Winter Ulcer Disease

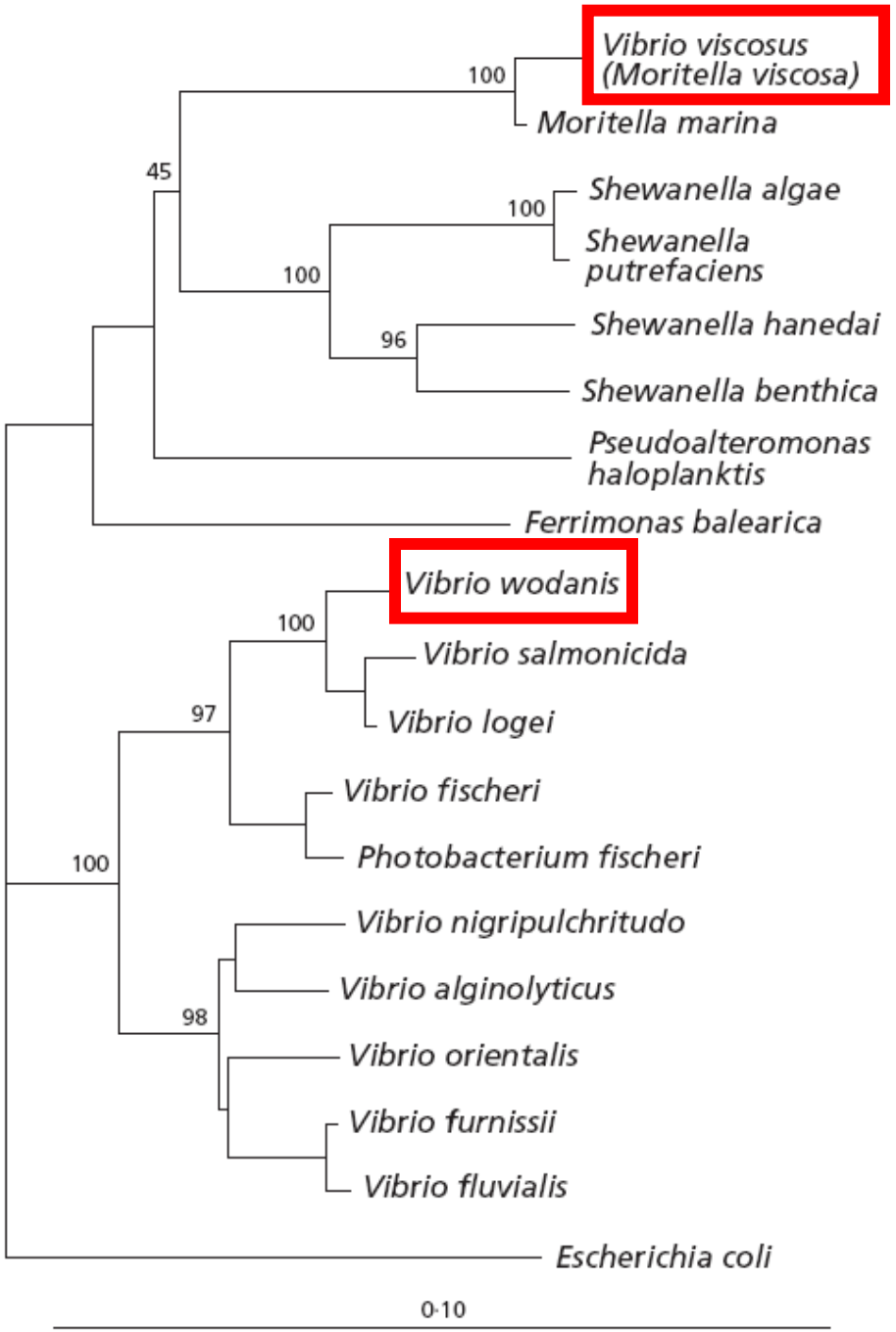
Photo: Brit Tørud

Aetiology

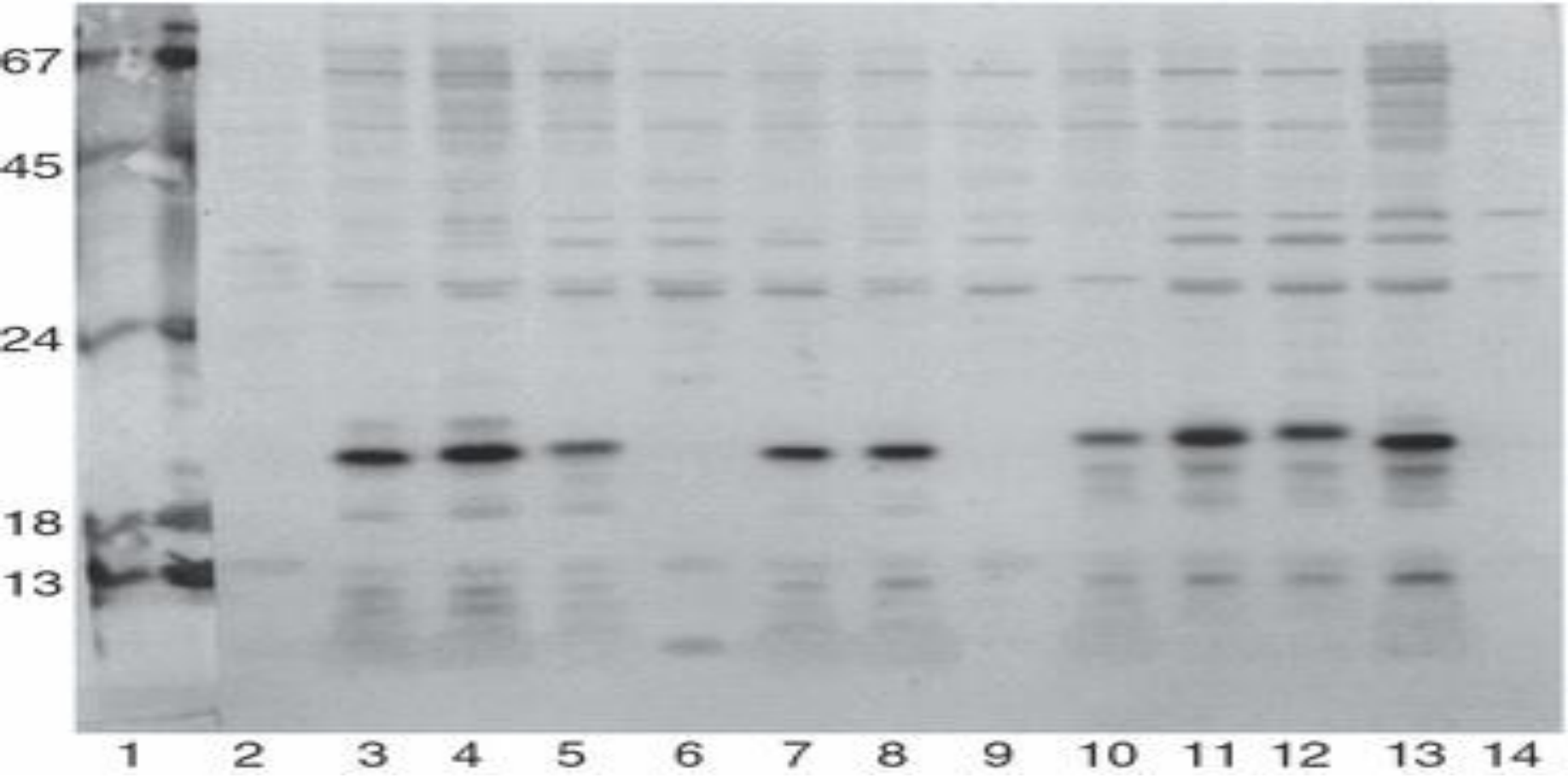
- ***Moritella viscosa***
  - Previously classified as *Vibrio viscosus*
  - Most closely related to *Moritella marina* (prev.*Vibrio marinus)*
- ***Aliivibrio* (*Vibrio*) *wodanis***
  - Named after the Norse god Wodan (as in Wednesday) = Odin (as in onsdag)
  - Most closely related to *V. logei*
    - 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



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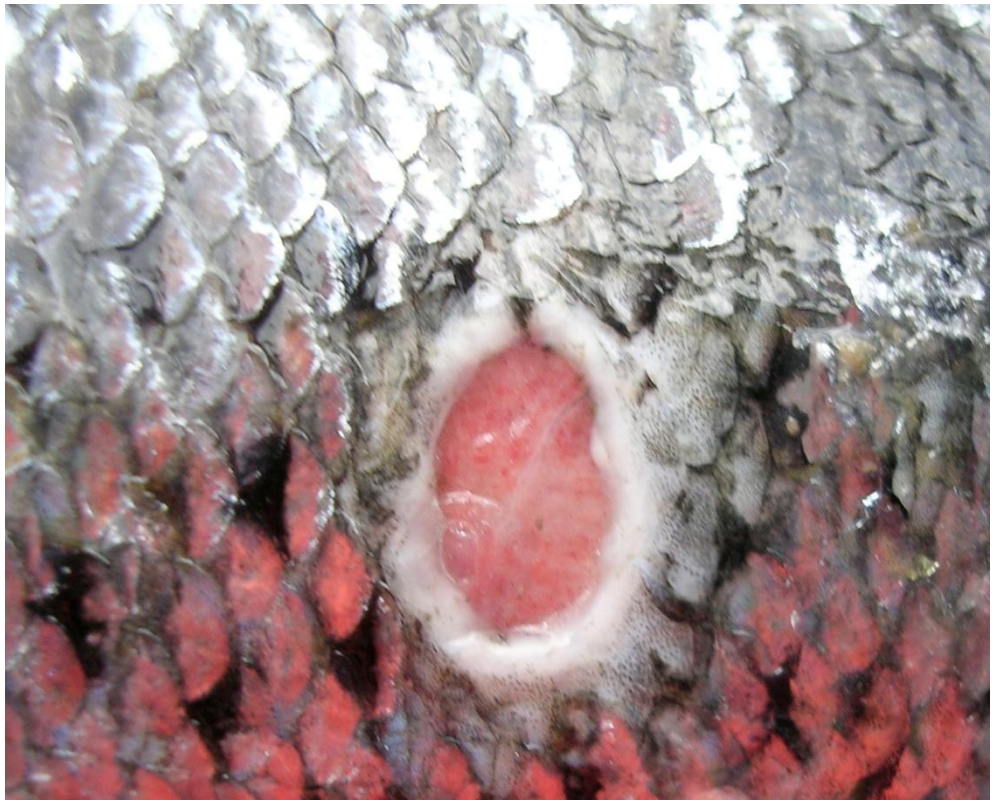
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2007.03639.x/full#f1

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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Post-doc Renate Johansen, Oslo / Copenhagen
Prof. Lone Gram, Copenhagen
Post-doc Anette Bauer Ellingsen, Oslo
PhD-student Christian Karlsen, Tromsø
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. (%)
Vibrio sp. 1 [a] ***Moritella viscosa***	1	7 (7)	13 (13)	3 (4)
Vibrio sp. 2 [b] ***Vibrio wodanis***	2	34 (33)	35 (34)	6 (9)
Both *Vibrio* sp. 1 and *Vibrio* sp. 2 [c]	5	60 (59)	29 (28)	0
Bacteria other than *Vibrio* sp. 1 and *Vibrio* sp. 2	0	0	0	4 (6)
No bacteria	0	1 (1)	25 (25)	54 (81)

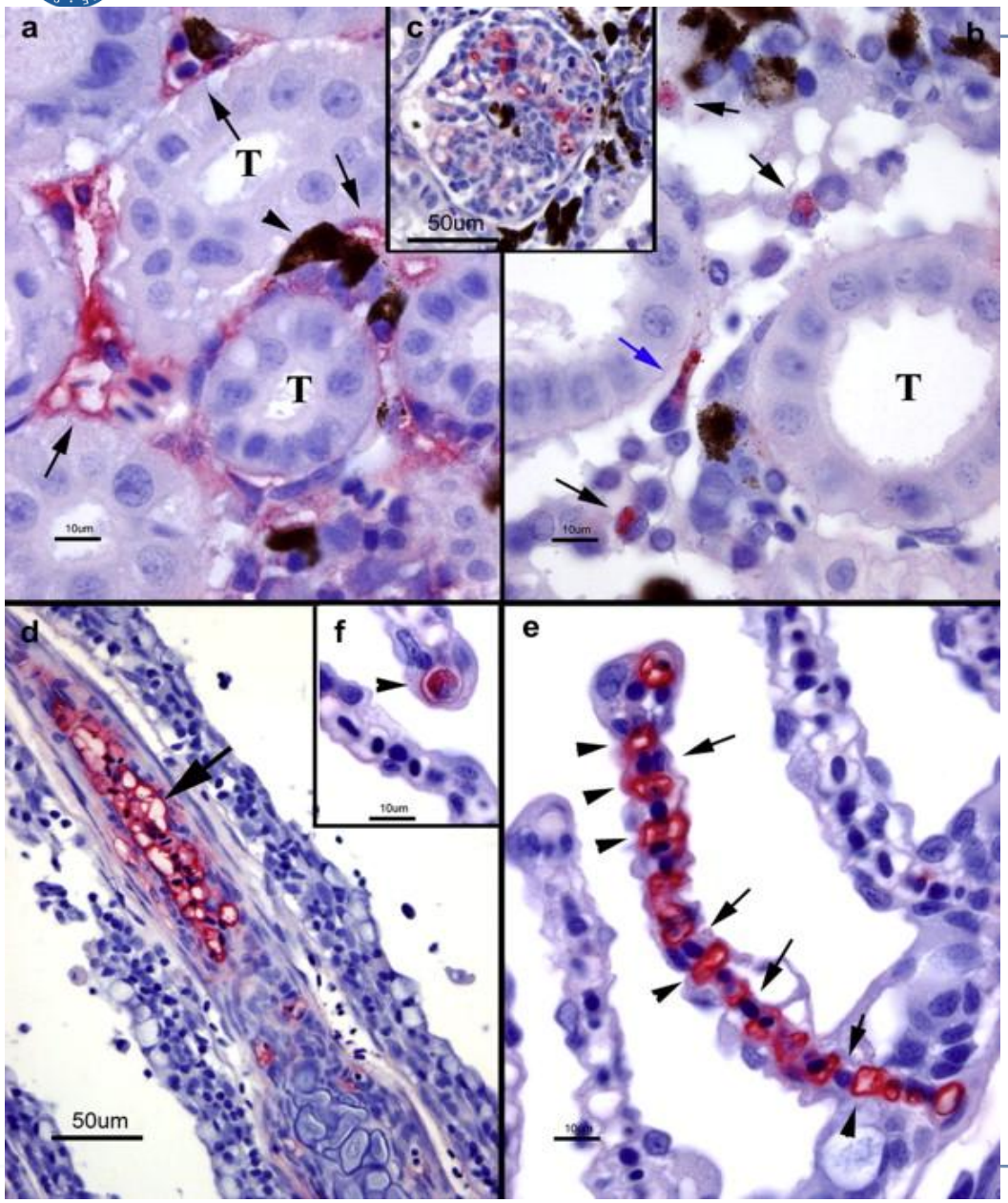
[a] Isolated in pure culture or mixed with bacteria other than *Vibrio* sp. 2
[b] Isolated in pure culture or mixed with bacteria other than *Vibrio* sp. 1
[c] Both vibrios present, sometimes mixed with other bacteria

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

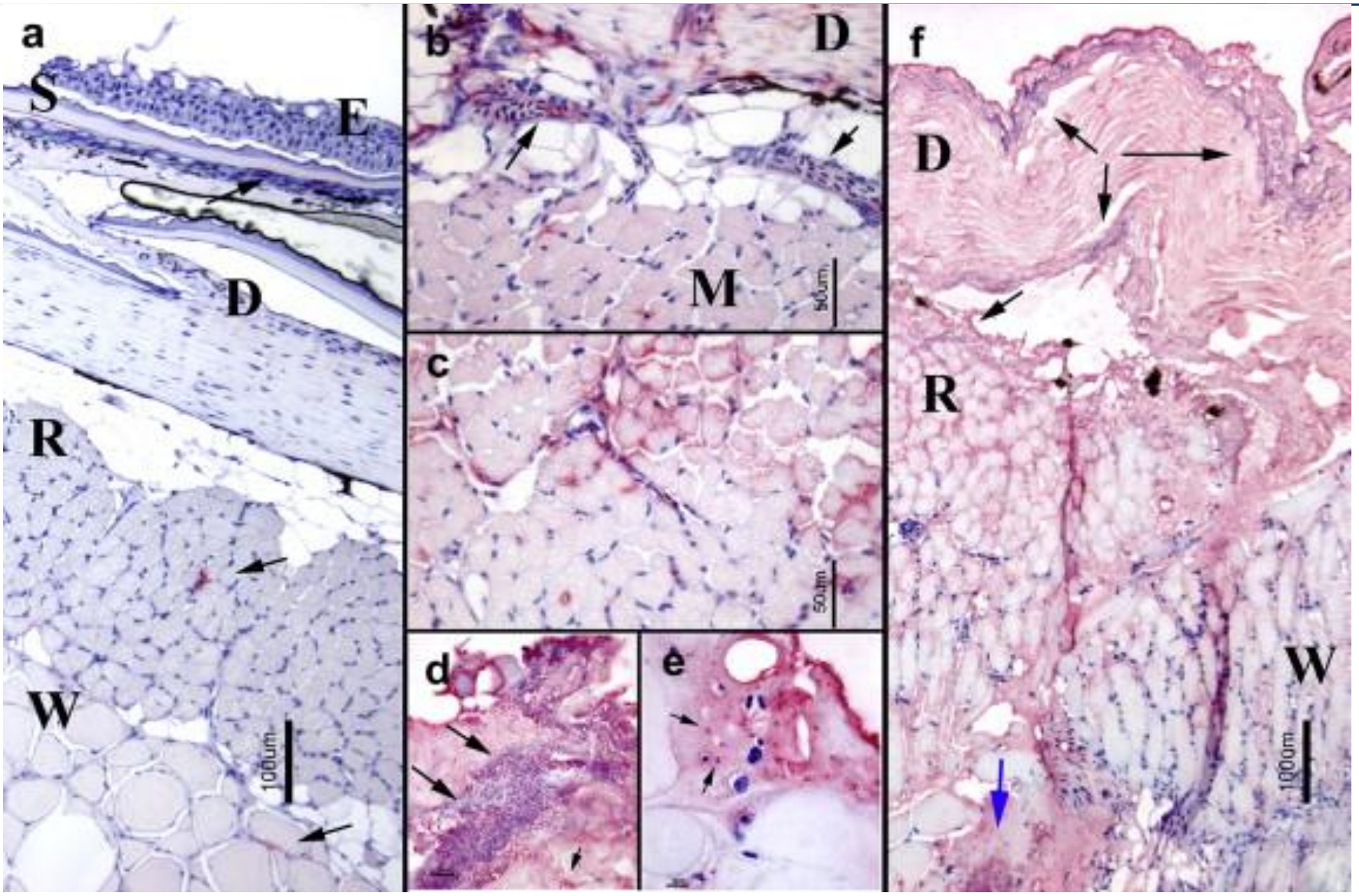
Photo: Brit Tørud

Pathology

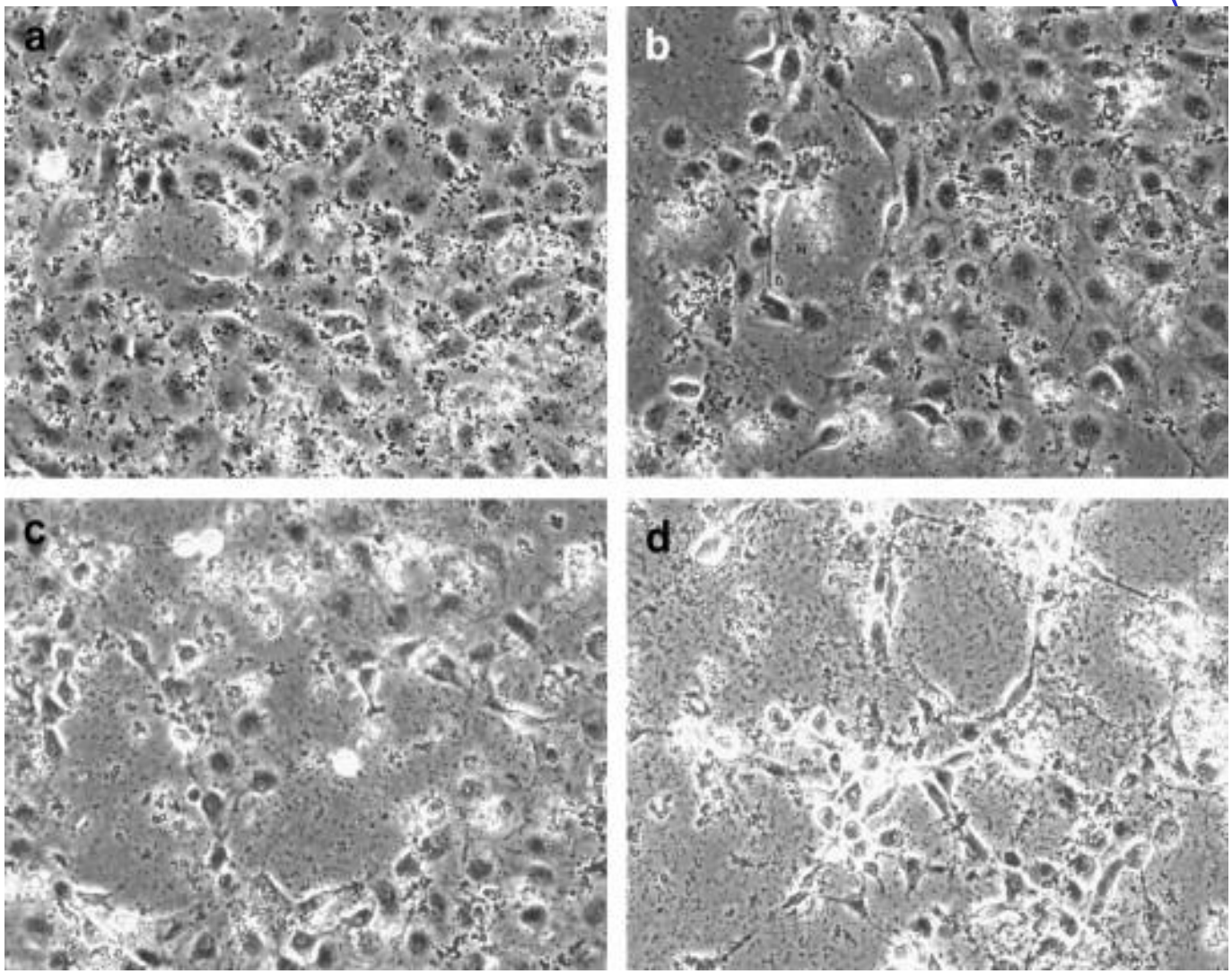
- **Little is known of the pathogenesis**
- **Ulcers**
- **Septicaemia**
  - Signs of circulatory failure like fluid in the abdominal cavity
  - Petecchial haemorrhages on intestinal organs etc.
  - Detection of bacteria both in kidney and ulcers
- **Possible to find bacterial septicaemia in fish with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



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

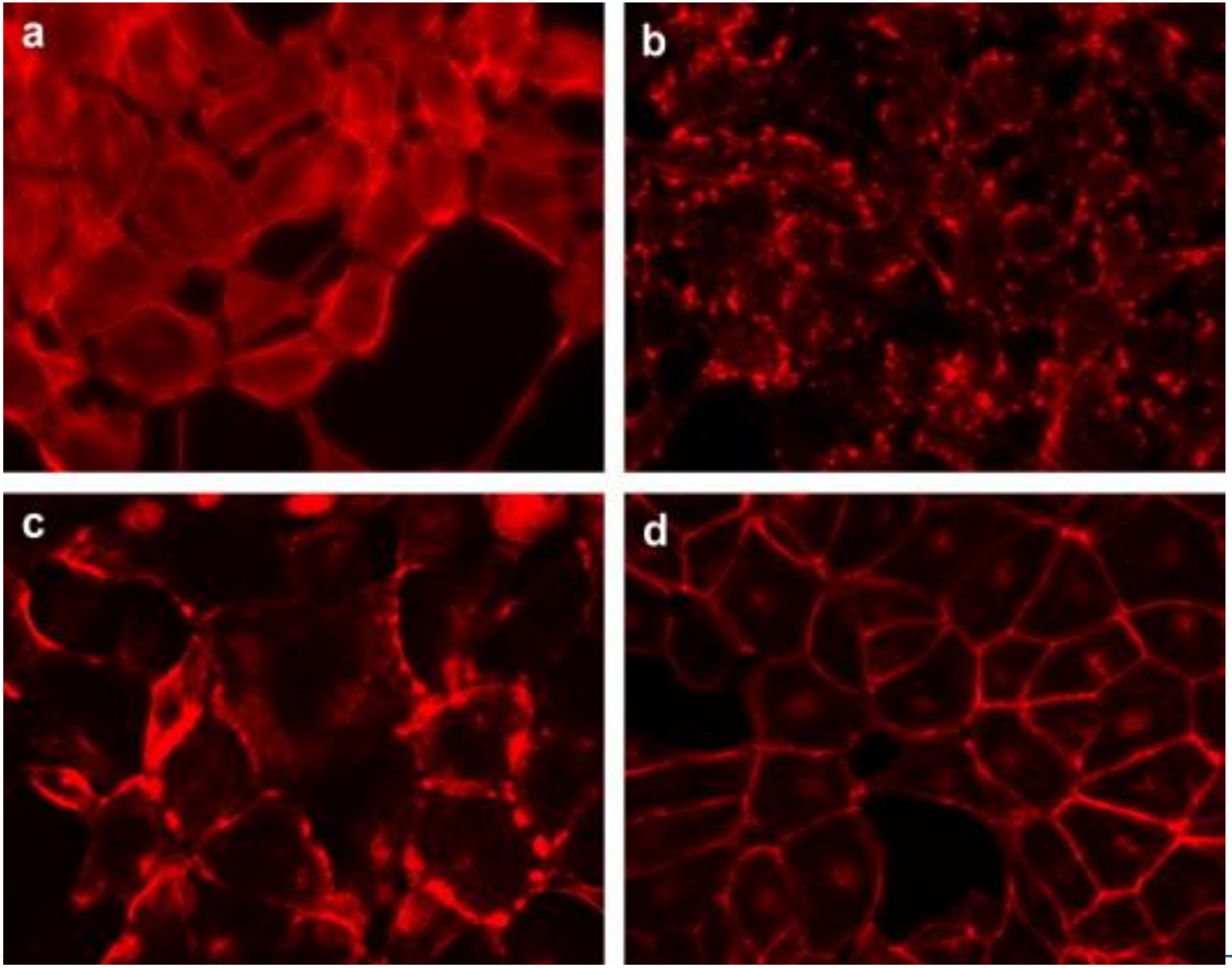

a
S
E
D
R
W
100um
b
D
M
50um
c
50um
d
e
f
D
R
W
100um

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

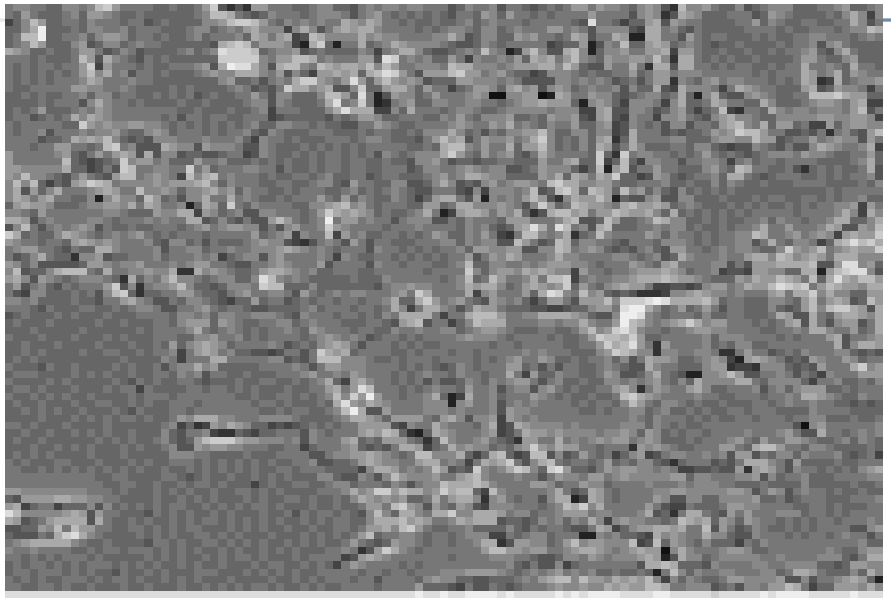


a. 2h
b. 4h
c. 8h
d. 14h

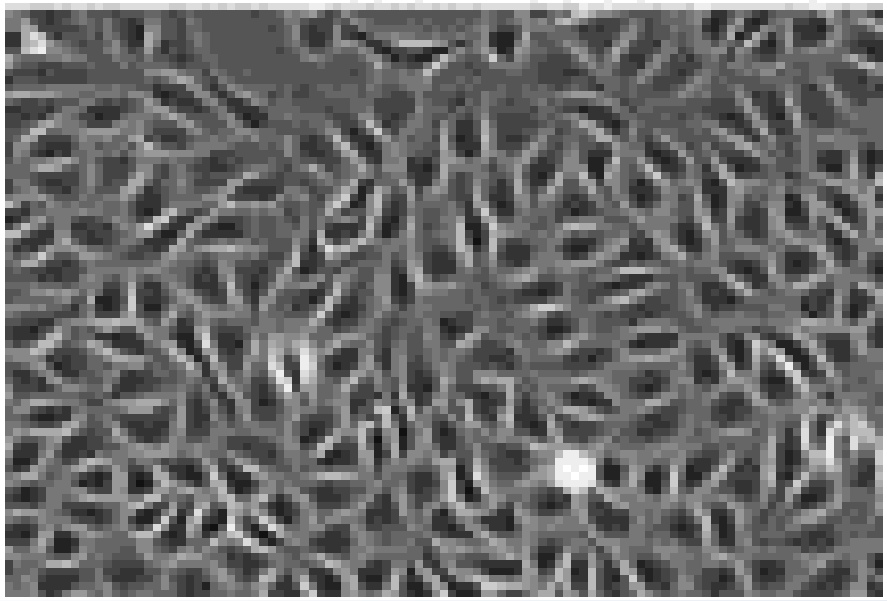
Actin staining of CHSE cytoskeleton *M. viscosa* infection



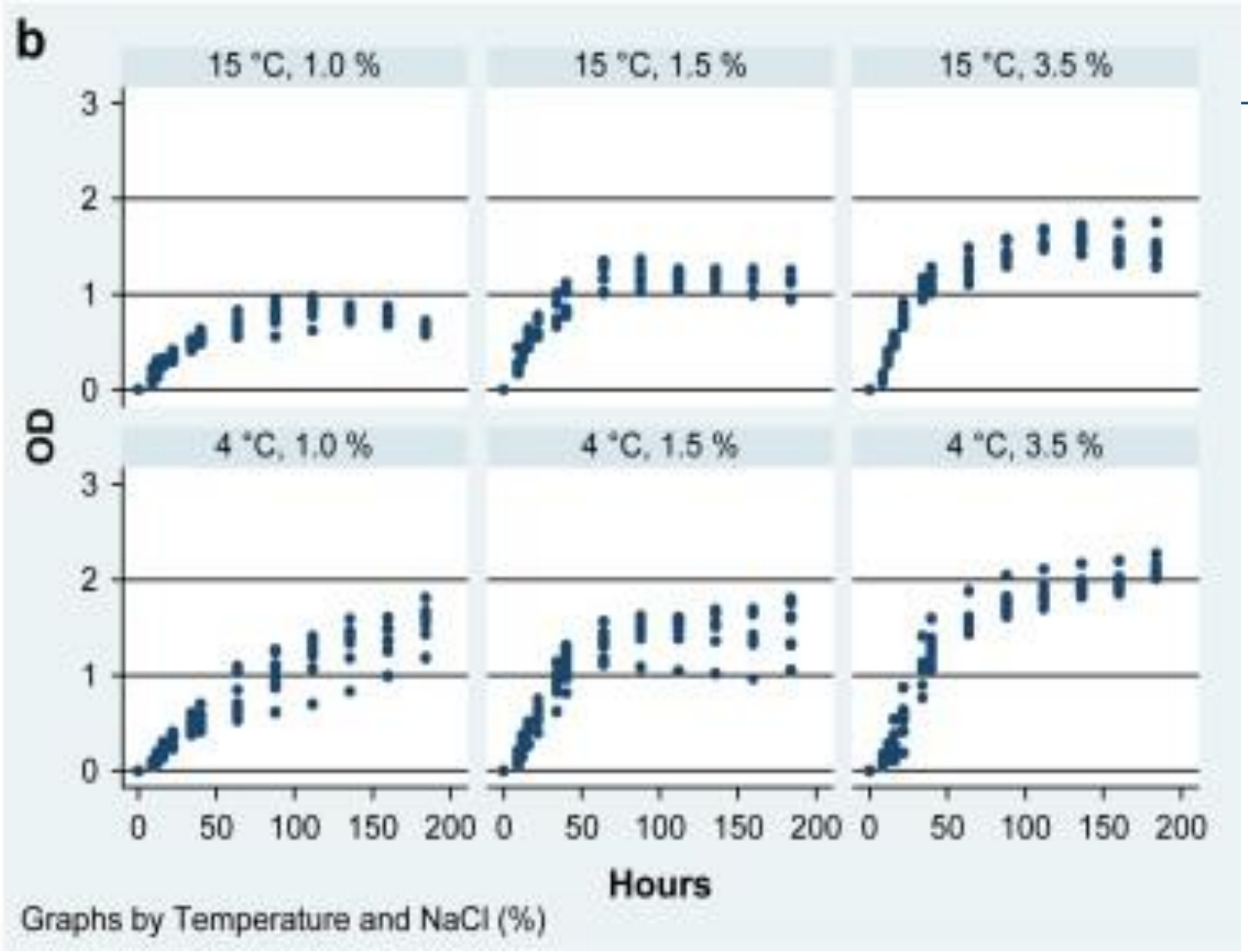
a. Control
b. 1h
c. 5h
d. 7h



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



Control


b
15 °C, 1.0 %
15 °C, 1.5 %
15 °C, 3.5 %
4 °C, 1.0 %
4 °C, 1.5 %
4 °C, 3.5 %
OD
0
1
2
3
0
50
100
150
200
Hours
Graphs by Temperature and NaCl (%)

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

	***M. viscosa* 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

Fish & Shellfish Immunology 26 (2009) 877–884

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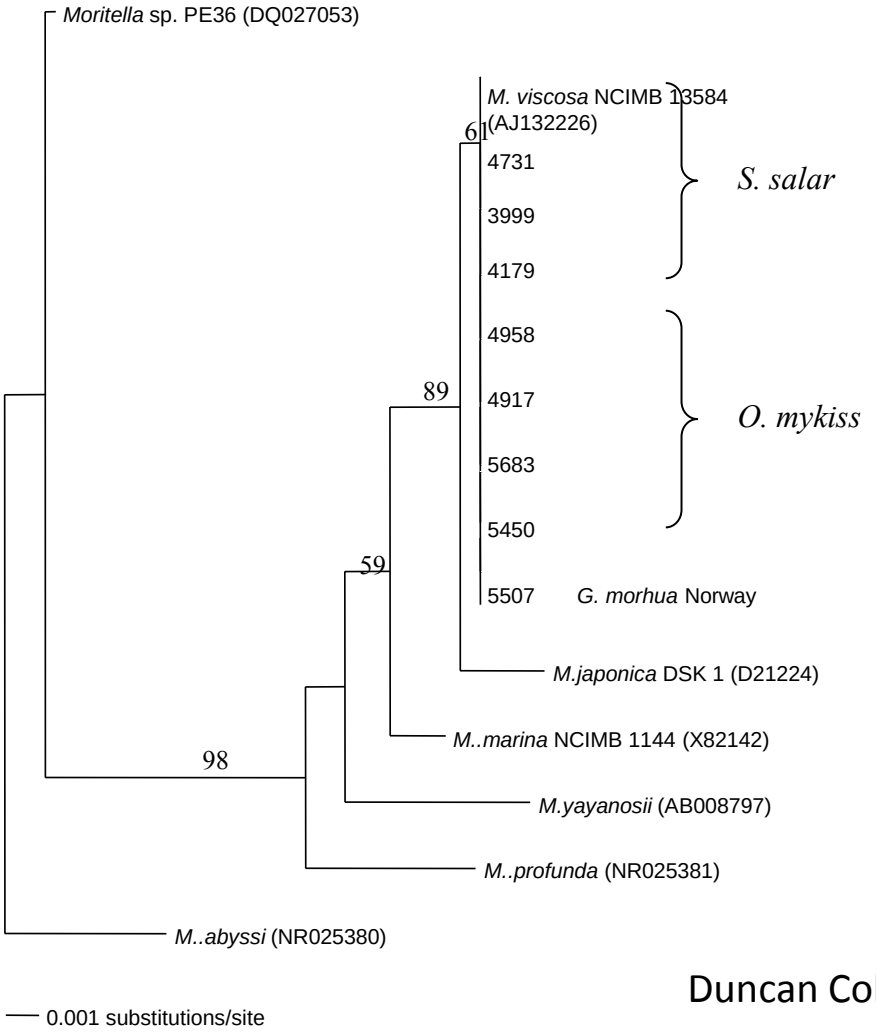
journal homepage: www.elsevier.com/locate/fsi

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.

M. viscosa – homogenous species? 16S rRNA gene analysis

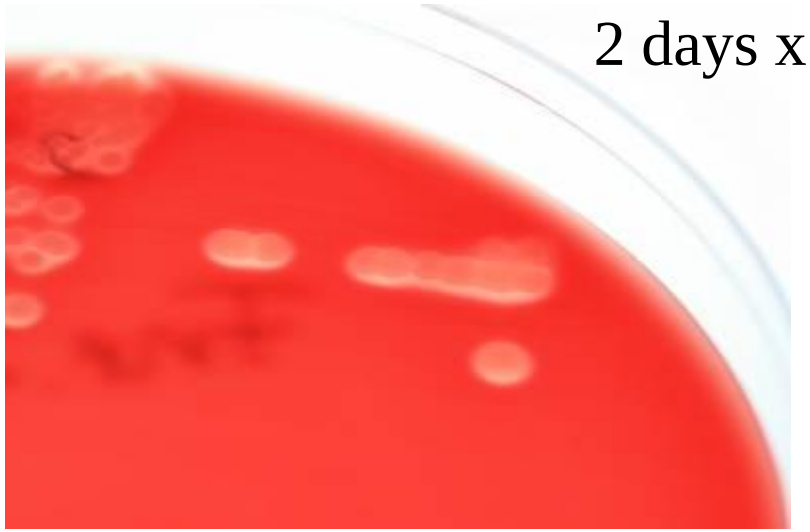


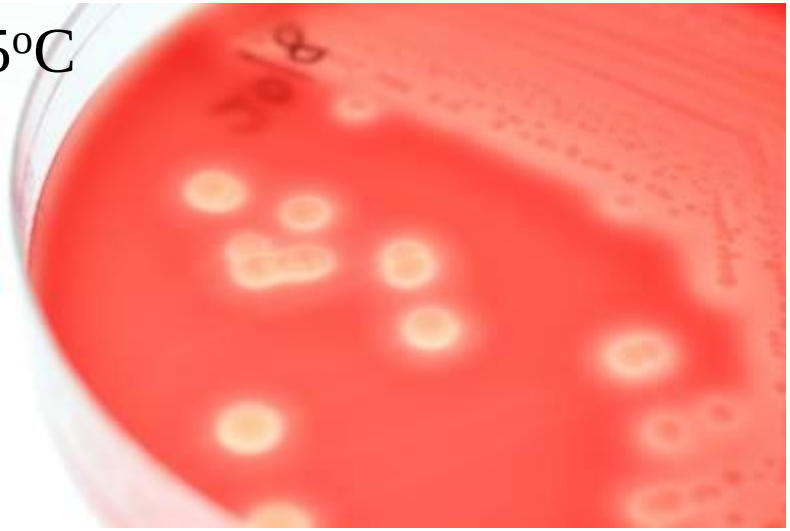
Duncan Colquhoun, Veterinærinstituttet

”typical” from Atlantic salmon

”atypical” from rainbow trout

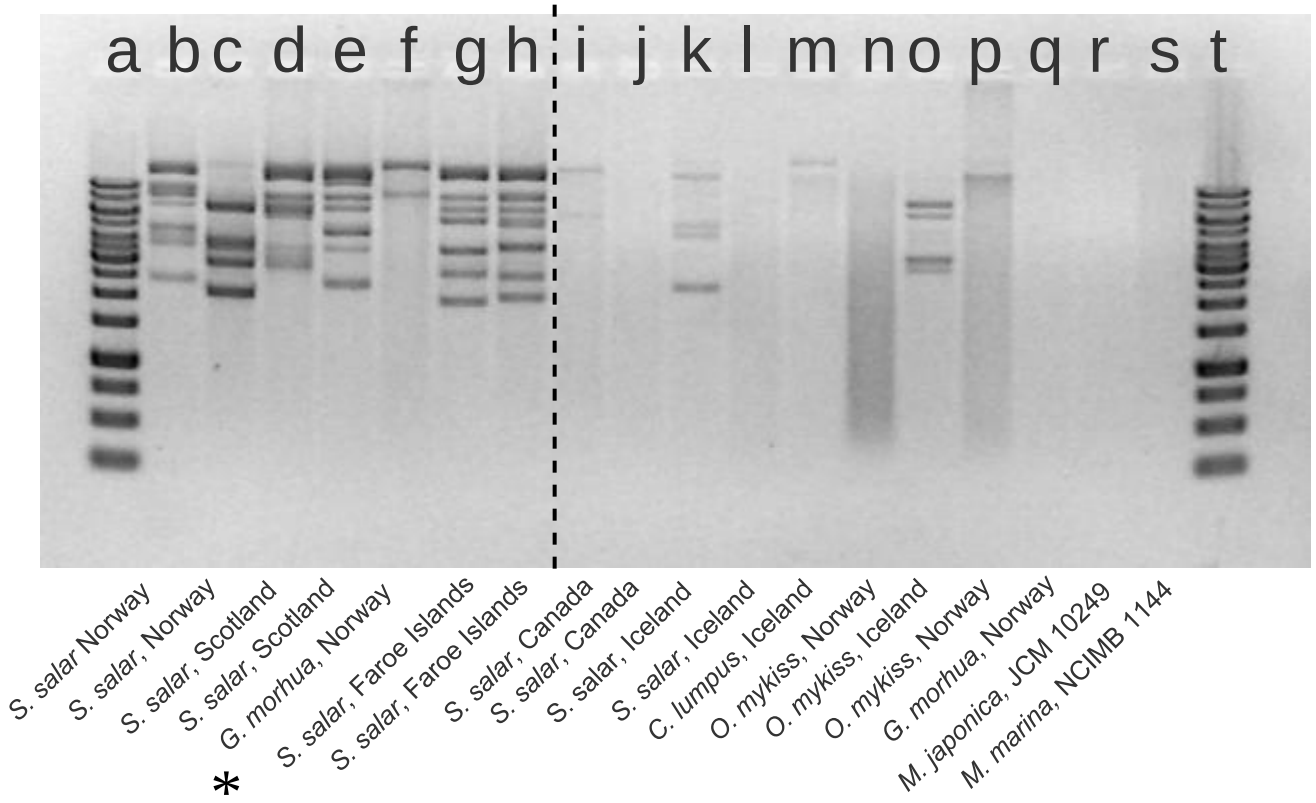
2 days x 15°C





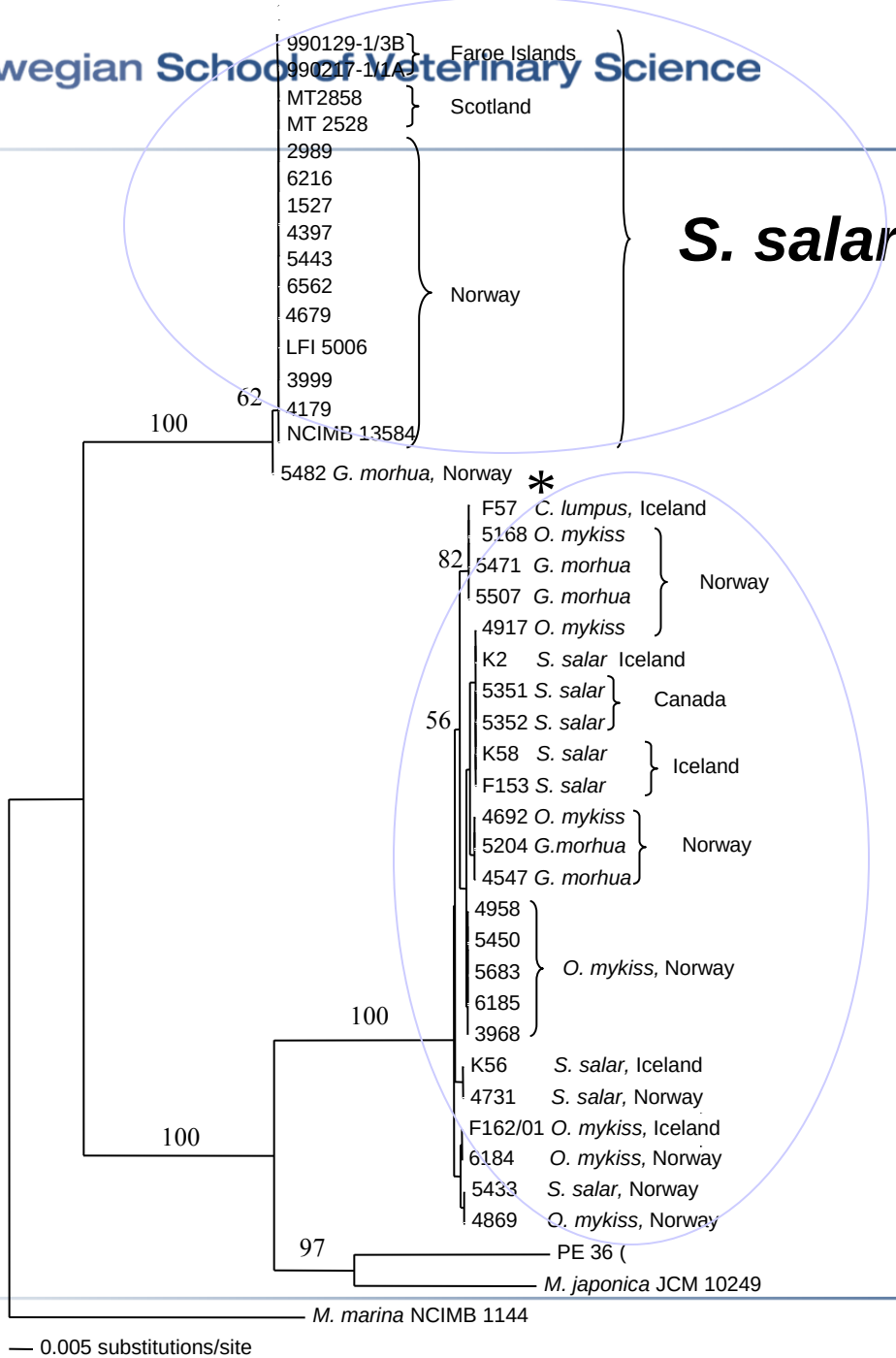
D. Colquhoun, Veterinærinstituttet

5 days x 15°C



D. Colquhoun, Veterinærinstituttet

gyrB

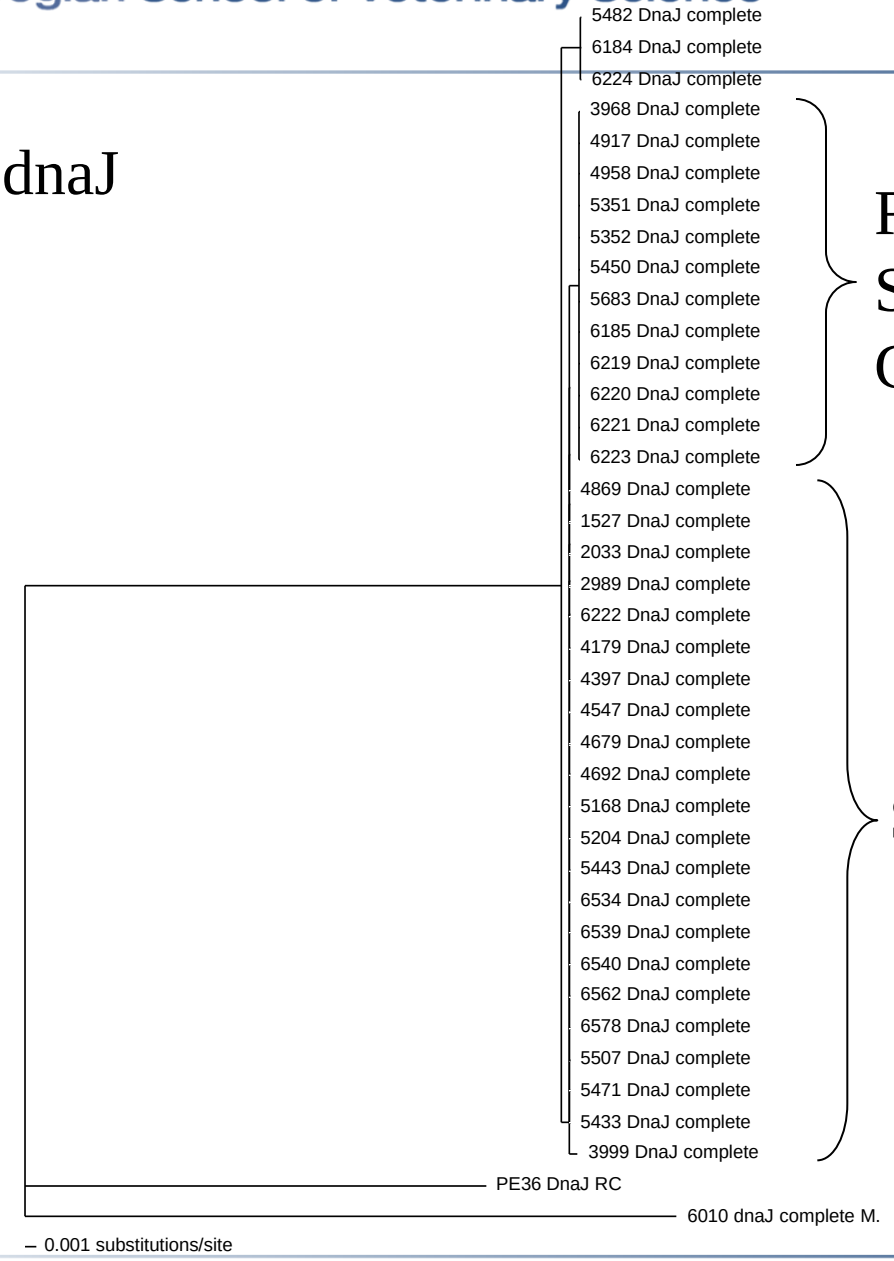


do **not** produce acid from mannose

most but **not all** produce acid from mannose

D. Colquhoun, Veterinærinstituttet

dnaJ

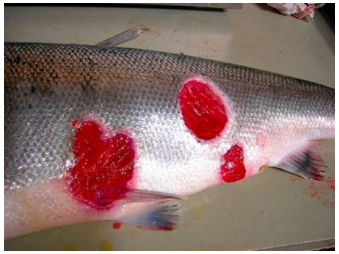


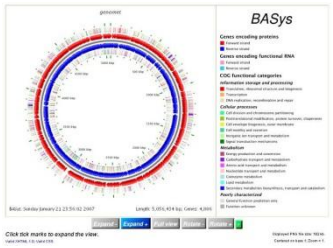
Rainbow trout
Salmon (Iceland and Canada)
Cod

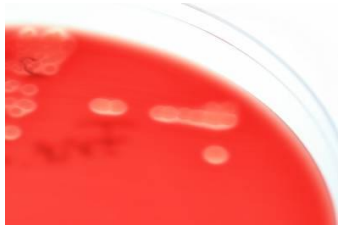
Salmon (Norway, Faroe Isles
Scotland)

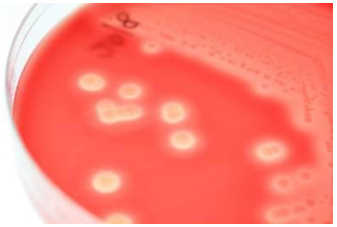
D. Colquhoun, Veterinærinstituttet

Moritella viscosa, winter ulcer









			Haemolysis	Prod. of acid from mannose	Prod. of lysine-decarboxylase
K58	*S. salar*	Iceland	+++	+	-
K2	*S. salar*	Iceland	+++	+	-
F153	*S. salar*	Iceland	+++	+	+
Vvi-11	*S.salar*	Canada	+++#	+	-
Vvi-7	*S. salar*	Canada	+++#	+	-
NVI 4917	*O. mykiss*	Norway	+++	+	-
NVI 4692	*O. mykiss*	Norway	+++	-	-
NVI 4547	*G. morhua*	Norway	+++	(-)	-
NVI 5204	*G. morhua*	Norway	+++	-	-
NVI 6185	*O. mykiss*	Norway	+++	+	+
NVI 3968	*O. mykiss*	Norway	+++	+	-
NVI 4958	*O. mykiss*	Norway	+++	+	-
NVI 5450	*O. mykiss*	Norway	+++	+	+
NVI 5683	*O. mykiss*	Norway	+++	+	-
NVI 5168	*O. mykiss*	Norway	+++	+	-
NVI 5507	*G. morhua*	Norway	+++	-	-
F57	*C. lumpus*	Iceland	+++	(-)	-
NVI 5471	*G. morhua*	Norway	+++	-	-
K56	*S. salar*	Iceland	++#	-	-
NVI 4731	*S. salar*	Norway	++#	+	-
F162/01	*O. mykiss*	Iceland	+++	-	-
NVI 6184	*O. mykiss*	Norway	+++	-	+
NVI 5433	*S. salar*	Norway	+++	-	-
NVI 4869	*O. mykiss*	Norway	+++	-	+
Moritella japonica JCM 10249			+	-	+
Moritella marina NCIMB 1144			+	+	-
990217-1/1A	Faroe Islands		+	-	+
990129-1/3B			+	-	+
MT 2858	Scotland		+	-	+
MT 2528			+	-	-
NVI 4679	*S. salar*	Norway	+	-	+
NVI 6216			+	(-)	-
NVI 1527			+	-	+
NVI 6562			+	(-)	-
NVI 3999			+	-	+
NVI 2989			+	(-)	-
NVI 5443			+	-	+
LFI 5006			+	-	+
NVI 4397			+	-	+
NVI 4179			+	-	-
NCIMB 13584			+	-	+
NVI 5482	*G. morhua*		+#	-	-
Moritella sp. PE 36 (NZ_ABCQ00000000)					

80, 100, 82, 95, 60, 52, 65, 98

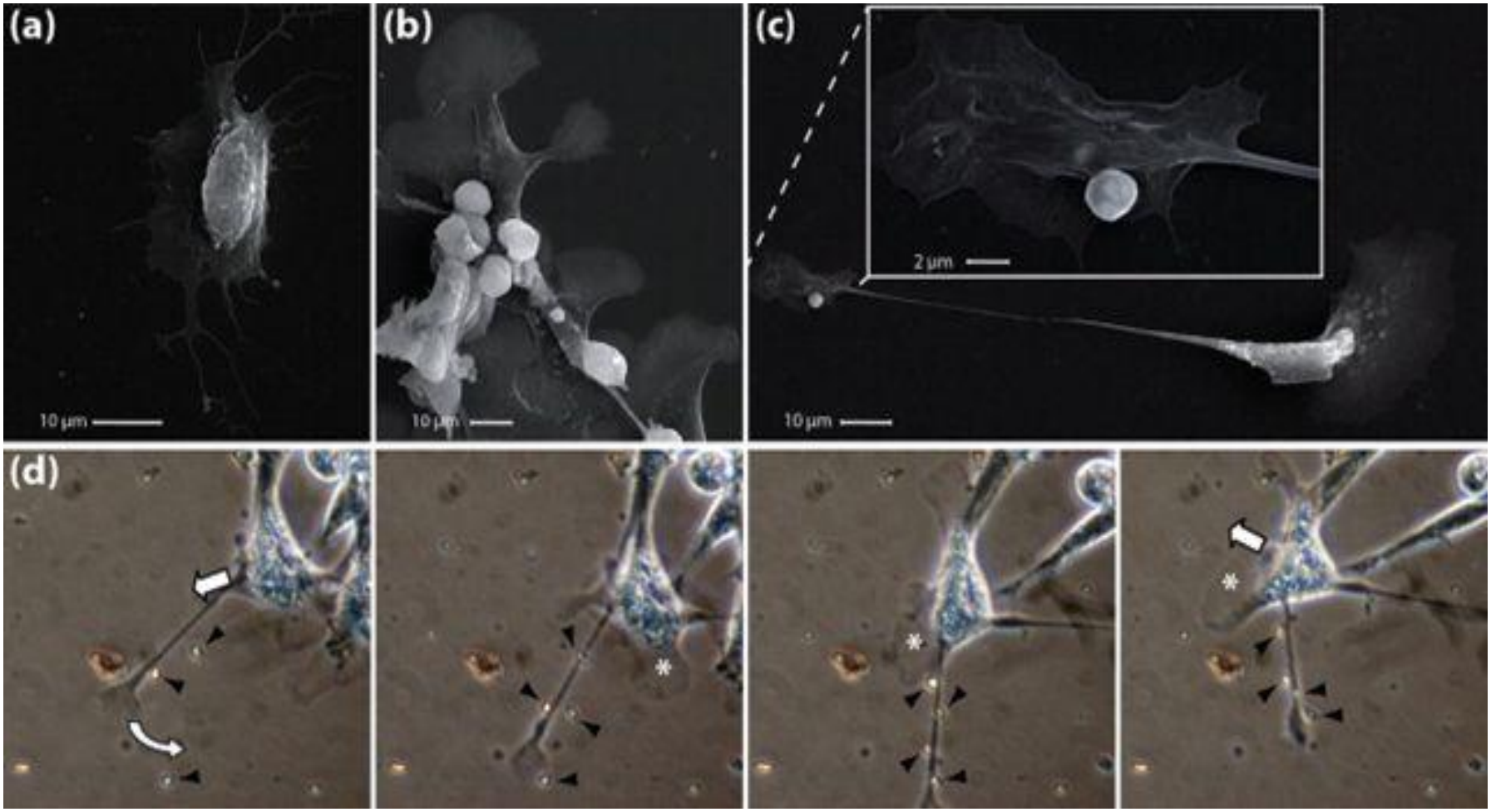
– 0.01 substitutions/site

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*

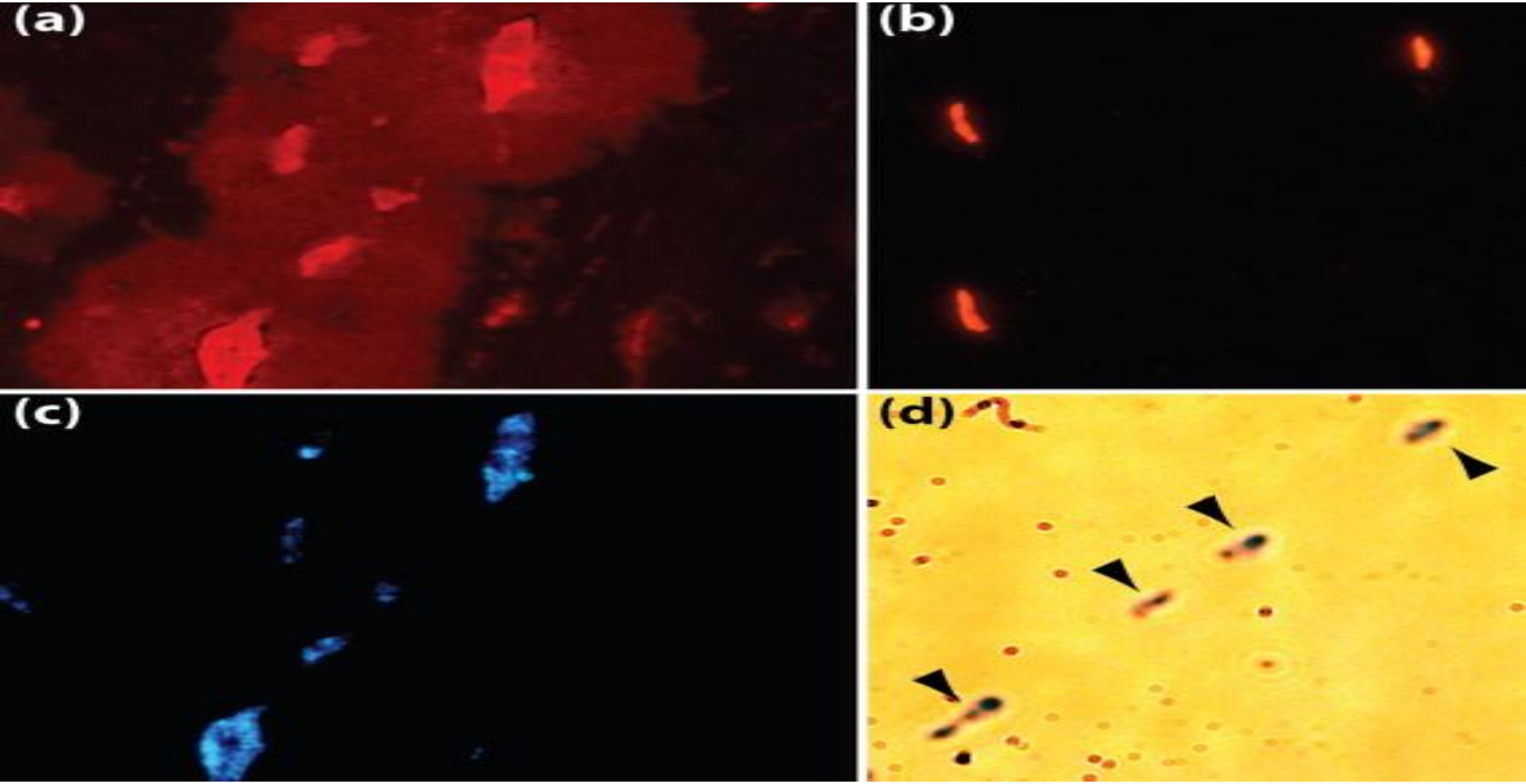
- Both isolates from the same kidney of one salmon that was diseased in an outbreak of Winter ulcer in 2006.
- Finalized 2012?

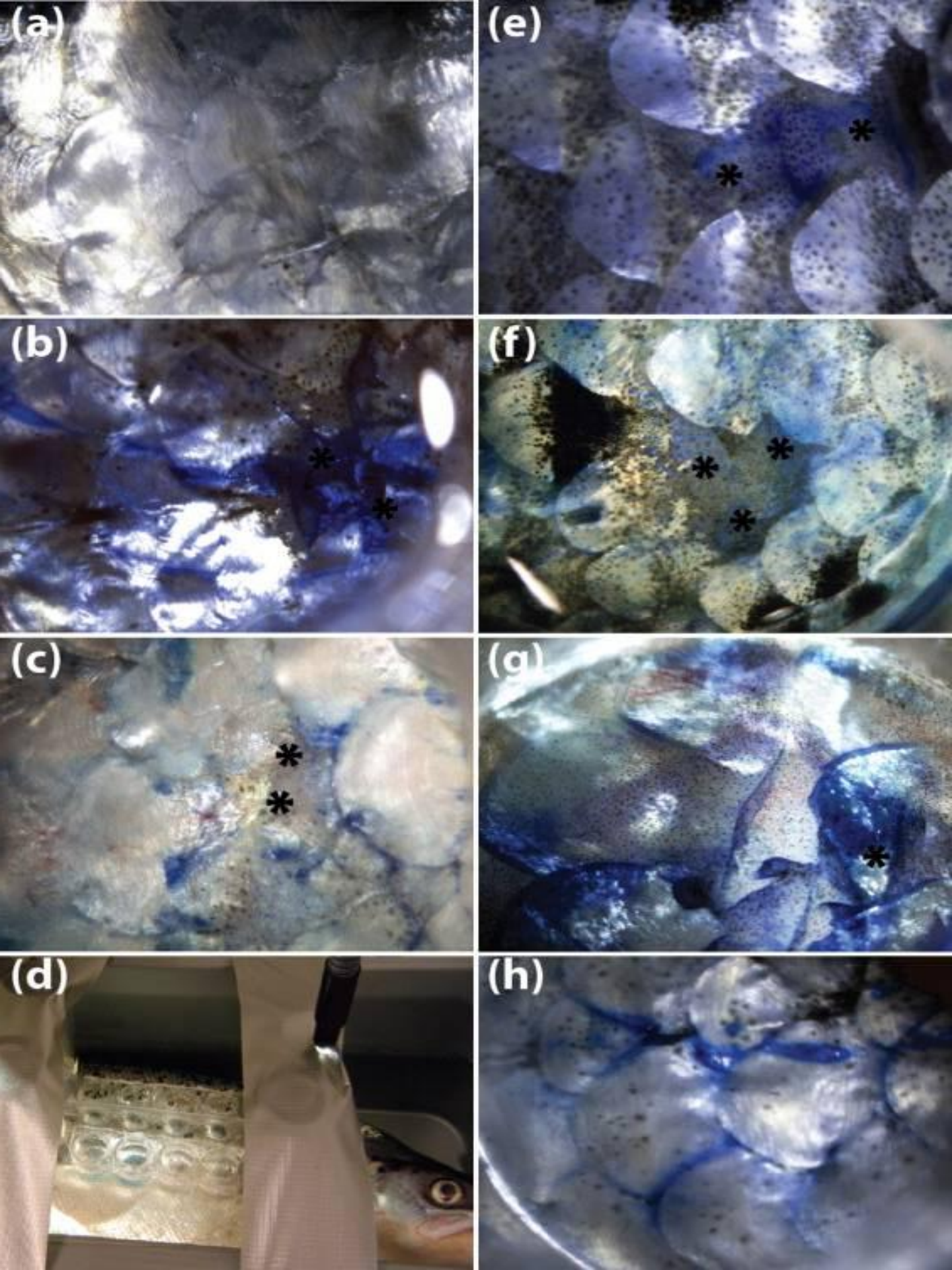
Keratocytes are cleaning and repairing the skin



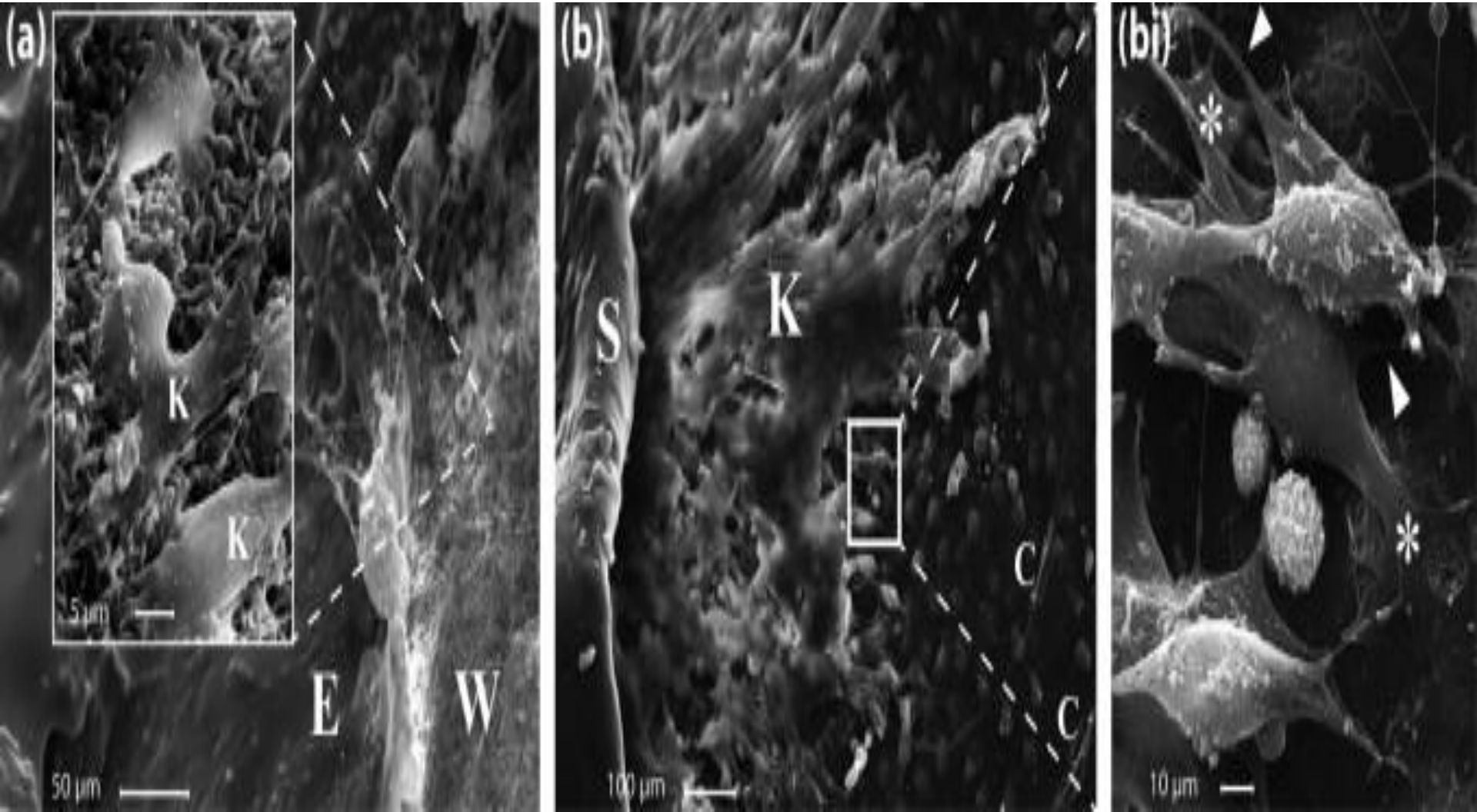
- Karlsen et al 2012 Veterinary Microbiology Volume 154, Issues 3–4, 27 January 2012, Pages 353–362

Moritella viscosa is excreting an immunogenic product




(a)
(e)
(b)
(f)
(c)
(g)
(d)
(h)

Scanning electron microscope of keratocytes

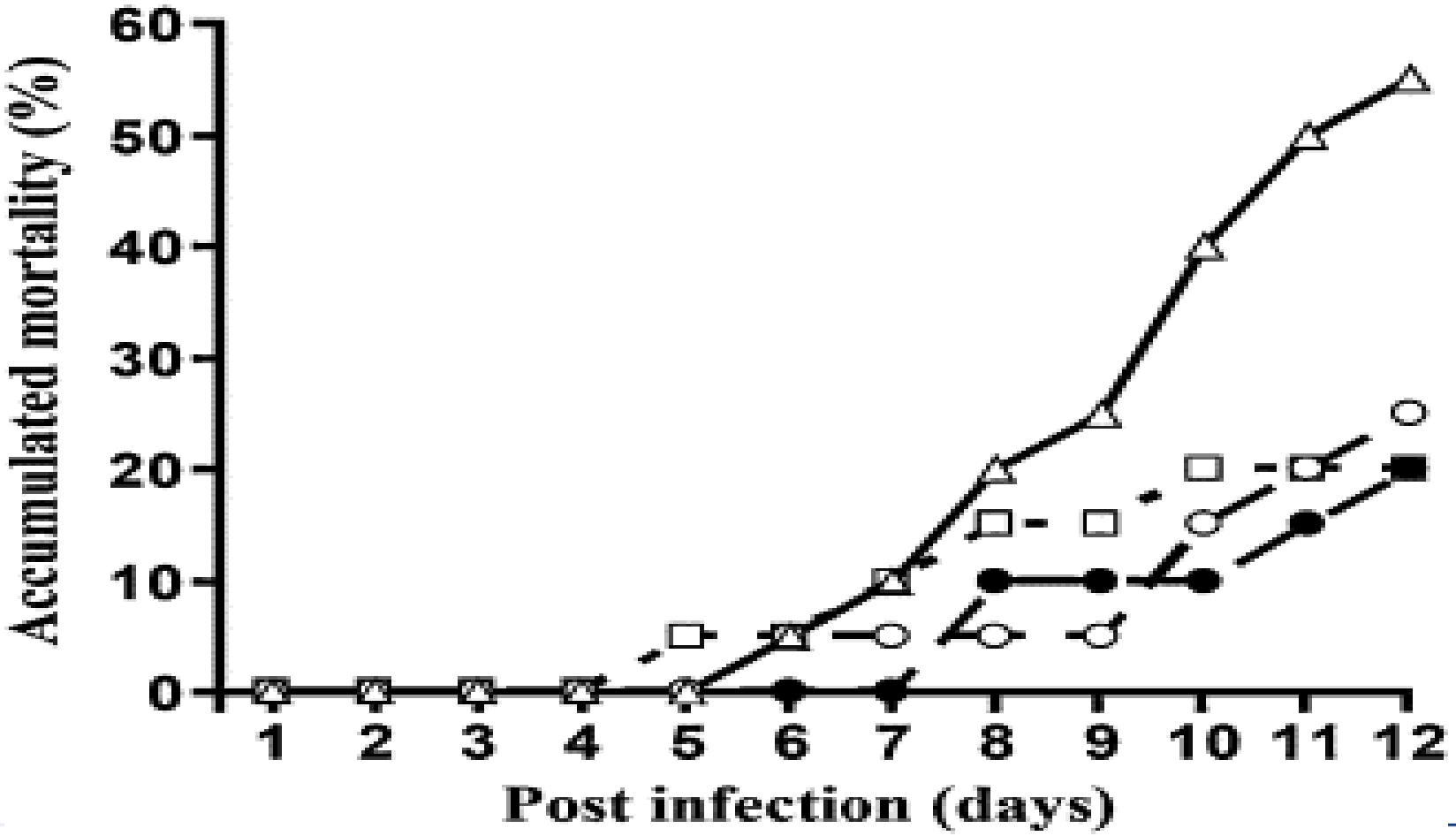


Port of infection

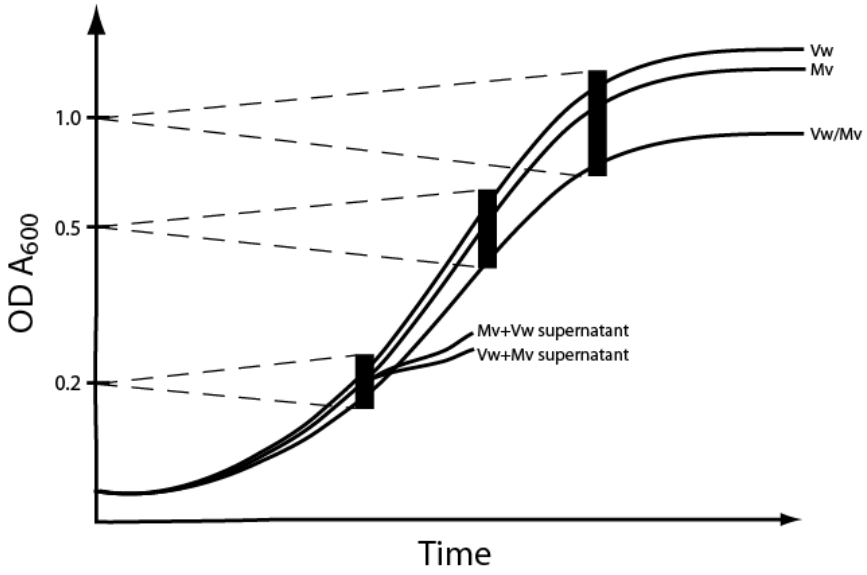
- For bacteria infecting fish little is known about the port(s) of infection
- Assuming that gills and or intestine are important
- For Winter ulcer a skin port should be expected.
- Most ulcers along the side line of the fish
- Is the side line a never discovered port of infection?
- Other unattended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

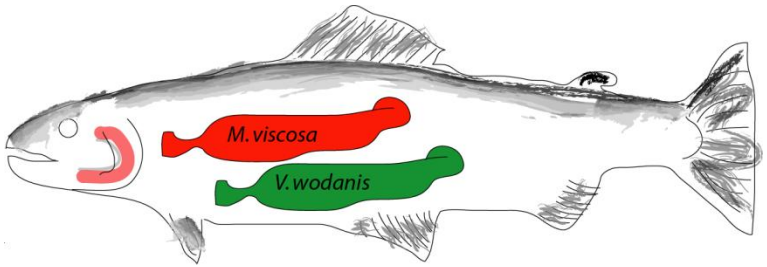
Port of infection, *Moritella viscosa*

Karlsen et al 2012



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





In vivo	*In vitro*	
M. viscosa	*V. wodanis*	(0.2, 0.5, 1.0)
V. wodanis	*M. viscosa*	(0.2, 0.5, 1.0)
M. viscosa + V. wodanis	*V. wodanis/M. Viscosa*	(0.5, 1.0)
M. viscosa/V. wodanis	*Vw + Mv supernatant*	(~0.2)
	Mv + Vw 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-thorugh-put 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.Nocht 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!