Vol. 97: 155–165, 2011
doi: 10.3354/dao02414

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 6

OPEN ACCESS

Vector potential of the salmon louse *Lepeophtheirus salmonis* in the transmission of infectious haematopoietic necrosis virus (IHNV)

E. Jakob[1,2,*], D. E. Barker[2], K. A. Garver[1]

[1]Fisheries & Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia V9T 6N7, Canada

[2]Fisheries & Aquaculture Department, Vancouver Island University, 900 Fifth Street, Nanaimo, British Columbia V9R 5S5, Canada

ABSTRACT: To better understand the role of vector transmission of aquatic viruses, we established an *in vivo* virus–parasite challenge specifically to address (1) whether *Lepeophtheirus salmonis* can acquire infectious haematopoietic necrosis virus (IHNV) after water bath exposure or via parasitizing infected Atlantic salmon *Salmo salar* and if so, define the duration of this association and (2) whether *L. salmonis* can transmit IHNV to naïve Atlantic salmon and whether this transmission requires attachment to the host. Salmon lice which were water bath-exposed to 1×10^5 plaque-forming units (pfu) ml^{-1} of IHNV for 1 h acquired the virus (2.1×10^4 pfu g^{-1}) and remained IHNV-positive for 24 h post exposure. After parasitizing IHNV-infected hosts (viral titer in fish mucus 3.3×10^4 pfu ml^{-1}) salmon lice acquired IHNV (3.4×10^3 pfu g^{-1}) and remained virus-positive for 12 h. IHNV-positive salmon lice generated through water bath exposure or after parasitizing infected Atlantic salmon successfully transmitted IHNV, resulting in 76.5 and 86.6% of the exposed Atlantic salmon testing positive for IHNV, respectively. In a second experiment, only salmon lice that became IHNV-positive through water bath exposure transmitted IHNV to 20% of the naïve fish, and no virus was transmitted when IHNV-infected salmon lice were cohabitated but restrained from attaching to naïve fish. Under laboratory conditions, adult *L. salmonis* can acquire IHNV and transmit it to naïve Atlantic salmon through parasitism. However, the ephemeral association of IHNV with *L. salmonis* indicates that the salmon louse act as a mechanical rather than a biological vector or reservoir.

KEY WORDS: Sea lice · *Lepeophteirus salmonis* · Infectious heamatopoietic necrosis virus (IHNV) · Disease vector · Virus transmission · Atlantic salmon · *Salmo salar*

INTRODUCTION

The rhabdovirus, infectious haematopoietic necrosis virus (IHNV), is an enzootic pathogen in many Pacific salmonid populations in western North America (Wolf 1988, Bootland & Leong 1999). The virus has been detected in both fresh- and saltwater environments and in wild and cultured fish populations. In British Columbia, Canada, IHNV has been detected in wild sockeye *Oncorhynchus nerka*, chinook *O. tshawytscha*, pink *O. gorbuscha* and chum *O. keta* salmon with disease outbreaks occurring mainly in juvenile sockeye salmon reared in freshwater (Williams & Amend 1976, Traxler & Rankin 1989). Nonetheless, the virus has also been associated with mass mortalities of farmed Atlantic salmon *Salmo salar* reared in the marine waters of British Columbia. Three major outbreaks of IHNV in farmed Atlantic salmon have been reported between 1992 and 2003 (St-Hilaire et al. 2002, Saksida 2006). Dur-

*Email: eva.jakob@dfo-mpo.gc.ca

ing the last epidemic (2001–2003) the average cumulative mortality of Atlantic salmon on infected farms was 58% (Saksida 2006), highlighting the significant economic cost of this disease.

Questions concerning the epidemiology of IHNV, such as the source of infection, mode of transmission, and mechanism that perpetuates the virus among salmonid populations, are yet to be fully addressed (Bootland & Leong 1999). Laboratory studies have clearly demonstrated water exposure as an effective mode of IHNV transmission to both Pacific and Atlantic salmon in salt- and freshwater (Traxler et al. 1993, Wolf 1988). Moreover, epidemiological investigation of the spatial and temporal occurrence of IHNV in saltwater-farmed Atlantic salmon suggest that waterborne transmission may have played a role in the spread of virus between farms located in close proximity to each other (Saksida 2006). However, alternative modes of IHNV transmission, including the potential for transfer by aquatic invertebrates, cannot be discounted.

The topic of piscine ectoparasites as vectors for pathogens has been reviewed by Cusack & Cone (1986), focusing on tissue feeding species with monoxenous life cycles. The authors hypothesize that while moving between hosts such species may contribute to dissemination of pathogens. With respect to IHNV, detections of aquatic rhabdoviruses in invertebrate species have suggested that non-finfish hosts may act as vectors and/or reservoirs of the virus and thereby have the potential to contribute to viral transmission. IHNV was isolated from both a leech *Piscicola salmositica* and a copepod *Salmincola* sp. (Mulcahy et al. 1990) while a related aquatic rhabdovirus, viral hemorrhagic septicemia virus (VHSV), was detected in a leech *Myzobdella lugubris* (Faisal & Schulz 2009) and from amphipod zooplankton *Diporeia* spp. (Faisal & Winters 2011). Few studies have actually evaluated the direct role of invertebrates in the transmission of aquatic viruses. To date, evidence supporting invertebrate viral transmission is limited to the transfer of spring viraemia of carp virus (SVCV) via the carp louse *Argulus foliaceus* and a leech *P. geometra* from infected to naïve carp *Cyprinus carpio* (Ahne 1978, 1985, Pfeil-Putzien 1978).

The salmon louse *Lepeophtheirus salmonis* (Krøyer) is a common marine ectoparasitic copepod of wild and farmed salmonids in the marine environment (Kabata 1979, Pike & Wadsworth 1999). Its life cycle consists of 5 phases and 10 stages. These include 2 free-swimming naupliar stages, 1 free-swimming infective copepodid stage, 4 attached chalimus stages, 2 preadult stages, and an adult stage (Kabata 1973). Although copepodids are the primary infectious stage, the motile preadult (male and female) and adult (male) stages have the ability to transfer between hosts held under high densities as shown under farmed and laboratory conditions (Ritchie 1997). Furthermore host switching of adult female *L. salmonis* has been observed within our laboratory trial utilizing stained salmon lice and fin- clipped fish (data not shown).

Isolation of the aquatic pathogens infectious salmon aneamia virus (ISAV, Nylund et al. 1993), salmonid alphavirus (SAV, Petterson et al. 2009), *Aeromonas salmonicida* (Nese & Enger 1993), *Tenacibaculum maritimum*, *Pseudomonas fluorescens* and *Vibrio* spp. (Barker et al. 2009) from *Lepeophtheirus salmonis* suggests that salmon lice are capable of harboring aquatic pathogens; however, their role in pathogen transmission has never been proven.

To this end we have established an *in vivo* virus–parasite challenge model utilizing the virus IHNV and the copepod *Lepeophtheirus salmonis* to examine whether adult female *L. salmonis* can act as reservoirs for IHNV and if they have the capacity to transmit IHNV to a naïve host. This was accomplished by performing 4 experimental challenges specifically addressing whether *L. salmonis* (1) can acquire IHNV from water bath exposure and, if so, define the duration of association; (2) can acquire IHNV directly from infected Atlantic salmon and, if so, define the duration of association; (3) can transmit IHNV to naïve Atlantic salmon after either water bath exposure to IHNV or after feeding on IHNV-infected Atlantic salmon; and (4) have to attach to the naïve Atlantic salmon in order to transmit the virus.

MATERIALS AND METHODS

Parasites

Adult female *Lepeophtheirus salmonis* (referred to as salmon louse/lice within this manuscript) were obtained from farmed Atlantic salmon during harvesting in British Columbia, Canada. Salmon lice were collected from the fish using forceps, placed in aerated buckets containing sand-filtered and UV-treated seawater and kept chilled in a cooling box for transportation. Depending on the density of lice and duration of transportation, multiple water changes were performed. Salmon lice were kept overnight between 4 and 9°C and used for infection trials on the next day. To ensure that the obtained *L. salmonis* were free of IHNV, subsamples were screened by

RT-PCR and tissue culture (TC) for the presence of IHNV as described below and/or a control group was established with *L. salmonis* parasitizing on naïve fish. This group also provided a control for parasitic effects on Atlantic salmon not exposed to IHNV.

Fish

Atlantic salmon (*Salmo salar*, Mowi strain) parr were moved from a hatchery with no previous occurrence of IHNV to the Pacific Biological Station, Nanaimo, British Columbia, Canada. Fish were held in ambient pathogen-free freshwater and subsequently smolted by increasing the seawater:freshwater ratio by ¼ every 4 to 5 d. Fish were fed daily, at 1.5% of their body weight, a semi-moist pelleted diet (BioOregon).

Virus amplification and quantification

The IHNV isolate (93-057) used in this study originated from a clinical outbreak of IHNV in 1993 on an Atlantic salmon net pen farm in the Discovery Islands, British Columbia. Based on partial nucleotide sequence of the glycoprotein gene, the virus phylogenetically groups into the enzootic U-genogroup (K. Garver unpubl. data). For transmission experiments, the virus was propagated on *Epithelioma papulosum cyprini* (EPC) cells following published methods (Fijan et al. 1983, Winton et al. 2010). Virus stocks were quantified using plaque assays as described by Traxler et al. (1997).

Detection of IHNV in salmon lice and fish

Tissue culture

Atlantic salmon kidney tissue was homogenized in minimum essential medium (MEM) supplemented with 4% fetal bovine serum and buffered with HEPES (MEM-4-HEPES+AB; Gibco) using a Stomacher (60 s, high speed) while salmon lice (either individual or pools of a maximum of 3 lice) were placed in 1.5 ml safe lock tubes (Eppendorf) containing a sterile metal bead and homogenized using a tissue lyser. Each homogenate was re-suspended in MEM-4-HEPES+AB to achieve a 5% dilution. Mucus swabs taken along the lateral line of the fish were placed in a tube containing 1 ml MEM-4-HEPES+AB. Kidney tissue, salmon lice and mucus homogenates were centrifuged at 2500 × *g* for 12 min. Supernatants were serial diluted (up to 10^5) in Hanks' balanced salt solution (HBSS; Gibco) and 100 µl of each dilution was inoculated in duplicate onto 12-well plates containing newly seeded (within 24 h) EPC cells pretreated with 7% polyethylene glycol (PEG) solution (20 000 molecular weight) in MEM-4. Cells were incubated at 15°C for 60 min and then overlaid with 0.85% methylcellulose and MEM-4-HEPES+AB (2 ml $well^{-1}$) for quantifying virus by plaque assay or with MEM-4-HEPES+AB (2 ml $well^{-1}$) to determine presence/absence of virus. Assay plates were examined twice weekly for viral plaque formation and cytopathic effect (CPE), fixed after 10 to 14 d post inoculation with 10% formalin and stained with a 0.1% crystal violet solution. Plaques were enumerated and reported as plaque forming units (pfu) ml^{-1} for mucus samples and pfu g^{-1} for kidney and salmon lice samples.

RT-PCR analysis

RT-PCR targeting the IHNV G-gene was utilized to determine virus presence in salmon lice homogenates and to confirm IHNV identity in all TC-positive kidney, mucus and salmon louse samples. Total RNA was extracted from either salmon lice homogenate or liquid cell culture supernatant samples using Trizol or Trizol LS reagents (Invitrogen), respectively. RT-PCR containing extracted RNA as template was performed following the confirmation method for IHNV (AFS-FHS 2010). If the first round PCR was negative, a second PCR using a nested primer set was performed following Emmenegger et al. (2000).

The positive PCR products (first round 693 bp; second round 483 bp) were sequenced using a fluorescent dye terminator cycle sequencing kit (Applied Biosystems BigDye Terminator version 3.1) using the first or second round PCR primers and following the manufacturer's protocols.

Salmon lice and IHNV challenges

Challenge 1. Can salmon lice acquire IHNV through water bath exposure and what is the duration of association?

Salmon lice (n = 63) were immersion challenged for 1 h in 500 ml aerated seawater (10°C) containing 1 × 10^5 pfu ml^{-1} IHNV. After 1 h static exposure, they

were transferred to a new beaker with flowing aerated sand-filtered and UV-treated seawater with a water temperature (T) of 10°C. At the time point of transfer (TP = 0), a subsample of 9 salmon lice (3 pools; 3 lice pool^{-1}) were removed and stored at −80°C. At 1, 12, 24, 36, 42, 48 h post virus-exposure; an additional 9 lice (3 pools, 3 lice pool $^{-1}$) were sampled and stored at −80°C until they were processed for virological analyses.

Challenge 2.1. Can salmon lice acquire IHNV after parasitizing virus-infected fish?

To generate IHNV-infected fish, 81 Atlantic salmon (mean wt = 306.1 g) were anaesthetized with 50 mg l^{-1} tricaine methanesulphonate (TMS) and intraperitoneal (i.p.)-injected with 500 µl 1 × 10^8 pfu fish^{-1} of IHNV. The IHNV-infected fish were randomly allocated into 2 1.8 m^3 aquaria. Fish (n = 41) in one tank represented the experimental treatment group and were exposed to 360 salmon lice (ratio of 9 lice fish^{-1}) while the other tank represented the IHNV-positive control, containing 40 i.p.-injected fish without salmon lice. As a mock challenge control, an additional 40 Atlantic salmon were i.p.-injected with 500 µl Hanks' balanced salt solution (HBSS) and exposed to 360 salmon lice (ratio 9 lice fish^{-1}). The average water temperature was 8.6°C during the challenge. All fish were checked daily for mortalities. The treatment groups exposed to salmon lice were terminated 9 d post-infection (dpi) while cumulative mortality of the IHNV-positive control group without salmon lice was monitored for 30 dpi. For sampling, fish were euthanized with TMS (200 mg l^{-1}), and salmon lice, mucus swabs, and kidney tissue from each fish were archived for virological analysis. Virus assays were performed on all kidney, mucus and salmon lice samples of the 2 salmon lice infected groups. Salmon lice of individual fish were pooled (up to 3 lice pool^{-1}). Of the IHNV-positive control group 25% of the fish were checked for the presence of virus in the kidney.

Challenge 2.2. What is the duration of virus association after parasitizing IHNV-infected fish?

Salmon lice (n = 130) were removed from IHNV-infected fish (7 dpi of Challenge 4.2, see below) and transferred into a beaker with aerated sand-filtered and UV-treated seawater (T = 10°C). Upon transfer, salmon lice were immediately sampled (TP0) with the remaining lice sampled thereafter every 6 h over a period of 54 h. At each time point, 9 salmon lice (3 pools; 3 lice pool^{-1}) were sampled and stored at −80°C until further processing.

Challenge 3.1. Can salmon lice transmit IHNV to Atlantic salmon after acquiring virus through water bath exposure?

To test whether salmon lice infected via water bath exposure can transmit the virus to naïve salmon, lice (n = 77) were immersed for 1 h in 1.6 × 10^5 pfu ml^{-1}. After the 1 h static immersion challenge, salmon lice were removed from the immersion bath and quickly rinsed in sand-filtered and UV-treated saltwater. From these 77 immersed salmon lice, a subsample of 9 lice (3 pools; 3 lice pool^{-1}) were removed and screened for the presence of IHNV using cell culture and RT-PCR. The remaining lice (n = 68) were manually distributed with sterile forceps to a ratio of 4 lice fish^{-1} to a 60 l tank containing 17 naïve Atlantic salmon (mean wt = 49.6 g). This challenge was terminated after 44 dpi (mean T = 9.2°C).

Challenge 3.2. Can salmon lice transmit IHNV to Atlantic salmon after acquiring virus through parasitizing IHNV-positive fish?

To generate IHNV-infected salmon lice through parasitism, generating IHNV-infected hosts was first necessary. A total of 92 Atlantic salmon (mean wt = 49.6 g) were anaesthetized with TMS (50 mg l^{-1}) and i.p.-injected with 100 µl 1 × 10^6 pfu fish^{-1} of IHNV. In each of three 60 l tanks, Atlantic salmon (n = 25, 25 and 15; mean wt = 49.6 g) were exposed to salmon lice at a ratio of 6 lice fish^{-1} while in a fourth tank, IHNV-infected fish (n = 27; mean wt = 49.6 g) were not exposed to salmon lice. At 1, 4 and 6 dpi, a 5 l water sample was removed from one of the IHNV + salmon lice tanks to determine the level of shed virus in the water. To collect the sample, water flow was stopped for 1 h after which 5 l was collected and immediately processed via ultrafiltration as described in Grant et al. (in press). At 7 dpi, fish in the IHNV + salmon lice challenge groups were euthanized (TMS 200 mg l^{-1}) and salmon lice were removed manually using sterile forceps. These salmon lice (n = 70) were collected in a beaker containing sand-filtered and UV-treated saltwater and then were added to a 60 l tank containing 15 naïve Atlantic salmon (mean wt = 49.6 g) in a ratio of 4

to 5 lice fish^{-1}. The challenge was terminated after 39 dpi (mean T = 9.2°C).

Moribund and dead fish of Challenges 3.1 and 3.2 were sampled daily. Virus assays were performed on all salmon lice, kidney and mucus samples of dead, moribund and challenge-surviving fish and were either processed immediately or stored at −80°C for subsequent processing.

Challenge 4.1. Is salmon lice attachment to their host required for virus transmission after acquiring IHNV through water bath exposure?

Salmon lice (n = 400) were immersion challenged for 1.5 h in a virus suspension of 2.5 × 10^6 pfu ml^{-1}. After 1.5 h, the salmon lice were removed from the immersion bath and subsequently rinsed with sand-filtered and UV-treated saltwater. The salmon lice were manually distributed with sterile forceps to a ratio of 9 lice fish^{-1} to each of four 60 l tanks containing 10 naïve Atlantic salmon (mean wt = 77.2 g) each. In 2 tanks, the salmon lice were freely able to attach to the naïve fish while in the other 2 tanks, the salmon lice were confined within a fine mesh box that prevented the attachment of the lice on the cohabiting fish. As a control to monitor the parasitic effect of salmon lice on their host, 10 naïve Atlantic salmon (mean wt = 77.2 g) were exposed to IHNV-free salmon lice (9 lice fish^{-1}). Additionally 10 naïve Atlantic salmon (mean wt = 77.2 g) were water bath exposed to1.1 × 10^4 pfu ml^{-1} IHNV to evaluate the susceptibility of the challenge fish to the utilized strain of IHNV.

Challenge 4.2. Is salmon lice attachment to their host required for virus transmission after acquiring IHNV through parasitism?

To generate IHNV-infected salmon lice through parasitism, generating IHNV-infected hosts was first necessary. Atlantic salmon (n = 140; mean wt = 77.2 g) were anaesthetized with TMS (50 mg l^{-1}) and i.p.-injected with 100 µl 7.9 × 10^7 pfu fish^{-1} of IHNV. Fish were equally divided into two 380 l tanks and exposed to salmon lice (8 to 10 lice fish^{-1}) and checked daily for mortalities. At 7 dpi, the challenge was terminated, the surviving fish were euthanized (TMS 200 mg l^{-1}) and the salmon lice were sampled from infected fish. Prior to salmon lice collection, a 10 l water sample was processed using ultrafiltration, which is described in Grant et al. (in press), to determine the titer of shed virus in the water. Of the 140 IHNV-infected fish, 20 were analyzed for the presence of IHNV in their kidney and mucus. The salmon lice removed from IHNV-infected Atlantic salmon, which were sampled either from moribund fish at 5 and 6 dpi (n = 32 and 44 lice, respectively) or from euthanized fish at 7 dpi (n = 364), were added to each of four 60 l tanks containing 11 naïve Atlantic salmon (mean wt = 77.2 g), resulting in a ratio of 10 lice fish^{-1}. To ensure successful viral transmission from the fish to the lice an additional sample of 12 salmon lice collected at 7 dpi (6 pools; 2 lice pool^{-1}) were analyzed for presence of IHNV, and a total of 130 salmon lice were utilized to examine the duration of virus association (Challenge 2.2). As in Challenge 4.1, in 2 tanks salmon lice were freely able to attach to the naïve fish while in the other 2 tanks, the salmon lice were confined within a fine mesh box that prevented lice attachment to the cohabiting fish.

Average water temperature in Challenge 4.1 and 4.2 was 9.0°C over the duration of the experiment. Moribund and dead fish were sampled daily, and virus assays performed on kidney, mucus and salmon lice samples were either processed immediately or stored at −80°C until processing. If skin lesions were present, skin scrapes were taken and Gram stained. Additionally, kidney swabs from these fish were plated onto tryptic soy agar (TSA, with and without salt) and incubated at 20°C. All treatment groups were terminated 30 dpi and virus assays were performed on kidney and mucus samples from each survivor of the challenge.

RESULTS

Challenge 1. Salmon lice acquisition of IHNV via water bath exposure and the duration of virus association

Salmon lice immersed in 1 × 10^5 pfu ml^{-1} of IHNV were positive for the virus up to 24 h post exposure. The virus was detected in 100% of the salmon lice pools following virus exposure (TP0) and at TP1, TP2 and TP24 by both RT-PCR and TC. Quantifiable virus titers could only be determined for samples up to 12 h. There was a successive reduction in infectious virus levels from 2.1 × 10^4 pfu g^{-1} at TP0 to 5.3 × 10^1 pfu g^{-1} at TP12 (Table 1). Generation of IHNV-positive salmon lice through water bath exposure was further demonstrated in Challenges 3.1 and 4.1 with virus titers at TP0 of 6.5 × 10^2 and 4.5 × 10^2 pfu g^{-1}, respectively.

Table 1. *Lepeophtheirus salmonis*. Challenges 1 and 2.2. Viral presence in salmon lice at various time points post infectious haematopoietic necrosis virus (IHNV) exposure via water bath immersion (exposure level of 1×10^5 pfu ml^{-1}) (Challenge 1) or through parasitizing IHNV-positive Atlantic salmon *Salmo salar* (exposure levels of 3.5×10^3 pfu ml^{-1} in water and GMT 3.3×10^4 pfu ml^{-1} in fish mucus) (Challenge 2.2). pfu: plaque-forming units; +: positive for IHNV; –: negative for IHNV; ns: no sample taken; TC: tissue culture; GMT: geometric mean viral titer

Time (h)	Water bath exposure to IHNV			Parasitizing IHNV-infected Atlantic salmon		
	PCR	TC	GMT (pfu g^{-1})	PCR	TC	GMT (pfu g^{-1})
Control	–	–	–	–	–	–
0	+	+	2.1×10^4	+	+	3.4×10^3
1	+	+	6.9×10^2	+	+	2.5×10^2
6	ns	ns	ns	+	+	–
12	+	+	5.3×10^1	+	+	–
18	ns	ns	ns	–	–	–
24	+	+	–	–	–	–
30	ns	ns	ns	–	–	–
36	–	–	–	–	–	–
42	–	–	–	–	–	–
48	–	–	–	–	–	–
54	ns	ns	ns	–	–	–

Challenge 2.1. Salmon lice acquisition of IHNV after parasitizing virus infected fish

In both the IHNV-infected salmon exposed to salmon lice and the IHNV-positive control group without salmon lice, mortalities began at 7 dpi. At 9 dpi, which was 2 d after the onset of mortality, the IHNV donor group was terminated. In total, 65 lice (35 pools) were recovered from 26 Atlantic salmon with a prevalence of infection (*P*) of 63.4% and a mean intensity (*I*) of 1.6 lice fish^{-1}. IHNV was detected in 94% (33 of 35) of the pooled salmon lice samples. Of the 26 IHNV-infected fish on which salmon lice were found, 100% were positive for IHNV in the mucus with a geometric mean titer (GMT) of 4.0×10^2 pfu ml^{-1} and a titer ranging from 2.0×10^1 to 8.3×10^3 pfu ml^{-1} (Table 2). Viral assays of the kidney tissue revealed 92.5% (37 of 41) of the Atlantic salmon injected with IHNV and exposed to salmon lice were positive for IHNV (GMT: 1.2×10^7; range: 1.8×10^3 to 2.1×10^9 pfu g^{-1}) (Table 2). No mortalities occurred among the naïve Atlantic salmon solely exposed to naïve salmon lice (IHNV-free control group) and all of the fish (kidney and mucus) and salmon lice pools tested negative for IHNV (Table 2). The IHNV-positive control group had a cumulative mortality of 70% at 30 dpi and 100% of the kidneys tested positive for IHNV (GMT: 1.4×10^7 pfu g^{-1}) (Table 2).

Challenge 2.2. Duration of virus association in salmon lice after parasitizing IHNV-infected fish

The duration of association of IHNV with salmon lice after parasitizing IHNV-infected Atlantic salmon hosts was examined using 130 salmon lice obtained from Challenge 4.2. IHNV concentrations in host mucus ranged from 9.0×10^2 to 1.4×10^5 pfu ml^{-1} (GMT: 3.3×10^4 pfu ml^{-1}). Up to 12 h after removal from infected hosts, salmon lice remained IHNV-positive. Equivalent virus detection was observed between RT-PCR and TC methods at all time points sampled. Quantifiable virus levels were only observed at TP0 (3.4×10^3 pfu g^{-1}) and at TP1 (2.5×10^2 pfu g^{-1}) following removal of infected hosts (Table 1).

Table 2. *Lepeophtheirus salmonis* and *Salmo salar*. Challenge 2.1. Salmon lice acquisition of infectious haematopoietic necrosis virus (IHNV) after parasitizing IHNV-infected Atlantic salmon. Prevalence of infection (*P*) and mean intensity (*I*) of salmon lice on Atlantic salmon and percent of IHNV-positive salmon lice (IHNV+) in Atlantic salmon kidney and mucus samples. dpi: days post-infection; CM: cumulative mortality; GMT: geometric mean viral titer; pfu: plaque-forming units; na: not applicable; ns: no sample taken

Challenge treatment groups	No. of fish	dpi	CM (%)	Salmon lice			Kidney		Mucus	
				P (%)	*I*	IHNV+ (%)	IHNV+ (%)	GMT (pfu g^{-1})	IHNV+ (%)	GMT (pfu ml^{-1})
IHNV-infected fish	40	30	70	na[a]	na[a]	na[a]	100	1.4×10^7	ns	ns
Naïve fish infected with naïve salmon lice	40	9	0	42.8	1.3	0	0	0	0	0
IHNV-infected fish infected with naïve salmon lice	41	9	10	63.4	1.6	94	92.5	1.2×10^7	100	4.0×10^2

[a]IHNV-positive control group. Fish not exposed to salmon lice

Challenge 3.1. IHNV transmission from salmon lice to naïve fish. Salmon lice virus positive through water bath exposure

IHNV-positive salmon lice were generated by 1 h immersion in an IHNV bath of 1.6×10^5 pfu ml^{-1}. Lice tested from 3 pools (3 lice pool^{-1}) were found all to be IHNV-positive (GMT: 6.5×10^2 pfu g^{-1}). The remaining salmon lice (n = 68) were used to infect naïve Atlantic salmon (n = 17) in a ratio of 4 lice fish^{-1}. Fish mortalities started at 9 dpi and subsided with a cumulative mortality of 70.6% on 35 dpi. Among the 17 fish, 13 (12 moribund, 1 survivor) tested positive for IHNV (76.5%) in kidney samples (GMT: 2.1×10^7; range: 4.7×10^5 to 7.4×10^8 pfu g^{-1}) and in the mucus (GMT: 1.5×10^3; range: 1.0×10^1 to 3.0×10^4 pfu ml^{-1}) (Table 3).

Challenge 3.2. IHNV transmission from salmon lice to naïve fish. Salmon lice virus positive through parasitizing IHNV-infected fish

IHNV-positive salmon lice were generated through parasitism on IHNV-infected Atlantic salmon. Tanks containing IHNV-infected salmon shed virus in the water at a titer of 2.3 pfu ml^{-1} at 1 dpi with increased titers of 1.2×10^2 and 7.9×10^1 pfu ml^{-1} observed at 4 and 6 dpi, respectively. Atlantic salmon mortality among the 3 groups of IHNV-infected fish exposed to salmon lice and the IHNV control group started at 3 dpi. Salmon lice collected from moribund and dead fish during the first 5 d of the challenge were screened for the presence of IHNV to ensure the transmission success from infected fish. IHNV was detected in 100% of the 14 lice pools (2 lice pool^{-1}) that were tested (GMT: 3.9×10^5; range: 2.6×10^4 to 8.2×10^6 pfu g^{-1}).

On 7 dpi, all remaining salmon lice were collected and the prevalence of lice infection in each of the 3 IHNV-infected fish + salmon lice groups were 68, 80 and 86.9% with mean intensities of 2.4, 2.8 and 4.7 lice fish^{-1}, respectively.

A total of 70 lice were recovered and transferred to a tank holding 15 naïve fish (4 to 5 lice fish^{-1}). The first mortalities of this group occurred at 7 dpi and subsided at 23 dpi with a cumulative mortality of 66.6% (10 of 15 fish). Among these 15 fish, 13 (9 moribund and 4 survivors) tested positive for IHNV (86.6%) in the kidney (GMT: 4.7×10^6, range: 1.5×10^5 to 2.2×10^8 pfu g^{-1}) and in the mucus (GMT: 5.5×10^3, range: 7.0×10^1 to 1.3×10^5 pfu ml^{-1}) (Table 3).

Challenge 4.1. IHNV transmission from attached vs. non-attached salmon lice to naïve fish. Salmon lice virus positive through water bath exposure

IHNV-positive salmon lice (n = 400) were generated by water bath exposure (1.5 h in 2.5×10^6 pfu ml^{-1}). From this exposure, samples of 6 pools (2 lice pool^{-1}) were found to be virus-positive with a GMT of 4.5×10^2 pfu g^{-1}. Remaining lice were transferred into the challenge tanks. In the duplicate tanks where salmon lice were prohibited from attaching to naïve Atlantic salmon, a cumulative mortality of 10%

Table 3. *Lepeophtheirus salmonis* and *Salmo salar*. Challenges 3.1. and 3.2. Salmon lice acquisition of infectious haematopoietic necrosis virus (IHNV) after parasitizing IHNV-infected Atlantic salmon and IHNV transmission to naïve Atlantic salmon through salmon lice virus positive through water bath exposure (Challenge 3.1) or after parasitizing IHNV-infected Atlantic salmon (Challenge 3.2). Prevalence of infection (*P*) and mean intensity (*I*) of salmon lice on Atlantic salmon and percent of IHNV-positive salmon lice (IHNV+) in Atlantic salmon kidney and mucus samples. dpi: days post-infection; CM: cumulative mortality; GMT: geometric mean viral titer; pfu: plaque-forming units; na: not applicable; ns: no sample taken

Challenge treatment groups	No. of replicates	No. of fish	dpi	CM (%)	Salmon lice *P* (%)	Salmon lice I	Salmon lice IHNV+ (%)	Salmon lice GMT (pfu g^{-1})	Kidney IHNV+ (%)	Kidney GMT (pfu g^{-1})	Mucus IHNV+ (%)	Mucus GMT (pfu ml^{-1})
IHNV-infected fish	1	27	7	37	na[a]	na[a]	na[a]	na[a]	100	2.1×10^8	ns	ns
IHNV-infected fish infected with naïve salmon lice	1	25	7	96	68	2.4	100	3.9×10^5	100	1.7×10^8	100	5.2×10^3
	2	25	6	56	80	2.8	na[b]	na[b]	ns	ns	ns	ns
	3	15	5	26	86.6	4.7	na[b]	na[b]	ns	ns	ns	ns
Naïve fish infected with salmon lice IHNV-positive through water bath exposure	1	17	44	70.6	29.4	1.6	50	2.4×10^3	76.5	2.1×10^7	76.5	1.5×10^3
Naïve fish infected with salmon lice IHNV-positive through parasitism	1	15	39	66.6	46.6	3.1	81.8	1.4×10^3	86.6	4.7×10^6	86.6	5.5×10^3

[a]IHNV-positive control group. Fish not exposed to salmon lice; [b]IHNV-positive salmon lice used to infect naïve fish

Table 4. *Lepeophtheirus salmonis* and *Salmo salar*. Challenges 4.1 and 4.2. Infectious haematopoietic necrosis virus (IHNV) transmission from attached and non-attached salmon lice to naïve Atlantic salmon using salmon lice that became virus-positive through water bath exposure (Challenge 4.1) or after parasitizing IHNV-infected Atlantic salmon (Challenge 4.2). Challenges were terminated at 30 d post-infection. Prevalence of infection (*P*) and mean intensity (*I*) of salmon lice on Atlantic salmon and percent of IHNV-positive salmon lice (IHNV+) in Atlantic salmon kidney and mucus samples. CM: cumulative mortality; GMT: geometric mean viral titer; na: not applicable; pfu: plaque-forming units

Challenge treatment groups	No. of replicates	No. of fish	CM (%)	Salmon lice		Kidney		Mucus	
				P (%)	*I*	IHNV+ (%)	GMT (pfu g^{-1})	IHNV+ (%)	GMT (pfu ml^{-1})
Naïve fish infected with naïve salmon lice	1	10	10	10	1	0	0	0	0
Naïve fish water bath exposed to IHNV	1	10	40	na[a]	na[a]	50	6.9×10^6	40	1.1×10^2
Naïve fish cohabitated with non-attached salmon lice IHNV-positive through water bath exposure	1	10	0	na[b]	na[b]	0	0	0	0
	2	10	10	na[b]	na[b]	0	0	0	0
Naïve fish infected with salmon lice IHNV-positive through water bath exposure	1	10	60	36	6.8	10	1.1×10^7	20	2.0×10^2
	2	10	20	0	0	0	0	0	0
Naïve fish infected with salmon lice IHNV-positive through parasitism	1	11	27.3	27	5	0	0	0	0
	2	11	54.5	72	4.9	0	0	0	0
Naïve fish cohabitated with non-attached salmon lice IHNV-positive through parasitism	1	11	0	na[b]	na[b]	0	0	0	0
	2	11	0	na[b]	na[b]	0	0	0	0

[a]Fish not exposed to salmon lice; [b]salmon lice prohibited from attaching to naïve fish

was observed in one of the challenge groups; however, all fish were tested negative for IHNV (Table 4).

Conversely, in one of the tanks where salmon lice were able to attach to Atlantic salmon ($P = 36\%$; $I = 6.8$), a cumulative mortality of 60% was observed. IHNV was transmitted to 2 fish with one fish testing positive in the kidney (1.1×10^7 pfu g^{-1}) and mucus (4.1×10^2 pfu ml^{-1}) and the second fish testing positive for IHNV in the mucus (1.0×10^2 pfu ml^{-1}) but not in the kidney (Table 4). In the duplicate tank where salmon lice were able to attach to Atlantic salmon, a cumulative mortality of 20% was observed; however, at the end of the challenge, none of the fish were infected with salmon lice and all fish were tested negative for IHNV (Table 4). In both groups where salmon lice were able to parasitize Atlantic salmon, fish showed minor to severe signs of discoloration and descaling. Severe skin lesions with exposed musculature located between and below the dorsal and adipose fin were present in 18% of the fish.

In the treatment group containing naïve Atlantic salmon exposed to naïve salmon lice, 1 fish died and 2 fish showed scale loss and skin lesions, but no IHNV was detected and no clinical signs of IHN disease were observed (Table 4). Among the IHNV-positive control group (Atlantic salmon water bath exposed to IHNV), the onset of mortality started at 14 dpi with a cumulative mortality of 40% (4 of 10). Of these 10 fish, 50% were tested positive for IHNV in the kidney (GMT: 6.9×10^6; range: 2.0×10^4 to 1.3×10^8 pfu g^{-1}) and 40% of the mucus samples were IHNV- positive (GMT: 1.1×10^2; range: 5.0 to 4.0×10^2 pfu ml^{-1}) (Table 4).

Challenge 4.2. IHNV transmission from attached vs. non-attached salmon lice to naïve fish. Salmon lice virus positive through parasitizing IHNV-infected fish

Tanks containing IHNV-infected Atlantic salmon (n = 140) shed virus in the water at a titer of 3.5×10^3 pfu ml^{-1} at 7 dpi. Among the 140 IHNV-infected Atlantic salmon a subsample of 20 fish were screened for the IHNV and found to be 100% virus-positive in the kidney (GMT: 3.4×10^8; range: 1.0×10^8 to 1.1×10^9 pfu g^{-1}) and the mucus (GMT: 3.3×10^4; range: 9.0×10^2 to 1.4×10^5 pfu ml^{-1}). A total of 452 salmon lice were recovered from the 140 IHNV-infected fish over the course of 3 d starting at 5 dpi. From these 452 salmon lice, a sample of 6 pools (2 lice $pool^{-1}$) were screened for virus and found to be 100% IHNV-positive (GMT: 8.5×10^2; range: 6.3×10^2 to 1.1×10^3 pfu g^{-1}). The remaining salmon lice were subsequently transferred to the challenge tanks. In the duplicate tanks where salmon lice were prohibited from attaching to naïve Atlantic salmon no mortalities occurred and all survivors tested negative for IHNV (Table 4). Duplicate treatment groups where salmon lice were able to attach to naïve Atlantic salmon resulted in a prevalence of infection of 27 ($I = 5$) and 72% ($I = 4.9$), respectively (Table 4). A cumu-

lative mortality of 27.3 and 54.5% was observed but all fish were tested negative for IHNV (Table 4). Minor to severe signs of discoloration and descaling were observed in fish of both replicates. Severe skin lesions with exposed musculature in an area between and below the dorsal and adipose fin were present in 27% of the fish.

Bacterial assays

Skin smears around the lesions showed the presence of typical opportunistic predominantly Gram-negative bacilli. Plated kidney swabs of fish with skin lesions formed mainly yellow colonies and to a lesser amount grey colonies. Bacteria identified using an API 20 NE test gave evidence for *Pasteurella* spp. (yellow colonies) and *Moraxella* spp. (grey colonies). The bacteria were not identified any further.

DISCUSSION

This is the first study to demonstrate the potential of adult *Lepeophtheirus salmonis* to acquire IHNV and to transmit the pathogen by parasitizing naïve Atlantic salmon under laboratory conditions. The duration of virus association with salmon lice appears to be related to the dose of viral exposure. Salmon lice that were water bath-exposed to IHNV at a dose of 1×10^5 pfu ml^{-1} acquired the virus at a titer of 2.1×10^4 pfu g^{-1} and remained IHNV-positive up to 24 h post exposure. However, when lice were exposed to lower levels of IHNV via parasitizing virus-positive fish (mucus titers of 3.3×10^4 pfu ml^{-1}), they obtained a lower viral titer of 3.4×10^3 pfu g^{-1} and harbored the virus only for 12 h. Interestingly, regardless of whether salmon lice acquired IHNV through water bath exposure or after parasitizing IHNV-infected fish, the duration of virus association with salmon lice never exceeded 24 h and viral titers rapidly diminished over time with infectious virus levels falling below detectable limits within 1 to 12 h. This ephemeral association of IHNV with salmon lice suggests that the virus is not replicating within the louse. To date we are not able to differentiate if the virus is externally and/or internally associated with the salmon lice. Attempts to localize the virus was unsuccessful and would require further investigation.

Similar observations were made by Ahne (1985) in transmission experiments for SVCV via *Argulus foliaceus* and *Piscicola geometra*. Both parasites successfully transmitted the virus to naïve carp, but virus multiplication did not occur in the vectors. On the other hand, Mulcahy et al. (1990) demonstrated that after feeding on IHNV-positive fish leeches *Piscicola salmositica* showed an increase in viral titers over time, suggesting that the virus can replicate within leeches. Compared to copepods, leeches are true blood feeders, perform multiple host switches and are a known vector for aquatic pathogens like *Trypanoplasma borelli* and *Cryptobia* (*Trypanoplasma*) *salmositica* (Kruse et al. 1989, Mehlhorn & Ruthmann 1992, Woo 1998), whereas salmon lice are primarily skin grazers and ingest blood opportunistically as a result of epidermal damaging (Pike & Wadsworth 1999).

Despite the short duration of IHNV association with salmon lice, virus-positive lice did transmit IHNV to naïve Atlantic salmon resulting in clinical disease symptoms and mortality. However, the success at which IHNV was transmitted to naïve hosts was variable and undoubtedly dependent upon numerous factors involving complex interactions of the host, pathogen(s), and environment. For example, in Challenges 3.1 and 3.2, IHNV was transmitted to 76.5 and 86.6% of exposed naïve fish, whereas, in Challenge 4.1 only 20% became infected with IHNV, and none of the fish were IHNV-positive in Challenge 4.2. The transmission success observed in Challenges 3.1 and 3.2 may be explained by higher viral titers associated with the salmon lice. As discussed above, salmon lice acquiring higher levels of IHNV harbor the virus for longer durations and thereby likely possess a greater potential to transmit the virus to their host. Moreover, differences in host susceptibilities may exist between challenges. Smaller and potentially more susceptible fish were utilized in Challenge 3 (mean wt = 49.6 g) compared to those in Challenge 4 (mean wt = 77.2 g). The size and age of a fish have been shown to be important factors affecting the virulence of IHNV among salmonids with decreasing mortality correlating with increasing age or size (LaPatra 1998, Bergmann et al. 2003).

Although the results of our study prove successful virus transmission via salmon lice, waterborne transmission is the natural pathway of virus dissemination of IHNV and, as described by Traxler et al. (1993), this is also possible in saltwater. IHNV is not dependent on salmon lice for transmission to its target host as would be the case in a true vector-dependent pathogen like *Trypanoplasma* spp. via its leech vector. However, under ideal conditions, i.e. a highly susceptible host and a high infectious dose carried by the parasite, *L. salmonis* could play a role in virus dissemination. Salmon lice infections have been shown

to increase cortisol levels in their fish hosts, indicating a stress response (Nolan et al. 1999, Pike & Wadsworth 1999, Bowers et al. 2000, Fast et al. 2006). If stressors persist for an extended period of time, fish may become immunosuppressed and therefore more susceptible to various pathogens (Pickering & Pottinger 1989, Bowers et al. 2000). Furthermore, Cusack & Cone (1986) hypothesized that a low transmission efficiency of a pathogen might be offset by parasite vectors especially for pathogens with short survival times outside the host. Although IHNV has proven stable in freshwater, in marine environments the survival time is shortened 2-fold (Barja et al. 1983). Moreover, dependent upon the microbial content and the presence of UV-radiation (natural sunlight), virus survival time in nature can be reduced from days to minutes (K. Garver unpubl. data). For IHNV, more studies are required to test the hypotheses that salmon lice infections would increase viral transmission efficiency.

An additional observation of our study was that most of the fish showed minor to severe signs of discoloration, scale loss and lesions. This is in accordance with previously reported effects of *Lepeophtheirus salmonis* infections on Atlantic and Pacific salmon (Jónsdóttir et al. 1992, Grimnes & Jakobsen 1996, Johnson et al. 1996). Severe lesions, as seen in Challenges 4.1 and 4.2, are known to cause osmotic stress and an increased susceptibility for secondary infections (Wootten et al. 1982, Jónsdóttir et al. 1992). This may explain the relatively high mortality in those salmon lice-infected groups. Furthermore, we isolated bacteria belonging to the genera *Pasteurella* and *Moraxella* from the kidney swabs of those fish with lesions. Bacteria of the family *Pasteurellaceae* are the causative agents of pasteurelliosis, have been reported from cultured and wild fish (Southgate 1993) and were first described from a clinical disease in farmed Atlantic salmon by Jones & Cox (1999). None of those bacterially induced symptoms were detected in groups where salmon lice were prevented from parasitizing naïve Atlantic salmon or among the IHNV control groups (no lice exposure). These observations further support the hypothesis that salmon lice activities on the host provide a port of entry for secondary infections.

In conclusion, results from this study indicate that under laboratory conditions adult *Lepeophtheirus salmonis* are able to acquire IHNV and transmit it to naïve fish through parasitism. However, the ephemeral association of IHNV with salmon lice gives evidence that *L. salmonis* act as a mechanical rather than a biological vector or reservoir.

Acknowledgements. This project was funded by a Natural Sciences and Engineering Research Council (NSERC) Strategic Projects Grant (STPGP 372605 – 08) awarded to D.E.B., K.A.G. and S.M.R.J. The authors thank Marine Harvest for providing Atlantic salmon and access to the fish farms, the staff of the Aquatic Animal Health section at the Pacific Biological Station for technical support and S. Johnson for providing valuable comments on this manuscript.

LITERATURE CITED

AFS-FHS (American Fisheries Society-Fish Health Section) (2010) FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2010 edn. AFS-FHS, Bethesda, MA

Ahne W (1978) Untersuchungsergebnisse über die akute Form der Bauchwassersucht (Frühlingsvirämie) der Karpfen. Fischwirt 28:46–47

Ahne W (1985) *Argulus foliaceus* L. and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). J Fish Dis 8:241–242

Barja JL, Toranzo AE, Lemos ML, Hetrick FM (1983) Influence of water temperature and salinity on the survival of IPN and IHN viruses. Bull Eur Assoc Fish Pathol 3:47–50

Barker DE, Braden LM, Coombs MP, Boyce B (2009) Preliminary studies on the isolation of bacteria from sea lice, *Lepeophtheirus salmonis*, infecting farmed salmon in British Columbia, Canada. Parasitol Res 105:1173–1177

Bergmann SM, Fichtner D, Skall HF, Schlotfeldt HJ, Olesen NJ (2003) Age- and weight-dependent susceptibility of rainbow trout *Oncorhynchus mykiss* to isolates of infectious haematopoietic necrosis virus (IHNV) of varying virulence. Dis Aquat Org 55:205–210

Bootland LM, Leong JC (1999) Infectious haematopoietic necrosis virus. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3. Viral, bacterial and fungal infections. CABI Publishing, Wallingford, p 57–121

Bowers JM, Mustafa A, Speare DJ, Conboy GA, Brimacombe M, Sims DE, Burka JF (2000) The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis.* J Fish Dis 23:165–172

Cusack R, Cone DK (1986) A review of parasites as vectors of viral and bacterial diseases of fish. J Fish Dis 9:169–171

Emmenegger EJ, Meyers TR, Burton TO, Kurath G (2000) Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Dis Aquat Org 40: 163–176

Faisal M, Schulz CA (2009) Detection of viral hemorrhagic septicemia virus (VHSV) from the leech *Myzobdella lugubris* Leidy, 1851. Parasit Vectors 2:45–48

Faisal M, Winters AD (2011) Detection of viral hemorrhagic septicemia virus (VHSV) from *Diporeia* spp. (Pontoporeiidae, Amphipoda) in the Laurentian Great Lakes, USA. Parasit Vectors 4:2–5

Fast MD, Muise DM, Easy RE, Ross NW, Johnson SC (2006) The effects of *Lepeophtheirus salmonis* infections on the stress response and immunological status of Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol 21:228–241

Fijan N, Sulimanovi D, Bearzotti M, Muzini D, Zwillenberg LO, Chlimonczyk S, Vautherot JF, de Kinkelin P (1983) Some properties of the *Epithelioma papulosum cyprini* (EPC) cell line from carp *Cyprinus carpio.* Ann Inst Pasteur Virol 134:207–220

Grant AAM, Jakob E, Richard J, Garver KA (in press) Concentration of infectious aquatic rhabdoviruses from freshwater and seawater using ultra-filtration. J Aquat Anim Health

Grimnes A, Jakobsen PJ (1996) The physiological effects of salmon lice infections on post-smolt of Atlantic salmon. J Fish Biol 48:1179–1194

Johnson SC, Blaylock RB, Elphick J, Hyatt KD (1996) Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. Can J Fish Aquat Sci 53:2888–2897

Jones MW, Cox DI (1999) Clinical disease in seafarmed Atlantic salmon (*Salmo salar*) associated with a member of the family Pasteurellaceae — a case history. Bull Eur Assoc Fish Pathol 19:75–78

Jónsdóttir H, Bron JE, Wootten R, Turnbull JF (1992) The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. J Fish Dis 15:521–527

Kabata Z (1973) The species of *Lepeophtheirus* (Copepoda: Caligidae) from fishes of British Columbia. J Fish Res Board Can 30:729–759

Kabata Z (1979) Parasitic copepoda of British fishes. The Ray Society, London

Kruse P, Steinhagen D, Körting W (1989) Development of *Trypanoplasma borreli* (Mastigophora: Kinetoplastida) in the leech vector *Piscicola geometra* and its infectivity for the common carp, *Cyprinus carpio.* J Parasitol 75: 527–530

LaPatra SE (1998) Factors affecting pathogenicity of infectious hematopoietic necrosis virus (IHNV) for salmonid fish. J Aquat Anim Health 10:121–131

Mehlhorn H, Ruthmann A (1992) Allgemeine Protozoologie. Gustav Fischer Verlag, Jena-Stuttgart

Mulcahy D, Klaybor D, Batts WN (1990) Isolation of infectious hematopoietic necrosis virus from a leech (*Piscicola salmositica*) and a copepod (*Salmincola* sp.), ectoparasites of sockeye salmon *Oncorhynchus nerka.* Dis Aquat Org 8:29–34

Nese L, Enger Ø (1993) Isolation of *Aeromonas salmonicida* from salmon lice *Lepeophtheirus salmonis* and marine plankton. Dis Aquat Org 16:79–81

Nolan DT, Reilly P, Wendelaar Bonga SE (1999) Infection with low numbers of the sea louse *Lepeophtheirus salmonis* induces stress-related effects in postsmolt Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 56: 947–959

Nylund A, Wallace C, Hovland T (1993) The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anaemia. In: Boxshall G, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. Ellis Horwood, Chichester, p 367–373

Petterson E, Sandberg M, Santi N (2009) Salmonid alphavirus associated with *Lepeophtheirus salmonis* (Copepoda: Caligidae) from Atlantic salmon, *Salmo salar* L. J Fish Dis 32:477–479

Pfeil-Putzien C (1978) Experimentelle Übertragung der Frühjahrsvirämie (spring viraemia) der Karpfen durch Karpfenläuse (*Argulus foliaceus*). Zbl Vet Med B 25:319–323

Pickering AD, Pottinger TG (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol Biochem 7: 253–258

Pike AW, Wadsworth SL (1999) Sea lice on salmonids: their biology and control. Adv Parasitol 44:233–337

Ritchie G (1997) The host transfer ability of *Lepeophtheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. J Fish Dis 20:153–157

Saksida SM (2006) Infectious haematopoietic necrosis epidemic (2001 to 2003) in farmed Atlantic salmon *Salmo salar* in British Columbia. Dis Aquat Org 72:213–223

Southgate P (1993) Disease in aquaculture. In: Brown L (ed) Aquaculture for veterinarians: fish husbandry and medicine. Pergamon Press, Oxford, p 91–130

St-Hilaire S, Ribble CS, Stephen C, Anderson E, Kurath G, Kent ML (2002) Epidemiological investigation of infectious hematopoietic necrosis virus in salt water net-pen reared Atlantic salmon in British Columbia, Canada. Aquaculture 212:49–67

Traxler GS, Rankin JB (1989) An infectious hematopoietic necrosis epizootic in sockeye salmon *Oncorhynchus nerka* in Weaver Creek spawning channel, Fraser River system, B.C., Canada. Dis Aquat Org 6:221–226

Traxler GS, Roome JR, Kent ML (1993) Transmission of infectious hematopoietic necrosis virus in seawater. Dis Aquat Org 16:111–114

Traxler GS, Roome JR, Lauda KA, LaPatra S (1997) Appearance of infectious hematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockeye salmon *Oncorhynchus nerka* during their migration and maturation period. Dis Aquat Org 28:31–38

Williams IV, Amend DF (1976) A natural epizootic of infectious hematopoietic necrosis in fry of sockeye salmon (*Oncorhynchus nerka*) at Chilko Lake, British Columbia. J Fish Res Board Can 33:1564–1567

Winton J, Batts W, De Kinkelin P, Le Berre M, Bremont M, Fijan N (2010) Current lineages of the *epithelioma papulosum cyprini* (EPC) cell line are contaminated with fathead minnow, *Pimephales promelas*, cells. J Fish Dis 33: 701–704

Wolf K (1988) Fish viruses and fish viral diseases. Cornell Universtiy Press, Ithaca, NY

Woo PT (1998) Protection against *Cryptobia* (*Trypanoplasma*) *salmositica* and salmonid cryptobiosis. Parasitol Today 14:272–277

Wootten R, Smith JW, Needham EA (1982) Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proc R Soc Edinb B 81:185–197

Editorial responsibility: V. Gregory Chinchar, Jackson, Mississippi, USA

Submitted: July 25, 2011; Accepted: September 19, 2011
Proofs received from author(s): November 15, 2011