Risk Factor Analysis for Sea Lice (Caligus rogercresseyi) Levels in Farmed Salmonids in Southern Chile

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ABSTRACT

Sea lice (*Caligus rogercresseyi*) are ectoparasitic copepods which affect salmon farming industry in southern Chile. Local farmers have reported increasing levels of infestation since 2004, reaching the highest levels in 2007. In response to this situation the National Fisheries Service, SERNAPESCA, developed a surveillance program, which first step consisted in a general survey across the salmon farms. This included counts of parasite loadings on fish, and measurement of several husbandry and environmental factors. The aim of this study is to identify risk factors for the observed levels of infestation, emerging from the factors registered at this stage. The information was analyzed using linear mixed models technique. The variables geographic zone, fish species, treatment one month before sampling, stocking density, fish weight and water salinity were significantly associated with sea lice burdens. Treatment two and three months before sampling, use of photoperiod in sea cages, and water temperature were not significant.

There was significant unexplained variation at all levels of aggregation, namely sub-zone, farm and cage level, being the farm level variation the greatest. Among the causes of such variation there could be several factors, including local water currents, differences in native hosts' distribution, different health status among centers and differences in potentially stressful practices or events. Future areas of research are proposed in order to improve the understanding and control of this disease.

KEYWORDS

Sea lice, Salmon Farming, Chile, Risk Factors, Lineal Mixed Effect Models, DGC test

INTRODUCTION

Sea lice are ectoparasitic copepods belonging to the genera *Lepeophtheirus* and *Caligus*. In Chile the predominant parasite specie is *Caligus rogercresseyi* which, according to the salmon farmer's monitoring, has shown an increase in the number of positive cages and in the intensity of infestation in the past few years. In response to this sanitary situation, the National Fisheries Service (SERNAPESCA) implemented an official surveillance program to asses the real situation of sea lice in the salmon farms in southern Chile, and to gather relevant information about environmental and husbandry conditions to allow the development of control strategies against this parasite (Hamilton-West et al., 2008). The objective of the present study is to analyze the data collected by this surveillance program, to evaluate the effect of the risk factors derived from husbandry and environmental conditions over the sea lice burdens in farmed salmonids in southern Chile, using the information gathered by SERNAPESCA in august, 2007.

Material and methods
The study was developed using the information from the Chilean Official Surveillance Program, in its stage of General Sea Lice Diagnostic (SLGD) carried out in august 2007 in the X and XI administrative regions (A.R.), which for purposes of this program were classified into 10 productive-environmental zones, and 36 sub zones. SLGD consisted in the random sampling of 10 fishes from each cage of every fish farm in activity and the counting of sea lice's life stages, which must be repeated every year. Nevertheless, as this was the first time such procedure was done, it was voluntary, obtaining data from 323 fish farms, including 5681 fish cages. From the data collected by the sampling, the mean parasite count per fish per cage was obtained. Also, data was collected about environmental and husbandry variables at the cage and farm level.

The parasite mean counts were transformed using a Logarithmic transformation (ln), in order to obtain a better approximation to normality of the residuals. Data was analyzed using lineal mixed effect models (LME), which take into account the nested nature of the data. The statistical software Infostat was used in all the statistical analysis. Fixed effects considered for the present study were water temperature and salinity, photoperiod in sea cages, average fish weight, stocking density, fish species, applied treatments up to three months before sampling, and the productive-environmental zone. To estimate possible differences between the levels of a categorical variable that were not directly compared with each other, an adjusted mean comparison test based on cluster analysis was done, using Di Rienzo, Guzmán y Casanoves test (DGC test) (Di Rienzo et al., 2002), with a significance level $p < 0.05$. Random effects considered in this study were the

productive-environmental sub-zones, fish farms nested inside the sub-zone, and the cage effect or error. Fixed effects were selected from the initial model using partial F-tests, removing those whose regression coefficients were not significant (p-value > 0.05) with a backward elimination procedure. Non significant variables that produced a change in the regression coefficients of the significant ones of 20% or more when removed, were retained in the model to adjust for confounding. Random effects were retained based on their confidence intervals.

Results

Data were collected from 270 fish farms and 4282 fish cages, which represents around 50% of the active fish farms in the country at the time. Missing data in any variable of interest was the main cause of data elimination (1281 fish cages), also data was eliminated due to data entry errors (118 fish cages). From the 36 sub-zones there is one, Colaco sub zone (Chiloé north zone), that was leaved out of the study because it was in fallowing during the sampling period. Table N° 1 shows the fixed effects retained in the final model.

Table N° 1. Fixed effects retained in the final model and associated statistics. Treatment_1: Treatment one month before sampling date; St_Density: stocking density.

	numDF	denDF	F-value	p-value
(Intercept)	1	3935	2.84	0.0922
Zone	9	26	4.06	0.0024
Species	2	3935	362.20	<0.0001
Treatment_1	2	3935	20.69	<0.0001
Salinity	1	3935	63.85	<0.0001
Weight	1	3935	40.75	<0.0001
St_Density	1	3935	6.06	0.0138
Temperature	1	3935	2.76	0.0967

Water temperature was retained in the final model despite not showing a significant association with mean parasite count, because it appeared to be a confounder on the productive-environmental zones. From the zones included in the model, those that presented a significant association with the transformed mean parasite count were Melinka (p = 0.03), Puerto Cisnes (p = 0.04), and Puerto Aysén (p = 0.03) zones; presenting transformed mean parasite counts of 1.21, 1.38, and 1.41 units less than the reference zone of Chiloé sur, respectively. These three zones did not show significant differences between them.

Rainbow trout and Atlantic salmon were associated to the highest mean parasite counts (p-value < 0.0001 for both), showing a transformed mean parasite count of 2.53 and 2.34 units higher than Pacific salmon (reference level), respectively. There were no significant differences between the two former.

Only the treatment applied one month before sampling exhibited a significant association with the transformed mean parasite count, showing negative regression coefficients for both the hydrogen peroxide baths and the in feed emamectin benzoate (p-value < 0.0001 for both), with values of 0.52 and 0.24 units less than untreated fish, respectively. When comparing the treatments' means, a paradoxical result is observed, given that the adjusted means of the treated groups are significantly higher than that of the untreated group. The cause of these results is probably due to the fact that in general treatment is applied in those cages where there are high parasite burdens. In the case of cages treated with hydrogen peroxide, the observation that this group mean is the highest should be associated with the fact that the use of this product is authorized in cases of high parasite infestation (Hamilton-West et al., 2008). Then it can be inferred that treatments applied one month before sampling date diminish the parasite burdens, but this reduction is not enough to revert the initial situation of high parasite loads. When comparing the regression coefficients of the hydrogen peroxide and the emamectin benzoate with each other by a contrast test based on the F distribution, the difference between them is significant. Therefore the hydrogen peroxide treatment appears to be more effective in the reduction of the mean parasite count than emamectin benzoate.

The stocking density, is significantly associated (p = 0.01) with higher mean parasite counts, with a regression coefficient of 0.01. Average fish weight and water salinity, also exhibited significant associations (p-values < 0.0001 for both) with higher mean parasite counts, showing regression coefficients of 0.07 and 0.11, respectively.

Random effect estimators and their 95% confidence intervals (95% CI) were all significant (CI did not include zero), meaning that in all hierarchy levels there is significant variability not explained by the fixed effects included in the final model. The sub-zone effect was the smallest, with a standard deviation (SD) of 0.55 units of transformed mean parasite count. The highest variability was at the fish farm level, with a SD of 1.26 units. The cage effect or error had a SD of 0.59 units.

Discussion

The results from the model permit to distinguish two major groups of zones: the first correspond to the zones located in the X A.R., that are associated with higher infestation intensities; the second correspond to the

zones located to the south, in the XI A.R. and are associated with lower infestation levels. From the fish farms that informed in the 2007 SLGD and whose information was incorporated in the present study, around a 69% (186 fish farms) were located in the X A.R, while the remaining 31% (84 fish farms) was located in the XI A.R. This way, the observed differences between the X and XI A.R. could be due to differences in the aggregation of the fish farms and in their geographic location, the latter associated to oceanographic factors like water depth, tidal range, water circulation patterns and flow rate, among others.

The observed resistance of Pacific salmon is probably related to nonspecific mechanisms of tissue response, similar to those observed in the response to *L. salmonis*. Another factor that could be involved is the sea lice preference for different host species (Pino-Marambio et al., 2007).

The results of the present study suggest that treatment effect, whether with hydrogen peroxide or emamectin benzoate, does not exceed one month. Bravo et al., (2008) in an in vitro sensibility assay, suggests a reduction in C. rogercresseyi sensibility to emamectin benzoate. This sensitivity lost could be related with the lower performance of this product when compared to hydrogen peroxide baths.

It can be suggested that fish exposed to higher densities present an elevated stress level, which stimulates cortisol release, increasing fish susceptibility to sea lice.

The effect of fish weight in the present study is probably related with the time of exposure (age of the fishes) and with the larger body surface that is exposed to the parasite in heavier fish.

The association between water salinity and mean parasite count would be explained by the negative effect of low salinity on larval stages survival (Gonzales & Carvajal, 2003).

The lack of significance of water temperature is similar to the results of other authors (Saksida et al., 2007; Revie et al., 2003), although this might be an effect of the nature of the present study, where data was collected at a specific time of the year (mid winter), making the temperature range to be reduced.

There are one or more factors not considered in the present study that would explain the observed variability in the mean parasite count at the sub-zone, fish farm and cage level. The greatest variability between fish farms could be due to differences in husbandry practices, local oceanographic conditions, native hosts' distribution, sanitary status of the fish farms, and genetic differences between the populations of different fish farms, among others.

CONCLUSSION

Sea lice epidemiology appears as a complex issue, and more research is needed in diverse areas, like in the response of the different hosts to infection, with the object of determine more precisely the differences in susceptibility between species and the responsible mechanisms; the stocking density, in order to establish an optimum range for this variable that minimizes the stress response; the development and incorporation of new treatments against this parasite that would permit the rotation of drugs, and the application of an integrated pest management program, to reduce the risk of resistance development. From the analysis of the random effects it is recognized the need of more knowledge about the environmental characteristics of the salmon farming area, like the water circulation patterns or the distribution of native hosts, among others, in order to understand the causes of the observed variability. Future research should also focus on the identification of spatial, temporal and spatial-temporal clusters, with the object of identifying in which areas and times there is a higher risk for this disease. For this it is necessary to incorporate to the existing surveillance system the use of geographical information systems (GIS) and spatial epidemiology tools.

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