Annual Report

The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2018

Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

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Authors
Kari Olli Helgesen, Tor Einar Horsberg, Attila Tarpai

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Summary

The number of medicinal treatments applied against salmon lice was reduced in 2018. This continued a yearly trend that started in 2015. Despite this, the level of resistance seen in salmon lice remained high in 2018. However, the tendency towards a reduction in the level of resistance that started in 2017, continued in 2018. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were generally widespread along the Norwegian coast. Less resistance was found towards hydrogen peroxide than towards the other medicines, but loss of sensitivity was indicated in several areas. 484 prescriptions for medicines against salmon lice were prescribed in 2018, which was a 38 percent reduction compared to 2017. The number of non-medicinal treatments increased with 21 percent, to 2023 reported treatments, in the same period. Non-medicinal methods for treatment and prevention were thereby the dominating methods for salmon lice control. Thermic delousing accounted for 68 percent of the non-medicinal treatments in 2018.

Introduction

Salmon lice (*Lepeophtheirus salmonis*) are considered one of the biggest health threats against both farmed and wild salmonids in Norway. Medicinal treatments have traditionally been used to control salmon lice in the fish farms, but the development of resistant parasites has reduced the efficacy of these treatments. Resistance towards chemotherapeutants in salmon lice has been reported from several countries, including Norway (1). The reports have been based on reduced treatment efficacy and/or results from toxicological or molecular resistance tests. Reduced sensitivity has been associated with local treatment intensity (2). Results from resistance testing have been used by the industry as a decision making tool in salmon lice management. However, until 2013 there was no comprehensive survey of the resistance status of *L. salmonis* in any country. To maintain salmon lice control, non-medicinal methods for treatment and prevention have become increasingly more important, to a large degree as a result of the resistance situation.

In order to get an overview of the resistance status of *L. salmonis* in Norway and the use of chemotherapeutants against salmon lice, The Norwegian Food Safety Authority established a surveillance program in 2013, which has continued since then (3). In the passive surveillance part of the programme, prescriptions for salmon lice treatments are summarised. In the active surveillance part, toxicological or molecular resistance tests are performed on salmon lice from approximately 70 salmon farms located along the Norwegian coast. The Norwegian Veterinary Institute is responsible for the planning, data collection and reporting components of the programme. Due to its current importance for salmon lice control, an overview of the use of non-medicinal treatments against salmon lice is also given.

Aims

The surveillance program aims to summarize the use of chemotherapeutants against salmon lice and to describe the resistance status in *L. salmonis* towards the most important of these chemotherapeutants in Norway.

Materials and methods

Passive surveillance

Prescriptions of medicines

Prescriptions of medicines applied for salmon lice treatments, from the Veterinary medicine register (VetReg), were summarised into 5 different categories. The medicines were subdivided into categories according to their mode of action and therefore most likely joint selection pressure towards resistance. The five categories were azamethiphos, pyrethroids (cypermethrin and deltamethrin), emamectin benzoate, hydrogen peroxide and flubenzurones (diflubenzuron and teflubenzuron). A prescription can be prescribed for treatments of some or all the fish cages in a farm. Hydrogen peroxide is used against salmon lice infestations, but also against amoebic gill disease at a lower concentration. In addition, some of the prescriptions for azamethiphos, pyrethroids, emamectin benzoate or hydrogen peroxide might have been prescribed for treatment of fish infested with the sea louse *Caligus elongatus*. All prescriptions of medicines with salmon lice as a possible indication were however included. This is due to the fact that all these treatments are likely to inflict a selection pressure for resistance in salmon lice, regardless of the treatment indication. The extracts from VetReg were performed 14.01.2019 (dd.mm.yyyy).

The farms without any prescriptions for salmon lice medicines were identified using the weekly reports of salmon lice to the Norwegian Food Safety Authority (extracted 04.03.2019) in addition to VetReg. Farms that during a year had reported the presence of adult female lice, but had no prescriptions issued for them in the same period, were regarded as farms without prescriptions.

Non-medicinal treatments

The number of non-medicinal treatments performed in Norwegian salmon farms was extracted from the weekly mandatory reporting of salmon lice data to the Norwegian Food Safety Authority 04.03.2019. Non-medicinal treatments include mechanical and thermic delousing, in addition to delousing in fresh water baths. The reports do not have data on the number of cages treated per week, and this can vary between one and all cages. The non-medicinal treatments were subdivided into different method-categories based on information automatically extracted from the free-text fields in the reporting form.

Reported sensitivity data

According to the current regulation on control of salmon lice in Norwegian aquaculture (4), there is mandatory reporting of suspected resistance and results from sensitivity tests. If resistance is suspected, the reason for suspicion is to be reported in one of 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 three categories: sensitive, reduced sensitivity, or resistant. Reported sensitivity data have not been summarised for 2018. This is due to the fact that these data are of limited value. There are farms where medicinal treatments are not applied and these will therefore most likely not report sensitivity data. This is despite the fact that resistance might have led to the absence of medicinal treatments. In addition, there are no objective criteria for the categorisation of the results from the sensitivity tests.

Data processing

Data processing and statistical analyses were performed in the statistical software R (5). Geographical processing and presentation of data was performed using ArcGIS (6).

Active surveillance

Bioassays

Seven fish health services along the Norwegian coast were engaged in 2018 to perform toxicological resistance tests (bioassays) on live parasites. The bioassay protocol was based on Helgesen *et al* 2013 and 2015 (7, 8) and had also been applied for the previous years of the surveillance programme (2013-2017). The protocol was standardised and similar for each substance. Identical stock solutions and identical equipment were used by all the fish health services. The locations (Figure 1) were chosen by the fish health services themselves inside a designated area.

L. salmonis from a maximum of 64 farms were tested with the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide. The bioassays were performed by exposing live parasites of motile stages, removed from the fish, for two different concentrations of each chemical plus a sea water control. The concentrations applied are presented in Table 1. After 24 hour exposure to the chemicals in seawater, salmon lice mortality in identified stages and genders (preadult I and II and adults; females and males) were noted as the test outcome. Salmon lice mortality at the low concentration was used to indicate the sensitivity status of the salmon lice population, with mortalities higher than 80 percent indicative of fully sensitive populations. Salmon lice mortality at high concentration was used to indicate the expected outcome of a subsequent treatment.

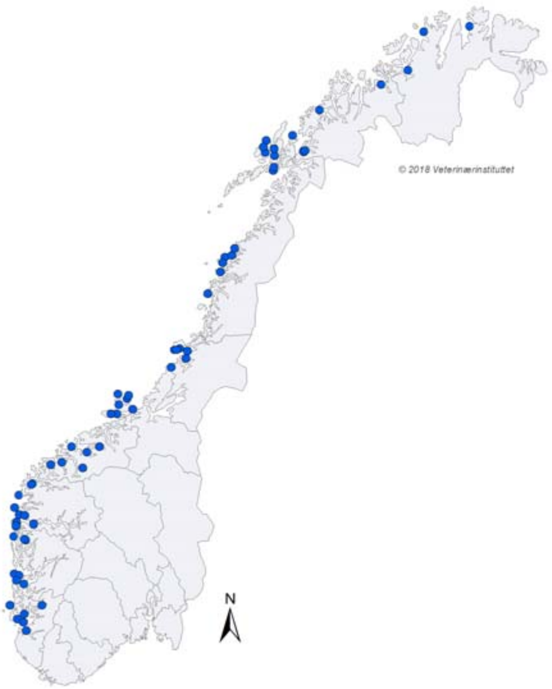


Figure 1. Locations of farms where salmon lice were collected for bioassays in 2018.

Table 1. Concentrations used in the exposed groups in the bioassays, in ppb (µg/l).

Substance category	Low concentration (ppb)	High concentration (ppb)
Deltamethrin	0.2	1
Azamethiphos	0.4	2
Emamectin benzoate	100	300
Hydrogen peroxide	120	240

Molecular resistance tests
Salmon lice infestation levels on farms in Vest-Agder in the far south of Norway had been low for several years. Performing bioassay in Finnmark, in the most northern part of Norway, had proven difficult in previous years. This was due to a combination of low lice levels and challenging logistics. In order to test lice from these areas for resistance, 30 lice were collected from each of three farms from Vest-Agder and two farms in Finnmark. Patogen Analyse AS analysed the genetic characteristics with regard to pyrethroid, azamethiphos and hydrogen peroxide resistance using PCR methodology. Test results were reported according to percentage of lice from each farm categorized as resistant or sensitive to pyrethroids; sensitive, intermediate resistant or resistant to azamethiphos; and as percent expected efficacy of a subsequent treatment for hydrogen peroxide.

Results and Discussion

Passive surveillance

Number of prescriptions
Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2018. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years, but this was somewhat decreased in 2015. The decrease was more prominent in 2016 and continued in 2017 and 2018. In 2018 the total number of prescriptions were reduced with 38 percent compared to the previous year. The decrease in the number of prescriptions from 2017 to 2018 was prominent for all substances/classes of substances, but most markedly for hydrogen peroxide (60 percent reduction) and flubenzurones (66 percent reduction). This reduction lowered the selection

pressure towards resistance, compared to 2017. Emamectin benzoate was the most commonly prescribed medicine, but the number of prescriptions was reduced, also for this substance, by 21 percent in 2018 compared to 2017.

Table 2. Number of prescriptions for the given substances/class of substances applied to control salmon lice in 2011 to 2018. The number of prescriptions was collected from VetReg 14.01.19. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.

Substance category	2011	2012	2013	2014	2015	2016	2017	2018
Azamethiphos	409	691	480	749	619	257	58	38
Pyrethroids	456	1155	1123	1043	662	276	80	55
Emamectin benzoate	288	164	162	481	523	608	348	274
Hydrogen peroxide	172	110	250	1009	1279	629	214	90
Flubenzurones	23	129	170	195	201	173	79	27
Total	**1348**	**2249**	**2185**	**3477**	**3284**	**1943**	**779**	**484**

Prescriptions per farm

The maps in Figure 2 sum up the total number of prescriptions per location in the period 2015 - 2018. Prescriptions were issued for 661 farms in 2015 with a mean of 5.0 prescriptions per farm that had prescriptions issues for them; for 623 farms in 2016 with a mean of 3.1 prescriptions per farm; for 435 farms in 2017 with a mean number of 1.8 prescriptions per farm; and for 289 farms in 2018 with a mean number of 1.7 prescriptions per farm. The reduction in the number of prescriptions from 2017 to 2018 was therefore mainly caused by a reduction in the number of farms which had prescriptions issued for them.

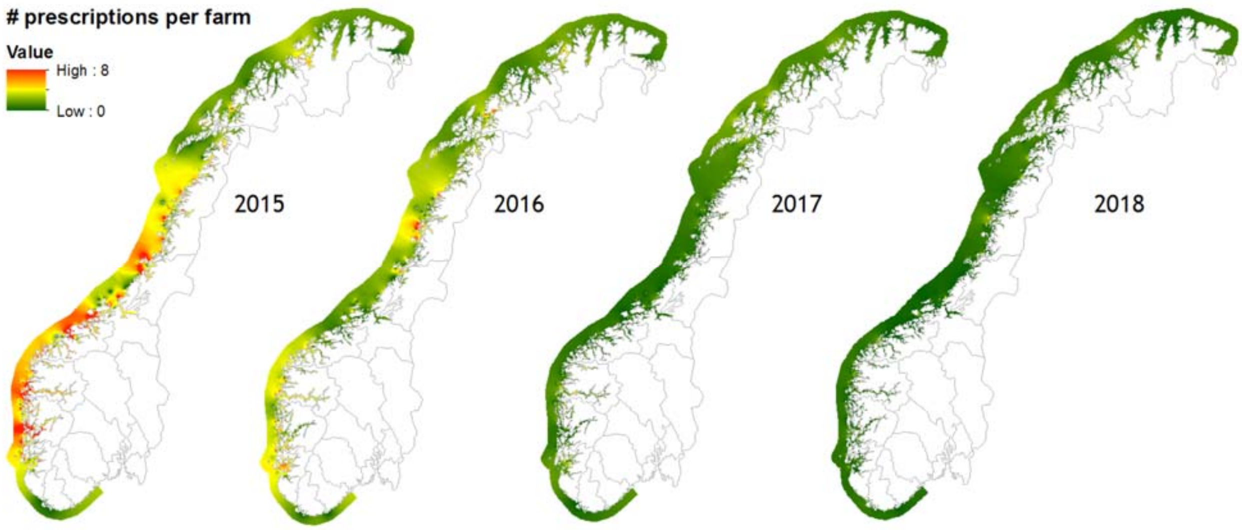


Figure 2. Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 8 prescriptions per location is expected, while dark green denote areas where the expectation of zero treatment is approached. The map layer was generated using the IDW function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighboring farm locations).

Azamethiphos was mainly used in five smaller sub-regions scattered along the coast. Pyrethroids were mainly used in Finnmark, Troms and some on the West Coast of Norway. Emamectin benzoate was mostly used on the West Coast and in Nordland. The use of flubenzurones and hydrogen peroxide was mainly restricted to the southwest (Figure 3).

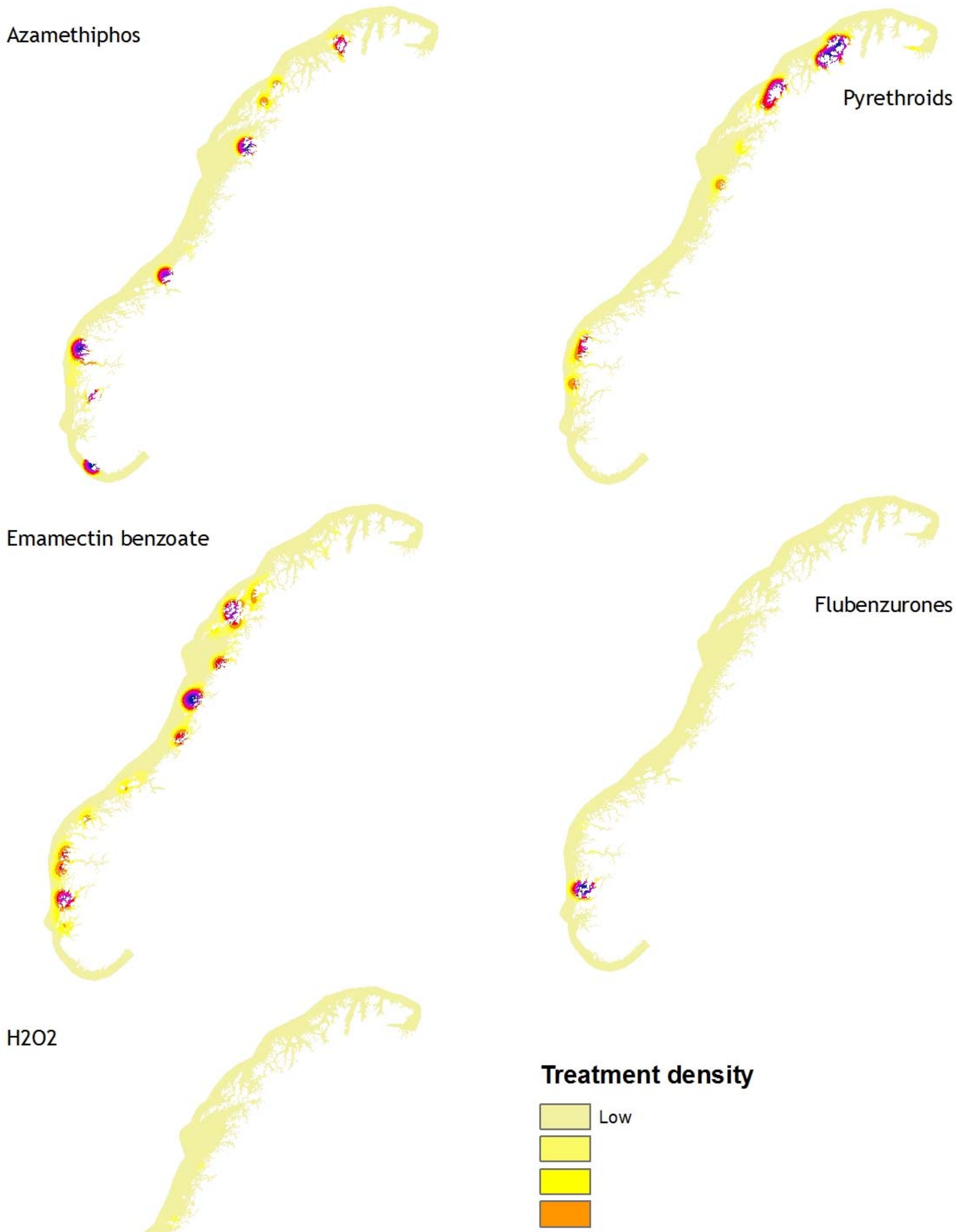


Figure 3. Kernel densities of prescriptions for five different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2018. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, where blue indicates relatively high intensities while yellow indicates relatively low densities.

Non-medicinal treatments

Table 3 summarizes the number of weeks farms have reported “mechanical treatments” in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority. The number of non-medicinal treatments increased 5.9 times in 2016 compared to 2015. The growth continued in 2017 with 42 percent increase in the number of non-medicinal treatments compared to 2016, and further in 2018 with 21 percent increase compared to 2017. 79 farms performed non-medical treatments in 2015, while the number had increased to 323 farms in 2016, 417 farms in 2017, and 484 farms in 2018. 68 percent of the non-medicinal treatments in 2018 were performed using thermic delousing. A study from 2017 showed genetic variation in the tolerance of warm water in salmon lice (9). The frequent use of thermic delousing inflicts a selection pressure favouring lice that can survive warm water treatments. This selection pressure was inflicted on a large geographic area, since the use of thermic delousing was frequent along most of the west coast of Norway, as well as in Trøndelag and parts of Nordland (Figure 4).

Table 3. Number of weeks when farms have reported non-medicinal treatments of salmon lice, in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority, from 2012 to 2018. The treatments were subdivided into four different categories. “Thermic delousing” summarizes treatments using warm water and “mechanical removal” summarizes treatments using water pressure or brushes. The number of treatments was collected from the register 04.03.19.

Treatment category	2012	2013	2014	2015	2016	2017	2018
Thermic	0	0	3	36	683	1244	1374
Mechanical removal	4	2	38	34	331	279	471
Fresh water	0	1	1	28	88	95	102
Other	132	108	136	103	77	55	76
Total	**136**	**111**	**178**	**201**	**1179**	**1673**	**2023**

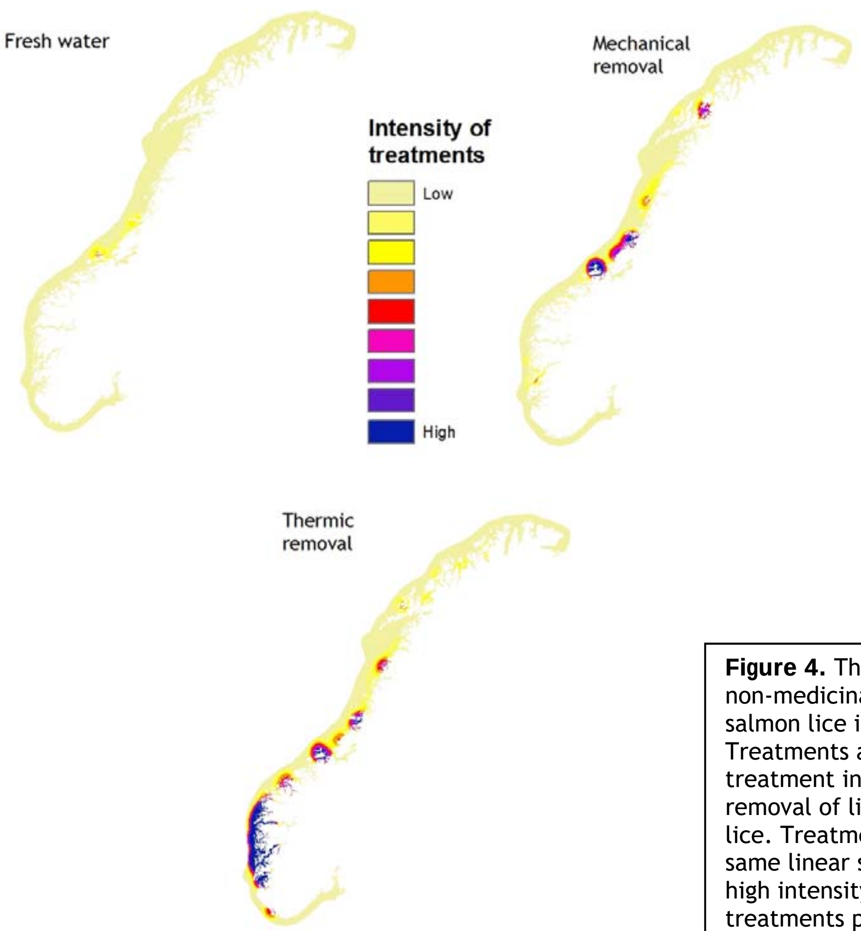


Figure 4. The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2018. Treatments are categorized into bath treatment in fresh water, mechanical removal of lice and thermic removal of lice. Treatment intensity is shown with the same linear scale in all three maps. The high intensity (blue) is equivalent to two treatments per 100 km^2 of water surface, while low intensity (light yellow) is equivalent to zero treatments.

Active surveillance

Altogether, 199 bioassays were performed on salmon lice from 64 different salmon farms along the cost (Figure 1). Of these, 49 farms were tested using azamethiphos, 48 farms using deltamethrin, 47 farms using emamectin benzoate and 55 farms using hydrogen peroxide (Table 6).

Table 6 shows that salmon lice mortalities were lower than 80 percent in the majority of locations tested at low concentrations for each substance. This indicates that reduced sensitivity to chemotherapeutants is widespread in salmon lice in Norwegian salmon farms.

Table 6. Number of bioassays with the two concentrations applied (low and high), subdivided by the test outcome (percent mortality among the included salmon lice).

Substance category	Number of tests	Percent mortality				
Low concentration		0-20 %	20-40 %	40-60 %	60-80 %	80-100 %
Azamethiphos	49	8	18	19	3	1
Deltamethrin	48	25	17	4	0	2
Emamectin benzoate	47	20	13	6	4	4
Hydrogen peroxide	55	2	17	20	9	7
High concentration						
Azamethiphos	49	3	16	20	10	0
Deltamethrin	48	1	11	15	12	9
Emamectin benzoate	47	5	6	11	13	12
Hydrogen peroxide	55	0	0	3	12	40

Table 7 shows that the salmon lice mortality results from low and high concentrations are significantly correlated. 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 significant, indicating consistency in the results from low and high concentration tests within farms.

Substance category	N	Spearman Correlation Coefficients
Azamethiphos	49	0.52
Deltamethrin	48	0.43
Emamectin benzoate	47	0.70
Hydrogen peroxide	55	0.45

Table 8. Results from molecular resistance test from five farms in Vest-Agder and Finnmark. The resistance levels are given as mean percentage of parasites categorized as sensitive or resistant towards pyrethroids or sensitive, intermediate resistant or resistant towards organophosphates. The number of farms tested in each county is given (n=).

Substance category Level of resistance	Vest-Agder (n=3)	Finnmark (n=2)
Pyrethroids		
Sensitive	30 %	24 %
Resistant	70 %	76 %
Organophosphates		
Sensitive	35 %	31 %
Intermediary	58 %	52 %
Resistant	8 %	17.5 %

Bioassay results are shown geographically and distributions of proportional mortality are given in box plots for azamethiphos (Figure 5), deltamethrin (Figure 6), emamectin benzoate (Figure 7) and hydrogen peroxide (Figure 8).

Low salmon lice mortalities in high concentration azamethiphos bioassays (Figure 5A), indicating that low treatment efficacy may be expected, were generally widespread. However, there were some variations in mortality between the different farms (Figure 5).

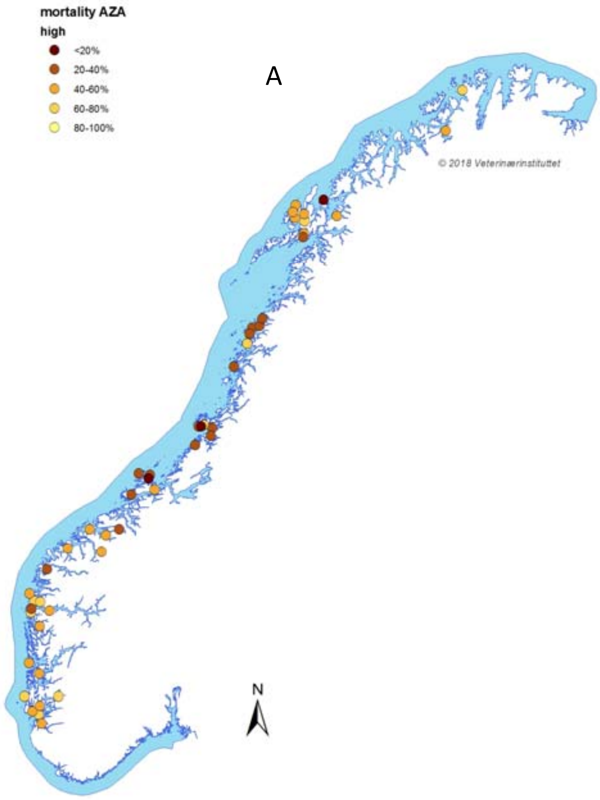
The low mortality in the low concentration deltamethrin bioassays (Figure 6B) indicates that reduced sensitivity to deltamethrin is widespread along the coast. Only two farms showed test mortalities exceeding 80 percent. In general, the results from the high concentration deltamethrin bioassays (Figure 6A) indicate that farms in most areas tested may expect low treatment efficacy.

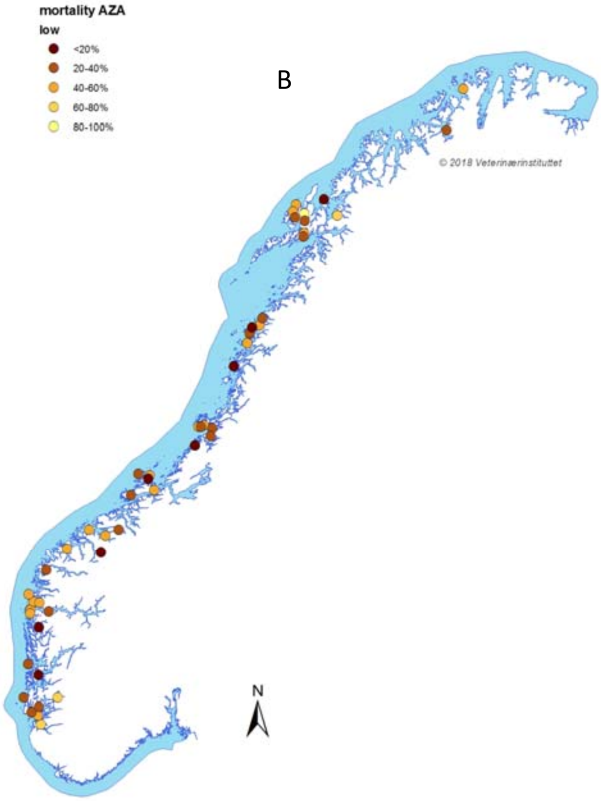
The low concentration emamectin benzoate bioassays showed that reduced sensitivity is widespread along the coast (Figure 7B). The high concentration emamectin benzoate bioassays (Figure 7A) showed that reduced treatment efficacy could be expected along most of the coast.

For hydrogen peroxide, results from the high concentration bioassays yielded generally higher mortalities than for the other substances tested. This means that better treatment results could be expected than from treatments with other the substances. The low concentration tests (Figure 8B) however showed low mortality in most areas, indicating loss of sensitivity to hydrogen peroxide.

The molecular tests of lice from the three farms in in Vest-Agder revealed a high percentage of pyrethroid resistant lice (Table 8). The increase in the presence of resistant lice seen from 2016 to 2017 (33-40 percent to 81 percent) (10, 11), were followed by the finding of 70 percent resistant lice in 2018. The effect of the deltamethrin treatments performed in the area in the autumn of 2016, after several years without medicinal treatments in this area, was by this finding also seen in 2018. The increase seen in organophosphate resistance continued, from 30-40 percent in 2016, to 50 percent in 2017 and 66 percent in 2018. The level of resistance towards pyrethroids seen in the molecular tests in Finnmark was increased compared to 2017; from 29 percent to 76 percent resistant parasites. The increase in organophosphate resistance was not as prominent in the same period; from 57 percent to 69.5 percent resistant parasites. The tests were not performed on lice from the same farms each year. Although the number of tests were limited, the trend seems to be an increased resistance frequency towards pyrethroids in Finnmark. The use of pyrethroids against salmon lice was also relatively high in this county in 2018 (Fig. 3).

Figure 9 display all high dose bioassay results for the four substances applied. The results indicate a reduction in resistance for all substances. This reduction is indicated to have started in 2017 for azamethiphos, deltamethrin and emamectin benzoate, and in 2018 for hydrogen peroxide.





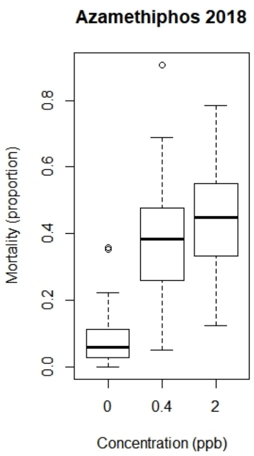
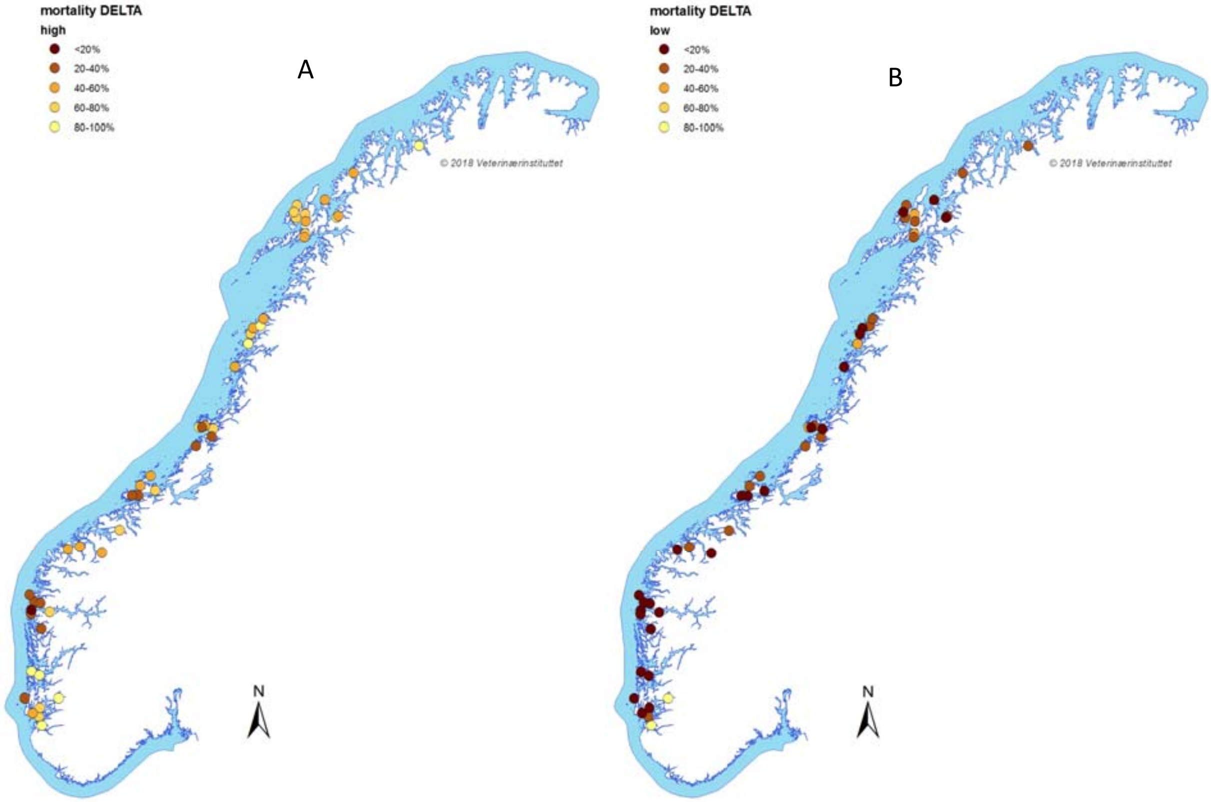


Figure 5. Maps showing categorical mortalities in bioassays with high (A) and low (B) azamethiphos concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).



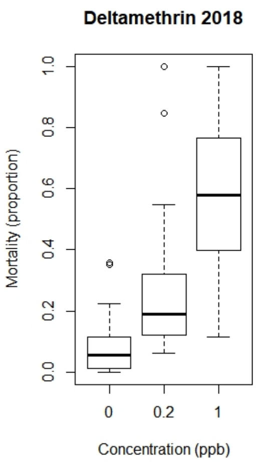
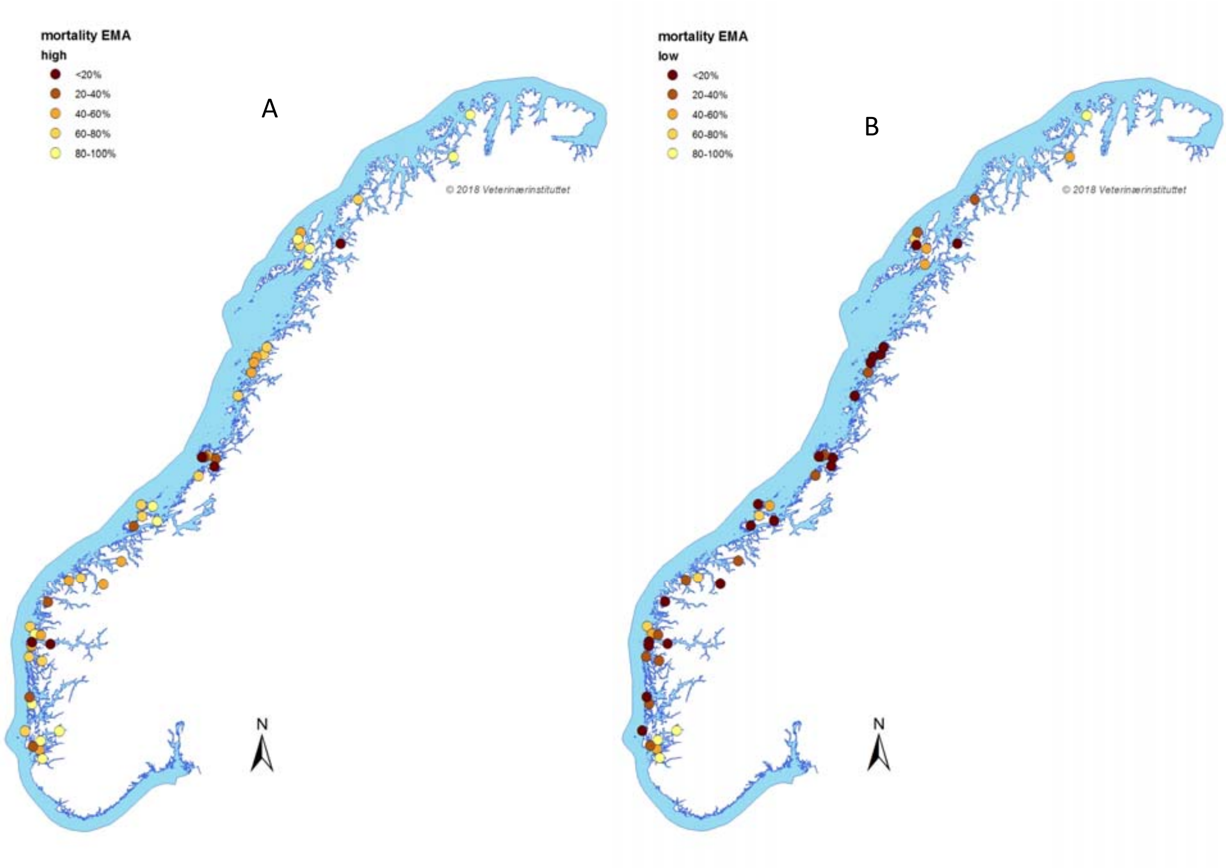


Figure 6. Maps showing categorical mortalities in bioassays with high (A) and low (B) deltamethrin concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).



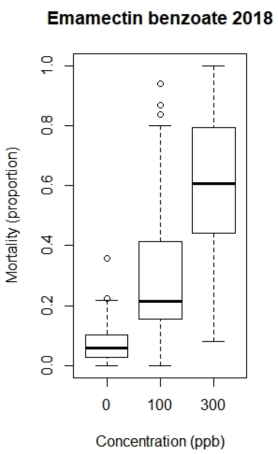
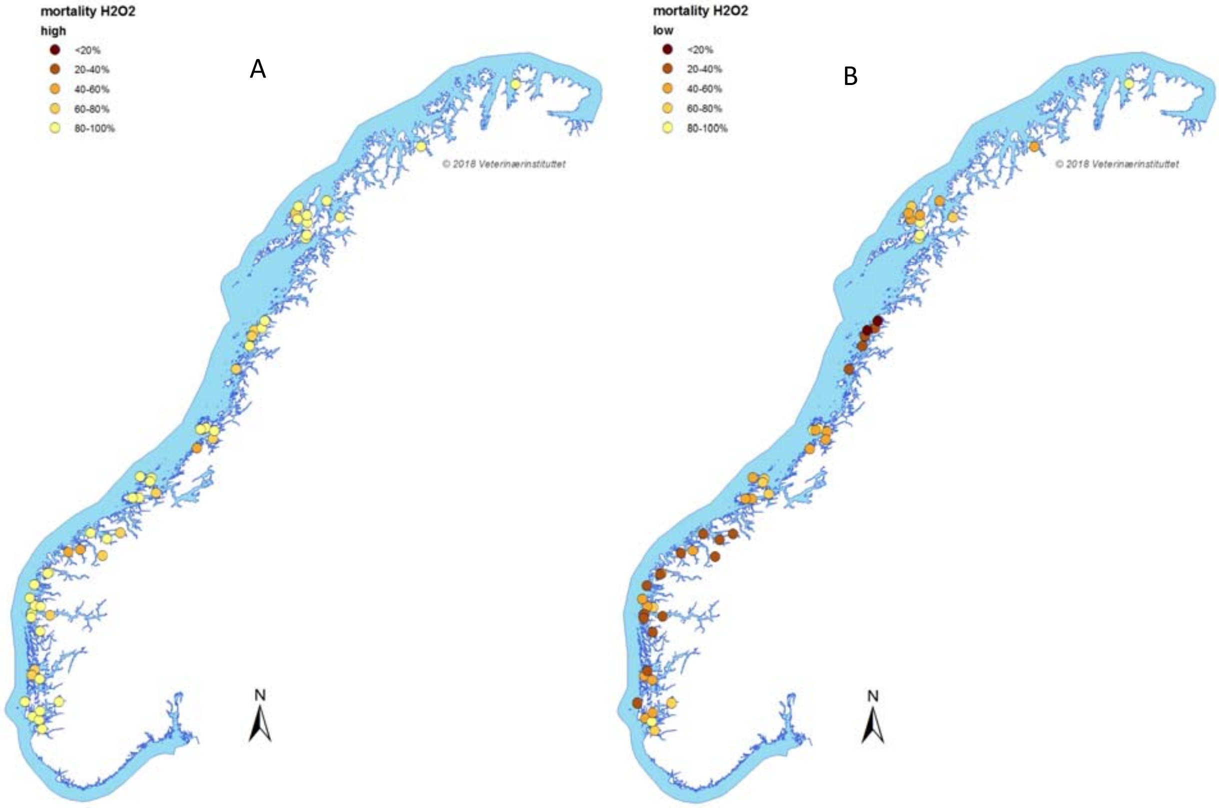


Figure 7. Maps showing categorical mortalities in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).



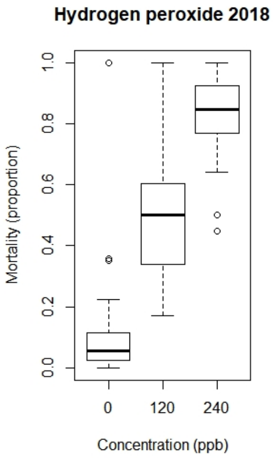


Figure 8: Maps showing categorical mortalities in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the three substances tested).

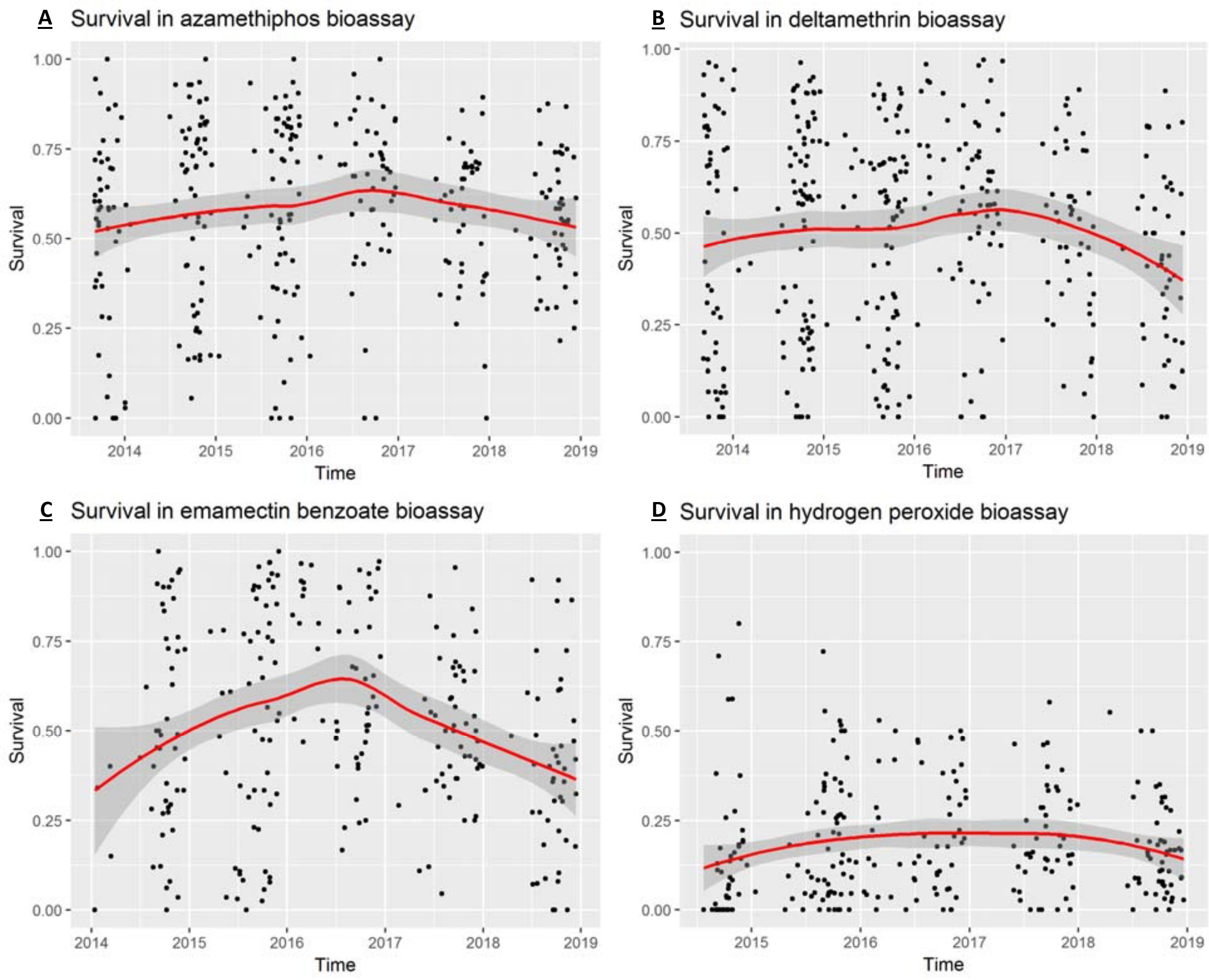


Figure 9. All bioassay results from exposure to azamethiphos (A), deltametrin (B), emamectin benzoate (C) and hydrogen peroxide (D) displayed as percent survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances. The red line is the spline best fitting the data and the dark grey area is the 95 percent confidence interval for the spline.

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Conclusions

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2018. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were generally widespread along the Norwegian coast. Less resistance was found towards hydrogen peroxide than towards the other medicines, but loss of hydrogen peroxide sensitivity was indicated in several areas. However, the results for all years of the surveillance program compiled indicate a somewhat reduced resistance level towards all four substances. The reduction was probably caused by the massive reduction in the number of medicinal treatments against salmon lice, which started in 2015. The indications of reduced resistance shown in this report is in accordance with the goals set by the government's action plan against resistance in salmon lice from 2017, saying that the development of resistance towards antiparasitics in salmon lice should be stopped or reversed compared to a 2015-level (12).

The number of prescriptions of medicines against salmon lice was reduced by 38 percent from 2017 to 2018. Compared to 2014, when the number of prescriptions peaked, the number was reduced by 86 percent. This reduction was most likely to a large degree caused by resistance. When resistance towards a medicine is present, the medicine is not prescribed due to expected low treatment efficacies. Another reason for the decrease in the number of prescriptions is the increase in the availability of non-medicinal treatments options. The reduction in prescriptions was valid for all substances/categories of substances.

The number of non-medicinal treatments increased with 21 percent compared to 2017. 2018 was the first year where more farms reported the use of non-medicinal methods than farms which had medicines against salmon lice prescribed for them; 484 vs. 289 farms. Thermic delousing was the dominating method with 68 percent of the non-medicinal treatments. Frequent treatment with a single method will most likely inflict a selection pressure towards more temperature tolerant salmon lice.

Although there are indications of a reduction of resistance from 2016 to 2018, the lice have for most substances not the sensitivity level needed to obtain effective treatments. The massive reduction in the use of medicinal treatments will not necessarily lead to fully restored sensitivity. One reason for this assertion is the relatively low frequency of sensitive parasites, which could possibly dilute the resistance genes in the absence of a selection pressure. Neither can salmon lice from wild migrating salmon be trusted as a source of purely sensitive lice, as these also carry resistance factors as shown for organophosphate resistance (13). The other reason is the continuous use of medicinal treatments, although at a lower intensity. The performed treatments will contribute to withhold a selection pressure towards resistance.

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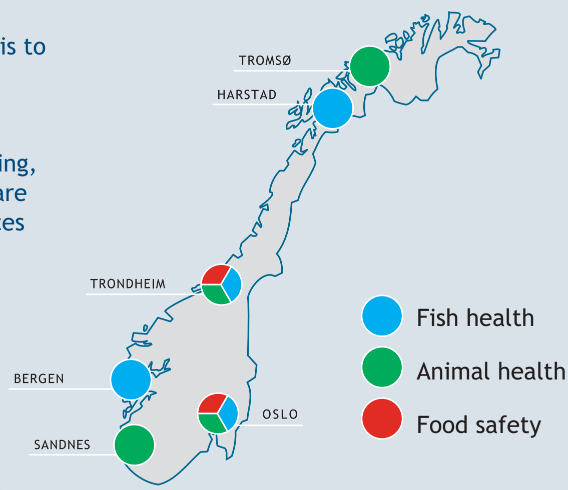

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Oslo postmottak@vetinst.no
Trondheim vit@vetinst.no
Sandnes vis@vetinst.no
Bergen post.vib@vetinst.no
Harstad vih@vetinst.no
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