Veterinærinstituttet
Norwegian Veterinary Institute

Rapport 8 - 2022

The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2021

REPORT 8/2022

The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2021

Authors

Kari Olli Helgesen, Tor Einar Horsberg, Leif Christian Stige, Attila Tarpai

Suggested citation

Helgesen, Kari Olli, Horsberg, Tor Einar, Stige, Leif Christian, Tarpai, Attila. The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2021. Surveillance program report. Veterinærinstituttet 2022. © Norwegian Veterinary Institute, copy permitted with citation

Quality controlled by

Edgar Brun, Director of Fish Health and Welfare, Norwegian Veterinary Institute

Published

2022 on www.vetinst.no

ISSN 1890-3290 (electronic edition)

Commissioned by / In collaboration with

Norwegian Food Safety Authority

Colophon

Cover design: Reine Linjer

Cover photo: Trygve Poppe

www.vetinst.no

Content

Summary

The number of prescriptions for anti-salmon lice medicines increased slightly in 2021 compared to 2020. The number of prescriptions has however been relatively stable since 2017. This is in contrast to the period 2014 to 2017, during which the number decreased by 78 percent. In 2021, for the first time in more than two decades, a new anti-salmon lice medicine from a new substance class was approved in Norway. The level of resistance seen in salmon lice towards most anti-salmon medicines remained high in 2021. There was however a tendency of reduced resistance towards deltamethrin and azamethiphos. This is despite an increased use of azamethiphos over the last three years. For hydrogen peroxide and emamectin benzoate a more stable resistance situation was seen. 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 reported farm treatment-weeks using non-medicinal treatments decreased by five percent, to 2822 weeks, from 2020 to 2021. This was the first decrease seen in the number of non-medicinal treatments per year. Non-medicinal methods for treatment and prevention were still the dominating methods for salmon lice control. Fresh water delousing, alone or in combination with other treatments, accounted for 15 percent of the non-medicinal treatments in 2021 (412 reported treatments-weeks). A field study of fresh water sensitivity was performed for the third time in the surveillance program in 2021, comparing the sensitivity levels of salmon lice from areas with low and higher frequency of fresh water treatments. The results showed slightly higher fresh water tolerance in lice from farms located in the higher fresh water usage areas, but the difference was smaller than what was seen in 2020.

Introduction

Salmon lice (*Lepeophtheirus salmonis*) are considered one of the biggest health threats against 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 antiparasitics 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 control with salmon lice, 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 antiparasitics 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 60 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 (4). If they are infested with salmon lice with increased fresh water tolerance the efficacy of their natural delousing strategy might decline. As in 2019 and 2020 a field study was therefore conducted in 2021, investigating the tolerance levels in salmon lice towards fresh water. Toxicological tests exposing lice to reduced salinity were 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 antiparasitics against salmon lice and to describe the resistance status in *L. salmonis* towards the most important of these antiparasitics in Norway. An additional aim 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 six different categories according to their mode of action and therefore most likely joint selection pressure towards resistance. The six categories were azamethiphos, pyrethroids (cypermethrin and deltamethrin), emamectin benzoate, hydrogen peroxide, flubenzurones (diflubenzuron and teflubenzuron) and imidacloprid (new substance from 2021). 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 and hydrogen peroxide might have been for treatment of fish infested with the sea louse *Caligus elongatus*. Similar to previous years all prescriptions of medicines with salmon lice as a possible indication were however included. This since all these treatments are likely to inflict a selection pressure for resistance in salmon lice due to co-infection of *L. salmonis and P. perurans* or *L. salmonis and C. elongatus*, regardless of the treatment indication. The extracts from VetReg were performed 28.01.2022 (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 28.01.2022) in addition to VetReg. Farms that during 2021 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 16.01.2022 from the weekly mandatory reporting of salmon lice data to the Norwegian Food Safety Authority. 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 was 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 2021 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

Eight fish health services along the Norwegian coast were engaged in 2021 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 was also used for the previous years of the surveillance programme (2013-2020). 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).

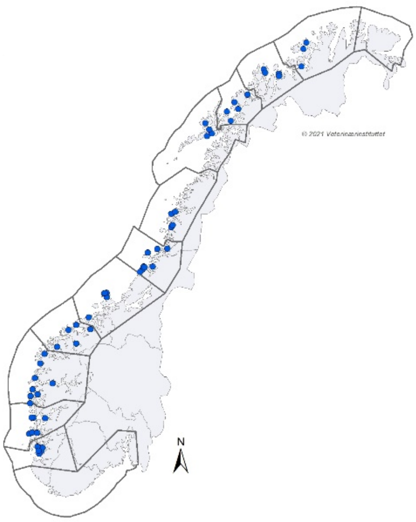


***Figure 1**. Locations of farms where salmon lice were collected for bioassays in 2021 (blue dots). The black lines subdivides Norway into 13 production zones.*

L. salmonis from 58 sites were subjected to bioassays. A total of 43-46 tests were conducted on each of the four 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 8 and 53 lice were used per group). When less than 10 lice were used per group the results were excluded (one low dose result was excluded). 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 considered indicative of a 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 ppb (µg/l) for deltamethrin, azamethiphos and emamectin benzoate and in ppm (mg/l) for hydrogen peroxide.*

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 one in the far south of Norway had been low for several years. In order to still test lice from this area for resistance, 30 lice were collected from each of two 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 eight fish health services performing the bioassays with chemical treatment agents along the Norwegian coast, were engaged in 2021 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: 0-10 freshwater treatments per production zone and year in 2019 and 2020) consisted of production zones 1, 8, 9, 10, 11, 12 and 13. Region two and three (higher usage of fresh water treatments: 17-70 freshwater treatments per production zone and year in 2019 and 2020) consisted of production zones 2, 3 and 4 (region two), and 6 and 7 (region three).

L. salmonis from 27 farms were tested: 13 from region one, six from region two and eight 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 per mille (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. A separate analysis was performed to investigate the development in mortality over the years in the higher use areas. Data from farms where the control group (salinity: 20 ‰) mortality exceeded 20 percent were excluded from the analysis, as these lice might have died from another reason than exposure to low salinity (data from one farm in region three were excluded). In addition, the result for one salinity level from another bioassay was excluded, as this salinity was not included in the protocol.

Results and Discussion

Passive surveillance

Number of prescriptions
Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2013 – 2021. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years; thereafter a decrease continued until 2018. There was a slight increase in 2021 compared to 2020; total increase in 2021 was 5 percent (36 prescriptions). There was an increase in the number of prescriptions for azamethiphos,

emamectin benzoate and imidacloprid (new class in 2021) in 2021 compared to 2020. Emamectin benzoate was the most commonly prescribed medicine, prescribed three 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 2021. The number of prescriptions was collected from VetReg 28.01.22. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.*

Substance category	2013	2014	2015	2016	2017	2018	2019	2020	2021
Azamethiphos	483	752	621	262	59	39	82	119	144
Pyrethroids	1 130	1 049	664	280	82	56	73	51	42
Emamectin benzoate	163	481	523	612	351	371	451	415	437
Flubenzurones	171	195	202	173	81	40	61	51	22
Hydrogen peroxide	255	1021	1 284	629	214	96	82	47	45
Imidacloprid	-	-	-	-	-	-	-	-	29
Total	**2 202**	**3 498**	**3 294**	**1 956**	**787**	**602**	**749**	**683**	**719**

Prescriptions per farm

Prescriptions were issued 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; for 371 farms in 2020 with a mean number of 1.8 prescriptions per farm; and for 413 farms in 2021 with a mean number of 1.7 prescriptions per farm. The number of active farms have increased with between five and 22 farms per year during the years 2018 to 2020. The slight increase in the number of prescriptions from 2020 to 2021 was therefore caused by an increase in the number of farms which had prescriptions issued for them.

Azamethiphos had four foci of frequent use; in production zone 3, 4, 9 and 10. Emamectin bencoate use was spread along most of the coast. The most frequent use of pyrethroids was seen in production zone 10, 11 and 12. The most frequent use of flubenzurones was found in production zone three, while the most frequent hydrogen peroxide usage was seen in production zones 10 and 11 (Figure 2). Almost all use of imidacloprid was in production zone six.

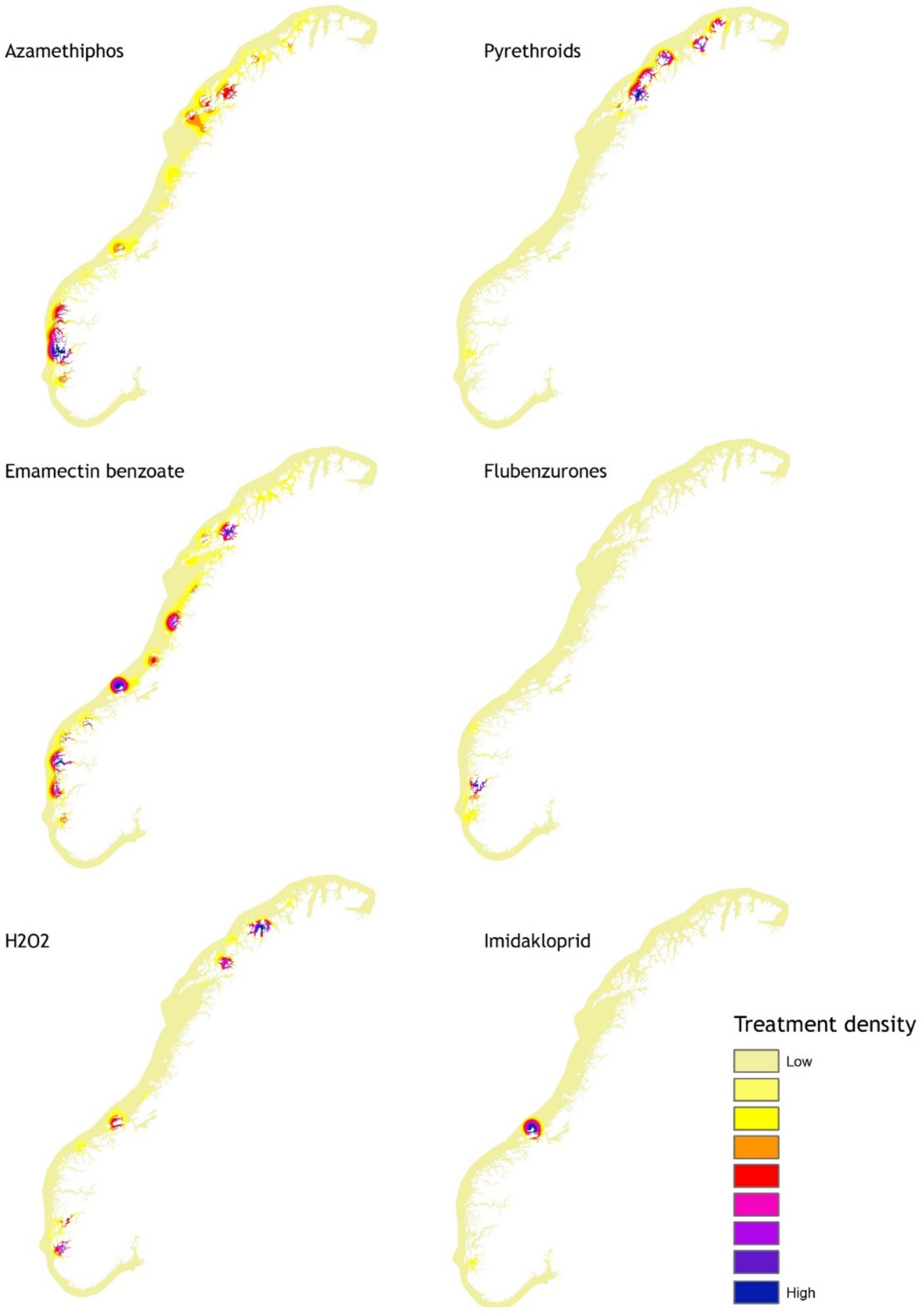


Figure 2*. Geographical distribution of the density of prescriptions per farm for six different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2021. Note that the kernel densities are not scaled equally between different substances so the densities reflect 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 decreased for the first time from 2020 to 2021, by five percent. This discontinued a trend of substantial yearly increases in the number of treatments, which started in 2016. A total of 573 farms performed non-medicinal treatments in 2020 and 580 farms in 2021. The 580 farms in 2021 reported between 1 and 22 treatment weeks, with an average of 4.9 weeks. Of the non-medicinal treatments in 2021, 55 percent were performed using thermal delousing alone or in combination with other non-medicinal methods. 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 2021 (Figure 3). Fresh water treatments have been performed more frequently every year since 2015. This is of special concern with regards to potential resistance development, since premature migration is a lice-coping strategy for the wild sea trout (4).

Table 3. *Number of weeks farms have reported non-medicinal treatments of salmon lice, in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority, from 2014 to 2021*[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 16.01.22.*

Treatment category	2014	2015	2016	2017	2018	2019	2020	2021
Thermal	3	36	685	1246	1330	1449	1723	1453
Mechanical	38	34	311	236	423	673	812	851
Fresh water	1	28	73	75	84	148	234	313
Thermal + Mech	0	0	12	42	35	56	57	30
Thermal + Fresh water	0	0	16	21	17	27	23	64
Mech + Fresh water	0	0	7	1	7	7	18	32
Thermal + Mech + Fresh water	0	0	0	0	1	0	0	3
Other	136	103	75	52	69	88	95	76
Total	**178**	**201**	**1179**	**1673**	**1966**	**2448**	**2962**	**2822**

[1]Deviations from the 2020 resistance report are caused by new combination categories, updated routines to identify incorrect reporting and type of treatment from free text in the report forms, and late incoming reports.

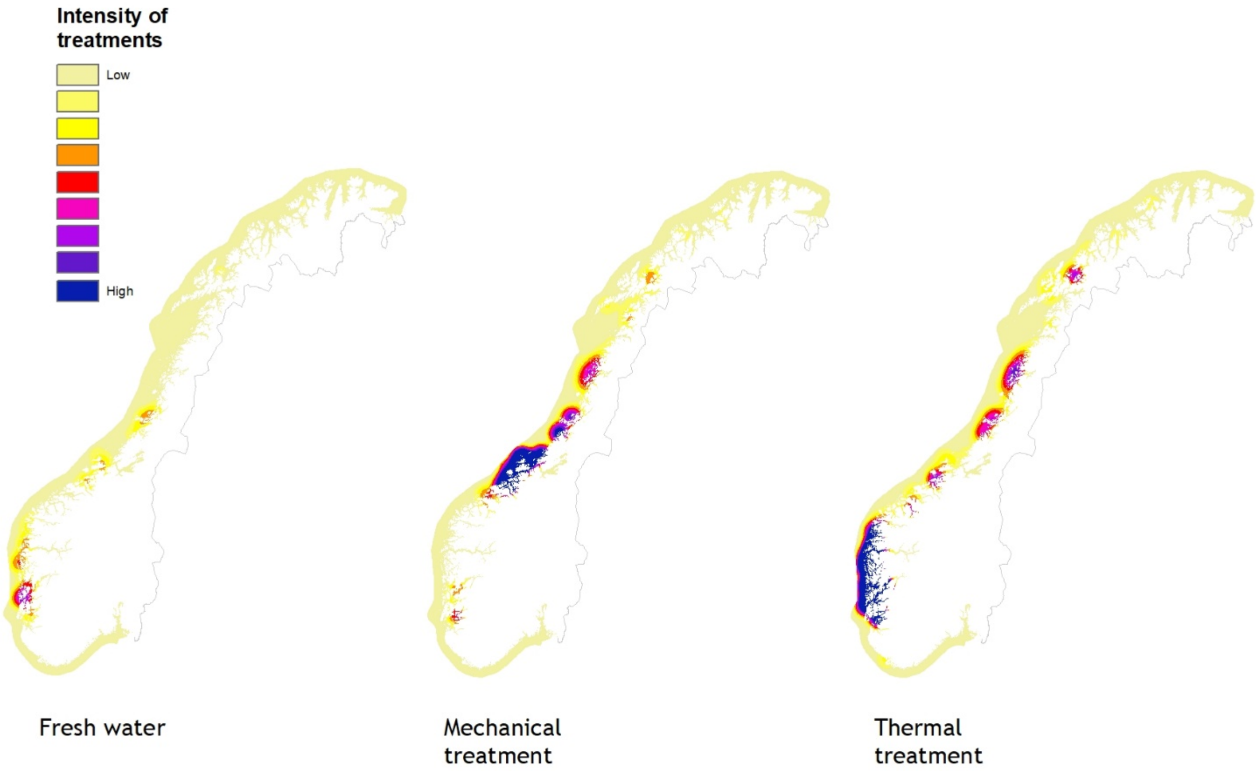


Figure 3. *The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2021. Treatments are categorized into bath treatment in fresh water, mechanical delousing and thermal delousing. Combination treatments are not included in the maps. Treatment intensity is shown with the same linear scale in all three maps. The high intensity (blue) is equivalent to two treatments per 100 km² of water surface, while low intensity (light yellow) is equivalent to zero treatments.*

Active surveillance

Altogether, 179 bioassays were performed on salmon lice from 58 different salmon farms along the coast (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 at low concentrations of the antiparasitics were lower than 80 percent in the majority of locations. This shows that reduced sensitivity to antiparasitics 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	45	7	13	20	3	2
Deltamethrin	43	22	10	5	5	1
Emamectin benzoate	46	24	11	2	5	4
Hydrogen peroxide	43	1	9	13	15	5
High concentration						
Azamethiphos	46	0	9	21	10	6
Deltamethrin	44	1	5	6	16	16
Emamectin benzoate	46	4	9	8	15	10
Hydrogen peroxide	43	0	0	3	14	26

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	45	0.43
Deltamethrin	43	0.34
Emamectin benzoate	46	0.63
Hydrogen peroxide	43	0.35

Bioassay 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).

Salmon lice mortalities were generally low in high concentration azamethiphos bioassays (Figure 4A), indicating that low treatment efficacy may be expected in most areas. However, there were some variations in mortality between the different farms (Figure 4).

The low mortality in the low concentration deltamethrin bioassays (Figure 5B) 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 5A) indicate that farms in most areas tested may expect low treatment efficacy, although 16 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 6B). The high concentration emamectin benzoate bioassays

(Figure 6A) 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 7B) however showed low mortality in some areas, indicating loss of sensitivity to hydrogen peroxide.

Figure 8 displays all high dose bioassay results for the four substances applied. The results indicate decreased resistance level in 2021 for deltamethrin and azamethiphos, continuing a trend since 2017. For emamectin benzoate and hydrogen peroxide the curves indicate a more stable resistance situation after a decrease seen in 2017 for emamectin benzoate and in 2018 for hydrogen peroxide.

The molecular resistance tests of lice from the two farms in production zone one revealed a divergent picture with regards to pyrethroid resistance; 22 percent lice with genetic marker indicating resistance were found in one farm and 62 percent in the other farm. In 2020 an average of 63 percent pyrethroid resistant lice were seen, 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), 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 one in the autumn of 2016, after several years without medicinal treatments in this area, was still seen in 2020. Most farms in production zone one were fallowed simultaneously in 2021 and no medicinal treatments were performed in this zone in 2021. Different sources to salmon lice infestation after restocking might explain the differences in resistance observed.

There was a substantial drop in organophosphate resistance (to 25 and 40 percent) in 2021, from 71 percent in 2020. Organophosphate resistance levels developed 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 and the decrease in 2021 indicates that the tested lice do not originate from the lice tested in 2020. The tests were not performed on lice from the same farms each year.

Table 6. *Results from molecular resistance test* from two farms *in production zone one. The resistance levels are given as percentage of parasites categorized as sensitive or resistant towards pyrethroids and sensitive, intermediate resistant or resistant towards organophosphates.* * *Lice collected January 2022.*

Substance category Level of resistance	Farm 1	Farm 2*
Pyrethroids		
Sensitive	78 %	38 %
Resistant	22 %	62 %
Organophosphates		
Sensitive	75 %	60 %
Intermediate	25 %	36 %
Resistant	0 %	4 %

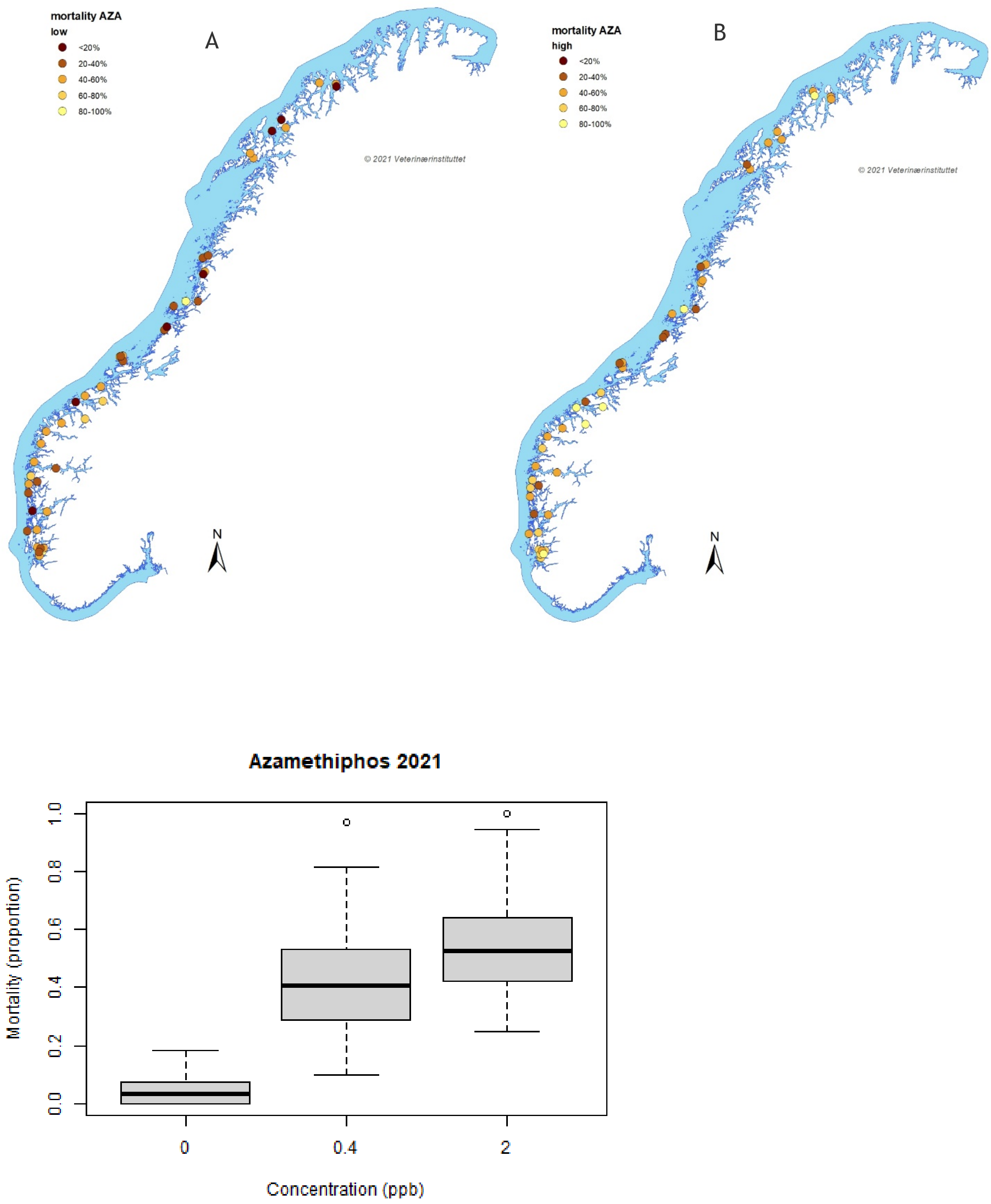
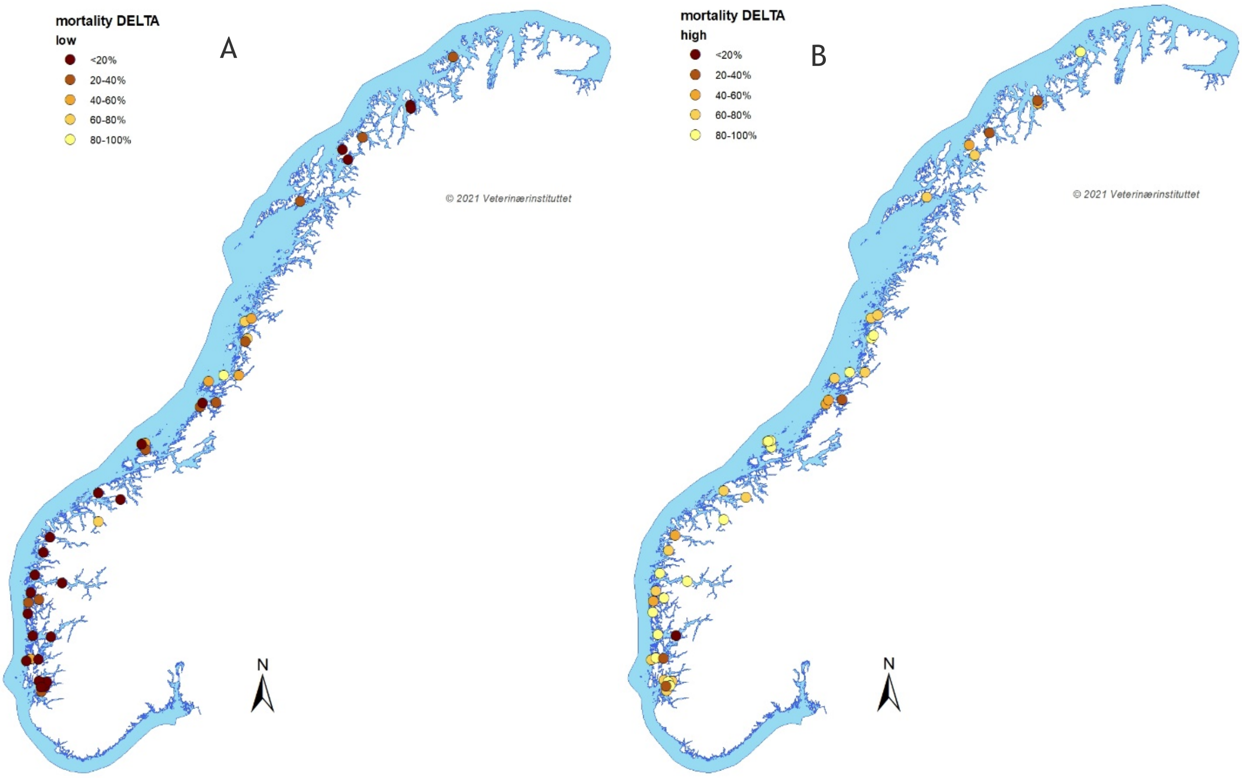


Figure 4. *Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) azamethiphos concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of azamethiphos (0, 0.4 and 2 ppb) (note that the control experiment is the same for the four substances tested).*



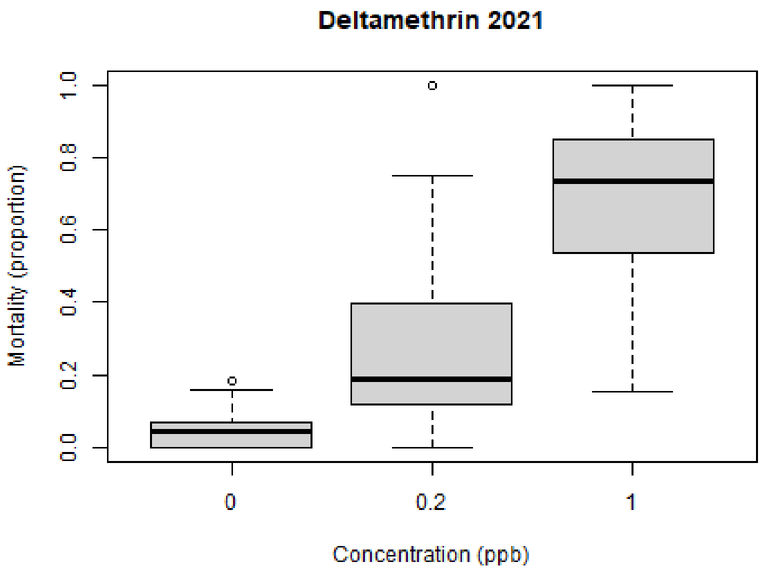
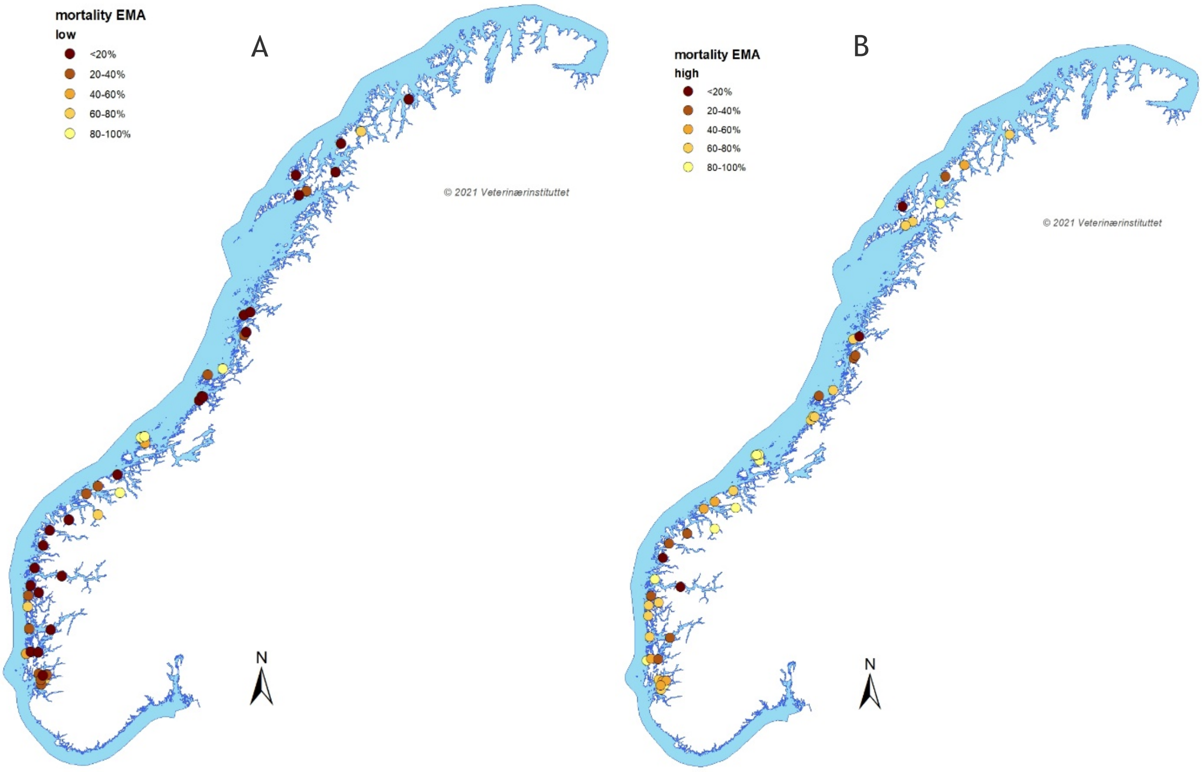


__Figure 5__. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) deltamethrin concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of deltamethrin (0, 0.2 and 1 ppb) (note that the control experiment is the same for the four substances tested).



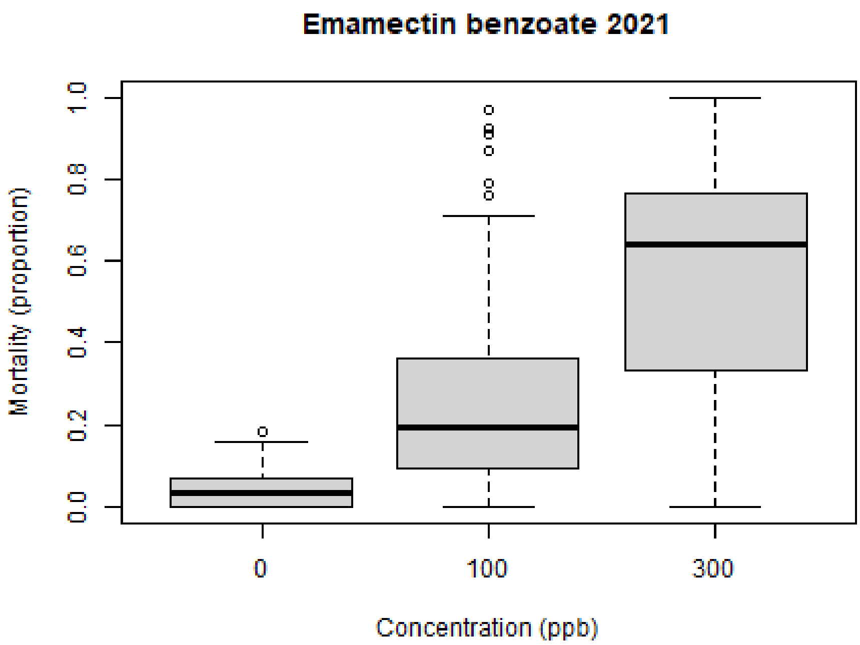


Figure 6. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of emamectin benzoate (0, 100 and 300 ppb) (note that the control experiment is the same for the four substances tested).

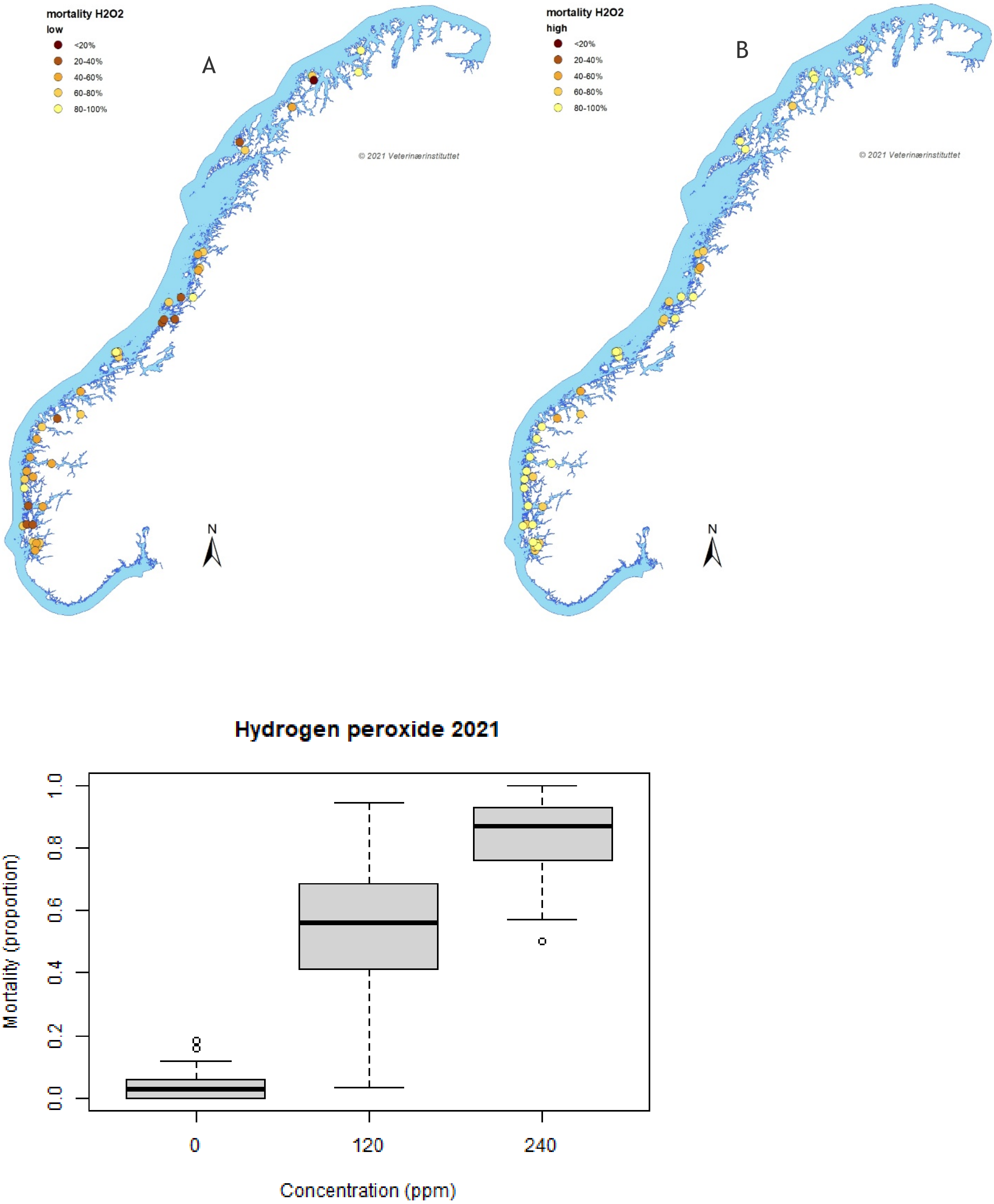


Figure 7*. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of hydrogen peroxide (0, 120 and 240 ppm) (note that the control experiment is the same for the four substances tested).*

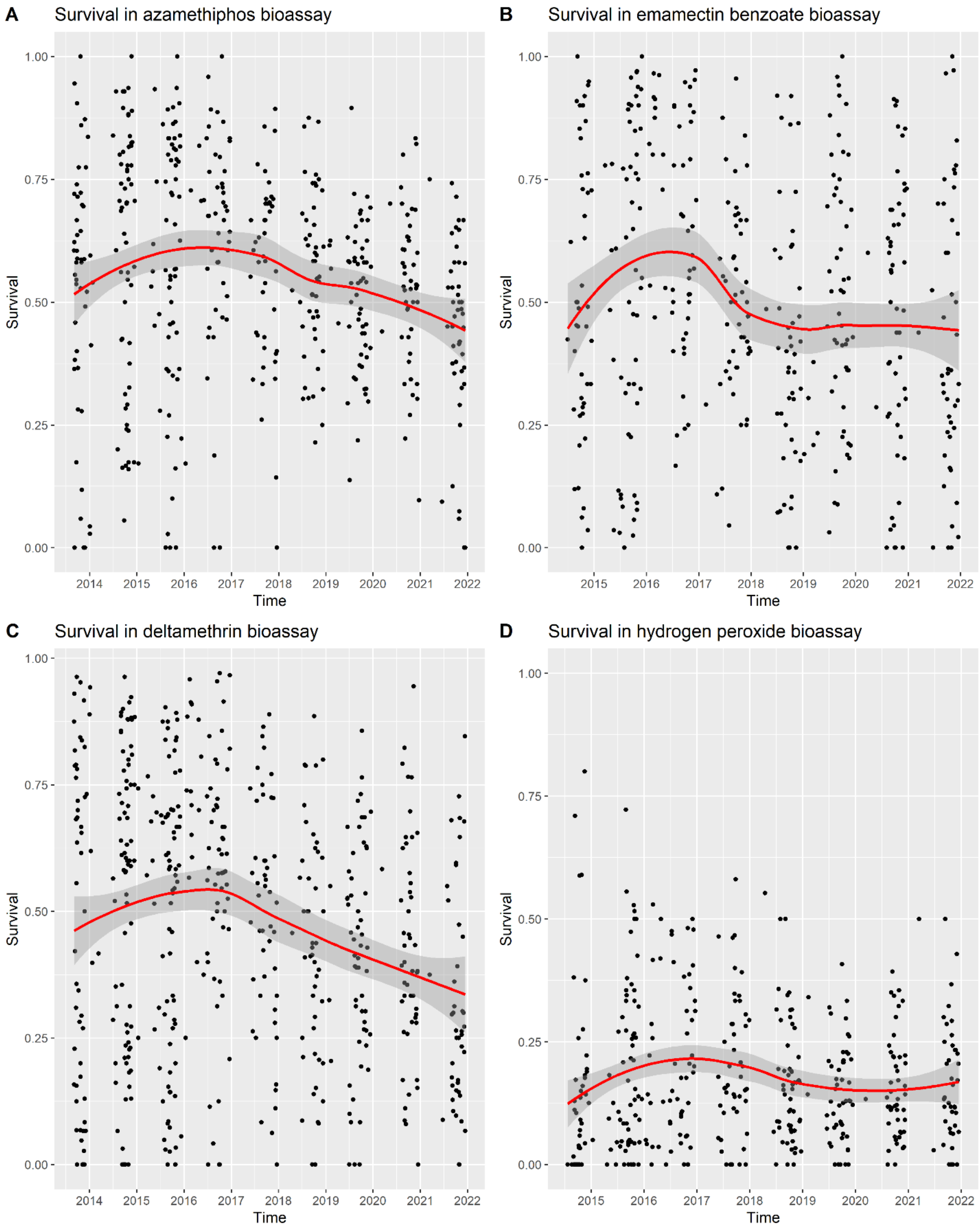


Figure 8. All bioassay results from exposure to azamethiphos (A), emamectin benzoate (B), deltamethrin (C) and hydrogen peroxide (D) displayed as percent survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances. The red line is the 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 bioassay salinities differed significantly (P=0.05) between areas in Norway with low (production zone 1, 8, 9, 10, 11, 12 and 13) or higher usage (production zone 2, 3, 4, 6 and 7) of fresh water bath treatment. Bioassay mortality also dependent on seawater salinity at the farm the day of lice sampling. When ‘low and higher usage areas’ was exchanged in the model with ‘the number of fresh water treatments at the farm in 2020 and 2021 combined’, this was also a significant explanatory variable. 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 two other significant variables from the 2021-analysis were not significant in the 2020-analysis. In a separate analysis, where all data from the areas with highest use of fresh water treatments from all years (2019-2021) were combined, no increasing tolerance towards low salinity was observed.

The mortality at the different concentrations used in the bioassay was slightly higher in the low usage area compared to the higher usage area. Lower salinity at the farm the day of lice sampling was associated with lower mortality in the bioassay. The data also showed an association between the number of freshwater treatments and tolerance towards fresh water. Figure 9 shows the predicted dose-response curves from the two areas and from the different farm salinities, based on the results from the logistic regression.

From this type of analysis it is not possible to conclude on the cause of the observed difference. The question of biological relevance cannot be answered through this analysis alone. 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. The difference in mortality was however not major and can be caused by another underlying difference between these two groups of farms that we do not have data on. Salinity at the farm the day of lice sampling showed a stronger association with the bioassay mortality. The causes of this association are uncertain, but we hypothesize that lice in areas with more frequent low-salinity events are better adapted to tolerate low salinity, either due to genetic factors (selection) or shorter-term physiological responses.

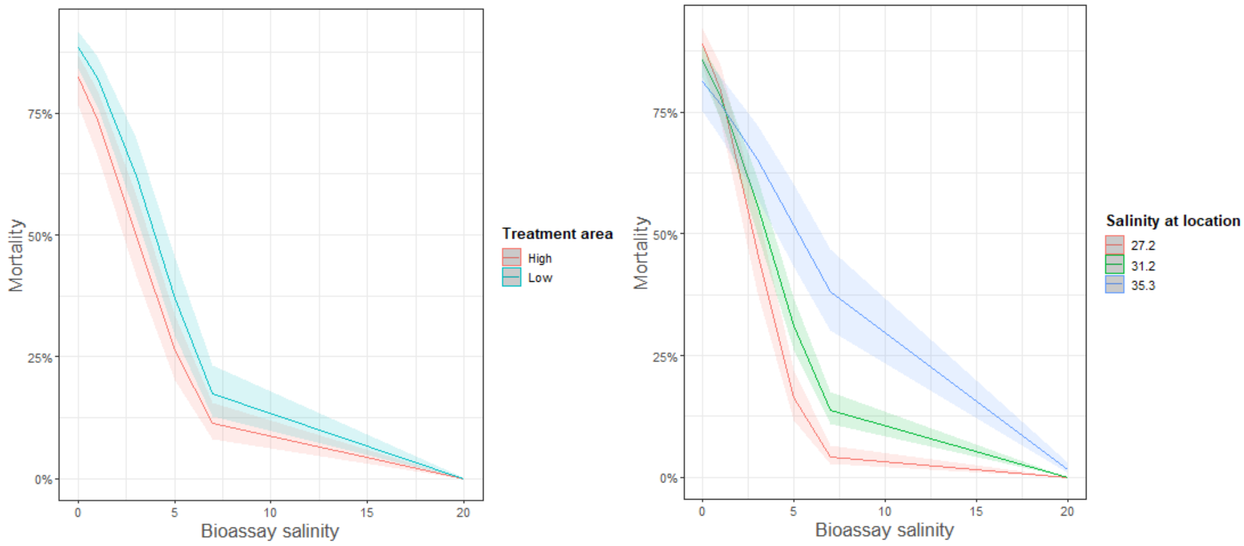


Figure 9*. Predicted results from a regression analysis of the effect of treatment area and salinity at the farm at lice sampling time on the mortality in fresh water bioassays. The left figure predicts the effect of treatment area where the blue line represents the predicted dose-response curve from a bioassay from areas with low treatment intensity and the red line represents the predicted dose-response curve from a bioassay from areas with higher treatment intensity. The right figure predicts the effect of salinity at the farm at lice sampling time where the red, green and blue lines represents the predicted dose-response curve from a bioassay where the lice came from a sea-water salinity of 27.2, 31.2 and 35.3 ‰, respectively. The lighter coloured area around each line is the 95 percent confidence interval for the lines.*

Conclusion

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2021. 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 reduction in the resistance level towards deltamethrin and azamethiphos, while a more stable resistance level was seen towards emamectin benzoate and hydrogen peroxide. This harmonizes well with the number of pyrethroid and emamectin benzoate treatments, which over the last three years has been reduced and kept relatively stable, respectively. The numbers of azamethiphos treatments have however increased for the past three years, while the numbers of hydrogen peroxide treatments have decreased over the last five years, without being reflected in the resistance development. The reduction in azamethiphos resistance seen might be due to more area-wise "hot-spots" of treatments and thereby resistance pressure, while the surveillance of resistance covers almost the entire coast. Furthermore, the azamethiphos use is still lower than in the period 2013-2016, and the continued reduction in resistance might also indicate that the lice population is still in a state of 'recovery' from the high resistance level developed in that period.

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 (13). 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.

The number of prescriptions of medicines against salmon lice increased by five percent from 2020 to 2021. Compared to 2014, when the number of prescriptions peaked, the number was however reduced by 79 percent. When resistance towards an antiparasitic medicine is present, the medicine is normally 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. Some of the explanation to the increase is the use in 2021 of the new anti-salmon lice medicine, for which good treatment efficacy should be expected. The use of this medicine started up with relatively few prescriptions (29 prescriptions). They were however almost all issued within a small area and the selection pressure for resistance in this area was therefore greater than what the overall figures indicate.

The number of reported farm treatment-weeks using non-medicinal methods decreased by 5 percent from 2020 to 2021. This discontinued a yearly trend since 2016 with increasing numbers of non-medicinal treatments per year. This could indicate that the industry has reached a plateau-level in the number of treatments necessary to control salmon lice, since the number of medicinal treatments have been kept relatively stable since 2017. In 2021, 580 farms reported the use of non-medicinal methods, while 413 farms had medicines against salmon lice prescribed for them. Thermal delousing was the dominating method with 55 percent of the non-medicinal treatments (alone or in combination). This percentage has been decreasing since 2018, while the actual number of thermal treatments were decreasing for the first time in 2021. It is however still at a high level (1550 weeks of treatment reported) and frequent treatment with a single method will most likely inflict a selection pressure towards more temperature tolerant salmon lice.

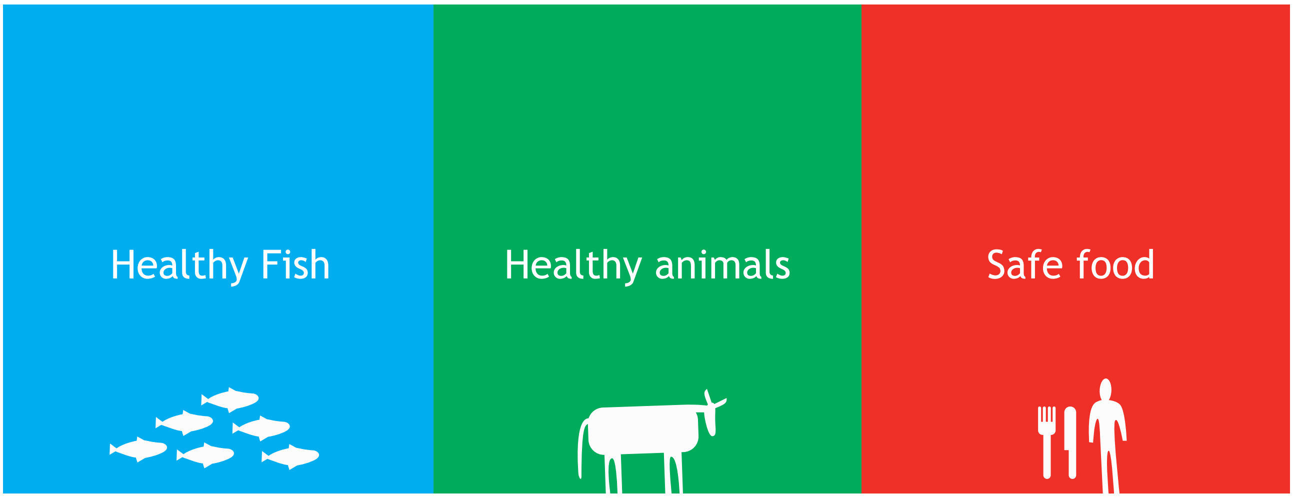
Fresh water bath treatments were the type of treatment that increased the most from 2020 to 2021. In 2021 freshwater was used alone or in combination with other non-medicinal methods in 412 weeks, which is 15 percent of the reported weeks of treatment. In 2021 a survey was conducted to look for differences in fresh water sensitivity levels between lice from low and higher usage areas of fresh water treatments. In this survey lice from farms in higher usage areas tolerated lower salinities slightly better than lice from lower usage areas. The tolerance also varied with varying salinities on the farms where the lice were sampled. This difference between areas were also seen, and to a greater extent, in the 2020-study, but not in the 2019-study. There was no sign of increasing salinity tolerance in the area with the highest usage of fresh water treatments over the years 2019-2021. 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.

Acknowledgment

The eight fish health services engaged in this program, has contributed significantly to the accomplishment of this survey. Thanks to:
Akvaplan-niva AS, Akvavet Gulen AS, Aqua Kompetanse AS, FoMAS – Fiskehelse og Miljø AS, HaVet AS, Marin Helse AS, STIM AS, Åkerblå AS. In addition, thanks to MOWI for salmon lice sampling.

References

1. Aaen SM, Helgesen KO, Bakke MJ, Kaur K, Horsberg TE. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol 2015; 31: 72-81.
2. Jansen PA, Grøntvedt RN, Tarpai A, Helgesen KO, Horsberg TE. Surveillance of the sensitivity towards antiparasitic bath-treatments in the salmon louse (*Lepeophtheirus salmonis*). PLOS ONE 2016; 11(2) DOI: 10.1371/journal.pone.0149006.
3. Grøntvedt RN, Jansen PA, Horsberg TE, Helgesen K, Tarpai A. The surveillance programme for resistance to chemotherapeutants in L. salmonis in Norway 2013. Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2013. Oslo: Norwegian Veterinary Institute 2014.
4. Halttunen E, Gjelland KØ, Hamel S, Serra-Llinares RM, Nilsen R, Arechavala-Lopez P, Skarðhamar J, Johnsen IA, Asplin L, Karlsen Ø, Bjørn PA, Finstad B. Sea trout adapt their migratory behaviour in response to high salmon lice concentrations. J Fish Dis 2018; DOI: 10.1111/jfd.12749.
5. Ministry of Trade, Industry and Fisheries. Regulation on control of salmon lice in aquaculture in Norway (In Norwegian: Forskrift om bekjempelse av lakselus i akvakulturanlegg). https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140?q=lakselus. Accessed: 24.02.20.
6. R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
7. ESRI, 2014. ArcGIS Desktop: Release 10.5. Redlands, CA: Environmental Systems Research Institute.
8. Helgesen KO, Horsberg TE. Single-dose field bioassay for sensitivity testing in sea lice, *Lepeophtheirus salmonis*: development of a rapid diagnostic tool. J Fish Dis 2013; 36: 261-272.
9. Helgesen KO, Romstad H, Aaen S, Horsberg TE. First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. Aquaculture reports 2015; 1:37-42.
10. Ministry of Trade, Industry and Fisheries. Regulation on production zones for salmonid aquaculture at sea (In Norwegian: Forskrift om produksjonsområder for akvakultur av matfisk i sjø av laks, ørret og regnbueørret). https://lovdata.no/dokument/SF/forskrift/2017-01-16-61. Accessed: 24.02.20.
11. Andrews M, Horsberg TE. Sensitivity towards low salinity determined by bioassay in the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Aquaculture 2020; 514: 734511.
12. Ljungfeldt LER, Quintela M, Besnier F, Nilsen F, Glover KA. A pedigree-based experiment reveals variation in salinity and thermal tolerance in the salmon louse, *Lepeophtheirus salmonis*. Evol Appl 2017;10:1007–1019.
13. Kaur K, Helgesen KO, Bakke MJ, Horsberg TE. Mechanism behind Resistance against the Organophosphate Azamethiphos in Salmon Lice (Lepeophtheirus salmonis). PLOS ONE 2015. https://doi.org/10.1371/journal.pone.0124220



Scientifically ambitious, forward-looking and collaborative- for one health!

Veterinærinstituttet
Norwegian Veterinary Institute

Ås | Trondheim | Sandnes | Bergen | Harstad | Tromsø

postmottak@vetinst.no
www.vetinst.no