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Original Article

Aquaculture-driven evolution: distribution of pyrethroid resistance in the salmon louse throughout the North Atlantic in the years 2000–2017

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The parasitic salmon louse, and its documented resistance to chemotherapeutants, represents the most persistent environmental challenge to global salmonid aquaculture. We used a genetic marker associated with pyrethroid resistance to analyse ∼15 000 lice collected from the North Atlantic in the period 2000–2017. The genotype associated with resistance was not detected in lice collected from throughout the North Atlantic in the year 2000 or 2002. However, by the year 2009 onwards, it was found in lice from fish farms throughout much of the North Atlantic. It was also found in modest frequencies in lice collected from wild Atlantic salmon captured off Greenland. The most recent samples displayed very high frequencies of the genotype associated with resistance, particularly in intensive aquaculture regions of Norway (>90%) and Scotland (>70%). These results closely align with observations from the field. We suggest that pyrethroid resistance first emerged in Europe just before or around the year 2000 and was thereafter dispersed throughout much of the North Atlantic where its increased frequency was driven by extensive pyrethroid use. Although the resistant genotype was not detected in lice from Canada, it is likely to occur in very low frequencies that would quickly increase if pyrethroids were to be used in that region.

Keywords: aquaculture, Atlantic salmon, copepod, genetic, North Atlantic, parasite, resistance

Introduction

All food producing systems are challenged by organisms that slow down or suppress production. Plant producers have pests and weeds to fight, while animal breeders are challenged by parasites and diseases. As a result, most industrial food production is dependent on chemicals to protect crops or stocks (Oerke, 2006; Alonso-Dìaz *et al.*, 2014). When pests or parasites develop resistance to chemotherapeutants, consequences can be severe for food production and security (Clark and Yamaguchi, 2001). Global salmonid aquaculture also experiences this challenge, and in marine net-pens where fish are reared, parasitic salmon lice (*Lepeophtheirus salmonis*) that have developed resistance to various chemotherapeutants constitute a major problem (Torrissen *et al.*, 2013; Aaen *et al.*, 2015; Taranger *et al.*, 2015; Murray *et al.*, 2016).

The salmon louse is an endemic ectoparasitic copepod in the North Atlantic and Pacific, specializing on salmonids (Kabata,

1979; Skern-Mauritzen *et al.*, 2014). Chemotherapeutants were used to control infestations of salmon lice in salmonid aquaculture already in the 1970s (Pike, 1989). Organophosphates were introduced first (Brandal and Egidius, 1979), followed by pyrethroids (Jakobsen and Holm, 1990), hydrogen peroxide (Johnson *et al.*, 1993), avermectins (Johnson and Margolis, 1993) and benzoylphenyl ureas (Erdal *et al.*, 1997; Ritchie *et al.*, 1997). Repeated use of a chemotherapeutant drives the development of resistance (Denholm *et al.*, 2002). Now, salmon lice display reduced sensitivity and/or resistance to all the chemotherapeutents used in commercial salmonid aquaculture, except the benzoylphenyl ureas (Aaen *et al.*, 2015; Helgesen *et al.*, 2015, 2019). Organophosphates were used almost exclusively until resistance became widespread (Jones *et al.*, 1992) and were replaced by pyrethroids in Norway and other European salmon-producing countries (Denholm *et al.*, 2002; Sevatdal *et al.*, 2005; Aaen *et al.*, 2015). The first commercial use of pyrethroids in Norwegian aquaculture was in 1994, and by 1999, ∼90% of the delousing treatments in Norwegian fish farms were based upon pyrethroids (Denholm *et al.*, 2002). However, reports of treatment failure were registered in some farms in one county in Norway by 2000 (Sevatdal and Horsberg, 2000, 2003). Indications of reduced sensitivity to pyrethroids were also found through bioassays conducted in Ireland in 2001 and in Scotland in 2002 (Sevatdal *et al.*, 2005).

Population genetic studies of the salmon louse in the Pacific (Messmer *et al.*, 2011) and Atlantic Ocean (Todd *et al.*, 2004; Tjensvoll *et al.*, 2006; Glover *et al.*, 2011) have revealed a species characterized by extensive gene flow across large regions. By combining population-genomics, linkage-mapping and haplotyping analysis in parts of the genome where selective sweeps had been identified, Besnier *et al.* (2014) demonstrated that resistance to the delousing chemotherapeutant emamectin benzoate (avermectin) most probably evolved in lice from a single farm source and was thereafter dispersed to lice throughout the North Atlantic in <11 years. Similarly, the *Phe362Tyr* mutation that causes resistance to organophosphates (Kaur *et al.*, 2015) has been found in lice from all regions of the North Atlantic, although multiple origins for organophosphate resistance were indicated (Kaur *et al.*, 2017). The same mutation responsible for organophosphate resistance has also been observed in high frequencies on lice collected on wild Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) in Norway, demonstrating that wild salmonids can both host and help disperse resistant lice (Fjørtoft *et al.*, 2017). A recent study on pyrethroid resistance from farmed and wild hosts in Norway, using the same marker of resistance as the present study, demonstrated the same tendencies (Fjørtoft *et al.*, 2019). These authors found that pyrethroid-resistant lice existed in high frequencies on wild sea trout and wild Atlantic salmon returning from the ocean. Collectively, these studies demonstrate that the salmon louse is a species in which resistance to chemotherapeutants can quickly emerge and disperse over vast distances. Studying the patterns of development and dispersal of resistance provides information to advise future management strategies as and when new chemotherapeutants become commercially available.

Although the exact mode of resistance is not understood, recent investigations have demonstrated that pyrethroid resistance in *L. salmonis* is maternally inherited via mitochondrial DNA (mtDNA) (Nilsen and Espedal, 2015; Carmona-Antoñanzas *et al.*, 2017; Bakke *et al.*, 2018). A patented mtDNA genetic marker that is closely associated with pyrethroid resistance in salmon lice has also been developed (Nilsen and Espedal, 2015). The marker patent included extensive phenotyping and genotyping analyses that collectively validate a non-causative but strong association between the genotype of lice at the developed marker and survival of lice in controlled studies as well as in the field (Nilsen and Espedal, 2015). This marker has been used to genotype ∼15 000 lice from ∼200 fish farms in the United Kingdom and Norway to test sensitivity of lice within cages prior to delousing. In addition, a set of lice samples spanning the entire North Atlantic in the period 2000–2017 have been genotyped with the marker. These data that provide a unique insight into the spatial and temporal patterns of pyrethroid resistance are presented here.

Methods

Overall study design

The study is based on the following two components: (i) a spatial–temporal analysis of pyrethroid resistance in 1462 lice collected from the North Atlantic in the period 2000–2017 to investigate resistance dispersal in the pan-Atlantic salmon louse population and (ii) a high-resolution analysis of pyrethroid resistance of >11 000 lice collected from commercial fish farms in Norway (2012–2015) and of >3500 lice collected from fish farms in Scotland (2014–2017) to investigate how resistance disperses locally under selection.

Genotyping

All of the lice in this study were genotyped using the patented marker for pyrethroid resistance (Nilsen and Espedal, 2015). Genotypes resulting from the analysis of this mtDNA marker are hereon referred to as resistant and sensitive, as mtDNA does not display recombination and thus heterozygote genotypes. While the marker does not cause pyrethroid resistance, extensive laboratory and field studies documented within the patent, comparing survival and genotype, demonstrate a strong association between the marker and the phenotype (Nilsen and Espedal, 2015).

All genotyping was performed by the commercial company PatoGen AS in their ISO accredited laboratory in Norway. In short, genotyping consisted of a reverse transcriptase real time/quantitative polymerase chain reaction (TaqMan) 5′-nuclease assay using the following primers and probe: forward primer: TTCTTACAGACAAAGCTAAAGCCACTA, reverse primer: AGTAACTCCTGCTCACATTCAACCT, and probe: CCCCCCC/TAACTTAT. A one-step amplification (45 cycles) was performed on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions. Resulting genotypes were scored as resistant or sensitive.

Spatial–temporal analysis of pyrethroid resistance throughout the North Atlantic 2000–2017

A total of 1462 lice collected from throughout the North Atlantic in the period 2000–2017 were genotyped. These samples included 753 lice that have been used in previous population genetic and genomic studies (Tjensvoll *et al.*, 2006; Glover *et al.*, 2011; Besnier *et al.*, 2014). The majority of these lice was sampled from fish farms in Northern Europe and Canada but also includes 31 salmon lice sampled from wild Atlantic salmon in Russia in 2000. In addition, 399 salmon lice were collected from the North Atlantic in 2016 and 2017. These included lice sampled from farms in Canada, Iceland, Ireland, Scotland, and the Faroe Islands, and lice sampled from wild Atlantic salmon captured on

the west coast of Greenland. Samples originating from farmed fish were collected by farm employees or by fish vet personnel during routine lice counts or sampling. Samples from wild salmon were collected by researchers from fish caught by local fishermen.

In Norway, >11 000 samples of lice were collected from fish farms in the period 2012–2015 (see below for full description). For the spatial–temporal analysis across the North Atlantic, we used salmon lice sampled from fish farms in 2015 from the same regions where we had samples from 2000 to 2009 to balance the design (Southern Norway $N = 38$, Western Norway $N = 2378$, Northern Norway $N = 746$, Finnmark $N = 149$).

A binominal generalized linerar model (GLM) with logit link function was fitted for the results from the North Atlantic.

$$\text{logit}(Y) = \alpha + \beta_1 T + \beta_2 S + \text{e}, \qquad \text{(Model 1)}$$

where Y is the frequency of the resistant genotype in each sample, T is the sampling year, and S is the sampling site.

To avoid numerical singularity when fitting the GLM, an epsilon equal to 0.001 was added to the observed frequency of resistant lice in all samples. This way, all observed frequencies were strictly greater than zero and the GLM algorithm converged correctly. A separate binominal GLM with logit link function was fitted for the samples from 2009 to test for differences between locations. For the regions where data from both 2009 and 2016/2017 were available, each region was tested separately for differences over time. Finally, for all samples from Norway, a separate model was fitted to test for variation in the frequency of resistance both for time and location. The pooled Norwegian data were compared to the frequency results from the other North Atlantic locations sampled in 2016 and 2017.

High-resolution screening of pyrethroid resistance in Norwegian fish farms in the period 2012–2015

A total of 11 326 salmon lice collected from 116 salmon farms along the Norwegian coast were genotyped. These were sampled in the period 2012–2015, and some farms were sampled several times both within and between years. These data were thereafter used to find the prevalence of the resistant genotype at the municipality and county levels.

All delousing treatments are reported to the Norwegian food safety authorities and are publicly available (BarentsWatch, 2017). The locations and sample dates of the batches of salmon lice collected from Norwegian fish farms in the period 2012–2015 were aligned to the information on treatments with the pyrethroids deltamethrin and cypermethrin. Immediately after a treatment, the prevalence of resistant salmon lice will be higher than what is representative for the region. To avoid skewness in this direction, a new indicator variable was added to the model, this new variable had a value of "1" for samples collected from farms that used pyrethroids within the last 4 weeks before the sample date and "0" otherwise. Four weeks is the approximate time for the emergence of one generation of salmon lice after the treatment, dependent on the temperature (Samsing *et al.*, 2016). In total, genotype results from 10 355 salmon lice sampled at 95 locations were retained for the analyses of spatial and temporal patterns in Norwegian farms.

The frequency of the resistant genotype in Norwegian farms was compared between years and regions.

$$\text{logit}(Y) = \alpha + \beta_1 T + \beta_2 S + \beta_3 I + \text{e}, \qquad \text{(Model 2)}$$

where Y is the frequency of the resistant genotype in each sample, T is the sampling year, and S is the sampling site. I is a binary indicator that is equal to 1 for all sampled farms that were treated with pyrethroids 4 weeks or less previous to sampling, and e is a vector of normally distributed residuals. More information on model estimates is given in Supplementary Table S1.

High-resolution screening of pyrethroid resistance in Scottish fish farms in the period 2015–2017

A total of 3532 salmon lice from 77 fish farms in Scotland were genotyped. Lice originating from the counties Western Isles (Eilean Siar), Highland, Argyll and Bute, and North Ayrshire were sampled between 2014 and 2017. For each batch of lice collected from a farm, the number of lice displaying the resistant genotype was reported. These data were used to find the frequency of the resistant genotype at marine management area and region levels.

All chemotherapeutant use in Scottish aquaculture is reported monthly to the authorities (Scotland's Aquaculture, 2018). By accessing the information on pyrethroid use for each sampled location, we were able to identify farms that had been treated within the same month or the month before the lice were sampled. As for the Norwegian farm data, a new indicator variable was added to the model, to identify samples from these farms. More information on model estimates is given in Supplementary Table S2. A total of 3292 lice from 58 locations remained, and all sampled between 2015 and 2017.

To investigate the development in the frequency of resistance over time and between regions within Scotland, resistance was modelled as a binary response (R/S) in a GLM with binominal family as in model 2.

Ethics approval

The salmon louse is not covered by the Norwegian Animal Welfare Act, nor by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, but the host of the salmon louse is.

Salmon lice sampled from 2012 to 2015 in Norway, 2014 to 2017 in Scotland, and 2016 and 2017 in the remaining North Atlantic were with one exception collected from farmed salmon. All sampling was conducted with the consent of the fish farmer and was thus not subject to further licencing. Most lice were sampled during routine lice counting and did not harm the fish. The Greenlandic salmon lice were sampled from wild Atlantic salmon caught and killed by local fishermen. Personnel from the National Oceanic and Atmospheric Administration Fisheries Service collected the lice. The researchers thus took the advantage of ongoing fishery activities and did not contribute to extra mortality on the wild Atlantic salmon stock. Given the design of the study, further consideration by an ethical committee was not necessary.

Results

Spatial–temporal analysis of pyrethroid resistance throughout the North Atlantic in the period 2000–2017

The resistant genotype was not detected in salmon lice sampled from wild Russian Atlantic salmon or farmed salmon in Norway in 2000, nor in lice sampled from fish farms in Canada, Scotland,

and Norway in 2002 (Figure 1). However, in 2009, it was found in 99 and 57% of the salmon lice sampled from farms in Shetland and Ireland, respectively. In the Norwegian samples collected from fish farms in 2009, the resistant genotype displayed a frequency of 68% in Northern Norway and 28% in Western Norway. By 2015, the resistant genotype was found in lice from fish farms in all parts of Norway, with up to 90% in the west. In the remaining North Atlantic, only samples from fish farms in Canada remained with no detection of the resistant genotype in lice sampled in 2017. In the Faroe Islands sample from fish farms in 2016, the resistant genotype only displayed a frequency of 3% and was not detected in the 2009 sample. In lice from Iceland, where delousing chemotherapeutants have never been used in the time line of relevance for the present study, the resistant genotype was found in 12% of the sampled lice from fish farms, while it was found in 20% of the lice sampled from wild Atlantic salmon in Greenland. In the Irish sample from 2016, the frequency of the resistant genotype had decreased significantly from the 2009 level of 57–21% ($df = 1$, $\chi^2 = 20.45$, $p = 6 \times 10^{-6}$), both samples obtained from farmed salmon. In Scotland, the frequency was 48% in lice sampled from fish farms in 2016. The full dataset is available in Supplementary Table S3.

There was statistically significant variation in the frequency of the resistant genotype between the locations sampled in 2009 ($df = 8$, $\chi^2 = 147.8$, $p < 2 \times 10^{-16}$). The sample from Canada had no lice displaying the resistant genotype and was considered as the reference point for further comparisons. The lice from the Faroes were not significantly different from the Canadian sample ($df = 1$, $\chi^2 = 0.98$, $p = 0.32$), but the samples from Ireland, Shetland, Northern Norway, and Western Norway differed significantly (respectively, $df = 1$, $\chi^2 = 103$, $p < 2 \times 10^{-16}$, $df = 1$, $\chi^2 = 332$, $p < 2 \times 10^{-16}$, $df = 1$,$\chi^2 = 284$, $p < 2 \times 10^{-16}$, $df = 1$, $\chi^2 = 45$, $p = 2 \times 10^{-11}$).

The frequency of the resistant genotype in the Norwegian samples increased in the time period 2009–2015 ($df = 1$, $\chi^2 = 204$, $p < 2 \times 10^{-16}$). Geography also contributed to variation, where southern Norway had a significantly lower frequency of the resistant genotype compared to Finnmark ($df = 1$, $\chi^2 = 6.0$, $p = 0.014$), while Northern Norway and Western Norway displayed significantly higher frequencies ($df = 1$, $\chi^2 = 95.9$, $p < 2 \times 10^{-16}$ and $df = 1$, $\chi^2 = 45.5$, $p = 1 \times 10^{-11}$). The frequency of the resistant genotype in the pooled Norwegian data from 2015 was higher (88%) than in all other North Atlantic locations sampled (27%) ($df = 1$, $\chi^2 = 712$, $p < 2 \times 10^{-16}$).

High-resolution screening of pyrethroid resistance in Norwegian fish farms in the period 2012–2015

The resistant genotype was detected in high frequencies in lice sampled from fish farms in all regions of Norway with intensive aquaculture and was also found in areas with low or minimal salmonid production in the southernmost and northernmost parts of the coast (Figure 2a). The frequency of the resistant genotype differed significantly between counties ($df = 8$, $\chi^2 = 864$, $p < 2 \times 10^{-16}$). The highest frequencies were found in the counties Hordaland, Møre og Romsdal, and Sør-Trøndelag, all of which had average frequencies >90% (Figure 2b). The frequency of the resistant genotype increased significantly over the 4-year period ($df = 1$, $\chi^2 = 157$, $p < 2 \times 10^{-16}$). For the year 2015, resistance was between 90 and 95% for all counties from Nordland to Hordaland (Figure 3). The full dataset is available in Supplementary Table S4. The farms that were treated 4 weeks or less previous to sampling had a significantly higher frequency of resistant genotypes compared to the farms that were not treated recently ($df = 1$, $\chi^2 = 9.45$, $p = 2 \times 10^{-3}$).

High-resolution screening of pyrethroid resistance in Scottish fish farms in the period 2015–2017

The resistant genotype was found in lice from farmed fish in all marine management areas sampled in Scotland (Figure 4a). At the farm level, the frequency of the resistant genotype ranged from 13 (Western Isles) to 100% (Strathclyde) (Supplementary

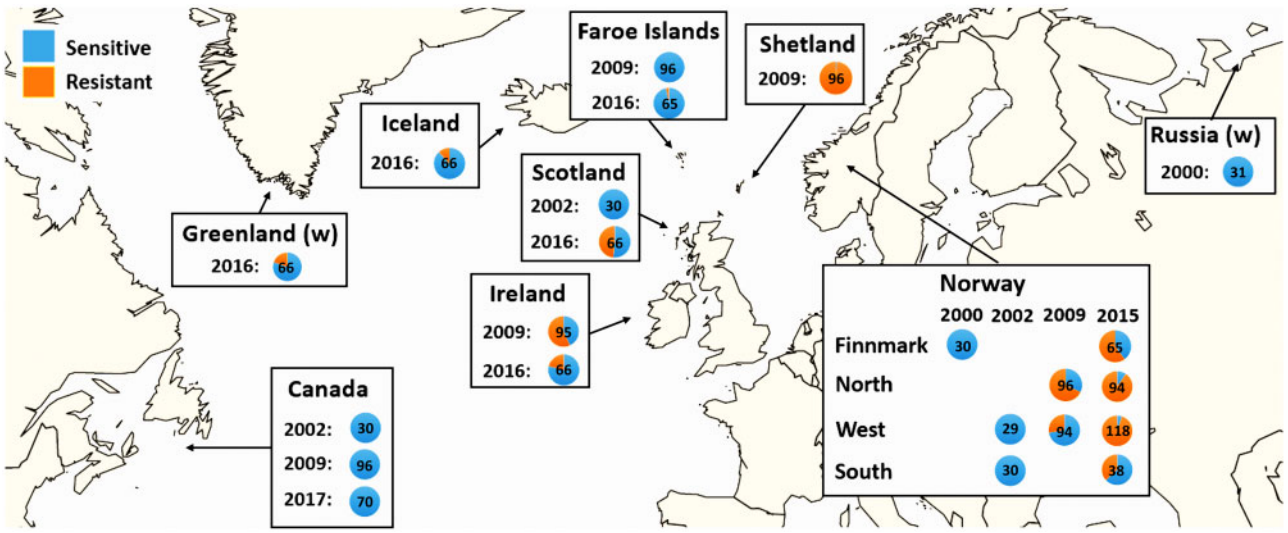

Figure 1. The observed frequency of the pyrethroid-resistant genotype in 1462 lice sampled in the period 2000–2017. Samples marked with (w) are from wild Atlantic salmon, and all others are from farmed salmon. The number inside the pie charts represents the sample size. The background map is derived from Global Administrative Areas (2017) and R packages (Becker and Wilks, 1993, 1995; Pebesma and Bivand, 2005; Bivand *et al.*, 2013).

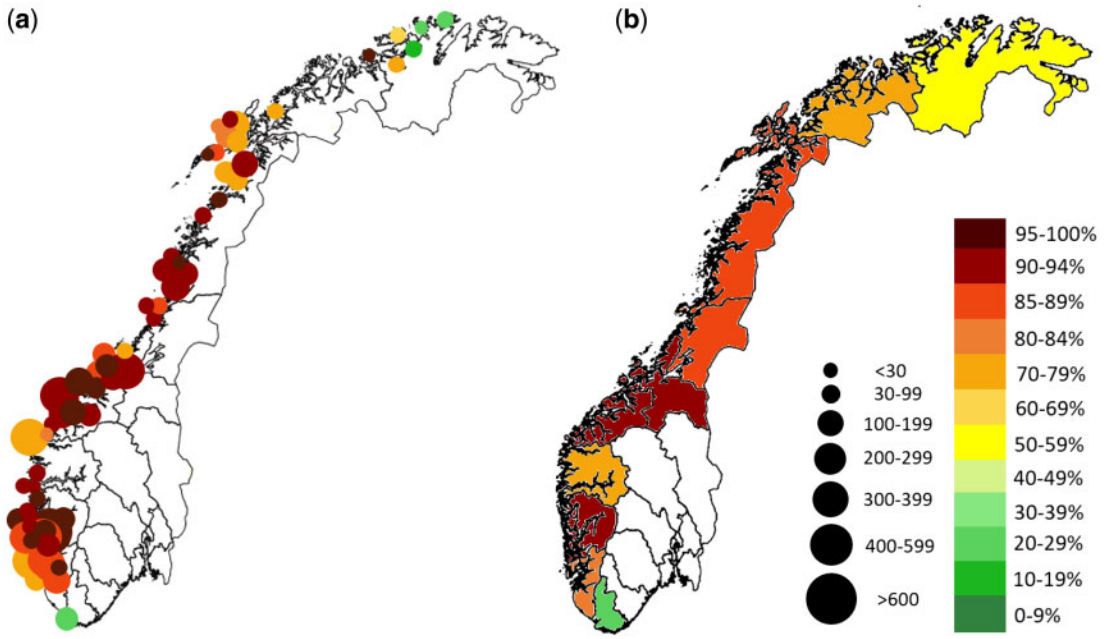

Figure 2. Frequency of the pyrethroid-resistant genotype in 10 355 lice sampled from Norwegian farms. (a) The frequency of the resistant genotype at the municipality level. (b) The frequency at the county level. The size of the circles in (a) indicates the number of lice analysed, and the colours in both (a) and (b) indicate the frequency of the resistant genotype in each sample. Lice were sampled in the period 2012–2015. The background map is derived from Global Administrative Areas (2017) and R packages (Becker and Wilks, 1993, 1995; Pebesma and Bivand, 2005; Bivand *et al.*, 2013).

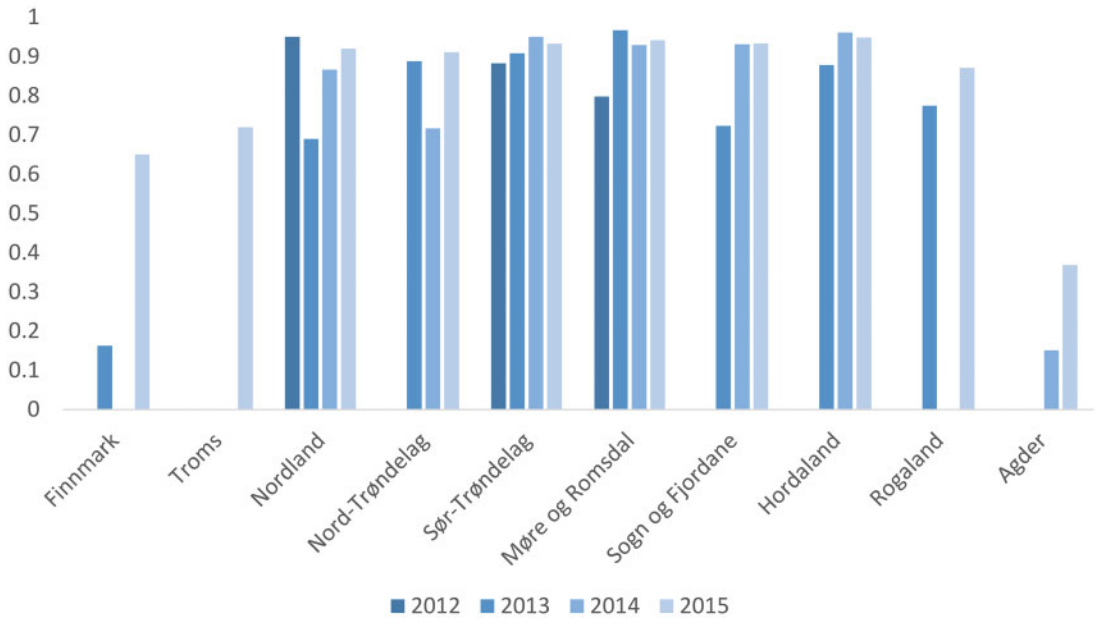

Figure 3. Frequency of the pyrethroid-resistant genotype in the counties along the Norwegian coast from north (Finnmark) to south (Agder) in the years 2012–2015.

Table S5). At the regional level, Western Isles had an average frequency of 35%, while Highland had 75% and Strathclyde had 79% (Figure 4b). The difference in frequency of the resistant genotype between the Western Isles and the two other aquaculture regions was significant ($df = 2$, $\chi^2 = 190$, $p < 2 \times 10^{-16}$), and also Highland and Strathclyde were significantly different from each other ($df = 1$, $\chi^2 = 5.2$, $p = 0.022$).

The frequency of the resistant genotype decreased from 2015 to 2017 when the whole dataset from Scotland was considered ($df = 1$, $\chi^2 = 28$, $p = 1 \times 10^{-7}$). When both time and region were considered, there was a significant increase in resistant genotype frequency in the Western Isles from 2015 to 2017 ($df = 1$, $\chi^2 = 8.4$, $p = 3 \times 10^{-3}$), while both Highland and Strathclyde had decreased frequencies. This trend was however not statistically

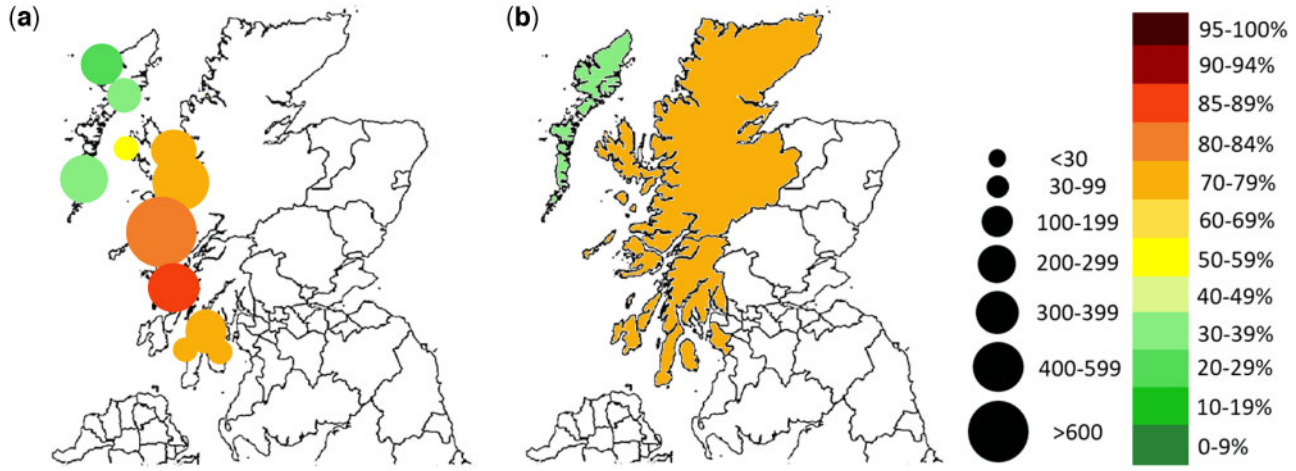

Figure 4. Frequency of the pyrethroid-resistant genotype in 3292 lice sampled from Scottish farms. (a) The frequency of the resistant genotype at the marine management area level. (b) The frequency at the regional level. The size of the circles in (a) indicates the number of lice analysed, and the colours in both (a) and (b) indicate the frequency of the resistant genotype in lice from each sample. Lice were sampled in the period 2015–2017. The background map is derived from Global Administrative Areas (2017) and R packages (Becker and Wilks, 1993, 1995; Pebesma and Bivand, 2005; Bivand *et al.*, 2013).

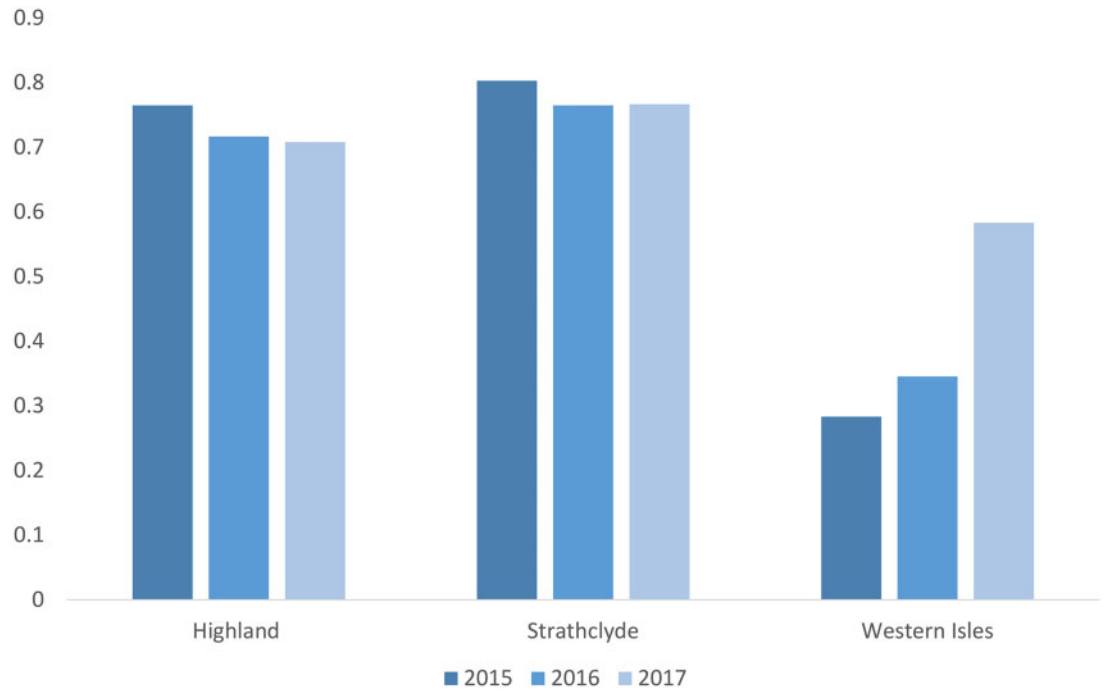

Figure 5. Frequency of the pyrethroid-resistant genotype marker in Scottish aquaculture producing regions in the years 2015–2017.

significant for either Highland ($df = 1$, $\chi^2 = 24.6$, $p = 0.10$) or Strathclyde ($df = 1$, $\chi^2 = 1.5$, $p = 0.21$) (Figure 5). The full dataset is available in Supplementary Table S5. The frequency of the resistant genotype was not significantly different between the recently treated farms and the other farms ($df = 1$, $\chi^2 = 0.57$, $p = 0.45$).

Discussion

This study presents the first spatial–temporal analysis of pyrethroid resistance in the salmon louse, the parasitic copepod that represents the most persistent challenge to environmentally sustainable global salmonid aquaculture (Taranger *et al.*, 2015). We used the recently developed pyrethroid resistance marker (Nilsen and Espedal, 2015) to genotype ~15 000 lice collected throughout the North Atlantic to investigate the development and dispersal of resistance in the period 2000–2017. The genotype associated with resistance was completely absent in all samples of lice collected throughout the entire North Atlantic up to and including the year 2002. However, the resistant genotype was observed throughout most of the European part of the North Atlantic by 2009 and, by 2017, displayed moderate-to-very high frequencies in lice from most regions of the North Atlantic. Based

upon all available evidence, we suggest that pyrethroid resistance emerged in Europe in the very late-1990s to early-2000s and was thereafter rapidly dispersed throughout the North Atlantic, driven by widespread pyrethroid use. While the resistant genotype was not detected in samples from Canada in this study, we suggest that it probably exists there in a very low frequency and that local use of pyrethroids would quickly lead to its rapid selection.

Emergence and dispersal of pyrethroid resistance throughout the Atlantic

The pattern in the development and dispersal of pyrethroid resistance throughout the North Atlantic, as revealed here (Figure 1), fits closely with observations of treatment failure and bioassays of sensitivity from the field (Sevatdal *et al.*, 2005; Whyte *et al.*, 2014; Helgesen *et al.*, 2019). One of the significant questions is whether resistance developed in one region and was thereafter rapidly dispersed to other regions of the North Atlantic, or alternatively, resistance developed in multiple farms and locations simultaneously?

In the case of emamectin benzoate resistance, conserved haplotypes across markers co-located on linkage group 5 of the *L. salmonis* genome, where at least part of emamectin benzoate resistance is located, demonstrated that resistance to this chemotherapeutant primarily emerged as a *de novo* mutation in one farm location and was thereafter dispersed rapidly to lice in the entire Atlantic (Besnier *et al.*, 2014). In contrast, a lack of conserved haplotypes across markers tightly linked with the *Phe362Tyr* mutation causing organophosphate resistance in lice (Kaur *et al.*, 2015) suggested that organophosphate resistance most likely originated in multiple farms and locations and was selected for more or less in parallel (Kaur *et al.*, 2017). Due to recombination, a nuclear single nucleotide polymorphism (SNP) under hitchhiking selection with a causative mutation will fade in its relationship with the associated phenotypic trait from one generation to the next. In contrast, the pyrethroid resistance marker used here (Nilsen and Espedal, 2015), while not the cause of resistance (Nilsen and Espedal, 2015; Carmona-Antoñanzas *et al.*, 2017; Bakke *et al.*, 2018), remains very tightly, albeit non-causatively, linked to resistance due to the lack of recombination in mtDNA. Therefore, the fact that the resistant genotype was not observed at all in any of the historical samples from 2000 to 2002 but was observed in high or very high frequencies in most of the samples from Europe by 2009 onwards, suggests that pyrethroid resistance, as for emamectin benzoate resistance may have primarily originated in a single location and was dispersed thereafter. This suggestion is also supported from the historical use of pyrethroids, and the reports of treatment failure, all of which point to an origin in Europe.

Pyrethroids were introduced and used extensively in European aquaculture from the late 1990s, but only used for a limited period in 2009/2010 in Atlantic Canada (Sevatdal *et al.*, 2005; Whyte *et al.*, 2014). By 2002, reduced sensitivity had been reported in farms in Norway, Ireland, and Scotland (Sevatdal and Horsberg, 2000, 2003; Sevatdal *et al.*, 2005). In our historical material, the resistant genotype was not detected before 2009, ~10 years after the first reports of treatment failure (Sevatdal and Horsberg, 2000) and then at frequencies >50% in Northern Norway and Ireland, and at 99% in the sample from Shetland. By 2017, the resistant genotype was found in all parts of the North Atlantic, except Canada. These findings indicate a strong selection for the resistant genotype on the European side, with a subsequent dispersal also to areas with no or little pyrethroid use. Resistant lice sampled from Icelandic farmed salmon and wild Atlantic salmon caught off Greenland are examples of this. Neither of these hosts have ever been treated with pyrethroids. In the 180 lice sampled throughout the North Atlantic by Tjensvoll *et al.* (2006) in 2000–2002, 158 different mtDNA haplotypes were found. This demonstrates a very high diversity in the mtDNA genome of the salmon louse at the time when pyrethroid resistance first emerged (Sevatdal and Horsberg, 2000, 2003; Sevatdal *et al.*, 2005). In comparison, the resistant genotype went from being completely absent in all of the lice originating from the study by Tjensvoll *et al.* (2006) in 2000–2002, to very high frequencies in most of the European samples by 2016. As the genetic marker used here is not the causative mutation for pyrethroid resistance (Nilsen and Espedal, 2015; Carmona-Antoñanzas *et al.*, 2017; Bakke *et al.*, 2018), our observations here indicate a primarily single origin for pyrethroid resistance. The alternative hypothesis would be that multiple lice independently obtained the causative *de novo* mutation simultaneously with the resistance-associated SNP genotype used here and were selected for in parallel in several regions. This hypothesis appears unlikely given the observed highly diverse mtDNA genome immediately prior to pyrethroid resistance emergence. However, unequivocal demonstration of this requires further analysis.

The resistant genotype was not detected in samples of lice from fish farms in Canada up to and including 2017. This is not evidence of genetic isolation of lice across the Atlantic Ocean but most likely reflects sampling intensity and the lack of pyrethroid use in that region. The study by Besnier *et al.* (2014) demonstrated that a mutation on linkage group 5, causing resistance to emamectin benzoate, was spread to both sides of the Atlantic Ocean in 11 years. However, emamectin benzoate was used in aquaculture both in Canada and Europe; thus, selection for resistance occurred on both sides of the Atlantic. With pyrethroids, the selection for resistance has only occurred in Europe, with the exception of the short period of usage on the Canadian side in 2009/2010. During this period, bioassays and lice counting before and after treatments were conducted in that region to monitor the effect of the compound (Whyte *et al.*, 2014). Even if the average effective concentration affecting half the population (EC 50) values from the bioassays were below the treatment concentration, an increase in mean EC 50 values from 2009 to 2010 was observed in Canada, which may suggest some very low (and undetected here) frequency of resistant lice in the short time-window of pyrethroid usage in that region (Whyte *et al.*, 2014).

The role of wild salmonids as vectors of pyrethroid-resistant salmon lice has been investigated in a recent study from Norway (Fjørtoft *et al.*, 2019). In that study, the frequencies of resistant lice on returning wild Atlantic salmon and wild sea trout were compared to the frequencies of resistant lice in salmon farms from the same regions. While there was no significant difference between the frequencies of resistant lice from wild sea trout and farmed salmon within a region, the wild Atlantic salmon returning from the ocean carried less resistant lice than the wild sea trout and the farmed salmon in the areas of intensive aquaculture (Fjørtoft *et al.*, 2019). These findings elude to the role of wild Atlantic salmon in dispersing resistant salmon lice. Lice that infect salmon post-smolts migrating from aquaculture regions are likely to carry the resistant genotype, while the returning adult

salmon carry a higher frequency of sensitive lice back to their regions of origin. The reason for this could be that there is a fitness-cost associated with the resistant genotype. Although this cannot be ruled out, it is likely that the reduced frequency of resistant salmon lice on returning wild Atlantic salmon is due to a dilution effect whereby they are infected on the high seas with sensitive lice originating from salmon that have migrated from areas without selection for pyrethroid resistance, for example from Canada. In most aquaculture-producing regions of the North Atlantic, the number of farmed Atlantic salmon outnumbers the number of wild Atlantic salmon. The dilution effect of the sensitive salmon lice carried back to the Norwegian coast is unlikely to be high as long as selection for resistance, through chemical usage, is still practised. However, if pyrethroid usage was to completely stop, then the sensitive lice carried by the returning wild salmon to intense farming areas will in time reduce the resistance levels. In the same manner, a low frequency of resistant lice carried to naive areas, such as Iceland and Canada, may, assuming a low cost of resistance, cause a surprisingly fast emergence of resistance if pyrethroids were introduced.

Pyrethroid resistance in Europe's primary salmon-producing countries: Scotland and Norway

The high-resolution screening of lice from fish farms in Norway and Scotland demonstrated that the frequency of lice carrying the resistant genotype is highest in areas of intensive aquaculture (Figures 2 and 4). This is most likely due to the intensive and ongoing inadvertent selection for resistance through repeated delousing treatments in aquaculture-dense regions. This is highlighted by the almost fixation of the resistant genotype in some of the most aquaculture intense regions (e.g. Western Norway) compared to lower frequencies in areas where pyrethroids have been used less (Finnmark), or not at all (Sørlandet) (Figure 3). In Scotland, this equates to the differences in resistance marker frequencies between the region Western Isles, with little aquaculture, and the regions Highland and Strathclyde, with more intensive aquaculture (Figure 5). The high frequencies of pyrethroid-resistant lice in these two major aquaculture areas demonstrate that these chemotherapeutants have a limited usefulness for delousing in these regions. This suggestion is supported by the reports that the number of pyrethroid treatments in aquaculture has plummeted from 1155 prescriptions in 2012 to 55 in 2018 in Norway (Helgesen *et al.*, 2019) and from 264 treatments in 2012 to 60 in 2018 in Scotland (Scotland's Aquaculture, 2018).

Management implications

In addition to genetic interactions between farmed escapees and wild conspecifics (Glover *et al.*, 2017), the salmon louse represents the most persistent challenge to environmentally sustainable salmon aquaculture (Torrissen *et al.*, 2013; Taranger *et al.*, 2015). In both the Pacific and Atlantic, salmon lice cause huge economic losses in the form of reduced productivity and treatment costs (Costello, 2009a; Iversen *et al.*, 2015) and constitute a challenge to wild salmonid survival for populations located in the proximity of farming dense regions (Birkeland and Jakobsen, 1997; Bjørn and Finstad, 2002; Gargan *et al.*, 2003; Costello, 2009b). While alternative control measures exist, development of resistance to chemotherapeutants increasingly challenges the industry's ability to control this parasite as chemotherapeutants have provided the primary mode of parasite control and probably will be important also in the future. Therefore, understanding the patterns of emergence and dispersal of resistance in this parasite is of utmost importance in the continued search for improved management strategies and to improve the effective life span of new emerging chemotherapeutants. This is illustrated by the results here and those from studies looking at emergence and dispersal of resistance to emamectin benzoate (Besnier *et al.*, 2014) and organophosphates (Kaur *et al.*, 2017). Collectively, these findings demonstrate that this parasite is highly capable of developing and dispersing resistance quickly. This evolutionary capacity is driven by very large population sizes, high amounts of gene flow over large distances (Glover *et al.*, 2011; Besnier *et al.*, 2014), rapid generation times, and that aquaculture represents the primary driver of salmon louse population dynamics in farming dense regions (Fjørtoft *et al.*, 2017, 2019). Furthermore, as cross-infection can occur on the open seas between wild salmon hosts (Jacobsen and Gaard, 1997), salmon from all parts of the North Atlantic can be infected with resistant lice in the open ocean where they meet and thus bring resistant lice back to their countries. As a result, a large fraction of this species is exposed to chemotherapeutants over time and the life span of any given chemotherapeutant is likely to be limited. Therefore, once resistance has developed, it will quickly reach high frequencies and disperse to other aquaculture areas as long as the chemotherapeutant is used frequently in multiple regions. As such, management plans aimed at prolonging the effective life of new and emerging chemotherapeutants need to be agreed upon internationally.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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Authors' contributions

HBF, AS, FN, PAB, A-KT, and KAG conceived and designed the study. HBF and KAG coordinated the work. HBF, FN, PGE, KAG, and VTA obtained salmon lice. FB conducted statistical analyses, while VTA conducted genotyping. HBF wrote the first draft of the manuscript together with KAG. All authors contributed to data interpretation, critically reviewed the drafts, and approved the final manuscript.

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