



Winter Ulcer Disease



Photo: Brit Tørud



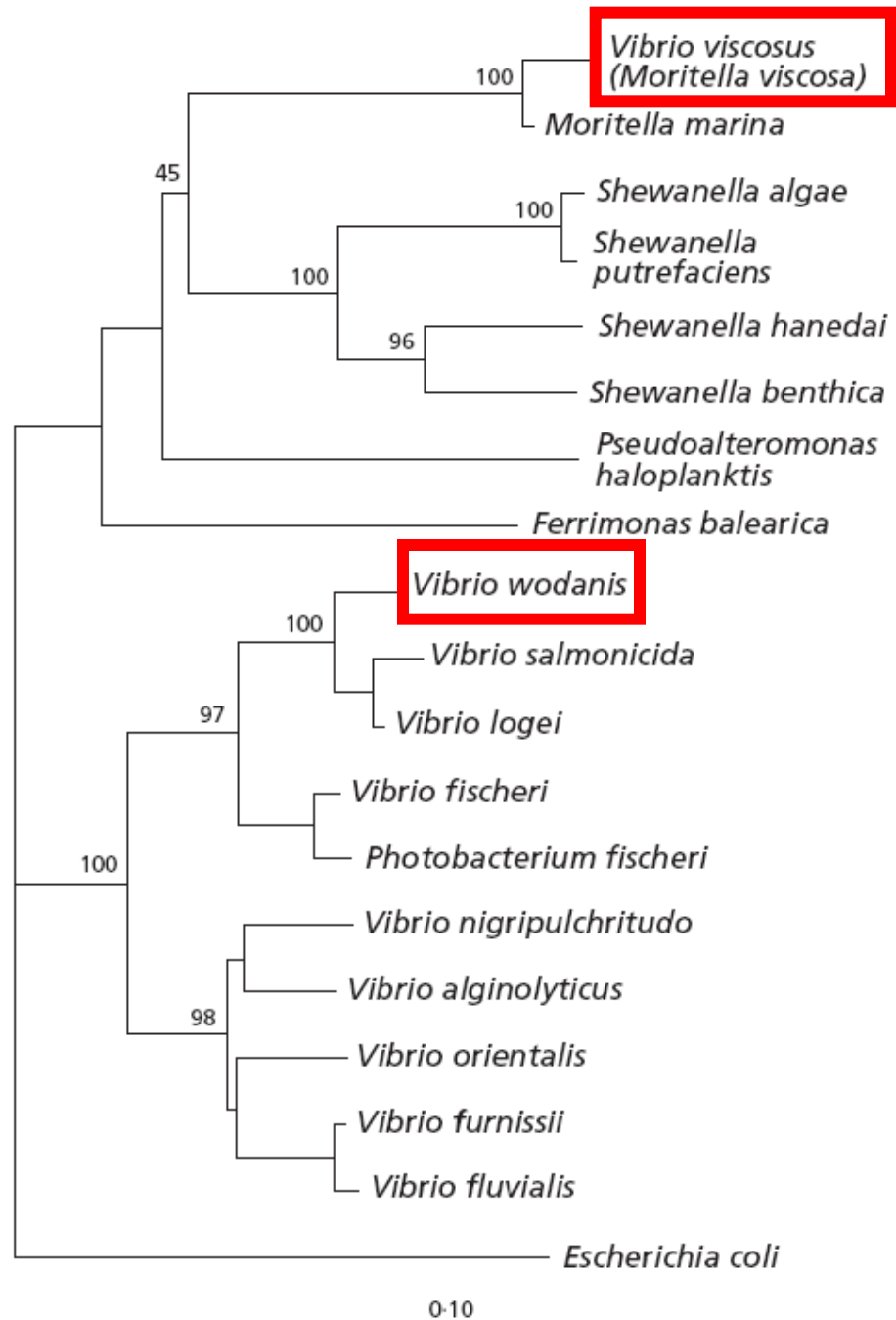
Aetiology

- ***Moritella viscosa***
 - Previously classified as *Vibrio viscosus*
 - Most closely related to *Moritella marina* (prev. *Vibrio marinus*)
- ***Aliivibrio (Vibrio) wodanis***
 - Named after the Norse god Wodan (as in Wednesday) = Odin (as in onsdag)
 - Most closely related to *V. logei*
 - Norse god Loge = Loke, Odin's blood brother
 - Heterogeneous both genetic and phylogenetic

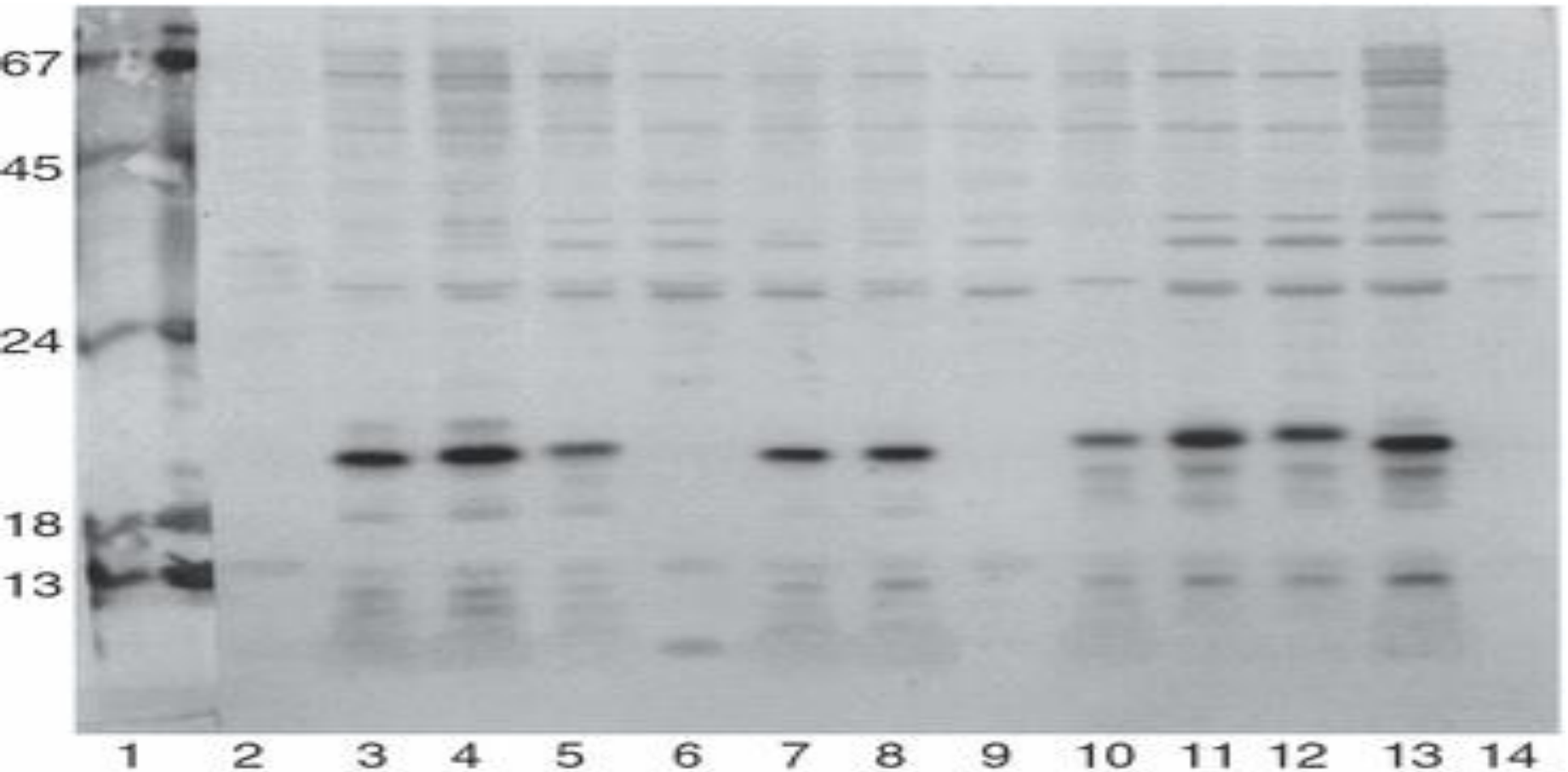


Phylogenetic tree

Lunder et al. 2000
Benediktsdottir et al 2000



Antigen profiles of the fish pathogen *Moritella viscosa* and protection in fish

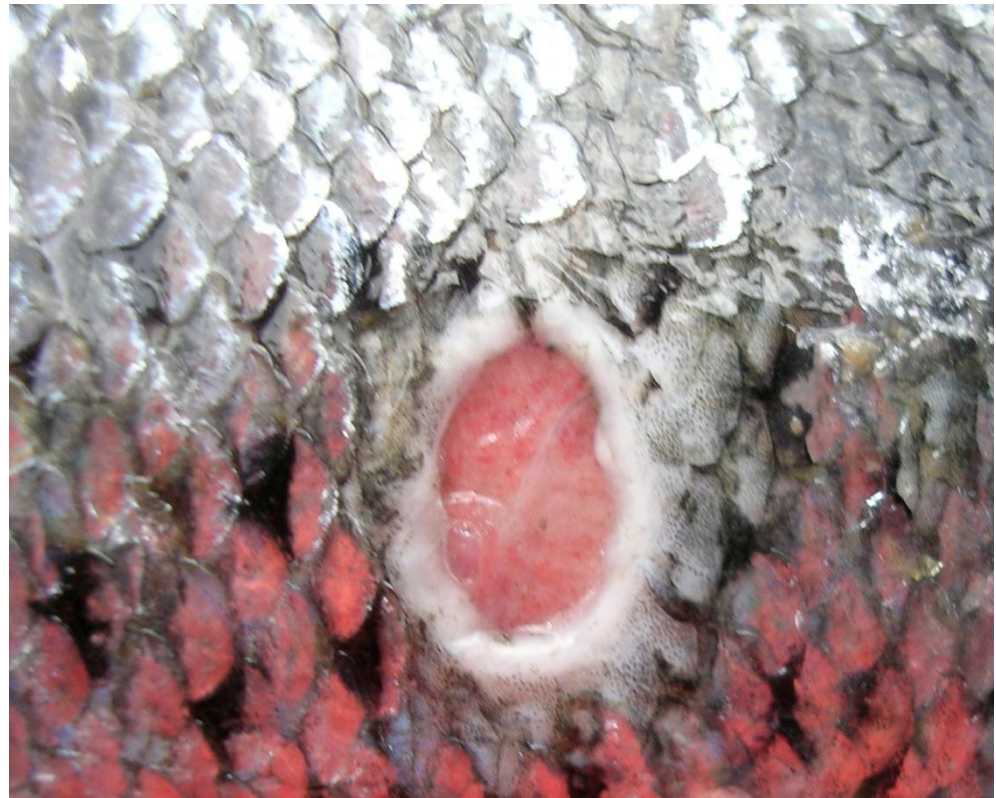




No adequate treatment

- **Antibiotics are not sustainable**
- **Almost all fish in Norway are vaccinated against *M. viscosa***
 - A bath challenge model for *M. viscosa* is established
 - Good protection in experimental studies
 - Poor protection in real life
- ***Aliivibrio (Vibrio) wodanis***
 - The bacteria is detected in most salmons with winter ulcers, but the significance is unknown

Ulcers open for additional infections, but normally only *M. viscosa* and *A. wodanis* are detected





Project:

The importance of bacterial interactions for winter ulcer and the potential use in control of fish diseases

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Prof. Lone Gram, Copenhagen
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Prof. Nils-Peder Willassen, Tromsø
Helene Mikkelsen, Tromsø
Bereket Tesfamichael, Oslo





Lunder et al. 1995

Table 2. Bacteria isolated from skin ulcers and/or kidneys in different individuals of *Salmo salar* from 8 different fish farms with winter ulcer

Bacteria	No. of fish farms	Fish with skin ulcers (n = 102)		Fish without skin ulcers (n = 67)
		Ulcers No. (%)	Kidney No. (%)	Kidney No. (%)
<i>Vibrio</i> sp. 1 ^a <i>Moritella viscosa</i>	1	7 (7)	13 (13)	3 (4)
<i>Vibrio</i> sp. 2 ^b <i>Vibrio wodanis</i>	2	34 (33)	35 (34)	6 (9)
Both <i>Vibrio</i> sp. 1 and <i>Vibrio</i> sp. 2 ^c	5	60 (59)	29 (28)	0
Bacteria other than <i>Vibrio</i> sp. 1 and <i>Vibrio</i> sp. 2	0	0	0	4 (6)
No bacteria	0	1 (1)	25 (25)	54 (81)

^aIsolated in pure culture or mixed with bacteria other than *Vibrio* sp. 2
^bIsolated in pure culture or mixed with bacteria other than *Vibrio* sp. 1
^cBoth vibrios present, sometimes mixed with other bacteria

Ulcers mainly on the side of the fish, mechanical factor?

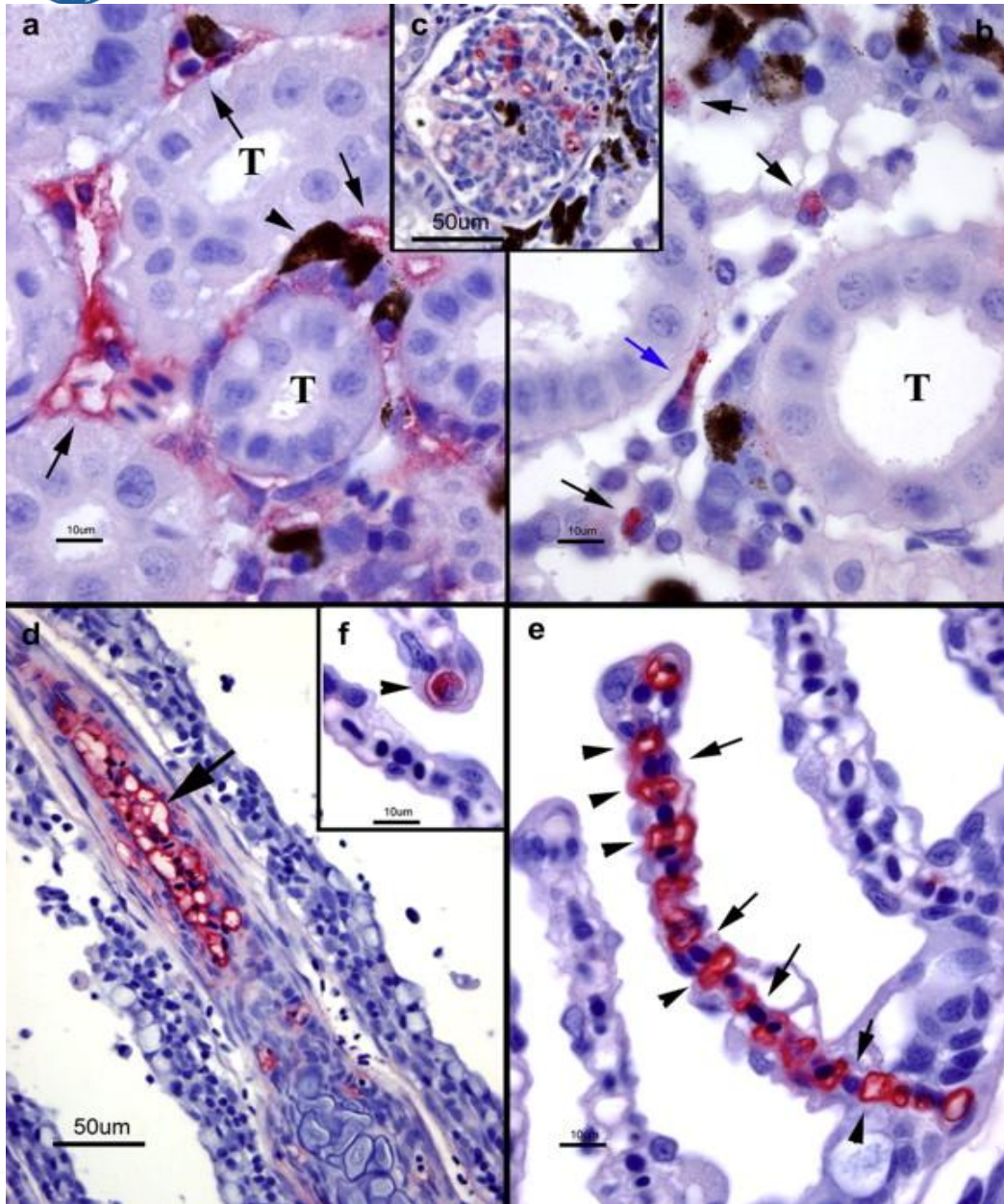


Photo: Brit Tørud

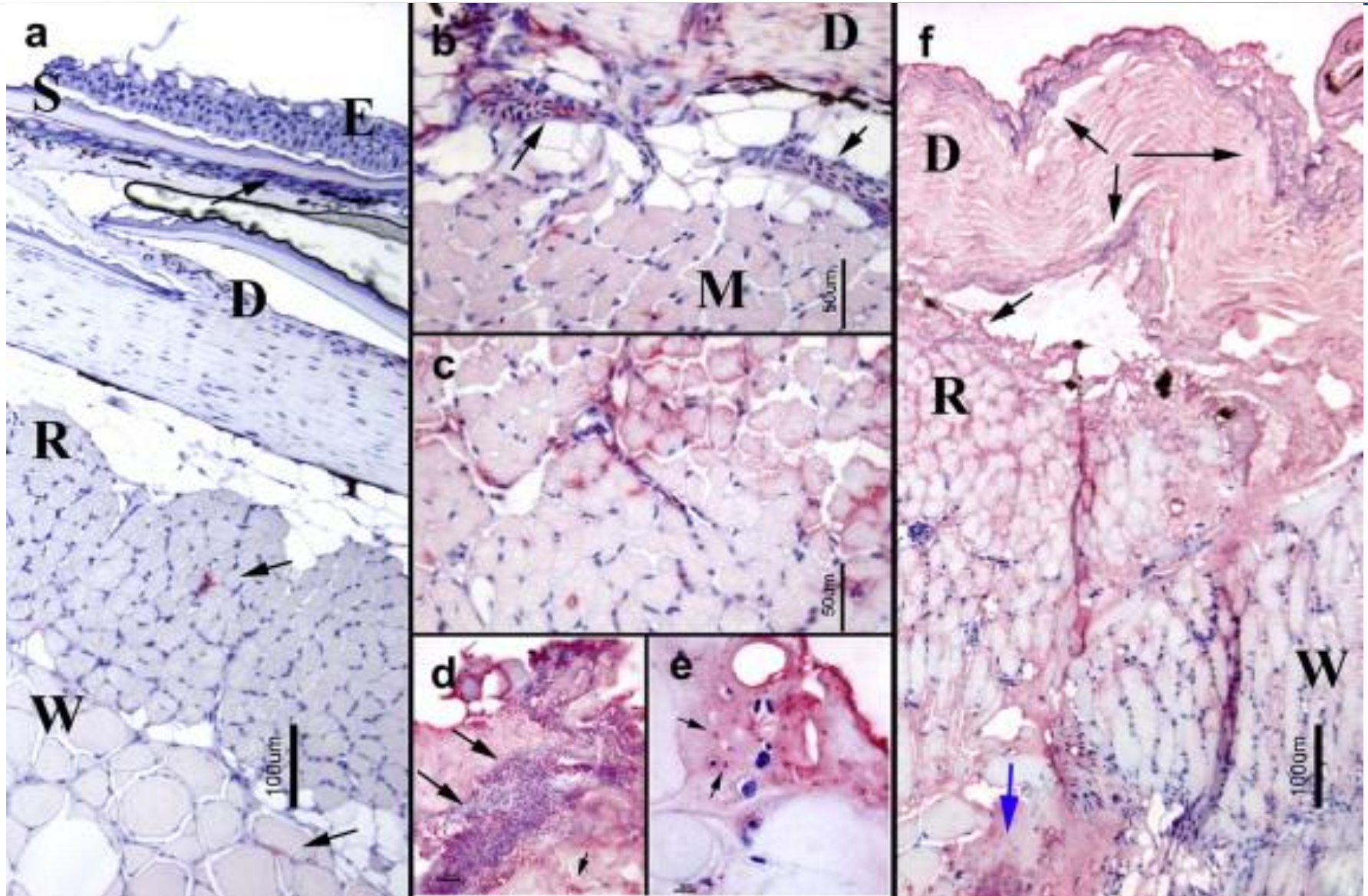


Pathology

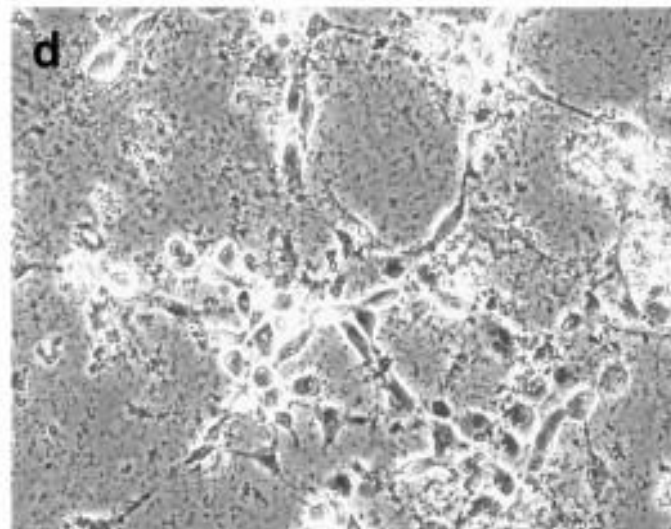
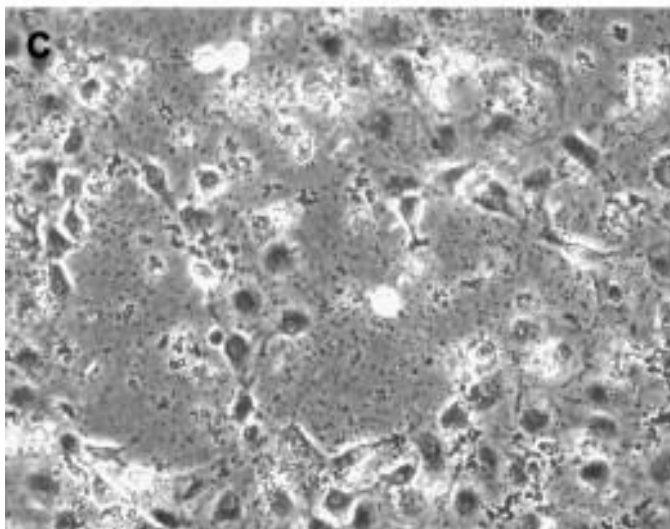
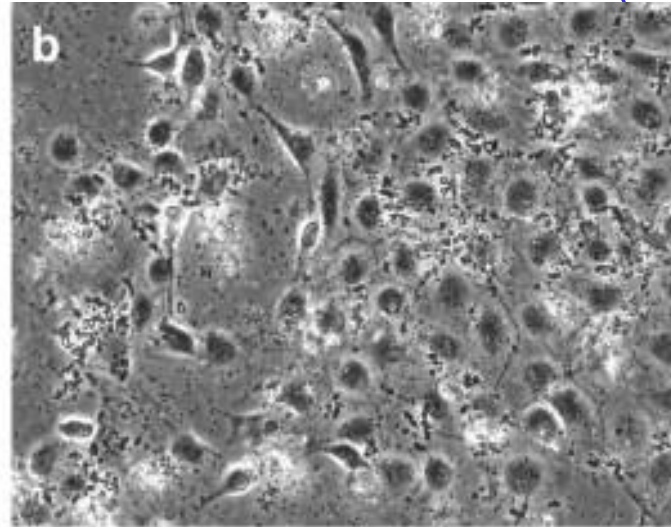
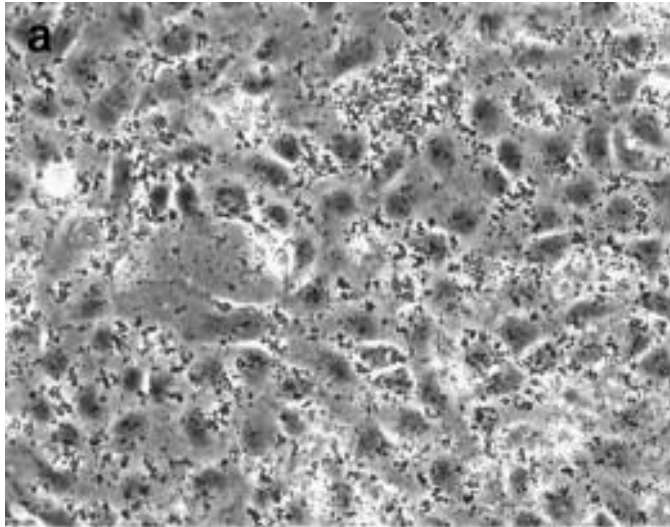
- **Little is known of the pathogenesis**
- **Ulcers**
- **Septicaemia**
 - Signs of circulatory failure like fluid in the abdominal cavity
 - Petechial haemorrhages on intestinal organs etc.
 - Detection of bacteria both in kidney and ulcers
- **Possible to find bacterial septicaemia in fish with no ulcers, but most of the diseased fish has ulcers**



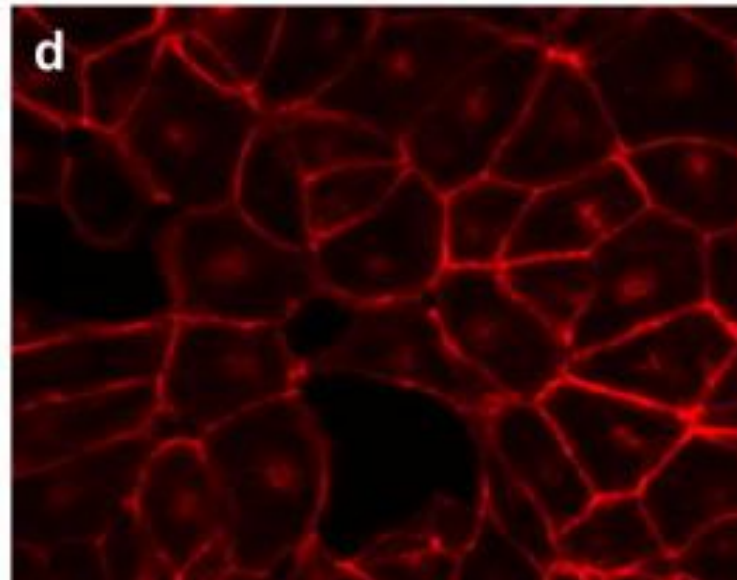
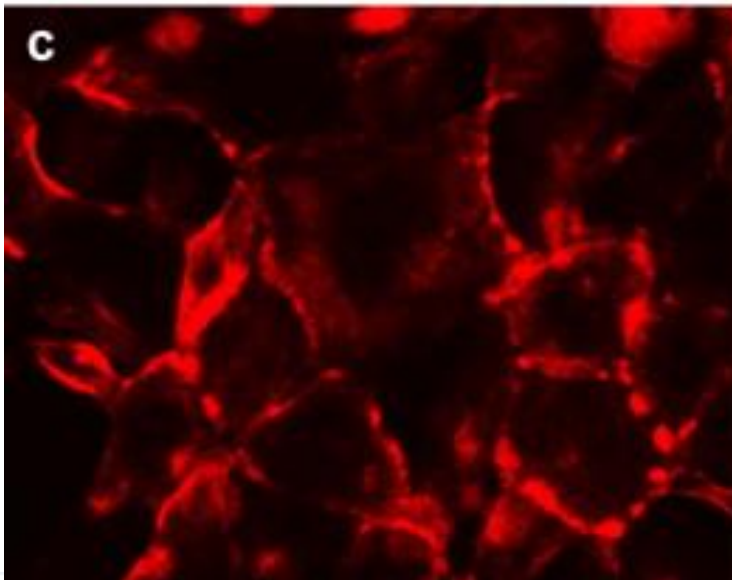
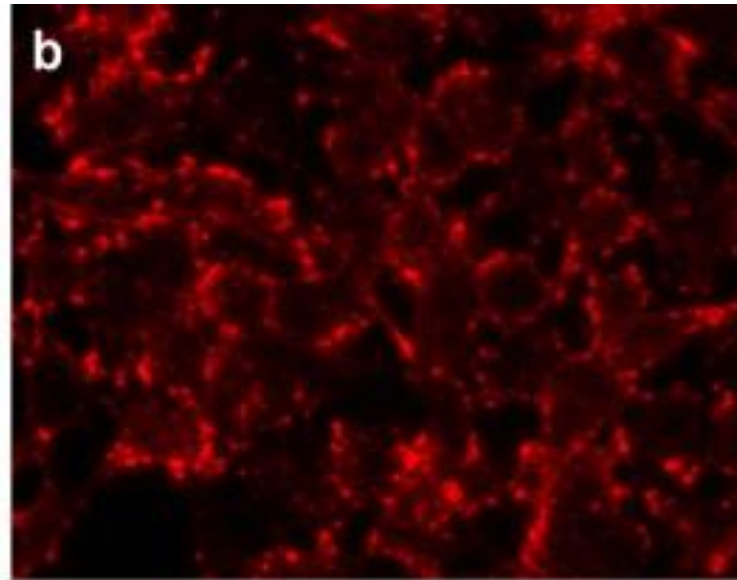
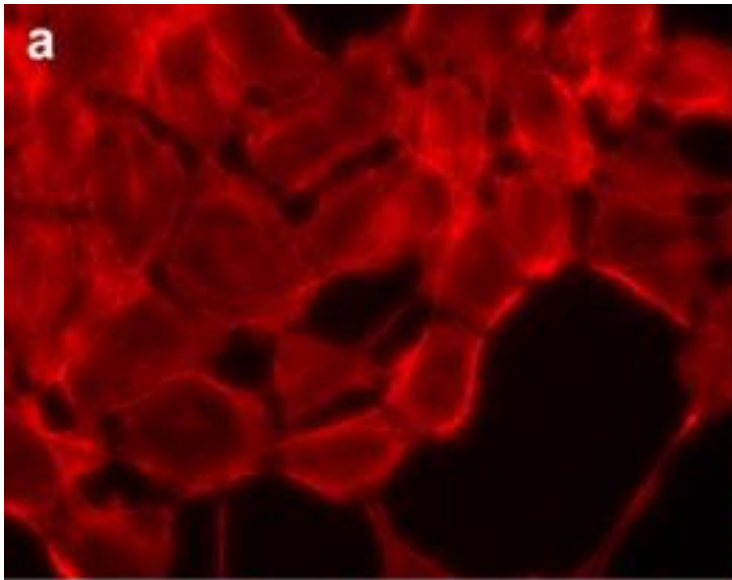
Immunohistochemistry of Atlantic salmon (50 g) challenged with *M. viscosa*



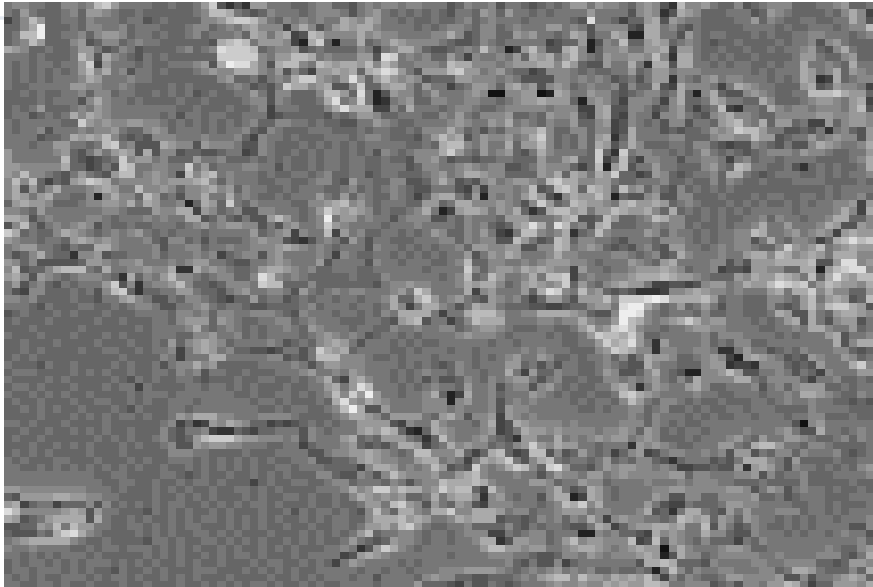
Cell culture infection with *M. viscosa* (CHSE cells)



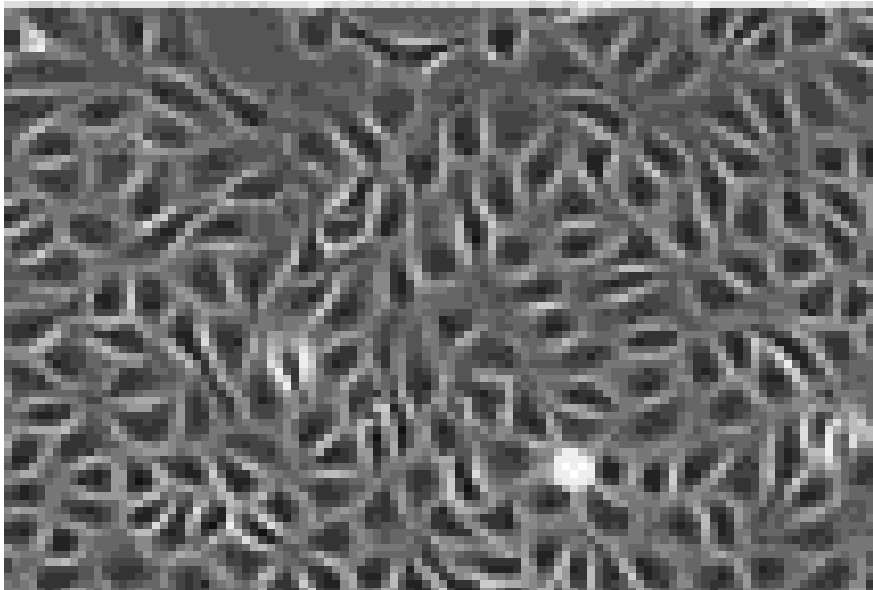
- a. 2h
- b. 4h
- c. 8h
- d. 14h



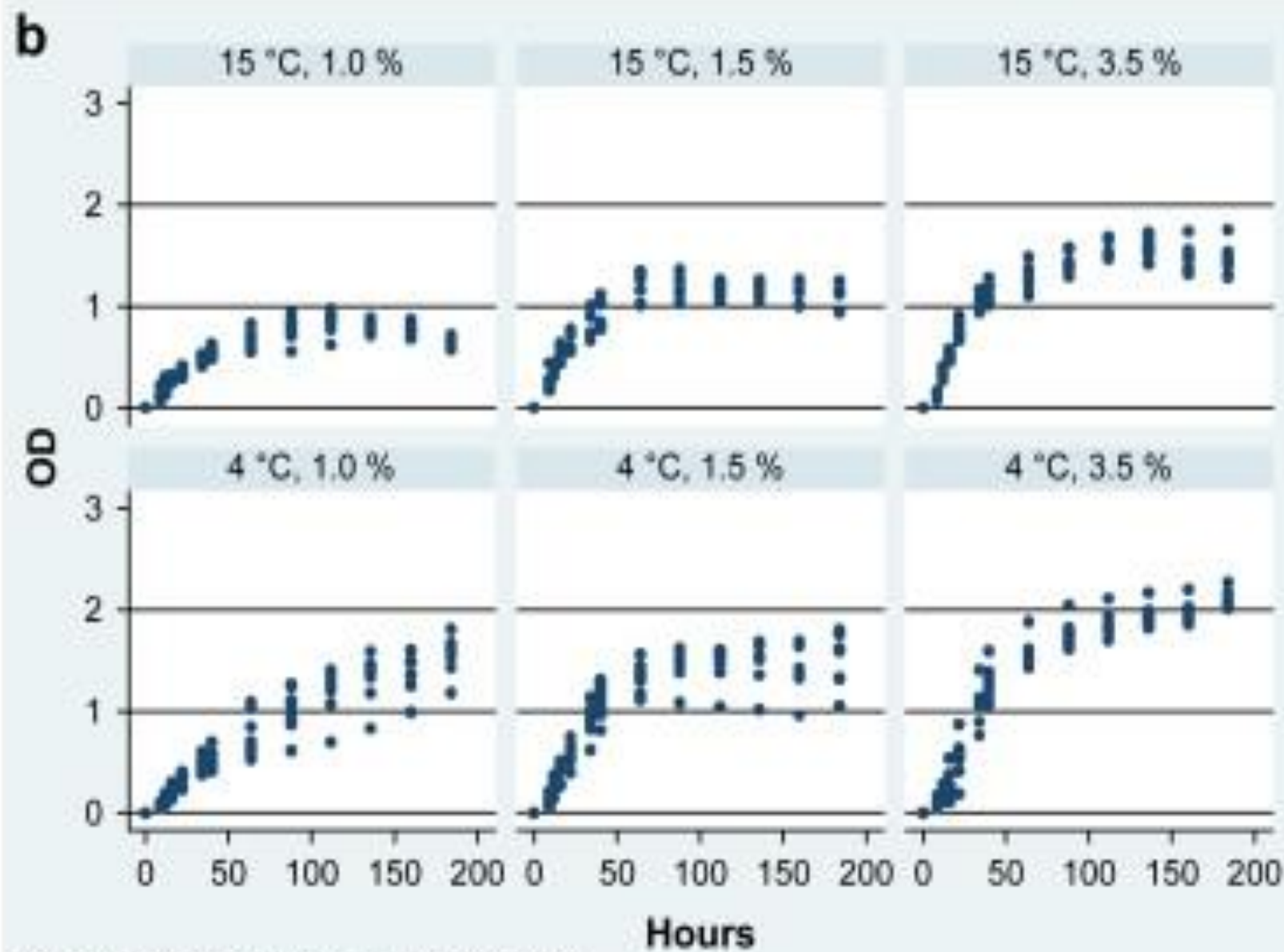
- a. Control
- b. 1h
- c. 5h
- d. 7h



CHSE cells 30 min after
addition of culture
supernatant of *M. viscosa*



Control



Graphs by Temperature and NaCl (%)



Toxins, secretion systems and colonization factors found in *M. viscosa* (Hege smith Tunsjø, MP)

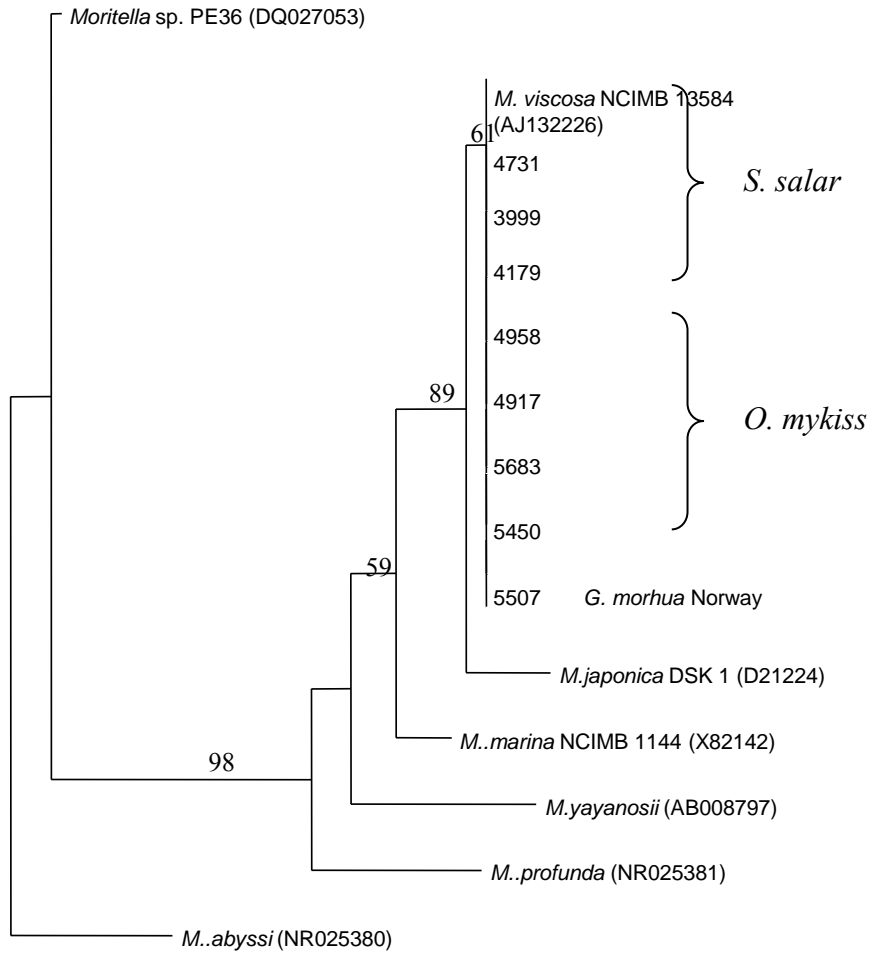
	<i>M. viscosa</i> T ^{88/478} (NVI/ScanVacc)
Exotoxins and degrading enzymes	Hemolysins RTX Proteases Phospholipase Lipases CNF Insecticidal toxin
Secretion systems	Type I Type II Type VI
Colonization factors	Polar flagella Lateral flagella Type IV pili



Atlantic salmon bath challenged with *Moritella viscosa* – Pathogen invasion and host response

M. Løvoll^{a,*}, C.R. Wiik-Nielsen^a, H.S. Tunsjø^b, D. Colquhoun^a, T. Lunder^c, H. Sørum^b, S. Grove^a

- PCR analysis suggested gills as the port of entry as DNA from *M. viscosa* was consistently detected from an early time point compared to muscle, skin and intestine samples.



— 0.001 substitutions/site

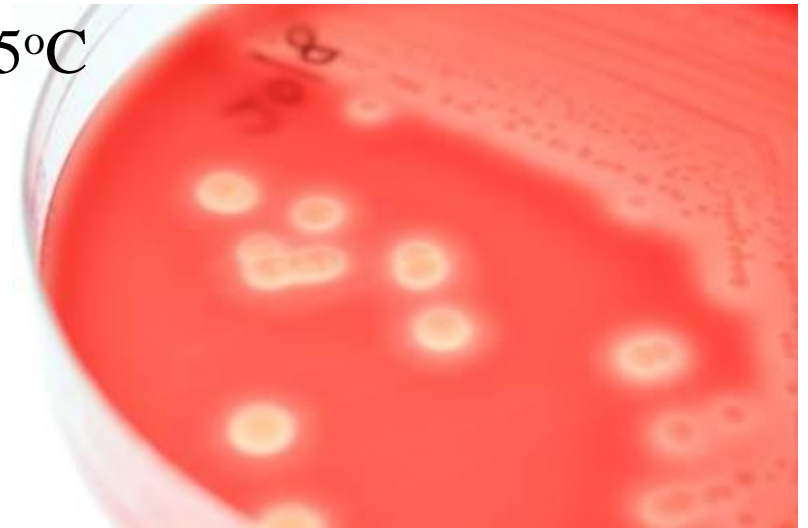
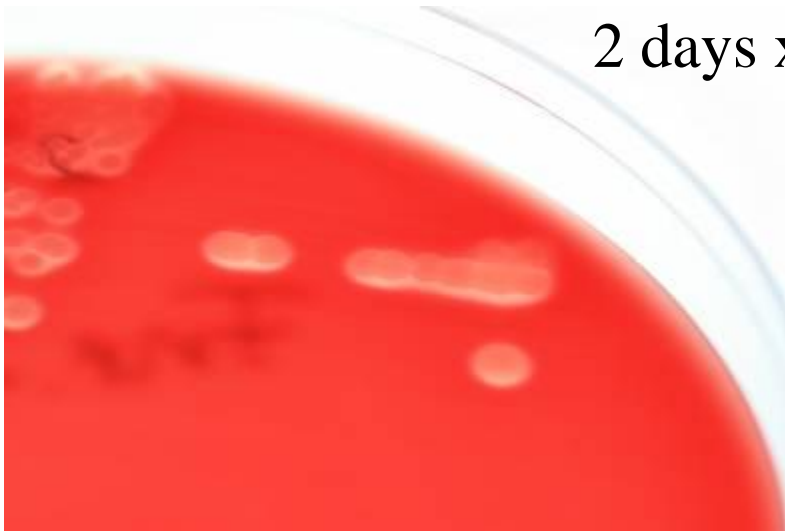


Norwegian School of Veterinary Science

”typical” from Atlantic salmon ”atypical” from rainbow trout

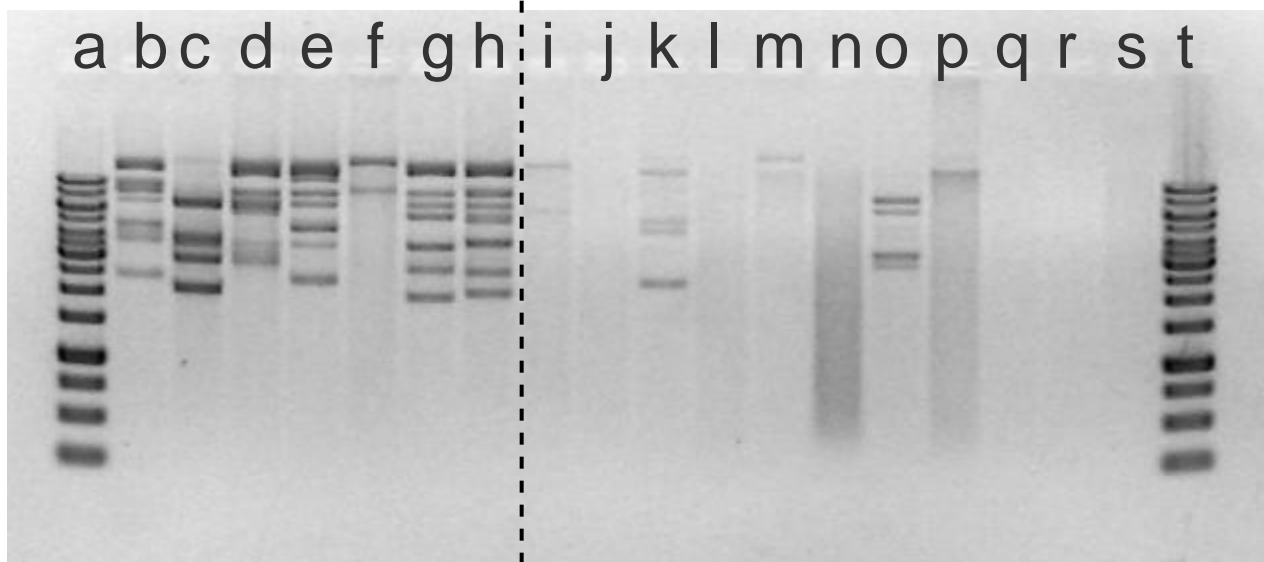


2 days x 15°C

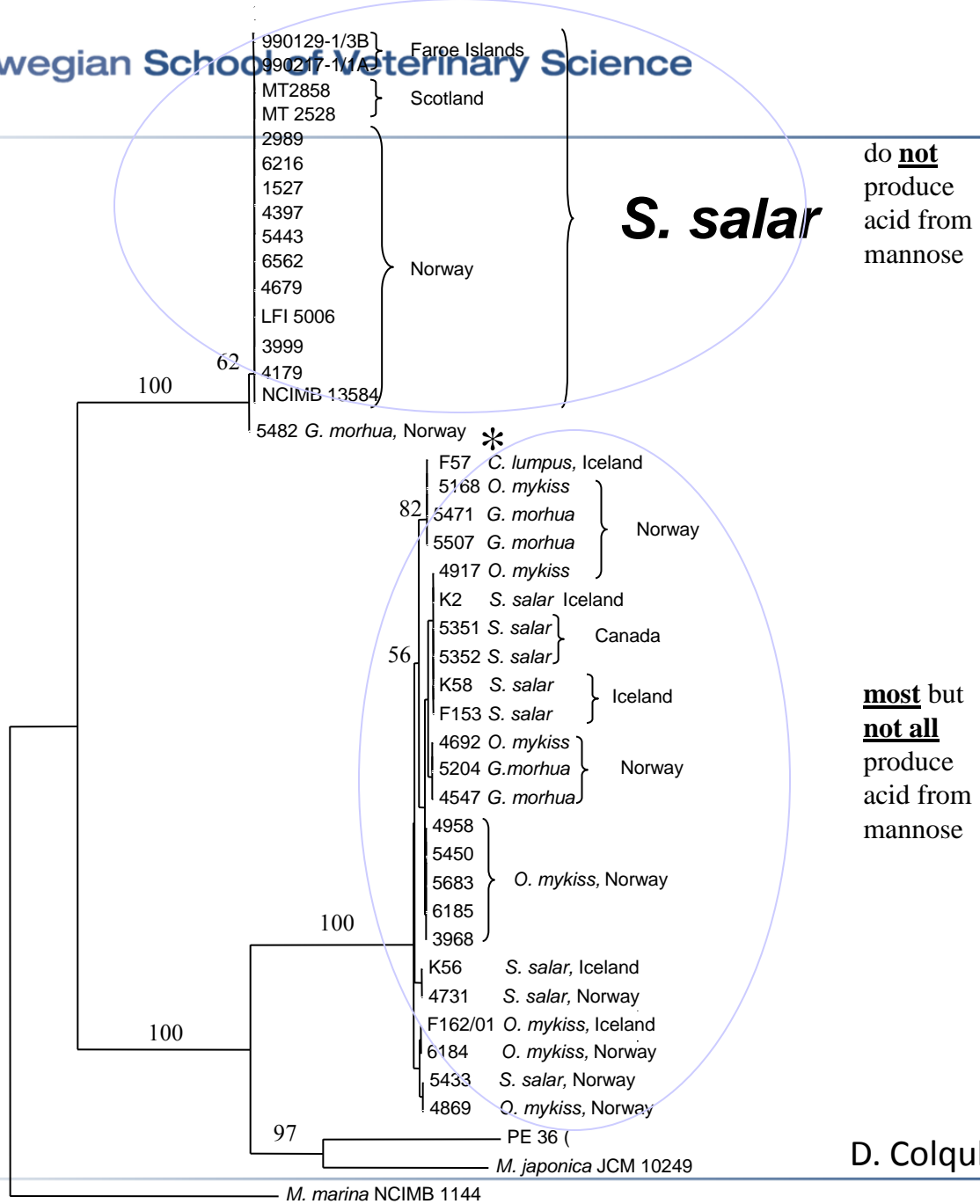


5 days x 15°C

D. Colquhoun, Veterinærinstituttet



- S. salar, Norway
- S. salar, Norway
- S. salar, Scotland
- S. salar, Scotland
- G. morhua, Norway
- S. salar, Faroe Islands
- S. salar, Faroe Islands
- S. salar, Canada
- S. salar, Canada
- S. salar, Iceland
- S. salar, Iceland
- C. lumpus, Iceland
- O. mykiss, Iceland
- O. mykiss, Norway
- O. mykiss, Iceland
- G. morhua, Norway
- M. japonica, JCM 10249
- M. marina, NCIMB 1144



— 0.005 substitutions/site



dnaJ



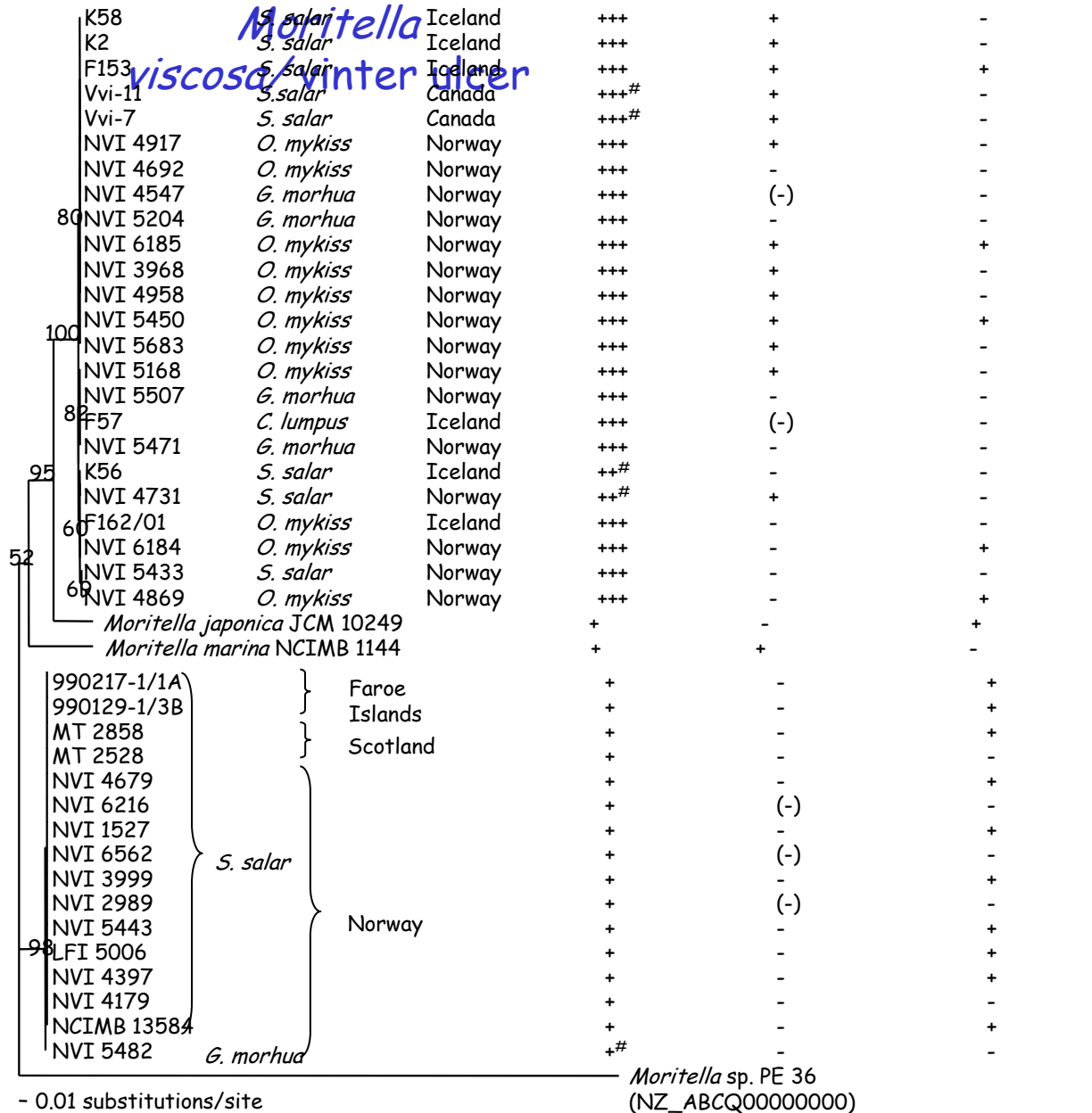
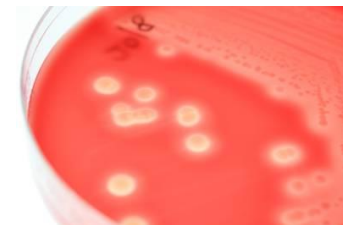
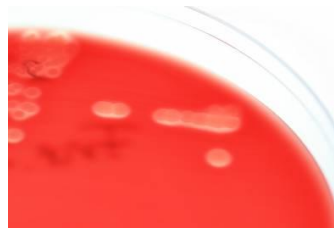
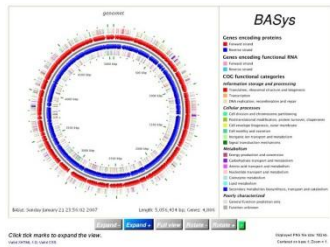
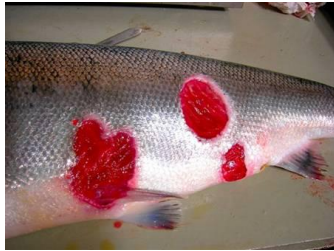
- 5482 DnaJ complete
- 6184 DnaJ complete
- 6224 DnaJ complete
- 3968 DnaJ complete
- 4917 DnaJ complete
- 4958 DnaJ complete
- 5351 DnaJ complete
- 5352 DnaJ complete
- 5450 DnaJ complete
- 5683 DnaJ complete
- 6185 DnaJ complete
- 6219 DnaJ complete
- 6220 DnaJ complete
- 6221 DnaJ complete
- 6223 DnaJ complete
- 4869 DnaJ complete
- 1527 DnaJ complete
- 2033 DnaJ complete
- 2989 DnaJ complete
- 6222 DnaJ complete
- 4179 DnaJ complete
- 4397 DnaJ complete
- 4547 DnaJ complete
- 4679 DnaJ complete
- 4692 DnaJ complete
- 5168 DnaJ complete
- 5204 DnaJ complete
- 5443 DnaJ complete
- 6534 DnaJ complete
- 6539 DnaJ complete
- 6540 DnaJ complete
- 6562 DnaJ complete
- 6578 DnaJ complete
- 5507 DnaJ complete
- 5471 DnaJ complete
- 5433 DnaJ complete
- 3999 DnaJ complete

PE36 DnaJ RC

6010 dnaJ complete M.

Rainbow trout
Salmon (Iceland and Canada)
Cod

Salmon (Norway, Faroe Isles
Scotland)



- 0.01 substitutions/site

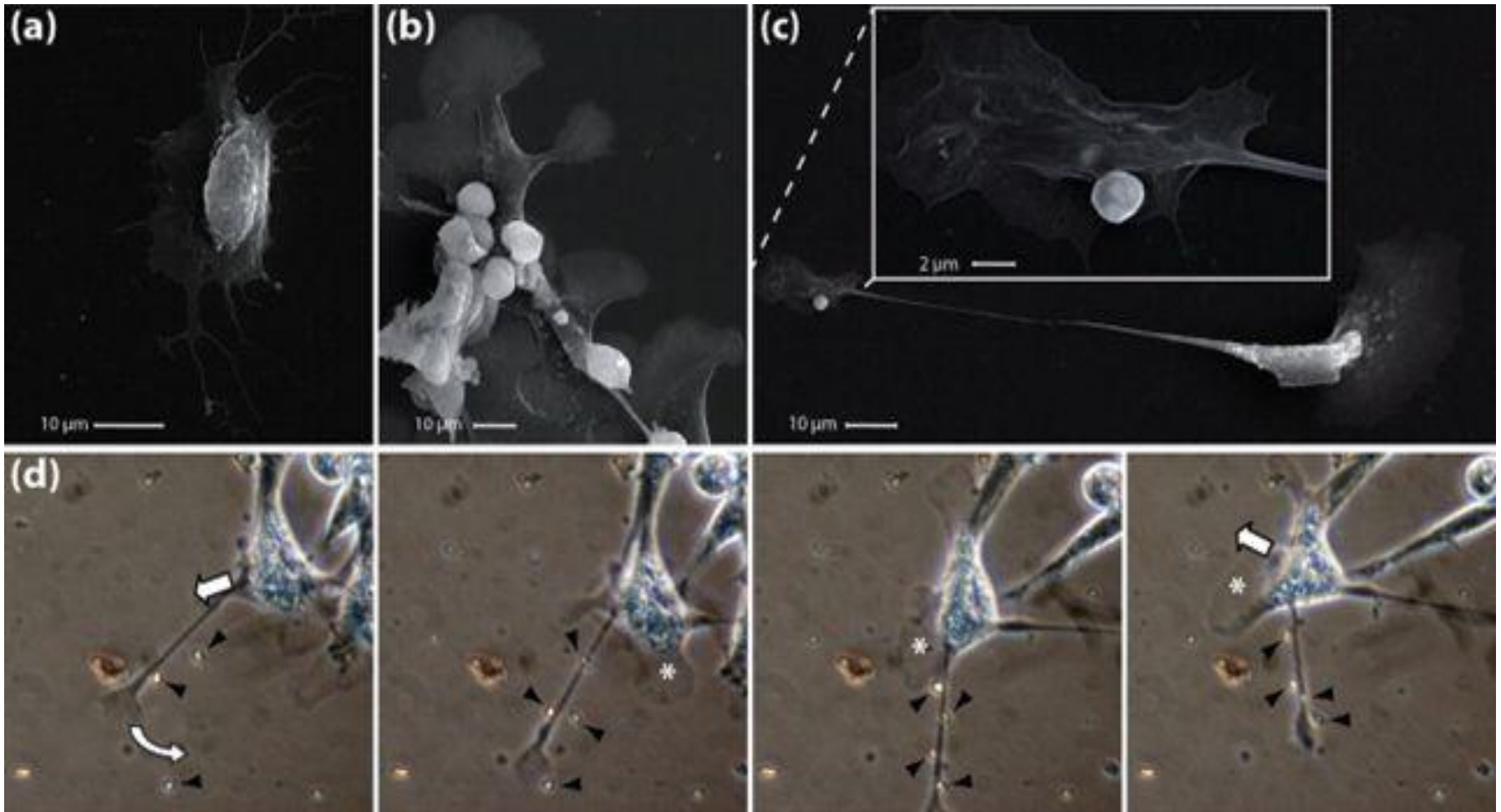
Grove S., Wiik-Nielsen C., Lunder T., Tunsjø Smith H., Reitan L.J., Martinussen A., Sørgaard M., Tandstad M., Olsen A.B. and Colquhoun D.J. (2010) Previously unrecognised division within *Moritella viscosa* isolated from fish farmed in the North Atlantic. *Diseases of Aquatic Organisms*. 93: 51-61.



Sanger sequencing of *Moritella viscosa* and *Vibrio wodanis*

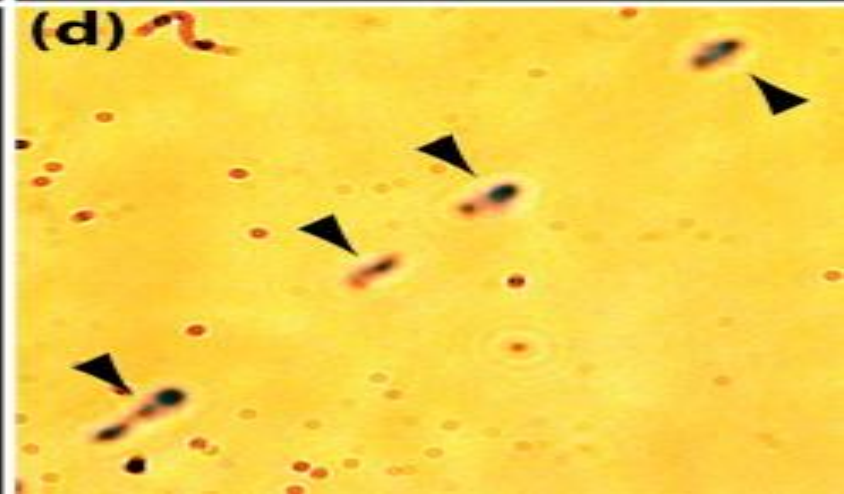
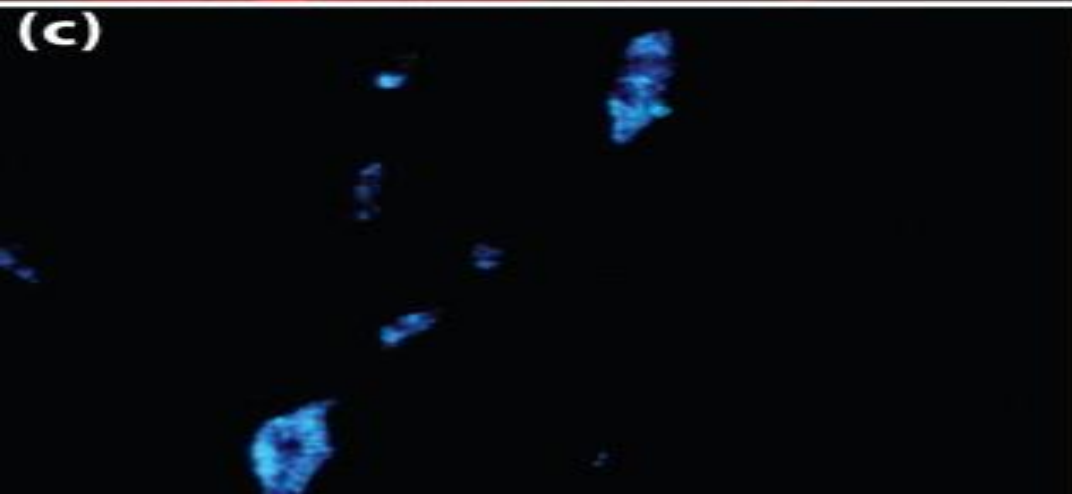
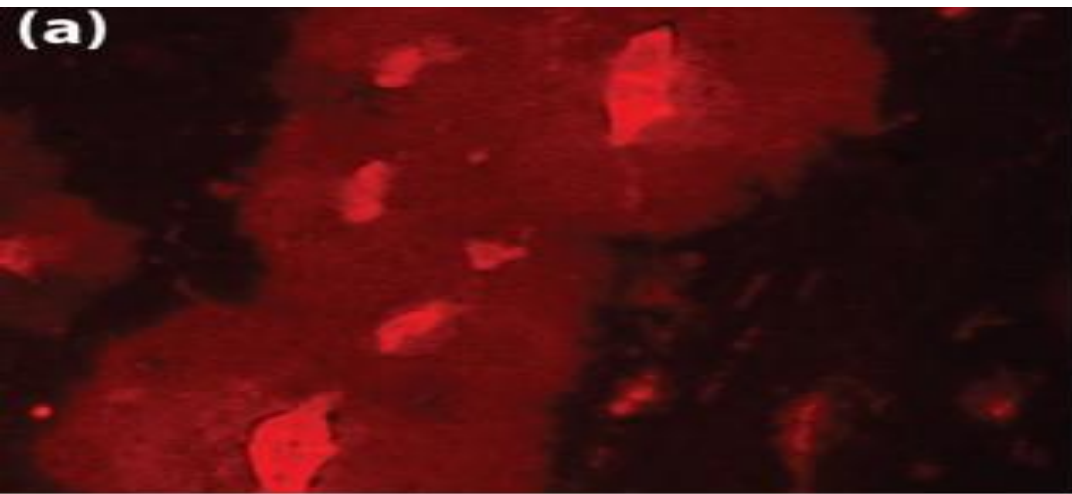
- Both isolates from the same kidney of one salmon that was diseased in an outbreak of Winter ulcer in 2006.
- Finalized 2012?

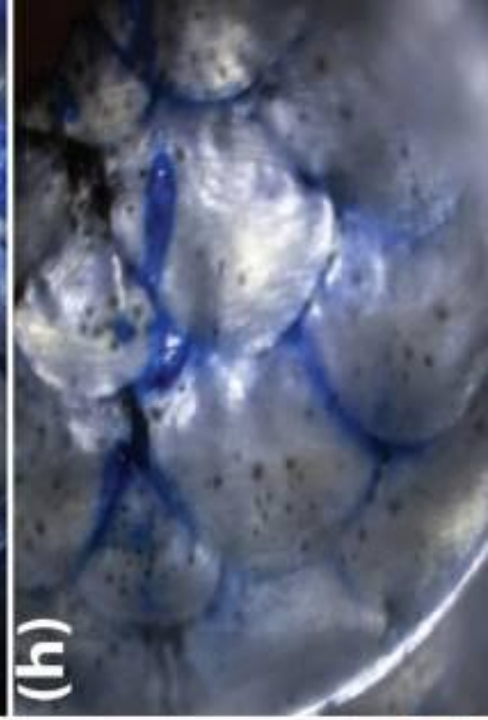
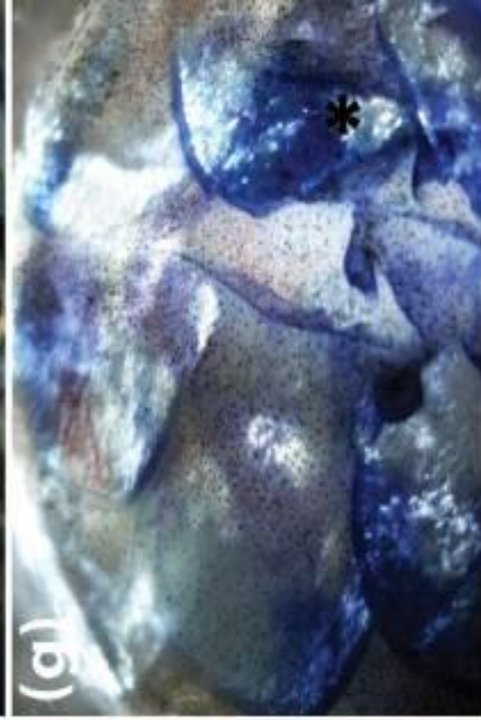
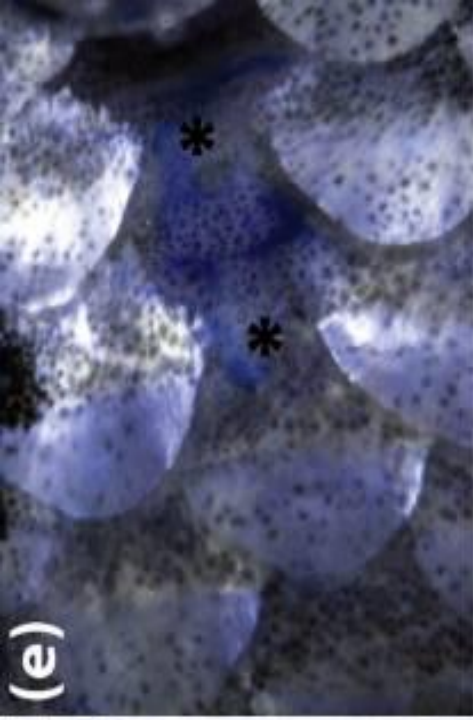
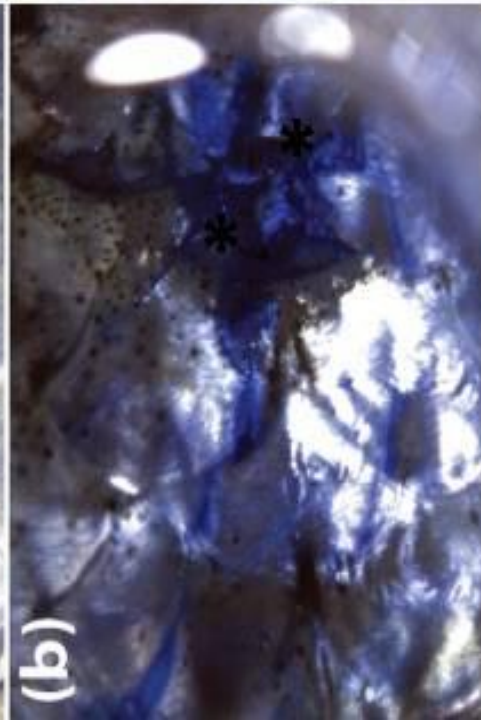
Keratocytes are cleaning and repairing the skin



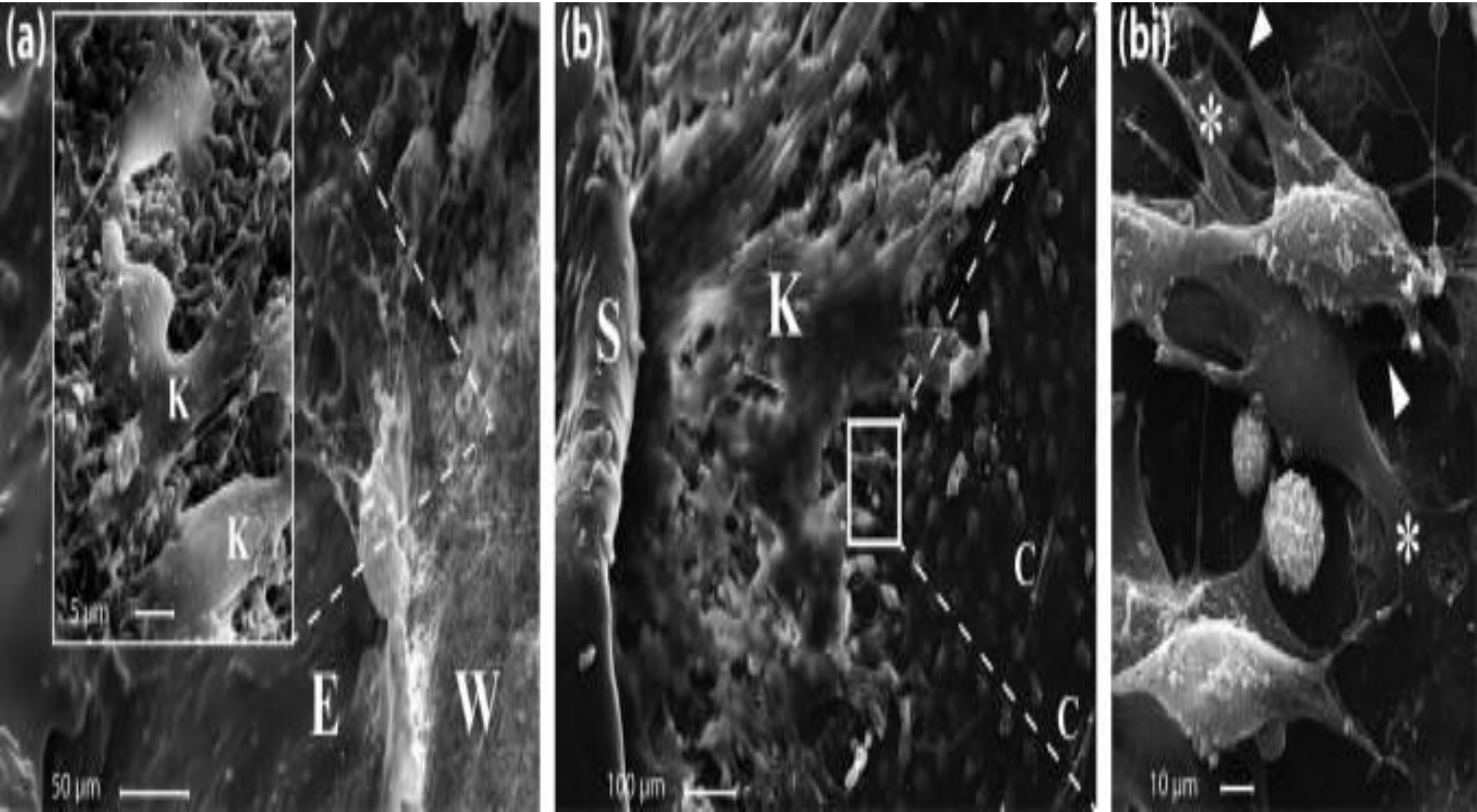
- [Karlsen et al 2012 Veterinary Microbiology Volume 154, Issues 3–4, 27 January 2012, Pages 353–362](#)

Moritella viscosa is excreting an immunogenic product





Scanning electron microscope of keratocytes





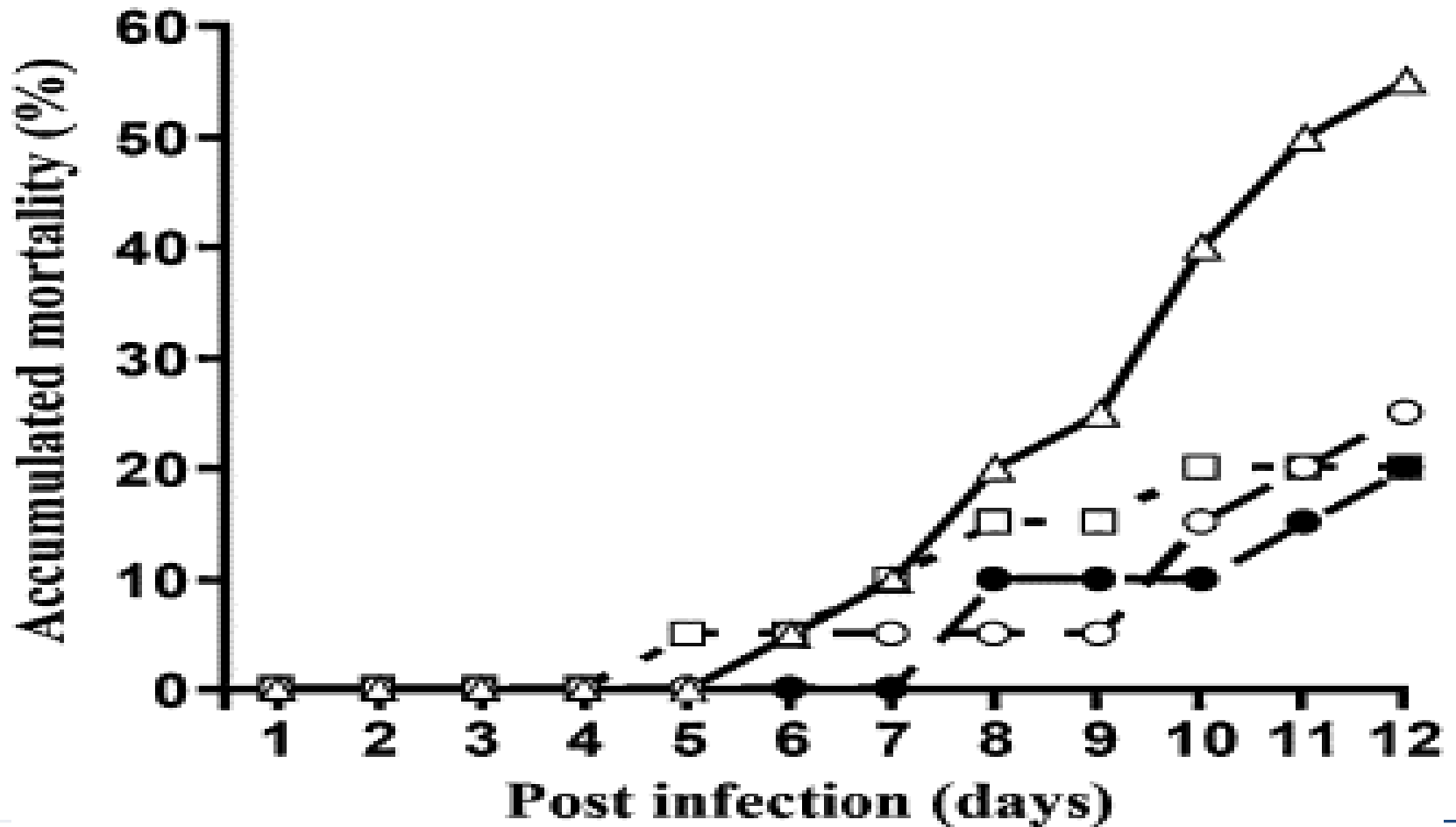
Port of infection

- For bacteria infecting fish little is known about the port(s) of infection
 - Assuming that gills and or intestine are important
 - For Winter ulcer a skin port should be expected.
 - Most ulcers along the side line of the fish
 - Is the side line a never discovered port of infection?
 - Other unattended ports like mucosa in the olfactory organ?
-



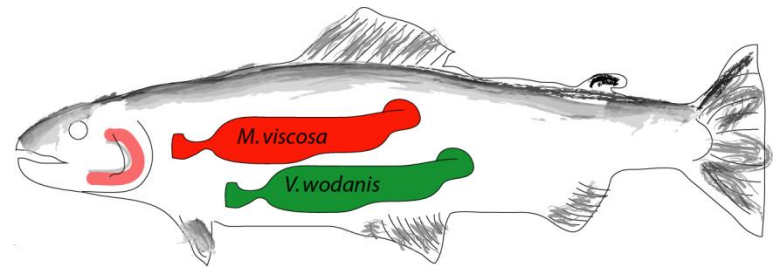
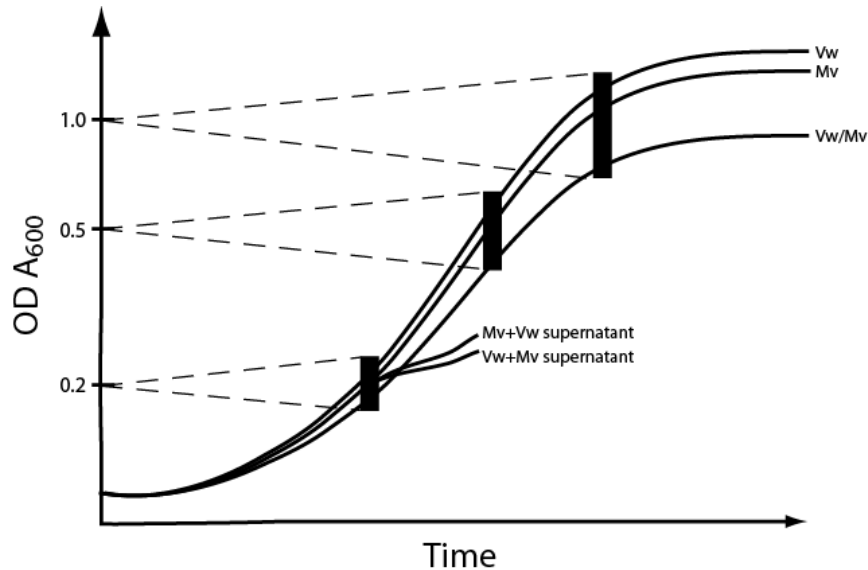
Port of infection, *Moritella viscosa*

Karlsen et al 2012





Co-cultivation of *M. viscosa* and *V. wodanis*



<i>In vivo</i>	<i>In vitro</i>
<i>M. viscosa</i>	<i>V. wodanis</i> (0.2, 0.5, 1.0)
<i>V. wodanis</i>	<i>M. viscosa</i> (0.2, 0.5, 1.0)
<i>M. viscosa</i> + <i>V. wodanis</i>	<i>V. wodanis</i> / <i>M. Viscosa</i> (0.5, 1.0)
<i>M. viscosa</i> / <i>V. wodanis</i>	<i>Vw</i> + <i>Mv</i> supernatant (~0.2)
	<i>Mv</i> + <i>Vw</i> supernatant (~0.2)



Coordinated Bacterial Virulence: Relevance in Winter ulcer.

- Funding 6,5 mill NOK for 3 years by FHF and NFR Aquaculture Program
- Objectives – Address several key virulence factors genome sequencing
- Knock-out mutations of putative virulence genes and testing in model systems and in the natural host
- Test the activity of the immune genes of the host as a response of the virulence factors
- High-throughput testing of mutants in cell cultures and bio-assays



Partners

- *National partners:*
- Associate Professor Hanne Winther-Larsen, UiOslo
- Senior Research Scientist Anne Tøndervik, SINTEF, Tr. Heim
- Professor Nils Peder Willassen, UiTromsø
- Professor Henning Sørum, NVH
- *International partners:*
- Professor Debra Milton, UiUmeå/Southern Res Inst, Alabama
- Professor Matthew Waldor, Harvard Medical School, Boston
- Senior Research Monica Hagedorn, Bern.Noct Inst, Hamburg
- Senior Research Sun Nyunt Whai, UiUmeå



To the funding bodies:

- Thank you for the trust, we will do our very best to reduce the winter ulcer losses by the knowledge gained previously combined with the novel approaches of COBACVIR!
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