

TriNation 2026 · Belfast

# A dual-readout approach to understand heart healing after PMCV infection in Atlantic salmon

*Collagen remodeling and cardiomyocyte gene signatures*

A. Gamil & Ø. Evensen

Norwegian University of Life Sciences (NMBU)

# Background: Cardiomyopathy Syndrome (CMS) in Atlantic Salmon

## DISEASE OVERVIEW

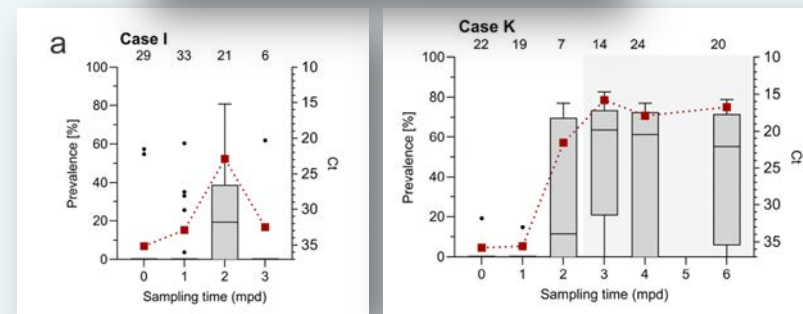
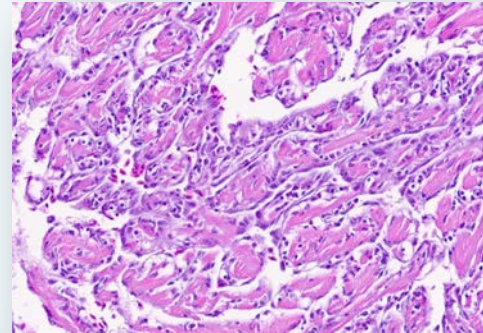
**Pathogen:** Piscine Myocarditis Virus (PMCV)

**Target tissue:** Spongy ventricular myocardium & atrium

**Lesion pattern:** Necrotizing myocarditis — mononuclear infiltration, necrosis

**Disease course:** Chronic; late production stages, high condition factor fish

**Outcome:** Poor resolution of lesions; atrial rupture; sudden death



Virus Evolution, 2024, 10(1), veae007  
DOI: <https://doi.org/10.1093/ve/veae007>  
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Research Article

Unraveling the genomic landscape of piscine myocarditis virus: mutation frequencies, viral diversity and evolutionary dynamics in Atlantic salmon

Ficheal Amono<sup>1</sup>, Turhan Markussen<sup>1</sup>, Vikash K. Singh<sup>2</sup>, Morten Lund<sup>3</sup>, Farah Manji<sup>4</sup>, Sunil K. Mor<sup>5</sup>, Øystein Evensen<sup>1</sup>

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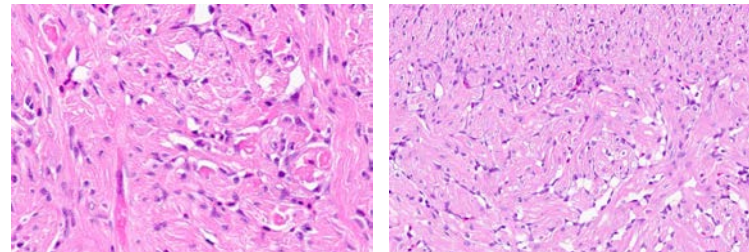
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## KEY KNOWLEDGE GAP



**Pancreas disease** – necrotic and inflammatory changes that resolve, resulting in a healed / regenerated heart

**HSMI** – similar trend as for PD

**PMCV/CMS** – poor resolution of lesions

Does the post-PMCV heart undergo **repair (fibrosis)**, or are the signs of **regeneration**?

# Study Aims & Two-Pronged Approach

*Aim: characterize matrix remodeling and cardiomyocyte gene signatures in PMCV-infected Atlantic salmon hearts*

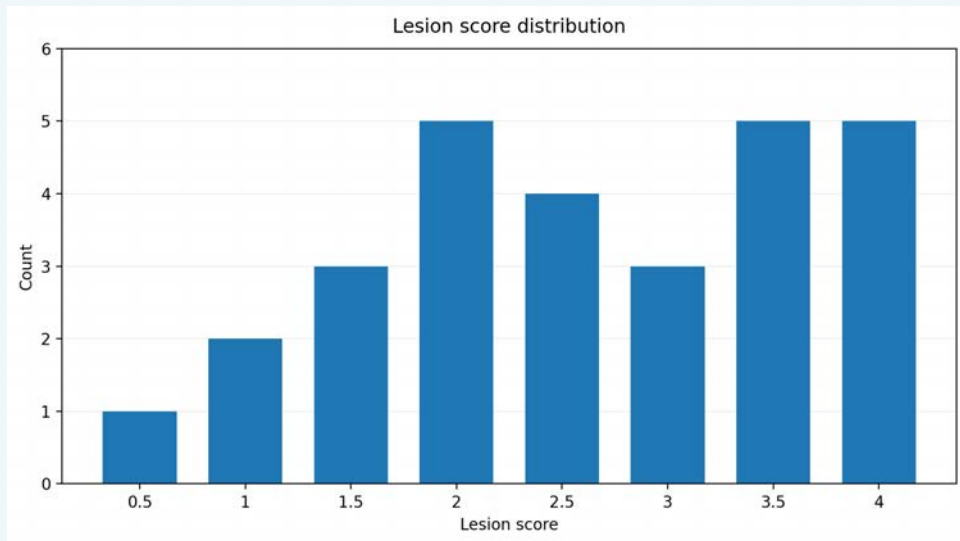
## ARM 1 — Histology & Special Stains

- PMCV-positive hearts (field material, n=50, qPCR-confirmed, CMS outbreak 2019)
- HE staining: score severity & distribution
- Special stains: Van Gieson/EVG, Masson's trichrome, Reticulin, Picrosirius red\*
- Polarized light microscopy: discriminate collagen type I vs III

## ARM 2 — Transcriptional Readouts

- Experimental PMCV challenge model
- Sampling at 4 wpc and 10 wpc (+ uninfected controls)
- Targeted qPCR panel:
- Phospholamban (pln) — cardiomyocyte homeostasis marker
- Notch signalling — candidate regenerative pathway

# ARM 1: Histopathological Findings — HE Staining (n = 50)



## Key finding

78% of fish scored  $\geq 2.0$   
No fish scored 0  
Study samples show typical  
necrotizing myocarditis pattern

## CHARACTERISTIC LESIONS

- Mononuclear inflammatory infiltration • Cardiomyocyte necrosis
- Endocardial hyperplasia • "Tube structures" (empty myocyte scaffolds)
- Focal connective tissue proliferation • Atrial involvement

**32%**

Regeneration signs  
(Study 1)

**21%**

Regeneration  
(Study 2)

**53%**

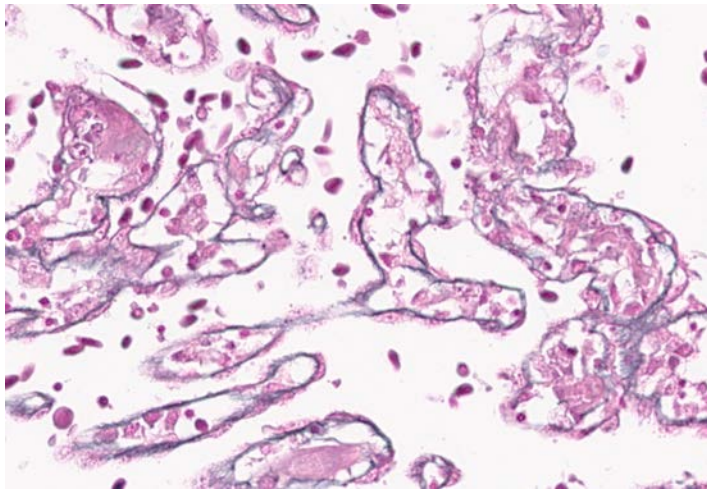
Repair signs  
(Study 1)

**18%**

Repair signs  
(Study 2)

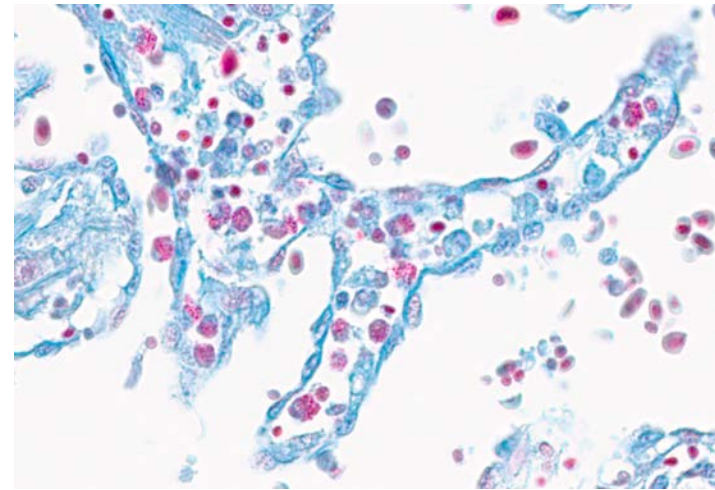
## Reticulin staining and Masson's Trichrome

### Reticulin staining

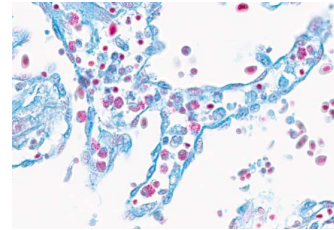
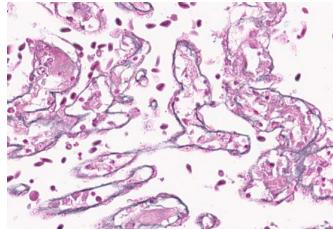


- The preserved reticulin-positive lining beneath the endocardial surface suggests maintenance of the subendocardial extracellular matrix scaffold

### Masson's Trichrome



- Collagen is blue, and the prominent color in the trabecular framework aligns with black reticulin outlines
- A collagen-rich scaffold, indicating remodeling during disease progression, or active fibroplasia as a response to myocyte loss



Together, the reticulin and trichrome patterns suggest

- Tissue repair proceeds on a retained extracellular matrix scaffold
- Persistent, mononuclear inflammation indicates ongoing remodeling rather than *restitutio ad integrum* (full recovery with no lasting damage)

# Picrosirius Red — Collagen Typing under Polarized Light

**Collagen I** → yellow–red birefringence    **Collagen III** → green birefringence    (*polarized light, cross-polarization*)

## Collagen I

- Orange-red birefringence is often associated with thicker, more tightly packed, more mature fibers
- Interpreted as type I–dominant or type I–enriched

## Collagen III

- Type III collagen fibers, early stage responses

## Relationship to Lesion Severity

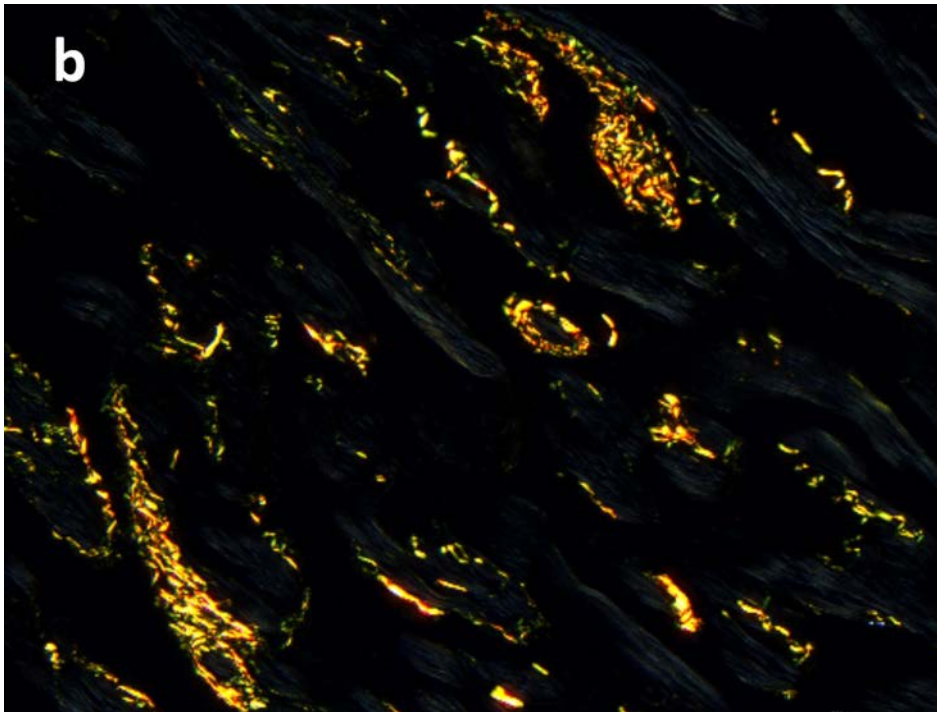
- In early/mild lesions, collagen III is more prominent (remodeling phase).
- In more severe/advanced lesions, collagen I predominates, suggesting transition to a persistent scar-like matrix rather than a transient, remodeled matrix

## Picrosirius Red — Polarized Light Microscopy - heterogeneous collagen remodeling

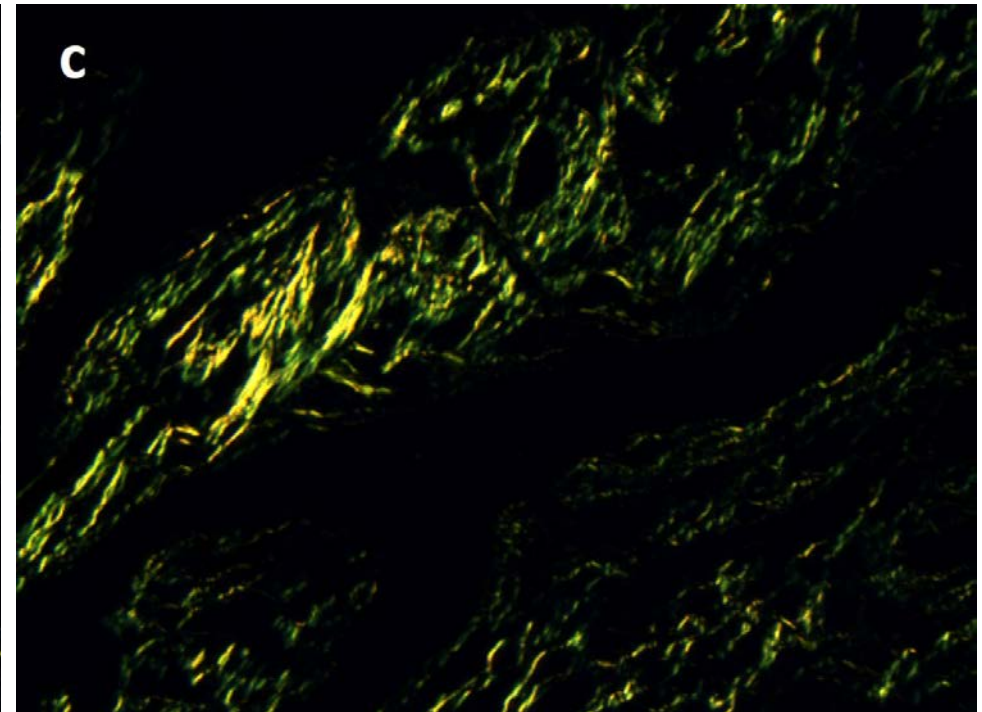
Type I collagen

Type III collagen

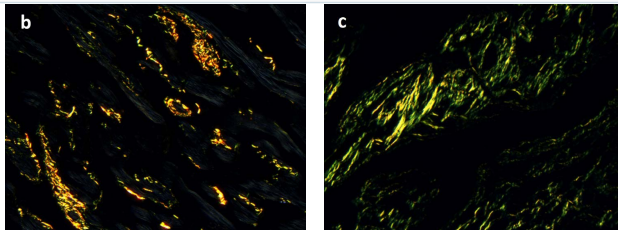
Background



Orange-red birefringent fibers indicate thick, densely packed type I collagen bundles. These are concentrated around vascular structures and in discrete linear deposits along the myocardium

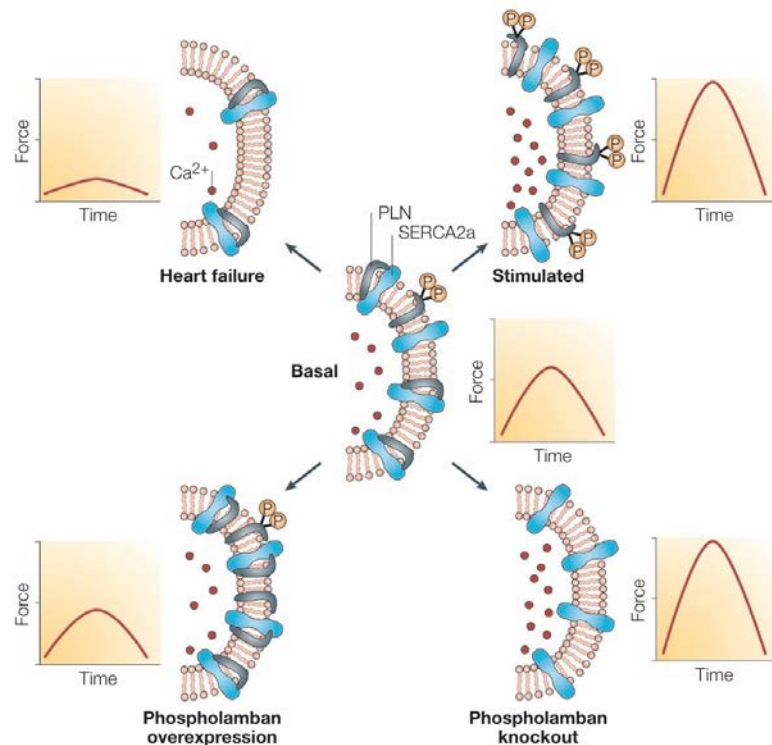


The signal is more dominated by green and yellow-green, indicating thin type III collagen fibers  
Pattern consistent with reactive interstitial fibrosis or early reparative matrix

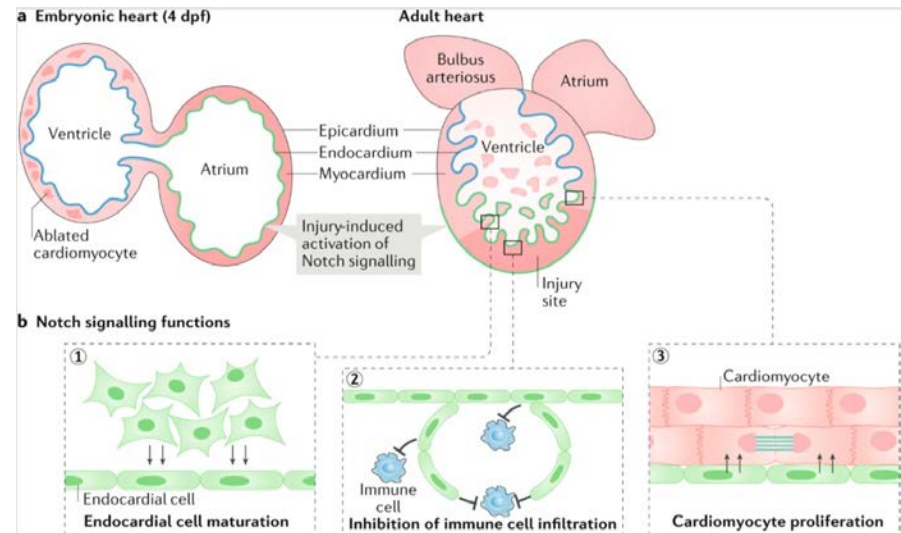


- Picosirius pattern - consistent with mixed collagen organization
  - concurrent deposition of mature compact collagen and a finer reticular collagenous matrix
- Remodeling rather than uniform mature scar formation
- Reticulin/Masson's trichrome and picosirius pattern
  - support persistence of a remodeled extracellular matrix scaffold, and mixed collagen architecture
- Repair-dominant chronic remodeling rather than regenerative restitution

## Gene regulation deep dive PLN and Notch



PLN – phospholamban: decreases  $\text{Ca}^{2+}$ -pump activity, thus making  $\text{Ca}^{2+}$  available for release during cardiac muscle contraction  
McLennan & Cranias Nat Rev Mol Cell Biol, 2003



Notch – promotes proliferation of cardiomyocytes post injury, and limits fibrosis, and promotes angiogenesis  
MacGrogan et al. 2018 Nat Rev Cardiol, 2018

# Transcriptional Readouts — Phospholamban & Notch Signalling

PMCV experimental challenge timeline



## Phospholamban (pIn)

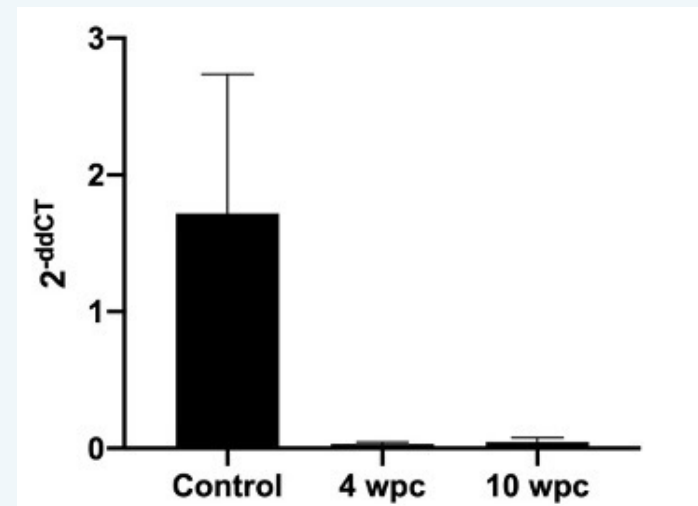
**Function:** Cardiomyocyte homeostasis / SR Ca<sup>2+</sup> cycling

Result: Significantly REDUCED in PMCV-infected fish vs controls at both 4 wpc and 10 wpc

### Interpretation:

Points toward a functional cardiomyopathy at the cellular level, not merely structural damage

## PLN expression in heart



⚠ *Caveat: bulk-tissue qPCR cannot resolve compartment-specific activation — spatial analyses needed*

# Transcriptional Readouts — Phospholamban & Notch Signalling



## Notch signalling

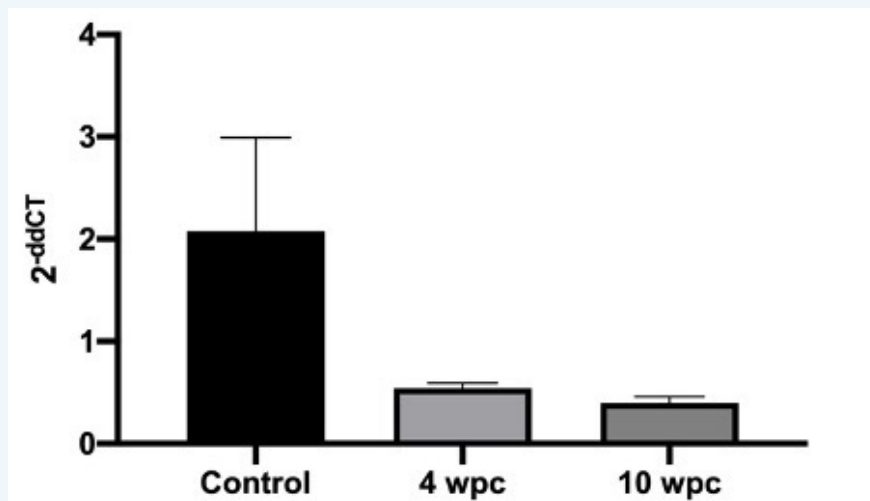
**Function:** Candidate regenerative signalling pathway

Result: Significantly REDUCED in PMCV-infected fish vs controls at both 4 wpc and 10 wpc

### Interpretation:

Suggests limited engagement of Notch-linked regenerative signaling at these post-infection stages, consistent with poor regenerative response to CMS

## Notch expression in heart



⚠ *Caveat: bulk-tissue qPCR cannot resolve compartment-specific activation — spatial analyses needed*

## Integrated Interpretation: Repair vs. Regeneration in CMS

- 1 PMCV infection drives the infected heart into a state of both regenerative failure (Notch suppression) and calcium-handling dysfunction (PLN loss)
- 2 Histochemical staining shows that heart tissue undergoes fibrotic remodeling
- 3
  - Sustained suppression of PLN and Notch expression over 10 wpc suggests no transition toward a mature regenerative program
  - Remains in a state of chronic inflammatory-fibrotic remodeling with incomplete functional restitution

# Relevance to the Salmon Farming Industry

*CMS is a major welfare and production loss driver in Norwegian and global salmon aquaculture*

## Prognosis



Ability to distinguish reversible / regenerative healing from persistent fibrotic repair in PMCV-affected hearts would strengthen clinical prognosis at the fish level.

## Harvest Timing



Quantitative matrix and gene-expression endpoints provide objective criteria for decisions around optimal harvest timing and loss-risk assessment.

## Intervention Endpoints



Measurable repair/regeneration readouts create endpoints to evaluate selective breeding programmes, vaccination strategies, and management mitigation.

## Welfare



Understanding why CMS hearts fail to regenerate — unlike PD and HSMI — is essential for developing meaningful fish welfare standards.

# Acknowledgments

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