THE HARDANGERFJORD SALMON LICE PROJECT – FINAL REPORT, 31.05.2010.

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Project summary
Wild sea trout and Atlantic salmon were sampled by a range of methods to investigate the level of infection by salmon lice in the Hardangerfjord basin (WP 1). In addition, sentinel cages were used to study spatiotemporal variability in infection pressure throughout the whole Hardangerfjord system. The results indicated that the infection levels were still too high in this fjord system and that there has been an increasing trend in infection pressure during the project period. In 2008, about 50% of both salmon smolts and sea trout were infected with so high levels of lice that negative effects on their physiology and ecology were expected. In 2009, the situation improved, especially for salmon smolts. Sea trout smolts collected from the smolt trap in River Guddal and treated chemically against salmon lice had almost twice as high return rate, although not significantly different (sea survival) from the untreated control group, which provides evidence of salmon lice induced mortality on sea trout in this area. Most strikingly is however the overall very low seawater survival of sea trout smolts (< 3 %). This altogether, especially when combined with the very low and reduced spawning populations in almost all sea trout and salmon rivers in the fjord, strongly indicate that the measures taken have not had a sufficient effect.

Fish health work in the fjord is to a large extent initiated and coordinated by the Hardanger Fish Health Network (HFN). In WP 2, HFN and researchers explored control options by coordinating "experimental" lice treatments and examining results by different lice counting methods. To this end the occurrence of salmon lice on c. 20 salmon farms covering all parts of the fjord were monitored by specially trained counting teams. Counting by teams was carried out from April to early autumn. In the first half of the project it was demonstrated that treating with emamectine benzoate (Slice) between November and January dramatically reduced lice population growth through the following spring. In the critical smolt run period very few adult female lice were found. Bath treatments in the same period did not give the same effect. This was supported by later analyses including more variables.

Monitoring guidelines valid up until August 2009 specified that a sample of at least 20 fish be taken from two pens, one presumably the most infected pen, and the other a random pen. The accuracy of this protocol was tested by examining data sets from sites where all pens had been sampled, and data from a large set of regular farmer two-pen countings. A comparison with similar data from Scotland was also carried out. Analyses indicated that there was no significant difference between the lice mean abundances in the presumed most infected and the random pens. Furthermore, for site mean abundance to be estimated, the clustered lice distribution within a site needs to be taken into account. Data simulations based on Hardanger and Scottish data show that more than two pens need to be sampled for reasonably accurate site mean: better few fish from many pens than many fish from few pens.

The physical environment in the Hardangerfjord (WP 3) varies on time scales ranging from hours up to many decades. The shorter time variations in currents and salinity affect the distribution of the planktonic salmon lice in the fjord system. On the inter-annual scale, we find relatively large differences in the environmental conditions between years. This influence the abundance and distribution of the salmon lice. Numerical model results shows that spreading of planktonic salmon lice can be rapid and potentially long distant. It is necessary to consider large regions in order to manage the salmon lice problem.

The analysis of historical farm data from the Hardangerfjord between 2004 and 2006 (WP 4) confirmed that a large number of factors were associated with an impact on chalimus and adult sea lice levels on farmed fish. These factors included zone, temperature, salinity, fish weight and

treatment. Detailed examination of the epidemiological patterns of sea lice infections on autumn and spring-stocked farms produced distinctive patterns and illustrated the importance of the strategic use of the two major treatments i.e. topical cypermethrin and in-feed emamectin benzoate. The sea lice difference equation simulation (*SLiDESim*) model previously used to describe and investigate the control of sea lice infections on salmon farms in Scotland was fitted using the autumn and spring-stocked profiles. A range of biological, environmental and production parameters were explored using the *SLiDESim* model to obtain the best fit to patterns. The modelling took account of the frequency and type of treatment practised on the farms. This predominantly consisted of in-feed treatments during the first year of production and bath treatments in the second year. The results showed that modelling could give a good first approximation to the observed Hardangerfjord lice patterns. However some features of the observed patterns were not captured and before the model can be used to evaluate optimal treatment strategies there is a need to further understand the underlying biological and environmental processes associated with lice populations.

Overall, the project has shown that the farmers have done a good job and, especially through coordinated spring delousing campaigns, managed to keep lice levels on farmed fish low throughout the important smolt run in May. Thereafter, infection levels in farmed fish has increased and in late summer and autumn, no further effect of the spring delousing has been seen (WP2). Furthermore, modelling of the physical environment as well as spread of pelagic lice larvae (WP3) has shown that spreading of planktonic salmon lice can be rapid and potentially long distant. It is therefore necessary to consider large regions in order to manage the salmon lice problem in the fjord system. In combinations with the sea lice difference equation simulation (*SLiDESim*) model (WP4), forecast models have the potential to be developed and used to evaluate optimal treatment strategies. This is urgently needed as the infection pressure on wild fish in the Hardangerfjord system has not, as expected, been reduced in the Hardangerfjord system throughout the total project period 2004-2009 (WP 4). The reason for this is probably the concurrent increase in farm fish production. When the project was started in 2004, farmed production was below 40 000 tons. When the project was ended in 2009, farmed production had increased to more than 70 000 tons. Any positive benefits of the strategic and coordinated delousing program, has therefore probably “been eaten” up by the increased production. At the moment, it’s therefore difficult to see how sustainable populations of wild salmonids can coexist with a farm production at current production levels and management practices.

Introduction

The Norwegian fish farming industry has shown a strong growth during the last years and in 2009 approximately 850 000 tonnes of farmed salmonids were produced. Salmon lice (*Lepeophtheirus salmonis* Krøyer) represent an important economical loss factor in Norwegian and international salmon farming industry and a constant threat to sustainable production if not controlled. Given the frequently high numbers of gravid salmon lice carried by the large numbers of cultured fish throughout the year, it is likely that the development of an aquaculture industry has lead to changes in the natural host-parasite relationship, and made possible the production of large quantities of the infective dispersal lice stages. As plankton, these larvae will drift and be dispersed over long distances, but apparently concentrate near the surface by day, and probably also near pycnoclines in stratified waters. The density of infective salmon lice stages are, therefore, likely to be greatest in inshore coastal areas and fjords that are subject to constrained tidal flushing. These locations are exploited by the farming industry as well as seaward migrating postsmolts.

Salmon lice epidemics have also been described as a problem for wild salmonids. Based on crude estimates of how many lice a smolt can tolerate, direct parasite-induced mortality of wild postsmolt sea trout has been estimated to 30-50 % in an area with intensive fish farming activity. Recent results also show that a load of 12-13 lice•fish^{-1} is found to be a consistent breakpoint across a range of physiological measures. The salmon lice problem on wild salmonids has increased during the last decade, and while no direct link has yet been established, increasing evidence suggests that the problem has a connection with the rapidly growing salmon farming industry. Measures have, therefore, been taken by the fish farming industry and Norwegian management authorities to reduce salmon lice levels in the fjords and coastal current.

It is indicated that the relative contribution of lice may vary between host species, geographic area, levels of farming activity and management practises. Furthermore, hydrographic conditions and current systems seem to be crucial in the dispersal of infective lice, and will determine the risks of re-infection of hosts. Currently such models are being tailored to model drift of infective lice stages. Epidemiological models combining lice population dynamics and dispersal potential are needed to understand the complex relationship between hosts and parasites. This can be done by using multi-factorial regression techniques to analyze data sets collected associated with varying lice infection pressure on farmed and wild fish. This model is the basis for investigating sea lice control at national and site level through veterinary interventions. This component requires extensive modelling and programming effort, and could also form the basis for exploring in more detail the ‘sources’ and ‘sinks’ of lice production as a means of better understanding the interaction between wild and farmed salmonids.

The Hardangerfjord which is the study area has the highest density of fish farms in Norway (approx. 70 fish farms in 2009), is 150 kms long and approx. 70 000 tonnes salmon is produced here. This system is an ideal study area because it is mainly affected by inner fjord dynamics, has important wild salmon and sea trout stocks affected by salmon lice, and nearly all salmon farms are cooperating through a fish health network.

During the project period we have gained much experience and information. However, although strategies for lice treatment in fish farms have been greatly improved through synchronized delousing in all farms in the fjord, we still experience episodes of too high lice infestation on wild salmonids in this fjord system. The reason for this may be the greater biomass of farmed fish in the Hardangerfjord system compared to other fjord systems still makes the prevailing lice levels in farms too high or that the prevailing environmental conditions (eg. salinity, temperature, currents) have led to favourable environmental conditions for the spread of lice from farms to wild salmonids despite the greatly improved measures taken. Therefore, it is vital to extend the time series to cover more of the different combinations of environmental and management factors which influence salmon lice levels on the different salmonid populations. This will enable us to reach a better understanding of interactions of salmon lice between wild and farmed fish in this fjord system and merge this into an epidemiological model combining data of lice population, environment and measures taken in the farming industry.

Project description

The different parts of the project have been achieved through four closely linked workpackages (WPs):

WP 1 – Salmon lice abundance on wild and escaped salmonids

- Gathering data of salmon lice abundance on all free swimming hosts in the fjord system: wild salmon and sea trout and escaped farmed salmon
- Evaluate the success of the local salmon lice management model in Hardangerfjorden through the infection level on wild salmonids

WP 2 - Optimised salmon lice monitoring and control strategies in farms

- Improve sea lice monitoring and management on individual farms and the region in general by fjord integrated pest management and synchronized delousing processes
- Evaluate the success of sea lice management strategies in Hardangerfjord through investigation of the infection level on farmed fish (and wild fish – WP1)

WP 3 - Understand physical oceanographical factors on salmon lice abundance and distribution in the Hardangerfjord - to quantify the abundance and distribution of salmon lice in the Hardangerfjord area based on the physical oceanographical and meteorological

conditions for a given salmon lice production (number of lice and origin).
- Estimate the time variations (seasonal/annual) of the sheltering brackish fjord water
- Estimate the total fjord distribution of salmon lice from different fish farming locations (farming strategies)

WP 4 - Development of a mathematical population model for the Hardangerfjord system
- Analyze data sets being collected at the Hardangerjord for possible risk factors associated with varying lice infection pressure
- Develop a mathematical population model for the Hardangerfjord system to enable the exploration of optimal lice control strategies

By combining results from WPs 1, 2, 3 and 4 we aim to further develop an ecological model system for the Hardangerfjord which can be used in management schemes aimed at minimising the risk of salmon lice infestation on wild and farmed fish stocks. Modelling may also give advice to the industry for optimal placement of fish farms within a fjord system. Results obtained in this project can be used for other fjord systems globally in management schemes aimed at minimising the risk of salmon lice infestation on wild and farmed fish stocks.

WP 1 – Salmon lice abundance on wild and escaped salmonids

Pål Arne Bjørn (IMR), Øystein Skaala (IMR), Steinar Kålås (RB) and Bengt Finstad (NINA).

Summary – WP 1
Wild sea trout (*Salmo trutta* L.) and Atlantic salmon (*S. salar* L.) were sampled by a range of methods to investigate the level of infection by salmon lice (*Lepeophtheirus salmonis*) in the Hardangerfjord basin and the effect of the measures taken. In addition sentinel cages were used to study spatiotemporal variability in infection pressure throughout the whole Hardangerfjord system. The results indicated that the infection levels were still too high in this fjord system and that there has been an increasing trend in infection pressure during the project period. In e.g. 2008, about 50% of both salmon smolts and sea trout were infected with so high levels of lice that negative effects on their physiology and ecology were expected. In 2009, the situation improved, especially for salmon smolts. Sea trout smolts collected from the smolt trap in River Guddal and treated chemically against salmon lice had almost twice as high return rate, although not significantly different (sea survival) as the untreated control group, which gives evidence of salmon lice induced mortality on sea trout in this area. Most strikingly is however the overall very low seawater survival (< 3 %). This altogether, especially when combined with the very low and reduced spawning populations in almost all sea trout and salmon rivers in the fjord, strongly indicate that the measures taken have not had a sufficient effect.

Introduction
Already in the early 1990'ies (Jakobsen et al. 1992), sea trout heavily infected with salmon lice were captured in the Hardangerfjord, and in consecutive years a discussion followed about the connection between the high infestation level observed on wild salmonids and the apparent severe decline in spawning populations in several rivers especially in the middle parts of the fjord system. It was further reported that sea survival of salmon populations from Hardanger was lower than for stocks from other regions of the Norwegian west coast, and that the Hardanger stocks failed to respond to an improvement in ocean climate and return to more normal levels from year 2000, as did several other stocks along the Norwegian coast. An improvement in the sea lice infection pressure through lower levels of female lice on farmed fish in the fjord was thought to be able to improve this situation. Low infection intensities in wild salmonids and improved seawater survival and spawning populations were therefore considered as the ultimate success factor for the Hardangerfjord salmon lice project.

Materials and methods
It is not straightforward to quantify precisely the infection pressure and mortality caused by salmon lice. The infection level on postsmolts and sea trout can give some indirect evidence, given that a representative sampling of fish populations and their infection level can be obtained. To try and compensate for limitations in sampling methods, an attempt was also made to assess the mortality caused by salmon lice by comparing survival of smolt groups treated chemically against lice to untreated control groups of smolt collected in the trapping facilities in River Guddal. We also used sentinel cages to study spatiotemporal differences in infection pressure and estimating changes in spawning stocks in a number of Hardangerfjord Rivers. During the project, salmon lice were counted on the most important wild species of salmonids in the fjord system. To accomplish this, a variety of gear (electrofishing, gill-netting, trawling) was chosen to capture specimens of various life stages from postsmolts to adults, throughout the summer season, and at different fjord regions (inner, middle, outer). However, the low abundance of the populations of sea trout and salmon in parts of the fjord, posed a problem for the sampling, as the catch per unit effort were low, particularly in the areas where the decline in the populations appeared to be strongest (se Bjørn et al. 2009, 2010ab for further details).

To investigate the mortality caused by salmon lice in the natural environment, part of the wild smolts from the trapping facility in River Guddal were treated chemically (Substance EX and injections with Slice) against lice before released back to the river. Another smolt group was used as untreated control and all fish were fin clipped to distinguish the two groups at return in the trap.

Results

Infection levels on post smolts of Atlantic salmon and sea trout captured by trawling

Catching representative samples of fish populations may be a challenge in general and particularly so when numbers and life stages of salmon lice are to be recorded on the specimens. Given that the numbers are representative, the observations indicate that mortality on salmon smolts caused by salmon lice is somewhat higher than in previous years. However, sea trout which is more stationary in the fjords compared to salmon, appears to accumulate higher numbers of salmon lice (**figure 1**). Smolts of sea trout are often larger than salmon smolts, and therefore it is expected that sea trout also will survive with slightly higher numbers of salmon lice. Still, it has been reported that even a few lice (between 0.1 and 0.3 lice per gram fish weight) will disturb the physiological homeostasis in small salmonids. In the worst year, more that 50 % of the trawl captured salmon and sea trout were above that level (se Bjørn et al. 2009, 2010ab for details).

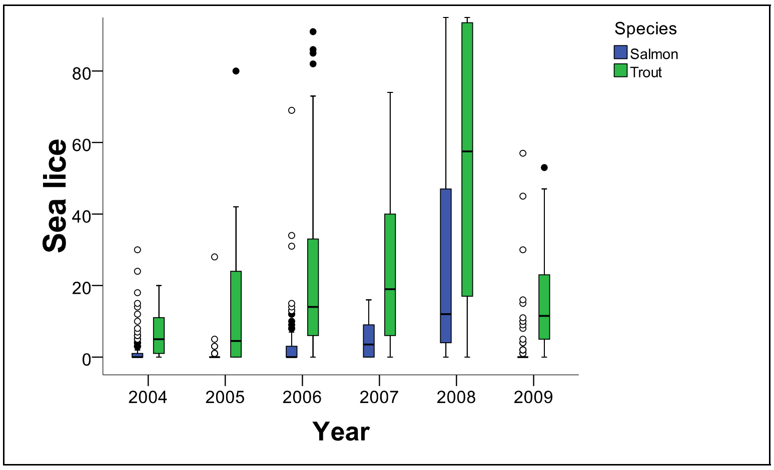

Figure 1. *Boxplot showing the median number of sea lice on all (abundance) captured fish by trawl in the outer part of Hardangerfjord 2004-2009.*

Infection levels in sea trout captured by gillnetting, and premature return to freshwater

To further assess the infection levels on sea trout, the following stations were sampled by gil-nets: Granvin (inner), Rosendal (middle) and Etne (outer) (**figure 2**). Typically, the number of lice varied much among the regions but also within individuals within a region. For example, in Granvin (inner region), there was very little lice on the fish both in 2008 and 2009. In Rosendal (middle region), the number of lice on sea trout was moderate in both 2008 and 2009. In Etne (outer region), the infection level was very high in 2008, and much lower in 2009. Generally, infection pressure in middle and outer regions is still too high, and many individuals are expected to be negatively influenced by this (se Bjørn et al. 2009, 2010ab for further details). This is also in accordance with observations of premature return to freshwater in the project period (Kålås et al. 2010), some of them intensively lice infected.

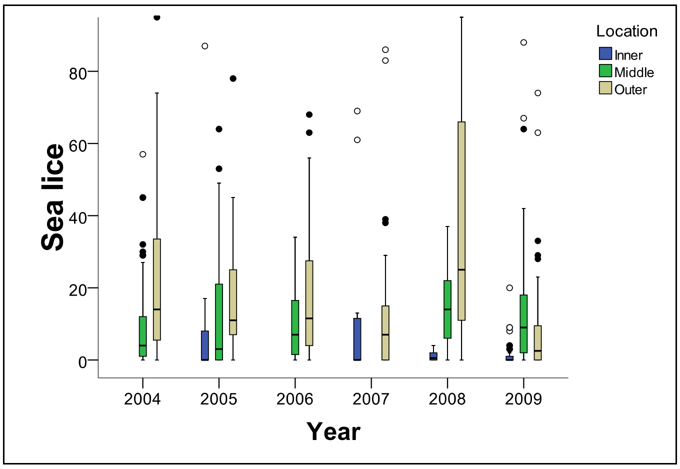

Figure 2. *Boxplot showing the median number of sea lice on all (abundance) captured sea trout by gillnets in inner, middle and outer parts of the Hardangerfjord 2004-2009.*

<u>Evidence for mortality caused by salmon lice: comparison of survival of smolt chemically treated against salmon lice and untreated controls</u>

The return rate, which is reflecting sea survival, was very low for both treated (3.0 %) and untreated (1.8 %) groups. However, the recapture rate of the treated smolt group, was somewhat higher (although not significantly) than that of the untreated control group after the two first sea migrations. Mean weight and condition factor were also higher in the treated group than in the control group, although not statistically significant. The total recapture rate was less than 3%, which is exceptionally low for sea trout. A high percentage of the trout captured in the upstream trap and in gillnet catches had open wounds behind the anal fin, an area of the body surface where lice often appear to concentrate. This observation further illustrates the damage inflicted by salmon lice. Together with the very low stock levels and no sign of improvement (Bjørn et al. 2010b), the results suggest that mortality on sea trout caused by salmon lice may be high in parts of the Hardangerfjord basin during the project period.

Discussion

The key goal in the Hardangerfjord salmon lice project was to lower the number of female salmon lice on farmed fish to such a level that infection pressure on both farmed but especially wild salmonids is sustainable. This has been done by synchronised and coordinated winter and spring delousing in most of the fish farms in the fjord system. Low infection intensities in wild sea trout and running salmon smolts as well as improved seawater survival and spawning populations were therefore considered as the ultimate success factor for the Hardangerfjord salmon lice project. Result from the long time surveillance program on wild salmonids, shows that no significant improvement in infection pressure have been seen throughout the project period 2004-2009. On the contrary, there seemed to be and increasing trend in infection pressure both on sea trout and running salmon smolts, although there is large spatiotemporal variability both between areas and between years. There is still premature return to freshwater to a number of Hardangerfjord rivers (e.g. Kålås et al. 2010), running Atlantic smolts and sea trout are still too heavily infected with lice, especially in middle and outer areas of the fjord (se Bjørn et al. 2008, 2009 for details), seawater survival is still low and there seems to be no improvement in spawning stocks in Hardangerfjord rivers (Bjørn et al. 2010b). Especially the outer and middle areas of the fjord seems to have too high infection pressure, while the situation in the inner part seems to be better (Bjørn et al. 2008, 2009). This is probably caused by a combination of more unfavourable conditions for lice (low salinity), fewer fish farms and probably also prevailing wind and current conditions bringing pelagic lice larvae outwards the fjord. The fish farmers have done a large effort and, most of the years, managed to keep number of female lice on farmed fish very low, especially during the smolt migratory periods (week 20-24, se WP 2 for details). It is therefore surprisingly that infection pressure from pelagic lice larvae, haven't shown the same reduction.

Some of this variability may be modulated by favourable conditions for lice through high winter and summer temperature and high salinity (Asplin et al. 2010). Most of the lack of improvement is however probably caused by the concurrent growth of the farmed biomass in the project period. When the Hardangerfjord salmon lice project was started in 2004, the production was below 40 000 tons. In 2009 this had increased to over 70 000 tons (Bjørn et al. 2010b). The positive effects of the strategic delousing programme have therefore probably been "eaten up" by the increase in production between 2004 - 2009.

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WP 2 - Optimised salmon lice monitoring and control strategies in farms

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Summary – WP 2

The purpose of WP2 was to optimise lice monitoring on farms; and to evaluate the success of sea lice management strategies in the Hardangerfjord through investigation of the infection levels of farmed fish. Fish health work in the fjord is to a large extent initiated and coordinated by the Hardanger Fish Health Network (HFN). In this workpackage, HFN and researchers explored control options by coordinating “experimental” lice treatments and examining results by different lice counting methods. To this end the occurrence of salmon lice on c. 20 salmon farms covering all parts of the fjord were monitored by specially trained counting teams. Counting by teams was carried out from April to early autumn. In the first half of the project it was demonstrated that treating with emamectine benzoate (Slice) between November and January dramatically reduced lice population growth through the following spring. In the critical smolt run period very few adult female lice were found. Bath treatments in the same period did not give the same effect. This was supported by later analyses including more variables.

Monitoring guidelines valid up until August 2009 specified that a sample of at least 20 fish be taken from two pens, one presumably the most infected pen, and the other a random pen. The accuracy of this protocol was tested by examining data sets from sites where all pens had been sampled, and data from a large set of regular farmer two-pen countings. A comparison with similar data from Scotland was also carried out. Analyses indicated that there was no significant difference between the lice mean abundances in the presumed most infected and the random pens. Furthermore, for site mean abundance to be estimated, the clustered lice distribution within a site needs to be taken into account. Data simulations based on Hardanger and Scottish data show that more pens than two needs to be sampled for reasonably accurate site mean: better few fish from many pens than many fish from few pens.

Methods

<u>Data collection</u>

A series of workshops for farmers and fish health personnel were arranged by HFN in the first half of the project (04-06), where methodology and suggestions for improvement were discussed. Important issues have been cleaner fish and how to integrate this approach with control by chemotherapeutants and strategic treatments in the fjord.

The HFN coordinated collection of lice data from farmers and organized sea lice counting by special teams. Counting teams and farm personnel/local veterinarians were trained in a standardized lice counting procedure and reported lice numbers to HFN using standard forms. Farm stocking data, environment and treatment history were sent from the farmers to HFN and the data are stored in their data base. The counting teams operated from April to early autumn. In 2004, lice were counted on 20 fish from all pens in 4 farms, and 80 fish in all pens in one farm. This was done to acquire data for simulation of different sample sizes in pens by Monte Carlo methods (Revie et al., 2007). In 2005-2009 c. 20 farms were visited by teams, whereas the other farms in the fjord counted and reported to HFN. The project had access to all data at HFN.

<u>Experimental strategic treatments</u>

Late Desember 2004, oral treatment using Slice (Emamectin) was carried out on 66% of the fish from the 2004 generation in 3 different periods. The rest of the fish were bath treated. The experiment was supported by Skretting, Biomar, EWOS and Schering Plough. Late December 2005, the oral treatment experiment was repeated, this time using Slice (Emamectin) on 67,3% of the fish from the 2005 generations in 3 different periods. The rest of the fish were treated as in 2004.

Protocol modelling

Sampling from two sites with high ICCs were simulated 1000 times, comparing the mean of each sample to the true mean of the dataset. In the case of *L. salmonis* mobiles at site S1 (ICC = 0.35) the effect of the sampling strategy of taking 20 fish from each of 2 cages was contrasted with the sampling strategy of taking 8 fish from each of 5 cages. In the case of *C. elongatus* mobiles (ICC = 0.39) at site S10 the effect of the sampling strategy of taking 6 fish from each of 3 cages was contrasted with the sampling strategy of taking 2 fish from each of 9 cages (Revie et al. 2007).

Results/Discussion

Strategic treatments

Results revealed new knowledge as to how low lice abundance can be maintained for when many sites in a fjord uses the same strategy. The experiment resulted in what we believe is a historically low lice infection pressure in the important spring period when the wild fish leave the rivers and swim through the fjord. This coincides with the stocking the spring generations cages (**figures 3 and 4**). The groups which did not follow the strategy carried nine times as much lice as the Slice-treated fish in the middle of May, which is the month of the spring run. Six sites used wrasse the following summer, and these did not need any further treatment until after October. Except for the spring period, the lice problem was greater in 2005 than the last two years. Some of the explanation seems to be the high salinity this winter, and the warm summer. A full account of the strategic treatments in the fjord can be found in Stigum Olsen (2005).

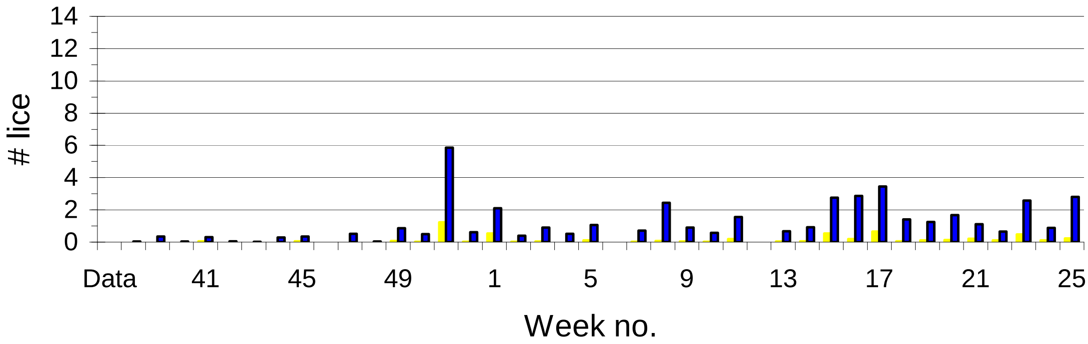

Figure 3. *Mean abundance of lice on 7 different farms using bath treatment or no treatment in the winter 2004-2005. Yellow bars: adult females, blue bars: total # lice.*

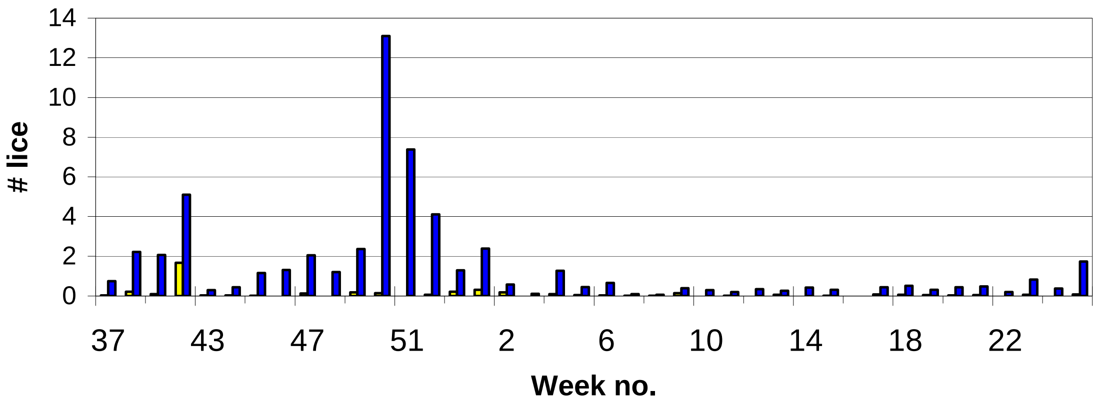

Figure 4. *Lice on 17 different farms using Slice winter treatment in the winter 2004-2005. The treatment lasted from Nov-Jan. Yellow bars: adult females, blue bars: total # lice.*

<u>Sea lice monitoring</u>
During 2004 counting data in farms included around 1000 fish every two weeks. A competition between the farms established to increase reporting of lice numbers proved to be a success. In 2005 the teams counted lice on 20 sites (including 3 generations of fish) between April and August. Collected data from 2004-2005 confirm low prevalence of lice in areas where the majority of sites follow the same strategy. Results showed that the lice problem was even smaller than 2004 for the farmed fish (2004 was also a very good year), see **figure 5**. As for 2003 and 2004 it looked like there was a higher abundance of lice in the outer part of the fjord, most likely because of the higher salinity in the area, but also due to lack of cooperation from the farmers in those parts. This was further investigated later, see below.

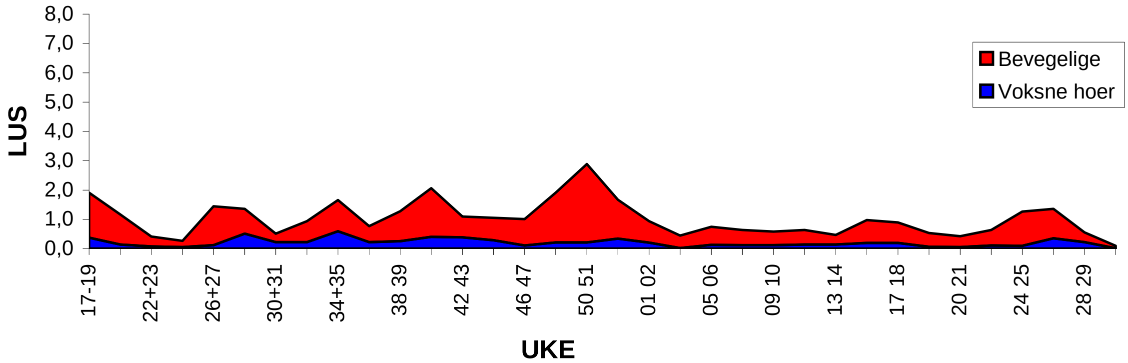

Figure 5. *The development of both mobile and female lice in the whole of the fjord 2004 and 2005*

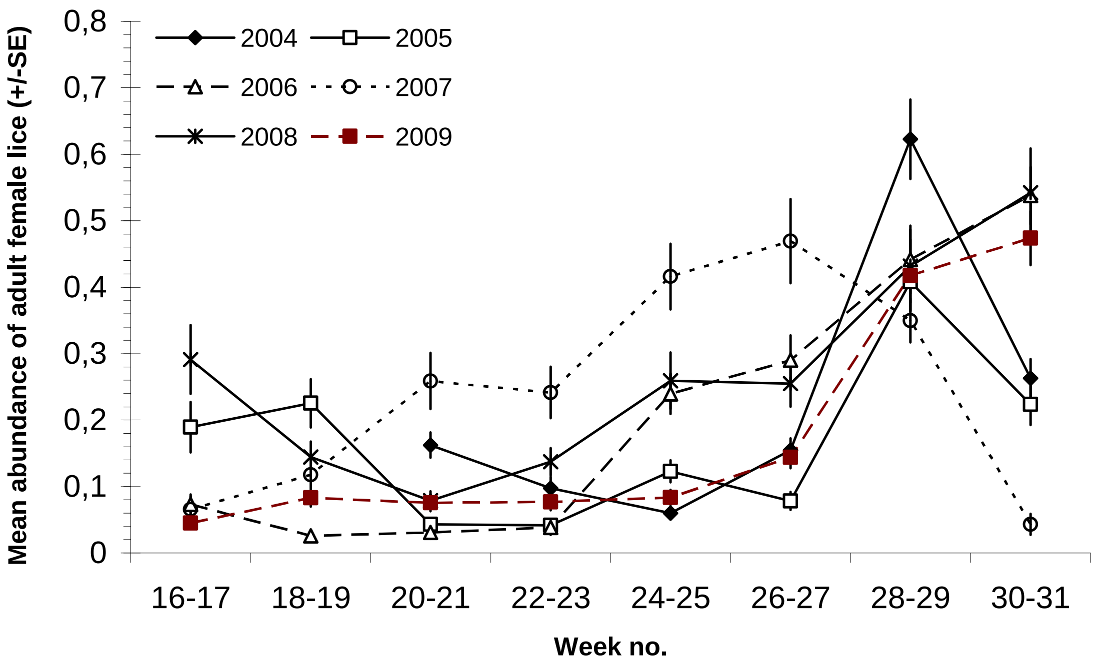

Figure 6. *Mean abundance of adult female salmon lice in the monitored salmon farms in the Hardanger fjord 2004-2009. n = 600-800 fish per data point.*

The strategic treatments in the winter continued after the “experiment” in 2004-2005, and results for 2005-2006 are reported by Stigum Olsen (2006). The results for this winter seemed somewhat less encouraging than the years before, possibly due to higher temperatures and salinity (see below). In the farms, for the whole project period, the development in the mean numbers of adult female lice follows mostly the same pattern, but there are some noteworthy differences between years. The HFN goal of 0.25 adult female lice per fish in the critical period between week 17 and 20 was reached in 2004 (data for week 20 only), 2005, 2006 and 2009, but not in 2007 and 2008 (**figure 6**). The year 2007 in particular was characterized by a higher mean in week 20-27, whereas 2004 exceeded the national lice limit in week 28-29. This limit was otherwise only exceeded in week 30-31 in 2006 and 2008. The HFN limit was breached in all years in week 28-29, showing that the effect of the strategic treatments in winter did not extend to this time point.

<u>Differences between inner and outer fjord</u>
Lice counts from 2004-2006 were used to investigate the differences in lice production between the inner and the outer fjord. The HFN zones B and C (north and east of the line between Ånuglo and Herøysund) and zones D and E (south and west) were used (see Heuch et al. 2009). In the analysis variables concerning the types and timing of lice treatments were included, as well as fish weight, salinity year and interaction terms. Exploratory data analysis showed that water temperature had no significant effect. Results clearly showed that the two innermost zones, B and C, had the lowest lice mean abundances, whereas the outermost zones, D and E, consistently had more lice. Differences in mean abundance of adult female lice between the zones were due to salinity differences, but treatments administered strategically also significantly reduced adult female lice abundance. Furthermore, the type of medicine used appeared to influence lice abundance, as did fish weight (Heuch et al. 2009). This supports the early results from the comparison between Slice and pyrethroids (**figures 3 and 4**, Stigum Olsen 2005).

<u>Lice counting protocols</u>
An MS Access database was designed for the study. The data from the lice counts were collected by the HFN and subsequently fed into the database. The clustering of lice on fish was examined using the data from the all pen counts. It was apparent that the lice abundances in the Hardanger data were much lower than in Scottish data used for comparison. Clustering between units within a data set can be quantified with the intra cluster coefficient ICC (Revie et al. 2007). Significant ICCs in the two data sets were found for prevalences down to 21%-23%, both for chalimus and for mobile stages. ICCs did tend to be larger at higher prevalences. Scottish data were used to model different sampling strategies at high ICCs (Revie et al. 2007, **figure 7**). The degree to which the sample means depart from the true mean is seen in a distribution plot of the deviations for each sampling strategy. The strategies use the same total number of sampled fish, but when fewer cages are sampled there is a greater chance of considerable deviation of the sample mean from the true mean. It is apparent that in most cases the estimate is 0-1 louse away from the true mean when more cages are sampled.

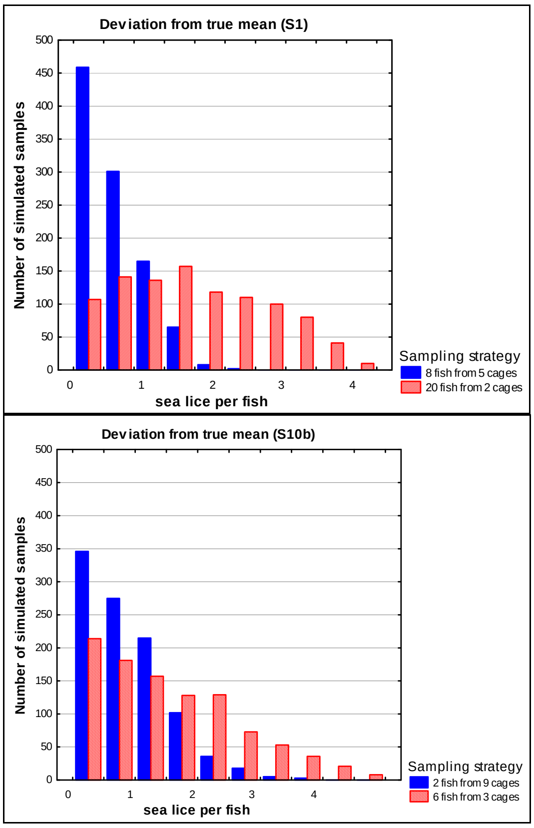

Figure 7. *Results from 1000 runs of a Monte Carlo simulation illustrating the difference in sampling strategies on likely deviations from the true mean in the presence of ICC for two farms in Scotland: site S1, ICC = 0.35 (L. salmonis mobiles); site S10b, ICC = 0.42 (C. elongatus mobiles). From Revie et al. (2007).*

The Norwegian regulations have demanded the reporting of lice from the presumed most infected pen on the site, on the assumption that this could be identified. Statistical analyses from the Hardanger data showed that the presumed "worst" pens (in terms of lice abundance) were frequently not worse than the other randomly chosen sampled pen on the site (Heuch et al. in preparation). The two reported pens therefore may be seen as two random pens. In the presence of significant clustering, however, such a sample of two pens form a site will give a poor estimate for the site mean abundance (**figure 7**).

The quality of farmer lice counts

The parallel collection of lice counts by farmers (FC) and by the dedicated "telleteams" (TT) gave the opportunity to compare the accuracy of the counters. A data set collected in 2004-2006 was examined. In addition to counting, the following variables were included in the analyses: Inner (zone B and C) and outer (zone D and E) fjord, year class of fish, time of data collection and interaction terms. The analyses were done separately for the three stadium groups: sessiles, mobiles and adult females. There were instances of one counter finding more parasites than the other, but generally, there were no significant differences between TT and FC (Heuch et al. in preparation).

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WP 3 – Understanding physical oceanographical factors on salmon lice abundance and distribution in the Hardangerfjord

Lars Asplin (IMR) and Anne D. Sandvik (IMR).

Summary – WP 3
The physical environment in the Hardangerfjord varies on time scales ranging from hours up to multi decadal. The shorter time variations in currents and salinity affect the distribution of the planktonic salmon lice in the fjord system. On the inter-annual scale, we find relative large differences in the environmental conditions between years. This influence the abundance and distribution of the salmon lice. Numerical model results shows that spreading of planktonic salmon lice can be rapid and potentially long distant. It is necessary to consider large regions in order to manage the salmon lice problem.

Introduction
Knowledge of the physical environment of the Hardangerfjord and its variability is important to understand the variability of the ecosystem. The abundance and distribution of salmon louse is influenced in various ways by the fjord water temperature, salinity and currents. The WP3 is rather extensively reported as the work has been done in a synergy between especially two other IMR projects: EPIGRAPH (fjord ecology) and Surveillance of Salmon Fjords. In addition to the knowledge exchange between various projects, information gained from WP3 has been extensively used as a basis for the role IMR has as a governmental advisor in e.g. questions related to salmon lice as a threat to wild fish.

Materials and methods
We have used both field observations and numerical model simulations to achieve information of hydrography and currents in the Hardangerfjord. We have also used a numerical model for growth and distribution of salmon lice to assess various distribution patterns.

Field observations
Observations of salinity and temperature (hydrography) were made primarily at eight fixed cross sections along the fjord from the mouth to about 140 km inwards. Usually the upper 30-50 m were measured with 3-5 stations at each cross-section. The measurements were made with a SAIV ctd sonde model SD204 (http://www.saivas.no). A total of 28 surveys have made hydrography measurements (**table 1**), although some surveys have used a reduced number of stations.

***Table 1**. Months where hydrography measurements were made in the Hardangerfjord.*

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004					x	x						
2005	x			x	x	x						
2006					x	x						
2007					x	x						
2008		x	x	x	x x	x	x		x		x	
2009		x	x	x	x	x	x		x	x		x

In addition to the dedicated surveys, we have measured hydrography from an observational buoy positioned in the middle part of the Hardangerfjord (N59 59.2, E5 55.2). This platform measures salinity and temperature as 10 minutes mean values from 3 and 10 m depths. The data are uploaded to the internet every hour (http://data.nodc.no/observasjonsboye).

Numerical model
We use a coupled numerical model system consisting of several sub-models and data sources to run the fjord model giving information on hydrography and currents in the Hardangerfjord. This model system uses an ocean model for open boundary conditions to the fjord model, a wind

model for atmospheric forcing and a hydrology model for riven runoff. The fjord model has horizontal resolution of 800 m, and we also run a version with 200 m horizontal resolution. In the vertical the resolution varies, but we resolve the upper ~20 m with 15 vertical levels to capture gradients from winds and freshwater runoff.

Driven by the results for currents and hydrography from the fjord model, we simulated spreading and growth of salmon lice (Asplin et al. 2010). The model salmon louse is given certain behavior, as diel migration and avoidance of water with salinity less than 24 ppt. In particular a period from May 2007 has been chosen to illustrate results from this model.

Results/Discussion

Understanding the variability of the physical environment can in many ways contribute to understand the abundance of the salmon lice in the Hardangerfjord. The values of temperature have an influence on the growth of the lice and the salinity influences the distribution of the planktonic salmon lice stages. The currents also influence the distribution of the planktonic salmon lice from the many sources, typically fish farms, throughout the fjord.

All the physical parameters vary on many time scales, going from hourly up through seasonally to inter-annually and multi decadal scales. The observed salinity at 3 m depth from the observational buoy in the middle of the Hardangerfjord serves as an example of variability on a shorter time scale (**figure 8**). The amplitudes can be many salinity units, and the changes are caused by either advection of water or a vertical shift of the halocline. Temperature and current has similar variability.

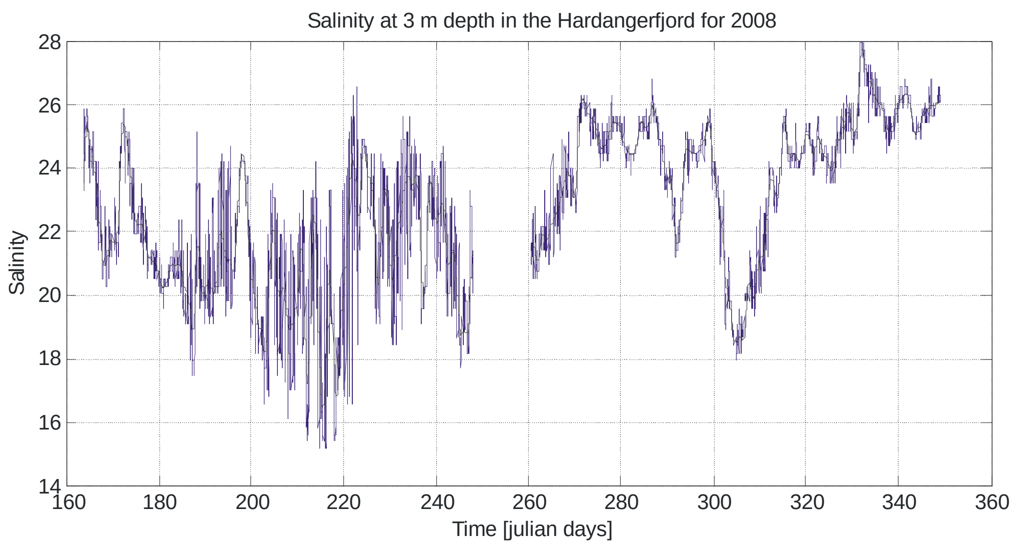

Figure 8. *Time series of salinity at 3 m depth from the Hardangerfjord buoy as 10 minute measurements from June to December 2008.*

The upper brackish layer in the Hardangerfjord will probably act as a shelter for migrating salmon smolt (Davidsen et al. 2008). This is created from freshwater runoff from more than 70 rivers in the fjord, and consists of a layer with salinity less than approximately 24 ppt. typically in a 5-10 m thick layer. The brackish layer contains the freshwater driven flow. We have found large inter-annual variations of the along-fjord extension of the brackish layer as well as absolute values of salinity and temperature of the layer, and measurements from mid June show a large extension in 2005, 2007 and 2008 and shorter in 2004, 2006 and 2009 (**figure 9**). The temperature of the brackish layer in June was relatively high in 2006, 2007 and 2008 while colder in 2005 and 2009.

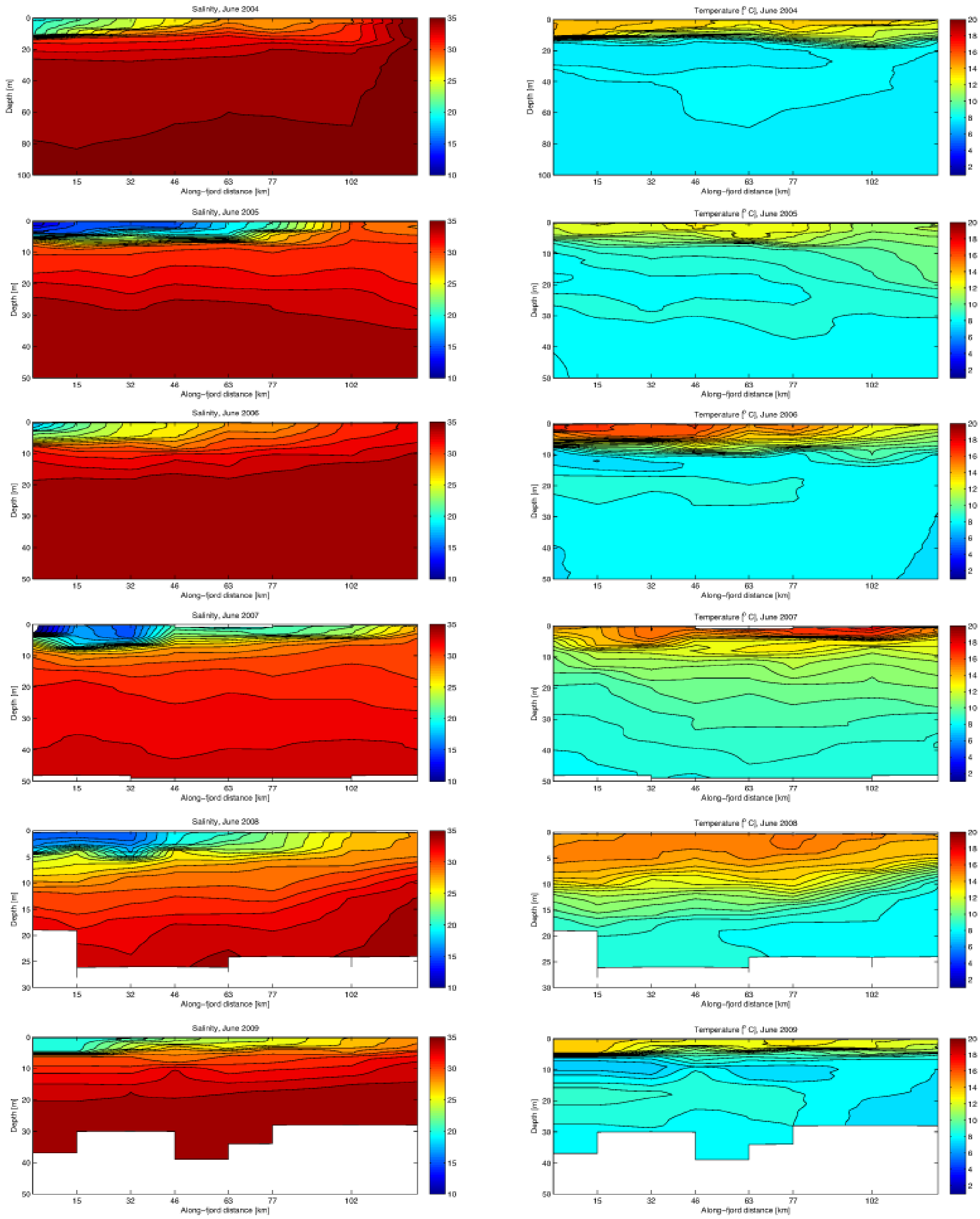

***Figure 9**. Along-fjord sections of salinity (left column) and temperature (right column) for the middle of June for the years 2004-2009 (2004 at the top, 2009 at the bottom).*

As to distribution and abundance of planktonic salmon lice, we have used realistic currents from April and May 2007 to simulate various scenarios (Asplin et al. 2010). The results are as previously found, and shows that the distribution might change rapidly (~2 km/hr), the lice might possibly spread far from its source (>100 km) and the spreading as a whole is variable (since the underlying parametres, like the currents, vary on many time scales form hours and upwards - including possibly longer periods of calm).

Today in the Hardangerfjord, more than 60 fish farm sites are distributed throughout the Hardangerfjord more or less uniformly. To illustrate a possible effect of clustering farms into larger units, we have made a comparison simulation. We have released model salmon lice from either 10 small farms or from 2 larger clusters of farms. 200 lice were released at each of the smaller farms, and 1000 from each cluster. The lice were released as a batch in the beginning of the experiment, starting at May 1, 2007 and running for 20 days. At the end of the simulation we have counted lice inside three control areas to get a view of geographical distribution (**figure 10**).

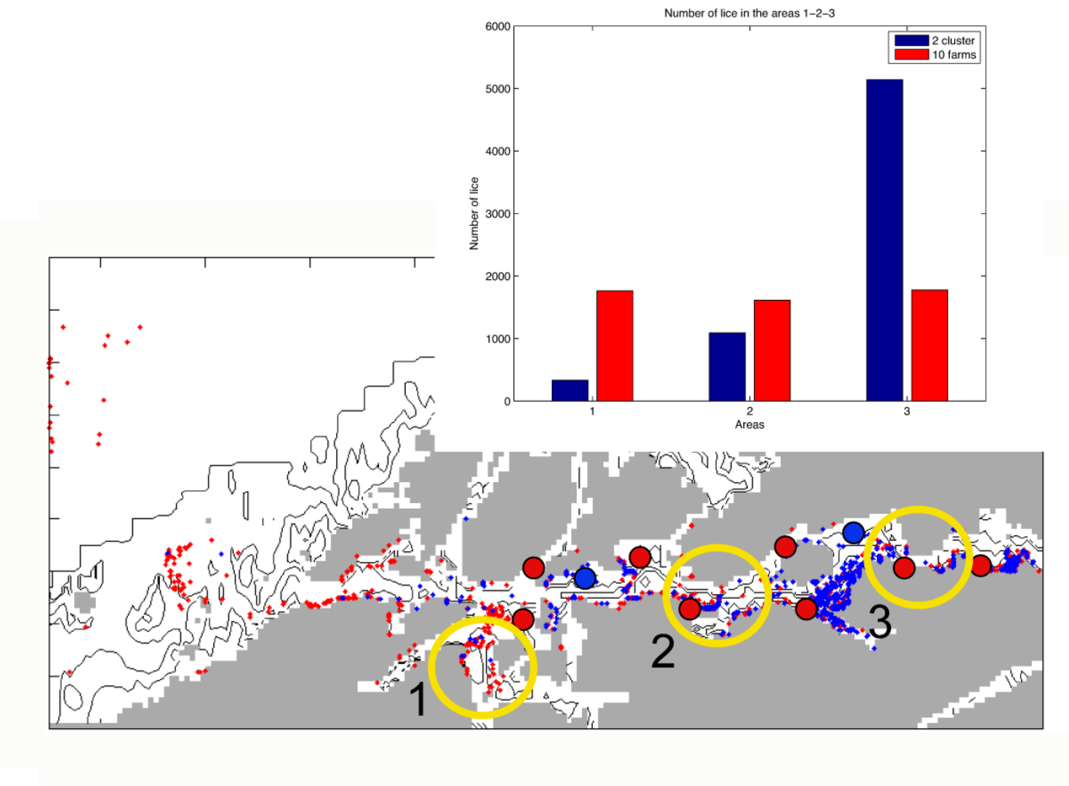

Figure 10. *Modelled distribution of salmon lice larvae at May 14, 2007. The red small dots are lice from the small farms (larger red dots) and the blue dots are lice from the two clusters of farms (larger blue dots). The yellow circles indicate position of control areas 1-3 where lice during the 20 days simulation have been counted. The result of the counts is shown in the bar diagram where the red bars represent number of lice from the 10 small farms and the blue bars represent number of lice from the two clusters.*

We see from the simulation that the equally spaced distribution of salmon farms will give an equally distributed abundance of salmon lice throughout the fjord (given the number of released lice is equal, which generally not is the case). The clustering of farms, especially in the inner part of the fjord, might retain lice in a more limited geographical area.

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WP 4 - Development of a mathematical population model for the Hardangerfjord system.

George Gettinby (UoS), Crawford Revie (UPEI), Fiona Lees (UoS), Chris Robbins (Grallator), Peter Andreas Heuch (NVI) and Bengt Finstad (NINA).

Summary – WP 4
The analysis of historical farm data from the Hardangerfjord between 2004 and 2006 confirmed that a number of factors were associated with an impact on chalimus and adult sea lice levels on farmed fish. These factors included zone, salinity, fish weight and treatment. Detailed examination of the epidemiological patterns of sea lice infections on autumn and spring stocked farms produced distinctive patterns and illustrated the importance of the strategic use of the two major treatments i.e. topical pyrethroids and in-feed emamectin benzoate. The sea lice difference equation simulation (*SLiDESim*) model previously used to describe and investigate the control of sea lice infections on salmon farms in Scotland was fitted using the autumn and spring-stocked profiles. A range of biological, environmental and production parameters were explored using the *SLiDESim* model to obtain the best fit to patterns. The modelling took account of the frequency and type of treatment practised on the farms. This predominantly consisted of in-feed treatments during the first year of production and bath treatments in the second year. The results showed that modelling could give a good first approximation to the observed Hardangerfjord lice patterns. However some features of the observed patterns were not captured and before the model can be used to evaluate optimal treatment strategies there is a need to further understand the underlying biological and environmental processes associated with lice populations.

Introduction
Sea lice infections on farmed salmon are a constant threat to fish welfare and a potential danger to wild salmon stocks which migrate to and from fjords within range of infected farms. The commercial viability of salmon production depends critically on maintaining salmon stocks with few lice infections. Each year Norwegian farms are faced with the task of how best to control the ubiquitous sea lice challenge and this generally means the constant application of expensive veterinary medicines. In the last decade sea lice levels on farms have been successfully controlled through the use of novel effective treatments (pyrethroids and emamectin benzoate) and the adoption of strategic regional control programmes to ensure adult female levels on fish are kept to a minimum. However, very little is known about the influence of management and environmental factors on sea lice levels at the farm level. It is imperative that the key factors be identified and their understanding used to improve the control of sea lice. This has been approached in a three stage process consisting of (i) statistical analysis of historical data for important risk factors, (ii) obtaining a clear understanding of the epidemiological patterns of sea lice on farmed salmon under different production systems and (iii) the construction of a mathematical model to explain the variation in patterns seen on farms. These activities have been possible in the Hardangerfjord II project because of collaboration with the other work programmes and the pooling of expertise and data from NVI, NINA and HFN. The monitoring and surveillance of infections on farms in WP 2 has been important to the WP4 work programme. Although the approach taken has been on a different spatial scale to the oceanic and meteorological approaches adopted in WP1 and WP3, where the focus is on the fjord and not the farms, the work programmes have been complementary.

Materials and methods
<u>Risk factors for sea lice infection.</u>
Historical mean sea lice counts for chalimus and adult stages on fish sampled at two weekly intervals across Hardangerfjord farms in the years 2004 to 2006 were assimilated and analyzed using a general linear model to establish the significance of the risk factors: zone (B/C, inner fjord, and D/E, outer fjord), water temperature and salinity, fish weight and treatment interventions. Details of methods have been reported in Heuch et al. (2009).

Sea lice profiles on farms

The Hardanger Fish Health Network (HFN) operates in the Hardangerfjord on the south-west coast of Norway. Sixteen of the 18 fish farming companies that operate in Hardangerfjord are members of this network and aim to keep mean lice abundance below agreed levels, particularly during the wild smolt run. Throughout each production cycle, farms in the Hardangerfjord supplied their stocking information, together with sea lice counts and treatment data to the HFN. This allowed lice abundance and sea lice treatment interventions to be monitored across the fjord on a continuous basis. Additionally, dedicated 'telle' teams carried out detailed counting of lice on farmed fish between April and September each year. Farm stocking, lice count and treatment data were available for 69 salmon farms in the Hardangerfjord between 2004 and 2007, however not all farms were stocked every year.

In order to insure that data were of suitable quality, data supplied to the HFN by each farm were cross-checked against data collected by the dedicated counting teams. Where discrepancies arose, farms were asked by the HFN to check their own records and to supply the correct information. Once each farm's data had been validated, the two data sets were merged to create one master dataset. It was not always possible to resolve all data issues and where data could not be verified they were not used.

Mathematical modelling of sea lice infection

The predictions of sea lice dynamics are based on a mathematical representation of the *L. salmonis* populations that has been described in Revie et al. (2005). The model is based on six interacting compartments that describe five key stages of *L. salmonis* development as well as the potential for external infective pressure, see **figure 11** which also shows the inter-compartmental interactions. Each compartment represents a density of lice per fish and is modelled using a delay differential equation to ensure that population dynamics maintain an appropriate biological development time.

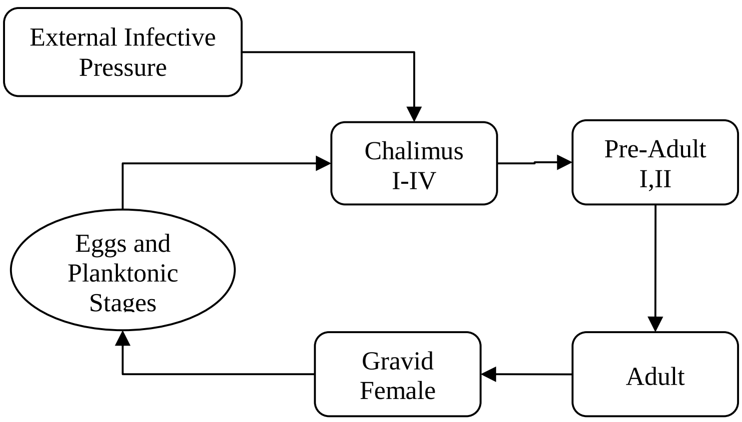


Figure 11. *Diagrammatic flow chart of the key stages of L. salmonis life-cycle associated with modelling the infestation of sea lice on Atlantic salmon farms.*

Results

Risk factors for sea lice infection

Table 2 shows the results of the GLM analyses for risk factors associated with adult female abundance. The results indicated that abundances were influenced by each of the factors fish weight (heavier fish had more lice), the zone (inner zones B and C had less lice than outer zones D and E), the salinity, and treatments given before and during the lice counting period.

Table 2. *GLM analyses of adult female L. salmonis lice counts, illustrating factors fitted in each model and their associated level of statistical significance.*

(a)	Factor	Levels	Type
	Mean fish weight	-	Continuous co-variate
	Site (Zone)	Sites 1 to 43	Random
	Zone	B, C, D, E	Fixed
	Year	2004, 2005, 2006	Fixed

Fitted linear model and significance associated with factors

sqrt(adult female *L. salmonis*) = weight [p = 0.000] + site [p=0.000] + zone [p = 0.007]
+ year [p = 0.015] + zone x year [p=0.000]

R-sq = 40.00%

(b)	Factor	Levels	Type
	Mean fish weight	-	Continuous co-variate
	Site (Salinity level)	Sites 1 to 51	Random
	Salinity level*	Low, Medium, High	Fixed
	Year	2004, 2005, 2006	Fixed

Fitted linear model and significance associated with factors

sqrt(adult female *L. salmonis*) = weight [p = 0.000] + site [p = 0.000] + salinity level [p = 0.000]
+ year [p = 0.020]

R-sq = 39.80%

(c)	Factor	Levels	Type
	Mean fish weight	-	Continuous co-variate
	Site (Salinity level)	Sites 1 to 51	Random

Salinity level*	Low, Medium, High	Fixed
Pre-treatment type	None, Pyrethroid, Emamectin benzoate	Fixed
Peri-treatment type	None, Pyrethroid, Emamectin benzoate, Both	Fixed

Fitted linear model and significance associated with factors

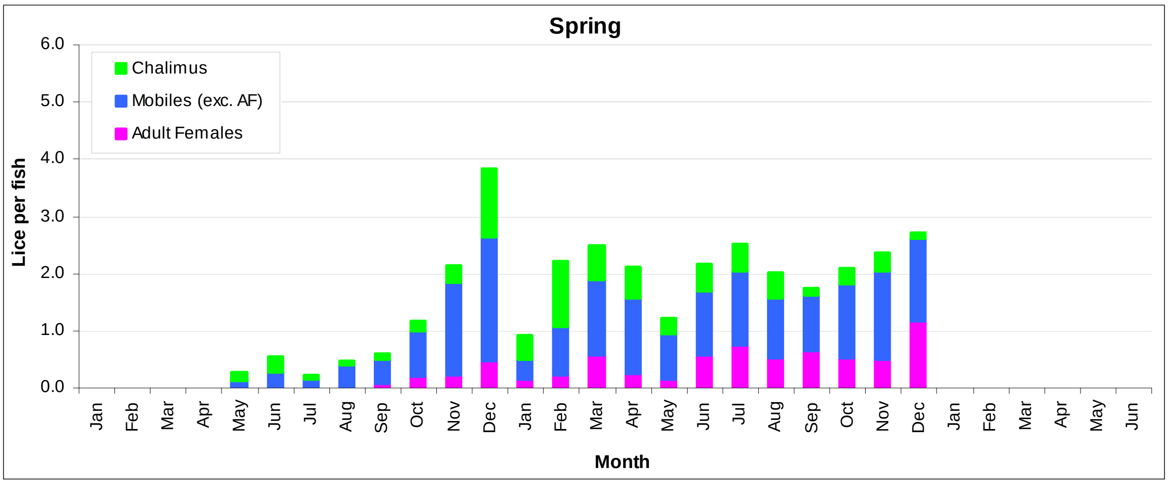
sqrt(adult female *L. salmonis*) = weight [p = 0.000] + site [p = 0.000] + salinity level [p = 0.024] + pre-treatment type [p = 0.001] + peri-treatment type [p = 0.011]

R-sq = 41.75%

* There were no medium salinity sites in 2005 and no low salinity sites in 2006.

<u>Sea lice profiles on farms</u>

The HFN merged datasets were summarized in order to build up a profile of lice abundance and treatment at each farm throughout each production cycle (Gettinby et al., in prep.). Most farms in the Hardangerfjord adhere to a single year class stocking pattern with either autumn or spring stocking, followed by a period of fallow. Two characteristic sea lice infestation profiles were developed; one for sites that stocked in the spring and one for sites that stocked in the autumn (**figure 12**). Mixed year class production cycles, and those where it was not possible to determine the year class of the smolts, were discounted from the profiles.



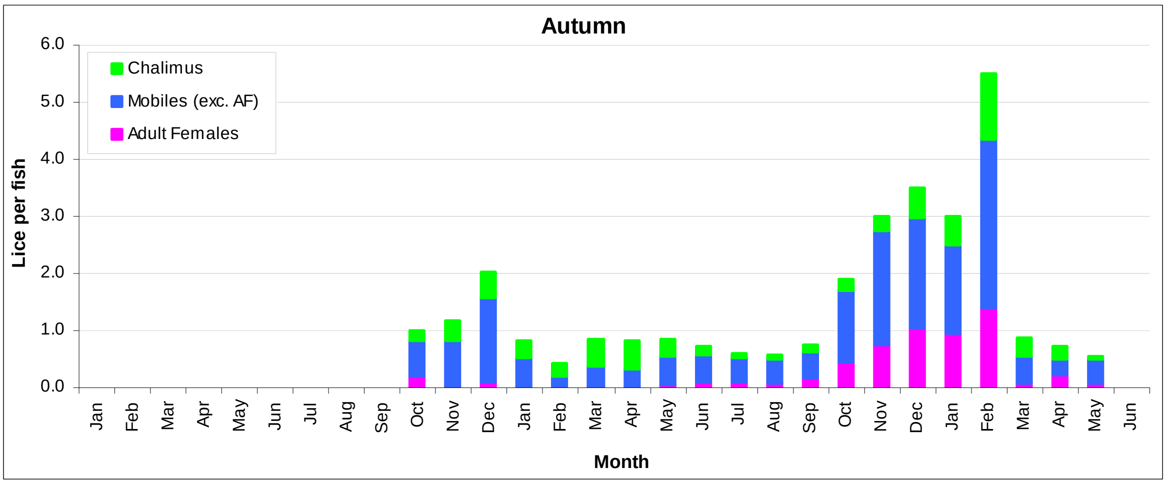


Figure 12. *Averaged levels of sea lice infection found on spring-stocked and autumn-stocked production cycles in Hardangerfjord farms between 2004 and 2007.*

The spring profile shows mean monthly lice abundance between May of the first calendar year of production and December of the second, based on 27 production cycles at 23 farms. The autumn profile is based on 24 production cycles at 21 farms and shows monthly lice abundance between October of the first year of production and May of the third calendar year.

Mathematical modelling of sea lice infection

The mathematical model was used to explore a range of biological parameters i.e. development times in the chalimus, pre-adult, adult and copepodid stages in combination with environmental factors including background infection, gravid female to chalimus ratio and survival of mobile and chalimus stages. The results indicate that a model very similar to that used to model sea lice infections on Scottish farms can be used to describe the patterns of sea lice observed on Norwegian spring stocked and autumn stocked farms. The model provides a fit to the general peaks and for the spring case the tendency for population growth towards the end of the cycle. However some features are not captured, such as the apparent mobile population oscillations between month 12 and month 18, and the increased chalimus levels between month 11 and month 15 for the spring stocked case.

Discussion

A study across many farms in the fjord confirmed that different sea lice levels were associated with factors such as zone, salinity etc. However such differences were small and may have been found to be significant because of the large sample sizes available for analysis.

The spring and autumn stocked farms were observed to have different patterns of sea lice counts on salmon during the first and second years of production, a novel, and somewhat unexpected finding. Spring stocked sites increased counts toward the end of the first year and on average remained high, whereas autumn stocked sites averaged low counts throughout most of the production cycle until the autumn of the second year when they rapidly escalated. These patterns required the identification of different parameters within the mathematical model to best explain the observed counts.

Examination of the modelling parameters indicated that stage development times for chalimus, preadults, adults, gravid females and egg to chalimus stages were unchanged for spring and autumn stocked farms, and no different to those previously adopted in models used to describe Scottish farms. Similarly, in these models sea lice attaching to fish were unable to successfully develop in the first 120 days of the production cycle. Survival rates of chalimus and mobiles were similar. However egg survival was such that fourfold more chalimus resulted from each gravid female on spring stocked fish compare to those on autumn stocked fish.

The findings suggest that a mathematical model at farm level has the potential to explain changes in the level of infection on salmon during the two-year production cycle. However more work is required to better estimate the parameters used in the model. Moreover, the model does not fully exploit the role of water temperature on stage development rates or the impact of salinity on survival. These are some of the areas in which further work will be needed if the model is to be validated and the effectiveness of treatment strategies investigated. This work has recently been completely for Scottish farms which shows that timing of treatment is critical and that even small changes in timing can lead to substantial gains in sea lice control Robbins et al. (2010). Such findings could equally apply to Norwegian farms and require further consideration.

Note it has not always been possible to fully analyze all the data available from the project as data assimilation, cleaning and analysing are lenghty processes. It would have been instructive to have investigated data collected in the final years for confirmation of reported findings and evidence of more recent changes.

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