Marine Environment & Health Series No. 34 2008

PANCREAS DISEASE IN FARMED SALMON - HEALTH MANAGEMENT AND INVESTIGATIONS AT IRISH FARM SITES 2005 - 2008

Neil Ruane

David Graham

Hamish Rodger

Marine Institute
Foras na Mara

PANCREAS DISEASE IN FARMED SALMON – HEALTH MANAGEMENT AND INVESTIGATIONS AT IRISH FARM SITES 2005 – 2008

NEIL RUANE[1], DAVID GRAHAM[2], HAMISH RODGER[3]
[1]Marine Institute, Oranmore, Co. Galway.
[2]Agri-Food & Biosciences Institute, Stormont, Belfast
[3]Vet-Aqua International, Oranmore, Co. Galway

September 2008

This project (Grant-aid Agreement No. ST/05/01) is carried out with the support of the Marine Institute and the Marine RTDI Measure, Productive Sector Operational Programme, National Development Plan 2000 – 2006.

YOUR PLAN – YOUR FUTURE

ISSN NO: 1649-0053

Marine Environment & Health Series, No. 34, 2008.

ACKNOWLEDGEMENTS

We wish to convey our thanks and appreciation to all the contributors and organisations who were involved in the collaborative research project on pancreas disease, summarised in this report.

A special note of appreciation is given to the Irish salmon farmers for their willingness to co-operate and support the work involved in this research project. We would specifically like to mention Gerry O Donoghue, Eugene Casey and the staff at Mannin Bay Salmon Co., Willie Ward and the staff at Eany Fish Products and Catherine McManus, Ashie Norris, Nigel Teape, Bernie Hegarty and the staff at Marine Harvest Ireland.

We also wish to acknowledge the ongoing support of Gordon Ritchie, Marine Harvest ASA and all the members of the Tri-Nation Pancreas Disease Group and Torunn Taksdal, National Veterinary Institute, Norway for her work and dedication as the external monitor for the project.

SUMMARY

This publication constitutes the final report for the research project ST/05/01 "Site investigations and disease management of the pancreas disease virus in Irish farmed salmon", funded under the NDP Marine RTDI Programme. Work undertaken within the project included longitudinal studies of rainbow trout and Atlantic salmon at sea following the course of infection, testing for vectors and reservoirs of the virus, molecular studies of the virus and an epidemiological investigation of pancreas disease in Ireland. Results have shown that although pancreas disease is endemic in marine farmed Atlantic salmon, no evidence of infection in rainbow trout farmed at sea was found. Serological and molecular based diagnostic methods were shown to be suitable for the screening of fish stocks for the presence of the virus. For the confirmation of clinical outbreaks, farm data and histopathological results should be included. The results also suggest that horizontal transmission of the virus may be the main route of infection between sites. The project also involved the technology transfer of molecular and serological diagnostic methods for pancreas disease between partners and the final chapter includes practical information on management of, and mitigation against, pancreas disease.

Pathologies such as pancreas disease, heart and skeletal muscle inflammation and cardiomyopathy syndrome, pose a serious threat to salmonid farming in Ireland, Scotland and Norway. Most significant among this group of diseases is pancreas disease, a viral disease affecting Atlantic salmon during the marine stage of the production cycle. From the first description of pancreas disease in farmed Atlantic salmon from Scotland in 1976 the disease has now become endemic in Ireland and parts of Norway and continues to be significant in Scotland. The causal agent of pancreas disease, a salmonid alphavirus, has now been characterised and a closely related subtype of the virus is known to cause sleeping disease in farmed rainbow trout on continental Europe and in the United Kingdom. The Irish salmon farming industry has estimated that pancreas disease has resulted in a total loss of turnover of €35 million with €12 million loss of profit in the years 2003-2004. The economic impacts are estimated to be in the range of €100 million per year in Norway. In Scotland, pancreas disease and related pathologies are increasingly responsible for significant losses in marine salmon farms but these have yet to be quantified.

LIST OF CONTRIBUTORS

Albert Girons, Vet-Aqua International, Oranmore Business Park, Oranmore, Co. Galway.
Amanda Browne, Department of Veterinary Science, Queen's University of Belfast, Veterinary Sciences Division, Stoney Road, Stormont, Belfast.
Claire Wilson, Department of Veterinary Science, Queen's University of Belfast, Veterinary Sciences Division, Stoney Road, Stormont, Belfast.
Damien Ó Ceallacháin, Meitheal Éisc Teo, Leitir Caladh, Co. na Gaillimhe.
David Graham, Veterinary Sciences Division, Agri-Food & Biosciences Institute, Stoney Road, Belfast.
Elena Fringuelli, Department of Veterinary Science, Queen's University of Belfast, Veterinary Sciences Division, Stoney Road, Stormont, Belfast.
Eugene Casey, Mannin Bay Salmon Co., Errislannan, Clifden, Co. Galway.
Fiona Geoghegan, Fish Health Unit, Marine Institute, Oranmore, Co. Galway.
Gordon Richie, Marine Harvest ASA, Stortingsgaten 8, Oslo, Norway.
Hamish Rodger, Vet-Aqua International, Oranmore Business Park, Oranmore, Co. Galway.
Karen McCrann, Mannin Bay Salmon Co., Errislannan, Clifden, Co. Galway.
Laura Murchie, Department of Veterinary Science, Queen's University of Belfast, Veterinary Sciences Division, Stoney Road, Stormont, Belfast.
Leo Foyle, Veterinary Sciences Centre, School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4.
Lorraine McCarthy, Fish Health Unit, Marine Institute, Oranmore, Co. Galway.
Louise Totten, Department of Veterinary Science, Queen's University of Belfast, Veterinary Sciences Division, Stoney Road, Stormont, Belfast.
Micheal Ó Cinneide, Marine Environment & Food Safety, Marine Institute, Oranmore, Co. Galway.
Neil Ruane, Fish Health Unit, Marine Institute, Oranmore, Co. Galway.
Sarah Culloty, Department of Zoology, Ecology & Plant Sciences, University College Cork, Distillery Field, North Mall, Cork.
Simon More, Centre for Veterinary Epidemiology & Risk Analysis, Veterinary Sciences Centre, School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4.
Stephen McCleary, Fish Health Unit, Marine Institute, Oranmore, Co. Galway.
Susie Mitchell, Vet-Aqua International, Oranmore Business Park, Oranmore, Co. Galway.
Tracy Clegg, Centre for Veterinary Epidemiology & Risk Analysis, Veterinary Sciences Centre, School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4.
William Ward, Eany Fish Products Ltd., Killybegs, Co. Donegal.

TABLE OF CONTENTS

1. Introduction 1
    1.1 The Tri-Nation Initiative 1
    1.2 PD in Ireland 2
    1.3 PD in Scotland 3
    1.4 PD in Norway 3

2. The *Salmonid alphavirus* 6
    2.1 The nature of the virus 6
    2.2 Discovery of the *Salmonid alphavirus* 7
    2.3 Pancreas disease diagnostics 7
    2.4 Diseases of similar pathology 8

3. Objectives of the research project: ST/05/01 10

4. Longitudinal Studies (WP 2) 11
    4.1 Rainbow trout 11
    4.2 Atlantic salmon S1's 11
    4.3 Discussion 17

5. Vectors & Reservoirs (WP 3) 19
    5.1 Experimental infection of mussels 19
    5.2 Testing sea lice 22
    5.3 Vertical transmission & broodstock testing 22
    5.4 Wild fish testing 23

6. Virus Sequencing Studies (WP 4) 25

7. Epidemiology (WP 5) 30
    7.1 Materials & Methods 30
    7.2 Results 31
    7.3 Data analysis 35
    7.4 Discussion 41

8. Strategies for PD Management & Mitigation (WP 6) 43
    8.1 Disinfection against SAV 43
    8.2 Management strategies for pancreas disease 43
    8.3 Recommendations for future research 44

References 46

List of Appendices
    Appendix I. Project outputs 50
    Appendix II Sampling protocol for longitudinal studies 51
    Appendix III A summary of disinfection data for SAV 52
    Appendix IV Tri-nation PD seminar agenda (September 2007) 53
    Appendix V Tri-nation PD seminar attendees (September 2007) 54
    Appendix VI Tri-nation PD seminar agenda (May 2008) 56
    Appendix VII Tri-nation PD seminar attendees (May 2008) 58

1. INTRODUCTION

Neil Ruane, Micheal Ó Cinneide, David Graham, Hamish Rodger, Gordon Ritchie

In response to the serious losses due to pancreas disease in the Irish salmon industry since 2002 (see section 1.2 below), the Marine Institute set up a *Pancreas Disease Research Group* in early 2004 to advise and suggest initiatives on research into the disease. The Institute in association with IFA Aquaculture, convened a seminar entitled "*Pancreas Disease in Ireland and Future Research Priorities*" in Galway, October 2004. From this meeting it became clear that pancreas disease was not only a problem for the Irish salmon industry, but was emerging as a significant problem in Norway and an international approach was needed to combat the disease.

1.1 The Tri-Nation Initiative

The Tri-Nation Initiative is a voluntary group consisting of personnel from the salmon farming industry, private veterinary practices, state agencies and third-level institutes from Ireland, Scotland and Norway. Representatives from England, France and the Faroe Islands have also been involved. The group allows participants to share current knowledge on pancreas disease and to debate future strategies for research and management of the disease. The group first convened at a seminar "*PD: similar pathologies and prevention*" in Bergen, February 2005 and at two follow-up seminars in Aberdeen, March 2005 and again in Bergen in May 2005. The aims of these seminars were to:

- Share knowledge and experience on pancreas disease and diseases with a similar pathology.
- Accelerate progress in developing specific control strategies.
- Identify knowledge gaps and future research strategies.

This led to the Tri-Nation Initiative being established with research and industry representation from each of the three countries. The group is built on four pillar activities: Steering, Research I (epidemiology), Research II (aetiology & longitudinal studies) & Research III (diagnostics). The objectives of the steering committee (Table 1.1) are:

- To harness new results with the aim of accelerating progress in the development of specific mitigation/control strategies.
- To encourage the presentation of new data and scientific findings to the committee (including industry representatives) at Tri-Nation meetings, before general release.
- Encourage the research co-ordinators to develop projects that are integrated and harmonised on a Tri-Nation basis.

The research working groups were responsible for drafting and submitting appropriate research proposals for funding in each of the Tri-Nation member countries. As a consequence of these efforts, eight projects on pancreas disease and diseases with similar pathologies have evolved under the Tri-Nation research programme umbrella, with the majority having started in January 2006 and running for 1-4 years. Updates on these research projects and new findings have been presented at the regular Tri-Nation seminars held in Oslo (February 2006), Galway (September 2006), Aberdeen (April 2007), Bergen (November 2007) and Galway (May 2008). It is planned that these current research projects will act as a precursor for additional and more integrated research proposals to the European Union under the Framework 7 and INTERREG programmes.

Table 1.1. Overview of the Tri-Nation Pancreas Disease Committee (2005 – 2008).

Groups	Ireland/N. Ireland	Scotland	Norway
Steering	N. Ruane (MI) D. Graham (AFBI) A. Norris (Industry)	R. Richards (UoS) D. Cox (Industry) C. Mitchell (Industry)	G. Ritchie (Industry) A. B. Olsen (NVI) A. Nylund (UoB) K. Maroni (FHL)
Epidemiology	H. Rodger (Industry)	A. Murray (FRS)	E. Brun (NVI) M. Sandberg (NSVS)
Aetiology	D. Graham M. McLoughlin (Industry)	H. Ferguson (UoS)	T. Taksdal (NVI) O. Brek (NVI) A. Nylund
Diagnostics	M. McLoughlin D. Graham	D. Cox D. Smail (FRS)	T. Taksdal

Marine Institute (MI), Agri-Food & Biosciences Institute (AFBI), University of Stirling (UoS), Fisheries Research Services (FRS), National Veterinary Institute (NVI), University of Bergen (UoB), Norwegian Seafood Federation (FHL), Norwegian School of Veterinary Sciences (NSVS).

1.2 Pancreas disease in Ireland

Pancreas disease emerged as a significant disease of farmed salmon in Ireland in the mid-1980's with 73% of marine sites affected by the disease in 1987 (Branson pers. comm., cited in McLoughlin *et al*., 1998). The disease is now considered endemic in Ireland, occurring on the majority of marine salmon sites each year and is the most significant infectious disease of Irish farmed salmon at present (Ruane *et al*., 2005; McLoughlin & Graham, 2007). In Ireland, the disease usually affects salmon in their first year at sea and for a detailed description of the clinical and histopathological signs of the disease the reader is referred to McLoughlin & Graham (2007).

The first major report on the disease in Ireland was produced by Wheatley *et al*. (1995) using data collected from 11 salmon farms over the period 1988 – 1992. This study showed that pancreas disease was the most significant factor determining mortality in Irish farmed salmon during their first year at sea within that period. In fact pancreas disease accounted for 51% of the total mortality recorded (Wheatley *et al*., 1995). A collaborative project between the Irish Salmon Growers Association and the Veterinary Sciences Division, Queen's University of Belfast developed a computerised management and disease information system for the intensive production of Atlantic salmon in Ireland (Menzies *et al*., 1996). Analysis of data from 1990 showed that 76.5% of marine sites were affected by pancreas disease that year with mortalities ranging between 0 – 45%. The database was then used to study the epizootiology of pancreas disease in Ireland between 1989 and 1994 (Crockford *et al*., 1999). Throughout the study, pancreas disease was recorded in over 70% of the marine sites. The mean annual mortality rates attributed to pancreas disease fell from *ca*. 30% in 1989 to *ca*. 10% in 1994 and was attributed to changes in management practices introduced in 1993, primarily single generation rearing and fallowing (Crockford *et al*., 1999).

The isolation of the virus causing pancreas disease (Nelson *et al*., 1995) resulted in the development of sensitive serological diagnostic tests. A serological survey of 17 marine sites in Ireland in 1995 showed that nine (52.9%) were antibody positive with only five of those (29.4%) reporting clinical signs of disease (McLoughlin *et al*., 1998). These studies (Crockford *et al*., 1999; McLoughlin *et al*., 1998) indicated that the incidence and severity of pancreas disease was reduced in the mid to late 1990's with estimated pancreas disease associated mortality on Irish farms being less than 10% in 2000 and 2001 (McLoughlin *et al*., 2003). In 2002 however, the

disease apparently re-emerged and 59% of Irish marine sites were affected by pancreas disease, with mortalities averaging 12% on affected sites (Table 1.2) (range 1 – 41%; McLoughlin *et al*., 2003).

Table 1.2. The prevalence and pancreas disease related mortality on Irish marine salmon sites, 1990 - 2007.

Year	sites affected (%)	PD related mortality (%)	Mortality range (%)	Average infection duration (days)
1990	76.5	20	0 – 45	127
1993	87	4	0 – 13	94.5
1994	70	13.5	1 – 63	112
2002	59	12	1 – 41	141
2003	62	19	2 – 27	
2004	86	15	4 – 35	
2005	80	10	1 – 31	147
2006	69	10	2 – 29	91
2007	91	23	5 – 45	

The Marine Institute commissioned further studies on the epidemiology of pancreas disease in Ireland in 2003 and 2004 which showed that the severity of the disease had in fact increased. In 2003, 62% of sites were affected with an average pancreas disease associated mortality of 18.8% (range 2 – 35%) which increased in 2004 to 86% of sites affected and 14.8% average mortality (range 4 – 35%; Rodger & Mitchell, 2007). The number of sites rearing salmon in Ireland declined in 2004, due to both economic and disease reasons and this may have had a confounding effect on the percentage of sites affected by pancreas disease (Rodger & Mitchell, 2007). Over the next two years, the number of sites affected by pancreas disease remained high in Ireland (80% in 2005; 69% in 2006) but the average mortality associated with the disease was reduced to just below 10% in both years (Rodger, 2007). The prevalence of the disease increased again in 2007 to > 90% of marine sites with an associated mortality of 23% (Rodger, 2008).

1.3 Pancreas disease in Scotland

In Scotland the number of pancreas disease cases diagnosed in recent years has apparently increased, along with the geographical spread of affected sites and the associated losses. In terms of the actual losses (number of fish) due to suspected pancreas disease, the numbers are relatively low i.e. *ca*. 3%. When losses are calculated in terms of biomass lost, pancreas disease can account for 11% of losses, the most significant among all infectious diseases (Murray & Kilburn, 2008). Although cases of suspect pancreas disease have been recorded throughout the year, a peak in the number of cases occurs during August and September (Murray & Kilburn, 2008). However, it is believed that outbreaks of pancreas disease are under-reported in Scotland and research initiatives into the epidemiology of pancreas disease and other diseases of similar pathology, such as cardiomyopathy syndrome, are currently underway.

1.4 Pancreas disease in Norway

Pancreas disease was first reported in Norway in the late 1980's (Poppe *et al*., 1989) and in the mid-1990's, serious outbreaks of pancreas disease were reported in Atlantic salmon and rainbow trout marine farms in western Norway (Olsen & Wangel, 1997; Christie *et al*., 1998). Prior to 2002, the majority of pancreas disease cases were limited to the Hordaland and Sogn og Fjordane counties in western Norway. However, in 2003 and 2004 there were reports of the disease occurring in the northern counties of Nordland, Troms and Finnmark (Brun *et al*., 2005; Karlsen *et al*., 2006) while the number of cases within the western and south western counties continued to spread and increase.

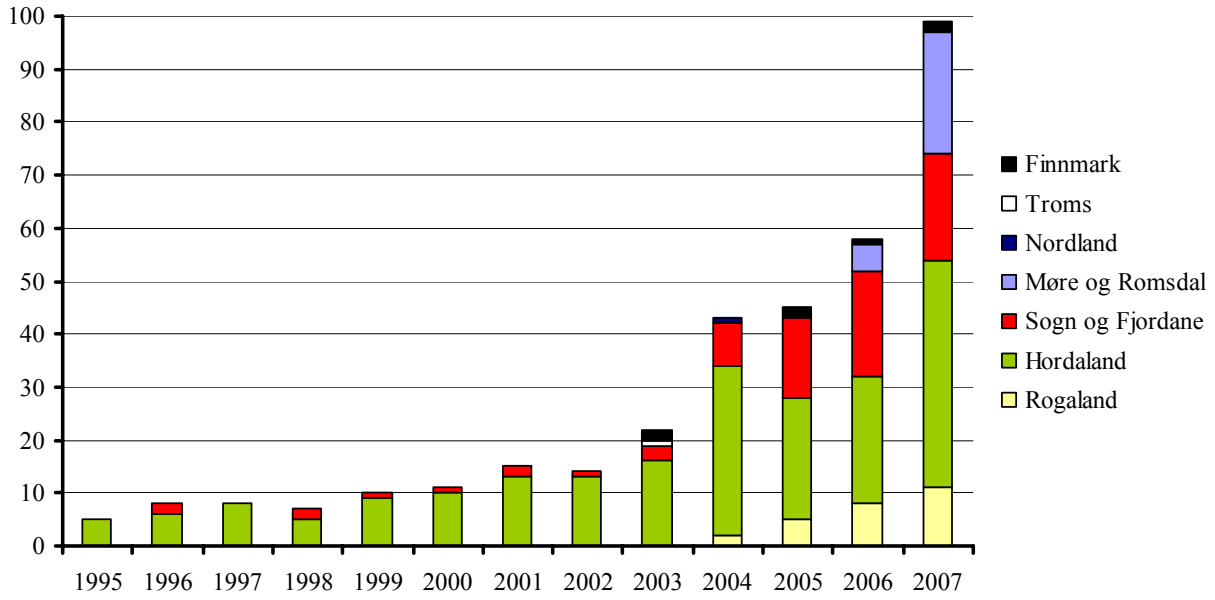

Figure 1.2. The number of pancreas disease cases, by county, in Norway from 1995 - 2007. Sources: National Veterinary Institute, Norway and the Norwegian Food Safety Authority.

Pancreas disease remains a problem for both sea reared Atlantic salmon and rainbow trout. The average time from sea transfer to outbreak of disease is approximately eight months, although outbreaks may be registered throughout the entire marine production phase. In Norway, pancreas disease can be diagnosed throughout the year, with higher numbers of cases reported between May and October (Olsen *et al*., 2007).

Table 1.3. The number of Norwegian fish farms with diagnosed infectious salmon anaemia (ISA), infectious pancreatic necrosis (IPN), pancreas disease (PD) and heart & skeletal muscle inflammation (HSMI), 1998 – 2007.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
ISA	13	14	23	21	12	8	16	11	4	7
IPN					174	178	172	208	207	165
PD	7	10	11	15	14	22	43	45	58	98
HSMI							54	83	94	162

Data sourced from the National Veterinary Institute, Norway (Skjelstad *et al*., 2008).

The genome of the virus causing pancreas disease in Norway has been sequenced and found to differ slightly from that causing pancreas disease in Ireland and Scotland and also from that causing sleeping disease in freshwater rainbow trout. It has therefore been termed Norwegian salmonid alphavirus (NSAV; Hodneland *et al*., 2005). Using an experimental challenge test, Christie *et al*. (2007) did not identify significant differences in the pathological, serological or virological responses of salmon infected with an Irish isolate and a Norwegian isolate of the virus. Some differences were found in a study of clinical signs and pathological findings of naturally occurring pancreas disease in Norway compared to findings reported for Ireland and Scotland (Taksdal *et al*., 2007). Compared with Irish outbreaks, the heart appeared to recover earlier and the loss of pancreatic tissues was more persistent in the reported cases.

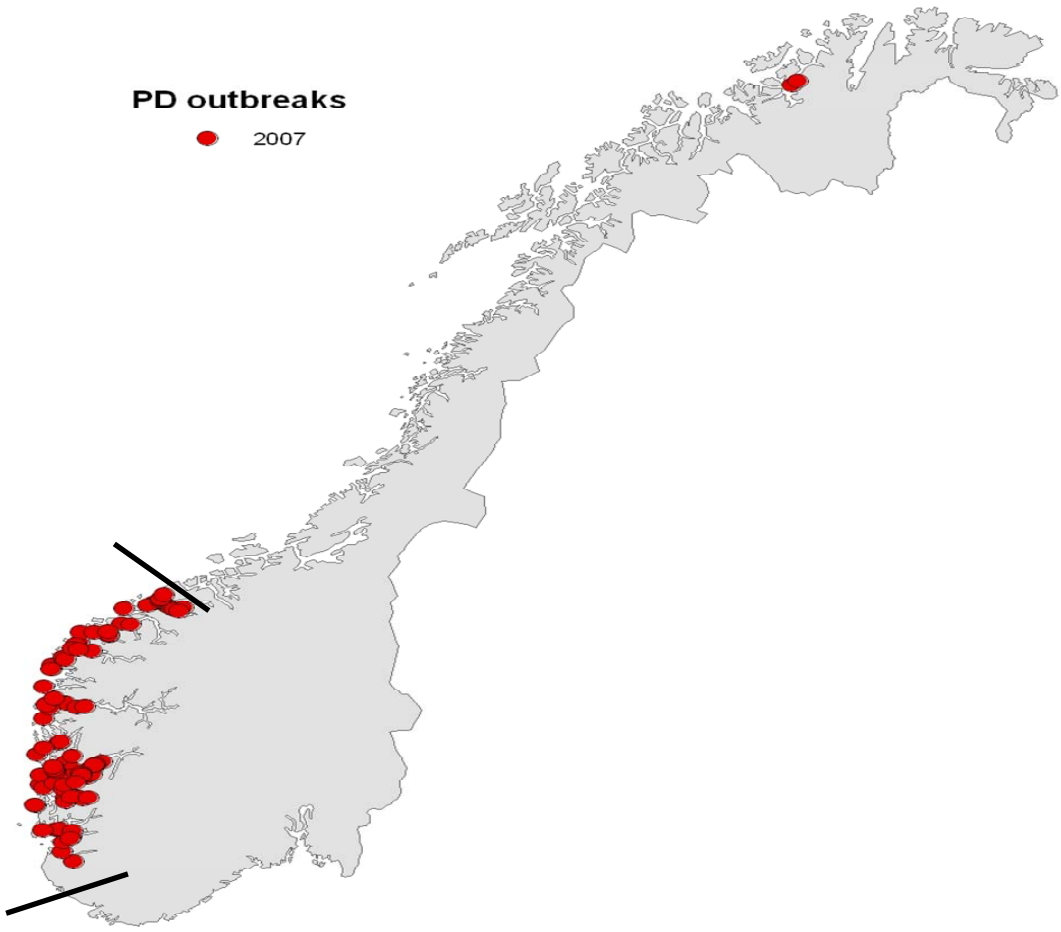

Figure 1.3. A map of Norway indicating the locations of the 2007 PD outbreaks and the proposed PD-zone in the south-west of the country (Olsen, 2008).

Since December 2007, the Norwegian authorities have made pancreas disease a List B disease, equivalent to List II diseases under Directive 91/67/EEC. This has led to the setting up of a geographical zone in the South-West of Norway within which the PD positive farms are located (Figure 1.3). The aim is to prevent and limit the spread of the virus, to combat the disease outside the zone and to prevent and control disease within the zone (Olsen, 2008).

2. THE *SALMONID ALPHAVIRUS*

Neil Ruane, David Graham, Hamish Rodger

2.1 The nature of the virus

Alphaviruses belong to the family *Togaviridae* and currently consists of 25 species which are closely related in structure and molecular characteristics. Members of the genus *Alphavirus* are typically maintained in natural cycles involving transmission by an arthropod vector among susceptible hosts. These vectors are primarily mosquitoes, but also include mites, ticks and lice (Strauss & Strauss, 1994; Waarts, 2004). Alphaviruses replicate in both arthropod vectors and vertebrate hosts, producing a persistent lifelong infection in arthropods, while leading to an acute, short infection in vertebrates (Strauss & Strauss, 1994). The diversity seen within the alphavirus species and subtypes is believed to be influenced by host mobility (Powers *et al*., 2001). Viruses that utilize reservoir hosts with limited mobility, such as small mammals, tend to be quite diverse and have non-overlapping distributions. The Venezuelan Equine Encephalitis virus complex, which primarily uses rodent hosts and a mosquito vector species with a limited flight range, occurs throughout the tropics, but the distributions of the various subtypes are discrete. The Ross River virus from Australia exhibits a similar epidemiological pattern. Viruses that use birds as their reservoir hosts, such as the Sindbis virus in Australia and the Western Equine Encephalitis virus in North America are less diverse, with each subtype occupying a greater geographical range (Sammels *et al*., 1999). The recently discovered *Salmonid alphavirus* (SAV), which has salmonid fish species as a host (Weston *et al*., 1999; Villoing *et al*., 2000a, is not known to have an arthropod host, although it is possible that the sea louse, *Lepeophtheirus salmonis* may play a role in the transmission of SAV (Petterson *et al*., 2007).

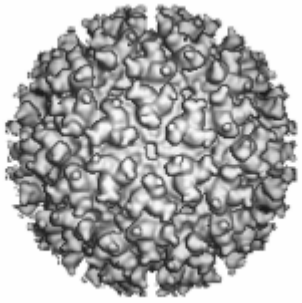

Figure 2.1. A reconstruction of a typical alphavirus (Sindbis virus; Waarts, 2004).

Alphaviruses are small (70 nm in diameter), spherical, enveloped viruses (Figure 2.1) with a genome consisting of a single strand of positive-sense RNA. The non-structural protein genes are encoded in the 5' two-thirds of the genome (nsP1 – nsP4), while the structural proteins are translated from a subgenomic mRNA from the 3' third of the genome (Figure 2.2).

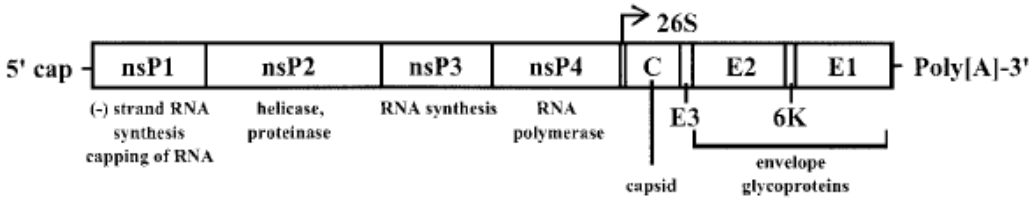

Figure 2.2. Organization of the *Alphavirus* genome with gene products and associated functions indicated (Powers *et al*., 2001).

Replication occurs within the cytoplasm of the infected cell and virions mature by budding through the plasma membrane where the virus-encoded surface glycoproteins E2 and E1 are assimilated. These proteins form the basis of many diagnostic tests e.g. neutralisation assays, with the E2 protein being the site of most neutralising epitopes and the E1 protein containing more conserved, cross-reactive epitopes (Powers *et al*., 2001).

2.2 Discovery of the *Salmonid alphavirus*

Nelson *et al*. (1995) reported the isolation of a virus from samples of pancreas disease-affected Atlantic salmon collected in the north-west of Ireland. The isolated virus showed physicochemical characteristics and morphology resembling members of the *Togaviridae*. Experimental infection of salmon post-smolts resulted in pathological changes consistent with pancreas disease and the authors proposed the name salmon pancreas disease virus (SPDV). In a follow on study, this virus was shown to be the causal agent of the disease (McLoughlin *et al*., 1996), a conclusion further supported by the isolation of the virus from salmon in Norway (Christie *et al*., 1998) and Scotland (Rowley *et al*., 1998) affected by pancreas disease. Weston *et al*. (1999) produced cDNA clones specific to SPDV RNA and after sequence analysis identified the virus as the first reported alphavirus in fish. An in-depth study of the biochemical characteristics of the virus showed that in terms of its protein and RNA composition, SPDV shared many of the characteristics of animal alphaviruses (Welsh *et al*., 2000). At the same time, Villoing *et al*. (2000a) characterised the sleeping disease virus (SDV, see Section 2.3) as an alphavirus and studies showed that both diseases were associated with similar histological lesions of the pancreas, heart and muscle in salmon and rainbow trout (Boucher & Baudin-Laurencin, 1996). Weston *et al*. (2002) showed that SPDV and SDV exhibited 91.1% nucleotide sequence identity over their complete genomes, with 95 and 93.6% amino acid identities over their non-structural and structural proteins, respectively. Virus neutralization tests performed with sera from experimentally infected salmon indicated that SPDV and SDV belonged to the same serotype. Therefore, it was proposed that SPDV and SDV were closely related isolates of the same virus species and the name *Salmonid alphavirus* was proposed (Weston *et al*., 2002). Genetic analysis of the *Salmonid alphavirus* from Norway revealed that it formed a distinct subtype, separate from both previously reported subtypes, named Norwegian salmonid alphavirus (Hodneland *et al*., 2005). Therefore classification of the virus was arranged into three subtypes (Hodneland *et al*., 2005; Weston *et al*., 2005; Karlsen *et al*., 2006):

SAV 1: causing pancreas disease in salmon in Ireland and Scotland
SAV 2: causing sleeping disease in freshwater rainbow trout
SAV 3: causing pancreas disease in salmon and marine rainbow trout in Norway

Work carried out within this project now suggests that there may be up to six different subtypes of the virus (Fringuelli *et al*., 2008). See 'Chapter 6 Virus Sequencing Studies' for more detail.

2.3 Pancreas disease diagnostics

Diagnosing pancreas disease on marine sites is based on clinical signs, gross pathology and laboratory diagnostic tools and techniques including histopathology, virus isolation, serological tests and molecular based techniques (McLoughlin & Graham, 2007).

Clinical signs associated with pancreas disease include a loss of appetite, lethargy, an increased number of faecal casts in the cages and increased mortality (McLoughlin *et al*., 2002). Affected fish are often unable to maintain their position in the water column due to muscle damage and are sensitive to any handling procedures. Prior to the isolation of the virus, histopathology was the main diagnostic tool for pancreas disease and still plays a major role in the study and diagnosis of the disease today (Murphy *et al*., 1992; McLoughlin *et al*., 2002; McLoughlin & Graham, 2007). The first histopathological signs are observed in the pancreas with acute necrosis of the pancreatic acinar cells soon after infection, closely followed by lesions in the heart. Skeletal muscle lesions tend to first appear 3 – 4 weeks after the appearance of pancreatic and heart lesions. On occasion, lesions in the kidney and brain have been observed (McLoughlin & Graham, 2007).

Figure 2.3. An Atlantic salmon with clinical pancreas disease. Note the lack of fatty tissue around the pyloric caecae and the presence of faecal casts in the intestine.

The first isolations of the virus used Chinook salmon embryo (CHSE-214) cells (Nelson *et al*., 1995) although the development of a cytopathic effect (CPE) was slow and more difficult to observe. Graham *et al*. (2007a) compared the efficacy of a number of cell lines in isolating the *Salmonid alphavirus*. They concluded that TO and BF-2 cell lines produced the highest titres and the optimum temperature for virus growth was 15^{o}C. The production of virus specific antibodies (Todd *et al*., 2001) led to the development of more specific and sensitive immunostaining techniques which has helped in the isolation of a greater number of virus isolates (Graham *et al*., 2003a; Jewhurst *et al*., 2004). However there is a need for the development of more SAV-specific antibodies to aid in the development of diagnostic tools such as ELISA tests and immunohistochemistry.

A number of sensitive molecular techniques for detecting SAV in fish tissues and serum have been developed. Conventional RT-PCR methods have been described and have proven very useful in providing samples for sequencing of the virus (Villoing *et al*. 2000b; Weston *et al*., 2005; Karlsen *et al*., 2006). More recently, real-time RT-PCR protocols have been described (Graham *et al*., 2006; Hodneland & Endresen, 2006; Christie *et al*., 2007). Real-time methods offer a rapid result without the need for post-amplification handling of amplicons, thus reducing risk of cross-contamination. In addition, these protocols can be further developed into quantitative methods which could measure viral load in samples.

2.4 Diseases of similar pathology

2.4.1 Sleeping Disease

Sleeping disease of farmed freshwater rainbow trout, *Oncorhynchus mykiss*, has been observed in France for many years, causing mortalities of up to 22% (Boucher & Baudin-Laurencin, 1994). The characteristic signs of the disease include the unusual behaviour of the fish 'sleeping' on their sides at the bottom of the tank. Histological observations of diseased fish show a chronological appearance of lesions in the pancreas, heart and finally in the skeletal muscle,

similar to the histopathological development of pancreas disease in salmon (Boucher & Baudin-Laurencin, 1996). A viral aetiology of sleeping disease had been suspected as virus-like particles were observed in purified homogenates from kidneys of diseased fish (Boucher *et al*., 1994). The virus was first isolated by Castric *et al*. (1997) and fully characterised as an alphavirus by Villiong *et al*. (2000a). Historically, the disease has been endemic in parts of France with up to 30% of trout farms in Brittany infected (Villoing *et al*., 2000a). A number of reports in recent years have shown that the disease has now spread to neighbouring countries. Sleeping disease was first diagnosed in rainbow trout in England and Scotland in 2002 (Branson, 2002) and confirmed by isolation of the *Salmonid alphavirus* (Graham *et al*., 2003b). Bergmann *et al*. (2005) reported the first isolation of the virus in Germany. The disease was also suspected to occur in Spain and Italy but the virus was never isolated due to a lack of diagnostic tools and the presence of infectious pancreatic necrosis virus. However, Graham *et al*. (2007b) reported the detection by virus isolation and/or RT-PCR of *Salmonid alphavirus* from rainbow trout exhibiting clinical signs of sleeping disease from both Spain and Italy, confirming earlier suspicions.

2.4.2 Heart and skeletal muscle inflammation

Heart and skeletal muscle inflammation (HSMI) is a disease of suspected viral aetiology first observed in Norwegian farmed salmon in 1999 (Kongtorp *et al*., 2004a; 2004b). The disease has now spread to all coastal counties between Vest-Agder and Finnmark, with 94 farms diagnosed with the disease in 2006 (Olsen *et al*., 2007). In 2007, the number of HSMI cases increased to 162 (Skjelstad *et al*., 2008). A disease resembling HSMI has also been reported in farmed salmon in Scotland (Ferguson *et al*., 2005). Diagnosis of HSMI is presently based on histological examination, characterised by significant lesions of the heart and red skeletal muscle in the absence of histopathological changes in the pancreas which is considered by some groups to differentiate it from pancreas disease (Kongtorp *et al*., 2004a; McLoughlin & Graham, 2007). Kongtorp *et al*. (2004b) demonstrated that HSMI was infectious through experimental transmission of the disease by intra-peritoneal injection of homogenised tissue and by cohabitation. HSMI has been diagnosed in salmon five months after transfer to sea (Kongtorp *et al*., 2006) but can also occur in fish three weeks post-transfer or in grower fish of 5 – 6 kg (Olsen *et al*., 2007). Thus, HSMI is a condition which can affect salmon at all stages of the marine production cycle. Mortalities due to HSMI may be variable but the high levels of morbidity, which can reach almost 100%, and prolonged nature of the disease are significant problems for the industry (Kongtorp *et al*., 2006). Although a virus has been isolated from fish with clinical HSMI and used in experimental challenge experiments to study the disease (Eliassen *et al*., 2004), no details have yet been published. Due to the increasing importance of the disease in Norway, the development of rapid and reliable diagnostic tools and an effective vaccine are major priorities for the industry.

2.4.3 Cardiomyopathy syndrome

Cardiomyopathy syndrome was first reported in farmed salmon in Norway in the mid-1980's (Ferguson *et al*., 1990) and subsequently in Scotland (Rodger & Turnbull, 2000). The condition typically affects large fast-growing salmon in their second year at sea. Compared to other diseases, such as pancreas disease, the fish frequently die with little reported evidence of prior clinical disease (Ferguson *et al*., 1990; Brun *et al*., 2003). The cause of the disease is unknown, although the involvement of an infectious agent is suspected. CMS has a serious economic impact on infected farms because it affects fish late in the production cycle when the expenditures incurred are high (Brun *et al*., 2003). Although early harvesting or reduced feeding are practised in an attempt to combat the disease, the lack of information relating to CMS leaves the producers with little or no real management strategies to prevent the problem.

3. OBJECTIVES OF THE RESEARCH PROJECT: ST/05/01

In 2002, the Marine Institute commissioned a study on the re-emergence of pancreas disease in the Irish farmed salmon industry (McLoughlin *et al*., 2003). This study had recommended that '*there should be a concerted national effort to continue to monitor for pancreas disease, its level of mortality and recording of the environmental and other livestock parameters associated with all farms*'. Following on from this, in 2005, a call for a strategic research proposal, under the NDP Marine RTDI Programme was announced to further investigate the continuing threat posed by pancreas disease to the Irish salmon farming industry. This publication constitutes the final report for that research project entitled "Site investigations and disease management of the pancreas disease virus in Irish farmed salmon – ST/05/01".

The project was divided into the following work packages, the results of which are outlined in the following sections.

- WP 1 Project Management – project coordination and integration.
- WP 2 Longitudinal studies – sites investigations of farmed populations of salmon and rainbow trout.
- WP 3 Vectors & Reservoirs – studies on the roles of mussels, sea lice and the freshwater phase of salmon rearing, in transmitting SAV.
- WP 4 Virus sequencing studies – molecular sequencing of SAV isolates from Ireland.
- WP 5 Epidemiology – a study determining the risk factors associated with pancreas disease outbreaks on marine sites.
- WP 6 Management & Mitigation – information dissemination and the provision of advice based on the research findings.

The goals of the project were:

- *To develop our understanding of how pancreas disease spreads within a site, in addition to the impact of infection* – Chapter 4 details longitudinal studies performed on marine sites in Ireland. Fish were sampled before, during and after outbreaks of pancreas disease and the infection studied using a range of diagnostic methods. Chapter 5 outlines testing performed on potential vectors and reservoirs of the virus (mussels from sea cages, sea lice, wild fish). Sequencing of viral isolates from Ireland and Scotland was also performed (Chapter 6) to gain an insight into the diversity of the virus and to provide information on the means of spread of SAV between farms.
- *To better understand the epidemiological factors which contribute to the introduction of SAV to a site* – a detailed epidemiological study of the 2005 and 2006 year class of salmon is outlined in Chapter 7.
- *To build national capacity in the area of screening and to establish early warning systems for pancreas disease* – during the project, technology transfer of diagnostic methods took place between Queens University Belfast and the Marine Institute. The Fish Health Unit at the Marine Institute now has expertise in serological and molecular based methods for the diagnosis of pancreas disease in fish serum and tissues.
- *To develop management tools and provide advice on mitigating factors for industry practitioners and veterinarians* – the results of the study have been collated and management practices for mitigating pancreas disease are outlined in Chapter 8.
- *To communicate the research findings to the Irish and European aquaculture industries* – throughout the project, results were presented at project meetings, PD Tri-Nation Group meetings and at international conferences such as the European Association of Fish Pathologists conferences. A full list of presentations and publications can be found in Appendix I.

4. LONGITUDINAL STUDIES (WP 2)

David Graham, Elena Fringuelli, Claire Wilson, Helen Rowley, Amanda Brown, Laura Murchie, Louise Totten, Eugene Casey, Karen McCrann, William Ward, Leo Foyle, Lorraine McCarthy, Neil Ruane

A series of longitudinal studies were planned within the project, with the intention of targeting S0 and S1 populations of salmon and also rainbow trout reared in seawater. The principle goals of this work package were to compare and contrast disease outbreaks in the different populations in terms of rates of spread, levels and duration of infection and the performance of different diagnostic tests, including virology, serology and real time RT-PCR, with a view to better understanding their relative strengths and weaknesses. All of the study sites were chosen on the basis of their previous histories of pancreas disease infection. In each study, sampling protocols and frequencies were agreed in advance with the farms. Details of the sampling protocol are shown in Appendix II.

4.1 Rainbow trout.

A longitudinal study was initiated on Eany Fish Farm, Donegal on 27th January 2006. This farm had previously seen significant problems with pancreas disease in salmon, but not in trout, farmed side by side. An extensive range of samples including tissues and blood from 20 fish, mussels and water were collected. Sampling was conducted at fortnightly intervals, except as dictated by weather or management requirements. The site also held S1 salmon. Therefore the sampling program was extended to include these also, although typically at 4-weekly intervals. Sampling continued until 14th June 2006, when the study was terminated as most of the fish had been harvested.

Sera were routinely tested on receipt in the laboratory for the presence of virus neutralizing antibodies or the SAV itself. Positive results on these tests would be used as evidence of infection and justify the time and expense of testing all of the samples collected using a wider range of techniques. However all of the samples tested negative, indicating that neither the salmon nor the rainbow trout populations had been challenged during the trial period.

A second longitudinal study was initiated on a population of rainbow trout that went to sea on 28th June 2006. The first samples were collected on 25th July 2006, with sampling scheduled for three week intervals. No salmon were on the site from this time forward, so only rainbow trout were sampled. Sampling and serological/virological testing continued until harvest the following summer, with the last samples collected on 20th April 2007.

Once again, no evidence of infection was found in this study. The reason for this is unknown. However, when the studies commenced, this was the only stocked fish farm in Donegal Bay. It is possible that the absence of stock from other farms in the Bay broke a cycle of infection whereby naïve fish going to sea were exposed to challenge from sick or recovered fish on other sites, with continual cycling of infection within the Bay. Evidence in support of this horizontal spread is presented below in this work package, and also in Chapter 6.

4.2 Atlantic salmon S1's.

Atlantic salmon of Fanad origin were transferred to the Hawk's Nest site of the Mannin Bay Salmon Company, Clifden, Co. Galway in April 2006. The average weight of these fish was 67g. One cage (H7), containing 30,000 fish, was selected as a sentinel cage for the duration of the study. Six other cages, holding an initial total of 214,500 S1's were also present on site. The Hawk's Nest site has a previous history of pancreas disease and it was anticipated that the sentinel fish would be exposed to the salmonid alphavirus from the production fish comrades or environment, in the same and surrounding cages, in natural conditions, until the end of the trial. This site experienced a pancreas disease infection from August to November 2005 during a similar trial involving Marine Harvest family fish (Ruane *et al*., 2005).

4.2.1. Sampling

At each sample date twenty fish were removed from the sentinel cage and placed in a bin of water containing MS-222 for transport to the farm laboratory. Prior to dissection, fish were weighed and measured. Fish were sampled for histology, virology, serology (Graham *et al.*, 2003a) and molecular diagnosis by real-time RT-PCR (Hodneland & Endresen, 2006). When possible, wild fish, sea lice and mussels from the cage were also taken for SAV detection by real-time RT-PCR. Additional information on the water temperature, cage mortalities and cause of death were also recorded throughout the study. A description of the sampling protocol is shown in Appendix II.

4.2.2. Results

Fish weight, water temperature & mortality levels

The growth of the fish as measured by the weight of the fish sampled is shown in Figure 4.1.

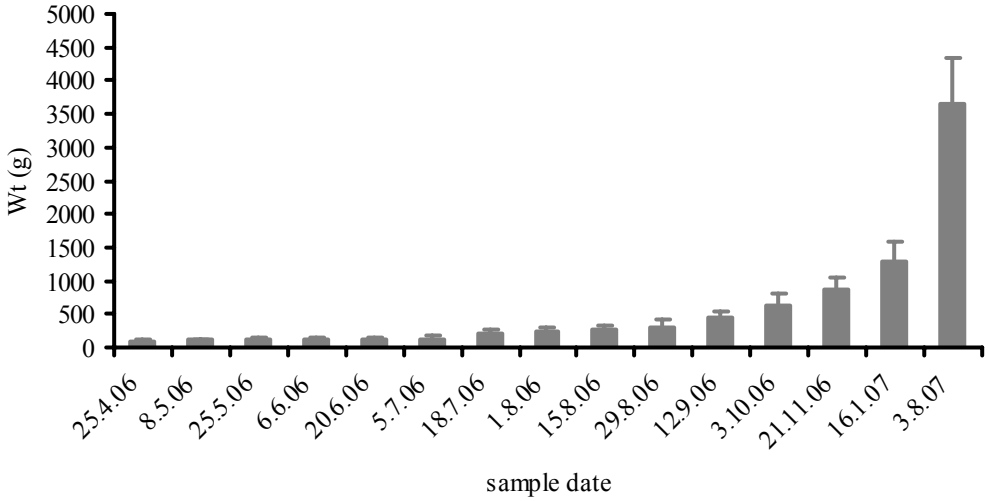

Figure 4.1. Growth of the sentinel Atlantic salmon used in the Mannin Bay study (April 2006 – August 2007). Fish were moved to the grower site in April 07.

The weekly mortalities and water temperature levels for the entire Hawk's Nest site are shown in Figure 4.2. The initial peak of mortality seen from week 9 – 16 (June and July) is primarily due to pancreas disease. A second minor peak was observed from week 19 – 25 (August) which was attributed to changes in the water quality and an increase in phytoplankton in the Bay. After week 51 the fish were moved to a grower site until harvest.

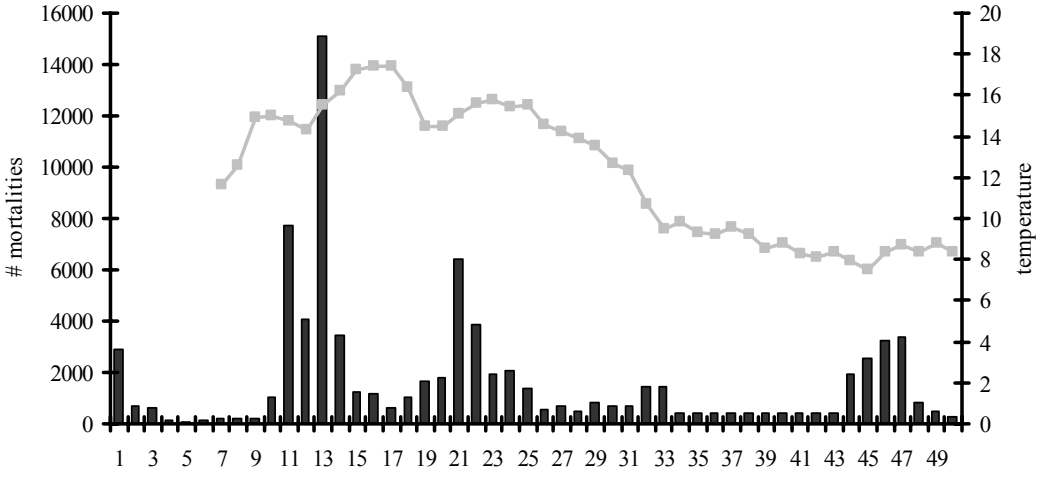

Figure 4.2. Weekly mortalities and water temperature at Hawk's Nest (April 2006 – April 2007). ■ mortalities; ■ temperature (^{o}C).

The causes of death of the mortalities were classified during the period of April to October 2007. Mortalities were classified according to the cause of death in the following manner: I Bird damage; II Seal Damage; III Runt; IV Chronic PD; V Acute PD; VI Decomposed; VII Other. Pancreas disease was first diagnosed on site on the 25th of May, seven weeks after transfer to sea (Figure 4.7). The greatest cause of death was from predator damage (bird and seal) accounting for 44% of the observed mortality. Pancreas disease accounted for 34% in total (Figure 4.3). It is possible that a percentage of the mortalities due to predators were in fact fish suffering from pancreas disease, therefore data should be read as estimates.

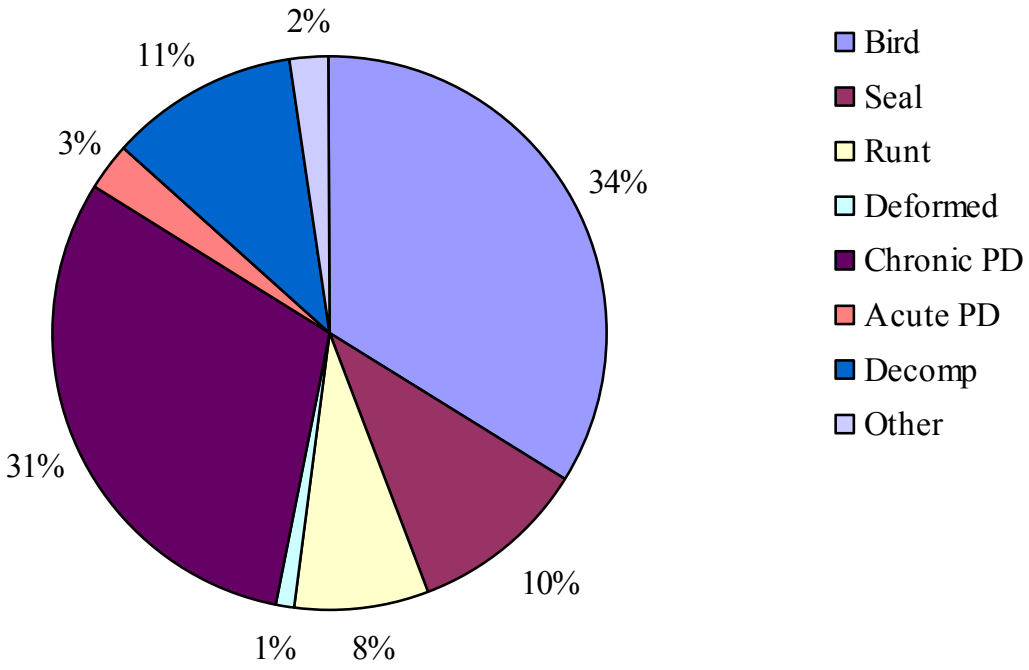

Figure 4.3. A breakdown of the cause of death of fish in the sentinel cage at the Hawk's Nest site from April 2007 – October 2007.

Histology

The first histopathological signs of pancreas disease were found on May 25th with acute necrosis of the exocrine pancreas and grade I necrosis of cardiomyocytes being observed. No signs of infection were seen in the muscle tissue at this stage. By June 6th, pancreatic acinar tissue was partially or totally absent from a number of fish. Necrosis was now observed in the heart and the red and white muscle tissues. By June 13th, severe histopathological changes were observed in almost all fish. In addition to this, hepatic pyknosis or inflammation consistent with a viral infection was also observed. A large number of sloughed enterocytes were observed in the caecae.

Histopathological changes associated with pancreas disease were observed until July 18th. At this time, a low level of necrotic acinar cells was observed in a small number of fish. Large numbers of inflammatory cells were observed in the myocardium and pancreatic fat suggesting that an immune response had been mounted against the infection. After this time, no significant changes associated with pancreas disease were observed for the remainder of the study, apart from chronic inflammation of the pancreatic fat in a number of fish on August 29th. The histological findings are summarised below in Figure 4.4.

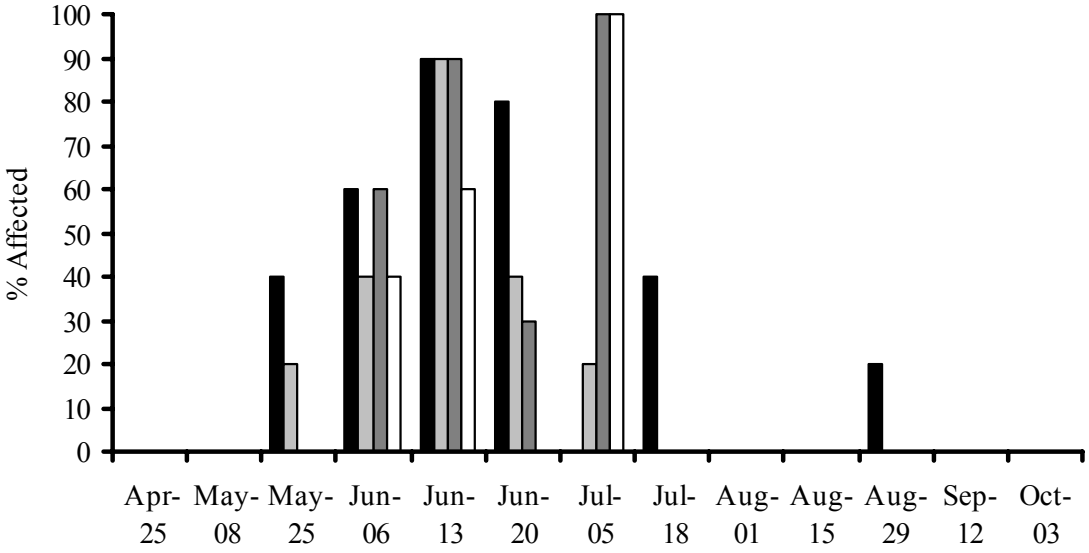

Figure 4.4. Histopathological changes in the sentinel fish during a natural outbreak of pancreas disease at Hawk's Nest. ■ pancreatic necrosis; ■ cardiomyopathy; ■ red muscle necrosis; □ white muscle necrosis.

Testing of sera for virus-neutralizing antibodies and virus.

The first virus-positive sera were detected on May 25th (coinciding with the first histopathological observations) with virus positive sera also detected on June 6th, but not thereafter. When using real-time RT-PCR to detect virus in the serum, positive samples were detected as early as May 8th with one positive fish also being detected on July 5th. Antibody positive fish were found on June 6th with the number of antibody positive fish increasing to >90% by June 20th. Antibodies were consistently found in the serum of 60 – 70% of the fish tested throughout the study even up to August of the following year (prior to harvesting), almost 15 months after the infection was first detected.

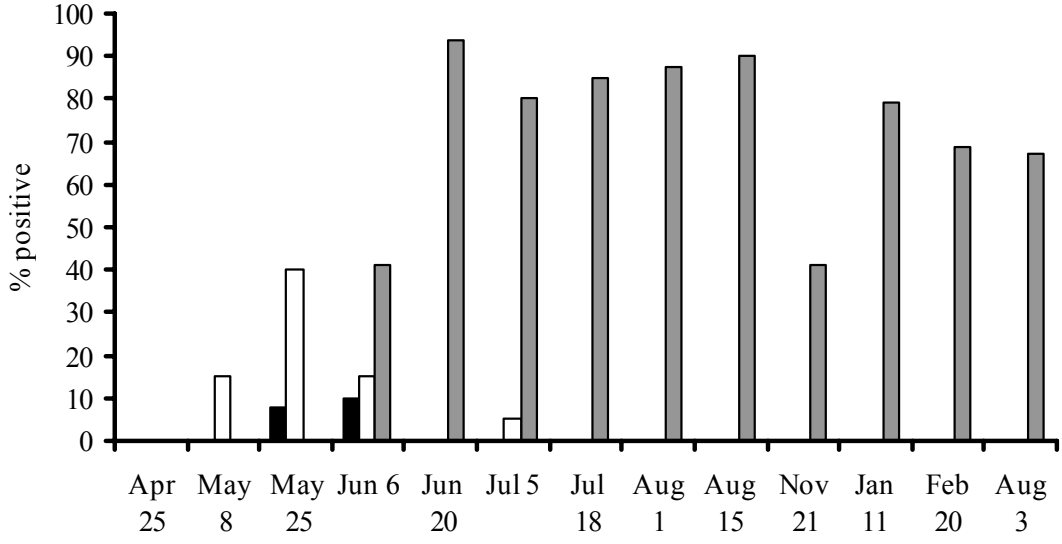

Figure 4.5. The percentage of fish positive for SAV using virus isolation on serum (■), real-time RT-PCR (□) and the percentage of antibody-positive sentinel fish(■) at Hawk's Nest.

Real-time RT-PCR

Heart, kidney, gill and brain tissue samples were removed from each fish during the longitudinal study and tested for the presence of SAV by a real-time RT-PCR procedure described by

Hodneland & Endresen (2006). All four tissues were first found to be positive for viral RNA on May 25th coinciding with the first histopathological signs and detection of the virus by isolation in cell culture. Although all four tissues were found to be positive throughout most of the sampling period, heart and gill tissues gave the highest level of virus positive samples in the study suggesting that these tissues are the most suitable for detection of fish previously exposed to the *Salmonid alphavirus*. Positive tissues were detected in a sample of fish taken in February 2007 but not at the next sampling in August 2007, prior to harvest, indicating that tissues can remain positive for virus for an extended period.

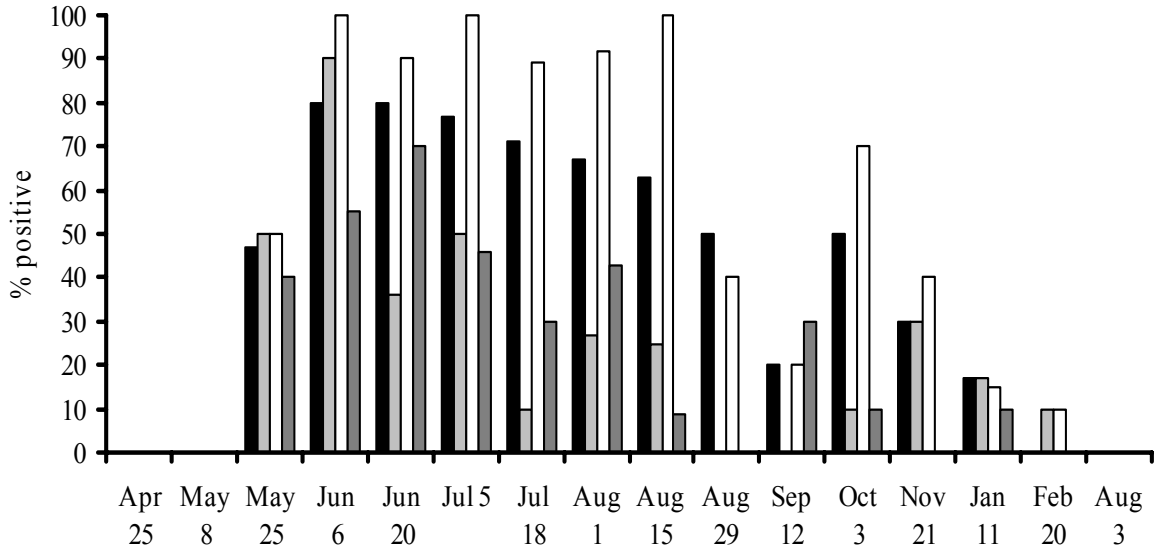

Figure 4.6. The percentage of sampled fish positive for SAV by real-time RT-PCR during the Hawk's Nest longitudinal study; heart (■), kidney (■), gill (□) and brain (■).

Virus isolation

A selected number of samples taken for virology were tested for virus by inoculating the supernatant from the tissue homogenates on *Epithelioma papulosum cyprini* (EPC) and bluegill fry fibroblast (BF-2) cell lines. Inoculated cultures were incubated at 15°C for 7 days before subcultivation onto fresh EPC and BF-2 cell monolayers for a further 7 days. RNA was extracted from the second-passage infected cell cultures using TRIzol® Reagent (Invitrogen) and used in a real-time RT-PCR assay for SAV as described by Hodneland & Endresen (2006).

After the second passage, aliquots of the cell culture media removed from the 24-well plates were inoculated onto fresh EPC and BF-2 cell monolayers on 96-well plates for a further 7 days. Detection of the *Salmonid alphavirus* was performed using an immunostaining method previously described by Graham *et al*. (2003a) using the monoclonal antibody 2D9 raised against SAV strain F93-125 (Todd *et al*., 2001).

The results are summarized in Table 4.1 below. The first positive samples detected by virus isolation were on May 25th and confirmed by using the real-time RT-PCR assay.

Table 4.1. Results of virus isolation, from tissue homogenates, of SAV on EPC and BF-2 cell lines. Data is shown as the number of positive wells out of three replicates after staining with a specific monoclonal antibody or as a positive or negative result by real-time RT-PCR.

Fish #	Sample Date	Sample Day	EPC Virus Isol.	EPC RRT-PCR	BF2 Virus Isol.	BF2 RRT-PCR
1	25-04-06	1	0/3	NEG	0/3	NEG
2	25-04-06	1	0/3	NEG	0/3	NEG
3	25-04-06	1	0/3	NEG	0/3	NEG
4	25-04-06	1	0/3	NEG	0/3	NEG
55	25-05-06	30	3/3	POS	2/2	POS
58	25-05-06	30	3/3	POS	3/3	POS
66	25-05-06	30	IPNV+	POS	IPNV+	POS
68	25-05-06	30	3/3	POS	3/3	POS
85	6-06-06	42	3/3	POS	3/3	POS
88	6-06-06	42	2/3	POS	3/3	POS
94	6-06-06	42	3/3	POS	0/3	POS
95	6-06-06	42	3/3	POS	1/3	POS
147	5-07-06	71	3/3	POS	0/3	POS
148	5-07-06	71	1/3	POS	0/3	POS
149	5-07-06	71	0/3	NEG	0/3	POS
150	5-07-06	71	0/3	NEG	0/3	POS
213	15-08-06	112	0/3	NEG	0/3	POS
214	15-08-06	112	0/3	NEG	0/3	NEG
218	15-08-06	112	0/3	NEG	0/3	NEG
223	15-08-06	112	0/3	NEG	0/3	NEG
261	12-09-06	140	0/3	NEG	0/3	NEG
265	12-09-06	140	0/3	NEG	0/3	NEG
269	12-09-06	140	0/3	NEG	0/3	NEG
270	12-09-06	140	0/3	NEG	0/3	NEG
286	3-10-06	161	0/3	NEG	0/3	NEG
295	3-10-06	161	0/3	NEG	0/3	NEG
302	3-10-06	161	0/3	NEG	0/3	NEG
303	3-10-06	161	0/3	NEG	0/3	NEG
314	21-11-06	210	0/3	NEG	0/3	NEG
315	21-11-06	210	0/3	NEG	0/3	NEG
316	21-11-06	210	0/3	NEG	0/3	NEG
317	21-11-06	210	0/3	NEG	0/3	NEG
337	16-01-07	266	0/3	NEG	0/3	NEG
344	16-01-07	266	0/3	NEG	0/3	NEG
346	16-01-07	266	0/3	NEG	0/3	NEG
348	16-01-07	266	0/3	POS	0/3	NEG

4.3 Discussion

An overview of the mortality figures in the sentinel cage and the key dates are shown in Figure 4.7. Pancreas disease was first confirmed on the site in a sample taken on May 25th simultaneously by histopathology, serology, virus isolation and by molecular methods, seven weeks after transfer of the sentinel fish to sea. The earliest indication of infection was the detection of virus-positive serum by real-time RT-PCR on May 8th just four weeks after transfer to sea. This contrasts to the disease outbreak of 2005, when pancreas disease was first suspected on the site on July 25th and confirmed on August 16th. In both cases, fish were transferred to sea in April (2005 and 2006).

In this study, the peak of mortalities due to pancreas disease occurred from mid-June until late July and is consistent with the histopathology results which show very little PD-associated changes after August 1st. Interestingly, viraemic sera were only found by isolation in cell culture on May 25th and June 6th suggesting a transient infection of the blood or low virus levels below the detection limit of the assay. Using a more sensitive molecular based assay, viraemic sera were detected over a slightly longer period, from May 8th up to July 5th (Figure 4.5). Nonetheless, virus was detectable in serum for a much shorter period than was found for a range of tissues by RT-PCR (Figures 4.5, 4.6). This indicates that viraemia is a transient event present only in the early stages of infection in an individual fish. Where infection moves through a cage rapidly, as was seen in this case, the same holds true at the population level. In such cases, the detection of viraemia may therefore be interpreted as current, active infection, whereas detection of viral RNA by RT-PCR in tissues could indicate either current disease or be indicative of a historical infection from exposure to virus that took place some months previously. The presence of clinical signs or findings from additional tests such as histopathology or testing of serum for virus can help distinguish between these two possibilities.

The long persistence of a detectable signal by RT-PCR in a range of tissues, particularly heart and gill, in this study is consistent with earlier findings from an experimental study (Christie *et al*., 2007) and suggests that these fish are carriers with a persistent or latent infection which poses a risk to naïve populations going to sea alongside recovered populations. This hypothesis is supported by findings from molecular strain typing on this site. The study population overlapped with a recovered population on this farm. Typing of the strains from both populations indicated that they were indistinguishable. This is consistent with the study population being infected from the recovered population and would explain the rapidity with which infection was acquired post transfer. Evidence from other sites also supports this local transmission of virus. If this hypothesis is correct, it has clear implications in terms of mitigation and control, with a requirement to avoid overlapping of naïve and recovered populations in time and space. Fallowing alone, which only addresses the persistence of infection in the environment and on equipment, would therefore be expected to be of limited use, particularly in bays with multiple sites.

Seroconversion was first detected on June 6th and the number of antibody positive fish increased throughout June, July and into August. Seropositive fish were still found in a sample taken in August of the following year just prior to harvesting of the fish. This would suggest that fish which have recovered from pancreas disease remain immune to reinfection for the remainder of the marine production cycle.

Between the period of April – October 2006, it was estimated that 34% of the mortalities in the sentinel cage were due to pancreas disease (compared with 75% in 2005; Ruane *et al*., 2005). However, 44% of the mortalities in this period were classified as being caused by predators (birds and seals) and it is likely that the majority of these fish were suffering from pancreas

disease. Thus the figures for cause of death are likely to underestimate the real number of pancreas disease related mortalities.

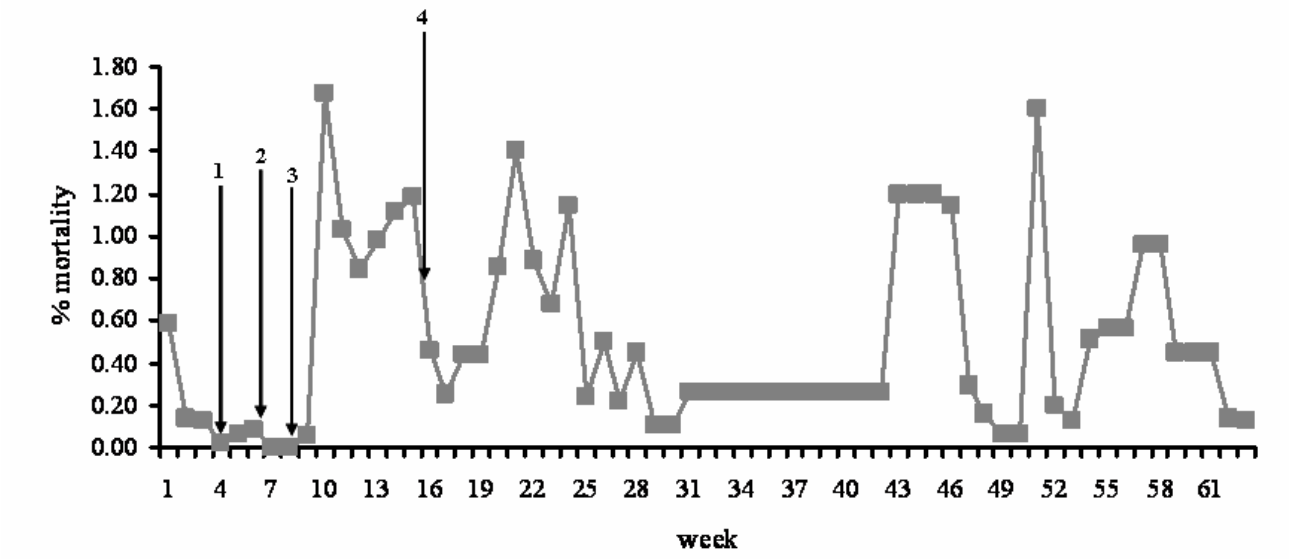

Figure 4.7. An overview of the weekly mortalities expressed as a percentage of the population in the sentinel cage during the pancreas disease longitudinal study.

1. Virus positive serum first detected in week 4 by molecular diagnosis.
2. First histopathological signs of disease recorded. First tissues tested positive for virus by molecular methods.
3. First seropositive fish detected.
4. Last recording of pancreas disease histopathological signs.

5. VECTORS & RESERVOIRS (WP 3)

David Graham, Elena Fringuelli, Claire Wilson, Helen Rowley, Sarah Culloty, Stephen McCleary, Neil Ruane

As described above, other alphaviruses of humans and other mammals have an epidemiology that typically involves an invertebrate vector such as mosquitoes in the transmission of infection. The identities of possible vectors and reservoirs of infection for SAV that may play a key role in introducing and maintaining infection on sites remain unknown. Within the project, emphasis was placed on the role of sea lice and mussels as possible invertebrate vectors. The approach proposed, as described in more detail below, centred on the collection of field samples during longitudinal studies coupled with an experimental infection of mussels. In addition, the relative importance of vertical (from parent to offspring) transmission relative to horizontal transmission were unknown.

The aims of this work package were therefore as follows:

- Conduct an experimental infection of mussels and test the samples to evaluate their role as a possible carrier species.
- Test lice/mussels from salmon farms for the presence of SAV as considered appropriate based on results of experimental/field studies.
- Test salmon broodstock in freshwater for SAV and the subsequent testing of their progeny throughout the culture cycle.
- Test wild fish for the presence of SAV.

5.1 Experimental infection of mussels.

An experimental infection of mussels (*Mytilus edulis*) was conducted in collaboration with the Department of Zoology, Ecology & Plant Sciences (ZEPS), University College Cork (UCC). It is possible that SAV may be detected in mussels due solely to bioaccumulation as a result of the feeding process. This is distinct from actual infection of the mussels, where the virus is capable of infecting tissues and replicating therein, resulting in production of new virus particles which can be transmitted onward to initiate new infections. The aim of this work was therefore to determine the level and duration of bioaccumulation of SAV or of the ability of the virus to actively replicate in the mussels. The experimental study was conducted over a 20 day period using wild mussels from County Cork. The mussels were brought into tanks and acclimatised to the hold conditions in seawater at 10ºC.

Five hundred mussels (*Mytilus edulis*) were obtained from Fastnet Mussels, Bantry on the 26th June 2007. The animals were allowed to acclimatise to a constant temperature of 10ºC over a ten day period. Mussels were held in aerated 50L aquaria in a constant temperature room throughout the study. Control and experimental tanks were kept separate. Animals were deemed healthy as no mortalities occurred and they continued to open their shells and filter during this period. The animals were fed on Phytoplex algal solution each day to ensure that filtration continued.

A sub-sample of 20 mussels was removed after this acclimation period on the day before challenge (D-1). Hepatopancreas (HP) and gill (G) were removed from each individual and separate samples placed in RNA*later*® and maintenance medium. These samples were then screened for SAV by real time RT-PCR to confirm the absence of adventitious infection or contamination, with negative results. The trial was conducted using SAV strain F02-143, isolated from marine Atlantic salmon in Connemara in 2002. The virus was grown in TO cells, and had an initial titre of $10^{7.05}$ $TCID_{50}$/ml.

For exposure to the virus, experimental animals (approximately 240) were placed in an aerated tank containing 3 L of seawater (enough to cover the shells and allow filtering to continue). A 30 ml volume of viral solution was added, giving a final concentration of $10^{6.0}$ $TCID_{50}$/ml and the animals were exposed for 24 hours. Previous experimental studies have shown that the half life of virus at 10ºC in seawater is >4 days (Graham *et al*., 2007c). Control animals (160) were placed in 3 L of water and 30 ml of control medium added and exposure also continued for 24 hours. During this time mussels in both groups were observed to have their shells opened and were filtering. After this time period both groups were washed in clean seawater (in separate systems) and placed in tanks of clean aerated seawater. Two control tanks were set up with 80 mussels each and three experimental tanks with 80 animals each. Animals were fed daily on Phytoplex and the trial continued for a period of 20 days.

Twenty experimental animals and 10 control animals were removed on days 1, 2, 3, 5, 7, 14 and 20 days post-exposure. Two samples of gills and hepatopancreas were removed from each animal and individually placed in RNA*later*® (for subsequent RT-PCR testing) and maintenance medium (for subsequent virus isolation). Samples of gills and hepatopancreas in maintenance medium were stored at -80ºC. Samples in RNA*later*® were stored at -20 ºC. The trial was terminated after 20 days of exposure.

Samples were initially tested for the presence of SAV by real time RT-PCR, a highly sensitive technique which detects the presence of the viral genetic material (RNA). Samples are tested over 40 reaction cycles. The lower the cycle number at which a sample becomes positive (the CT value), the higher the relative level of RNA present in the initial sample. None of the control samples tested positive. In the exposed mussels, 40% of gill samples (Figure 5.1) and 60% of hepatopancreas samples (Figure 5.2) were positive at day 0 (i.e. immediately after challenge). On day 1, these figures had declined to 20% and 10% respectively. Samples from gills on days 2, 3, 5 and 7 were all negative. On days 2 and 3, 20% and 10% of hepatopancreas samples were positive. All hepatopancreas samples tested on days 5 and 7 were negative. CT values in both gills and hepatopancreas were consistently high (Figures 5.1 and 5.2), suggesting low levels of RNA present. Testing was conducted on 10 mussels from the exposed group on days 0, 1, 2, and 3. 20 mussels were tested on days 5 and 7. Either 5 or 10 control mussels were tested on each of these days. Based on these initial findings, no further testing was carried out.

The results indicate that under the trial conditions used the mussels did not become infected with SAV, with subsequent replication of the virus. Rather, the short duration of positivity, associated with high CT values, is consistent with the positive signals detected being due to bioaccumulation/contamination of the shellfish with virus during the initial exposure, with subsequent leaching/decay rapidly resulting in negative results. In conclusion, the results suggest that mussels are not a reservoir for SAV infection for farmed salmon. Based on these results, no testing of field samples was conducted.

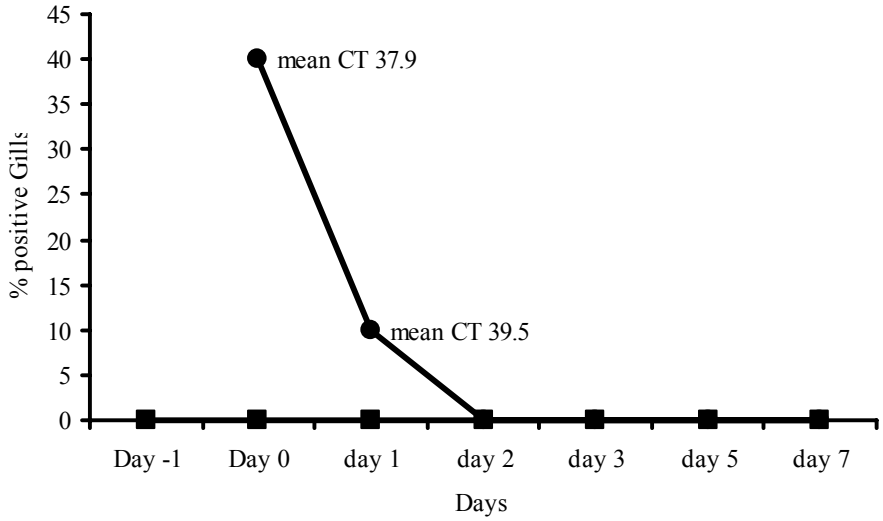

Figure 5.1. Real time RT-PCR results for gill tissues from mussels (*Mytilus edulis*) experimentally exposed to SAV (•) and unexposed controls (■), showing the mean CT values of positive shellfish.

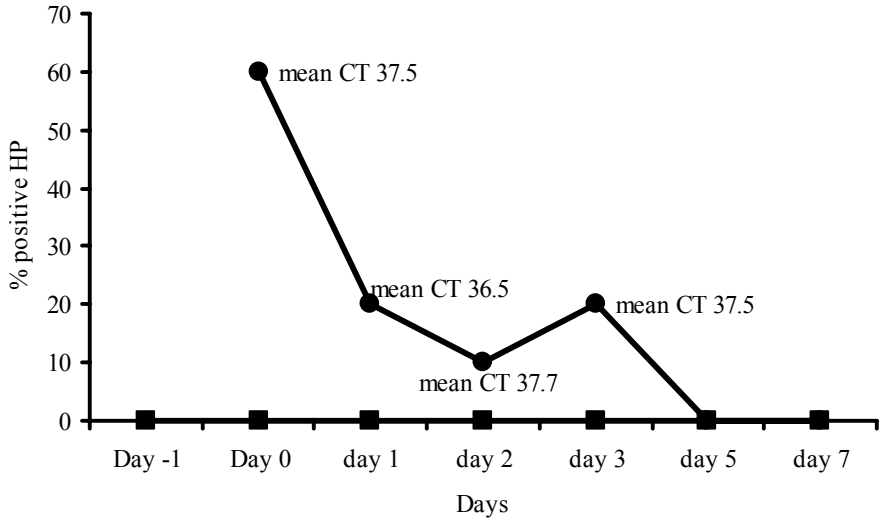

Figure 5.2. Real time RT-PCR results for hepatopancreas tissues from mussels (*Mytilus edulis*) experimentally exposed to SAV (•) and unexposed controls (■), showing the mean CT values of positive shellfish.

5.2 Testing sea lice

During the course of the S1 longitudinal study (Section 4.2), lice were collected from farmed salmon on a number of occasions. These were received as either individual lice or pools collected on the same day. The numbers of lice submitted were limited, reflecting in part the low numbers of lice present at some sampling points. These were tested for SAV by real time RT-PCR. Details of lice tested and results are shown below (Table 5.1).

Table 5.1. Details of real time RT-PCR testing of lice for SAV from S1 longitudinal study.

Case ref	Sample ref	Date	Single or pool	Result
F06-236	3165	27/07/06	Single	Neg
F06-236	3175	01/08/06	Single	Neg
F06-236	3207	12/09/06	4 pools of 5 lice	Neg
F06-236	3218	03/10/06	4 pools of 5 lice	Neg
F06-281	-	21/11/06	4 pools of 5 lice	Neg

Based on these results, no testing of lice from other sites was carried out. It is recognised that the lice available for testing were largely collected some time after infection occurred in the study population (May/June). Therefore, it is likely that there were few or no viraemic fish present when the tested samples were collected. The precise role of lice in the epidemiology of SAV infection therefore remains to be clarified, and would be worthy of further study in future work. Indeed, SAV has recently been reported by RT-PCR in lice in a Norwegian study (Petterson *et al*., 2007).

5.3 Vertical transmission and broodstock testing.

At the start of the project, the possibility of there being a freshwater reservoir of infection in salmon, based on vertical transmission from broodstock through eggs, milt and reproductive fluids, had been proposed. Studies were therefore designed to explore the significance of this in the Irish context. An initial sampling of broodstock was conducted at Fanad (Marine Harvest Ireland) in December 2005. Based on an agreed sampling protocol, serum, tissues and milt or ovarian fluid (n = 67) were collected from broodstock. Sera were subjected to serological and virological testing. Heart tissue and reproductive fluids were tested by real time RT-PCR. Four sera were found to contain neutralizing antibodies. None were viraemic. All tissues and reproductive fluids also tested negative.

Fish derived from this broodstock population also served as the basis for subsequent longitudinal studies. These were sampled several times in freshwater (FW) and bloods tested for evidence of antibodies or virus. Part of this population went to sea in November 2006 as S0 fish, while the remainder went to sea as S1 fish in spring 2007. Details of the FW testing are given in Table 5.2 below.

Table 5.2. Details of FW testing of Fanad fish for SAV and associated antibodies.

REFERENCE	DATE	No. fish	Antibody pos	Virus pos
F06-12032	25/08	20	0	0
F06-14312	19/10	19	0	0
F06-15253	09/11	20	0	0
F07-4501	16/03	20	0	0

Additional testing of juveniles in freshwater was carried out on other sites, based on routine surveillance samples. In total, 80 sera from 7 separate submissions were tested for virus and antibody, with negative results.

In December 2006, a further round of broodstock testing was conducted at Fanad. This time a total of 100 female broodstock were sampled, with blood, ovarian fluids, and heart tissue collected. Virus neutralizing antibodies were detected in 14 sera, while none of the sera were viraemic. All of the ovarian fluids and hearts tested negative by real time RT-PCR for SAV. Based on these results, no evidence for broodstock infection/vertical transmission was found.

It is concluded that vertical transmission is not an important feature of the epidemiology of SAV infections in salmon farming in Ireland. Rather, fish seem to acquire infection during the marine phase of production, either from other farmed salmon, or from an as yet unidentified carrier/reservoir host(s). This finding is consistent with the results of molecular studies described in Chapter 6.

5.4 Wild fish testing.

Marine fish

As part of the S1 longitudinal study (see Section 4.2) a small number of fish were caught by rod and line near the sentinel cage during the period of the pancreas disease outbreak. In total, kidney, heart and gill tissues were removed from one mullet and 11 mackerel and placed in RNA*later*® for subsequent analysis by real-time RT-PCR. All samples tested negative which would suggest that these fish species do not transmit the virus, particularly as analysis of these tissues from salmon give positive results for up to 12 months after infection. However the number of fish sampled was very small and future studies should focus on a larger sample size and test a greater number of fish species.

Freshwater fish

As part of a study investigating the health status of wild freshwater fish, the Fish Health Unit screened over 1,200 fish from eight rivers in 2007. Tissue samples (in pools of five) were tested for the *Salmonid alphavirus* using conventional virological methods using EPC and BF-2 cell cultures. No evidence of SAV was found. In addition to this, gill tissue was taken from each fish and stored in RNA*later*® for subsequent analysis by real-time RT-PCR. A number of samples from one river gave a positive reading and an illustration of a retesting of ten positive samples is shown below in Figure 5.3.

Work is currently ongoing to amplify the product and have it sequenced which will be necessary in order to show that the samples are actually positive for the *Salmonid alphavirus*.

Wild fish potentially play an important role in the epidemiology of a number of important fish viral diseases, such as infectious salmon anaemia (Raynard *et al*., 2001; Plarre *et al*., 2005) and infectious pancreatic necrosis (Wallace *et al*., 2008). It is therefore highly probable that a wild fish reservoir for the *Salmonid alphavirus* exists and a comprehensive monitoring of wild fish should be initiated in order to determine if the virus is limited to salmonid species or if other fish species are involved. As the virus is also the causal agent of sleeping disease in rainbow trout in freshwater, detection of the virus in freshwater would appear to be possible. Nylund *et al*. (2003) detected the Norwegian subtype of the salmonid alphavirus in freshwater farmed Atlantic salmon suffering from haemhorrhagic smolt syndrome. At this stage the prevalence of the virus in wild stocks is unknown and the risk, if any, to aquaculture facilities remains to be quantified. As the virus was not isolated in these samples but only detected by real-time PCR, this would indicate that the levels were extremely low and may represent a natural background level in wild fish.

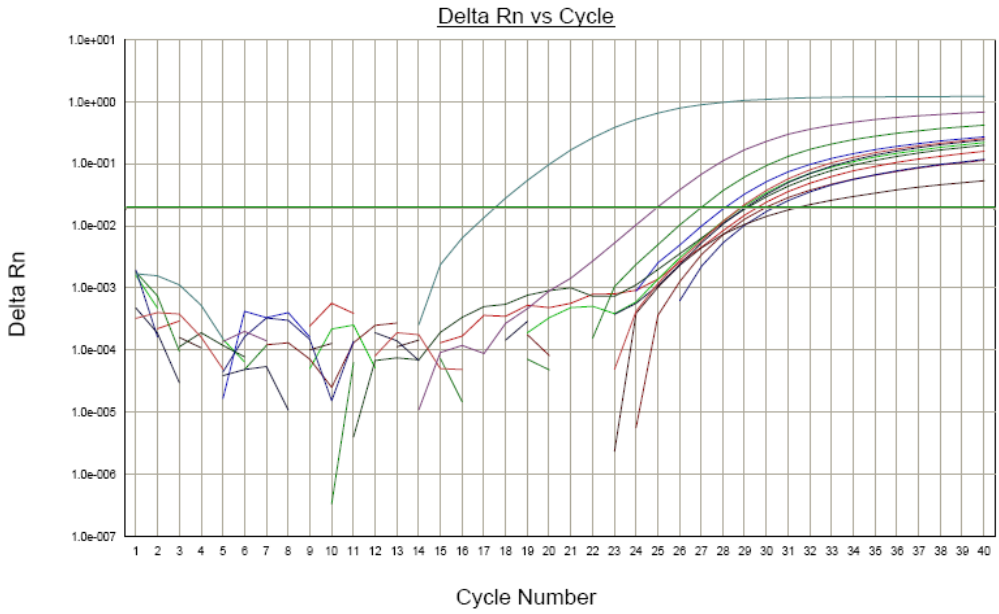

Figure 5.3. Graphic presentation of ten gill tissue samples from freshwater brown trout tested for the salmonid alphavirus by real-time RT-PCR. The samples with the lowest Ct values (*ca*. 18 and 25 are positive controls). Gill tissues show Ct values from 27 – 32.

6. VIRUS SEQUENCING STUDIES (WP 4)

Elena Fringuelli, David Graham

At the start of the project, only limited published genome sequence data was available for SAV. Such information has allowed the degree of variation between viruses to be studied. This has important implications for areas such as vaccine efficacy, virus virulence, ability to trace the source of outbreaks and the design of diagnostic tools. From previous work carried out at the AFBI laboratory a large library of viruses was available for examination, primarily from salmon in Ireland, but also from other species (rainbow trout) and countries (England, Scotland, France, Italy, Spain). This library, complemented by further isolates made during this study and from other routine diagnostic work in the laboratory, formed the basis of this work package.

Initial work concentrated on the design of the sequencing reactions to be used. The focus of these studies was to identify the most variable (and therefore informative) regions of the genome using available data from subtypes 1, 2 and 3, and then to develop RT-PCR assays to amplify these genome segments. These studies showed regions of nsP3 and E2 (non-structural and structural proteins respectively) to be the most variable, with nucleotide variability of up to 25.1% and 12.3% between strains respectively. Primers were therefore designed and assays developed to amplify 490 and 516bp products of nsP3 and E2 respectively. These assays were then used to generate sequence data from a total of 48 strains. Details of these are given in Table 6.1.

Table 6.1. Details of virus strains used in the sequencing study.

								accession number	
Virus	Year	Country	Company	farm	Source	Species	Mort. rate	nsP3	E2
F93-125	1993	Ireland	A	1	CHSE-214 cells	AS	na	AJ316244	AJ316244
F-2111(1)	2002	Ireland	A	1	serum	AS	na	EF675503	EF675551
F91-115 (A3)	1991	Ireland	A	1	serum	AS	na	EF675515	EF675563
F91-116 (P1-6)	1991	Ireland	A	1	serum	AS	na	EF675516	EF675564
F04-44 (10)	2004	Ireland	B	2	serum	AS	15%	EF675514	EF675560
F06-186 (5)	2006	Ireland	B	3	serum	AS	na	EF675517	EF675561
F03-123 (3)	2003	Ireland	C	4	serum	AS	18%	EF675500	EF675548
F04-183 (A1)	2004	Ireland	C	5	serum	AS	23%	EF675511	EF675559
F05-118 (5)	2005	Ireland	C	6	serum	AS	15%	EF675510	EF675558
F06-227 (20)	2006	Ireland	D	7	serum	AS	< 5%	EF675506	EF675554
F05-27 (13)	2005	Ireland	D	8	serum	AS	20%	EF675507	EF675555
F 1045/96	1996	Ireland	D	9	CHSE-214 cells	AS	na	EF675499	EF675547
F06-182 (55)	2006	Ireland	E	10	serum	AS	na	EF675501	EF675549
F05-184 (5)	2005	Ireland	E	11	serum	AS	na	EF675502	EF675550
F05-238 (9)	2005	Ireland	E	12	serum	AS	< 5%	EF675505	EF675553
F06-43 (4)	2006	Ireland	E	13	serum	AS	10%	EF675508	EF675556
F02-194 (11)	2002	N. Ireland	F	14	serum	AS	na	EF675504	EF675552
F05-190 (38)	2005	Ireland	G	15	serum	AS	35%	EF675509	EF675557
F05-294 (3)	2005	Ireland	H	16	serum	AS	6%	EF675512	EF675562
F03-209 (3)	2003	Scotland	I		serum	AS	na	EF675513	EF675565
F06-243 (4)	2006	Scotland	I	17	serum	AS	0%	EF675518	EF675566
F06-17 (9)	2006	Scotland	I	18	serum	AS	9%	EF675521	EF675568
F07-02	2007	Scotland	I	19	serum	AS	na	EF675522	EF675571
F06-241(1)	2006	Scotland	I	20	serum	AS	0%	EF675524	EF675572
F06-267(9)	2006	Scotland	I	21	serum	AS	0%	EF675526	EF675574
F06-290 (6)	2006	Scotland	I	22	serum	AS	<1%	EF675534	EF675582
F06-290 (8)	2006	Scotland	I	23	serum	AS	< 1%	EF675535	EF675583
F06-139 (33)	2006	Scotland	L	24	serum	AS	15%	EF675519	EF675569
F06-93 (37)	2006	Scotland	L	25	serum	AS	< 15%	EF675520	EF675567
F06-41 (54)	2006	Scotland	L	26	serum	AS	< 15%	EF675523	EF675570
F04-224 (17)	2004	Scotland	M	27	serum	AS	< 5%	EF675525	EF675573
F05-310 (10)	2005	Scotland	M	28	serum	AS	< 5%	EF675527	EF675575
F05-310 (18)	2005	Scotland	M	29	serum	AS	< 5%	EF675528	EF675576
F05-124 (5)	2005	Scotland	M	27	serum	AS	< 5%	EF675529	EF675577
S49p	1995	France			CHSE-214 cells	RT	na	AJ316246	AJ316246
EE37		France			heart	RT	na	EF675530	EF675578
VF03 (p4)	2002	France			CHSE-214 cells	RT	na	EF675531	EF675579
F02-85 (9)	2002	Scotland			CHSE-214 cells	RT	na	EF675532	EF675580
F06-119 (7)	2006	Scotland			serum	RT	na	EF675536	EF675581
F04-212 (5)	2004	England			serum	RT	na	EF675537	EF675585
F02-67 (18)	2002	England			serum	RT	na	EF675533	EF675584
F04-08 (6)	2004	Spain			serum	RT	na	EF675538	EF675586
F04-08 (18)	2004	Spain			serum	RT	na	EF675539	EF675587
F04-198 (29)	2004	Italy			serum	RT	na	EF675540	EF675588
F05-105 (12)	2005	Italy			serum	RT	na	EF675541	EF675589
F04-198 (22)	2004	Italy			serum	AS	na	EF675542	EF675590
SavH20/03	2003	Norway			heart/kidney	AS	na	AY604235	AY604235
SavH10/02	2002	Norway			CHSE-214 cells	AS	na	AY604236	AY604236
PD97-N3	1997	Norway			CHSE-214 cells	AS	na	AY604237	AY604237
SavSF21/03	2003	Norway			heart/kidney	AS	na	AY604238	AY604238
F04-170 (6)	2004	Norway			serum	AS	na	EF675543	EF675591
F04-170 (8)	2004	Norway			serum	AS	na	EF675544	EF675592
F04-170 (3)	2004	Norway			serum	AS	na	EF675545	EF675593
F04-170 (7)	2004	Norway			serum	AS	na	EF675546	EF675594

The sequence data generated for the two gene segments, along with published data for reference strains were then used to construct phylogenetic trees to examine the relatedness of the strains (Figures 6.1, 6.2).

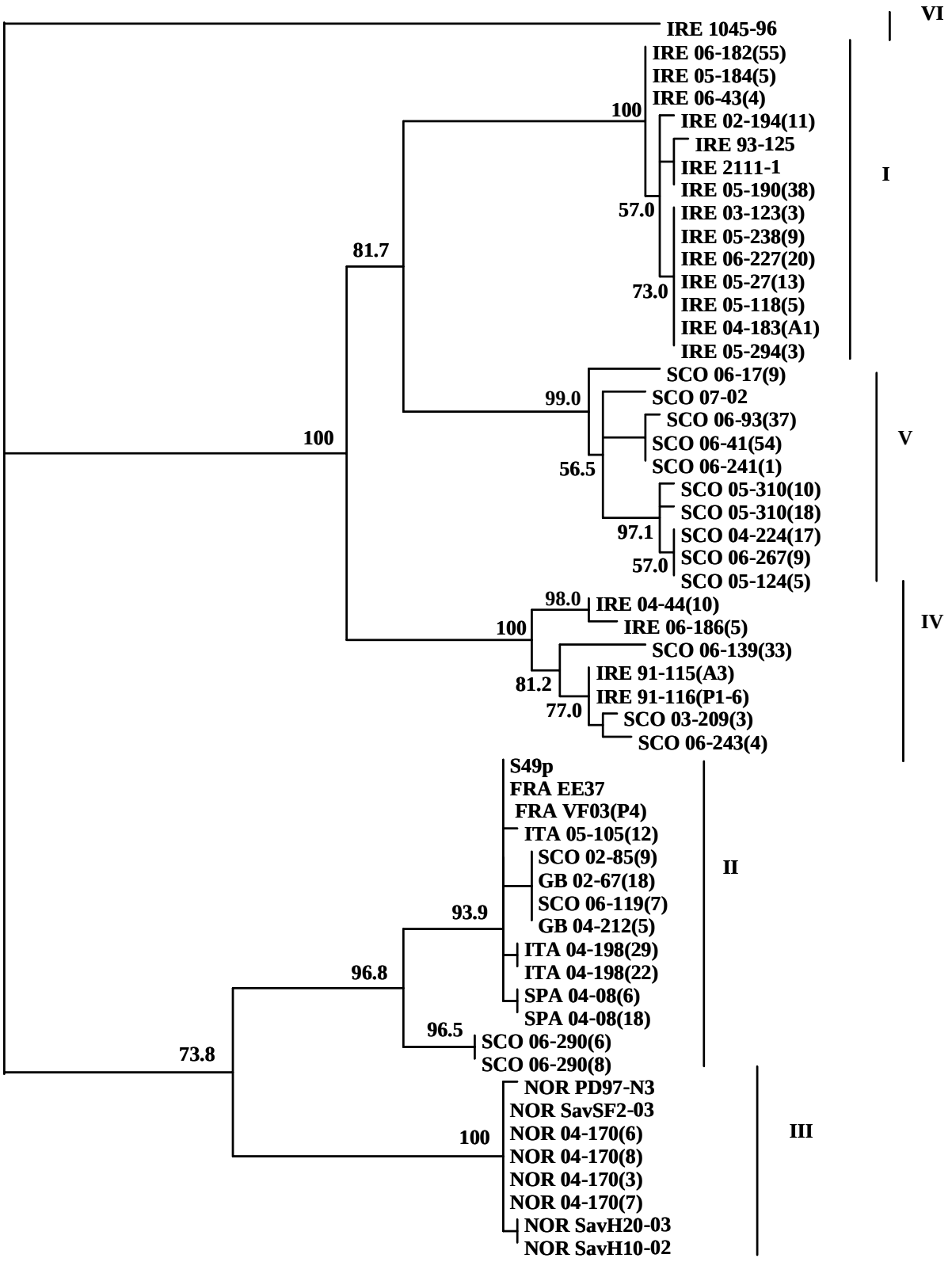

Figure 6.1. Phylogenetic relationship between SAV strains based on nucleotide sequence comparisons of an nsP3 gene fragment. Percentages of bootstrap values are given at each node. Branch lengths are informative and drawn to scale.

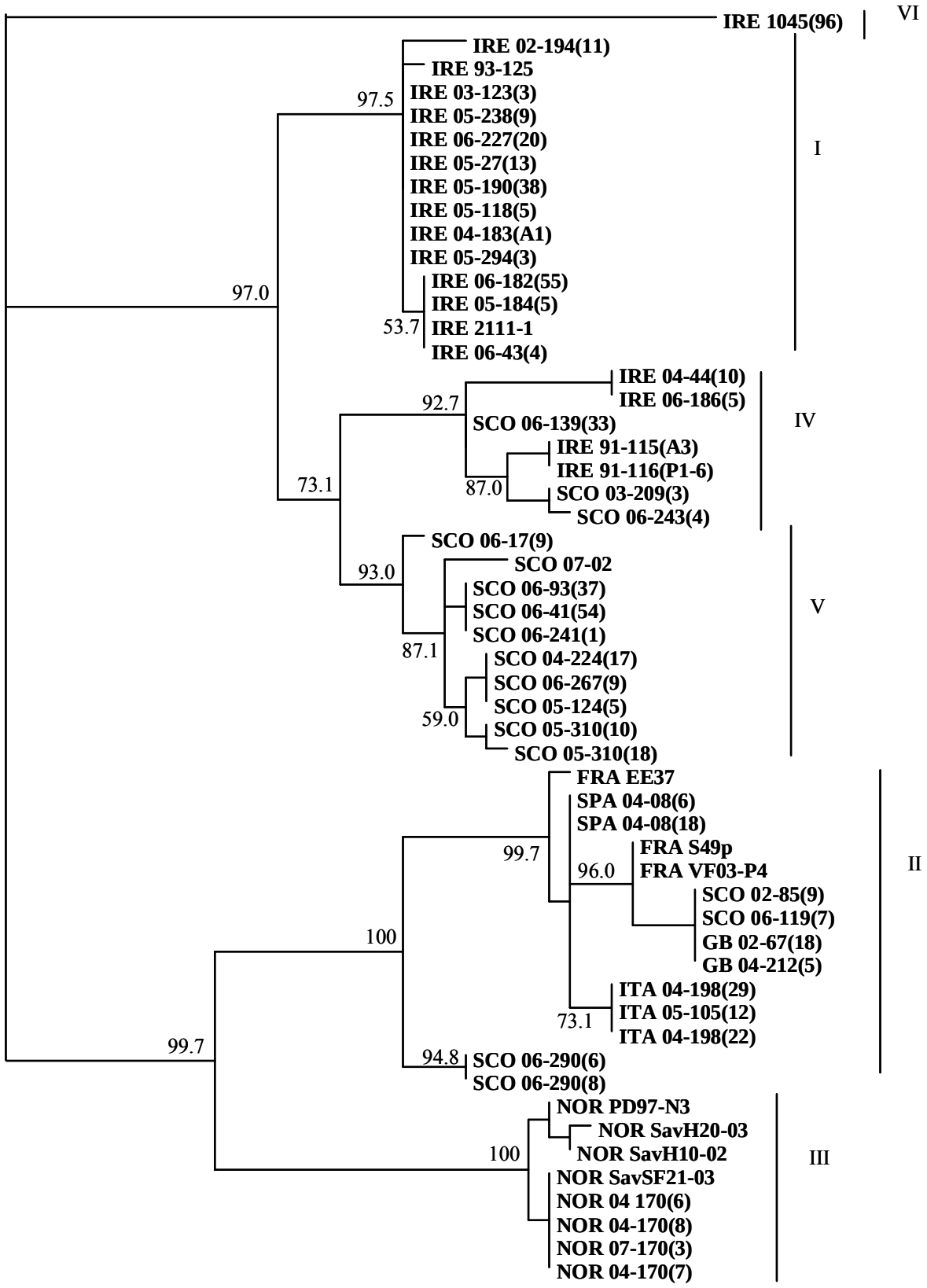

Figure 6.2. Phylogenetic tree showing phylogenetic relations between salmonid alphaviruses strains based on nucleotide sequence comparisons of E2 gene fragment. Percentages of bootstrap values are given at each node. Branch lengths are informative and drawn to scale.

There was good agreement between both trees, with the classification of each strain remaining consistent within both clusters. Examination of these data revealed a number of new findings.

- Firstly, the study identified three previously unrecognised subtypes of the virus based on the clustering of strains. These have been designated subtypes 4, 5 and 6 (SAV4, SAV5, SAV6).
- SAV4 was found in Ireland and Scotland. Strains 91-115 and 91-116 (from 1991) came from archived field material held by AFBI. These are the oldest strains from which sequence data is available. It is noteworthy that these strains are from the same farm in Donegal where the reference strain of virus (F93-125) was isolated in 1993. F93-125 is a subtype 1 virus, demonstrating the presence of two different subtypes on the farm in a two year period.
- SAV5 was found in Scotland only.
- SAV6- only one isolate (F/1045/96) fell into this new subtype. This came from an outbreak of PD in Connemara in 1996.
- Prior to this study, SAV2 had only been reported from rainbow trout in freshwater. For the first time, SAV2 was detected in marine farmed salmon on one site in Scotland. Follow-up work, has since demonstrated the presence of SAV2 on another Scottish salmon farm, plus identified an old (1990s) Scottish isolate as SAV2, indicating that this subtype has been present for some time. This suggests that the freshwater cases may have derived from a marine source.
- SAV2 strains were also confirmed in farmed FW rainbow trout in Spain and Italy during the course of this work. This discovery formed the basis of a published paper (Graham *et al.* 2007b). This is consistent with a single/limited introduction of infection into freshwater, with subsequent dissemination within the industry, including the UK, where it was first reported in 2002.

Further analyses of strains by location indicated that in Scotland, SAV subtypes 2, 4 and 5 were present (Figure 6.3; subsequent work has also revealed the presence of SAV1). In Ireland SAV1 and SAV4 predominate, with the single isolation of SAV6 also, although this has not been detected recently. It was evident that SAV4 isolates were restricted to Donegal, whereas SAV1 strains were concentrated in Connemara.

The data were considered in two ways for evidence in support of either horizontal or vertical transmission. Firstly, where clusters of strains with identical or very similar sequences were found, evidence of geographical clustering was looked for. This was evident in several instances (strains within dotted circles, Figure 6.3). This data showed that in a number of locations, including Mannin Bay, Mulroy Bay, Kilkieran Bay and the Hebrides, there was clustering of related/identical strains over time on different farms and even between different companies. This provides strong circumstantial evidence for local horizontal circulation of these strains between farms over several years, either through the water or by other means. In several cases where this was investigated further, evidence indicated that populations of recovered and naïve fish had been in the water together in these locations, suggesting that the former may have served as the ultimate source of infection for the latter. This is supported by the findings of the longitudinal surveys, where persistence of viral RNA for extended periods of time was demonstrated (Chapter 4).

In addition, analysis of strains from Kilkieran Bay showed the presence of multiple isolates with identical sequences (Figures 6.1, 6.2, 6.3). The sources of input fish for these sites were either Scotland or Donegal. If infection had been introduced vertically, it would logically be expected that the strains present would reflect those predominant in the areas from where the fish were sourced. This is clearly not the case in this instance. This data has been accepted for publication (Fringuelli *et al*., 2008).

In conclusion, the results from this work package have considerably extended our knowledge on the variability of SAV strains and the epidemiology of infection. This work in turn raises a number of issues that require further consideration. These include:

- Impact of sequence variability on diagnostic tools (serological, virological, molecular).
- Impact of strain subtypes on vaccine efficacy.
- Transmission routes of infection between farms.
- Investigation of wildlife (vertebrate or invertebrate) reservoirs of infection.
- Application of molecular epidemiology as an aid to outbreak investigations and identification of risk factors that could support control/mitigation.

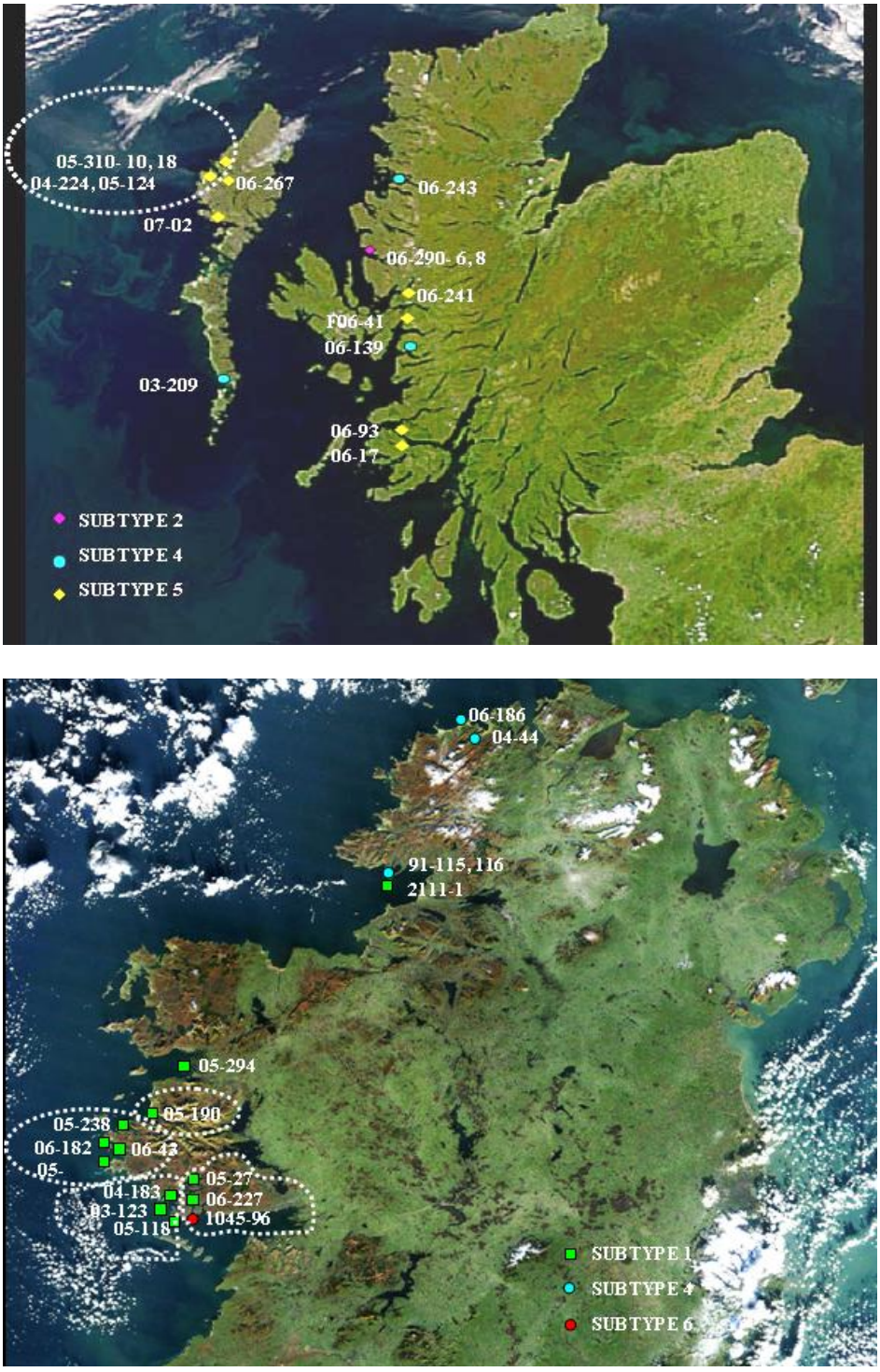

Figure 6.3. Distribution of Scottish and Irish isolates by subtype and location. Those within dotted circles are clustered phylogenetically.(Background image courtesy NASA (www.nasa.gov))

7. EPIDEMIOLOGY (WP5)

Hamish Rodger, Susie Mitchell, Albert Girons, Tracy Clegg, Simon More.

In the present work data from two generations of salmon at sea (2005 and 2006) have been analysed. For selected variables, data from four different generations (2003, 2004, 2005 and 2006) have been used to test for significant associations. This study builds on those investigations previously reported (McLoughlin *et al*., 2003, Rodger & Mitchell, 2007).

7.1 Materials & Methods

Survey population

Populations targeted for this study were commercially reared Atlantic salmon (*Salmo salar*) from farm sites in the northwest, west and the southwest of Ireland. Two subsequent generations were sampled: 2005 year-class, fish hatched in early 2004 that went to sea in late 2004 as S0s and in early 2005 as S1s, as well as 2006 year-class, fish hatched in early 2005 that went to sea in late 2005 as S0s and early 2006 as S1s.

Collection of data

Data was collected using a detailed questionnaire for each site. The questionnaire consisted of open ended and closed questions covering a number of areas. Information on the stock was collected including fish number, date of transfer at sea, fish weight at time of PD outbreak, stocking density, strain of fish and smolt type. Management information gathered involved whether fallowing was employed, feeding rate prior to and during an outbreak of PD, whether fish were moved during their life cycle, existence of other PD-positive sites in the same bay and vaccination history. Information on PD and other diseases was also collected, including time of outbreak of PD (where appropriate), number of days from transfer to sea to PD outbreak, number of weeks to reach peak mortality, number of weeks PD present in site, as well as percentage of mortality caused by specific disorders, PDV vaccination status (for 2005 year-class), average lice levels during production cycle, previous history of PD in the site, estimated loss of growth due to PD and site geographical region and proximity to a fish processing plant. Environmental data such as seawater temperature during the whole production cycle was also gathered.

Diagnosis of PD

A farm was categorised as positive for PD where there had been a diagnosis of the clinical disease with laboratory test confirmation, i.e. veterinary examination with recording of typical clinical signs plus either PD histopathology and/or the detection of salmonid alphavirus (SAV) through virus isolation or SAV antibodies. The start date for an outbreak of PD on each site was taken as the date when clinical signs were first observed. The end date for an outbreak was taken as the date when mortalities returned to background levels and clinical signs of PD were no longer observed. Where a farm was confirmed with PD, the estimated loss of growth due to PD was given as the percentage of thin, poor condition fish in the pens that were, as a result, downgraded at harvest.

Water temperatures

A water temperature profile for the whole production cycle was put together for each site for 2005 and 2006. Marine Institute water temperature figures were used when available. In those cases where this data was not available, farm records were used. The water temperature profile for each farm was used to calculate the water temperature at the time of a PD outbreak, as well as the number of days that fish were exposed to water temperatures between 10 and 15ºC after the diagnosis of PD.

Sea lice
Sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) burdens on farmed salmon are monitored regularly in each generation of fish at all marine sites in Ireland. Each population is inspected at least monthly by Marine Institute staff and these figures were used for the purposes of this study.

Feeding rate
Farm records supplied the feeding rate data, which was expressed as a percentage of body weight, in the month prior to confirmation of a PD outbreak and these were assessed in relation to PD mortality levels.

Data analysis
Data was summarised, entered and sorted into different categories using Microsoft Excel. Due to the nature of the data and the small sizes, non-parametric statistic tests were used for most of analysis of the collected data. SPSS, Version 5 (SPSS Inc., Chicago, IL) was the statistical software used. For categorical data (occurrence and impact of PD, smolt type, fallowing, history of PD, livestock movement, strain, bay and company) a percentage of inputs was calculated for each variable in relation to either PD status (positive or negative) and PD impact (high impact when mortality ≥10%, versus low impact, when mortality <10%). Significance in relationships between groups was assigned when $P < 0.05$ in all analysis. Odds ratios were calculated for variables in relation to occurrence of PD. Fisher's Exact Test was used to determine any significant differences. Continuous variables (stocking densities, time to outbreak, temperature at PD outbreak, feeding rate, and days at 10 - 15ºC risk window) were analysed using Wilcoxon Two sample test.

7.2 Results
Number of inputs and fish
Information was gathered from 29 separate populations of salmon at sea from two different generations or year-classes. The 2005-year class was constituted by 15 inputs, while 2006-year class included information from 14 different inputs. The number of salmon that went to sea is summarised in Table 7.1.

Table 7.1. Number of study populations and fish going to sea by smolt type.

Year	No. of S0s populations (No. of fish)	No. of S1s populations (No. of fish)	Total inputs (No. of fish)
2005	6 (1771197)	9 (3638276)	15 (5409473)
2006	6 (1936087)	8 (3675828)	14 (5611915)
2005 & 2006	12 (3707284)	17 (7314104)	29 (11021388)

These figures represent all the marine salmon farms in Ireland which transferred fish to sea from autumn 2004 to spring 2006. Basic farm data was collated from all marine sites. However, more detailed records with regard to feeding rates, weights, mortality numbers, etc. were not available for a low number of farms.

Incidence of PD
In 2005, 12 out of 15 (80%) populations reported PD outbreaks. For 2006, one of the farms had an unknown PD status and 9 out of 13 (69%) farms reported PD outbreaks. The average total mortality due to all diseases was 34.8% (range 16% to 90%) for 2005 and 34.7% (range 10.5% to 100%) for 2006. Mortality due to PD on affected farms was 9.8% (range 0.7% to 31%) for 2005 and 9.6% (range 2.1% to 29%) for 2006. In addition to mortalities due to PD, the percentage of runts due to PD was 2.8% (range 1.6% to 7.5%) for 2005 and 1.7% (range 2%-

14%) for 2006. Average weight of a fish at the time of PD outbreak was 764g in 2005 and 660g in 2006. When fish were differentiated by smolt type, average body weight for 04 S0s was 960g (range 282-1681g) and 1134g (range 334-2200g) for 05 S0s. For 2006 year-class average body weight for 05 S1s was 635g (range 202-1120g) and 283g (range 138-484g) for 06 S1s. The number of operating sites, percentage of sites that reported a PD outbreak, and percentage of mortality due to PD for the period 2002 to 2006 are featured in Figures 5 and 6. Total and PD-mortality figures for 2005 and 2006 year-class by smolt type in comparison with data from 2003 and 2004 year-class are presented in Figure 7.3.

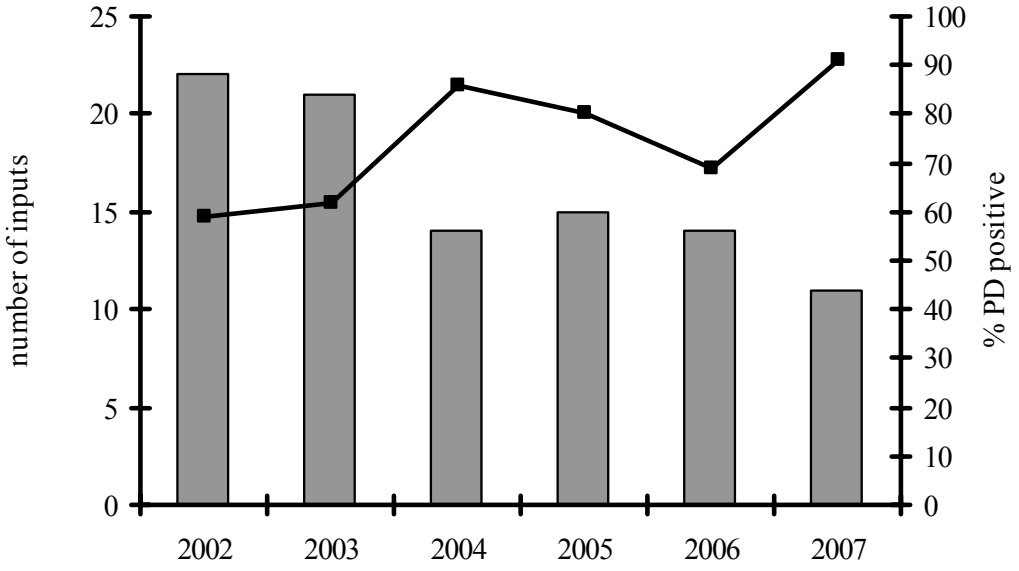

Figure 7.1. The number of smolt inputs (bars) and the percentage of PD positive sites (line) in Ireland from 2002 – 2007.

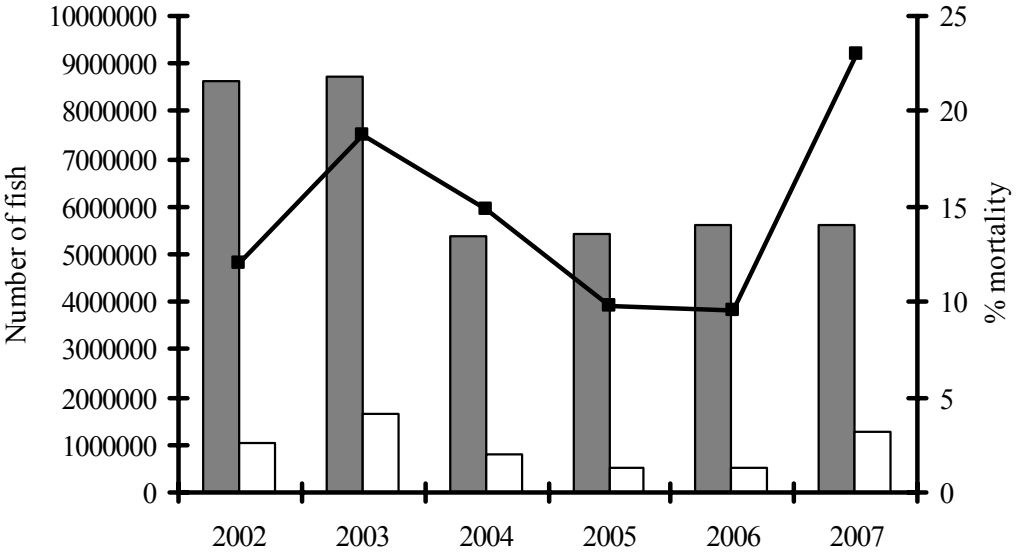

Figure 7.2. Number of fish going to sea (■), number of fish which died because of PD (□) and average percentage of mortality due to PD (■) during the period 2002-2007.

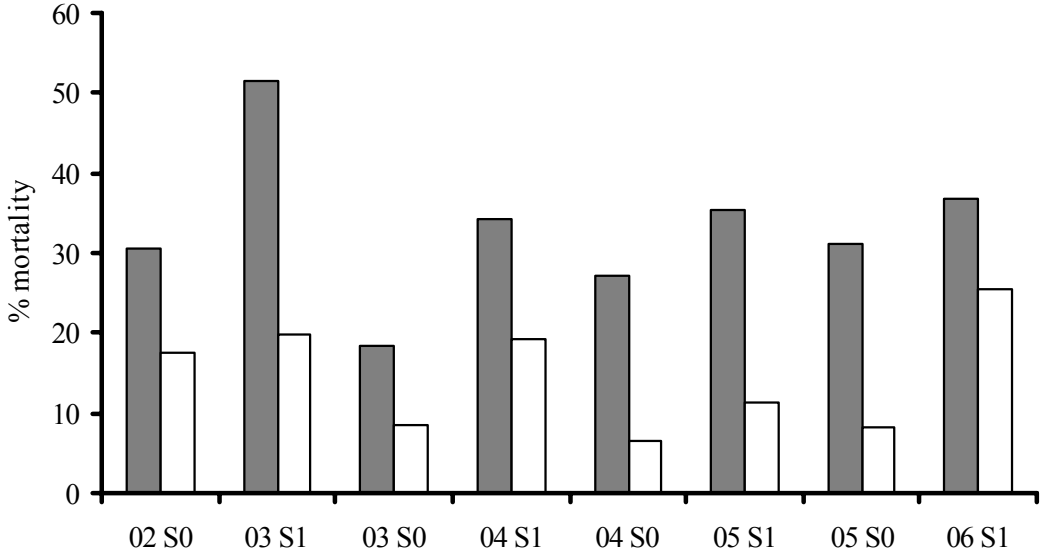

Figure 7.3. Total mortality (■) and PD-specific mortality (□) for 2003, 2004, 2005 and 2006 year-class detailed by smolt type.

Seasonality

The month that outbreaks occurred in 2005 and 2006 varied between sites, with more than half of the outbreaks (63%) occurring between June and November (Figure 7.4). When examining data for the last five years, there is a higher concentration of number of cases in early summer and late summer/early autumn than in any other period of the year (Figure 7.5).

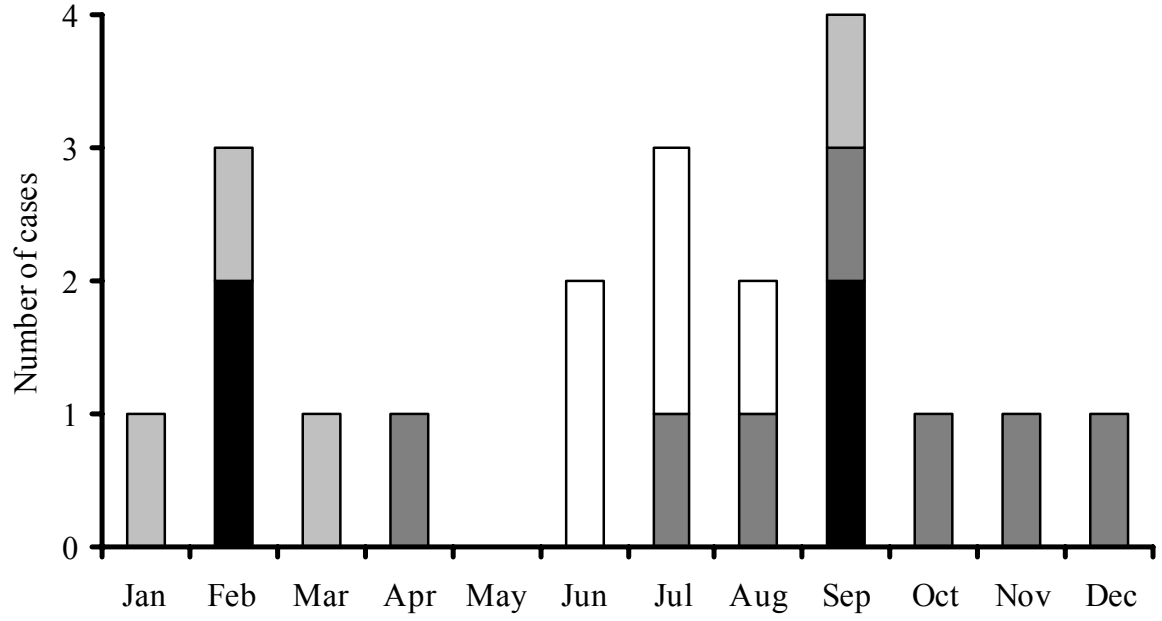

Figure 7.4. A breakdown of the month when PD was first diagnosed in each of the sea sites for 2005 and 2006 by smolt type; ■ 2004 S0; ■ 2005 S1; ■ 2005 S0; □ 2006 S1.

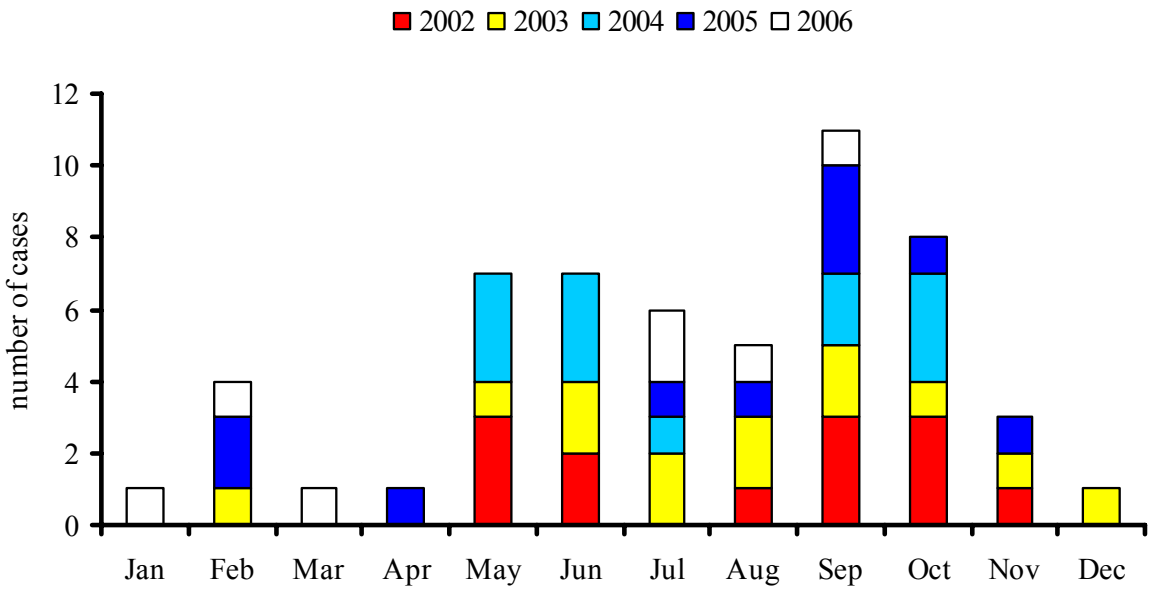

Figure 7.5. The month when PD was first diagnosed in Ireland during the period 2002 to 2006.

Duration of PD outbreaks

In 2005, the average time that fish spent at sea from transfer to PD outbreak was 243 days (range 116-423 days) and 174 days (range 72-263 days) in 2006. The average time for an affected farm to reach peak or highest mortalities in an outbreak was 15 weeks (range from 1 to 32 weeks) in 2005 and 5 weeks (range 3 to 6) in 2006 after the first signs of disease. The average time that PD persisted as a problem in a site was 21 weeks (range 12 to 32 weeks) in 2005 and 13 weeks (range 6 to 20 weeks) in 2006. Mortality patterns often followed a bell-shaped distribution, consistent with the infectious nature of the disease.

Water temperatures

A water temperature profile was produced for every site for 2005 and 2006 using data collected by the Marine Institute or the farm itself. Temperature measurements were done between 5 and 10 metres of depth. It was a common feature for years 2005 and 2006 to get water temperatures over 10ºC during the second half of April or early May and over 15ºC in early/mid July. During August water temperatures could drop some days below 15ºC in some sites. It was not until the second half of September or early October that water temperatures consistently dropped below 15ºC and it was not until late November, or early December in some sites, that water temperatures dropped below 10ºC.

Main causes of mortality

Total mortality figures for the 2005 and 2006 generations were recorded by cause of mortality. Gill problems were the group of disorders causing the highest percentage of mortality in the Irish salmon industry [2005: 15.8% (range 1.4% to 89%) and 2006: 15.1% (range: 2% to 85%)]. PD was the most significant infectious disease in terms of mortality during the study period. The main causes of mortality for each generation are represented in Figures 7.6 and 7.7.

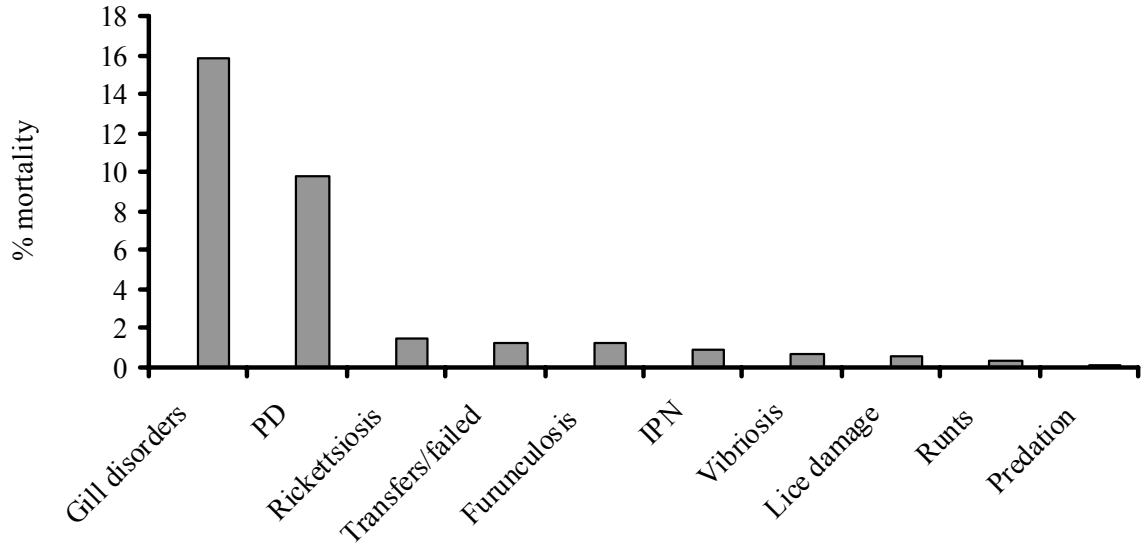

Figure 7.6. Average mortality due to specific causes for all marine Atlantic salmon stocks in Ireland in 2005.

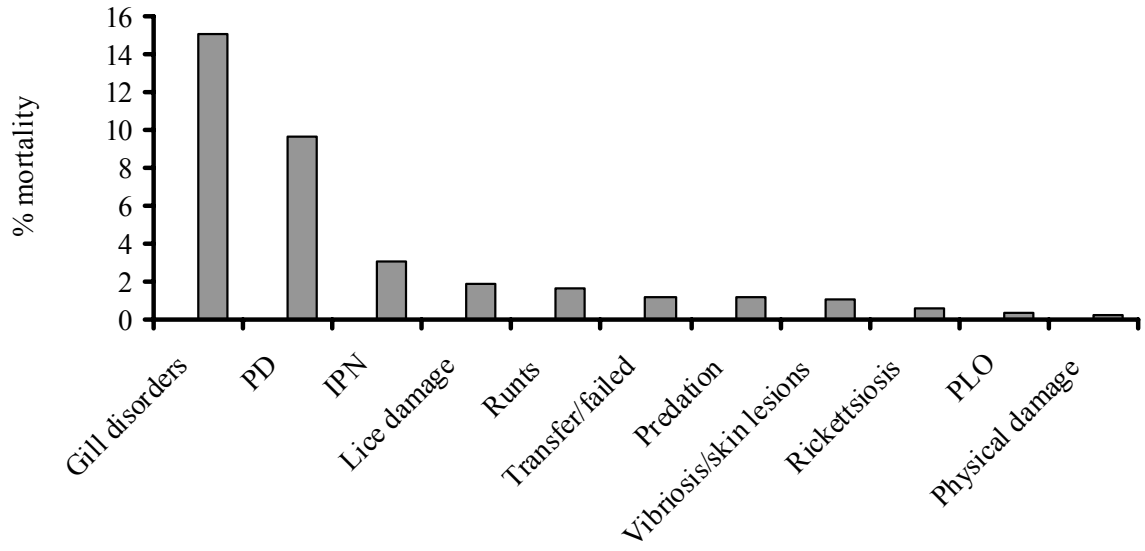

Figure 7.7. Average mortality due to specific causes for all marine Atlantic salmon stocks in Ireland in 2006.

7.3 Data analysis

Data from 2005 and 2006 generation have been analysed. When possible, data from a total of four different generations (2003, 2004, 2005 and 2006) have been used to test for any statistically significant differences in terms of occurrence of PD and its impact (high impact for PD mortality levels ≥10% and low impact for PD mortality levels <10%). For the purpose of the analysis of univariate statistics, all sites and years were assumed to be independent. In order to give a better idea of strength of relation between a variable and occurrence or impact of PD, odds ratio was calculated for those categorical variables with *p*-values that were significant or close to significant. Special attention to water temperatures was taken following the recommendations from a previous study (Rodger & Mitchell, 2007).

7.3.1. CATEGORICAL DATA ANALYSIS

Occurrence and impact of PD between different generations

In 2003, 14 PD outbreaks were reported out of 21 (67%) different inputs of salmon. In 2004, 12 out of 14 (86%) different populations suffered PD. In 2005, the proportion of inputs that suffered PD was 12 out of 15 (80%), and in 2006 it was 9 out of 13 (69%) in 2006. There has been limited variation in the percentage of sites affected by PD and this was not significantly different between these four different years (Fisher's exact, $P = 0.583$). The percentage of farms having high impact of PD was also not significantly different between these four different generations [2003 = 11/14 (79%), 2004 = 6/12 (50%), 2005 = 6/12 (50%) and 2006 = 4/9 (44%). Fisher's exact, $P = 0.287$].

Smolt type

Data from 4 different generations of salmon (2003 to 2006) was taken into account. Twenty out of 23 inputs (87%) of S0 salmon and 27 out of 40 inputs (68%) of S1 salmon reported PD outbreaks. No statistically significant differences were found (Fisher's exact test, $P = 0.133$). In terms of PD impact, borderline differences were found (OR = 3.56, Fisher's exact test, $P = 0.072$). 8 out of 20 populations of S0 PD-affected fish (40%) suffered high impact of PD, while 19 out of 27 populations of S1 PD-affected fish (70%) presented with high impact outbreaks.

Fallowing

Use of fallowing in these four generations of salmon at sea was analysed. No statistically significant differences were found in terms of PD occurrence or impact. A total of 37 out of 47 (79%) farms using fallowing were PD-positive and 10 out of 16 (63%) farms not using fallowing were PD-positive (Fisher's exact, $P = 0.319$). Twenty two out of 37 (60%) farms using fallowing had high impact of PD and 5 out of 10 (50%) farms not using fallowing had high impact of PD (Fisher's exact, $P = 0.723$).

History of PD

Borderline differences in terms of occurrence of PD were found between sites with or without previous history of PD in 2003, 2004, 2005 and 2006 (40 out of 50 sites (80%) with previous history of PD suffered PD and 13 out of 25 sites (52%) with no previous history of PD suffered PD (OR = 3.43, Fisher's exact, $P = 0.075$)). No statistically significant differences were found for impact of PD between both groups (24 out of 40 sites (60%) with previous history of PD suffered high impact of PD and 2 out of 7 sites (29%) with no previous history of PD suffered high impact of PD (Fisher's exact, $P = 0.118$)).

Livestock movement

Highly significant differences were found in percentage of PD affected inputs between groups of fish that were and were not moved during the production cycle in 2003, 2004, 2005 and 2006 (34 out 38 (90%) inputs that were moved during the production cycle had a PD outbreak and 13 out of 25 (52%) inputs that were not moved during the production cycle had a PD outbreak (OR = 7.85, Fisher's exact, $P = 0.001$)). No statistically significant differences were found in terms of PD impact: 19 out of 34 (56%) inputs that were moved during the production cycle suffered high impact of PD and 8 out 13 (62%) inputs that were not moved during the production cycle suffered high impact of PD (Fisher's exact, $P = 1.00$).

Strain

Data from 2003 to 2006 and data from 2005 and 2006 only were used to compare occurrence of PD and impact of PD between a total of 4 different strains of salmon. No statistically significant differences were found. Results are illustrated in Table 7.2.

Table 7.2. Percentage of inputs that suffered PD and high impact of PD for 2003 to 2006 period and only for 2005 and 2006.

	03, 04, 05 and 06 data				05 and 06 data			
STRAIN	% PD	P	% High mortality	P	% PD	P	% High mortality	P
X	80	0.213	68	0.089	75	1	58	0.387
All other types	63		33		75		33	
Y	62	0.076	56	1	69	0.67	33	0.387
All other types	84		58		80		58	
Z	75	1	67	1	75	1	67	0.586
All other types	75		57		75		44	
Other	80	1	50	1	67	1	50	1
All other types	74		58		76		47	

Bay and company

Data collected from 2003, 2004, 2005 and 2006 generation of salmon going to sea was used to calculate percentage of inputs per bay and per company that were PD-positive, and which proportion of them suffered a high impact of PD (Table 7.3 and 7.4). Interpretation of 2003 to 2006 data for PD presence demonstrated a significant association with company (P= 0.01) and bay (P = 0.02). No statistically significant differences were found between companies and bays, in terms of PD presence and impact, using 2005 and 2006 data.

Table 7.3. PD outbreaks and its impact per company

	03,04,05,06 generation			05 and 06 generation		
Company	% PD +ve	% high impact	inputs	% PD +ve	% high impact	Inputs
A	67	50	3	100	0	1
B	100	100	4	100	100	2
C	0	0	1	-	-	-
D	0	0	1	-	-	-
E	33	100	3	0	0	1
F	80	50	5	67	50	3
G	100	50	4	100	0	2
H	83	47	23	73	50	11
I	100	57	7	100	50	4
J	50	100	2	0	0	1
K	0	0	2	-	-	-
L	50	50	4	67	50	3
M	50	0	2	-	-	-
	Pr = < P 0.0111	Pr = < P 0.7261	Total=61	Pr = < P 0.3961	Pr = < P 0.7168	Total=28

Table 7.4. PD outbreak and its impact per bay

Bay	03,04,05,06 generation			05 and 06 generation		
	% PD +ve	% high impact	inputs	% PD +ve	% high impact	Inputs
N	100	0	1			
O	57	50	7	75	33	4
P	100	80	5	100	67	3
Q	20	100	5	0	0	1
R	33	0	3			
S	82	56	11	67	25	6
T	100	100	4	100	100	2
U	100	100	1			
V	0	0	1			
W	100	67	6	100	50	4
X	77	38	17	63	40	8
	Pr = < P 0.0208	Pr = < P 0.2677	Total=61	Pr = < P 0.4607	Pr = < P 0.7438	Total=28

Table 7.5. Categorical data statistical analysis results from 2003, 2004, 2005 and 2006 generation of salmon at sea

	% PD	**P**	% High mortality	**P**
SMOLT				
S0	87	0.133	40	0.072
S1	68		70	
YEAR				
2003	67	0.583	79	0.287
2004	86		50	
2005	80		50	
2006	69		44	
FALLOWING				
Yes	79	0.319	60	0.723
No	63		50	
HISTORY OF PD				
Yes	80	0.075	63	0.118
No	54		29	
LIVESTOCK MOVEMENT				
Yes	90	0.001	60	1.000
No	52		62	

Table 7.6. Categorical data statistical analysis results from 2005 and 2006 generation of salmon at sea

	% PD	**P**	% High mortality	**P**
SMOLT				
S0	73	1	25	0.183
S1	77		62	
YEAR				
2005	80	0.67	50	1
2006	69		44	

7.3.4. CONTINUOUS DATA ANALYSIS

Input number

No statistically significant differences were found when numbers of fish transferred to sea in each input from autumn 2002 to spring 2006 were compared (average for PD-positive sites = 361629 and average for PD-negative sites = 255500. Wilcoxon two sample test, $P = 0.347$). However, highly statistically significant differences were found when average input numbers of PD-positive and PD-negative sites for only 2005 and 2006 generation were compared (PD-positive = 378201 and PD-negative = 191328. Wilcoxon two sample test, $P = 0.008$). Significant differences in average input number were also found between populations that suffered high and low impact of PD from 2003, 2004, 2005 and 2006 generation (high impact = 450000 and low impact = 271344, Wilcoxon two sample test, $P = 0.049$). No statistically significant differences were found when the same was done with PD-positive populations from 2005 and 2006 generation (high impact = 492458 and low impact = 280000, Wilcoxon two sample test, $P = 0.359$).

Stocking density

Two different measurements from four different generations of fish (2003 to 2006) were used to evaluate stocking densities: fish/m^2 and kg of fish/m^3. Significant statistical differences were found in average stocking densities (kg of fish/m^3) at time of PD outbreak and average stocking densities at a similar time of PD-negative populations (6.4 kg/m^3 in PD positive sites and 2.9 kg/m^3 in PD-negative sites (Wilcoxon two sample test, $P = 0.016$). No statistically significant differences were found when average stocking densities in terms of fish/m^2 from PD-positive and PD-negative sites at a similar time were compared (85 fish/m^2 in PD positive sites and 124 fish/m^2 in PD-negative sites. Wilcoxon two sample test, $P = 0.146$). Average stocking densities from populations that suffered high and low impact of PD were compared. No statistically significant differences were found (high impact 90.1 fish/m^2 and low impact 65 fish/m^2. Wilcoxon two sample test, P=0.101. High impact = 6.2 fish/m^3 and low impact = 6.6 kg fish/m^3. Wilcoxon two sample test, P=0.468).

Days to outbreak

Populations from four different generations (2003 to 2006) that suffered from PD were used to calculate average number of days from transfer to sea until first signs of PD appeared. No statistically significant differences were found between groups of high and low impact of PD (high impact = 134 and low impact = 264. Wilcoxon two sample test, $P = 0.256$). The same occurred when data from 2005 and 2006 was used (high impact = 128 and low impact = 253, Wilcoxon two sample test, $P = 0.244$).

Feeding rate

Average feeding rates (% body weight) 4 weeks prior to detection of PD were compared between populations of salmon that suffered high and low impact PD between 2003 and 2006. No statistically significant differences were found between them (high mortality = 1.1 and low mortality = 0.9. Wilcoxon two sample test, $P = 0.35$).

Current speeds

No statistically significant differences were found when current speeds from PD-positive and PD-negative populations, as well as current speeds from high and low PD-impact sites were compared (PD-positive = 38.6 cm/s and PD-negative = 10 cm/s, Wilcoxon two sample test, P=0.170. High impact = 47.5 cm/s and low impact = 38.5 cm/s. Wilcoxon two sample test, $P = 0.457$).

Temperature at time of PD outbreak

No differences were found between average temperatures at time of PD outbreak for sites that suffered high and low PD impact during 2005 and 2006 (high impact = 13.8ºC and low impact = 15.5ºC. Wilcoxon two sample test, $P = 0.363$).

Days in 10 to 15ºC risk window

Using water temperature profiles for each site for 2005 and 2006, the number of days that seawater was within temperatures of ≥10ºC and ≤15ºC in each site was calculated. Highly statistically significant differences were found between average number of days that PD-positive sites were in the risk window in comparison with PD-negative sites (PD positive = 129 days and PD negative = 119 days. Wilcoxon two sample test, $P = 0.008$). Highly statistically significant differences were also found between groups of high and low PD impact (high impact = 139 days and low impact = 122 days. Wilcoxon two sample test, $P = 0.007$). Tables 7.7 and 7.8 summarize results from the statistical analysis of continuous variables.

Table 7.7. Continuous data statistical analysis results from 2003, 2004 2005 and 2006 generation salmon at sea

	Median		*P*	Median		*P*
	PD positive	PD negative		Low mortality	High mortality	
Stocking density (fish/m^2)	85	124	0.146	65	90.1	0.101
Stocking density (kg fish/m^3)	6.4	2.9	0.016	6.6	6.2	0.468
Input number	361629	255500	0.347	271344	450000	0.049
Days to outbreak	_	_	_	264	134	0.259
Feeding rate (% BW)	_	_	_	0.9	1.1	0.35

Table 7.8. Continuous data statistical analysis results from 2005 and 2006 generation salmon at sea

	Median		*P*	Median		*P*
	PD positive	PD negative		Low mortality	High mortality	
Days at risk window (10-15ºC)	129	119	0.008	122	139	0.007
Current speeds (cm/s)	38.6	10	0.1695	38.5	47.5	0.457
Input number	378201	191328	0.0057	280000	492458	0.359
Temperature at PD outbreak	_	_	_	15.5	13.8	0.363
Days to outbreak	_	_	_	264	134	0.244

7.4 Discussion

Similar surveys investigated the epidemiology of PD in Ireland in 2002 (McLoughlin *et al*., 2003) and for the period 2003-2004 (Rodger & Mitchell, 2007). The present survey aims to add to information regarding the epidemiology of PD, as well as recording aspects of fish health status in the Irish salmon industry during the period 2005 to 2007. Since the apparent re-emergence of PD in Ireland in 2002, the number of operating sites has decreased from 23 to 13 in 2006. As highlighted by Rodger & Mitchell (2007), the low number of sites in the study is an impediment to achieving results that are statistically significant. In order to reduce this problem, this study attempted to include as much data from as many different years as possible. Univariate statistics were calculated based on the assumption that all sites and years were independent.

The number of sites reporting PD outbreaks increased from 59% (13/22) in 2002 to 86% (12/14) in 2004. Data from 2006 year-class, where 69% (9/13) of sites reported PD, suggests a decrease in percentage of farms suffering PD. In terms of PD impact, the highest average mortality due to PD was reached in 2003 (18.8%) while average mortality due to PD for 2005 and 2006 was 9.8% and 9.6% respectively. Data discussed above is featured in Figures 7.1 and 7.2. Although these figures show a decreasing trend in number of sites suffering PD and its impact in the period 2004 to 2007, these differences were not statistically significant.

Although PD does not affect the farms synchronously, the number of PD outbreaks reported during the warmer months of the year (June to November) was higher than at any other time. Looking at historical data (Figure 7.5), a biphasic presentation was evident with peaks of reported number of PD outbreaks in early summer and early autumn. It is possible that the influence of certain environmental factors, such as seawater temperature, might have an effect on the presentation of outbreaks through the year. In Ireland during 2006, 4/9 (44.4%) outbreaks occurred in early summer, with one outbreak reported in August and another in September. No biphasic pattern was observed. Interestingly, summer of 2006 was considered to be the warmest since 1995, when seawater temperature was above 15ºC from 24th June to 24th September.

Pancreas Disease outbreaks in 2006 were shorter and occurred more acutely. While in 2005 the average length of a PD outbreak was 21 weeks (range 12-32), while in 2006 it was only 13 weeks (range 6-20). Similarly, in 2005 mortalities peaked 15 weeks after first signs on average (range 1-32) and only after 5 weeks in 2006 (range 3-6). The PD associated mortality in many outbreaks followed the bell shaped curve consistent with an infectious disease. However, in some cases more complex curves were present which may reflect multiple factors affecting the transmission and impact of the virus on the population or the influence of secondary and/or concurrent diseases.

The *Salmonid alphavirus* does not grow well *in vitro* above 15ºC, and high water temperatures have been considered to have a suppressing role in the presentation of PD outbreaks (McLoughlin *et al*., 1998). Using water temperature profiles for each site in 2005 and 2006, the number of days that seawater temperature ranged between 10 and 15ºC was calculated after PD diagnosis. This temperature range was considered as a risk window for Atlantic salmon to manifest clinical PD and/or present with a higher level of mortality due to PD. Statistically significant differences were found between PD-positive and PD-negative sites (P = 0.008) and between sites that suffered high and low PD impact (P = 0.007). Results suggested that the higher the number of days salmon spent in that risk window, the higher was the risk of occurrence and severity of PD. This variable alone or in combination with other variables e.g. fish movement, may be helpful for modelling a PD outbreak and predict the likelihood of occurrence and its impact for a particular site. This observation may be of significance and is consistent with reports of lower pathogenicity of SAV subtype 2 at 10°C compared to 14°C

(Moriette *et al*., 2006) and as suggested by Graham *et al*., (2007c) low temperatures may be more conducive to sub-clinical spread of the virus, despite prolonged viral survival times.

More than 15% of fish going to sea in Ireland died of gill problems during 2005 and 2006. However, PD remains the single most significant infectious disease affecting marine salmon farms in Ireland. In agreement with previous observations (Rodger & Mitchell, 2007), data from 2003 to 2006 had significant differences between bays ($P = 0.01$) and companies ($P = 0.02$) with a higher prevalence of PD in those bays and companies located in the western counties of Ireland (Counties Galway and Mayo). No significant differences were found in terms of PD impact for the 2003-2006 period. When data only from 2005 and 2006 was analysed in the same way, no statistical differences were found between bays and companies in terms of prevalence of PD and its impact. These findings suggest an endemic situation of PD in Ireland.

The stress known to be associated with transporting fish led to livestock movement being considered as a potential risk factor for PD. Previous studies showed a positive relationship between moving fish during the production cycle and the occurrence of PD. Analysis including data from 2003 to 2006 generation showed that fish that were moved during the production cycle were 7.85 times more likely to suffer PD ($P = 0.001$). However, caution in interpreting this observation is advised as many companies started to operate summer rearing sites (which entailed livestock movement) as a result of recurrent PD outbreaks, hence this observation may be a confounding factor.

The four year data also indicates that sites which contract PD and have a higher number of fish are more at risk from a higher mortality from PD than those with a lower number of fish on site (the high percentage mortality PD sites had mean numbers of 450,000 fish compared to those with low percentage mortality which had a mean of 271,344 fish). Although it is not established what levels of the virus are required to give rise to a clinical manifestation of PD, it has been found that levels of SAV subtypes 1 and 2 in the serum of infected salmonids can be as high as 10^{10} $TCID_{50}$/50µl (Jewhurst *et al*., 2004) and it can be considered that with higher numbers of fish viraemic and multiplying SAV, leading to higher viral shedding and subsequent accumulation in the microenvironment of a sea pen is more likely to lead to critical levels of viral loading in a farm, which will then overwhelm the natural defences of the fish and be one of the factors that leads to more significant clinical disease.

The results reported from this survey assist in the expansion of the knowledge of the epidemiology of SAV in farmed salmon. However, practical application of this knowledge is more complex and requires application on a site specific or company basis.

8. MANAGEMENT & MITIGATION OF PANCREAS DISEASE (WP6)

Neil Ruane, David Graham, Hamish Rodger

8.1 Disinfection against *Salmonid alphavirus*

Biosecurity protocols play an important role in preventing disease outbreaks and the spread of pathogens both on and between farms. In order to develop effective protocols, fundamental information on the biophysical properties of the pathogen in question needs to be determined. In the case of the *Salmonid alphavirus*, a study was undertaken by the Irish Salmon Growers Association in conjunction with the Veterinary Sciences Division of Queen's University Belfast to determine the influence of pH, temperature and common disinfectants on virus survival. The results of this research have been published (Graham *et al*., 2007c; 2007d) and the following section is a brief overview of the results from that study (see also Appendix III).

Results from the study showed that there was rapid inactivation of the virus (within five minutes of exposure), both at a pH of 4 and 12. Inactivation of the virus also occurred after 1 h at a temperature of 60^{o}C showing that the virus is susceptible to extremes of pH (low and high) and to high temperatures. Therefore, treatment and disposal of dead fish during an outbreak of pancreas disease using the common practices of ensiling (low pH), alkaline hydrolysis (high pH) or composting (high temperatures) can effectively inactivate the virus.

A range of common disinfectants (Virex, Virkon-S, Halamid (Chloramine-T), Fam 30 and Buffodine) were also tested for their ability to inactivate the virus. All disinfectants were found to be effective under most of the conditions tested, although some (Fam 30, Buffodine and Halamid) had a reduced efficacy in the presence of organic matter emphasising the importance of thorough cleaning prior to the application of a disinfectant.

8.2 Management strategies for pancreas disease

The following sections list some practical measures which are recommended for avoiding infection and/or reducing the impact of pancreas disease on a site.

Monitoring for pancreas disease.

- A range of diagnostic tools (histological, serological, molecular) are now available to monitor populations for infection and regular screening of the farm stock is advised. Use of these tools allows infection to be rapidly identified and appropriate mitigation factors put in place, prior to a major mortality event.
- Monitor the sea lice burden (weekly) and treat before clinical problems develop.

Recommendations specific to pancreas disease.

- Maintain a high level of site biosecurity with emphasis on personnel, visitors and equipment. Evidence to date suggests that horizontal rather than vertical transmission is responsible for the spread of infection in Ireland.
- If recovered populations are indeed a source of infection for naïve fish then efforts should be made to avoid overlapping these populations at a farm or bay level. Adherence to single bay management strategies are therefore encouraged.
- Fast fish for 5 to 10 days on a pen-by-pen basis if pancreas disease is detected at an early stage.
- If myopathies are significant then fish should be fasted during periods of spring tides/periods of strong currents.
- Mortality removal should be frequent (ideally daily with use of lift-up type systems).

Recommendations for the mitigation of pancreas disease.

- Specialised PD diets or functional foods should be utilised (when at risk or after confirmation of PD) which are more easily digestible, have higher micronutrient (antioxidants and vitamins) levels as well as immunostimulants.
- Select strains of fish suitable for sea site.
- Vaccinate all stock against pancreas disease (results of new vaccine pending).

General recommendations.

- Each site should adhere to the recommendations in their Fish Health Management Plan.
- Maintain a high standard of pen environment i.e. keep nets clean and maintain optimal oxygen levels (aerate or oxygenate during periods of low dissolved oxygen).
- Feed fish to tables or set farm percentage and not to appetite i.e. do not allow fish to gorge themselves.
- Where possible avoid management practices that will stress sick fish (e.g. movements, net changes)
- Mortality removal should be frequent (ideally daily with use of lift-up type systems).

8.3 Recommendations for future research

The Pancreas Disease Tri-nation group carried out a recent exercise to identify knowledge gaps and proposed areas of future research with regards to pancreas disease and the *Salmonid alphavirus*. Outputs from this exercise together with specific recommendations from this project are shown below.

Table 8.1. Areas for future research related to pancreas disease.

Objectives	RTDI Requirements	Key Outputs
Selective breeding for PD resistance.	Identification of susceptible/resistant traits. Benchmarking resistant families through sentinel trials.	Production of families/strains resistant to SAV infection.
Improved diagnostics.	Production of more antibodies specific to SAV. Development of practical and rapid diagnostic tests. Work on standardisation, validation and accreditation of diagnostic methods. Application of microarray technologies.	Increased availability of diagnostic tests for the rapid identification of SAV infections.
Increased knowledge of the salmonid alphavirus.	Increased knowledge on the virulence/pathogenicity of virus sub-types through sequencing and infection studies. Improvement of experimental transmission models. Improved knowledge of the disease cycle (transmission mechanisms, reservoirs, role of wild fish, disease triggers). Survival of the virus in seawater and freshwater.	Informed vaccine development and management and mitigation plans.
Improve prevention and/or treatment of the disease.	Development of more effective vaccines. Investigations into the effectiveness of PD diets and immunostimulants. Epidemiological studies to include meta-analysis from different countries, molecular epidemiology, modelling. Effectiveness of area management strategies.	Reduction of losses during and outbreak of pancreas disease.

A review of the information gathered in the course of this research project leads to the following conclusions.

- Pancreas disease is endemic in marine farmed Atlantic salmon in Ireland, but no evidence of infection in rainbow trout has been found.
- Screening of fish stocks for pancreas disease should be based on serological and/or molecular based methods.
- Confirmation of a clinical outbreak of pancreas disease should be based on site mortality figures, histopathology and serological and/or molecular methods as confirmatory tests.
- Seawater temperatures may play an important role in determining the length and severity of a pancreas disease outbreak.
- Survivors of a pancreas disease outbreak remain antibody positive against the virus for the remainder of the production cycle, thus are unlikely to get reinfected.
- Survivors of a pancreas disease outbreak can potentially act as a source of infection for naïve fish.
- No evidence was found to suggest that sea lice and mussels can transmit the virus.
- Although vertical transmission of the virus cannot be ruled out, it is likely that horizontal transmission of the virus between fish and adjacent sites is the primary route of infection.
- Strict biosecurity protocols and an adherence to the principles of single bay management are recommended.

REFERENCES

Bergmann, S. M., Castric, J., Brémont, M., Riebe, R. & Fichtner, D. **2005.** Detection of sleeping disease virus (SDV) in Germany. EAFP 12th International Conference, Copenhagen. Book of Abstracts O-150.

Boucher, P. & Baudin-Laurencin, F. **1994.** Sleeping disease of salmonids. *Bulletin of the European Association of Fish Pathologists* 14: 179-180.

Boucher, P. & Baudin-Laurencin, F. **1996.** Sleeping disease and pancreas disease comparative histopathology and acquired cross-protection. *Journal of Fish Diseases* 19: 303-310.

Boucher, P., Castric, J. & Baudin-Laurencin, F. **1994.** Observation of virus-like particles in rainbow trout *Oncorhynchus mykiss* infected with sleeping disease virulent material. *Bulletin of the European Association of Fish Pathologists* 14:215-216.

Branson, E. **2002.** Sleeping disease in trout. *The Veterinary Record* 150: 759-760.

Brun, E., Poppe, T., Skrudland, A. & Jarp, J. **2003.** Cardiomyopathy syndrome in farmed Atlantic salmon *Salmo salar*: occurrence and direct financial losses for Norwegian aquaculture. *Diseases of Aquatic Organisms* 56: 241-247.

Brun, E., Olsen, A. B. & Rørvik, L. **2005.** Factors associated with outbreaks of pancreas disease in farmed Atlantic salmon. EAFP 12th International Conference, Copenhagen. Book of Abstracts O-147.

Castric, J., Baudin-Laurencin, F., Brémont, M., Jeffroy, J., Le Ven, A. & Béarzotti, M. **1997.** Isolation of the virus responsible for sleeping disease in experimentally infected rainbow trout *Oncorhynchus mykiss*. *Bulletin of the European Association of Fish Pathologists* 17: 27-30.

Christie, K. E., Fyrand, K., Holtet, L., & Rowley, H. M. **1998.** Isolation of pancreas disease virus from farmed Atlantic salmon, *Salmo salar* L., in Norway. *Journal of Fish Diseases* 21: 391-394.

Christie, K. E., Graham, D. A., McLoughlin, M. F., Villoing, S., Todd, D. & Knappskog, D. **2007.** Experimental infection of Atlantic salmon *Salmo salar* pre-smolts by i.p. injection with new Irish and Norwegian salmonid alphavirus (SAV) isolates: a comparative study. *Diseases of Aquatic Organisms* 75: 13-22.

Crockford, T., Menzies, F. D., McLoughlin, M. F., Wheatley, S. B. & Goodall, E. A. **1999.** Aspects of the epizootiology of pancreas disease in farmed Atlantic salmon *Salmo salar* in Ireland. *Diseases of Aquatic Organisms* 36: 113-119.

Eliassen, T. M., Solbakk, I. T., Evensen, Ø. & Gravningen, K. **2004.** Isolation of heart and skeletal muscle inflammation virus (HSMIV) from salmon. Presented at the 6th International Symposium on Viruses of Lower Vertebrates, Hakodate, Japan.

Ferguson, H. W., Poppe, T. & Speare, D. J. **1990.** Cardiomyopathy in farmed Norwegian salmon. *Diseases of Aquatic Organisms* 8: 225-231.

Ferguson, H. W., Kongtorp, R. T., Taksdal, T., Graham, D. & Falk, K. **2005.** An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, *Salmo salar* L., with observations on myocardial regeneration. *Journal of Fish Diseases* 28: 119-123.

Fringuelli, E., Rowley, H.M., Wilson, J.C., Hunter, R., Rodger, H. & Graham, D.A. **2008.** Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. *Journal of Fish Diseases* (submitted).

Graham, D. A., Jewhurst, V. A., Rowley, H. M., McLoughlin, M. F. & Todd, D. **2003a.** A rapid immunoperoxidase-based virus neutralization assay for salmonid alphavirus used for a serological survey in Northern Ireland. *Journal of Fish Diseases* 26: 407-413.

Graham, D. A., Rowley, H. M., Walker, I. W., Weston, J. H., Branson, E. J. & Todd, D. **2003b.** First isolation of sleeping disease virus from rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom. *Journal of Fish Diseases* 26: 691-694.

Graham, D. A., Taylor, C., Rodgers, D., Weston, J., Khalili, M., Ball, N., Christie, K. E. & Todd, D. **2006.** Development and evaluation of a one-step real-time reverse transcription polymerase chain reaction assay for the detection of salmonid alphaviruses in serum and tissues. *Diseases of Aquatic Organisms* 70: 47-54.

Graham, D. A., Wilson, J. C., Jewhurst, H. & Rowley, H. M. **2007a.** Cultural characteristics of *Salmonid alphavirus* (SAV) – influence of cell line and temperature. EAFP 13th International Conference, Grado. Book of Abstracts P-218.

Graham, D. A., Rowley, H. M., Fringuelli, E., Bova, G., Amedeo, M., McLoughlin, M. F., Zarza, C., Khalili, M. & Todd, D. **2007b.** First laboratory confirmation of salmonid alphavirus infection in Italy and Spain. *Journal of Fish Diseases* 30: 569-572.

Graham, D. A., Staples, C., Wilson, C. J., Jewhurst, H., Cherry, K., Gordon, A. & Rowley, H. M. **2007c.** Biophysical properties of salmon alphaviruses: influence of temperature and pH on virus survival. *Journal of Fish Diseases* 30: 533-543.

Graham, D. A., Cherry, K., Wilson, C. J. & Rowley, H. M. **2007d.** Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *Journal of Fish Diseases* 30: 269-277.

Hodneland, K. & Endresen, C. **2006.** Sensitive and specific detection of *Salmonid alphavirus* using real-time PCR (TaqMan®). *Journal of Virological Methods* 131: 184-192.

Hodneland, K., Bratland, A., Christie, K. E., Endresen, C. & Nylund, A. **2005.** New subtype of salmonid alphavirus (SAV), *Togaviridae*, from Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* in Norway. *Diseases of Aquatic Organisms* 66: 113-120.

Jewhurst, V. A., Todd, D., Rowley, H. M., Walker, I. W., Weston, J. H., McLoughlin, M. F. & Graham, D. A. **2004.** Detection and antigenic characterization of salmonid alphavirus isolates from sera obtained from farmed Atlantic salmon, *Salmo salar* L., and farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 27: 143-149.

Karlsen, M., Hodneland, K., Endresen, C. & Nylund, A. **2006.** Genetic stability within the Norwegian subtype of salmonid alphavirus (family *Togaviridae*). *Archives of Virology* 151: 861-874.

Kongtorp, R. T., Taksdal, T. & Lyngøy, A. **2004a.** Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 59: 217-224.

Kongtorp, R. T., Kjerstad, A., Taksdal, T., Guttvik, A. & Falk, K. **2004b.** Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L.: a new infectious disease. *Journal of Fish Diseases* 27: 351-358.

Kongtorp, R. T., Halse, M., Taksdal, T. & Falk, K. **2006.** Longitudinal study of a natural outbreak of heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 29: 233-244.

McLoughlin, M. F. & Graham, D. A. **2007.** Alphavirus infections in salmonids – a review. *Journal of Fish Diseases* 30: 511-531.

McLoughlin, M. F., Nelson, R. T., Rowley, H .M., Cox, D. I. & Grant, A. N. **1996.** Experimental pancreas disease in Atlantic salmon *Salmo salar* post-smolts induced by salmon pancreas disease virus (SPDV). *Diseases of Aquatic Organisms* 26: 117-124.

McLoughlin, M. F., Rowley, H. M. & Doherty, C. E. **1998.** A serological survey of salmon pancreas disease virus (SPDV) antibodies in farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 21: 305-307.

McLoughlin, M. F., Nelson, R. T., McCormick, J. I., Rowley, H. M. & Bryson, D. G. **2002.** Clinical and histopathological features of naturally occurring pancreas disease in farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 25: 33-43.

McLoughlin, M. F., Peeler, E., Foyle, K. L., Rodger, H. D., Ó Ceallacháin, D. & Geoghegan, F. **2003.** An epidemiological investigation of the re-emergence of pancreas disease in Irish farmed Atlantic salmon (*Salmo salar* L.) in 2002. Marine Institute, *Marine Environment & Health Series* No. 14.

Menzies, F. D., McLoughlin, M. F., Wheatley, S. B. & Goodall, E. A. **1996.** Development of a computerized information retrieval system for Atlantic salmon, *Salmo salar* L., production. *Aquaculture Research* 27: 183-190.

Moriette, C., LeBerre, M., Lamoureux, A., Lai, T.-H. & Bremont, M. **2006.** Recovery of a recombinant salmonid alphavirus fully attentuated and protective for rainbow trout. *Journal of Virology* 80: 4088 – 4098.

Murphy, T. M., Rodger, H. D., Drinan, E. M., Gannon, F., Kruse, P. & Korting, W. **1992.** The sequential pathology of pancreas disease in Atlantic salmon farms in Ireland. *Journal of Fish Diseases* 15: 401-408.

Murray, A. & Kilburn, R. **2008.** Update on pancreas disease in Scotland. Presented at the 8th Tri-Nation Pancreas Disease Seminar, Galway, 7 – 8 May, 2008.

Nelson, R. T., McLoughlin, M. F., Rowley. H. M., Platten, M. A. & McCormick, J. I. **1995.** Isolation of a toga-like virus from farmed Atlantic salmon *Salmo salar* with pancreas disease. *Diseases of Aquatic Organisms* 22: 25-32.

Nylund, A., Plarre, H., Hodneland, K., Devold, M., Aspehaug, V., Aarseth, M., Koren, C. & Watanabe, K. **2003.** Haemorrhagic smolt syndrome (HSS) in Norway: pathology and associated virus-like particles. *Diseases of Aquatic Organisms* 54: 15-27.

Olsen, A. B. **2008.** Update on pancreas disease in Norway. Presented at the 8th Tri-Nation Pancreas Disease Seminar, Galway, 7 – 8 May, 2008.

Olsen, A. B. & Wangel, C. **1997.** An outbreak of PD-like disease in rainbow trout (*Oncorhynchus mykiss*) in Norway. EAFP 8th International Conference, Edinburgh. Book of Abstracts P-62.

Olsen, A. B., Bornø, G., Colquhoun, D. J., Flesjå, K., Haldorsen, R., Mo, T. A., Nilsen, H., Skjelstad, H. R. & Hjeltnes, B. **2007.** The health situation in salmonid fish 2006. In "*The Health Situation in Farmed Fish in Norway 2006*", a report by the National Veterinary Institute, Norway.

Petterson, E., Santi, N. & Sandberg, M. **2007.** Can sea lice function as a vector for transmission of *Salmonid alphavirus* subtype 3 in Atlantic salmon? EAFP 13th International Conference, Grado. Book of Abstracts P-19.

Plarre, H., Devold, M., Snow, M. & Nylund, A. **2005.** Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway. *Diseases of Aquatic Organisms* 66: 71-79.

Poppe, T., Rimstad, E. & Hyllseth, B. **1989.** Pancreas disease of Atlantic salmon (*Salmo salar* L.) post-smolts infected with infectious pancreatic necrosis virus (IPNV). *Bulletin of the European Association of Fish Pathologists* 9: 83-85.

Powers, A. M., Brault, A. C., Shirako, Y., Strauss, E. G., Kang, W., Strauss, J. H. & Weaver, S. C. **2001.** Evolutionary relationships and systematics of the alphaviruses. *Journal of Virology* 75: 10118-10131.

Raynard, R. S., Murray, A. G. & Gregory, A. **2001.** Infectious salmon anaemia virus in wild fish from Scotland. *Diseases of Aquatic Organisms* 46: 93-100.

Rodger, H. **2007.** Pancreas disease in Ireland: epidemiological investigations. Presented at the 6th Tri-Nation Pancreas Disease Seminar, Aberdeen, 19 - 20 April, 2007.

Rodger, H. **2008.** Update on the PD situation in Ireland. Presented at the 7th Tri-Nation Pancreas Disease Seminar, Galway, 7 - 8 May, 2008.

Rodger, H. & Turnbull, T. **2000.** Cardiomyopathy syndrome in farmed Scottish salmon. *The Veterinary Record* 146: 500-501.

Rodger, H. & Mitchell, S. **2007.** Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. *Journal of Fish Diseases* 30: 157-167.

Rowley, H. M., Doherty, C., McLoughlin, M. F. & Welsh, M. D. **1998.** Isolation of salmon pancreas disease virus (SPDV) from Scottish farmed salmon. *Journal of Fish Diseases* 21: 469-471.

Ruane, N., Rodger, H., Graham, D., Foyle, L., Norris, A., Ratcliff, J., Murphy, K., Mitchell, S., Staples, C., Jewhurst, H., Todd, D., Geoghegan, F. & Ó Cinneide, M. **2005.** Research on pancreas disease in Irish farmed salmon 2004/2005 – current and future initiatives. Marine Institute, *Marine Environment & Health Series* No. 22.

Sammels, L. M., Lindsay, M. D., Poidinger, M., Coelen, R. J. & Mackenzie, J. S. **1999.** Geographic distribution and evolution of Sindbis virus in Australia. *Journal of General Virology* 80: 739-748.

Skjelstad, H. R., Bornø, G., Flesjå, K., Hansen, H., Nilsen, H., Wasmuth, M. A. & Hjeltnes, B. **2008.** *Helsesituasjonen hos laksefisk 2007*. National Veterinary Institute Report Series, Norway.

Strauss, J. H. & Strauss, E. G. **1994.** The alphaviruses: gene expression, replication and evolution. *Microbiological Reviews* 58: 491-562.

Taksdal, T., Olsen, A. B., Bjerkås, I., Hjortaas, M. J., Dannevig, B. H., Graham, D. A. & McLoughlin, M. F. **2007.** Pancreas disease in farmed Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Norway. *Journal of Fish Diseases* 30: 545-558.

Todd, D., Jewhurst, V. A., Welsh, M. D., Borghmans, B. J., Weston, J. H., Rowley, H. M., Mackie, D. P. & McLoughlin, M. F. **2001.** Production and characterisation of monoclonal antibodies to salmon pancreas disease virus. *Diseases of Aquatic Organisms* 46: 101-108.

Villoing, S., Béarzotti, M., Chilmonczyk, S., Castric J. & Brémont, M. **2000a.** Rainbow trout sleeping disease virus is an atypical alphavirus. *Journal of Virology* 74: 173-183.

Villoing, S., Castric, J., Jeffroy, J., Le Ven, A., Thiery, R. & Brémont M. **2000b.** An RT-PCR-based method for the diagnosis of the sleeping disease virus in experimentally and naturally infected salmonids. *Diseases of Aquatic Organisms* 40: 19-27.

Waarts, B.L. **2004.** Cell entry mechanisms of alphaviruses: receptor interaction and membrane fusion in a liposomal model system. PhD Thesis, Rijksuniversiteit Groningen.

Wallace, I. S., Gregory, A., Murray, A. G., Munro, E. S. & Raynard, R. S. **2008.** Distribution of infectious pancreatic necrosis virus (IPNV) in wild marine fish from Scottish waters with respect to clinically infected aquaculture sites producing Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 31: 177-186.

Welsh, M., Weston, J., Borghmans, B. J., Mackie, D., Rowley, H., Nelson, R., McLoughlin, M. & Todd, D. **2000.** Biochemical characterization of salmon pancreas disease virus. *Journal of General Virology* 81: 813-820.

Weston, J. H., Welsh, M. D., McLoughlin, M. F. & Todd, D. **1999.** Salmon pancreas disease virus, an alphavirus infecting farmed Atlantic salmon, *Salmo salar* L. *Virology* 256: 188-195.

Weston, J., Villoing, S., Brémont, M., Castric, J., Pfeffer, M, Jewhurst, V., McLoughlin, M., Rødseth, O. M., Christie, K. E., Koumans, J. & Todd, D. **2002.** Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequencing analysis, monoclonal reactivity, and cross-infection. *Journal of Virology* 76, 6155-6163.

Weston, J. H., Graham, D. A., Branson, E., Rowley, H. M., Walker, I. W., Jewhurst, V. A., Jewhurst, H. L. & Todd, D. **2005.** Nucleotide sequence variation in salmonid alphaviruses from outbreaks of salmon pancreas disease and sleeping disease. *Diseases of Aquatic Organisms* 66: 105-111.

Wheatley, S. B., McLoughlin, M. F., Menzies, F. D. & Goodall, E. A. **1995.** Site management factors influencing mortality rates in Atlantic salmon (*Salmo salar* L.) during marine production. *Aquaculture* 136: 195-207.

APPENDIX I. Project Outputs

Pancreas disease meetings supported by the project ST/05/01.

Meeting	Date	Place
Initial project meeting	15.11.2005	MI, Galway
Tri-nation	01.02.2006	Oslo
Project	26.05.2006	MI, Galway
Tri-nation	21-22.09.2006	MI, Galway
Project	06.12.2006	MI, Galway
Tri-nation	19-20.04.2007	Aberdeen
Project	31.05.2007	MI, Galway
Tri-nation	15-16.11.2007	Bergen
Final project meeting/Tri-nation	7-8.05.2008	MI Galway

Presentations/Posters

Fringuelli, E., Rowley, H. M., Wilson, J. C., Hunter, R., Rodger, H., Ruane, N. M. & Graham, D. A. Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV). EAFP 13th International Conference, 17 – 22 September, Grado. Book of Abstracts O-113.

Graham, D. A., Fringuelli, E., Wilson, J. C., Ruane, N. M., Foyle, L. & Rowley, H. M. Application of multiple diagnostic tests to a prospective longitudinal study of infection with *Salmonid alphavirus* – a comparative study. EAFP 13th International Conference, 17 – 22 September, Grado. Book of Anstracts P-29.

Graham, D. A., Wilson, J. C., Jewhurst, H. & Rowley, H. M. Cultural characteristics of *Salmonid alphavirus* (SAV) – influence of cell line and temperature. EAFP 13th International Conference, 17 – 22 September, Grado. Book of Abstracts P-218.

Publications

Fringuelli, E., Rowley, H.M., Wilson, J.C., Hunter, R., Rodger, H. & Graham, D.A. **2008**. Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. Journal of Fish Diseases (in press).

Graham, D. A., Rowley, H. M., Fringuelli, E., Bova, G., Amedeo, M., McLoughlin, M. F., Zarza, C., Khalili, M. & Todd, D. **2007**. First laboratory confirmation of salmonid alphavirus infection in Italy and Spain. Journal of Fish Diseases 30: 569-572.

Graham, D. A., Wilson, C. J., Jewhurst, J. L. & Rowley, H. M. 2008. Cultural characteristics of salmonid alphaviruses (SAV) – influence of cell line and temperature. Journal of Fish Diseases (in press).

Rodger, H. & Mitchell, S. **2007**. Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. Journal of Fish Diseases 30: 157-167.

Ruane, N., Rodger, H., Graham, D., Foyle, L., Norris, A., Ratcliff, J., Murphy, K., Mitchell, S., Staples, C., Jewhurst, H., Todd, D., Geoghegan, F. & Ó Cinneide, M. **2005**. Research on pancreas disease in Irish farmed salmon 2004/2005 – current and future initiatives. Marine Institute, Marine Environment & Health Series No. 22.

APPENDIX II Sampling protocol for longitudinal studies.

Sampling was conducted every 14 days, from each set of 20 fish, the following samples were collected:

Sample Type	Sample ID	Molecular	Virology	Histology
Heart	H	√	√	√
Anterior kidney	K	√	√	√
Brain	B	√	√	√
Pyloric caecae	C	√	√	√
Pseudobranch	P	√		√
Gill	G	√	√	√
Lateral line muscle	M			√
Serum	S	√	√	
Lice	LC (*Caligus*) LL (*Lepeophtheirus*)	√		

PROTOCOL

Sampling tissues to RNA*later*® (molecular)

- From each fish take a sample of heart approximately 0.5cm x 0.5cm x 0.5cm in size.
- Place in a 1.5 mL plastic tube containing 1 mL RNA*later*® (supplied pre-dispensed).
- Label with sample identifier (H), FHU reference number and fish number.
- Repeat for other tissues, using a separate tube for each tissue.
- If lice are present on an individual fish, sample a maximum of 10 lice. If both species are present, place in separate vials, labelled with fish number and species and the species of lice as given above.

Sampling tissues for virus isolation and histology

- Virology:
    - From each fish take a sample of heart tissue (>0.5g [1cm x 1cm x 0.5cm) and place in a falcon tube containing 4 mL transport medium and label with FHU reference number and fish number.
    - Repeat for other tissues, placing all tissues from the same fish in the **same** tube.
- Histology
    - From first 5 fish (10 if clinical signs) sample as above, placing all tissues from each fish in the same formalin pot. Pyloric caecae/pancreas, heart and muscle will be processed initially, with the other samples stored until PD has been diagnosed.

Sampling serum

- Take 0.5 – 1 mL blood from each fish.
- Allow red cells to clot/settle (e.g. 4ºC overnight).
- Centrifuge sample and remove serum to a blood tube labelled with FHU reference number and fish number.
- NB Avoid haemolysis during sample collection/processing.

Shellfish

10 mussels should be taken from each cage from which fish are being sampled on a given day, placed in a labelled bag (cage, species, date).

Storage

Formalin samples should be stored in the dark at room temperature.
The remainder of the samples should be stored at –20ºC or below.

APPENDIX III. A summary of disinfection data for Salmonid alphavirus based on Graham *et al*. (2007c; 2007d).

Method	Dose	Result
Heat	60°C for 1 hour	Inactivation
Acid	pH 4 for 5 mins	Inactivation
Alkaline	pH 12 for 5 mins	Inactivation
Peroxygen compounds (Virex, Virkon*)	0.5 – 2%; 4 or 10°C	Inactivation (5 – 30 mins)
Chloramine T (Halamid) [$]	0.5 – 2%; 4 or 10°C	Inactivation (5 – 30 mins)
Iodophors (FAM 30, Vanodine)[$]	1/600 – 1/125; 10°C	Inactivation (30 mins)
Buffered iodophors (Buffodine)[#, $]	1 – 2%;10°C	Inactivation (30 mins)

* Forms a precipitate when dissolved in seawater, therefore no testing was performed in seawater.

[#] Not tested in seawater.

[$] Reduced efficacy in the presence of organic material.

APPENDIX IV. Tri-Nation Seminar Agenda (September 2006).

5th Tri-Nation Seminar on Pancreas Disease & Related Pathologies
20 – 21 September 2006
North Atlantic Drift Auditorium
Marine Institute, Galway, Ireland

20 September 2006

Introduction

(G. Ritchie, Marine Harvest ASA; M. Ó Cinnéide, Marine Institute) 09.15 – 10.00
- minutes from Oslo Tri-Nation Meeting, 1 Feb 2006
- PD update 2006 + meeting objectives

Session I. Project Updates & Practical Outputs

I.I Epidemiology (E. Brun, National Veterinary Institute; M. Sandberg, Norwegian School of Veterinary Science) 10.00 – 11.15
- inc. update on Norway, Scotland
- Ireland (H. Rodger, Vet-Aqua Int.)

I.II Aetiology & Longitudinal Studies (D. Graham, AFBINI) 11.30 – 12.45
- inc. Marine Harvest study (C. Wallace, Marine Harvest Scotland)

I.III General Diagnostics (H. Sindre, National Veterinary Institute) 12.45 – 13.30
- inc. SARF project in Scotland (J. Costa, Stirling Univ.)

Lunch 13.30 – 14.30

Session II. Update on Related Pathologies & Research Projects (N. Ruane, MI)

Recent Norwegian projects to support management and control of PD
Ase-Helen Garseth (VESO) 15.00 – 15.30

Salmon Heart Diseases: HSS, HSMI, CMS, and SAV disease
Are Nylund (University of Bergen) 15.30 – 16.00

Research update on Heart & Skeletal Muscle Inflammation
Oystein Evensen (Norwegian School of Vet. Sci.) 16.15 – 16.45

21 September 2006

Session II (cont). Update on Related Pathologies & Research Projects (N. Ruane, MI)

Heritability of mortality in response to a natural PD challenge in salmon post-smolts
Ashie Norris (Marine Harvest Ireland) 9.15 – 9.45

Biophysical properties of SAV
David Graham (AFBINI/QUB) 9.45 – 10.15

Vertical transmission studies of SPDV
J. Castric (AFSSA, France) 10.15 – 10.45

Q-PCR assay for gene expression studies during SAV infection in salmonid cell lines
Marius Karlsen (University of Bergen) 10.45 – 11.15

Session III: Future Projects & Funding (N. Ruane MI; B. Hjeltnes, National Veterinary Institute) 11.30 – 13.30
- discussion on FP7 funding
- other projects/focus areas/issues
- conclusions & actions

Lunch 13.30 – 14.30

Session IV: Wrap Up (G. Ritchie MH; M. O Cinnéide MI)
- milestone reporting; overview, publication in trade press 14.30 – 15.30
- next meeting

APPENDIX V. Tri-Nation Seminar Attendees (September 2006).

	Name	Institute/Company
1	Gordon Ritchie	Marine Harvest ASA
2	Neil Ruane	Marine Institute, Ireland
3	Micheal O Cinneide	Marine Institute, Ireland
4	Fiona Geoghegan	Marine Institute, Ireland
5	Catherine Henderson	Marine Institute, Ireland
6	David Graham	Agri-Food & Biosciences Institute, N. Ireland
7	Helen Rowley	Agri-Food & Biosciences Institute, N. Ireland
8	Heather Jewhurst	Agri-Food & Biosciences Institute, N. Ireland
9	Kim Cherry	Agri-Food & Biosciences Institute, N. Ireland
10	Claire Wilson	Agri-Food & Biosciences Institute, N. Ireland
11	Tracy Clegg	University College Dublin, Ireland
12	Leo Foyle	University College Dublin, Ireland
13	Hamish Rodger	Vet-Aqua International, Ireland
14	Albert Girons	Vet-Aqua International, Ireland
15	Ashie Norris	Marine Harvest Ireland
16	Marian McLoughlin	Aquatic Vet. Services, N. Ireland
17	Olav Breck	Marine Harvest Norway
18	John Finlay	Nutreco
19	Andy Reeve	AquaGen/Nor West Aqua, Scotland
20	Ronald Roberts	University of Idaho/Landcatch, Scotland
21	Chris Mitchell	Landcatch, Scotland
22	Ed Branson	Fish Veterinary Society, England
23	David Bruno	Fisheries Research Services, Scotland
24	Trevor Hastings	Fisheries Research Services, Scotland
25	Kjell Maroni	Norwegian Seafood Federation
26	Are Nylund	University of Bergen, Norway
27	Marius Karlsen	University of Bergen, Norway
28	Kjartan Hodneland	University of Bergen, Norway
29	Kim Thompson	University of Stirling, Scotland
30	Janina Costa	University of Stirling, Scotland
31	Øystein Evensen	Norwegian School of Veterinary Science
32	Jeanette Castric	AFSSA, France
33	Karen Elina Christie	Intervet, Norway
34	Marianne Sandberg	Norwegian School of Veterinary Science
35	Edgar Brun	National Veterinary Institute, Norway
36	Brit Hjletnes	National Veterinary Institute, Norway
37	Hilde Sindre	National Veterinary Institute, Norway
38	Christian Wallace	VESO Vikan, Norway
39	Åse Helen Garseth	VESO Trondheim, Norway
40	David Stone	CEFAS, England
41	Dave Cockerill	Marine Harvest Scotland
42	Alasdair MacLennan	Marine Harvest Scotland
43	Chris Wallace	Marine Harvest Scotland
44	Carol Cox	Marine Harvest Scotland
45	Catherine McManus	Marine Harvest Ireland
46	Karen McCrann	Mannin Bay Salmon Company, Ireland
47	Joe McElwee	Irish Salmon Growers' Association

48	Abdon Ryan	Celtic Atlantic, Ireland
49	Damien Ó Ceallachain	Meitheal Eisc Teo., Ireland
50	Daragh Ó Fhlatharta	Eisc Ui Fhlatharta, Ireland
51	Louise Collins	Bord Iascaigh Mhara, Ireland
52	Joe Lee	Meitheal Eisc Teo., Ireland
53	Patrick Smith	Schering Plough Aquaculture, England
54	Declan Clarke	National University of Ireland, Galway
55	Gery Flynn	Inshore Ireland
56	Aengus Parsons	Marine Institute, Ireland
57	Laura Hogan	Marine Institute, Ireland
58	Michelle Geary	Marine Institute, Ireland
59	David Swords	Marine Institute, Ireland

APPENDIX VI. Tri-Nation Seminar Agenda (May 2008).

8th Tri-Nation PD(+) seminar
7 – 8 May, 2008
Marine Institute, Galway
Seminar Programme

Wednesday May 7

Introduction and overview of the Tri-Nation consortium	9.15 – 9.30
Neil Ruane (Marine Institute) & Gordon Richie (Marine Harvest ASA)	

Session 1. Scene setting (Chair: Neil Ruane)

"Update on pancreas disease in Scotland"	
Sandy Murray, FRS Aberdeen	9.30 – 9.50
"Update on pancreas disease in Ireland"	
Hamish Rodger, Vet-Aqua International	9.50 – 10.10
"Update on pancreas disease in Norway"	
Anne Berit Olsen, National Veterinary Institute, Norway	10.10–10.30

Session 2. Research Presentations (Chair: David Graham)

"Descriptive results from the Norwegian cohort study"	
Mona Jansen, Norwegian School of Veterinary Sciences	10.30–11.00
COFFEE 11.00 – 11.30	
"A longitudinal study of PD in Ireland"	
David Graham, Agri-Food & Biosciences Institute	11.30–11.50
"Modelling risk factors for outbreaks of HSMI and PD in Norway"	
Anja Kristoffersen, NVI	11.50–12.10
"Modelling sea currents and risks of disease transmission among aquaculture sites"	
Hildegunn Viljugrein, NVI	12.10–12.30
"Isolation of Salmonid alphavirus from sea lice"	
Marianne Sandberg, NSVS	12.30–12.50
"PD work at the Institute of Aquaculture, University of Stirling: An Update"	
Tharangani Herath, Kim Thompson, Alexandra Adams, Randolph Richards	12.50–13.10
LUNCH 13.10 – 14.00	
"Experimental transmission of cardiomyopathy in Atlantic salmon"	
David Bruno, FRS Aberdeen	14.00–14.20
"Experimental transmission of CMS in Atlantic salmon"	
Camilla Fritsvold, NVI	14.20–14.45
"Studies on experimental transmission of HSMI"	
Ruth Torill Kongtorp, NVI	14.45–15.10
Open discussion	15.10–15.30
COFFEE 15.30 – 16.00	

Extra Session. Gill Pathologies (Chair: Gordon Ritchie)

An extra session will be included to discuss the issues related to gill pathologies and interest in developing a similar Tri-Nation approach to dealing with the problem will be sought.

Presentations & open discussion	16.00–17.30

Thursday May 8

Session 3. Open session: Industry Research Projects (Chair: Gordon Richie)

"Radical change is inevitable to combat PD"
Cato Lyngoy, Marine Harvest Norway 9.15 – 9.35

"Unexpected challenges with real-time PCR analyses on PDV-vaccinated fish"
Vidar Auspehaug, Patogen 9.35 – 9.55

"PD vaccination: additional methods for efficacy evaluation"
Petter Frost, Intervet 9.55 – 10.15

"React PD: dietary mitigation"
Charles McGurk, Skretting. 10.15–10.35

"Assessing the efficacy of a PD diet"
Adel El-Mowafi, EWOS Innovation; Marianne Sandberg, NSVS 10.35–10.55

COFFEE 10.55 – 11.20

Session 4. Expert panel (Chair: Micheal Ó Cinneide)

An expert panel will be put together to answer questions from the industry in relation to pancreas disease. 11.20–12.20

"Closing discussion" Gordon Richie (Marine Harvest ASA) & Micheal Ó Cinneide (Marine Institute) 12.20–13.15

LUNCH 13.15 – 14.00

APPENDIX VII. Tri-Nation Seminar Attendees (May 2008).

	Name	Institute/Company
1	Gordon Ritchie	Marine Harvest ASA
2	Neil Ruane	Marine Institute, Ireland
3	Micheal Ó Cinneide	Marine Institute, Ireland
4	Kathy Henshilwood	Marine Institute, Ireland
5	Oonagh McMeel	Marine Institute, Ireland
6	Stephen McCleary	Marine Institute, Ireland
7	Cathy Hickey	Marine Institute, Ireland
8	Lorraine McCarthy	Marine Institute, Ireland
9	Marcel Curé	Marine Institute, Ireland
10	Hamish Rodger	Vet Aqua International, Ireland
11	Susan Mitchell	Vet Aqua International, Ireland
12	Louise Henry	Vet Aqua International, Ireland
13	David Graham	Agri-Food & Biosciences Institute, N. Ireland
14	Marian McLoughlin	Aquatic Veterinary Services, N. Ireland
15	Iain Shaw	National University of Ireland, Galway
16	Tom Doyle	University College Cork, Ireland
17	Damien Ó Ceallachain	Meitheal Eisc Teo., Ireland
18	Joe Lee	Meitheal Eisc Teo., Ireland
19	Abdon Ryan	Celtic Atlantic, Ireland
20	Gery Flynn	Inshore Ireland
21	Isabel Valera	Bord Iascaigh Mhara, Ireland
22	Cato Lyngøy	Marine Harvest Norway
23	Anne Berit Olsen	National Veterinary Institute, Norway
24	Edgar Brun	National Veterinary Institute, Norway
25	Torunn Taksdal	National Veterinary Institute, Norway
26	Camilla Fritsvold	National Veterinary Institute, Norway
27	Ruth Torill Kongtorp	National Veterinary Institute, Norway
28	Hildegunn Viljugrein	National Veterinary Institute, Norway
29	Marianne Sandberg	Norwegian School of Veterinary Science
30	Mona Jansen	Norwegian School of Veterinary Science
31	Anja Kristoffersen	Norwegian School of Veterinary Science
32	Linda Andersen	University of Bergen, Norway
33	Kjell Maroni	Norwegian Seafood Federation
34	Rudi Seim	Immunocorp, Norway
35	Petter Frost	Intervet, Norway
36	Andre Bratland	Havbruksinstituttet, Norway
37	Vidar Auspehaug	Patogen, Norway
38	Magnus Devold	Patogen, Norway
39	Charles McGurk	Skretting, Norway
40	Adel El-Mowafi	EWOS Innovation, Norway
41	Marie Egenberg	Pharmaq, Norway
42	David Bruno	Fisheries Research Services, Scotland
43	Patricia Noguera	Fisheries Research Services, Scotland
44	Sandy Murray	Fisheries Research Services, Scotland
45	Randolph Richards	University of Stirling, Scotland
46	Darren Green	University of Stirling, Scotland
47	Kim Thompson	University of Stirling, Scotland
48	Chris Mitchell	Landcatch, Scotland
49	Chris Gould	Schering Plough Aquaculture, England

MEHS Publications

1. O'Donohoe, G., Hensey, M., O'Connor, B. (2000) Assessment of Water Quality Data from Kilkieran Bay, Co. Galway.

2. McGovern, E., Rowe, A., McHugh, B., Costello, J., Bloxham, M., Duffy, C., Nixon, E. (2001) Trace Metal and Chlorinated Hydrocarbon Concentrations in Shellfish from Irish Waters, 1997-1999.

3. Coyne, R., Smith, P., Moriarty, C. (2001) The Fate of Oxytetracycline in the Marine Environment of a Salmon Cage Farm.

4. McGovern, E., Monaghan, E., Bloxham, M., Rowe, A., Duffy, C., Quinn, A., McHugh, B., McMahon, T., Smyth, M., Naughton, M., McManus, M., Nixon, E. (2002) Winter Nutrient Monitoring of the Western Irish Sea - 1990 to 2000.

5. Minchin, D., Lucy, F., Sullivan, M. (2002) Monitoring of Zebra Mussels in the Shannon-Boyle Navigation, Other Navigable Regions and Principal Irish Lakes, 2001 & 2002.

6. Minchin, D. (2003) Monitoring of Tributyl Tin Contamination in Six Marine Inlets using Biological Indicators.

7. Glynn, D., Tyrrell, L., McHugh, B., Rowe, A., Costello, J., McGovern, E. (2003) Trace Metal and Chlorinated Hydrocarbon Concentrations in Shellfish from Irish Waters, 2000.

8. Tyrrell, L., Glynn, D., Rowe, A., McHugh, B., Costello, J., Duffy, C., Quinn, A., Naughton, M., Bloxhan, M, Nixon, E., McGovern, E. (2003) Trace Metal and Chlorinated Hydrocarbon Concentrations in Various Fish Species, Landed at Selected Irish Ports, 1997-2000.

9. Telfor, T. & Robinson, K. (2003) Environmental Quality and Carrying Capacity for Aquaculture in Mulroy Bay Co. Donegal.

10. Glynn, D., Tyrell, L., McHugh, B., Rowe, A., Monaghan , E., Costello, J., McGovern, E. (2003) Trace Metal and Chlorinated Hydrocarbon Concentrations in Shellfish from Irish Waters, 2001.

11. Grehan, A., Long, R., Deegan, B., O'Cinneide, M. (2003) The Irish Coral Task Force and Atlantic Coral Ecosystem Study Report on Two deep-Water Coral Conservation Stakeholder Workshops held in Galway in 2000 and 2002.

12. McHugh, B., Glynn, D., Nixon, E., McGovern, E. (2003) The Occurrence and Risk Assessment of the Pesticide Toxaphene in Fish from Irish Waters 2003.

13. Tyrrell, L., Glynn, D., McHugh, B., Rowe, A., Monaghan, E., Costello, J., McGovern, E. (2003) Trace Metal and Chlorinated Hydrocarbon Concentrations in Various Fish Species Landed at Selected Irish Ports, 2001.

14. McLoughlin, M.F., Peeler, E., Foyle, K.L., Rodger, H.D., O'Ceallachain, D., Geoghegan, F. (2003) An Epidemiological Investigation of the Re-emergence of Pancreas Disease in Irish Farmed Atlantic Salmon (Salmo Salar L.) in 2002.

15. Cronin, M., Cusack, C., Geoghegan, F., Jackson, D., McGovern, E., McMahon, T., O'Beirn, F., O'Cinneide M., Silke, J. (2004) Salmon Mortalities at Inver Bay and McSwynes Bay Finfish Farms, County Donegal, Ireland during 2003.

16. Glynn, D., Tyrrell, L., McHugh, B., Monaghan, E., Costello, J., McGovern, E. (2004) Trace Metal and Chlorinated Hydrocarbon Concentrations in Shellfish from Irish waters, 2002. .

17. Werner, A. Kraan, S.(2004) Review of the Potential Mechanisation of Kelp Harvesting in Ireland.

18. Tyrrell, L., Twomey, M., Glynn, D., McHugh, B., Joyce, E., Costello, J., McGovern, E. (2004) Trace Metal and Chlorinated Hydrocarbon Concentrations in Various Fish Species Landed at Selected Irish Ports, 2002.

19. (2005) Proceedings of the 5th Irish Shellfish Safety Scientific Workshop.

20. Tyrrell L., McHugh B., Glynn D., Twomey M., Joyce E., Costello J., McGovern E. (2005) Trace Metal Concentrations in Various Fish Species Landed at Selected Irish Ports 2003.

21. Silke J., O Beirn, F., Cronin M. (2005) *Karenia mikimotoi:* An Exceptional Dinoflagellate Bloom in Western Irish Waters - Summer 2005.

22. Ruane, N., Graham, D., Foyle, L, Norris, A., Ratcliff, J., Murphy, K. Mitchell, S. Staples, C., Jewhurst, H., Todd, D., Geoghegan, F. & O'Cinneide, M. (2005) Research on Pancreas Disease in Irish Farmed Salmon 2004/2005 - Current and Future Initiatives.

23. (2006) Proceedings of the 6th Irish Shellfish Safety Scientific Workshop.

24. Cronin M., McGovern E., McMahon T. Boelens R. (2006) Guidelines For The Assessment of Dredge Material For Disposal In Irish Waters.

25. Boyle B., Tyrrell L., McHugh B., Joyce E., Costello J., Glynn D., McGovern E. (2006) Trace Metal Concentrations in Shellfish from Irish Waters, 2003.

26. Tlustos C., McHugh, B., Pratt. I., Tyrrell L., McGovern E. (2006) Investigation into Levels of Dioxins, Furans, Polychlorinated Biphenyls and Brominated Flame Retardants in Fishery Produce in Ireland.

27. (2007) Proceedings from the 7th Irish Shellfish Safety Workshop.

28. Hess P., McCarron P, Rehmann N., Kilcoyne J., McMahon T., Ryan G., Ryan, M.P., Twiner M. J., Doucette G.J., Satake M., Ito E., Yasumoto T. (2007) Isolation and Purification of AZAs from Naturally Contaminated Materials, and Evaluation of their Toxicological Effects (ASTOX).

29. Culloty S. & Mulcahy M. (2007) *Bonamia ostrea* in the Native Oyster Ostrea edulis: A Review.

30. Ruane N., Geoghegan F., O'Cinneide M. (2007) Infectious Pancreatic Necrosis Virus and its Impact on the Irish Salmon Aquaculture and Wild Fish Sectors.

31. Maguire J. A., Knights T. Burnell G., Crowe, T., O'Beirn, F., McGrath, D., Ferns M., McDonough, N., McQuaid N., O'Connor, B., Doyle R., Newell C., Seed R., Smaal, A., O'Carroll, T., Watson L., Dennis J., O'Cinneide M. (2007) Management Recommendations for the Sustainable Exploitation of Mussel Seed in the Irish Sea.

32. O'Mahony C., Sutton G., McMahon T., Ó'Cinnéide M., Nixon E. (2008) Issues and Recommendations for the Development and Regulation of Marine Aggregate Extraction in the Irish Sea.

33. McMahon T., Deegan B., Silke J., O'Cinneide M. (2008) Proceedings of the 8th Irish Shellfish Safety Workshop.

www.marine.ie

ISSN 1649 0053

HEADQUARTERS & LABORATORIES

MARINE INSTITUTE
Rinville
Oranmore
Co. Galway
Tel: +353 91 387 200
Fax: +353 91 387 201
Email: institute.mail@marine.ie

MARINE INSTITUTE REGIONAL OFFICES

MARINE INSTITUTE
80 Harcourt Street
Dublin 2
Tel: +353 1 476 6500
Fax: +353 1 478 4988

MARINE INSTITUTE
Furnace
Newport
Co. Mayo
Tel: +353 98 42300
Fax: +353 98 42340