

# Pancreas disease – factors influencing transmission and infection

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Thanks to.....

Research council of Norway:

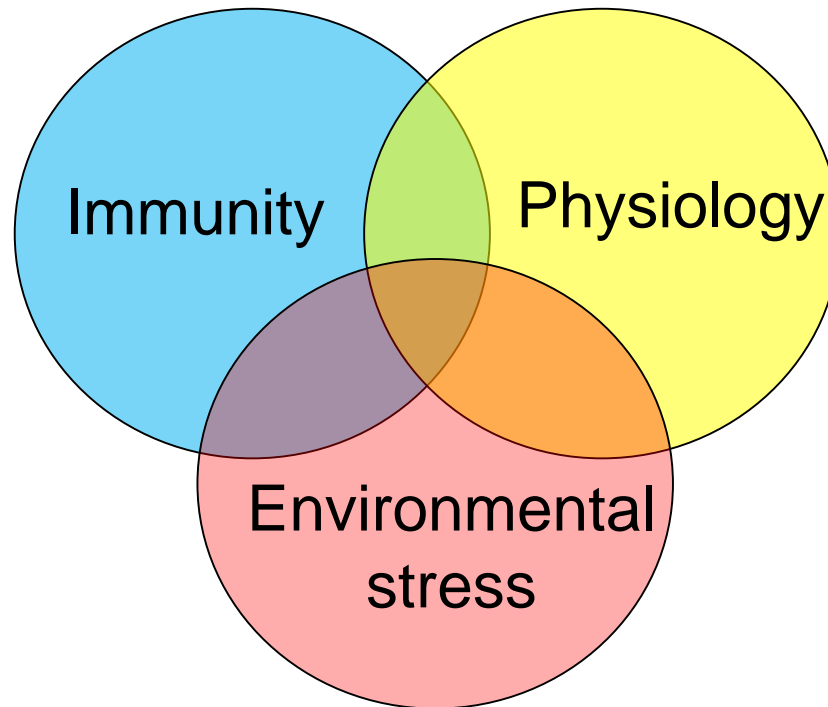
Toppforsk research Grant 224885/E40, MitSAV

Ministry of trade, industry and fisheries through Aquaculture  
program, IMR

NORAD

# Projects: MitSAV and PATHOS

1. Understand the effects of physiological and environmental factors on SAV infectivity, latency, and survival, and how these relate to the development of the clinical symptoms of PD
2. Develop methods and tools to better understand the immune response of Atlantic salmon to SAV3



# Objectives

1. Develop tools and assays esp SAV challenge model in post-smolt stages
2. Characterise the effect of sub-optimal physiological and environmental parameters on host, pathogen and host-pathogen interaction
3. Characterise the role of specific immune cells, and immune response during a SAV infection
4. Investigate the SAV susceptibility and immune response in both diploid and triploid Atlantic salmon
5. Study correlation between SAV3 infection and microbiome of salmon

# Factors influencing salmon-SAV interaction

## Salmon:

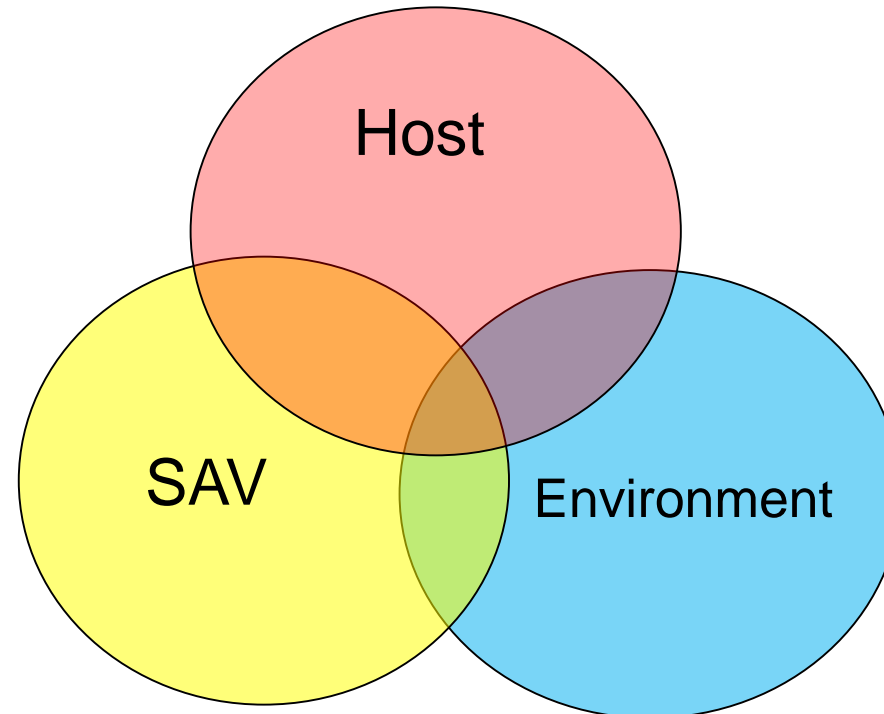
- Physiology
- Innate and adaptive immune responses
- Genetics and Ploidy

## SAV:

- Sub-type and isolate
- Virulence
- Infectivity and dose
- Host specificity
- Survival in and outside host

## Environment/other factors:

- Time
- Temperature
- Organic load
- Oxygen
- UV radiation
- Microbiome

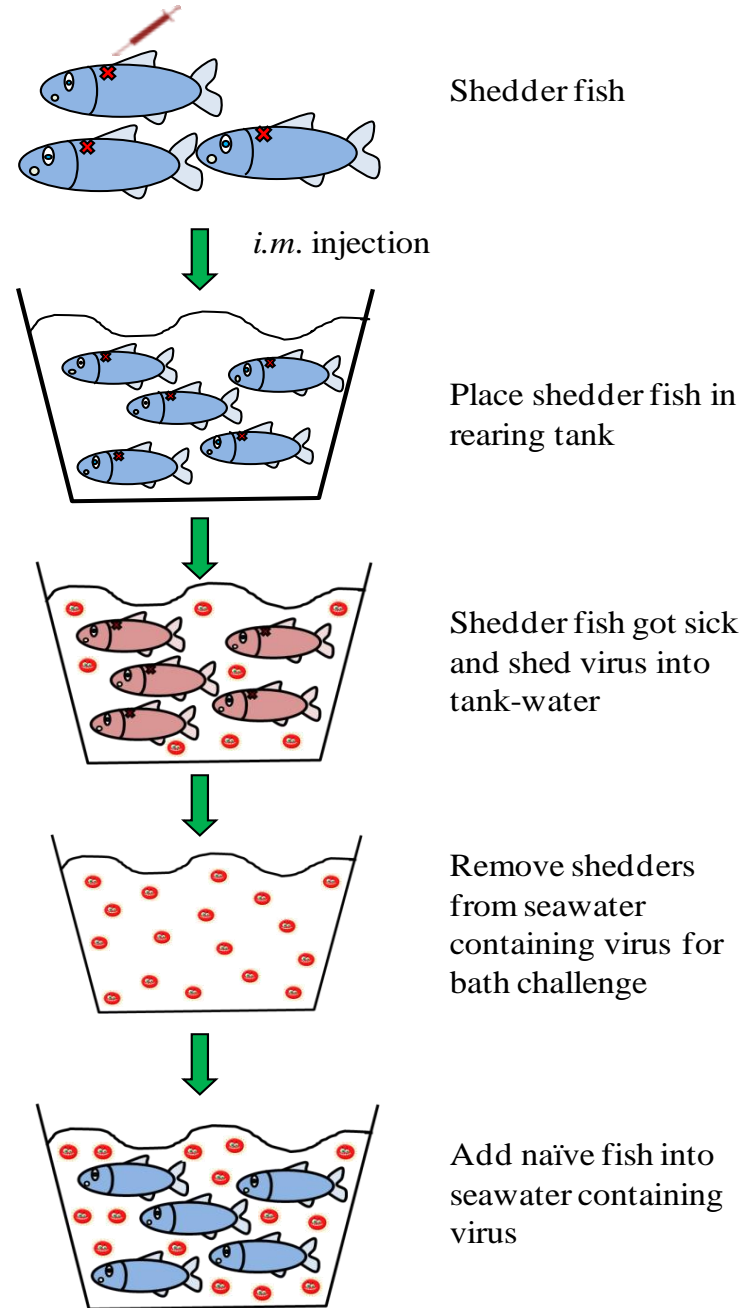


# Objective

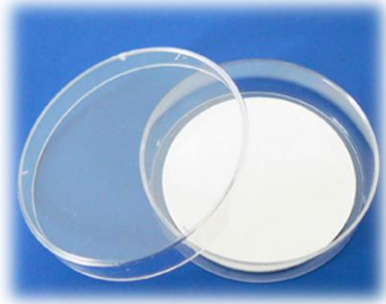
1. Develop tools and assays esp SAV challenge model in post-smolt stages
  - Establish bath challenge model with SAV3 in post-smolts
  - Optimise SAV analysis method for seawater

# Bath challenge model

- Natural infection route
- Defined time of infection
- Dose quantitated by RT-qPCR and TCID50



# Optimised method for quantitating SAV in water



Place filter upside down into petri dish



Add L-15+FCS medium



Shake for 15 min



Collect the eluent



RT-qPCR

+

50% end-point tissue culture infectious dose (TCID<sub>50</sub>)



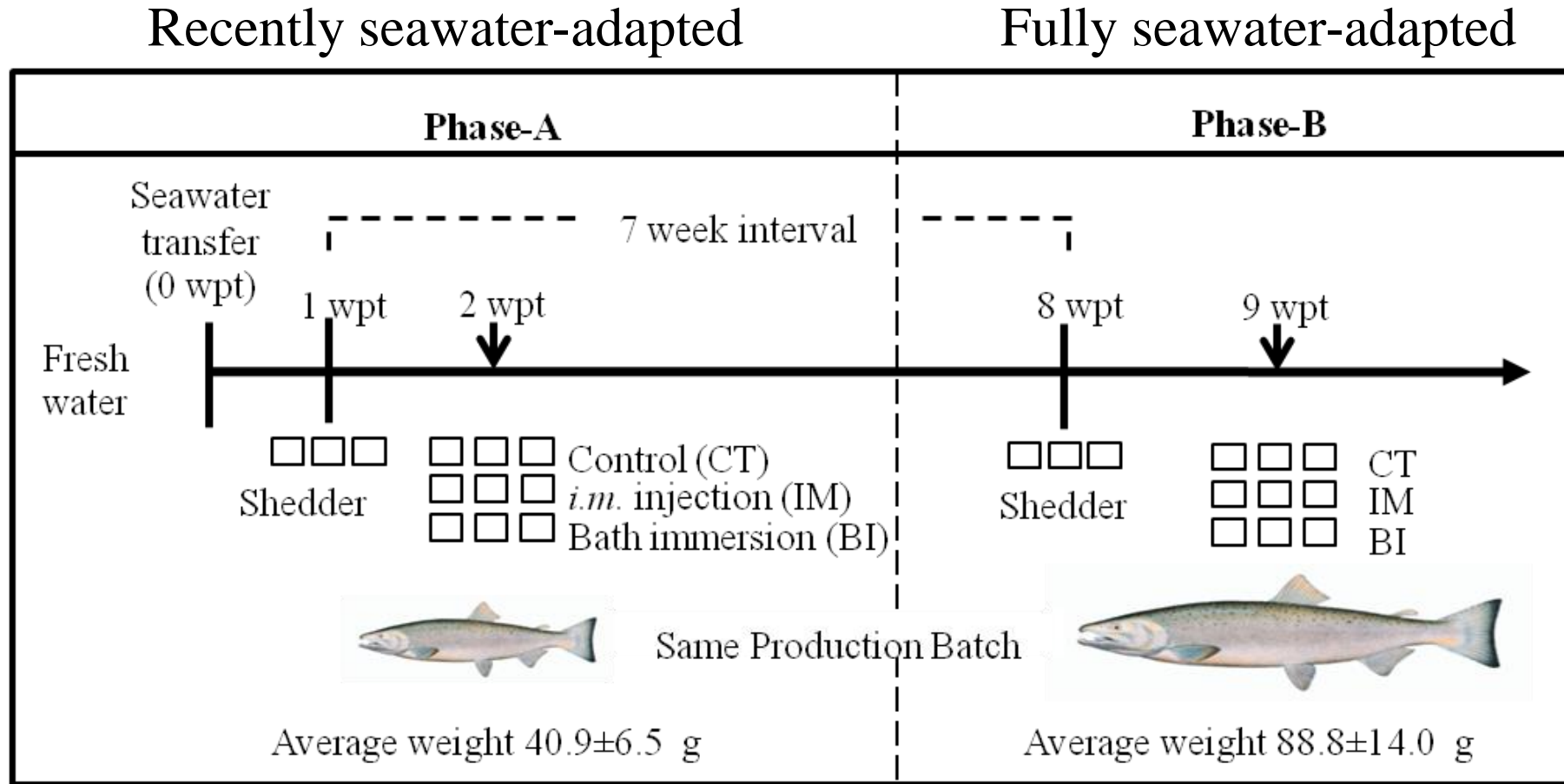


# Objective

2. Characterise the effect of sub-optimal physiological and environmental parameters on host, pathogen and host-pathogen interaction

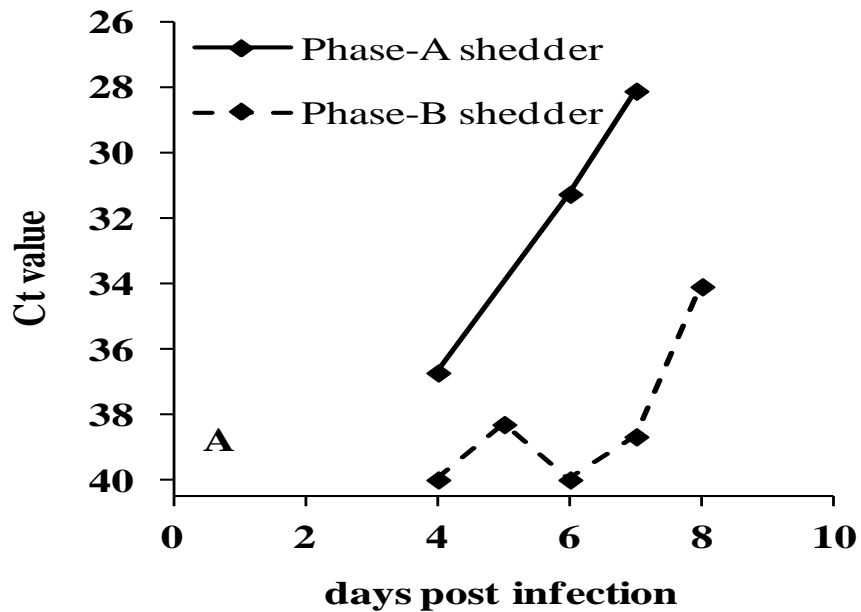
- Susceptibility of salmon at two different time-points post sea water transfer
- Impact of viral dose on infection dynamics
- Survival of SAV at different temperatures and in different “medium”

# Susceptibility to SAV3

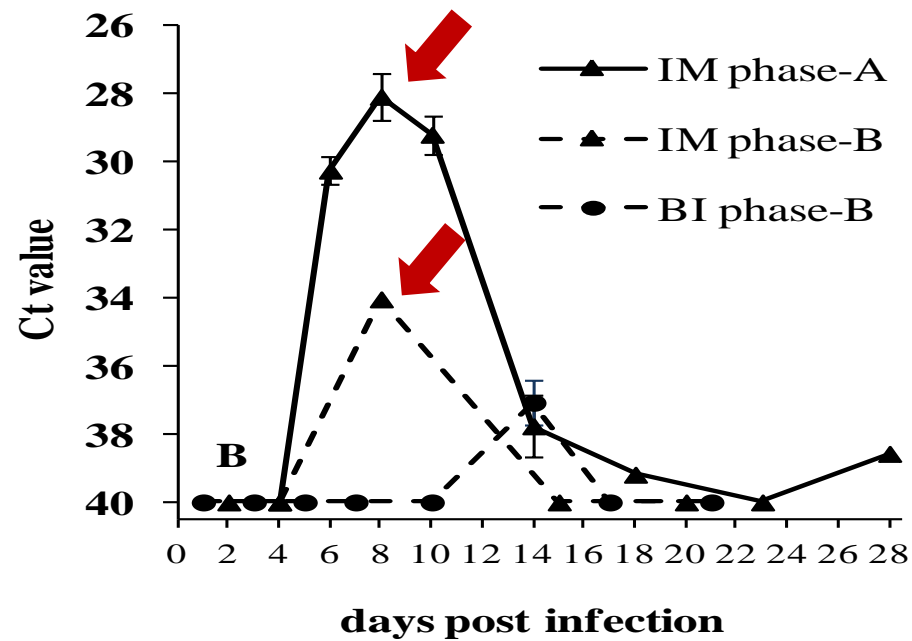


# Viral shedding in tank-water – RT-qPCR

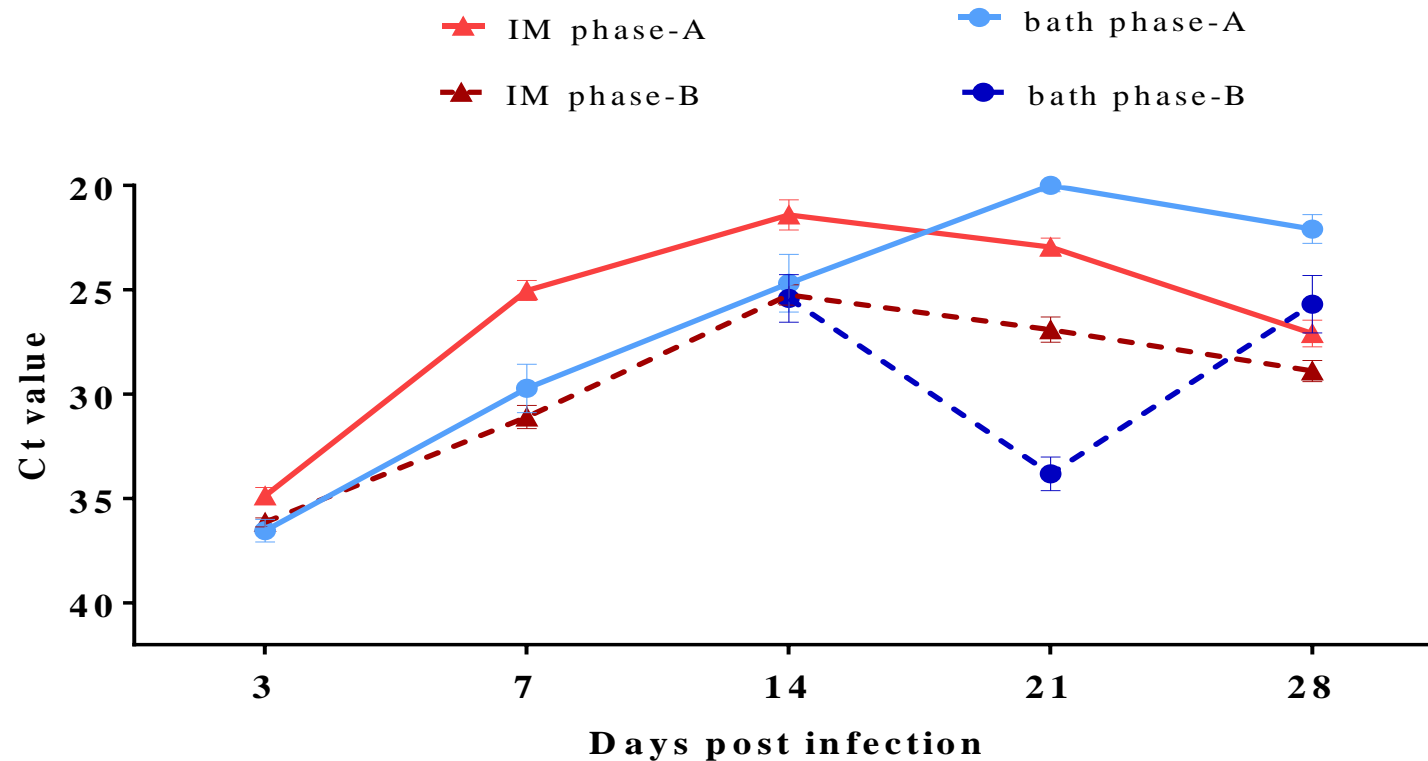
Shedding from shedders



Shedding from fish bath challenged in shedder water

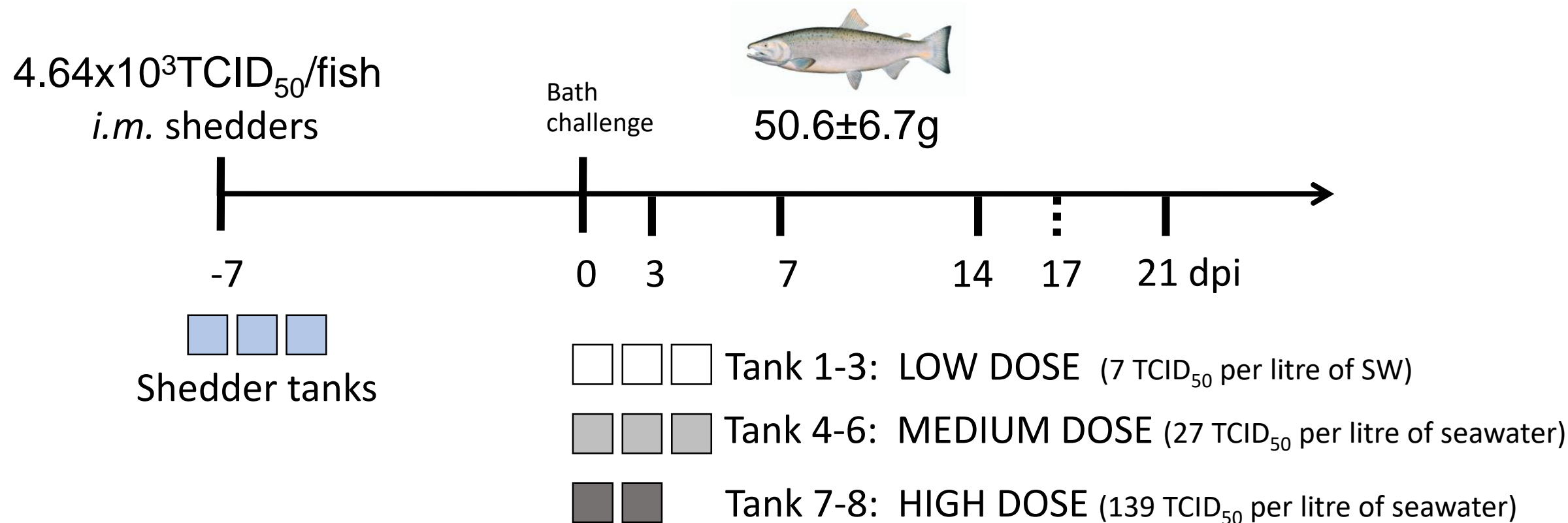


# Time for adaptation to seawater - susceptibility



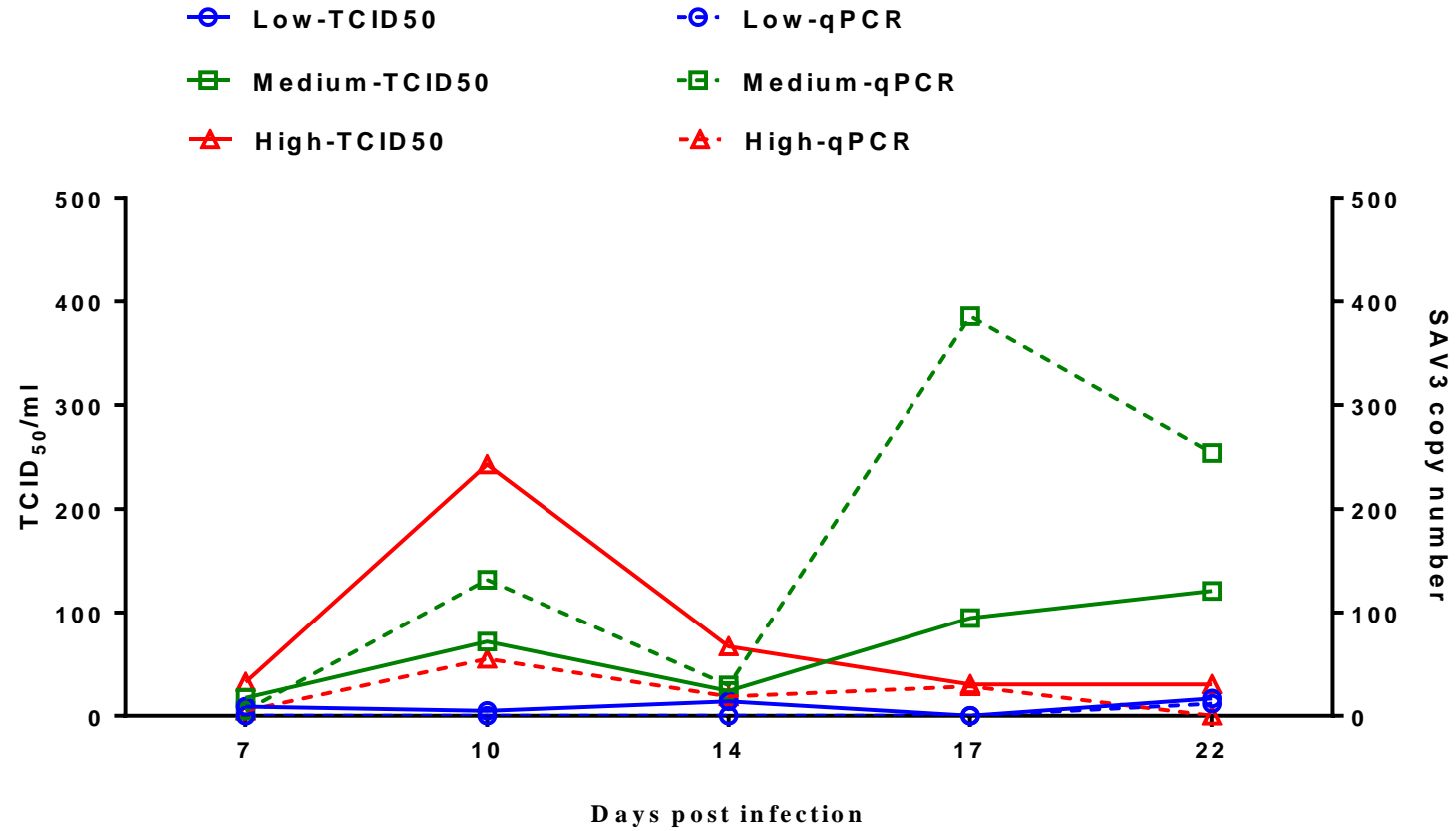
Phase B: Lower prevalence, Lower viral loads and lower shedding

# Dose study - Experimental setup



- 75 fish per 150-L tank
- Seawater temperature 12 °C
- Salinity 34.5 ppt
- Flow rate 300 L/h

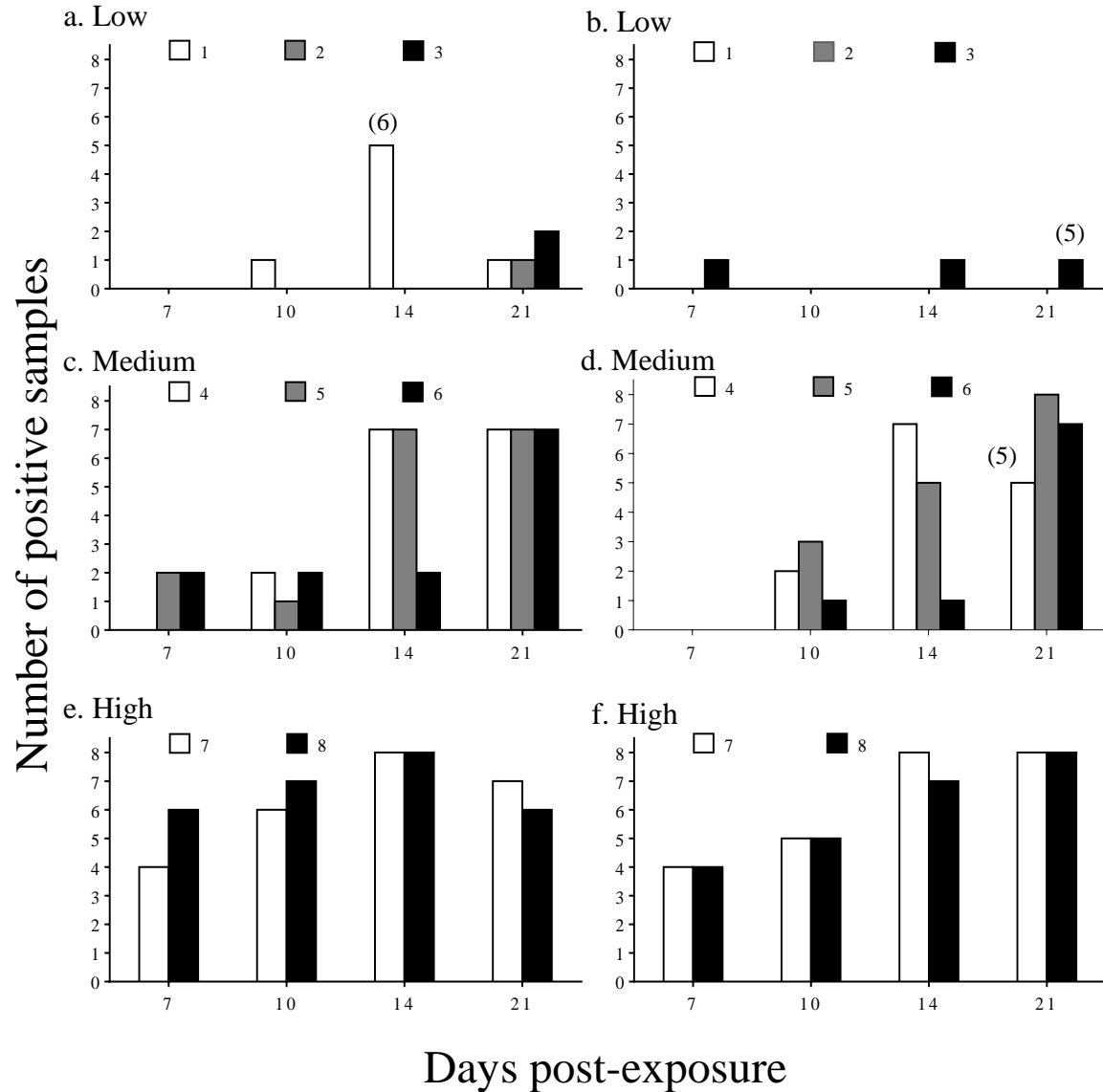
# Shedding in water



# Prevalance

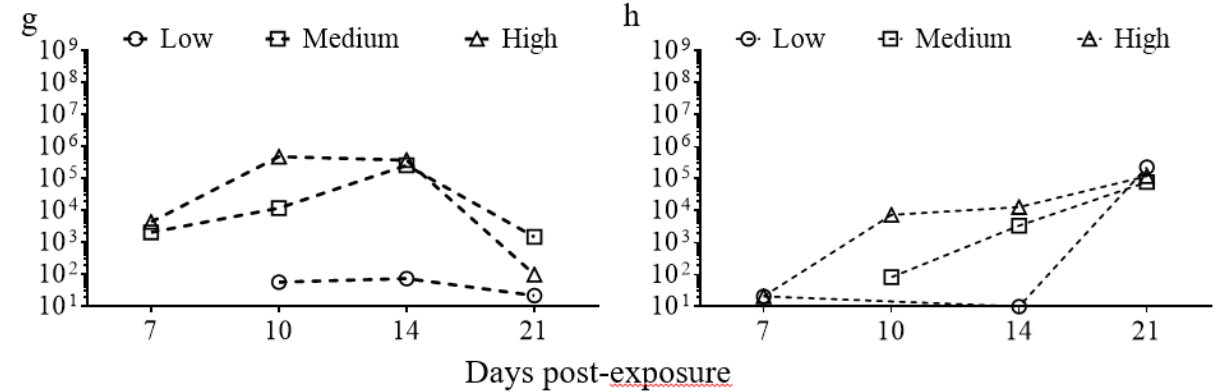
Serum

Heart




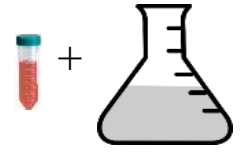


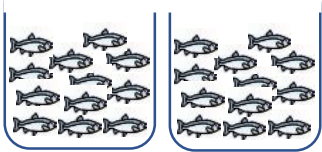

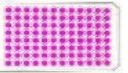

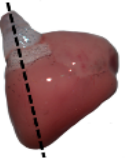


# Dose study

## Viral loads



# Survival of SAV3

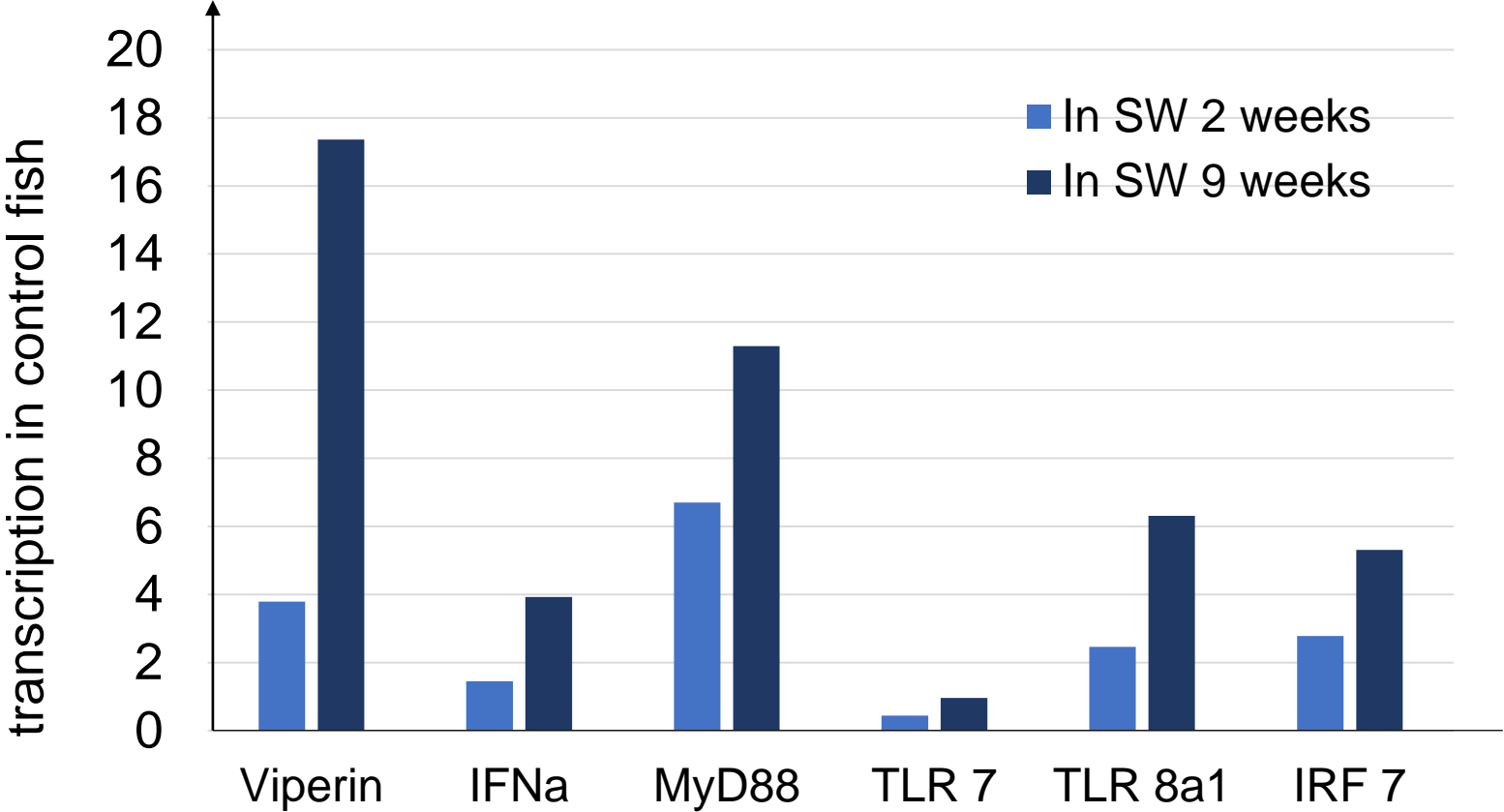
Exp.	Virus source	Incubation	Sampling	Analysis
A	 Cell cultured virus	 6°C 12°C 16°C	1 aliquot processed after 0, 1, 2, 3 and 4 weeks	→ RNA extraction → RT-qPCR → End-point dilution 
B	 Cell cultured virus diluted in natural seawater	 4°C 10°C 16°C 2 x 2 L flask	2 x 200 mL water filtered/concentrated after 0, 1, 3, 7, 10 and 15 days	→ RNA extraction → RT-qPCR → End-point dilution 
C	 Seawater containing SAV3 by shedders	 6°C 12°C 16°C 25 L container	1 container processed after 0, 1, 2, 3 and 4 weeks  2 x 1 L water filtered/concentrated	→ RNA extraction → RT-qPCR → End-point dilution 
			23 L bath challenge of 6 fish  	After 3 weeks hearts sampled  RT-qPCR 



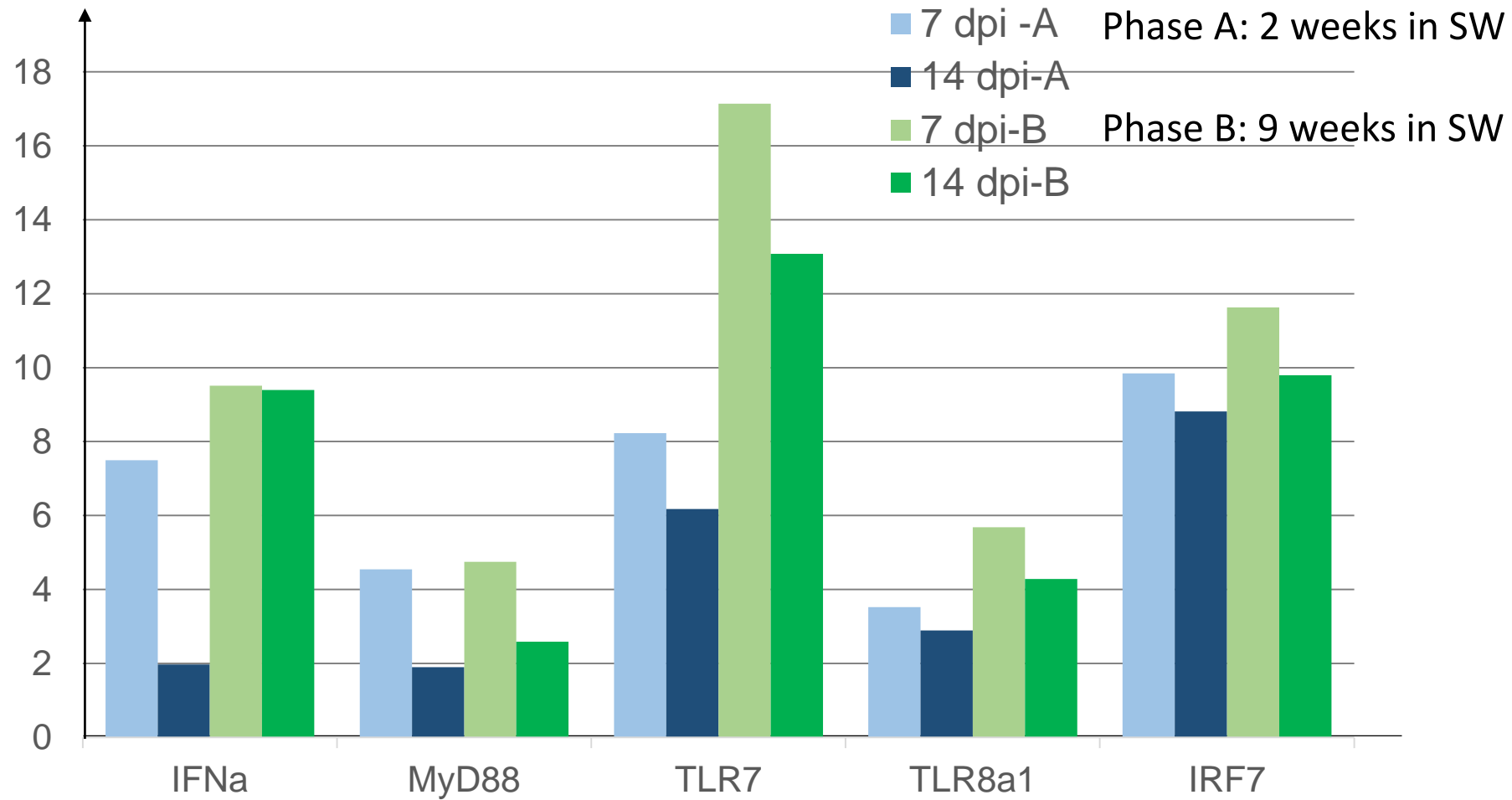
# Objective

3. Characterise the role of specific immune cells, and immune response during a SAV infection
  - Gene regulation of innate and adaptive genes in the salmon at two phases
  - Immune cells in SAV affected tissues

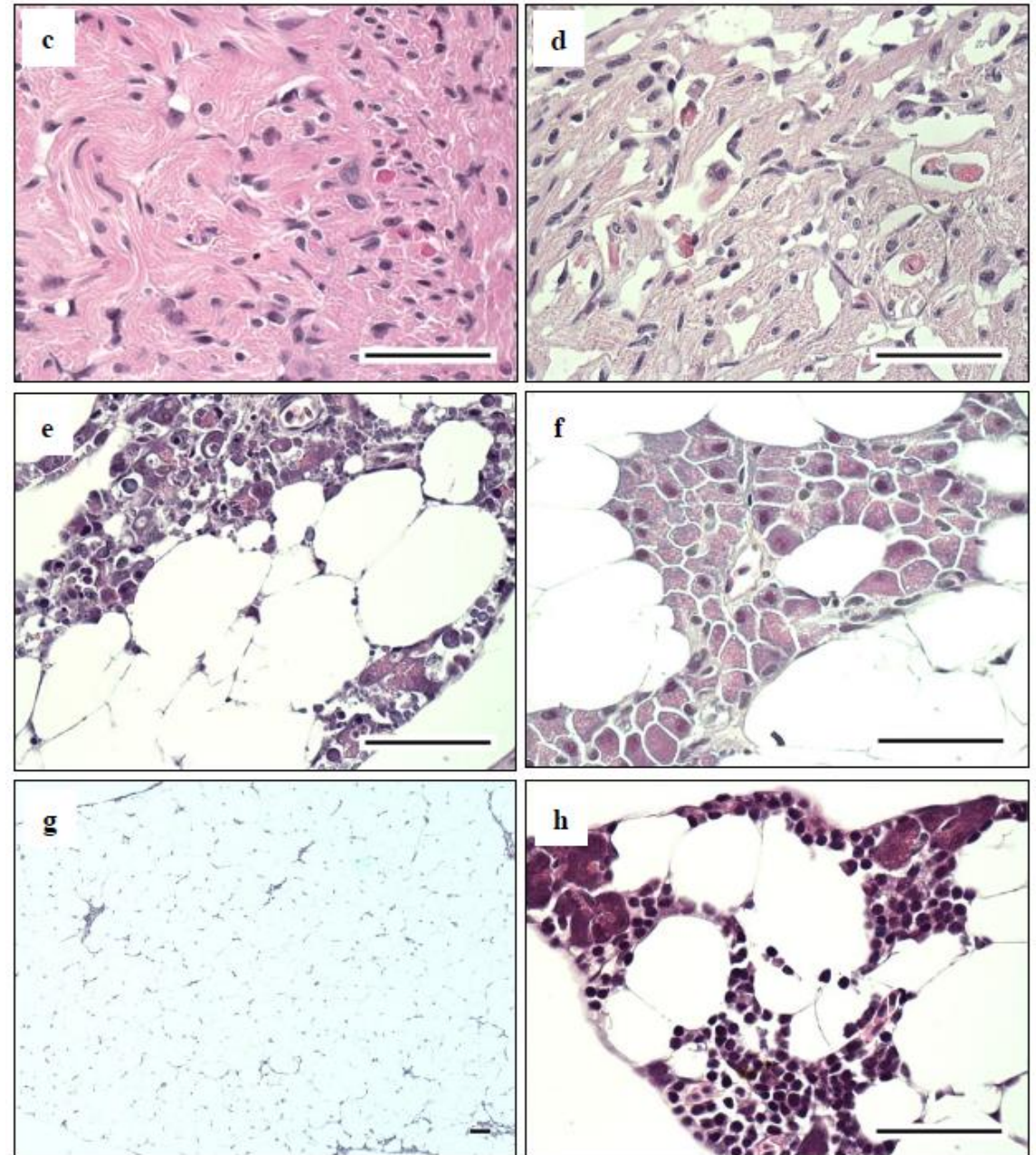
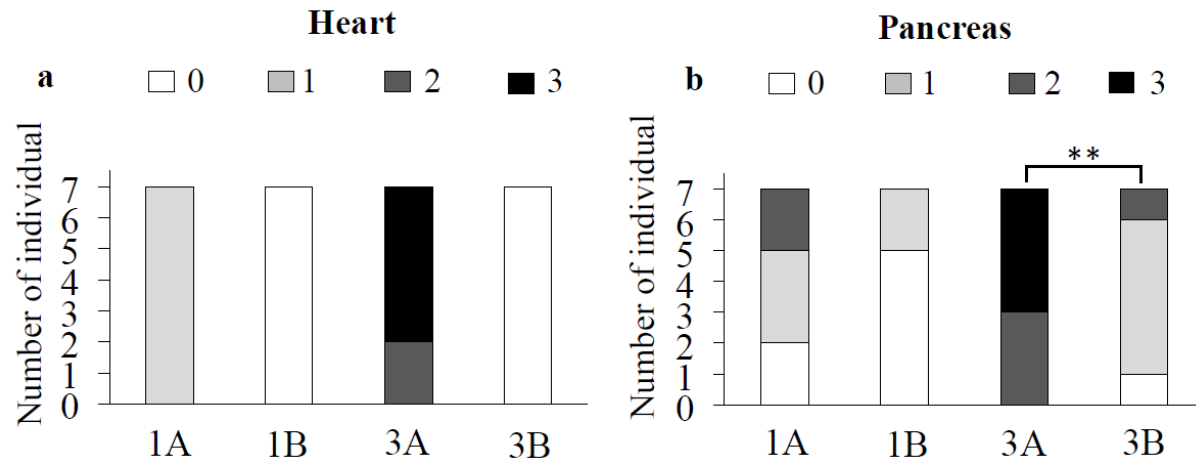
# Innate immune genes in head kidney of non-infected fish



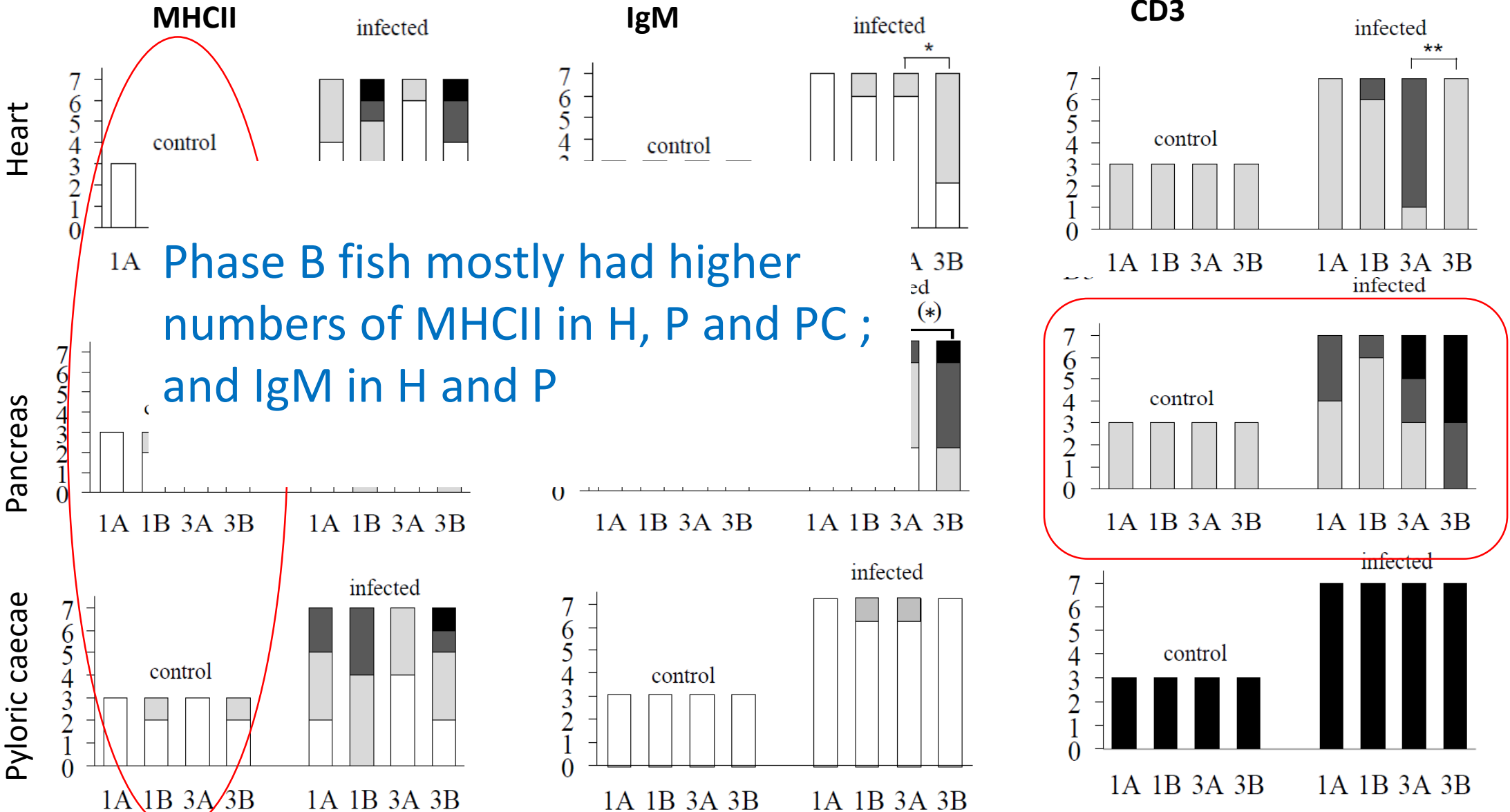
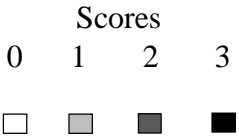
# Innate immune genes in head kidney of SAV3 infected salmon



# Affected tissues -histology



# Cells in affected tissues -IHC



Phase B fish mostly had higher numbers of MHCII in H, P and PC; and IgM in H and P

# Objective

4. Investigate the SAV susceptibility and immune response in both diploid and triploid Atlantic salmon

# Atlantic salmon

AquaGen<sup>®</sup> QTLinnOva<sup>®</sup> IPN/PD

Diploids



76.4±12.2 g

Triploids



71.6±19.0 g

Presentation by Noelia Nunez Ortiz

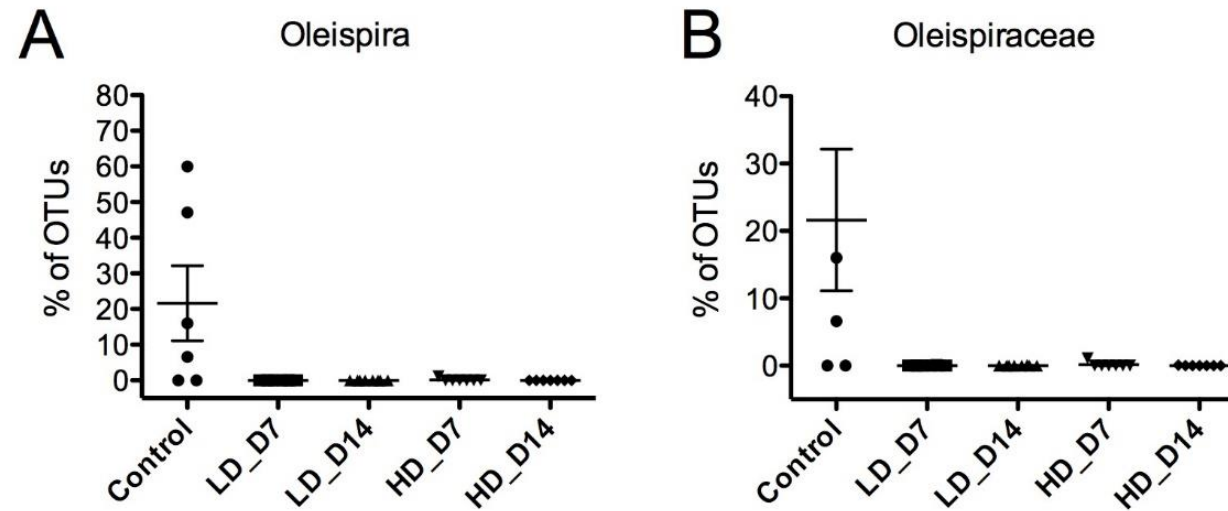
# Sub-topic

5. Study correlation between SAV3 infection and microbiome of salmon

- Co-relation between microbiome and dose of SAV3 in salmon skin
- Compare gill microbiome in triploid and diploid salmon

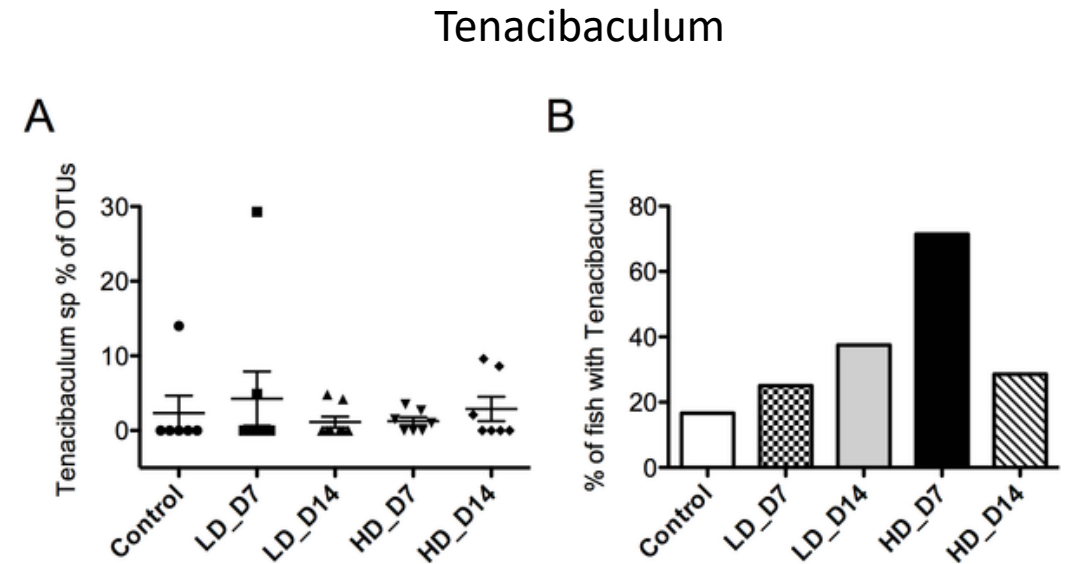
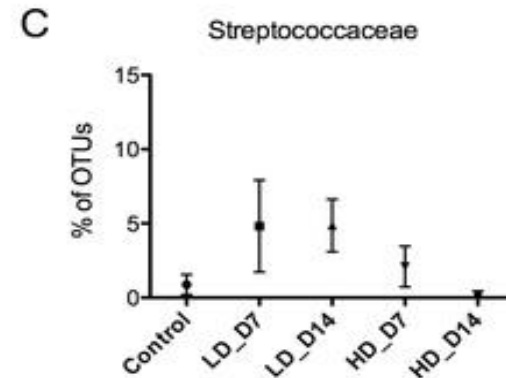
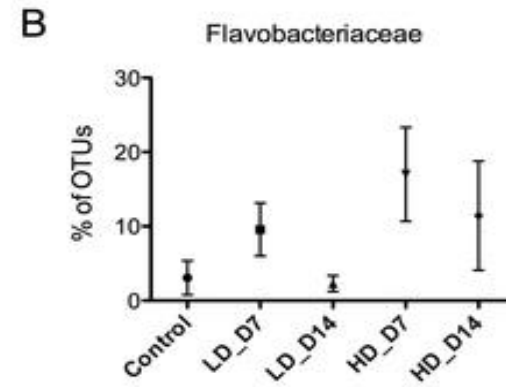
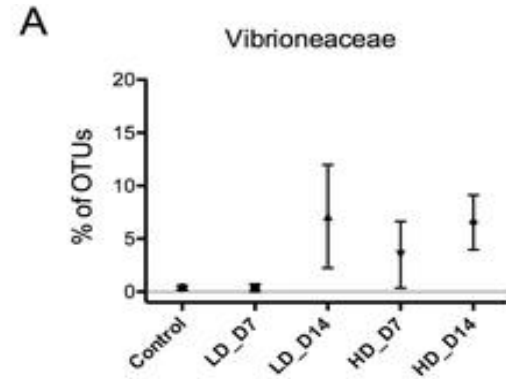


# Microbiome from Dose study- Skin



SAV3 infection results in losses of beneficial bacteria in Atlantic salmon skin

# Skin dysbiosis - microbiome



**SAV-induced skin dysbiosis is characterized by expansion of potentially pathogenic taxa**

# Factors influencing salmon-SAV interaction - summary

## Salmon:

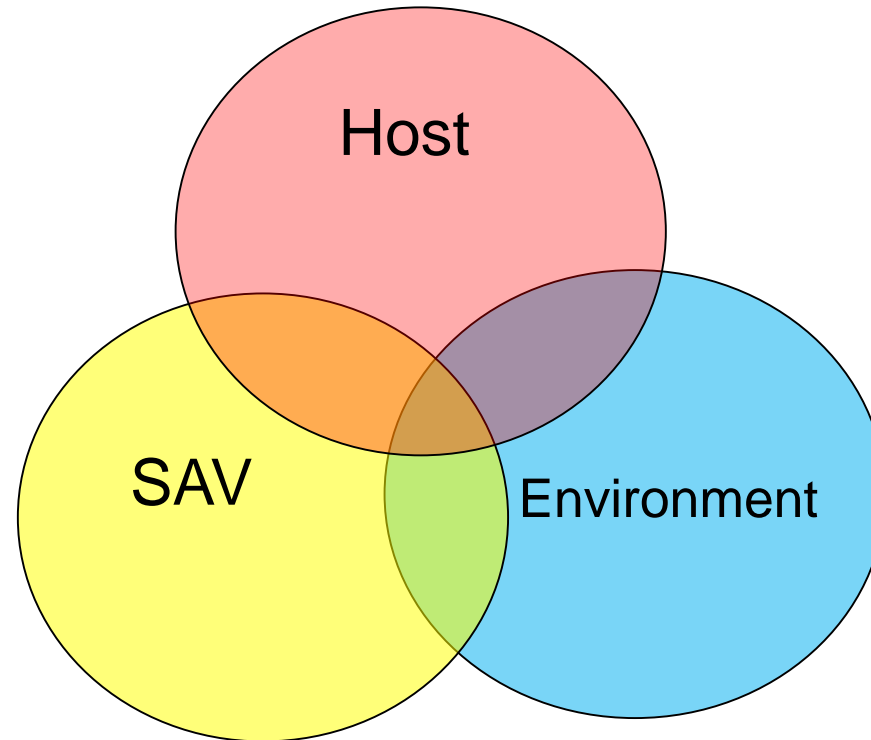
- **Physiology**
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## SAV:

- Sub-type and isolate
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- **Infectivity and dose**
- Host specificity
- **Survival in and outside host**

## Environment/other factors:

- **Time**
- **Temperature**
- Organic load
- Oxygen
- UV radiation
- **Microbiome**



# Thanks to..

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