AN EPIDEMIOLOGICAL INVESTIGATION OF THE RE-EMERGENCE OF PANCREAS DISEASE IN IRISH FARMED ATLANTIC SALMON (*SALMO SALAR* L.) IN 2002

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McLoughlin, M.F., [1] **Peeler, E.,** [2] **Foyle, K.L.,** [3] **Rodger, H.D.,** [3]
O'Ceallachain,D. [4] **and Geoghegan, F.** [5]

3. Aquatic Veterinary Services, 35 Cherryvalley Park, Belfast, BT5 6PN.
3. Centre for Environment, Fisheries and Aquaculture Science, Weymouth, DT4 8UB
3. Vet-Aqua International, Oranmore Business Park, Oranmore, Co. Galway
3. Muir Gheal Teo., Lettermore, Co. Galway
3. Fish Health Unit, Marine Institute, Abbottstown, Dublin

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EXECUTIVE SUMMARY

In the early 1990's pancreas disease (PD) was shown to be the most significant cause of mortality in farmed Atlantic salmon (*Salmo salar* L.) in Ireland. For the last several years PD was a sporadic and relatively minor cause of losses in Ireland. In 2002, PD re-emerged as a serious cause of mortality and production loss in Irish Atlantic salmon. In order to identify factors associated with this re-emergence of PD in Ireland, an in-depth epidemiological investigation was carried out on all marine sites. The period under investigation was from October 2001 until June 2003.

In the previous two production cycles PD associated mortality on Irish farms was recorded as less than 10 % in all farms which experienced PD. Fifty-nine percent of marine sites containing 01 generation salmon experienced PD in 2002. The average PD associated mortality recorded on affected sites was 12% with a range of 1 to 42%. PD commenced on most sites between March and October 2002. The average duration of a PD outbreak was 141 days (range 61-288). PD associated mortality was high in individual cages and on some individual farms and this was a similar pattern to that observed in 1990-94.

S0 smolts were 3 times more likely to get PD than S1 smolts but there was no difference in PD associated mortalities, once they got PD. Farms which moved fish during their marine production cycle were 6 times more likely to get PD than those which did not move fish. There was a strong association between the use of tow boats and the risk of occurrence of PD for farms which moved fish during marine production cycles. Sites stocking lower overall numbers of fish (<250,000) and or lower numbers of fish per cage (<20,000) were less likely to get 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 also indicate site or specific environmental factors may be very important and remain to be defined.
No differences in PD-associated mortality were found between groups of fish vaccinated with mineral-oil or non-mineral oil vaccines. The impact of the effect of fallowing was contradictory to previous understanding of breaking disease cycles. Single bay fallowing may be more beneficial. Alternatively other factors may be more significant for re-infection than fallowing, i.e. Proximity to and/or lack of treatment of effluent from processing plants.

Average monthly temperatures were less than 15^{0}C for 49 weeks in 2002, compared to 42 weeks in 2001 and predicted 40 weeks in 2003. These lower seawater temperatures are more likely to allow SPDV replication and growth in a poikilothermic animal such as the fish, and may mean that virus challenge was greater in 2002 than in previous years.

Shared net cleaning activities did not appear to increase the risk of PD. Farms which did not lend or share equipment with other farms were at a greatly reduced risk of PD. Farms that used contract divers were at increased risk of getting PD. Farms which had dedicated teams for each site also had a significantly reduced risk of getting PD.

These observations serve to emphasise the importance of biosecurity and disinfection in the control of infectious diseases.

The exact reason/s for the disease re-emerging in 2002/3 and causing such significant losses in some regions remain to be defined; however, from the results of the survey a number of important recommendations can be made. High standards of biosecurity on farms and in bays need to be adhered to on an individual basis. These should involve:

a) The avoidance of equipment sharing or movements of livestock between infected and uninfected farms.
b) The avoidance of livestock movements of infected fish to uninfected areas/bays where previously unaffected fish are being held, (where these would be summer site to winter site movements, consideration should be given to all other farms in a bay.
c) Separate diving staff for each farm, and separate farm staff on each sea site.
d) S0 sites with a history of severe PD should consider switching to S1 production cycle.
e) Farm sites with a history of severe PD should give consideration to reducing the number, or eliminating altogether, livestock movements i.e. site to site movements (even within the same water body). This may require more flexibility in licence requirements.
f) Sites or bays with a severe history of PD should consider fallowing the water body and, on restocking use less fish per cage and fewer fish in the site in total.
g) Sites or bays with a history of smolt strain susceptibility to PD should consider avoidance of this strain for future restocking.
h) Smolts going into a site with a history of PD should be vaccinated with a PD vaccine.
i) The recommendations listed should be considered on an individual and bay management basis by farm managers with their vets and farm health personnel.
As a result of the survey, and from experience with pancreas disease in Ireland over the past few years, it is apparent that a number of areas require further investigation. These are as follows:

3) There should be a concerted national effort to continue to monitor for pancreas disease, its level of mortality and recording of the environmental and other livestock parameters associated with all farms.
3) Investigation of smolt strain susceptibility to salmon pancreas disease virus (SPDV). This should involve tank trials with the smolt strains used or available in Ireland.
3) Investigation of the biophysical properties of SPDV. This knowledge will enable workers to know how to kill the virus and the best methods of disinfection and cleaning. The investigation should also establish how long the virus can survive in seawater and dead fish.

A search for potential vectors or reservoirs of SPD virus, such as sea lice, wild fish, shellfish and other forms of marine life would greatly enhance our understanding of the natural history and the control of PD. The role of surviving fish as carriers of the virus also requires investigation.

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INTRODUCTION

In the early 1990's pancreas disease (PD) was shown to be the most significant cause of mortality in Irish farmed salmon (Wheatley *et al*., 1995, Menzies *et al*., 1996, Crockford *et al*., 1999). At that time the aetiology of PD was uncertain but in 1995 it was conclusively shown that PD was caused by a virus subsequently named salmon pancreas disease virus (SPDV), (Nelson *et al*., 1995, McLoughlin *et al*., 1996). It has since been further classified and named salmonid alphavirus (Weston *et al*., 1999, Weston *et al*., 2002). The clinical and histopathological features of naturally occurring pancreas disease in farmed Atlantic salmon in Ireland were described by Murphy *et al*., 1992 and McLoughlin *et al*., 2002.

The original epidemiological studies of PD in Ireland indicated that mortality rates up to 48% have occurred in farmed Atlantic salmon in their first year in the sea on some individual fish farms (Menzies *et al*., 1996). PD was recorded in over 70% of marine sites monitored and the majority of PD outbreaks occurred during August to October (Crockford *et al*., 1999). A serological survey for the presence of SPDV antibody in 1996 revealed 53% of the sites (9/17) were positive and 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). This pattern persisted until 2002 when there was a serious increase in both the incidence and severity of PD reported on farmed Atlantic salmon marine sites in Ireland.

In order to identify factors associated with this re-emergence of PD in Irish farmed salmon, an in-depth epidemiological investigation was carried out on all marine sites. In trying to identify the cause of a disease from epidemiological studies the concept of time, place and individual is paramount, i.e. why did a particular disease occur in a particular animal or group of animals at a particular time in a particular place? Epidemiological study designs are chosen so that predictor variables are measured in affected and non-affected "units". These "units" may be cells, animals, pens, farms or countries. The association between these predictor variables and disease is then examined. Where associations are demonstrated then risk factors for disease can be identified. A risk factor is a predictor variable associated with the disease. However the identification of a risk factor does not imply causation. The strength, consistency, temporality and dose response effect of the risk factor add weight to it being causal but only an intervention study where the risk factor is added or omitted can confirm this. Individual or univariate analysis of the relationship between a predictor variable and disease may result in an association being demonstrated. However it is dangerous to read too much into univariate analyses as factors which have been ignored, not measured or not seen as important may have a confounding effect. Multivariate analysis is used to try to provide estimates of association adjusted for the effect of confounding factors is a much more accurate and powerful epidemiological tool, but was of limited application in this snapshot survey of a single production cycle.

This report aims to describe the occurrence and severity of PD in Irish farmed salmon in 2002-2003 and to identify risk factors associated with the re-emergence of severe PD. Finally, recommendations are made on how PD can be managed to reduce its serious impact on the health, welfare and productivity of Irish farmed salmon.

MATERIALS AND METHODS

2.1 Survey population

The target population in the survey was the commercially reared Atlantic salmon (*Salmo salar L.*) population in the Republic of Ireland. A single generation of fish was chosen: those hatched at the start of 2001. These subsequently went to sea, either before the end of the year as 01GS0's (2001 generation S-zeros or S0's) or after the New Year as 01GS1's (2001 generation S-ones or S1's). All the fish of this generation were accounted for, with the exception of those covered by a single unreturned questionnaire. The fish were reared in sixteen freshwater sites, and were sent to sea in twenty-three different sites owned by fourteen companies. This amounted to twenty-three different inputs into the sea between the S0 and S1 transfers, these inputs are referred to as "farms" throughout this report. Throughout the course of the production cycle, up until the completion of the survey, a total of 42 sea sites were used.

2.2 Data collection

Data were collected through an interview administered questionnaire. The survey consisted of a questionnaire in 14 sections, of open-ended and closed questions covering stock and vaccination details; water quality and environmental data; dietary, growth and performance data; health, disease, pancreas disease and treatment data; and information on biosecurity. This questionnaire was then distributed to all relevant members of the Irish salmon farming industry, involving both fresh water and seawater stages of production. One questionnaire was allocated to each input of fish into the sea from a single generation, and the appropriate number of surveys was sent to all participants with a cover letter explaining the planned approach for data collection. To help with interpretation of questions and consistency of data retrieval, one of the aquaculture veterinarians arranged to visit each sea farm a number of weeks after sending out the survey, to assist the respondent in completing each of the questionnaires.

Data were collected between the 2nd April and 4th June 2003 when fourteen visits were made to the sea farms. Questionnaires were filled-in to varying degrees at the time of the veterinary visit, depending on the farm. A small percentage was almost entirely filled in, or had been left entirely unattempted, while the majority of questionnaires were partially filled. Average completion time for an individual questionnaire was approximately 4 hours. Twenty-three questionnaires were sent out, and twenty-two were collected at the end of the survey. No single questionnaire was completed entirely, either as a result of unavailability of information, or perceived commercial sensitivity. Freshwater hatcheries were contacted by letter and in some cases by telephone. It was not necessary to conduct any veterinary visits to assist in completion of the freshwater details.

2.3 Statistical analysis

The data were entered and sorted in "Access" (Microsoft) and analysed using STATA release 8 (Stata Corporation, College Station, USA, 2003). Fisher's exact, chi-squared test and Student's t-test were used to test for unconditional associations between the occurrence of PD and farm level binary and continuous variables, respectively. Odds ratios (OR) were also calculated to assess the strength of the association between the occurrence of PD and farm-level variables; if there is no association the OR is 1, the greater the departure from 1 the stronger the association between factor and disease (>1 positive association, <1 negative association). Questionnaires from twenty-two farms provided farm level data. At the cage level the mortality due to PD and total mortality were calculated as the number of fish dying from PD or in total, as a percentage of the total number of fish stocked in the cage.

A chi-squared test was used to test the associations between an outbreak of PD and biosecurity measures. Results are presented for association were the probability that the association arose by chance was less than or equal to 0.20. The odds ratios, with 95% confidence limits, are presented as a measure of the strength of the association.

RESULTS

3. Results–Farm Level Data

3.1 Quantitative and qualitative description of PD occurrence in 2002

Twenty-two questionnaires were returned which cover 3,010,204 S0 smolts (01G S0) on 8 sites and 5,630,860 S1 smolts (01GS1) on 14 sites in the 2001 generation. Each questionnaire covered a single input of smolts. This survey covers 8,651,064 fish in 173 cages at 22 input sites. Thirteen sites (59%) reported PD in the 01 generation smolts during the calendar year 2002. Seven of the 13 sites with a PD outbreak had experienced a PD outbreak in previous generations of fish on these sites. Nine sites had not recorded any evidence of PD in this production cycle at the time when the questionnaires were completed (April–May 2003).

The average PD related mortality recorded on affected sites, calculated by aggregating cage level data for each farm, was 12 % (Table 14)(page.15) with a range from 1 to 42 %. PD commenced on affected sites between March and October 2002 (Figure 1) with an average duration of 141 days and a range from 61 to 288 days. On average the PD outbreak lasted 123 days in a cage with a range from 33 to 345 days. Fifty percent of outbreaks lasted between 64 and 177 days. The mean number of days between the first and last cage becoming infected was 62 (SD 71), which ranged from 0 to 236 days. There was a large variation between farms in the seawater temperature (mean 13.8^{0}C, range 9 to 16^{0}C when PD started) size of fish affected (mean 798g, range 250 to 1764g), weeks at sea, (mean 24 weeks, range 8 to 43), percentage of stock and cages affected between farms. While some individual cages on some sites had very high PD associated mortalities, 50 % of all PD affected cages had mortality levels less than 5 %. Farms with high levels of mortality also showed a high level of between cage variations.

All but one farm diagnosed PD on clinical signs, and 8 had the diagnosis confirmed by a laboratory. Seven out of 10 farmers (70%) considered that spread had been contiguous between cages, i.e. it spread directly between adjacent cages. The main clinical signs of PD were reduced feeding (100% farms), faecal casts (100%) and lethargic swimming. Faecal casts generally appeared as the feeding level reduced. On the majority of farms (77%) feeding was reduced before PD was diagnosed, and the drop in feeding was sudden (50%). In most cases (10) feeding decreased between 4 and 14 days before PD (mean 7) was diagnosed. Farmers used reduced feeding and faecal casts as the first indication of PD. On most farms the fish were growing at average rate (50%) or above average rate (42%) before PD was diagnosed. Increasing feed intake and improved feeding behaviour were the main signs of recovery.

Seventy percent of farms had fresh mortalities examined by a vet or submitted to a laboratory. Sudden death syndrome or severe chronic PD with severe skeletal and heart lesions was observed on only 3 farms (23%).

Most farmers (74%) estimated that sea lice infections were non-existent or mild at the time of the PD diagnosis; none considered sea lice infections to be severe.

Following a PD diagnosis the most common intervention was to starve affected cages (38%) or all cages (23%) on site. Two of the five farmers that starved affected cages thought it was beneficial. Three farmers (23%) used a special diet; one considered that it improved the situation. The mean percentage of post PD runts was 6.8% ranging from 1 to 31.5%.

There was no consensus about factors contributing to the PD outbreak, although unspecified stressors were suggested by some farms.

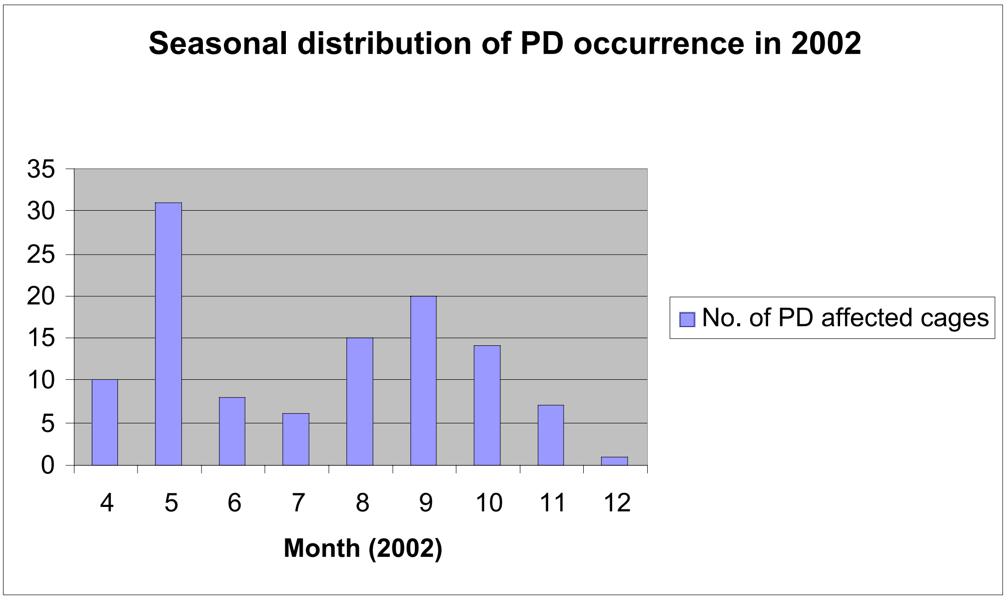


Figure 1 Seasonal distribution of PD occurrence in 2002

3.2 Investigation of farm level factors associated with the occurrence of PD in 2002

3.2.1 Smolt suppliers

The number of freshwater smolt suppliers supplying a single input varied from one to six. Most inputs (12) used one freshwater smolt supplier, six used two, two used three and two used more than three. There was no association between PD and the number of smolt suppliers used. A total of 16 freshwater smolt producers supplied the inputs in this survey, 13 of which supplied one to three inputs, and only two supplied more than three inputs. None of the inputs supplied by 4 freshwater sites reported PD; all of the inputs supplied by 8 freshwater smolt suppliers reported PD (Table 1). Four freshwater smolt suppliers supplied PD positive and negative inputs.

Four strains of Atlantic salmon smolt were used; Salmon strains A and B were the most popular. The majority of farms used one or two strains (10 and 7, respectively) and only four used three or more strains (Table1).

Table 1: PD status of marine sites and smolt strains supplied for each freshwater smolt supplier

Freshwater site	**Marine sites supplied**			**Smolt strain**			
	Total	**PD negative**	**PD positive**	**A**	**B**	**C**	**D**
1	1	0	1	**X**	**X**		
2	7	2	5	**X**			
3	3	0	3	**X**			
4	2	0	2		**X**		
5	3	0	3		**X**		
6	1	0	1	**X**	**X**		
7	4	2	2	**X**		**X**	
8	2	1	1	**X**	**X**		
9	1	1	0		**X**		
10	2	2	0	**X**	**X**	**X**	**X**
11	3	2	1	**X**	**X**		
12	3	0	3		**X**		
13	2	0	2	**X**			
14	2	2	0				**X**
15	2	2	0		**X**		
16	2	0	2	**X**	**X**		

3.2.2 Smolt type

Fourteen farms used S1 generation (01GS1) and 8 farms used S0 (01GS0) smolts. Sites using S0 smolts were found to be three times more likely to get PD than S1 smolt inputs (OR=3 P = 0.25 Fisher exact) (Table 2). Six companies reared both S0 and S1 generation fish but only in four companies did these smolt types share sites.

Table 2: Smolt type vs. PD occurrence

Total sites	Smolt type	Pancreas Disease	
22		**No**	**Yes**
8	**S0**	2	6
14	**S1**	7	7

3.2.3 Transfer dates

The earliest transfer date was 25/9/01, and transfers took place over the following 196 days. SO's were transferred from 25/09/01 to 31/12/01. S1's were transferred from 1/01/02 to 25/04/02. The six SO inputs that were transferred during October all suffered PD the following year, whereas the two inputs made from the end of November through December 2001 did not suffer PD. There was no association between PD and time of transfer for S1 inputs.

3.2.4 Farm populations

The average site population during 2002 was 392,859, range 60,000 to 807,000 (SD 194,397). Farms that suffered a PD outbreak had more fish in 2002 compared with unaffected farms, means of 206,207 compared with 452,849, respectively (P = 0.08, t-test).

3.2.5 Marine site movements

Farms which used more than one site during the marine production cycle (i.e. moved fish) were more likely to suffer a PD outbreak (Table 3) (OR 6.88, P=0.064 [Fisher's exact]).

Table 3: Number of sites used per production cycle vs. PD occurrence

Number of sites	Category	Pancreas disease	
		NO	**YES**
1	**all inputs**	5	2
>1	**22**	4	11
1	**S0**	2	1
>1	**8**	0	5
1	**S1**	3	1
>1	**14**	4	6

The risk of PD occurrence was considerably higher for farms which made seawater to seawater movements (OR 4.4, *P* = 0.17 Fisher's exact), compared to farmers that did not move their smolts or growers while at sea (Table 4).

Table 4: Sea-water to sea-water movements vs. PD status

Smolt type	Sea-site to sea-site transfer	Pancreas disease	
		NO	YES
all fish (22 sites)	NO	4	2
	YES	5	11
S0 8	NO	2	1
	YES	0	5
S1 14	NO	2	1
	YES	5	6

The main reasons for making seawater to seawater moves are summer to winter sites which are mainly offshore to inshore movements, three farms made inshore to offshore movements and other cited licence requirements. No movements were made because of disease outbreaks, algal blooms or jellyfish swarms.

The method of seawater movement was investigated and indicated that six farms used a well boat, eight used a tow boat and one used both methods for seawater movements. There was a strong association between using a tow boat and the risk of PD occurrence (OR = 14, *P* = 0.09 Fisher's exact) i.e. tow boat movements increased the risk of PD occurrence; it was not linked however to higher mortalities due to PD (Table 5).

Table 5: Use of tow boat Vs. PD status

Boat type	Pancreas disease	
	NO	YES
Tow boat	2	7
Well boat	4	1

3.2.6 Marine site description & location

Most sites used one type of cage (14 sites) or two types of cage (7). The designs used were Polar (16 sites), Bridgestone-Dunlop (8) and steel (6). The majority of cages were circular. Most cages were between 80 and 100m in circumference. The nets for all the cages were made of nylon. The majority of cages were within 1 km of the shore. Most of the cages were situated in either exposed (12 of 27) or sheltered locations (9 of 27) (Table 6). PD outbreaks were positively associated with a sheltered location (OR 5.6, *P*=0.1), but there was no association between an exposed location and PD (OR = 0.67 *P*=0.7).

Table 6: PD status of marine sites by location

Sheltered	**Pancreas disease**	
	NO	**YES**
NO	8	5
YES	2	7

Nineteen of the 22 farms described the seabed below the cages as level. The prevailing wind direction was south or south-west (14 farms) or north or north-west (5 farms). On 7 farms the prevailing wind direction was the most problematic direction. There was no association between wind direction and occurrence of PD.

3.2.7 Cage rotation and fallowing

Cage location was rotated on six sites, and the cages were moved between 5 and 100 metres. There was a non-significant positive association between rotating cages and PD (OR = 2, *P*=0.64). Only 2 of the six farms rotated cages every year. Eighteen farms provided information on fallowing. The fallowing period before smolts were added varied from no fallowing (3 inputs) to greater than 6 months. Thirteen farms fallowed for more than 60 days before adding smolts to the site, which was highly associated with PD (OD 11.67, *P*=0.27). Only two farms harrowed the site during the fallow period. Only 3 farms did not fallow during the year before the smolts were added, and none of these farms suffered from PD. Six farms fallowed their input site for 5 months or more, whilst 7 fallowed for between 3 and 4 months and 5 fallowed for between 1 and 2 months. There appeared to be a positive association for both S0 and S1 smolts between duration and fallowing and PD (Table 7), all inputs which has been left fallow for 5 months or more had PD.

Table 7: Number of sites by fallowing period, smolt type and PD

Fallowing	**S0 (PD positive)**	**S1 (PD positive)**
none	1 (0)	2 (0)
<1 month	0 (0)	0 (0)
1-2 months	2 (1)	3 (1)
3-4 months	2 (2)	5 (2)
5-6 months	2 (2)	1 (1)
>6	0 (0)	3 (3)

This result may indicate that fallowing of specific water bodies (single bays) may be more important for disease control than fallowing of specific sites, or there may be other confounding factors.

3.2.8 *Net cleaning*

All farms mechanically cleaned their nets. Fifteen farms took their nets to a cleaning station used by other farms, however this was not strongly associated with an increased risk of PD (P = 0.64, Fisher's exact). The interval between net cleaning was 73 days (range 28 to 180); the interval for farms with and without PD was 69 and 79, respectively. Anti-foulants were used on 14 farms.

3.2.9 Water quality and environmental factors

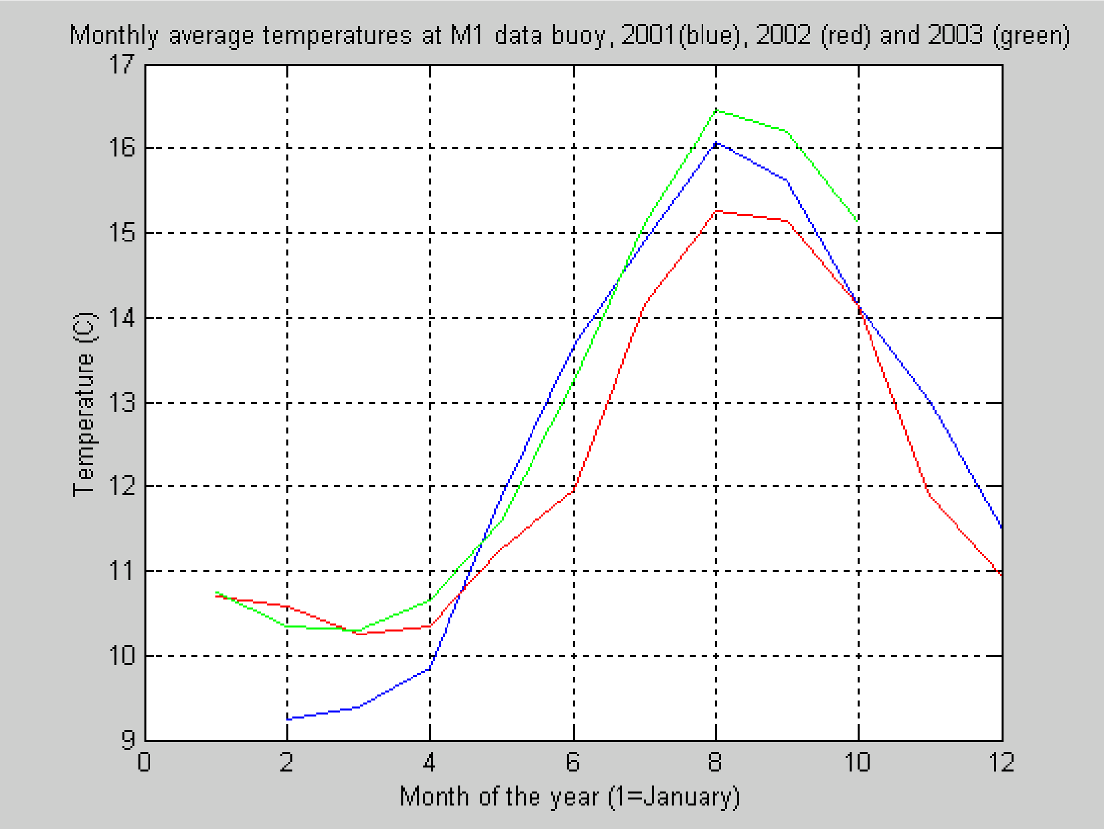
All farms monitored water temperature and most (16) did so daily and at a depth of between 0 and 5 m. A maximum-minimum thermometer was used by only one farm. The lowest water temperatures recorded were 4.7^{0}C and 6.0^{0}C in 2001 and 2002, respectively. The highest water temperatures were 19 and 18.5^{0}C in 2001 and 2002, respectively. Monthly average temperatures for 2001, 2002 and to-date in 2003 were obtained from a Marine Institute monitored buoy in the Bantry area, which were found to be similar to those recorded in the North of Donegal [Malin] (Figure 2). This data showed that the average monthly temperature in 2002 only exceeded 15^{0}C for a few weeks in September while in 2001, it was above 15^{0}C from early July to mid September and in 2003 from early July until the end of September. The significance of these findings is that coldwater fish viruses including SPD virus do not grow well at temperatures about 15^{0}C but ideal conditions are in the temperature range 10-14^{0}C which persisted for most of 2002. The mean water temperatures on affected sites at the start of a PD outbreak were 11^{0}C in March-May, 15.1^{0}C in June–August and 14.6^{0}C in September to November 2003.

All farms recorded dissolved oxygen, using Oxyguard (14) or YSI (7) [Yellow Springs Incorporated, Ohio]. A considerable range of values were recorded on some farms during a year. Overall the minimum value was 4 mg/L and the maximum 12 mg/L, the biggest difference was on farm W (4 to 10.5mg/L), on average the difference between the minimum and maximum values was 3.7mg/L. Six farms did not have records of maximum and minimum oxygen values during the year. None of the farms recorded diurnal variation in oxygen.

Half of the farms (11) measured salinity. There was little variation between the farms in the maximum recorded salinity during the year (around 34 parts per thousand) but the minimum value ranged from 16 to 34 ppt. Fifteen farms monitored ammonia, nitrite and nitrate every month or less frequently.

Seventeen farms monitored for algal blooms, and 8 reported that there was evidence of blooms in the vicinity during the last 18 months, mostly in August and September. There was no association between algal blooms and the occurrence of PD. Five farms reported losses due to jellyfish in 2001 and 3 in 2002.

Figure 2: Average monthly temperature for 2001, 2002 and 2003



3.2.10 Dietary Information and feed management

Ten farms used one type of feed, 11 used 2 and one farm used 4 types. Type A was the most commonly used manufacturer, followed by B and C (Table 8).

Table 8: Feed manufacturers distributed by farm

Manufacturer	Number of farms
A	12
B	7
C	5
D	4
E	3
G	2
F	2

Fourteen farms used immunostimulants. Feeding immunostimulants appeared to be associated with PD (OR = 2.50), probably because they are more likely to be used if PD occurs, however the association was not significant (P = 0.340, Fisher's exact). Feeding by hand or a combination of hand and automatic feeding was most commonly used (Table 9.). All but one farm using automatic feeders checked them daily. Most farms (15) feed fish 2 or 3 times per day (Table 10).

Table 9: Feeding method distributed by farm

Feeding method	Number of farms
Hand	10
Automatic	4
Hand & automatic	10
Demand fed	0
Continuous	0
Other	8

Table 10: Feeding frequency distributed by farm

Feeding frequency per day	Number of farms
2	7
3	8
4	3
>4	4

Most farms feed their fish to appetite at both the time of the survey and in the first month post-transfer (Table 11).

Table 11: Feeding policy distributed by farm

Period	Feeding policy (number of farmers)		
	To appetite	Below appetite	Slightly overfeed
Present	19	2	1
First month post transfer	15	0	7

Slightly overfeeding in the first month post-transfer appeared to be protective for PD (OR = 0.38), however the association was not significant ($P = 0.39$). Eight farmers had changed the feed type since the previous cycle. On the majority of farms less than or equal to 5 feeding days were missed in the 2002 generation (Table 12). All farms monitored feed intake daily by cage, but only 11 monitored feed wastage (generally weekly – 9 farms) and no-one operated a feed retrieval system.

Table 12: Missed feeding days in 2002 generation distributed by farm

Missed feeding days	Number of farms
≤5	11
6-10	7
11-20	3
21+	1

3.2.11 Growth and performance

Most farms (14) weighed a sample of fish from every cage monthly although some farms weighed every two weeks (3) or every 2 months (5). Nearly all farms used feed and a hand net or a box net to catch fish to weigh. Eighteen farms weighed fish singly, and 7 weighed batches. Eight farms weighed less than 50 fish and 12 weighed between 50 and 100 fish. Eleven farms graded fish and used either an out-of-water grader farms and or a well-boat.

The mean transfer loss (mortalities within 4 weeks of transfer) was 2.34% and ranged from 0.1% to 8% (n = 17). Most farms thought that transfer losses were the same or higher than previous years (n = 17). S0 smolt inputs suffered lower transfer losses compared to S1 smolt inputs (1.8% versus 2.7%). The mean post-transfer runting / failed smolt percentage was 4.4% (range 0.1 – 13.6, n = 16) and 50% of the farms (8) had a sample examined by a vet. S0 smolt inputs had less runting/failed smolt losses compared with S1 smolts (2.7 versus 5.4%).

3.2.12 Harvest weight and time to harvest

Most inputs had a target harvest weight of between 2 – 4 kg or >4 Kg (12 and 8, respectively). S0 inputs were more likely to have a target weight of >4 kg (4 of 8) compared with S1s (4 of 14). A target weight of more than 4 kg was strongly associated with PD (OR 9.33, P = 0.07 Fisher's exact) (Table 13). The positive association was found for both S0 and S1.

Table 13: Target harvest weight by PD

Target slaughter wt (kg)	Category	Pancreas Disease	
		NO	YES
<4kg	all inputs	8	6
≥4kg		1	7
<4kg	S0	2	2
≥4kg		0	4
<4kg	S1	6	4
≥4kg		1	3

Sites which experienced PD, on average harvested 96 days or 3 months earlier than sites which did not have PD ($P = 0.03$, t-test). It was also found that the harvest period for farms with PD was longer than those that didn't i.e. 142 days compared with 70 days ($P = 0.06$, t-test).

3.2.13 Health and disease data

All farms carried out health monitoring checks weekly (6), monthly (12) or quarterly (5) either by using in house staff (15), a veterinarian (18) or other professionals (4). All farms used a veterinarian at some time, but on most farms (18) on fewer than 6 occasions per year.

Mortalities were collected either weekly (9) or twice weekly (13), and were counted per cage on all farms. On most farms (16) mortalities were ensiled**.** Fish were generally held for less than two days before ensiling. Only 3 farms regularly checked the efficacy of the ensiler. The ensiled material was spread on agricultural land or sub-soil injected. In the past, mortalities from 4 farms had been used as creel bait; however, this had taken place on only one farm in 2002.

Thirteen farms reported PD in 2002 of which 8 provided estimates of accumulated mortality, which ranged from 1% to 41%. Seven farms considered that stress was associated with the occurrence of PD. The other main causes of mortality in this study period are summarised in Table 14. There are few differences between S0 and S1 smolt inputs other than for failed smolts, recorded by only 2 of 8 S0 inputs compared with 8 of 14 S1 inputs.

Table 14: Causes of disease and mortality during marine cycle 2001/2

Disease	Number of inputs affected (n=22)	S0 inputs affected (n=8)	S0 inputs affected (n=14)	Mean accumulated mortality % (range)
PD	13	6	7	**12 (1-42)**
IPN	0			
Furunculosis	1	0	1	**1**
Atypical Aeromonas	0			
ERM	0			
Vibriosis	6	3	3	**<0.5**
Winter wound	4	2	2	**<1**
Rickettsia	4	1	3	**3 (<0.5 – 10)**
Proliferative kidney disease (PKD)	1	0	1	**0.5**
PCLS	0			
Cataracts	7	3	4	**1 (0 – 4)**
Jelly fish Kill	6	2	4	**1 (<0.1 – 4)**
Algal Blooms	8	3	5	**3 (0.1 – 7)**
Brain parasite	0			
Transfer losses	18	7	11	**3 (0.1 – 8)**
Failed smolts	11	2	9	**5 (1 - &)**
Predation	14	6	8	**0.7 (0.1- 2)**
Storm damage	5	2	3	**0.5**

In 2002, 17.5% of the total smolt input had succumbed to infectious disease problems, and overall losses of 31.7%. PD had caused 68% of the losses from infectious disease.

Trends in total mortality and mortality associated with PD, and production losses due to PD from 2000 to 2002 are given in Tables 15 and 16, respectively. These data reveal that the situation worsened considerably in 2002 compared with the previous two years.

Table 15: Trends in farm-level mortality from 2000 to 2002

Year	Total mortality %				PD associated mortality %				
	<10	10-25	26-50	n	0	<10	10-20%	26-50%	n
2000	8	8	0	16	10	4	0	0	14
2001	11	6	1	18	12	3	0	0	15
2002	5	10	4	19	7	5	5	1	18

Table 16: Trends in farm-level production losses due to PD from 2000 to 2002

Year	none	<10%	10-25%	26-50%	n
2000	6	1	1	0	8
2001	6	1	2	1	10
2002	3	3	3	0	9

3.2.14 Biosecurity

Twenty farms provided information on biosecurity, of which 11 had, and 9 had not experienced a PD outbreak.

Given the small sample size (20 farms) the lack of statistical significance at $P<0.05$ is not surprising. It is interesting to note that a number of strong associations with biosecurity measures were identified (Table 17).

Table 17: Biosecurity measures associated with pancreas disease outbreak

Q	Measure	P*	OR	95% CL for OR	
7	Equipment not lent to other farms	0.16	0.15	0.00	2.01
13	Different employees for each generation	0.05	11.25	0.83	180.10
15	Predator netting used	0.20	4.2	0.43	55
24	Contract divers used	0.16	4.2	0.43	55

*from Fisher's exact or chi-squared test.

Farms that shared staff between sites were at a much higher risk of PD than those using dedicated staff (OR 11.25). Farms that did not lend equipment to other farms were at a greatly reduced risk of PD (OR 0.15). Farms that used contract divers were at increased risk of PD (OR 4.2). Both lending equipment, using the same staff on a number of sites and using contract divers are potential routes for the introduction of the virus. The biological explanations for the other associations are less clear. It is possible that farms experiencing PD improve biosecurity, i.e. used different staff or different generations. This leads to positive associations between disease occurrence and good biosecurity.

Bivariate analyses have been presented. These simple comparisons may lead to biased estimates or if two or more of the variables, which are themselves associated, constitute determinants of disease. Thus a multivariate analysis is required to assess the role of a given determinant whilst controlling for other determinants.

4. Results – Cage level data

4.1 Cage production data

Data from 173 cages on 22 sites was used in this cage level analysis. The number of cages per input ranged from 2 to 22. Seventy nine cages on 9 sites used SO and 84 cages on 11 sites were stocked with S1. The main smolt strains used were the A (103 cages on 14 farms) and B (46 cages on 10 farms). Nine farms kept only strain A smolts, 2 only B strain and a further 5 kept both strains (Table 18).

Table 18: Smolt strains kept by farm

Smolt strains kept			
A only	B only	A and B	Other
9	2	5	6

The stocking level per cage varied greatly between farms from 8,000 to 128,000 fish per cage (mean 54,505); however, there was relatively little variation within a farm.

4.2 Pancreas disease cage data

Pancreas disease was reported in 13 sites of the 22 farms (65 cages of S0 smolts and 53 cages of S1 smolts). All cages on infected farms were reported to be affected. The start dates for the outbreaks occurred between March and October 2002. The duration of an outbreak on a farm varied from 61 to 288 days (mean 141). On average an outbreak lasted 123 days in a cage, and ranged between 33 and 345 days. The average mortality was 12% of the number of fish originally stocked, but this varied greatly between farms from <1% to 42%. Fifty percent of cages had mortality levels less than or equal to 5%. Farms with high levels of mortality also showed a high level of between cage variations.

4.3. Total site mortality.

Total mortality data were not available from 2 farms (A and F). The mean cage-level total mortality was 13.2% and it ranged between cages from 0 to 57.8%. Most farms showed considerable variation in mortality between cages, a few recorded 4-5 fold differences. This needs further investigation.

4.3.1 Post PD runting

Data on post PD runting was available from 13 of the 22 sites. The mean percentage of runts per cage was 8.1%. This varied from 1 to 31.5% between farms. The variation within a farm also varied considerably. Some farms recorded all cages having low levels of runting (1 or 2%) and others recorded large differences between cages. A more accurate estimate of runting could be obtained by recording harvesting runting data.

4.4 Investigation of associations between cage-level factors and PD associated mortality

The association between PD associated mortality and the following cage level factors were investigated:

3. Strain of smolt
3. Smolt type (S0 versus S1)
3. Marine rearing region
3. Number of fish initially stocked
3. Month of seawater transfer
3. Average weight at start of PD outbreak
3. Month of outbreak
3. Vaccine type used

Continuous variables were categorised and the association between the percentage of fish dying from PD and the categorised variables was investigated. The strength and direction of the association was used to create categorical variables for inclusion in a multivariate negative binomial regression. The distribution of the number of fish dying from PD best fitted a negative binomial distribution. Farm was modelled as a random effect. Data was collected from a total of 173 cages. PD was reported in 118 cages on 13 farms.

4.4.1 Association between PD mortality and smolt strain

Preliminary unconditional bivariate analysis indicated an association between salmon strain and level of PD associated mortality. Fifty-five percent of cages stocked with strain A smolts had levels of PD mortality greater than 5%, compared with 40% for strain B smolts (Table 19). The mean PD associated mortality in strain A smolts was 12.4%, compared with 7.0% in strain B smolts (Table 19). However, many farms only stocked one strain, and thus other farm level factors may account for this association. Strain A and B smolts were both kept on 6 farms where PD was recorded. The differences in mortality between A and B smolts were small on all farms where both strains were stocked with the exception of farms C and O (Table 20).

Table 19: Comparison of PD associated mortality between A and B smolt strains

Smolt strain	n	Mean PD mortality	Range	Percent of cages with PD mortality >5%
A	73	12.4	0.29 – 53	55
B	30	7.0	0.58 – 31	40

n = number of cages

Table 20: Comparison of PD associated mortality in strain A compared with Strain B smolts kept on the same farm

Farm	A		B	
	PD mortality	Number of cages	PD mortality	Number of cages
B	20.6	1	16.2	1
C	35.0	10	11.1	3
D	12.6	9	15.5	2
E	4.3	8	3.1	4
F	0.4	1	0.8	3
H	3.4	3	3.3	3
O	32.2	3	20.3	4

4.4.2 Association between PD mortality and smolt type

While there was a strong greater risk of getting PD in S0 stock (Table 2), there was no difference between the PD mortality observed in S0 and S1 smolts (Table 21).

Table 21 Mean PD associated mortality distributed by smolt type (S0 versus S1)

Smolt type	Number of cages	Mean PD associated cage-level mortality (%)
S0	68	11.1
S1	46	10.1

4.4.3 Regional variation in PD associated mortality

The farms fall into 3 regions: a Northern group, a Western and a South-Western region. There were strong regional differences in both the farm level occurrence of PD and the level of mortality on PD affected farms (Table 22 & 23).

Table 22: PD outbreaks and PD associated mortality by region in 2001 generation

Region	Number PD positive sites (total number of sites)	Mean PD associated cage-level mortality (%)	
		mean	range
North	6 (10)	6.6	0.30 – 40.6
West	7 (7)	14.4	0.4 – 53.8
South-west	0 (5)		

Table 23: PD associated mortality by region and smolt strain in 2001 generation

Region	Mean PD associated cage-level mortality (%)			
	A		B	
	n	mean	n	mean
North	38	6.6	14	7.4
West	35	18.7	16	6.6
South-west	0		0	

4.4.4 Association between PD mortality and number of fish stocked

Cages stocked with fewer than 20,000 smolts suffered lower levels of PD associated mortality compared with cages with higher initial stocking levels [2.1% compared with 9.9-13.3%] (Table 24).

Table 24: Mean PD associated mortality distributed by number of fish stocked per cage

Number of fish stocked ('000)	Number of cages	Mean PD associated cage-level mortality (%)
≤20	17	2.1
21-40	54	13.3
41-80	19	11.8
>80	24	9.9

4.4.5 Association between PD mortality and weight at start of outbreak

Salmon under 500 g at the start of the outbreak incurred higher levels of PD associated mortality compared with heavier fish [16.9% versus 9.9%] (Table 25).

Table 25: Mean PD associated mortality distributed by mean weight at start of outbreak

Mean weight (g) at start of the outbreak	Number of cages		Mean PD associated cage-level mortality (%)	
	S0	S1	S0	S1
1-500	3	10	8.1	19.5
501-750	41	14	7.5	10.5
751-1000	5	12	30.6	4.6
1001-1250	3	5	21.9	8.7
>1250	16	5	12.4	4.9

4.4.6 Association between PD mortality and month of outbreak

There was no clear associated between PD mortality and month of outbreak (Table 26).

Table 26 Mean PD associated mortality distributed by month of outbreak (start)

Month of outbreak (2002)	Number of cages	Mean PD associated cage-level mortality (%)
April	10	17.0
May	31	6.0
June	8	16.4
July	6	17.7
August	14	12.3
September	20	13.5
October	14	3.7
November	7	12.7

4.4.7 Association between PD mortality and seawater transfer month

The first transfers took place in October 2001, followed by transfers in January 2002. The PD associated mortality of fish transferred in October 2001, (S0) was considerably higher than transfers in all other months (Table 27). Outbreaks of PD in cages stocked in October 2001 took place between 22.4.02 and 30.11.02.

Table 27 Mean PD associated mortality distributed by freshwater to seawater transfer month

Month	Number of cages (total number of cages)	Mean PD associated cage-level mortality (%)
Oct 01	27 (28)	*21.1
Nov 01	0 (0)	-
Dec 01	0 (8)	-
Jan 02	4 (6)	0.8
Feb 02	7 (8)	15.0
Mar 02	42 (59)	* 8.9
Apr 02	5 (7)	20.7

* The majority of fish were transferred in these 2 months.

4.4.8 Association between PD mortality and vaccine type used

The majority of smolts in the 2001 generation were vaccinated against furunculosis and vibriosis and two vaccine types were used in most of the vaccinated stocks. One vaccine contained a mineral oil adjuvant (vaccine A) and the other a non-mineral oil adjuvant (vaccine B). The PD mortality rate in fish vaccinated with vaccine A and vaccine B were similar (Table 28). Some cages were vaccinated with both vaccines and the remaining cages (36) were vaccinated with other types of vaccine.

Table 28: Mean PD associated mortality distributed by type of PD vaccination

Type of PD vaccination	**Number of cages**	**Mean PD associated cage-level mortality (%)**
Vaccine A (oil adjuvant)	57	10.6
Vaccine B (non-mineral adjuvant)	20	8.9
Others and combinations	36	11.4

4.5 A multivariate model for PD associated mortality

Simple bivariate associations between the occurrence of PD and the level of mortality associated with PD and possible risk factors have been explored in this report. These estimates may lead to biased or misleading results if the risk factors themselves are associated, a problem known as confounding. As a result, statistical models are required which examine the data for significant associations, whilst taking into account the effect of other risk factors. These models are known as multivariate. In this model the outcome was the number of fish dying from PD per cage, which was modelled as a negative binomial outcome, using the number of fish stocked at the outset. Variables with a significance probability less than or equal to 0.1 were retained in the model (Table 30). Two variables were retained in the final model: weight at the start of the outbreak ≤ 600 g and transfer month October 2001. Input (modelled as random effect) was highly significant. The association between PD mortality and other variables was accounted for by the input effect and thus were not retained in the final model. The rate ratios indicate that both independent variables in the final model increased the level of PD associated mortality by approximately 1.5 times.

Table 30: Results of random effect model for PD associated mortality

Independent variables	Rate ratio	P	95% Confidence interval for rate ratio	
weight at start of PD outbreak ≤ 600 g	1.51	<0.01	1.11	2.05
Transfer month October 2001	1.52	0.07	0.95	2.41

(Farm modelled as a fixed effect, baseline: cages stocked after October 2001, average weight of fish at time of outbreak >600 g)

This multivariate analysis confirms that S0 smolts are 1.5 times more likely to get PD than S0 smolts.

DISCUSSION

The response rate to the 2002 PD survey was 95%. This very high response rate to a very detailed and lengthy questionnaire indicated the concern of the salmon producers about the re-emergence of PD in Ireland. As with all retrospective surveys, it is impossible to get all the requested information from all sites and it is important to realise that this report is only looking at one generation of fish in a single production year. It is therefore a less powerful investigation than that undertaken previously in Ireland from 1989 to 1994 (Menzies *et al*., 1996, Wheatley *et al*., 1995). The 1990's epidemiological study investigated disease occurrence in 49 smolt inputs totalling 17.8 million smolts in 509 cages over a 5 year period. The current study covers 22 smolt inputs totalling 8.6 million smolts placed in 165 cages and its strength is that it reviewed data from the majority of farmed salmon in Ireland in 2002.

Clinical signs
The clinical signs, mainly reduction in feeding, faecal casts and lethargy were reported in 100% of PD outbreaks in 2002. These signs were similar to those previously reported by Murphy et al., 1992 and McLoughlin et al., 2002. Three sites suffered from what is still termed Sudden Death Syndrome (SDS) as described by Rodger et al., 1991. This condition is now widely recognised as severe chronic PD where fish die suddenly from severe skeletal and variable heart lesions 6-8 weeks after initial SPD virus infection. The factors associated with severe chronic PD (SDS), requires in-depth investigation on a site by site basis.

Incidence of PD
In 1987, 73% of sea sites in Ireland were affected by PD (Branson 1988). In 1990, 94.1 % of Irish marine sites (27) reported PD and sudden death syndrome resulting in 0-45% mortality (Menzies *et al*., 1996). A serological survey for the presence SPDV antibody in 1996 revealed 53% of the participating sites (9/17) were positive indicating SPD virus challenge had occurred and not all positive sites had recognised clinical signs of PD indicating sub-clinical infection or relatively mild PD outbreaks at that time (McLoughlin *et al*., 1998). Fifty-nine percent of Irish marine sites containing the 01 generation salmon experienced PD in 2002.

PD associated mortalities
In the previous two salmon production cycles (2000 and 2001) the estimated PD associated mortality on Irish farms was less than 10% on PD affected sites. PD associated mortality in 2002 ranged from 1-41% with an average of 12% mortality across 13 affected sites. There was a significant downward trend in the mortalities in Irish farmed salmon due to disease from 1989 to 1994 (p<0.05), with percentage mortalities due to PD falling from 30% in 1989 to 10% in 1994, p=<0.01).The PD associated mortality in the same period ranged form 4-30% with considerable variation between years (Crockford *et al*., 1999). The highest percentage mortality recorded on an individual site in 1994 was 63%, while the highest recorded PD associated mortality on an individual site in 2002 was 48%. PD associated mortality was high in individual cages and on some individual farms in 2002. This observation needs to be further investigated at farm level. Crockford *et al*., 1999 also described significant differences in PD mortality rates between sites rather than between years and suggested that this demonstrated the importance of site management factors in decreasing losses and improving disease control.

These quantitative figures indicate that the incidence (number of new cases in a given population occurring during a certain period) has not significantly increased since the mid 1990's but the current losses due to PD are very similar to those recorded in the late 1980's and early 1990's when PD was the most serious cause of mortality and economic loss to the Irish farmed salmon industry. It can be concluded therefore that the incidence and nature of PD *per se* has not changed markedly but on individual sites it has re-emerged as a major problem, where it may previously been a sub-clinical or a minor or undiagnosed problem.

Seasonality and duration of PD

Between 1989 and 1994, 43 outbreaks of PD were recorded with 57% of these starting in the three month period from August to October of the smolts first year in the sea. In 2002, PD outbreaks started from March to October. In previous studies, which dealt exclusively with spring S1 smolt inputs there was a very highly significant correlation between the date when a PD outbreak occurred and the percentage mortality during the outbreak (r=0.53). The PD mortality rates were higher when PD occurred earlier in the year (May-July). The duration of the PD outbreak also tended to be longer when they occurred earlier in the year i.e. from May onwards (Crockford *et al*., 1999). The main difference between the 1990's data and 2002 would be the increased use of S0 generation fish that would be at sea from October of the previous year and would be a source of susceptible hosts in the following spring when rapid growth and rising temperatures among other factors may act as a trigger for PD. This study indicated that while S0 were 3 times more likely to get PD than S1 smolts there was no difference in PD associated mortalities once PD occurred.

The mean length of a PD outbreak (i.e. the date PD was recognised to the date when feeding, behaviour and mortality rates returned to normal) between 1989 and 1994 was 112 days (SE 7.7, n=37 range 79 to 149) compared to 141 days recorded in 2002 with a range from 61 to 288 days (n=13). The duration of a PD outbreak on site could be dictated by a complex interaction of a number of factors including total number of fish on site, number of fish per cage, distance between cages, hydrographical conditions, disease management and biosecurity activities.

Stock numbers and stocking density

Sites stocking lower overall numbers of fish (<250,000) and or lower numbers of fish per cage were less likely to get PD. The explanation for this observation is probably a complex interaction between the host, the environment and the PD virus. If you have less susceptible hosts in a given unit, then the likelihood of the critical viral challenge threshold necessary to cause clinical disease may not be reached. Husbandry and care may also be better on smaller units.

Production effects of PD

Sites which had PD, tended to start harvesting earlier (emergency harvest) and the harvest period was twice as long in PD affected sites. Emergency harvest is often undertaken in harvest size fish following SPDV infection in order to avoid potential losses from severe heart and skeletal muscle lesions (SDS). The longer harvest period probably reflects the poor feeding response during and for some time after a PD outbreak which results in poor growth, although compensatory growth is sometimes noted. The full economic impact of PD not only included mortalities but also a significant impact on growth.

Smolt type

S0 smolts were 3 times more likely to get PD than S1 smolts. There could be number of factors which have interacted or combined to increase the risk of PD. The S0 generation fish would be at sea from October of the previous year and would be a source of susceptible hosts in the following spring when rising temperatures and rapid growth among other factors may act as a trigger for PD. Site characteristics, strain type and the fact that there was more seawater to seawater transfers in S0 fish which may also increase the risk of PD.

An interesting general observation was that S0 smolts had less transfer losses and less incidence and severity of failed smolts when compared to S1 smolts. This fact may deserve further investigation, perhaps the critical size and timing of SO smolt production produces a good sized smolt and a more homogenous population with a very narrow transfer window compared to the wider spread of size and transfer period usually associated with S1 smolt inputs.

It was also noted that slight overfeeding post transfer in an effort to ensure that all smolts came onto food quickly after transfer to sea appeared to protect against PD.

Smolt Strain

Increased susceptibility to PD with specific smolt strain was identified on individual sites but was not consistently seen across all affected sites. The level of PD associated mortality in individual smolt strains was very different between on-growing regions with fish grown in Donegal having a mean PD associated cage level mortality of 6% compared to 14.4 percent in Mayo and Galway and zero in the south-west during the period of this study. This may reflect site or rather specific environmental factors which remain to be defined.

Smolt suppliers

The number of smolt suppliers used per site had no association with occurrence of PD. In Norway, studies on risk factors associated with infectious salmon anaemia (ISA) and infectious pancreatic necrosis (IPN) in sea-cultured Atlantic salmon, consistently showed that the greater the number of smolt suppliers, the greater the risk of developing both these diseases (Jarp *et al*., 1995, Jarp & Karlsen 1997). It may be that more data points are required to really test this hypothesis in the Irish industry. Similar smolt supply patterns were noted between 1989 and 1994 with sites recorded as being stocked with different smolt strains from several different hatchery sources with up to 5 different groups of fish held on the one site (Crockford *et al.,* 1999). It is worth noting that the increased risk of infectious disease associated with multiple suppliers is supported by epidemiological studies of infectious diseases in other animals (Webster *et al*., 1985, Pritchard *et al*., 1989, Thomsen *et al*. 1992).

Seawater to seawater movements and the mode of transfer

Farms that moved fish during their marine production cycle were 6 times more likely to get PD than those that did not move fish. There was a strong association between the use of tow boats and the risk of occurrence of PD for farms which moved fish during marine production cycles. This is a relatively new practice in the Irish industry and would not have been recorded in early surveys. These movements are obviously a source of stress to the fish. Fish perceive disturbance and handling as a general and potentially dangerous stress. The nervous system detects the threat, and almost instantly, adrenaline is released into the bloodstream. This hormone is followed closely by others such as cortisol, all of which go to work preparing the fish for its reaction, e.g. escape. The result is that blood glucose, red blood cell counts, heartbeat and ventilation rate all increase, and digestive processes may cease temporarily. Once triggered, even by a very transient stress, the sequence plays through, although in rough proportion to the severity and duration of the stressor.

While a fish may not suffer disadvantage from briefly increased blood glucose or red blood cell counts (both of which serve primarily to enhance the fish's ability to escape from danger), other stress response components have significant drawbacks. Loss of appetite can persist following a handling stress and normal reproductive functions may be suppressed for a considerable time. Adrenaline disturbs ion transport at the gill membrane, where only a scant few cell layers separate the world within from the surrounding environment. Fish maintain their blood chemistry markedly different from the water that surrounds them, both in fresh water and in sea water. Adrenaline and cortisol both cause temporary changes in gill permeability which in fresh water allows dilution of the blood by excessive entry of water, and vice versa in normal sea water. Consequently, blood levels of calcium, magnesium, sodium and other vital electrolytes are pushed out of normal operating ranges for as much as 24 hours after a brief stress. The burden of trying to restore physiological and metabolic order consumes valuable stored energy, which may leave the fish less capable of fighting pathogens or adapting to new temperatures or reduced oxygen availability. Cortisol elevation itself suppresses immune system function and a frequent sign of poor handling practices in fish is a subsequent infection.

It would therefore be useful to investigate ways of mitigating the stress response during movements. In chinook salmon undergoing simulated transport in small containers, exclusion of light reduced the hormonal response to stress by about 25% over unprotected fish. Other key factors of habitat quality, particularly oxygen content, should also be kept appropriate to the particular fish species' needs in all waters to which they will be exposed. For water temperature, a broad rule of thumb in fish is to restrict temperature changes to one or two degrees centigrade per day, which allows physiological compensation to take place.

Like us, fish maintain their blood and tissues at a salt composition intermediate between fresh water and normal seawater. As they pass large volumes of water over their gills, they must constantly expend some energy to maintain the imbalance, except when in brackish water of osmotic pressure similar to their blood (i.e. 12 to 15 ppt salinity). The provision of brackish water surroundings when fish are likely to be stressed has definite advantages, both in conserving energy for physiological emergency purposes and in countering the electrolyte disturbances associated with stress-elevated adrenaline and cortisol levels described above. The presence of salts softens the side effects of the hormones released during the initial stress response, and in the case of chloride and other monovalent anions, can also directly suppress cortisol secretion.

The use of brackish water in the manner described above may have one further advantage, that of killing or restraining some pathogenic organisms which may otherwise exploit a fish's temporary distress to invade and establish an infection. (Ostrander, G. K. 2000).

The use of well boats with a more controlled environment may help to reduce some transfer stress, if fish movements cannot be avoided.

Fallowing

The results on the effect of fallowing were contradictory. Only 3 farms did not fallow prior to smolt input and none of them got PD. Ten out of 13 farms which fallowed for >60days all got PD. Previous studies that were conducted prior to the widespread use of fallowing sites between fish populations, indicated that fallowed years had significantly lower total mortality than un-fallowed years ($p<0.05$). The same study also recorded a reduction in mortalities due to PD when fallowing was practised but this was not significant (Wheatley *et al*., 1995). A combination of single generation rearing (35% pre- 1993, 94% in 1993 and 1994) and fallowing of sites (26% pre-1993, 94% in 1993 and 1994 may have contributed to the significant reduction in mortality during 1993 and 1994 (13.6%) compared to mortality rates in the previous 5 years of 27.6% (Crockford *et al*., 1999). It is generally agreed that fallowing marine sites is beneficial for the environment (Gowan 1990) and the health of the fish. There are a number of ways in which waste deposition can affect fish health such as oxygen depletion (Brown *et al.,* 1987) and release of hydrogen sulphide (Braaten *et al.,* 1983). Fallowing has been demonstrated to reduce numbers of *Lepeophtheirus salmonis* on newly introduced fish for several months after stocking (Bron *et al*., 1993). While low sea lice were reported by farms in 2002 and while sea lice have not been shown to be vectors for SPD virus they have been found to be possible vectors for other important viral infections of salmon such as ISA (Nylund *et al*., 1993). A search for potential vectors or reservoirs of SPD virus, such as sea lice, wild fish, shellfish and other forms of marine life would greatly enhance our understanding of the natural history of the PD virus.

The findings regarding fallowing in this survey need further investigation. It may be that single bay fallowing may be more beneficial than individual site fallowing. Alternatively other factors may be more significant for re-infection than fallowing, i.e. proximity to and/or lack of treatment of effluent from processing plants or sea lice levels. It is interesting to note that the incidence of PD is higher in areas where up to recently processing plants were not treating their effluent for fish pathogens.

Previously, total mortality was significantly lower in single generation sites compared to multiple generation sites, but while vibriosis and PD mortality were lower in single generation sites compared to multiple generation sites, these differences were not found to be significant (Wheatley *et al*., 1995). While the majority of sites post 1993 in Ireland have practised single generation rearing with a fallow period between site usage, a small number still have multiple generation sites where PD infection is constantly present. (M. McLoughlin, personal communication).

Site location

Other than geographical location no significant risk factors were identified regarding production site location. Sites in Mayo and Galway suffered greater losses than other regions. Exposed sites were not found to be any more at risk than sheltered sites, however, sheltered sites were found to be positively associated with PD. Offshore farms at deep sites with good water exchange have previously been assumed to be less at risk from PD than inshore sites (Anonymous, 1993). This is a similar finding to that reported by Wheatley *et al*., 1995 who found no relationship between site depth and the severity of disease outbreaks on Irish sea farms. Other factors such as the site hydrography may be more important in determining the severity of disease outbreaks.

No analysis was carried out on the occurrence and severity of PD outbreaks in adjacent sites. Further data points (production years) would be required to fully interpret this type of data. Jarp & Karlsen (1997) found that the risk of ISA infection increased by 8.0 if a site was situated closer than 5km to another ISA-positive site as compared to the risk when the site was more than 5km away. A site location within 5km from a salmonid processing plant gave an ISA odds ratio of 13.0 compared to a site location further away. Disinfecting the waste water from the slaughtering and processing plants seemed to prevent the transmission of ISA. These authors recommend a minimum distance of 5 km between sea sites to minimise the risk of seawater transmission.

Type of vaccine used and PD associated mortalities

The farms raised a hypothesis that there was a difference in PD susceptibility depending on which type of vaccine adjuvant was used. The majority of smolts in the 2001 generation were vaccinated against furunculosis and vibriosis and two vaccine types were used in most of the vaccinated stocks. One vaccine contained a mineral oil adjuvant (vaccine A) and the other a non-mineral oil adjuvant (vaccine B). The PD mortality rate in fish vaccinated with vaccine A and vaccine B were similar i.e. mineral oil adjuvanted vaccines (A) 10.6%, non-mineral oil vaccine (B) 8.9%. It was felt that mineral oil vaccines may stimulate a greater non-specific immune response. While this study was not set up to investigate this theory in depth, analysis of the data does not support the hypothesis.

Biosecurity

Shared net cleaning activities did not appear to increase the risk of PD indicating that net cleaning and disinfection was satisfactory at communal cleaning stations. Farms which did not lend or share equipment with other farms were at a greatly reduced risk of PD. Farms that used contract divers were at increased risk of getting PD. Farms which had dedicated teams for each site also had a reduced risk of getting PD. These observations serve to emphasize the importance of biosecurity and disinfection in the control of infectious diseases. Transmission of SPDV and other pathogenic organisms may be reduced by restricting movement of equipment, boats and personnel between growing sites as is practised in other types of intensive animal production (Moore 1992, Wheatley *et al*., 1995).

Salmon pancreas disease virus update.

PD is caused by a virus subsequently named salmon pancreas disease virus (SPDV), (Nelson *et al.* 1995, McLoughlin *et al.* 1996). It has since been further classified and named salmonid alphavirus (Weston *et al.,* 1999, Welsh *et al*., 2000, Weston *et al.*

2002). No antigenic or serological differences between salmon pancreas disease virus (SPDV) isolated in the mid 1990s in Ireland and current SPDV isolates have been found. (Weston *et al.*, 2003, Jewhurst *et al.*, 2003). No antigenic differences have been found between SPDV isolated in Ireland, Scotland and Norway (Weston *et al.,* 2003). This suggests that salmon pancreas disease virus has not changed antigenically in the last 10 years and that a new, more virulent SPD virus has not emerged.

Recently an SPDV isolate was made associated with PD–like signs and lesions in rainbow trout reared in the sea in Norway, confirming experimental evidence that rainbow trout are susceptible to salmon pancreas disease virus as well as sleeping disease virus. This finding may have implications for putting rainbow trout into PD positive salmon sites or alongside salmon.

There is currently an EU project (QLK2-CT-00970) seeking to improve our understanding of the development, diagnosis and epidemiology of PD in salmon and sleeping disease (SD) in rainbow trout. Two years of this 42 month project are completed and significant advances have been made in the rapid isolation of SPD virus, RT-PCR techniques and serological diagnosis. Recently some success has been achieved in the use of immunohistochemical diagnosis of both PD and SD. (Taksdal *et al.*, 2003, Boscher, *et al.*, 2003)

PD vaccine development has been on-going for the last 8 years and a number of field trials have been conducted in Ireland, Scotland and Norway (McLoughlin *et al.*, 2003). Currently a monovalent PD vaccine is available for general use in Ireland. The vaccination strategy requires the PD vaccine to be given at least 3 weeks prior to any bacterial vaccines as it would appear that if given in combination with bacterial antigens or after the bacterial vaccines that efficacy is reduced. Vaccination is an important part of disease control strategies in aquaculture, as it has in human and domestic animal medicine. Fish vaccinology, especially fish viral vaccine development is still in its infancy and it will be some years before the perfect vaccination programme will be developed.

Fish vaccines work by stimulating the immune system to recognise a specific antigen or organism, so that if they are exposed to a natural challenge that the immune system is primed and ready to react to fight off the challenge. There are 6 important components in this defence mechanism, the immune response of the fish, the duration of immunity, the temperature of the water, the pathogen challenge dose, the efficacy of the vaccine and the vaccination technique. In all populations there will be a natural variation in the response to any vaccine with a small percentage of what are termed non-responders, who will remain susceptible to challenge. The success of a vaccination programme depends on total population vaccination to reduce and even in some cases eradicate the pathogen (e.g. smallpox). When PD vaccination is carried out in all fish within a coastal zone, optimum levels of protection are expected.

General Discussion

Pancreas disease is considered by far the most significant disease affecting Irish salmon farming. The exact reason/s for the disease re-emerging in 2002/3 and causing such significant losses in some regions remain to be fully defined; however this snapshot survey has identified some significant trends and findings. These are summarised in the conclusions and recommendations.

All diseases are multi-factorial, which means that there is a complex interaction between the host, the environment and the pathogenic agent in order for the disease to manifest itself. A simple example would be the exposure of the staff on a fish farm to the common cold virus; this would not result in everyone developing a nasal discharge and headache. Genetic susceptibility, stress, immunity, age and sex may influence the occurrence of the disease. In this example the common cold virus is essential and is known as a necessary cause. The other factors are contributory causes. All diseases may be viewed as having necessary and contributory factors which link together in a web of causation.

Effective control and prevention hinges on a complete understanding of PD transmission and persistence and the natural immune response to field challenge. Current and future research should go some way to answering these outstanding questions. From a dynamic point of view, infectious diseases can be divided into two main classes, those that are endemic, i.e. locally persistent within their host populations, and those that are not. Furunculosis would exemplify the former and influenza exemplifies the latter.

Non-endemic, that is epidemic diseases occur when there are sufficient susceptible hosts (fish) exposed to a critical number of infectious agents (virus) under the influence or not of environmental or managerial stress. So epidemic diseases are introduced into the host population, break out and burn out locally. Such diseases only persist through epidemics (Barlett, 1956). Many infectious diseases have a natural peak and trough incidence pattern such as observed recently in seal morbillivirus, which was first recognised in 1988 (Kennedy et al., 1988) and there were a number of years where infection and deaths were low followed recently by another epidemic peak in northern European seals. This cyclic nature of certain infectious diseases is a result of natural variation and interaction in the number of infected or carrier animals or critical population size, the disease challenge threshold (virus load), maternal or innate immunity, suitable environment and very often stressors. Complex models have been developed for some human infectious diseases e.g. measles in order to predict the measles epidemics in children (Finkenstadt *et al.*, 2002). Overall the evidence would suggest that PD is an endemic disease in Irish coastal waters with a complex causal web. It must be assumed that all susceptible smolts put to sea in Ireland are at risk from pancreas disease.

CONCLUSIONS

- The clinical signs, duration and spread of PD were very similar to previously recorded PD outbreaks in Ireland.
- Fifty-nine percent of marine sites containing 01 generation (S0 and S1) salmon experienced PD in 2002.
- The average PD associated mortality recorded on affected sites was 12% with a range of 1 to 42%.
- In the previous 2 production cycles PD associated mortality on Irish farms was recorded as less than 10 % in all farms which experienced PD.
- PD commenced on most sites between March and October 2002.
- The average duration of a PD outbreak was 141 days (range 61-288).
- The average time for all cages on site to become infected was 62 days (range 0 to 236 days).
- While PD associated mortality was high in individual cages and on some individual farms this was a similar pattern to that observed in 1990-94.
- S0 smolts were 3 times more likely to get PD than S1 smolts but there was no difference in PD associated mortalities once they got PD.
- Sites which had PD tended to start harvesting earlier (emergency harvest) and the harvest period was twice as long in PD affected sites (reduced growth performance).
- Farms which moved fish during their marine production cycle were 6 times more likely to get PD than those which did not move fish.
- There was a strong association between the use of tow boats and the risk of occurrence of PD for farms which moved fish during marine production cycles.
- Sites stocking lower overall numbers of fish (<250,000) and or lower numbers of fish per cage (<20,000) were less likely to get 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 indicate site or specific environmental factors may also be very important and need to be defined.
- No differences in PD-associated mortality were found between groups of fish vaccinated with mineral-oil or non-mineral oil vaccines.
- The impact of the effect of fallowing was contradictory. Only 3 farms did not fallow prior to smolt input and none of them got PD. Ten out of 13 farms which fallowed for >60days all got PD. Single bay fallowing may be more beneficial.
- Alternatively other factors may be more significant for re-infection than fallowing, i.e. proximity to and/or lack of treatment of effluent from processing plants.
- Average monthly temperatures were less than 15^{0}C for 49 weeks in 2002, compared to 42 weeks in 2001 and predicted 40 weeks in 2003. These lower seawater temperatures are ideal for SPDV replication and growth in a poikilothermic animal such as the fish, and may mean that virus challenge was greater in 2002 than in previous years.

- Shared net cleaning activities did not appear to increase the risk of PD indicating that net cleaning and disinfection was satisfactory at communal cleaning stations.
- Farms which did not lend or share equipment with other farms were at a greatly reduced risk of PD.
- Farms that used contract divers were at increased risk of getting PD
- Farms which had dedicated teams for each site also had a significantly reduced risk of getting PD.
- These observations serve to emphasis the importance of biosecurity and disinfection in the control of infectious diseases.

RECOMMENDATIONS

7.1 Recommendations with regard to avoidance of PD and reducing the impact of the disease outbreaks

Pancreas disease remains the most significant disease affecting Irish salmon farming. The exact reason/s for the disease re-emerging in 2002/3 and causing such significant losses in some regions remain to be defined; however, from the results of the survey a number of important recommendations can be made. These recommendations may help to avoid pancreas disease or reduce its impact in the case of an outbreak. The recommendations are as follows:

c) High standards of biosecurity on farms and in bays need to be adhered to on an individual basis. These should involve:
) The avoidance of equipment sharing or movements of livestock between infected and uninfected farms
) The avoidance of livestock movements of infected fish to uninfected areas/bays where previously unaffected fish are being held (where these would be summer site to winter site movements, consideration should be given to all other farms in a bay)
) Separate diving staff for each farm
) Separate farm staff on each sea site

c) S0 sites with a history of severe PD should consider switching to a S1 production cycle
c) Farm sites with a history of severe PD should give consideration to reducing the number, or eliminating altogether, livestock movements i.e. site to site movements (even within the same water body).
c) Sites or bays with a severe history of PD should consider fallowing the water body and, on restocking use less fish per cage and fewer fish in the site in total.
c) Sites or bays with a history of smolt strain susceptibility to PD should consider avoidance of this strain for future restocking.
c) The recommendations listed should be considered on an individual and bay management basis by farm managers with their vets and farm health personnel.

7.2 Additional recommendations outwith the survey

c) Smolts going into a site with a history of PD should consider vaccination with a PD vaccine.

7.3 Areas for further investigation

As a result of the survey, and from experience with pancreas disease in Ireland over the past few years, it is apparent that a number of areas require further investigation. These are as follows:

3) There should be a concerted national effort to continue to monitor for pancreas disease, its level of mortality and recording of the environmental and other livestock parameters associated with all farms.
3) Investigation of smolt strain susceptibility to salmon pancreas disease virus (SPDV). This should involve tank trials with the smolt strains used or available in Ireland.
3) Investigation of the biophysical properties of SPDV. This knowledge will enable workers to know how to kill the virus and the best methods of disinfection and cleaning. The investigation should also establish how long the virus can survive in seawater and dead fish.
3) A search for potential vectors or reservoirs of SPD virus, such as sea lice, wild fish, shellfish and other forms of marine life would greatly enhance our understanding of the natural history and the control of PD. The role of surviving fish as carriers of the virus also requires investigation

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GLOSSARY

Commercial company	The commercial unit responsible for the fish at sea. A commercial company is a fish farm in normal parlance, and it may own, or be responsible for more than one input. For example, a commercial company may have an SO population of fish and an S1 population of fish in the sea, and in this survey would have received two questionnaires to cover both inputs if they were of the relevant generation. There were 14 commercial companies in the survey.
Farm	See Input.
Generation	The generation of fish covered by this survey, which was those salmon eggs hatched in early 2001, i.e. 01 Generation or 01G. See SO and S1.
Input	Synonymous with "farm" in this survey. For the purposes of this survey, "input" and "farm" were taken as a population of either SO or S1 smolts bought by a commercial company, and transferred to sea to a single input-site. The transfer may have taken place over a few days or a number of weeks, and more than one freshwater site may have provided fish for a single input. A single questionnaire was allocated to each input. Fish that subsequently moved to different on-growing sites remained covered by the same questionnaire. For example, 500,000 S1's go to sea in the smolt site of farm ZZ. This is a single input of fish which got one questionnaire. There were 22 inputs of fish to sea, or "farms", covered by this survey.
Site	A sea site used in the production of Atlantic salmon. In this survey, a commercial company would send SO and/or S1 smolts to sea at a certain site. This input of fish would usually then be moved to subsequent sites as the production cycle progressed.
SO	Synonymous with S_ and 01GSO; smolts that are ready to transfer to sea from freshwater before the New Year of their first winter.
S1	Synonymous with 01GS1; smolts that transfer to sea from freshwater after the New Year of their first winter; many transferred in the spring.

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