Spatial and non-spatial risk factors associated with cage-level distribution of infectious salmon anaemia at three Atlantic salmon, *Salmo salar* L., farms in Maine, USA

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Abstract

The distribution of infectious salmon anaemia (ISA) was examined among 80 cages from three Atlantic salmon grow-out farms in Maine, USA that were stocked with smolts from a single hatchery. Cage-level disease was broadly defined as one or more moribund fish testing positive for infectious salmon anaemia virus (ISAV) by RT-PCR and a second confirmatory test (IFAT, culture or genotype sequence). Spatio-temporal and cage-level risks were explored using logistic regression and survival analysis. Non-spatial risk factors associated with ISA, or shortened survival time to disease, included increased predation, trucking company choice for smolt transfers, a finely-sedimented benthic substrate, and smaller average size of smolts at stocking. Univariable analysis identified the time-dependent spatial factor ‘adjacency to newly infected cages’ to be predictive of new infection in neighbouring cages 11–12 weeks later. However, none of the spatial factors, or their lags retained relevance in multiple-variable models. The results suggest a diffuse distribution of virus exposure throughout infected sites, with host-susceptibility factors probably influencing disease manifestation in individual cages. The narrow focus of the current study may limit application of the findings to other sites and year-classes. However, these data support the relevance of husbandry efforts to optimize fish health in regions affected by ISAV.

Keywords: Atlantic salmon, binary time-series cross-sectional data, epidemiology, infectious salmon anaemia, risk factors, spatio-temporal.

Introduction

Infectious salmon anaemia (ISA) is a disease of viral aetiology with substantial economic significance to farmed Atlantic salmon, *Salmo salar* L., operations in Maine, USA (Bouchard, Brockway, Giray, Keleher & Merrill 2001), New Brunswick, Canada (Mullins, Groman & Wadowska 1998; Lovely, Dannevig, Falk, Hutchin, MacKinnon, Melville, Rimstad & Griffiths 1999) and Norway (Thorud & Djupvik 1988), the United Kingdom (Rodger, Turnbull, Muir, Millar & Richards 1998) and the Faroe Islands (Anonymous 2000). ISA is caused by an orthomyxovirus (Falk, Nomork, Rimstad, Mjaaland & Dannevig 1997) capable of surviving in seawater from days to weeks, on a temperature and titre dependent basis (Giray, Bouchard, Brockway & Merrill 2004). ISA manifests and spreads in Atlantic salmon with varying degrees of morbidity and mortality (Hammell & Dohoo 2005a). Several genotypes of infectious salmon anaemia virus (ISAV) have been detected in Maine, and many more in neighbouring New Brunswick. These may be broadly classified into North American and...
European genotypes (Ritchie, Cook, Melville, Simard, Cusack & Griffiths 2001; Cook-Versloot, Griffiths, Cusack, McGeachy & Ritchie 2004). Maine isolates that have been successfully cultured on cell lines, or were associated with mortality in the field, have so far been sequenced as a North American type using segment 6 or segment 8 primers (ISA Program, unpublished data). Salmon farms in Maine have also evidenced sporadic detections by molecular assays of a European genotype of ISAV; however, this genotype (HPR0) has not yet been associated with disease in the USA (ISA Program, unpublished data).

Atlantic salmon farmed in Maine are typically held in large floating net pens clustered in groups within designated lease sites (farms). The farms are generally sited in relatively cold, well-flushed, coastal waters, and may be separated from neighbouring farms in Maine or nearby New Brunswick, Canada, by as little as 0.5 km. Transmission of disease between sites is a concern for farms situated within a region with pronounced tidal mixing of water. Hydrographic relationships between farms can explain a small, but significant, proportion of the spatio-temporal pattern of disease incidence within the Quoddy region of Maine and New Brunswick (Ellis, Gustafson, Giray, Robinson, Marenghi & Merrill 2006; Gustafson, Ellis, Beattie, Chang, Dickey, Robinson, Marenghi, Moffett & Page 2007). Similarly, proximity to processing plants, or to ISA diseased farms, has repeatedly been named a risk factor in various epidemiological studies from the European Union, Canada and the USA (Vagholm, Djupvik, Willumsen, Tveit & Tangen 1994; Jarp & Karlsen 1997; Gustafson, Ellis & Bartlett 2005; McClure, Hammell & Dohoo 2005a). Conversely, informal observations of disease occurrence among cages within a site suggest an apparently random pattern of spread showing little concordance with spatial configurations (ISA Program, personal observations). Risk factor studies based on previous ISA epizootics in New Brunswick suggest the relevance of husbandry practices in cage-level susceptibility to ISA (Hammell & Dohoo 2005b; McClure et al. 2005a).

However, the spatio-temporal movement of clinical disease among cages within a farm has received little, if any, previous research attention.

Infectious salmon anaemia spread is managed in Maine by separation of year-classes and management zones, strict biosecurity practices, and the early detection and swift removal of newly infected cages [USDA APHIS (US Department of Agriculture Animal and Plant Health Inspection Service) Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002]. However, ISA has been annually recurrent in the Cobscook Bay region of Maine since 2001 (Ellis et al. 2006). Industry-endorsed ISA control strategies in Maine also promote husbandry practices aiming to reduce exposure and susceptibility to disease-causing situations in general, such as integrated pest management programmes, low-density stocking, environmental quality, heightened biosecurity, minimal handling of fish and vaccination when products are available and efficacious. It is within this context of strong management and disease control practices that we evaluate factors of continued relevance to cage-level disease transmission in the Cobscook Bay region of Maine from 2003 to 2005.

We conducted a study of spatial and non-spatial factors associated with cage-level risk of ISA on infected farms. Our study differs from previous risk factor studies in two ways. First, it provides a direct evaluation of spatial factors governing disease transmission throughout infected sites. Secondly, it provides an update of non-spatial risk factors important to farms already implementing management practices, as informed by existing knowledge of ISA epidemiology.

**Methods**

**Subject selection**

This retrospective evaluation was based on all cages in Cobscook Bay, Maine, that were stocked with Atlantic salmon smolts in the spring of 2003. The study fish were comprised of a single year-class cohort raised in a single Canadian hatchery, which were distributed to 80 marine cages (representing three distinct marine farms) over a 9-day period at the end of May. Smolt transfer and production variables (i.e. risk factors), as well as disease response, varied (and were measured) at the cage-level. The category of site (i.e. marine farm) was included as a blocking variable to account for unmeasured site-level variation (such as might be expected with different on-site managers, different equipment, etc.). However, all three marine grow-out sites in this study were situated in the same bay, and fell under uniform company ownership and upper-level management policies. Cage-level disease and production information from stocking to
harvest were evaluated. The Cobscook Bay region is considered a high-risk zone for ISAV exposure, where ISAV infection and disease outbreaks have been centred in Maine in previous years (Ellis et al. 2006). Cobscook Bay is also contiguous with areas of Atlantic salmon culture in NB, Canada that have experienced disease attributed to ISAV since 1996 (Hammell & Dohoo 2005b).

**Surveillance programme**

Surveillance protocols described in the US Department of Agriculture, Animal and Plant Health Inspection Service’s (APHIS) ISA Programme Standards [USDA APHIS (US Department of Agriculture Animal and Plant Health Inspection Service) Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002], were implemented in cooperation with Maine’s Department of Marine Resources (DMR) and the salmon industry. All Atlantic salmon cages in this ISAV high-risk region of Maine were visually inspected by APHIS-accredited veterinarians at least twice-monthly (weather-dependent), from 3 weeks post-stocking to harvest, for any indications of clinical disease or increased mortality suggestive of ISA. During ISA surveillance inspections, moribund fish were collected by dip-net or diver, with samplers aiming for 10 fish per farm per site visit. Necropsies were performed by a veterinarian or APHIS ISA Programme trained and approved technician; gross pathologies were noted and tissue samples submitted for ISAV testing. Surveillance sampling included kidney tissue sections for RT-PCR using the 1D/2 primer set (Mjaaland, Rimsstad, Falk & Dannevig 1997; Blake, Bouchard, Keleher, Opitz & Nicholson 1999; Office International des Epizooties 2003), and impression smears utilized for IFAT testing (Falk, Namork & Dannevig 1998; Office International des Epizooties 2003), for all submissions. For samples associated with a previous history of ISAV detection or clinical signs consistent with ISA, kidney, heart and spleen sections were also collected for virus culture on Chinook salmon embryo (CHSE-214), salmon head kidney (SHK-1) and Atlantic salmon kidney (ASK) cell monolayers (Lannan, Winton & Fryer 1984; Dannevig, Brudeseth, Gjoen, Rode, Nergeland, Evensen & Press 1997; Bouchard, Keleher, Opitz, Blake, Edwards & Nicholson 1999). Reported estimates for diagnostic test sensitivity and specificity, respectively, are 0.93 and 0.98 for RT-PCR (McClure, Hammell, Stryhn & Dohoo 2005b), 0.79 and 0.96 for IFAT (McClure et al. 2005b), and ≥0.84 and ≥0.90 for virus isolation (Nerette, Dohoo & Hammell 2005). Follow-up genotyping was requested for most surveillance samples found to be positive by RT-PCR assays. North American and European genotypes were differentiated, for initial RT-PCR positives from a cage, by segment 8 sequencing (Mjaaland et al. 1997), or segment 6 sequencing of the highly polymorphic region (HPR) of the haemagglutinin gene (Cook-Versloot et al. 2004). Classification of HPR0 vs. non-HPR0 genotypes in remaining RT-PCR positives was determined by comparison of RT-PCR fragment lengths obtained by primer amplification of the HPR, with HPR0 and non-HPR0 controls (C. Giray, personal communication). The consistent detection of only two HPR genotypes in the USA (HPR0 of European strain type and HPR4 subtypes of North American strain type) allowed these inferences to be made with good confidence when sequence analysis was not possible. The regular and consistent approach to site and fish inspection provided a uniform basis for comparison of disease incidence dates between cages and sites.

**Case definition**

Animal and Plant Health Inspection Service’s ISA Programme definition of an infected cage [USDA APHIS (US Department of Agriculture Animal and Plant Health Inspection Service) Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002] is met if any two fish from a given cage test positive by any two specified ISAV detection assays, including RT-PCR, cell culture and/or IFAT. This designation is a precursor to qualification for potential indemnification if cages are eradicated under a State of Maine-mandated kill order. However, because early removal of positive cages is recognized as a means of improved disease control (Gustafson, Ellis, Hawkins, Moore, Robinson & MacPhee 2006), cages are often emptied voluntarily by producers prior to meeting the criteria for a formal declaration of infection. Consequently, to minimize mis-classification of positive cages emptied before formal diagnosis, we used the less restrictive case definition of disease as any cage having one or more moribund fish found positive by RT-PCR, and substantiated in the same fish by corroborating (3+ or 4+) IFAT, cell culture, or North American genotype results.
Risk factor data collection

Cage-specific information for the following variables were completed from industry production records: site, stocking weight (g), trucking company (companies may differ by tank size, oxygenation control and biosecurity practices, for example), initial post-transfer 42-day cumulative per cent mortality; predation pressure (measured as average predation-attributed mortalities per days-in-water), mortality of ‘pinheads’ (fish failing to acclimatize and grow properly post-transfer from fresh- to seawater), benthic deposition index (a measure of substrate type under the cage), whether the cage had been selected for routine bi-weekly lice counts (consisting of dip-netting five apparently healthy fish for manual lice counts), and whether a European genotype (HPR0, presumed to be non-pathogenic) had ever been detected in the cage.

Benthic deposition index was based on a subjective ranking scored by a private environmental assessment team (MER Inc., Brunswick, ME, USA) responsible for assessment of site benthic and environmental quality for the State of Maine. MER evaluates the benthic quality of active farmed salmon cage systems in Maine semi annually, acquiring dive data of the site perimeter and a series of line transects under the cage systems. At the time of the study, MER personnel were not aware of the cage-level infection status of any of the sites. MER personnel were asked to collectively rank the depositional pattern of cages on a scale from 1 to 10, with a ranking of 1 assigned to a heavily eroded (i.e. high-energy) benthos, and 10 for a finely-sedimented (i.e. highly depositional) benthos.

Spatial variables considered for possible association with cage-level survival included the number of newly infected adjacent cages, the number of harvests of adjacent infected cages, and the number of harvest vessel visits to any (infected or non-infected) adjacent cage. Spatial data, as well as the outcome variables, were revised (updated) at biweekly intervals. Each biweekly entry was distinct from the others, e.g. infections and harvests were only counted in the interval in which they were first observed. Non-spatial explanatory variables, in contrast, were not considered time-dependent.

Statistical analysis

Variables were evaluated for their associations with two separate dependent variables: (i) cage-level occurrence of pathogenic ISA (as defined in the case definition), and (ii) cage-level survival-time to pathogenic ISA. The Kolmogorov–Smirnov test was used to assess distribution normality. Non-spatial univariable associations with the dependent variable ‘ISA occurrence’ were evaluated using Chi-squared or, when appropriate, Fisher’s exact tests for categorical variables, and the Mann–Whitney U-test for continuous variables. Individual variable association with the dependent variable ‘survival time to ISA’ was evaluated through univariable Cox proportional hazards analysis, which was extended to time-dependent covariates for the spatial factors. Selection of factors for inclusion in multiple-variable analyses was based on results from individual variable analysis. Variables with a P-value ≤ 0.25 were selected for inclusion in multiple-variable analyses.

Spatial data took the form of a time-series cross-sectional (TSCS) dataset (Beck 2001; Beck & Katz 2004; Shi, Macinko, Starfield, Xu, Regan, Politzer & Wu 2004). Neighbouring cage infection and harvest status, and harvest vessel visits to neighbouring cages (whether infected or not), were recorded for each of the 80 (cross-sectional) cages at biweekly (time-series) intervals. Temporal dependence is common with TSCS data, but can be mitigated or modelled directly, e.g. using TSCS regression for models with continuous (Beck 2001; Beck & Katz 2004; Shi et al. 2004), or Cox proportional hazards with time-dependent covariates for binary (Tucker & Beck 1997; Beck, Katz & Tucker 2001), dependent variables. Because of the probable delay between exposure from a neighbouring cage and any resulting new infection, we first conducted univariable evaluations of the lagged impact of neighbouring cage status (infection and harvest), with lags offset from the outcome measure in biweekly intervals from 0 to 16 weeks. Statistically significant (P < 0.05) lag periods for the spatial variables (neighbouring cage infection, harvest and vessel visit status) were included in following multiple-variable Cox proportional hazards models.

Multivariable logistic regression was used to assess the combined statistical impact of relevant non-spatial predictors on susceptibility of a cage to infection with ISA. The logistic regression model took the form logit(\(p\)) = \(\alpha + x'b\), where \(\alpha\) is the intercept parameter and \(b\) is a vector of slope parameters (SAS® Institute Inc. 2003). Cox’s proportional hazards analysis was used to determine the impact of spatial and non-spatial independent
variables on survival time to infection for individual cages. The proportional hazards model took the form $\lambda(t,x) = \lambda_0(t)e^{\beta x}$, where $\lambda_0(t)$ is an unspecified baseline hazard function, $x$ is a vector of covariate (including time-dependent) values and $\beta$ is a vector of unknown regression parameters (SAS® Institute Inc. 2003). Non-infected cages were censored at harvest. Multi-variable model-building for both logistic and survival analyses proceeded in a backwards stepwise elimination process, with the alpha for removal set at 0.10, until only significant variables (Wald-test $P < 0.05$) remained. We evaluated Spearman correlations between predictive variables with an alpha of 0.01. Two-way interactions between predictors were examined for statistical relevance using the Wald statistic. Logistic-regression model fit was assessed using Hosmer-Lemeshow and Pearson Chi-squared statistics. The proportional hazards assumption was evaluated by the Supremum test for proportional hazards.

Results

A total of 1473 moribund fish were submitted from the 80 study cages for routine surveillance testing during the course of the study. RT-PCR results were available for almost all (1472) of the submissions, IFAT results were available for 1464 samples and cell culture results were available for 483 samples. There were 154 RT-PCR positive samples from 56 different cages. Of these, 8.1% (12 of 149) were positive by IFAT, and 57.8% (78 of 135) culture-tested RT-PCR positives from 35 different cages were positive by culture. Genotype data were available for 121 of the 154 RT-PCR positives: 116 (from 48 different cages) were positive by IFAT, and 57.8% (78 of 135) culture-tested RT-PCR positives from 11 different cages were positive by IFAT. Genotype data were available for 121 of the 154 RT-PCR positives: 116 (from 48 different cages) were positive by culture. Genotype data were available for 121 of the 154 RT-PCR positives: 116 (from 48 different cages) were positive by IFAT. The study population was characterized by the following cage-level statistics: smolt weight (median 94.5 g, range 77–144 g), predation pressure per day (median 0.08 predation-attributed mortalities, range 0–22), number of adjacent cages (median 5, range 3–8) and benthic deposition index (median 4, range 1–6). Fifty two of the 80 study cages met our case definition of disease (at least 1 moribund fish positive by RT PCR and at least one confirmatory test), and 21 of these 52 cages (40.4%) were removed within 2 weeks of the case definition date. Days to ISA detection (per case definition) from marine stocking ranged from 341 to 805 days (median 488). European genotype status was finalized for 56 of the 80 study cages (by completing genotype analysis for all submissions from a cage, until either a European detection was made or all RT-PCR samples had been typed). Six of these 56 cages produced European (HPR0, presumed non-pathogenic) ISA detections. Alternate samples from five of the six European genotype cages also tested positive for North American genotypes. North American and European genotypes were sequenced from the same fish (and same tissue sample) in a single case.

Univariable analysis of non-spatial risk factors for cage-level disease identified stocking weight, benthic deposition index, trucking company and number of adjacent cages for further evaluation ($P \leq 0.25$) by multiple-variable logistic regression. None of the factors were significant at an alpha of 0.05. Though not significant in the univariate analysis, we forced ‘farm’ into the multiple-variable logistic regression model to account for variation attributed to unmeasured farm-level factors.

Multi-variable logistic regression (Table 1) retained only two of these variables, stocking weight and benthic-deposition index (in addition to site) in the final model of risk factors for ISA ($P = 0.035$, $-2 \log L = 90.78$, AIC 100.78). Interactions ($P > 0.10$) were not statistically significant. The model predicts a 1.61-fold (1/0.62) greater odds of infection with every 10 g increment decrease in average stocking weight, and a 1.79-fold greater odds of infection with every step increase in the 10-point scale representing depositional character of the ocean bottom under the cage (with greater deposition associated with greater risk). The Hosmer–Lemeshow (Chi-squared 5.83, d.f. 8, $P = 0.69$) test found no reason to reject the fit of the model.

Univariable proportional hazards analysis of individual non-spatial and spatio-temporal factors revealed a statistically significant ($P < 0.05$) relationship between timing of new outbreaks and adjacent cage occurrence of new infection (at an 11–12 week lag). Additional spatial (new adjacent infections with a 15–16 week lag, harvests of adjacent infected cages with a 7–8 and 9–10 week lag, and harvest vessel visits to adjacent cages with a 5–6, 7–8 and 9–10 week lag) and non-spatial (predation pressure and cage type) variables were identified for inclusion ($P < 0.25$) in multivariable Cox proportional hazards models.
Multi-variable survival analysis of selected spatial and non-spatial factors identified only two variables mutually predictive of survival time to infection (Table 2): predation pressure and trucking company \((P = 0.003; \text{AIC } 357.64, -2 \log L 353.64)\). The interaction term was not statistically significant \((P > 0.10)\). We did not retain ‘farm’ variables in the survival analysis, as doing so worsened model fit and did not improve predictive significance. The Kolmogorov-type Supremum test for the proportional hazards assumption found no evidence of assumption violations for either predictive variable (predation pressure max. absolute value 1.399, replications 1000, seed 19, \(P = 0.12\); trucking company max. absolute value 0.740, replications 1000, seed 19, \(P = 0.575\)). Furthermore, though the predation pressure data were bounded on the left by zero and skewed to the right, a log transformation did not improve the fit (AIC 359.38, \(-2 \log L 355.38\), predicted significance (Wald-test of global null hypothesis, \(P = 0.005\)) or approximate relevance (hazard ratio for trucking company = 0.415, \(P = 0.006\); hazard ratio for log predation pressure = 0.789, \(P = 0.044\)) of the model.

**Discussion**

A key finding of this study was the limited relationship between cage adjacency and timing of disease. Spatio-temporal relationships have been described in the movement of ISAV between farms (Gustafson *et al.*, 2007). However, in this within-farm cage-level study, direct neighbours of infected cages were not influenced temporally by the timing of new infection, infected-fish harvests or harvest-vessel visits at neighbouring cages. Newly infected neighbouring cages held some predictive significance for new outbreaks 11–12 weeks later in a univariable analysis. However, none of the spatial variables achieved lasting statistical relevance in multiple-variable models. This limited statistical impact on neighbouring cages may reflect the benefits of an extremely aggressive removal strategy. Our case definition was dated to time of moribund fish collection, rather than laboratory report, yet a full 40% of positive cages were emptied in the same 2 week interval as initial detection. These extremely aggressive removals require trusted relations with industry veterinarians (whose clinical observations may precede formal diagnosis and/or set the stage for rapid response), rapid turnaround of all relevant laboratory results, and the ability to mobilize vessels and crews for harvest with little advance notice. It is possible that stronger spatial patterns might be observed in less aggressively managed settings.

The lack of a strong spatio-temporal pattern in the current study suggests a relatively diffuse exposure to ISAV throughout the sites, with subsequent manifestation of disease under certain local (cage-level) conditions. Fish condition variables were associated with disease risk. An elevated risk of disease in cages stocked with lower-weight smolts, along with the presumptive influence of trucking company choice on cage survival-time to infection, suggests that even the early condition of smolts may influence disease occurrence on grow-out sites. Smolt size may reflect, to some degree, extent of smoltification (Thorpe, Talbot & Villarreal 1982). Small fish with a developing or...
compromised osmoregulatory ability may experience reduced immune function upon entering sea water (Barton, Schrech, Ewing, Hemmingsen & Patino 1985; Glover, Skar, Christie, Glette, Rudra & Skaala 2006). Smolt delivery companies vary by truck transfer-tank size, oxygenation capacity and biosecurity implementation. Biosecurity practices may have influenced the current findings. For example, a marine outbreak of enteric redmouth disease (caused by Yersinia ruckeri, a Gram-negative bacterial species) was later inferred to be related to improper disinfection of the trucking company’s transfer tanks.

At the grow-out site, predation pressure (measured as average number of predation-attributable mortalities per days-in-water) also influenced a cage’s survival time, with higher levels of predation associated with earlier outbreaks. Seals and seabirds appear to prey more heavily on some cages than others, an anecdotal phenomenon perhaps related to distance to roosting sites and ledges, human activity, health status of fish in that cage and/or cage conformation and tidal exposure. The number of mortalities attributed to predation may reflect stress or injuries sustained by fish during predation events, either of which could conceivably influence susceptibility to ISAV infection, or to recrudescence of a latent infection with subsequent shedding of virus. The finding of elevated risk of disease in cages overlying more finely-sedimented benthos (perhaps reflecting increased particle residence time and/or pooling of currents) might relate to duration of exposure to water-borne ISAV. The deposition ranking could also reflect improved fish condition associated with better water exchange. European (presumed non-pathogenic) ISAV genotype detections neither improved nor diminished risk of, or time of development to, ISA.

Farms evaluated during this study demonstrated strong management practices and great attention to fish health. Consequently, our case definition of disease was relatively broad, aiming for very early detection. At this early level of detection, cages generally provide very few, if any, outward clinical appearances of illness, beyond a low number of moribund fish targeted for routine surveillance sampling. Case definitions were further assigned by fish collection date, rather than by laboratory report dates that typically followed sample submission by 4–7 days (with culture results and sequencing reports about 7–28 days post-submission). Consequently, the fact that a full 21 of 52 case-defined cages were removed within 14 days of the case definition date confirms an extremely aggressive approach by farmers to ISA management. This early detection and early response context contrasts to varying degrees with cage-level risk factor studies conducted elsewhere (Hammell & Dohoo 2005b; McClure et al. 2005a), and may help to explain why certain previously defined risk factors were not identified in the present study. Similarly, our benthic deposition indices may or may not apply to other contexts. Our region of study was centred in a single bay with relatively uniform flushing, salinity and temperatures. Minor differences within this system may have accounted for some of the risk of disease. Nevertheless, it remains to be seen whether similar benthic associations will apply to a more general and objective framework. Hydrological data collected by current meters at each site, or an objective ranking of benthic type by core samples and sediment analysis, could contribute to future studies looking more closely at this issue.

The present study identifies host-related risk factors that appear to influence cage-level susceptibility to ISAV under highly responsive management conditions. Two of these factors pertain to decisions and/or conditions occurring very early in the production cycle, either at or during transfer to marine sites from the hatchery (average smolt size and smolt transfer company); others factors apply to characteristics associated with the marine setting (deposition and predation). Spatial adjacency factors showed only limited association with timing of ISA outbreaks. Consequently, results suggest a relatively generalized pattern of virus exposure to and throughout a site, with localized outbreak patterns better described by factors influencing host susceptibility. This study supports the assumption held by regulators and industry that husbandry practices strongly orientated to good biosecurity and general fish health help to minimize disease onset and spread on farms exposed to ISAV.

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References


USDA APHIS (US Department of Agriculture Animal and Plant Health Inspection Service) Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association (2002). *Infectious Salmon Anemia Program Standards* pp. 51. Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association, Riverdale, MD.


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