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Space–time cluster analysis of the non-pathogenic infectious salmon anemia virus (HPR0 ISAV) in Chile, 2011–2012

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ABSTRACT

In 2007, Chile reported its first case of infectious salmon anemia (ISA), caused by ISA virus (ISAV), in sea-cage reared Atlantic salmon. By 2010, the ISA disease outbreak was under control, but similarly to other countries, Chile continues to occasionally detect the non-pathogenic variant of ISAV (HPR0 ISAV). The Chilean salmon industry has been including gill samples in their routine ISAV monitoring program to improve the detection of HRP0 ISAV, which provides an opportunity to better assess the distribution of HPR0 ISAV and describe its occurrence in the Chilean marine aquaculture industry.

A risk and a relative risk surface map were generated for the predominant regions of the Chilean aquaculture industry, and demonstrated areas of increased risk to HPR0 ISAV for the entire surveillance period. The observed occurrence and seasonal patterns of HPR0 ISAV were similar to those previously reported in Chile and in other countries. Further, spatiotemporal cluster analysis showed that the areas with a high-risk of HPR0 ISAV changed during the surveillance period (highly prevalent and causing non-clinical transient infections), and cases in clusters were initially observed within companies, followed by neighboring farms affecting other companies.

Monitoring HPR0 ISAV in marine phase salmon production highlighted possible transmission patterns within the Chilean aquaculture industry and identified higher-risk areas associated with circulating orthomyxoviruses.

1. Introduction

In 2007, Chile reported its first case of infectious salmon anemia (ISA) in sea-cage reared Atlantic salmon (*Salmo salar*) (Godoy et al., 2008). This disease is caused by ISA virus (ISAV), an aquatic orthomyxovirus that was first isolated in Norway in 1984 (Thorud and Djupvik, 1988) and has since been reported in Canada, Scotland, the Faroe Islands, and the United States of America (OIE, 2013). This viral disease is an OIE-listed pathogen, which causes high mortality in Atlantic salmon and subclinical infections in rainbow trout (Olsen et al., 2012). Several variants of ISAV have been identified and these are distinguished by their highly-polymorphic region (HPR) of the haemagglutinin-esterase (HE) gene (Mjaaland et al., 2002). Although most variants cause clinical disease in Atlantic salmon, there is one variant type that only causes transient (subclinical) gill infection, and is not associated with mortality in this species of fish (Christiansen et al., 2011; Lyngstad et al., 2011). This virus is referred to as HPR0 ISAV because it consists of the full length HPR of the HE gene (Cunningham et al., 2002; Mjaaland et al., 2002).

Several management strategies were implemented in 2007 (Resolution 1670, SERNAPESCA 2007) to control the spread of ISAV within the Chilean industry, including new regulations on marine and fresh water management practices, fallowing, processing, restricted fish movements, and equipment disinfection (SalmonChile, personal communication). By 2010, Chile was reporting very few cases of ISAV (Godoy et al., 2013; Kibenge et al., 2012), but like other countries that have experienced ISA outbreaks, Chile occasionally detects the non-pathogenic variant of ISAV in both their fresh and saltwater Atlantic salmon farms (Kibenge et al., 2012).

Surveys in the Faroe Islands and Norway suggest that HPR0 ISAV is present at a relatively high prevalence in individual fish and farms (Christiansen et al., 2011; Lyngstad et al., 2011, 2012). The presence of HPR0 ISAV is also reported in Scotland and Canada (Cook-Versloot et al., 2004; McBeath et al., 2009). Molecular analyses of variants of ISAV in Norway and Chile (Godoy et al., 2013; Lyngstad et al., 2011, 2012; Markussen et al., 2008) support the hypothesis that virulent forms of ISAV sporadically emerge from HPR0 ISAV (Mjaaland et al., 2002). Due to this, it is important to describe and understand the distribution of HPR0 ISAV and, although not an objective of this study, identify risk factors that may be associated with the suggested emergence of pathogenic HPR deleted ISAV from HPR0 ISAV (EFSA AHAW, 2012).

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Surveillance of HPR0 ISAV has been problematic in that infected fish show no clinical signs and do not succumb to the infection, making it difficult for producers to identify an infection, thus requiring frequent testing. Farmed Atlantic salmon (*S. salar*) are generally considered to be the susceptible host since natural disease outbreaks of ISA have only been reported in this species; however, experimental infections with ISAV have been described in other salmonids and non-salmonid fish (Rimstad et al., 2010). In contrast to the pathogenic ISAV, an HPR0 ISAV infected site will usually clear the pathogen within 4 months (range 2–9 months) of its emergence (Christiansen et al., 2011). The Chilean salmon industry has been including gill samples from Atlantic salmon in their routine ISAV monitoring program since November 2010, which most likely increases the probability to detect HPR0 ISAV in their marine aquaculture industry. The objective of this study was to describe, using surveillance data routinely collected by the aquaculture industry, the occurrence of HPR0 ISAV and identify high-risk HPR0 ISAV areas in Chile between May 2011 and October 2012 in Atlantic salmon.

2. Materials and methods

A retrospective study of Atlantic salmon farms in Chile between May 2011 and October 2012 was carried out to determine high-risk areas for HPR0 ISAV. Several maps were created to show the results, and an overview of the analytical steps was to: 1) generate a risk map describing the spatial heterogeneity in HPR0 ISAV prevalence, 2) run a purely spatial cluster analysis to reveal areas with higher than expected prevalences of HPR0 ISAV for the surveillance period of 18 months, which was used to inform the 3) exploratory relative risk map, comparing spatial risk to the regional expected risk, and 4) detect high-risk clusters of HPR0 ISAV over six 3-month periods during the study, by using two alternate methods to calculate distances between farms (Euclidian and seaway), using geo-coordinates as reported by in the surveillance database.

2.1. Data

We assessed farm data for fish species (Atlantic salmon, *S. salar*; coho salmon, *Oncorhynchus kisutch*; rainbow trout, *Oncorhynchus mykiss*), location, company, and HPR0 ISAV status (determined from PCR result), between May 2011 and October 2012. Data were provided by the Instituto Tecnológico del Salmón (INTESAL), the research institution that belongs to the biggest salmon grower association in Chile, SalmonChile. Farm-level data during the study period were obtained from the industry-based sea lice monitoring program which requires all active farms to report sea lice information on a weekly basis. INTESAL obtained test results for HPR0 ISAV from the publically available results of the National Services for Fish and Aquaculture (SERNAPESCA, Servicio Nacional de Pesca y Acuicultura) ISAV surveillance program. Under the ISAV surveillance program, all active marine farms in Chile, meaning any farm site with fish in sea-cages during any period of the study, are required to submit 30 freshly dead (less than 12 h) or moribund fish from cages with the highest mortalities. Each active farm must submit samples every three months (or sooner given prior ISAV test result) for PCR testing for ISAV, as per regulations set by SERNAPESCA (Resolution 1577 of 2011; www.sernapesca.cl). Only farms with Atlantic salmon were included in the disease distribution maps and space–time analyses, because they are considered to be the only fish species susceptible to HPR0 ISAV (EFSA AHAW, 2012).

Samples of gills, heart, and kidney were screened for ISAV using real-time RT-PCR with TaqMan® probe and primers targeting ISAV gene segment 8, as described by Snow et al. (2006). All real-time RT-PCR positive samples were further genetically sequenced by RT-PCR products obtained using segment 6 (HPR) primers, as previously described (Cunningham et al., 2002; Godoy et al., 2013; Kibenge et al., 2009; Lyngstad et al., 2011), to identify the non-pathogenic HPR0 ISAV strain. Diagnostic sensitivities and specificities were not available for this diagnostic test; therefore all prevalence and risk estimates are based on apparent values.

2.2. Statistical analyses

Data handling and descriptive statistics were performed using Stata 11 (Stata, 2009), while spatial data management and mapping were conducted in QGIS (QGIS Development Team, 2009). Kernel density intensities were generated for farms stocking Atlantic salmon and for HPR0 ISAV positive farms, using the 'spatstat' package version 1.31-0 (A. and Turner, 2005) in R (R Development Core Team, 2011) and implemented within the SEXTANTE plugin (Olaya, 2013) in QGIS. These kernel density intensities used isotropic Gaussian kernel with the same fixed bandwidth, as determined from the coordinate ranges from the farms stocking Atlantic salmon ($(1/8) \times \min(x_{range}, y_{range})$). Risk and relative risk maps were generated with the kernel density intensities, as described by Berke (2005), after identifying purely spatial clusters in SaTScan (Kulldorff, 1997). Briefly, risk maps were created by dividing the raster map estimates (derived from kernel density intensities) for the number of HPR0 ISAV positive farms per square kilometer by the raster map estimates for the number of salmon farms at risk per square kilometer; the end product estimates the number of HPR0 ISAV positive farms per 100 Atlantic salmon farm at risk per square kilometer. The relative risk maps were created by dividing the risk map raster estimates by the background risk, as defined by the overall regional prevalence from the population at risk outside significant high-risk areas; therefore the relative risk map estimates the spatial prevalence ratio.

Retrospective analyses for clustering of HPR0 ISAV in both space and time were performed using the Bernouilli model available in SaTScan (Kulldorff, 1997), with a maximum temporal window set at 90% (maximum available option) of the study periods (Kulldorff et al., 2006), allowing clusters to remain present over most of the study period. Both Euclidean (elliptical scanning window set at a maximum spatial cluster size of 20% of the population at risk, and was selected as the largest sample size with no change in the pattern of clustering) and seaway distances (organized as a neighborhood matrix) were included in the retrospective space and time analyses. The seaway distances were calculated with the 'gdistance' package, version 1.1-4 (van Etten, 2012) in R, to produce a large matrix of seaway distances between each farm; the data were re-organized into a neighborhood matrix (Kulldorff, 2009), where farms were sorted on their seaway distances from one another. Farm sites were stratified by geographical regions (X, XI, and XII; see Fig. 1) due to their relative spatial isolation (i.e. >80 km separation; pathogens are unlikely to spread over such large distances in Chile (Kristoffersen et al., 2013; Rees et al., 2014). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Description of the surveillance data

Weekly surveillance data for HPR0 ISAV were available from May 2011 to October 2012, and were aggregated into six 3-month periods, based on the sampling schedule for the ISAV surveillance program; therefore each active farm site was sampled once per period: (1) May 1–July 31, 2011; (2) August 1–October 31, 2011; (3) November 1–January 31, 2012; (4) February 1–April 30, 2012; (5) May 1–July 31, 2012; and (6) August 1–October 31, 2012. Not all farms contributed to all six 3-month periods, due to harvesting and fallowing; nevertheless the vast majority of farms (80%) contributed to a minimum of 3 periods; in total, there were 43, 55, 104, 112, 111, and 63 individual farms that contributed from 1 to 6 periods, respectively. Overall, the number of active sites participating in the surveillance program

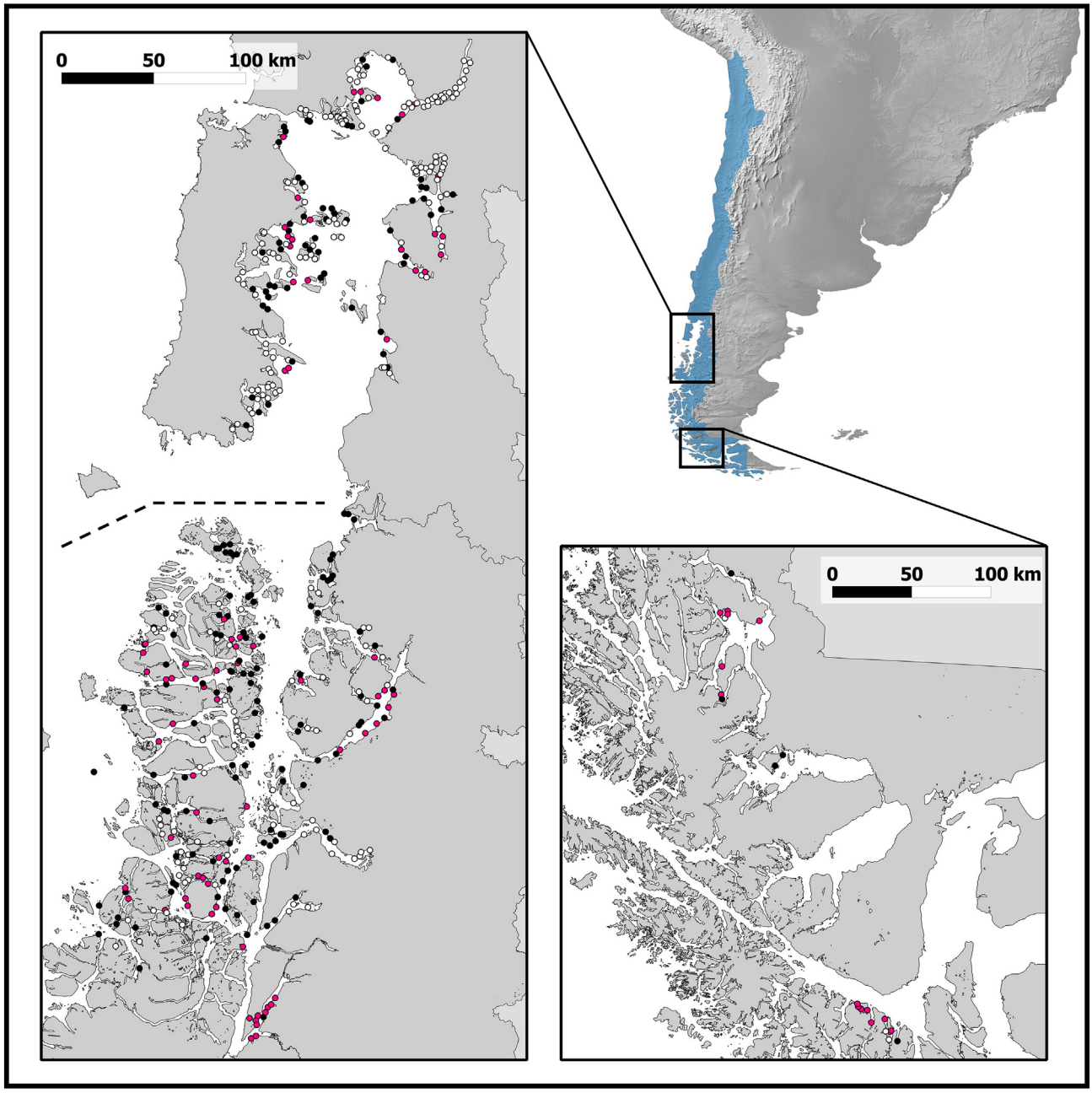

Fig. 1. Aquaculture farm sites in regions X (top left, separated by dashed-line), XI (bottom left, separated by dashed-line), and XII (bottom right) along the coastline of Chile between May 2011 and October 2012. Pink dots represent Atlantic salmon (*Salmo salar*) farms that tested positive for HPR0 ISAV, black dots represent those farm sites that stocked Atlantic salmon, and white dots represent those farms that stocked either coho salmon (*Oncorhynchus kisutch*) or rainbow trout (*Oncorhynchus mykiss*) during the study period.

increased over time, with 250 sites in the first time period and a maximum of 344 sites for the last time period. Table 1 summarizes the number of HPR0 ISAV positive farms from the number of active farms over the six time periods, between the three geographical regions.

The proportion of HPR0 ISAV, for susceptible farm sites (farms stocking Atlantic salmon) during the entire study, in region X was similar to region XI (8.3%, and 8.4%, respectively), while it was twice as high for region XII (18.9%, Table 1). Although the overall proportions were relatively similar for both northern regions (X and XI), there was a large variation in 3-month period proportions between them (Table 1). For example, the 3-month period prevalence of HRP0 ISAV between November 2011 and January 2012 was only 3.4% in region X, while it was 8.1% in region XI; in contrast, the following time period (February and April 2012) observed a prevalence of 15.7% in region X, while it was only 5.5% in region XI. The overall prevalence of HPR0 ISAV for all farm sites (including non-susceptible farms) was greater in region XI compared with region X (6 [95% CI 4 to 8] cases per 100 farms at risk versus 3 [95% CI 2 to 5], respectively), which reflects the differences in proportions of susceptible to non-susceptible fish species. Farms stocking Atlantic salmon represented 38.8% of the farms in region X, and 71.4% in region XI.

Region XII was excluded from further spatial and spatio-temporal analyses due to sparse data; however, the proportion of HPR0 ISAV farms was very high for the active farm sites in this region (18.9%), with the majority of sites stocking Atlantic salmon (82.2%).

Table 1
Summary of all active farms during the study period which stocked either Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), or rainbow trout (*Oncorhynchus mykiss*), by time period (1–6) and geographical region (X, XI, or XII). Proportions (%) of HPR0 ISAV positive farms (+) from those farms stocking Atlantic salmon (n) are also summarized by time period (1–6) and geographical region (X, XI, or XII).

	All farms (n)				Farms with Atlantic salmon (+/n)			
Time period	All regions	Region X	Region XI	Region XII	All regions	Region X	Region XI	Region XII
1	250	116	121	13	33/130	6/35	20/84	7/11
May–July 2011					(25.4%)	(17.1%)	(23.8%)	(63.6%)
2	291	134	143	14	15/158	3/50	10/97	2/11
Aug.–Oct. 2011					(9.5%)	(6.0%)	(10.3%)	(18.2%)
3	311	136	160	15	11/181	2/59	9/111	0/11
Nov.–Jan. 2012					(6.1%)	(3.4%)	(8.1%)	(0%)
4	315	128	170	17	15/189	8/51	7/124	0/14
Feb.–April 2012					(7.9%)	(15.7%)	(5.6%)	(0%)
5	335	137	181	17	17/197	6/52	9/131	2/14
May–July 2012					(8.6%)	(11.5%)	(6.9%)	(14.3%)
6	344	154	176	14	6/210	1/65	2/132	3/13
Aug.–Oct. 2012					(2.9%)	(1.5%)	(1.5%)	(23.1%)
Total	1846	805	951	90	97/1,065	26/312	57/679	14/74
					(9.1%)	(8.3%)	(8.4%)	(18.9%)

3.2. Risk, spatial clustering, and relative risk mapping for HPR0 ISAV positive sites

The risk, spatial cluster detection, and relative risk maps for HPR0 ISAV positive sites in region X and XI, during the period of May 2011 and October 2012, are shown in Fig. 2. The cluster analysis (purely spatial with no temporal component), performed for each region, demonstrated one marginally non-significant cluster in the eastern part of region X ($P = 0.134$), and one significant cluster in the southern part of region XI ($P = 0.006$).

Region X had 24 positive sites out of the 83 active farm sites (28.9%) across all 6 time periods with Atlantic salmon, and there was some indication of spatial clustering, although not significant, that included 5 positive sites. Since the cluster was not significant, the calculations for the relative risk map included all susceptible farms ($n = 83$), thus giving the same average background risk of 28.9% (24/83) as the regional prevalence. In this region (X), there were two farms with at least 2 time periods with HPR0 ISAV infections, of which only one farm tested positive for 2 consecutive 3-month periods.

Region XI had 55 positive sites out of the 170 farm sites (32.4%) with Atlantic salmon, and the significant cluster included 15 positive sites out of the 17 sites present (88.2%), thus giving an average background risk of 26.1% ([(55–15)/(170–17)]; (Berke, 2005)). Similarly to region X, region XI had two farms that tested positive for at least 2 time periods with HPR0 ISAV, of which one farm was positive for 2 consecutive time periods.

3.3. Spatiotemporal clustering and seasonal variation of HPR0 ISAV positive sites

The spatial clustering of HPR0 ISAV infected sites changed over time (Fig. 3), where the areas with higher risk of infection started in May 2011 in three areas—two in region XI, and one non-significant ($P = 0.120$) area in region X. The large cluster of infected farms in the southern area of region XI persisted for approximately one year (May 2011 until April 2012), while the other two clusters disappeared within 3 months. One very small, non-significant ($P = 0.091$), and short-lived cluster was present in the western area of region X, between February 2012 and April 2012, which included 4 farms. After accounting for seaway distances, the very small and short-lived non-significant cluster in the western area of region X was also present, but was less significant (not shown on map, $P = 0.118$) and included only three farms. The non-significant cluster in the eastern area of region X that included two large estuaries disappeared completely after accounting for seaway distances. Both significant clusters in region XI were similar spatiotemporally after accounting for seaway distances (shown in Fig. 3), and both remained highly significant (most southerly, $P = 0.005$; most easterly, $P < 0.001$).

The risk for a site to be HPR0 ISAV positive was significantly associated with season, as defined by the closely matched time periods (Winter, May–July; Spring, August–October; Summer, November–January; Autumn, February–April), where sites were 2.40 times more likely to be positive for HPR0 ISAV in winter (May–July) than any other season ($P < 0.001$, Chi^2 test; 95% CI, 1.65–3.50).

4. Discussion

HPR0 ISAV occurred frequently during the study period, with overall farm-level prevalences between 3 and 25% and an average of 9% within aggregated 3-month periods in Chile (Table 1, and Fig. 2), which is consistent with other countries reporting HPR0 ISAV (Christiansen et al., 2011; Lyngstad et al., 2012; McBeath et al., 2009). In Scotland, HPR0 ISAV was detected in 4 of 36 (11%) Atlantic salmon sites (McBeath et al., 2009). In Norway, ISAV was detected in 23% of fish groups (tested only once), where more than half of these were confirmed HPR0 and due to the absence of clinical ISA, they were all assumed to be HPR0 (Lyngstad et al., 2012). In the Faroe Islands, the annual fish-level prevalence of HPR0 ISAV from gill samples varied between 9.6 and 15.1% over a 3-year period, with an overall fish-level prevalence of 11.9% (Christiansen et al., 2011). However, Christiansen et al. (2011) also reported that 100% of fish cohorts experienced HPR0 infection in the Faroe Islands during the marine phase of production, suggesting that regional prevalence estimates are likely dependent on the farm-level sampling frequency and protocol, which may be a reflection of the surveillance program sensitivity. Additionally, our observation of seasonally differing prevalences was consistent with previous reports in Chile and in the Faroe Islands (Christiansen et al., 2011; Godoy et al., 2013).

The spatiotemporal analysis detected two significant (and two marginally non-significant) HPR0 ISAV clusters within the aquaculture industry in Chile between May 2011 and April 2012. The finding that HPRO ISAV clusters in space and time suggests that with the given distribution of farms during the surveillance period, the risk of HPR0 ISAV was higher in these areas than other locations in the study. Pathogen transmission through water is a biologically plausible explanation for spatiotemporal clustering of HPR0 ISAV. Transmission through short seaway distances is supported by a Norwegian study suggesting that there was a higher probability of having local transmission of HPR0 ISAV compared to transmission over longer distances (Lyngstad et al., 2012). The prevalence of HPR0 ISAV within an infected farm is typically high (Christiansen et al., 2011) which likely generates a large pathogen load that can be spread through water and onto other farms.

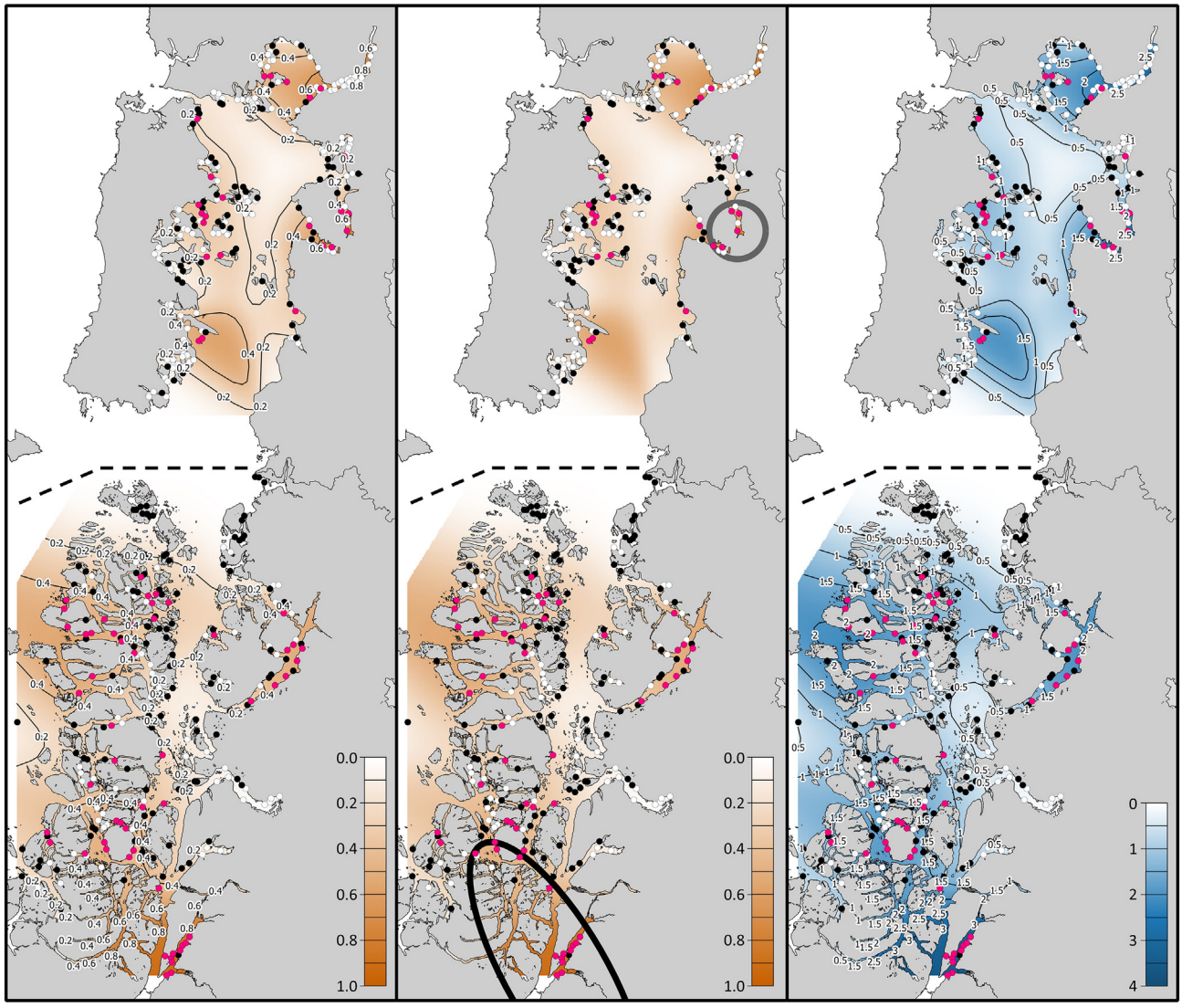

Fig. 2. Risk map (left), purely spatial clusters (center), and relative risk map (right) for HPR0 ISAV positive sites in regions X (top) and XI (bottom; separated by dashed lines) between May 2011 and October 2012. Pink dots represent Atlantic salmon (*Salmo salar*) farms that tested positive for HPR0 ISAV, black dots represent those farm sites that stocked Atlantic salmon, and white dots represent those farms that stocked either coho salmon (*Oncorhynchus kisutch*) or rainbow trout (*Oncorhynchus mykiss*) during the study period. The color gradients for the risk and relative risk maps are linearly proportional to their respective values, as indicated by contour lines. The light grey circle demarcates a non-significant cluster ($P = 0.134$), while the black ellipse demarcates a significant cluster ($P = 0.006$).

The population of wild salmon is assumed to be limited in Chile; therefore, it is likely that horizontal transmission comes from virus originating in farmed fish and shed in the marine environment.

We were able to trace infected farms to individual companies within clusters, and found that one company had the majority of infections within each cluster for the first time period, and if the infection persisted within a cluster, the proportion changed to include other companies. In Chile, many companies buy smolts from different freshwater operations, and HPR0 ISAV has been previously detected by PCR in Atlantic salmon in the freshwater phase showing no clinical signs of disease (Vike et al., 2009; SalmonChile, personal communication); similarly, HPR0 ISAV has been detected in freshwater operations (juvenile salmon not exposed to marine environment) in Norway (Lyngstad et al., 2012) and the Faroe Islands (Christiansen and Østergaard, 2011). Regardless of the exact source of transmission, the role of companies has previously been identified as a contributing factor in the transmission of ISAV in Chile (Mardones et al., 2009, 2014), and future studies should continue to investigate the effect of companies and commercial compartmentalization (Zepeda et al., 2008) in the aquaculture industry on disease transmission.

The spatiotemporal clustering we found in this study corroborates with findings from other density-dependent infectious disease models in Chile, suggesting that farm-to-farm spread of pathogen occurs within the industry (Kristoffersen et al., 2013), and also corroborates with findings from other ISAV studies supporting horizontal transmission (Aldrin et al., 2010, 2011; Lyngstad et al., 2008, 2011; Mardones et al., 2009; Scheel et al., 2007).

One limitation to this study was that the surveillance program tested fish on farms approximately every 3 months, so it is possible that some cases were not detected due to the transient nature of the subclinical infection with HPR0 ISAV. Christiansen et al. (2011) found that HPR0 ISAV infections within farm sites peaked after 2 months (range 1–5 months) and were no longer detectable in populations after an additional 2 months (range 1–4 months), meaning that the overall time interval to detect an infection on a farm was approximately 4 months (range 2–9 months)—which is greater than our 3-month surveillance interval. We only had 3 farms that tested positive for two consecutive 3-month periods during the course of our surveillance period, out of the 97 positive results, indicating that most of the on-farm HPR0 ISAV detections probably lasted less than 3 months. The aggregated

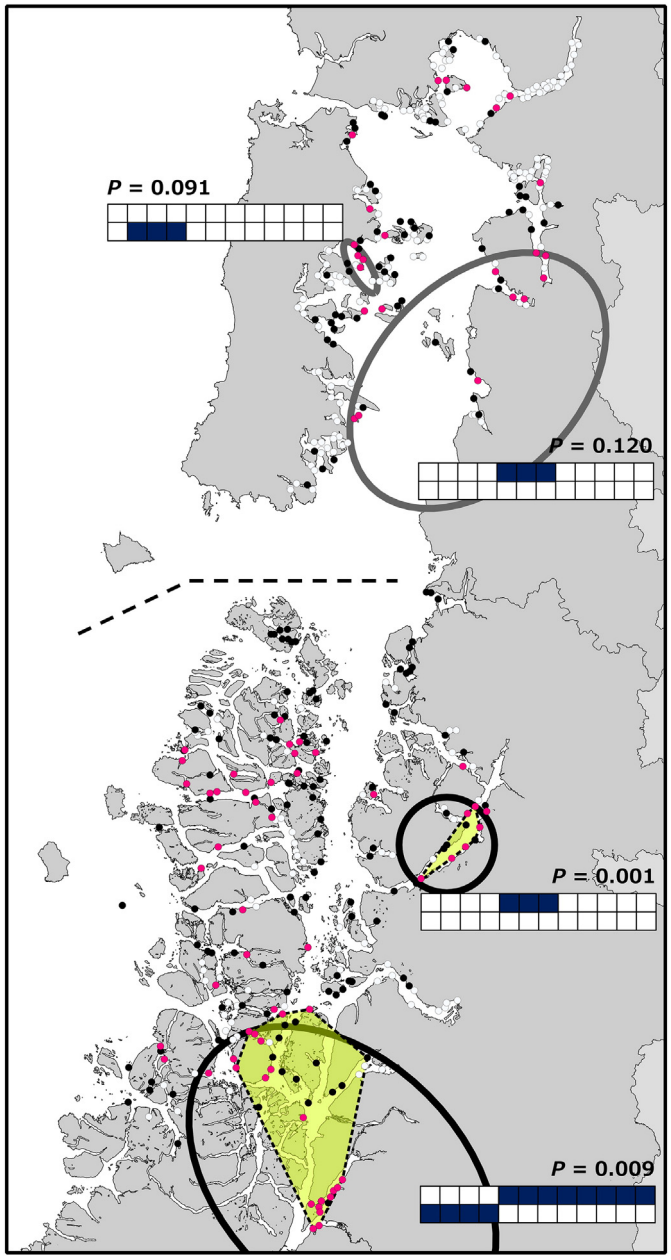

Fig. 3. Spatiotemporal clustering of HPR0 ISAV positive Atlantic salmon (*Salmo salar*) farm sites for region X (top) and region XI (bottom; separated by dashed lines) between May 2011 and October 2012. The clustered farms are delineated by ellipses for Euclidean distance calculations (black line includes significant clusters, and grey line includes marginally non-significant clusters), and by yellow shaded areas (significant clusters) for seaway distance calculations. The temporal component of the clusters is represented by the 12 × 2 grid, where each row indicates one year (top = 2011, bottom = 2012) and each cell represents one month; for example, the bottom cluster started in May 2011 and ended in April 2012. Pink dots represent farms that tested positive for HPR0 ISAV, black dots represent those farm sites that stocked Atlantic salmon, and white dots represent those farms that stocked either coho salmon (*Oncorhynchus kisutch*) or rainbow trout (*Oncorhynchus mykiss*) during the study period.

3-month period was selected to reflect the surveillance sampling scheme, and given the few number of farms that were positive for HPR0 ISAV for 2 consecutive time periods, it would have been better to reduce the sampling time period for HPR0 ISAV testing. We speculate that a more intensive sampling regimen with shorter time periods would probably increase the observed prevalence of HPR0 ISAV, as reported in the Faroe Islands (Christiansen et al., 2011), and better reflect the circulation patterns of the virus.

In April 2013, two outbreaks of ISA occurred in close proximity to each other, in the northwestern area of region XI. Based on genetic sequencing from the ISA outbreak isolates and from the surveillance program HPR0 ISAV isolates, Godoy et al. (2013) found evidence that ISA may be directly linked to the presence of HPR0 ISAV in Chile, as was previously theorized (EFSA AHAW, 2012). Although the authors found one large cluster in region X, using a permutation space–time statistic with HPR0 ISAV cases (reported between May 4, 2011 and April 22, 2013), this was not the location where the recent ISA outbreak occurred. In contrast to using only HPR0 ISAV case farms, our study included all susceptible farms that were present during the surveillance period, aggregated within 3-month periods to reflect the ISAV surveillance program sampling regimen, allowed for elliptical scanning windows, and assessed both Euclidean and seaway distances. With the added information from susceptible farms, allowing for risk and relative risk estimates, we determined that the two recent ISA outbreak sites were located in areas with relatively high HPR0 ISAV prevalences (52 and 34%, respectively) and with estimated relative risks of 2.01 and 1.29, respectively. Although the two farms with ISA were not located within significant space–time clusters of HPR0 ISAV in our analyses, they were located within a marginally non-significant cluster (Euclidean distance $P = 0.193$, seaway distance $P = 0.163$; not shown on map).

The ISAV surveillance program is valuable in gaining knowledge about the distribution of HPR0 ISAV, and can therefore be used to identify risk factors associated with the circulation of HPR0 ISAV. For example, we observed higher prevalences of HPR0 ISAV in several estuaries, where farms are more likely to be hydrodynamically connected due to tidal patterns and fresh water run-off. An important distinction to make is that these risk factors and higher risk areas to HPR0 ISAV are not necessarily going to identify risk factors driving the transition from HPR0 ISAV to the virulent form of ISAV; however, they may be useful in finding new cases of virulent ISAV if both pathogenic and non-pathogenic forms of ISAV share common risk factors.

It is reasonable to assume that seaway distance better reflects disease transmission patterns in water than Euclidian distance. For the most part, results from both Euclidean and seaway distance models were similar, with the exception of two estuaries in the eastern area of region X, where the largest differences between the two methods of measurements were observed. This was not surprising considering the large seaway distance (approximately 100 km from the two most proximal farms) with a relatively short Euclidean distance between the two estuaries (less than 13 km from the two most proximal farms). In the future, hydrodynamic information could be used to inform the neighborhood matrix, rather than seaway distances. For the moment, hydrodynamic models are scarce in Chile; however, this information would better reflect the connectivity between farms and improve our estimates of disease risk transmission in coastal waters.

The vast majority of HPR0 ISAV detections do not evolve into virulent ISAV; therefore the ISA surveillance programs should continue to target clinical fish (dead and moribund), and focus on management risk factors, such as high density production areas, shared management, mixed generations of fish in an area, etc. Nevertheless, there is epidemiological merit in monitoring HPR0 ISAV infections in marine and freshwater operations to better understand transmission patterns and risk factors associated with circulating orthomyxoviruses, be it pathogenic or non-pathogenic. Additionally, the use of gene sequence data on all HPR0 ISAV isolates from the current surveillance program would improve the understanding of ISAV evolution and transmission, and may help determine driving forces in the suggested transition from HPR0 to pathogenic ISAV.

5. Conclusion

Based on the ISAV surveillance program in Chile, we were able to estimate the risk and relative risk of HPR0 ISAV on Atlantic salmon

farms in Chile in time and space. At the spatial and temporal resolution of the testing available, we demonstrate that HPR0 ISAV infections cluster in space and time, which supports the hypothesis that this pathogen is spread by water.

Further, if the non-pathogenic HPR0 strain of ISAV has a risk of mutating to a virulent form of the virus, it may be useful to identify areas where it is prevalent to increase surveillance and the probability of early detection of virulent forms of the virus. The earlier the virulent form of this virus is detected on a farm, the earlier control measures can be implemented, and the less likely it will spread to other farms.

We recognize that risk factors and higher risk areas to HPR0 ISAV do not necessarily identify risk factors driving the suggested transition from HPR0 ISAV to the pathogenic form of ISAV; however, they may be useful in finding new cases of virulent ISAV if both pathogenic and non-pathogenic forms of ISAV share common risk factors.

Conflict of interest

There were no known conflicts of interest.

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