The Royal Society of Edinburgh

# THE SCIENTIFIC ISSUES SURROUNDING THE CONTROL OF INFECTIOUS SALMON ANAEMIA (ISA) IN SCOTLAND

**A Report of the Royal Society of Edinburgh Working Party on Infectious Salmon Anaemia**

**June 2002**

***Introduction by Sir William Stewart FRS FRSE, President of the Royal Society of Edinburgh***

The Council of the Royal Society of Edinburgh (RSE) established this Working Party to examine the scientific issues associated with the control of infectious salmon anaemia (ISA).

The RSE, as Scotland's National Academy, seeks to provide independent advice on policy issues of importance to Scotland, by drawing upon the expertise of its multidisciplinary fellowship of men and women of international standing. The decision by the Council of the Royal Society to set up this independent Working Party on infectious salmon anaemia was taken following requests by sections of the Scottish salmon farming industry.

This report by Sir Roderick MacSween and his Working Party is a contribution to an ongoing debate about ISA. I wish to express my gratitude to Sir Roderick for his able Chairmanship of the Working Party and to all Working Party members.

***Foreword by Sir Roderick MacSween, FRSE***

From a small number of experimental farm units on the West Coast in the 1960s, the Scottish salmon farming industry has expanded exponentially. It now makes a major contribution to the Scottish economy, producing fish for the food chain and also rearing broodstock genetically selected to improve the yield and quality of fish and for supply to other countries in which salmon farming is an important industry.

Atlantic salmon both in the wild and in fish farms are subject to a number of infectious diseases. The first outbreak of so-called infectious salmon anaemia occurred in Norway in 1984. In 1998/1999 a number of farms in Scotland were affected by the virus and the European Commission was required to develop and introduce methods to control the disease. This has been successful and there have been no further outbreaks in Scotland since 1999. This is in contrast to Norway and Canada where the disease has been controlled but not eradicated.

The culling methods employed in Scotland do have economic consequences for the farming industry and, in addition, such methods were seen as a threat to the broodstock industry representing, as they did, a big exercise and investment in genetic selection.

The Royal Society of Edinburgh determined that it would be important to examine the scientific basis from which the European Commission's proposals were drawn, which it could do from a wholly independent position, drawing on the expertise of its own Fellowship.

This paper is the result of the deliberations of the Working Party set up by the Society. It has obtained evidence both from industry and from those regulating the control of infectious salmon anaemia and other fish diseases; evidence has also been obtained from all countries in which there is a salmon farming industry and in which disease control measures have been introduced. The group hopes that its findings and recommendations will be seen as a contribution to ensure the future welfare of the salmon industry.

I thank all members of the group (listed in Annex 1) for their efforts and for their contributions, based on their own individual areas of expertise, both to the discussions and to the drafting of the final report. I also thank those who gave oral evidence or submitted comments to the Working party (listed in Annex 2). The group owes a special debt of gratitude to Dr Marc Rands (Research Officer) who not only undertook all the secretariat work but also contributed to some of the research which was required.

**Summary and Recommendations**

1. Salmon farming is a major Scottish industry with a world-wide reputation for excellence and quality, whose continued well-being depends on the production of healthy, high quality fish whilst protecting the environment.

2. However, in 1998 a viral disease of farmed Atlantic salmon, infectious salmon anaemia (ISA), appeared in Scotland for the first time, although it had previously been reported in salmon from Norway and Canada. In Scotland, the disease was quickly brought under control and, following the last outbreak in Scotland in 1999, new measures for detecting and controlling the disease were proposed by the European Commission, in line with a precautionary approach. This involved the development, by member states, **of an extended withdrawal scheme tailored to fit the circumstances of the individual farm so that, in a remote location with low mortalities or few clinical signs, a cage-by-cage clearance programme based on mortality triggers would be allowed. Alternatively, an infected farm close to other uninfected farms would be required to be cleared as soon as possible**. A withdrawal scheme was submitted by the UK (Scotland) and approved through EU Decision 2001/186/EC in February 2001. The Working Party welcomes the general approach taken by the Scottish Executive and the EU to control ISA. In accepting the Scottish withdrawal scheme, however, the Commission laid down an alternative criterion for diagnosing ISA, namely the isolation and identification of ISA virus from any fish on the farm, without signs of clinical disease or pathological findings.

3. Following concerns from the Society's Fellowship and the Scottish salmon farming industry about the possible long-term impact of the withdrawal scheme on Scottish fish farming, including the broodstock industry, the Royal Society of Edinburgh (RSE), as Scotland's National Academy, set up a Working Party on ISA to investigate the scientific issues surrounding the disease and its control. These included consideration of the reliability of detection methods, effectiveness of culling as a means of control, comparison of regimes for control in other countries and the prevalence/incidence and nature of the ISA virus in wild and farmed salmon.

4. The Working Party consulted widely and took account of the many relevant inputs made (see Annex 2 for a list of those who submitted evidence). At the outset, the Working Party considered it to be important to distinguish between ISA as a disease and infection by the ISA virus. **On the basis of the evidence made available to us, we conclude that clinical outbreaks of ISA have been eliminated in Scotland**. The eradication policy, coupled with improvements in fish husbandry and management, has achieved this. **We further conclude that the detection of virus by currently available laboratory methods in the absence of clinical or pathological features, does not necessarily indicate ISA disease or predict that an ISA disease outbreak will occur** (paragraphs 11, 17, 20).

5. There has been anecdotal and unconfirmed evidence that the ISA virus is prevalent in open waters of Scotland as distinct from in and around fish farms, and limited and fragmentary evidence that it occurs also in other species of fish (paragraph 13, 14, 52). **We conclude that it is impossible to establish on the basis of presently available evidence whether this is the case or whether the virus is exotic to EU waters**. We note that the Fisheries Research Services (FRS) survey of the presence of ISA virus in wild salmon and other fish has been stopped. **We recommend that the survey for ISA virus in wild fish be re-established** (paragraph 52). **We further recommend that there should be extended**

**surveillance of Scottish salmon farms for the ISA virus to determine whether, in the absence of the disease, the virus is still present**.

6. In order to control and manage ISA, accurate and rapid diagnosis of the disease and detection of the virus are crucial. **The diagnostic methods used by the FRS have been recognised internationally as the best available**. However, there is a need for on-going external quality assurance and **we believe that to provide assurance on quality control at the FRS laboratory, there needs to be a regular exchange of samples between national and international accredited laboratories** (paragraphs 54-55). **Access to such laboratories should also be available to industry. We recommend a policy of openness and transparency between the salmon industry and those investigating and regulating ISA and other fish diseases**

7. We note the development of new and more reliable virus detection techniques such as nucleic acid sequence based amplification (NASBA) and real time fluorescent reverse transcriptase - polymerase chain reaction (RT-PCR) (Paragraphs 28, 54). Additional resources will be required for the full application of these techniques in ISA and other fish diseases. We also note that there is currently little knowledge of the serology of the ISA virus. Further research in this area is a necessary prerequisite to assessing the possible efficacy of vaccination and for the possible screening for ISA infection (paragraph 56). Vaccination has been adopted in Canada as an aid to the control of ISA and **we recommend that efforts to develop and improve ISA vaccines be undertaken** (paragraph 57).

8. **Overall, the FRS must remain at the international forefront of diagnostic service provision within the EU and internationally. We recommend that the FRS in Scotland receives the necessary additional resources required to upgrade its technological and diagnostic base so that it remains a world leader with respect to ISA. We further recommend that the EU designate the FRS as the first point of reference for suspected ISA disease and that this should be reflected by Scottish Executive and EU funding of the laboratory**.

9. It is important that continued effort be sustained on surveillance for ISA in Scotland because there is always the possibility that ISA may recur. **We believe that the current eradication policy through withdrawal be continued in confirmed cases of ISA** (paragraphs 50-51). **We also recommend that the scientific criteria for the category "suspicion" of ISA should be re-examined and clearly defined** (paragraphs 53, 59). In the event of suspicion, the recommendations of the Joint Government/Industry Working Group on ISA provides a good basis for management of a potential outbreak (paragraphs 51, 52).

10. **We are fearful that current policy disadvantages Scotland's salmon broodstock industry**. The procedures advocated by the Joint Government/Industry Working Group on ISA do not well address the broodstock industry's needs and could result, in certain circumstances, in serious loss of its unique gene pool and resulting serious financial damage. **We recommend that the regulators and the broodstock industry together address the changes necessary to the current regulations to take account of the needs of the Scottish broodstock industry** (paragraph 59).

11. Finally, We note that the current eradication policy has operated without financial compensation for fish slaughter. We are concerned that this may restrict robust surveillance

and **we recommend that the provision of financial compensation for slaughtering of fish be re-examined** (paragraph 58).

## Introduction

1. Salmon farming is a major industry in Scotland. In 2000, 90 companies operating 346 sites produced an estimated 128,959 tonnes of adult fish for human consumption. Infectious salmon anaemia (ISA) is a viral disease of farmed Atlantic salmon which first appeared in Scotland in 1998. The RSE set up the Working Party on ISA following concerns from the Fellowship and the Scottish salmon farming industry about the impact on Scottish fish farming of the ISA withdrawal scheme approved through EU Decision 2001/186/EC in February 2001. There were also concerns centred on the possibility that withdrawal could be triggered by the detection of the ISA virus without the presence of any signs of the disease. Furthermore, it was suggested that a background level of ISA virus in the wild could result in low level, but repeated, ISA virus infection of farmed salmon without causing disease.

2. The remit of the Working Party was, therefore, to investigate the scientific issues surrounding the scheme submitted by the United Kingdom for the withdrawal of all fish in Scottish farms infected with ISA virus, required under EU Directive 2000/27/EC(2), including the reliability of detection methods, effectiveness of culling as a means of control, comparison of regimes for control in other countries and the prevalence/incidence and nature of ISA virus in wild and farmed salmon. The members of the RSE Infectious Salmon Anaemia Working Party are detailed in Annex 1.

## Salmon farming in Scotland

3. The farming of Atlantic salmon in Scotland started in the 1960s with a small number of experimental sites in sea lochs on the west coast. The industry developed slowly during the 1970s, when various husbandry difficulties concerning cage design, nutrition, breeding and disease were tackled. By the end of the 1970s, the industry had started to expand at an almost exponential rate and this expansion continued until the end of the century. However, as the industry developed, the number of companies contracted and more and more of the total production was controlled by large companies. During this period the average farm size increased from 85 tonnes in 1985 to 355 tonnes in 1995 and by 2000 there were 90 companies (120 in 1995) operating 346 sites producing an estimated 128,959 tonnes of adult fish for consumption. Fifteen companies accounted for 74% of this production. Data for Scottish fish farms are prepared annually for the Scottish Executive by its agency, Fisheries Research Services (e.g. Stagg and Allan 2001). The Working Party was made aware of the environmental implications of salmon farming but noted that, although it merited investigation, it was not within the current remit of the group.

4. The rearing cycle for production in salmon farming attempts to mirror the natural life cycle of the fish, but at the same time attempting to speed up the development at all stages. Although the original fish came from the wild, almost all the adult fish now used for broodstock have been farmed for several generations and selected for particular traits such as rapid growth. Broodstock fish are kept in sea water but moved into fresh water prior to spawning, which is carried out manually between October and January, stripping first the female fish of eggs and then fertilising these with milt from the males. The fertilised eggs are kept in cool running water until they hatch. About midway during incubation, when they become ‘eyed’, they are relatively robust and can withstand transportation at this stage if necessary. After hatching, the fry are kept in tanks in fresh water and fed on an artificial diet. As they grow and develop into parr, they may be moved to larger floating cages in lochs. After about 6, 12, 18 or 24 months, depending on production requirements, they

develop into the silvery smolt stage when they are transferred into cages in the sea. Here, growth rate increases and, on a diet of specialised feed pellets, they grow to marketable size in about two years.

5. Broodstock farms are also an important component of salmon farming. In 1999/00 the number of eggs laid down to hatch was in excess of 78.5 million. In 2000, the number of sites holding broodstock was 18 (27 in 1991) and from these 17,854 female fish were stripped to produce some 125 million eggs, an average yield of some 7,000 eggs per fish. Almost all eggs for the production of Scottish farmed salmon were derived from Scottish farmed stocks, with only 6% coming from non-Scottish stocks (just over 5 million in total with over 4.5 million of these coming from Iceland). In addition, just under 3.5 million parr and smolts were imported from EU member states. In 2001, the export of eggs to other countries within the EU decreased by 56% to under 7 million, whilst exports to Chile decreased by 40% to just over 10 million. Most eggs obtained from wild stock (50,000 in 2000) are held and hatched for wild stock enhancement, in co-operation with wild fisheries interests (e.g. rod and line fishing). In 2000, 60 companies were engaged in the freshwater production of salmon smolts and 279 freshwater sites were registered, producing 45,583,000 smolts (22,404,000 in 1991). The number of smolts put to sea cages was 45.2 million, which included smolts imported from England and the Republic of Ireland.

**Infectious salmon anaemia**

6. Infectious salmon anaemia (ISA) is a viral disease of farmed Atlantic salmon (*Salmo salar*). ISA is the designation of the disease recommended by the Office International des Epizooties (OIE) Fish Disease Commission, with which the disease was officially registered in 1990. The first recorded outbreak of the disease occurred in Norway in 1984 (Thorud and Djupvik 1988) when it was referred to as Brennes syndrome due to the location of the outbreak in the Brennes region; the outbreak was associated with a mortality of 80%. The disease subsequently spread, the pattern of spread suggesting it was contagious. The incidence of the disease peaked in Norway in 1991. The aetiological agent was identified as an orthomyxovirus-like enveloped virus in 1995 using a cell line established from Atlantic **s**almon **h**ead **k**idney (SHK-1) (Dannevig *et al.* 1995).

7. Following the outbreak in Norway, ISA was first suspected in Canada in 1996 in the New Brunswick area. It was, however, initially reported as haemorrhagic kidney syndrome because the pathological findings were different from Norwegian ISA, with lesions occurring in the kidney rather than the liver (Bryne et al. 1998, Mullins *et al.* 1998). The virus was subsequently detected in Nova Scotia in 1999 (Ritchie *et al.* 2001), in the Faroe Islands in 2000 (OIE 2000), in Maine, USA, (OIE 2001) and at a Coho salmon farm in Chile in 2001(Kibenge *et al.* 2001).

8. The first outbreak of the disease in the European Union (EU) occurred in a salmon farm in Loch Nevis in May 1998 (Roger *et al.* 1998). From 1998 to 1999 it spread to a total of 11 farms and was suspected in a further 25 farms on the Scottish west coast mainland, Skye, Orkney, Shetland and the Western Isles. The last confirmed outbreak was in Shetland in 1999. However, a survey for ISA virus in farmed salmonid fish continued to show the presence (albeit declining) of the virus in farm sites in marine surveillance zones, other marine sites and freshwater sites in 2000 (Stagg *et al.* 2001).

9. The European Commission Scientific Committee on Animal Health and Animal Welfare recently concluded that there was no evidence for risk to man (Scientific Committee on Animal Health and Animal Welfare 2000) for the following reasons:
   - the virus is inactivated by pH values below 4.5 and if humans ate infected fish, the pH of gastric secretions, being below 2.0, would rapidly inactivate the virus.
   - the virus does not replicate *in vitro* at temperatures of 25$^{o}$C or above and therefore it is unlikely that the virus could replicate in the human body.
   - the virus appears not to be able to replicate in human cells.

**The Infectious Salmon Anaemia (ISA) virus and the epidemiology of the disease**

10. Infectious salmon anaemia virus is an Orthomyxovirus-like virus, similar to the influenza viruses of humans, mammals and birds. It is the only recorded orthomyxovirus disease of fish and determination of nucleotide sequences places it in a separate genus with a long evolutionary separation from the influenza viruses (Krossoy *et al.* 1999). The virus is a single stranded enveloped ribonucleic acid (RNA) virus of spherical shape and about 100 nm in diameter (Falk *et al.* 1997).

11. By comparing the nucleic acid sequences from the different viral isolates, it is possible to estimate the relatedness of different virus stains. Two separate strains of ISA virus have been identified in Scotland (Stagg *et. al* 2001); two different strains in Canada (Ritchie *et al.* 2001), one of which caused no characteristic ISA virus-related pathology in salmon; one in Chile (Kibenge *et al.* 2001) and a number of different ISA virus isolates in Norway.

12. The Scottish strains, although not identical with, were more closely related to the Norwegian virus, than the New Brunswick Canadian virus (Cunningham and Snow 2000). Estimates of the mutation rates of PB1 on segment 2 of ISA virus suggests that the New Brunswick, Canadian virus diverged from Norwegian isolates over 100 years ago (Krossøy *et al.* 2001). This is important because it is unlikely that the appearance of ISA virus in Canada was the direct result of recent importation of infected fish from Norway, or introduction of a Norwegian strain of the ISA virus by naturally infected wild fish populations (Blake *et al.* 1999).

13. Importantly, the ISA virus can be carried by other salmonid species such as brown trout (*Salmo trutta*), sea trout (*S.trutta*) (Raynard *et al.* 2001), rainbow trout (*Oncorhynchus mykiss*) and, to a lesser extent Arctic charr (*Salvelinus alpinus*) (Snow *et al.* 2001), without the fish showing clinical signs of the disease. Unconfirmed positive tests for the virus have also been reported in Coho salmon (*Oncorhynchus kisutch*) (Kibenge *et al.* 2001), the European eel (*Anguilla Anguilla*) (Raynard 2000), wild plaice (*Hippoglossoides platessoides*) (Mjaaland *et al.* in press) and possibly Atlantic herring (*Clupea harengus*) (Mullins *et al.* 1999) and haddock (*Melanogrammus aeglefinus*) (Mjaaland *et al.* in press).

14. In Scotland there is putative evidence of ISA virus in salmon parr, juvenile brown trout, sea trout and eel (Raynard 2000), both in marine and fresh water. It is notable that the eel and sea trout were found in areas close to farms infected with ISA virus, while the salmon parr and juvenile brown trout were found in fresh water in areas distant from salmon farms. The detection of the virus or its nucleic acid, however, cannot in isolation be equated with ISA. Methods of detecting ISA virus and criteria for establishing its presence are considered later (paragraphs 17-28).

15. Infection can be spread by fish-to-fish contact, through seawater, by transport of equipment and by organic material, especially blood and processing water (Jarp and Karlsen 1997). The infection spreads slowly and the virus is considered to be of low virulence, although mortality can be high in some outbreaks of the disease. An outbreak within a farm usually develops within 1-3 weeks, often following the stock being stressed, and may be restricted to one or two fish cages. Most ISA outbreaks occur during spring or early summer, while a minor peak is reached in autumn/winter months.

16. In tests on salmon, the ISA virus was not transmitted from infected parents through their gametes to their offspring (Melville and Griffiths 1999). Thus, importantly, there is no evidence of vertical spread.

**The diagnosis of ISA**

***Clinical and Pathological features***

17. There have been variations in the patterns of ISA outbreaks with differences in the pathological findings and their severity; these may depend upon the dose of virus, virus strain and pathogenicity, age and immune status of the salmon, and extraneous factors such as season and temperature. Experimentally infected fish show an incubation time of 10-20 days before the onset of clinical signs, although on salmon farms some fish may harbour the ISA virus for weeks or months before developing the disease (Mjaaland *et al.* in press). Cumulative mortality during an outbreak of ISA varies from insignificant to moderate but some farms may suffer losses of 90%. It is suspected, albeit not proven, that on fish farms, the pathology of the infection may vary with the genetic strain of salmon (Ferguson 2002: personal communication) and, in experimental studies, mortality can vary between 10 – 100% depending upon the genetic background of the salmon (Nylund *et al.* 1995). This is significant given there is compelling evidence for structuring of Atlantic salmon and brown trout into distinct reproductive populations and for genetic differentiation and local adaptation (Youngson *et al.* in press). We conclude that the detection of virus by laboratory methods (IFAT, PCR or virus isolation) in the absence of clinical or pathological features, does not necessarily indicate or predict ISA disease.

18. Classically, ISA virus-infected fish appear lethargic and many keep close to the walls of the fish cages. In the terminal stages, diseased fish may sink to the bottom of the cage. The further development of the disease varies and up to 12 months can pass before clinical ISA has spread to all the cages in a fish farm. In the acute form of the disease there is a rapid development and high mortality. Prominent external signs are pale gills, exophthalmos (protrusion of the eyeballs) and sometimes intra-orbital bleeding and skin haemorrhages. The fish become severely anaemic with haematocrit values below 10% and as low as 5%.

19. The (pathological) macroscopic features comprise ascites (the collection of fluid in the cavity of the abdomen), a dark liver and dark foregut, enlarged spleen, congestion of the intestinal wall and petechiae (small local haemorrhages) in the adipose tissue and swim bladder (Thorud 1991). Histologically, the major finding is multifocal haemorrhagic liver necrosis which may become confluent; there is sinusoidal dilation and congestion and blood-filled spaces may be seen. These hepatic changes are highly characteristic of ISA but are not individually pathognomonic.

20. In the chronic form of the disease there is a slow increase in mortality over several months. The pathological features are not so severe, with less marked ascites but haemorrhages in

the skin and swim bladder may be more marked. The anaemia is less severe and the liver appears pale or yellowish (Evensen *et al.* 1991).

21. In the New Brunswick outbreak in Canada, the disease was characterised by renal interstitial haemorrhage with tubular casts and necrosis; gross liver lesions were rarely observed. This led to the original diagnosis of haemorrhagic kidney syndrome (HKS), the aetiology of which, on the basis of virus isolation, was subsequently shown to be due to ISA virus (Lovely *et al.* 1999). Similar kidney changes have been found in some archival material from early Norwegian outbreaks (Lovely *et al.* 1999).

22. In Chile, ISA virus has been detected in Coho salmon diagnosed with Icterus disease, with affected fish having pale gills and severe anaemia, pale liver and gall bladder and mild enlargement of the spleen (Kibenge *et al.* 2001). It has not yet been identified, however, whether the clinical signs of Icterus are due to the ISA virus present or another aetiology (Hästein, written evidence).

23. Anaemia is an absolute in making the diagnosis but this, in common with the other clinical and pathological signs, shows varying degrees of severity. The diagnosis of ISA may be based solely on clinical and pathological findings when all the criteria are fulfilled (OIE Diagnostic Manual), but diagnostic methodology varies between Norway, Canada and the EU (see paragraphs 31-50). When the clinical and pathological findings raise suspicion of ISA, the diagnosis is confirmed by positive laboratory findings for ISA virus.

***Laboratory Tests***

*Virus isolation*

24. Virus isolation represents the 'gold standard' for ISA confirmation. However, early attempts to isolate the ISA virus in commercial fish cell lines were unsuccessful and the virus was only isolated approximately 10 years after the initial outbreak of ISA in Norway, using a new cell line from Atlantic salmon head kidney, SHK-1 (Dannevig and Thorud 1999). There are now a number of other cell lines in use (Devold *et al.* 2000, Kibenge *et al.* 2000, Sommer and Mennen 1997, Wergeland and Jakobsen 2001). However, virus isolation is not routinely used for detection because the test takes time, the virus can often be difficult to grow and it may not give a cytopathic effect even if present.

*Indirect fluorescent antibody test (IFAT)*

25. Indirect fluorescent antibody testing is based on monoclonal antibodies raised against viral protein antigens, notably the haemagglutinin molecule on the surface of the virus (Rimstad *et al.* 2001). The bound antibodies are detected by secondary fluorescently-labelled antibodies enabling them to be seen as green dots with a fluorescent microscope. Key factors influencing the reliability of the method include the integrity of the sample, the amount of virus in the sample, the specificity of the first stage antibody and the correct use of known negative and positive controls.

*Reverse transcriptase - polymerase chain reaction (RT-PCR)*

26. This test detects specific sequences of the viral RNA genome, i.e. either whole viral RNA or particular fragments of degraded viral RNA, whether from live viruses or dead viruses. The RT-PCR test uses viral RNA extracted from tissues or cells to produce complementary DNA

by reverse transcriptase (RT). The complementary DNA is then amplified by repeated duplications through a polymerase chain reaction (PCR) and the final product is visualised on an agarose gel (Mjaaland *et al.* 1997). RT-PCR can be used in combination with IFAT to confirm ISA diagnosis.

27. The laboratory procedures are crucial for the reliability of RT-PCR. The specificity of the primers used is of key importance. For example, primers amplifying a conserved region of the complementary DNA (cDNA) may detect all viruses within the orthomyxoviridae, not just ISA (resulting in false positive results), while primers amplifying variable regions of the cDNA may only amplify cDNA from a single virus isolate (possibly missing other ISA strains). Other important factors are:
    - the choice of controls (negative, positive and/or internal controls) that run in parallel with the samples during each analysis;
    - the organisation of the laboratory to avoid contamination between samples from different locations and from amplified products from earlier analysis;
    - the laboratory skills of the persons performing the analysis;
    - the procedures used for sampling tissue, in particular avoiding excessive degradation of viral RNA;
    - the number of parallel samples tested separately;
    - the number of PCR-positive fish from a single location required for confirmative diagnosis;
    - the test sensitivity, specificity and predictive values.

    Without these protocols in place, there is a risk of erroneous findings with both false positive and false negative results.

28. Newer methods for detecting ISA virus, including real time fluorescent PCR and **n**ucleic **a**cid **s**equence **b**ased **a**mplification (NASBA), are currently being developed. These techniques have the advantages of being able to use more than one primer and so may identify more than one strain of ISA virus per sample; they will also operate with sealed reaction vessels throughout the analysis, thereby reducing the chances of contamination; they are likely also to be able to indicate the amount of virus present.

**European legislation for ISA disease control**

29. In 1991 ISA was classified as a List 1 disease (Council Directive 91/67/EEC (as amended)). This required it to be eradicated through control measures initially prescribed in Council Directive 93/53/EEC, comprising the immediate withdrawal of all fish from an infected farm where official confirmation of infection by laboratory examination or clinical and post-mortem examination had taken place. Withdrawn fish are slaughtered and, if of a marketable size, can be sold for human consumption.

30. In the light of experience gained during the outbreak in Scotland in 1998, the European Commission developed proposals for a more pragmatic way to control ISA and to allow efficient control of the disease whilst safeguarding as much as possible the interests of the infected farms. This new directive, Council Directive 2000/27/EC, amended Council Directive 93/53/EEC in two important ways: (a) the requirement for immediate withdrawal of fish was replaced by approval of phased removal of fish over a period of time allowing, in some cases, the salmon to grow to marketable size before slaughter; (b) the prohibition of use of vaccines for List 1 diseases was removed.

31. In May 2000 the European Commission carried out a mission to Scotland to assess the situation following the ISA outbreaks (European Commission Mission 2000). Their report recommended to the Scottish authorities that they should reconsider the decision-making processes for the confirmation of infection by ISA virus, with virus isolation in cell culture leading to confirmation of the infection, regardless of the clinical severity of disease on a farm. *At that time, the procedure adopted for diagnosis of ISA in Scotland, based on Council Directive 93/53/EEC, required all of the following criteria to be satisfied: clinical disease, typical macroscopic findings including evidence of anaemia, typical histological findings and evidence of infection (Comments of the Competent Authority to European Commission Mission 2000).*

32. However, in February 2001 the European Commission accepted a withdrawal scheme submitted by the UK (Scotland), later amended to include England, Scotland and Wales, in response to the European Commission amendment to Council Directive 93/53/EEC (Commission Decision 2001/186/EC). *In accepting the Scottish withdrawal scheme, the Commission laid down an alternative criterion for diagnosing ISA, namely the isolation and identification of ISA virus from any fish on the farm, without signs of clinical disease or pathological findings.* This was, however, also an option outlined in Council Directive 93/53/EEC and was consistent with approaches taken for human and animal diseases.

33. The withdrawal scheme itself (although not the new diagnostic criteria) was introduced following widespread consultation with industry, fishery and wildlife interests. *In confirmed cases of ISA* a withdrawal scheme is tailored to fit the circumstances of the individual farm so that, in a remote location with low mortalities or few clinical signs, a cage by cage clearance programme based on mortality triggers would be allowed. On the other hand, an infected farm close to other uninfected farms would be required to be cleared as soon as possible. In the case of *widespread mortality* (where the disease has spread or is spreading rapidly through the farm) withdrawal and clearance of the farm is required as soon as practicable, whereas in the case of *significant mortality* (usually >0.05% per cage per day) withdrawal is undertaken within the part of the farm affected. Farms or parts of farms with mortality attributed to ISA but consistently just below 0.05% per day may also require to be cleared. An obligation is placed on the farmer to keep daily records of mortality rate in each cage and to send these on a weekly basis to inspectors at the FRS laboratory in Aberdeen. The farmer must comply with the individual withdrawal scheme, imposed by powers vested in the Scottish Ministers and enforced by a fine of up to £5000. As in the previous directive, restocking is not allowed until the farm has been fallowed for 6 months following withdrawal of the last fish.

34. To prevent spread of disease, a category of "*Farms Under Official Suspicion of ISA*" is also defined by one of the following three criteria:
    i) the occurrence of the clinical signs of ISA (as specified in paragraph 31);
    ii) at least two positive results from each of more than one of the laboratory tests;
    iii) there has been transfer of live fish into the farm from a site where ISA is confirmed or where there are reasonable grounds to suspect ISA was present.

35. Where there is "*suspicion of ISA*", based on the above criteria, under the Diseases of Fish Act 1937,1983, a thirty-day notice will be placed on the farm, complemented by a gate notice imposing a range of restrictions on the movement of fish, eggs or gametes, and people and vehicles entering or leaving the farm without the authorisation of the official service (e.g. the Fish Health Inspectorate). Investigations on the fish farm will continue until either suspicion is revoked (i.e. reasonable evidence of an alternative cause is found or no

further evidence of ISA is found for a period of six months) or ISA is confirmed. The thirty-day notice can be extended up to a maximum of 60 days whereupon, if suspicion continues, a Designated Area Order (DAO) will be placed introducing long term containment measures.

36. Following confirmation or suspicion of an outbreak, a programme of regular inspections is established. Within the control zone a minimum of 12 inspections are carried out in the first year. In the second year and in farms within the surveillance zone a minimum of six inspections are done. This is continued until all farms in the control and surveillance zones have been fallowed and disinfected. (NB. Under current industry codes of practice all farms with or without disease must be fallowed after each production cycle). After the two-year period, in the absence of further outbreaks, clinical inspections may be reduced to two per year for a minimum of two years. For a clinical inspection all production units are examined together with any available samples of dead, or sick fish. Where there are no recent mortalities or fish showing clinical signs of ISA, no samples are required. If clinical signs consistent with ISA are observed, a minimum of ten fish should be sampled taking as many abnormal or sick fish as available but making up numbers with healthy fish. Samples will be subject to laboratory examination as in paragraphs 31, 32, 34.

**Regimes for ISA disease control in other countries**

37. The general approach is that summarised in the Diagnostic Manual for Aquatic Animal Disease (2000) published by the Office International des Epizooties (OIE).

    "*The incidence of ISA may be greatly reduced by implementation of general legislatory measures regarding the movement of fish, mandatory health control, and slaughterhouse and transport regulations, as well as specific measures including restrictions on affected, suspected and neighbouring farms, epizootiological studies, enforced sanitary slaughtering, generation segregation ('all in/all out'), and disinfection of offal and wastewater, etc., from slaughterhouses and fish processing plants."*

*Norway*

38. Given the numerous fish farms distributed along its extensive coastline and many rivers populated by migratory salmonids, Norway regards eradication of the ISA virus as an impractical goal. Instead, it has opted for minimisation of the disease. The Norwegian Animal Health Authority, with the Regional Veterinary Officers and District Veterinary Officers bear the main responsibility for administration of the Fish Disease Act. As soon as there is any suspicion of ISA, blood and tissue samples are taken from five suspect fish for histological examination. Kidney imprints and kidney tissue stored in transport-medium are also taken from at least ten fish for IFAT analysis and virus isolation, respectively, to the National Veterinary Institute. Restrictions banning trade are placed on the farm on suspicion of an outbreak of ISA.

39. Diagnosis is confirmed on the basis of one of the following alternatives (Dannevig, written evidence):
    (i) Solely on clinical and pathological findings: e.g. clinical signs/mortality and typical macroscopic findings, anaemia and typical histopathological findings. All criteria (as set out in the OIE Diagnostic Manual for Aquatic Animal Diseases) should be fulfilled.

(ii) Positive IFAT and suspicious clinical and pathological findings: e.g. detection of ISA virus by IFAT on tissue imprints, and suspicious histopathological findings or haematological findings.

Note: ISA diagnosis is not confirmed by detection of virus by IFAT or RT-PCR alone without there being clinical or pathological findings.

40. Sampling is carried out twice at an interval of one month before the Regional Veterinary Officer can consider lifting the suspicion of ISA.

41. Once ISA is confirmed on a farm, fish are withdrawn on a cage-by-cage basis once mortality reaches 0.05% per cage per day. Assessment is on a case-by-case basis in relation to the infection risk to other farms/sites. Following infection, the premises and equipment are cleaned and disinfected and left fallow for six months.

42. Since 1984 there have been continuing outbreaks of ISA in Norway, culminating in over 95 outbreaks in 1990. The number of outbreaks has since declined, but in 2001 there were 21 separate outbreaks of ISA.

*Atlantic Canada*

43. The disease, first formally diagnosed in 1997, is controlled by a programme similar to that applied in Norway, under the New Brunswick Aquaculture Act (Provincial) (Olivier, written evidence). Fish health, unusual clinical signs and pathology are assessed by veterinarians, who visit sites every six to eight weeks, monitored by the Provincial government. Diagnosis of ISA is based on evidence of positive IFAT in one or more fish sampled during surveillance visits, supported by evidence of veterinary examination and laboratory evidence of virus (RT-PCR or culture)

44. Samples for laboratory analysis can be sent to a variety of Government or university laboratories. When ISA is confirmed on a farm, fish are withdrawn on a cage-by-cage basis, once mortalities exceed 0.05% per cage per day. However, the majority of cage depopulation occurs at a lower mortality level. Vaccination against ISA is permitted.

45. A farm is considered ‘suspect’ as a result of one positive RT-PCR result for ISA virus, or two positive IFAT results for ISA virus, without any other corroborating evidence. In such cases, no direct action is taken but the attending veterinarian, or the Provincial staff, will likely be more cautious and may follow the site more closely.

*Pacific Canada*

46. ISA has not been detected on Pacific coastal farms but there is increased vigilance and advanced planning for rapid containment of infection and stock removal should an outbreak be detected

*USA*

47. The US Department of Agriculture has developed a contingency plan to combat the disease which first occurred in Maine during 2001. Control will be achieved through surveillance, vaccination and good operating practice. Funding will be provided for a control programme that will include diagnostic procedures, surveillance, epidemiological studies and disinfection. Funding is available to compensate fish farmers and to train veterinarians.

*The Faroe Islands*

48. In the Faroes (where the first outbreak was in 2000), withdrawal is again on a cage-by-cage basis and, while they had one outbreak in 2000, they had a further five in 2001, all with genetically similar strains of the virus.

*Chile*

49. No information is available at present.

## Conclusions

50. The outbreak of ISA in 1998 and 1999 undoubtedly caused substantial distress and damage to the Atlantic salmon farming industry in Scotland. However, the prompt and decisive steps taken by the authorities to control the outbreak and eliminate the disease, especially the extensive culling involved and the improved codes of practice for fish husbandry, were ostensibly successful in that no further outbreaks have occurred since 1999. We recommend that the current eradication policy be continued in confirmed cases of ISA.

51. It would be complacent, however, to assume that ISA will not, or cannot recur in Scottish salmon farms, not least because the disease continues to occur regularly in Norwegian and North American salmon farms, albeit under different regulatory regimes from those operated in Scotland. In addition, although the point source of the ISA outbreak in Scotland was quickly identified and the ways in which the disease spread to other fish farms in Scotland were readily elucidated and understood, precisely how the outbreak occurred and, above all, where the ISA virus responsible for the disease originated from remain, very largely, matters of conjecture. There is evidence that the site where the outbreak occurred was being intensively farmed and was in an area where extensive processing of both wild and farmed fish was taking place. Elevated biomass generated by high stocking densities compounded by substantial inputs of fish waste into relatively restricted waters are, self evidently, conducive to outbreaks of disease. The Report of the Joint Government/Industry Working Group on ISA rightly stresses the importance of good farm husbandry in disease prevention and lists numerous practical ways of ensuring high standards of husbandry.

52. Of particular relevance to where the ISA virus outbreak in Scotland originated is the issue of whether the virus is exotic to Scottish waters (or EU waters), as is currently the official position of the European Commission, or whether it is endemic. The majority of those presenting evidence to the Working Party were of the opinion that the ISA virus was endemic in Northwest European, including Scottish, waters. However, convincing evidence for the presence of the ISA virus in wild salmon, in other salmonids or in other marine species is, at best, fragmentary; this in part is due to technical difficulties in detecting the virus. Tagging studies have shown that Scottish and Norwegian fish share feeding grounds around the Faroe islands (ICES 1986) and a portion of Scottish fish share feeding grounds around Greenland with fish from Canada and the USA (Moller Jensen 1980). Thus, it is theoretically possible for the ISA virus to be spread by wild salmon. However, we conclude that there is no evidence, at present, that the presence of the ISA virus in wild fish was or is a significant factor in the outbreak of ISA in farmed salmon in Scotland. Some respondents to the Working Party rightly emphasised that the lack of evidence for the ISA virus or ISA is not absolute evidence for their absence in wild fish. However, in the absence of scientific data it is impossible to establish whether the virus is exotic to the EU. We recommend that the survey for ISA virus in wild fish be re-established.

53. An issue which caused concern during the ISA outbreak in Scotland and which is central to the proper understanding of the disease, is the reliability of the methods used for diagnosis and for detecting the virus. The Working Party noted the variability of the clinical signs of ISA, especially in its early stages when correct diagnosis is vital to ensure that appropriate action is promptly taken. The best example of clinical variability is the marked difference between the Norwegian (and Scottish) and the Canadian forms of ISA, even though the disease is caused by an identical or very similar virus. Such variability of clinical and pathological features places a premium on the reliability of laboratory tests for confirming

the presence of the virus. These tests comprise virus isolation, IFAT for detecting virus antigen in tissues, and RT-PCR for detecting viral RNA in tissues.

54. While IFAT was a relatively well established technique at the time of the ISA outbreak in Scotland, RT-PCR was, and is still being developed. Therefore, the weight that should be given to positive RT-PCR tests in suspected cases of ISA was questioned by some respondents to the Working Party, especially in view of the possibility of false positives. Despite this concern, there is no evidence that ISA virus was wrongly identified on the basis of false positive RT-PCR tests in the Scottish outbreak and the diagnostic methods used by the Fisheries Research Services have been those recognised internationally as the best available. Expert evidence presented to the Working Party made it clear that the precision, accuracy and reliability of RT-PCR has developed significantly since the technique was first applied in the Scottish outbreak. In addition, EU-funded research involving Scottish academic and government agency scientists is in progress to apply new technologies such as real time fluorescent RT-PCR and NASBA to more reliably detect ISA virus. There is a need, however, for external quality assurance of the diagnostic methodologies, facilitated by exchange between national and international accredited laboratories. Access to such laboratories should also be available to industry.

55. It is noteworthy that the practice in diagnosing ISA in Canada is to utilise expert laboratories in both universities and Government to conduct laboratory tests, often simultaneously. Adoption of such practice in Scotland would alleviate some concerns that, currently, a single Government laboratory is responsible both for diagnosing ISA and for controlling the disease.

56. The Working Party noted that there has been little research on the serology of ISA virus infection in salmon and, thus, evidence of current or past infection is not available. Serological testing would enable extended epidemiological studies to be carried out both in wild and farmed salmon, with implications for the endemic/exotic virus controversy. We recommend that serological methods of screening for ISA infection be developed.

57. In 2000, vaccines were used at 114 sites to vaccinate 45.8 million fish, mainly to provide protection against furunculosis, but fish at some sites were vaccinated against enteric redmouth disease (ERM), *Vibrio* bacteria and infectious pancreatic necrosis (IPN). There is a need to develop appropriate vaccines against the ISA virus to broaden the options for containing and controlling outbreaks of the disease. This is very important in situations where culling is particularly detrimental to the industry, most notably in the case of broodstock fish which are of defined genetic make-up and essentially irreplaceable. We recommend that efforts to develop and improve ISA vaccines be undertaken.

58. The Working Party was aware that an overriding issue for the salmon farming industry was the lack of compensation for culled fish. There is a concern that, in the continuing absence of compensation, motivation for increasing scientific understanding of ISA and its possible future impact on the industry is inhibited. There may also be a view that, because there have been no outbreaks of ISA in Scotland since 1999, the problem has been solved and there is no need to re-examine the issue of compensation. However, evidence presented to the Working Party indicated that it seems likely that salmon farmers may now not readily volunteer mildly sick fish for veterinary inspection, given the possibility that, if ISA virus is detected, this will have serious economic consequences. It is also likely that farmers will not readily volunteer fish as part of a survey to establish the prevalence of the ISA virus in Scotland. Such attitudes militate against advancing the scientific understanding of ISA.

59. The situation in broodstock farms where there is complete containment of individual stocks in land-based cages warrants separate consideration. There are a number of points which are relevant to ISA, in terms of its control and its legislation: (i) the increased period for slaughter for ISA-infected fish, introduced by the new withdrawal scheme, does not ameliorate the impact of slaughter on broodstock and smolts, which cannot be brought to market; (ii) broodstock salmon are valuable, not only financially, because of the high costs of running broodstock units, but also genetically as a resource for the whole salmon farming industry; (iii) the situation in relation to disease is unlike that pertaining to fish in seawater cages (which are open to the sea and to other cages in close proximity) in that there is strict containment of individual stocks with high standards of hygiene. If disease affected one stock, it is unlikely that cross-infection of other stocks would occur; (iv) in the absence of vertical transmission of ISA virus, broodstock eggs will be ISA virus-free, although this does not eliminate the possibility of viral contamination. We believe that in the event of confirmation and suspicion of ISA, the procedures within the Joint Government/Industry Working Group on ISA do not well address the needs of the salmon broodstock industry where stocks can be isolated and decontamination measures put in place. Attention should be given to risk assessment protocols, agreed in collaboration with the regulators and the broodstock industry.

60. A thorough scientific understanding of ISA and its causative virus is vitally important for those involved in farming. They must also have full confidence both in the scientific methodology for diagnosis, and in the mechanisms for disease control. In the interest of all concerned, we recommend a policy of openness and transparency between industry and those investigating and regulating ISA and other fish diseases.

**Annex 1: Membership of the RSE Infectious Salmon Anaemia Working Party**

*Chair* : **Sir Roderick MacSween FRSE**: Emeritus Professor of Pathology, University of Glasgow

*Members*: **Professor Ian Aitken**: Scientific Director, Edinburgh Centre for Rural Research

**Professor Peter Maitland FRSE**: Independent Research Consultant, Fish Conservation Centre

**Professor Imants Priede FRSE**: Professor of Zoology, University of Aberdeen

**Professor Stuart Reid FRSE**: Professor of Veterinary Informatics and Epidemiology, University of Glasgow Veterinary School and University of Strathclyde

**Professor John Sargent FRSE**: Professor of Nutritional Biochemistry, Institute of Aquaculture, University of Stirling

**Sir William Stewart FRS FRSE**: President of the Royal Society of Edinburgh

*Secretary*: **Dr Marc Rands:** Research Officer, Royal Society of Edinburgh

***Annex 2: List of organisations and individuals who submitted or gave evidence***

Mr Allan Berry, Knapdale Seafarms Limited

Dr B. Dannevig, Office International des Epizooties (OIE) expert for Infectious Salmon Anaemia

Professor Hugh Ferguson, Professor of Diagnostic Pathology & Microbiology, University of Stirling, and formerly Professor of Pathology, Ontario Veterinary College, Canada

Dr Roar Gudding, Deputy Director, National Veterinary Institute, Norway

Professor Tore Hästein, Head of the OIE Reference Laboratory for ISA, National Veterinary Institute, Norway

Mr James Lithgow, Chairman, Lithgows Ltd

Dr Alasdair McVicar, Senior Policy/Program Advisor, Department of Fisheries and Oceans Canada

Mr Angus Morgan, Chairman of the Scottish Salmon Industry's ISA Commercial Group

Dr Gilles Olivier, Manager Aquaculture Division, Department of Fisheries and Oceans Canada

Mr Gordon Rae, Technical Director, Scottish Quality Salmon

Dr Hugh Reid, Head of the Division of Virology, Moredun Research Institute

Professor Randolph Richards, Director of the Institute of Aquaculture, University of Stirling

Professor Ronald Roberts, Scientific Advisor to Lithgows Group Board

Mr Ronnie Soutar, former Production Manager for Hydro Seafood GSP ltd, and former President of the UK Fish Veterinary Society

Dr Ron Stagg, Deputy Director, Marine Laboratory Aberdeen

Mr Treves-Brown, Fish Veterinary Society

Dr Malcolm Windsor, Secretary, The North Atlantic Salmon Conservation Organisation

Members of the Working Party also visited the Fisheries Research Service Marine Laboratory in Aberdeen (courtesy of Dr Ron Stagg) and Ormsary Fish Farm, a broodstock farm (courtesy of Mr James Lithgow)

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