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The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2015

Veterinærinstituttet
Norwegian Veterinary Institute

Surveillance programs for terrestrial and aquatic animals in Norway

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The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2015

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Results obtained in this surveillance program show that the number of prescriptions of medicines used against salmon lice is still high but reduced compared to 2014. Reduced sensitivity and resistance towards the medicines tested in bioassays are generally widespread along the coast, but seem less prominent in the far south (Agder). Compared to the surveillance in 2014, there seems to be a further loss of sensitivity towards deltamethrin, azamethiphos and emamectin benzoate in Finnmark. The results for hydrogen peroxide were generally better than for the other medicines, but loss of sensitivity was indicated in several areas. A survey on resistance in salmon lice collected from wild salmonids in Norway in 2014 by the Institute of Marine Research, show that resistance towards antiparasitics is widespread also in parasites on wild salmonids.

Introduction

Resistance towards chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (also referred to as sea lice), has been reported from several countries (1, 2) including Norway (3). Episodes of reduced treatment efficacy, along with extensive field sensitivity testing of *L. salmonis* against pyrethroids, emamectin benzoate and azamethiphos by the use of six-dose toxicological tests (3,4), has brought about concerns of reduced sensitivity against the available chemotherapeutants. However, reporting of results from this extensive sensitivity testing has not been mandatory until 2013 and a comprehensive survey of the resistance status in Norway was first reported in 2014 (5). The reduced sensitivity has been associated with local treatment intensity (6).

In order to obtain a survey of the resistance status of *L. salmonis* in Norway, and the use of chemoterapeutants that are believed to influence this status, The Norwegian Food Safety Authority established a surveillance program in 2013. The program summarizes reported data from the industry on drug use and *L. salmonis* sensitivity (passive surveillance), and present a collection of sensitivity data from approximately 75 salmon farm locations along the Norwegian coast (active surveillance). For this year's report a survey on resistance in 839 parasites collected from wild salmonids in Norway in 2014, were also included, in order to add more knowledge to the interactions between wild and farmed salmonids.

Aim

The surveillance program aims to summarize the use of various anti-salmon lice chemotherapeutants in salmon farming and to describe the sensitivity/resistance status in *L. salmonis* towards the most important of these chemotherapeutants in Norway.

Materials and methods

Passive surveillance

Veterinary medicine register data
The Norwegian Veterinary Institute (NVI) has received monthly extracts from the Veterinary medicine register (VetReg) that cover prescriptions coupled to treatment of fish. These data are summarized into 5 different categories of substances used to control salmon lice infestations. In total over the years 2011 – 2015 there were 12 528 prescriptions coupled to these categories of substances and to a specific farm site.

The five categories of substances are in the following termed azamethiphos (named in the register: Azamethiphos, Salmosan Vet, Trident Vet, Azasure Vet), pyrethroids (named in the register: Alpha Max, Betamax vet, Cypermethrin or Deltamethrin), emamectin benzoate (named in the register: Emamectin benzoate or Slice vet), hydrogen peroxside and flubenzurones (named in the register: Diflubenzuron, Ektobann vet, Releeze vet or Teflubenzuron). Table 2 summarizes the number of prescriptions per substance category and year.

No quantification of the use of different substances is presented since the units used in VetReg vary substantially, e.g. between kg, g, l and ml for the same substance. It should also be noted that there may be a degree of underreporting of prescriptions since these are manually reported by wholesale businesses.

Reported sensitivity data
In the current regulation on the control of salmon lice in aquaculture in Norway (FOR-2012-12-05-1140), effective from 1.1.2013, there is a disclosure of mandatory reporting on suspected resistance and results from sensitivity tests. If resistance is suspected, the reason for suspicion is to be reported in one of the 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 the three categories: sensitive; reduced sensitivity; or resistant. Reported data have been summarized as part of the passive surveillance.

Active surveillance

Performance of simplified bioassay tests
In the performance of the active surveillance, 11 fish health services along the Norwegian coast were engaged to carry out a newly developed simplified field bioassay (7, 8) for sensitivity testing of *L. salmonis*. The simplified bioassay was standardised, with the same protocol employed for each substance and by the use of identical stock solutions and identical equipment by all the fish health services. The simplified bioassay is less time consuming and the number of salmon lice required is less, than in the six-dose bioassay. Performing sensitivity testing using this protocol would presumably make it possible to achieve reliable and comparable sensitivity results from a larger number of locations than if the traditional bioassay protocol was chosen. The locations (Figure 3) were chosen by the fish health services themselves inside a designated area.

L. salmonis from a maximum of 82 farm locations (Table 5) were tested against the four chemotherapeutants deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide. The simplified field bioassays were performed with two different concentrations (low and high) and a control. After 24 hours of exposure to the chemical in sea water, salmon lice mortality in identified stages and genders (preadult I and II and adults; females and males) were noted as the test outcome. Salmon lice mortality in the low concentration tests was used to indicate the sensitivity status of the salmon lice population, with mortalities higher than 80% indicative of fully sensitive populations (as shown in preadult parasites in (7)).

Salmon lice mortality in the high concentration tests was used to indicate the expected outcome of a subsequent treatment.

Performance of molecular tests of resistance
Salmon lice infestation levels on farms in Vest-Agder in the far south of Norway are known to be low. In order to sample lice from such farms, lice were collected at slaughtering from fish originating from one farm in Vest-Agder. Patogen Analyse AS analysed the genetic characteristics with regard to pyrethroid, azamethiphos and hydrogenperoxide resistance using PCR methodology. Test results were reported according to percentage of lice from each farm categorized as resistant or sensitive for deltamethrin, and sensitive, showing reduced sensitivity or resistant for azamethiphos. Molecular testing of pyrethroid resistance was also conducted on a sample of salmon lice from one of the farms in Nord-Trøndelag which had previously been tested in bioassays.

Table 1: High and low concentrations used in the simplified bioassay tests.

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

Results and Discussion

Passive surveillance

VetReg data
Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2015. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years, but this has somewhat decreased in 2015. The decrease in prescriptions can especially be seen for azametifos and pyrethroids. However, the number of prescriptions of hydrogen peroxide and emamectin benzoate increased in 2015 compared with 2014. As the amounts prescribed could not be calculated, the VetReg data could not be validated against sales data from wholesalers http://www.fhi.no/artikler/?id=117980 .

Table 2: Number of prescriptions for the given category of substances used to control salmon lice during 2011 - 2015.

Substance category	2011	2012	2013	2014	2015
Azamethiphos	409	691	480	749	616
Pyrethroids	456	1155	1123	1043	660
Emamectin benzoate	288	164	162	481	522
Hydrogen peroxide	172	110	250	1009	1270
Flubenzurones	23	129	170	195	201
Sum	1348	2249	2185	3477	3269

The maps in Figure 1 sum up the total number of prescriptions per location during 2013 - 2015. In 2013 there were prescriptions reported from 642 farms, with a mean number of prescriptions per farm of 3.4; 679 farms in 2014 with a mean of 5.1 prescriptions per farm; and 661 farms in 2015 with a mean of 4.9 prescriptions per farm, respectively.

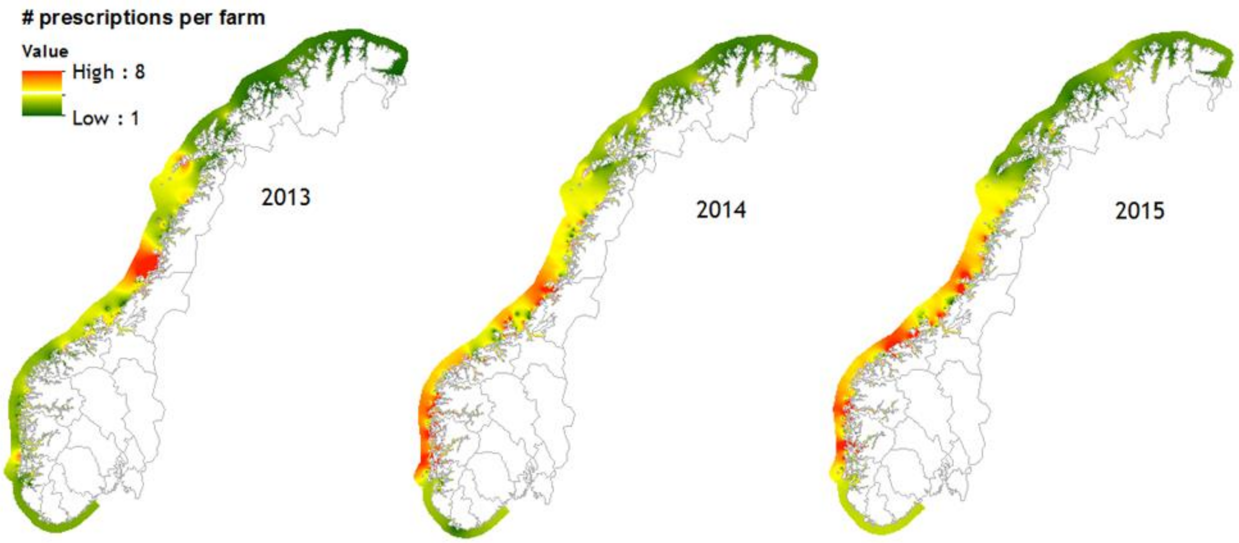


Figure 1: Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 8 prescriptions per location is expected, while dark green denote areas where the expectation of no treatment is approached. The map layer was generated using the IDW function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighbouring farm locations).

Azamethiphos, pyrethroids and emamectin benzoate were used in in most production intense areas along the coast. The use of flubenzurones and hydrogen peroxide was more restricted to the southwest and less so to the coast of Nord-Trøndelag (Figure 2).

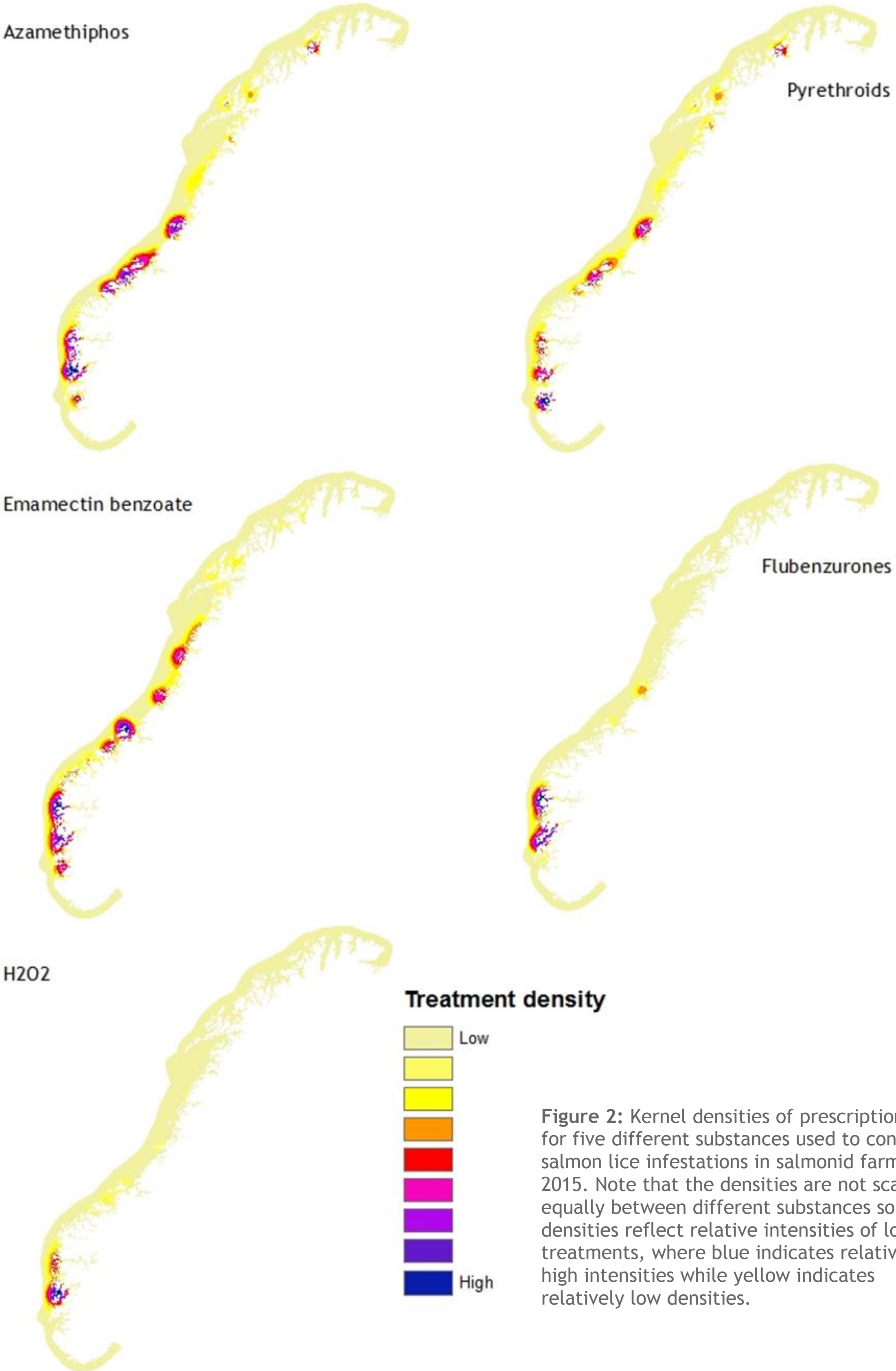


Figure 2: Kernel densities of prescriptions for five different substances used to control salmon lice infestations in salmonid farms in 2015. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, where blue indicates relatively high intensities while yellow indicates relatively low densities.

Reported sensitivity data

Table 3. The number of reports from sensitivity studies within the three categories of reported sensitivity status.

Substance category	2014			2015		
	Sensitive	Reduced sens.	Resistant	Sensitive	Reduced sens.	Resistant
Azamehtiphos	29	33	19	1	26	1
Emamectin benzoate	7	9	3	3	20	0
Flubenzurones						
Hydrogen peroxide	3	5	1	7	14	0
Pyrethroids	25	60	8	3	34	3
Total	**64**	**107**	**31**	**14**	**94**	**4**

With regard to the sensitivity status reported from sensitivity tests, there are no obvious trends in the data (Table 3). The number of reports due to suspicion of resistance was comparable to that of 2014 (Table 4).

Table 4. The number of reports due to suspicion of resistance. The reports are categorized with respect to suspected reasons for resistance (1 = bioassay results; 2 = treatment effect; 3 = situation in the area).

Substance category	2013			2014			2015		
	1	2	3	1	2	3	1	2	3
Azamethiphos	15	11		25	52	2	11	37	1
Emamectin benzoat	1	1		21	2			16	
Flubenzurones									
Hydrogen peroxide		5	1	3	10			25	
Pyrethroids	16	23	2	31	66		12	36	1
Total	**32**	**40**	**3**	**80**	**130**	**2**	**23**	**114**	**2**

Active surveillance

Altogether, 267 high concentration and 261 low concentration simplified bioassay tests were performed on salmon lice from 84 different salmon farm locations along the cost (Figure 3). Of these, 62 farms were tested for azamethiphos, 74 farms for deltamethrin, 55 farms for emamectin benzoate and 61 farms for hydrogen peroxide (Table 5).

The table shows that salmon lice mortalities were lower than 80% in the majority of locations tested at low concentrations for each substance. This indicates that reduced sensitivity to chemotherapeutants in salmon lice is widespread in Norwegian salmon farming.

Table 5. *Classification of mortality results from low and high concentration bioassay tests. The Number of tests column refers to the number of tests conducted (some test were duplicated on the same farm) at different farm locations. Column numbers denote the number of tests that fell within the high, intermediate or low mortality classifications for each drug and test-concentration. Note that in 2 bioassays conducted with azamethiphos, 2 bioassays with deltamethrin and 2 bioassays with emamectin benzoate, low concentration tests were not performed.*

Substance category	Locations tested	Number of tests	Mortality classification (number of observations)		
Low concentration			High (> 80 %)	Intermediate (80 - 33 %)	Low (< 33 %)
Azamethiphos	*60*	*62*	*0*	*20*	*42*
Deltamethrin	*72*	*80*	*10*	*17*	*53*
Emamectin benzoate	*53*	*55*	*4*	*13*	*38*
Hydrogen peroxide	*61*	*64*	*12*	*31*	*21*
High concentration			High (> 90 %)	Intermediate (90 - 33 %)	Low (< 33 %)
Azamethiphos	*62*	*64*	*4*	*32*	*28*
Deltamethrin	*74*	*82*	*11*	*44*	*27*
Emamectin benzoate	*55*	*57*	*8*	*25*	*24*
Hydrogen peroxide	*61*	*64*	*27*	*36*	*1*

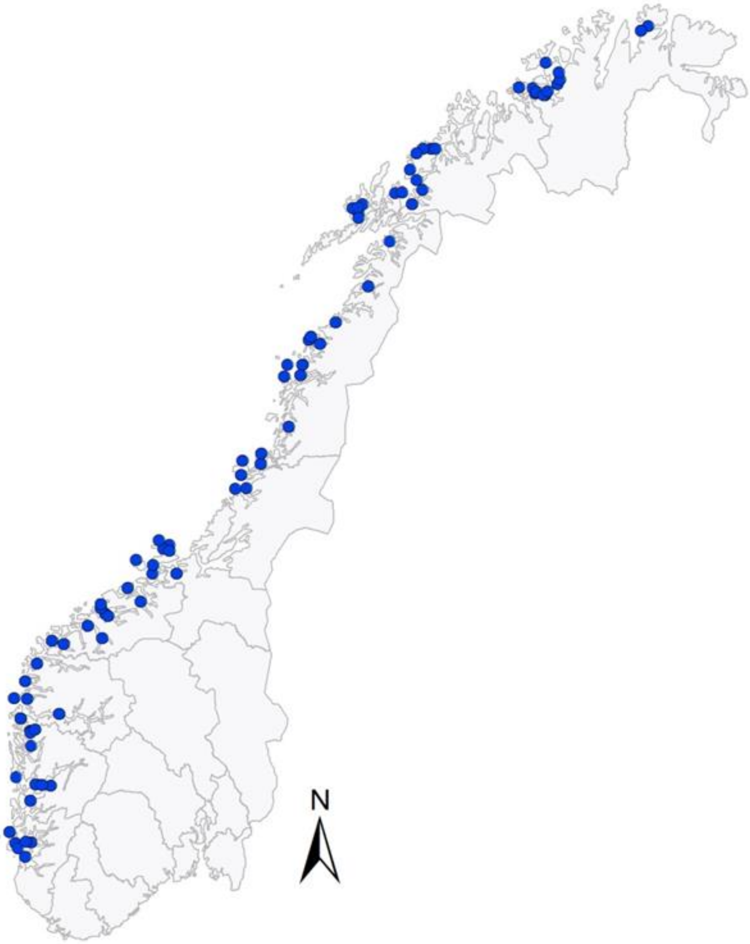


Figure 3: Locations of farms where salmon lice were collected for simplified bioassay testing in 2015.

Table 6 shows that the salmon lice mortality results from low and high concentrations are significantly correlated. These correlations show that the results from low and high concentration tests are consistent.

Table 6. *Spearman Correlation Coefficients between mortality proportions in the low and high concentration bioassay tests on farms. The correlation coefficients are all relatively high and significant, indicating consistency in the results from low and high concentration tests within farms.*

Substance category	N	Spearman Correlation Coefficients
Azamethiphos	62	0.61
Deltamethrin	80	0.65
Emamectin benzoate	55	0.71
Hydrogen peroxide	64	0.71

Test results are shown geographically in maps together with box plots showing the distribution of proportional mortality for azamethiphos (Figure 4), deltamethrin (Figure 5), emamectin benzoate (Figure 6) and hydrogen peroxide (Figure 7).

For low concentration azamethiphos tests (Figure 4 B), no farm with salmon lice test-mortalities exceeding 80% (indicative of fully sensitive populations) were located. Low salmon lice mortalities in high concentration azamethiphos tests (Figure 4A), indicating that low treatment efficacy may be expected, were generally widespread. However, there were variations in mortality when lice from different farms were exposed to high concentration of azamethiphos (Figure 4).

In general, the results from the high concentration deltamethrin tests (Figure 5A) indicate that several areas may expect low treatment efficacy. The boxplots showing the distribution of proportional mortalities in low and high concentration deltamethrin showed large variations between tests, indicating that reduced sensitivity is common and that low treatment efficacy often is to be expected. The low concentration deltamethrin tests (Figure 5B) indicate that reduced sensitivity to deltamethrin is widespread along the coast. The unexpected results showing high mortality in both low and high concentration test in area Nord-Trøndelag, indicating a salmon lice population sensitive towards pyrethroids, was not confirmed in molecular tests performed on the same sample of salmon lice from one of the farms. This molecular test revealed a high percentage of lice being resistant to pyrethroids, i.e. 90%. Further surveillance of sensitivity in this area may elucidate the mismatch between test results.

The low concentration emamectin benzoate tests showed that reduced sensitivity is widespread along the coast, but varies considerably with generally higher mortalities in areas south of Trøndelag and in Rogaland (Figure 6B). The high concentration emamectin tests (Figure 6A) resulted in comparably high mortalities in some farms in Mid Norway and Rogaland, but varying mortality in the rest of the country. The box plots of proportional mortality in high and low concentration tests, showed large variability, but indicated that reduced sensitivity and low treatment efficacy may be expected for emamectin as well as for azametiphos and pyrethroids.

For hydrogen peroxide, results from the high concentration tests yielded in general higher mortalities than for the other substances tested. Compared to results from previous years (9), however, mortalities seem reduced in several areas, except for Finnmark and Rogaland (Figure 7A). The low concentration tests (Figure 7B) show low mortality in Nord-Trøndelag and parts of Nordland, indicating loss of sensitivity to hydrogen peroxide.

The molecular tests of lice from the southern one farm in Vest-Agder revealed a higher percentage of lice being sensitive to pyrethroids (63%) than resistant (37%). For azamethiphos the percentage of the lice being sensitive was reported to be less (44%) than the percentage of salmon lice with reduced sensitivity (56%). Compared to results from molecular tests performed in 2014 (9), this indicates that the sensitivity to azametiphos and pyrethroids in lice from the southernmost farms in Norway is reduced. Molecular testing of hydrogenperoxid sensitivity revealed an estimated treatment efficacy of 70%.

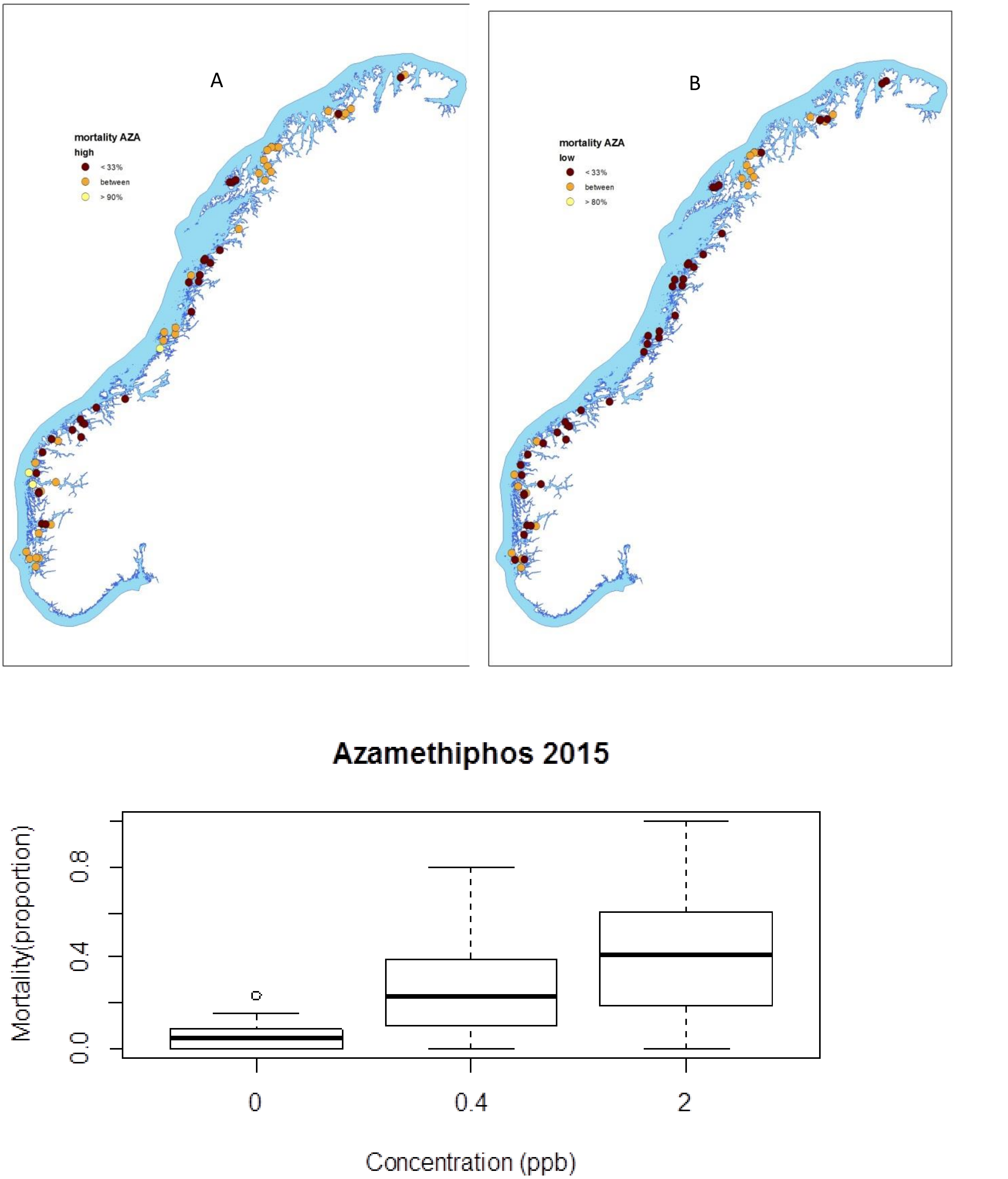
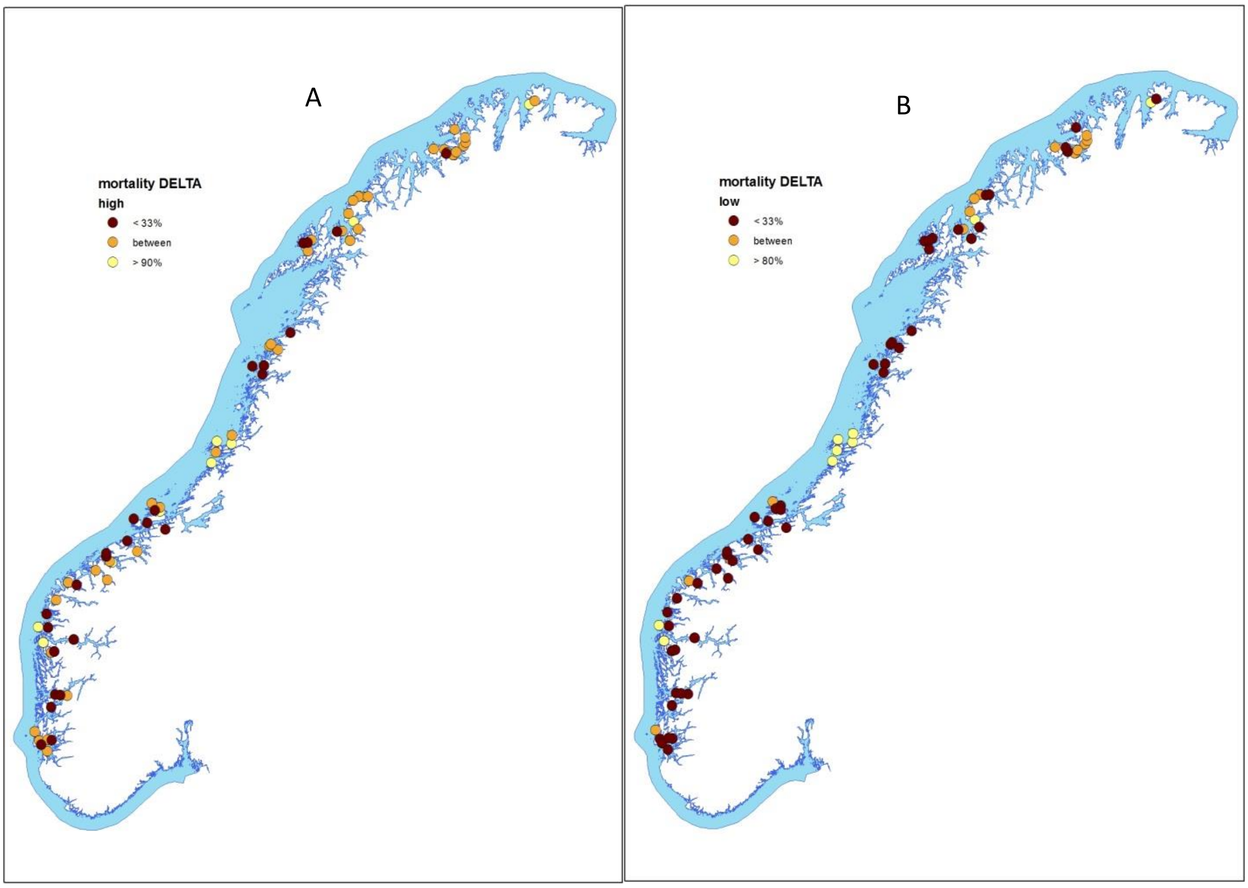


Figure 4. Maps showing categorical mortality in bioassays with high (A) and low (B) *azamethiphos* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).



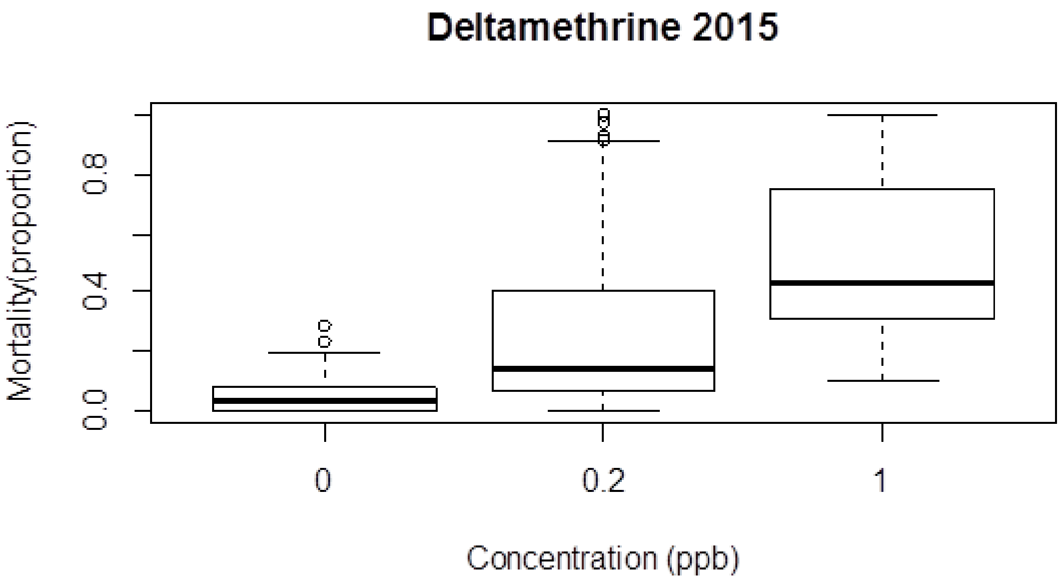
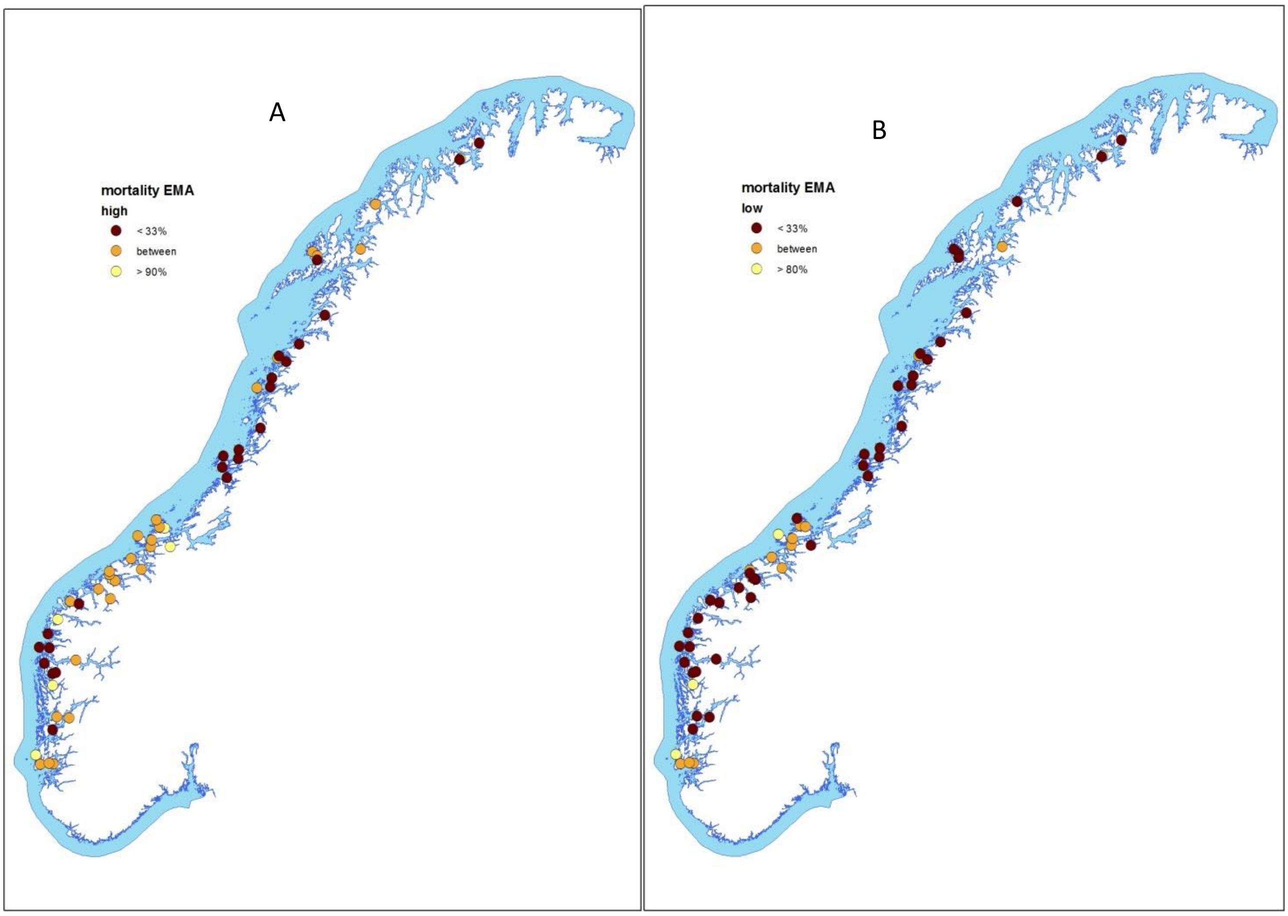


Figure 5. Maps showing categorical mortality in bioassays with high (A) and low (B) *deltamethrin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two. The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).



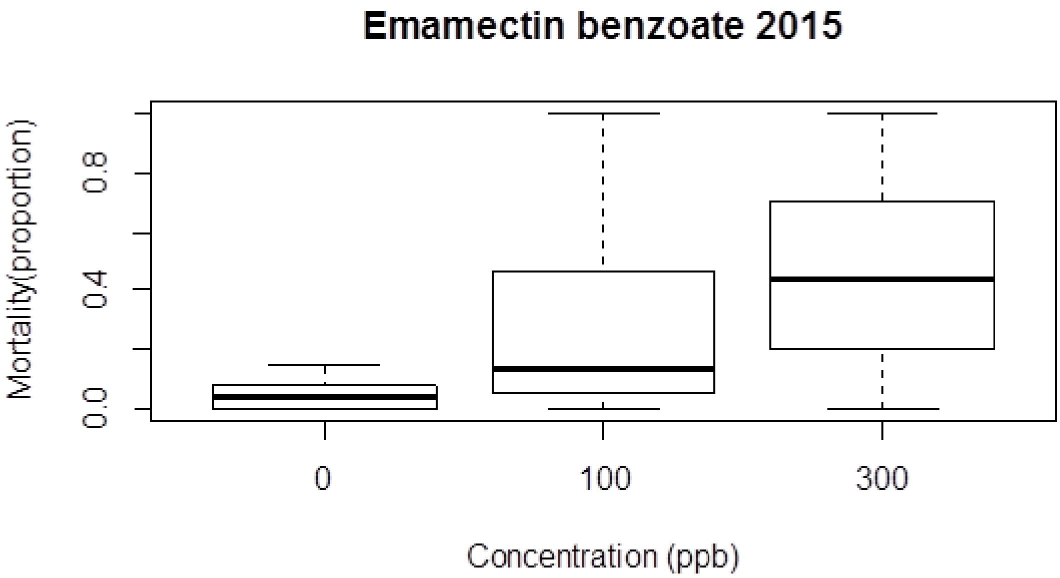


Figure 6. Maps showing categorical mortality in bioassays with high (A) and low (B) *emamectin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend). The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the four substances tested).

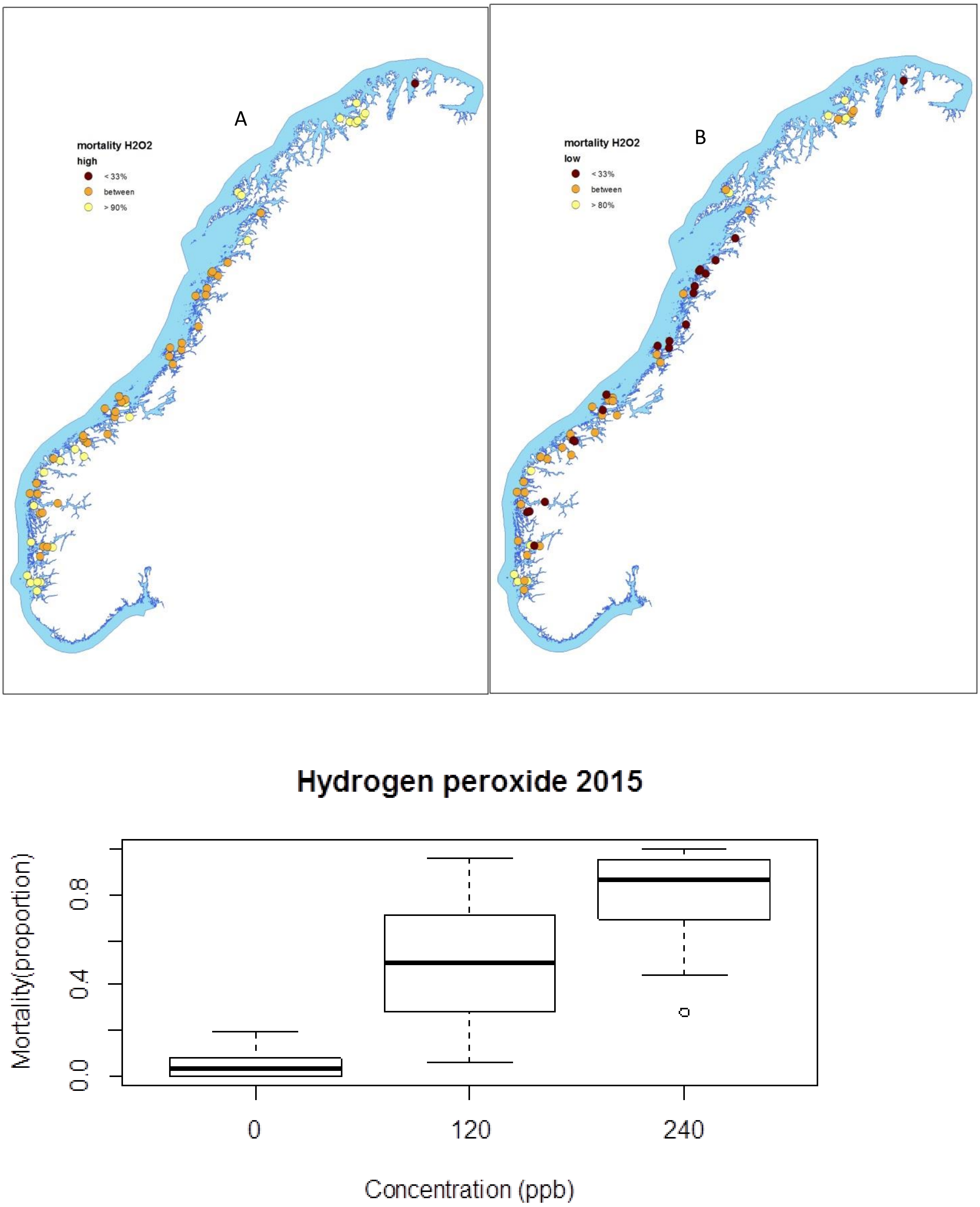


Figure 7: Maps showing categorical mortality in bioassays with high (A) and low (B) hydrogen peroxide concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (see figure legend). The boxplot shows the distribution of proportional mortalities for all tests (note that the control experiment is the same for the three substances tested).

Organophosphate and pyrethroid resistance in salmon lice from wild salmonids

In 2014 organophosphate and pyrethroid resistance were tested by molecular tests (Patogen Analyse AS) in salmon lice collected from wild Atlantic salmon and sea trout. This was performed as part of the national surveillance program on salmon lice on wild salmonids, carried out by the Institute of Marine Research. Table 7 summarizes the frequency of resistant salmon lice on wild adult salmonids.

Table 7: *Frequency of organophosphate and pyrethroid resistance in salmon lice, collected from wild Atlantic salmon and sea trout, in Norway in 2014. Data received from Helene B. Fjørtoft[12], Kevin A. Glover[23], Frank Nilsen[2], Pål Arne Bjørn[3] and Anne Stene[1] ([1]NTNU in Ålesund, [2]University of Bergen- Sea Lice Research Centre, [3]Institute of Marine Research)*

Fish species	Number of salmon lice	Number of sites	Organophosphate resistant	Pyrethroid resistant
Atlantic salmon	307	7	25%	27%
Sea trout	532	11	58%	73%

The parasites were collected from several sites in Norway and the regional differences in resistance will be further analysed and published by the research group.

The overall results showed that a great proportion of the salmon lice collected from wild salmonids were resistant to antiparasitics. The level of organophosphate resistance expected in a novel population of salmon lice is below five percent (10). This indicates that salmon lice on wild salmonids originate from salmon lice that have experienced selection by antiparasitics, i.e. they originate from salmon farms. Salmon lice from sea trout were more resistant compared to those collected from Atlantic salmon. This is probably a result of the more costal habitat of sea trout, and thereby a more close contact with salmon farms.

Salmon lice genes are exchanged throughout the Atlantic Ocean (11, 12). This includes resistance alleles for emamectin benzoate resistance (12) and therefore probably resistance alleles coding for other types of antiparasitic resistance. The studies by Glover et al. (2011) and Besnier et al. (2014) were conducted on parasites of farm origin. The current data from wild salmonids may provide an explanation to the gene exchange. The resistance detected in salmon lice from wild Norwegian Atlantic salmon may both originate and spread to other parts of the Atlantic Ocean, through common Atlantic salmon feeding grounds. This also includes introducing resistance to new areas of Norway. Salmon lice from wild salmonids may therefore partly explain the spread of resistance alleles in Norway. This in addition to salmon lice larvae transported by costal currents and well boats carrying live salmonids.

Conclusions

The total number of prescriptions of substances used to control salmon lice infestations showed a moderate decrease in 2015 compared to 2014. Compared to earlier years the use of chemotherapeutants against salmon lice is still high in Norway.

No clear trends in the reported sensitivity data were observed.

In the active surveillance, the low and high dose concentration tests were significantly correlated, showing consistent results. Reduced sensitivity and resitance to chemotherapeutants was generally found to be widespread along the coast. This also includes areas in the northernmost part of Norway where *L. salmonis* were found to be mostly sensitive in 2013. Salmon lice from the southernmost area (Agder) genotyped for resistance towards azamethiphos, pyrethroids and hydrogen peroxide, demonstrated a higher level of resistance than in other areas or in 2014 (9).

The overview of results from molecular testing of salmon lice from wild salmonids shows that a large proportion of the salmon lice on wild salmonids are resistant to antiparasitics. This indicates that a significant proportion of salmon lice on these fish originate from salmon farms, and that further gene exchange and spread of resistance alleles may happen throughout the Atlantic Ocean.

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The primary mission of the NVI is to give research-based independent advisory support to ministries and governing authorities. Preparedness, diagnostics, surveillance, reference functions, risk assessments, and advisory and educational functions are the most important areas of operation. The Institute has its main laboratory in Oslo, with regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø, with about 330 employees in total.

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Norwegian Veterinary Institute

The Norwegian Food Safety Authority (NFSA) is a governmental body whose aim is to ensure through regulations and controls that food and drinking water are as safe and healthy as possible for consumers and to promote plant, fish and animal health and ethical farming of fish and animals.

We encourage environmentally friendly production and we also regulate and control cosmetics, veterinary medicines and animal health personnel. The NFSA drafts and provides information on legislation, performs risk-based inspections, monitors food safety, plant, fish and animal health, draws up contingency plans and provides updates on developments in our field of competence. The NFSA comprises two administrative levels, five regions in addition to the head office, and has some 1250 employees. The NFSA advises and reports to the Ministry of Agriculture and Food, the Ministry of Trade, Industry and Fisheries and the Ministry of Health and Care Services.

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