

**DTU**



# Transmission pathways of Piscine orthoreovirus (PRV-3) and Infectious Pancreatic Necrosis Virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*) at farm level

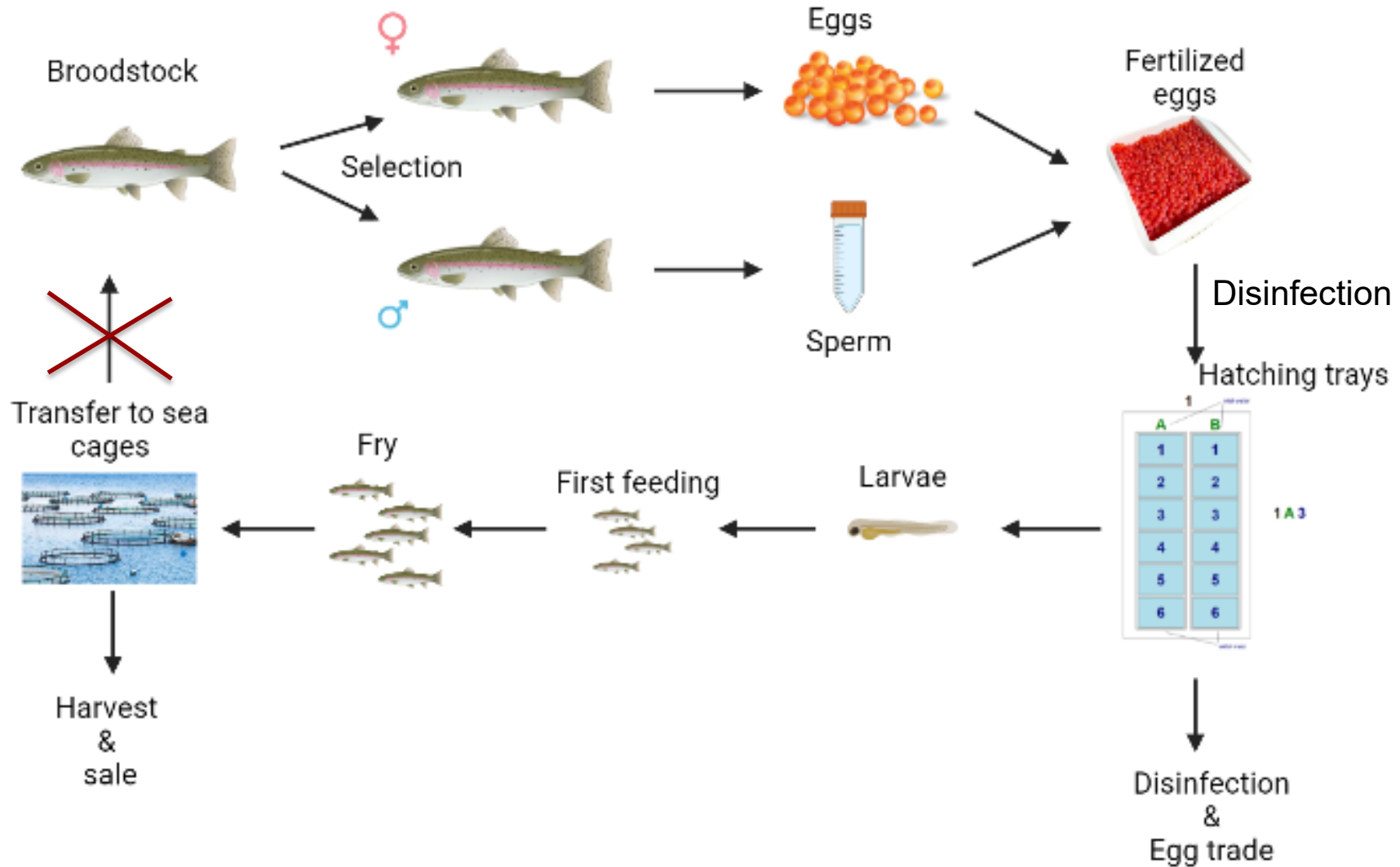


We had Eleni -a Master student writing her thesis on this topic





# Rainbow trout (*Oncorhynchus mykiss*)

NOT in Denmark  
VHS in wild  
marine species

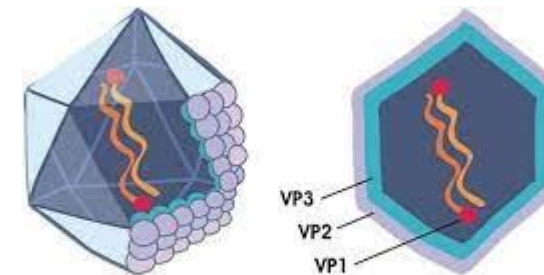
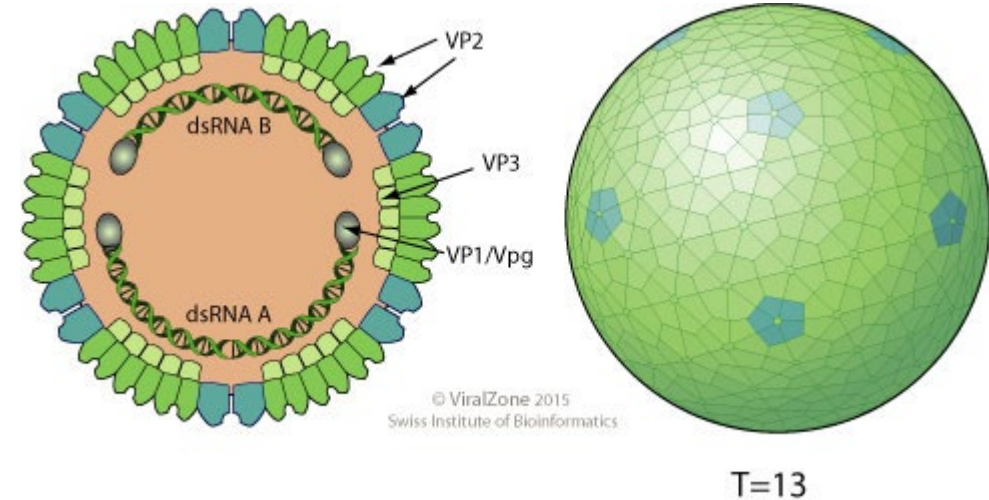


# Transmission routes

 <p><b>Horizontal</b></p>	 <p><b>Vertical</b></p>	
<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> (furunculosis)	<b>Egg-associated</b>	<b>True vertical/ Intra ovum</b>
<i>Vibrio anguillarum</i> (vibriosis)	(all horizontal if no proper disinfection)	<i>Renibacterium salmoninarum</i> (BKD)
<i>Yersinia ruckeri</i> (enteric redmouth syndrome)		Infectious pancreatic necrosis virus (IPN)
viral haemorrhagic septicaemia virus (VHS)		<b>PRV-3???</b>
Infectious haematopoietic necrosis virus (IHN)		
<i>Flavobacterium psychrophilum</i> (rainbow trout fry syndrome)		
<b>PRV-3</b>	<b>PRV-3 ???</b>	

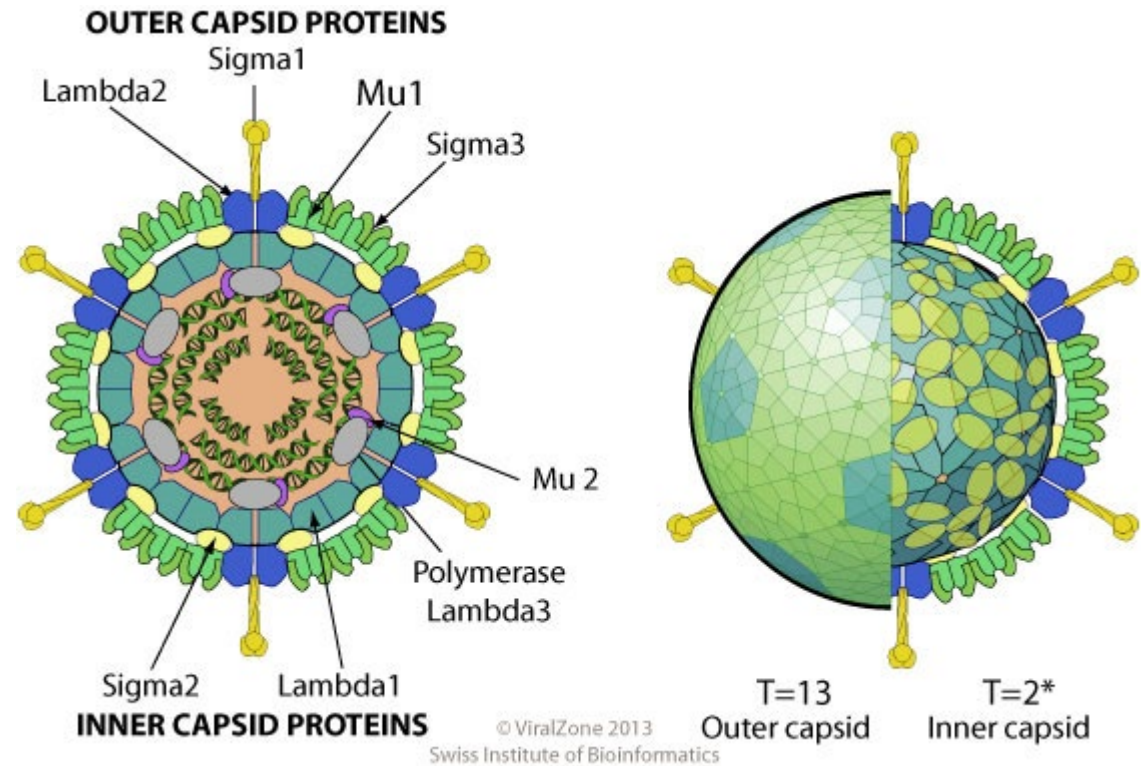
# Infectious pancreatic necrosis virus (IPNV)

- Birnaviridae family
- Genus Aquabirnavirus
- dsRNA
- Non-enveloped
- 2 RNA segments
- Different strains of IPNV
- True vertical transmission



# *Piscine orthoreovirus (PRV)*

- Reoviridae family
- dsRNA
- 10 linear segments (23 kb)
- Non-enveloped
- 3 genotypes
- Cannot grow in cell-culture
- Target red blood cells
- PRV-1 & PRV-3 are transmitted horizontally
- Vertically?

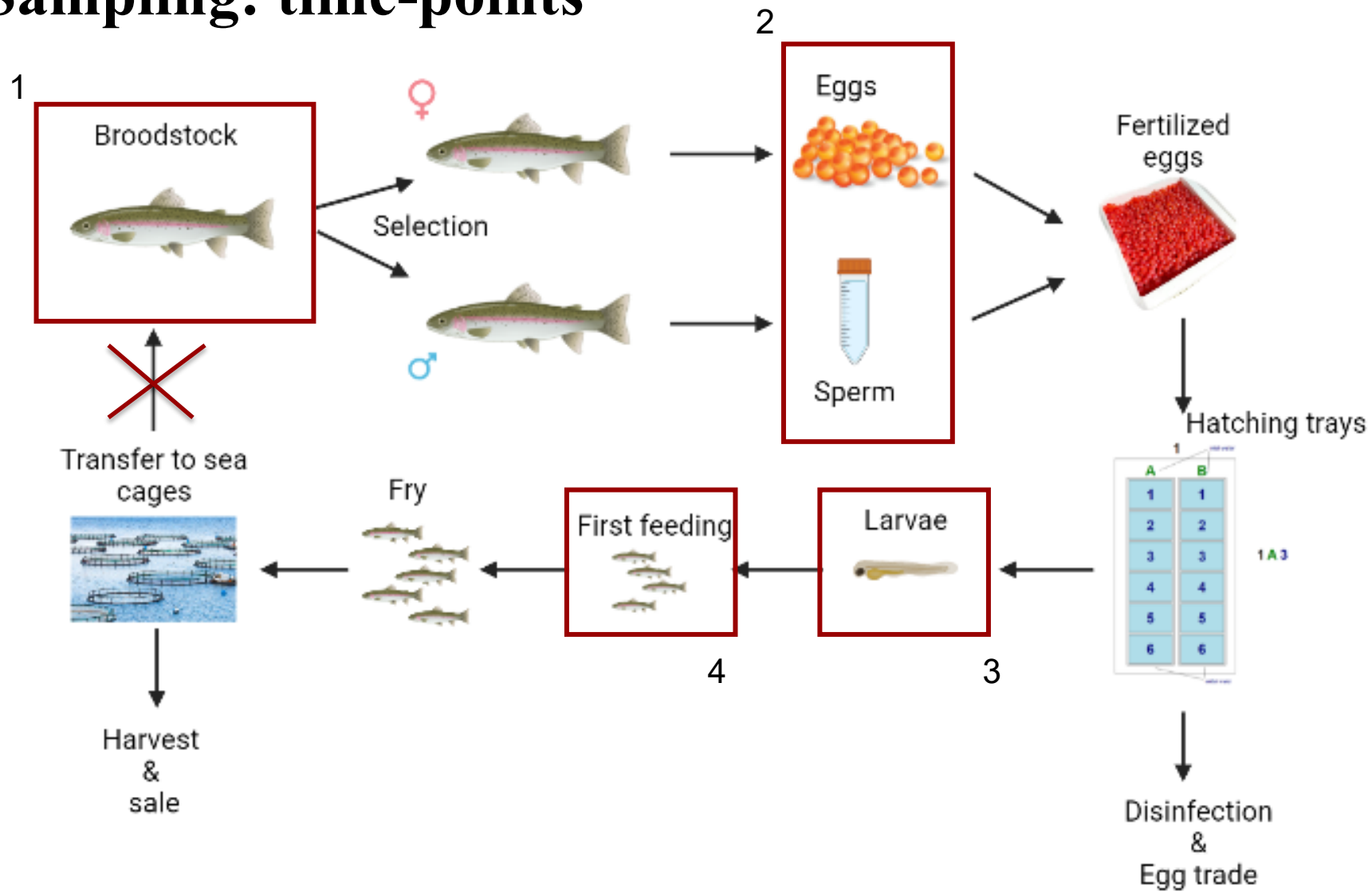


Genotype	Species	Disease
PRV-1	Atlantic salmon	HSMI*
PRV-2	Coho salmon	EIBS**
PRV-3	Rainbow trout	HSMI-like

\*HSMI: Heart and Skeletal Muscle Inflammation

\*\*EIBS: Erythrocytic Inclusion Body Syndrome

# Sampling: time-points





# Broodstock- blood sampling

Broodstock and Juveniles are reared in RAS system with demonstrated presence of PRV-3

28	1st year spawners
29	1st year spawners
31	3rd year spawners
32	3rd year spawners
33	2nd year spawners
34	2nd year spawners

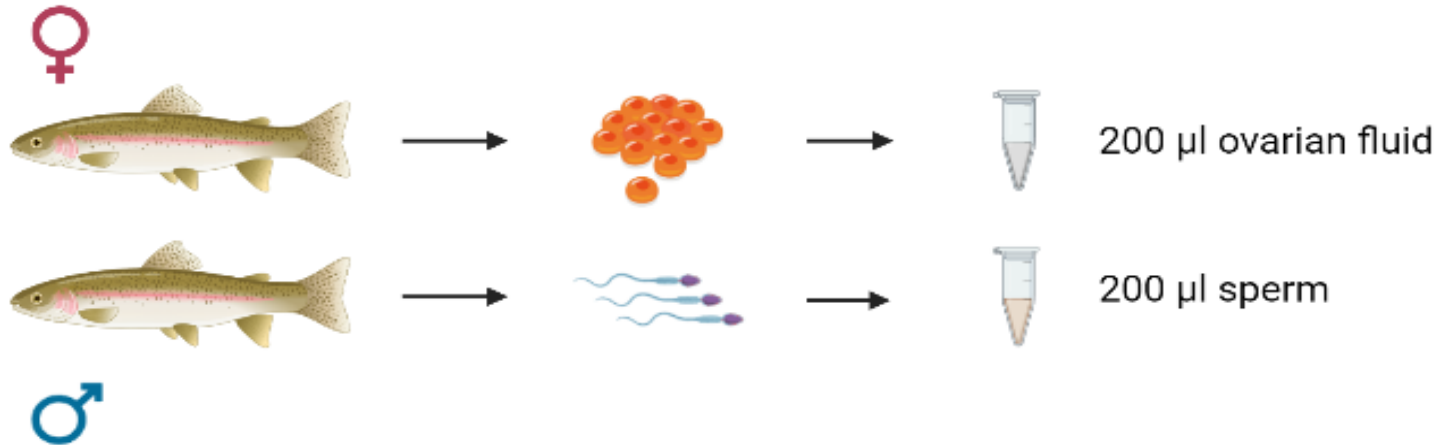
- 20 females/group
- 5 males
- 2x5 pools of juveniles performing poorly



# Broodstock- gonadal fluids

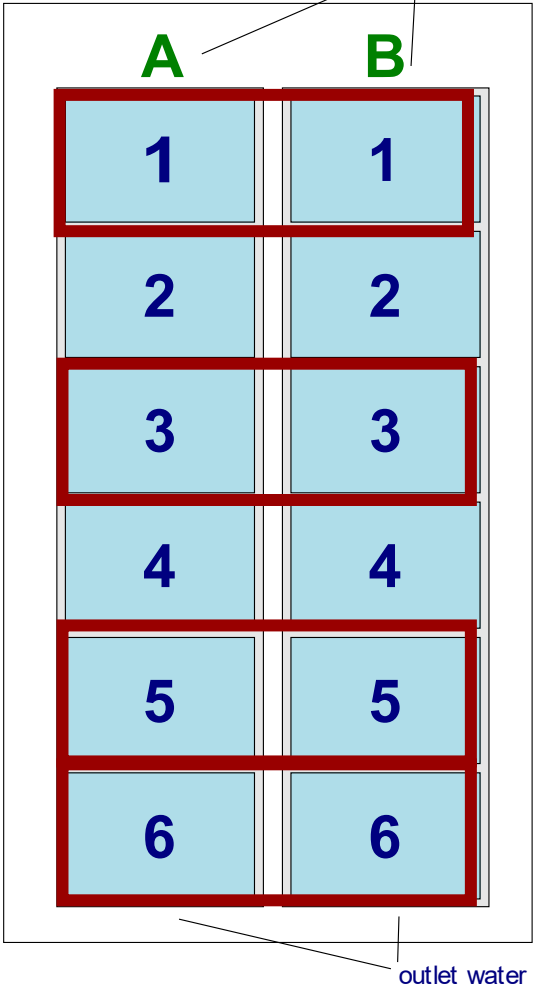
28	1st year spawners
29	1st year spawners
31	3rd year spawners
32	3rd year spawners
33	2nd year spawners
34	2nd year spawners

- 20 females from 2<sup>nd</sup> year spawners
- 5 males



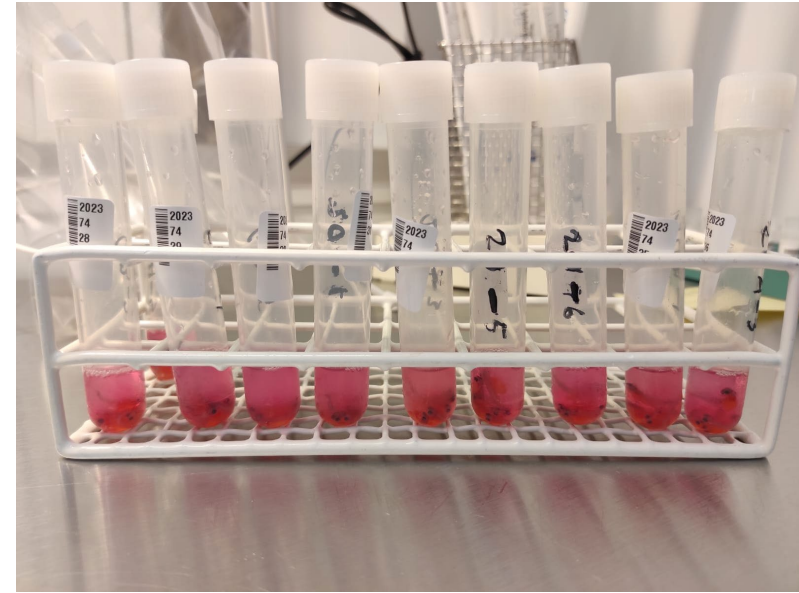
eggs- larvae (yolk sac) – larvae first feeding are in another facility with borehole water

1 inlet water



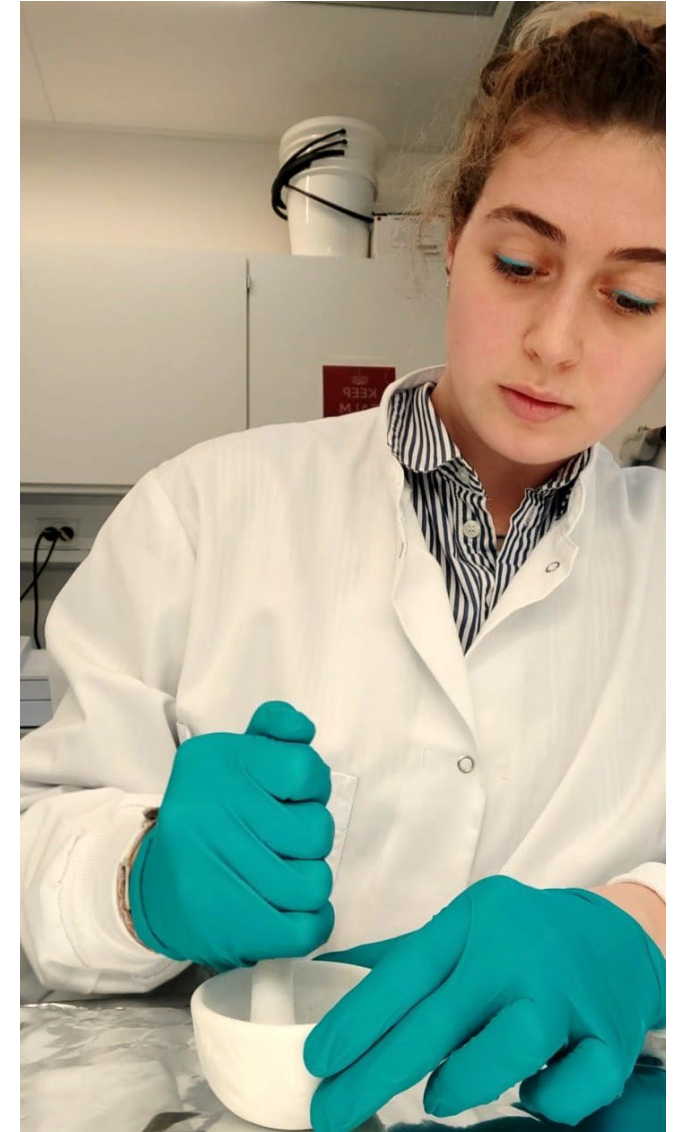
- Hatching trays (2 groups)
- 86 pools → 66(group 1) & 20(group 2)
- 5 fish/pool
- MEM → prevention of bacterial growth
- Grinded
- Dilution with Lysis buffer

1 A 3

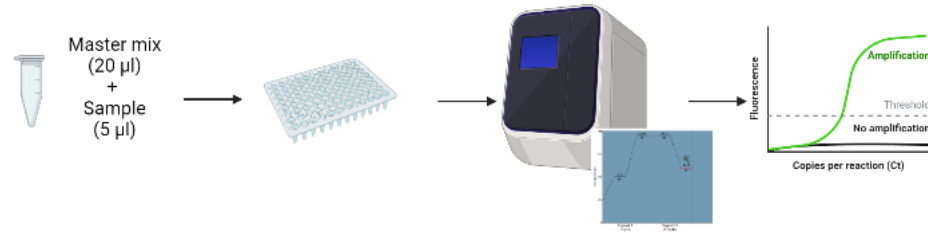


# First feeding

- transferred to 15 concrete tanks
- 45 pools
- 3 pools / tank
- 10 fish / pool
- 13 tanks from group 1
- 2 tanks from group 2
- Grinded + lysis buffer



# RT-qPCR

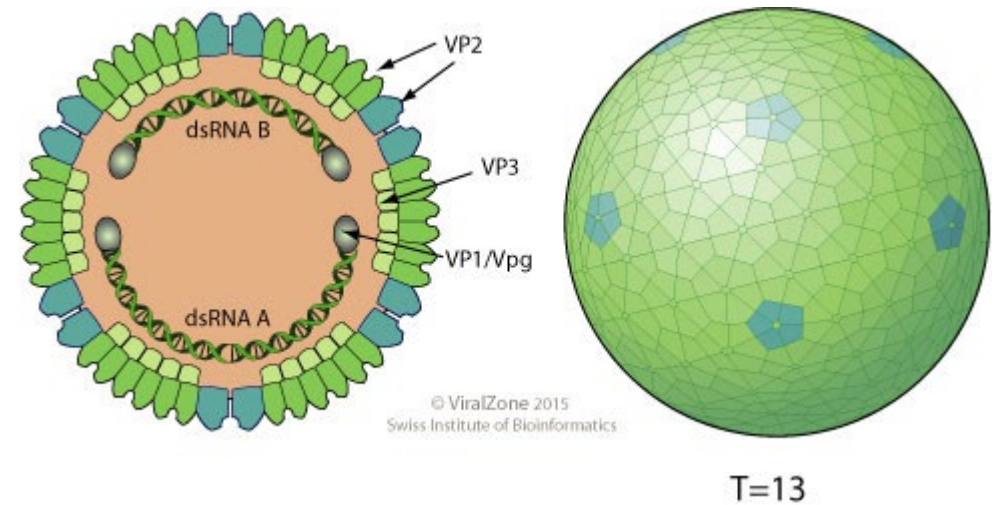
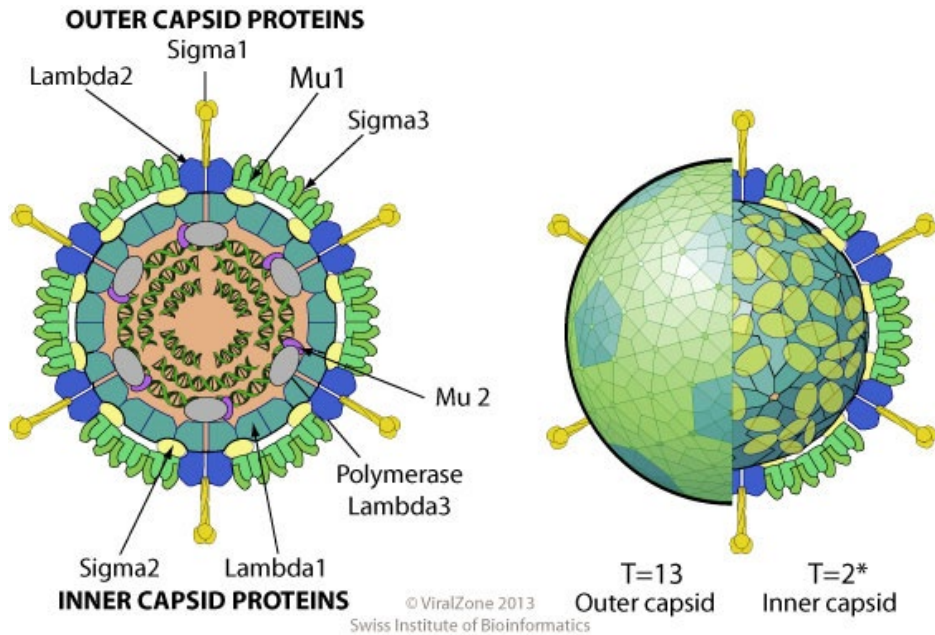


## PRV-3

- Inability to grow in cell-culture
- Primers bind on the L1 segment of the virus (Sørensen et al., 2017)

## IPNV

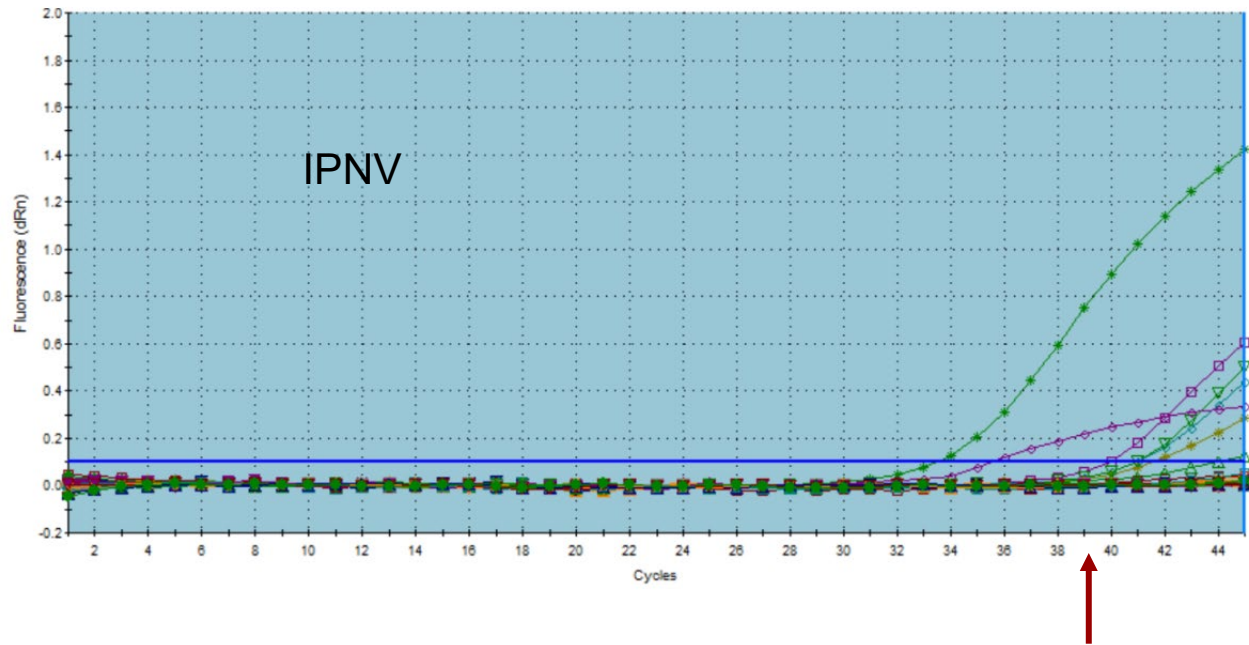
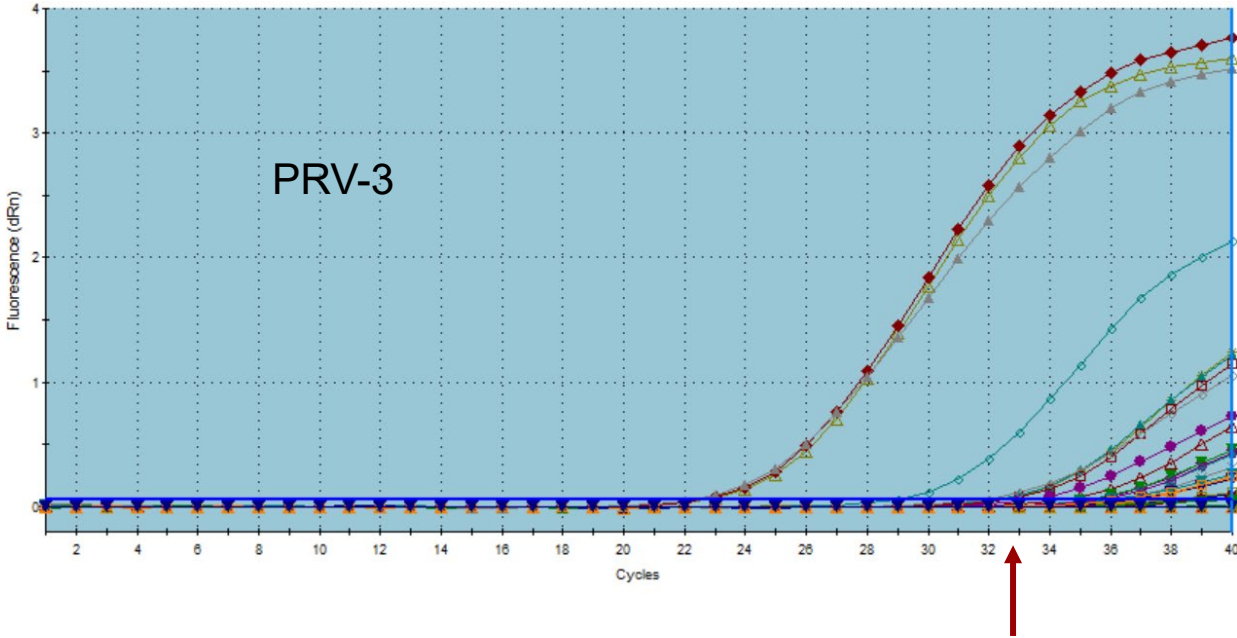
- VP1\_tapia bind on capsid gene
- SP\_VP2 targets major structural protein



# Broodstock- blood samples

Criteria for result evaluation

1. Controls
2. Sigmoidity
3. 
 <35 Ct → positive  
 35 Ct < sample < 40 Ct → suspect  
 >40 Ct → negative



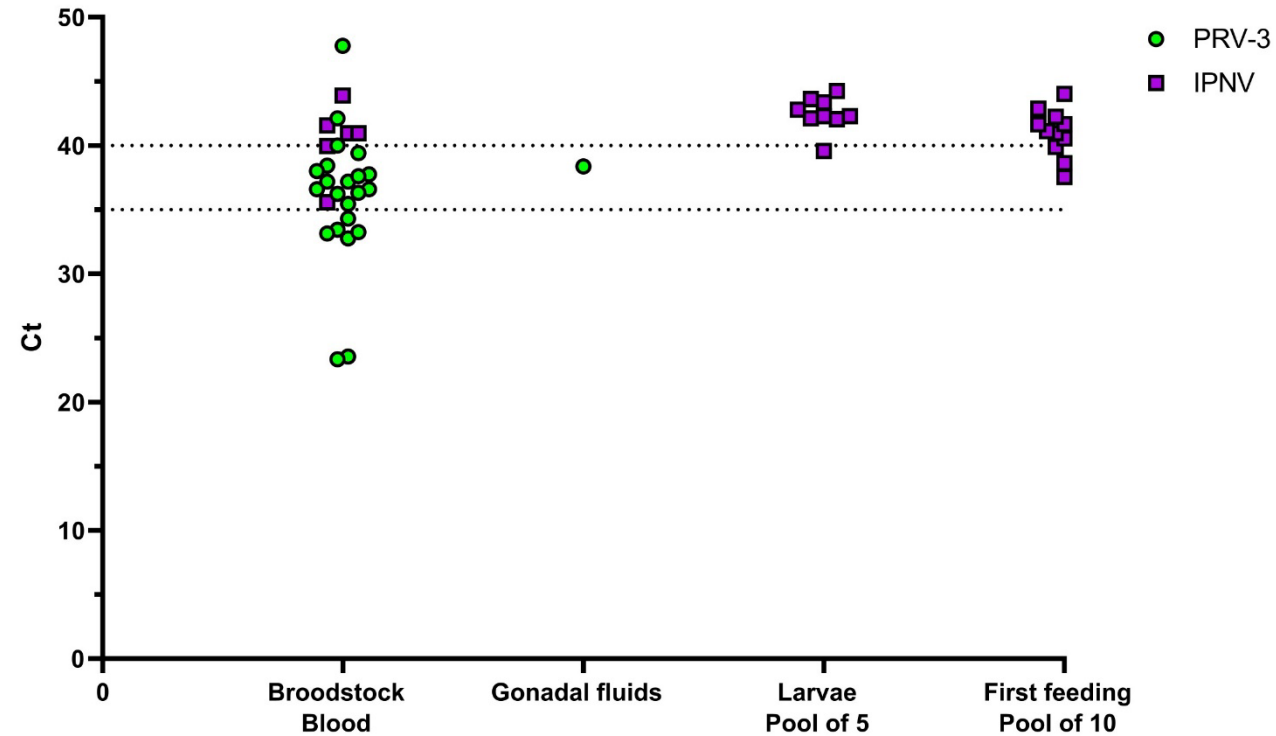
# Overall results

<35 Ct → positive

35 Ct < sample < 40 Ct → suspect

>40 Ct → negative

Ct values of PRV-3 and IPNV



Time-points	PRV-3	IPNV
Broodstock_blood (Week 0)	<p>5/65 ≤ 35 Ct</p> <p>35 Ct &lt; 13/65 ≤ 40 Ct</p> <p>2/65 &gt; 40 Ct</p>	<p>0/65 ≤ 35 Ct</p> <p>35 Ct &lt; 2/65 ≤ 40 Ct</p> <p>4/65 &gt; 40 Ct</p>
Ovarian fluid / sperm (Week 7)	All negative	All negative
Larvae (Week 14)	All negative	<p>0/86 ≤ 35 Ct</p> <p>35 Ct &lt; 1/86 ≤ 40 Ct</p> <p>8/86 &gt; 40 Ct</p>
First feeding (Week 20)	All negative	<p>0/45 ≤ 35 Ct</p> <p>35 Ct &lt; 4/45 ≤ 40 Ct</p> <p>11/45 &gt; 40 Ct</p>



The initial idea → experimental setup in DTU, 2 groups of eggs, disinfecting one group and evaluate the efficacy of iodine against PRV-3.

The shift → direct sampling from the farm in the early life stages of rainbow trout.

# IPNV detection

- Health status – no officially free IPN
- Only one assay showed detection with very high Ct values
- Multiple detection

Blood from broodstock: 9%



Gonadal fluids: 0%

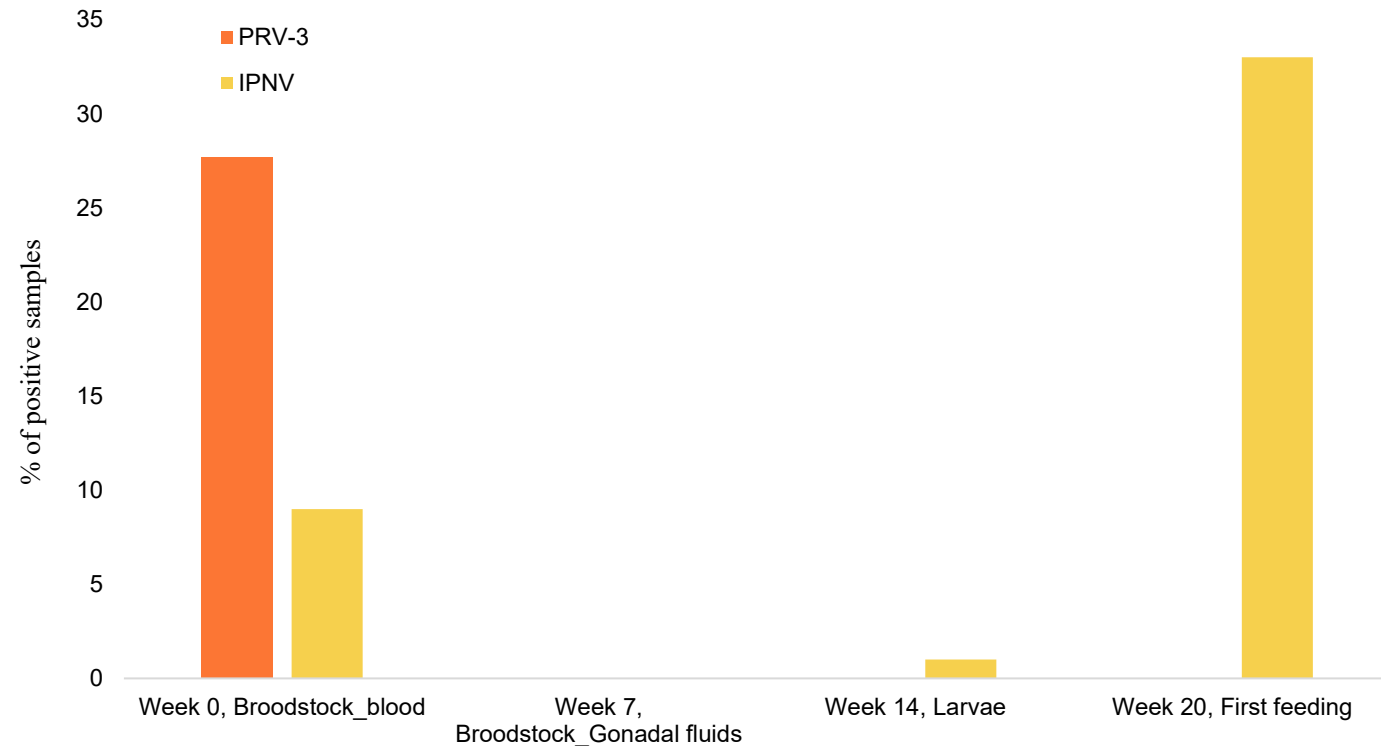


Larvae: 10%



First feeding: 33%

Prevalence of PRV-3 & IPNV in different life stages





# Prevalence of PRV-3

Blood of broodstock: 7.7-27.7%



2<sup>nd</sup> year class 20-68%



Gonadal fluids: Testing the hypothesis 20% prevalence → NO positive samples

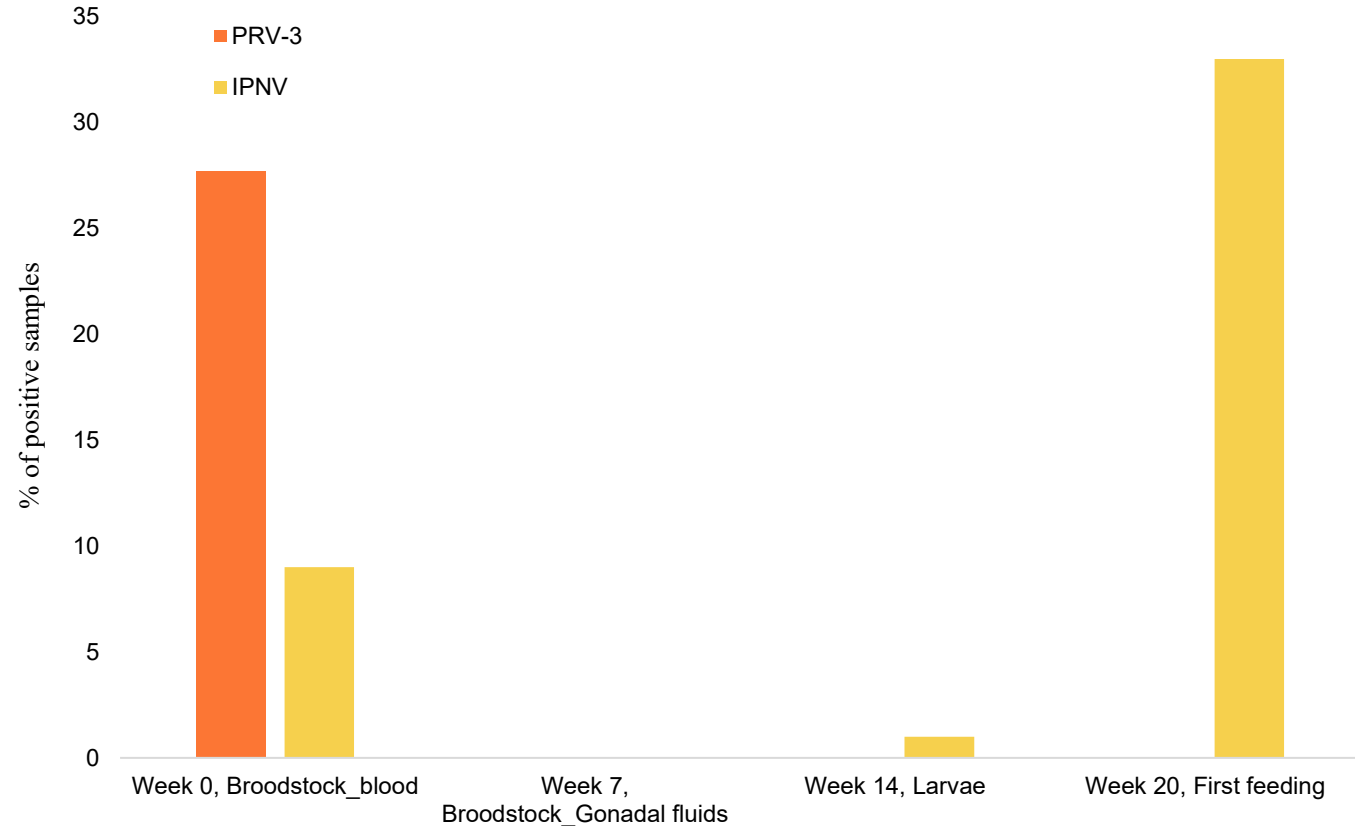


Larvae: Setting low estimated prevalence (3-4%) → NO positive samples



First feeding: based on the sample size, estimated prevalence 7% → NO positive samples

Prevalence of PRV-3 & IPNV in different life stages



Sample tupe	Prevalence
Broodstock- blood	7.7-27.7%
Broodstock- gonadal fluids	0% or <20%
Larvae	0% or <3-4%
First feeding	0% or <7%

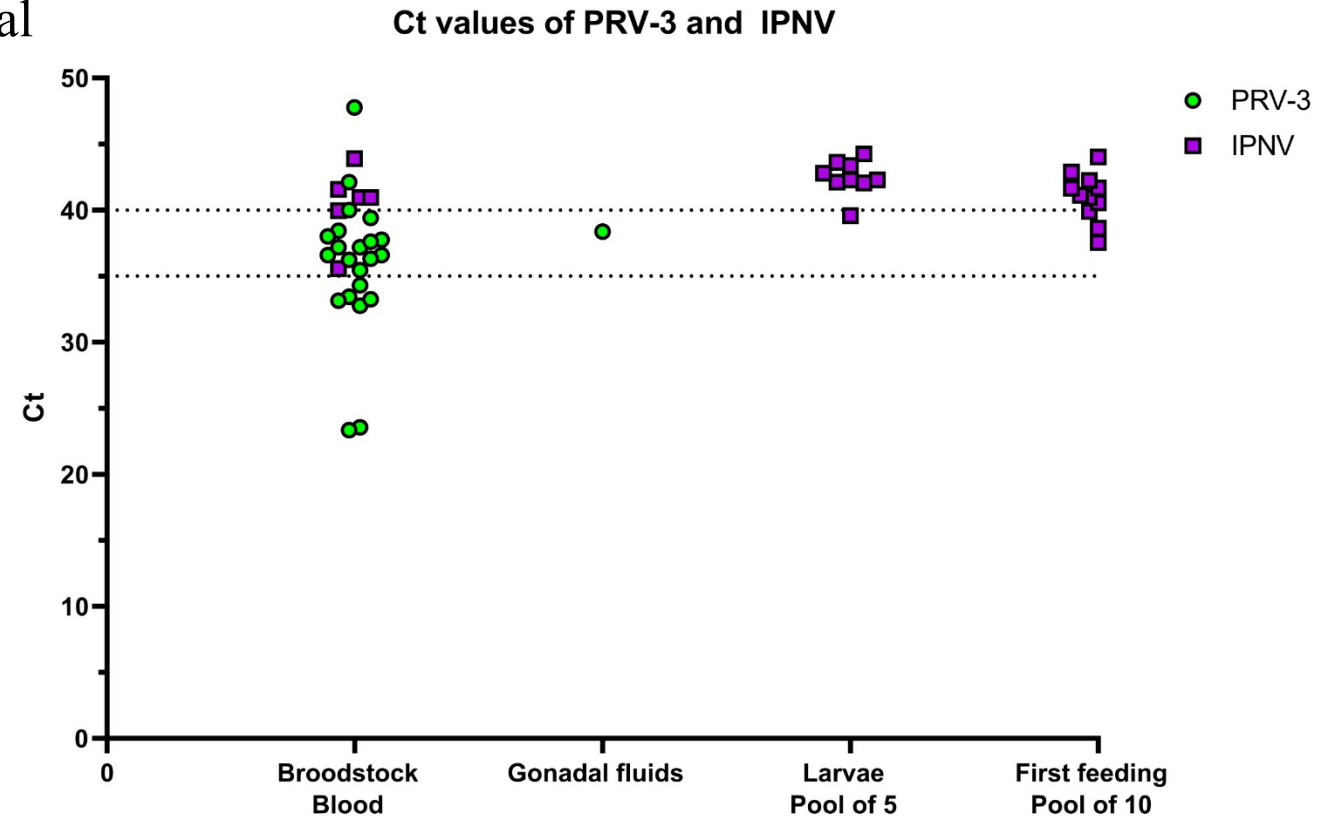
# Vertical transmission?

## » PRV-3

- PRV-3 found only in blood, and highly perfused tissue
- Eggs aren't
- Possibility of blood contamination during stripping
- From an experimental point of view, relatively low viral load in brood stock (what if viremic state?)
- Prevalence tested in larvae < 3% (1%)
- Effect of pooling

## » IPNV

- IPNV true vertical transmitted
- Preliminary data seems to show transfer of virus from broodstock to larvae
- Uncertainty related to diagnostic sensitivity of the assay



## Conclusion

- Vertical transmission does not appear to be a likely route of transmission, even in the absence of disinfection
- This is also consistent with personal experience and anecdotal reports
- Disinfection is always important in supporting biosecurity measures and limiting contamination risks

# RNA extraction



# Disinfection

- Kills pathogens on the outside of the eggshells
- Only formalin against *saprolegnia* sp. → non disinfected
- Risk of blood contamination while stripping. Virus might be found outside the eggshell
- Washing and disinfecting the eggs limits the risk
- Other pathogens in aquaculture facilities
- Standard procedure of disinfection in Denmark

Iodophor (50-100 ppm)



10 min

