

# *VisuaLice*

## Population Interpretation of Passive Sea Lice Monitoring

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*A report to Havbruksinstituttet AS and FHF Norway*

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## Executive Summary

Regular and accurate sea lice monitoring is a vital component to any effective integrated pest management regime targeted against one of the most costly ectoparasitic pathogens associated with modern salmon aquaculture. In most regions with substantial cultured salmon production, sea lice (mainly *Lepeophtheirus salmonis* and various *Caligus* species) continue to be one of the most important fish health concerns. Even in regions where significant infestations tend not to be experienced, such as British Columbia or the far north of Norway, it is important to monitor sea lice levels to mitigate any potential negative impacts for wild salmon.

In addition to regular monitoring, the recent emergence in a number of regions of tolerance to certain chemotherapeutants has emphasised the importance of methods to obtain accurate sea lice estimates before and after treatment to properly assess the efficacy of any medication being used so as to gain early warning of tolerance issues. However, to date, this involves a manual process which is time consuming and dependent for its accuracy on the skill of the individual carrying out the count and their ability to access a range of sea pens. Crowding fish within pens to select a representative sample also imposes stress on these fish. Because of the time required only a small number of fish can be sampled. However as lice numbers have been driven down over the past decade, increasingly large samples are required to maintain the statistical reliability of any population-level estimates.

The use of underwater imaging has therefore been proposed as an automated and passive counting system which can offer the benefits of enhanced repeatability and accuracy, larger sample sizes, continuous monitoring, lower costs and lower levels of disturbance to the fish. It was proposed to develop such a system as part of the *VisuaLice* research project discussed in this report. To assess the relative value of this novel approach in comparison to traditional manual counting it was proposed that a computer model be set up to simulate various scenarios. The results from field trials and experiments would be used to parameterise such a model and therefore enable the exploration of a range of farm settings, cage configurations, pre- and post-treatment conditions, in a simulation. This provides a more efficient way to illustrate the utility of the automated passive monitoring approach, rather than setting up a whole raft of field-based experiments.

A computer model to simulate such scenarios was created by the researchers at UPEI and is discussed in the following pages. Unfortunately the field-based trials of the underwater imaging system were largely unsuccessful and so many of the key parameters required to complete the modelling activities and generate realistic scenarios and outcomes could not be fully explored. Nevertheless it is hoped that the framework constructed will be of value in the future and the researchers involved plan to publish key documentation around the modelling activities so that colleagues have the opportunity to learn from their initial explorations.

## Note on status of UPEI input into the *VisuaLice* project

Researchers at the Centre for Veterinary Research (CVER) were contracted to become involved with the Eurostars E4721 (*VisuaLice*) project in 2011. Their particular remit was to, “explore how the novel data sets generated can be best exploited. UPEI will use statistical population modelling using real world scenarios based on historical lice data from Norwegian and Scottish farms”. It was therefore expected that the novel data would be made available, “provided by the equipment trials will also be used to examine how valid comparisons can be made between lice infestation data generated by existing manual inspection and data generated by the new technology”.

Unfortunately, as is always the risk when developing new methods and equipment, there were challenges with the field trials to such an extent that it became clear than the research planned at UPEI would have to move ahead in the absence of the “novel data” expected, and UPEI created a framework to evaluate such, as and when they might become available.

The situation in mid-2011 was summarised in a note from Thorvaldur Pétursson of Vaki Aquaculture Systems who was acting as the project manager for the overall Eurostars E4721 (*VisuaLice*) project. In this he states:

"Trials in Macrihanish in November 2011 gave us further valuable information but they also showed that the attenuation of the UV wave lengths that we are using is far higher in costal seawater than we anticipated. Though, this gave us quantification of parameters that we needed to improve, we were close to the physical and practical limitations of the light source we were using... There are however still some technical challenges to be solved relating to the shaping of the light. This has, unfortunately, delayed our progress. It is not a failure and we are still on a track though we are delayed. *This of course means that we have not been able to produce the data that UPEI needs for their part of the project.* UPEI has spent time and effort in preparing the data processing and creation of the models and are waiting for us to advance the hardware development and data collection."  
(7th May, 2012. Italics mine)

As noted, this meant that our ability to fully progress the modelling and data analyses aspects of the project were compromised. Nevertheless a reasonable amount of effort had been expended and a simulation framework had been constructed to allow for the exploration of various sampling strategies. It was agreed with the sponsoring partners, Havbruksinstituttet and FHF Norway, that the delays and lack of progress in supplying novel data sets meant that the full research contract could not be completed. Instead it was agreed that 50% of the contracted work could be funded in the form of a scientific report that summarised the creation of the simulation ‘workbench’, together with some sample outputs of the types of results and scenarios that could be addressed by the approach adopted. The main report, which follows, provides a summary of this research activity and it is hoped that in the future this will form the basis of a scientific publication; one of the original goals associated with UPEI’s involvement in this project.

# Counting sea lice on Atlantic salmon farms – an individual based modelling framework to allow for the exploration of comparative approaches

## Introduction

Sea lice are naturally occurring ectoparasitic copepods that transmit directly between hosts in their planktonic larval stage and, to a much lesser degree, in the adult motile stage. They occur frequently in marine salmonid farms, the most common species in Norway and Scotland being *Lepeophtheirus salmonis*, with the generalist *Caligus elongates* occurring at lower abundances (Bjorn & Finstad 2002, Revie et al 2002, Heuch et al 2003, Lees et al 2008) . Infestations can cause significant morbidity and mortality (Costello 2009) and can be costly in terms of loss of fish, chemical treatment and are environmentally damaging when they infect wild salmon (Costello 2009, Krkošek et al 2005).

In a number of countries, including Norway, sea lice infections in farmed salmon are regulated using a maximum threshold in abundance of mobile stages. Historically, farmers in Norway were required to count sea lice on salmon at regular intervals and report the highest mean count in a month (Jansen et al 2012). However, over the past few years and in common with changing practices in other salmon-producing regions, the requirements for counting have become more rigorous with weekly counts on a larger proportion of cages now being the norm (Heuch et al 2011, Jimenez et al 2012, Kristoffersen et al 2013). Despite these increased requirements, sea lice assessment on farmed fish is still carried out manually. It is a time-consuming activity; its accuracy depends on the skill of the counter who must have physical access to the sea pens to carry out any counting exercise (Heuch et al 2011, Elmoslemany et al 2013). In addition manual counting can cause stress to the fish as they may be crowded into a small area in order to select a sample. Only relatively small sample sizes can be inspected due to limits on time. This can be particularly problematic when lice levels are low, which can be the result of limited infection pressure or highly effective treatment, as larger samples are required in order to gain reliable estimates of the lice abundance. Indeed in such circumstances prevalence may be a better and more easily estimable measure than abundance (Baillie et al 2009), but again a fairly large sample of fish is required to gain accurate prevalence estimates.

In light of these realities there is a reasonably urgent need to consider automated methods that could be used to assist with sea lice counting procedures on salmon farms. Following on from some earlier pilot work in this area a research partnership has been formed to explore the use of automated imaging systems for this task and the development of novel tools to be made available to the aquaculture industry. This collaboration has been taking place under the auspices of the Eurostars project 'VisuaLice E!4721' and involves Vaki Aquaculture Systems (Iceland), Marine Harvest (Scotland/Norway), Silsoe Livestock Systems (UK) and the University of Prince Edward Island (Canada). Ultimately the portable imaging frames being developed will support the automatic detection and counting of sea lice on salmon. The frames can be suspended at various depths in the salmon net pens so that the fish are able to swim through them. Images of the fish are collected and the data passed to a central computer which over a period of time will be able

to build up an estimate of the sea lice population. Feasibility studies were conducted in 2010 and 2011 on salmon in a tank-based setting. This work demonstrated that underwater images had the potential to detect lice on individual salmon.

Companies involved in developing the equipment plan to make it available to the aquaculture industry. Such an automatic passive system to count sea lice would facilitate enhanced repeatability and accuracy, larger sample size, continuous monitoring and a lower level of disturbance to the fish. It therefore has the potential to provide more detailed estimates of the lice population dynamics (rates of population change, short term population variations and enhanced accuracy of prevalence estimation) on which to base intervention and targeted treatment decisions. Savings should be significant to industry both in terms of reduced manpower and better targeted treatments.

A key question that must be answered relates to the likely reliability of using such a method of counting and knowing how the data generated will compare to those obtained by manual counting methods. To investigate this issue the research partners at the University of Prince Edward Island (UPEI) used data generated by a computer simulation, based on an individual based model of a salmon production cycle, to assess how various sampling techniques may influence estimates of parasite infection in farmed salmon populations.

## **Aim**

To compare the efficacy of different lice sampling strategies, by creating simulations of both manual counting and automated counting approaches. These may be used to assess how the various monitoring strategies would estimate the true lice population within a simulated cage of fish (for which the true sea lice population will be known).

## **Methods**

### *The model*

A stochastic individual based model was developed to simulate individual Atlantic salmon within a cage on a typical salmon farm over a two-year production cycle. The model includes a population of sea lice *L. salmonis*, which can parasitise the salmon hosts. The lice develop through a simplified four-stage sea lice life-cycle (Figure 1). The stages that are represented in the model are eggs, planktonic copepodid, juveniles, and adults (including gravid females). Copepodids are free living, while juvenile (chalmus and pre-adults) and adults are attached to individual fish; eggs are attached to gravid females until they hatch. An additional parameter models the introduction of copepodids from external sources (e.g. other farms or from wild fish). The parasite burden on each fish can be tracked throughout a two year production cycle. In reality lice develop through a greater number of stages, however, for simplicity, the developmental stages of two nauplii (I and II) were represented within a planktonic copepodid stage, while the four chalmus stages (I, II, III and IV) plus the pre-adult stages are represented by a juvenile stage.

### *Parameterisation*

The model was parameterised using various estimates from the scientific literature (see Table 1). The model sea lice have temperature-dependent development and reproductive rates (Stien et al 2005). Each life stage experiences mortality based on a stage-specific mortality rate. A probability parameter determines the lice gender as well as the successful attachment of copepodids to salmon.

### *Water temperature*

Temperature was modelled as a function of time in days by fitting water temperature data from Scotland from four different years with an oscillatory sinusoidal curve [Equation 1] where  $a$  is the mean temperature,  $b$  is the magnitude parameter, and  $c$  is a phase shift parameter. (For the empirical data considered:  $a = 13.17$ ,  $b = -8.14$ ,  $c = 184$ ). Using these values, the temperature curve simulates temperature from spring over one year and therefore begins at the time when salmon smolts are typically transferred to salt water cages.

[Equation 1]

$$T(\text{time}) = a + b * \sin^2\left(\pi * \frac{(\text{time} + c)}{365}\right)$$

### *Lice development*

Development of each lice life stage was modelled by accounting for two phases of development – a minimum development time ( $\tau$ ) e.g. minimum number of days an individual spends as an egg, followed by a time taken to complete development ( $\nu$ ) e.g. time taken for eggs to complete hatching once hatching begins. These values were estimated from published studies that investigated development of *L. salmonis* across temperatures ranging from approximately 8 to 14°C, in particular those summarised in (Stien et al 2005).

Minimum development time ( $\tau$ ) (in days) was modelled using a modification of Belehrádek's formula (Equation 2), where  $\tau_{i,j}$  is the minimum required development time for individual  $i$  in stage  $j$  at temperature  $T$ .  $\beta_1$  is a shape parameter and  $\beta_2^{-2}$  is the average  $\tau$  at 10°C.

[Equation 2]

$$\tau_{i,j}(T) = (\beta_1/T - 10 + \beta_1\beta_2)^2$$

The mean and standard deviation for  $\beta_1$  and  $\beta_2$  were estimated for development of eggs, nauplii, chalimus, pre-adults and adult females by (Stien et al 2005), (Table 1). Variation in developmental rates was incorporated by selecting values from a normal distribution – also estimated by (Stien et al 2005). Model development rate of eggs to copepodid, chalimus to adults and female adults to gravid females followed this temperature-dependent development equation, using the stage specific parameters.

The model did not incorporate the nauplii stage or the pre-adult stage. Development rate of eggs to copepodid was therefore represented as the sum of the development rate of eggs to nauplii and nauplii to copepodid. Similarly, development of chalimus to adults incorporated the development rate of chalimus to pre-adults and pre-adults to adults. Chalimus developed into male and female adults according to a probability of 0.5. There is evidence that the minimum development time to pre-adult and to adults differs between gender and therefore male development rate was higher than the female development rate.

The stage specific time taken to complete development ( $v$ ) was added to the minimum development time to represent the time taken for development after the minimum developmental time ( $\tau$ ). These values were also estimated from (Stien et al 2005), and are shown in Table 1.

The total rate of development in the model for each stage was calculated assuming that 0.995 of individuals develop to the next stage. The rate was therefore  $-\ln(0.005)/(\tau + v)$ .

### *Mortality*

Life stage specific mortality rates were constant instantaneous values (Table 2) since there was insufficient data available with which to determine how these parameters vary with temperature. As the model does not incorporate the detailed nauplii or pre-adult stages, the mortality rate for copepodids was estimated using the product of the mortality rate of the nauplii and copepodid stages. Similarly, mortality for juveniles incorporated the mortality of the various chalimus and pre-adult stages. Salmon hosts are presumed not to experience any mortality throughout the production cycle.

### *Infection rate*

Salmon are exposed to free-living copepodid that are either the offspring of louse on the salmon farm (internal infection) or that come from a source external to the farm (external infection). Environmental conditions such as water currents, local sea lice management, distance between farms, number of salmon on a farm and presence of wild salmon in the region can influence the rate of infection from internal or external sources (Costello 2009, Saksida et al 2011, Jansen et al 2012). External infection rate was estimated from (Tully & Nolan 2002, Revie et al 2005). Since there is little information with which to estimate the external infection pressure, a range of values was tested in the model. This was represented as a number of copepodid entering the population per day. Internal infection pressure was the result of gravid female fecundity along with egg development and survival rates. There is little information about the rate at which copepodid are successful at finding and attaching to a salmon host. In reality the attachment process clearly depends on host density and local environmental conditions. Estimates of attachment rates of copepodids span values of 0.25, 0.81 and 0.98 lice fish<sup>-1</sup> day<sup>-1</sup> in a cage study by (Tully & Nolan 2002), (Table 2). Current tests used a value of 0.95 (95% of copepodids attached to a host) based on these estimates, but sensitivity analyses will be carried out in the next phase to investigate how sensitive the models are to these assumptions.

### *Sea lice fecundity*

Clutch size of gravid female lice has been estimated to be between 70 and 583. The size of the second clutch tends to be larger than the first, and number of eggs appears to be independent of temperature. Average clutch size was calculated based on these estimates as 331 eggs per clutch.

In reality sea lice eggs in one egg string develop concurrently and complete development within approximately 65 hours of the string being produced (Johnson & Albright 1991). However in the model gravid female egg production was represented as a constant daily rate. This method was used because the model simulated a population of lice, rather than identifiable individual adult lice that could be assigned two egg strings each.

To account for a low probability of sexual reproduction at low parasite intensity, adult females only developed to gravid females when they were attached to a fish where there was also a male adult louse present. This represented for the observation that at low lice levels female lice are less likely to encounter a male louse.

#### *Egg viability*

Egg viability decreases with decreasing temperature, however there was little data to estimate a temperature dependent parameter. Egg viability was therefore assigned a constant value of 85% based on estimates from previous studies (Table 2).

#### *Starting conditions*

The simulations have currently been run using 'virtual cages' of 5000 salmon. This number can be easily increased once the researchers have decided what the more interesting parameters to explore are. (Larger numbers will result in longer simulation runs and larger data sets to post-process so it makes sense to have a bit more focus before engaging in these larger trials.) None of the salmon are parasitised on entry to the model, indeed there are no free-living stages present at the start of the production cycle. The infestations that arise are a consequence of copepodids that initially arrive from external sources entering the model at a user-determined rate of external infection.

#### *Chemical treatment to reduce parasite infection*

The effect of treatment is simulated using an instantaneous mortality, representative of topical bath treatments which cause instant knock-down. Treatment reduces the number of chalimus and mobile lice populations when the total number of mobiles is an average of more than four per fish. The rate of reduction, i.e. the treatment efficacy, can be defined for a given treatment and applies to all lice on all fish. The sensitivity of sea lice population dynamics to different treatment efficacies can be investigated by changing the estimates of efficacy.

#### *Software implementation*

The model was implemented as a bespoke piece of computer software using the *Visual Basic* (Version 6, Microsoft Corporation, Seattle, Washington, USA) programming environment. It uses the day as the basic time step and can simulate fish and their infestation with lice over the period of a two year production cycle.

#### *Data output*

The results from the model are exported as a structured text file and may be saved or exported to statistical analysis software. Output parameters can be determined by the user. Since the model runs on a continuous daily basis and many calculations use probability and rate functions, the population numbers are reported as single values. These are converted to integers when the model has completed running by rounding to the nearest whole number.

## Data Analysis and Results

Simulations were also run to test the effect of various treatment interventions on the mean numbers of mobile lice per fish so that simulated estimations would not only address the issue of routine surveillance but could also be used to compare the accuracy of treatment efficacy estimations. Three different scenarios were tested: a bath treatment was applied when the mean number of mobile lice per fish reached 1 lice, 2 lice or 4 lice respectively. To investigate the effect of different counting strategies estimates of the mean number of lice per fish were carried out using different approaches. Counts of lice were carried out on random samples of: 10 fish, 20 fish, 50 fish, 100 fish, 1000 fish or 5000 fish (the total population). To provide some illustrative results these counts were taken on day 365 of the first year of the production cycle, but clearly the sampling could be simulated on any day. In addition it would be possible to simulate daily counts (something that would be possible with the automated counting technology) and compare the estimates derived from the approach to those gained from the more normal weekly counting methods used in manual counting. In addition the presumed accuracy on each individual fish counted is the same irrespective of whether a human or machine is carrying out the task. Once again this could clearly be altered but the researchers currently had no data on which to set up parameters indicating the relative accuracy of these different methods. (It is hoped that some initial indication of such values will be forthcoming from the pilot trials being carried out by other partners in the *VisuaLice* project.)

As far as initial results are concerned, the model mostly provided the types of outcome that would be expected. Though it was somewhat surprising that the variance components of the simulation were less differentiated than might initially have been expected.

As the treatment trigger level was increased the mean number of lice per fish obviously also increased (Figure 2). [Note, the lack of difference between panels (b) and (c) is likely due to the fact that a treatment took place late in the first year and the lice levels are still just 'recovering' from this intervention. Simulating more sample points would provide some clarity on this issue.] It can also be seen that as the sample size increased (from 10 to 5000 fish) the confidence intervals for the estimate of number of mobiles per salmon decreased (Figure 2). However, the reduction - particularly after 100 fish - was not that marked; the researchers are still investigating the cause of this slightly unusual outcome. (As can be seen from some of the later figures this was not always the case.)

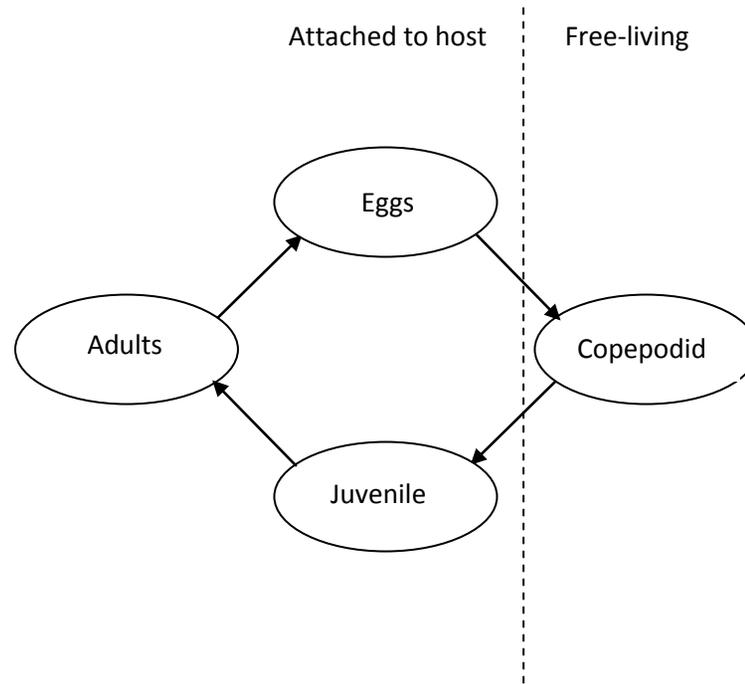
Simulations were run to test the influence of the external infection pressure on lice infection. Four different scenarios were tested: an external infection pressure of 1000, 2000, 4000 or 5000 copepodids entered the lice population per day. As the external infection pressure increased, the mean number of lice per fish also increased (Figure 3). Once again the counts were simulated to happen of day 365 of the first year of production and the results were collected for scenarios involving random samples of 10, 20, 50, 100, 1000 and 5000 fish. There were some anomalous results in terms of estimates of mean with some scenarios (e.g. [a] and [c]) showing a declining estimate of the mean value, while others (e.g. [d]) showing the opposite trend. It was not expected that the estimated mean would vary much and certainly not in a consistent manner (i.e. the result from panel [b] were more like those expected). It may be that the relatively small

number of replicate runs (N=10) is the cause of these anomalies and larger numbers of replicates will be tried once the focus of the simulation studies has been more clearly determined. What was relatively clear and consistent was that the confidence intervals around the estimated mean number of mobiles per salmon decreased as the sample size increased (Figure 3).

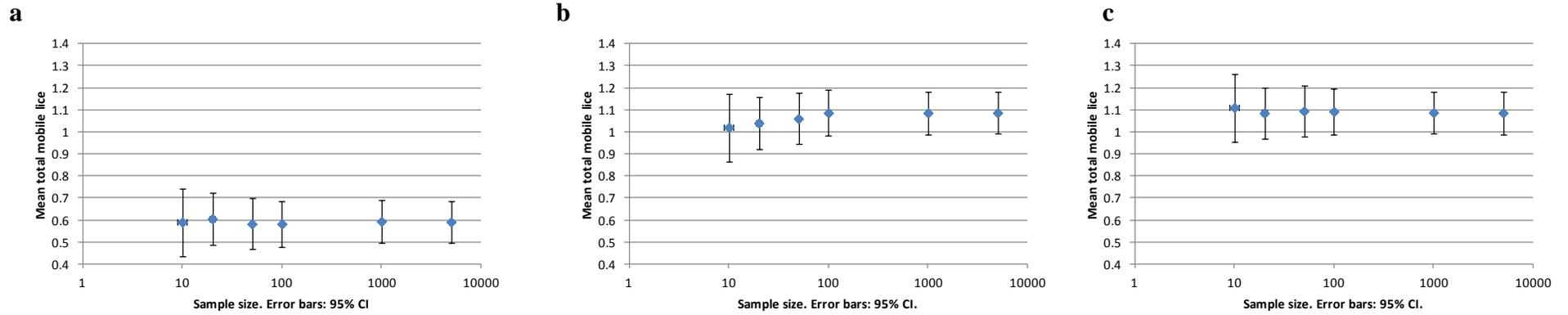
To investigate the potential sources of variability it was decided to look at the distribution of mobile lice on the *whole* simulated salmon population at a fixed point in the production cycle under different assumptions. Once again this was carried out for day 365 of the simulated run and the results from four different simulations are shown in Figure 4. While there were differences between these model runs, as the images suggest, they were somewhat less varied than we had expected. Once again the researchers are investigating the simulation code and in particular the daily 'rounding' adjustments that are made to see whether this is having an undue influence on the range of values resulting from the various simulation runs.

The distributions which were observed (Figure 4) indicated that as the external infection pressure increased so did the resultant levels of lice observed, assuming similar treatment trigger threshold, which is as would be expected. However, what was not expected was the fact that - particularly at these relatively low mean levels of infestation - there were so few fish which did not have any lice present on them. This varies dramatically from the over-dispersed distributions that are typically seen in empirical data and while some over-dispersion can be seen in the scenarios with higher external infection pressure (4000 and 5000 copepodids per day in Figures 4d and 4e respectively), this does not appear to reflect the range of values that would be seen in a typical cage of salmon. (Or at least not what we see in empirical data based on samples of much lower numbers of fish.) Once again this is a subject that requires further investigation of the model coding structures.

**Figure 1. Simplified life cycle of *L. salmonis* used in the individual-based model**



**Figure 2. Estimates and Confidence Intervals on the mean total mobiles per fish based on different treatment trigger levels**  
 Average number of mobile lice per fish to trigger a treatment: a=1, b=2, c=4.  
 (Ten replicates were simulated per figure. A constant external infection pressure of 2000 copepodids per day was used in all cases.)

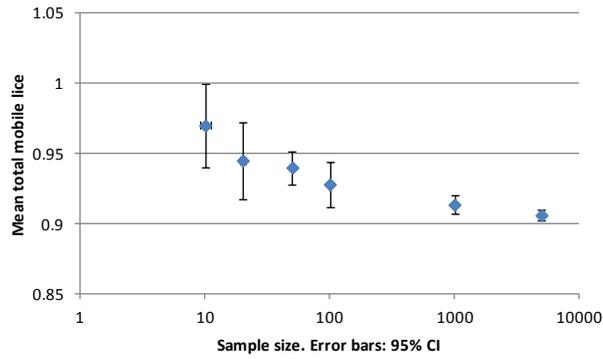


**Figure 3. Estimates and Confidence Intervals on mean total mobiles per fish under different external infection pressure**

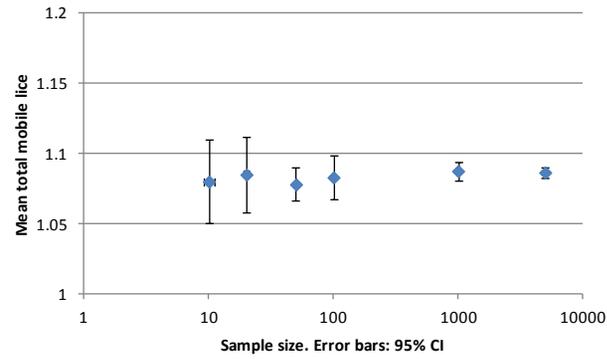
External infection pressure was varied from: a=1000, b=2000, c=4000, d=5000 copepodid per day.

(Three were ten replicates pre figure. A constant treatment trigger level was set at a mean of 4 mobile lice per fish.)

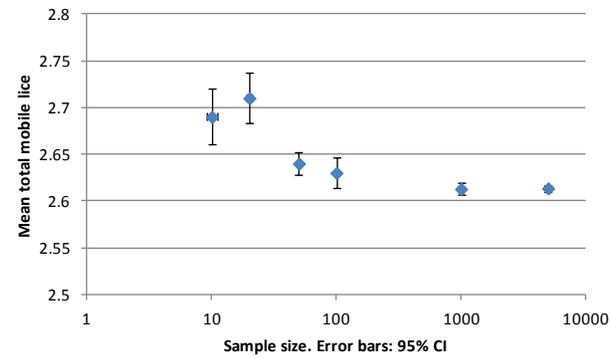
**a**



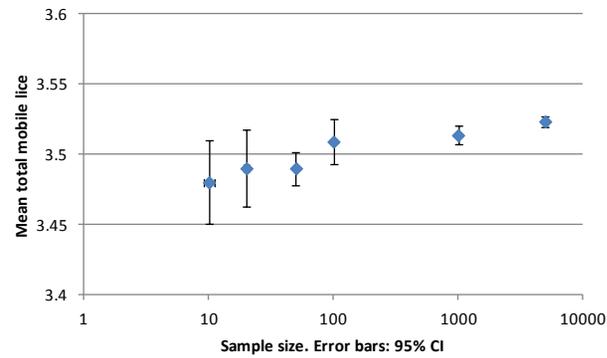
**b**



**c**



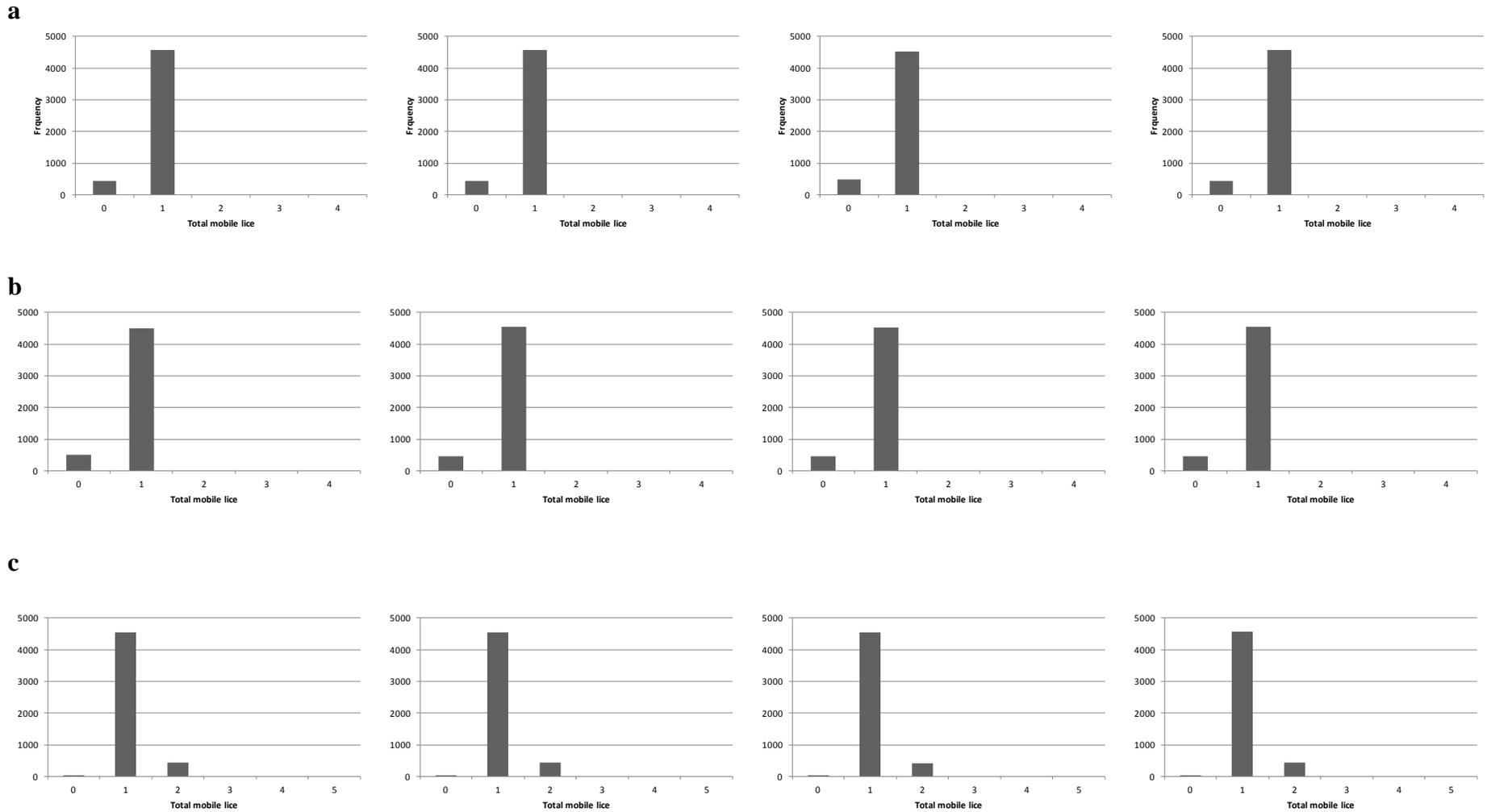
**d**



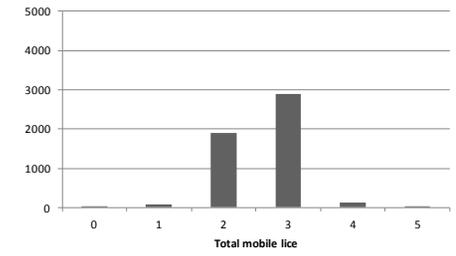
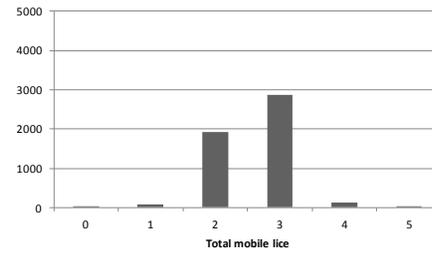
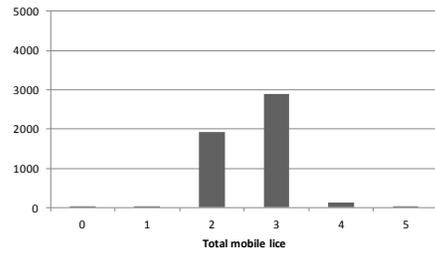
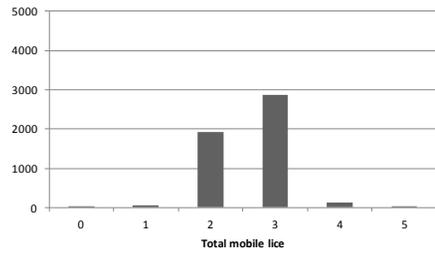
**Figure 4. Parasite infestation of each member of the population of 5000 fish as simulated at day 365 of the production cycle**

The four 'columns' of graphs represent different replicates of the same model with the same parameter combination. The five different scenarios modelled represent different combinations of external infection pressure and treatment trigger levels.

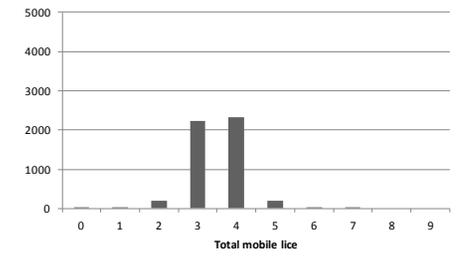
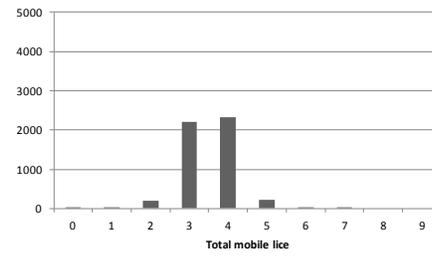
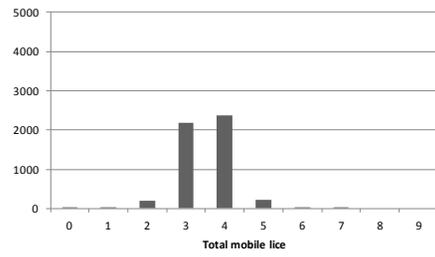
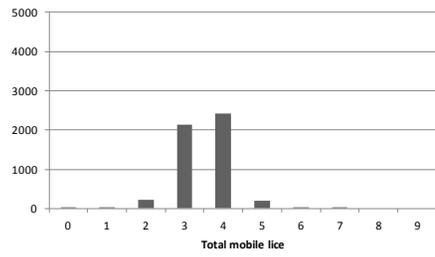
(External infection pressure: a=1000, b=1000, c=2000, d=4000, e=5000. Mean mobile lice to trigger a treatment: a= 2, b=4, c=4, d=4, e=4.)



**d**



e



**Table 1. Parameter values for temperature dependent development rates of sea lice (*Lepeophtheirus salmonis*) from Stein et al. (2005).**

<b>Life Stage transition</b>	<b><math>B_1</math> (Standard Error)</b>	<b><math>B_2</math> (Standard Error)</b>	<b>v time take to complete development (days)</b>
Eggs – Pre-infective nauplii	41.98 (2.85)	0.338 (0.012)	2.7 <sup>a</sup>
Pre-infective nauplii – infective copepodid	24.79 (1.43)	0.525 (0.017)	No information
Chalimus – Pre-adult female	74.7 (33.64)	0.246 (0.007)	4.2
Chalimus - Pre-adult male	74.7 (33.64)	0.255 (0.007)	3.7
Pre-adult female – adult female	67.47 (20.36)	0.197 (0.006)	15.8 <sup>b</sup>
Pre-adult male – adult male	67.47 (20.36)	0.177 (0.006)	11.3
Adult female – gravid female <sup>c</sup>	41.98 (2.85)	0.338 (0.012)	2.7

<sup>a</sup> Egg development rate after the minimum development time. The probability of egg development has been estimated to be 0.995 in 2.7 days. This was converted to a probability of development by (Stien et al 2005) as follows:  $-\log(0.005)/2.7=2 \text{ ind}^{-1} \text{ day}^{-1}$ . The rate used in our individual based model was calculated so that  $\geq 0.995\%$  of eggs had developed after 3 days. The rate used was 0.83.

<sup>b</sup> Estimates of preadult instantaneous development ranged from 0.33 to 0.34 for females and 0.33 to 0.80 for males. These equate to a development time (assuming that 0.995 of preadults develop) in the range of 15.6 to 16.1 days for females and 6.6 to 16.1 days for males. Mean values of 15.8 and 11.3 days were selected to reflect that females tend to develop more slowly than males.

<sup>c</sup> It was estimated (Heuch et al 2000) that the time taken for an adult female louse to develop a new pair of eggs strings is similar to the time from egg string extrusion to egg hatching. Females become gravid females if they are attached to a fish where there is also a male louse present.

**Table 2. Parameters estimates for constants used in the model**

<b>Parameter</b>	<b>Parameter value</b>	<b>Parameter value reported in the literature</b>	<b>Literature source</b>
Egg mortality	0.419 Egg mortality = 0.3 Nauplii mortality = 0.17		Stien et al, 2005
Egg Viability	0.756	50-92.5% (Mean = 75.6%)	Heuch, Nordhagen, & Schram, 2000; Ritchie, Mordue, Pike, & Rae, 1993; Johnson & Albright, 1991
Copepodid Mortality <sup>b</sup>	0.22 ind/day <sup>-1</sup>		Stien et al, 2005; Johnson & Albright, 1991
Infection rate – probability that copepodid will attach to a salmon	0.5		
Gender selection – proportion of copepodid that become female at time of development to chalimus	0.5		
Chalimus Mortality	Preadult male mortality = 0.073 <sup>c</sup> Preadult female mortality = 0.053 Chalimus mortality = 0.0063 Combined mortality rate = 0.069	0.018-0.18 (mean=0.073) 0.035-0.074 (mean=0.053) 0.002-0.01 (mean=0.0063)	Stien et al, 2005
Adult Male Mortality	0.087	0.03-0.06 (mean=0.045)	
Adult Female Mortality	0.14	0.02-0.04 <sup>a</sup> (mean=0.03)	
Gravid Female Mortality	0.14		Stien et al, 2005
Eggs per egg string <sup>d</sup>	331	70-583 eggs (mean 331)	Ritchie, 1993; Heuch et al, 2000 Johnson & Albright, 1991; Ritchie 1993, Tully & Whelan, 1993; Heuch et al, 2000

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Treatment efficacy	0.95
Treatment trigger	1-4
External copepodid source	1000-5000
Number of salmon	5000

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<sup>a</sup> Stien et al (2005) indicate that the estimates of adult mortality from some studies contained little data. These are best estimates, but could be higher.

<sup>b</sup> The proportion of nauplii that survive to develop to copepodids has been estimated at 50%, translated to a mortality rate of 0.17ind/day based on the knowledge that nauplii have a residence time of 4 days. The mean survival time of copepodids has been estimated at 4.6 days, which suggests a mortality rate of 0.22 ind day<sup>-1</sup>.

<sup>c</sup> Since female and male pre-adult mortality were very similar, we used one value (a mean) for preadult mortality. This was combined with chalimus mortality rate to give a total mortality rate for the model chalimus.

<sup>d</sup> Gravid females produce two egg strings, the second tends to produce more eggs than the first. The mean per egg string was 331 eggs. The rate of egg production per gravid female was calculated as a proportion of eggs produced per day for the number of days that the louse spends as a gravid female. Time spent as a gravid female =  $\tau_e + \nu_e$ . The rate was therefore  $331/\tau_e + \nu_e$ .

## Discussion

Clearly it was initially hoped that the provision of various parameter estimates from the passive monitoring equipment would allow the models to be re-run under a wide range of scenarios to provide feedback to *VisuaLice* project partners on the likely performance of such an approach. As it became clear that these data were not going to become available within the time-frame of the research activities documented here, it was important to create some output to illustrate the utility of such simulated outcomes. In particular we sought to simulate the likely accuracy estimates of any automated approach – rather than focussing on sample size issues – but in the absence of empirical data it was not possible to validate such estimate, beyond the internal validation that can be provided by undertaking a range of sensitivity analyses.

Temperature dependent parameters were included in the model when there was sufficient evidence to include such variations. While there is limited data to suggest that some parameters may be temperature dependent e.g. attachment rate of copepodids (Tucker et al 2000), there was not enough information to fully estimate many potentially temperature dependent parameters and in these cases the parameters were simply represented by constants. In all of the simulations currently reported a single instantiation of water temperatures has been used – based on typical sea water characteristics from the west coast of Scotland. It is relatively straightforward to simulate alternative sea water temperature profiles, though the research team have found that a few degrees of difference from these typical north-east Atlantic temperatures does not result in significant differences in the modelled outcomes.

In summary, there is a pressing need to develop automated methods that could be used to assess sea lice numbers on farmed salmon. A computer model has been developed that can provide an efficient way of comparing such an automated passive monitoring approach with the more traditional manual counting methods. When additional data becomes available, it will enable an effective *in-silico* assessment of such novel automated counting approaches under a range of farm settings, cage configurations, and pre-/post-treatment conditions.

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