Epidemiological analysis of the active surveillance programme for *Piscirickettsia salmonis* of the National Fisheries and Aquaculture Service of Chile

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Summary

Piscirickettsia salmonis is the causative agent of piscirickettsiosis, a disease that causes significant economic losses in salmonid sea farms in Chile. The objective of this study was to determine and describe the geographical distribution, seasonality and time period when *P. salmonis* was first detected in farms studied under the active surveillance programme for piscirickettsiosis of the National Fisheries and Aquaculture Service of Chile (SERNAPESCA), which was conducted from January 2013 to March 2017. A 0.28% prevalence of piscirickettsiosis was determined in freshwater fish and one of 58.1% in sea farms. The prevalence of *P. salmonis* was 61.1% in the Aysén region, 59.8% in the Los Lagos region, 5.1% in the Los Ríos region and 3.0% in the Magallanes region. In Los Lagos and Aysén, eight clusters of sea farms, were identified, in space and time, as having a positive diagnosis of *P. salmonis*, whereas, in Magallanes, none were identified, confirming the absence of horizontal transmission or spread of the agent in this geographical area. A seasonal variation was found in the monthly

prevalence of *P. salmonis*, with increases in *Salmo salar* and *Oncorhynchus mykiss* in summer and autumn, and in *Oncorhynchus kisutch* in winter, spring and summer. It was determined that the average time required to detect the agent after fish had been transferred to the sea was 105 days (minimum, 7 days; maximum, 351 days), and no differences were found either between regions or species. Thus the results obtained from the active surveillance programme have helped to increase knowledge of the epidemiology of *P. salmonis*.

Keywords

Active surveillance programme – Chile – Diagnosis – *Piscirickettsia salmonis* – Piscirickettsiosis – SERNAPESCA.

Introduction

Piscirickettsiosis (salmonid rickettsial septicaemia, or SRS) is one of the leading health problems affecting the marine aquaculture of salmonids in Chile, as it causes high mortality rates and generates economic costs in relation to its prevention and control in the main species of farmed salmonids (1, 2). *Piscirickettsia salmonis*, the causative agent of the disease, is a facultative intracellular bacterium that is predominantly coccoid in shape, unencapsulated, highly fastidious and measures approximately 0.2–1.5 µm in diameter (3). An *in silico* pan-genome analysis of *P. salmonis* identified two genogroups whose respective reference strains were EM90 and LF89 (2). *Piscirickettsia salmonis* has been isolated in Australia; British Columbia, Canada; Ireland; Norway and Scotland (4, 5). However, it is in Chile that piscirickettsiosis has had the heaviest animal health and economic impacts. In Chile, the disease has been diagnosed in the main species of farmed salmonids, in wild salmonids and in wild non-salmonids (1, 6). The agent's main route of transmission is horizontal, with vertical transmission having been confirmed *in vitro* only, specifically in *Oncorhynchus mykiss* (7, 8). Even though a preliminary diagnosis of piscirickettsiosis in salmonids can be made on the basis of external and internal macroscopic signs (1, 9), a number of molecular techniques, solid and liquid culture media and serological tests for the

confirmatory diagnosis of *P. salmonis* have been developed and refined, including ones based on immunofluorescence (10).

In December 2012, Chile launched its Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (PSVCSRS), aimed at early detection, case tracking and the application of timely and gradual control measures (11), all of which allows active surveillance of the disease. The objective is to systematically collect information that accurately represents the status of the disease in a population of known size by providing access to the calculation of rates and prevalence (12). The aim of this study was to determine and describe the geographical distribution, seasonality and time period when *P. salmonis* was first detected in farms under the PSVCSRS programme of the National Fisheries and Aquaculture Service of Chile (SERNAPESCA).

Materials and methods

Active surveillance programme

For this study, the databases of the PSVCSRS programme (11) and the General Health Programme for Mortality Management (PSGM) and its classification system (13) were used, which are stored in SERNAPESCA's information system for the control of aquaculture (SIFA). The PSVCSRS programme calls for active surveillance of the agent in farms through targeted sampling of fish and standardised laboratory testing at a set frequency. In freshwater farms, sampling is performed no sooner than 30 days prior to the transfer of fish to sea farms. Here, the first sampling is carried out after a period of 30 days has elapsed following completed sea transfer, with subsequent sampling performed every two months until the end of the production cycle, except in farms located in the Magallanes region, where it is performed every four months. Samples are taken from a minimum sample size of 15 fish (SERNAPESCA criterion) and the diagnostic technique used is polymerase chain reaction (PCR), as per SERNAPESCA technical standards LABD/NT 1 (14) and LABD/NT 2 (15). Furthermore, any results from farms using sample sizes, sampling frequencies or diagnostic techniques (bacterial culture or immunofluorescence) other than those specified must be reported to

SERNAPESCA and are taken into account in the programme's active surveillance. The diagnostic laboratories notify the analysis results to SIFA on a weekly basis (11). The PSGM programme database provided the data for the average weight of fish reported in the general descriptive results for freshwater farms and sea farms in Magallanes with a diagnosis of *P. salmonis,* and for the breakdown of sea farm clusters with a positive diagnosis of the agent. The free software R (version 3.3.3, www.r-project.org) was used for data management and statistical analysis.

Study area and distribution of farms

Chile has a monthly average of 311 active sea farms engaged in ongrowing Atlantic salmon (*Salmo salar*) (71% of farms), rainbow trout (*Oncorhynchus mykiss*) (11% of farms) and Coho salmon (*Oncorhynchus kisutch*) (18% of farms). These farms stretch from the Los Ríos region to Magallanes. Regions with sea farms are sub-divided into groups of salmonid farm sites (salmonid concession groups, or ACS). Los Lagos has 22 ACS, Aysén has 37 and Magallanes has 12, whereas Los Ríos has none. Each ACS has a 24-week production period, followed by a three-week fallow period (16). There is an annual average of 180 active freshwater farms, stretching from the Metropolitan region to Magallanes, with the main aquaculture areas being Araucanía and Los Lagos.

Case definition

For the general descriptive analysis, a case was defined as a report that included at least one diagnosis of *P. salmonis* by PCR, indirect fluorescent antibody test (IFAT) or bacterial culture of a sample taken from a facility producing any of the three main species of salmonids. Excluded from the analysis was any report *a)* missing the farm code, date of sampling or number of fish analysed; *b)* noting the presence of experimental activity or non-salmonid species; or *c)* noting the use of non-reference laboratory samples (such as sediment). For the general descriptive analysis related to freshwater production and production in the Magallanes region, reports that included a positive diagnosis were examined to ascertain the number of fish with and without infection.

For the spatial analysis, a case was defined as any sea farm producing any of the three main species for which there was at least one monthly laboratory report (taking the date of sampling as a reference) that included a *P. salmonis* diagnosis. The regions analysed were Los Lagos, Aysén and Magallanes, between January 2013 and March 2017. The population at risk was that of active sea farms with a negative diagnosis of *P. salmonis*, which produced any of the three main species. The regions and period analysed were those indicated above.

General descriptive analysis

An exploratory and descriptive analysis was made of the laboratory reports submitted to SIFA under the PSVCSRS programme between January 2013 and March 2017. The variables analysed in each of the reports were the origin of the water (seawater or fresh water), geopolitical area (region) and species (*S. salar*, *O. mykiss* or *O. kisutch*).

Spatial analysis

A spatial scan test was used to examine the random distribution of cases in space and time (17) and to perform a Poisson space–time analysis to compare the monthly incidence rate of cases within a space–time window against the rate for farms at risk situated outside the window (18). With regard to space, the window was moved across the study regions and ranged from 0 km to 10 km in radius, in accordance with Rees *et al.* (19), who stated that this distance posed a risk for the spread of piscirickettsiosis from a neighbouring farm with the disease. With regard to the duration of piscirickettsiosis outbreaks, established by Jakob *et al.* (20), a maximum time window of four months was set. It was decided that the window should start in January 2013 (launch of the PSVCSRS programme), in a monthly time cluster, taking into account the fact that there was a high incidence of the disease in the population, and areas with high incidence rates were sought. The geographic coordinates (latitude/longitude) of each farm studied were obtained from SERNAPESCA's national aquaculture register. Monte Carlo replications ($n = 999$) were performed to determine the distribution of the test statistic, the distribution of the likelihood ratio

and the associated *p* value (17). All the analyses were conducted using SaTScan (version 7.0.1, www.satscan.org) with a significance level of $p < 0.05$. The open source software QGIS (version 2.14.8, www.qgis.org) was used to view the results.

Time series analysis

A descriptive and decomposition analysis was performed, by species and region, of monthly prevalence series in sea farms with a diagnosis of *P. salmonis*. The series represents the number of farms that constituted cases for spatial analysis in Los Lagos and Aysén between January 2013 and March 2017, divided by the total number of sea farms that conducted monthly analyses for *P. salmonis* in these regions during the same period under the PSVCSRS programme, in the form of a percentage. The *ts* and *decompose* functions in R were used to describe and obtain the seasonality index.

Survival analysis

The survival time was defined as the interval of days from the date of transfer of fish to the sea until the date of sampling with a diagnosis of *P. salmonis* within a single production cycle of a sea farm. This analysis used the case definition applied to the general descriptive analysis conducted for Los Lagos and Aysén between July 2014 and March 2017. The analysis included farms which *a)* transferred vaccinated fish; *b)* did not detect the agent in fresh water; *c)* did not employ antibiotics during the evaluation period; and *d)* had the production objective of ongrowing (not smoltification or breeding). The median survival time was determined using the Kaplan–Meier curve and the log-rank test, to ascertain differences between regions and species. All the statistical analyses were performed in R using the *survfit* and *survdiff* functions, with a significance level of $p < 0.05$.

Results

Descriptive analysis

A total of 13,440 reports were analysed, of which 1,894 (14.1%) related to freshwater farms and 11,546 (85.9%) to sea farms.

Piscirickettsia salmonis was detected in 0.28% (*n* = 90) of the total number of fish sampled in fresh water (*n* = 32,293). In 1.2% (*n* = 22) of the total number of reports on freshwater farms, the agent was diagnosed using PCR, and at least one report with a positive diagnosis was made per year (except for 2014). All these reports with positive diagnoses came from ten hatcheries located in four regions (Biobío, Los Ríos, Los Lagos and Aysén). The sources of water for the hatcheries were brackish water (mix of fresh water and seawater) in 68% of cases and fresh water (including springs, rivers, streams and lakes) in 32%. The agent was detected in all farmed species (*S. salar, O. mykiss* and *O. kisutch*). The median of the average weights at the time of diagnosis was 153 g (Table I).

Table I

Description of reports on fish hatcheries with a diagnosis of *Piscirickettsia salmonis*. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

Report no.	Farm	Lab.[a]	Date of sampling	Water source	Region	Species	Weight (g)[b]	Sample[c]	Technique[d]	Total fish[e]	Fish (+)[f]
1	B	3	23 Jan. 2013	Lake	Los Lagos	*O. kisutch*	73.5	Organ	PCR	15	15
2	H	2	19 Feb. 2013	Spring	Aysén	*O. kisutch*	6,500[h]	Organ	PCR	2	2
3	D	3	29 Apr. 2013	Stream	Los Ríos	*O. mykiss*	30.5	Organ	PCR	15	15
4	F	1	13 Aug. 2015	Brackish [g]	Los Lagos	*S. salar*	69.6	Organ	PCR	5	1
5	G	1	28 Sept. 2015	Brackish [g]	Los Lagos	*S. salar*	422.6	Organ	PCR	15	3
6	I	5	30 Oct. 2015	Brackish [g]	Aysén	*S. salar*	75.2	Organ	PCR	13	3
7	I	5	3 Nov. 2015	Brackish [g]	Aysén	*S. salar*	82.4	Organ	PCR	26	1
8	I	5	11 Nov. 2015	Brackish [g]	Aysén	*S. salar*	72.1	Organ	PCR	10	2
9	G	1	16 Nov. 2015	Brackish [g]	Los Lagos	*S. salar*	334.2	Organ	PCR	3	1
10	G	1	2 Nov. 2016	Brackish [g]	Los Lagos	*S. salar*	417.0	Organ	PCR	8	8
11	I	5	10 Jun. 2016	Brackish [g]	Aysén	*S. salar*	94.9	Organ	PCR	20	1
12	I	5	11 Jul. 2016	Brackish [g]	Aysén	*S. salar*	89.4	Organ	PCR	14	1
13	I	5	14 Jul. 2016	Brackish [g]	Aysén	*S. salar*	89.4	Organ	PCR	12	5
14	I	5	21 Jul. 2016	Brackish [g]	Aysén	*S. salar*	97.8	Organ	PCR	51	2

15	I	5	29 Jul. 2016	Brackish [g]	Aysén	*S. salar*	92.0	Organ	PCR	18	1
16	I	5	5 Aug. 2016	Brackish [g]	Aysén	*S. salar*	88.8	Organ	PCR	7	1
17	I	5	14 Sept. 2016	Brackish [g]	Aysén	*S. salar*	110.5	Organ	PCR	21	5
18	J	5	22 Sept. 2016	River	Los Ríos	*S. salar*	NIA	Organ	PCR	33	4
19	I	5	8 Oct. 2016	Brackish [g]	Aysén	*S. salar*	125.5	Organ	PCR	10	1
20	E	4	10 Nov. 2016	River	Los Lagos	*O. mykiss*	136.0	Organ	PCR	15	6
21	A	4	16 Nov. 2016	River	Biobío	*O. mykiss*	136.0	Organ	PCR	15	6
22	C	4	30 Jan. 2017	River	Los Lagos	*S. salar*	169.0	Organ	PCR	15	6

a) Diagnostic laboratory
b) Average weight of fish
c) Includes samples of brain, liver and/or kidney
d) Includes techniques developed by the laboratory itself or already published
e) Total number of fish sampled
f) Number of fish with a positive diagnosis
g) Mix of fresh water and seawater in the water supply
h) Broodstock sample

NIA: no information available
O. mykiss: *Oncorhynchus mykiss*
PCR: polymerase chain reaction
S. salar: *Salmo salar* L

Of the total number of reports on sea farms (*n* = 11,546), *P. salmonis* was detected in 62.1% of reports relating to *S. salar*; 53.4% of reports relating to *O. kisutch*; and 40.6% of reports relating to *O. mykiss*. The study by region showed 5.1% positive diagnoses for piscirickettsiosis in the Los Ríos region, 59.8% in Los Lagos, 61.1% in Aysén and 3.0% in Magallanes (Table II).

Table II
Number of reports on sea farms by region and species. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

Region	Positive diagnosis (+)	Total	Percentage (+)
Los Ríos	6	118	5.1
Los Lagos	3,366	5,625	59.8
Aysén	3,326	5,441	61.1
Magallanes	11	362	3.0
Species	**Positive diagnosis (+)**	**Total**	**Percentage (+)**
S. salar	5,268	8,484	62.1
O. kisutch	616	1,518	40.6
O. mykiss	825	1,544	53.4
Total	6,709	11,546	58.1

O. kisutch: Oncorhynchus kisutch
O. mykiss: Oncorhynchus mykiss
S. salar: Salmo salar

Piscirickettsia salmonis was detected in 2.3% (*n* = 120) of the fish sampled in Magallanes (*n* = 5,207). All the reports for Magallanes with a positive diagnosis for piscirickettsiosis related to 2013 and 2014 and to six farms, and were detected by PCR in organ samples in three laboratories. A total of 88.9% of the positive reports for

piscirickettsiosis related to *S. salar* and 11.1% to *O. mykiss*; the average weight of the fish at the time of diagnosis was 1,948 g (Table III).

Table III
Sea farms in Magallanes with a positive diagnosis of *Piscirickettsia salmonis*. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

Farm	Month	Year	Lab.[a]	Species	Weight (g)[b]	Sample[c]	Technique[d]	Fish (+)[e]
5	March	2013	1	*S. salar*	557	Organ	PCR	72
5	May	2013	1	*S. salar*	835	Organ	PCR	15
2	May	2013	2	*S. salar*	548	Organ	PCR	8
1	June	2013	1	*O. mykiss*	310	Organ	PCR	6
6	June	2013	2	*S. salar*	1,948	Organ	PCR	5
6	July	2013	2	*S. salar*	3,945	Organ	PCR	2
5	June	2014	1	*S. salar*	5,329	Organ	PCR	3
3	June	2014	1	*S. salar*	5,958	Organ	PCR	3
4	November	2014	3	*S. salar*	2,569	Organ	PCR	6

a) Diagnostic laboratory
b) Average weight of fish
c) Includes samples of brain, liver and/or kidney
d) Includes techniques developed by the laboratory itself or published
e) Number of fish with a positive diagnosis

O. mykiss: *Oncorhynchus mykiss*
PCR: polymerase chain reaction
S. salar: *Salmo salar*

Spatial analysis

There were cases in all the active ACS in the Los Lagos and Aysén regions; however, 54.5% of the active ACS were in Magallanes. The Poisson space–time model identified eight clusters of farms at risk of *P. salmonis*; five in Los Lagos and three in Aysén (Fig. 1). In Magallanes, no significant clusters were detected ($p > 0.05$) either in space or in time (Fig. 2). Of the two primary clusters, the first cluster

(C1) occurred in the period between October 2015 and January 2016 and spread from a single infected farm (with its centroid at 42°33′50.4″S, 73°29′42.0″W) to cover a final radius of 9.56 km, ultimately involving 12 *S. salar* and *O. kisutch* farms belonging to ACS 9A and ACS 10, with average weights, at the start of the period and by ACS, of 389 g and 3,116 g, respectively. The second cluster (C6) occurred in the period between April 2013 and June 2013 and spread from a single infected farm (with its centroid at 45°27′14.4″S, 73°48′25.2″W) to cover a final radius of 6.81 km, ultimately involving seven *S. salar* farms belonging to ACS 22D and ACS 24, with average weights, at the start of the period and by ACS, of 658 g and 3,681 g, respectively (Table IV).

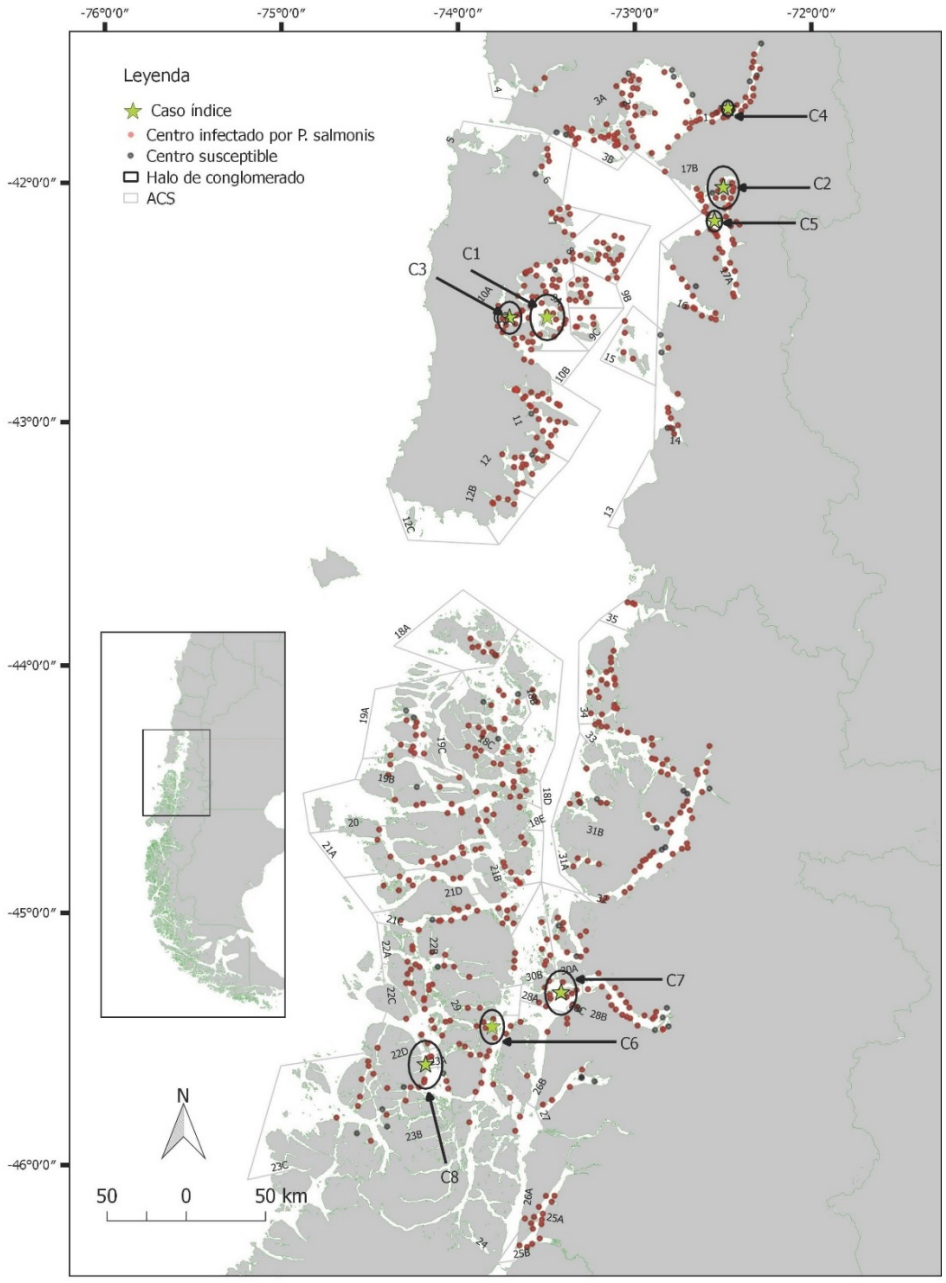


Fig. 1

Spatial distribution and clusters in space and time of salmonid sea farms with a positive diagnosis in Los Lagos and Aysén. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

ACS: salmonid concession group
C1–8: Clusters 1 to 8
P. salmonis: *Piscirickettsia salmonis*

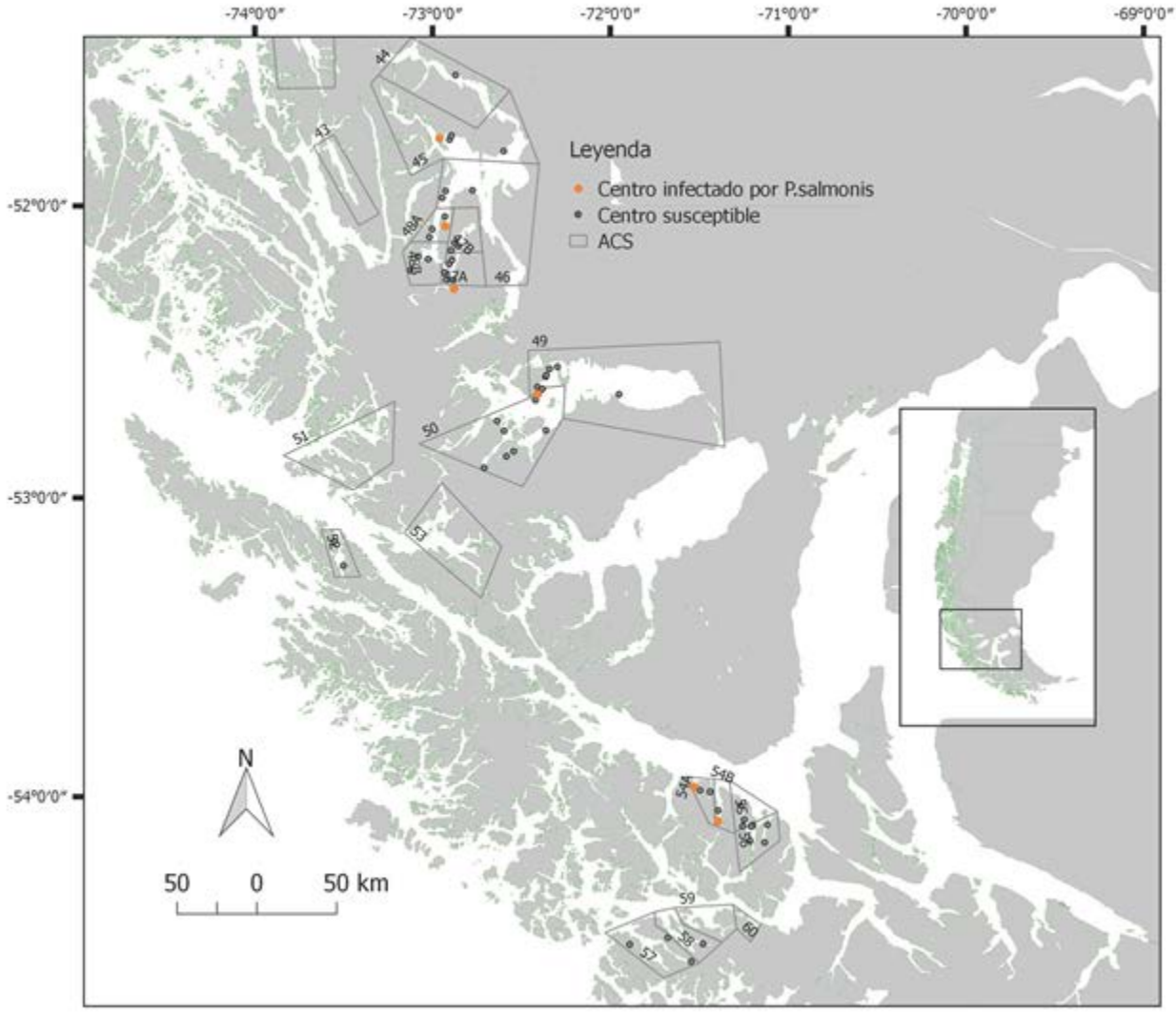


Fig. 2

Spatial distribution of salmonid sea farms in Magallanes with a positive diagnosis. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

ACS: salmonid concession group

P. salmonis: *Piscirickettsia salmonis*

Table IV

Breakdown of sea farm clusters with a positive diagnosis of *Piscirickettsia salmonis*. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

Cluster	Type of cluster	Period of the cluster	Radius (km)	RR	Chi² *p*-value	ACS involved	Number of farms involved	Species	Average weight (g) of fish
C1	Primary[a]	Oct. 2015–Jan. 2016	9.56	3.3	0.001	9A	2	*S. salar*	389
						10A	7	*S. salar*	3,355
							3	*O. kisutch*	2,877
C2	Secondary[b]	Nov. 2013–Feb. 2014	9.00	4.3	0.001	17B	4	*O. kisutch*	2,155
							7	*O. mykiss*	1,132
C3	Secondary	Jun. 2015–Sept. 2015	6.73	3.6	0.002	10A	2	*S. salar*	1,407
							6	*O. kisutch*	1,220
						10B	1	*S. salar*	2,517
							1	*O. kisutch*	1,129
C4	Secondary	Sept. 2013–Dec. 2013	3.37	6.7	0.023	1	4	*O. mykiss*	1,494
C5	Secondary	Mar. 2015–Jun. 2015	4.41	6.5	0.035	17A	3	*S. salar*	2,719
C6	Primary	Apr. 2013–Jun. 2013	6.81	6.7	0.003	22D	5	*S. salar*	658
						24	2	*S. salar*	3,681
C7	Secondary	Mar. 2013–Jun. 2013	8.83	3.9	0.004	28A	5	*O. mykiss*	1,918
							4	*S. salar*	2,086

							1	*O. kisutch*	1,918
C8	Secondary	Mar. 2014–Jun. 2014	9.56	4.2	0.031	22D	1	*S. salar*	4,082
						23A	3	*S. salar*	984
						23B	2	*S. salar*	5,270

a) Primary: hierarchically most likely cluster
b) Secondary: secondary cluster

ACS: salmonid concession group
C1–8: clusters 1 to 8
O. kisutch: *Oncorhynchus kisutch*
O. mykiss: *Oncorhynchus mykiss*
RR: relative risk
S. salar: *Salmo salar*

Time series analysis

Farms producing *S. salar* had an average monthly prevalence of 67.6% (95% confidence interval [CI]: 70.5–64.7), while those producing *O. mykiss* had a prevalence of 58.9% (95% CI: 54.3–63.5), and those producing *O. kisutch* had a prevalence of 43.8% (95% CI: 37.8–49.8) (Fig. 3). The seasonal index for *S. salar* showed a slightly higher-than-average number of cases in the months of January, February, March, April, May, June, November and December, and a slight lower number in July, August, September and October. In the case of the *O. mykiss* species, seasonality was found in February, March, April and June, and a decrease in the months of May, July, August, September, October, November and December. The seasonal index for the *O. kisutch* species showed an increase in January, June, August, September, October, November and December, and a decrease in February, March, April, May and July (Fig. 4).

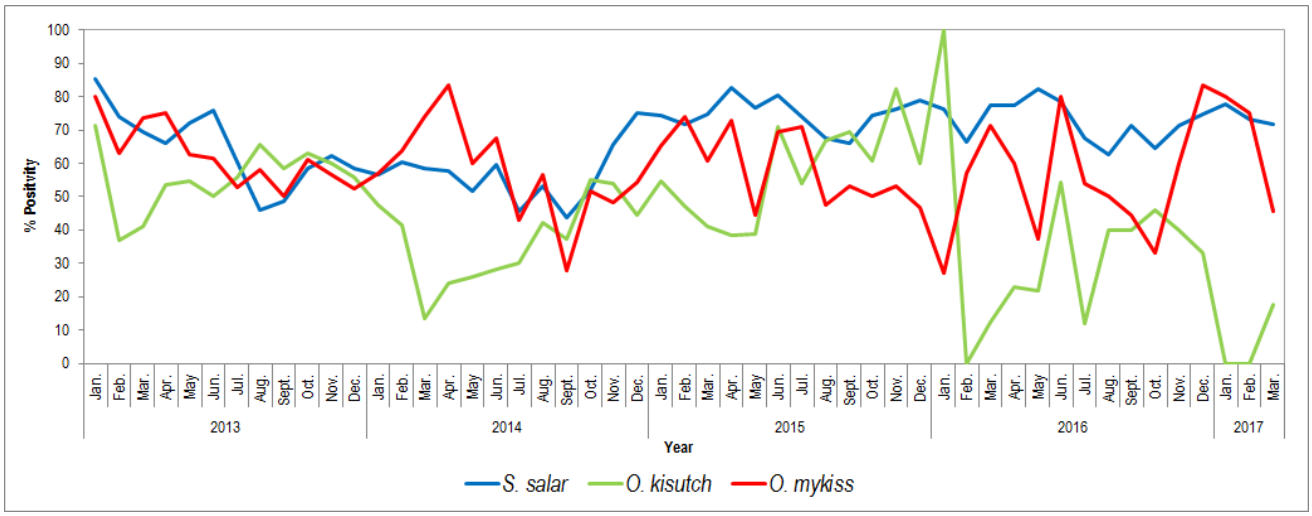


Fig. 3

Monthly prevalence of piscirickettsiosis in farms in Los Lagos and Aysén with a positive diagnosis, by species. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

O. kisutch: Oncorhynchus kisutch
O. mykiss: Oncorhynchus mykiss
S. salar: Salmo salar

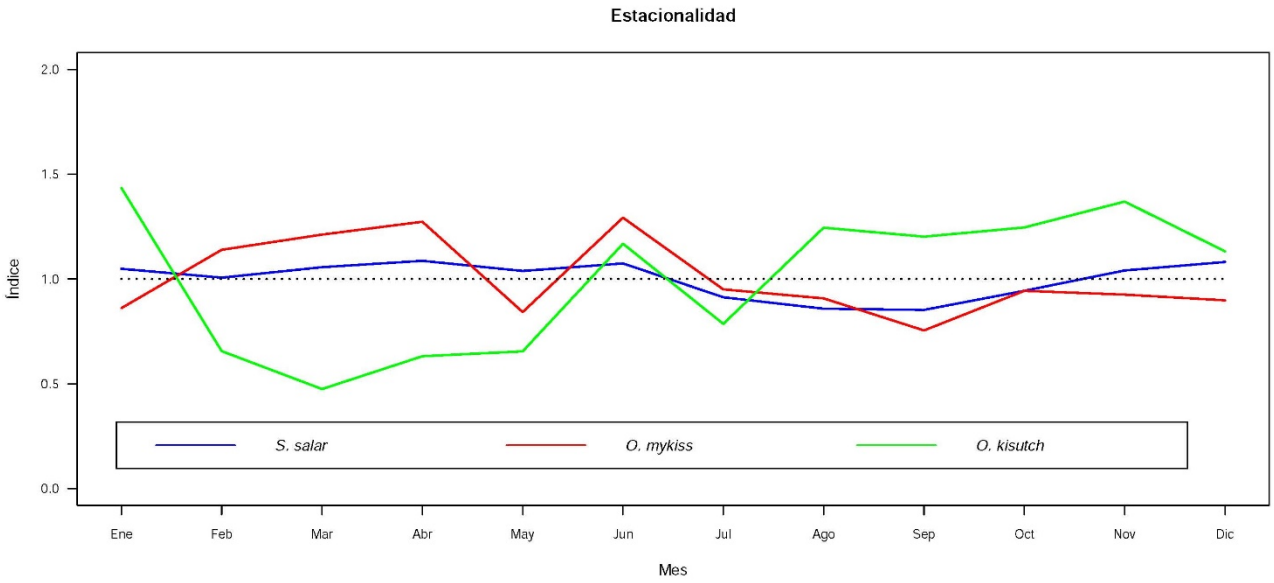


Fig. 4

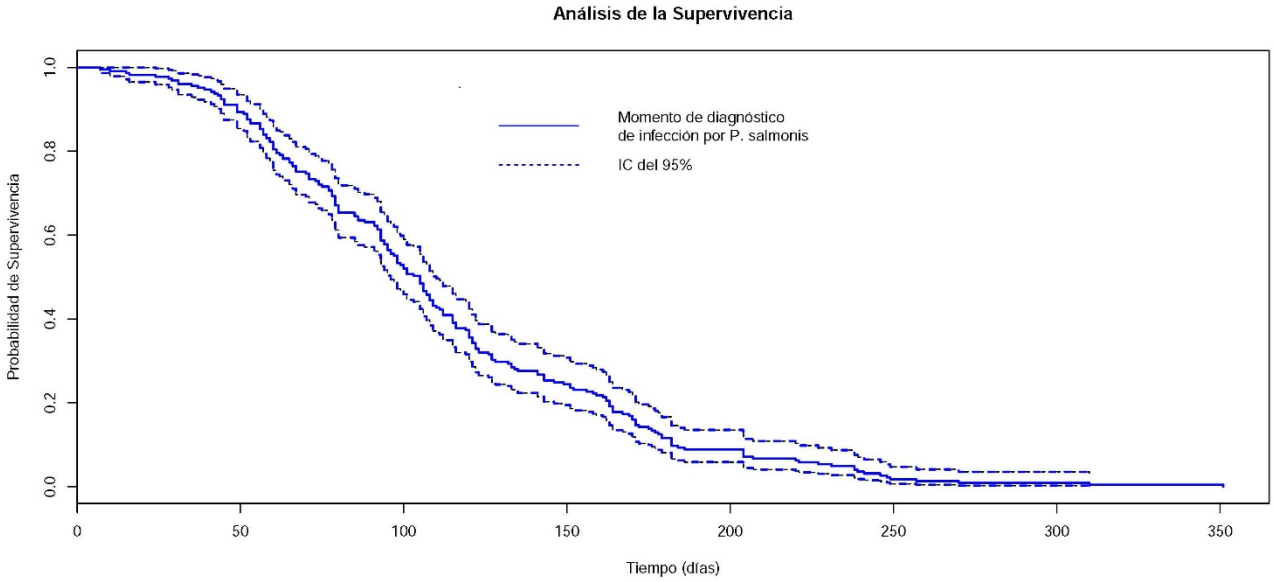
Monthly prevalence of piscirickettsiosis in sea farms in Los Lagos and Aysén with a positive diagnosis, by species. Specific Health Programme for the Surveillance and Control of Piscirickettsiosis (January 2013–March 2017)

O. kisutch:Oncorhynchus kisutch
O. mykiss:Oncorhynchus mykiss
S. salar: Salmo salar

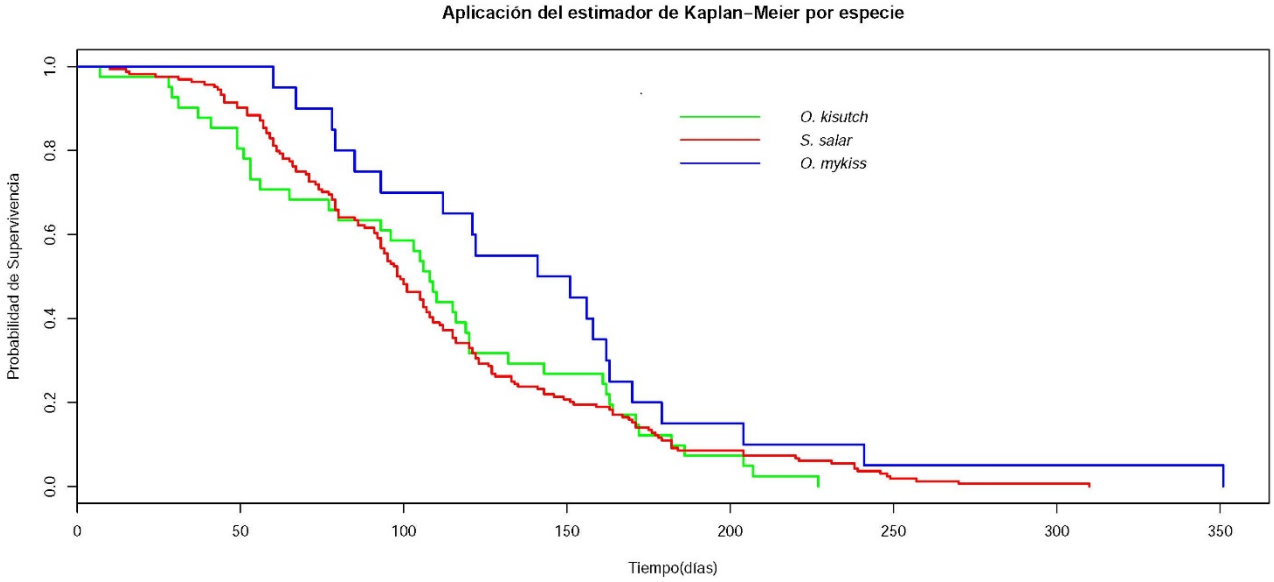
Survival analysis

A total of 225 production cycles were analysed and the median overall survival time, from the first diagnosis, was found to be 105 days, with a minimum of 7 days and a maximum of 351 days. The Kaplan–Meier survival curve determined that, in the case of *S. salar*, median survival was 99 days (95% CI: 93–107), in the case of *O. kisutch*, it was 108 days (95% CI: 93–120) and in the case of *O. mykiss*, it was 146 days (95% CI: 112–170); there were no significant differences between species ($p = 0.130$). In the comparison of regions, the Kaplan–Meier curve showed that the median time in the case of Los Lagos was 100 days (95% CI: 93–110) and in the case of Aysén it was 106 days (95% CI: 95–119); there were no significant differences between regions either ($p = 0.319$) (Fig. 5).

Overall



Species



Region

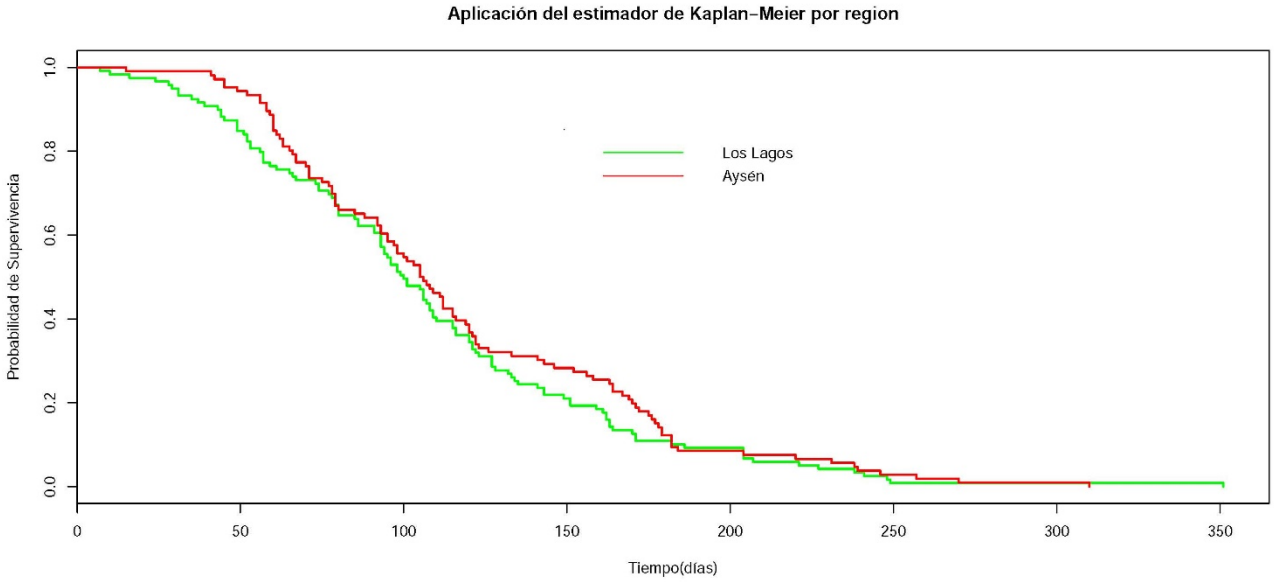


Fig. 5

Kaplan–Meier curves (95% CI) from the time of first diagnosis of *Piscirickettsia salmonis* infection in sea farms in general, as well as by species and region

CI: confidence interval
K–M: Kaplan-Meier
O. kisutch: *Oncorhynchus kisutch*
O. mykiss: *Oncorhynchus mykiss*
P. salmonis: *Piscirickettsia salmonis*
S. salar: *Salmo salar*

Discussion

A low prevalence (0.28%) of *P. salmonis* was found in fish farmed in fresh water, which correlates with the small number of outbreaks of piscirickettsiosis in salmonids farmed in this environment (10, 21) and could be explained by the rapid instability of the bacterium in fresh water (22). Detection of the agent in hatchery fish may be associated with production practices that increase the risk of infection, such as supplying non-disinfected seawater; entry, from seawater, of carrier animals that may be used for breeding; detecting the vaccine agent after fish have been vaccinated; or using low-specificity, low-sensitivity diagnostic tests.

There is a high percentage of positive diagnoses in sea farms, which is associated with the pervasiveness of *P. salmonis* in marine areas and the high frequency of outbreaks in this environment (20, 22, 23). This could be explained by the properties of the bacterium itself, enabling it to survive and replicate in this environment. Authors have described how *P. salmonis* forms a biofilm structure that allows it to survive in the sea under adverse environmental conditions and even in the absence of fish for up to 40 days (24, 25). There are several biological reservoirs of *P. salmonis*, such as crustaceans and free-living marine molluscs (10, 26). Infected fish excrete the bacterium via bile, faeces and urine (27). A 9.8% prevalence of piscirickettsiosis diagnoses has been determined in wild and feral fish, with the Patagonian blenny (*Eleginops maclovinus*) and Chilean silverside (*Odontesthes regia*) showing the highest levels of infection (28). Feral fish are a source of the bacterium, especially those found close to farms, as is the case with farmed fish, whether healthy, dying or dead as a result of the disease (10, 29). However, the role of aquatic organisms, as biological or mechanical vectors, in sustaining the agent at sea is yet to be determined.

All three species studied are highly susceptible to infection, as described by Murray and Peeler (29), who point out that these species are at high risk of contracting piscirickettsiosis. However, the disease presents differently depending on the species affected. Jakob *et al.* (20) report that an outbreak in *O. mykiss* affects more cages, is more extensive and has a higher mortality rate than an outbreak in *S. salar*, which could be explained by differences in the virulence of *P. salmonis* from one species to another (19). There are serotypes and genetic variants with a preference for a specific host. Saavedra *et al.* (30) report that the genotype of strain EM-90 shows a marked preference for infecting *S. salar*, whereas the genotype of the LF-89 strain is detected in *S. salar*, *O. mykiss* and *O. kisutch*. It was determined *in vitro* that, in *S. salar*, EM-90 caused infection with a shorter time to death and a higher cumulative mortality rate than LF-89 (31). However, there is a lack of information on the species-specific pathogenicity, prevalence and geographical distribution of the different serotypes and genogroups. Such information is important in establishing control strategies for the disease (26).

Los Lagos and Aysén are the main regions with a diagnosis and farm clusters, which is associated with the high prevalence of farms with the disease in these regions. This is possibly owing to the fact that these two regions account for more than 90% of the country's ongrowing (32) and have a high concentration of farms (sometimes less than 10 km apart, a factor conducive to rapid spread of the bacterium), coupled with circumstances favourable for the bacterium's growth and survival (environmental conditions and water temperatures), as has been proven experimentally by the synergetic effect of temperatures of approximately 14°C and high population density (20 kg/m^3) on the occurrence of piscirickettsiosis (33). In contrast, in the Magallanes region, a low prevalence (3.0%) and no clusters of infected farms were found, which is related to the absence of outbreaks and spread of the disease between sea farms in the region (32). The low prevalence found in Magallanes might be explained by the entry of fish, from another endemic geographical area, that are carriers or are sub-clinically infected. The absence of clusters, which suggests zero spread, could be due to the region's production and environmental conditions, such as the low number/geographical concentration of sea farms and low water temperature (32), as it has been proven *in vitro* that the disease does not occur when fish are kept in seawater below 7.5–8.5°C because temperatures of <10°C slow down the bacterium's growth (34, 35). However, given that salmonid farming has increased in Magallanes in recent years, it is important to develop preventive health measures to reduce the risk of the agent's entry and spread in the region.

The seasonal index of piscirickettsiosis diagnosis in *S. salar* and *O. mykiss* increases in the summer–autumn period, which accords with the findings published by Rees *et al.* (19) of a correlation, in both species, between higher temperatures and a higher piscirickettsiosis incidence rate. This could be explained by the fact that, in Los Lagos and Aysén, the average maximum summer temperature at the sea surface is 14–15°C (32), which could cause stress in fish and increase feeding, as well as aiding survival of the bacterium, which has optimal replication *in vitro* at 15–18°C (35). Certain strains of genogroup 1 (G1) have been found to grow optimally at 16–19°C, whereas some genogroup 2 (G2) strains grow optimally at 19–22°C (36). Branson and

Nieto Díaz-Muñoz (37) report outbreaks of the disease after periods of significant water temperature variation, as in autumn and spring. A point of note is that the disease preferentially affects animals weighing over 1 kg and that, in *S. salar*, the first outbreak occurs on average 8.3 months into the production cycle and, in *O. mykiss*, 6.6 months into the cycle (20, 32). During the above-mentioned seasons, the concentration of farms producing high biomass in the water therefore increases. A possible reason why this production group has a higher risk of infection could be weakened passive immunity to the agent from the stress caused by high stocking densities, leading to skin damage, sea lice (*Caligus)*-type parasitic infections and viral diseases. Furthermore, seasonality in *O. kisutch* explains the first epizootic of the disease described in Chile in 1989, in which outbreaks occurred from autumn to mid-winter and again in spring and early summer (38). This might be due to the seasonality of the annual production cycle and to the fact that stocking is carried out preferably between January and April, and harvesting between December and February.

In the Los Lagos and Aysén regions, the first diagnosis of *P. salmonis* in sea farms producing one of the three species occurred after a minimum of seven days, a maximum of 351 days and an average of 105 days (3.5 months) as from the start of the production cycle, which agrees with the finding by Gaggero *et al.* (39) that the disease occurs 1.5–3 months after the start of the seawater production cycle. However, Bravo (21) reported that the infection began a maximum of two weeks following seawater transfer, and Jakob *et al.* (20) reported that the disease had already been detected two months after the start of the production cycle. Early infection at sea may be explained by defective *P. salmonis* vaccination of fish in hatcheries, possibly related to the vaccine serotype, the type of antigen inoculated (live, attenuated or other) or improper vaccine administration contrary to the manufacturer's instructions. This is associated with the sea transfer of groups of fish that have failed to attain optimum physiological, morphological or behavioural conditions (smolt quality) and which are therefore more susceptible to infection. Accordingly, consideration could be given to the stress caused during sea transfer and to poor fish adaptation and acclimatisation to the sea. Indeed, Jakob *et al.* (20)

reported that these factors were related to the high cumulative mortality rate during the early weeks of ongrowing, a situation that correlates with extremely severe piscirickettsiosis outbreaks during the production cycle, and it has been determined that fish introduced in spring–summer are more exposed to high *P. salmonis* loads than those introduced in autumn–winter.

Conclusions

The results obtained from active surveillance carried out under the PSVCSRS programme have helped to further knowledge of the epidemiology of *P. salmonis* in Chile. A low prevalence of the agent has been detected in hatchery fish, confirming that diagnosis of the bacterium takes place mainly during the ongrowing stage at sea and in the three commercially important species. In sea farms, infection occurs in the early stages and, in the case of *S. salar* and *O. mykiss*, seasonality is associated mainly with the summer–autumn seasons, while in the case of *O. kisutch*, it is associated mainly with the autumn–spring–summer seasons. The spatial distribution of the disease is concentrated chiefly in two regions, Los Lagos and Aysén. In Magallanes, disease risk is low owing to the infrequent detection of the agent and the absence of spread to neighbouring farms.

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