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The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2014

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Results obtained in the surveillance program for resistance to chemotherapeutants in salmon lice show a pronounced increase in prescribed medicines used as delousing agents. Furthermore, reduced sensitivity and resistance to the medicines tested in bioassays are generally widespread along the coast, but seem less prominent in the far north and far south. Compared to the surveillance in 2013, however, there seems to be a loss of sensitivity to deltamethrin and azamethiphos in Finnmark. The results for hydrogen peroxide were generally better than for other medicines, but loss of sensitivity was indicated in areas in Hordaland and Trøndelag.

Introduction

Resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (also referred to as sea lice) has been reported from several countries (Jones, Sommerville & Wotten 1992; Lees et al. 2008; Roth et al 1996) including Norway (Sevatdal & Horsberg 2003; Sevatdal *et al*. 2005). Episodes of reduced treatment effect, along with extensive field sensitivity testing of *L. salmonis* against pyrethroids, emamectin benzoate (EMB) and azamethiphos by the use of six-dose toxicological tests (Sevatdal & Horsberg 2003; Bravo *et al*. 2008; Westcott et al. 2008), 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 (Grøntvedt et al. 2014).

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).

Aim

The surveillance program aims to summarize the use of various chemotherapeutants in salmon farming and to describe the resistance status against the most important of these chemotherapeutants in *L. salmonis* 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 – 2014 there were 8274 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), hydrogenperoxside 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 performance of the active surveillance, 11 fish health services along the Norwegian coast were engaged to carry out a newly developed simplified field bioassay (Helgesen & Horsberg 2013, Helgesen et al. 2015) 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 (fig. 3) were chosen by the fish health services themselves inside a designated area.

L. salmonis from a maximum of 78 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, the salmon lice mortality in identified stages and genders (preadult I and II and adults; females and males) were noted as the test outcome. The salmon lice mortality in the low concentration was used to indicate sensitivity status of the salmon lice population, with salmon lice mortality higher than 80% in parasites indicative of a fully sensitive population (as shown in preadulte parasites in Helgesen and Horsberg 2013).

In the active surveillance for 2014 we included tests using hydrogen peroxide. This is due to an increased use of this therapeutant to control salmon lice, as well as the first reports of loss of sensitivity of salmon lice to hydrogen peroxide treatments (Helgesen et al. 2015).

The salmon lice mortality in the high concentration was used to indicate the degree of reduced sensitivity and the expected outcome of a subsequent treatment, with salmon lice mortality higher than 90% indicative for an expected treatment efficacy of 90% or more.

Performance of molecular tests of resistance
Salmon lice infection 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 slaughter from fish originating from two farms in Vest Agder. Patogen Analyse AS analysed the genetic characteristics with regard to detamethrin and azamethiphos 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, reduced sensitivity or resistant for azamethiphos.

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

Note that the high concentration emamectin benzoate was reduced from 500 ppb to 300 ppb in 2014 compared to 2013 (Grøntvedt et al. 2014). This was done to better predict treatment efficacy.

Results and Discussion

Passive surveillance

VetReg data
Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2014. Pronounced increases in the total number of prescriptions were registered in 2014 compared to earlier years. Increases were especially large for hydrogen peroxide, flubenzurones and emamectin benzoate. As the amounts prescribed could not be calculated, they could also not be validated against sales data from wholesalers http://www.fhi.no/artikler/?id=114175 . Thus, the results should be interpreted with care.

Table 2: Number of prescriptions for the given category of substances used to control salmon lice during 2011 - 2014.

Substance category	2011	2012	2013	2014
Azamethiphos	451	617	448	747
Pyrethroids	501	1005	1065	1042
Emamectin benzoate	245	50	47	481
Hydrogen peroxide	167	60	68	977
Flubenzurones	22	62	26	193
Sum	1386	1794	1654	3440

The maps in figure 1 sum up the total number of prescriptions per location during 2013 and 2014. In 2013 there were prescriptions coupled to 560 farm locations, with a mean number of prescriptions per farm of 2.95 (range 1 – 16). Comparable numbers for 2014 were 679 farm locations, with a mean of 5.05 prescriptions per farm (range 1 – 23), respectively.

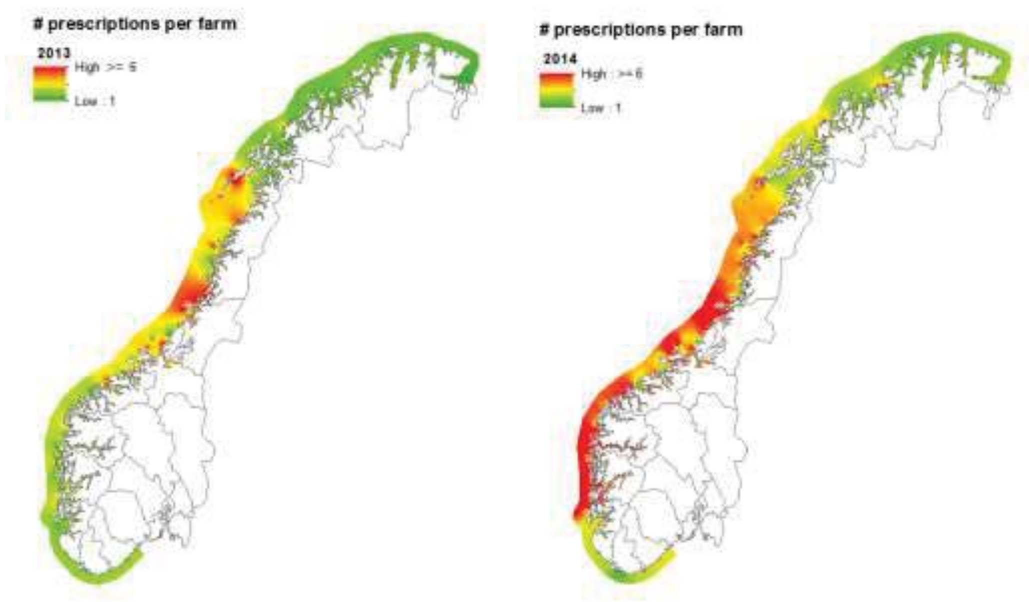


Figure 1: Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 6 prescriptions per location is expected, while dark green denote areas where the expectation of one treatment is approached. The map layer was generated using the IDW function in ArcGIS spatial analyst (accounting for prescriptions from 50 nearest neighbour farm locations). Farms with 0 prescriptions were not part of the data.

The use of azamethiphos and pyrethroids show much the same spatial distribution. The use of emamectin benzoate seems to be distributed comparatively more northerly. The use of hydrogen peroxide is restricted to smaller areas, especially in the South-West and on the coast of Nord Trøndelag. The flubenzurones are used mostly on the south west coast.

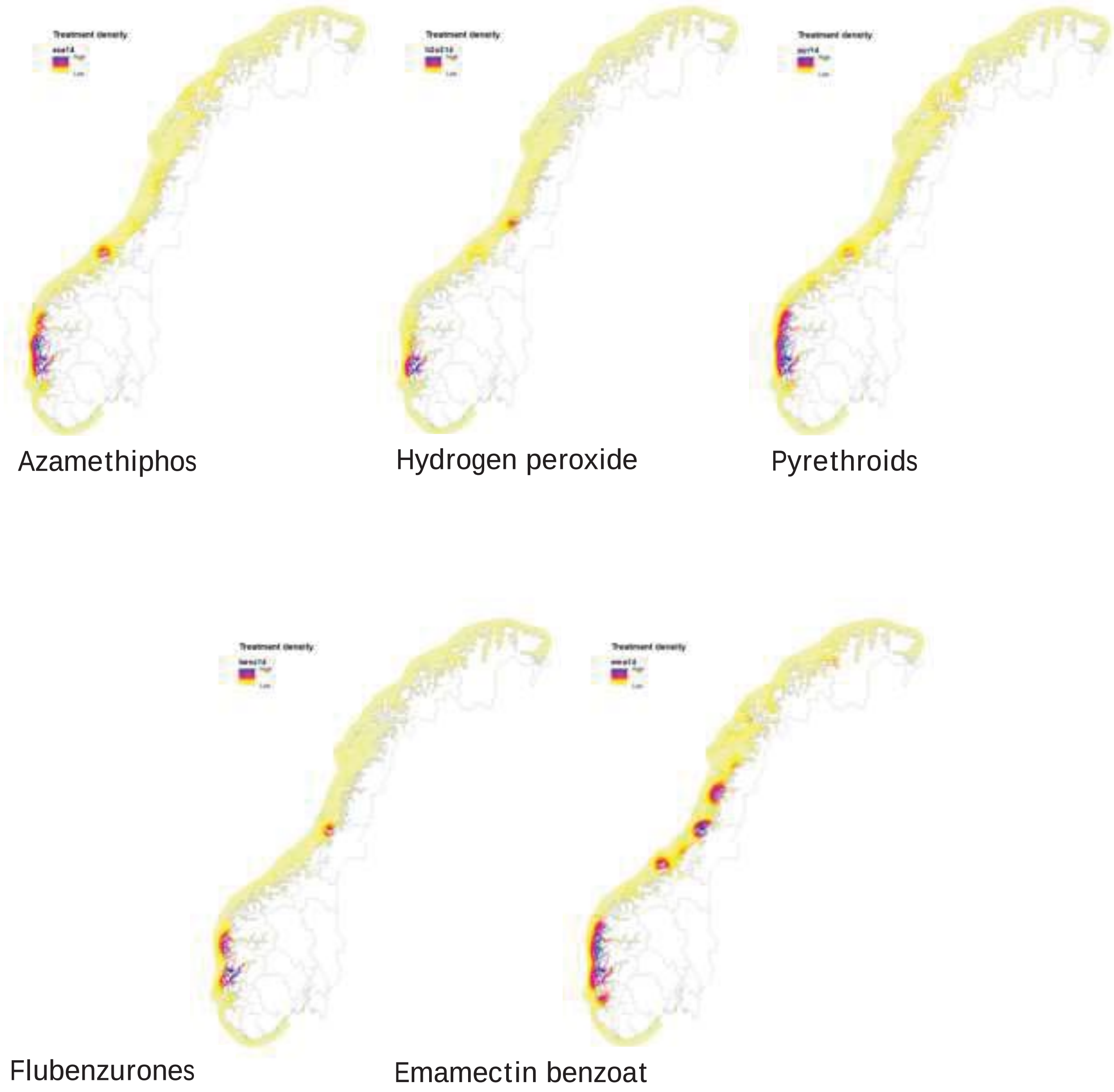


Figure 2: Kernel densities of prescriptions for five different substances used to control salmon lice infestations in salmonid farms in 2014. Note that the densities are not scaled equally between different substances so the densities reflect relative intensities of local treatments, where blue indicates 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	2013			2014		
	Sensitive	Reduced sens.	Resistant	Sensitive	Reduced sens.	Resistant
Azamehtiphos	18	28	15	29	33	19
Emamectin benzoat	3	6	2	7	9	3
Flubenzurones		3				
H2O2		5		3	5	1
Pyrethroids	43	52	8	25	60	8
Total	**64**	**94**	**25**	**64**	**107**	**31**

With regard to the sensitivity status reported from sensitivity tests there are no obvious trends in the data. The number of reports due to suspicion of resistance showed a pronounced increase in 2014 compared to earlier years.

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; 9 = other unspecified).

Substance category	2012				2013			2014		
	1	2	3	9	1	2	3	1	2	3
Azamethiphos	18	8	8		15	11		25	52	2
Emamectin benzoat	9	9	46	1	1	1		21	2	
Flubenzurones	1	1								
H2O2						5	1	3	10	
Pyrethroids	31	12	1	3	16	23	2	31	66	
Total	**59**	**30**	**55**	**4**	**32**	**40**	**3**	**80**	**130**	**2**

Active surveillance

Altogether, 230 high concentration and 230 low concentration simplified bioassay tests on salmon lice from altogether 90 different salmon farm locations along the cost (figure 3). Of these, 59 farms were tested for azamethiphos, 78 farms for deltamethrin, 48 farms for emamectin benzoate and 45 farms for hydrogen peroxide.

Table 5 summarizes the outcome of all simplified bioassays according to mortality classification. Differences in mortality rates between genders and/or developmental stages are not presented in the table. For pyrethroids and azamethiphos, this variation was low, but higher for emamectin benzoate. The categories are high mortality (>80% for low concentration and >90 % for high concentration tests), intermediate mortality and low mortality (< 33% mortality for both low and high concentration tests) for each substance.

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.

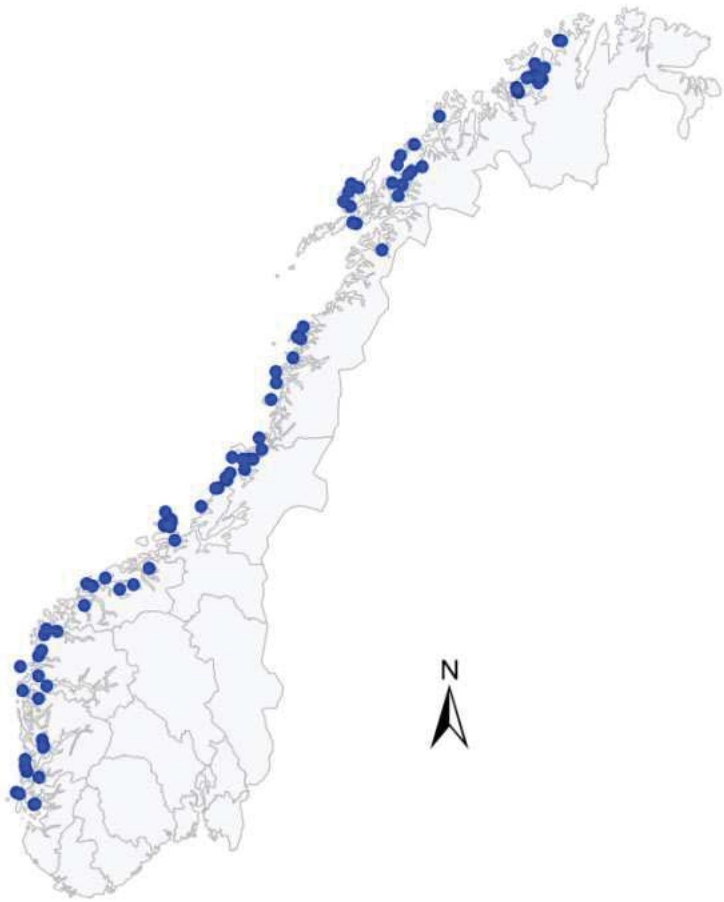


Figure 3: Locations of farms where salmon lice were collected for simplified bioassay testing in 2014.

Table 5. Classification of mortality results from low and high concentration bioassay tests. The Total column refers to the number of tests conducted at different farm locations (except for deltamethrin where tests were duplicated on four farms, conducted at 74 different farms and hydrogen peroxide where tests were duplicated in two farms, conducted at 43 farms). Column numbers denote the number of tests that fell within the high, intermediate or low mortality classifications for each drug and test-concentration.*

Substance category		Mortality classification (number of observations)		
Low concentration	Total	High (> 80 %)	Intermediate (80 - 33 %)	Low (< 33 %)
Azamethiphos	59	1	18	40
Deltamethrin	78	4	17	57
EMB	48	1	17	30
H2O2	45	6	26	13
High concentration	Total	High (> 90 %)	Intermediate (90 - 33 %)	Low (< 33 %)
Azamethiphos	59	1	26	32
Deltamethrin	78	6	43	29
EMB	48	4	28	16
H2O2	45	23	20	2

Table 6 shows that the salmon lice mortality results from low and high concentrations are significantly correlated, with highest correlations for azamethiphos and pyrethroids. 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 are highly significant, indicating consistency in the results from low and high concentration tests within farms.*

Substance category	N	Spearman Correlation Coefficients	Prob > |r| under H0: Rho=0
Azamethiphos	59	0.81	< 0.001
Deltamethrin	77	0.71	< 0.001
EMB	49	0.80	< 0.001
H2O2	45	0.56	< 0.001

The geographic location of farms where tests were performed and the distribution of mortality results are shown in maps and box plots for azamethiphos (Figure 4), deltamethrin (Figure 5), emamectin benzoate (Figure 6) and hydrogen peroxide (Figure 7). As in table 5, differences in mortality rates between genders and/or development stages are not shown in the figures. For low concentration azamethiphos tests (Figure 4 B), the only farm with salmon lice test-mortalities exceeding 80 % (indicative of fully sensitive populations) was in Finnmark in the far north. Low salmon lice mortalities in high concentration azamethiphos tests (Figure 4A) were found especially in the areas Nordland, Trøndelag and partly Hordaland. Low treatment efficacy may thus be expected in these areas. The boxplots showing the distribution of proportional mortalities in low and high concentration azamethiphos experiments showed large variations between tests, indicating that reduced sensitivity is common and that low treatment efficacy often is to be expected.

For deltamethrin, mortalities were comparably high in high concentration tests in Finnmark (Figure 5A). In general, however, the results from the high concentration deltamethrin tests indicate that several areas can expect low treatment efficacy. The low concentration deltamethrin tests (Figure 5B) indicate that that reduced sensitivity to deltamethrin is widespread along the coast.

The low concentration emamectin benzoate tests (Figure 6B), showed that reduced sensitivity is widespread, but varies considerably (boxplot). The high concentration emamectin tests (Figure 6A) resulted in comparably high mortalities in the north, but varying mortality in the rest of the country.

For hydrogen peroxide, results from the high concentration tests yielded reasonably high mortalities in general, but reduced mortalities in an area in Mid-Norway and in the southernmost tested farms. The low concentration tests corroborated the results of the high concentration tests, especially by low mortalities in farms located in Mid-Norway (Figure 7).

The molecular tests of lice from the southern two farms in Vest Agder revealed a high percentage of lice being sensitive to deltamethrin, ***i.e.*** 85% for both farms. Also for azamethiphos a high percentage of the lice were sensitive, i.e. 76% and 78%, respectively. Reduced sensitivity to azamethiphos was reported from 20 and 18% of the lice, respectively. This indicates that lice from the southernmost farms in Norway generally are sensitive to chemotherapeutants.

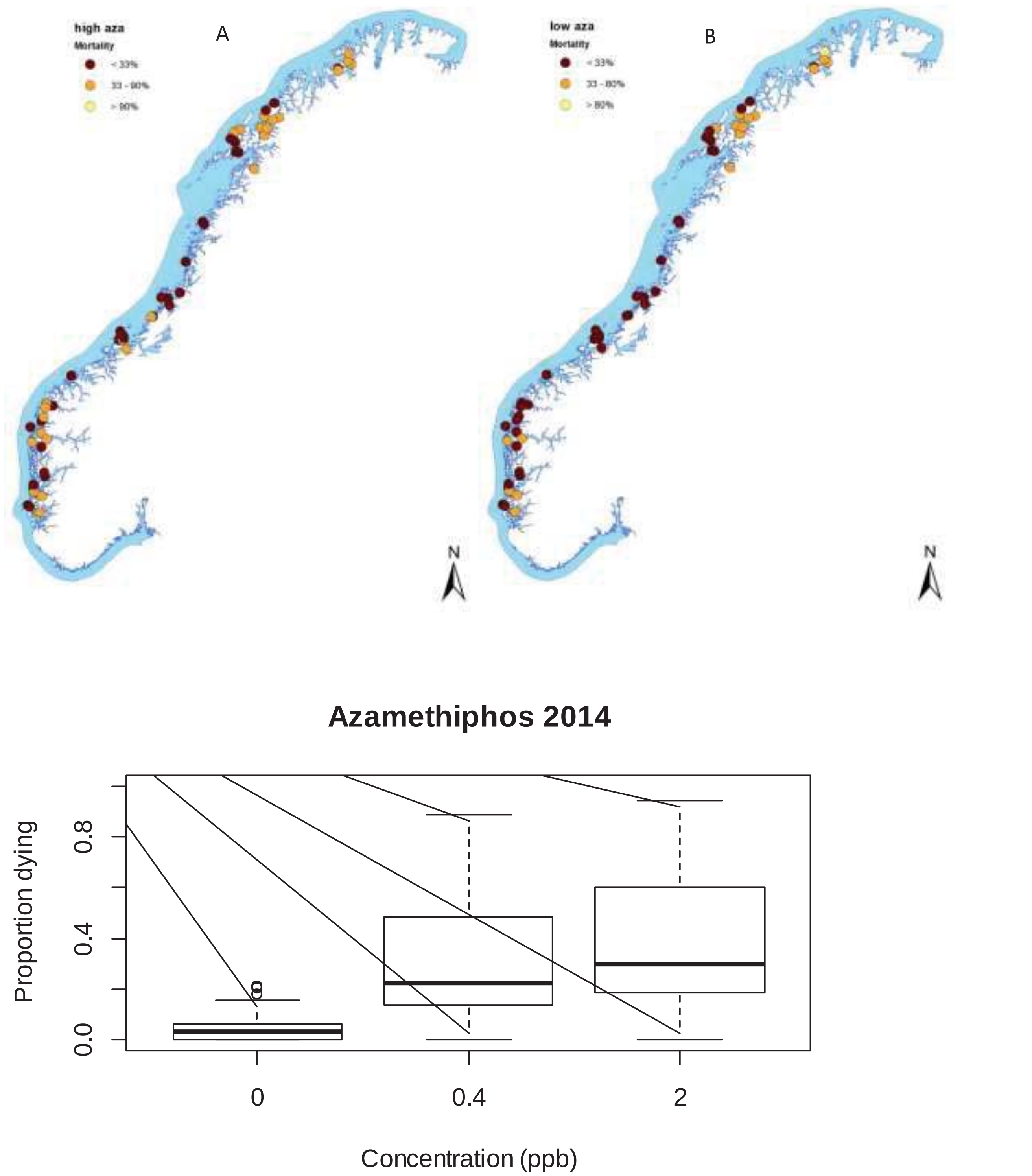
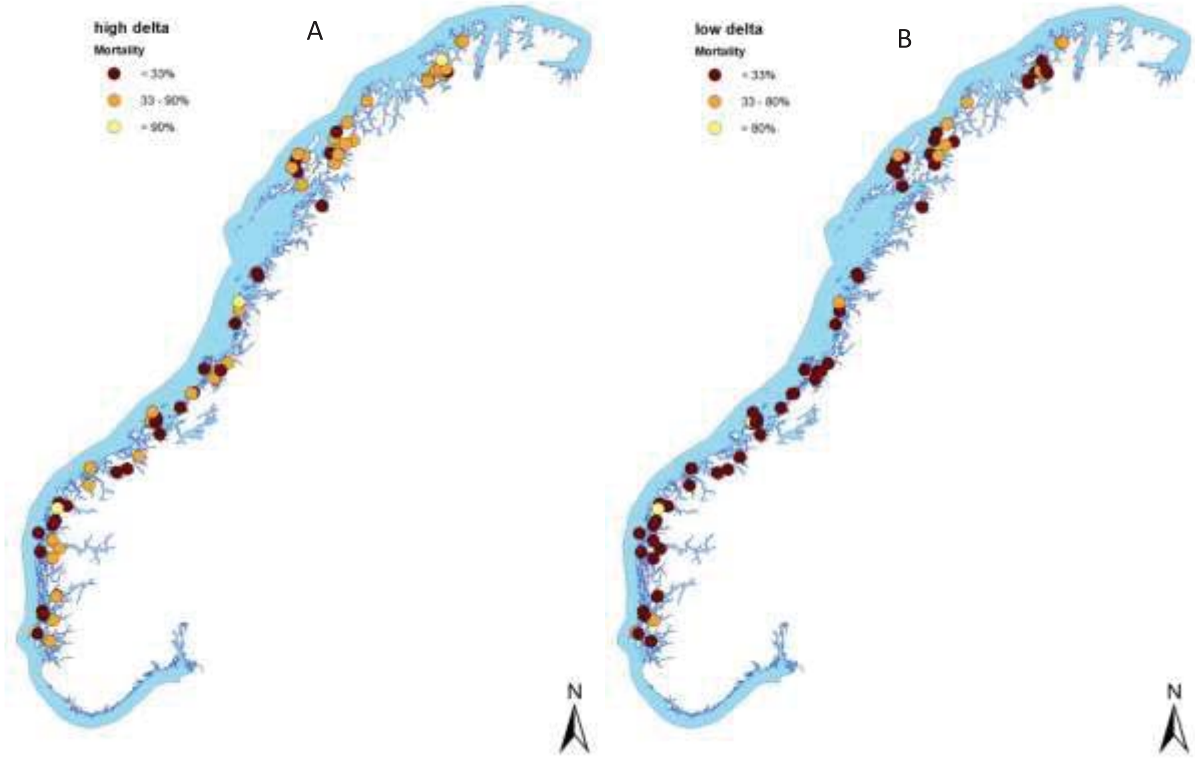


Figure 4. Maps showing categorical mortality in bioassay tests high (A) and low (B) *azamethiphos* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (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).



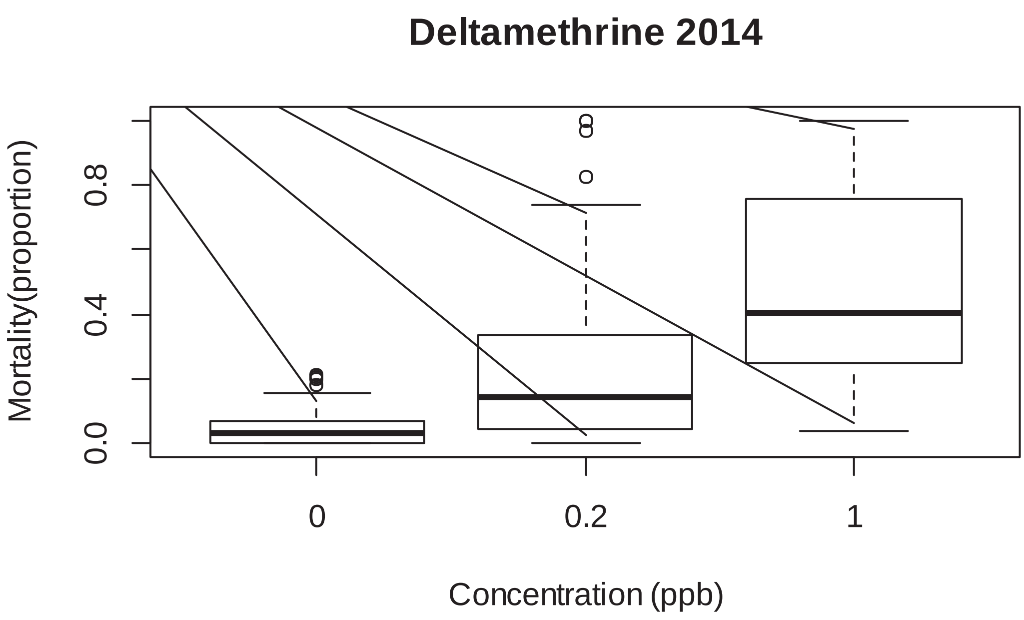


Figure 5. Maps showing categorical mortality in bioassay tests with high (A) and low (B) *deltamethrin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots denote mortalities between the two (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).

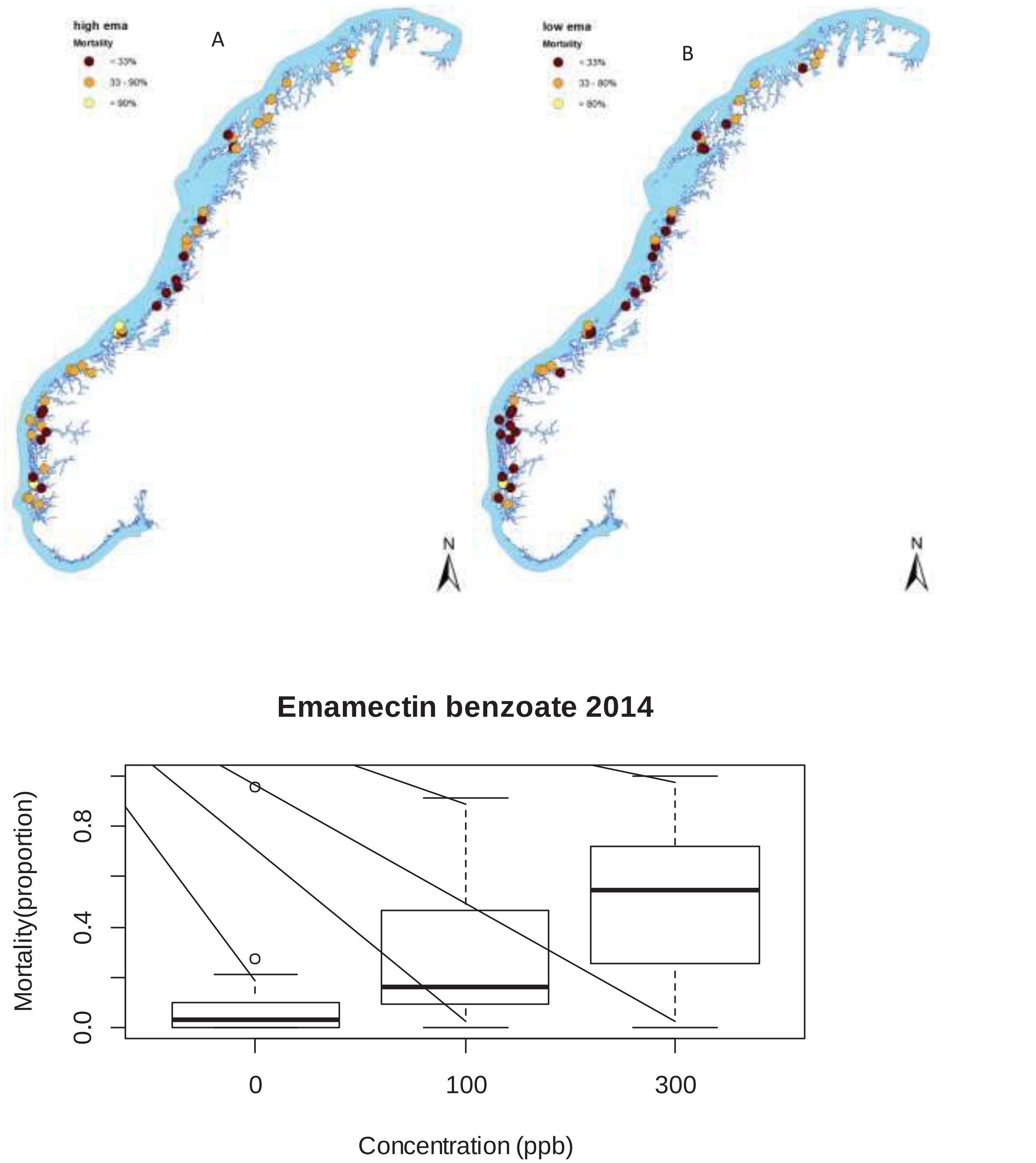


Figure 6. Maps showing categorical mortality in bioassay tests with high (A) and low (B) *emamectin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots 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).

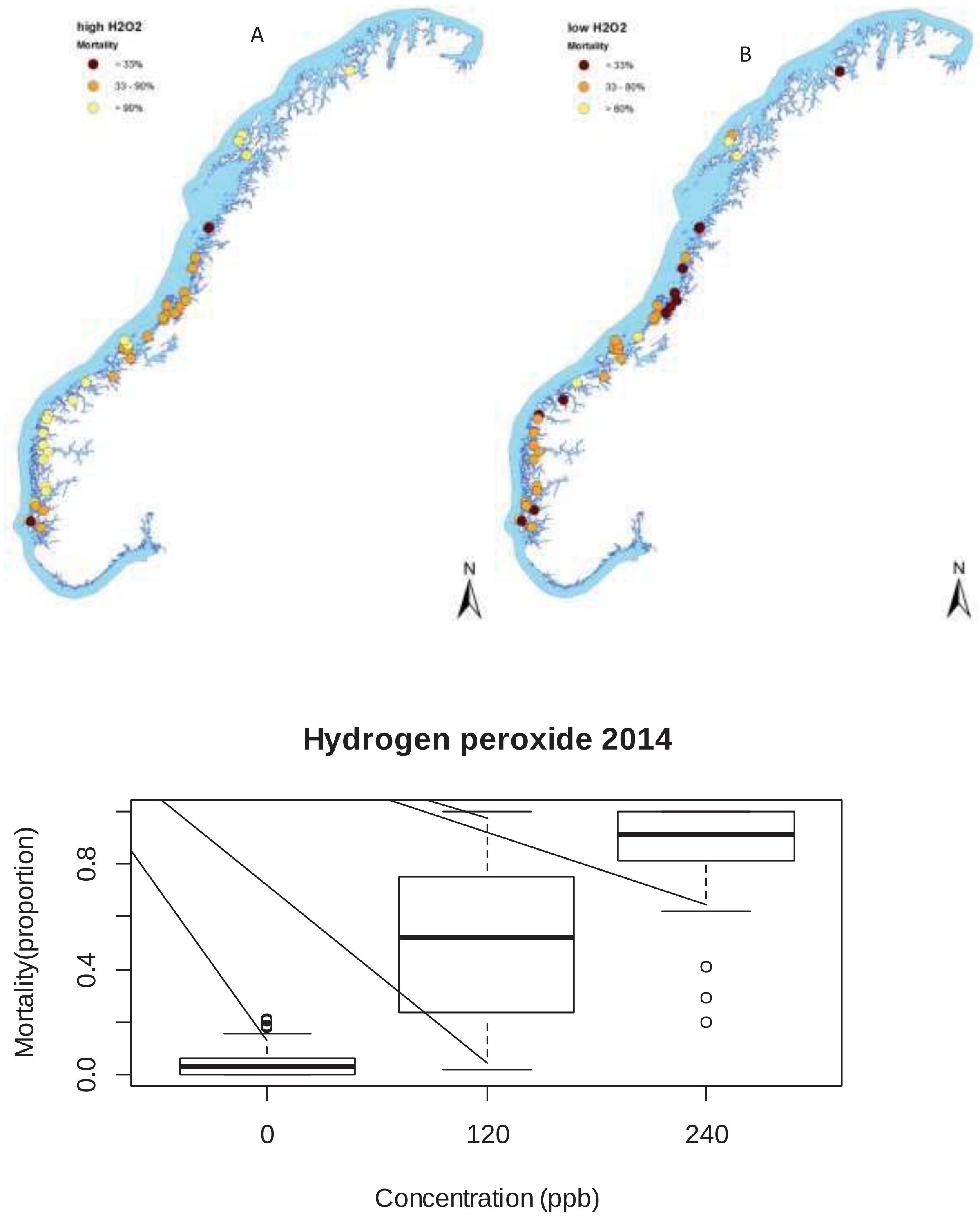


Figure 7: Maps showing categorical mortality in bioassay tests with high (A) and low (B) *hydrogen peroxide* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange 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).

Conclusions

Passive surveillance

VetReg data

- The total number of prescriptions of substances used to control salmon lice infections showed a pronounced increase in 2014 compared to the years 2011 – 2013. The coverage of the VetReg data of the total use of chemotherapeutica to control salmon lice is, however, uncertain.
- Increases were especially pronounced for hydrogen peroxide, flubenzurones and emamectin benzoate.
- The use of azamethiphos and pyrethroids showed much the same spatial distribution
- The use of emamectin benzoate has a comparably more northerly distribution
- Hydrogen peroxide use is restricted to smaller areas, whereas flubenzurones are used mostly on the south west coast

Reported sensitivity data

- No clear trends in the reported sensitivity data were observed.

Active surveillance

The program has succeeded in collecting sensitivity data along the coast. Implementation of standardized simplified bioassays has given comparable salmon lice mortality results from the test locations making it possible to assess the sensitivity status of salmon lice to azamethiphos, pyrethroids, emamectin benzoate and hydrogen peroxide along most of the Norwegian coast.

The survey shows that reduced sensitivity is widespread. The area with results indicating comparably sensitive salmon lice populations was in Finmark in the far north, although also here reduced sensitivity to different chemotherapeutants is indicated. Bioassay tests from the southernmost areas of salmon farming were not undertaken. Instead genotyping of parasites for azamethiphos and pyrethroid resistance markers was undertaken, demonstrating a low level of these resistance markers in this area.

Salmon lice mortalities in high concentration azamethiphos tests showed that low treatment efficacies can be expected especially in the areas northern Nordland/southern Troms, Trøndelag and Hordaland. For deltamethrin, salmon lice mortalities in high concentration tests indicate that several areas can expect low treatment efficacy, although the mortalities in high concentration tests varied a lot.

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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.

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The NFSA advises and reports to the Ministry of Agriculture and Food, the Ministry of Fisheries and Coastal Affaires and the Ministry of Health and Care Services.

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