Red mark syndrome in rainbow trout

Jacob G. Schmidt

Red mark syndrome in rainbow trout

Problem Background Project Model Agent Response Future

Red mark syndrome in rainbow trout

Starting c. 2010 Danish fish farmers started reporting red marks

2015 questionnaire: 1 of 3 farms with RMS. Increasing.

Now most important disease in DK aquaculture (alongside BCWD)

The fish looks sick

RMS is very difficult to manage

"Slow disease"

Red mark syndrome in rainbow trout

Red mark syndrome in rainbow trout

Likely a disease endemic to western North America, where it is known as strawberry disease and affects *Oncorhynchus* sp. First reports are from middle of 20th century.

One report from France in mid-80s, but apart from this the first European RMS reports are from the UK c. 2003.

The disease has since spread to many European countries as well as Iran and Chile.

Red mark syndrome in rainbow trout

The disease has been proposed to be associated with an adenovirus, *Flavobacterium psychrophilum* and a Rickettsia-like organism (RLO).

The first association with an RLO was made in 2008. This has since been considered the most likely candidate for a causative agent but a definite connection has not been made.

The RLO is now known to be more closely related to the recently described family Midichloriaceae, and is thus now referred to as Midichloria-like organism (MLO).

Red mark syndrome in rainbow trout

- **Firmly establish what causes RMS**
  - Developing an infection model
  - Diagnostics
  - NGS
- **Describe RMS symptom development**
  - Histology: H&E, IHC, ISH
  - Images
- **Manipulating the disease**
  - Testing different factors such as antibiotics and glucocorticoids

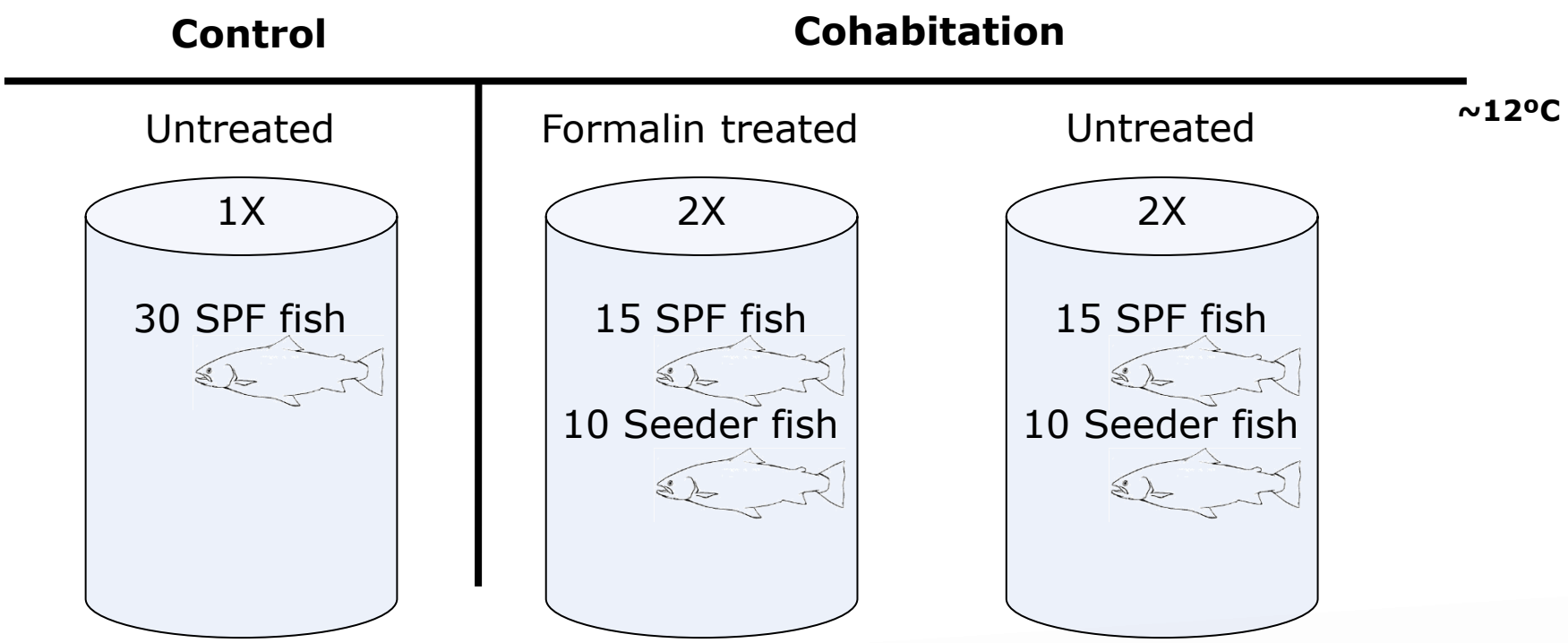
Red mark syndrome in rainbow trout

- **We first aimed at a cohabitation model of infection.**

- RMS-affected fish brought in from Møborg fish farm (~170 g).
- 3 fish were screened for vira, bacteria and parasites.
  - Vira: None (on cell culture)
  - Bacteria: Few (on BA, TYA and CHAB). *F. psychrophilum*.
  - Parasites: Few. Only *Ichthyobodo necator* detected.
- Cohabitation with own SPF fish (~90 g)
- All fish were PIT-tagged prior to cohabitation.

Red mark syndrome in rainbow trout

- **We first aimed at a cohabitation model of infection.**



Red mark syndrome in rainbow trout

- **Infection facilities.**

Red mark syndrome in rainbow trout

DTU

- **Seeders**

Red mark syndrome in rainbow trout

- **Problems**
- After 16 days of cohabitation SPF fish started to die. Over the next two weeks 11 out of 60 cohabitants died or were killed.
  - Probably a mix of mostly bacterial factors – sometimes infection of tagging site.
  - *F. psychrophilum* was the only bacterial fish pathogen detected from deceased or moribund fish – and only in small numbers.
  - No virus detected.
  - Two fish had fungal infection.

Red mark syndrome in rainbow trout

- **More problems**
- Just as the fish started to recover, Ich infection was detected.
- Salt treatment
- 4 fish were killed due to heavy infection (several hundred trophonts).
- No further mortality.

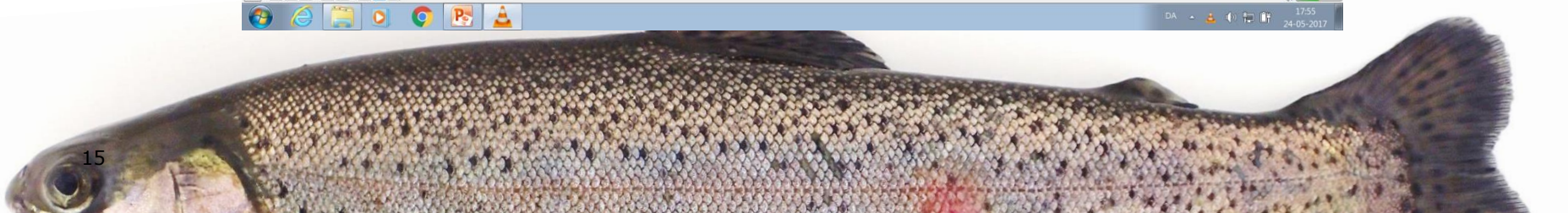
Red mark syndrome in rainbow trout

- **Eureka moment**
- Just as the Ich infection was wearing off the fish developed lesions consistent with early RMS symptoms

(~550 degree days)

Red mark syndrome in rainbow trout

- **Symptoms developing nicely**



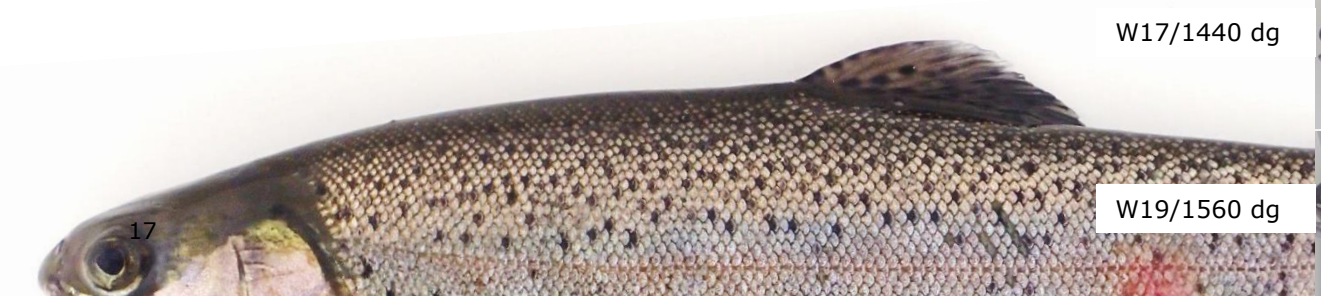
Red mark syndrome in rainbow trout

- **Cohabitants after ~900 degree days at 12 ºC**

Red mark syndrome in rainb

Problem Background Model Project Agent

- **Cohabitant 518999**



W0/0 dg

W7/420 dg

W9/740 dg

W10/850 dg

W12/970 dg

W14/1150 dg

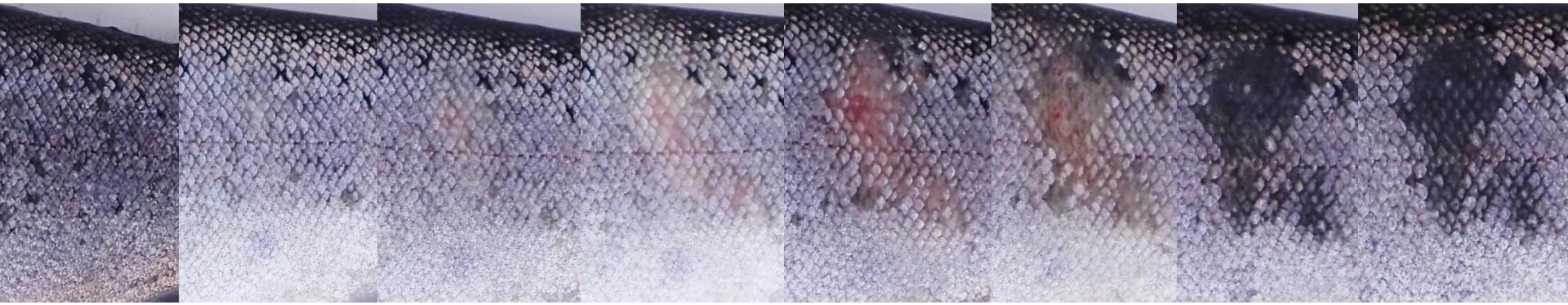
W16/1350 dg

W17/1440 dg

W19/1560 dg

Red mark syndrome in rainbow trout

- **Cohabitant 518999**

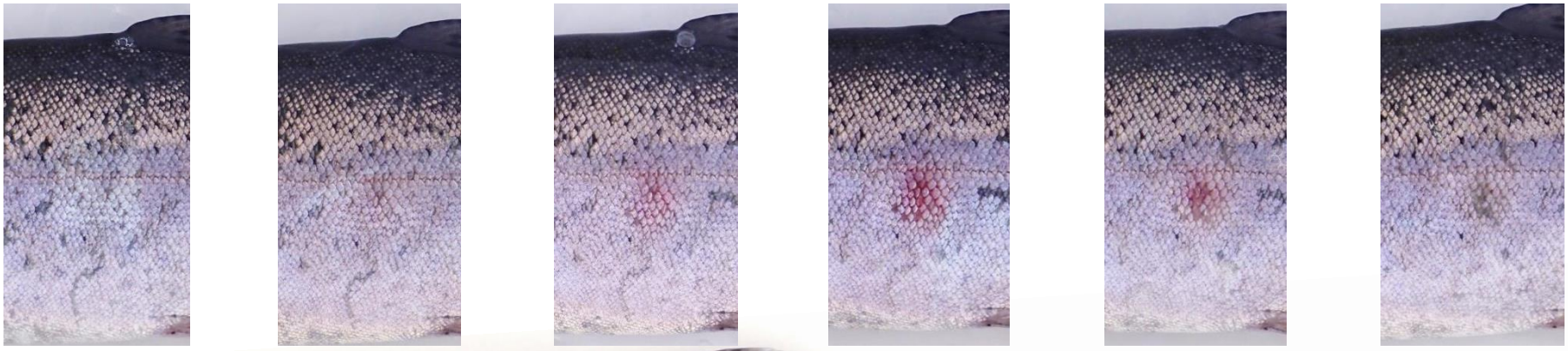


Red mark syndrome in rainbow trout

- **Cohabitation model established**
  - Over the past year we have successfully transferred RMS to 5 passages of new cohabitants by using the old cohabitants as seeders for new SPF cohabitants
  - Ich is eradicated
  - *F. psychrophilum* is still in the system although it causes only very occasional mortality now.

Red mark syndrome in rainbow trout

- **Other infection approaches:**
  - Injection of tissue homogenate (organs and skin)
    - Hoping to avoid *F. psychrophilum*
  - Limited success. Only few symptoms, and mostly at injection sites.
  - Still *F. psychrophilum*.



Red mark syndrome in rainbow trout

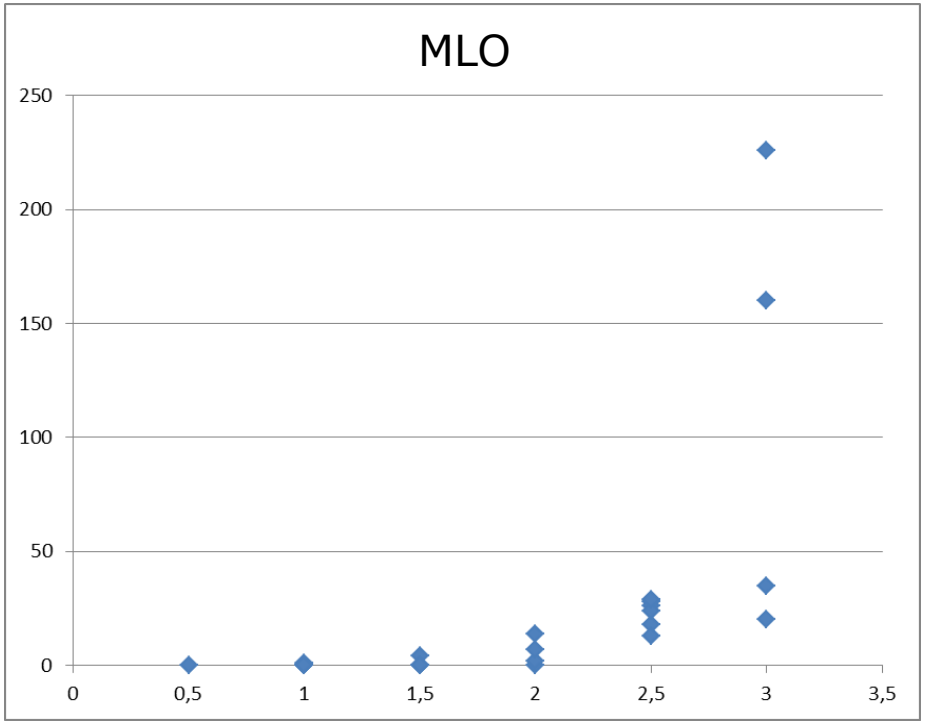
- **Other infection approaches:**
  - Indirect cohabitation
  - Also successful, but fewer lesions, longer incubation time.
  - Actually seems to resemble farm situation somewhat better
  - For experimental purposes, direct cohabitation more applicable

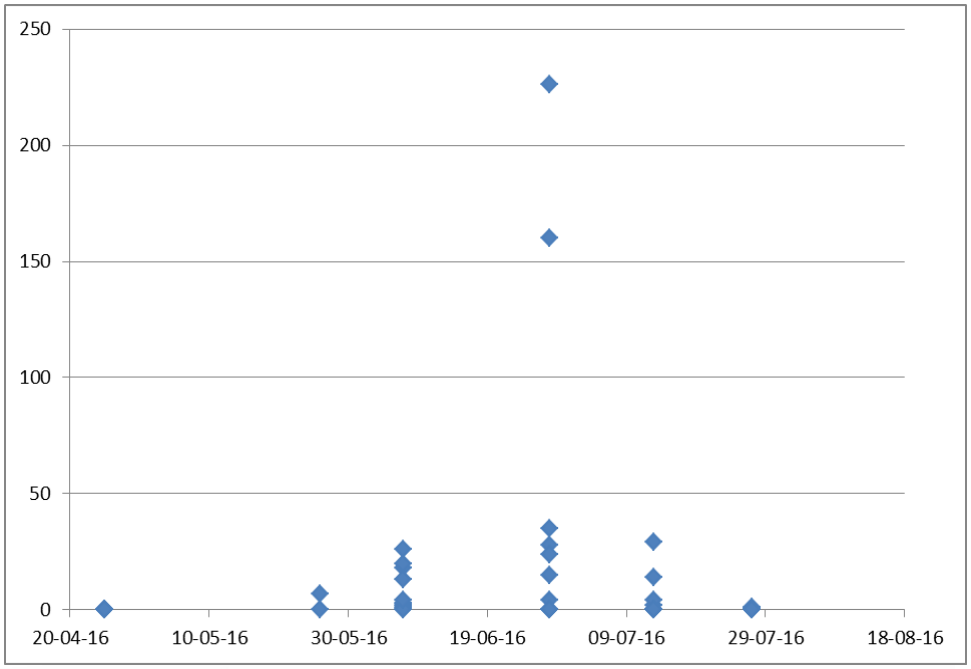
Red mark syndrome in rainbow trout

- **Diagnostics:**
  - No virus detected
    (no CPE on RTG, BF-2 and EPC)
  - No obvious pathogenic bacteria detected on agar plates
    (BA, TYA, CHAB) – apart from *F. psychrophilum*
  - MLO detected with specific qPCR and with 16S rDNA NGS
    - Correlation with disease

Red mark syndrome in rainbow trout

- **Diagnostics:**



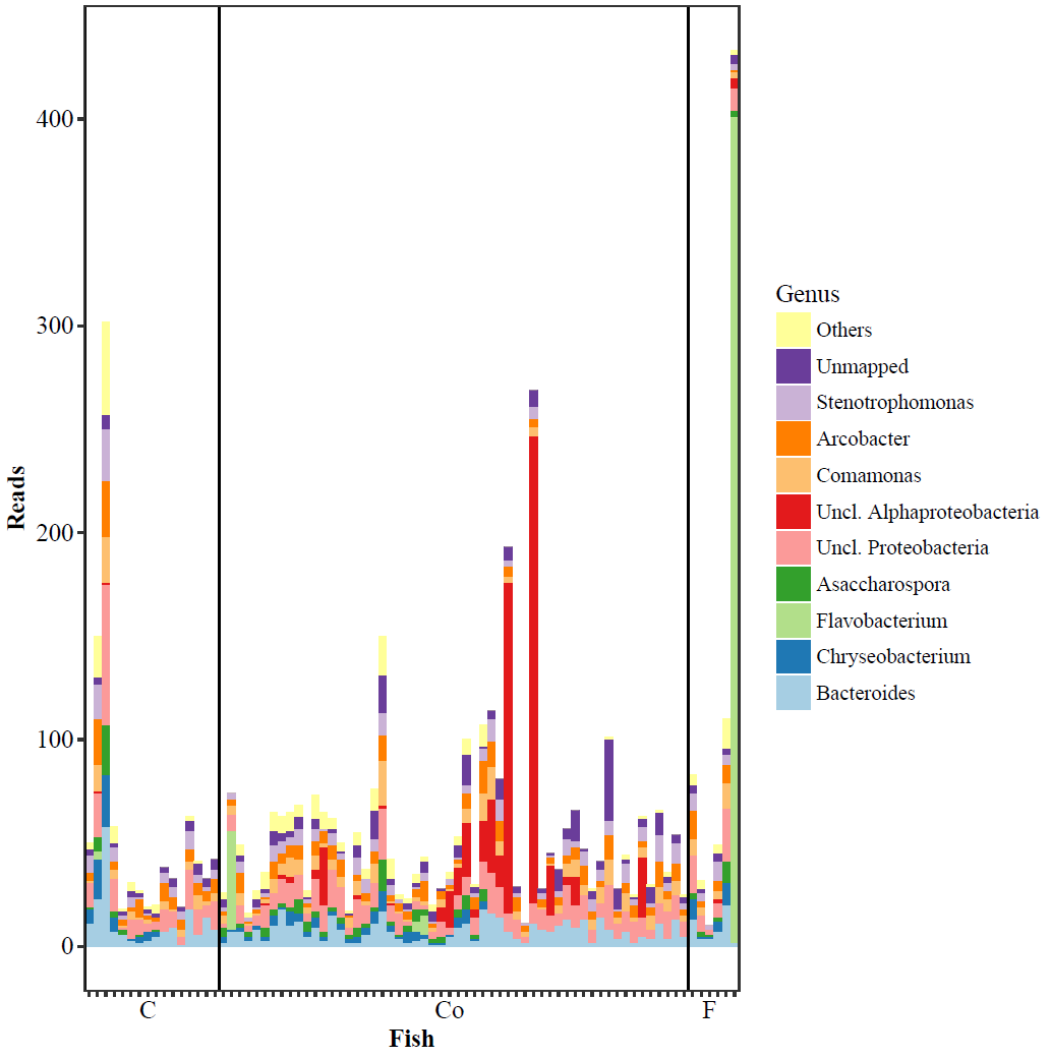


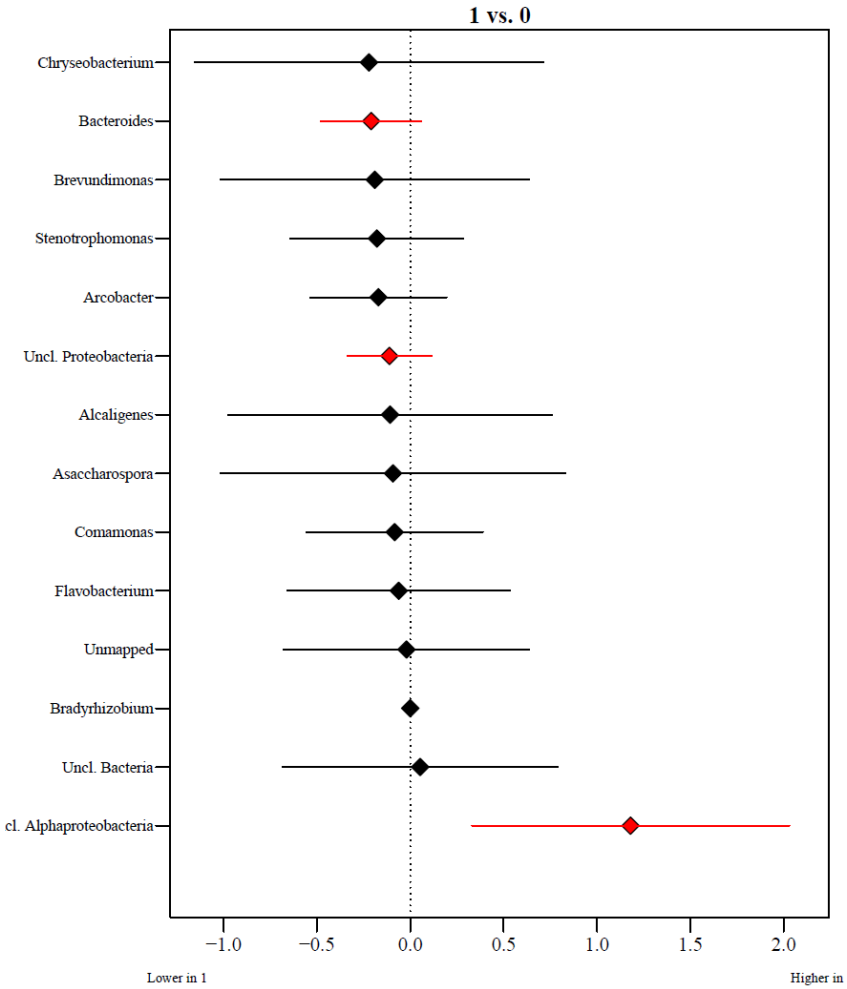
Red mark syndrome in rainbow trout

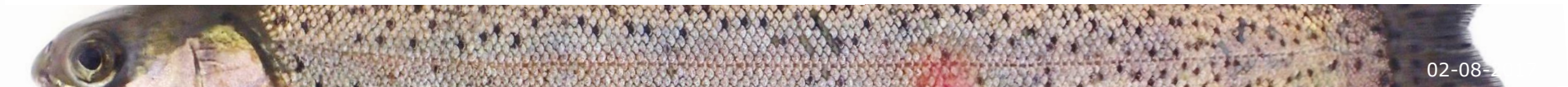
- **Diagnostics:**
  - Next generation sequencing of bacterial 16S rDNA further shows that MLO is the only bacterial group that correlates with RMS lesions

Red mark syndrome in rainbow trout

Problem Background Project Model **Agent** Response Future



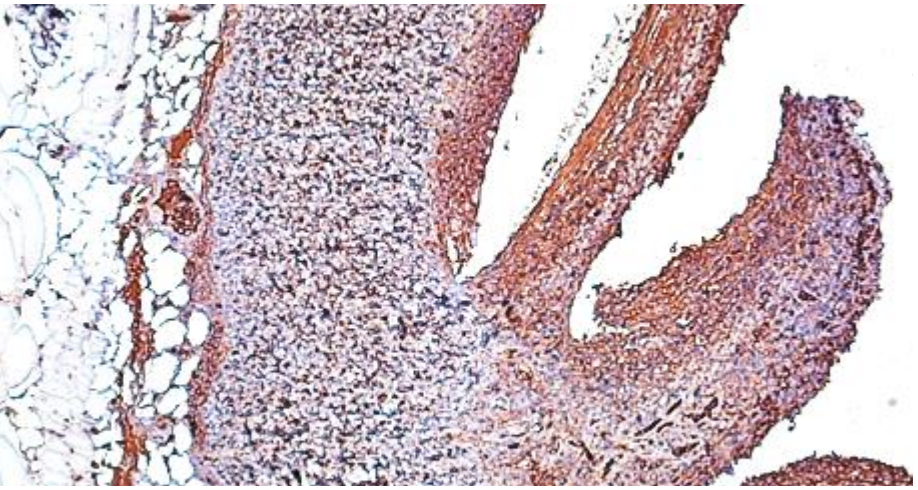




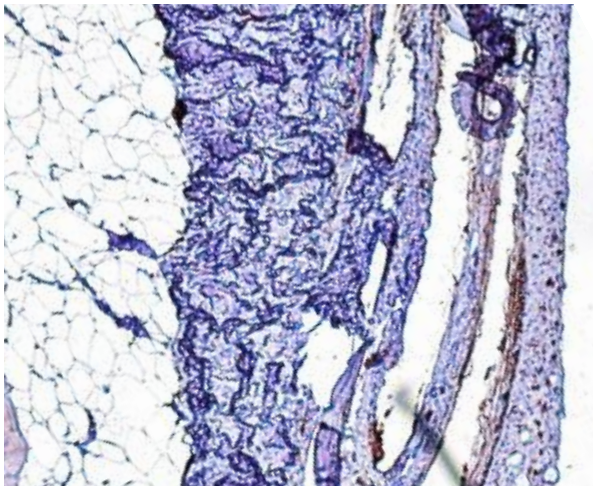
Red mark syndrome in rainbow trout

- **Immunohistochemistry: IgM**

RMS

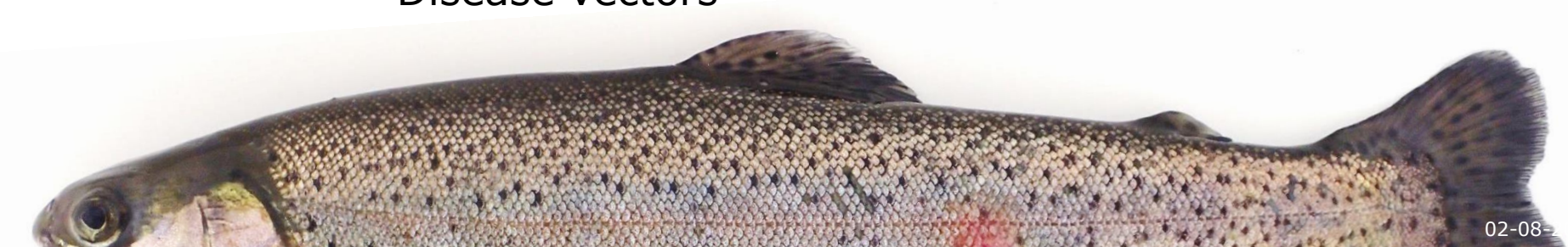


Control



Red mark syndrome in rainbow trout

- **In this project:**
  - Fish farms:
    - NGS
    - Questionnaires
  - At DTU:
    - Continuous optimization of the infection model
    - RMS experiments with the model
      - Antibiotics
      - Glucocorticoids
      - Fish size
      - Disease vectors



Red mark syndrome in rainbow trout

- **Future RMS projects:**
  - WGS
  - Vaccine development
  - ETC!

Red mark syndrome in r

Problem Background Project Model Future

The end