In search for a new vaccine against the salmon louse (*Lepeophtheirus salmonis*)

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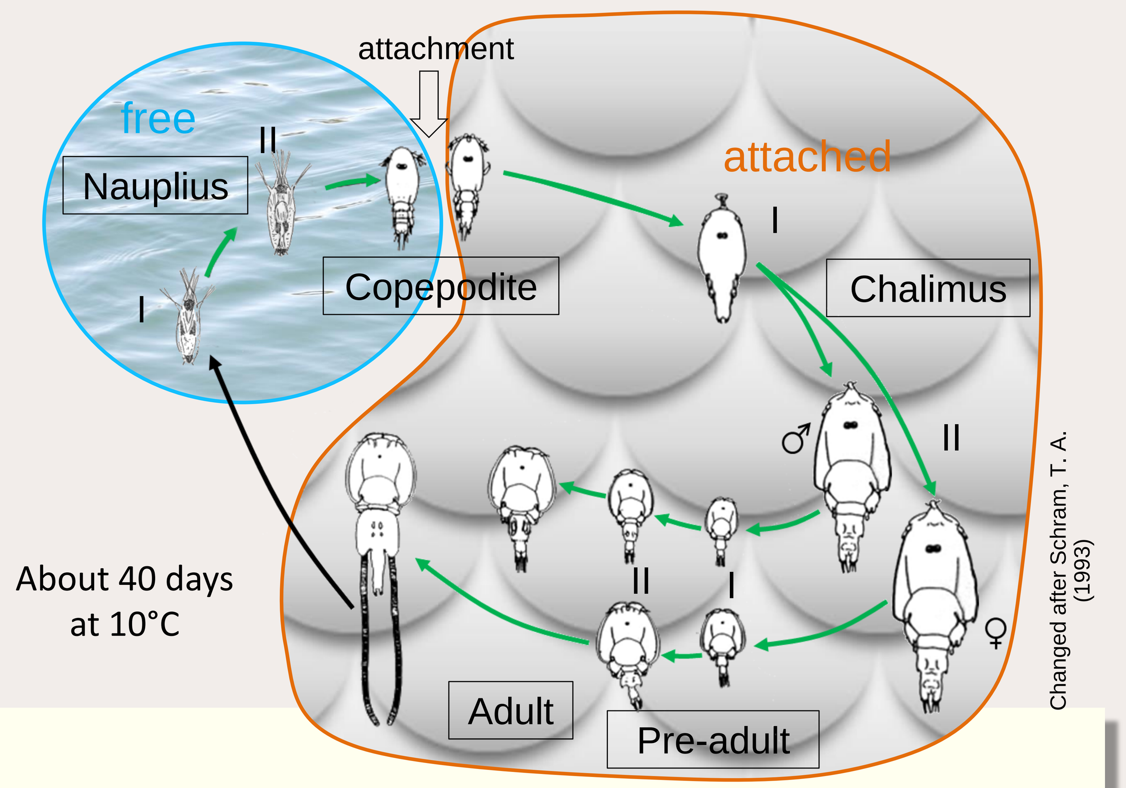
Atlantic Salmon infected with salmon lice


Adult female *L. salmonis*

Lepeophtheirus salmonis

The salmon louse is an ectoparasitic copepod, feeding on salmonid fish. Its life cycle is characterized by a high reproduction rate required for the low density of natural occurring hosts. However, the introduction of fish farming in the northern hemisphere with high densities of Atlantic salmon standing in coastal regions all year round has changed the host availability leading to an increased number of reproductive lice making *L. salmonis* a threat for wild salmon and sea trout and a major problem to the fish farming industry.

Live cycle of *L.salmonis*

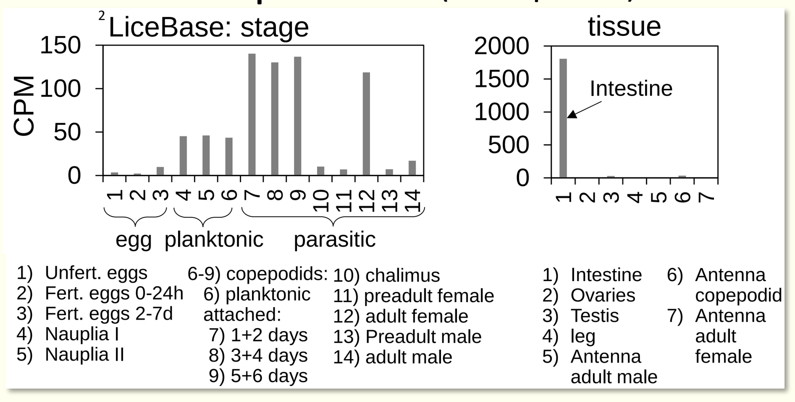


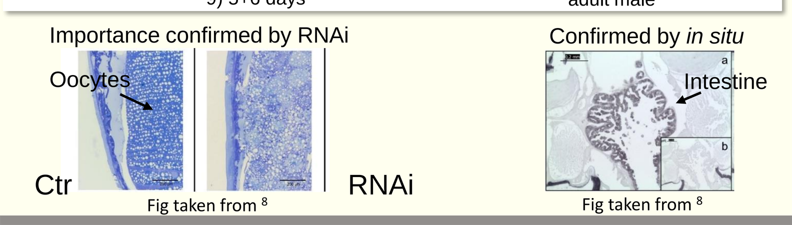
Introduction

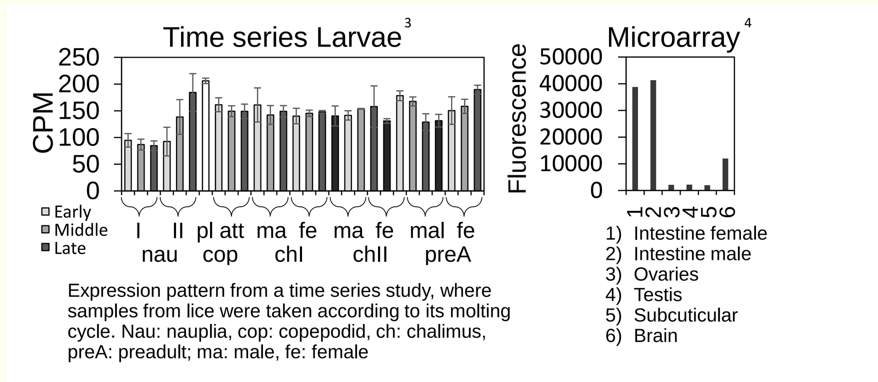
Today both chemical and mechanical delousing methods against the salmon louse are used, which resulted in both resistance against common delousing agents as well as shows negative impact on the environment and fish welfare. The demand for the development of new solutions is high. Use of fish vaccines against bacteria and virus diseases has lead to strong reduction of medicine use in aquaculture. Also a vaccine against the ectoparasitic salmon louse would be the best preventive measure to combat the parasite. However, to develop vaccines against parasites[1], especially ectoparasites is challenging. Until now there is only one commercial available vaccine against an metazoan ectoparasite.

Gene expression

- ❑ Gene expression (Example #1)







Genes were chosen according to expression pattern:

- In the intestine
- Expressed in parasitic lice

#1 Ferritin[8]
#2 Scavanger receptor[9]
#3 mixture dry protein
#4 Chitin synthase[10]
#5 V-type proton ATPase subunit
#6-8 Sodium/potassium-transporting ATPase subunit

Identification of suitable antigenic targets

Problem

Generally demanding to develop vaccines against ectoparasites

- ❑ Limited **contact area** between parasite and the immune system of its host compared to bacteria, virus or enteroparasites
- ❑ Mechanism for **suppression of hosts immune defence** (e.g. antigenic variation; secreted bioactive molecules counteracting the host's haemostatic, inflammatory and immune mechanisms) have to be bypassed

Requirement

- ❑ associated with some **vital function** of the louse
  - ❑ Antilice effects expressed as lice or egg/offspring mortality, decreased engorgement or egg string length, or inhibition of moulting
- ❑ Type of antigen:
  - ❑ **Exposed antigens** that are secreted in saliva during attachment and feeding on a host (proteins or peptides synthesized in the salivary glands, taken up at feeding site by host dendritic cells)
  - ❑ **Concealed antigens** that are normally hidden from host immune mechanisms (encounters immunoglobulins, typically found on the gut wall and interact with specific immunoglobulins taken up in the blood meal)
- ❑ Has to **trigger the host immune defence**

Approaches

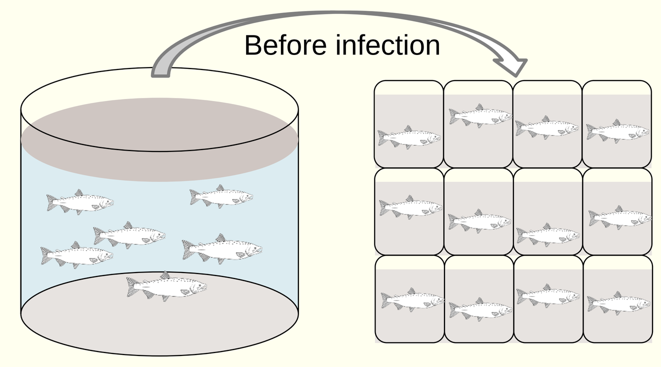
Investigating the site of expression

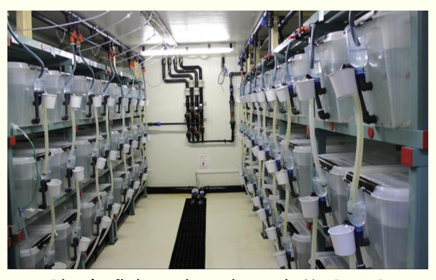
- ❑ Gene expression data as source for candidate choice (LiceBase[2], earlier RNA seq- and microarray experiments, e.g. time series[3], microarray tissue[4], microarray maturation and egg production[5])
- ❑ Confirmation with in situ

Importance

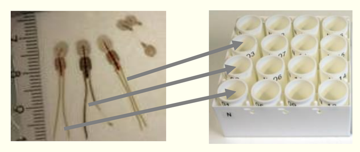
- ❑ RNA interference studies[6,7]
  - ❑ Gene knock-down of target gene and evaluation of fitness of the louse

Experimental set-up




Single fish tanks: LiceLab [11], SLRC


Hatching board: LiceLab, SLRC

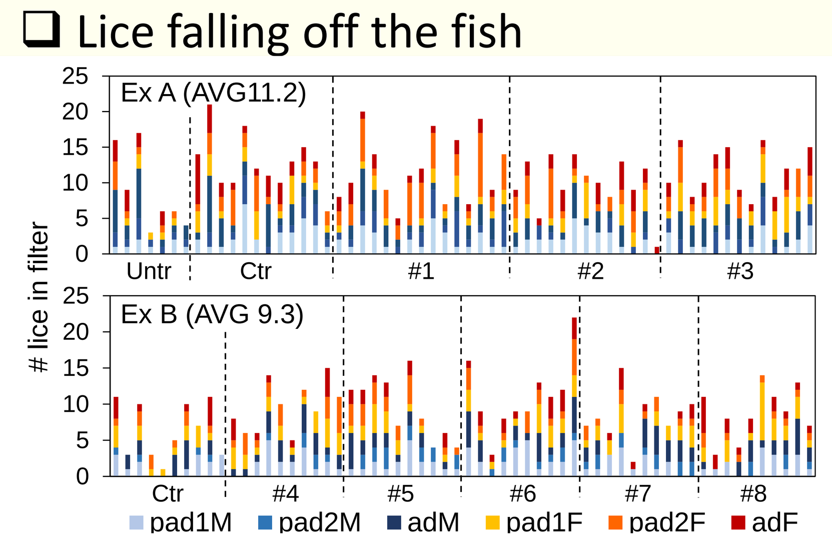


- ❑ Vaccination and smoltification in common tank (individually marked fish), infection trial in single fish tanks
- ❑ Evaluation of reproduction
  - ❑ Length measurement, hatching success

Results

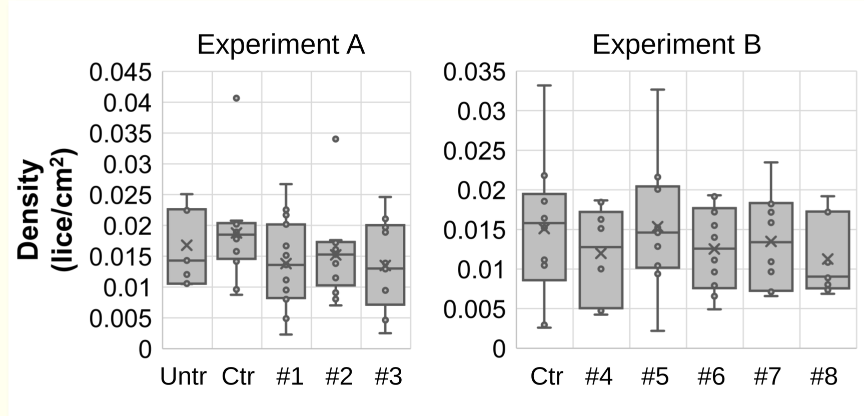
Lice numbers

- ❑ Lice falling off the fish



- ➢ No significant differences between groups in the amount of lice falling from the fish

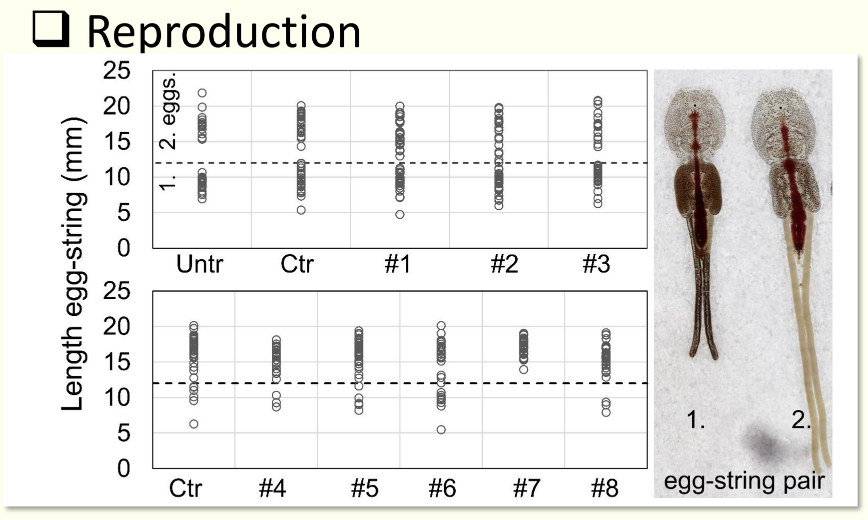
- ❑ Density of lice at sampling



- ➢ No reduction could be seen, except a minor effect for test vaccine #2 against control (but not untreated). High variation in lice numbers lead to low statistical power.

Other lice parameters

- ❑ Reproduction

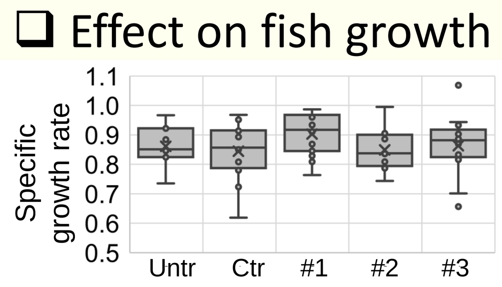


- ❑ Engorgement

- ➢ No significant difference between groups in:
  - ➢ Egg string length
  - ➢ Hatching success
  - ➢ Engorgement of blood

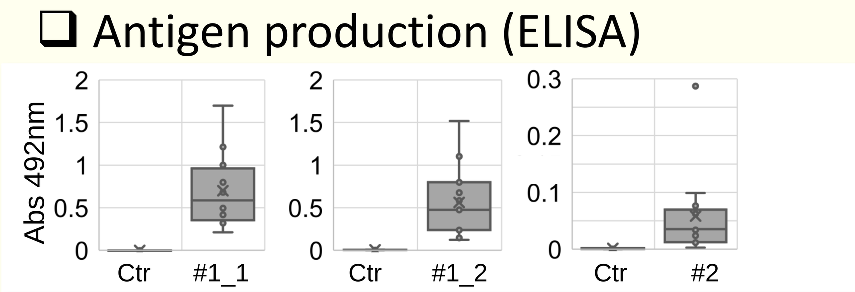
Fish parameter

- ❑ Effect on fish growth



- ➢ The vaccine did not have an effect on fish growth

- ❑ Antigen production (ELISA)



Conclusions

- ❑ Targets were chosen which fulfilled the ad hoc requirements.
- ❑ Test vaccines were investigated in vaccine trials.
- ❑ However, none of the targets showed the desired effect on lice survival or reproduction of the salmon louse under given circumstances.
- ❑ High variability in lice numbers lead to low power of the experimental set-up.

ACKNOWLEDGEMENTS

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Sea Lice Research Centre

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