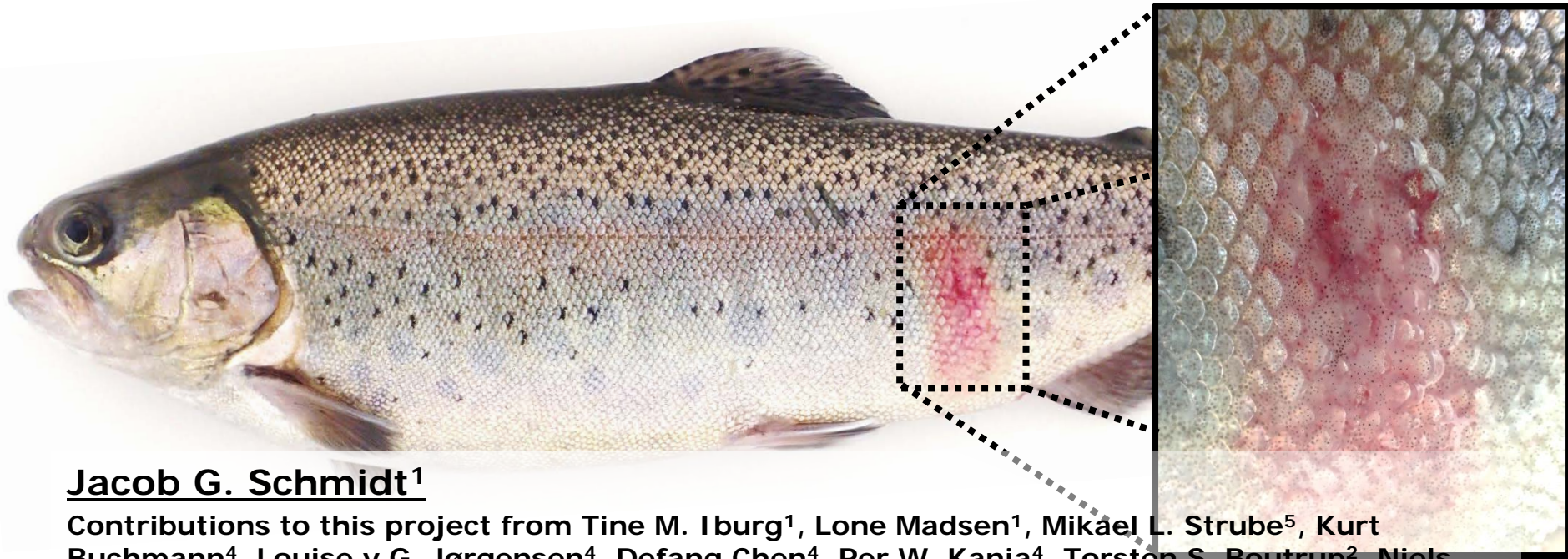


Update on Red Mark Syndrome



Jacob G. Schmidt¹

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...and financially:

Henriksens Fund and

European Maritime and Fisheries Foundation



Problem and background

Recap from AW21

New and on-going



Farmer perception

Diagnostic

Establishing infection model

Immune response

Describe disease development

Size

Antibiotics

Causative agent

Brown trout

Problem and background

Recap from AW21

New and on-going

Since the first reports around the turn of the millennium RMS has been an increasing problem for European rainbow trout farmers

In Denmark approximately every third farm report RMS problems

Presently considered among the most important diseases in Danish freshwater rainbow trout farming (alongside RTFS and PRV3) in spite of very limited mortality, because:

Affects large fish

(Well nobody wants to eat you)

The fish looks sick

RMS is very difficult for farmers to manage (new and slow)

But I feel fine!

I'm okay with that!



Problem and background



Recap from AW21



New and on-going



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New and
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Typically symptoms in 10-30% of fish in an affected pond/raceway

Infection “rolling” through population over several months

Stress-related – sorting/transport

No mortality or growth retardation

Management problems – customer dissatisfaction, downgrading,
loss of market shares.

Occurs in traditional earth ponds, recirculated farms, organic...



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Problem and background



Recap from AW21



New and on-going



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**Problem and
background**

**Recap from
AW21**

**New and
on-going**

Start 41d/490dd

61d/730

84d/1010dd

110d/1300dd





Background: Red mark syndrome (RMS) is an infectious disease so far found to only affect salmonids in the *Oncorhynchus* genus. Macroscopically, the distinguishing feature of the disease is the appearance of raised, red blotches up to several centimeters in diameter. These are mostly seen on the flanks, but may appear almost anywhere on the body, including the fins. RMS has a serious impact on rainbow trout farming in Denmark. Increasing evidence points to an intracellular bacterium as the causative agent of RMS.



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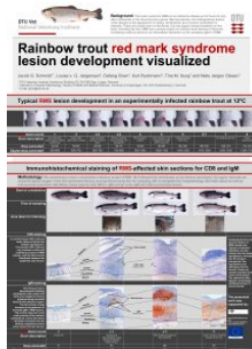
THE FISH NECROPSY MANUAL

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Poster Prizes at 18th EAFP Conference in Belfast, Northern Ireland-UK in September 2017



Jacob Schmidt, Jørgensen L., Chen D., Buchmann K., Iburg T. & Olesen N.

Rainbow trout red mark syndrome lesion development visualized.

[Click here to download it](#)

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Methodology: We established a direct cohabitation infection model of RMS. We followed fish individually as the lesions developed. At regular intervals we acquired digital images of the fish (presented above), and sacrificed some fish for sampling. We investigated the histopathology with H&E stains as well as immunohistochemistry with MABs raised against CD8, MHCII, IgM and IgT (only IgM and CD8 is presented below).

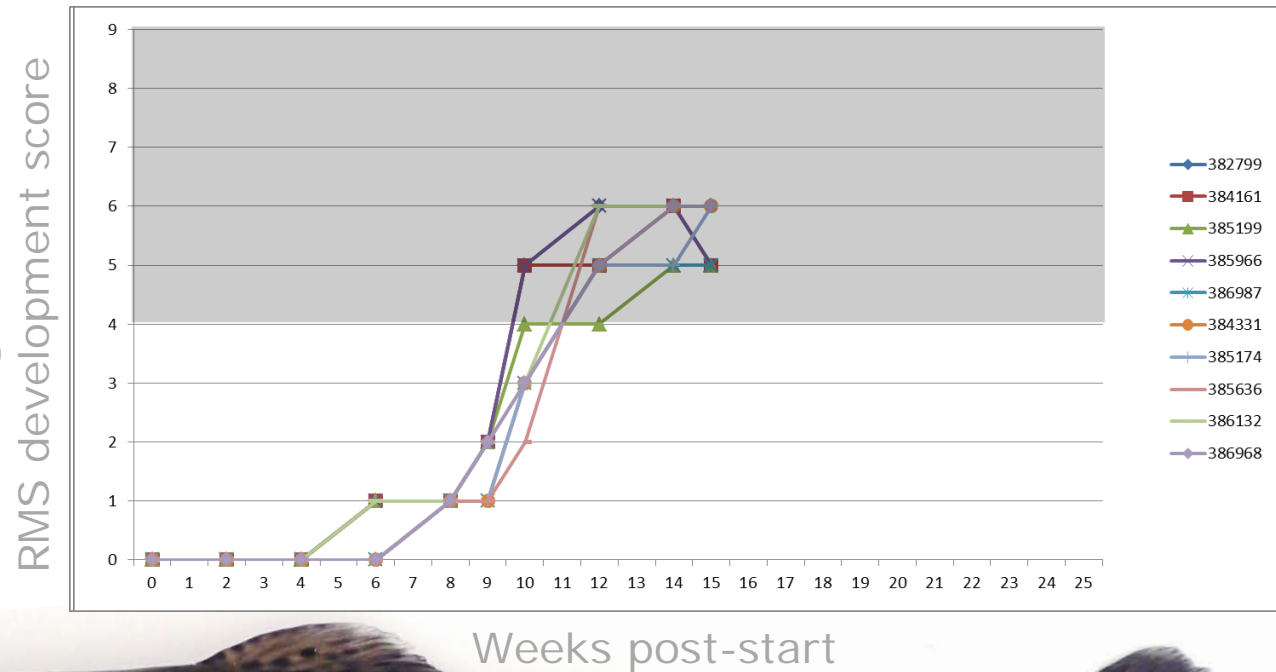
Problem and background

Recap from AW21

New and on-going

- Infection pressure:
 - Differences in disease manifestation
 - Timing and development of RMS lesions

Direct cohabitation
(high infection pressure)



Problem and background

Recap from AW21

New and on-going

- Infection pressure:
 - Differences in disease manifestation
 - Numbers and severity of lesions



Problem and background

Recap from AW21

New and on-going

- Infection pressure:
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We have implemented a diagnostic tool to detect MLO (modified qPCR from Cafiso et al 2016)

Thus we can now evaluate RMS by

- visual disease manifestation through image capture
- as well as by measuring MLO 16S rDNA copies in tissue samples.

We applied these two methodologies to a cohabitation experiment where we detected MLO in different tissues over the course of infection.



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In a similar experiment we investigated the development of the immune response in RMS skin lesions.

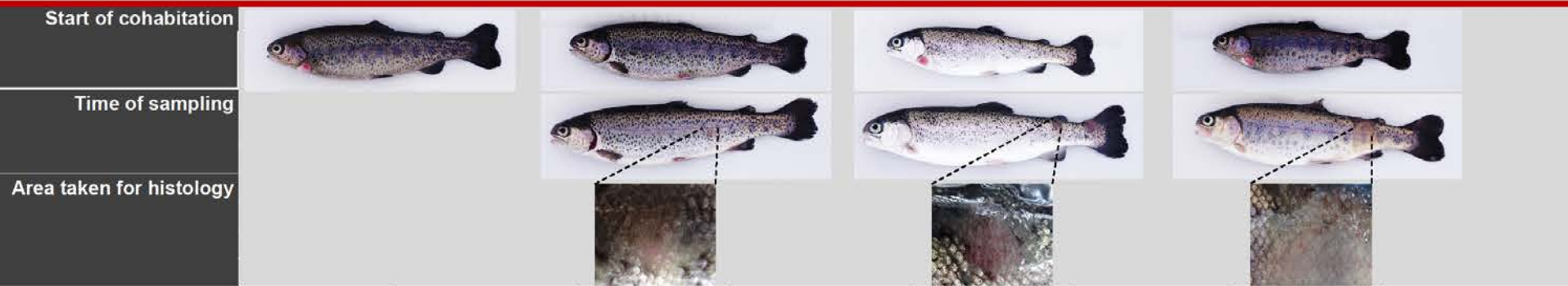
We looked at the expression of an array of immune genes by qPCR as well as presence of CD8, MHCII and the three immunoglobulin isotypes by IHC.

To much info to go into here, but one thing that was quite noticeable was the three immunoglobulin isotypes, which were all increased in RMS lesions – expression and IHC.



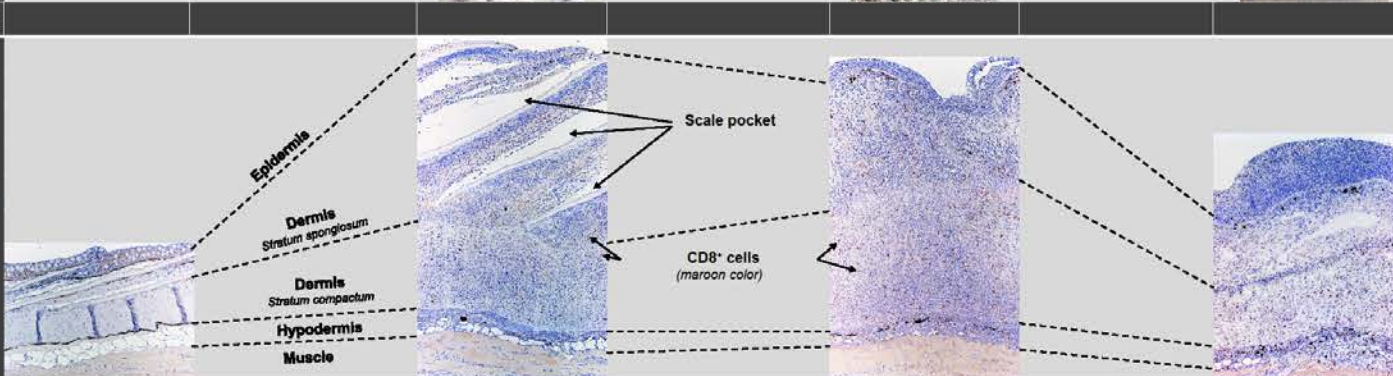
Immunohistochemical staining of RMS-affected skin sections for CD8 and IgM

Methodology: We established a direct cohabitation infection model of RMS. We followed fish individually as the lesions developed. At regular intervals we acquired digital images of the fish (presented above), and sacrificed some fish for sampling. We investigated the histopathology with H&E stains as well as immunohistochemistry with MAbs raised against CD8, MHCII, IgM and IgT (only IgM and CD8 is presented below).



CD8 staining

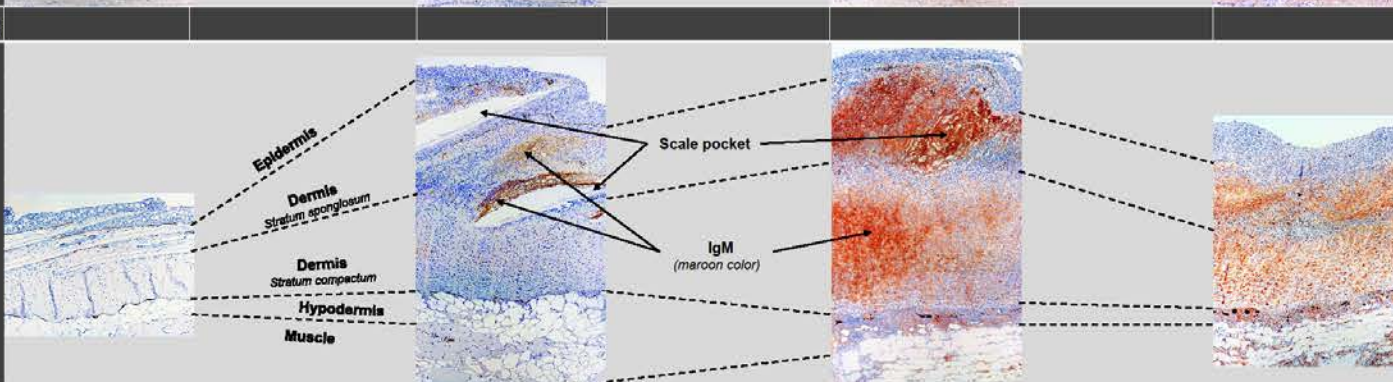
In control tissue CD8+ cells are rarely observed below the stratum spongiosum. Conversely CD8+ cells are very common in skin and underlying muscle of RMS lesions, and are fairly evenly distributed between the epidermis and muscle.



Histologically the most obvious feature of RMS-related skin change is extensive infiltration of cells initially into the stratum spongiosum, and later into the stratum compactum, the hypodermis and ultimately into the muscle. Scales are usually resorbed at the center of the lesion. The epidermis often appears surprisingly unaffected except in severe lesions.

IgM staining

The timing of lesion development following cohabitation with seeder fish hints at an adaptive immune response being mounted towards the infectious agent. The IHC shows a large increase in the presence of secreted IgM in RMS lesions. This is first observed in the scale pockets of the stratum spongiosum, and as the lesion develops it extends into the stratum compactum and to some extent into the muscle.



The presented work was supported by:

Henrik Henriksens fond and The European Maritime and Fisheries Fund

See European Union

RMS lesion score	0	2-3	4-5	6
Short description	Control skin	Swelling and haemorrhaging. Scale resorption starting in centre of lesion	RMS symptoms peaking or early resolution	Lesion resolving. Swelling reduced, brown hue.
Days post-start	0	61	82	97
Degree-days post-start	0	730	980	1160

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Thanks. And remember the EURL fish website!

