SZENT ISTVÁN EGYETEM
ÁLLATORVOS-TUDOMÁNYI KAR, BUDAPEST

Institute for Fish Diseases
Department of Veterinary Pathology

DELOUSING METHODS AND THE RESISTANCE TO CHEMOTHERAPEUTANTS IN SALMON LICE (*LEPEOPHTHEIRUS SALMONIS*) IN NORWAY

Diploma work
Completed in partial fulfilment of the Veterinary Medicine Diploma course of the Faculty of Veterinary Medecine, Budapest.

By
Malin Røren
Szent István Univeristy, Budapest

Supervisor, Dr. Baska Ferenc

2015

Table of contents

Abstract

During my time as a student I come across many interesting cases but this one case about the salmon lice problem in Norway I couldn't let go. The reason it became so interesting was because when I first heard about this problem, I did not realize how big Norway was in exporting salmon. Later after reading different articles and talking to different fish biologist about the subject, I found it more and more interesting and now I want to become a fish veterinarian who works directly with this problem. There have been many different solutions to the problem but it is still not perfect because of development of resistance against the chemotherapautics used. I hope that I can joint this battle in the future and maybe make a difference.

Introduction

The salmon louse, *Lepeophtheirus salmonis*

The salmon louse is not really a louse at all. It is a tiny parasitic crustacean that lives by gnawing at the skin of the salmon. Wounds and scrapes that the salmon louse cause, makes the salmon more vulnerable to infections, and can also cause serious problems with the salt balance in the body. Sea salt is leaking in through the holes in the skin, and the salmon cannot seem to get rid of the salt in the body fast enough. If they become infected with too many lice, the salmon can simply die of their injuries.

The salmon louse goes through several stages of development. When the small larvae first come out of their nits, they can float in the water up to several weeks. After a few days they are ready to find a host, and with their hooks, they attach themselves to the first salmon fish swimming past. When attached, the salmon louse starts to gnaw at the skin, and after four or five weeks they are sexually mature. Female lice begin to produce eggs and continue to spew amounts of them as long as she gets to be in peace. If many fish in a shoal is infected with such adult females, the waters in large areas will soon be packed with new larvae in search for more fish skin to eat. This is also how the wild salmons along the coast and in the fjords get infected.

If we shall avoid that both wild and domesticated salmon get sick and die, it is important to keep the number of sexually mature female lice at a low level. Today fish farmers are obligated to ensure that there is no more than one such louse for every other salmon. If there are more, the salmon must be treated. Smolt and juveniles can get a special feed with substances that protect against lice. If the fish are grown, the entire cage is wrapped in tarpaulin and treated with chemicals that kill the lice.

Delousing is however expensive and can be a strain for the fish and for the environment. Therefore it is important to find a balance that improves the situation for all parties. In the longer term perhaps vaccines, resistant salmon stroke, better placement of plants and joint delousing take over for chemicals used today.

Figure 1. The salmon louse, ***Lepeophtheirus salmonis.*** Foto: Scanpix 2011

The Norwegian fish farms

Almost all Norwegian farmed salmon is exported. In 2013, Norway exported salmon to almost 100 countries worldwide, and the total export value for salmon and trout was NOK 42.2 billion. The total export value of Norwegian seafood was over 61 billion. EU is the biggest market for Norwegian salmon, while is the France's largest single market, followed by Poland and Russia. Climatic conditions and a long coastline with cold, clear water and great water exchange make it particularly beneficial to conduct aquaculture in Norway. Along the coast, generations reaped by the sea, and fish production through aquaculture is a natural extension of this tradition. Today Norway is the world's largest producer of Atlantic salmon.

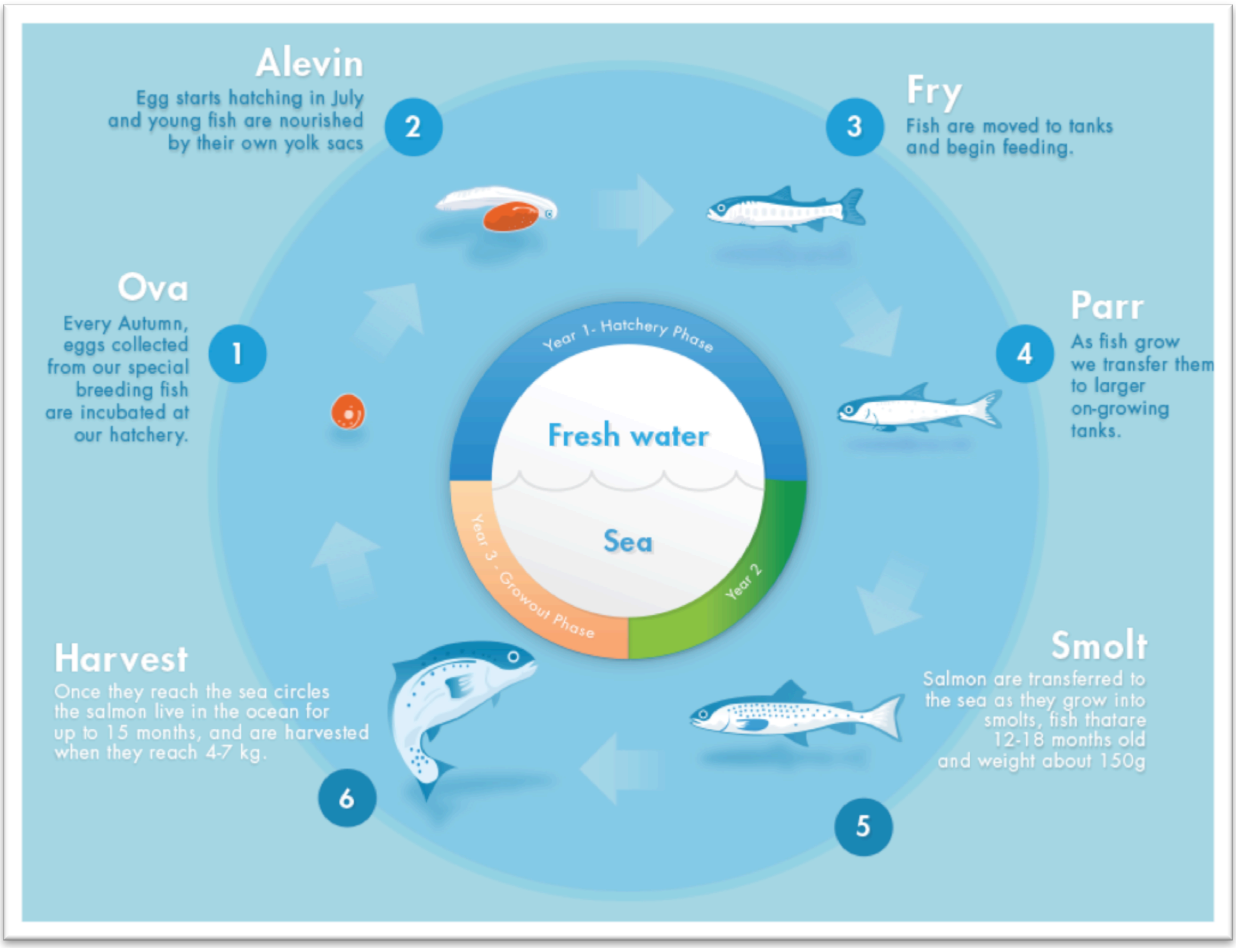


Figure 2. The life cycle of the Atlantic salmon.

Salmon is the dominant and most important commercial species in Norwegian aquaculture despite it all started with the trout in 1959. Since 1998, Norway has more than tripled the production of salmon, to about one million tones. The outlook for the coming decades indicates that both demand and production of salmon will increase significantly in the future.

The Norwegian salmon's success rests on the results from controlled food production and thus represents a steady supply to the markets. While many other ingredients from both land and sea included fasting seasons, the fresh salmon stand on the menu year round. Seafood from Norway is also associated with food safety and strict quality standards at all stages.

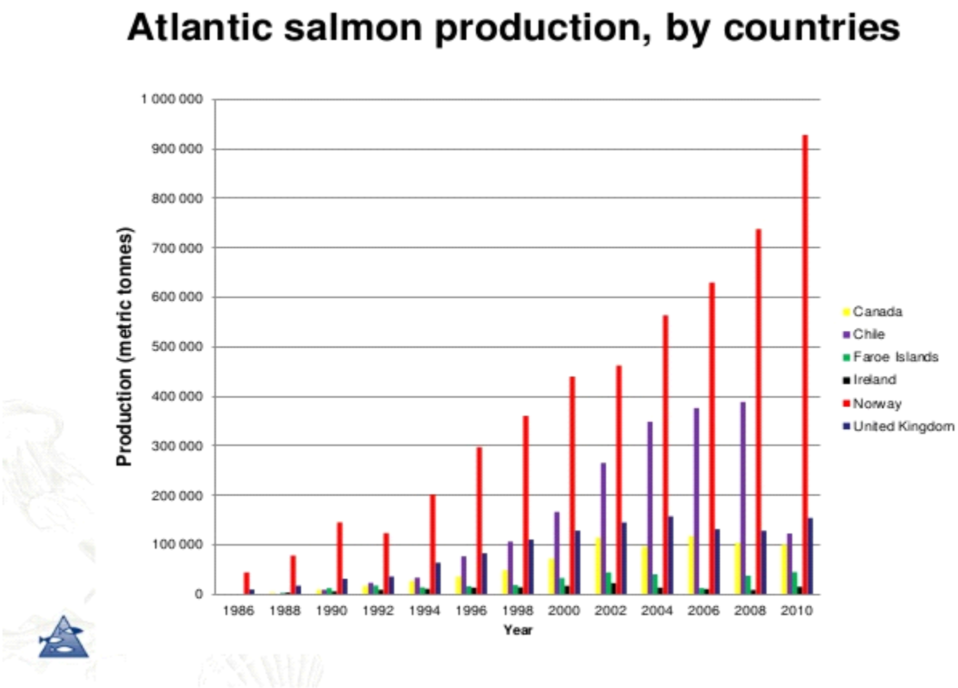


Figure 3a.

Production
Farmed salmon: World

	2006	2007	2008	2009*	2010*
	(1000 tonnes)				
ATLANTIC SALMON					
Norway	600	725	790	880	900
Chile	370	355	360	180	95
UK	125	140	145	150	160
Canada	115	110	110	120	140
Faeroe Is.	13	20	25	30	35
Australia	16	20	20	20	22
Ireland	15	15	15	15	18
USA	10	12	12	15	20
Others	3	3	3	5	5
Total	**1267**	**1400**	**1480**	**1415**	**1395**
PACIFIC SALMON					
Japan	10	10	10	10	10
Chile	115	120	113	120	135
Canada	10	8	7	7	7
New Zealand	10	10	10	10	10
Total	**145**	**148**	**140**	**147**	**162**
Gr. Total	**1412**	**1548**	**1620**	**1559**	**1557**

Source: GLOBEFISH AN 12201
* estimate

Figure 3b.

Lifecycle of *L.salmonis*

The salmon lice have a life cycle that consists of seven stages. The first two stages, nauplius larvae, are free-living, while the third stage infects the fish. On all these three stages the lice are less than 1 mm long. The later stages do the harm to the fish. The adult males are 5-6 mm long, and females 10 to 12 mm. They also have a tail like egg-string, so that the total length is nearly 30 mm. The adult individuals of lice mate on the host. They need only one host to develop into sexually mature individual. When the eggs of the females are fully developed, they are spawned out of the so-called egg-string before the small nauplius is hatching.

Generation time is temperature dependent approximatly 20 weeks at 5° C and 6 weeks at 15 ° C. After the eggs have hatched the lice develop through seven stages of moulting between each stage. The egg hatch and release the first of three free-swimming stages, naupilus 1 and naupilus 2 stage that becomes copepodites, which locates and infects fish. The first two spreads passively with water and the flow conditions will therefore determine how large an area they spread over. This stages, which can last for several weeks at low temperatures, may spread the lice over many kilometers. Copepodites is the infective stage, and goes from being free-living to become a parasite on fish when they find a host. It then undergoes two sessile stages ,chalimus 1-2, that stuck to the fish by means of a cords secured in the fish skin. Finally remaining two movable half adult stages (pre-adult 1 and 2, male or female) before the lice ends up moving adult lice. Pre-adult 1 and 2 and adult lice are moving around the fish, and it is these that make the greatest harm.

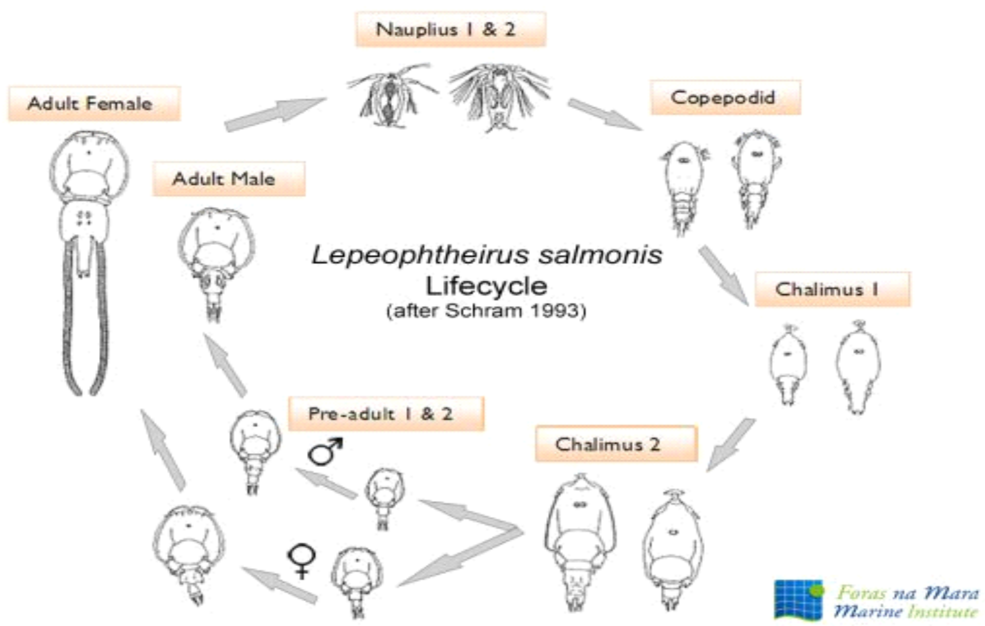


Figure 4a. Lepeophtheirus salmonis Lifecycle, after Scham 1993.

At the end stages of the parasites they feed on the salmon themselves by eating the skin, subcutaneous tissue, blood and tissue fluid. The damage that occurs depends on the development stage and number of individuals. The greatest damage is caused by the pre-adult and adult stage. Greatly affected fish is emaciated, and damage to the skin can go so deep that the head bones are exposed. A secondary infection with bacteria and fungi aggravates the condition, and without treatment, mortality is high. Salmon lice are also relatively common among wild salmon in the sea, but it falls off after a time when the salmon goes up in freshwater.

During migration from the rivers the salmon smolts can become infected with the free-living stages of salmon lice. Such infections can lead to increased mortality, and it has been shown that infected salmon spend longer time in the ocean before migrating back to spawn in the rivers.

Salmon lice are widespread in all oceans on the northern hemisphere. It is common along the coasts of Norway. According to the Institute of Marine Research recent research has shown that there are big differences between salmon lice from the Pacific ocean and the Atlantic ocean. They can now be considered as two subspecies, but because it is possible to cross the two, the inherit substances is not so different that it concerns two species. Salmon lice from the Atlantic ocean belongs to the race Lepeophtheirus salmonis salmonis, and from the Pacific ocean subspecies Lepeophtheirus salmonis onchorhynchi.

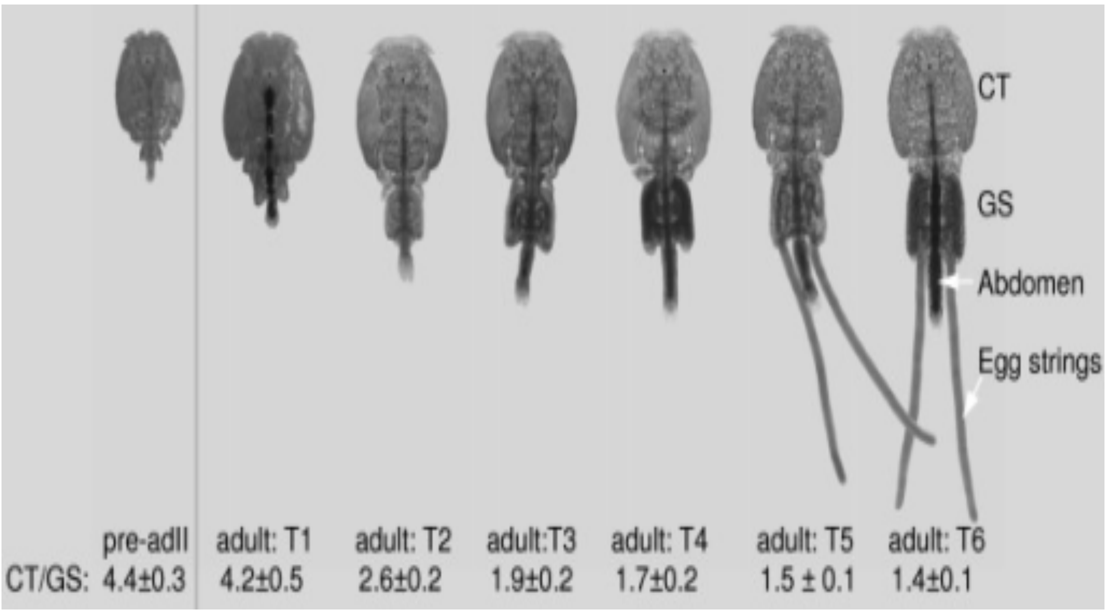


Figure 4b. Post molting growth of adult female ***L. salmonis.***

The different delousing methods

As mentioned, the ectoparasitic salmon lice, causes skin lesions followed by osmo-regulatory problems and thereby exposes the salmon for secondary infections. Damages and losses caused by the salmon lice infestation currently represent one of the largest health and economic problem within Norwegian aquaculture. High prevalence of salmon lice considered also as a serious threat to wild populations of salmon. Use of drugs to combat salmon lice in fish farms has raised questions among others environmentally toxic effects. To avoid the development of resistance to salmon lice means it essential to ensure that these are used optimally and that treatment only happens on indication.

To combat salmon lice we have biological methods (wrasse) and chemical methods (use of drugs). Mechanical methods such as sprinkling are also tested. Preparations against lice are presented in Table 1. When choosing which preparation to use against lice, it is important to take into consideration, that it is preferred that the preparation we use have the least harmful effects under similar circumstances. The veterinarian needs environmental documentation and professional assessment of the different medicinal products environmental effects, to make a good decision on which preparation to use.

Table 1. Drugs used against salmon lice in Norway.

Classification	Active ingredient	Trade name
Pyrethroids	**Cypermethrin** **Cis-cypermethrin** **Deltamethrin**	**Excis vet.** **Betamax vet.** **Alpha Max**
Pyrethrum	**Pyrethrum extract**	**Py-sal vet.**
Organic phosphorus compounds	**Azamethiphos**	**Salmosan vet.** **Trident Vet.** **Azasure Vet.**
Chitin inhibitors	**Diflubenzuron** **Teflubenzuron**	**Lepsidon vet.** **Ektobann vet.** **Releeze vet.**
Avermectins	**Emamectin**	**Slice vet.**
Disinfectant	**Hydrogenperoxide**	**Akzo Nobel.**

Development of resistance

To avoid the development of resistance to salmon lice, it is essential to ensure that these preparations are used optimally and that the treatment only happens on indication.

It is not unlikely that Norway may face a real resistance problem in the delousing agents in the near future. Unilateral and extensive use of bath treatment pyrethroids has dominated lice treatment in recent years. A lesson learned from similar therapy areas in other animal species suggests that just such practices (unilateral therapy) may contribute to creating a resistance situation.

General conditions

Good corporate hygienic principles like the separation of the different generations and fallowing are important prerequisites to defeat the salmon lice. When performing different procedures, for example fish handling by the well boat, the salmon lice may fall off the fish. If this happens it is important to filter the lice out and destroy them. According to regulations on biosecurity measures at the fish slaughterhouses, processing plants, etc. all the sewage from the butcheries must pass through a screening device and be treated so that it does not involve the danger of infection. To achieve an effective fight against the salmon lice it is important that water from unloading sites are drained to the sewer and that the requirements for the wastewater treatment at the slaughterhouses is observed. Documentation of the lice levels made in accordance with the guidelines from Statens dyrehelsetilsyn (State Animal Health Department),taking into account the total number of lice, the different lice species and the frequency of lice in various developmental stages. These guidelines are considered the minimum requirements for documentation of the lice levels. Wrasse should always be used where this is possible, otherwise the drug treatment is used if the incidence of lice exceeds the upper recommended limit, and preferably before the lice develop into adult stages.

Figure 5. Well boat

Control of salmon lice infestation on salmon < 500 g, in individual plants.

After the smolt is set out into the salt water cage, the wrasse is used wherever and whenever this is possible. Geographical (climatic) constraints in wrasse occur. The following recommendations assume that wrasse is used (where possible) as an integral part of combating lice.

Early treatment of smolt

If there is a moderate salmon lice infestation composed of only larvae (or some pre-adult stages and adult males) immediately after release (early June), the oral treatment is used if their appetite is good. The risk of IPN(infectious pancreatic necrosis) outbreaks at this time is an important argument to avoid stressing of the fish, and it is therefore preferred to give the oral treatment instead of the bath treatment. If there is an occurrence of the other sea lice species *Caligus elongatus*, the emamectin should be chosen over the chitin inhibitors.

Summer treatment of smolt

In late July or early August, the effect of winter / spring delousing decline and the level of lice increases. If there is a large salmon lice infestation, or if sexually mature females begins to emerge as an indication that the wrasse are unable to keep the level of lice below the minimum limit, it is necessary with drug therapy in addition to the usage of wrasse. If the wrasse is not used, the threshold limit for usage of drug therapy will be lower; total number of 2-5 louse per fish. If oral therapy is indicated, emamectin is considered to be the first choice. It should be noted that there is not yet a broad practical and clinical experience using emamectin in Norway, and that the drug has not yet received marketing authorization. Emamectin have several benefits like operating characteristics and that the composition is easy to administer and have long-lasting effects. The drug also has better efficacy against the other sea lice species ,*Caligus elongatus*, than bath treatment. The addition of a new drug with a new antiparasitic mechanism to the therapeutic arsenal will help to reduce the risk for the development of resistance to pyrethrins / pyrethroids. It shall be mentioned that emamectin has a long withdrawal period, that the maximum effect is achieved only 14 days after initiation of treatment, and that the environmental impacts are not fully investigated. For all the oral treatment, it is assumed that the fish has no other diseases which decrease their appetite and feed intake. If there are big fish in the same locality (which should not occur upon completion of the separation of the different generations), this must be included in the selection of the therapy due to the long withdrawal period for emamectin. If the bath treatments are indicated, the pyrethroids are the first choice. Factors supporting bath treatment, is for example, very large salmon lice infestation that require rapid onset of effect or if the fish have decreased appetite.

Delousing before winter

Depending on geographic conditions, delousing before winter has to be carried out at somehow different times, but for most of the country November / December will be a relevant period for the delousing. In the therapy recommendation there is a difference between the treatment of autumn transferred and the spring transferred smolts.

Autumn transferred smolt

First choice is the oral treatment with emamectin. This provides long effect throughout the winter, and is a non-stressful treatment. If the fish have decreased appetite the bathe treatment with pyrethroid must be selected.

Spring transferred smolts

First choice is bath treatment with pyrethroids. This is preferred before the oral treatment with emamectin due to the long withdrawal time of emamectin and fish size at this time.

Winter and early spring delousing

First-line treatment at this time is bath treatments with pyrethroids. One should be cautious with all handling and processing of the fish at sea temperatures under 6 ° C and be aware that there is a lower margin of safety using pyrethroids at low temperatures.

Guidelines for bath treatment of smolt

<u>General criteria for the selection of bath treatment :</u>

• High total number of lice (all moving lice stages included sexually mature females)

> 10 per fish.

• Significant incidence of sexually mature females.

• Illness that reduce the fish's appetite.

Preparation options :

Pyrethroids are the first choice for bath treatment since they have an effect on all lice stages and have a relatively low toxicity on fish. Experiences from the field indicate that cypermethrin have limited effect on adult females lice. The bad effect of azametifos (organic phosphorous insecticide) compared with pyrethroids allows this only to be considered as a last choice by bath treatment, unless there is manifest or suspected resistance to pyrethroids.

Treatment

Good oxygenation must always be ensured with all the bath treatments. Whole tarpaulin is used in the cages up to 96 meters in diameter, if possible. The effect of this procedure is well documented. If you cannot use full enclosure with the tarpaulin and you have to wear skirts, the cages needs to be lined up, and the skirts must be at least two meters deeper than the bottom of the note. Comparative studies of the effect after bathing treatment with use of skirt and whole tarpaulin is not published. If there is poor water exchange at the site, it must be considered to set an upper limit on the number of bathing treatments per day. In addition, one must take into account local current directions, to avoid accumulative overdose in individual cages. Use of well boat is limited by availability, cost and time required for implementation of delousing. The well boat is considered primarily if it can be used simultaneously with other operations in addition to delousing. The time required for treatment of the whole plant must be taken into account and the well boat delousing is not suitable for constructions where the delousing believed to extend over more than 2-3 days.

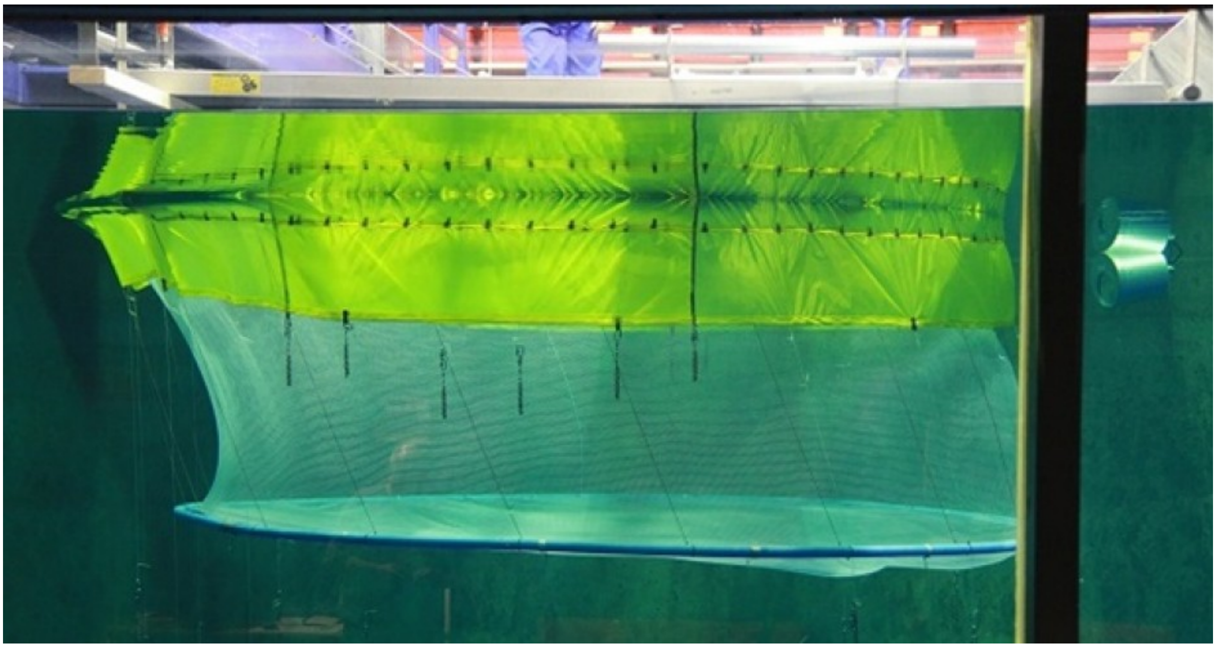


Figure 6. Skirts in the note . Photo : Sintef

Control of salmon lice infestation on salmon > 500 g, in individual plants.

In the following part of the therapy recommendation, we distinguish between two fish sizes:

• Fish between 500 g and 1 kg

• Fish > 1 kg

Treatment of fish between 500 g and 1 kg

When treating in the spring / summer the first choice should be the wrasse (all species) if there is access to grazing wrasse. If drug therapy is used, the first choice should be bath treatment with pyrethroids. The second choice is the use of oral agents.

Treatment of fish> 1 kg

For treatment against salmon lice in fish of this size, only the use of bath treatment is applicable. This also applies to the treatment of the other sea lice species ,*Caligus elongatus.*

Preparation options

The firsthand choice is pyrethroids. This choice is justified by efficiency, retention periods, price and that the pyrethroids are effective against most of the salmon lice developmental stages. In case of resistance to pyrethroids, other preparations have to be chosen. This is a situation that can be expected to occur in the future based on experience from treatment with pyrethroids in other animal species and in humans. In such a situation the following process for the choice of the preparation is recommended:

1) For fish that is relevant to slaughter within a period of 60-120 days it is recommended to use the bath treatment with azametifos.

2) For fish that is not likely to be slaughtered within the next 120 days the oral agents are recommended.

3) Hydrogen peroxide is perceived as a reserve composition (must not be used at temperatures> 13 ° C).

Whit the use of these treatments the zoo sanitary situation of the installations must be taken into consideration.

Treatment

The bath treatment should preferably be carried out in closed units as a well boat or in whole bag (tarpaulin). The second hand selection of method for implementation of bathing therapy is the use of skirts. It lacks evidence of the extent to which recommended treatment concentrations are maintained at skirt treatment. Treatment in open nets or incomplete treatment of plant is completely unacceptable.

Dosage

It is recommended to follow the dosages compared to the relative treatment like it is specified in the approved SPC.

Follow-up treatment

The effect of the treatment should be monitored by lice counts 7-10 days (depending on temperature) after completion of treatment.

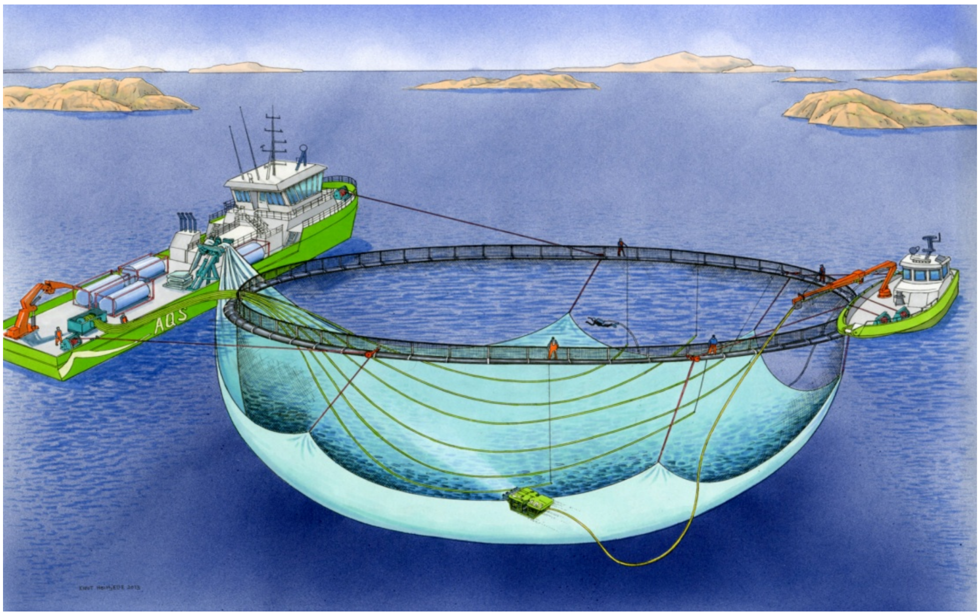


Figure 7. Delousing with bath threatment in Hydrogen Peroxide.

Strategic combat against salmon lice attack in Norway.

The long term goal for combating salmon lice in Norway should be that the aquaculture industry shall not give rise to infective stages of salmon lice (copepodites) free in the water column in the deployment of smolt and emigration of wild fish. Both of these situations take place around the same time in the various geographical regions. This means that it must not occur sexually mature females in the period 1-2 months before this. The only way to achieve this is for the expert group's perception through a clarification of existing "Goals 5 'in the' National action plan against lice in salmon": *It shall be carried out coordinated delousing along the coast during the cold season.*

Indication for delousing

No treatment should take place without prior indication to treat. Indication shall be in the “cage level” and if it is an indication of the cage level, the entire locality should be treated. “Blot treatment” of individual cages in a locality is not desirable and could contribute to the development of resistance. In addition, the blot treatment can lead to the re-infestation of treated fish originating from separate plant (own infection). Nevertheless, situations may arise where one can consider not treating some cages due to the absence of lice.

Nationally organized times for delousing

There should be a larger regional or preferably national delousing (unless there are indications) in November / December in connection with the decline in sea temperature. For northern regions it may be necessary to implement this as early as October. This will lead to a sharp decrease in the egg production and in addition cause the fish to meet the winter "lice free". National spring delousing should be carried out in March / April (if the indication exists) and organized centrally starting in the south and successive progression northward. Regional working groups should coordinate the implementation of this and determine the end date for the delousing. Nationally organized delousing in the summer is considered less relevant. However efforts should be coordinated locally for the delousing at this time of the year.

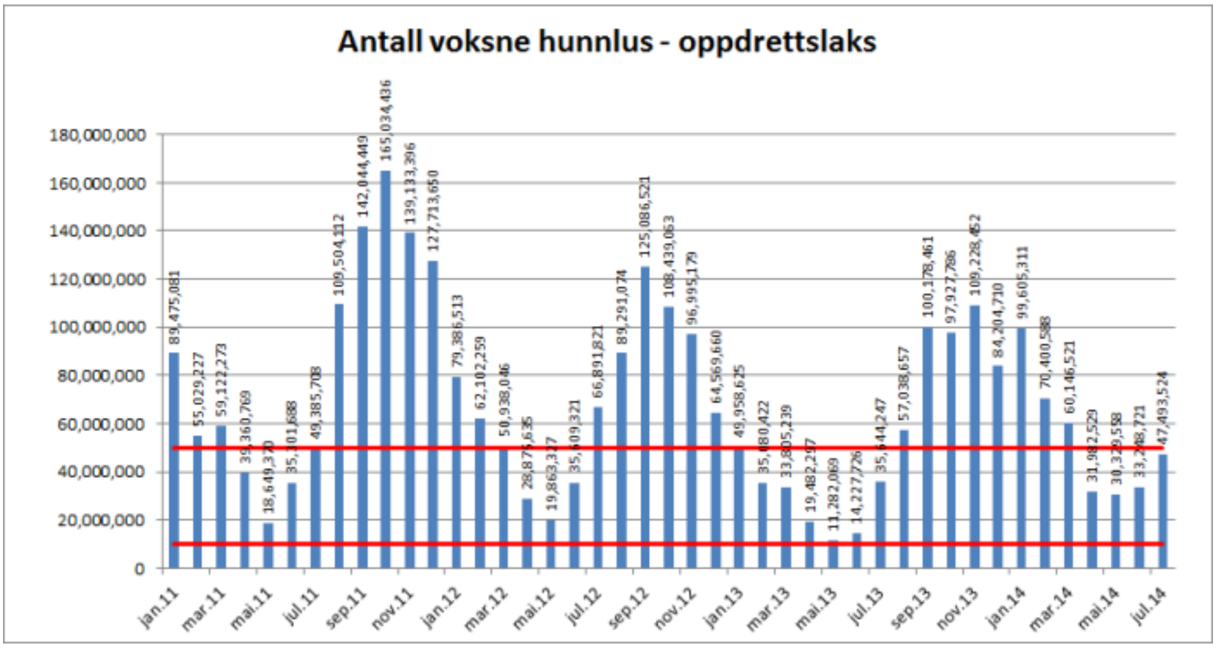


Figure 8 . Number of female louse in farmed salmon 2011-2014

Treatment regions

It is inappropriate to follow veterinary county boundaries in connection with implementation of a coordinated central delousing at the national level. Efforts should be made so most possible regions are processed simultaneously in connection with such measures.

Measuring results

It is essential to keep a continuous record of the lice amount throughout the year. Guidelines for how to do this are provided by the State Animal Health department and it is considered as a minimum requirement for documentation of the lice levels. Registration of lice occurrence on wild fish needs to be improved compared to today. Without a good practice for records of this it is not possible to document any effects of centrally implemented measures.

Limits on regional measures

Cages that have in average:

- 0,2 sexually mature female lice per fish

  or

- 3 lice (all moving lice stages included sexually mature females)

  per fish should be treated, also harvest fish.

The above values are an expert group's professional recommendation for the limits of how the regional measures optimally should be. Eventually, one should consider the implementation of these limits in existing regulations on salmon lice. Limits as stated in the regulations should be lowered gradually. However, this presupposes that the infection pressure in the plants will built gradually down and that there is acceptable evidence that this kind of action leads to positive effects on wild fish and that there are effective delousing agents available. To succeed in organized salmon lice control it is essential that the same action level also is conducted for large and harvestable fish.

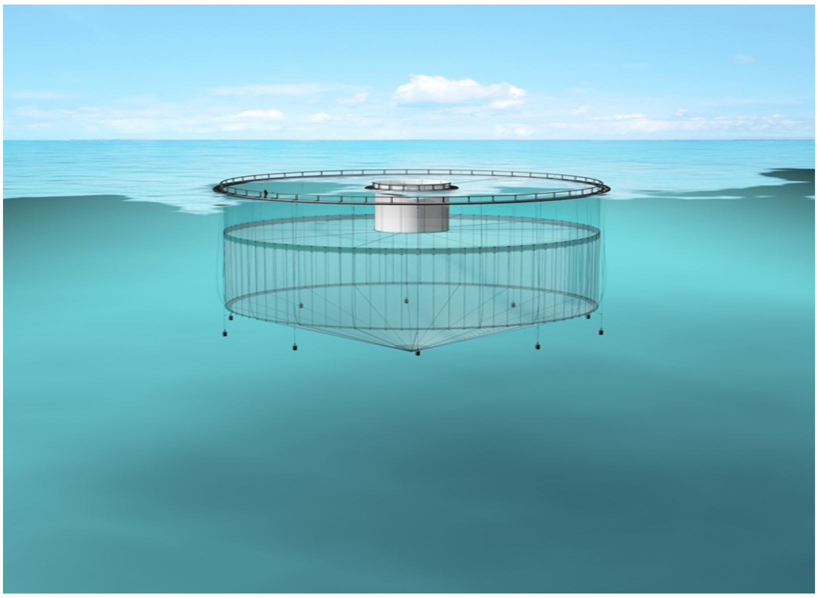


Figure 9. One merd .

Pharmacology of antiparasitic agents used against salmon lice.

A variety of factors affect the incidence of salmon lice in fish farms ; water exchange on the locality, distance from other facilities, salinity, temperature, number of year classes in the locality, fallowing procedures, different notes, fish density, health status, hygiene, operational procedures etc. Dealing with the salmon lice problem can therefore vary between systems and

/ or regions. Optimal control strategies, based on the concept "Integrated Pest Management" takes into account all these conditions, and set drug treatment in a broader context where optimization of operational circumstances player a key role.

To combat salmon lice it has been – and it is partly still - used both biological methods (clean fish: goldsinny, rock cook, corkwing, cuckoo and ballan wrasse) and chemical agents which can be grouped as antiseptics (formalin and hydrogen peroxide), organic phosphorus insecticides (metriphonate, dichlorvos and azametifos) pyrethrins and pyrethroids (pyrethrum extract, cypermethrin, deltamethrin), chitin synthesis inhibitors (diflubenzuron and teflubenzuron) and one avermectin (emamectin).

Figure 10. Salmon infected with salmon lice. Photo : Bengt Finstad

Wrasse

Wrasse has already been an option for a decade instead of using the parasitic chemicals. Use of clean fish can be viewed both as a treatment and as a preventive measure to reduce the use of drug delousing. It has been found that wrasse from our waters can feed on parasites of other fish. Especially goldsinny and rock cook are effective clean fish, but also corkwing and cuckoo has attempted shown to be useful. These species used for the salmon which is the first year in the sea, and graze mainly the adult females of the species Lepeophtheirus salmonis.

Experiments with ballan wrasse have shown that this species can be used also on larger fish on their second year in the lake. In addition, cleaning fish also feed on fouling in the cage wall. In periods of low lice amounts and thus little food for cleaning fish it is important to feed particularly the ballan wrasse to prevent them from damaging the salmon around and on the eyes.

Wrasse species are more thermophilic than salmon, and there is sometimes a considerable high mortality during the winter. Wrasse used today is caught wild fish. An important question is that wild wrasse may bring infection with them in to the plant. It should therefore be a goal that salmon lice must be combated with farmed wrasse and not wrasse with unknown health status.

Figure 11 a. Ballan wrasse are ideal for eating lice off the salmon. Credit : Anne Mette Kirkemo.

Figure 11b. Wrasse eating lice off the salmon.

Antiseptics

Hydrogen peroxide is the antiseptic agent which in recent years has been used against salmon lice. Formalin has admittedly also been used occasionally, but not the last ten years.

Hydrogen Peroxide

Hydrogen peroxide is used in bathing treatment against salmon lice. It is heavier than water, and is added through a perforated plastic tube towed forward and back of the cage surface. The fish is usually crowded together under the treatment to reduce the amount as much as possible. The cage is surrounded by a dense plastic tarpaulin.

Hydrogen peroxide is probably taken up in the parasite in the same way as water. Under the influence of a catalyst (for example the enzyme catalase, metals, heat) decomposes hydrogen peroxide to water and oxygen gas. When this happens within the tissue (such as in the salmon lice) it blast the tissue structures apart. In parasites that fall off the fish under delousing with hydrogen peroxide, such gas bubbles are found, especially in the gut. In many cases, however, the parasite is not killed, it only let go of the fish, but can later attach itself again.

Hydrogen peroxide has only effect on moving salmon lice (pre-adult and adult). Typically, 80-85% of the lice in impressionable stages fall off during the processing. The attached larval stages does not seem to be affected in an appreciably degree.

Hydrogen peroxide is a strong oxidizing agent, and in concentrated solutions it is very highly corrosive. For delousing a solution of 50% is used. The usual dose at temperatures below 8 ° C: 1.7 kg hydrogen peroxide per m3 water for about 20 minutes. At higher water temperatures the dosage is 1.3-1.5 kg hydrogen peroxide per m3 water for about 20 minutes. The agent is not used in water at temperatures above 13 ° C, because the safety margin is too small. At approximately 10 ° C it is required approximately 250 liters of 50% hydrogen peroxide solution for the treatment of one net bag with output volume of 500 m3, crowded together to approximately 100 m3 during treatment. By misuse sometimes severe gill damage and significant mortality can be seen. It is not introduced withholding deadlines by treatment with hydrogen peroxide, and the substance is also considered not to require any MRL-value.

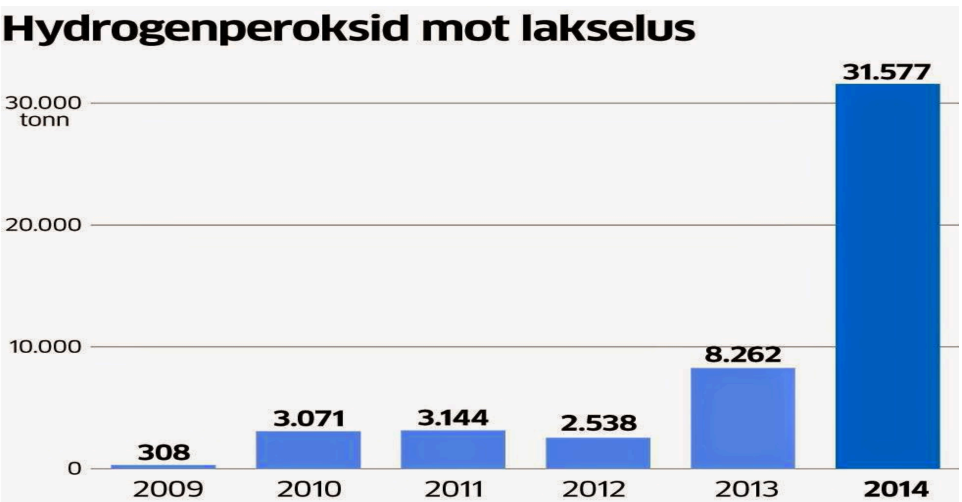


Figure 12. Hydrogen peroxide used against salmon lice.

Organophosphorus insecticides.

In Norway three organophosphorus insecticides has been used to combat salmon lice: metriphonate, dichlorvos and azametifos. Today only azametifos have authorization for use against lice. The treatment method is bath and consists of tarpaulin placed outside the usual note. The tarpaulin can be dense or open at the bottom ("skirt"). Thereafter agent in the right concentration is added. The calculation of the treatment volume can be difficult.

Organophosphates are fat soluble and are taken up by the parasite via the hydrophobic chitin layer and via parasite gills. They are also taken up in the fish, mainly through the gills. They are distributed to all tissues and organs, including the central and autonomic nervous system and neuromuscular end plates. Organophosphates have an inhibitory effect on the enzyme acetylcholine esterase.

The inhibition causes the transmitter substance acetylcholine not to break down, providing the stimulation, followed by blocking of the relevant receptors. Organophosphates bind almost irreversibly to the enzyme so that regeneration of the activity is mainly done by the new enzyme that is synthesized. The selective toxicity depends on that the fund quickly penetrates the parasite, while this takes longer in the fish. The relevant organophosphates have higher affinity for the parasites acetylcholinesterase than for the fishes. Finally, organophosphates are more rapidly metabolized to inactive products in the fish than in the parasite. Yet it is reported several cases with partially high mortality of salmon by organophosphate treatment.

Organophosphates act on adults (adult) and half-fiction (pre-adult) lice. Around 95% of the impressionable stages normally fall off during the first few hours after the treatment. However treated salmon will still be infected with the younger sessile chalimus stages, as the drug only have minor effect (approximately 25% mortality) on them. This does that the salmon must be treated again when the chalimus stages have evolved into pre-adult and adult. The dosage depends on the temperature in the ocean. Azamethiphos is more potent than dichlorvos, and less toxic to the user. Azamethiphos (Salmosan) is used in the concentration of 0.1 mg per liter of water with a treatment time of 20-40 minutes (the longest processing time at low temperature).Slaughter deadline is seven days. In the EU, azametifos received an MRL value for the muscle and the skin in natural proportions of 100 micrograms / kg.

Pyrethrins and pyrethroids

Pyrethrum is an extract from the plant Chrysanthemum cincerariaefolium. This extract contains naturally occurring pyrethrins and is a commonly used insecticide. In recent years it has, together with piperonyl butoxide, in small scale been used to treat farmed salmon for salmon lice, preferably in conjunction with procedures where the fish need to be addressed from the sea for weighing, sorting and similar.

Pyrethroids are synthetic or semisynthetic analogs of pyrethrins with similar pharmacological abilities. Pyrethroids are more potent and stable than the naturally produced pyrethrins.

The treatment method for pyrethroids like cypermethrin and deltamethrin are the baths and usually consists of a tarpaulin placed outside the normal note, in the same way as organophosphates.

The funds are relatively fat soluble and penetrates fast in the parasites cuticula / gills. They are also taken up in the fish, mainly over the gills. They are distributed to all tissues and organs, including the parasite's nervous system. They work on the peripheral nerves they by preventing Na + channels in the nerve cells to close normally after depolarization. The nerve cells' ability to is disturbed and thereby leads to coordination failure, hyperactivity, paralysis and death. Pyrethroids are very toxic for fish, but even more toxic for lice. It is this margin which is utilized therapeutic.

Pyrethrins and pyrethroids also act on the sessile chalimus stages. The toxicity is higher for pre-adults than for adult stages (most pronounced for cypermethrin). The treatment effectiveness is normally 95-99% of pre-adult and adult stages, somewhat lower at chalimus stages (about 85%). The adult and adolescent lice fall off the salmon a good while after treatment so the effect can only be recorded after 1-2 days.

Cypermethrin (Excis) is dosed with 5 micrograms per liter of sea water in one hour. Deltamethrin(Alpha Max) is dosed with 3 micrograms per liter of sea water in 30 minutes. The withdrawal time for both are three days, but for the new formulation of cypermethrin, (Betamax), the retention period is currently seven days. In the EU, cypermethrin received an MRL value for muscle and skin in natural proportions of 50 mcg / kg. Deltamethrin has not yet received any MRL value for fish.

Avermectins

Among avermectins the benzoate salt of Emamectin is granted exemption from approval for use against salmon lice in Norway. In Scotland, Ireland and Canada, ivermectin is also used. Emamectin is used blended in feed. It is taken up from the fish's intestines, and distributed to the appropriate tissues, including blood and skin slime. The bioavailability is not known. The excretion is slow compared other drugs used against salmon lice. The mechanism of action is believed to be similar as with other avermectins. Avermectins gives an elevated membrane permeability above chloride ions in invertebrates. This gives disturbance in signal transmission in and between the nerve cells. Avermectins is effective against both chalimus- ,pre-adult and adult stages of the salmon lice. The salmon lice impact can be reduced by around 90% or more in all stages compared with untreated controls. The effect is seen only after approximately one week, and lasts for up to ten weeks after concluding a cure. Emamectin (Slice) is dosed with 50 micrograms / kg daily for seven days. Preliminary detention deadline is 120 days. In the EU, emamectin have received an MRL 100 mcg / kg for muscle and skin in natural proportions.

Figure 13. Three adult females and a male louse (rightmost). Photo: T. Poppe.

Chitin synthesis inhibitors

Chitin is a polymer made up of units of the amino sugar D-glucosamine. The exoskeleton(shell) to insects and crustaceans consists of, large quantities of chitin. Chitin synthesis inhibitors diflubenzuron and teflubenzuron are used somewhat against salmon lice.

The funds is blended in the feed. It is sucked up from the intestine and distributed to the blood and tissues, including skin slime layer. Here come the parasites in contact with them. Bioavailability in salmon is approximately 30% for diflubenzuron, and approximately 10% for teflubenzuron. The substances have been in the media spotlight because one of the metabolites that may be formed by diflubenzuron, para-chloro-aniline, can be carcinogenic. When test animals are given large amounts of diflubenzuron this metabolite can only be detected in tiny amounts. However, this metabolite have not been detected by metabolism studies with radioactive marked diflubenzuron in salmon.

The substances are blocking normal production of chitin and thus the shell formation. The exact mechanism of action is not been completely clarified. Lice moult between the various stages of development and therefore will be affected by these drugs. All larval stages and the pre-adult are influenced (up to 90% mortality), but the means has no effect on the adult lice, since these do not undergo more shell changes. The dosage of teflubenzuron (Ektobann) is 10 mg / kg daily for seven days. Diflubenzuron (Lepsidon) are dosed with 3 mg / kg daily for 14 days. Currently, the retention period for both substances is 60 days, and independent of the water temperature. The EU has given diflubenzuron one MRL value for muscle and skin in natural proportions of 1000 micrograms / kg, while teflubenzuron has received an MRL-value of 500 micrograms / kg.

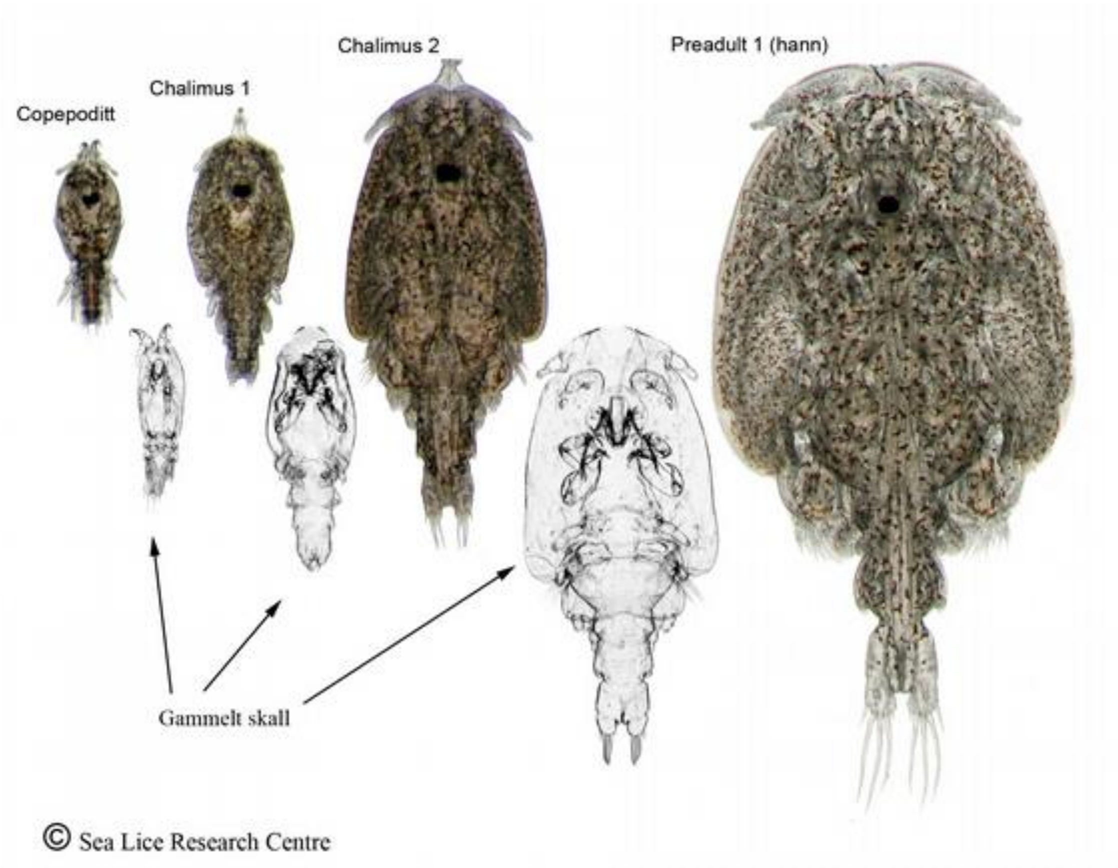


Figure 14. Showing the old exoskeleton (shell) after moulting.

Resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway.

Results obtained in the surveillance program for resistance to chemotherapeutants in salmon lice show a pronounced increase in prescribed medicines used as delousing agents. Furthermore, reduced sensitivity and resistance to the medicines tested in bioassays are generally widespread along the coast, but seem less prominent in the far north and far south. Compared to the surveillance in 2013, however, there seems to be a loss of sensitivity to deltamethrin and azamethiphos in Finnmark. The results for hydrogen peroxide were generally better than for other medicines, but loss of sensitivity was indicated in areas in Hordaland and Trøndelag.

Resistance to chemotherapeutants in salmon lice, Lepeophtheirus salmonis (also referred to as sea lice) has been reported from several countries including Norway .Episodes of reduced treatment effect, along with extensive field sensitivity testing of L. salmonis against pyrethroids, emamectin benzoate (EMB) and azamethiphos by the use of six-dose toxicological tests has brought about concerns of reduced sensitivity against the available chemotherapeutants. However, reporting of results from this extensive sensitivity testing has not been mandatory until 2013 and a comprehensive survey of the resistance status in Norway was first reported in 2014 . In order to obtain a survey of the resistance status of L. salmonis in Norway, and the use of chemoterapeutants that are believed to influence this status, The Norwegian Food Safety Authority established a surveillance program in 2013. The program summarizes reported data from the industry on drug use and L. salmonis sensitivity (passive surveillance), and present a collection of sensitivity data from approximately 75 salmon farm locations along the Norwegian coast (active surveillance). The surveillance program aims to summarize the use of various chemotherapeutants in salmon farming and to describe the resistance status against the most important of these chemotherapeutants in L. salmonis in Norway.

Materials and methods

Passive surveillance:

Veterinary medicine register dataThe Norwegian Veterinary Institute (NVI) has received monthly extracts from the Veterinary medicine register (VetReg) that cover prescriptions coupled to treatment of fish. These data are summarized into 5 different categories of substances used to control salmon lice infestations. In total over the years 2011 – 2014 there were 8274 prescriptions coupled to these categories of substances and to a specific farm site.

The five categories of substances are in the following termed azamethiphos (named in the register: Azamethiphos, Salmosan Vet, Trident Vet, Azasure Vet), pyrethroids (named in the register: Alpha Max, Betamax vet, Cypermethrin or Deltamethrin), emamectin benzoate (named in the register: Emamectin benzoate or Slice vet), hydrogenperoxside and flubenzurones (named in the register: Diflubenzuron, Ektobann vet, Releeze vet or Teflubenzuron). Table 3 summarizes the number of prescriptions per substance category and year.

No quantification of the use of different substances is presented since the units used in VetReg vary substantially, e.g. between kg, g, l and ml for the same substance. It should also be noted that there may be a degree of underreporting of prescriptions since these are manually reported by wholesale businesses.

Reported sensitivity data

In the current regulation on the control of salmon lice in aquaculture in Norway (FOR-2012-12-05-1140), effective from 1.1.2013, there is a disclosure of mandatory reporting on suspected resistance and results from sensitivity tests. If resistance is suspected, the reason for suspicion is to be reported in one of the four categories: results from bioassays; reduced treatment efficacy; the situation in the area; or other reasons. The sensitivity data are to be reported in one of the three categories: sensitive; reduced sensitivity; or resistant. Reported data have been summarized as part of the passive surveillance.

Active surveillance:

Performance of simplified bioassay tests:

In performance of the active surveillance, 11 fish health services along the Norwegian coast were engaged to carry out a newly developed simplified field bioassay for sensitivity testing of L. salmonis. The simplified bioassay was standardized, with the same protocol employed for each substance and by the use of identical stock solutions and identical equipment by all the fish health services. The simplified bioassay is less time consuming and the number of salmon lice required is less than in the six-dose bioassay. Performing sensitivity testing using this protocol would presumably make it possible to achieve reliable and comparable sensitivity results from a larger number of locations than if the traditional bioassay protocol was chosen. The locations (fig. 17) were chosen by the fish health services themselves inside a designated area. L. salmonis from a maximum of 78 farm locations (Table 6) were tested against the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide. The simplified field bioassays were performed with two different concentrations (low and high) and a control. After 24 hours of exposure to the chemical in sea water, the salmon lice mortality in identified stages and genders (preadult I and II and adults; females and males) were noted as the test outcome. The salmon lice mortality in the low concentration was used to indicate sensitivity status of the salmon lice population, with salmon lice mortality higher than 80% in parasites indicative of a fully sensitive population

In the active surveillance for 2014 we included tests using hydrogen peroxide. This is due to an increased use of this the repentant to control salmon lice, as well as the first reports of loss of sensitivity of salmon lice to hydrogen peroxide treatments. The salmon lice mortality in the high concentration was used to indicate the degree of reduced sensitivity and the expected outcome of a subsequent treatment, with salmon lice mortality higher than 90% indicative
for an expected treatment efficacy of 90% or more.

Performance of molecular tests of resistance

Salmon lice infection levels on farms in Vest Agder in the far south of Norway are known to be low. In order to sample lice from such farms, lice were collected at slaughter from fish originating from two farms in Vest Agder. Patogen Analyse AS analysed the genetic characteristics with regard to detamethrin and azamethiphos resistance using PCR methodology. Test results were reported according to percentage of lice from each farm categorized as resistant or sensitive for deltamethrin, and sensitive, reduced sensitivity or resistant for azamethiphos.

Table 2: High and low concentrations used in the simplified bioassay tests

Substance category	Low concentration (ppb)	High concentration (ppb)

Deltamethrin	0.2	1
Azamethiphos	0.4	2
Emamectin benzoate	100	300
Hydrogen peroxide	120	240

Note that the high concentration emamectin benzoate was reduced from 500 ppb to 300 ppb in 2014 compared to 2013. This was done to better predict treatment efficacy.

Results

Passive surveillance

Table 3 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2014. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years. Increases were especially large for hydrogen peroxide, flubenzurones and emamectin benzoate. As the amounts prescribed could not be calculated, they could also not be validated against sales data from wholesalers.Thus, the results should be interpreted with care.

Table 3: Number of prescriptions for the given category of substances used to control salmon lice during 2011 - 2014.

Substance category	2011	2012	2013	2014
Azamethiphos	451	617	448	747
Pyrethroids	501	1005	1065	1042
Emamectin benzoate	245	50	47	481
Hydrogen peroxide	167	60	68	977
Flubenzurones	22	62	26	193
Sum	1386	1794	1654	3440

The maps in figure 15 sum up the total number of prescriptions per location during 2013 and 2014. In 2013 there were prescriptions coupled to 560 farm locations, with a mean number of prescriptions per farm of 2.95 (range 1 – 16). Comparable numbers for 2014 were 679 farm locations, with a mean of 5.05 prescriptions per farm (range 1 – 23), respectively.

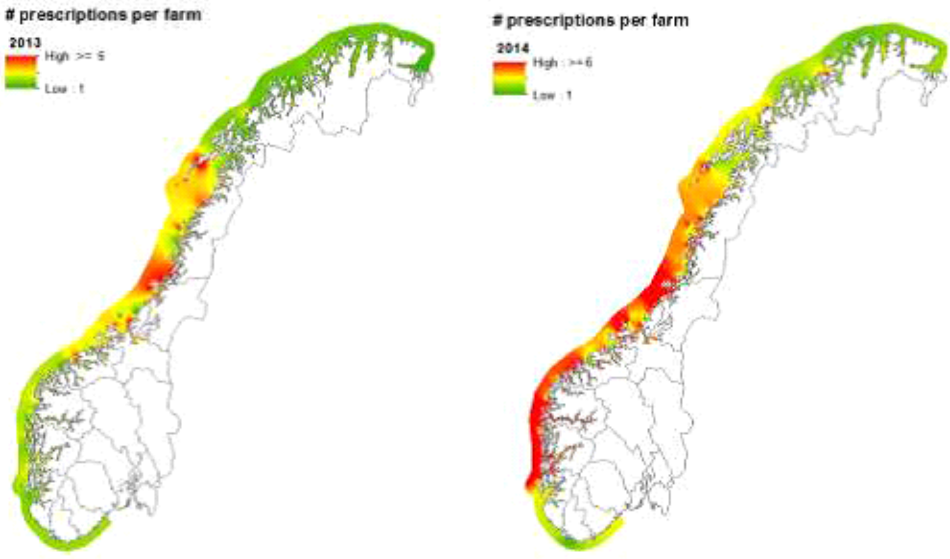
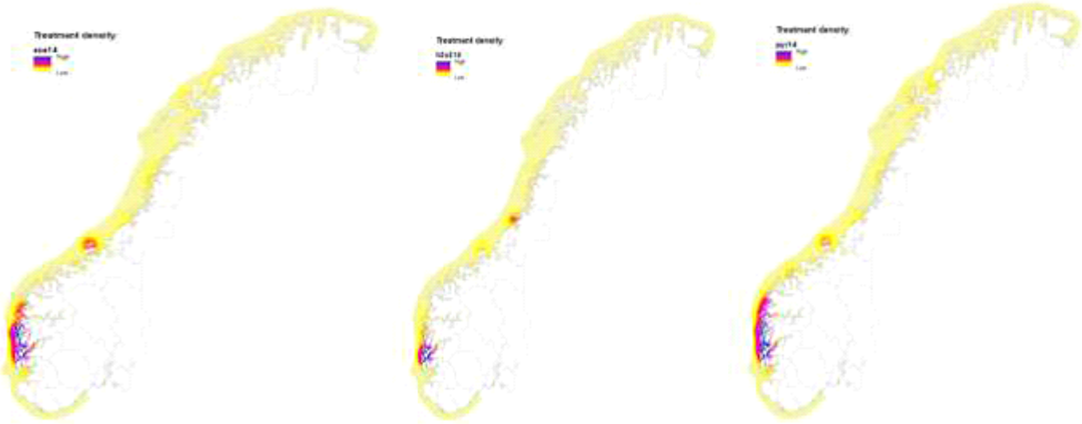
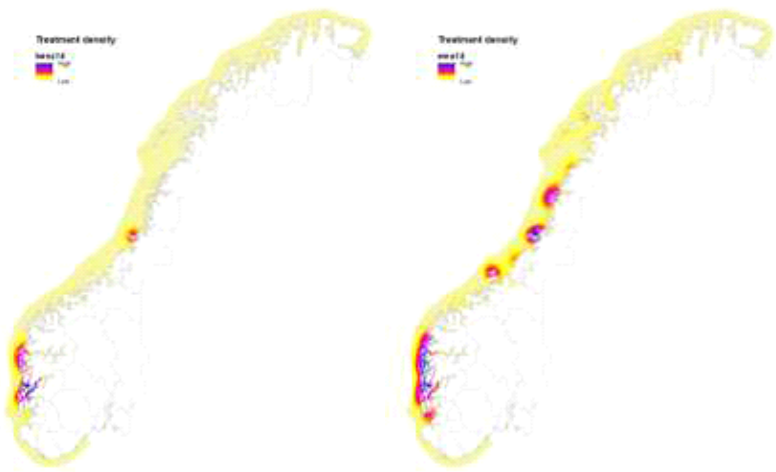


Figure 15: Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 6 prescriptions per location is expected, while dark green denote areas where the expectation of one treatment is approached. The map layer was generated using the IDW function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighbour farm locations). Farms with 0 prescriptions were not part of the data.

The use of azamethiphos and pyrethroids show much the same spatial distribution. The use of emamectin benzoate seems to be distributed comparatively more northerly. The use of hydrogen peroxide is restricted to smaller areas, especially in the South-West and on the coast of Nord Trøndelag. The flubenzurones are used mostly on the south west coast.(Yellow : Low, Blue : High)



Azamethiphos Hydrogen peroxide Pyrethroids



Flubenzurones Emamectin benzoat

Figure 16: Kernel densities of prescriptions for five different substances used to control salmon lice infestations in salmonid farms in 2014. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, where blue indicates relatively high intensities while yellow indicates relatively low densities.

Reported sensitivity data

Table 4. The number of reports from sensitivity studies within the three categories of reported sensitivity status.

	2013			2014		
Substance category	Sensitive	Reduced sens.	Resistant	Sensetive	Reduced sens.	Resistant
Azamehtiphos	18	28	15	29	33	19
Emamectin benzoat	3	6	2	7	9	3
Flubenzurones		3				
H2O2		5		3	5	1
Pyrethroids	43	52	8	25	60	8
Total	**64**	**94**	**25**	**64**	**107**	**31**

With regard to the sensitivity status reported from sensitivity tests there are no obvious trends in the data. The number of reports due to suspicion of resistance showed a pronounced increase in 2014 compared to earlier years.

Table 5. The number of reports due to suspicion of resistance. The reports are categorized with respect to suspected reasons for resistance (1 = bioassay results; 2 = treatment effect; 3 = situation in the area; 9 = other unspecified).

	2012				2013			2014		
Substance category	1	2	3	9	1	2	3	1	2	3
Azamehtiphos	18	8	8		15	11		25	52	2
Emamectin benzoat	9	9	46	1	1	1		21	2	
Flubenzurones	1	1								
H2O2						5	1	3	10	
Pyrethroids	31	12	1	3	16	23	2	31	66	
Total	**59**	**30**	**55**	**4**	**32**	**40**	**3**	**80**	**130**	**2**

Active surveillance

Altogether, 230 high concentration and 230 low concentration simplified bioassay tests on salmon lice from altogether 90 different salmon farm locations along the cost (figure 17). Of these, 59 farms were tested for azamethiphos, 78 farms for deltamethrin, 48 farms for emamectin benzoate and 45 farms for hydrogen peroxide. Table 6 summarizes the outcome of all simplified bioassays according to mortality classification. Differences in mortality rates between genders and/or developmental stages are not presented in the table. For pyrethroids and azamethiphos, this variation was low, but higher for emamectin benzoate. The categories are high mortality (>80% for low concentration and >90 % for high concentration tests), intermediate mortality and low mortality (< 33% mortality for both low and high concentration tests) for each substance. The table shows that salmon lice mortalities were lower than 80% in the majority of locations tested at low concentrations for each substance. This indicates that reduced sensitivity to chemotherapeutants in salmon lice is widespread in Norwegian salmon farming.

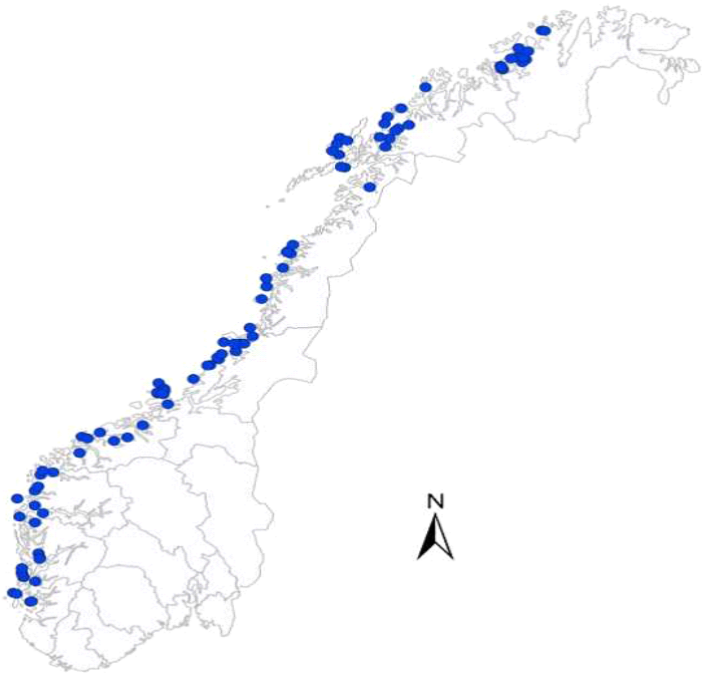


Figure 17: Locations of farms where salmon lice were collected for simplified bioassay testing in 2014.

Table 6. Classification of mortality results from low and high concentration bioassay tests. The Total column refers to the number of tests conducted at different farm locations (* except for deltamethrin where tests were duplicated on four farms, conducted at 74 different farms

and hydrogen peroxide where tests were duplicated in two farms, conducted at 43 farms). Column numbers denote the number of tests that fell within the high, intermediate or low mortality classifications for each drug and test-concentration.

Substance category		Mortality classification (number of observations)		
Low concentration	Total	High (> 80 %)	Intermediate (80 – 33 %)	Low (< 33 %)
Azamethiphos	59	1	18	40
Deltamethrin	78	4	17	57
EMB	48	1	17	30
H2O2	45	6	26	13
High concentration	Total	High (> 90 %)	Intermediate (90 – 33 %)	Low (< 33 %)
Azemethiphos	59	1	26	32
Deltamethrin	78	6	43	29
EMB	48	4	28	16
H2O2	45	23	20	2

Table 7 shows that the salmon lice mortality results from low and high concentrations are significantly correlated, with highest correlations for azamethiphos and pyrethroids. These correlations show that the results from low and high concentration tests are consistent.

Table 7. Spearman Correlation Coefficients between mortality proportions in the low and high concentration bioassay tests on farms. The correlation coefficients are all relatively high and are highly significant, indicating consistency in the results from low and high concentration tests within farms.

Substance category	N	Spearman Correlation Coefficients	Prob > |r| under H0: Rho=0
Azamethiphos	59	0.81	< 0.001
Deltamethrin	77	0.71	< 0.001
EMB	49	0.80	< 0.001
H2O2	45	0.56	< 0.001

The geographic location of farms where tests were performed and the distribution of mortality results are shown in maps and box plots for azamethiphos (Figure 18), deltamethrin (Figure 19), emamectin benzoate (Figure 20) and hydrogen peroxide (Figure 21). As in table 7, differences in mortality rates between genders and/or development stages are not shown in the figures. For low concentration azamethiphos tests (Figure 18 B), the only farm with salmon lice test-mortalities exceeding 80 % (indicative of fully sensitive populations) was in Finnmark in the far north. Low salmon lice mortalities in high concentration azamethiphos tests (Figure 18A) were found especially in the areas Nordland, Trøndelag and partly Hordaland. Low treatment efficacy may thus be expected in these areas. The boxplots showing the distribution of proportional mortalities in low and high concentration azamethiphos experiments showed large variations between tests, indicating that reduced sensitivity is common and that low treatment efficacy often is to be expected.

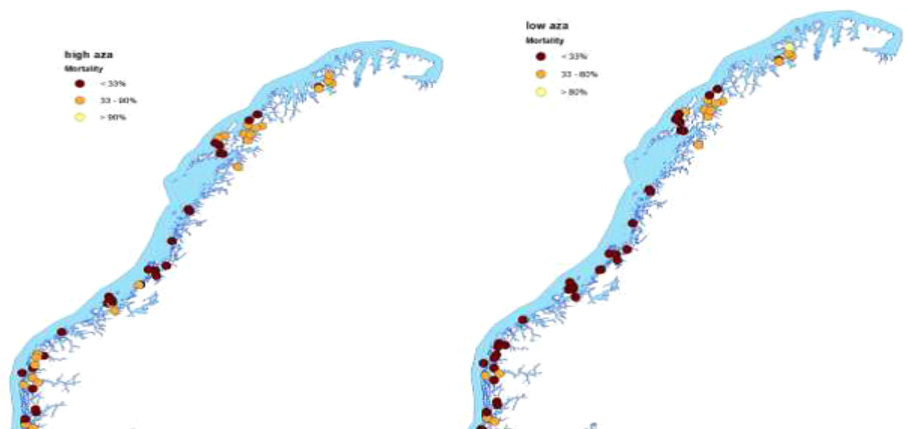
For deltamethrin, mortalities were comparably high in high concentration tests in Finnmark (Figure 19A). In general, however, the results from the high concentration deltamethrin tests indicate that several areas can expect low treatment efficacy. The low concentration deltamethrin tests (Figure 19B) indicate that that reduced sensitivity to deltamethrin is widespread along the coast.

The low concentration emamectin benzoate tests (Figure 20B), showed that reduced sensitivity is widespread, but varies considerably (boxplot). The high concentration emamectin tests (Figure 20A) resulted
in comparably high mortalities in the north, but varying mortality in the rest of the country.

For hydrogen peroxide, results from the high concentration tests yielded reasonably high mortalities in general, but reduced mortalities in an area in Mid-Norway and in the southernmost tested farms. The low concentration tests corroborated the results of the high concentration tests, especially by low mortalities in farms located in Mid-Norway (Figure 21).

The molecular tests of lice from the southern two farms in Vest Agder revealed a high percentage of lice being sensitive to deltamethrin, i.e. 85% for both farms. Also for azamethiphos a high percentage of the lice were sensitive, i.e. 76% and 78%, respectively. Reduced sensitivity to azamethiphos was reported from 20 and 18% of the lice, respectively. This indicates that lice from the southernmost farms in Norway generally are sensitive to chemotherapeutants.

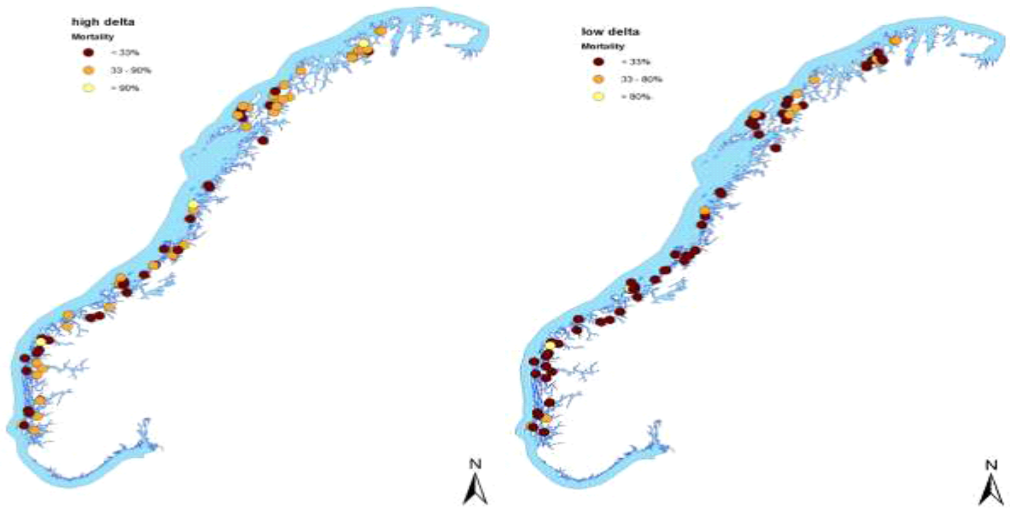
A B



Azamethiphos 2014

Figure 18. Maps showing categorical mortality in bioassay tests high (A) and low (B) azamethiphos concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend).

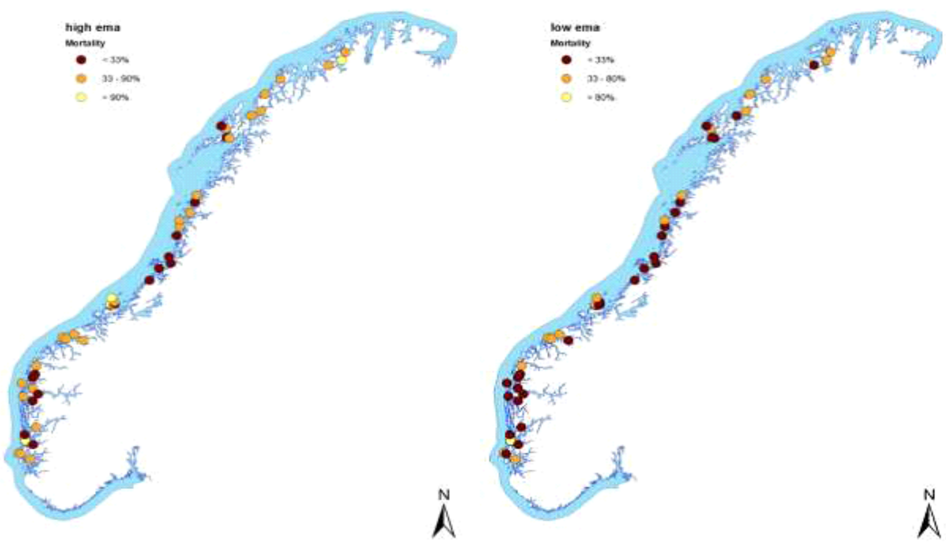
A B



Deltamethrine 2014

Figure 19. Maps showing categorical mortality in bioassay tests with high (A) and low (B) deltamethrin concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend).

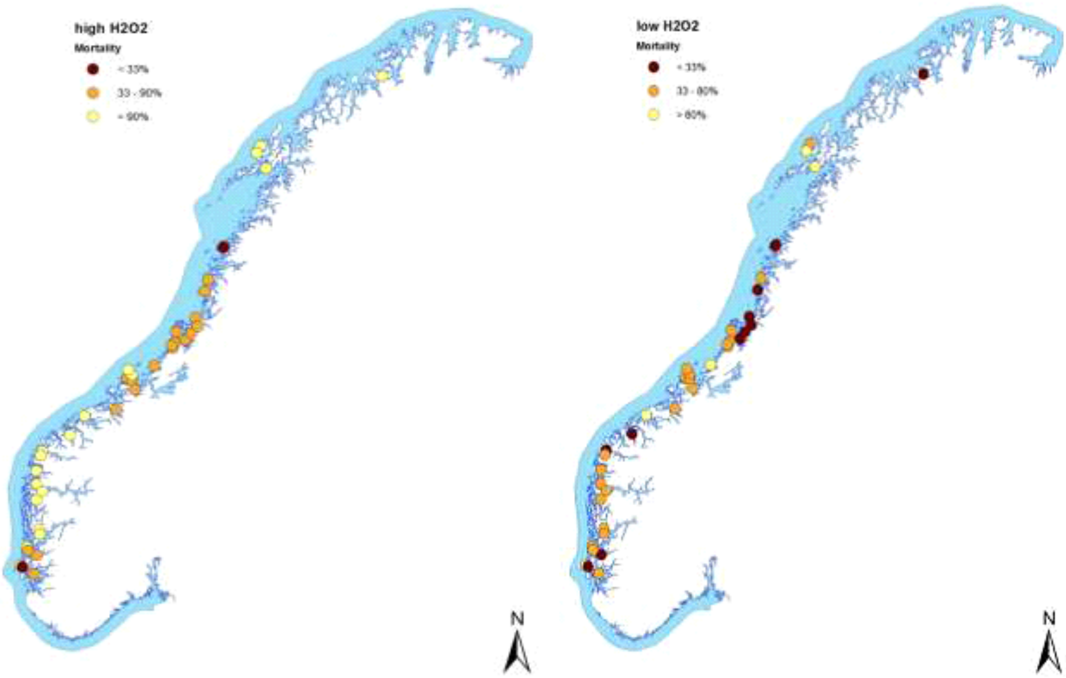
A B



Emamectin benzoate 2014

Figure 20. Maps showing categorical mortality in bioassay tests with high (A) and low (B) emamectin concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend).

A B



Hydrogen peroxide 2014

Figure 21: Maps showing categorical mortality in bioassay tests with high (A) and low (B) hydrogen peroxide concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend).

Results

Passive surveillance :

- The total number of prescriptions of substances used to control salmon lice infections showed a pronounced increase in 2014 compared to the years 2011 – 2013. The coverage of the total use of chemotherapeutica to control salmon lice is, however, uncertain.
- Increases were especially pronounced for hydrogen peroxide, flubenzurones and emamectin benzoate.
- The use of azamethiphos and pyrethroids showed much the same spatial distribution
- The use of emamectin benzoate has a comparably more northerly distribution.
- Hydrogen peroxide use is restricted to smaller areas, whereas flubenzurones are used mostly on the south west coast.

Active surveillance :

The program has succeeded in collecting sensitivity data along the coast. Implementation of standardized simplified bioassays has given comparable salmon lice mortality results from the test locations making it possible to assess the sensitivity status of salmon lice to azamethiphos, pyrethroids, emamectin benzoate and hydrogen peroxide along most of the Norwegian coast.
The survey shows that reduced sensitivity is widespread. The area with results indicating comparably sensitive salmon lice populations was in Finmark in the far north, although also here reduced sensitivity to different chemotherapeutants is indicated. Bioassay tests from the southernmost areas of salmon farming were not undertaken. Instead genotyping of parasites for azamethiphos and pyrethroid resistance markers was undertaken, demonstrating a low level of these resistance markers in this area.
Salmon lice mortalities in high concentration azamethiphos tests showed that low treatment efficacies can be expected especially in the areas northern Nordland/southern Troms, Trøndelag and Hordaland. For deltamethrin, salmon lice mortalities in high concentration tests indicate that several areas can expect low treatment efficacy, although the mortalities in high concentration tests varied a lot.

Conclution

My personal opinion is that it is very important to put the spotlight on our salmon lice problem in Norway. We have to encourage and teach our fish farmers and our veterinarians about these problems so they can work for healtier animals. We as humans, who are in control of the breeding and with that then are responsible for the outcome. The fish farmers, veterinarians and the consumers, all want a healty animal and I hope that all the fish farm societies not only in Norway but around the world do whats best for the fish to continue to improve their conditions and not the opposite.
The worlds population is continuously growing and Norway have a vision to produce more and more fish to satisfy the increasing demand. We have a huge responsibility to continue the sustainable development of the farmed salmon.

Acknowledgements

I would like to thank Baard Johannessen with the University of Oslo, department of biology and toxicology and with EcoLice for introducing me to this problem and for showing me some guidelines during this writing.

I would also like to thank my friends and family who has been there with all the things around the writing. Everything from a room and a desk till dinner and a listening ear.

And ofcouse a thank you till my supervisor Doctor Baska Ferenc for support and advise during the writing process.

References:

ARTICLES

- **ESPEDAL, P. G., GLOVER, K. A., HORSBERG, T. E. AND NILSEN, F.** Emamectin benzoate resistance and fitness in laboratory reared salmon lice (Lepeophtheirus salmonis)
  In-text: (Espedal et al., 2013)
  Bibliography: Espedal, P., Glover, K., Horsberg, T. and Nilsen, F. (2013). Emamectin benzoate resistance and fitness in laboratory reared salmon lice (Lepeophtheirus salmonis). *Aquaculture*, 416-417, pp.111-118.

- **GRØNTVEDT R.N., JANSEN P.A., HORSBERG T.A., HELGESEN K. AND TARPAI A.** The surveillance programme for resistance to chemotherapeutants in *L.salmonis* in Norway 2013. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2013*. Oslo: Norwegian Veterinary Institute 2014.

- **HELGESEN K.O. & HORSBERG T.E.** (2013) Single-dose field bioassay for sensitivity testing in sea lice, *Lepeophtheirus salmonis*: development of a rapid diagnostic tool. Journal of Fish Diseases 36, 261-272.

- **JONES M.W., SOMMERVILLE C. & WOTTEN R.** (1992) Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. Journal of Fish Diseases 14, 197-202.

- **KAUR, K., HELGESEN, K. O., BAKKE, M. J. AND HORSBERG, T. E.** Mechanism behind Resistance against the Organophosphate Azamethiphos in Salmon Lice (Lepeophtheirus salmonis)
  In-text: (Kaur et al., 2015)
  Bibliography: Kaur, K., Helgesen, K., Bakke, M. and Horsberg, T. (2015). Mechanism behind Resistance against the Organophosphate Azamethiphos in Salmon Lice (Lepeophtheirus salmonis). *PLOS ONE*, 10(4), p.e0124220.

- **ROTH M., RICHARDS R.H., DOBSON D.P & RAE G.H.** (1996) Field trial on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda:Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). Aquaculture 140, 217-239.

- **SEVATDAL S. & HORSBERG T.E.** (2003) Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Kroyer) against the pyrethroid deltamethrin using bioassays and probit modelling. Aquaculture 218, 21-31.

- **SIVERTSGÅRD, R., THORSTAD, E. B., ØKLAND, F., FINSTAD, B., BJØRN, P. A., JEPSEN, N., NORDAL, T. AND MCKINLEY, R. S.** Effects of salmon lice infection and salmon lice protection on fjord migrating Atlantic salmon and brown trout post-smolts
  In-text: (Sivertsgård et al., 2007)
  Bibliography: Sivertsgård, R., Thorstad, E., Økland, F., Finstad, B., Bjørn, P.,Jepsen, N., Nordal, T. and McKinley, R. (2007). Effects of salmon lice infection and salmon lice protection on fjord migrating Atlantic salmon and brown trout post smolts. *Hydrobiologia*, 582(1), pp.35-42.

- **TREASURER, J. W., WADSWORTH, S. AND GRANT, A.**
  Resistance of sea lice, Lepeophtheirus salmonis (Kroyer), to hydrogen peroxide on farmed Atlantic salmon, Salmo salar L.
  In-text: (Treasurer, Wadsworth and Grant, 2000)
  Bibliography: Treasurer, J., Wadsworth, S. and Grant, A. (2000). Resistance of sea lice, Lepeophtheirus salmonis (Kroyer), to hydrogen peroxide on farmed Atlantic salmon, Salmo salar L. *Aquaculture Research*, 31(11), pp.855-860.

- **WESTCOTT J.D., STRYHN H., BURKA J.F. & HAMMEL K.L.** (2008) Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate. Diseases of Aquatic Organisms 79, 119-131.

WEB PAGES

- **FJORDLAB.NO** Legemiddel
  In-text: (Fjordlab.no, 2016)
  Bibliography: Fjordlab.no, (2016). *Legemiddel*. [online] Available at: http://www.fjordlab.no/legemiddel.htm [Accessed 28 Jan. 2016].

- **INSTITUTE OF MARINE RESEARCH** Sea lice
  In-text: (Institute of Marine Research, 2016)
  Bibliography: Institute of Marine Research, (2016). *Sea lice*. [online] Available at: http://www.imr.no/temasider/parasitter/lus/lakselus/en [Accessed 28 Jan. 2016].

- **LAKSEFAKTA.NO** Laksefakta
  In-text: (Laksefakta.no, 2014)
  Bibliography: Laksefakta.no, (2014). *Laksefakta*. [online] Available at: http://www.laksefakta.no/ [Accessed 28 Jan. 2016].

- **SYSTEMS, E.** Lakselus / Faktabank / Hjem - Veterinærinstituttet
  In-text: (systems, 2015)
  Bibliography: systems, e. (2015). *Lakselus / Faktabank / Hjem - Veterinærinstituttet*. [online] Vetinst.no. Available at: http://www.vetinst.no/Faktabank/Lakselus [Accessed 28 Jan. 2016].

- **SYSTEMS, E.** Fisk - Lakselus, resistens / Helseovervåking / Hjem - Veterinærinstituttet
  In-text: (systems, 2016)
  Bibliography: systems, e. (2016). *Fisk - Lakselus, resistens / Helseovervåking / Hjem - Veterinærinstituttet*. [online] Vetinst.no. Available at: http://www.vetinst.no/Helseovervaaking/Fisk-Lakselus-resistens [Accessed 28 Jan. 2016].

- **VALMOT, O.** Ny metode knekker lakselusa
  In-text: (Valmot, 2016)
  Bibliography: Valmot, O. (2016). *Ny metode knekker lakselusa*. [online] Teknisk Ukeblad. Available at: http://www.tu.no/klima/2011/10/13/ny-metode-knekker-lakselusa [Accessed 28 Jan. 2016].

- **WERKMAN M, E. A.** The effectiveness of fallowing strategies in disease control in salmon aquaculture assessed with an SIS model. - PubMed - NCBI
  In-text: (Werkman M, 2016)
  Bibliography: Werkman M, e. (2016). *The effectiveness of fallowing strategies in disease control in salmon aquaculture assessed with an SIS model. - PubMed - NCBI*. [online] Ncbi.nlm.nih.gov. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21040988 [Accessed 28 Jan. 2016].

- **WWF.NO** Ordinære lusemilder mot lakselus
  In-text: (Wwf.no, 2016)
  Bibliography: Wwf.no, (2016). *Ordinære lusemilder mot lakselus*. [online] Available at : http://www.wwf.no/dette_jobber_med/hav_og_kyst/havbruk/parasitter_og_sjukdommer/ordinare_lusemilder_mot_lakselus/ [Accessed 28 Jan. 2016].

PICTURES

- **Figure 1. : EAA-EUROPE.ORG - EAA**
  In-text: (Eaa-europe.org, 2016)
  Bibliography: Eaa-europe.org, (2016). - EAA. [online]
  Available at: http://www.eaa-europe.org/resolutions/ [Accessed 30 Jan. 2016].

- **Figure 2. : TASSAL.COM.AU** Tassal Tasmanian Salmon | Our Salmon
  In-text: (Tassal.com.au, 2016)
  Bibliography: Tassal.com.au, (2016). *Tassal Tasmanian Salmon | Our Salmon*. [online] Available at: https://www.tassal.com.au/sustainability/our-salmon/ [Accessed 30 Jan. 2016].

- **Figure 3a and 3b. : BEHANDLING AV LAKS MOT LAKSELUS.** Legemiddelverket
  In-text: (Behandling av laks mot lakselus, 2016)
  Bibliography: Behandling av laks mot lakselus, (2016). Legemiddelverket. [online] Available at: http://www.legemiddelverket.no/Veterinaermedisin/terapianbefalinger/Documents/Behandling%20mot%20lakselus%20i%20oppdrettsanlegg.pdf [Accessed 30 Jan. 2016].

- **Figure 4a. : MARINE.IE** Life cycle of the Salmon Louse | Marine Institute
  In-text: (Marine.ie, 2016)
  Bibliography: Marine.ie, (2016). Life cycle of the Salmon Louse | Marine Institute. [online] Available at: http://www.marine.ie/Home/site-area/areas-activity/aquaculture/sea-lice/life-cycle-salmon-louse [Accessed 30 Jan. 2016].

- **Figure 4b. : : EICHNER, C., FROST, P., DYSVIK, B., JONASSEN, I., KRISTIANSEN, B. AND NILSEN, F. Salmon louse (Lepeophtheirus salmonis) transcriptomes during post molting maturation and egg production, revealed using EST-sequencing and microarray analysis**
  In-text: (Eichner et al., 2008)
  Bibliography: Eichner, C., Frost, P., Dysvik, B., Jonassen, I., Kristiansen, B. and Nilsen, F. (2008). Salmon louse (Lepeophtheirus salmonis) transcriptomes during post molting maturation and egg production, revealed using EST-sequencing and microarray analysis. *BMC Genomics*, 9(1), p.126.

- **Figure 5. : ACEAQUA.NO** Brønnbåt | ACE – AquaCulture Engineering
  In-text: (Aceaqua.no, 2016)
  Bibliography: Aceaqua.no, (2016). *Brønnbåt | ACE – AquaCulture Engineering*. [online] Available at: http://aceaqua.no/om-ace/fotoalbum/_7003979/ [Accessed 30 Jan. 2016].

- **Figure 6. : FORSKNING.NO** Mindre lus med skjørt rundt merdene
  In-text: (Forskning, 2016)
  Bibliography: Forskning, (2016). *Mindre lus med skjørt rundt merdene*. [online] forskning.no. Available at: http://forskning.no/oppdrett/2015/04/mindre-lus-med-skjort-rundt-merdene [Accessed 31 Jan. 2016].

- **Figur 7. : KYST.NO**
  I dette fylket ble det behandlet flest ganger mot lus – det var ikke i Midt-Norge
  In-text: (No, 2016)
  Bibliography: No, K. (2016). *I dette fylket ble det behandlet flest ganger mot lus – det var ikke i Midt-Norge*. [online] Kyst – SalmonView. Available at: http://kyst.no/nyheter/i-dette-fylket-ble-det-behandlet-flest-ganger-mot-lus-det-var-ikke-i-midt-norge/ [Accessed 31 Jan. 2016].

- **Figur 8. : SJØMAT, N.** Sjomatnorge.no Økt bruk av legemidler mot lakselus
  In-text: (Sjømat, 2016)
  Bibliography: Sjømat, N. (2016). *Sjomatnorge.no Økt bruk av legemidler mot lakselus*. [online] Sjomatnorge.no. Available at: http://sjomatnorge.no/okt-bruk-av-legemidler-mot-lakselus/ [Accessed 31 Jan. 2016]

- **Figur 9. : EGERSUND, G.** Snorkelmerd og lusetube - Egersund Group
  In-text: (Egersund, 2016)
  Bibliography: Egersund, G. (2016). *Snorkelmerd og lusetube - Egersund Group*. [online] Egersundgroup.no. Available at: http://www.egersundgroup.no/nyheter/snorkelmerd-og-lusetube [Accessed 31 Jan. 2016].

- **Figure 10. : FORSKNING NO** Avl kan minske lakselusproblemer
  In-text: (Forskning No, 2016)
  Bibliography: Forskning No, (2016). *Avl kan minske lakselusproblemer*. [online] forskning.no. Available at: http://forskning.no/fisk-fiskehelse-oppdrett-stub-stub/2008/04/avl-kan-minske-lakselusproblemer [Accessed 31 Jan. 2016].

- **Figure 11a and 11b . : SCIENCEDAILY** New approach to combating sea lice: Wrasse to the rescue.
  In-text: (ScienceDaily, 2016)
  Bibliography: ScienceDaily, (2016). *New approach to combating sea lice: Wrasse to the rescue*. [online] Available at: http://www.sciencedaily.com/releases/2010/04/100423215021.htm [Accessed 30 Jan. 2016].

- **Figure 12. : KVISTAD, A. Lusedata**
  In-text: (Kvistad, 2016)
  Bibliography: Kvistad, A. (2016). *Lusedata*. [online] Lusedata. Available at: http://lusedata.no/ [Accessed 31 Jan. 2016].

- **Figure 13. : HAVBRUK** Nyheter - HAVBRUK
  In-text: (Havbruk, 2016)
  Bibliography: Havbruk, (2016). *Nyheter - HAVBRUK*. [online] Forskningsradet.no. Available at: http://www.forskningsradet.no/prognett-havbruk/Nyheter/PrevenT_pa_intens_kunnskapsjakt/1253983909165 [Accessed 31 Jan. 2016].

- **Figur 14. : SLRC- SEA LICE RESEARCH CENTER .**Nye resultat endra lakselusa sin livssyklus
  In-text: (slrc, 2016)
  Bibliography: slrc, (2016). *Nye resultat endra lakselusa sin livssyklus*. [online] Havforskningsinstituttet. Available at: http://www.imr.no/nyhetsarkiv/2013/september/nye_resultat_endra_lakselusa_sin_livssyklus/nb-no [Accessed 31 Jan. 2016].

- **Figure 15 and 16. : HAVFORSKNINGINSTITUTTET.** Hvordan spres lakselusa?
  In-text: (Havforskninginstituttet, 2016)
  Bibliography: Havforskninginstituttet, (2016). *Hvordan spres lakselusa?*. [online] Havforskningsinstituttet. Available at: http://www.imr.no/temasider/parasitter/lus/lakselus/90683/nb-no [Accessed 1 Feb. 2016].

- **Figure 17. : JANSEN, P.** Smittepress fra lakselus
  In-text: (Jansen, 2016)
  Bibliography: Jansen, P. (2016). *Smittepress fra lakselus*. [online] Available at: http://europharma.no/wp-content/uploads/downloads/lofotseminar/presentasjoner/Jansen.pdf [Accessed 31 Jan. 2016].

- **Figure 18-21. : DIN LAKS** AKVAKULTUR PRODUSERER ENORME MENGDER LAKSELUS
  In-text: (Din laks, 2015)
  Bibliography: Din laks, (2015). *AKVAKULTUR PRODUSERER ENORME MENGDER LAKSELUS*. [online] DinLaks. Available at: http://www.dinlaks.org/2015/09/08/akvakultur-produserer-enorme-mengder-lakselus-2/ [Accessed 1 Feb. 2016].

- **Table 1 : : BEHANDLING AV LAKS MOT LAKSELUS.** Legemiddelverket
  In-text: (Behandling av laks mot lakselus, 2016)
  Bibliography: Behandling av laks mot lakselus, (2016). Legemiddelverket. [online] Available at: http://www.legemiddelverket.no/Veterinaermedisin/terapianbefalinger/Documents/Behandling%20mot%20lakselus%20i%20oppdrettsanlegg.pdf [Accessed 30 Jan. 2016].

- **Table 2-7 . :NSD.UIB.NO** Søkeresultat - nettsider - Forvaltningsdatabasen - NSD
  In-text: (Nsd.uib.no, 2016)
  Bibliography: Nsd.uib.no, (2016). Søkeresultat - nettsider - Forvaltningsdatabasen - NSD. [online] Available at: http://www.nsd.uib.no/polsys/forvaltning/resultat.html?q=lakselus [Accessed 29 Jan. 2016].