

# Impact of Vaccination Temperature on Atlantic Salmon Resistance to Infectious Salmon Anemia Virus

Yan-Ru Chen<sup>1</sup>, Manuel Soto-Dávila<sup>1</sup>, Taylor Wheatley<sup>1</sup>, Ruth Quispe<sup>1</sup>, Shona K. Whyte<sup>1</sup>, Shawna L. Semple<sup>1</sup>, Reza Ghanei-Motlagh<sup>1</sup>, Eyesun Fajei<sup>1</sup>, Eleanor Glahn<sup>1</sup>, Frederick von Rönge<sup>2</sup>, Andrew Swanson<sup>2</sup>, and Mark D. Fast<sup>1</sup>

<sup>1</sup> Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE

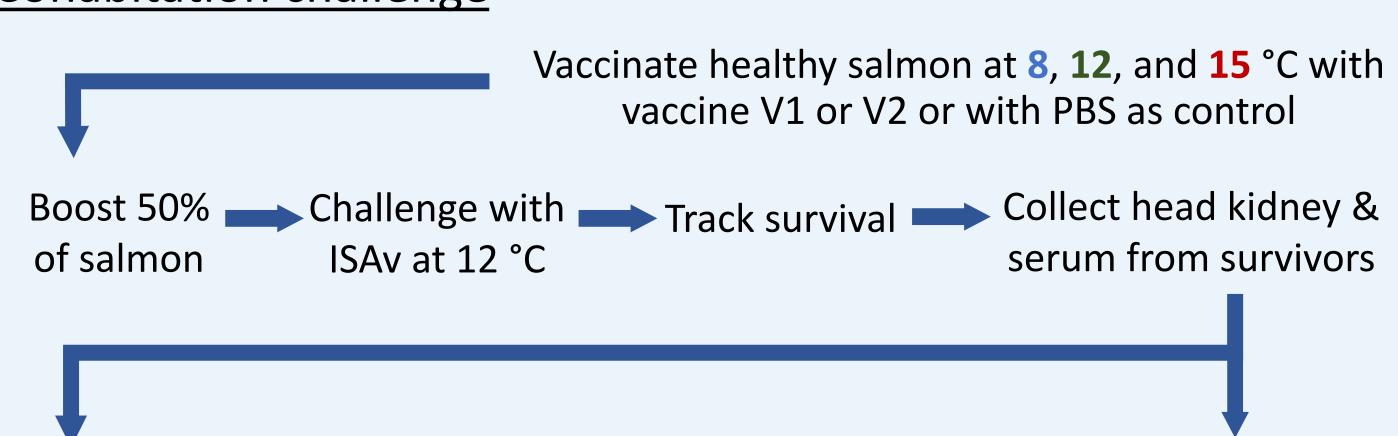
<sup>2</sup> Cooke Aquaculture Inc., Saint John, NB, Canada

#### **INTRODUCTION**

- Aquaculture accounts for > 50% of global aquatic animal protein production.
- Infectious salmon anemia virus (ISAv) affects Atlantic salmon aquaculture, and commercial vaccines have limited efficacy.
- As ectotherms, salmon have an optimal physiological temperature of 12-15°C.
- In aquaculture, vaccination occurs across a wide thermal range, thus low temperatures may compromise protection against ISAv.
- Aims: 1) to assess the impact of vaccination temperature on protection;
  - 2) to determine whether lower IgM production is associated with higher viral load and lower survival outcomes.

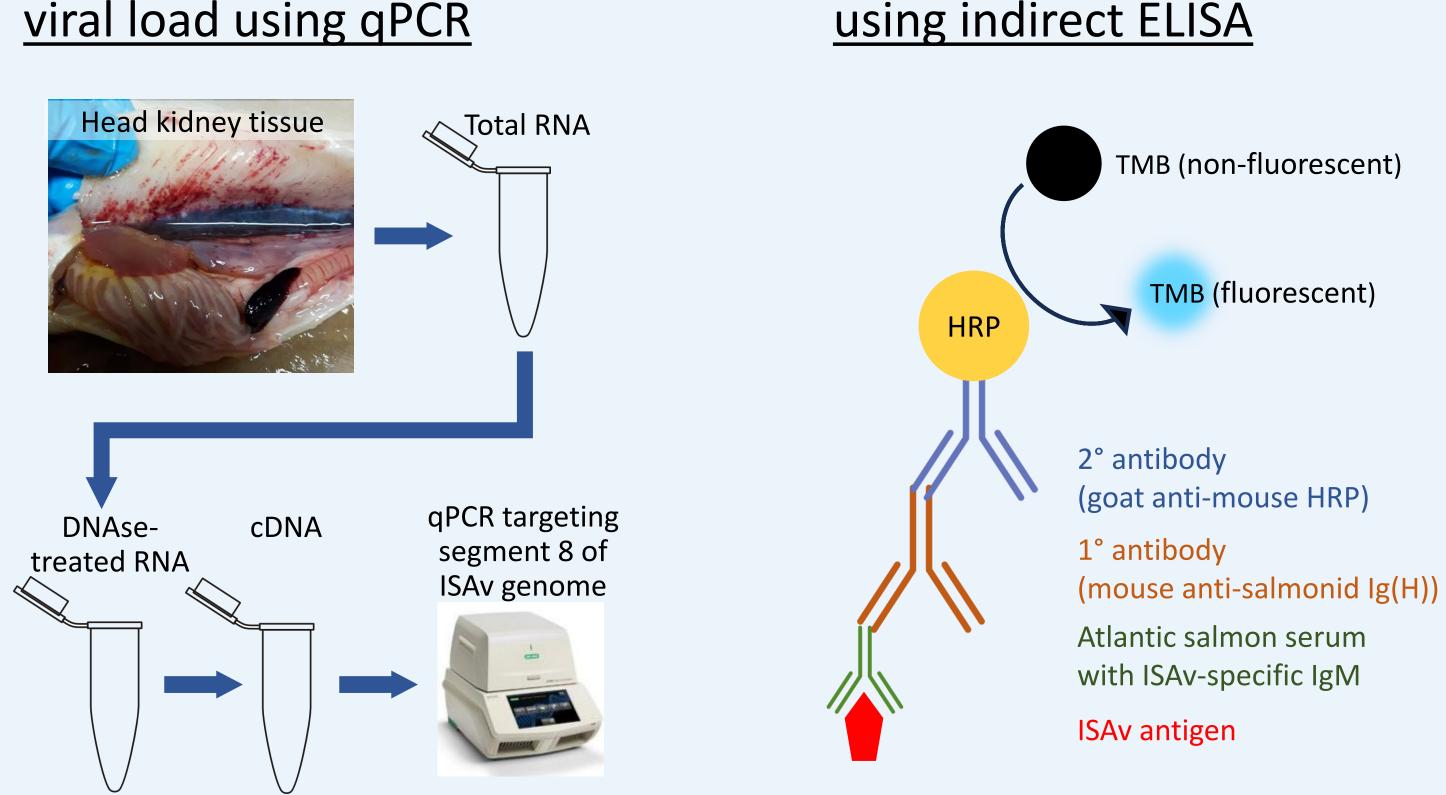
#### **METHODS**

#### Cohabitation challenge



Detection of serum IgM

## Quantification of head kidney viral load using qPCR



#### Statistical analysis

- Kaplan-Meier analysis was performed to estimate survival probabilities over time.
- A two-way ANOVA was conducted for IgM detection using temperature and vaccine treatment as independent variables, followed by Tukey's multiple comparisons test to determine significant differences between groups, p < 0.05.

#### **RESULTS**

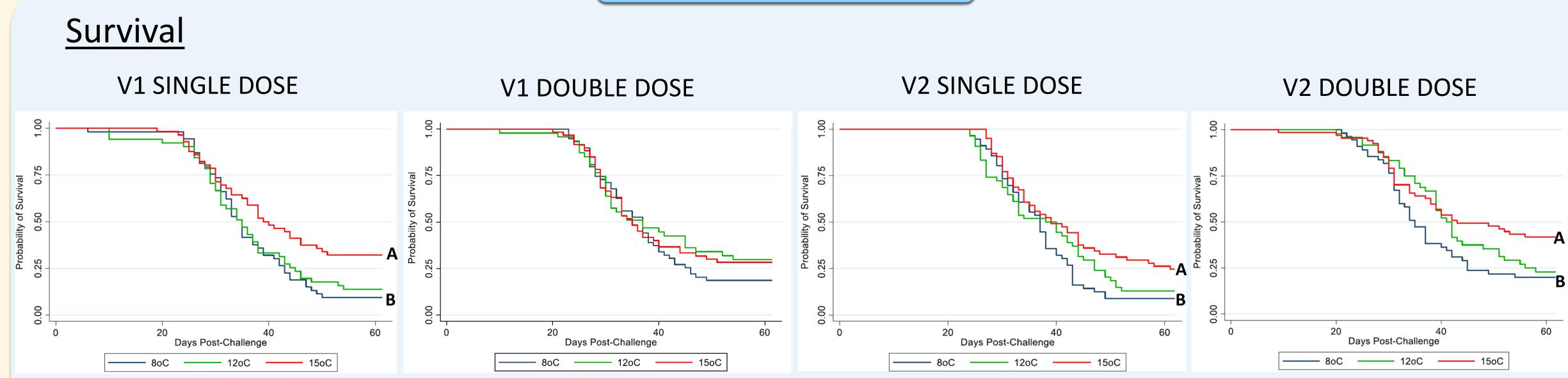


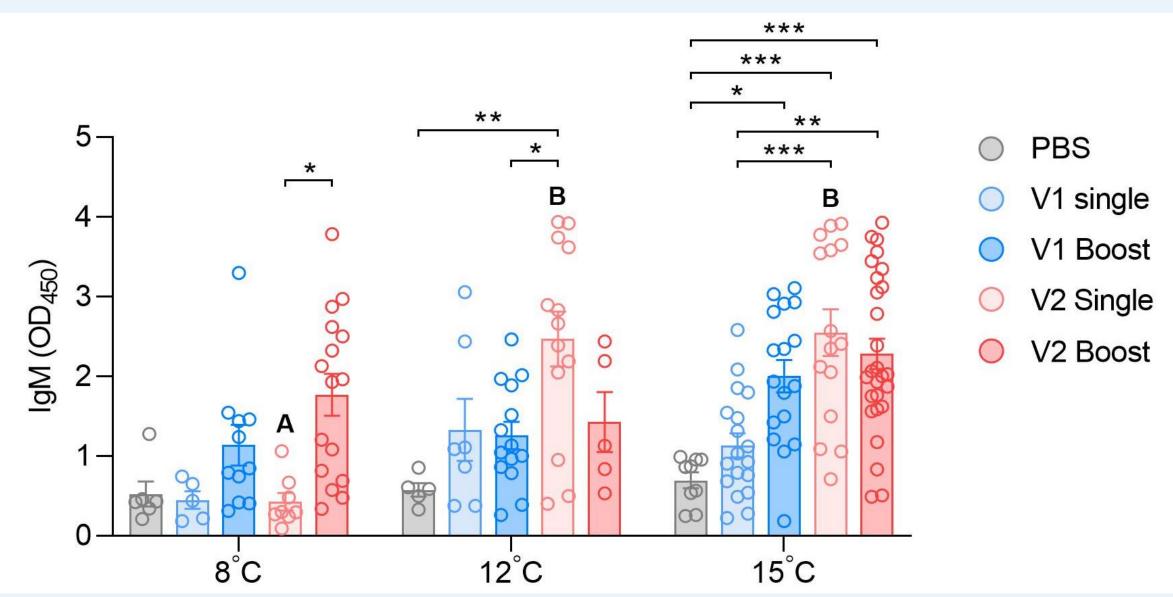
Figure 1. Kaplan-Meier survival analysis from the cohabitation challenge. Atlantic salmon were exposed to infected donor fish (ISAv-HPR4, TCID50:  $1x10^5$ mL) at a 7.5:1 cohabitant-to-donor ratio. Letters (A, B) indicate a significant difference in survival probabilities from different vaccination temperatures under the same vaccine treatment, p < 0.05.

### Viral load

Temperature/ Vaccine	<b>8 °C</b> (n = 35)		<b>12 °C</b> (n = 35)		<b>15 °C</b> (n = 40)	
	ISAv+	ISAv-	ISAv+	ISAv-	ISAv+	ISAv-
PBS (control)	1	5	0	5	0	8
V1 single	0	5	0	7	0	8
V1 double	2	6	1	7	1	7
V2 single	0	8	0	7	0	8
V2 double	1	7	2	6	0	8

**Table 1.** ISAv load in head kidney tissue from 110 survivor fish, assessed by qPCR. Samples with Ct values ≥34 were considered ISAv-negative, while those with Ct values <34 were classified as ISAv-positive. In this study, positive samples contained between 2.84 and 895 viral copies per μL of cDNA.

#### ISAv-specific IgM production



**Figure 2.** Indirect ELISA analysis of total IgM levels in serum from 110 survivor fish. Asterisks indicate significant differences in IgM levels between vaccine treatments at the same temperature (\*\*\*p < 0.001; \*\*p < 0.01; \*p < 0.05). Letters (A, B) denote significant differences in IgM levels between vaccination temperatures within the same vaccine treatment.

#### CONCLUSION

- Survival: Atlantic salmon vaccinated at 15°C with V1 (single dose) or V2 (single dose or boosted) had significantly higher survival than salmon vaccinated at 8°C.
- Viral load: Out of 8 survivors with a detectable head kidney ISAv load, 7 were vaccinated at lower temperatures (8 or 12°C), whereas only 1 was vaccinated at the highest temperature (15°C).
- IgM production: Survivors vaccinated at 15°C with either V1 (boosted) or V2 (single dose or boosted) had significantly higher ISAv-specific IgM levels compared to PBS controls.

#### **HIGHLIGHTS**

Overall, vaccination at 15°C correlated with higher survival and, among survivors, higher IgM production and lower probability of detectable head kidney viral load. Our findings highlight the importance of temperature in optimizing vaccine efficacy and improving aquaculture sustainability.