Journal of Fish Diseases 2003, **26,** 511–520

Relative resistance of Pacific salmon to infectious salmon anaemia virus

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Abstract

Infectious salmon anaemia (ISA) is a major disease of Atlantic salmon, *Salmo salar*, caused by an orthomyxovirus (ISAV). Increases in global aquaculture and the international movement of fish made it important to determine if Pacific salmon are at risk. Steelhead trout, *Oncorhynchus mykiss*, and chum, *O. keta*, Chinook, *O. tshawytscha*, coho, *O. kisutch*, and Atlantic salmon were injected intraperitoneally with a high, medium, or low dose of a Norwegian strain of ISAV. In a second challenge, the same species, except chum salmon, were injected with a high dose of either a Canadian or the Norwegian strain. Average cumulative mortality of Atlantic salmon in trial 1 was 12% in the high dose group, 20% in the medium dose group and 16% in the low dose group. The average cumulative mortality of Atlantic salmon in trial 2 was 98%. No signs typical of ISA and no ISAV-related mortality occurred among any of the groups of *Oncorhynchus* spp. in either experiment, although ISAV was reisolated from some fish sampled at intervals post-challenge. The results indicate that while *Oncorhynchus* spp. are quite resistant to ISAV relative to Atlantic salmon, the potential for ISAV to adapt to *Oncorhynchus* spp. should not be ignored.

Keywords: anaemia, orthomyxovirus, salmon, virus.

Introduction

Infectious salmon anaemia (ISA) is an important disease of Atlantic salmon, *Salmo salar* L., reared in marine net pens in parts of Europe and North America. Outbreaks of ISA have occurred in Norway since 1984 (Thorud & Djupvik 1988) and, more recently, the disease has been reported to occur among salmonid fish in Scotland (Rodger, Turnbull, Muir, Millar & Richards 1998; Stagg, Bruno, Cunningham, Hastings & Bricknell 1999; Turnbull 1999), Canada (Mullins, Groman & Wadowska 1998; Bouchard, Keleher, Opitz, Blake, Edwards & Nicholson 1999; Lovely, Dannevig, Falk, Hutchin, MacKinnon, Melville, Rimstad & Griffiths 1999), the United States (Bouchard, Brockway, Giray, Keleher & Merrill 2001), Chile (Kibenge, Gárate, Johnson, Arriagada, Kibenge & Wadowska 2001a) and the Faroe Islands (Anonymous 2000). In August 2002, the causative virus (ISAV) was isolated for the first time in Ireland from seawater-reared rainbow trout, *Oncorhynchus mykiss* (Walbaum), showing no clinical signs of the disease (A. McVicar, personal communication).

Pathological changes due to ISA in Atlantic salmon are characterized by severe anaemia, leucopenia, petechiae in the viscera, ascites, haemorrhagic necrosis of liver and kidney, and congestion of the liver, spleen, kidney and foregut (Thorud & Djupvik 1988; Evensen, Thorud & Olsen 1991; Thorud 1991; Simko, Brown, MacKinnon, Byrne, Ostland & Ferguson 2000; Jones & Groman 2001). The disease is listed in the International Aquatic Animal Health Code of the Office International des Epizooties as a significant disease for which control measures are appropriate. Management of the disease is regulated within European Union member nations by Directive 93/53/EEC and includes eradication of a confirmed diseased population, surveillance, containment and fallowing. Norway, Canada and the United States have implemented similar regulations which restrict the movement of fish and eggs from areas where

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ISA is present and also include sanitary measures, surveillance, containment, disinfection, fallowing, and in some cases, eradication of the diseased population (Hastings, Olivier, Cusack, Bricknell, Nylund, Binde, Munro & Allan 1999; US Department of Agriculture, Animal and Plant Health Inspection Service 2002). Commercial vaccines against ISA became available in 2001 and are likely to be implemented in management strategies although their efficacy in the prevention of ISA is still being evaluated.

The aetiological agent of ISA is an enveloped virus, approximately 100 nm in diameter, which buds from polymorphonuclear leucocytes and endothelial cells lining the heart and blood vessels (Hovland, Nylund, Watanabe & Endresen 1994; Nylund, Krossøy, Watanabe & Holm 1996). The viral genome consists of eight segments of negative-stranded RNA ranging from 1.0 to 2.4 kb in size (Mjaaland, Rimstad, Falk & Dannevig 1997; Clouthier, Rector, Brown & Anderson 2002). The biochemical, physiochemical and morphological properties of ISAV are similar to those of influenza viruses (Falk, Namork, Rimstad, Mjaaland & Dannevig 1997) and, based on the complete cDNA sequence of the PB1 gene coding for the viral polymerase, it has been suggested that ISAV belongs to a new genus within the *Orthomyxoviridae*. Krossøy, Hordvik, Nilsen, Nylund & Endresen (1999) suggested the new genus be named *Aquaorthomyxovirus*; however, the International Committee on Taxonomy of Viruses has recently proposed the name *Isavirus* (http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm). Sequence comparisons of multiple isolates of ISAV have revealed two genetically distinguishable lineages of ISAV: a North American strain and a European strain with somewhat greater variation among isolates within the European clade (Blake, Bouchard, Keleher, Opitz & Nicholson 1999; Cunningham & Snow 2000; Inglis, Bruce & Cunningham 2000; Devold, Falk, Dale, Krossoy, Biering, Aspehaug, Nilsen & Nylund 2001; Kibenge, Kibenge, McKenna, Stothard, Marshall, Cusack & McGeachy 2001b; Krossøy, Nilsen, Falk, Endresen & Nylund 2001; Ritchie, Cook, Melville, Simard, Cusack & Griffiths 2001).

Clinical outbreaks of ISA occur in seawater-adapted Atlantic salmon reared in marine net pens. However, ISAV has also been identified in wild Atlantic salmon (Nylund, Kevenseth & Krossøy 1995a), sea trout, *Salmo trutta* L., (Raynard, Murray & Gregory 2001a) and Atlantic herring, *Clupea harengus harengus* L. (A. Nylund, personal communication) although no clinical signs of the disease were present. Sea trout (Nylund, Alexandersen, Løvik & Jakobsen 1994; Nylund, Alexandersen, Rolland & Jakobsen 1995b; Nylund & Jakobsen 1995; Rolland & Nylund 1998), brown trout, *Salmo trutta* L. (Snow, Raynard & Bruno 2001), Arctic char, *Salvelinus alpinus* (L.) (Snow *et al.* 2001), and rainbow trout (Nylund, Kvenseth, Krossøy & Hodneland 1997; Snow *et al.* 2001) represent possible natural reservoirs of ISAV because the virus is able to propagate in these species without producing clinical disease, while saithe, *Pollachius virens* (L.), a species commonly associated with marine net pens, was shown not to be a likely reservoir (Snow, Raynard, Bruno, van Nieuwstadt, Olesen, Lovold & Wallace 2002). Recently, Kibenge *et al.* (2001a) reported isolation of ISAV from farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in Chile. However, these clinically diseased fish exhibited markedly different pathology from typical ISA and although the disease has been associated with an orthomyxovirus, it is reported that the disease in Chilean coho is of multifactorial origin (Smith, Larenas, Contreras, Cassigoli, Venegas, Rojas, Guajardo, Troncoso & Macias 2002).

Studies on the susceptibility of various fish species to ISAV have focused on those endemic to areas where ISA has been diagnosed. Because of increases in global aquaculture and the international movement of fish, as well as reports of isolation of ISAV from fish cultured in areas of the world where the virus was not formerly known to exist, it was important to determine if stocks of Pacific salmon, *Oncorhynchus* spp., were at risk from a potential introduction of ISAV into western North America. In this study, we compared the susceptibilities of Pacific and Atlantic salmon challenged with Norwegian and Canadian isolates of ISAV.

Materials and methods

Virus strains and cell lines

The CCBB strain of ISAV was isolated from infected Atlantic salmon reared at a commercial aquaculture company in Back Bay, New Brunswick, Canada (Clouthier *et al.* 2002) and the Bremnes strain was isolated from infected Atlantic salmon reared near Bergen, Norway (Krossøy *et al.* 2001).

The isolates were propagated in the salmon head kidney (SHK-1) cell line (Dannevig, Falk & Namork 1995). Cell monolayers were inoculated with 100 μL of thawed ISAV stock and cultures were incubated for 1 h at 15 °C to allow for virus adsorption. Leibowitz's L-15 medium supplemented with L-glutamine (4 mM), penicillin (100 IU mL^{-1}), streptomycin (100 μg mL^{-1}), and 2-mercaptoethanol (40 μM) was then added and cells were further incubated at 15 °C. Cell culture fluid containing ISAV was collected after the appearance of cytopathic effect (CPE) and aliquots of virus stock were frozen at −70 °C for later use. The Atlantic salmon kidney (ASK) cell line (Devold, Krossoy, Aspehaug & Nylund 2000) was used for virus titration. Serial 10-fold viral dilutions of virus stocks were inoculated on 96-well plates containing ASK monolayers and the 50% tissue culture infective dose ($TCID_{50}$) was determined.

Stocks of fish

During the late spring and summer of 2001, Atlantic salmon (Penobscot stock) were obtained from the Greenlake National Fish Hatchery, Ellsworth, ME, USA; chum, *O. keta* (Walbaum), and coho salmon were obtained from the Quilcene National Fish Hatchery, Quilcene, WA, USA; and fall chinook salmon, *O. tshawytscha* (Walbaum), and winter steelhead trout, *O. mykiss* (Walbaum), were obtained from the Makah National Fish Hatchery, Neah Bay, WA, USA. The fish were approximately 5–10 cm in length when received and were held in separate aquaria provided with pathogen-free fresh water at 10 °C. All fish were fed daily with a moist salmon diet until used in the experimental challenges.

Experimental trials

In preparation for the experimental challenges, the ability of free chlorine to inactivate ISAV was determined (data not shown). The aquatic Biosafety Level 3 (BL-3) laboratory at the Western Fisheries Research Center was set up to provide a series of 18 L aquaria supplied with pathogen-free, fresh water at 10 °C. All effluent from the BL-3 laboratory was batch-treated with chlorine at appropriate levels. Frozen stocks of virus were thawed and the titre determined in order to provide challenge doses of known titre when needed. The first trial was conducted in the autumn of 2001 (November–December) when the fish were approximately 8–13 cm in length. The second trial was conducted 4 months later when the fish had grown to 11–20 cm in length. Temperatures ranged from 9.9 to 11 °C during the course of the first experiment and from 9.3 to 12 °C in the second experiment.

Trial 1

Yearling chum salmon, steelhead trout, chinook salmon, coho salmon and Atlantic salmon were moved into the BL-3 laboratory and distributed into tanks. Duplicate groups containing 25 fish per species per treatment were injected intraperitoneally (i.p.) with 0.1 mL of specific dilutions of stock virus to provide fish with a high (10^7 $TCID_{50}$), medium (10^5 $TCID_{50}$) or low (10^3 $TCID_{50}$) dose of the Bremnes strain of ISAV. Control fish received 0.1 mL of minimal essential medium (MEM). A separate group of 25 fish of each species was challenged with the high dosage of ISAV and held in a tank to be sampled for histology and virus titration. Tanks were checked daily for dead or moribund fish and the first three fish dying in any replicate were dissected for recording of gross clinical signs of disease and for determination of viral titre. Five fish from each experimental group were randomly sampled at 13 and 26 days post-challenge, and at the conclusion of the experiment, 33 days post-challenge. Sampled fish were killed with an overdose of MS-222. Two fish were placed in Davidson's fixative for histology and three fish were dissected for recording of gross clinical signs of ISA and for determination of viral titre. Kidney, spleen and occasionally liver tissue were removed and diluted 1:3 (w/v) in L-15 medium, supplemented as before. The tissue was homogenized and serial 10-fold dilutions were inoculated onto ASK monolayers in 96-well plates. Plates were incubated at 15 °C and the $TCID_{50}$ g^{-1} of tissue was estimated to the nearest whole $\log_{10}$.

Trial 2

Duplicate groups of 25 coho, chinook and steelhead were injected i.p. with 0.1 mL of specific dilutions of stock virus to provide fish with a high (10^7 $TCID_{50}$) dose of ISAV-Bremnes or with ISAV-CCBB. Controls received MEM. Duplicate groups of 25 Atlantic salmon were injected i.p. with a high dose (10^7 $TCID_{50}$) of ISAV-CCBB or MEM.

A third group of 25 fish of each species was injected with a high dose (10^7 $TCID_{50}$) of ISAV-CCBB for titre and histology samples with the exception of the Atlantic salmon where only three Atlantic salmon remained for creation of the histology/titre group. Tanks were checked daily for dead or moribund fish and the first four to five fish dying in any replicate were dissected for recording of gross clinical signs of disease and for determination of viral titre. Random samples of five fish from each experimental group of Pacific salmon challenged with the CCBB strain of ISAV were collected 15 and 22 days post-challenge, and at the conclusion of the experiment, 28 days post-challenge. At each sampling date, two fish were placed in fixative and the three remaining fish were dissected and tissues collected for viral titration, as before. For Atlantic salmon challenged with the CCBB isolate, two fish were sampled on day 15 post-challenge for determination of viral titre and one fish was sampled at the conclusion of the challenge because only three fish were used in this group.

Results

Trial 1

Cumulative per cent mortalities from ISA-challenged fish are shown in Table 1. The only mortalities among *Oncorhynchus* spp. in this trial occurred in the first two days following injection. No clinical signs were noted and these fish were judged to have died from stress associated with movement and challenge. Mortalities in the Atlantic salmon groups were low, ranging from 8 to 28%. In one of the Atlantic salmon low-dose ISAV groups and in both of the medium-dose ISA groups, a few fish died immediately following injection. Mortality in the first low-dose ISA group ceased at day 14 post-injection and in the medium-dose replicates, at days 30 and 33, respectively. The second low-dose ISAV group of Atlantic salmon showed mortality commencing on day 29 and ending on day 30. In both Atlantic salmon high-dose ISAV groups, all mortality occurred between days 24 and 31. None of the Atlantic salmon mortalities from the first two days post-injection exhibited signs of ISA; however, all the remaining Atlantic salmon mortalities exhibited pathological changes consistent with clinical ISA and those that were positive for viral isolation on the ASK cell line had titres ranging from 10^3 to 10^8 $TCID_{50}$ g^{-1} (Table 2). Some virus was recovered from chum, coho and Atlantic salmon as well as from steelhead sampled randomly at 13 days post-injection (Table 2). No ISAV was recovered from any fish sampled at 26 days post-injection or in fish sampled at the end of the first trial at 33 days post-injection. No mortalities occurred in any of the control groups during trial 1.

Trial 2

There were no mortalities in the experimental groups of Pacific salmon during trial 2 with the exception of two chinook salmon that died the day following injection in one tank of fish injected with the Bremnes strain. Cumulative per cent mortality in the Atlantic salmon groups challenged with ISAV (CCBB) reached 96 and 100% (Fig. 1). No mortality was recorded in any of the control groups of Pacific salmon throughout the trial. The Atlantic salmon control groups had 0 and 20% mortality (Table 3). The mortality in the replicate of the Atlantic salmon controls began 22 days post-challenge and was due to an aggressive fungal infection. No gross clinical signs of ISA were observed and the fish tested negative for virus.

Titres were calculated for the first four or five Atlantic salmon that died in each replicate of the CCBB challenge (Table 4). High titres were obtained from these fish (ranging from $10^{4.7}$ to

Table 1 Cumulative per cent mortality among five salmonid species following intraperitoneal injection of three different doses of a Norwegian strain of infectious salmon anaemia virus

	Low 1	Low 2	Medium 1	Medium 2	High 1	High 2	Control 1	Control 2
Chum	8[a]	4[a]	12[a]	12[a]	0	0	0	0
Coho	0	0	0	0	0	0	0	0
Chinook	0	0	0	0	0	0	0	0
Steelhead	0	0	0	0	0	0	0	0
Atlantic	12	12	28	16	12	8	0	0

[a] All mortalities occurred at 1 day post-injection.

Table 2 Approximate titres in five salmonid species challenged with three doses of a Norwegian strain of infectious salmon anaemia virus in trial 1

			Virus titre ($TCID_{50}$ g^{-1})	
	ISAV dose	Days post-infection	In selected fish dying during the trial	In fish randomly sampled
Atlantic salmon	High	13		10^5
	High	13		10^4
	High	13		10^3
	High	26		BDL
	High	26		BDL
	High	26		BDL
	Medium	20	10^6	
	Medium	23	BDL	
	Medium	24	10^8	
	High	24	10^8	
	High	24	10^8	
	Medium	25	10^8	
	Medium	25	10^3	
	High	25	10^3	
	High	25	10^3	
	Low	29	BDL	
	Medium	30	BDL	
	High	31	BDL	
	High	33		BDL
	High	33		BDL
	High	33		BDL
Chinook salmon	High	13		BDL
	High	13		BDL
	High	13		BDL
	High	26		BDL
	High	26		BDL
	High	26		BDL
	High	33		BDL
	High	33		BDL
	High	33		BDL
Chum salmon	High	13		10^5
	High	13		10^5
	High	13		10^4
	High	26		BDL
	High	26		BDL
	High	26		BDL
	High	33		BDL
	High	33		BDL
	High	33		BDL
Coho salmon	High	13		10^3
	High	13		BDL
	High	13		BDL
	High	26		BDL
	High	26		BDL
	High	26		BDL
	High	33		BDL
	High	33		BDL
	High	33		BDL
Steelhead trout	High	13		10^3
	High	13		BDL
	High	13		BDL
	High	26		BDL
	High	26		BDL
	High	26		BDL
	High	33		BDL
	High	33		BDL
	High	33		BDL

BDL, below detection limit of the cell culture assay.

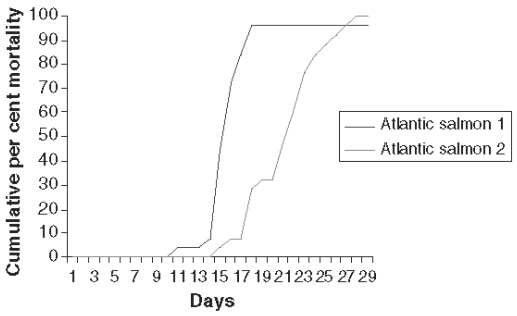

Figure 1 Cumulative per cent mortality in two replicate groups of Atlantic salmon challenged with ISAV strain CCBB by intraperitoneal injection in trial 2.

$10^{8.6}$ $TCID_{50}$ g^{-1}) confirming they had died from ISA. Atlantic salmon randomly sampled at 15 days post-challenge also had high titres of ISAV-CCBB indicating they were actively infected. Low-level titres of ISAV were detected in one coho and one steelhead sampled at day 15 (Table 4), however, these levels may also have been due to residual challenge virus that had not yet been cleared. No titres were detected from any of the Pacific salmon sampled at 22 days post-challenge or at the conclusion of the experiment, 28 days post-challenge. The one Atlantic salmon survivor from an ISA challenge group was killed at the conclusion of the experiment and was used for virus isolation. The ISAV titre in the survivor was $10^{6.8}$ $TCID_{50}$ g^{-1} suggesting the infection was still active in this last remaining fish and that the challenge had been unusually severe. Gross pathology observed in all Atlantic salmon mortalities was consistent with findings typically associated with ISA.

Discussion

Here we report the results of a controlled laboratory challenge of four species of Pacific salmon with two strains of ISAV. While the intraperitoneal route of challenge does not represent a natural form of infection (Nordmo 1997) and other challenge routes have been shown to be useful for ISAV (Jones & Groman 2001; Mikalsen, Teig, Helleman, Mjaaland & Rimstad 2001; Raynard, Snow & Bruno 2001b), it is an effective method for presenting a naïve animal with a known amount of virus, especially at a high dose. Here, we used a relatively severe challenge dose of ISAV delivered by intraperitoneal injection to show that Pacific salmon were considerably more resistant to ISAV compared with their Atlantic counterparts. Although our findings suggest Pacific salmon are quite resistant to the strains we used, ISA has been reported to occur in farmed coho salmon in Chile (Kibenge *et al.* 2001a). However, Smith *et al.* (2002) have suggested that the disease associated with the orthomyxovirus recovered from Chilean coho salmon may be of multifactorial origin.

Trial 1 resulted in lower than expected mortality in the Atlantic salmon challenged with the Norwegian strain of ISAV. Our initial concern was that the virus titre was inaccurately calculated or that it declined during our injection procedures, although the virus stock was maintained on ice during injection. To eliminate the possibility of experimental error, the titre of the frozen virus stock was reconfirmed and the virus dilutions used for challenge were retested. Titres of frozen stocks were the same on retesting and the material remaining from the challenge preparations that were held at 4 °C for several weeks had declined only twofold. Although it is also possible that the ISAV-Bremnes used in our trial had been attenuated during laboratory culture, it now seems more likely that the low mortality in Atlantic salmon was a result of the trial being carried out in the autumn, a seasonal effect that has been observed previously (K. Falk, personal communication; A. Nylund, personal communication). Unfortunately, insufficient Atlantic salmon remained in our stock group to establish challenge groups for the Bremnes isolate in trial 2; however, our results suggest that seasonal differences in the susceptibility of Atlantic salmon to ISAV may need to be taken into account in future trials.

Table 3 Cumulative per cent mortality among four salmonid species following intraperitoneal injection of a high dose of the Bremnes or CCBB isolates of infectious salmon anaemia virus

	Bremnes 1	Bremnes 2	CCBB 1	CCBB 2	Control 1	Control 2
Coho	0	0	0	0	0	0
Chinook	0	8[a]	0	0	0	0
Steelhead	0	0	0	0	0	0
Atlantic	NA	NA	96	100	0	20

NA, insufficient Atlantic salmon remained to establish these groups.
[a] All mortalities occurred at 1 day post-injection.

Table 4 Titres in four salmonid species challenged with a high dose of the CCBB isolate of infectious salmon anaemia virus in trial 2

	Days post-infection	Virus titre ($TCID_{50}$ g^{-1}) In selected fish dying during the trial	In fish randomly sampled
Atlantic salmon	10	$10^{4.7}$	
	13	$10^{5.2}$	
	14	$10^{5.5}$	
	14	$10^{4.8}$	
	14	$10^{4.8}$	
	15	$10^{8.6}$	
	15	$10^{8.0}$	
	15		$10^{5.8}$
	15		$10^{4.8}$
	15		$10^{5.0}$
	17	$10^{5.4}$	
	17	$10^{5.6}$	
	28		$10^{6.8}$
Chinook salmon	15		BDL
	15		BDL
	15		BDL
	22		BDL
	22		BDL
	22		BDL
	28		BDL
	28		BDL
	28		BDL
Coho salmon	15		$10^{2.5}$
	15		BDL
	15		BDL
	22		BDL
	22		BDL
	22		BDL
	28		BDL
	28		BDL
	28		BDL
Steelhead trout	15		$10^{1.0}$
	15		BDL
	15		BDL
	22		BDL
	22		BDL
	22		BDL
	28		BDL
	28		BDL
	28		BDL

BDL, below detection limit of the cell culture assay.

Nonetheless, disease was induced in the Atlantic salmon in trial 1 and mortality due to ISA began in the latter half of the experimental period as expected. No disease signs were observed in any of the chum, coho, chinook or steelhead. During the course of the first trial, virus was isolated from all the species tested, although titres from Atlantic salmon and chum salmon tended to be one to threefold higher than titres obtained from chinook, steelhead or coho. The higher titres in chum salmon may indicate that this species is slightly more susceptible than the other Pacific salmonids tested or they may be due to stresses associated with being held in fresh water. Chum salmon are normally in the sea long before the age of fish used in our trials and these fish showed the least tolerance to our injection procedures. In fact, the chum salmon were not included in the second trial because the stock fish had begun to experience losses prior to the second challenge.

The second trial resulted in mortality curves that were much more typical for experimental ISA infections in Atlantic salmon (Jones, MacKinnon & Groman 1999; Raynard *et al.* 2001b). The onset of mortality occurred at 11 and 15 days post-challenge in the replicate groups of Atlantic salmon. All Atlantic salmon mortalities in these two replicates exhibited gross pathology typical of ISA; however,

the replicate with the earlier onset of disease had a few fish with a fungal infection of the caudal peduncle. The earlier onset of mortality in that one replicate may have resulted from a co-infection with the fungus, although it could not be determined if the fungus predisposed the fish to ISAV or if ISAV suppressed the resistance to the fungus. No fungus was observed in any of the other experimental groups and the outcome was ultimately the same in both replicate tanks of Atlantic salmon. There were no mortalities among the chinook, steelhead or coho. Virus was reisolated from one steelhead and one coho randomly sampled at 15 days post-challenge, although titres were low (10^1 and $10^{2.5}$ $TCID_{50}$ g^{-1}, respectively) when compared with the three Atlantic salmon sampled on this date which were all positive for ISAV with titres ranging from $10^{4.8}$ to $10^{5.8}$ $TCID_{50}$ g^{-1}.

These experiments demonstrated that the Pacific salmon species, chum, coho, chinook and steelhead, were relatively resistant to ISAV when compared with Atlantic salmon. Thus it appears that Pacific salmon species are at relatively low risk should ISA spread to the west coast of North America where these species are endemic. Our results also suggest that Pacific salmon may offer an attractive option for aquaculture in areas where ISA is problematic. However, salmonids such as rainbow trout, brown trout and sea trout have been reported to be carriers of ISAV, and the reisolation of ISAV from the Pacific salmon species used in these trials indicates that it would be unwise to overlook the possibility of ISAV replicating in, or establishing a carrier status among these species should they be exposed to the virus. Furthermore, the haemagglutinin gene of ISAV shows substantial diversity among isolates that may be associated with antigenic variation or recombination (Devold *et al.* 2001; Kibenge *et al.* 2001b; Cunningham, Gregory, Black, Simpson & Raynard 2002) and such variation may result in evolution of strains with differences in host range, virulence or immune response to vaccines.

Acknowledgements

The authors thank Fred Trasko of the US Fish and Wildlife Service (USFWS) Greenlake National Fish Hatchery, Larry Telles of the USFWS Quilcene National Fish Hatchery and Al Jensen of the USFWS Makah National Fish Hatchery for providing the fish used in these experiments. We also thank John Coll, Ray Brunson and Joy Evered of the USFWS; Jan Glecker and Marcia House of the Northwest Indian Fisheries Commission; and Bill Batts of the Western Fisheries Research Center for assistance in coordinating and conducting these experiments. Dr Are Nylund provided the Norwegian isolate of ISAV and Deborah Bouchard and Bill Keleher provided the Canadian isolate of ISAV, SHK-1 cells and technical advice. The authors are indebted to Dr Curt Endresen who provided thoughtful advice and technical review of this manuscript.

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Received: 20 February 2003
Accepted: 8 April 2003