Spatial and temporal heterogeneity of infectious hematopoietic necrosis virus in Pacific Northwest salmonids

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Abstract

The aquatic rhabdoviral pathogen infectious hematopoietic necrosis virus (IHNV) causes acute disease in juvenile fish of a number of populations of Pacific salmonid species. Heavily managed in both marine and freshwater environments, these fish species are cultured during the juvenile stage in freshwater conservation hatcheries, where IHNV is one of the top three infectious diseases that cause serious morbidity and mortality. Therefore, a comprehensive study of viral genetic surveillance data representing 2,590 field isolates collected between 1958-2014 was conducted to determine the spatial and temporal patterns of IHNV in the Pacific Northwest of the contiguous United States. Prevalence of infection varied over time, fluctuating over a rough 5-7 year cycle. The genetic analysis revealed numerous subgroups of IHNV, each of which exhibited spatial heterogeneity. Within all subgroups, dominant genetic types were apparent, though the temporal patterns of emergence of these types varied among subgroups. Finally, the affinity or fidelity of subgroups to specific host species also varied, where UC subgroup viruses exhibited a more generalist profile and all other subgroups exhibited a specialist profile. These complex patterns are likely synergistically driven by numerous ecological, pathobiological, and anthropogenic factors. Since only a few anthropogenic factors are candidates for managed intervention aimed at improving the health of threatened or endangered salmonid fish populations, determining the relative impact of these factors is a high priority for future studies.

1. Introduction

Pacific salmonid fish are an important cultural and ecological resource in the Pacific Northwest of North America. Due to overfishing and habitat loss, all but one of the six species of Pacific salmonids are cultured to some extent in conservation hatcheries. Among hatchery populations, the rhabdoviral pathogen infectious hematopoietic necrosis virus (IHNV) is a particular burden (Wolf 1988; Bootland & Leong 1999). IHNV

primarily infects sockeye and kokanee salmon (migratory and freshwater life history types of *Oncorhynchus nerka*, respectively), Chinook salmon (*O. tshawytscha*), and steelhead and rainbow trout (migratory and freshwater life history types of *O. mykiss*, respectively). In these hosts IHNV typically causes acute disease and mortality only in juvenile fish, while infection in adults is usually asymptomatic. Both juvenile and adult fish can transmit IHNV horizontally through water. Within hatcheries, transmission from infected adults to progeny via egg-associated virus is greatly reduced or eliminated by egg disinfection (Amend 1975; Meyers 1998). As observed in captive fish, acute IHN disease in juveniles is often associated with epidemic mortality, which can be up to 90% (Groberg 1983a; Groberg 1983b; LaPatra et al. 1993a; LaPatra et al. 1993b; Bootland & Leong 1999).

Established methods for IHNV molecular epidemiology are based on the sequence of a variable portion of the viral glycoprotein gene, the 303 nt midG region. Analysing virus isolate midG sequence genotype data together with epidemiological case data allows inference of probable transmission sources, and some estimates of secondary transmission risk. This method has been used to define recent emergence events and support effective control measures to mitigate losses due to IHN disease (Breyta et al. 2013; Breyta et al. 2016b). A previous molecular epidemiology study of 393 IHNV isolates collected throughout the entire range of IHNV in North America revealed three phylogenetic genogroups designated U, M, and L (Kurath 2003). U genogroup viruses are found in Alaska, Western Canada, Puget Sound, Coastal Washington, and the large Columbia River basin that extends throughout much of Washington, Oregon, Idaho, and Montana. M genogroup viruses are also found in the Columbia River basin, as well as sporadic emergence events in coastal Washington (Breyta et al. 2013) and Oregon (this paper). L genogroup viruses have only ever been detected in California and the southern Oregon coastal region. Subsequent phylogenetic analyses have identified six subgroups within the M genogroup, designated MA-MF (Troyer et al., 2003), two subgroups within the U genogroup, designated UC and UP (Black thesis 2015), and two subgroups within the L genogroup, designated L1 and L2 (Kelley et al. 2007).

U and M group IHNV have coexisted within the large complex Columbia River basin for decades (Garver et al. 2003). During that time, M group viruses caused serious

disease impacts to conservation hatchery populations of steelhead trout and farm populations of rainbow trout (Garver et al. 2003; Troyer & Kurath 2003; Breyta et al. 2016b). These M group viruses are not uniformly distributed throughout the Columbia basin, despite the fact that the *O. mykiss* host species can be found in all parts of the watershed. Within one portion of the basin, dominant M virus types have been observed to exhibit serial emergence and displacement patterns, prompting investigations into possible mechanisms (Kell et al. 2014; Breyta et al. 2016a). Viruses in the U genogroup are also not uniformly distributed, and a recent study revealed population genetic structure (subgroups UP and UC) within the U group IHNV viruses in the Columbia River basin (Black 2015). In this region the UC subgroup is mostly associated with Chinook salmon and steelhead trout, and the UP subgroup is associated with sockeye salmon (Black thesis 2015). While mortality in Chinook salmon due to UC viruses has not been reported at levels similar to that of steelhead trout infected with M group IHNV, disease outbreaks have occurred in juvenile Chinook salmon, and the impact of UC viruses in Chinook salmon conservation programs is not well characterized.

In order to determine the spatial and temporal distribution of IHNV subgroups and dominant genetic types in this region, an updated comprehensive molecular epidemiology analysis focusing on IHNV in the Columbia basin, coastal Oregon, coastal Washington, and Puget Sound was conducted. Compared to earlier studies, some IHNV subgroups were still detected, while others were no longer evident (MA, ME, MF) (Troyer & Kurath 2003). The analysis revealed that all subgroups exhibited heterogeneity in temporal and spatial occurrence, despite the availability of host species throughout the study area. Variation in host-specific patterns was observed for different virus subgroups, and both specialist and generalist viral strategies were observed. Several dominant virus genotypes were identified within the U and M genogroups, and specific focus sites with high levels of disease impacts were observed. Within this complexity there are novel insights with potential for contributions to targeted control strategies in the future.

2. Methods

2.1 Virus Isolates. The molecular biology program at the USGS Western Fisheries Research Center (WFRC) provides midG sequence analysis of IHNV field isolates as a

technical assistance service to fisheries managers throughout the Pacific Northwest. The data generated is maintained in a database at WFRC. This database has a freely accessible internet version at <http://gis.nacse.org/ihnv/>.

IHNV isolates and diagnostic records were obtained from fish health laboratory staff from the United States Fish and Wildlife Service, Washington Department of Fish and Wildlife, Northwest Indian Fisheries Commission, Oregon Department of Fish and Wildlife, Idaho Department of Fish and Game, and from the archival collection at the WFRC. Samples were taken from dead, dying, or asymptomatic juvenile fish or from asymptomatic adult fish and processed for virus isolation in cell culture using standardized protocols (American Fisheries Society Fish Health Section Blue book). Virus culture supernatants of low passage number (≤ 2 passages for the majority of isolates) were sent to WFRC for analysis and archival storage at -80°C . The 2590 IHNV isolates in this study were collected from a total of 252 different sites within the study region and represented 1469 distinct IHNV positive fish cohorts. These samples came from steelhead or rainbow trout (*O. mykiss*), Chinook salmon (*O. tshawytscha*), sockeye or kokanee salmon (*O. nerka*), coho salmon (*O. kisutch*), chum salmon (*O. keta*), Atlantic salmon (*Salmo salar*), or unknown hosts.

2.2 Bias and coding by detection events Fish cohorts at hatcheries are tested by agency staff for IHNV infection primarily under three different testing schemes: routine screening of asymptomatic adult fish when they are spawned, occasional screening of asymptomatic juvenile fish, and diagnostic testing of juvenile fish suffering mortality or exhibiting signs of IHNV infection. When positive samples from these testing strategies are submitted for viral genetic typing, two kinds of sampling bias may occur. One is when few samples are submitted for sequence analysis from specific geographic areas because the presence of IHNV in those fish cohorts is not unusual, and a greater number are submitted from fish cohorts that are unexpectedly positive. This pattern creates a geographic surveillance-pressure bias: fish cohorts from areas with newly emergent IHNV are typed more heavily while fish in IHNV endemic areas are sampled and typed less intensely. The second form of bias is in the number of IHNV isolates submitted for typing. For example, one incident of juvenile epidemic IHN disease may be represented by only one or two isolates, while another may be represented by as many as thirty. If

IHNV detections were reported by the raw number of typed isolates, these two sampling biases would mask important patterns. To correct this second form of bias, isolates have been coded into positive fish cohorts (by collection site, age, species, and seasonal timing,(Breyta et al. 2013)). After genetic typing, fish cohorts for which all virus isolates had identical sequence types were each assigned to one ‘detection event’. If more than one sequence type was detected among multiple isolates from one cohort, a separate event was assigned for each sequence type detected (Breyta et al., 2013; Breyta et al, 2016). In the current report, cohorts with more than one virus type are described as having a ‘dominant variant’ and ‘non-dominant variant(s)’.

2.3 Viral RNA extraction and sequence analysis Viral genomic RNA was extracted from 200-500µL of virus culture supernatant with TriReagent (Sigma) according to manufacturer’s directions with tRNA (Promega) added to aid in RNA precipitation. Reverse transcription and amplification of a ~550bp fragment containing the midG region was performed as previously described (Emmenegger et al. 2000) in one 50 µL reaction using avian myeloblastosis virus (AMV) reverse transcriptase, Taq polymerase, IHNV-specific primers and 30 cycles of amplification. The PCR product was purified away from amplification components using Strataprep PCR purification columns (Agilent Technologies), and 0.5 µL was used in each of two 10 µL Big Dye Terminator (Applied Biosystems) sequencing PCR reactions, using the same forward and reverse primers as above and 30 cycles as previously described (Emmenegger et al. 2003).

2.4 Phylogenetic analysis Sequence data was assembled, edited, and trimmed to the established midG 303nt fragment (Emmenegger et al. 2003; Garver et al. 2003; Kurath et al. 2003; Troyer & Kurath 2003) using Sequencher software v4.9. Consensus midG sequences for each virus isolate were aligned in Clustalx (Qt/QMake), and manually inspected and corrected for artefactual gap insertion. The term ‘genotyping’ is used to describe this process, and the term ‘sequence type’ or ‘genetic variant’ is used to describe individual sequences (haplotypes) as described previously (Breyta et al. 2013). Sequence types based on midG sequences are designated in the format mG###, where ### indicates a randomly assigned 3 digit number that is specific to a sequence, and U, M, or L is added at the end to indicate the major IHNV genogroup (e.g. type mG001U falls within the U genogroup in phylogenetic analyses). Newly generated sequences and all

previously known USD sequences detected in North American IHNV were used for phylogenetic analysis. Taxa were individual sequences representing each midG haplotype, associated with the first year of its detection. Phylogenetic analysis was performed using coalescent Bayesian MCMC methods as implemented in the BEAST software package v1.8.0 (Drummond et al. 2002; Drummond & Rambaut 2007). Results were analysed, annotated, and drawn using the complementary suite of programs, including Tracer v1.5 and FigTree v1.3.1 (Rambaut & Drummond 2003; Rambaut & Drummond 2007; Rambaut 2009). Dated taxa were used with a relaxed uncorrelated lognormal molecular clock prior, with a gamma-distributed rate prior extrapolated from the range of known error rates of RNA-dependent RNA polymerases (Drummond et al. 2006; Biek et al. 2006; Drummond & Suchard 2010). The relaxed clock was used for the midG analysis after it was confirmed that the strict clock could be rejected (95% confidence interval of the coefficient of variation and standard deviation of the relaxed clock did not span zero) and the relaxed clock yielded a positive Bayes Factor in comparison to the strict clock. No specific inferences were made directly from the molecular clock parameter. The phylogenetic tree is derived from three separate MCMC analyses of 150 million generations that each achieved convergence and good mixing, and were subsequently combined, summarized, and analyzed.

3. Results

3.1 Geographic and genetic scope of study

Within the US, the Columbia River basin occupies large portions of Washington, Oregon, Idaho, and Montana states. Washington and Oregon also have significant numbers of smaller non-Columbia watersheds, those that drain directly to the Pacific Ocean, that are also included in this analysis. Within this geographic region, a total of 2,590 isolates of IHNV collected from salmonid fish hosts between 1958-2014 have been genetically typed (Table 1). Of these isolates, only 2% were detected before 1980, and 77% were detected during 2000-2014 (Figure 1). Even when corrected for sampling bias by using only one representative of each virus detection event (see methods), 65% of typed cohorts were sampled between 2000-2014 (Figure 1). Of the 2590 isolates in this study, 94% originated from one of three major salmonid species: steelhead or rainbow trout (*O. mykiss*, 44%), Chinook salmon (*O. tshawytscha*, 32%) or sockeye or kokanee

salmon (*O. nerka*, 18%). Virus isolates from Washington and Idaho states were approximately equally distributed between either the U or M genogroup (Table 1). In Oregon state, typed IHNV isolates were found in all three genogroups, though U was more abundant than M, and L detections were the great minority. Isolates from Montana were the least numerous and consisted entirely of U group virus in kokanee salmon (freshwater *O. nerka*).

Among the 2,590 isolates, there were 322 genetic types. Coalescent phylogenetic analyses of these haplotypes revealed strong support for all previously described North American IHNV genogroups and subgroups (Figure 2). U genogroup types were either part of UC or UP subgroups. M genogroup types were primarily of MB, MC, and MD subgroups, with small numbers of types in subgroups MA, ME, and MF. L genogroup types were the least numerous. As expected from previous analyses, the L genogroup was clearly monophyletic (posterior probability 0.97), and the U and M genogroups shared a strongly supported common ancestral node (posterior probability 0.97). U genogroup taxa formed a polytomy with this common ancestral node, while the M genogroup was derived from that node as a monophyletic group (posterior probability 0.79). The larger subgroups within the major genogroups were supported as follows: UC 0.89, MB 0.76, MC 1.0, and MD 1.0. Smaller subgroup support values were MA 1.0, ME 1.0, and MF 1.0.

The phylogeny also revealed one previously unrecognized subgroup within the M genogroup, designated ‘MH’ (Figure 2, asterisk). This new MH subgroup includes several types previously described as part of the MN subgroup, but not all. The remaining MN types do not form a well-supported clade, instead these types form a polytomy with the ancestral node of the M genogroup. The MH subgroup had a posterior probability of 0.86 and contained 7 taxa. The most commonly detected type in the MH subgroup was type mG007M, which first appeared in 1980 and was associated with a significant emergence in steelhead trout that involved disease outbreaks at many sites in the lower Columbia region. This type was repeatedly detected until 1994, and was then displaced by another dominant MD genotype (Breyta et al. 2013; Kell et al. 2014). The six additional taxa in the MH subgroup were detected between 1981 and 1992, also in the lower Columbia region. The overlapping geographic and temporal signal of these six

types, along with their phylogenetic signal of high probability of relatedness to mG007M, indicates that they are variants of mG007M. It was not previously known that type mG007M produced genetic variants to a similar degree as later major types (Breyta et al. 2013).

3.2 Complex patterns of IHNV viruses in Washington, Oregon, Idaho, and Montana

There was a temporal pattern in which the numbers of typed virus isolates, and the bias-corrected numbers of virus-positive fish cohorts, peaked every 5-7 years (Figure 1). There was a notable increase in the number of typed isolates and cohorts in 2002, and another increase in the number of typed isolates in 2009 that corresponded to a more gradual increase in the number of positive cohorts. The increase in numbers of positive cohorts in 2002 was driven by samples originating from Columbia River basin steelhead/rainbow trout and Chinook salmon (Figure 3 panels A and C). The increase in 2009 was partially driven by isolates originating from coastal steelhead trout cohorts (Figure 3, panel B), due to documented emergence events (Breyta et al., 2013). Peaks in coastal sockeye salmon in 2011-2013 were also apparent (Figure 3). In general, maximum years had high numbers of typed adult and juvenile cohorts, while minimum years had low numbers of typed adult cohorts, and few or no typed juvenile cohorts (Figure 3). This periodic pattern appeared in both U and M viruses, although the signal was strongest for U viruses in Columbia River basin Chinook salmon and M viruses in Columbia River basin steelhead/rainbow trout.

Geographic spread of M group IHNV was also apparent. Before 2004, there was only one detection of M group IHNV outside the Columbia River basin, (Emmenegger et al. 2000; Breyta et al. 2013)(Figure 3B). Starting in 2004, M group IHNV emerged in several coastal populations of Chinook salmon and steelhead trout. In coastal waters of Oregon, type mG110M was found in Chinook salmon in 2004 and 2006, and in steelhead/rainbow trout during 2005-2007. In the coastal waters of Washington, type mG110M (and its non-dominant variant type mG168M) was found in Chinook salmon during 2007-2009 (Figure 3D), at the beginning of the larger emergence of types mG110M and mG139M and their variants in coastal Washington steelhead trout during 2007-2011 (Breyta et al. 2013). In both coastal Oregon and coastal Washington the most

significant impact of these emergence events in terms of juvenile fish disease occurred mostly in steelhead/rainbow trout, not Chinook salmon (compare Figure 3B and D).

There was also a pattern of host specificity, apparent in all regions and all years. Steelhead/rainbow trout in both the Columbia River basin and coastal/Puget Sound waters had far more M group IHNV detected than U group, in both adult and juvenile fish (Figure 3, top row, orange bars). Chinook and sockeye salmon both had far more U group IHNV detected in fish of any age and in either region (Figure 3, middle and bottom row panels). When Chinook salmon were found to have M group viruses it was most commonly detected in asymptomatic adult fish and in years when large numbers of sympatric steelhead trout cohorts had M group viruses, suggesting spillover from steelhead trout. The same spillover pattern of M viruses in sockeye salmon was evident in the Columbia River basin. In addition to this broad pattern of host specificity, there was a more nuanced pattern of host specificity among U and M subgroups that is described below.

3.3 Distribution of subgroups in the landscape

Throughout the study landscape heterogeneity was evident in which IHNV subgroup was predominant in each hydrobiological area, and the host species in which they were most commonly found. The hydrological landscape can be divided by natural drainage and by what virus subgroups were dominant, making 12 distinct regions (Figure 4 and Table 2). Of these, only three regions had only U or M subgroup viruses. Within the Puget Sound, only UP subgroup viruses were found with one exception, detection of an MH type in 1986 (region 12 in Figure 4A and Table 2). These Puget Sound UP viruses were primarily detected in sockeye/kokanee salmon. The second region was the upper Columbia River basin, which is defined as the portion of the watershed that is no longer open to fish migration due to the barrier of Chief Joseph dam, including sites in both Washington and Montana (region 4, Figure 4B). This region had a very low number of typed cohorts, accurately reflecting a low number of positive cohorts overall. Viruses in this area were also UP viruses, entirely in kokanee salmon (Table 2). The third region with only one genogroup of IHNV was the upper Snake River region, which is similarly restricted for fish migration due to Oxbow dam, and includes the intensive rainbow trout farming industry in the Hagerman Valley (region 8, Figure 4A). In this region, only M

subgroup viruses were detected, and it was the only area where all 7 M subgroups have been detected (Table 2) (Troyer & Kurath 2003). Furthermore, all known detections of MN types occurred in this region (Table 2). MN types are the most basal, least derived members of the M group (Figure 2, red box) (Kurath et al. 2003). In the upper Snake, detections of M subgroup viruses occurred primarily in rainbow trout and to a lesser extent steelhead trout (Table 2).

Throughout the rest of the Columbia River basin, U and M subgroup viruses co-occurred, and there were distinct patterns of which dominated where. The Columbia River and its largest tributary, the Snake River, were each divided into middle and lower sections, with a major tributary watershed in each (Figure 4B, Columbia River regions 1-3 and Snake River is regions 5-7). The Columbia River regions 1 and 2 had the greatest numbers of typed cohorts of any region in the current study, and contained the most viral diversity. These regions were also the location of 52% of all detections of MH subgroup viruses (Figure 2). Comparing the four segments of the Columbia River, the burden of IHNV (number of typed events) generally decreased moving upstream (Table 2). The proportional presence of U viruses increased moving upstream, with UC viruses increasing in prevalence from region 1 to 2 and predominating in the central two regions, and UP viruses increasing between regions 3 and 4. At the same time the proportional presence of MD viruses decreased moving upstream. In these regions, steelhead trout was slightly more often positive than Chinook salmon. In contrast, sockeye salmon were the least commonly positive in anadromous regions, but kokanee salmon (also *O. nerka*) was the only positive host in the landlocked upper Columbia River, even though trout species, including rainbow trout, exist in the landlocked waters as well.

Comparing the four Snake River regions (Table 2 regions 5-8), the greatest prevalence of IHNV was in the lower Snake, and the lower three regions had similar subgroups of viruses detected. However, the proportional presence of UC decreased moving upstream, and the proportional presence of MB and MC increased moving upstream. Within the lower three regions of the Snake River, Chinook salmon were the most commonly positive, while the majority of isolates in the upper Snake River were from rainbow trout.

The coastal regions are divided into south and north Oregon coasts and the Washington coast (regions 9-11, respectively, in Figure 4B and Table 2). The south Oregon coast had very few typed positive cohorts, all of which originated from Chinook salmon. Among these cohorts, L genogroup was most common, but there was one detection of UC subgroup virus. No L group viruses were detected in the north Oregon coast, but UC and MD viruses were found in a 3:1 ratio. The host species with these viruses was mostly steelhead and rainbow trout, followed by Chinook salmon. The Washington coast differed in that there were more typed cohorts (nearly 2x the two Oregon coast regions combined) and there were no UC viruses detected. Instead, Washington coast had a mix of MD and UP viruses, which were primarily found in steelhead trout and sockeye salmon, respectively. The MD virus detections in the Washington coast region were twice as common as UP detections, due to the emergence of these viruses between 2007-2011 (Breyta et al. 2013).

In all 9 regions where U and M, or U and L genogroup viruses co-existed, only two subgroups were observed in the majority of events, despite all seven U or M subgroups being detected at some time (Figure 4B, regions 1-3, 5-7, 9-11). Furthermore, within each region the two dominant subgroups were never found in equal proportions. Instead, one subgroup was found in an average of 75% of detections (range 67-89%) while the second was in an average of 20% of detections (range 6-33%), a 3.5-fold difference (Figure 4G). In all cases, the hydrobiological region where each subgroup formed the primary proportion was adjacent to the region where it was the secondary dominant proportion (Figure 4C-F).

3.4 Host specificity of subgroups

While the general host specificity of U and M genogroups was consistent with previous reports, greater detail was evident for the five dominant U and M subgroups. Here host specificity was measured as natural field incidence, or the proportion of each subgroup detected in different host species in cohorts of any age fish (Table 3). This was also refined by considering only detections in juvenile fish as a proxy for disease (Table 3). The three M subgroups MB, MC, and MD all occurred predominantly in *O. mykiss* hosts, but differed in which host life history types (migratory steelhead trout or freshwater rainbow trout, both *O. mykiss*) were infected most often. Within subgroups

MB and MC, 82% and 79% of all detections, respectively, occurred in rainbow trout, with similar proportions when only detections in juvenile fish were considered. In contrast, 71% of all MD subgroup viruses were found in steelhead trout, and 77% of MD viruses from juvenile fish were also in steelhead trout (Table 3). None of the three M subgroups were detected in significant numbers of events (either all fish or juvenile fish) in sockeye salmon (1%, 0%) or Chinook salmon (13%, 2%), although spillover of M subgroup types was more common in Chinook salmon than in sockeye/kokanee salmon (Table 3).

In contrast, the U subgroups UP and UC were not specific for the *O. mykiss* host species. The detection of UP types was most common in sockeye and kokanee salmon, with only rare (<7% in detections of any age fish, <2% of juvenile fish) detections in steelhead/rainbow trout or Chinook salmon. Appearance of UC types was primarily in Chinook salmon (62%, 59%), but a large proportion of UC viruses were also found in steelhead (30%, 30%), and only a small proportion was detected in sockeye/kokanee salmon (5%, 6%) (Table 3). Thus the two U subgroups differed in host species specificity, with the UC subgroup being unique among the five dominant U or M subgroups in occurring in relatively high abundance in two different host species.

3.5 Dominant virus types

Among the genetic types detected, several dominant types were identified that were widely detected in time and space. Among the 160 U group virus types, 8 types (6%) were responsible for 72% of all detection events (Figure 5A). Similarly among the 139 detected M group virus types, 7 types (5%) were responsible for 60% of all detection events (Figure 6A).

Within U genogroup, there were 5 dominant UC virus types and 3 dominant UP types that were each detected in 5 or more years, and at 5 or more sites (Figure 5A). In both subgroups there was a general temporal pattern of the older dominant types being detected for many years and continuing concurrently with more recently emerged dominant types (Figure 5B). Among UC viruses, type mG001U has been detected in a total of 28 years between 1973-2011. These detections are dispersed throughout the hydrobiological range of UC viruses (Figure 4C). Type mG032U was first detected in 1988 so its temporal range falls entirely within that of mG001U (Figure 5B), and its

geographic range is almost as dispersed. The three remaining dominant UC types were all first detected in 2005 and have been detected every year since (Figure 5B). Types mG147U and mG174U were first detected in the lower Snake region (Figure 4A, region 5) and have remained there, dominating detections there and in the middle Snake, with one exception for each type. Type mG147U was detected once in the north Oregon coast in 2007, and type mG174U was detected in the same region in 2011, both in adult Chinook salmon. In the lower Snake region, type mG174U and numerous phylogenetically related variants (Figure 2, orange box) are more commonly detected than mG147U and its variants (Figure 2, blue box). Type mG151U was first detected in the Salmon River region (Figure 4A, region 7) in 2005. This type and its variants (Figure 2, yellow box) became the dominant UC type in the lower and middle Columbia River region (Figure 4A, regions 2-3), with continued but sporadic detections in the middle Snake River region (Figure 4A, region 6). These three more recently emerged UC types had smaller geographic footprints than the older dominant UC types, and with only two exceptions they were limited to the range where UC is dominant (Figure 4C).

Among UP viruses, the types mG002U and mG003U have the longest time span of detection, beginning in 1984 and 1974, respectively (Figure 5B). They also have the widest geographic dispersal, being detected in Puget Sound and the Washington coast, and also beyond the study area in Alaska and British Columbia (Kurath et al., 2003; K. Garver, pers. comm.). Type mG050U was first detected in 1988 in the middle Columbia River region, and was not detected again until 2009-2013 when it emerged and became dominant in the Puget Sound and Washington coast regions. One additional UP type, mG265U, first emerged in 2012 and has since been detected in 2013-2014 at a total of 9 sites. While this means that type mG265U does not yet meet the aforementioned dominant type criteria, it is included here because it continues to be detected in 2015 (data not shown), and it has a uniquely broad host distribution that suggests it may become dominant. Type mG265U has been detected in six different species/types of Pacific salmonids (sockeye and kokanee salmon, Chinook salmon, rainbow and steelhead trout, and chum salmon), and in symptomatic juveniles of three of those species (sockeye salmon, Chinook salmon, and rainbow trout). Out of all other known IHNV types, only type mG003U has been detected in this broad distribution of species, with and without

disease signs. However, type mG003U attained this host distribution in a minimum of 23 years, while type mG265U has done it in only 4 years, making it a genotype to be monitored.

Within the M genogroup, there were 7 dominant types from 5 different subgroups that were each detected in 4 or more years and at 5 or more sites (Figure 6B). These types were dominant in two different regions, however. The dominant types of the upper Snake River, types mG009M (MB), mG008M (MC), and mG010M (MA), were all detected roughly concurrently, in the 1990s (Figure 6B), and almost exclusively detected in the rainbow trout farming portion of the upper Snake River region 8. Genetic surveillance of virus isolated from this region during 1980-1999 (Troyer et al. 2000; Troyer & Kurath 2003) was 3-fold higher than during 2000-2014, so it is possible that these types have continued to circulate but have not been sampled.

In contrast, types mG007M (MH), mG111M (MD) and mG110M (MD) were all primarily detected in the lower Columbia and lowest Columbia/Willamette regions, but they did not occur concurrently (Figure 4A). Instead, a pattern of serial emergence and displacement was evident for these three types (Figure 6B) (Garver et al. 2003; Kell et al. 2014; Breyta et al. 2016a). A fourth dominant M type, mG139M (MD), also emerged in the lower Columbia region in 2003 but did not displace mG110M there. All 4 of these types also appeared outside the lower Columbia region, but only types mG110M and mG139M became dominant elsewhere. Type mG110M became dominant in steelhead trout of the Oregon coast from 2005-2007 and of the Washington coast during 2007-2009. Type mG139M became dominant in steelhead trout of the lower Snake region during 2008-2010 (Breyta et al. 2016b), and in the Washington coast during 2010-2011, apparently displacing mG110M there (Breyta et al. 2013). These virus types have been extensively characterized for replication fitness, virulence, and infectivity as potential drivers of viral displacement (Kell et al. 2014; Breyta et al. 2016a). More recently, there were no M group IHNV types detected in 2014, echoing the submergence of M virus detections during 2000-2001 (Figure 6B).

3.6 Incidence of disease is not homogenous in the landscape

While salmon and trout migrate throughout the freshwater environment, adult populations eventually concentrate spatially to spawn, either at natural sites or hatcheries.

Within the study area, genetic surveillance was conducted on samples from 252 unique sites or complexes (a complex is a collection of discrete sites that are related by management and fish movement, like a hatchery and its adult fish trap). Of these sites, 231 (92%) were a hatchery, complex, or fish farm, and non-hatchery/farm sites comprised only 3% of the detection events in this report. Among all sampled sites in the current study, virus was not uniformly detected. Ten sites comprised 26% of detections with reported signs of IHN disease in juvenile fish, and each site had 10 or more cases of juvenile disease. These ten hatchery or farm sites (4% of collection sites) accounted for 31% of all detections and were designated ‘focus sites’ a-j (Figure 7). There was one focus site in each Puget Sound, lower Snake, middle Snake River, north Oregon coast, and lowest Columbia Willamette regions (Table 4). The lower Columbia River region had two focus sites and the upper Snake River region had three focus sites, all of which were rainbow trout farms that have not been sampled since 1998 (Table 4). For all but one of the focus sites, the viruses detected correlated completely with the dominant virus subgroup(s) and host types of their region (Table 4). The only exception was in the lower Columbia River region, where more UC than MD viruses were detected, occurring in steelhead trout and Chinook salmon. One of the two focus sites in this region only had virus isolates from steelhead trout, and it had more MD than UC viruses, as is typical for this host species (site e, Table 4).

4. Discussion

Genetic typing of viruses isolated over a period of 35 years from naturally occurring IHN virus infections in Pacific salmonid fish of the waters of Washington, Oregon, Idaho, and Montana have revealed that IHN virus is spatially, temporally, and ecologically diverse. Despite the sympatric migration of multiple host fish species within the extensive Columbia River basin, the heterogeneous nature and distribution of IHN virus subgroups has been maintained for decades. Only a few drivers of these patterns can be identified here, leaving much to be learned.

The temporal heterogeneity in numbers of typed fish cohorts may indicate true fluctuations in IHN virus prevalence, but the possible contribution of surveillance biases must also be considered. One kind of surveillance bias was identifiable in the current

data, namely variable numbers of samples typed from positive fish cohorts. This was controlled for by transforming the data into the numbers of positive cohorts instead of numbers of virus isolates. This corrected for known cases of this surveillance bias, such as the increase in the number of isolates typed in 2007-2009, during detailed investigation of emergence events on the Washington coast (Breyta et al. 2013). A second kind of surveillance bias would be variation in the number of fish cohorts sampled by the fish health agencies over time. In general, agency testing has been consistent for adult fish populations in our study area since the 1980s. Since adult fish cohorts comprise 84% of the isolates in our data set we conclude that during 1980-2014 there was a reasonably stable surveillance pressure overall, but this does not rule out changes from year to year that may be important. Our knowledge, and thus our confidence in this assessment, is highest during the more recent period from 2000-2014, allowing us to infer that the temporal peaks observed in 2002-2004 and 2009-2011 reflected actual increases in field prevalence of virus. Since temporal maximums and minimums occurred every 5-7 years over the entire 34-year dataset, it is plausible that they represent real variations in IHNV field prevalence. Drivers of these fluctuations may have included impacts on general fish health from variation in natural factors like ocean conditions, stream conditions, or by variations in anthropogenic factors associated with fish culture. The fact that minimum years were characterized by having few or no positive juvenile cohorts and maximum years had high numbers of both positive adult and juvenile cohorts, suggests that adults were an infection source for juveniles. One possible driver of the temporal pattern in adult fish is persistence of infection from the juvenile stage. However, since the saltwater residence time for the three species differs slightly (2-3 years for sockeye salmon, 3-4 years for Chinook salmon, and 1-3 years for steelhead trout), and since all members of a single juvenile cohort do not migrate back to spawn at the same time, this hypothesis is difficult to assess under the current analysis. Future possible studies to better understand the natural or anthropogenic factors that may have contributed to periodic increases in IHNV prevalence and disease burden would inform management efforts to reduce IHN disease and mortality.

Some level of spatial heterogeneity of IHNV, such as the punctate signals of highest IHN disease incidence at hatcheries, is expected given the life history of fish as well as

concentrating effect of hatcheries. In addition, the majority of surveillance in this report is hatchery-based, and wild fish are under-sampled in this study. What is not clear is why only 4% of hatcheries in this study account for 26% of all reported IHN disease events. If factors at these specific sites that contributed to this signal can be identified, such as the number of species reared, the size of the programs, or water supply conditions, then targeted strategies for overall improvements to IHN disease burden can be developed. A recent example of this was the successful reduction of IHN disease in steelhead trout at a national hatchery when it was shown that delaying exposure of young fish to river water avoided the primary transmission source of IHNV disease (Breyta et al. 2016b).

The drivers of spatial heterogeneity of virus subgroups may also stem in part from hydrological ranges of fish species, as the different subgroups have distinctly different host affinities. For example, the number of sockeye/kokanee salmon populations are greatest in the Washington coast, Puget Sound, and the upper Columbia River basin, and it is in these regions that the UP subgroup is dominant. Therefore the sockeye/kokanee may maintain UP incidence at higher levels in those regions, even in the coastal regions where Chinook salmon and steelhead trout are also found. The numbers of sockeye/kokanee salmon populations are next most numerous in the middle Columbia River region, where UP viruses were found but to a lesser extent than were UC viruses. The number of sockeye or kokanee salmon populations are fewest in the lower segments of the Columbia River and the Snake River regions 5-7, and the species is not found at all in the Oregon coast regions. These regions had no detections of UP viruses, instead viruses of the UC and MD subgroups were dominant. It is not clear if the high numbers of populations of Chinook salmon and steelhead/rainbow trout in these regions means that their UC and MD viruses suppressed UP incidence, or if other factors are more important.

The differences between the U virus subgroups in the coastal Washington and north coastal Oregon regions may be driven by host species distribution as well. Viruses of UC subgroup only occurred in coastal Oregon where there are nearly no sockeye/kokanee salmon. There were no UC viruses detected in coastal Washington despite the presence of their most common host species, Chinook salmon and steelhead trout. However, since sockeye/kokanee salmon are present in coastal Washington, it may be that UP viruses

circulating in coastal Washington, and occasionally detected in Chinook salmon, protected those fish from infection with UC viruses. Laboratory studies show that IHN viruses from each subgroup can infect each of these major host species, and these infections can trigger cross-protective immune responses (Engelking & Leong 1989; LaPatra et al. 1993c; Corbeil et al. 2000; Garver et al. 2005; Kurath et al. 2010). This protective phenomenon may only operate in the coastal waters where overall burden of IHN is relatively low because UP viruses do not seem to have a protective effect for local Chinook salmon in the mid-Columbia region, where Chinook salmon are more frequently found to have UC viruses. As a caveat to this interpretation that applies to the absence of any subgroup throughout this study, it is possible that low levels of UC in coastal Washington or UP in coastal Oregon may have occurred undetected.

Another feature of the North coastal Oregon region 10 is that it was affected by the same ‘tide’ of M group IHN emergence that occurred in coastal Washington in 2007. However, it occurred slightly earlier, caused fewer juvenile infections, and involved the emergence of only one MD type (mG110M) instead of the three (types mG111M, mG110M, and mG139M) observed in coastal Washington. These three types first emerged in the Columbia basin, and of the three, type mG110M was the most widely and frequently detected (Breyta et al. 2013; Breyta et al. 2016a). The detection of only type mG110M in coastal Oregon waters might imply that only the most prominent MD type was able to emerge there, or it may be due to regional differences in surveillance, or mechanism(s) that make the fish of Washington and Oregon coasts different, such as different marine migration patterns.

One factor that may have influenced the distribution of subgroups within the Columbia River basin was the anthropogenic impacts of dams and fish farms. Hydroelectric dams installed between 1955-1961 completely block natural fish migration from the two most inland reaches of the Columbia River basin (regions 4 and 8). In these regions the distribution of IHN subgroups was the most limited. In the upper Columbia region 4 the only viruses detected were UP viruses, and in the upper Snake region 8, only M subgroup viruses were detected. Since UP viruses were also detected in the adjoining middle Columbia region 3, it is possible that the presence of UP viruses in kokanee salmon of the upper Columbia region 4 predated the dams that now prevent anadromous

ocean migration. This is consistent with current theory that U group IHNV in sockeye salmon is the ancestral virus: host relationship (Kurath et al. 2003). The same could have occurred in the upper Snake region 8, except that this region has very few kokanee salmon populations (which are monitored for IHNV, and not found infected to date) but is home to intensive rainbow trout farming. Given this dramatic man-made shift in host availability, it is possible that the vast expansion of M group IHNV in that area displaced U group IHNV below surveillance detection limits.

Emergence patterns of the specific dominant virus types differed among subgroups. Comparing the three subgroups for which surveillance data was most consistent over time, dominant MD viruses demonstrated a serial pattern of emergence and displacement, while UC and UP viruses did not. Instead, dominant UP and UC viruses tended to persist for a very long time throughout the hydrobiological range of the subgroup, and newer dominant types displaced older types only on a local scale, within specific hydrobiological regions. For instance, type mG174U is a UC type that largely displaced mG001U within the lower Snake region 5, and type mG151U largely displaced mG001U in the lower and middle Columbia regions 2-3. Since the analysis here revealed that UC subgroup viruses have a larger footprint than MD, this may allow UC types looser competitive constraints. Similarly the selection pressures for UP viruses may be different than those of MD viruses, due to their extensive range that includes the fish of British Columbia, Alaska, and Russia, suggesting that UP viruses may transmit across oceanic ranges more successfully than UC or MD viruses (Kurath et al. 2003; Rudakova et al. 2007).

In addition to landscape-level differences, there were interesting differences apparent in the ecological strategies demonstrated by UP, UC, and MD subgroup viruses inferred from host species affinities of natural infections. Theory suggests that pathogens fall on a specialist-generalist continuum of host species affinity, utilizing different benefits and costs associated with each end of the spectrum. In practical terms, specialist viruses are most fit and occur predominantly in a single host species, while generalist viruses maintain fitness and occur in more than one host species (Remold 2012; McGee & Remold 2013). Both UP and MD subgroups displayed high fidelity for a single host species, while UC viruses frequently infected two host species (Table 3). The percent

distribution of detection events among the three hosts *O. nerka*: *O. tshawytscha*: *O. mykiss* was 87:7:7 for UP viruses, and 1:13:86 for MD viruses. In contrast, the percent distribution among the same species was 6:62:32 for UC viruses. If we consider only detection events in juvenile fish where disease impacts occur, the ratios are similar, with 90:2:7 for UP, 0:2:98 for MD, and 5:59:30 for UC. This clearly shows that UP viruses are specialists for *O. nerka*, MD viruses are specialists for *O. mykiss*, and UC viruses are generalists that access both *O. mykiss* and *O. tshawytscha*. One implication of this is that steelhead trout suffer disease caused by viruses of two different subgroups, MD and UC, while sockeye salmon and Chinook salmon are affected by viruses of only one subgroup. Another implication is that the selective pressures on the more generalist UC viruses are likely to be different than those acting on UP and MD viruses, which may be detectable via future genetic analysis. Analyses of complete genomes of specific isolates from each of these lineages may reveal more and is currently underway.

These results reveal that IHNV exists in the freshwater environment of the Columbia River basin and surrounding watersheds in a complex patchwork of spatial dominance, host species affinity, and temporal fluctuation. While it is likely that these complex patterns are due to a combination of natural host-pathogen dynamics, anthropogenic factors, and ecological conditions like climate and water quality, the relative influence of any one factor is unknown. Since anthropogenic factors can potentially be changed to benefit fish health in a directed, reasoned way, elucidating more about how the numerous contributing factors drive patterns in IHNV incidence is a promising way to provide direction for future management changes.

Acknowledgements

This work was supported by the U.S. Geological Survey (USGS), the U.S. Fish and Wildlife Service (USFWS) FONS program, and USDA grant 2012-67015-19960 as part of the joint USDA-NSF-NIH Ecology and Evolution of Infectious Disease program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Table 1. Geographic, temporal, genetic, and species distributions of IHNV isolates in this report.

State of origin ^a	Year span	# isolate (#cohorts)			Hosts most commonly positive
		U	M	L	
Washington	1973-2014	445 (275)	402 (175)	0 (0)	Steelhead/rainbow trout, Chinook salmon, sockeye/kokanee salmon
Idaho	1972-2014	491 (153)	472 (210)	0 (0)	Steelhead/rainbow trout, Chinook salmon
Oregon	1958-2014	310 (235)	138 (105)	17 (11)	Steelhead/rainbow trout, Chinook salmon
Montana	2014-2015	2 (2)	0 (0)	0 (0)	Kokanee salmon

^a There were 195 isolates of unknown state or province origin. Isolates from Washington state may originate within the Columbia River basin or from coastal or Salish Sea watersheds. Isolates from Oregon may originate within the Columbia River basin or from coastal watersheds.

^b 'cohorts' reflect bias correction to show the number of typed positive fish cohorts rather than the number of typed isolates (see methods).

Table 2. Hydro-biological range and host species distribution of major U and M subgroups. Number of typed events is shown, from a total of 2590 isolates collected between 1958-2014.

(map #) Hydro-biological region ^a	# events ^b	subgroup, (exceptions) ^c	majority ^d	# cohorts per host species ^b			
				Sockeye or Kokanee salmon	Chinook salmon	Steelhead trout	Rainbow trout
1 Lowest Columbia and Willamette R	185	MD	71%	1	24	89	18
		UC	20%	3	12	19	3
		MH		1	2	8	1
		UP		1	2	1	
2 Lower Columbia R	242	UC	72%	19	92	61	3
		MD	25%		4	52	4
		MH				5	
		MB			1	1	
3 Mid Columbia R	57	UC	69%	4	28	4	
		UP	33%	8	7	4	
		(MB)				1	
		(MD)		1			
4 Upper Columbia R	5	UP	100%	5			
5 Lower Snake R	246	UC	75%		98	43	1
		MD	17%		7	22	3
		MH		n.s.		6	
		MC			3	2	1
		UP			2	1	
6 Mid Snake R	93	(ME)				1	
		UC	86%		60	19	2
		MD	6%		1	4	1
		MH		n.s.		4	
		ME			2		
7 Salmon R	31	(MB)				1	
		UC	71%	3	17	2	
		MB	13%		2	1	1
		MC	7%		1	1	
		(MD)				1	
		(ME)			1		
8 Upper Snake R	69	(MH)				1	
		MC	35%			3	21
		MB	30%			3	18
		MD				2	6
		MA					7
		MH		n.s.	1	2	
		ME				1	1
		MF					2
9 S. OR Coast	9	MN					2
		L	89%		8		
10 N. OR Coast	32	UC	11%	n.s.	1		
		MD	75%		3	13	8
11 WA Coast	79	UC	25%	n.s.	3	5	
		MD	67%		4	46	3
12 Puget Sound	95	UP	33%	17	1	7	1
		UP	94%	89	1		1
		UC		1	2		
		(MH)					1

^a See Figure 4 for map of hydro-biological regions. R, River.

^b Tally of total events and per species reflect 90% of all detections, remaining isolations occurred in Atlantic salmon, chum salmon, and coho salmon; n.s. no spawning population of host species present in this region.

^c Exceptions are defined as 1 event; subgroups are listed in order of prevalence within each region.

^d majority is defined as the natural observed breakpoint of subgroup(s) that comprised $\geq 89\%$ of typed cohorts.

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Table 3. Distribution of five dominant IHNV subgroups among host species.

Host species ^a	# all events (% of total), # juvenile events (% of total) ^b				
	MB	MC	MD	UC	UP
<i>O. nerka</i>	0 (0),	0 (0),	2 (1%),	31 (6%),	228 (87%),
	0 (0)	0 (0)	0 (0)	4 (5%)	37 (90%)
<i>O. mykiss</i> -RT	50 (82%),	48 (79%),	49 (15%),	10 (2%),	4 (2%),
	10 (77%)	11 (65%)	20 (21%)	4 (5%)	2 (5%)
<i>O. mykiss</i> -ST	8 (13%),	9 (15%),	230 (71%),	154 (30%),	13 (5%),
	3 (23%)	6 (35%)	74 (77%)	22 (30%)	1 (2%)
<i>O. tshawytscha</i>	3 (5%),	4 (7%),	43 (13%),	319 (62%),	18 (7%),
	0 (0)	0 (0)	2 (2%)	44 (59%)	1 (2%)

^a *Oncorhynchus nerka* (*O. nerka*) includes both sockeye salmon (anadromous) and kokanee salmon (non-anadromous) because these are similarly managed populations in fish hatcheries, while *O. mykiss* is separated into rainbow trout (*O. mykiss*-RT) and steelhead trout (*O. mykiss*-ST) because these are, in general, differently managed populations, either fish farms or hatcheries, respectively. *O. tshawytscha* is Chinook salmon.

^b the number of events in juvenile fish is shown separately as a general indicator of the incidence of disease events, which occur almost exclusively in juvenile fish.

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Table 4. Focus sites of high disease prevalence and relationship to virus occurrence within their hydrobiological regions.

Site ^a	Years sampled	<u>Focus site</u>	Region # ^c	<u>Hydro-biological region</u>
		Virus subgroups & hosts ^b		Virus subgroups & hosts ^d
a	1984-2014	UC > MD in Chinook, steelhead	5	UC > MD in Chinook, steelhead
b	1999-2013	UC > MD in Chinook	6	UC > MD in Chinook, steelhead
c	1975-2014	UC in Chinook, steelhead	2	UC > MD in steelhead, Chinook
d	1994-2014	UP in sockeye, coho	12	UP in sockeye
e	1981-2013	MD > UC in steelhead	2	UC > MD in steelhead, Chinook
f	1991-2008	MD > UC in steelhead/rainbow, Chinook	1	MD > UC in steelhead, Chinook
g	1991-2014	MD > UC in steelhead, Chinook	10	MD > UC in steelhead, rainbow
h	1988-1998	MB, MC in rainbow	8	MC, MB in rainbow
i	1982-1998	MB, MC in rainbow	8	MC, MB in rainbow
j	1988-1998	MB, MC in rainbow	8	MC, MB in rainbow

^a Focus sites a-j are as in figure 7

^b IHN subgroups that predominate at each focus site are shown. An inequality sign (>) indicates two subgroups are detected in unequal proportions at some sites, while other sites have two subgroups in similar proportions, or single subgroups. The two most common host species at each site are listed in order of frequency of virus detection; Chinook, Chinook salmon; steelhead, steelhead trout; coho, coho salmon; rainbow, rainbow trout; sockeye, sockeye salmon.

^c hydrobiological region from figure 4 where the focus site is located

^d IHN subgroups that predominate in each hydro-biological region are shown, with proportions indicated as in footnote b above. The two most commonly infected hosts in the region are shown (from table 2), in order of frequency of virus detection.

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Figure 1. Number of typed isolates (grey) and cohorts (red) per year, 1980-2014.

Figure 2. Coalescent phylogenetic analysis of all 322 currently known North American IHNV genetic types (haplotypes) based on the 303 nt midG region. Branch lengths indicate substitutions/site/year, and circles at nodes are scaled so that the closer the posterior probability is to 1.0, the larger the circle; root node posterior probability is 1.0. Nodes with small circles should be interpreted as node-less branches polytomous at the next well-supported node to the left. Subgroups are bracketed and labeled at right, with the newly recognized subgroup MH indicated by an asterisk. Red box highlights the 7 taxa designated MN, the M group taxa least derived from M group ancestral node. Specific dominant taxa and their variants are indicated by colored boxes: mG174U orange; mG151U yellow; mG147U blue.

Figure 3. Number of typed IHN positive cohorts of fish between 1980-2014. Detection of U (blue) or M (orange) genogroup IHN types are shown in the three most common host species: steelhead/rainbow trout (top row, A-B); chinook salmon (middle row, C-D); or sockeye salmon (bottom row, E-F). Detection is indicated for adult (solid bars) or juvenile (hatched bars) fish in either the Columbia River basin (left, A, C, E) or coastal and Puget Sound waters (right, B, D, F), where there are fewer fish cohorts overall (note different axis scales in the two regions).

Figure 4. Rivers and hydrographic units of Washington, Oregon, and Idaho states. The Columbia and Snake Rivers are shown in heavy black, tributary rivers are shown in thin black. Hydrographic units delineate freshwater drainage and are color coded into hydro-biological regions first by shared drainage to either the Columbia River, the Snake River, or directly to the Pacific Ocean, and second by distribution of IHN subgroups/subgroups. The uncolored regions are either arid and lack salmonid culture, or are watersheds not connected to the Columbia River basin. The Columbia River watershed includes portions of the Canadian British Columbia province, which lies across the northern border of WA state, and the US state of Montana which lies east of Idaho, so the map of the watershed goes over the state lines of WA and ID. A) Hydro-biological regions are as follows: 1 lowest Columbia River and Willamette, 2 lower Columbia River, 3 middle Columbia River, 4 upper Columbia River, 5 lower Snake River, 6 middle Snake River, 7 Salmon River, 8 upper Snake River, 9 south Oregon coast, 10 north Oregon coast, 11 Washington coast, and 12 Puget Sound. B-E) The four dominant subgroups of IHN are shown in each panel, where the dark color indicates that subgroup is found in an average of 75% of detections and the light color indicates it is found in an average of 20% of detections. F) Bio-hydrographic regions are color coded and labeled for how the dominant IHN subgroup(s) overlap.

Figure 5. A) Temporal recurrence and spatial spread of U genogroup virus types. Every type is indicated by a triangle; where many types have the same x,y values the shape is heavier. The natural breakpoint used to define major types is detection in 5 or more sites and 5 or more years. B) Timeline of incidence of each of the 8 major types. Type mG265U was detected in 9 locations and 3 years, but its temporal incidence is indicated in panel B.

Figure 6. A) Temporal recurrence and spatial spread of M genogroup virus types. Every type is indicated by a diamond; where many types have the same x,y values the shape is heavier. The natural breakpoint used to define major types is detection in 5 or more sites and 4 or more years. B) Timeline of incidence of each of the 7 major types.

Figure 7. Distribution of numbers of typed isolates by collection site, originating either from any fish (black) or from fish reported to have IHN disease (pink), ordered on numbers of all isolates. Out of the 252 collection sites in this report, only the 39 sites with ≥ 10 isolates are shown. The scale is set to best visualize the numbers of disease events, so the number of isolates from the first site, which is greater than 80 typed isolates is indicated above. The 10 sites with ≥ 10 cases of reported disease events are lettered, and site specific details of IHNV subgroups and primary host species are indicated for each site in Table 4.