Fred Kibenge - RE: Re: Paper

From: Fred Kibenge
To: Simon.Jones@dfo-mpo.gc.ca
Date: 14/11/2011 11:28 AM
Subject: RE: Re: Paper
CC: Laura Richards; Mark Higgins; Mark Saunders; Molly Kibenge; Stewart ...

Dear Simon,

Thank you for your input on the publication question and for the information about the Cohen Commission.

For your information, this data (with your qualifiers below) is being relayed to CFIA as part of their on-going investigation. Our lab is currently making preparations for participation in this process and will disclose this work notwithstanding its age. I think that this historical data may also clarify some of the issues around recent ISAV testing in BC.

As you likely know, the OIE Manual of Diagnostic Tests for Aquatic Animals 2010 (http://www.cabi.org/ac/default.aspx?site=162&page=3325) in the chapter on ISAV (http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.3.05_ISA%20.pdf) lists both criteria for suspicion and for confirmation of ISAV infection. This manuscript, which describes a targeted surveillance with significant prevalence rates of suspected infection in specific Pacific salmon species, accompanied by virus sequence data, may assist the CFIA.

Thanks.

Fred.

>>> "Jones, Simon" <Simon.Jones@dfo-mpo.gc.ca> 11/4/2011 8:35 PM >>>

Dear Molly,

Thanks for the message and for asking permission to submit the draft manuscript for publication.

Your email is timely. Recent events in BC concerning the alleged detection of ISA virus in wild Pacific salmon brought to mind the research you conducted while you were an NSERC Visiting Fellow at the Pacific Biological Station, and some of the questions it raised.

You may recall that Fish Health staff at DFO disagreed that your data supported the conclusion that ISA virus, whether asymptomatic or otherwise, occurred in the salmon you examined. For example, all attempts to isolate the virus into cell culture failed. As you are aware, the OIE case definition for ISAV infection requires either the isolation and identification of the virus into cell culture from two independent samples taken on two separate occasions, or the isolation and identification of ISAV in cell culture plus RT-PCR or serologic confirmation from tissue preparations. You may further recall that an independent laboratory was unable, on more than one occasion, to reproduce your RT-PCR results on the same samples. In my opinion, it will be very important to better understand the disagreement in laboratory results and to better test the hypothesis of "Asymptomatic ISAV" before moving towards publication.

On the related issue, CFIA is now conducting confirmatory testing of more recent samples from Pacific salmon in which initial positive RT-PCR results for ISAV were obtained. In addition, the Cohen Commission will reconvene for two days in December to hear evidence on ISA virus in British Columbia. I will wait to hear the outcome of these processes before further discussion on a 7-year-old manuscript. Consequently, I do not give permission

to submit this work, whether in this manuscript or any other, for publication.

With my best wishes,

Simon

Dr. Simon R.M. Jones
Aquatic Animal Health Section
Pacific Biological Station
Fisheries and Oceans Canada
3190 Hammond Bay Road
Nanaimo, British Columbia
V9T 6N7, Canada

Tel: 250 729 8351
Fax: 250 756 7053
E-mail: simon.jones@dfo-mpo.gc.ca

http://www.pac.dfo-mpo.gc.ca/sci/aqua/profiles/jones_e.htm

From: Molly Kibenge [mailto:Mkibenge@upei.ca]
Sent: November 4, 2011 7:19 AM
To: Jones, Simon
Cc: Fred Kibenge
Subject: Fwd: Re: Paper

Dear Simon,

I hope all is well in Nanaimo. It has been long since we last corresponded.

I am writing to follow up on the work I did in your lab during my post-doc at DFO-PBS, Nanaimo. As you recall we prepared a draft manuscript (attached). I am writing to ask your permission to submit it for publication as soon as possible. I would like to submit it to Diseases of Aquatic Organisms or Journal of Fish Diseases. What do you think?

Please let me know. If you have additional changes, please send them along and I will then proceed from there.

Please say hello to the family.

Best regards,

Molly.

Asymptomatic infectious salmon anaemia in juvenile *Oncorhychus* species from the North West Pacific Ocean

Kibenge Molly. T [1*], Jones Simon[1], Traxler Garth[1], Kibenge Frederick. S.B[2]

[1]Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia, V9T 6N7, Canada,

[2] Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada CIA 4P3.

Running title: Surveillance of wild pacific salmon for viruses.

*Corresponding Author: Dr. Molly Kibenge;

E-mail: KibengeM@pac.dfo-mpo.gc.ca; Tel: (250) 729-8380;

Fax: (250) 756-7053

Abstract

Juvenile chinook (*Oncorhynchus tshawytscha*), chum (*O. keta),* coho (*O. kisutch),* pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) from the West Coast of Vancouver Island, Southeast Alaska, and the Bering Sea were surveyed between August 2002 and April 2003 for infectious salmon anaemia virus (ISAV). Spawning sockeye from the Cultus Lake and Kokanee from Lois Lake, BC population was also sampled. Pooled or individual tissues were tested by RT-PCR, nucleotide sequencing and virus isolation. ISAV segment 8 was amplified from 34 of 121 (28%) chinook and 15 of 88 (17%) pink salmon caught off the west Coast Vancouver Island and southeast Alaska. ISAV segment 8 was also amplified from all 64 spawning sockeye and one cultured Atlantic salmon. The 220bp RT-PCR products were 94% to 98% homologous with Canadian ISAV isolates and 92% to 93% with European ISAV isolates. A product of 377 bp was obtained with Segment 7 ORF1 products were obtained in 5 chinook fish and the nucleotide sequence corresponded to ISAV segment 7 ORF2 products and was 95.7% identical to NBISA01 control isolate (Canadian isolate) and 99.7 % identity to an ISAV isolate 810/9/99 from Norway. ISAV segments 2, 6 and full opening frame for segment 8 were not amplified nor was ISAV isolated onto SHK or CHSE and ASK-2 cells. These results lead us to conclude that an asymptomatic form of ISA occurs among some species of wild Pacific salmon in the north Pacific.

KEYWORDS: Oncorhynchus, wild Pacific salmon, ISAV, Surveillance

Introduction

Viruses are important pathogens in marine-farmed fish however their importance as pathogens in wild stocks and the roles played by wild fish as reservoirs of infections are poorly understood. Infectious salmon anemia (ISA) is a severe disease of marine farmed Atlantic salmon, *Salmo salar* caused by an Orthomyxovirus-like virus, (Mjaaland *et al*., 1997) The disease is characterized by severe anemia, leucopenia, ascites and hemorrhagic liver necrosis and kidney necrosis. The disease has affected marine farmed Atlantic salmon in Norway since 1984 (Thorud and Djupvik, 1988). More recently the disease has been diagnosed in marine farmed Atlantic salmon in Eeastern Canada (New Brunswick and Nova Scotia), Scotland, eastern USA (Maine), and the Faroe Islands (1998, Mullin *et al*., Ritchie *et al*., 2001, 1998, Rogers *et al*., 1998, Bouchard *et al*., 2001, Anonymous 2000). ISAV was isolated from farmed coho salmon in Chile (Kibenge *et al*., 2001). The virus has also been found in health farmed rainbow trout (Anon 2002). Although, *Oncorhynchus* species have not been shown to be affected by the natural disease and experimental infection, virus was recovered from chum, coho and steelhead trout after inoculation with a very high virus concentration of 10^7 $TCID_{50}$ (Rolland and Winton, 2003). Also experimental infection with high concentration ($TCID_{50}$ over 10^7 /200µl) of selected ISAV isolates caused mortality in rainbow trout (*Oncorhynchus mykiss*) (McWilliams *et al*., 2003, Kibenge unpublished data) indicating that some of *Oncorhynchus* species could be affected by ISAV and also serve as reservoirs of

Comment [DFOE1]:

Comment [DFOE2R1]:

the virus. Natural and experimentally infected sea trout (*Salmo trutta)* and brown trout (*Salmo trutto)* become asymptomatic ISAV carriers and transmit the virus to Atlantic salmon by cohabitation (Raynard *et al*., 2001, Nylund and Jakobosen, 1995; Nylund *et al.*, 1995; Nylund *et al*., 1997). Also ISA virus was isolated from asymptomatic wild Atlantic salmon (Olivier G, 2002, Raynard *et al*., 2001) Arctic char (*Salvelinus alpinus*) (Snow *et al*., 2001), pollock (*Pollachius virens*) from ISA infected cages and cod (MacLean *et al*., 2002). Thus wild marine fish stock may act as the virus reservoir and transmit the virus to farmed Atlantic salmon. Nylund *et al*., (2003) hypothesized that wild salmonids species are the source of benign ISAV isolates which become virulent after mutation either in the wild or in the farmed Atlantic salmon after transmission based on the analysis of the highly polymorphic region (HPR) of ISAV haemagglutinin gene. (Nylund *et at.,* 2003). The Northwest Pacific coast of Canada and the United States are the only regions where marine Atlantic salmon farming is practised that are still free of ISA disease. Because routine screening for the virus has been mostly done by virus isolation on cell culture, there is a possibility that low levels of virus in fish tissues or presence of non-pathogenic virus strains could go undetected. Although there is increased pathogen surveillance studies they are directed to culture fin fish. Therefore for this study, molecular biology technique of reverse transcription-polymerase chain reaction (RT-PCR) was added to diagnostic methods for investigation of ISAV in wild pacific salmon species. In this study five

Pacific salmon species were examined for the presence of infectious salmon anemia (ISAV) virus.

Materials and methods

Fish, tissue collection and storage.

Juvenile chinook (*Oncorhynchus tshawytscha*), chum (*O. keta),* coho (*O. kisutch),* pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) from the West Coast of Vancouver Island, Southeast Alaska, and the Bering Sea were surveyed between August 2002 and April 2003 for infectious salmon anaemia virus (ISAV). Spawning sockeye from the Cultus Lake and Kokanee salmon from the Lois Lake were also sampled (Table 1). Organ tissues (head kidney, heart, spleen and liver) were harvested from partially thawed high sea pacific salmon and from freshly sacrificed sockeye, Kokanee and Atlantic salmon from Cultus Lake, Lois Lake and PBS respectively. Two of the 8 Atlantic salmon sampled had been on salt water for a while the remaining 6 were only exposed to salt water for 2 weeks for the purpose of fungal treatment. Location and the weights of fish are shown in Table .1 in the results section. The samples were stored and -80C°individually or pooled until they were analyzed for virus by RT-PCR and cell culture.

Cells and Virus propagation.

Chinook salmon embryonic cells (CHSE-214) cells were grown in 24 or 48 well plates using Eagle's minimum essential medium supplemented with 10% fetal

bovine serum (FBS), 2mM L-glutamine (Invitrogen Life Technologies) while salmon head kidney (SHK-1) and Atlantic salmon kidney (ASK-2) cells monolayers were grown using Leibowitz's L-15 medium (Dannevig *et a*l., 1997) The tissues were macerated and homogenised in presence of HBSS supplemented with 10% antibiotic/mycotic solution. The homogenate was then clarified by centrifugation at 3000 rpm for 15 minutes was inoculate on cell monolayer and incubated for 1hr at 15 °C to allow virus adsorption. After which MEM or L-15 supplemented with 4% FBS, 1% antibiotic/mycotic solution (Invitrogen Life Technologies) and HEPES (Sigma) was added to the wells. Wells of uninfected cells were left as negative controls and the plates were returned to the 15°C incubator. The cell monolayers were checked for development of CPE using inverted microscope. Normally ISAV causes CPE in CHSE-214 cells by 15 days while it may take up to 21 days for CPE to develop in SHK-1 cells.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR).

Samples of kidney, liver, spleen and heart were harvested from individual fish and were either stored and analysed separately or pooled. The tissues were then macerated in presence of Hank's balanced salt solution (HBSS) with 10% antibiotic solution. Total RNA was extracted from 300 µl of tissue homogenate or cell culture lysate in case of the control IHNV and VHSV using the Trizol LS Reagent (Invitrogen, Canada) following the manufacturer's protocol. The RNA pellet was dissolved in 50 µl RNase free dionized water. The one step reverse transcription-polymerase chain reaction (RT-PCR) was performed using 1 µl (1 µg-2 µg) of RNA in a 25 µl volume reaction using one step RT-PCR kit (Qiagen). RT-PCR was performed in PTC-200DNA engine Peltier thermal cycler (MJ Research Inc.) Cycling conditions for all viruses consisted of once cycle of cDNA synthesis at 50°C for 30 minutes followed by 15 minutes at 95°C to activate the Hot Star Taq DNA polymerase and inactivate the reverse transcriptase. This was followed by 40 cycles, each consisting of denaturation at 95 °C for 30s, annealing at 61 °C for 45s in case of for ISAV and extension at 72 °C for 90s with final extension at 72 °C for 10 minutes. The primer sequence used in the RT-PCR for ISAV amplification of 220 bp fragment of segment 8 of the virus (Devold *et al.*, 2000, Kibenge *et al.*, 2001), and the nucleotide sequence for both the sense (342-363 nt) and antisense (532-552 nt) are (5'GAA GAG TCA GGA TGC CAA GAC G 3', 5'GAA GTC GAT GAT TCG CAG CGA 3') respectively. The samples

sent to AVC were also analyzed with segment 7 ORF1 and segment 7OR2 primers. The two opening frames share the reverse primer and the primer nucleotide sequence and were designed using ISAV Accession # AF328627 (Ritchie *et al.,* 2002). Here are the sequences for both sense and the antisense primers are: ISAV SEG7 (ORF1) FOR (23 mer from 1-23nt) 5'-ATG GAT TTC ACC AAA GTG TAT GG-3' , and ISAV SEG7 (ORF1) REV (23 mer from 881-903) 5'-TCACATTCTGAAGTGAAGTCCAG-3' and for the segment 7 ORF2 the sense primer is (82 mer from 1-63/590-608 nt) 5'-ATG GAT TTC ACC AAA GTG TAT GGT GTG CTG GTT GAC CAA CTA AAA CTT CAC GGA AAA GAC AAG GTG GCT TCT TTC CTG TCG G-3'. The PCR products were resolved by electrophoresis on 1.5% agarose gel with 0.01 mg ethidium bromide and visualized using the imaging system ( ). In samples that had a PCR product of 220 bp, amplification of the other ISAV gene segments (2, 6 and ORF1 and 2 for segment 8 proteins) was attempted. In the beginning of the study we included ISAV positive control to make sure that the reactions were working properly. We obtained positive RNA control from Atlantic Veterinary College (AVC) Virology Research laboratory (Charlottetown, PEI).

Cloning, sequencing and sequence analysis

The RT-PCR products were purified from the agarose gel using either Roche or Qiagen PCR purification kit and then cloned into pCR®II vector using a TOPO TA cloning kit (Invitrogen Life Technologies) in preparation for sequencing. Clones were screened for inserts by PCR and then positive ones were

sequenced using big dye terminator sequencing kit (Applied Biosystems). The nucleotide sequences were trimmed and assembled using the Sequencer program (Gene Codes Corporation, Ann Arbor, MI) Nucleotide sequence pairwise comparison was performed using the BLAST program and Multialignment with the known segment 8 nucleotide sequences was carried out using multiple sequence alignment program (Corpet, 1988).

Results

Virus Isolation: No virus was isolated from any sample using SHK-1, CHSE-214 and ASK-2 cell lines.

RT-PCR and sequence analysis:

A 220 base-pair product was produced in the ISAV segment 8 RT-PCR assay of 116 of 520 (22.3%) samples. Table 1 shows the source and number fish samples tested with ISAV segment 8 primer. The number in the brackets denotes fish that had the expected PCR product of 220 bp. Alt least 2 clones for each PCR product was sequenced.

Control ISAV NBISA01.

The RT-PCR product (220bp) was clone and sequenced and nucleotide sequence of our product (193 bp) was 99% identical to the original sequence of the same isolate in the GenBank.

Chinook: Of 121 samples analyzed with segment 8 primers, 31 had a 220 bp PCR product. The AVC Virology lab identified an additional 3 positive samples

bringing the positive to 34(28%). DNA sequence from two samples was not that of ISAV (Table 2). Those that were sent to AVC that turned out positive were also tested with segment 7 primers and all positive products were sequenced. The Pacific salmon DNA inserts had an identity of 94% identity with NBISA01 +ve while among there was a homology ranging between 97% to 100%. All the samples that showed a positive product with segment 8 primers were analyzed with other ISAV genes (segment 2(PB1), segment 6 (HA) segment 7 both open reading frames and segment both open reading frames 1 and 2). The ones we tried here were all negative but from AVC virology lab samples (Table 2) had PCR product of 377 bp. This product corresponded with ISAV segment 7 ORF2 products and had an identity of 99.7% and 95.8% with ISAV isolates 810/9/99 from Norway and NBISA01 from New Brunswick, respectively.

Comment [J3]: Please tidy up. You are talking about segment 7 then 8 then 7 again. Include the AVC analysis after describing what we found here.

Pink: Out of 89 fish and all the tissues organs were pooled, 15 (17%) were RT-PCR positive with ISAV segment 8 primers. DNA sequence of 213 bp from one fish was had 97% identity to NBISA01 and most other North American Isolates and 92 % identity to European Isolates.

Atlantic salmon (salt water): RNA extracted from the heart of one fish was positive with segment 8 primer and PCR product of 211bp had 98% identity to most Canadian ISAV isolates including NBISA01 isolate in the 202 bp that were overlapping and 93% identical to European isolates in 197 nucleotides.

Sockeye: Although all Cultus lake sockeye samples had correct size PCR product, most of the products could not be clone, however fish # 7890C product was successfully cloned and sequenced and all three clones had a product of 241 bp. The nucleotide sequence of these inserts had identity to ISAV only in the primer sequence.

However when all DNA inserts were aligned, using hierarchial clustering of nucleotides sequence (Corpet, 1988), they fell into three groups, with the inserts from Atlantic salmon, one from chinook (sample HS 2002 38 IVI02-124-019P), pink and the control NBISAV01 in one group, the other chinook samples ((HS200238-ISEA124-005L and (HS200238-IVI07-124-001h) fell into another group and the sockeye inserts had their own group although this group is close to the second group with the chinook samples.

Chum and coho salmon samples were all negative. ISAV segments 2 (PB1), 6 (HA) and full opening frame for segment 8 (NS1 and NS2) were not amplified nor was ISAV isolated onto SHK or CHSE cells. The expected RT-PCR products with the other ISAV genes used were obtained but they were obtained

Segment 7 ORF1 and ORF2.

Five samples (Table 2) from Chinook salmon had PCR product of approximately 400bp with segment 7 ORF1 primers, while the positive control NBISA01 had an insert of 903 bp corresponding to segment 7 ORF1. These products were cloned and sequenced, the sequence obtained (377bp) corresponded with that of

segment 7ORF2 nucleotide sequence. All chinook salmon samples the DNA inserts were 99.7% identical and corresponded to the ISAV Segment 7 ORF2 product. They had a 95.8% identity with the NBISA01 +ve control, and 99.7% identity with ISAV strain 810/9/99 from Norway. Segment 7 products were not amplified in any other fish samples.

Table 1: Pacific salmon species analyzes for the presence of ISAV segment 8 primers

Salmon Species (weight)	Location and number of samples examined (positive by RT-PCR with segment 8 primers)											
	PBS	**LL**	**ISEA**	**IVI**	**VI**	**QCS**	**E P**	**DE**	**F I**	**BA**	**CL**	**T I**
Chinook (200-350g)			37 (10)	40(22)	8 (1)	9(3)				22(0)		
Chum (150-250g)				5(0)	14(0)		17(0)	52 (0)				30(0)
Coho (100-200g)				16 (0)						20 (0)		
Pink (150-250g)					24(4)		19 (4)	40(6)	2 (1)			3(0)
Sockeye (100g-200g)					19(0)			20 (0)			64(64)	
Kokanee[a]		50(0)										
Atlantic (100-150g) SW	2 (1)											
Atlantic (18-20g) FW	7(0)											

PBS=Pacific Biological Station, LL=Lois Lake, ISEA=Inside East Alaska, IVI= Inside Vancouver Island (inlets), VI+ Along the coast line of Vancouver Island, EP= Estavan point, DE- Dixon Entrance, FI= Forrester Island, TI= Triangle Island, BA =Broughton area, CL=Cultus Lake

[a]Weight unknown.

Table 3: High Sea Chinook, pink and cultured Atlantic salmon and Adult sockeye samples checked for ISAV using RT-PCR, with segment 8 and 7 primers and sequenced.

Sample	Segment 8 primers (F5/R5)	Segment 7 ORF1 primers
Chinook		
HS 2002 38 IVI02-124-019P*	+ ve (~220bp)	- ve
HS200238 IVI08-124-004L*	+ ve (~220bp)	+ ve (~400bp)
HS200238 IVI08-124-003L*	weak + ve (~220bp)	-ve
HS200238 IVI17-124-20P*	+ ve (200pb)	Not done
HS200238 ISEA06-124-007P*	+ ve (~220bp)	+ ve (~400bp)
HS200238 ISEA02-124-009H*	- ve	+ ve (~400bp)
HS200238ISEA23-124-005L*	+ ve (~220bp)	+ ve (~400bp)
HS200238V101-124-002H$^{\$}$	+ve (200bp)	-ve
HS200238IVI07-124-001H$^{\$}$	+ve (200bp)	-ve
Pink		
HS200304 EP04-108-001P$^{\$}$	+ve	-ve
Cultus Lake Sockeye		
7890C P (tag #)$^{\$}$	+ve	-ve
Atlantic Salmon 1H$^{\$}$	+ve	-ve
NBISA01 +ve control	+ ve (~220bp) $^{\$}$	+ ve (903bp) *

HS=high sea, 200238: year and # of boat run, IVI08=the area and 124 is a code for chinook and 108 is for pink and the last # is fish as they were caught. For example HS200238 IVI02 124-19 means that chinook salmon was caught in year 2002 on the 38 run, Inside Vancouver Island (inlets) and was numbered 19.

-ve denotes no PCR product seen; + ve denotes PCR product (size in base pairs) seen.
* denotes samples that were sent to AVC Virology Research laboratory and sequenced by ACGT laboratory, $^{\$}$ Products sequenced (6) here at PBS in Genetics lab:

Discussion.

Infectious salmon anaemia or ISA virus has not been detected in Pacific North west coast of Canada or USA. In this investigation we were able to detect ISAV segment 8 and segment 7 PCR products in juvenile chinook, pink, spawning sockeye and culture Atlantic salmon using RT-PCR. Sequence analysis of some of the product reveal a homology of 94-99% and 92-93% to the North American and European ISAV isolates, respectively. The fact that segment 8 and segment 7ORF2 sequences amplified in some samples were not 100% identical to the control NBISAV01 rules out contamination during sample processing. These results are not unique since Raynard et al. (2001) also reported detection of ISAV by RT-PCR in wild fish in Scotland without isolating the virus by cell culture even though they were able to amplify the full segment 8 and 2. Thus our results may imply that wild pacific salmon species have a non-pathogenic ISAV isolate or just non-infective particle of ISAV. It has been speculated from analysis of ISAV haemagglutinin gene that the wild salmon may harbour a non pathogenic ISAV isolate with no mutation in this gene and that after this ISAV isolate mutates by deletions it becomes pathogenic to Atlantic salmon (Nylund *et al*., 2003). Thus pacific salmon species could harbour non pathogenic ISAV that has not yet experienced any mutations thus is unable to infect cultured Atlantic salmon in the area. The fact that were able to amplify ISAV segment 8 sequences in salt water cultured Atlantic salmon which did not have ISA symptoms may attest to that fact and could also indicate that ISAV nucleotide are present in the sea water.

In experimental infection of *Oncorhynchus species*, steelhead trout*, (O. mykiss)*, chum (*O.keta*), chinook (*O. tshawytscha*) and coho (*O. kitsutch),* virus was recovered 13-15 d p.i only in fish that were given a very high dose although no disease was produced in any of these fish while the same viral doses cause mortality in Atlantic salmon show that the virus might be able to survive in these Pacific salmon species, therefore could act as carriers of ISAV (Rolland and Winton, 2003). In natural environment, the viral titre may be very low thus making it un detectable. We were not able to detect any nucleotide sequences in chum and coho in this study. Other diagnostic methods such as IFAT and should be used along with RT-PCR (Snow *et al*., 2003).

From these results, failure to isolate ISAV on cell culture combined with failure to amplify other genes of ISAV from juvenile pacific salmon species leads to the conclusion that there is anon-pathological ISAV isolate in the Pacific North west coast. In future use fresh material and in vivo experiments and use of serological methods may provide a different perspective.

References:

Anonymous (2000). Infectious salmon anaemia in the Faroe Islands. Disease information, Office of International des Epizooties **13**: 53-59.

Anonymous (2002). Salmon virus detected in Clew Bay fish farm. The Irish Times. www.ireland .com, August 12, 2002.

Bouchard D, Brockway K, Giray C, Keleher W, Merril P.L. (2001). First report of Infectious salmon anaemia (ISA) in the United States. Bull. Eur. Assoc.Fish Pathol. 21:86-88.

.

Corpet F. (1988). Multiple sequence alignment with hierarchical clustering Nucl. Acids Res., 16 (22): 10881-10890.

Devold M, Krossøy B, Aspehaug V, Nylund A. (2000). Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. Dis. Aquat. Org 40: 9-18.

Kibenge FSB, Garate ON, Johnson G, Arrigada R, Kibenge MTJ, Wadowska D. (2001). Isolation and identification of infectious salmon anaemia virus (ISAV) from coho salmon in Chile. Dis Aquat Org 45(1): 9-18.

MacLean S.A, Bouchard D.A, Ellis SK. (2003). Survey of non salmonids marine fisheries for detection of infectious salmon anaemia virus and other salmonid pathogens. In: Miller Otis, Cipriano, Rocco C., tech. coords. International response to infectious salmon anaemia: prevention, control, and eradication: proceedings of a symposium 3-4 September 2002, New Orleans LA, Tech. Bull.1902. Washington, DC: US Department of Agriculture, Animal and Plant Health Inspection Service, U.S Department of Interior, U.S Geological Survey; U.S, Department of Commerce, National Marine Fisheries service 135-143.

McWilliams C, Kibenge F.S.B, Johnson G. (2003). Fatal Infectious haemorrhagic disease in rainbow trout experimentally infected with infectious salmon anaemia virus (ISAV). Annual Fish Health & Western Fish Disease Conference, Seattle, WA, U.S.A July 13-17 July 2003.

Mullins J.E, Groman D, Wadowska D. 1998 Infectious salmon anaemia in salt water Atlantic salmon (*Salmo salar* L.) in New Brunswick, Canada. Bull. Eur. Assoc. Fish. Pathol. 18: 110-114.

Nylund A, Jakobsen P. (1995). Sea trout as a carrier of infectious salmon anemia virus. J Fish Biol 45: 174-176.

Nylund A, Alexandersen S, Rolland JB, Jakobsen P. (995). Infectious salmon anemia virus (ISAV) in brown trout. J Aquat Animal Health 7:236-240.

Raynard R.S, Murray AG, Gregory A. (2001). Infectious salmon anaemia virus in wild fish in Scotland. Dis Aquat org 46: 93-100.

Ritchie, R. J, Bardiot A, Melville K, Griffiths S, Cunningham C.O, and Snow M. (2002). Identification and characterization of the genomic segment 7 of the infectious salmon anaemia virus genome. Virus Res. 84:161-70.

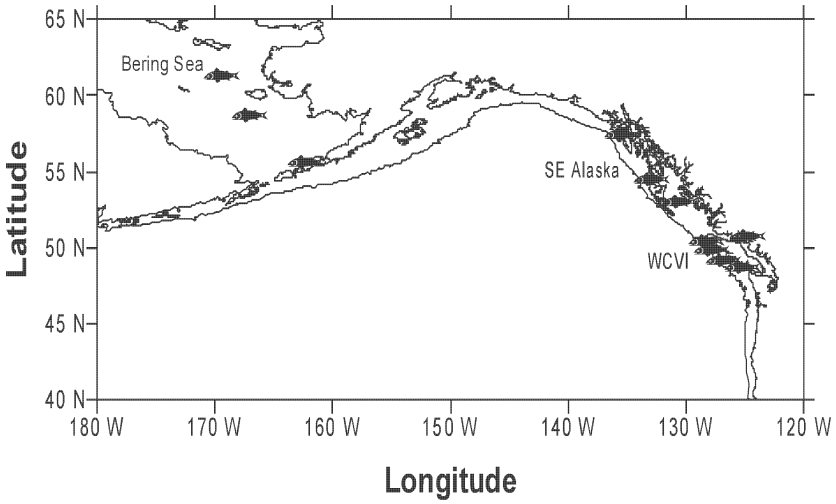
Ritchie R.J, Cook M, Melville K, Simard N, Cusack R, Griffiths S. 2001. Identification of infectious salmon anaemia in Atlantic salmon from Nova Scotia (Canada): evidence for functional strain differences. Dis Aquat Org 44: 171-178.

Olivier G. (2002). Disease interaction between wild and cultured fish-perspective from the American Northeast (Atlantic Provinces). Bulletin of the European Fish Pathologist Association 22; 102-109.

Snow M, Raynard R.S, Murray A.G, Bruno D.W, King J.A, Grant R, Bricknell I.R, Bain N, Gregory A. (2003). An evaluation of current diagnostic tests for the detection of infectious salmon anaemia virus (ISAV) following experimental water-borne infection of Atlantic salmon *Salmo salar* L. J. Fish Dis 26: 135-145.

Snow M, Raynard R.S, Bruno D.W. (2001). Comparative susceptibility of Arctic char (*Salvelinus alpinus)* rainbow trout (*Oncorhynchus mykiss)* and brown trout (*Salmo trutto*) to the Scottish isolate of infectious salmon anaemia virus. Aquaculture 196: 47-54.

Thorud K.E, Djupvik H.O. (1988). Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.) Bull. Eur.Assoc.Fish.Pathol.8:109-111.
High sea pacific salmon sampling


65 N
60 N
55 N
50 N
45 N
40 N
Latitude
Bering Sea
SE Alaska
WCVI
180 W
170 W
160 W
150 W
140 W
130 W
120 W
Longitude