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The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2020

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The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2020

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Content

Summary

The number of medicinal treatments applied against salmon lice decreased slightly in 2020 compared to 2019. The number of treatments has been relatively stable since 2017. This is in contrast to the period 2014 to 2017, during which the number decreased by 78 percent. The level of resistance seen in salmon lice remained high in 2020. The tendency towards a reduced resistance level seen from 2017 to 2019 for deltamethrin, azamethiphos and hydrogen peroxide, were exchanged in more flattened curves for 2020. For emamectin benzoate the tendency towards increased resistance seen in 2019, continued in 2020. Resistance towards deltamethrin, azamethiphos and emamectin benzoate was 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. The number of non-medicinal treatments increased by 21 percent, to 2983 reported treatment weeks, from 2019 to 2020. Non-medicinal methods for treatment and prevention were thereby the dominating methods for salmon lice control. Fresh water delousing, alone or in combination with other treatments, accounted for 9 percent of the non-medicinal treatments in 2020 (281 reported treatments). A field study of fresh water sensitivity was performed for the second time in the surveillance program in 2020, comparing the sensitivity levels of salmon lice from areas with low and higher frequency of fresh water treatments. The results showed higher fresh water tolerance in lice from farms located in the higher fresh water usage areas.

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 emergence 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 applied 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.

The use of fresh water for delousing is of particular concern to the authorities, partly due to the wild sea trout's (*Salmo trutta*) use of fresh and brackish water for delousing and thereby vulnerability for salmon lice with increased fresh water tolerance (4). As in 2019 a field study was therefore conducted in 2020, investigating the tolerance levels in salmon lice towards fresh water. Toxicological tests exposing lice to reduced salinity was conducted on lice from farms in areas with low and higher use of fresh water for delousing during the previous years.

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. An additional aim starting from the 2019-program is to see if fresh water tolerance varied between salmon lice from areas with low and higher use of fresh water bath treatments.

Materials and methods

Passive surveillance

Prescriptions of medicines

Prescriptions of medicines applied for salmon lice treatments, from the Veterinary prescription 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 issued 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 (infection with *Paramoeba perurans*) at a lower concentration. In addition, some of the prescriptions for azamethiphos, pyrethroids, emamectin benzoate or hydrogen peroxide might have been 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 due to co-infection *L. salmonis*/*P. perurans* or *L. salmonis*/*C. elongatus*, regardless of the treatment indication. The extracts from VetReg were performed 04.02.2021.

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 04.02.2021) in addition to VetReg. Farms that during 2020 reported the presence of adult female lice, but had no prescriptions issued for them in that year, 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 21.01.2021. These numbers represent the number of weeks farms have reported the use of such treatments. Non-medicinal treatments include mechanical and thermal delousing, in addition to delousing in fresh water baths. Delousing using water pressure and/or brushing technology were regarded as mechanical while delousing using temperate water was regarded as thermal. The reports do not have data on the number of cages treated per week, and this can vary between one and all cages in a farm. 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 (5), 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 2020 in this report. This is due to the fact that these data are regarded to be 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 (6). Geographical processing and presentation of data was performed using ArcGIS (7).

Active surveillance

Bioassays

Seven fish health services along the Norwegian coast were engaged in 2020 to perform toxicological resistance tests (bioassays) on live parasites against chemical treatment agents. The bioassay protocol was based on Helgesen et al 2013 and 2015 (8, 9) and had also been applied for the previous years of the surveillance programme (2013-2019). 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 production zone. Norway’s 13 production zones are given by regulation (10) and shown in Figure 1 (numbered 1 to 13 from south to north).

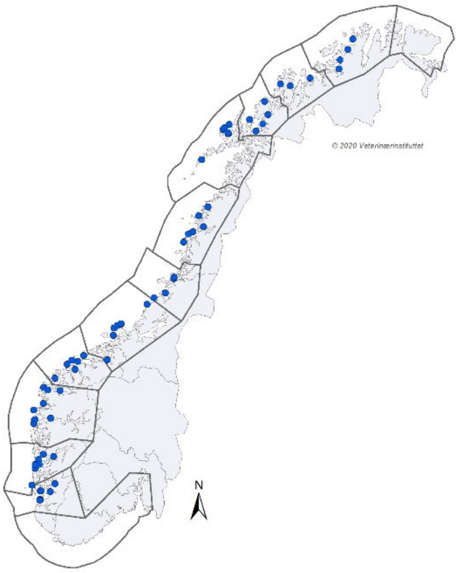


***Figure 1**: Locations of farms where salmon lice were collected for bioassays in 2020 (blue dots). The black lines subdivides Norway into 13 production zones.*

L. salmonis from between 47 to 52 farms were tested with the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide (lice from 62 farms were tested with one or more substances). 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 (between 10 and 60 lice were used per group). 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. Lice were regarded as dead if they were not able to attach to the surface of a container. This was used to indicate that they would not be able to stay attached to a fish and therefore not survive. The mortality at the low concentration was used to indicate the sensitivity status of the salmon lice population. Higher than 80 percent mortality was indicative of fully sensitive populations. The percentage affected 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 ppm (mg/l) for hydrogen peroxide and in ppb (µg/l) for deltamethrin, azamethiphos and emamectin benzoate.*

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

Molecular resistance tests
Salmon lice infestation levels in farms in production zone 1 in the far south of Norway had been low for several years. In order to test lice from this area 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 to pyrethroids; sensitive, intermediate resistant or resistant to azamethiphos; and as percent expected efficacy of a subsequent treatment for hydrogen peroxide.

Fresh water bioassays
The same seven fish health services along the Norwegian coast were engaged in 2020 to perform toxicological resistance tests (bioassays) on live parasites against low salinity. The bioassay protocol was based on Andrews and Horsberg 2020 (11). The locations were chosen by the fish health services themselves inside one of three regions. Region one (low usage of fresh water treatments) consisted of production zones 1, 8, 9, 10, 11, 12 and 13. Region two and three (higher usage of fresh water treatments) consisted of production zones 2, 3, 4, 6 and 7. Compared to the 2019-programme production zones 2, 3 and 4 (region three) were included in the survey.

L. salmonis from 25 farms were tested; eight from region one, eight from region two and nine from region three. The bioassays were performed by exposing live parasites of motile stages, removed from the fish, for water of six different salinities; 0, 1, 3, 5, 7 and 20 percent (control). After 24-hour exposure, salmon lice mortality, grouped according to stages and genders, was noted as the test outcome.

The results were analysed using a logistic regression to see if there were differences in salinity tolerance between lice from areas with low and higher usage of fresh water bath treatments. Data from farms where the control group (salinity: 20 ‰) mortality exceeded 20 percent were excluded from the analysis (data from four farms were excluded).

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 – 2020. 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. There was an increase in the number of prescriptions in 2019, but a slight decrease again in 2020; total decrease was 9 percent (66 prescriptions). There was a decrease for all substance groups except for azamethiphos, for which 37 more prescriptions were issued in 2020 than in 2019. Emamectin benzoate was the most commonly prescribed medicine, prescribed 3.5 times as often as the second most prescribed medicine (azamethiphos).

Table 2: Number of prescriptions for the given substances/class of substances applied to control salmon lice in 2011 to 2020. The number of prescriptions was collected from VetReg 04.02.21. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.

Substance category	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Azamethiphos	418	695	483	752	621	262	59	39	82	119
Pyrethroids	460	1 163	1 130	1 049	664	280	82	56	73	51
Emamectin benzoate	294	169	163	481	523	612	351	371	451	415
Flubenzurones	24	133	171	195	202	173	81	40	61	51
Hydrogen peroxide	179	110	255	1021	1 284	629	214	96	82	47
Total	**1 375**	**2 270**	**2 202**	**3 498**	**3 294**	**1 956**	**787**	**602**	**749**	**683**

Prescriptions per farm

The maps in Figure 2 sum up the total number of prescriptions per location in the period 2017 - 2020. Prescriptions were issued for 623 farms in 2016 with a mean of 3.1 prescriptions per farm; for 437 farms in 2017 with a mean number of 1.8 prescriptions per farm; for 344 farms in 2018 with a mean number of 1.7 prescriptions per farm; for 390 farms in 2019 with a mean number of 1.9 prescriptions per farm; and for 371 farms in 2020 with a mean number of 1.8 prescriptions per farm. The number of active farms have increased with between 12 and 29 farms per year during the years 2016 to 2020. The decrease in the number of prescriptions from 2019 to 2020 was therefore both caused by a decrease in the number of farms which had prescriptions issued for them and a decrease in the number of prescriptions per farm. The decrease was not explained by decreasing numbers of active farms.

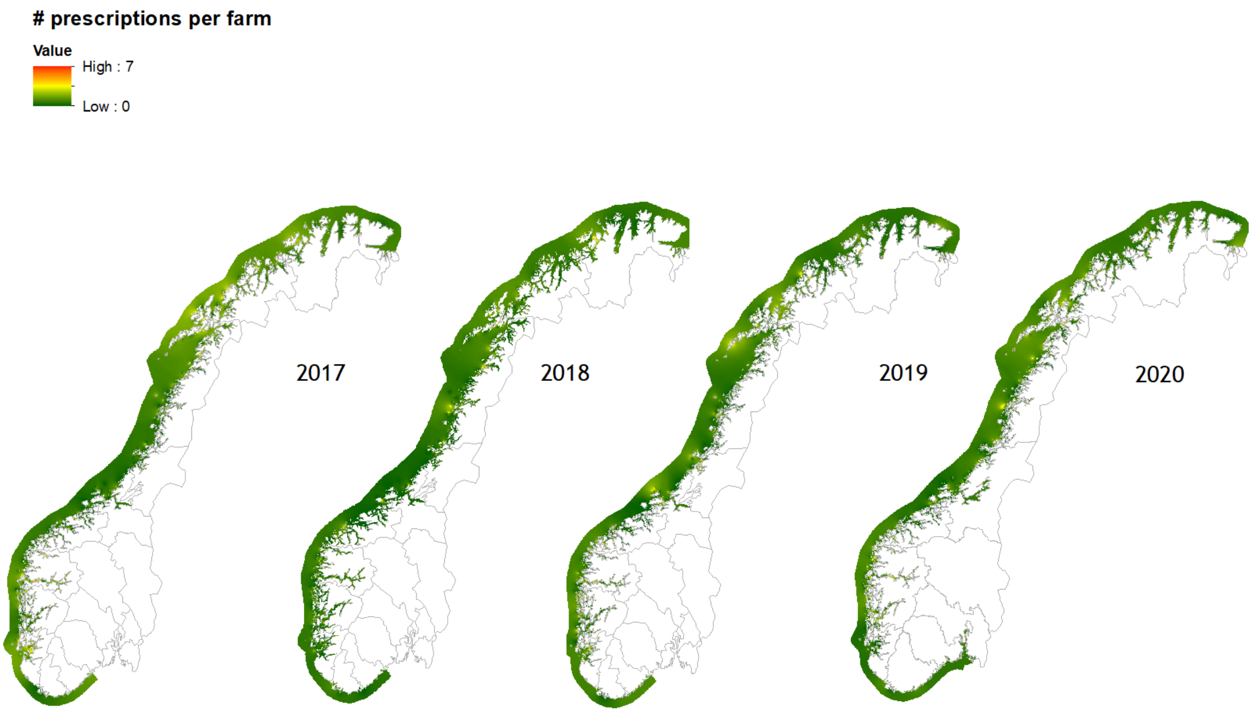


Figure 2: Geographical distribution of prescriptions at farm level, used to control salmon lice from 2017 to 2020. Dark red denote areas where more than seven prescriptions per location is expected, while dark green denote areas where the expectation of zero treatment is approached. The map layer was generated using the Inverse distance weighted (IDW) function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighbouring farm locations).

Azamethiphos had four foci of dense use, in production zone 3, 4, 8 and 9. Emamectin bencoate use was spread along most of the coast. The most dense use of pyrethroids was seen in production zone 3 and 10. The most dense use of flubenzurones was found in production zone 3, while the most dense hydrogen peroxide usage was seen in production zone 10 (Figure 3).

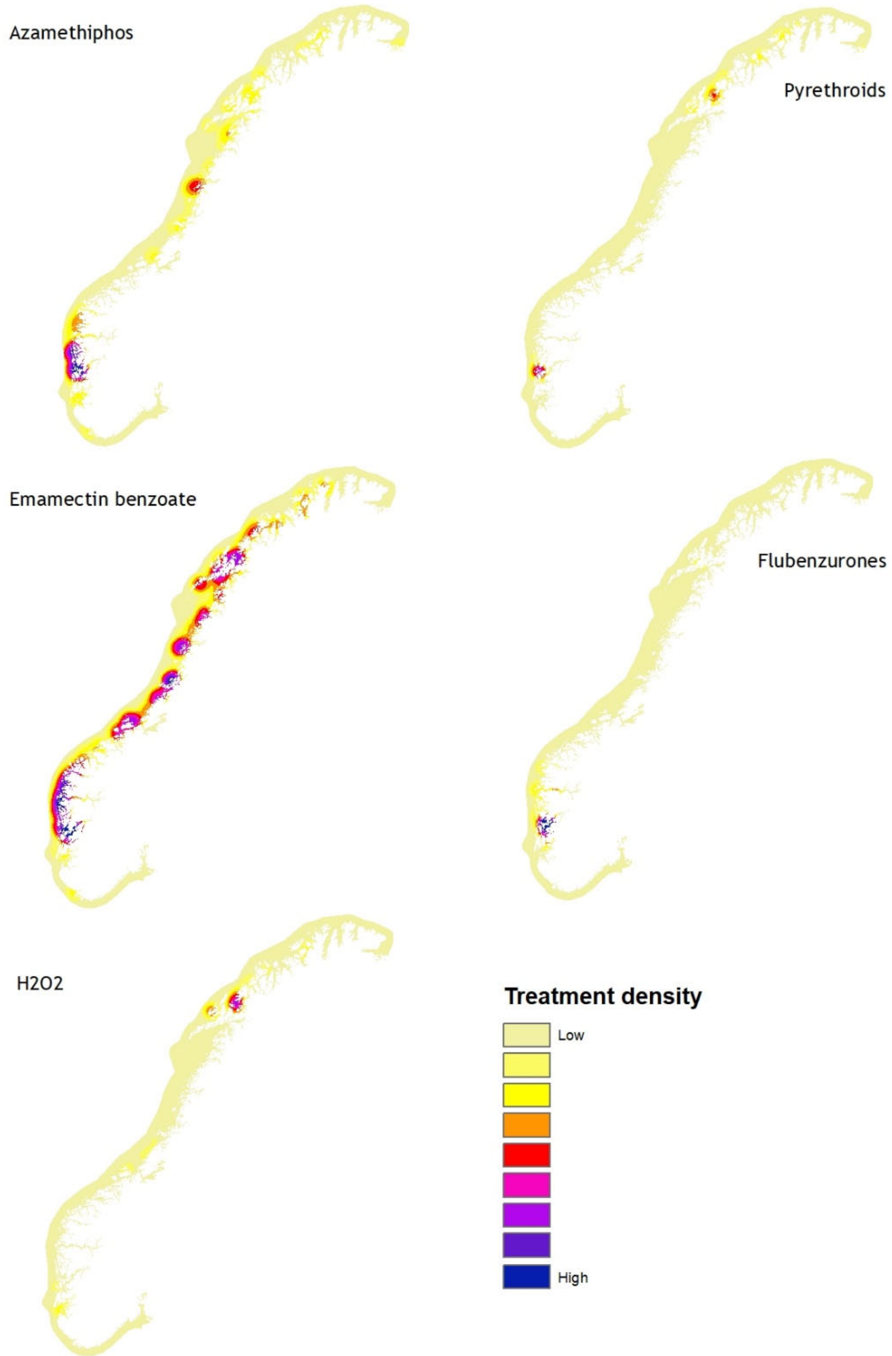


Figure 3*: Figure 3. Geographical distribution of the density of prescriptions per farm for five different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2020. Note that the kernel densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments. Blue indicates relatively high intensities while yellow indicates relatively low densities.*

Non-medicinal treatments

Table 3 summarizes the number of weeks farms have reported non-medicinal treatments in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority. The number of non-medicinal treatments increased by 21 percent from 2019 to 2020. This continued a trend of substantial yearly increases in the number of treatments, which started in 2016. 323 farms performed non-medicinal treatments in 2016, 417 farms in 2017, 483 farms in 2018, 563 farms in 2019 and 573 farms in 2020. The 573 farms reported between 1 and 26 treatment weeks, with an average of 5.2 weeks. 61 percent of the non-medicinal treatments in 2020 were performed using thermal delousing (18 treatments were substracted from the thermal/mech+fresh water category in table 3 since these were mech+freshwater). A study from 2017 showed genetic variation in the tolerance of warm water in salmon lice (11). The frequent use of thermal delousing inflicts a selection pressure favouring lice that can survive warm water treatments. This selection pressure was inflicted on a large geographic area in 2020 (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 2020[1]. The treatments were subdivided into categories. "Thermal" summarizes treatments using temperate water and "mechanical" (abbr. "Mech") summarizes treatments using water pressure or brushes. "Fresh water" is fresh water bath treatments. The combination categories are reports on the use of more than one type of treatment. An example from the category "other" are reports not containing a description of the method used. The number of treatments was collected from the register 21.01.21.*

Treatment category	2012	2013	2014	2015	2016	2017	2018	2019	2020
Thermal	0	0	3	36	684	1 247	1 355	1 463	1736
Mechanical	4	2	38	34	312	236	428	673	816
Fresh water	0	1	1	28	73	75	87	150	238
Thermal + Mech	0	0	0	0	12	42	38	58	57
Thermal/Mech + Fresh water	0	0	0	0	23	22	25	34	43
Other	132	108	136	103	75	51	69	87	93
Total	**136**	**111**	**178**	**201**	**1 179**	**1 673**	**2 002**	**2 465**	**2983**

[1]Deviations from the 2019 resistance report are caused by new combination categories, updated routines to identify type of treatment from free text in the report forms and late incoming reports.

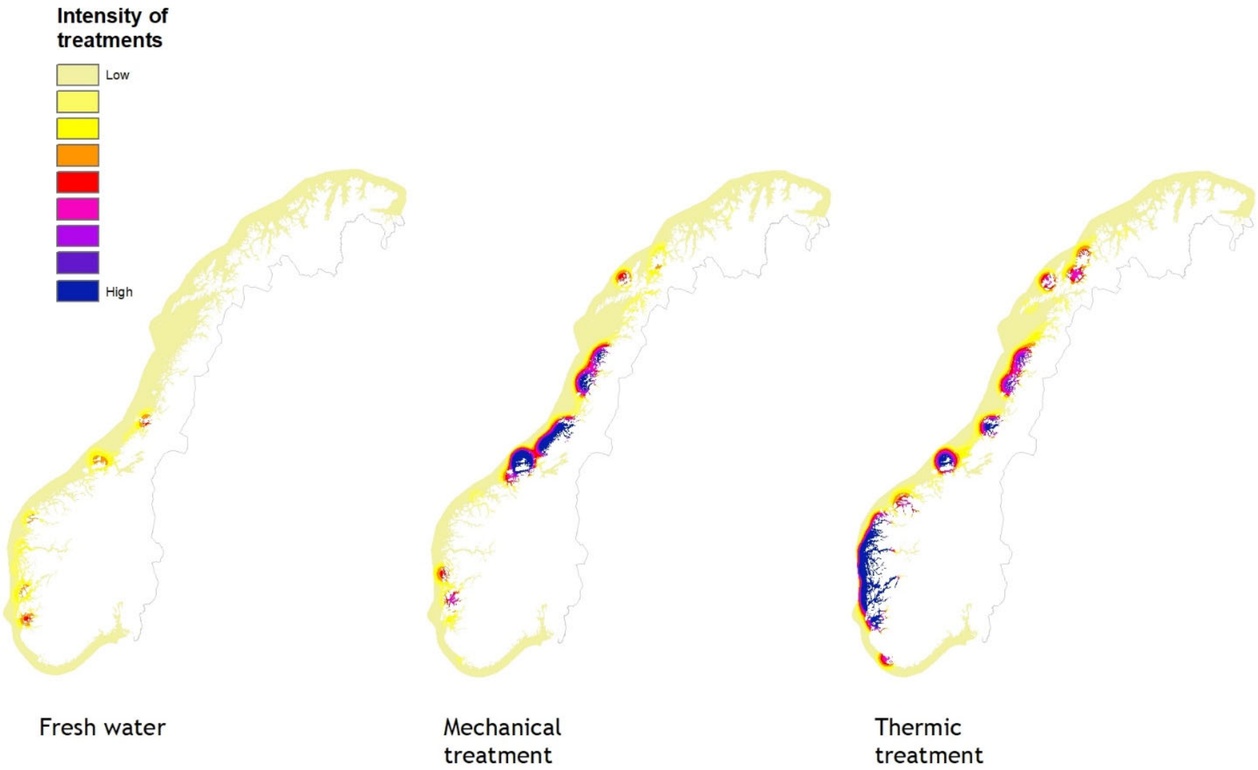


Figure 4*: The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2020. Treatments are categorized into bath treatment in fresh water, mechanical delousing and thermal delousing. Combination treatments are not included in the maps. Treatment intensity is 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, 200 bioassays were performed on salmon lice from 62 different salmon farms along the cost (Figure 1). The number of farms tested using the different substances and concentrations are listed in table 4.

Table 4 shows that salmon lice mortalities were lower than 80 percent in the majority of locations tested at low concentrations for each substance. This shows that reduced sensitivity to chemotherapeutants is widespread in salmon lice in Norwegian salmon farms.

***Table 4**: 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	4	21	20	3	0
Deltamethrin	52	27	17	5	2	1
Emamectin benzoate	52	25	12	10	2	3
Hydrogen peroxide	47	3	13	16	12	3
High concentration						
Azamethiphos	47	2	14	21	9	1
Deltamethrin	51	2	9	13	19	8
Emamectin benzoate	52	6	13	11	12	10
Hydrogen peroxide	48	0	0	0	15	33

Table 5 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 5**: Spearman Correlation Coefficients between mortality proportions in the low and high concentration bioassay tests on farms (N: number of bioassays included in each test).*

Substance category	N	Spearman Correlation Coefficients
Azamethiphos	47	0.49
Deltamethrin	51	0.42
Emamectin benzoate	53	0.85
Hydrogen peroxide	47	0.56

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).

Salmon lice mortalities were generally low in high- concentration azamethiphos bioassays (Figure 5A), indicating that low treatment efficacy may be expected in most areas. 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 one farm 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, although eight farms showed test mortalities exceeding 80 percent at this concentration.

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) additionally showed that reduced treatment efficacy could be expected along most of the coast.

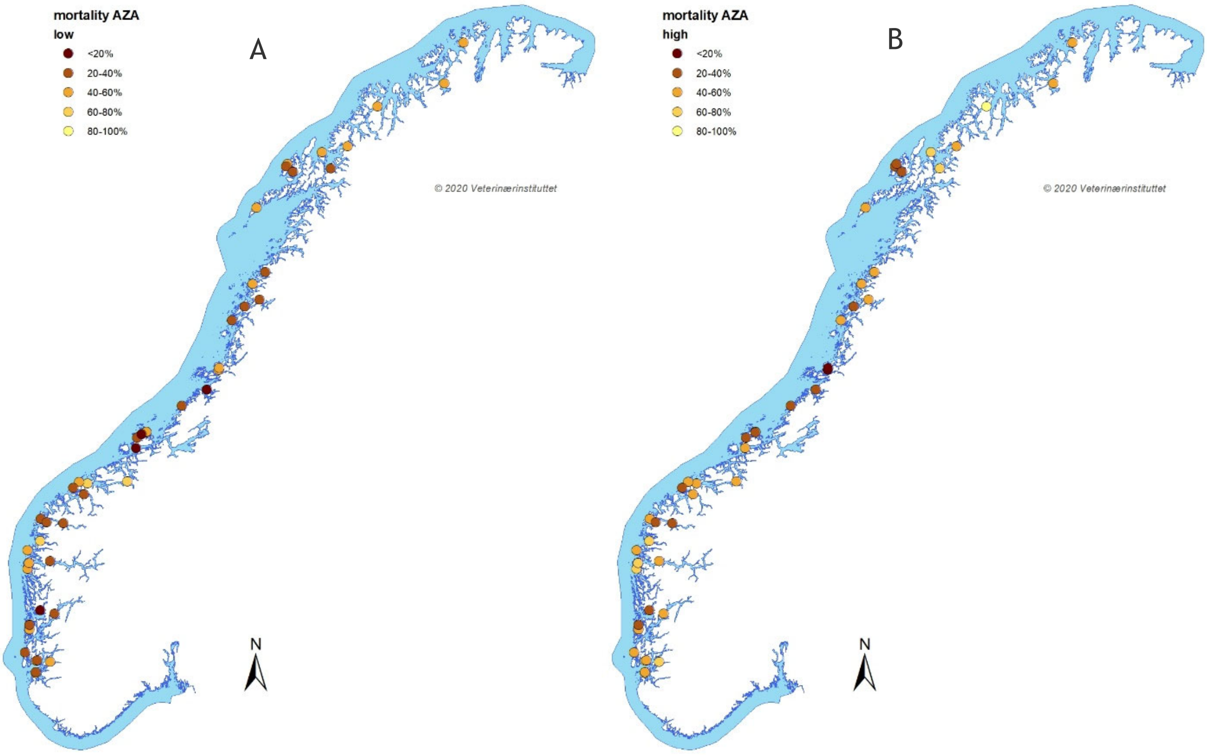
For hydrogen peroxide, results from the high concentration bioassays yielded generally higher mortalities than for the other substances tested. This means that better treatment results could be expected than from treatments with the other substances. The low concentration tests (Figure 8B) however showed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

Figure 9 displays all high dose bioassay results for the four substances applied. The results indicate a similar level of resistance as in 2019 for all substances, except emamectin benzoate. The reduction in resistance indicated to have started in 2017 for azamethiphos, deltamethrin and emamectin benzoate, and in 2018 for hydrogen peroxide thereby seem to have stopped. The figure indicate that the increase in resistance level for emamectin benzoate, which started in 2019, continued in 2020.

The molecular tests of lice from the three farms in production zone 1 revealed an average of 63 percent pyrethroid resistant lice, which was similar to the level seen in 2019 (59 percent) (Table 6). These figures follow a history of increase in the presence of resistant lice from 2016 to 2017 (33-40 percent to 81 percent) (13, 14), and then a slight reduction to 70 percent resistant lice in 2018. It is likely that the effect on the resistance level from the deltamethrin treatments performed in production zone 1 in the autumn of 2016, after several years without medicinal treatments in this area, was still seen in 2020. This is under the assumption that there is limited lice infestation from other production zones to zone 1, given the long distance to active farms in other zones. There was a further increase in organophosphate resistance levels after one year of recession, from 30-40 percent in 2016, to 50 percent in 2017, 66 percent in 2018, 62 percent in 2019 and 71 percent in 2020. The increase in 2020 was expected as two treatments with organophosphates were performed in 2020. The tests were not performed on lice from the same farms each year.

Table 6*: Results from molecular resistance test from three farms in production zone 1. The resistance levels are given as percentage of parasites categorized as sensitive or resistant towards pyrethroids and sensitive, intermediate resistant or resistant towards organophosphates.*

Substance category Level of resistance	Farm 1	Farm 2	Farm 3
Pyrethroids			
Sensitive	30 %	37 %	45 %
Resistant	70 %	63 %	55 %
Organophosphates			
Sensitive	23 %	33 %	29 %
Intermediary	53 %	59 %	57 %
Resistant	23 %	7 %	14 %



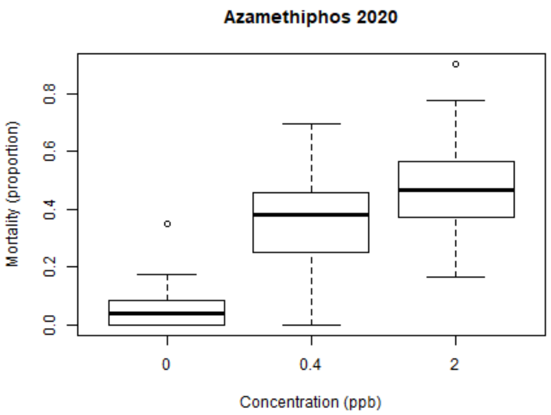


Figure 5*: Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) azamethiphos concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of azamethiphos (0, 0.4 and 2 ppb) (note that the control experiment is the same for the four substances tested).*

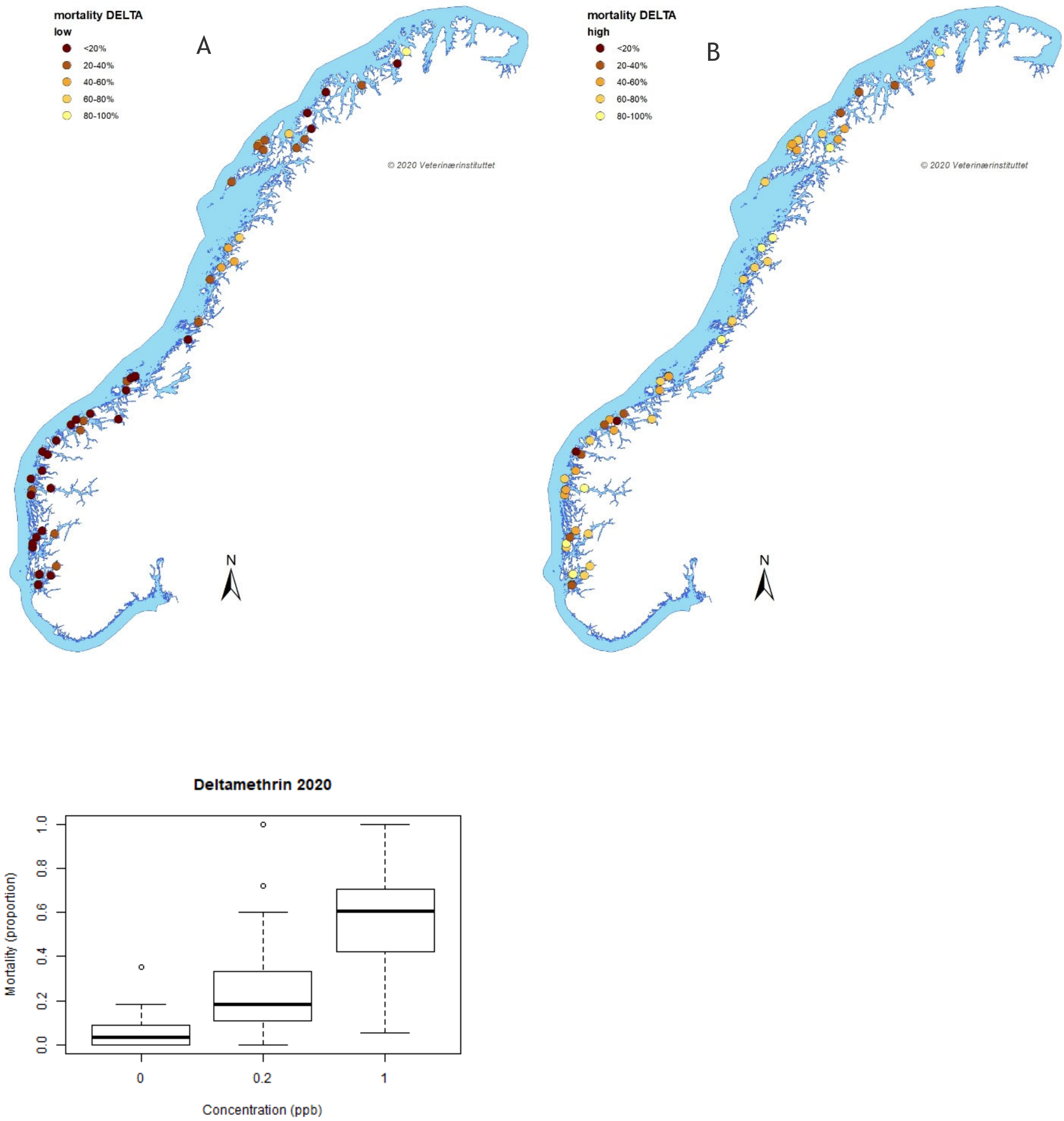


Figure 6*: Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) deltamethrin concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of deltamethrin (0, 0.2 and 1 ppb) (note that the control experiment is the same for the four substances tested).*

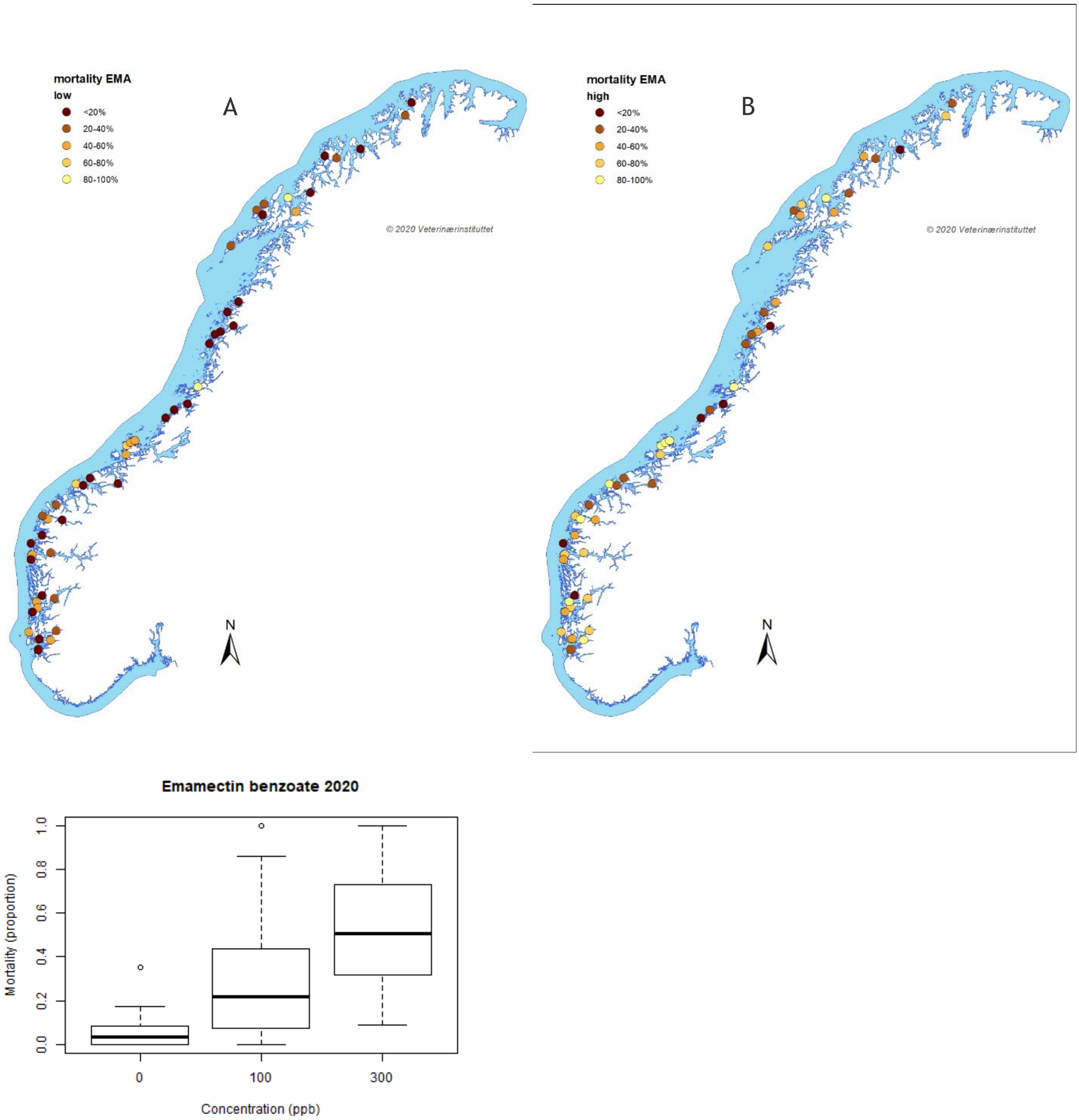


Figure 7*: Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of emamectin benzoate (0, 100 and 300 ppb) (note that the control experiment is the same for the four substances tested).*

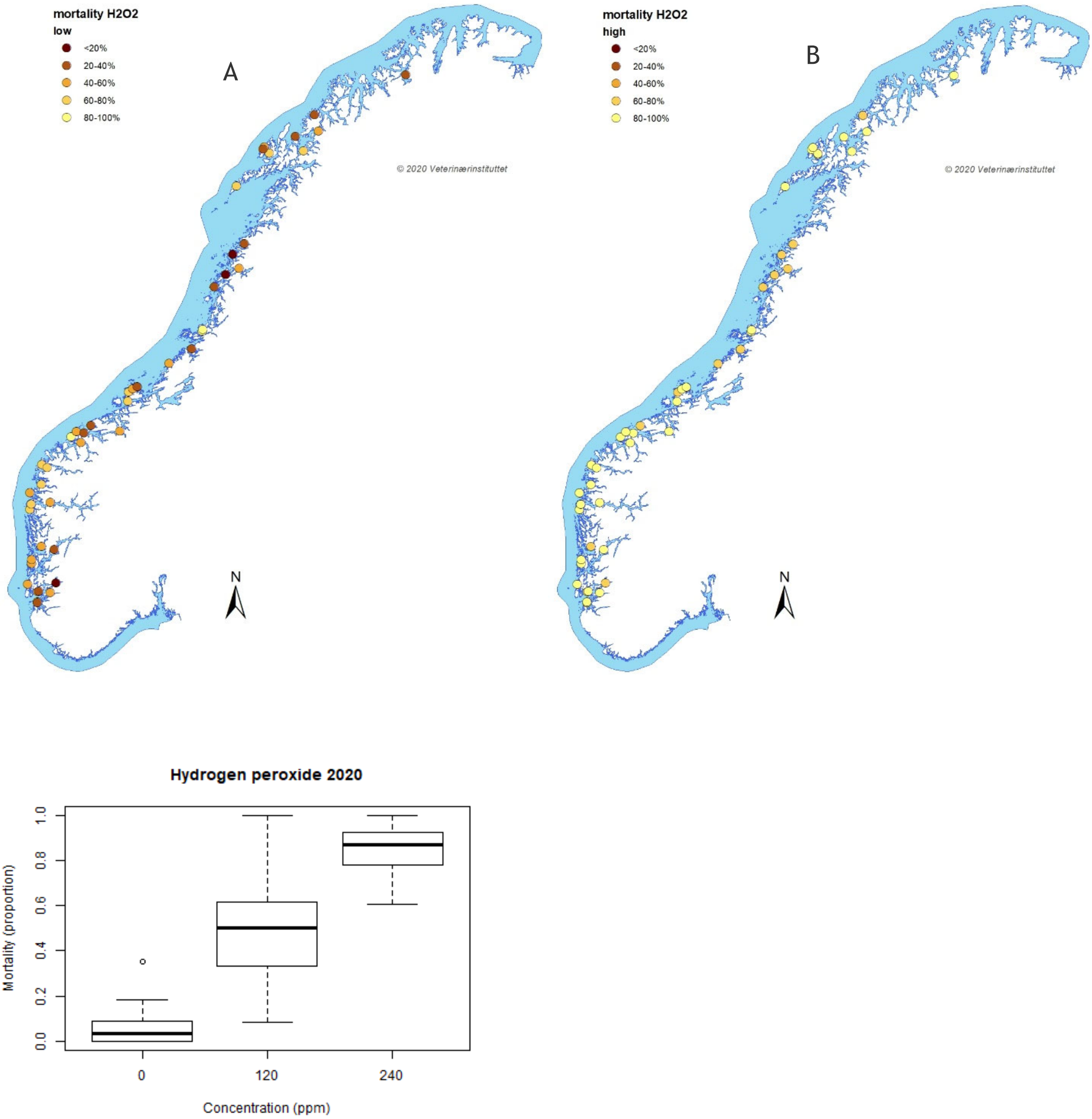


***Figure 8**: Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of hydrogen peroxide (0, 120 and 240 ppm) (note that the control experiment is the same for the four substances tested).*

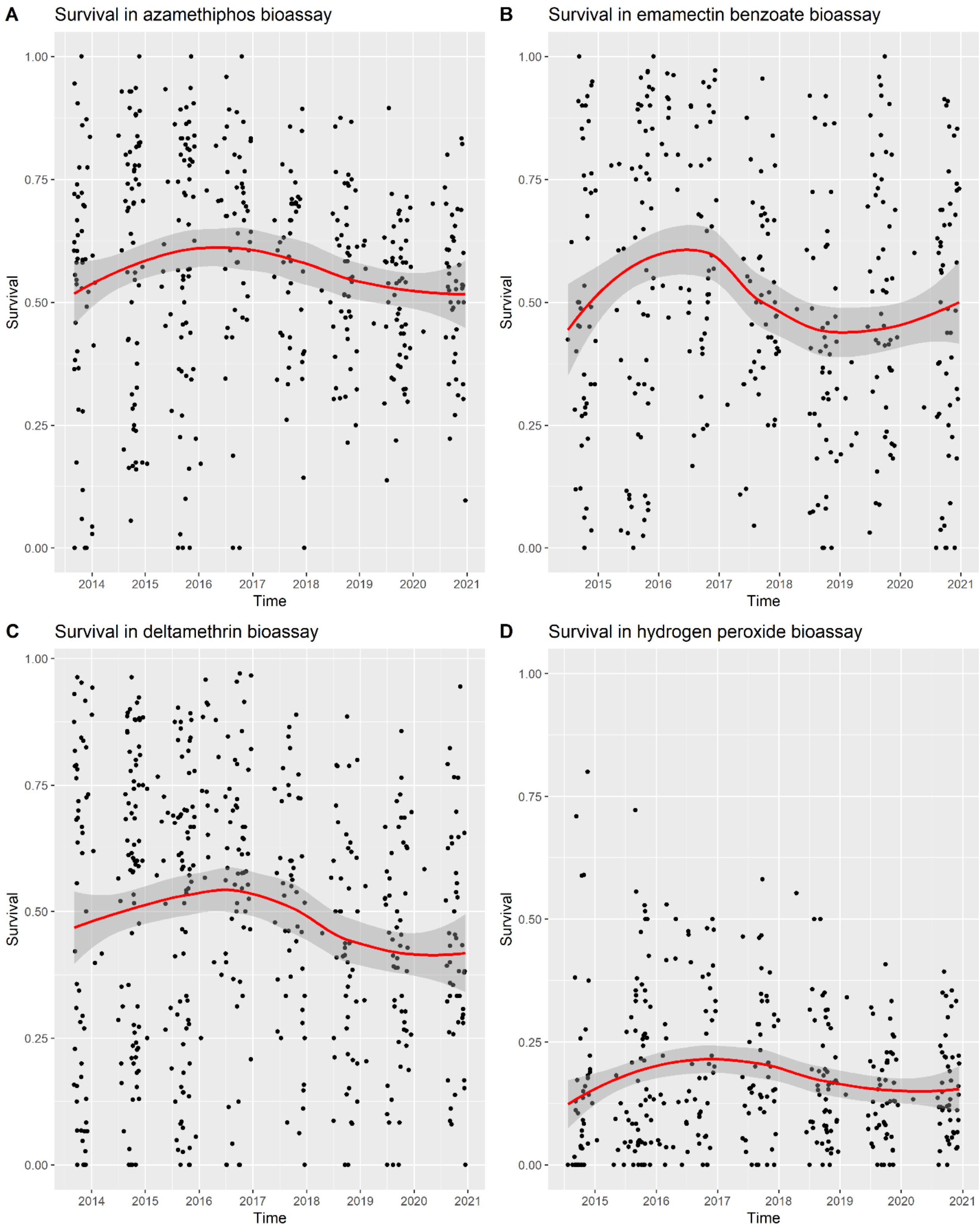


Figure 9: All bioassay results from exposure to azamethiphos (A), emamectin benzoate (B), deltamethrin (C) and hydrogen peroxide (D) displayed as percent survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances. The red line is the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

Fresh water bioassays

In the logistic regression of the fresh water bioassay results, the mortality at the different salinities differed significantly between low or higher usage (of fresh water bath treatment) areas in Norway. The mortality at the different concentrations used in the bioassay was higher in the low usage area compared to the higher usage area. All production areas included in the study except 2, 3, 4, 6 and 7 were called low usage areas. Figure 10 displays the modelled results from all bioassays except the four assays where control group mortality exceeded 20 percent. Farms A-H are from a low use area of fresh water bath treatments, while farms I-Y are from a higher use area. The modelled curve from farm T is deviating from the rest of the farms, because the results from this farm did not show constantly increasing mortality by decreasing salinity. Figure 11 shows the predicted dose-response curves from the two areas based on the results from the logistic regression.

The fresh water bioassay survey conducted in 2020 showed significantly lower tolerance towards fresh water in the low use area compared to the higher use area ($p<0.001$). The same conclusion was drawn in a sub-analysis where data from farm T was excluded. The same conclusion was also drawn when seawater salinity at the site the same day was included as an explanatory variable in the analysis. From this type of analysis it is not possible to conclude on the cause of the observed difference. The question of biological relevance can also not be answered through this analysis alone. The low treatment group only consisted of data from 5 farms, which also weakens the strength of the conclusion. The significant difference in tolerance between the two groups of farms can however be used to form the hypothesis that fresh water bath treatments have led to more low salinity tolerant lice. This hypothesis can further be explored in other types of studies.

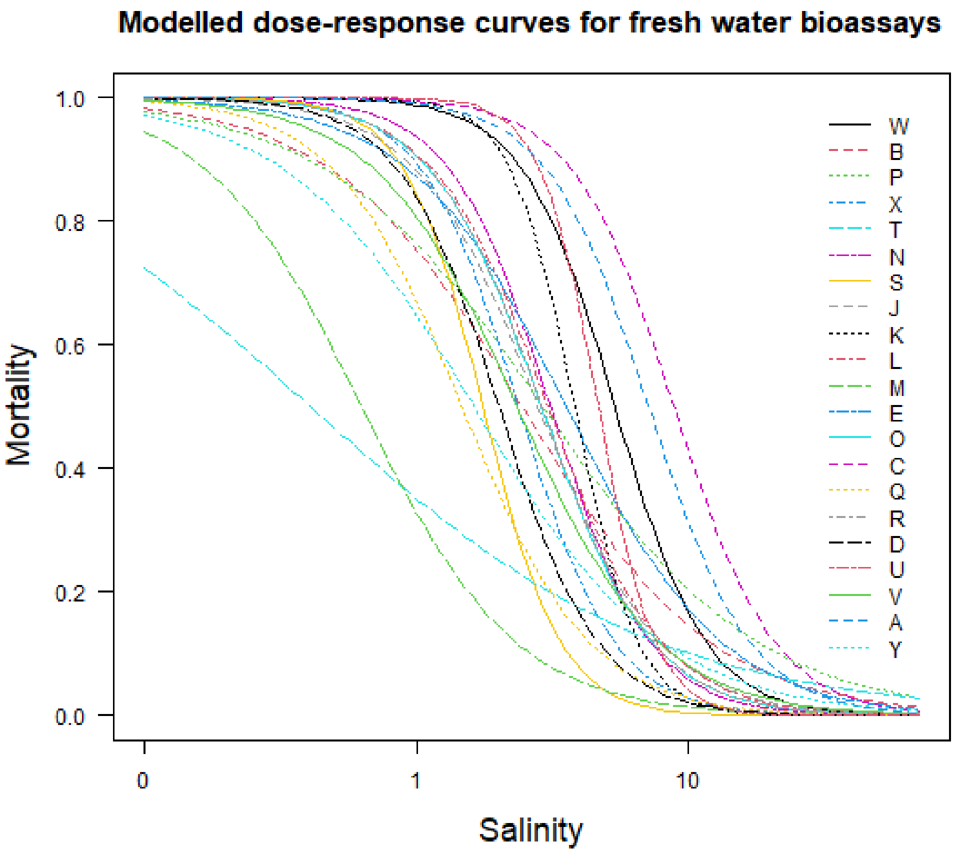


Figure 10: *The best fitted dose-response curves are modelled for all fresh water bioassays. Farm F, G, H and I were excluded due to control-group mortality exceeding 20 percent. Farms A-H are from a low use area for fresh water treatments, while farms I-Y are from a higher use area.*

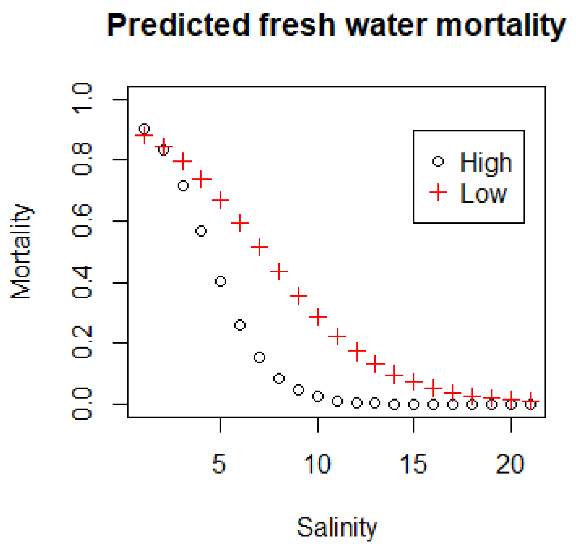


Figure 11: *Predicted results from a regression analysis of the effect of treatment area on the mortality in fresh water bioassays. The red crosses represent the predicted dose-response curve from a bioassay from areas with low treatment intensity and the black circles represent the predicted dose-response curve from a bioassay from areas with higher treatment intensity.*

Conclusion

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2020. Resistance towards deltamethrin, azamethiphos and emamectin benzoate was generally widespread along the Norwegian coast. Less resistance was found towards hydrogen peroxide than towards the other medicines, but reduced hydrogen peroxide sensitivity was indicated in several areas. The results for all years of the surveillance program compiled indicate a similar resistance level in 2020 as was seen in 2019 towards all tested substances, except for emamectin benzoate. The number of medicinal treatments with these substances in 2020 could possibly explain this, as it also was at a similar and relatively low level as was seen in 2019. The level of emamectin benzoate resistance seemed to increase somewhat in 2020 compared to 2019. There was no increase in the use of emamectin benzoate in 2020, but the number of treatments remained high (415 prescriptions).

The number of prescriptions of medicines against salmon lice decreased by 9 percent from 2019 to 2020. Compared to 2014, when the number of prescriptions peaked, the number was reduced by 80 percent. This reduction was most likely 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 increased availability of non-medicinal treatment options. The reduction in prescriptions since 2014 was substantial for all substances/categories of substances, except for emamectin benzoate where a smaller reduction was seen.

The number of non-medicinal treatments increased by 21 percent from 2019 to 2020. In 2020, 573 farms reported the use of non-medicinal methods, while 371 farms had medicines against salmon lice prescribed for them. Thermal delousing was the dominating method with 61 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.

There were indications of a reduction of resistance from 2016 to 2019, but this did not seem to continue in 2020. This was despite a slight reduction in the overall number of medicinal treatments. Fully restored sensitivity is most likely unrealistic to obtain, even with very few medicinal treatments. One reason for this assertion is the history of organophosphate resistance in Norway. The same mutation that was found in lice from 1998 causes resistance today, despite no treatments with organophosphates between 2000 and 2007 (15). This indicates that resistance alleles have survived eight years without selection pressure. 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.

Fresh water bath treatments are performed at a moderate level in Norway, but the use increased by 53 percent from 2019 to 2020 (281 treatments in total). In 2020 a survey was conducted to look for differences in fresh water sensitivity levels between lice from low and higher usage areas of fresh water treatments. In this study lice from farms in higher usage areas tolerated lower salinities better than lice from lower usage areas. The same difference was not seen in the 2019-study, possibly showing a development towards more low salinity tolerant lice. Since wild sea trout use fresh and brackish water for delousing, such a development is unwanted also from a wild fish perspective. The limited number of farms included in the study and the relatively small difference between the two groups however makes it difficult to draw strong conclusions from this survey.

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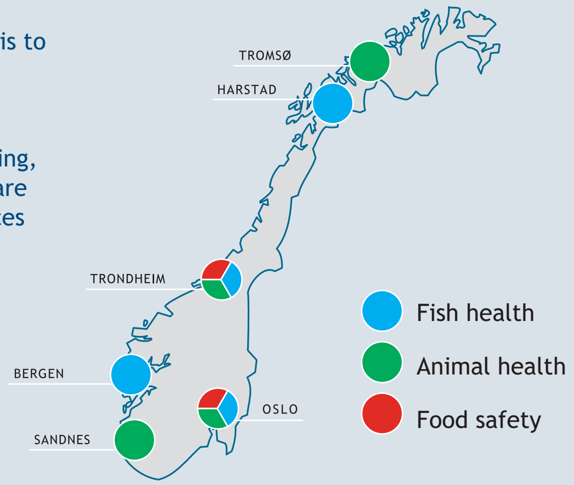

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