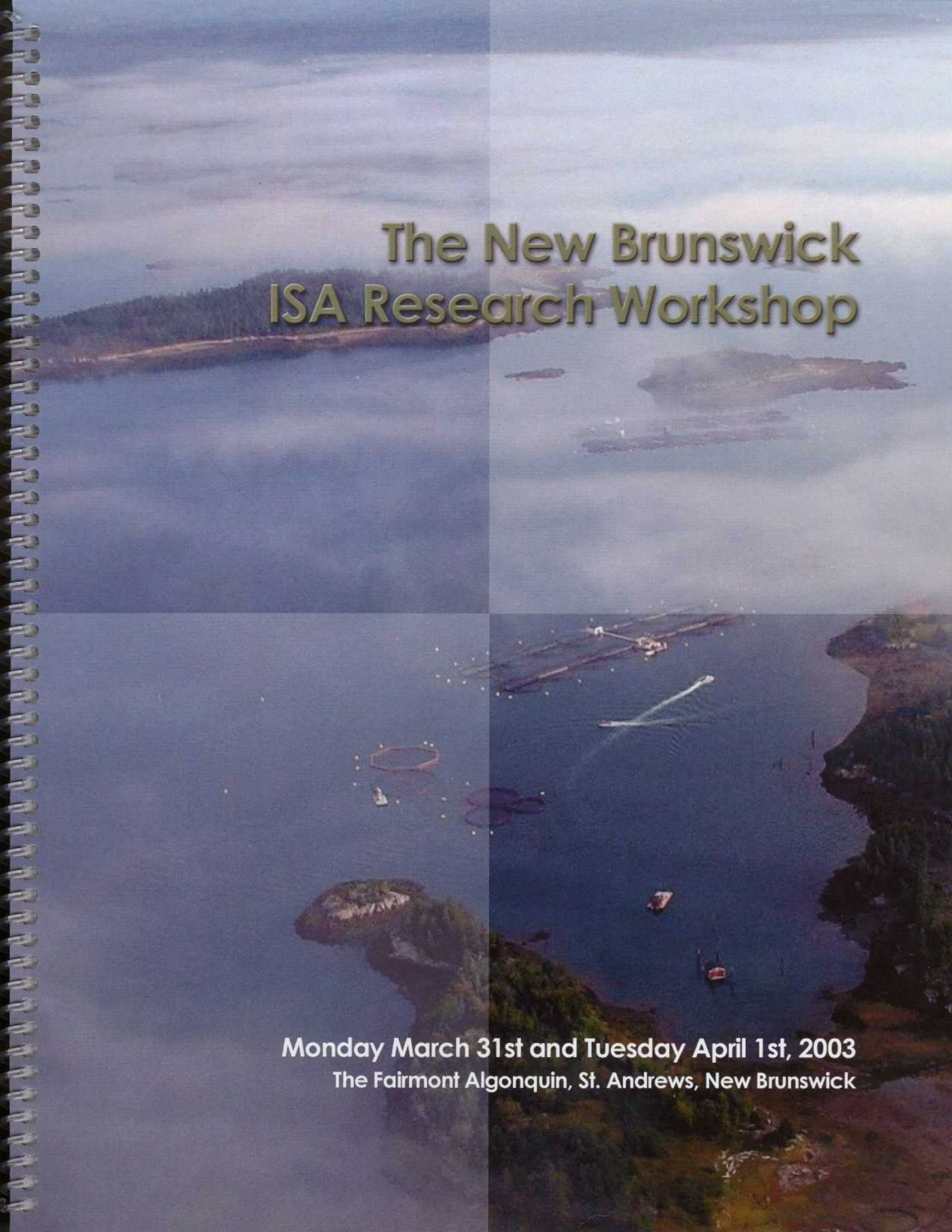

The New Brunswick ISA Research Workshop

Monday March 31st and Tuesday April 1st, 2003

The Fairmont Algonquin, St. Andrews, New Brunswick

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Workshop Overview

Infectious Salmon Anemia (ISA) continues to pose a significant challenge to the salmon farming industry in New Brunswick. A key element of the effective prevention and management of ISA is a sound understanding of the disease and how it interacts with fish populations and the environment. This understanding is furthered by research.

The purpose of this workshop is to bring the key stakeholder groups together - industry, fish health professionals, funding agencies, regulators and researchers - to identify and prioritize specific research needs with regard to Infectious Salmon Anemia (ISA). This workshop will also serve to bring key stakeholders up to date on what research is presently available, what research is ongoing and what research is needed.

The objective of Day 1 of this workshop is to identify the general areas of priority for ISA research.

The objective of Day 2 of this workshop is to identify specific projects and programs within the priority areas that will contribute to the more effective management of ISA. The identification of specific research priorities will serve as reference for stakeholders when evaluation potential research projects and programs.

Sponsors

Department of Agriculture Fisheries & Aquaculture - www.gnb.ca

Department of Fisheries & Oceans/Aquaculture Collaborative Research & Development Program - www.pac.dfo-mpo.gc.ca

New Brunswick Salmon Growers Association - www.nbsga.org

Aqua Health Ltd. - www.aquahealth.co.uk

Card's Aquaculture Products Ltd. - www.cardsaqua.com

Corey Feed Mills Ltd. - www.corey.ca

Future Nets & Supplies Ltd. - www.futurenets.net

Micro Technologies Inc. - www.microtechnologies.biz

Research & Productivity Council - www.rpc.ca

Shur-Gain/Maple Leaf Foods - www.mlfi.com

Skretting - www.skretting.com

Organizing Committee

Jamey Smith - NBSGA

Bev Bacon - Research & Productivity Council

Brian Glebe - DFO

Larry Hammell - Atlantic Veterinary College

Mark Moore - NB DAFA

Sandy McGeachy - NB DAFA

Gilles Olivier - DFO

1. Background

Infectious Salmon Anemia (ISA) continues to pose a significant challenge to the salmon farming industry in New Brunswick. A key element of an effective ISA prevention and management strategy is a sound understanding of the dynamics of the disease and how it interacts in the environment. This understanding is furthered by research.

While it is generally agreed by key decision makers that further research is required to more effectively manage ISA, specific areas of research and the priority which they should be accorded, need to be established.

In late March 2003, key stakeholder groups came together for a two-day workshop in St. Andrews, New Brunswick to map out new directions with regard to research needs to assist in combating Infectious Salmon Anemia (ISA).

2. Workshop Objectives

The primary objective of the workshop was to bring industry, regulators, fish health professionals, funding agencies and researchers together to identify specific ISA research priorities. The identification of specific research programs and projects will point the way to the future and serve as a much-needed reference for stakeholders when evaluating research proposals. Hopefully, this will in-turn lead to the most effective use of research funds applied to ISA issues.

The secondary objective of this workshop was to bring key stakeholders up to date on the present status of ISA research (both in North America as well as in Norway and Scotland) – identify what research is available, what research is ongoing and what areas need further investigation.

3. Priorities Supporting Effective ISA Research

During the workshop, several key issues were identified that, while related to effective ISA research, were not in themselves ISA research projects or programs. While it was beyond the scope of this workshop to elaborate on these issues, these "other" priorities have been included in this report because they were felt to be essential to the overall effectiveness of ISA research in New Brunswick.

3.1.1 Ongoing Review of ISA Management Strategy

The need to have a critical review and analysis of the existing ISA management strategies was repeatedly emphasized during the workshop by various participants. It was felt that an ongoing process of review and assessment of ISA management strategies and policies was essential to making improvements in ISA management. In many cases, strategy and policy decisions will directly influence what will be considered research priorities. Although this point received much support, it was decided that this workshop was not the venue to decide how this was to be addressed.

3.1.2 Communications Plan

A communications plan to disseminate new ISA research information amongst the research community, as well as between the research community, industry, veterinarians, government and other stakeholders was identified as a critical component of an effective research program. Various means of accomplishing this objective were discussed, such as the re-establishment of an ISA Research Committee, a literature compilation and the development of a web-based source for ISA research communications. Although communication was identified as an important issue, it was agreed that the creation of such a plan was outside of the scope of the workshop.

3.1.3 ISA Database

A recurring theme throughout the workshop was the need for an effective database to facilitate ISA research in a variety of areas. Certainly all projects that were identified as retrospective studies would greatly benefit from having access to a standardized database. Similarly, all prospective field-based studies would be much more efficient if such a database is established. The existence of an effective fish health / production database would not only benefit ISA research, but research in many other areas.

3.1.4 Other Areas

Other areas such as genetic improvement for ISAV resistance and supersmolt were briefly discussed, however, it was felt that these areas were either of lesser importance or would be more appropriately dealt with by other groups.

4. ISA Research Priorities

The ISA research priorities that were identified were classified into four program areas:

i) Epidemiology / Risk Management
ii) Diagnostic Testing
iii) ISA Vaccine Development / Evaluation
iv) Other

It is important to note that the above research areas are not exclusive of one another. Research projects may address issues in more than one area simultaneously.

4.1.1 Epidemiology / Risk Management

The following eight specific project areas have been identified within "Epidemiology / Risk Management" as representing priorities for ISA research. It was concluded that a better understanding in each of these areas would lead to better decision-making and hence more effective management of ISA. Critical management decisions and control strategies, including depopulation and biosecurity, should be strengthened by additional research in these areas.

A. **Viral Transmission Field Studies** – retrospective and prospective collection of relevant data to determine primary modes of ISAV transmission under production conditions. A better understanding of the primary means of ISAV spread within and between sites will provide critical management information to key stakeholders.

B. **ISAV Infection Prevalence Studies** – prospective investigation (possibly some aspects could be retrospective) of prevalence of infection in various populations at risk, including outbreak cages, low mortality cages at positive sites and cages at undetected sites / areas. Understanding prevalence of ISAV infection in these various populations will provide information about transmission (contributing to Viral Transmission Studies 4.1.1 - A) and allow for better decision-making with regard to depopulation and other management strategies. It will also provide information about the relative success of the containment of virus for a given management and control strategy.

C. **Risk Factor Studies** - retrospective review of data collected by farms regarding environmental, husbandry, and host factors and associations with outbreak cages, with and without elevated mortality. Prospective monitoring of risk factors suspected of being causally related to ISA outbreaks, such as sea lice burdens, and associations with infection levels (overlapping with Prevalence Studies, Strain Studies, and Quantitative RT-PCR Studies) and mortality

patterns. As well, monitoring of mortality patterns is essential to the investigation of possible viral strain differences in virulence under production conditions (see 4.1.1 – D, ISA Virus Strain Studies).

D. ISA Virus Strain Studies - variation and virulence of ISAV isolates in New Brunswick are potential explanations for differing clinical disease experiences within the industry and should be considered when addressing transmission and prevalence studies (4.1.1A and 4.1.1B). Correlation of the strains identified with mortality patterns (as identified above in Risk Factor Studies) is needed to determine if known New Brunswick strains of ISAV differ in virulence. Laboratory typing of the important genomic sequences contributing to the virulence is needed.

E. Environmental Persistence Studies – determination of the infective potential of ISAV under various environmental conditions would contribute to information about transmission between sites and also to decisions regarding such issues as fallow periods, biosecurity standards and control programs.

F. Pathogenesis Studies – characterize the infective dose of ISA for farmed Atlantic salmon and the stages of disease progression within individual fish. This information should then form the basis for studies of disease progression in populations of fish, including viremic periods and probability of pathogen shedding from asymptomatic fish.

G. Sea Lice Management Studies - Lice monitoring and field studies which support optimal sea lice management related to ISA control policies, including studies involving therapeutant withdrawal times

H. Economic Evaluation of ISA Management Options – The economic impact of a number of ISA management options is unknown to key stakeholders. Understanding the economic consequences of different approaches to ISA management would assist farmers, policy makers, fish health professionals and others in making critical decisions around managing this disease. The application of economic principles to known scientific information would provide this much needed financial perspective. Economic evaluation of control policies would reflect and be closely linked to research in areas such as strain variation and virulence, infection prevalence, infection detection, viral transmission field studies and risk factor studies. Economic predictive models would assist decision makers in areas such as depopulation, control and containment measures and biosecurity standards.

4.1.2 Diagnostic Testing

A. **Infection Detection Studies** - a continuation of the prospective investigation of diagnostic test interpretation based on their association with infection, clinical disease and mortality. The development and use of rapid, on-site testing methods for ISA should be considered.

B. **Strain Detection Studies** - development of diagnostic tests to differentiate ISAV strains in New Brunswick may be important with regard to decisions regarding control policies. This research is closely linked to 4.1.1 D. listed above – ISA Virus Strain Studies. If it is determined that the strains of ISAV in New Brunswick vary in their virulence, it will be necessary to differentiate these strains using effective diagnostic tests. Rapid and cost-effective strain identification will be an important tool in determining appropriate action in an infected population.

C. **Use of Quantitative PCR**. The development of quantitative PCR techniques would allow the monitoring of levels of ISA in a cage or site. Understanding the viral load and the changes in viral load for a population may serve as a predictive tool for managers. This research is closely linked to 4.1.1 F – Pathogenesis Studies and to 4.1.1 C – Risk Factor Studies.

4.1.3 ISA Vaccine Development / Evaluation

A. **Vaccine Efficacy Observational Studies** – a retrospective and prospective investigation of the field efficacy of vaccination based on vaccination as a risk factor in observational studies.

B. **Vaccine Clinical Field Trials** – although these types of studies will pose a challenge in that clinically infected fish must undergo depopulation under the current ISA management program in NB, these studies provide much needed information with regard to vaccine efficacy against infection, clinical disease, and mortality. This issue is very important to policy decisions regarding ISA control.

C. **Response to Vaccination Studies** – various questions need to be answered in this respect – what are the results of various differences in vaccine application (water temperature at time of vaccination, need for immersion or injection booster, degree days required, etc.). This may be addressed by observational studies to determine factors that are associated with effectiveness of vaccines and then clinical field trials to further assess the causal nature of the vaccine response.

Dr. Are Nylund
Institute of Fisheries and Marine Biology
University of Bergen
Bergen, Norway

Dr. Nylund is a professor at the Institute of Fisheries and Marine Biology at the University of Bergen. Dr. Nylund has worked extensively with molecular genetics and fish pathology. Dr Nylund developed the RT-PCR for ISAv used by many researchers and diagnostic laboratories. Dr. Nylund has also completed work on sea lice and their implications on the transmission of ISAv. Dr. Nylund has been with the University of Bergen since 1980.

Other research areas of interest to Dr. Nylund include study of parasites and viral iseases. Dr. Nylund has published many scientific papers and written chapters dealing with sea lice, ISA and other parasitic and viral diseases. Dr. Nylund also participated in our first workshop on ISA in New Brunswick in 1997.

ISA in Norway

Are Nylund
Institute of Fisheries and Marine Biology
University of Bergen, Norway

History of infectious salmon anaemia (ISA)

The first officially registered outbreak of infectious salmon anaemia (ISA) started in November 1984 (Thorud and Djupvik, 1988). It was a chronically proceeding disease among Atlantic salmon (*Salmo salar*) parr in a hatchery on the West Coast of Norway (Bremnes). Affected salmon were lethargic with pale gills, fin rot, exophthalmia, and haemorrhages in the anterior eye chamber and abdominal skin. Other clinical signs were a dark, pale or yellowish liver, a dark and swollen spleen, congested intestinal walls, petechia in periviceral fat, acites fluid, oedemas, haemorrhages in the swim bladder wall, and muscular haemorrhages (**Figure 1**). About 80% of the parr in the hatchery died during that winter and spring. Judging from the pathological findings, "Hitra disease" was suspected, however, no bacteria were isolated from diseased fish and oxytetracycline did not reduce mortality. In the following years, a disease with similar clinical and pathological signs occurred in smolt and adult salmon in marine farms that had received smolt form this hatchery.

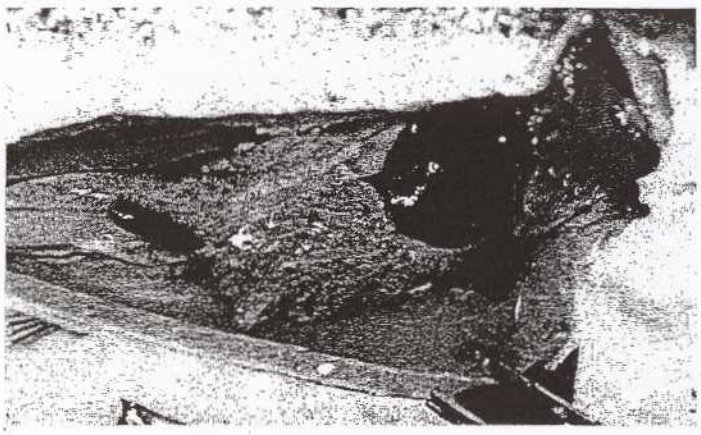


Figure 1. Atlantic salmon showing the "classical" signs of ISA.

Norway is the leading salmon (*Salmo salar*) producer in the world and the potential for increased production in the coming years is large. However, the production of Atlantic salmon in the eighties and the first three years of the nineties were to a large extent dominated by diseases and the industry was close to a collapse. One important factor that changed the development in a positive direction was the successful fight against the dominating salmon diseases (*Vibrio salmonicida* cold water vibriosis, *Aeromonas salmonicida* furunculosis and ISA). The salmon lice, *Lepeophtheirus salmonis*, bacterial kidney disease (*Renibacterium salmoninarum*), salmon pancreas disease virus (SPDV), infectious pancreatic necrosis virus (IPNV), cardiomyopathy, hemorrhagic smolt syndrome are still causing some problems, in addition there were minor problems with some parasites (*Ichthyobodo* spp., trichodinids, *Spironucleus*, *Parvicapsula*) and bacteria (epitheliocystis and winter ulcers, *Vibrio wodanis* and *Moritella viscosa*). One of the reasons for the large problems in the eighties was the lack of proper diagnostic methods for some of the diseases (cold water vibriosis, ISA and cardiomyopathy).

The first official registration of ISA came at very bad time for Norwegian salmon production. One problem was an ongoing discussion of the causes of "Hitra disease". Some insisted it was caused by malnutrition (Poppe *et al.* 1986) while others believed it was a bacterial disease (Totland et al. 1988;) and they even managed to culture the bacteria that was later named *Vibrio salmonicida* (cold water vibriosis). The dispute about the causes of "Hitra disease" goes back to the end of the seventies, and later studies have indicated that the reason for the disagreement was probably that "Hitra disease" encompassed at least three different diseases; cold water vibriosis, cardiomyopathy and ISA. There are several indications of this in articles published in Norwegian (cf. *Frisk Fisk* brochure from this period, i.e. published reports from the Norwegian Research council). In some electron microscopic studies of tissues from salmon with "Hitra disease", the authors were not able to detect any bacteria and described pathological changes that later studies have shown to be associated with ISA. Even as late as in 1987 the three disease were confused. One farm in Northern Norway was given the diagnosis Bremnes syndrome (i.e. ISA), but this was later changed to cardiomyopathy. The reason for the confusion was of course the lack of a safe diagnostic. The first indication of ISA

can be found as early as in 1977 (Poppe *et al.* 1983). The authors give a review of haemorrhagic syndrome of Atlantic salmon in Norwegian aquaculture. The first case of haemorrhagic syndrome was registered in September/October 1977 in a fish farm close to Hammerfest, Northern Norway. They were not able to isolate any infective agent and therapy with oxytetracyclin did not influence the mortality. The same disease was also registered in 1978, in 1979 it was found in other areas in Northern Norway. Several explanations were given for this syndrome (stress, environmental stress, genetics, water temperature, malnutrition, virus (VHS, but it was not possible to isolate this virus), bacteria (it was not always possible to isolate bacteria, but in some farms *Vibrio* spp. were found), and physical factors. Studies of tissues from fish with haemorrhagic syndrome have later revealed the presence of ISA virus particles in some of these tissues (pers. obs).

ISA was first named "Bremnes syndrome" after the locality of the first appearance. This was later changed to Salmon anaemia syndrome (SAS). However, protest from an airline company made another change of name necessary and in 1990 ISA was recognised by the International Office of Epizootics.

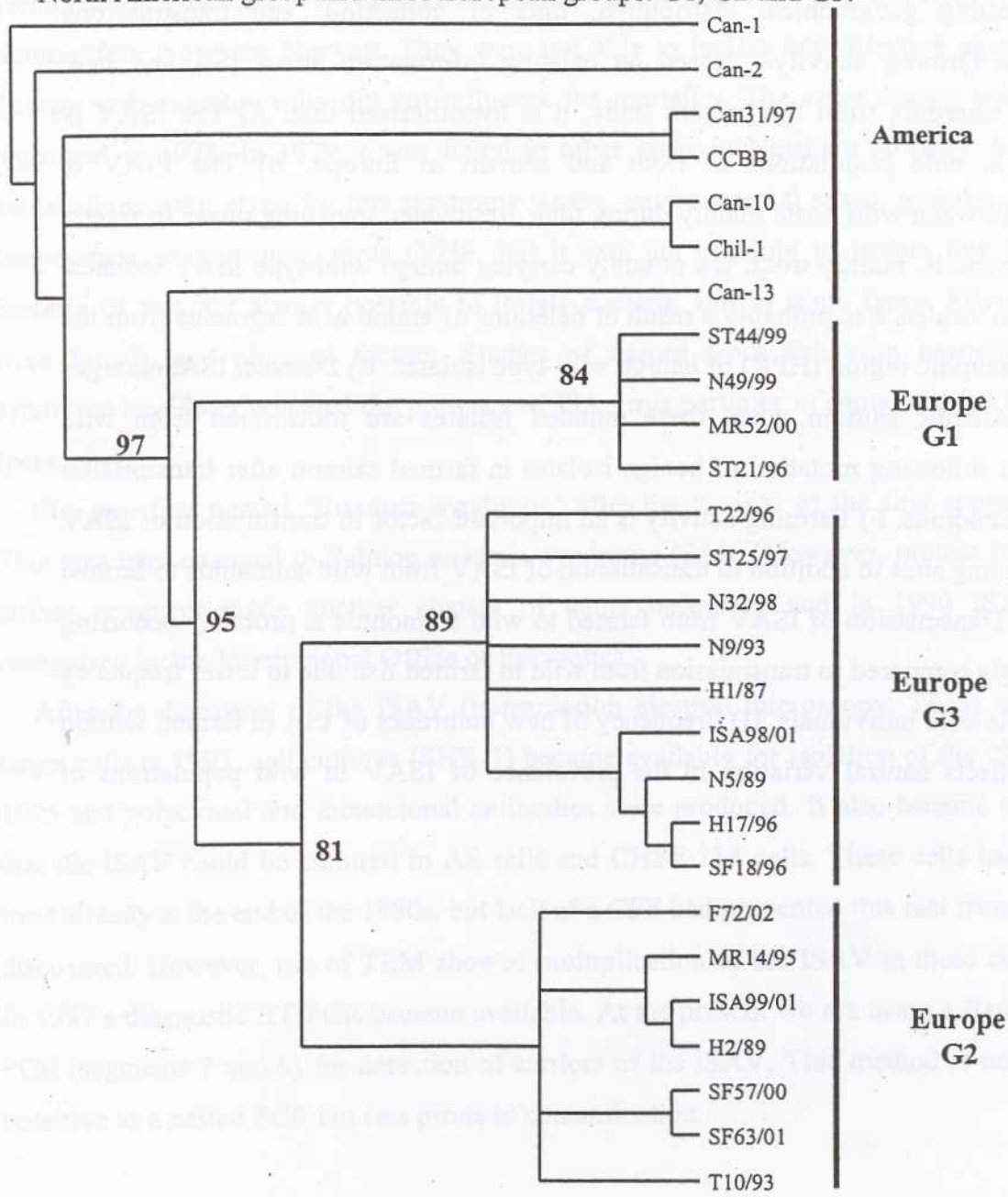
After the discovery of the ISAV (transmission electron microscopy, TEM) and the target cells in 1993, cell cultures (SHK-1) became available for isolation of the ISAV in 1995 and polyclonal and monoclonal antibodies were produced. It also became evident that the ISAV could be cultured in AS cells and CHSE-214 cells. These cells had been tried already at the end of the 1980s, but lack of a CPE had prevented this fact from being discovered. However, use of TEM showed multiplication of the ISAV in these cultures. In 1997 a diagnostic RT-PCR became available. At the present we are using a Real Time PCR (segments 7 and 8) for detection of carriers of the ISAV. This method is nearly as sensitive as a nested PCR but less prone to contamination.

A new hypothesis

Molecular methods have become important tools in the study of diversity, dissemination and maintenance of viral diseases. The nucleotide sequences of the hemagglutinin gene (HA) from 70 ISAV isolates have know been analysed for phylogenetic relationship and the average mutation rate of nucleotide substitutions has

been calculated. The isolates constitute two major groups, one European and one North American group (**Figure 2**). The isolate from Chile is closely related to the North American isolates. The European isolates can be further divided into three separate groups reflecting geographical distribution, time of collection, and transmissions connected to farming activity. Based on existing information about ISA and new information emerging from the present study, it is hypothesised that: A) The ISAV is maintained in wild populations of trout and salmon in Europe. B) The ISAV is transmitted between wild hosts mainly during their freshwater spawning phase in rivers. C) Wild salmonids, mainly trout, are possibly carrying benign wild-type ISAV isolates. D) Change in virulence is probably a result of deletions of amino acid segments from the highly polymorphic region (HPR) of benign wild-type isolates. E) Disease, ISA, emerges in farmed Atlantic salmon when these mutated isolates are transmitted from wild salmonids or following mutation of benign isolates in farmed salmon after transmission from wild salmonids. F) Farming activity is an important factor in transmission of ISAV between farming sites in addition to transmission of ISAV from wild salmonids to farmed salmon. G) Transmission of ISAV from farmed to wild salmonids is probably occurring less frequently compared to transmission from wild to farmed fish due to lower frequency of susceptible wild individuals. H) Frequency of new outbreaks of ISA in farmed salmon probably reflects natural variation in the prevalence of ISAV in wild populations of salmonids.

Figure 2. Clustering of ISAV isolates based on sequence analysis of HA gene 5`-end (surface tail region - St, nt 1 - 1002). The unrooted quartet puzzling tree topology with support values for the major internal branches are given. The isolates resolve into one North American group and three European groups: G1, G2 and G3.



Dr. Carey Cunningham

Dr. Carey Cunningham is the Molecular Genetics Group Leader for the Fisheries Research Services Marine Laboratory in Aberdeen, Scotland. FRS is the National Reference Laboratory for Fish and Mollusk Diseases for Scotland, as part of the European Community network of Reference Labs within the EC Fish Health Program.

The Molecular Genetics group carries out research and diagnosis, applying molecular methods to topics in fisheries and aquaculture. This work includes research on ISAv and other pathogens of concern such as Gyrodactylus parasites and IPNv. Dr. Cunningham has been with the Marine Laboratory since 1984 and studied for her first degree in Molecular and Cell Biology while carrying out work on various fish pathogens. She obtained her Ph.D. from the University of Aberdeen where she studied molecular methods to distinguish species of Gyrodactylus. Dr. Cunningham has also coordinated and participated in projects with several European projects, funded by the European Community Framework Program covering Research.

FISHERIES RESEARCH SERVICES

ISAV in Scotland: History and Research

Carey Cunningham
FRS Marine Laboratory, Aberdeen, Scotland

Notifiable Diseases

•List I

•Infectious salmon anaemia

•List II

•Viral haemorrhagic septicaemia
•Infectious haematopoietic necrosis

•List III

•Furunculosis (*Aeromonas salmonicida*)
•Enteric redmouth (*Yersinia ruckeri*)
•Bacterial kidney disease (*Renibacterium salmoninarum*)
•Infectious pancreatic necrosis
•Spring viraemia of carp
•*Gyrodactylus salaris*

Establishment & Maintenance of Zones

- Knowledge of the industry
  - Registration of farms
- Surveillance programme
  - Passive & Active
- Establish which species susceptible
- Rules stop import from lower status farms
  - Within EU or From 3rd countries
- Action to deal with outbreaks
  - Directive 93/53/EC

ISA outbreak in Scotland

- 5 May 1998, Notified suspect ISA
  - Movement restrictions
  - Confirmation, culling, ensiling
- 35 more suspect sites, 10 confirmed

Control Directive (93/53/EC)

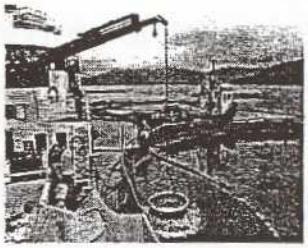
- Required for ISA (exotic) and for List II diseases if MS wishes to maintain approved zone
- Applies to any fish shown to be infected
- Actions on suspicion (to prevent spread)
- Actions on confirmation (to eliminate infection)
- Notification (early warning) to other member sates and the OIE

Actions on suspicion (Article 5)

- Census
- Movement restriction and biosecurity
  - live and dead fish
  - People and equipment
- Disposal of dead fish controlled
- Epizootic investigation
- Zoning

Actions on confirmation (Article 6)

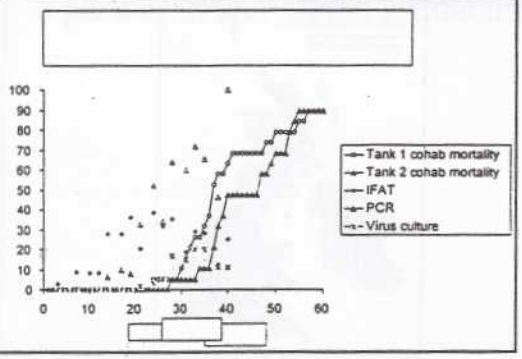
- Withdrawal of fish
- Disposal or marketing of all fish
- Disinfection and cleaning
- Fallowing
- Surveillance of adjacent farms in established zones



Detection of ISAV by PCR

- PCR product 155bp
  - Short fragment - sensitivity, sample integrity
  - Conserved region of segment 8
- Rapid
- Expensive
- False positives - negative controls at all steps
- False negatives - positive control

Diagnostic methods



EC project ISA 1.1.02 - 31.12.04

Development and standardisation of diagnostic methods and aspects of the epidemiology of ISA

- Institute of Aquaculture, Stirling, Scotland
- Fisheries Research Services, Aberdeen, Scotland
- National Veterinary Institute, Oslo, Norway
- University of Bergen, Norway
- RPC, Fredericton, Canada
- Danish Veterinary Laboratory, Arhus, Denmark

EC project: workpackages

- Characterise genome, compare sequences, develop primers, recombinant peptides
- Produce and characterise antibodies
- Real-time PCR and NASBA
- Immunohistochemistry and histopathology
- ELISA, serology and aspects of epidemiology
- Standardisation of diagnostic protocols and recommendations for control policies

ISAV genes (March 2003)

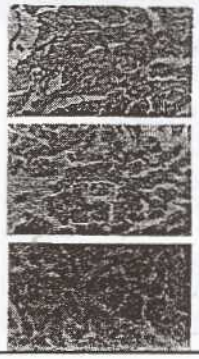
Protein	ISAV	'flu
Polymerase	1	1
Polymerase	2	2
Nucleoprotein	3	5
Polymerase	4	3
Fusion	5	6
Haemagglutinin	6	4
Non-structural	7	8
Matrix	8	7

IFAT detection of ISAV

Antibody production

- Segment 6 cloned in baculovirus vector
- Cloning segments 5 and 6 in DNA vaccine vector
- Phage display library panned with peptides from segment 5
  - One potential binder being analysed further

In situ hybridisation



Segment 8

Segment 2

Negative control

Epizootiology

ISAV in Scotland

1. The role of FRS and EC Fish Health
2. ISA outbreak in Scotland
3. ISAV Research in Scotland

Role of FRS

- Implementation of Legislation
  - Inspection & testing
  - Disease control
  - Import/export
  - National reference laboratory
- Advice and support
  - Government
  - Industry
- Develop legislation
- Welfare

Mark Moore DVM, MBA
Maritime Veterinary Services
St. George, New Brunswick

Mark has been involved with the aquaculture industry since 1985 and is presently an associate of Maritime Veterinary Services, providing fish health management services to salmon farmers in Eastern Canada and Maine. Mark was previously Manager of Fish Health for NB DAFA (Dept. Agriculture Fisheries and Aquaculture) and continues to work with the Department in the development of fish health policy and programs.

Mark started working in the aquaculture field in 1985 on a trout farm in Ontario and in the summer of 1988, he worked with one of the pioneer aquaculture vets in Norway. He graduated from the Ontario Veterinary College, University of Guelph in 1989 (DVM) and from the Richard Ivey School of Business, University of Western Ontario, 1993 (MBA). Mark was a partner in Jail Island Aquaculture from 1993 to 2001.

ISA in New Brunswick
Putting It In Perspective

Dr. Mark Moore DVM
New Brunswick Department Agriculture Fisheries and Aquaculture

ISA in New Brunswick

Putting it in Perspective

New Brunswick ISA Research Workshop
March 31st, 2003
Mark Moore DVM
Maritime Veterinary Services

Objective

To provide an overview of ISA in New Brunswick - both historically and our present situation.

From the information presented, to provide you with a context from which you can view ISA, its impacts and the relative merits of targeted research.

Overview

- A Brief History of ISA in New Brunswick
- Where are we now? - present status of ISA
- Year to Year Comparison of ISA (1999 - 2002)
- Impacts of ISA
  - production #'s
  - economic #'s
- Risk Factors

History of ISA in New Brunswick

- **Industry Overview**
  - **salmon farming in NB started 25 years ago**
  - **presently 96 farms**
  - **annual production: 40,000 MT / $240 million**
  - **5 companies operate / control approx 68 farms**
  - **approximately 31 farming companies (contract growers, affiliates, independent)**

ISA in NB - A Brief History

- First detected in summer of '96
- elevated mortality of unknown cause at three sites
- identified as ISA in summer '97
- varying degrees of losses in all year classes since '96
- $40 million in compensation over the past 6 years
- signficant challenge to the NB salmon farming industry

ISA Status - 2001 Year Class

- 1.1 million fish eradicated in the 2001 YC
- 22 sites were identified as positive
- areas affected - BMA's 5, 7, 8, 9, 10 and 19
- 6 of 23 sites in BMA's 8, 9 and 10 **remained negative**

ISA Status - 2002 Year Class

- approximately 1.7 million fish destroyed to date
- at 8 sites (L'Etete & Deer Island West)
- 4 of the positive sites now empty
- 4 sites remain positive

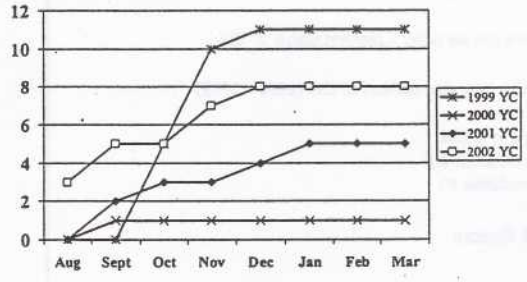
Historical Distribution of ISA Infection in New Brunswick

Year Class	# Positive Sites	# Fish Depopulated	Average # Fish Depopulated per Site
1995	4 (estimate)	N/A	N/A
1996	17 (estimate)	N/A	N/A
1997	25	1,667,000	79,000
1998	16	1,190,000	74,000
1999	24	1,637,000	68,000
2000	9	222,000	28,000
2001	15	1,050,000	67,000
2002	8	1,680,000	210,000

- 1995, 1996 and some of 1997 numbers are estimates due to unknown aetiology

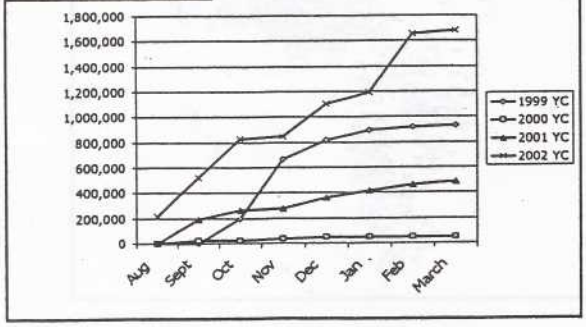
Comparison - # of ISA Positive Sites

1999 to 2002 Year Class



Comparison - # Fish Eradicated

2001 vs 2002 Year Class



Summary - ISA Status

- onset of ISA earlier in the 2002 YC than in previous years
- 2002 YC more advanced than any other year class at this point in the production cycle
- limited to 8 sites in the Western Deer Island and L'Etere areas

Impact of ISA in New Brunswick

- Production #'s
  - →7.5 million fish eradicated since 1997 due to ISA
  - →average loss
    - 1.2 million fish / year
    - 11% of total annual production
  - →this year (2002 YC)
    - estimate 2.1 million fish (20% of annual production) will be lost if no new sites become positive

Impact of ISA in New Brunswick

- Financial #'s
  - →over $40 million in compensation paid out over the past 6 years
  - →direct out-of-pocket losses to NB farmers
    - average $7.0 to $8.0 million per year
    - approx $45 million since 1997

Impact of ISA in New Brunswick

- Financial #'s
  - →opportunity cost / lost revenue
    - loss of production volume (depopulation, mortality)
    - erratic supply to market (unplanned harvests, oversupply) impacts price
    - market impact affects **all fish**, not just ISA + fish
  - →estimated annual loss (gross $ sales)
    - average 1997-2002: $35 - $40 million
    - estimated 2002 YC: $60 - $70 million
    - (assumptions: 10lb avg, $1.75/lb US, market impact - $.10/lb)

Risk Factors - ISA in New Brunswick

- →The Source - Infected Fish
- →The Spread
  - →overlapping of year classes (exposure of smolt)
  - →harvest vessels (harvest practices, movement)
  - →wharves (cross-contamination)

Summary

- ISA is a major challenge to the NB salmon farming industry
- since 1997, ISA has caused an average annual loss of 1.2 million fish / lost revenue: $35 million
- this year (2002 YC) ISA will cause the highest losses since its onset, > 2.0 million fish or $65.0 million in lost revenue

Dr. Dougie McIntosh
Molecular Biology Group
Research and Productivity Council (RPC)
Fredericton, New Brunswick

- Honours Degree In Microbiology, (1987)
- PhD. (1990) Thesis title: *Bacterial Fish Pathogens; with emphasis on morphological variation in Aeromonas salmonicida*
- Both awarded by Heriot-Watt University, Edinburgh, Scotland

Present Employment:
Fish Health Microbiologist at RPC: My current research is focused on the application of molecular biotechnology to the commercial development of improved fish pathogen diagnostic (Biosensors) and treatment (Probotics) approaches. ISA is among the conditions under investigation.

Research Experience:

From Nov 1990 to Jan 1992, I was contracted by Aquaculture Vaccines Limited (AVL) to undertake the development of novel vaccines for the protection of salmonids against furunculosis, at HWU.

During 1992, I held a Fellowship awarded by The Royal Society (London) to investigate the role of *Vibrio alginolyticus* in gastro-enteritis of turbot (Scopthalmus maximus) larvae. This study involved the biochemical and molecular characterization of extracellular proteases and cytotoxins and was performed in the Department of Microbiology, at The University of Barcelona, Spain.

I returned to Scotland (HWU) in 1993, to take up the position of Senior Research Scientist on an European Union funded project entitled; *New in vitro approaches for fish protection in aquaculture*. This project was based upon the use of tissue culture and molecular biology techniques for the study of pathogenicity mechanisms of the intracellular fish pathogen *Renibacterium salmoninarum* and the host response to infection.

In February 1996 I was contracted by the Department of Biological Sciences, University of Plymouth, England as a Senior Research Fellow, to develop research on the evaluation and improvement of PCR-based detection systems for *R. salmoninarum*. This research involved the comparison of different extraction and amplification techniques in order to develop a rapid, reliable and economical means of pathogen detection, which could be used routinely in Ministry of Agriculture (MAFF) laboratories.

From April 1997 until November 2002 I was employed as Visiting Research Fellow at the Oswaldo Cruz Institute (FIOCRUZ), in Rio de Janeiro. My research focused on the development of recombinant vaccines based on *Mycobacterium bovis* BCG; for the control of both human and animal diseases including, whooping cough, schistosomiasis, Dengue, babesia, anaplasma.

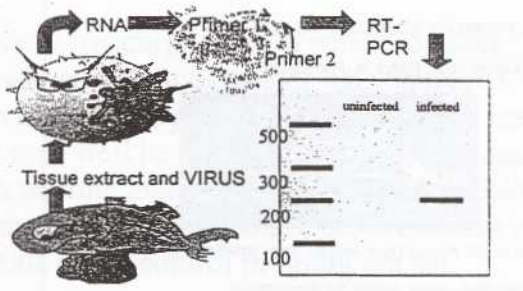
USE, INTERPRETATION AND IMPROVEMENT OF DIAGNOSTIC TESTS FOR ISAV:

Laboratory Perspective

Dougie McIntosh, Rachael Ritchie, Marcia Cook,
Rebecca Liston, Eric Johnsen, Sherry Vincent,
Erin Macdonald, Steve Griffiths

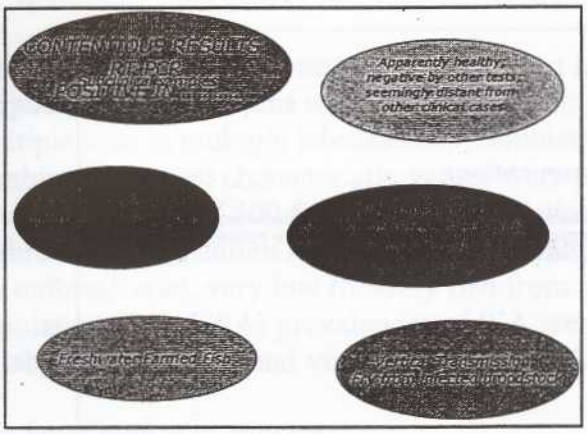
Molecular Biology Group
Research and Productivity Council (RPC)
Fredericton

Schematic Representation of RT-PCR Technique



SICK FISH

Sensitivity: 1-10 viral targets per reaction
"POSITIVE RESULT DOES NOT MEAN VIRUS IS INFECTIVE"



Assay validation

"A detection technique can only be of value if it measures what it is suppose to measure" Hiney & Smith 1997.

- *In vitro/test tube models*
- Seeded samples
- Experimentally infected animals

Used to define assay specificity and sensitivity

- *Extrapolation of data from above models to field samples is not a straight forward process!*
- Virus in different parts of these samples will be at various stages of replication, host immune responses, variable levels of target.
- Mutual Comparative Validation (RT-PCR, IFAT, Culture)
- Predictive validation (Link results of RT-PCR and other methods with probability of clinical ISA disease)

Trying to Understand the results of RT-PCR

- *High Prevalence Population : Highly Infected Fish*

  Consecutive sampling will result in consistent positive results

- *Low Prevalence Population : subclinical or carrier infections*

  Consecutive sampling of material from a single tissue sample may result in contradictory results

- *Lab Perspective: THESE ARE NOT FALSE POSITIVES!*

In a well established lab false positives or negatives should not occur, due to the incorporation of appropriate controls and confirmatory testing

- *Important to view the results from the whole sample of fish*

NOTE: Freeze/thawing cycles may result in the liberation of more viral particles allowing greater access to target in subsequent tests.

HETEROGENEITY OF TARGET DISTRIBUTION IN TISSUE SAMPLES

Tissue from clinically infected fish

Diagnostic assay POSITIVE

Tissue from "CONTENTIOUS" source

Diagnostic assay NEGATIVE
Disperse target concentration
Degradation of target in necropsied tissue

Minimizing Target Loss

CHOOSE APPROPRIATE SOURCE OF TISSUE

- Head Kidney, Gill, Heart in Parr/Smolt

- Blood or Gill Mucus (non-lethal) from market fish
- Ovarian fluid tissue debris for Broodstock
- Increase chances of target acquisition e.g. crude cell fractionation. Useful for intracellular pathogens (McIntosh *et al*, 1996)

IFAT

- Immunological method, uses anti-ISAV antibodies
- Identification of fluorescent specks in a tissue imprint
- Generally considered to be highly subjective/operator dependent particularly when examining sub-clinically infected fish
- 2 dimensional rather than 3 dimensional
- Head kidney should be targeted in subclinical cases
- In clinical cases kidney tissue may be so damaged as to yield *negative fluorescence*

FALSE NEGATIVE

Viral Culture in Susceptible Cell lines

- Detection of viable, replicating virus
- Generally regarded as the definitive/'Gold standard' method
- Looking for holes in the carpet!
- Seeing is believing!
- Needs a critical mass
- Viral replication may go undetected

- *Possible Improvements:*
- Combine culture and IFAT, look for replication by microscopy (FALK,1998)
- Modify sample preparation e.g Lymphocyte enrichment

Communication:

Ensure free exchange of information between field staff, testing centers, clinicians and legislative authorities to enable results to be put into perspective

Continuing thanks and appreciation to:

New Brunswick Salmon Growers Association
ACRDP
NBDAFA (Sandi, Kim)
AVC (Larry, Pascale, Ian, Dave)
MVS (Dan, Leighanne, Mark)
DFO Moncton (Gilles, Nellie, Anne-Margaret)
Stolt (Shirley , Dean, Mark)
NAVL (Allison, Mike, Kira).

MOLECULAR BIOLOGY GROUP

The Evaluation of Diagnostic Tests for ISA

Ian Dohoo, Pascale Nerette, Larry Hammell, Carol McClure
University of PEI

Any infectious disease control program relies on diagnostic (screening) tests on which to make decisions about control actions such as depopulation, movement restrictions etc. The choice of test(s) to use for a control program needs to be tailored to the objectives of the program. The best tests to use in a program aimed at eradication of an agent may well be different than the tests best suited for a control program which has minimization of economic losses as its goal. The objective of this research was to determine the characteristics of commonly used diagnostic tests for ISA in order that a rational testing program can be devised once the goals of the control program are set.

The four key test characteristics examined were:

- repeatability - the ability of a test to give consistent results within a laboratory
- reproducibility - the consistency of results from the same test from different laboratories
- sensitivity - the proportion of infected fish that give a positive test result
- specificity - the proportion of negative fish that give a negative test result

Since no "gold standard" test (ie a perfect test in all situations) was available for comparison purposes, test evaluations were carried out using multiple samples from each fish submitted to multiple tests in multiple laboratories. Sophisticated statistical analyses were used to determine what combination of test characteristic values were most likely to have given the complete set of results obtained. A total of 500 fish, from 5 populations expected to have: high (moribund fish in an outbreak cage), medium (healthy fish in an outbreak cage), low (healthy fish from another cage on the outbreak site), very low (healthy fish from sites not affected by outbreaks) and zero (fish from a population free of ISA) prevalences of ISA were sampled. Test evaluated to date include the IFAT (1 lab), PCR (2 labs), and virus isolation (VI) (2 labs).

The key findings to date are:

- IFAT - very specific (ie very few false positives) but sensitivity only about 70%
- VI - very specific (no false positives) and more sensitive than anticipated (>90%)
- PCR - relatively low repeatability and reproducibility
  - quite different results from the two labs (high sens./low spec., or moderate sens./high spec.)

Future research plans include:

- evaluation of combinations of tests interpreted in series or in parallel
- estimation of pen-level sensitivity and specificity (results from groups of fish)
- evaluation of additional tests
  - ELISA - Norway
  - extend evaluation of VI
- further investigate one unusual result. Approximately 25% of samples from healthy fish from a cage adjacent to an outbreak cage were PCR+. If these are true positives, the finding has important implications for the current control program (depopulating only outbreak cages). If the results are false positives, it means the PCR has a much lower specificity in this population of fish than it shows in all other populations (which is hard to explain).
- thorough evaluation of assumptions underlying the statistical analyses.

Marcia Cook M.Sc.
Molecular Biology Group
Research and Productivity Council (RPC)
Fredericton, New Brunswick

Marcia Cook has worked in the aquaculture field since 1996. After completing her M.Sc. degree from the University of New Brunswick she joined the Food, Fisheries and Aquaculture group at the Research and Productivity Council (RPC), Fredericton, NB, Canada. At RPC, Marcia is involved with disease surveillance and diagnostic work and a variety of research projects related to the local and international aquaculture community.

ISAV Strains in Canada

While the initial focus of infectious salmon anemia virus (ISAV) management concentrated on surveillance for developing infections and the fallowing of affected sites, it has since been realized that the characterization of discrete isolates may also play an important role in reducing the impact of the disease within Atlantic Canada. Research has since been directed at the identification of specific markers for discrete strains in order to monitor the spread of disease, to contribute to vaccine development and to aid in management decisions such as depopulation. Generally attention has been directed to variability associated with the nucleotide and amino acid sequence of the hemagglutinin protein, specifically a highly polymorphic region (hpr), that European colleagues have suggested may enable the differentiation between disease causing strains and benign strains. In contrast to Europe, only a few hpr variants have been recorded in Canada. Nevertheless, two isolates have been identified in New Brunswick and Nova Scotia which are nearly identical to the possibly benign HPR0 variant identified in Europe. A brief overview will be given on what is currently known about ISAV strains in Canada and suggestions for future analysis with specific reference to disease management in this area will be given.

ISAV Strains in Canada

Molecular Biology Group,
Research and Productivity Council (RPC), Fredericton, NB
Bev Bacon, Steve Griffiths, Rachael Ritchie, Dougie McIntosh, Marcia Cook, Sherry Vincent, Rebecca Liston, Eric Johnsen, Erin MacDonald

Development of Disease

Importance of Strain Identification

- monitor the spread of disease
- vaccine development
- management decisions

ISAV Genetics

- Single stranded –ve RNA virus
- 8 segments
- Orthomyxo-like virus

PB2
PB1
NP
PA
RDE
HA
NS
MX

ISAV Characterization

- most focus has been placed on the genetic characterization of the ISAV hemagglutinin, especially the hpr

- in Europe numerous hpr's have been identified, most of which have been associated with disease

ISAV Characterization

- whether there is a relation between hpr's and disease progression is uncertain

- cell culture replication and cytopathic effect have been reported to vary between viruses from different hpr groups
(Mjaaland et al, (2002),Virology 304:379-391)

ISAV Characterization in New Brunswick

- no comprehensive effort has been made to look at ISAV variants in the Bay of Fundy region and to associate field data with them (ie. mortality, geographic location, etc.)

Hpr Variants in Canada

		St	Highly polymorphic region (HPR)	TMR
New Brunswick	NB280	TDV	MNRVDAI LGVMQVEQPETSVPEWIFISM	GV
	NB508	TDV	MNRVDAIPPQL EWIFISM	GV
	1330/1390	TDV	MNRVDAIPPQLMQT X	GV
	980712-1	TDV	KIRVDAIPPQLMQTFNTMQVEQPETSVLEWIFISM	GV
Nova Scotia	6-6	TDV	KIRVDAIPPQLMQTFNTMQVEQPATSVLEWIFISM	GV
	7035-1	TDV	KIRVDAIPPQLMQT FISM	GV

Current Work

- currently typing ISAV from positive sites in the Bay of Fundy during winter 2002/2003

- also typing those sites/cases considered "unique"

- attempting to gather information from the field to combine with the lab data

- using a combination of RT-PCR/DGGE technology and sequencing; considering portions of segments 6 (hpr), 7, 8

DGGE

ds DNA fragments

↓

gel with ascending gradient of chemical denaturant

↓

electrophorese

↓

differential separation of fragments based on their melting behavior

Goal

To develop a diagnostic assay for use in surveillance programs capable of providing quick, reliable results for ISAV detection while concurrently providing strain identification.

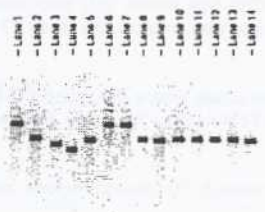
Typing of 5 New Brunswick Isolates by RT-PCR/DGGE Based on Segment 6 Sequence

Reference isolates:

Lane 1	NB280
Lane 2, 8, 13	NB508
Lane 3	Norway
Lane 4	Scotland
Lane 5, 9, 14	Nova Scotia

5 NB isolates:

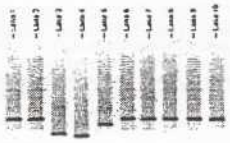
Lane 6	NB877
Lane 7	NB028
Lane 10	NB049
Lane 11	NB002
Lane 12	NB458



Typing of 5 New Brunswick Isolates by RT-PCR/DGGE Based on Segment 7 Sequence

Reference isolates:
Lane 1 NB280
Lane 2 NB508
Lane 3 Norway
Lane 4 Scotland
Lane 5 Nova Scotia

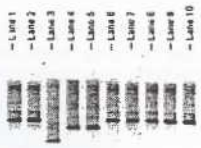
5 NB Isolates:
Lane 6 NB877
Lane 7 NB028
Lane 8 NB049
Lane 9 NB002
Lane 10 NB458



Typing of 5 New Brunswick Isolates by RT-PCR/DGGE Based on Segment 8 Sequence

Reference isolates:
Lane 1 NB280
Lane 2 NB508
Lane 3 Norway
Lane 4 Scotland
Lane 5 Nova Scotia

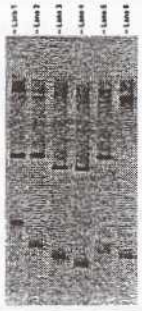
5 NB Isolates:
Lane 6 NB877
Lane 7 NB028
Lane 8 NB049
Lane 9 NB002
Lane 10 NB458



Typing of ISAV Isolates by RT-PCR/DGGE Based on Segment 6 and 7 Sequences

Reference isolates:

Lane 1 NB280
Lane 2 NB508
Lane 3 Norway
Lane 4 Scotland
Lane 5 Nova Scotia
Lane 6 NB1330



Future

- Type ISAV at aquaculture sites

- Associate ISAV strains with field data (ie. mortality, location, time period, etc)

- What about wild fish?

Future

- Is the HPR0-type isolate truly unculturable? Can we alter techniques so we can grow and study it? Is it truly benign?

- Are there cases where IFAT and RT-PCR detect HPR0-type ISAV?? How does this affect depopulation decisions?

- Determine/verify the genetic regions which really do affect virulence.

Acknowledgments

Colleagues:

Department of Fisheries and Marine Biology, University of Bergen
National Veterinary Institute, Norway
Norwegian School of Veterinary Science
FRS Marine Laboratory, Scotland

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New Brunswick Department of Agriculture, Fisheries and Aquaculture (NBDAFA)

ACRDP Program of the Department of Fisheries and Oceans

New Brunswick Salmon Growers Association (NBSGA)

Aqua Health Ltd, a division of Novartis

Brian Glebe

Currently Salmon Aquaculture Research Scientist with the Aquaculture Section at the Dept of Fisheries and Oceans Biological Station in St. Andrews.
Formerly, Manager of the Atlantic Salmon Broodstock Development Program, a genetic improvement program administered by the Huntsman Marine Science Centre for a consortium of New Brunswick salmon growers.
Previously, Freshwater Production Manager for Heritage Salmon in Blacks Harbour.
Other employers have included the Atlantic Salmon Federation, The University of New Brunswick, Jail Island Salmon, Mariculture Products and Briden Consultants
Has a degree in Agriculture from the University of Guelph and a PhD from McGill University

ISA Vaccines- Lab Perspective

Brian Gieg, Wilfred Young-lai, Kim Salonius, Alison McKinnon, Sharon Gauthier, Eric Anderson
ISA Research Frontiers Workshop
2003

Control Measure for ISAV

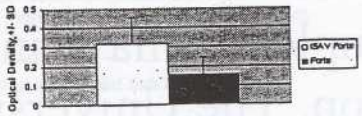
Management by vaccination has thus far been limited to use in the Bay of Fundy

Facilities

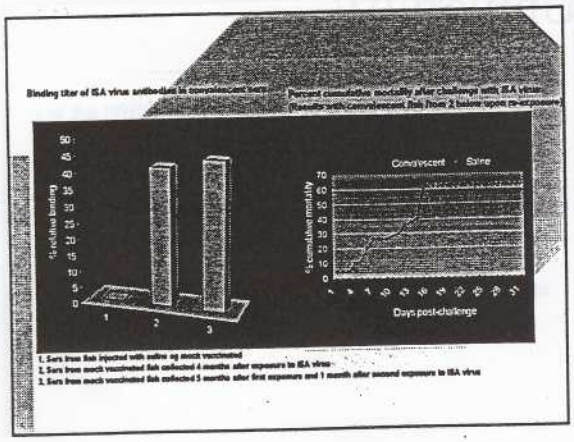
DFO Quarantine Lab

- Injection challenge
- Cohabitation challenge

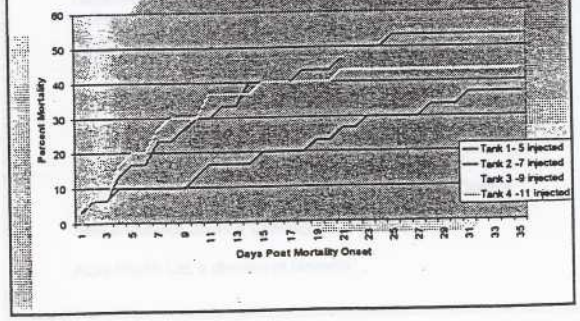
Mean Specific Serum Antibody Titres For ISAV

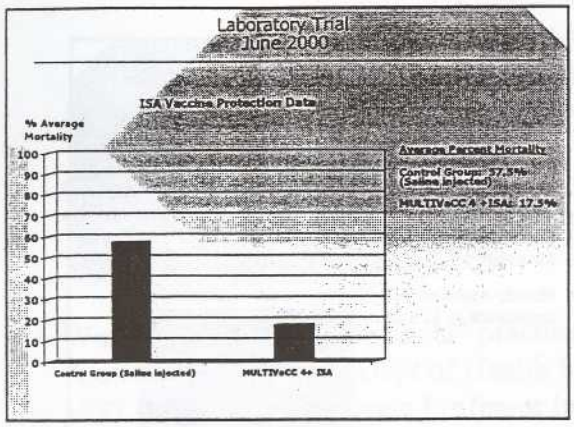


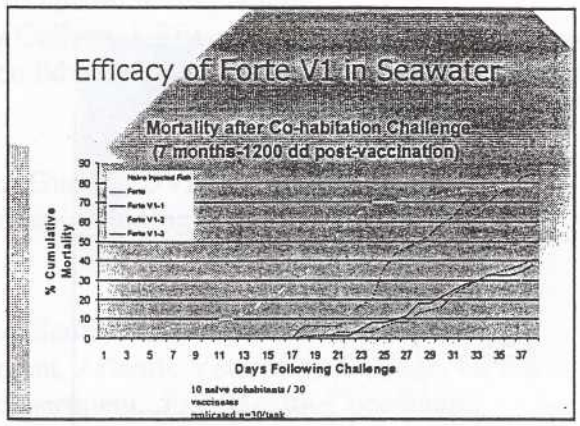
p<0.01, N=10

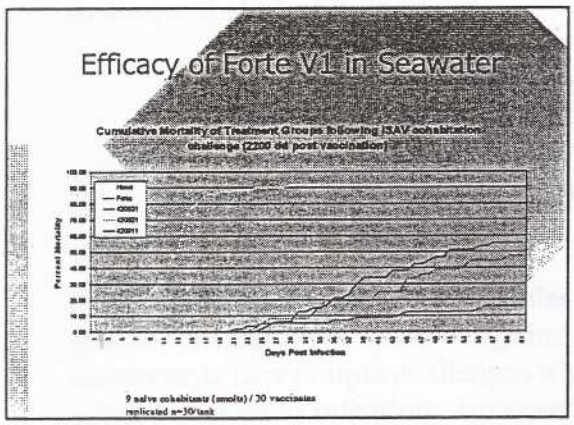


Cumulative Mortality of Unvaccinated (30) Fish when Exposed to Infected Cohabs









Summary of Relative Percent Survivals

	Forte V1-1 RPS	Forte V1-2 RPS	Forte V1-3 RPS	Forte Control (mortality)
800 dd	81.1	75.5	94.6	74.0 %
1200 dd	55.1	59.8	55.2	80.6 %
1500 dd	63.6	76.2	0.0	78.6 %
2200 dd	0.0	51.8	65.5	47.0 %

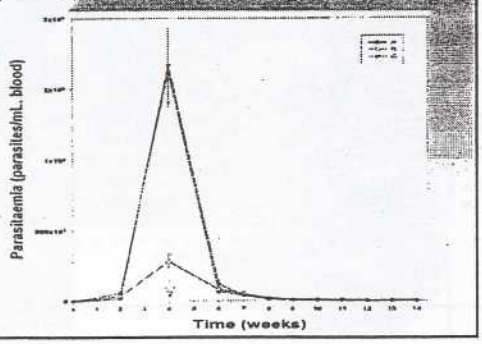
Recombinant Vaccines

- Virus genes that direct synthesis of antigenic proteins put into bacteria
- Proteins synthesized by bacteria then used to vaccinate fish
- Effectiveness of 5 diffferent ISA recombinant vaccines being studied
- Initial challenge RPS of 72% comparable to conventional vaccines

Molecular Approach

- Select Resistent Salmon Strain- AVC Trials indicated ASBDP strain most resistant
- Mark Families- Challenge with Pathogen/Vaccine
- Isolate Good General Immunity Responders
- Establish Quantitative Trait Loci for this Trait by Microsatellite Markers
- Select for Trait in Breeding Program

Parasitaemia of Vaccinated/Challenge Family Groups



Genes Associated with ISA Resistance

- Variation in family immune response to vaccination and ISA resistance linked to gene or QTL variation for selection
- Marker Assisted Selection to increase disease resistance in SJ strain

Further work

- Studies to support shorter onset of protection and effect of temperature on development of protection
- Formulation changes to improve the vaccine efficacy, including a re-formulation to a 0.1 ml dose
- Further investigation as to the infectivity of latent carriers and to what extent this state and potential for shedding exists in vaccinated fish
- Continued research into the protective antigens that may lead to a new technology vaccine
- Vaccine effectiveness for triploids and grilse
- Genetic improvement for immune response
- Evaluation of potential reduced risk of depopulation due to vaccination

Larry Hammell DVM, MSc

Associate Professor, Dept. of Health Management
Atlantic Veterinary College, UPEI
Charlottetown, Prince Edward Island

Obtained a BSc (Biology) in 1984 from Univ of Guelph, DVM in 1988 from Ontario Veterinary College (Univ of Guelph), and MSc (epidemiology) in 1992 from Atlantic Veterinary College, (Univ of PEI).

Worked in private "traditional" practice in Nova Scotia from 1988- 1990. Started as Assistant Professor in Dept of Health Management, Atlantic Veterinary College, UPEI, in 1992. Currently Associate Professor in same department. Fish Health Coordinator for AVC 1996-202.

research interests: health & production monitoring, disease surveillance, risk factor studies, and clinical field trialss

Summary

ISA Vaccine Assessment – Field Perspective

Field trials do not control the variables as strictly as laboratory trials and therefore require special considerations when designing and implementing studies. ISA vaccine assessments face unique challenges when depopulation prevents following groups past an initial detection of infection. However, it is crucial to long term, cost effective control policies to quantify the protection expected from vaccines under field conditions. A recent field trial was performed using individual fish in one study cage that went through an ISA outbreak in the summer of 2002. It showed limited to undetectable differences in survival between ISA vaccinated and non-ISA vaccinated groups through an ISA outbreak. Further vaccine clinical field trials must be done to evaluate vaccines under different environmental and husbandry conditions.

ISA Vaccine Assessment Field Perspective

Larry Hammell, Holly Beaman

Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, PEI

Do we need field trials?

- **In addition to asking whether it is ethical in the light of current knowledge to plan a randomized trial in which some ... will not be offered the new measure,**
- **it is also necessary to ask whether it is ethical not to plan a randomized trial**
- **since failure to do so may subject the population as a whole to the perpetuation of an ineffective program**

Hill 1952

If it does not harm, the possibility of protection is worth the small cost of vaccination

Based on lab evidence that vaccines *likely* protect, the cost of a rigorous field trial(s) may outweigh the benefits of knowledge generated

Particularly when depopulation practices prevent assessment of impact on mortality

(as opposed to impacts on probability of depopulation)

We *need* big picture thinking

- **Industry & government policy must choose the most cost effective and sustainable *policy* toward ISA control**
- ***If* vaccination reduces mortality during outbreaks to manageable levels, depopulation may not be the most cost effective control**

Big picture

- **Policy decisions must be based upon quantified assessments of vaccine effectiveness**
  - (and other control practices)
- **It no longer becomes just an individual company decision about cost**

Lab vs field trials

- **Lab trials attempt to control all / most variables except vaccine status**
  - Issues arise about the *representativeness* of the fish used (one source non-randomly selected, often "wrong" size or season, etc)
  - Environmental and husbandry variables are standard for fish in study, but not necessarily representative of production situations
- **Clear conclusions can be made about different responses within constraints of lab conditions**

Lab vs field trials

- **Field trials attempt to maintain similar influence of uncontrollable variables on study groups**
  - Issues in data collection, sampling, management decisions / compliance, storms, etc
  - Environmental & husbandry variables influence study results – but these variables also influence effectiveness of vaccine under conditions of intended use

Other field trial issues

- **Outcomes of interest**
  - Cost related to mortality is primary interest for ISA
  - Infection and transmission of new cases is primary interest of depopulation program
  - Presents a conflict for field trials (more than lab trials)

Other field trial issues

50% vacc : 50% no-vacc is best
(67% vacc : 33% no-vacc is acceptable)

- **Unit of concern**
  - Vaccination decisions are made at site level
  - Randomizing sites to ISA vaccine or no-ISA vaccine presents too large a cost if no-ISA vaccine sites have increased mortality and transmission
  - Randomizing cages to ISA / no-ISA vaccine presents a large risk for other cages at site
  - Randomizing individual fish to ISA / no-ISA vaccine presents other challenges of sampling and herd immunity

Other field trial issues

Generalization:
representative sites / cages / fish
sample size (getting the *average* response)
Randomization: reducing biases of uncontrollable variables
Blinding: reducing the biases of expectation

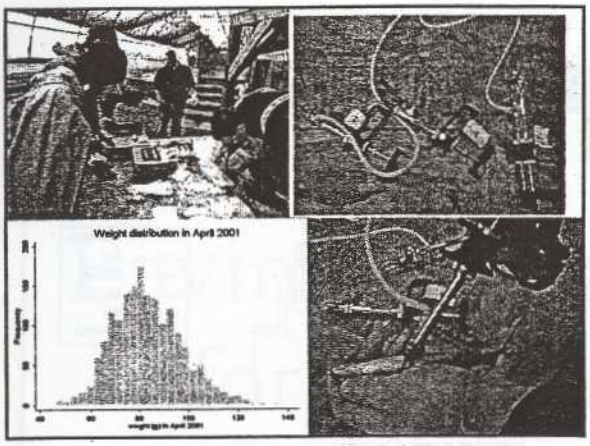
Decisions to be made by industry & government

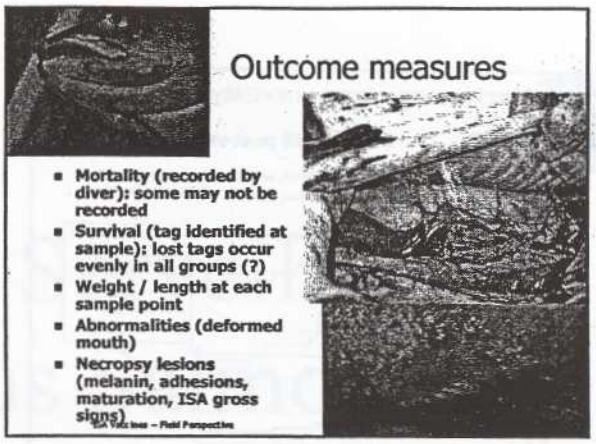
- **Is the field effectiveness of ISA vaccination important to policy decisions over the long term?**
- **If so, how rigorous a trial is needed?**
- **And, who will bear the cost of a multi-site, rigorous trial?**
  - Vaccine sales are small relative to cost of the policies

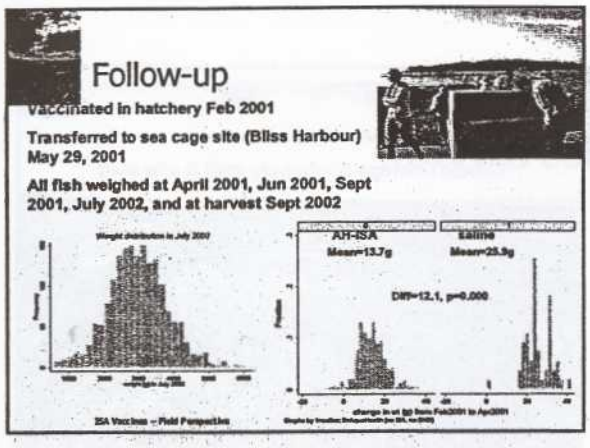
Quick summary of ISA field trial (Feb01 – Sep02)

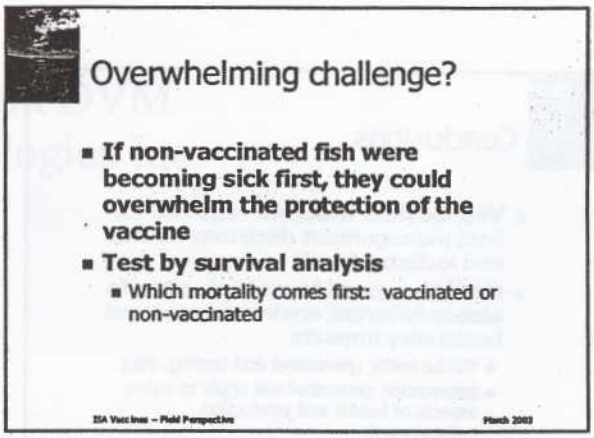
- **Used individual fish as unit**
- **One study cage containing 3187 PIT tagged individuals**
- **Randomized to vaccines in hatchery:**
  - AH+ISA, AH-ISA, AH+BKD, saline
  - BA+ISA, BA-ISA, saline

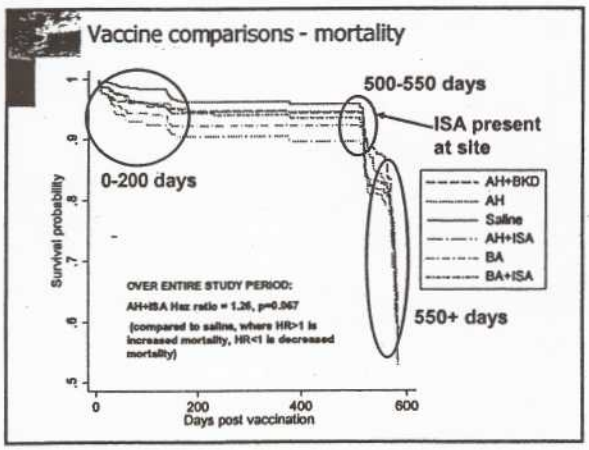
AH+ISA	AH-ISA	BA+ISA	BA-ISA	AH+BKD	Saline
480	455	660	480	450	659

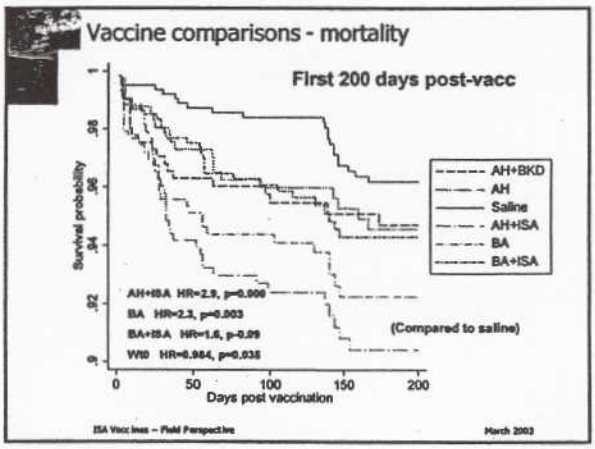

Weight distribution in April 2001


Outcome measures
Mortality (recorded by diver): some may not be recorded
Survival (tag identified at sample): lost tags occur evenly in all groups (?)
Weight / length at each sample point
Abnormalities (deformed mouth)
Necropsy lesions (melanin, adhesions, maturation, ISA gross signs)

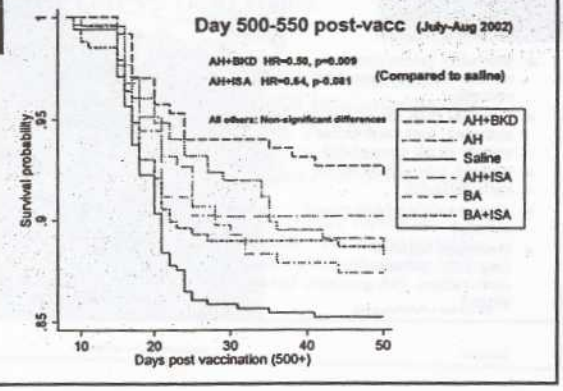

Follow-up
Vaccinated in hatchery Feb 2001
Transferred to sea cage site (Bliss Harbour) May 29, 2001
All fish weighed at April 2001, Jun 2001, Sept 2001, July 2002, and at harvest Sept 2002
AH-ISA
Saline
Meanr=13.7g
Meanr=25.9g
Diff=12.1, p=0.000


Overwhelming challenge?
If non-vaccinated fish were becoming sick first, they could overwhelm the protection of the vaccine
Test by survival analysis
Which mortality comes first: vaccinated or non-vaccinated
March 2003

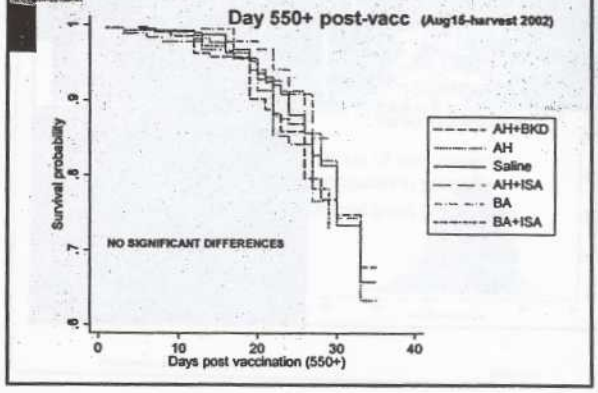

Vaccine comparisons - mortality
500-550 days
ISA present at site
0-200 days
550+ days
AH+BKD
AH
Saline
AH+ISA
BA
BA+ISA
OVER ENTIRE STUDY PERIOD:
AH+ISA Haz ratio = 1.26, p=0.067
(compared to saline, where HR>1 is increased mortality, HR<1 is decreased mortality)
Survival probability
Days post vaccination


Vaccine comparisons - mortality
First 200 days post-vacc
AH+BKD
AH
Saline
AH+ISA
BA
BA+ISA
AH+ISA HR=2.9, p=0.000
BA HR=2.3, p=0.003
BA+ISA HR=1.6, p=0.09
WI0 HR=0.984, p=0.035
(Compared to saline)
Survival probability
Days post vaccination
March 2003

Vaccine comparisons - mortality



Vaccine comparisons - mortality



Conclusions

- **Vaccine field trials are required for best management decisions at site and industry levels**
- **Need to expand to multiple sites to assess different environmental and husbandry impacts**
  - Will be costly (personnel and testing, etc)
  - Information generated will apply to many aspects of health and production management

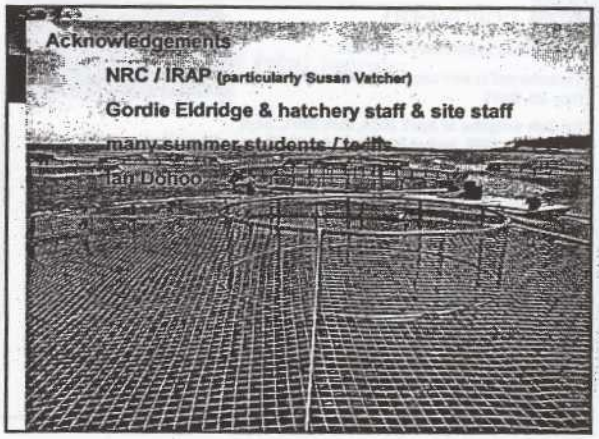
Acknowledgements

NRC / IRAP (particularly Susan Vatcher)

Gordie Eldridge & hatchery staff & site staff

many summer students / techs

Ian Dohoo



Environmental Surveillance for Infectious Salmon Anemia Virus

Peter L. Merrill DVM
Micro Technologies Inc

USDA APHIS ISA Program

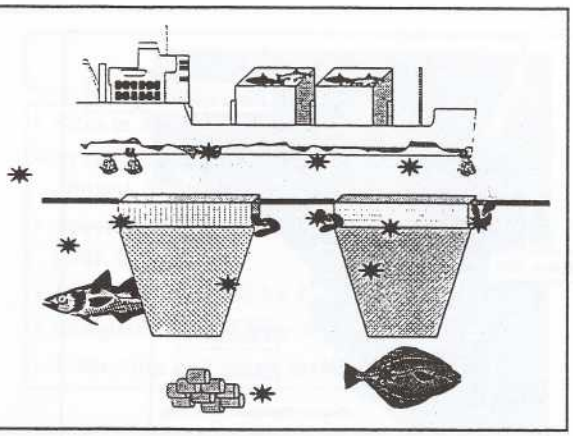
Environmental Surveillance for Infectious Salmon Anemia Virus

Stephen K. Ellis, DVM USDA APHIS VS

Peter L. Merrill, DVM Micro Technologies, Inc.

ISA/ISAV Research Priority Summit

3/31/2003 St. Andrews NB



USDA Cumulative Environmental Sampling Jan 02-Jan 03

	0/1			0/1
	2/52	0/12	0/24	0/32
			0/5	1/30
	0/4			
	0/8			0/6
	0/6			1/36
	0/8			1/39
	7/38	0/2	0/6	1/14
	0/6			
	2/11			1/2
	0/7		0/5	0/1
	0/7			
	0/15		0/2	0/2
	0/6		1/5	0/1
	0/2			
				0/6
Totals=15	11/171	0/14	1/47	5/170

Can ISAV Be Detected*:

- In marine water samples??
- On pontoons/nets??
- In wild fish populations??
- In sediment under cages??
- In algae or barnacles??
- In shellfish??
- On boat decks and hulls??

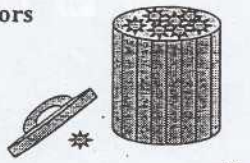
* *In vitro* or *in vivo*

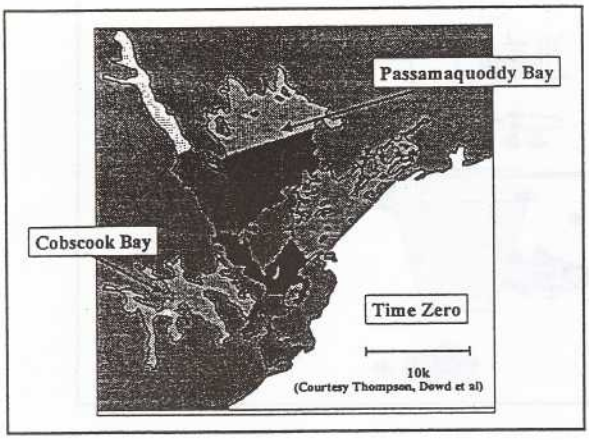
Environmental Sampling Goals

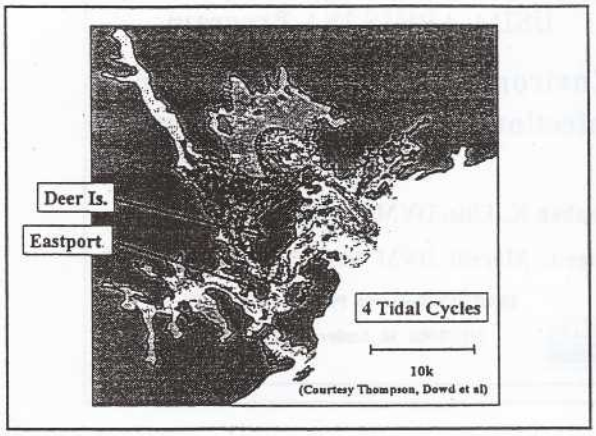
- Distribution of virus
- Quantity of virus
- Qualities of virus
- Changes over time
- Cost effectiveness
- Timeliness
- Risk assessment/Epizootiology
- Fit into overall surveillance program

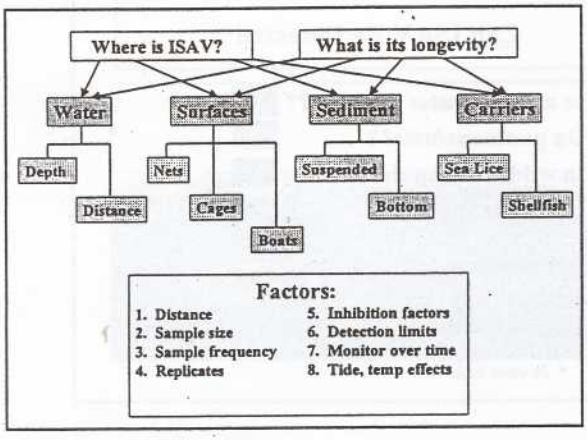
Environmental Reservoirs

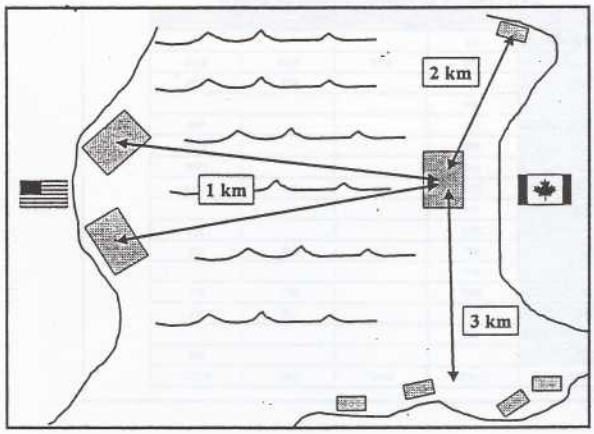
- Possible/optimal sample sources
- Detectability factors
- Prevalence
- Longevity
- Viability
- Infectivity
- Reliability factors
- Facilitative, mitigating or inhibiting factors

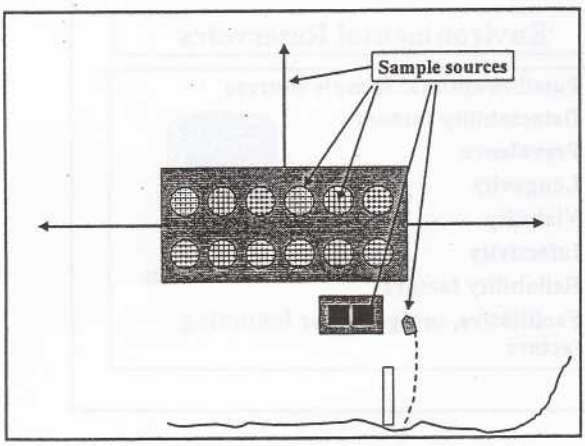


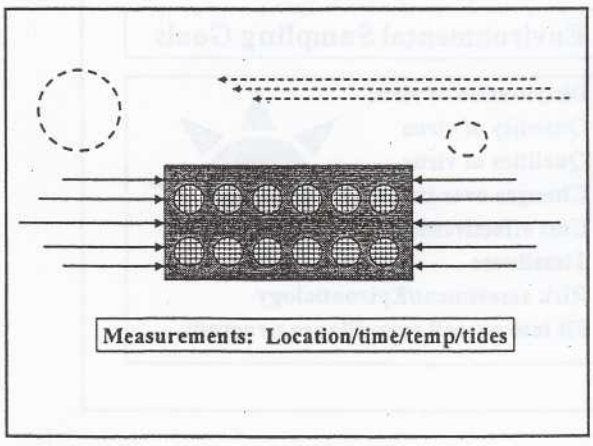

Passamaquoddy Bay
Cobscook Bay
Time Zero
10k
(Courtesy Thompson, Dowd et al)


Deer Is.
Eastport
4 Tidal Cycles
10k
(Courtesy Thompson, Dowd et al)


Where is ISAV?
What is its longevity?
Water
Surfaces
Sediment
Carriers
Depth
Distance
Nets
Cages
Boats
Suspended
Bottom
Sea Lice
Shellfish
Factors:
1. Distance
2. Sample size
3. Sample frequency
4. Replicates
5. Inhibition factors
6. Detection limits
7. Monitor over time
8. Tide, temp effects


2 km
1 km
3 km


Sample sources


Measurements: Location/time/temp/tides

Water Testing Specifics

- Sample collection (amount, location)
- Transit time and conditions
- Filter types
- pH effects
- Temp effects
- Incubation time
- Presence/longevity/viability

Seawater testing

- Sites in Maine and NB
- Previously and currently positive and/or clinical (Categories 2, 3, 4 & 5)
- Previously and presumptively negative (Cat. 1, 2 and 6)
- Locations marked by GPS coordinates
- Sampled serially over time and tidal cycles
- Collection and assay techniques

Technique Summaries: Fish

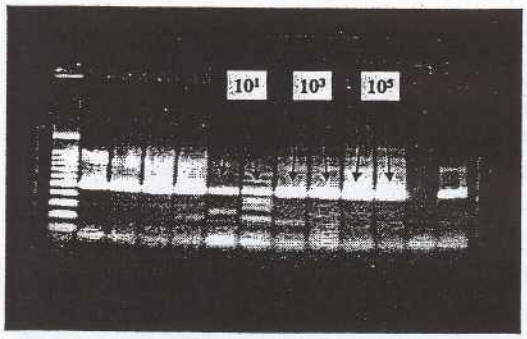
- At least 10 fish per cage
- VI (pooled) and PCR (ind.)
- CHSE, SHK and ASK lines; 1D/2 primers
- Kidney and blood
- Excellent correlations

Technique Summaries: Seawater

- 2 liter samples
- Various distances
- Electronegative filter concentration
- Filters, eluates and filtrates tested by VI and/or PCR
- Direct and dilutions
- Results

Technique Summaries: Seawater

- Spiked SW samples: dilution series
- Detection limits:
  - VI: 10^5 virions/2 liters
  - PCR: 10^2 virions/2 liters
- (VI confirmed with PCR)
- PCR products sequenced



Results Summaries: Seawater

- Only PCR + results
- Positives found up to 1.5 km from site
- Tide/current effects?
- Detection limits and interpretations

Technique Summaries: Swabs

- Pontoons, nets, boat decks/hulls, tarps
- 900 cm^2 surface area used
- PBS for culture; EtOH for PCR
- Positives (by PCR only) on many surfaces

Technique Summaries: Sea Lice

- Tested in correlation with host fish
- Topical disinfection with bleach
- VI and PCR tests used
- Homegenates diluted 1:10 in PBS
- Strong + lice/ + fish correlations
- Also: PCR + lice on – fish

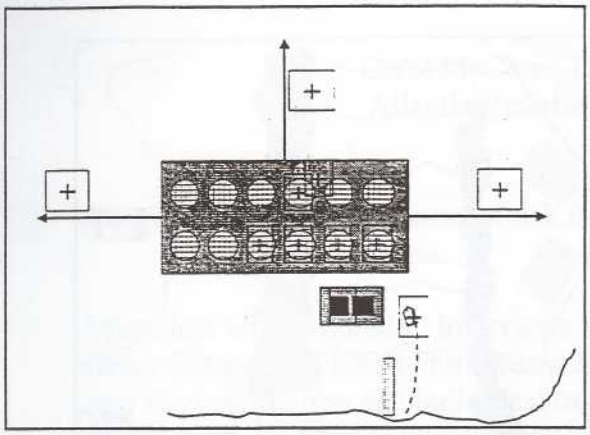
Technique Summaries: Mussels

- Collected from clinically positive cages
- Various organs and whole tissue homogenates tested by VI and PCR
- Absence of detectable levels of virus
- Possible inhibiting factors
- More research needed

Technique Summaries:Sediments

- Mini-cores used for sample collection below cages
- Upper 2 mm of sediment tested by VI and PCR
- No virus obtained
- Inhibiting factors?
- Research ongoing

Clinical Site Water Sampling 2003				
Sample Dist.	1/15/03 (OG)	1/30/03 (JI)	2/19/03 (TC)	3/5/03 (OG)
0 m SE	Pos*	Neg	Neg	Pos*
100 m SE	Pos*	Neg	Neg	Pos*
500 m SE	Pos*	Neg	Pos*	Neg
1 km SE		Neg	Pos*	Neg
1.5 km SE		Neg	Pos*	
2 km SE		Neg	Neg	
0 m WSW		Neg	Neg	Neg
100 m WSW		Neg	Neg	Neg
500 m WSW		Pos*	Neg	Neg
0 m NW		Pos*	Pos*	Pos*
100 m NW		Pos*	Pos*	Neg
500 m NW		Pos*	Neg	Neg
1 km NW		Neg	Neg	



Clinical Site Sampling Cumulative Results Jan-Mar 2003

15 January 2003

Sample Type	C10		C8		C6	
	VI (Pooled)	PCR	VI (Pooled)	PCR	VI (Pooled)	PCR
Kidney	6/6	28/28	6/6	25/28	6/6	29/29
Blood	4/6	28/28	5/6	26/28	5/6	29/29
Lice	0/5	14/14	0/1	10/10	N/a	13/13
Seawater	0/3	3/3	0/3	3/3	0/3	3/3
Swabs	0/3	3/3	0/3	3/3	0/3	3/3

Clinical Site Sampling Cumulative Results Jan-Mar 2003

19 February 2003

Sample Type	C10		C8		C6	
	VI (Pooled)	PCR	VI (Pooled)	PCR	VI (Pooled)	PCR
Kidney	10/10	10/10	10/10	10/10	10/10	10/10
Blood	10/10	10/10	10/10	10/10	10/10	10/10
Lice	N/a	N/a	N/a	N/a	N/a	N/a
Seawater	0/1	0/1	0/1	0/1	0/1	1/1
Swabs	0/1	1/1	0/1	1/1	0/1	1/1

Clinical Site Sampling Cumulative Results Jan-Mar 2003

5 March 2003

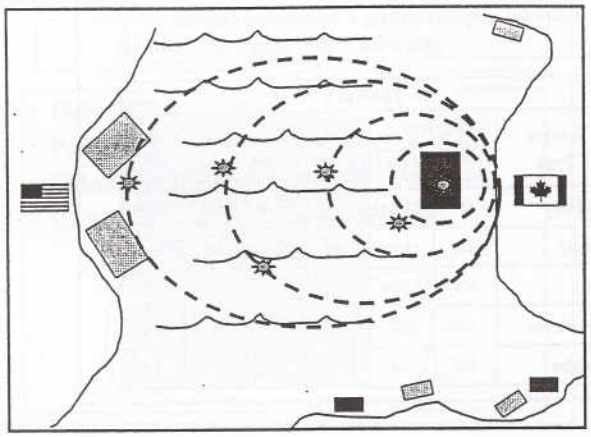
Sample Type	C12		C9		C6	
	VI (Pooled)	PCR	VI (Pooled)	PCR	VI (Pooled)	PCR
Kidney	(2/2)	1/10	(2/2)	9/10	(2/2)	5/10
Blood	(1/2)	1/10	(2/2)	8/10	(2/2)	5/10
Lice		2/3		2/2		1/1
Seawater		1/1		0/1		1/1
Swabs		1/1		1/1		1/1

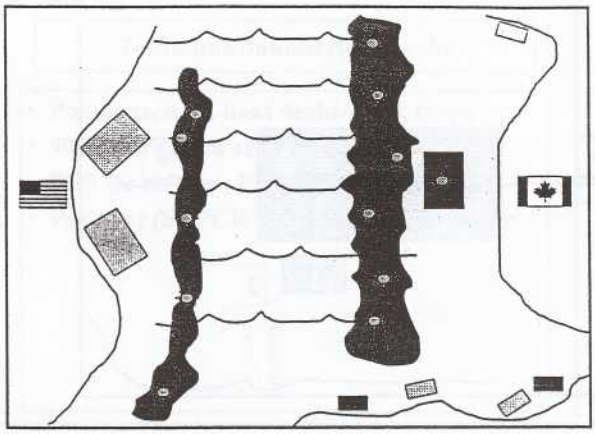
NMFS Wild Fish Testing

- Cod
- Haddock
- Pollock
- Flounder
- Herring
- Mackerel
- Alewives
- Lumpfish
- 1800+ fish were tested

Environmental Sampling: Q & A

- PCR products regularly sequence as ISAV
- Positives rarely confirmed by culture
- Sensitivity/specificity
- False positives/false negatives
- Inhibiting factors?





Preliminary results of the 2002 ISA Risk Factor Study

Carol McClure, Larry Hammell, and Ian Dohoo
Atlantic Veterinary College, University of PEI

Objective: To identify current risk factors for ISA in the New Brunswick Atlantic Salmon farms.

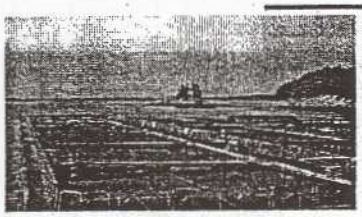
Materials and Methods: Interviews were conducted with site managers or owners of farms that stocked smolt in the years 2000 and 2001. Interview questions were divided into site information and individual cage information. In addition there was a section on hatchery information to which the hatchery managers responded. Analysis of the data included multivariable logistic regression and was performed using the Stata 7 software.

Preliminary Results: The participation rate was 97.6% (83 of the 85 qualifying farms). Factors that are associated with an increase the risk of ISA at a site include: having a neighbor farm with ISA less than 2 km from your farm, having greater than or equal to 5% mortalities in the first 30 days post-smolt transfer, weight sampling fish one or more times per month, sites that have cages that are nine meters or less in depth, and having dry feed delivered by the feed company or contract company boat. Factors that are associated with an increase in the risk of ISA in a cage include: cages that are nine meters or less in depth, cages that have less than 9 meters of water underneath the bottom of the cage at low tide, cages that are estimated to have 1000 or more Harbour Pollock present in the cage, and cages that were treated less than twice for sea lice. Hatchery risk factors that are associated with an increase in the risk of ISA in a cage include: fish that were not vaccinated for ISA, fish that were given immersion vaccines, and fish that had low degrees-days post vaccination prior to entry into sea water.

Current research and conclusion: Preliminary results will be investigated further by merging all site, cage, and hatchery data and analyzing them as a unit. The time at which cages became infected needs to be identified to properly analyze the importance of having a farm with ISA within 2 km of your farm. Significant risk factors can be used to recommend ISA control protocols and as preliminary information for forming hypotheses for experimental studies.

Carol McClure is currently a Ph.D. candidate in aquaculture epidemiology. She received her DVM in 1993 from Cornell University. After working as a mixed animal practitioner, she pursued a MSc. degree at the University of Wisconsin. Before returning to school for her Ph.D., she worked as an instructor at Cornell University and at the Atlantic Veterinary College.

Preliminary results from the 2002 ISA Risk Factor Study



Carol McClure
ISA Workshop
March 31, 2003

Introduction
Observational Studies

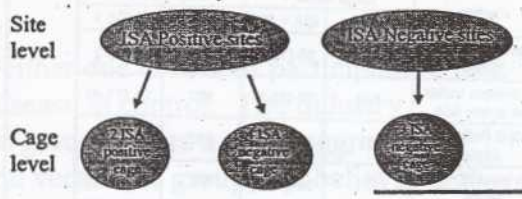
- Designed to
  - Collect valuable data by observing
  - Without having special treatments or medications
  - Without having special holding facilities
- Good in cases where an experimental study would be complicated and expensive
- More like real life than a laboratory study
- Unfortunately can not control any factors like in a lab study

Risk Factor Studies

- Risk factor studies are observational studies
- Significant risk factors
  - Are associated with an increase chance of fish having ISA
  - Can be used to recommend ISA control protocols
  - May be used to form hypotheses for future research

ISA Risk Factor Study 2002

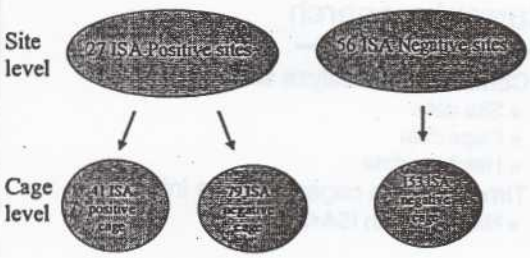
- Objective: to identify risk factors associated with a cage or site becoming ISA positive
- Case-control study for 2000 and 2001 YC



Data

- Data collection
  - Interviews with site manager or owner
  - Telephone and fax survey with hatchery managers
  - Record collection
- Data analysis
  - Unconditional associations
  - Multivariable logistic regression

Data collection



Results

- Information collected from
  - 83 of the 85 qualifying farms (97.6% participation)
  - 273 cages
- Information analyzed in groups
  - Site factors (74 different factors)
  - Cage factors (37)
  - Hatchery factors (14)

Site risk factor data
Results

Factor	Site factor +		Site factor -	
	number	ISA + (%)	number	%ISA +
ISA neighbor within 2 km	47	26 (55.3)	36	1 (2.8)
Dry feed delivered	26	15 (57.7)	56	12 (21.4)
Cage depth 9m or less	58	22 (37.9)	23	3 (13.0)
Post transfer mortalities>5%	22	10 (45.5)	38	10 (26.3)
Weight sample 1+ per month	44	18 (40.9)	39	9 (23.1)

Cage risk factor data
Results

Factor	Cage factor +		Cage factor -	
	number	ISA + (%)	number	%ISA +
Cage depth 9m or less	195	35 (18.0)	78	6 (7.7)
≥ 5 meters under net at low tide	143	27 (18.9)	123	14 (11.4)
≥ 1000 Pollock in cage	31	10 (32.3)	187	28 (15.0)
Lice treatment <2 times	171	30 (17.5)	96	11 (11.5)

Hatchery risk factor data
Results

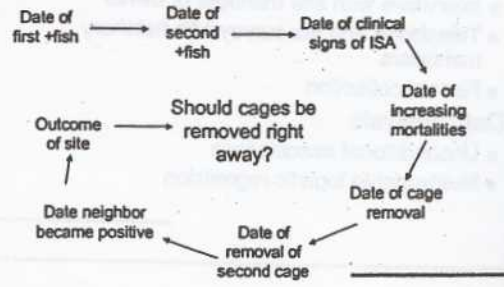
Factor	Cage factor +		Cage factor -	
	number	ISA + (%)	number	%ISA +
ISA vaccine	145	19 (13.1)	91	21 (23.1)
Immersion vaccine	80	17 (21.4)	154	21 (13.6)

Factor	ISA + cages		ISA - cages	
	number	avg	number	avg
Degree days	28	612	144	688

Current research

- Combine and analyze simultaneously
  - Site data
  - Cage data
  - Hatchery data
- Time at which cages became infected
  - Neighbor with ISA<2 km

Future research
observational studies



Larry Hammell DVM, MSc

Associate Professor, Dept. of Health Management
Atlantic Veterinary College, UPEI
Charlottetown, Prince Edward Island

Obtained a BSc (Biology) in 1984 from Univ of Guelph, DVM in 1988 from Ontario Veterinary College (Univ of Guelph), and MSc (epidemiology) in 1992 from Atlantic Veterinary College, (Univ of PEI).

Worked in private "traditional" practice in Nova Scotia from 1988- 1990. Started as Assistant Professor in Dept of Health Management, Atlantic Veterinary College, UPEI, in 1992. Currently Associate Professor in same department. Fish Health Coordinator for AVC 1996-202.

research interests: health & production monitoring, disease surveillance, risk factor studies, and clinical field trialss

Summary

ISA Control & Management Decisions

ISA control policies do not appear to be working either due to lack of participation, lack of appropriate policy, or an impossibly difficult disease to control. The industry members have diverged into two main camps: one group desires a more aggressive approach with removal at earliest signs of infection versus the group that believes that management of mortalities is possible despite infection with ISA virus. The decisions must be made at the industry level to refine and improve the surveillance, compliance, and control of ISA. The applied research program to support the chosen policies must be defined and targeted to help justify that we are pursuing the most cost-effective and sustainable methods of ISA control.

Attendees

Backman, Steve - Skretting
Bacon, Bev - Research & Productivity Council
Beaman, Holly - University of PEI
Beattie, Mike - Skretting
Beecroft, Rob - Immunal Presica Antibodies Ltd
Brown, Glenn - Cooke Aquaculture Ltd.
Caines, Jennifer - North Atlantic Cod Farms
Carpenter, Arthur - Deer Island Salmon Inc
Carpenter, Jamie - Sussex Salmon Inc.
Chang, Blythe - Biological Station, St. Andrews
Cleghorn, Kathy - DAFA
Cook, Marcia M.Sc - Research & Productivity Council, Fredericton
Coombs, Karen - DAFA, St. George NB
Corey, Lee - Corey Feed Mills Ltd.
Cunningham, Carey - Fisheries Research Services Marine Laboratory (FRS), Aberdeen, Scotland
Cusack, Roland - NS DAFA
Davage, Chris - Stolt Seafarm Inc.
Delaney, Jennifer - DAFA
Demmings, Carolyn - Bank of Montreal, Saint John
Dohoo, Ian - University of PEI
Drost, Terry - Corey Feed Mills Ltd.
Ellis, Steve - USDA Gov.
Frye, Stacey - West Isles Seafood
Gagne, Nellie - DFO, Moncton
Gagne, Evie - Cooke Aquaculture Ltd.
George, Sheldon - Aqua Buyers Group
Giray, Cem - Micro Technologies Inc., Maine
Glebe, Brian - DFO, St. Andrews
Griffiths, Steve - Research & Productivity Council
Gustafson, Lori - USDA Gov.
Halse, Nell - NB Salmon Growers Association, Letang, NB
Hammell, Larry - University of PEI
Hosian, Alisha - International Aquaculture Vet Services
House, Betty - Heritage Salmon Ltd.
Hutchin, Lynn - DAFA, St. George NB
Jackson, Tim - NRC-IRAP
Kennedy, Eddy - DFO, Office of Sustainable Aquaculture
Kibenge, Fred - University of PEI
Kohler, Chris - DAFA, St. George NB
Lipsett, Kim - DAFA, Fredericton
MacDonald, Linda - ACOA, Saint John, NB

Attendees

MacKinnon, Allison - Aqua Health in PEI
McPhee, Dan - Maritime Veterinary Services, St. George
Madill, Hugh - DAFA, St. George NB
Marcoux, Ernie - Marsh Canada Ltd.
McClure, Carol - University of PEI
McCray, Michelle - NB Salmon Growers Association, Letang, NB
McGarry, Alison - DAFA, Fredericton
McGeachy, Sandi - DAFA, Fredericton
McGrattan, Stan - Aqua Fish Farms Inc.
McIntosh, Dougie - Research & Productivity Council
Menton, Don - Resident of St. Andrews
Merrill, Peter - Micro Technologies Inc., Maine
Merritt, Rob - DAFA NB Lab
Moore, Mark - Deptartment Agriculture, Fisheries & Aquaculture DAFA, St. George
Mullins, Julia - Heritage Salmon Ltd.
Nerette, Pascal - University of PEI
Nyland, Are - Institute of Fisheries & Marine Biology, University of Bergeen, Norway
O'Halloran, John - Aquaculture Vet Services
Olivier, Gilles - DFO, Moncton
Page, Fred - DFO, St. Andrews
Purdy, Lloyd - Jail Island Salmon
Robinson, Teresa - USDA
Roland, Jill - US Deptartment of Agriculture
Sephton, Tom - Biological Station, St. Andrews
Smith, Jamey - NB Salmon Growers Association, Letang, NB
Smith, Sybil - NB Salmon Growers Association, Letang, NB
Smith-Miner, Karen - Novartis
Solonnius, Kira - Aqua Health in PEI
Stanley, Trevor - Skretting
Steine, Nils - Atlantic Salmon of Maine
Stewart, Ian - Ocean Legacy
Taylor, Tom - Shur Gain
Thorpe, Bruce - DAFA St. George
Vinneau, Doreen - Downeast Plastics, Cap Pele, NB
Waterstrat, Paul - Maine Department of Marine Resources
Whelan, Daryl - DAFA, Nfld
Wright, Laurie - DAFA, NB
Yason, Carmencita - University of PEI
Young-Lai, Wilfred - Biological Station, St. Andrews