THE REPORT OF THE AQUACULTURE HEALTH JOINT WORKING GROUP ON INFECTIOUS PANCREATIC NECROSIS IN SCOTLAND

Submitted to the Deputy Minister of the Environment and Rural Development of the Scottish Executive, Scottish Quality Salmon (SQS), the Shetland Salmon Farmers Association (SSFA), the British Marine Finfish Association (BMFA), the Orkney Fish Farmers Association (OFFA), the Western Isles Aquaculture Association (WIAA) and other members of the Scottish aquaculture industry.

Citation:

Anon. 2003. Final report of the Aquaculture Health Joint Working Group sub-group on infectious pancreatic necrosis in Scotland. Aberdeen: Fisheries Research Services. 90p.

Published by Fisheries Research Services

ISBN 0-95 464 90 0-0-1

Fisheries Research Services is an agency of the Scottish Executive

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FOREWORD

Infectious pancreatic necrosis (IPN) has become of increasing economic importance to the Atlantic salmon farming sector in the North Atlantic and beyond. Although particularly significant in the marine environment, where it has become a serious cause of acute mortality in Atlantic salmon smolts shortly after introduction to sea water, IPN can also cause very considerable mortality in fresh water, particularly in the vulnerable fry stages.

Whilst adult marine finfish appear resistant to the disease, an increasing number of marine finfish species are known to be capable of carrying infection, and in some cases can suffer mortality due to IPN during juvenile development.

In the light of the increasing impact of IPN in Scottish aquaculture, the apparently ubiquitous nature of the IPN virus in the marine environment and the virus' resistance to physical and chemical inactivation, the Aquaculture Health Joint Working Group established an IPN Sub-Group to review the currently available scientific information on the disease in salmonids and other species, to determine its prevalence and virulence in Scotland and to provide recommendations on risk reduction measures and on the control regime for IPN. In Scotland IPN is currently notifiable in some species but not in all.

This report is submitted to the Deputy Minister of Environment and Rural Development of the Scottish Executive, Scottish Quality Salmon (SQS), the Shetland Salmon Farmers Association (SSFA), the British Marine Finfish Association (BMFA), the Orkney Fish Farmers Association (OFFA), the Western Isles Aquaculture Association (WIAA) and other members of the Scottish aquaculture industry. It represents a comprehensive review of IPN with particular reference to Scotland and should serve as a useful reference for regulators, aquaculturists and indeed anyone with an interest in fish health.

The risk reduction measures recommended in this report are consistent with those contained in *A Code of Practice to Avoid and Minimise the Effect of Infectious Salmon Anaemia* (Anon, 2000)[3] and are commended to industry for adoption. Government is encouraged to adopt a regulatory framework that promotes good husbandry practice whilst recognising that IPN virus is widespread, if not ubiquitous, in the marine environment.

I would like to thank all the contributors and editors who have given so willingly of their time in delivering such a comprehensive document.

Finally, it is the wish of the IPN Sub-Group that this document is dedicated to the memory of Gordon Rae, co-editor and active participant in the Group, who died so prematurely just as we concluded our work. Gordon's knowledge, enthusiasm and rare humour touched all of us in the course of this task. Most important to Gordon was that contributions such as this would help in the development of a sustainable and vibrant industry supporting the communities of rural Scotland.

Alan A. Stewart
Chairman,
IPN Sub Group of the Aquaculture Health Joint Working Group
December 2003

GLOSSARY OF ACRONYMS AND ABBREVIATIONS

AHJWG	Aquaculture Health Joint Working Group
AMA	Area Management Agreement
Bus stop deliveries	The practice of delivering smolts to more than one location from a single supplier in one journey by wellboat.
DAO	Designated Area Order – under the Diseases of Fish Act 1937 (as amended) to place movement of fish and foodstuffs under control of the official service in the case of a notifiable disease.
ELISA	Enzyme-linked immunosorbent assay
Ensiled waste	The maceration and preservation in formic acid of dead fish and factory offal at a pH of less than 3.9.
Enzootic	Animal disease peculiar to or constantly present in a specific geographical area.
Epizootic	Outbreak of disease temporarily affecting a large number of animals within a particular region or geographic area.
Extra-ovum	Outwith the egg.
FITC	Fluorescein isothiocyanate
FRS	Fisheries Research Services, comprising: the Marine Laboratory Aberdeen and the Freshwater Fisheries Laboratory Pitlochry
FW	Fresh water
Gametes	Ova and sperm
IFAT	Indirect fluorescent antibody test
Intra-ovum	Within the egg
IPN	Infectious pancreatic necrosis
IPN virus	Infectious pancreatic necrosis virus
ISA	Infectious salmon anaemia
ISA virus	Infectious salmon anaemia virus
OIE	Office Internationale des Epizooties
OFFA	Orkney Fish Farmers Association

RT-PCR	Reverse transcriptase polymerase chain reaction
S½	A salmon smolt that is less than one year old and which goes to sea in the autumn.
S1	A salmon smolt that is approximately one year old and which goes to sea in the spring.
SDP	Standard Disinfection Procedures
SEPA	Scottish Environment Protection Agency
SEERAD	Scottish Executive Environment and Rural Affairs Department (formerly The Scottish Office Agriculture, Environment and Fisheries Department).
SQS	Scottish Quality Salmon
SSFA	Shetland Salmon Farmers Association
SSGA	Scottish Salmon Growers Association
SW	Sea water
Tidal excursion	The distance a particle (of water) will move over one tidal cycle.
Vertical transmission	The transmission of a pathogen within the contents of the gametes i.e. from parents to offspring.

ACKNOWLEDGEMENTS

The AHJWG would like to acknowledge the contribution of the following individuals and organisations:

Linda Leith and Keith Mutch of Fisheries Research Services for typing and formatting the report.

Alan Stewart, the late Gordon Rae, Trevor Hastings and Sarah Heath for editing the report.

Fisheries Research Services, the Scottish Executive Environment and Rural Affairs Department and Scottish Quality Salmon for accommodating the Sub Group and the editors for their meetings.

CHAPTER 1: INTRODUCTION

1.1 Establishment of the Infectious Pancreatic Necrosis (IPN) Sub Group

Infectious pancreatic necrosis (IPN) is caused by an aquatic birnavirus. The virus was the first fish virus to be isolated and is endemic in most parts of North America, Europe, Japan and South America. The disease IPN has been known in Scotland since 1971 when the first outbreak was reported at a rainbow trout farm in Loch Awe.[5] Historically IPN has been associated with high mortality in first feeding salmonid fry and subsequent low mortality in parr up to the smolt stage. More recently however, in Norway then in Scotland, it has become a serious cause of acute mortality in Atlantic salmon (*Salmo salar*) smolts 7-12 weeks after introduction to sea water. This has become of increasing economic importance in both Norway and Scotland.

Surveillance conducted by FRS has revealed the presence of IPN virus in an increasing proportion of Atlantic salmon farms in sea water (*See* Chapter 3 of this report). For example, in October 2000 some 45% of marine salmon farms had movement restrictions for IPN. The corresponding figure for salmon farms in fresh water was 8%. The situation in rainbow trout (*Oncorhynchus mykiss)* and brown trout (*Salmo trutta)* farms is uncertain as routine surveillance for IPN virus has not been conducted in trout farms since 1995.

IPN virus has been demonstrated to affect a wide range of fish species[44] including:

- Atlantic salmon (*Salmo salar L .)*
- Rainbow trout (*Oncorhynchus mykiss*)
- Brown trout (*Salmo trutta*)
- Arctic charr (*Salvelinus alpinus*)
- Halibut (*Hippoglossus hippoglossus*)
- Cod (*Gadus morhua*)
- Haddock (*Melanogrammus aeglefinus*)
- Turbot (*Scophthalmus maximus*)

All of these species are either currently farmed or in various stages of commercial development in Scotland. Infectious pancreatic necrosis can cause serious mortalities in juvenile marine finfish but the disease has not been reported in older fish. The overall impact of IPN in marine fish species is not known.

In light of the increasing impact of IPN in Scottish aquaculture, the Aquaculture Health Joint Working Group established a sub group with the following Terms of Reference:

- To review the current science of IPN in salmonids and other species.
- To determine the occurrence, virulence and economic significance of IPN.
- To review and make recommendations on the status and control regime for IPN.

This report describes the current state of knowledge regarding the transmission, prevalence, detection and diagnosis, and control of IPN in all farmed species in Scotland. A number of recommendations are made which it is intended should complement the

recommendations contained in the *Final Report of the Joint Government-Industry Working Group on Infectious Salmon Anaemia (ISA).*[4]

1.2 Membership and Participation in the IPN Sub Group

Chairman: Mr Alan Stewart
Chief Executive Landcatch Ltd.

Vice Chairman: Dr Ronald Stagg
Deputy Chief Executive Fisheries Research Services.

Members:

Dr Tony Ellis, Fisheries Research Services.

Mr Andrew Grant, Fish Veterinary Society.

Dr Trevor Hastings, Fisheries Research Services.

Mr Christopher Mitchell, Landcatch Ltd.

Dr Pauline Munro, Fisheries Research Services.

Dr Sandy Murray, Fisheries Research Services.

Mr Gordon Rae, Scottish Quality Salmon.

Mr David Sandison, Shetland Salmon Farmers Association.

Mr Richard Slaski, British Marine Finfish Association.

Dr David Smail, Fisheries Research Services.

Dr John Webster, Scottish Quality Salmon.

Secretariat: Mr Paul Shave and Dr Wendy-Louise Smith, Scottish Executive Environment and Rural Affairs Department.

The following also contributed to the work of the group by attendance at various meetings as experts on specific matters.

Mr Edward Branson, Fish Veterinary Society.

Dr Torunn Taksdal, The National Veterinary Institute, Oslo.

1.3 Schedule of Meetings of the IPN Sub Group

17 October 2000	Marine Laboratory, Aberdeen
19 December 2000	Marine Laboratory, Aberdeen
2 July 2001	Marine Laboratory, Aberdeen
25 September 2001	Marine Laboratory, Aberdeen
6 December 2001	Pentland House, Edinburgh
5 February 2002	Durn, Perth
5 March 2002	Open Forum in Marriott Hotel, Inverness
24 May 2002	Durn, Perth
26 July 2002	Durn, Perth

CHAPTER 2: IPN, THE VIRUS AND THE DISEASE

IPN virus belongs to the complex genus *Aquabirnavirus,* members of which have been isolated from many species of fish and shellfish from virtually all parts of the world. There are two serotypes which are important for European aquaculture at present. They are serotype Sp, which causes mortality in farmed salmonids and halibut, and serotype Ab, which has been associated with mortality in farmed turbot. The host range is not very well known. The Sp serotype has been associated with clinical disease in farmed Atlantic salmon, rainbow trout, eel (*Anguilla anguilla)* and carp (*Cyprinus carpio* and others) and has been isolated from many wild freshwater fish species without clinical disease. In farms, mortalities occur mainly in the fry (or juvenile) stages but in recent years, Atlantic salmon post-smolts have suffered considerable mortalities from IPN, especially in Norway and Scotland. The virus can set up a persistent infection of apparently healthy populations of all age groups of fish, in both fresh and sea water environments. Such asymptomatic carriers may shed virus and are considered to be a source of horizontal transmission of IPN virus. Furthermore, in some species of trout, it has been shown that carrier broodstock can vertically transmit the virus to progeny. Little is known about the nature of the carrier state, what determines why some fish become carriers, how some fish clear the virus and why some die of IPN.

The development of vaccines has been problematic mainly for economic reasons because the cost of producing inactivated viral vaccines from virus grown in fish tissue culture cell lines is very high. Recently, improved methods of virus culture and recombinant vaccine production are leading to trials of more affordable vaccines. However, it is too early to say how effective these will be in controlling the disease or the reservoirs of infection associated with carrier fish.

In the absence of fully authorised, effective vaccines, methods of controlling IPN have focused on management, sanitary and regulatory areas. However, IPN virus is a very tough virus compared to many others. It can withstand desiccation and can survive in both fresh and sea water for considerable periods, thus horizontal transmission in the environment occurs very readily. It is quite temperature resistant, for instance while ISA virus is inactivated at 55^{o} C for five minutes, IPN virus requires heating to about 80^{o} C for two hours to achieve significant inactivation. IPN virus is also resistant to low pH. Its tolerance of high temperatures and low pH enables it to survive in ensiled waste and to pass through the gut of birds and mammals. Its safe disposal is therefore compromised and the possibility of the virus being spread by piscivorous (fish-eating) predators complicates containment. It is about 100 times more resistant to ultraviolet radiation than some other viruses, for example, infectious haematopoietic necrosis (IHN) virus, making it very difficult and costly to sterilise hatchery water supplies by this method.

IPN virus is sensitive to some commonly used disinfectants and these can be successfully used to inactivate it on the surface of eggs and equipment in fresh water. However, in some species it has been shown that the virus can enter eggs at the time of fertilisation where it is inaccessible to disinfection and thus can be transmitted vertically. Many disinfectants such as iodophors have reduced activity in sea water and their efficacy in disinfecting eggs in the marine environment remains to be proven.

For further information on the nature of the virus the reader is referred to reviews by Reno[44] and Smail and Munro[57].

CHAPTER 3: A DESCRIPTION OF THE REGIONAL AND TEMPORAL PATTERNS OF IPN VIRUS PREVALENCE IN SCOTTISH SALMON FARMS 1996-2002

This chapter considers Fisheries Research Services (FRS) sampling records from Scotland in the period 1996-2002 to evaluate regional and temporal variations in the prevalence of IPN virus in the Scottish salmon farming industry.

Prevalence values are obtained from the fraction of fish samples tested for IPN virus that are positive. It should be noted that sampling may be biased to sites that are suspected of being IPN virus positive, so prevalence may be a little higher than the true proportion of farms positive. For the analysis of patterns, any bias should not affect the assessment of the relative distributions of IPN virus. In some cases there were relatively few data. Error ranges were applied to these by including the maximum and minimum values whose 95% ile equals the observed value, given the number of samples available.

Most of the data available concerned salmon, although the FRS database includes some data on other farmed fish. Briefly, freshwater species tended to have much lower prevalence of IPN virus than seawater species. However, rainbow trout had relatively high prevalence of IPN virus in fresh water, although less than in sea water. Brown trout, on the few data available, had very low prevalence in both fresh and sea water.

Salmon are the major subject of the database and are, therefore, suitable for detailed analysis of patterns of IPN virus prevalence. For salmon, in freshwater sites IPN virus prevalence is lower than in sea water sites with overall prevalence values of 13% and 55% respectively. The prevalence shows increasing trends in both fresh and sea water (Fig. 1), so that by 2002 prevalence in fresh water was 26% and in sea water 82%. The increases in fresh water were 3% year^{-1} ($p = 0.012$, $r^2 = 0.75$) and in sea water 7.6% year^{-1} ($p= 0.002$, $r^2 =0.85$).

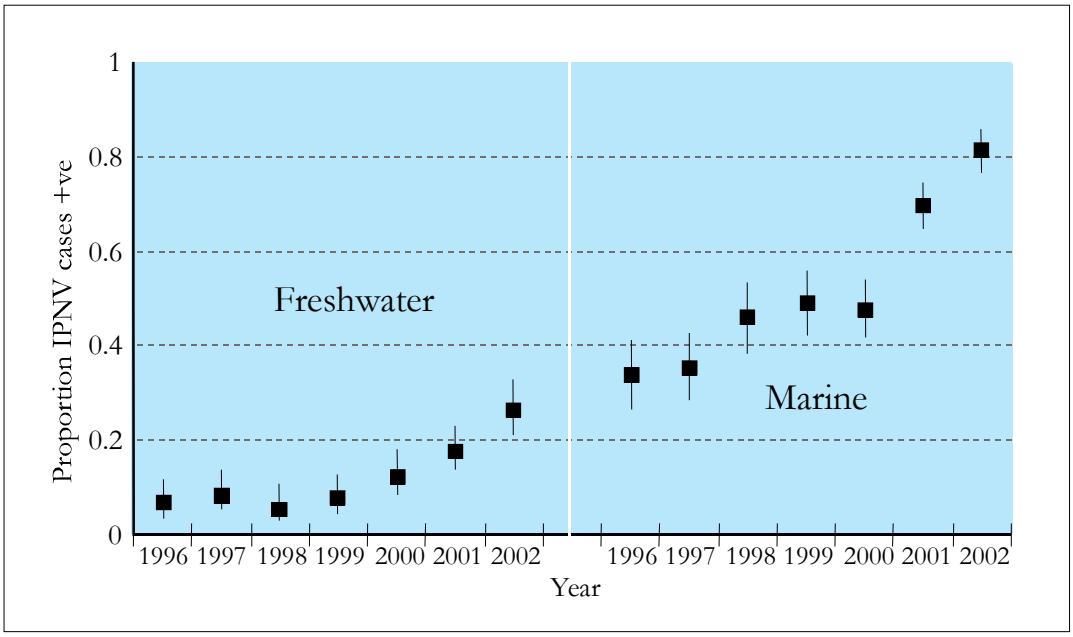


Figure 1. IPN virus prevalence in Scottish salmon production site by sector in 1996-2002. Bars show 95%iles as described in text.

3.1 Regional Variation

These data are broken down into the regions of northern and southern mainland Scotland, Shetland, Orkney and the Outer Hebrides. Northern and southern regions of the mainland are split using the Ordnance Survey's 800 km north line, which lies roughly between Mallaig and Aberdeen. Shetland has larger than average mean IPN virus prevalence in both fresh and sea water (χ^2 $p < 0.001$). The Outer Hebrides and northern mainland Scotland both have highly significant lower than average IPN virus prevalence in sea water ($p < 0.001$) and the northern mainland has a significantly low prevalence in fresh water ($p < 0.05$). However, as shown below, region differences declined in the later years of the analysis as the regional averages all became high.

Given that average prevalence in Shetland is so different from other areas, it is worth looking separately at the temporal patterns of prevalence of IPN virus in salmon production sites in the five regions previously specified.

In freshwater production sites year-to-year variation in IPN virus prevalence shows a pattern of increases (Fig. 2). These increases are statistically significant in all regions except northern Scotland ($p = 0.14$). The increase appears to have picked up since 1998. Prevalence in Shetland in fresh water is higher than in other areas, it is also increasing more rapidly than elsewhere.

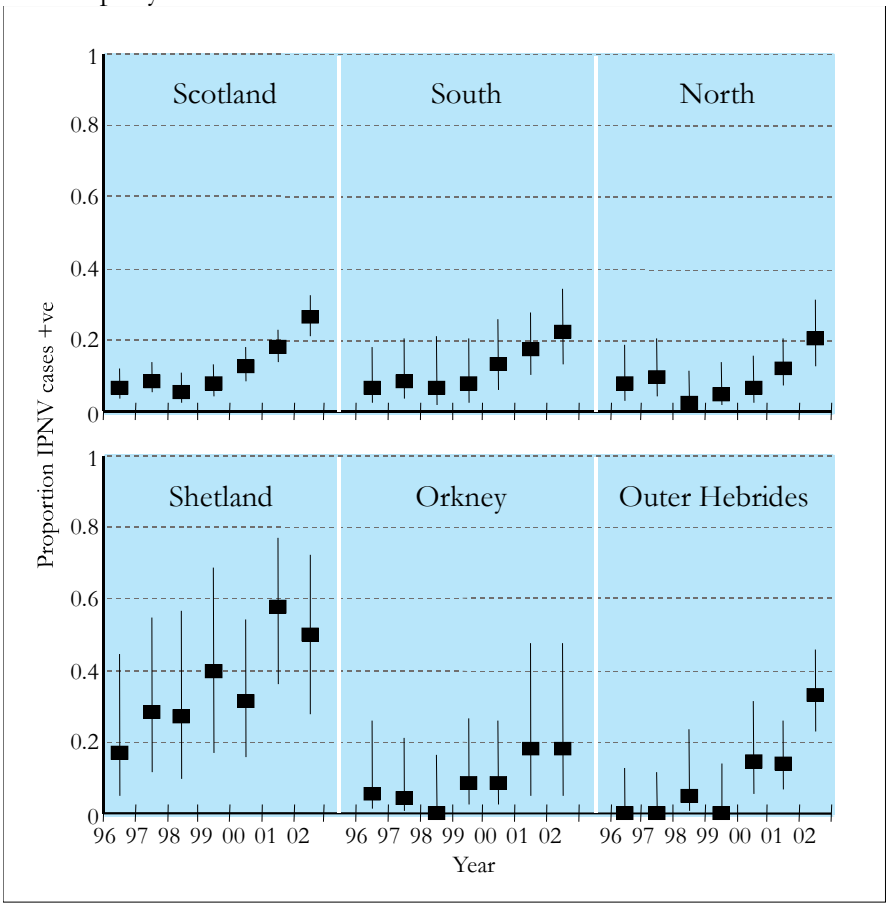


Figure 2. IPN virus prevalence in Scottish fresh water salmon sites by year (1996-2002) for all Scotland and for regions. The regions are: northern mainland Scotland, southern mainland Scotland, Shetland, Orkney and the Outer Hebrides. Bars as in Figure 1.

In seawater sites there is clear evidence of increasing prevalence of IPN virus in all regions, except southern mainland Scotland (Fig. 3). Here the regression is not significant, but is negative. In most regions, particularly the Outer Hebrides, these increases are from very low levels so IPN virus prevalence remains moderate. However, in Shetland IPN virus prevalence was initially high so by 1999 it had become almost ubiquitous and further large increases became impossible.

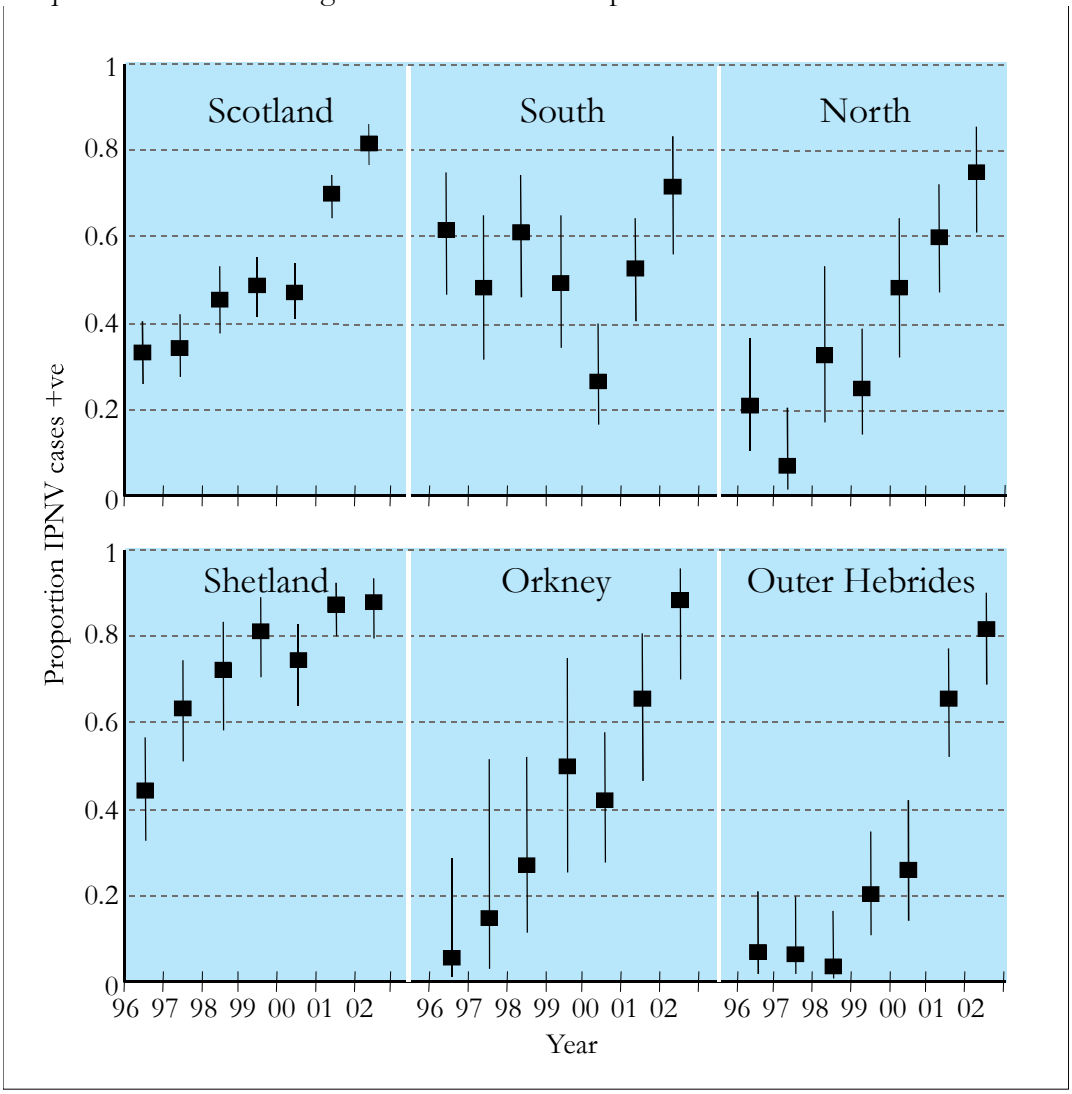


Figure 3. IPN virus prevalence in Scottish marine salmon production sites by year (1996-2002) for all Scotland and by region. The regions are: northern mainland Scotland, southern mainland Scotland, Shetland, Orkney and the Outer Hebrides. Bars as in earlier figures.

In most regions there is a decline, or at least a reduction in the rate of increase, of IPN virus prevalence in 1999 or 2000. This decline may be related to the management of the ISA outbreak of 1998-99. Mainland regions show a response in 1999, while island regions respond in 2000.

Towards the end of the time series IPN virus prevalence in sea water had become very similar in most regions. This is particularly striking when annual region means and standard deviation between means are plotted (Fig. 4). In proportional data, standard

deviation is related to $\sqrt{[p \times (1 - p)]}$, so variance would be expected to rise until 2001, when p exceeds 0.5 and a slight decline is expected. However, standard deviation shows a tendency to fall and the large drop seen in 2001 and 2002 is not expected. The analysis indicates that not only is IPN virus becoming more abundant in Scotland, but that regional differences are disappearing.

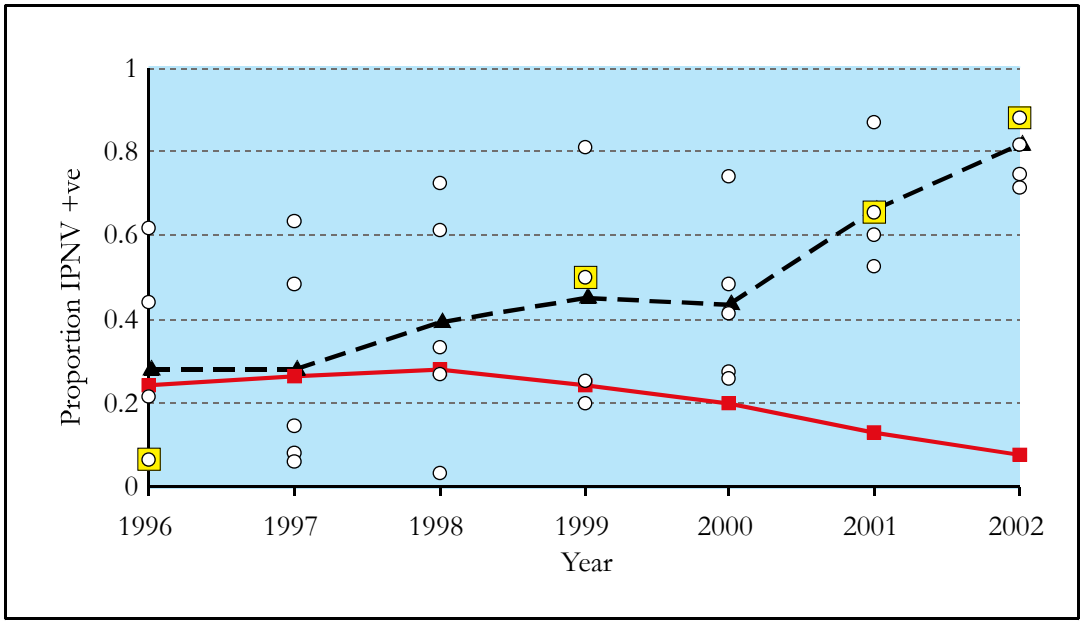


Figure 4. Prevalence by year of IPN virus in sea water in the Scottish salmon producing regions. Thin dashed line is mean of annual regional prevalence and thick solid line is the standard deviation about the mean. Circles denote individual regional means, squares are where two coincide.

3.2 Seasonal Patterns

Reported cases of IPN virus show peak values in summer (Fig. 5). However, this is also the period in which most samples were taken. When effort is discounted by looking at prevalence the signal is very weak (Fig. 6). A particularly high case load from 2001 partly reflects the coincidence of high prevalence with the period of maximum sampling. IPN virus prevalence would appear to be higher in summer, but winter values are based on very few data and are highly uncertain.

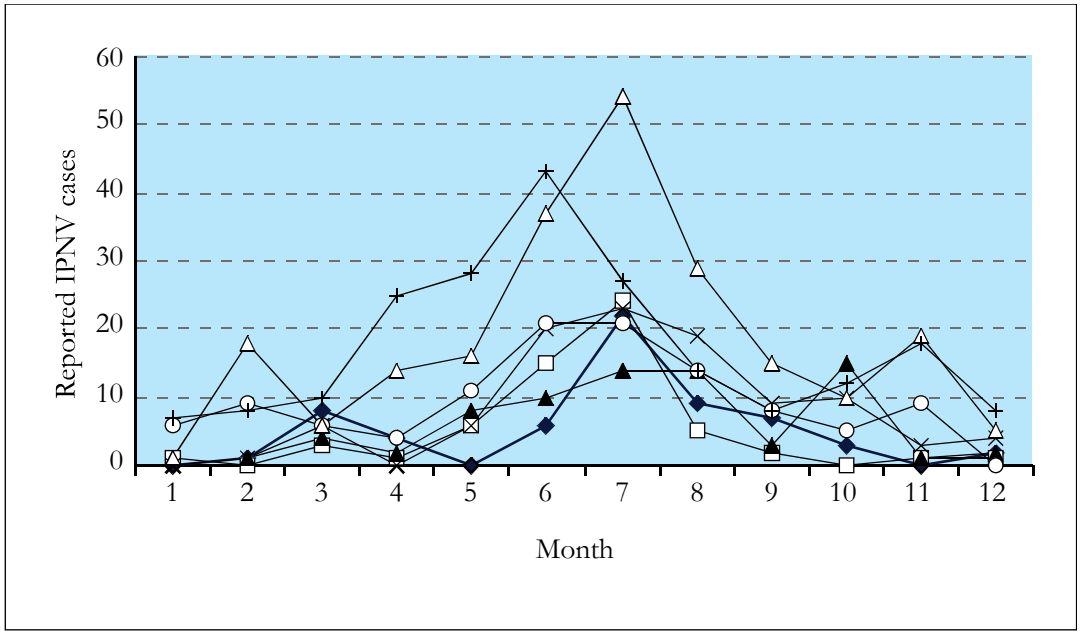


Figure 5. Monthly recorded cases of IPN virus in marine sites Scotland 1996-2002 (years shown separately). Symbols: 1996 ♦solid diamonds; 1997 □ hollow squares; 1998 ▲ solid triangles; 1999 × diagonal crosses; 2000 ◊ hollow diamonds; 2001 Δ hollow triangles; 2002 + vertical crosses.

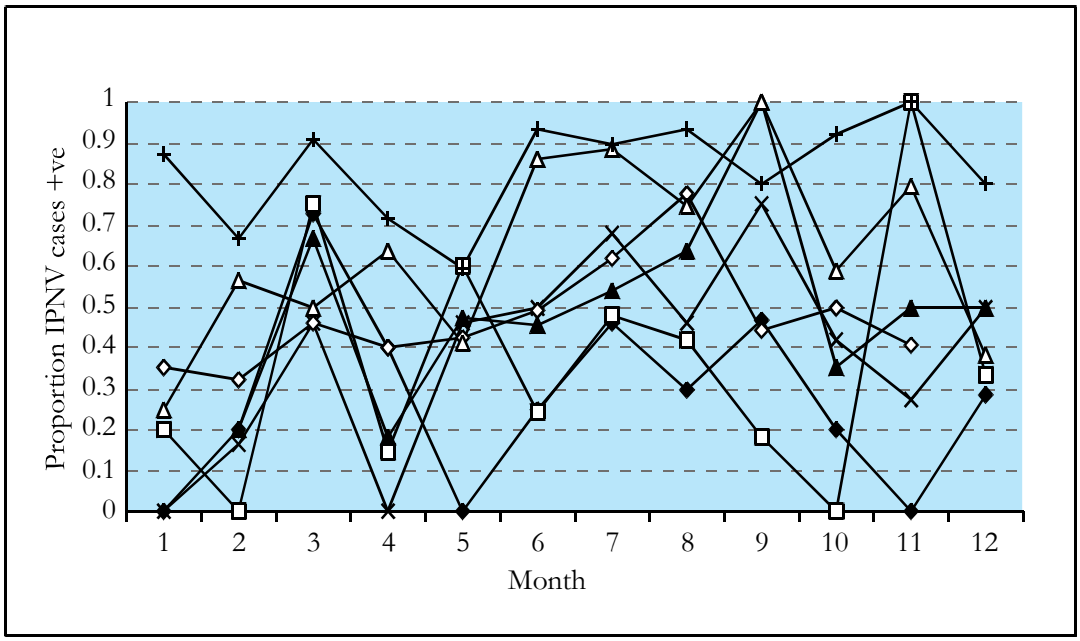


Figure 6. Monthly recorded prevalence of IPN virus in marine sites in Scotland 1996-2002 (years shown separately). Symbols as Fig. 5.

3.3 A Multi-Level Model

There is variation between IPN virus prevalence in sea water and fresh water, between regions, years and possibly seasons. It would be useful to assess the relative significance of these components in controlling the variation observed among samples. An approach which allows this variance to be broken down is multi-level modelling using the statistical analysis package MlwiN.[41]

A multi-level model of the prevalence of IPN virus was set up by dividing the data into marine and freshwater sites and these were in turn divided into the five regions discussed earlier. For each of the regions there were data on the annual level of IPN virus prevalence and these were divided into three periods of four months (January to April, May to August and September to December). This model has been run with 1996–2002 data.

The structure of variance was examined at the different levels assuming a binomial data distribution. This is necessary because at the seasonal level data sets may consist of very few samples, especially seasonal periods 1 and 3, and therefore normal distribution cannot be assumed. Variance at these levels is listed below:

	Variance
Level 1, sea/fresh:	0.413 ± 0.510
Level 2, region:	0.370 ± 0.239
Level 3, year:	0.534 ± 0.123
Level 4, seasonal:	0.057 ± 0.050

In earlier analyses of data up to 2001, the largest contributor to variance was the difference between regions because of the very high prevalence in Shetland and low prevalence in the Outer Hebrides prior to 2001.[35] However, regional variation now contributes relatively little to overall variance as all regions have similar high prevalence. In the more recent data the contribution of annual variation has become dominant. This variation is highly important because it reflects the trends of increasing prevalence observed in most regions, and the year-to-year variation (without trend) in the southern Scottish mainland area. The difference between marine and fresh water environments is also a moderately large source of variation. Its contribution is very uncertain because there are only two classes. Seasonal variance in IPN virus prevalence is very small and uncertain.

Regional variation was historically more important than it now is, as there were large regional differences in the 1990s that had largely disappeared by 2002 (Fig 4). Earlier analysis of part of the data (1996-2000 and 1996-2001) showed that the contribution of this level to overall variance is declining rapidly. Similarly, the role of year-to-year differences in overall variance is increasing as prevalence increases.

3.4 Conclusions

IPN virus has become very widespread in Scottish salmon farms over the period 1996-2002. Prevalence shows large variation, IPN virus being most common (88% prevalence) in Shetland marine sites in 2002 and undetected in fresh water sites in the Outer Hebrides in several years. Prior to 2001, this variation was largely controlled by differences between regions, with the effect of difference between fresh water and sea water environments and year-to-year differences being of secondary significance. Recently, the role of regional differences has declined, while that of inter-annual variation has increased. Seasonal differences in IPN virus prevalence are small in spite of large differences in case numbers.

Year-to-year differences are highly significant in that, except in the southern mainland, this variance reflects a trend of increasing IPN virus prevalence at an annual rate of 3% in fresh water and 7.6% in sea water, but local increases are sometimes faster than this.[35]

In Orkney, the northern mainland and particularly the Outer Hebrides, these increases are from low to moderately high levels. However, in Shetland the initial prevalence was not low, so IPN virus has become almost ubiquitous in sea water by 2000. By 2002 very high prevalence had been reached in marine waters in almost all areas. In fresh water sites the prevalence of IPN virus also shows rapid increase, which is faster in Shetland than in fresh water sites elsewhere. More statistical analysis of the data for 1996-2001 is available elsewhere.[35]

This analysis is a description of the FRS Fish Health Inspectorate's data set, and biases with respect to real IPN virus prevalence may result from the different purposes for which these data were collected. However, levels of prevalence correspond closely to the number of farms under movement restriction (*See* Chapter 1). Moreover, the patterns obtained from these data should be buffered against such biases, being dependent on relative prevalence. These patterns of declining regional variation and a general, but not universal, increase in prevalence of IPN virus are striking.

CHAPTER 4: VERTICAL TRANSMISSION

Vertical transmission denotes germ-line associated transmission of virus *via* ova or milt. Vertical transmission of IPN virus has been demonstrated in rainbow trout and brook trout but to date has not been proven in Atlantic salmon.

4.1 Risk Factors

There are three potential ways in which infected broodstock may transmit IPN virus to their progeny:

- True (*intra-ovum*) vertical transmission within the contents of the gametes;
- *Extra-ovum* transmission on the surface of the gametes and in natural secretions and excretions from the parents, for example, ovarian fluids, seminal fluids or mucus;
- Transmission *via* contamination from infected water, personnel, clothing and equipment associated with stripping broodfish and fertilising ova.

There is evidence of true vertical transmission, both intra-ovum and sperm-mediated in brook trout and rainbow trout.[16] These authors also showed evidence against the efficacy of disinfectants at the time of fertilisation. This result demonstrated that virus attached to spermatozoa can evade disinfection. This experiment was carried out using high titres of cultured virus and may not represent virus titres in farmed fish.

4.1.1 Risk assessment

In general terms the literature suggests that the probability of vertical transmission to the gametes is proportional to the virus levels in the gonads. In brook trout it was found that virus titres over 10^4 $TCID_{50}$/ml in ovarian fluid were correlated with the isolation of IPN virus from egg homogenates. Therefore 10^4 $TCID_{50}$ /ml in ovarian fluid may represent a threshold of positive transmission in brook trout.[63]

For *extra-ovum* transmission of virus, the probability of virus transmission is considered to be much lower because virus could be inactivated in the external milieu. Normal disinfection precautions for eyed-eggs should remove and destroy any virus particles loosely bound to the hardened egg shell.

IPN virus has been reported to be vertically transmitted in brook trout,[11,8] rainbow trout[18] and Arctic char.[2] IPN virus is known to bind to milt *via* the sperm head[33] and this is the likely mechanism whereby the virus enters the egg *via* the micropyle at the point of fertilisation.[18] In addition, Ahne *et al* [1] showed that in experimental infection of eyed rainbow trout eggs, IPN virus could adsorb to the hardened shell of the eggs and remain infective, acting as a source of infection for sac-fry.

Although it has not been conclusively shown in salmon, there should be a presumption that there is a significant risk of vertical transmission in that species, especially *via* the *intra-ovum* route.

Evidence for vertical transmission of IPN virus in Atlantic salmon may be considered under the following headings:

(a) Experiments carried out at FRS Marine Laboratory.[56]
(b) Virus titre analysis (*See* Appendix I).
(c) Norwegian experience.

a) Experiments carried out at FRS Marine Laboratory

- No experiments have demonstrated transmission from parent to offspring in salmon.
- Attempts to infect female broodfish by injection of virus resulted in the ovarian fluid of only one of 30 broodfish becoming infected at a low titre.
- Testes and ovaries of salmon can be infected experimentally.
- Micro-injection of IPN virus into the perivitelline space of dry-fertilised eggs showed that virus will survive for up to four months.

b) Virus titre analysis

Experiments with trout show that high virus titres are important in vertical transmission *via* the milt[18,8] and circumstantial evidence indicates this may also apply in the case of ovarian fluid.[9]

Two observations may be made:

- The sensitivity of virus detection is not very critical for it is the high titre fish that need to be detected.
- Titre/frequency analysis on a small data set of ovarian fluid cell sonicates predicted about 1% of samples to have titres of 10^4 pfu/ml or greater (*See* Appendix I). This may indicate a risk of vertical transmission.

c) Norwegian experience

Recent experience of IPN in Norway has been provided by Taksdal (*See* Appendix II) and is summarised below.

- IPN has effectively been deregulated in Norway.
- There is a general lack of broodstock testing in Norway.
- There is a high incidence of IPN in fresh water.
- Sea water is used in some hatcheries in Norway.
- The disinfectant regimes for hatcheries are generally inadequate to prevent IPN virus entering from sea water.

The onus on proof for vertical transmission in salmon remains. There are two areas which will lead to an advance in understanding:

- An expansion of the IPN virus titre/frequency data set for Atlantic salmon broodstock.
- Examination of the role of experimentally infected males in vertical transmission.

4.2 Conclusion

It has been demonstrated that IPN is vertically transmitted in several salmonid species. Although vertical transmission has not been proven in salmon specifically, some of the experimental evidence supports the notion of IPN virus entry and survival in eggs. As a precautionary approach the presumption should be that the virus is vertically transmitted. There is no information to advise on the risk of vertical transmission of IPN in non-salmonid species at this time.

4.3 Risk Reduction Measures

Practical preventative measures for Atlantic salmon currently incorporate the following:

- Broodstock testing at the level of 30 fish per site to determine whether or not a farm is infected;
- 100% broodstock testing with disposal of all potentially infected egg stocks to identify infected individuals in infected farms;
- Separate water supplies for all egg batches;
- Strict hygiene at broodstock sites to avoid cross-contamination of egg lots;
- Egg disinfection immediately post-fertilisation and again pre-hatch.

4.3.1 *Recommendations*

Research should be undertaken to establish conclusively and as a matter of urgency whether vertical transmission of IPN virus occurs in Atlantic salmon. Priorities for research are:

(a) An expansion of the IPN virus titre/frequency data set for Atlantic salmon broodstock.

(b) Examination of the role of experimentally infected males in vertical transmission.

Research should be undertaken to establish whether vertical transmission of IPN virus occurs in marine finfish species.

Until the absence of vertical transmission of IPN virus in Atlantic salmon is demonstrated, all Atlantic salmon broodstock should be tested for IPN virus and ova from infected parents destroyed.

CHAPTER 5: HORIZONTAL TRANSMISSION AND SOURCES OF INFECTIOUS PANCREATIC NECROSIS VIRUS

5.1 Introduction

This chapter describes the current understanding of horizontal transmission of IPN virus and the potential reservoirs and vectors of infection.

5.2 Horizontal Transmission

Horizontal transmission of IPN can be defined as the lateral spread of IPN virus. During an epizootic of IPN, virus is shed from dead and moribund fish into the waters around the farm. Infected fish will serve as a reservoir and exert an infectious pressure on other susceptible individuals of the same species or other species.

The virus may be transmitted in and between the freshwater and marine environments by a variety of routes, and involving various reservoirs and vectors, which are discussed below.

The proportion of fish that are infected within a farm will vary and can change over time. The rate of detectable infection in a population may also vary considerably at different times of year.[49] In 2000, the proportion of fish in nine Scottish Atlantic salmon broodstock sites that had detectable IPN virus infection at time of spawning ranged from 0% to 65% (FRS unpublished data).

Recent work by Bowden *et al.*[10] suggests that the infectious dose of IPN virus may be very low. In their IPN virus challenge model, mortalities occurred in Atlantic salmon smolts infected with IPN virus by intraperitoneal injection (IP) at a dose of only 10 $TCID_{50}$ per fish. IPN virus was also transmitted to naïve smolts cohabiting with the IP-injected fish.

The processes of infection and transmission of IPN can be considered to be interactive. It is likely that virus can travel from the primary host (for example, salmon or trout) to the various reservoirs and vectors of infection and *vice versa*. Farmed fish may be the most important reservoir of IPN virus in the environment. However, other potential reservoirs include wild fish, species cohabiting with farmed fish, sediments under fish farm cages, ectoparasites, shellfish, plankton, crustaceans, birds and mammals. The potential reservoirs and vectors of IPN virus are discussed in greater detail below.

The activities of fish farming staff are also likely to be important in the transmission of IPN. Fish farm equipment that comes into contact with fish, such as hand nets, grading machinery, harvest bins and mortality containers, pose a risk of the transmission of disease if they are transferred between tanks, ponds or cages or between fish farms.

The transporting of fish and the equipment associated with this activity, such as vehicles, wellboats[36] and other vessels, also pose a significant risk. Other fish farm activities, such as harvesting and processing of fish, can lead to the spread of disease if risk reduction methods are not employed. The risks associated with these activities are described below.

5.3 Reservoirs and Vectors

There is a wide range of potential reservoirs and vectors and these will be considered below in order of perceived importance.

5.3.1 Live farmed fish and fish used for stock enhancement

5.3.1.1 *Risk Factors*

The prevalence of IPN virus infection in freshwater Atlantic salmon farms in Scotland is low, but increasing and clinical outbreaks of disease are occurring with increasing frequency. In general, the risk associated with farmed salmon in fresh water is low compared with that of farmed salmon in sea water. Salmon post smolts in marine waters around mainland Scotland as well as the Northern Isles appear to show an increasing prevalence of infection and associated incidence of clinical IPN. The indications are that IPN virus replicates in these fish to very high titres of $\log_{10}$ 9-10 $TCID_{50}$ /g.[54]

The emerging scenario from experimental work is that healthy smolts are very susceptible to IPN virus if challenged within six weeks of sea water.[10] In the cage environment secondary infections such as *Vibrio* may serve to weaken host defences and further compromise the health of post-smolts.

Viral titres in carrier fish can vary markedly. IPN virus titres in viscera of rainbow trout can range from $10^{0.85}$ to $10^{4.2}$ $TCID_{50}$ g^{-1},[65] while those in brook trout have been reported to range from $10^{0.85}$ to $10^{6.5}$ $TCID_{50}$ g^{-1}.[43] Titres of a similar order were reported in the kidney of Atlantic salmon parr in fresh water.[55] Viral titres in tissues can be markedly higher during episodes of clinical disease. For example, Smail *et al* [54] reported median kidney virus titres of $10^{4.51}$ and $10^{9.04}$ $TCID_{50}$ g^{-1} in the kidney of healthy and diseased Atlantic salmon post-smolts respectively.

There is a clear potential for wild salmonids to transmit IPN virus in the same way as farmed salmonids. It follows that a risk of transmission of the virus both to wild and farmed salmonids may arise as a consequence of IPN infected or untested adult fish being used for the production of juveniles which are subsequently released into the wild during the restocking of rivers and other fishery enhancement activities.

5.3.1.2 *Risk Reduction Measures*

Where IPN is suspected or confirmed on a farm, FRS, acting for the Scottish Ministers, may serve movement controls in the form of a Thirty Day Notice (TDN) or a Designated Area Order (DAO) under The Diseases of Fish Acts 1937 and 1983. These movement controls are described in more detail in Chapter 7 of this report.

Movement of live fish or gametes subject to a TDN or DAO is not permitted without the prior approval of the Scottish Executive Environment and Rural Affairs Department (SEERAD). A risk assessment is conducted before any movement of live fish is permitted.

All hatcheries and other facilities used in the production of salmonids for restocking and enhancement of fisheries should be registered and subjected to the same control regime as commercial fish farming facilities.

Vaccination has proven to be effective in controlling a number of bacterial diseases of fish, including vibriosis, enteric redmouth and furunculosis, and offers a potential means of controlling IPN. A number of IPN vaccines have undergone trials but their effectiveness in controlling the disease is as yet unproven.

Different populations of rainbow trout can vary significantly in their susceptibility to IPN.[40] The impact of IPN could be significantly reduced if IPN-resistant families of Atlantic salmon can be developed.

Atlantic salmon smolts are particularly susceptible to IPN in the weeks following transfer to sea water. It would seem important therefore to apply high standards of husbandry in the period prior to and following sea water transfer.

5.3.1.3 *Recommendations*

Effective vaccines against IPN should be developed for use in Atlantic salmon and marine fin fish species.

IPN-resistant strains of Atlantic salmon and other marine fin fish species should be developed.

The highest standards of husbandry should be ensured especially prior to and when transferring smolts to sea and in the ensuing weeks when they are highly susceptible to disease.

A risk assessment should be conducted before any movement of live fish takes place, either under voluntary Code of Practice or a regulatory regime.

All hatcheries and other facilities used in the production of salmonids for restocking and enhancement of fisheries should be registered and subjected to the same control regime as commercial fish farming facilities.

5.3.2 Mortalities

5.3.2.1 *Risk Factors*

As with other diseases of fish, IPN-infected mortalities may promote the spread of IPN if they are not promptly removed and disposed of in an appropriate manner. The disposal of mortalities is controlled under Regulation (EC) No 1774/2002 of the European Parliament and of the Council. Under this Regulation, fish that die other than being slaughtered for human consumption, including fish killed to eradicate an epizootic disease, are categorised as Category 2 material. Such material must be disposed of by rendering or incineration. There is also provision for ensiling or composting under conditions yet to be established and approved by the European Commission.

Unlike the ISA virus, IPN virus is not inactivated at acid pH. Ensiled mortalities should therefore still be regarded as high-risk waste and potentially infected with IPN virus until final disposal by an approved method takes place.

Survivors of an acute IPN infection may become persistent carriers. Therefore, fish from infected populations that have apparently died of other causes may still pose a risk of transferring IPN virus to uninfected stocks.

Equipment used to remove or transport dead fish poses a high risk of transferring IPN virus to healthy fish.

5.3.2.2 *Risk Reduction Measures*

The prompt removal and safe disposal of dead and moribund fish can help prevent the spread of disease. Tanks and cages should be inspected daily for evidence of dead or moribund fish.

If possible, separate hand nets and mortality containers should be available for individual tanks or cages of fish. IPN virus is well preserved by freeze-drying[64] and it is probable it can survive desiccation. Therefore, equipment that comes into contact with mortalities should be thoroughly cleaned and disinfected after use. Iodophors are suitable disinfectants against IPN virus. IPN virus is also susceptible to formaldehyde and to alkaline pH.

Bins used to store dead or ensiled fish should be leak-proof and bins and vehicles used to transport dead or ensiled fish should be cleaned and disinfected after use.

Divers could inadvertently spread infection between farms. Where possible they should use site-specific gear. Alternatively, they should disinfect their equipment thoroughly between diving operations on different sites.

Good husbandry and management practice, employing methods of disease prevention and control that are known to be effective against other diseases, are likely to be effective in the prevention and control of IPN infection.

5.3.2.3 *Recommendations*

Further development of systems for monitoring and removing mortalities from fish farm tanks and cages is desirable.

Alternative methods for the safe, cost-effective disposal of mortalities should be investigated, for example composting or improvements to ensiling.

Tanks and cages should be inspected daily for evidence of dead or moribund fish.

Mortalities should be removed from tanks and cages without undue delay and disposed of in accordance with EC Regulation 1774/2002 or any subsequent legislation.

Where practicable, farms should not share mortality ensiling points. Where farms cannot avoid sharing ensiling points, particular care should be taken to prevent transfer of pathogens between the farms.

Where practicable, separate hand nets and mortality containers should be available for individual tanks or cages of fish.

Equipment used to remove or transport dead fish should be disinfected after use.

Divers should use site-specific gear or disinfect their equipment thoroughly between diving operations on different sites.

5.3.3 Escaped fish

5.3.3.1 *Risk Factors*

Even with good levels of containment it is possible that some fish will escape at some time from commercial fish farms. Escaped fish have the potential to shed virus into the aquatic environment some distance from the farm from which they escaped.

Several factors will contribute to the level of disease risk associated with escapes:

- the likelihood of an escape event occurring on an infected farm;
- the level of IPN virus infection on the farm;
- the level of clinical disease present on the farm;
- the dispersal of escaped fish in relation to susceptible wild and farmed fish populations.

These factors were considered in the *Final Report of the Joint Government/Industry Working Group on Infectious Salmon Anaemia (ISA).*[4]

It is considered that escaped fish that are suffering from clinical disease are likely to be more susceptible to predation than healthy carriers. By virtue of their weakened condition, fish that are suffering from clinical disease will not be likely to survive and travel as great a distance as healthy carriers. However, it is possible that piscine predators themselves may become infected or that avian or mammalian predators may become passive vectors of infection. In a three-year study of the distribution and prevalence of IPN virus in wild fish in a Scottish freshwater loch, there was evidence that wild adult brown trout and perch (*Perca fluvialis*) may have become infected by feeding on rainbow trout that had escaped from a nearby.[34]

5.3.3.2 *Risk Reduction Measures*

The recommendations contained in "*What to do in the event of an escape of fish from a fish farm*" (*See* http://www.scotland.gov.uk/library5/environment/escape.pdf) and the *Code of Practice on the Containment of Farmed Fish, Official Notification Following the Escape of Fish, and Possible Measures to be Employed to Attempt Recapture*, (*See* http://www.scottishsalmon.co.uk/pdfs/contain.pdf) should be followed and any requirements of legislation should be taken into account. Cage security inspections should be increased immediately IPN is confirmed on a site and a net inspection programme should be followed until all fish are removed from the cages.

Efforts can be made to recapture escaped fish. Particular effort should be directed at attempts to recapture or prevent escaped sexually mature salmon entering river systems in line with any methods proposed by the Farmed Fish Escapes Working Group.

5.3.3.3 *Recommendations*

Under an amendment of the Registration of Fish Farming and Shellfish Farming Businesses Order (1985), fish farmers must notify the Scottish Ministers of any escapes or suspected escapes of farmed fish. The guidance provided in "What to do in the event of an escape of fish from a fish farm" should be followed.
(*See* http://www.scotland.gov.uk/library5/environment/escape.pdf***)***

The Code of Practice on the Containment of Farmed Fish, Official Notification Following the Escape of Fish, and Possible Measures to be Employed to Attempt Recapture should be followed.
(http://www.scottishsalmon.co.uk/pdfs/contain.pdf***)***

Cage security inspection should be reviewed immediately IPN is confirmed on a site and a net inspection programme, if not already in place, should be followed until all fish are removed from the cages.

Particular effort should be directed at attempts to recapture escaped salmon which are, or are likely to become, sexually mature in line with any methods proposed by the Farmed Fish Escapes Working Group.

Further research is needed to establish the longevity of infection in escaped fish, the risk of predators of escaped fish acting as vectors of infection, and the disease risk posed to wild fish and other farmed fish stocks.

5.3.4 Water and sediments

5.3.4.1 *Risk Factors*

It is recognised that farms stocked with fish suffering from IPN, or carrying IPN virus, shed virus particles into the water (Table 5.1).

Table 5.1. Measurements of IPN virus levels in water holding IPN virus-infected rainbow trout

PFU/ml *	Comments	Reference	Virus transmitted to uninfected fish
10^5	Experimental infection	Dorson & Torchy, 1981[17]	Yes
10^4	Hatchery effluent	Munro et al. 1976[34]	Likely
10^1	Hatchery effluent	McAllister & Bebak (1997)[29]	Unknown
10^{-1}	Downstream effluent	McAllister & Bebak (1997)[29]	Unknown

*PFU/ml = plaque forming units/ml

Rainbow trout hatchery effluent virus levels have been measured at 10 pfu/ml[29] to 10^4 pfu/ml.[34] Concentrations of IPN virus of 10^{-1} pfu/ml water have been reported downstream of IPN infected rainbow trout hatcheries.[29] It is not known whether virus infection can be established at concentrations below 10^2 pfu/ml.

Survival of IPN virus in fresh, estuarine and sea water was reported as long-lived with an inactivation profile of approximately 3 $\log_{10}$ over three weeks,[62] equivalent to a 10-fold reduction per week at 15°C. Survival of IPN virus outside the host is longer at lower temperatures. IPN virus survived for 147 days in Tris-glycine acid buffer, pH 3.8 at 4 °C but was undetectable at 71 days at 20 °C.[52]

Infected post-smolts may shed low amounts of virus *via* the faeces up to six weeks after bathing infection and up to eight weeks after feeding infection (Smail, unpublished data). It is probable that IPN virus will enter sediments but the ability of the virus to survive in this environment is unknown.

5.3.4.2 *Risk Reduction Measures*

Intakes and effluents to and from land-based sites can be disinfected to inactivate IPN virus using ultraviolet (UV) and/or ozone treatment although this is not thought to be practicable for on-growing.

Single year class stocking is a method used by salmon farmers to break cycles of disease. Farmers are currently implementing the recommendations of *A Code of Practice to Avoid and Minimise the Impact of Infectious Salmon Anaemia (ISA)*[3] and the objectives of the Tripartite Working Group by forming Area Management Agreements whereby farms in management areas will synchronise fallowing, whereby farms are emptied of fish and equipment is disinfected. A fallowing period of six weeks to six months is employed for ISA, according to circumstances. However, the effectiveness of this regime for IPN is unknown.

5.3.4.3 *Recommendations*

Water intakes to and effluents from land-based farms should be disinfected where practicable.

In cage culture a fallowing policy should be established, based on risk assessment.

Within land based facilities, farmers should be encouraged to maintain cohort separation, and cleaning and disinfection should be undertaken every time ponds or tanks are vacated and prior to restocking.

Research is needed into shedding rates of IPN virus from infected fish, survival of IPN virus in water and sediments, and establishing the minimum effective dose of the virus for susceptible species and agegroups.

Research is needed into the effect of fallowing on environmental levels of IPN virus.

5.3.5 Equipment and transportation of fish

5.3.5.1 *Risk Factors*

It is recognised that the biggest risk associated with the use of transport and other fish farm equipment lies with contamination from the fish rather than the hardware itself. Numerous fish farming activities, such as grading, mortality removal and fish transport using wellboats, lorries or helicopter buckets, involve direct contact between fish and the equipment used. Net washing is another area of potential risk, especially where nets from different farms are washed at a single location.

5.3.5.2 *Risk Reduction Measures*

Farm operations should be managed to minimise the number of fish movements. Fish farm equipment should be site-specific as far as practicable. Where transfer between sites is unavoidable, cleaning and disinfection must be carried out. There is not a great deal of published information on the range of disinfectants that are effective against IPN virus but it has been established that hypochlorite- and iodophore-based disinfectants are effective.[13,19] It is important that all fish farm staff are made aware of the importance of cleaning and disinfection routines, and that they are trained in carrying out the procedures. They should also be kept informed of the health status of fish in their care.

The cleaning and disinfection procedure for wellboats is well documented (*Field Guide - Disinfection with Regard to ISA Virus* [22] and *A Code of Practice to Avoid and Minimise the Impact of Infectious Salmon Anaemia* [3] and has three stages as it applies to ISA. These procedures can be adapted for the control of IPN (Table 5.2). The most rigorous protocols are required when leaving an infected farm for a new area and on entry to Scottish waters.

The inter-site movement of any vessels should be kept to a minimum and where such movements are unavoidable, suitable disinfection procedures should be followed.

Wellboats should operate with valves closed within a 5 km range of any fish farm.

Table 5.2: Disinfection stages required for wellboats according to operational circumstance

Operational circumstance	Stage 1*	Stage 2*	Stage 3*
Arriving from outwith UK waters other than from EU waters with equivalent zone status†	x	x	x
Moving from any harvesting operations to smolt transfer operations	x	x	x
Moving from one farm to any other farm in sea water.	x	x	
General deliveries (non-fish)	x		

* Stages 1, 2 and 3 as defined in *A Code of Practice to Avoid and Minimise the Impact of Infectious Salmon Anaemia*.[4] Details are provided in Appendix VI. † As defined in Directive 91/67/EEC.

5.3.5.3 *Recommendations*

If nets from different farms are washed at a common location, care should be taken to avoid cross-contamination and transfer of infection between nets from different farms.

Where possible, equipment should be site-specific. Where movements of equipment between sites is unavoidable, the equipment must be thoroughly cleaned and disinfected.

Staff should be trained in cleaning and disinfection routines.

Staff and other relevant parties (for example, fish transporters) should be kept informed on the health status of fish in their care.

Farm operations should be managed to minimise the number of fish movements.

Movements of vehicles, wellboats and equipment between farms should be kept to a minimum.

Further research is required to identify suitable, safe disinfectant products effective against fish pathogens.

Wellboats should operate with valves closed within a 5 km range of any fish farm.

5.3.6 Harvesting operations and processing plants

5.3.6.1 *Risk Factors*

Fish may be transported live for harvesting *via* wellboat and pumped directly on-shore, or killed on a harvest barge or similar facility and transported in bins by boat or by road to the processing plant. Wellboats, vehicles, harvest bins and pallets, particularly wooden pallets which are difficult to disinfect, may pose a risk of the spread of infection. Harvest stations are considered to pose a particularly high risk.

Spillage may occur during the routine tipping of harvest bins or during an accidental release, for example, where a harvest bin bursts or falls, or in the event of a road accident. This could result in contamination of personnel or vehicles and seepage to groundwater or water courses. Harvesting in adverse weather conditions increases the level of risk associated with harvesting operations.

Primary processing plants are often situated close to production areas and deal with the gutting, grading and packing of fish for onward transport. Secondary processing plants may be located closer to final markets and deal with a variety of further processing operations, such as filleting and smoking. Both types of processing plants may unwittingly deal with fish infected with pathogens, including IPN virus.

Vehicles used to transport fish pose a risk if they are not thoroughly disinfected before leaving the plant. Other vehicles may also become contaminated, unless there are separate entrances and exits and designated parking areas for staff or visitors' cars and vehicles transporting fish.

All liquid effluent from processing plants could pose a risk of spread of disease if it is not satisfactorily disinfected before disposal. Seepage of potentially infected material to groundwater or water courses must be avoided.

Waste solids from processing plants may pose a risk if they are not stored and disposed of safely. Rodents or birds could become contaminated and act as vectors for disease. The use of fish heads as bait, in creels for example, must be avoided. Due to the risk of viscera and other solid waste from processing operations being contaminated with IPN virus, the use of such material in agricultural fertiliser is not recommended without prior treatment to render the material safe. Current methods of ensiling do not inactivate IPN virus (see 5.3.2.1). Therefore, ensiled viscera from processing plants should still be regarded as high risk waste with respect to IPN until further treatment by heating.

Eviscerated fish and raw primary processed products, such as fillets, are generally deemed to pose a lower risk of transfer of disease than whole fish. However, melt water from ice used to cool fish products and washing water may pose a risk.

5.3.6.2 *Risk reduction measures*

Whenever possible, harvesting operations should be carried out in good weather conditions. Killing tables should be equipped with sides high enough to prevent escapes or have a net positioned to capture any escaped fish. A tarpaulin under the killing table will contain blood spillage and splashing.

Wellboats, particularly the wells and fish pumping equipment, should be disinfected after transporting fish to a processing plant and especially between shuttle runs to different fish farm sites. If live fish are transported to a processing plant the wellboat should operate with valves closed within a 5 km radius of the plant. Other vessels or vehicles used to transport slaughtered fish must be disinfected before leaving a processing plant. Pick-ups of harvested fish from more than one fish farm site on route to a processing plant should be avoided. Plastic pallets are preferred since wooden pallets are difficult to disinfect.

Spillage of fish and blood and ice water during transport of slaughtered fish should be minimised by ensuring harvest bins are not over filled. Harvest bin liners should be used (although it should be noted that there are disposal problems associated with the use of bin liners). Harvest bins should have close fitting lids secured by ties. Vehicles used to transport fish to processing plants should be equipped with disinfectant and the driver should be aware of the operating procedures required in the event of a spillage. Spillage at a processing plant should be dealt with promptly. Any fish should be cleared away and disinfectant applied. Standing surfaces should be of a non-porous material, rather than tarmac, to prevent seepage.

If possible, there should be separate entrances and exits and parking areas for staff and visitors cars and vehicles used to transport fish or fish products. Staff should be educated so that they understand the risks. Protective clothing, boots, etc. should not leave the processing plant except under containment on route to the laundry for example. Access to 'dirty' areas should be restricted. A high standard of hygiene should be in operation at processing plants at all times.

All blood water effluent from processing plants should be subject to a disinfectant treatment before disposal. Ideally, processing plants should be located at least five kilometres or one tidal excursion, whichever is greater, away from fish farms, since disinfection does not guarantee elimination of the pathogen. Filtration followed by sodium hypochlorite treatment is an effective means of disinfecting blood water, as is ozonation. However, discharge of hypochlorite into the marine environment is not generally approved by the Scottish Environment Protection Agency (SEPA). UV is effective if the blood water is sufficiently diluted but relatively high doses are required to inactivate IPN virus compared to other viruses such as ISA virus.

Viscera, heads and frames must be stored in secure containers to prevent access by rodents and birds. Since frames and viscera from IPN-infected fish farms cannot be distinguished from material coming from non-infected farms, all processing waste should be treated as high risk and should be disposed of in an approved manner, as specified in EC Regulation 1774/2002 and any subsequent legislation. Vehicles and equipment contaminated with melt water from ice used to cool fish products should be disinfected in an appropriate manner to avoid the spread of infection.

5.3.6.3 *Recommendations*

Continued research into safe, cost-effective methods of disinfection of blood water and solid waste from processing plants is required.

Killing tables should be equipped with sides high enough to prevent escapes or have a net positioned to capture any escaped fish. A tarpaulin

under the killing table will contain blood spillage. Whenever possible, harvesting operations should be carried out in good weather conditions.

Wellboats transporting live fish to a processing plant should operate with closed valves when operating within a 5 km range of any fish farm.

Off-loading bays at processing plants must be equipped with a waterproof apron, draining to a collection point and should be surrounded by a bund or similar structure.

Drainage from 'dirty' areas must feed into a disinfection facility.

A disinfectant spray or wheel bath must be available to treat vehicles leaving a processing plant.

Wellboats or other vessels should be disinfected after visiting a processing plant, particularly between shuttle runs to different fish farm sites.

Full protective clothing must be provided for staff and should be kept on site, except for laundering in which case it must be properly contained for transport. Rubber overalls must be disinfected in a soak bath.

Plastic pallets should be used where possible and these should be disinfected before leaving the processing plant. Wooden pallets should be for 'single use' only.

Harvest bins must be cleaned and disinfected before leaving the processing plant. Clean bins must be stored in a specified area away from dirty areas.

Access to dirty areas should be restricted.

Processing area surfaces should be waterproof and amenable to disinfection. All drainage from these areas must feed into a disinfection facility.

All liquid effluent from processing operations must be disinfected before disposal or discharge.

All viscera and other solid waste must be treated in an appropriate manner to prevent the spread of disease, meeting in all circumstances the requirements of EC Regulation 1774/2002 and any subsequent legislation.

5.3.7 Divers

5.3.7.1 *Risk Factors*

Divers are potential mechanical vectors of IPN virus if they move their gear between farms. Dive suits and other diving gear may become contaminated by direct contact with fish or from other potential sources of infection such as sea water in the vicinity of infected fish.

5.3.7.2 *Risk Reduction Measures*

The *Field Guide to Disinfection with Regard to ISA Virus* [22] describes disinfection procedures for diving equipment specifically in relation to ISA virus. In the absence of a published guide relating to disinfection with regard to IPN virus, the following risk reduction measures may be used:

Remove organic material from dive suits and other diving equipment.

Disinfect suits and other diving equipment by immersing in fresh water containing iodophore (minimum 100mg/l free iodine) for 20 minutes. Rinse with clean fresh water and dry thoroughly.

Hypochlorite is an effective disinfectant against IPN virus but its discharge into the marine environment is not generally approved by SEPA.

This specific disinfection routine for divers should be documented and audited by fish farm staff on each occasion that divers are employed.

5.3.7.3 *Recommendations*

Divers should use site-specific gear where possible. If the movement of diving gear is unavoidable it should be thoroughly cleaned and disinfected between operations on different farms.

The Field Guide to Disinfection with Regard to ISA Virus should be re-written to include procedures for effective IPN virus disinfection.

5.3.8 Fish farm personnel and visitors

5.3.8.1 *Risk Factors*

Fish farm staff and visitors can carry pathogens inadvertently between farms on their clothes and footwear.

5.3.8.2 *Risk Reduction Measures*

Ideally, staff should not operate on more than one site. Where this is not possible movements of staff between farms should be minimised. Enquiries should be made as to the recent activities of prospective visitors to determine the level of risk associated with allowing them on site.

Strict hygiene and disinfection regimes should be followed at all times. Footbaths of fresh disinfectant should be available at the entrances to and exits from farms.

Site-specific protective clothing and boots should be available.

5.3.8.3 *Recommendations*

Access to farms by visitors should be minimised where possible. When access is necessary, site-specific protective clothing and boots should be available for use by farm visitors.

Research is required to identify alternative, safe disinfectants active against IPN virus in the presence of organic matter.

5.3.9 Wild fish

5.3.9.1 *Risk Factors*

IPN virus has been isolated from numerous freshwater and marine fish species.[44] Although many isolations have been from farmed fish, or from wild fish in the vicinity of infected farms, evidence of IPN virus infection has also been detected in wild salmonid fish where there was no known contact with hatchery-reared fish (FRS, unpublished data). However, the level of virus shedding from wild salmonid fish is unknown.

Serogroup B birnavirus has been isolated from a range of wild marine fish species in the North Sea, and it has been suggested that the virus may be enzootic in flatfish in Danish coastal waters.[50] Although no similar study of birnavirus prevalence and distribution in wild marine fish has yet been conducted in Scottish coastal waters, IPN virus has been isolated from saithe (*Pollachius virens*) and pollack (*Pollachius pollachius*) in the vicinity of a marine salmon farm in Shetland (FRS, unpublished data).

A study was carried out on the distribution and prevalence of IPN virus in wild fish, principally mature brown trout, in Loch Awe.[34] After an IPN outbreak at a Loch Awe rainbow trout farm in 1971, further epizootics occurred from 1972-75. In the nearby loch, IPN virus was detected in salmonid and non-salmonid fish but the low prevalence (range 0.2-2.5% for both) and the absence of detection after 1977 indicated that the infection was not enzootic in the wild fish in the loch. IPN virus was, therefore, not self-sustaining as a natural infection in the wild fishery in the absence of the source of virus from the rainbow trout farm.

5.3.9.2 *Risk Reduction Measures*

Where possible, water supplies should be disinfected to reduce pathogen loading.

Where possible, wild fish should be excluded from the farm environment.

5.3.9.3 *Recommendations*

Where practicable, water intakes to and effluents from land-based farms should be disinfected.

Measures should always be taken at water intakes to minimise the risk of ingress of wild fish. Twin sets of screens should be used so that wild fish cannot get access to the farm when the screens are being cleaned.

Research is required to establish the prevalence and distribution of IPN virus in wild fish in Scottish waters.

5.3.10 Cohabiting species

5.3.10.1 *Risk Factors*

Saithe or other species may enter and become established in salmon cages. In addition, goldsinny wrasse (*Ctenolabrus rupestris*) and other wrasse species may be deliberately introduced to salmon cages with the aim of controlling sea lice.

Saithe and goldsinny wrasse have been shown to be susceptible to infection with IPN virus.[24] However, it is not known whether other non-salmonid cohabiting fish replicate IPN virus.

As farmers experiment with co-cultivation of different species there is a potential risk of transfer of infection between species. It should be noted that another sub group of the AHJWG, the New Species Interactions Sub Group, is working in this area.

5.3.10.2 *Risk Reduction Measures*

Where wild wrasse are to be introduced into salmon cages for the purposes of sea lice control, such fish should be locally caught if possible.

5.3.10.3 *Recommendations*

Where wrasse are to be introduced into salmon cages for the purposes of sea lice control, such fish should be locally caught if possible.

An epizootiological study of IPN virus in cohabiting species is required.

5.3.11 Sea lice and other ectoparasites

5.3.11.1 *Risk Factors*

There are no reports in the literature on the potential of sea lice or other ectoparasites to transmit IPN. Both *Lepeophtheirus salmonis* and *Caligus elongatus* have, however, been shown to transmit infectious salmon anaemia.[38,37]

IPN virus has been found in blood leucocytes.[60] Sea lice ingest blood cells as they graze on the fish skin, hence they could take up IPN virus. IPN virus could pass through the sea louse digestive system and be excreted in the waste products, or it could be passed to other fish *via* food debris on the mouth parts. The tendency of pre-adult and adult sea lice to move between hosts could result in the transmission of IPN between fish farm sites.

5.3.11.2 *Risk Reduction Measures*

Regular sea lice inspections should be conducted to select the optimal timing for treatments and to verify efficacy. The use of effective medicines is important since resistance to some, notably hydrogen peroxide, has been found.

Procedures recommended in the National Treatment Strategy for the Control of Sea Lice (SSGA, 1998) and by Rae [42] should be adopted.

5.3.11.3 *Recommendations*

Experimental work is required to determine whether sea lice can transmit IPN virus from infected fish to naïve fish.

Farmers should commit to effective control of sea lice and should adopt procedures recommended by the National Treatment Strategy for the Control of Sea Lice (SSGA, 1998) and by Rae. [42]

Pharmaceutical companies and licensing authorities should endeavour to increase the availability of safe, efficacious and environmentally acceptable sea lice medicines.

5.3.12 Shellfish, plankton and crustaceans

5.3.12.1 *Risk Factors*

The aquatic birnaviruses isolated from shellfish, plankton and crustaceans have been termed "IPN virus-like". [31] Isolated IPN virus from the faeces and pseudofaeces of scallops (*Pecten maximus*) and from prawns (*Pandalus borealis* and *Palaemon elegans*) which grazed on dead IPN virus-contaminated scallops. However, it is not known whether such hosts can actually replicate IPN virus or whether they act as passive carriers. The risks to marine cage sites may be influenced by the presence or abundance of carrier shellfish in the vicinity.

The ability of planktonic organisms, such as rotifers (*Brachionus plicatilis*), to harbour IPN-like virus may be significant as these organisms are widely used in the culture of juvenile pre-weaned marine species some of which (for example, halibut and cod) are susceptible to IPN virus.[32] However, it is not known if rotifers can carry IPN virus virulent to halibut. It was concluded that the rotifer birnavirus showed unique biophysical and biochemical characteristics within the birnavirus group. [12]

The literature suggests that IPNV enjoys a diverse range of hosts especially amongst crustaceans and molluscs.

Larger marine crustacean hosts include the shore crab (*Carcinus maenas*) and harbour crab (*Macropipus depurator)* [27] as well as the freshwater crayfish (*Astacus astacus*).[25]

Shellfish hosts include the American oyster (*Crassostrea gigas*), the European oyster (*Ostrea edulis*), the mussel (*Mytilus edulis*) [27] and Tellina, (*Tellina tenuis).*[26]

Clearly, with such a wide range of potential host species, the capacity for IPN to be sustained in both the marine and freshwater environments is considerable. Studies on

the freshwater crayfish have shown that not only can the virus still be isolated from this animal one year after the original infection, but that IPN virus is excreted into the water continuously.[25] This virus shedding was demonstrated to cause subsequent infection in cohabiting rainbow trout fry.

5.3.12.2 *Risk Reduction Measures*

The siting of shellfish farms and finfish farms may have to be reviewed if it is shown that the former are significant reservoirs of IPN virus.

Fouling on fish farm cages should be regularly removed as a precautionary measure.

5.3.12.3 *Recommendations*

Research is required to ascertain if wild crustaceans and molluscs acquire IPN virus from infected salmon populations.

Research is required to ascertain if and for how long IPN virus replicates in molluscs and crustaceans.

Research is required to ascertain if molluscs and crustaceans can transmit IPN virus to salmon and other fish species.

Fouling on fish farm cages should be regularly removed as a precautionary measure.

5.3.13 Birds

5.3.13.1 *Risk Factors*

Birds are regular and persistent visitors to aquatic installations and there have been some investigations into their role as vectors of aquatic pathogens. It has been shown, for example, that piscivorous birds including corvids, herons and kingfishers predating on rainbow trout fry infected with IPN virus can excrete live IPN virus in their faeces.[30] These authors suggest that this represents a significant risk of virus transmission. IPN virus has also been isolated from blackheaded gulls (*Larus ridibundus*).[20]

All birds that frequent fish farms should be considered as potential risks for the transfer of disease.

5.3.13.2 *Risk reduction measures*

Aquaculture installations can be made less attractive to birds by eliminating access to mortalities and feed.

Freshwater installations can be netted to deny access to larger birds such as herons.

The selective use of scarers and decoys plus occasional permitted shooting can contribute to discouraging birds from visiting fish farms. None of these, however, are successful when used on their own.

5.3.13.3 *Recommendations*

Access to mortalities and fish food should be prevented as these are attractive to birds.

Effective measures should be in place to minimise access by birds to freshwater and marine farms.

Research to improve knowledge of the prevalence of IPN virus among seabirds where IPN is enzootic would help in the understanding of the epizootiology of the disease.

5.3.14 Mammals

5.3.14.1 *Risk Factors*

Predators and scavengers that frequent fish farms have potential to act as vectors of infection. IPN virus has been shown to survive passage through the gastro-intestinal tract of mink fed IPN virus-infected fish.[58] Smail *et al* [53]passaged IPN virus through the gut of cows in an attempt to assess the suitability of IPN virus-containing fish silage as an animal feed. IPN virus was detectable in cattle faeces up to three days after feeding. This demonstrates the ability of the virus to survive passage through the mammalian gut.

Apart from this work, there is limited detailed published material regarding mammals as vectors of IPN virus. Other mammals that interact with fish farms include rodents, mink, otters, seals and cetaceans. Humans should be regarded as mechanical vectors but are unlikely to be true carriers.

5.3.14.2 *Risk Reduction Measures*

Good site hygiene should reduce incentives for vermin to visit farms.

The use of predator nets and permitted anti-predator devices should be encouraged.

5.3.14.3 *Recommendations*

Knowledge of the prevalence of IPN virus among mammals where IPN is enzootic would help in the understanding of the epizootiology of the disease.

Vermin should be discouraged from fish farms by preventing access to fish food and maintaining good hygiene procedures.

Although our knowledge of the propensity of mammals to act as vectors of IPN virus is limited, a precautionary approach is recommended. Thus, permitted anti-predator methods should be used where possible.

5.3.15 Feed

5.3.15.1 *Risk Factors*

Feed is a potential source of infection if it is made using unprocessed fish or ingredients that carry the pathogen or if it becomes contaminated.

Dry feed is manufactured in the UK to specifications defined by the United Kingdom Agricultural Supply Trade Association (UKASTA), which has developed the UKASTA Feed Assurance Scheme (UFAS). UFAS is a strict code of practice for feed manufacturers to produce and supply safe feed to livestock producers. Parallel schemes operate in other countries.

5.3.15.2 *Risk Reduction Measures*

The use of unpasteurised fish as feed should be avoided.

5.3.15.3 *Recommendations*

Unpasteurised fish should not be used as feed.

CHAPTER 6: DETECTION AND DIAGNOSIS OF INFECTIOUS PANCREATIC NECROSIS (IPN)

6.1 Introduction

This chapter focuses on the main diagnostic techniques for IPN in Atlantic salmon. Clinical signs, histopathology and field tests all have a role to play but the gold standard in diagnosis for IPN is virus isolation using cell culture with serological or genetic identification of virus.

6.2 Clinical Disease

In Atlantic salmon fry, the clinical signs of IPN include sudden loss of appetite, trailing faecal casts, weak swimming and drifting. In parr, the clinical signs are similar, with dark skin pigmentation and sudden loss of appetite.

In Atlantic salmon post-smolts, the clinical signs are variable and must not be confused with a generalised failed smolt syndrome.[59] IPN can present in poorly adapted smolts[51,54] and also in fast-growing thriving smolts (C. Mitchell, unpublished observation). Very high titres of virus (eg 10^9 $TCID_{50}$/g tissue) and gross pancreas pathology are associated with all such fish.

6.3 Histopathology and Immunohistochemistry

IPN can be presumptively diagnosed by histopathology and diagnosed with certainty by immunohistochemistry.

IPN virus causes very specific lesions of focal necrosis in the pancreas and occasionally the liver and kidney. The pancreas lesions can be gross in extreme cases and more than 50% of the exocrine pancreas can be affected.[54] There is often a marked sloughing of the intestinal epithelium of the gut with a typical catarrhal exudate filling the gut lumen.

Immunostaining of histological sections using polyclonal antibody as first described by Evansen and Rimstad[21] is a valuable confirmatory test for IPN. It can be used retrospectively on archived tissue blocks. This technique has also found use in detection of IPN virus in halibut yolk-sac larvae.[6] It is important that the procedure is well validated at the outset and that positive and negative control sections are run in every test.

6.4 Field Kits

A popular IPN test kit in Norway and Scotland is the co-agglutination kit. This is based on the use of antibody-coated *Staphylococcus aureus* to detect IPN virus in Atlantic salmon tissues.[61] The test has a detection limit of 10^5 infectious units per ml of tissue homogenate. It is useful in the presumptive diagnosis of IPN in clinically diseased fish but is not of practical use for detection of carriers where virus levels may be much lower.

Field ELISA kits have also been developed but have largely remained in the research sector. The sensitivity of these systems varies from 10^3-10^6 pfu/ml.[15,46]

6.5 Virus Isolation Using Cell Culture

IPN virus can be cultured in a variety of established fish cell lines eg BF-2, CHSE-214 and RTG-2, producing fast cytopathic effects often within 48 hours. High titres are produced after 4-7 days incubation at 15°C and virus identification tests can then follow. The sensitivity of the test is approximately 100 pfu/g of tissue.

6.6 Virus Identification Using Serology

The methods of choice in most National Reference Laboratories of the EU member states are ELISA, neutralisation and immunofluorescence.[39]

The most common ELISA system uses a polyclonal antibody to capture the virus. The captured virus is recognised by a secondary antibody from a different species or a monoclonal antibody. This in turn is recognised by a third antibody conjugated to an enzyme which produces a colour reaction.

Identification of the virus by neutralisation also requires the use of specific antibodies. There are two methods of performing the neutralisation test. Varying virus concentration against a constant amount of antiserum is the quickest to carry out and gives a virus identification result in four days. Varying the antiserum concentration against a constant amount of virus is slower and requires the virus titre to be previously determined.

The immunofluorescence test is performed by fixing virus-infected cells and incubating them with a polyclonal or monoclonal antibody to IPN virus. A secondary antibody conjugated with a dye, which fluoresces at a specific wavelength of visible light, is then used to visualise the viral antigen.

6.7 Virus Identification Using Molecular Genetic Methods

IPN virus can be identified by the recognition of viral-specific genetic sequences. Polymerase chain reaction (PCR) assays have been reported for cultured virus and also for organ extracts. Rimstad *et al* [45] described a nested PCR for the cultured IPN virus isolates Sp, Ab and VR-299 with a detection limit of 1ng of genomic RNA. In addition, Blake *et al* [7] reported a reverse transcriptase PCR (RT-PCR) for detection of genomic RNA in kidney and spleen samples from carrier fish with a sensitivity equal to virus isolation by cell culture.

6.8 Non-Lethal Testing Methods

Available methods comprise assay of serum for virus-specific antibodies and virus isolation from blood leucocytes. In addition a novel antigen-staining method is reported for the detection of IPN virus on milt.

In both rainbow trout and Atlantic salmon, antibody detection by either ELISA or neutralization will indicate previous infection at the population or individual level but will not indicate current virus status at the time of sampling. In a study of experimentally infected and farm populations of rainbow trout it was shown[14] that some 30% of fish were virus-positive by culture in contrast to 71% antibody positive by ELISA or serum neutralisation. In two populations of persistently infected Atlantic salmon post-smolts,

antibody neutralising titre and virus titre showed no positive or negative correlation.[55] Whilst antibody assay by ELISA in particular can be a useful serological screening method for populations, it cannot replace sampling and isolation procedures for infectious virus where the objective is to determine the current virus status of the individual or population.

In both rainbow trout and Atlantic salmon, leucocytes can be co-cultured with fish cell cultures to isolate IPN virus.[66,48] Furthermore, in Atlantic salmon the use of the mitogen phytohaemagglutinin to stimulate cell division in cell suspensions from sampled tissues enhanced the isolation rate from persistently infected fish.[28]

A novel technique for the detection of IPN virus on milt has been described in rainbow trout.[47] Cells were stained using a polyclonal antibody and FITC-labelled conjugate and the fluorescent signal detected in a cell analyser. This direct virus-labelling cell analysis correlated well with virus isolation, which takes several weeks, and gave the great advantage of speed of detection.

The techniques of co-cultivation, mitogen stimulation and direct virus-labelling cell analysis are not yet validated or sufficiently developed to be cost effective tools for routine virus screening and diagnosis.

6.9 Summary

When Atlantic salmon fry or post-smolts present with clinical signs of IPN, the virus can be detected by the use of the co-agglutination field kit. IPN can also be presumptively diagnosed by histopathology and confirmed by immunohistochemistry. The gold standard in diagnosis is virus isolation by cell culture and virus identification by serology or genetic analysis (PCR). Non-lethal testing methods exist but none of these have proved suitable for reliable detection and diagnosis of infection in individual animals.

6.10 Recommendation

It is recommended that research is undertaken to optimise existing techniques and to develop and validate alternative techniques, especially non-destructive testing methods, for detection and diagnosis of IPN virus.

It is recommended that research is undertaken to establish if strains of IPN virus differ in virulence, and if so to establish diagnostic methods capable of differentiating between virulent and avirulent strains in order to facilitate control of virulent strains.

CHAPTER 7: CURRENT CONTROLS FOR IPN

7.1 Notification of IPN

IPN is categorised as a List III disease under Annex A of EU Council Directive 91/67/EEC (as Amended). List III diseases are present within the EU and are regulated under national control programmes within each Member State. In Great Britain IPN is a notifiable disease under The Diseases of Fish Acts 1937 and 1983. Any person who has the right to take fish from inland waters, or has care of inland waters, must notify the Scottish Ministers in writing if they suspect the waters are infected with IPN. Similarly, any person who owns or possesses a fish farm cage in marine waters, or is employed for the purposes of having care of a fish farm cage in marine waters, must notify the Scottish Ministers in writing if they suspect the waters are infected with IPN. Fisheries Research Services (FRS) Fish Health Inspectorate act on behalf of the Scottish Ministers and can be contacted at the following address:

FRS Marine Laboratory
PO Box 101
375 Victoria Road
Aberdeen AB11 9DB

Tel: 01224 295525 Fax: 01224 295620
e-mail: fishhealth@marlab.ac.uk.

In 1995, a review of the controls for IPN concluded that trout and other freshwater fish, except for salmon, should be exempt from official controls for IPN. The reasons given were:

- IPN was widespread in trout farms. At least 40% of trout farms, the vast majority of which were located in England and Wales, compared with 22% of salmon farms in Scotland, were infected.
- IPN was considered to have a relatively low economic impact in trout since outbreaks were sporadic and not as systematic and severe as in salmon.
- The British Trout Association (BTA) were in favour of relaxation of the controls.
- The Scottish Salmon Growers Association (SSGA) took the view that controls for IPN should be maintained in salmon.

The then Minister for Agriculture and Fisheries approved the recommendations of the review. Thus, since 1995, controls for IPN have not been enforced in trout, and there is no requirement to notify the FRS Fish Health Inspectorate (FHI) if IPN is suspected on a trout farm, unless there are other species of fish on the same farm. If IPN is suspected on a farm with any other species of fish, including marine finfish, FRS FHI must be informed.

7.2 Inspection and Testing for IPN

Regular inspections for IPN are carried out by FRS FHI. Samples will be taken if the Inspectorate is notified that the waters are suspected of being infected with IPN, in order to confirm or rule out the presence of infection. Routine samples are only taken from salmon farms. Where possible, to minimise the number of fish sampled from a farm, testing is carried out in conjunction with the sampling programme for the purpose of

maintaining the status of Great Britain as an approved zone within the European Union for the List II diseases, VHS and IHN. All salmon farms holding broodstock are inspected twice each year and 30 fish are sampled once a year.

Freshwater salmon farms not holding broodstock are inspected and sampled once a year. The sample size is 30 fish because, statistically, that gives a 95% confidence level that a carrier will be sampled if the disease is present in 10% of the population, regardless of the size of the farm. Marine salmon farms not holding brood stock are inspected once a year and 30 fish are sampled every second year.

The use of salmon brood fish infected with IPN is prohibited. Where IPN-infected Atlantic salmon brood stock populations are subject to official controls, the parent fish must be tested for IPN and the gametes or eggs from fish that test positive for IPN must be destroyed. Since trout are exempt from official controls for IPN the brood fish are not tested.

Marine finfish species, such as halibut and cod, have not been subject to the same compulsory broodstock testing regime as salmon since testing for IPN is lethal to the fish. These marine species have only recently begun to be cultured and the brood fish are extremely valuable, particularly since they may spawn many times throughout their lives.

7.3 Thirty Day Notices and Designated Area Orders

If there are reasonable grounds for suspecting that any inland or marine waters are infected, or may become infected, with IPN, the Ministers may designate those waters in order to prevent the spread of the disease. A Thirty Day Notice (TDN) or a Designated Area Order (DAO) may be served on any person who is the occupier of inland waters or any person who carries on the business of fish farming in marine waters situated in the designated area.

A TDN is a temporary notice that may be served as a precautionary measure, while an investigation is conducted to confirm or rule out the presence of IPN or any other notifiable disease. No live fish, or eggs of fish, may be taken into or out of a fish farm and, in addition, no foodstuff for fish may be taken out of a fish farm that is situated in the waters specified in a TDN, without the permission of the Ministers. Applications for permission for movements should be sent to FRS FHI at least 14 days in advance.

A TDN lapses after 30 days but a second TDN may be served when it is not possible to confirm or rule out the presence of IPN within 30 days or, when action to remove infected stocks and disinfect the farm has not yet been completed. A second TDN extends the period under which the movement controls are effective from 30 days to 60 days from the date on which the first TDN was served. The details of a TDN are not published.

A DAO may be made when the presence of IPN has been confirmed and no action has been taken to remove the stocks and disinfect the farm or, when it is not possible to confirm or rule out the presence of IPN within the period specified in a TDN. DAOs are published in the Edinburgh Gazette.

DAOs give the Ministers powers to:

- prohibit the movement of live fish or eggs, or foodstuff for fish, into or out of a farm, without the permission of the Ministers;
- serve notices requiring the removal of dead and dying fish, and the disposal of such fish by a specified method;
- serve notices (in the case of ISA, VHS or IHN) requiring action to eradicate the disease, including the slaughter of all the fish on a farm.

Trout are exempt from official controls for IPN, but movement restrictions are applied to farms stocked with any other species of fish where the waters are suspected of being infected with IPN. In such cases movement restrictions would not apply to any trout held on the farm.

7.4 Revocation of Thirty Day Notices and Designated Area Orders

The restrictions imposed by a TDN will normally be lifted immediately if tests undertaken by FRS FHI are negative, or if all the fish are removed and the farm is disinfected under the supervision of FRS FHI. A second TDN may be served to allow time to complete the clearance and disinfection work.

The timing of the revocation of a DAO will depend upon the action taken to remove the infection. A DAO will be revoked if all the fish are removed and the farm is disinfected under the supervision of the FHI. Where only the infected stocks are removed but the farm still has fish on site, movement restrictions may be lifted following a programme of testing carried out by the FHI. Normally, this will entail two samples of 150 fish, taken at least three months apart. If the results of both samples are negative, a DAO revocation Order will be made. DAO revocation Orders are published in the Edinburgh Gazette.

If no action is taken to remove the infected stocks, other than the removal of dead or dying fish, a DAO will not be revoked until negative results have been obtained from at least two samples of 150 fish per year over a two year period, starting from one year after the last positive identification of IPN virus.

Where IPN-infected Atlantic salmon brood fish are subject to a TDN or DAO, providing the egg batches have been held in isolation, the movement restrictions will be revoked when all the test results are complete, infected parent fish, their eggs and gametes have been destroyed and the tanks, or other facilities that held the infected stock, have been disinfected.

7.5 Movements of Live Fish on or Off a Farm Subject to a Thirty Day Notice or a Designated Area Order

7.5.1 Movements of brood fish from sea water to fresh water

Where brood fish are subject to movement restrictions due to IPN, granting permission will only be considered for the transfer of apparently healthy populations with no recent history of clinical disease. Movement restrictions, usually in the form of a TDN, will be

applied to the receiving freshwater site or hatchery. Written confirmation from the receiving fish farmer that he is aware of the disease status of the farm of origin of the fish will be required and he may have to comply with specific conditions. Where approval is granted, conditions may be applied with regard to transport arrangements. For example, there may be a requirement to prohibit any discharge of water *en route* and to disinfect vehicles or equipment after use.

7.5.2 Movements of smolts from fresh water to sea water

Movements of smolts from IPN-infected hatcheries or freshwater lochs to marine farms will not normally be permitted except when the receiving site is itself subject to a DAO, or where the receiving site is located in the vicinity of other farms subject to a DAO for IPN such that the movement will not affect the health status of the receiving waters. Permission may also be granted where there is deemed to be no significant risk to other farmed stocks or wild fish populations within the vicinity of the receiving site. Granting permission will only be considered for the transfer of apparently health populations with no recent history of clinical disease.

Written confirmation from the receiving fish farmer that he is aware of the disease status of the farm of origin of the fish will be required and he may have to comply with specific conditions. Where approval is granted, conditions may be applied with regard to transport arrangements. For example, there may be a requirement to prohibit any discharge of water *en route* and to disinfect vehicles or equipment after use.

7.5.3 Movements of growers between seawater farms

Due to the high risk of disease transfer associated with sea water to sea water fish movements there is a general presumption against them. Where the receiving farm or neighbouring farms are subject to a DAO for IPN movements may be permitted. Permission may also be granted where there is deemed to be no significant risk to other farmed stocks or wild fish populations within the vicinity of the receiving site. The guidelines laid down in *A Code of Practice to avoid and Minimise the Impact of Infectious Salmon Anaemia* [3] will be considered when applications for permission to carry out sea water to sea water fish movements are assessed. Granting permission will only be considered for the transfer of apparently healthy populations with no recent history of clinical disease.

Written confirmation from the receiving fish farmer that he is aware of the disease status of the farm of origin of the fish will be required and he may have to comply with specific conditions. Where approval is granted, conditions may be applied with regard to transport arrangements. For example, if a wellboat is used it may have to operate with closed valves and be disinfected afterwards.

7.5.4 Movements of fish for harvest

Fish slaughtered for harvest are not subject to the restrictions posed by a DAO for IPN so they can be freely moved off a farm. All live fish movements, however, require written permission, even if the fish are being transported directly to a processing plant. Permission may be granted for multiple return journeys taking place over a period of time and may be subject to specified conditions. For example, wellboats transporting live fish should operate with the valves closed within 5 km of another fish farm and the processing plant may be required to disinfect the blood water effluent.

7.5.5 Frequency of movements of fish subject to movement restrictions

Every application for permission to move fish is subject to a risk assessment conducted by FRS FHI. The Inspectorate provides a recommendation to the Scottish Executive Environment and Rural Affairs Department (SEERAD) as to whether the movement should be approved or refused based on the risk assessment. Where proposals present an unacceptable disease risk, SEERAD will consider alternative solutions. Over the period from 30th September 2000 to 1st October 2001, the FHI received 195 applications for permission to move fish (not all due to IPN). Permission was refused in 7% of cases.

CHAPTER 8: VACCINATION AGAINST INFECTIOUS PANCREATIC NECROSIS

8.1 Introduction

Several vaccines against Infectious pancreatic necrosis are currently undergoing field trials in marine sites in Scotland, Norway and Chile. However, the efficacy of these vaccines in protecting against mortality in post-smolts is still uncertain because of the lack of reliable lethal challenge models. Until the latter become available for experimental testing of the vaccines, or the field trials produce compelling evidence for their efficacy, it is not possible to know how effective they are.

Vaccines currently under field trials include killed whole virus vaccines, and recombinant vaccines produced in *Escherichia coli* or yeast. They are incorporated into existing commercial vaccines against a variety of bacterial diseases and delivered by injection some months before smolts are transferred to sea water.

8.2 Potential Uses of IPN Vaccines

8.2.1 Reduction of mortalities

In salmonids, mortalities occur from IPN in first-feeding fry and in Atlantic salmon post-smolts during the first three months following transfer to sea. Exposure of fish to IPN virus outside these periods of susceptibility does not appear to result in any mortality but many of the fish become asymptomatic carriers of the virus. It is not known if such carriers shed the virus and pose a risk to susceptible fish. Atlantic halibut fry are very susceptible to IPN with high mortality occurring. Older fish are not susceptible to the disease but it is not clear if they can carry the virus. Injection vaccination is impractical for first-feeding fry so its only potential use is in Atlantic salmon post-smolts.

8.2.2 Reduce vertical transmission from carrier broodstock

In the UK, salmon broodstock from known carrier populations are tested for IPN virus by culture methods. This testing is expensive and results in the destruction of eggs from infected parents. If vaccines could prevent broodstock from becoming IPN virus carriers, large savings could be made. A similar situation probably applies to halibut brood fish. It should be noted, however, that although vertical transmission of IPN virus has been shown to occur in a number of salmonid species (*See* Chapter 4), it is not known whether it occurs in marine finfish species.

There is some evidence that vaccinated salmon pre-smolts do not become carriers of the virus following experimental challenge 10 weeks after.[23] Little is known about the carrier state of IPN virus and much work is needed to establish how effective vaccination is in eliminating or preventing the carrier state. Vaccination is never 100% effective and there will always be a few individual fish which do not respond to the vaccine. Such fish may still become carriers and while vaccination may markedly reduce the number of carrier broodstock it is unlikely to eliminate them completely. Thus the need to test broodstock may not be eliminated but the wastage of eggs from carrier parents would be greatly reduced.

8.2.3 Vaccination and legislative control

There are currently no fully licensed vaccines for IPN. However there is strong interest in developing efficacious vaccines, particularly for dealing with outbreaks of IPN in post-smolts. Animal Test Certificates (ATCs) have been granted by the Veterinary Medicines Directorate (VMD) for field trials of two vaccines although the efficacy of these is not yet proven. There is a general presumption in the animal health field that vaccination and statutory regulation (through movement restriction and/or stamping out) are mutually exclusive control methods. The latter relies on testing to identify infected populations and a potential consequence of vaccination is that the detection of infected individuals might be compromised during routine screening. Vaccination is rarely 100% effective and, whilst it may be sufficient to raise the level of immunity in a population to prevent the propagation of an epidemic, a small proportion of non-vaccinated or non-responding fish in the vaccinated population, might still remain infectious. For IPN the biggest threat this will pose is to the control regime applied to prevent vertical transmission and the occurrence of IPN in fresh water. In such cases infected populations might not be detected and could potentially be used as brood stock, or moved to sites with brood stock, with the potential for vertical transmission of infection. A solution to this problem would be to prevent vaccinated fish from being used as brood stock or to 100% test all brood stock from a vaccinated population to ensure no infected individuals are used for the production of gametes.

To allow the development and testing of vaccines a temporary solution has been arrived at with the VMD to deal with this potential difficulty. Thus, a condition of the ATC is that vaccinated fish are only permitted to be transferred to marine sites with an existing restriction (DAO) for IPN. If any fish held on those sites are used as brood stock they will be subject to the standard requirement for 100% testing for IPN with destruction of ova from infected parents.

Effective vaccination may of course reduce infection pressure and in the longer-term result in IPN becoming much more rare which would also reduce the potential for vertical transmission.

8.2.4 Reduction of infectious pressure

Potentially, vaccination could have the most far-reaching beneficial effects, but it is difficult to predict. If vaccination prevents mortality in post-smolts and assuming that moribund and dead fish shed more virus than asymptomatic carriers, there will be a reduction in the amount of virus being shed into the marine environment. It is not known how much virus, if any, is shed by carriers, but the viral titres in carrier fish are much lower than in fish suffering clinical disease (*See* Chapter 5). Furthermore, if vaccinated fish are more able to eliminate virus, there will be fewer carriers in the population. With a reduction in the amount of IPN virus being shed from farms, there should be less risk of transmission of the virus to other neighbouring farms and to wild fish. If the presence of IPN virus could be reduced to levels below those required to transmit and infect susceptible fish, improvements in fish health may be expected.

8.3 Conclusions

It is clear that effective vaccines against IPN would be very useful but the efficacy of existing vaccines remains to be proven. Once proven, the effect of vaccines on the carrier state needs to be assessed as well as identifying the risk posed by carriers in relation to vertical and horizontal transmission of the virus. The potential risk that vaccination could compromise statutory disease controls is discussed.

8.4 Recommendations

Effective vaccines against IPN should be developed for use in Atlantic salmon and marine fin fish species.

The effects of vaccines on the carrier state, and the risk posed by carriers in relation to vertical and horizontal transmission of the virus, should be assessed.

CHAPTER 9: RECOMMENDATIONS FOR FUTURE REGULATORY CONTROLS

9.1 Introduction

Under the current regulatory regime the prevalence of IPN has remained relatively low in freshwater salmon farms and relatively high in marine salmon farms for a number of years. However, the prevalence of infection has been increasing in recent years, especially in the Northern and Western Isles. Cases of clinical disease also appear to be increasing both in freshwater and marine sites, and outbreaks of disease on marine salmon farms are no longer confined to Shetland. Disease outbreaks are now also occurring in some marine finfish hatcheries. In short, prevalence of infection is increasing in marine and freshwater farms and all of the evidence also points to an increase in the spread and incidence of clinical disease. Therefore, the current policy has had limited effectiveness in preventing the spread and increase in incidence of the disease or infection.

Three options exist for future control of IPN:

- to relax or remove all statutory controls;
- to tighten statutory controls;
- to consider if there is a requirement to introduce new Codes of Practice for disease control.

In considering these options the working group reviewed the role of the current legislation in the context of disease control. For a fish disease to be considered appropriate for control by the fish health legislation currently operating in Scotland the following criteria should be satisfied:

- the disease should be exotic or have a restricted distribution;
- the disease has potential to make a significant socio-economic impact;
- there is no suitable alternative method of control.

There are additional grounds for control by legislation if the disease has potential for impact on wild populations.

In considering the first criterion for a disease with a widespread distribution the risk of an outbreak arising from farm activities would have to be considerably greater than the risks from other sources of the agent if the disease was to be regulated.

The implications of the different options received careful consideration by the working group and they considered all of these criteria in the context of sea water to sea water (SW-SW), fresh water to fresh water (FW-FW), sea water to fresh water (SW-FW), and fresh water to sea water (FW-SW) transfers of fish for all species.

9.2 Sea Water to Sea Water Movements

9.2.1 Background

Existing controls have had little impact on the prevalence of IPN virus which is now almost ubiquitous in sea water sites for all species, nor have the controls reduced the incidence and impact of clinical disease which is also increasing.

SW-SW movements of live fish are controlled by DAOs in some 70% of seawater sites, and such movements are only permitted if they are consistent with the ISA Code of Practice. Movement of clinically diseased fish is prohibited under statute. If SW-SW movements are deregulated, an alternative mechanism is required to minimise the risk of spread of disease by SW-SW movements. A mandatory or enforceable Code of Practice would achieve this aim.

9.2.2 Recommendations

The current regulatory regime should be maintained as an interim measure. SW-SW movements of live fish should only be permitted in accordance with the ISA Code of Practice.

Codes of Practice should be developed for all industry sectors to minimise the spread of infectious diseases and the likelihood of disease outbreaks.

Government should introduce regulatory measures to ensure the implementation of the Codes of Practice referred to in 9.2.2.2.

Current regulations prohibiting the movement of clinically diseased fish should be maintained.

9.3 Fresh Water to Fresh Water Movements

9.3.1 Background

Salmonid fry are highly susceptible to IPN. The prevalence of infection in freshwater salmon farms has increased during the last six years but remains relatively low compared with the situation in the marine environment. The prevalence of infection in wild salmonid fish is also low. It is believed that statutory controls have contributed to maintaining a low prevalence of infection in fresh water and few outbreaks of disease. It is proposed that current statutory controls should be retained and implemented more rigorously to prevent further spread of the virus and if possible reduce the prevalence and impact in fresh water.

The prevalence of IPN virus in trout farms is not presently known but when last recorded (1995) was of the order of 40%. Despite deregulation of IPN in trout there is no evidence of an impact on other sectors of fish farming or on wild fish.

9.3.2 Recommendations

IPN in fresh water should remain under the current legislative control regime.

A survey should be carried out of the prevalence of IPN virus in farmed and wild fish to inform whether legislative control of IPN should be extended to all sectors in fresh water, including trout and restocking hatcheries.

Current regulations prohibiting the movement of clinically diseased fish should be maintained.

9.4 Sea Water to Fresh Water Movements

9.4.1 Background

There is a perceived risk of IPN virus infection to fish in the fresh water environment because the prevalence of infection in sea water is high and the prevalence in fresh water is still low. There is a risk of horizontal transmission from broodstock moved from sea water to fresh water and held prior to stripping, and IPN disease outbreaks in fry because of the potential for vertical transmission.

9.4.2 Recommendations

Current legislative controls on the movement of live fish from sea water to fresh water should be maintained.

Government should introduce new regulations controlling movement of any fish from sea water to fresh water [for example, between coastal and continental zones].

A Code of Practice should be developed governing sea water to fresh water movements of live fish and the holding and testing of broodstock.

9.5 Fresh Water to Sea Water Movements

9.5.1 Background

The prevalence of IPN virus infection in sea water is high. Therefore there is no advantage in regulating movements of fish from fresh to sea water.

9.5.2 Recommendations

Movements of live fish from IPN virus infected freshwater sites to sea water should be permitted, irrespective of the IPN status of the site in sea water.

Current regulations prohibiting the movement of clinically diseased fish should be maintained.

CHAPTER 10: SUMMARY OF RECOMMENDATIONS

10.1 ***Research should be undertaken to establish conclusively and as a matter of urgency whether vertical transmission of IPN virus occurs in Atlantic salmon. Priorities for research are:***

(a) An expansion of the IPN virus titre/frequency data set for Atlantic salmon broodstock.

(b) Examination of the role of experimentally infected males in vertical transmission.

10.2 ***Research should be undertaken to establish whether vertical transmission of IPN virus occurs in marine finfish species.***

10.3 ***Until the absence of vertical transmission of IPN virus in Atlantic salmon is demonstrated, all Atlantic salmon broodstock should be tested for IPN virus and ova from infected parents destroyed.***

10.4 ***Effective vaccines against IPN should be developed for use in Atlantic salmon and marine fin fish species.***

10.5 ***IPN-resistant strains of Atlantic salmon and other marine fin fish species should be developed.***

10.6 ***The highest standards of husbandry should be ensured especially prior to and when transferring smolts to sea and in the ensuing weeks when they are highly susceptible to disease.***

10.7 ***A risk assessment should be conducted before any movement of live fish takes place, either under voluntary Code of Practice or a regulatory regime.***

10.8 ***All hatcheries and other facilities used in the production of salmonids for restocking and enhancement of fisheries should be registered and subjected to the same control regime as commercial fish farming facilities.***

10.9 ***Further development of systems for monitoring and removing mortalities from fish farm tanks and cages is desirable.***

10.10 ***Alternative methods for the safe, cost-effective disposal of mortalities should be investigated, for example composting or improvements to ensiling.***

10.11 ***Tanks and cages should be inspected daily for evidence of dead or moribund fish.***

10.12 ***Mortalities should be removed from tanks and cages without undue delay and disposed of in accordance with EC Regulation 1774/2002 or any subsequent legislation.***

10.13 ***Where practicable, farms should not share mortality ensiling points. Where farms cannot avoid sharing ensiling points, particular care should be taken to prevent transfer of pathogens between the farms.***

10.14 ***Where practicable, separate hand nets and mortality containers should be available for individual tanks or cages of fish.***

10.15 ***Equipment used to remove or transport dead fish should be disinfected after use.***

10.16 ***Divers should use site-specific gear or disinfect their equipment thoroughly between diving operations on different sites.***

10.17 ***Under an amendment of the Registration of Fish Farming and Shellfish Farming Businesses Order (1985), fish farmers must notify the Scottish Ministers of any escapes or suspected escapes of farmed fish. The guidance provided in*** **"What to do in the event of an escape of fish from a fish farm" should be followed.**
http://www.scotland.gov.uk/library5/environment/escape.pdf

10.18 ***The Code of Practice on the Containment of Farmed Fish, Official Notification Following the Escape of Fish, and Possible Measures to be Employed to Attempt Recapture should be followed.***
http://www.scottishsalmon.co.uk/pdfs/contain.pdf

10.19 ***Cage security inspection should be reviewed immediately IPN is confirmed on a site and a net inspection programme, if not already in place, should be followed until all fish are removed from the cages.***

10.20 ***Particular effort should be directed at attempts to recapture escaped salmon which are, or are likely to become, sexually mature in line with any methods proposed by the Farmed Fish Escapes Working Group.***

10.21 ***Further research is needed to establish the longevity of infection in escaped fish, the risk of predators of escaped fish acting as vectors of infection, and the disease risk posed to wild fish and other farmed fish stocks.***

10.22 **W*ater intakes to and effluents from land-based farms should be disinfected where practicable.***

10.23 ***In cage culture a fallowing policy should be established, based on risk assessment.***

10.24 ***Within land based facilities, farmers should be encouraged to maintain cohort separation, and cleaning and disinfection should be undertaken every time ponds or tanks are vacated and prior to restocking.***

10.25 ***Research is needed into shedding rates of IPN virus from infected fish,***

survival of IPN virus in water and sediments, and establishing the minimum effective dose of the virus for susceptible species and age groups.

10.26 Research is needed into the effect of fallowing on environmental levels of IPN virus.

10.27 ***If nets from different farms are washed at a common location, care should be taken to avoid cross-contamination and transfer of infection between nets from different farms.***

10.28 ***Where possible, equipment should be site-specific. Where movements of equipment between sites is unavoidable, the equipment must be thoroughly cleaned and disinfected.***

10.29 ***Staff should be trained in cleaning and disinfection routines.***

10.30 ***Staff and other relevant parties (for example, fish transporters) should be kept informed on the health status of fish in their care.***

10.31 ***Farm operations should be managed to minimise the number of fish movements.***

10.32 ***Movements of vehicles, wellboats and equipment between farms should be kept to a minimum.***

10.33 ***Further research is required to identify suitable, safe disinfectant products effective against fish pathogens.***

10.34 ***Wellboats should operate with valves closed within a 5 km range of any fish farm.***

10.35 ***Continued research into safe, cost-effective methods of disinfection of blood, water and solid waste from processing plants is required.***

10.36 ***Killing tables should be equipped with sides high enough to prevent escapes or have a net positioned to capture any escaped fish. A tarpaulin under the killing table will contain blood spillage. Whenever possible, harvesting operations should be carried out in good weather conditions.***

10.37 ***Wellboats transporting live fish to a processing plant should operate with closed valves when operating within a 5 km range of any fish farm.***

10.38 ***Off-loading bays at processing plants must be equipped with a waterproof apron, draining to a collection point and should be surrounded by a bund or similar structure.***

10.39 ***Drainage from "dirty" areas must feed into a disinfection facility.***

10.40 ***A disinfectant spray or wheel bath must be available to treat vehicles leaving a processing plant.***

10.41 ***Wellboats or other vessels should be disinfected after visiting a processing plant, particularly between shuttle runs to different fish farm sites.***

10.42 ***Full protective clothing must be provided for staff and should be kept on site, except for laundering in which case it must be properly contained for transport. Rubber overalls must be disinfected in a soak bath.***

10.43 ***Plastic pallets should be used where possible and these should be disinfected before leaving the processing plant. Wooden pallets should be for 'single use' only.***

10.44 ***Harvest bins must be cleaned and disinfected before leaving the processing plant. Clean bins must be stored in a specified area away from dirty areas.***

10.45 ***Access to dirty areas should be restricted.***

10.46 ***Processing area surfaces should be waterproof and amenable to disinfection. All drainage from these areas must feed into a disinfection facility.***

10.47 ***All liquid effluent from processing operations must be disinfected before disposal.***

10.48 ***All viscera and other solid waste must be treated in an appropriate manner to prevent the spread of disease, meeting in all circumstances the requirements of EC Regulation 1774/2002 and any subsequent legislation.***

10.49 ***Divers should use site-specific gear where possible. If the movement of diving gear is unavoidable it should be thoroughly cleaned and disinfected between operations on different farms.***

10.50 ***The Field Guide to Disinfection with Regard to ISA Virus should be re-written to include procedures for effective IPN virus disinfection.***

10.51 ***Access to farms by visitors should be minimised where possible. When access is necessary, site-specific protective clothing and boots should be available for use by farm visitors.***

10.52 ***Research is required to identify alternative, safe disinfectants active against IPN virus in the presence of organic matter.***

10.53 ***Where practicable, water intakes to and effluents from land-based farms should be disinfected.***

10.54 ***Measures should always be taken at water intakes to minimise the risk of ingress of wild fish. Twin sets of screens should be used so that wild fish cannot get access to the farm when the screens are being cleaned.***

10.55 ***Research is required to establish the prevalence and distribution of IPN virus in wild fish in Scottish waters.***

10.56 ***Where wrasse are to be introduced into salmon cages for the purposes of sea lice control, such fish should be locally caught if possible and should not be released from the site at the end of the production cycle.***

10.57 ***An epizootiological study of IPN virus in cohabiting species is required.***

10.58 ***Experimental work is required to determine whether sea lice can transmit IPN virus from infected fish to naïve fish.***

10.59 ***Farmers should commit to effective control of sea lice and should adopt procedures recommended by Rae.[42] and in the National Treatment Strategy for the Control of Sea Lice (SSGA, 1998) and*** http://www.scottishsalmon.co.uk/pdfs/sealice.pdf

10.60 ***Pharmaceutical companies and licensing authorities should endeavour to increase the availability of safe, efficacious and environmentally acceptable sea lice medicines.***

10.61 ***Research is required to ascertain if wild crustaceans and molluscs acquire IPN virus from infected salmon populations.***

10.62 ***Research is required to ascertain if and for how long IPN virus replicates in molluscs and crustaceans.***

10.63 ***Research is required to ascertain if molluscs and crustaceans can transmit IPN virus to salmon and other fish species.***

10.64 ***Fouling on fish farm cages should be regularly removed as a precautionary measure.***

10.65 ***Access to mortalities and fish food should be prevented, as these are attractive to birds.***

10.66 ***Effective measures should be in place to minimise access by birds to fresh water and marine farms.***

10.67 ***Research to improve knowledge of the prevalence of IPN virus among seabirds where IPN is enzootic would help in the understanding of the epizootiology of the disease.***

10.68 ***Knowledge of the prevalence of IPN virus among mammals where IPN is enzootic would help in the understanding of the epizootiology of the disease.***

10.69 ***Vermin should be discouraged by preventing access to fish food and maintaining good hygiene procedures.***

10.70 ***Although our knowledge of the ability of mammals to act as vectors of IPN virus is limited, a precautionary approach is recommended. Thus, permitted anti-predator methods should be employed where possible.***

10.71 ***Unpasteurised fish should not be used as feed.***

10.72 ***It is recommended that research is undertaken to optimise existing techniques and to develop and validate alternative techniques, especially non-destructive testing methods, for detection and diagnosis of IPN virus.***

10.73 ***It is recommended that research is undertaken to establish if strains of IPN virus differ in virulence, and if so to establish diagnostic methods capable of differentiating between virulent and avirulent strains in order to facilitate control of virulent strains.***

10.74 ***Effective vaccines against IPN should be developed for use in Atlantic salmon and marine fin fish species.***

10.75 The effects of vaccines on the carrier state, and the risk posed by carriers in relation to vertical and horizontal transmission of the virus, should be assessed.

10.76 The current regulatory regime should be maintained as an interim measure. SW-SW movements of live fish should only be permitted in accordance with the ISA Code of Practice.

10.77 Codes of Practice should be developed for all industry sectors to minimise the spread of infectious diseases and the likelihood of disease outbreaks.

10.78 Government should introduce regulatory measures to ensure the implementation of the Codes of Practice referred to in 9.2.2.2.

10.79 ***Current regulations prohibiting the movement of clinically diseased fish should be maintained.***

10.80 ***IPN in fresh water should remain under the current legislative control regime.***

10.81 ***A survey should be carried out of the prevalence of IPN virus in farmed and wild fish to inform whether legislative control of IPN should be extended to all sectors in fresh water, including trout and restocking hatcheries.***

10.82 ***Current regulations prohibiting the movement of clinically diseased fish should be maintained.***

10.83 ***Current legislative controls on the movement of live fish from sea water to fresh water should be maintained.***

10.84 ***Government should introduce new regulations controlling movement of any fish from sea water to fresh water [for example, between coastal and continental zones].***

10.85 A Code of Practice should be developed governing sea water to fresh water movements of live fish and the holding and testing of broodstock.

10.86 Movements of live fish from IPN virus infected fresh water sites to sea water should be permitted, irrespective of the IPN status of the site in sea water.

10.87 Current regulations prohibiting the movement of clinically diseased fish should be maintained.

APPENDIX I

IPN Titre Frequency Data

By Dr Sandy Murray, Fisheries Research Services

Data are available on IPN titres obtained from 100 fish kidneys sampled in each of March, June and August of 1996, together with a smaller sample of 40 fish for which kidneys, ovarian fluid and the sonicate of cells from ovarian fluid were sampled.

The objective of this study was to establish what the existing data show as to the possibility of fish with high ovarian fluid titre entering the brood stock. Titres of over 10^4 pfu in ovarian fluid are known to be capable of transmitting IPN vertically to the eggs of brook trout. Since hatchery offspring are dispersed widely among fish farms, the presence of infected fish in hatcheries represents a serious potential risk to the fish production industry.

The first analysis is of the 300 fish whose kidneys were sampled in 1996, since this data set is the largest and so most likely to produce statistically meaningful results.

The specific objective is to find the frequency with which fish have given titres of IPN in their kidneys, within the data available. To do this, the fish have been sorted in order of the size of their IPN titre. Fish with titres greater or equal to the largest titre are expected to occur at a 1/100 frequency, the 10th largest titre or greater should be the 1/10 frequency and so on. Many of the fish lack IPN or have titres that are at or below the detection limits used. Inclusion of these would distort the distribution pattern, so in practice only 12 to 18 fish whose IPN titres are above the detection limit are available in the monthly samples. The data are also combined to obtain a set of 43 fish with meaningful titres, and to extend the frequency range to 1/300.

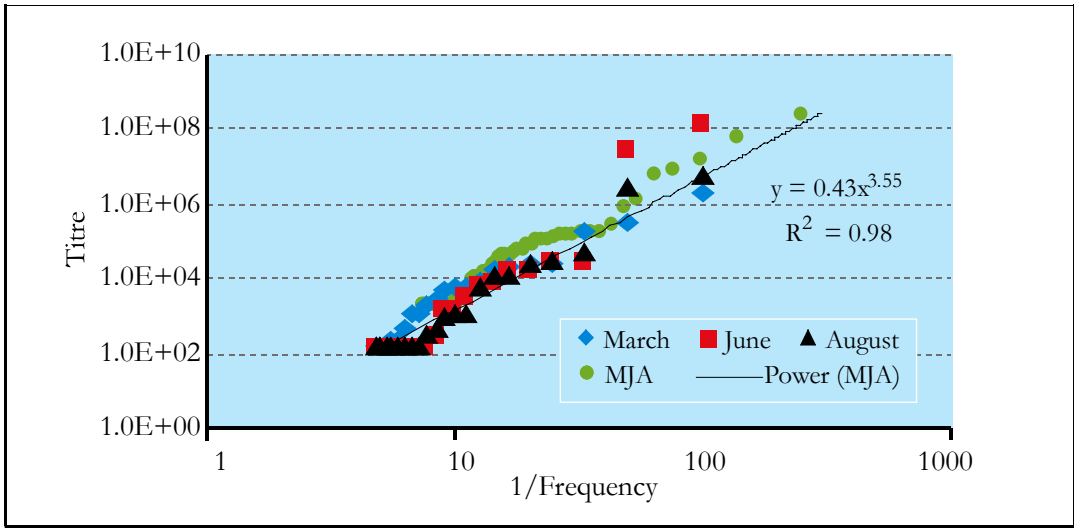


Figure 1: Log-log plot of titre of IPN from kidneys of fish, versus inverse frequency of occurrence. Both separately and combined, these data sets all support a slope of approximately 3.5x, ie for each order of magnitude reduction in frequency there are 3.5 order increase in IPN titre.

The frequency distribution shows little scatter over about two log units of frequency, and between the data sets. This means that there is no sign of a decline in the trend in IPN samples and you would expect that if a sample were increased 10 fold then the largest titre would increase by 3.5 orders of magnitude. This means that in large populations when IPN is present one must expect some fish to have very high IPN titres in their kidneys.

Good though this fit is, a more detailed examination shows that it is not the entire picture. The IPN titre levels found at frequencies of less than approximately 1/30 appear to be heading towards saturation at a kidney titre of 10^5 pfu.

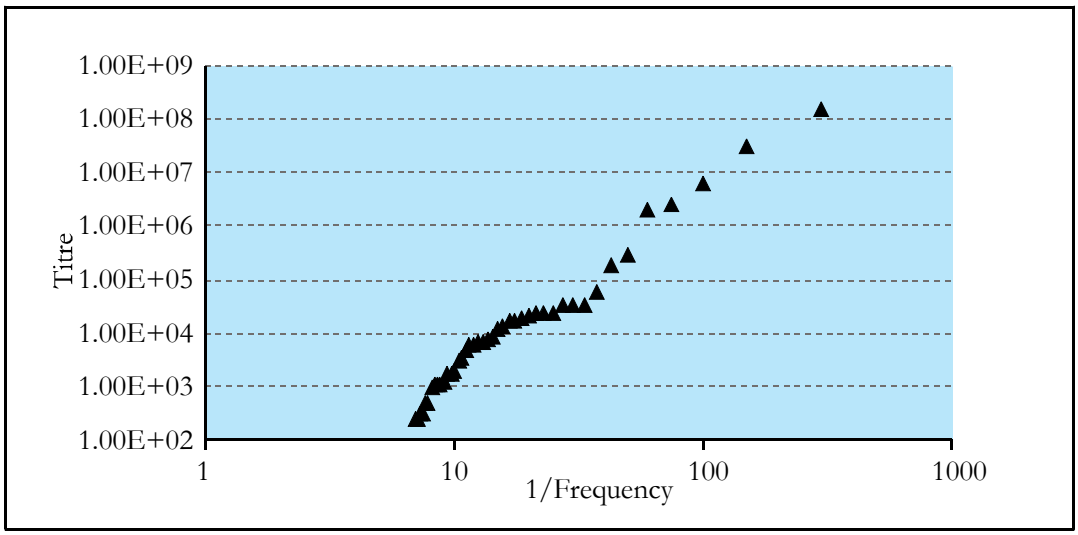


Figure 2: Log-log plot of titre of IPN from kidneys of fish, versus inverse frequency of occurrence in combined data set.

In the remaining 3% of fish the slope increases again and much higher titres are found. Arguably, but not statistically, there may be signs that this later part of the curve is also saturating. The regression is slightly steeper than for the bulk of the cure at 3.9, which might argue for fish with very large titres to be slightly more frequent than the regression on all titres that are greater than the resolution predicts. However, if the saturation is genuine there may be a maximum titre which could be as low as 10^9, no matter how large the sample. Clearly, there must be some ultimate level at which the titre saturates, if only because some IPN level must be lethal to the fish. The sample of very large doses is too small for further meaningful analysis.

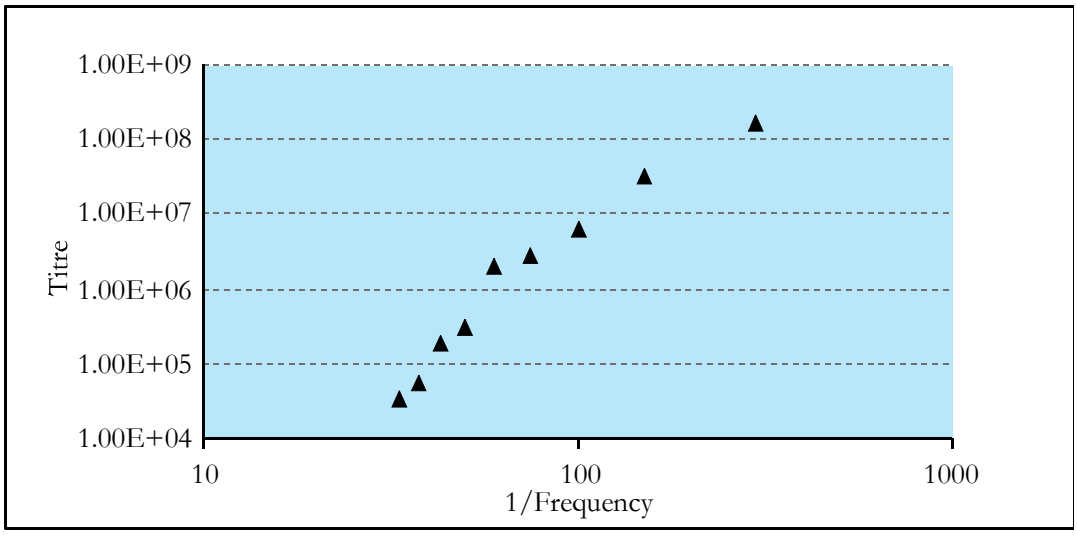


Figure 3: Log-log plot of titre of IPN from kidneys of fish, versus inverse frequency of occurrence in combined data set, for nine highest titres.

The data examined suggest that in any large infected population, some small fraction of the fish will have very large IPN kidney titres. How does this translate into ovarian fluid titre?

In the second data set of 40 fish, the curve of frequency of titre size is of a similar shape to that obtained earlier, with a tendency to saturate at a titre of about 10^5 pfu. However, in this case many more fish have moderate IPN titres, so the prevalence of infection is much higher. Similar variation exists within the first data set, albeit on a smaller scale, with more low to moderate IPN titre fish being detected in March than on the other occasions. The pattern appears broadly similar to the previously examined data set, except that detectable levels of titre are much more prevalent. If so, we would occasionally expect to see fish with very high kidney titres, but this sample is too small to provide much chance of detecting such fish.

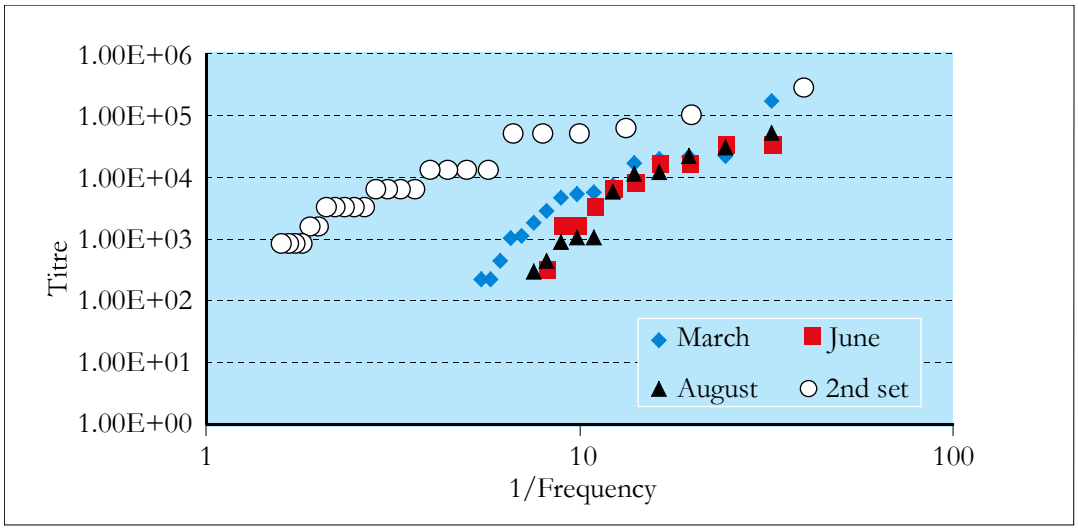


Figure 4: Inverse frequency distribution of IPN titre in fish kidneys for the two data sets, excluding frequencies of <1/40.

Unfortunately, there is a lack of data on the ovarian fluid IPN titre, only three samples having detectable levels of IPN. So instead we look at the frequency of IPN titre levels in the cell sonicate from ovarian fluid. Titre levels were not resolved finely, which means that, apart from detection limit, there are only four recorded levels of IPN. The distribution does appear to be log linear, but it is still possible that the curve is saturating and so high titres may be very rare. The regression is 0.85 with standard error (SE) of 0.2, which means the IPN titres are much lower than for kidney titres. However, if the regression holds, about 1% of the samples to have titres of 10^4 pfu, (10,000) this may indicate a serious danger of vertical transmission of infection but titres in fluid appear to be lower. As IPN titre was above detection in ovarian fluid in only three cases, comparison is difficult, but as IPN is encountered less often in the fluid than in the cell sonicate, we would expect a lower number of titres of 10^4 in the fluid.

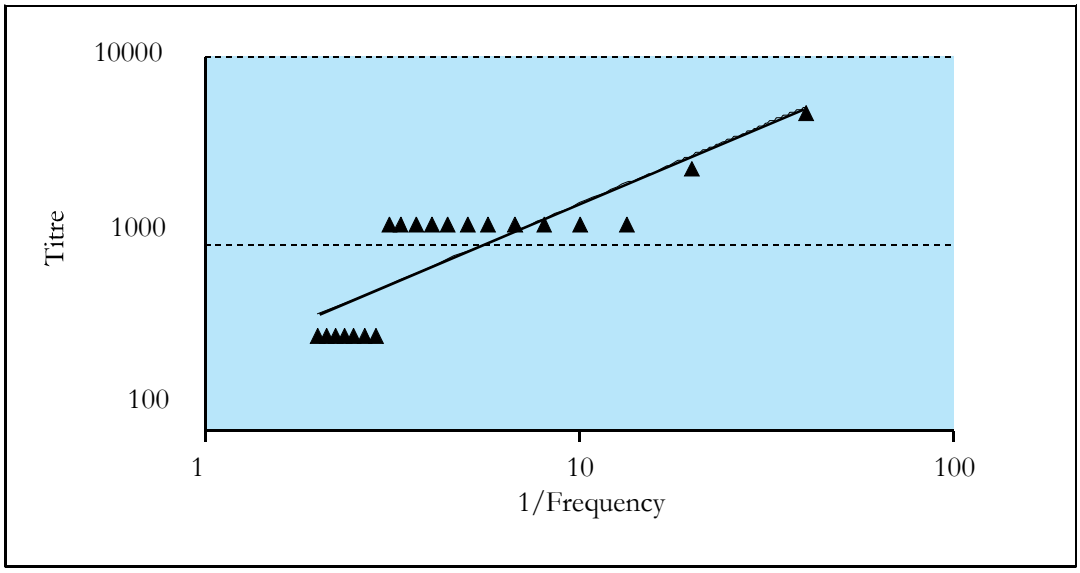


Figure 5: Inverse frequency distribution of IPN titre from cell sonicates from ovarian fluid. Regression titre=230×inverse frequency$^{0.85}$, $r^2 = 0.71$.

If IPN is present in ovarian cell sonicate at the potentially dangerous levels of 10^4 pfu in 1% of fish, then a sample size of 300 would give a 95% chance of locating at least one fish with such a high titre. Such a sample size should give a good indicator of the pattern of IPN titres present at lower frequency. A sample size of 600, would give a 95% chance of detecting such a high pfu, even if their abundance had halved, and would give a 45% chance of locating a 10^4 pfu titre, even if it were present in only 0.1% of the fish. Such a sample would better allow for uncertainty as to the variation in high titre between populations. At the other extreme, a sample of 230 would give a 90% chance of locating a 10^4 pfu if these were present in 1% of the population, this can be regarded as a minimum size for such an analysis.

There are insufficient data to provide an analysis of ovarian fluid titres, only three samples out of 40 exceed the detection limits. It would seem that a much larger sample size than is currently available would be required for analysis. However, if three titres above detection limit were found we would expect that sample sizes of 230, 300 and 600 required to analyse cell sonicates would give 17, 22 and 45 ovarian fluid samples above detection limits. Because we have only three positive samples, there is a large range of possible variation in the actual proportion of fish with ovarian fluid titres in excess of the

detection limits. The 90% range that could produce the observed 3 is 0.8 to 7.75 in the 40 samples, so there is a 5% chance that the larger samples could contain as few as 5, 6 or 12 results that are above detection limits. This is a worst case and it is likely that the large samples of 230-600 required for the ovarian fluid cell sonicate analysis will produce a statistically useful number of ovarian fluid titres, allowing the basic distribution pattern to be deduced. However, given the observed pattern of kidney IPN titres, with a small number of fish with much higher titres than would be predicted from 97% of the fish, it is possible that the pattern will be incomplete and that large titres will not be detected, or predictable. If necessary, the pattern that emerges from a study of 230-600 fish can be used to design a study to look into these rare high titre fish.

The titre levels in the kidneys show only a very weak relationship with titres in the ovarian fluid. The highest ovarian fluid cell sonicate level, 3.7, was associate with IPN titre levels below detection limits in both ovarian fluid and in the kidney. It is therefore not possible to predict ovarian fluid titres from kidney titres, although fish with high kidney titres are more likely to have high ovarian fluid titres.

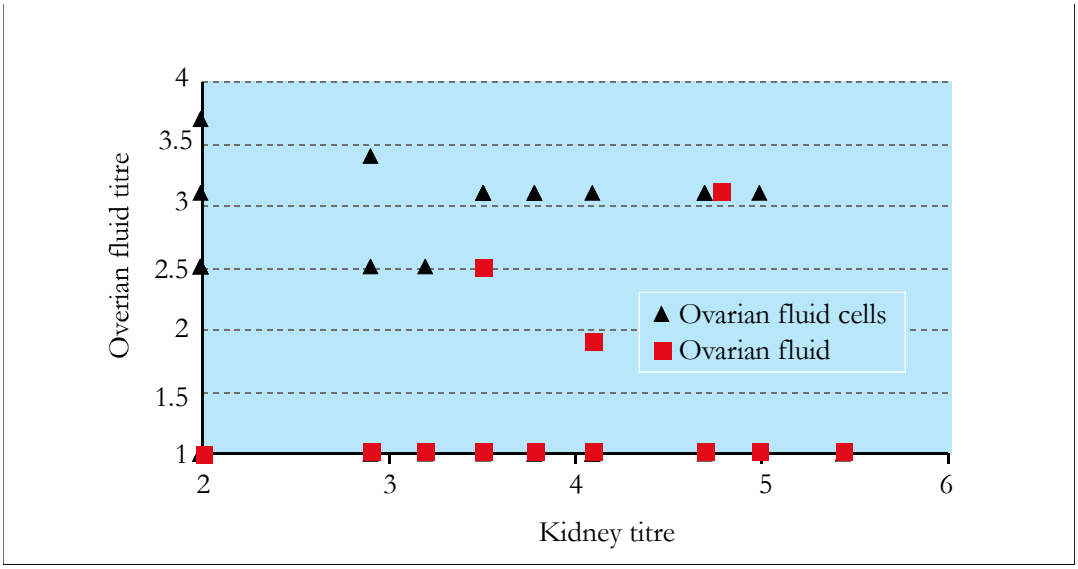


Figure 6: Relationship between IPN titres in fish kidneys and ovarian fluids and ovarian fluid cell sonicates in log pfu units. Axes are the limits of detection.

In conclusion, in an IPN infected population small numbers of fish are expected to have very high IPN titres in their kidneys. The pattern of increase in titre is not entirely constant, with an increase at low frequency of titre levels, that is, a few fish have much higher levels of IPN than would appear to be case from the trend among 97% of the fish. There is evidence that the pattern may be similar for cell sonicate from ovarian fluid, but this is based on rather little, poorly resolved, data. The data from the ovarian fluid itself are too sparse for analysis. There appears to be only a weak relationship between kidney titres and ovarian fluid titres of IPN. Further analysis would require a sample of 230-600 fish to obtain statistically useful results. However, given the pattern observed in kidney titres, some very high titres may be present at low frequency and these high titres may not be apparent, or predictable, except from very large samples.

APPENDIX II

Aquaculture Health Joint Working Group. IPN Sub-group Meeting, Marine Laboratory, 17th October 2000

Presentation by Dr T Taksdal, National Veterinary Institute, Oslo, Norway

Infectious pancreatic necrosis (IPN) in Atlantic salmon, Norwegian experience

The speaker concentrated on the disease IPN rather than the virus (IPNV). The main sources of information on the Norwegian experience are:
Epidemiological studies by T. Bruheim *et al.*, National Veterinary Institute, Trondheim, Norway (in Norwegian).
Epidemiological studies by J. Jarp *et al.*, National Veterinary Institute, Oslo, Norway
Infection experiments by T. Taksdal *et al.*

The IPN history and present situation:

IPNV was first isolated in Norway in 1975. The first outbreak of the disease was diagnosed in 1985. During the next few years, there were only a few outbreaks. However, during 1988-1991, a marked increase in number of IPN outbreaks occurred, mainly in post-smolts. Since then, there have been disease outbreaks in about 30-40% of hatcheries (freshwater) and in 40-70% of seawater fish farms annually.

1998 was the *'annus horribilis'*. In a study of four counties in mid-Norway: Sogn & Fjordane, Møre & Romsdal, Sør-Trøndelag, and Nord-Trøndelag in 1998, outbreaks of IPN were diagnosed in 83% of the sea water sites. The mean mortality in affected fish groups was 16.3%, whereas the losses in unaffected groups were 4.9%.

In a similar study of the situation in 1999, IPN was diagnosed in 74% of the seawater sites. The mean mortality in affected fish groups was 11.2%, whereas the losses in unaffected groups were 4.5%.

Do the vaccines against IPN work in the field?

In 1998, 66.2% of the smolt groups in the four counties already referred to, were vaccinated against IPNV. This affected neither prevalence of outbreaks of IPN nor mean losses.

In 1999, 75% of the smolt groups in these four counties were vaccinated against IPNV. This *seemingly* enhanced both the prevalence of IPN and the mean losses. However, the fish in farms or areas where the mortality and losses due to IPN are 'acceptable' are less inclined to use these vaccines. The vaccines may thus gain a false bad reputation. On the other hand, if the vaccines really protect against IPN in field, one would expect that the effect could be demonstrated in such a study. The studies in 1998 and 1999 covered 90/84 seawater sites, and 245/297 smolt groups, respectively.

Risk factors connected to outbreaks of IPN in sea water:

Higher risk: Collection of smolts from several hatcheries, 'Historic IPN' on the site, Geography, Transportation (possibly stress mediated).
Lower risk: Higher weight at sea water transfer, age (2-yearlings).
No connection found: Smoltification measured as plasma chloride, the use of smolts from own hatched fry or purchased fry, other diseases.
A lot of factors are under question. However, as the conditions in the fish farms changes rapidly, the influence of the different factors are also changing over a short time, thus making some results from epidemiological studies of limited use.

IPN in fresh water:

Disease outbreaks occur in fry and parr throughout the year. It is most frequent in small fish below 25 grams and most frequent in summer (April to August). Risk factors: hatching substrate, low temperature at hatch, purchase of fry, introduction of sea water into the hatchery (which is common in Norway) and 'historic IPN'. Whether an outbreak of IPN in fresh water protects against subsequent outbreaks in sea water has been heavily debated and the experiences from different fish farms/fish groups are contradictory.

Infection trials:

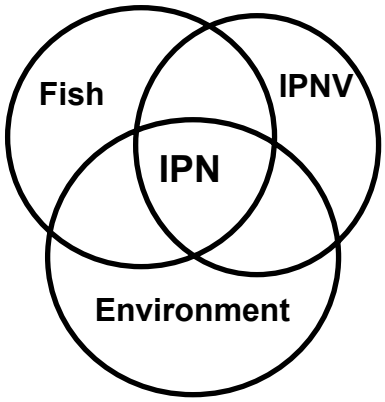
Infection trials are important for the development and evaluation of vaccines and for research aimed at establishing preventive measures.

A challenge model using fry and virulent IPNV serotype Sp. has been established and is easily reproducible when the fry are young enough. It is used for testing of fry of different origin regarding their susceptibility to IPN and testing of virulence of different strains of IPNV. However, the model is not useful for the development of vaccines, as the fish used are too small.

Experimental bath challenge of Atlantic salmon smolts at sea water transfer with virulent IPNV serotype Sp. usually induces outbreaks of IPN. However, mortality varies and more work is needed to establish a challenge model that is more reproducible and which gives mortality high enough for vaccine evaluation. Two research stations in Norway (VESO Vikan AkvaVet and Fiskeriforskning) perform infection trials with IPN in smolts on a commercial basis.

The results of other experiments indicate that outbreaks of IPN, at least in some cases, are stress related. Outbreaks of IPN were induced in covertly infected Atlantic salmon post-smolts by lowering the water level for ten minutes twice a week. This indicates that environmental stress may be important for development of disease outbreaks.

Factors to consider to control IPN and for infection experiments:



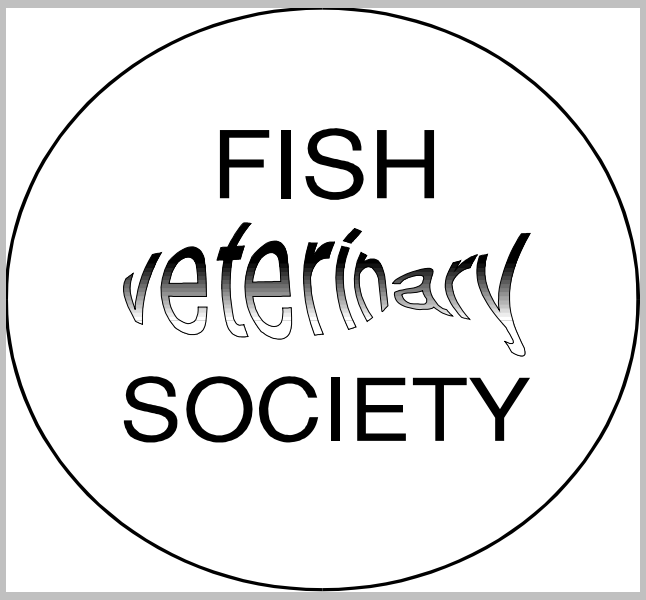
- The fish
  - Fish strain, age, physiology
  - Vaccination
- Environment:
  - Stress & husbandry
  - Temperature?
- IPNV
  - Avoidance (vertical and horizontal transmission)
  - Viulence of the strain of IPNV

In conclusion:

If the Norwegian situation regarding IPN can be avoided, it will probably be beneficial to the industry, although preventive measures are expensive. The overall costs of outbreaks of IPN to the fish farming industry in Norway have been estimated at approximately £30 million yearly (NVI, Trondheim 1994, in Norwegian) or 60 million USD yearly (Christie 1997). However, the unpredictability regarding production losses caused by IPN is possibly more serious for single fish farms than the calculations of economic losses to the industry may indicate.

Our knowledge of the disease, and especially of the covert infection, is still insufficient for the control of the disease.

APPENDIX III



A survey conducted by the
Fish Veterinary Society (FVS)
on behalf of the
Aquaculture Health Joint Working Group (AHJWG)
Infectious Pancreatic Necrosis (IPN) sub-group

AUGUST 2001

1. Background

At a meeting of the IPN sub-group held on 17th October 2000, it was proposed that information be collected on the incidence of clinical IPN in Scottish salmon farming. The objective of this survey was to clarify the incidence and economic cost of the disease. A supplementary section addressed other related issues and species and is reported separately.

2. Method

Information was collected only from veterinarians providing services to salmon farmers, in absolute confidence using a standardised questionnaire. Neither the veterinarians questioned nor individual farms were identified in the survey which was carried out either by face-to-face interview or received as a written response.

Twelve potential respondents were identified and of these 9 provided detailed information. The remaining three stated that their involvement with salmon farmers is presently minor.

For questions requiring a quantitative response, the value attached to each individual's response is equated with the number of farms for which services are provided. Responses to questions inviting opinion are presented *verbatim*.

The survey covers the period from January 2000 to March 2001. Each respondent was asked to exclude information covering the 6 months immediately preceding the date of their own response and to consider only the previous 12 month period.

This report will be presented to the IPN sub-group to be included in the groups` report to the Aquaculture Health Joint Working Group (AHJWG). A copy of the report will be made available to the sponsors, respondents and to the Committee of FVS. The supplementary survey will be presented to the IPN sub-group for their consideration.

3. Presentation

The data gathered from the survey are presented in tabular form, under three main category headings:

- Salmon
- Control and Research
- Non-Salmonid Species

4. Interpretation

No detailed interpretation of the basic data has been undertaken.

MAIN IPN QUESTIONNAIRE

SALMON

Question 1	GENERAL	
Code	**Question**	**Answer**
Q1.1	How many salmon farming companies do you provide with veterinary services?	**66**
Q1.2	How many freshwater farms does this represent? (*'Farm' is taken to be a Premises registered under DoFA*)	**91**
Q1.3	How many marine farms does this represent? (*'Farm' is taken to be a premises registered under DoFA*)	**150**

Question 2	FRESH WATER	
Code	**Question**	**Answer**
Q2.1 - A	In the last year has IPNV been diagnosed at any farm?	**Yes**
Q2.1 - B	If yes, at how many farms?	**15**

Q2.1 - C	If yes, what was the IPN status of the parent fish? As a % of All Cases Known Positive Known Negative Known Positive and Negative (***NB*** *A broodstock population with both +ve and –ve*) Status Unknown *(information not available or not disclosed to the respondent)*	 **20%** **33%** **7%** **40%**
Q2.2	Has the diagnosis of IPN been associated with clinical signs, morbidity and Mortality attributable to IPNV? As a % of All Cases Clinical Signs Histopathology Virus isolation Virus isolation only Intercurrent disease? Other (specify)	 **73%** **80%** **80%** **7%** **-** **-**
Q2.3	What size of fish were affected and what were the losses? **SIZE OF FISH** / **MONTH AFFECTED** / **%** < 5 g — April — 60% 5 – 10 g — May — 7% 10 – 15 g — July — 20% > 15 g — September — 13% (***NB*** *In some cases a high loss may reflect deliberate culling of known infected stock*)	**Losses** **20-100%** **< 5%** **< 5%** **< 5%**
Q2.4	Have IPNV infected smolts been transferred to sea?	**Yes**
Q2.5	Have you been able to identify risk factors for clinical IPN? If yes detail • High IPNV positivity rate in parent fish • Broodstock held in Shetland • Use of recirculation in infected hatcheries • Unprotected water source • Exposure of small fish • Cage rearing in migratory lochs	

Question 3	SEA WATER	
Code	**Question**	**Answer**
Q3.1 - A	In the last year has IPNV been diagnosed at any farm?	**Yes**
Q3.1 - B	If yes, at how many farms	**35**
Q3.1 - C	If yes, what was the IPN status of the parent fish? As a % of All Cases Known Positive Known Negative Known Positive and Negative (***NB*** *A broodstock population with both +ve and –ve*) Status Unknown *(information not available or not disclosed to the respondent)*	 **9%** **3%** **0%** **88%**
Q3.2	Has the diagnosis of IPN been associated with clinical signs, morbidity and Mortality attributable to IPNV? As a % of All Cases Clinical Signs Histopath Virus isolation Virus isolation only Intercurrent disease?	 **49%** **51%** **34%** **29%** **9%**
Q3.3	What size of fish were affected and what were the losses? **SIZE OF FISH** **MONTH** 60 – 300 G * (***** *The majority of losses were suffered within the three months following transfer and included out of season and "conventional" smolts. Losses were highly variable & unpredictable*)	**Losses** **1 – 30%**
Q3.4	Have you been able to identify risk factors for clinical IPN? If yes, detail • Smolt origin • Long transportation • Stress • Transfer to Shetland • Poor smolt quality • IPNV positive smolts History of IPN at site Wild reservoirs Intercurrent disease Stock type Mixed year classes Proximity of other sites	

Question 4 OTHER COMMENTS
• Broodstock testing (allied with effective vaccination) is a valuable control measure. • There is a risk that present legislation might encourage hatcheries to move fish that they suspect might be IPNV positive before investigation by the official service. • Legislation may encourage farmers not to seek revocation of DAOs on seawater sites to provide security in case infected smolts have to be moved. • Movements between seawater sites should be considered on a case by case basis.

SUPPLEMENTARY IPN QUESTIONNAIRE

CONTROL

Question	CONTROL	
Code	Question	Answer

<table>
<tr><td>Q1.1</td><td>In the experience of the farms under your care, do you believe IPN is changing in significance in fresh water? If yes, please justify
(NB Six of the nine respondents commented that there has been an apparent increase in cases of IPN in both fresh and seawater since March 2001; this period was not included in the survey)</td><td>9 out of 12 said – Yes

• More isolations, particularly in lochs
• More clinical cases</td></tr>
<tr><td>Q1.2</td><td>In the experience of the farms under your care, do you believe IPN is changing in significance in sea water? If yes please justify
(NB The situation in Shetland is reported to be consistent with previous years)</td><td>8 out of 12 said – Yes

• Increasing morbidity and mortality associated with IPN especially in weeks 6-18 post transfer. This change has been particularly marked on the mainland</td></tr>
<tr><td>Q1.3</td><td>IPN can be controlled in a number of ways. In the table below please rate for freshwater farms, each of the control methods according to the scale (5 - highly relevant; 0 - of no relevance)

DISINFECTION OF OVA

DESTRUCTION OF PROGENY OF IPNV POSITIVE PARENT FISH

VACCINATION (assuming significant protection)

MOVEMENT CONTROLS

HUSBANDRY MEASURES</td><td>(NB The value entered in each cell is the numbers of farms serviced by the respondents who chose that category in the scale. The figure in brackets is the number of respondents favouring that option. The total number of freshwater farms captured in the survey is 91)

<table>
<tr><td>37 (2)</td><td>6 (1)</td><td>48 (6)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>14 (3)</td><td>46 (5)</td><td>0</td><td>0</td><td>0</td><td>31 (1)</td></tr>
<tr><td>30 (1)</td><td>6 (1)</td><td>15 (3)</td><td>0</td><td>40 (4)</td><td>0</td></tr>
<tr><td>77 (6)</td><td>7 (1)</td><td>7 (2)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>14 (3)</td><td>71 (5)</td><td>6 (1)</td><td>0</td><td>0</td><td>0</td></tr>
</table>
0</td></tr>
<tr><td>Q1.4</td><td>Are there any other controls that should be considered?</td><td>• Protected water supply
• Biosecurity
• Controlled movement of personnel
• Control of wild fish movement
• Surveillance of wild fish
• Vaccination before stocking into migratory waters</td></tr>
</table>

Question	CONTROL - CONTINUED	
Code	Question	Answer
Q1.5	IPN can be controlled in a number of ways. In the table below please rate for **seawater** farms, each of the control methods according to the scale (5 - highly relevant; 0 - of no relevance)	***(NB The value entered in each cell is the numbers of farms serviced by the respondents who chose that category in the scale. The figure in brackets is the number of respondents favouring that option. The total number of freshwater farms captured in the survey is 150)***

DISINFECTION OF OVA	51 (2)	0	31 (2)	28 (1)	40 (4)	0
DESTRUCTION OF PROGENY OF IPNV POSITIVE PARENT FISH	1 (1)	0	30 (2)	69 (5)	0	50 (1)
VACCINATION (assuming significant protection)	120 (7)	28 (1)	2 (1)	0	0	0
MOVEMENT CONTROLS	1 (1)	30 (2)	50 (1)	40 (4)	29 (1)	0
HUSBANDRY MEASURES	3 (2)	78 (2)	40 (4)	29 (1)	0	0

0

Q1.6	Are there any other controls that should be considered?	• **Vaccination** • **Biosecurity** • **Fallowing** • **Effective sea lice control**

Question 2 RESEARCH – What do you consider should be priorities for Research?
(***NB*** *The number of respondents favouring each topic is indicated)*
• **Vaccines (8/12)**
• **Egg disinfection/vertical transmission (7/12)**
• **Stock susceptibility (5/12)**
• **Diagnostics (4/12)**
• **Immuno-modulation (2/12)**
• **Significance of intercurrent disease on IPN virulence (2/12)**
• **Heterogeneity of isolates (1/12)**

SUPPLEMENTARY IPN QUESTIONNAIRE

NON-SALMONID SPECIES

Question	GENERAL	
Code	Question	Answer
Q1.1	How many non-salmonid marine fin fish farming companies do you provide with veterinary services?	7
Q1.2	How many hatcheries does this represent?	7
Q1.3	How many grow-out farms does this represent?	9

Question	HATCHERY	
Code	Question	Answer
Q2.1 - A	In the last year has IPNV been diagnosed at any hatchery?	Yes
Q2.1 - B	If yes, at how many hatcheries?	1
Q2.1 - C	If yes, what was the IPN status of the parent fish? As a % of All Cases **Known Positive** **Known Negative** **Known Positive and Negative** (***NB*** *a broodstock population with both +ve and –ve*) **Status Unknown** *(information not available or not disclosed to the respondent)*	 - - - 100%
Q2.2	Has the diagnosis of IPN been associated with clinical signs, morbidity and mortality attributable to IPNV? As a % of All Cases **Clinical Signs** **Histopath** **Virus isolation** **Virus isolation only** **Intercurrent disease?** **Other (specify)**	 100% 100% 100% - - Water Quality
Q2.3	What size of fish were affected and what were the losses? **SIZE OF FISH** — **MONTH** — **Losses** < 2.5 g — August — 100% < 5 g — August — Variable (***NB*** *These all relate to the one case*) > 5 g — August — <None	
Q2.5	Have IPNV infected juveniles been transferred to sea?	No
Q2.6	Have you been able to identify risk factors for clinical IPN? If yes, detail • **Poor water quality** • **Proximity of IPNV positive salmon farm** • **Unprotected water supply** • **Exposure of small fish**	

Question	GROW-OUT FARMS	
Code	Question	Answer
Q3.1 - A	In the last year has IPNV been diagnosed at any grow-out farm?	**No**
Q3.1 - B	If yes, at how many hatcheries?	-
Q3.1 - C	If yes, what was the IPN status of the parent fish? As a % of All Cases **Known Positive** **Known Negative** **Known Positive and Negative** (***NB*** *A broodstock population with both +ve and –ve*) **Status Unknown** *(information not available or not disclosed to the respondent)*	 - - - -
Q3.2	Has the diagnosis of IPN been associated with clinical signs, morbidity and mortality attributable to IPNV? **Clinical Signs** **Histopath** **Virus isolation** **Virus isolation only** **Intercurrent disease?** **Other (specify)**	 - - - - - -
Q3.3	What size of fish were affected and what were the losses?	-
Q3.5	Have IPNV infected juveniles been transferred to sea?	-
Q3.6	Have you been able to identify risk factors for clinical IPN? If yes, detail • **No Comments Received**	

Question 4 OTHER COMMENTS
• **Implications for salmonids of adjacent infected non-salmonid marine fin fish farms** • **Anomalies in notification arrangements for IPN between species** • **Wild fisheries inadequately regulated**

APPENDIX IV

Survey Of Incidence Of IPN On Scottish Quality Salmon (SQS) Member Farms In 2001

Nine members responded to a request for information about the incidence of IPN in 2000 S0 and 2001 S1 smolt stocks. The responses covered thirty sites (1 FW and 29SW). Eighteen SW sites recorded mortalities due to IPN and the severity is shown below. Twenty-three different stocks/sources of fish were identified and there is no obvious correlation between stock origin and incidence of IPN (except at five sites where IPN infected fish were moved to sea. Average mortality was 5.3% but some were S1 and some were S0, see below). These results are similar to previous findings showing that IPN is widespread and can cause significant losses.

Characteristic	Average mortality	Range
2001 S1 (49 stocks)	3.7%	0.3 – 30%
2000 S0 (9 stocks)	5.8%	0.6 – 25%
Vaccinated (5 stocks)	16%*	2.0 – 30%

* Vaccinated stocks may have been placed where a challenge was known to exist.

APPENDIX V

Summary Information From Shetland Salmon Farmers Association (SSFA) Infectious Pancreatic Necrosis (IPN) Survey Of 2001 Year Class

The historical incidence of IPN is something which has not been consistently measured in great detail up until now. First records of clinical IPN were in 1990 in Shetland, having previously been a disease associated with hatchery and freshwater phases. In that year up to 45% of farms were IPN infected and some were losing up to 35% of stock. However, this was not the only disease issue and mortality levels in general were much higher than nowadays.

Mortality levels due to IPN have been consistently high in Shetland ever since and were particularly bad in 1998 and 1999. The SSFA conducted a mortality survey in 2000, the main conclusion of which was that IPN was not as severe as in previous years. No particular reasons could be identified for this, however it is fair to say fish health in general and growth and survival rates in particular were very good.

Discussions within the scientific community and the veterinarians in 1999/2000 pointed towards the potential existence of a slightly different strain of IPN virus in Shetland. The SSFA at this time lobbied for funding to continue with a research programme based at the FRS Marine Laboratory in Aberdeen to look at the efficacy of vaccines under development and the diagnostic tools necessary to determine virulent strains of the virus.

The mortality levels in late 2000 smolts were high, and when the spring smolt intake was completed the SSFA decided that a thorough survey would help develop the economic impact information useful to the IPN sub group of the AHJWG. It was also thought that this information might further the case for increased research priority and funding.

The scope of the survey was more detailed than those conducted previously, and covered farms on a site by site basis. The survey gathered information on source of smolts (both company and hatchery) losses (number and percentage), intake dates, vaccination status, which stock suffered the first outbreak, transfer problems, insurance claims and all general comments.

The objectives of the survey were to gain information to be shared by the Shetland producers to inform their knowledge of the local situation, and to then widen this out to the rest of Scotland through all appropriate channels.

Summary of survey results

Total input of smolts in spring 2001 was 14,250,000, a slight increase on year 2000 due to lifting of ISA fallowing restrictions.

Total IPN losses were 1,429,000, representing 10% of total intake. The 2000 S1/2 losses were 487,000 representing approx 16% of total.

Losses happened at 30 sites altogether and varied from 1% to 80% of stocks. However, the mid range loss is 20-30%.

Losses began mainly six to eight weeks post transfer, as has been the norm. However in a few cases this was quicker at four-five weeks.

Anecdotally most farmers observe that it is the healthier looking fish that are lost.

In sites holding smolts from more than one source the losses varied but in general, once mortality started, it spread to the whole stock.

The loss equates to an immediate cash value approaching £2 million and to this must be added collateral loss in effect on commercial viability of remaining stock, i.e. when the numbers remaining make the whole site less viable commercially.

There is also an issue concerning insurance risk and it remains to be seen how this will be affected in the future. In many cases losses are below claim level and even when they are above claim level insurance only covers a small part of the actual loss to the farmer.

Appendix VI

Disinfection Stages Required For Wellboats Under Different Operating Circumstances

Extracted from *A Code of Practice to Avoid and Minimise the Impact of Infectious Salmon Anaemia (ISA)*

Operational circumstances	Stage 1	Stage 2	Stage 3
Arriving from outwith UK waters other than from EU waters with equivalent zone status*	X	X	X
Operating within a Surveillance Zone	X		
Leaving a Surveillance Zone on shuttle returns	X		
Leaving a confirmed or suspicious site for any location	X	X	X
Leaving a Surveillance Zone for a new operating location	X	X	X
Operating between sites on shuttle returns	X		
Leaving an existing site to start at a new site	X	X	
General deliveries (non-fish)	X		

NB not all sites will have equal status within a Surveillance Zone

Stage 1 (daily hygiene when working with fish)

Brush/clean solids from all surfaces.

Hot-water pressure clean (with detergent) the following areas:

- deck;
- wells;
- equipment;
- protective clothing;
- pumps.

Following the instructions given in the Disinfection Guide.

Stage 2

Complete stage 1 and carry out the following additional tasks:

- internally inspect and disinfect the fish pump** and remove and clean all organic material from it before carrying out the normal disinfection procedure;
- steam clean and disinfect with iodophor, the deck, well and hull above the waterline;

- complete the checklist (Appendix 1 of Code of Practice);
- sign the checklist (Appendix 1 of Code of Practice) with duplicates for each party. Copies should be retained at reception site for auditing.

Stage 3

Complete all of Stages 1 and 2 and carry out the following additional tasks:

- Slip the vessel, clean and disinfect the hull below the waterline;
- Every wellboat operator should carry out an assessment of the design of each of their wellboats, with regard to the practicalities of efficient cleaning and disinfection. Each wellboat should have its own copy of the current edition of the SDP[2], including any supplements, to take account of particular design features;
- Bus stop deliveries may only be made to an empty site or series of empty sites. This does not preclude delivery to a site containing fish as long as the vessel does not subsequently proceed to another site;
- Wellboats must travel closed (ie no water exchange) when located within 5 km of any fin fish farm site;
- Ballast water must not be discharged within 5 km or one tidal excursion (whichever is greater) of a farm site. This means that ballasting and pump cleaning need to be part of a vessel's passage plan, and are sequential operations;
- Compliance with the above procedures should be audited by the receiving site management using the wellboat movement records, the disinfection logs and the corresponding fish movement records.

*As defined in Directive 91/67/EEC
**The design of pumps must enable routine inspection and disinfection to take place.

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