INFECTIOUS PANCREATIC NECROSIS VIRUS AND ITS IMPACT ON THE IRISH SALMON AQUACULTURE AND WILD FISH SECTORS

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SUMMARY

Infectious pancreatic necrosis (IPN) is an economically significant viral disease of salmonid fish worldwide. Infectious pancreatic necrosis is categorised as a List III disease under Annex A of EU Council Directive 91/67/EEC. List III diseases are present within the EU and up to 2004 were regulated under national control programmes within each member state.

The disease was first described in freshwater trout in North America in the 1950's (Wood *et al*., 1955) and has been reported in Europe since the early 1970's (Ball *et al*., 1971). Initially, IPN was regarded as a serious disease affecting rainbow trout fry and fingerlings (Roberts & Pearson, 2005). However as the salmon farming industry began to expand during the 1970's, incidence of IPN disease in salmon also increased with the result that IPN is now widespread in the salmon farming industry in both Norway and Scotland. The economic loss due to the disease is large and outbreaks may occur in Atlantic salmon juveniles in fresh-water and in post-smolts after transfer to sea-water.

Historically in Ireland, isolations of the IPN virus have been rare and occasional outbreaks have occurred in both rainbow trout and Atlantic salmon facilities. The Marine Institute and its predecessor, the Fisheries Research Centre, have been testing farmed and wild fish for disease pathogens since the mid 1980's. The first reported clinical outbreak of IPN in Atlantic salmon occurred in 2003. However in 2006 severe outbreaks in a number of freshwater salmon hatcheries occurred which were all linked to imports from a specific single source. To date, clinical outbreaks of IPN in Ireland have been associated with imports of infected ova and their subsequent movement within the country. This report reviews the prevalence of the IPN virus in the Irish salmon farming industry and also in wild fish from selected rivers. It describes the steps taken by the industry to control the disease in 2006 and aims to provide some practical solutions to reduce the prevalence of the virus in farmed and wild fish and to prevent future outbreaks of the disease.

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FOREWORD

In early 2003, the Marine Institute asked Dr. Marian McLoughlin, a specialist in Fish Health, to write an article for the trade press on ""***IPN Knocking on our Door***". By the time the article was published in Aquaculture Ireland (June/July 2003), the title of Marion's article had to be updated to "***Infectious Pancreatic Necrosis has Arrived on Ireland's Shore***".

This report sets out to document the sequence of events and the lessons leaned since 2003 in the management of IPN in the Irish aquaculture industry. During this time, staff from the Fish Health Unit of the Marine Institute were active in providing detailed scientific advice to the regulatory bodies (DCMNR) and industry on the management of IPN, with a special focus on the following topics:

- Policy implications of taking up the Additional Guarantee (AG) for IPN, which was granted by the EU Commission in early 2004, under Article 13 of Directive 91/67/EEC as well as disease implications if IPN was uncontrolled.
- Drafting and evolution of an IPN Code of Practice
- Risk Assessment approach to the testing and imports into Ireland of salmonid eggs
- Risk Assessment on the management of clinical or sub clinical IPN at Irish hatchery and seawater sites.
- Advice on biosecurity and husbandry measures on sites infected with IPN, to minimize the risk to wild or farmed fish in the general vicinity.

The management approaches taken in the absence of a legal framework for the control of IPN were not always successful and the Irish industry has been adversely impacted by losses due to IPN. This report seeks to capture the evolution of IPN in Ireland and to provide a context for the way in which it was managed in Ireland in 2006.

As with each of our scientific publications, our aim is to make the information available to the research community, to fish health professionals and to a broader audience of fish farmers and wild fish interests.

We look forward to working with industry and policy makers to ensure that a robust framework of monitoring, import analysis and risk management is developed to minimize the risks from infectious disease on the Irish aquaculture and inland fisheries sectors.

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1. THE STATUS OF IPN IN IRELAND & GLOBAL AQUACULTURE

1.1 Ireland

In late 2003, several Member States including Ireland and the UK, applied to the EU Commission for Additional Guarantees (AG) for certain diseases, including infectious pancreatic necrosis (IPN). In early 2004, the EU Commission granted Ireland the AG for IPN under Article 13 of Directive 91/67/EEC. This AG would allow the Irish Authorities to control IPN and to legally request certification of freedom from IPN for imports of live fish and ova. At the same time, the UK Authorities had also applied for an AG for IPN which was not granted. As a result, acceptance of the AG by Ireland would have led to the establishment of a trade barrier between the two countries.

Following discussions with industry, it was agreed in February 2004 that the Department of Communications, Marine and Natural Resources (DCMNR) would not pursue the IPN AG, provided an IPN Code of Practice was devised and implemented. A number of drafts of the Code of Practice were circulated to industry by the Marine Institute (MI), the last of which was distributed in July 2004 (Appendix I). Consensus on the implementation of the IPN Code was not achieved. However through the Irish Fish & Shellfish Health Advisory Committee (IFSHAC) it was agreed to work on the development of a generic Fish Health Code of Practice incorporating a range of diseases including IPN. In the meantime, industry decided to manage IPN in consultation with their veterinary practitioners. The MI continues to encourage adherence to the principles of the draft IPN Code of Practice and recommends that adequate testing and bio-security measures are implemented ahead of any importation of live fish or ova. The use of a 'management cell' approach to importations has also been put forward. Such a platform would have representation from industry, DCMNR and the MI.

In Ireland, wild and farmed fish have been tested for the presence of disease pathogens since the mid 1980's. Despite the ongoing problems associated with IPN in both Norway and Scotland, the IPN virus was rarely isolated in Irish salmon and trout farms (Figure 1; Appendix II), and no clinical signs of disease were officially reported for farmed salmon[1]. The first clinical outbreak, reported to the Marine Institute, occurred in the spring of 2003 in salmon fry which resulted in the fish being culled. Information on the isolation of the virus and the number of reported clinical outbreaks are shown below[2].

In 2006, clinical outbreaks of IPN occurred in five salmon hatcheries (Figure 2). All five hatcheries had imported ova from a single source in Scotland. Industry (consisting mainly of those affected by IPN) sought advice from the MI and four meetings were held to discuss information on the disease, the current situation and possible alternative sources of ova/fish for the future. These meetings were held at the Marine Institute, Galway on May 15, May 31, June 9 and June 21, 2006. Since that time, all outbreaks were controlled through a combination of increased biosecurity, regular removal of mortalities and culling of infected tanks and risk assessments prior to the movement of fish. Mobile hatchery units were provided by BIM to allow infected hatcheries to be cleaned, disinfected and fallowed.

[1] Isolation of the IPN virus does not always result in a clinical outbreak of IPN disease.

[2] Data is shown from 1993 as this represents the first year of testing under the EU Directive 91/67 which governs disease monitoring in aquaculture (although diagnostic samples were regularly tested prior to 1993). Data for 2007 is correct as of July 2007.

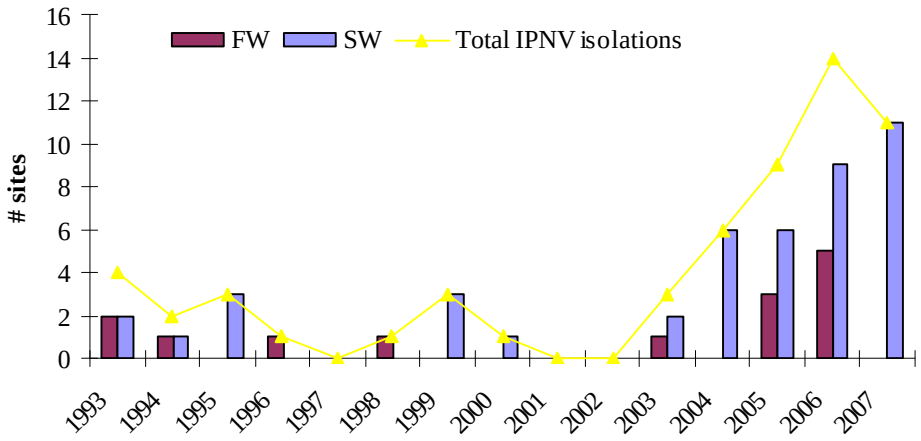

Figure 1. Incidence of the IPN virus in Ireland (1993 – 2007). The figure details the number of IPNV isolations in freshwater (FW) and marine (SW) Atlantic salmon sites.

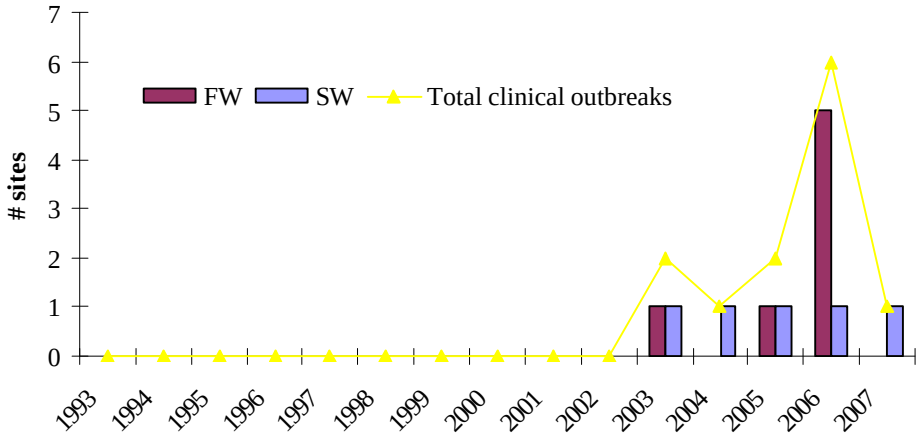

Figure 2. The number of reported clinical outbreaks of IPN in Irish salmon farms, 1993 – 2007. The figure details the number of clinical cases in freshwater (FW) and marine (SW) Atlantic salmon sites.

For 2007, no ova were imported from Scotland and alternative sources were located. This has resulted in no IPN virus isolations from freshwater sites thus far in 2007. Marine isolations of the virus showed a slight increase and are possibly due to the movement of the virus as the 2006 fish are moved to the marine sites. As there has been only one reported outbreak of IPN in 2007 it would appear that the management procedures implemented have had a positive effect on preventing serious losses due to the disease.

1.2 Scotland

IPN was first diagnosed in Scotland in 1971 at a rainbow trout farm in Loch Awe (Ball *et al.*, 1971) and has since become widespread in trout farms throughout the UK and is increasing in prevalence in both freshwater and marine salmon facilities (Anon., 2003a). From 1989, losses began to occur in the Shetland Isles and throughout the rest of Scotland during the 1990's (Smail *et al.*, 1992). Under the Diseases of Fish Acts 1937 and 1983 those involved in the aquaculture industry or who have care of water bodies were obliged to notify the Scottish Ministers if they suspected that waters became infected with IPNV. In 1995, a review of the controls for IPN concluded that trout and other freshwater fish, except salmon, should be exempt from official controls. This was mainly due to the high prevalence of IPNV in trout farms (including England and Wales) and the low economic impact of IPN for the trout industry (Anon., 2003a). However, the Scottish Salmon

Growers Association took the view that controls for IPN should be maintained in salmon, as only 22% of Scottish salmon farms were infected with IPNV at that time. This meant that salmon freshwater facilities had to undergo regular inspections for IPN by the Fisheries Research Services (FRS) Fish Health Inspectorate. Controlling the spread of IPN was mainly carried out through the issuing of a Thirty Day Notice or a Designated Area Order. Once issued, no live fish or ova could be taken into or out of aquaculture facilities within that area until a full investigation or eradication of the disease had been completed (Anon., 2003a).

When the UK failed to obtain an AG for IPN under Commission Decision 2004/453/EC, the Scottish Executive having no legal basis for controlling IPN, revoked movement controls on salmon farms with IPN. This effectively led to the deregulation of IPN in Scotland from early 2005. The FRS has kept records of IPN prevalence from 1996 – 2002. Although the prevalence of the virus in freshwater sites is lower than in sea water sites, prevalence has increased for both freshwater and sea water sites throughout this period. In freshwater, prevalence increased from < 10% in 1996 to 26% in 2002 while in sea water sites the prevalence went from ~ 30% up to 82% in the same period (Murray *et al*., 2003). The most affected area is Shetland (Smail *et al*., 2006) where the virus is assumed to be ubiquitous in the marine environment. With regard to clinical outbreaks of IPN, a similar increasing pattern has been observed. In 2002, 39 cases were recorded in Scotland, up from one case in 1996 and six in 1990 (Bruno, 2004). A survey by the Shetland Salmon Farmers Association in 2001 showed an average loss due to IPN of 20 – 30% in salmon post-smolts, which equated to an immediate cash value for that year of £2M, but this was seen as an underestimation (Anon., 2003a).

1.3 Norway

According to a report published by VESO (Anon., 2003b), IPN has been described as the biggest health problem for the Norwegian aquaculture industry and a real possibility exists that cases of clinical IPN outbreaks have been under reported. Statistics released by the National Veterinary Institute of Norway showed that in 2005 there were 208 cases of clinical IPN, compared to 35 cases of pancreas disease (Bornó *et al*., 2006). In Norway, IPN is listed as a Group B (II) disease, which means that it is notifiable and restrictions on movement of fish apply. Detection of the IPN virus alone does not trigger restrictions for any fish farm, this can only occur when clinical signs of disease are found. The reason for this is that the IPN virus is widespread in Norwegian aquaculture production leading to frequent virus detections without any signs of clinical disease.

The IPN virus was first isolated in Norway in 1975 (Håstein & Krogsrud, 1976), from rainbow trout in freshwater. From the mid 1980's severe mortalities due to IPN were seen in Atlantic salmon post-smolts. Throughout the 1990's a number of epidemiological studies were carried out on Norwegian hatcheries (see Anon, 2003b). IPN outbreaks were recorded in 30 – 40% of all hatcheries, with the majority of outbreaks occurring between March and July. Over half of the outbreaks affected parr less than 20g in weight. During the period 1994 – 2000, 40 – 70% of all seawater sites experienced IPN outbreaks with an average mortality ranging between 10 – 20%. As a result of the open national market for smolt purchase and distribution in Norway, it is possible that all salmon sea farms harbour positive carriers. The estimated annual cost to the Norwegian aquaculture industry is €75 – 100 M (Anon, 2003b).

1.4 Other countries

The IPN virus is widespread throughout the salmon and trout farming countries of northern Europe (Ariel & Olesen, 2002) although Sweden and parts of Finland are declared IPN-free zones (Commission Decision 2004/453/EC). In southern Europe, the virus is known to occur in Spain (Cutrin *et al*., 2000) and has been isolated from rainbow trout in Greece (Varvarigos & Way, 2002) and Turkey (Candan, 2002). In North America, IPN is endemic in New England and in the Canadian Maritime Provinces. However the virus has generally not been found to be a major source of mortality of salmon and is managed under national Fish Health Protection Regulations in Canada. A Buhl strain of IPNV (belonging to the West Buxton A1 serotype) was isolated from clinical rainbow trout in Mexico (Ortega *et al*., 2002) and the IPN virus has been known to occur in Chile for many years (McAllister & Reyes, 1984). Crane *et al*. (2000) isolated an aquatic birnavirus closely related to the IPN virus from asymptomatic farmed Atlantic salmon in Australia.

- IPN is a serious problem for Norwegian and Scottish salmon aquaculture
- Due to the widespread nature of the virus, IPN has effectively been deregulated in Scotland since 2005 and is currently regulated at the clinical outbreak level in Norway
- The first reported clinical outbreak of IPN in farmed salmon in Ireland occurred in salmon fry in 2003
- Six clinical outbreaks were reported in 2006 in Ireland, five of these were in freshwater facilities
- Due to a concerted effort by industry and state agencies, no IPN outbreaks have occurred in freshwater in 2007

2. A REVIEW OF IPN RESEARCH

2.1 Characteristics of the IPN virus

Infectious pancreatic necrosis virus (IPNV) is a member of the Genus *Aquabirnavirus*, family *Birnaviridae*. Aquatic birnaviruses have a wide host range infecting many species of fish. Apart from salmonids they have been isolated from fish belonging to over 32 different families, 11 species of molluscs and four crustacean families (Hill & Way, 1995).

The genome of IPNV consists of two segments of double stranded RNA, enclosed within a single-shelled icosahedral capsid measuring 60 nm in diameter (Dobos, 1995). Genomic segment A encodes all the structural (VP2 and VP3) and non-structural proteins whereas Segment B encodes the viral RNA-dependent RNA polymerase (VP1). Most aquatic birnavisuses, regardless of host or geographic location, are antigenetically related and belong to a single serogroup A. Serogroup A has been divided into nine serotypes: A1 – A9 (Romero-Brey *et al*., 2004). The A1 serotype contains most of the isolates from the United States (reference strain West Buxton); serotypes A2 to A5 are primarily European isolates (reference strains Sp, Ab, Hecht and Tellina). Serotypes A6 to A9 occur in Canada (reference strains C1, C2, C3 and Jasper). Serogroup B comprises one serotype isolated from molluscs (Hill & Way, 1995). Blake *et al*. (2001) compared the nucleotide and deduced amino acid sequences of a number of aquabirnaviruses representing the nine serotypes mentioned above. These aquatic birnaviruses clustered into six genogroups and generally correlated with geographical origin and serological classification.

Within the various serotypes/genogroups, there is a high degree of antigenic variability and differences in the virulence and pathogenicity among the strains. Virulence of the IPNV has been associated with segment A (Sano *et al*., 1992) and in particular with the viral protein 2 (VP2) structural protein (Bruslind & Reno, 2000; Shivappa *et al*., 2004). Viral protein 2 is a major capsid protein and is responsible for the production of type-specific monoclonal antibodies. It has been hypothesised that variations in the amino acid residues of this protein may be associated with changes in virulence (Shivappa *et al*., 2004). In fact, by a comparison of the deduced amino acid sequences of various field isolates exhibiting different mortality in Atlantic salmon fry, the putative motifs involved in virulence of IPNV Sp strains have been proposed. Virulent strains typically have residues threonine, alanine, threonine/alanine, and tyrosine/histidine at positions 217, 221, 247 and 500 of the VP2 gene (Santi *et al*., 2004). Further work has shown that virulent isolates possess residues Thr217 and Ala221; moderate to low virulent strains have Pro217 and Ala221; and strains containing Thr221 are almost avirulent, irrespective of the residue at position 217 (Song *et al*., 2005). These findings are summarised in Table I.

Table 1. Key amino acid positions in the VP2 protein and their association with virulence (Santi *et al*., 2004; Song *et al*., 2005).

	217	221	247	500
Virulent	T	A	T/A	T/H
Moderate/Low	P	A	-	-
Avirulent	-	T	-	-

(-) implies that virulence is independent of the amino acid residue at that position.
T (Threonine); A (Alanine); Y (Tyrosine); H (Histidine); P (Proline).

2.2 Pathology

Freshwater Stage

IPN outbreaks may occur in juvenile salmon up to four weeks after the onset of exogenous feeding. Mortalities at this stage can be as high as 90% and infected fry may show typical signs such as a darkening of the skin colour, swimming high in the water column or lying on their side and hyperventilating (Roberts & Pearson, 2005). Histopathological features include a severe necrosis of the pancreatic acinar cells along with necrosis of the intestinal mucosa and the liver.

Mortalities at the parr stage tend not to be as severe as with fry and are normally in the order of 10 – 20%. Distinctive features include a dark colouration, abnormal swimming behaviour (whirling, lying on the side and swimming with the head pointing upwards) and hyperventilating. The liver of infected fish is generally a pale yellowish colour and the intestine contains white/yellow exudates often referred to as 'catarrhal'. These symptoms are accompanied by extensive necrosis of the pancreatic acinar tissue, extensive necrosis of the liver tissue often characterised by loss of all cellular architecture (Roberts & Pearson, 2005). The intestinal mucosa often remains intact, although focal necrosis can be common.

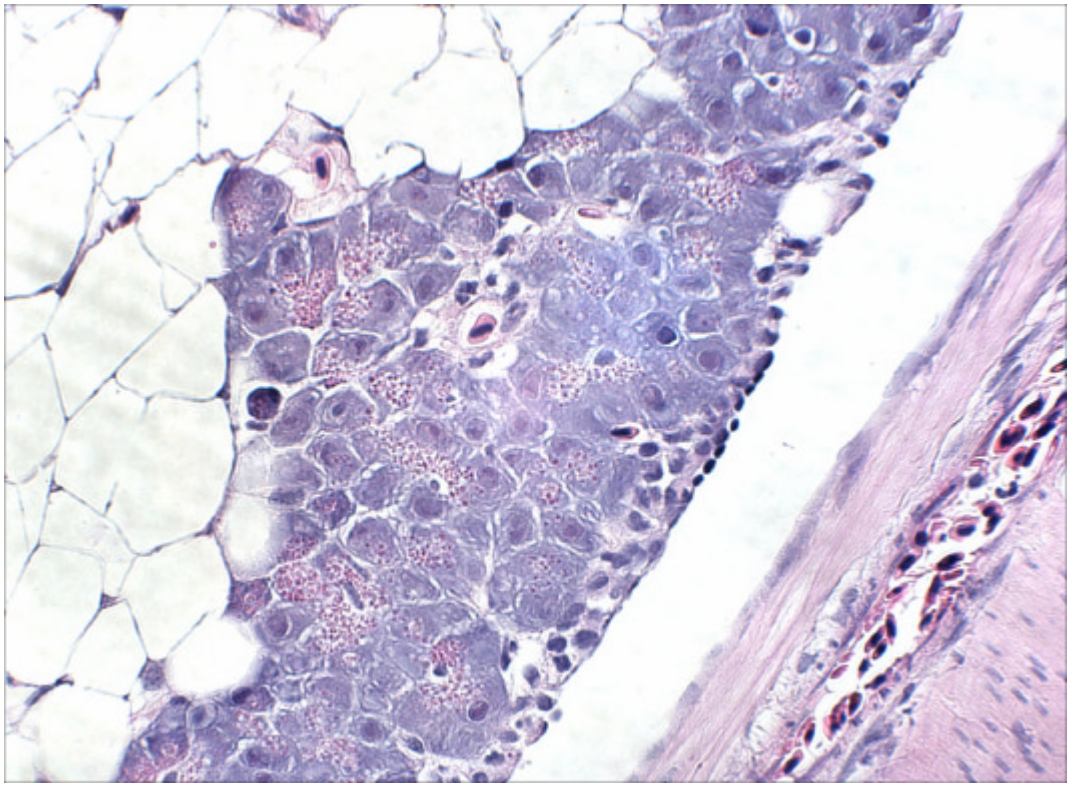

Figure 3. Early stage pyknotic acinar cells in an IPN infected Atlantic salmon post-smolt.

Marine Stage

Fish which have recovered from a freshwater outbreak of IPN do not generally succumb to a second infection of IPN once transferred to sea. A small number of apparently healthy fish however, fail to feed and die off a couple of weeks post transfer. If smolts undergo an IPN infection at sea, it is generally 2 – 3 months after transfer to sea (Bruno, 2004). Losses may reach more than 50%, however typical ranges are between 10 – 25% (Roberts & Pearson, 2005). Fish may stop feeding, exhibit abnormal swimming behaviour and a high number of moribund fish are often observed. No obvious signs can be observed on internal organs, apart from a pale liver and extensive 'catarrhal' exudate in a gut devoid of food. Histological signs are similar to those found in infected parr, but are often more acute. The liver can show severe focal necrosis with necrotic areas commonly found in the haemopoietic tissue of the kidney. The pancreatic acini are largely destroyed and sections

are characterised by numerous pyknotic nuclei (Figure 4). The intestine can lose the mucosal epithelium or alternatively appear intact with large numbers of individual McKnight cells being expelled through the mucosa. McKnight cells are characteristic of IPN infections and are apoptotic mucosal epithelium cells (McKnight & Roberts, 1976). Survivors of an IPN outbreak generally show fibrous replacement of the exocrine pancreas (Bruno, 2004).

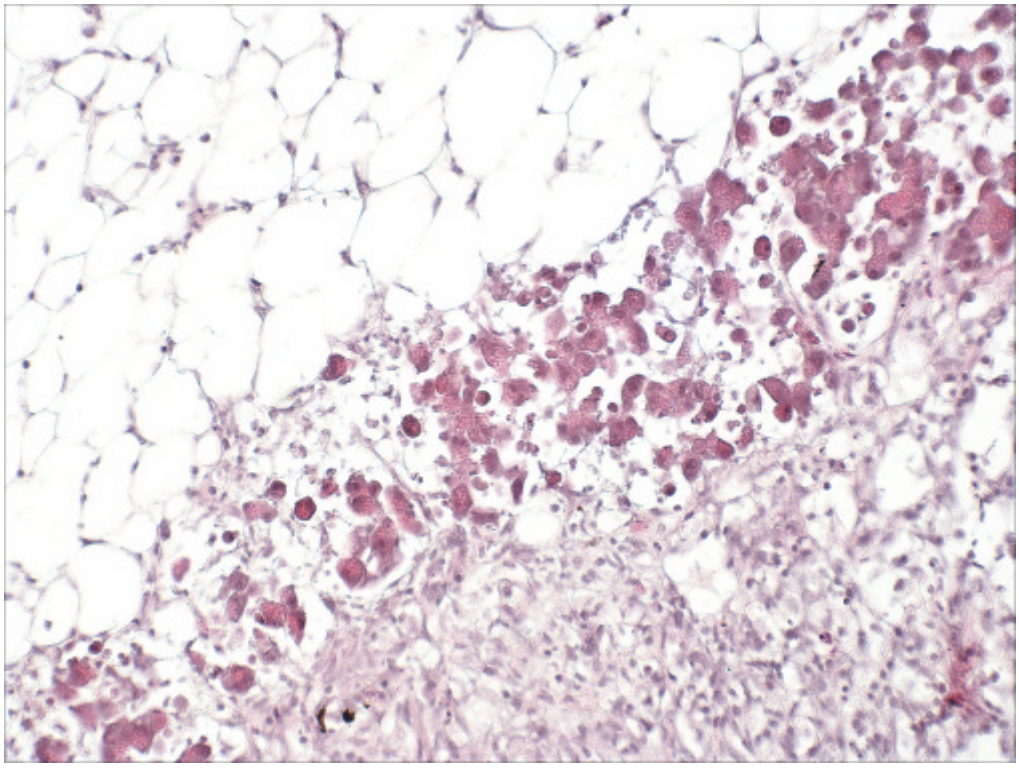

Figure 4. Acute pancreatic necrosis in an IPN infected Atlantic salmon post-smolt.

2.3 Host response

Teleost fish are equipped with an innate antiviral defence mechanism based on the production of interferon. Interferon's are proteins which are produced by virus infected cells and transported through the blood to stimulate other cells to produce interferon and other anti-viral proteins such as Mx protein (Robertsen, 2006). It has been shown that replication of IPNV is inhibited by Atlantic salmon Mx1 protein (Larsen *et al*., 2004) although the exact mechanism of inhibition is unknown; it is believed that Mx proteins can disrupt viral replication in infected cells. Although it has been shown that interferon and Mx proteins are produced in Atlantic salmon infected with IPNV (McBeath *et al*., 2007) no correlation between survival and the immune response has been found to date (Lockhart *et al*., 2007). Studies with rainbow trout showed that innate IPNV inhibitors in the serum showed characteristics similar to those of lectins and consistent inhibition was not obtained until the rainbow trout had reached the age of 23 weeks post-hatch suggesting, that newly hatched fry were more susceptible to infection (Park & Reno, 2005). Antiviral cytotoxic cells, capable of destroying virus infected cells, are also present in teleost fish and play an important role in any immune response to viral infection (Ellis, 2001).

The specific immune response is developed later in the course of infection and is dependent on temperature. This stage of the immune response is characterised by the production of specific antibodies by B-cells against the pathogen. A challenge experiment with Atlantic salmon (Rønneseth *et al*., 2006) showed that IPNV infection did not alter the level of B-cells, but led to a significant reduction in the level of neutrophils. Although these interactions between the virus and the immune system of the fish host are poorly

understood, suppression of the immune system would prolong the survival of the virus. Some studies have shown that the IPN virus has suppressive effects on macrophage respiratory burst activity (Johansen & Sommer, 1995a) and B-cell proliferation (Novoa *et al*., 1996). While much work is still needed in the area of host-pathogen interactions, it appears that the virus has evolved elaborate mechanisms to protect itself whilst in the host cells.

2.4 Transmission

Vertical Transmission

Vertical transmission denotes germ-line associated transmission of virus via ova or milt and can occur through extra- or intra-ovum transmission. Extra-ovum transmission on the surface of the gametes can be readily stopped through the proper disinfection procedures and a thorough biosecurity procedure should adequately protect the site from infection. However intra-ovum transmission could prove to be a greater risk and can only be controlled through rigorous testing of broodstock.

Vertical transmission by the intra-ovum route is known to occur in salmonid species such as rainbow trout (Ahne & Negele, 1985) and brook trout (Bootland *et al*., 1991). Evidence also exits which suggests that the seminal fluid of rainbow trout can transmit the virus resulting in infection of the progeny (Ahne, 1983). Research on brook trout showed that the sampling of reproductive products and the visceral organs (pyloric caecae/pancreas and kidney) should be carried out to determine the carrier state of potential broodstock (Bootland *et al*., 1991). Therefore in the case of brook trout, even if the reproductive products of IPNV-carrier fish are free from the virus, these fish should not be used for spawning purposes as the potential for vertical transmission of IPNV to progeny exists. To date, true vertical (intra-ovum) transmission has not been shown to occur in Atlantic salmon. Laidler (2002) described a number of studies investigating vertical transmission in Atlantic salmon. It was shown that the IPN virus could be transferred to the egg via infected milt and by experimentally infecting female broodstock with high doses. However, the virus did not persist in the eggs and was not found two weeks after the experimental infections.

Horizontal Transmission

Horizontal transmission of IPN can be defined as the lateral spread of the IPN virus. It is well known that survivors of an IPN outbreak can become lifelong carriers of the virus resulting in those fish being an important reservoir of virus. During an outbreak of IPN, virus is shed from dead and moribund fish into the waters around a farm, as well as in the faeces and urine of asymptomatic carriers (Hill, 1982; Mangunwiryo & Agius, 1988). Infected fish can act as reservoirs without showing clinical signs of disease and recent studies have shown that the virus in these asymptomatic carrier fish is associated with leucocytes in the blood and kidney (Johansen & Sommer, 1995b; Munro *et al*., 2006).

Survival of the virus in the water is an important aspect of horizontal transmission, in that the virus needs to remain infective for a suitable length of time in order to reach and infect a susceptible host. Virus levels between $10 – 10^4$ pfu mL^{-1} (Munro *et al*., 1976; McAllister & Bebak, 1997) have been found in the water effluent of IPN infected rainbow trout hatcheries. Although one can expect a dilution effect in a river system, these ranges of virus titres have induced mortalities in salmon under experimental conditions (Bowden *et al*., 2002). Survival of the virus in fresh and marine water has been studied by a number of authors and it has been shown that temperature plays a significant role in determining the length of time the virus remains infective. Barja *et al*. (1983) reported that IPN virus remained infective for up to 20 days in freshwater at 15°C, but only 15 days at 20°C. In a

similar study, Toranzo *et al.* (1983) showed inactivation of the virus after 17 days at 15°C and 9 days at 20°C. The virus appears to be more stable in marine water which will have consequences for the persistence of infections on marine sites. Temperature (15 or 20°C) had no effect on the survival of the virus in seawater with reported survival of 20 days and 14-17 days reported by Barja *et al*. (1983) and Toranzo *et al*. (1983) respectively.

In a study by Dorson & Torchy (1981), rainbow trout alevins were infected with the IPN virus at 6, 10 and 16°C. The lowest mortality occurred at 16°C and infecting fish at 5°C only delayed the onset of mortality. When the alevins were moved from 10 to 16°C before infection, mortality was reduced, but not when fish were moved from 16 to 10°C. This suggests that thermal manipulation of the water may help in controlling IPN outbreaks as has been reported for another viral disease, infectious haematopoetic necrosis (Amend, 1976). It is believed that thermal manipulation works by assisting the onset of the fish immune response and/or attenuating the virus.

A range of vectors and reservoirs can transfer virus between freshwater and sea water sites inc. wild fish, birds & mammals, transport equipment and farmed escapes. Piscivorous birds predating on rainbow trout fry infected with IPN were shown to excrete IPNV in their faeces (McAllister & Owens, 1992). The IPN virus was also detected in faeces 72 h after feeding a contaminated fish silage mixture (with normal grass silage) to cows (Smail *et al*., 1993b). The virus was not detected in the faeces after four days. Sea lice have been shown to act as vectors of infectious salmon anaemia virus (Nylund *et al*., 1994), however it is not known if they also transmit IPNV.

2.5 Methods of Detection

The IPN virus may be isolated during routine sampling of fish showing no clinical signs of disease reflecting a carrier state in the fish previously exposed to the virus at some stage in the production cycle. The screening procedure for IPNV is based on virus isolation in cell culture, followed by a secondary confirmatory test e.g. neutralisation, ELISA, RT-PCR etc.). For a full diagnosis of clinical disease on site, reporting of virus isolation should be combined with information on mortalities, visible signs of disease and histopathological findings.

Fish material suitable for virological examination includes:

- Asymptomatic fish (healthy): organs (liver, kidney, spleen, brain)
- Broodstock: milt, ovarian fluid, organs[3] (e.g. kidney)
- Clinically affected fish: alevins < 4 cm (whole body); fry 4 – 6 cm (viscera); fish > 6 cm (organs)

The presence of the virus can be confirmed in a sample within days, depending on the level of the virus titre in the original sample. Correct storage of the sample material is an important factor. The IPN virus is known to be stable under most storage conditions e.g. temperatures from -80°C to +20°C; salinities from 0 - 40‰. Mortensen *et al*. (1998) showed that repeated freezing and thawing of a sample resulted in a reduction of the virus titre which was greater in samples stored at -80°C than at -20°C. A five log reduction in titre was seen after ten freeze-thaw cycles in samples stored at -80°C, compared with a two log reduction in those stored at -20°C. It was also recommended that samples should be inoculated onto cell lines immediately after homogenisation.

[3] It is recommended to use kidney testing when screening broodstock due to the higher detection level of IPNV positive fish (see Appendix III).

Standard protocols at the Fish Health Unit, MI, recommend that the samples are inoculated onto cell cultures for a seven day period and passed onto a fresh cell line for a further seven day period (14 days if necessary). Therefore confirmation of the virus may take between one and three weeks. The effectiveness of screening for any fish pathogen will rely considerably on the sensitivity and precision of the diagnostic procedures applied. In a study initiated by the EU Community Reference Laboratory in 1995 and involving 11 laboratories, the sensitivity of a range of common cell lines to IPNV (amongst others) was examined (Lorenzen *et al*., 1999). It was found that the most sensitive cell lines for the isolation of IPNV were BF-2 (fibroblast cell line from caudal trunk of bluegill *Lepomis macrochirus* fry; Wolf *et al*., 1966) and CHSE-214 (epitheloid cell line from Chinook salmon *Oncorhynchus tshawytscha* embryo; Fryer *et al*., 1965) cells.

A number of studies have been carried out in recent years developing new rapid methods for the detection of IPNV. A staphylococcal coagglutination (COA) test was evaluated by Taksdal & Thorud (1999). The COA test was found to be suitable for detecting outbreaks of IPN. However covert infections were not detected as the limit of detection of the test was 10^5 $TCID_{50}$ mL^{-1}. A similar COA test was found to be unsatisfactory by Rodriguez Saint-Jean *et al*. (2001) who compared six diagnostic methods for the detection of IPNV. They found that RT-PCR and flow cytometry were the most sensitive methods followed by immunofluorescence and immunoperoxidase methods. In recent years the use of molecular tools for detecting fish pathogens has increased and a range of polymerase chain reaction (PCR) based assays are available for detecting IPNV (Taksdal *et al*., 2001; Barlic-Maganja *et al*., 2002; Shivappa *et al*., 2004)

A full detailed account of the standard methods used for detection of IPNV can be found in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE, 2003). However it must be noted that IPN is no longer listed by the OIE and the information available has not been updated since 2003.

- A wide range of IPN virus isolates exist ranging from avirulent to highly virulent
- IPN can cause significant mortalities in both the freshwater and marine stages of the production cycle
- Vertical transmission of IPNV is known to occur in some salmonid species, however true intra-ovum transmission has not been scientifically proven in Atlantic salmon
- The IPN virus can be spread from farm to farm by infected fish, farm personnel & equipment, and through the water

3. IPNV IN FARMED FISH

3.1 The prevalence of IPNV in Ireland, 1993 – 2007

This section deals with the prevalence of IPNV in Irish farmed salmon over the period 1993 – 2007*. From the data it can seen that up until 2003, all isolations of IPNV in Atlantic salmon were non-clinical (see Figures 1 & 2). Post 2003, all clinical outbreaks of IPN occurred in hatcheries or marine sites which had imported ova/fish from a single source. A list of all IPNV isolations is shown below.

Table 2. A list of isolations of IPNV in Ireland, 1993 – 2007*. Results are based on diagnostic tests carried out by the Fish Health Unit, Marine Institute.

Year	FHU ref.	FW/SW	Species	Year	FHU ref.	FW/SW	Species
1993	484	FW	Salmon	2005	2921	SW	Salmon
	561	SW?	Salmon		2925	FW	Salmon
	631	SW	Salmon		2926	SW	Salmon
	640	SW	Salmon		2931	SW	Salmon
1994	737	SW	Salmon	2006	n/a	FW	Salmon
1995	885	SW	Salmon		3202	FW	Salmon
	957	SW	Salmon		3088	FW	Salmon
	892/3	SW	Salmon		n/a	FW	Salmon
1996	1050	SW	Salmon		n/a	FW	Salmon
1998	1619	FW	Salmon		3102	SW	Salmon
1999	1703	SW	Salmon		3104	SW	Salmon
	1768	SW	Salmon		3107	SW	Salmon
	1719	SW	Salmon		3108	SW	Salmon
2000	1935	FW	Brown trout		3109	SW	Salmon
	2017	FW	R. trout		n/a	SW	Salmon
	1902	SW	Salmon		3117	SW	Salmon
2003	2427	FW	Salmon		3118	SW	Salmon
	2463	SW	Salmon		3135	SW	Salmon
	2492	SW	Salmon	2007*	3340	SW	Salmon
2004	2723/53	SW	Salmon		3345	SW	Salmon
	2709/30	SW	Salmon		3346	SW	Salmon
	2758/59/60	SW	Salmon		3350	SW	Salmon
	2711/32	SW	Salmon		3351	SW	Salmon
	2713/31	SW	Salmon		3356	SW	Salmon
	2712/33	SW	Salmon		3357	SW	Salmon
2005	2853/2927	SW	Salmon		3359	SW	Salmon
	2868/88	FW	Salmon		3360	SW	Salmon
	2882	FW	Salmon		3361	SW	Salmon
	2916	SW	Salmon		3365	SW	Salmon
	2917	SW	Salmon				

*Results for 2007 are correct as of July 2007.

Sequencing of Irish IPNV Isolates

In order to determine the relatedness of the virus isolates from Irish salmon farms, viral RNA was extracted from cell scrapes which had visible CPE (indicating virus growth) using the TRIZOL method. A one step RT-PCR kit (Qiagen) with the primers A-Sp500F and A-Sp1689R were used to generate an 1100 kb PCR product covering the VP2 and VP3 coding region of segment A (Santi *et al*., 2004). The PCR product was used for direct sequencing by a commercial company (Sequiserve). A number of isolates had also been sequenced in collaboration with the Norwegian School of Veterinary Science.

As mentioned previously (Section 2.1), mortalities due to IPN can vary depending on a number of factors including the virus strain. Virulence of the IPN virus has been associated with VP2 and the amino acid sequence of this protein has been used to identify whether an isolate of the virus is virulent or not (Song *et al*., 2005; Table I). In general, the amino acid

threonine when located at position 217 is associated with virulence, while proline at this position is found only among isolates of a lower virulence (Santi *et al*., 2004). To date, no IPNV isolates with the highly virulent motif (e.g. TATY) have been found in Ireland. All Irish isolates have the PAAH/Y or PTAY motifs which according to (Song *et al*., 2005) are either moderate to low virulent or avirulent isolates. However, Smail *et al*. (2006) showed that a Scottish isolate (975/99) was highly virulent both in the field and in experimental infection trials. The amino acid motif of this strain has Pro217 and Ala221 which would classify it as a strain of low virulence according to Santi *et al*. (2004). Clearly the host-virus interactions determining virulence will depend on a range of factors other than the genotype of the virus.

Sequencing of the Irish IPNV isolates has shown that they are all closely related (consensus sequences range from 98 – 100 % similarity); however they can be subdivided into two groups (Figure 5). The grouping on the right of Figure 5 consists of one hatchery (FW2) and four marine sites (SW 1, 4, 5 & 12). These sites were stocked with salmon which originated from Irish stock (although SW1 and SW12 have stocked both Irish and imported stock) and no IPN mortalities have been recorded. The isolation of the virus on the site FW2 (F3286P11_FW2_2007) was from broodstock fish which may have picked up the virus at sea. The remaining group on the left side of Figure 5 were stocked with salmon which had originated from outside Ireland (primarily UK) and a number of these sites have reported losses due to IPN. The exception is site SW6, a recently reopened site stocked with Irish stock. Although a number of IPN virus isolations were recorded in the bay during the 1990's, it has been fallow for the last three years. Therefore the origin of the virus isolate remains unknown at this time.

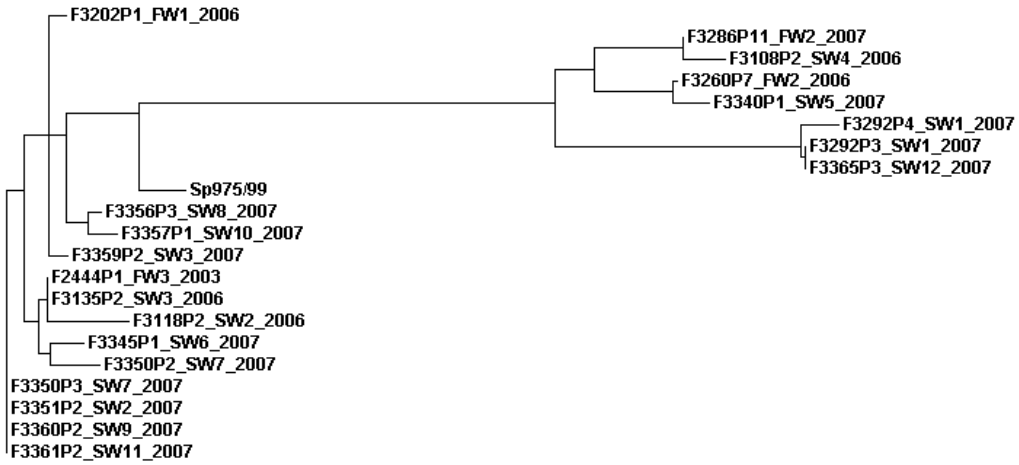

Figure 5. A phylogram illustrating the relatedness of IPNV isolated from Atlantic salmon production facilities in Ireland. Each isolate is identified by the MI reference number followed by, freshwater (FW) or marine site (SW) site and year of isolation. The sequences are also compared with a previously published sequence of IPNV Sp975/99 from Scotland.

It should be noted however that many sites have stocked fish from sources both within and outside Ireland in recent years which could result in both isolate subtypes coexisting.

3.2 Modelling the Spread of IPNV in Ireland

This section was written in collaboration with Dr. A. G. Murray, Fisheries Research Services Marine Laboratory, Aberdeen, Scotland.

Historically, Ireland has been free of IPN disease. However in the last number of years the incidence of the virus has been steadily increasing together with reports of clinical outbreaks of disease (Figures 1 & 2). The aim of this section is to use data collected through routine inspections by the Fish Health Unit to analyse the spread of the IPN virus within the salmon farming industry, utilising a model developed for a similar analysis of Scottish data (Murray, 2006a). Modelling the spread of disease and disease causing pathogens has been extensively applied to human diseases (Anderson & May, 1991) and has been a major focus in agriculture (Kao, 2002). It is only in recent years that modelling has been used to describe the spread of aquatic diseases (Murray *et al*., 2001; Murray, 2006a; 2006b).

Under EU Directive 91/67 all Irish freshwater hatcheries and smolt sites are routinely sampled annually for the presence of diseases listed in the Directive, including IPN. The time period analysed in this study is from 1994 – 2006, divided into freshwater and marine sites (Table III). Isolations of the virus prior to 1994 have not been included in the model as prevalence in Ireland was very low.

Table 3. Surveillance results for IPNV from Irish salmon farms 1994 – 2006.

Year	Freshwater sites	Positives	Marine sites	Positives
1994	26	1	7	0
1995	30	0	22	3
1996	28	0	26	1
1997	36	0	23	0
1998	29	1	25	0
1999	25	0	20	3
2000	30	0	23	1
2001	26	0	23	0
2002	24	0	23	0
2003	22	1	21	2
2004	21	0	14	6
2005	19	3	17	6
2006	16	5	16	9

This data was collected using samples from over 18,000 fry/parr in freshwater and 8,600 salmon from marine sites. The majority of samples consisted of 30 fish in pools of ten or five; however sample size could vary up to 150 fish and an individual site may have been sampled more than once per year. Any sample from a site containing at least one positive pool meant that the site was regarded as positive for IPNV for that year. The screening procedure for the identification of IPNV positive samples was primarily based on virus isolation on cell culture (CHSE-214, BF-2 or EPC) followed by a confirmatory ELISA test (OIE, 2003).

Results

IPNV has been detected in Ireland in most years since 1994 (Figure 1) however, until 2004 the number of virus isolations was small. In 2006 prevalence exceeded 60% of marine sites and 30% of freshwater sites (Figure 6). Simulation follows this observed pattern with the simulated prevalence very low initially and only exceeding a prevalence of 10% in 2002 for marine sites and after 2004 for freshwater sites.

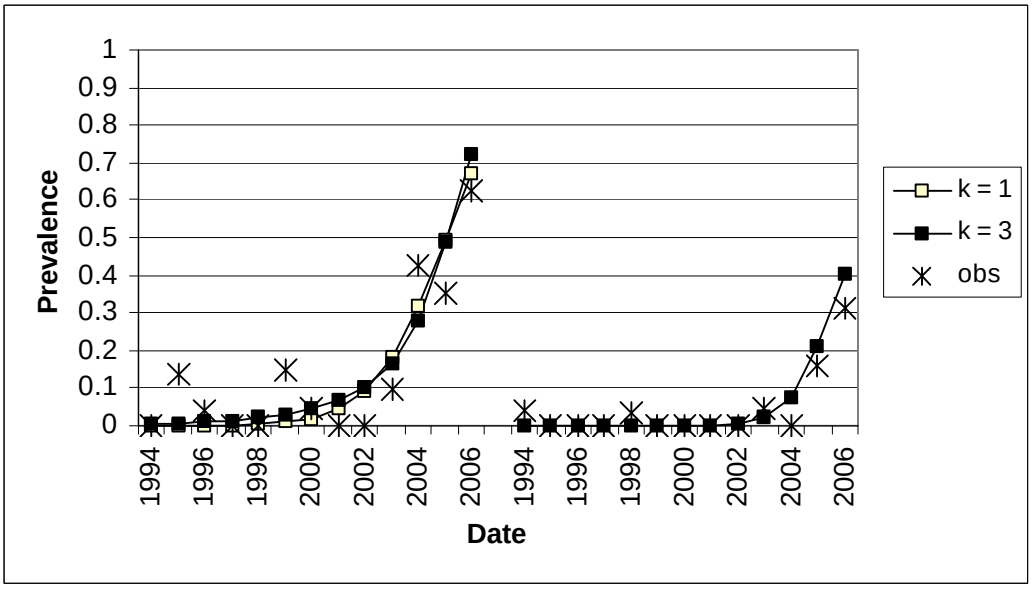

Figure 6. Observed (obs) and simulated modelled prevalence (k = theoretical number of ova/smolt sources) of IPNV in Ireland for both freshwater and marine sites. The figure demonstrates that the model fits well with the observed data and so can be used to predict future prevalence and the impact of control measures.

Modelled transmission required to fit the observations can be understood in terms of the increase ratio R_0 (Reno, 1998). This parameter represents the number of infective contacts a site has before it is harvested (Table IV) and an explanation of the model is given in Appendix IV. In order to eradicate a pathogen R_0 must be reduced below 1, which suggests transmission in both freshwater and marine water would have to be more than halved to eradicate IPNV. For population dependent transmission R_0 will be reduced to very low levels if production in Ireland continues to fall, however if it stabilises at 2006 levels (close to 1994 production) then R_0 will be close to 1994 levels i.e. > 1. The initial prevalence of infection is extremely small, particularly in freshwater (9.5×10^{-8}).

Table 4. Model R0 values for Ireland under population independent (PI) and population dependent (PD) forms. 1994 is the initial year of the run when normalised production = 1, 2001 = peak year (normalised production = 2.01); mean = mean 1994 – 2005.

Case	Marine, *k* = 1	Marine, *k* = 3	Freshwater
Ireland PI	2.24	1.66	2.39
Ireland PD 1994	1.77	1.40	2.25
Ireland PD 2001	3.55	2.81	4.51
Ireland PD mean	2.14	1.92	3.07

Overall infection pressure on marine sites is that generated when *k* = 1 (*k* being the number of smolt sources), however if marine sites really use multiple sources of smolts then simulation with *k* > 1 incorporates explicitly transmission between marine sites and the effect of use of multiple sources. The remaining R_0 is thus the true inter-site transmission, if the correct value of *k* is used. If the model run is extended, prevalence is predicted to

rapidly rise to around 60% of freshwater and 90% of marine sites by around 2008/09, stabilising thereafter (Figure 7). If controls are introduced then reduction of b_m (transmission in the marine environment) is quite ineffective and a reduction of k is only marginally more effective. The most effective single strategy is control of b_f (transmission in the freshwater environment) and this is the only strategy affecting freshwater prevalence. A combination of all these controls is by far the most effective at reducing IPNV, as was found for Scotland (Murray, 2006a).

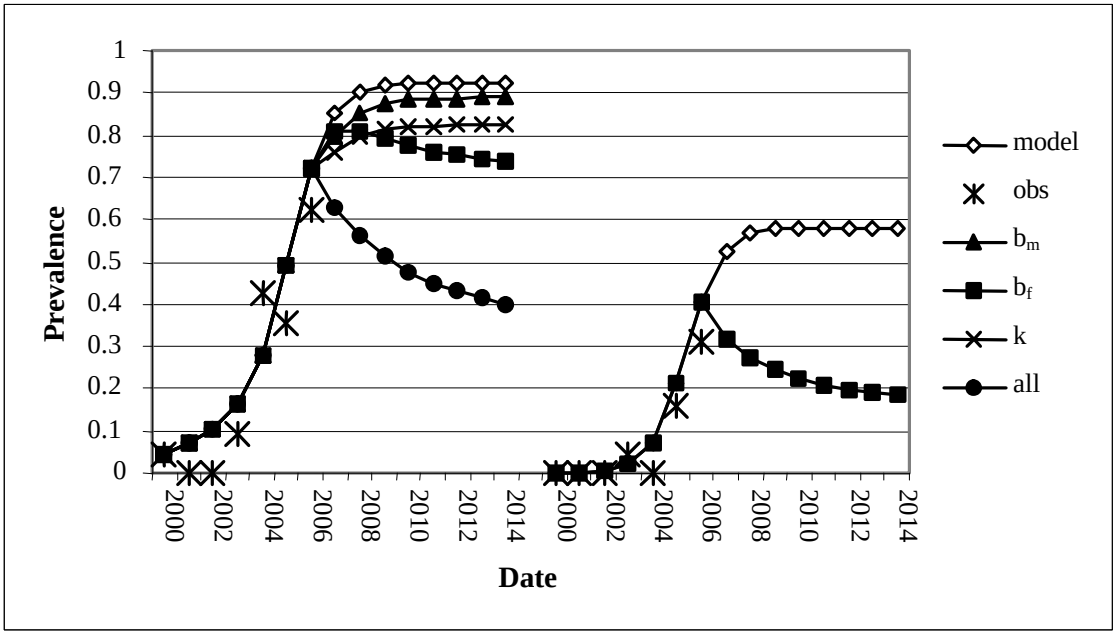

Figure 7. Projected national prevalence of farms infected with IPNV 2000 – 2014 with a change of control policy after 2006. Legend: no further controls (◇); 50% reduction in marine transmission (▲); 50% reduction in freshwater transmission (■); reduction of k to 1 (**x**); introduction of all 3 controls (●).

The Role of Imports

As shown above (Table IV), the R_0 values for Ireland are very high particularly in freshwater (2.39 against 1.41 in Scotland; Murray, 2006). This suggests continuing input of infected ova/fish which allowed the prevalence to increase faster than expected due to endogenous spread. To determine whether inputs were important for system dynamics the model was modified to include inputs into freshwater and marine sites (Ruane *et al.*, in prep.). The model was then rerun and the predicted prevalence was calculated using the same scenarios as shown in Figure 7. The main difference was that the model now predicted prevalence based on whether the number of infected ova/fish imports was low or high (Figure 8A-D).

When the number of infected imports is low, the model predicts that in freshwater (Figure 8A) stopping infected imports would not affect IPNV prevalence, but reducing freshwater transmission would dramatically reduce the prevalence. In the marine sites (Figure 8C) a moderate reduction in prevalence would occur if freshwater transmission was cut by 50% or smolt source was reduced to one. When the number of infected imports is high then stopping the imports will have a significant effect on prevalence in the freshwater (Figure 8B) and marine sites (Figure 8D).

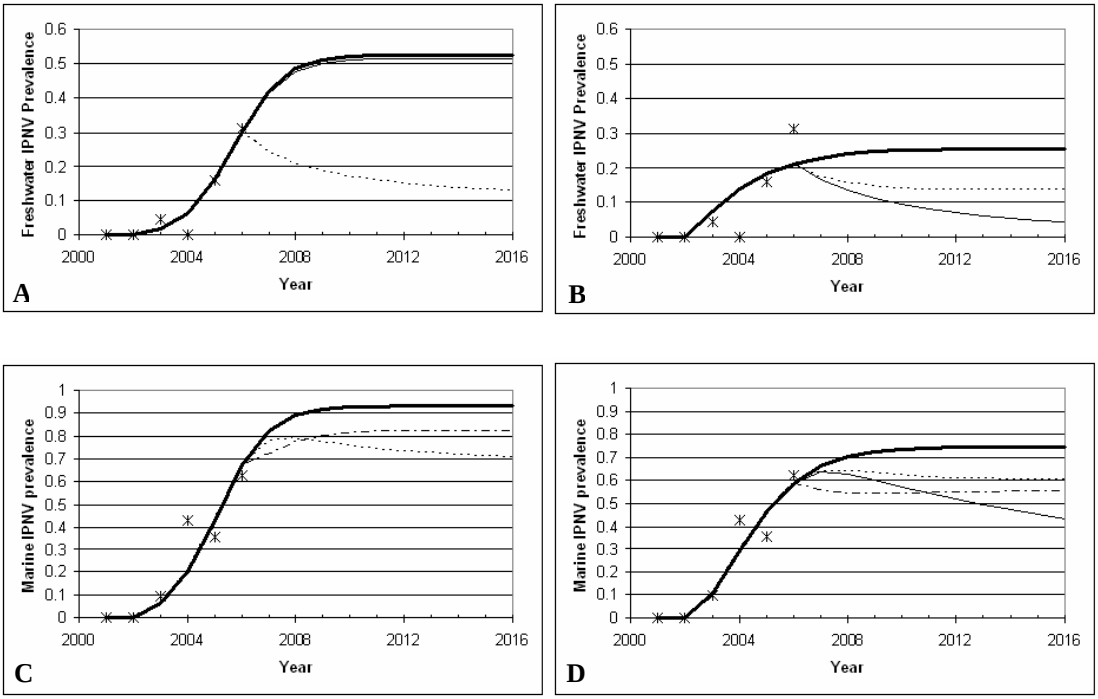

Figure 8. Projected prevalence of IPNV in freshwater and marine sites, 2000 – 2016, assuming a low (A; C) or high (B; D) input of infected ova/fish. Future scenarios are 1) no change (thick line); 2) stopping infected inputs (thin line); 3) reducing FW transmission by 50% (dotted line); 4) reducing smolt sources to 1 (dashed line).

Discussion

IPNV prevalence in Ireland has historically been low, but has been increasing rapidly over the last 3 – 4 years (Figure 5). IPNV was detected in Ireland in 1994 in a freshwater site and subsequently in marine sites in 1995–96. This suggests that IPNV prevalence increased in Ireland in the mid 1990's, however it failed to become established. The model implies that a later increase became established around 2001. This increase may be related to observed isolations of IPNV going back to 1998 as a low prevalence infection with a small number of cases spreads stochastically, rather than deterministically as in this model. If no action was taken, simulation suggests that IPNV prevalence could increase to a maximum of 60% of freshwater and 90% of marine sites over the next 2 – 3 years (Figures 7 & 8).

Once the virus became established it spread in a similar way to the Scottish case. Irish R_0 values were slightly higher for marine spread and higher still for freshwater spread. As a result prevalence is estimated to reach somewhat higher values than were estimated for Scotland, although similar high prevalence's are being reached in Shetland in both fresh and marine waters (Murray *et al*., 2003).

The estimated transmission rate in Ireland is moderately higher than that in Scotland. The difference between them may be an artefact of limited data, because of the very low prevalence up to 2003 and the small size of the Irish industry; there are only eleven reported cases from freshwater sites, of which only nine postdate 2000. It is also possible that the smaller Irish industry is more strongly connected leading to more mixing. However, the most realistic explanation is the importation of infected ova/fish which is known to have occurred and this has appeared in the model as increased transmission.

If transmission is population dependent, then the decline in Irish salmon production that occurred from 2001 – 2005 could have implications for the future of IPNV. If the decline in farm numbers continues, then it is possible that IPNV could disappear. This is based on the assumption that decline will lead to the few remaining farms being physically isolated by increasing distance. Alternatively, decline could lead to farms occupying a smaller area and not changing their distance from the nearest neighbouring farm (a known risk factor for IPN). Such geographical contraction would effectively lead to population independent transmission. The fact that in Ireland IPNV could spread during a period of declining production (when the increased prevalence of IPNV occurred in Scotland during a time of increasing production) does suggest population independence is the best model for the Irish situation.

In contrast to both the Scottish and Norwegian industries, incidence of clinical IPN in Ireland is thought to be related to importation of infected ova and smolts. This spread into Ireland may be regarded as part of a general southern spread of the IPN virus (Roberts & Pearson, 2005) aided by a change in broodstock monitoring programmes from kidney to ovarian fluid testing. The model predicts that if the importation of infected ova/fish were large, then cutting them off would be sufficient to dramatically reduce the prevalence of the virus in freshwater sites. This would in turn lead to a gradual reduction in marine site prevalence. If the increased prevalence of IPNV is due to endogenous spread (and not due to imports) then cutting freshwater transmission by 50% would have the most beneficial effects.

By far the most effective strategies to reduce the prevalence of the virus in Ireland is to cut the importation of infected ova/fish coupled with reducing the transmission of the virus in the freshwater and marine environments (i.e. strict biosecurity protocols).

- The first reported clinical outbreak of IPN in Irish farmed Atlantic salmon occurred in 2003
- Two main sub-groups of the IPN virus have been found in Ireland
- In 2006 prevalence of the virus exceeded 60% of marine sites and 30% of freshwater sites
- Modelling suggests that IPN virus prevalence could increase to 90% of marine sites and 60% of freshwater sites, if no further action is taken
- Clinical IPN in Ireland is clearly related to the importation of infected ova and smolts
- Importing IPNV free ova/smolts together with strict biosecurity measures on freshwater and marine sites are the main steps to be taken in controlling the disease in Ireland

3.3 Potential Economic Impact

In 2006, there were six reported cases of clinical IPN in Ireland, five in freshwater and one on a marine site. Table V below lists the total number of fish lost to IPN and also the number of fish culled as a result of preventative measures taken to minimise the spread of the disease.

Table 5. Number of mortalities due to IPN and fish culled in six Irish salmon rearing units in 2006.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
Mortalities	350,000	100,000	-	400,000	10,000	50,000	910,000
Fish culled	300,000	150,000	300,000	-	-	-	750,000

Therefore, in total the number of farmed fish lost to the disease was 1,650,000 for 2006. Had these fish survived and were sold at between €4.41/kg to €5.23/kg based on average 2006 market prices at an average harvest size of 4 kg, with an ideal 90% survival (Browne *et al*., 2007), they would have been worth €26.2 - €31 M. This figure represents a gross value which does not take account of a range of production costs such as staff time, feed etc. Thanks to a collaborative effort by ISGA, BIM and the MI, replacement disease-free stocks and temporary hatching facilities were sourced and installed (while existing facilities were disinfected and fallowed), thereby substantially reducing the loss sustained by industry due to the 2006 IPN outbreak. Actual costs such as replacement eggs, freshwater hatching space replacement, veterinary advice, culling, disposal, disinfection and fallowing and increased workload on farm staff, amounted to approximately €1.2 M (ISGA, pers. comm.). This number does not include a further 327,000 fry culled by the ESB Fisheries which were destined for restocking purposes.

- The potential value of the fish lost due to the 2006 IPN outbreak was estimated at €26 - 31M
- A collaborative effort by the industry, MI and BIM resulted in the actual cost of the outbreak being significantly lower *ca.* €1.2 M

4. IPNV IN WILD FISH

4.1 Freshwater Studies

The first major report investigating the prevalence of IPNV in wild freshwater fish was produced by Munro *et al*. (1975). This study was initiated after an IPN outbreak at a Loch Awe rainbow trout farm in Scotland in 1971. The virus was detected at a low prevalence (0.2 – 2.5%) in both salmonid and non-salmonid fish. No clinical signs of disease were found in any fish and all isolates were of the Sp serotype. The highest number of positive pools was found in the area surrounding the farm and the majority of these were from rainbow trout. As rainbow trout were not present in the system prior to the opening of the farm it was believed that these were escapees. The virus was also found in brown trout and Atlantic salmon plus a small number of non-salmonid species (minnow, perch and lamprey). The infected hatchery was subsequently closed (for unrelated reasons) and after 1977 no further isolations of the virus were found indicating that the virus was not self-sustaining in the wild once the source of infection was removed (Anon., 2003a). From 1992 to 2003, 7,553 wild salmonids from freshwater were tested by the FRS in Scotland for the presence of IPNV. Yearly prevalence ranged from 0 – 1.8% with an average for the entire period of ~ 0.5%, despite IPNV prevalence in farmed salmon increasing to about 30% in freshwater sites (Murray, 2006). Although the study did not target specific sites in relation to infected hatcheries, there was no apparent association between the presence of positive samples and the proximity to a salmonid hatchery (Stuart Wallace, FRS pers. comm.).

In Norway, approximately 3,000 salmon and trout have been sampled from different rivers between 1991 – 2002 (Anon., 2003b). In 1991 and 1992, IPNV positive fish were found in one third of the rivers tested while no infected fish were found during the period 1995 – 1999. Two rivers were found positive in 2000 and one in 2001. In the last year of the study in 2002, two positive salmon were detected in one river; however both fish were classified by fish scale analysis as escaped farmed salmon. Similar to the Scottish studies, these findings indicate that the prevalence of IPNV in wild fish is low (95% confidence limits: 0.02 – 5.5%) despite the high prevalence and increasing outbreak frequency in farmed fish experienced during the late 1990's.

In contrast to these studies, Bandín & Dopazo (2006) have reported a much higher prevalence of IPNV in wild salmon broodstock caught for a restocking programme in Galicia, Spain in 2004 and 2005. Using molecular methods to detect the virus, IPNV was found in 39% of salmon caught in 2004 and 51% in 2005. All fish caught were asymptomatic carriers showing no signs of disease. At this stage, it is not known whether the virus is more prevalent in that region or if the higher prevalence is due to the use of a more sensitive method of virus detection.

4.2 Marine Studies

Isolations of aquatic birnaviruses (often termed IPNV) from marine fish species have been numerous over the years. In a study carried out by the Marine Institute in 1998 off the coast of Ireland, 355 marine fish were tested for the presence of aquatic viruses. Birnavirus was isolated from haddock *Melanogrammus aeglefinus*, plaice *Pleuronectes platessa* and dab *Limanda limanda*. An extensive survey of over 30,000 marine fish from Scotland showed that the prevalence of IPNV is extremely low in wild marine fish (average 0.15%; Wallace *et al*., 2005). In total nine different marine fish species tested positive for IPNV, the majority being flatfish (dab, plaice and lemon sole *Microstomus kitt*) suggesting a possible role of these fish species as vectors or reservoirs of the virus. The prevalence of IPNV positive fish increased the closer they were sampled to an infected marine site.

Prevalence of IPNV among wild fish caught at a marine farm ranged between 1 – 8%, fish caught 1 – 3 km from the farm ranged from <1 – 3% and fish caught > 5 km from a farm ranged from 0 – 0.5%. Titre data from this study indicated that in 43 out of 45 isolations, the level of IPNV was below the limit of titration. Using genetic sequence analysis, all the isolates from this study (with the exception of one) were similar to the European reference strain Sp. The exception was one isolate related to the Canadian reference strain C1.

Romero-Brey *et al*. (2004) recovered several isolates of aquatic birnaviruses from different species of wild fish from the Flemish Cap, Newfoundland. Using nucleotide sequence data from the isolates it was shown that they were closely related to the West Buxton reference strain of IPNV, common to North America. In the seas around America, the virus has been isolated from Atlantic menhaden, *Brevoortia tyrannus* (Stephens *et al*., 1980) and Southern flounder, *Paralichthys lethostigma* (McAllister *et al*., 1983). Birnavirus have been isolated from numerous marine fish and shellfish species around Japan (Isshiki *et al*., 2001) and from flounder *Rhombosolea tapirina,* cod *Pseudophycis* sp., dogfish *Squalua megalops* and ling *Genypterus blacodes* in Tasmania, Australia (Crane *et al*., 2000).

Using experimental infection trials, Isshiki *et al*. (2001) infected five different species of fish with seven different strains of birnavirus and one IPNV strain (AM-98). The results showed that not all virus strains resulted in mortality. In fact, virulence was dependent upon virus strain and fish species suggesting that the host-parasite relationship is highly evolved.

4.3 Presence of IPNV in wild fish in Ireland

The Fish Health Unit regularly screens marine and freshwater wild fish for pathogens, including IPNV. From the period 1994 – 2005, *ca.* 4,000 fish were tested for a range of aquatic pathogens (Appendix V).

In 2006, five hatcheries reported clinical outbreaks of IPN in Atlantic salmon fry. It was therefore decided to conduct a targeted sampling of the rivers where the hatcheries were located. The rivers sampled during 2006 were: River Lee, Co. Cork; River Screebe, Co. Galway; River Poulmounty, Co. Carlow; River Carrigahorig, Co Tipperary and the River Burrin, Co. Carlow (Table 6)[4]. These rivers will be sampled again in 2007 and the survey will be expanded to include river catchments which do not have any fish production facilities on them.

2006 Sampling

Sampling of the rivers was carried out in conjunction with ESB Fisheries, NUI Galway, Central Fisheries Board, South Western Regional Fisheries Board (RFB), Western RFB, Shannon RFB and the Southern RFB. All sites in each river were electrofished using a GFT Safari Research Surveyor (models 660-D or 550-D) backpack battery powered sets. The units deliver a 100Hz pulsed DC current of a maximum of 400 V. For the sampling purposes of these studies the voltage was set in the 180 – 240 V range (maximum current < 1 amp). The cathode, a metal cable, was trailed and the operator held the anode, a metal ringed net with an insulated handle. No stop nets were used as the areas being fished were from a selected fixed point in an upstream direction towards a behavioural barrier (such as a large/fast riffle area). All species of fish were retained in a holding bin. The main target

[4] The SRFB acknowledge that large numbers of salmon and trout were removed from rivers in an effort to contain or eradicate the virus. The Board "*feels that such action while necessary, is a very serious course of action and should not be taken lightly. Particularly, at present on the Barrow system where salmon stocks are not meeting their conservation limits and both angling and commercial exploitation of the stock is prohibited*".

species were salmonid fish, namely Atlantic salmon and brown trout. A maximum of 30 fish per site were sampled, where the number of salmonid fish were less than this, other species such as pike, *Esox lucius* and perch, *Perca fluviatilis* were also sampled (Table 6).

A total of 380 fish were sampled from five rivers (Table VI). Pools of tissue (spleen, pyloric caecae and kidney or brain) from between one and ten fish were placed in cooled plastic tubes containing 2.5 mL of MEM medium for transport back to the laboratory. In the laboratory, the screening procedure for the identification of IPNV positive samples was primarily based on virus isolation on cell culture (BF-2 and EPC) followed by a confirmatory ELISA test (OIE, 2003). Histological samples were taken from fish from the Carrigahorig during the second sampling period at that river on 3rd October and from the Burrin on the 29th November. However no histopathological signs of IPN disease were found in any fish.

Table 6. A list of the samples taken from five Irish rivers for IPNV testing during 2006.

Sample ID	River	Location	Date	Pools	Virus Isolation
F3166/06	Lee	Carrigadrohid	24/07/06	1. Salmon (1)	NEG
				2. Eel (10)	NEG
				3. Pike (10)	NEG
F3167/06	Lee	Inniscarra	24/07/06	1. Salmon (10)	NEG
				2. Salmon (10)	NEG
				3. Salmon (10)	NEG
				4. Eel (4)	NEG
F3172/06	Screebe	Below hatchery (50 – 100 m)	31/07/06	1. Salmon (5)	NEG
				2. Salmon (5)	NEG
				3. Salmon (5)	NEG
				4. Salmon (5)	NEG
				5. Salmon (5)	NEG
				6. Trout (4)	NEG
F3173/06	Screebe	Above Lough Screebe	31/07/06	1. Salmon (5)	NEG
				2. Salmon (10)	NEG
				3. Salmon (10)	NEG
				4. Trout (5)	NEG
F3187/06	Poulmounty	Below hatchery (20 – 100 m)	18/08/06	1. Salmon (5)	NEG
				2. Salmon (5)	NEG
				3. Salmon (5)	NEG
				4. Salmon (5)	NEG
				5. Salmon (5)	NEG
				6. Salmon (5)	NEG
				7. Trout (1)	NEG
F3203/06	Carrigahorig	Above hatchery (~ 200 m)	11/09/06	1. Trout (5)	NEG
				2. Trout (5)	NEG
				3. Trout (5)	NEG
				4. Trout (5)	NEG
				5. Trout (5)	NEG
				6. Trout (5)	NEG
F3204/06	Carrigahorig	Below hatchery (0 – 200 m)	11/09/06	1. Salmon (5)	**POS**
				2. Salmon (5)	NEG
				3. Salmon (5)	**POS**
				4. Trout (5)	NEG
				5. Trout (5)	**POS**
				6. Trout (5)	NEG
F3219/06	Carrigahorig	Above hatchery (~ 200 m)	03/10/06	1. Trout (5)	NEG
				2. Trout (5)	NEG
				3. Trout (5)	NEG
				4. Trout (5)	NEG
				5. Trout (5)	NEG
				6. Trout (5)	NEG
F3220/06	Carrigahorig	Below hatchery	03/10/06	1. Trout (5)	NEG

		(0 – 200 m)		2. Trout (5)	NEG
				3. Trout (5)	NEG
				4. Trout (5)	NEG
				5. Trout (5)	NEG
				6. Salmon (3)	NEG
F3221/06	Carrigahorig	Below hatchery (200-500 m)	03/10/06	1. Perch (1)	NEG
				2. Perch (3)	NEG
				3. Pike (1)	NEG
				4. Pike (1)	NEG
				5. Pike (3)	NEG
F3222/06	Carrigahorig	Below hatchery (>500 m)	03/10/06	1. Pike (1)	NEG
				2. Pike (1)	NEG
				3. Trout (1)	NEG
				4. Perch (5)	NEG
F3233/06	Burrin	Below hatchery (0 – 200 m)	10/10/06	1. Salmon (5)	**POS**
				2. Salmon (5)	**POS**
				3. Salmon (5)	**POS**
				4. Salmon (5)	NEG
				5. Salmon (5)	NEG
				6. R. Trout (3)	NEG
F3261/06	Burrin	Above hatchery outflow	29/11/06	1. Salmon (2)	**POS**
				2. Trout (4)	**POS**
F3262/06	Burrin	Below hatchery (0 – 100 m)	29/11/06	1. Trout (5)	**POS**
				2. Salmon (3)	**POS**
				3. Salmon (4)	**POS**
				4. R. Trout (1)	NEG
				5. Trout (3)	NEG
F3263/06	Burrin	Below hatchery (100 – 300 m)	29/11/06	1. Trout (5)	NEG
				2. Salmon (5)	**POS**
				3. Trout (5)	NEG
				4. Salmon (5)	**POS**
				5. Salmon (4)	**POS**
F3264/06	Burrin	Below hatchery (1 km)	29/11/06	1. Trout (5)	**POS**
				2. Trout (5)	NEG
				3. Trout (5)	NEG
				4. Trout (5)	NEG
				5. Trout (5)	NEG
				6. Salmon (1)	NEG

2007 Sampling

Thus far in 2007 the Burrin, Poulmounty and Carrigahorig Rivers have been sampled in conjunction with the Regional Fisheries Boards and the details are shown below in Table 7. Fish were sampled by electrofishing and the IPN virus was tested in pools of tissue as described above. Presence of the virus was also tested using molecular methods; therefore a sample of gill tissue was removed from each fish and stored individually in 1.5 mL plastic tubes containing RNA*later*® (Ambion). RNA was extracted from gill tissue using the TRIZOL method. A one step RT-PCR kit (Qiagen) with the primers IPNV-1 and IPNV-2 were used to detect the presence of the IPN virus (Santi *et al*., 2005).

Table 7. A list of the samples taken from Irish rivers for IPNV testing to date in 2007.

Sample ID	River	Location	Date	Pools	Virus Isolation	PCR positive
F3333/07	Burrin	Below hatchery (0 – 100 m)	01/05/07	1. Trout (5)	NEG	2/5
				2. Trout (5)	NEG	2/5
				3. Trout (4)	NEG	1/4
F3334/07	Burrin	Below hatchery (100 – 200 m)	01/05/07	1. Trout (5)	NEG	2/3
				2. Trout (5)	NEG	4/5
				3. Trout (2)	NEG	0/2
F3335/07	Burrin	Above hatchery outflow (500 m)	01/05/07	1. Trout (5)	NEG	5/5
				2. Trout (5)	NEG	5/5
				3. Trout (4)	NEG	4/4
F3336/07	Burrin	Below hatchery (1 km)	01/05/07	1. Trout (5)	NEG	5/5
				2. Trout (5)	NEG	5/5
				3. Trout (5)	NEG	4/5
				4. Trout (5)	NEG	5/5
F3337/06	Poulmounty	Below hatchery (250 - 300 m)	02/05/07	1. Salmon (2)	NEG	2/2
				2. Trout (5)	NEG	4/5
				3. Trout (5)	NEG	5/5
				4. Trout (5)	NEG	3/5
F3338/06	Poulmounty	Below hatchery (50 – 100 m)	02/05/07	1. Trout (5)	**POS**	5/5
				2. Trout (5)	NEG	5/5
				3. Salmon (3)	**POS**	3/3
				4. Trout (5)	NEG	5/5
				5. Trout (4)	NEG	4/4
F3363/07	Poulmounty	Upstream of hatchery	28/05/07	3 pools (15 salmon)	NEG	5/9
				22 pools (110 trout)	NEG	11/45
		Downstream of hatchery		4 pools (20 salmon)	NEG	nd
				1 pool (5 trout)	NEG	nd
F3364/07	Burrin	Upstream of hatchery inlet	29/05/07	21 pools (105 trout)	NEG	24/65
		Downstream of hatchery inlet		9 pools (45 trout	NEG	nd
				1 pool (1 salmon)	NEG	1/1
F3375/07	Carrigahorig	Upstream of hatchery (200 m)	28/06/07	16 pools (80 trout)	NEG	4/15
F3376/07	Carrigahorig	Downstream of hatchery (0 – 200 m)	28/06/07	13 pools (65 trout)	NEG	0/10
				1 pool (2 salmon)	NEG	0/2
				1 pool (1 pike)	NEG	0/1

nd: not determined

Results

Carrigahorig River, Co. Tipperary

In the Carrigahorig sample, taken on 11th September 2006 (60 fish), no positive pools were found above the hatchery. However, from a sample taken directly below the hatchery two salmon and one trout pool tested positive. As no salmon were found above the hatchery and in the absence of any restocking of salmon in the River in recent years (D. Doherty, ESB Fisheries, pers. comm.) it is believed that the salmon found below the hatchery were escapees. The fact that one pool of trout also tested positive implies that some form of horizontal transmission of the IPNV had occurred. There would appear to be two possible modes of transmission. Firstly, the escaped salmon could have already been asymptomatic carriers of the virus and passed it on directly to the trout or secondly, untreated discharge water from the hatchery could have acted as a source of the virus infecting both the salmon and trout in that area[5].

A follow-up sample of the river on 3rd October (85 fish) failed to show up any positive samples. Again, only trout were found in the sample taken above the hatchery but only three salmon were found below the hatchery. Samples taken further downstream of the hatchery showed only coarse fish (pike and perch) with only one trout being caught > 500 m downstream of the hatchery. On 28th June 2007, 148 fish were sampled from the river. Eighty brown trout were sampled upstream of the hatchery, while downstream, two salmon parr, one pike and 65 brown trout were sampled. All samples were negative for IPNV by virus isolation. Fifteen samples from above and 13 from below the hatchery were also tested for the virus by RT-PCR. Four trout samples from above the hatchery tested positive while all samples from below, including both salmon, were negative.

Burrin River, Co. Carlow

On 10th October 2006, the River Burrin was sampled (28 fish) and 3/5 salmon pools were IPNV positive. This River would appear to have a healthy population of wild salmon and brown trout, with a small number of larger (200 – 500 g) rainbow trout also resident in the River[6]. In this case it is difficult to ascertain whether the positive salmon samples were actual farmed escapes or not, although all fish sampled were taken from within 200 m of the hatchery outflow. Interestingly, even though IPN was historically a disease of rainbow trout in freshwater, no virus was found in the rainbow trout sampled from this river.

A follow-up study took place on November 29th 2006 (72 fish), to determine whether the virus is still present in the River and if it is localised to the area immediately downstream of the hatchery. Four sample sites were located along the River: 1) upstream of the hatchery outflow; 2) immediately below the hatchery; 3) 100 – 300 m below the hatchery and 4) *ca.* 1 km below the hatchery. Fish were sampled in pools of five (to a maximum of 30 fish per site) for virology and a sub-sample for histological analysis. Salmon remained the species of choice for sampling although a smaller number of brown trout and rainbow trout were also taken. IPNV was isolated from fish taken at all four sampling sites and included Atlantic salmon and brown trout, but not rainbow trout (Table 6). No histopathological signs of disease were seen in the histology samples.

The River was again sampled on May 1st 2007 (60 fish) and the four sample sites (which included one above the hatchery inflow) all tested negative for the virus by virus isolation. However, no salmon were caught and the pools all consisted of brown trout tissue samples.

[5] The SRFB feel that "*the matter of effluent discharge from hatcheries to rivers, that may carry a virus is a major conern and should be examined under licensing*".

[6] The SRFB note that " *this species is not indigenous to the river and we can only assume that these are escapes (escapees may be a breach of licence conditions)*".

RNA was extracted from the gill tissues of all the fish sampled and tested by PCR for the presence of IPNV genetic material. In this case, 44 out of 58 fish were positive and included fish from each of the four sample sites (Table 7). On the 29th May 2007, 151 fish were sampled from the river and all tested negative for IPNV by virus isolation. Sixty five gill samples were taken for analysis by RT-PCR and 24 were positive (one salmon and 23 brown trout).

Poulmounty River, Co. Carlow

The Poulmounty River, downstream of the hatchery, was first sampled on August 18th 2006 and the sample, which consisted of 30 salmon and one trout, was negative for IPNV by virus isolation. The River was again sampled on May 2nd 2007 (39 fish) and this time one pool of salmon and one pool of trout were both positive. All fish sampled were also tested for IPNV by PCR and in this case 36 out of 39 fish were positive. As with the Burrin River data, this also suggests that the Poulmounty fish had previously been exposed to the virus even though the hatchery on that river had been fallowed for six months prior to the sample on May 2nd.

The Poulmounty River was again sampled on the 28th May 2007 in which samples from 125 fish upstream of the hatchery and 25 fish from below were submitted for virological examination. All samples tested negative for IPN virus by virus isolation. Fifty four gill samples were taken for analysis by RT-PCR and 16 were positive (five salmon and eleven brown trout).

Conclusion

As the IPN virus was detected in both the Carrigahorig and Burrin Rivers it is possible that the resident fish were infected by virus in the water shed from the infected fish within the hatcheries. Due to the response of the industry the source of the virus has been removed as shown by the fact that the virus was not detected by virus isolation in the Carrigahorig and Burrin Rivers in 2007. The virus was isolated in one sample from the Poulmounty River in 2007, but not in subsequent samples. It is possible that it has not been able to establish itself in the resident fish populations. The significance of PCR positive results in the absence of virus isolation is difficult to ascertain. However, the PCR positive results would indicate that the fish in the river had previously been exposed to the virus, but that virus levels in these fish are low and would suggest a carrier state in these fish rather than active infection.

Sequencing of IPNV Isolates from Wild Fish

In order to determine the relatedness of the virus isolates from the wild fish and those from farms, viral RNA was extracted from cell scrapes which had visible CPE (indicating virus growth) using the TRIZOL method. A one step RT-PCR kit (Qiagen) with the primers A-Sp500F and A-Sp1689R were used to generate a 1100 kb PCR product covering the VP2 and VP3 coding region of segment A (Santi *et al*., 2004). The PCR product was used for direct sequencing by a commercial company (Sequiserve).

The relatedness of the IPN virus isolated from salmon and brown trout from the three rivers is shown above in Figure 9. All river isolates cluster closely together along with isolates from two freshwater salmon hatcheries (FW1 and FW3). Both hatcheries FW1 and FW2 have imported salmon ova in recent years and reported clinical IPN in 2006. The relatedness of the sequences from the three rivers and the isolates from FW1 and FW3 suggest that it may be the same virus which was found in the wild fish. This would support the hypothesis of horizontal transmission of the virus from an infected hatchery to the wild fish. It must be noted however that no signs of disease were noted in any wild fish and the existence of a 'wild-type' IPN virus in wild fish can not be ruled out.

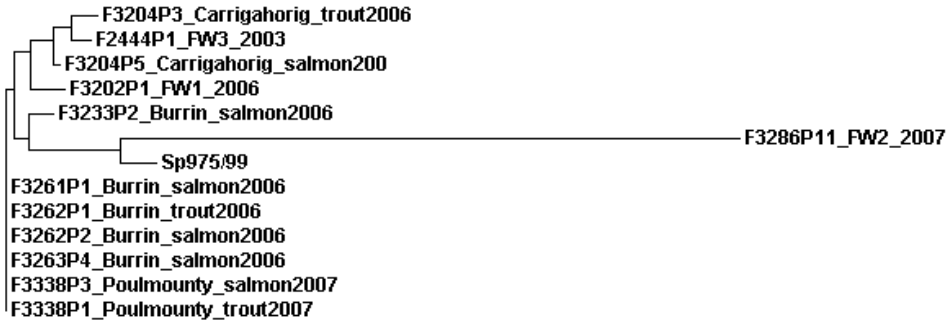

Figure 9. A phylogram illustrating the relatedness of IPNV isolated from wild Atlantic salmon and brown trout in the Burrin, Poulmounty and Carrigahorig rivers. The sequences are also compared with a previously published sequence of IPNV Sp975/99 from Scotland and three Irish salmon hatcheries (F3202P1_FW1; F3286P11_FW2 & F2444P1_FW3).

The hatchery site (FW2) has not reported losses due to IPN and stocks only salmon broodstock from within Ireland. From Figure 9, it does not appear that the source of the virus in the wild fish was this hatchery. The Irish isolates are closely related to a Scottish isolate (975/99) from a marine site in the Shetland Isles (Smail *et al*., 2006). Isolate 975/99 is a Sp serotype associated with high levels of mortalities both on the farm (Smail *et al*., 2006) and in experimental challenge models (Bowden *et al*., 2002).

It is apparent that a sampling regime testing wild fish for the presence of aquatic pathogens should be further developed. Future IPNV isolates from farmed and wild fish in Ireland should be sequenced in order to develop an overview of their relatedness and would provide valuable information on epidemiological studies of IPNV in Ireland. This is necessary not only to determine whether a threat exists to the wild fish populations due to disease outbreaks in aquaculture facilities, but also to determine the risk to farmed fish of pathogens which may exist in the wild.

- Despite the wide spread occurrence of the IPN virus in Scottish and Norwegian salmon farms, no significant effect on wild fish has been found in those countries
- Of the five rivers with infected hatcheries, three had IPN virus positive fish in the vicinity of the facilities
- Testing of wild fish for aquatic pathogens should be further developed in order to identify the risks for both wild and farmed fish

5. CONTROL OF IPN IN AQUACULTURE

5.1 Risk Factors

Through epidemiological studies, a number of reports have identified risk factors associated with outbreaks of clinical IPN. The following section will provide an overview of these factors as identified by the studies which were carried out in Norway and Scotland. As the results from these studies often depend upon the situation at a particular place and time, they may not be directly relevant to the Irish situation, but can highlight probable areas of concern.

Jarp *et al*. (1994) carried out an epidemiological study of 124 Norwegian sea-sites holding salmon post-smolts which had been transferred to sea between 1st April and 1st August 1991. In 39.5 % of the sites IPN was diagnosed. The risk of clinical IPN was significantly associated with the purchase of smolts from several hatcheries. It is generally believed that increasing the number of smolt sources also increases the chance of introducing the virus to the site. An association between the age of the smolts at sea transfer and mortality was also found, with S1 smolts being more susceptible than S2's. Two other factors identified were the geographic location of the site and the mode of smolt transport from the hatchery, suggested to be due to the stressful effects of transportation.

In Scotland, a preliminary study was carried out using data collected by questionnaire from 103 marine sites, during 2001 and 2002 (Murray *et al*., 2004). Of the 103 sites, 40 were classified as cases (clinical IPN) and 63 were controls. A strong association was found in the type of smolt (S1 or S½) used on site. As with the Norwegian study, clinical IPN in Scotland was strongly associated with S1 smolts, however it was believed that this may, at least in part, be due to the seasonality of IPN outbreaks occurring just after transfer to sea of the S1 smolts. A short distance between sites (< 3 km) was also strongly associated with IPN. Further analysis of the data collected showed that the risk of IPN progressively decreased as distance increased up to at least 8 km. The number of hatcheries that a site used as sources for smolts was shown to be associated with clinical IPN. A site receiving smolts from only one source was much less likely to have clinical IPN. Sites using two or three sources had an increased, but similar, probability of IPN with the risk increasing again for sites using four and five sources. Frequency of removal of mortalities was found to have a small but significant association with IPN. A larger number of control sites (i.e. sites with no clinical IPN) had either daily removal or removal every 1 to 3 days. Frequent removal of dead fish was found to have a significant effect on preventing outbreaks of infectious salmon anaemia (ISA) in Norway (Jarp & Karlsen, 1997). Other factors identified included, the total number of smolts transferred to a site and the method of transfer (road transfer being less associated with IPN than other methods).

Although both cases (Jarp *et al*., 1994; Murray *et al*., 2004) dealt with a limited number of marine sites, useful risk factors can be identified to help reduce the impact of IPN on marine sites. Both studies showed that by increasing the number of freshwater smolts sources, the possibility of having a clinical IPN outbreak also increases. In both studies, S1 smolts appeared to be more susceptible to IPN than S2's (Jarp *et al*., 1994) or S½'s (Murray *et al*., 2004). The underlying reason behind this is not clear. Being older S2 smolts may have a better developed immune system offering protection to the fish; however this would not explain the fact that S½'s appeared to be less affected by IPN in Scotland. A confounding effect of the time the smolts are put to sea would most likely explain this effect. S1 smolts are put to sea in early spring and IPN outbreaks typically occur in late spring/early summer. Smoltification is a highly stressful event for salmon, coupled with the stress associated with smolt transport and adaptation to life in the marine environment; the

smolts are likely to be severely immunocompromised during this period and more susceptible to disease-causing pathogens (Maule *et al*., 1987; Carey & McCormick, 1998). The Scottish study identified distance between sites as a risk factor for IPN. It was shown that if the distance between marine sites is greater than 10 km then the risk of IPN is much lower. This finding is most likely related to the horizontal transmission of the virus by vectors, or by equipment, people or vehicles moving between closely located sites. The method of smolt transport was also shown to be a risk factor in both studies. Transport by road would appear to be the best method, which may be due to the lower stress associated with this method or a reduced risk of transferring the virus.

5.2 Biosecurity

The IPN virus is very difficult to kill. It can withstand desiccation and survive for long periods of time in both sea and freshwater (Desautels & MacKelvie, 1975; Wedemeyer *et al*., 1978). The IPN virus requires heating to 80^{o}C for two hours to achieve significant inactivation. The virus is also resistant to low pH. Its tolerance to high temperatures and low pH enables it to survive in ensiled waste (Smail *et al*., 1993a) and to pass through the gut of birds and mammals (McAllister & Owens, 1992; Smail *et al*., 1993b).

It is also extremely resistant to ultraviolet radiation causing further complications with regard to the sterilization of water. Øye & Rimstad (2001) showed that UVC irradiation of 1188 J m^{-2} resulted in a 99.9% inactivation of IPNV, compared with 33 J m^{-2} for the ISA virus and 7.9 J m^{-2} for viral haemorrhagic septicaemia (VHS) virus. The dose required for a similar inactivation of IPNV in wastewater was even higher at 3367 J m^{-2}. It is however sensitive to a number of commonly used chlorine and iodine based disinfectants which can be used to inactivate the virus on equipment and the surface of eggs (Amend & Pietsch, 1972; Desautels & MacKelvie, 1975).

Disinfectants with peroxygen compounds such as Virkon have been shown to be effective against the IPN virus using *in vitro* laboratory trials. In the absence of organic contamination a 1% and 2% w/v solution worked with an efficacy of > 99.9999% after a one minute exposure time. In the presence of organic contamination a similar level of efficacy was reached after 10 minutes (Torgersen, 1991).

5.3 Vaccination

The majority of licensed fish viral vaccines are based upon inactivated antigens formulated in oil emulsions. These antigens are produced by growing virus in cell lines followed by inactivation or by the recombinant expression of protective antigens. Inactivated vaccines are non-replicating and non-infective and therefore are generally best delivered by injection (Biering *et al*., 2005). The development of vaccines for fish diseases has been problematic. The smaller market for aquatic animals compared with the much larger terrestrial animal market means that the costs of producing inactivated viral vaccines are relatively high. In addition to this, oral vaccines against fish viral diseases, which would provide a stress-free method of vaccinating fish of any age, are rare as high costs are associated with developing carrier compounds to protect the vaccine against the digestive system.

In Norway, some 80% of smolts are vaccinated before going to sea (Anon, 2003b). Indications are that some of the IPN vaccines will reduce mortality; however confusion remains to a large degree caused by the lack of proper scientific study design, analysis and quality assurance of available data leading to inconsistent results from field trials. Some studies do provide promising results on the protective effects of vaccines, such as those using recombinant VP2 proteins (Frost & Ness, 1997; Frost *et al*., 1998). Although the

development of reproducible experimental challenge protocols have been reported (Stangeland *et al*., 1996; Bowden *et al*., 2002), their limited availability has been a problem in testing vaccine efficacy.

In Norway, three manufacturers sell IPN vaccines; Pharmaq AS and Aqua Health/Novartis produce multivalent vaccines containing inactivated whole-virus antigen, whereas Intervet Norbio use the VP2 protein as their IPN antigen (Biering *et al*., 2005). Pharmaq have recently licensed the ALPHA-JECT™ 2-2 vaccine for use in the UK which is a combination vaccine against IPN and furunculosis. This vaccine is currently used in Ireland under an AR-16 for exports to Scotland and is the only vaccine available in the country at the moment. In 2006, Schering Plough licensed the first oral IPN vaccine (AquaVac™ IPN Oral) for use in Chile.

The main risk factors associated with IPN in Scottish and Norwegian marine sites are:

- Presence of IPNV infected fish
- More than one source of smolts
- Mode of transport
- Use of S1 smolts
- Distance between sites of < 10 km

Disinfectants with the following properties have been shown to be useful (see Appendix VII for more details):

- chlorine and iodophore based disinfectants
- alkaline solutions > pH 12
- temperatures > 60^{o}C
- formalin based

Water may be treated for virus inactivation with (see Appendix VII):

- UV > 1200 J m^{-2}
- Ozone 0.1 – 0.2 mg L^{-1}

Vaccines

- There is a general belief that IPN vaccines offer protection; however a need for more independent scientific studies exists.
- The ALPHA-JECT™ 2-2 vaccine is the only IPN vaccine available in Ireland under an AR-16 licence.

6. MANAGEMENT STRATEGIES FOR IPN

6.1 IPN Code of Practice

Due to the high prevalence of IPNV in the Scottish farming industry, regulatory controls of IPN have effectively been removed with the exception of controls regarding non-movement of clinically infected fish. The Irish situation however represents a different case, in that all clinical outbreaks of IPN have been associated with imports. If the imports from infected sites are stopped, the disease could potentially be eradicated. A practical solution to the problem would be the development of a Code of Practice dealing with IPN and in particular with the screening of broodstock/ova for IPNV and fish movements. A draft Code of Practice has already been drawn up (Annex I). In the context of the broader Fish Health Code of Practice, the IPN Code should be updated. Inherent in the Code should be strict recommendations regarding the movement of IPNV positive fish between freshwater sites within the country (i.e. only sites previously found positive for IPNV should accept IPNV positive fish)[7]. In addition to this, a national salmon broodstock programme has been proposed to provide disease free ova for the salmon industry and the industry should be encouraged to promote this if it is found to be economically feasible.

As with most viral diseases of fish, there is no method of curing the disease, once found on a site. Therefore the best way of dealing with diseases such as IPN should be based on preventative measures i.e. keeping the virus out. Murray (2006b) used data collected by the FRS for the period 1996 – 2003 on the prevalence of IPNV in Scottish salmon farms to determine whether the virus persists on particular farms. The study showed that infection of salmon farms with the IPN virus was transient and highly dynamic. The data suggests that the virus has a turnover period of two years on marine sites and four years in freshwater sites. This supports suggestions that control measures for IPN should be targeted at freshwater sites (as the introduction of IPNV infected smolts is a major risk factor) and that an area management approach is more suitable for marine sites. The importance of strict biosecurity protocols should not be underestimated. A site specific biosecurity and water quality improvement plan introduced into the Marine Harvest Norway, Bessaker site in 2000/01 resulted in the successful eradication of the virus (G. Ritchie, Marine Harvest, pers. comm.).

6.2 Recommendations for freshwater sites[8]

- All Atlantic salmon broodstock should be tested for IPN virus and ova from infected parents destroyed as outlined in the IPN Code of Practice (Annex I).
- An audit of conditions in the receiving hatchery e.g. biosecurity protocols, should be carried out prior to the introduction of stock.
- Proper biosecurity measures and disinfection protocols need to be adhered to, bearing in mind the ability of the virus to survive adverse conditions.
- In the event of a disease outbreak, all clinically affected fish should be culled, in an effort to reduce infection pressure within each hatchery.

[7] The ISGA feel that "*the only realistic approach to the problem is to manage the disease, not the virus in co-operation with the MI and DCMNR and in the context of having access to sufficient sites to on-grow fish through an entire generation on a single site thereby also tackling the other main fish health issues of PD and sea lice. In addition, any CoP must have sufficient flexibility to isolate clinical outbreaks down to single units such as tanks or cages on a farm to prevent unnecessary culling or stress to other fish on-site*".

[8] The SRFB "*feel that the recommendations for freshwater sites do not mirror the seriousness of the situation*". The Board "*welcomes industry, but our native stock and water quality must be protected at all times*". In addition, the Board recommend "*a co-operative approach to the management of wild stock. A quality assurance system must be put in place to ensure no infection is transferred from the facility to the receiving waters*".

- If an immediate eradication policy is not to be followed, survivors of IPN infections may be on-grown. Such on-growing should be considered on IPN positive sites only and be subject to Risk Assessment. These Risk Assessments should be carried out by the industry veterinarian in consultation with scientific experts (MI/UCD) and the risk management measures recommended in each Assessment should be agreed with DCMNR, MI and where relevant, with the local Regional Fisheries Boards[9].
- Biosecurity measures must be stepped up on all infected sites. Every effort should be taken to contain the disease on the infected site and to treat the effluent from the site to a degree where it does not pose a threat to either wild or farmed fish in the general vicinity.
- Producers in the UK and elsewhere should be encouraged by the Irish industry to get involved in a 2 year testing programme for BKD. This would increase the potential number of IPN and BKD-free ova/smolt sources.
- Proper procedures preventing farmed escapes should be documented, monitored and strictly adhered to.
- Containment protocols should be adhered to on IPN infected freshwater hatcheries.

6.3 Recommendations for marine sites[10]

- The highest standards of husbandry should be employed, particularly prior to and after the transfer of smolts to sea.
- Good husbandry should be practiced at all times in an effort to minimise stress levels, thereby decreasing infection pressure and ultimately minimising the impact of the disease.
- Where possible sites should be stocked with smolts from only one source.
- Where producers are importing smolts, vaccination prior to transport is recommended.

6.4 Recommendations in Relation To Wild Fish

- The current study determining the presence of aquatic pathogens in wild fish should be expanded to cover a greater range of rivers[11].
- As infected sites can act as a source of the IPN virus, screening of wild fish in the vicinity of an infected site should be carried out on a regular basis.
- All fish produced for restocking purposes on an infected site should be culled in the event that the IPN virus has been isolated, irrespective of the presence of disease signs.

[9] The WRFB recommend that "*once a site has been determined as infected in a cycle that entire site must be flagged as a potential carrier and **no** transfers to disease free sites should be permitted. The Board feel very strongly about such a recommendation being an essential safeguard to wild fish*".
[10] The ISGA state that "*without a clear policy implemented by the DCMNR which allows producers the freedom to farm fish to the highest standards by providing adequate fallowing sites and overruling the restrictions imposed by ALAB, none of the aspirations in section 6.3 are achievable*".
[11] The SRFB state that "*the removal of any salmonids from any part of a river for any reason must be taken seriously*".

A review of all the information gathered thus far leads to the following conclusions:

- Prevalence of the IPN virus has increased dramatically in the Irish salmon farming industry, particularly in the freshwater sector
- Serious losses have occurred due to IPN in 2006
- There are two main sources of the virus in Ireland, one avirulent strain which appears to be endemic in Ireland and one associated with imports
- All clinical cases of IPN so far can be traced back to imported stock
- In order to significantly reduce the prevalence of the virus, control measures should consist of a combination of strict biosecurity measures in freshwater and importation of IPN-free ova/smolts
- The reliance of the industry on imports represents a high risk with regard to IPN (and other infectious diseases); insisting on proper broodstock testing or sourcing ova/fish from IPN free sources is imperative
- Based on the 2006 experience the IPN Code should be reviewed in the context of developing a National Fish Health Code of Practice
- The effective management of infectious diseases in farmed fish is greatly enhanced by practices such as adequate fallowing and separation of generations
- The importation of ova from alternate IPNV-free sources has resulted in no virus isolations from hatcheries in 2007

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APPENDIX I

This draft IPN Code of Practice dates from 2004 and has since been superseded by the drafting of the Fish Health Code of Practice.

MARINE INSTITUTE / IRISH SALMON GROWERS ASSOCIATION UPDATED CODE OF PRACTICE FOR THE CONTROL OF IPN IN IMPORTED SALMON STOCKS

July 2004

1 PURPOSE

This paper aims to define an agreed IPN testing protocol, which will be used by Irish salmon producers who wish to import live fish or ova from other Member States, or EEA countries, such as Norway or Iceland.

This Code of Practice contains updated advice on IPN and outlines the current legislative situation in relation to IPN and BKD, with the objective of reaching a formal understanding between the Marine Institute, DCMNR and ISGA.

2 BACKGROUND

In March 2003, the Marine Institute met with the Board of the ISGA, to discuss the need for a Code of Practice to control the importation of ova from sources, which might be infected with IPN and/or BKD. The paper presented at that meeting is attached as a separate document. The proposed Code of Practice was subsequently discussed with DCMNR and ISGA in May 2003 and on a number of occasions since then, at meetings of the Irish Fish & Shellfish Health Advisory Committee (***IFSHAC***), which includes MI, DCMNR, ISGA representatives and fish vets.

At the latest meeting of ***IFSHAC***, held on May 7th 2004, it was decided that the draft Code of Practice should be updated and re-circulated to ISGA members, with a view to its rapid implementation.

3 PREVIOUS SITUATION IN RELATION TO INTRACOMMUNITY TRADE

Until 2004, the EU Commission had not recognised Ireland's application for Additional Guarantees in relation to certain List 3 diseases. As a consequence, when ova or live fish were imported from the UK for example, they were certified as coming from a zone, which was free from IHN and VHS. Assurances that the zone/site/parents of origin were free from IPN and/or BKD were received on an informal basis from the Marine Laboratory in Aberdeen or from the CEFAS Laboratory in Weymouth.
DCMNR/MI had no legal powers to insist on certification of freedom from these diseases, but since imports were mainly of ova, from a limited number of hatcheries, this informal system worked well for many years.

4 CHANGES IN THE TRADING PATTERN

Since early 2003, there have been some changes in the trading pattern outlined above. For market and strain preference reasons, live fish have begun to be imported from Scotland, and the Norwegian market in ova and live fish has opened up following the EU lifting of Safeguard Measures which had been in place against Norway, for some years. These changes make it even more important to protect the Irish Industry from the serious fish diseases, IPN and BKD.

5 ADDITIONAL GUARANTEES (AGs)

In 1993, Ireland and a number of other member States (MS) made applications to the EU Commission for recognition of freedom from certain diseases and for the establishment of control programmes for other diseases, appearing on List 3 of Council Directive 91/67/EEC. These applications were not dealt with until mid 2003, when the Commission requested that MS should submit updated applications for certain List 3 diseases if they so wished.

Ireland submitted applications for recognition of freedom from the List 3 diseases BKD, *G. salaris* and SVC, and for the application of a Control Programme for IPN. Early in 2004, indications were received from the Commission that they were willing to grant Additional Guarantees to Ireland in respect of all four diseases. Although this would have granted Ireland the highest health status in Europe, and provided sound protection in terms of the introduction of the diseases mentioned above, following a meeting with Industry it became clear that if Ireland went ahead with the AG for IPN there could be serious trade problems for the Irish industry. This was due to the fact that the UK was not granted an AG with respect to IPN, thus making it impossible for us to import from that country. This would of course have caused enormous problems for many Irish companies, who wish to import from Scotland for reasons relating to genetic diversity, strain preference, cost, etc.

An additional factor in DCMNR's decision not to accept the AG was the fact that the EU Commission insisted that in the event of IPNV being isolated in a MS, which was running a Control Programme, the disease must be eradicated. This would be done without compensation, either from the EU or from the MS government, Since IPNV has been isolated regularly, but infrequently in Ireland for many years (mainly as a result of sub-clinical infection), DCMNR/MI consulted with ISGA and decided that all things considered, it would be more prudent to forego the AG for IPN, *provided* an MI/industry led Code of Practice was put in place.

Ireland was granted AGs for BKD and *G. salaris* (and SVC in cyprinids), and as a result, any new Code of Practice will cover IPN only. Certification in relation to BKD and *G. salaris* will be provided by the Competent Authority in the country of origin.

It is clear that the only way to protect against the introduction of IPNV through trade, is to design and implement a Code of Practice which will be used as a guide by all farmers wishing to import live fish and ova from within the EU and/or from EEA countries such as Norway and Iceland. DCMNR/MI should be fully consulted in relation to each proposed import. It is proposed that a "Case Management" structure should be established, to decide on import applications. The structure should be comprised of representatives from industry, MI and DCMNR.

6 GENERAL PRINCIPLES OF FISH HEALTH MANAGEMENT AS OUTLINED BY THE OIE

When deciding what type of testing regime should be put in place at the farm/zone of origin, it is important to examine the standards described by the ***OIE (World Organisation for Animal Health)*.**

According to the OIE, a comprehensive approach to fish health management and control requires:

- Assessment of the fish health status of animals in production site based on inspections and standardized sampling procedures followed by laboratory examinations conducted according to standard methods (e.g. those outlined in the OIE Diagnostic Manual or in a relevant EU Commission Decision).
- The constraint of stocking only with aquatic animals having a health status higher than, or equal to that of the stock already on the farm.
- Eradication of the disease where possible, by slaughtering infected stocks, disinfecting facilities and re-stocking with fish from approved disease free sources.
- Notification by every country of its particular requirements, besides those provided for in the OIE Code, for the importation of aquatic animals and aquatic animal products.

7 OPTIONS (Freedom vs. Individual Parent Testing)

Although it is always preferable to source stock from a site, which has a history of freedom from the pathogen of interest, this may not be easy to achieve for IPN, because of the widespread prevalence of this virus on many sites in Scotland and Norway. It is recognised that for commercial reasons, individual farmers may wish to import from sites, which have a history of IPN. **It is recommended that a system of individual parent testing should be employed in this instance** (see below).

8 INSPECTIONS / TESTING FOR IPN

Inspections and laboratory testing prior to export should be carried out either by the Competent Authority or by a laboratory recognised and accredited by the Competent Authority in the country of origin.

9 ESTABLISHMENT OF DISEASE FREE STATUS

The sampling procedures and diagnostic regime to be employed for the establishment of the disease status of a given site is outlined in Chapter 1.1 of the OIE Diagnostic Manual. The basic principles are as follows:

- A fish culture unit must be inspected twice a year for 2 years at the appropriate life stage of the fish and at times of the year when temperature and season offer the best opportunity for observing clinical signs and isolating pathogens. On each occasion any species of fish which is listed in the OIE Code, as susceptible to IPN, must be collected in order to detect a prevalence of infection equal to, or higher than 2% at a confidence level of 95%. In most cases, this will equate to 150 fish twice per year.
- During this two-year period, the fish production unit may only receive fish from a unit whose health status has already been approved and is equal to, or higher than that of the receiving facility.

10 MAINTENANCE OF HEALTH STATUS

According to the OIE regime, once a production unit has been recognised to be free of certain diseases listed in the Code as a result of twice yearly inspection over a period of two years, these twice-yearly inspections must continue, however, the sample size may be reduced from 150 to 30.

Moribund fish

- Observed during inspection visits must, however, be collected for further laboratory examination.
- The fish production unit may only receive fish having a health status equal to, or higher than, the status previously established for this site.

However, because of the fact that IPN is now widespread in the marine environment in Norway and in certain parts of Scotland, the Marine Institute would recommend that a sampling regime of 150 fish twice per year should be maintained even after the completion of the initial two-year testing programme, when importing from such areas.

11 INDIVIDUAL PARENT TESTING

As outlined above, ova may be imported from IPN positive sites provided the exporting company has established a programme of individual parent testing which promotes the immediate disposal of ova from IPN positive parents as soon as laboratory results become available. It may be necessary to ask the supplier to provide an assurance that there is strict separation of ova in the hatchery to ensure that ova from IPN negative parents do not become infected accidentally in the hatchery.

Staff from the Marine Laboratory (FRS) in Aberdeen, carry out an annual audit of broodstock facilities in Scotland. Where eggs are purchased from Scotland, it is recommended that details of the most recent audit carried out by FRS personnel be requested, in order to ensure that the highest standards of hygiene are met in the Broodstock Unit/hatchery and therefore to avoid the accidental introduction of the virus with eggs/live fish.

However, although IPN now rates more highly in terms of mortality and financial loss than any other disease currently present in important salmon producing countries such as Scotland or Norway; for trade and other reasons, Ireland has elected not to accept an AG for this disease. The net result is that MI/DCMNR have no legal powers to regulate the importation of salmonid stocks,

which might be infected with IPNV. Under the current legislation, the only way that this can be regulated is through the implementation of an Industry led Code of Practice with respect to imports. This issue has become more important of late, since trading patterns have changed and new markets have recently opened up to the Irish industry.

Without an AG or a Code of Practice in relation to IPN, it is very likely that the prevalence and severity of the disease in Ireland will increase in the short to medium term, resulting in negative consequences for the Irish industry as a whole, and perhaps also impacting on the wild salmonid sector.

DCMNR/MI and ISGA expect members to adhere fully to the Code of Practice outlined above, in order to prevent the widespread introduction of IPNV into Ireland, a country which has had a relatively trouble free history with this disease to date.

13 INTERNAL MOVEMENTS OF OVA

The same conditions should apply to internal movements of ova as apply to imported ova i.e. either purchase from a site which has been shown to be free of IPN, using the criteria laid down by the OIE, or request individual parent testing, where ova from positive parents are discarded appropriately.

14 ADDITIONAL WORK

Although the first important task in relation to IPNV is to establish and implement a Code of Practice to deal with the importation of live fish and ova, a "Phase 2" Code should follow in the short term. The scope of the second phase should establish an agreed Industry/Government response in terms of dealing with the following instances:

- Clinical case of IPN in freshwater
- Clinical case of IPN in sea water
- Sub-clinical case of IPN in freshwater
- Sub-clinical case of IPN in sea water

It is proposed that a joint Industry/Government Working Group should be established to develop the "Phase 2" Code. We would ask the ISGA to indicate their support for such a move and to put forward members to sit on the Group.

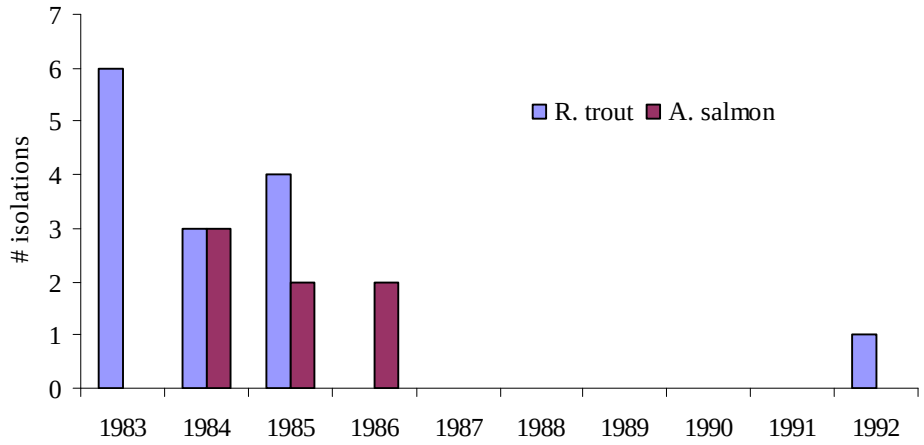
15 IMPORTS FROM THIRD COUNTRIES

Imports from countries outside of the EU/EEA are covered by Commission Decisions 2003/858/EC and 2004/454/EC. Importation is only permitted from countries outlined in Annex 1 of 2004/454/EC and must be accompanied by a Movement Document based on the model outlined in Annex III of the same Decision.

Both the MI and DCMNR welcome the opportunity to advise industry with respect to safeguarding against the importation of IPNV, and we look forward to working closely with ISGA on implementing, and in due course reviewing this IPN Code of Practice.

APPENDIX II

MARINE INSTITUTE IPNV ISOLATIONS (FARMED FISH) 1983 - 1992



This figure shows the number of isolations of the IPN virus from rainbow trout and Atlantic salmon farms (FW + SW) in Ireland, between 1983 and 1993.

NOTE: After 1993, IPNV was isolated on one rainbow trout facility in 2000.

APPENDIX III

MARINE INSTITUTE ATLANTIC SALMON BROODSTOCK TESTING DATA

Information is shown below on broodstock testing for IPNV in Ireland from 2004 – 2006. Detection of the IPN virus is based on isolation in cell culture followed by a confirmatory ELISA test. The table outlines information from the testing of sample pools (ovarian fluid, milt or kidney/brain/heart) from between 1 – 10 fish. It can be seen that the highest number of positive isolations occurs in organ samples submitted for testing. When all samples are taken together, the average prevalence of IPNV positive broodstock fish remains less than 3%, however this is biased due to the higher number of ovarian fluid samples tested and the prevalence clearly depends upon the tissues used for sampling.

% positive pools	Ovarian fluid	Milt	Organs
2004	0.2	0	18.8
2005	0	0	1.6
2006	0.3	0	21.4
Totals	0.2	0	13.9

APPENDIX IV

MODELLING THE SPREAD OF IPNV IN IRELAND

The model

This appendix contains background information on the model used to generate the data presented in Section 3.2.

In order to understand the dynamics of emergence that the IPNV data display, a model developed for the Scottish industry was used (Murray, 2006a). The basic structure of the model is an SI model, under which the population *N* is divided into the proportion of the population that is in uninfected susceptible farms (*S*) and the proportion in infected farms (*I*): as these are proportions $N = S + I = 1$. All the fish in *S* are uninfected, but most fish in *I* may not be infected, however the population in *I* is treated as a single unit with random mixing of infected and uninfected fish (Figure 10).

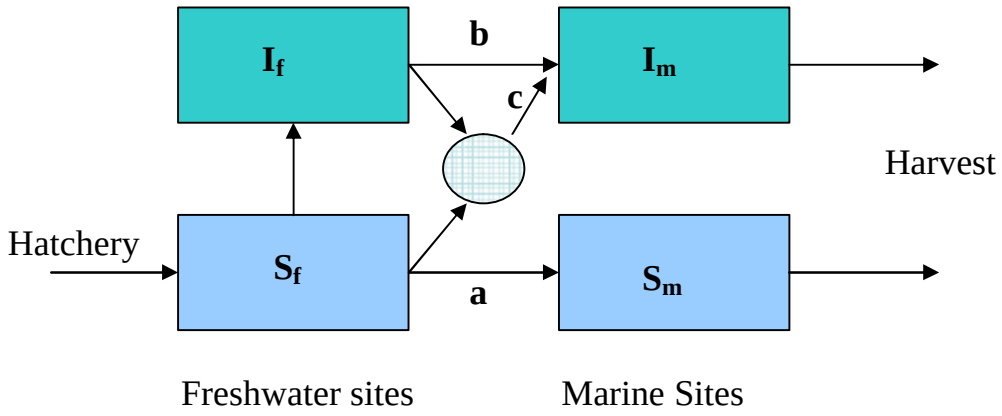


Figure 10. Model structure used for the analysis of the spread of IPNV in Ireland. S = susceptible (uninfected), I = infected, f = freshwater, m = marine. INPV-free fry are input to freshwater sites (Sf), where they may become infected (If). After 1 year, marine sites receive smolts from 1 to 3 freshwater sites. A marine site receiving only from uninfected smolts will be uninfected (a), a site receiving only infected smolts is infected (b), if both infected and uninfected smolts are received then these are mixed (hashed circle) and the marine site is infected (c). IPNV-free marine sites can become infected during the 18 month period prior to harvest.

Fish are input to freshwater as susceptible ova/fry (S_f) where they may pick up infection by exposure to infected populations (I_f) using the formula $b_f S_f I_f$ (Anderson & May, 1979). After one year they are transferred to marine sites, these marine sites may receive smolts from multiple freshwater sites and if any one of them is infected the receiving marine site is also infected (*X*). If the population is not infected it may pick up infection within the marine environment $b_m S_m I_m$.

The proportion of marine sites receiving infected smolts *X* is determined as:

$$X = I_f k/(1 + I_f(k - 1))$$

Where *k* = number of smolt sources.

The model may be run in population independent form, or it may be made population dependent allowing for increased rates of contact between closer farms. As S and I are

proportions and so S + I = 1, in each environment transmission rates must be multiplied by relative population for a given year m_y if this to be applied (m_y = 1 for population independent transmission).

Thus, the model is therefore based on the following formulae:

$$dS_f/dt = s - m_y b_f S_f I_f - sS_f$$
$$dI_f/dt = m_y b_f S_f I_f - sI_f$$
$$dSm/dt = h(1 - X) - m_y b_m S_m I_m - hS_m$$
$$dI_m/dt = hX + m_y b_m S_m I_m - hI_m$$

The parameters *s* and *h* are rates of turnover, the inverse of the time spent on freshwater and marine sites respectively.

Model Parameterisation

To run the model requires giving it appropriate parameter values which may be dependent on the structure of the industry. The model assumes that fish spend 1 year in freshwater and 18 months at sea, therefore the turnover parameters $s = 1$ and $h = 0.6667$ yr^{-1}.

The number of sources of smolts used by marine sites is *k* and different scenarios use *k* of 1 to 3.

As salmon production in Ireland peaked in 2001 a simple trend cannot be fitted for the relative population parameter m_y. Instead, for a given year *y*, the value of m_y is fitted from observed production p_y using p_y/p_{1994}. This is extended, on under two assumptions; that production continues to decline at the rate observed for 2001 – 2005, or that production will stabilise at the 2005 level. Production could increase, but it will be shown that even stabilisation will lead to IPNV becoming widespread.

The values of the parameters b_f and b_m are adjusted to optimise the fit of the modelled values of I_{f0} and I_{m0} to the observation by systematic exploration and minimising the sum of square differences between the model and observations. The initial prevalence of infection is also adjusted to optimise this fit. As the freshwater component of the model is independent of the marine component it is possible to fit the freshwater component first and thus only b_f and I_{f0} need to be adjusted, allowing a systematic search through parameter space. When the freshwater component has been fitted the marine component is fitted for which b_f and I_{f0} have been optimised.

Once the model has been fitted to the existing data it can be projected to predict future development of IPNV prevalence in Ireland. Scenarios are applied whereby IPNV is followed for eight years with no change in parameters or where b_f or b_m are cut by 50%, or *k* is cut from 3 to 1. The scenarios are used to investigate possible control policies and are similar to those used previously for Scotland (Murray, 2006a).

APPENDIX V

MARINE INSTITUTE WILD FISH TESTING 1994 – 2005

Year	# fish	# pools	Virus identified
1994	204	31	-
1995	174	36	-
1996	204	32	-
1997	448	73	Rhabdovirus (pike/perch)
1998	495	78	Aquabirnavirus
1999	163	21	Eel virus X
2000	208	50	-
2001	201	55	-
2002	429	49	-
2003	430	68	-
2004	537	95	-
2005	849	107	Rhabdovirus (perch)
Total	4,342	695	

APPENDIX VI

CERTIFICATION OF SALMON OVA IMPORTS DEC 2005 – FEB 2006

At the MI/ISGA meeting to discuss IPN on 21st June 2006 it was noted that seven Irish hatcheries received ova from the suspected source of infection in Scotland. Irish hatchery 1 received ova from site A and was not affected by IPN. The remaining six hatcheries received ova from site B and four suffered losses due to clinical IPN. Hatchery A did not experience any problems with IPN.

All imports of ova were certified free of VHS and IHN according to EU regulations and free of *G. salaris* and BKD under the additional guarantee granted to Ireland. As there is no statutory control of IPN, DCMNR/MI does not have certification in relation to this pathogen although some farms are in possession of documentation outlining testing of broodstock prior to shipment.

Document Reference	Source	Hatchery	# Ova	Date	Certified free	Additional Guarantee
MD05/048A	A	1	267,800	Dec 28	IHN/VHS	BKD/*G. salaris*
MD06/001A	B	2	280,000	Jan 13	IHN/VHS	BKD/*G. salaris*
MD06/002A	B	3	600,000	Jan 13	IHN/VHS	BKD/*G. salaris*
MD06/003A	B	2	750,000	Jan 19	IHN/VHS	BKD/*G. salaris*
MD06/004A	B	4	82,400	Jan 25	IHN/VHS	BKD/*G. salaris*
MD06/005A	B	5	309,000	Feb 2	IHN/VHS	BKD/*G. salaris*
MD06/006A	B	3	430,000	Feb 2	IHN/VHS	BKD/*G. salaris*
MD06/008A	B	6	463,500	Feb 2	IHN/VHS	BKD/*G. salaris*
MD06/009A	B	6	360,500	Feb 9	IHN/VHS	BKD/*G. salaris*
MD06/010A	B	4	10,000	Feb 9	IHN/VHS	BKD/*G. salaris*
MD06/011A	B	7	412,000	Feb 23	IHN/VHS	BKD/*G. salaris*

APPENDIX VII

GENERAL INFORMATION ON DISINFECTION

Disinfectant	Dose	Result	Reference
Heat	60°C for 8 h 80°C for 10 min	Inactivated Inactivated	Whipple & Rohovec, 1994
Heat	60°C for 30 min	3 $\log_{10}$ reduction	MacKelvie & Desautels, 1975
Peroxy compounds	0.2 – 2% w/v for 1 min (no organic loading) 1 – 2% w/v for 10 min (with organic loading)	6 $\log_{10}$ reduction 6 $\log_{10}$ reduction	Torgersen, 1991
Acid	pH 3.8 – 4.3 for 14 d	No effect	Whipple & Rohovec, 1994
Acid	pH 3.8 for 147 d	0.5 – 2 $\log_{10}$ reduction	Smail *et al.*, 1993a
UV	1850 – 3000 J m^{-2}	3 $\log_{10}$ reduction	MacKelvie & Desautels, 1975
UV	1188 J m^{-2}	3 $\log_{10}$ reduction	Øye & Rimstad, 2001
UV	1220 J m^{-2}	3 $\log_{10}$ reduction	Liltved *et al.*, 1995
Chlorine	25 – 40 ppm for 30 min	Inactivated	Desautels & MacKelvie, 1975
Chlorine	0.7 mg l^{-1} for 2 min	Inactivated	Wedemeyer *et al.*, 1978
Ozone	90 mg/(lxh) for 0.5 – 10 min	Inactivated	Wedemeyer *et al.*, 1978
Ozone	0.1 – 0.2 mg l^{-1} for 1 min	4 $\log_{10}$ reduction	Liltved *et al.*, 1995
Iodophore	32 mg l^{-1} for 5 min	Inactivated	Amend & Pietsch, 1972
Iodophore	35 mg l^{-1} for 5 min	Inactivated	Desautels & MacKelvie, 1975