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A metapopulation model reveals connectivity-driven hotspots in treatment resistance evolution in a marine parasite

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In salmon aquaculture, the sustainable management of salmon lice (*Lepeophtheirus salmonis*) is limited by the adaptive capacity of the parasite. This is evident in the repeated evolution of pesticide resistance in the salmon louse population. To better prepare for resistance, we constructed a numerical metapopulation model that predicts the evolutionary dynamics of lice across an interconnected farm network. This model integrates within-farm population dynamics and between-farm louse dispersal, the latter using outputs from a state-of-the-art particle-tracking model. Distinct from previous metapopulation models, it also simulates spatial and temporal genetic variation arising from selection. The model was parameterized to simulate the evolution of resistance to the pesticide azamethiphos on farms in southern Norway. It successfully reproduced the rapid (within 10 years) evolution of azamethiphos resistance following extensive delousing treatments. It also identified strong spatial patterns in resistance, with regions of high farm connectivity being potential hotspots of louse adaptation. Rates of infestation and evolution were significantly reduced when highly connected farms were excluded from the simulation, compared to when low-connectivity or random sites were excluded. This model can be a valuable tool for coordinating pest management at a regional scale, in a way that slows or prevents the spread of resistance.

Keywords: Salmon, lice, evolution, model, resistance, aquaculture.

Introduction

The evolution of pesticide resistance has been extensively documented in terrestrial agriculture (Georghiou and Saito, 1983; Brattsten *et al.*, 1986; Kaplan and Vidyashankar, 2012; Knolhoff and Onstad, 2014). It has long been recognized that pest populations have the ability to adapt to the novel pressures faced in farm environments—despite this, it is only recently that such evolutionary thinking has been widely applied to the growing commercial aquaculture sector (Nowak, 2007; Mennerat *et al.*, 2010; Sundberg *et al.*, 2016). Parasites and pathogens present a major challenge to aquaculture (Blaylock and Bullard, 2014; Lafferty *et al.*, 2015), and efforts to control these pests are undermined by the evolution of resistant strains.

This is perhaps best exemplified by the repeated evolution of pesticide resistance in the salmon louse, *Lepeophtheirus salmonis*, which is a major pest in the aquaculture of Atlantic salmon, *Salmo salar* (Torrissen *et al.*, 2013; Aaen *et al.*, 2015). Salmon lice are ectoparasites that cause severe pathologies in salmon at high infestation densities (Wagner *et al.*, 2008; Fjelldal *et al.*, 2019). At the start of their life cycle, lice are free-living—they drift in the plankton, surviving on endogenous energy reserves until they encounter a host (Tucker *et al.*, 2000a; Samsing *et al.*, 2016). In this larval phase, lice can disperse great distances on ocean currents to infest farmed and wild salmonids (Myksvoll *et al.*, 2020; Sandvik *et al.*, 2020; Johnsen *et al.*, 2021). To mitigate the environmental, welfare, and economic impacts of louse outbreaks, a diverse array of management strategies have been used in salmon aquaculture (Coates *et al.*, 2021a). Chemical treatments have been the predominant method of control, but over the last two decades, lice in the Atlantic have developed resistance to four of the five types of chemical (Aaen *et al.*, 2015).

As salmon lice develop resistance to a treatment, outbreaks can become more severe and more frequent treatments are needed to control them (which can have adverse fish welfare outcomes; Overton *et al.*, 2019). A considerable amount of resources are spent in developing replacement methods of control (Brakstad *et al.*, 2019)—which may themselves have only a limited lifespan. Integrated pest management, whereby various methods of control and prevention are coordinated across farms in an evolutionarily informed manner, can slow or even prevent the evolution of resistance (Peshin *et al.*, 2009; Barzman *et al.*, 2015; McEwan *et al.*, 2016). However, this requires the ability to predict how louse populations across an interconnected farm network will respond to the selection pressures imposed by these strategies.

Numerical models provide an opportunity to predict population dynamics and evolution in a system under a range of different scenarios. In an agricultural setting, models are used

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to explore how pest adaptation is affected under different conditions, and to determine which farming strategies are most effective at preventing resistance (MacKenzie and Bishop, 2001; Kemper *et al.*, 2013; Onstad *et al.*, 2013). A number of population dynamics models have been specifically created to predict the trajectory of louse infestations and the effect of management strategies on salmon farms (e.g. Revie *et al.*, 2005; Groner *et al.*, 2013; Rittenhouse *et al.*, 2016; Kragesteen *et al.*, 2019; Jeong *et al.*, 2021). Some include an evolutionary component within the model to predict how different scenarios (such as various treatment regimes, the size of wild host refugia, or wild host migration) influence the evolution of pesticide resistance within farms (Bateman *et al.*, 2020; Kreitzman *et al.*, 2018; McEwan *et al.*, 2015, 2016; Murray 2011). Such models focus only on population dynamics within individual farm sites, or treat all farms in the area as a single population.

To model population dynamics across a larger geographic scale, lice can be considered as part of a metapopulation structure: each salmon farm supports a self-contained louse population, with movement of lice between farms occurring during the planktonic larval stage. The *L. salmonis* metapopulation can, therefore, be simulated by running multiple single-farm models concurrently, with an additional component that mediates the movement of larvae between farms. This is the approach used by Aldrin *et al.* (2017, 2019), who constructed metapopulation models using the extensive datasets available for Norwegian salmon farms. These models were used to simulate the demographic response of the louse population to the many on-farm processes occurring during a salmon production cycle. Off-farm processes—the movement of louse larvae between farms—was parameterized as a function of the distance between farms (Aldrin *et al.*, 2017, 2019).

Larval dispersal is determined by a complex interplay between hydrodynamics and larval life history. In their metapopulation model, Toorians and Adams (2020) parameterized the larval migration using the output of a particle-tracking model. Such models offer the opportunity to predict larval dispersal between real-world farm locations (Murray and Gillibrand, 2006; Adams *et al.*, 2016; Johnsen *et al.*, 2016; Myksvoll *et al.*, 2018). This approach combines hydrodynamic data (such as ocean currents, temperature, and salinity) and biological parameters (including larval development, swimming behaviour, and mortality) to predict the transport and survival of planktonic larvae through time across a study area. Applications for these models include real-time monitoring of outbreaks (Myksvoll *et al.*, 2018), informing legislation for farm production quotas (Vollset *et al.*, 2014; Myksvoll *et al.*, 2020; Sandvik *et al.*, 2020), evaluating the role of farm placement on louse connectivity (Samsing *et al.*, 2017, 2019) and, recently, assessing possible selection pressures on larvae by barrier cages (Coates *et al.*, 2021b). The infestation levels predicted for a location using such dispersal models agree strongly with those observed in sentinel cages and in wild salmon (Johnsen *et al.*, 2021; Myksvoll *et al.*, 2018; Sandvik *et al.*, 2016, 2020).

Previous metapopulation models have either excluded genetic variation within the louse population (Aldrin *et al.*, 2017, 2019; Kragesteen *et al.*, 2019; Toorians and Adams, 2020), or excluded demographic variation between farms (Bateman *et al.*, 2020; Kreitzman *et al.*, 2018; Murray, 2011). Here, we construct a discrete-time, stage-structured metapopulation model for *L. salmonis* that also incorporates genetic variation. To our knowledge, this is the first attempt to model evolutionary dynamics in salmon lice across a network or salmon farms. The parameters for larval movement between farms were set using the outputs from a Norwegian lice dispersal model (Samsing *et al.*, 2017). This allowed the model to capture the distinct spatial and temporal patterns in gene flow through a Norwegian farm network. Our goal was to produce a functioning evolutionarily dynamic population model that simulates spatial and temporal patterns of resistance across a large salmon-farming region. Simulations can be run under a range of farming scenarios, to identify sites of rapid evolution, and to determine how management strategies might be best coordinated at these hotspots to mitigate louse resistance. In this paper, we develop the model and test its capacity to recapitulate the rapid evolution of resistance to the chemical treatment azamethiphos, as observed in the Norwegian louse population (Kaur *et al.*, 2017; Fjørtoft *et al.*, 2021).

Methods

Azamethiphos resistance as a case study

To test the model, we set the parameters to simulate the evolution of azamethiphos resistance on Atlantic salmon (*S. salar*) farms in southern Norway. Azamethiphos is a bath-administered parasiticide, belonging to a class of chemical compounds known as organophosphates. It was introduced to salmon aquaculture in 1994 to replace dichlorvos, another organophosphate. Dichlorvos resistance had already been recorded at this time (Jones *et al.*, 1992) and reports of failed azamethiphos treatments emerged soon after (Roth *et al.*, 1996). Bioassays indicate that high levels of resistance to this chemical is now widespread in the Atlantic *L. salmonis* population (Grøntvedt *et al.*, 2015; Kaur *et al.*, 2015; Myhre Jensen *et al.*, 2017).

Resistance has been linked to a single mutation in the *Phe362Tyr* gene coding for acetylcholinesterase (AChE), which is a target site for organophosphates (Kaur *et al.*, 2015, 2016). A total of two alleles at this locus—the wild-type susceptible allele (S) and the mutant resistant allele (R)—have a significant, co-dominant effect on survival following azamethiphos treatment (Kaur *et al.*, 2015; Myhre Jensen *et al.*, 2017). The mutation was likely already present in the louse population at low frequency before the introduction of organophosphates, and was driven to high frequencies across multiple locations following widespread use of the chemical (Kaur *et al.*, 2017).

The evolution of azamethiphos resistance along the Norwegian coastline was a suitable starting point to test our model for several reasons. First, since resistance is largely determined at a single biallelic locus, only three louse genotypes were required, which is relatively simple to model. Second, survival of the three genotypes after azamethiphos exposure could be parameterized directly from bioassay data (Kaur *et al.*, 2015; Myhre Jensen *et al.*, 2017). Finally, model outputs could be compared against actual survey data tracking the frequency of the resistant gene in the louse population in Norway over the course of a decade (Fjørtoft *et al.*, 2017, 2021; Kaur *et al.*, 2017).

Model overview

Populations of lice were located at distinct salmon farming sites, with movement of lice between farms occurring via the parasite's dispersive larval stage. Wild hosts were excluded

from the model, since farmed salmonids comprise > 99% of the hosts available to lice in Norway (Dempster *et al.*, 2021). Each discrete time-step, t, represented 1 week. The model tracked the number of lice as they progressed week-by-week through four life stages on salmon farms.

Each week, individual farm sites were assigned a temperature according to the time of year and location. These temperatures were the means recorded for each farm over 5-week periods, using the data available at BarentsWatch (2022; for full description, see Supplementary Material S1). This allowed for broad seasonal and spatial variation in temperature, which has a significant influence on louse development (Hamre *et al.*, 2019).

The transition of lice through life stages across time-steps was simulated using a matrix population model (Groner *et al.*, 2014, 2016; Toorians and Adams, 2020). We developed this model by nesting three levels of organization: first with a simple stage-structured model, which was then nested within a model for multiple genotypes, which was itself expanded into a metapopulation model to account for multiple farms. We will describe the model in steps, starting with a single-farm, single-genotype model and progressing to the full model, which includes multiple farms, multiple genotypes, and azamethiphos treatments.

Life stages

Lice were grouped into four life stages, b, representing the major stages of the parasite's life cycle (Hamre *et al.*, 2019). The life stages (in order of increasing age) were: "larva" (representing the free-living stages), "chalimus", "pre-adult", and "adult". These are notated here as $b = \{L, C, P, A\}$, respectively. At each time-step, a proportion of lice progressed to the subsequent life stage (i.e. larvae to chalimus, chalimus to pre-adult, and pre-adult to adult). Adult lice remained in the adult stage and produced new larva every t. For a single farm, the number of lice at a time-step is given by the column vector $\mathbf{N}$, where each row corresponds to the number of lice (n) of each life stage. With just one genotype (g), the number of lice at $t + 1$ is given by $\mathbf{N}$ at the previous time-step, multiplied by a transition matrix, $\mathbf{S}$

$$\mathbf{N}_{t+1,g} = \mathbf{S}_g \cdot \mathbf{N}_{tg} = \begin{bmatrix} 0 & 0 & 0 & fu_g \\ dv & (1-s)*(1-\mu_C) & 0 & 0 \\ 0 & s*(1-\mu_C) & (1-s)*(1-\mu_P) & 0 \\ 0 & 0 & s*(1-\mu_P) & 1-\mu_A \end{bmatrix} \cdot \begin{bmatrix} n_L \\ n_C \\ n_P \\ n_A \end{bmatrix}_{tg}. \quad (1)$$

The parameter s is the proportion of lice that transition to the next life stage in one time-step. The value of s is calculated according to the temperature on the farm at t, using the equation given in Hamre *et al.*, (2019) for temperature-dependent development rate (see Supplementary Material S2 for details). The proportion of lice remaining in the same life stage is, therefore, 1–s. A proportion of lice (0.162 for adults and pre-adults; 0.014 for chalimus) were also lost through a background weekly mortality rate, μ, estimated from Stien *et al.*, (2005; see Supplementary Material S2). The parameter f represents the mean number of larvae produced per adult per week. The value for f was calculated according to the temperature at t (as reproductive rate is temperature-dependent), using the equation given in Johnsen *et al.*, (2020; see Supplementary Material S2). The parameter u_g is the proportion of larvae produced that carry the genotype, g (in this example with one ubiquitous genotype, $u_g = 1$).

For simplicity, we did not differentiate sexes in our model and instead assumed a 1:1 sex ratio (see Supplementary Material S2). We used values for s and μ that were intermediate between those for males and females. As there is an average of one egg-string per adult, the mean reproductive output per adult (f) is half the output per female.

Of the larvae produced at t in this single-farm model, a proportion (dv) re-infest the farm and survive to become chalimus at $t + 1$. Here, d is the proportion of larvae returning to the farm of origin as infective copepodids (the probability of "self-recruitment" of farms), and v is the proportion of those copepodids that successfully re-enter a cage and attach to a host. Values for d were determined by the particle-tracking model (below). It is near impossible to empirically determine a value for v; we decided on $v = 0.05$ (see Supplementary Material S2).

Genotypes

In addition to life stages, b, lice were also grouped according to genotype, g, at a single, biallelic locus. This locus represented the *Phe362Tyr* marker gene for azamethiphos resistance. Genotypes are notated here as $g = \{RR, RS, SS\}$, where R is the resistant (mutant) allele and S the susceptible (wild-type) allele.

The number of larvae of each genotype (n_{Lg}) produced was calculated according to the Hardy–Weinberg principle: the expected proportions of RR, RS, and SS offspring are p^2, $2pq$, and q^2, respectively, where p and q are the frequencies of the R and S alleles in the parent population (Falconer and Mackay, 1996). The Hardy–Weinberg principle assumes non-assortative mating of adults and no selective pressure over this reproductive period.

For example, the total number of RR larvae produced by the adults on a farm at $t + 1$ can be given by

$$\begin{aligned} n_{L,t+1,RR} &= fp^2 n_{At} \\ &= fp^2(n_{AtRR} + n_{AtRS} + n_{AtSS}), \end{aligned} \quad (2)$$

where n_{At} is the total number of adults (across all genotypes) at the farm.

To capture this, Equation (1) was expanded to block matrix form as

$$\mathbf{L}_{t+1} = \mathbf{G} \cdot \mathbf{L}_t = \begin{bmatrix} \mathbf{S}_{RR} & \mathbf{F}_{RR} & \mathbf{F}_{RR} \\ \mathbf{F}_{RS} & \mathbf{S}_{RS} & \mathbf{F}_{RS} \\ \mathbf{F}_{SS} & \mathbf{F}_{SS} & \mathbf{S}_{SS} \end{bmatrix} \cdot \begin{bmatrix} \mathbf{N}_{RR} \\ \mathbf{N}_{RS} \\ \mathbf{N}_{SS} \end{bmatrix}_t, \quad (3)$$

where $\mathbf{L}$ is the number of lice across all life stages and genotypes, and $\mathbf{G}$ is the matrix for stage and genotype transitions. Within $\mathbf{G}$, the matrices $\mathbf{S}_g$ are the same as in Equation (1), but with u_g equal to either p^2, $2pq$ or q^2; for $\mathbf{S}_{RR}$, $\mathbf{S}_{RS}$, and $\mathbf{S}_{SS}$, respectively. New values for p and q (allele frequencies in the adult population) are calculated at each time-step. The matrices $\mathbf{F}_g$ within $\mathbf{G}$ are

$$\mathbf{F}_g = \begin{bmatrix} 0 & 0 & 0 & fu_g \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, \quad (4)$$

again where u_{RR}, u_{RS} and u_{SS} is p^2, $2pq$ and q^2, respectively.

Farms

The matrix model was expanded to include multiple farm sites, with movement of lice between sites occurring during the larval stage. The model is given by

$$\mathbf{P}_{t+1} = \mathbf{M} \cdot \mathbf{P}_t, \tag{5}$$

where **P** is the louse metapopulation, composed of the louse populations, **L**, at each farm, *i*

$$\mathbf{P}_t = \begin{bmatrix} \mathbf{L}_1 \\ \mathbf{L}_2 \\ \vdots \\ \mathbf{L}_i \end{bmatrix}_t, \tag{6}$$

and **M** is the block matrix

$$\mathbf{M} = \begin{bmatrix} \mathbf{G}_1 & \mathbf{C}_{21} & \cdots & \mathbf{C}_{i1} \\ \mathbf{C}_{12} & \mathbf{G}_2 & \cdots & \mathbf{C}_{i2} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{C}_{1i} & \mathbf{C}_{21} & \cdots & \mathbf{G}_i \end{bmatrix}, \tag{7}$$

with the number of rows and columns equal to the number of farms in the simulation. On the leading diagonal of **M** is the transition matrix $\mathbf{G}_i$ for each farm. These capture within-farm processes (survival, development, reproduction, and larval self-recruitment). The matrices $\mathbf{G}_i$ are equal to **G** in Equation (3), but with unique self-recruitment values (d) for each farm: d_{ii}, which is the probability of dispersing from farm *i* back to farm *i*. Unique values for p and q are also calculated for each farm site, according to the total number of adults at that site.

The rest of **M** contains connectivity matrices, $\mathbf{C}_{ji}$, which represent the probability of larval dispersal from farm *j* (corresponding to columns in **M**) to farm *i* (corresponding to rows in **M**). The matrix $\mathbf{C}_{ji}$ is given by

$$\mathbf{C}_{ji} = \begin{bmatrix} \mathbf{D}_{RR} & \mathbf{Z} & \mathbf{Z} \\ \mathbf{Z} & \mathbf{D}_{RS} & \mathbf{Z} \\ \mathbf{Z} & \mathbf{Z} & \mathbf{D}_{SS} \end{bmatrix}_{ji}, \tag{8}$$

and where $\mathbf{D}_g$ is

$$\mathbf{D}_g = \begin{bmatrix} 0 & 0\,0\,0 \\ d_{ji}v & 0\,0\,0 \\ 0 & 0\,0\,0 \\ 0 & 0\,0\,0 \end{bmatrix}_g. \tag{9}$$

$\mathbf{D}_g$ contains d_{ji}, which is the probability of larvae (of genotype *g*) dispersing from farm *j* to farm *i*. The rest of $\mathbf{C}_{ji}$ is filled with **Z**, which represents a 4 × 4 matrix containing zeros (as larval dispersal of one genotype does not influence recruitment of the other genotypes).

The metapopulation in our simulation comprised 537 farm sites, representing active farms in southern Norway (58.4–66.4°N), as included in the connectivity analysis by Samsing *et al.* (2017). The outputs of a particle-tracking model (Samsing *et al.*, 2017) were used to parameterize dispersal in our population model. Samsing *et al.* (2017) simulated larval transport between 537 farm sites in southern Norway over two periods: November–February, and April–July. The probability of a particle released from any farm *j* successfully dispersing to any farm *i* was calculated from the output of the particle-tracking simulation and used as d_{ji} in our model. We used the April–July d_{ji} values for weeks 11–38 in our simulation, and the November–February values for the remaining weeks. Dispersal probabilities for each season were averaged over the 6 years (2009–2014) that were simulated by Samsing *et al.* (2017).

Table 1. Parameter values for the treatment component of the model. The proportion survival of each genotype to azamethiphos treatments (x_g) are for adult and pre-adult lice (larvae and chalimus are unaffected). Azamethiphos treatments and forced harvests are applied to farms where louse abundance (adults fish^{-1}) exceeds the trigger level.

Parameter	Value
RR azamethiphos survival (x_{RR})	0.8
RS azamethiphos survival (x_{RS})	0.2
SS azamethiphos survival (x_{SS})	0.01
Azamethiphos trigger (summer—winter)	1 adults fish^{-1}
Azamethiphos trigger (spring)	0.4 adults fish^{-1}
Forced harvest trigger	2 adults fish^{-1}

In this full model, the number of chalimus of a given genotype at farm *i* (n_{Cig}) is determined by the number of larvae (of that genotype) successfully dispersing from every farm *j* and infesting farm *i*. This is given by

$$n_{C,t+1,i,g} = \sum_j n_{Ltjg} d_{ji} v, \tag{10}$$

where *j* includes the target farm, *i*, to account for self-recruitment (d_{ii}).

Treatments

The final metapopulation model included an additional function that simulated delousing treatments in response to high infestations. This function was applied after the matrix multiplication component [that is, once $\mathbf{P}_{t+1}$ was calculated according to Equation (5)]. At each time-step, azamethiphos treatments were applied only to farms where the total abundance of adult lice (across genotypes) exceeded 1 louse fish^{-1}, or 0.4 lice fish^{-1} during spring (Table 1). These treatment triggers were estimated from the reported louse abundances and treatment records in the Norwegian farm database (see Supplementary Material S3). The limits of 1 and 0.4 adults fish^{-1} match Norway's legal limits of 0.5 and 0.2 adult females fish^{-1} (Sandvik *et al.*, 2021), assuming a 1:1 sex ratio in adults.

These abundances were converted to the total number of adults that trigger a treatment on a farm, k_i, by multiplying abundance by the estimated number of hosts at a farm, h_i. That is, $k_i = 0.4h_i$ in spring, and $k_i = h_i$ during the rest of the simulation. The number of hosts per farm was kept constant for simplicity, and was calculated as the maximum allowed biomass for that farm (as provided by Samsing *et al.*, 2017) divided by the mean weight of salmon at harvest (4.5 kg; Barrett *et al.*, 2022). This assumes that farms stock enough fish so that biomass limits are not exceeded once the fish are fully grown. This is likely to be an underestimation of the number of hosts on a farm at any point in time if the farm stocks additional salmon to account for mortality during the production cycle.

At each time-step, following the matrix multiplication in Equation (5), the total number of adults at each farm ($n_{A,t+1,i}$) was compared against the lice limit for that farm, k_i. If $n_{A,t+1,i} < k_i$, then no treatment was administered. If $n_{A,t+1,i} > k_i$, then additional mortality was applied to that

farm to simulate azamethiphos treatment. In this instance, the numbers of adults and pre-adults of each genotype ($n_{A,\,t+1,\,i,\,g}$ and $n_{P,\,t+1,\,i,\,g}$) were multiplied by x_g, which is the proportion that survive azamethiphos, according to genotype. Values for x_g were estimated from bioassay data for the three genotypes, with $x_{RR} = 0.8$, $x_{RS} = 0.2$, and $x_{SS} = 0.01$ (Myhre Jensen *et al.*, 2017; Table 1). There was in fact 0% survival of the SS genotype in these bioassays, but we allowed for a more lenient 1% survival in our simulation. There was no additional mortality for chalimi and larvae, irrespective of genotype, since chalimi are not removed by azamethiphos (Roth *et al.*, 1996; Whyte *et al.*, 2016) and larvae are presumed to be outside of farms during treatment.

Farm records (BarentsWatch, 2022) show that farms harvest salmon shortly after adult louse abundance reaches very high levels (although it may be that farms sometimes avoid delousing—thus resulting in higher abundances—in the weeks leading up to a planned full harvest). Our model included a second lice limit for each farm of 2 adults fish^{-1} (Table 1). Farms exceeding this limit were forced to "harvest", which, therefore, resulted in 100% mortality of all lice. This assumption was included to reflect harvesting of farms in response to uncontrolled infestations, but also harvesting as part of the regular production cycle. It was also added to dampen excessive lice levels once azamethiphos resistance became common.

Simulations

Simulations were run for 10 years. The start of the simulation was nominally representative of the year 2009, when azamethiphos was re-introduced to Norway after a decade of its absence (Coates *et al.*, 2021a). To determine the number of lice present at the start of the simulation, the average louse abundance on each farm in the first week of the year was calculated from the 2012–2020 farm infestation database (BarentsWatch, 2022). These records are for "chalimus", "motile", and "adult female" lice, which were used as substitutes for chalimus, pre-adult and adult stages in the model. Abundances were multiplied by h_i to convert them into the total number of lice of each stage.

These starting lice were then portioned into the three genotypes. It was assumed that the mutation for azamethiphos resistance was already present in the louse population at low frequencies before this chemical was introduced (Kaur *et al.*, 2017). At each farm, 1% of lice were assigned the RS genotype, and the remaining individuals the SS genotype. We did not include any RR individuals at the start of the simulation. This equated to a starting R allele frequency of 0.005 per farm.

The simulations were run on R (version 4.0.3).

Post hoc analyses

The vector $\mathbf{P}_t$ captures the number of lice of each genotype in the metapopulation through time. The frequency of the R allele, both at the farm level and for the entire population, was calculated to track the evolution of resistance. The number of treatments applied at each farm and time-step was also recorded from the simulation. We used Spearman's rank correlations to explore the relationships between resistance (the frequency of the R allele at the end of the simulation), treatment frequency, farm connectivity, average yearly temperature, and farm size (h_i). As a metric of farm connectivity, we summed all d_{ji} values for each farm (excluding the probability of self-recruitment, d_{ii}) as a measure of larval "influx" from surrounding farms (Toorians and Adams, 2020).

To assess whether our predictions fall within reasonable bounds for farms, we compared model outputs with a database of louse abundance and delousing frequency, as reported for actual farms in the study area 2012–2021 (BarentsWatch, 2022). The adult genotype frequencies predicted by the model were compared against the data given in Kaur *et al.* (2016). These data are the proportions of the three genotypes at the *Phe362Tyr* gene in lice sampled from Norwegian farms 2012–2014.

Additional simulations adjusting connectivity

To assess the effect of farm placement and connectivity on louse evolutionary dynamics, we ran additional simulations in which 25% of the farm sites were excluded from the model. In one simulation, the least-connected 25% of farms (those with the lowest larval influx) were removed. In another, the most-connected 25% were removed. In the final three simulations, the farms removed were randomly selected. The models were otherwise parameterized as above, and the outputs assessed in the same way as the original simulation.

Results

Resistance through time and space

Our model predicted azamethiphos resistance to become widespread in the louse population over the course of 10 years. Resistance evolved most rapidly in the Hordaland region in the south-west of Norway, and the Nordland region in the northernmost part of the study site. An increase in the frequency of R followed as a wave of resistance out from these areas through time (Figures 1 and 2). A high level of evolved resistance was associated with farms supporting a large louse population (Figures 1 and 2).

R frequency (p)

The frequency of the R allele across the entire adult metapopulation (p) was calculated at each time-step. The value for p increased from 0.5% very gradually in the first 2 years. It then rose dramatically to 50% over years 3–4, and reached 90% after 7 years (Figure 3a). The rate of evolution slowed as R approached fixation, with $p = 96\%$ at the end of the simulation. The gene frequency of R within individual farm sites at the end of the simulation ranged $p = 0 - 1$; these values were only weakly correlated with farm size (Figure 4).

Louse numbers

There was a distinct seasonal cycle in louse numbers, with a decline over spring, followed by population growth occurring during summer and autumn (Figure 3b). The seasonal trends in mean adult louse abundance predicted by our model matched well with observed louse infestations recorded by farms in the study area (Figure 5). The mean adult abundance across farms gradually increased through time: it cycled around ~0.25 adults fish^{-1} during the first 3 years, as opposed to ~0.4 adults fish^{-1} in the final years. Spatial patterns in louse abundance were also observed (Figure 1). Infestations tended to be lower on farms in the northern half of the study area—particularly in the Møre og Romsdal region (the middle latitudes of the study area, approximately 62.5°N)—and at the southernmost tip of Norway.

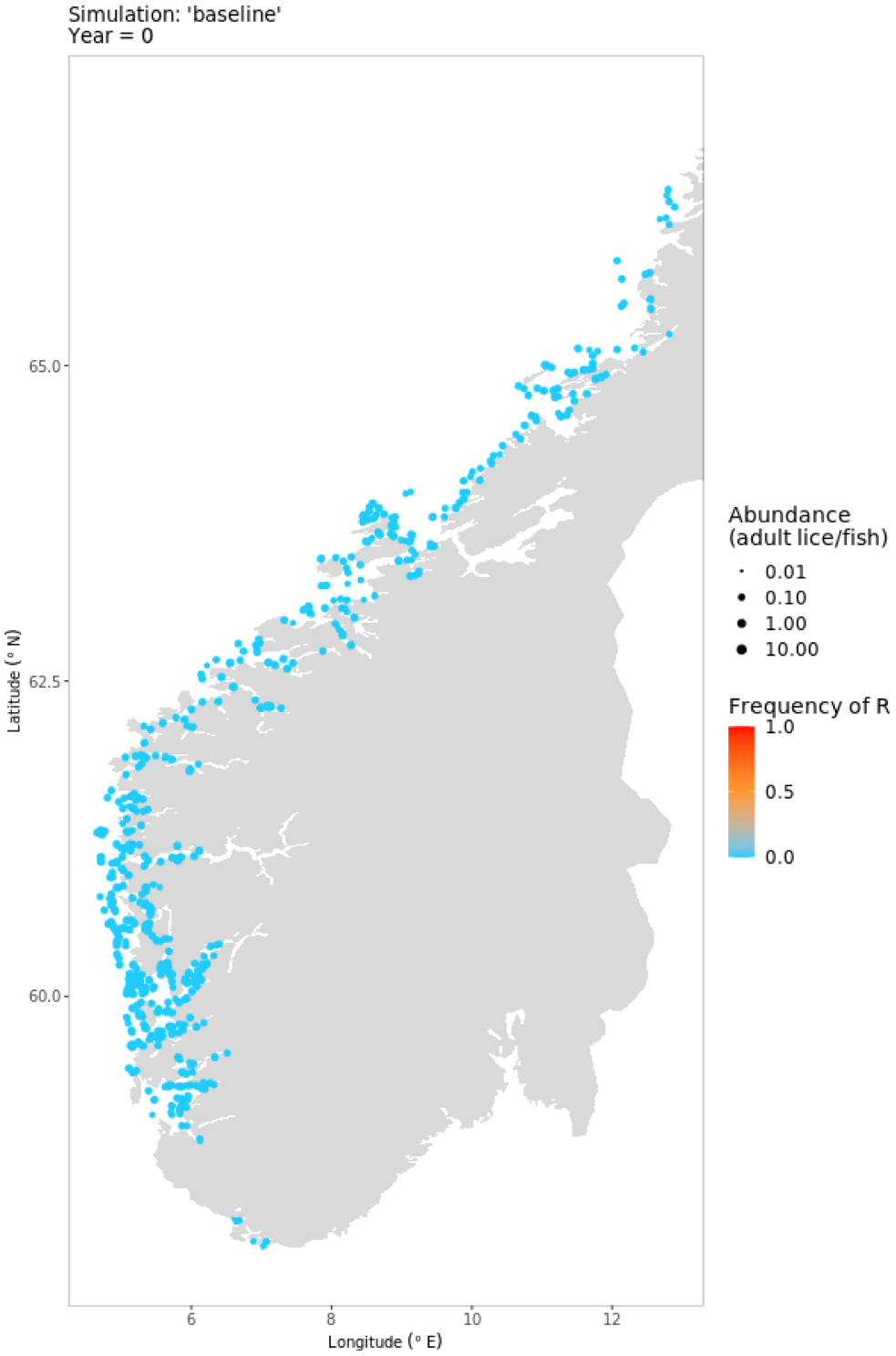

Figure 1. Figure animation can be accessed at: https://cloudstor.aarnet.edu.au/plus/s/WnqoSnovnv6qjmq/download. Simulated population of adult *L. salmonis* on farms in southern Norway over 10 years of selection by azamethiphos treatments. Colour indicates the frequency of the resistant R allele in adults on each farm (low = blue; high = red). Size of point indicates the abundance of adults (lice fish^{-1}) at a farm.

Treatment frequency

There was a seasonal pattern in treatment frequency, with spikes in treatments occurring at the start of spring, when the lice maximum lice limit dropped from 0.5 to 0.2 adult females fish^{-1}. This trend matched closely with farm records from the study area, although the spike in treatments occurred 2–3 weeks earlier in the farm dataset (BarentsWatch, 2022; Figure 5). The total number of treatments each week was similar to the BarentsWatch farm database for the first years of the simulation. In later years of the simulation, however, weekly treatment frequency was twice as high as in the farm database, most notably during the spring period of low lice limits (Figure 5). There was an almost fivefold increase in the number of azamethiphos treatments per year over the course of the

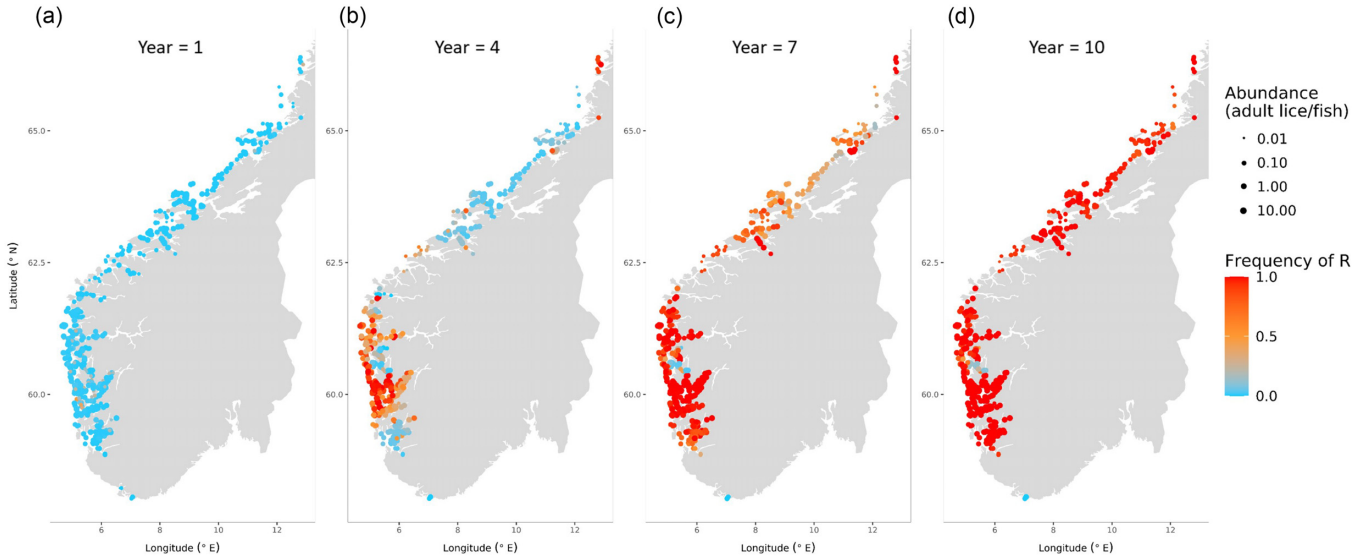

Figure 2. Population of adult *L. salmonis* on farms in southern Norway at 1, 4, 7, and 10 years into the simulation [(a)–(d), respectively]. Colour indicates the frequency of the resistant R allele in adults on each farm (low = blue; high = red). Size of point indicates the abundance of adults (lice fish^{-1}) at a farm.

simulation (853 to 4051; Figure 3c). A total of 24936 azamethiphos treatments were administered over the 10 years of the simulation. These treatments were limited to 59% of the sites (317 farms). In comparison, delousing treatments (chemical baths and mechanical delousing) recorded for actual farms in our study area over 2012–2021 (BarentsWatch, 2022) were limited to 74% of farms in the database. In both the Norwegian database and in the simulation, the untreated farm sites were distributed evenly along the length of the coastline in the study area. As in the farm records, there was a long-tailed distribution in treatment frequency across farms in our model: of the farms in the simulation that were treated at least once, one-third (106 farms) were treated 1–10 times over 10 years, another third (108 farms) were treated 11–100 times, and the remaining third received between 102 and 337 treatments.

Adult abundance exceeded 2 lice fish^{-1}, triggering a forced harvest, a total of 1036 times in the simulation. These occurred at 70 farms, and were most frequent at smaller farm sites (biomass $\leq$ 780 t). A total of 32 forced harvests were applied after the first time-step, since the starting adult abundances (taken from BarentsWatch, 2022) already exceeded our threshold of $>$ 2 lice fish^{-1}. A total of eight of these farm sites did not receive any additional treatments following the initial harvest. As with azamethiphos treatments, forced harvest became more frequent as the simulation progressed (65 in the first year, 155 in the final year).

Post hoc analysis

There was a strong positive correlation between winter and summer values for larval influx (Figure 4). A moderate to strong, positive correlation was predicted between the number of treatments, resistance (p over final year), larval influx (at both seasons) and average temperature. There was a moderate, negative correlation between the number of treatments and farm size.

Of the 56 genotype frequencies (the proportion of RR, RS, and SS lice at a single site) recorded by Kaur *et al.* (2016) for farms in the same region, 50 were reproduced (proportions within $\pm$ 0.05) by the metapopulation model. That is, proportions of the three genotypes predicted by the model fell well within the range of those recorded in the population in 2012–2014 (Kaur *et al.*, 2016). Of the six genotype proportions that were not observed in our model, five had very high frequencies of the RS genotype (73–100%) and the other had 50% each of RR and RS lice.

Manipulating connectivity

When the 25% least-connected farms were excluded from the model, the eco-evolutionary dynamics of lice at the metapopulation level were not substantially impacted. The rate of evolution and treatment frequency were similar to the full model (Figure 3). Removing farms with low larval influx slightly increased the mean adult abundance per farm (Figure 3b), since these farms had lower infestation rates in the full simulation. In contrast, there was a marked effect of removing the most-connected farms. In this simulation, the R allele took much longer to spread through the population (p = 56% after 10 years), and the mean infestation and treatment levels were suppressed (approximately 0.1 adults fish^{-1} and 50 treatments per year; Figure 3). When farm removal was random, these populations measures were somewhere between half and equal to that of the full simulation.

Discussion

Evolution of resistance

We constructed a discrete-time, stage-structured metapopulation matrix model that successfully simulated the rapid evolution of azamethiphos resistance in Norwegian salmon lice. Within the space of 10 years, the frequency of the R allele increased from very low to very high levels throughout the study area in response to regular treatments. This is consistent with the evolution of organophosphate resistance that was observed to occur in the Atlantic (Kaur *et al.*, 2017). The model predicted resistance to evolve most rapidly and reached higher frequencies in the south-west (Hordaland) and north

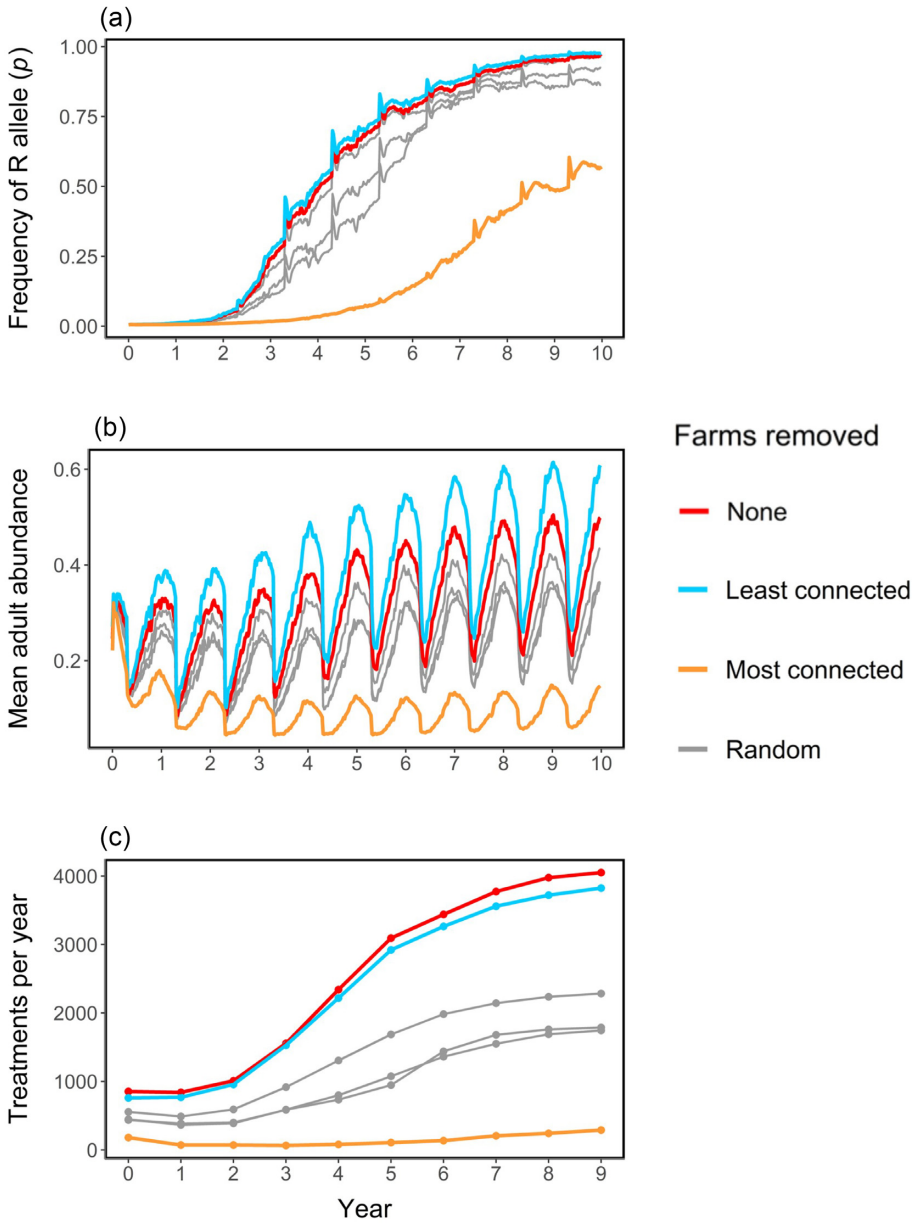

Figure 3. Summary of the entire louse metapopulation in the study area through time: in the full simulation (red), and in simulations with either the least connected (blue), most connected (orange), or random (grey) 25% of sites removed. (a) The frequency of the R allele (*p*) in the adult louse metapopulation; (b) the mean adult abundance (lice fish^{-1}) on farms; and (c) The number of azamethiphos treatments applied to across farms each year.

(Nordland) of our study area. This outcome is also consistent with the spatial distribution of the R allele recorded in the population in 2012–2014 (Kaur *et al.*, 2016). A wave of resistance was borne northwards from the Hordaland hotspot along the current that travels south to north along the Norwegian coastline (Figure 1). Because of the direction of this current, the dispersal of lice from resistant populations southwards into more susceptible populations was limited.

The Hordaland area is a high-density region of aquaculture, containing approximately 35% of the farm sites and 30% of the hosts in our simulation. Farm density is tightly bound to louse connectivity, and there is a particularly high degree of larval migration between farm sites in the Hordaland cluster (Samsing *et al.*, 2017, 2019). Removing farms according to their connections with surrounding sites had a much more pronounced effect on population dynamics than when farms were removed randomly. When the most highly connected sites were excluded, the remaining cluster of farms in the Hordaland region (which still comprised approximately 26% of the farms in the study area) still acted as an evolutionary hotspot (Figure 6), but the rate at which the resistant allele spread through the metapopulation was delayed by at least 4–5 years. In this scenario, the evolution of resistance was also stymied in the north of the study area by the removal of well-connected farms in that region (Figure 6). In contrast, reducing farm numbers by 25% had a minimal effect at the metapopulation level if those sites were the most isolated ones in the network.

High farm connectivity facilitates gene flow of the R allele from resistant to susceptible populations. Further, farms receiving a greater influx of lice from surrounding sites are more likely to require treatments to keep infestations in check

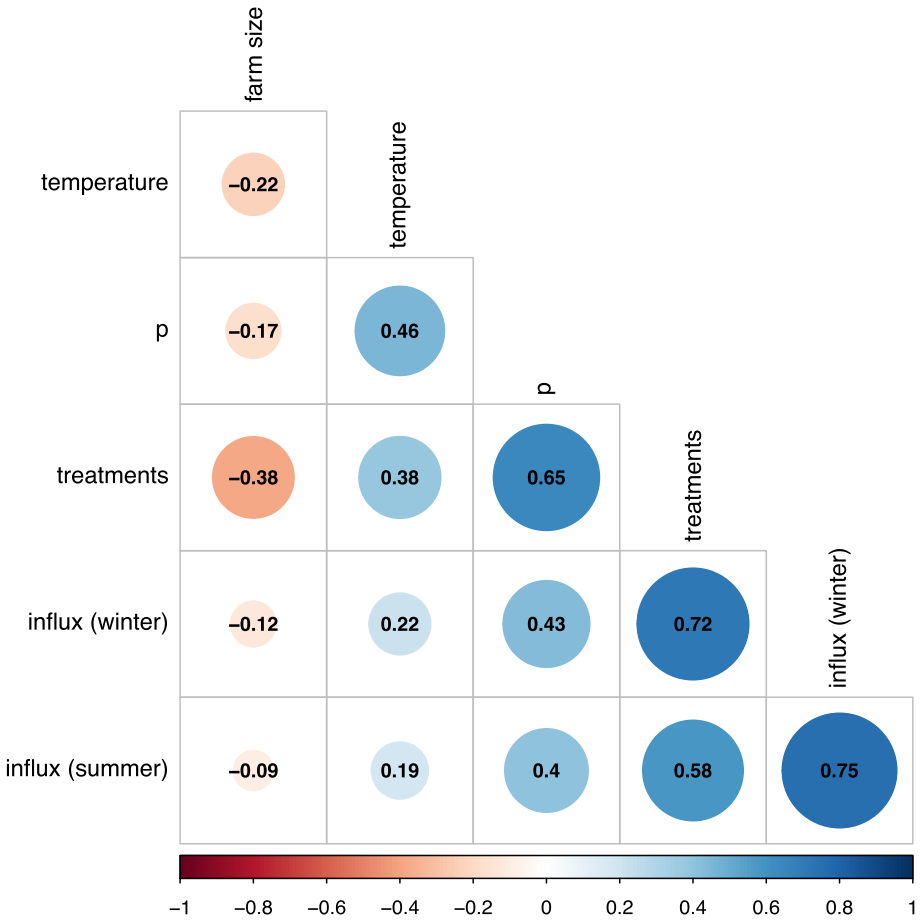

Figure 4. Spearman's rank correlation matrix for farm variables: mean yearly temperature, the average frequency of the R allele (*p*) in the final year of the simulation, the number of azamethiphos treatments applied over the simulation, larval influx (sum of dispersal probabilities from neighbouring farms, in winter and summer), and the number of hosts per farm (farm size). Numbers are correlation coefficients, circle size indicates the strength of the correlation and colour indicates the direction of correlation (blue = positive, red = negative).

(Figures 3 and 4). As depicted in the model, there is a strong, positive feedback between treatment frequency and louse resistance: more treatments leads to stronger selection for resistance, and as resistance becomes more widespread in the population, farms must be treated more often to keep lice levels low. This explains the concurrent rise in resistance, abundance, and treatment frequency in the population through the simulation, especially from the fourth year onwards (Figure 3).

Our model captured the seasonal trend in treatment frequency observed in farm records, in particular the spike at the beginning of spring (Figure 5a). The spring treatments in the model were a reactionary response to the louse limit falling from 1 to 0.4 adults fish^{-1} during this period. In reality, farmers can pre-empt the changing lice limits and adjust treatment regimes accordingly in the weeks leading up to spring. As such, the rise in treatment frequency observed in the farm data (Figure 5) is more gradual than in our simulation, and the spike in treatments occurs slightly before the change in lice limits.

The seasonal fluctuation in louse abundance in our model also matched that observed in the farm database (Figure 5b) and in other population models (Groner *et al.*, 2014). A reduction in louse numbers during the spring treatment period was followed by rapid population growth over summer. Population growth slowed over winter due to temperature effects on

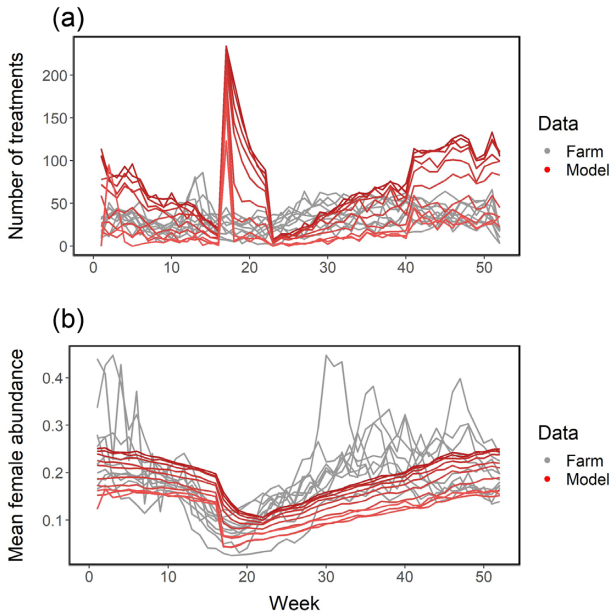

Figure 5. (a) Number of delousing treatments over the course of a year, recorded on farms in the years 2012–2021 (grey) and from the model simulation from year 1 (light red) to year 10 (dark red). (b) Mean adult female abundance (lice fish^{-1}) across farms over a year, reported on farms 2012–2021 (grey) and from the simulation (red).

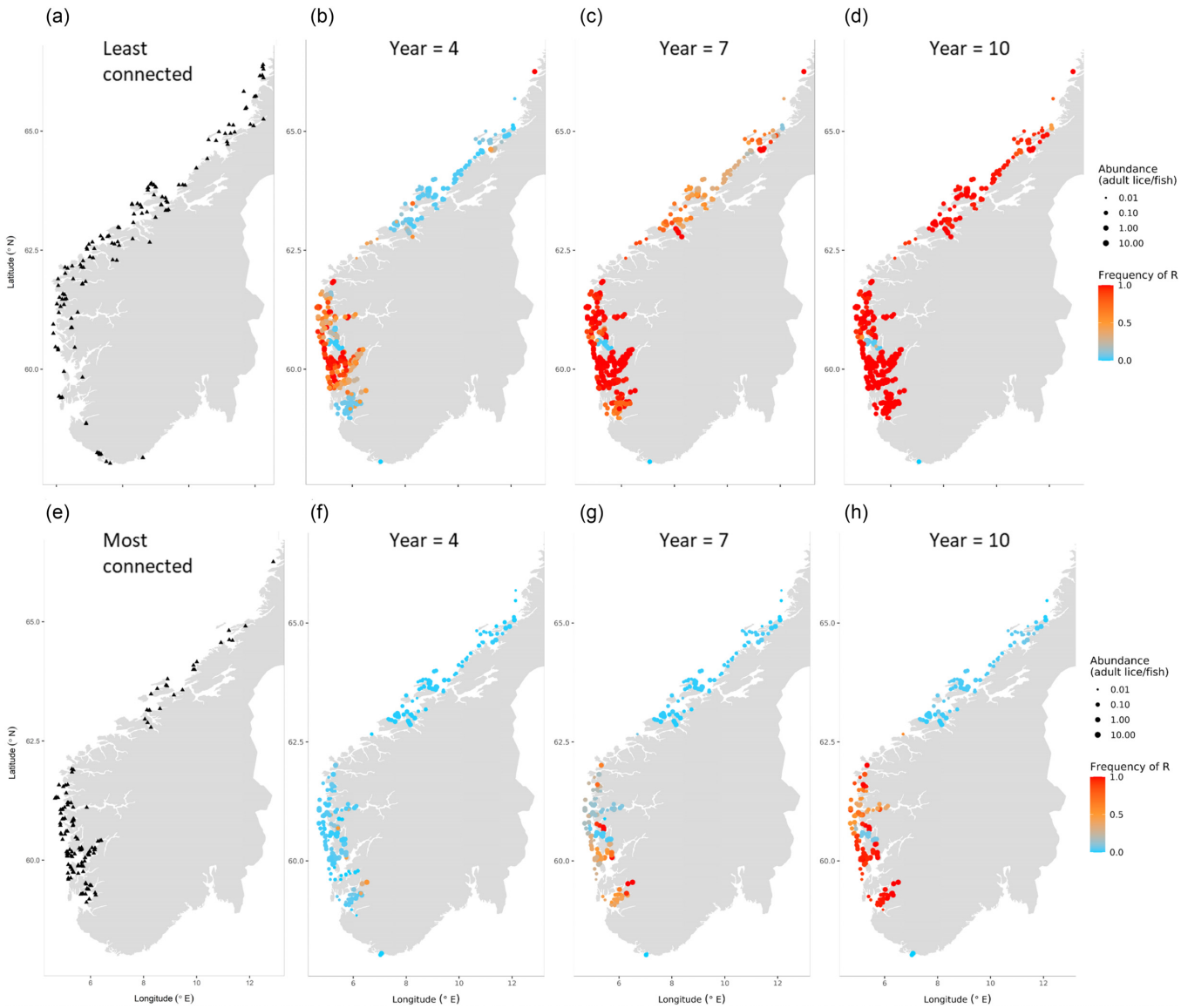

Figure 6. (a) Location of the 25% least-connected (lowest larval influx) farm sites. (b)–(d) Adult abundance (lice fish^{-1}) and frequency of resistance (p) on farms when the least-connected sites were removed from the simulation, at 4, 7, and 10 years, respectively. (e) Location of the 25% most-connected farm sites. (f)–(h) Adult abundance and frequency of resistance on farms when the least-connected sites were removed from the simulation, at 4, 7, and 10 years.

development and reproductive rates. Faster development during the warmer months meant a shorter generation time and a higher net reproductive output as the time between clutches was reduced. On the other hand, higher temperatures also shortened the infective window available for copepodids in the particle-tracking model, thus reducing farm connectivity (Samsing *et al.*, 2017). Nevertheless, the population growth over summer in the model indicated that the improved development and survival of attached stages outweighed the reduced dispersal.

Genotype frequencies

Some of the earliest *L. salmonis* samples genotyped for the *Phe362Tyr* mutation indicate that the resistant allele had already reached a high frequency (50%) in the Norwegian population by 1998. By this time, azamethiphos had already been used in Norway for 4 years, and other organophosphates for two decades before that (Grave *et al.*, 1991). This is concordant with the rapid speed at which resistance spread in our model. As a consequence of resistance, from 1999 to 2008 there was virtually no organophosphate use in Norway, and the frequency of the R allele declined over this period. In 2009, when azamethiphos was reintroduced, the frequency of the R allele was at approximately 10% in some areas (Kaur *et al.*, 2017). We compared the genotype frequencies recorded in 2012–2014 (corresponding to 4–6 years after the reintroduction of azamethiphos in Norway, following a decade of absence) with those predicted by our model for the same regions of Norway. To accurately validate our model results, gene frequency data with a higher spatial and temporal resolution are needed, ideally covering the decade immediately following organophosphate introduction. Nonetheless, using the 2012–2014 snapshot as a guide, our model results fall well within the window of gene frequencies expected

across the different regions. The observed genotype proportions that did not closely match our simulations involved very high frequencies of heterozygotes. This may be due to the R allele already being at a higher frequency in 2009, before azamethiphos was re-introduced and drove selection for homozygotes.

Limitations

The goal of our study was to expand upon previous single-farm models (McEwan *et al.*, 2016) and focus on evolution at the metapopulation scale in response to the selective pressures of farm treatments. As such, some of the more complex population and farm dynamics not concerned with genetic variation (e.g. those included in Aldrin *et al.*, 2017, 2019) were, for simplicity, omitted from our model. For example, azamethiphos was the only treatment available to farms in our simulation (plus a "forced harvest" under higher abundances). In reality, over the past 20 years Norwegian farms have had at their disposal up to five types of chemicals with different modes of action, in addition to the non-chemical methods available more recently (Coates *et al.*, 2021a). Single-farm models have demonstrated that when multiple treatments are applied in combination, the evolution of resistance can be slowed or even halted (McEwan *et al.*, 2016). Despite the many control options available in the Atlantic, resistance to multiple chemotherapeutants has nevertheless rapidly evolved in parallel (Besnier *et al.*, 2014; Helgesen *et al.*, 2017; Fjørtoft *et al.*, 2021).

Farms in Norway are required to fallow for 2 months after harvest; including fallow periods in the model would effectively remove nodes in the farm network at different points in time. This is expected to change patterns in larval influx and gene flow. However, given the high connectivity of farms (Samsing *et al.*, 2017), we would not expect major changes in louse gene flow unless fallowing was synchronized across all farms at key sites (Werkman *et al.*, 2011; Samsing *et al.*, 2019). The timing of stocking after a fallow period might also have an effect; stocking farms in spring can mean more treatments are required than autumn stocking, since the average temperature (and hence louse development) during the production cycle is higher (Jeong *et al.*, 2021).

Other complexities not included in our model include variation in the number and size of hosts on farms through the production cycle, and the partitioning of farms into multiple cages, which could be treated independently (whereas whole farms were treated as a single unit in our model). Including these factors may have influenced the frequency of treatments, and hence the pace of evolution, since mean abundance (lice fish^{-1}) increases more rapidly in cages with fewer hosts (on larger farms, infestation pressures is spread more sparsely across hosts), resulting in treatment triggers being reached sooner. Also, the treatment regimes imposed by our model—in particular forced harvests whenever louse abundance was > 2 adults fish^{-1}–were generally stricter than those observed on farms from 2012 to 2020. The highest abundance on a farm in our simulation (before any treatments were applied) was 14 adults fish^{-1}. This corresponds to 7 adult females fish^{-1}, assuming a 1:1 sex ratio. In comparison, the highest female abundance recorded in the 2012–2020 dataset was 29 adult females fish^{-1} (although this appears to be an outlier, the next highest abundances were still extreme, at 14 adult females fish^{-1}; BarentsWatch, 2022). Across all farms in our 10-year simulation, there were 20 instances of abundances > 5 adult females fish^{-1}, compared to 199 instances of this in the farm dataset. Farm records also show high infestations (> 1 adult female fish^{-1}) being maintained for several weeks at a time due to inefficient treatments, whereas in our model, forced harvests (with 100% louse mortality) ensured swift control of outbreaks. In other words, our simulated farms reacting more quickly and precisely to follow the legally mandated lice limits than actually occurs in reality.

The current model is also limited by the spatial resolution of the particle-tracking model used to parameterize larval dispersal. We used an existing model that combines real-world oceanographic data with louse life history, giving us our best guess at broad dispersal patterns along the Norwegian coastline. However, whilst this model predicts the probability of infective lice reaching the vicinity of a farm, estimating the proportion of these lice that are transported into a salmon cage would require a different, fine-scale hydrodynamics model.

Future directions

Although the present study focused on simulating the evolution of azamethiphos resistance, this model provides the foundation for simulating evolutionary dynamics in *L. salmonis* under diverse scenarios. For example, rather than beginning the simulation with a low frequency of resistance throughout the population, a mutation can be introduced at a single location, as is thought to have been the case for emamectin benzoate resistance (Besnier *et al.*, 2014). Resistance can evolve and disperse more rapidly from some sites than from others, and this model could allow such hotspots for adaptation to be identified. Our simulations removing sites based on connectivity were just the first step towards disentangling the roles of farm density and connectivity on evolution. Further explorations combining this model with network analysis methods (Samsing *et al.*, 2017, 2019) could identify key sites that, if removed at certain times, could restrict gene flow to localized parts of the population, and hence disrupt the dispersal of resistant alleles.

The various parameters in our model can be adjusted to explore their relative effect on population and evolutionary dynamics. For example, fitness trade-offs to resistance can be included, such as reduced survival or fecundity for carriers of the R allele. Azamethiphos resistance has not been associated with any strong fitness costs (Fallang *et al.*, 2004; Fjørtoft *et al.*, 2017), but trade-offs may occur for resistance mechanisms to other management strategies. How large a fitness cost is required to significantly slow adaptation can then be identified. Alternatively, the treatment survival for each genotype (x_g) can be adjusted in different scenarios to explore the interplay between selection strength and treatment efficacy. Azamethiphos is highly effective against susceptible lice but much less so for resistant strains, resulting in strong selection. Other treatments may have weaker selection, but be less effective against lice overall. The model allows us to search for scenarios generating a balance between the two, where adaptation is slowed whilst still keeping infestations under control.

Another parameter that can be adjusted under different scenarios is the lice level that triggers treatment. In Norway, farms are required to immediately treat infestations if they ex-

ceed 0.5 adult females fish^{-1}, or 0.2 during the spring wild smolt migration. These limits have been implemented to prevent major outbreaks, and to reduce the infestation pressure on wild smolts during spring (Johnsen *et al.*, 2021). However, these limits are applied uniformly across Norway and do not consider the variability in farm density and host biomass in different areas. The mandate for regular delousing has garnered some criticism from farmers, as treatments can themselves be costly. It has been estimated that farms will break even from a treatment once infestations reached 7–10 lice (any stage) per fish, but farms are often required to treat below this level (Abolofia *et al.*, 2017). Optimal treatment thresholds—at the individual farm and the regional level—are dependent on many factors, including site connectivity, farm profit, policy adherence of neighbouring farms, and the risk of transmission to wild populations; metapopulation models can help to tease apart these interactions (Kragesteen *et al.*, 2019; Sandvik *et al.*, 2020). Our model can be used to balance these factors with the need to reduce selection pressure and avoid the rapid evolution of resistance. The strong effect of connectivity should also influence decisions around the establishment of new farm sites: adding new farms increases metapopulation connectivity, and hence accelerates the evolution of resistance at a regional level.

Whilst azamethiphos targets the pre-adult and adult lice, management strategies that affect earlier life stages can also be simulated. These do not have to be restricted to chemical treatments. Since 2015, the salmon farming industry has shifted away from pesticides in favour of non-chemical alternatives, although there are concerns that lice could adapt to these as well (Coates *et al.*, 2021a). This model can be used to simulate preventative methods, for example, such as physical barriers to lice or the stocking of parasite-resistant hosts (Barrett *et al.*, 2020). By continuously acting on lice in earlier life stages, preventative strategies can significantly influence population dynamics (Jeong *et al.*, 2021). Such methods may also impose their own selective pressures on the population, which could be included in our model (Coates *et al.*, 2021a, b). The model can also be used to assess the risk of louse resistance to management strategies that are still under development. For example, as gene-edited louse-resistant salmon are developed, model simulations can help gauge, which resistance mechanisms are more likely to drive counter-adaptations in lice, and how stocking of gene-edited salmon can be coordinated across farms in a way that slows any louse evolution (Robinson *et al.*, 2022).

In addition, the model can be expanded to include multiple types of treatments and/or genotypes. New genotypes arise from additional alleles at the one locus, or from a second gene at a new locus. With the latter, the model can be used to simulate multiple selective pressures on different genes, and examine the interplay between them. These pressures could be two different treatments, each selecting certain resistant genes. With this approach, the model can be used to identify how different management strategies can be coordinated across the farm metapopulation to slow the evolution of resistance (McEwan *et al.*, 2016).

Conclusion

This is, to our knowledge, the first use of a metapopulation model to simulate gene flow and evolutionary dynamics in salmon lice. The model captures the broad population dynamics and evolution of azamethiphos resistance expected from real-world data. Our results support the growing evidence that salmon lice have the potential to rapidly adapt to management strategies. Our model also reveals a striking spatial pattern to the spread of resistance, with the Hordaland and Nordland regions of Norway appearing to be hotspots for louse evolution. This is driven by the density and connectivity of farms, and so similar patterns are likely to emerge during the evolution of resistance to other control methods as well. The strong spatial effect in our results highlights the need to coordinate management strategies at a regional scale, rather than a farm-by-farm basis, to slow or avoid louse adaptation. Additionally, evolutionary hotspots are likely to be particularly good sites for monitoring the early stages of resistance to future control methods. The long-term efficacy of pest management in aquaculture is dependent on understanding evolutionary processes. Metapopulation modelling promises to be a versatile tool for predicting the rise and spread of resistance under various scenarios. In particular, it can be used to identify how management strategies might be coordinated across a network of sites to restrict pest adaptation—in salmon farming, and in highly-connected aquaculture and agriculture systems more generally.

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Supplementary data

Supplementary material is available at ICESJMS online.

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Authors' contributions

A.C.—conceptualization, methodology, and writing; N.R.—conceptualization and writing; T.D.—conceptualization and writing; F.S.—resources, software, and writing; I.J.—resources, software, and writing; B.P.—conceptualization, methodology, and writing.

Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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