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The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2023

REPORT 23/2024

The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2023

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Content

Sammendrag

Det ble skrevet ut 702 resepter på lakselusmidler i 2023. Dette var en liten nedgang fra 2022 (7 prosent). Antallet resepter har holdt seg på omtrent samme nivå siden 2017. Dette i motsetning til perioden 2014 til 2017, der antallet årlige resepter ble redusert med 77 prosent. Bruken av imidakloprid mot lus, som fikk markedsføringstillatelse i Norge i 2021, var moderat i 2021, 2022 og 2023, med henholdsvis 25, 91 og 51 resepter. Resistensnivået mot de fleste lakselusmidlene som ble testet holdt seg høyt også i 2023. Nivået var stabilt eller litt stigende, med et forbehold om at det fra 2022 ble testet lus fra færre oppdrettsanlegg. Det har vært en økt bruk av azametifos de siste fem årene, emamektinbenzoat-bruken har vært stabil, mens bruken av pyretroider og hydrogenperoksid har gått ned. Resistens mot azametifos, deltametrin og emamektinbenzoat var utbredt langs norskekysten. Det ble funnet mindre resistens mot hydrogenperoksid, men tap av sensitivitet ble sett flere steder. Det ble rapportert 17 prosent færre uker med medikamentfri avlusning i 2023 sammenliknet med 2022; 2609 i 2023 mot 3145 uker i 2022. Medikamentfri avlusning var fortsatt den dominerende behandlingsmetoden. Ferskvannsbehandling, alene eller i kombinasjon med andre metoder, ble rapportert for 22 prosent av ukene med medikamentfri avlusning i 2023 (576 behandlingsuker). Ferskvannssensitivitet ble inkludert i overvåkningsprogrammet i 2019 og resultatene ble sammenliknet med behandlingshistorikken på anlegget. Resultatene viste lavere mortalitet på lus i resistenstestene ved moderate saliniteter jo flere ganger anlegget hadde behandlet mot lus de siste to årene. Dette var tydeligst ved eksponering for salinitet på 7 promille. Den samme forskjellen ble ikke sett ved de laveste salinitetene i testen (0 og 1 promille). Imidaklopridsensitivitet ble inkludert i overvåkningsprogrammet for andre gang og resultatene viste en naturlig variasjon i imidaklopridsensitivitet.

Summary

702 prescriptions for anti-salmon lice medicines were issued in 2023. This was a slight decrease compared to 2022 (7 percent). 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 77 percent. The use of imidacloprid, which received marked authorization in 2021, was moderate in 2021, 2022 and 2023, with 25, 91 and 51 prescriptions respectively. The level of resistance seen in salmon lice towards most anti-salmon lice medicines, remained high in 2023. The resistance level was generally stable or slightly increasing for all substances tested, but of notice is the reduced numbers of tested farms the two last years. Over the last five years, the use of azamethiphos has been increasing, the emamectin benzoate use has been relatively stable, while the use of pyrethroids and hydrogen peroxide has decreased. 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 from 3145 in 2022 to 2609 in 2023 (17 percent decrease). 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 22 percent of the non-medicinal treatments in 2023 (576 reported treatments-weeks). Fresh water sensitivity has been included in the surveillance program since 2019 and the results were compared with the treatment history of the farms. The results showed lower mortality of lice in the resistance tests at moderate salinities by increasing number of fresh water treatments the two last years. This was most prominent at a salinity of 7 ppt. The same difference was not seen at the lowest test-salinities (0 and 1 ppt). Imidacloprid bioassays were performed as part of the surveillance program for the second time and the results indicate a natural variation in imidacloprid sensitivity.

Introduction

Salmon lice (*Lepeophtheirus salmonis*) is 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 was responsible for the planning, data collection and reporting components of the programme. Due to its 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 the sea trout 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 2022, fresh water bioassays were therefore conducted in 2023, investigating the tolerance levels in salmon lice towards fresh water.

To obtain a base-level of imidacloprid sensitivity and look for results deviating from this base level, imidacloprid bioassays were performed along most of the Norwegian coast in 2023, as it also was 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. The surveillance program for resistance in salmon lice will not continue in 2024.

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 with the farms' fresh water treatment history.

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 their joint selection pressure towards resistance. The six categories were azamethiphos, pyrethroids (cypermethrin and deltamethrin), emamectin benzoate, hydrogen peroxide, flubenzurones (diflubenzuron and teflubenzuron) and imidacloprid. VetReg data were downloaded 15.03.2024. VetReg contains data reported to the Norwegian Food Safety Authority (NFSA) of all medicines used for farmed fish. Pharmacies report their sales to fish farms and fish health personnel (FHP), and the FHP report their own use of medicines.

All records for medicines against salmon lice were initially selected from VetReg. Thereafter, records for pharmacies sales to FHP and FHP reporting use were investigated. No records were found of FHP buying the medicines they thereafter reported used. The record of FHP reporting use were therefore believed to be double reporting of prescriptions (pharmacies should be the source of this information when the medicines are delivered to a fish farm) and therefore excluded. The reports of pharmacies sales to FHP were also excluded as FHP's reported use should be the source of this use data (no reports of FHP first buying and then using were found). Thereafter all records of the same substance category for the same fish farm issued on the same date were only accounted for once. Multiple reports of a prescription of the same medicine to the same fish farm at the same time were merged, as this probably was due to different package sizes being included to fill the prescriptions. Records without farm identification were included. This will most likely lead to a slight overestimation of the number of treatments as some of these are most likely also records that would have been excluded had the information on fish farm been known.

Prescriptions 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 may 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.

Active farms, defined as farms that during a year 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 02.02.2024).

Non-medicinal treatments
The number of non-medicinal treatments performed in Norwegian salmon farms, was extracted 16.01.2024 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, whilst 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 or from a drop-down menu in the reporting form. The template's segment for reporting method was changed from free-text to drop-down menu during 2023.

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
Five fish health services along the Norwegian coast were engaged in 2023, 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 (8, 9) and was also used for the previous years of the surveillance programme (2013-2022). 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 area. Norway's 13 production areas are given by regulation (10) and shown in Figure 1 (numbered 1 to 13 from south to north).

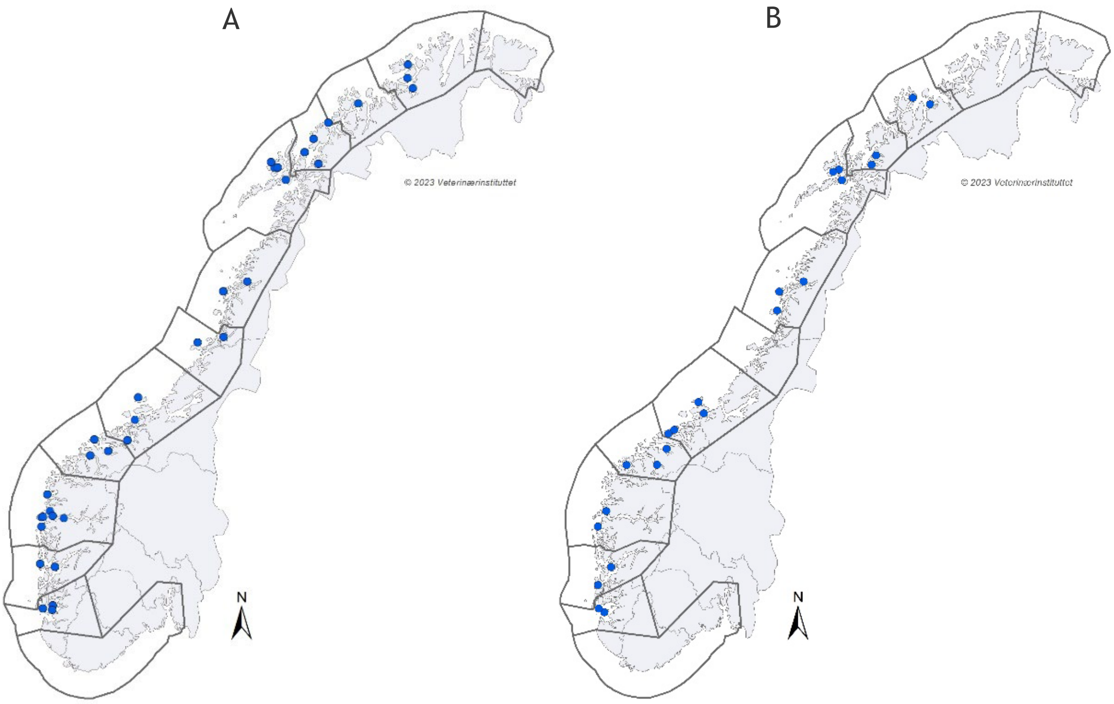


Figure 1*: Locations of farms where salmon lice were collected for bioassays against medicines in 2023 (blue dots). The black lines subdivides Norway into 13 production areas. A show the bioassays against deltamethrin, emamectin benzoate, azamethiphos and hydrogen peroxide, while B show the location of the imidacloprid bioassays.*

L. salmonis from 34 sites were subjected to bioassays. Between 20 and 32 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 11 and 51 lice were used per group). When less than 10 lice were used per group the results were excluded (none were excluded in 2023). 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 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 (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

Fresh water bioassays

The salinity bioassay protocol was based on Andrews and Horsberg 2020 (11). The locations were chosen by the fish health services themselves, inside a given production area. All were asked to aim for conducting bioassays in farms that had previously conducted fresh water treatments and if such farms were unavailable, to choose other farms.

L. salmonis from 23 farms were tested: one from production area 11, two from production area 3, 8, 9 and 10, three from production area 2 and 5, and four from production area 6. 10 of the farms had not performed fresh water treatments in 2022 or 2023, six of the farms had treated once, five of the farms had treated twice, one of the farms had treated four times and one had treated six times. Treatments were counted from the weekly reports to the NFSA; fresh water treatment alone or in combination with other methods were included. Consecutive weeks were regarded as one treatment. The bioassays were performed by exposing live motile salmon lice, removed from the fish, for water of six different salinities: 0, 1, 3, 5, 7 and 20 parts per thousand (ppt). 20 ppt was the control-group. 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 (R-package lme4 (12)) to see if there were differences in salinity tolerance between lice from farms dependent of the number of fresh water bath treatments the previous two years and the sea water salinity at the farm the test-day. Predictions from the model were made using R-package sjPlot (13) and displayed using ggplot2 (14). A separate analysis was performed to investigate the same research question combining all data (2019-2023), where year was added to the analysis as a possible explanatory variable. Data from farms where the control group (salinity: 20 ‰) mortality exceeded 20 percent were excluded from the analysis, as these lice may have died from other causes than exposure to low salinity. Data from one farm in production area 6 and one in production area 10 were excluded.

Dose-response curves were modelled for all years (2019-2023) 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 (15). Data from farms where the control group (salinity: 20 ‰) mortality exceeded 20 percent were excluded from the analysis.

Imidacloprid bioassays

The imidacloprid bioassay protocol was based on a protocol developed by Aaen and Horsberg (16). 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 area. They were asked to choose farms that had treated with imidacloprid if possible. Figure 1B shows the location of all imidacloprid bioassays.

L. salmonis from 23 farms were tested: two assays from production area 2, 3, 4, 10 and 11, three from production area 5, 8 and 9 and four from production area 6. 15 of the farms had not treated with imidacloprid in 2022 or 2023, six had treated once and two had treated twice. Of note is that some of the imidacloprid reports to VetReg was without fish farm given (39 in 2022, none in 2021 and 2023). These treatments could therefore not be associated with any bioassay result. Treatments were counted as number of prescriptions issued for the relevant farm. The bioassays were performed by exposing live motile salmon lice, 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 concentration immobilising 50 percent of the lice (LC50-values) were calculated, using a two parameter log-logistic model from the package drc in R (15). Data from all farms were included.

Results and Discussion

Passive surveillance

Number of prescriptions
Table 2 summarizes the number of prescriptions covering each substance or class of substances over the years 2013 – 2023. A pronounced increase 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 in 2023 compared to 2022. Emamectin benzoate was the most commonly prescribed medicine. The number of imidacloprid-prescriptions for 2022 must be considered with some care as information on fish farm was not provided for 39 records and possible duplicates could therefore not be identified for these entries.

Table 2: Number of prescriptions for the given substances/class of substances applied to control salmon lice in 2013 to 2023. The number of prescriptions was collected from VetReg 15.03.24. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.

Substance category	2013[1]	2014[1]	2015[1]	2016[1]	2017[1]	2018[1]	2019[1]	2020[1]	2021[1]	2022[1]	2023[1]
Azamethiphos	478	732	612	261	59	38	72	111	141	232	278
Pyrethroids	1115	1023	645	274	80	53	62	50	40	29	18
Emamectin benzoate	150	431	468	523	322	297	368	396	380	344	313
Flubenzurones	158	168	180	156	74	32	47	47	20	23	26
Hydrogen peroxide	236	826	1025	567	203	94	76	46	45	35	16
Imidacloprid	-	-	-	-	-	-	-	-	25	91[2]	51
Total	**2137**	**3180**	**2930**	**1781**	**738**	**514**	**625**	**650**	**651**	**754**	**702**

[1]Changes compared to previous reports come from updated historical data, thereby including more late-coming reports, and improved method to detect duplicates.
[2]For 39 of these prescriptions, possible duplicates could not be identified.

Prescriptions per farm
Prescriptions were issued for 430 farms in 2023 with a mean number of 1.6 prescriptions per farm. This was almost identical to 2022 with 422 farms, with a mean number of 1.6 prescriptions per farm. The annual number of active farms has varied from 817 to 849 during the years 2018 to 2023.

Azamethiphos and emamectin benzoate use was spread along most of the coast. The most frequent use of pyrethroids was seen in production area 10. The most frequent use of flubenzurones was found in production area 4, while the most frequent hydrogen peroxide usage was seen in production area 6. Imidacloprid was also most frequently used in production area 6 (Figure 2).

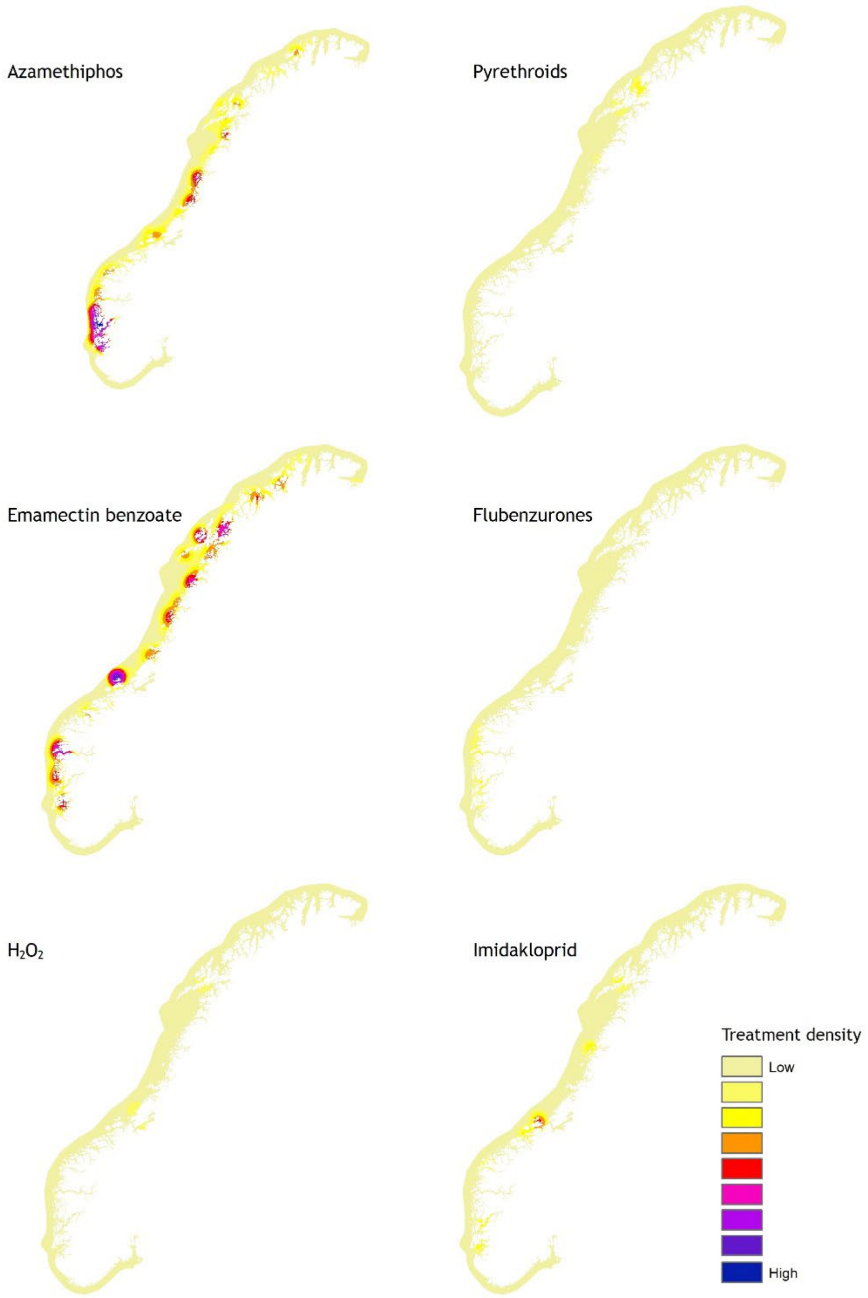


***Figure 2**: Geographical distribution of the density of prescriptions per farm for six different substances or classes of substances, used to control salmon lice infestations in salmonid farms in 2023. Note that the kernel densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments. Blue indicates relatively high intensities, whilst 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 by 17 percent from 2022 to 2023. This discontinued a yearly trend of substantial yearly increases in the number of treatments from 2016 to 2022, with an exception of 2021. A total of 599 farms performed non-medicinal treatments in 2023. The 599 farms in 2023 reported between 1 and 26 treatment weeks, with an average of 4.4 weeks. Of the non-medicinal treatments in 2023, 45 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 (17). The frequent use of thermal delousing imposes a selection pressure, favouring lice that can survive warm water treatments. This selection pressure was inflicted on a large geographic area in 2023, but the use was most frequent in production areas 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 infested wild sea trout (5). Of note in 2023 is also the increase in the use of a combination of methods in the same week. This concerned 11 percent of the weeks in 2022 and 17 percent in 2023.

***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 2023. 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 18.02.2024.*

Treatment category	2015	2016	2017	2018	2019	2020	2021	2022	2023
Thermal	36	685	1245	1327	1447	1723	1456	1357	888
Mechanical	34	311	236	423	674	823	862	1074	980
Fresh water	28	73	75	84	148	220	286	225	186
Thermal + Mech	0	12	42	35	56	59	30	47	59
Thermal + Fresh water	0	16	21	17	27	20	63	141	227
Mech + Fresh water	0	7	1	7	7	24	56	153	151
Thermal + Mech + Fresh water	0	0	0	1	0	1	5	9	12
Other	103	75	52	69	87	92	72	139	106
Total	**201**	**1179**	**1672**	**1963**	**2446**	**2962**	**2830**	**3145**	**2609**

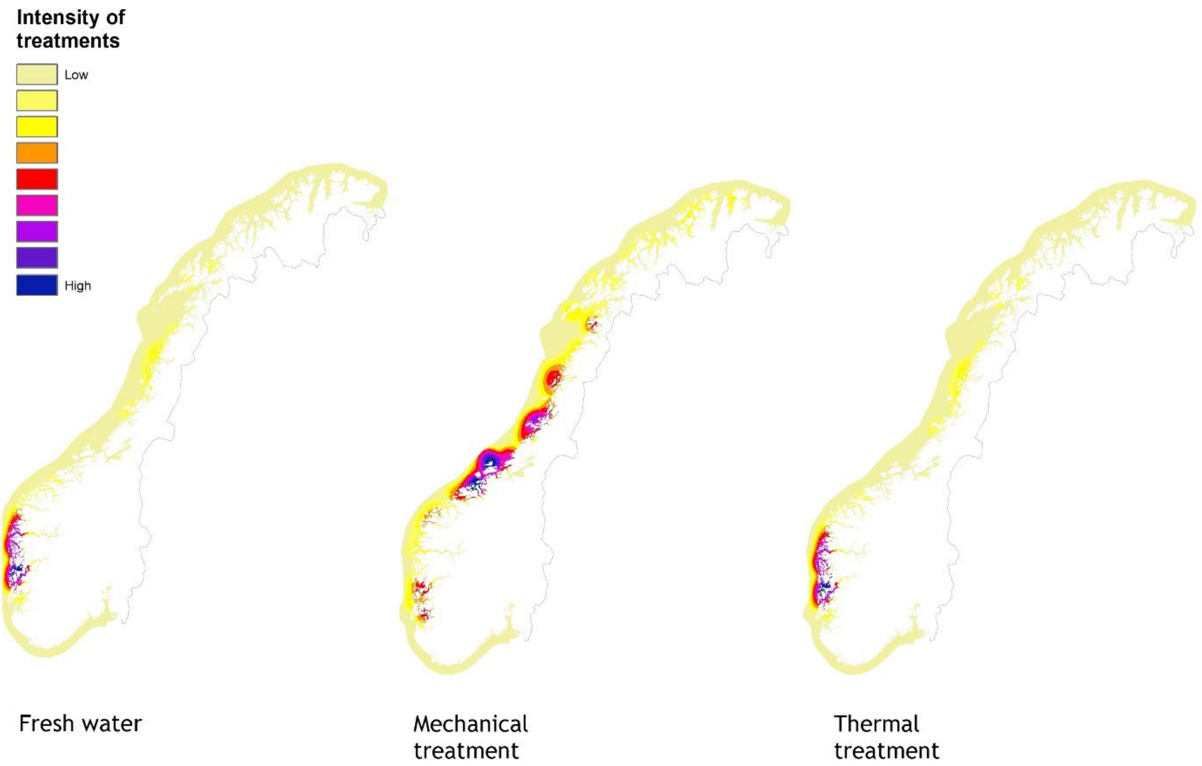


Figure 3*: The intensity (kernel density) of non-medicinal treatments used against salmon lice in salmon farms in 2023. Treatments are categorized into bath treatment in fresh water, mechanical delousing and thermal delousing. Combination treatments are included in both/all respective categories. Treatment 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, 99 bioassays were performed on salmon lice from 34 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	24	1	8	12	3	0
Deltamethrin	23	11	7	3	1	1
Emamectin benzoate	32	25	3	1	3	0
Hydrogen peroxide	20	1	4	7	8	0
High concentration						
Azamethiphos	24	0	4	16	3	1
Deltamethrin	23	2	2	7	10	2
Emamectin benzoate	32	7	3	11	6	5
Hydrogen peroxide	20	0	0	2	3	15

Table 5 shows the correlation between salmon lice mortality results from low and high concentrations, which were significantly correlated for azamethiphos and deltamethrin. The reduction in number of tests to approximately half the tests performed prior to 2022 contributes to the differences between substances 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 Coefficient
Azamethiphos	24	0.56
Deltamethrin	23	0.54
Emamectin benzoate	32	0.14
Hydrogen peroxide	20	0.15

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 intermediate in high concentration azamethiphos bioassays (Figure 4B), indicating that low treatment efficacy, for treatments according to the SPC, 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 two farms and 10 farms with between 60 and 80 percent mortality.

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 lower 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 a relatively stable or slightly increasing resistance level in 2023 for all substances tested, compared to the last couple of years. Of note is however the reduced number of bioassays included in the 2022- and 2023-programs compared to previous years, which adds uncertainty to the conclusions.

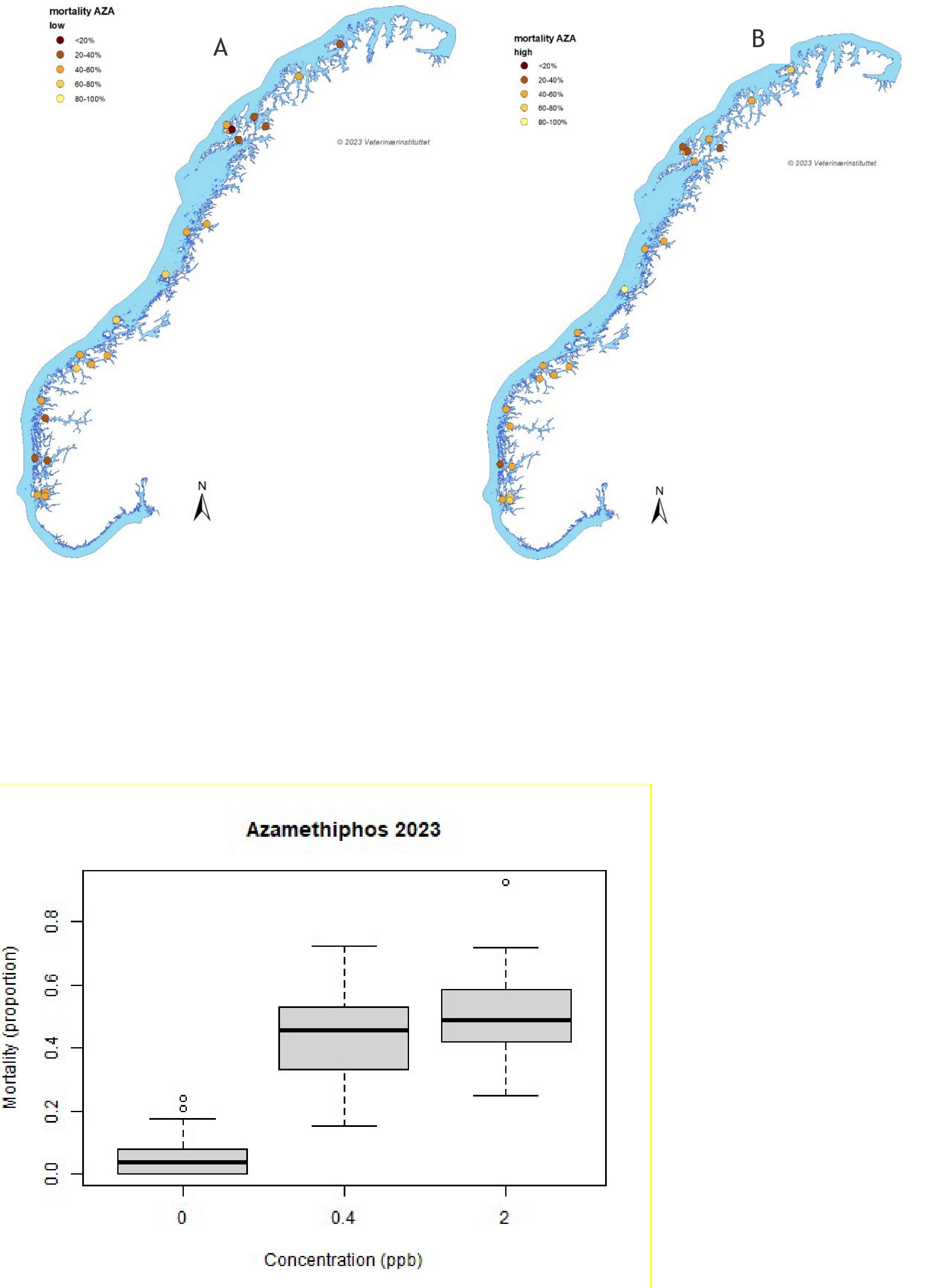


Figure 4: *Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) azamethiphos concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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

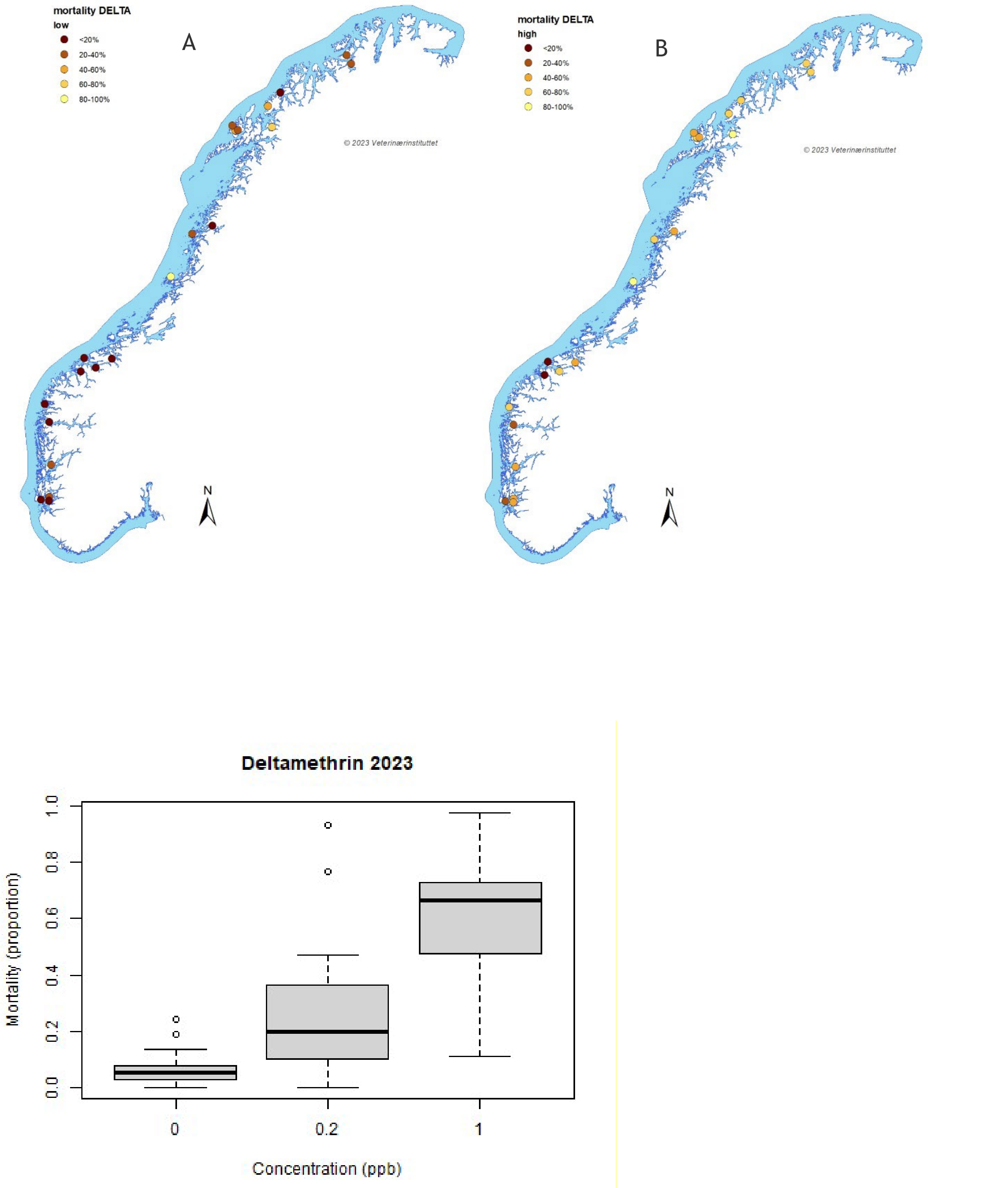
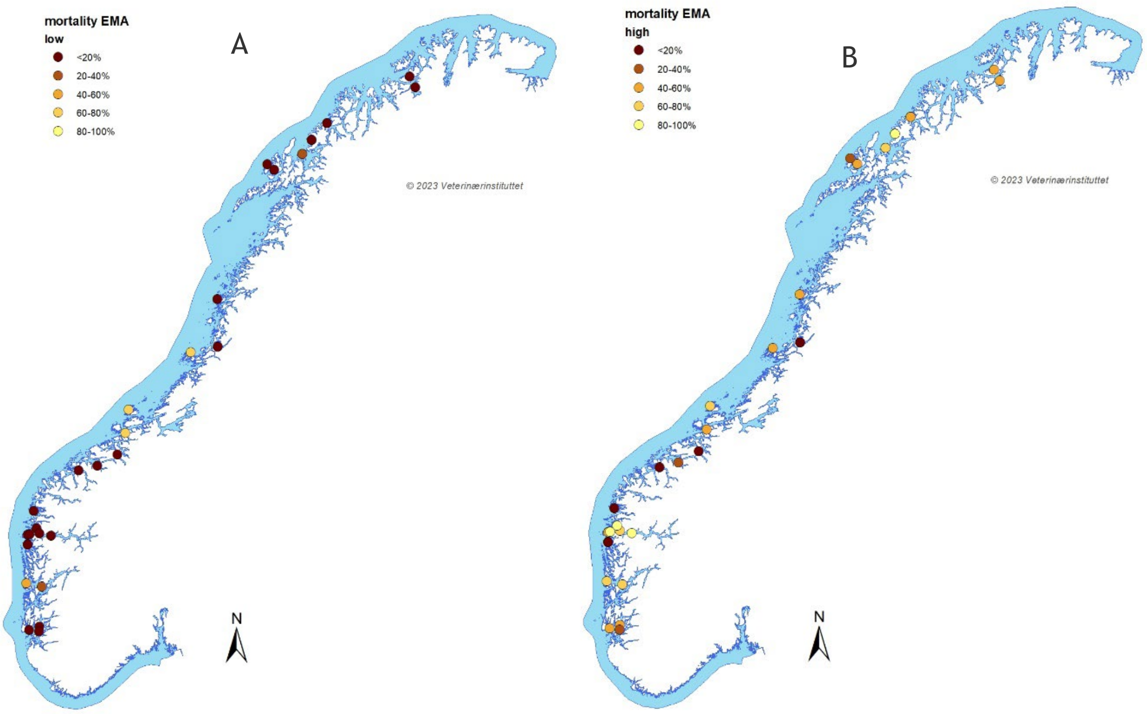


Figure 5: *Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) deltamethrin concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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



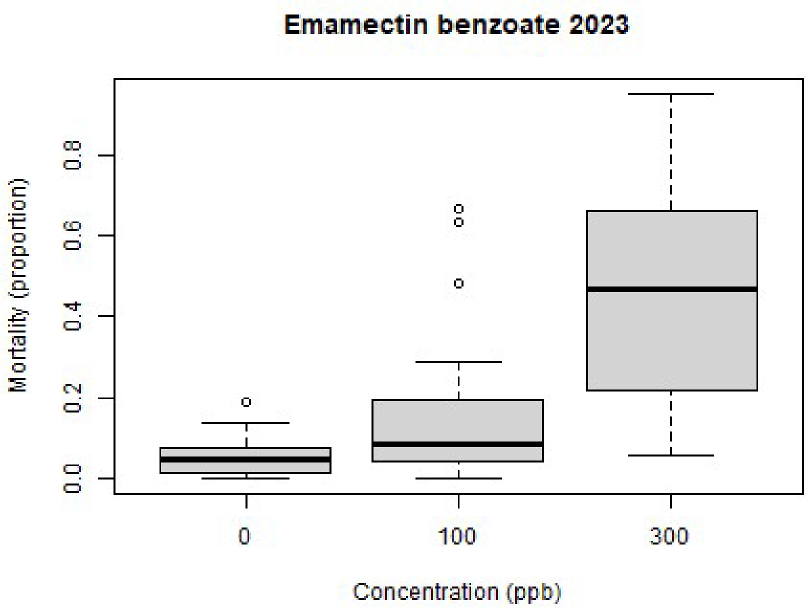
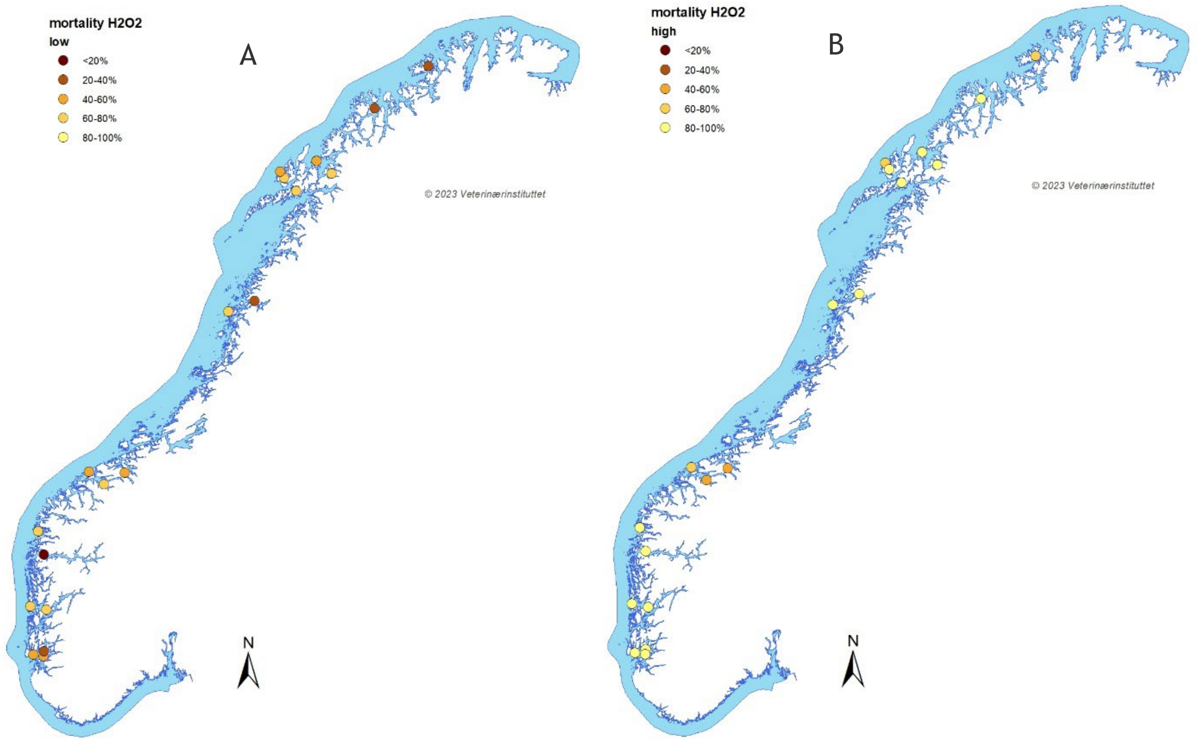


Figure 6: *Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) emamectin benzoate concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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



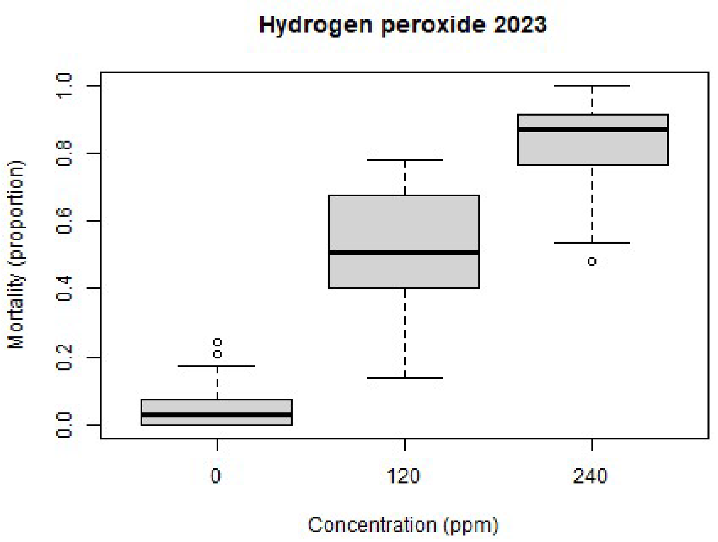


Figure 7*: Maps showing proportional mortalities of salmon lice in bioassays with low (A) and high (B) hydrogen peroxide concentrations. The colours of the dots indicate different levels of mortality. The darkest colours are indicative of the lowest mortality. The boxplot shows the distribution of mortalities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

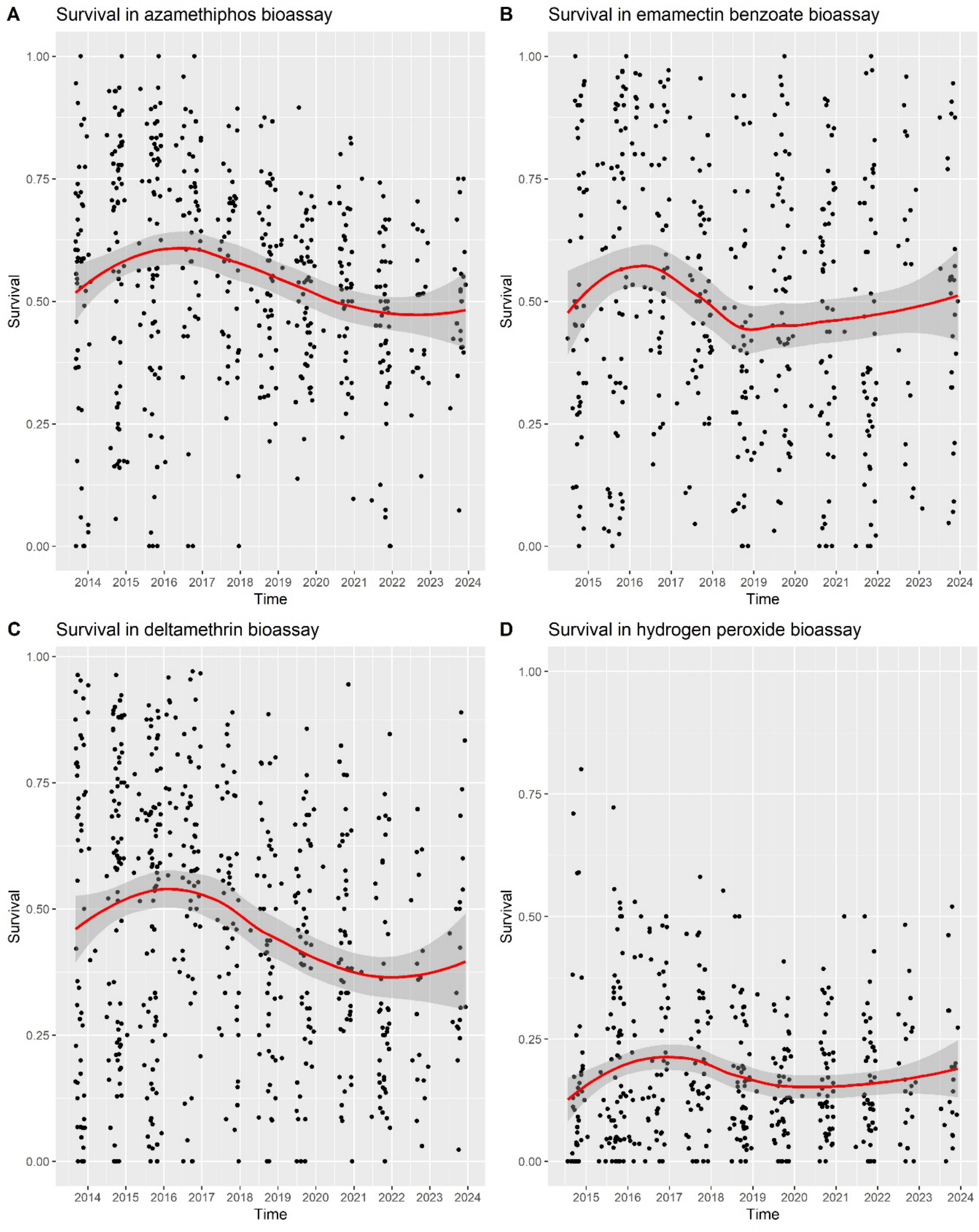


***Figure 8**: All bioassay results from exposure to azamethiphos (A), emamectin benzoate (B), deltamethrin (C) and hydrogen peroxide (D) displayed as proportion of survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances and that 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 2023, the mortality in the bioassays did not differ significantly (significance level set to be P=0.05) between farms dependent of their treatment history of fresh water bath treatment in 2022 and 2023. The bioassay mortality did neither differ significantly dependent of the sea-water salinity at the farm. There were however significant interactions between the effects of number of treatments and bioassay salinity and between the effects of salinity at the farm level and bioassay salinity. Specifically, the results indicated that lice tolerated moderate reductions in salinity better the more often the farm had treated over the last two years, whereas mortality was similarly high at the lowest salinities. The difference in mortality was most prominent at a salinity of 7 ppt and not seen at 0, 1 and 20 ppt (Figure 9). Further, the results indicated that lice tolerated moderate reductions in salinity better the lower the salinity level at the farm on the test date was. This was not the case for the highest and lowest bioassay salinities (Figure 10). Figure 9 shows the predicted dose-response curves based on number of treatments while Figure 10 shows the same based on farm salinity; both based on the results from the logistic regression.

This result is similar to what was seen in the 2022-data, except for the additional interaction effect between salinity at the farm level and bioassay salinity on mortality. The same effects were seen in a separate analysis, where all data from all years (2019-2023) were combined. The results indicated a higher mortality at moderate salinities for the most recent years. There was no trend towards lower mortality over time.

From the modelled dose-response curves for fresh water bioassays for all years (2019-2023), the concentration immobilising 50 percent of the lice (LC50) were calculated per farm. The median LC50-value was 2.58 ppt salinity and the 10^{th} and 90^{th} percentile LC50-values were 0.61 and 5.58 ppt respectively. In the study by Andrews and Horsberg (12) LC50 was calculated to be 2.8 ppt for pre adult II. The lowest calculated LC50 value from our data was 0.0019 ppt; based on results from a farm tested in 2023. There was no survival in the 0 ppt (fresh water) group in this test. Ideally this farm should be retested in order to see if the result is repeated.

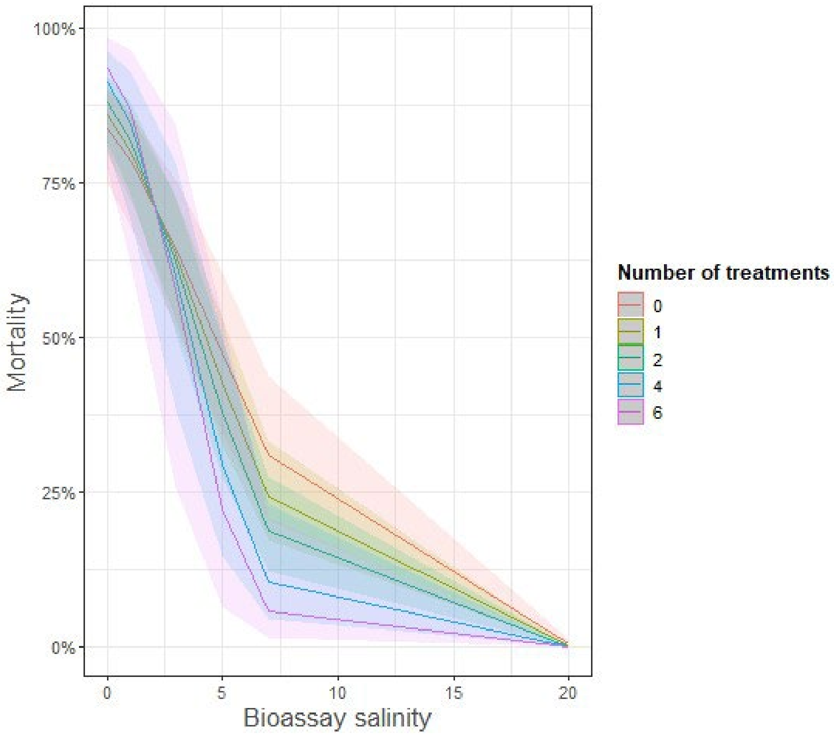


Figure 9*: Predicted results from a regression analysis of the effect of number of fresh water treatments on the mortality in fresh water bioassays in 2023. The figure shows the significant interaction effect of number of treatments and bioassay salinity (in ppt). The various coloured lines represent predicted dose-response curves from a bioassay from various numbers of treatments at farm level performed in 2022 and 2023. The lighter coloured area around each line is the 95 percent confidence interval for the lines.*

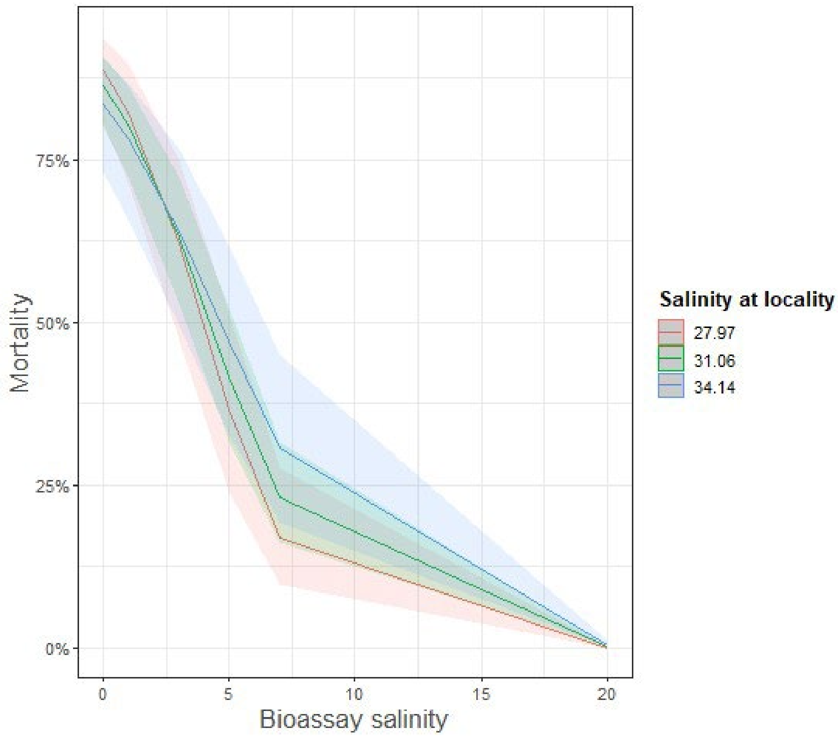


***Figure 10**: Predicted results from a regression analysis of the effect of farm sea water salinity on the mortality in fresh water bioassays in 2023. The figure shows the significant interaction effect of farm salinity and bioassay salinity (in ppt). The three coloured lines represent predicted dose-response curves from a bioassay from three different farm salinities. 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 10th and 90th percentile LC50-values were 0.007 and 0.14 respectively. The corresponding figures in 2022 were 0.06, 0.03 and 0.14 mg/L. The figures from 2023 are also similar to what was previously observed in one salmon lice strain by Aaen and Horsberg (16); LC50 (90 % CI) was 0.098 mg/L (0.074-0.149). Imidacloprid has been used for salmon lice treatments for only 2.5 years and good treatment efficacies have been observed (18). The variation in LC50-values are therefore most likely reflecting natural variation in salmon lice imidacloprid susceptibility. There were no extreme LC50-values in this dataset (maximum value 0.20 mg/L).

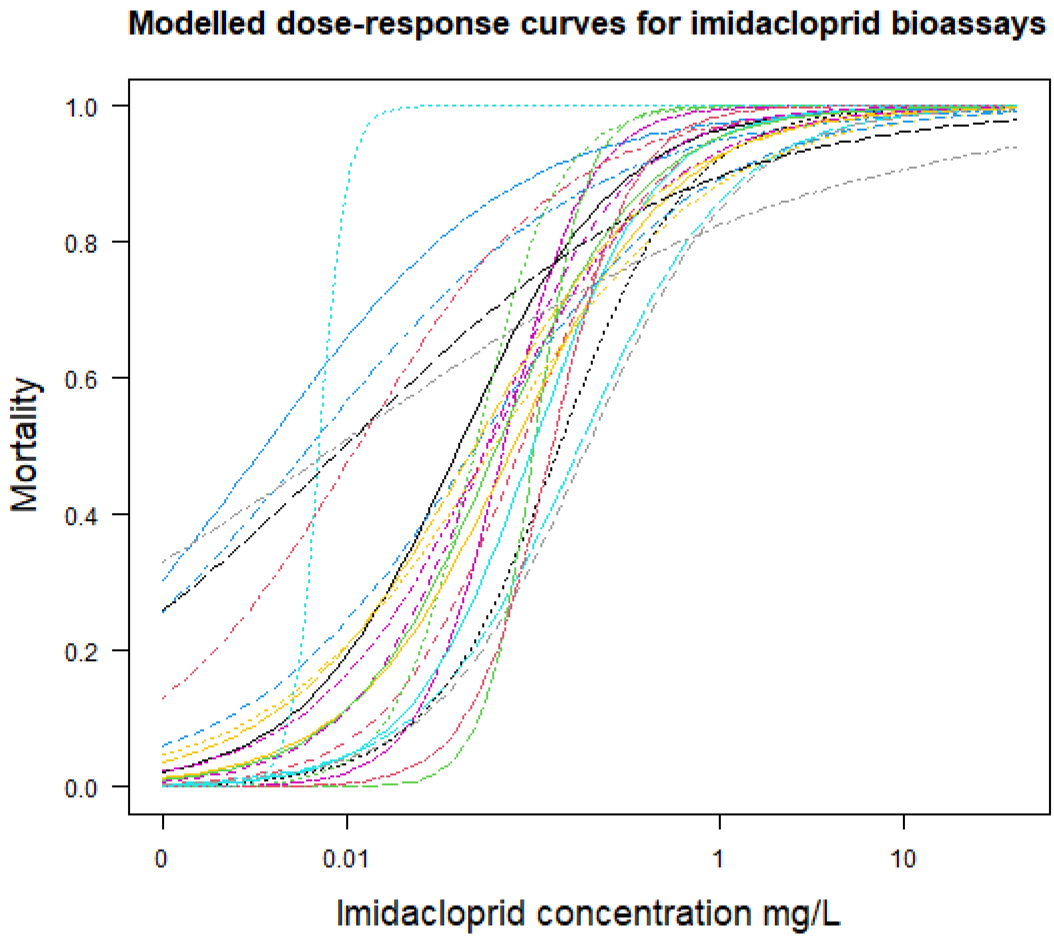


Figure 10: *Modelled dose-response curves from the imidacloprid bioassays. Each line represent one bioassay.*

Conclusion

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2023 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 compounds, but reduced hydrogen peroxide sensitivity was indicated in some areas. The results for all years of the surveillance program compiled indicate a stable or slightly increased resistance towards the four tested substances over the last couple of years. In the paper by Aldrin et al (18) they found low (26 to 50 percent in average) effectiveness from treatments with azamethiphos, deltamethrin and emamectin benzoate. The average effectiveness of hydrogen peroxide treatments was 74 percent.

The relatively similar resistance development despite variations in the utilisation between the different compounds over the years, is somewhat unexpected. This finding indicates that for the low-use compounds (pyrethroids and hydrogen peroxide), resistance is not a very costly trait for the lice. For emamectin benzoate, the use has been relatively high over years, while azamethiphos use has increased in the latest years. Off-label use, in the form of higher dosage or longer treatment time is common for emamectin benzoate treatments and prolonged holding time is used for azamethiphos treatments (19, 20). The latter is difficult to know the extent of, since holding time is not reported in public records. Off-label use is most likely chosen to overpower reduced efficacy due to resistance. For azamethiphos, resistance seems not to have increased in the period 2021-2023 in spite of increased use of the compound. If this is the case, salmon lice might need an additional resistance mechanism in order to better survive treatments. Azamethiphos treatment effectiveness from the study by Aldrin et al (18) did not show any signs of overpowering resistance, but the holding time of the treatments included in that study is not known. Practical application of the medication, such as concentration and treatment time, that have an impact on resistance development should ideally be reported. This may also be important knowledge for other reasons, such as fish welfare and environmental impact.

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 (21). 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 found was still at a presumably high level. This probably, as in 2022, describes part of the natural variation in imidacloprid sensitivity and can be used as a reference in future imidacloprid resistance surveillance. A potential future resistance development towards imidacloprid is expected to happen by the introduction or selection of an inheritable trait that gives the lice higher tolerance towards imidacloprid than what was seen in the 2022 and 2023-surveillance program. The use of imidacloprid remained at a moderate level in 2023 (51 prescriptions). 18 of these were however issued for farms in one production 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 from 3145 in 2022 to 2609 in 2023 (17 percent decrease). This discontinued a trend of yearly increases, ongoing since 2016, with an exception of 2021. In 2022, 599 farms reported the use of non-medicinal methods, while 430 farms had medicines against salmon lice prescribed for them. Thermal delousing was the dominating method with 45 percent of the non-medicinal treatments (alone or in combination). This percentage has been decreasing since 2018. It is however still frequent (1186 weeks of treatment reported), and frequent treatment with a specific 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; 17 percent of the weeks in 2023 compared to 11 percent in 2022 and five percent in 2021.

Fresh water bath treatments were used more often in 2023 compared to 2022. In 2023 fresh water was used alone or in combination with other non-medicinal methods in 576 weeks, which is 22 percent of the reported weeks of treatment. To the survey among fish-health personnel for the Fish health report, five (of 102) reported incidences of unexpected low treatment efficacies of fresh water treatment (22). In 2023 salinity tolerance in bioassays were compared to the fresh water treatment history of the farm and sea-water salinity at sampling day, and the results indicated that lice tolerated moderate reductions in salinity better the more often the farm had treated over the last two years. This was most prominent at a salinity of 7 ppt. For all years compiled no development towards higher low-salinity tolerance was seen. The tested lice from one farm in 2023 showed especially high tolerance to low salinities. This should though be interpreted with caution, as bioassays are prone to errors. Such tests with results deviating considerably from the average should be repeated, and preferably small-scale treatment tests should be performed on the lice population. Since wild sea trout use fresh and brackish water for delousing, a development towards low-salinity tolerance would be unwanted, also from a wild fish perspective.

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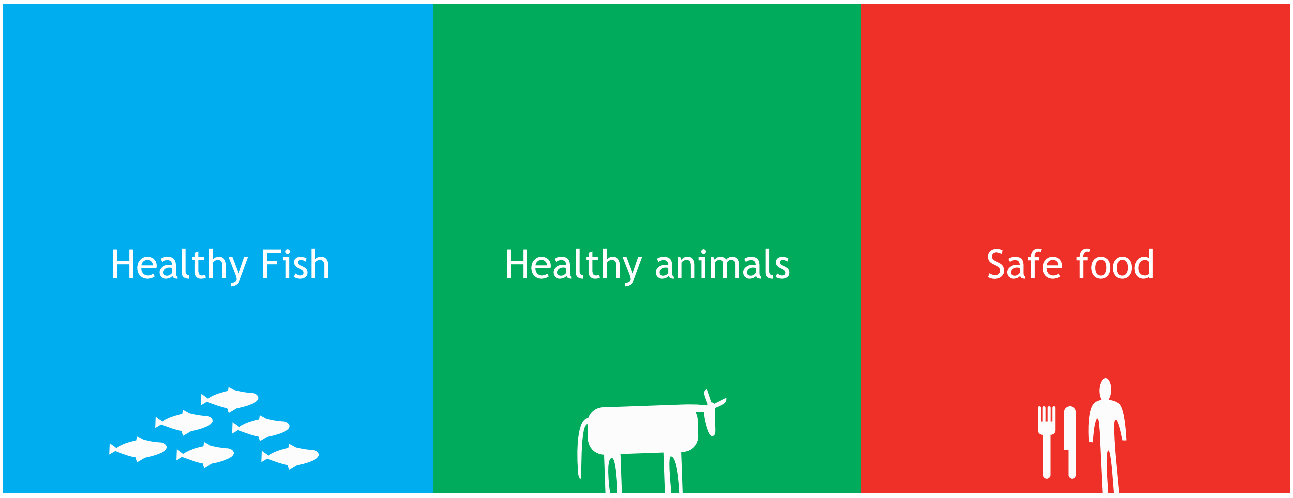

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