RESEARCH ON PANCREAS DISEASE IN IRISH FARMED SALMON 2004/2005 – CURRENT AND FUTURE INITIATIVES

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ABSTRACT

Pancreas Disease is the most significant single infectious disease affecting marine salmon farms in Ireland. The first epidemiological studies of Pancreas Disease (PD) in Ireland in the early 1990's indicated that significant losses occurred in farmed Atlantic salmon in their first year at sea on some individual farms (Menzies *et al*., 1996). A serological survey for the presence of Salmon Pancreas Disease Virus (SPDV) antibody in 1996 revealed that 53% of Irish sites were positive, but that not all positive sites had recognised clinical signs of PD. This indicated a relatively low incidence and severity of PD at that time (McLoughlin *et al*., 1998) and this pattern persisted throughout the remainder of the 1990's. In 2002, a serious increase in both the incidence and severity of PD was reported on farmed Atlantic salmon marine sites in Ireland. An epidemiological survey of Irish sites for 2002 revealed that 59% of sites reported an outbreak of PD, with mortalities averaging 12% (range 1 – 42%; McLoughlin *et al*., 2003). A recent study using data collected for 2003 and 2004 indicated that PD occurred in 62% and 86% of sites respectively. The average mortality due to PD on affected farms was 18.8% in 2003 and 14.8% in 2004 (Rodger & Mitchell, chapter 2).

In response to these significant losses due to PD a range of research initiatives was launched in Ireland and was supported by the Marine Institute. This document gives an overview of the most recent findings from the current projects, carried out in 2004/2005 and also provides an overview of the various actions in Scotland and Norway, where PD has also become a disease of economic significance in salmon aquaculture.

TABLE OF CONTENTS

1. BACKGROUND INFORMATION

1.1 Introduction

Pancreas disease, a viral disease of Atlantic salmon, was first recorded in Scotland in 1976 (Munro *et al*., 1984). It has since been diagnosed in countries where Atlantic salmon, the target species, is intensively reared, including the USA (Kent & Elston, 1987), Norway (Poppe *et al*., 1989) and Ireland (Murphy *et al*., 1992). Some cases were also reported in France and Spain (Raynard *et al*., 1992). During the early 1990's PD was a serious problem for the salmon farming industry in Ireland with up to 50% mortality occurring (Menzies *et al*., 1996). This was followed in later years by a reduction in the clinical severity of the disease, though mild persistent infections were found (McLoughlin *et al*., 1998). Since then, PD has re-emerged as a significant cause of mortality in the marine phase of production of the Irish salmon industry (McLoughlin *et al*., 2003a). In 2002 59% of sites reported an outbreak of PD with attributed mortalities on sites ranging between 1 and 42%. Continued losses of this magnitude threaten the economic viability of the industry, which can be seen by the decline in production in the finfish sector for a fourth consecutive year (Parsons, 2005).

In response to these serious losses in the salmon industry since 2002, the Marine Institute set up a *Pancreas Disease Research Group* in early 2004 to advise and suggest initiatives on PD research. The Marine Institute in association with IFA Aquaculture, convened a seminar on "*Pancreas Disease in Ireland and Future Research Priorities*" in Galway in October 2004. It became clear from this meeting that PD was also becoming a significant issue for the Norwegian industry.

1.2 Evolution of the Tri-Nation Pancreas Disease Initiative

Following contact with Dr. Gordon Ritchie, the Fish Health Technical Manager at Marine Harvest International (MHI) in Norway, the Marine Institute supported a delegation of eight researchers and industry representatives to take part in the seminar on "*PD: similar pathologies and prevention*" in Bergen on February 7 – 8, 2005. This seminar brought together some 50 participants (research scientists, regulators and industry managers) from Norway, Scotland and Ireland to share current knowledge on PD and related conditions (HSMI, SD) and to debate future strategies for research and management of PD. Two follow on workshops also attended by researchers from Ireland, Scotland and Norway, were held in Aberdeen, on March 1 – 2, 2005, and in the National Veterinary Institute, Bergen on May 3, 2005, as part of the Tri-Nation Research Initiative.

The Irish industry has estimated that PD has resulted in a total loss of turnover of €35 million with €12 million loss of profit in the years 2003 – 2004. However, pancreas disease has also emerged as a serious threat to the sustainability of salmon farming in Scotland and Norway. In Scotland, PD and related pathologies are increasingly responsible for significant losses in marine salmon farms and recently these have been quantified as up to €0.5 million per PD affected site. Impacts are estimated to be in the range of €7 – 13 million per year in Norway.

The importance of PD and similar conditions, plus the urgent need for additional scientific knowledge was highlighted through this series of seminars held in 2004 and early 2005. The seminars promoted the sharing of knowledge and experiences, at an international level, on the alphavirus that causes PD and its relationship with similar pathologies such as

- Sleeping Disease (SD) of rainbow trout
- Heart and Skeletal Muscle Inflammation (HSMI)
- Sudden Death Syndrome (SDS)
- Cardiomyopathy Syndrome (CMS)

The seminars concluded that there were significant gaps in the scientific knowledge on PD and similar pathologies. Consequently there was an urgent need for an internationally combined and

extensive research effort. This led to the establishment of the **Tri-Nation PD Co-ordination Committee**, consisting of a Steering Group and three research Working Groups. Each group contains representatives from science and industry from Ireland, Scotland and Norway with the aim of defining future research strategies and the development of specific control strategies. Representatives identified the following main focus areas for future research on a Tri-Nation basis:

- Aetiology of the disease. Studies to clarify the relationships between PD, HSMI and CMS and to determine if these conditions are related. Such work would include longitudinal studies at production sites, serology, experimental transmission and a refinement of diagnostic criteria.
- Epidemiology. Studies to further investigate the key aspects of the epidemiology in farmed salmonids. This work would include case control studies on prevalence, risk factors and performing structured cross-sectional surveys using a combination of serology/virus isolation/RT-PCR to provide an accurate Tri-Nation baseline of prevalence.

1.3 Update on Research within the Tri-Nation Initiative

Appropriate research proposals have been developed in each of the Tri-Nation member countries for funding. While the main focus of such proposals will be directed toward national investigations, each will encourage dialogue and exchange of ideas, results and recommendations to industry in Ireland, Scotland and Norway. This international tier can provide a model for added value in European aquaculture and fish health research.

Ireland

Throughout 2004, a number of projects focussing on PD in Ireland have received support from the Marine Institute:

Epidemiological Survey 2003 – 2004 (see chapter 2)

The Marine Institute funded an epidemiological study on PD, carried out by Vet-Aqua International, Galway. This report was based on a detailed questionnaire survey of farm management at all Irish sites in 2003 and 2004. The survey builds upon a previous survey that was funded by the Marine Institute on the 2002 generation of fish and presented at the BRADÁN conference of the Irish Salmon Growers Association in Autumn 2003 (McLoughlin *et al*., 2003a).

Smolt Susceptibility Trial – Landcatch UK (see chapter 3)

Landcatch UK, have a successful breeding programme for resistance to Infectious Pancreatic Necrosis and are currently applying those techniques to breeding for PD resistance. A project entitled "*Pancreas Disease Sentinel Testing – Ireland*" was run in partnership with Irish salmon producers Muir Gheal Teo and Muir Acmhainni Teo, Co. Galway and received partial support from the Marine Institute.

Smolt Susceptibility Trial – Marine Harvest Ireland (see chapter 3)

Marine Harvest Ireland are currently running a smolt susceptibility trial in partnership with the Mannin Bay Salmon Company, Co. Galway and have also received partial funding by the Marine Institute.

Biophysical Properties of the PD Virus (see chapter 4)

This is a one year research programme carried out by Queens University Belfast, in association with the Irish Salmon Growers Association and is funded under the Marine RTD Applied Industry measure.

Pathogenesis of the PD Virus (see chapter 5)
The Marine Institute is funding a PhD programme entitled "*Pathogenesis of the Salmon Pancreas Disease Virus*" at the Faculty of Veterinary Medicine, UCD and includes close collaboration with both Queens University Belfast and the Dublin Institute of Technology.

Site Investigations and Disease Management of the Pancreas Disease Virus in Irish Farmed Salmon (see chapter 6)
This two year project has recently received funding under the NDP Marine RTDI Strategic Programme measure and involves the Marine Institute, Queens University Belfast and Vet-Aqua International. This study aims to increase our knowledge on the epidemiology of PD, diagnostic capabilities, disease development and management and mitigation.

Scotland
Research in Scotland into PD and related pathologies is currently being carried out by Marine Harvest Scotland and the Fisheries Research Service (FRS).

Marine Harvest Scotland
Longitudinal studies are currently being carried out in the Sound of Mull in conjunction with Marine Harvest, FRS and the Department of Agriculture & Rural Development, Northern Ireland (DARDNI). This project is ongoing and has received partial funding from Scottish Quality Salmon (SQS).

Together with Schering Plough and Skretting, Marine Harvest is currently running a trial to investigate the effect of feeding Ergosan™ on the outbreak of PD in Atlantic salmon.

Fisheries Research Service
An epidemiological study will be carried out on a number of Scottish sites by the FRS. In addition to this, an analysis of the Marine Harvest Scotland database, including mortality and attributed causes of mortality, will be used to examine regional, environmental and husbandry factors associated with that mortality.

Samples from Atlantic salmon farms will be collected and tested to determine the prevalence of PD infection in the Scottish industry. Sampling will also be conducted at freshwater farms in order to establish if PD is present during the freshwater rearing stages. These projects have received funding from the Scottish Executive Environment and Rural Affairs Department (SEERAD) and are expected to begin in 2006.

A two-year project funded by the Scottish Aquaculture Research Forum (SARF) on "*Aetiology and epidemiology of PD, HSMI and CMS in Scotland*" started in November 2005 and is run by the FRS and the Institute of Aquaculture, University of Stirling. The main objective of this study is to establish whether PD, HSMI or CMS are due to the same causative agent, presenting a different set of symptoms and pathologies or are they three separate diseases.

Norway

Within the framework of the Tri-Nation Initiative, research into PD and related activities in Norway is taking place at a number of institutions including Marine Harvest Norway, The National Veterinary Institute and Veterinary Science Opportunities (VESO®).

National Veterinary Institute

Current work on PD focuses on the improvement and validation of diagnostic methods e.g. real time RT-PCR and longitudinal studies on Norwegian sites. In addition to this, a current proposal on "*Pancreas disease in Atlantic salmon and rainbow trout; pathogenesis and risk factors*" has recently been approved for funding by the Norwegian Science Council and is set to begin in 2006.

VESO®

Sponsored by the Fishery and Aquaculture Industry Research Fund, VESO are reviewing current knowledge and aim to produce a leaflet making recommendations on general and specific mitigation measures against viral diseases including PD. This project is being carried out in conjunction with Marine Harvest and the Norwegian Fish Farmers Association and is to be completed by February 2006.

2 PANCREAS DISEASE IN IRELAND: EPIDEMIOLOGICAL SURVEY RESULTS FOR 2003 AND 2004

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2.1.Introduction

Pancreas disease aetiology & clinical disease

Pancreas disease (PD) is an infectious viral disease of marine stage farmed Atlantic salmon (*Salmo salar*) that was first described by Munro *et al*. (1984) in Scotland. The disease has been reported in Ireland (1984), Norway (1985), France (1986), USA (1987) and Spain (1989) (Kent & Elston, 1987, Raynard *et al*., 1992) with the most serious impacts from the disease reported in Ireland, Norway and Scotland.

The causal agent of the disease is an atypical alphavirus, the salmon pancreas disease virus (SPDV), of which there appear to be at least two subtypes (Hodneland *et al*., 2005, Weston *et al*., 2005); one subtype is associated with PD in Scotland and Ireland and another is associated with PD in Norway. A third salmonid alphavirus, very closely related to SPDV, and known as the sleeping disease (SD) virus, causes a similar condition in rainbow trout (*Oncorhynchus mykiss*) in freshwater and is reported in France, Italy, Germany and the UK (Castric *et al*., 1997, Bergmann *et al*., 2005). The name salmonid alphavirus (SAV) has been proposed for these closely related viral isolates (Weston *et al*., 2002).

Infection with these viruses can lead to clinical disease due to acinar pancreatic necrosis and fibrosis as well as a range of myopathies (cardiac, skeletal, oesophageal) (Ferguson *et al.*, 1986, Rodger *et al.*, 1994, McLoughlin *et al*., 2002). Clinically affected fish present with lethargy, anorexia and increased mortality. Mortalities can reach over 40% in affected pens and failure to thrive is a further consequence of the disease resulting in poor condition, thin fish that are susceptible to parasitism and secondary bacterial disease (Figure 1).

Figure 1: Three salmon from a pen affected by pancreas disease (PD) in Ireland with the top two fish exhibiting low condition factor, typical of those affected by chronic PD.

In a farm affected by PD, the majority of fish will exhibit pancreatic regeneration and recovery and appetite then returns. However, in fish affected by the later developing and longer lasting myopathies, further mortalities can then ensue when the fish exhibit a strong feeding response (Rodger *et al*., 1991), probably due to either cardiac muscle exhaustion, further exertional

rhabdomyolysis or both. Such fish may present as either lethargic circling fish in the water column or appear exhausted lying on the pen floor (Rodger *et al*., 1991, McLoughlin *et al*., 2002). These fish will be vulnerable to physical damage and predation. Concurrent with the return to feeding, a population of non-feeding fish will emerge in the pen or farm and these are the fish in which the acinar pancreatic tissue has not regenerated. These fish can survive in the pens but fail to thrive and become thin and dark in appearance and often carry a heavy sea lice burden. These are often referred to as runts, "poor doers" or chronically affected-PD fish.

The level of mortality on PD affected farms varies significantly with some experiencing a mortality of less than 5% for the whole cycle whereas other farms may lose up to 40% of all stock over a three or four month period. The percentage of PD runts that are present in a farm at the end of the growth cycle also varies significantly, although it has been the author's observation that farms which have a low mortality due to PD often have a higher percentage of PD runts when compared to those with high percentage mortality i.e. greater than 10%. Sub-clinical disease has also been observed where fish close to harvest are confirmed as positive to SPDV antibodies, but have not had a reported clinical outbreak or suspicion of disease, although in some cases growth and performance may have been less than expected (Rodger, personal observation, Graham *et al*., 2005a).

Epidemiology of PD

The first description of some epidemiological aspects of PD in Scotland was undertaken by McVicar (1987). In this early description it was recorded that PD outbreaks were only a feature of marine sites and that there was no association with freshwater environment or stock. Further outbreaks were reported to occur in either post-smolts, one sea winter fish or two sea winter fish but that no seasonal trend was obvious, although peak prevalence was in late July through to early September. The disease was observed to be persistent on sites, infecting new inputs of smolts every year, and fish that recovered from the disease did not develop disease again. By the end of 1985 the disease was recorded in 19% of the salmon farms throughout Scotland (McVicar, 1987). Investigations in Ireland were undertaken between 1989 and 1994 and indicated that of the 43 outbreaks recorded on the farms from which data was collated, 57% occurred in the three month period August to October inclusive or 17 to 32 weeks post-transfer (Crockford *et al.*, 1999). Analysis of variance of mortality rates during PD outbreaks which occurred on six marine sites over a five year period showed that mortality rates varied significantly between sites ($P< 0.001$), but that it did not vary significantly between the years over the time period. The mortality rate during PD outbreaks ranged from 0.1 to 63% and mortality rates were significantly higher when PD outbreaks occurred earlier in the year. The mean length of a PD outbreak was 112 days. There was no correlation between PD mortality rate and smolt input weight, initial stocking density and transfer mortality.

Site management factors which significantly reduced production losses were fallowing of the site for at least three weeks before smolt input, single generation rearing on the site, slaughtering of fish away from the site and avoidance of farm staff movement between farm sites (Wheatley *et al*., 1995). The more recent epidemiological investigation of PD in Ireland in 2002 was undertaken in response to the re-emergence of serious outbreaks of PD after a relatively quiescent period (McLoughlin *et al*., 2003a). This study concluded that the clinical signs, duration and spread of the PD were very similar to those previously described in Ireland and 59% of the sites surveyed (13/22) had PD outbreaks confirmed. The average PD associated mortality was 12% with a range from 1 to 42%. Outbreaks commenced on most farms between March and October and the average duration of an outbreak was 141 days (range 68 to 288 days). The average time for all pens on site to become infected was 62 days (range 0 to 236 days). In the 2002 survey S0 smolts were three times more likely to succumb to PD than S1 populations and farms that moved their fish to another site during their production cycle were six times more likely to have an outbreak of PD when compared to those which did not. Sites

stocking lower overall numbers of fish (<250,000) and/or lower numbers of fish per pen (<20,000) were less likely to succumb to PD. Smolt strain susceptibility was identified on individual sites, but not consistently across the country, indicating that site and management factors may also be involved. There appeared to be a regional difference for strain susceptibility to the level of PD mortality which may have indicated site or specific environmental factors that may be important and these remained to be defined.

Longitudinal serological surveys of salmon in two farms in Ireland through 2002 –2003 which contracted PD revealed that in the populations monitored there was a progressive increase in SPDV seroprevalence to 90 – 100%, typically accompanied by rises in antibody titres and that seroprevalence remained high through to the end of the study period or to harvest (Graham *et al*., 2005b).

The Norwegian experience of PD has been investigated through a case control study between 1999 and 2002 (Brun *et al*., 2005). Sites with PD outbreaks were positively associated with well boat movements and a lack of fallowing before sea transfer. Whether the fish were S0 or S1 stocks was not associated with PD outbreaks. There appeared to be a protective effect with regard to PD outbreaks when the feeding regime was in discrete meals rather than continuous feeding. As in the Irish experience there was reportedly a large variation in mortality in PD affected farms with 6% of the farms losing less than 5% stock due to PD, but between 13% and 14% of the farms having losses of greater than 20% due to PD. The average duration of an outbreak was reported to be 10 weeks, however, some continued for 24 weeks.

One of the recommendations of the 2002 study in Ireland was that there should be a concerted national effort to continue to monitor for the presence of pancreas disease, its level of mortality, and that the recording of environmental and other livestock parameters be maintained for further epidemiological investigations (McLoughlin *et al*., 2003a). In response to this recommendation further epidemiological investigations were undertaken by the authors of this report in 2005 into PD outbreaks in Ireland during 2003 and 2004. The objectives of this investigation were to:

a) Assess the occurrence and severity of pancreas disease in farmed salmon in Ireland in 2003 and 2004
b) Investigate and identify possible risk factors associated with an outbreak of PD, as well as those that affect the severity of the outbreak
c) To be able to highlight or recommend areas for further investigation and research

2.2. Materials & Methods

Survey population

The population surveyed in this study was commercially reared Atlantic salmon reared in sites in the northwest, west and the southwest of Ireland. Two subsequent generations were examined. Fish hatched in early 2002 went to sea in two populations, in late 2002 as S0's and in early 2003 as S1's (collectively referred to as "2003 generation" in this report). Fish hatched in early 2004 also went to sea in two populations as above (S0's and S1's, collectively "2004 generation"). S0's are produced by artificial manipulation of day length.

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 of management. Information on the stock was collected including fish number, fish weight at time of outbreak of PD, stocking density, strain of fish, and smolt type. Information on the management of the sites included, whether sites were organic or conventional, whether fallowing was employed, feeding rate prior to and during an outbreak of PD, what method of holding in freshwater had been employed, whether the fish were moved in the sea during their life cycle and if they were, what time interval was there between this movement and the outbreak of disease and finally, was there another PD positive site in the same bay. Information on PD and other diseases was also collected including time of outbreak of PD where appropriate, percentage mortality due to PD, what other diseases affected the livestock and the percentage mortality caused by specific disorders, vaccination status and type of vaccine, lice levels at time of outbreak, previous history of PD in the site and finally estimated loss of growth due to PD.

The information in some cases was accurate, but in other cases it was vague and consisted of best estimates by farmers. To help with the interpretation of the questionnaires and to ensure data retrieved was as accurate as possible, there was extensive liaison between one of the aquaculture veterinarians at the practice carrying out the survey and the farmers targeted by the investigation.

Diagnosis of PD

A farm was categorised as positive for PD where there had been a diagnosis of the disease with laboratory test confirmation i.e. veterinary examination and recording of typical clinical signs plus either PD histopathology and/or the detection of salmonid alphavirus (SAV) through virus isolation or SAV antibodies (McLoughlin *et al*., 2002, Graham *et al*., 2003). 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 that had failed to thrive in the pens and were as a result downgraded at harvest.

Sea lice

Sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) burdens on farmed salmon are monitored throughout the year in each generation of fish at each marine site in Ireland, with each population being inspected on 14 occasions through the year. The figures for each site are published annually by the Marine Institute and these were used for the purposes of this study for each respective year (O'Donohue *et al*., 2004, O'Donohue *et al*., 2005). The lice burden for each site was recorded at a period four to six weeks prior to any outbreak of PD and these compared to those farms without PD (at equivalent high risk periods for PD which were taken to be early summer [May/June] or early autumn [September/October]) and similarly the lice burdens in those sites which had a high mortality due to PD (>10%) were compared to those which had a low mortality (≤10%) due to PD. The lice burden was categorised as either high (total lice no. > 1) or low (total lice no. ≤ 1) for each lice species.

Vaccination against PD
A limited number of doses of a PDV vaccine (Norvax Compact PD™, Intervet Ireland Ltd.) were available through a special Animal Remedies Regulation licence (AR16 licence) for the 2003 and 2004 cycles of fish. PDV vaccinated fish were compared to unvaccinated fish where they were reared in the same sites.

Feeding rate
The feeding rate, as a percentage of body weight, in the month prior to any confirmation of a PD outbreak was assessed and this categorised as either high (>1%) or low (≤ 1%) where figures were available. Further, whether the feeding rate was high or low prior to a PD outbreak was assessed against the eventual impact of the PD (high or low level mortalities).

Preliminary Data analysis
The data was summarised, entered and sorted into different categories using Microsoft Excel. Non-parametric statistics were used in the preliminary analysis of the data collected. The odds ratio (OR), where appropriate, was calculated for each variable in relation to occurrence of PD or more often mortality *level* due to PD, for instance high (>10% mortality) versus low (≤ 10% mortality). In situations where one of the inputs into the 2 X 2 contingency tables was zero, 0.5 was added to each input to enable the calculation to be carried out (Thrusfield, 1995). The OR is a useful, simple measure of the strength of association between two parameters. If there is no association the OR = 1. The greater the departure from 1 the stronger the association between the factor in question and the mortality level, or the occurrence of disease (>1 = positive association, <1 = negative association) (Thrusfield, 1995). The variables assessed were dichotomous in all cases. Where there were continuous data sets the variables were transformed by categorisation beforehand to become dichotomous. The OR in this study was used to indicate if there was an increased probability of high mortality occurring in relation to the variables listed in Table 1. Once the OR was calculated, the Fishers Exact test was used to uncover if there was significance in the associations. Significance in relationships between groups was assigned when $P < 0.05$.

Table 1. Variables examined for association in the survey.

Table of Variable Factors	
1. Smolt type (S1 or S0)	9. Organic or conventional farm
2. No. of fish on site	10. PDV vaccination
3. Strain of fish	11. Movement of fish to another site
4. Stocking density (kg/m^3 and fish/m^2)	12. Freshwater site (pens or tanks)
5. Other PD positive site in the same bay	13. Weight of fish at time of outbreak
6. Fallowing of site	14. Proximity to processing plant
7. Lice levels prior to outbreak	15. Feeding rate around PD outbreak
8. Previous history of PD	

Further Data Analysis
Further analysis was carried out using the statistical software SPSS (SPSS Inc., Chicago, IL). The data, which had previously been converted to a dichotomous format for preliminary analysis of a number of the different variables (sea lice levels, stocking density, etc.) was reanalysed in continuous format. The data set for each individual variable was assessed to establish if it was normally distributed using Kolmogorov-Smirnov and Shapiro-Wilk tests. In cases where the data was normal, parametric tests such as the Student's t-test and correlation analysis were carried out, in an attempt to identify relationships between sets of variables. For data that was not normal, Mann-Whitney U and Mann-Whitney exact tests (for small sample sizes) were used to establish if there was a relationship between mortality levels and particular continuous variables. Chi-square analysis was also appropriate for some variables.

2.3. Results

Number of inputs and fish in survey
Information was gathered over two years from 35 separate populations. Table 2 illustrates the breakdown in year and smolt type.

Table 2. Number of study populations and fish going to sea by smolt type and year.

Year	No. of populations – S0s (no. fish)	No. of populations – S1s (no. fish)	Total inputs (no. fish)
2003	7 (2,308,000)	14 (6,420,808)	21 (8,728,808)
2004	5 (1,551,500)	9 (3,832,324)	14 (5,383,824)

These figures represent all the marine salmon farms in Ireland in 2003 and 2004. Basic farm data was collated from all marine sites operating in 2003 and 2004, however, more detailed records with regard to feeding rate, weights, mortality numbers, etc. were not available for a low number of units.

Incidence of PD
In 2003, 13 out of 21 (62%) populations reported outbreaks of PD, in 2004 the figure was 12 populations out of 14 (86%). The average mortality due to PD on affected farms was 18.8% (SD +/- 8.3, range 2 - 27) in 2003 and 14.8% (SD+/-11.6, range 4 - 35) in 2004. The time of year that outbreaks occurred varied between sites, with most outbreaks occurring in either early summer (May/June) or early autumn (September/October). This is illustrated in Figure 2.

A number of factors at the different sites were investigated in an attempt to establish if there was an association between them and the levels of mortality observed with PD infection. Some of the populations in the study experienced substantially lower mortality due to PD then others, and attempts were made to correlate this with different measurable variables in the study. Since a large percentage of sites in the survey experienced outbreaks of PD, this discrepancy in mortality was used as an arbitrary measure when carrying out statistical analysis instead of the more common approach of using disease presence/absence as an arbitrary measure. However, the latter was used for some of the variables when appropriate.

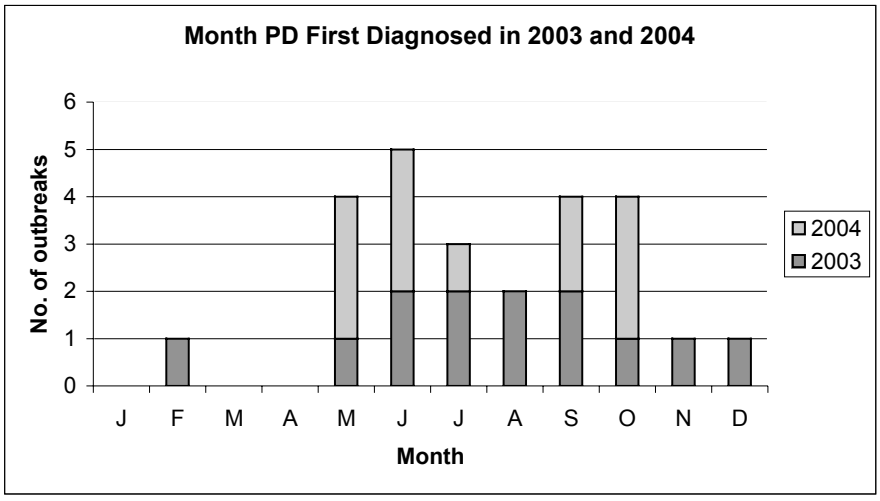

Figure 2. Month PD was first diagnosed in each of the sea sites for 2003 and 2004.

In addition to the mortalities, the average estimated loss of growth due to PD was 11.4%, with a range from 1.1 to 34.0%, over the two years studied. The average time for an affected farm to reach peak or highest mortalities in an outbreak was eight weeks after the first signs of disease and the time to reach this peak ranged from four to 12 weeks. The majority of farms experienced mortality patterns against time that presented in a bell-shaped or sigmoid distribution, both

typical of exposure to an infectious agent and also both typical of direct horizontal transmission (Klontz, 1993). Typical examples of these are shown in Figures 3 and 4.

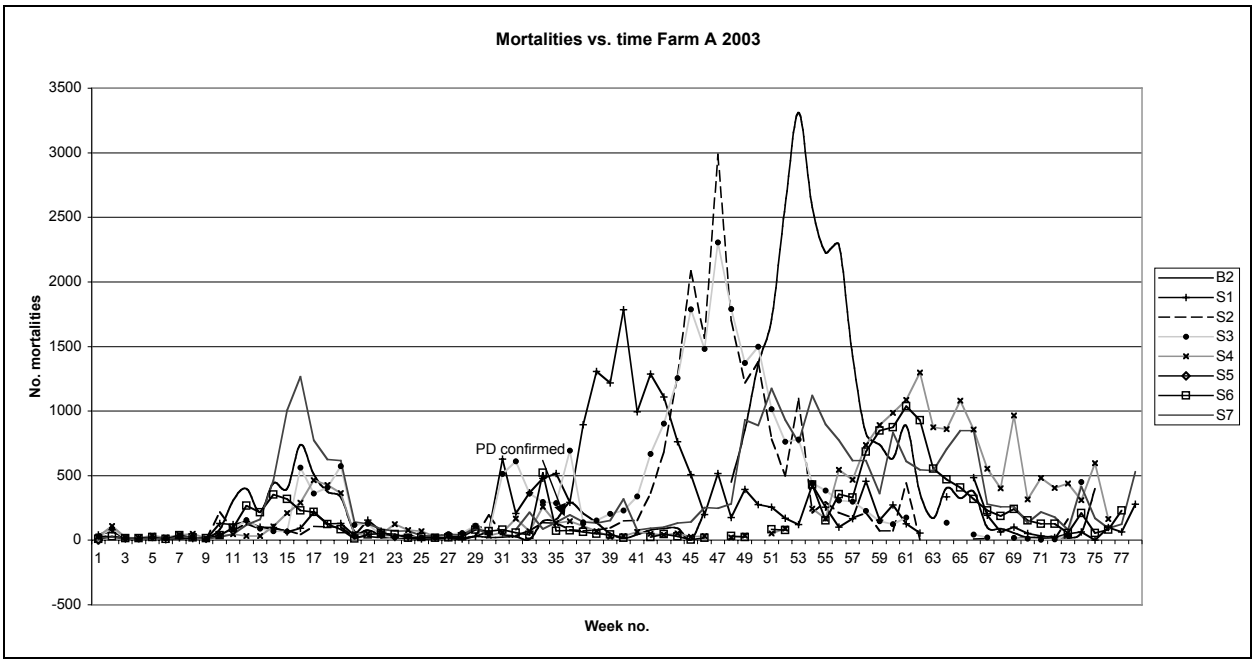

Figure 3: Mortalities against time in different pens in Farm A in 2003.

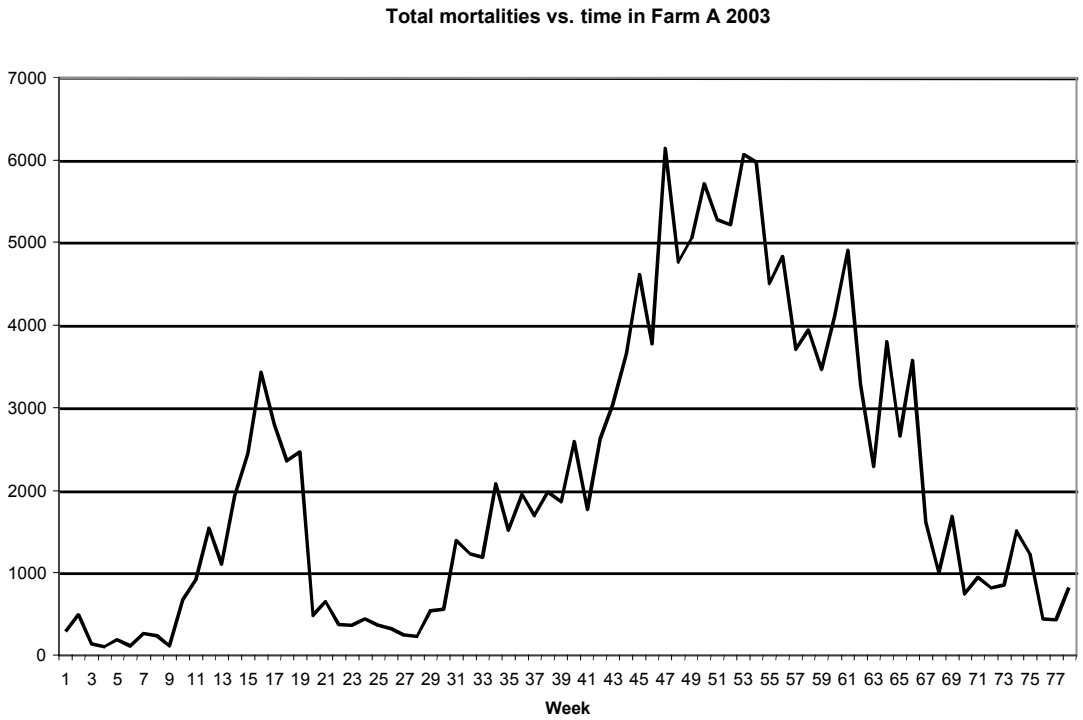

Figure 4: Mortalities against time in Farm A in 2003, showing bell-shaped curve after PD confirmation in week 35.

Results by variable

1. Smolt type

When both 2003 and 2004 are combined, 12 of the total inputs to the sea were S0's and 23 were S1's. The odds ratio was calculated comparing occurrence of PD and then subsequent mortality levels due to PD between the two groups. The summary details are outlined in Tables 3 through to 7 and Figure 5.

Table 3. Smolt type and occurrence of PD, 2003.

2003	PD	No PD
S0	6	1
S1	7	7

Table 4. Smolt type and categorised PD mortality levels, 2003.

2003	PD – High Mortality	PD – Low Mortality
S0	5	1
S1	6	1

In the 2003 generation, those sites using S0 smolts were more likely to get PD than those with S1s (OR = 6, $P = 0.17$) although this was not statistically significant. There was no significant difference in the levels of mortality between the two smolt types (OR = 0.8, $P = 1.0$)

Table 5. Smolt type and PD occurrence, 2004.

2004	PD	No PD
S0	5	0
S1	7	2

Table 6. Smolt type and PD mortality level, 2004.

2004	PD – High Mortality	PD – Low Mortality
S0	4	1
S1	4	3

There was no significant difference between smolt types and susceptibility to PD in 2004 inputs (OR = 3.7, $P = 0.51$). There was no significant difference in the levels of mortality between the two smolt types (OR = 3.0, $P = 0.58$). When both years were combined there was no significant association of presence of PD (OR = 7, $P = 0.11$) or impact of PD (OR = 1.8, $P = 0.66$) with smolt type and the figures are shown in Table 7.

Table 7. Smolt type and occurrence of PD in 2003 and 2004.

2003 & 2004 combined	PD positive	PD negative
S0	11	1
S1	14	9

The average weight of the S0s when they succumbed to PD was 1262g and this compared to 572g for the S1s reflecting the increased economic impact of PD outbreaks in S0s when compared to S1s (Table 13).

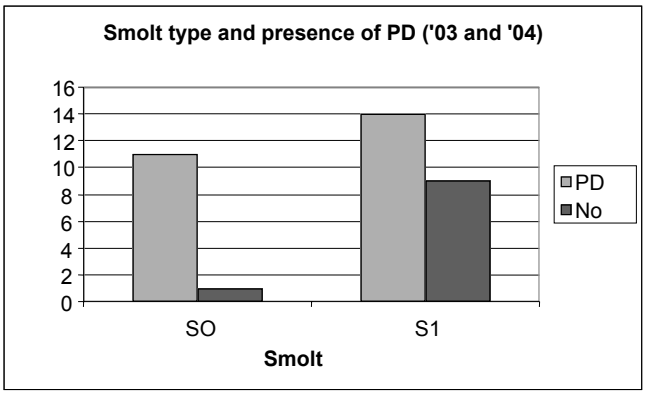

Figure 5: Number of sites and PD status vs. smolt type.

2. Number of fish in site

Although there was an indication that there was an increased risk of PD (OR = 1.1, $P = 1.0$), or a greater impact from PD if it occurred (OR = 3.5, $P = 0.2$), if the number of fish on the site was greater than 250,000, this was not statistically significant and figures are shown in Tables 8 and 9 and Figure 6.

The data for 2003 and 2004 were assessed in continuous format using Student's t-tests and Pearson correlations to establish if there was a relationship between disease occurrence and fish number on site, and also with high or low mortality where appropriate. No significant associations were uncovered.

Table 9: No. of sites with greater or less than 250,000 fish and level of impact of PD in 2003 and 2004.

No. fish per site	PD positive	PD negative
>250,000	18	7
<250,000	7	3
No. fish per site	**PD high mortality**	**PD low mortality**
>250,000	13	5
<250,000	3	4

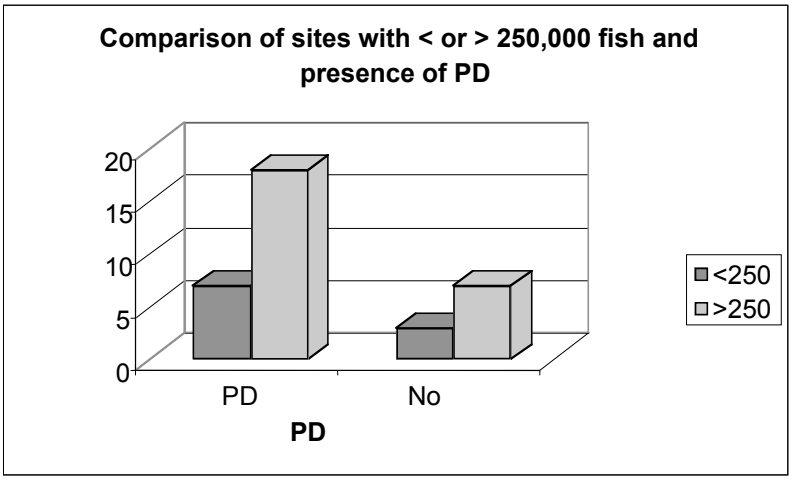

Figure 6: Number of sites with greater or less than 250,000 fish and their PD status in 2003/4.

3. Strain of Fish.

There were two strains of salmon smolt that comprised the majority of the salmon farmed in Ireland in 2003 and 2004 (designated X and Y for survey confidentiality). Some of the sites in the survey farmed more then one strain in both years of the survey. These different strains were usually reared in separate pens, but there was not sufficient accurate mortality data on a pen-by-pen basis to look at this variable in most of the sites. Instead, the variable was assessed using detailed data that was available on a cage-by-cage basis for four of the sites, designated A, B, C and D where in each of these sites the stocking levels, pen type, vaccine status and feed type were all equivalent in the pens compared i.e. the only considered significant variable was the strain of fish. Details are shown in Table 10 and Figure 7.

Table 10. Strain specific mortality from four sites, 2003.

Site	**Percentage mortality due to PD**		**Number of pens**	
	Strain X	Strain Y	Strain X	Strain Y
A	34.7	21.7	4	4
B	24	1.25	1	4
C	25	4	1	1
D	59	34	3	1
Average (+/-SD)	**35.7 (+/- 16.3)**	**15.3 (+/- 15.4)**		

The percentage mortality due to PD appeared substantially higher in strain X than strain Y in the sites examined. Analysis was subsequently carried out on a farm by farm basis. Strain effect on susceptibility to disease was investigated by comparing the mortality levels experienced by each as a result of PD on the four farms and results are shown in Table 11.

Table 11. Comparison of strain associated mortality on PD affected farms.

	Percentage mortality		Odds ratio	Chi-square *P*-value
	Strain X	Strain Y		
Farm A	34.7	21.7	2.3	$P < 0.001$
Farm B	24	1.25	25.7	$P < 0.001$
Farm C	25	4	8	$P < 0.001$
Farm D	59	34	2.9	$P < 0.001$

In all cases there was a significant difference between the PD associated mortality of the two strains. The odds ratio indicates the likelihood of increased mortality with strain X in each case. The chi square *P*-values were calculated using actual fish numbers instead of percentages to make the analysis more accurate. Further in the case of farms A and D, more detailed data on strain and PD vaccination status was available. The results are detailed in Table 12. The association of strain X with significantly higher mortality levels then Strain Y remained strong.

Figure 8 displays the mortality pattern in one farm when two strains of fish are compared. From the data in Figure 8 it is apparent that both strains of fish are affected by PD, however, strain Y is affected to a lesser extent in terms of number and later in time i.e. when the fish are larger.

Table 12. Comparison of PDV vaccinated or unvaccinated fish and strain specific mortality.

	PDV vaccinated-strain X vs strain Y		PDV unvaccinated-strain X vs strain Y	
	Odds ratio	Chi-square *P*-value	Odds ratio	Chi-square *P*-value
Farm A	2.8	$P < 0.001$	3.0	$P < 0.001$
Farm D	2.2	$P < 0.001$	n/a	n/a

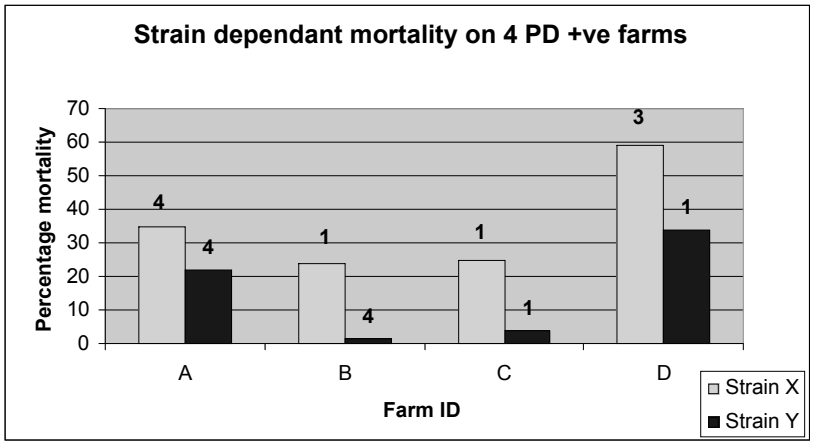

Figure 7: Percentage mortality due to PD on four farms holding two strains of fish in 2003 (no. of pens in each category at top of each bar).

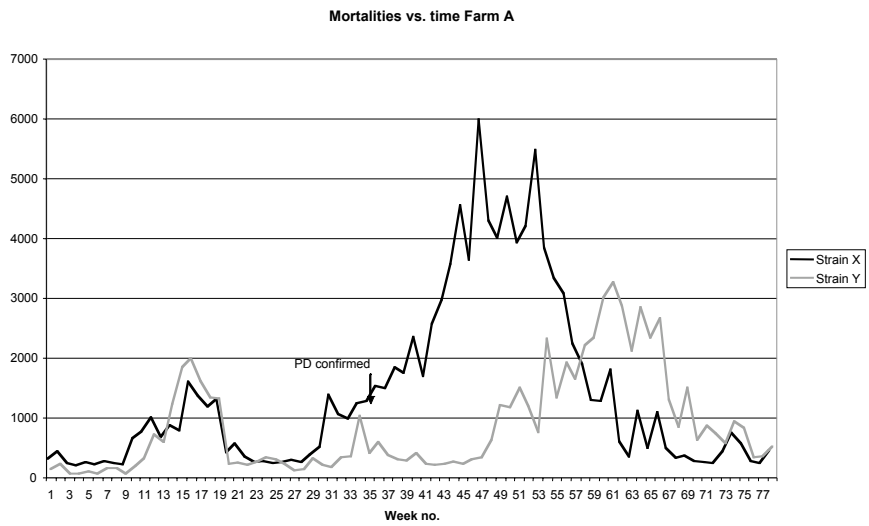

Figure 8: Number of mortalities in two strains of fish in Farm A in 2003 against time.

4. Stocking density

Stocking densities in the form of number of fish/m^2 and kg of fish/m^3 were examined as possible significant causes of variation in mortality levels in the PD affected sites. No significance was uncovered in this association for either the 2003 (fish/m^2: OR = 1.5, P = 1.0; kg fish/m^3: OR = 0.83, P = 1.0) or 2004 (fish/m^2: OR = 1.13, P = 1.0; kg fish/m^3: OR = 0.2, P = 0.47) inputs.

Stocking density data was normal in distribution but no significant relationship between stocking density and mortality level was uncovered for either year when examined using stocking density as a continuous variable (2003 Mann Whitney Exact, P = 0.64, 2004 T-test, P = 0.48).

5. Presence of another PD positive site in the same bay

In 2003, the OR indicated there was an increased likelihood of high mortality due to PD (OR = 4.5) with another positive site in the same bay, but that this was not significant (P = 0.42). In 2004 the OR indicated the opposite was true (OR = 0.67) but this was also not significant (P = 1.0). The presence of a PD positive site in the same bay and the occurrence of PD was positively associated indicated by the OR, but was not significant (2003: OR = 1.11, P = 1.0; 2004: OR = 5, P = 1.0).

6. Fallowing of sites and its effect on occurrence of PD and mortality levels

No significant associations were calculated between fallowing and mortality level due to PD. The OR for both years of the survey indicated an increased likelihood of high mortality with fallowing but the P values were not significant (2003: OR = 2.6, P = 1.0; 2004: OR = 5, P = 0.47). The OR indicates an increased likelihood of PD occurring in conjunction with fallowing the sites, and the 2003 result had statistical significance (2003: OR = 24, P = 0.01; 2004: OR = 21, P = 0.07).

7. Lice levels at time of outbreak

L. salmonis

In 2003 there was an increased likelihood of PD if there was a high *L. salmonis* burden present, and when this data was analyzed in a continuous format there was a significant association between *L. salmonis* level and the incidence of PD (Mann Whitney, P = 0.037). There was no apparent association with lice burden and PD in 2004 (OR = 0.33, P = 0.5) by either method of analysis. When the data from the two years was combined the likelihood of association remained but was not statistically significant (OR = 1.6, P = 0.44). In 2003, there was a decreased likelihood of high PD mortality with a high *L. salmonis* burden (OR = 0.17, P = 0.46). This was not apparent when the 2004 data was analyzed (OR = 4, P = 0.52). When this variable was assessed as continuous in association with mortality level, no significant relationships were uncovered.

C. elongatus

Categorical analysis was not carried out on this lice species as some of the inputs were too small to allow the statistical tests to produce meaningful results. The data was analysed in its continuous format both in association with mortality level and occurrence of PD but no significant relationships emerged.

8. Previous history of PD in a site

A previous history of PD in a site increased the likelihood of the reoccurrence of disease in both years, but was only statistically significant in 2004 (2003: OR = 3.3, $P = 0.33$; 2004: OR = 38, $P = 0.03$). Previous history was also associated with an increase in the level of mortality both years, but was not significant (2003: OR =10, $P = 0.29$; 2004: OR = 2.5, $P = 1.0$).

9. Organic or conventional farming methods

There was an increased likelihood of high mortality due to PD on organic farms during both years, however it was not significant (2003: OR =1.47, $P = 1.0$; 2004: OR = 6, $P = 0.49$).

10. Vaccination against PDV

The number of PDV vaccinated pens that could be compared to unvaccinated pens on the same sites was very limited and when all farms were compared vaccination appeared not to confer any significant resistance to occurrence of PD in 2003. In 2004 vaccination appeared to have a protective effect (OR = 5, $P = 0.4$), however, it was not significant. However, when specific sites holding both vaccinated and unvaccinated fish were examined and compared and taking into account the different strains of fish there was an apparent benefit from PD vaccination and these findings are shown in Figures 9-13 and Table 12. In one farm (Farm A) where PDV vaccinated and unvaccinated fish of two strains could be compared, there was an indication of the more rapid reduction in PD mortalities in vaccinated fish but the more significant impact of strain over vaccination status and results (Figures 11 and 12). Another farm (Farm D) also had low numbers of comparable pens of PDV vaccinated and unvaccinated fish and vaccinated fish had a lower mortality pattern compared to unvaccinates (Figure 13). The pattern of mortalities in this farm also presented with the two peak mortality pattern often seen with PD where the second wave of mortalities can occur after pancreatic tissue and appetite recover but myopathies remain severe (previously referred to as sudden death syndrome).

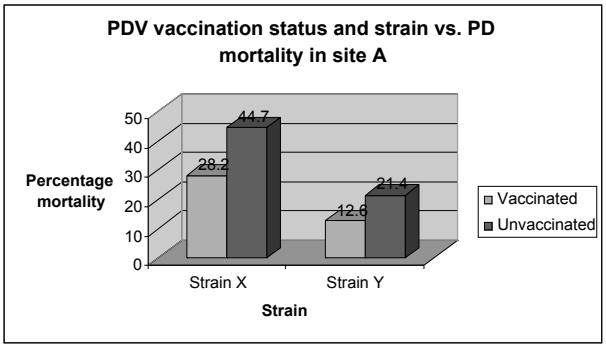

Figure 9: PDV vaccinated and unvaccinated fish of two different strains in site A in 2003.

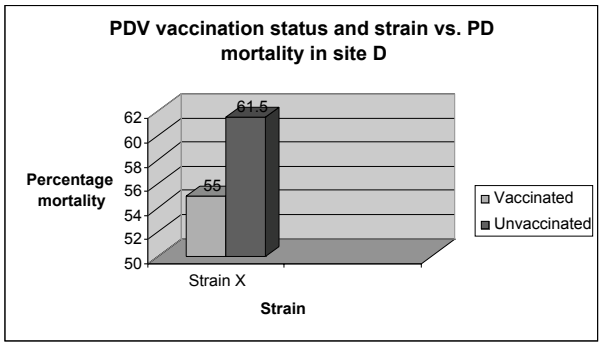

Figure 10: PDV vaccinated and unvaccinated fish of one strain of fish and resultant PD mortality in site D in 2003.

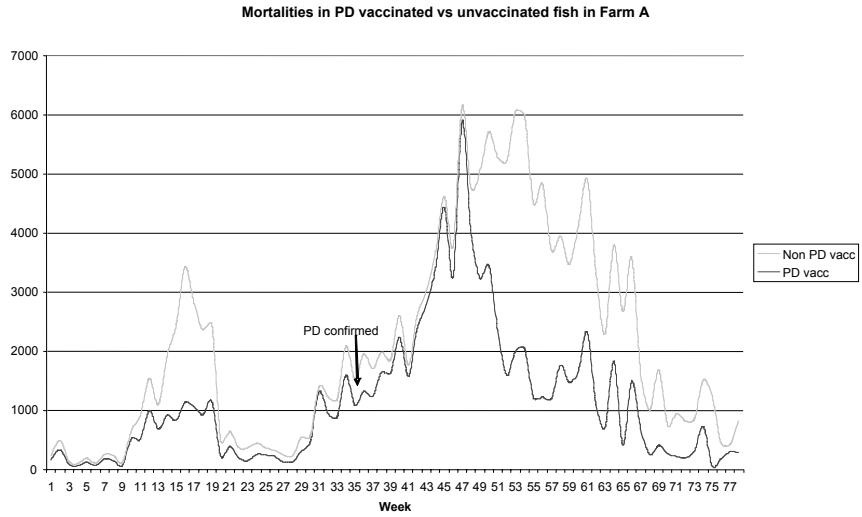

Figure 11: Mortalities in Farm A in PDV vaccinated and unvaccinated fish in 2003 showing more rapid decline in mortalities in vaccinated fish.

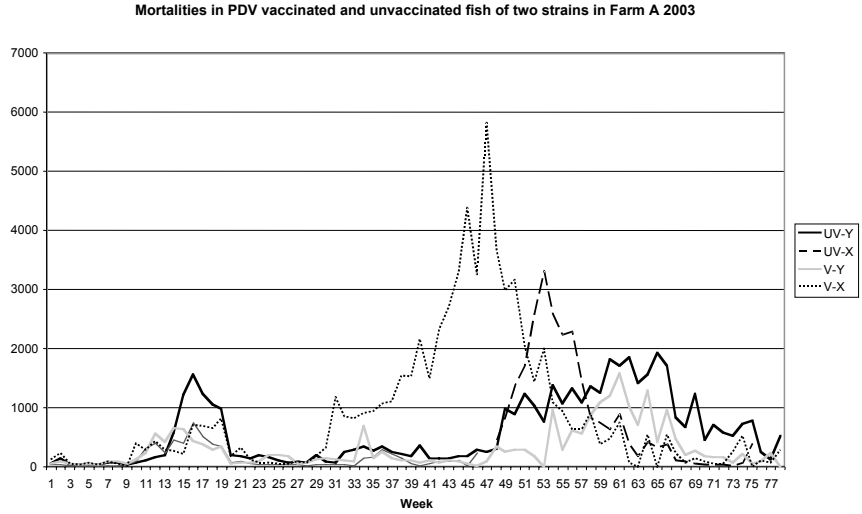

Figure 12: Mortalities in PD vaccinated (V-Y and V-X) and unvaccinated (UV-Y and UV-X) fish of two strains in Farm A, 2003 (PD confirmed in week 35).

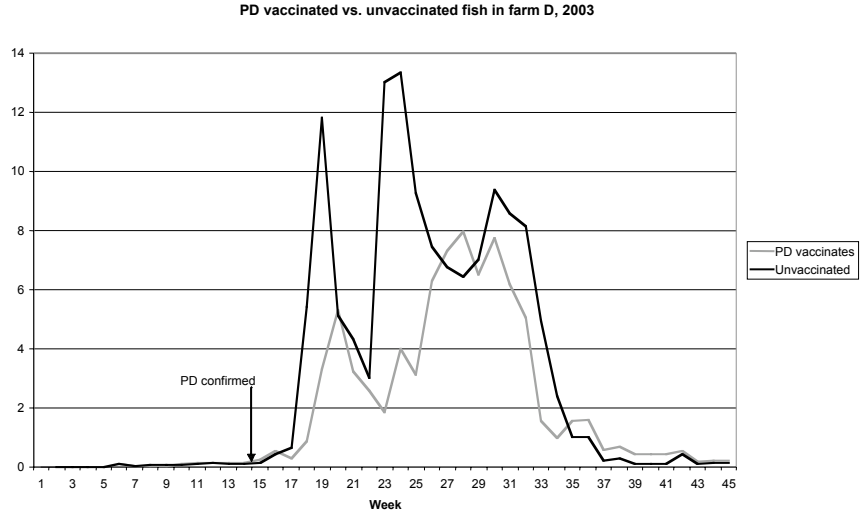

Figure 13: Mortalities in PD vaccinated and unvaccinated fish in comparable pens in Farm D, 2003.

11. Movement

Fish movement and occurrence of PD was significantly associated in 2003 (OR = 16, P <0.001). This was not significant in 2004 (OR = 13.6, P = 0.11). Fish movement and its effect on PD mortality level was also examined and nine sites moved fish during their marine life cycle in each of the years of the survey. There was a trend towards an increase in the likelihood of high mortality with movement, but this was deemed not significant for both years (2003: OR =2.7, P = 1.0; 2004: OR = 8.6, P = 0.2). An attempt was made to compare the two most common methods of transfer (wellboat or towing) and their association with mortality but the sample was too skewed towards towing for accurate analysis.

12. Method of holding in freshwater sites

Fish were segregated into those which were held solely in tanks during their freshwater life cycle versus those held in a both tanks and pens during different stages. In both years there was an increase in the likelihood of high mortality due to PD in fish that spent time in pens, but this was not significant (2003: OR = 4.2, P = 0.49; 2004: OR = 9, P = 0.22).

13. Fish weight at outbreak

The average weight of fish at the time of a PD outbreak was 986g (± 665g) and the details are shown in Table 13.

Table 13. Average weights at time of PD outbreaks.

	Average weight (g)	Range (g)
S0s 2003	1469	743 – 2300
S1s 2003	717	200 – 1800
S0s 2004	1054	372 – 1800
S1s 2004	427	200 - 700
Average weight (g)	986	
Average weight S0s (g)	1262	
Average weight S1s (g)	572	

The variable of fish weight at outbreak of PD was examined to see if there was any correlation between it and levels of mortality. In 2003 there was an increased likelihood of high mortality (OR = 4.23), and in 2004 the opposite was the case (OR = 0.6). Neither figure was statistically significant (2003: P = 0.36, 2004: P = 0.64). The data available for fish weight had a normal distribution, but the sample size of fish experiencing "low mortality" was too small to use a t-test accurately. A non-parametric Mann – Whitney exact test was used instead. The information was analysed to assess if there was a relationship between fish weight at time of disease outbreak and levels of mortality, to attempt to identify what life stage suffered the most dramatic effects of disease. There was no significant association uncovered between mortality level and size of fish (Mann-Whitney Exact, P =0.51). Pearson correlation plotting mortality percentage against body weight also failed to show a relationship in the data. There were no significant results for 2004 (Mann-Whitney Exact, P=0.76).

14. Proximity to processing plant

Farm sites within a 4km radius of a fish processing plant were compared to those distant from any processing plant. For both years the OR suggested an increase in the likelihood of high mortality due to PD for sites positioned near processing plants, but these results failed to show statistical significance (2003: OR =1.2, P = 1.0; 2004: OR = 3, P = 0.58). Occurrence of disease and proximity to a plant was also analyzed. No significant relationships were uncovered (2003: OR =0.7, P = 1.0; 2004: OR = 2, P = 1.0).

15. Feeding rate at time of PD outbreak

In 2004 there was an increased likelihood of high mortality due to PD when the fish were fed at >1% (high feeding rate) than when fed at low feeding rate (<1%) prior to any PD outbreak. This,

however, was not statistically significant (OR = 9, $P = 0.2$). The opposite was true of 2003 (OR = 0.33, $P = 1.0$). There was data missing from a number of farms in 2003 so the calculation may not be a fully accurate reflection of what was occurring in that year.

Feeding and mortality data were assessed and deemed to be normal for 2003 and 2004. The Pearson correlation test was carried out but this failed to return a significant relationship between these two variables (2003: $P = 0.304$, 2004: $P = 0.144$).

Mortality due to different diseases including PD

Pancreas disease was the single most significant cause of mortality in all generations in both years, apart from the S1s in 2003 that experienced high losses due to gill disorders, predominantly in the North West (Cronin *et al.* 2004). The collective data is illustrated in Figures 14 through to 17. Pancreas disease associated mortality ranged from 2% to 35 % in 2003 with an average of 18.8% and from 4% to 35 % with an average of 14.8% in 2004.

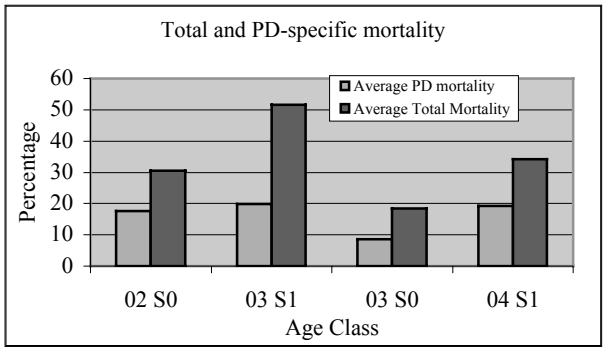

Figure 14: Total mortality and PD associated mortality by year and smolt type for all sites in the survey.

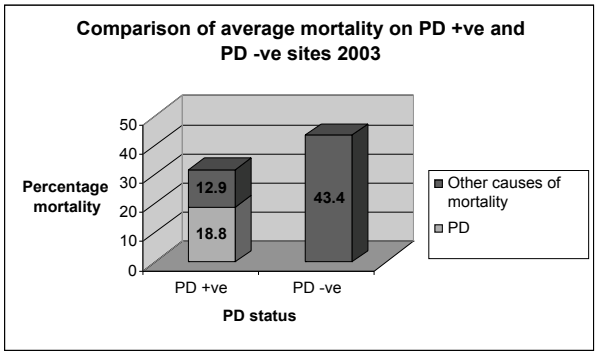

Figure 15: Average mortalities for marine farms in Ireland in 2003 grouped according to PD status.

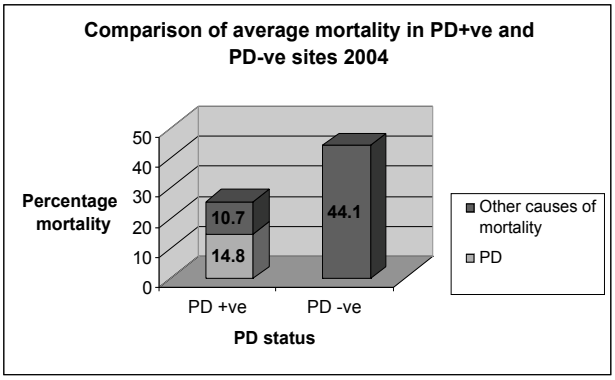

Figure 16 Average mortalities for marine farms in Ireland in 2004 grouped according to PD status

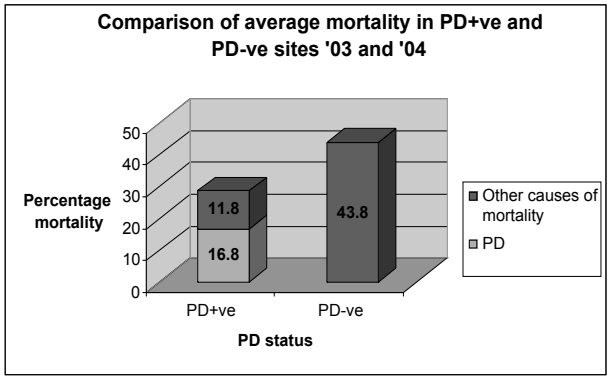

Figure 17: Average mortalities for marine farms in Ireland in 2003 and 2004 grouped according to PD status.

A number of other diseases contributed to overall mortality levels. The general proportional mortality to each is summarized and displayed in Figures 12 through to 16. Total mortality to all diseases varied from 3.5 to 94% in specific populations in 2003 and from 8 to 70% in 2004. The average mortality in salmon farms to all causes in the marine environment was 44.3% in 2003 and 18.9% in 2004.

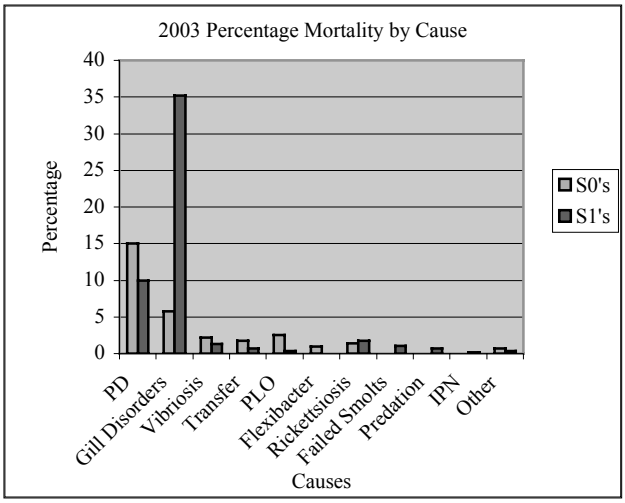

Figure 18: Percentage mortality for all diseases experienced by S0's and S1's in 2003 ("Other" category includes lice damage, etc.).

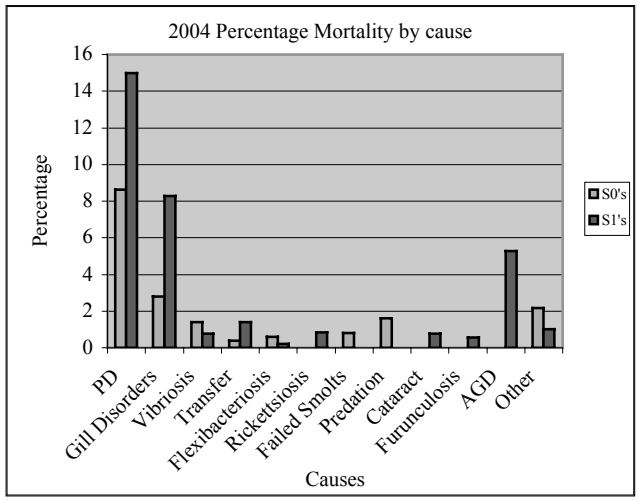

Figure 19: Percentage mortality on a cause by cause basis for S0's and S1's in 2004 (AGD = amoebic gill disease).

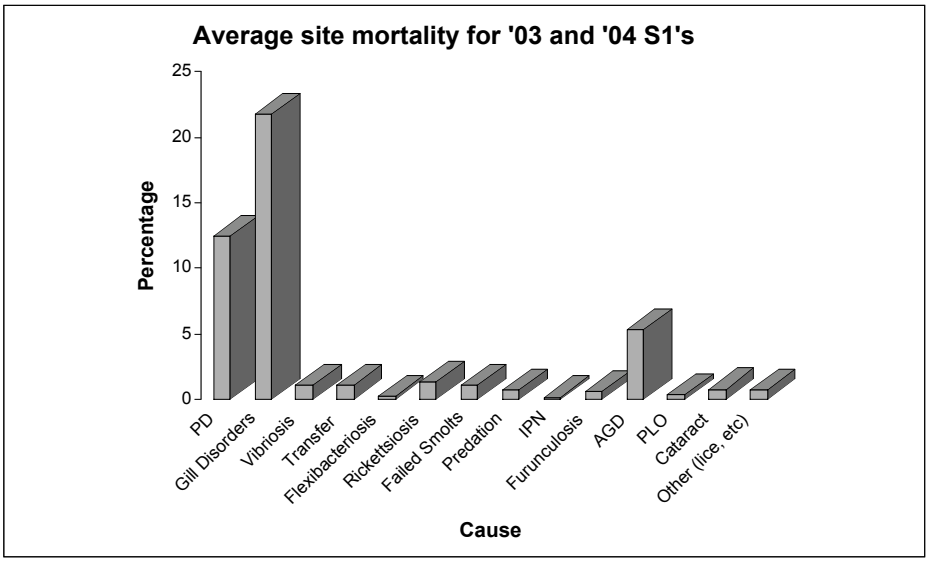

Figure 14. Average S1 mortality due to specific diseases in 2003 and 2004.

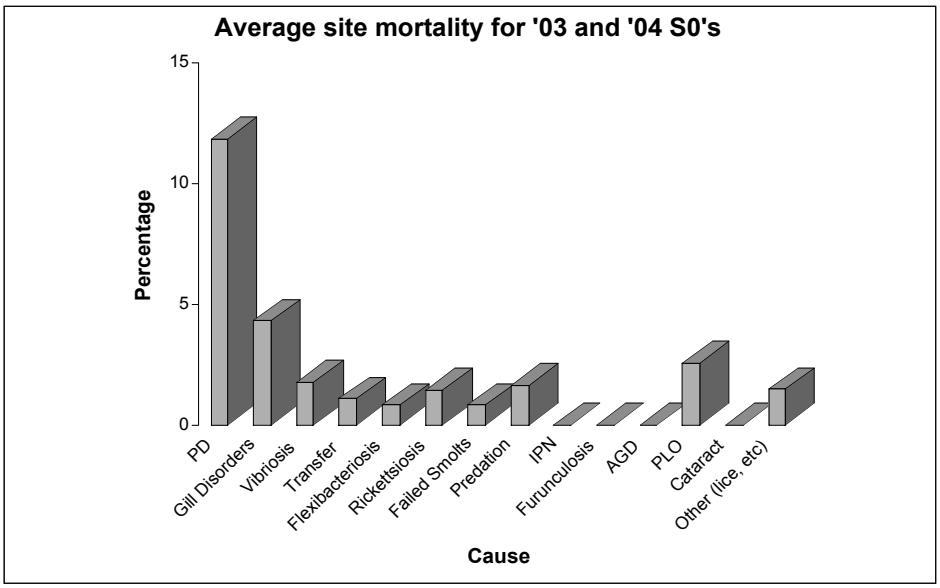

Figure 15. Average S0 mortality due to specific diseases in 2003 and 2004.

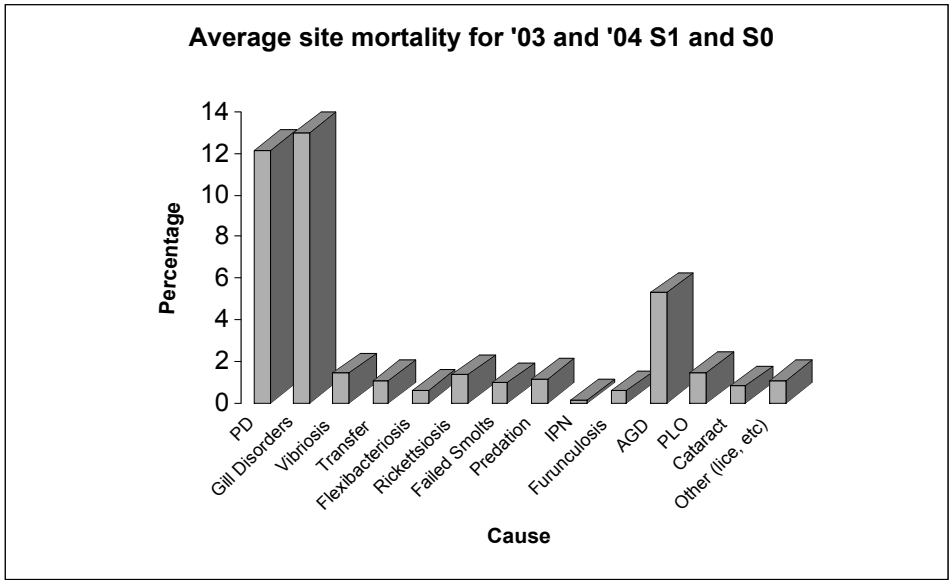

Figure 16. Average mortality due to specific diseases for all stocks in 2003 and 2004.

Regional assessment

The Western counties (Galway and Mayo) are most severely affected by PD, in terms of mortalities, with all sites in these regions positive in both study years and average mortalities two to three times higher than affected farms in other regions. The summary figures are shown in Table 14.

Table 14. Regional incidence and mortality due to PD in 2003 and 2004.

Region (year)	No. sites	No. PD +ve (%)	Average mortality due to PD
North (2003)	9	4 (44)	9.6
West (2003)	8	8 (100)	24.3
South (2003)	4	1 (25)	12
North (2004)	6	6 (100)	9.7
West (2004)	6	6 (100)	19.9
South (2004)	2	0 (0)	NA

2003

Cross tabulation of the three main regions North, West and South revealed a significant difference existed between them in terms of occurrence of PD (Fishers Exact Test, $P = 0.013$). Testing paired comparisons was carried out between regions, accounting for multiple comparisons by using the Bonferroni Correction to modify the P value (0.05 / 3: New $P = 0.02$). This uncovered a relationship of borderline significance between North and West ($P = 0.029$) and between South and West ($P = 0.018$).

2004

The difference between the mortality levels as opposed to occurrence of PD was more amenable to this type of analysis for 2004 data. A significant difference was uncovered when all three regions were compared using cross tabulation ($P < 0.01$). No paired comparisons returned significant values.

2.4. Discussion

The survey was carried out in an attempt to increase the current knowledge available on the epidemiology of PD in Ireland. A similar survey was carried out in 2002 in response to the recent re-emergence of PD as a major disease problem in Ireland (McLoughlin *et al*., 2003a). The 2002 survey did not uncover the exact reason for the re-emergence of the disease, but it did produce recommendations to help avoid PD or reduce its impact, in case of an outbreak.

Incidence and mortality due to PD

There was a very dramatic decline in the number of sites rearing salmon in Ireland in 2004 and this was due to economic as well as disease issues. Although the survey results had 100% of all farms, due to the low number operating and the high prevalence of PD (12/14 sites positive), analytical national epidemiology was of limited value.

The current study covered two production cycles with 21 inputs to the sea in 2003 (8.7 million smolts) and 14 inputs in 2004 (5.4 million smolts). In 2003, 13 out of 21 (62%) sites reported outbreaks of PD; in 2004 the figure was 12 sites out of 14 (86%). These figures show an increase in the number of sites affected by PD since the previous survey in 2002, when the figure was calculated to be 59% (McLoughlin *et al*., 2003a), however, it is the reduction in the number of operating sites that has given this apparent increase in prevalence. The average mortality due to PD on affected farms was 18.8% in 2003 and 14.8% in 2004. This compares with 12% from the previous survey. In six farms studied through 1990 to 1994 the mean PD mortality levels ranged from 3.8% to 16.8% (Crockford *et al*., 1999). The large range in mortality due to PD in both years of this investigation (2003, 2%-35%; 2004, 4%-35%) indicates site management, environmental or other factors may play an important role in the impact of the disease. As in 2002, the regional association with presence and impact of PD showed that farms in the Western counties (Galway and Mayo) were all affected by PD in both years and that the mortality due to PD was two to three times higher than farms affected in other regions (Table 14). This could not be explained by the variables examined and may reflect specific environmental conditions or viral risk factors or mixtures of these that remain to be determined.

The time of year that outbreaks occurred varied between sites, but there were two distinct high risk periods for contracting PD; either the early summer or early autumn. This characteristic biphasic disease peak is typical of PD and has been observed in Norway (Brun *et al*., 2005) and also in the previous study in Ireland (McLoughlin *et al*., 2003a). The reason for this is unknown. It may be due to a number of interacting factors such as feeding rate, temperature, or environmental conditions resulting in increased metabolic demands for the fish, making them more vulnerable or susceptible to disease. In laboratory cell culture the virus is reported to grow most readily between 10°C and 14°C and does not grow well above 15°C (McLoughlin *et al*., 1998). This is possibly the most likely reason for the characteristic outbreak pattern, with Irish sea temperatures being in this zone in early summer and early autumn. Further research in this area is required to make an attempt to plot spatial sea temperature in conjunction with individual outbreaks over time and in specific locations. The graphic representations of PD mortality against time indicate a typical pattern of infectious disease and specifically one which is transmitted horizontally i.e. it is not consistent with vertical transmission.

Smolt type and strain

Although sites using S0 smolts were apparently more likely to get PD than those with S1 smolts in 2003, in 2004 there was no significant difference. When the years were combined there was also no significant difference between S0 or S1s in contrast to the 2002 study but consistent with the studies in Norway (Brun *et al*., 2005). No significant difference in mortality level between the two smolt types was uncovered in this study.

Analysis of data from four sites was used to compare strain of fish and susceptibility. There was a highly significant difference ($P < 0.001$) between mortality levels experienced by the two strains in each of the four farms. All four farms were located in the Western region.

Stocking density and fish weight

The reason these factors were investigated was because with current knowledge on disease transmission and susceptibility of fish at different life stages, higher mortality levels would be expected with high stocking density (increased disease transmission) and younger fish (less immune competence). However, no significant associations were found between either levels of mortality or occurrence of disease.

Previous history of PD in a site, presence of another PD positive site in the same bay or proximity to a processing plant.

If PD had been present on a site in previous years and if another PD-positive farm was present in the same water body, an increase in the incidence of PD, or mortality due to it, may be expected as a result of an increased infective challenge, or dose, from the virus. A similar association could be expected if a processing plant in close proximity to a fish farm site discharges untreated processing effluent, which would be likely to contain infective material. In 2004, previous history of PD in a site was a significant factor in reoccurrence of the disease. In regard to all three above factors there were no other significant associations uncovered, either with occurrence of disease or mortality levels. There was an increased likelihood of high mortality in association with each of the three factors however (see OR's in *Results*). The lack of statistical significance in these situations may be due to the low number of units / inputs in the study.

Vaccination of fish against PD

Pancreas disease vaccines have been undergoing development for the past 10 years and a number of field trials have been conducted in Ireland, Scotland and Norway (McLoughlin *et al*., 2003b). The vaccine currently available to Irish farms is monovalent in nature. Vaccination is generally an important disease control strategy in intensive systems such as aquaculture, with many effective bacterial vaccines on the market for the last number of years. Vaccination against PD would be expected to confer resistance to the disease or at least substantially reduce mortality. On first examination the results for both years of the study suggest that PDV vaccination is of limited benefit, however, these results may have occurred due to the fact that only farms that had a previous history of significant PD decided to vaccinate. However, in sites where vaccinated fish could be compared to unvaccinated there was an apparent more rapid decline in PD mortalities in vaccinated fish when compared to unvaccinated (Figure 11), although the strain of fish may be a more significant factor (Figure 12) compared to vaccine. Further work in the area of alternative vaccine development and investigation into the efficacy of the currently available vaccine needs to be carried out.

Fallowing

It is generally accepted that fallowing marine sites is beneficial for the environment and the health of the fish. A previously reported study indicates that in the years following fallowing there had been significantly lower total mortality when compared to unfallowed years ($P<0.05$). The same study (Wheatley *et al*., 1995) also indicated that a reduction in mortalities due to PD occurred with fallowing but this result was not significant. In the current study, the OR calculated for both years of the survey indicated there was an increased likelihood of PD occurring and of elevated mortality levels associated with fallowing. None of these results were statistically significant, however in 2003, the increased likelihood of PD occurring in conjunction with fallowing approached significance ($P = 0.07$). These counterintuitive results may be due to the fact that only farms that previously recently experienced severe PD decided to fallow their sites, or that those factors other than breaking the disease cycle and accumulation of infective material through carrier fish, etc. are more important in giving rise to a severe PD

outbreak. It was also apparent through the study that although specific sites may have been fallowed, the bay or whole rearing area would not be totally fallowed of all salmon livestock i.e. other farms were maintaining stock in the same water body and this may negate or reduce any possible benefit of specific site fallowing.

Movement of fish, method of holding at freshwater sites and organic vs conventional farms
Movement was selected as a possible factor that may be associated with outbreak of disease or increased mortality, due to the elevation in stress levels in the fish. The timing of fish movement was recorded in relation to the incidence of PD. Fish movement and occurrence of PD were significantly associated in 2003 ($P = 0.02$) but were not in 2004. Fish movement and its effect on PD mortality level was further examined and although there were indications that there was an association with movement and high mortalities due to PD for both years, these were not statistically significant. Future temporal and spatial analysis of this data may uncover some interesting epidemiological aspects of disease. There was an increased likelihood of high mortality in fish that spent part of their life cycle in open water pens as opposed to just tanks. This was not significant however. Increased high mortality was also the trend in organic versus conventional farms, but also lacked significance.

Lice burden at time of PD outbreak
The data from 2003 showed a significant association with salmon lice (*L. salmonis*) burden and outbreaks of PD, but not with the severity of the PD outbreak. There were no associations with data for 2004. A high lice burden may stress the fish and could result in increased susceptibility to PD. In addition, or alternatively, lice could act as a vector for the PD virus. Terrestrial alphaviruses are transmitted between vertebrates by mosquitoes and certain other haematophagous arthropods and have a wide host range and a worldwide distribution (Murphy *et al*., 1995). The recent finding of an alphavirus from a louse (*L. macrorhini*) on elephant seals has added to speculation that sea lice could act as vectors or transmitters of alphavirus to salmonids (Linn *et al*., 2001). In addition, in the cell-mediated immune response in fish, the classical division of T cells remains to be proven. However, it could be hypothesized that with an increased parasite burden there may be stimulation of the TH-2 side of the immune response, making the fish more susceptible to viral infections, as the TH-1 side of the response (responsible for countering bacterial and viral infection) is down regulated in these situations (Roitt *et al*., 1998). Furthermore, an increased cortisol level due to increased parasitism could also leave the fish more vulnerable to viral infection.

Feeding rate at time of outbreak.
No statistical significance was uncovered in this association, however there was an increasing likelihood of high mortality (OR = 9) with fish fed at high feeding rate (>1%) in 2004. When questioned, farmers generally felt that feeding fish at a lower then normal rate during the time of PD outbreak resulted in a reduced mortality level. It has also been observed that during a PD outbreak, and especially during the second wave of mortalities associated with myopathies (Figure 13) fasting the fish or holding back on feed will have a marked reduction on mortality rate (Rodger, personal observation). This area requires further investigation, because if it has a significant impact on mortality it would be a valuable management tool to reduce the impact of the disease.

2.5. Conclusions & Recommendations

Pancreas disease remains the most significant single infectious disease affecting marine salmon farms in Ireland. However, mortality due to other conditions, in particular gill disorders were also very significant in 2003 and 2004. Mortalities caused by PD are particularly severe in the farms in the western counties of Galway and Mayo, but the disease also causes a significant impact in Donegal.

The two periods of highest risk to succumb to PD during the year are in the early summer (May to June) and early autumn (September to October), although outbreaks can occur out with these periods. During PD outbreaks the peak mortalities are reached on average eight weeks after the first clinical signs. The average loss of growth in PD affected fish was estimated at 11.4%, for the two years studied.

There appeared to be some indications of association with PD, or the severity of mortalities due to PD, for the following factors:

- >250,000 fish in a site
- Use of a specific strain of fish in the Counties Galway and Mayo
- Presence of another PD positive farm in the water body
- A previous history of PD on the farm
- Moving livestock to another sea site during the marine rearing phase
- High lice burden
- High feeding rate prior to a PD outbreak
- Being located <4km from a fish processing plant

However, due to low numbers of each category some of these variables were not statistically significant in association for each year.

It is recommended that further investigations into this data and future years be undertaken, specifically for the variables highlighted. Environmental data for each farm, in particular water temperature will also be worthwhile assessing in light of the regional differences.

These identified risk factors that have an indicated association with PD outbreaks, may lead to an increased severity in an outbreak but require further investigations through national clinical trials and international epidemiology. Qualitative or descriptive epidemiology indicates and emphasizes that further research is required into the areas of; developing strains of salmon that are tolerant of PD, improving vaccine types and strategies, investigating the role of sea lice in PDV epidemiology and screening of lice for PDV, conducting clinical trials into feeding strategies to combat the impact of PD and conducting investigations into sea temperature profiles for each sea site in Ireland and associations with the severity of PD outbreaks.

Further management procedures recommended

In addition to the recommendations for further research outlined above, the following farm management recommendations are suggested to minimise the impact of PD in Ireland:

a) Feeding during the high risk periods (May to June and September to October) should be strictly controlled at specific pre-set feeding rate percentages (less than 1% body weight). This implies accurate fish number and sample weight data on a pen-by-pen basis as well as accurate feed quantity data.
b) If PD is confirmed the feeding rates should be held at base maintenance levels and fish not allowed to gorge or feed to appetite when appetite returns. If mortalities increase dramatically, a period of fasting (7 to 10 days) followed by a slow return to maintenance ration over two to three weeks on a pen-by-pen basis is also suggested.
c) New sites should not be sited near (<4km) fish processing plants.
d) All fish processing plants should ensure that all effluent and discards are treated or sterilized to reduce the risk of virus survival.

e) Adequate and secure treatment of PD mortalities should be routine to ensure the risk from this infectious material is minimised.
f) Bays should be fallowed prior to restocking.
g) Avoid moving or grading fish when affected by PD.
h) Mortality removal from pens should be frequent (daily during high mortality periods) and ideally involve a component such as the automatic compressor driven system that reduces the risk of dissemination of infectious material during the removal process.
i) If PDV vaccination is to be used on site, all fish should be vaccinated to ensure the benefits of maximum herd protection and prevention of disease.
j) Regular screening of fish populations for PDV and/or typical pathologies prior to movements, grading, etc. should be standard practise.

The Marine Institute, Galway is acknowledged for financially supporting this investigation. The salmon farmers of Ireland are thanked for their time and patience in data collation. Professor Simon More, Ms. Tracy Clegg and Mr. Dan Collins of the Faculty of Veterinary Medicine, University College Dublin, are thanked for advice on veterinary epidemiology and statistics.

3. SMOLT SUSCEPTIBILITY TRIALS

LANDCATCH TRIAL
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MARINE HARVEST TRIAL
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The following chapter details the current data collected from smolt susceptibility trials using Landcatch UK stock, carried out between December 2004 and July 2005 at Muir Gheal Teo and Mui Achmhainni Teo, and Marine Harvest stock carried out between February and November 2005, at the Mannin Bay Salmon Co. Galway (Figure 1).

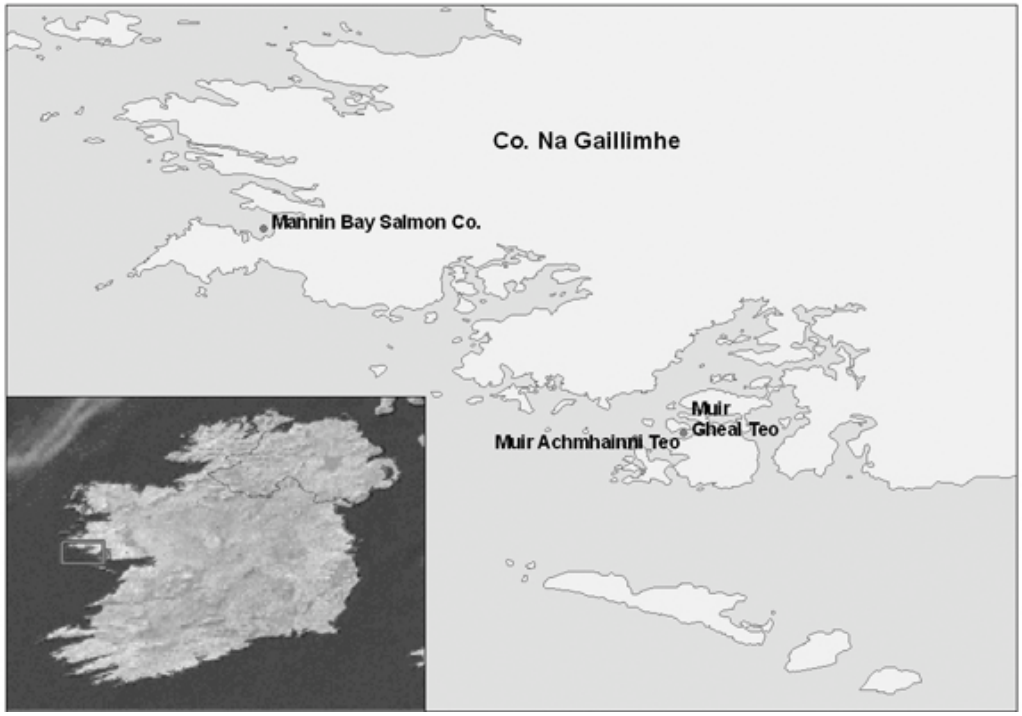


Figure 1: Map of the sites used in both the Landcatch UK and Marine Harvest Ireland trials.

3.1. Landcatch UK trial

Description

On December 22, 2004 two populations of 5,000 fish representing approximately 180-200 families from the Landcatch hatchery at Ormsary, Scotland, were each put to sea into two commercial salmon farming sites; Dinish (Muir Achmahainni Teo) and Knock (Muir Gheal Teo) in Co. Galway. These sites had previous histories of salmon pancreas disease and it was anticipated that the experimental fish would be exposed to the salmon pancreas disease virus from the production fish comrades or the environment, until they were removed from the sea at the end of the trial. The fish were placed into 70 m polar circle cages alongside 2003 generation S0 fish that were still suffering mortalities due to PD. The trial ended on 14th August 2005. During this period, numbers of mortalities were counted, sampled and categorised into 4 groups

(Table 1 & Figure 2). From each mortality the adipose fin was clipped and placed into ethanol/EDTA tubes for DNA analysis. A sub-sample was collected for histopathology and serology to assess the PD status of the fish and to establish an initial diagnosis of pancreas disease. Histopathology was employed and veterinary visits conducted to determine changes in mortality trends, to facilitate mortality categorisation. During the trial, fish were fed four times per day by hand and received two slice and two bath treatments.

Muir Gheal Teo

1. No fish were recorded as suffering from initial transfer mortalities or subsequent trauma. However, during the subsequent two months some fish exhibited cataracts and failed to thrive.
2. Four bloods were taken on the February 22, 2005 one of which was viraemic.
3. No subsequent serology or histology has demonstrated the presence of the virus or virus-induced pathology.
4. Some gliosis (focal inflammation in the brain tissue) was seen on two occasions, in each case, in single fish.
5. The final serology sample taken on the July 21, 2005 showed an absence of viraemia or seroconversion in all samples.

Since the single viraemia positive in February, there was no further definite sign of PD infection in the fish. Serology and histology samples were taken in late July to assess seroconversion and pathological change, but results were negative on all samples.

Muir Achmhainni Teo

Muirachmhainni Teo. incurred a considerable number of transport mortalities (397 alone on the December 22 & 23, 2004).

Cataracts became a significant issue in the first 1-2 months of the trial resulting in poor fish being the main cause of mortality through January. Mortalities dipped in February before rising again in March. These consisted mainly of thin failed smolts. During this period, some fish exhibited lateral ulcerative lesions, but the problem did not affect the population, seeming only to be associated with the failed fish, and numbering around 17-18 individuals only.

1. Histology remained unremarkable until April 12 when single rounded acinar cells were seen among a majority of normal cells.
2. Over the next 3 weeks, rounded acini without overt pyknosis increased until a presumptive diagnosis of SPD was offered by histology on May 7. Two days later, nineteen blood samples were taken, and two of these were shown to be viraemic (had not yet seroconverted -antibody negative).
3. Histology samples taken on May 17 showed 3 fish with acute PD, while the other 3 in the sample exhibited some red muscle involvement.
4. Subsequent histology and serology samples confirmed the progression of the infection up to the end of the trial (June 30).
5. 274 fish were marked down as category 4 (“other”) during May and June. In the absence of any other significant clinical findings during this period, it is most likely that these fish suffered from the effects of PD.
6. Fish were removed on the August 11.

Since diagnosis of SPDV infection on May 9 (viraemia +ve), routine sampling yielded good histological examples of the infection, ranging from acute to chronic cases. These samples proved necessary in assigning groups of mortalities to the various categories, and continue to provide an archive for further immunocytochemistry studies.

Despite this, uncertainty existed when groups of fish were being assigned to mortality categories in the absence of veterinary input. Groups of fish were categorised as group 4 (“other”) and some

of these have been shown to be PD fish from histology. These definite PD fish have been included in the total for PD in column 1 above.

It seems likely that many of the group 4 fish from the end of May onwards were affected with PD, and died as a result. No record is made of fish suffering from gill lesions and no evidence of gill lesions or any other significant infection was seen in the histology samples. These fish are included in column 2 (Likely PD). Finally, fish continued to be sampled in July (DNA) in the absence of histology. These were assigned to group 4 by the sampler. No report of other disease was made during this period and again it seems likely that these suffered from the affects of PD. These are included in column 3 (July).

Table 1. Numbers of mortalities assigned to categories 1-4

Mortality Category	Muirachmhainni Teo.	Muirachmhainni Teo. (likely PD fish)	Muirachmhainni Teo. (including July)	Muir Gheal Teo.
Input number	5000			5000
1 - Accidental / trauma	227	227	227	0
2 - Failed	130	130	130	107
3 - PD	56	164	363	0
4 - Other	382	274	382	328
	795	**795**	**1102**	**435**
Number removed	452			890
Difference	**3753**			**3675**

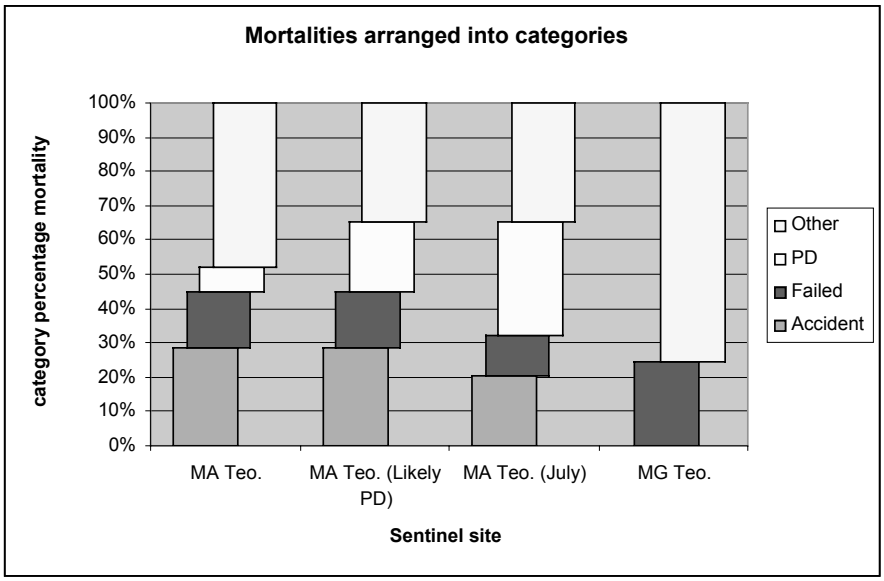


Figure 2: Percentage mortalities arranged into four categories (see Table 1) during the Landcatch sentinel trial.

Comments and Observations

It became apparent when the fish were removed from the sea, that there was a discrepancy in both sites between the number of fish that were put to sea in December and the combined number of fish that died and were removed from the sea at the end of the trial.

No conclusive explanation has been found for this. Input numbers may have been inaccurate, losses may have been incurred during the trial, or a combination of these factors may have resulted in fewer than expected fish at the end of the trial. Discussions arising since the

discoveries of the missing fish have focused on (avian) predation as the main reason for losses incurred. There was no record or indication of an escape event. While there had been some lack in visualising the fish from the surface, particularly in Muir Gheal Teo, in the last few weeks, it was not felt that the populations had suffered any losses. Diver reports did not estimate numbers, but did report fish behaving relatively normal.

Failure of clinical disease in Muir Gheal Teo or any indication of sub-clinical disease or infection through serology, was unexpected. The appearance of a viraemic fish in February remained the only such +ve result. This may have been an obscure clinically affected fish, or an obscure laboratory result, and the significance is unclear.

Based on observations and findings from these sentinel trials, future improvements might include:

1. Marking and mixing of trial fish with same generation production fish in a co-habitation trial.
2. Employment of a suitably experienced staff member responsible for the day-to-day management of the project.
3. The daily removal and sampling of mortalities by that staff member.
4. Trial cages should be completely sealed with small mesh bird nets and a thorough anti-predator strategy implemented.

Acknowledgement is due to the Marine Institute for covering the cost of veterinary input and sample processing; to the Marine Institute and the Vet School, UCD for histology; Veterinary Sciences Division, Stormont for serology; to Landcatch Natural Selection for providing the stock, and to Damien O'Ceallacháin, Noel Lee and the staff of both farms for assisting in the trial and for facilitating access to the cages.

3.2 Marine Harvest Ireland Trial

Tagging:

A total of 6,000 Fanad family fish from 150 families were electronically PIT tagged during two weeks in February 2005 (Figure 3). Fish were anaesthetized in small batches, (c.25), a small incision was made between the pelvic fins and the PIT tags were inserted into the belly cavity manually. These fish were concomitantly weighed and their adipose fin removed for genotyping before being returned to an oxygenated tank for recovery. All fish recovered well and no mortalities were observed immediately following the procedure. Tanks were also checked for tags prior to emptying and although tag loss has previously been reported as a problem, none were found.

The 6,000 tagged family fish were transferred from Altan, Co. Donegal to Hawks Nest site of the Mannin Bay Salmon Company, Clifden Bay, Co. Galway on April 28, 2005, together with 24,000 untagged fish. This site has a previous history of PD and it was anticipated that the sentinel fish would be exposed to the salmon pancreas disease virus from the production fish comrades or the environment, until the end of the trial. The sentinel fish were maintained in one cage, at Hawks Nest, for the duration of the trial. Six other cages, holding an initial total of 214,500 S1's were also present on site.

Routine Sampling

Observations of behaviour and feeding response were made on a daily basis. Specifically, inappetent, lethargic fish hanging at the net were watched for, along with the presence of white faecal casts.

Initially, mortalities were collected once a week, by diving. By mid-June an uplift system was in place and from then on the mortalities were collected every day. Each mortality from the sentinel cage was checked for the presence of a tag (identified by a PIT tag scanner or by the absence of

the adipose fin), the tagged fish were then scanned into the database, clinical observations made and they were assigned a code denoting the suspected cause of death (see Table 2). Untagged fish were also counted and assigned a cause of death, although this was based solely on the external condition of the fish.

Further to the above, five tagged fish per week were removed for histology and blood sampling. Pyloric caecae, heart and skeletal muscle were taken as a matter of course. Additionally, any other tissue showing signs of pathology, most notably gills, were sampled when deemed necessary. When PD was suspected on site, routine sampling was increased to 8-10 fish per week until presence of the virus was confirmed.

Samples were stored in buffered formalin for approximately 24 hours before processing and slide preparation either in the Marine Institute, Galway or in the Department of Veterinary Pathology, UCD. Blood samples were chilled and settled overnight before the sera were removed. Analysis for the presence of PD virus and antibodies was carried out by the Fish Disease Unit, Department of Agriculture & Rural Development, Northern Ireland.

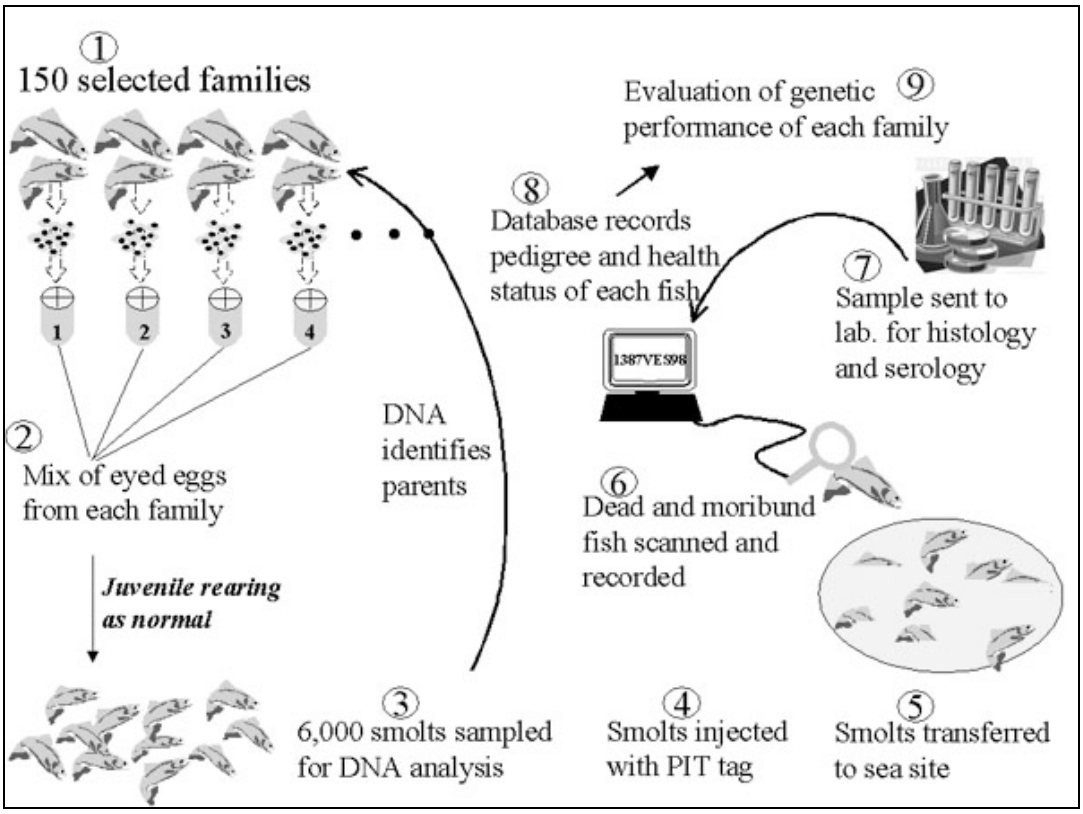


Figure 3: A schematic diagram of the experimental set-up for the Marine Harvest smolt susceptibility trial.

Table 2. Cause of death and corresponding code.

Code	Cause
1	Tagging related mortality before transfer to sea.
2	Lesions, mainly on flanks and dorsally around adipose fin – due to late sea transfer.
3	Other non-PD eg. seal, failed smolt, non-PD pathology. (Details recorded in each case.)
4	Routine histology sampling.
5	Tag damage (obsolete).
6	PD
7	Chronic heart failure stage of PD.

Criteria for Assigning Cause of Death

Once PD was confirmed in the sentinel cage, mortalities were assigned Code 6 (PD) where there were no other signs of pathology externally or internally, and where there were yellow/white casts or where the gut was empty. In each case notes were made for each individual fish and a score of the extent of pyloric fat was kept. Code 7 was assigned to large feeding fish that showed no gross internal or external pathology – this code was only used once PD had been present for some weeks.

Food Consumption and Fish Condition

At the time of tagging the average weight of the fish was 64.5g, on going to sea it was 80g. The data below (Figure 4) were taken from the fish sampled weekly for histological examination. Care must be taken when interpreting the data as the sample size was small (between 5 and 10 fish) resulting in a large variability in the data. However they do serve to give an indication of growth over the summer months. As of early November, the variation in weight of the sentinel fish ranged between 200g and >600g.

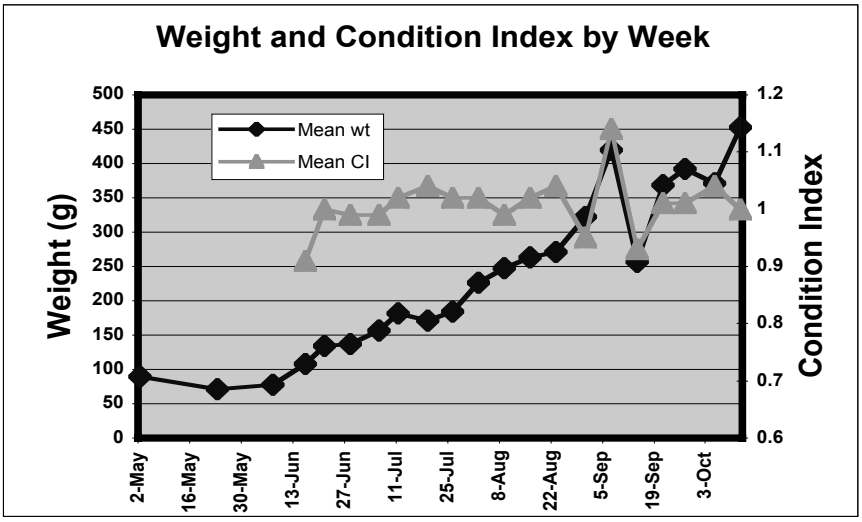


Figure 4: Weight and condition index of salmon sampled during the trial from May to October, 2005. The Condition Index was calculated as (weight / (length)^3)*100.

Summary of Mortalities

Up to the 6th November the number of mortalities recorded was 7,256. Of these, 832 have been tagged fish, which equates to 11.5% of all fish retrieved being tagged. Tagged fish initially made up 20% of the sentinel population and so this indicates an under representation of tagged fish in the mortalities. It is unlikely that this due to a problem in identifying the tagged versus untagged fish and is more likely due to a loss of tags in the field, primarily to seal predation.

Approximately 1020 seal damaged fish have been retrieved and for the great majority of these, only the head and fore-body remained. This is reflected in the fact that only 39 tags were collected from seal damaged fish, less than four percent of the total. It is also likely that some tags were lost from decomposed fish and that a percentage of fish have not been retrieved at all. It is also unknown how many, if any, tags were lost from fish prior to their move to sea.

The first mortality peak, during May, was post-transfer to sea and due to skin lesions as a result of over-smoltification and mechanical damage during transport. There were also a large number of failed smolts in the first two months. Mortality was low between mid-June and mid-August, until PD deaths began. Pancreas disease was first suspected on site on the July 25, but a diagnosis was not offered for the sentinel fish until August 16.

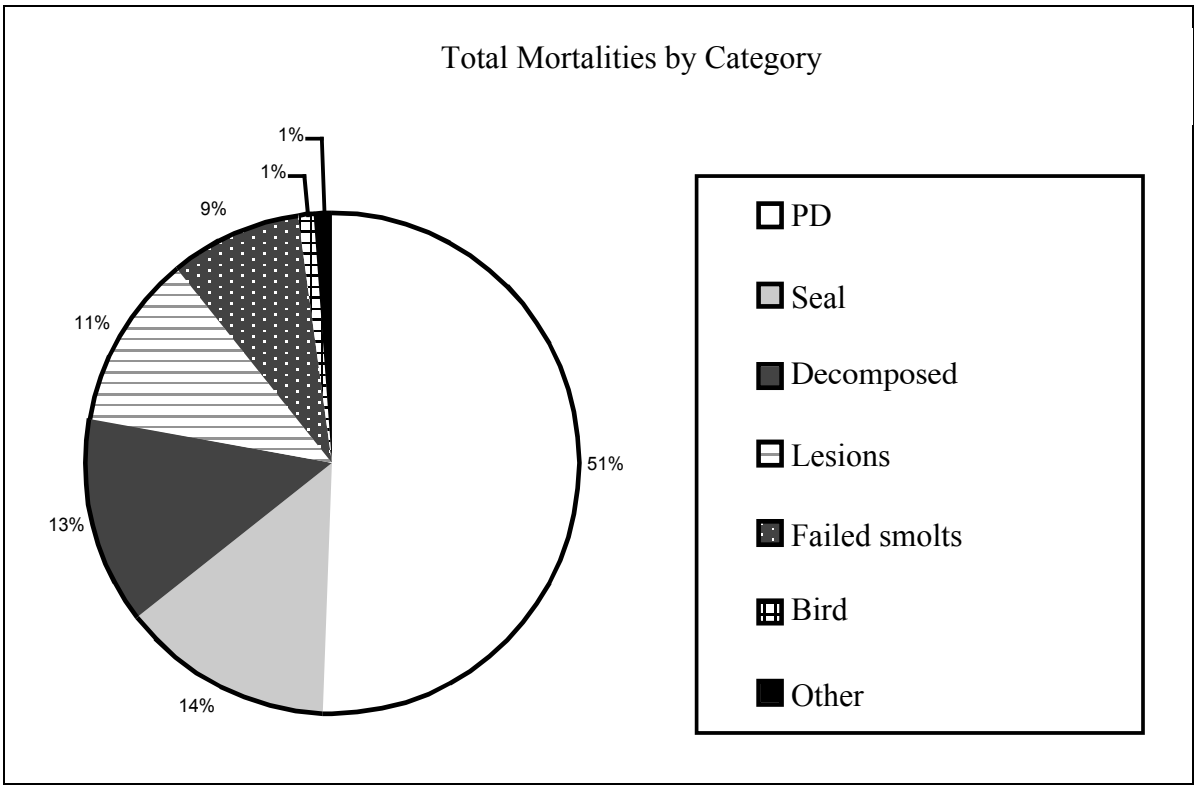


Figure 5. A breakdown of the causes for mortality in salmon during the trial.

Of total mortalities, 51% were due to pancreas disease – Figure 5. It is also likely that fish recorded as seal damaged and decomposed, (which are essentially unassigned), were also for the most part PD deaths. No seal damage was observed on live fish, which has previously been indicative that only dead fish are being taken (Eugene Casey, pers comm). Figure 6 gives a detailed breakdown of the causes of death of the tagged fish, week by week (approximately), for the duration of the trial.

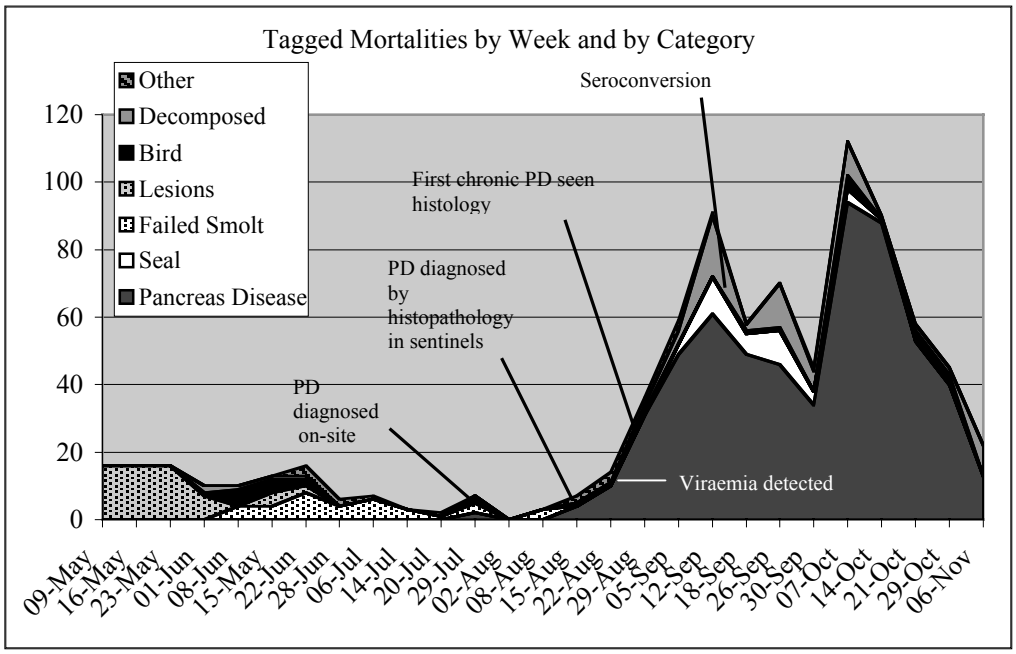


Figure 6: The progression of PD during the trial with the key dates on PD detection and diagnosis indicated.

Progression of Pancreas Disease during the trial

The contiguous cages exhibited signs consistent with pancreas disease around July 26 (histopathology and serology), and within the following two weeks, histology from the sentinel cage displayed signs of increased cardiac cellularity (endocardium). The second week (August 9), had fish exhibiting signs of early infectious challenge: cardiac changes and individual acinar cells appearing pyknotic in the pancreas. In retrospect this was likely the first indication of early pancreas disease; however, typical PD histopathology was not seen until the following week (16^{th}), when a diagnosis based on histology of acute PD was offered. Nine days later, the first viraemic fish was detected, confirming the diagnosis. Interestingly, within two weeks of histological diagnosis, the first chronic PD fish was seen (August 31). One month from histological diagnosis, the first evidence of antibodies to the virus was seen: seroconversion on the September 16. Various stages of PD (acute, sub-acute, chronic) were seen on histology in the subsequent weeks until the end of the trial at the end of October. The final sample taken on November 3 revealed some fish with what appeared to be subclinical PD, exhibiting minor changes to the pancreatic fat, heart and muscle, or perhaps evidence of some other clinical insult (2 of these fish had significant gill pathology).

In August, some fish samples exhibited caecal mucosal sloughing, with pyknosis seen in the mucosal epithelium occasionally. This mucosal sloughing was seen in some of the acute PD fish. Two vials of tissue, representing 6 fish sampled on August 31, were taken for virological screening, and found to be negative for IHNv, VHSv and IPNv. The sloughing appeared to stop around the end of August. There is a chance that this sloughing represented a concurrent infection, or possibly it was a sequel to acute PD infection. There was no continued effect; neither was any mortality directly attributable to it.

Key Dates:

- July 26: contiguous cages displaying signs of PD (sentinels remain free)
- August 9: first suggestion of pancreatic changes
- August 16: first diagnosis of PD offered from histopathology
- August 25: first confirmation of SPDV viraemia from infected fish: definitive diagnosis of PD
- August 31: first chronic PD seen
- September 16: first antibody detected: first seroconversion

Temperatures have ranged between 12ºC and 18ºC, with no periods of sustained high temperatures. Mortalities were unremarkable for the first month, with some bacterial lesions described likely arising from mechanical damage and transport stress; by early July losses were being attributed to the lesion problem and failed smolts. Mortalities remained relatively low throughout July (540/30000 representing 1.8%). This period coincided with a *Karenia* sp. bloom (latter half June through to early August), which may account for some of the losses. Gills were sufficiently irritated to exhibit haemorrhage while being anaesthetised for examination and secondary changes were described on histological examination. Since the end of July a cataract problem was described in the fish, initially mostly grade 1-2, but with the percentage of >grade 2 increasing into September and October (when >grade 2 was 13.6% and 15% respectively).

Table 2. The extent and severity of cataracts in the sentinel fish. (Source: Vet-Aqua International)

Date	No. sampled	% with cataracts	% cataracts > 2.2
16/08/05	13	77	0
02/09/05	26	65	8
04/10/05	17	77	18
02/11/05	11	91	27

3.3. A Note on Selective Breeding For Resistance to Pancreas Disease

Of the 6,000 fish transferred to the Clifden Bay test site, 5,710 fish have been assigned to parents. The number of fish in each family group is an important statistic in estimating the variance components of any trait. This is particularly true when the percentage of fish that express a trait (here, for example, survival/susceptibility to disease) is either very high or very low.

All the fish in the present study originate from a family mating scheme whereby 147 dams were mated (either in a nested or factorial design) to 60 sires (each sire either mated to one, two or three dams). This resulted in 147 dam families (full sib groups) and 60 sire families (half sib groups). From the 5,710 fish assigned to parents so far, only one sire family and two dam families were not represented. From the remainder of the families, there was an average of **40 offspring in each dam family** and **95 offspring in each sire family**. There was a wide spread in the number of offspring in sire and dam families as demonstrated in the graphs below. However, there were only 3 sire families and 15 dam families where there were fewer than 10 offspring represented in the sample of 5,710 (~200 fish not assigned to any family=3%).

By November there were 815 fish recorded as mortalities in the Elvis database. From these over half are definite PD mortalities. From the remainder a significant proportion of fish may be re-classified as PD mortalities but at the time of sampling there was some doubt as to the cause of death. A further 250 mortality samples are being re-genotyped and these will be included into the PD mortality group once their identity is confirmed.

Fish from this trial can be classified into three groups; fish which survived the trial, mortality as a result of PD and mortality as a result of other causes. Figure 5 shows the mortality for each family from overall mortality and PD mortality as a proportion of the number of smolts input

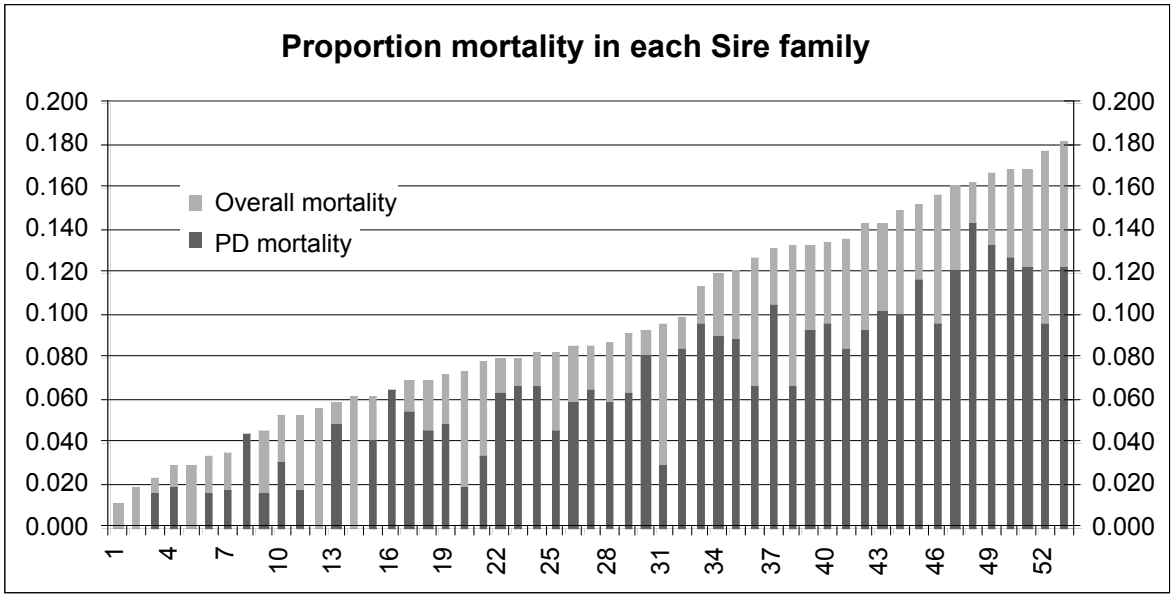
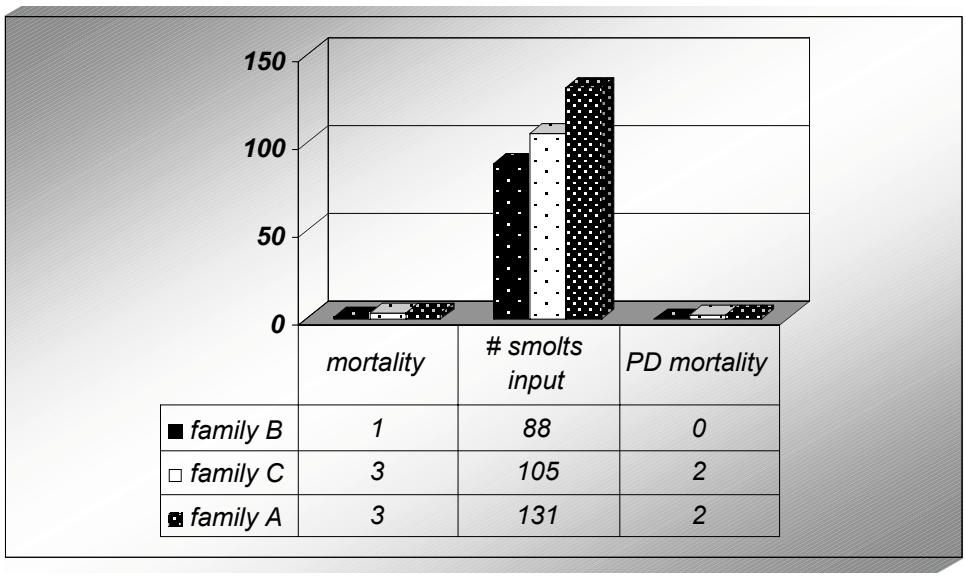


Figure 7: Proportion of mortality in each sire family during the trial as a result of overall mortality and definite PD mortality.

In general, overall mortality was proportionate to mortality due to definite PD. This could be because some or most of the mortality recorded as non-PD was in fact a result of PD, but it was not possible to say this for definite at the time. For example, some mortalities are recorded as being the result of seal damage but seals mostly do not kill healthy fish and these fish could have been suffering from PD infection before the seal damage. There is no serious cause of mortality in this trial which is independent of PD infection. If this was the case we would see many

instances of families with high mortality but without high PD mortality and vice versa. We did not observe this in this trial.
A number of families in the trial exhibited no, or very low numbers of, mortalities despite having large number of smolts per family input into the trial. Figure 8 shows three such families (Labelled A, B and C) where both overall mortality and PD mortality was low.



	mortality	# smolts input	PD mortality
■ family B	1	88	0
□ family C	3	105	2
▪ family A	3	131	2

Figure 8: Mortality, number of smolts input and PD mortality for 3 families

Already, there is a significant family 'effect' on both overall mortality and PD mortality. Genetic analysis can begin when all mortalities have been collected. This analysis will allow for the estimation of the amount of genetic variation and hence the heritability in these families for resistance to a PD infection as well as for overall mortality. Following this, potential broodstock can be identified from these top performing families and used for the production of eggs which will also exhibit superior resistance to PD.

Acknowledgement is due to the Marine Institute for covering the cost of veterinary input and sample processing; to the Marine Institute and the Vet School, UCD for histology; Veterinary Sciences Division, Stormont for serology; to Marine Harvest Ireland for provision of the stock and tags, and to Gerry O'Donaghue, Eugene Casey and the staff of Mannin Bay Salmon Co. Ltd. for assisting in the trial and for facilitating access to the cages.

4 EVALUATION OF SELECTED BIOPHYSICAL PROPERTIES OF SALMON PANCREAS DISEASE VIRUS (SPDV)

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4.1 Background

Although pancreas disease (PD) in Atlantic salmon has been recognised for many years, it is only within the last decade that SPDV has been isolated from affected fish and shown to be the causal agent. Following infection, SPDV causes sequential pathology of the pancreas, heart and skeletal muscle. While there is evidence of infection with SPDV on Irish farms throughout the 1990's, the clinical severity of these outbreaks typically tended to be mild. However, severe PD has now re-emerged as a significant cause of mortality in the marine phase of production of the Irish salmon industry, with attributed mortalities exceeding 50% on some sites. The economic impact of such losses has already contributed to a reduction in the number of smolts going to sea during 2004 and continued losses of this magnitude threaten the economic viability of the industry. The factors contributing to this re-emergence are presently unknown, but a recent study commissioned by the Marine Institute has made an initial epidemiological investigation of possible contributory factors and recommended areas requiring further research.

While the understanding of SPDV at the molecular and biochemical level has improved considerably in recent years, there is still a marked lack of knowledge in relation to the biophysical properties of the virus and one of the recommendations of the report is that studies should be undertaken in this area. This recommendation has recently been reinforced by an *Ad Hoc* Pancreas Disease Working Group consisting of government, industry and academic representatives and brought together by the Marine Institute. Knowledge of the biophysical properties of SPDV is required in two areas to assist industry in dealing with the current problems caused by SPDV;

1. Firstly, there is an urgent need for studies to confirm the effectiveness or otherwise of commonly used disinfectants against SPDV.
2. Secondly, there is a need for fundamental knowledge on the influence of temperature on the survival of SPDV in a range of different environments.

Such knowledge is essential to gaining a better understanding of the epidemiology of infections on farms and to the drawing up of effective control measures. Thus a short survival time in seawater under typical Irish conditions implies that direct site to site transfer through tidal excursions, well boats etc. poses a relatively small risk to the introduction of infection. On the other hand, long survival times indicate a high risk that possibly may negate the effectiveness of other biosecurity measures or the use of fallow periods. In addition, knowledge of the effect of temperature on the survival of SPDV in diagnostic samples such as serum is currently unknown. Recent improvements in virus isolation techniques now mean that this can be a useful rapid diagnostic technique. However, transport or storage of samples under sub-optimal conditions could unwittingly lead to false negative results and the subsequent failure to implement appropriate management practices to minimise the impact of infection.

In December 2005, the Marine Institute awarded the Irish Salmon Growers Association (with Queen's University Belfast as the research performer) a research contract (IND/04/12) to explore selected biophysical properties of (SPDV). This document serves as an interim report on the research conducted under this contract. Results presented are provisional and may be subject to change.

Objectives & Targets

- Objective 1. Investigate the susceptibility of SPDV to a range of chemical disinfectants and pH levels.
- Target 1. Identification of disinfectants effective against SPDV, information on the impact of acidity and alkalinity on the survival of SPDV and recommendations for their use.
- Objective 2. Determine the survival of SPDV at different temperatures and in different media under different organic loads.
- Target 2. Gain knowledge of the survival of SPDV in field situations and consider the epidemiological implications of the results. Provide recommendations of optimum conditions for sample collection, transport and storage to ensure accuracy and validity of diagnostic testing.

4.2 Materials & Methods.

Virus strains.

Two strains of SPDV grown in chinook salmon embryo (CHSE-214) cells are being used: F93-125 (10^{th} passage), the reference strain of SPDV, and F02-143 (5^{th} passage). Both viruses are subtype 1 strains of salmonid alphavirus, the predominant strain present in Irish salmon.

Virus titration

To determine the amount of viable virus in a given sample at a given time point, serial 10-fold (log) dilutions were prepared and tested as previously described (Jewhurst *et al*., 2004), except that 50_l volumes of each dilution were inoculated into 8 separate wells of a tissue culture microtitre plate. Titres were calculated according to the method of Karber (1931) and results expressed as 50% tissue culture infectious doses ($TCID_{50}$) i.e. the highest dilution of the sample that would infect cells 50% of the times it was inoculated.

4.3. Objective 1: pH testing

Inorganic Acid Testing (pH 4, pH 12)

Testing was performed to determine the susceptibility of both SPDV strains to pH 4.0 and 12.0 in the presence of organic material. For both virus strains, three replicates of culture medium containing 5% foetal bovine serum (FBS) and 37.5mg/ml bovine serum albumin (BSA) were adjusted to pH 4.0 with 1N hydrochloric acid (HCl) at 4ºC. 0.5 ml of each virus was then added to each of three replicate tubes and mixed (time 0). Immediately aliquots were removed from each replicate, neutralized to pH 7.2 with 1M sodium hydroxide (NaOH) and titrated. Aliquots were likewise removed and tested after 1 day and after 7 days. At the same time, and following the same protocol, both viruses were inoculated into culture medium containing 5% foetal bovine serum and 37.5mg/ml BSA that had been adjusted to pH 12.0 with NaOH. At 0, 1 and 7 days test and control samples were removed, neutralized as necessary to pH 7.2 using HCl, and titrated. Controls for each virus consisted of parallel triplicate inoculations into the same media at pH 7.2.

Results are summarized in Table 1. Over the seven day trial period, the controls (pH 7.2) for both strains showed minimal loss of viable virus, with a high degree of reproducibility between replicates at each time point. In contrast, exposure to either pH 4.0 or 12.0 resulted in very rapid inactivation, with virus levels falling to below detectable levels at the first test point. Although the starting titres of the two viruses used were different, both behaved similarly in terms of susceptibility to high and low pH.

Table 1. Results of pH 4.0 (HCl) and 12.0 survival testing for F93-125 and F02-143.

		Day		
Virus	pH	0	1	7
F93-125 p10	4.0	-**	-	-
F93-125 p10	4.0	-	-	-
F93-125 p10	4.0	-	-	-
F93-125 p10	12.0	-	-	-
F93-125 p10	12.0	-	-	-
F93-125 p10	12.0	-	-	-
F93-125 p10	7.2	5.625*	5.5	5.75
F93-125 p10	7.2	5.75	5.625	5.625
F93-125 p10	7.2	5.625	5.5	5.5
F02-143 p5	4.0	-	-	-
F02-143 p5	4.0	-	-	-
F02-143 p5	4.0	-	-	-
F02-143 p5	12.0	-	-	-
F02-143 p5	12.0	-	-	-
F02-143 p5	12.0	-	-	-
F02-143 p5	7.2	4.5	4.5	5
F02-143 p5	7.2	4.5	4.5	5.125
F02-143 p5	7.2	4.375	4.625	5

*reciprocal log titre ($TCID_{50}$/50µl).
** less than the limit of detection of the titration (<0.42 TCID50/50µl)

Organic Acid Testing (pH 4, pH5, pH6)
Based on the results of this initial testing showing rapid inactivation at low pH with an inorganic acid, a further round of testing was performed to examine the effects of using formic acid at a range of acidic pH levels (4.0, 5.0 and 6.0). The same protocol was used except that the timing of the first sample was standardized to 5 minutes, after addition of each virus to the acidified or control medium.

The results of this testing are shown in Table 2. Compared to the pH 4.0 data generated with HCl, both strains survived somewhat better in the presence of formic acid, with both strains being detectable after 5 minutes exposure. Nevertheless, the titre of both strains at this point had been reduced by around 4 logs, equivalent to 99.99% inactivation. No viable virus was detectable for either strain after one day at pH 4.0.

Exposure to pH 5.0 also resulted in viral inactivation, although at a notably slower rate. Thus at day 0 (five minutes) there was no loss of titre for either virus. After 1 day, there was partial inactivation of both strains, which was completed at 7 days. Exposure to pH 6.0 had no effect on either strain of virus, with titres not declining through the study period and being similar to the control values at each sample point.

Table 2: Survival testing for F93-125 and F02-143 at a range of acidic pH levels in the presence of formic acid.

		Day		
Virus	pH	0 (5 mins)	1	7
F93-125 p10	4.0	1.625*	-**	-
F93-125 p10	4.0	1.375	-	-
F93-125 p10	4.0	1.625	-	-
F93-125 p10	5.0	5.5	2.625	-
F93-125 p10	5.0	5.625	2.625	-
F93-125 p10	5.0	5.625	2.75	-
F93-125 p10	6.0	5.625	5.625	5.625
F93-125 p10	6.0	5.625	5.625	5.625
F93-125 p10	6.0	5.625	5.5	5.75
F93-125 p10	7.2	5.625	5.75	6.0
F93-125 p10	7.2	5.625	5.75	6.0
F93-125 p10	7.2	5.625	5.75	6.0
F02-143 p5	4.0	0.5*	-	-
F02-143 p5	4.0	-**	-	-
F02-143 p5	4.0		-	-
F02-143 p5	5.0	4.25	3.0	-
F02-143 p5	5.0	4.25	2.875	-
F02-143 p5	5.0	4.125	2.75	-
F02-143 p5	6.0	4.125	4.0	4.625
F02-143 p5	6.0	4.125	4.0	4.625
F02-143 p5	6.0	4.25	4.0	4.625
F02-143 p5	7.2	4.25	4.375	4.5
F02-143 p5	7.2	4.25	4.5	4.5
F02-143 p5	7.2	4.125	4.5	4.5

*reciprocal log titre ($TCID_{50}$/50µl).
** less than the limit of detection of the titration (<0.42 TCID50/50µl)

4.4. Objective 2: Temperature testing

Survival at 60°C

This experiment was conducted to determine the susceptibility of the virus strains to heat at inactivation 60°C in liquid media in the presence of organic matter. A similar protocol to that used for the pH testing was followed, except that the replicate aliquots were heated to 60°C before addition of virus. Samples were then held at this temperature before aliquots were removed and titrated after 5 minutes. Controls consisted of parallel samples held at 4°C. Results to date are shown in Table 3.

Survival Over Time at a Temperature Range

In the absence of data on the survival of SPDV at different temperatures, the first experiment was conducted using cell-culture grown virus with intensive sampling over a 60 day period. Multiple aliquots of each virus were dispensed and held at 4°C, 10°C, 15°C and 20°C. Initially sub-samples were removed daily for titration, reducing subsequently alternate days and then twice weekly. Due to the large numbers of samples to be tested on each sample day, each titration was performed only once, rather than in triplicate.

Table 3. Survival testing for F93-125 and F02-143 at 60 and 4°C.

Virus	Temp.	Time (5 mins)
F93-125 p10	60°C	-**
F93-125 p10	60°C	-
F93-125 p10	60°C	-
F93-125 p10	4°C	5.875*
F93-125 p10	4°C	5.75
F93-125 p10	4°C	5.75
F02-143 p5	60°C	-**
F02-143 p5	60°C	-
F02-143 p5	60°C	-
F02-143 p5	4°C	4.625*
F02-143 p5	4°C	4.625
F02-143 p5	4°C	4.625

*reciprocal log titre ($TCID_{50}$/50µl).
** less than the limit of detection of the titration (<0.42 TCID50/50µl)

The results are shown in Figure 1. Overall, the rate of decline in the titre for both F93-125 and F02-143 was inversely proportional to temperature, with more than 13% of F93-125 surviving at 4°C after 60 days (>3% for F02-143). With the exception of the day 60 sample at 20°C from F02-143, viable virus was detectable in all other samples at all time points.

When the half life ($t_{1/2}$) of the decline of the viral titres at each temperature was calculated, they were found to decrease with temperature (Table 4). Values were very similar between strains except for data at 4°C.

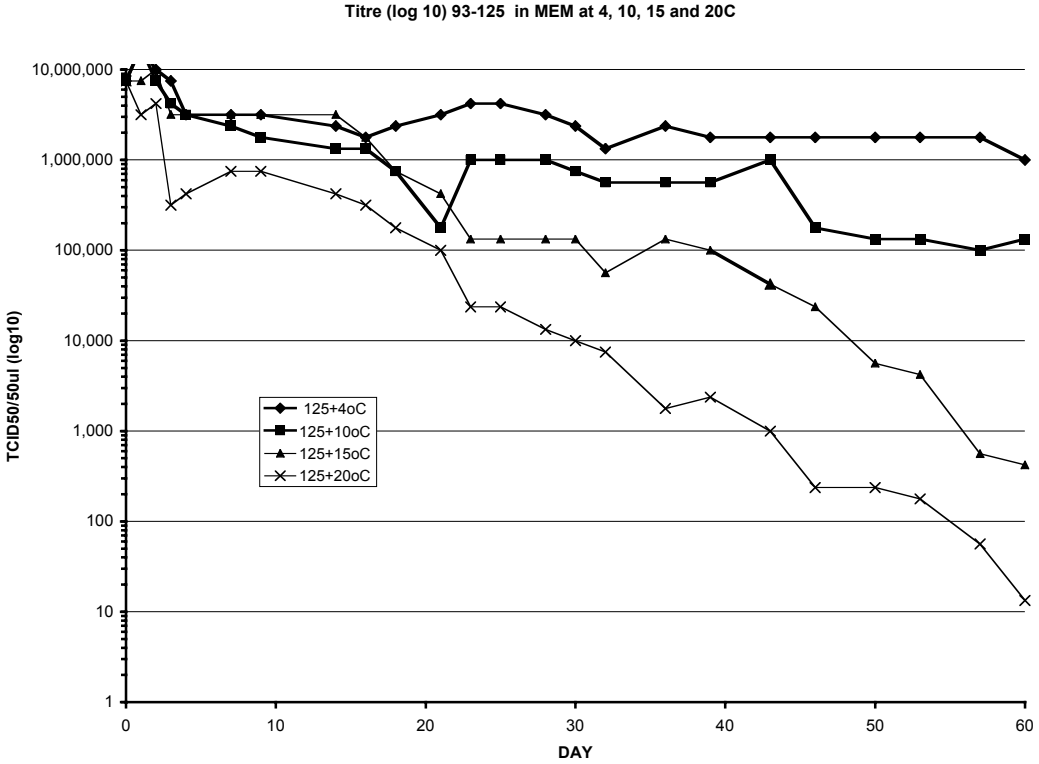

Figure 1: Decline in titre of F93-125 at 4°C, 10°C, 15°C and 20°C

Table 4. $t_{1/2}$ values (days) for each virus at each temperature

Temperature	F93-125	F02-143
4°C	25.3	13.6
10°C	10.5	9.7
15°C	4.6	4.3
20°C	3.5	3.6

4.5. Conclusions & Future Work

The results so far shed light on the usefulness of extremes of pH or temperature for inactivation of SPDV. Results of similar testing on other fish viruses belonging to virus families has sometimes shown evidence of variation in susceptibility between strains. However the similarity of the results with both strains tested suggest that the findings can be extrapolated to other strains. SPDV was found to be rapidly inactivated at high (12.0) and low (4.0) pH levels. This suggests that approaches to disinfection or waste disposal based on these methods are likely to be effective. Typically this would involve either ensiling or alkaline hydrolysis. There was some evidence that SPDV was inactivated slightly less rapidly by formic acid when compared to HCl. Nevertheless, inactivation with formic acid was complete for both strains tested after 24 hours.

SPDV was also found to be rapidly inactivated at 60°C, suggesting that methods of cleaning or waste disposal, such as composting, that involve heat can be expected to render inactive any virus present. Previous testing has found that the levels of virus in the serum of infected fish can be as high as $10^{10}TCID_{50}$/50µl (Jewhurst *et al*., 2004). Given the rate of inactivation seen with heat or pH it is anticipated that even these high levels would be inactivated within a relatively short period. However, further validation under field conditions would be required to confirm this.

The initial results indicate that SPDV can survive for extended periods, albeit with a gradual reduction in titre over time. Viral survival was clearly temperature related, with survival ($t_{1/2}$) most prolonged at low temperatures. These results suggest that SPDV would survive for extended periods in the aquatic environment. This supports the hypothesis that virus could be transmitted between farms without direct or indirect human or animal intervention.

Testing of both viruses will continue using full strength and half strength sea water, at different temperatures and under organic loads. At a final stage the survival of the virus exposed to different disinfectants will be conducted. This will aid in establishing protocols for disinfection testing and the application of this to a range of aquaculture disinfectants.

This study was funded by the Marine Institute under the Marine RTD Applied Industry measure (IND/04/12).

5 PATHOGENESIS OF THE SALMON PANCREAS DISEASE VIRUS

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5.1. Objectives

In partnership with the Marine Institute, a new Fellowship post was created in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, UCD, entitled "*Lectureship in Fish Pathology and Marine Sciences*". Postgraduate research was included in the scope of the Fellowship. Studies leading to a PhD, in the area of pathogenesis of the salmon pancreas disease virus (SPDV) commenced in October 2004.

The stated objectives for the research project are:

- Description of pathology associated with SPDV
- Optimisation of an *in situ* hybridisation probe
- Application of probes to investigate the role of survivors and potential chronic carriers

5.2 Key Outcomes & Current Progress

The descriptive pathology will focus on the various methods of visualising virus and virus-associated material giving a more complete picture of the infection process. From the smolt susceptibility trials run in 2005 a large number of samples have been collected and will now be used to describe the pathology and development of PD within these fish.

The ability to trace the nucleic acid material from the SPDV allows the visualisation of the viral infection once it enters the host fish. Data can be gained on points of entry, the development of the disease, the effect of the disease on bodily tissues and the mechanisms of damage and timing of the course of disease. In order to achieve this, a range of techniques (*in situ* hybridisation, immunocytochemistry and immunohistochemistry) are currently being developed and validated in conjunction with the Veterinary Sciences Division, Stormont and the School of Biological Sciences, Dublin Institute of Technology.

Examination of survivors and cohabitants for the presence of viral antigen and nucleic acids will assist the understanding of the dynamics of the virus in the local environment and allow a better understanding of the survivability of the virus in the population throughout the production cycle. A subset of the fish population may be affected chronically. Normal or runt-type fish can be associated with some presentations of this infection. But it is not known the extent to which these survivors and runts contribute to the perpetuation of the virus in the local environment.

6. NEW RESEARCH - SITE INVESTIGATIONS AND DISEASE MANAGEMENT OF THE PANCREAS DISEASE VIRUS IN IRISH FARMED SALMON

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6.1. Background

Based on information presented at the Tri-Nation seminars, gaps in the knowledge on pancreas disease (PD) were discussed and research priorities identified. These fell into three groupings - epidemiology, aetiology/longitudinal studies and diagnostic methods. This proposal seeks to develop research programmes and advance our knowledge in these areas, as part of both a National and European research initiative on PD. The Marine Institute envisages that this integrated approach to disease research and management, could provide a template for the development of fish health and welfare strategies with which to manage the impacts of other viral diseases in farmed finfish.

The Marine Institute published a call for research proposals in "*Site Investigations and Disease Management of the Pancreas Disease Virus in Irish Farmed Salmon*" in early 2005. A consortium was formed which included the Marine Institute, Queen's University of Belfast and Vet-Aqua International and which submitted a joint proposal in August 2005. This proposal for a Strategic project has been externally evaluated and was approved for funding by the Marine Institute Board in November 2005.

The objective of this strategic project is to determine the aetiology, lifecycle, environmental and farm risk factors of the PD virus in Irish salmon farms. This will be accomplished by means of longitudinal studies, vector and reservoir investigations, epidemiology and viral sequence studies. The project will build Irish research capacity in the area of viral disease and molecular biology; it will involve partners from the aquaculture industry and will form part of a tri-nation research programme on PD in Norway and Scotland.

The objectives of the project will be reached by achieving the following goals:

- to better understand the epidemiological factors that contribute to the introduction of salmonid alphavirus (SAV) to a site
- to develop our understanding of how PD spreads within a site, in addition to the impact of the infection
- to build national capacity in the area of screening and to establish early warning systems for PD
- to develop management tools (including a Code-of-Practice) and provide advice on mitigating factors for industry practitioners and vets
- to communicate the research findings to the Irish and European aquaculture industries in a timely fashion.

6.2. Project Plan

The project will consist of six work packages. The role of project coordination and integration will be carried out in WP 1, **Project Management**. Site investigations will be carried out by means of a series of **Longitudinal Studies** (WP 2) and research into the **Vectors and Reservoirs** (WP 3) of PD. These site specific studies will be complemented by laboratory based **Virus Sequencing Studies** (WP 4) and a two-year **National Epidemiological Study** (WP 5),

which will be key to the development of management plans for individual sites. All of the research findings will be reviewed and distilled into strategies for PD **Management and Mitigation** (WP 6), which will be disseminated on a regular basis to industry partners in Ireland and Europe.

Work Packages

WP 1 Project Management (Marine Institute)

The Marine Institute will organise meetings of the PD Steering Group where individual partners will present progress reports on individual components of the project. The Research Manager will act as a liaison between the partners through regular contact by e-mail/telephone keeping each partner regularly informed as to the progress of the others.

WP 2 Longitudinal Studies (Queens University Belfast)

Through farm trials, the pattern of disease development will be followed in a set population of fish, over a defined period of time. This will improve our understanding and interpretation of diagnostic results, predict outbreaks and advise on management strategies.

WP 3 Vectors & Reservoirs (Queens University Belfast)

This work package will explore the role of lice and mussels in the epidemiology of PD outbreaks through experimental studies and farm-based sampling. The role of broodstock and the freshwater phase of salmonid culture in the epidemiology of PD will also be investigated.

WP 4 Virus Sequencing (Queens University Belfast)

Pancreas disease virus isolates collected during the longitudinal study in WP 2 will be genetically sequenced in order to determine the variability between different isolates. Isolates found in other sites not involved in WP 2, or from Norway and Scotland will also be sequenced.

WP 5 Epidemiological Studies (Vet-Aqua International)

This work package will involve the development and submission of a detailed questionnaire to each farm in Ireland and its subsequent analysis. Industry partners will also take part in trials where fish will be held under different management regimes (e.g. feeding regime, use of immunostimulants) within the same site.

WP 6 PD Management & Mitigation (Marine Institute)

The Marine Institute will communicate research findings to the industry to facilitate monitoring, reviewing and verification of PD management techniques e.g. feeding strategies, stress management, lice control, stocking densities. This will be accomplished through six-monthly meetings of the PD steering committee and also the Tri-nation meetings, to which industry partners will be encouraged to attend. The Marine Institute will also co-ordinate seminars and workshops to relay information to the industry. A Code-Of-Practice on the management of PD will be published, based on the outcomes of research and circulated both within Ireland and abroad.

6.3. Expected Outcomes

Through the proposed work packages, further information will become available on possible risk factors, the spread of the PD virus (through vectors, reservoirs, survivability in seawater) and on any possible role of the freshwater phase. This information will feed into a practical document and Code-of-practice on coping with PD in salmon farms. The development of rapid molecular diagnostic tools and the transfer of the technology to the Marine Institute will allow PD to be added to the current suite of diseases screened for in Ireland. Virus sequence studies will allow for the determination of a possible relationship between viral sequence and pathogenicity, and the ability to trace the source of a virus as well as provide vital information for future vaccine development.

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