Experimental infection of rainbow trout with *Renibacterium salmoninarum*, the causative agent of Bacterial Kidney Disease

Alejandra Villamil Alonso
Niccolò Vendramin
Lone Madsen
Argelia Cuenca

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Renibacterium salmoninarum

Gram-positive, slow-growing diplobacillus

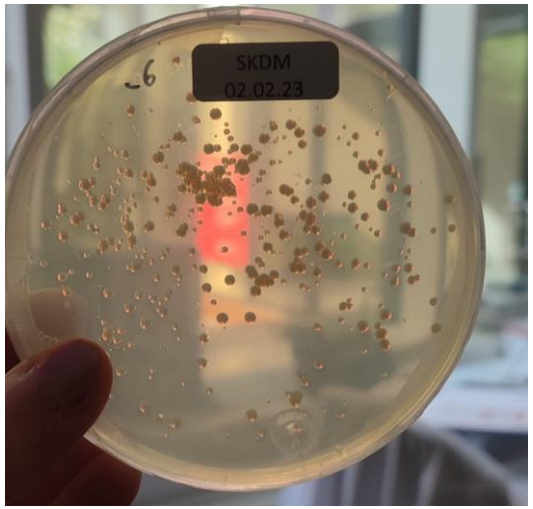
Facultative intracellular bacterium

Replicates within fish macrophages

Preferred temperature: 15° C

Causative agent of **bacterial kidney disease in salmonids**

57 protein secreted by MSA



Diagnostic methods:

- ELISA (p57)
- PCR (*msa2*)
- BACTERIA CULTURING (SKDM agar)

Bacterial Kidney Disease (BKD)

Scotland, "Dee Disease"

1930s

North America

1935

Skjern Å, South Jutland; 1997

Bull. Eur. Ass. Fish Pathol. 17(3/4),140, 1996.

FIRST DEMONSTRATION OF *RENIBACTERIUM SALMONINARUM* / BKD IN DENMARK

E. LORENZEN[1], N.J. OLESEN[1], H. KORSHOLM[2], O.E. HEUER[1] & Ø. EVENSEN[3]
[1] Danish Veterinary Laboratory, Hangøvej 2, DK-8200 Århus N, Denmark, [2] Danish Veterinary Service, Skomagervej 3B, DK-7100 Vejle, Denmark, [3] National Veterinary Institute, PO Box 8156 Dep., N-0033 Oslo, Norway

Abstract

A clinical outbreak of BKD was demonstrated in Denmark in March 1997. Subsequently *R. salmoninarum* was found in other 5 rainbow trout farms. The bacterium was demonstrated by immunohistochemistry in situ and later isolated from kidney samples from fish with more or less pronounced pathological lesions. The diagnostic methods applied are described and considerations about a survey for BKD discussed.

Horizontal and **vertical** transmission

Difficult treatment (poor effect of antibiotics)

Importance of **surveillance programs** to control the disease!

BOX 14

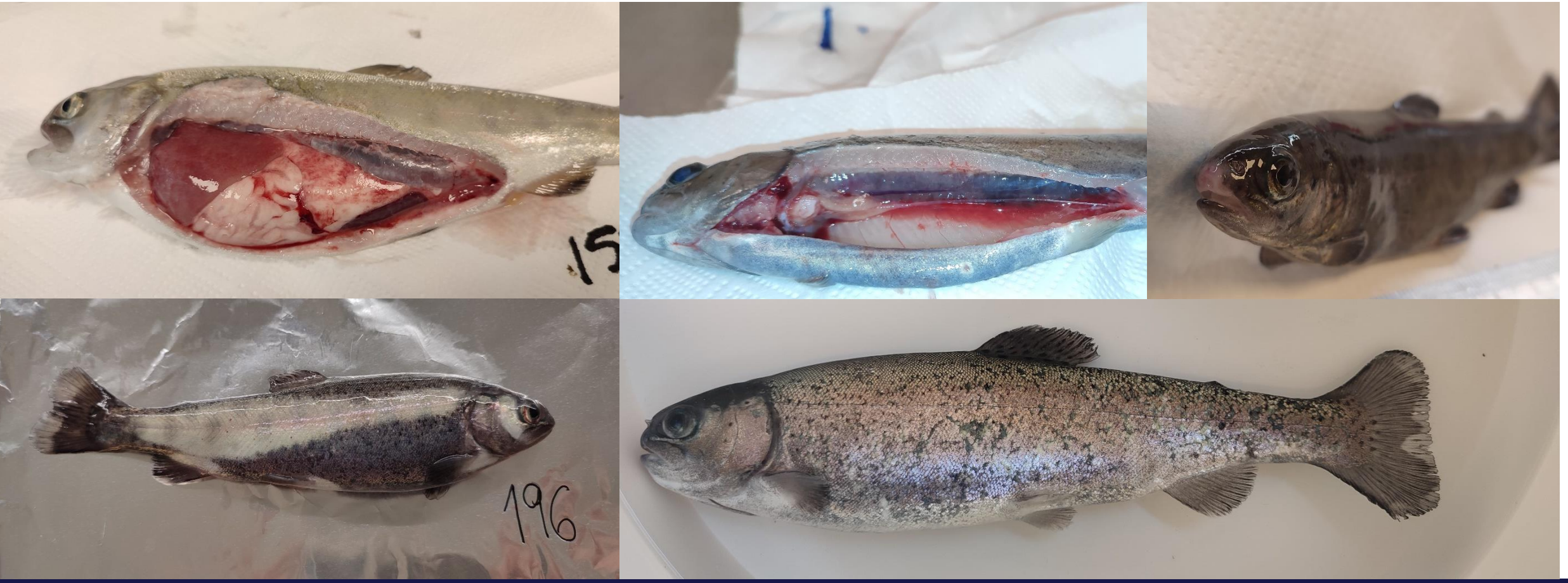
National disease control plan for infectious pancreatic necrosis virus and bacterial kidney disease in freshwater fish farms

Infectious pancreatic necrosis virus (IPNV) and bacterial kidney disease (BKD) are present in Denmark. Ongoing surveillance is conducted for IPNV and BKD, and breeding and production farms can be registered as IPNV-free and BKD-free by the DVFA. Denmark has 25 freshwater fish farms registered as being IPNV-free and 15 freshwater fish farms as being BKD-free (Executive Order No. 1492 og 12 December 2019 on the surveillance and registration of IPN and BKD). These farms were also recognised by the EU as being free from the diseases in question (Commission Implementing Decision (EU) 2021/260).

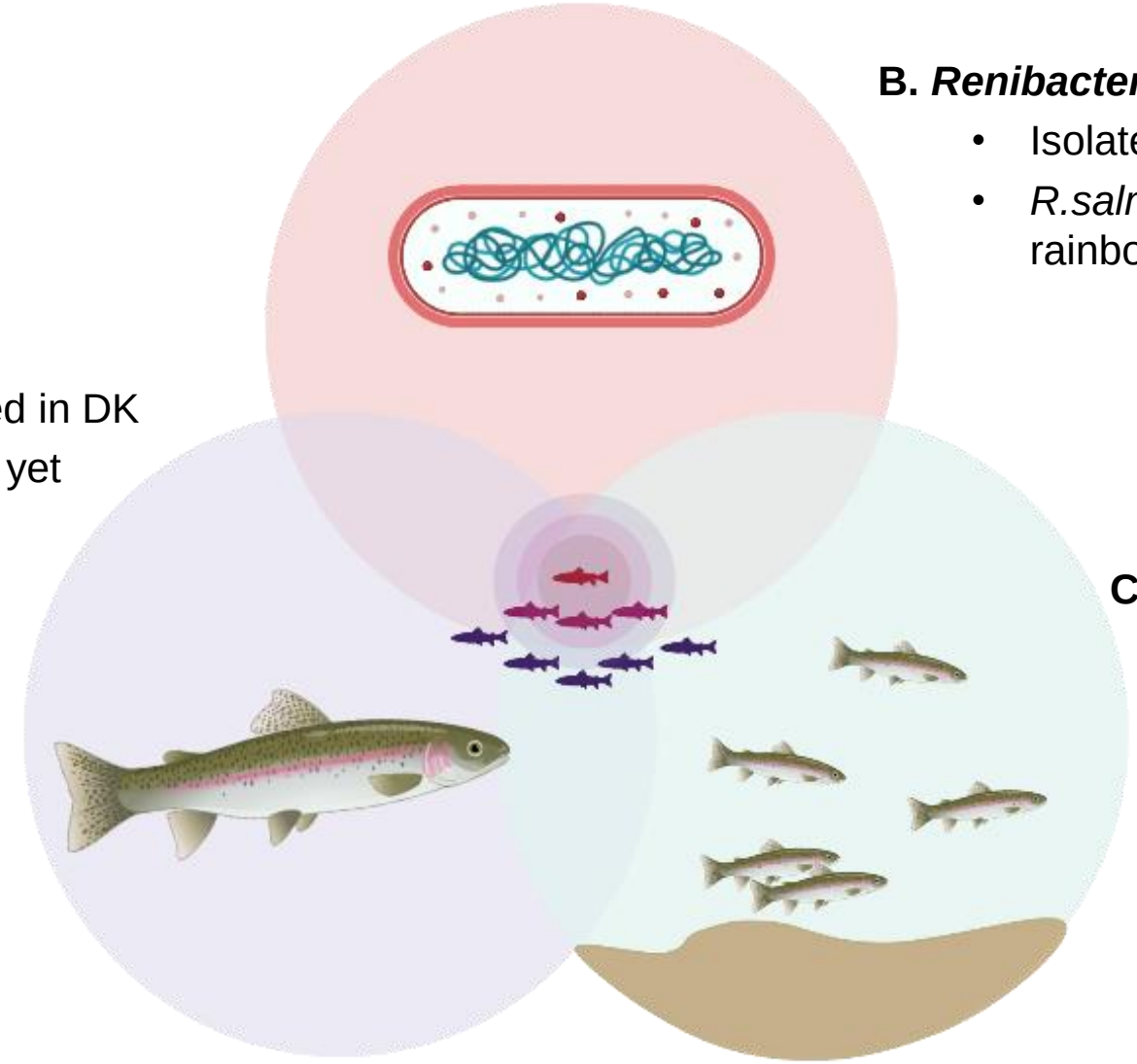
Targeted surveillance is conducted at aquaculture production businesses (APBs) registered as free from IPN and/or BKD. Those APBs are inspected and sampled twice a year if the fish are reared at broodstock farms and once a year if they are reared at production farms. For each inspection, a sample of 30 fish is collected for virological examination for IPNV and another sample of 30 fish for bacteriological examination for BKD.

Bacterial Kidney Disease (BKD)

slow chronic disease



Host-Pathogen-Environment



A. Rainbow trout

- Main species farmed in DK
- Infection model not yet established

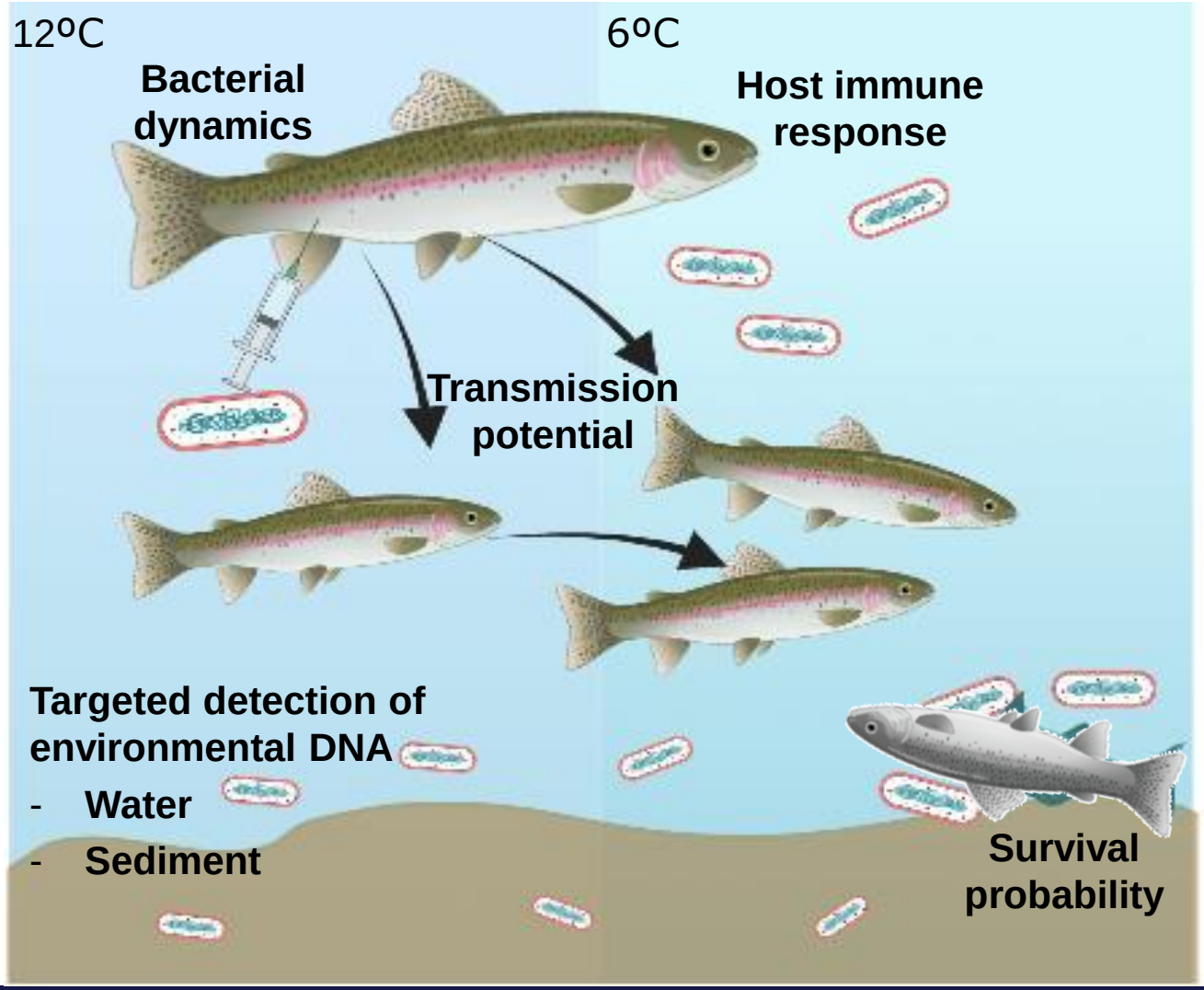
B. *Renibacterium salmoninarum*

- Isolated from Danish farms for years!
- *R.salmoninarum* 2021-1446/4 isolated from rainbow trout

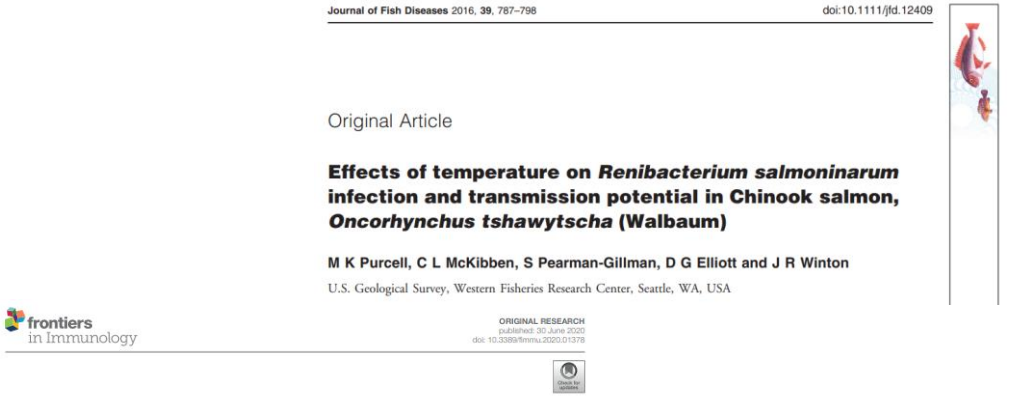
C. Recirculating aquaculture systems

- Temperature
- Water quality (CO_2, salinity…)

OVERVIEW



Our study aimed to characterize disease kinetics and host survival through *in-vivo* cohabitation challenge at 6°C and 12°C in RAS


Journal of Fish Diseases 2016, 39, 787–798 doi:10.1111/jfd.12409

Original Article

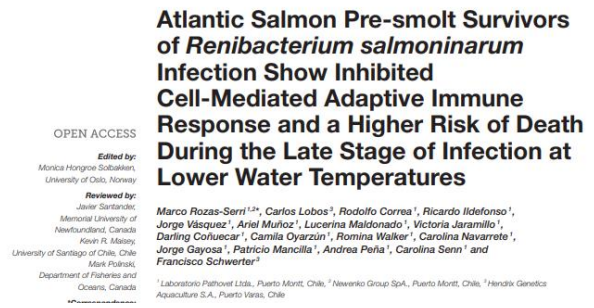
Effects of temperature on *Renibacterium salmoninarum* infection and transmission potential in Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum)

M K Purcell, C L McKibben, S Pearman-Gillman, D G Elliott and J R Winton

U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA, USA

frontiers in Immunology

ORIGINAL RESEARCH


OPEN ACCESS

Atlantic Salmon Pre-smolt Survivors of *Renibacterium salmoninarum* Infection Show Inhibited Cell-Mediated Adaptive Immune Response and a Higher Risk of Death During the Late Stage of Infection at Lower Water Temperatures

EXPERIMENTAL DESIGN

6 RAS tanks

~60 gr SPF rainbow trout/tank

20 "shedders"
40 "cohabitants"

Bacteria prepared for infection; two doses:

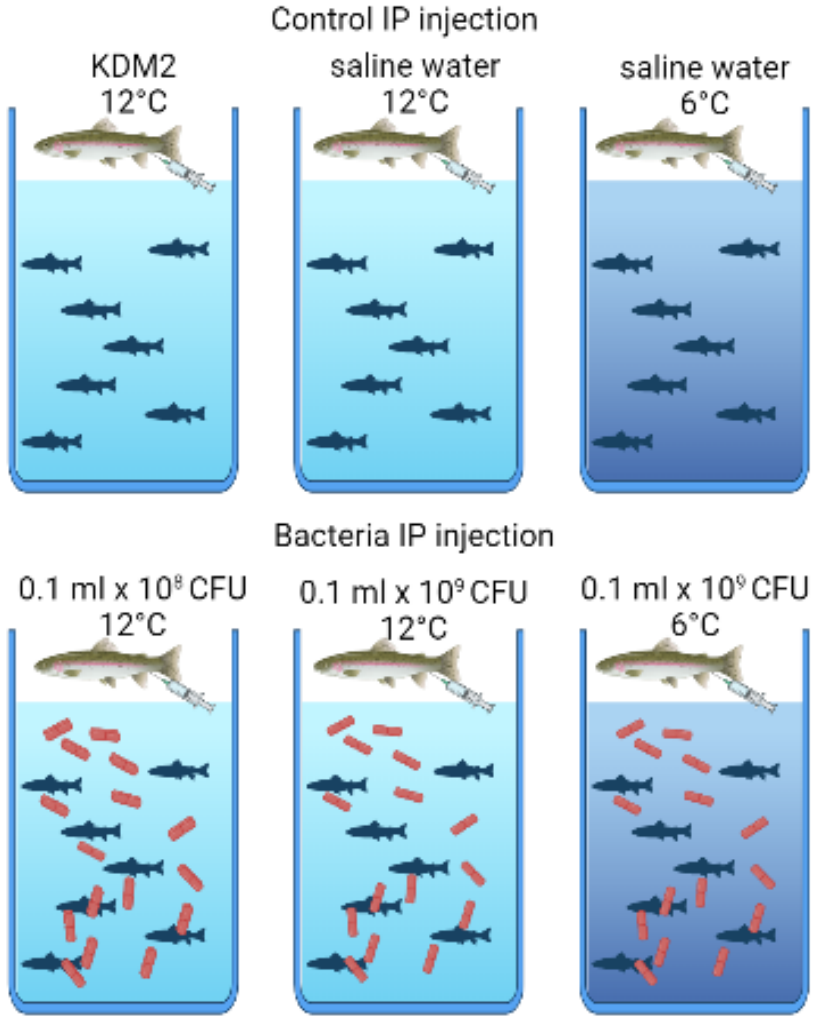
10^8 CFU/fish
10^7 CFU/fish

Influence of temperature?

6ºC vs 12ºC

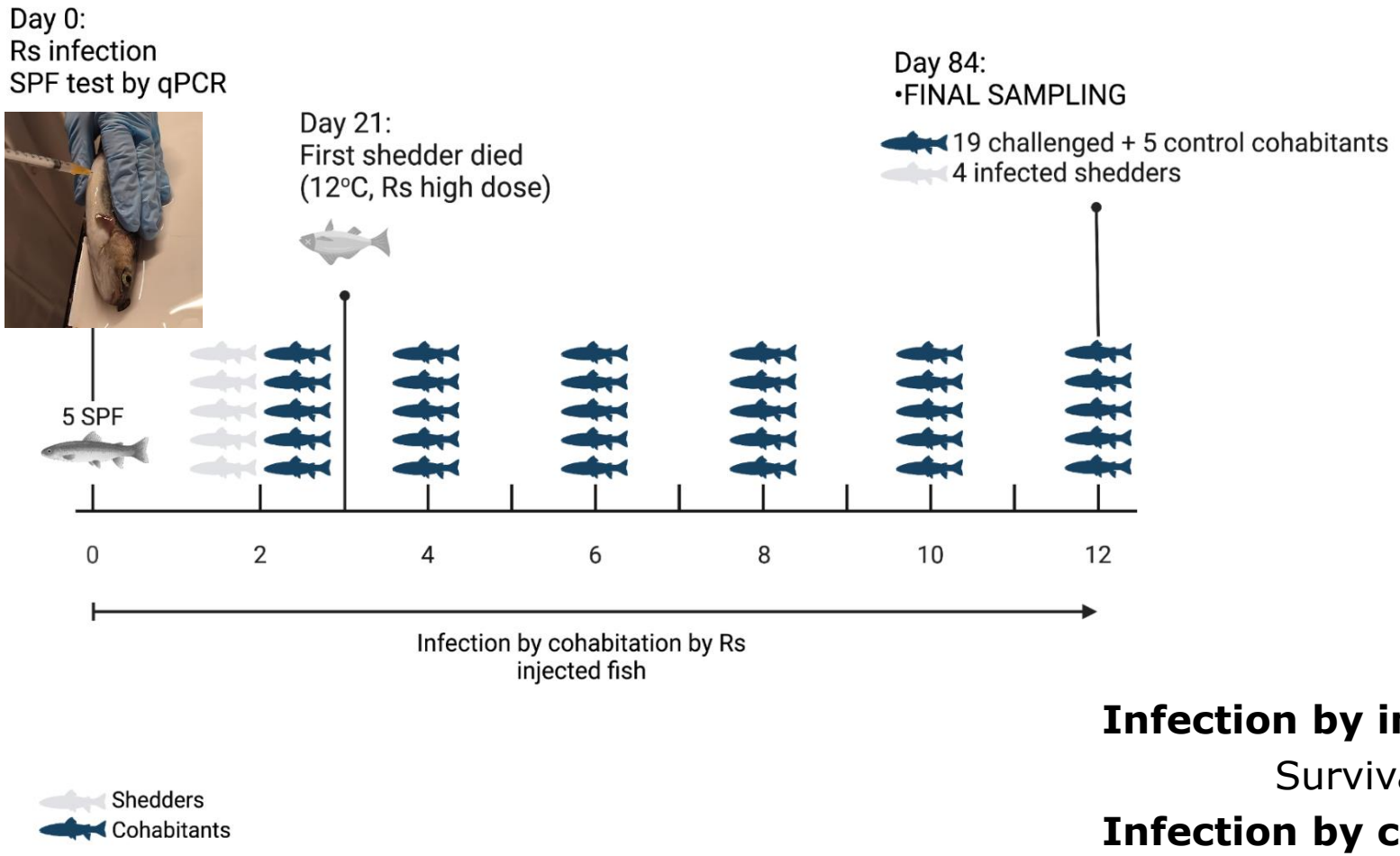
Challenge methods

Infection by injection
Infection by cohabitation



***IN-VIVO* CHALLENGE**

EXPERIMENTAL DESIGN



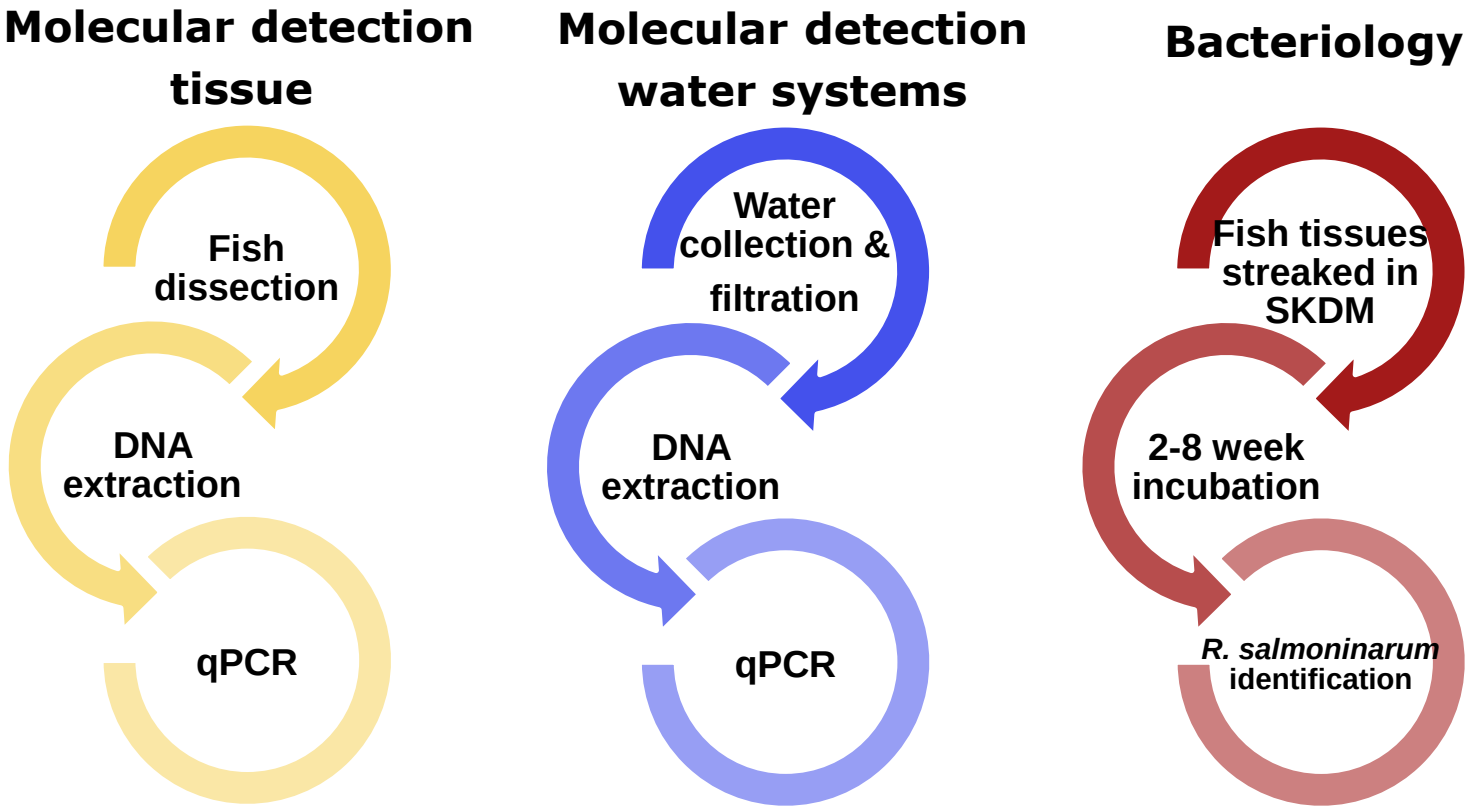
Infection by injection

Survival

Infection by cohabitation

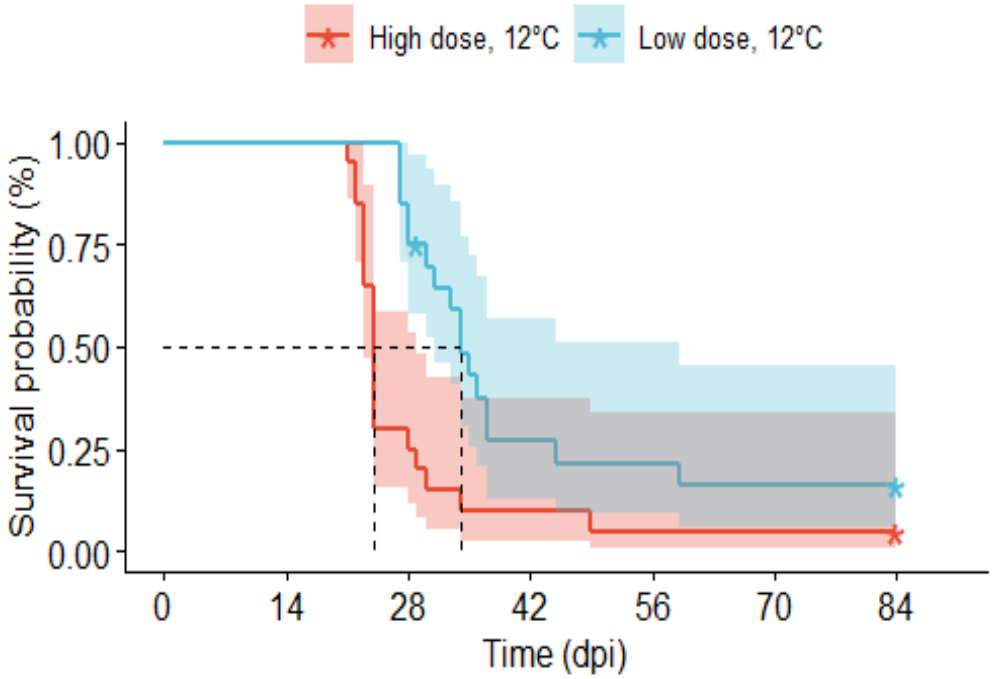
Disease onset and transmission

METHODS

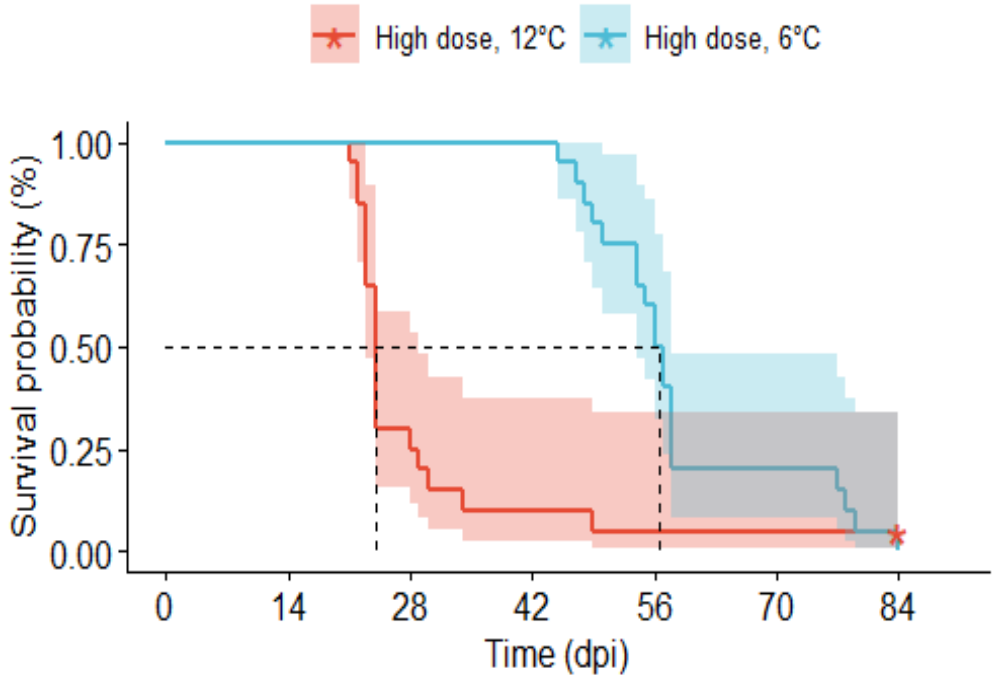

Molecular detection tissue
Fish dissection
DNA extraction
qPCR
Molecular detection water systems
Water collection & filtration
DNA extraction
qPCR
Bacteriology
Fish tissues streaked in SKDM
2-8 week incubation
R. salmoninarum identification

RESULTS: SURVIVAL PROBABILITY OF SHEDDERS INJECTED WITH *R. SALMONINARUM*

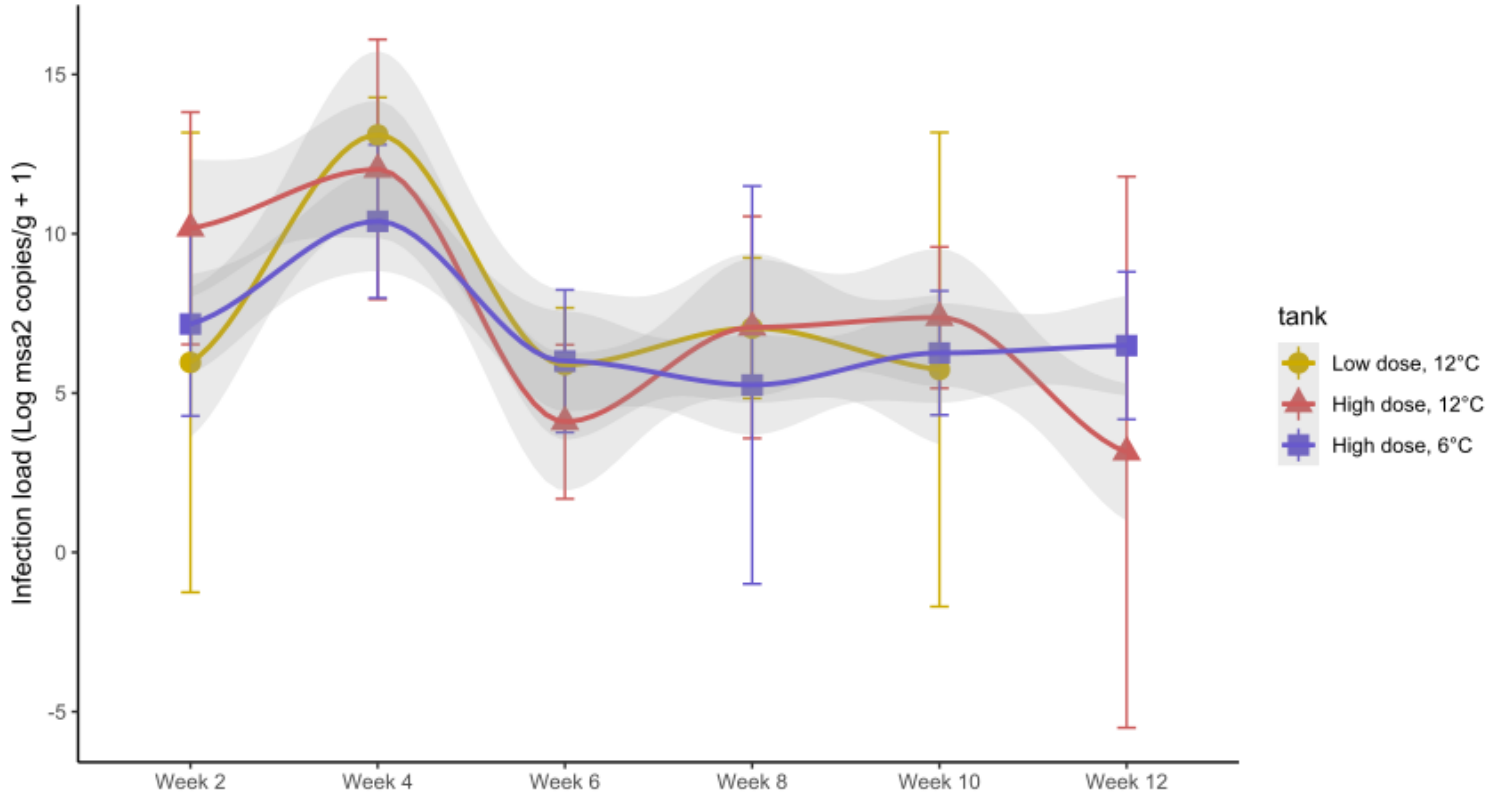
Dose effect



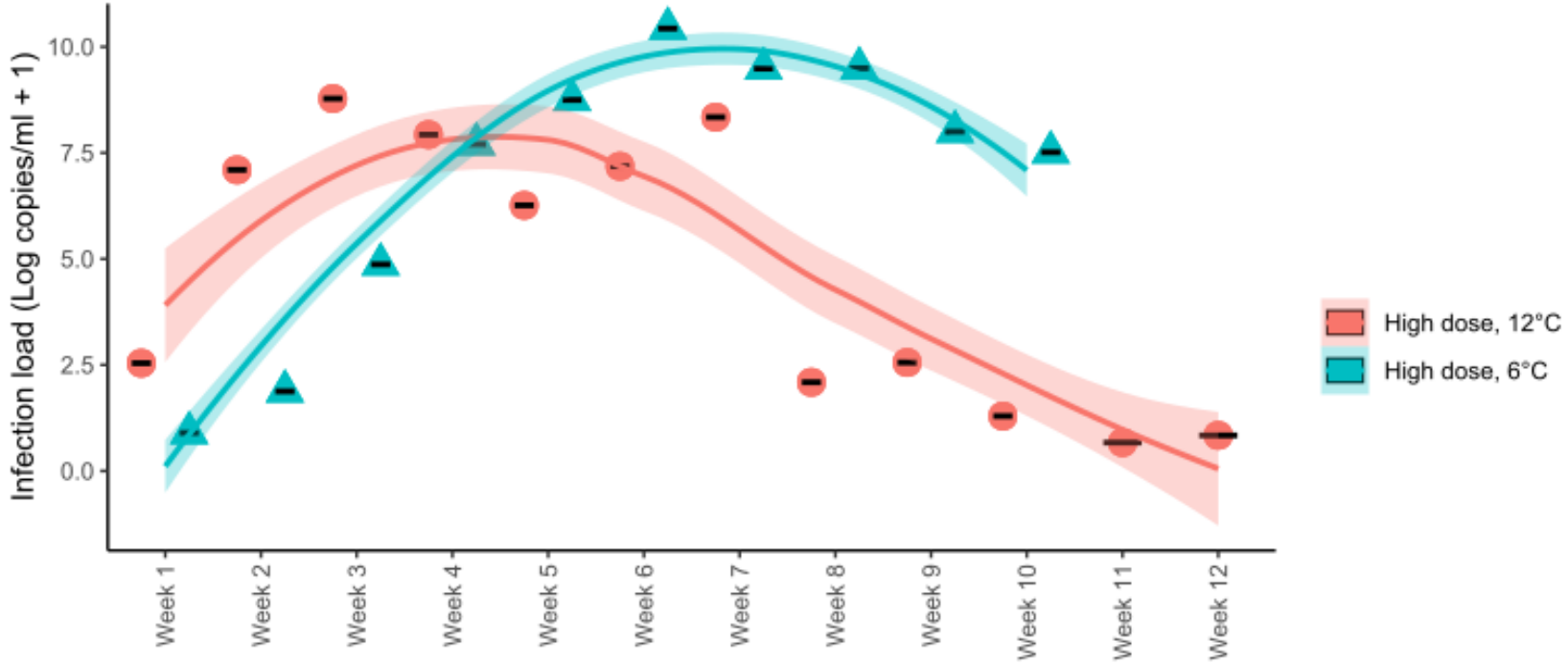
Temperature effect



RESULTS: *R. SALMONINARUM* DYNAMICS IN KIDNEY TISSUE OF COHABITANTS



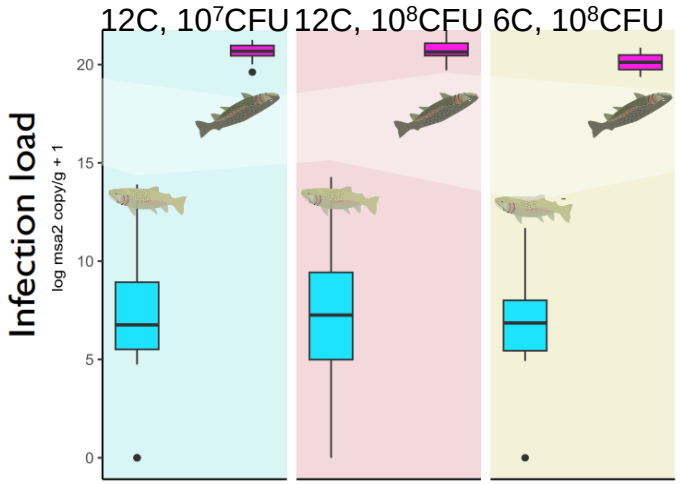
RESULTS: *R. SALMONINARUM* eDNA LEVELS IN WATER SYSTEMS



TO SUM UP…

Challenge by injection: survival analysis

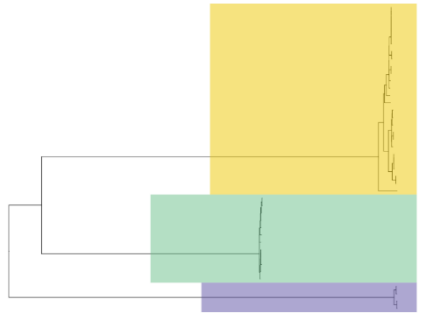
- All cohabitants survived
- Shedders from all groups experienced reduced survival
- Survival was not dependent on temperature under the given infection doses



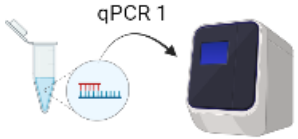
Challenge by cohabitation:

- Infection was established within 14 days by cohabitation with injected fish.
- Peak of infection at week 4, with similar levels at 12° C and 6° C.
- Temperature did not greatly influence BKD progression under these experimental conditions
- *R. salmoninarum* can be detected in water at all stages of the disease!

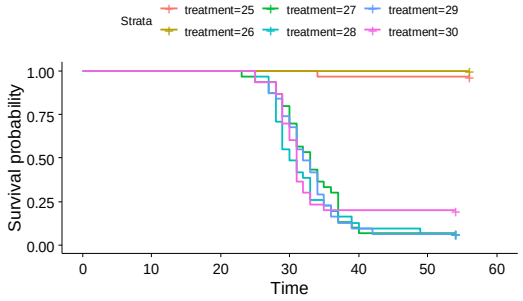
MOLECULAR TRACING OF *R. SALMONINARUM* IN DK AND NORWAY THROUGH WGS ANALYSIS



IMPROVED DIAGNOSTIC METHODS



KAFREA: Pathogenesis of BKD in Rainbow trout with the introduction of an environmental stressor (increased CO2 levels; CO2 25mg/L water)



HOST-PATHOGEN INTERACTIONS: PROFILING THE IMMUNE RESPONSE OF TROUT UPON INFECTION


THANK YOU
FOR YOUR
ATTENTION ☺