Vol. 121: 15–28, 2016
doi: 10.3354/dao03030

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 31

Susceptibility of ocean- and stream-type Chinook salmon to isolates of the L, U, and M genogroups of infectious hematopoietic necrosis virus (IHNV)

Daniel G. Hernandez[1,2], Maureen K. Purcell[2], Carolyn S. Friedman[1], Gael Kurath[2,*]

[1]University of Washington, School of Aquatic and Fishery Sciences, Box 355020, Seattle, WA 98195, USA
[2]US Geological Survey, Western Fisheries Research Center, 6505 Northeast 65th St., Seattle, WA 98115, USA

ABSTRACT: This study examined the susceptibility of Chinook salmon *Oncorhynchus tshawytscha* to viral strains from the L, U, and M genogroups of infectious hematopoietic necrosis virus (IHNV) present in western North America. The goal of this investigation was to establish a baseline understanding of the susceptibility of ocean- and stream-type Chinook salmon to infection and mortality caused by exposure to commonly detected strains of L, U, and M IHNV. The L IHNV strain tested here was highly infectious and virulent in both Chinook salmon populations, following patterns previously reported for Chinook salmon. Furthermore, ocean- and stream-type Chinook salmon fry at 1 g can also become subclinically infected with U and M strains of IHNV without experiencing significant mortality. The stream-type life history phenotype was generally more susceptible to infection and suffered greater mortality than the ocean-type phenotype. Between the U and M genogroup strains tested, the U group strains were generally more infectious than the M group strains in both Chinook salmon types. Substantial viral clearance occurred by 30 d post exposure, but persistent viral infection was observed with L, U, and M strains in both host populations. While mortality decreased with increased host size in stream-type Chinook salmon, infection prevalence was not lower for all strains at a greater size. These results suggest that Chinook salmon may serve as reservoirs and/or vectors of U and M genogroup IHNV.

KEY WORDS: Rhabdovirus · *Oncorhynchus tshawytscha* · Subclinical infection · Virus persistence · Fish disease · Columbia River Basin

INTRODUCTION

Infectious hematopoietic necrosis virus (IHNV) is an enveloped, single-stranded, negative-sense RNA virus that causes acute, systemic disease in salmonids. Genetic sequencing of a 303 nucleotide region within the glycoprotein gene (mid-G) from hundreds of virus isolates has identified 3 genetic subgroups of IHNV designated U (upper), M (middle), and L (lower) for their relative geographic occurrence in western North America (Garver et al. 2003, Kurath et al. 2003). Each genogroup of IHNV contains many individual isolates, and genogroup-specific patterns of host specificity have been observed. Isolates in the U genogroup of IHNV are primarily virulent for sockeye salmon *Oncorhynchus nerka* (Garver et al. 2006) and occur as 2 phylogenetic subgroups designated U-P (Pacific) and U-C (Columbia River Basin) (Black 2015). Isolates in the M genogroup are primarily virulent for rainbow trout *O. mykiss* and steelhead trout (anadromous *O. mykiss*) (Garver et al. 2006) and can be separated into 6 subgroups, viz. M-A through M-F (Troyer & Kurath 2003). Isolates in the L genogroup are virulent in juvenile Chinook salmon *O. tshawytscha* of northern California and southern Oregon and form 2 sub-

*Corresponding author: gkurath@usgs.gov

groups designated L-I and L-II (Bendorf et al. 2007, Kelley et al. 2007). While IHNV is detected frequently in up-migrating adult salmonids, the majority of disease is expressed in juvenile fish at propagation facilities. Epidemics of IHNV can result in losses of up to 90% (Bootland & Leong 1999), making IHNV the most threatening viral pathogen to cultured Pacific salmonids.

Chinook salmon populations of the US Pacific Northwest are genetically diverse with expressed phenotypic differences in behavioral patterns, life history, and geographical distribution (Quinn 2005). Two juvenile life history patterns have been defined (Gilbert 1913, Healey 1991). Stream-type Chinook salmon reside in rivers for a full year prior to seaward migration, whereas ocean-type ('sea-type' in Gilbert 1913) migrate to the ocean within their first year of life. These juvenile life history types are linked to a suite of environmental conditions affecting growth rate, and in many cases, they co-vary with adult return migration timing and the spatial distribution of spawning (Taylor 1990a,b, Brannon et al. 2004). Because ocean- and stream-type Chinook salmon are sympatric with other salmonid species throughout many streams of the Pacific Northwest, defining their interactions with virus strains of the dominant IHNV genogroups is critical in understanding their potential role in the ecology and epidemiology of IHNV.

The Columbia River Basin (CRB), which spans portions of the US states of Washington, Oregon, Idaho, Montana, Wyoming, Utah, and Nevada, and the Canadian province of British Columbia, is host to a number of Pacific salmon, trout, and charr species (Fulton 1970, Wydoski & Whitney 2003) that are potential hosts for IHNV. In the CRB, strains of IHNV from both the U and M genogroups are found throughout much of the system (Garver et al. 2003), where disease outbreaks that occur in sockeye salmon are typically caused by U group IHNV, and disease in steelhead/rainbow trout is typically caused by M group IHNV (Garver et al. 2003, Breyta et al. 2013, 2016). Chinook salmon are the dominant salmonid species propagated and present in the CRB (Columbia River DART Database, cbr.washington.edu/dart/). While juvenile Chinook salmon in the CRB experience little disease due to IHNV, the virus is frequently detected in adults at spawning. Consistent presence of IHNV in adult Chinook salmon has led us to hypothesize that this species may function as a reservoir and/or vector of IHNV in the CRB and perhaps facilitate transfer of the virus to other susceptible salmonid species.

Prior to this investigation, information regarding the interaction of Chinook salmon with IHNV was based mostly on observed disease epidemics caused by IHNV isolates from the L genogroup in juvenile Chinook salmon of California hatcheries. The virulence of various IHNV isolates has also been explored in laboratory experiments using juvenile Chinook salmon of southern Oregon and northern California (LaPatra et al. 1993, Bendorf 2010). The investigation presented here used controlled laboratory challenge experiments to define the survival, infection prevalence, viral load, and viral persistence of L, U, and M genogroup representative strains of IHNV in ocean- and stream-type Chinook salmon of the Pacific Northwest. Chinook salmon fry were exposed to selected strains of IHNV by immersion, and infection prevalence and viral load were quantified at 7 and 30 d post exposure (dpe) using both a plaque assay and reverse transcriptase quantitative PCR (RT-qPCR). Further, stream-type Chinook salmon were tested for survival, infection, and viral persistence at a larger size. The goal of this investigation was to establish a baseline understanding of the susceptibility of ocean- and stream-type Chinook salmon to L, U, and M IHNV strains.

MATERIALS AND METHODS

Viral strains

Five IHNV strains representing the 3 genogroups (L, U, and M) were selected for investigation (Table 1). Strain FR0031, representing the L geno-

Table 1. Infectious hematopoietic necrosis virus (IHNV) strains used in experimental challenges

Virus	Genogroup-subgenogroup	Host	Isolation site	Year of isolation
FR0031	L-II	Chinook salmon	Feather River Hatchery, CA	2000
BLK94	U-P	Sockeye salmon	Baker Lake, WA	1994
RB1	U-C	Steelhead trout	Round Butte Hatchery, OR	1975
220-90	M-B	Rainbow trout	Hagerman Valley, ID	1990
DW09	M-D	Steelhead trout	Dworshak Hatchery, ID	2009

group, was isolated from a diseased California juvenile Chinook salmon and was included in this study as a virulent positive control. Strain BLK94, representative of the U-P (Pacific) subgenogroup (Black 2015), was selected for its high virulence in sockeye salmon (Garver et al. 2006). Further, BLK94 is a reference strain representing the IHNV sequence type most commonly detected outside the CRB (mid-G type mG002U) (Emmenegger & Kurath 2002, Kurath & Breyta 2013). Strain RB1, also highly virulent in sockeye salmon (Garver et al. 2006), was selected as a U-C (CRB) subgenogroup representative that has the IHNV sequence type most commonly detected throughout the CRB (mid-G type mG001U) (Garver et al. 2003). Strain 220-90 (subgroup M-B) is an M genogroup representative that has high virulence in rainbow trout (mid-G type mG009M) (LaPatra et al. 1994, Garver et al. 2006). Strain DW09 (subgroup M-D) was included for its high virulence in steelhead trout and as a representative of the mG139M sequence type that has been recently dominant in the Snake River system of the CRB and emerged during 2010/2011 on the Washington coast (Breyta et al. 2013, 2016, Kell et al. 2014). Sources of these strains have been described by Garver et al. (2006) and Kell et al. (2014). Each virus strain was propagated on epithelioma papulosum cyprini (EPC) cells in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, and viral titers were determined as previously described (Batts & Winton 1989).

Fish

All fish rearing and experiments were conducted at the USGS Western Fisheries Research Center (WFRC) wet lab in Seattle, WA, using single-pass, flow-through, sand-filtered, and UV-treated fresh water from Lake Washington. Two Chinook salmon populations, representative of the ocean and stream life history phenotypes, were selected for viral challenges. Ocean-type Chinook salmon, returning to the Soos Creek Salmon Hatchery (Puget Sound), were artificially spawned at the WFRC from male and female gametes of 20 mating pairs. Soos Creek is a tributary of the Green River in central Puget Sound, and this Chinook salmon population is representative of the ocean life history type in this region. For the stream-type Chinook salmon, approximately 2000 fertilized eggs, from a total of 84 adult mating pairs, were obtained from the Little White Salmon National Fish Hatchery in the CRB. All eggs were obtained from females that had been screened and found negative for *Renibacterium salmoninarum*, a vertically transmitted bacterial pathogen that is common in Chinook salmon (Munson et al. 2010). Ocean- and stream-type Chinook salmon eggs were incubated and hatched at the WFRC, where they were reared to approximately 1 g (referred to hereafter as 'fry') at a constant temperature of 10°C and fed daily with a semi-moist pellet diet (BioOregon) at a rate of 1.0 to 2.0% body weight. Stream-type Chinook salmon were subsequently reared to an average size of 28 g for testing at a larger size.

Virus exposures

All viral challenges were conducted at a constant water temperature of 10°C to mimic general conditions of the CRB and allow comparison of results with previous challenges in rainbow trout (Garver et al. 2006), steelhead trout (Breyta et al. 2014), and sockeye salmon (Garver et al. 2006). Three controlled laboratory challenges were performed on Chinook salmon.

In Expt 1, ocean-type Chinook salmon at an average weight of 0.98 g were exposed to each of 5 viral strains (Table 1) or a mock control treatment (virus-free media), creating 6 treatment groups. Triplicate groups of 20 fry were challenged by static immersion for 1 h in 1 l of laboratory water containing 2×10^5 plaque-forming units (PFU) ml^{-1} of a designated virus strain, or mock control, as previously described by Garver et al. (2006). After static immersion, independent flow-through water was resumed to each tank at a final volume of 5 l. These triplicate groups were monitored daily over the course of 30 d for mortality, morbidity, and clinical signs of infection. All deceased fish were removed and stored at −80°C, and a minimum of 20%, selected to include fish from all treatments over time, were later tested by plaque assay (Batts & Winton 1989) for the presence and quantity of IHNV to confirm virus as the likely cause of mortality. Cumulative percent survival (CPS) for each treatment was calculated as the average CPS among triplicate groups of 20 fish exposed to L, U, and M strains of IHNV, and virulence was compared by survival analyses as described below. For each treatment group, a fourth replicate group of 20 fish was challenged, as described above, and 10 fish were sampled at 7 dpe to assay for subclinical infection. Fish of these sampled groups were euthanized with 240 mg l^{-1} of buffered tricaine methanesulfonate (Western Chemical) and individually stored at −80°C until processing. Thirty dpe, a total of 10 surviving

fish per treatment, selected randomly from the 3 replicate tanks, were also sampled for assay of persistent infection.

In Expt 2, stream-type Chinook salmon at an average weight of 1.23 g were challenged using the same methods described above.

In Expt 3, stream-type Chinook salmon at an average weight of 28.1 g were exposed to 1 of 3 viral treatments by batch immersion. Treatment groups in this experiment were viral strains FR0031 (L genogroup), RB1 (U genogroup), DW09 (M genogroup), and the mock control. There were 3 replicate tanks treatment^{-1}, with 25 fish tank^{-1}. The virus treatment concentration was 2×10^5 PFU ml^{-1} in a total of 10 l to accommodate increased fish size. After a 1 h static immersion, single flow-through laboratory water was resumed, and tanks held a total volume of 38 l. Fish were monitored daily, and virulence was assessed as described above. Kidney and spleen tissues from deceased fish were excised, pooled for each fish, and stored at −80°C. As above, a minimum of 20% were later tested by plaque assay to confirm virus-related mortality. Seven dpe, 5 fish were sampled from each tank, totaling 15 fish treatment^{-1}. Kidney and spleen tissues were aseptically excised from each sampled fish and stored at −80°C for later processing. Thirty dpe, 5 surviving fish were sampled from each replicate, for a total of 15 fish treatment^{-1}, for assay of persistent infection.

Virus quantification assays

Viral plaque assay and RT-qPCR measure different indicators of viral presence in a host. Plaque assay quantifies infectious viral titer in PFU g^{-1} of fish (Batts & Winton 1989), while RT-qPCR quantifies viral RNA copy number g^{-1} of fish (viral load) as a proxy for IHNV infection (Purcell et al. 2006). In order to compare each metric of virus infection in 1 g Chinook salmon exposed to IHNV, both assays were carried out in each fish sampled from Expts 1 and 2. Viral titers of fish sampled 7 and 30 dpe were determined using plaque assay of whole fish homogenates as previously described by Batts & Winton (1989), with the exception that fish were diluted 1:4 (weight:volume) in MEM (with no serum) for homogenization. RNA was also extracted from fish homogenates by diluting 500 µl of the homogenate 1:2 in a guanidine-based denaturing solution (Wargo et al. 2010) within 3 min of homogenization. RNA was then extracted as outlined by Wargo et al. (2010). Viral load was determined using the IHNV glycoprotein (G) gene RT-qPCR assay described by Purcell et al. (2006). All RT-qPCR assays were run in duplicate, and absolute quantification standards based on transcript RNA were included on each qPCR plate as previously described by Wargo et al. (2010). For the purpose of our analysis, samples were considered positive if both replicate wells reached the RT-qPCR threshold by 40 cycles (C_t). Samples for which both replicate wells did not reach the threshold by 40 C_t were considered negative by the test. If 1 replicate well reached 40 C_t while the other did not, the test result was considered suspect, but interpreted as a negative test result for statistical analyses. Given this criterion, 38 samples yielded suspect test results but were assigned a negative test result. The calculated average detection limit of the G IHNV RT-qPCR assay in whole fry homogenates was 7272 copies g^{-1} of fish (log 3.86 copies) based on our ability to detect a minimum of 2 viral RNA copies well^{-1} in the transcript RNA standard curves. The detection limit for the plaque assay was 100 PFU g^{-1} of fish, calculated as the virus concentration that would result in 1 plaque formed in 1 of the 2 duplicate wells at the lowest dilution plated.

In the combined kidney and spleen tissues excised from individual 28 g stream-type Chinook salmon exposed to IHNV, only the RT-qPCR assay was carried out. Tissues were weighed and homogenized in a guanidine-based denaturing solution at a dilution of 1:8 as outlined by Wargo et al. (2010), with the exception that homogenization was done in Whirl-Pak® bags using a roller rather than a stomacher. While the majority of tissue samples weighed ≥1 g, some samples collected 30 dpe were too small for processing as individual fish, so tissues from 2 fish were pooled in 13 instances. For the purpose of statistical analysis, fish tissues processed individually or as a pool from the tissues of 2 fish represented a single data point. For kidney and spleen samples, the detection limit of the G IHNV RT-qPCR assay was 4545 copies g^{-1} of fish tissue (log 3.65 copies), which differed from the detection limit in whole fry due to differences in dilutions during sample processing.

Statistical analyses

Survival curves for triplicate groups of 20 fry in each of the 5 viral treatments or mock control were estimated using the Kaplan-Meier method (SigmaPlot, version 13). No significant differences were found for replicate tanks within treatments except for the FR0031 treatment in 1 g stream-type Chinook salmon. In this treatment, 1 of the 3 replicate tanks

differed significantly in survival ($p = 0.023$; see Table 2), but repeated analysis using individual replicates did not change the statistical outcome of the data set. Subsequent analyses were therefore conducted using data pooled from the replicate tanks within each treatment. Survival curves were compared within and between experiments with a log-rank test where, if the log-rank test for the survival curves was greater than would be expected by chance ($p < 0.001$), there was a significant difference between survival curves. To identify the viral treatment group that differed, multiple pairwise comparisons using the Holm-Sidak method were carried out with an overall significance level of $\alpha = 0.05$ (SigmaPlot, version 13). Significant differences in infection prevalence were determined using Fisher's exact test with Bonferroni corrected p-values to account for multiple pairwise comparisons. In Expts 1 and 2, a p-value ≤ 0.0125 was considered significant, while in Expt 3, $p \leq 0.025$ was considered significant. Analysis of variance (ANOVA) and Tukey's HSD test were used to assess differences in mean log viral load in positively infected fish from distinct viral treatment groups. Pearson's product moment correlation analysis was used to measure the linear correlation of viral titer (by viral plaque assay) and viral load (by RT-qPCR) for each juvenile fish that was positive by 1 or both techniques, using combined data from fish sampled at 7 and 30 dpe.

RESULTS

Survival of ocean- and stream-type Chinook salmon fry exposed to L, U, and M strains of IHNV

Ocean-type Chinook salmon fry in the positive control L genogroup FR0031 treatment showed an average CPS of 63%, with kinetics illustrated in Fig. 1a. The onset of mortality occurred at 7 dpe with a steep decline in survival between Days 7 and 11, followed by more gradual losses through the end of the 30 d monitoring period. Clinical signs of disease including exophthalmia, skin darkening, and hemorrhaging were observed in approximately one-third of the fish prior to death (Table 2). Mortality occurred in the RB1 treatment at 17 and 22 dpe, resulting in an average CPS of 97% for this treatment. Although there was no mortality (average 100% CPS) in the other 3 treatments, clinical signs similar to those described above were observed in a small number of fish in the BLK94, RB1, and DW09 treatments (approximately 1 to 5 fish treatment^{-1}; Table 2). The mock-exposed fish also had an average CPS of 100%. The log rank statistic for the survival curve of fish in the FR0031 virus treatment differed significantly ($p < 0.001$) from those of fish in all other virus treatments and the mock control (Fig. 1a). There were no significant differences among treatment groups other than FR0031. A subset of 7 of 24 fish

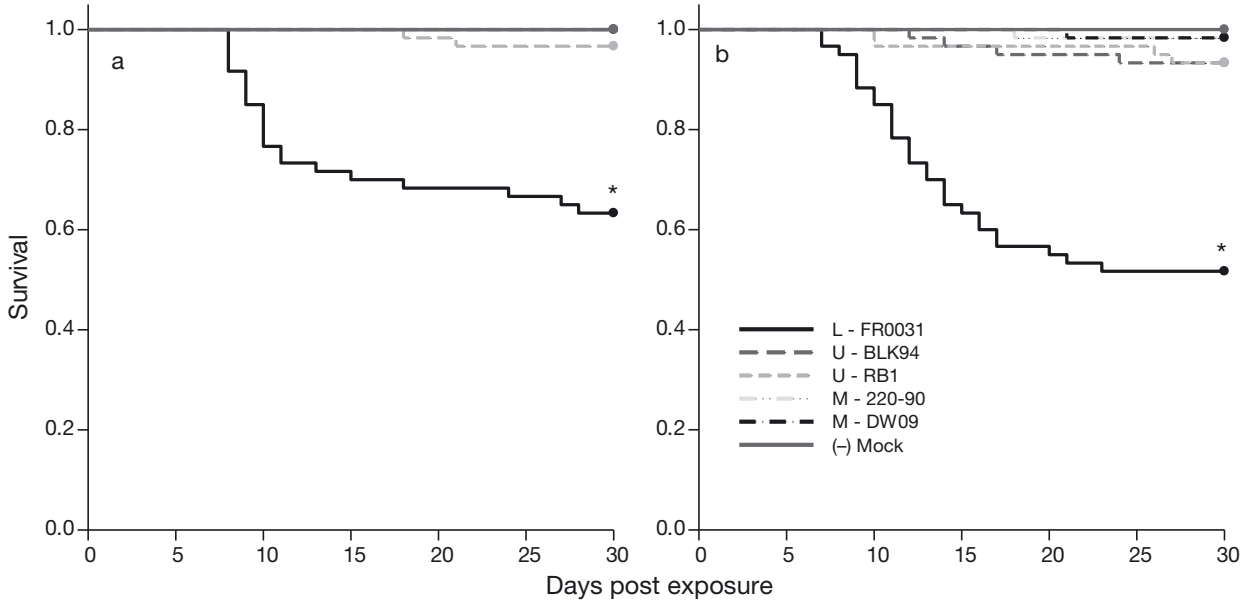

Fig. 1. Daily cumulative proportion survival of 1 g (a) ocean- and (b) stream-type Chinook salmon *Oncorhynchus tshawytscha* exposed by immersion to 5 strains of infectious hematopoietic necrosis virus (IHNV; strain names and genogroups are given in the key). Data are pooled from triplicate groups of 20 fish treatment^{-1}. Data overlap on the 100% survival line for several virus treatments. Asterisks indicate that fish in the positive control L genogroup (FR0031) treatment had significantly lower survival than fish in the other treatments ($p < 0.001$). No other significant differences were found among virus treatments

Table 2. Summary of results from 3 experiments in which Chinook salmon *Oncorhynchus tshawytscha* were exposed to various strains of IHNV and then monitored for both survival (cumulative percent survival, CPS) and infection, measured as viral load by RT-qPCR and viral titer by plaque assay (PA). The number of observations of clinical signs during daily monitoring of each replicate tank in each treatment is shown. Note that this is not necessarily equivalent to the actual number of fish that developed signs because the same fish may have been observed on sequential days. dpe: days post exposure; na: not applicable, as the PA was not conducted for samples from 28 g fish

Host Virus (genogroup)	CPS			Clinical signs	No. positive/total tested			
	Individual tank	Mean	SEM		7 dpe		30 dpe	
					RT-qPCR	PA	RT-qPCR	PA
Ocean-type Chinook, 1g								
FR0031 (L)	55, 60, 75	63	6.01	5, 6, 8	7/10	10/10	2/5	1/5
BLK94 (U)	100, 100, 100	100	0.00	0, 1, 2	0/10	1/10	1/10	0/10
RB1 (U)	100, 100, 90	97	3.33	0, 0, 1	5/10	3/10	0/10	0/10
220-90 (M)	100, 100, 100	100	0.00	0, 0, 0	2/10	2/10	1/10	0/10
DW09 (M)	100, 100, 100	100	0.00	0, 2, 3	0/10	0/10	2/10	1/10
Mock	100, 100, 100	100	0.00	0, 0, 0	0/10	0/10	0/5	0/5
Stream-type Chinook, 1g								
FR0031 (L)	32, 47, 74	51	12.29	11, 16, 19	9/10	9/10	3/8	3/8
BLK94 (U)	90, 95, 95	93	1.67	2, 2, 5	5/10	6/10	0/10	0/10
RB1 (U)	90, 95, 95	93	1.67	1, 2, 2	5/10	9/10	2/10	1/10
220-90 (M)	95, 95, 100	97	1.67	0, 0, 2	2/10	2/10	0/10	1/10
DW09 (M)	95, 100, 100	98	1.67	0, 2, 2	3/10	3/10	0/10	0/10
Mock	100, 100, 100	100	0.00	0, 0, 0	0/10	0/10	0/5	0/5
Stream-type Chinook, 28g								
FR0031 (L)	67, 80, 87	78	5.85	2, 2, 5	14/15	na	6/13[a]	na
RB1 (U)	100, 100, 100	100	0.00	0, 0, 0	4/15	na	0/9[a]	na
DW09 (M)	100, 100, 100	100	0.00	0, 0, 0	3/15	na	2/13[a]	na
Mock	100, 100, 100	100	0.00	0, 0, 0	0/15	na	0/11[a]	na

[a]Some samples from 28 g fish were tested as pools of tissue from 2 fish (see 'Materials and methods')

that died during challenge were tested, and all were found positive for virus by plaque assay with an average titer of 5.13×10^6 PFU g^{-1} (SEM = 0.64), indicating virus infection as the cause of the observed mortality.

In stream-type Chinook salmon fry, the L genogroup FR0031 treatment caused slightly higher, but not significantly different, mortality than in ocean-type fry, resulting in an average CPS of 51%, with kinetics shown in Fig. 1b. Mortality began at 6 dpe and continued through 16 dpe, plateauing at 22 dpe. Clinical signs of disease, as described above, were observed in approximately two-thirds of the fish (Table 2). The other 4 viral treatments each had low mortality. The U genogroup treatments BLK94 and RB1 each had an average CPS of 93%, and the M genogroup treatments 220-90 and DW09 each had an average CPS of 98%. Clinical signs, as described above, were observed in a small number of fish in each of the U and M viral treatments (at most 2 to 11 fish treatment^{-1}; Table 2). There was no mortality in mock-exposed fish. As observed in ocean-type Chinook fry, only the survival curve for the FR0031 treatment differed significantly (Fig. 1b) when compared to all other treatment groups. Differences in survival among the U and M viral treatments were not statistically significant. A subset of 9 of 41 fish that died were tested, and all were found positive for virus by plaque assay with an average titer of 4.1×10^6 PFU g^{-1} of fish (SEM = 0.54).

Virulence comparisons between the 2 Chinook salmon populations were performed using survival data from each virus treatment. The log rank statistic for each pair of survival curves indicated that only the BLK94 virus treatment differed significantly ($p = 0.043$) between ocean- and stream-type Chinook salmon fry (Fig. 1).

Infection of ocean- and stream-type Chinook salmon fry at 7 dpe

Among ocean-type fry in the positive control L genogroup FR0031 treatment, 100% of the fish sampled were infected at 7 dpe when assessed by viral plaque assay (Fig. 2a). Using this method, infection occurred in 10 to 30% of fish in the U genogroup BLK94 and RB1 treatments, and in 0 to 20% of fish in

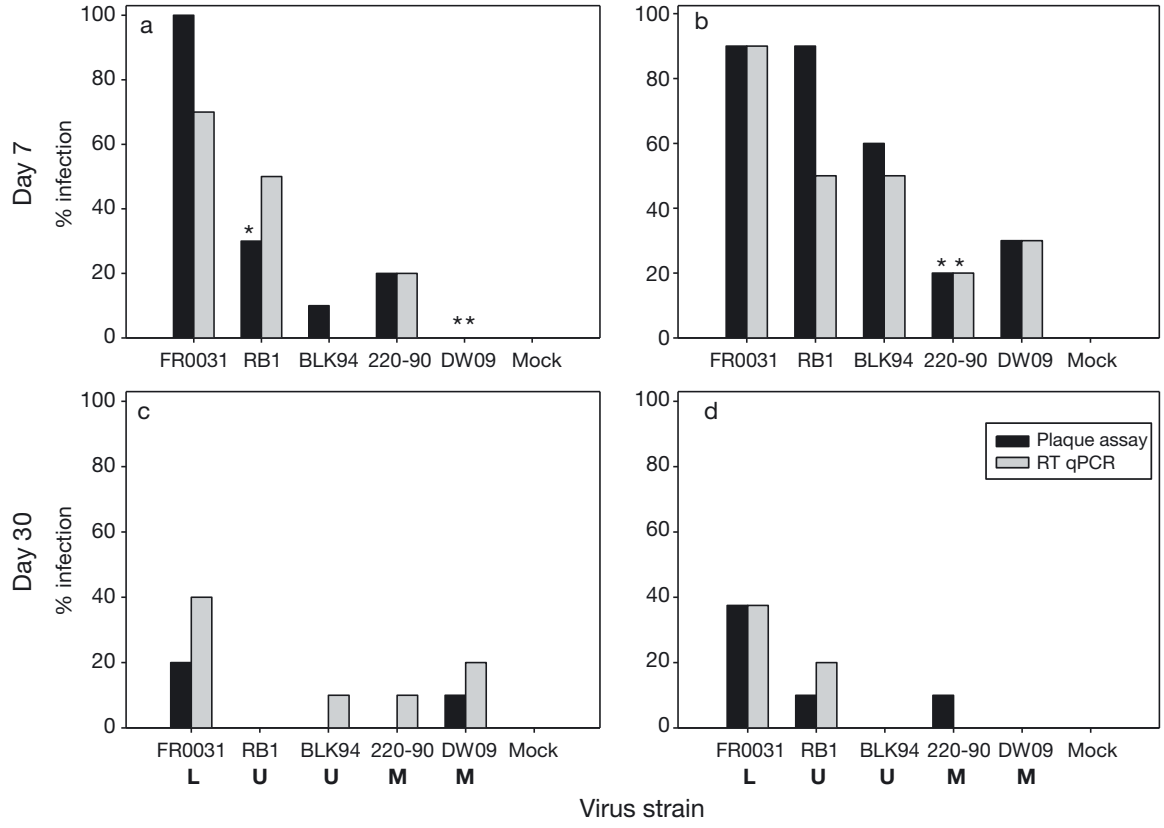

Fig. 2. Frequency of infection of 1 g (a,c) ocean- and (b,d) stream-type Chinook salmon *Oncorhynchus tshawytscha* sampled 7 d (a,b) and 30 d (c,d) post exposure to 5 L, U, and M IHNV isolates. Ten fish per treatment group were sampled and processed by plaque assay and RT-qPCR, with the exception of the FR0031 treatment groups at 30 d, where 5 ocean- and 8 stream-type fish were processed. Asterisks denote significant differences ($p < 0.0125$) from the positive control FR0031 treatment tested by the relevant assay

the M genogroup 220-90 and DW09 treatments. When assayed by RT-qPCR, infection was detected in 70% of ocean-type fry in the FR0031 treatment, 0 to 50% of fish in the BLK94 and RB1 treatments, and 0 to 20% of fish in the 220-90 and DW09 treatments. When compared to the positive control FR0031 treatment, infection prevalence in ocean-type fry was significantly lower ($p < 0.0125$) for all other viral treatments groups tested at 7 dpe by viral plaque assay. By RT-qPCR, infection prevalence was significantly lower ($p < 0.0125$) only in the BLK94 and DW09 treatments where no infection was detected.

In stream-type Chinook salmon, 90% of the fish in the FR0031 treatment were infected at 7 dpe when assayed by viral plaque assay (Fig. 2b). Infection prevalence ranged from 60 to 90% in the BLK94 and RB1 treatments, and 20 to 30% in the 220-90 and DW09 treatments. When assayed by RT-qPCR at 7 dpe, infection prevalence in the FR0031, 220-90, and DW09 virus treatments was the same as determined by plaque assay, and prevalence in the BLK94 and RB1 treatments was slightly lower, at 50%. At 7 dpe, infection prevalence differed significantly from the FR0031treatment only in the 220-90 treatment group ($p = 0.0055$).

Persistence of infection in ocean- and stream-type Chinook salmon fry at 30 dpe

At 30 dpe, infection prevalence in ocean-type Chinook fry in the FR0031 L genogroup treatment was 20% when assayed by viral plaque assay (Fig. 2c). By this assay, infection prevalence in the DW09 (M) treatment was 10%, and no infection was detected in the BLK94, RB1, or 220-90 viral treatments. When assayed by RT-qPCR, 40% of ocean-type fry in the FR0031 treatment were infected at 30 dpe, and infection prevalence ranged between 0 and 20% in the U and M viral treatments.

In stream-type Chinook salmon fry in the FR0031 treatment, infection prevalence was 37.5% at 30 dpe,

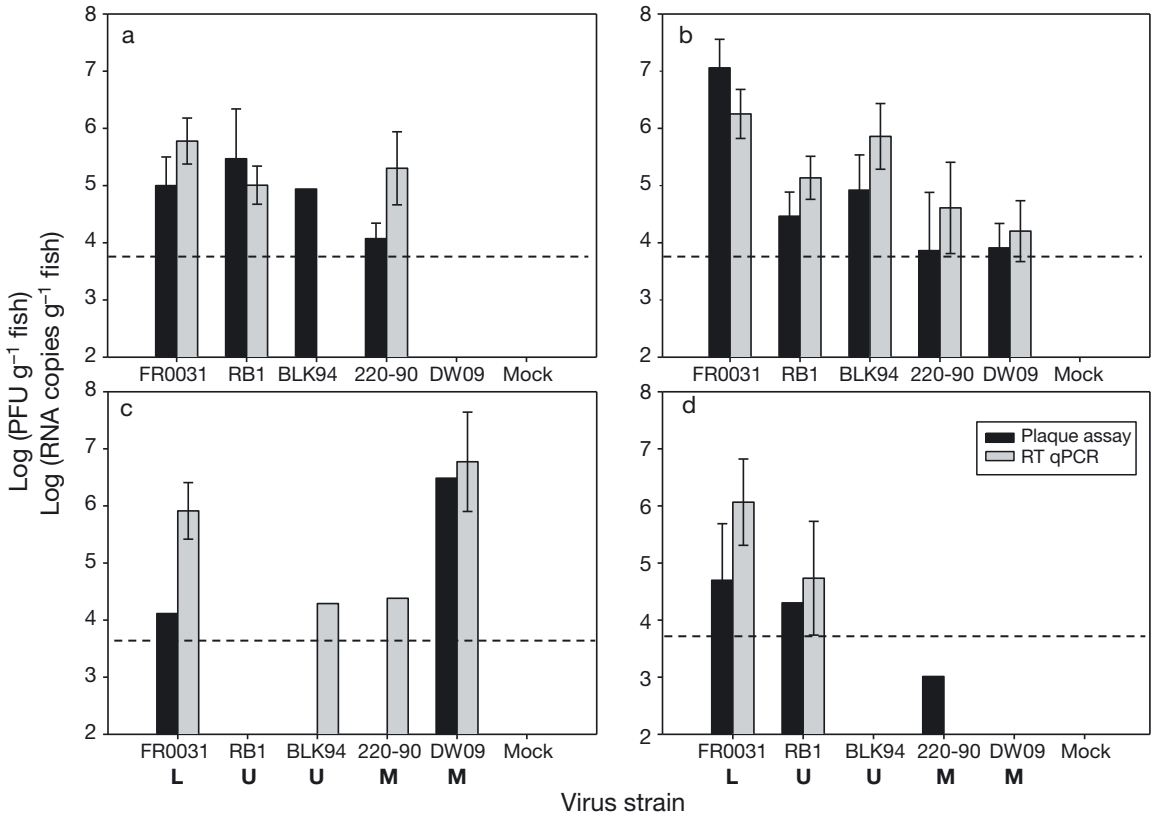

Fig. 3. Mean (± SE) viral load of 1 g (a,c) ocean- and (b,d) stream-type Chinook salmon *Oncorhynchus tshawytscha* infected 7 d (a,b) and 30 d (c,d) post exposure to 5 L, U, and M IHNV isolates. Viral quantities were determined by plaque assay (plaque-forming units, PFU g^{-1}) and RT-qPCR (viral RNA copies g^{-1}). Only virus-positive fish were used to determine averages. Where there are no error bars, data represent a single fish as detailed in Table 2. The detection limit for each assay is denoted by the dashed horizontal lines

and prevalence ranged between 0 and 10% in the U and M viral treatments when assayed by viral plaque assay (Fig. 2d). When assayed by RT-qPCR, infection prevalence in the FR0031 treatment was 37.5%, as with viral plaque assay. By this assay, infection prevalence in the RB1 (U) treatment was 20%, and no infection was detected in the BLK94, 220-90, and DW09 viral treatments at 30 dpe. Pairwise comparisons using Fisher's exact test did not find variation in infection prevalence between viral treatments to be significant within or among Chinook salmon hosts tested at 30 dpe.

Quantity of virus in ocean- and stream-type Chinook salmon fry at 7 and 30 dpe

Ocean- and stream-type Chinook salmon fry positively infected with L, U, or M IHNV strains had viral titers ranging between 10^3 and 10^7 PFU g^{-1} of fish as determined by plaque assay and between 10^4 and 10^6 viral RNA copies g^{-1} of fish by RT-qPCR (Fig. 3). Among ocean-type fry positive at 7 dpe with L, U, and M virus strains, little variation was noted among mean log viral titers or mean log viral loads (Fig. 3a). In stream-type fry, however, viral titers and viral loads were highest in fish with the FR0031 strain and lowest in fish with the M strains, although the differences were not significant (Fig. 3b). Overall, fewer fish were infected at 30 dpe (Fig. 3c,d); however, viral titers and viral loads were not significantly lower than those observed at 7 dpe. ANOVA of positively infected fish indicated no significant differences in viral titer or viral load among viral treatments, within or between Chinook salmon hosts at both 7 and 30 dpe.

Survival of stream-type Chinook salmon exposed at a greater size to L, U, and M strains of IHNV

In 28 g stream-type Chinook salmon, average CPS in the FR0031 treatment was 78% (Fig. 4). The onset of mortality occurred at 11 dpe, with a decline in sur-

vival between Days 11 and 18, followed by gradual losses through the end of the 30 d monitoring period. Clinical signs of disease including exophthalmia, skin darkening, and some hemorrhaging were observed prior to death in the majority of fish that died (Table 2). There was no mortality in the RB1 (U) and DW09 (M) viral treatments or the mock control group. The survival curve of the FR0031 treatment differed significantly ($p < 0.001$) from those of the U and M viral treatments and mock control (Fig. 4). All fish that died were tested and found positive for virus by plaque assay with an average titer of 1.82×10^4 PFU g^{-1} of combined kidney and spleen tissues (SEM = 1.17).

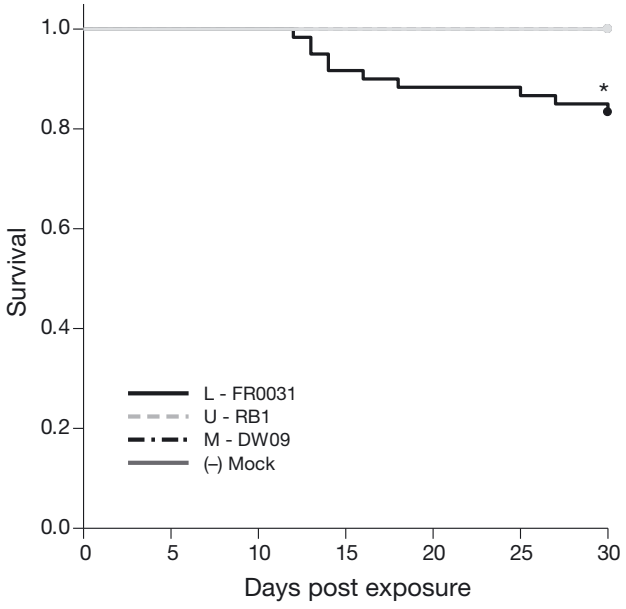

Fig. 4. Daily cumulative proportion survival of 28 g stream-type Chinook salmon *Oncorhynchus tshawytscha* exposed by immersion to 3 strains of IHNV. Data shown are pooled from triplicate groups of 20 fish treatment^{-1}. There was 100% survival in fish in the RB1 (U) and DW09 (M) virus treatments (lines overlap at 100%). Fish in the positive control FR0031 L genogroup treatment had significantly lower survival than fish in the other treatments (*$p < 0.001$)

Infection of stream-type Chinook salmon of a greater size at 7 and 30 dpe

At 7 dpe, 93% of the 28 g stream-type Chinook salmon in the FR0031 (L) treatment were infected when assayed by RT-qPCR (Fig. 5a). Infection prevalence was 26% in the RB1 (U) treatment and 20% in the DW09 (M) treatment. When compared to fish in the positive control FR0031 viral treatment, infection prevalence was significantly less ($p < 0.025$) in both the U and M treatment groups at 7 dpe, and prevalence did not differ significantly between the U and M treatment groups. At 30 dpe, infection prevalence of 28 g stream-type Chinook salmon in the FR0031 treatment was 46% (Fig. 5b). There was no detectable infection in the RB1 treatment, while infection prevalence in the DW09 strain was 15%. There were no significant differences in infection prevalence between any of the treatment groups at 30 dpe.

Mean log viral loads at 7 dpe were highest in fish infected with the FR0031 strain; however, little variation was observed in mean log viral loads of fish

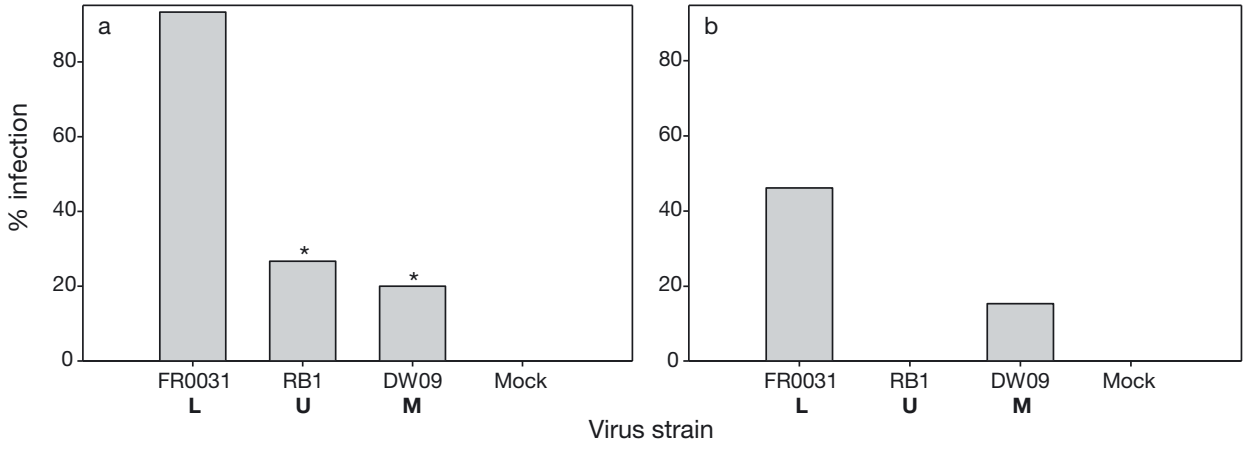

Fig. 5. Frequency of infection of 28 g stream-type Chinook salmon *Oncorhynchus tshawytscha* sampled (a) 7 d and (b) 30 d post exposure to L, U, and M strains of IHNV by immersion. Samples consisted of pooled kidney and spleen tissues processed by RT-qPCR. Day 7 data represent groups of 15 fish processed individually, and Day 30 data are groups of 9 to 13 fish processed individually or as pools of 2 fish. Asterisks denote significant differences ($p < 0.025$) from the positive control FR0031 treatment

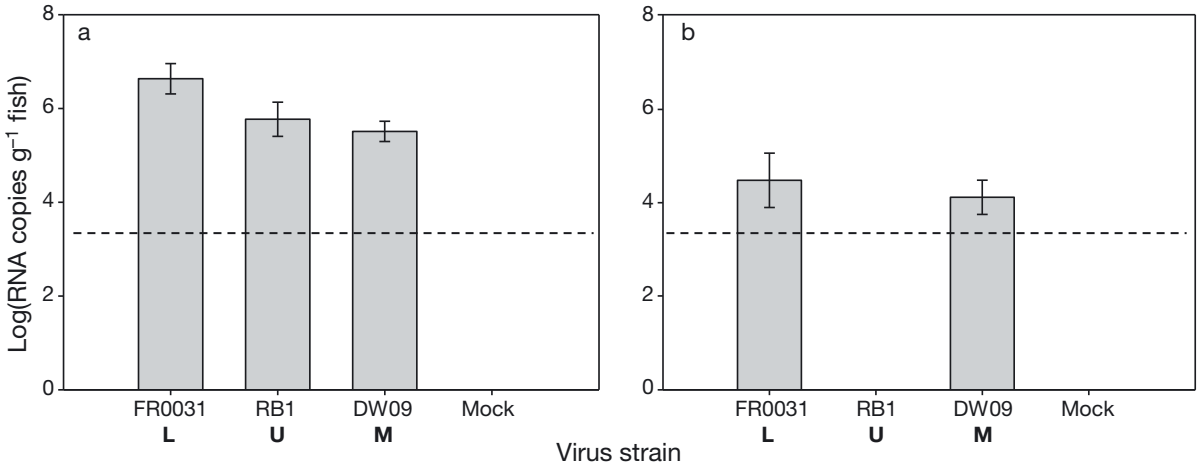

Fig. 6. Mean (± SE) viral load of 28 g stream-type Chinook salmon *Oncorhynchus tshawytscha* infected (a) 7 d and (b) 30 d post exposure to L, U, and M IHNV isolates. Viral load was determined by RT-qPCR in viral RNA copies g^{-1} of fish tissue. Only virus-positive fish were used to determine averages. The detection limit for the RT-qPCR assay is denoted by the dashed horizontal lines

infected with the U or M strains of IHNV (Fig. 6a). While viral loads were higher at 7 dpe than at 30 dpe (Fig. 6), there were no significant differences in mean log viral loads among viral treatment groups between the 2 time points.

Correlation of plaque assay titers and RT-qPCR viral load

Among the total of 223 fry from Expts 1 and 2 that were tested by both plaque assay and RT-qPCR, 41 were positive by both assays, and 8 were positive by RT-qPCR but negative by plaque assay. Another 11 samples were positive by plaque assay but negative by RT-qPCR; however, 9 of these 11 samples were classified as suspect by RT-qPCR (1 replicate well tested positive and the other tested negative, see 'Materials and methods'). Viral titer and viral load estimates from individual fish that were positive by both viral plaque assay and RT-qPCR indicated a significant positive correlation coefficient ($r = 0.644$, $p < 0.005$). This was only slightly different from the value calculated when samples that were positive by only 1 of the techniques were also included ($r = 0.648$, $p < 0.005$; Fig. 7).

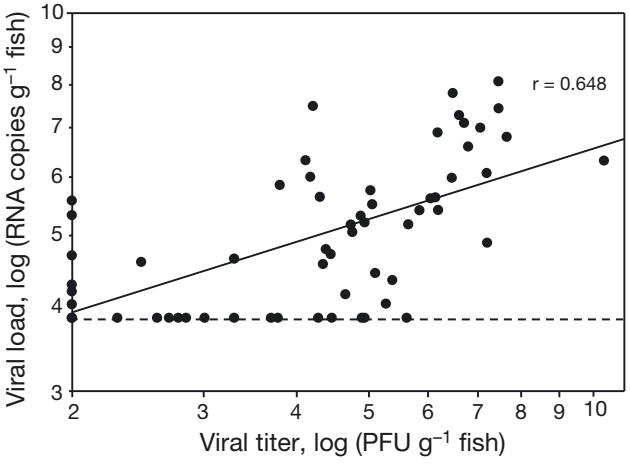

Fig. 7. Correlation between viral quantities determined by plaque assay and by RT-qPCR ($r = 0.648$). Data shown are for individual ocean- and stream-type Chinook salmon *Oncorhynchus tshawytscha* exposed to IHNV at 1 g and assayed for virus by both methods at 7 or 30 d post exposure (see Fig. 2). Log plaque-forming units (PFU) g^{-1} of fish was determined by plaque assay and log viral RNA copies g^{-1} of fish was determined using the IHNV RT-qPCR assay. The lower limits of detection were 100 PFU g^{-1} of fish (log 2, *x*-axis) and 7272 RNA copies g^{-1} of fish (log 3.86, *y*-axis; dashed line). Eight samples that were positive by RT-qPCR and not by plaque assay are displayed on the *y*-axis. Eleven samples that were positive by plaque assay but not by RT-qPCR, as well as 5 samples that were positive by RT-qPCR, but below the calculated average for the detection limit, are displayed on the dashed line

DISCUSSION

Variation in the virulence of IHNV strains was previously reported in a foundational study using isolates originally differentiated by electropherotype (now known to include U, M, and L strains) in controlled challenges of juvenile Chinook salmon and steelhead trout (LaPatra et al. 1993). Subsequent investigations have described the host-specific viru-

lence and the infectivity of U and M genogroup strains of IHNV in rainbow trout (Garver et al. 2006, Peñaranda et al. 2009, Wargo et al. 2010), steelhead trout (Breyta et al. 2014), and sockeye salmon (Garver et al. 2006, Purcell et al. 2009, Wargo et al. 2010). More recently, virulence of virus strains representing the L genogroup has been tested in California Chinook salmon (Bendorf 2010). Altogether, these studies have broadened our understanding of the host specificity of IHNV in western North America. The data presented here expand on this knowledge by defining the survival, infection prevalence, viral load, and viral persistence of L, U, and M genogroup representative strains of IHNV in ocean- and stream-type Chinook salmon.

In rainbow trout, viral exposures with M genogroup isolates of IHNV resulted in high mortality, whereas exposures with U genogroup isolates resulted in low mortality (Garver et al. 2006). In sockeye salmon, the converse was observed, where exposure to U isolates of IHNV resulted in high mortality and M isolates caused low mortality (Garver et al. 2006). While both U and M type viruses were able to enter and infect both sockeye salmon and rainbow trout, in each host the more virulent isolate replicated faster, to higher levels, in a higher proportion of the fish, and persisted longer, than the less virulent virus (Peñaranda et al. 2009, Purcell et al. 2009).

In our investigation, the higher virulence of the L genogroup strain was consistent with the losses observed in the field during epizootic events in juvenile Chinook salmon of California hatcheries, and with previously reported L genogroup virulence challenges (Bendorf 2010). Among the U and M strains tested here, the observation of low or no mortality also mimicked general field patterns where Chinook salmon generally show little disease when infected with U or M group IHNV (Garver et al. 2003, Kurath & Breyta 2013). In stream-type Chinook fry, the onset of mortality in the U strain treatments was earlier than in the M strain treatments. While not statistically significant, the U strains also resulted in measurably higher mortality than the M strains.

Infection prevalence at 7 dpe, as determined by both viral plaque and RT-qPCR assays, indicated greatest susceptibility of both ocean- and stream-type Chinook fry to the L genogroup strain of IHNV. Both infection assays indicated that ocean- and stream-type Chinook fry could also become infected with U and M strains of IHNV. Although not statistically significant, a trend of higher susceptibility to infection with U genogroup strains than M strains was noted in both Chinook salmon types when sampled at 7 dpe. This pattern was consistent among stream-type Chinook salmon exposed both at 1 and 28 g.

When infected, quantities of virus in ocean-type Chinook fry were generally comparable among viral treatments at 7 dpe. However, in stream-type Chinook fry, a trend was observed in which the highest virus levels were found in the L virus treatment group, with moderate levels in the U virus treatments, and lower levels in the M virus treatments. As with infection prevalence, this pattern was consistent among stream-type Chinook salmon exposed both at 1 and 28 g. Although no significant differences were noted in viral quantities among IHNV strains tested at 7 dpe, it is possible that differences in kinetics may have occurred at earlier times in infection. While the pattern of higher infectivity and virulence with U strains than with M strains was not statistically significant in Chinook salmon as it was in sockeye salmon (Purcell et al. 2009), the observed trend may be biologically relevant and should be tested with larger sample numbers in the future.

In both Chinook salmon populations, infection prevalence was lower at 30 dpe than at 7 dpe. While not completely concordant, both virus quantification assays detected persistence of L, U, and M strains of IHNV in small numbers of both ocean- and stream-type Chinook salmon. Notably, persistent infections at 30 dpe showed virus quantities similar to those in fish positive at 7 dpe. While infection with an L genogroup strain of IHNV may persist in ocean-type Chinook salmon for as long as 216 dpe (Bendorf 2010), here infections with U and M strains of IHNV are shown to persist in the 2 dominant Chinook salmon life history phenotypes. Altogether, the isolation of infectious L, U, and M IHNV strains from both ocean- and stream-type Chinook salmon at 30 dpe supports previously published evidence for the possibility of a carrier state for IHNV in Chinook salmon (St-Hilaire et al. 2001).

Size-dependent susceptibility to IHNV has been previously observed in stream-type Chinook salmon (LaPatra 1989) and other salmonid hosts (LaPatra et al. 1990), where increased host size and/or age was associated with decreased mortality upon virus exposure. Consistent with these previously published reports, 28 g stream-type Chinook salmon in the L IHNV treatment had increased survival relative to 1 g fish, although this difference was not statistically significant. While infection prevalence at 7 dpe was lower in 28 g fish in the U and M viral treatments when compared to 1 g stream-type Chinook salmon, infection of fish in the L virus treatment was equal in

the 2 size classes. While infection prevalence decreased over the course of 30 d, viral loads were not significantly less than reported at 7 dpe. Thus, results from Expts 2 and 3 support previous reports of size-dependent susceptibility of Chinook salmon to IHNV-related mortality, but we did not observe a size-dependent decrease in susceptibility to infection for the L virus. At the 28 g size chosen to represent out-migrating juveniles, stream-type Chinook salmon can become subclinically infected with L, U, and M strains of IHNV.

One of the goals of our experimental design was to compare viral plaque assay and RT-qPCR as indicators of viral infection. Previously published correlation analyses between viable IHNV quantities in infected fish tissues measured by plaque assay and viral load by RT-qPCR showed a strong correlation ($r = 0.94$) and an average ratio of 8.3×10^3 genome copies per PFU in infected fish tissue (Purcell et al. 2006). In our investigation, we observed a significant but moderate correlation ($r = 0.648$), and our average ratio of viral RNA copies to PFU was 74:1. Although the 2 studies are not strictly comparable, it is notable that our ratio was approximately 100-fold lower than the previously published relationship. This difference in ratio may be due to differences in fish species, experimental design, RNA extraction, or RT-qPCR methods. Here, whole fish were homogenized in MEM to preserve the ability to detect viable virus prior to a dilution in a guanidine-based denaturing solution for RNA extraction, while Purcell et al. (2006) homogenized tissues directly in a commercial RNA extraction kit. In general, the RT-qPCR assay has been reported to have increased sensitivity relative to viral plaque assay. Consistent with this, in our study, we found that 8 of the 60 samples tested by both methods were positive by RT-qPCR but not by plaque assay. However, we also had 11 instances where samples were positive by plaque assay but negative by RT-qPCR. Nine of these 11 samples actually had 'suspect' results in the RT-qPCR (1 positive and 1 negative in the duplicate wells), suggesting that the viral copy number in these samples was near the detection limit of the assay. Altogether, the data suggest an overall low sensitivity of our RT-qPCR assay relative to that of Purcell et al. (2006). It is possible that homogenization in MEM prior to the guanidine-based extraction may have resulted in a reduced RNA yield due to RNA degradation. Nevertheless, the ability to re-isolate culturable virus from Chinook salmon exposed to IHNV demonstrated that the fish were infected with viable virus of all 3 genogroups. The positive correlation observed between viable viral titers and viral loads supports the use of the G gene IHNV RT-qPCR assay as a higher throughput technique for determining IHNV prevalence and infection levels in fish.

The observations outlined in this investigation confirm that Chinook salmon fry and larger juveniles can be infected with U and M strains of IHNV, maintain infections for at least 30 d, and not experience high levels of mortality. Such observations mirror field patterns of IHNV detection in Chinook salmon (Garver et al. 2003, Kurath & Breyta 2013). Altogether, the data presented here support the hypothesis that Chinook salmon may be reservoirs and/or vectors of U and M genogroup IHNV. Such a reservoir role would not be unique to IHNV. In the ecology of viral hemorrhagic septicemia virus (VHSV) in the US Great Lakes, the round goby *Neogobius melanostomus* and yellow perch *Perca flavescens* have been identified as species that may be subclinical carriers of VHSV transmissible to many other fish species (Bain et al. 2010). Although IHN disease in juvenile CRB Chinook salmon is relatively infrequent, it has been observed occasionally and can cause substantial losses (Kurath & Breyta 2013), such as during a 1995 epidemic in a federally threatened stream-type Chinook population (W. Groberg pers. comm.). While further studies are needed to better understand the role that Chinook salmon may play in the ecology and epidemiology of IHNV in western North America, this study is the first to characterize the susceptibility of juvenile Chinook salmon to U and M IHNV in a controlled laboratory setting.

Acknowledgements. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1256082, the United States Geological Survey, the United States Fish and Wildlife Service, the Puget Sound Anglers (Fidalgo Chapter), the University of Washington Graduate Opportunities & Minority Achievement Program (GOMAP), and the University of Washington College of the Environment under the Galen and Helen Maxfield Endowed Fisheries Fellowship and the Robert E. Resoff Endowed Scholarship. We thank Mike Wilson of the Soos Creek Salmon Hatchery and Casey Risley of the Little White Salmon National Fish Hatchery for providing us with fish for these controlled laboratory studies. The manuscript was considerably improved as a result of the insightful comments of Thomas P. Quinn and James R. Winton. Use of any trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government. All animal experiments were approved by the University of Washington Institutional Animal Care and Use Committee.

LITERATURE CITED

Bain MB, Cornwell ER, Hope KM, Eckerlin GE and others (2010) Distribution of an invasive aquatic pathogen (viral hemorrhagic septicemia virus) in the Great Lakes and its relationship to shipping. PLoS ONE 5:e10156

Batts W, Winton J (1989) Enhanced detection of infectious hematopoietic necrosis virus and other fish viruses by pretreatment of cell monolayers with polyethylene glycol. J Aquat Anim Health 1:284–290

Bendorf CM (2010) Phylogenetic analysis and virulence characteristics for Chinook salmon, rainbow trout, and steelhead of L genogroup infectious hematopoietic necrosis virus (IHNV) from the Feather River, California. PhD thesis, University of California Davis, CA

Bendorf CM, Kelley GO, Yun SC, Kurath G, Andree KB, Hedrick RP (2007) Genetic diversity of infectious hematopoietic necrosis virus from Feather River and Lake Oroville, California, and virulence of selected isolates for chinook salmon and rainbow trout. J Aquat Anim Health 19:254–269

Black A (2015) Geographic and host factors shape the evolution of a newly recognized subgroup within the U genogroup of the fish rhabdovirus IHNV. MSc thesis, University of Washington, Seattle, WA

Bootland L, Leong J (1999) Infectious haematopoietic necrosis virus. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3. CAB International, New York, NY, p 57–121

Brannon EL, Powell MS, Quinn TP, Talbot A (2004) Population structure of Columbia River basin Chinook salmon and steelhead trout. Rev Fish Sci 12:99–232

Breyta R, Jones A, Stewart B, Brunson R and others (2013) Emergence of MD type infectious hematopoietic necrosis virus in Washington State coastal steelhead trout. Dis Aquat Org 104:179–195

Breyta R, Jones A, Kurath G (2014) Differential susceptibility in steelhead trout populations to an emergent MD strain of infectious hematopoietic necrosis virus. Dis Aquat Org 112:17–28

Breyta R, Samson C, Blair M, Black A, Kurath G (2016) Successful mitigation of viral disease based on a delayed exposure rearing strategy at a large-scale steelhead trout conservation hatchery. Aquaculture 450:213–224

Emmenegger EJ, Kurath G (2002) Genetic characterization of infectious hematopoietic necrosis virus of coastal salmonid stocks in Washington State. J Aquat Anim Health 14:25–34

Fulton LA (1970) Spawning areas and abundance of steelhead trout and coho, sockeye, and chum salmon in the Columbia River Basin—past and present. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Washington, DC

Garver KA, Troyer RM, Kurath G (2003) Two distinct phylogenetic clades of infectious hematopoietic necrosis virus overlap within the Columbia River basin. Dis Aquat Org 55:187–203

Garver KA, Batts WN, Kurath G (2006) Virulence comparisons of infectious hematopoietic necrosis virus U and M genogroups in sockeye salmon and rainbow trout. J Aquat Anim Health 18:232–243

Gilbert CH (1913) Age at maturity of the Pacific coast salmon of the genus *Oncorhynchus*. Bull Bur Fish 32: 1–22

Healey M (1991) Life history of chinook salmon (*Oncorhynchus tshawytscha*). In: Groot C, Margolis L (eds) Pacific salmon life histories. University of British Columbia Press, Vancouver, BC, p 311–394

Kell AM, Wargo AR, Kurath G (2014) Viral fitness does not correlate with three genotype displacement events involving infectious hematopoietic necrosis virus. Virology 464-465:146–155

Kelley GO, Bendorf CM, Yun SC, Kurath G, Hedrick RP (2007) Genotypes and phylogeographical relationships of infectious hematopoietic necrosis virus in California, USA. Dis Aquat Org 77:29–40

Kurath G, Breyta R (2013) Molecular epidemiology of aquatic pathogens—infectious hematopoietic necrosis virus (MEAP—IHNV) database. US Geological Survey. Available at gis.nacse.org/ihnv

Kurath G, Garver KA, Troyer RM, Emmenegger EJ, Einer-Jensen K, Anderson ED (2003) Phylogeography of infectious haematopoietic necrosis virus in North America. J Gen Virol 84:803–814

LaPatra SE (1989) Strain differentiation and detection of infectious hematopoietic necrosis virus. PhD dissertation, Oregon State University, Corvallis, OR

LaPatra S, Groberg W, Rohovec J, Fryer J (1990) Size-related susceptibility of salmonids to two strains of infectious hematopoietic necrosis virus. Trans Am Fish Soc 119:25–30

LaPatra SE, Fryer JL, Rohovec JS (1993) Virulence comparison of different electropherotypes of infectious hematopoietic necrosis virus. Dis Aquat Org 16: 115–120

LaPatra SE, Lauda KA, Jones GR (1994) Antigenic variants of infectious hematopoietic necrosis virus and implications for vaccine development. Dis Aquat Org 20: 119–126

Munson AD, Elliott DG, Johnson K (2010) Management of bacterial kidney disease in Chinook salmon hatcheries based on broodstock testing by enzyme-linked immunosorbent assay: a multiyear study. N Am J Fish Manag 30: 940–955

Peñaranda MMD, Purcell MK, Kurath G (2009) Differential virulence mechanisms of infectious hematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*) include host entry and virus replication kinetics. J Gen Virol 90: 2172–2182

Purcell MK, Hart SA, Kurath G, Winton JR (2006) Strand-specific, real-time RT-PCR assays for quantification of genomic and positive-sense RNAs of the fish rhabdovirus, Infectious hematopoietic necrosis virus. J Virol Methods 132:18–24

Purcell M, Garver K, Conway C, Elliott D, Kurath G (2009) Infectious haematopoietic necrosis virus genogroup-specific virulence mechanisms in sockeye salmon, *Oncorhynchus nerka* (Walbaum), from Redfish Lake, Idaho. J Fish Dis 32:619–631

Quinn TP (2005) The behavior and ecology of Pacific salmon and trout. University of British Columbia Press, Vancouver

St-Hilaire S, Ribble C, Traxler G, Davies T, Kent ML (2001) Evidence for a carrier state of infectious hematopoietic necrosis virus in chinook salmon *Oncorhynchus tshawytscha*. Dis Aquat Org 46:173–179

Taylor EB (1990a) Environmental correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J Fish Biol 37:1–17

Taylor EB (1990b) Variability in agonistic behaviour and salinity tolerance between and within two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha*, with contrasting life histories. Can J Fish Aquat Sci 47: 2172–2180

Troyer RM, Kurath G (2003) Molecular epidemiology of infectious hematopoietic necrosis virus reveals complex virus traffic and evolution within southern Idaho aquaculture. Dis Aquat Org 55:175–185

Wargo AR, Garver KA, Kurath G (2010) Virulence correlates with fitness *in vivo* for two M group genotypes of Infectious hematopoietic necrosis virus (IHNV). Virology 404:51–58

Wydoski RS, Whitney RR (2003) Inland fishes of Washington. American Fisheries Society, Bethesda, MD

Editorial responsibility: Mark Crane, Geelong, Victoria, Australia

Submitted: January 22, 2016; Accepted: June 8, 2016
Proofs received from author(s): August 6, 2016