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Evaluating the effect of synchronized sea lice treatments in Chile

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ABSTRACT

The sea louse is considered an important ectoparasite that affects farmed salmonids around the world. Sea lice control relies heavily on pharmacological treatments in several salmon-producing countries, including Chile. Among options for drug administration, immersion treatments represent the majority of antiparasitic control strategies used in Chile. As a topical procedure, immersion treatments do not induce a long lasting effect; therefore, re-infestation from neighbouring farms may undermine their efficacy. Synchronization of treatments has been proposed as a strategy to improve immersion treatment performance, but it has not been evaluated so far. Using a repeated-measures linear mixed-effect model, we evaluated the impact of treatment synchronization of neighbouring farms (within 10 km seaway distance) on the adult lice mean abundance from weeks 2 to 8 post-treatment on rainbow trout and Atlantic salmon farms in Chile, while controlling for external and internal sources of lice before the treatments, and also for environmental and fish-related variables. Results indicate that treatment synchronization was significantly associated with lower adult lice levels from weeks 5 to 7 after treatment. This relationship appeared to be linear, suggesting that higher levels of synchronization may result in lower adult sea lice levels during these weeks. These findings suggest that synchronization can improve the performance of immersion delousing treatments by keeping sea lice levels low for a longer period of time. Our results may be applicable to other regions of the world where immersion treatments are widely used.

1. Introduction

Sea lice are parasitic copepods that affect farmed and wild salmonids in the marine phase and are considered one of the main health challenges for the salmon industry worldwide (Costello, 2006; Burka et al., 2012). In Chile, the sea lice species of concern is *Caligus rogercresseyi*. Heavy infections with other sea lice species have been associated with skin damage (Joínsdoíttir et al., 1992; Nolan et al., 1999), chronic stress and, possibly, increasing susceptibility to secondary infections (Johnson et al., 2004; Revie et al., 2009; González et al., 2015). Infections are thought to increase costs on farms due to reduced fish growth, reduced feed conversion efficiency, administration of chemotherapeutants, and reduced marketability due to skin lesions (Costello, 2009; Liu and Bjelland, 2014).

Globally, the most common tool for controlling sea lice is the use of antiparasitic drugs (Igboeli et al., 2014; Bravo et al., 2015); however, in recent years treatment failures have been reported in most salmon-producing regions (Sevatdal and Horsberg, 2003; Sevatdal et al., 2005; Bravo et al., 2008; Lees et al., 2008; Jones et al., 2012). This situation has motivated investigations of the performance of anti-lice treatments, revealing that one cause of treatment failure is the low sensitivity of sea lice to certain chemicals (Sevatdal and Horsberg, 2003; Sevatdal et al., 2005; Bravo et al., 2008). More recently, research has focused on improving drug administration methods with immersion treatments (i.e. baths) (Corner et al., 2011), which involve complex procedures at the farm.

Sea lice re-infestation from external sources is a factor that can reduce the length of time that treatments are effective for by rapidly increasing the lice levels immediately post-treatment. This may be exacerbated in the case of immersion treatments, which do not provide long lasting residual effects, as do some in-feed treatments such as emamectin benzoate (EMB). Several studies

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have found that external sources of lice are significantly associated with sea lice abundances at the farm level (Jansen et al., 2012; Aldrin et al., 2013; Kristoffersen et al., 2014). Moreover, a recent study conducted in Chile concluded that the infection pressure from neighbouring farms was greater than that coming from within the farm itself (Kristoffersen et al., 2013). Thus, in the context of immersion sea lice treatments in Chile, external sources of sea lice may seriously limit the duration of the treatment effect associated with these products.

One treatment strategy that addresses external sources of sea lice is coordinated treatments. The rationale behind this approach is to interrupt the sea lice life cycle at all farms at the same time, which should minimize the exchange of copepodids among farms after the treatments and keep the sea lice levels low over time (Ritchie and Boxaspen, 2011). Coordinated sea lice treatments have been implemented in many salmon producing regions around the world (Rae, 1999; Jackson, 2011; Revie, 2011; Ritchie and Boxaspen, 2011; Saksida et al., 2011). In Norway, Ireland, Scotland, and the western coast of Canada, coordinated delousing treatments are performed at specific times of the year (once or twice a year, usually in winter and spring) to reduce gravid sea lice on farms and transmission to out-migrating juvenile wild salmonids in the spring (Rae, 1999; Jackson, 2011; Revie, 2011; Ritchie and Boxaspen, 2011; Saksida et al., 2011). These procedures have been referred to as strategic coordinated treatments, as they target specific lice stages at specific times of the year.

In Chile, coordinated treatments are aimed at improving treatment performance; to that end, treatment coordination is encouraged all year round by establishing coordinated windows of 7 days of duration every 2 weeks (approximate) for each of the eight administrative macro-zones in the country (SERNAPESCA, 2012). Within each macro-zone, salmon farms are grouped into neighbourhoods, in which farms are required to coordinate certain management strategies. Neighbourhoods are delimited by epidemiologic, oceanographic, operational, and geographic criteria by the government authority (Subpesca, 2011). Because treatments in Chile need to be carried out in a relatively short period of time, the term "synchronized" is a better descriptor of the activity than "coordinated". At the time of this study, synchronized treatments in Chile were optional unless the parasite level on a farm surpassed 9 mobile lice per fish. Farms with this level of lice, and neighbouring farms within 5 nautical miles with more than 6 mobile lice per fish, were required to treat within the synchronization window (SERNAPESCA, 2012).

There are no published studies that have evaluated the effect of treatment synchronization on sea lice levels over time. The Chilean context, which involves monthly voluntary synchronized treatments, weekly sea lice monitoring, and a large number of fish farms, offers a unique opportunity to evaluate treatment synchronization at the farm level, while controlling for external sources of lice and factors that affect the sea lice abundance at the farm itself. The objective of this research was to assess the duration of the effect of synchronized treatments on sea lice levels while controlling for the initial treatment effect on farms and other potential confounders.

2. Materials and methods

2.1. Study location

Our study was conducted in Los Lagos and Aysén regions (41°28′ to 46°18′S) in southern Chile. This area consists of a 500 × 150 km system of small channels, fjords, and islets, which contains approximately 90% of the salmon farming activity in the country. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) were the most commonly grown species on approximately 70% of the active farms, in 2012–2013, while Coho (*Oncorhynchus kisutch*) and Chinook salmon (*Oncorhynchus tshawytscha*) represented the rest.

2.2. Data and study period

Data originated from the Chilean salmon farming association's (SalmonChile) sea lice monitoring program, which collects and manages information on approximately 90% of salmon farms located in the study area. Each participating farm reports *C. rogercresseyi* counts for juvenile (chalimus I–IV), mobile adults (including non-gravid females), and gravid female stages on 10 fish from each of four pens (40 fish in total) on a weekly basis. Weekly sea lice assessments are performed by farmers following the protocols described in the Specific Sanitary Program for Surveillance and Control of Caligidosis (SERNAPESCA, 2012), which is run by the Chilean government. Information about delousing treatments is also reported to SalmonChile's database, specifying the product used and the start/finish dates of the procedure at the farm level. Environmental data, such as water temperature and salinity, and production information, such as number of fish and average fish weight, are also collected on a weekly basis. We restricted the data in our study from January 2012 to September 2013 because of a change in the method of reporting treatments in the database that occurred in late 2011. Farms rearing Coho and Chinook salmon were not included in the analysis because these farms are not required to report lice abundance as frequently as the other species, due to their relative low susceptibility to *C. rogercresseyi* infections (Bravo, 2003; Yatabe et al., 2011).

2.3. Selection of treatment events

Treatment events for this study were selected from all immersion treatments reported through SalmonChile's sea lice monitoring program during the study period. Each treatment event was followed in time, either until a new delousing treatment occurred or to a maximum of eight weeks post-treatment. Our study was restricted to treatments performed with the topical drugs azamethiphos and synthetic pyrethroids (deltamethrin and cypermethrin), because these drugs were the most common used in Chile during the study period (R. Ibarra, Intesal-SalmonChile, pers. comm.). Treatments performed with more than one drug were not included in this study because they are not a common practice in Chile and are not promoted by the industry (J. Mancilla, Marine Harvest Chile, pers. comm.). In addition, we included only treatment procedures lasting up to one week, in order to avoid exceptionally long procedures. Finally, we excluded treatment events carried out on farms with no neighbouring farms within 10 km seaway distance, because the treatment synchronization effect could not be assessed if farms did not have neighbours. Delousing treatments were carried out by the farmers based on their own criteria, following the manufacturers' directions.

2.4. Study design and outcome variable

The study design was structured as a retrospective cohort study. Our outcome of interest was the adult *C. rogercresseyi* mean abundance at the farm level after a delousing treatment, starting from the second week and up to the eighth week after the procedure. We did not include the first week post-treatment in the outcome because we were not interested in modeling the drop of sea lice levels right after the treatment, given that another study recently addressed this issue on *C. rogercresseyi* (Arriagada et al., 2014). We chose adult *C. rogercresseyi* as the outcome, because this life stage appeared to be more sensitive to synthetic pyrethroids than the juvenile stages (Arriagada et al., 2014), and because both

pyrethroids and azamethiphos are authorized for their use on adult stages of *C. rogercresseyi* (SAG, 2016). Adult *C. rogercresseyi* includes male and non-gravid and gravid female lice. The mean adult lice abundance (Rózsa et al., 2000) was based on the total 40-fish sample reported every week through the sea lice monitoring program, maintained by SalmonChile.

2.5. Treatment synchronization variable

For each immersion treatment at the farm of interest that met our inclusion criteria, we set a synchronization window, delineated by a period of time starting three days before the treatment and finishing three days after treatment completion. Any neighbouring farms within 10 km seaway distance from the farm of interest that treated their fish for sea lice within this period of time were considered synchronized with the farm of interest.

The treatment synchronization intensity (TSI) was determined as follows: for each immersion treatment (g) included in our study, we weighted neighbouring farms (j) within 10 km seaway distance of the treatment farm (i) that reported any delousing treatment (immersion or oral) within the corresponding synchronization window, where each weight was determined from the seaway distance d_{ij} and a Gaussian kernel density ($w_{10k}(d_{i,j})$). This procedure has been previously used by other authors for modeling the external sea lice pressure (Jansen et al., 2012; Kristoffersen et al., 2013). Seaway distances were calculated using the *gdistance* package in R v.3.0.1 (www.r-project.org). Geographic coordinates for each farm were provided by SalmonChile. In formulae, the treatment synchronization intensity for a given treatment event (TSI_g) was calculated as follows:

$$TSI_g = \sum_{j \in R(g)} w_{10k}\left(d_{i,j}\right) \quad , i = farm\,(g)\,, \tag{1}$$

where $R(g)$ is the spatio-temporal synchronization window drawn for each treatment (g).

The Gaussian kernel weights ($w_{10k}(d_{i,j})$) for each neighbouring farm (j) relative to the farm of interest (i) were calculated as follows:

$$w_{10k}\left(d_{i,j}\right) = \frac{1}{\sqrt{2\pi\tau}} e^{-\frac{1}{2\tau^2} {d_{ij}}^2} \tag{2}$$

with the bandwidth τ given as one-fourth of the total distance plus one, i.e. $\tau = (2^*10+1)/4 = 5.25$, a default setting for kernel functions in the R language.

Because TSI did not consider the total number of farms in the area, we included the total number of active neighbouring farms, around the farm of interest, as a predictor in our model, called maximum synchronization potential (MSP). MSP was calculated as the sum of kernel-weighted distances between neighbouring farms with sea lice and the farm of interest within a 10 km seaway distance, similar to the way TSI was calculated, but including all the neighbouring farms irrespective of whether they treated or not.

2.6. Selection of other predictors

2.6.1. Other off-farm predictors

We accounted for external infectious pressure before treatment synchronization by including the reproductive potential of neighbouring farms within 30 km seaway distance from the farm of interest. This variable was expressed as the sum of gravid female mean abundances ($GF_{j,t(g)-1}$) at neighbouring farms (j) one week before the treatment (g) and within 30 km seaway distance of the treatment farm (i); each of those were also weighted by seaway distance and a Gaussian kernel density ($w_{30k}(d_{i,j})$) with a bandwidth determined as previously described. The neighbouring farm's reproductive potential (NRP_g) was calculated as follows:

$$NRP_g = \sum_{j \in A_i} GF_{j,t(g)-1} w_{30k}\left(d_{i,j}\right), \; i = \text{farm(g)}, \tag{3}$$

where A_i is the area included within the 30 km seaway distance from the treatment farm of interest (i). This distance was selected based on other studies that have found this to be a good average distance of influence for sea lice in Chile and Western Canada (Kristoffersen et al., 2013; Rees et al., 2015).

2.6.2. On-farm predictors

The internal source of lice was represented by the mean gravid female lice one week before the immersion treatment. In addition, we evaluated a group of treatment-related variables, including the drug used in the treatment procedure (azamethiphos or pyrethroid), time (in days) that it took to treat the entire farm, and previous delousing treatments during four weeks before the treatment under evaluation. Water salinity and temperature, and fish-related variables such as fish weight, total number of fish, fish biomass, and stocking density during the follow-up period, were included in the model building process as well.

2.7. Statistical models

The natural log ($\log_e$) of adult lice mean abundance at week t after treatment g was modeled using a linear mixed-effects model with neighbourhood, farm, and treatment event as random effects, and a correlation structure to account for the repeated measures (weeks) within each treatment event. We added 0.01 to the mean abundance of adult lice before the log transformation in order to avoid losing data because of zeros. The model equation was expressed as:

$$\ln\left(Y_{gt} + 0.01\right) = X_{gt}\beta + u_g + u_{farm(g)} + u_{neighb(g)} + \varepsilon_{gt} \tag{4}$$

where Y_{gt} is the adult lice mean abundance, X_{gt} is the vector for fixed effects, β is the corresponding coefficient vector, while u_g, $u_{farm(g)}$, and $u_{neighb(g)}$ are random effects for treatment event, farm, and neighbourhood, respectively, all assumed to be independent and normally distributed, with mean zero and corresponding variances. Errors (ε_{gt}) were assumed to be correlated due to repeated observations in time and, consequently, this equation component was modeled with a suitable correlation structure, allowing for the expected decay in correlation while increasing the time steps, as explained below.

2.8. Model building process and model validation

The model was built using the stepwise backward elimination approach, starting from a maximum model that included all the predictors described above. Variables with the highest p-values (Wald test) were removed from the model one at a time until all predictors were below the significance threshold ($p \leq 0.05$), unless the removal of a potential confounding variable induced a substantial change (>20%) in the coefficients of the other predictors. When two explanatory variables were highly correlated ($|r| > 0.7$), only one of the predictors was retained in the model. The choice between them was based on known biological effect and Akaike Information Criterion (AIC).

Time was included as a categorical variable (week 2–8), and we included interaction terms between week and predictors that could plausibly have a time-varying effect. These predictors were TSI, MSP, NRP, gravid female mean abundance one week before treatment, and adult and juvenile mean abundance one week after

treatment. In cases where the interaction term was not significant (Wald test $p < 0.05$), we evaluated the predictor on its own significance. Because the biology of sea lice suggests treatment synchronization should not impact adult lice levels before week 4 (given that, in the most favourable environmental scenario, the louse needs at least 25 days to develop from egg to adult stage), we opted to compute the Wald test for the effect of TSI on weeks 4 to 8.

If the single term was not significant, we removed the predictor from the final model. MSP was always included with TSI, regardless of its significance, because the latter represents the maximum potential of treatment synchronization among neighbouring farms in the 10-km area and, thus, we considered it essential for interpretation of the TSI effect.

The model was fitted using maximum likelihood (ML) estimation. Correlation between error terms was evaluated by exploring different correlation structures, such as first order autoregressive (AR(1)), Toeplitz, and unstructured with equal and unequal variances. We chose the best fitting correlation structure based on parameter significance, model parsimony, and whether the estimated ρ (rho) had an expected decay across time steps. Model comparisons involving hierarchical and correlation structure were based on the likelihood ratio test and AIC. The assumption of linearity between continuous predictors and the outcome was inspected by running mean and locally-weighted smoothing curves (lowess) between the standardized residuals and each continuous predictor retained in the final model. Random effects with variance estimates equal to zero were dropped from the final model. The homoscedasticity assumption for random effects was inspected by plotting standardized residuals versus predicted values, while the normality assumption was assessed with Q–Q plots using the standardized residuals and predicted best linear unbiased predictors (BLUPs) for random effects. The impact of poorly fitted observations (defined by having standardized residuals numerically >3) on the final model coefficients was evaluated by rerunning the final model without these observations. We also explored the impact of using different offsets before log-transforming the outcome; to that end, we performed a Box-Cox procedure (Venables and Ripley, 1999), in which we tried different offset values ranging from 0.0001 to 2.5. The final model was also fitted using robust standard errors as a sensitivity analysis for the mixed model assumptions. All statistical analyses were performed with Stata IC 13 (StataCorp LP).

2.9. Model predictions

We used the final model to predict adult lice levels during the follow-up period, under different scenarios. The treatment synchronization variable was assessed by comparing the scenarios of null and high synchronization intensity. In all cases, predictions were made assuming that treatments were performed in the Aysén region, using pyrethroids, and the treatment synchronization intensity (TSI) was not greater than the maximum synchronization potential (MSP), while the rest of the predictors were set at their mean values.

3. Results

3.1. Descriptive analysis

Out of 1811 immersion treatments reported in the Salmon-Chile's Sea Lice Monitoring Program during the study period, 706 (39%) fulfilled our inclusion criteria. These treatments were

Table 1
Descriptive statistics for selected variables included in the model building process.

Variable	Mean	Median	Standard deviation	Range
Number of neighbouring farms within a 10 km seaway distance[a] (excluding farms with Coho and Chinook salmon, and farms with no neighbours within 10 km seaway distance)	2.61	2	1.51	1–8
Duration of the treatment procedure in the farm (days)[a] (limited to 7 days)	3	3	1.36	1–7
Water temperature (°C)[b]	10.90	10.80	1.44	7.40–16.70
Water salinity (ppt)[b]	30.92	31.00	2.19	12.00–36.00
Mean fish weight (kg)[b]				
Atlantic salmon	2.65	2.50	1.51	0.16–9.41
Rainbow trout	1.95	1.98	0.72	0.39–5.75
Fish number ('000)[b]				
Atlantic salmon	741.9	778.0	277.2	1.1–6234.2
Rainbow trout	799.3	854.6	288.2	28.3–1291.1
Fish biomass (ton)[b]				
Atlantic salmon	1768.1	1635.3	1069.0	4.0–7174.1
Rainbow trout	1482.0	1532.9	782.5	30.9–6908.9
Stocking density (kg/m^3)[b]				
Atlantic salmon	7.30	6.93	4.05	0.27–22.30
Rainbow trout	6.50	6.55	2.05	1.24–13.10
Gravid female lice mean abundance one week before treatment[a]	4.13	3.00	5.60	0.00–91.98
Adult lice mean abundance one week after treatment[a]	4.24	2.25	11.21	0.00–281.98
Juvenile lice mean abundance one week after treatment[a]	3.92	1.66	10.57	0.00–157.08
Treatment synchronization intensity (TSI)[a]	0.054	0.046	0.056	0.000–0.252
Maximum synchronization potential (MSP)[a]	0.108	0.093	0.066	0.013–0.372
Neighbouring reproductive potential (NRP)[a]	0.475	0.308	0.648	0.016–7.716

[a] Variables recorded as a single value for all weeks within a treatment event (n = 706).
[b] Variables recorded as one value per week within a treatment event (n = 2278).

Table 2
Descriptive statistics for the adult lice mean abundance across different levels of selected predictors.

Variable	Levels	Mean	Median	90% range	n
Week post-treatment (follow-up period)	2	7.70	4.28	0.55–19.05	704
	3	8.76	5.45	0.93–23.80	557
	4	9.74	5.85	0.75–25.65	368
	5	9.86	5.04	0.70–38.40	256
	6	8.30	5.13	0.48–24.40	171
	7	9.19	5.00	0.58–24.60	127
	8	6.87	4.55	0.33–20.50	95
Number of neighbouring farms within a 10 km seaway distance (excluding farms with Coho and Chinook salmon, and farms with no neighbours within 10 km seaway distance)	1	7.88	5.00	0.50–28.18	653
	2	6.17	4.65	0.75–17.75	613
	3	9.29	5.16	0.69–21.75	420
	4+	11.51	5.08	0.68–44.33	592
Drug	azamethiphos	8.09	4.60	0.33–28.70	165
	pyrethroids	8.67	4.98	0.70–23.20	2113
Duration (days) of the treatment procedure in the farm (limited to 7 days)	≤2	8.73	4.25	0.53–24.73	971
	3–4	7.70	5.34	0.75–21.95	986
	4–7	11.14	5.93	1.25–28.80	321
Water temperature (°C)	<9	4.90	4.55	0.08–10.60	163
	9–13	9.04	4.98	0.70–26.68	1977
	>13	7.06	5.43	1.15–22.60	138
Water salinity (ppt)	<21	5.57	0.25	0.00–45.30	17
	21–30	9.48	5.90	0.70–28.90	866
	>30	8.13	4.55	0.70–19.63	1395
Species	Atlantic salmon	8.96	5.03	0.70–26.83	1928
	Rainbow trout	6.80	4.54	0.50–14.38	350
Stocking density (kg/m^3)	<5	5.54	3.88	0.45–12.95	746
	5–10	8.83	5.10	0.78–22.23	1008
	>10	12.63	6.34	1.63–51.35	524
Gravid female lice mean abundance one week before treatment	0–3	4.45	3.58	0.38–10.53	1138
	3–6	8.51	6.16	1.33–23.23	870
	>6	26.62	8.51	1.80–112.38	270
Adult lice mean abundance one week after treatment	0–3	4.45	3.50	0.45–10.48	1425
	3–6	9.01	6.73	2.05–24.55	562
	>6	28.33	9.60	3.95–109.60	291
Juvenile lice mean abundance one week after treatment	0–3	5.13	3.85	0.50–12.63	1553
	3–6	8.59	6.64	1.85–21.23	404
	>6	25.59	8.50	2.15–107.40	321

performed on 227 farms located in 46 neighbourhoods across two fish farming regions in Chile. Six-hundred and four (604) sea lice treatments were carried out on Atlantic salmon and 102 on rainbow trout. During the study period the farms treated, on average, six times per production cycle, with 23% of farms treating 10 or more times. In total, 2278 weekly sea lice measurements were included in our analysis. Fish included in the study weighed, on average, 2.56 kg, though weights ranged from 0.16 to 9.41 kg. Water temperature ranged between 7.4 and 16.7 °C, with a mean of 10.9 °C (Table 1).

When the adult lice mean abundance was averaged at different levels of the predictors included in our analysis (raw data), distinct patterns were observed (Table 2). For example, the adult lice level increased from week 2 to 5 post-treatment. Raw data also showed that, in general, the adult lice levels increased when the farm of interest was surrounded by a greater number of neighbouring farms. Other factors that appeared positively associated with sea lice levels post-treatment were sea lice abundance one week before the procedure, stocking density, treatment duration, and rearing Atlantic salmon (instead of rainbow trout). Higher adult lice levels were observed at intermediate levels of both water temperature (9–13° C) and water salinity (21–30 ppt).

Treatment synchronization intensity (TSI) ranged from 0.00 to 0.25, averaging 0.05, while the maximum synchronization potential (MSP) values ranged from 0.01 to 0.37, with a mean of 0.11. In the case of NRP, the minimum and maximum values were 0.02 and 7.7, respectively, with a mean of 0.48 (Table 1).

3.2. Multivariable analysis

3.2.1. Treatment synchronization variable

The final model showed that as TSI increased (i.e. as the number of neighbouring farms treating during our defined synchronization window increased), the adult lice levels at the farm of interest decreased at weeks 5, 6, and 7 post-treatment ($p = 0.009$, $p = 0.038$, and $p = 0.038$, respectively). The lice levels during the initial 2 to 4 weeks post-treatment were not significantly different for farms that had a high or low number of neighbour farms synchronizing their sea lice treatments (Table 3). The total effect of TSI in weeks 4 to 8 was significant ($p = 0.019$).

As the value of TSI ranged between 0 and 0.25 units (Table 1), we chose to interpret the coefficient for a change in 0.25 units, which represents a high synchronization scenario. Accordingly, we set the influence of neighbouring farms (MSP) at 0.25 units. Because TSI incorporates several factors, a specific TSI value can be achieved by different combinations of these factors. For example, 0.25 TSI units could be achieved if a farm had four neighbouring farms within 3.2 km that treated. Under the high synchronization scenario (TSI = 0.25), the predicted adult lice levels at weeks 5, 6, and 7 post-treatment were, respectively, 50%, 60%, and 70% lower than the null synchronization scenario (TSI = 0.00) (Fig. 1), after controlling for the other predictors in the model.

The number of rainbow trout or Atlantic salmon farms in the synchronization area (MSP) was associated with greater adult lice levels during the follow-up period, although not significantly so

Table 3
Multivariable model. Coefficient estimates, standard errors, and *p*-values for explanatory variables in the final model for the log adult *C. rogercresseyi* mean abundance from week 2 to week 8 post-treatment, on Atlantic salmon and rainbow trout farms in Chile (n = 2278).

Variable name	Estimate	Standard error	*p*-value	95% confidence interval	
Fixed effects parameters					
Intercept	−0.300	0.182	0.099	−0.656	0.056
Week after treatment (week 2 as reference)			**<0.001**		
3	0.555	0.046	**<0.001**	0.465	0.646
4	0.395	0.078	**<0.001**	0.244	0.548
5	0.669	0.087	**<0.001**	0.499	0.838
6	0.740	0.131	**<0.001**	0.483	0.998
7	0.832	0.187	**<0.001**	0.466	1.199
8	0.645	0.173	**<0.001**	0.307	0.983
Treatment synchronization intensity (1w10k) (TSI)	−0.267	0.496	0.591	−1.239	0.706
TSI * week			0.051		
3	−0.187	0.419	0.656	−1.008	0.635
4	−0.024	0.780	0.975	−1.505	1.553
5	−2.509	0.954	**0.009**	−4.379	−0.640
6	−3.392	1.631	**0.038**	−6.589	−0.195
7	−4.482	2.162	**0.038**	−8.719	−0.245
8	−1.280	1.936	0.508	−5.074	2.513
Neighbourhood reproductive potential pre-treatment (30 km) (NRP)	0.190	0.036	**<0.001**	0.119	0.261
Maximum synchronization potential (10 km) (MSP)	0.749	0.414	0.070	−0.062	1.559
Log of gravid female mean abundance one week before treatment	0.014	0.030	0.656	−0.046	0.073
Log of gravid female mean abundance one week before treatment * week			**<0.001**		
3	0.008	0.031	0.794	−0.052	0.068
4	0.322	0.050	**<0.001**	0.224	0.420
5	0.255	0.054	**<0.001**	0.149	0.360
6	0.187	0.079	**0.018**	0.033	0.342
7	0.275	0.128	**0.032**	0.023	0.527
8	0.374	0.121	**0.002**	0.137	0.611
Log of adult mean abundance one week after treatment	0.490	0.027	**<0.001**	0.436	0.543
Log of adult mean abundance one week after treatment * week			**<0.001**		
3	−0.258	0.027	**<0.001**	−0.312	−0.204
4	−0.311	0.047	**<0.001**	−0.404	−0.218
5	−0.327	0.053	**<0.001**	−0.430	−0.224
6	−0.325	0.077	**<0.001**	−0.476	−0.174
7	−0.322	0.094	**0.001**	−0.506	−0.139
8	−0.304	0.084	**<0.001**	−0.469	−0.140
Log of juvenile mean abundance one week after treatment	0.183	0.026	**<0.001**	0.133	0.233
Log of juvenile mean abundance one week after treatment * week			**0.033**		
3	0.058	0.026	**0.028**	0.006	0.109
4	−0.067	0.046	0.140	−0.157	0.022
5	−0.033	0.053	0.531	−0.137	0.071
6	−0.016	0.079	0.838	−0.171	0.139
7	0.025	0.096	0.796	−0.164	0.214
8	−0.055	0.085	0.517	−0.222	0.111
Region (Los Lagos as reference)	0.175	0.048	**<0.001**	0.082	0.269
Drug used in the on-farm treatment (azamethiphos as reference)	0.206	0.088	**0.019**	0.034	0.379
Water temperature	0.048	0.014	**0.001**	0.021	0.076
Stocking density	0.017	0.006	**0.002**	0.006	0.028
Random effects parameters					
Farm	0.006	0.011	0.266	<0.001	0.182

Residual structure[a]

week	2	3	4	5	6	7	8
2	0.353						
3	0.556	0.439					
4	0.300	0.516	0.742				
5	0.223	0.438	0.534	0.693			
6	0.076	0.237	0.316	0.503	1.050		
7	0.172	0.317	0.398	0.451	0.538	1.366	
8	0.166	0.290	0.453	0.417	0.361	0.761	0.930

[a] Variances in the diagonal; correlations in the rest of the cells.

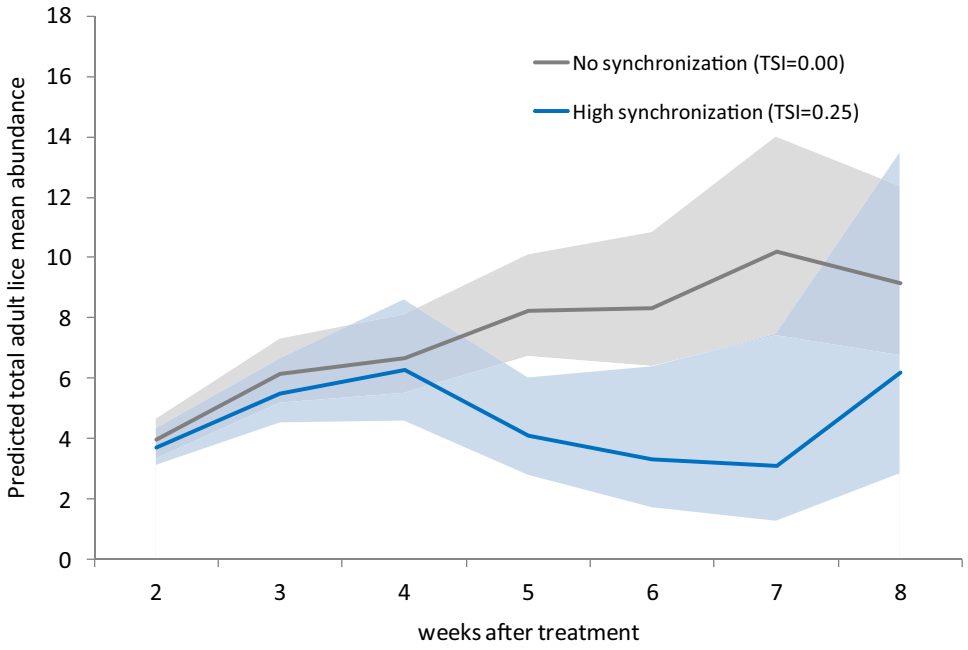

Fig. 1. Predicted adult lice abundances for different treatment synchronization intensities. Predicted adult lice mean abundance and its 95% CI by week after treatment under no (TSI = 0.00) and high treatment synchronization (TSI = 0.25) among neighbouring farms within our synchronizing window. Maximum synchronization potential (MSP) was set at 0.25, region was Aysén and the drug was pyrethroids; while the rest of predictors were set at their mean values. Predictions are estimated from the final linear mixed effects model presented in Table 3.

($p = 0.070$). The interaction between MSP and weeks was less significant ($p = 0.141$). Also, the 3-way interaction term between TSI, weeks, and MSP was not significant ($p = 0.115$).

3.2.2. Other off-farm predictors

We found a very significant positive association between NRP one week before the treatment synchronization and the adult lice levels during the follow-up period ($p < 0.001$), but neither the interaction of NRP with time, nor the 3-way interaction with TSI and week were significant ($p = 0.395$ and 0.129, respectively). One extra unit of NRP (which could represent, for example, a farm with four neighbouring farms at 2.5 km, each with an average of 10 gravid female lice per fish) impacted the farm of interest with 21% more adult lice during the follow-up period.

3.2.3. On-farm predictors

Our model indicated that the abundance of both adult and juvenile lice one week after treatment had a positive impact on the adult lice level during the follow-up period. In both cases, the effect significantly varied across weeks, although adult lice showed a stronger variation in time ($p < 0.001$) than juvenile lice ($p = 0.033$). Similarly, the level of gravid females one week before the treatment was significantly associated with post-treatment adult lice abundance and it varied across the follow-up period ($p < 0.001$).

We found that the abundance of adult lice during the follow-up period was significantly higher, across all weeks, when a farm treated with pyrethroids versus azamethiphos ($p = 0.019$). The interaction between drug and weeks was not significant ($p = 0.102$). Current treatment duration and previous delousing treatments on the same farm during four weeks before the treatment being evaluated did not show a significant impact on the adult lice level during the follow-up period (treatment duration $p = 0.866$; previous treatment $p = 0.298$).

Among the four fish-related variables we tested, fish number, fish weight, and stocking density were significantly associated with our outcome; however, because they were correlated to each other, we chose stocking density, as it produced a model with the lowest AIC. This predictor and water temperature were positively associated with the outcome.

3.2.4. Residual structure

The best fit model had a structured correlation and unequal variances of the residuals (Table 3). In general, the variability increased over weeks, and the correlation between pairs of weeks showed a slow decay as the time step increased (see residual correlation matrix at the end of Table 3).

3.2.5. Model fit

Standardized residuals for the lowest level of our model (weeks) showed some departure from normality, which was driven by a group of observations (n = 27) with extreme negative residuals (standardized residuals <−3). After fitting a model without these observations, no substantial changes to the model coefficients were observed, so we retained the full dataset in our final model. The variance of standardized residuals appeared to be constant across fitted values (i.e. homoscedastic). We also tried a different offset before log-transforming the outcome (i.e. 1.3) after performing a Box-Cox procedure. The fit of this model was better among negative residuals (data not shown). Coefficients and significance of this model did not change substantially, compared to the model shown, with an offset of 0.01. After running the final model shown in Table 3 using robust standard errors, the inference remained essentially the same (data not shown).

4. Discussion

We evaluated the effect of synchronized sea lice treatments on the abundance of adult *C. rogercresseyi* on farms 2 to 8 weeks post-treatment, adjusting for treatment effect on the farm, external sources of lice prior to treatment, and other factors affecting post-treatment sea lice levels. The number of neighbouring farms within a 10 km radius that treated for lice within three days of the farm of interest's treatment (TSI) significantly affected the level of lice post-treatment. Our study suggests that the greater the

number of farms that treated within this synchronization window, the lower the levels of adult lice at weeks 5–7 post-treatment. This finding suggests the synchronization of treatments with neighbouring farms may have resulted in a reduction in the number of infective copepodids in the area.

The pattern of predicted lice abundance over time (Fig. 1) may be explained by the sea lice life stages that are targeted by bath treatments. The predicted increase in adult lice in the early weeks after treatment suggests that, despite the treatment, juvenile lice were still present on fish after treatment. In an earlier study, researchers found that pyrethroids are less effective against juvenile stages of *C. rogercresseyi* (Arriagada et al., 2014); therefore, the increase in adult sea lice during weeks 2 to 4 after treatment may be the consequence of surviving juvenile lice that have evolved into adult stages. The drop in adult lice levels at weeks 5, 6, and 7 on farms that had some level of treatment synchronization with their neighbours, compared to those that did not, suggests the external source of juvenile lice at the farm of interest was interrupted or substantially reduced approximately 4–5 weeks prior. Coincidentally, this is the length of time that *C. rogercresseyi* eggs need to develop into adult stages at 11° C (approximately 32 days) (Bravo, 2010). Because over half the adult sea lice on fish do not survive more than 30 days on fish in a laboratory setting (Bravo, 2010), we hypothesize that, a sudden reduction in the source of sea lice, as is expected with treatment synchronization, would lead to a significant drop in adult sea lice counts within 5–7 weeks post-treatment, as was observed in our study.

Although there was a trend suggesting that the difference in sea lice abundance between the two treatment synchronization scenarios continued beyond 7 weeks, this difference was not statistically significant (Table 3). The number of farms retained in our study for the entire monitoring period (week 2–8 post treatment), was quite low (Table 2). Many farms dropped out of our monitoring time series because they applied a new sea lice treatment before 8 weeks post treatment. Although this selection bias, which would have reduced our ability to detect a synchronization effect, was present throughout our time series, it was more likely to occur at the end of the monitoring period when lice levels were higher. Since our model predictions suggests unsynchronized farms attained the critical treatment threshold in Chile of 9 lice per fish (SERNAPESCA, 2012) at week 7 post treatment, it is likely that beyond this time point selection bias was greater for these farms than for farms that synchronized treatments with their neighbours. This potential differential selection bias makes it difficult to interpret the comparison between synchronized and unsynchronized farms beyond 7 weeks and limits our ability to make any definitive statements about the duration of the effect of synchronization. Nonetheless, the overall finding that synchronization of sea lice treatments between farms within 10 km had an effect on lice abundance is difficult to dispute, as our model findings are biologically consistent with the life cycle of *C. rogercresseyi.*

During the study period, the Chilean legislation mandated the harvest of fish when the mean adult lice per fish reached 9 or greater for three consecutive weeks (SERNAPESCA, 2012). Predictions from our model indicate that when delousing treatments are performed individually (without synchronization) the adult lice level on an average farm may exceed this threshold around the 5th week after treatment, when other predictors in our model were set at the average industry values. By contrast, when all farms with lice-susceptible fish (i.e. rainbow trout and Atlantic salmon) within the 10 km seaway distance joined the treatment synchronization, the treatment threshold, on average, would not be exceeded until after the 8th week post-treatment. This delay in the increase of parasites could mean delaying the next treatment on a farm, which may reduce operating costs. It is worth mentioning, however, that treatment synchronization may, theoretically, synchronize egg production in an area and the consequence of this is unknown, especially in regards to the development of resistance to chemotherapeutants.

Our model confirmed that the neighbouring farms' infectious pressure, expressed in our case as NRP, had a positive association with sea lice abundance at the farm level, which suggests a direct link between the area's gravid female level and our outcome. The influence of the infectious pressure on sea lice abundance has been studied by other researchers for both *C. rogercresseyi* and *L. salmonis*, with results similar to ours (Jansen et al., 2012; Kristoffersen et al., 2013). We expected to have a significant interaction between NRP and time (weeks), as the effect of NRP on post-treatment lice levels should not be of the same magnitude over the entire 8-week observation period; however, this interaction was not significant ($p = 0.395$). We hypothesize this may be because of the correlation between NRP values across consecutive weeks, meaning that the single NRP value we have for each treatment is actually a surrogate variable for NRP values some weeks before. The high autocorrelation value (rho = 0.85) observed for NRP supports this hypothesis.

Our final model included the number of active fish farms in the area before the treatment synchronization (MSP), to control for the number of farms that could have treated at the same time as the farm of interest. By including NRP, in addition to MSP, in our final model, we also controlled for the overall level of lice in the area, which helped adjust for farms that may have had many neighbours, but no lice.

We expected that the levels of NRP and MSP might modify the effect of TSI; however, neither of these two three-way interactions (TSI, weeks, and NRP or MSP) was significant. We may not have had sufficient power to assess these interactions, or the high level of correlation between these predictors over time (NRP = 0.85 and MSP = 0.89) may have contributed to the lack of significance of the 3-way interactions on our outcome measurement.

A potential source of bias that may have affected our results was that, in some cases, our synchronizing window matched the government's treatment synchronization window. In these cases, synchronization was performed at the neighbourhood level (~40 km diameter), so the actual number of farms joining the coordinated procedure may have been greater than the farms we included in our 10 km seaway window. This situation could have produced a stronger synchronization effect than the actual effect of the 10 km synchronization window.

Another potential source of bias in this study includes the possibility that there were un-reported sea lice treatments. We observed extreme negative residuals due to lice levels of zero (or very close) at week 3 or greater during the follow-up period. Reductions in sea lice levels, with no management intervention, are considered unusual as, theoretically, sea lice increase exponentially over time (Krkošek et al., 2010). We attributed this to non-reported delousing treatments during the follow-up period. Because farms with more neighbours tend to have higher lice counts (Kristoffersen et al., 2013) and these were more likely to have un-reported treatments, this effect would have biased our findings towards the null. Other potential reasons for unexpected sea lice reductions are salinity changes, and biomass reduction (harvest).

Acute decreases in salinity have been associated with reduced *L. salmonis* counts in farmed salmon in British Columbia (Arriagada et al., 2016), while fish harvesting could theoretically reduce the host-density threshold for sustaining disease (Frazer et al., 2012). To control for the effect of these factors we included temperature, salinity, and stocking density on the day of sea lice sampling in our initial model. Salinity did not vary greatly in our dataset and was not significant, so it was removed from the final model, but other factors such as region and farm effect were retained, which likely capture the effect of this predictor and other spatially correlated variables, such as sea lice resistance to chemotherapeutants.

Recent studies have reported the presence of populations of *C. rogercresseyi* with low sensitivity to deltamethrin, cypermethrin and azamethiphos in the study area, using bioassays (Helgesen et al., 2014; Marín et al., 2015). Because we did not have access to this type of information for our analysis, we could not control for the potential effect of sea lice resistance to chemotherapeutants. We were however, able to include random farm, and neighbourhood effects, in addition to a region effect, and the neighbours' sea lice reproductive potential (NRP), which may have inadvertently explained the variance in sea lice abundance associated with a treatment resistance phenomenon. Further, areas with lice that were less sensitive to chemotherapeutants likely had higher lice counts and, consequently, were more likely to have synchronized treatments on neighbouring farms, which would have biased our results towards the null (i.e. reduced the treatment synchronization effect). Even if there is a resistance issue in parts of Chile, on average synchronization still appears to reduce lice counts on farms for a longer period of time than unsynchronized treatments.

This study provides the first report of data to suggest that synchronization of sea lice bath treatments on fish farms in close proximity improves the duration of the treatment effect. Our final model, which included TSI, MSP, and NRP, explained sea lice treatment dynamics on farms and the average effect of treatment synchronization among neighbours; however, this is only the first step in understanding the effect of synchronization of antiparasitic treatments at the area level. Future research should explore alternative ways of expressing treatment synchronization and refine the measurement by incorporating, for example, the efficacy of treatments at neighbouring farms. Future research should also consider assessment of other synchronization window sizes (e.g. 20 km) and treatment trigger thresholds (e.g. >3 adult lice) to determine the synchronization settings that have the greatest impact on sea lice levels over time, as well as the effect on the overall treatment frequency during the entire fish production cycle. The logistics of synchronizing sea lice treatments on multiple farms owned by different companies will also need to be taken into consideration. We believe this study provides a solid foundation for these future studies.

5. Conclusion

Anti-parasitic treatment failures can be due to low sensitivity of sea lice to drugs, inadequate drug administration procedures, and/or re-infestation from external sources of sea lice, such as infected neighbouring farms. Our study provides, for the first time, evidence that the synchronization of delousing treatments within 10 km seaway distance may improve treatment effect. In particular, our results suggest that the synchronization of treatments within a 10-km seaway distance window reduces the adult lice levels on farms at weeks 5, 6, and 7 post-treatment, delaying the increase of lice levels for at least 3 weeks. We also observed a linear relationship between the intensity of synchronization and the reduction of sea lice burdens, suggesting that full synchronization of farms within the synchronizing window produced better results than both partial and no synchronization. It is important to note that, despite synchronization, the adult lice levels steadily increased up to 4 weeks after treatment. This may be because juvenile lice are not killed and free swimming larval stages are not affected by the immersion treatments, and highlights the possibility that a single bath treatment, when there are juvenile lice on fish and free swimming lice larvae, will not successfully eliminate all lice on farms in open net-pen aquaculture.

Conflict of interest

The authors declare that they have no conflict of interest.

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