The surveillance programme for resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway 2019
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Content

Summary ...................................................................................................................... 3
Introduction .................................................................................................................. 3
Aims ........................................................................................................................... 3
Materials and methods ............................................................................................... 4
Results and Discussion ............................................................................................... 6
Conclusions .................................................................................................................. 18
Acknowledgement ......................................................................................................... 18
References ................................................................................................................... 19

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Summary

The number of medicinal treatments applied against salmon lice increased slightly in 2019 compared to 2018. This discontinued a yearly trend of reduction that started in 2015. The level of resistance seen in salmon lice remained high in 2019. However, the tendency towards a reduced resistance level that started in 2017, continued in 2019 for deltamethrin, azamethiphos and hydrogen peroxide. For emamectin benzoate there was a tendency towards increased resistance in 2019, unlike the previous two years. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were 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 prescriptions for medicines against salmon lice was 698 in 2019, which was a 16 percent increase compared to 2018. The number of non-medicinal treatments increased by 23 percent, to 2462 reported treatments, in the same period. Non-medicinal methods for treatment and prevention were thereby the dominating methods for salmon lice control. Fresh water delousing accounted for 7 percent of the non-medicinal treatments in 2019 (172 reported treatments). A small field study of fresh water sensitivity was performed in the surveillance program in 2019, comparing the sensitivity levels of salmon lice from low and higher fresh water usage areas. No difference in the sensitivity levels between the farms from these two areas was detected.

Introduction

Salmon lice (Lepeophtheirus salmonis) are considered one of the biggest health threats against both farmed and wild salmonids in Norway. Medicinal treatments have traditionally been used to control salmon lice in the fish farms, but the emergence of resistant parasites has reduced the efficacy of these treatments. Resistance towards chemotherapeutants in salmon lice has been reported from several countries, including Norway (1). The reports have been based on reduced treatment efficacy and/or results from toxicological or molecular resistance tests. Reduced sensitivity has been associated with local treatment intensity (2). Results from resistance testing have been used by the industry as a decision making tool in salmon lice management. However, until 2013 there was no comprehensive survey of the resistance status of L. salmonis in any country. To maintain salmon lice control, non-medicinal methods for treatment and prevention have become increasingly more important, to a large degree as a result of the resistance situation.

In order to get an overview of the resistance status of L. salmonis in Norway and the use of chemotherapeutants against salmon lice, The Norwegian Food Safety Authority established a surveillance program in 2013, which has continued since then (3). In the passive surveillance part of the programme, prescriptions for salmon lice treatments are summarised. In the active surveillance part, toxicological or molecular resistance tests are performed on salmon lice from approximately 70 salmon farms located along the Norwegian coast. The Norwegian Veterinary Institute is responsible for the planning, data collection and reporting components of the programme. Due to its current importance for salmon lice control, an overview of the use of non-medicinal treatments against salmon lice is also given.

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

Aims

The surveillance program aims to summarize the use of chemotherapeutants against salmon lice and to describe the resistance status in L. salmonis towards the most important of these chemotherapeutants in Norway. An additional aim in 2019 was to see if fresh water tolerance varied between salmon lice from areas with low and higher use of fresh water bath treatments.
Materials and methods

Passive surveillance

Prescriptions of medicines

Prescriptions of medicines applied for salmon lice treatments, from the Veterinary prescription register (VetReg), were summarised into five different categories. The medicines were subdivided into categories according to their mode of action and therefore most likely joint selection pressure towards resistance. The five categories were azamethiphos, pyrethroids (cypermethrin and deltamethrin), emamectin benzoate, hydrogen peroxide and flubenzurones (diflubenzuron and teflubenzuron). A prescription can be issued for treatments of some or all the fish cages in a farm. Hydrogen peroxide is used against salmon lice infestations, but also against amoebic gill disease (infection with Paramoeba perurans) at a lower concentration. In addition, some of the prescriptions for azamethiphos, pyrethroids, emamectin benzoate or hydrogen peroxide might have been for treatment of fish infested with the sea louse Caligus elongatus. All prescriptions of medicines with salmon lice as a possible indication were however included. This is due to the fact that all these treatments are likely to inflict a selection pressure for resistance in salmon lice due to co-infection L. salmonis/P. perurans or L. salmonis/C. elongatus, regardless of the treatment indication. The extracts from VetReg were performed 13.01.2020.

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 18.02.2020) in addition to VetReg. Farms that during 2019 reported the presence of adult female lice, but had no prescriptions issued for them in that year, were regarded as farms without prescriptions.

Non-medicinal treatments

The number of non-medicinal treatments performed in Norwegian salmon farms was extracted from the weekly mandatory reporting of salmon lice data to the Norwegian Food Safety Authority 18.02.2020. These numbers represent the number of weeks farms have reported the use of such treatments. Non-medicinal treatments include mechanical and thermal delousing, in addition to delousing in fresh water baths. Delousing using water pressure and/or brushing technology were regarded as mechanical while delousing using temperate water was regarded as thermal. The reports do not have data on the number of cages treated per week, and this can vary between one and all cages in a farm. The non-medicinal treatments were subdivided into different method-categories based on information automatically extracted from the free-text fields in the reporting form.

Reported sensitivity data

According to the current regulation on control of salmon lice in Norwegian aquaculture (4), there is mandatory reporting of suspected resistance and results from sensitivity tests. If resistance is suspected, the reason for suspicion is to be reported in one of four categories: results from bioassays, reduced treatment efficacy, the situation in the area, or other reasons. The sensitivity data are to be reported in one of three categories: sensitive, reduced sensitivity, or resistant. Reported sensitivity data have not been summarised for 2019 in this report. This is due to the fact that these data are of limited value. There are farms where medicinal treatments are not applied and these will therefore most likely not report sensitivity data. This is despite the fact that resistance might have led to the absence of medicinal treatments. In addition, there are no objective criteria for the categorisation of the results from the sensitivity tests.

Data processing

Data processing and statistical analyses were performed in the statistical software R (6). Geographical processing and presentation of data was performed using ArcGIS (7).
Active surveillance

Bioassays
Seven fish health services along the Norwegian coast were engaged in 2019 to perform toxicological resistance tests (bioassays) on live parasites against chemical treatment agents. The bioassay protocol was based on Helgesen et al 2013 and 2015 (8, 9) and had also been applied for the previous years of the surveillance programme (2013-2018). 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 (9).

Figure 1. Locations of farms where salmon lice were collected for bioassays in 2019.

*L. salmonis* from a maximum of 79 farms were tested with the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide. 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. 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. The percentage of salmon lice visibly affected by the exposure to the low concentration was used to indicate the sensitivity status of the salmon lice population. Higher than 80 percent was indicative of fully sensitive populations. The percentage affected at high concentration was used to indicate the expected outcome of a subsequent treatment.

Table 1. Concentrations used in the exposed groups in the bioassays, in ppm (mg/l) for hydrogen peroxide and in ppb (µg/l) for deltamethrin, azamethiphos and emamectin benzoate.

<table>
<thead>
<tr>
<th>Substance category</th>
<th>Low concentration</th>
<th>High concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltamethrin</td>
<td>0.2 ppb</td>
<td>1 ppb</td>
</tr>
<tr>
<td>Azamethiphos</td>
<td>0.4 ppb</td>
<td>2 ppb</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>100 ppb</td>
<td>300 ppb</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>120 ppm</td>
<td>240 ppm</td>
</tr>
</tbody>
</table>
Molecular resistance tests
Salmon lice infestation levels in farms in Vest-Agder in the far south of Norway had been low for several years. In order to test lice from this area for resistance, 30 lice were collected from each of three farms. Patogen Analyse AS analysed the genetic characteristics with regard to pyrethroid, azamethiphos and hydrogen peroxide resistance using PCR methodology. Test results were reported according to percentage of lice from each farm categorized as resistant or sensitive to pyrethroids; sensitive, intermediate resistant or resistant to azamethiphos; and as percent expected efficacy of a subsequent treatment for hydrogen peroxide.

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

*L. salmonis* from 15 farms were tested; seven from region 1 and eight from region 2. The bioassays were performed by exposing live parasites of motile stages, removed from the fish, for water of six different salinities; 0, 3, 5, 7, 10 and 20 ‰ (control). After 24-hour exposure, the percentage of salmon lice affected, grouped according to stages and genders, were 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 the two regions. The results were also modelled to find the dose-response curves best fitting the data from each farm. Data from farms where the control group (salinity: 20 ‰) exceeded 20 % were excluded from the analysis (Two farms were excluded).

Results and Discussion

Passive surveillance

Number of prescriptions
Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 - 2019. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years, but this was somewhat decreased in 2015. The decrease was more prominent in 2016 and continued in 2017 and 2018. There were an increase in the number of prescriptions of all substance groups in 2019 except hydrogen peroxide; total increase was 16 percent. Emamectin benzoate was the most commonly prescribed medicine, prescribed more than five 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 2019. The number of prescriptions was collected from VetReg 13.01.20. Pyrethroids include cypermethrin and deltamethrin. Flubenzurones include diflubenzuron and teflubenzuron.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Azamethiphos</td>
<td>418</td>
<td>695</td>
<td>483</td>
<td>752</td>
<td>621</td>
<td>262</td>
<td>59</td>
<td>39</td>
<td>82</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>460</td>
<td>1 163</td>
<td>1 130</td>
<td>1 049</td>
<td>664</td>
<td>280</td>
<td>82</td>
<td>56</td>
<td>73</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>294</td>
<td>169</td>
<td>163</td>
<td>481</td>
<td>523</td>
<td>612</td>
<td>351</td>
<td>371</td>
<td>424</td>
</tr>
<tr>
<td>Flubenzurones</td>
<td>24</td>
<td>133</td>
<td>171</td>
<td>195</td>
<td>202</td>
<td>173</td>
<td>81</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>179</td>
<td>110</td>
<td>255</td>
<td>1 021</td>
<td>1 284</td>
<td>629</td>
<td>214</td>
<td>96</td>
<td>77</td>
</tr>
<tr>
<td>Total</td>
<td>1 375</td>
<td>2 270</td>
<td>2 202</td>
<td>3 498</td>
<td>3 294</td>
<td>1 956</td>
<td>787</td>
<td>602</td>
<td>698</td>
</tr>
</tbody>
</table>
Prescriptions per farm
The maps in Figure 2 sum up the total number of prescriptions per location in the period 2016 - 2019. Prescriptions were issued for 623 farms in 2016 with a mean of 3.1 prescriptions per farm; for 437 farms in 2017 with a mean number of 1.8 prescriptions per farm; for 344 farms in 2018 with a mean number of 1.7 prescriptions per farm; and for 365 farms in 2019 with a mean number of 1.9 prescriptions per farm. The increase in the number of prescriptions from 2018 to 2019 was therefore both caused by an increase in the number of farms which had prescriptions issued for them and an increase in the number of prescriptions per farm.

Figure 2. Geographical distribution of prescriptions at farm level, used to control salmon lice from 2016 to 2019. Dark red denote areas where more than eight prescriptions per location is expected, while dark green denote areas where the expectation of zero treatment is approached. The map layer was generated using the Inverse distance weighted (IDW) function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighboring farm locations).

Azamethiphos was mainly used on the west coast and in the northern part of Norway. Pyrethroids were mostly used in three smaller sub-regions scattered along the coast. Emamectin benzoate was used along most of the coast. The use of flubenzurones was predominantly restricted to the southwest, while most of the usage of hydrogen peroxide took place in western Finnmark, southern Troms, Trøndelag and the southwest coast of Norway (Figure 3).
Figure 3. Geographical distribution of the density of prescriptions for five different substances or classes of substances used to control salmon lice infestations in salmonid farms in 2019. 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 “mechanical treatments” in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority. The number of non-medicinal treatments increased 5.9 times in 2016 compared to 2015. The growth continued in 2017 with 42 percent increase in the number of non-medicinal treatments compared to 2016, further in 2018 with 20 percent increase compared to 2017 and further again in 2019 with a 23 percent increase compared to the previous year. 323 farms performed non-medical treatments in 2016, 417 farms in 2017, 483 farms in 2018 and 563 farms in 2019. 59 percent of the non-medicinal treatments in 2019 were performed using thermal delousing. 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 2019, since the use of thermal delousing was frequent along most of the west coast of Norway, as well as in Trøndelag and parts of Nordland (Figure 4).

Table 3. Number of weeks when farms have reported non-medicinal treatments of salmon lice, in the weekly mandatory salmon lice reports to the Norwegian Food Safety Authority, from 2012 to 2019. The treatments were subdivided into four different categories. “Thermal delousing” summarizes treatments using temperate water and “mechanical removal” summarizes treatments using water pressure or brushes. “Fresh water” is fresh water bath treatments. 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 18.02.20.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>36</td>
<td>685</td>
<td>1 247</td>
<td>1 355</td>
<td>1 461</td>
</tr>
<tr>
<td>Mechanical removal</td>
<td>4</td>
<td>2</td>
<td>38</td>
<td>34</td>
<td>331</td>
<td>279</td>
<td>471</td>
<td>738</td>
</tr>
<tr>
<td>Fresh water</td>
<td>0</td>
<td>1</td>
<td>28</td>
<td>88</td>
<td>96</td>
<td>104</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>132</td>
<td>108</td>
<td>136</td>
<td>103</td>
<td>75</td>
<td>51</td>
<td>72</td>
<td>89</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>111</td>
<td>178</td>
<td>201</td>
<td>1 179</td>
<td>1 673</td>
<td>2 002</td>
<td>2 462</td>
</tr>
</tbody>
</table>

Active surveillance
Altogether, 224 bioassays were performed on salmon lice from 79 different salmon farms along the coast (Figure 1). Of these, 57 farms were tested using azamethiphos, 59 farms using deltamethrin, 56 farms using emamectin benzoate and 52 farms using hydrogen peroxide (Table 4).

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

Table 4. Number of bioassays with the two concentrations applied (low and high), subdivided by the test outcome (percent mortality among the included salmon lice).

<table>
<thead>
<tr>
<th>Substance category</th>
<th>Number of tests</th>
<th>Percent mortality</th>
<th>Percent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-20 %</td>
<td>20-40 %</td>
</tr>
<tr>
<td><strong>Low concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azamethiphos</td>
<td>57</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>59</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>56</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>52</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>High concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azamethiphos</td>
<td>57</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>59</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>56</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5 shows that the salmon lice mortality results from low and high concentrations are significantly correlated, with a possible exception of azamethiphos (p-value = 0.06). 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).

<table>
<thead>
<tr>
<th>Substance category</th>
<th>N</th>
<th>Spearman Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azamethiphos</td>
<td>57</td>
<td>0.25</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>59</td>
<td>0.34</td>
</tr>
<tr>
<td>Emamectin benzoate</td>
<td>56</td>
<td>0.66</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>52</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Bioassay results are shown geographically and distributions of proportional mortality are given in box plots for azamethiphos (Figure 5), deltamethrin (Figure 6), emamectin benzoate (Figure 7) and hydrogen peroxide (Figure 8).

Low salmon lice mortalities in high concentration azamethiphos bioassays (Figure 5A), indicating that low treatment efficacy may be expected, were generally widespread. However, there were some variations in mortality between the different farms (Figure 5).
The low mortality in the low concentration deltamethrin bioassays (Figure 6B) indicates that reduced sensitivity to deltamethrin is widespread along the coast. Only two farms showed test mortalities exceeding 80 percent. In general, the results from the high concentration deltamethrin bioassays (Figure 6A) indicate that farms in most areas tested may expect low treatment efficacy, although ten farms showed test mortalities exceeding 80 percent at this concentration.

The low concentration emamectin benzoate bioassays showed that reduced sensitivity is widespread along the coast (Figure 7B). The high concentration emamectin benzoate bioassays (Figure 7A) additionally showed that reduced treatment efficacy could be expected along most of the coast.

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

The molecular tests of lice from the three farms in Vest-Agder revealed an average of 59 percent pyrethroid resistant lice, which was a decrease compared to 2018 (Table 6). These figure follows a history of increase in the presence of resistant lice from 2016 to 2017 (33-40 percent to 81 percent) (13, 14), and then a slight reduction to 70 percent resistant lice in 2018. The effect on the resistance level from the deltamethrin treatments performed in Vest-Agder in the autumn of 2016, after several years without medicinal treatments in this area, was thereby still seen in 2019, although reduced from 2017 and 2018. There was no further increase in organophosphate resistance levels, from 30-40 percent in 2016, to 50 percent in 2017, 66 percent in 2018 and 62 percent in 2019. An increase was not expected as treatments with organophosphates were performed in 2018, but not in 2019. The tests were not performed on lice from the same farms each year.

Table 6. Results from molecular resistance test from three farms in Vest-Agder. The resistance levels are given as mean percentage of parasites categorized as sensitive or resistant towards pyrethroids or sensitive, intermediate resistant or resistant towards organophosphates.

<table>
<thead>
<tr>
<th>Substance category</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>34 %</td>
<td>40 %</td>
<td>48 %</td>
</tr>
<tr>
<td>Resistant</td>
<td>66 %</td>
<td>60 %</td>
<td>52 %</td>
</tr>
<tr>
<td>Organophosphates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>39 %</td>
<td>43 %</td>
<td>32 %</td>
</tr>
<tr>
<td>Intermediary</td>
<td>50 %</td>
<td>43 %</td>
<td>41 %</td>
</tr>
<tr>
<td>Resistant</td>
<td>11 %</td>
<td>13 %</td>
<td>27 %</td>
</tr>
</tbody>
</table>

Figure 9 display all high dose bioassay results for the four substances applied. The results indicate a reduction in resistance for all substances, except emamectin benzoate. This reduction is indicated to have started in 2017 for azamethiphos, deltamethrin and emamectin benzoate, and in 2018 for hydrogen peroxide. In 2019 a new increase in the resistance level was seen for emamectin benzoate compared to the previous year.
Figure 5. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) azamethiphos concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of azamethiphos (0, 0.4 and 2 ppb) (note that the control experiment is the same for the four substances tested).
Figure 6. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) deltamethrin concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of deltamethrin (0, 0.2 and 1 ppb) (note that the control experiment is the same for the four substances tested).
Figure 7. Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) emamectin benzoate concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of emamectin benzoate (0, 100 and 300 ppb) (note that the control experiment is the same for the four substances tested).
Figure 8: Maps showing proportional mortalities of salmon lice in bioassays with high (A) and low (B) hydrogen peroxide concentrations. The colors of the dots indicate different levels of mortality. The darkest colors are indicative of the lowest mortality. The boxplot shows the distribution of mortalities at three concentrations of hydrogen peroxide (0, 120 and 240 ppm) (note that the control experiment is the same for the four substances tested).
Figure 9. All bioassay results from exposure to azamethiphos (A), emamectin benzoate (B), deltamethrin (C) and hydrogen peroxide (D) displayed as percent survival per high dose assay. Note that comparable results are not available for the exact same period for all four substances. The red line is the spline best fitting the data and the dark grey area is the 95 percent confidence interval for the spline.
Fresh water bioassays
In the logistic regression of the fresh water bioassay results, the mortality at the different salinities did not differ significantly between low or higher usage (of fresh water bath treatment) areas in Norway. Figure 10 display the modelled results from all bioassays except the two assays where control group mortality exceeded 20 percent. Farms C-G are from a low use area of fresh water bath treatments, while farms H-0 are from a higher use area. The modelled curve from farm H is deviating from the rest of the farms. The results from this farm did however not show constantly increasing mortality by decreasing salinity, and the curve can therefore not represent the data perfectly.

The fresh water bioassay survey conducted in 2019 did not show signs of fresh water tolerant salmon lice. The somewhat varying dose-response curves are therefore believed to represent the normal variation in fresh water tolerance in salmon lice. The presence of increased fresh water tolerance in Norway can however not be excluded based on this survey for two main reasons: 1) The protocol’s ability to separate between tolerant and sensitive lice has not been documented in laboratory studies, as known tolerant lice have not yet been identified. 2) The 15 farms included in this study was a limited number of farms and none of the farms were chosen based on suspicion of increased tolerance.

![Modelled dose-response curves for fresh water bioassays](image)

Figure 10. The best fitted dose-response curves are modelled for all fresh water bioassays. Farm A and B were excluded due to control-group mortality exceeding 20 percent. Farms C-G are from a low use area for fresh water treatments, while farms H-0 are from a higher use area.
Conclusions

Results obtained in this surveillance program show that the level of resistance in salmon lice remained high in 2019. Resistance towards deltamethrin, azamethiphos and emamectin benzoate were 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. However, the results for all years of the surveillance program compiled indicate a somewhat reduced resistance level towards all tested substances, except for emamectin benzoate. The reduction was probably caused by the massive reduction in the number of medicinal treatments against salmon lice, which started in 2015. For emamectin benzoate, there was an increase of 14 percent in the number of prescriptions between 2018 and 2019. The level of resistance also increased in this period.

The number of prescriptions of medicines against salmon lice was increased by 16 percent from 2018 to 2019. Compared to 2014, when the number of prescriptions peaked, the number was reduced by 80 percent. This reduction was most likely mainly caused by resistance. When resistance towards a medicine is present, the medicine is not prescribed due to expected low treatment efficacies. Another reason for the decrease in the number of prescriptions is the increased availability of non-medicinal treatments options. The reduction in prescriptions since 2014 was valid for all substances/categories of substances, but to a much lesser extent for emamectin benzoate.

The number of non-medicinal treatments increased with 23 percent compared to 2018. In 2019 563 farms reported the use of non-medicinal methods, while 365 farms had medicines against salmon lice prescribed for them. Thermal delousing was the dominating method with 59 percent of the non-medicinal treatments. Frequent treatment with a single method will most likely inflict a selection pressure towards more temperature tolerant salmon lice.

Although there are indications of a reduction of resistance from 2016 to 2019, the lice have for most substances not the sensitivity level needed to obtain effective treatments. The massive reduction in the use of medicinal treatments will not necessarily lead to fully restored sensitivity. One reason for this assertion is the relatively low frequency of sensitive parasites, which could possibly dilute the resistance genes in the absence of a selection pressure. Neither can salmon lice from wild migrating salmon be trusted as a source of purely sensitive lice, as these also carry resistance factors as shown for organophosphate and pyrethroid resistance (15, 16). The other reason is the continuous use of medicinal treatments, although at a lower intensity. The performed treatments will contribute to withhold a selection pressure towards resistance.

Fresh water bath treatments are performed at a moderate level in Norway, but predominately in two areas; in Trøndelag and on the southwest coast. In 2019 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. Such differences were not detected.

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References


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