Norwegian University of Life Sciences

Infection dynamics and genetic variability of the virus over the course of infection

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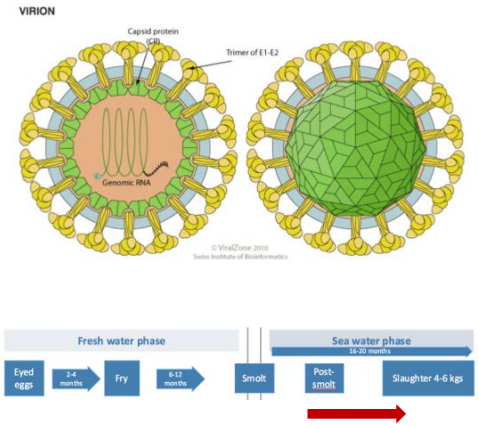
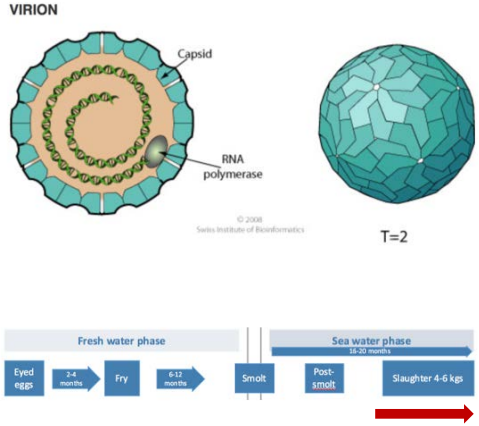
TRINATION meeting 13 -15 March 2018

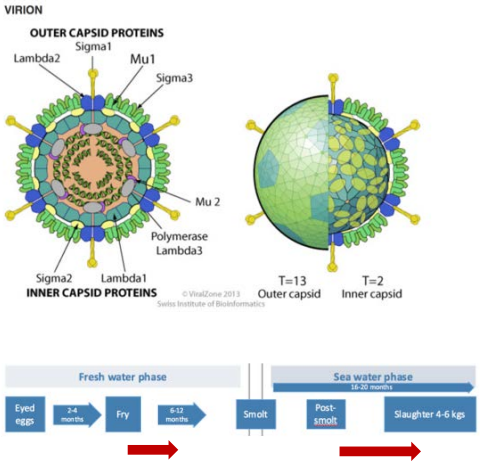
- Alphavirus (PD)
- *Togaviridae*
- 66 nm
- Membrane-bound
- Virus genome: single-stranded ssRNA virus (+ strand)

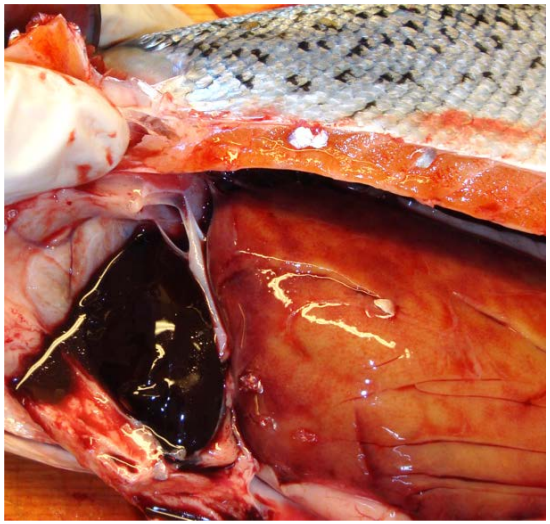


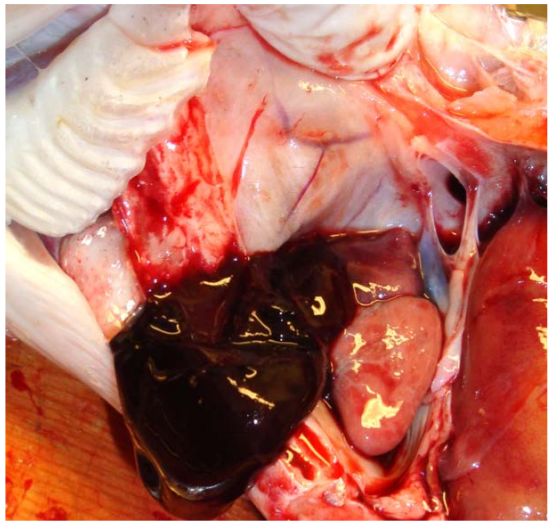
- Totivirus (CMS)
- *Totiviridae*,
- 50 nm
- Naked
- Virus genome: dsRNA virus



- Piscine orthoreovirus (HSMI)
- Reoviridae
- 55 nm
- Naked
- Virus genome: dsRNA virus

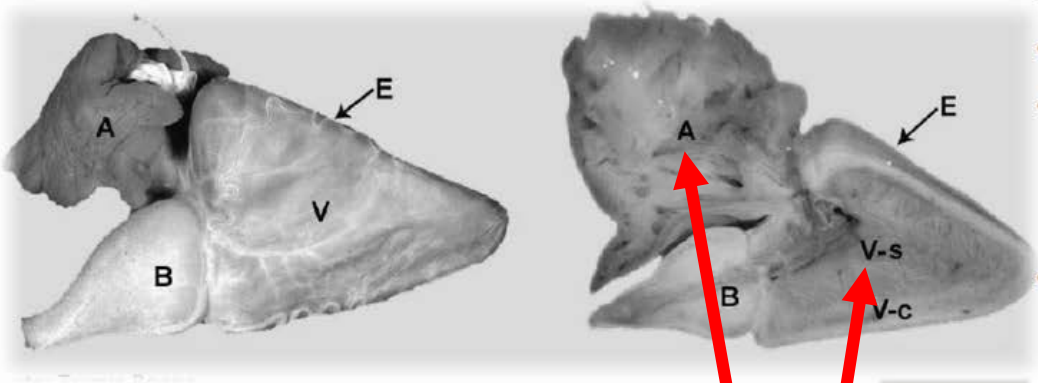


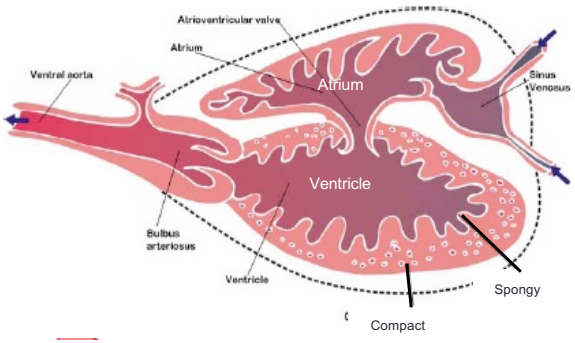




Cardiomyopathy syndrome (CMS)

Cardiomyopathy syndrome (CMS) – affected parts of heart

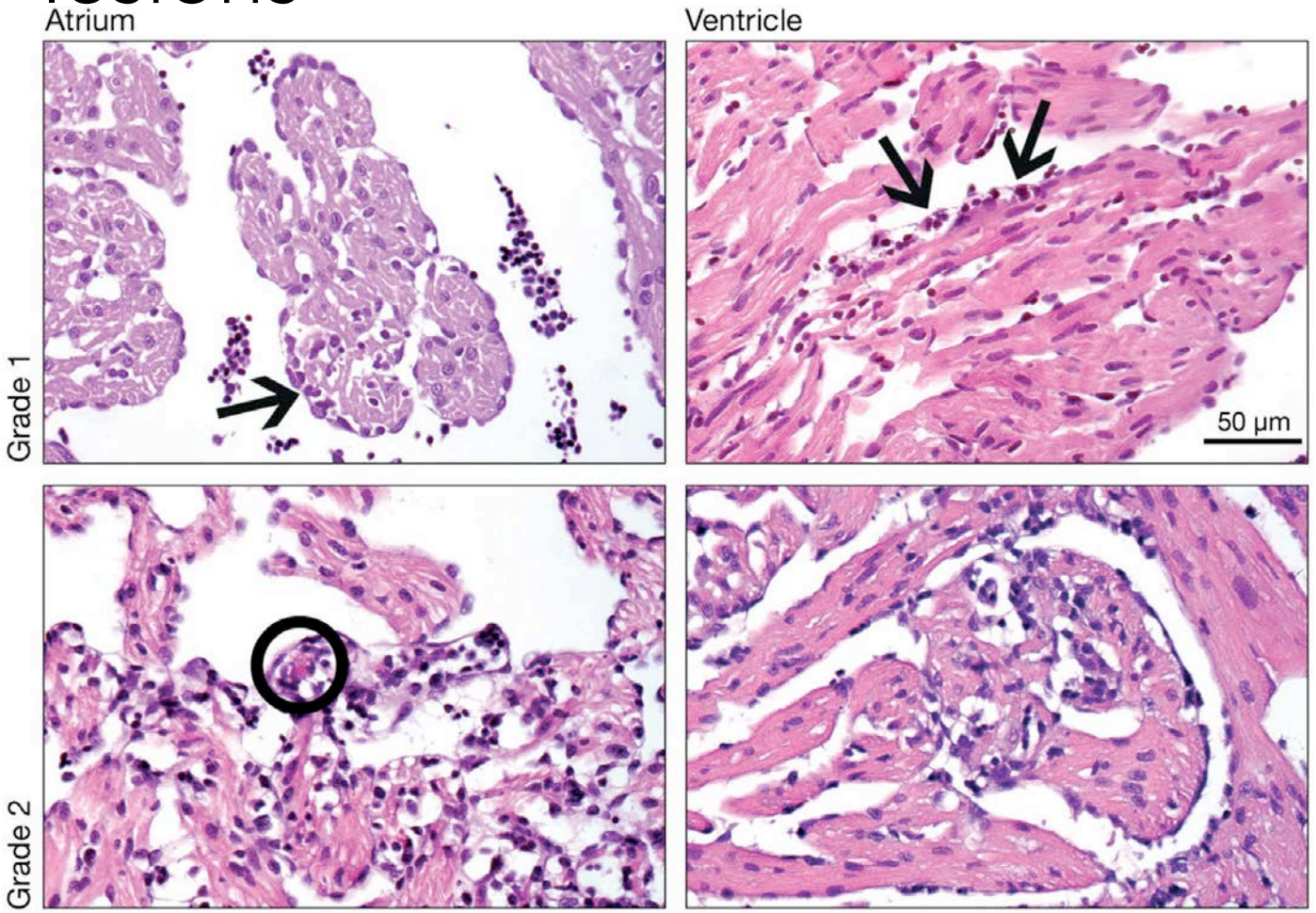




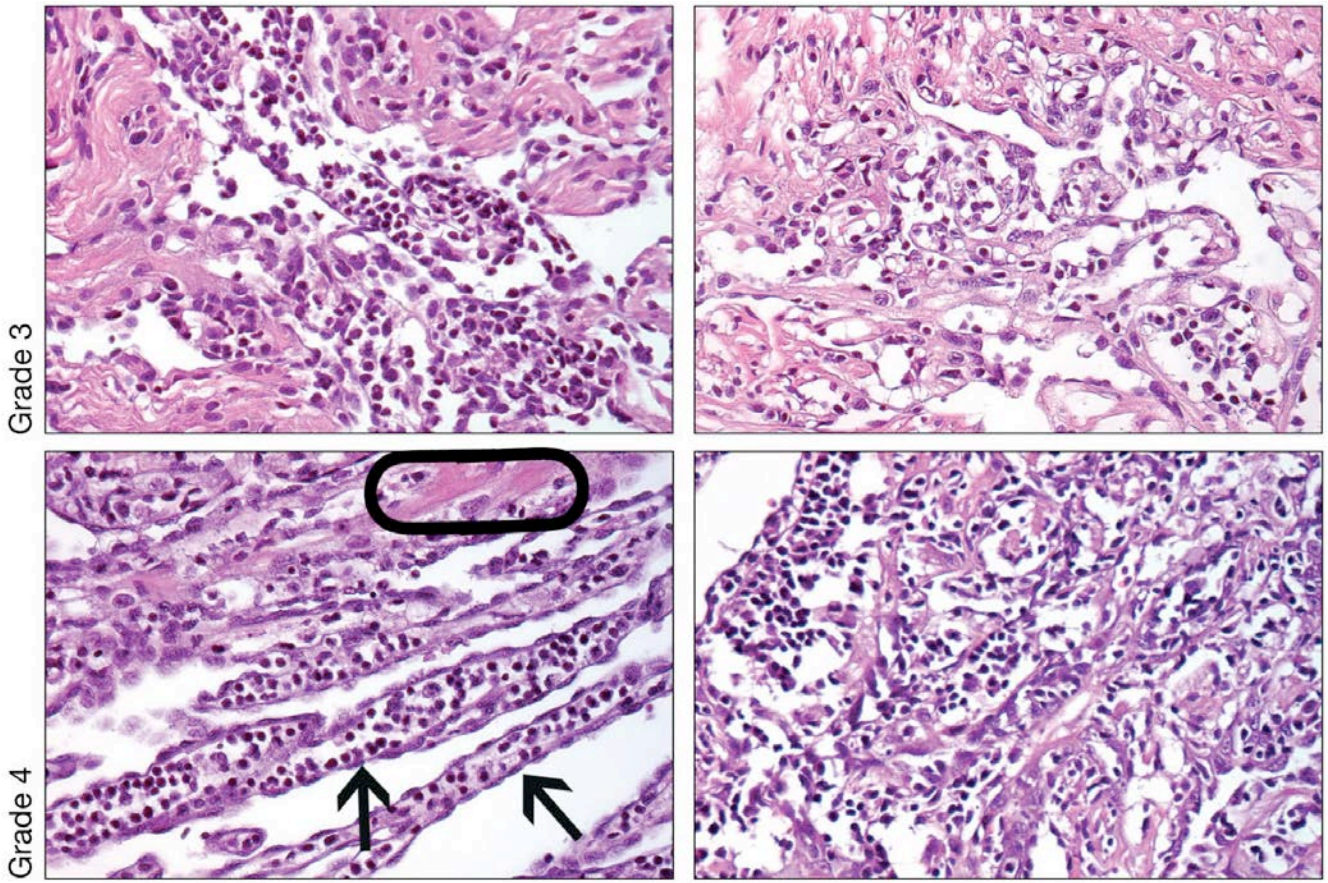
- A: Atrium
- B: Bulbus arteriosus
- V: Ventricle
  - V-s, spongious ventricle
  - V-c, compact ventricle
- E: Epicard

Parts of the heart affected by CMS

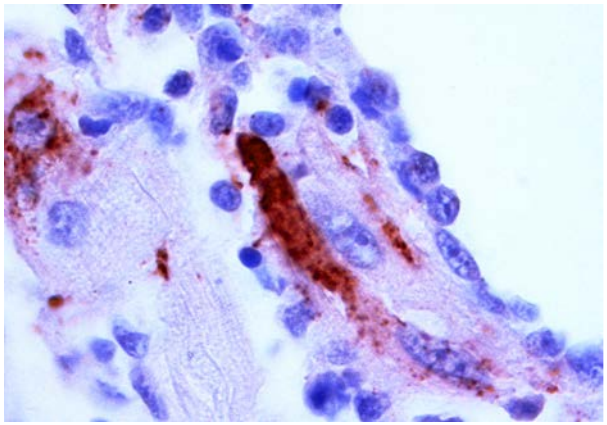
Histological classification of lesions



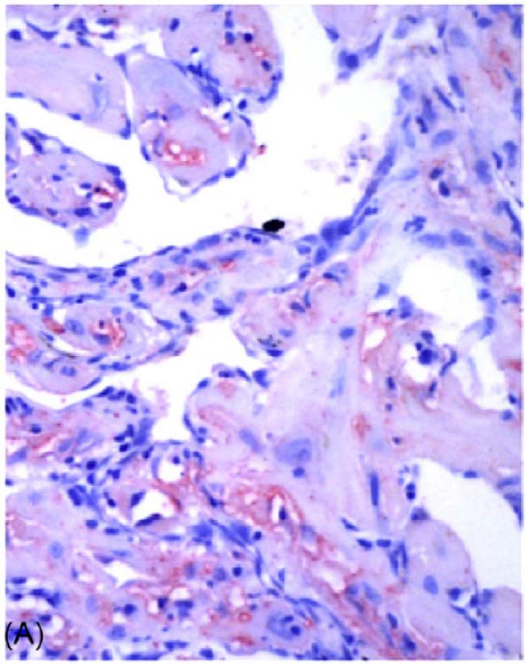
Fritsvold et al. (2009) Dis Aquat Org. 87: 225–234



Fritsvold et al. (2009) Dis Aquat Org. 87: 225–234



In situ hybridization PMCV



IHC PMCV
(ORF3 specific)

- Detection of viral genome by ISH or viral antigen/proteins (IHC)
- Simultaneous detection of pathology and the “footprint” of the virus provides good documentation that the damage seen is caused by the virus

Haugland O, Mikalsen AB, Nilsen P, Lindmo K, Thu BJ, Eliassen TM, Roos N, Rode M, Evensen O. J Virol. 2011;85(11):5275-86

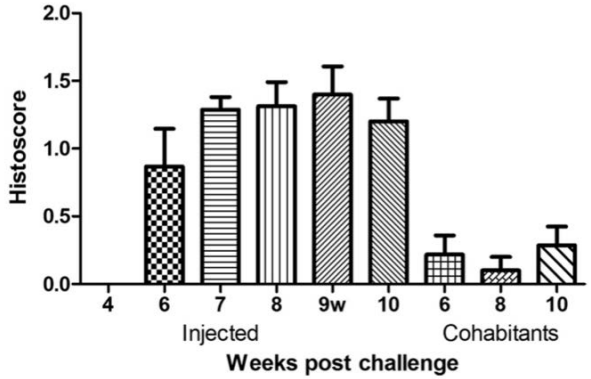


FIG. 8. Histoscores for injection-challenged (Injected) and cohabitation-challenged (Cohabitants) salmon at different time postchallenge. No changes were observed by 4 weeks for the injected group, with gradual increases from 6 weeks up to 9 weeks. Fish challenged by cohabitation were collected at 6, 8, and 10 weeks postchallenge. Lower scores were found for this group, but distinct changes were present. Average values plus standard errors of the mean (SEM) are shown (n = 6 to 7 individuals per time point).

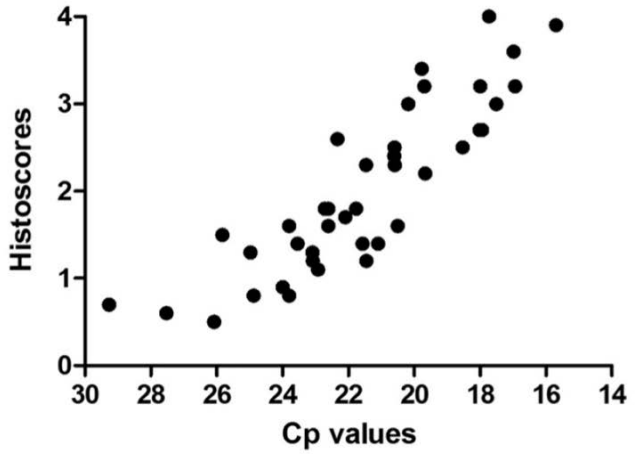
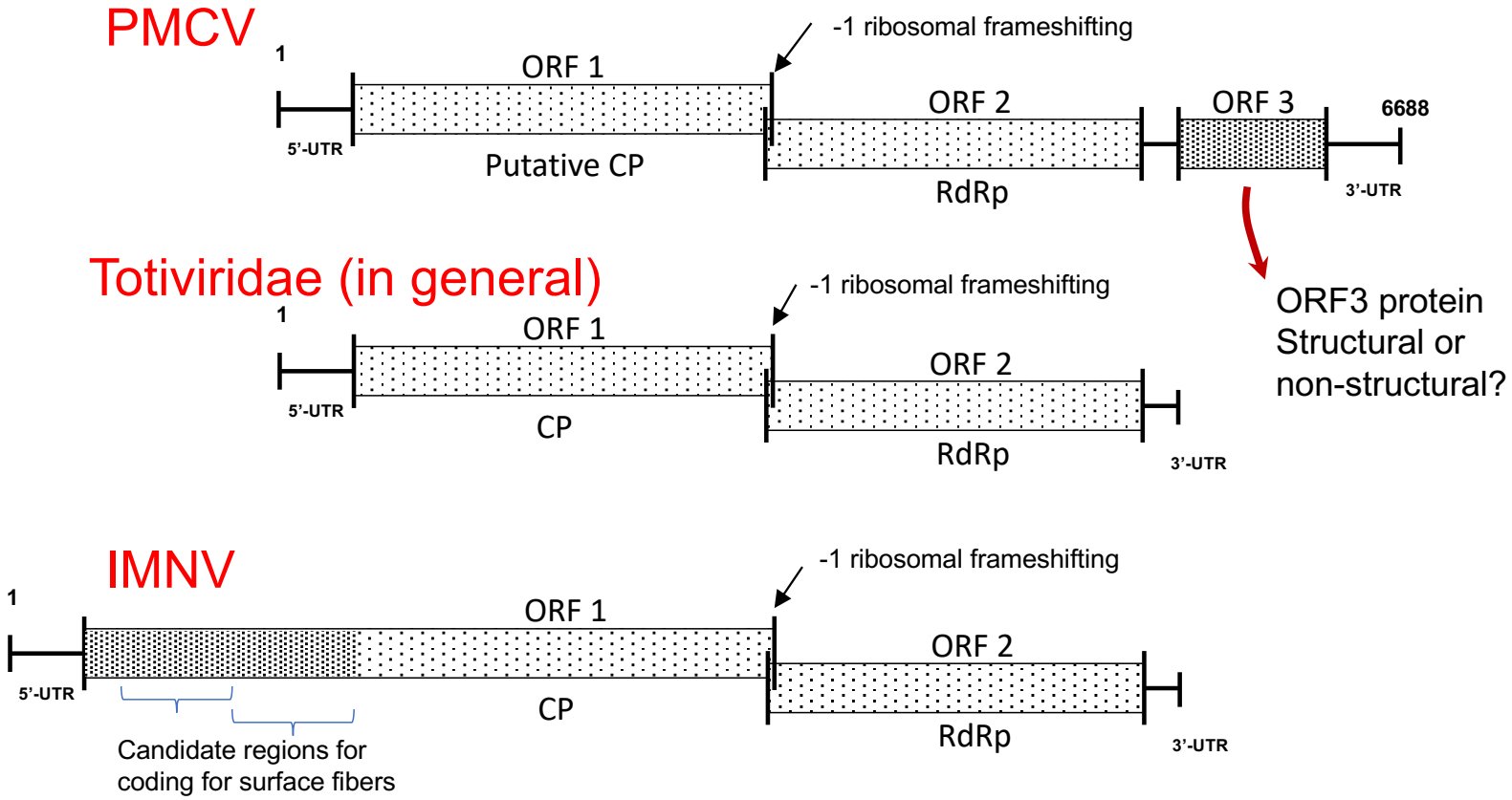


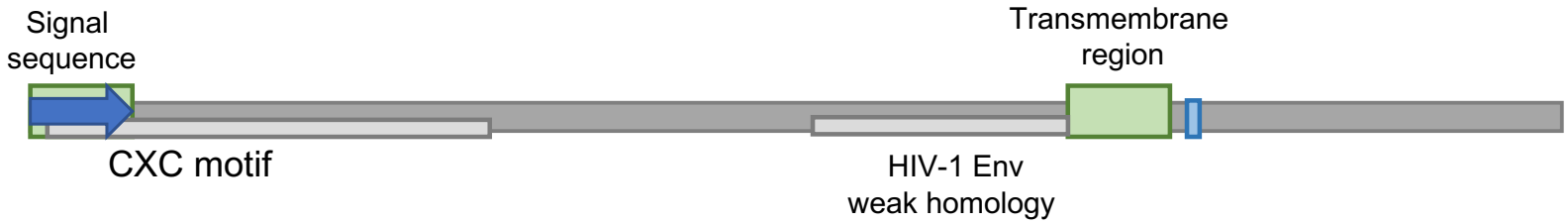
FIG. 10. Bivariate plot showing the relationship between the histoscore of the spongious part of the heart ventricle and the virus load (expressed as Cp values) in heart tissue for sample 1 (n = 40).

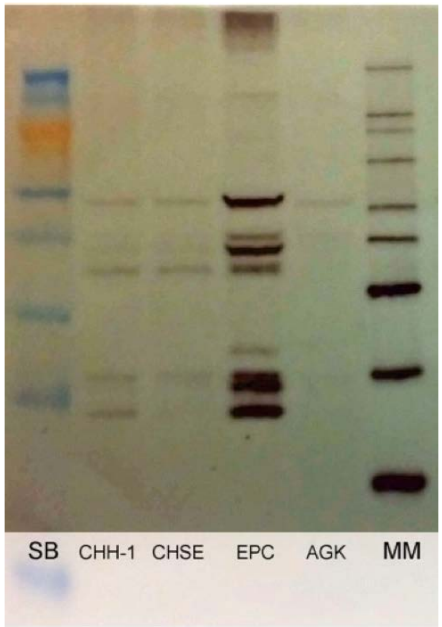
Horizontal spread and correlation between viral load and histoscores

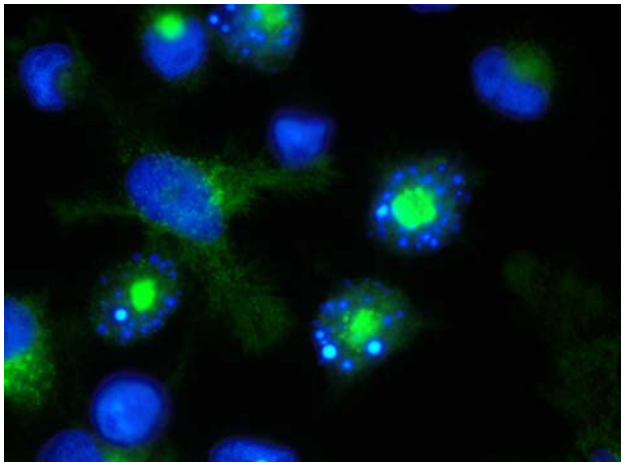
Piscine myocarditis virus (PMCV)



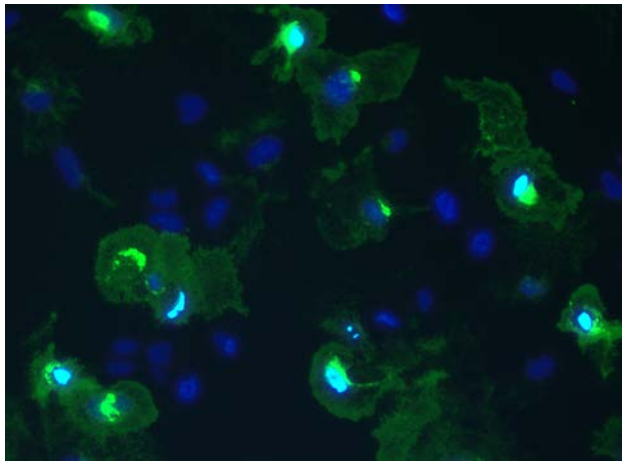
ORF3 putative domains







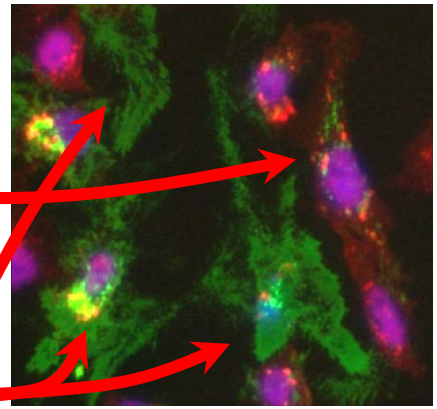
Fragmented nuclei



Dissolved cells/cell shadows

Transient expression w/fluorescent C-terminal tag – ORF3

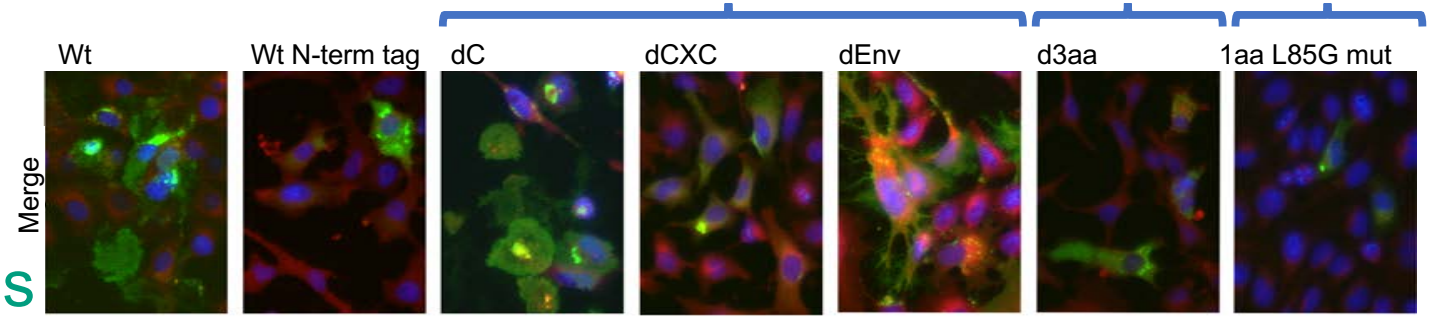
- In a smaller fraction of the cells/early time points
  - protein expressed as dense perinuclear granules
- Dominant in the cultures/late time points
  - accumulations of protein structures with various size and morphology
  - indications of hemisphere shaped, stretched out morphology with fiber-like protrusions or dispersed in the well at lower concentrations
  - characteristic of this is the non-existent relation to cell membranes or nucleus



- The results suggest that the high expression of the protein were destructive to the cell in a decomposing manner leaving only the protein behind in extreme cases

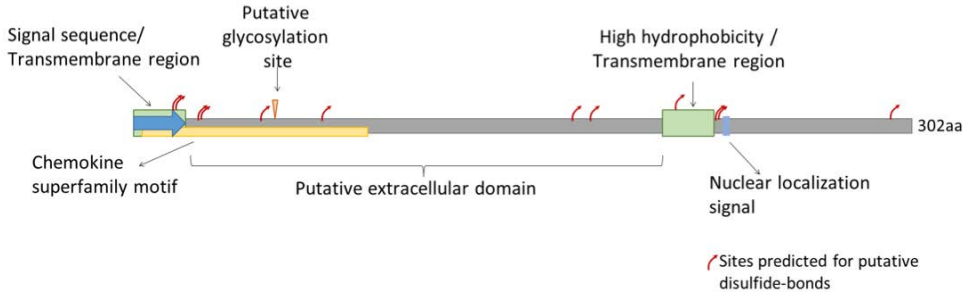
Transient expression

w/ deletions/mutations

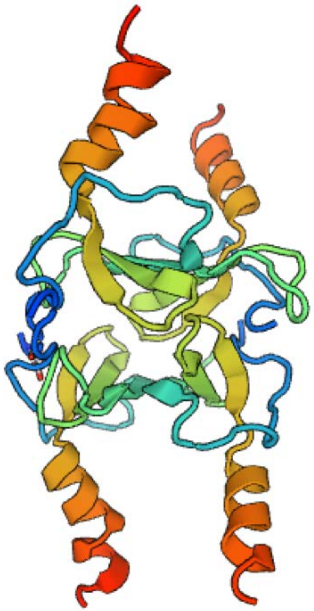


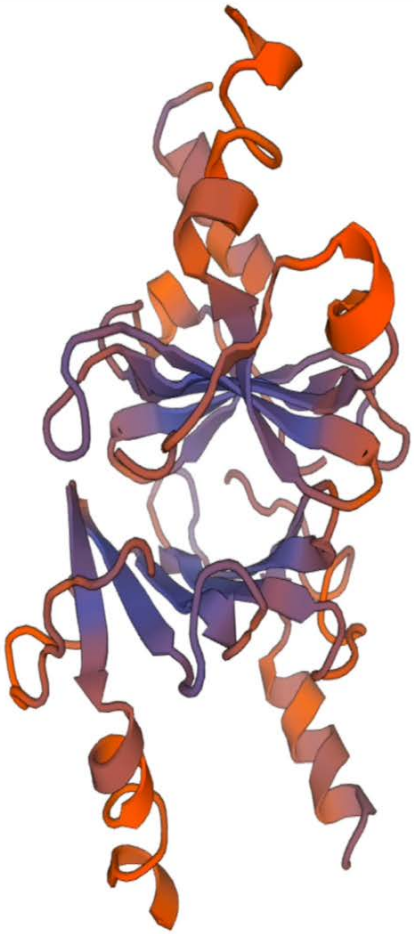
- The signal sequence (N-terminal) is important for the activity
- The region between the signal sequence and a high hydrophobicity region is important
- The C-terminal seems to play lesser of a role for the activity

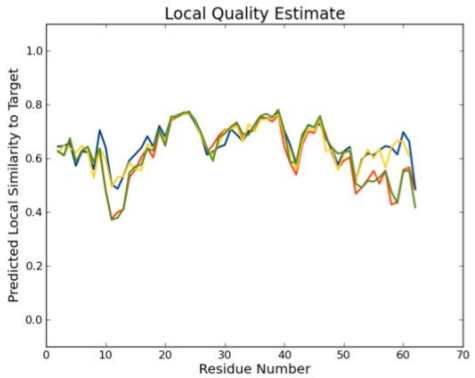
Schematic overview of protein and *in silico*-predicted regions



Functionality





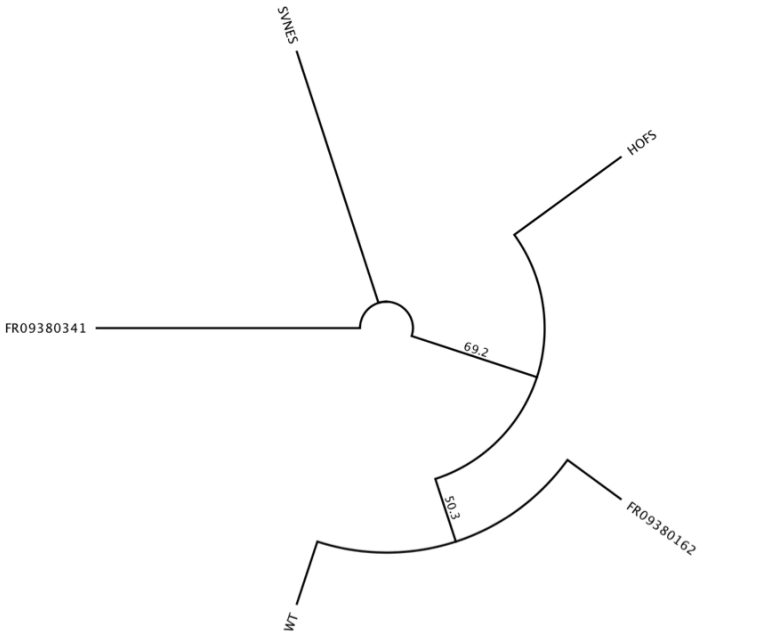


Crystal Structure of CXCL4L1

Sampling (field)

- Coordinated sampling with CMS-Epi project
  - Sampling during
    - Early stage infection without clinical signs of disease
    - Clinical signs of disease (classical CMS changes)
    - Different time during the infection cycle
- Sample examination
  - Sequencing of the ORF1 and ORF3 gene (including the ORF – intergenic region)

Sequence data



ORF3 positions (as)

13	22	24	46	60	78	84	87	88	97	114	191	205	222	242	243
L	V	M	R	G	E	I	K	T	R	F	A	M	V	A	E
.	.	.	.	R	G	V	Q	.	Q	.	.	.	I	V	.
.	I	.	K	.	.	.	.		.	.	.	.	.	.	Q
.	.	.	.	R	G	V	Q	.	Q	.	.	.	I	V	.
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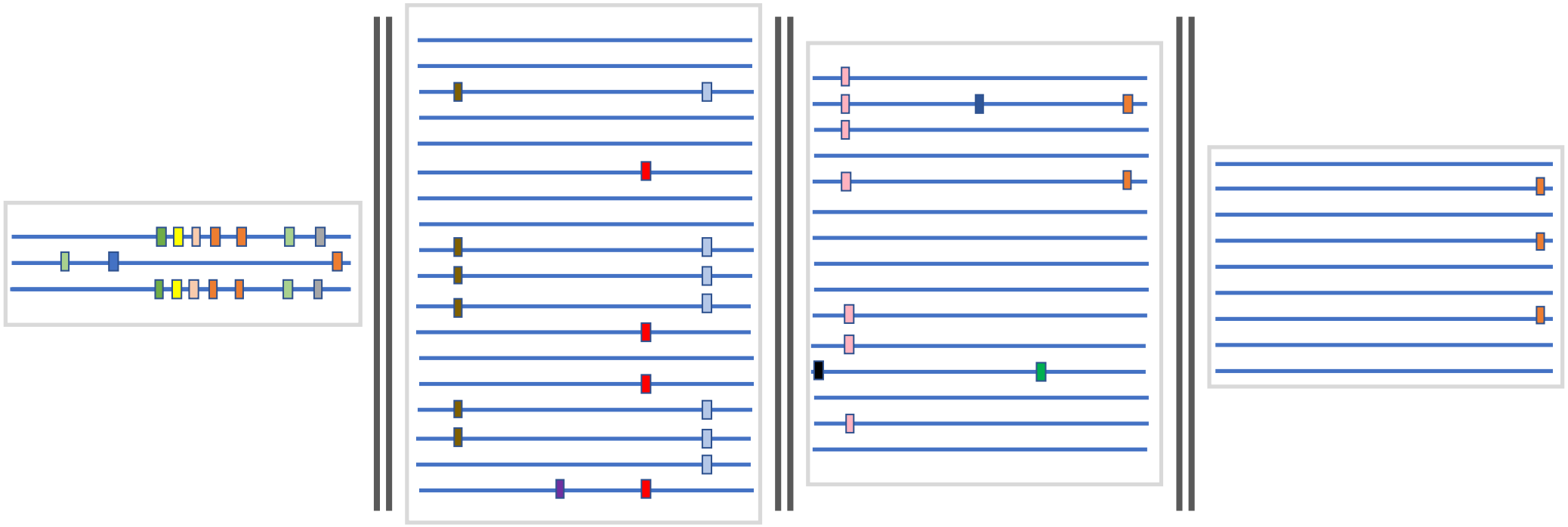
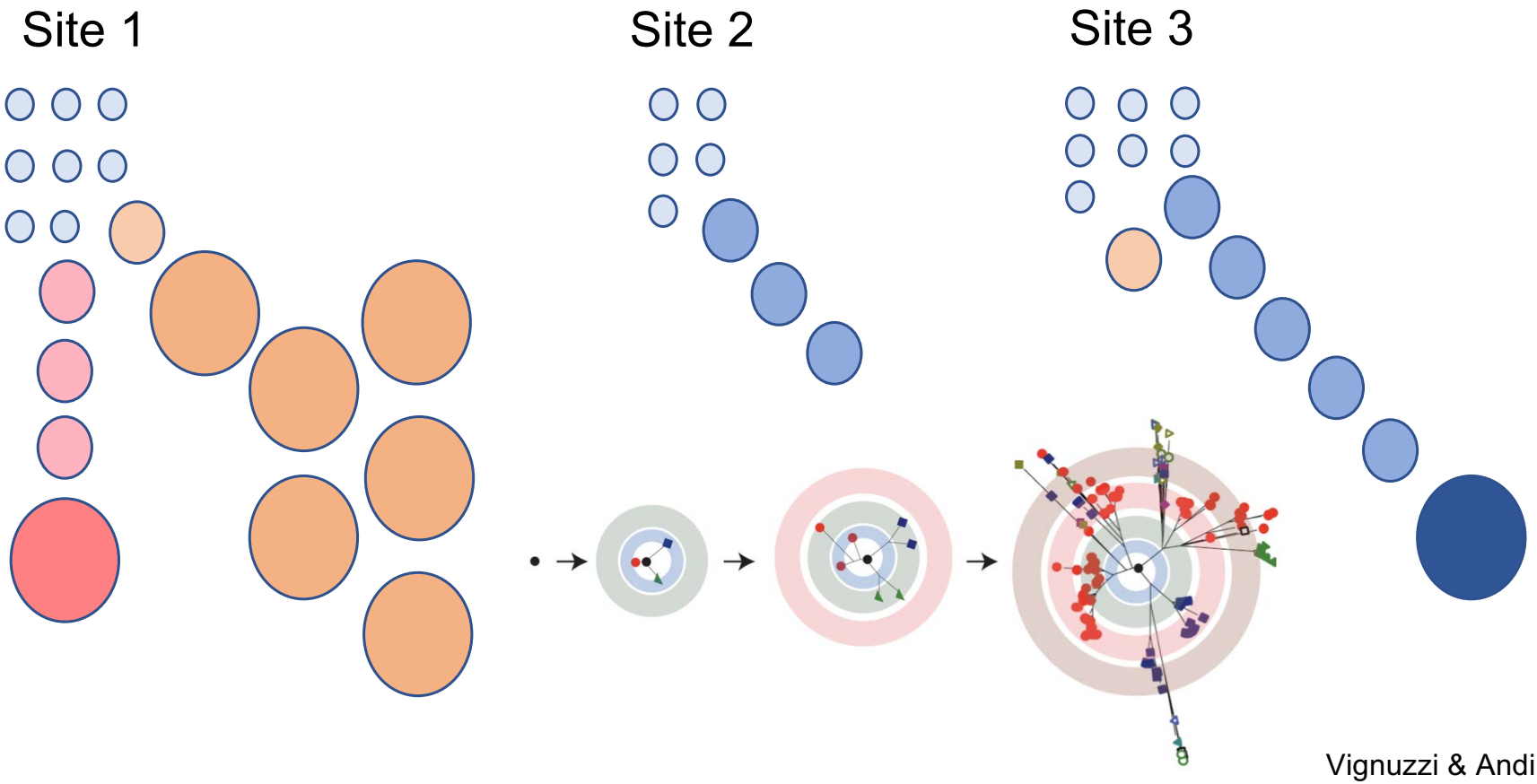
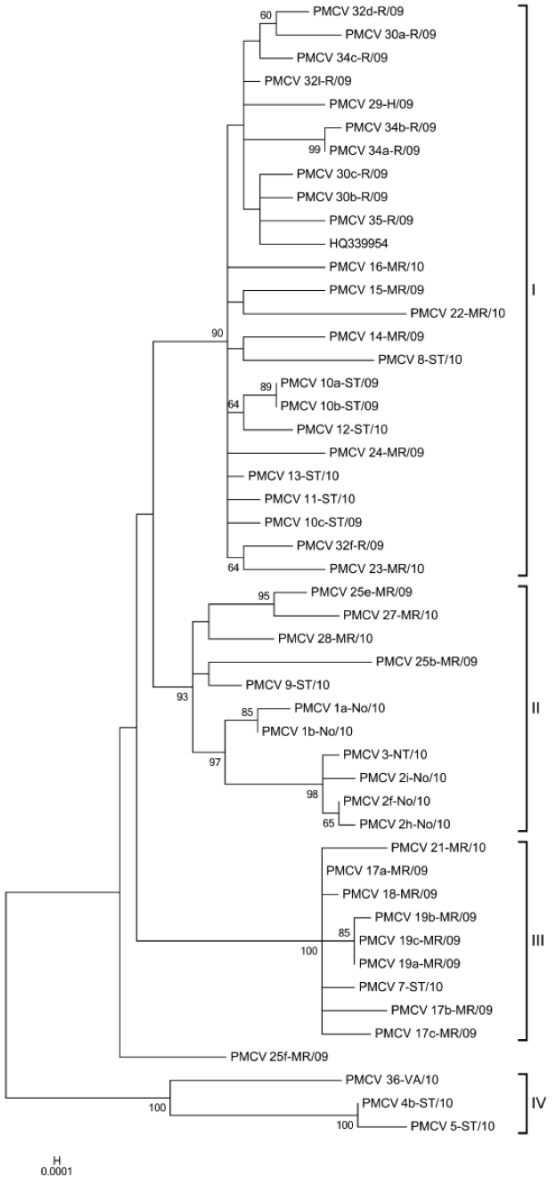


Sequence distribution of ORF3 at different sites

Mutant spectra (ORF3) in different groups of salmon (field data)



Vignuzzi & Andino 2013



- Variation within is equal/comparable to variation between
- Potential hot-spots in ORF1 and ORF3

Wiik-Nielsen et al. 2013

Results (summary)

- We find the ALV708 sequence in all fish examined

13	22	24	46	60	78	84	87	88	97	114	191	205	222	242	243
L	V	M	R	G	E	I	K	T	R	F	A	M	V	A	E

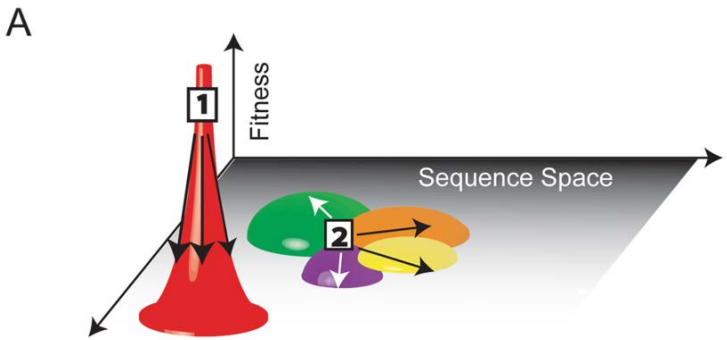
- Genome diversification in infected fish
  - Normal event during viral replication – leads to formation of a complex composition of **virus clouds** referred to as quasispecies
  - Quasispecies theory is a mathematical framework that was initially formulated to explain the evolution of life in the "precellular RNA world"

Briones and Domingo, 2008; Domingo, 2000; Ruiz-Jarabo et al., 2000).

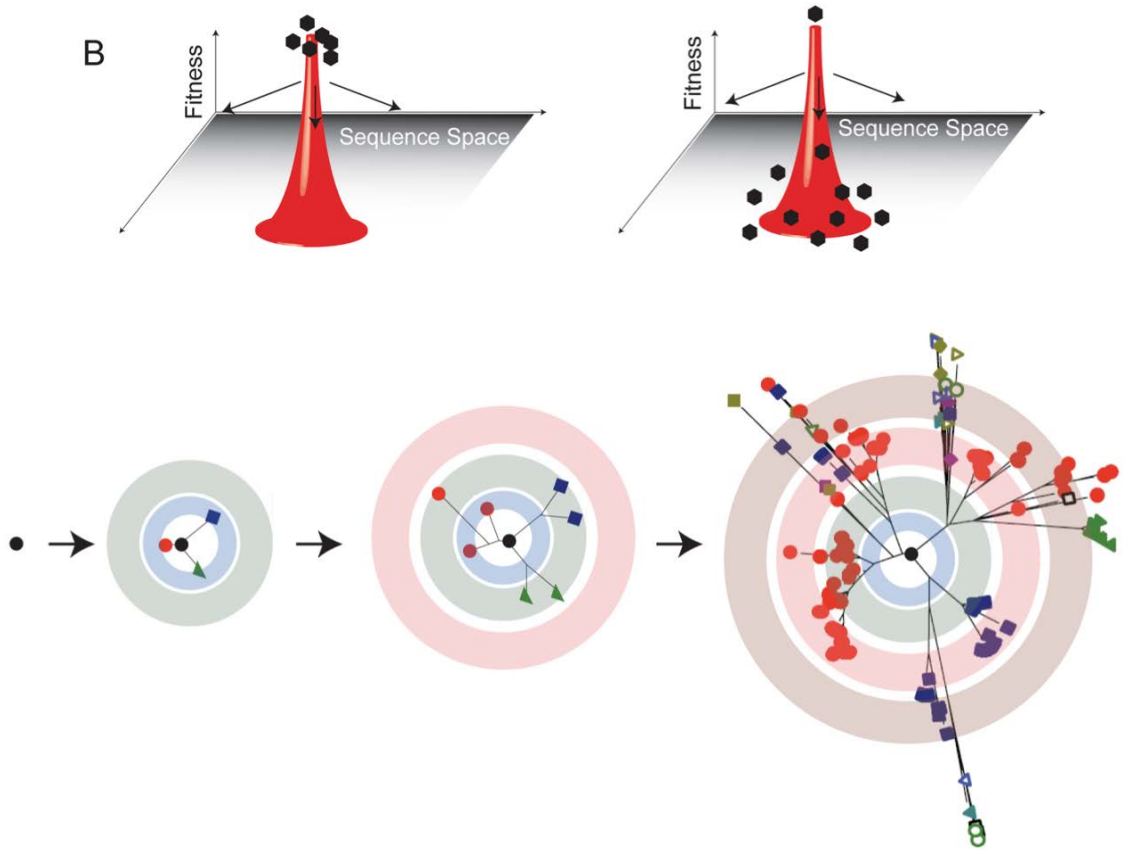
- This allows a viral population to respond more effectively to selective constraints
- The evidence just summarized is consistent with the idea that viral quasispecies can act as a unit of selection

Questions

- Infection mechanisms
  - The wt (ALV708) sequence is always present – importance?
  - Recent “jump” into Atlantic salmon?
  - Narrow sequence space?
    - RNA viruses experience a permanent conflict between pressure to change imposed by high mutation rates and the need to restrict variation due to their ancestral complexity and dependence on cellular functions
    - Limited exploration of the sequence space in the “new” host?
  - Mechanisms of selection (neutral or positive)
    - A given species of a quasi-species distribution might be more suited to replicate in a (new) environment – results in emergence of new variants

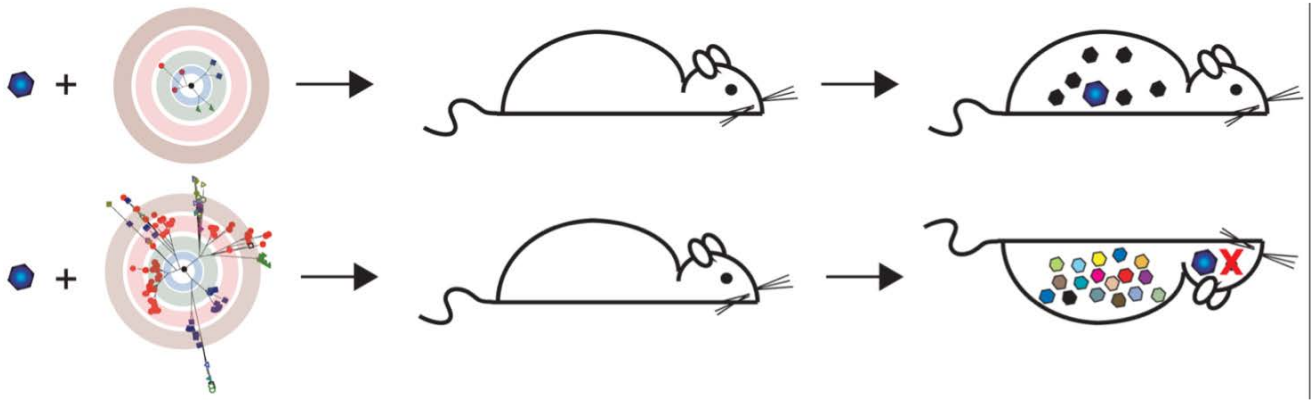


Infection mechanisms



- Is the infection of salmon a «bottleneck event», *i.e.* only a few genotypes are able to infect salmon?
- Diversification post infection

Or is infection dependent on collaboration within virus populations?

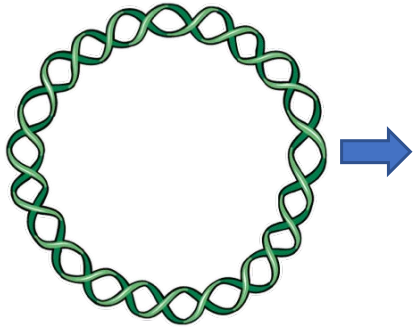


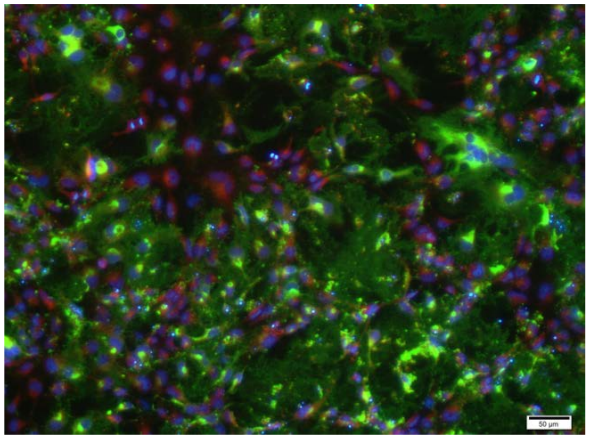
Vignuzzi et al. 2006 *Nature* **439**, 344–348.

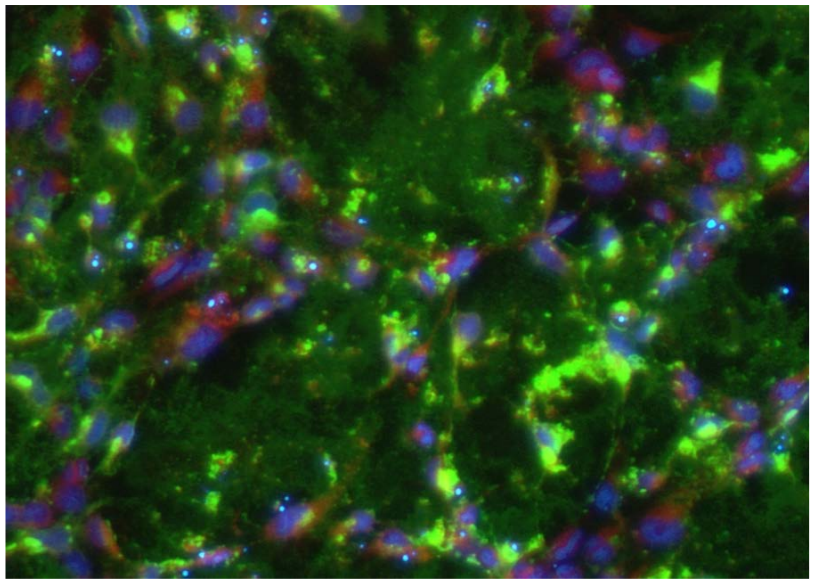
- «Collaboration within the viral quasispecies» ?
- Infection progression
  - Primary replication in kidney/spleen
  - Target organ – ventricle (spongious part)

Functional importance of mutant strains

ORF3^{wt} variant

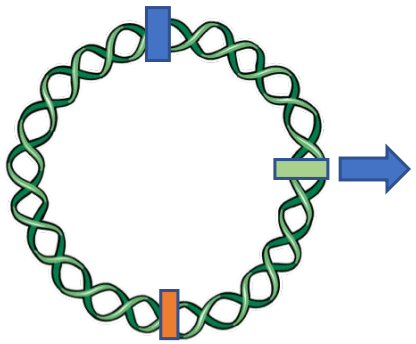


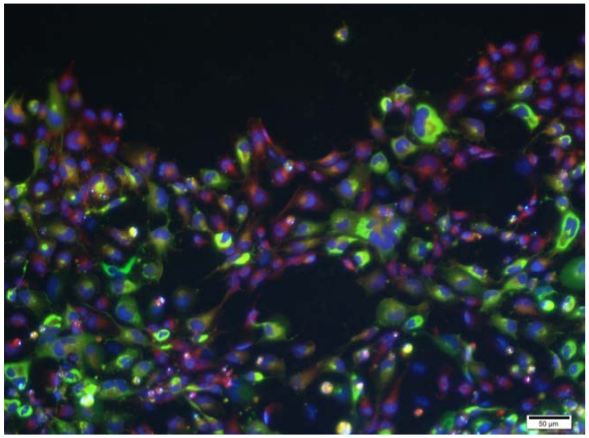


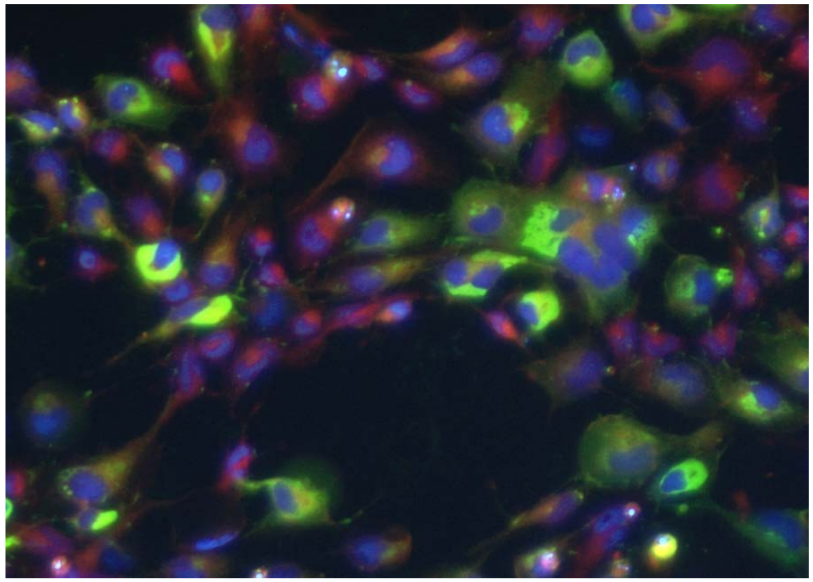


Naturally occuring non-virulent variants

ORF3^{mut}







Gaps

- Is the ORF3-protein a virulence marker/associated with virulence?
- UTR region involvement in initiation of transcription (efficiency)

Output of sir_graph (©)
mfold_util 4.7

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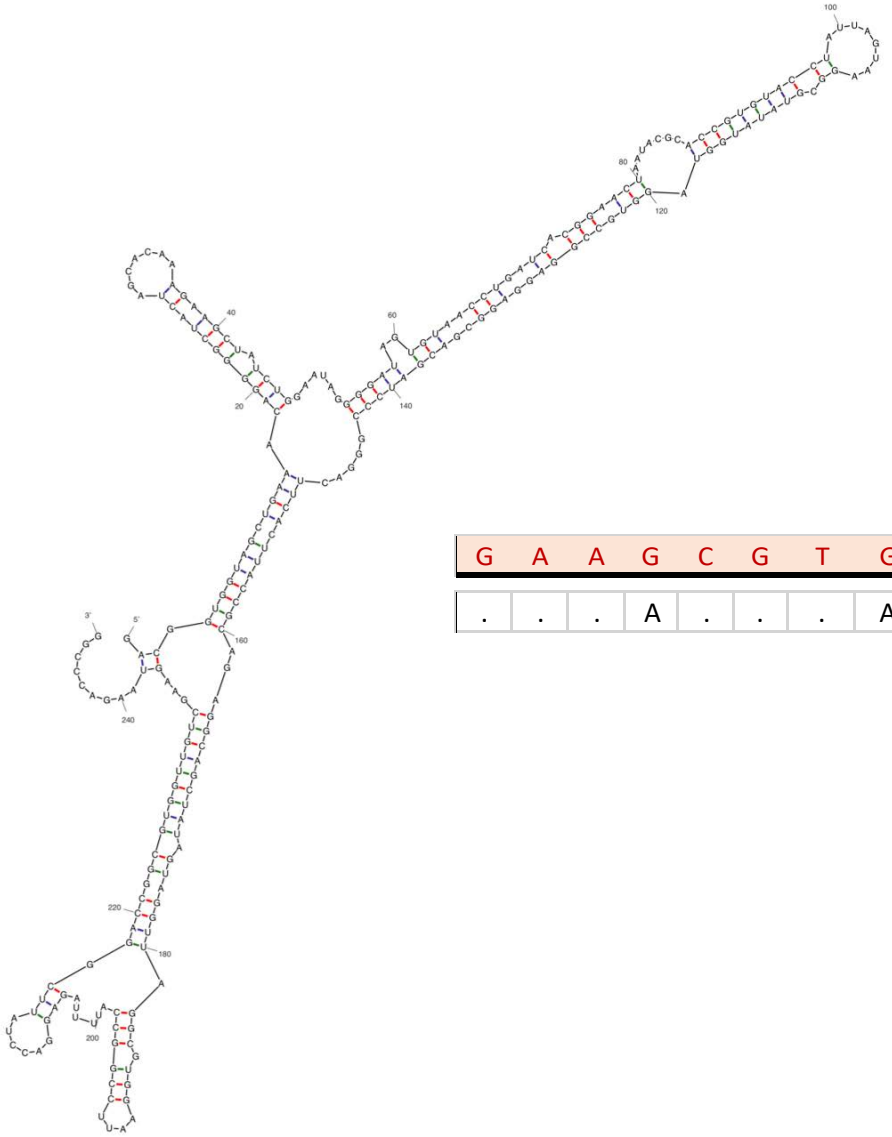

G	A	A	G	C	G	T	G	G	A	A	G	T
.	.	.	A	.	.	.	A	A	.	.	.	C

dG = -69.65 [Initially -74.90] UTR-ORF3

Output of sir_graph (©)
mfold_util 4.7

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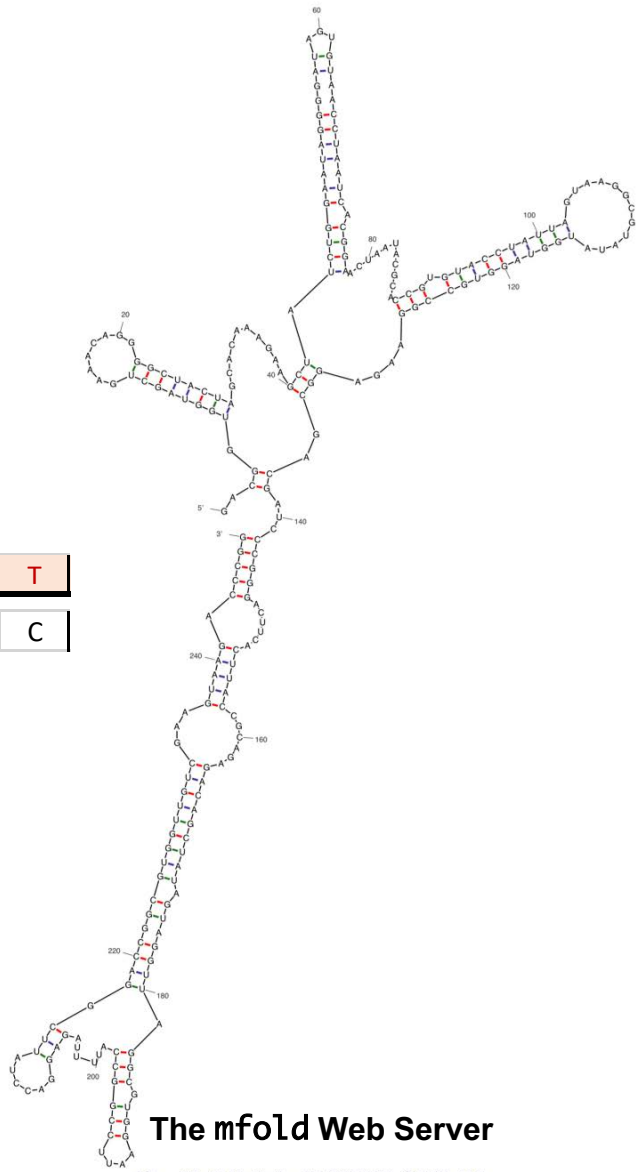

The mfold Web Server

dG = -68.45 [Initially -75.00] UTR-ORF3-413

Gaps

- Is the ORF3-protein a virulence marker/associated with virulence?
- UTR region involvement in initiation of transcription (efficiency)
- Is cytotoxicity *in vitro* a proxy for *in vivo* virulence?
- Approach
  - Heart pathology associated with different ORF3 variants
  - Development over time – quasispecies composition
  - Experimental challenge

Acknowledgments

- Funded by FHF Project no. 901179 «Kardiomyopatisyndrom (CMS) - påvisning av egenskaper hos *piscint myokarditvirus* som forklarer opptreden av klinisk sykdom i ulike faser av lakseproduksjonen»
- Collaborators
  - CMS-EPI and Britt Bang-Jensen as PI