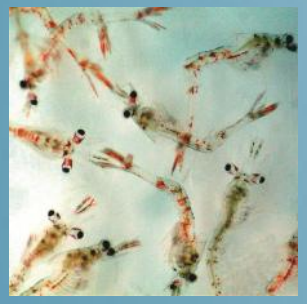
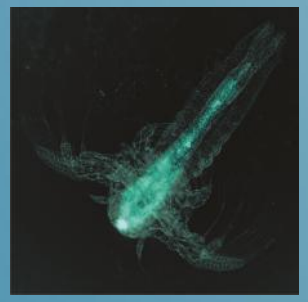
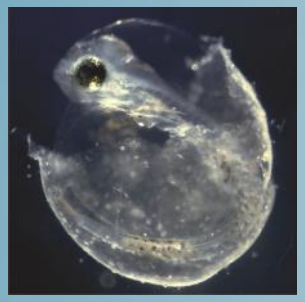
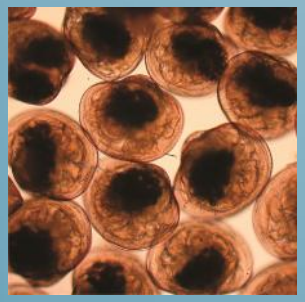




larvi 2013

6th fish & shellfish larviculture symposium

Larval challenge protocols: the quest for virulence and verification

Øivind Bergh

ghent university, belgium, 2-5 september 2013


INSTITUTE OF MARINE RESEARCH
HAVFORSKNINGSINSTITUTTET

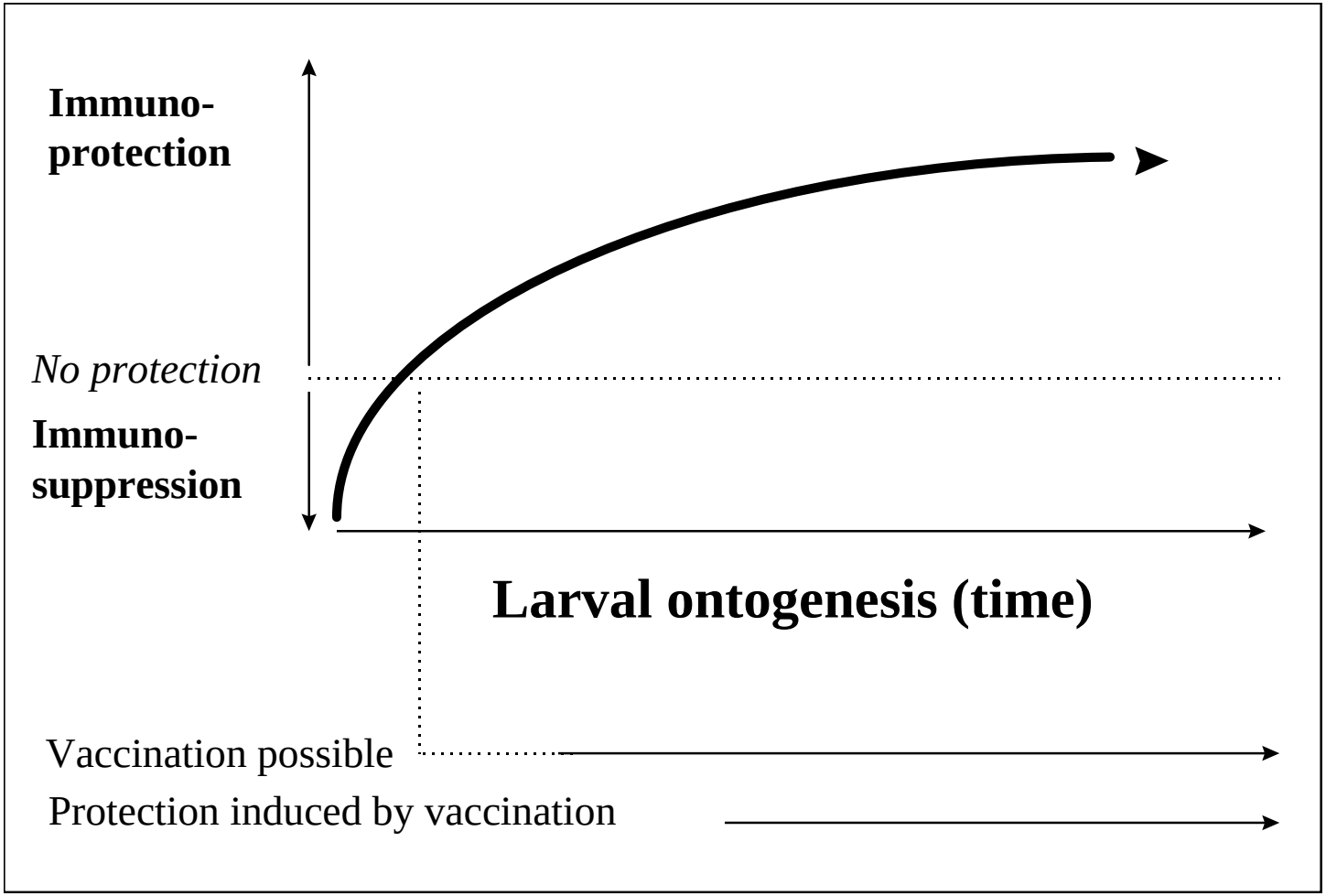
Experimental challenge
The quest for virulence – and protection

Øivind Bergh
Institute of Marine Research, Bergen, Norway

Why challenge?

- **Basic questions:**
  - Is ”the bug” virulent?
  - How does it affect the host?
  - How does it enter the host?

- **Applied**:
  - How can we protect the host
  - How can we verify that the protection is effective?

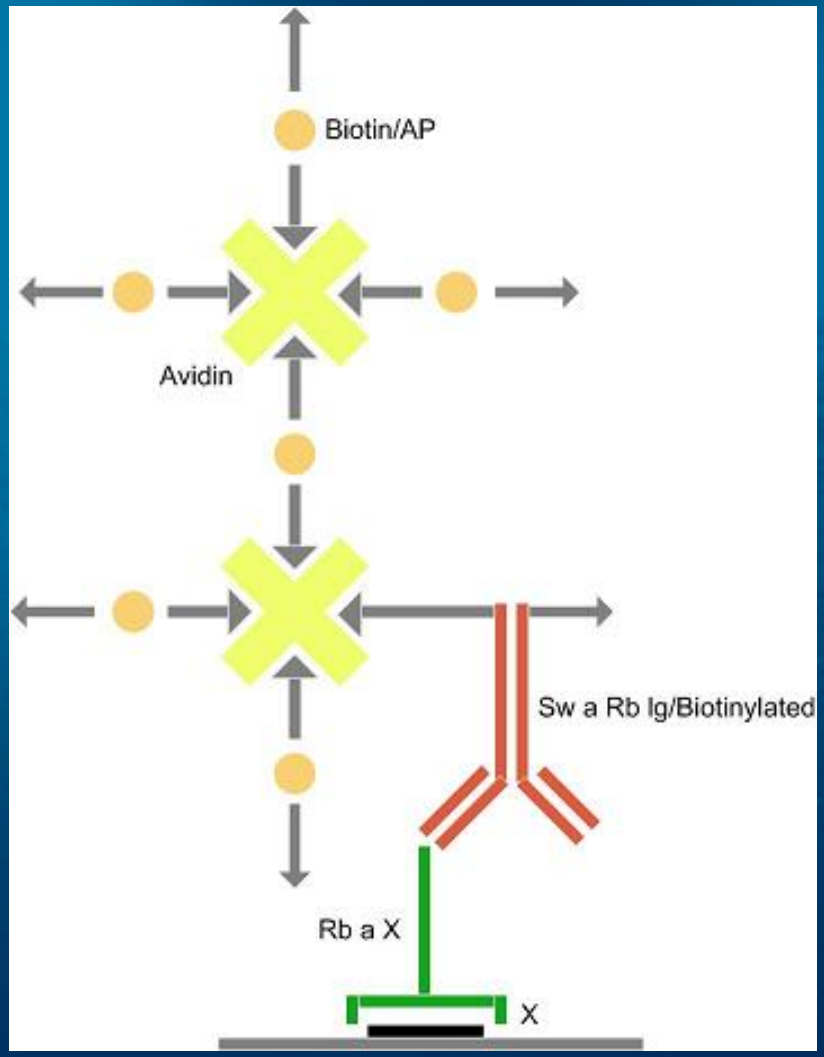


Vadstein, Mo and Bergh 2004

Challenge experiments on yolk sac larvae

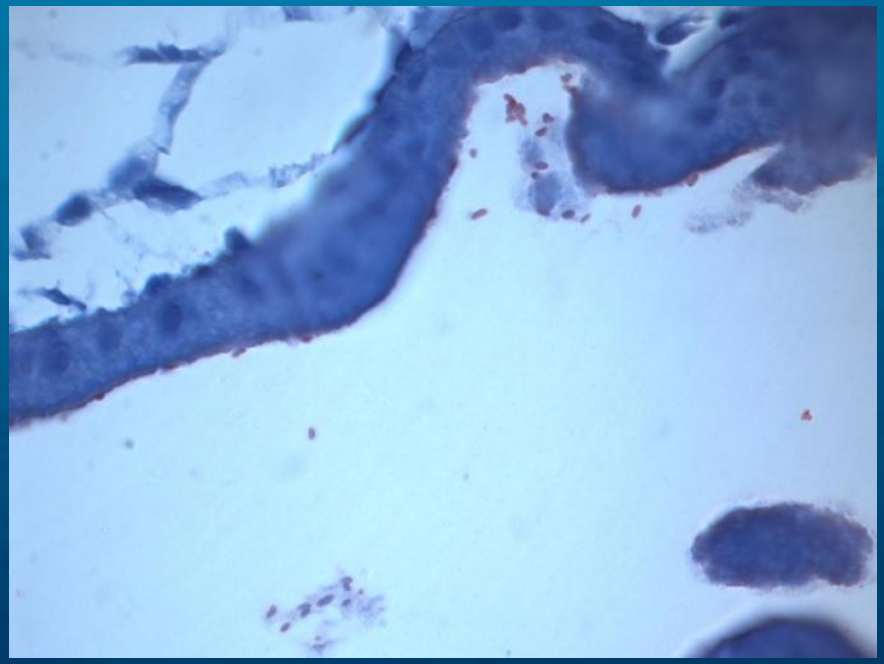
- Rearing of larvae in multiwell dishes
- 72 independent parallel wells
- One egg/larvae per well
- Larvae hatches in well, lives until end of yolk sac period
- Protocol developed from various challenge experiments during two decades:
  - –Bergh et al. 1991 J. Fish Dis.
  - –Sandlund et al. 2010 Dis. Aquat. Org.

Immunohistochemistry

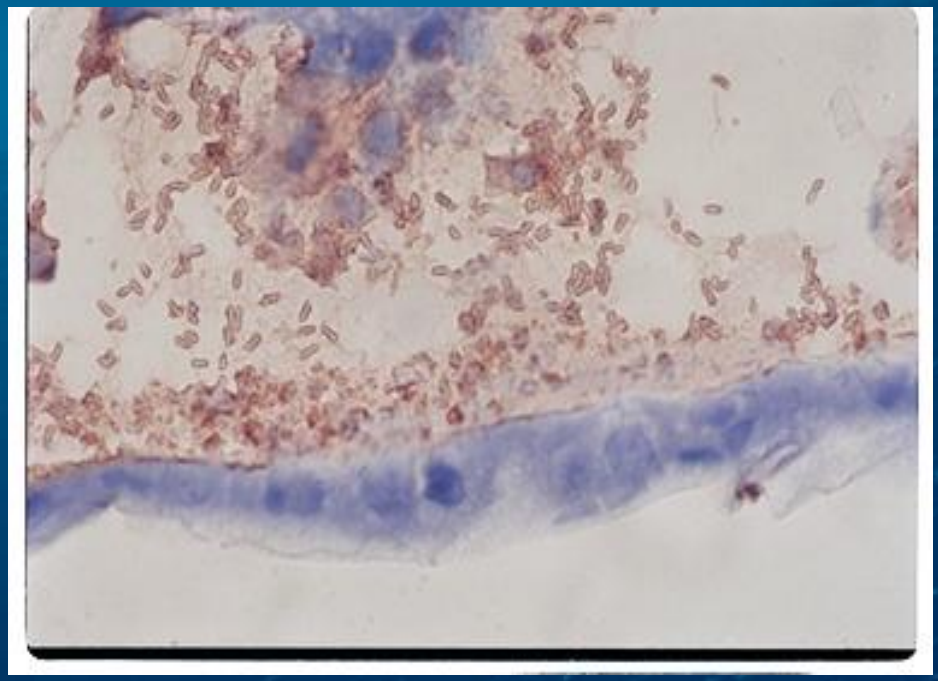


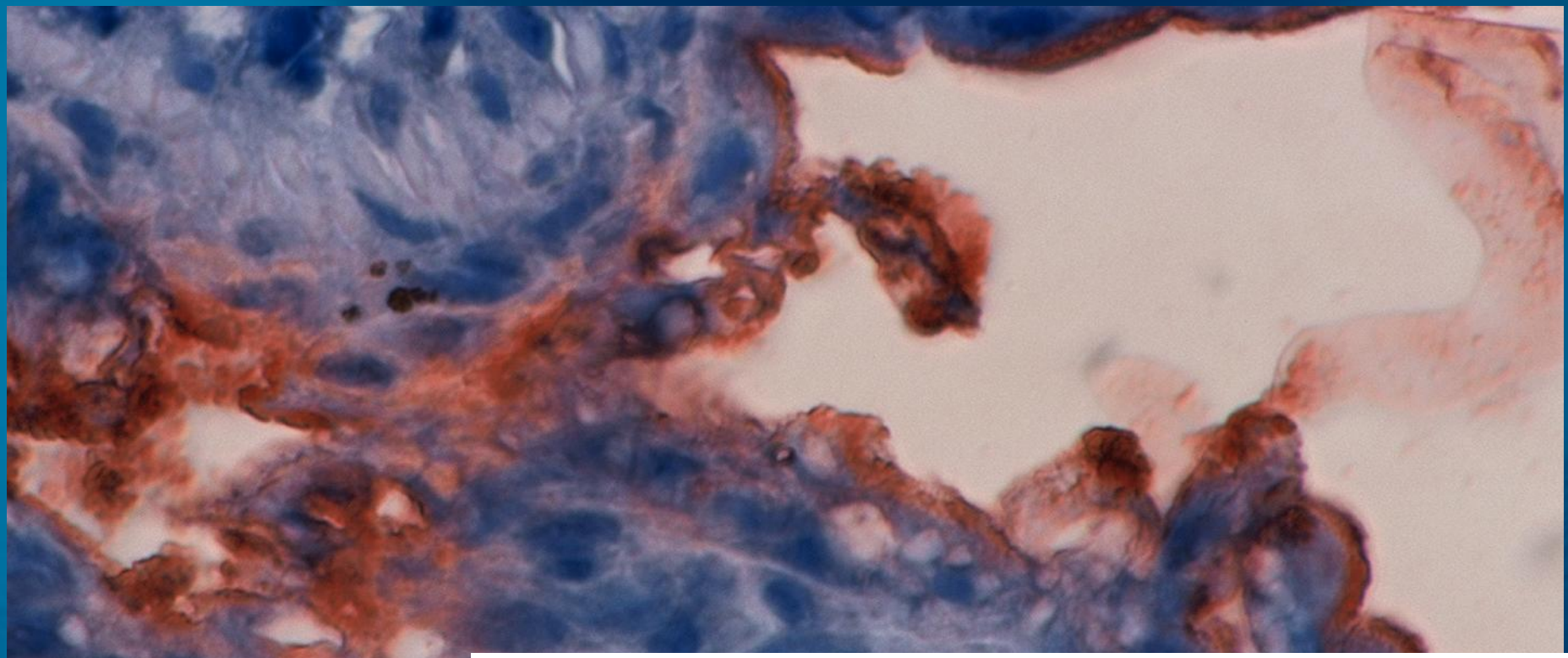
Immunohistochemistry – yolk sac larvae

Intestinal ephithelium - cod



Epidermis - halibut



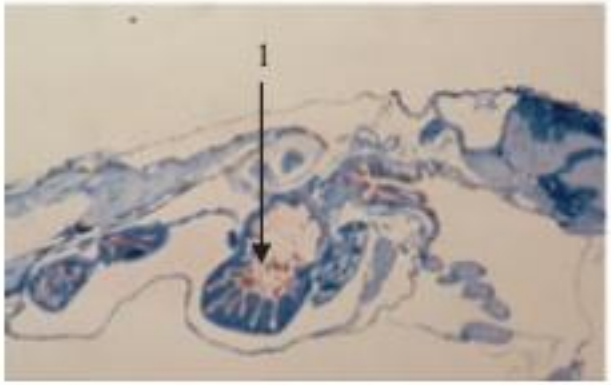


Journal of Fish Diseases 2012, 35, 153–167 doi:10.1111/j.1365-2761.2011.01331.x

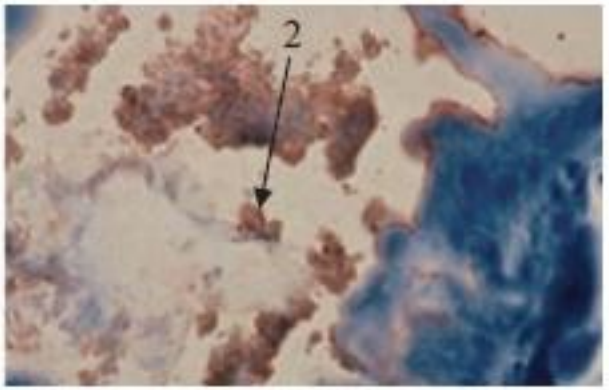
An aerolysin-like enterotoxin from *Vibrio splendidus* may be involved in intestinal tract damage and mortalities in turbot, *Scophthalmus maximus* (L.), and cod, *Gadus morhua* L., larvae

H L Macpherson[1], Ø Bergh[2,3] and T H Birkbeck[1]

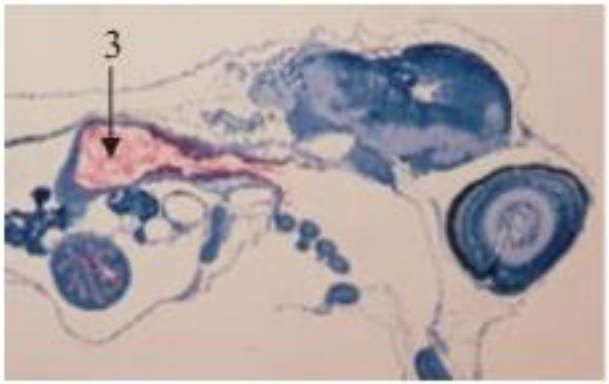
1 Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK
2 Institute of Marine Research, Bergen, Norway
3 Department of Biology, University of Bergen, Norway



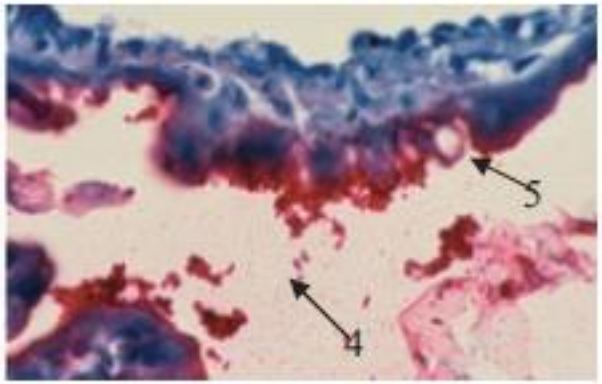
(a1) 100× magnification.



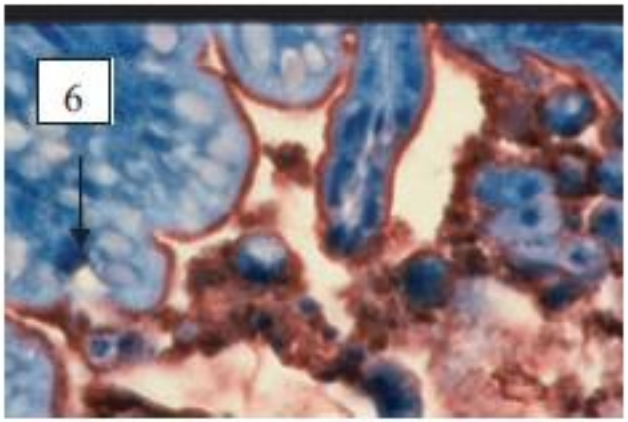
(a2) 1000× magnification.



(b1) 100× magnification.



(b2) 1000× magnification.



(b3) 1000× magnification.

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3 Department of Biology, University of Bergen, Norway

- Wild type *V. splendidus* causes mortality
- **Aerolysin negative mutant** could not be separated from negative control

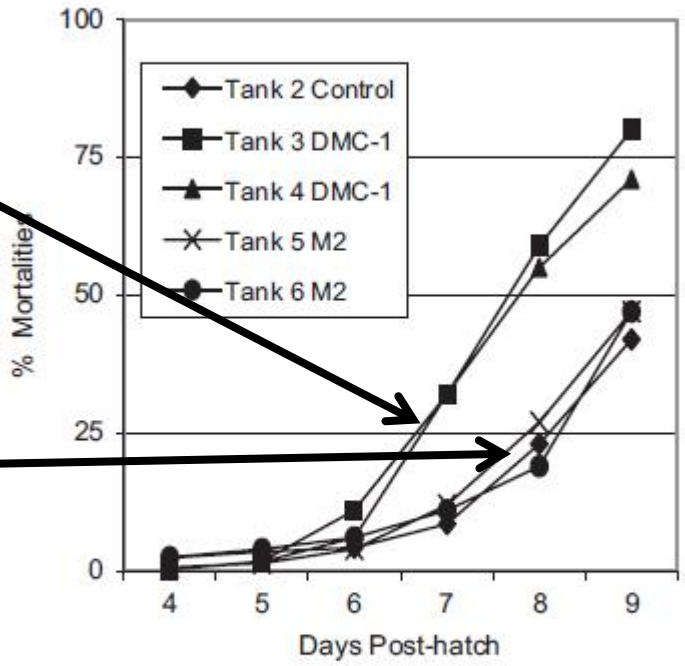

Figure 4 Turbot larvae first-feeding trials with wild-type *Vibrio splendidus* DMC-1 and haemolysin-negative mutant *V. splendidus* DMC-1-M2. Turbot larvae were challenged on days 4 and 5, with challenge bacteria added to the live food rotifers to give a final bacterial concentration of 3×10^4 cfu mL^{-1} in each challenge tank.

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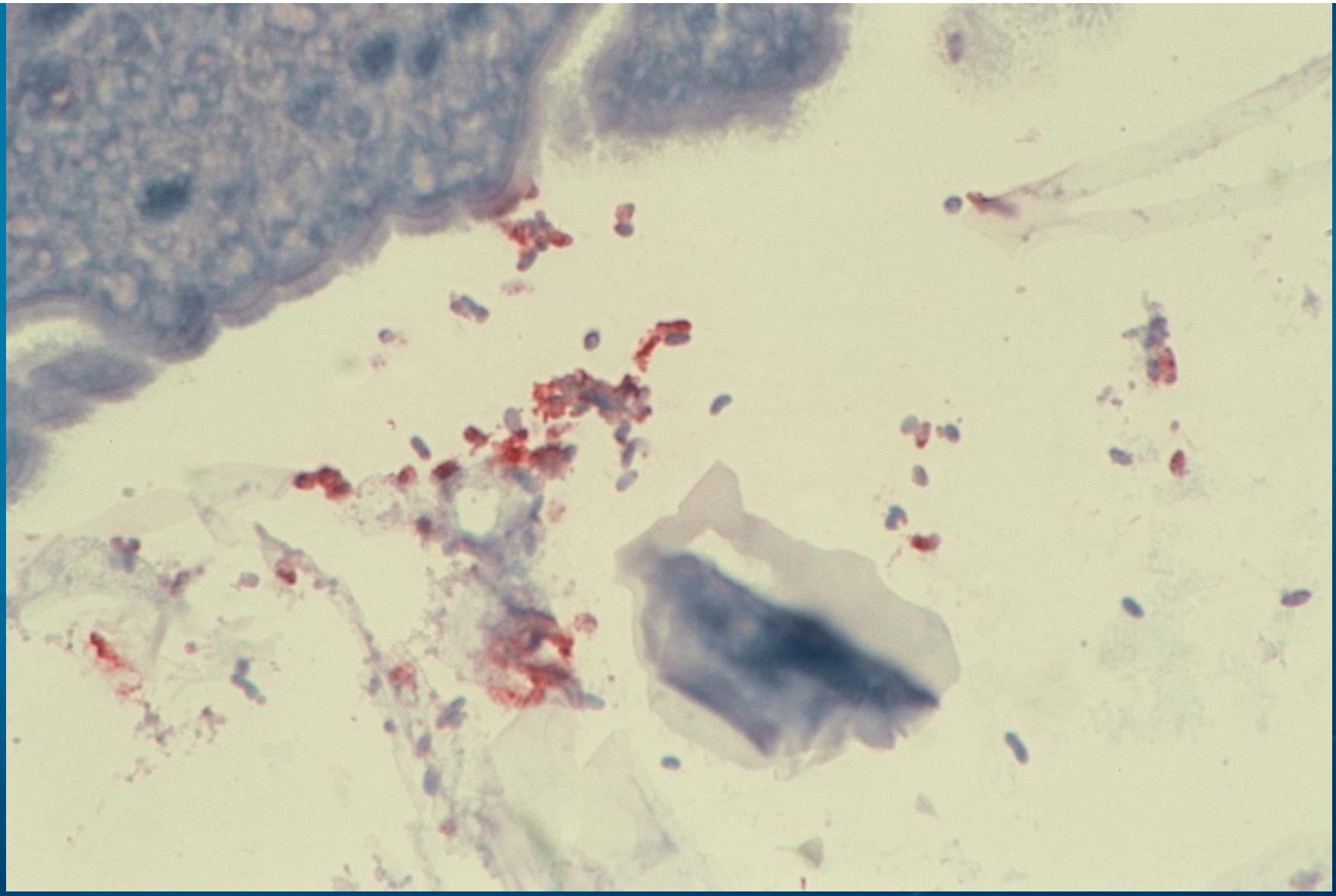
Probiotic effect in vivo of *Roseobacter* strain 27-4 against *Vibrio (Listonella) anguillarum* infections in turbot (*Scophthalmus maximus* L.) larvae

Miquel Planas [a,*], María Pérez-Lorenzo [a], Mette Hjelm [b], Lone Gram [b], Ingrid Uglenes Fiksdal [c], Øivind Bergh [c], José Pintado [a]

[a] *Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Galicia, Spain*
[b] *Danish Institute for Fisheries Research, Department of Seafood Research, Søltofts Plads, c/o Technical University of Denmark Bldg. 221, DK-2800 Kgs. Lyngby, Denmark*
[c] *Institute of Marine Research, PO Box 1870, N-5817, Bergen, Norway*

Received 3 May 2005; received in revised form 17 November 2005; accepted 19 November 2005

Addition of Roseobacter (Rugeria) 27-4 positively affected survival



Note: probiotics in lumen
– NOT attached to epithelium

Vol. 89: 29–37, 2010
doi: 10.3354/dao02176

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

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OPEN ACCESS

Comparative susceptibility of turbot, halibut, and cod yolk-sac larvae to challenge with *Vibrio* spp.

Nina Sandlund[1,*], Odd M. Rødseth[1,4], Dag H. Knappskog[2], Ingrid Uglenes Fiksdal[1], Øivind Bergh[1,3]

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[3]Department of Biology, University of Bergen, PO Box 7803, 5020 Bergen, Norway

[4]*Present address:* Aqua Gen AS, Havnegata 9, 7010 Trondheim, Norway

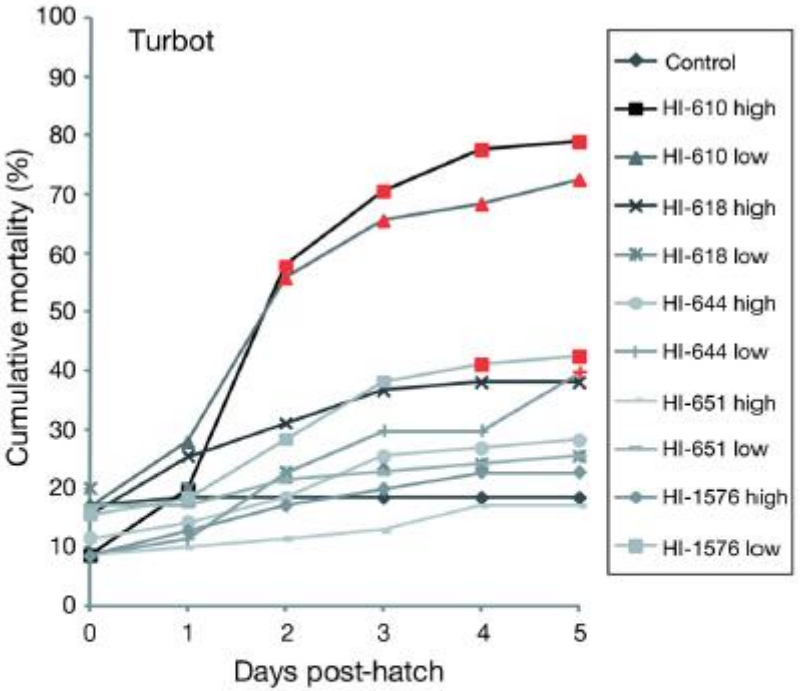

Fig. 1. *Scophthalmus maximus*. Cumulative percentage mortality of turbot larvae challenged with the bacterial strains HI-610, HI-618, HI-644, HI-651, and HI-1576 (see Table 1 for bacterial strains used. High: challenge dose 10^6 CFU ml^{-1}, low: challenge dose 10^4 CFU ml^{-1}, control: unchallenged larvae. Day 0: day of hatching. Red symbols: mortality rates significantly different from the control ($p < 0.01$ Bonferroni correction)

Comparative mortality

5 different *Vibrio* spp

3 x *V. anguillarum*
V. splendidus
V. salmonicida

3 different fish larvae: turbot, halibut, cod

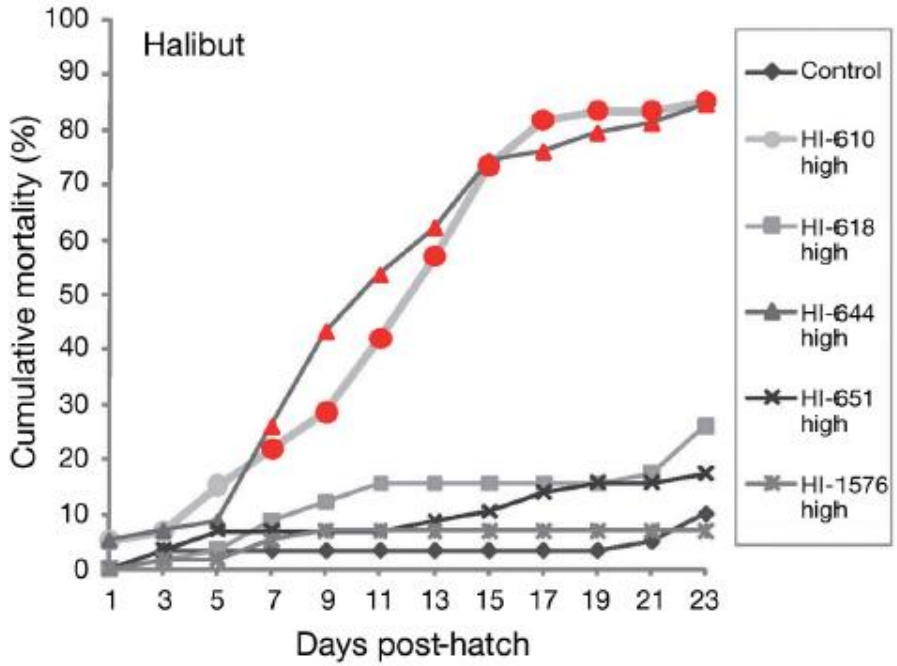

Fig. 2. *Hippoglossus hippoglossus*. Cumulative percentage mortality of halibut larvae challenged with the bacterial strains HI-610, HI-618, HI-644, HI-651, and HI-1576 (see Table 1 for bacterial strains used). High: challenge dose 10^6 CFU ml^{-1}, control: unchallenged larvae. Red symbols: mortality rates significantly different from the control ($p < 0.01$ Bonferroni correction)

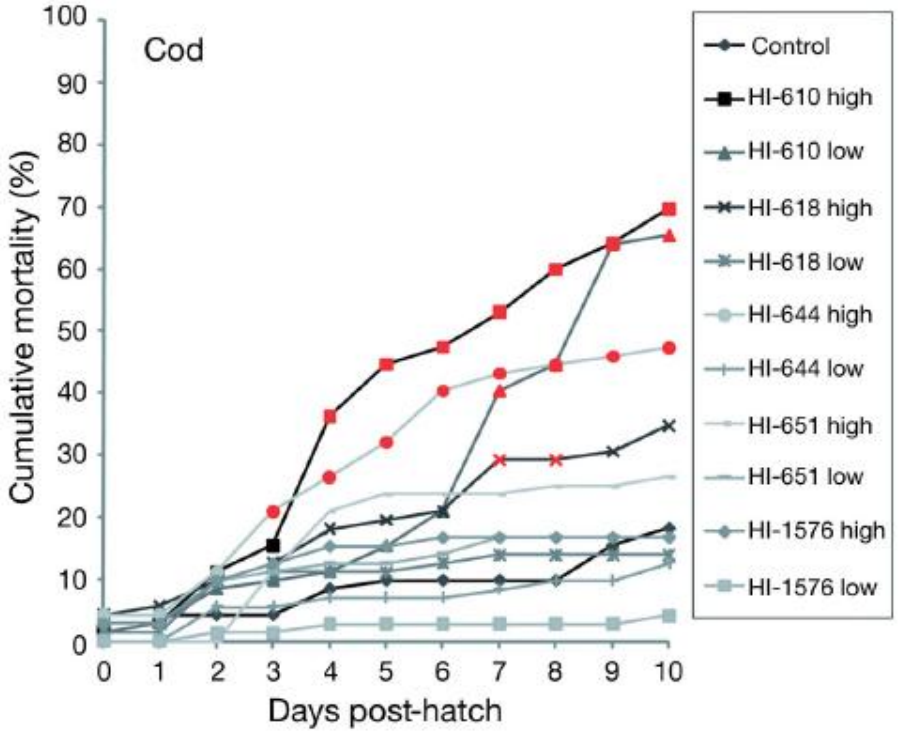

Fig. 3. *Gadus morhua*. Cumulative percentage mortality of cod larvae challenged with the same bacterial strains and doses as in Fig. 1. Day 0: day of hatching. Red symbols: mortality rates significantly different from the control group ($p < 0.01$ Bonferroni correction)

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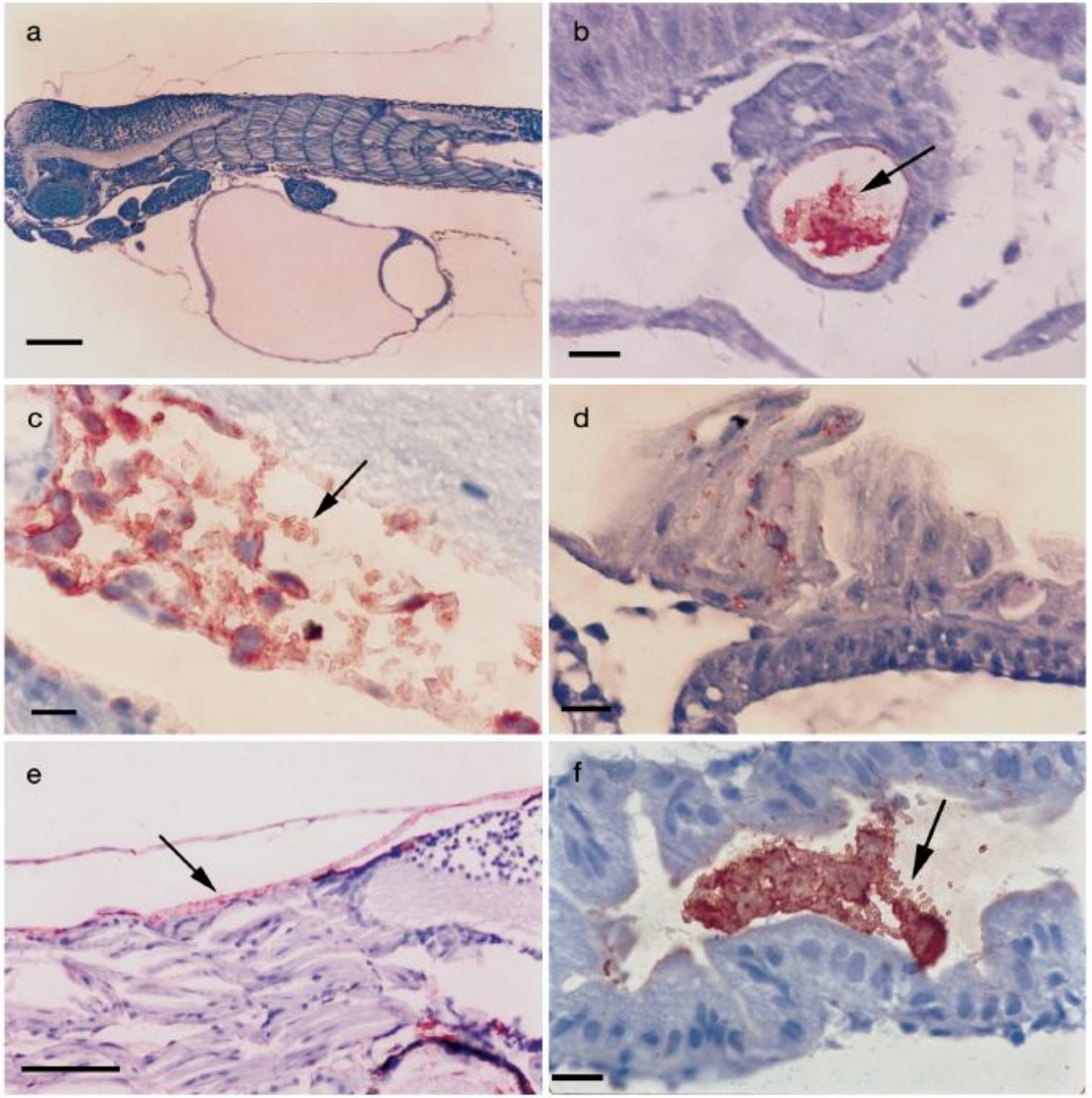
a
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Vol. 81: 203–217, 2008
doi: 10.3354/dao01934

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published September 24

OPEN ACCESS

Screening and characterisation of potentially pathogenic bacteria associated with Atlantic cod *Gadus morhua* larvae: bath challenge trials using a multidish system

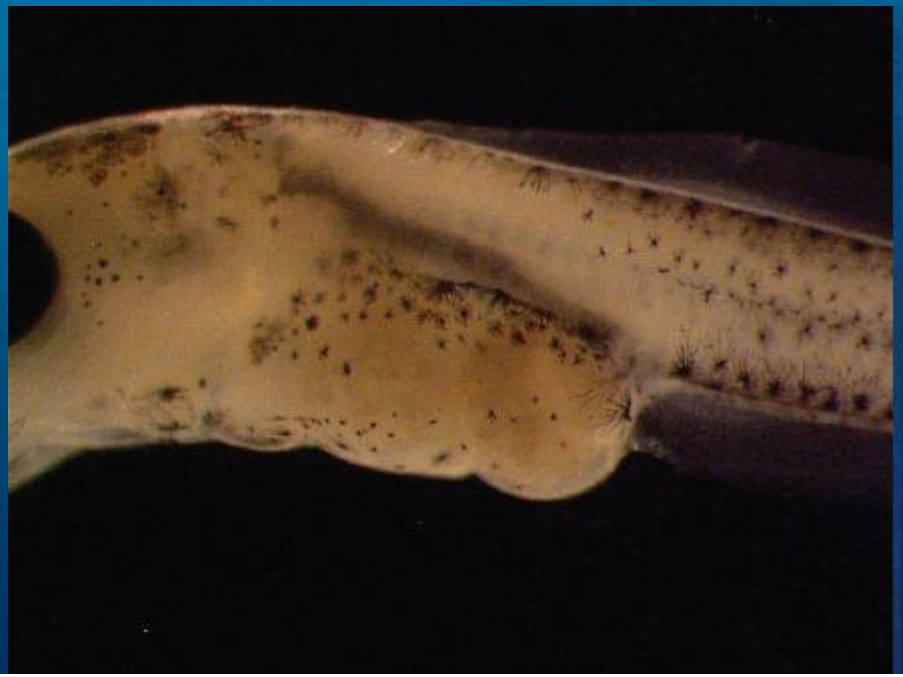
Nina Sandlund*, Øivind Bergh

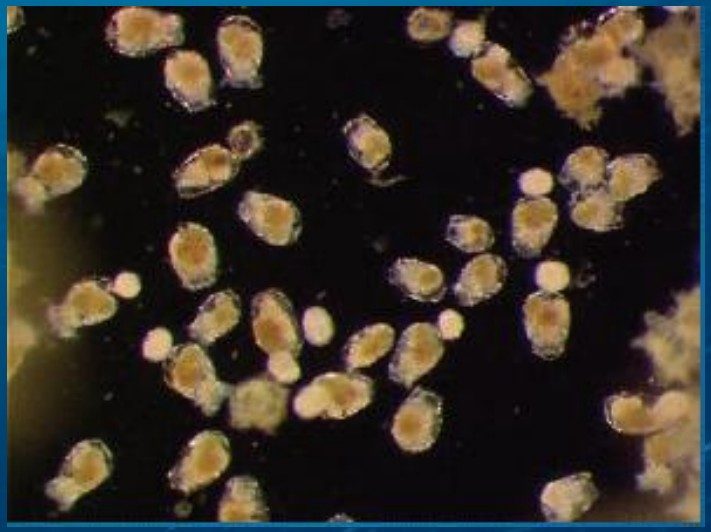
Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway

Most bacteria associated with larvae are opportunists, not able to cause mortality alone

V. anguillarum is a ”true” pathogen

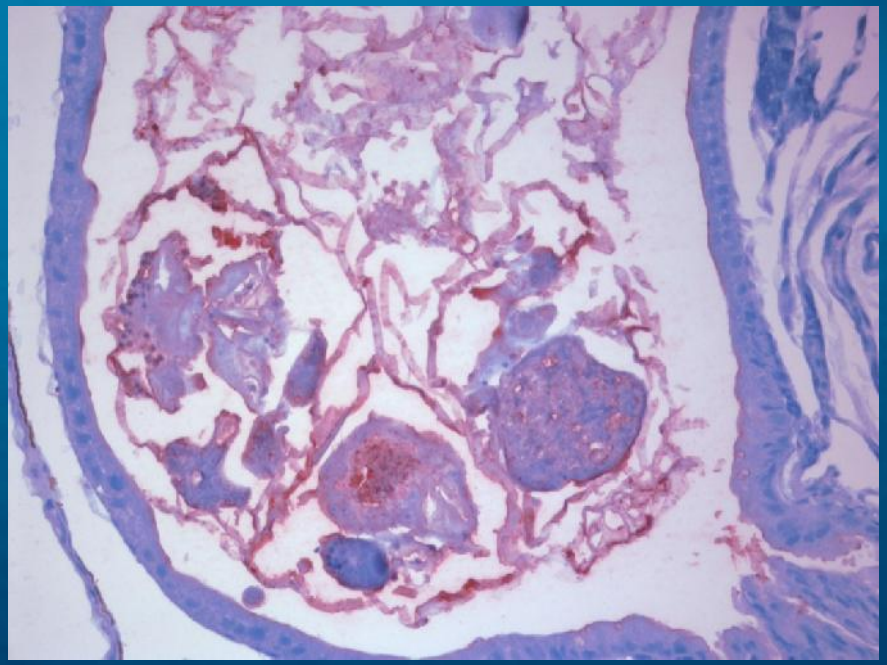
Challenges via live feed
= oral administration



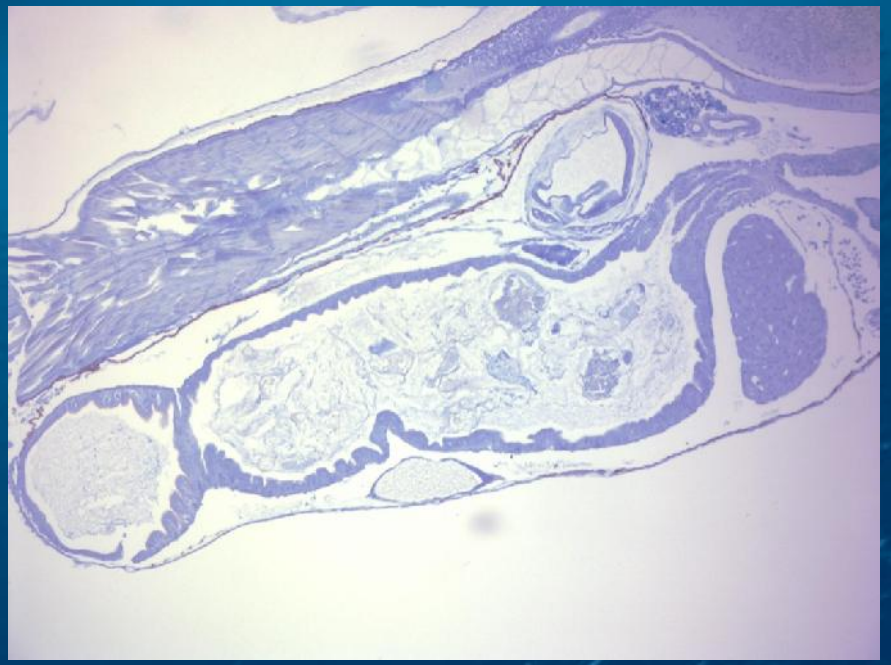


Challenge with *V. logei*

Intestine 24 h post challenge



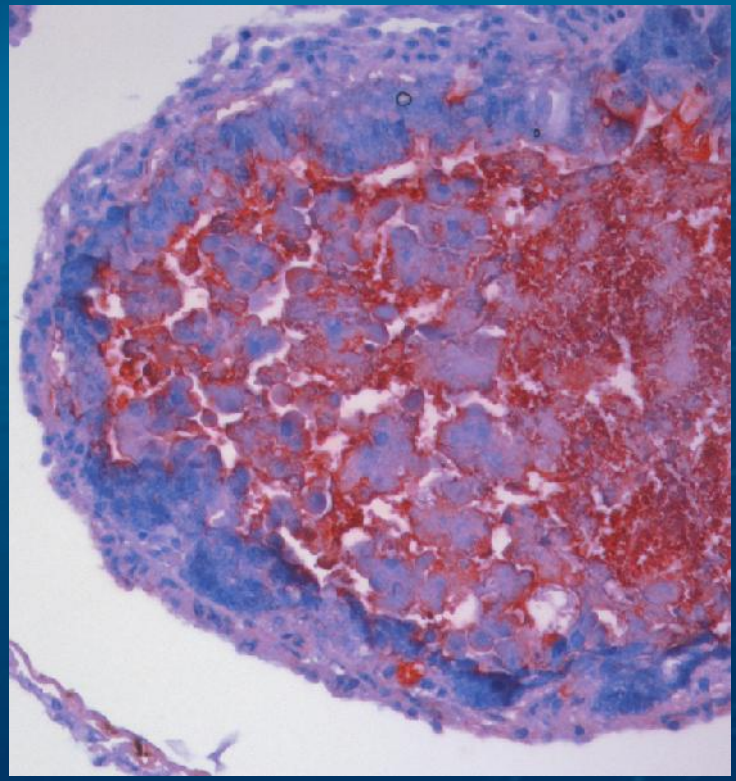
72 h post challenge



V. splendidus

Gills

Intestine



Vol. 69: 163–173, 2006 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published April 6

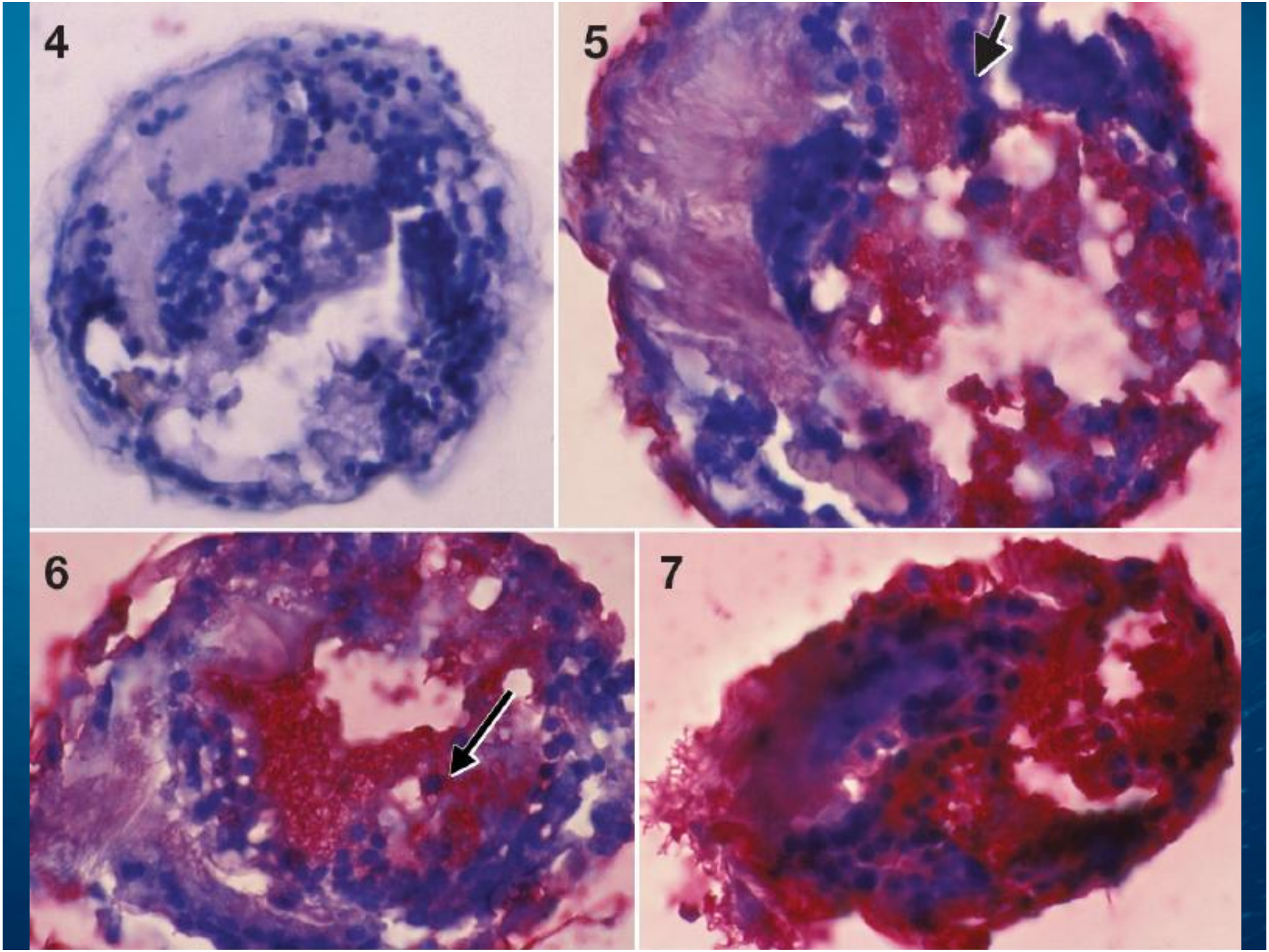
OPEN ACCESS

Immunohistochemistry of great scallop *Pecten maximus* larvae experimentally challenged with pathogenic bacteria

Nina Sandlund[1,*], Lise Torkildsen[1], Thorolf Magnesen[2], Stein Mortensen[1], Øivind Bergh[1]

[1]Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway
[2]Centre for Studies of Environment and Resources, University of Bergen, PO Box 7800, 5020 Bergen, Norway

- Immunohistochemstry: as in fish larvae
- Experimental setup: as in fish larvae
  - However – 10-20 larvae/well
- Mortality: read as absence of motility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Thank you for your attention!