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A retrospective assessment of the effect of fallowing on piscirickettsiosis in Chile

Derek Price [a,*], Rolando Ibarra [b], Javier Sánchez [a], Sophie St-Hilaire [a]

[a] Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE C1A 4P3, Canada
[b] Instituto Tecnológico del Salmón, INTESAL-SalmonChile, Av Juan Soler Manfredini 41, OF 1802, Puerto Montt, Chile

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ABSTRACT

Piscirickettsiosis is an endemic disease of farmed salmonids in Chile, and is the main cause of infectious disease-related losses in the Chilean aquaculture industry. Inconsistent results with the use of vaccines and antimicrobials have led producers and government to search for alternative control measures. Fallowing sites between fish production cycles has been mandated by the government authority, but the effectiveness of this practice for preventing this disease has not been assessed under field conditions. We used a discrete-time survival analysis model to evaluate the effect of the duration of the fallow period on the hazard of piscirickettsiosis during the first 24 weeks of the production cycle on farms between September 2009 and August 2015. We compared the hazard of piscirickettsia for production cycles on farms that did and did not report the disease immediately before fallowing in the preceding cycle. We controlled for potential confounders, including external infectious pressure from neighboring farms. Our results showed that for both Atlantic salmon and rainbow trout there was no significant difference between the hazard of SRS for farms that reported the disease in the previous cycle and the comparison group, when these farms fallowed for more than three months. Shorter fallow periods were only assessed in rainbow trout, and findings indicate that the hazard of piscirickettsiosis is higher for farms with a recent history of the disease than for farms without a recent history of the disease prior to fallowing. These results suggest that fallowing for three months may be adequate to lower the exposure of *P. salmonis* from one production cycle to the next; however, our study also suggests there is a high hazard of observing SRS on farms, regardless of fallowing.

1. Introduction

Piscirickettsia salmonis is the causative agent of piscirickettsiosis (Fryer et al., 1990), also known as Salmonid Rickettsial Septicaemia (SRS). *P. salmonis* and other rickettsia-like organisms have been described in several different species of fish worldwide (Rozas and Enríquez, 2014). Since its first description in Chile in 1989 (Bravo and Campos, 1989), SRS has been a difficult disease to manage due to inconsistent results with control strategies such as antimicrobial treatments and vaccination (Bravo and Midtlyng, 2007; Rozas and Enríquez, 2014). In recent years, SRS has been the most prevalent infectious disease in Chilean salmonids in marine farms (Sernapesca, 2016a,c, 2015) and the main contributor to antimicrobial use in the Chilean aquaculture industry (Sernapesca, 2016b).

Establishing a fallow period between production cycles, where all animals are removed from the farm and all equipment is cleaned and disinfected, is a common strategy to reduce or eliminate pathogenic organisms to control disease transmission between production cycles in intensive animal farming. In salmon farming, the use of farm and area-level fallowing has been proposed as a strategy to reduce transmission of infectious pathogens between production cycles (Bron et al., 1993; Kilburn et al., 2012; McVicar, 1987; Murray, 2006; Rae, 2002; Werkman et al., 2011; Wheatley et al., 1995).

In Chile, Olivares and Marshall (2010) found that after a period of 50 days, once all fish were removed from a farm experiencing SRS, *P. salmonis* was no longer detectable in the seawater column around the farm; however, the effectiveness of fallowing to reduce the risk of SRS or delay the onset of the disease in marine farms has not been assessed.

Farm-level fallowing was made mandatory in Chile in response to the series of infectious salmon anemia outbreaks that started in July 2007 (Sernapesca, 2007). This regulation has evolved and, currently, farm-level as well as area-level fallowing strategies are in place (Sernapesca, 2009). Under this regulation, management areas, also known as neighborhoods, were defined. All companies with farms in a neighborhood must establish a common production period, usually of 24 months in duration. Farms within management areas can stock their fish at any time, but at the end of the production period, all farms must fallow for at least three months. Farms are allowed to introduce more than one crop of fish within a production period, but they have to fallow for a minimum of one month between crops. This is

* Corresponding author at: Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3, Canada.
E-mail address: dprice@upei.ca (D. Price).

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frequently done on farms with rainbow trout (*Oncorhynchus mykiss*) or coho salmon (*O. kisutch*) because their grow-out periods are short, but it is rarely achievable with the longer periods needed to grow Atlantic salmon (*Salmo salar*). Our objective was to determine if the duration of the fallowing period used by farms was associated with the probability of reporting SRS in the subsequent production cycle.

2. Materials and methods

We estimated the hazard of SRS from the 5th to the 24th week of production on seawater farms that fallowed for different lengths of time. We compared the hazard of the disease on farms that reported SRS mortality during the last four weeks of the previous production cycle, over time. We also compared this hazard to the hazard of disease over time on farms that did not report SRS in the last four weeks of their previous production cycle.

2.1. Data source

Companies report pen-level data to Intesal-SalmonChile on a weekly basis. These reports include, for example, species, the total number of fish at the start of the week, average weight, average water temperature, and SRS-specific mortality. For our analysis, Intesal-SalmonChile provided data aggregated at the farm level for the period starting in September 2009 and ending in August 2015.

We identified the start and end of fish production cycles on farms using the periodicity between weekly reports, the number of fish, and the weight of the fish on a farm. We calculated the fallow period between production cycles for each farm in our dataset. We only included sites with production cycles that had fallow periods of <48 weeks. Farms that fallow for exceptionally long periods were excluded from our analysis because this practice is not normal in the Chilean industry and often farms with long fallow periods are not in operation for various reasons, including general poor performance.

In Chile, all fish mortality on farms must be classified by trained personnel according to the criteria established by Sernapesca (2012). We identified our outcome of interest as the first week in each production cycle where the farm reported mortality attributed to SRS in the weekly mortality database. We assumed a minimum 4 week period was required from time of *P. salmonis* exposure to the clinical manifestation SRS based on the incubation period derived from laboratory studies (Strand and Midtlyng, 2007). We assumed if SRS was reported prior to this period, exposure to the bacteria occurred prior to transfer to the farm in question; therefore, these production cycles were excluded from the analysis. We also could not determine the time lagged predictors for these production cycles to include them in our analysis.

We only observed production cycles for up to 24 weeks post seawater transfer because we felt that if the fish had not developed SRS during this period, from exposure to *P. salmonis* originating from the previous production cycle, they were not likely to do so. Also, once a farm developed SRS only the first report of SRS contributed to the analysis.

The occurrence of SRS in the last four weeks of the previous cycle was determined for all production cycles analyzed in this study. Because we had production cycles that did not have SRS-related mortality during the last 4 weeks of the previous production cycle on the same farm, we were able to compare the effect of the duration of the fallow period between production cycles on farms with a history of SRS (i.e. declared SRS at the end of the previous production cycle) and farms without a history of this disease.

2.2. Statistical analysis

We choose to model the hazard of reporting SRS for the first time in a production cycle using a discrete-time survival analysis based on a mixed logistic regression model, with random effects for farm and neighborhood. Mathematically, our model was represented as:

$$logit(h_{ij}) = \beta_0 + \alpha_j + \beta X_{ij} + u_{neighborhood(i)} + v_{farm(i)}$$

where $logit(h_{ij})$ is the hazard of reporting SRS in the ith production cycle during the jth time interval (4 week periods), conditional on being present at the start of said interval. β_0 is the baseline hazard, α_j is the effect of the time interval j, and β represents the effect of all fixed predictors X in our model, including those we interacted with time. Our random effects for neighborhood and farms within a neighborhood are represented by u and v, respectively.

To assess time varying effects, such as what we would expect from different lengths of fallow periods, on farms with a recent history of SRS prior to fallowing and farms that did not have SRS prior to fallowing (comparison group), we forced a three-way interaction between the SRS status in the last four weeks of the previous cycle, the length of fallow in months, and our time variable. The overall significance of higher order terms in the models were assessed using Wald tests.

To reduce the number of categories for our time variable, we aggregated weekly reports into four-week intervals. For each time interval, we averaged water temperature and recorded whether SRS was reported during this period. Also, because *P. salmonis* has been shown to transfer between farms within a 10 km seaway distance (Rees et al., 2014), we controlled for external sources of infection by including the maximum number of farms that reported SRS during the four-week period that ended four weeks prior to the second week of the current interval, within a 10 km seaway distance of the farm of interest.

Our initial model included the following predictors: the time interval when the outcome was measured, fallow duration in months, the history of SRS in the previous production cycle, the three-way interaction of the above terms, the maximum number of neighbors within 10 km in the previous four-week interval, and average water temperature during the current four-week interval. We dropped predictors that were not significant ($P < 0.1$) in our analysis, with the exception of our variable of interest (fallow time) and its interaction.

We fitted separate models for each species because Atlantic salmon and rainbow trout farms had different minimum fallow lengths, were managed differently, and may have had different susceptibility to *P. salmonis*; however, we did not take into consideration the species of the neighbors in our count of infected farms for our measure of external infection pressure in our analysis.

For Atlantic salmon, we explored the role of temperature by creating spline terms with a single knot at 10 °C, because the relationship between temperature and SRS appeared to be different above and below this temperature point. In our trout model, temperature was treated as a single continuous variable.

In the case of rainbow trout, which had a significant number of farms with fallow periods <3 months, we conducted an additional analysis to compare the probability of reporting SRS prior to 12 weeks post seawater entry on sites that fallowed for <3 months to those that fallowed longer than 3 months. This analysis was performed using a mixed-effects logistic model controlling for potential confounders. All statistical analyses were done using Stata® 13.1.

3. Results

3.1. Descriptive statistics

In total, 1176 production cycles were identified, but 504 of them were not analyzed because they were the first production cycle in our dataset for the farm in question and we had no information on their previous production cycle with which to determine the fallow period. These cycles were only used to determine SRS occurrence in the last 4 weeks prior to the fallow period and the duration of the fallow before the subsequent group of fish were transferred to the farm. In addition, 214 cycles were discarded because they fallowed the site for longer

Table 1
Number of production cycles by Species, SRS mortality occurrence within study period, and previous cycle SRS history.

Species	SRS in previous cycle	Censored (no SRS)	Failed (SRS)	Total
Atlantic Salmon	No	85 (40.9%)	123 (59.1%)	208 (66.9%)
	Yes	35 (34.0%)	68 (66.0%)	103 (33.1%)
Rainbow trout	No	37 (55.2%)	30 (44.8%)	67 (59.3%)
	Yes	12 (26.1%)	34 (73.9%)	46 (40.7%)
Total		169 (39.9%)	255 (60.1%)	424 (100%)

than 48 weeks. Twenty-one cycles were excluded because they reported SRS prior to our observation period and, therefore, did not meet our minimum 4-week incubation period post seawater transfer. Another 13 production cycles did not have mortality information or information on the status of SRS on the farm; therefore, they could not be included in our analysis. The remaining 424 production cycles in the final dataset consisted of 311 Atlantic salmon production cycles from 223 sites, and 113 rainbow trout production cycles from 88 sites, located in Los Lagos and Aysén regions.

Of the 311 Atlantic salmon production cycles analyzed, 103 (33.12%) were from farms that reported SRS at the end of the previous cycle, while 208 (66.88%) occurred in farms that did not. During the study period, SRS was reported within the first 24 weeks of seawater entry in 68 of 103 (66.02%) cycles with a history of SRS, compared to 123 of 208 (59.13%) in the comparison group (Table 1). Of the 113 rainbow trout cycles in our study, 46 (40.71%) production cycles occurred on farms with a recent history of SRS, while 67 (59.29%) cycles were on farms did not report SRS in the last four weeks of the previous cycle. SRS mortality was reported in 34 of 46 (73.91%) cycles that had a recent history of SRS prior to fallowing. Among the comparison group, SRS mortality was reported within our observation period in 30 of 67 (44.78%) cycles (Table 1).

Overall, the probability of SRS on Atlantic salmon farms increased over time, regardless of the length of the fallow period or the history of SRS on a farm (Fig. 1). In rainbow trout, the pattern was similar, with the exception that at week 16 there seemed to be a decrease in the hazard of the disease; however, this drop was observed in farms with and without a history of SRS and did not persist after this time point (Fig. 1). We found that the three-way interaction between fallow duration, history of SRS in the previous cycle, and month of production was not significant for either species ($P = 0.21$ in Atlantic salmon, and $P = 0.82$ in rainbow trout) (Table 2 and Figs. 1 and 2).

In Atlantic salmon, where we could only assess fallow periods of 3 months or longer, the probability of SRS over the 24 weeks evaluated was not inversely associated with the time of fallow (Fig. 2). In fact, in some cases, the hazard of SRS was higher when sites were fallowed longer (Fig. 2); however, this relationship was not statistically significant and was stronger for farms that did not have a recent history of SRS in their previous cycle.

In trout, although not statistically significant, there was a positive trend observed during the initial period of observation (<12 weeks into the production cycle), where shorter fallow periods tended to have a higher hazard of SRS; this relationship appeared only on sites with a history of SRS in the preceding cycle (dashed line in Fig. 2). Using a mixed-effect logistic model, where observations from the first 5 to 12 weeks post seawater entry were dichotomized and fallow length was split into fewer than 3 months and greater than or equal to 3 months, a significant interaction between farms with a history of SRS and short fallow periods was detected ($P < 0.05$). That is, farms that fallowed for fewer than 3 months and had a history of SRS in the previous production cycle had a higher probability of reporting SRS within this period after stocking (Table 3).

For Atlantic salmon farms, we found that the hazard of SRS was significantly associated with the number of infected neighbors within 10 km in the previous month ($P = 0.04$); more specifically, having 3 or more infected neighbors within 10 km one month prior increased

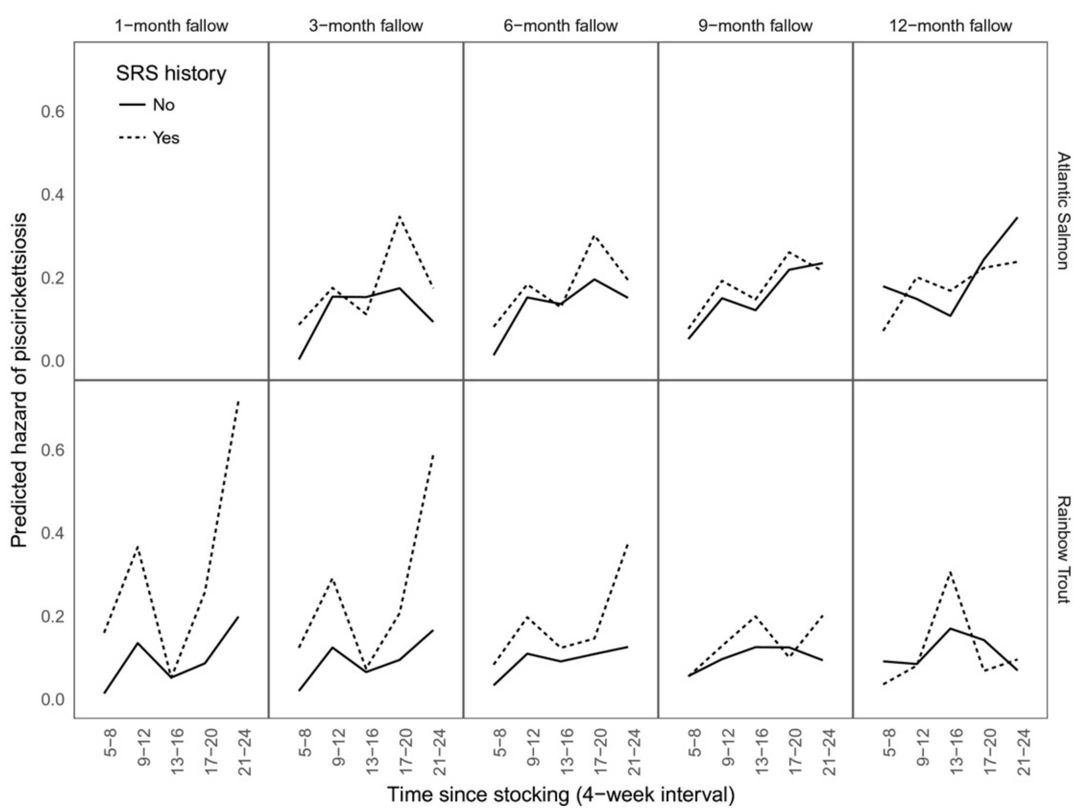

Fig. 1. Predicted hazard of reporting SRS mortality for farms with and without a history of SRS for each 4-week interval, by species and selected fallow durations.

Table 2
Table of coefficients, standard error (SE), and *P*-values for Atlantic salmon and Rainbow trout models.

Term	Atlantic salmon			Rainbow trout		
	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value
Intercept	−14.68	4.64		−6.93	1.72	
Fallow length	0.46	0.16		0.18	0.16	
SRS history						
No	(Reference)					
Yes	4.79	1.89		2.89	1.47	
SRS history: fallow	−0.49	0.21		−0.32	0.23	
4-Week interval						
5–8 weeks	(Reference)					
9–12 weeks	5.40	1.66		2.59	1.45	
13–16 weeks	5.51	1.69		1.39	1.59	
17–20 weeks	5.39	1.71		1.99	1.59	
21–24 weeks	4.25	1.83		3.12	1.56	
Interval: fallow						
5–8 weeks	(Reference)					
9–12 weeks	−0.47	0.18		−0.22	0.19	
13–16 weeks	−0.51	0.18		−0.06	0.20	
17–20 weeks	−0.42	0.18		−0.12	0.21	
21–24 weeks	−0.28	0.20		−0.28	0.21	
SRS history: interval						
5–8 weeks	(Reference)					
9–12 weeks	−4.71	2.13		−1.46	1.74	
13–16 weeks	−5.46	2.29		−2.97	2.19	
17–20 weeks	−3.49	2.19		−1.40	2.01	
21–24 weeks	−3.64	2.50		−0.39	2.14	
SRS history: interval: fallow			0.21*			0.82*
5–8 weeks	(Reference)					
9–12 weeks	0.51	0.24		0.20	0.28	
13–16 weeks	0.59	0.27		0.39	0.32	
17–20 weeks	0.37	0.26		0.13	0.32	
21–24 weeks	0.35	0.29		0.14	0.34	
Infected neighbors within 10 k			0.04*			0.02*
0	(Reference)					
1	−0.29	0.25	0.25	0.39	0.39	0.32
2	0.52	0.35	0.13	0.38	0.50	0.44
≥3	0.89	0.45	0.05	1.37	0.45	<0.01
Temperature ≤ 10	0.75	0.45	0.10			
Temperature > 10	0.29	0.09	<0.01			
Temperature				0.22	0.08	<0.01

* Overall *P*-values obtained using Wald tests.

the hazard of reporting SRS mortality by 2.43 times ($P < 0.05$). In rainbow trout farms, this relationship was also significant ($P = 0.02$). Having three or more neighbors led to an increase of 3.94 times the hazard of reporting SRS, compared to farms with no neighbors ($P < 0.01$). Having 1 or 2 neighbors with SRS also increased the hazard of SRS; however, this was not significant for either species ($P = 0.25$ and $P = 0.13$ in Atlantic salmon, $P = 0.32$ and $P = 0.44$ in rainbow trout).

We found that temperature was significantly associated with the hazard of the first report of SRS. For trout, we used a linear term to capture the effect of temperature ($P < 0.01$), while for Atlantic salmon we found a better model fit when the temperature was described using two spline terms, with a knot at 10 °C. At temperatures lower than or equal to 10 °C, we did not see an association between temperature and SRS ($P = 0.10$); but, when water temperature was >10 °C, the hazard of reporting SRS increased significantly ($P < 0.01$).

4. Discussion

In our study, 60.1% (255 out of 424) of all productions cycles reported SRS within 5 and 24 weeks post stocking (Table 1). To determine whether the probability of the first report of SRS was associated with the length of time the farm was fallowed, we estimated the hazard of SRS detection for each 4-week interval between the 5th to the 24th week of a production cycle of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), and we compared this hazard on farms that reported SRS mortality during the last four weeks of the previous production cycle to the hazard on farms that did not. We chose to compare these two groups of farms because fallowing should only affect the hazard of SRS on a farm with a history of the disease prior to the fallowing period. Because the probability of manifesting SRS depends on the exposure dose of *P. salmonis* (Garcés et al., 1991), and this agent is known to degrade in seawater (Lannan and Fryer, 1994; Olivares and Marshall, 2010), we hypothesized that the longer the fallow period after the occurrence of SRS, the lower the infective dose would be to the new crop of fish, and the longer it should take to develop clinical SRS. If fallowing for 3 months, as is typically done on Atlantic salmon farms, is adequate to eliminate *P. salmonis* from the environment we would not see any additional benefit from fallowing for a longer period.

To test our hypothesis, we modeled the hazard of SRS over time for different fallow periods (i.e. interaction between production month and fallow period) on farms with a known history of SRS just prior to fallowing. In Atlantic salmon, where we could only assess periods of fallow of three months or longer, we observed no difference in the hazard of SRS, regardless of the length of time the farm fallowed, which suggested no additional benefit to fallowing longer than 3 months. Either fallowing is not effective at all, which would be unlikely given the bacteria does not survive and replicate in saltwater for an extended period of time (Lannan and Fryer, 1994; Olivares and Marshall, 2010), or fallowing for 3 or more months is sufficient to remove *P. salmonis* associated with the previous cycle, and there are other sources of *P. salmonis* infection for fish in Chile.

To further explore the effect of fallowing, we compared the hazard of SRS over time for sites which should have had higher levels of *P. salmonis* from the previous cycle (because they reported the disease within four weeks of fallowing) to sites less likely to have *P. salmonis* from the previous cycle because there were no reports of SRS within the month prior to fallowing. In Atlantic salmon, we did not detect a difference in the hazard of SRS in these two groups of farms, even when we varied the time window for SRS in the cycle prior to fallowing (results not shown). The robustness of this finding provides further evidence that fallowing for 3 months is likely adequate to reduce the hazard of SRS from the bacteria originating in the previous cycle.

We were not able to assess fallow periods shorter than three months in Atlantic salmon farms because this is seldom feasible with the current regulation in Chile; however, we were able to assess short fallow periods in rainbow trout, and the trend in the data suggested fallowing fewer than 3 months was associated with a higher hazard of SRS (Fig. 2). When we analyzed the fallow period as a continuous predictor in our rainbow trout models, our three-way interaction was not significant; however, it is possible that we lacked statistical power to assess this term, given we only had 113 production cycles in our analysis. When we evaluated the association between fallow periods shorter and longer than 3 months and the probability of SRS early in the production cycle (i.e. 5 to 12 weeks post stocking) for farms with and without a history of the disease in the previous cycle, we detected a significant positive association between a short fallow period and the disease; this relationship was only detected in the group of farms with a history of SRS (Table 3). This suggests bacteria may not always be eliminated from sites that are fallowed for fewer than three months, which is also consistent with Olivares and Marshall (2010), who detected *P. salmonis* in seawater samples for up to 40 days.

Another possible explanation for the results of the short fallow periods in our rainbow trout analysis may be the fact that rainbow trout farms that fallow fewer than three months start their new production cycles in the middle of a neighborhood production cycle, so there are often active farms in the area when the new year class of fish is introduced to the site. To control for bias from confounding between the fallow period and *P. salmonis* infected neighbors, we controlled for infected neighboring farms in our models by including a predictor that captured the number of infected neighbors four to six weeks prior to disease onset on the farm of interest. We confirmed the findings of Rees et al. (2014), that farms with a high number of infected neighbors had a significantly higher hazard of SRS than farms without infected neighbors within 10 km (Tables 2 and 3). Although we found that only 3 or more infected neighbors increased the hazard significantly, we observed a dose response with the number of infected neighbors and the hazard of SRS. We may not have completely controlled for the neighbor effect in our analyses, as this relationship is complex and may depend on the mortality level (i.e. magnitude of exposure), the distance, and the species of fish on neighboring farms. Despite this limitation, the significant neighbor effect detected in this study, regardless of the neighbor distance and lag times to infection used in our analyses (results not shown), should deter producers from introducing naïve fish on farms when neighbors within 10 km are infected with this pathogen.

Given we likely did not fully control for infection pressure from neighboring farms in our analysis, it is possible that the association we observed between short fallow periods and the hazard of SRS in our logistic regression model for rainbow trout is partially due to exposure to *P. salmonis* from neighboring sites and, therefore, our findings should be considered preliminary. Further research is necessary to characterize the precise level of exposure from infected neighbors and to differentiate this source of infection from the bacteria that may be carried-over from one production cycle to another when a farm is fallowed for fewer than 3 months. Empirical data collected during the fallow period would help clarify the survival of the bacteria in sediment under farm sites during short fallow periods when neighboring farms are not undergoing outbreaks of SRS.

Other sources of infection for newly introduced fish in our study include infected wild fish and exposure prior to seawater transfer. The role of wild fish populations in the spread of the SRS has not been established, but *P. salmonis*, phylogenetically related to isolates from farmed salmonids, has been identified in wild and feral fish sampled in the vicinity of farms (Contreras-Lynch et al., 2015; García et al., 2016). It is possible that infected wild fish populations dwelling in management areas during the fallow period maintain the pathogen in the environment. We could not control for exposure to wild fish, so it is possible that not including this source of infection biased our results; however, we believe that it would have resulted in a bias towards finding a significant interaction between fallowing and previous history of SRS on the hazard of disease, and this was not observed. Our study suggests if wild fish are infected from the previous cycle prior to fallowing a site, the level of *P. salmonis* is not higher on farms with different fallow

Table 3
Table of coefficients, *P*-values, and Odds ratios (OR) for the dichotomized rainbow trout mixed-effects logistic model.

Term	Coefficient	SE	*P*-value	OR	OR 95% conf. int
Intercept	−2.71	1.23			
Fallow length					
≥3 months	(Reference)				
<3 months	0.10	0.67			
SRS history					
No	(Reference)				
Yes	0.01	0.64			
SRS history: fallow length					
≥3 months	(Reference)				
<3 months	1.85	0.93	<0.05	6.34	1.02–39.62
Infected neighbors within 10 k					
0	(Reference)				
1	0.49	0.59	0.41	1.63	0.51–5.19
2	−0.70	1.13	0.53	0.50	0.05–4.52
≥3	1.79	0.62	<0.01	5.98	1.78–20.06
Temperature	0.01	0.10	0.92	1.01	0.82–1.24

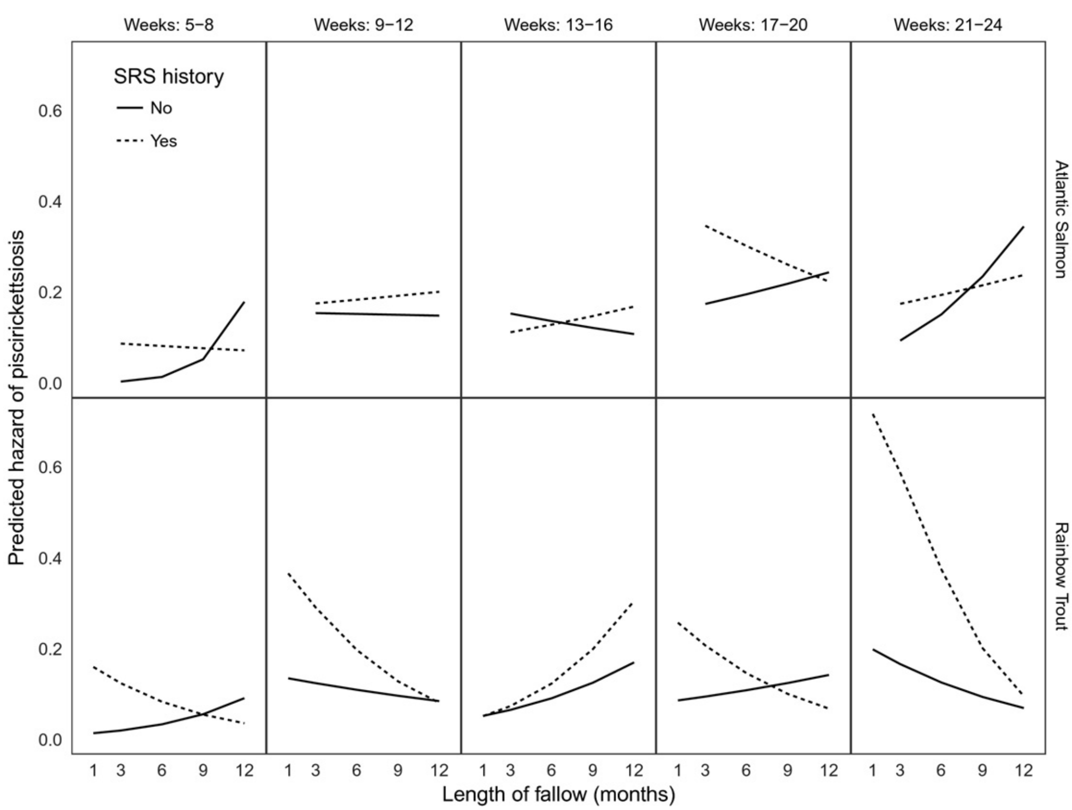

Fig. 2. Predicted hazard of reporting SRS mortality for farms with and without a history of SRS for each fallow duration by species and 4-week interval after seawater entry.

periods or farms with and without a history of SRS in the previous production cycle.

Although fish in our study could have been exposed to *P. salmonis* prior to seawater transfer, either while in fresh water (Bravo, 1994; Gaggero et al., 1995), or at a brackish water smoltification site, this agent is rarely detected in the mandatory government testing conducted before the transfer of fish to seawater sites. For example, in 2015 out of 5966 samples tested in fresh water within 30 days prior to seawater transfer, *P. salmonis* was never detected, and during the first half of 2016, *P. salmonis* was only detected in 24 out of 3780 (0.6%) Atlantic salmon smolts and 9 out of 760 (1.2%) rainbow trouts (Sernapesca, 2016c). We excluded the few production cycles that developed SRS within 4 weeks of seawater transfer to reduce the likelihood of including fish that may have been exposed to *P. salmonis* prior to seawater transfer. We felt this was an adequate minimum incubation period for fish, given the level of bacteria on seawater farms is unlikely to be higher than what is used in laboratory bath challenges, which report a similar time to disease onset (Strand and Midtlyng, 2007). Although the exclusion of these production cycles may have reduced our ability to find an effect of fallowing on early disease onset, only 5 of 21 groups of fish that were excluded from our analysis were from farms with a history of SRS on their sites prior to fallowing. Further, all these farms were fallowed, on average, for 6 1/2 months. Given our sample size, the exclusion of these production cycles would not likely have changed our overall conclusions.

An interesting trend in our Atlantic salmon model, which also suggests the source of *P. salmonis* for farms in Chile is not directly linked to the presence of bacteria from the previous cycle, was that sites with long fallow periods (i.e. longer than 9 months), regardless of their previous SRS status, appeared to have a higher hazard of SRS as time in seawater increased (Fig. 2). Because this trend (not statistically significant) occurred on sites with no recent history of SRS prior to fallowing, we do not believe this increase in the hazard of SRS is related to *P. salmonis* from the previous cycle. Further, the relationship observed is the opposite of what would be expected (i.e. we expected a shorter the fallow period to lead to a higher the probability of developing SRS earlier in the production cycle, given the natural decay of this bacteria in seawater). It is possible that sites that are fallowed for long periods are problematic sites, and farmers avoid using them for as long as they can. Further research is required to confirm this trend and test our hypothesis that long fallows are associated with poor performing farms.

5. Conclusions

The hazard of SRS in both Atlantic salmon and rainbow trout was high throughout our study, but the hazard was not significantly different for farms that fallowed for 3 or more months, whether they had a recent history of SRS or not. Given the consistency in our findings, regardless of the time lags used to capture the exposure from the previous cycle and neighbors, we are confident that the results of our study are robust and that fallowing for 3 months is likely adequate to reduce the hazard of SRS from a carry-over effect of bacteria originating in the previous production cycle. We are less confident that fallowing fewer than 3 months, which occurred with rainbow trout, is not associated with a higher hazard of reporting SRS within the first three months of sea-water entry, because when we grouped farms with a recent history of SRS in their previous cycle that fallowed for fewer than 12 weeks, we found a significant increase in the probability of reporting SRS within 5 to 12 weeks post-stocking. Given the significant neighbor effect detected in our analyses and the fact that most farms fallowing for fewer than 3 months have active farms within 10 km, mitigating the secondary spread of this bacteria between neighboring farms could reduce the incidence of SRS in the industry, and could help clarify the association between fallowing for fewer than 3 months and the hazard of SRS. Overall, it would appear that the hazard of SRS on farms is quite high within the first 24 weeks, but this is likely not because of bacteria originating directly from the previous production cycle within the farm, when farms are fallowed for 3 or more months.

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