5 degrees of separation -

does a reduced propagation temperature for SAV contribute towards higher titers and increased challenge model mortality?

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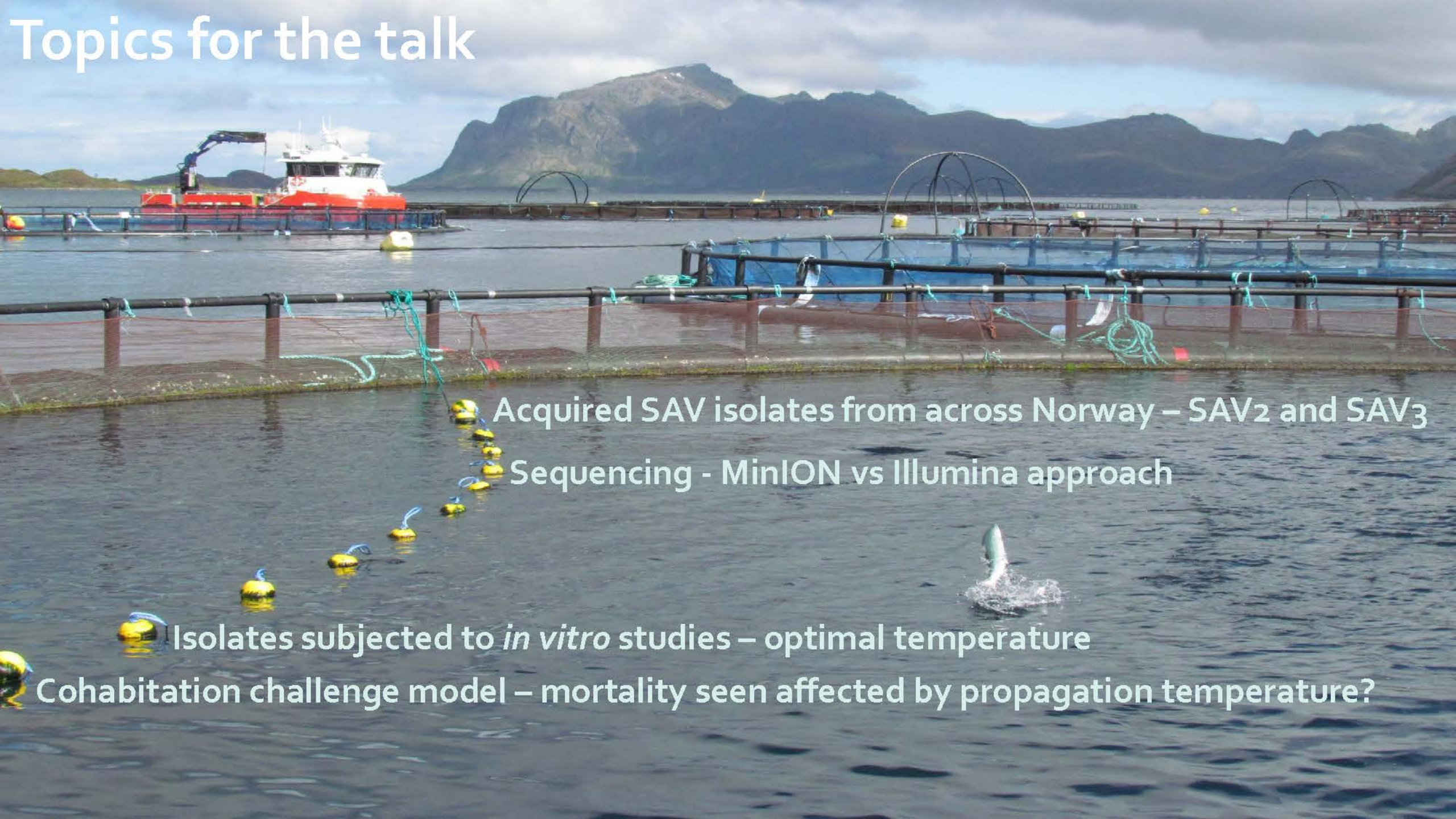
Topics for the talk



- Acquired SAV isolates from across Norway – SAV2 and SAV3
- Sequencing - MinION vs Illumina approach
- Isolates subjected to *in vitro* studies – optimal temperature
- Cohabitation challenge model – mortality seen affected by propagation temperature?

Handling of heart tissues from confirmed PD outbreaks

Tissue homogenates were inoculated on CHH-1 cells

→ cultures with high SAV transcript levels were chosen

Passaged until visible CPE → 2-7 passages

Selected some isolates for sequencing

	Heart tissue	Cell culture	Passage #
SAV3	VX01	VX07	7
	VX02	VX08	3
	VX03	VX09	5
	-	VX13	4
SAV2	VX04	VX10	3
	VX05	VX11	2
	VX06	VX12	3

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Illumina Sequence Capture vs. MinION Genome Sequencing

The consensus sequences for Illumina and MinION were identica

- MinION

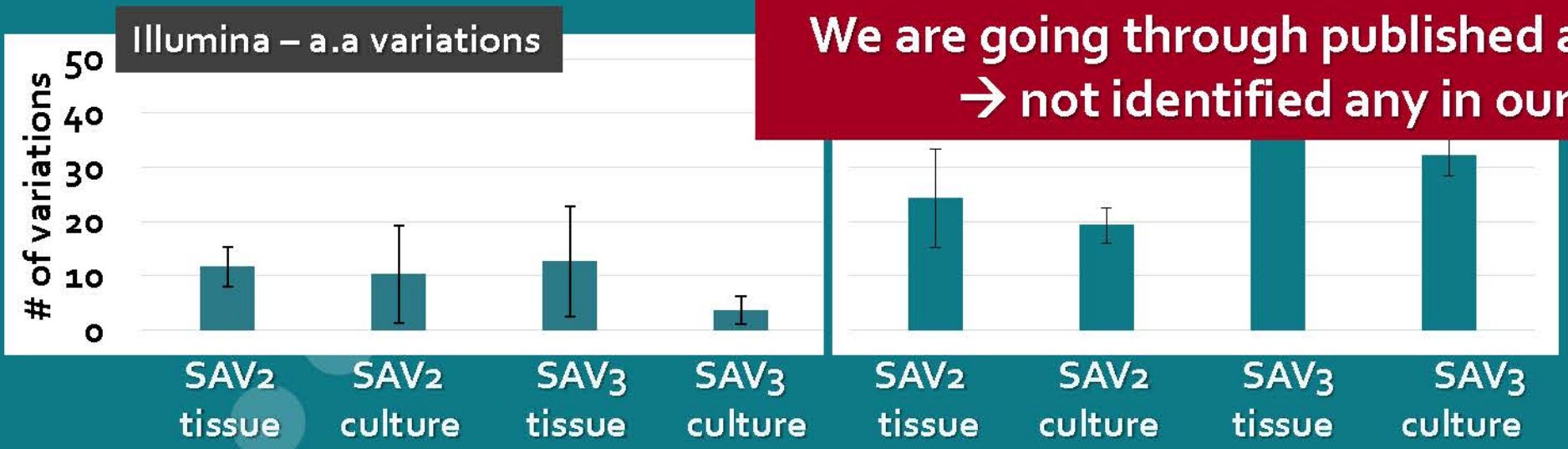
Phylogentically the parallel tissue and cell culture samples grouped together

- **The subtypes fall into distinct clusters**
- **In general very similar sequences with few genetic variations**

MinION seem to overestimate single nucleotide variations

- Illumina showed limited SNV's in both tissues and cultures
- SAV2 indicates less variation than SAV3
- Bottleneck effect; reduced variation in cultured samples than tissues

We are going through published amino acid mutations → not identified any in our sequences yet



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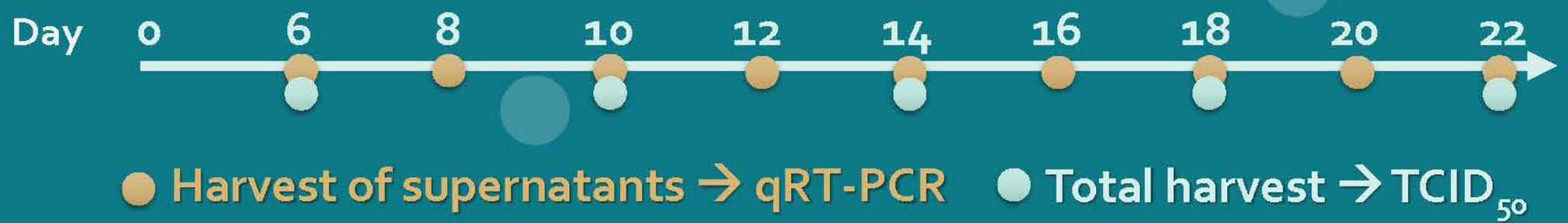
In vitro characterization of SAV2 and 3 isolates

SAV2: VX010
SAV3: VX07, VX08 and VX09

Optimal culture conditions verified by SAV transcripts & $TCID_{50}$



- Incubation at 10, 15 and 18°C
- Three independent experiments

→ present data from individual experiments

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In vitro characterization of SAV isolates

Incubation at 10°C gave higher SAV transcript levels than 15 and 18°C

Infectivity were in general negatively affected over time at 15°C

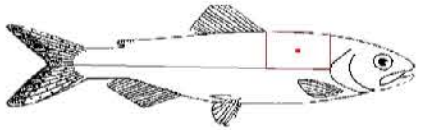
Cultivation at 10°C gave highest titer for both SAV2 and SAV3

SAV3 challenge model development

Sea water challenge

VX13

i.m. injection of shedders



50:50 shedder/cohab

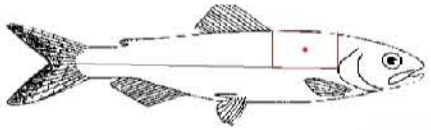
n(total) = 50 fish/group

LOW MEDIUM HIGH

Sea water challenge

VX13

i.m. injection of shedders



50:50 shedder/cohab HIGH

20:80 shedder/cohab HIGH HIGH+

n(total) = 50 fish/group

Bath challenge

n(total) = 60 (50:50) HIGH

As described by Jarungsriapisit et al 2016

Husbandry at both challenges:

24:0 (L:D)

>33‰ and 12°C

80% oxygen saturation

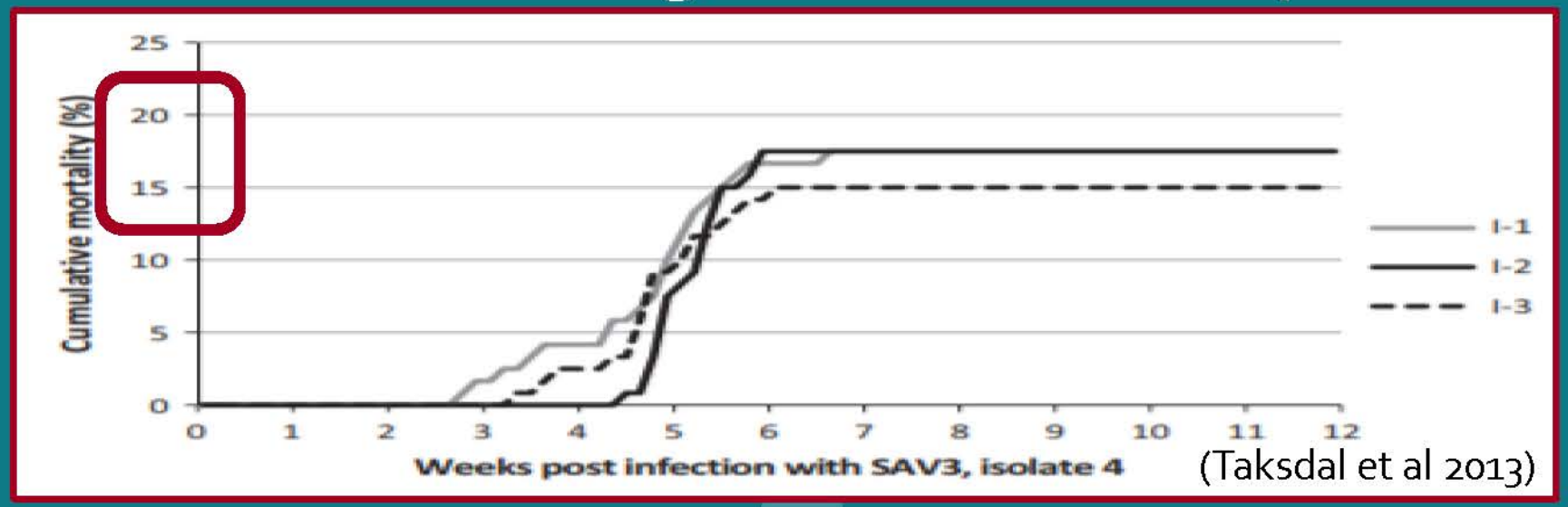
VX13 has given mortality in a sea water challenge model (Taksdal et al 2013)

→ could we repeat that?

Moribund fish sampled for qRT-PCR of heart tissue

Challenge terminated after 6-8 weeks with sampling for histology and qRT-PCR (n=10/group)

Sea water challenge model development



(Taksdal et al 2013)

Table 3 Cumulative mortality in Atlantic salmon carrier fish injected with three isolates of salmonid alphavirus (SAV) 2 and three isolates of SAV 3 and in sham-injected fish in control tanks

	SAV 2			SAV 3			
Viral isolate number	1	2	3	4	5	6	Control
Number of dead fish	0	0	0	1	1	1	1
Percentage mortality	0	0	0	0.3	0.3	0.3	0.3

Differences:

Dose? Our low dose is lower

Propagation? CHSE vs CHH-1

Temperature? 15 vs 10°C

Shedder ratio? 50:50 vs 20:80

Lower cumulative mortality for the second challenge – any indications in the PD infection status?

Conclusions

Norwegian SAV2 and SAV3 isolates

- SAV3 yields higher TCID50 than SAV2
- SAV3 show slightly higher genetic variation than SAV2
  - both in tissues and after propagation in cell culture

For both SAV2 and SAV3, propagation at 10°C was superior to 15 and 18°C

- Higher SAV transcript levels and titers at 10°C
- Reduction of infectious particles with increase in temperature

SAV3 challenge models induced mortalities between 35-60%

- Similar mortality rates in both cohabitation and bath challenge models
- Has been shown previously for the same isolate (Taksdal et al. 2013)
  - ***May cultivation temperature play a role?***

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The literature show that temperature is important – the degree of separation is ±5° ?

Moriette et al (2006) – sleeping disease virus

- *In vivo* challenge after propagation at 10 and 14°C of a WT and recombinant SD virus
- Increased mortality for the strains propagated at 14°C for both WT and rSD

Taksdal propagate at 15°C – we at 10°C → variation in cumulative mortality

- Not only propagation temperature that contribute;
  - fish
  - challenge doses and challenge model
  - onset of challenge after smoltification

We have an abundance of sequencing data to dig through…

- Compare our isolates to previously known mutations that may be relevant for infectivity
  - Sequence samples from 10 and 15°C cultivated samples - genetic differences?

Thank you for your attention!

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