

The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2022



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Suggested citation

Helgesen, Kari Olli, Horsberg, Tor Einar, Stige, Leif Christian, Tarpai, Attila, Norheim, Kari. The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2022. Surveillance program report. Veterinærinstituttet 2023. © Norwegian Veterinary Institute, copy permitted with citation

Quality controlled by

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

Published

2023 on www.vetinst.no
ISSN 1890-3290 (electronic edition)
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Commissioned by / In collaboration with

Norwegian Food Safety Authority



Colophon

Cover design: Reine Linjer Cover photo: Colourbox www.vetinst.no

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Summary

The number of prescriptions for anti-salmon lice medicines were the same in 2022 as in 2021. The number of prescriptions has 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 use of this agent, with the active substance imidacloprid, was moderate in both 2021 and 2022, with 29 and 54 prescriptions respectively. The level of resistance seen in salmon lice towards most anti-salmon medicines remained high in 2022. There was however a tendency of reduced resistance towards deltamethrin and azamethiphos. This is despite an increased use of azamethiphos over the last four 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 increased by 11 percent, to 3145 weeks, from 2021 to 2022. This continued a yearly trend of increase seen since 2016 with an exception in 2021. 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 17 percent of the non-medicinal treatments in 2022 (528) reported treatments-weeks). A field study of fresh water sensitivity was performed for the fourth time in the surveillance program in 2022, comparing the sensitivity levels of salmon lice from areas with low and higher frequency of fresh water treatments. The results did not show significantly higher fresh water tolerance in lice from farms located in the higher fresh water usage areas. For the first year, imidacloprid bioassays were performed as part of the surveillance program and the natural variation in imidacloprid sensitivity was described.

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 and farm density (2, 3). Results from resistance testing have been applied by the industry as a decision support 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 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 (4). 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 (30 from 2022) 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 (5). If they are infested with salmon lice with increased fresh water tolerance, the efficacy of their natural delousing strategy might decline. As in the years 2019 to 2021, a field study was therefore conducted in 2022, 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.

To obtain a base-level of imidacloprid sensitivity (new substance from 2021), 30 imidacloprid bioassays were performed along most of the Norwegian coast in 2022. This was the first time since the start of the surveillance program it was possible to monitor the sensitivity towards an active substance that was novel to salmon lice.

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. 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 13.01.2023 (dd.mm.yyyy).

Active farms, defined as farms that during 2022 reported the presence of adult female lice, were identified using the weekly mandatory farm reports of salmon lice to the Norwegian Food Safety Authority (extracted 18.01.2022).

Non-medicinal treatments

The number of non-medicinal treatments performed in Norwegian salmon farms was extracted 16.01.2023 from the weekly salmon lice reports. 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.

Data processing

Data processing and statistical analyses were performed in the statistical software R (7). Geographical processing and presentation of data was performed using ArcGIS (8).

Active surveillance

Bioassays

Five fish health services along the Norwegian coast were engaged in 2022 to perform toxicological resistance tests (bioassays) on live parasites. The deltamethrin, emamectin benzoate, azamethiphos and hydrogen peroxide bioassay protocol was based on Helgesen et al. 2013 and 2015 (9, 10) and was also used for the previous years of the surveillance programme (2013-2021). 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 A) were chosen by the fish health services themselves inside a production zone. Norway's 13 production zones are given by regulation (11) and shown in Figure 1 (numbered 1 to 13 from south to north).

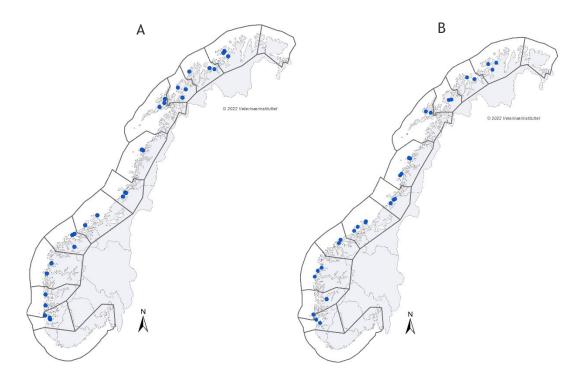


Figure 1: Locations of farms where salmon lice were collected for bioassays against medicines in 2022 (blue dots). The black lines subdivides Norway into 13 production zones. A show the bioassays against deltamethrin, emamectin benzoate, azamethiphos and hydrogen peroxide, while B show the location of the imidacloprid bioassays.

L. salmonis from 29 sites were subjected to bioassays. Between 18 and 23 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 6 and 59 lice were used per group). When less than 10 lice were used per group the results were excluded (two low dose result were 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 performed according to the medicine's summary of product characteristics (SPC).

Table 1: Concentrations used in the exposed groups in the bioassays, in ppb $(\mu g/l)$ 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

Fresh water bioassays

The salinity bioassay protocol was based on Andrews and Horsberg 2020 (12). The locations were chosen by the fish health services themselves inside one of three regions. Region one (low usage of fresh water treatments: 2-12 freshwater treatments per production zone and year in 2020 and 2021) consisted of production zones 1, 8, 9, 10, 11, 12 and 13. Region two and three (higher usage of fresh water treatments: 21-106 freshwater treatments per production zone and year in 2020 and 2021) consisted of production zones 2, 3 and 4 (region two), and 6 and 7 (region three).

L. salmonis from 28 farms were tested: 14 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 two farms in region one and two in region three were excluded).

Imidacloprid bioassays

The imidacloprid bioassay protocol was based on a protocol developed by Aaen and Horsberg (13). Identical stock solutions and identical equipment were used by all the fish health services. The locations were chosen by the fish health services themselves inside a given production zone. Figure 1 B shows the location of all imidacloprid bioassays.

L. salmonis from 30 farms were tested: two or three assays from each of the production zones 2-12. The bioassays were performed by exposing live parasites of motile stages, removed from the fish, to seawater with imidacloprid, in the concentrations: 0 (control), 0.01, 0.04, 0.1, 0.4 and 1 mg/L. After 24-hour exposure, salmon lice mortality, grouped according to stages and genders, was noted as the test outcome.

Dose-response curves were modelled and the values immobilising 50 percent of the lice (LC50-values) were calculated using a two parameter log-logistic model from the package drc in R (14). Data from all farms were included, despite the fact that mortality in one of the control groups (0 mg imidacloprid/L) exceeded 20 percent (it was 21 percent).

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 - 2022. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years; thereafter a decrease continued until 2018. Since 2017 the number of prescriptions has been relatively stable. There was an increase in the number of prescriptions for azamethiphos and imidacloprid in 2022 compared to 2021. Emamectin benzoate was the most commonly prescribed medicine.

Table 2: Number of prescriptions for the given substances/class of substances applied to control salmon lice in 2013 to 2022. The number of prescriptions was collected from VetReg 13.01.23. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.

Substance category	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Azamethiphos	483	752	621	262	59	39	82	119	144	212
Pyrethroids	1 130	1 049	664	280	82	56	73	51	42	25
Emamectin benzoate	163	481	523	612	351	371	451	415	437	371
Flubenzurones	171	195	202	173	81	40	61	51	22	22
Hydrogen peroxide	255	1021	1 284	629	214	96	82	47	45	35
Imidacloprid	-	-	-	-	-	-	-	-	29	54
Total	2 202	3 498	3 294	1 956	787	602	749	683	719	719

Prescriptions per farm

Prescriptions were issued for 412 farms in 2022 with a mean number of 1.7 prescriptions per farm. This was almost identical to 2021 when 413 farms had prescriptions issued for them with a mean number of 1.7 prescriptions per farm. The number of active farms have increased with between zero and 22 farms per year during the years 2018 to 2022.

Azamethiphos and emamectin benzoate 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 3, while the most frequent hydrogen peroxide usage was seen in production zones 5, 6 and 9. Imidacloprid was most frequently used in production zones 6 and 7 (Figure 2).

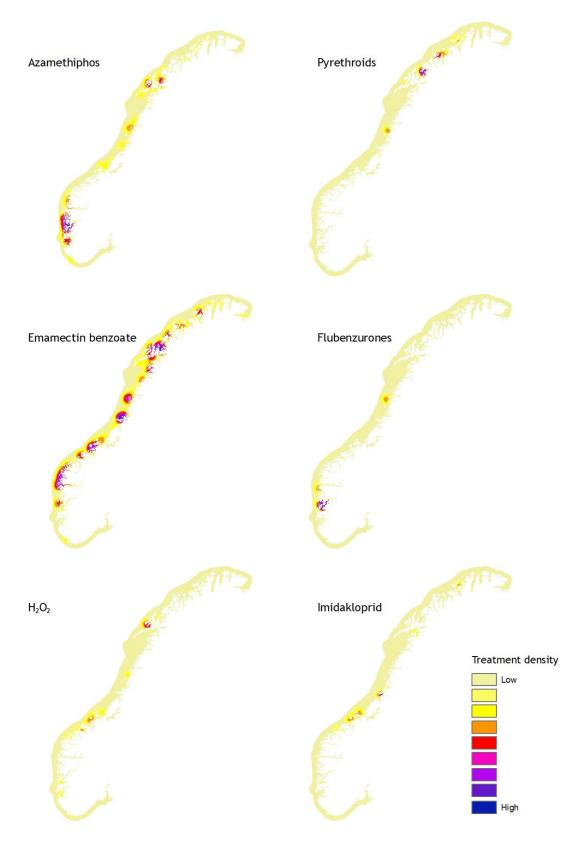


Figure 2: Geographical distribution of the density of prescriptions per farm for six different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2022. Note that the kernel densities arenot scaled equally between different substances so the densities reflect 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 11 percent from 2021 to 2022. This continued a yearly trend of substantial yearly increases in the number of treatments which started in 2016, with an exception of 2021. A total of 601 farms performed non-medicinal treatments in 2022. The 601 farms in 2022 reported between 1 and 27 treatment weeks, with an average of 5.2 weeks. Of the non-medicinal treatments in 2022, 49 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 (15). 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 2022, but the use was most frequent in production zones 3 and 4 (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). Of note in 2022 is also the increase in the use of a combination of methods in the same week. This concerned five percent of the weeks in 2021 and 11 percent in 2022.

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 2015 to 2022¹. 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 16.01.23.

Treatment category	2015	2016	2017	2018	2019	2020	2021	2022
Thermal	36	685	1245	1327	1447	1723	1456	1357
Mechanical	34	311	236	423	674	823	861	1074
Fresh water	28	73	75	84	148	220	286	225
Thermal + Mech	0	12	42	35	56	59	30	47
Thermal + Fresh water	0	16	21	17	27	20	63	141
Mech + Fresh water	0	7	1	7	7	24	56	153
Thermal + Mech + Fresh water	0	0	0	1	0	1	5	9
Other	103	75	52	69	87	92	73	139
Total	201	1179	1672	1963	2446	2962	2830	3145

¹Deviations from the 2021 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.

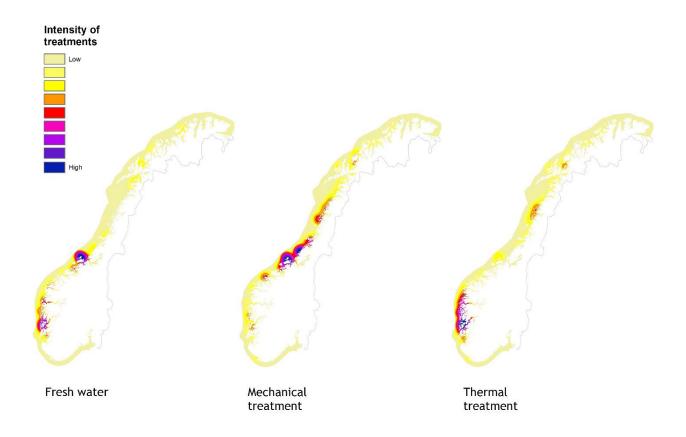


Figure 3: The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2022. Treatments are categorized into bath treatment in fresh water, mechanical delousing and thermal delousing. Combination treatments are not included in the maps. Treatment intensity is 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, 81 bioassays were performed on salmon lice from 29 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	er of Percent mortality						
Substance category	tests	0-20 %	20-40 %	40-60 %	60-80 %	80-100 %		
Low concentration								
Azamethiphos	21	1	7	11	2	0		
Deltamethrin	18	9	3	3	2	1		
Emamectin benzoate	19	12	2	3	0	2		
Hydrogen peroxide	21	1	5	8	3	4		
High concentration								
Azamethiphos	23	0	5	7	10	1		
Deltamethrin	18	0	3	3	5	7		
Emamectin benzoate	19	4	5	2	4	4		
Hydrogen peroxide	21	0	0	1	7	13		

Table 5 shows the correlation between salmon lice mortality results from low and high concentrations, which were significantly correlated for deltamethrin and emamectin benzoate. The reduction in number of tests to approximately half the tests performed previous years contributes to the differences between substance in the strength of the correlation, because random errors become more influential.

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	21	0.16
Deltamethrin	18	0.67
Emamectin benzoate	19	0.50
Hydrogen peroxide	21	0.36

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 4B), indicating that low treatment efficacy may be expected in most areas. However, there were some variations in mortality between the different farms (Figure 4).

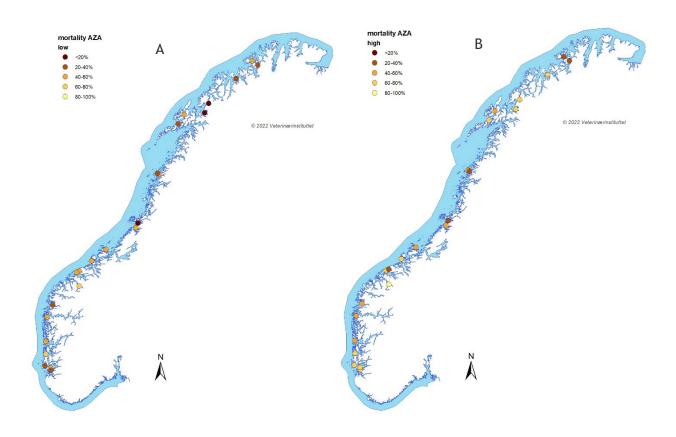
The low mortality in the low concentration deltamethrin bioassays (Figure 5A) indicates that reduced sensitivity to deltamethrin is widespread along the coast. Only one farm showed test mortalities exceeding 80 percent. The results from the high concentration deltamethrin bioassays (Figure 5B) were more diverse, with mortalities exceeding 80 percent in seven farms.

The low concentration emamectin benzoate bioassays showed that reduced sensitivity is widespread along the coast (Figure 6A). The high concentration emamectin benzoate bioassays

(Figure 6B) 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 7A) 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 2022 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. Of note is however the reduced number of bioassays included in the 2022-program compared to previous years, which adds uncertainty to the conclusions.





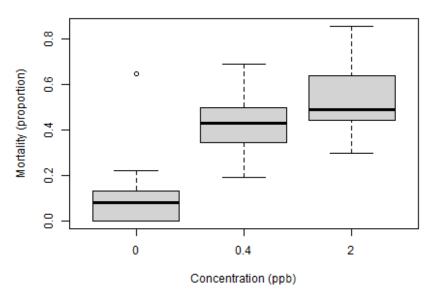
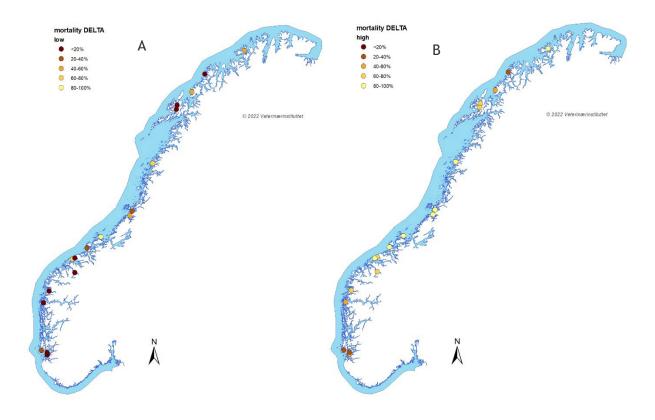


Figure 4: Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) azamethiphos concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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



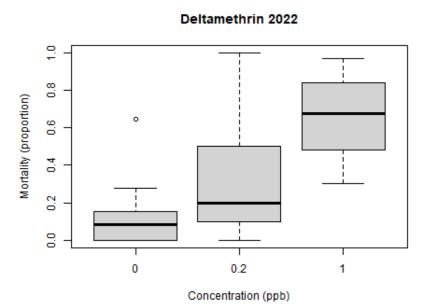
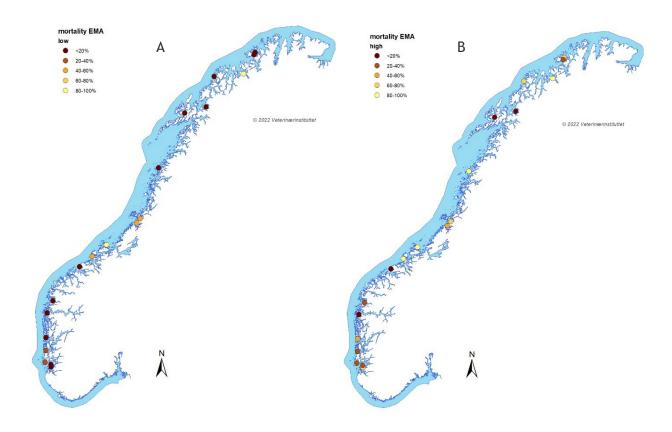


Figure 5: Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) deltamethrin concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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



Emamectin benzoate 2022

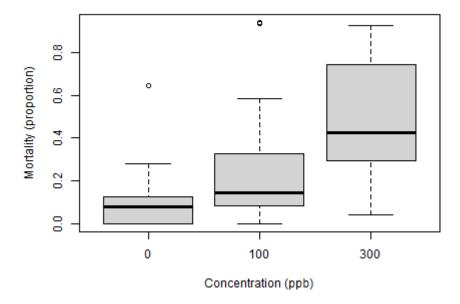
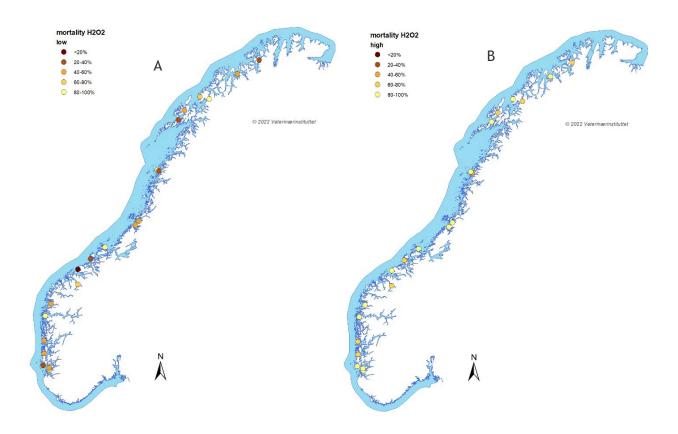


Figure 6: Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) emamectin benzoate concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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



Hydrogen peroxide 2022

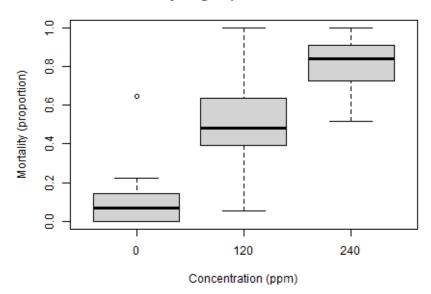


Figure 7: Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) hydrogen peroxide concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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

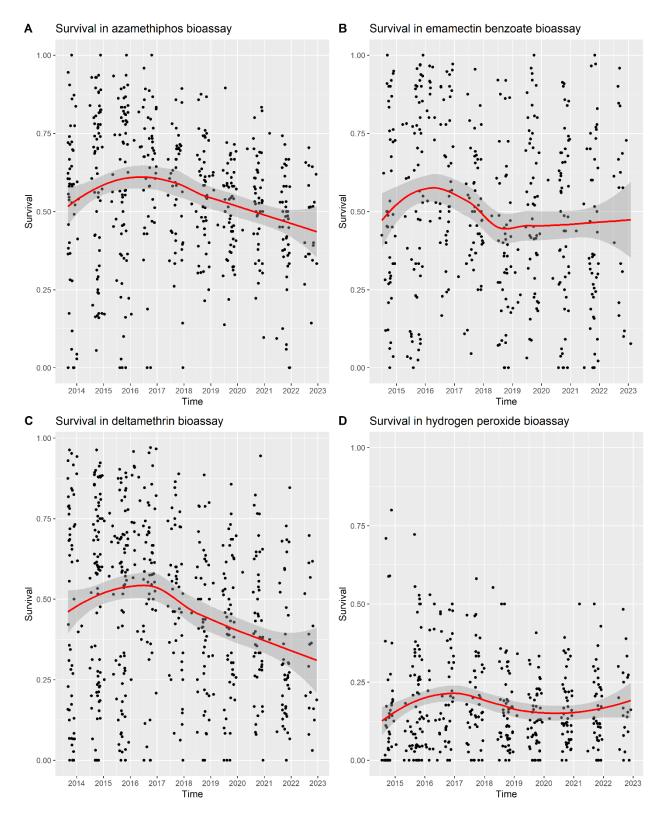


Figure 8: All bioassay results from exposure to azamethiphos (A), emamectin benzoate (B), deltamethrin (C) and hydrogen peroxide (D) displayed as proportion of survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances and that the number of data points were approximately halved from 2022. 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 from 2022, the mortality in the bioassays did not differ significantly (significance level set to be P=0.05) between areas in Norway with low (production zone 1, 8, 9, 10, 11, 12 and 13) compared to higher usage (production zone 2, 3, 4, 6 and 7) of fresh water bath treatment. When 'low and higher usage areas' was exchanged in the model with 'the number of fresh water treatments at the farm in the last two years', this was neither a significant explanatory variable. There was however a significant interaction between the effects of treatment area and bioassay salinity. Specifically, the results indicated that lice in the high treatment area tolerated moderate reductions in salinity better than lice in the low treatment area, whereas mortality was similarly high at the lowest salinities (Figure 9). Figure 9 shows the predicted dose-response curves from the two areas, based on the results from the logistic regression.

This result differed from what was seen in the 2021-data, when significant effects of both treatment area and salinity at the farm were found. In a separate analysis, where all data from the areas with highest use of fresh water treatments from all years (2019-2022) were combined, no increasing tolerance over years towards low salinity was observed.

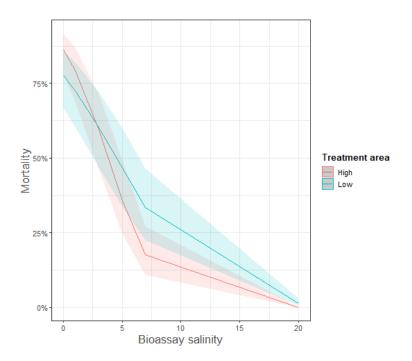


Figure 9: Predicted results from a regression analysis of the effect of treatment area on the mortality in fresh water bioassays. The figure shows the significant interaction effect of treatment area and bioassay salinity (in per mille), where the blue line represents the predicted dose-response curve 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 lighter coloured area around each line is the 95 percent confidence interval for the lines.

Imidacloprid bioassays

From the modelled dose-response curves for imidacloprid bioassays, the concentration immobilising 50 percent of the lice (LC50) were calculated per farm. The median LC50-value was 0.06~mg/L and the 10^{th} and 90^{th} percentile LC50-values were 0.03~and~0.14~mg/L

respectively. This is similar to what was previously observed in one salmon lice strain by Aaen and Horsberg (13); LC50 (90 % CI) was 0.098 mg/L (0.074-0.149). Imidacloprid has been used for salmon lice treatments for only 1.5 years and good treatment efficacies have been observed, to the best of our knowledge. The variation in LC50-values are therefore most likely reflecting natural variation in salmon lice imidacloprid susceptibility. The more extreme LC50-values in this dataset (minimum value 0.003 and maximum value 0.44 mg/L) could possibly also represent natural variation, but these values could also be caused by bioassays being susceptible to human errors. The assays with these more extreme values should therefore ideally have been repeated in order to decide which of these two categories these results fall into.

Modelled dose-response curves for imidacloprid bioassays

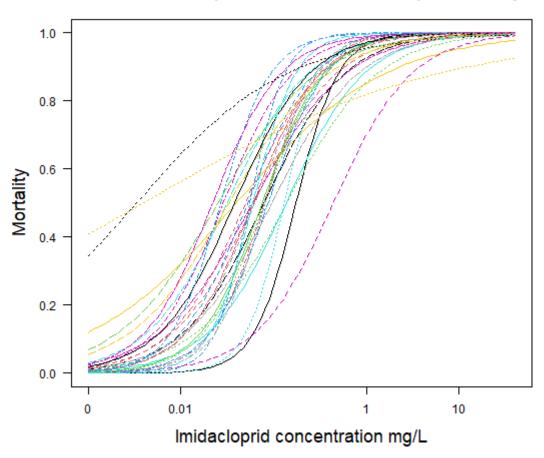


Figure 10: Modelled dose-response curves from the imidacloprid bioassays. Each line represent one bioassay.

Conclusion

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2022 towards the medicines that have been used for decades; deltamethrin, azamethiphos and emamectin benzoate. Resistance towards these substances was generally widespread along the Norwegian coast. Less resistance was found towards hydrogen peroxide than towards the other of these 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 numbers of pyrethroid and emamectin benzoate treatments, which over the last three years have, respectively, been reduced and kept relatively stable. The numbers of azamethiphos treatments have however increased for the past four years, while the numbers of hydrogen peroxide treatments have decreased over the last seven years, without being reflected in the resistance development.

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 (16). 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 imidacloprid sensitivity level of presumably imidacloprid sensitive lice from 30 farms along the coast was detected. This probably describes part of the natural variation in imidacloprid sensitivity and can be used as a reference in future imidacloprid resistance surveillance.

The number of prescriptions of medicines against salmon lice have remained relatively stable since 2017. 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 and imidacloprid which was new to the marked in 2021. The use of imidacloprid remained at a moderate level in 2022 (54 prescriptions). Almost half of the prescriptions were however issued in two production zones and the selection pressure for resistance in these areas was therefore greater than what the overall figures indicate.

The number of reported farm treatment-weeks using non-medicinal methods increased by 11 percent from 2021 to 2022. This continued a yearly trend since 2016, with an exception of 2021, with increasing numbers of non-medicinal treatments per year. In 2022, 601 farms reported the use of non-medicinal methods, while 390 farms had medicines against salmon lice prescribed for them. Thermal delousing was the dominating method with 49 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 and kept steady in 2022. It is however still at a high level (1554 weeks of treatment reported) and frequent treatment with a single method will most likely inflict a selection pressure towards more temperature-tolerant salmon lice. Of note is also the increase in combination of treatments in one farm in one week; 11 percent of the weeks in 2022 compared to five percent in 2021. The causes and possible consequences of this should be further explored.

Fresh water bath treatments were used more often in 2022 compared to 2021. In 2022 freshwater was used alone or in combination with other non-medicinal methods in 528 weeks, which is 17 percent of the reported weeks of treatment. In 2022 a survey was conducted to look for differences in sensitivity towards low salinity between lice from low and higher usage areas of fresh water treatments, but no strong difference in tolerance was observed. In the same survey in 2021, lice from farms in higher usage areas tolerated lower salinities slightly better than lice from lower usage areas. There was no sign of increasing low salinity tolerance in the area with the highest usage of fresh water treatments over the years 2019-2022. Since wild sea trout use fresh and brackish water for delousing, such a development would be unwanted also from a wild fish perspective. The limited number of farms included in the study makes it difficult to draw strong conclusions from this survey.

Acknowledgment

The five fish health services engaged in this program, has contributed significantly to the accomplishment of the surveillance. Thanks to:

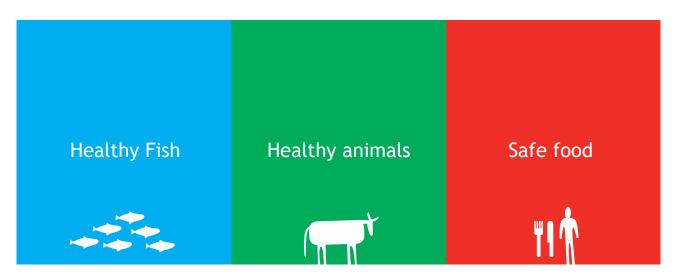
Akvavet Gulen AS, Aqua Kompetanse AS, HaVet AS, STIM AS and Åkerblå AS.

In addition thanks to all fish farms which have provided practical assistance to collect lice.

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. Coates A, Robinson N, Dempster T, Samsing F, Johnsen I, Phillips BL. A metapopulation model reveals connectivity-driven hotspots in treatment resistance evolution in a marine parasite. ICES Journal of Marine Science 2022; 79, 2682-2696. DOI: 10.1093/icesjms/fsac202.
- 3. 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.
- 4. 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.
- 5. 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.

- 6. 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.
- 7. 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/. R version 4.0.3 (2020-10-10)
- 8. ESRI, 2014. ArcGIS Desktop: Release 10.5. Redlands, CA: Environmental Systems Research Institute.
- 9. 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.
- 10. 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.
- 11. 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.
- 12. Andrews M, Horsberg TE. Sensitivity towards low salinity determined by bioassay in the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Aquaculture 2020; 514: 734511.
- 13. Aaen SM, Horsberg TE. A screening of multiple classes of pharmaceutical compounds for effect on preadult salmon lice *Lepeophtheirus salmonis*. Journal of Fish Diseases 2016. doi:10.1111/jfd.12463.
- 14. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. PLOS ONE 2015 10(12). E0146021.
- 15. 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.
- 16. 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



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