Annual Report

The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2016

Veterinærinstituttet
Norwegian Veterinary Institute

The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2016

Content

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Summary

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2016. There was, however, a marked reduction in medicinal treatments against salmon lice. The number of prescriptions of medicines used against salmon lice was reduced by 41 % from 2015 to 2016.The total number of salmon lice treatments was reduced by 10 %. The reduction in medicinal treatments was compensated by a more than six-fold increase in the use of non-medicinal salmon lice treatments. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were generally widespread along the Norwegian coast. Less resistance towards hydrogen peroxide were found than towards the other medicines, but loss of sensitivity was indicated in several areas.

Introduction

The Norwegian Food Safety Authority is responsible for implementing the surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*). The programme, which was started in 2013, is based on summarising prescriptions for salmon lice treatments and reports of resistance (passive surveillance), as well as performing toxicological and molecular resistance tests (active surveillance). The Norwegian Veterinary Institute is responsible for the planning, data collection and reporting components of the programme.

Salmon lice 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 tools. 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.

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 (3). Since then, the program has annually summarized reported data from the industry on prescriptions for salmon lice treatments and reports of resistance (passive surveillance) and presented a collection of sensitivity data from approximately 75 salmon farms located along the Norwegian coast (active surveillance).

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). Table 2 summarises 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 is no possibility to check whether all prescriptions are reported, but knowledge from prescriptions for antibiotics for fish, indicates that this is the case (Kari Grave, personal comment).

Non-medicinal treatments
The number of non-medicinal treatments performed in Norwegian salmon farms in 2016 is included in table 2. The data 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 as well as delousing in fresh water baths. The reports do not include data on the number of cages treated per week, and this will vary between one and all cages.

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 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 different categories.

Active surveillance

Bioassays
Eight fish health services along the Norwegian coast were engaged in 2016 to perform toxicological resistance tests (bioassays) on live parasites. The bioassay protocol was based on Helgesen *et al* 2013 and 2015 (5, 6) and had also been applied for the surveillance programme in 2013, 2014 and 2015. 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 3) were chosen by the fish health services themselves inside a designated area.

L. salmonis from a maximum of 80 farms (Table 5) were tested against the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide. The bioassays were performed by exposing live parasites for two different concentrations of each chemical plus a 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 low concentration was used to indicate the sensitivity status of the salmon lice population, with mortalities higher than 80 % indicative of fully sensitive populations. Salmon lice mortality at high concentration was used to indicate the expected outcome of a subsequent treatment.

Molecular resistance tests
Salmon lice infestation levels on farms in Vest-Agder in the far south of Norway had been low for several years. In order to test lice from such farms for resistance, 30 lice were collected from each of three farms. 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 for deltamethrin, sensitive, intermediate resistant or resistant for azamethiphos, and as expected efficacy of a subsequent treatment for hydrogen peroxide. Molecular testing of resistance was also conducted on salmon lice from one farm in Finnmark which had previously been tested in bioassays.

Table 1. High and low concentrations used 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

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 – 2016. Hydrogen peroxide prescriptions were included whether or not the prescriptions were against salmon lice. This is due to the fact that all hydrogen peroxide treatments will inflict a selection pressure for resistance in salmon lice, regardless of the treatment indication. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years, but this was somewhat decreased in 2015. In 2016 the decrease was more pronounced with 41 % reduction in the total number of prescriptions compared to 2015. The decrease in prescriptions was especially prominent for azamethiphos, pyrethroids and hydrogen peroxide. The number of prescriptions of emamectin benzoate increased in 2016 compared to 2015. As the amounts prescribed could not be calculated, the VetReg data could not be validated against sales figures from wholesalers (https://www.fhi.no/hn/legemiddelbruk/fisk/2016-salg-av-lakselusmidler-er-synkende/). The number of non-medicinal treatments was increased by more than six-fold from 2015 to 2016. The total number of treatments was therefore decreased by 10 %.

Table 2. Number of prescriptions for the given substances/category of substances and numbers of reported non-medicinal treatments applied to control salmon lice during 2011 – 2016.

Substance category	2011	2012	2013	2014	2015	2016
Azamethiphos	409	691	480	749	616	257
Pyrethroids	456	1155	1123	1043	660	275
Emamectin benzoate	288	164	162	481	522	607
Hydrogen peroxide	172	110	250	1 009	1 270	629
Flubenzurones	23	129	170	195	201	173
Sum	1 348	2 249	2 185	3 477	3 269	1 941
Non-medicinal treatments		136	110	176	185	1 174
Total number of treatments	1 348	2 385	2 295	3 653	3 454	3 115

The maps in Figure 1 sum up the total number of prescriptions per location in the period 2014 - 2016. Prescriptions were issued for 679 farms in 2014, with a mean number of 5.1 prescriptions per farm; for 661 farms in 2015 with a mean of 4.9 prescriptions per farm; and for 662 farms in 2016 with a mean of 3.1 prescriptions per farm. The reduction in the number of prescriptions from 2015 to 2016 was therefore caused by a reduction in numbers per farm and not in the total number of farms. 78 farms performed non-medical treatments in 2015, while the number had increased to 322 farms in 2016.

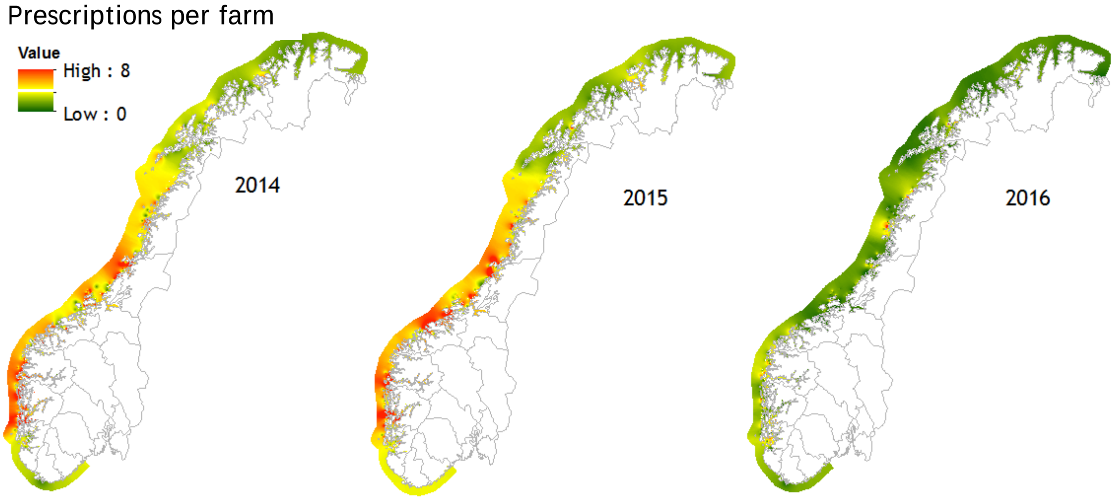

Figure 1. Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 8 prescriptions per location is expected, while dark green denote areas where the expectation of no treatment 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 three areas: southwest, south in Nordland and south in Troms. Pyrethroids were used in the same areas, but there was also a scattered use around the rest of the coast. Emamectin benzoate was used in all of the most production intense areas along the coast. The use of flubenzurones and hydrogen peroxide was mostly restricted to the southwest (Figure 2).

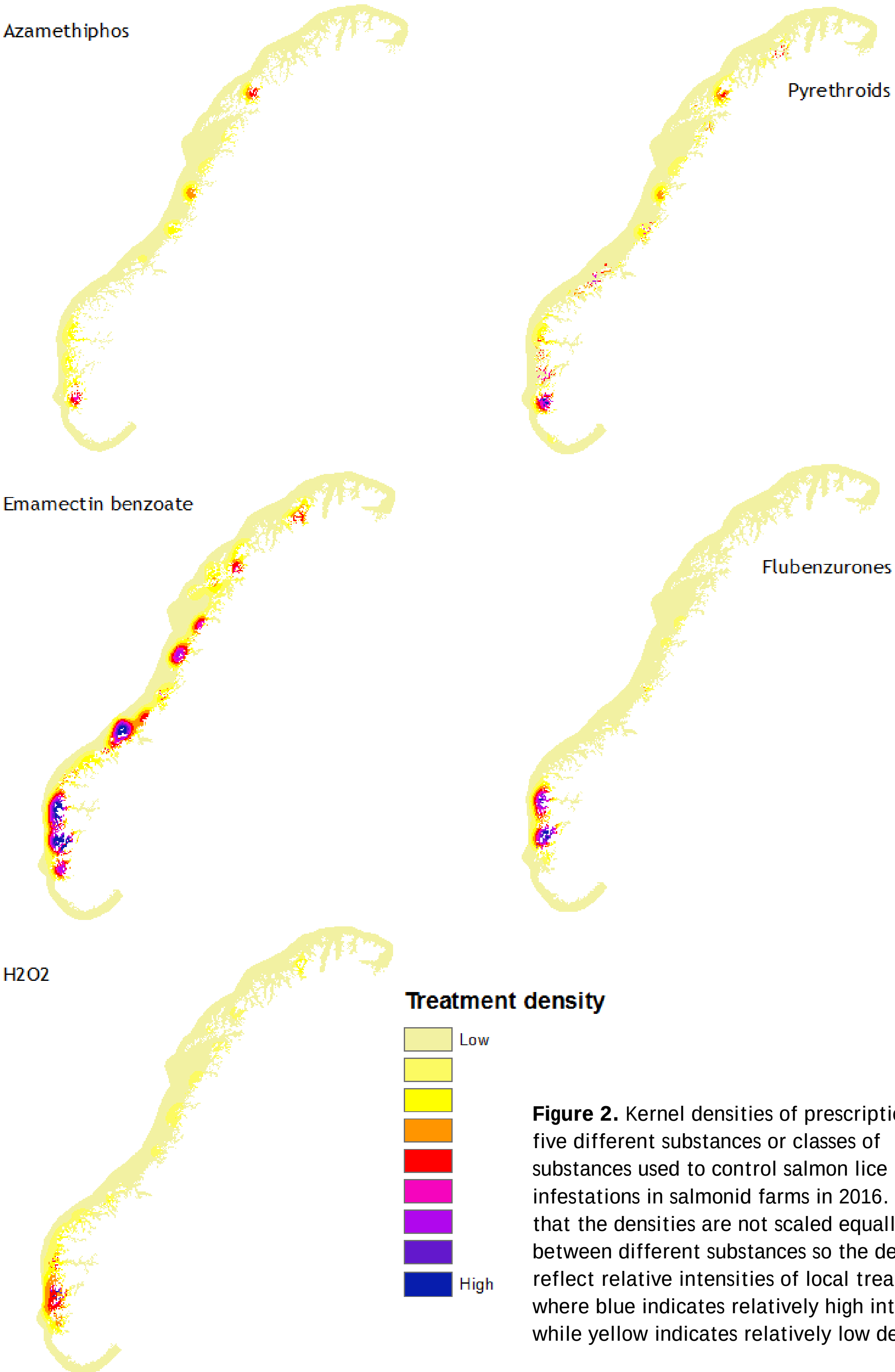

Figure 2. Kernel densities of prescriptions for five different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2016. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, where blue indicates relatively high intensities while yellow indicates relatively low densities.

Reported sensitivity data

Table 3. The number of reports from sensitivity studies within the three categories of reported sensitivity status.

Substance category	2015			2016		
	Sensitive	Reduced sensitivity	Resistant	Sensitive	Reduced sensitivity	Resistant
Azamethiphos	3	37	3	5	37	8
Emamectin benzoate	4	30	1	6	12	2
Flubenzurones				1	0	0
Hydrogen peroxide	8	19	0	18	24	1
Pyrethroids	5	48	5	11	42	10
Total	**20**	**134**	**9**	**42**	**115**	**21**

Table 3 and 4 summaries the reported resistance from the weekly salmon lice data. 178 reports from sensitivity studies were given, which is approximately one per 11 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 4. 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).

Substance category	2014			2015			2016		
	1	2	3	1	2	3	1	2	3
Azamethiphos	25	52	2	13	43	1	6	13	4
Emamectin benzoate	21	2		1	16		2	16	
Flubenzurones									
Hydrogen peroxide	3	10		1	25		4	5	1
Pyrethroids	31	66	4	15	43	1	7	11	3
Total	**80**	**130**	**6**	**30**	**127**	**2**	**19**	**45**	**8**

Active surveillance

Altogether, 226 bioassays were performed on salmon lice from 80 different salmon farms along the cost (Figure 3). Of these, 49 farms were tested using azamethiphos, 71 farms using deltamethrin, 52 farms using emamectin benzoate and 55 farms using hydrogen peroxide (Table 5).

Table 5 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.

Figure 3. Locations of farms where salmon lice were collected for bioassays in 2016.

Table 5. 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				
		0-20 %	20-40 %	40-60 %	60-80 %	80-100 %
Low concentration						
Azamethiphos	48	21	17	6	2	2
Deltamethrin	71	44	18	2	2	5
Emamectin benzoate	52	33	12	4	3	0
Hydrogen peroxide	55	12	12	15	13	3
High concentration						
Azamethiphos	49	10	23	11	1	4
Deltamethrin	71	13	25	17	9	7
Emamectin benzoate	52	17	11	17	6	1
Hydrogen peroxide	55	0	0	11	13	31

Table 6 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 6. 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	48	0.74
Deltamethrin	71	0.50
Emamectin benzoate	52	0.64
Hydrogen peroxide	55	0.57

Test results are shown geographically and distributions of proportional mortality are given in box plots for azamethiphos (Figure 4), deltamethrin (Figure 5), emamectin benzoate (Figure 6) and hydrogen peroxide (Figure 7).

For low concentration in azamethiphos bioassays (Figure 4 B), two farms with salmon lice test-mortalities exceeding 80 % (indicative of fully sensitive populations) were found. Lice from one of these farms, located in Finnmark, were also tested with molecular methods. In this test 39 % of the lice were sensitive towards azamethiphos. Further surveillance of sensitivity in this area may elucidate the mismatch between test results. Low salmon lice mortalities in high concentration azamethiphos bioassays (Figure 4A), indicating that low treatment efficacy may be expected, were generally widespread. However, there were some variations in mortality when lice from different farms were exposed to high concentration of azamethiphos (Figure 4).

The low mortality in the low concentration deltamethrin bioassays (Figure 5B) indicates that reduced sensitivity to deltamethrin is widespread along the coast. Five farms, however, showed test mortalities exceeding 80 %. One of these farms in Finnmark was tested for the molecular marker for pyrethroid resistance, but only 40 % were found to be sensitive. Further surveillance of sensitivity in this area may elucidate the mismatch between test results. In general, the results from the high concentration deltamethrin bioassays (Figure 5A) indicate that several areas may expect low treatment efficacy.

The low concentration emamectin benzoate bioassays showed that reduced sensitivity is widespread along the coast (Figure 6B). The high concentration emamectin benzoate bioassays (Figure 6A) resulted in higher mortalities in the farms in Finnmark and Rogaland compared to the rest of the country. The box plots of proportional mortality in high and low concentration tests, showed large variability, but indicated that reduced sensitivity and low treatment efficacy may be expected for emamectin benzoate as well as for azamethiphos and pyrethroids treatments.

For hydrogen peroxide, results from the high concentration bioassays yielded generally higher mortalities than for the other substances tested. The low concentration tests (Figure 7B) showed low mortality in Sør-Trøndelag, Nord-Trøndelag and parts of Nordland, indicating loss of sensitivity to hydrogen peroxide.

The molecular tests of lice from the three farms in in Vest-Agder revealed a higher percentage of lice being sensitive to pyrethroids than resistant (33-40 % resistant lice). For azamethiphos 30-40 % of the lice in the three tests were reported to be resistant (heterozygote or homozygote resistant). Compared to results from molecular tests performed in 2014 (7), this indicates that the sensitivity to azamethiphos and pyrethroids in lice from the southernmost farms in Norway was reduced. The reduction had however not evolved further from 2015 (8), but the samples were collected prior to the medicinal treatments that were performed in the area in the fall 2016. Molecular testing of hydrogen peroxid sensitivity was performed for two of the farms and gave an estimated treatment efficacy of 91 and 62 %.

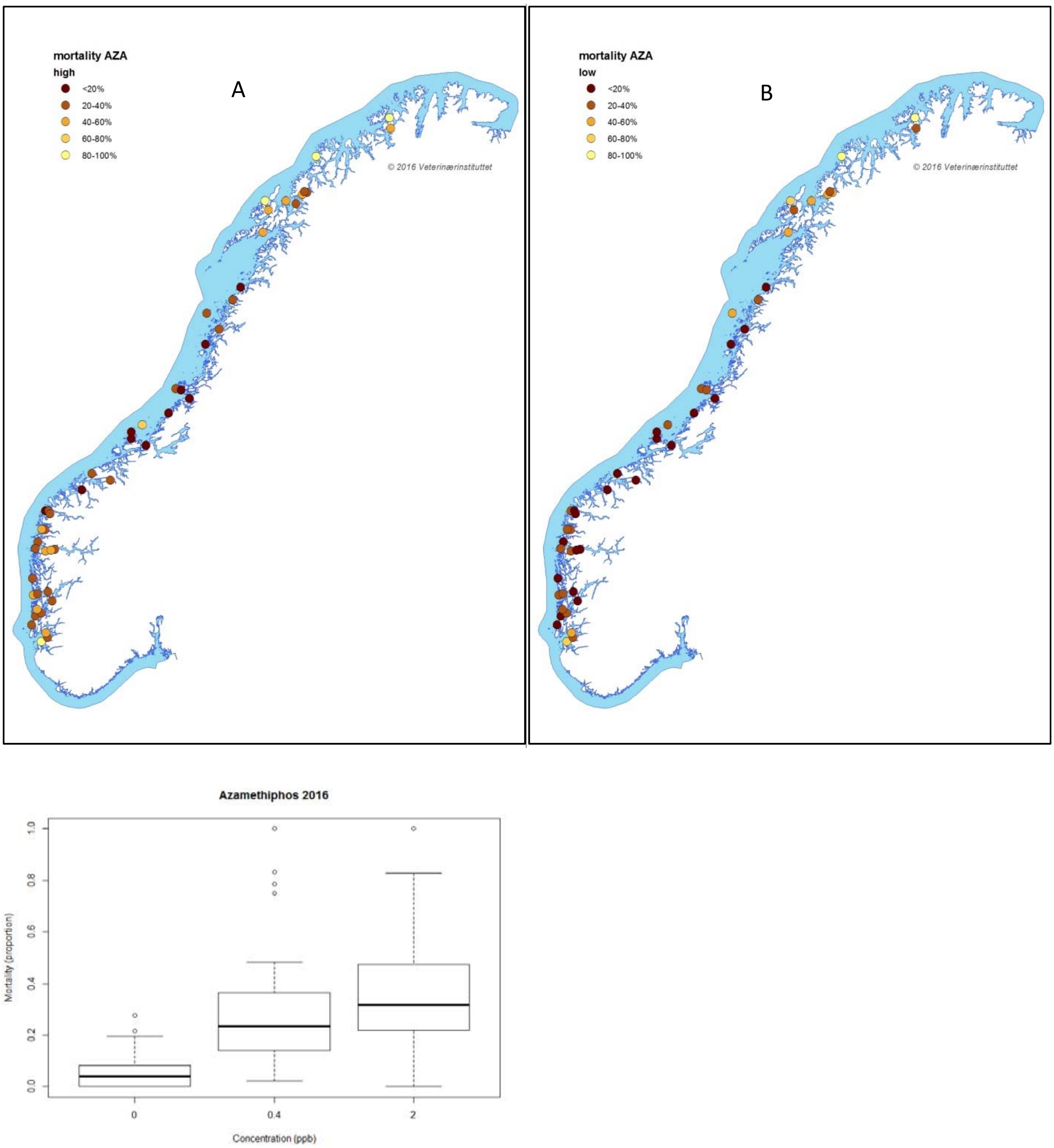

Figure 4. Maps showing categorical mortalities in bioassays with high (A) and low (B) azamethiphos concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).

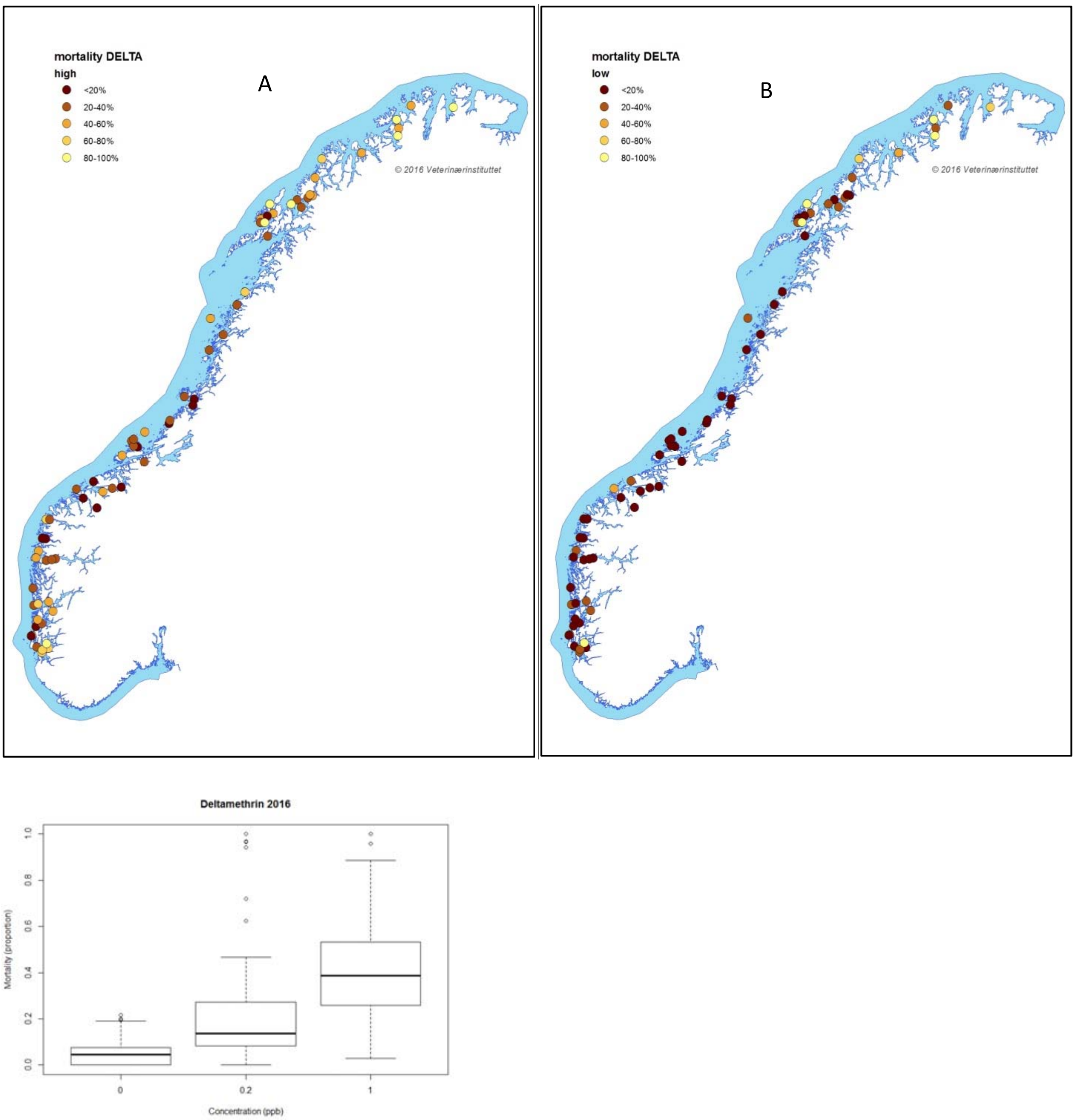

Figure 5. Maps showing categorical mortalities in bioassays with high (A) and low (B) deltamethrin concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all tests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

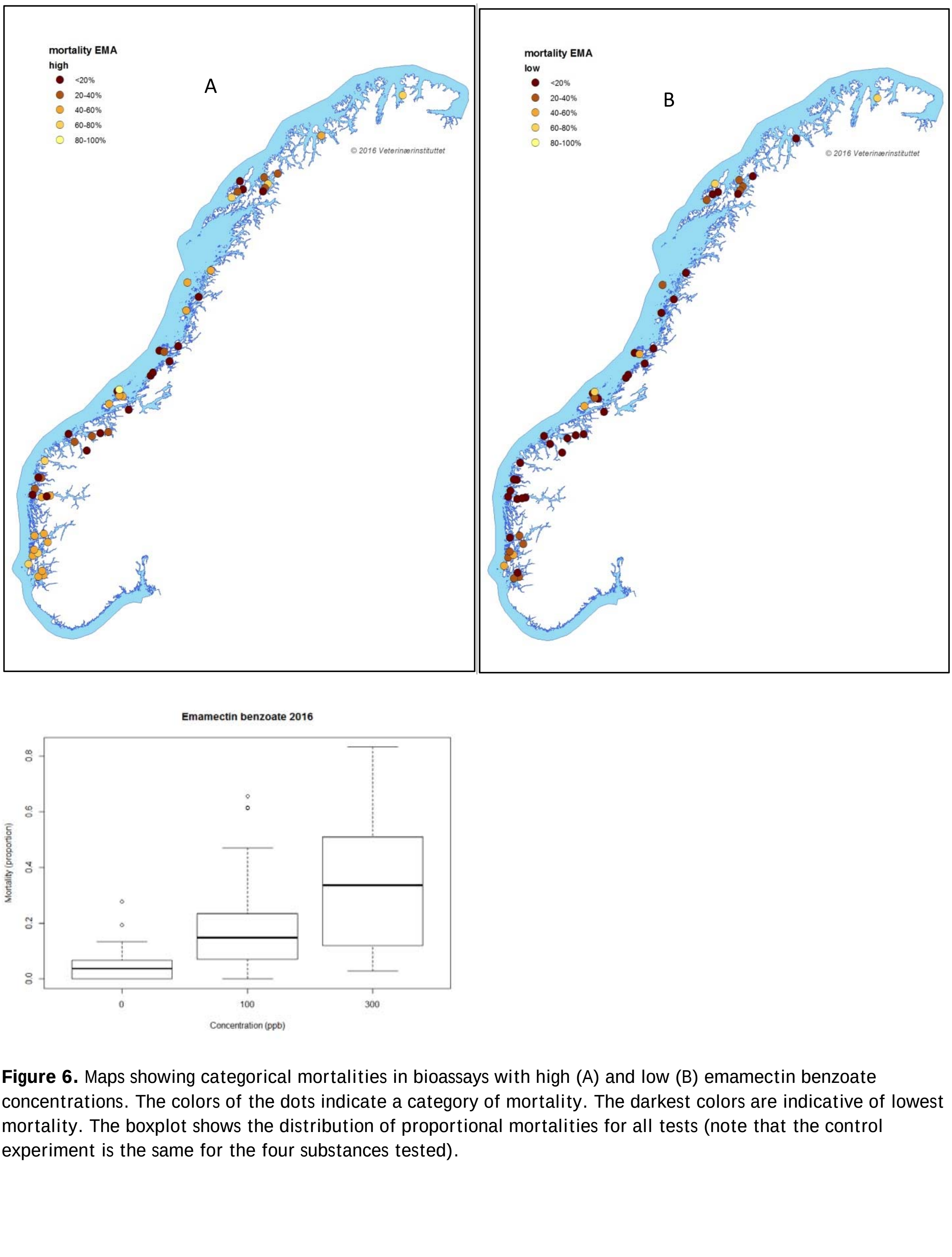

Figure 6. Maps showing categorical mortalities in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all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

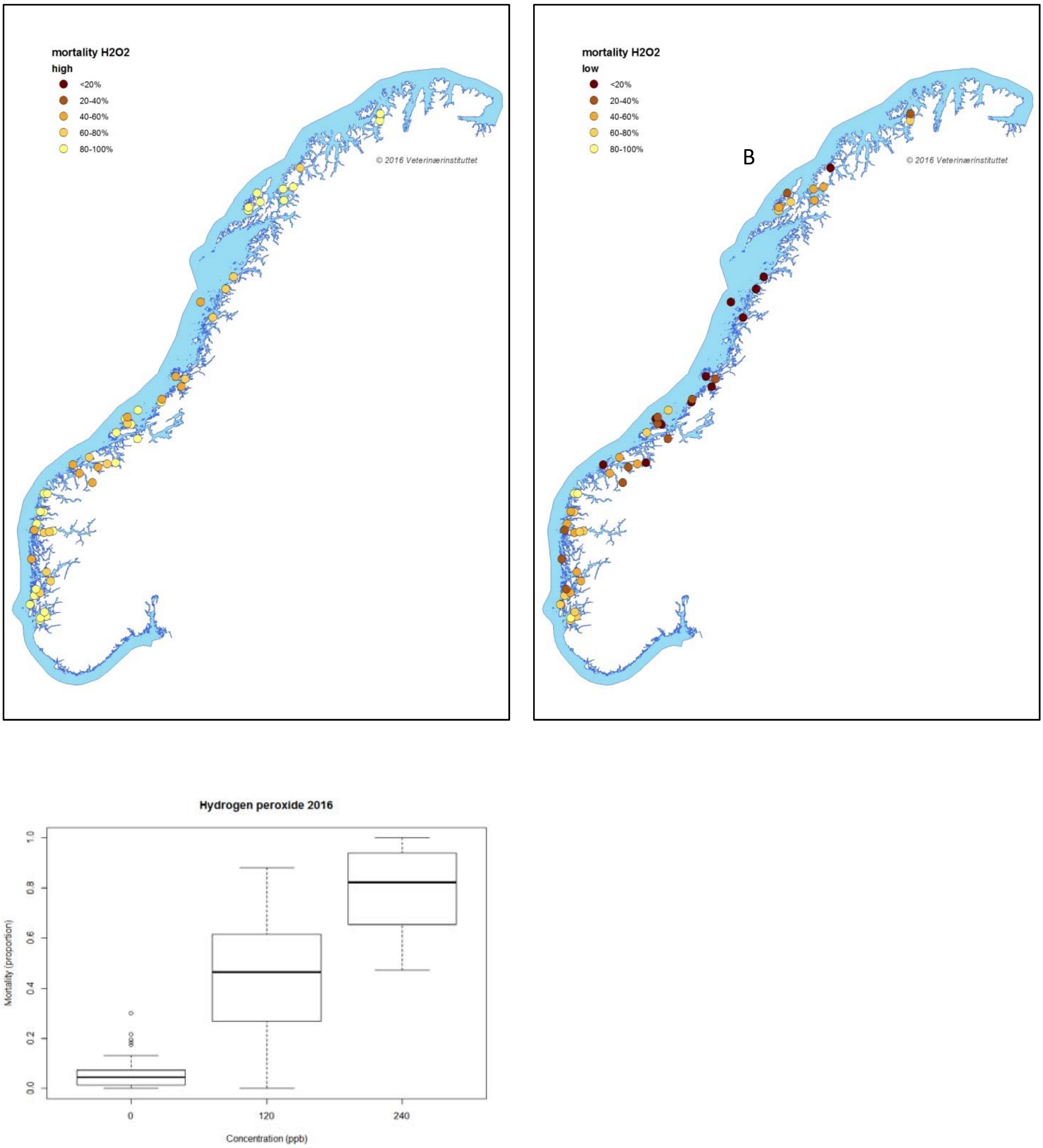

Figure 7: Maps showing categorical mortalities in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colors of the dots indicate a category of mortality. The darkest colors are indicative of lowest mortality. The boxplot shows the distribution of proportional mortalities for all 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 2016. 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.

The number of prescriptions of medicines against salmon lice was reduced by 41 % from 2015 to 2016. 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 except for emamectin benzoate. This is despite the fact that emamectin benzoate resistance was frequent in the same areas where the medicine was prescribed. Emamectin benzoate is applied to treat salmon lice infestations, in particular infestations with salmon lice larvae. The efficacy on the larvae stage is said to be present also against larvae from resistant salmon lice. The use in areas with resistant lice will however select for more emamectin benzoate resistant parasites. This might also include more resistant larvae.

The reduced use of medicinal treatments cannot be expected to give a major reduction in resistance. The reason for this is the lack of sensitive parasites that could possibly have diluted the frequency of resistance genes in the absence of selection pressure. The other reason is the continuous use of medicinal treatments, although at a lower intensity. The performed treatments will contribute to withhold and increase the frequency of resistance. Salmon lice management must therefore, in the absence of new medicines, rely more on prevention and non-medicinal treatment alternatives.

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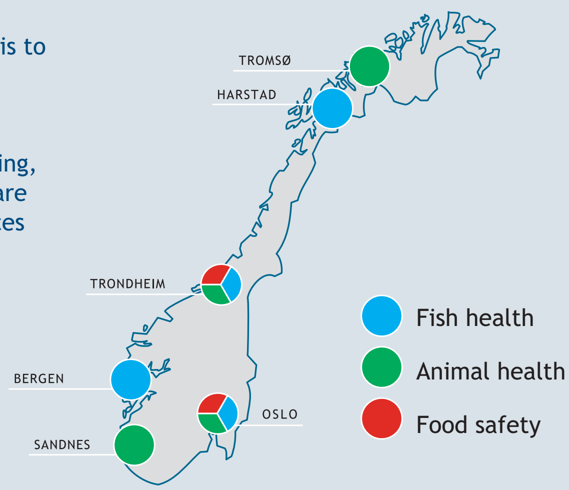

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