Annual Report

The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2017

Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2017

Content

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Summary

The number of medicinal treatments applied against salmon lice was reduced in 2017. This continued a yearly trend that started in 2015. Despite this, the level of resistance seen in salmon lice remained high in 2017. 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. 750 prescriptions for medicines against salmon lice were prescribed in 2017, which was a 61 percent reduction compared to 2016. The number of non-medicinal treatments increased with 47 percent, to 1,759 reported treatments, in the same time period. Non-medicinal methods for treatment and prevention were thereby the dominating methods for salmon lice control. Thermic delousing accounted for 74 percent of the non-medicinal treatments in 2017.

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, partly 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 and reports of resistance are summarised. In the active surveillance part, toxicological or molecular resistance tests are performed on salmon lice from approximately 75 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 was included in the report for 2017.

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 five 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 in a farm. Hydrogen peroxide is used against salmon lice infestations, but also against amoebic gill disease at a lower concentration. All hydrogen peroxide prescriptions were included. This is due to the fact that all hydrogen peroxide treatments are likely to inflict a selection pressure for resistance in salmon lice, regardless of the treatment indication.

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. 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 data have been summarised as part of the passive surveillance. These data are however 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 caused the lack 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

Eight fish health services along the Norwegian coast were engaged in 2017 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 (2013-2016). 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.

Figure 1. Locations of farms where salmon lice were collected for bioassays in 2017.

L. salmonis from a maximum of 68 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 sea water, 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.

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 Troms and 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 two farms from Vest-Agder, two farms in Troms and four 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 treatments

Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2017. 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 pronounced in 2016 and continued in 2017, with 61 percent reduction in the total number of prescriptions compared to the previous year. The massive decrease in the number of prescriptions was prominent for all substances/classes of substances. This reduction lowered selection pressure towards resistance, compared to 2016. Emamectin benzoate was the most commonly prescribed medicine, but the number of prescriptions was reduced, also for this substance, by 48 percent in 2017 compared to 2016.

Table 2. Number of prescriptions for the given substances/class of substances applied to control salmon lice in 2011 to 2017. The number of prescriptions was collected from VetReg 16.01.18.

Substance category	2011	2012	2013	2014	2015	2016	2017
Azamethiphos	409	691	480	749	619	257	58
Pyrethroids	456	1 155	1 123	1 043	662	276	80
Emamectin benzoate	288	164	162	481	523	608	319
Hydrogen peroxide	172	110	250	1 009	1 279	629	214
Flubenzurones	23	129	170	195	201	173	79
Total	**1 348**	**2 249**	**2 185**	**3 477**	**3284**	**1 943**	**750**

Prescriptions per farm

The maps in Figure 2 sum up the total number of prescriptions per location in the period 2015 - 2017. Prescriptions were issued for 661 farms in 2015 with a mean of 5.0 prescriptions per farm; for 623 farms in 2016 with a mean of 3.1 prescriptions per farm; and for 423 farms in 2017 with a mean number of 1.8 prescriptions per farm. The reduction in the number of prescriptions from 2016 to 2017 was therefore both caused by a reduction in the number of prescriptions per farm and by the total number of farms which had prescriptions issued for them.

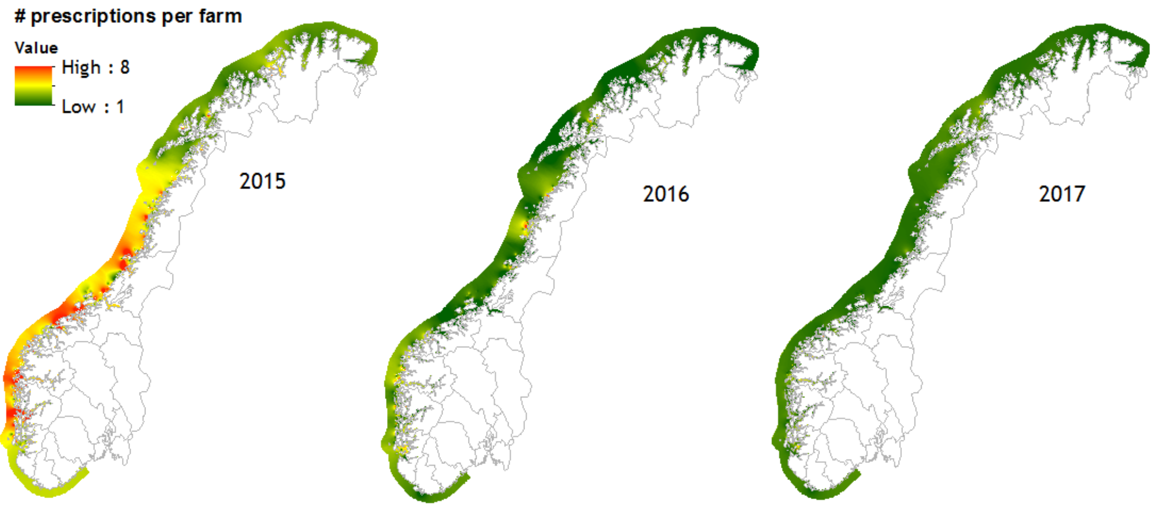


Figure 2. Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 8 prescriptions per location is expected, while dark green denote areas where the expectation of one treatment 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 northern Norway: north in Nordland and in Finnmark. Pyrethroids were used in the same region, but mostly in Troms. Emamectin benzoate was mostly used on the West Coast. The use of flubenzurones and hydrogen peroxide was mainly restricted to the southwest (Figure 3).

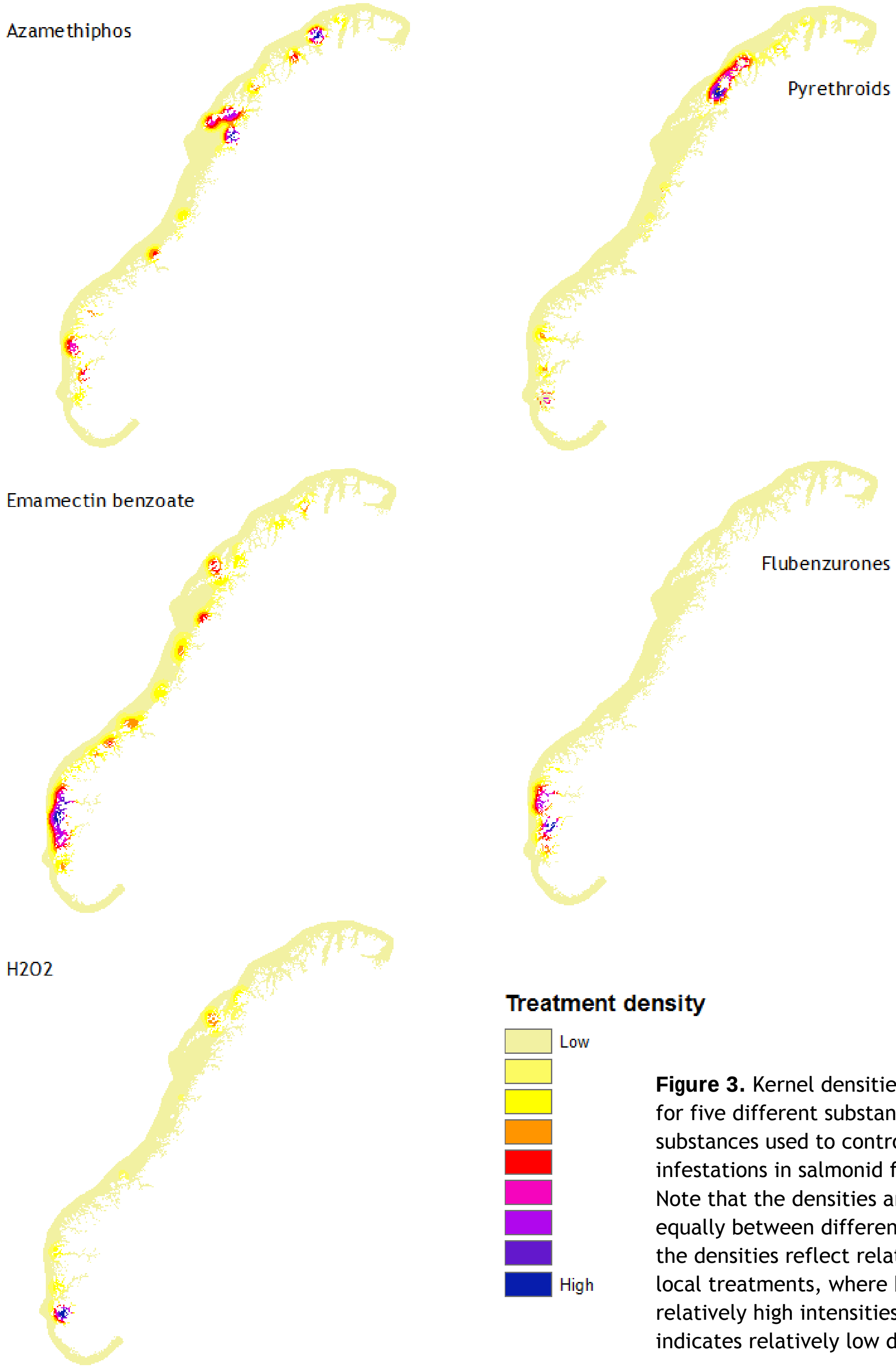


Figure 3. Kernel densities of prescriptions for five different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2017. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, 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 5.5 times in 2016 compared to 2015. The growth continued in 2017 with 47 percent increase in the number of non-medicinal treatments compared to 2016. 79 farms performed non-medical treatments in 2015, while the number had increased to 323 farms in 2016 and further to 416 farms in 2017. 74 percent of the non-medicinal treatments in 2017 were performed using thermic delousing, and this method accounted for almost the entire increase in the use of non-medicinal treatments in 2017 compared to 2016. 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 favoring 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 2017. 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 08.02.18.

Treatment category	2012	2013	2014	2015	2016	2017
Thermic	0	0	3	42	700	1 308
Mechanical removal	4	6	40	38	333	293
Fresh water	0	0	2	31	88	96
Other	132	109	140	106	76	62
Total	**136**	**115**	**185**	**217**	**1 197**	**1 759**

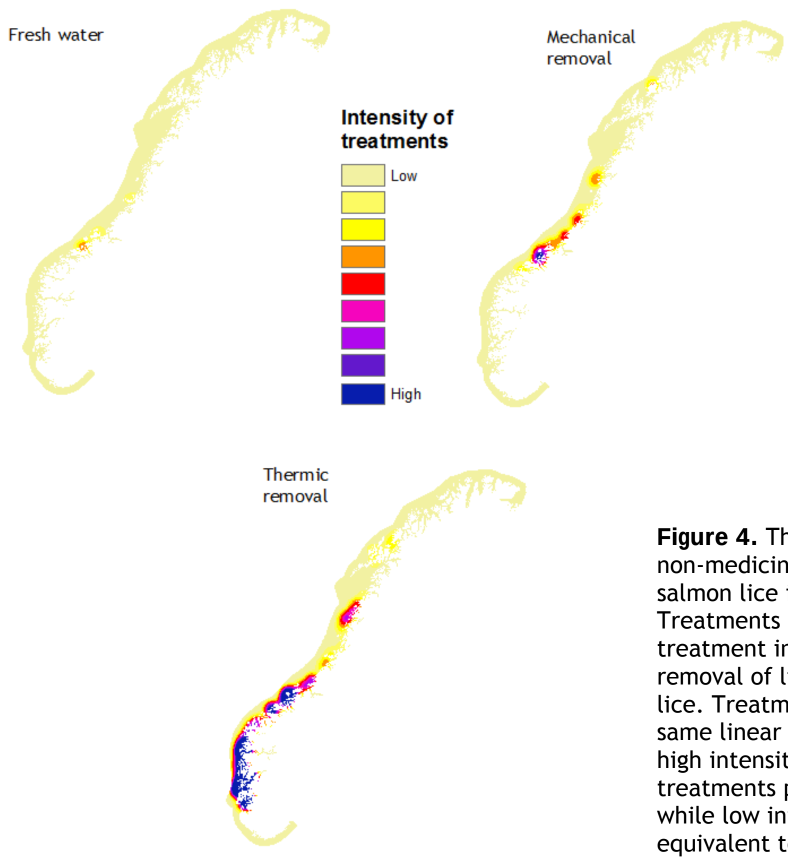


Figure 4. The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2017. Treatments are categorized into bath treatment in fresh water, mechanical removal of lice and thermic removal of lice. Treatment intensity is shown with the same linear scale in all 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.

Reported sensitivity data

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

Substance category	2016			2017		
	Sensitive	Reduced sensitivity	Resistant	Sensitive	Reduced sensitivity	Resistant
Azamethiphos	5	37	8	3	25	4
Emamectin benzoate	6	12	2	4	8	2
Flubenzurones	1	0	0	0	0	0
Hydrogen peroxide	18	24	1	17	13	0
Pyrethroids	11	42	10	5	28	4
Total	**41**	**115**	**21**	**29**	**74**	**10**

Table 4 and 5 summaries the reported resistance from the weekly salmon lice data. 113 reports from sensitivity studies were given, which is approximately one per 6.6 prescriptions of medicinal treatment. These were not from a random selection of farms and no objective criteria were given for the different groups. The data were therefore difficult to infer from.

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 efficacy; 3 = situation in the area; 9 = other).

Substance category	2015				2016				2017			
	1	2	3	9	1	2	3	9	1	2	3	9
Azamethiphos	13	43	1	0	6	13	4	0	7	4	7	2
Emamectin benzoate	1	16	0	0	2	16	0	0	1	3	0	2
Flubenzurones	0	0	0	0	0	0	0	1	0	0	0	0
Hydrogen peroxide	1	25	0	0	4	5	1	0	1	0	0	1
Pyrethroids	15	43	1	0	7	11	3	0	7	0	5	3
Total	**30**	**127**	**2**	**0**	**19**	**45**	**8**	**1**	**16**	**7**	**12**	**8**

Active surveillance

Altogether, 195 bioassays were performed on salmon lice from 68 different salmon farms along the cost (Figure 1). Of these, 44 farms were tested using azamethiphos, 50 farms using deltamethrin, 53 farms using emamectin benzoate and 48 farms using hydrogen peroxide (Table 6). Table 6 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 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	44	10	24	7	3	0
Deltamethrin	50	31	11	5	1	2
Emamectin benzoate	53	25	18	9	1	0
Hydrogen peroxide	48	4	15	17	6	6
High concentration						
Azamethiphos	44	3	19	12	8	2
Deltamethrin	50	4	10	20	10	6
Emamectin benzoate	52	3	11	20	15	3
Hydrogen peroxide	48	0	0	4	16	28

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	44	0.68
Deltamethrin	50	0.59
Emamectin benzoate	52	0.49
Hydrogen peroxide	48	0.54

Table 8. Results from molecular resistance test from eight farms in Vest-Agder, Troms 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=2)**	**Troms (n=2)**	**Finnmark (n=4)**
Pyrethroids			
Sensitive	19 %	29,5 %	29 %
Resistant	81 %	70,5 %	71 %
Organophosphates			
Sensitive	50 %	23 %	43 %
Intermediary	37,5 %	67 %	46 %
Resistant	12,5 %	10 %	11 %

Test 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).

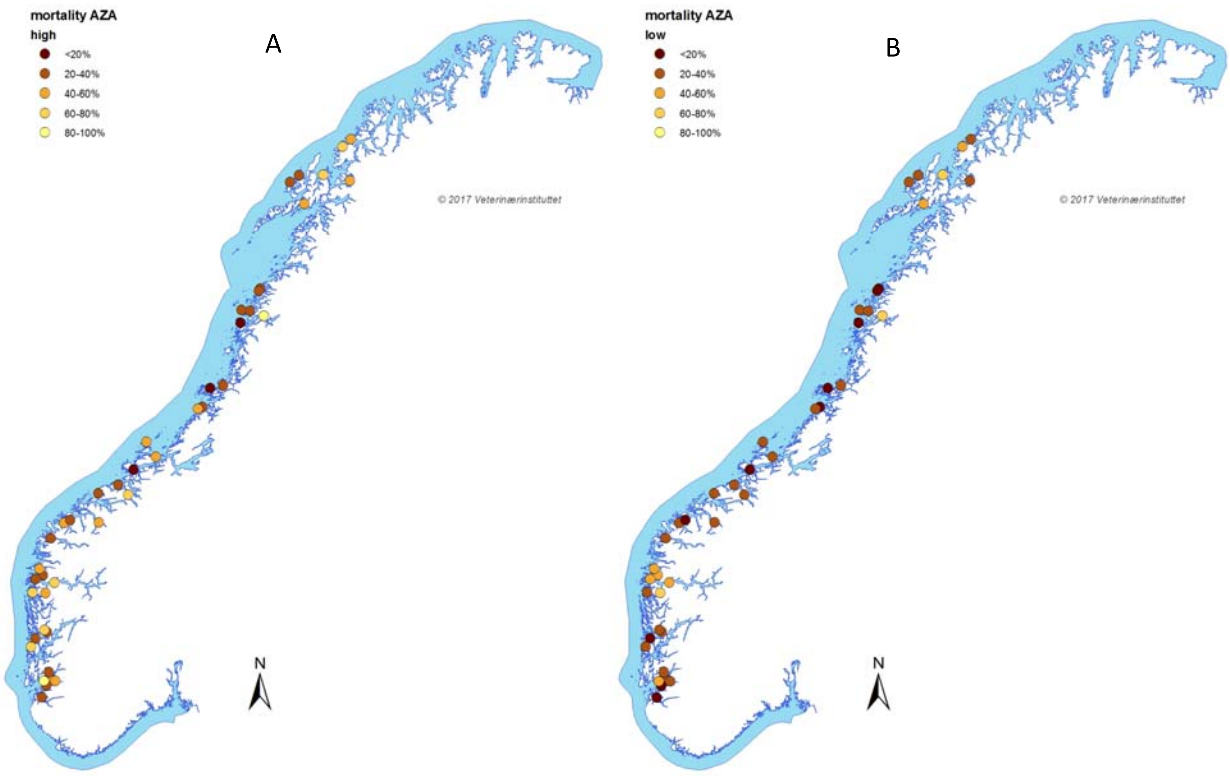
Low salmon lice mortalities in high concentration azamethiphos bioassays (Figure 5A), indicating that low treatment efficacy may be expected, were generally widespread. However, there were some variations in mortality between the different farms (Figure 5).

The low mortality in the low concentration deltamethrin bioassays (Figure 6B) indicates that reduced sensitivity to deltamethrin is widespread along the coast. Six farms, however, showed test mortalities exceeding 80 %. Two of these farms were in Finnmark and were also tested for the molecular marker for pyrethroid resistance, but only 40 and 23 % were found to be sensitive in this test. The different results could be explained by differences in the selected groups for the two different resistance tests, or by one of the possible sources for errors in either method. In general, the results from the high concentration deltamethrin bioassays (Figure 6A) indicate that farms in most areas 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 all along 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 the other 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 two farms in in Vest-Agder revealed a high percentage of pyrethroid resistant lice (Table 8). The increase in the presence of resistant lice from 2016 to 2017 (33-40 % to 81 %) (10), could possibly be explained by deltamethrin treatments performed in the area in the autumn of 2016, after several years without medicinal treatments in that area. The increase seen in organophosphate resistance was not as prominent (30-40 % to 50 %). The level of resistance towards pyrethroids seen in the molecular tests in Troms and Finnmark were similar (29 %), while the lice tested from Troms showed somewhat higher resistance towards organophosphates (77 % in Troms and 57 % in Finnmark).



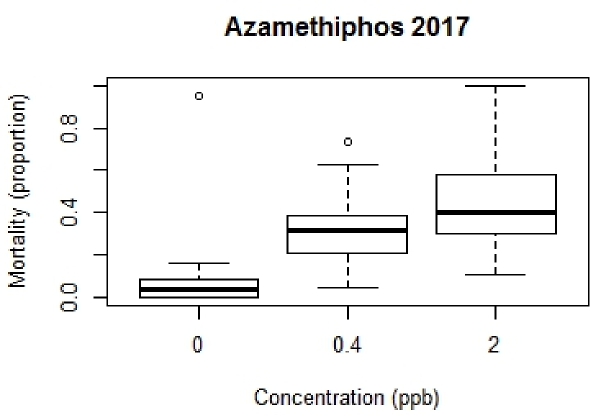


Figure 5. Maps showing categorical mortalities in bioassays with high (A) and low (B) azamethiphos concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests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

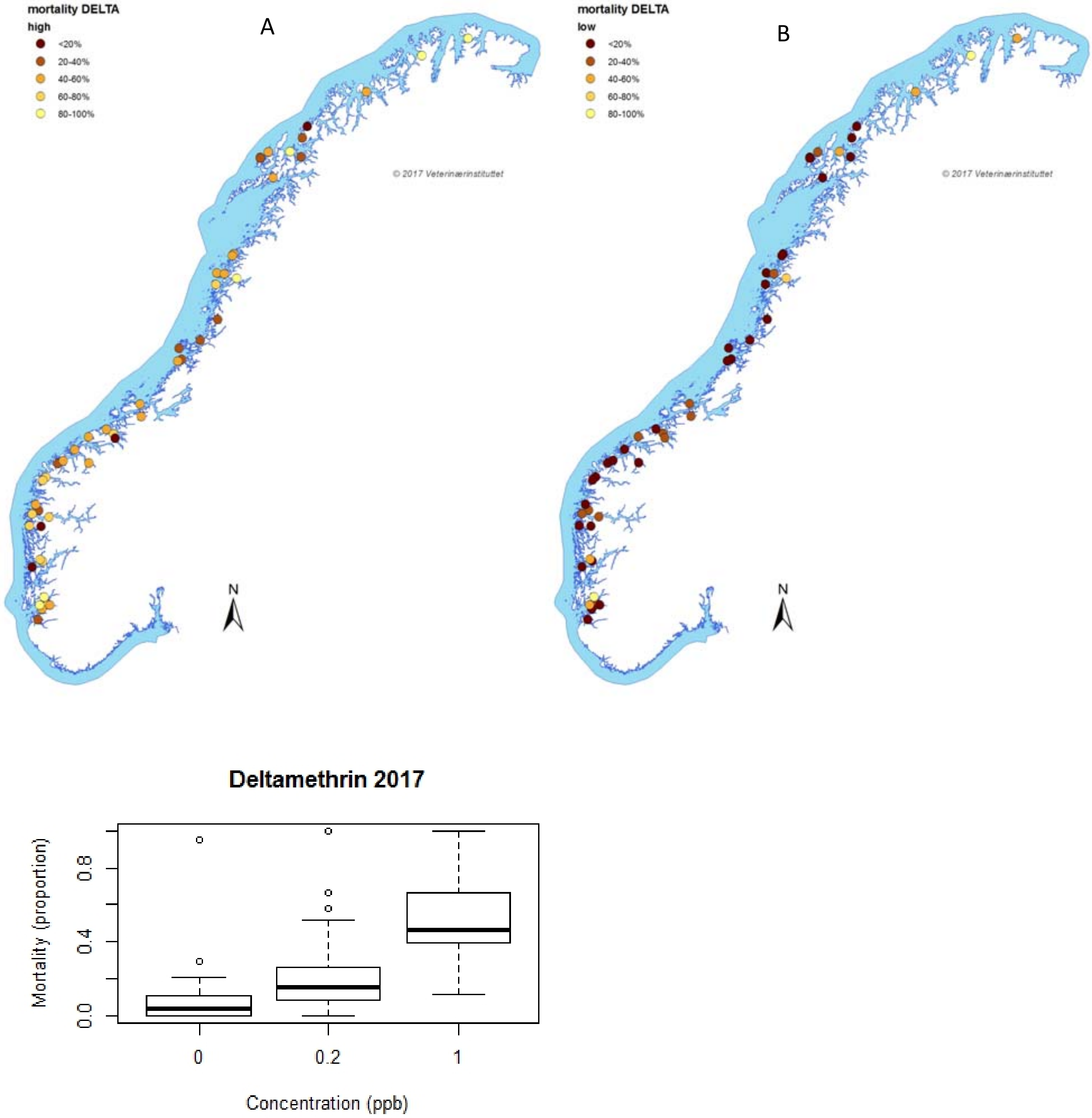


Figure 6. Maps showing categorical mortalities in bioassays with high (A) and low (B) deltamethrin concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests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

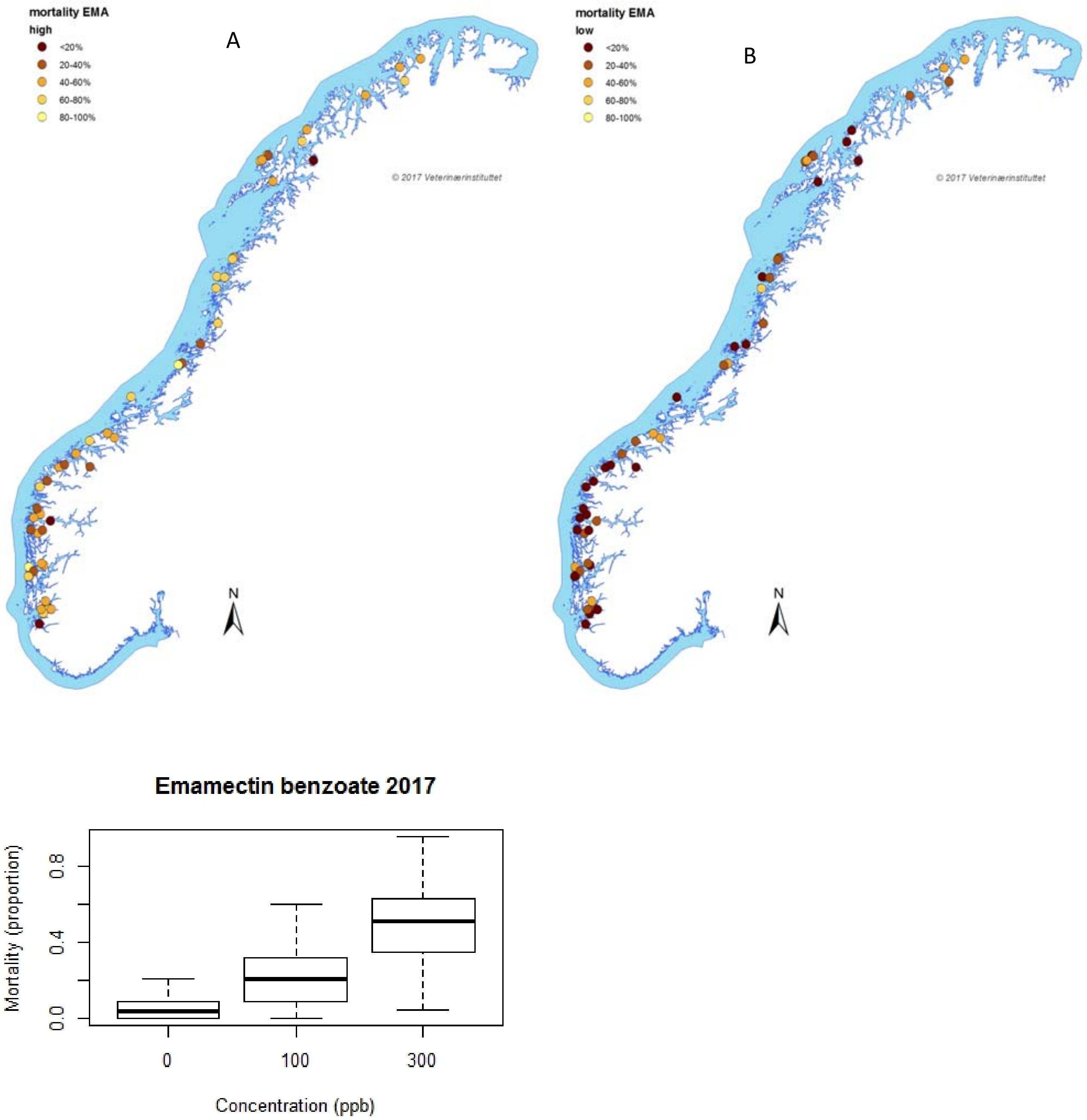


Figure 7. Maps showing categorical mortalities in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all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

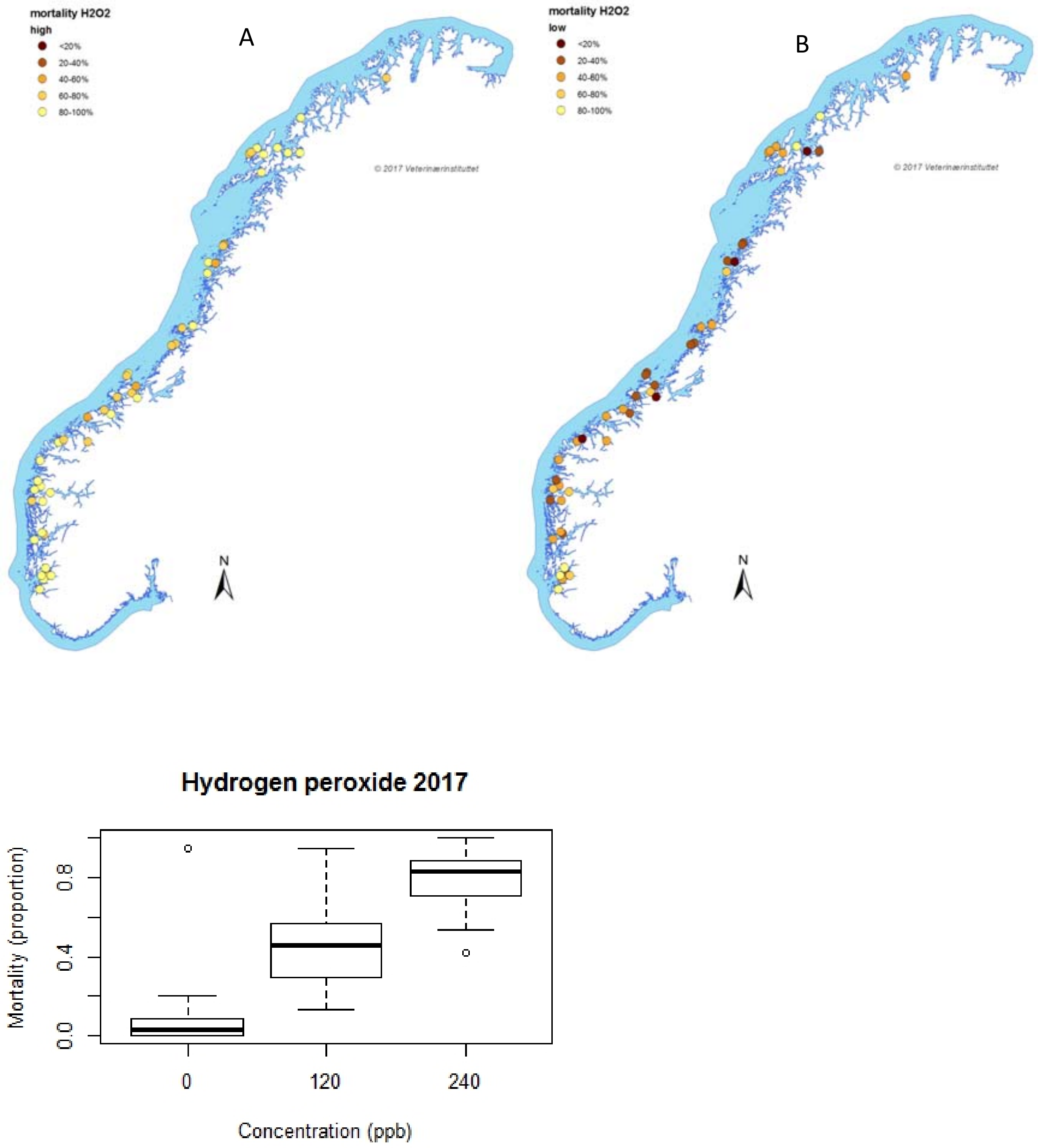


Figure 8: Maps showing categorical mortalities in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all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

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In addition, thanks to Marine Harvest for salmon lice sampling.

Conclusions

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2017. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were generally widespread along the Norwegian coast. Less resistance were found towards hydrogen peroxide than towards the other medicines, but loss of hydrogen peroxide sensitivity was indicated in several areas. This was despite the fact that the number of medicinal treatments against salmon lice was markedly reduced.

The number of prescriptions of medicines against salmon lice was reduced by 61 % from 2016 to 2017. Compared to 2014, when the number of prescriptions peaked, the number was reduced by 78 %. This reduction was most likely partly 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 47 % compared to 2016. This increase was almost entirely comprised of an increase in thermic delousing. This method was used for 74 % of the non-medicinal treatments against salmon lice. Frequent treatment with a single method will most likely inflict a selection pressure towards more temperature tolerant salmon lice.

The reduced use of medicinal treatments will not necessarily lead to major reductions in resistance for all substances. One reason for this assertion is the low frequency of sensitive parasites, which could possibly have diluted 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 shown for organophosphate resistance (11). The other reason is the continuous use of medicinal treatments, although at a lower intensity. The performed treatments will contribute to withhold and possibly increase the frequency of resistance.

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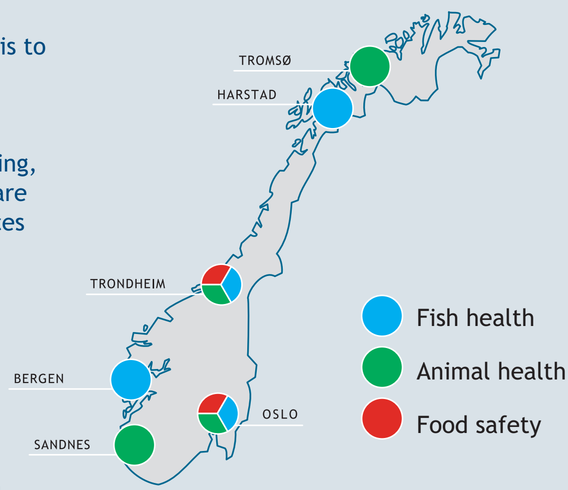

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