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Vaccine for Saprolegnia

**ParaFishControl Workshop “North European Fish
Parasite Management Strategies in Aquaculture Farms”**

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Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)



Three trials in Atlantic salmon:

- ▶ Two DNA vaccine trials – reverse vaccinology
- ▶ One protein homogenate trial – conventional vaccinology

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Reverse vaccinology hypothesis:

- ▶ target virulence factors like SpSsp1 (*S. parasitica* secreted serine protease 1)
 - Upregulated expression in pre-infective stages (i.e. germinating cysts & mycelia) ✓
 - Carry signal peptide ✓

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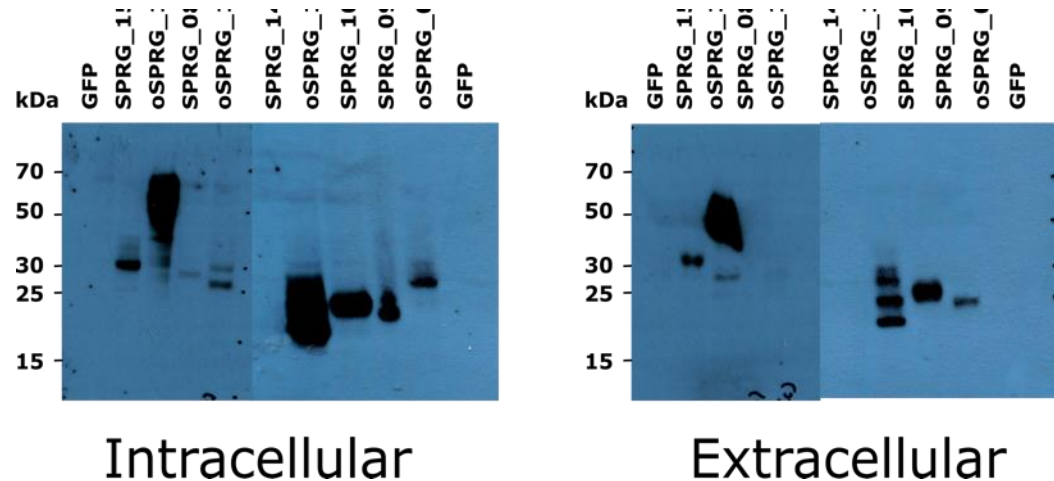
Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)

Reverse vaccinology: Development of DNA vaccines

- ▶ 15 targets + SpSsp1 cloned into appropriate vector

HEK239

In vitro expression
of DNA vaccines in
HEK293 cells

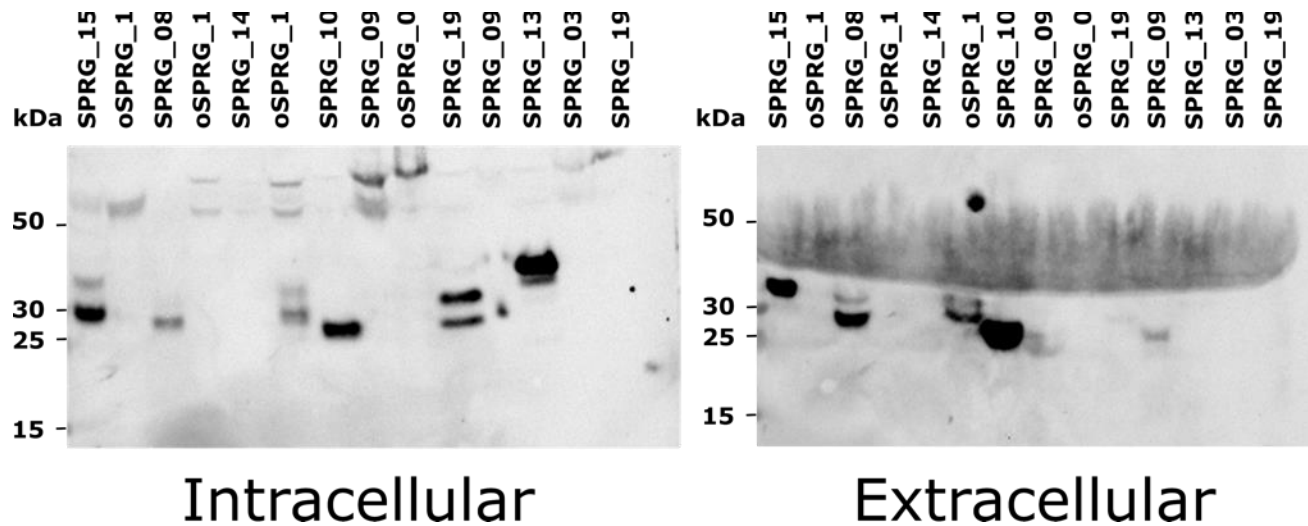


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Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)

In vitro expression
of DNA vaccines in
RTG-2 cells

constructs



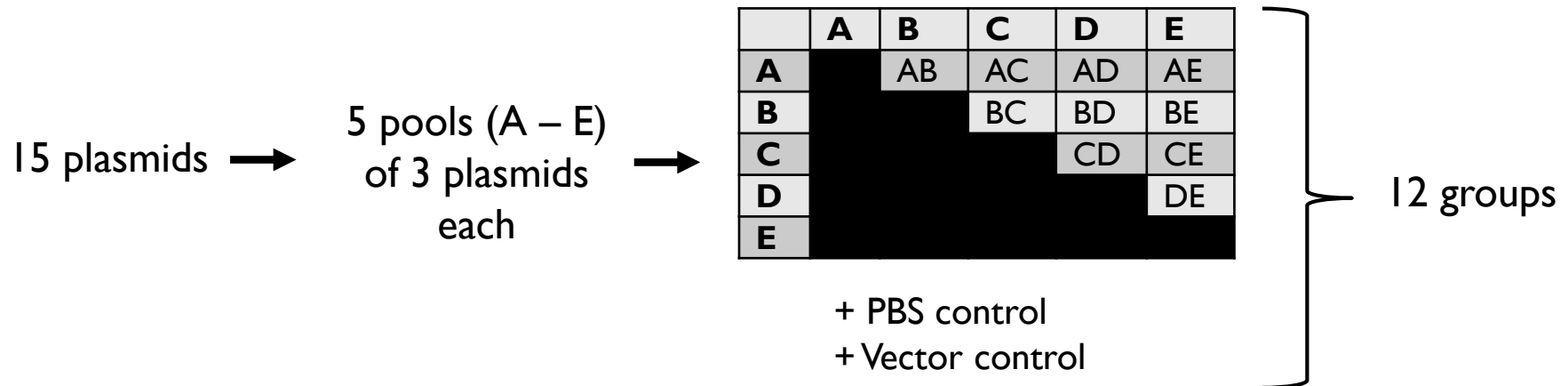
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Trial 1: 15 targets in matrix design

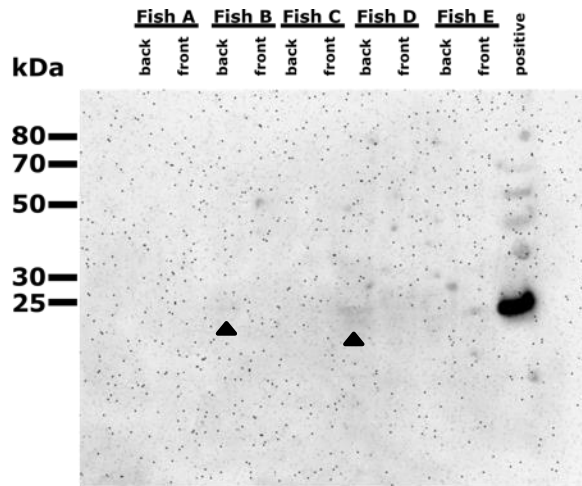
- ▶ Atlantic salmon pre-smolts
- ▶ 12 groups of 35 fish each (25 at the time of challenge)
- ▶ 6 DNA vaccines per fish; 2.5 µg of each plasmid
- ▶ Fish challenged 2 months post vaccination



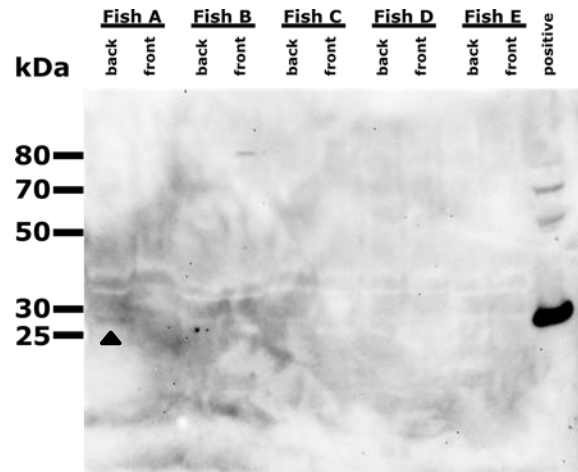


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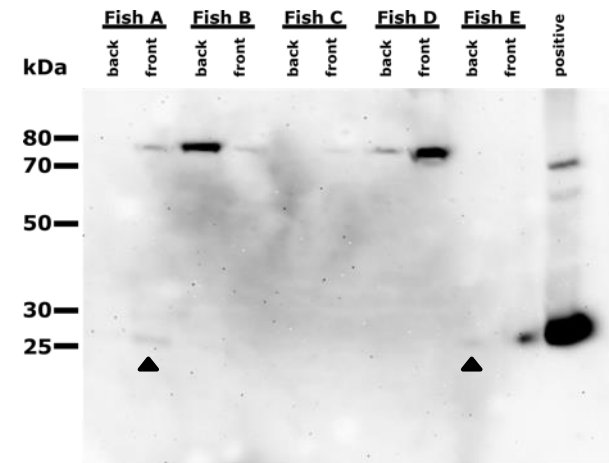
Trial 1: *In vivo* expression of *S. parasitica* proteins in vaccinated salmon myocytes



Group 1 (AB); 20 min



Group 3 (AD); 15 min



Group 10 (DE); 15 min

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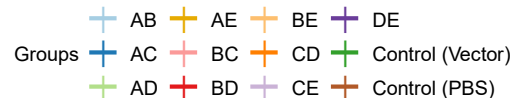
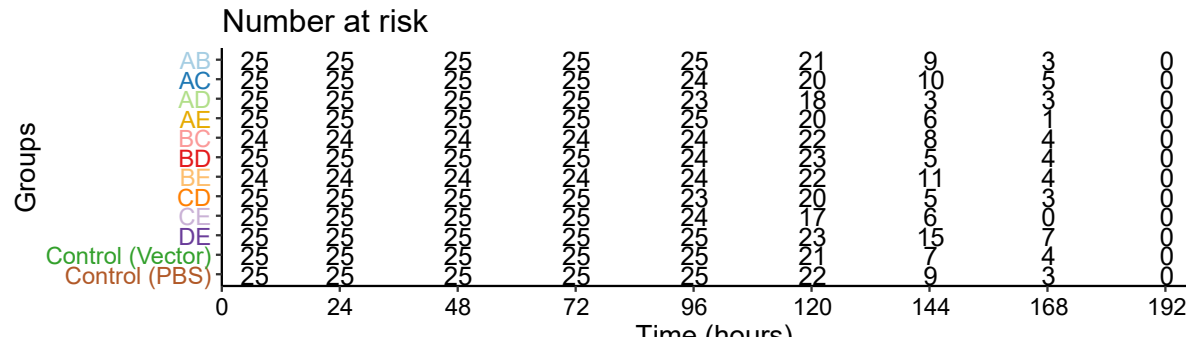
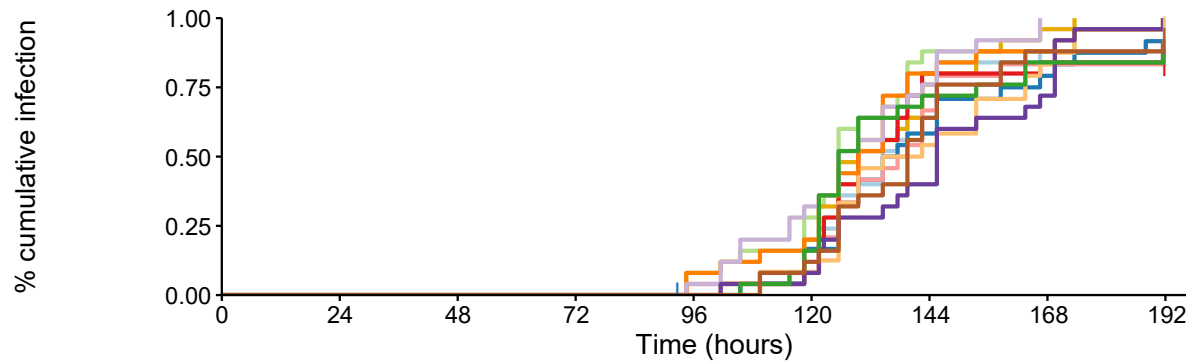
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Trial 1: Challenge



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Trial 2: SpSsp1

- ▶ Atlantic salmon pre-smolts
- ▶ 2 groups of 35 fish each (25 at the time of challenge)
- ▶ 1 DNA vaccine per fish
- ▶ Fish challenged 2 months post vaccination

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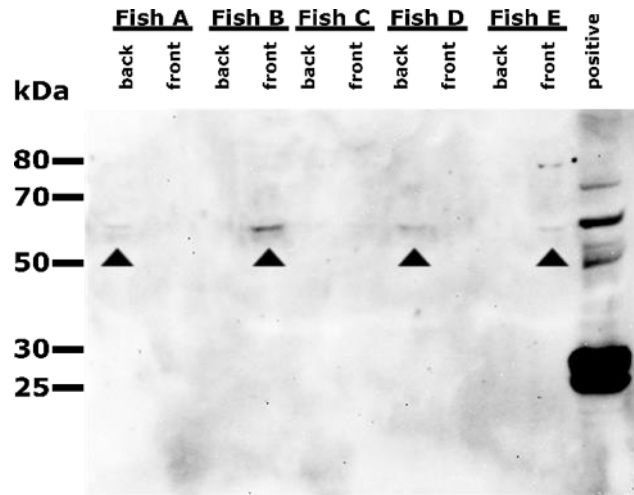




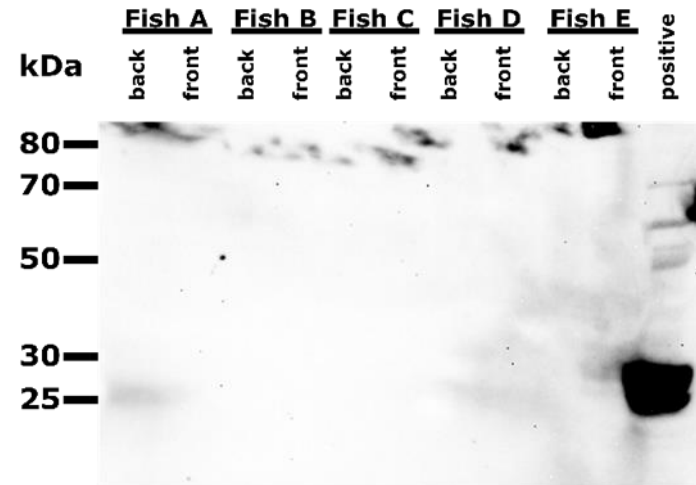
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Trial 2: *In vivo* expression of SpSsp1 in vaccinated salmon myocytes



Group SpSsp1, 15 min exposure



Group Vector, 15 min exposure

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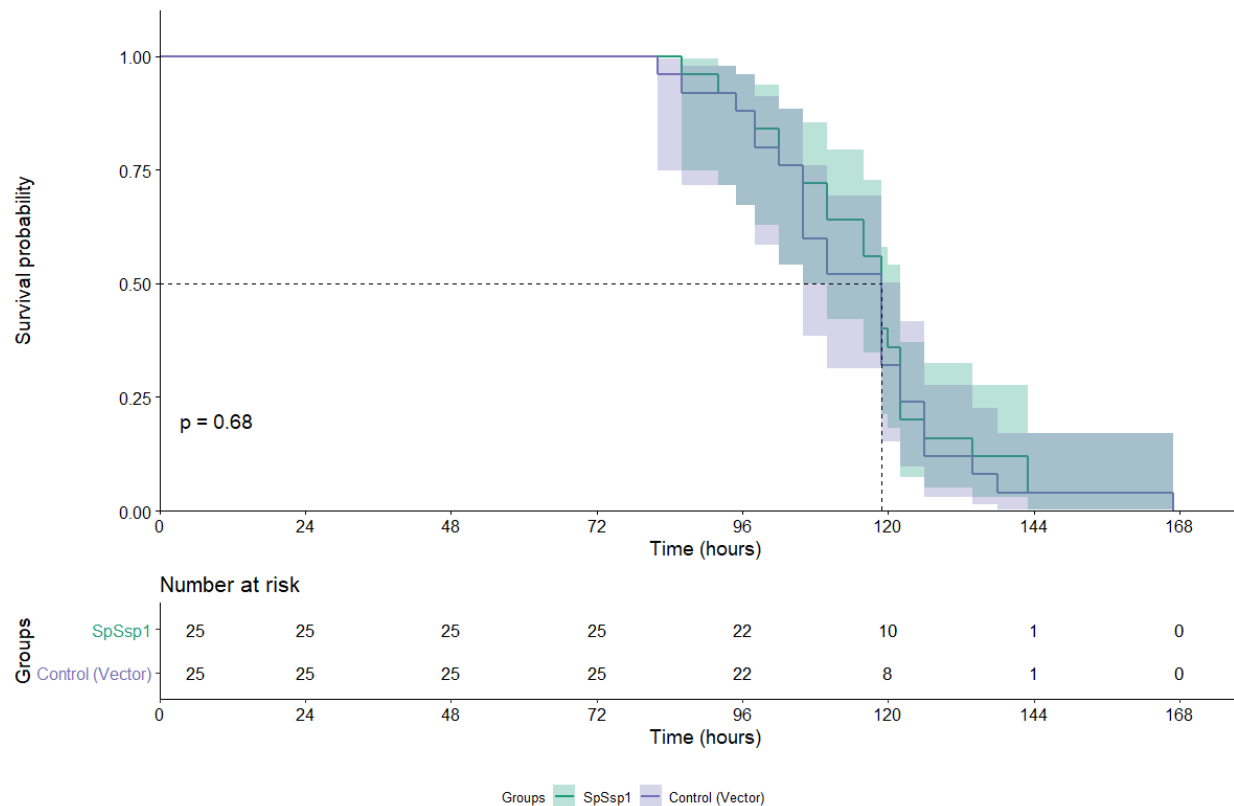


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Trial 2: Challenge – course of infection



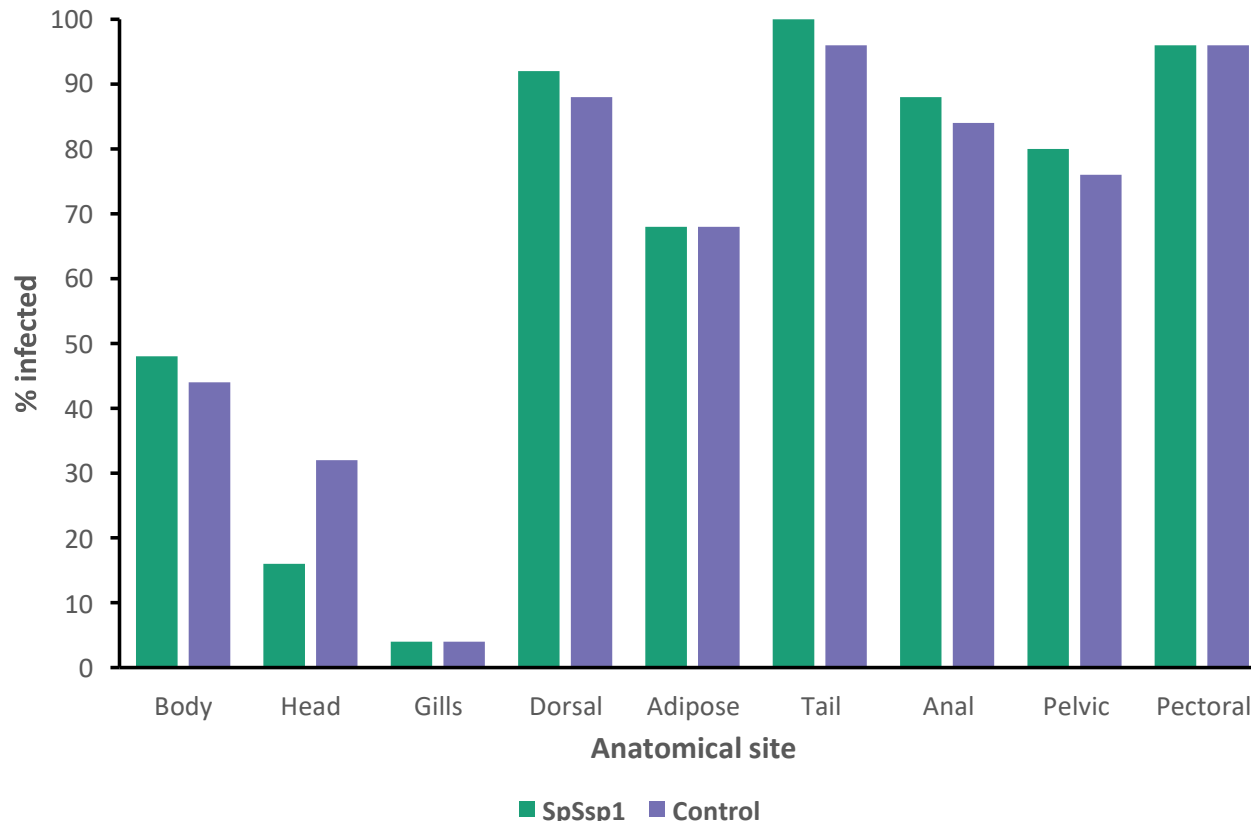


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Trial 2: Challenge – clinical manifestation

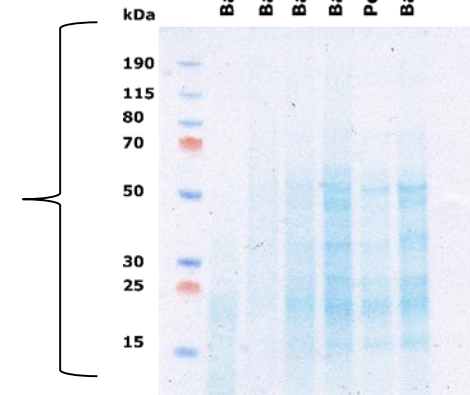
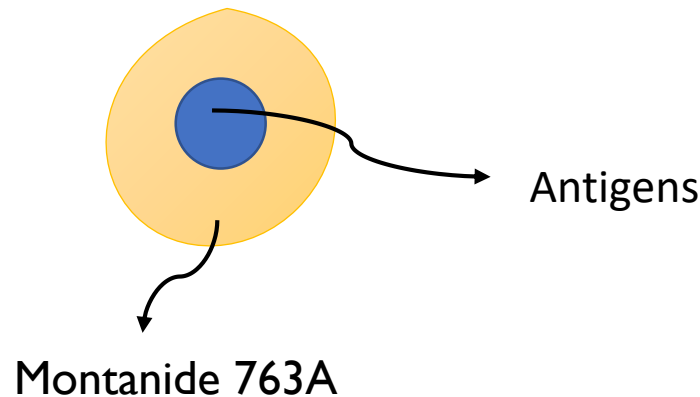




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Trial 3: protein homogenate vaccine

- ▶ Atlantic salmon pre-smolts
- ▶ 4 groups of 35 fish each (25 at the time of challenge)
- ▶ 0.2 mL adjuvanted vaccine; 50 μg protein per fish
- ▶ Fish challenged 2 months post vaccination



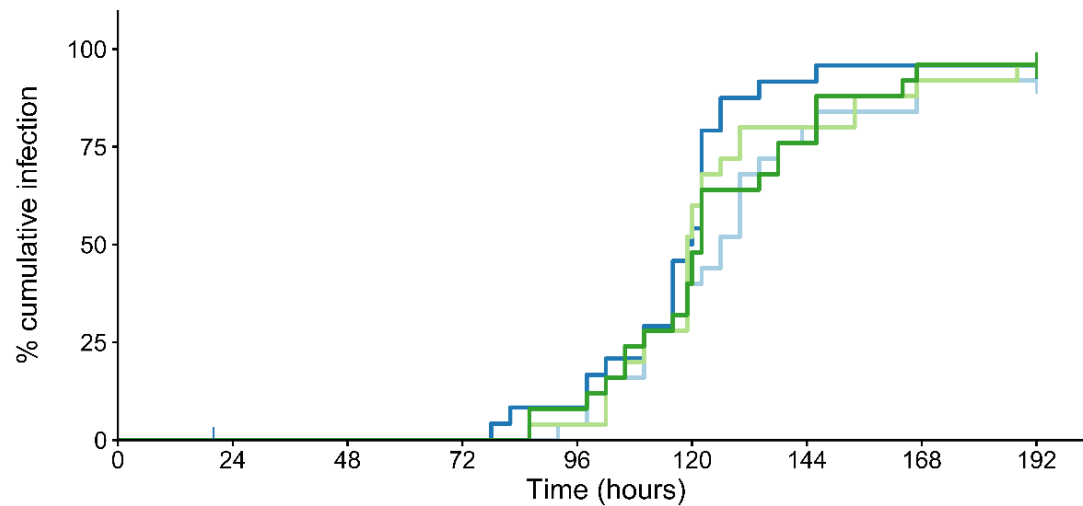


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Trial 3: Challenge – course of infection



| | | Number at risk | | | | | | | | |
|--------------------|-------|----------------|----|----|----|----|-----|-----|-----|-----|
| Groups | | 0 | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 |
| | Prime | + | 25 | 25 | 25 | 25 | 24 | 15 | 5 | 2 |
| Prime+boost | + | 25 | 24 | 24 | 24 | 22 | 12 | 2 | 1 | 1 |
| Control (Adjuvant) | + | 25 | 25 | 25 | 25 | 24 | 12 | 5 | 2 | 1 |
| Control (PBS) | + | 25 | 25 | 25 | 25 | 23 | 15 | 6 | 1 | 1 |

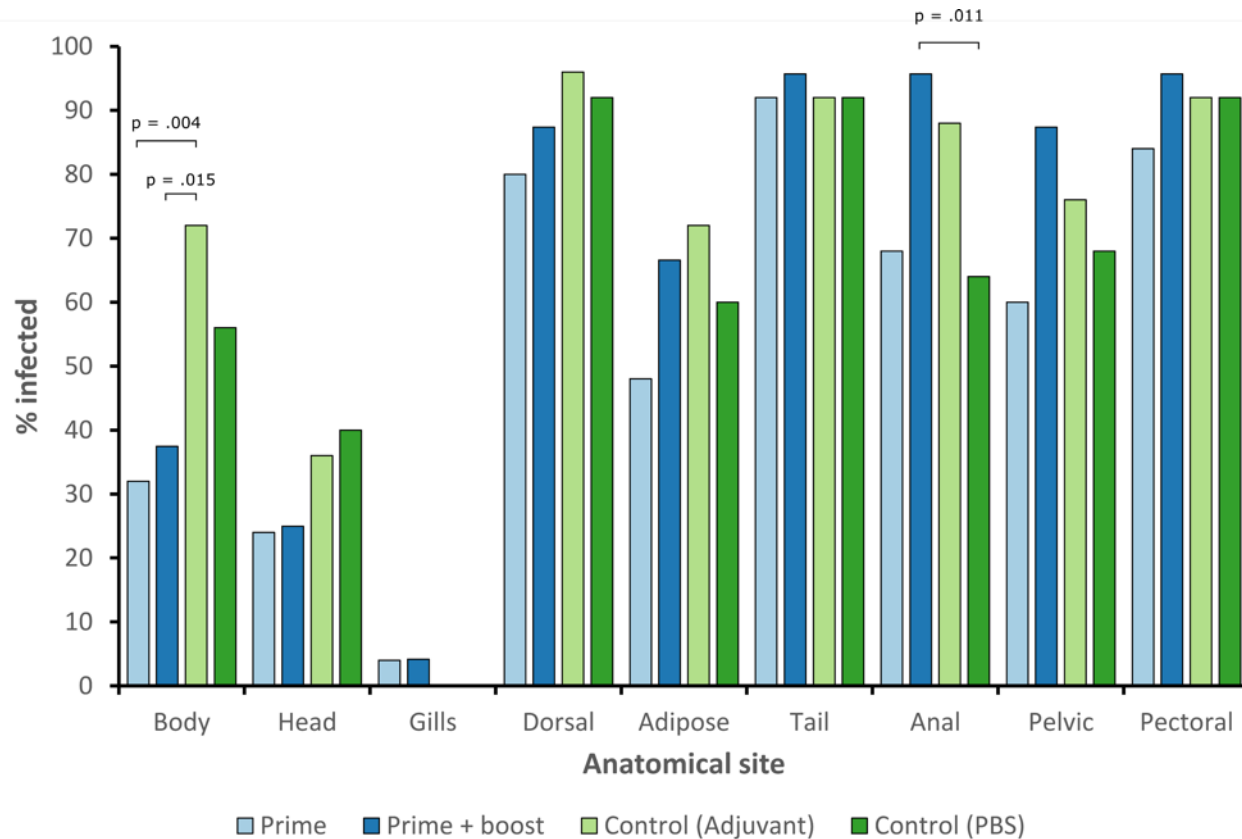
Groups + Prime + Prime+boost + Control (Adjuvant) + Control (PBS)





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Trial 3: Challenge – clinical manifestation



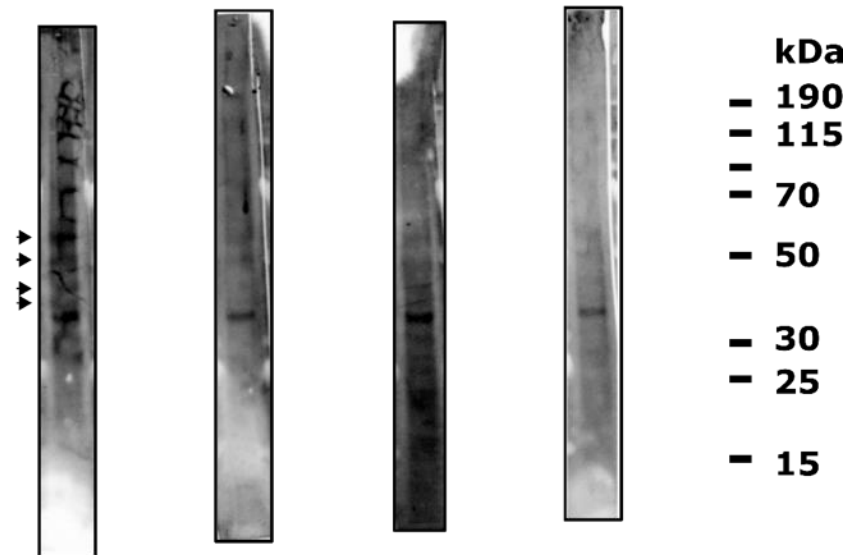


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Trial 3: Detection of anti-*Saprolegnia* IgM antibodies

| pool anti-serums (1:500) | Prime group | Prime/boost group | Adjuvant only control group | PBS control group |
|--------------------------|-------------|-------------------|-----------------------------|-------------------|
| anti-salmon IgM (1:34) | + | + | + | + |
| Anti-mouse-HRP (1:2500) | + | + | + | + |



Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)



In summary:

- ▶ Establishment of a robust challenge model
- ▶ Detection of *S. parasitica* gene expression in salmon myocytes after DNA vaccination
- ▶ Results do not support the SpSsp1 hypothesis
- ▶ Protein homogenate vaccine did not protect against infection, but strong evidence of disease mitigation
- ▶ Protein homogenate vaccine triggered the production of IgM

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Targets

| Pool | Target gene* | Size** (kDa) | Predicted or known function |
|------|--------------|--------------|-----------------------------|
| A | SPRG_X | 28.8 | Endonuclease |
| | oSPRG_Y | 23.72 | ? |
| | SPRG_Z | 16.4 | Elicitin |
| B | SPRG_A | 28.5 | RNase |
| | SPRG_B | 24 | ? |
| | SPRG_C | 21.8 | ? |
| C | SPRG_D | 27.7 | ? |
| | SPRG_E | 23.3 | ? |
| | SPRG_F | 10 | ? |
| D | SPRG_G | 25.9 | ? |
| | oSPRG_H | 22.7 | ? |
| | SPRG_I | 20.7 | Elicitin |
| E | SPRG_J | 24.1 | ? |
| | oSPRG_K | 22.4 | Cellulose binding domain |
| | SPRG_L | 14 | ? |

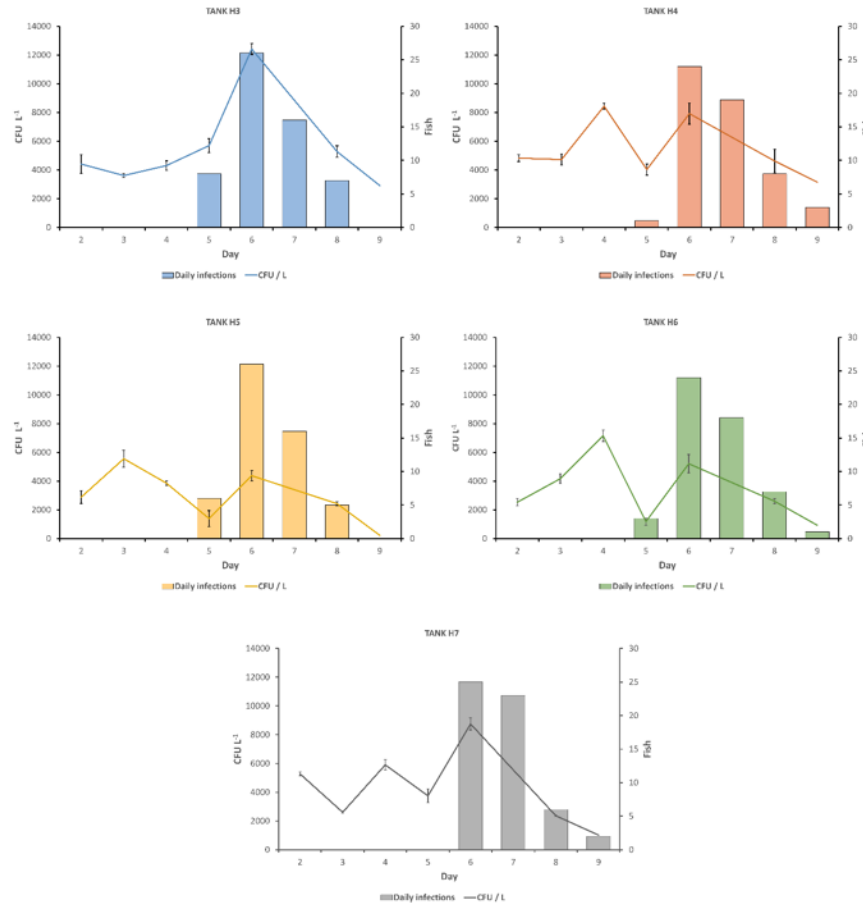
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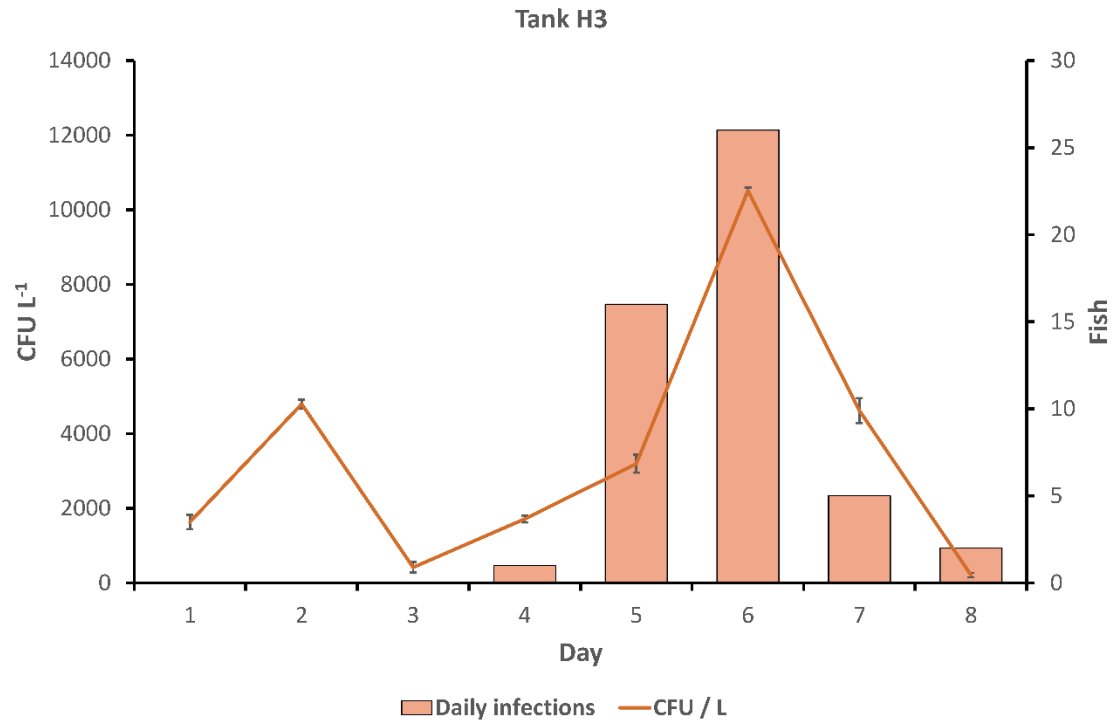
Zoospore counts in tanks – trial 1





Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)

Zoospore counts in tanks – trial 2





Task 3.1 Development of vaccines for *Saprolegnia* (M12-M56)

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Zoospore counts in tanks – trial 3

