**Norwegian University of Life Sciences**
Faculty of Veterinary Medicine and Biosciences
Centre for Epidemiology and Biostatistics

# Final report:

# Validating the sea lice reproduction model

# (FHF project # 900901)

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# Sammendrag

Hovedmålet med studien var å validere en reproduksjonsmodell for lakselus, *Lepeophtheirus salmonis,* ved å undersøke om vi fant en sammenheng mellom klekking og abundans voksne hunnlus i merden. For å oppnå dette samlet vi inn lus fra lokaliteter som ikke hadde blitt kjemisk behandlet mot lakselus det samme året og som hadde lave lusenivåer. Totalt ble 319 voksne hunnlus med eggstrenger samlet inn fra 2743 fisk fra 53 merder på 9 lokaliteter. Totalt klekket ca. 55 % av eggstrengene. Ingen assosiasjon mellom klekking og abundans av voksne hunnlus med eggstrenger i merd ble påvist, vi fant heller ikke noen variasjon i klekkerate mellom lokaliteter og merder. Den lave klekkeraten underbygger ikke modellen presentert av Heuch og Mo (2001), hvor det antas at alle hunnlusene gjennomgår en vellykket reproduksjon. Det ble funnet at fargen på eggstrengene kunne predikere klekking av eggene. Det lave antallet lokaliteter som ble prøvetatt vil påvirke utfallet av en slik studie da styrken på analysene blir lav.

Et sidefunn i studien var den store variasjonen i lusetall mellom merdene på ett og samme anlegg. Mer enn 65 % av variasjonen i abundans voksne hunnlus var på merdnivå og en så stor andel kan være av betydning når man utarbeider kontrollstrategier.

Vi foreslår videre studier med et større prøvemateriale for å kunne bedømme om det faktisk er en sammenheng mellom lusetall og klekkerate, og at andre faktorer som potensielt kan påvirke klekkesuksess også blir undersøkt i feltdata.

# Summary

This study aimed to validate a salmon louse, *Lepeophtheirus salmonis,* reproduction model by investigating a potential relationship between hatching success and adult female lice abundance. To achieve this goal, lice were collected from sites which had not been chemically deloused within the same production cycle and with low lice levels. A total of 319 gravid lice were collected from 9 sites, sampling from a total of 53 pens and 2743 fish. Overall approximately 55% of the egg strings hatched. No association between hatching success and adult female louse abundance was found; and no significant difference was observed when comparing variation in hatching rates between pens and farms. The overall low hatchability does not substantiate the model presented by Heuch and Mo (2001) which assumes that all adult females successfully reproduce.

It was found that using egg string colour was a viable method to predict hatching. The low number of sampled pens may have reduced the power of analysis of the salmon lice model.

Additionally we found a large variation in the abundance of adult female lice between pens at the same farm. More than 65% of the variation was at pen level, with rest at the farm level, and such a large proportion may be important when designing intervention plans.

We suggest that further studies should be undertaken, using a larger study material to be able to evaluate whether there actually is an association between hatching and lice abundance, such a study should also include analysis of other factors that potentially may affect hatchability in the field.

## Application of study

The study was expected to give results that can be applied in practical control of sea lice. The overall low hatching of lice eggs before the first treatment is an important finding that should be confirmed and included in models over sea lice population growth. The high variation of lice abundance between pens before the first lice treatment may be used as an incentive to alter intervention tactics from treating all the pens simultaneously to early cage-wise intervention to minimize internal infection between the pens.

A definite association between the lice abundance and hatching would have given a good reason for the lag phase seen at the beginning of the sea water period in many farms. This association could neither be confirmed nor refuted in this study.

## Organizing the work

- The project group consisted of:
    - Melanie Andrews – project leader
    - Arnfinn Aunsmo
    - Marit Stormoen
- Steering group consisted of:
    - Per Gunnar Kvenseth – Villa Arctic
    - Ragnhild Aukan – Lerøy Midt
    - Bjarne Johansen – Nordlaks Oppdrett
    - Kjell Maroni - Observer / Contact FHF
- FHF responsible has been Kjell Maroni

# Introduction

Salmon lice remain one of the main challenges facing the Norwegian aquaculture industry, with numerous studies conducted to improve overall understanding of the lice biology. One study was recently conducted by the Norwegian School of Veterinary Science (NVH) resulting in a sea lice reproduction model (Stormoen *et al.* 2013). This model indicated that at low lice levels there are a progressively smaller proportion of the female lice population available for fertilization and reproduction. This contrasts with the generally accepted view that a linear relationship exists between the number of mature sea lice and reproduction (Heuch and Mo, 2001). This new model contrasts to such an extent from established views that further work should be conducted in order to validate the model when using field data. If applicable it will improve our understanding of the salmon lice biology and contribute to understanding infection dynamics within and between farms.

Several publications have focused fully or partially on the Ally effect in salmon lice (*Lepeophtheirus salmonis*) (Krkosek *et al*. 2012; Stormoen *et al.* 2013). The Ally effect describes the lag in population growth due to a lack of mates at low abundance levels for organisms with a sexual reproduction. Understanding the hatching success rate of *L. salmonis* and other parasitic copepods is important when designing treatment and control strategies. Such knowledge would greatly improve the efficacy when conducting treatments, however very little has been done to address this question.

Successful mating and mate finding are important factors to consider when designing control methods, such behaviour has been observed and described in a number of caligid species. In particular males of *L. salmonis* have been observed to have mate preferences

with virgin adult females being selected over preadult II females, followed by preadult I females (Hull *et al.* 1998; Pert *et al.* 1996). Male *L. salmonis* have been observed displaying mate searching and testing behaviour soon after mating thus indicating that males may mate with multiple females (Ritchie et al. 1996). This is most likely aided by the relatively strong swimming ability of the male salmon louse which was found to be more mobile than the adult female (Hull *et al.* 1998). The ability to move between hosts may greatly increase the mating success rates in areas with low louse levels (Ritchie, 1997). Once mated female caligids have been observed to produce a number of generations of egg strings, for example both *L. salmonis* and *C. rogercresseyi* females have been observed to produce 11 generations within 74 days after a single mating (Heuch *et al.* 2000; Bravo, 2010). This resulted in significant capability to increase the number of infectious copepodids when the environmental conditions are suitable.

Environmental conditions, particularly water temperature and salinity, play a major role in caligid survival and hatching success rate. Observations of the effect of water temperatures on egg developmental times describes that at low temperatures of 2°C it took the eggs 45 days to hatch, whilst those held at 10°C took 8 days (Boxaspen and Næss, 2000). The authors surmised that salmon lice populations have adapted to the differing seasonal environmental conditions, this would in turn affect the control measures conducted on site. The combination of favourable environmental conditions and mating success would ensure the release of a large number of copepodids into the surrounding environment. Much speculation has been made about the transfer of copepodids between cultured and wild Atlantic salmon in the vicinity of farms, with suggestions that the copepodids attach to a salmon soon after they enter the sea (Heuch, 1995; Penston and Davies, 2009). To

determine whether this was the case a number of studies were conducted to determine the effect that salinity may have on the infective stage (Heuch, 1995; Bricknell et al., 2006). These studies found that copepodids experienced reduced survival at salinities below 29 ppt and actively avoided areas of salinity below 27 ppt, suggesting that copepodids have the ability to congregate in the more favourable areas.

A better understanding of the hatching success rate of *L. salmonis* would improve the control and treatment in farming operations by providing better knowledge of when and how to treat the farm site. This in turn would optimize the number of treatments per site, which in turn would reduce overall production costs. This project focused on determining the hatching success rate of *L. salmonis* from areas of varying lice levels.

# Project elements and aims

The aim of the study was to validate the reproduction model described in Stormoen et al. (2013) by investigating the possible association between hatching and lice levels. Further we sought to investigate the possibility of assessing the fertilization of sea lice by visual examination of the presence of a seminal pack. The project was initially designed to have two work packages, *WP1* to validate the reproduction model, and *WP2* to conduct sensitivity analysis of sea cage fertility assessments. However, as the project progressed we combined the tasks in order to improve efficiency and replicability. Therefore we present combined materials and methods, results and discussion.

# Materials and Methods

## *Field work*

During autumn 2013 nine Norwegian seawater farms with Atlantic salmon (*Salmo salar*) were visited. To be included in the study the farms needed to have low lice levels and not have been chemically deloused within the same production cycle. At each farm we aimed to collect 50 adult female lice with egg-strings, 10 from each of five pens. All farms had the previous week been below the treatment threshold for delousing (i.e. below 0.5 adult females at farm level). Low lice levels throughout central Norway during late summer/early autumn made it difficult to achieve the aim of collecting as many lice as needed in one day's operation. To finish within a reasonable time we made a cut-off at examining 100 fish from one pen. A total of 53 pens and 2743 fish were examined from the nine farms (Figure 1). A total of 319 adult female lice with egg-strings were collected from 38 pens. The remaining 15 pens had so low lice levels it wasn't possible to find enough adult females with egg strings to include them in the study. The lice were visually inspected on site for the presence or absence of a seminal pack (Figure 2) before being placed individually into a 100 ml container with seawater and transported back to the laboratory.

Figure 1. Map of Norway with the 9 sampled sites marked by a blue dot.

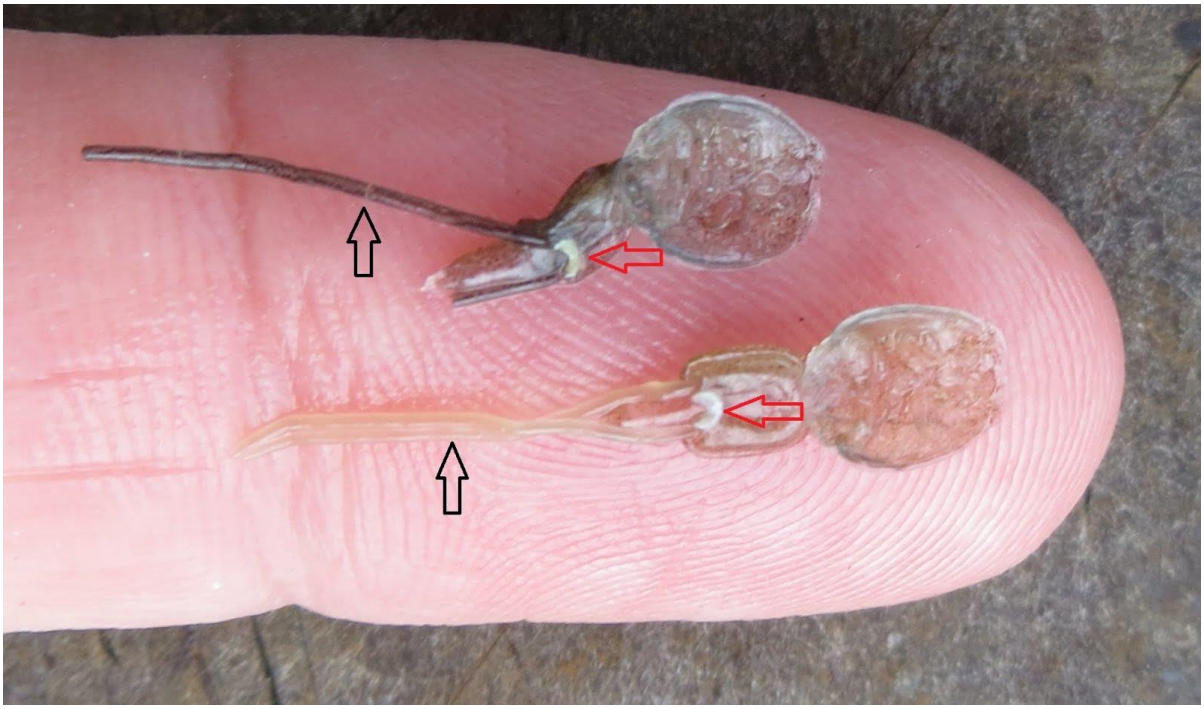

Figure 2. Adult female L. salmonis with visible seminal packs (red arrows) and marked differences in egg string colours (black arrows).

## *Experimental setup*

Upon arrival at the laboratory the lice were individually placed in a petri dish where the egg strings were gently removed using fine forceps. Using a macro lens the ventral side of the louse was photographed to determine whether a seminal pack was present or absent.

The colour of the egg strings was recorded on arrival, with a gradient ranging from white (less developed) to dark brown (near hatching). Each egg-string was placed in a 10x3cm hard plastic tube with mesh floor and a small mesh window to ensure water circulation. The experimental setup is illustrated in Figure 3 and 4. The tubes were placed in a grid in a 15L

tank with artificial seawater (Red Sea© Salt) with a salinity of 33.5 ppt, mixed according to the instructions from the manufacturer. Two containers were connected, and to a circulation system and a cooler, resulting in a 30L system. The pump had a capacity of pumping approximately 0.7l per second. Water temperature was set to 10°C but due to the small volume of water in each tank the water temperature was affected by the room temperature and thus fluctuated by plus-minus one degree.

We had the capacity of hatching lice from two farms in parallel in two identical setups. The eggs were checked for hatching daily for 10 days and tubes with nauplii were removed from the setup.

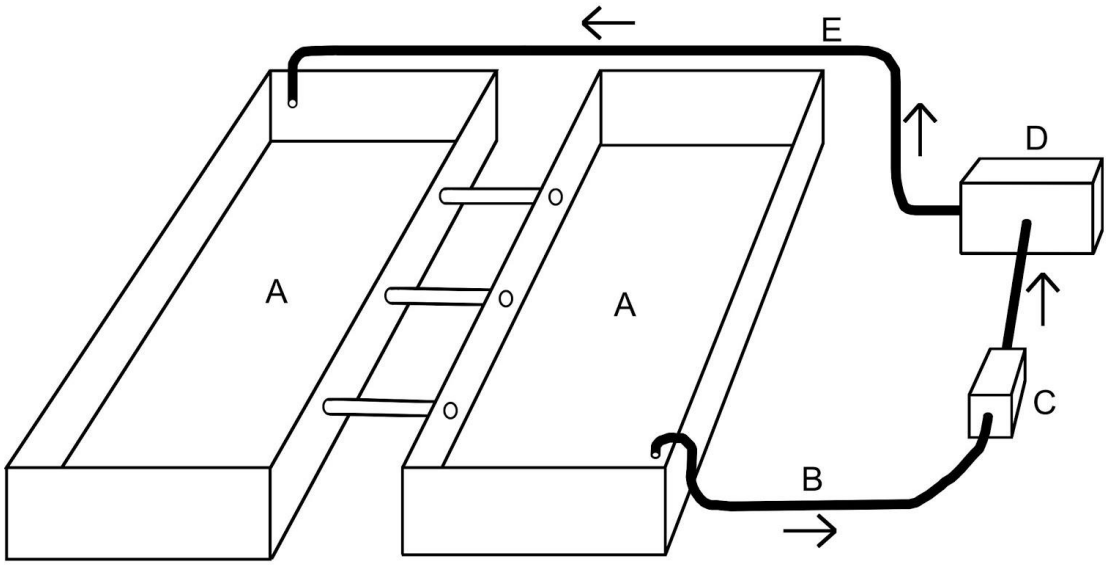


Figure 3. Experimental *L. salmonis* hatching setup schematic. A are the two 15l tanks, B is outflow tube, C is the circulation pump, D is the cooler and E is the inflow tube.

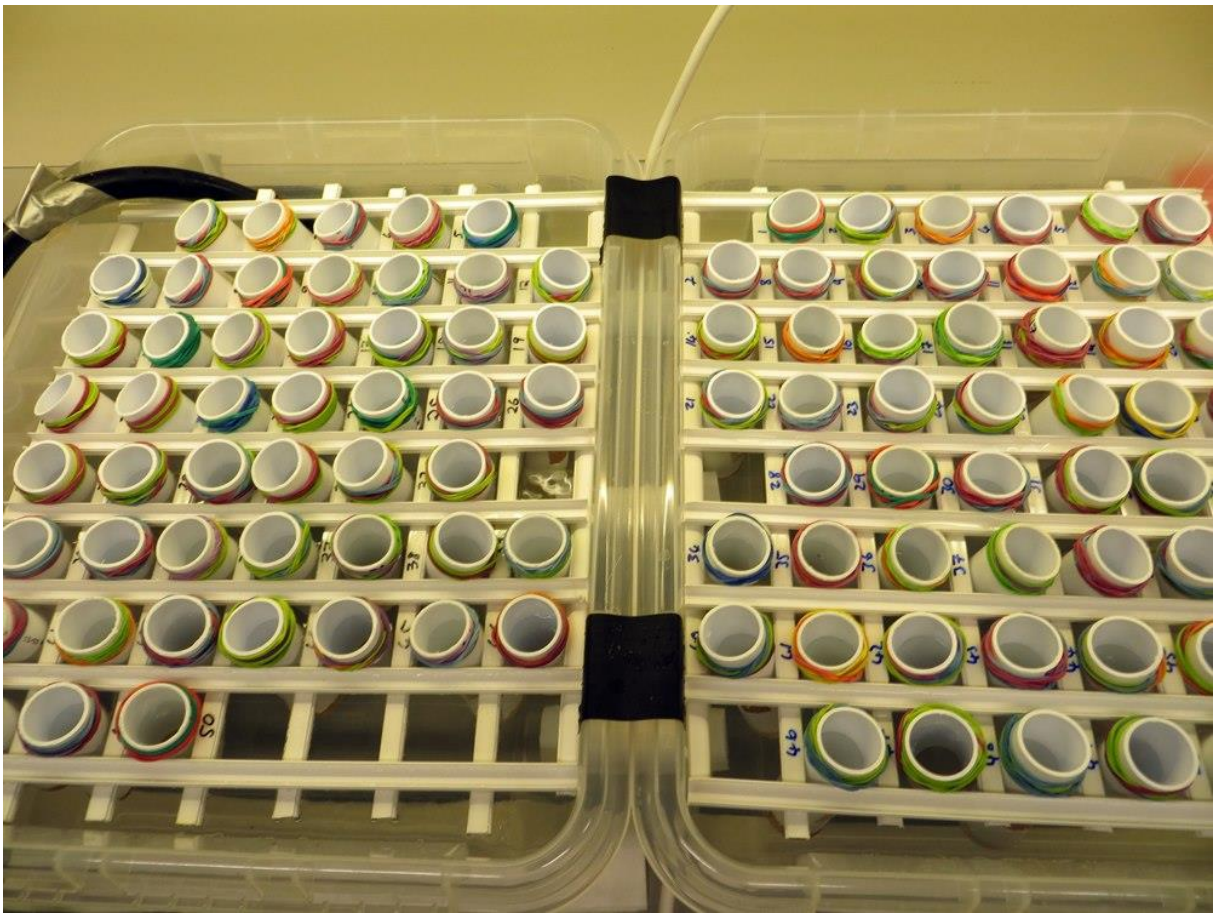

Figure 4. Experimental *L. salmonis* hatching setup picture

## *Data Management*

After sampling a follow up collection of data from the farms was completed, this included pen level lice counts prior to sampling, water temperature and cleanerfish usage. The historical data was delivered as excel sheets and was imported to Stata 12 and merged with the experimental data to form one dataset.

## *Statistical analysis*

All statistical analysis was performed in Stata 12. First descriptive analysis of the data was performed. To investigate the factors associated with hatching a logistic regression using hatching yes/no as an outcome and examining pen, adult female lice, the presence of wrasse in the pen (yes/no), percentage of wrasse in the pen, registered sea water temperature, “pairs” of adult female lice and adult males or mobiles on the same fish, egg string colour at collection and lab fertility testing as predictors. The model was a multilevel logistic regression model with clustering at farm level.

There were a lot of missing data in both the temperature and percentage wrasse variables. A new variable for both temperature and percentage wrasse was generated where we sought to use the value that originated as close to the sampling date as possible. If we had data from the sampling week it was used, if this was missing, we used the week before sampling and so on. There were no values used older than two weeks before sampling.

First an univariable analysis was done, significant factors and factors regarded as theoretically important were included in the multivariable analysis.

A multilevel Poisson model with counted adult female lice on fish level was fitted with clustering on both pen and farm level to investigate at which level the main variation in lice counts were found.

The lab fertilization test was examined for sensitivity and specificity using tools found on the AusVet© website (AusVet Animal Helath Sevices).

# Results

The summary statistics revealed a large variation in the abundance of adult female lice between pens at the same farm (Table 1). Only two farms had actually exceeded 0.5 adult female lice at the week of sampling, while 34.5% of the pens exceeded 0.5 adult female lice in average. To verify this finding a multilevel Poisson regression looking at the adult female lice counts on fish level, clustered at pen and farm, was constructed. It showed that 66.2% of the variation was at pen level.

| Farm no. | No. pens sampled, no. of examined pens in bracket | Farm average adult female lice | Range pen average adult female lice | Percent hatch at farm level, pen level range in bracket |
|---|---|---|---|---|
| 1 | 5 (6) | 0.27 | 0.08 – 0.59 | 52 (30 – 70) |
| 2 | 6 (6) | 1 | 0.09 – 1.47 | 44 (0 – 70) |
| 3 | 4 (8) | 0.20 | 0 – 1.17 | 30 (20 – 40) |
| 4 | 5 (5) | 0.66 | 0.03 – 3.23 | 68 (50 – 100) |
| 5 | 5 (8) | 0.46 | 0 – 2.38 | 70 ( 60 – 80) |
| 6 | 3 (6) | 0.31 | 0 – 1.23 | 71 (50 – 80) |
| 7 | 2 (5) | 0.04 | 0 – 0.11 | 82 (72.7 – 100) |
| 8 | 3 (6) | 0.18 | 0.05 – 0.34 | 30 (0 – 100) |
| 9 | 5 (6) | 0.46 | 0.20 – 0.80 | 47 (16.6 – 70) |
| Sum | 38 (53) | | 0 – 3.23 | 54.2 |

Table 1. Summary statistics of farms and pens examined, with adult female abundance and percentage hatching both at farm and pen level. Adult female lice could not be collected from all 53 pens due to too low abundance levels.

Overall of the 319 adult females with eggstrings, 54,2% hatched on either side (173 strings), there were a good consistency between the two egg strings and for approximately 79% both hatched or didn't hatch.

An association between hatching and the abundance of adult female lice could not be found using a logistic regression model with clustering at pen and farm level. When plotting the percent hatching against the adult female abundance level one can see that some farms follow the trend of low hatching at low abundance level, but no convincing overall trend could be seen when adding a trend curve (Figure 5). A similar analysis based on adult males could not be done as the counts of adult males were deemed of too poor quality.

No difference could be found between pen and farm level when it came to variation in hatching, both levels explained approximately 8% of the variation (ICC). When performing an univariable analysis only examination for fertilization in the lab and egg colour at sampling came out as significant predictors for hatching. Of these the egg colour at sampling explained 34% of the variation (ICC at pen level), meaning that egg colour may be an important factor when wanting to predict whether the eggs will hatch or not. Other factors tested were adult female abundance, sea water temperature, presence of wrasse and presence of possible pairs on the fish. Results of the multilevel, univariable logistic regression model are summarized in table 2.

| | OR | P-value | ICC – Farm level | ICC – Pen level |
|---|---|---|---|---|
| Null Model | 1.20 | 0.391 | 0.0804 | 0.0808 |
| Lab fertilization test | 2.19 | 0.002 | 0.0764 | 0.0874 |
| Adult female lice | 1.00 | 0.978 | 0.0802 | 0.0806 |
| Temperature | 0.86 | 0.311 | 0.0774 | 0.0774 |
| Wrasse | 1.08 | 0.183 | 0.0729 | 0.0765 |
| Egg colour | 2.28 | <0.001 | 0.3070 | 0.3421 |
| Pair | 1.12 | 0.643 | 0.0820 | 0.0825 |

Table 2. Results of the univariable logistic model. Or = Odds Ratio, ICC= Intracluster correlation coefficient. In the univariable analysis each variables effect on hatching was tested, such an analysis does not take into consideration the effect the different variables may have on each other.

A multivariable model was fitted using lab fertilization test, egg colour, adult female lice, pair, temperature and wrasse as predictors. The effect of the lab testing was no longer significant while the colour of the egg strings continued being significant. No other predictors could be found to significantly affect hatching.

The lab fertilization test was examined for sensitivity and specificity using hatching as the gold standard, thereby assuming that all fertilized egg strings hatched. The lab test had a 67% sensitivity and a 51% specificity on a 95% confidence level.

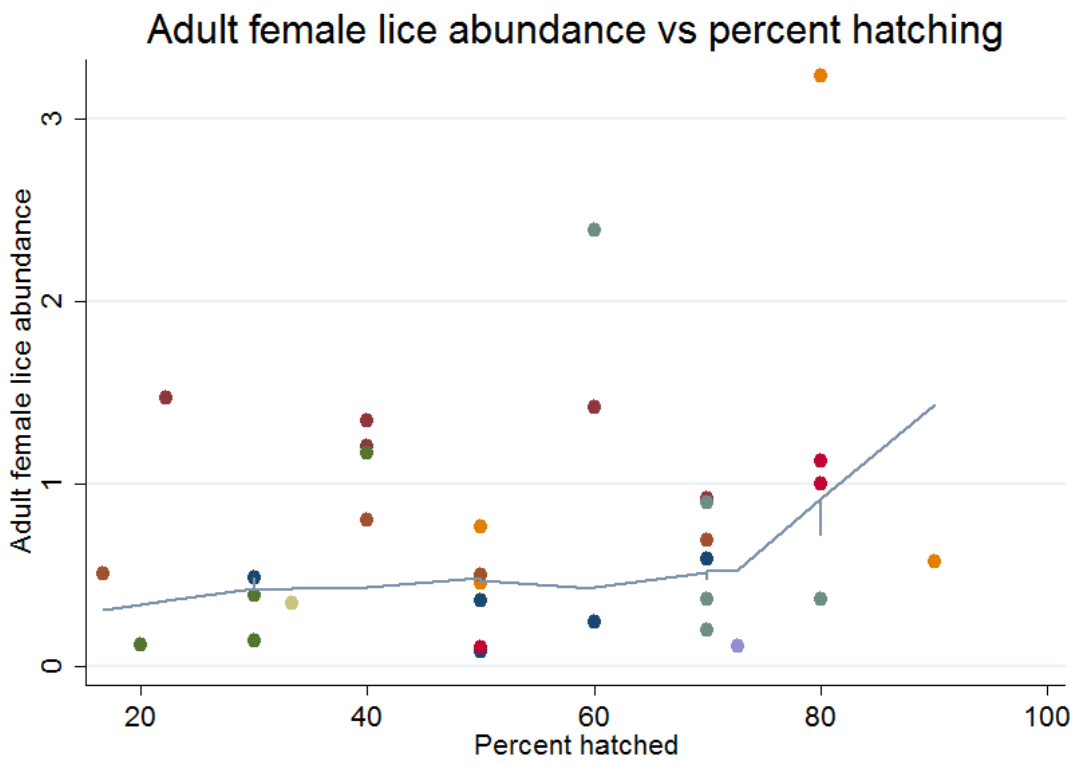


Figure 5. Scatter plot of percent hatched (x-axis) and adult female abundance (y-axis) from each pen. Each farm has a unique colour and each dot represents one pen. The lowest curve (blue line) shows the trend in the data on a pen level.

# Project summary and discussion

The aim of the study was foremost to attempt finding an association between hatching and adult female lice abundance in a pen. No such association could be found in the material examined here. An overall hatching of approximately 55% of the eggs was found. There was a good consistency between hatching of the left and right egg string, suggesting that the set up was not the cause of the overall relatively low hatchability. Furthermore, lab examination for seminal pack as well as egg string colour was found to significantly predict

whether the egg string would hatch or not. The low hatchability can therefore be assumed to reflect the situation out in the farms. This study could however not clarify any reasons for the low hatchability.

The low hatching rate does not substantiate the model presented by Heuch and Mo (2001), where it is assumed that all adult females reproduce successfully. The reproduction of sea lice in a farm setting with low abundance is likely to differ from that observed in tank trials with a large amount of lice available at all times and optimal environmental factors for the lice. As egg production is highly significant when estimating the total infection pressure generated by a pen or a farm it is important to further allude to this phenomenon.

The study had a relatively low power due to too few sampled pens, a real association between adult female lice abundance and hatching may exist, but could not be proven. Other possible reasons for the relatively low hatching rate, such as salinity, temperature and mate finding abilities should be examined in the future. It is known that wrasse target larger lice when feeding (Deady et al., 1995), this may further skew the distribution of stages in a farm setting.

An additional finding in the study was the high variation in lice numbers between pens at the same farm site. As described, more than 65% of the variation in adult pen female abundance was at pen level. Such a large difference between pens may be important when designing the intervention plans for a site as the local infection pressure generated from pens with a high adult female abundance may cause the abundance in the remaining pens to increase faster than necessary. It should be noted that as all these samples were taken

before the first medical delousing of a production cycle it is probable that the variation between the pens will be less after all pens are treated simultaneously. Wrasse is known to target the largest lice in a population, further the efficiency of cleaner fish is known to be altered by the amount of soiling on the net pens, the size of the salmon, water temperature and the amount of cleaner fish in the pen (Costello, 1996; Kvenseth, 1996; Deady et al., 1995; Groner et al., 2013). These factors may vary somewhat between pens though we would expect the largest variation being between sites. The mortality level of cleaner fish is known to be high and very variable, if the actual amount of cleaner fish varies greatly between pens at one farm it may exacerbate the variation in lice abundance.

# Further research

As hatchability is a vital factor when estimating the infection pressure generated by a farm, a larger study investigating hatching at different lice abundance levels and simultaneously investigating other factors that may influence the hatching from many different farms and over different seasons should be undertaken.

# Acknowledgements

We would like to thank Jostein Moulder Pettersen, Ola Brynildsrud and Dr. Ruth Cox for the invaluable help with sampling, as well as all the personnel at the farming sites for their friendly and helpful assistance. We would further like to thank Dr. Peter Alestrøm for providing us with space in his laboratory.

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