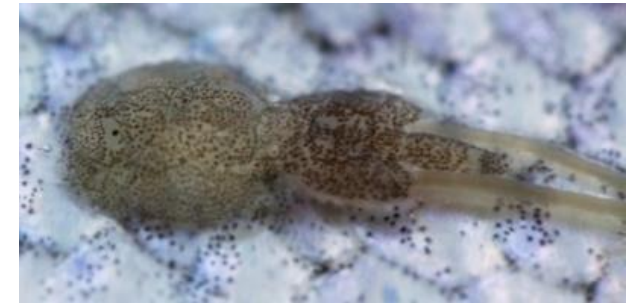




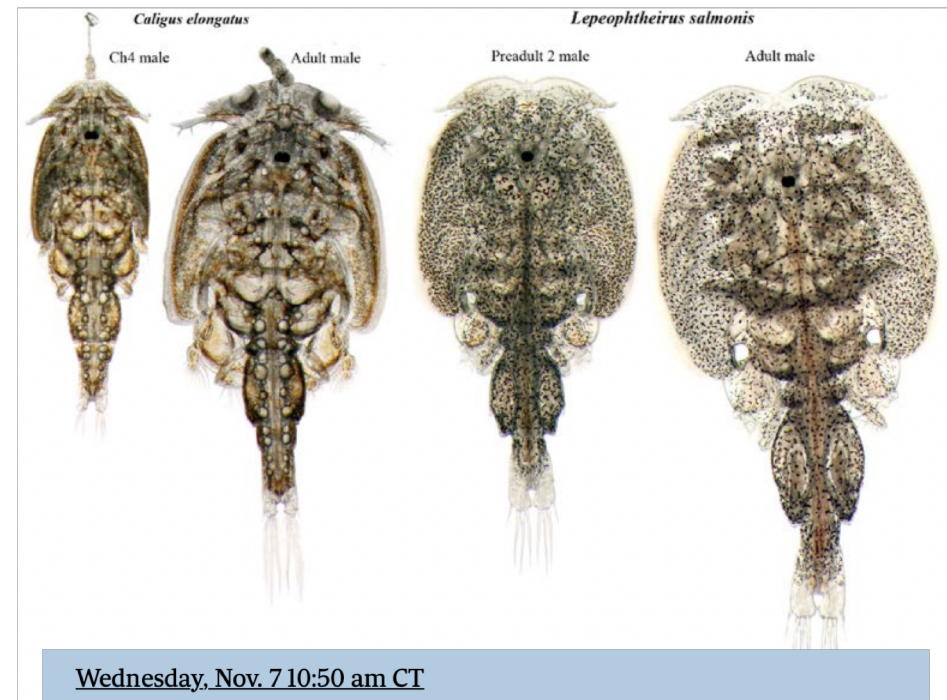
Vaksiner - hvor står vi? Hva er mulighetene?

Øystein Evensen, NMBU



Approaches

- Whole lice antigens
 - Different development stage of sea lice used to immunize salmon
- Recombinant antigens
 - Concealed antigen strategy
- Methods used to identify potential antigen candidates
 - RNAi
 - Phenotypic effects (and genotypic / transcript level)
 - RNAi as an entry strategy
- “Reverse vaccinology”
 - Learn from the host



Wednesday, Nov. 7 10:50 am CT

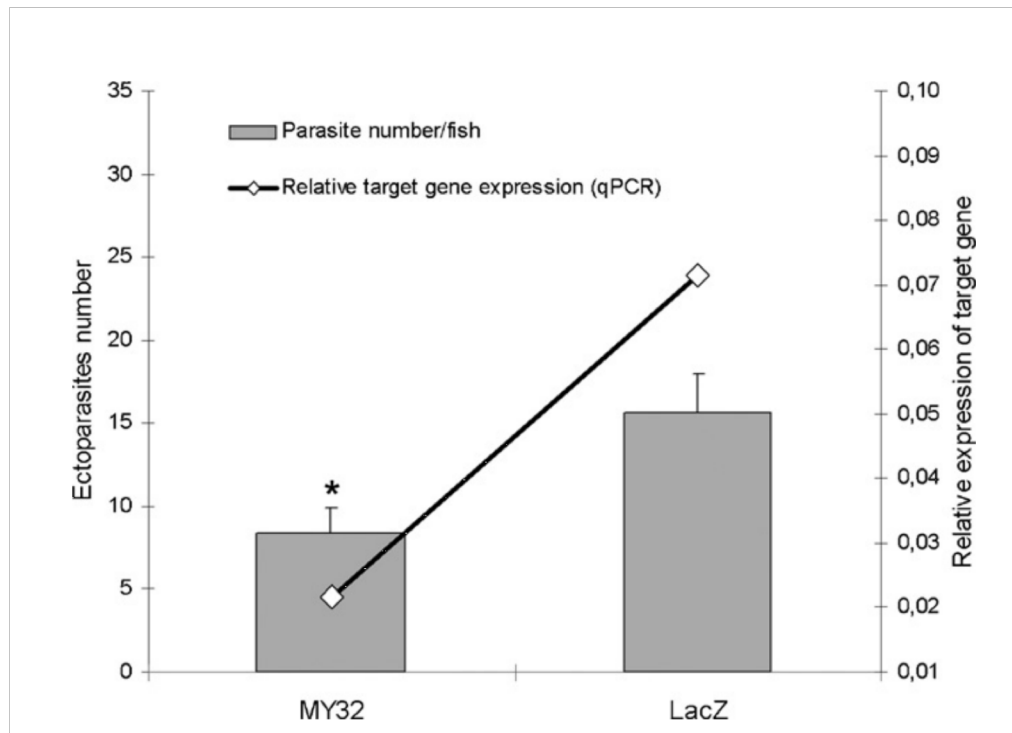
Reverse vaccinology shows promise in targeting Chile salmon lice disease

At least one prototype treatment targeting the "caligidosis" disease affecting Chilean salmon farming will be trialed in the field under commercial aquaculture conditions.

Vaccination attempts against sea lice

- One of the earliest studies was made by Stone and colleagues (Raynard et al. 2002)
 - immunised Atlantic salmon with crude whole-body extracts of *L salmonis*
 - specific antibody response to several louse antigens
 - antibody responses were non-protective when fish were challenged with adult and pre-adult *L salmonis*
- Expression libraries
 - Antigens were selected using monoclonal and polyclonal mouse antibodies to screen expression libraries. The screening antibodies were selected by immuno-histochemistry against *L salmonis* gut lining, mouth parts and soluble extracts
 - Female *L salmonis* on immunised Atlantic salmon showed a reduced proportion of egg strings that matured
 - 2–7% maturity compared with 34–46% in controls
 - greater number of egg strings were deformed - 33–98% compared to 8–56% in controls
- No commercial vaccine is currently available for piscine parasitoses, although experimental vaccines have been assayed against *I. multifiliis*, *Cryptobia salmositica* and scuticociliates. The known information points to the need for integrated studies of the mechanisms involved in protection, in order to choose the optimum antigen candidates, adjuvants and formulations (Alvarez-Pellitero 2008).

RNAi – functionality testing



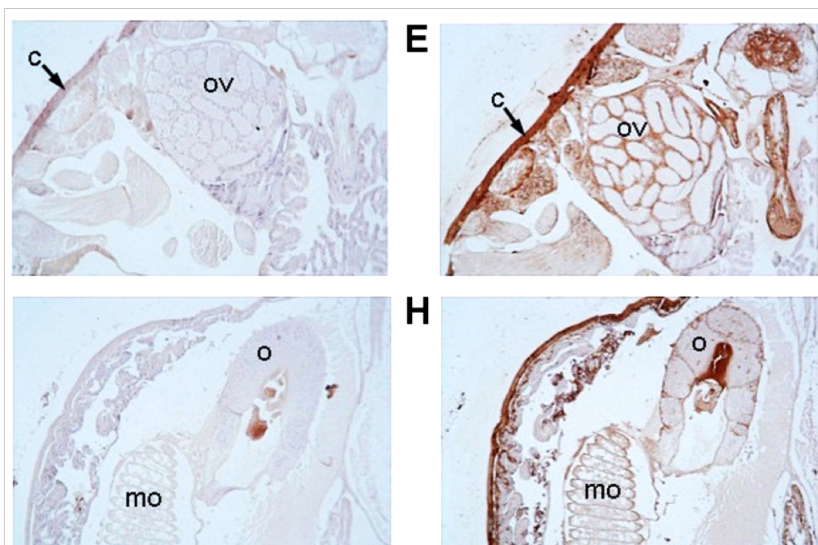
RNA interference experiment

- *Caligus rogercresseyi* copepodids – siRNA(my32-dsRNA or LacZ-dsRNA)
- 100 copepodids were used for infection
- 5 days after the experimental infestation number of lice estimated
- Quantitative real time PCR was carried out using either my32-dsRNA or LacZ-dsRNA treated sample cDNA from 100 soaked lice in conjunction with primers specific for actin or my32.
- Relative expression was determined as number of my32 molecules/number of actin molecules.

Table 2.

Results of vaccination with recombinant my32 in *Salmo salar* and challenge with *Caligus rogercresseyi*.

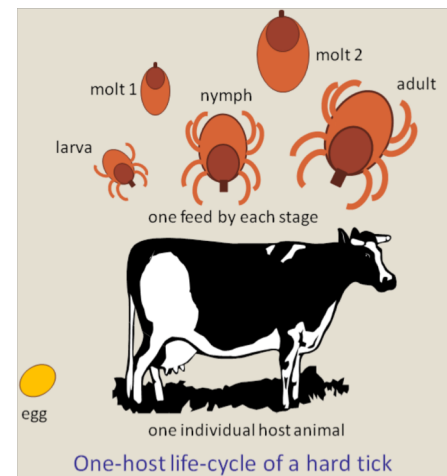
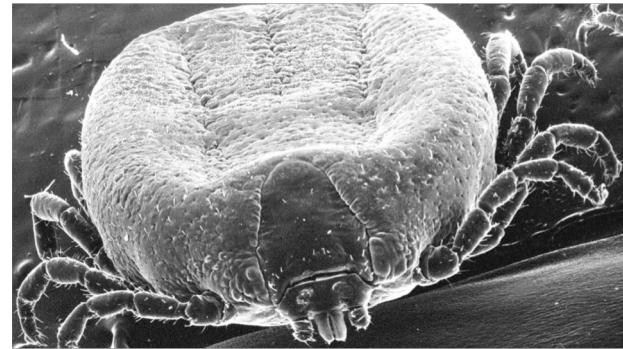
Sampling day post-challenge	Sample size	Experimental groups	Average number of parasites per fish ^c	Number of fish per group ^d	Inhibition of infestation ^e	Statistical analysis
10	5	Placebo ^a	13 ± 3	25	–	$p < 0.01$ ^f
		Vaccine ^b	23 ± 5	25	–	
24	20	Placebo ^a	37 ± 10	20	–	$p < 0.0001$ ^f
		Vaccine ^b	16 ± 7	20	57%	

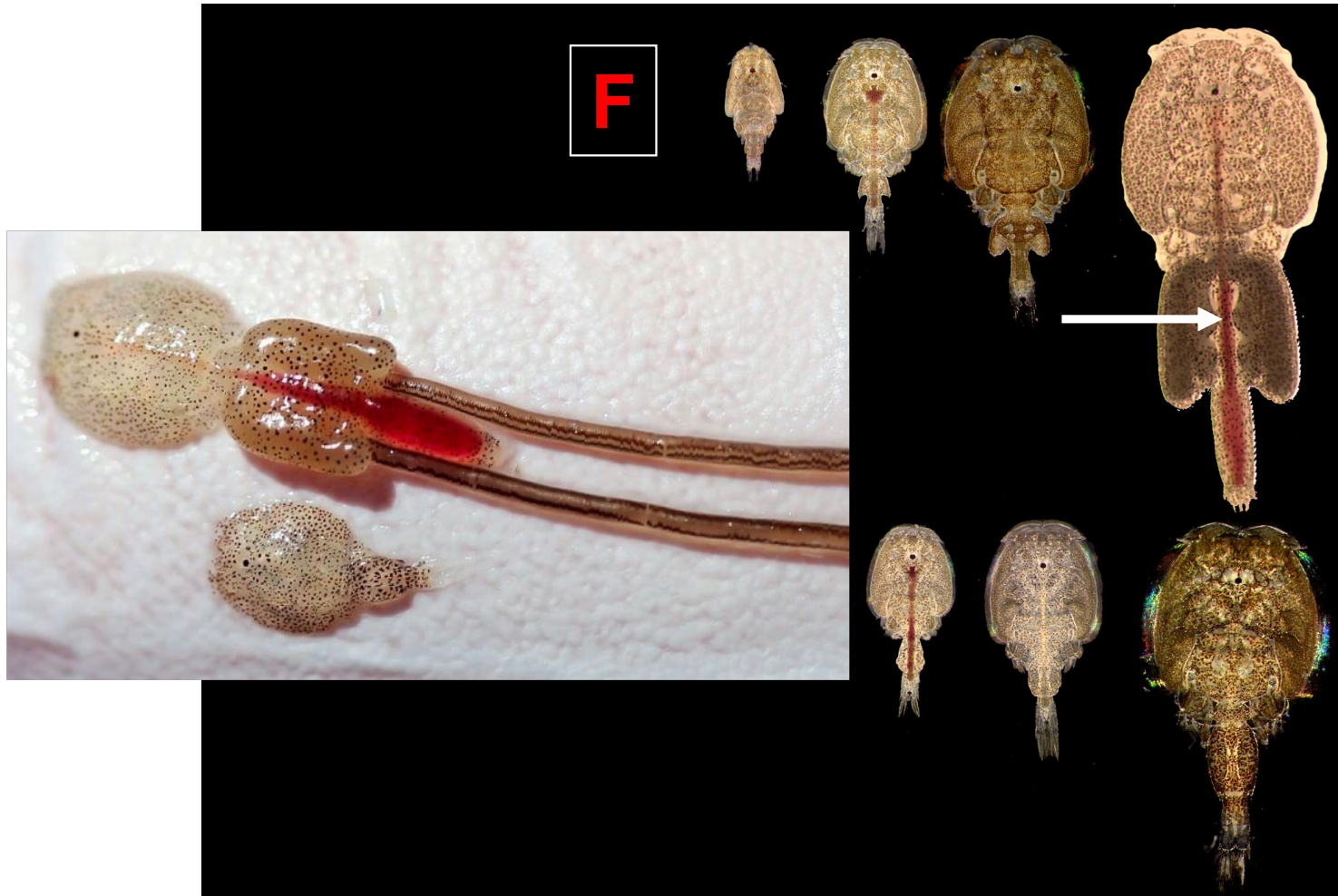


Summarized – a concealed antigen strategy

Boophilus microplus

- Exposed and hidden antigens have been tested
- The concept that has worked best is the “hidden antigen” – meaning the antigen is originally “hidden” for the immune system (not seen)
- The approach is to make it “visible” to the immune system
- The approach has been to use a mid-gut membrane-bound protein of the cattle tick



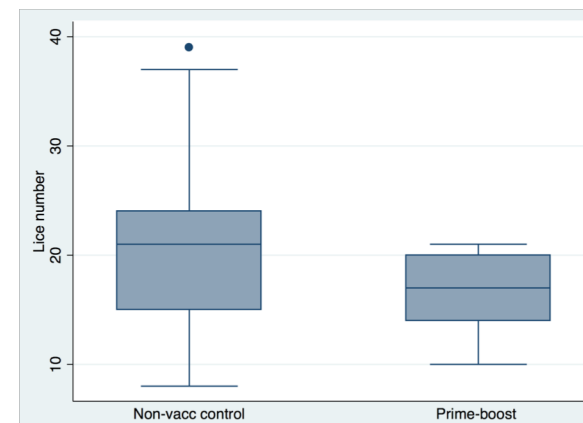
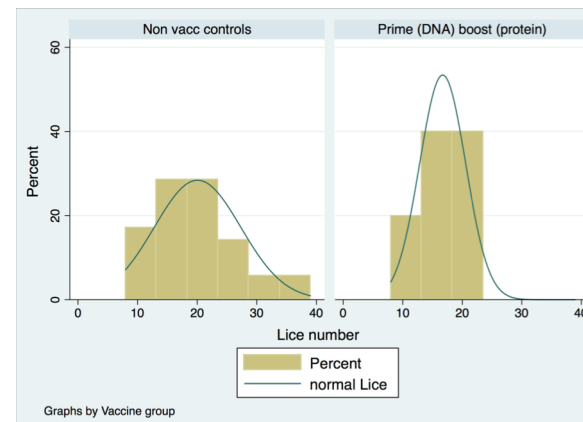


What has been achieved so far using the concealed antigen approach?

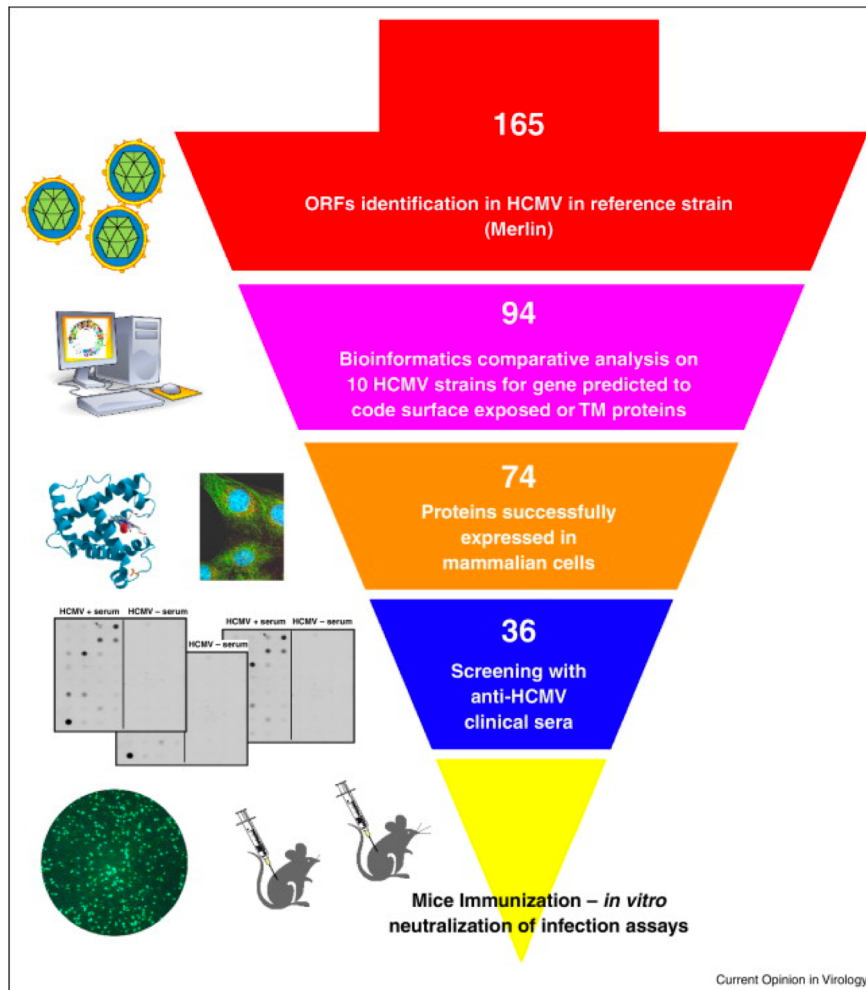
- Very little
 - Carpio et al. 2011 best results so far – RPS of 57%
 - Results have been difficult to replicate/reproduce
- Is the concealed antigen strategy a viable approach?
 - Blood meal is taken too late for antibodies to have an effect on viability and maturation?
 - Digestion in lice different from other ectoparasites?
 - Redundancy when it comes to digestive enzymes?

Vaccination modalities

- Vaccination modalities
 - Adjuvanted vaccines
 - AIO₄
 - Water in oil emulsions
 - DNA vaccines
 - Combination of DNA and subunit/protein
 - Live vaccines
 - Not tested (so far)



19% reduction

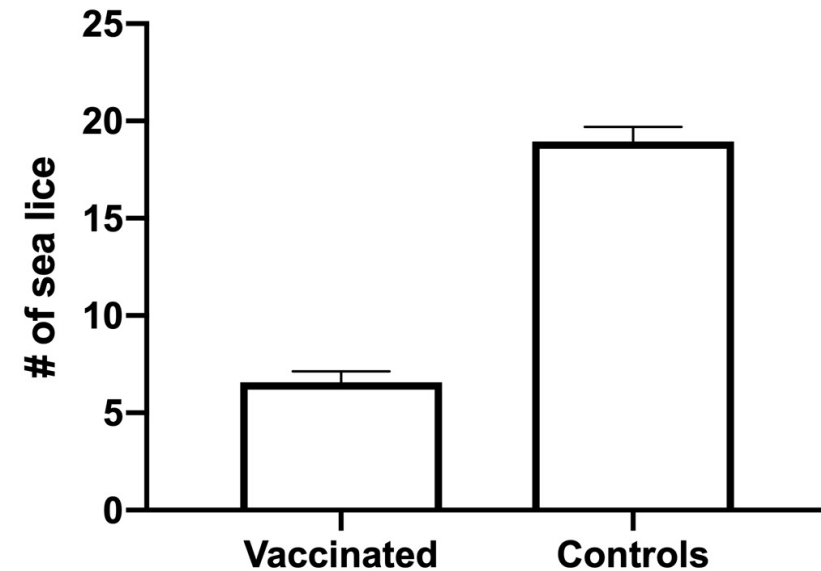


- Reverse vaccinology – is it a viable strategy for identifying candidate antigens in lice?
- Challenge
 - Very high number of ORFs (proteins)
 - Which to choose?
- Selection through immunization of salmon?
 - Reverse selection?

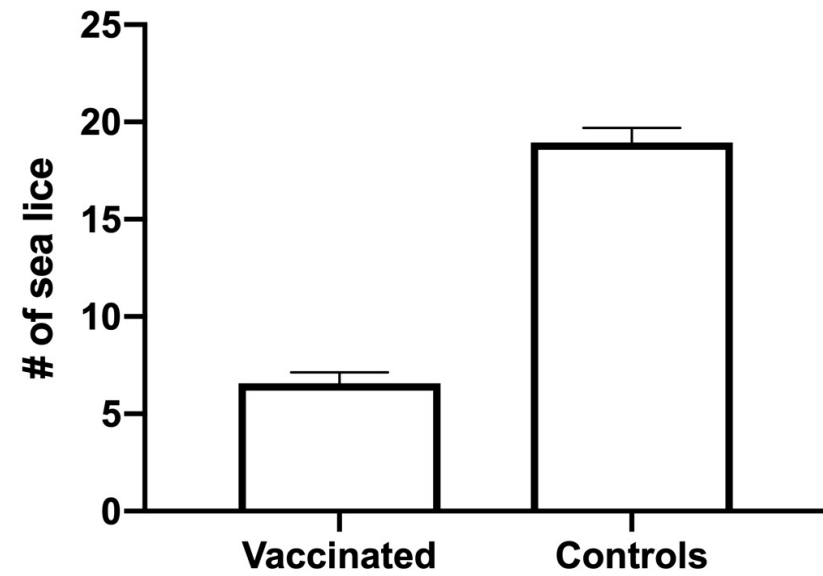
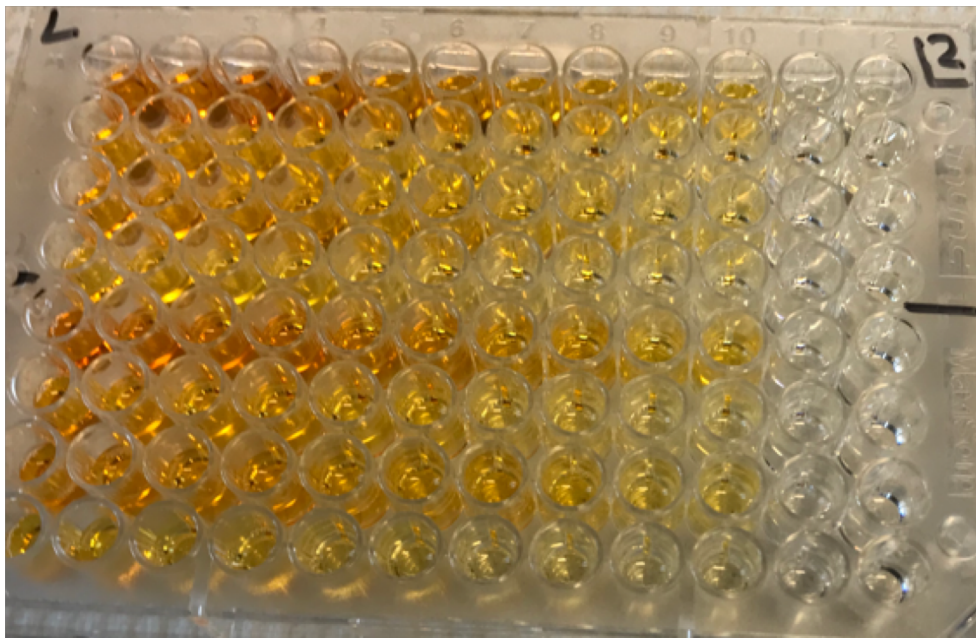


Approach

- Lice of different development stages
- Formulation that elicits strong humoral antibody responses
- Preferably a protective response
- Experimental challenge
 - 30 copepodids/fish
 - Counting of total # of lice at 28 days



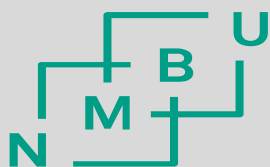
Learn from the host





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