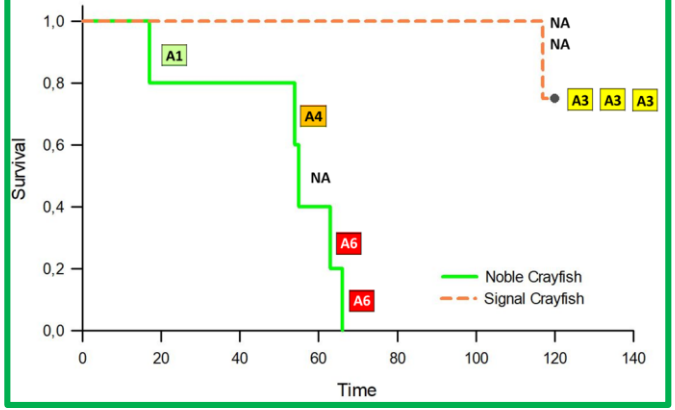
- Signal crayfish N = ~5
- No ultrasound

Control

- Noble crayfish N = ~5
- Signal crayfish N = ~5
- No ultrasound

Results co-habitation

Control – noble + signal



Single species controls = OK

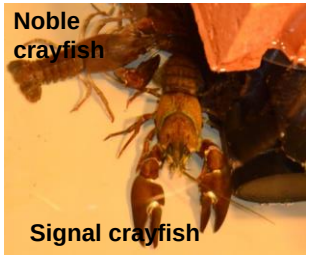
Control tank co-habitation = OK

100% noble crayfish mortality

- *A. astaci* prev 60%, A6

80% signal crayfish survival

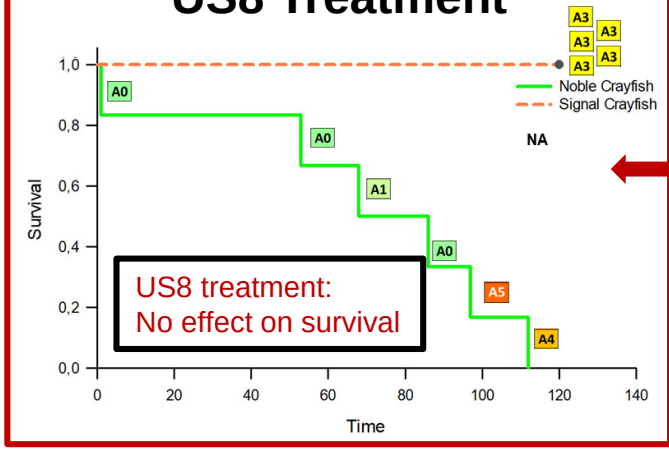
- *A. astaci* prev 70%, A3



Classification *A. astaci* agent levels (Vrålstad et al 2009)

A7	Exceptionally high
A6	Very high
A5	High
A4	Moderate
A3	Low
A2	Very low
A1	Trace (detected >LOD)
A0	Not detected

US8 Treatment



Co-habitation US treatment

US8

100% noble crayfish mortality

100% signal crayfish survival

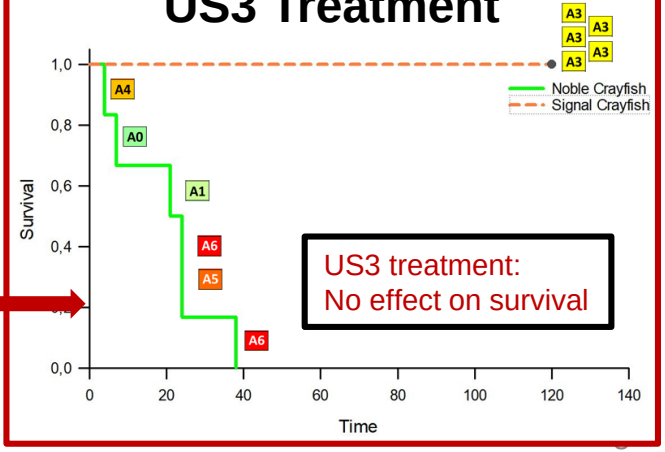
***US8: A. astaci* prevalence**

- 30% in noble crayfish
- 100% in signal crayfish

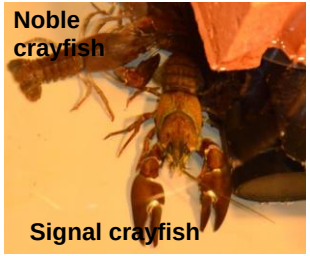
***US3: A. astaci* prevalence**

- 60% in noble crayfish
- 100% in signal crayfish

US3 Treatment



Results – ultrasound US5



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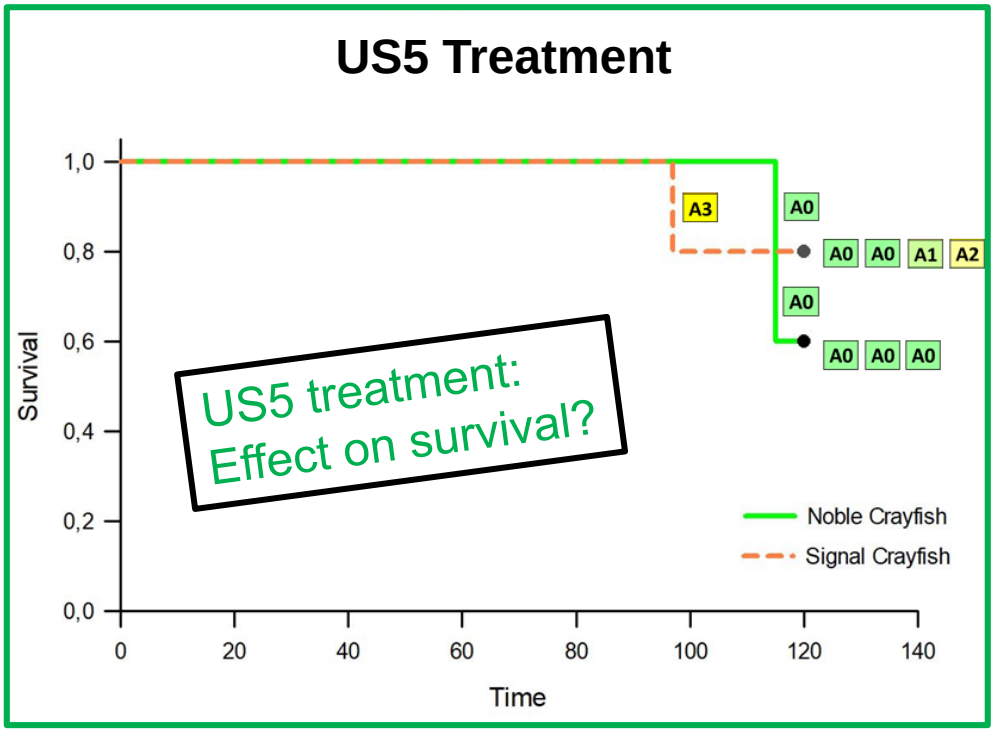
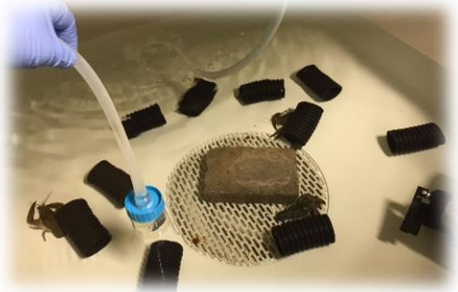


- 60% noble crayfish survived
  - 0% *A. astaci* prevalence
- 80% All signal crayfish survived
  - *A. astaci* prevalence 40 %
  - Highest agent level A3
- High survival in nobles, no *A. astaci*
- *BUT: A. astaci* prevalence and infection load low in the signal crayfish….

Moulting experiments with signal crayfish and US5 treatment

- Ultrasound treatment US5 - a promising candidate
- Experiment set up in Oslo from July 4th to August 30th 2018
- Signal crayfish treated with UL5 in a period of expected moults

Can ultrasound reduce or clear *A. astaci* infection in signal crayfish during moulting?



Experimental design moulting - US5 treatment

Treatment US5

- Signal Crayfish N = 16
- Duration = 8 wk
- Mean temp = ~14° C

Treatment US5

- Signal Crayfish N = 14
- Duration = 8 wk
- Mean temp = ~13° C

Control

- Signal Crayfish N = 16
- Duration = 8 wk
- Mean temp = ~14° C

Control

- Signal Crayfish N = 12
- Duration = 8 wk
- Mean temp = ~14° C

Fed with green peas every 3rd or 4th day

Results moulting experiments

- Control Exp1 – signal crayfish
  - 50% mortality, 12.5 % moulting
  - *A. astaci* prev. 100%, mean A5
- Control Exp2 – signal crayfish
  - 12.5 % mortality, **44% moulting**
  - *A. astaci* prev.100%, mean A4

Classification *A. astaci* agent levels (Vrålstad et al 2009)

A7	Exceptionally high
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A3	Low
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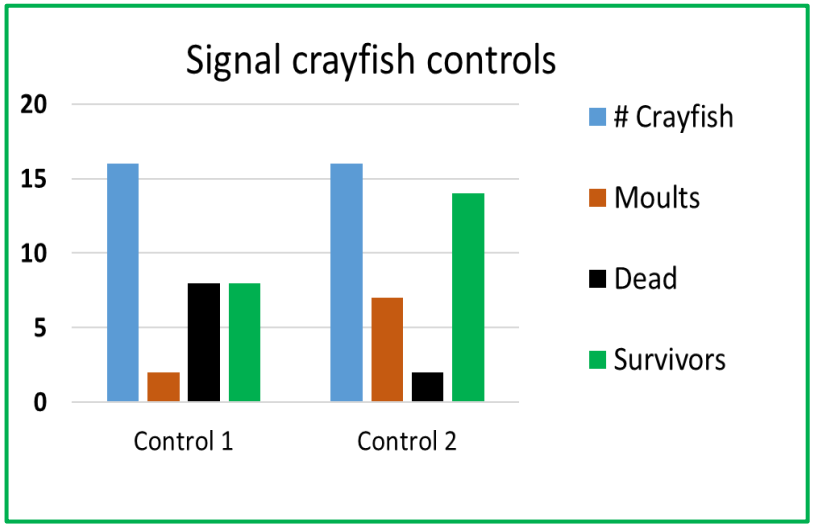
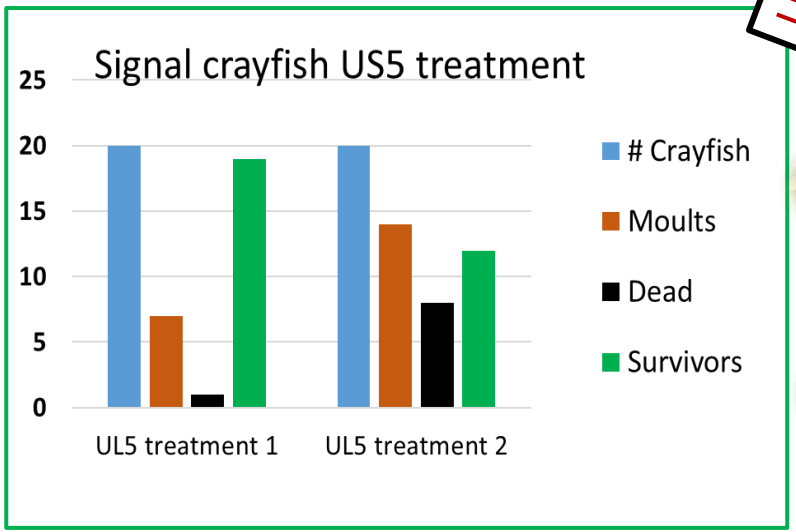
A0	Not detected

- US5 Exp1 – signal crayfish
  - 5% mortality, 35 % moulting
  - ***A. astaci* prev. 100%, mean A5**
- UL5 Exp2 – signal crayfish
  - 40 % mortality, **70 % moulting**
  - ***A. astaci* prev. 100%, mean A5**

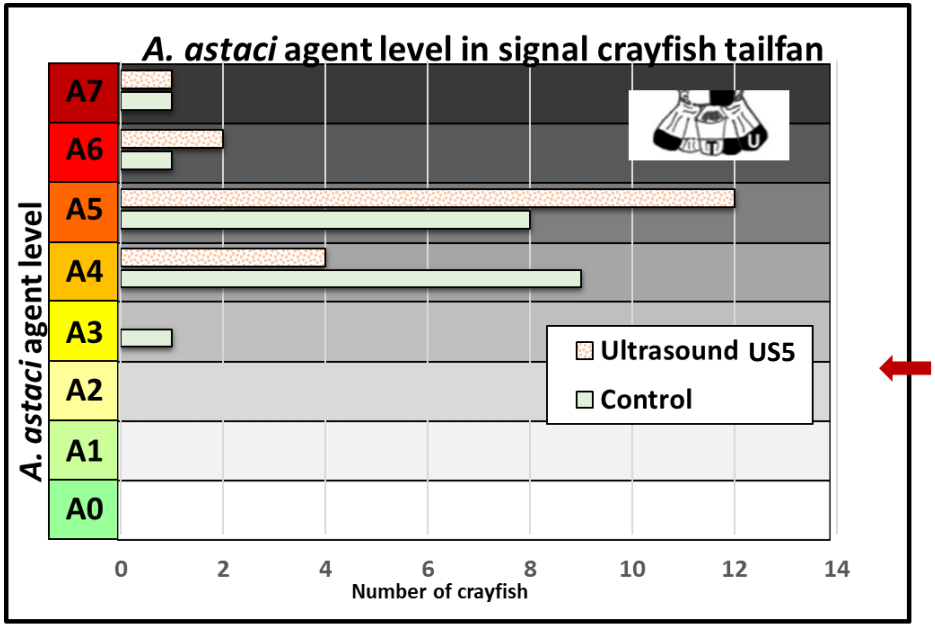
US5 treatment: No effect



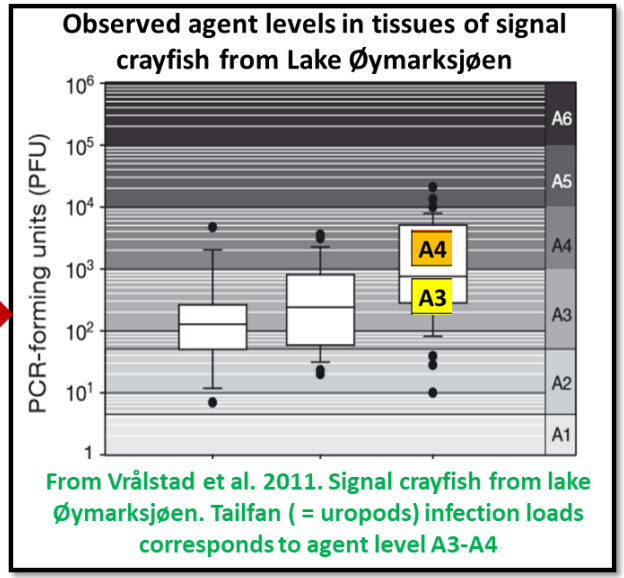


A. astaci agent levels

- Agent level high in control- & US-treated crayfish
  - Slighly higher in the US5 treatment than the control
  - Higher than normally observed in the wild

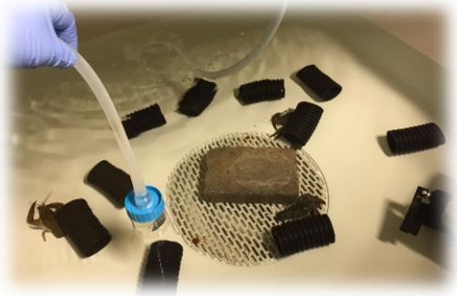


Crayfish originating from the same lake



Challenge experiments with *A. astaci* and noble crayfish

- FOTS approved 3 replicate experiments autumn 2017
- Challenge experiments at the NVI/NMBU aquarium facility
  - Noble crayfish from Hvaler crayfish farm
  - Challenged with *A. astaci* isolate VIO4853
    - Exp1 ~10 spores/ml, Exp2 & Exp3 ~100 spores/ml
  - Duration 7-9 weeks
  - Treatments: US1, US3, US4, US5, US6, US8, US11 (7 US programs)
  - Three replicate experiments:
    - 1: Des 2017 – Feb 2018, 2: March –May 2018, 3: Oct - Des 2018

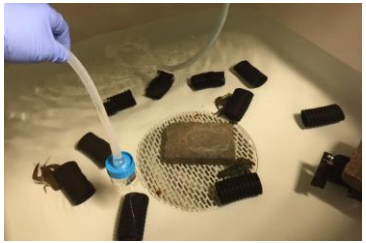


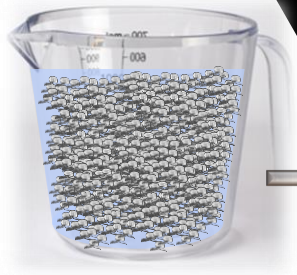
Mattilsynet

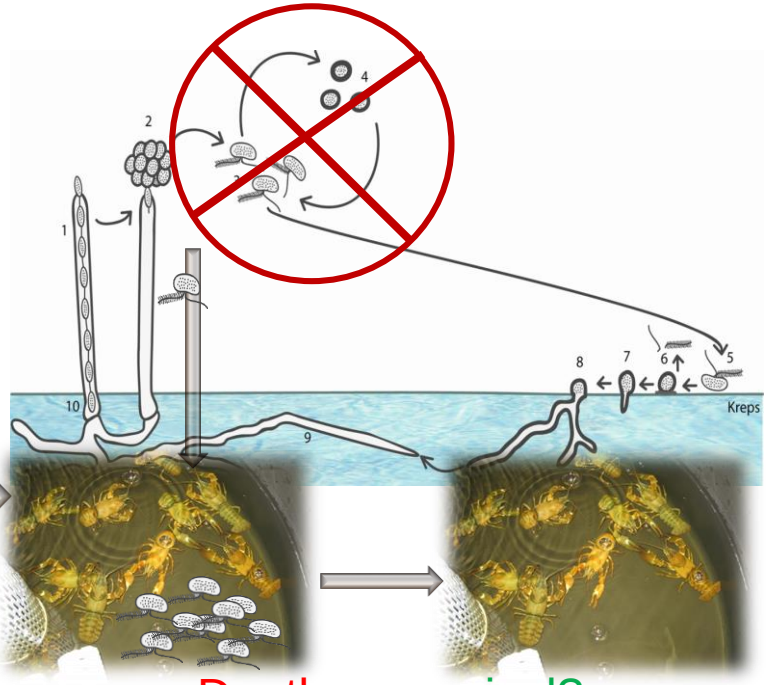
FOTS
Forsøksdyrforvaltningens tilsyns- og søknadssystem

FOTS id nr. 13033

- Ultrasonic exposure of *A. astaci* in challenge experiments with noble crayfish
  - Test ultrasonic treatment and effect on infection and survival rates
  - Assess survival rates of noble crayfish and infection load in tissues







Death or survival?

Experimental design – *A. astaci* spore challenge of noble crayfish

Treatment US3/US1/US6

- Noble Crayfish, N = 10
- *A. astaci spores,* 10/100 spores/ml

Treatment US8/US4/US11

- Noble Crayfish, N = 10
- *A. astaci spores,* 10/100 spores/ml

Treatment US5 (the promising)

- Noble Crayfish, N = 10
- *A. astaci spores,* 10/100 spores/ml

Control survival

- Noble Crayfish, N = 10
- No US

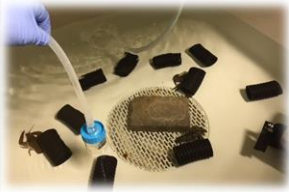
Control infection

- Noble Crayfish, N = 10
- *A. astaci spores,* 10/100 spores/ml
- No US

3 replicate experiments:

1. 10 ° C, fed every 3rd day
2. 18 ° C, fed every 3rd day
3. 18 ° C, fed every 3rd day

US start2 h before adding spores



Results – Replicate experiment 1

Treatment UL3/UL1/UL6
- Noble Crayfish, N = 10
- *A. astaci spores*, 10/100 spores/ml
- UL started 2 h before adding spores

Treatment UL8/UL4/UL11
- Noble Crayfish, N = 10
- *A. astaci spores*, 10/100 spores/ml
- UL started 2 h before adding spores

Treatment UL5
- Noble Crayfish, N = 10
- *A. astaci spores*, 10/100 spores/ml
- UL started 2 h before adding spores

Control survival
- Noble Crayfish, N = 10
- No UL

Control infection
- Noble Crayfish, N = 10
- *A. astaci spores*, 10/100 spores/ml
- No UL

3 replicate experiments:
1. 10 ° C, fed every 3rd day
2. 18 ° C, fed every 3rd day
3. 18 ° C, fed every 3rd day

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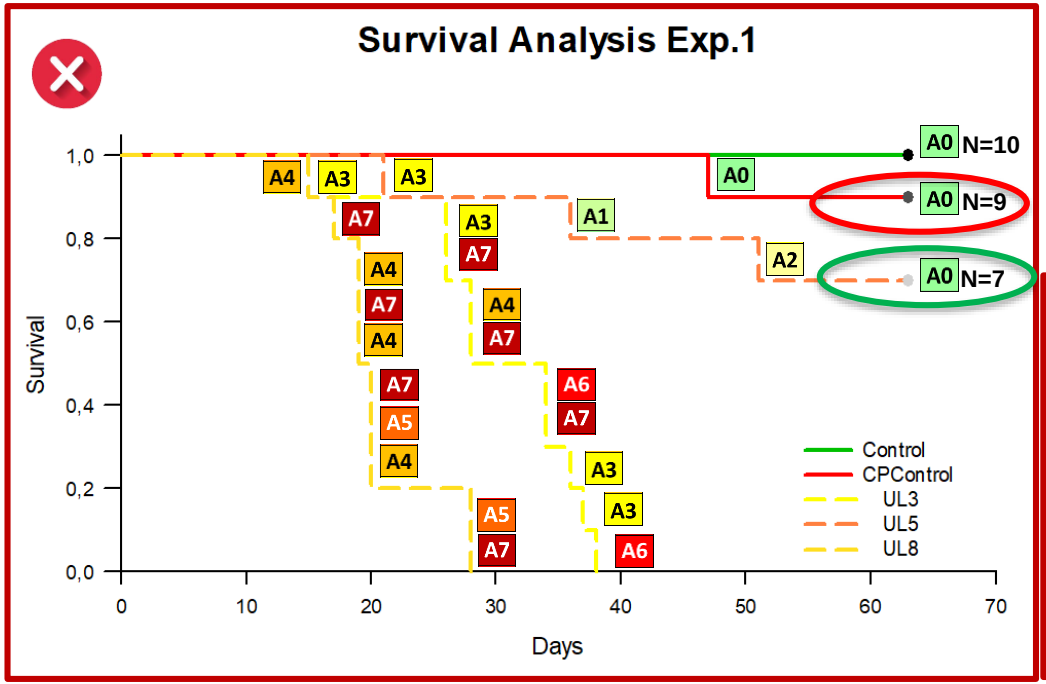

Non-conclusive

- **The challenged control crayfish unaffected – control not OK**
- The crayfish in US5 almost unaffected
- **Effect of US5 on survival or not?**
- **All noble crayfish died in US3/US1/US6/US8/US4/US11**
  - *A. astaci* prevalence 100 %
  - Highest agent level A7 (exceptionally high)

Results – Replicate experiment 2-3

Controls OK - Eksperiments conclusive ✓

No tested US treatment has effect on survival ✗

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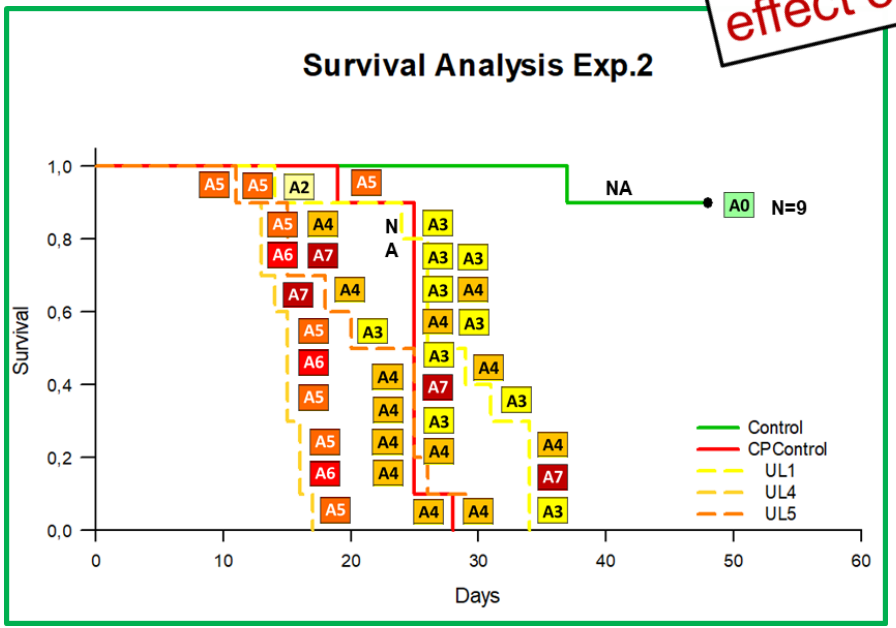

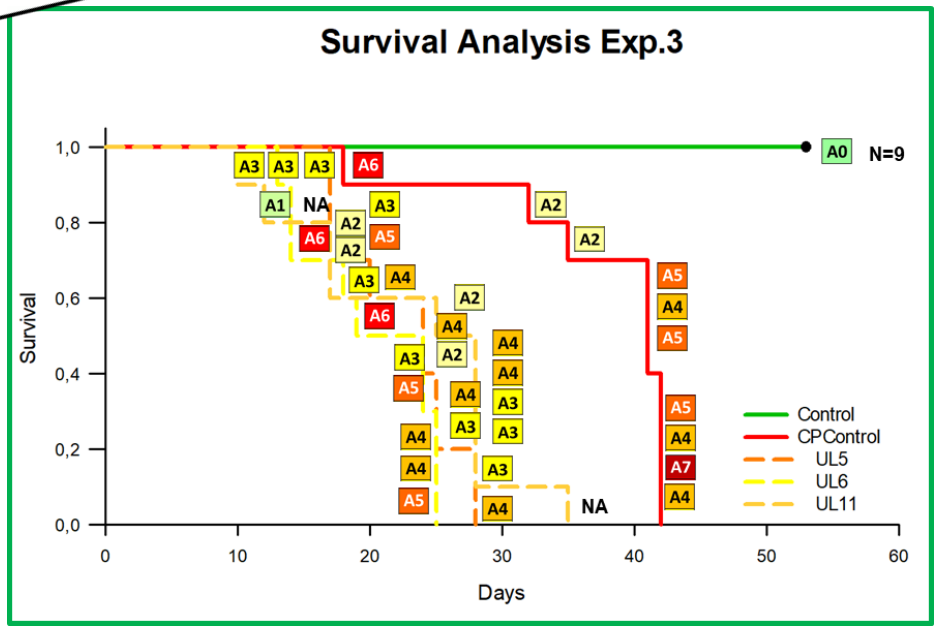

Conclusions

- **Ultrasound treatment US5 showed initially some promising (but inconclusive) tendencies**
- **However - the final experiments were clear**
  - **We did not find a specific ultrasound program that was detrimental to viable zoospores *A. astaci***
  - **The tested ultrasound treatments did not protect the noble crayfish from *A. astaci* infection and subsequent mortality**
  - **The hypothesis that ultrasould treatment will prevent *A. astaci* re-infection of carrier signal crayfish was not supported**




Thanks to

The Research Council of Norway

- SLU Aqua - facilities and helping with the co-habitation experiment
- Norwegian Food Safety Authority – FOTS approval
- KASA crayfish farm (Hvaler) providing noble crayfish
- LGSOUND for equipments and councelling

SLU

Mattilsynet

KASA KREPSEOPPDRETT

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