



Understanding sources of sea lice for salmon farms in Chile



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ABSTRACT

The decline of fisheries over recent decades and a growing human population has coincided with an increase in aquaculture production. As farmed fish densities increase, so have their rates of infectious diseases, as predicted by the theory of density-dependent disease transmission. One of the pathogen that has increased with the growth of salmon farming is sea lice. Effective management of this pathogen requires an understanding of the spatial scale of transmission. We used a two-part multi-scale model to account for the zero-inflated data observed in weekly sea lice abundance levels on rainbow trout and Atlantic salmon farms in Chile, and to assess internal (farm) and external (regional) sources of sea lice infection. We observed that the level of juvenile sea lice was higher on farms that were closer to processing plants with fish holding facilities. Further, evidence for sea lice exposure from the surrounding area was supported by a strong positive correlation between the level of juvenile sea lice on a farm and the number of gravid females on neighboring farms within 30 km two weeks prior. The relationship between external sources of sea lice from neighboring farms and juvenile sea lice on a farm was one of the strongest detected in our multivariable model. Our findings suggest that the management of sea lice should be coordinated between farms and should include all farms and processing plants with holding facilities within a relatively large geographic area. Understanding the contribution of pathogens on a farm from different sources is an important step in developing effective control strategies.

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1. Introduction

As salmon farming around the world responds to an expanding market the number of farms and the number of fish on farms increases. An indirect effect of this growth has been an increase in the potential for the spread of density-dependent infectious pathogens. Density-dependent transmission is observed in many

pathogen–host systems (Anderson and May, 1991, Grenfell and Dobson, 1995), including sea lice on salmon farms.

In Chile, the predominant sea lice species infecting farmed fish is *Caligus rogercresseyi*, and since 2008 there has been a bi-weekly government-mandated monitoring program to assist in the management of this ectoparasite (Bravo, 2003; Hamilton-West et al., 2012).¹ This program is used for early detection of sea lice infections on farms and for implementation and monitoring of control strategies.

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¹ As of May 2012 all farmers are required report sea lice abundance weekly.

The extent and frequency of the data collected as part of an industry-wide monitoring program provides a unique opportunity to assess pathogen movement within a vast aquatic system where there are large sentinel populations of animals.

The control and prevention of sea lice on salmon farms requires targeting adult female sea lice which are the source of copepodids, the parasite's infective stage. Juvenile lice on fish originate from either existing adult sea lice on fish within the same farm or from adult lice on fish outside the farm (i.e. external sources). Salmon farmers in Chile and elsewhere have been treating fish on their farms to reduce the number of adult sea lice and the risk of re-infection. Despite efforts to prevent re-infection of sea lice within farms, many industries around the world struggle to control this parasite, suggesting that treatments are not working effectively and/or external sources of lice play an important role in maintaining elevated lice levels on farms. Identifying and controlling external sources of infection has been a challenge for farmers partially because the two to three week time delay between egg production and the infective stage provides sufficient time for the larvae to disperse from their hatching site (Boxaspen, 2006; Amundrud and Murray, 2009; Foreman et al., 2009).

The role of neighboring farms as a source of infective copepodids for farms has become more of a concern as aquaculture industries grow in size. Several recent studies have detected a strong association between the sea lice levels on farms and the abundance of fish hosts in the surrounding area (Krkosek et al., 2011; Jansen et al., 2012). Anecdotal evidence of this phenomenon in Chile occurred between 2007 and 2009 when there was a large reduction in sea lice levels on farms from a mean abundance of 10 adults per fish in 2007 to 1.8 in 2009 (SalmonChile), which coincided with the large decrease in biomass on farms and in farming areas at the same time. It also appears that sea lice numbers have begun to rise again in the last two years as the industry has increased in size (with a mean of 3.0 adults per fish in 2011; SalmonChile). This trend has not been assessed objectively controlling for confounders, such as changes in treatment strategies over time, but it is consistent with the host density-dependent nature of sea lice.

Another management practice that has not been assessed and may be hampering area-wide sea lice control efforts is the movement of infected fish to holding facilities for harvesting. Some processing plants have fish holding facilities to which fish are transported and held for up to 5 days prior to harvest. These holding facilities provide the processing plants with flexibility for scheduling transport and harvest of fish, but they may also increase the risk of pathogen introduction into an area. Although a number of researchers have evaluated risk factors for sea lice (Zagmutt-Vergara et al., 2005; Yatabe et al., 2011), none have examined the role of these holding facilities.

Another factor which may affect sea lice infections and has not been evaluated under field conditions is the presence of shellfish facilities in close proximity to salmon farms. Researchers in North America have shown that bivalves can filter the free swimming life stages of *Leptheophthrius salmonis* (Davenport et al., 2000; Lehané and Davenport, 2006; Molloy et al., 2011). The idea of using

an integrated multi-trophic aquaculture system, which includes culturing bivalves and salmon in close proximity, has been proposed as a means of reducing nitrogenous waste and sea lice on finfish farms (MacDonald et al., 2011; Molloy et al., 2011). Chile's salmon industry, which has over 800 finfish leases and grows over 221,000 tons of Atlantic salmon, 203,000 tons of rainbow trout, and 154,000 tons of coho salmon annually, shares the coastline with 1200 shellfish leases that produce approximately 280,000 tons of shellfish per year. The natural co-existence of these two large industries provides an opportunity to evaluate the relationship between bivalves and *C. rogercresseyi* on fish farms under field conditions. The goals of this study were to better understand the sources of sea lice and the factors affecting lice infection pressure on salmon farms in Chile to improve mitigation efforts.

2. Methods

2.1. Study area and data

We assessed 443 Atlantic salmon and rainbow trout marine farms in Chile's most intense aquaculture regions (between 41°S and 47°S; Fig. 1). Our study did not include farms from the extreme south (south of 47°S) or those producing coho and chinook because these farms had no sea lice, or had low counts with very little variation. The mean temperature and salinity for our study area were 11.2°C and 29 (parts per thousand [ppt]), respectively (Table 1).

All data for this study were obtained through the Chilean salmon farmers association (SalmonChile <http://www.salmonchile.cl>) sea lice electronic monitoring program. Farmers reported on a weekly (occasionally biweekly) basis the mean number of sea lice per fish (abundance) based on a sample of approximately 40 fish collected from four cages. Sea lice were identified by several life-stage categories, but for the purpose of this study we only examined counts for juvenile lice and gravid females. "Juveniles" were defined as all chalimus stages of *C. rogercresseyi*. Gravid females were adult lice with attached egg strings. Average abundances for these two stages of lice were calculated based on their total counts divided by the number of fish examined at a given sample point. The abundance of juvenile sea lice was used as our outcome variable in the statistical analysis because it represents the earliest stage of infection. We used farm data collected between January 2009 and April 2012.

SalmonChile also provided point coordinate locations for all Atlantic salmon and rainbow trout farms and fish processing plants with fish holding facilities. The coordinates of shellfish leases were provided by the government of Chile (Servicio Nacional de Pesca y Acuicultura), the governing body for aquaculture in Chile, though no information was available as to the exact periods over which these leases had been active.

2.2. Description of the variables used in the statistical analysis

Although we were primarily interested in sources of sea lice for fish farms we included a number of factors that

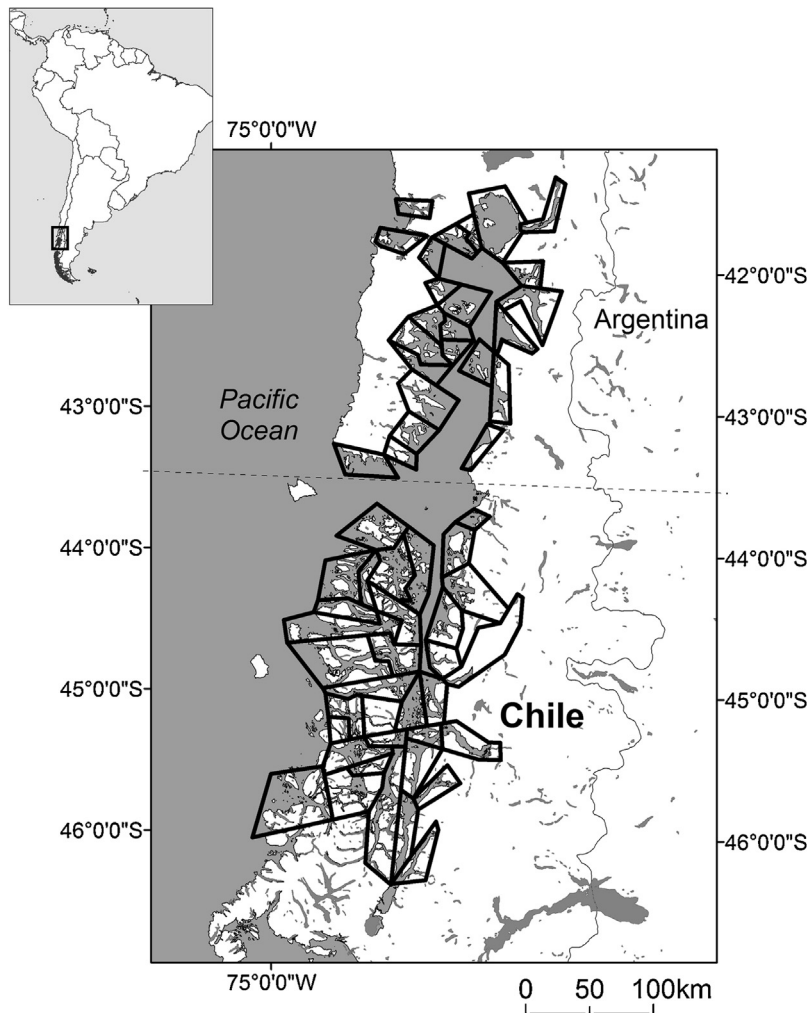


Fig. 1. General location of farms included in our study and farm neighborhood areas (outlined in black) in Chile.

could potentially be associated, indirectly or directly, with the abundance of juvenile sea lice on a fish farm in our model to correct for potential confounding. Factors were divided into three categories for organizational purposes: (1) potential sources of sea lice for the farm; (2) environmental factors affecting survival of sea lice; and (3) farm management factors potentially affecting the abundance of sea lice on fish.

2.2.1. Potential sources of sea lice

We estimated the exposure to infective copepodids originating from within the farm from the mean abundance of reported gravid sea lice on the farm two weeks prior (GF_{t-2}). The two-week lag time approximated the time required for eggs to develop into the copepodid stage at the mean sea water temperature in Chile (Gonzalez and Carvajal, 2003; Bravo, 2010).

To assess the external sources of sea lice infection on farms, we included variables representing sea lice infection from fish holding facilities at processing plants and from neighboring farms. We determined seaway distances between all farms, and between farms and holding

facilities at processing plants using the *gdistance* package in R (www.r-project.org). The direction and force of the water currents could not be incorporated in this measurement because the data were not available; however, our estimate of seaway distances corrected for land masses between sites.

The infection pressure on farm i at time t from neighboring farms ($LGF_{i,t}$) was determined by summing the potential output of lice from neighbors weighted by their distance to the farm. The potential output of sea lice at farm j was computed by multiplying the abundance of gravid sea lice two weeks prior on each of the neighboring farms ($GF_{j,t-2}$) by the number of fish ($N_{j,t-2}$). The weights ($w(d_{i,j})$) were obtained from the seaway distance ($d_{i,j}$) and a Gaussian kernel density with a band width equal to approximately one fourth of the total width, similar to what was used by Jansen et al. (2012). The resulting formula was

$$LGF_{i,t} = \sum_{j \in A_i} GF_{j,t-2} w(d_{i,j}) N_{j,t-2}$$

where A_i was the area included within the kernel's range.

Table 1

Descriptive statistics of the explanatory parameters retained in the top ranked AIC model and their relationship with the outcome variable, abundance of juvenile sea lice.

	Mean value for parameter	90% range for parameter	Parameter levels	Mean abundance of juvenile sea lice for different parameter levels	90% range of juvenile sea lice abundance for different parameter levels
Juvenile sea lice at $t-1$	1.32	0–4.40	0–0.20 0.20–0.70 0.70–1.58 >1.58	0.12 0.66 1.26 3.32	0.00–0.52 0.13–1.62 0.30–2.70 0.53–9.14
Gravid female lice on farm at $t-2$	1.17	0–3.82	0–0.1 0.10–0.73 0.73–1.52 >1.52	0.39 1.10 1.43 2.43	0.00–1.97 0.06–3.48 0.18–3.58 0.23–7.53
Gravid female lice in surrounding 30 km area $\times 10^5$ at $t-2$	0.96	0–2.94	0–0.20 0.20–0.64 0.64–1.34 >1.34	0.45 1.15 1.52 2.23	0.00–2.05 0.00–3.58 0.10–4.43 0.20–7.08
Salinity (ppt)	29	18–33	<20 20–30 >30	0.13 0.87 1.53	0.00–0.81 0.00–3.13 0.10–4.10
Temperature ($^{\circ}\text{C}$)	11.2	8.9–14.5	<10 10–13 >13	1.23 1.46 1.05	0.00–3.63 0.00–4.88 0.00–4.00
Distance (km) to nearest holding facility	98.7	10.1–198.4	<5 5–10 10–20 >20	2.42 2.45 2.24 1.17	0.17–11.70 0.00–6.64 0.05–8.08 0.00–3.87
Distance (km) to nearest shellfish lease	70.0	0.28–238.2	<0.5 0.5–1 1–2 >2	1.94 1.53 1.23 1.23	0.00–7.18 0.00–4.97 0.00–4.85 0.00–3.93
Average weight (kg)	1.91	0.27–4.79	<0.5 0.5–1 >1	0.85 1.02 1.55	0.00–3.05 0.00–3.33 0.00–5.05
Region			South North	1.26 1.43	0.00–4.10 0.00–4.90
Species			Rainbow trout Atlantic salmon	1.29 1.38	0.00–4.43 0.00–4.45
Treatment at $t-2$			No Yes	1.18 2.31	0.00–3.95 0.15–7.58
Number of fish on farm ($\times 10^6$)	0.76	0.19–1.21	<0.60 0.60–0.78 0.78–0.96 >0.96	1.63 1.37 1.35 1.01	0.00–5.55 0.00–4.26 0.00–4.73 0.00–3.45

To assess the distance where the effect of neighboring farms no longer contributed significantly to juvenile sea lice on farms we evaluated the output of lice from the neighbors at varying maximum distances (i.e. kernel ranges) of 5, 10, 20, 30 and 40 km. Missing values for gravid sea lice counts or number of fish on a site were imputed by taking the average of values reported at $t-1$ and $t-3$ whenever these were present. This resulted in a total of 1460 imputed values for $\text{GF}_{j,t-2}$ and $N_{j,t-2}$.

The sea lice exposure from fish holding facilities could not be quantified because these facilities were not included in the monitoring program. Hence, we assessed the exposure from these sites by including the farm's seaway distance to the nearest holding facility. Similarly we

included the seaway distance to the nearest shellfish farm among the predictors of interest.

2.2.2. Environmental factors

Salinity (ppt) and temperature ($^{\circ}\text{C}$) were estimated at the farm level using a 3-week rolling average at times $t-2$, $t-1$, and t . Some farms did not report values for temperature and salinity. To fill in these missing data (77 temperature and 1952 salinity values) we imputed values from farms within a weighted seaway distance of 40 km using a Gaussian kernel function for the same week.

There are two distinct geographic regions in our study area that are separated by a large flow of water (Fig. 1). Farms were categorized into either of these regions based

on their latitude; north ($\leq 43.5^\circ\text{S}$ latitude) or south ($> 43.5^\circ\text{S}$ latitude).

2.2.3. Farm management factors and fish characteristics

Factors pertaining to the management practices on a farm included number of fish on a farm, the mean weight of the fish at week t (a proxy for the age of the fish), the species of fish, sea lice treatment, and the number of juvenile sea lice the week prior ($t - 1$). We included the latter to control for the carry-over effect of lice on a farm from one week to the next as the weekly abundance of juvenile lice modeled was intended to represent “new” infections (Gonzalez and Carvajal, 2003; Bravo, 2010).

The predominant form of sea lice treatment in Chile during our study period was bath treatment using pyrethroids, which could potentially affect juvenile sea lice on fish. Therefore we considered whether there was a sea lice treatment on a farm two weeks prior ($t - 2$) in our study. A two-week lag period for sea lice treatment was used because it can take more than a week to complete a bath treatment on a farm and the effect on juveniles is sometimes delayed a few days. Further, given the life cycle of sea lice, new infections from adults within the farm should not re-occur within two weeks if the treatment was successful.

2.3. Statistical model

We used a multivariable two-part random effects model (Tooze et al., 2002; Liu et al., 2008) to determine factors affecting the mean abundance of juvenile sea lice at farm i for week t ($Y_{i,t}$). This model permitted us to account for a substantial proportion of zeros (i.e., $Y_{i,t} = 0$) in the dataset (12.4%). We modeled the odds of the mean juvenile abundance being greater than zero (part I) using a logistic regression model, and the mean juvenile sea lice abundance (part II) given that counts were greater than zero using a gamma regression model. Both parts of the model included farm random effects to account for repeated measures within the farms. Potential autocorrelation between weekly measures of juvenile sea lice was accounted for by including a time-lagged outcome (Y_{t-1}) among the predictors.

In summary the model equations were

$$\text{logit}(\pi_{i,t}) = X_{i,t}^I \beta^I + u_{1i} \quad \text{and} \quad \ln(\mu_{i,t}) = X_{i,t}^{II} \beta^{II} + u_{2i}$$

where $\pi_{i,t} = P(Y_{i,t} > 0)$, is the probability of a non-zero abundance, and $\mu_{i,t} = E(Y_{i,t} | Y_{i,t} > 0)$ is the mean non-zero abundance, modeled by a gamma distribution with a constant shape parameter. The pairs of random effects for farms were modeled as $[u_1, u_2] \sim N([0, 0], [\sigma_1^2, \rho \sigma_1 \sigma_2, \sigma_2^2])$. The model was implemented in SAS PROC NLMIXED (SAS® 9.1.3 for Windows, SAS Institute Inc., Cary, NC) and estimated by adaptive Gaussian quadrature, as previously described (Tooze et al., 2002; Liu, 2010).

2.3.1. Model building

We explored several models to determine the most appropriate combinations of predictors, as well as forms of the predictors (i.e. binary or continuous on either

original or log scale) to include in our model. Predictors were included in or excluded from the analysis simultaneously in both parts of the model. Two time-lagged sea lice count variables (juvenile sea lice and gravid sea lice) were included as binary predictors in Part I and as log scale continuous predictors in Part II of the model. We added 0.1 to the predictors prior to the log transformation to avoid losing data with the value zero. Salinity was assessed both on original scale and on restricted scales with lower cut-offs at 15, 20 or 25 ppt and an upper cutoff of 30 ppt, based on known biological effects of salinity on sea lice (Bricknell et al., 2006; Bravo et al., 2008). Distance to a shellfish lease was dichotomized at seaway distances of 0.5, 1 and 2 km from the farm to determine the best model fit. Similarly, distance to nearest holding facility was also explored in binary versions at distances of 5, 10, and 20 km.

Model selection used Akaike's information criterion (AIC). Models with a difference in AIC, $\Delta\text{AIC} \leq 4$, from the top ranked model provided effectively equal support (Burnham and Anderson, 2004).

As a diagnostic tool, we compared the observed number of farms with no sea lice (abundance = 0) to the expected number of zero values across the dataset, as predicted by part I of the model. For part II of the model, the Anscombe residuals (McCullagh and Nelder, 1989) were inspected visually by plotting them against all explanatory variables and the fitted values. For farms with continuous observations for more than 15 weeks, the autocorrelation of the Anscombe residuals within each farm was calculated. We also assessed unexplained seasonal variation by fitting a sinusoidal function with a period of 52 weeks in both parts of the model.

The top ranked model was re-fitted after standardizing the quantitative predictors to enable a direct comparison of the magnitudes of effects for these predictors. We also computed from the regression coefficients the odds ratio (Part I) or mean ratio (Part II) to aid in their quantitative interpretation, by exponentiating the unstandardized coefficients. For predictors on the natural log scale, the coefficient was multiplied by $\ln(2) = 0.693$ prior to exponentiation in order to achieve a ratio corresponding to a doubling in the predictor.

3. Results

There were 19,714 farm weekly sea lice reports in our dataset between January 2009 and April 2012 from Atlantic salmon and rainbow trout farms. When the mean abundance of juvenile sea lice on farms was summarized by individual predictor variables, several dose response trends emerged (Table 1). Juvenile sea lice apparently increased with increasing (1) numbers of gravid females on farms two weeks preceding, (2) numbers of gravid females two weeks prior in the surrounding area within 30 km of a farm, (3) salinity, and (4) weight of the fish (Table 1). There was a decreasing trend in the abundance of juvenile sea lice as the distance to a holding facility increased. These patterns were also present in the AIC top ranked multivariable model (Tables 2 and 3).

Table 2

Parameter coefficient estimates, standard errors, *p*-values, and odds/mean ratios for the explanatory variables in the AIC top ranked model for abundance of juvenile sea lice on Atlantic salmon and rainbow trout farms in Chile. Part I of the model indicates the probability of the abundance of juvenile sea lice being greater than zero, and part II models the expected abundance of juvenile sea lice given that a farm has this stage of sea lice. All reference for dichotomous variables was “no” unless otherwise stated.

Variable name	Coefficient estimate	Standard error	<i>p</i> -value	Standardized coefficient estimate	Odds ratio or mean ratio ^a (95% CI)
Part I logit model					
Intercept	−0.069	1.001		0.841	
Juveniles <i>t</i> − 1	2.907	0.111	<0.001	2.907	18.3 (14.72, 22.73)
Gravid females <i>t</i> − 2	1.704	0.115	<0.001	1.704	5.49 (4.38, 6.88)
Log ₁₀ of # of gravid females weighted by distance within 30 km	0.798	0.076	<0.001	0.820	1.74 (1.57, 1.93)
Salinity	0.160	0.020	<0.001	0.546	1.17 (1.13, 1.22)
Log ₁₀ of distance to nearest holding facility	−0.547	0.138	<0.001	−0.454	0.68 (0.57, 0.83)
Log ₁₀ of weight	0.125	0.071	0.079	0.103	1.09 (0.99, 1.20)
Region (North as reference)	−1.088	0.248	<0.001	−1.088	0.34 (0.21, 0.55)
Temperature	−0.104	0.026	<0.001	−0.170	0.90 (0.86, 0.95)
Number of fish on farm	0.382	0.164	0.020	0.127	1.47 (1.06, 2.02)
Treatment <i>t</i> − 2	0.489	0.250	0.050	0.483	1.63 (1.00, 2.66)
Part II continuous model					
Intercept	−0.263	0.178		−0.218	
Log ₁₀ of number of juvenile sea lice <i>t</i> − 1	0.661	0.007	<0.001	0.794	1.58 (1.57, 1.60)
Log ₁₀ of number of gravid females <i>t</i> − 2	0.043	0.006	<0.001	0.053	1.03 (1.02, 1.04)
Log ₁₀ of # of gravid females weighted by distance within 30 km	0.160	0.008	<0.001	0.168	1.12 (1.10, 1.13)
Salinity	0.028	0.004	<0.001	0.092	1.03 (1.02, 1.04)
Log ₁₀ of distance to nearest holding facility	−0.076	0.025	0.002	−0.070	0.95 (0.92, 0.98)
Log ₁₀ of weight	0.094	0.008	<0.001	0.075	1.07 (1.06, 1.08)
Region (north as reference)	−0.203	0.050	<0.001	−0.203	0.82 (0.74, 0.90)
Temperature	−0.007	0.004	0.064	−0.012	0.99 (0.986, 1.00)
Number of fish	−0.110	0.024	<0.001	−0.034	0.90 (0.86, 0.94)
Treatment <i>t</i> − 2	0.189	0.015	<0.001	0.189	1.21 (1.17, 1.24)
Log ₁₀ of shape parameter	0.877	0.010		0.877	
Farm stand deviation (Part I)	0.300	0.016		1.142	
Farm stand deviation (Part II)	1.163	0.120		0.299	
Farm correlation	0.394	0.084		−0.384	

^a Ratios are for a one unit change or for a doubling when the predictor was on a log scale.

3.1. Multivariable model

3.1.1. Sources of sea lice

We found the abundance of juvenile sea lice on farms was sensitive to the spatial scales of the predictor variables representing external infection pressures. Neighboring farms, on average, impacted sea lice abundance up to a distance of 30 km (Table 4). For every doubling in the exposure pressure from neighboring farms within the 30 km of

a farm there was, on average a 1.12 time increase in the mean (non-zero) abundance of sea lice, after controlling for the other predictors in the model (Table 2). Likewise, the logistic regression component of our model suggested that when the exposure on neighboring farms increased, the probability of having lice on a farm also increased (Table 2). The standardized coefficient for the infection pressure of sea lice from within a farm (0.053) was smaller than that of the infection pressure from the surrounding farms (0.168)

Table 3

Difference in the AIC, Δ AIC, between the top ranked model in Table 2 (AIC = 32,355, with a *K* = 26) and a model excluding indicated explanatory variable. The higher the Δ AIC values the higher the AIC for the revised model. *K* is the number of parameters in the nested model (note: we removed variables from both components of the model simultaneously).

Description of the change made to the top ranked model (AIC = 32,355)	Δ AIC	<i>K</i>
Excluded juvenile sea lice one week prior	8101	24
Excluded gravid females	260	24
Excluded sea lice exposure pressure from neighboring farms	474	24
Excluded treatment two weeks prior	155	24
Excluded mean fish weight (kg)	135	24
Excluded salinity	101	24
Excluded number of fish on farm	24	24
Excluded region	24	24
Excluded log ₁₀ of the distance to the nearest holding facility	16	24
Excluded log ₁₀ of the temperature	14	24
Included species	2	28
Included whether there was a shellfish lease within 2 km of the site	1	28

Table 4

Difference in the AIC, Δ AIC, between the top ranked model in Table 2 (AIC = 32,355) and various forms of different variables. The top ranked model had 26 variables. All model changes were undertaken in both parts of the model simultaneously.

Variable	Description of the change made to the variable in the top ranked model (AIC = 32,355) ^a	AIC	Δ AIC
Kernel weighted intensity of gravid female lice at $t - 2$ for different distances from the farm (LGF)	Top ranked model includes log(LGF) at 30 km	32,255	0
	Replacing with log ₁₀ (LGF) at 20 km	32,370	15
	Replacing with log ₁₀ (LGF) at 40 km	32,371	16
	Replacing with log ₁₀ (LGF) at 10 km	32,548	193
	Replacing with log ₁₀ (LGF) at 5 km	32,684	329
	Model excluding all forms of this variable	32,829	474
Salinity	Top ranked model includes Salinity_{20,30} (t)	32,355	0
	Replaced with Salinity _{15,30} (t)	32,368	13
	Replaced with Sal _{25,30} (t)	32,375	20
	Model excluding all forms of this variable	32,456	101
Seaway distance to nearest holding facility	Top model includes log₁₀(distance to holding facility)	32,355	0
	Replaced with binary form (within 20 km)	32,356	1
	Replaced with binary form (within 5 km)	32,364	9
	Replaced with binary form (within 10 km)	32,367	12
	Model excluding all forms of this variable	32,339	16
Seaway distance to nearest shellfish lease	Top rank model excludes this variable	32,355	0
	Included presence of shellfish lease within 0.5 km	32,359	4
	Included presence of shellfish lease within 1 km	32,357	2
	Included presence of shellfish lease within 2 km	32,356	1

^a The form of the variable in the top ranked model is presented in bold for comparison purposes.

in the continuous model (Table 2). The increase in the abundance of juvenile sea lice was by a factor of 1.03 for each doubling in abundance of gravid sea lice at $t - 2$. In comparison, there was a 1.12 time increase in the juvenile sea lice abundance for each doubling in the exposure pressure from neighboring farms (Table 2). This direct comparison of the standardized coefficients for these two predictors could not be done in the logistic regression model because gravid females within the farm was evaluated as a dichotomized variable; however, farms with gravid females two week prior, had on average, approximately 5.5 times the odds of having juvenile sea lice, after controlling for the other predictors in the model (Table 2).

The association between the level of gravid female lice on neighboring farms and a farm's abundance of juvenile sea lice two weeks later was a function of the neighborhood size (Table 4). The model's AIC gradually improved as the neighborhood size was increased to 30 km (Table 4). Including neighbors within 40 km of farms did not improve the model (Table 4).

The other variable that represented external sea lice infection pressure on farms, proximity to holding facilities, was also maintained in our final model. The farther the distance to the nearest holding facility, the less likely a farm was to have juvenile sea lice (OR = 0.68) (Table 2). When the distance to the nearest holding facility was halved, the odds of having any juvenile sea lice was 1.5 (1/0.68) times higher and, on average, there was a 1.05 (i.e. 1/0.95) increase in the abundance of juvenile sea lice (Table 2). The log scale of this parameter, compared to other forms evaluated, produced the model with the lowest AIC (Table 4).

Juvenile sea lice appeared to decrease as the distance to the nearest shellfish lease increased when no other variables were considered (Table 1). However, after accounting for other predictors the distance to the nearest shellfish

lease did not contribute to the abundance in juvenile sea lice (Δ AIC ≤ 4 ; Tables 3 and 4).

3.1.2. Environmental factors

All three environmental parameters tested in our analysis were retained in our final model based on our AIC criterion (Table 3). However, the standardized coefficient estimate for temperature was relatively small, particularly in Part II of our model (Table 2). The effect of salinity, among our different threshold models, was best described using a lower threshold effect of 20 ppt and an upper threshold of 30 ppt (Table 4). Region, which accounted for broad-scale area effects, was still significantly associated with the abundance of juvenile sea lice on farms (Table 2). The farms in the Southern region had, on average, lower levels of lice even after accounting for other predictors in the model (Table 2).

3.1.3. Farm management factors and fish characteristics

Our proxy for the duration of time at sea for fish, average fish weight, had a positive association with our outcome, abundance of juvenile sea lice (Table 2). Farms that treated for sea lice two weeks prior had, on average, a higher odds of having juvenile sea lice after controlling for the other predictors in both components of our statistical model (Table 2). Including this variable in the model had no effect on the other parameter estimates (changes were only observed at the second decimal place on the standardized parameter estimates), suggesting this variable was not a confounder.

The predictor in our final model that had the strongest impact on the abundance of juvenile sea lice on a farm was the abundance of juvenile sea lice on the farm the week before (Table 2). When the species of fish (Atlantic salmon

vs rainbow trout) was included in the model it did not decrease the AIC so it was dropped from our final model.

3.1.4. Model fit

The observed number of weekly reports with no sea lice was similar to the sum of the predicted number of zero values (2444 observed vs 2427 predicted). This suggested the logit component of our model (part I) fit the data well. No patterns were observed from plotting the fitted residuals from part II of the model against all explanatory variables and the fitted values, suggesting that the gamma regression model also fit the data well. Further, evaluation of the residuals from the subset of data containing farms that had 15 weeks of consecutive sea lice reports (75.8% of all the data) had less than 2.9% autocorrelation, suggesting no temporal pattern in the residuals. When seasonal effect was modeled using a sinusoidal function in both parts of our model, the AIC value only decreased by three and the parameter estimates for the other predictors did not change. We therefore concluded that any seasonal effect was sufficiently controlled for by including the salinity, temperature, and region parameters without the need to explicitly model season.

4. Discussion

We used a two-part random effects model to assess potential sources of juvenile *C. rogercresseyi* infection on Atlantic salmon and rainbow trout farms in Chile. Both variables in our model representing external sources of sea lice were positively associated with juvenile lice on farms, and the associations were dose dependent. The greater the exposure potential from neighboring farms within 30 km, the higher the abundance of juvenile sea lice. Similarly farms closer to processing plants with fish holding facilities, had a higher abundance of juvenile sea lice than farms farther away from these plants, and the closer the farms were to these facilities the greater the effect, even after controlling for potential confounders such as other sources of lice and environmental factors.

The average effect of holding facilities on juvenile sea lice abundance appeared to be lower than the average effect of the infection pressure from neighboring farms. We may have underestimated the effect of holding facilities in our study because we did not have data available to differentiate between weeks when there were fish with sea lice and when there were no fish or fish with no lice present at these facilities; however, it is likely that the infection pressure from neighboring farms is a stronger driver of juvenile sea lice than proximity to holding facilities because of the 10-fold higher number of fish at grow-out facilities in comparison with temporary holding facilities (data from SalmonChile). Further, fish at holding facilities are usually harvested within one week. *Caligus rogercresseyi* can produce egg strings every 4–6 days, depending on water temperature (Bravo, 2010). Therefore, if fish are infected with mature lice when they arrive at the holding facility there is only sufficient time to release one set of egg strings before they are harvested.

Surprisingly, in our final model the standardized coefficient estimate for the infection pressure from neighboring

farms was also larger than the coefficient for the abundance of gravid females within the farm, which suggests that the role of this external source of lice was greater than the sources within the farm (Part II continuous model, Table 2). There are at least two biologically plausible explanations for this finding. First it is possible, given that it takes between 1 and 2 weeks for eggs to reach the infective stage, that by the time copepodids originating within a farm have developed, they have been flushed away from the original site via water currents. It is also possible that producers control the level of re-infection on their farms relatively well by treating adult sea lice, but they do not control treatments on their neighbors farms.

The salmon industry reported relatively good efficacy of sea lice treatments against adult lice in 2011 (i.e. 70% reduction in adult sea lice one week post-bath treatments; SalmonChile), but similar to the findings of other researchers (Revie et al., 2003; Lees et al., 2008a), we found a positive association between treatment and juvenile sea lice abundance. This suggests that the treatments, on average, are not effectively controlling juvenile sea lice on farms. This finding could be due to a number of reasons such as treatment failure due to resistance or poor product application, or use of a product that has no effect on the juvenile stages. It could also reflect the fact that farms that treat for sea lice are generally in areas that have high levels of lice, and the use of bath treatments, which were the most common types of sea lice treatments in our study, do not prevent new infections from external sources. A better understanding of the effect of farm and neighborhood level treatments on juvenile stages of sea lice is essential in order to control infections with this parasite.

Also consistent with external sources of sea lice having a stronger association with juvenile sea lice abundance on a farm than internal sources was the fact that the number of fish on the farm was negatively associated with the abundance of juvenile lice on that farm (Table 2). Given the host density-dependent nature of sea lice infections and the fact that we used the abundance of gravid females on a farm (not the absolute number of gravid females) as the predictor variable, if the source of juvenile lice was predominantly the fish within the farm we would have expected the opposite relationship. However, if the source of sea lice was predominantly external to the farm, and therefore independent of the number of fish on the farm, then the greater the number of fish on a farm exposed to a finite number of copepodids from external sources, the lower the infection pressure on *individual* fish (i.e. abundance). This dilution effect has been described in other host–pathogen systems (Randolph and Dobson, 2012) including *C. elongatus* infections on Scottish fish farms where that species, in contrast to *L. salmonis*, is seen to be primarily driven by external infection pressure (Revie et al., 2002b).

Based on this study, the average distance of the “neighbor effect” in Chile is greater than currently estimated by some of the neighborhood designations (Fig. 1). Given our model, which controlled for environmental factors affecting sea lice, as well as farm and fish characteristics, it appears that, on average, farms within 30 km of each other should be managed for sea lice concurrently to reduce

infection levels. Logistically, it is unlikely that it will be feasible to synchronize sea lice treatments over such a large area given the spatial distribution of farms; therefore, a sea lice control strategy that could be investigated in the future is the use of buffer zones to break up larger management areas. One approach to creating these buffer zones without losing production may be to use coho salmon, a farmed species that is less susceptible to sea lice infections (Fast et al., 2002; Bravo, 2003). It should be noted that this strategy may not work for controlling other density-dependent pathogens if they do not share the same susceptibility patterns.

It is important to remember that the critical average threshold distance to manage farms, determined in this study, was based on sea lice data collected between 2009 and 2012 and did not include hydrological data. Therefore if the number of fish on farms and their proximity to one another changes, the infective zone around farms will also change.

Another management strategy that has been proposed for controlling sea lice is the use of multi-trophic aquaculture using bi-valves. We evaluated whether fish on farms close to a shellfish lease were less likely to be infected with juvenile sea lice. The basic trend between distance to a shellfish lease and juvenile sea lice on a farm was the opposite of what was expected, given laboratory and field observations in Canada with *Lepeophtheirus salmonis* (Davenport et al., 2000; Lehane and Davenport, 2006; Molloy et al., 2011) (Table 1). When we accounted for confounding factors in the multivariable analysis we found this variable did not improve the model, based on AIC model selection techniques. This result may be because the species of sea lice in Chile is of a different size than reported in the laboratory studies, and thus may not be filtered in a similar manner as the species found in Canada. It is also possible that areas where farms co-exist with shellfish leases are located in sheltered areas and therefore have reduced water flows. We did not have information on the water current near farms to evaluate the possible confounding effect between water flow (i.e. flushing potential) and shellfish leases, but this should be further investigated.

Of arguable importance, the assessment of proximity to shellfish leases on juvenile sea lice evaluated in this study was limited by lack of data on whether or not shellfish leases were active over the three years of data collection. Therefore, we likely underestimated the impact of shellfish leases, if there truly was an effect. It should also be noted that approximately 97% of the shellfish leases were in the Northern region, so a proportionally larger number of the control group (farms farther than the specified distance to a shellfish lease) came from the Southern region. However, we controlled for region within the model. In conclusion, our data analysis does not support the hypothesis that shellfish leases significantly reduce the level of sea lice on Chilean salmon farms; however, it should be reiterated that the data where such that it cannot be concluded with certainty that this association does not exist without further evaluation.

All other variables included in our model had previously been demonstrated to be associated with sea lice by other researchers (Revie et al., 2003; Yatabe et al., 2011;

Jansen et al., 2012). We refined some of the predictors in the previously published sea lice models; for example, we included salinity, but instead of using a continuous variable to describe this parameter we used a continuous variable with upper and lower threshold effects that described the biological effect on sea lice (Bravo et al., 2008). We evaluated three lower cut-offs and found that a lower limit of 20 ppt and upper limit of 30 ppt best explained our data.

The effect of temperature in our model was relatively modest, especially in the case of part II, which modeled expected abundance of juvenile lice. The sea water temperatures in Chile where the study sites were located were well within the optimal growth range for sea lice (90% temperature range 8.9–14.5 °C) (Table 1). The lack of a strong temperature impact on population level sea lice abundance mirrors results from Scotland where similar sea water temperature profiles were experienced (Revie et al., 2003; Robbins et al., 2010). This is in contrast to finding from Norway (Jansen et al., 2012) where a broader range of water temperatures was likely the reason that this parameter was found to be important (Heuch et al., 2003).

Farms in the Southern region of our study were less likely to have sea lice and, on average, a lower abundance of juvenile sea lice than farms in the north, after controlling for the other predictors in the model (Table 2). The regional difference could have been due to risk factors that were different between the two regions, and for which we did not have data available to assess (e.g. the sea lice contributions of wild fish populations and the role of ocean hydrology on sea lice dispersion).

Our analysis was consistent with other studies with regards to the positive relationship between the weight of fish and sea lice abundance (Jansen et al., 2012). It has been proposed by other researchers that the older the fish, the more time it has had to accumulate lice and the larger the surface area for attachment, thus the heavier the infection loads (Revie et al., 2002a; Lees et al., 2008b; Baillie et al., 2009; Heuch et al., 2009; Yatabe et al., 2011; Jansen et al., 2012). It is also possible that as the weight of the fish in pens increases so does the stocking density and this results in less water movement through the system.

Although our two-part model addressed the issue of modeling a dataset with numerous zero counts and appears to fit the juvenile sea lice data in Chile, the parameter estimates are still averages for the industry, which may limit its application to individual farms. The model is also only valid for the range of data from which it was developed. The estimated effects of the explanatory variables mostly behaved as predicted and were consistent with previous models of adult sea lice in Chile and in other parts of the world. It is possible that our model could have been improved by further refining our explanatory variables, for example, by including information on the role of wild fish or ocean hydrology. Some of the unexplained variation in our model may also have been due to the variation in the estimates of the juvenile sea lice counts on farms. The early attached stages of sea lice are difficult to identify. However, even with this potential source of error our model fit the data relatively well, suggesting we included the most important predictors of juvenile sea lice.

5. Conclusion

To enable the sustainable growth of salmon salt water net-pen aquaculture it will be necessary to manage sources of density-dependent pathogens such as sea lice. Although the spread of sea lice from neighboring farms and holding facilities has been suspected (Stucchi et al., 2011; Jansen et al., 2012), this is the first analysis in Chile that has tested this hypothesis. We provide strong evidence for a temporal and dose–response relationship between counts of juvenile sea lice on farms and gravid female lice at neighboring sites two weeks prior. Farms closer to either holding facilities or to other farms with sea lice had, on average, a higher abundance of juvenile sea lice than those more distant from them, suggesting sea lice sources are not confined to the farm-level. In this study a “neighbor effect” was detected, on average up to 30 km away; however, the scale over which external sources of sea lice play a role in infections depends, in part, on the intensity of farming at the regional level (e.g. the distance between farms and the number of infected fish on farms), which may change over time.

Conflict of interest

The authors declare that they have no competing interests.

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