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The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2013

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Introduction

Resistance to chemotherapeutants in salmon lice, *L. 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 azametiphos 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 has been lacking.

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 traits (passive surveillance), and present a collection of sensitivity data from approximately 50 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 2013.

Materials and methods

Passive surveillance

Veterinary medicine register data

The 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 sea lice infestations. In total over the years 2011 – 2013 there were 4834 prescriptions coupled to these categories of substances and to a specific farm site.

The five categories of substances are in the following termed azametiphos, 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 or Teflubenzuron). Tabell 1 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 sea 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) 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 sea lice required is fewer 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 62 farm locations were tested against the three chemotherapeutants deltamethrin, azametiphos and emamectin benzoate. 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 sea lice mortality in identified stages and genders (preadult I and II and adults; females and males) were noted as the test outcome. The sea lice mortality in the low concentration was used to indicate sensitivity status of the sea lice population, with sea lice mortality higher than 80% in parasites indicative of a fully sensitive population (as shown in preadulte parasites in Helgesen and Horsberg 2013).
The sea lice mortality in the high concentration was used to indicate the degree of reduced sensitivity and the expected outcome of a subsequent treatment, with sea lice mortality higher than 90% indicative for an expected treatment efficacy of 90% or more.

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

Medicament	Low concentration (ppb)	High concentration (ppb)
Deltamethrin	0.2	1
Azametiphos	0.4	2
Emamectin benzoate	100	500

Results and Discussion

Passive surveillance

VetReg data

Table 2 summarizes the number of prescriptions covering each substance/class of substances over the years 2011 – 2013, showing an increase in the number of prescriptions for pyrethroids and a reduction in the number of prescriptions for emamectin benzoat. For the other categories of substances there were less obvious trends over time. As the amounts prescribed could not be calculated, they could also not be validated against sales data from wholesalers (http://www.fhi.no/eway/default.aspx?pid=239&trg=Area_7064&Main_6157=6263:0:25,6201&MainContent_6263=7064:0:25,6201&Area_7064=6178:109416::0:7065:1:::0:0). Thus, the results should be interpreted with care.

Table 2: Number of prescriptions for the given category of substances used to control sea lice during 2011 - 2013.

Substance category	2011	2012	2013
Azametiphos	451	617	448
Pyrethroids	501	1005	1065
Emamectin benzoat	245	50	47
Hydrogenperoxide	167	60	68
Flubenzurones	22	62	26
Sum	1386	1794	1654

The maps in figure 1 sum up the total number of prescriptions per location during 2012 and 2013. In 2012 there were prescriptions coupled to 548 farm locations, with a mean number of prescriptions per farm of 3,27 (range 1 – 17). Comparable numbers for 2013 were 560 farm locations, with a mean of 2,95 prescriptions per farm (range 1 – 16), respectively

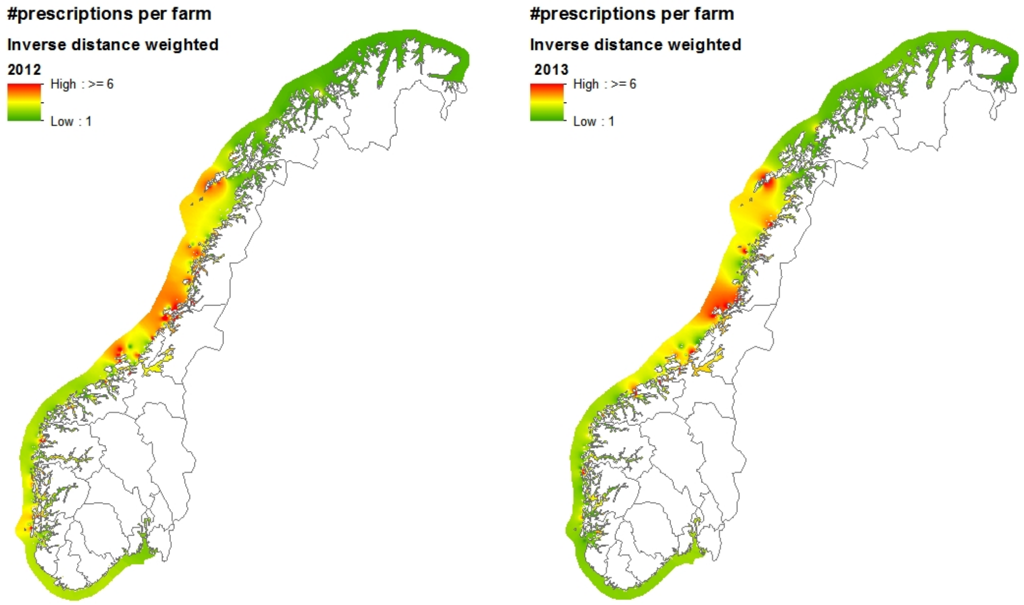
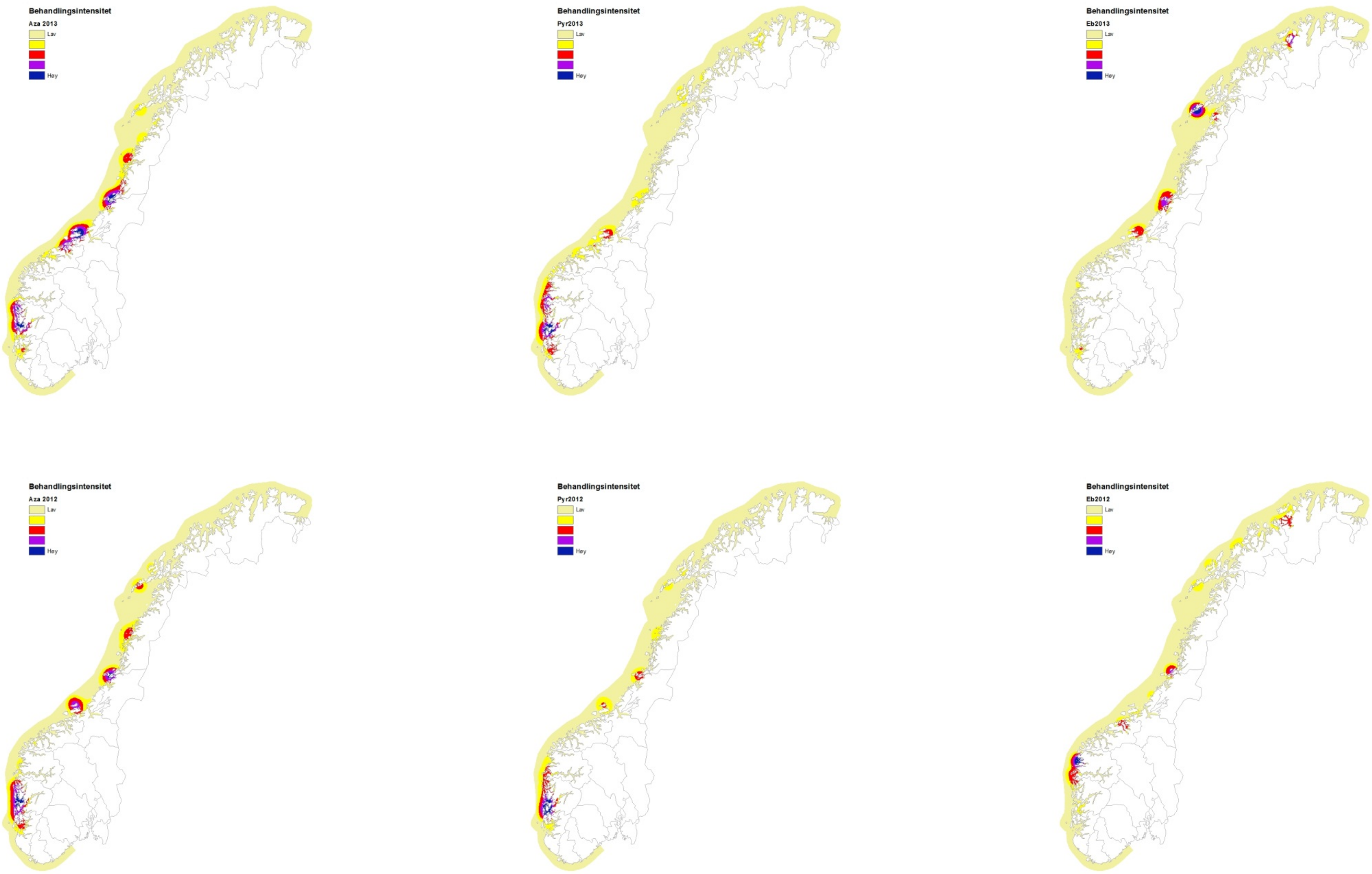


Figure 1: Inverse distance weighted (IDW) interpolation of the number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 6 prescriptions per location is expected, while dark green denote areas where the expectation of one treatment 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.

2013

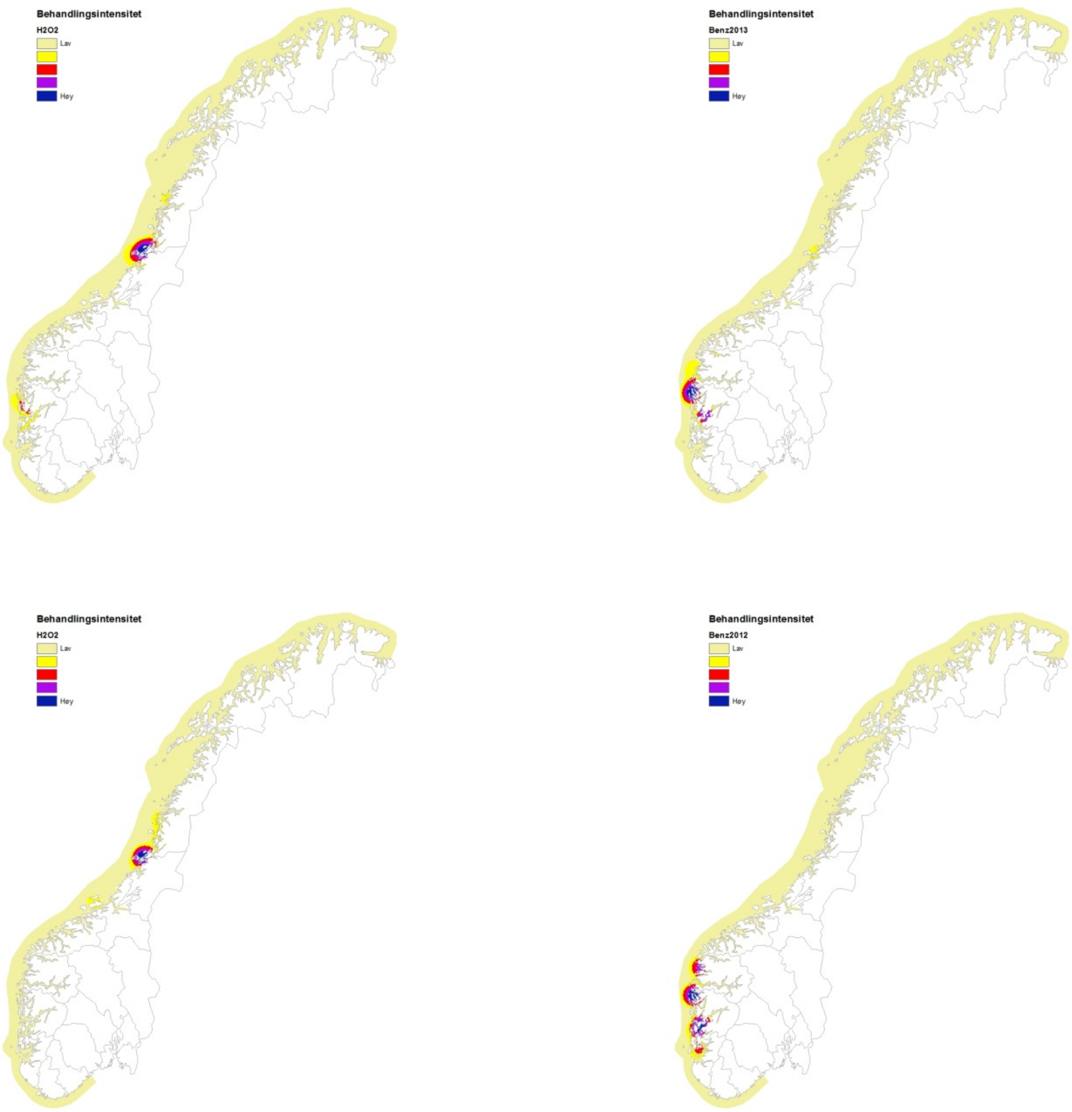
2012



Azametiphos

Pyrethroids

Emamectin benzoat



2013

2012

H_2O_2

Flubenzurones

Figure 2: Kernel densities of prescriptions for five different substances used to control sealice infestations in salmonid farms in 2012 (lower panel) and 2013 (top panel). 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.

The use of azametiphos and pyrethroids show much the same spatial distribution. The use of emamectin benzoate seems to be distributed comparatively more northerly. H2O2 use is restricted to smaller areas, especially on the coast of Nord Trøndelag, whereas the flubenzurones are used mostly on the south west coast.

Reported sensitivity data

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

	2012			2013		
Categories	**Sensitive**	**Reduced sens.**	**Resistant**	**Sensitive**	**Reduced sens.**	**Resistant**
Azametiphos	55	18	4	18	28	15
Emamectin benzoat	3	56	4	3	6	2
Flubenzurones		4			3	
H2O2					5	
Pyrethroids	17	127	26	43	52	8
Totalsum	**75**	**205**	**34**	**64**	**94**	**25**

With regard to the sensitivity status reported from sensitivity tests there are no clear trends in the data. Sensitivity for azametiphos seem shifted towards reduced sensitivity and resistance in 2013 compared to 2012, whereas the opposite seems to apply for pyrethroids.

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).

	2012				2013		
Categories	**1**	**2**	**3**	**9**	**1**	**2**	**3**
Azametiphos	18	8	8		15	11	
Emamectin benzoat	9	9	46	1	1	1	
Flubenzurones	1	1					
H2O2						5	1
Pyrethroids	31	12	1	3	16	23	2
Total	**59**	**30**	**55**	**4**	**32**	**40**	**3**

Active surveillance

Altogether, 145 simplified bioassay tests on sea lice from 62 different salmon farm locations along the cost (figure 3) were performed for azametiphos (50 tests, 2 repeated locations), deltametrin (56) and emamectin (39).

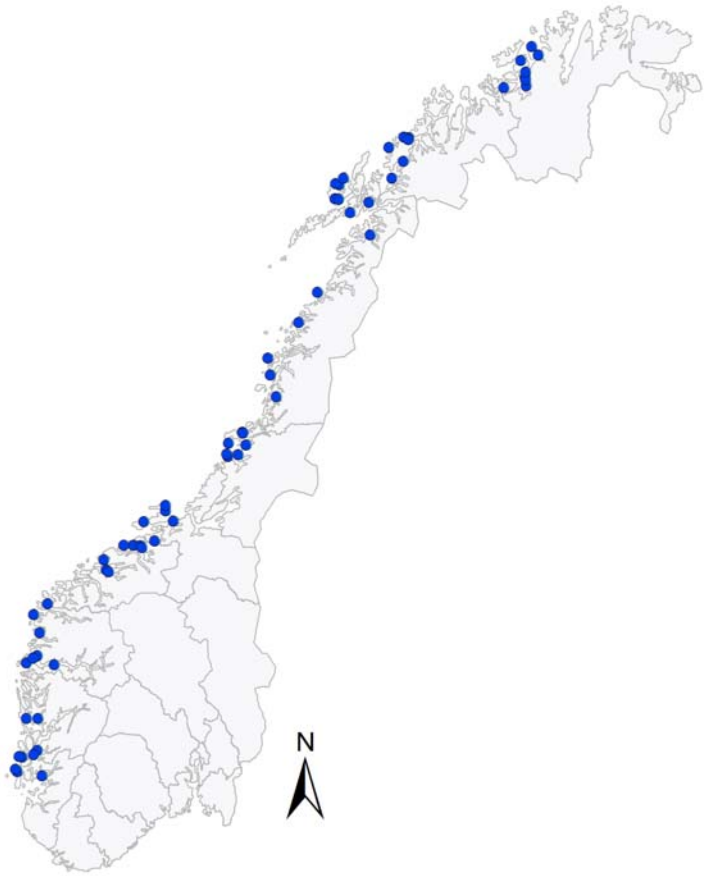


Figure 3: Locations of farms where sea lice were collected for simplified bioassay testing.

Table 5 summarizes the outcome of all the 145 simplified bioassays according to categories of mortality. Differences in mortality rates between genders and/or developmental stages are not presented in the table. For pyrethroids and azametiphos, 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 sea 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 sea lice is widespread in Norwegian salmon farming. Table 6 shows that the sea lice mortality results from low and high concentrations are significantly correlated, with highest correlations for azametiphos and pyrethroids. These correlations show that the results from low and high concentration tests are consistent.

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

Also for deltamethrin, the only area with highly sensitive sea lice was in Finnmark County (figure 5A). Sea lice mortalities in high concentration deltamethin tests (Figure 5B) indicates that several areas can expect low treatment efficacy, although the mortalities in high concentration tests varied a lot, as shown in the box plot.

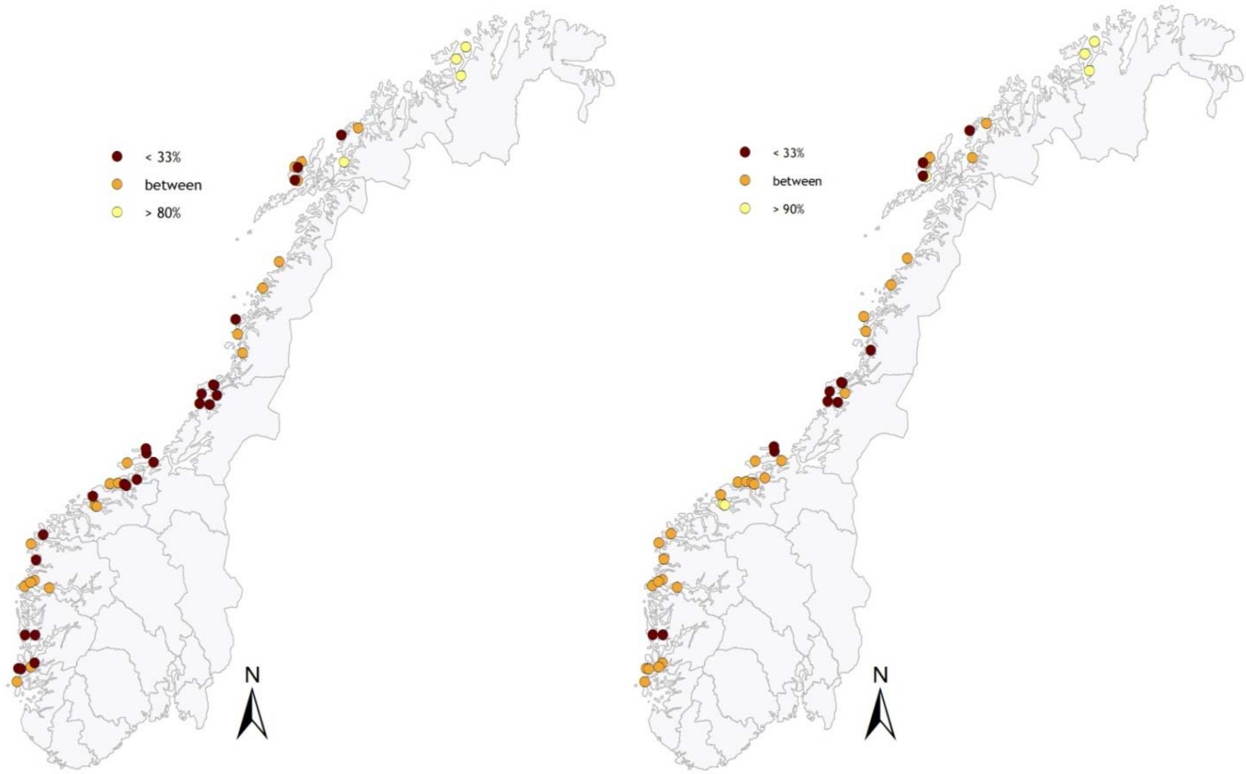
The low concentration emamectin benzoate tests (Figure 6A), showed that reduced sensitivity is widespread, but varies considerably (boxplot). One explanation for the variations in emamectin sensitivity may be variable sensitivity between developmental stages and genders. The high concentration emamectin tests (Figure 6B) resulted in comparably high mortalities. We suspect, however, that the emamectin dose in these assays was set too high compared to the doses in a full scale treatment, resulting in an underestimation of the frequency of resistant populations.

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 azametiphos where tests were duplicated on two farms, conducted at 48 different farms). Column numbers denote the number of tests that fell within the high, intermediate or low mortality classifications for each drug and test-concentration.*

		Mortality classification (number of observations)		
Low concentration	**Total**	**High (> 80 %)**	**Intermediate (80 – 33 %)**	**Low (< 33 %)**
Azametiphos	50*	5	20	25
Deltamethrin	56	8	8	40
EMB	39	2	10	27
High concentration	**Total**	**High (> 90 %)**	**Intermediate (90 – 33 %)**	**Low (< 33 %)**
Azametiphos	50*	7	29	14
Deltamethrin	56	12	23	21
EMB	39	12	25	2

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.

		N	**Spearman Correlation Coefficients**	**Prob > |r| under H0: Rho=0**
Azametiphos		50	0.75	< 0.001
Deltamethrin		56	0.72	< 0.001
EMB		39	0.50	= 0.001



A B

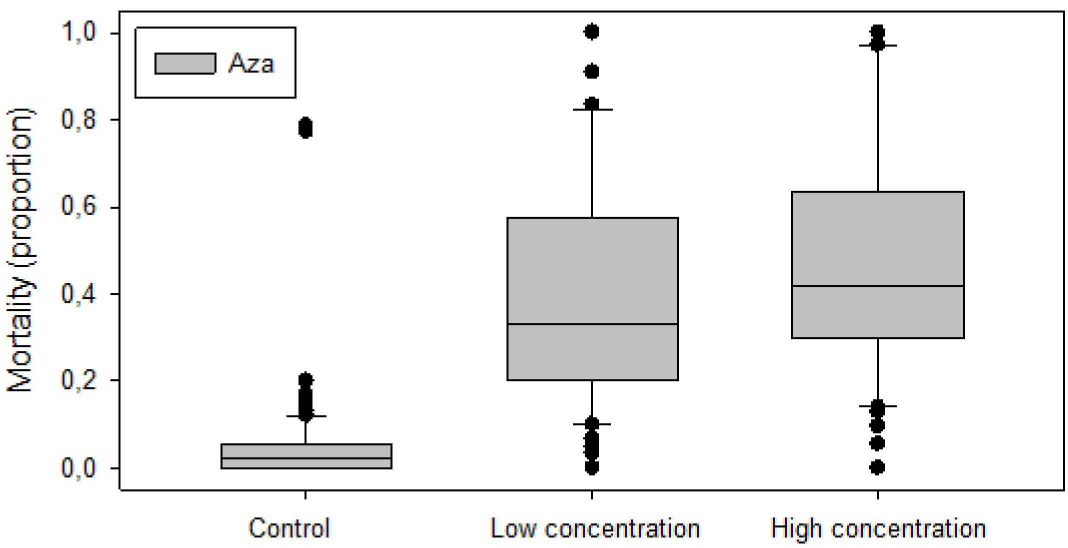
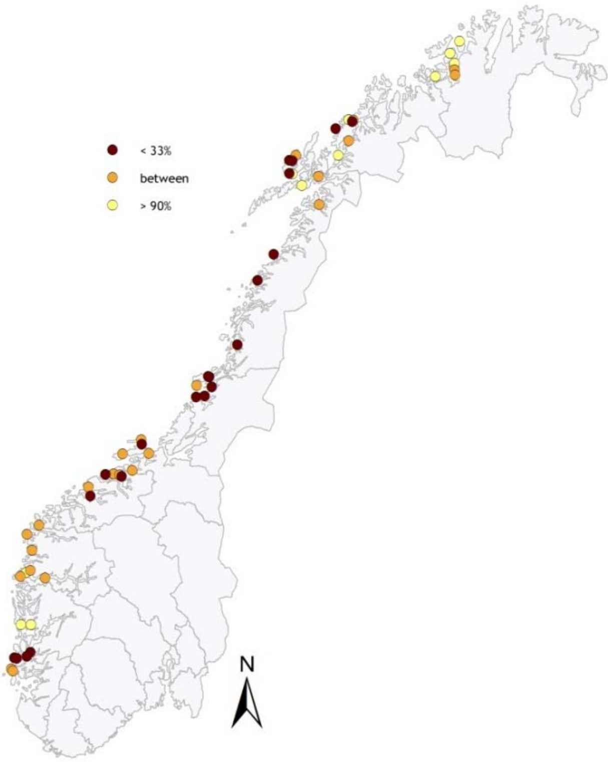
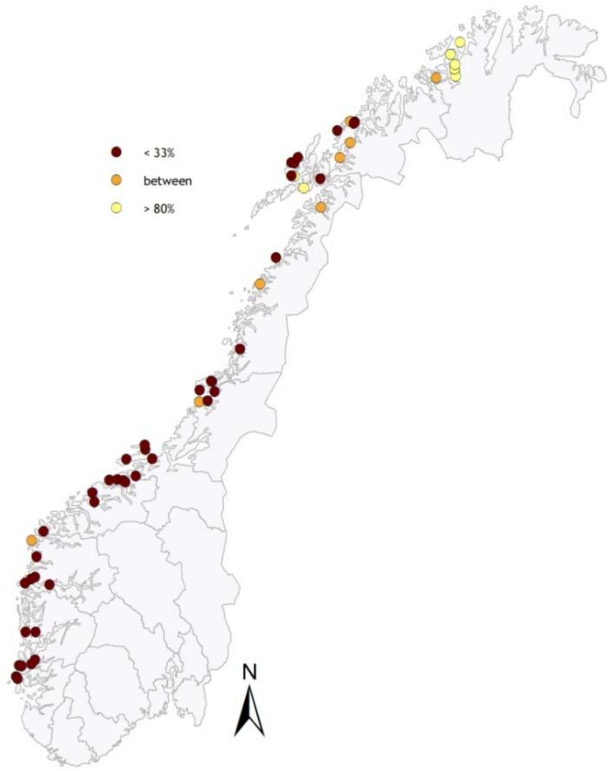


Figure 4: Maps showing categorical mortality in bioassay tests with low (A) and high (B) *azametiphos* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots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



A



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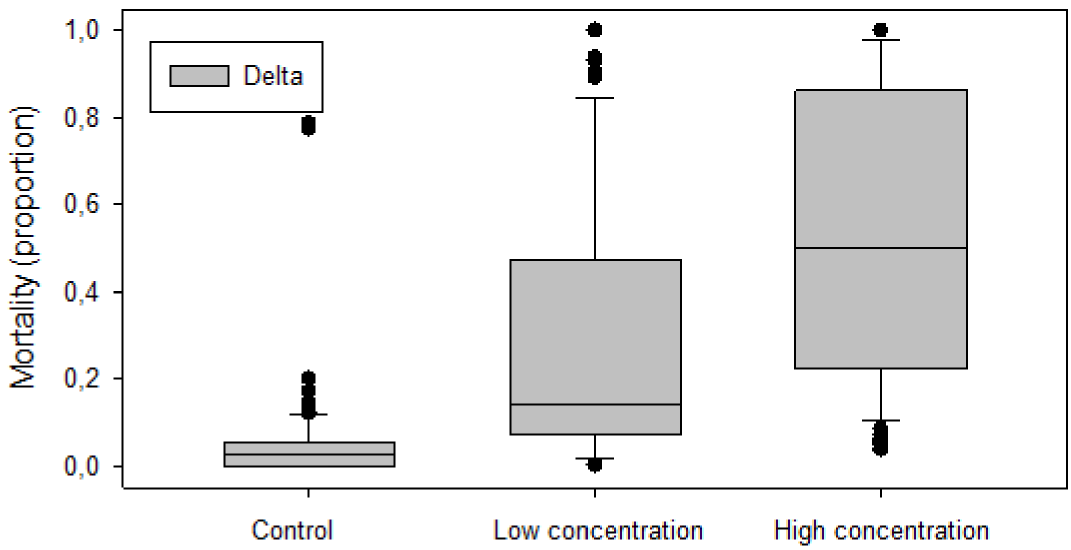

Figure 5: Maps showing categorical mortality in bioassay tests with low (A) and high (B) *deltamethrin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots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

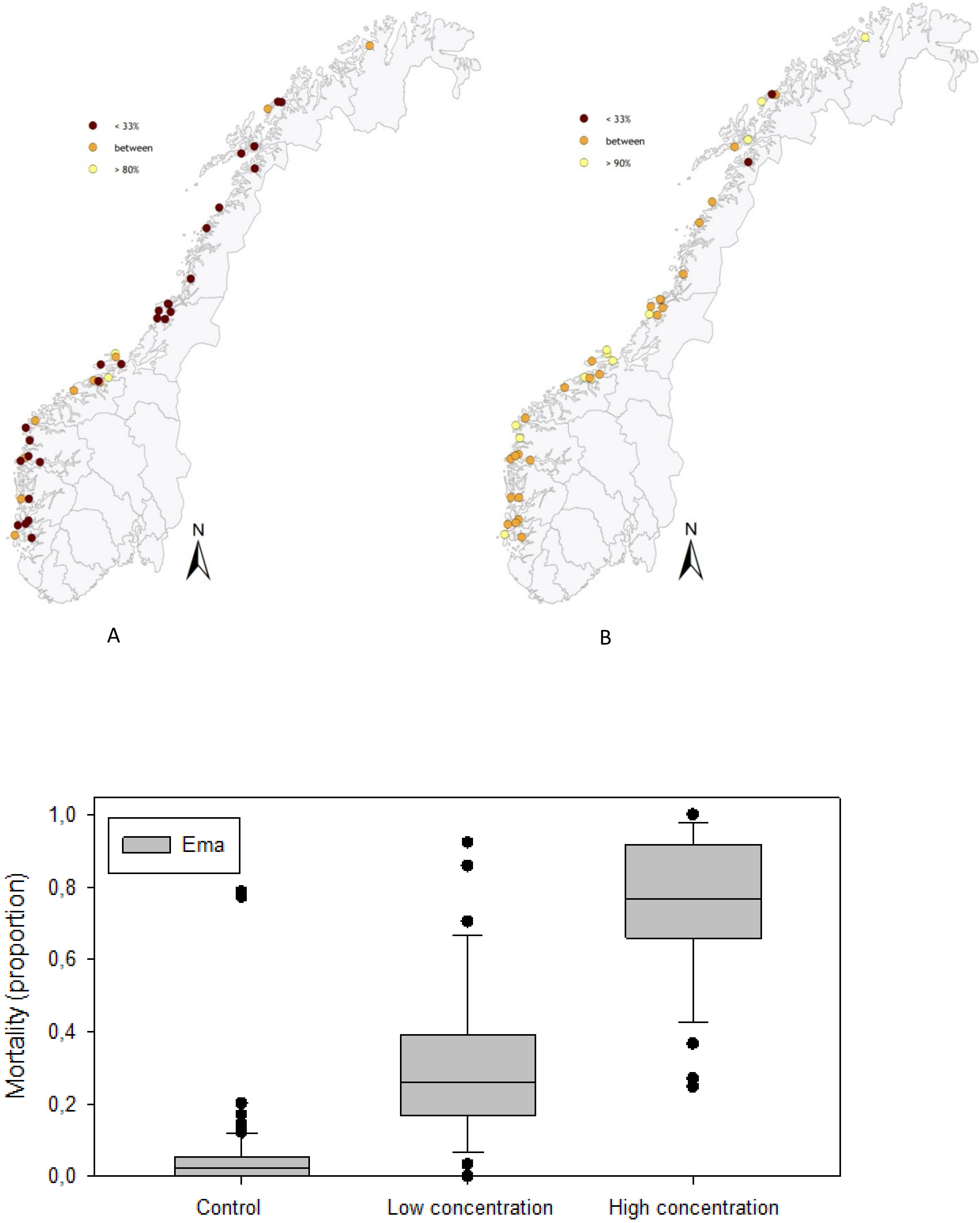

Figure 6: Maps showing categorical mortality in bioassay tests with low (A) and high (B) *emamectin* concentrations. Dark brown dots denote tests where less than 33% of the lice died, yellow dots denote mortalities in excess of 80% (low concentration) or 90% (high concentration tests) and orange dots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

Conclusions

Passive surveillance

VetReg data

- The total number of prescriptions of substances used to control salmon lice infections did not change notably over the years 2011 – 2013. The coverage of the VetReg data of the total use of chemotherapeutica to control salmon lice is, however, uncertain.
- Summarizing the numbers of prescriptions for different substances gave an increase in pyrethroid prescriptions and a reduction in emamectin benzoate prescriptions over the years 2011 – 2013.
- The use of azametiphos and pyrethroids showed much the same spatial distribution
- The use of emamectin benzoate has a comparably more northerly distribution
- Hydrogenperoxide 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 azametifos, pyrethroids and emamectin benzoate along most of the Norwegian coast.

The survey shows that reduced sensitivity is widespread. The only area with results indicating sensitive sea lice populations was in Finmark County in the far north. Tests from the southernmost areas of salmon farming were not undertaken, implying uncertain sensitivity status in the south.

Salmon lice mortalities in high concentration azametiphos 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. For emamectin benzoate, we expect that the high dose chosen for the tests may underestimate the frequency of resistant populations.

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