# Pilchard orthomyxovirus (POMV) - An emerging pathogen in farmed Atlantic salmon in Australia

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**ACDP Fish Diseases Laboratory**

# Australian Centre for Disease Preparedness

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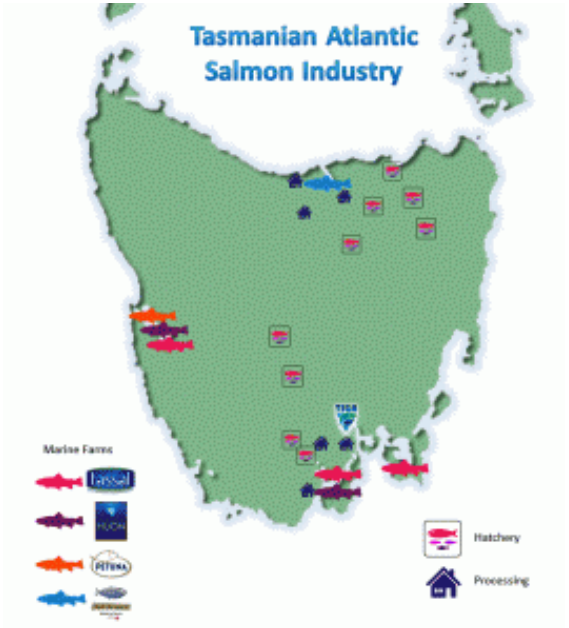
**ACDP Fish Diseases Laboratory**

- Diagnosis of exotic and emerging diseases of aquatic animals (fish/molluscs/crustaceans)
- Technical advice and training
- Research and Development
- OIE Reference Laboratory
  - AbHV
  - EHNV
  - Ranavirus
  - YHV1

# Atlantic salmon farming in Tasmania

- Atlantic salmon imported from Canada
- Populations established in 1960s
- Stock moved to Tasmania in 1980s
- Highest-value aquaculture fishery in Tasmania
- Output worth > $AU 800 million

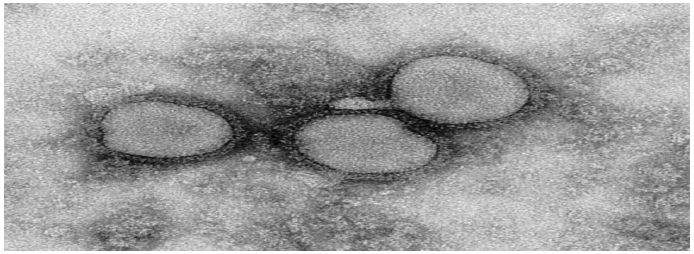

https://www.abc.net.au/




https://www.oceanwatch.org.au/

# Pilchard orthomyxovirus (POMV)

- POMV is a unique orthomyxovirus isolated from wild pilchards and farmed Atlantic salmon in Australia
- Salmon orthomyxoviral necrosis (SON) disease in farmed Atlantic salmon in Tasmania, caused by infection with POMV
- Pathological changes associated with SON result in sufficient damage to cause disease
- Since 2012 many outbreaks of SON disease in Tasmanian Atlantic salmon farms
- In severe outbreaks, cumulative stock losses of up to 50% of fish in individual pens have occurred



# POMV - 2020 Publications

## ACDP

Vol. 130: 35–50, 2020
https://doi.org/10.3354/dao03470
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published online April 30

OPEN ACCESS

### Pilchard orthomyxovirus (POMV). I. Characterisation of an emerging virus isolated from pilchards *Sardinops sagax* and Atlantic salmon *Salmo salar*

Peter G. Mohr[1,*], Mark St. J. Crane[1], John Hoad[1], Lynette M. Williams[1], David Cummins[1], Matthew J. Neave[1], Brian Shiell[1], Gary Beddome[1], Wojtek P. Michalski[1], Grantley R. Peck[1], Francisca Samsing[2], James W. Wynne[2], Sandra G. Crameri[1], Alexander D. Hyatt[1], Nicholas J. G. Moody[1]

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## DPIPWE Centre for Aquatic Animal Health and Vaccines

Vol. 130: 51–68, 2020
https://doi.org/10.3354/dao03469
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published online April 30

### Pilchard orthomyxovirus (POMV). II. Causative agent of salmon orthomyxoviral necrosis, a new disease of farmed Atlantic salmon *Salmo salar*

Scott E. Godwin*, Richard N. Morrison, Graeme Knowles, Martine C. Cornish, Dane Hayes, Jeremy Carson

Centre for Aquatic Animal Health and Vaccines, Department of Primary Industries, Parks, Water and Environment, Launceston, Tasmania 7250, Australia

TSGA

FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

## Agriculture and Food

Fish and Shellfish Immunology 105 (2020) 415–426
Contents lists available at ScienceDirect
Fish and Shellfish Immunology
journal homepage: www.elsevier.com/locate/fsi

Full length article

### Comparative transcriptome analysis of pilchard orthomyxovirus (POMV) and infectious salmon anaemia virus (ISAV)

Francisca Samsing [a,*], John Hoad [b], Peter Mohr [b], Megan Dearnley [b], James W. Wynne [a]

[a] CSIRO Agriculture and Food, Livestock and Aquaculture, Castray Esplanade, Battery Point, Tasmania 7004, Australia
[b] CSIRO Australian Centre for Disease Preparedness (ACDP) (formerly Australian Animal Health Laboratory, AAHL), 5 Portarlington Rd, East Geelong, Victoria 3220, Australia

Received: 1 July 2020 | Revised: 26 August 2020 | Accepted: 27 August 2020
DOI: 10.1111/jfd.13269

ORIGINAL ARTICLE

Journal of Fish Diseases — WILEY

### Seawater transmission and infection dynamics of pilchard orthomyxovirus (POMV) in Atlantic salmon (*Salmo salar*)

Francisca Samsing[1] | Megan Rigby[1] | Hedda K. Tengesdal[1,2] | Richard S. Taylor[1] | Daniela Farias[1] | Richard N. Morrison[3] | Scott Godwin[3] | Carla Giles[3] | Jeremy Carson[3] | Chloe J. English[4] | Roger Chong[4] | James W. Wynne[1]

*pathogens* — MDPI

*Article*

### Transcriptome Response of Atlantic Salmon (*Salmo salar*) to a New Piscine Orthomyxovirus

Francisca Samsing [1,*], Pamela Alexandre [2], Megan Rigby [1], Richard S. Taylor [1], Roger Chong [2] and James W. Wynne [1,*]

[1] CSIRO Agriculture and Food, Hobart 7004, Australia; Megan.Rigby@csiro.au (M.R.); Richard.Taylor@csiro.au (R.S.T.)
[2] CSIRO Agriculture and Food, Brisbane 2601, Australia; Pamela.Alexandre@csiro.au (P.A.); Roger.Chong@csiro.au (R.C.)
* Correspondence: Francisca.samsingpedrals@csiro.au (F.S.); james.wynne@csiro.au (J.W.W.)

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check for updates

# POMV - Incidental isolations

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## South Australia (1998)

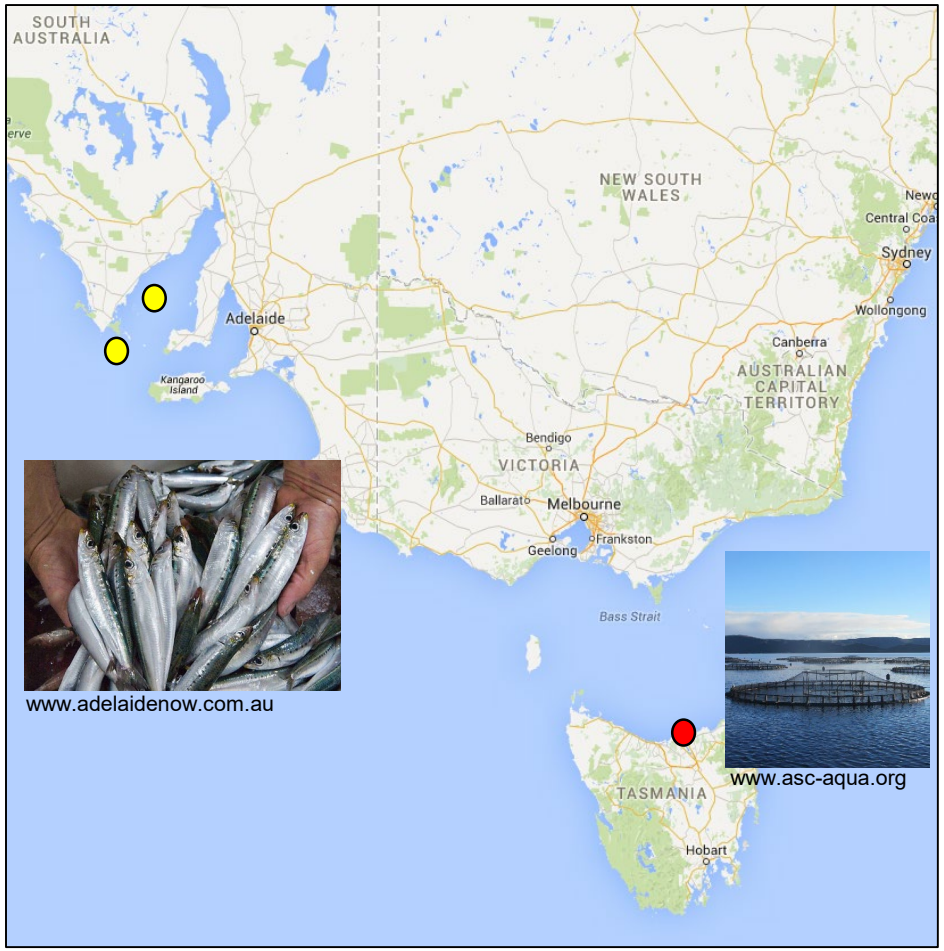
- Healthy pilchards
- Pilchard cell line development
- Pilchard herpes investigation

## Tasmania (2006)

- Healthy farmed Atlantic salmon
- Routine health surveillance

## South Australia (2007)

- Healthy pilchards
- Pilchard herpes investigation



# POMV - Identification pre-2015

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1) Virus Isolation

2a) Morphologically distinct from ISAV

- Transmission electron microscopy

2b) Antigenically distinct from ISAV

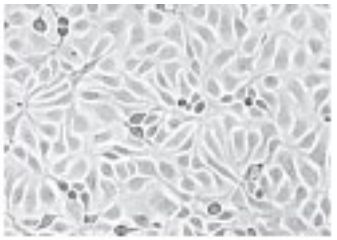
- ISAV-specific monoclonal antibody

2c) Genetically distinct from ISAV

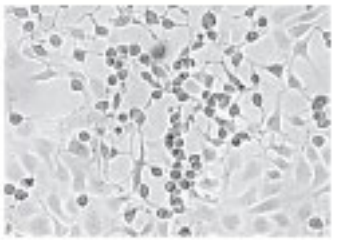
- All ISAV-specific PCR tests negative

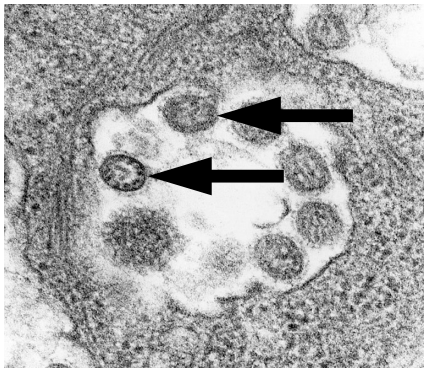
CHSE-214/POMV 98-01382

0 dpi

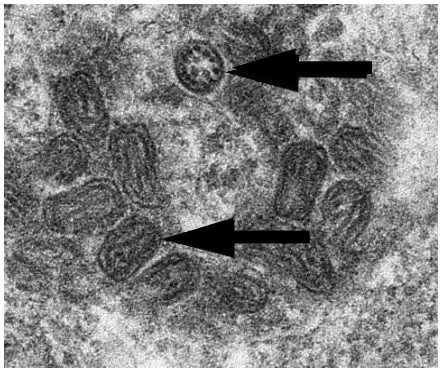


4 dpi





POMV from pilchards (ACDP EM)



POMV from Atlantic salmon (ACDP EM)

Confirm:

- Orthomyxovirus
- Not ISAV

# POMV – Next Generation Sequencing

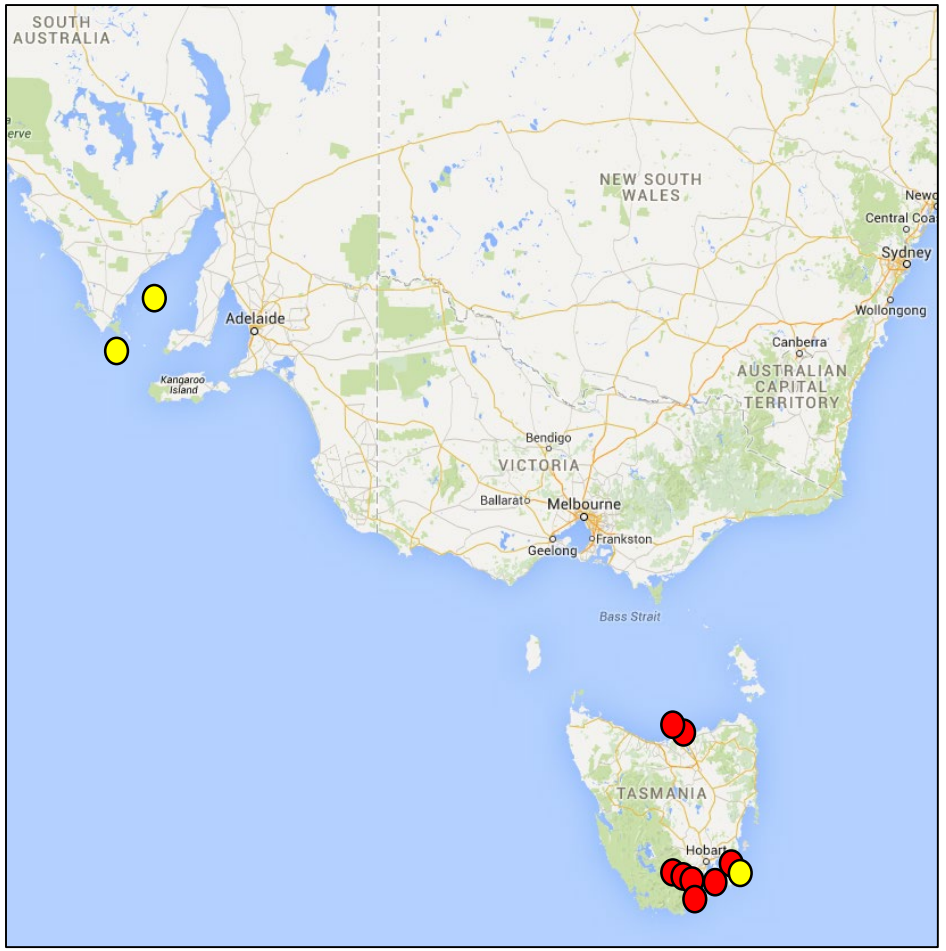
ACDP

## Wild Pilchards

| Id. | Location | Health Status |
|---|---|---|
| 98-01382 | Spencer Gulf (SA) | Healthy |
| 07-01002 | Port Lincoln (SA) | Healthy |
| 13-03672* | Nubeena (TAS) | Healthy |

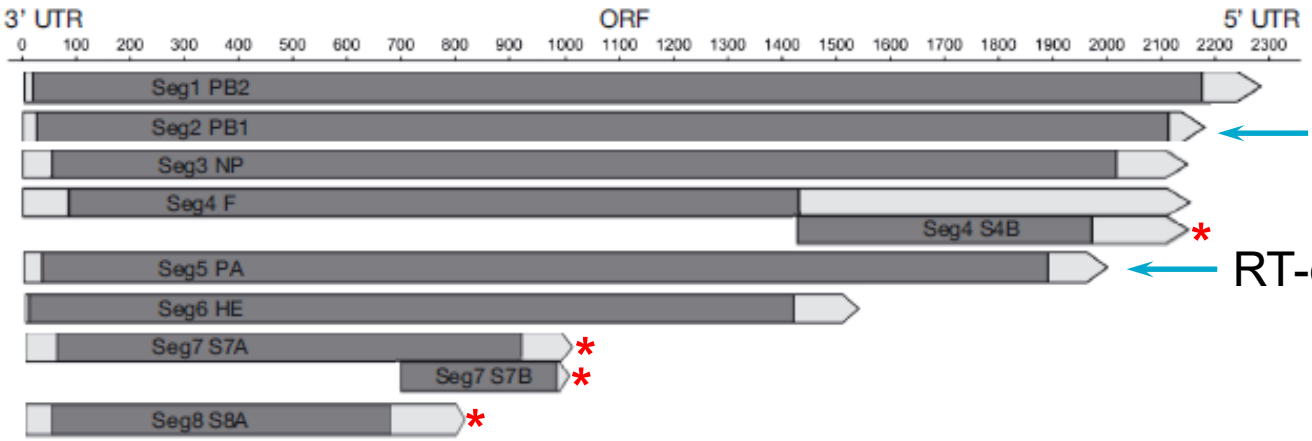
## Farmed Atlantic Salmon

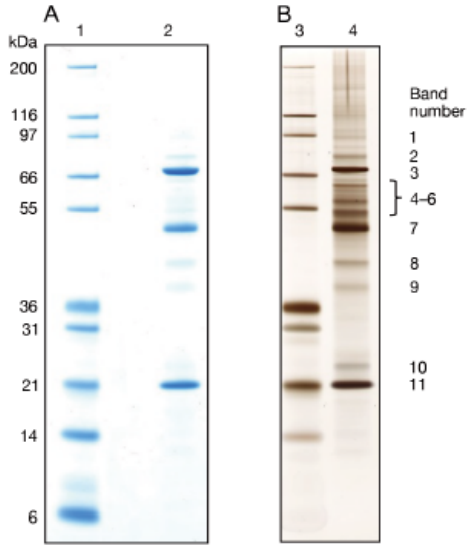
| Id. | Location | Health Status |
|---|---|---|
| 06-04216 | Tamar (TAS) | Healthy |
| 12-01390 | Huon (TAS) | Diseased |
| 12-02055 | Dover (TAS) | Diseased |
| 12-02935 | Bruny Is. (TAS) | Diseased |
| 13-01407 | Nubeena (TAS) | Diseased |
| 13-02097 | Huon (TAS) | Diseased |
| 13-03566 | Tamar (TAS) | Diseased |
| 14-01514 | Huon (TAS) | Diseased |



# POMV – Genome Assembly

ACDP A&F




| POMV protein | Band no. | Amino acid sequence coverage (%) |
|---|---|---|
| PB1 | 1 | 43 |
| | 2 | 25 |
| PB2 | 1 | 44 |
| | 2 | 30 |
| NP | 3 | 78 |
| | 4 | 51 |
| | 5 | 55 |
| F | 7 | 39 |
| | 8 | 38 |
| | 9 | 36 |
| S4B | 10 | 23 |
| PA | 3 | 49 |
| HE | 6 | 57 |
| | 7 | 79 |
| S8A | 10 | 43 |
| | 11 | 91 |

# POMV – Genome Comparison

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POMV 98-01382 amino acid pair-wise comparison:

| Id. | PB2 | PB1 | NP | F | S4B | PA | HE | S7A | S7B | S8A |
|---|---|---|---|---|---|---|---|---|---|---|
| **POMV – Pilchards** | | | | | | | | | | |
| 07-01002 | 99.5% | 100% | 100% | 99.8% | 99.4% | 99.8% | 100% | 100% | 98.2% | 100% |
| 13-03672 | 99.5% | 99.7% | 100% | 100% | 98.9% | 99.8% | 100% | 100% | 98.2% | 100% |
| **POMV – Atlantic salmon** | | | | | | | | | | |
| 06-04216 | 99.9% | 100% | 98.8% | 99.8% | 98.9% | 99.7% | 99.4% | 99.7% | 99.1% | 100% |
| 12-01390 | 99.5% | 100% | 100% | 99.8% | 99.4% | 99.8% | 100% | 100% | 98.2% | 100% |
| 12-02055 | 99.5% | 100% | 100% | 99.6% | 99.4% | 99.8% | 100% | 99.3% | 98.2% | 100% |
| 12-02935 | 99.5% | 100% | 100% | 100% | 99.4% | 99.8% | 100% | 100% | 98.2% | 100% |
| 13-01407 | 99.5% | 100% | 100% | 100% | 99.4% | 99.8% | 100% | 100% | 98.2% | 100% |
| 13-02097 | 99.9% | 100% | 98.8% | 100% | 99.4% | 99.8% | 100% | 99.3% | 98.2% | 100% |
| 13-03566 | 99.9% | 100% | 98.8% | 100% | 98.9% | 99.8% | 100% | 99.7% | 99.1% | 100% |
| 14-01514 | 99.9% | 100% | 98.8% | 100% | 98.9% | 99.8% | 100% | 100% | 98.2% | 100% |

Nucleotide pair-wise comparison of ORFs: 95.8% – 99.8% identity

CSIRO

# POMV – Unique genus?

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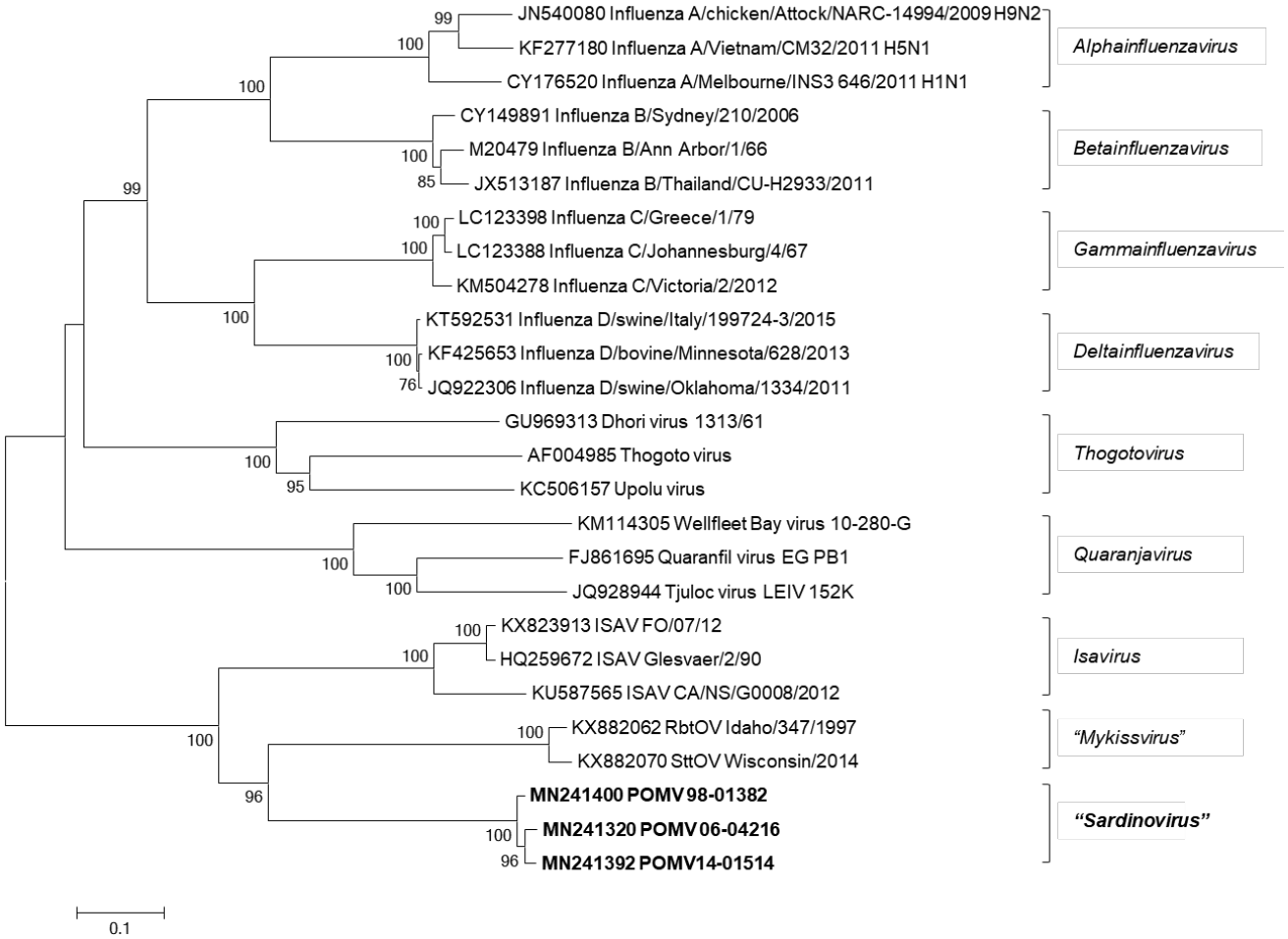



Figure 4 Phylogenetic relationships of POMV *PB1* gene and representative members of other *Orthomyxoviridae* genera.

# POMV – Gross Pathology

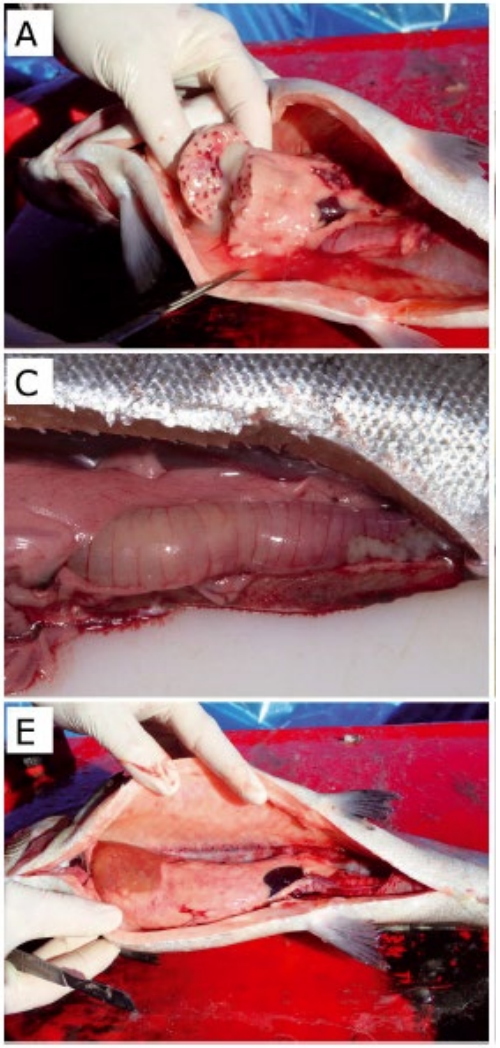
Farmed Atlantic salmon;

*External*

- Petechial haemorrhages - ventral skin
- Abdominal palpation - expelling mucus

*Internal*

- Splenomegaly
- Mucus - stomach and gastrointestinal tract
- Petechiae – visceral fat and peritoneal surfaces

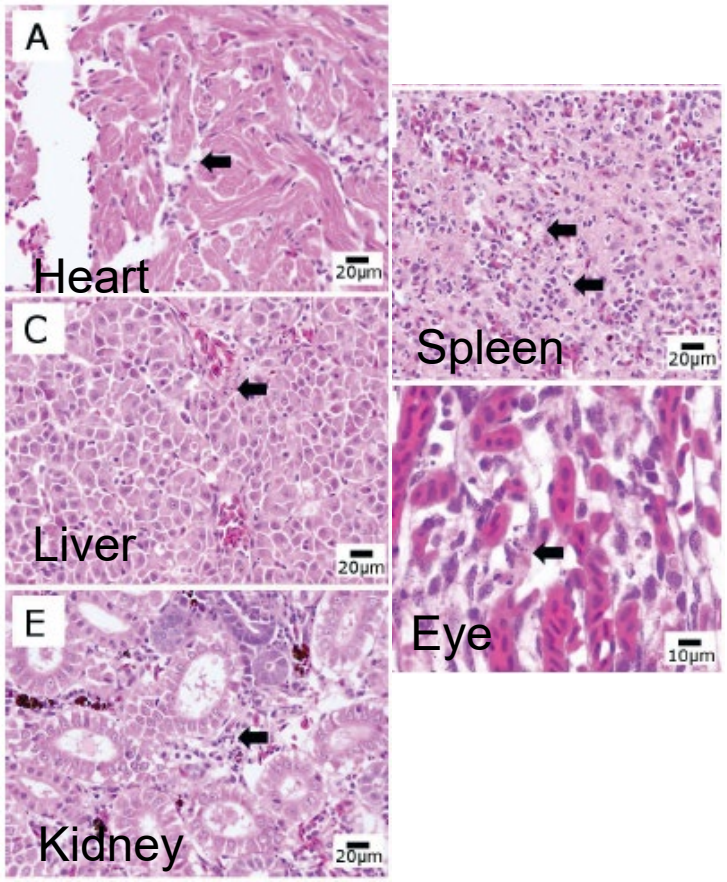


# POMV – Histopathology

Farmed Atlantic salmon;

*Necrotic changes in a range of organs*

- Heart - necrotising myocarditis
- Liver - mild multifocal hepatocellular necrosis apoptosis of individual hepatocytes
- Kidney - interstitial necrosis of renal haematopoietic tissue
- Spleen - exhibited splenitis with lymphocytolysis and lymphoid depletion
- Eye - necrotising posterior uveitis



# POMV - Pathogenicity

CAAHV

## Controlled infection trials

- cell culture-derived POMV
- significant morbidity
- Atlantic salmon fry, pre-smolt and post-smolt

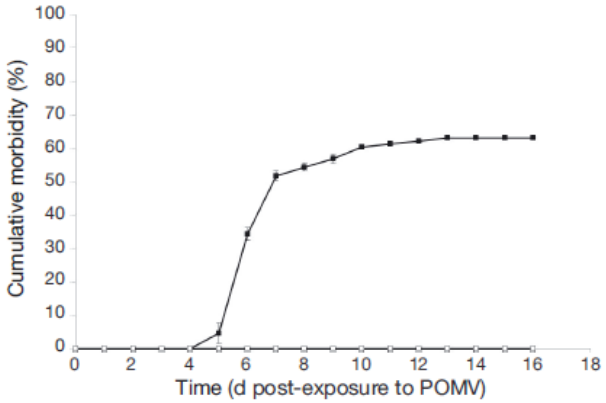




Fig. 4. Pathogenicity of pilchard orthomyxovirus (POMV) in Atlantic salmon fry exposed by immersion. Points represent the mean cumulative morbidity across duplicate tanks. Bars are SEM. Fry exposed to (■) supernatant of Atlantic salmon kidney (ASK) culture infected with POMV (TCFV 0067-0003) and (□) uninfected ASK culture

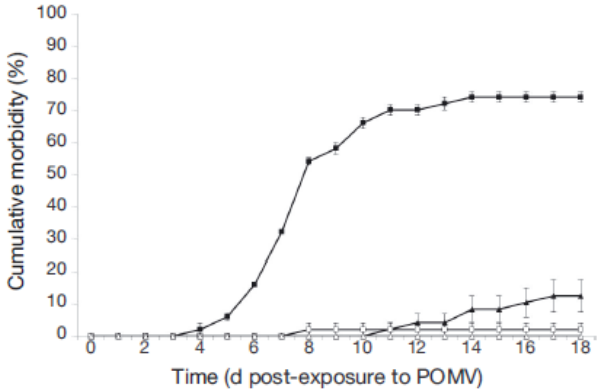



Fig. 5. Pathogenicity of pilchard orthomyxovirus (POMV) in pre-smolt Atlantic salmon challenged by intraperitoneal injection or cohabitation in freshwater. Points represent the mean cumulative morbidity across duplicate tanks. Bars are SEM. (■) Fish injected with 200 µl supernatant of CHSE-214 culture infected with POMV (TCFV 0067-0003). (▲) Cohabitant fish housed with POMV-injected fish. (□) Negative control fish injected with 200 µl supernatant of uninfected CHSE-214 culture. (△) Cohabitant fish housed with negative control fish

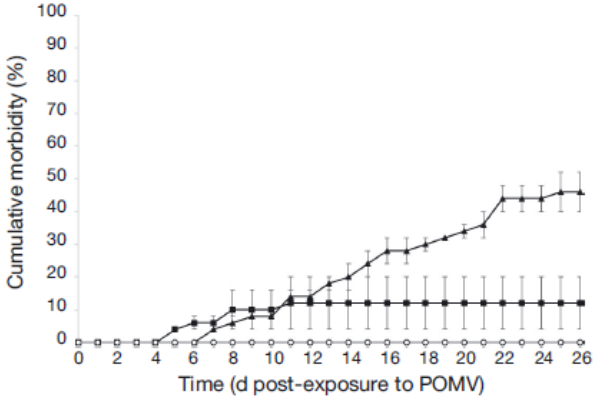


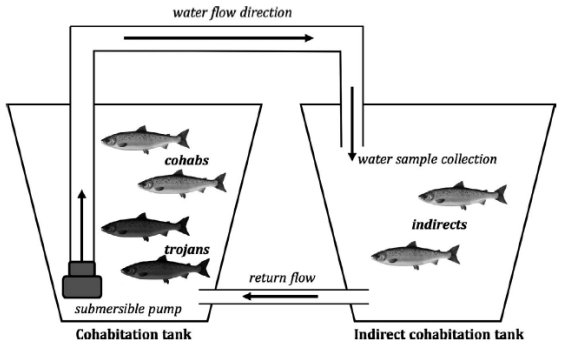

Fig. 6. Pathogenicity of pilchard orthomyxovirus (POMV) in post-smolt Atlantic salmon challenged by intraperitoneal injection or cohabitation in seawater. Points represent the mean cumulative morbidity across duplicate tanks. Bars are SEM. (■) Fish injected with 200 µl supernatant of CHSE-214 culture infected with POMV (TCFV 0067-0003). (▲) Cohabitant fish housed with POMV-injected fish. (O) Negative control fish injected with 200 µl supernatant of uninfected CHSE-214 culture, and cohabitant fish housed with negative control fish

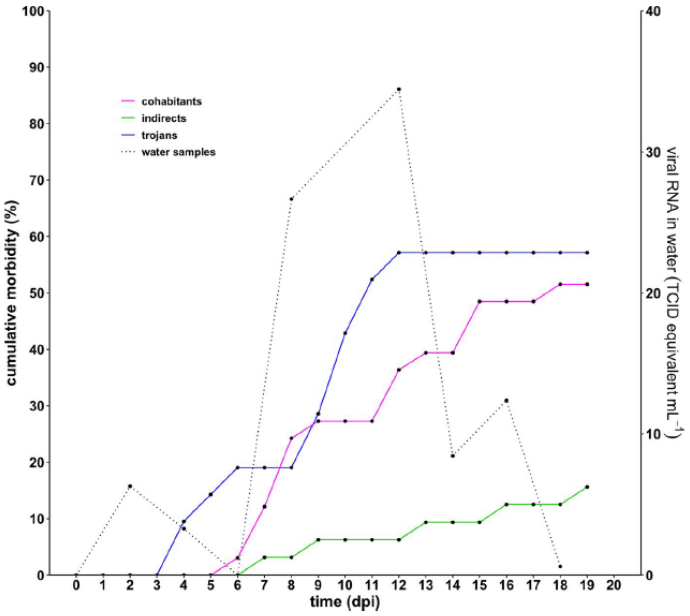
CSIRO

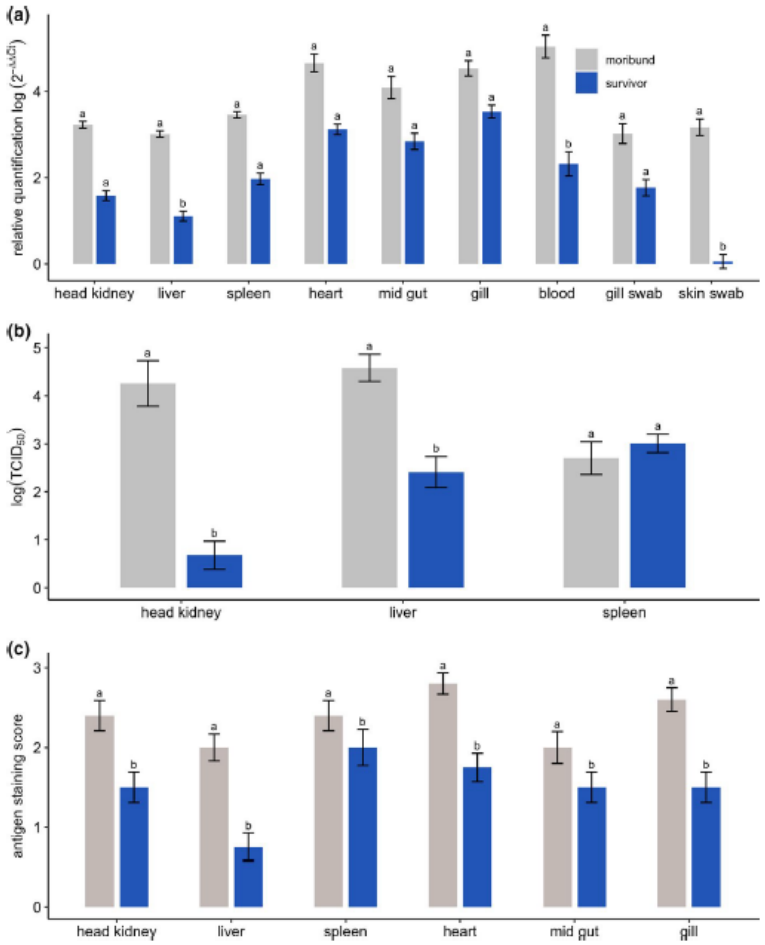
# POMV – Infection dynamics

A&F

CAAHV

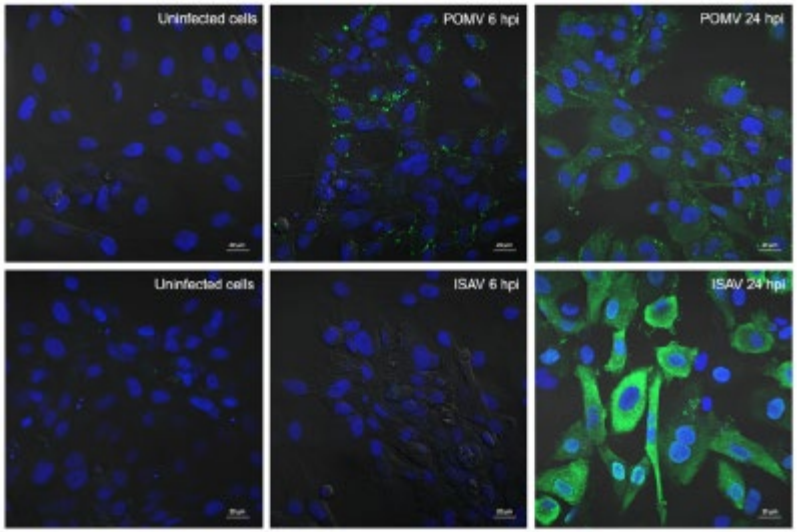


# POMV – *In vitro* transcriptomics

ACDP A&F




## Induced innate antiviral responses

- Early up-regulation of pathogen recognition receptor genes
- Triggering downstream interferon responses
- Strong induction of antiviral response genes

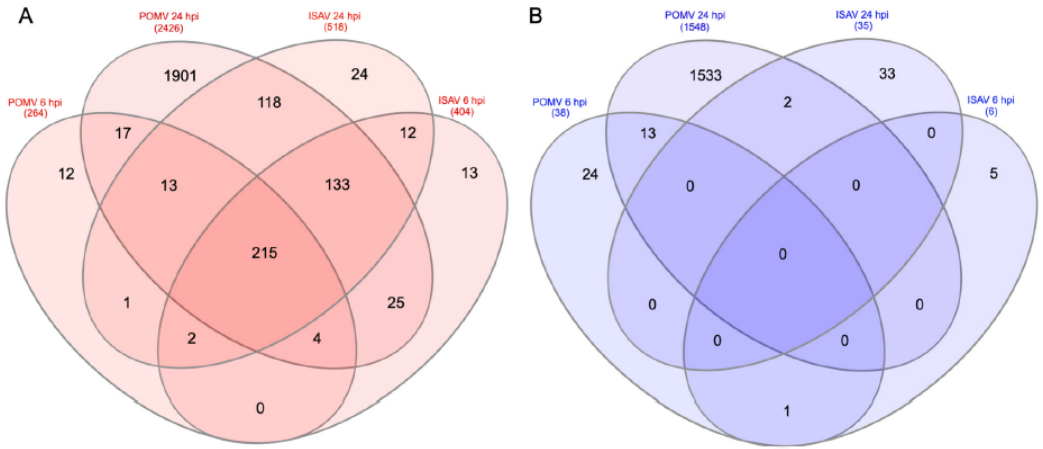



Fig. 2. Venn diagram summary of differentially expressed genes ($log_2$ fold-change > 2) at 6 and 24 h post infection (hpi) in Atlantic salmon kidney (ASK) cells infected with POMV and ISAV. A) Up-regulated and B) down-regulated genes.

# POMV – *In vivo* transcriptomics

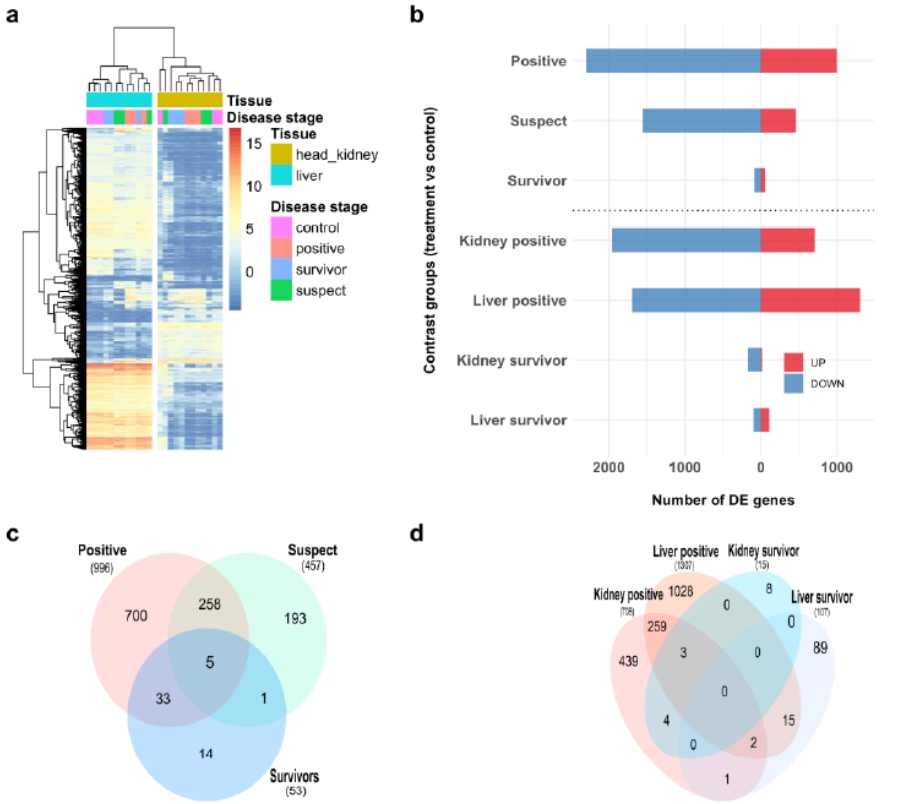



**Figure 3.** Host gene expression profiles of Atlantic salmon (*Salmo salar*) challenged with POMV: (a) Heatmap showing hierarchical clustering of normalized gene expression profiles for the top 1500 genes with the highest variance in liver and head kidney of control fish (fish sampled pre-challenged), moribund POMV-positive fish (real-time PCR cycle threshold (CT) value < 38 in both tissues), moribund POMV-suspect fish (either no CT or a CT ≥ 38) and survivors (fish exposed to POMV, but still alive at the end of trial with no clinical

Atlantic salmon infected with POMV;

- Strong innate immune response in both moribund and survivor fish
- Upregulation of pathogen recognition receptors
- Induction of interferon-stimulated and major histocompatibility complex genes
- Moribund fish had a dramatic induction of pro-inflammatory cytokines

# POMV – Vaccine development

Commissioned by the TSGA, funded by FRDC, TSGA and DPIPWE, and the R&D was undertaken by the DPIPWE-CAAHV

## Vaccine potency data

- Cohabitation challenge in seawater at a 1:1 Trojan:cohabitant ratio

## Certovac®

- First licensed viral vaccine for fish in Australia
- Large scale use across the Tasmanian Atlantic salmon farming industry

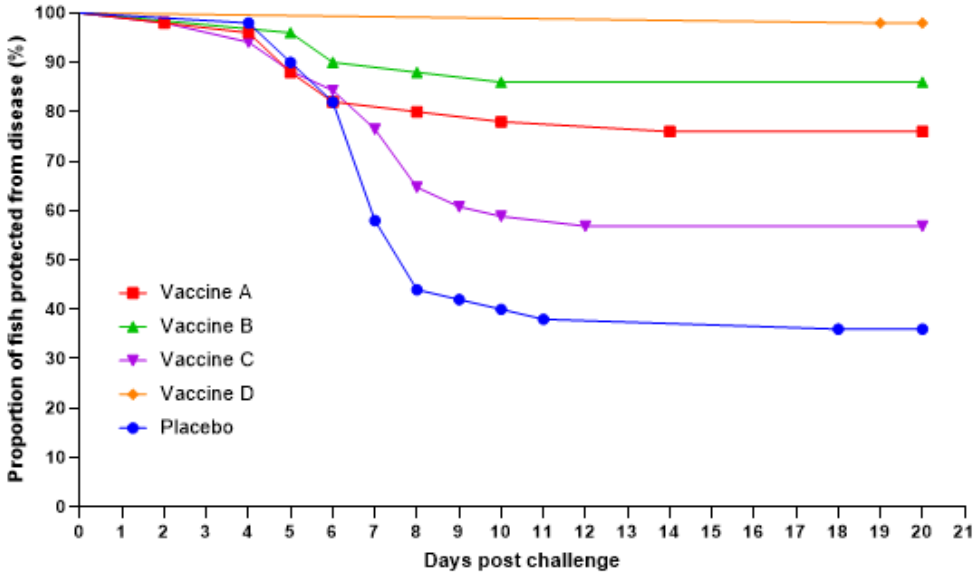



Figure 5. Protective effects of POMV vaccines in an *in vivo* vaccine trial. Groups of Atlantic salmon were immunized with one of four prototype POMV vaccines and then challenged by exposure to infectious POMV. Prototype vaccine D provided almost complete protection against POMV challenge and has led to the development of the commercial vaccine Certovac®.

*Animal Health Surveillance Quarterly (Q3 2020)– Animal Health Australia; Mohr PG and Godwin SM (in press)*

# Acknowledgements

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- CSIRO AFDL - Aquatic Diagnostic Capability Team
- DPIPWE - Centre for Aquatic Animal Health and Vaccines
- CSIRO - Agriculture and Food
- Tasmanian Salmon Growers Association
- Fisheries Research and Development Corporation

# THANK-YOU

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