
INSTITUTE OF MARINE RESEARCH
HAVFORSKNINGSINSTITUTTET

Prospects for probiotics with Atlantic halibut lavae

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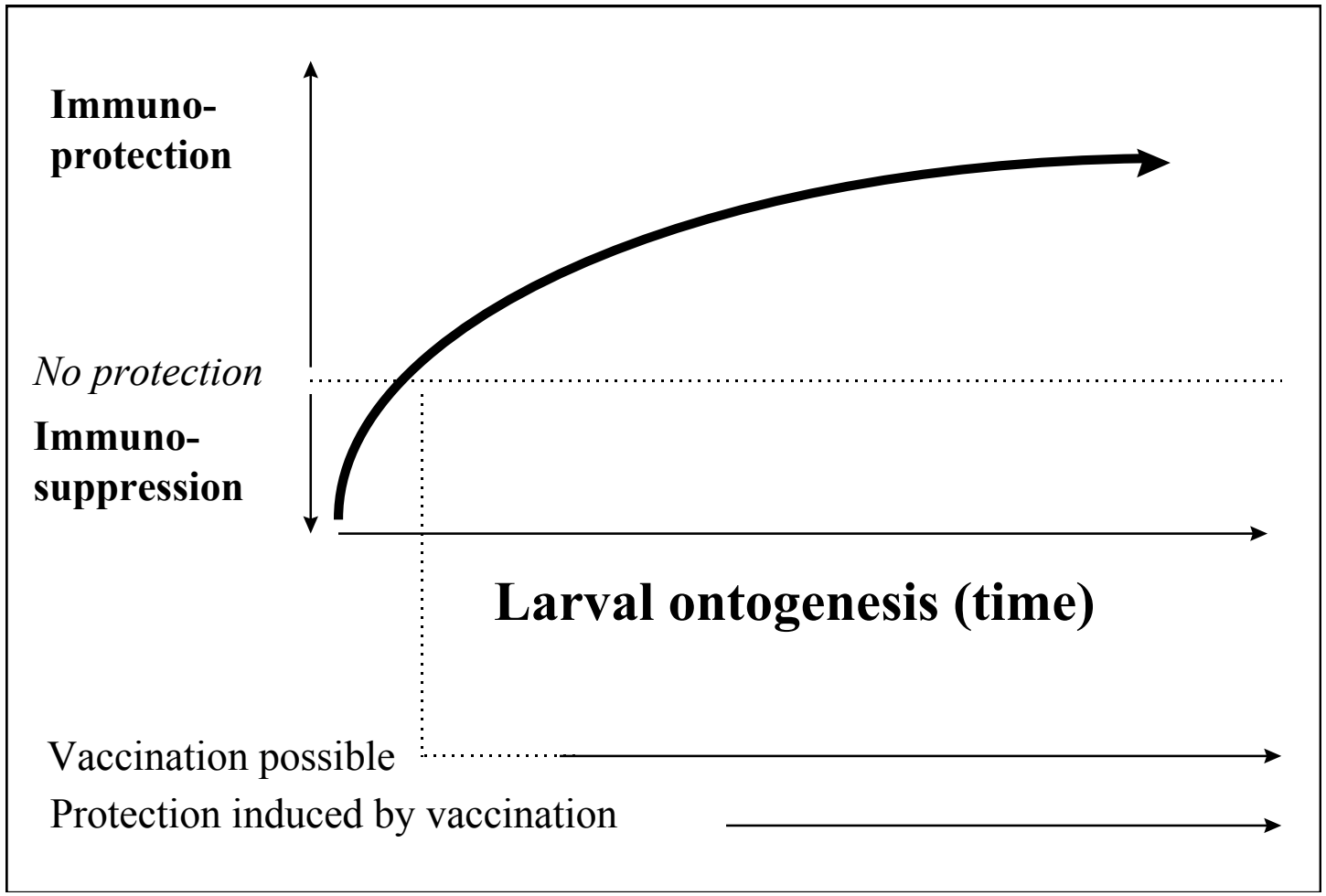
What is probiotics

- Beneficial bacteria which when added to the water or the food affects the health of the host in a positive way
- Often, but not always related to suppression of pathogens



Vadstein, Mo and Bergh 2004

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?

Collaborators: PROAQUA

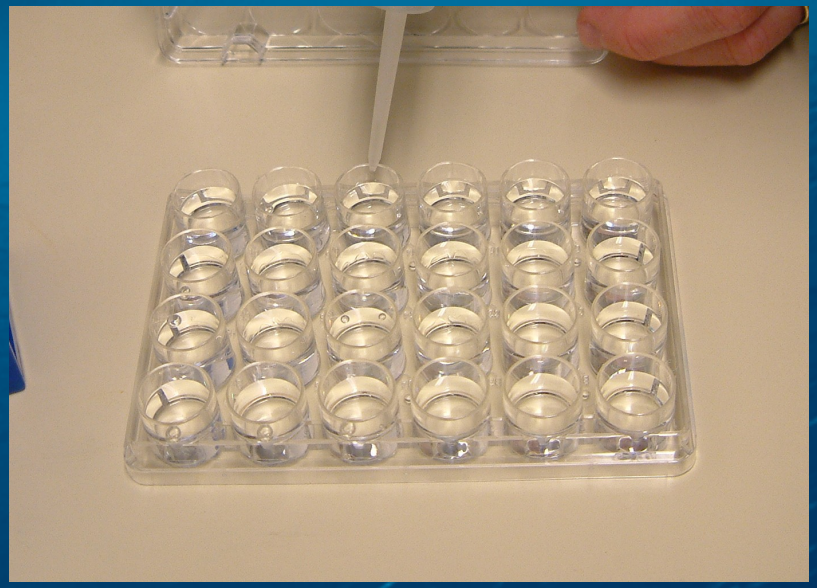
- Danish Technological University
- University of Bergen
- HMRC – Crete

Sequencing of community

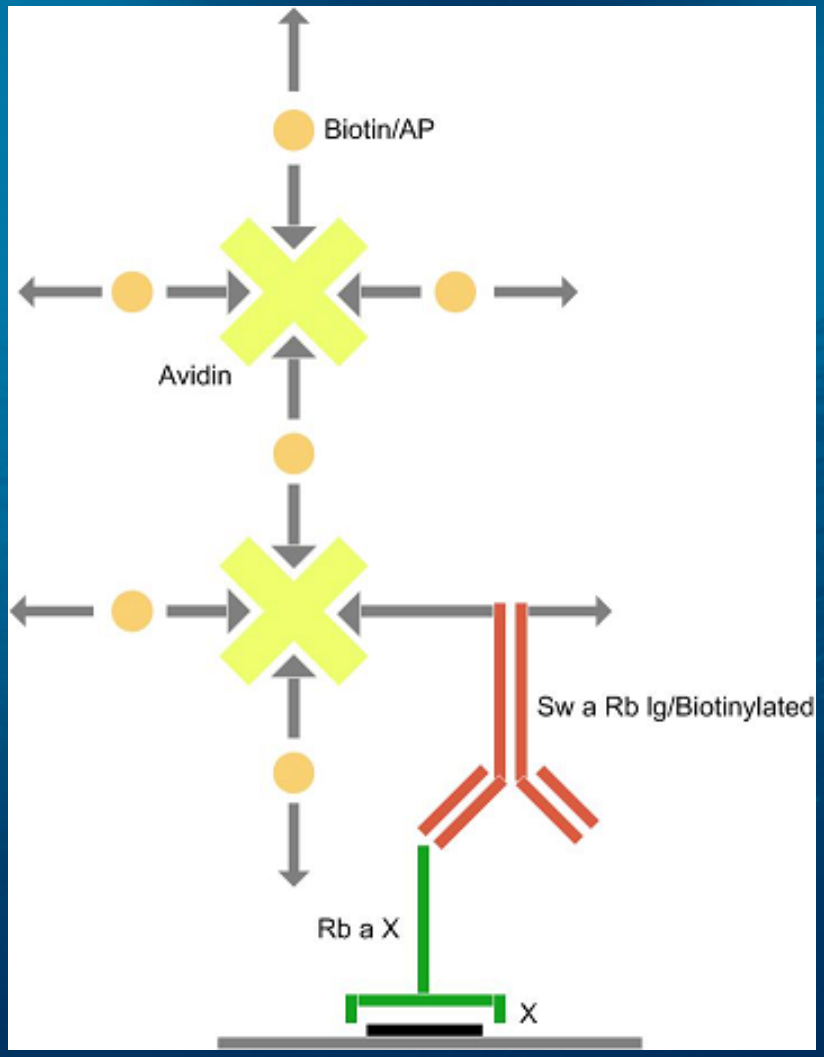
- To be performed by Dr. Nina Sandlund

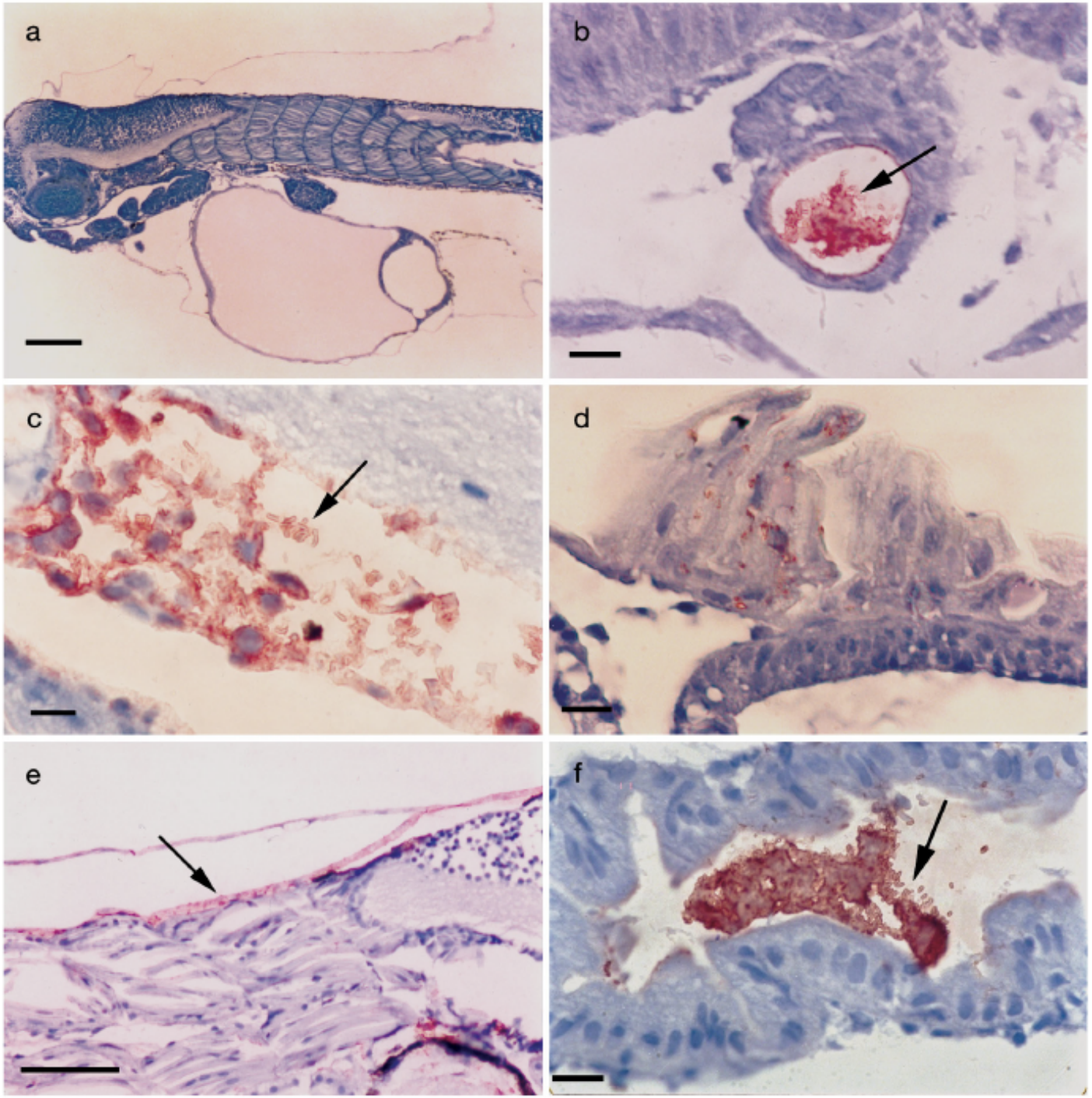
Challenge experiments on yolk sac larvae

- 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.



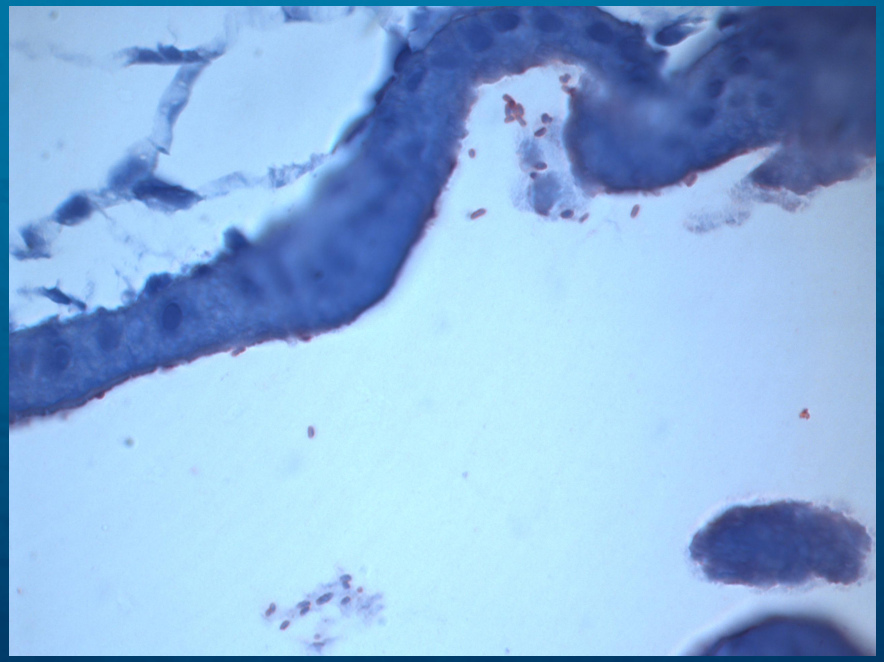
Immunohistochemistry



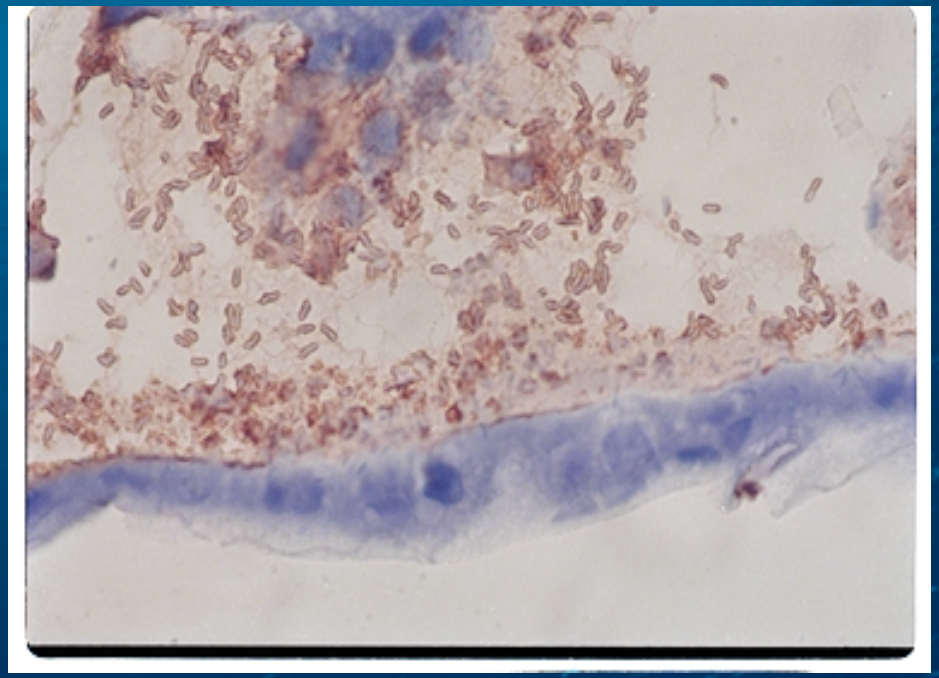

a
b
c
d
e
f

Immunohistochemistry – yolk sac larvae

Intestinal ephithelium - cod



Epidermis - halibut



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

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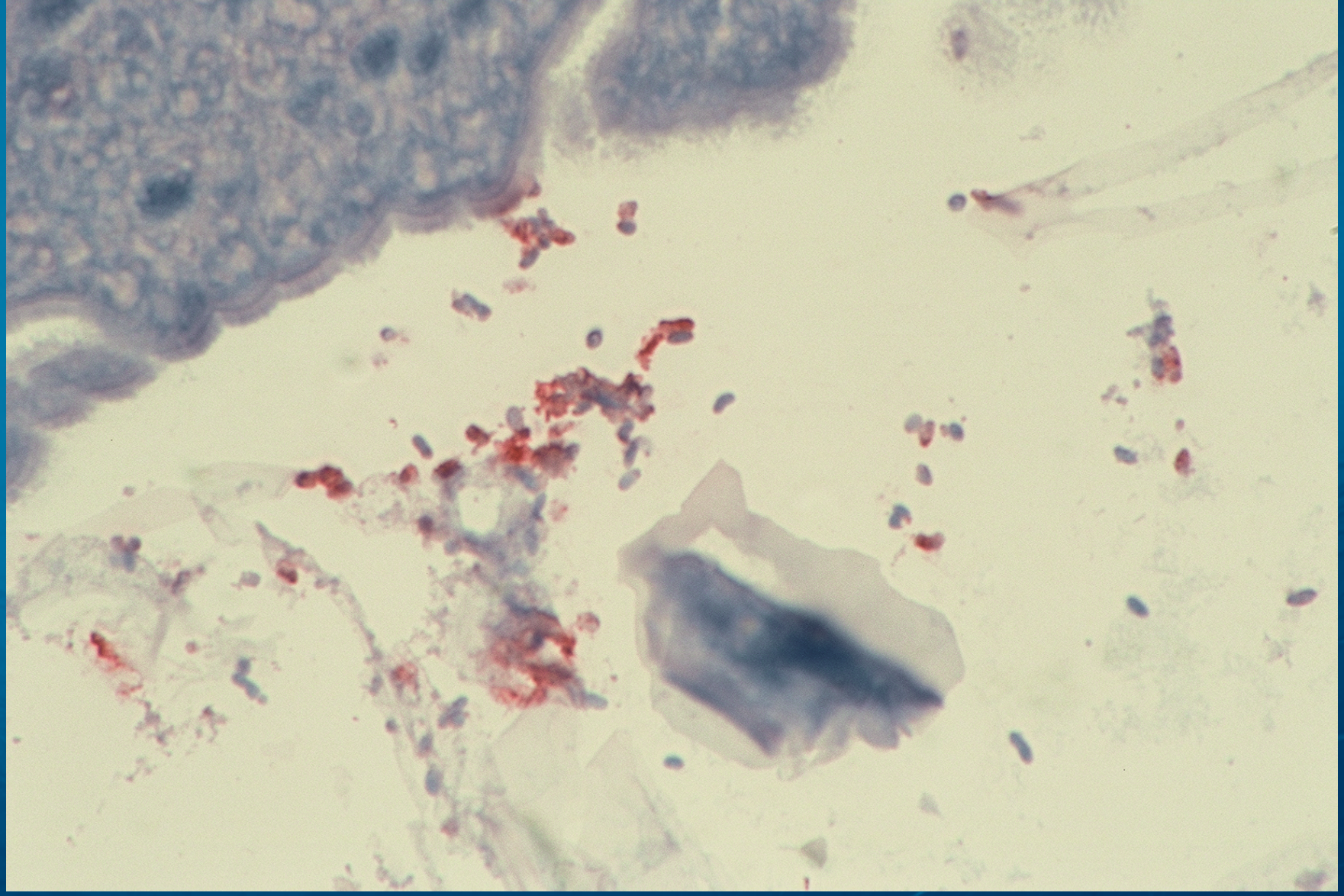
Addition of Roseobacter(Rugeria) 27-4 positively affected survival



Note: probiotics in lumen – NOT attached to epithelium

Comparative mortality pilot

5 different *Vibrio* spp

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

3 different fish larvae: turbot, halibut, cod

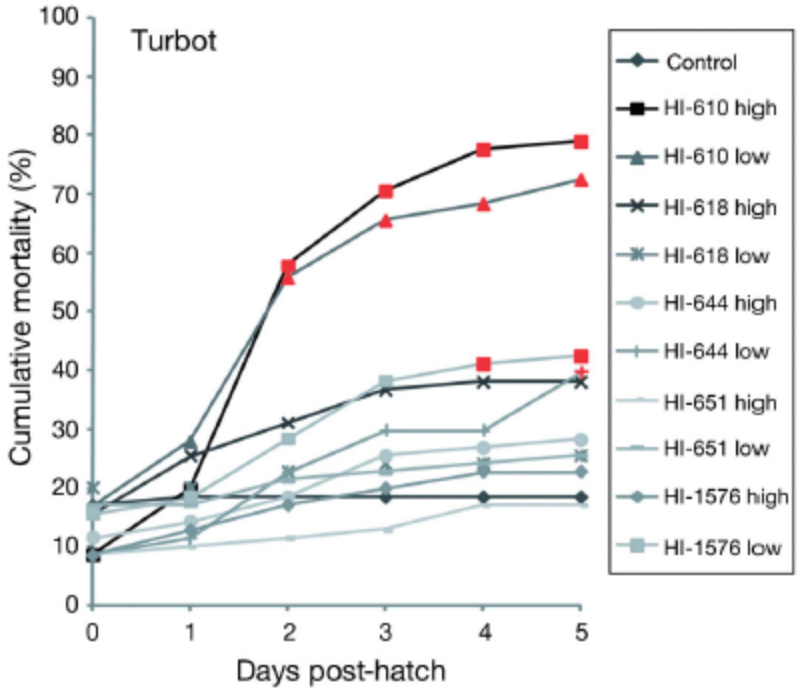


Fig. 1. *Scophthalmus maximus*. Cumulative percentage mortality of turbot larvae challenged with the bacterial strains HI-610, HI-618, HI-644, HI-651, and HI-1576 (see Table 1 for bacterial strains used. High: challenge dose 10^6 CFU ml^{-1}, low: challenge dose 10^4 CFU ml^{-1}, control: unchallenged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

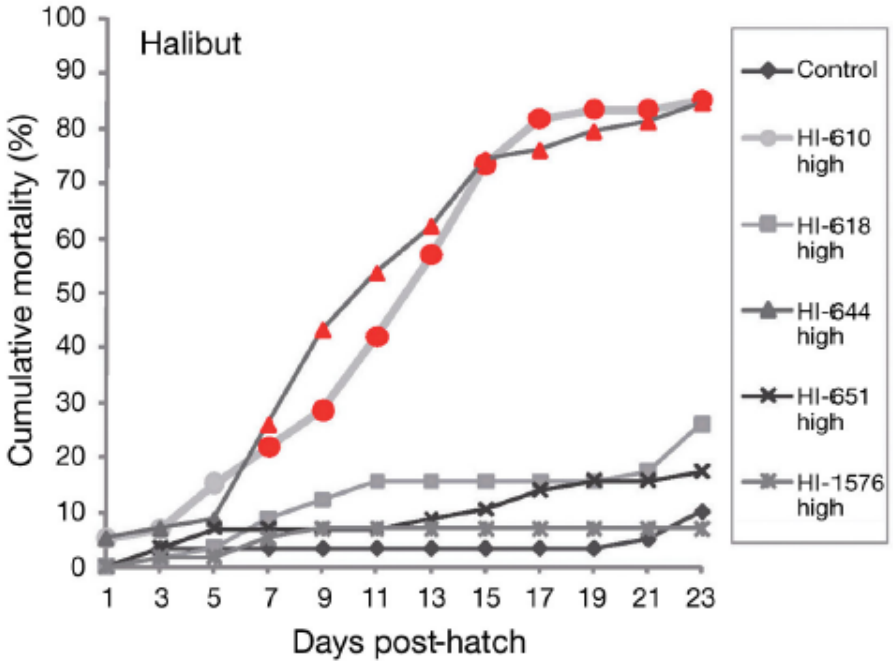


Fig. 2. *Hippoglossus hippoglossus*. Cumulative percentage mortality of halibut larvae challenged with the bacterial strains HI-610, HI-618, HI-644, HI-651, and HI-1576 (see Table 1 for bacterial strains used). High: challenge dose 10^6 CFU ml^{-1}, control: unchallenged larvae. Red symbols: mortality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

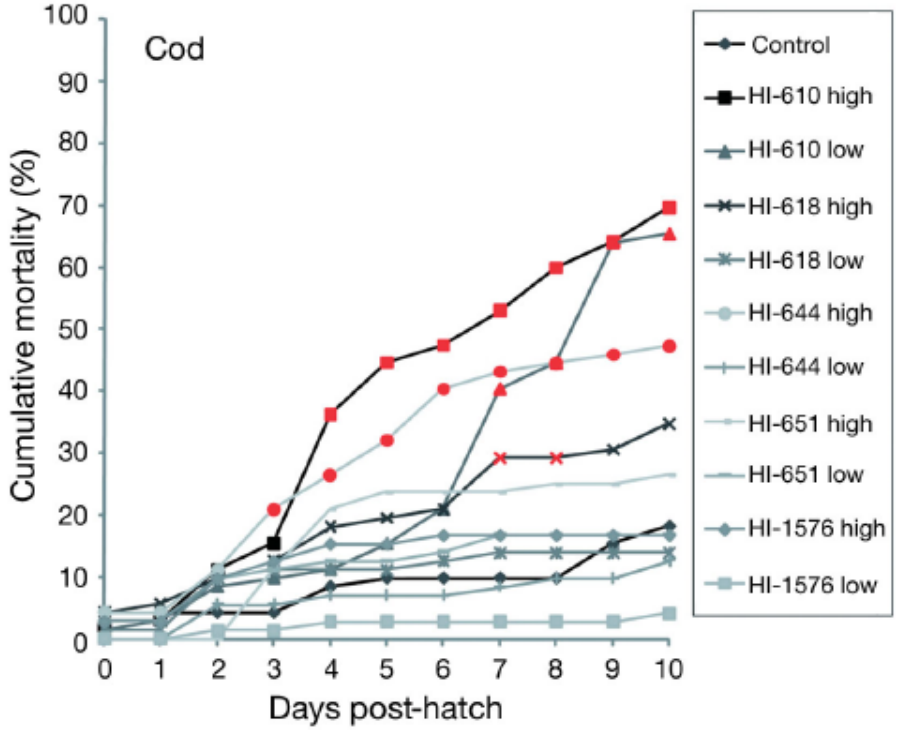
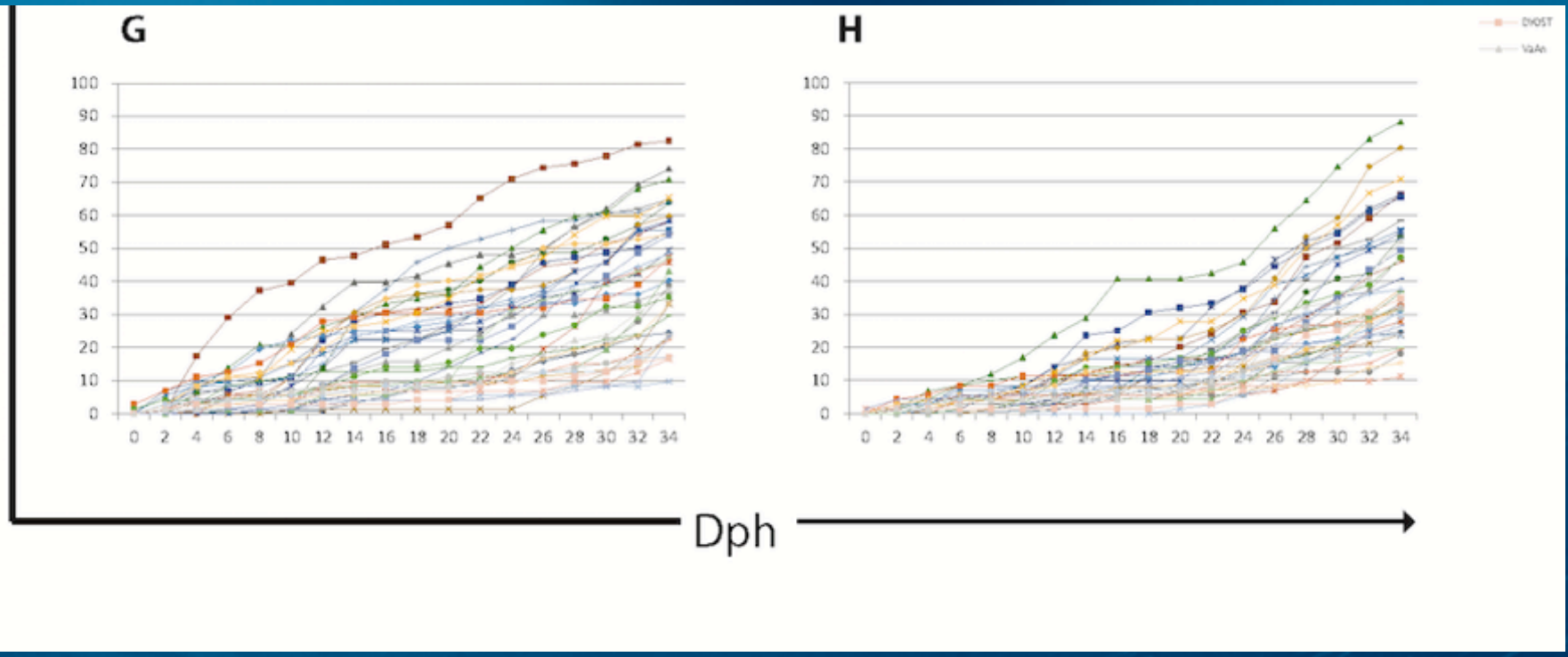


Fig. 3. *Gadus morhua*. Cumulative percentage mortality of cod larvae challenged with the same bacterial strains and doses as in Fig. 1. Day 0: day of hatching. Red symbols: mortality rates significantly different from the control group ($p < 0.01$ Bonferroni correction)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80
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40
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20
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0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Dph

Table 1. Bacterial strains and plasmids.

Strain or plasmid	Genotype or relevant markers
Strains	
P. gallaeciensis BS107 (DSM17395)	Wild type
P. gallaeciensis BS107-Pda8	CDS104961::EZ-Tn5, Kan^R
P. gallaeciensis dsRed	MiniTn7(Gm^R)$P_{A1/04/03}$*DsRed*Express-a
V. anguillarum NB10	Serotype O1, cm^R, PA1/04/03-RBSII-*gfp*mut3*-T1
V. anguillarum HI610	Serotype O2α
Plasmids	
EZ-Tn5™ Transposome	EZ-Tn5<R6Kγori, Kan^R>Tnp
pAKN132	miniTn7(Gm)$P_{A1/04/03}$DsRedExpress-a
pUX-BF13	Helper plasmid: Tn7 transposase proteins
pPDA11	*tdaCp::gfp* ligated into broad host range vector pRK415

doi:10.1371/journal.pone.0043996.t001

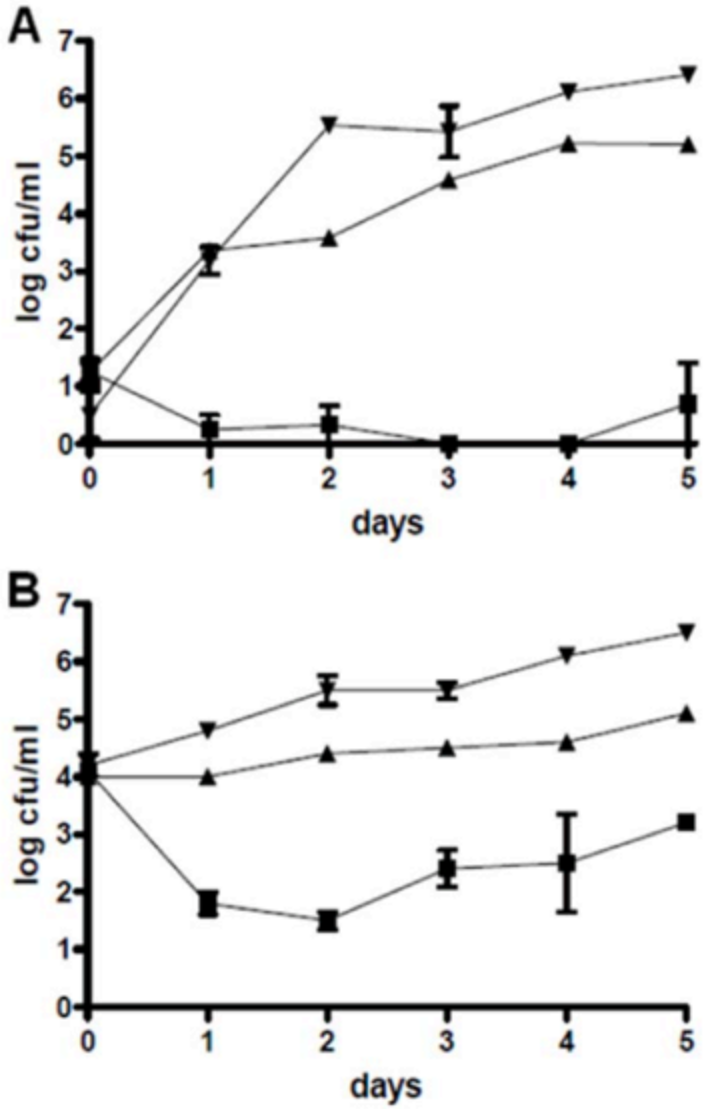

Figure 3. Reduction of *V. anguillarum* in cultures of *Tetraselmis suecica* by *Phaeobacter gallaeciensis*. Colony-forming units of *V. anguillarum* inoculated at 10^1 cfu/ml (A) and at 10^4 cfu/ml (B) in presence of *P. gallaeciensis* wild type (■), in presence of the *P. gallaeciensis* TDA-negative mutant (▲), and in the monoxenic control (▼).

doi:10.1371/journal.pone.0043996.g003

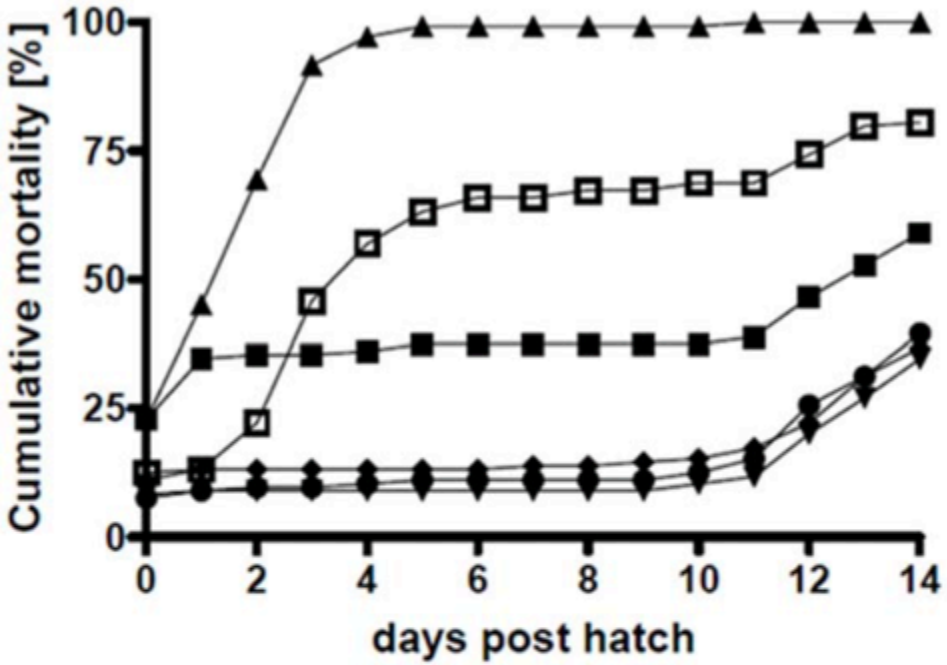

Figure 7. Mortality of cod larvae during the challenge trials. Mean values of two independent triplicate experiments. The single-larvae cultures were simultaneously inoculated with *P. gallaeciensis* wild type and *V. anguillarum* (T5, ●), or with the TDA-negative mutant of *P. gallaeciensis* and *V. anguillarum* (T6, □). Unexposed larvae and larvae exposed to single bacterial strains acted as controls: Negative Control (T1, ■), only *V. anguillarum* (T2, ▲), only *P. gallaeciensis* wild type (T3, ▼), and only *P. gallaeciensis* TDA-negative mutant (T4, ◆).
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Screening and characterisation of potentially pathogenic bacteria associated with Atlantic cod *Gadus morhua* larvae: bath challenge trials using a multidish system

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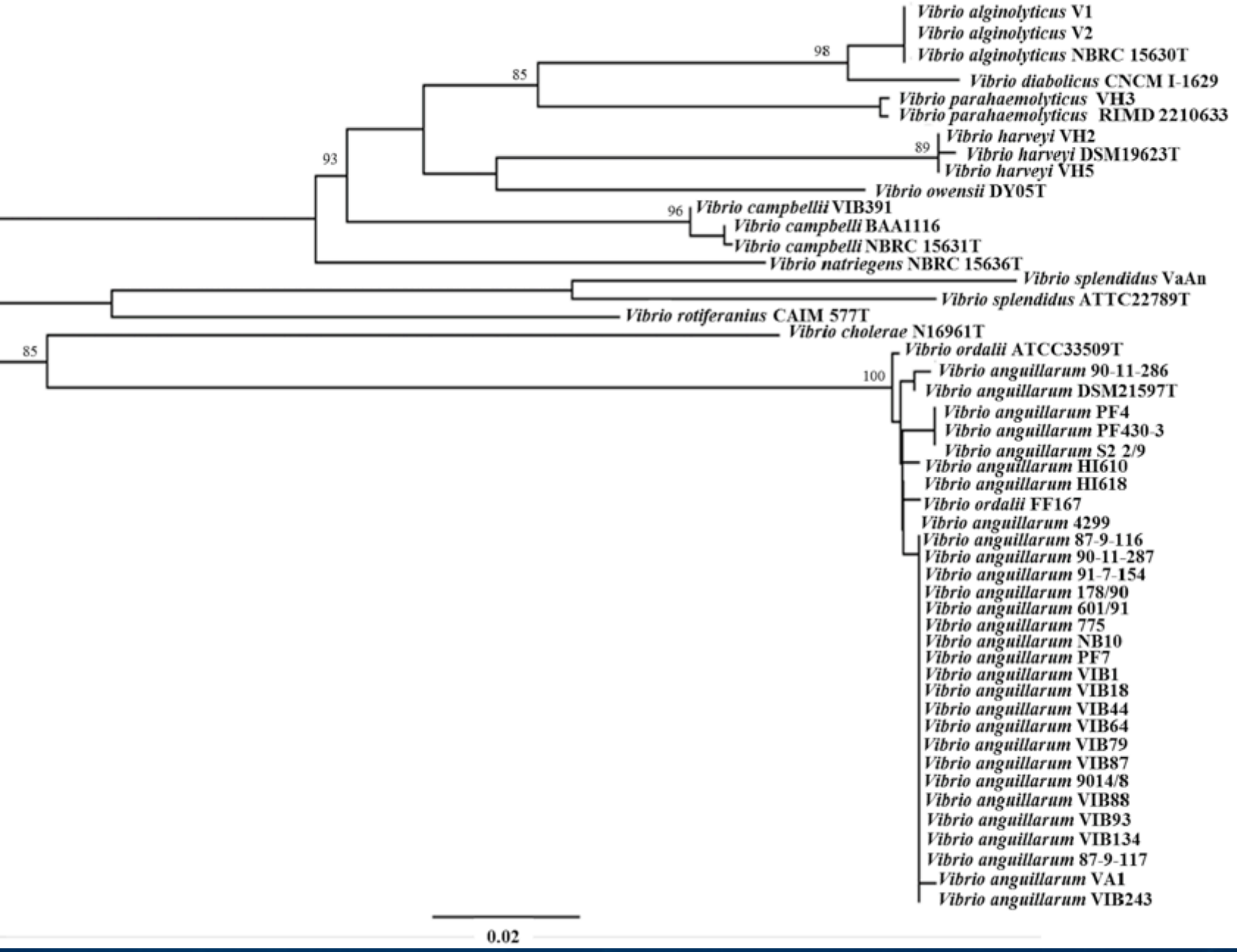
Most bacteria associated with larvae are opportunists, not able to cause mortality alone

V. anguillarum is a ”true” pathogen


Vibrio alginolyticus V1
Vibrio alginolyticus V2
Vibrio alginolyticus NBRC 15630T
Vibrio diabolicus CNCM I-1629
Vibrio parahaemolyticus VH3
Vibrio parahaemolyticus RIMD 2210633
Vibrio harveyi VH2
Vibrio harveyi DSM19623T
Vibrio harveyi VH5
Vibrio owensii DY05T
Vibrio campbellii VIB391
Vibrio campbelli BAA1116
Vibrio campbelli NBRC 15631T
Vibrio natriegens NBRC 15636T
Vibrio splendidus VaAn
Vibrio splendidus ATTC22789T
Vibrio rotiferanius CAIM 577T
Vibrio cholerae N16961T
Vibrio ordalii ATCC33509T
Vibrio anguillarum 90-11-286
Vibrio anguillarum DSM21597T
Vibrio anguillarum PF4
Vibrio anguillarum PF430-3
Vibrio anguillarum S2 2/9
Vibrio anguillarum HI610
Vibrio anguillarum HI618
Vibrio ordalii FF167
Vibrio anguillarum 4299
Vibrio anguillarum 87-9-116
Vibrio anguillarum 90-11-287
Vibrio anguillarum 91-7-154
Vibrio anguillarum 178/90
Vibrio anguillarum 601/91
Vibrio anguillarum 775
Vibrio anguillarum NB10
Vibrio anguillarum PF7
Vibrio anguillarum VIB1
Vibrio anguillarum VIB18
Vibrio anguillarum VIB44
Vibrio anguillarum VIB64
Vibrio anguillarum VIB79
Vibrio anguillarum VIB87
Vibrio anguillarum 9014/8
Vibrio anguillarum VIB88
Vibrio anguillarum VIB93
Vibrio anguillarum VIB134
Vibrio anguillarum 87-9-117
Vibrio anguillarum VA1
Vibrio anguillarum VIB243
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89
93
96
85
100
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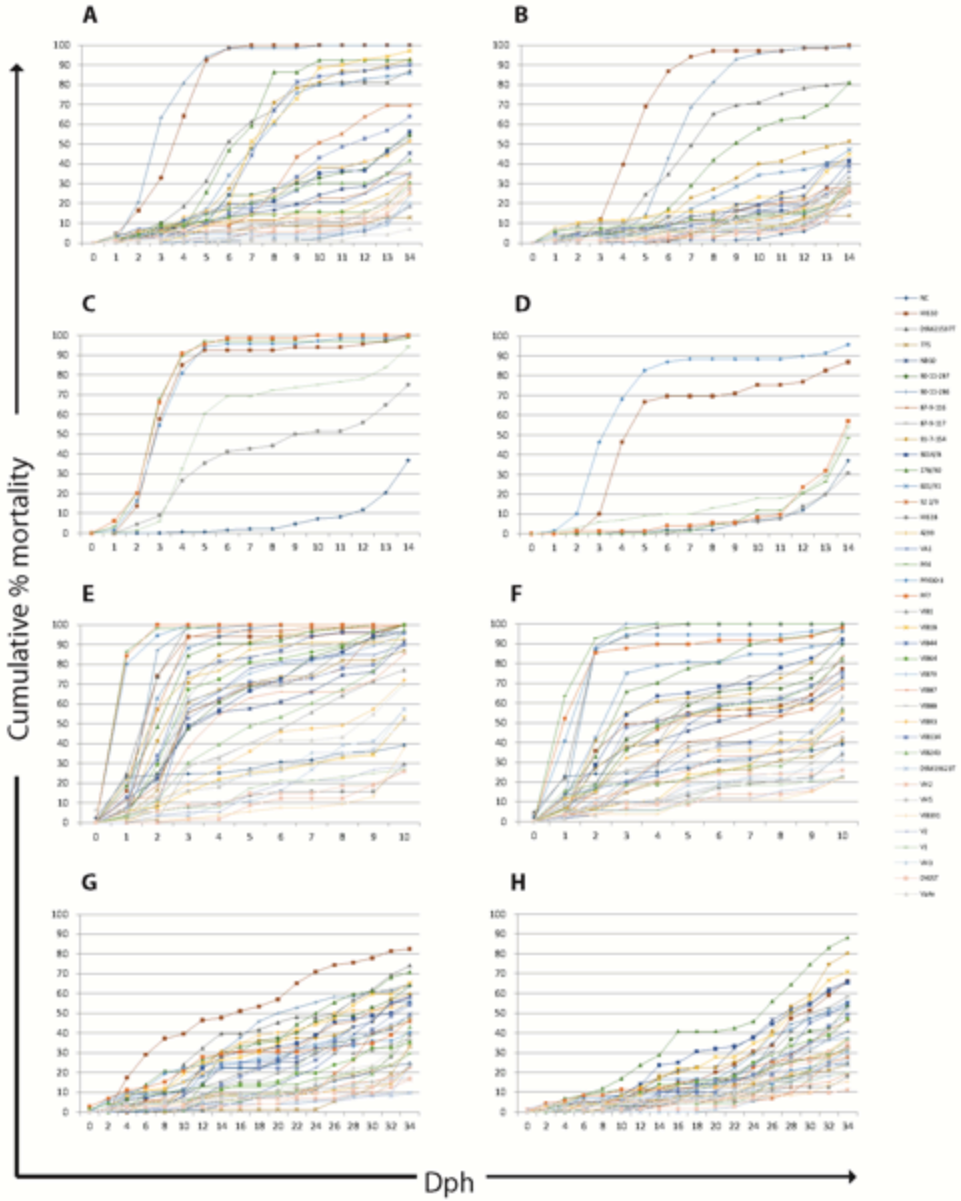
A
B
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Cumulative % mortality
Dph

Comparative assessment of *Vibrio* virulence in marine fish larvae

Anita Rønneseth[1+], Daniel Castillo[2+], Paul D'Alvise[3+,6], Øyvind Tønnesen[1], Gyri Haugland[1], Torben Grotkjær[3], Kirsten Engell-Sørensen[4], Louise Nørremark[4], Øivind Bergh[5], Heidrun I. Wergeland[1] and Lone Gram[3]

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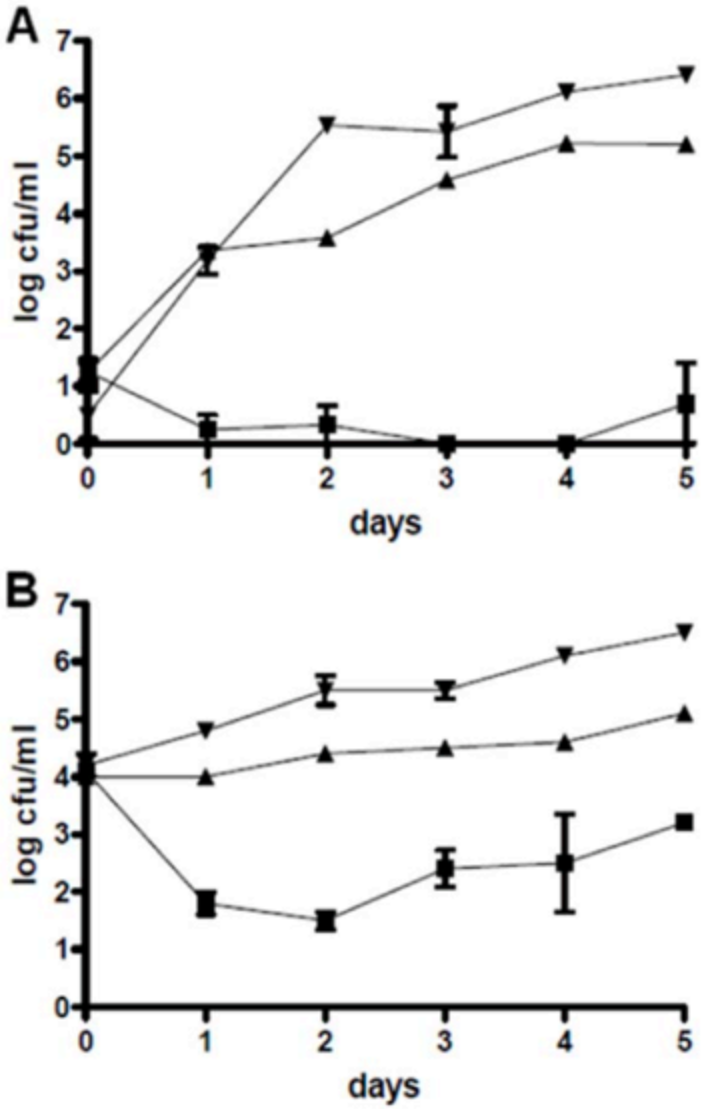

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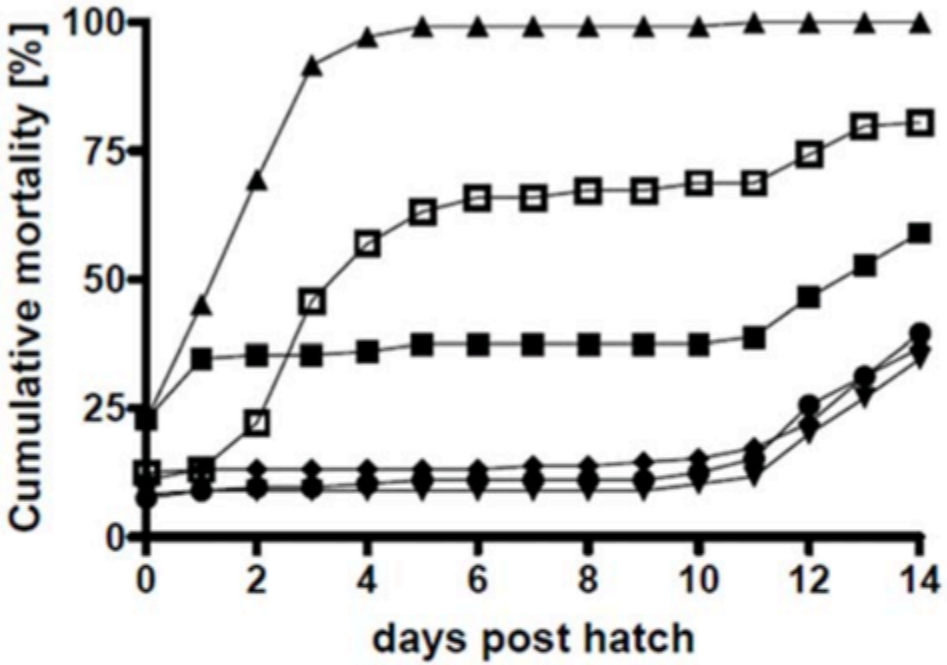
doi:10.1371/journal.pone.0043996.g003



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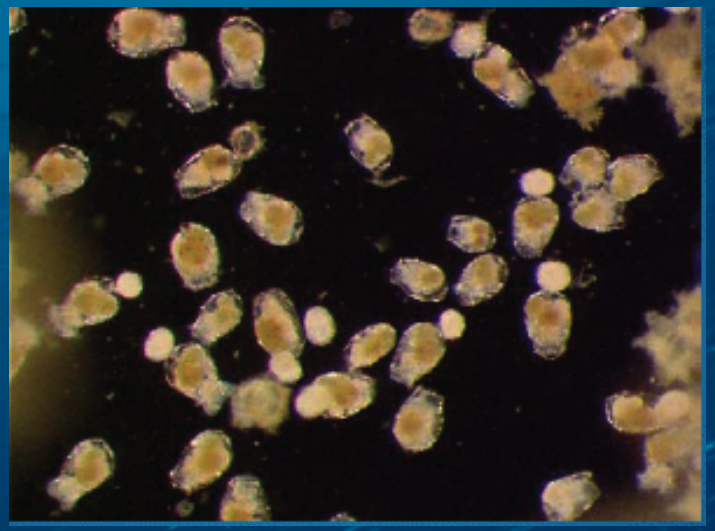
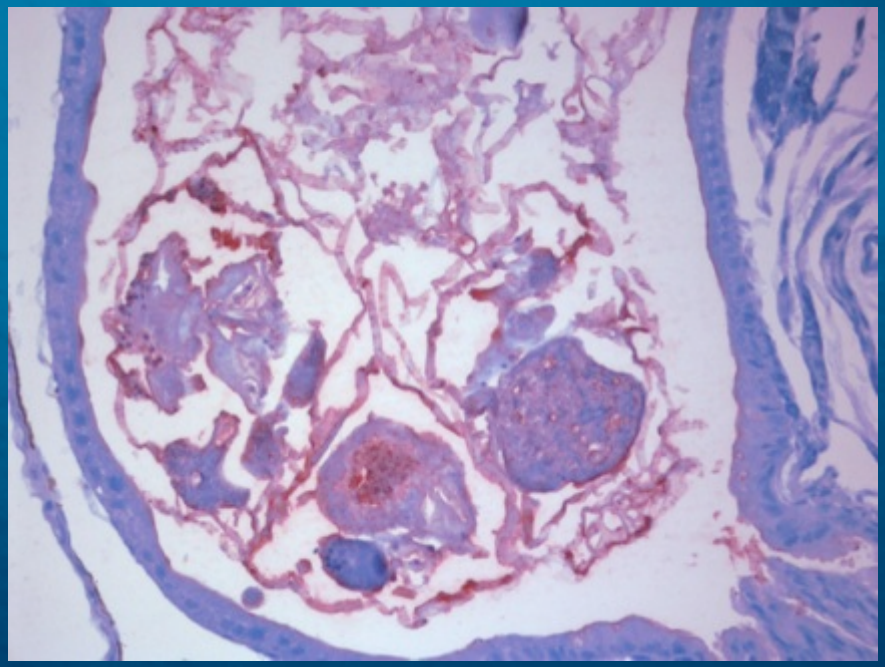
doi:10.1371/journal.pone.0043996.g007

Challenges wia live feed = oral administration

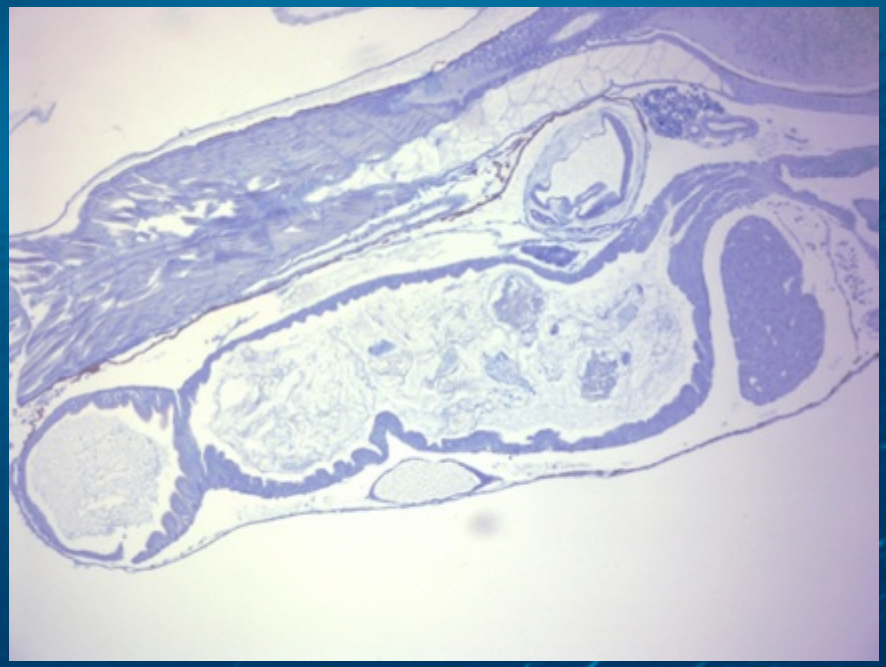


Challenge with *V. logei*

Intestine 24 h post challenge

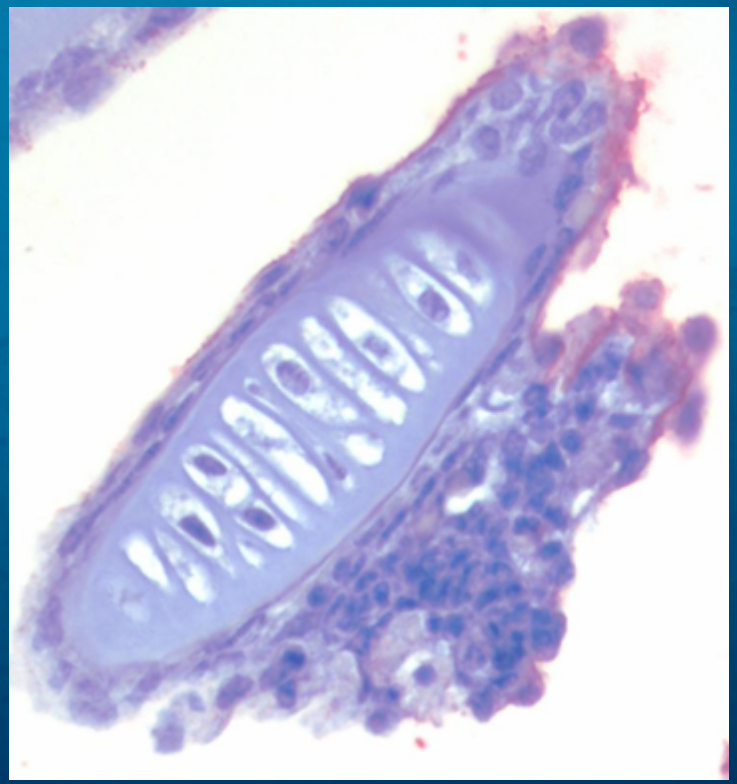


72 h post challenge

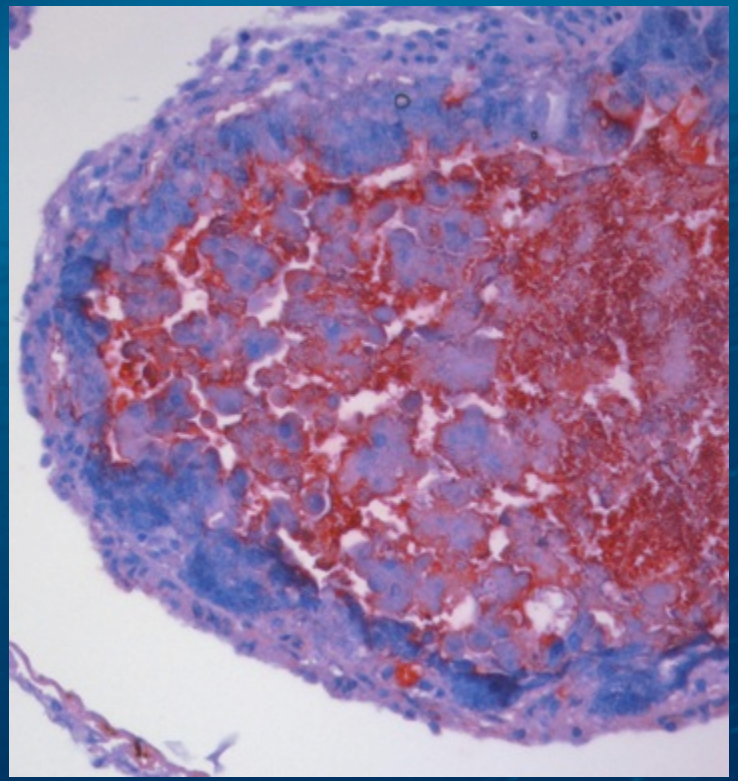


V. splendidus

Gills



Intestine

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