**Area contact networks and the spatio-temporal spread of infectious salmon
anemia virus (ISAV) in Chile**

L. Gustafson^{1*}, M. Remmenga¹, O. Sandoval del Valle², R. Ibarra³, M. Antognoli¹, A. Gallardo²,
C. Rosenfeld⁴, J. Doddiss⁴, R. Enriquez Sais⁵, E. Bell⁶, M. Lara Fica²

¹USDA APHIS VS Centers for Epidemiology and Animal Health, Surveillance Design and Analysis, 2150 Centre
Ave, Fort Collins, CO, 80526-8117, United States

²Sernapesca, Chile's National Fisheries and Aquaculture Service, Victoria 2832, Valparaiso, Chile,

³SalmonChile, Department of Fish Health, Instituto Tecnológico del Salmon, Av. Juan Soler Manfredini 41, OF
1802, Puerto Montt, Chile

⁴Instituto de Medicina Preventiva Veterinaria Facultad de Ciencias Veterinarias Universidad Austral de Chile
Campus Isla Teja s/n, Valdivia, Chile.

⁵Lab de Biotecnología y Patología Acuática Facultad de Ciencias Veterinarias Universidad Austral de Chile
Campus Isla Teja s/n, Valdivia, Chile

⁶Colorado Department of Public Health and Environment, 4300 Cherry Creek Drive South, Denver, CO 80246,
United States

* Corresponding author: lori.l.gustafson@aphis.usda.gov; +1 970 494 7297 (phone); +1 970 494 7174 (fax)

Abstract

Area management, the coordination of production and biosecurity practices across neighboring farms, is an important disease control strategy in aquaculture. Area management in aquaculture escalated in prominence in response to outbreaks of infectious salmon anemia (ISA) internationally. Successes in disease control have been attributed to the separation achieved through area-level synchronized stocking, fallowing, movement restrictions, and fomite or pest control. Area management, however, is costly; often demanding extra biosecurity, lengthy or inconveniently timed fallows, and localization of equipment, personnel, and services. Yet, this higher-order organizational structure has received limited epidemiologic attention.

Chile's National Fisheries and Aquaculture Service instigated area management practices in response to the 2007 emergence of ISA virus (ISAV). Longitudinal data simultaneously collected allowed retrospective evaluation of the impact of component tenets on virus control. Spatiotemporal analyses identified hydrographic linkages, shared ports, and fish transfers from areas with recent occurrence of ISAV as the strongest predictors of virus spread between areas, though specifics varied by ISAV type (here categorized as HPR0 for the non-virulent genotypes, and HPRv otherwise). Hydrographic linkages were most predictive in the period before implementation of enhanced biosecurity and fallowing regulations, suggesting that viral load can impact spread dynamics. HPR0 arose late in the study period, so few HPRv events were available by which to explore the hypothesis of HPR0 as progenitor of outbreaks. However, spatiotemporal patterns in HPRv occurrence were predictive of subsequent patterns in HPR0 detection, suggesting a parallel, or dependent, means of spread. Better data precision, breadth and consistency, common challenges for retrospective studies, could improve model fit; and, for HPR0, specification of diagnostic test accuracy would improve interpretation.

46

47 **Key words**

48 Infectious salmon anaemia, spatiotemporal analysis, bay management, epidemiology, fish health

49

50 **Introduction**

51 The emergence of infectious salmon anemia virus (ISAV), first in Norway in 1984 (Thorud
52 and Djupvik, 1988), then North America, Scotland, Faroe Islands, and most recently Chile
53 (Godoy et al., 2008), precipitated a new era in marine salmon farming. ISA disease typically
54 presents as septicemia with various combinations of lethargy, anemia, exophthalmia, ascites and
55 hemorrhagic lesions in affected fish. ISAV's potential to cause severe mortality and impacts to
56 trade, and, ironically, its responsiveness to mitigations following early detection, advanced a
57 shift from site to regional organization of farming activities. Termed neighborhoods, bay
58 management areas, regions, or zones, we hereafter refer to geographic clusters of marine farms
59 as areas, and strategies for their coordination as area management.

60 Through area management, farms in common waters operate with shared principles and
61 constraints and shared attention to preserving fish health and environmental quality in their
62 region. While much of this coordination is industry-led, responsible use of any common
63 resource is best managed under regulations that ensure consistent and fair practice. Thus,
64 governments may set and enforce standards for disease prevention, reporting, and response;
65 fostering natural 'breaks' in host exposure to pathogens through uniform fish density limits,
66 stocking dates, fallowing periods, traffic patterns, biosecurity, or integrated pest management

67 routines, for example. On the whole, regional coordination of disease response has been
68 successful, and credited with the reduction or resolution of ISA disease within years of its
69 emergence (Ellis et al., 2006; Murray et al., 2010; Christiansen et al., 2011; Alvia et al., 2012).
70 As such, area management appears a mainstay of marine salmonid farming in many locations
71 (Chang et al., 2007; CoGP Management Group, 2010; USDA APHIS et al., 2010; Rimstad,
72 2011; Kristoffersen et al., 2013).

73 ISAV control, however, is complicated by the need for continued vigilance given concern
74 about whether non-clinical variants (HPR0) could be progenitors or harbingers of future
75 outbreaks, versus sequelae or corollaries to outbreak strains (Kibenge et al., 2007; EFSA, 2012;
76 Kibenge et al., 2012). Termed HPR0, referring to a non-deleted highly polymorphic region
77 (HPR) of segment 6 of the ISAV genome, these non-clinical variants (also typically
78 characterized by an insertion in segment 5) are not implicated in ISA pathology and not
79 cultivable on cell culture (Kibenge et al., 2009; Godoy et al., 2013), but are considered possible
80 evolutionary progenitors of ISAV outbreak variants (HPRv). Often first observed during
81 resolution of an outbreak, HPR0 typically persists, and at times is quite prevalent (Kibenge et al.,
82 2009; Christiansen et al, 2011; Lyngstad et al., 2011; Vanderstichel et al., 2015), even after
83 apparent extirpation of HPRv. Consequently, though area management is generally successful in
84 the control of ISA, complete eradication of ISAV including HPR0 has proven difficult. Further,
85 re-emergence of ISA, though at a much reduced level, sometimes follows resolution of an initial
86 epidemic (Godoy et al., 2013). Whether explained by genetic shifts from non-virulent (HPR0)
87 variants, unidentified reservoirs, un-mitigated pathways of introduction, or some combination,
88 remains undetermined and signifies the need for research at broader scales.

89 This tenuous success of area management is also hard-earned. Single year-class farming,
90 synchronized fallows, and restrictions on activities involving multiple bays are costly and
91 laborious. Sound practices demand dedicated personnel, equipment and vessels, shared
92 information, and creative solutions to ensure continuous market availability of an otherwise
93 punctuated supply of product. And, improperly defined or enforced management areas incur
94 these costs without benefits of real protection. Consequently, studies to discern the relevance
95 and proper functioning of components of area management are crucial. A number of novel
96 epidemiologic studies examine disease risk on and between farms (Scheel et al., 2007; Lyngstad
97 et al., 2011; Mardones et al., 2014; Rees et al., 2014; Rosenfeld et al., 2015). Yet, field data to
98 explore the efficacy of mitigations on pathogen spread between areas are limited in scope or
99 baseline, often obstructed by missing data for key variables (e.g., records of transits between
100 areas), lack of statistical ‘controls’ (e.g., areas absent a particular mitigation), or time required
101 for retrospective analyses. Expert elicitations (Gustafson et al., 2014) or simulations (Salama
102 and Rabe, 2013) have been used for decision support. However, evaluation of field data, when
103 those data become available, remains an important standard.

104 Empirical data collected by Chile’s National Fisheries and Aquaculture Service (Sernapesca),
105 with local knowledge of area connectivity, afforded the unique opportunity to examine ISAV
106 control at an area management scale. The objective of this longitudinal spatiotemporal study
107 was to assess the predictive impact of contact networks on observed patterns of ISAV spread
108 between marine areas in Chile.

110 **Methods**

2.1 Study Region and Period

The emergence of ISAV (Genotype I) in Chile was confirmed and declared by Sernapesca in July 2007 based on results of investigations initiated June 2007 (Godoy et al., 2008). This study covered all areas farming Atlantic salmon (*Salmo salar*) between June 2007 and December 2012 in Chile's marine Regions de los Lagos and Aysén (Figures 1 and 2;

http://www.sernapesca.cl/index.php?option=com_content&task=view&id=128&Itemid=293).

This period covers the emergence of ISAV in Chile (Godoy et al., 2008), as well as a period of temporary resolution of ISA disease dominated instead by detections of a non-virulent ISAV variant termed HPR0 (Godoy et al., 2013). Sanitary regulations for aquaculture in Chile (RESA D.S. No.319-01), updated in March 2009 (e.g., mandating a rolling implementation of enhanced biosecurity and synchronized fallowing), also evolved throughout the study period (Sernapesca, 2001). Thus, early in the study, geography and site-level biosecurity were key facets supporting area separation; later in the study, enhanced biosecurity standards improved their distinction.

We analyzed HPRv data over the full time period. However, the 2009 revisions likely instigated a gradual reduction in circulating levels of virus in the environment, and consequent exposure pressures. Thus, we also analyzed the HPRv dataset piecemeal for the periods preceding- versus starting- April 2009, with the goal to identify whether pathways changed in prominence following enhanced mitigations. In contrast, because the first designation of HPR0 occurred after March 2009, the HPR0 evaluation comprises only a single segment of the study period. In all cases, we analyzed predictors of ISAV HPRv and ISAV HPR0 occurrence separately. Thus, we examined the dataset in 4 ways: (1) HPRv over the full period (months 1-67), (2) HPRv pre-2009-regulations (months 1-22), (3) HPRv post-2009-regulations (months 23-

67), and (4) HPR0 over a final segment of the study period (months 33-67). March 2009 corresponds to month 22 of the study.

All active Atlantic salmon (ATS) farming sites in the study regions and period were included, where active was defined as 4 or more months of continuous operation. Marine sites typically hold fish from sea transfer as smolts through to harvest; although some estuary sites rear smolts prior to transfer to higher salinity locations. A typical ‘production cycle’ for ATS in the seawater stage is 21 months in duration, followed by a 3 month fallow. We considered a cohort of fish to be those of a single site and production cycle. The term ‘site’ is used to describe an individual marine farm rearing ATS; the term ‘area’ refers to a collection of neighboring sites distinguished from other areas by location and contact networks. Study area delineations match those currently employed by Sernapesca, described locally as barrios. Lastly, we used the term ‘ISAV incidence’ to describe counts of ATS cohorts (sites/production cycles) newly meeting Sernapesca’s ISAV case definitions (ISAV designations) in a given month and area. Site names, ownership and address were withheld or coded by Sernapesca to protect confidentiality.

2.2 ISAV Surveillance and Constraints

Routine surveillance for ISAV followed Chile’s federal program standards (http://www.sernapesca.cl/index.php?option=com_remository&Itemid=246&func=fileinfo&id=7438). Marine sites were visited monthly, or more frequently, by licensed veterinarians under private or federal (Sernapesca) affiliation. At each visit a minimum of 30 fish were collected, selected from at least 3 cages. Tissue pools from 1-3 fish of kidney, heart, and (starting July 2008) gill tissues were submitted to official laboratories in Chile for diagnostic testing using real-

time reverse-transcription-polymerase chain reaction (RT-qPCR) and confirmatory sequencing. All laboratories employed TaqMan probes (Snow et al., 2006) for RT-qPCR starting December 2008 (a Sybr green probe was used in some laboratories prior). Moribund fish or fresh mortalities were targeted when possible.

As noted earlier, this study covers a dynamic period encompassing broad changes in the ISAV landscape, from detection, response, and resolution of the initial ISAV clinical outbreaks to the appearance and apparent ubiquity of an HPR0 variant. Thus, surveillance program standards also evolved, including a 2011 revision of Sernapesca's criteria for the designation of ISAV cases (see below). To address this natural drift in detection capacity, we applied or acknowledge the following rules and constraints. (1) The outcome variables estimate apparent rather than actual ISAV occurrence, as counts were not adjusted for system or diagnostic test sensitivity and specificity. (2) We used Sernapesca's 2011 revised definitions to standardize ISAV designations throughout. (3) Except in the case of cross-variant prediction (specifically, HPRv as a predictor of HPR0), we evaluate only short-term lags (≤ 5 months) and minimize comparisons over broad time intervals. And, (4) we report incidence of new ISAV designations as *counts*, rather than *rates*, as the rate denominator (number of susceptible cohorts) was only indirectly tracked during the study period. In summary, this study examines temporal relationships between apparent ISAV incidence in marine areas and their contact networks, where incidence measures counts of cohorts newly meeting Sernapesca 2011 ISAV case definitions (designations) in a given month.

2.3 Study Variables

2.3.1 Outcome Variables (ISAV Incidence)

New ISAV designations were tallied by area, month, and type (HPR0 or HPRv). ISAV HPR0 refers to ISAV genotypes considered non-virulent, absent any deletions in the HPR of segment 6. ISAV HPRv refers to any ISAV genotype containing deletions in the HPR of segment 6. While sequencing more of the genome would help differentiate higher-order degrees of relatedness within or across these groupings (Nylund et al., 2003; Devold et al., 2006; Kibenge et al., 2009; Godoy et al., 2013), full sequencing data were unavailable for most designations. Consequently, phylogenetics were not further explored in this study.

ISAV designations were based on Sernapesca's case definitions for ISAV HPR0 and ISAV HPRv. ISAV designations were applied to sites where two or more fish (collected 48 days or less apart) from any one or more cages with samples screening positive by RT-qPCR also tested positive by sequencing; or (especially prior to 2011 when sequencing was less common) if RT-qPCR results were corroborated by mortality, clinical or pathological signs consistent with ISA. Sernapesca ascribed ISAV designations only to initial findings of HPR0 and HPRv in a given site and production cycle. HPRv designations led to depopulation of affected net-pens, and sometimes the entire site. HPR0 designations led to increased frequency of surveillance. Cage-level (within site) data were not available for analysis. Consequently, outcome variables track initial ISAV findings in a given site and production cycle (cohort). However, a single site might accumulate multiple designations, each for a different cohort, over the course of the study.

ISAV HPRv incidence per area and month was the outcome variable for HPRv analyses; ISAV HPR0 incidence per area and month was the outcome variable for HPR0 analyses. ISAV incidence here describes the count of ISAV case designations newly ascribed in any given area

and month. As Sernapesca only ascribed designations to the first occurrence per cohort, this count is measured in cohorts. Harvest, stocking and depopulation dates by which to differentiate production cycles for non-affected sites were unavailable for design of a comparable rate denominator. Thus, our ISAV incidence variables capture the count (not rate) of new (rather than existing) ISAV occurrences in each time-space block. To address this deficit, we scripted a control variable (susceptible sites, see below) as a general proxy for area ISAV susceptibility. A control variable was included in all multiple regression models.

2.3.2 Control Variables

Control variables addressed variability in area susceptibility to ISAV infection. Though stocking and harvest dates were not available, Sernapesca did track ATS biomass, abundance, sea lice and transfer data through mandated monthly industry reports. ATS biomass here refers to units of million kg fish per 5,000 hectares (kg/hectare), and ATS abundance here refers to units of 100,000 fish per 5,000 hectare (fish/hectare), where five thousand hectares approximates the size of the smallest management area in Chile. However, reliability of biomass, abundance and sea lice reporting varied throughout the study period. Consequently, we created a simplified summary variable, ‘susceptible sites’, which tallied, by area, the number of ATS sites active (non-zero biomass) in a given month. Biomass records suggesting abbreviated occupancy (< 4 months in a row) were questioned and (1) discussed individually with Sernapesca staff, and (2) zeroed if confirmation of occupancy through other records was not possible. Overlap in several datasets provided the opportunity to check and resolve discrepancies. For example, if a site’s reported biomass was zero in a given month, but fish transfer records or sea lice data confirmed

site activity, and Sernapesca staff agreed, we labeled biomass ‘missing’ (rather than zero) and classified the site ‘susceptible’ for that period. Alternative species (e.g., *Oncorhynchus mykiss*, *O. kisutch*, or *O. tshawytscha*) were not included in these calculations.

2.3.3 Contact Networks between Areas

The predictive impact of ISAV incidence in marine contact networks on ISAV incidence in each area was analyzed through time. Contact networks for a given area were defined as constellations of areas with shared access to its ports, processing plants, or acopios (holding areas for live fish prior to processing), shared hydrographics, or sources for fish transfers. These five contact networks form the basis of our evaluation.

Hydrographic networks were estimated by Sernapesca and Intesal representatives in discussion with local subject matter experts. Water circulation is modeled for only limited portions of Chile’s coastline (Olivares et al., 2015). Connectivity was thus based on working knowledge of prevailing hydrographic patterns. Experts were asked to judge relative extent of connectivity between areas within a typical diurnal period. Specifically, they were asked to list, for each area, those areas they considered to be within 1 day reach in time, and ‘upstream’ per direction of prevailing currents. The same experts scored connectivity for all areas. Thus, decisions about how to gauge distance, time, or seasonal flux were applied with consistency.

Areas known or permitted by Sernapesca to use ports, processing plants, or acopios in a given (‘destination’) area were denoted its ‘suppliers’. Contact networks reflect allowed (rather than actual) usage patterns for ports, processing plants or acopios. Defined networks remained consistent throughout the study period, and thus did not capture volume or seasonality of use. In

contrast, dates and direction of transits moving fish between areas for further grow-out were individually tracked in industry reports to Sernapesca (RESA D.S. No. 319-01); thus, ‘fish transfers’ captures both frequency and seasonality of fish shipments. Transfer variables include movements of all farmed salmon or trout species (ATS, *Oncorhynchus mykiss*, *O. kisutch*, or *O. tshawytscha*), providing both origin and destination were marine study areas.

Though most network memberships were constant, their ISAV incidence was time-variant (evaluated monthly). Predictive variables were created for each destination area by summing ISAV incidence across its network suppliers, each month. For example, for a given destination area, the predictive variable ‘processing plant suppliers’ is the monthly sum of ISAV incidence across all areas authorized to use its processing plants. Similarly, ‘acopio suppliers’ sums ISAV incidence across areas authorized to use its acopios, ‘port users’ across areas authorized to use its ports, ‘neighbor’ across areas considered ‘upstream’ hydrographically of the destination area, and ‘fish suppliers’ across (marine) areas sourcing its fish for stocking or further grow-out. Transfers or contacts within area (self-transfers) were excluded from these counts.

2.4 Data Analysis

Data were cross-sectional and time-variant, covering 56 cross-sections, i.e., areas (21 in Region de los Lagos; 35 in Region Aysén), and 67 time intervals, i.e., months. Using SAS (version 9.4) statistical software, we ran time-series cross-sectional (TSCS) regression analyses to identify predictors of ISAV HPR0 and ISAV HPRv incidence over these 56x67 area-month observations. We explored univariate relationships between area-month HPRv incidence with the sum of HPRv incidence in ‘neighbor’, ‘acopio supplier’, ‘plant supplier’, ‘fish transfer

supplier', and 'port user' areas the same month as well as 1-5 months prior (lags). We also explored univariate HPRv relationships with area incidence of HPR0 up to 5 months prior. Number of susceptible sites, sea lice counts, and two measures of density (kg/hectare and fish/hectare) were similarly evaluated. However, sea lice counts were excluded from multiple regression as these data were only sporadically reported during the study period. To prepare for multiple regression, we explored whether region was a significant predictor ($\alpha = 0.05$) of the outcome variable, in which case further analyses were conducted separately for Region de los Lagos and Region Aysén to best discern regional differences. We repeated analyses with area-month HPR0 incidence as the outcome variable. For HPR0 analyses, however, we carried lags of HPRv incidence out to 37 months. This extended lag period was made possible by the delayed initial appearance of HPR0 in the study region.

Multiple regression models were fit to the TSCS data, modeling HPRv and HPR0 separately. As variable lags were tightly correlated, we identified the most significant lag (evaluated out 5 months for most variables), or 3-month rolling sum or average of lags, for each predictive variable. We used stepwise forward selection to identify the most parsimonious model, entering the single most important lag for the next selected variable (based on R-square values, given p-values ≤ 0.05) at each intermediary step. Model fit was judged, and best models chosen, from adjusted R-square statistics. The most important lag of the outcome variable (representing an area's prior ISAV incidence) was included in the predictive model to address autocorrelation. We excluded interaction terms; though many were statistically significant, they added only incremental strength and little interpretive value to the models. We used Fuller and Battese specifications assuming random time and space components in all TSCS regression models.

To discern connectivity ‘hot-spots’, we identified areas whose outbreaks were repeatedly associated through time with outbreaks in ‘upstream’ or ‘supplier’ areas. This was done by running the multiple regression models on each area separately and noting which, if any, predictive factors were statistically significant through time ($\alpha=0.05$). Individual area analyses included 35 and 67 time periods for HPR0 and HPRv, respectively. Results are reported as the proportion of areas showing a statistically significant association for a given factor.

Results

3.1 Data Summary

Fifty-six areas actively farmed ATS in one or more sites during some or all of the 67-month study period. The number of susceptible ATS sites per area varied over the study period (Figure 3). During the full study period, 96% of study areas detected ISAV HPRv or ISAV HPR0 at some point in time. A total of 227 HPRv and 125 HPR0 site designations were accumulated during the study period. HPRv occurred at some point in 95% of areas in Region de los Lagos and 80% in Region Aysén; and HPR0 occurred in 81% of areas in Region De Los Lagos and 80% in Region Aysén. HPRv incidence was concentrated during months 2 to 39 of the study period (99%), with the remainder occurring in months 53-54 (Figure 4). HPR0 ISAV was detected only during the latter portion of the study, from months 33 to 65 (Figure 5). We were able to carry lags for all predictive variables, except sea lice, 1-5 months back (t-5) for any point in the study. Sea lice data were available for month 9 of the study onward, thus lagged (t-5) sea lice data were only available starting month 14.

309

310 3.2 Univariate Analyses and Modeling Decisions

311 Univariate analyses on ISAV HPRv and ISAV HPR0 incidence (separately) identified
312 significant associations with the ISAV status of ‘neighbor’ areas, or areas sourcing processing
313 plant (‘plant’) users, ‘acopio users’, live fish transfers (‘fish suppliers’), and ‘port users’ in the
314 same month and/or 1-5 months previous (termed lags, Tables 1-2). ISAV incidence was also
315 associated with number of susceptible sites for both ISAV types.

316 Correlation analyses found tight relationships between processing plant and acopio predictive
317 variables, and moderate relationships between those and port variables (Table 3). Comparative
318 univariate TSCS regressions on base variables (i.e., without lags) gave processing plant the
319 smallest R^2 of the three for both HPRv (0.0459, 0.0262, and 0.0236 for port, acopio and plant
320 respectively) and HPR0 (0.0096, 0.0037, and 0.0039 for port, acopio and plant respectively)
321 prediction. We thus eliminated processing plant variables from further modeling.

322 Neither current nor previous HPR0 status was predictive of HPRv incidence for the short lag
323 periods (1-5 months) examined. However, because HPR0 occurred for the first time much later
324 in the study period, we were able to examine its relationship with the occurrence of HPRv up to
325 37 months (lags) prior. In this case, univariate analyses for ISAV HPR0 prediction revealed
326 statistically significant relationships with HPRv occurrence months 30-33 prior.

327 Region was found to be statistically significant in HPRv analysis (except the post-2009-
328 regulation period), but insignificant for HPR0. Consequently, multiple regression models were
329 built for Region de los Lagos and Region Aysén separately for HPRv (except the post-2009-
330 regulation period), but combined for HPR0 analysis.

331

332 3.3 HPRv Multiple Regression Analyses

333 The multiple regression model that best fit HPRv incidence in Region de los Lagos by area-
334 month included: (1) the area's prior HPRv incidence (summed over the preceding three months),
335 (2) the number of susceptible sites in the area (three months prior), (3) the sum of HPRv
336 incidence (two months prior) in areas using its ports, (4) the sum of HPRv incidence (one month
337 prior) in areas sourcing any fish transfers for continued grow-out, and (5) the sum of HPRv
338 incidence (one month prior) in neighbor areas considered 'upstream' hydrologically (Table 4).
339 The best fit multiple regression model describing HPRv incidence in Region Aysén by area-
340 month named similar predictors (different lags) with the exception of the port variable (Table 4).

341 April 2009 marked the beginning of a transition to synchronized fallowing, enhanced
342 processing plant biosecurity, and restricted acopio use during outbreaks. Separate analyses on
343 periods pre- and post-transition suggest that site volume and hydrographics dominate as
344 predictors of spread in the initial stage of the study (Table 5), while movement patterns, e.g., via
345 fish transfers and port usage, predominate later in the study (Table 6). Acopio and HPR0
346 predictors exert negative, but significant, effects in the later stages of the outbreak.

347

348 3.5 HPR0 Multiple Regression Analyses

349 The best multiple regression model for HPR0 incidence in the combined regions by area-
350 month included: (1) the area's prior HPR0 incidence (1 month prior), (2) the average number of
351 susceptible sites in the area (over the current and prior three months), (3) the total ATS fish per

hectare in the area (two months prior), (4) the sum of HPR0 incidence (the same month) across areas using its ports, (5) the sum of HPR0 incidence (1 month prior) across areas considered ‘upstream’ hydrologically, and also (5) HPRv incidence in the area (31 months prior) (Table 7).

3.6 Regression Model Explanatory Power

Though predictive variables were highly significant (low p values), the models explained only a portion of spatiotemporal spread (adjusted R-square range 8-30%). Described models explained the most variability in HPRv in Region de los Lagos, less for HPRv in Region Aysén or HPR0 in the combined regions, and extremely little for HPRv post-2009-regulations.

3.7 ‘Hot Spot’ Analyses

Follow-up analyses on individual areas identified those responsible for a substantial portion of predictive strength in TSCS regressions. Table 8 displays the proportion of areas with repeated associations between ISAV status and area connectivity measures over time ($p < 0.05$, full study period). Additional areas may also contribute to the predictive strengths of TSCS regression models; this table simply highlights those with sufficient repetition of associated events over time to independently demonstrate significance. Numerators represent the count of areas showing a statistically significant pattern of association, over time, between the predictor and ISAV incidence. Denominators represent the total number of areas exhibiting that predictive factor. For example, if 7 areas made their ports available for use by vessels originating in other

areas (and experienced HPRv during the study), and HPRv incidence in originating areas recurrently preceded HPRv events in 2 of those 7, the reported proportion is 2/7.

Discussion

Area management is often coined to improve disease prevention and response in aquaculture and reduce disease impacts to fish, industries and environment. However, constraining - as it does - product supply and work force continuity, and demanding replication of many services, area management is also difficult to orchestrate and costly to maintain. Epidemiologic studies of disease spread identify strengths and weaknesses in disease control programs (Peeler and Taylor, 2011). In the aquatic environment, pathogen transmission is often attributed to large-scale processes such as water currents (Gustafson et al., 2007; Murray et al., 2010), vessel transit (Murray et al., 2002; Mardones et al., 2009) or wild fish movements (Arechavala-Lopez et al., 2013). Traditionally, however, field studies evaluate site-to-site rather than area-to-area disease spread dynamics, which can make it difficult to characterize higher order patterns or controls.

Our spatiotemporal study of ISAV spread throughout Chile's Regions de los Lagos and Aysén was geared to specifically examine the efficacy of area-level controls in aquaculture. Several factors predicted monthly area incidence of ISAV HPRv in the current study. These included (1) the area's prior HPRv incidence, (2) the number of susceptible sites in the area, (3) HPRv incidence in neighbor (hydrographically 'upstream') areas, (4) HPRv incidence in areas sourcing transfers of live fish for further grow-out, or (5) for Region de los Lagos, HPRv incidence in areas known to utilize its ports. The most predictive lag for each variable differed,

but typically ranged 1-3 months prior. Results direct local attention to extant exposure pathways and geographic areas that might benefit from revised boundaries, mitigations, or additional research, and inform area management design for other countries or diseases.

The relative shortage of area management evaluations preceding this study may result from data structure and retrieval issues. Applied uniformly, area mitigations often leave few to none for statistical controls. Further, large datasets, especially those collected routinely and evaluated in retrospect, may be lacking in quality, as reliance on multiple producer reports, turnover of personnel responsible for the data, limited scope, or few opportunities (during emergency response) for field validation and quality control, are typical complications.

Data quality varied in our study as well. The outcome variable ISAV HPRv incidence, based on routine veterinary site visits and mandated testing at all sites, is likely highly dependable. In comparison, the contact networks generating many of the predictive variables (also counts of ISAV incidence) were coarse, were based on expected rather than observed patterns of use. However, scripted through consensus by local experts, these are likely well-informed. In contrast, the control variable for area susceptibility was inferred through a combination of measures, each complicated by missing data. Redundancies across datasets allowed us to form a proxy variable (susceptible sites) inferring site activity from either non-zero biomass reports or documented production activities (e.g., sea lice surveys or fish transfers). Related reporting errors should occur most often in ISAV negative sites under the least scrutiny. However, described imprecisions in variable classification reduce model clarity and sensitivity to existing trends. This study suggests future benefit from refined tracking of aquaculture production activities, e.g., stocking and harvest dates, freshwater contacts, and traffic flow between areas;

purposive inclusion of redundancies across some variables; and periodic field validation of random entries to strengthen data quality.

Our study spans the pre- and post- adoption of March 2009 federal regulations mandating enhanced biosecurity. Enhancements included, among others, all-in all-out farming, synchronized fallows, restrictions on fish movements and egg imports, reductions on total biomass; and also standards for the treatment of wastes and effluents from processing plants, mortality collections and harvest operations (Alvial et al., 2012). Early voluntary adoption of best practices by the industry staged the groundwork for these regulations (Alvial et al., 2012); and mandates were implemented in stages, with uniform adoption achieved by October 2012. Thus, prior to April 2009, areas relied predominantly on geography and site-level biosecurity to maintain epidemiologic separation from other areas; and, starting April 2009, enhanced biosecurity standards applied at the area level gradually strengthened their separation.

Hydrographic patterns were most predictive in the early period of study. Waterborne spread of pathogens between marine sites or areas has been implicated in other parts of the world (Scheel, I. et al., 2007; Gustafson et al., 2007). And space-time cluster analyses support a hydrographic influence, also, on site-level transmission of ISAV in Chile (Mardones et al., 2009; Godoy et al., 2013; Vanderstichel et al., 2015). Vast distances separate many Atlantic salmon production areas along Chile's extensive coastline (Figures 1, 2), however hydrographic connectivity also predicted spread between areas in the current study. Hydrographic dispersion models are only available for select regions in Chile (Olivares et al., 2015). Our study complements these modeling efforts by identifying areas with apparent consequential connectivity (per disease events repeatedly linked in time) to their neighbors. Results prioritize

locations for future research, e.g., hydrographic modeling or field investigation of alternate explanations for the observed spatial connectivity (e.g., wild fish, sea lice, birds, other fomites).

The influence of hydrographics diminished following 2009 regulations. A sustained reduction in HPRv incidence tracked a sharp decline in susceptible sites (Figures 3, 4), giving credence to the benefits of area management. Reduced biomass and optimized biosecurity should lessen virus loads in the environment, and could explain the reduction in relevance of transmission pathways, including the reach of waterborne spread. These observations also support macrozone concepts that adjust the size of management areas according to pathogen environmental persistence (Rimstad and Mjaaland, 2002) and stage of outbreak.

Fish transfers were more common in advance of 2009 regulations. Later movements were scarce, e.g., non-ATS species, or smolts transferred from estuarine holdings. However, fish transfer variables remained statistically significant in the later period, suggesting the few transfers that did occur were predictive of subsequent outbreaks. Earlier studies associate site risk of disease with intensity of incoming fish shipments (Murray et al., 2002; Mardones et al., 2014). Our data suggest that fish transfers also impose risks to the encompassing area.

Port risks were most significant in the Region de los Lagos region, and later period, of study. Commercial ports are few in Chile; thus, port-equipped areas share their land access with vessels originating from outer locations, neighboring or distant. While standard practices separate harvest vessel from routine port landings in time or space, the current study suggests other risks remain. Related variables (processing plant and acopio suppliers) were significant in univariate analyses, but did not improve multiple regression fit or prediction. Lack of independence may explain the parsimony. Acopios, temporary holdings for fish pre-processing, and processing

plants are often located in vicinity of ports. Ports, however, experience added risks of smolt and feed delivery, vessel cleaning, fisheries and other business ventures. And, while regulations improved biosecurity of harvest and processing operations, and constrained access to farms from areas deemed ISAV-free, port biosecurity was not similarly addressed.

HPR0 was not generally predictive of HPRv incidence in our study. As 97% of the HPRv events occurred prior to initial detection of HPR0, statistical relevance was not expected. However, in the single model (Table 6) in which HPR0 was a predictor of HPRv status, the two variables were negatively associated. In other words, where HPR0 shows any predictive effect at all for HPRv, it is a slightly protective one. This contrasts with earlier reports of HPR0 detections preceding the emergence of phylogenetically-related HPRv, as in two 2013 cases of ISA in Chile (Godoy et al., 2013), retrospective field study in Norway (Lyngstad et al., 2011), and the documentation of co-occurring HPR0 and HPRv variants of similar genetics in individual fish (Cardenas et al., 2014). However, our findings offer context suggesting that HPRv emergence from HPR0 is likely a low probability event. Model fit, and predictive variable strength, were much reduced post-2009 biosecurity regulations. Consequently, cautious interpretation suggests the need for further field study of the epidemiologic relationship between HPRv and HPR0 variants, and management factors that may influence their co-evolution.

ISAV establishment may have been density-dependent in the studied outbreak. The control variable ‘susceptible sites’ bounds maximum ISAV incidence. However, whether it also reflects volume of farming activities that could introduce pathogens (Arechavala-Lopez et al., 2013) or some undefined carrying capacity which (when exceeded) could raise disease susceptibility, is unknown. Specific, but less reliable, biomass variables dropped out of most models. At the time of study, federal regulations limited fish numbers per cage, but not cage numbers per site.

Models addressing carrying capacity or optimum stocking densities to advise aquaculture siting may ultimately benefit disease control (Salama and Rabe, 2014).

Analyses for HPR0 were least conclusive. Similar patterns between HPRv and HPR0 occurrence suggest parallel mechanisms of spread. In fact, a predictor for HPR0 occurrence was HPRv occurrence in the same area, years prior. However, HPR0 models were of limited strength; perhaps because this variant was captured with less accuracy. The sensitivity of RT-PCR for HPR0 is to date undescribed. HPR0 is non-cultivable; and confirmatory tests (sequencing) utilize screening test products and thus do not meet the independence assumption required for latent-class sensitivity estimation (Pouillot et al., 2002). Further, studies show HPR0 tissue tropism preference for gill over kidney (Christiansen et al., 2011); and gill was not sampled in the initial period of study. Consequently, it is difficult to decipher whether absence of detections truly signifies HPR0 absence; and, whether a positive finding indicates new introduction or simply chronic low-level infection (Kibenge et al., 2008; Godoy et al., 2013). These uncertainties will restrict interpretation of HPR0 field studies until more is known about the importance and persistence of the virus in both host and environment.

Imprecise variable classification, as noted earlier, limits the predictive strength of all models in this study. However, low R^2 values suggest key predictors may also be missing from the analyses. Undescribed variables here include, among others: the health and distribution of feral salmonids in the study region (Rosenfeld et al., 2009; Gonzalez et al., 2011; Miranda et al., 2012; Olmos and Schulze, 2013); the distribution of secondary processing plants (e.g., for value-added processing of salmonids, primary processing of molluscs, or other fisheries harvests); patterns of sea lice infestation (Valdes-Donoso et al., 2013; Kristoffersen et al., 2013; Godoy et al., 2013); the impact of management practices or other co-infections on ISAV susceptibility (Godoy et al.,

2013; Rees et al., 2014); and the impact of invertebrate fauna, benthic or environmental conditions on virus persistence (Gonzalez et al., 2011). Additionally, exposure via land-based or freshwater activities, e.g., broodstock lineage (Vike et al., 2009; Marshall et al., 2014), secondary service providers, hatcheries or other holdings, deserves further study (Gustafson et al., 2014).

The natural refinement of surveillance and response strategies seen through time and experience with emerging pathogens also confound retrospective analyses. We expect that initial ISAV events were slower to detect and confirm than those occurring later. Similarly, we expect that viral loads were better contained following implementation of 2009 revisions to sanitary regulations; and resulting relevance of transmission pathways would vary through time. However, such business process improvements should reduce, rather than exaggerate, measured associations. Thus, we expect our findings are conservative. Periodic evaluations of field data shed light on epidemiologic drivers of disease emergence and resolution. They also provide opportunities to improve data repositories, variables tracked, and their verification procedures.

Acknowledgements

This work was supported by the U.S. Department of State through interagency agreement with USDA APHIS VS CEAH under the U.S.-Chile Environmental Cooperation Agreement. Extensive in-kind support was also provided by Chile's Sernapesca, SalmonChile-Intesal, and Universidad Austral de Chile. We also thank the many industry stakeholders, university and government representatives in Chile that facilitated site visits, shared information and promoted discussions that provided important direction for this study.

References

- Alvial, A., Kibenge, F., Forster, J., Burgos, J.M., Ibarra, R., St-Hilaire, S. 2012. The recovery of the Chilean salmon industry: The ISA crisis and its consequences and lessons. The Global Aquaculture Alliance. 83 pp.
http://www.gaalliance.org/cmsAdmin/uploads/GAA_ISA-Report.pdf
- Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Sempere, J.T., Uglem, I., Mladineo, I. 2013. Reared fish, farmed escapees and wild fish stocks – a triangle of pathogen transmission of concern to Mediterranean aquaculture management. *Aquaculture Environment Interactions* 3, 153-161.
- Cardenas C, Carmona M, Gallardo A, Labra A, Marshall SH: Coexistence in field samples of two variants of the infectious salmon anemia virus: a putative shift to pathogenicity. *Plos One* 2014, 9:e87832.12.
- Chang, B.D., Page, F.H., Losier, R.J., Lawton, P., Singh, R. and Greenberg, D.A. 2007. Evaluation of bay management area scenarios for the southwestern New Brunswick salmon aquaculture industry: Aquaculture Collaborative Research and Development Program final project report. *Can. Tech. Rep. Fish. Aquat. Sci.* 2722: v+69p.
- Christiansen, D.H., Ostergaard, P.S., Snow, M., Dale, O.B., Falk, K. 2011. A low-pathogenic variant of infectious salmon anemia virus (ISAV1 - HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (*Salmo salar* L.) in the Faroe Islands. *J. Gen. Virol.*, 92, 909–918.

549 CoGP (Code of Good Practice) Management Group. 2010. A code of good practice for Scottish
550 finfish aquaculture. <http://bit.ly/z5LzYv>. Accessed 10 Feb 2015.

551 Devold, M., Karlsen, M., Nylund, A. 2006. Molecular sequence analysis of the fusion protein
552 from infectious salmon anaemia virus (ISAV) isolates: evidence of recombination and
553 reassortment. J Gen Virol 87, 2031-2040.

554 EFSA Panel on Animal Health and Welfare (AHAW): Scientific Opinion on infectious salmon
555 anaemia (ISA). EFSA Journal 2012, 10:2971–2992.

556 Ellis, S., L. Gustafson, C. Giray, T. Robinson, F. Marengi, P. Merrill. 2006. Hydrographics
557 and the epidemiology of ISA: Findings from a high-risk region in Maine and New
558 Brunswick. Bulletin of the Aquaculture Association of Canada. 106(1): 44-51.

559 Godoy, M.G., Aedo, A., Kibenge, M.J., Groman, D.B., Yason, C.V., Grothusen, H., Lisperguer,
560 A., Calbucura, M., Avendano, F., Imilan, M., Jarpa, M., Kibenge, F.S. 2008. First
561 detection, isolation and molecular characterization of infectious salmon anaemia virus
562 associated with clinical disease in farmed Atlantic salmon (*Salmo salar*) in Chile. BMC
563 Vet Res 4, 28.

564 Godoy, M.G., Kibenge, M.J.T., Suarez, R., Lazo, E., Heisinger, A., Aguinaga, J., Bravo, D.,
565 Mendoza, J., Llegues, K.O., Avendano-Herrera, R., Vera, C., Mardones, F., Kibenge,
566 F.S.B. 2013. Infectious salmon anaemia virus (ISAV) in Chilean Atlantic salmon
567 (*Salmo salar*) aquaculture: emergence of low pathogenic ISAV-HPR0 and re-emergence
568 of virulent ISAV-HPRΔ: HPR3 and HPR14. Virology Journal 10, 344-361.

569 Gonzales, R.R., Ruiz, P., Llanos-Rivera, A., Cruzat, F., Silva, J., Astuya, A., Grandon, M., Jara,
570 D., Aaburto, C. 2011. ISA virus outside the cage: ichthyofauna and other possible
571 reservoirs to be considered for marine biosafety management in the far-southern
572 ecosysmtes of Chile. *Aquaculture* 318, 37-42.

573 Gustafson, L.L., Ellis, S.K., Beattie, M.J., Chang, B.D., Dickey, D.A., Robinson, T.L.,
574 Marengi, F.P., Moffett, P.J., Page, F.H. 2007. Hydrographics and the timing of
575 infectious salmon anemia outbreaks among Atlantic salmon (*Salmo salar* L.) farms in the
576 Quoddy region of Maine, USA and New Brunswick, Canada. *Preventive Veterinary*
577 *Medicine* 78, 35-56.

578 Gustafson, L., Antognoli, M., Lara Fica, M., Ibarra, R., Mancilla, J., Sandoval del Valle, O.,
579 Enriquez Sais, R., Perez, A., Aguilar, D., Madrid, E., Bustos, P., Clement, A., godoy,
580 M.G., Johnson, C., Remmenga, M. 2014. Risk factors perceived predictive of ISA spread
581 in Chile: Applications to decision support. *Preventive Veterinary Medicine* 117, 276-
582 285.

583 Kibenge, F.S.B., Godoy, M.G., Wang, Y., Kibenge, M.J.T., Cherardelli, V., Mansilla, S.,
584 Lisperger, A., Jarpa, M., Larroquete, G., Avendano, F., Lara, M., Gallardo, A. 2009.
585 Infectious salmon anaemia virus (ISAV) isolated form the ISA disease outbreaks in Chile
586 diverged from ISAV isolates from Norway around 1996 and was disseminated around
587 2005, based on surface glycoprotein gene sequences. *Virology Journal* 6, 88-104.

588 Kibenge, F.S.B., Godoy, M.G., Fast, M., Workenhe, S., Kibenge, M.J.T. 2012. Countermeasures
589 against viral disease of farmed fish. *Antiviral Research* 95, 257-281.

590 Kristoffersen, A.B., Rees, E.E., Stryhn, H., Ibarra, R., Campisto, J.-L., Revie, C.W., St-Hilaire,
 591 S. 2013. Understanding sources of sea lice for salmon farms in Chile. Preventive
 592 Veterinary Medicine 111, 165-175.

593 Lyngstad, T.M., Hjortaa, M., Kristoffersen, A., Markussen, T., Karlsen, E., Jonassen, C.,
 594 Jansen, P., 2011. Use of molecular epidemiology to trace transmission pathways for
 595 infectious salmon anaemia virus (ISAV) in Norwegian salmon farming. Epidemics 3, 1-
 596 11.

597 Mardones, F.O., Perez, A.M., Carpenter, T.E. 2009. Epidemiologic investigation of the re-
 598 emergence of infectious salmon anemia virus in Chile. Diseases of Aquatic Organisms
 599 84, 105-114.

600 Mardones, F.O., Martinez-Lopez, B., Valdes-Donoso, P., Carpenter, T.E., Perez, A.M. 2014. The
 601 role of fish movements and the spread of infectious salmon anemia virus (ISAV) in
 602 Chile, 2007-2009. Preventive Veterinary Medicine 114, 37-46.

603 Marshall et al. 2014. Bona fide evidence for natural vertical transmission of infectious salmon
 604 anemia virus in freshwater brood stocks of farmed Atlantic salmon (*Salmo salar*) in
 605 Southern Chile. Journal of Virology 88, 6012-6018.

606 Miranda, P., Matus V., Olmos, P., Schulze, F. 2012. Evaluación y seguimiento de la situación
 607 sanitaria de especies silvestres en agua dulce y mar. Monografía. Asesoría Integral para
 608 la pesca y acuicultura, Subsecretaria de Pesca y Acuicultura e Instituto de Fomento
 609 Pesquero IFOP. Chile. <http://biblioteca.ifop.cl/F>

610 Murray, A.G., Smith, R.J., Stagg, R.M. 2002. Shipping and the spread of infectious salmon
611 anemia in Scottish aquaculture. *Emerging Infect Dis* 8, 1-5.

612 Murray, A.G., Munro, L.A., Wallace, I.S., Berx, B., Pendrey, D., Fraser, D., Raynard, R.S.
613 2010. Epidemiological investigation into the re-emergence and control of an outbreak of
614 infectious salmon anaemia in the Shetland Islands, Scotland. *Diseases of Aquatic*
615 *Organisms* 91, 189-200.

616 Nylund, A., Devold, M., Plarre, H., Isdal, E., Aarseth, M. 2003. Emergence and maintenance of
617 infectious salmon anaemia virus (ISAV) in Europe: a new hypothesis. *Diseases of*
618 *Aquatic Organisms* 56, 11-24.

619 Olivares G.R., Sepulveda, H.H., Yannicelli, B. 2015. Definition of sanitary boundaries to
620 prevent ISAV spread between salmon farms in Southern Chile based on numerical
621 simulations of currents. *Estuarine, Coastal and Shelf Science* 158, 31-39.

622 Olmos, P., Schulze, F. 2013. Evaluación y seguimiento de la situación sanitaria de especies
623 silvestres en agua dulce y mar. Monografía. Asesoría Integral para la pesca y acuicultura,
624 Subsecretaria de Pesca y Acuicultura e Instituto de Fomento Pesquero IFOP.
625 Chile. <http://biblioteca.ifop.cl/F>

626 Peeler, E.J., Taylor, N.G.H. 2011. The application of epidemiology in aquatic animal health –
627 opportunities and challenges. *Veterinary Research* 42, 94-109.

628 Rees, E.E., Ibarra, R., Medina, M., Sanchez, J., Jakob, E., Vanderstichel, R., St-Hilaire, S. 2014.
629 Transmission of *Piscirickettsia salmonis* among salt water salmonid farms in Chile.
630 *Aquaculture* 428-429, 180-194.

631 Rimstad, E., Mjaaland, S. 2002. Infectious salmon anaemia virus: An orthomyxovirus causing
632 an emerging infection in Atlantic salmon. *Acta Pathologica, Microbiologica et*
633 *Immunologica Scandinavica (APMIS)* 110, 273-282.

634 Rimstad, E. 2011. Examples of emerging virus diseases in salmonid aquaculture. *Aquaculture*
635 *Research* 42, 86-89.

636 Rosenfeld, C., Enríquez, R., Romero, A., Leal, C., González, M., Rolack., H., Molina, S .2009.
637 Determinación de prevalencia de los virus de la Anemia Infecciosa del Salmón (ISA) y
638 de la Enfermedad Pancreática (PD), en la población de peces silvestres y asilvestrados de
639 la Región de La Araucanía, De Los Ríos, De Los Lagos, Aysén y Magallanes con
640 concesiones de salmonicultura. Servicio Nacional de Pesca. Chile. Informe del estudio.
641 Secretaria de Pesca y Acuicultura y Sernapesca.
642 www.bcn.cl/obtienearchivo?id=documentos/10221.../Informe...UACH.

643 Rosenfeld, C., Doddiss, J., Gustafson, L., Remmenga, M., Antognoli, MC., Johnson, C., Gallardo,
644 A., Lara, M., Sandoval, O., Ibarra, R., Henriquez, R. 2015. Spreading factors in
645 Infectious Salmon Anemia virus (ISA) outbreak in southern Chile. ISVEE Meeting
646 November 3-7 Merida, Mexico.

647 Salama, N.K.G., Rabe, B. 2013. Developing models for investigating the environmental
648 transmission of disease-causing agents within open-cage salmon aquaculture.
649 *Aquaculture Environment Interactions* 4, 91-115.

650 Scheel, I., Aldrin, M., Frigessi, A., Jansen, P.A. 2007. A stochastic model for infectious salmon
651 anemia (ISA) in Atlantic salmon farming. *Journal of the Royal Society Interface* 4, 699-
652 706.

653 Sernapesca. 2001 D.S. N° 319 de 2001 Reglamento de medidas de protección, control y
 654 erradicación de enfermedades de alto riesgo para las especies hidrobiológicas.
 655 (Actualizado D.S. N° 4 de 2013). Ed by C. National Fisheries
 656 Service. <http://www.subpesca.cl/institucional/602/w3-propertyvalue-50853.html>
 657

658 Snow, M, McKay, P., McBeath, A.J.A., Black, J., Doig, F., Kerr, R., Cunningham, C.O.,
 659 Nylund, A., Devoid, M. 2006. Development, application and validation of a Taqman
 660 real-time RT-PCR assay for the detection of infectious salmon anaemia virus (ISAV) in
 661 Atlantic salmon (*Salmo salar*). Dev. Biol. Standardization 126: 133-145.

662 Stene, A., Viljugrein, H., Yndestad, H., Tavnorpanich, S., Skjerve, E., 2014. Transmission
 663 dynamics of pancreas disease (PD) in a Norwegian fjord: aspects of water transport,
 664 contact networks and infection pressure among salmon farms. Journal of Fish Diseases
 665 37, 123-134.

666 Thorud, K., Djupvik, H.O. 1998. Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.).
 667 Bull Eur Assoc Fish Pathol 8, 109-111.

668 USDA APHIS Veterinary Services, Maine Department of Marine Resources, Maine Aquaculture
 669 Association. 2010. Infectious Salmon Anemia Program Standards.
 670 [http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/isa_st](http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/isa_standards.pdf)
 671 [andards.pdf](http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/isa_standards.pdf). 59 pp. Accessed 10 Feb 2015.

672 Valdes-Donoso, P., Mardones, F.O., Jarpa, M., Ulloa, M., Carpenter, T.E., Perez, A.M. 2013.
 673 Co-infection patterns of infectious salmon anaemia and sea lice in farmed Atlantic

674 salmon, *Salmo salar* L., in southern Chile (2007-2009). Journal of Fish Diseases 36,
675 353-360.

676 Vanderstichel, R., St-Hilaire, S., Ibarra, R., Lyngstad, T.M., Rees, E., Medina, M.H. 2015.
677 Space-time cluster analysis of the non-pathogenic infectious salmon anemia virus (HPR0
678 ISAV) in Chile, 2011-2012. Aquaculture 437, 120-126.

679 Vike, S., Nylund, S., Nylund, A., 2009. ISA virus in Chile: evidence of vertical transmission.
680 Arch Virol 154, 1-8.

681 Vike, S., Duesund, H., Andersen, L., Nylund, A. 2014. Release and survival of infectious
682 salmon anaemia (ISA) virus during decomposition of Atlantic salmon (*Salmo salar* L.).
683 Aquaculture, 420-421, 119-125.

684 Werkman, M., Murray, A.G., Munro, L.A., Turnbull, J.F. and Green, D.M. 2014. Seasonality in
685 live fish movements and its effects on epidemic dynamics. Aquaculture 418-419, 72-78.

686

Table 1: Results of HPRv univariate analyses for Region de los Lagos and Region Aysén. Lags describe the interval (number of months) between the predicted outcome (HPRv incidence) and the measurement of the predictive variable. For example, significant *p* values for sea lice lags 0, 3 and 5 denotes that HPRv incidence in a given month correlates with sea lice counts in the same month, as well as 3 and 5 months prior. Sea lice measures were only available for a portion of the dataset.

Region	Variable	Most predictive lags ($p \leq 0.001$)	Less predictive lags ($0.001 < p \leq 0.05$)	Non-significant lags ($p > 0.05$)
Lagos	Prior HPRv	1,2,3	4,5	
	Prior HPR0			0,1,2,3,4,5
	Sea lice	0	3,5	1,2,4
	Susceptible sites	0,1,2,3,4,5		
	Fish per hectare ¹	0,1,2,3,4,5		
	Fish kg per hectare ²	0,1,2,3,4,5		
	HPRv in 'fish supplier' areas	1,4	1,2,5	0,3
	HPRv in 'port user' areas	0,1,2,3,4,5		
	HPRv in 'acopio user' areas	0,1,2,3,4,5		
	HPRv in 'plant user' areas	0,1,2,3,4,5		
	HPRv in 'neighbor' areas	0,1,2,3,4		5
Aysén	Prior HPRv	1,2,3,4		5
	Prior HPR0			0,1,2,3,4,5
	Sea lice	0	3,5	1,2,4
	Susceptible sites	0,1,2,3,4,5		
	Fish per hectare ¹	2,3,4,5	1	0
	Fish kg per hectare ²	2,3,4,5	0,1	
	HPRv in 'fish supplier' areas	0	3,4	1,2,5
	HPRv in 'port user' areas			0,1,2,3,4,5
	HPRv in 'acopio user' areas		5	0,1,2,3,4
	HPRv in 'plant user' areas		5	0,1,2,3,4
	HPRv in 'neighbor' areas	0,2,4,5	1,3	

¹ Fish per hectare is measured in units of hundred-thousand fish per 5000 hectares.

² Fish kg per hectare is measured in units of one million kilogram (kg) of fish per 5000 hectares.

Table 2: Results of HPRO univariate analyses for Region de los Lagos and Region Aysén. Lags describe the interval (number of months) between the predicted outcome (HPRO incidence) and the measurement of the predictive variable. Sea lice measures were only available for a portion of the dataset.

Region	Variable	Most predictive lags ($p \leq 0.001$)	Less predictive lags ($0.001 < p \leq 0.05$)	Non-significant lags ($p > 0.05$)
Lagos	Prior HPRO	1		2,3,4,5
	Prior HPRv			0-37
	Sea lice		*insufficient data	
	Susceptible sites	0,1,2,3,4,5		
	Fish per hectare ³	0,1,2,3,4,5		
	Fish kg per hectare ⁴	0,1,2,3,4,5		
	HPRO in 'fish supplier' areas	1,4	2,5	0,3
	HPRO in 'port user' areas	0,1,2,3,4,5		
	HPRO in 'acopio user' areas	0,1,2,3,4,5		
	HPRO in 'plant user' areas	0,1,2,3,4,5		
	HPRO in 'neighbor' areas	0,1,2,3,4		5
Aysén	Prior HPRO	1	2	3,4,5
	Prior HPRv	30,31,32,33		0-29, 34-37
	Sea lice			0,1,2,3,4,5
	Susceptible sites	0,1,2,3,4,5		
	Fish per hectare ³	2,3,4,5	1	0
	Fish kg per hectare ⁴	2,3,4,5	0,1	
	HPRO in 'fish supplier' areas	0	3,4	1,2,5
	HPRO in 'port user' areas			0,1,2,3,4,5
	HPRO in 'acopio user' areas		5	0,1,2,3,4
	HPRO in 'plant user' areas		5	0,1,2,3,4
	HPRO in 'neighbor' areas	0,2,4,5	1,3	

³ Fish per hectare is measured in units of hundred-thousand fish per 5000 hectares.

⁴ Fish kg per hectare is measured in units of one million kilogram (kg) of fish per 5000 hectares.

Table 3: Pearson correlation coefficients for the full HPRv study period (67 months, 56 areas, n=3752), and the HPRO study (35 months, 56 areas, n=1960). Predictive variables for HPRv measure HPRv incidence in the described contact network; and predictive variables for HPRO measure HPRO incidence in the described contact network. Asterisks indicate statistically significant relationships ($p < 0.001$). Plant stands for processing plant.

Study	Predictors	Processing plant user	Acopio user	Port user	Fish supplier	Neighbor
HPRv	Plant user	1.0				
	Acopio user	0.9749*	1.0			
	Port user	0.6721*	0.7252*	1.0		
	Fish supplier	0.0600*	0.0567*	0.0823*	1.0	
	Neighbors	0.3209*	0.3238*	0.2924*	0.2516*	1.0
HPRO	Plant user	1.0				
	Acopio user	0.9505*	1.0			
	Port user	0.6735*	0.7261*	1.0		
	Fish supplier	-0.0054	-0.0056	-0.0050	1.0	
	Neighbors	0.1432*	0.1540*	0.1103*	-0.0101	1.0

Table 4: Multiple regression model for spatio-temporal incidence of ISAV HPRv in Region de los Lagos and Region Aysén. Variables describe characteristics (susceptible sites) or HPRv counts (prior HPRv) in the area in question, or the sum of HPRv counts in its described contact network (port user, fish supplier, neighbor). Fitted lag describes which month or combination of months was most predictive and thus represented in the model. Sum areas refers to total HPRv counts across areas in a contact network. Region de los Lagos model R square = 0.3081, adjusted R square = 0.3056, CS (cross sections, number of areas) = 21, TS (time series, number of months) = 67. Region Aysén model R square = 0.1570, adjusted R square = 0.1556, CS = 35, TS = 67.

Region	Predictors	Estimate	Standard Error	t value	p value	Fitted lag
Lagos	Intercept	-0.0254	0.0124	-2.05	0.0410	
	Prior HPRv	0.1258	0.0124	10.88	< 0.0001	Sum, 0-3 months prior
	Port user	0.0243	0.00396	6.14	< 0.0001	Sum areas, 2 months prior
	Susceptible sites	0.0152	0.00244	6.21	< 0.0001	Number, 3 month prior
	Fish supplier	0.0378	0.0143	2.64	0.0084	Sum areas, 1 month prior
	Neighbors	0.0137	0.00622	2.20	0.0278	Sum area, 0-1 month prior
Aysén	Intercept	-0.0176	0.00884	-1.99	0.0462	
	Prior HPRv	0.0564	0.0101	5.58	< 0.0001	Sum, 0-3 months prior
	Fish supplier	2.1843	0.1594	13.70	0.0084	Sum areas, current month
	Susceptible sites	0.0140	0.00162	8.63	< 0.0001	Number, 3 months prior
	Neighbors	0.0247	0.00507	4.87	< 0.0001	Sum areas, 0-2 months prior

Table 5: Multiple regression model for spatio-temporal incidence of ISAV HPRv in Region de los Lagos and Region Aysén before April 2009. Variables describe characteristics (susceptible sites) or HPRv counts (prior HPRv) in the area in question, or the sum of HPRv counts in its contact network (remaining variables). Fitted lag describes which month or combination of months was most predictive and thus represented in the model. Sum areas refers to the sum of HPRv counts across areas in a contact network. Region de los Lago model R square = 0.2400, adjusted R square = 0.2350, CS (cross sections, number of areas) = 21, TS (time series, number of months) = 22. Region Aysén model R square = 0.2092, adjusted R square = 0.2051, CS = 35, TS = 22.

Region	Predictor	Estimate	Standard Error	t value	p value	Fitted lag
Lagos	Intercept	-0.0901	0.0540	-1.67	0.0958	
	Prior HPRv	0.1446	0.0193	7.51	< 0.0001	Sum, 0-3 months prior
	Susceptible sites	0.0307	0.00501	6.12	< 0.0001	Number, current month
	Neighbors	0.0381	0.0195	1.95	0.0514	Sum areas, 1 month prior
Aysén	Intercept	-0.0143	0.0282	-0.51	0.6107	
	Prior HPRv	0.0372	0.0175	2.13	0.0337	Sum, 0-3 months prior
	Fish suppliers	3.5191	0.3566	9.87	< 0.0001	Sum areas, current month
	Susceptible sites	0.0188	0.0033	5.69	< 0.0001	Number, 1 month prior
	Neighbors	0.1168	0.0264	4.43	< 0.0001	Sum areas, 4 months prior

Table 6: Multiple regression model for spatio-temporal incidence of ISAV HPRv in combined regions in the period starting April 2009. Variables describe characteristics (susceptible sites) or ISAV counts (prior HPRv, prior HPRO) in the area in question, or the sum of HPRv counts in its contact network (remaining variables). Sum areas refers to the sum of HPRv counts across areas in a contact network. Fitted lag describes which month or combination of months was most predictive and thus represented in the model. Model R square = 0.0792, adjusted R square = 0.0770, CS (cross sections, number of areas) = 56, TS (time series, number of months) = 45.

Variable	Estimate	Standard Error	t value	p value	Fitted lag
Intercept	-0.0039	0.00405	-0.96	0.3393	
Prior HPRv	0.0591	0.0107	5.52	< 0.0001	Number, 4 months prior
Susceptible sites	0.0066	0.00098	6.69	< 0.0001	Number, 5 months prior
Fish suppliers	0.9541	0.1075	8.87	< 0.0001	Sum areas, current month
Port users	0.1075	0.0227	4.74	< 0.0001	Sum areas, 1 month prior
Acopio users	-0.0415	0.0130	-3.20	0.0014	Sum areas, 2 months prior
Prior HPRO	-0.0177	0.00833	-2.13	0.0335	Number, 4 months prior

Table 7: Multiple regression model for spatio-temporal incidence of ISAV HPR0 in combined regions. Variables describe characteristics (susceptible sites, fish per hectare), HPR0 incidence (prior HPR0) or HPRv incidence (prior HPRv) in the area in question or HPR0 incidence in its contact areas (remaining variables). Fitted lag describes which month or combination of months was most predictive and thus represented in the model. Sum areas refers to the sum of HPR0 counts across areas in a contact network. Model R square = 0.1183, adjusted R square = 0.1156, CS (cross sections, number of areas) = 56, TS (time series, number of months) = 35.

Variable	Estimate	Standard Error	t value	p value	Fitted lag
Intercept	-0.0338	0.0112	-3.02	0.0026	
Prior HPR0	0.1554	0.0225	6.90	< 0.0001	Sum, 1 month prior
Prior HPRv	0.0605	0.0156	3.88	0.0001	Sum, 31 months prior
Susceptible sites	0.0217	0.00339	6.39	< 0.0001	Average, 0-3 months prior
Neighbors	0.0456	0.0125	3.65	0.0003	Sum areas, 1 month prior
Fish per hectare ⁵	0.0020	0.00059	3.46	0.0006	Number, 2 months prior
Port users	0.0417	0.0177	2.35	0.0188	Sum areas, current month

Table 8: Individual area analysis, showing the proportion of areas with repeated associations between ISAV status and identified predictors. Predictors include live fish transfers for grow-out (transfers), shared water systems (neighbors) or shared ports (ports). Results are grouped by variable, though the evaluated lag may vary by Region and ISAV type. For example, 'transfers' for HPRv sums the number of areas where this variable was predictive. Sums include areas in either Region de los Lagos or Region Aysén, though the TSCS-identified lag was 'last month' for the former and 'this month' for latter Region.

	Proportion, transfers p < 0.05	Proportion, neighbors p < 0.05	Proportion, ports p < 0.05
HPRv	5/11	19/44	2/7
HPR0	-	13/41	1/7

⁵ Fish per hectare is measured in units of hundred-thousand fish per 5000 hectares.

Figure 1: Map of Region de los Lagos in Chile. Numbered polygons correspond to management areas (also known as barrios).

Figure 2: Map of Region Aysén in Chile. Numbered polygons correspond to management areas (also known as barrios).

Figure 3: Susceptible sites per area, by month. Mean and maximum number of active sites farming ATS in a given month calculated over 56 areas. A mean of 5 for a particular month suggests a total of 56×5 ATS farms were in operation in Chile's Regions de los Lagos and Aysen during that month of the study period. The arrow points to the date of revised sanitary regulations (March 2009). Minimum values, not charted, were typically zero.

Figure 4: Area ISAV HPRv designations by month. Mean, maximum and sum of designations, by month, across the 56 management areas in Chile's Regions de los Lagos and Aysen. Month 1 corresponds to June 2007. Month 67 corresponds to December 2012. The arrow points to the date of revised sanitary regulations (March 2009). Minimum values, not charted, were typically zero.

Figure 5: Area ISAV HPRO designations by month. Mean, maximum and sum of designations, by month, across the 56 management areas in Chile's Regions de los Lagos and Aysen. Month 1 corresponds to June 2007. Month 67 corresponds to December 2012. The arrow points to the date of revised sanitary regulations (March 2009). Minimum values, not charted, were typically zero.