Fish health: Immune system and vaccine development

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Vaxxinova Norway and IMR

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Workshop on Atlantic halibut aquaculture, 11.-12. September

Thanks to colleagues at IMR and Vaxxinova

Colleagues at Austevoll research station

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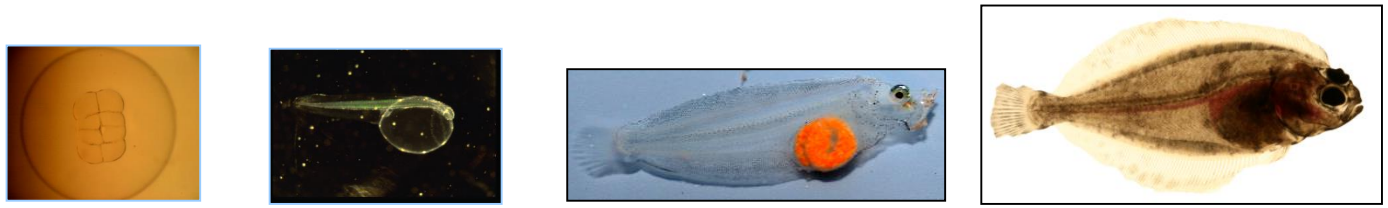
Prevention strategies

Knowledge about the immune system and disease/pathogen:

- When?
- How?
- For which pathogens?



Egg

Adult

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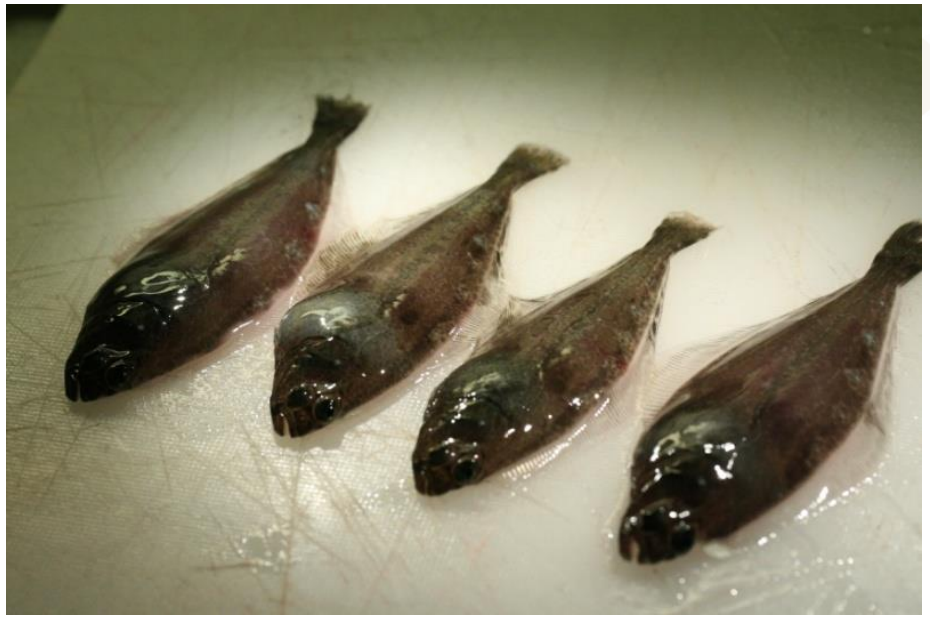
Diseases in halibut

- Bacterial
- Viral
- Parasties



Parasites

- Myxosporidia –High mortality in 2015, no detection since 2016
- *Ichthyobodo spp. (*"Costia"*),Trichodina* sp
- Combined with- bacterial gill health problems

Formalin treatment

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Diseases

Disease	2012	2013	2014	2015	2016	2017
Notifiable						
VER	1	1	0	0	0	0
Non-notifiable						
Furunculosis	1	-	-	X?	9	7
IPNV				2		
Moritella viscosa	X	-	-	-	-	-
ARV		X?	X			

- Vibriosis, IPN and atypical furunculosis: three most important diseases
- Atypical furunkulosis - Can be difficult to completely get control over in landbased facilities

Fiskehelserapportene, VI

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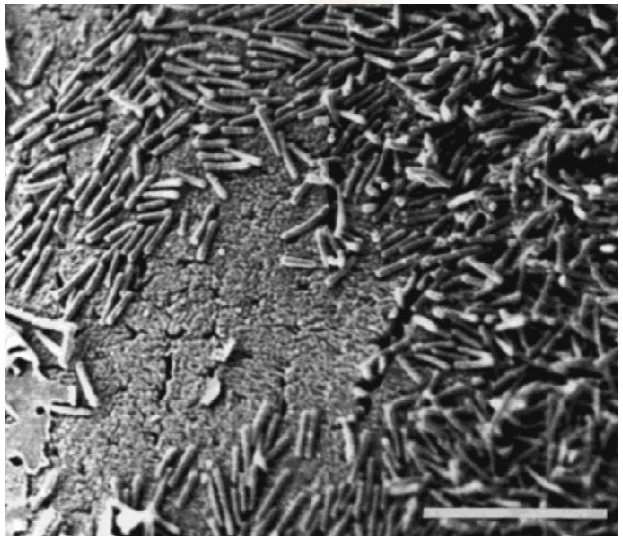
Bacterial diseases

- Atypical *Aeromonas salmonicida – type II*
- *Vibrio logei*
- Vibrio (Allivibrio) wodanis
- *Vibrio splendidus*
- *Vibrio tapetis*



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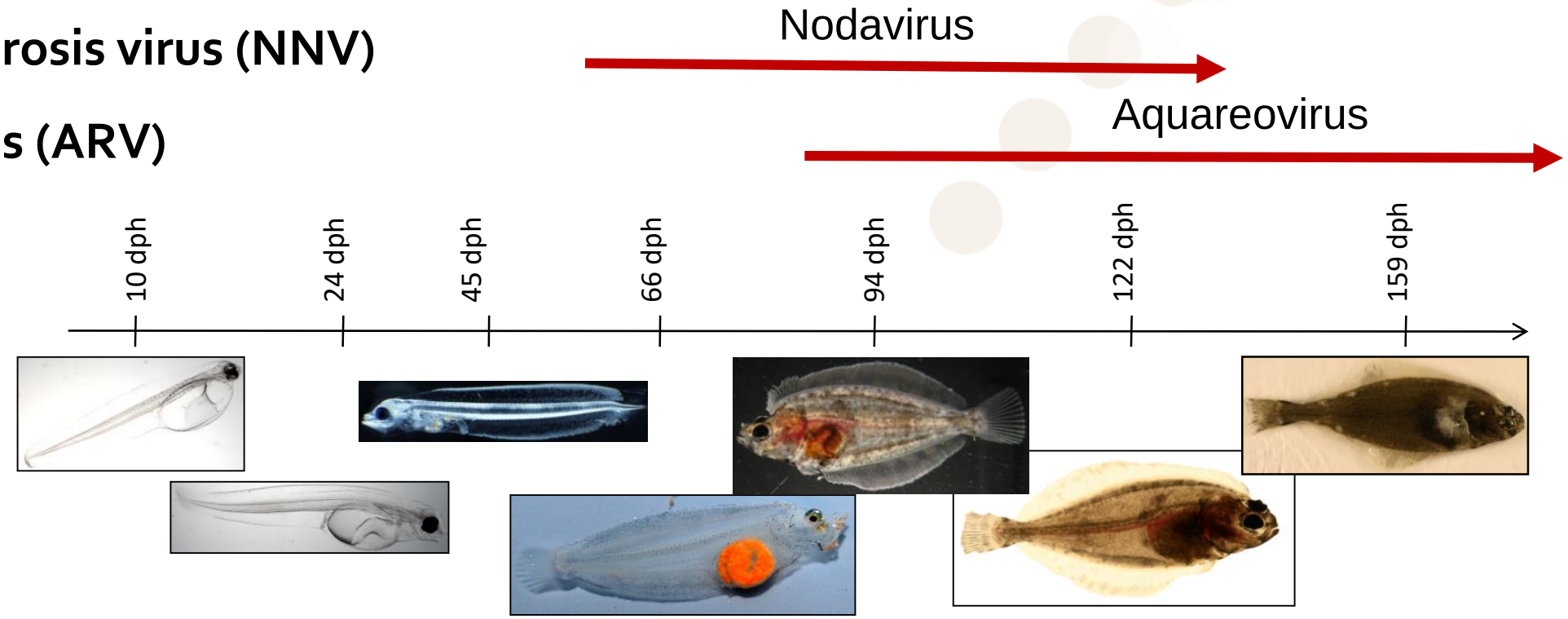
Viral diseases

- Viral haemorrhagic septicaemia virus (VHSV)
- Infectious pancreatic necrosis virus (IPNV)
- **Nervous necrosis virus (NNV)**
- **Aquareovirus (ARV)**



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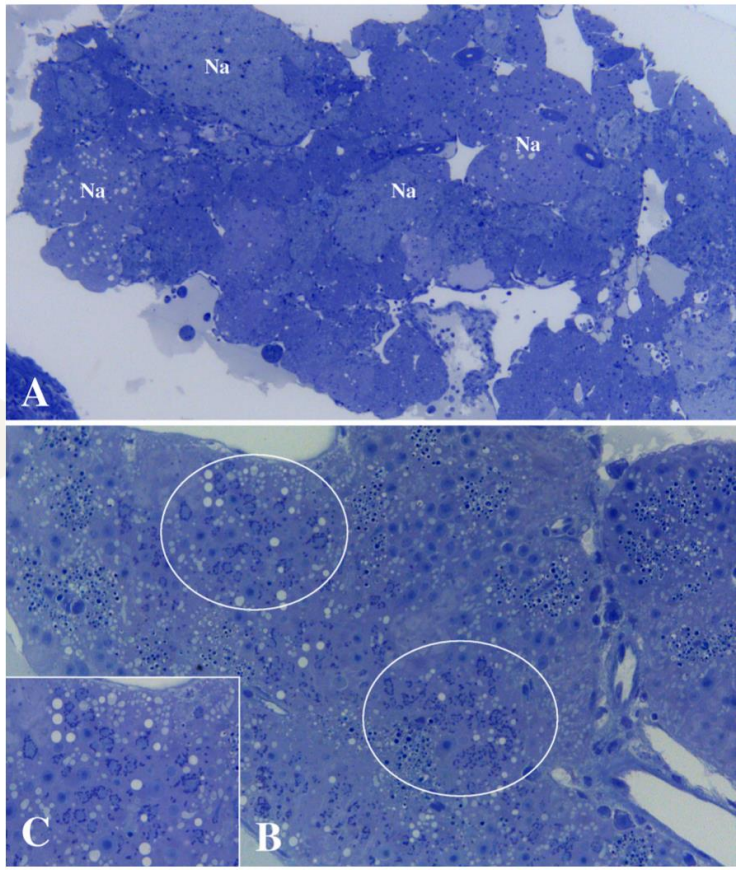
Aquareovirus (AHRV)

- Norway:
  - First aquareovirus from a marine coldwater fish spp.
  - Clinical signs: Lethargy, darkening
  - Necrosis in the liver and pancreas
  - 90–100 days after fertilization
  - 80-90% mortality

- Canada (1998): 100 mg – 1 g, ~56% mortality, +bacterial infections
- Scotland (2003): Weaned, 1000 dd, >95% mortality



Cusack et al, 2001, Ferguson 2003, Blindheim et al, 2015

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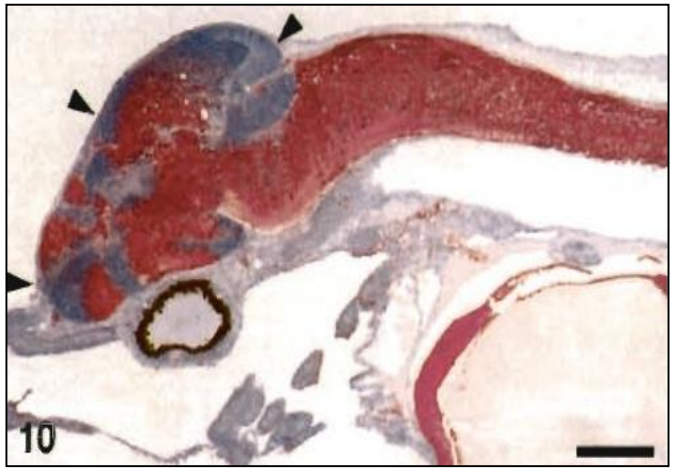
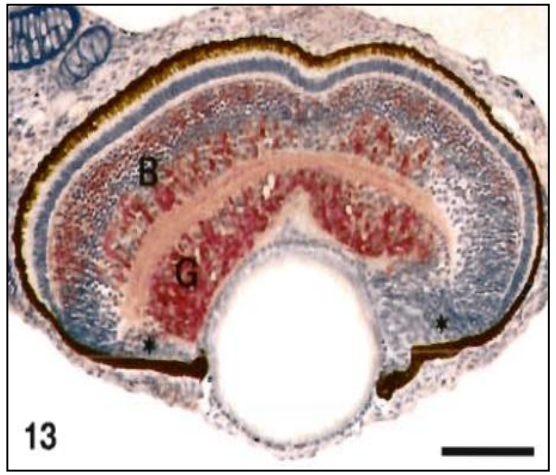
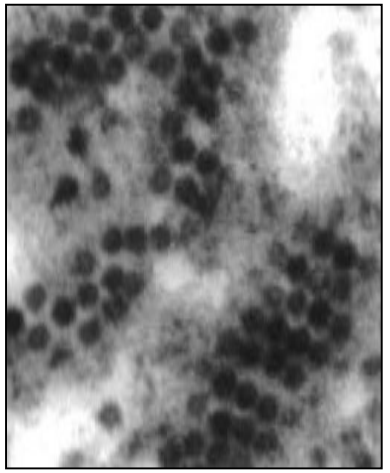
Nervous necrosis virus (NNV)

- Betanodavirus, *Nodaviridae* family
- Viral encephalopathy and retinopathy (VER)
- Central nervous system (CNS) and retina
- Abnormal swimming pattern and loss of appetite
- Larvae and juvenile stages affected by VER

Size: 25-30 nm
Genom: RNA1 3100nt
RNA2 1400nt







Grotmol et al. 1999: Bath challenged halibut yolk-sac larvae

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Pathogen transmission

- Horisontal
- Vertical

- Screening of broodstock

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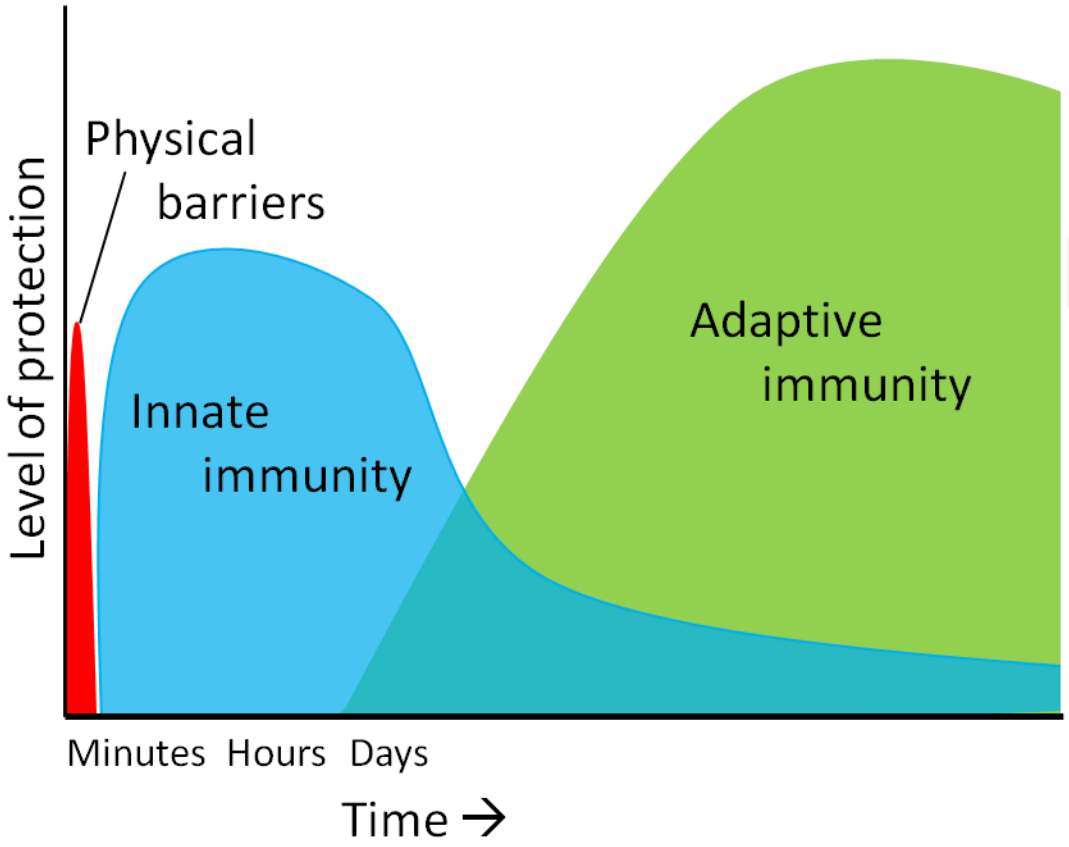
Immunity : Innate and Adaptive



- Innate immunity
  → non-specific

- Adaptive immunity
  → specific
  → memory

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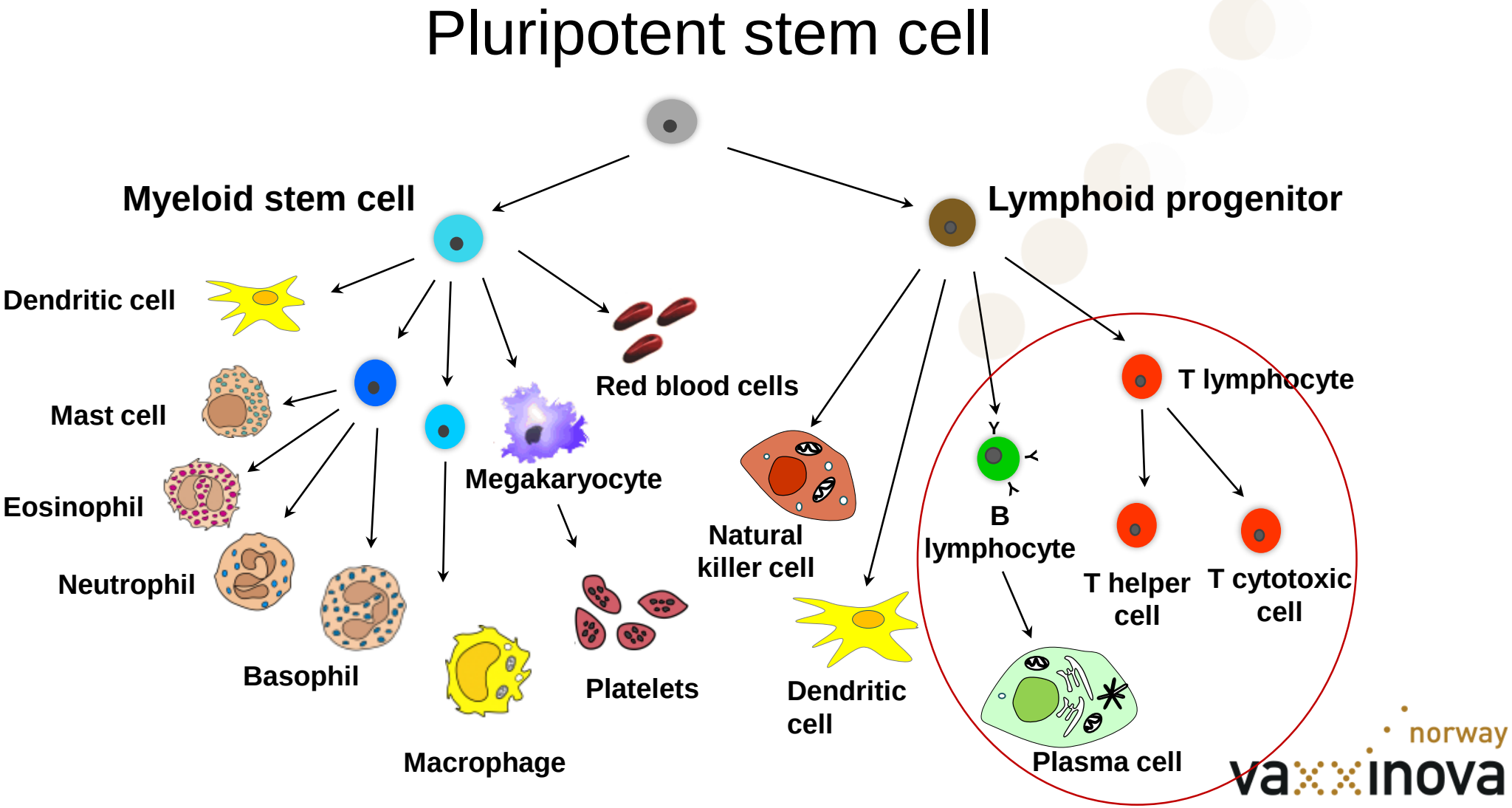
The cells in the immune system



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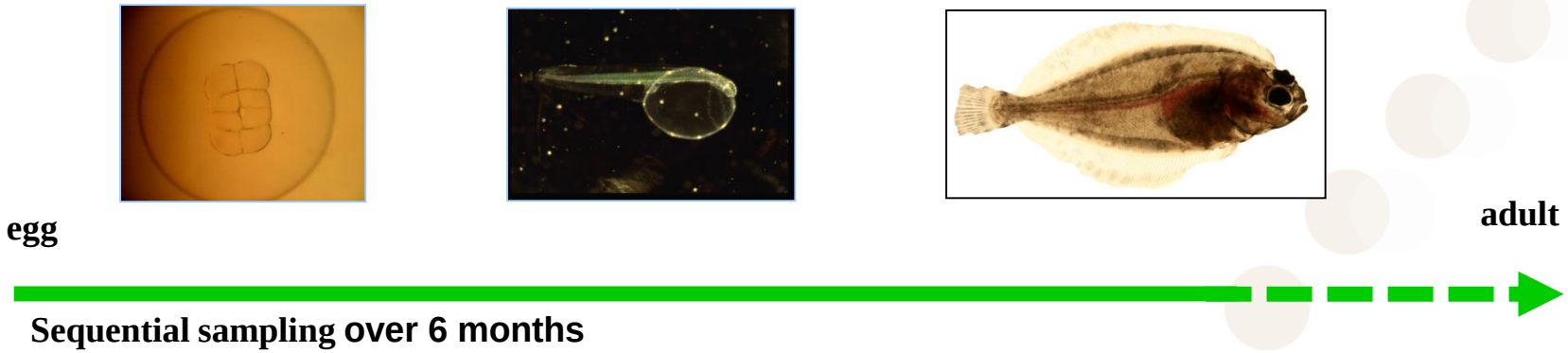
Development of immune system



- Developement of lymphoid organs
- Adaptive immunity:
  - Detection of specific B- and T-cell markers – RNA
  - Detection of IgM - protein

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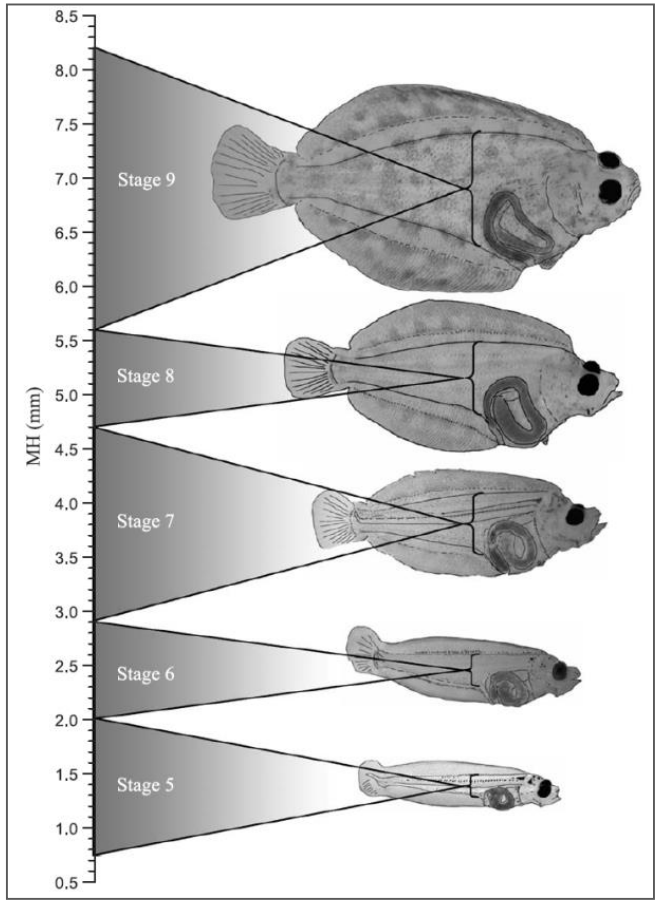
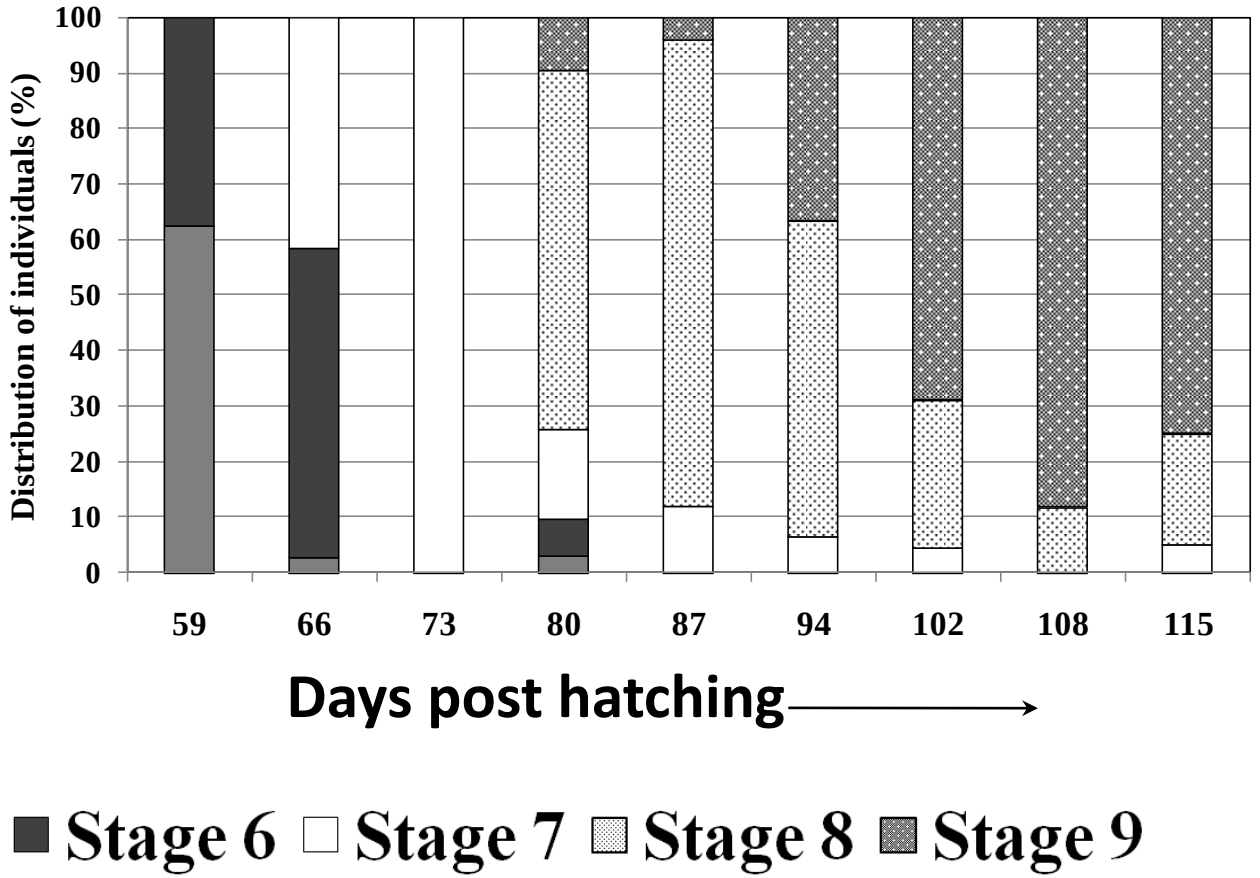
Halibut larvae during metamorphosis



Sæle et al, 2004



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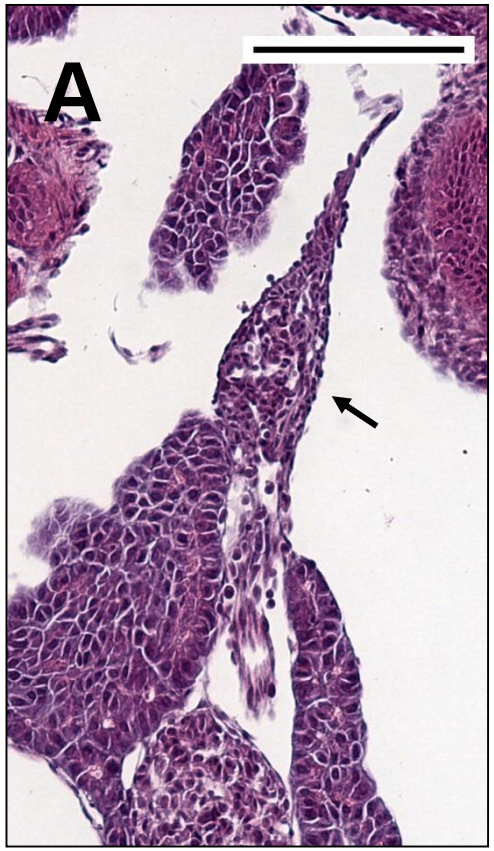
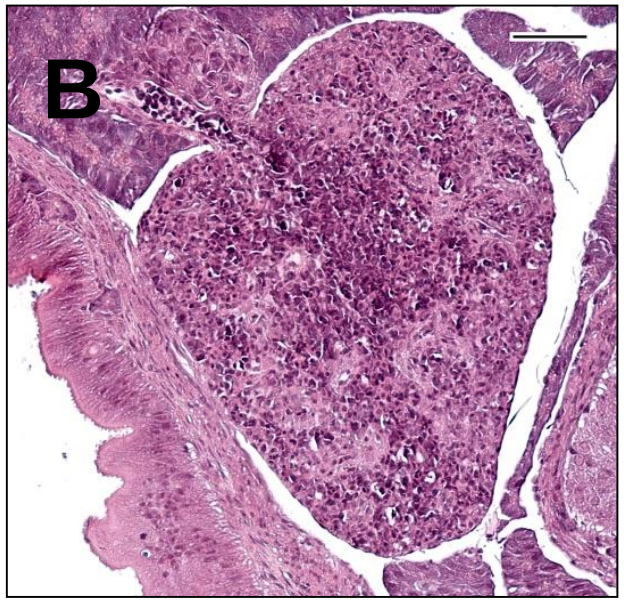
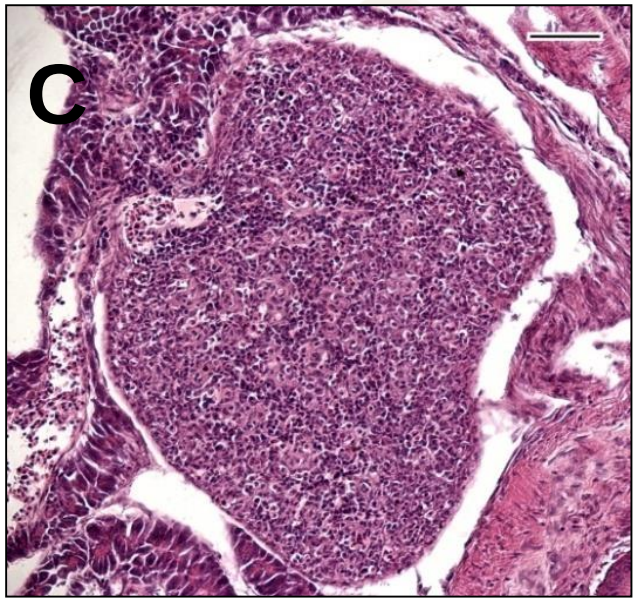
Development of Spleen



59 dph



80 dph



94 dph

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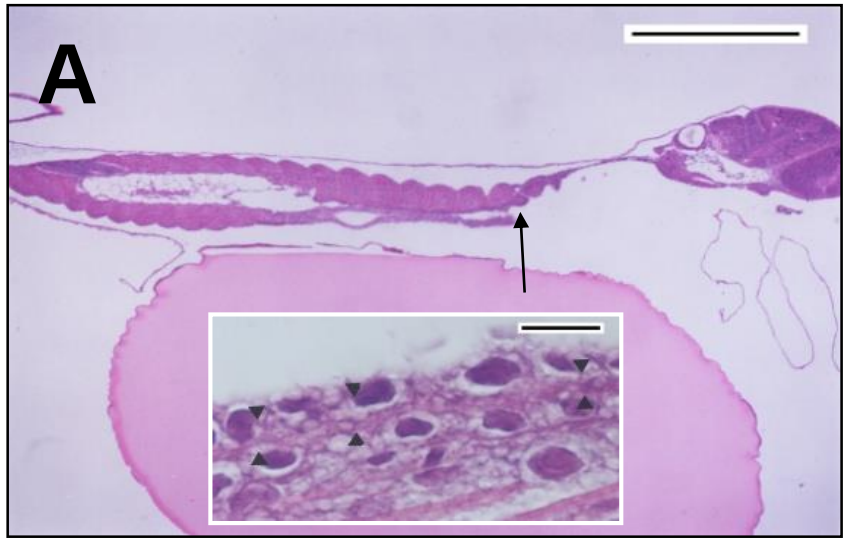
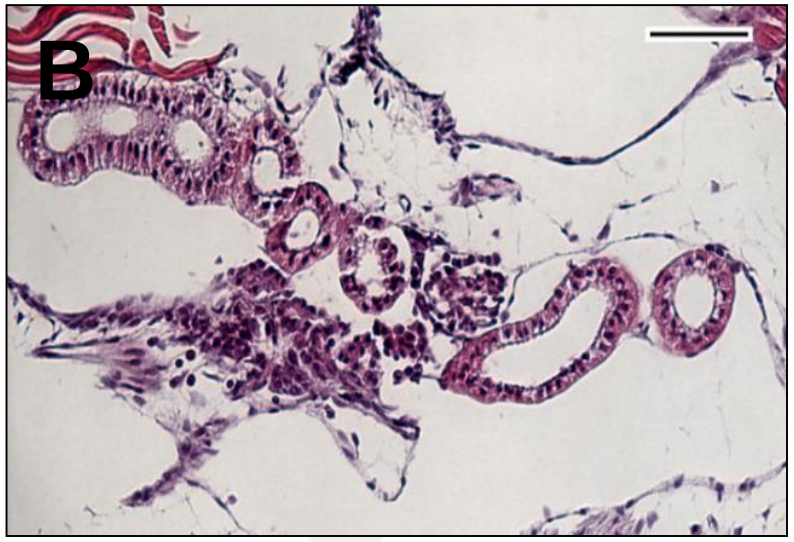
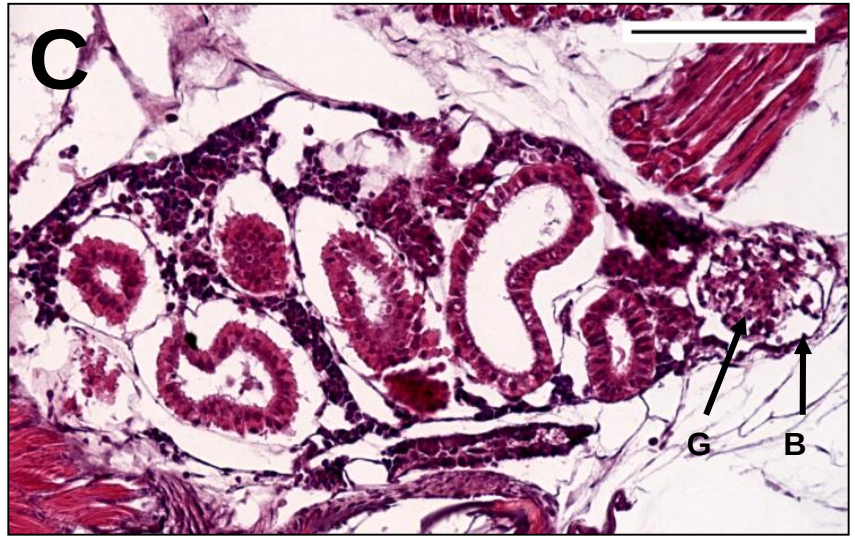
Development of anterior kidney



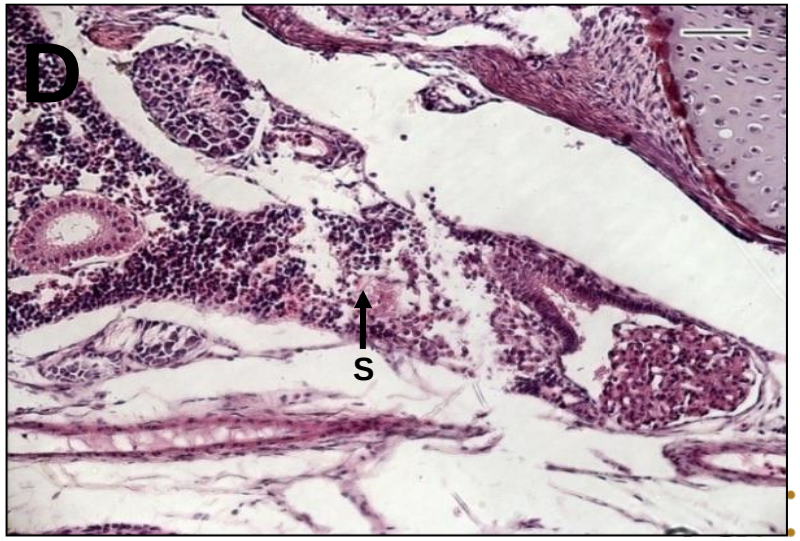
1 dph



49 dph



66dph



94 dph

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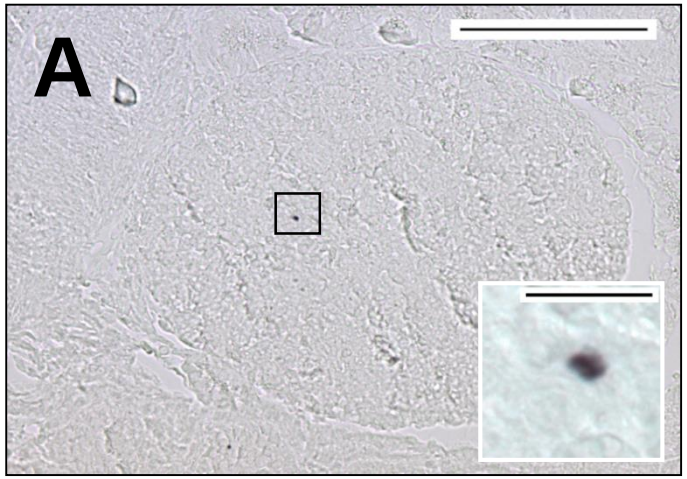
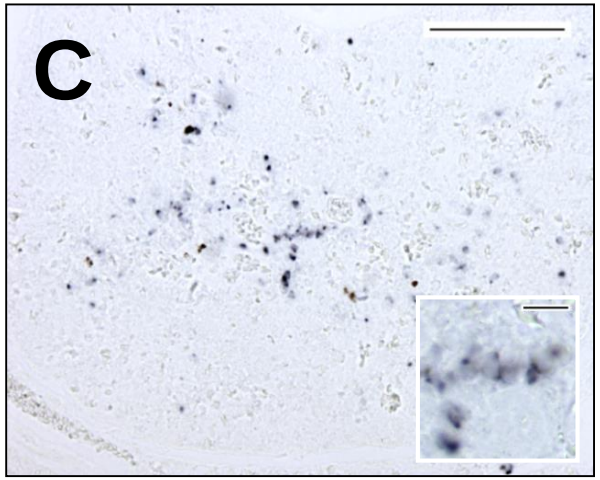
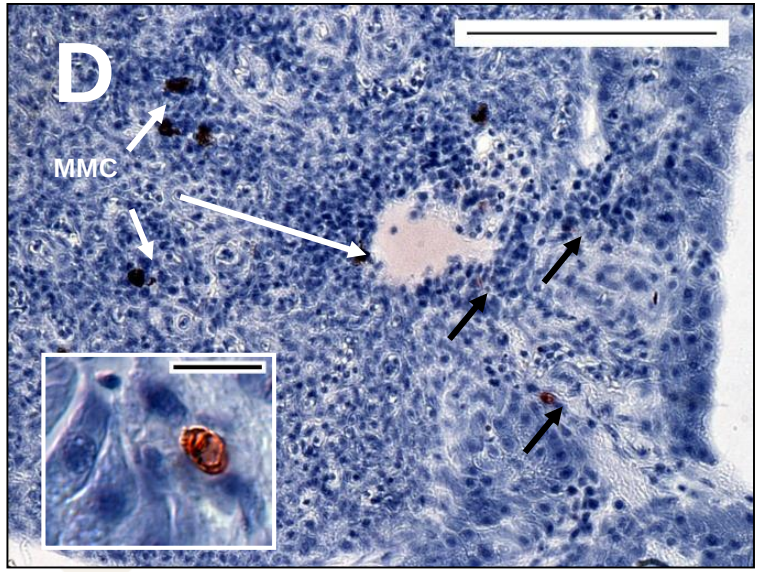
In situ hybridization and IHC - IgM

Spleen



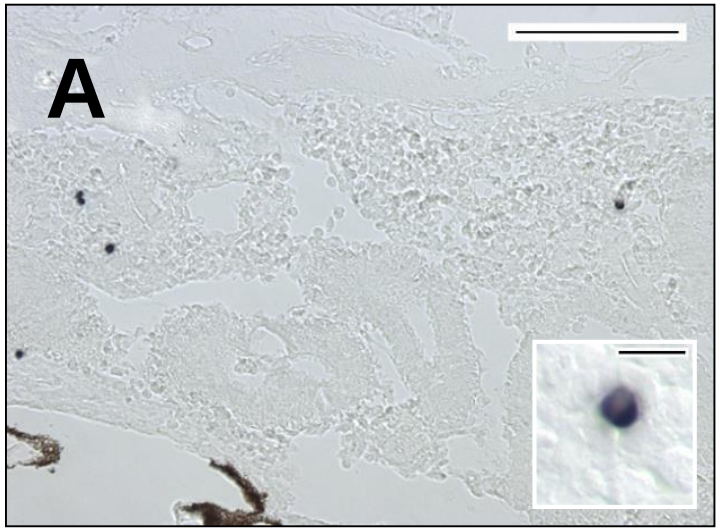


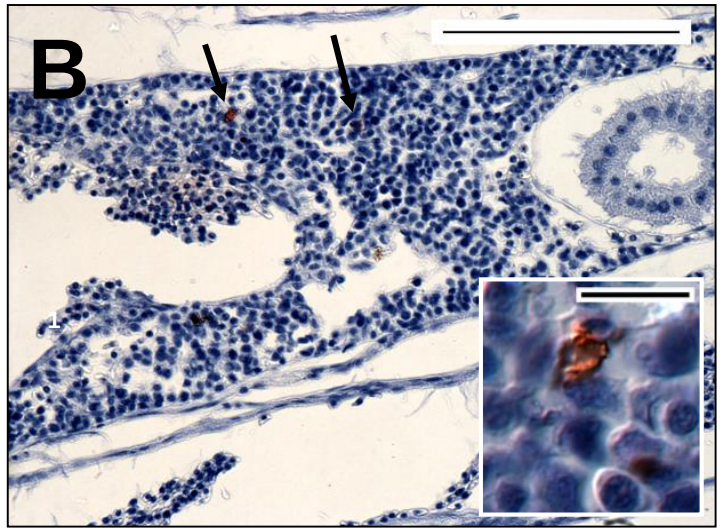


66 dph

94 dph

HK





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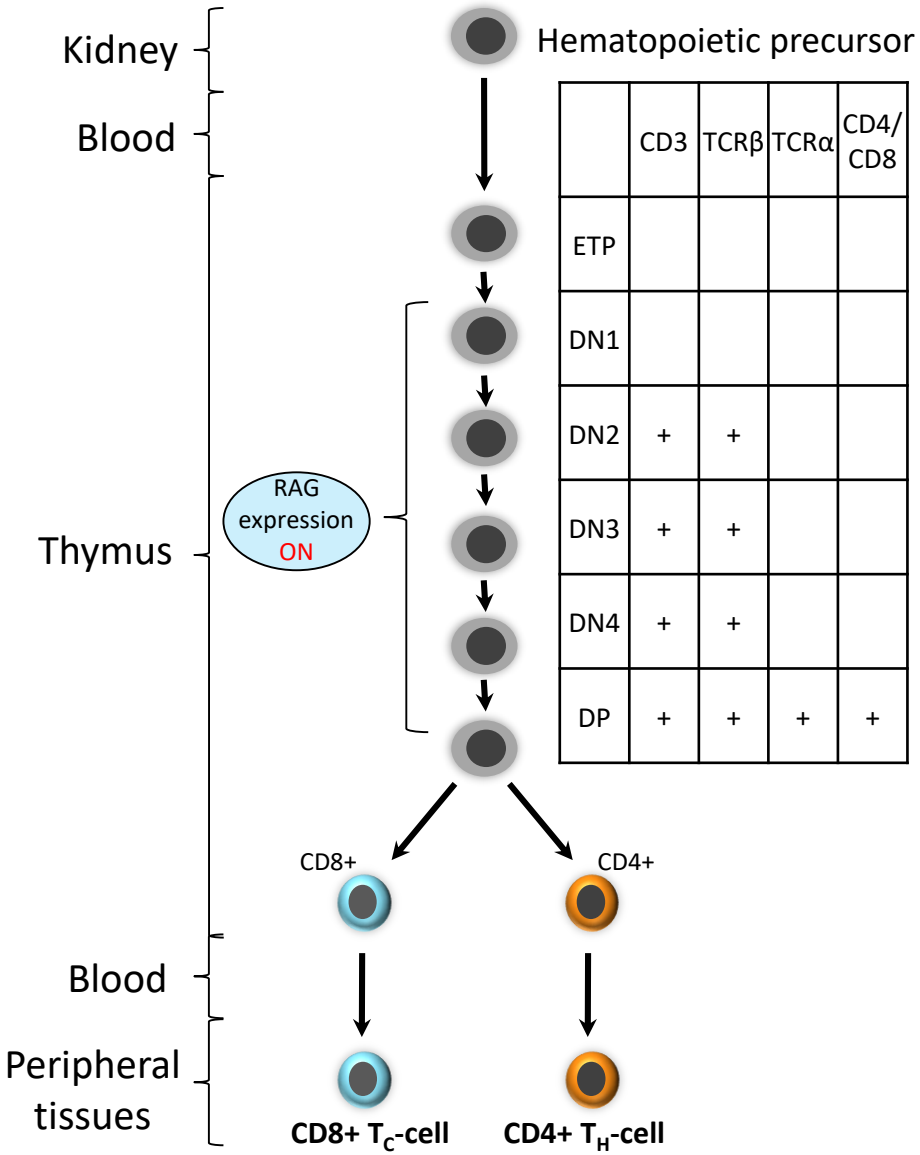
T-cell development



	CD3	TCRβ	TCRα	CD4/ CD8
ETP				
DN1				
DN2	+	+		
DN3	+	+		
DN4	+	+		
DP	+	+	+	+

Halibut timeline:

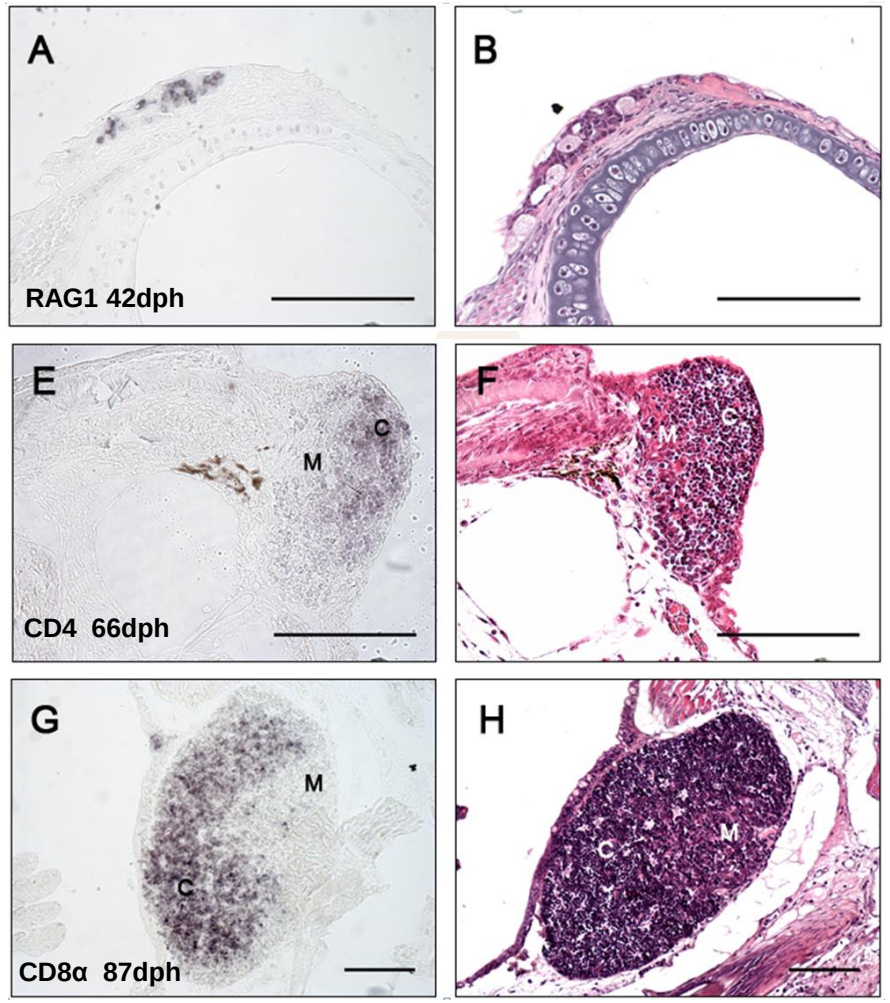
33 dph

42 dph

66-73 dph

87 dph

In situ hybridisation, thymus:



Immunocompetence ?

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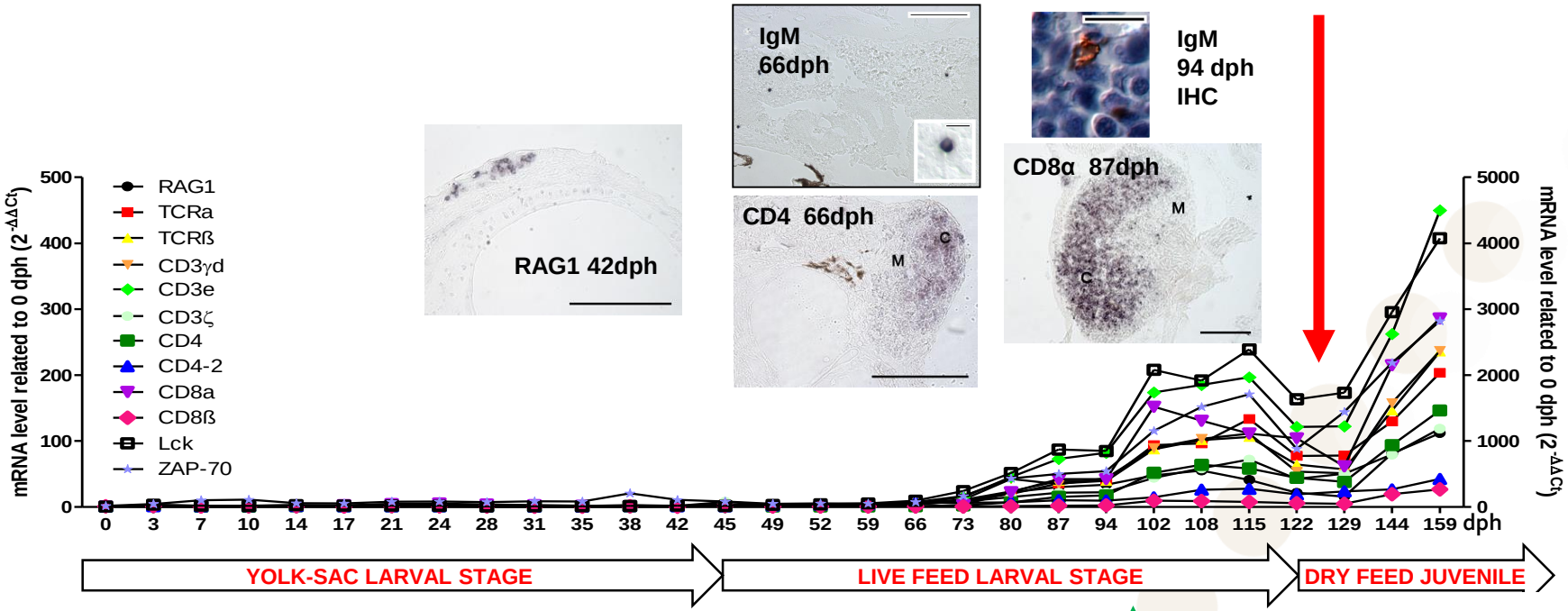
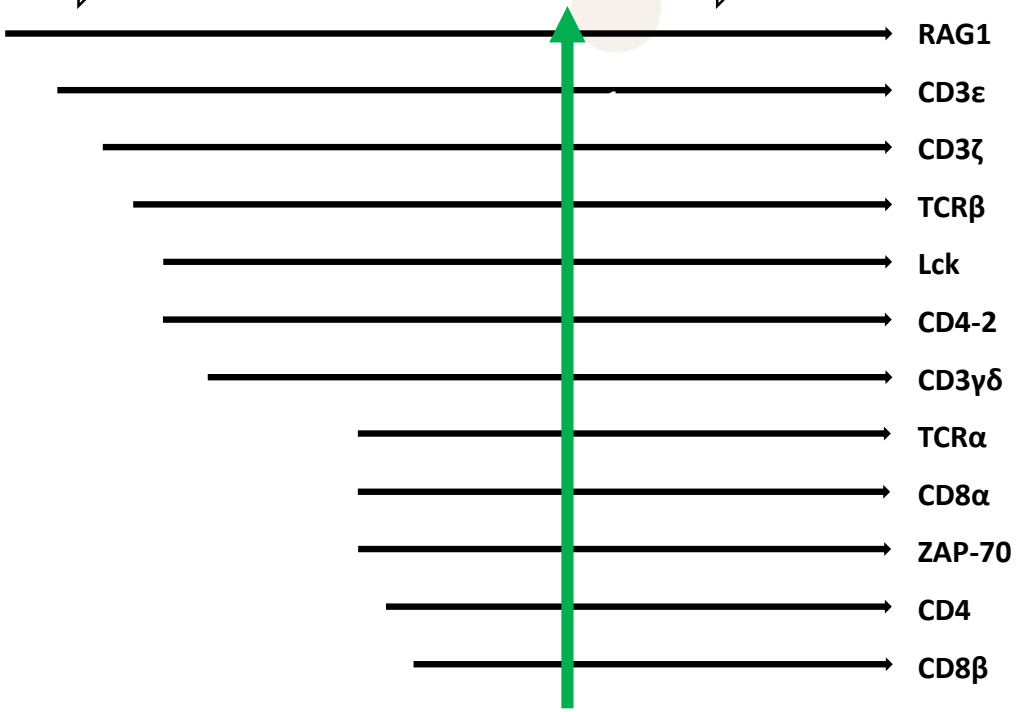
Figure modified from Kindt, 2007





Immunity development

Patel et al, 2009;
Øvergård et al 2011

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Vaccine trials - Laboratory

Trials:

1. Larvae ~70 dph – tolerance trial
2. Juveniles (25 g) – recombinant recCP by injection
3. Larvae/juveniles (100 dph) – several different formulations – oral and injection

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Vaccine and tolerance trial -larvae

Larvae ~70 dph, recCP, no adjuvant



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Unfortunately….

- 99% larvae died – handling stress!!
- Other methods!?
  - Trials with bath treatment
  - Trials with oral uptake
  - ……and analyse uptake of vaccine

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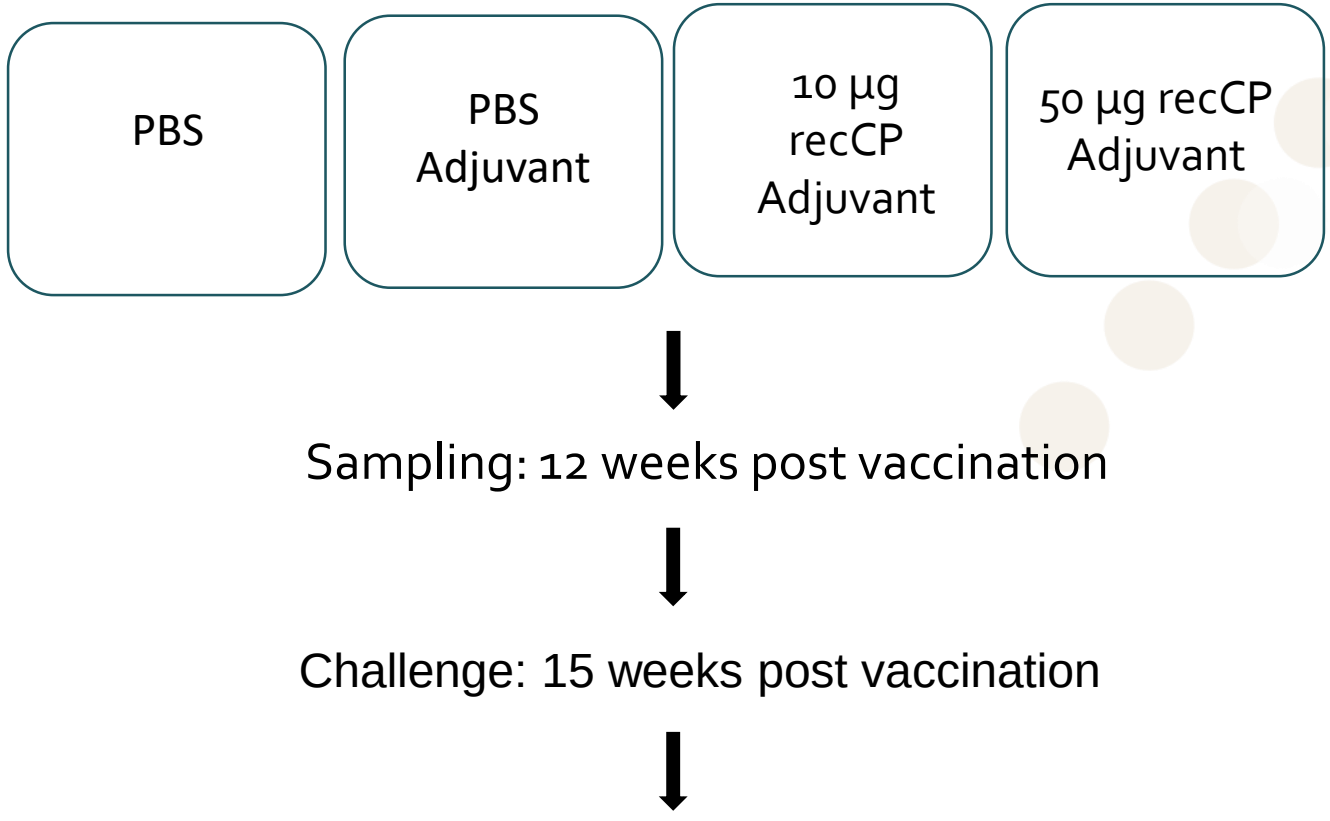
Assess protection against nodavirus

Juveniles (25 g)



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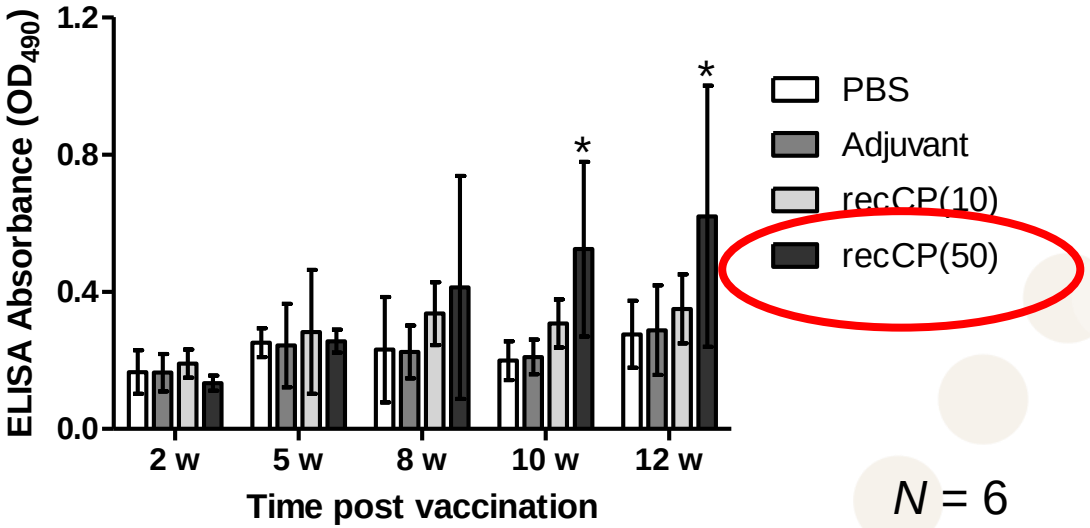
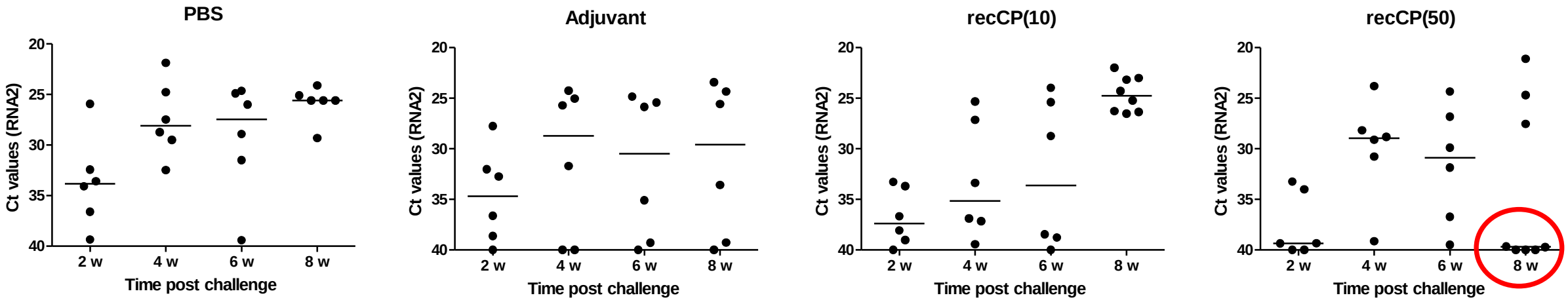
Ab production and protection



N = 6

Nodavirus RNA2



WP 26 Atlantic halibut

Objective: Determine the effect of delivering recombinant capsid protein during late larval stages on protection to nodavirus (VNN)

Task 26.1 Production of VNN capsid protein

Task 26.2 Monitor and assess immune response and protection

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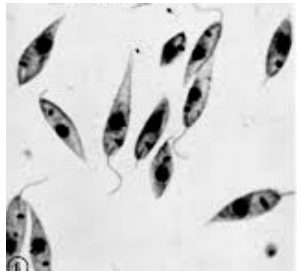
Assessment of vaccine

Production of antigen Protein expression in different systems

E.coli

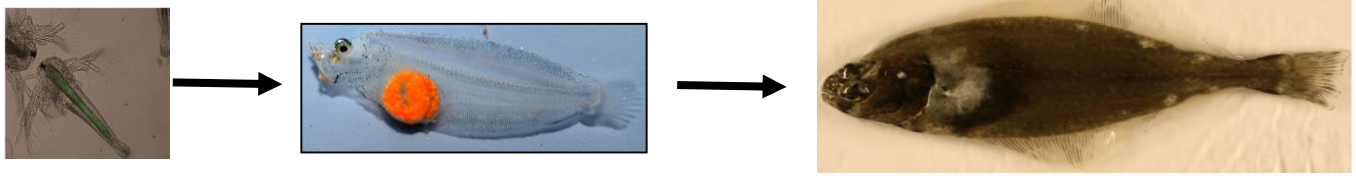


Leishmania tarentolae

Pichia pastoris

Tobacco leaves

Delivery and challenge



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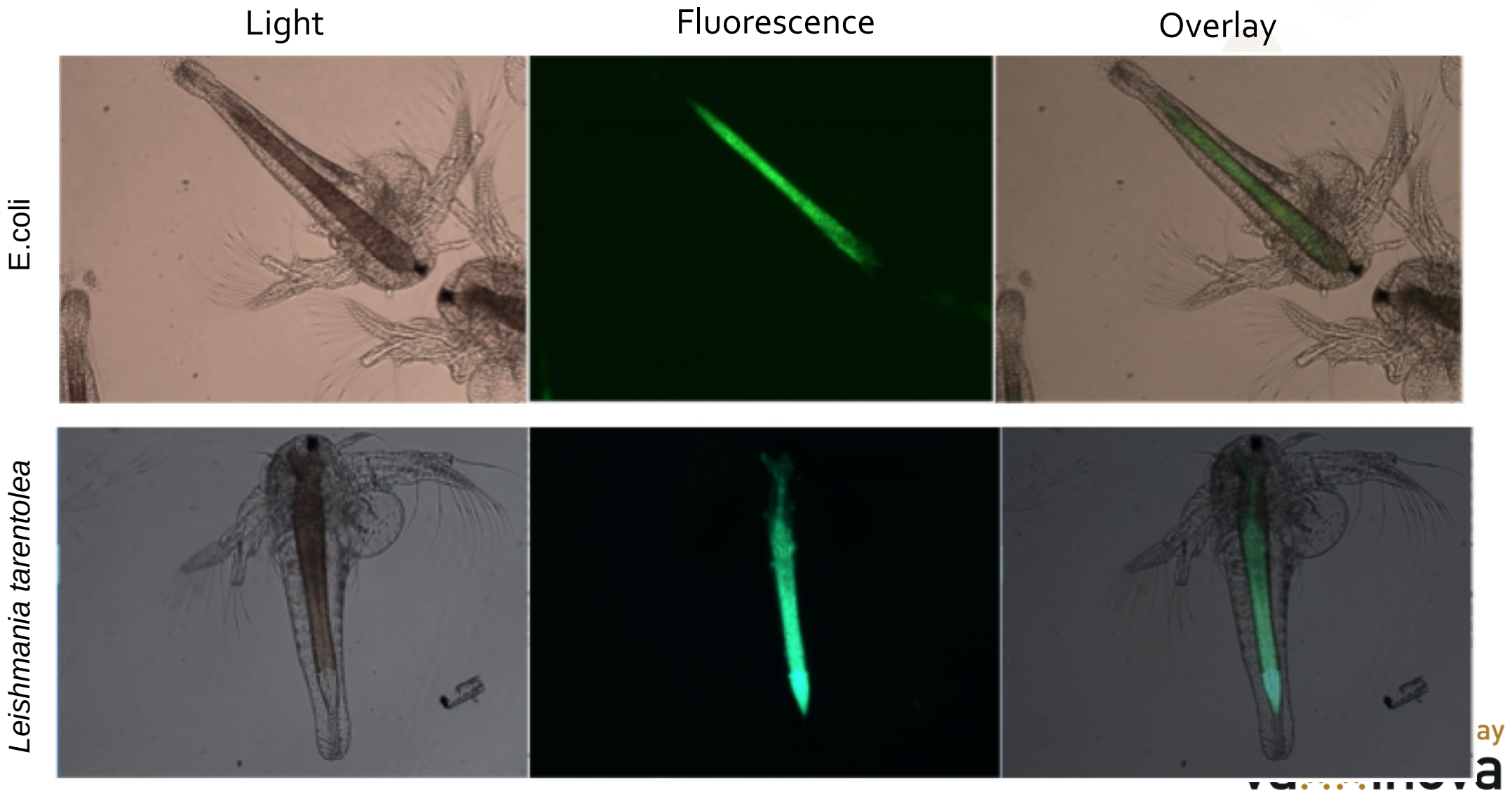
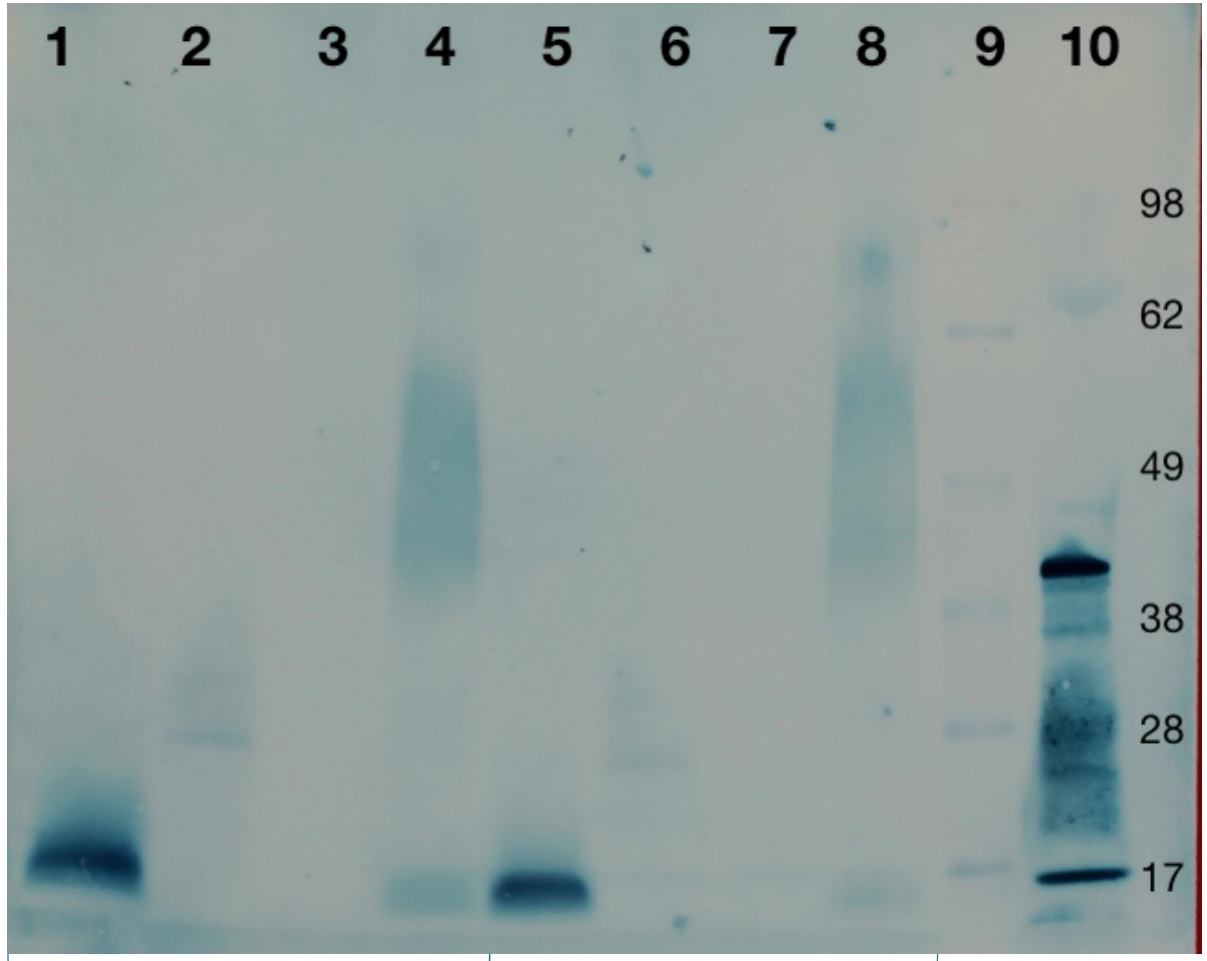
Test with GFP expressing organisms

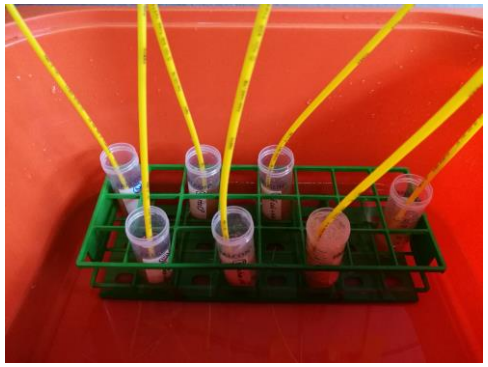


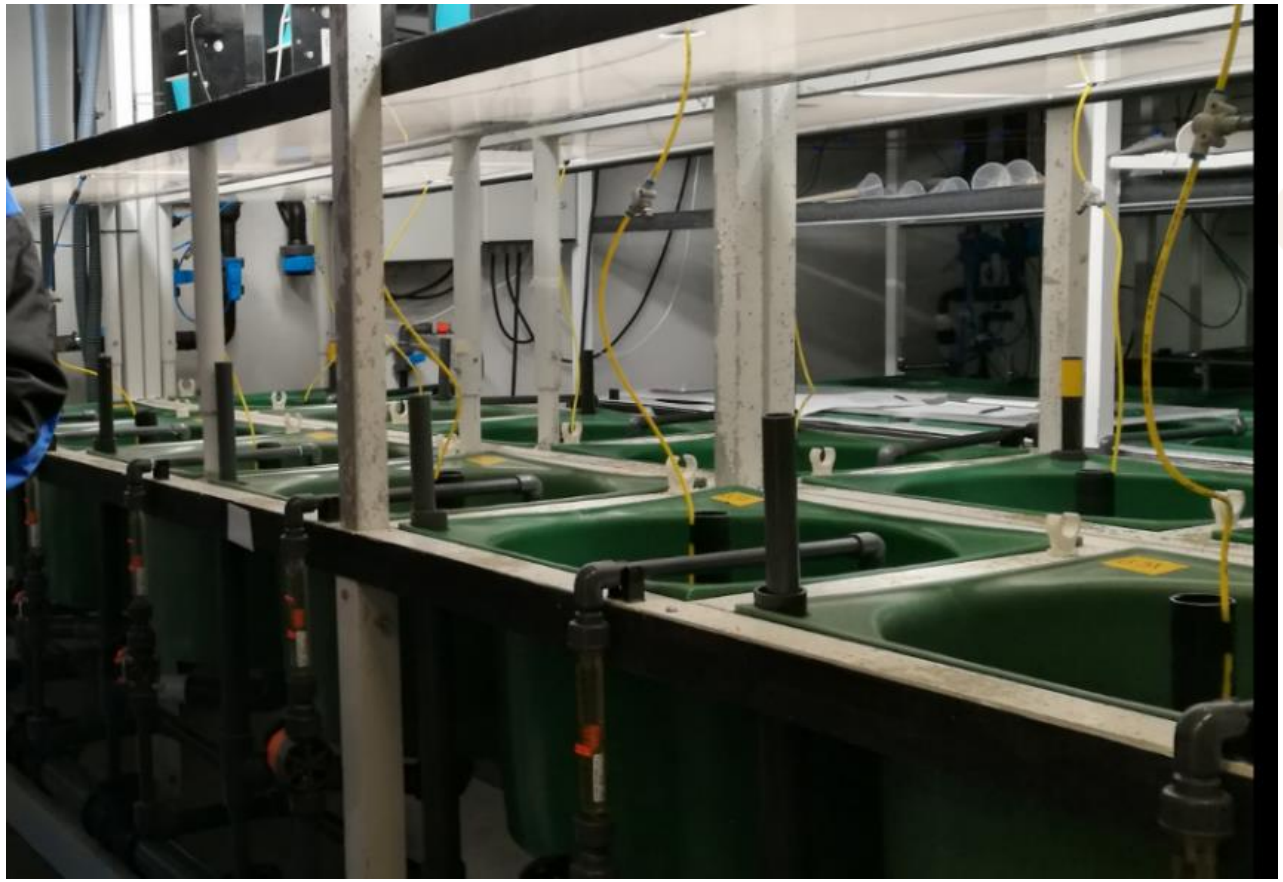
Uptake of protein by Artemia

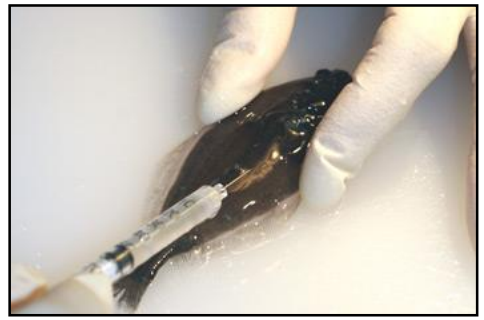


1. purified recCP expressed in *E. coli*
2. *E. coli* expressing capsid protein
3. *L. tarentolae* expressing capsid protein
4. *Pischia* expressing capsid protein.

Lab trial – oral and injection delivery







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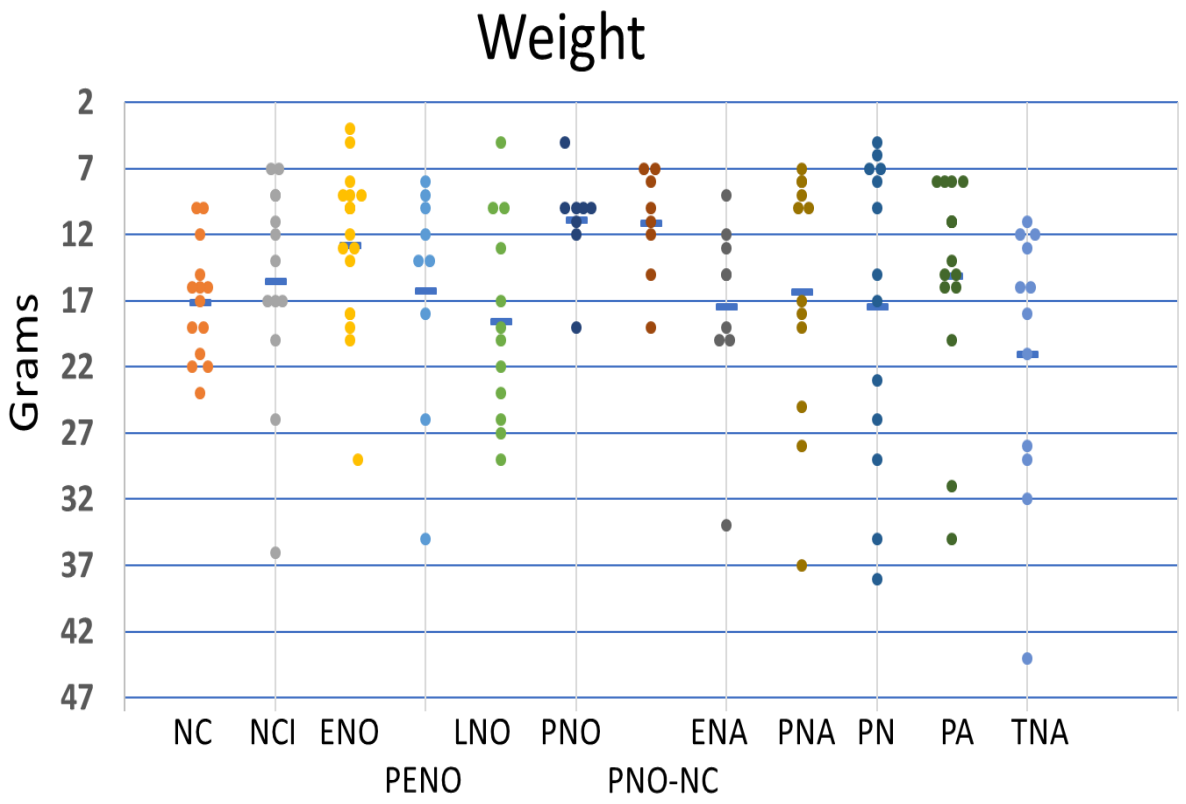
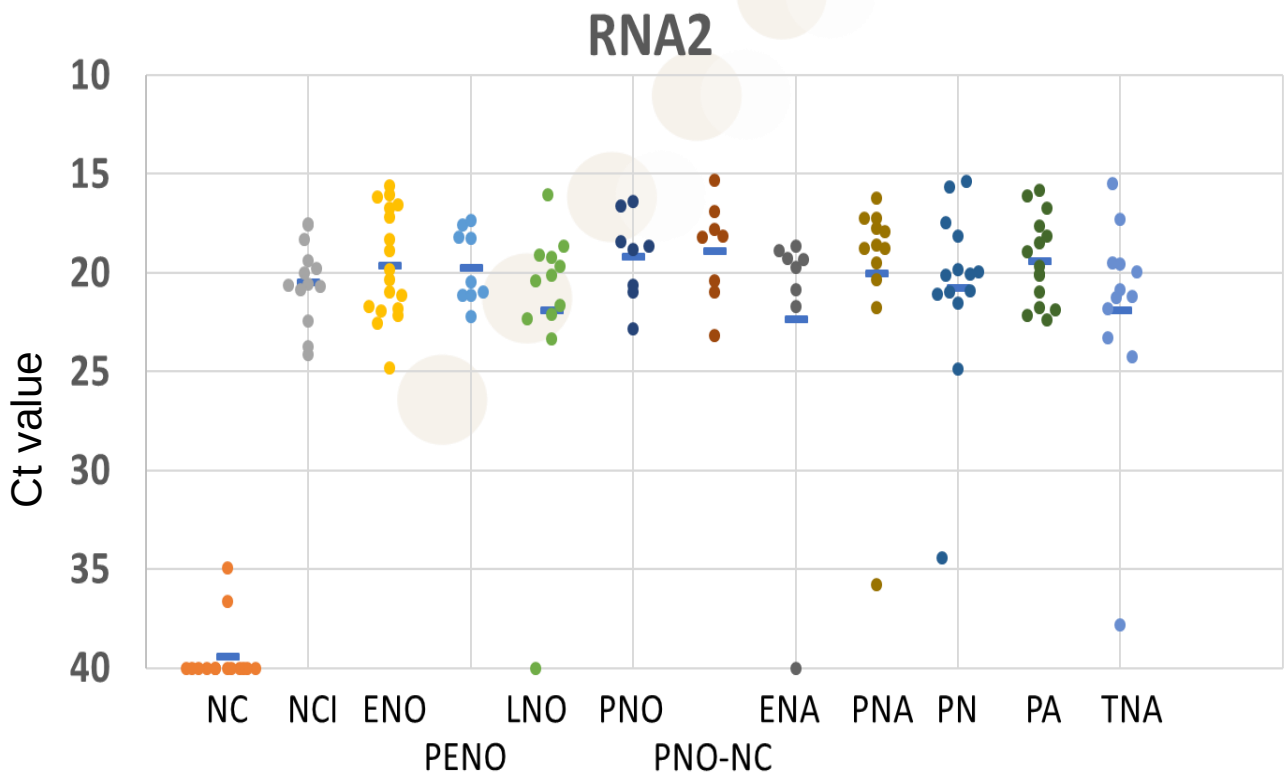
Protection?





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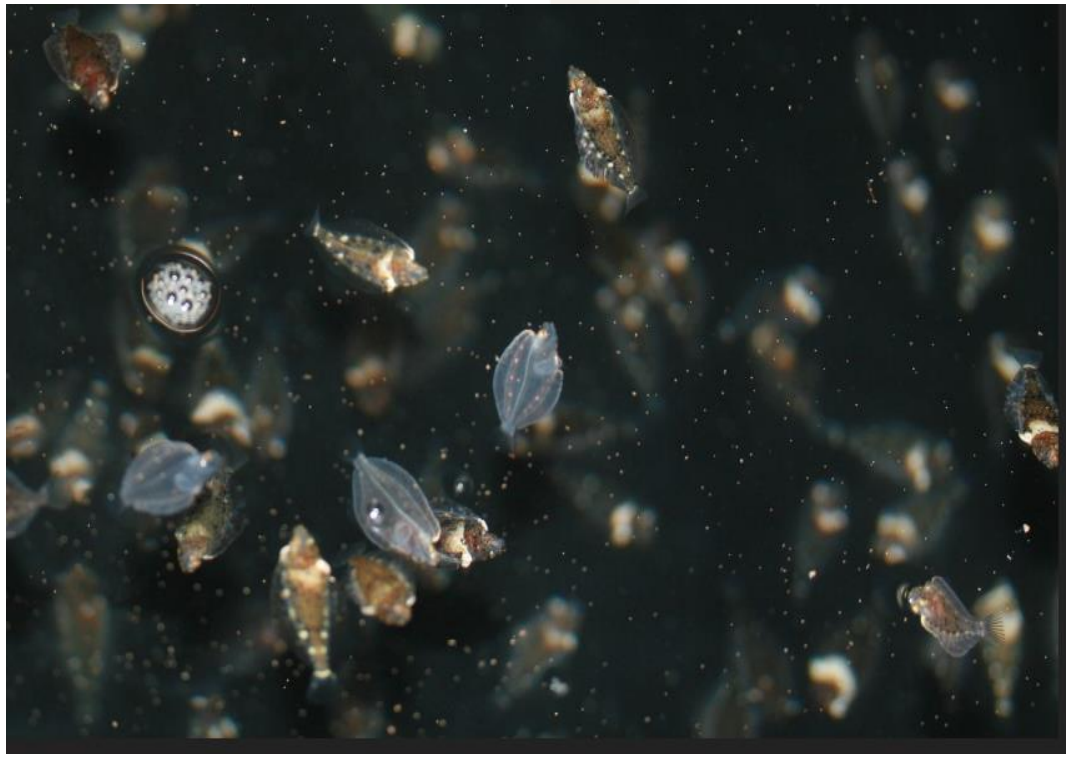
Notes from lab trials

- Vaccination trials should not be initiated before 100 dph
- A balanced diet during development could be important
- Best practice – hygiene during early phases

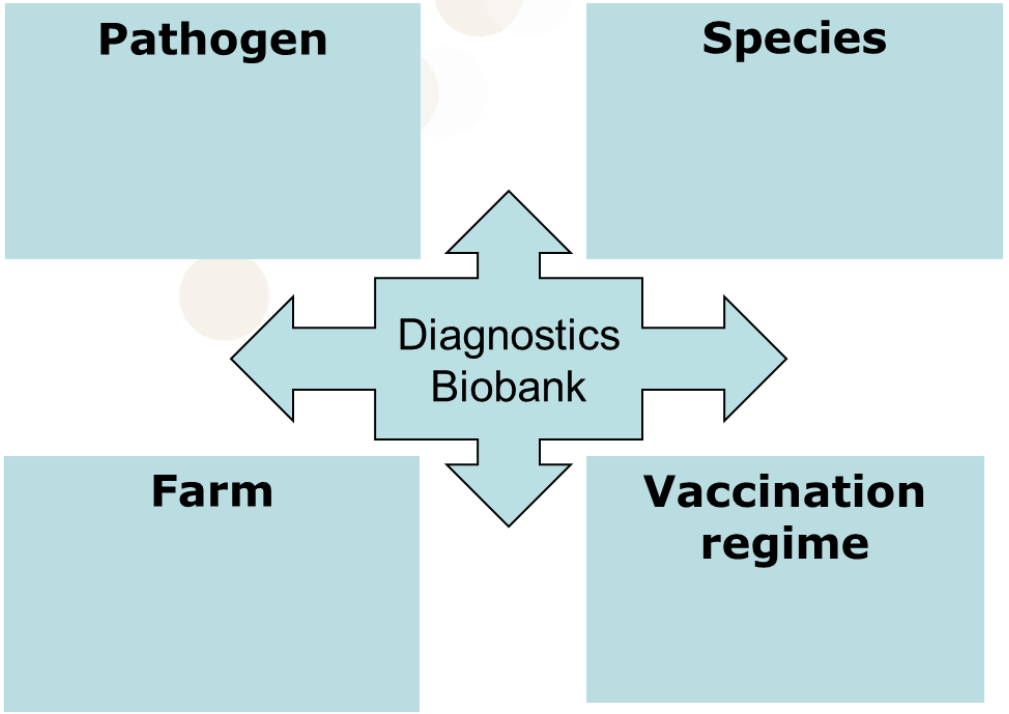


- Both *E.coli* and *L.tarantolae* are filtered from seawater and taken up by Artemia

Vaccines in practice – Administration and strategy at site

Depending on the situation at the specific farm

- Which pathogen is causing disease? Choosing the right isolate
- Farm/farm history….
- When is protection needed (onset and duration of immunity)?
- Handling of fish – stress
- Effect vs. stress and side effects
- Choose strategy (Bath, immersion/dip, injection)



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Vaccine as a solution

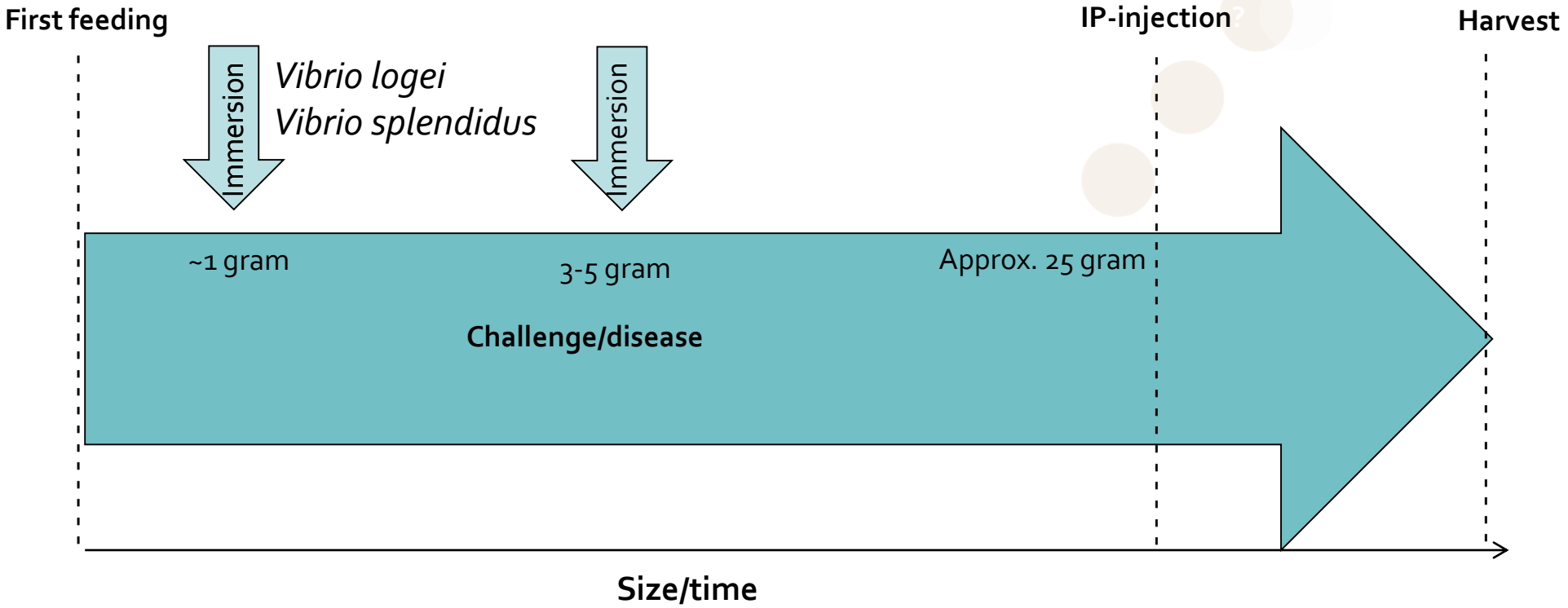
Relevant bacterial diseases

- Atypical *Aeromonas salmonicida*
- *Vibrio logei*
- *Vibrio splendidus*
- *Vibrio tapetis*

Example from use at a commercial site

Bath, immersion/dip, injection

Atypical *Aeromonas salmonicida*
Vibrio logei



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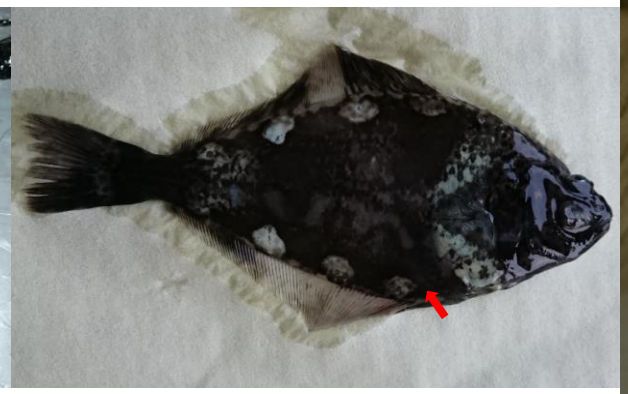
Site of injection

Intraperitoneal injection (i.p)

- Site of injection is close to the basis of the ventral fins posterior to the urogenital papilla
- The dose is deposited in the buccal cavity.



Site of injection

Intramuscular injection (i.m)

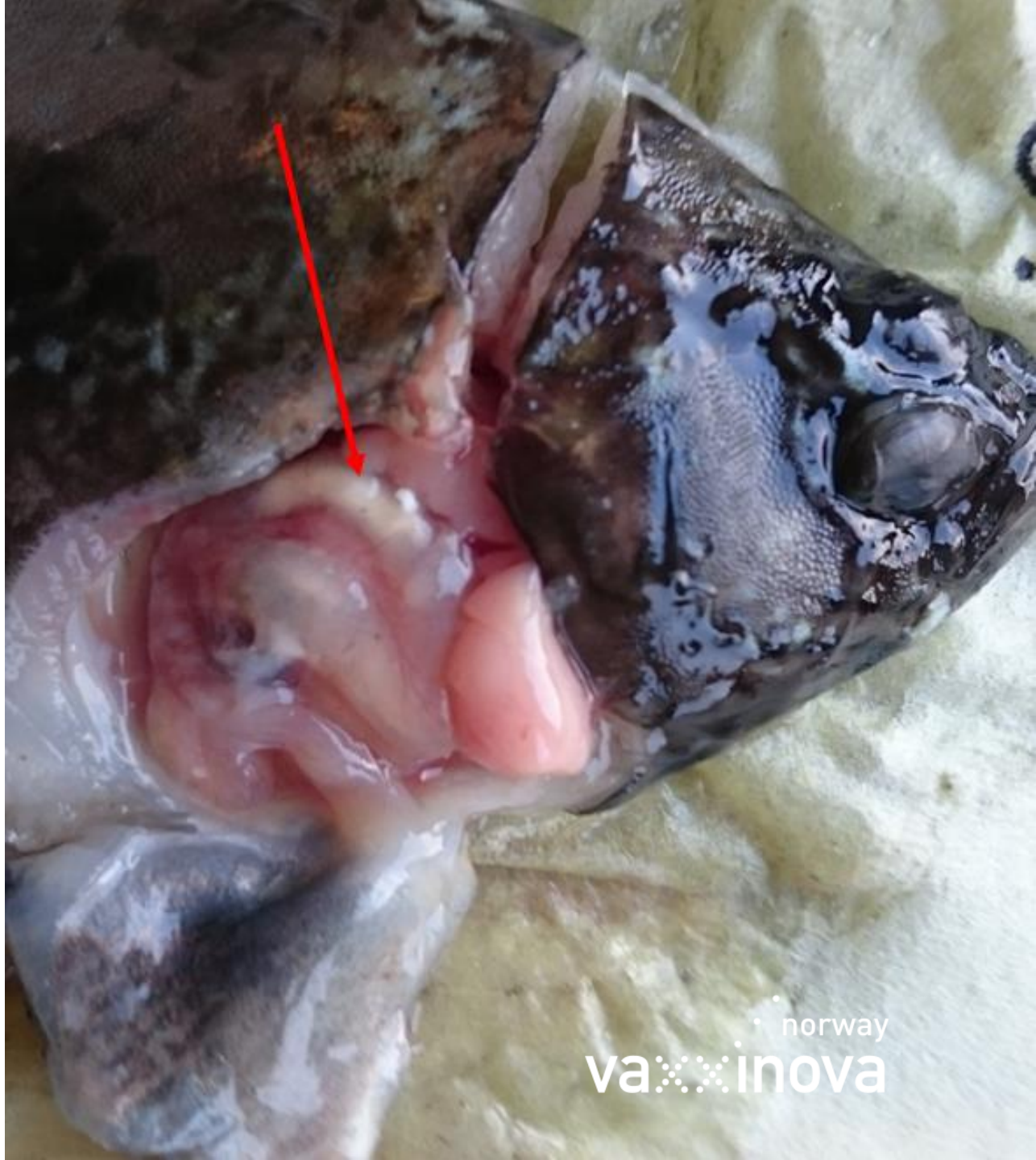
- Half way between the basis of the dorsal fin and the lateral line.
- Only done in trials!

Challenges

Vaccine injected into the intestine

- Experienced up to 20% with vaccine in the intestine
- Loss of herd immunity
- Disease, and lack of vaccine effect
- Need a solution

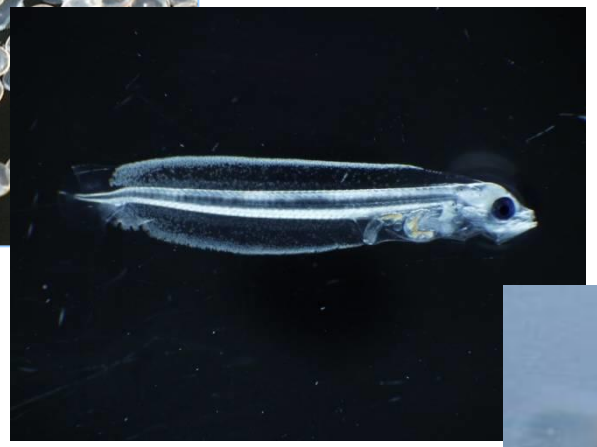
- Ongoing trial to check for safety – Trial ends on 20.Sep



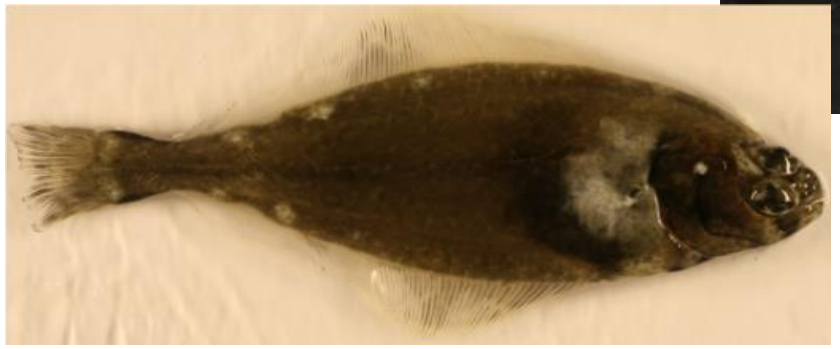
In need of a solution!

Change: administration, adjuvant, regime

- Injection site (i.p, i.m)? Is it safe?
- Adjuvant (water, oil)
- *A.salm* needs oil adjuvant for duration of immunity
- Two injections, different adjuvant?
  - Water based i.m injection, small fish
    - Oil based i.p injection, large fish
- Theory in lab (ILAB) vs reality at site!



Thank you!



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