Epidemiological Investigations into an Outbreak of Viral Haemorrhagic Septicaemia (VHS) in Yorkshire, United Kingdom

First Report

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National Control Centre for VHS

Centre for Environment, Fisheries and Aquaculture Science (Cefas).

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1 Introduction

1.1 Approved zone status for Great Britain

Great Britain (GB) has long-standing fish disease control legislation, originating with the introduction of the Diseases of Fish Act, 1937, which prohibited the importation of live salmonids into Great Britain (England, Wales and Scotland). The implementation of the single European market for trade and the adoption of Council Directive 91/67/EEC provided a free internal market for trade in fish and shellfish, subject to certain animal health protection measures. Those measures include the designation of "approved zones" that have been documented to be free from specified List II diseases (viral haemorrhagic septicaemia [VHS] and infectious haematopoietic necrosis [IHN]). Member States having approved zone status for either or both of these diseases are protected from their introduction by preventing importation of live fish, uneviscerated dead fish and ova of susceptible species from non-approved zones.

Great Britain was granted Approved Zone status for VHS and IHN in 1992 (EC Decision 92/538/EEC) on the grounds of its historic freedom from these diseases and its long history of fish disease controls.

1.2 History of VHS in Great Britain

In 1994, the first recorded outbreak of VHS in GB was diagnosed at a single land-based turbot farm on the Island of Gigha in Scotland. The island and an area around it were formally suspended from the GB Approved Zone. The farm was subject to immediate clearance, disinfection and fallowing, and a programme of surveillance was established to re-establish approved status for the suspended area.

Approved zone status for VHS was again established for the whole of GB in 2000 (EC Decision 2000/188/EC), following the successful completion of the surveillance programme.

In May 2006, the UK suspended its approved status for VHS for Great Britain following the identification of VHS at a single trout farm in England. The VHS Fish Disease Contingency Plan for Great Britain was immediately implemented and a national control centre established at the Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS) in Weymouth. Action was taken as set out in Articles 8 and 9 of Directive 93/53 and Annex B Section D of Directive 91/67. The epidemiological investigations carried out to determine if there had been any further spread of the disease and to identify the origin of infection are the subject of this report.

2 The outbreak

2.1 Background

Nidderdale Trout Farm, situated on the River Nidd (part of the Greater Yorkshire Ouse river catchment, see Map A) in North Yorkshire is an intensive rainbow trout farm producing fish exclusively for the table market with an annual production of around 140 tonnes. The fish farm has been subject to official controls for bacterial kidney disease (BKD) since May 2005.

The farm buys fingerlings for on-growing to market size, with all fish leaving the farm dead for processing at a separate unit. The fish farm abstracts water from, and discharges back into the River Nidd. In 2006 all stocks on site were obtained as fingerlings from a single supplier. Nidderdale Trout Farm has been inspected on an annual basis as required under Council Directive 91/67/EEC and tested for VHS every second year. The last monitoring visit prior to the disease outbreak took place on 14 March 2006, when a sample of thirty rainbow trout, sampled from across the farms fish stocks, tested negative for VHS virus (VHSV).

On 22 May 2006 the fish stocks at Nidderdale Trout Farm were examined by a Cefas Fish Health Inspector at the request of the fish farmer because of unresolved chronic mortalities of rainbow trout on the site. Examination of fish farm records showed an increased mortality of stocks starting in March 2006 (see Appendix 2A). Samples taken by two private fish health specialists contracted by the fish farm failed to produce a conclusive diagnosis and the fish had not responded to therapeutic treatment for suspected bacterial infection.

Inspection of the fish farm stocks revealed fish showing clinical signs of disease, and a series of samples were taken for a range of diagnostic tests to be undertaken at the Cefas Weymouth Laboratory. VHS was confirmed on 26 May 2006.

The National Control Centre (NCC) was immediately established at Cefas (Weymouth Laboratory) to implement the VHS contingency plan and met regularly to co-ordinate the epidemiological investigations and the control measures designed to combat the disease.

2.2 Diagnosis and confirmation of VHS

Samples of brain, spleen and kidney were inoculated on to the following cell lines, bluegill fry (BF-2), Chinook salmon embryo (CHSE-214) and *Epithelioma papulosum cyprini* (EPC) and incubated at 15ºC. After three days incubation a cytopathic effect was observed on all cell lines including those protected with antiserum against infectious pancreatic necrosis virus. An ELISA test gave a presumptive positive test for VHSV and this was confirmed using an RT-PCR assay and sequence analysis.

Confirmation of VHSV was performed using a two-step RT-PCR test similar to that described in the OIE Manual of Diagnostic Tests for Aquatic Animals 2006, with the exception that the OIE recommended primers were substituted with a primer set used routinely at the Cefas Weymouth Laboratory (Stone *et al.,* 1997; Dixon *et al.,* 2003). Using the VHSV-specific primer set VHSVR1 and VHSVF3, the appropriately sized product was generated when using RNA extracted from the diagnostic tissue culture supernatant and from the original tissue homogenate from the disease rainbow trout fry. Sequence analysis of the 468 base pair (bp) product confirmed that the virus was VHSV, and subsequent phylogenetic analysis based on the complete G-gene sequence (1524 bp) using Neighbor-Joining methods assigned the isolated virus to the VHSV genogroup Ia. Identification of the virus was confirmed by the EU Community Reference Laboratory for Fish Diseases, Aarhus, Denmark.

2.3 Clearance and disinfection of the index site

Cefas Fish Health Inspectors inspected the fish farm on 27 May 2006, established footbaths and vehicle disinfectant facilities and supervised a humane cull of the remaining 19 tonnes of fish stocks on site. The cull was completed on 28 May 2006, with all fish removed dead from the fish farm to a government-approved animal waste processing plant for rendering and subsequent incineration as required under the Animal By-Products Regulations 2003.

Approximately 10 tonnes of ensiled fish waste material that had accumulated on the farm during the mortality event and over the previous 12 months was also removed and treated in the same manner.

After the culling of the fish stocks, the water supply to the fish farm was interrupted and the site drained. The fish farm was then disinfected under the supervision of the Cefas Fish Health Inspectorate (FHI). This process took approximately four weeks, and involved pressure washing the holding facilities, dismantling pipe-work, the application of sodium hydroxide and detergent or quicklime, and the removal of the treated silt. Disinfection of the fish farm was completed on 28 June 2006 (Appendix 2A).

2.4 Fallowing of the site

Following completion of the disinfection, the fish farm remained fallow and dry with no water supply to the site for over 4 months.

3 Investigations into possible spread

3.1 Other fish farms in England and Wales

During the year preceding the VHS outbreak, Nidderdale Trout Farm received fingerlings for on growing from a single fish farm. This fish farm was located in the River Wharfe catchment adjacent to the River Nidd. The supplying farm imports rainbow trout ova from Denmark, and supplies a large number of fish farms with fingerlings in both England and Scotland. Fish from the supplying farm were tested at the 225 fish sample level by virus isolation immediately after the confirmation of VHS on Nidderdale Trout Farm and subsequently at the 150 fish level during the survey of all other trout farms in the Yorkshire Ouse river catchment (Appendix 2B provides a timeline for control measures and farmed and wild fish surveillance undertaken). In both cases the fish tested negative for VHSV. Tests were also conducted on wild fish sampled from the River Wharfe just below the fingerling supplier, as an additional test on whether VHSV had been previously present on the farm. VHSV was not detected in the sample of 12 grayling, 84 brown trout, and 4 rainbow trout by either virus isolation or RT-PCR test (a summary of results from all wild fish testing is provided in Appendix 3)

One other fish farm is situated on the River Nidd, approximately 2 miles upstream of Nidderdale Trout farm, and partially protected from migration of wild fish by a weir between the two farms. This site produces rainbow trout exclusively for the table market. There are no fish farms downstream of Nidderdale Trout Farm. The upstream fish farm was sampled on 27/28 May 2006 at the 225 fish level, and tested by the virus isolation method. The test was negative for VHSV.

Subsequently all fish farms (33 in total) holding susceptible species in the Yorkshire Ouse river catchment and contiguous adjacent catchments (see Map B) were tested for VHSV by the virus isolation method through two complete sampling rounds (150 fish per sampling visit). All tests yielded negative results.

3.2 Fish farms in Scotland

There were no known epizootic connections to Scotland through live fish movements from the infected site (Nidderdale Trout Farm had movement restrictions in place for BKD). Contact testing (back to the beginning of 2006) was conducted in relation to the site that supplied Nidderdale Trout farm with live rainbow trout. Recent fish movements had also been made from that fingerling producer to two Scottish fish farm sites

On 28 May 2006, inspections by the Fisheries Research Services Fish Health Inspectorate (FRS FHI), Aberdeen were conducted at both Scottish sites, and examinations made for presence of any clinical signs of disease. Samples were taken from 300 fish, concentrating on stock from Nidderdale Trout Farm's supplier, but inclusive of other stocks on site. Samples were taken for virus culture from both sites. The samples from both sites were returned to the laboratory for processing on 29 May 2006.

FRS FHI reviewed the records on file of Scottish fish farm sites, which were known to regularly stock rainbow trout from England. No other sites were found to have a link to Nidderdale Trout Farm's supplier through live fish movements during the time period of investigation.

The third fish farm was inspected for clinical signs of disease and statutory sampling from 300 fish was conducted on 30 May 2006 samples were taken for virus culture. Tissue samples were submitted for virus tests on 31 May 2006. The samples proved negative for VHSV.

3.3 Wild fish in the River Nidd

Calculations based on the worst-case scenario for the maximum concentration of VHS virus leaving Nidderdale Trout Farm in the effluent water at the height of the outbreak shows it to be lower than would be expected to trigger large-scale infections in wild fish in the River Nidd in the vicinity of the farm. Taking river mean flow volume data into account for the period of the outbreak, it is calculated that by 5 miles downriver of the farm there would have been a maximum of 0.1 infectious virus/ml in the water. This is very unlikely to be a sufficient dose to cause infection in the resident wild fish at that point and even less so further downriver.

Extensive wild fish sampling (electro-fishing) was conducted on the River Nidd by specialised fishery experts from the Environment Agency (see Appendix 3 for details of species and sample sizes) and tested for VHSV by Cefas. The virus was isolated from a single pooled sample of 7 grayling captured immediately below the outlet of the affected farm a few days after VHS was confirmed on Nidderdale Trout Farm. However, tests on pooled samples of brown trout captured at the same point were negative for the virus. Grayling, brown trout and rainbow trout taken from the river upstream of the affected farm have all tested negative for VHSV . The identity of the virus isolated from the wild grayling was confirmed to be VHSV using the RT-PCR method described above. The virus was shown to share 100% nucleotide identity with the original Nidderdale Trout Farm VHSV isolate over the 1524 bp of the complete glycoprotein gene.

In a second sampling round (Appendix 2B) on wild fish upriver and downriver of Nidderdale Trout Farm, the sample size was increased. Pools of 5 fish were tested by the virus isolation method and organs of individual fish were tested separately by the RT-PCR method. This was done to maximise the sensitivity of detection and to allow a more precise estimate of the prevalence of the virus within the wild fish population. No fish were found positive for VHSV

by the virus isolation method but a single grayling, taken less than 1 mile downriver of the farm outlet, proved VHSV positive by RT-PCR. All fish sampled upriver of the farm were again negative for the virus.

In a third round of sampling, grayling, brown trout and pike were collected further downriver (approximately 7, 18 and 35 miles) of the affected farm to assess if there had been any downriver spread of infection within the wild fish population. All tests proved negative for VHSV.

At the time of the third round of sampling, the water temperature of the river had risen above 14ºC. At water temperatures above 14ºC, the immune system in salmonid fish species, particularly trout, becomes more effective at preventing infection or eliminating infection with VHSV. Thus, tests for the detection of VHSV in fish exposed to the virus become less reliable above 14ºC, meaning that negative results cannot be regarded as definitive.

3.4 Wild fish in the River Clyde

On confirmation of the disease, Cefas immediately informed FRS that fish from Nidderdale trout Farm had been found to be infected with VHSV and that some had previously been harvested and sent dead on ice to the parent company's processing plant in Scotland The processing plant is situated close to the River Clyde so an investigation was carried out by FRS into the potential for the pathogen to have spread to fish farms and to wild fish within the River Clyde catchment.

Wild fish sampling took place on 8 June and 13 June 2006 at two locations near to the outflow of the Daldowie Sewage Treatment Works which processes wastewater from the processing plant. A total of 600 fish were sampled, targeting susceptible species where possible. Samples were taken for virus culture. Species sampled included eel (440), minnow (57), stone loach (52), brown trout (35), gudgeon (13), flounder (5), stickleback (3) and salmon (immature) (1). All the tests proved negative for VHSV.

No farmed fish were sampled from the Clyde, although there are farms in the catchment as the farms are isolated from the Daldowie Sewage Treatment Works by impassable natural barriers and so were not exposed to a significant risk of infection.

3.5 Fish movements from the affected farm

Nidderdale Trout Farm produces fish exclusively for the table market with almost no live fish movements having been made from the site. Since May 2005, the farm was subject to statutory movement controls for BKD, which prevented any live fish movements off site. However, between 1 January 2005 and the start of the BKD movement restrictions, one movement of live fish off site took place on 14 March 2005 (Appendix 2C provides a chronology of events in the 10 months prior to outbreak of VHS at Nidderdale Trout Farm). The movement was to another on-growing site of the parent company. This single movement of live fish in 2005 is considered to be prior to the introduction of VHSV onto site (see section 6.1.2). Therefore the possibility of VHSV spread via live fish movements from the site was discounted.

Between January and late May 2006 eight deliveries of dead uneviscerated fish were made from the site to the parent company's processing plant in Scotland. Of those, three deliveries

(16 April, 27 April and 25 May) were made after the presumed onset of VHS related mortalities, so potentially could have included fish carrying VHSV.

Biosecurity measures at the processing plant were assessed by the Fisheries Research Services (FRS) laboratory in Aberdeen, Scotland. FRS concluded that the factory was generally well run and secure, consignments of fish are processed in an organised and traceable manner as are processes of cleaning and disinfection. However, areas of risk were identified and recommendations to improve biosecurity were made.

Effluent water from the processing plant is treated in a sewage treatments works then discharged into the River Clyde. FRS has subsequently sampled 600 wild fish from the River Clyde close to the treated water discharge (see section 3.4) all fish tested negative for VHSV by virus isolation.

Investigations were also undertaken by FRS into the biosecurity procedures adopted by the haulage company that transports the dead fish to the processing plant with a view to assessing the potential for disease transfer from the processing plant. Procedures were in place for the disinfection of the fish transporters and fish holding facilities between consignments of fish. Although the discharge of ice slurry on fish farms by the transporters could inherently pose a potential risk of pathogen transfer, given the biosecurity practices at the processing plant it was concluded that the likelihood that VHSV was transferred in this way is low.

Waste from the processing plant was sent to a second processing facility in Scotland. This unit was also inspected by FRS staff. FRS concluded that the movement of waste to this site, cleaning and disinfection of equipment and vehicles, subsequent rendering and effluent treatment appeared to offer low risk in survival and transmission of fish pathogens.

3.6 Live fish transporters

Nidderdale Trout Farm had movement restrictions in place for BKD, so no live fish movements had been made from the site since 26 May 2005. Contacts for lorries transporting live fish consignments following deliveries to Nidderdale Trout Farm between January and the end of May 2006, were traced. Two farms were identified by this exercise, however no indication of presence of VHSV was noted on these sites during inspection and sampling visits undertaken.

4 Control measures

4.1 Initial controls and designated area

On confirmation of the disease at Nidderdale Trout Farm on 26 May 2006, the Cefas FHI immediately issued 30-day Holding Notices to a total of 33 farms in the Greater Yorkshire Ouse catchment (Appendix 2B). These temporary notices prohibited the movement of live fish to or from any of the farms. Farms subject to control were those on the five river catchments draining into a shared estuary, (the Humber estuary). The River Nidd is part of the Greater Yorkshire Ouse catchment that contains 5 river catchments:

- River Ouse (including the Rivers Ure, Swale and Nidd)
- River Wharfe
- Rivers Aire and Calder

- River Don
- River Derwent

The 5 river catchments are shown in Map B

A Designated Area Order was issued by Defra on 1 June 2006 to place statutory fish movement controls across all five river catchments. This Order placed controls on the movement of live and dead fish into and out of the designated area, and by extending specific controls to all of the farms in the area, imposed controls on movements between sites within the designated area. Anyone wishing to move fish within the designated area had to seek prior permission from Cefas FHI.

All movements of fish, live or dead, from Nidderdale Trout Farm were prohibited, and the entire stock slaughtered on site. The carcasses were disposed of in strict accordance with the Animal By Products legislation. Movements of live fish into and from the one other farm on the River Nidd and dead uneviscerated fish from that farm, and from the river or other fishery waters within the Greater Yorkshire Ouse catchment were also prohibited, pending the completion of an epidemiological investigation into the outbreak at Nidderdale Trout Farm.

Some trade in fish within and from the designated area was re-established immediately without risk of VHS spread. Permission was given for the retail sale of ornamental fish within the area and to movements of such fish into and out of the area, once Cefas FHI had confirmed that no such fish were kept in facilities connected to the five watercourses subject to controls.

Movements of dead eviscerated fish from the designated area for further processing or direct consumption were permitted, as were movements of whole dead fish for processing and eviscerated fish for direct consumption within the designated area.

Further fish movements were permitted following the collection of epidemiological information about the apparent disease status of salmonid farms within the designated area. After the completion of one round of negative testing for VHSV, the farms were given permission to move fish between farms, and to fishery waters within the same individual river catchment. No transfers of live fish between any of the five controlled river catchments, or movements of live salmonids out of the designated area were permitted at this time: only dead and eviscerated fish were allowed to be moved out of the designated area. All such movements of fish had to be pre-authorised by Cefas FHI. After completion of a second round of negative tests on the farms, there was further relaxation of movement controls within the designated area. Live fish movements, between farms on different river catchments within the designated area, except for the infected catchment, were allowed on a case-by-case basis.

4.2 Re-designation of the infected zone

On the basis of the following epidemiological data gathered after the disease outbreak, the VHS National Control Centre (NCC) made recommendations to Defra on the re-definition of the infected and controlled areas:

There was evidence from two successive rounds of testing that VHS was not present on any of the farms within the Derwent, Wharfe, Aire and Don river catchments (see Map B). Testing on wild fish indicated that there was a very low level of infection, detectable only in the grayling (*Thymallus thymallus*) population in the River Nidd in the immediate vicinity of

Nidderdale Trout Farm. There was evidence that the infection had not spread to the populations of susceptible species further down the River Nidd sub-catchment following sampling and negative testing of wild grayling, brown trout and pike 7, 18 and 35 miles downstream.

Evidence was presented to the NCC of:

- physical obstacles to fish migration and dispersal within the River Nidd sub-catchment,
- very low numbers of migratory salmonid species present in the affected river catchment and
- large distances between farms in adjacent river catchments

Considering the combination of these factors, it was concluded by the NCC that there was only a very low risk of transfer of VHSV from the known area of infection from the River Ouse catchment to the four adjacent river catchments.

Based on this assessment, the NCC concluded that the five adjacent river catchments upstream of their normal tidal limits (NTL, [see map B]) could be treated as epidemiologically-distinct zones. As a result the area subject to control for VHS was re-defined as follows:

- The catchment of the River Ouse upstream of its normal tidal limit would continue to be considered VHS-infected on the basis of evidence of infection in susceptible wild fish populations immediately adjacent to the site of the original disease outbreak.

- Individual farms within the River Ouse catchment would continue to be subject to movement controls appropriate for farms in a zone seeking approval for VHS.

- The other four river catchments (Derwent, Wharfe, Aire and Don) could be considered to be VHS free, as there was no evidence of the disease on any farms, no evidence of VHS spread to wild fish in the tidal river reaches between these river catchments, and negligible risk of direct transfer of the virus by movements of infected susceptible species from the River Nidd. The NCC concluded that these four catchments could be safely returned to the rest of the GB approved zone.

4.3 Buffer zone

In order to monitor any risk of transfer of VHS from the designated River Ouse catchment to the other four river catchments, and therefore into the GB approved zone, the NCC further decided that a buffer zone comprising the shared estuary of the five rivers downstream of their respective normal tidal limits should be established. Targeted surveillance of the wild fish populations will be carried out in this zone, in the vicinity of the tidal limit of each river. This should provide advance warning of the potential spread of the virus to uninfected areas, through the wild fish population. In addition there would be an increased level of farm inspection and sampling within the four catchments draining into this buffer zone. The location of the re-designated area and position of the buffer zone is shown in Map C

5 Risk mitigation measures

There is little if anything that can be done in practice to remove the low residual risk that VHSV may remain established in wild fish populations in the River Nidd and possibly be spread by migrating fish species, particularly sea trout, to the other rivers in the Yorkshire Ouse catchment area. If this were to occur, there is a risk that one or more farms taken out of the designated area could become infected and spread the disease via live fish trade to other farms elsewhere in GB.

However, tests for VHSV on 465 brown trout from the River Nidd have all been negative. Experimental challenge infections conducted by Cefas using the VHSV isolate from Nidderdale Trout Farm have determined that brown trout are significantly less susceptible to the virus than are rainbow trout. These data suggests that given the low doses of virus exiting the infected farm, brown trout are unlikely to have become infected, thus, reducing the risk level to 'negligible' for sea trout spreading VHSV from the River Nidd to the other zones. Furthermore, two subsequent rounds of tests on wild fish in the River Nidd, upstream and downstream of the Nidderdale Trout Farm, carried out during October and November when water temperatures fell below 14ºC proved negative for VHSV.

The best practical approach will be to monitor the field situation carefully to allow the earliest possible detection of VHSV infection in wild and farmed fish, followed by the immediate implementation of counter-measures should this occur. Enhanced surveillance will include both passive (farmer observations and reporting) and active (official inspections of farms and sampling/testing of wild fish) including:

- Wild fish at sampling points in the 'buffer zone' separating the five zones in the current designated area will be subjected to surveillance and tests for VHSV at intervals through the autumn, winter and spring when water temperatures are below 14ºC. Absence of positives will strengthen confidence that the virus is not being spread to the other rivers by wild fish migrating from the River Nidd.

- Enhanced risk-based surveillance for VHS will be carried out on trout farms in the four non-infected adjacent zones when water temperatures falls below 14ºC, particularly those farms at most risk of the virus being introduced by migrating wild fish in the river near their inlets.

- All farmers in the current designated area and in the rest of GB will be given guidance and encouraged to increase biosecurity measures to reduce any risk of future introduction of VHSV.

All farmers are required to notify Cefas, Weymouth (for England and Wales) or FRS, Aberdeen (for Scotland) of any escapes of rainbow trout, unusual mortalities or any signs of disease without delay and farms in the Greater Yorkshire Ouse catchment are also required to submit weekly records of routine mortalities to Cefas.

- The Environment Agency will report any mortalities in wild fish populations to Cefas.

- Any suspicions of VHS anywhere in GB will be immediately investigated by the Cefas FHI and VHS testing undertaken.

It is concluded by the NCC that these measures will provide adequate safeguards for minimising the extent and impact of any natural spread of VHS from the River Nidd via wild fish should this extremely low likelihood event occur.

6 Investigations into origin of infection

6.1 Identification of the likely time of introduction of VHSV onto Nidderdale Trout Farm

6.1.1 Onset and spread of VHS on the farm

Mortality data provided by Nidderdale Trout Farm were analysed to identify when VHS related mortalities started and how the disease spread within the farm. Low-level mortalities (*ca*. 5 mortalities / day in raceways holding market size fish) had been recorded prior to the presumed VHS-related increase in mortality. The cause of increased mortalities was initially investigated by commercial fish health services. *Yersinia ruckeri*, the gram-negative bacterium causing Enteric Redmouth Disease in rainbow trout, was isolated from Nidderdale Trout Farm fish in late April 2006. Some of the mortalities may have been due to this disease.

Mortalities increased towards the end of March 2006 (Appendix 2A) in the C-unit raceways located at the bottom of the water-use chain (See Figure 1). This unit contained near-market size rainbow trout and includes four raceways, C1-C4. The first increase in mortality was seen in C3 on 18 March 2006. This raceway was harvested on 21 March; therefore no daily mortalities were recorded for several weeks until after this unit was restocked on 16 April. The next raceway to be affected was C1, with a noticeable increase in mortalities around 30 March. Raceways C2 and C4 followed around 10 April (Figure 1).

Mortalities in the B-unit, containing medium sized fish (usually smaller than 200g), and receiving first use water, started around mid April 2006.

The fry channels (A-unit), also received first-use water and were located above the C-unit and parallel to the B-unit. This unit was empty until 23 March 06 when it was stocked with fingerlings. No unusual mortalities were noted in these fish until they were transferred to the B-unit (end of April 2006) (Figure 1).

The C-unit was graded twice in March (2 and 21 March). The smallest fish were back-graded into raceway C1, raceways C3 and C4 received large fish and C2 received medium grade fish. Almost complete reshuffling of the C-unit would have occurred, facilitating spread among the C raceways. Among the C raceways, C1 would have contained those fish most susceptible to the development of VHS, since these were the smallest and slowest growing fish.

6.1.2 Estimation of time point of introduction of VHSV onto the farm

Estimation of the likely time of introduction of the virus to the site is complicated by the following:

a) Fish mortalities occur on fish farms on a daily basis and are not necessarily related to the presence of obligate pathogens. An indication of something unusual happening would be a noticeable *increase* in daily mortality.

b) VHS may have overlapped with the presence of another disease within the population, making it more difficult to determine the onset of VHS-related mortalities.
c) Incubation time, rate of spread within a population, mortality rate, and time between infection and death depend on the strain of virus, water temperature and virus concentration.
d) Several gradings of, and harvests from, the C raceways took place between beginning of March and the end of May 2006. The populations within the C raceways were therefore rearranged and substantial numbers of fish were removed. This would have affected the development of mortalities.
e) The mortality records and grading reports were provided by the farmer. For some raceways, substantial discrepancies between numbers of fish reported as mortalities and the number of fish provided in grading reports were found. Therefore the data provided by the farmer may not be sufficiently reliable for analysis. A certain level of variation between fish numbers in grading reports and mortality data can be explained by the high number of animals the farmers are dealing with. Fish numbers in grading reports are estimates based on total weight of fish from a unit and average individual fish weight, based on a sample. Therefore a higher accuracy would be expected from daily-recorded mortalities.

The VHSV isolated from Nidderdale Trout Farm has been tested in challenge experiments with rainbow trout at Cefas Weymouth Laboratory and has been classified as highly virulent. However, between December 2005 and a few days before the noticeable increase of mortalities on 30 March 2006, water temperatures were within the range 3-5°C. Such low water temperatures can be expected to lead to a delay in expression of clinical VHS in infected fish, spread of VHS within a population, amplification of the virus within affected animals, and a reduced rate of release of VHSV from infected rainbow trout.

The average survival time of 25-35cm rainbow trout challenged with $10^{4.5}$ $TCID_{50}$/ml of a highly pathogenic viral strain at 3.5-4.5°C was reported as 16 days (Neukirch, 1992). The paper published by Neukirch is the only available scientific publication providing average times from exposure to VHSV to death of the fish at such low temperatures and was used by Cefas to estimate the likely time window for the introduction of VHSV onto Nidderdale Trout Farm. The mortality data on Nidderdale Trout Farm were analysed using a modelling approach. Assuming that early mortalities were due to VHS and that incubation times / times to mortality were as seen in high level exposure to VHSV ($10^{4.5}$$TCID_{50}$/ml), the introduction of VHSV was estimated to have occurred between mid January and early March 2006 (see Appendix 2C).

As outlined above, several factors complicate the estimation of the time of introduction of VHSV onto Nidderdale Trout Farm. Currently, there is no information available on incubation times and mortality rates for low-level VHSV-challenge at low temperatures. If the initial challenge was lower than $10^{4.5}$ $TCID_{50}$/ml (which is likely), the time window would probably have to be extended further (Appendix 2C). Also, the possibility of a dormant infection in the fish population on site over the winter has to be taken into account.

6.2 Analysis of potential pathways of introduction of VHSV onto Nidderdale Trout Farm

The mortality data provided by the farmer suggest that VHSV was introduced into the C-unit raceways containing near market size fish, situated at the bottom end of the water-use chain. The fact that mortalities started at the bottom end of the water-use chain in fish regarded as

less susceptible to VHSV (due to their size) compared to younger fish, suggests that the introduction of VHSV was directly into these raceways and not via contaminated water from upstream of the farm.

Although the mortality data suggest that contamination of the incoming water supply with VHSV by sites upstream is unlikely, such routes have been considered and investigated (e.g. section 6.2.1.3). A summary of other potential pathways for the introduction of VHS onto Nidderdale Trout Farm is presented in Figure 2 and discussed in detail below:

6.2.1 Live fish

6.2.1.1 Declared live fish supplies onto Nidderdale Trout Farm

Nidderdale Trout Farm received fingerlings for on-growing from only one fish farm during the year preceding the VHS outbreak. This fish farm is located on the River Wharfe, which is adjacent to the River Nidd in the Yorkshire Ouse catchment. The supplying farm imports rainbow trout ova from Denmark, and supplies a large number of fish farms with fingerlings in both England and Scotland. Fish from the supplying farm were tested at the 225 fish sample level by virus isolation immediately after the confirmation of VHS at Nidderdale Trout farm and subsequently at the 150 fish level during the survey of farms in the Yorkshire Ouse river catchment. In both cases the fish tested negative for VHSV.

Samples from wild fish were also taken from the River Wharfe just below the fingerling supplier, as an additional test on whether VHSV had been previously present on this farm. These included 12 grayling, 84 brown trout, and 4 rainbow trout, all tested negative by virus isolation and RT-PCR (see section 3.3 and Appendix 3).

6.2.1.2 Undeclared live fish supplies onto Nidderdale Trout Farm

A theoretical route of introduction of VHSV to Nidderdale Trout Farm is through an undeclared movement of live fish from an infected source. However, no evidence of such an introduction could be found despite extensive investigations.

6.2.1.3 Supplies of live fish to farm upstream of Nidderdale

One other rainbow trout farm is located 4 miles upstream of Nidderdale Trout Farm. This farm received live fish from farms other than Nidderdale Trout Farm both for processing and on-growing. Fish delivered for processing were kept in raceways until slaughter, and water from these fish and those for on-growing was discharged into the River Nidd. According to information provided by the farmer, the last delivery of live fish onto this site before the VHS outbreak at the end of May 2006 was made in September 2005.

All live fish deliveries were from sites within the UK, which are regularly tested for VHS as required for maintaining approved zone status. Since, according to mortality records provided by the farmer, no unusual mortalities were recorded in the fish delivered, or in fish, already on site, the risk of introduction of VHS onto the farm from these fish is considered negligible. In addition, this route would present an “upstream” route and would therefore have been more likely to have induced initial mortalities at Nidderdale Trout Farm in units receiving first-use water and holding medium-sized fish (the B-unit).

Therefore this potential source is considered to be very unlikely. Fish from this upstream farm where tested as part of the two rounds of negative VHS testing undertaken in May and June 2006 and wild fish in the river directly below the site also tested negative for VHS.

6.2.1.4 Stocking of live fish into the River Nidd upstream of Nidderdale Trout Farm

Fish are stocked into the River Nidd by an angling club that owns the fishing rights for stretches above, and a short distance below Nidderdale Trout Farm. Two releases of susceptible fish species into the River Nidd were made in 2006 prior to the increased mortality on Nidderdale Trout Farm at the end of March. One of these involved stocking upstream of the affected fish farm with 1800, 30cm diploid brown trout on the 7 January from a fish farm located outside of the Greater Yorkshire Ouse catchment. The stocking of fish downstream of Nidderdale Trout Farm involved 1100, 550g brown trout on 14 March 2006. However, all 465 brown trout tested during the course of an extensive wild fish sampling programme in the River Nidd were found to be negative for VHSV (see section 3.3).

6.2.2 Fish products (dead fish)

6.2.2.1 Smokery

A smokery that processes a variety of food products including fish from the UK and abroad is located approximately 1.5 miles upriver of Nidderdale Trout Farm, immediately adjacent to the River Nidd. Several fish species are processed, including various marine species, but also rainbow trout. The smokery had received small quantities of fish from outside the UK.

Records of fish consignments received up to 6 months prior the identification of VHSV on Nidderdale Trout Farm were checked. Sixteen consignments of rainbow trout were received between 17 November 2005 and 30 March 2006. All except one were from the rainbow trout farm upstream of Nidderdale Trout farm which tested negative for VHSV. One consignment, comprising 20 kg of either gutted or filleted rainbow trout originating from a Member State where VHSV is known to be present, was delivered to the smokery on 24 November 2005. Information provided by Inspectors for Fish Diseases in the country of origin, revealed that this particular consignment could have been infected with VHSV.

Investigations were undertaken to assess working practices at the smokery, identify routes of waste disposal, and assess the potential for accidental contamination of watercourses. Testing of water discharge points from the smokery was undertaken. A surface drain, located immediately outside the doors leading into the preparation room of the smokery, was found to discharge into the River Nidd. On the day the smokery was inspected by Cefas staff, small amounts of fish waste were found on the grid of the drains. This suggests that limited amounts of fish waste and effluents from the smokery could have been discharged into the River Nidd.

The smokery records indicated that only the single consignment (detailed above), may have originated from an infected source, however the VHSV recovered from Nidderdale trout farm was not a genetic match for the virus affecting this source. In addition, the virus concentration that would have been generated if parts (e.g. 20 g) of the consignment had

been discharged into the River Nidd was determined and considered to be insufficient to cause direct waterborne infection of fish at Nidderdale Trout Farm.

Wild fish species susceptible to VHSV (e.g. brown trout or grayling) in close proximity to the smokery's discharge channel during the period when the "suspect" material was processed may have been exposed to the virus. Fish scavenging infected material would be most likely to become infected, since these would be exposed to a relatively high dose of the virus. If any wild fish had become infected, they may not have developed disease until stressed (for example by spawning or extreme environmental events; both grayling and brown trout spawn, or have recently spawned between January and March).

To explain the onset of mortality due to virus from a wild fish source occurring first in the C-unit, which receives water that has previously passed through the A- or B-units, infected wild fish would have had to linger near the farm inlet supplying the A-unit before 23 March 2006, when this facility was stocked with fingerlings from another farm (see Figure 1).

This chain of events is highly unlikely. However, the delivery of trout detailed above to the smokery is the only recorded event, where potentially VHSV-infected material was brought in close proximity to Nidderdale Trout Farm.

6.2.2.2 Processing site at upstream fish farm

The processing facility at the rainbow trout farm above Nidderdale Trout Farm mainly received live fish for processing. The evaluation of the live fish route for an introduction of VHS onto Nidderdale Trout Farm is discussed above (6.2.1.3). This processing facility was also used by another fish farm to process their own fish. These fish originated from a farm within the River Ouse catchment and were dead on arrival. This farm was sampled as part of the two rounds of testing in May and June 2006 following the VHS outbreak and tested negative for VHSV.

6.2.2.3 Undeclared movements of dead fish

Similar to undeclared movements of live fish (see 6.2.1.2.), undeclared movements of dead fish present a theoretical route. However, there is no evidence that such movements occurred.

6.2.3 Fish waste movements onto Nidderdale Trout Farm

Between September 2005 and 20 April 2006, two fish farms delivered fish waste (mortalities) to Nidderdale Trout Farm for ensiling. Both farms are located in the Greater Yorkshire Ouse catchment and tested negative for VHSV in the sampling programme following the disease outbreak (end of May and June 2006).

Fish waste was initially treated in an ensiler (providing an acid environment) and was subsequently transferred to intermediate bulk containers (IBCs). The ensiler and the IBCs were located *ca.* 30 m from the raceways, close to the farm entrance, so vehicles delivering waste would not have accessed an area close to the raceways. The chance of any spillage or contamination occurring near the raceways is deemed extremely low.

Furthermore, the majority of the waste would have originated from the rainbow trout farm upstream of Nidderdale Trout Farm, which is considered a low risk contact (see 6.2.1.3.).

6.2.4 Mechanical transmission

Various routes of introduction of VHSV by mechanical transmission were considered:

6.2.4.1 Live fish transport vehicles

Five deliveries of live fish were made to Nidderdale Trout Farm from a single fish farm between April 2005 and the end of May 2006. Since the supplying site subsequently tested free of VHS and the mortalities at Nidderdale Trout Farm did not start in fry recently transferred, introduction of VHSV by these fish or by contaminated transport water appears highly unlikely.

6.2.4.2 Dead fish transports for processing

All fish produced at Nidderdale Trout Farm were collected by a haulage company and processed at one processing plant in Scotland. Between January 2006 and the end of March 2006, fish were harvested and transported to Scotland on five occasions. Three further transports took place after the presumed onset of VHSV related mortalities at the end of March 2006. The processing plant in Scotland receives eviscerated trout for further processing from across Europe. Fish processed at the plant included rainbow trout from more than one other Member State where VHSV is known to be present.

Within the likely window of infection there was a harvest of 8.5 tonnes of fish at Nidderdale Trout Farm on 6 March 2006. These fish were shipped to the parent company's processing plant, in Scotland. During this process, harvest bins containing ice were transported to the farm from the processors. Each bin could hold 0.5 tonnes of fish meaning that 17 bins of ice would have been brought onto site. During the harvest process, harvest bins are taken off the lorries and sited next to the unit in which mortality due to the virus first occurred (the C-unit). The bins are filled with water to create an ice slurry, and the dead fish are then allocated to the bins. Once full of fish, bungs at the bottom of the bins are removed and the water is allowed to drain out so the fish in the bins are layered throughout the ice. Substantial quantities of water leaving these bins are likely to enter the common channel supplying the C-unit. This provides a potential, albeit low risk, route for the virus to have entered the UK as the processing plant receives fish from more than one country in mainland Europe, and provides a possible explanation for how the virus could have directly entered the C-unit.

Fish arriving at the processing plant from mainland Europe are transported in plastic boxes not harvest bins, thus removing the chance that contaminated harvest bins could have been taken to Nidderdale Trout Farm. A different haulage company is used in the UK to that used to transport trout from mainland Europe meaning that direct transfer via lorries is unlikely. However, it is not impossible that infected products may have contaminated ice used within the processing plant. Biosecurity at the processing plant was assessed by FRS FHI in Scotland. Minor biosecurity deficiencies were identified, but biosecurity was generally assessed as good. These factors would suggest the likelihood of transmission via this route is unlikely, and would depend on a failure in biosecurity.

The processing plant receives very large quantities of fish each year, with transfer of vehicles and equipment between many sites. It has never been implicated in a disease incident of this kind before. However, it may be that the way in which harvests are conducted at Nidderdale trout farm allows completion of a low risk chain of events (i.e. the transfer of virus via ice slurry) allowing the introduction of infectious material.

6.2.4.3 Fish waste movements

Vehicles delivered fish waste to Nidderdale Trout Farm from 2 other farms. The assessment of these farms was made earlier (see section 6.2.3.). The likelihood of these farms being the source of the virus is deemed negligible.

6.2.4.4 Other vehicles

Other vehicles that have visited Nidderdale Trout Farm include: feed lorries, staff vehicles, fish health advisers, and sales agents. Visits by other vehicles were not reported. These vehicles are most unlikely to have had any direct contact with VHSV elsewhere. It is possible that a vehicular contact was made with the smokery. However, such a movement is not known to have occurred.

6.2.4.5 Sharing of equipment with other farms

Nidderdale Trout Farm bought equipment from a farm in Scotland, which was closing down. The equipment, consisting of numerous nets, bins, feeders and pipework, was brought onto site approximately 12 months before the VHS outbreak and was disinfected before use on the farm. The equipment was disinfected before being used. No outbreak of VHS was reported from the supplying farm. Therefore sharing of equipment is considered an unlikely route.

6.2.4.6 Contacts via personnel /visitors

In the months preceding the onset of VHSV related mortalities at Nidderdale Trout Farm, it can be assumed that the farm was visited by a number of people (feed suppliers, sales representatives, etc.). Due to the absence of any other known outbreak of VHS in the UK, such people would have had to introduce the virus from abroad. Due to the limited survival time of the virus in dry environments, this route is therefore considered unlikely.

6.2.4.7 Birds

a) Introduction of VHSV from continental Europe

Migratory birds were assessed to present a negligible risk for the introduction of VHSV. The farm is located in North Yorkshire and therefore a considerable distance from locations where VHS is known to be present (e.g. distance to Denmark is ca. 700 km). Considering flight durations, survival of VHSV on either the birds' body surface or within the digestive tract and analysis of flight routes proved migratory birds to be an unlikely route of transmission.

b) Local bird movements

Local bird movements may represent a possible route, if infected/ contaminated material was accessible to birds at a close distance to the affected farm. One location that might have received contaminated material is the smokery, which is 2 km away from Nidderdale Trout Farm. According to the records kept by the smokery, only one delivery made in November 2005 could have potentially carried VHSV. Birds would need to have had access to waste. Such material would have been available only in very small quantities (such as the waste material found outside of the smokery when visited by Cefas staff). In addition, Nidderdale Trout Farm is protected against access by birds by predator netting, making birds an unlikely route of introduction.

6.2.4.8 Angling activities

Angling takes place along the entire River Nidd, including the stretches above Nidderdale Trout Farm. Since no pike fishing takes place in the region above the farm, introduction of contaminated bait is considered a negligible risk. Introduction via contaminated angling equipment again is considered a negligible risk due to the low numbers of virus that would be introduced.

6.2.4.9 Further potential low risk routes of introduction

A number of other potential routes of introduction of VHSV onto a farm have been assessed, such as leisure activities (other than angling i.e. canoeing), in the River Nidd, or introduction of aquatic plants. However, the risk of transmission of VHSV by such routes was rated as negligible, due to the small amounts of virus that would be introduced via such a route.

6.2.5 Other routes

6.2.5.1 Wild fish

Wild fish were assessed as a potential route of introduction. There are two avenues through which introduction of VHSV from wild fish could occur:

a) via infection of wild fish through material released from the smokery (see 6.2.2.1)

and

b) through migrating wild fish.

A review of scientific information was carried out to assess the extent of wild fish migration from and to the River Nidd. The only wild fish species present in the River Nidd known to migrate long distances is the sea trout (anadromous brown trout). In order to become infected with a freshwater strain of VHSV, as was isolated from fish at Nidderdale Trout Farm, sea trout would have had to migrate to coastal areas of continental Europe. The number of sea trout present in the River Nidd is low (the number of sea trout returning to the River Nidd for spawning every year was estimated to be between 10 and 100). The number of sea trout migrating up to or above Nidderdale Trout Farm is likely to be even lower and possibly none will migrate this far. This is due to a number of weirs present in the River Nidd below Nidderdale Trout Farm.

The virus load that might be carried by such fish is unknown. Migration stress may induce clinical VHS, though it is unclear whether such fish would succumb to the disease before reaching their home waters.

Altogether, it was concluded that infection arising from introduction of VHSV via wild fish is most unlikely.

6.2.5.2 Intentional introduction of VHSV onto site

Sabotage was considered. Although the farmer did not have reason to suspect any deliberate hostile action against his farm and no independent evidence for this was identified during the epidemiological investigation, this route cannot be completely ruled out.

6.3 Problems in identifying the source of infection

- Comprehensive mortality data were not always available or not readily provided by the farms involved.

- Although VHS is one of the best-studied diseases in fish, significant knowledge gaps exist (such as incubation time, time to death and mortality rates at low dose challenge and low temperature, minimal infectious dose, etc.).

- Some information provided by the industry was contradictory. Reliability of previously undocumented information is questionable.

- Unusually high levels of mortality at Nidderdale Trout Farm were not reported to Cefas until two months after their onset.

- Documentation of the trade of fish products from European sources is not freely available to Cefas, as there is currently no legal requirement to provide these to official services.

- Trade in fish products of VHS-susceptible species into England and Wales are not fully documented and the few existing records were not available to Cefas. Tracing imports of potentially infected fish products from abroad is therefore difficult, if not impossible.

6.4 Summary of investigations into the source of the VHS outbreak

- The source of VHSV has not yet been identified but potential routes of introduction have been recognised. These include contacts with the fish processing plant in Scotland that deals with imported trout, potential release of VHSV from imported product processed at the smokery, or some undeclared or unrecorded fish movements of either live or dead fish.

- The likelihood of introduction of VHSV via processors is deemed to be low. The likelihood of undeclared fish movements is unknown.

- There were significant deficiencies in the biosecurity of the smokery upstream of Nidderdale Trout Farm

7 Conclusions

7.1 Spread of the disease

- It is likely that the VHS outbreak at Nidderdale Trout Farm was an isolated event without any spread to other farms or to wild fish (except to a few grayling in the River Nidd in close vicinity to the farm's discharge), because:

  - all salmonid fish farms within the Greater Yorkshire Ouse river catchments were tested twice for VHSV following the outbreak at Nidderdale Trout Farm. On both occasions, all farms tested negative.

  - there is no indication that VHSV may have occurred on any other farm elsewhere within GB.

  - several hundred VHS-susceptible wild fish were sampled up- and downstream of the affected farm shortly after VHS was diagnosed. VHSV detected in only two small samples of grayling.

  - further wild fish were sampled in October and November 2006 when water temperatures were again below 14ºC. VHSV was not isolated from any of these wild fish.

  - wild fish samples were also collected from the River Clyde in Scotland, into which the treated effluents of the processing plant, which processed fish from the infected farm after the presumed onset of VHS-related mortalities were discharged. All wild fish sampled tested negative for VHSV.

7.2 Origins of the disease

- The origin of the VHS outbreak on Nidderdale Trout Farm in Yorkshire, England has not yet been identified.

- Potential routes have been recognised, but conclusive evidence has not been established for any particular one.

- Ongoing molecular epidemiological investigations may allow the likely geographical origin to be narrowed down by comparison of the genetic sequence of the English VHSV-isolate with isolates from across Europe.

- The introduction appears to be the result of a combination of an unusual and improbable sequence of events. Otherwise, it would be expected that outbreaks would have occurred on previous occasions. This makes the identification of the origins of the virus difficult to establish with any certainty.

8 Recommendations

- Bio-security of fish farms and processing plants and the risks of introduction and spread of exotic pathogens via processing plants needs to be reviewed. This could include risk of pathogen spread via transport vehicles, shared equipment, materials (e.g. fish waste) and personnel.

- The potential risks of pathogen introduction to free areas via different types of fish products currently considered safe needs reviewing.

- Due to the potential disease risks, full documentation and traceability of fish and fish product movements is an essential requirement.

9 References

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10 Figures

Figure 1. Layout of Nidderdale Trout Farm and onset of mortalities across the farm.

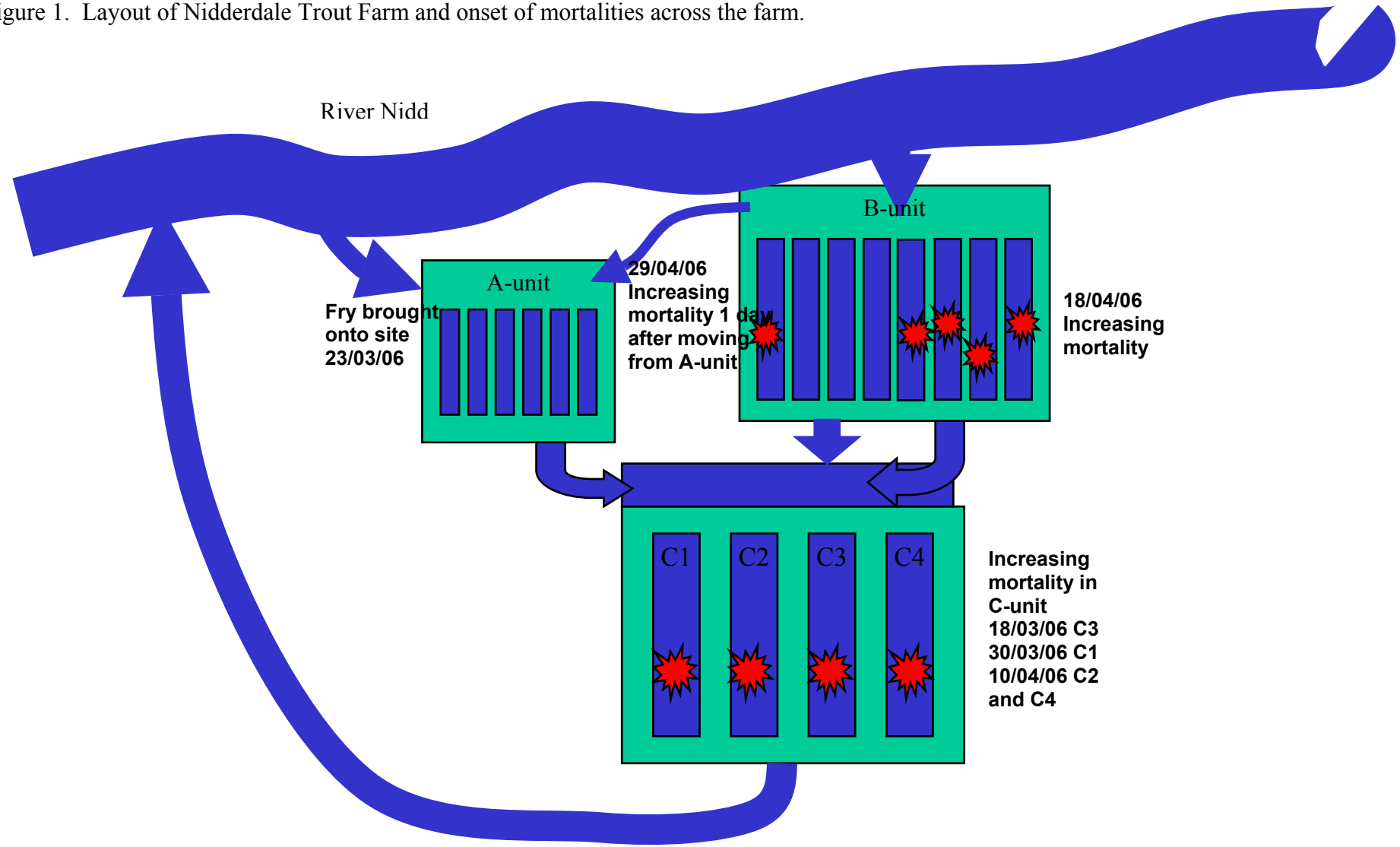
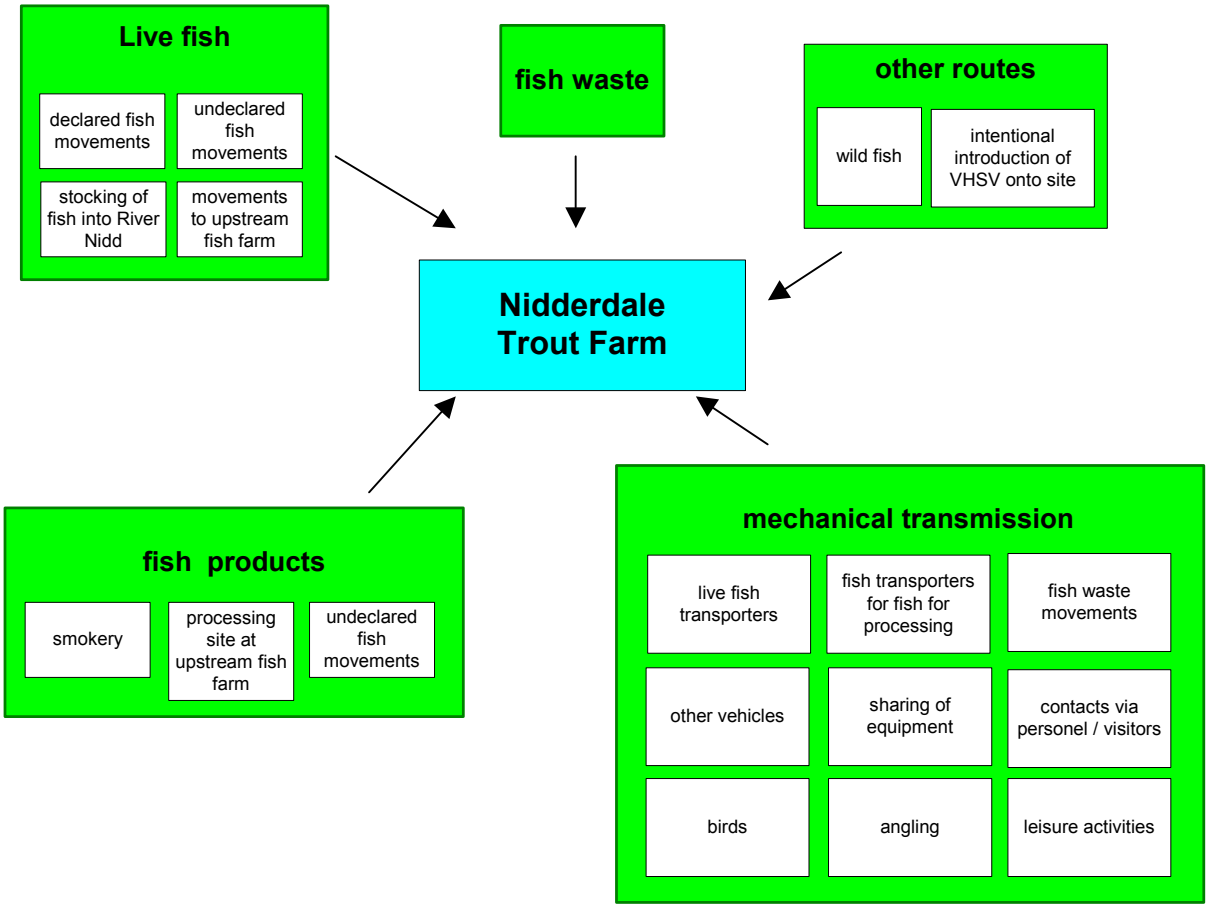


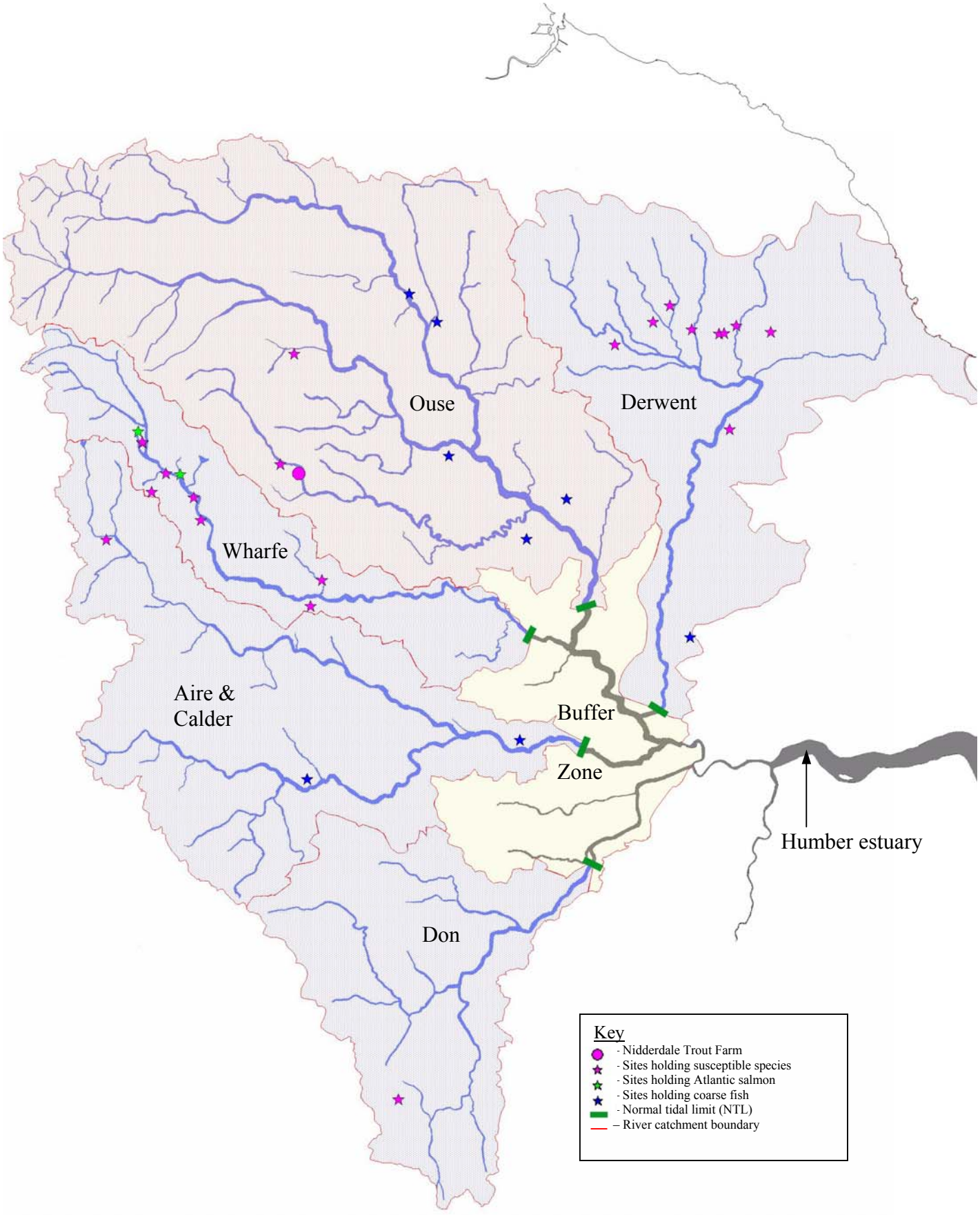
Figure 2. Potential pathways of introduction of VHS onto Nidderdale Trout Farm



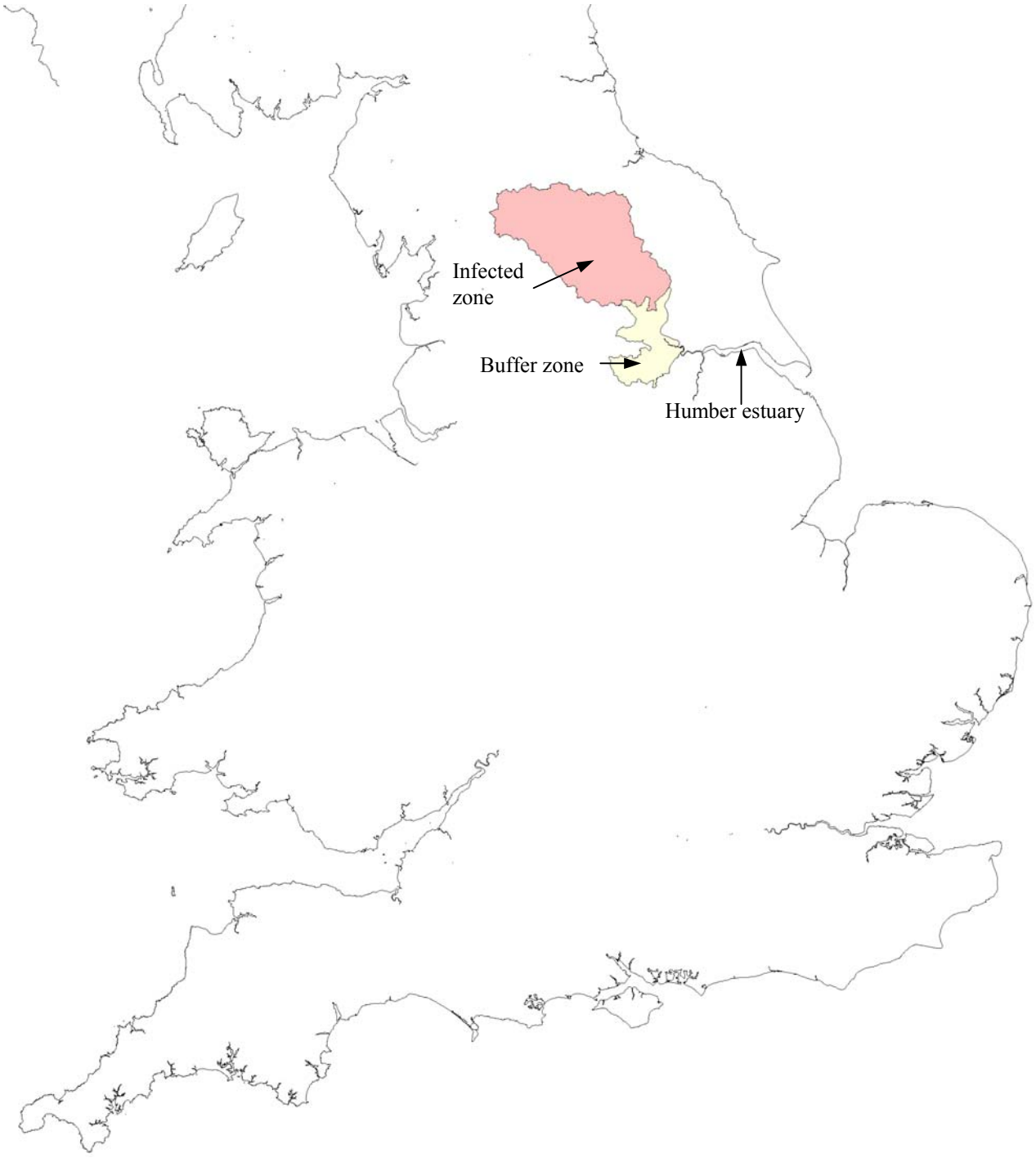
Appendix 1. Maps

A. The Greater Yorkshire Ouse river catchment

B. Detail of the five river catchments within the Designated Area

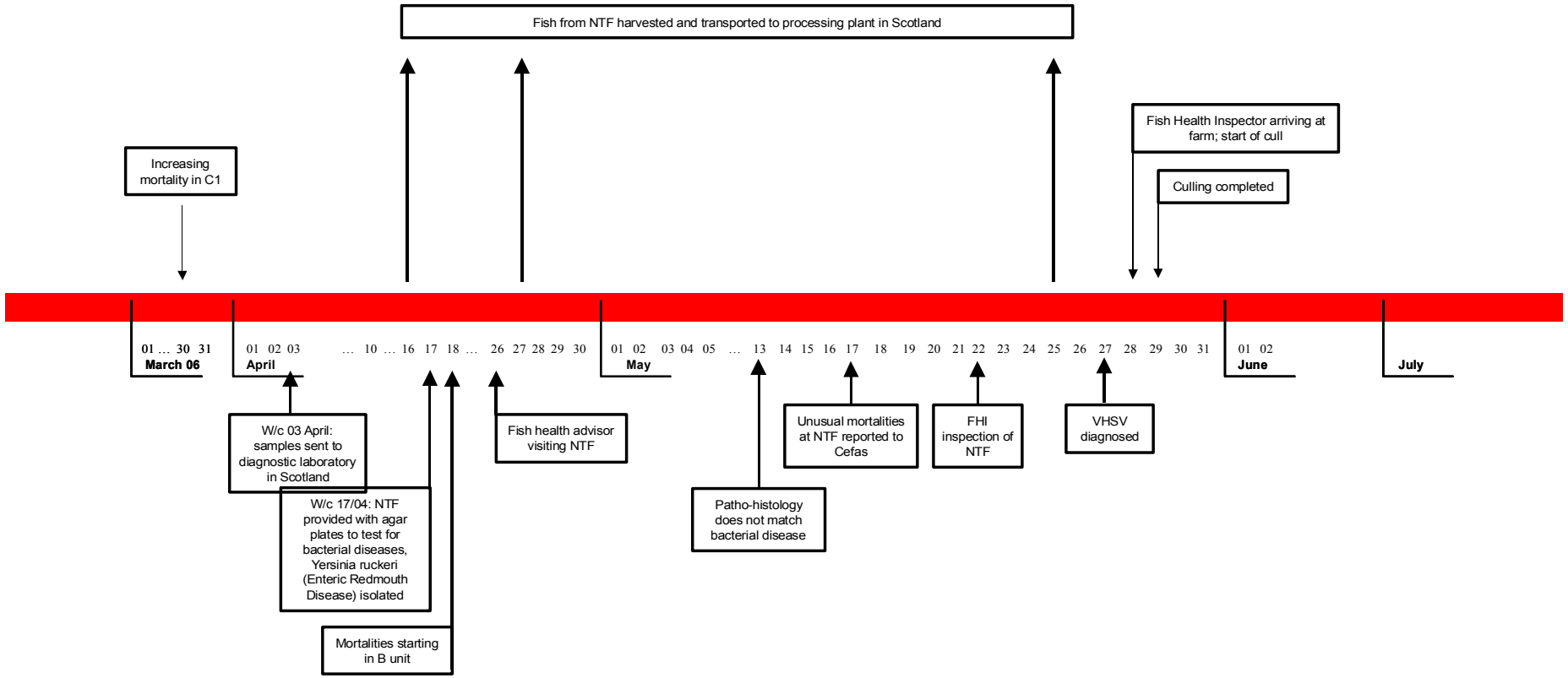


C. Area excluded from re-established approved zone for VHS (Commission Decision 2006/674/EC)

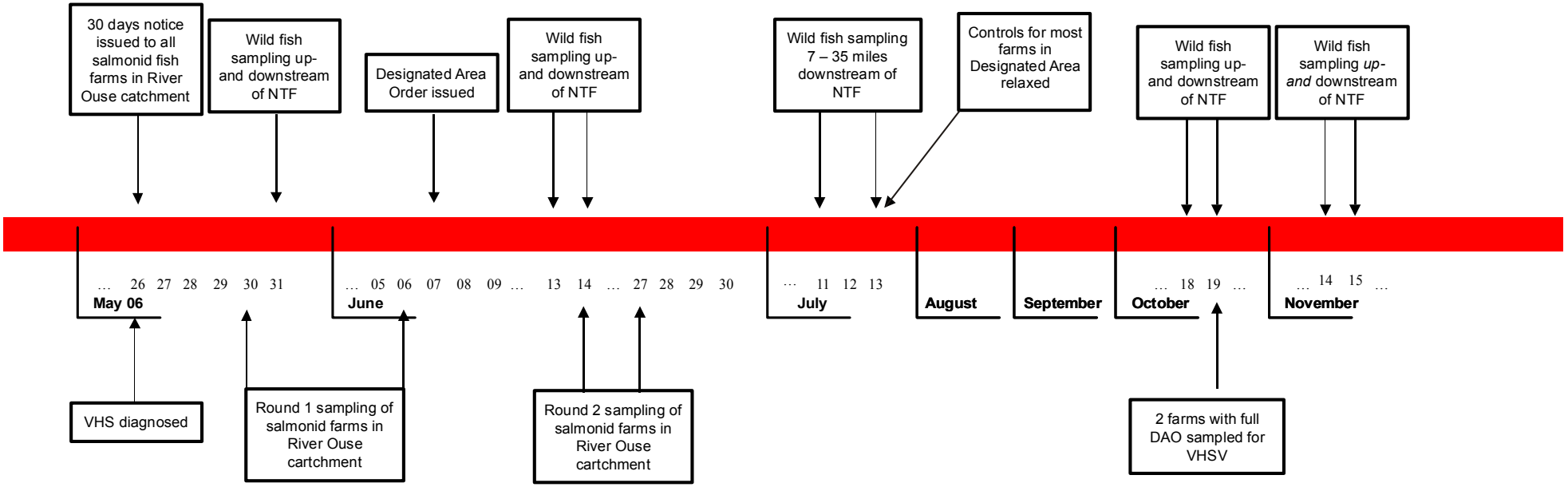


Appendix 2. Chronology diagrams

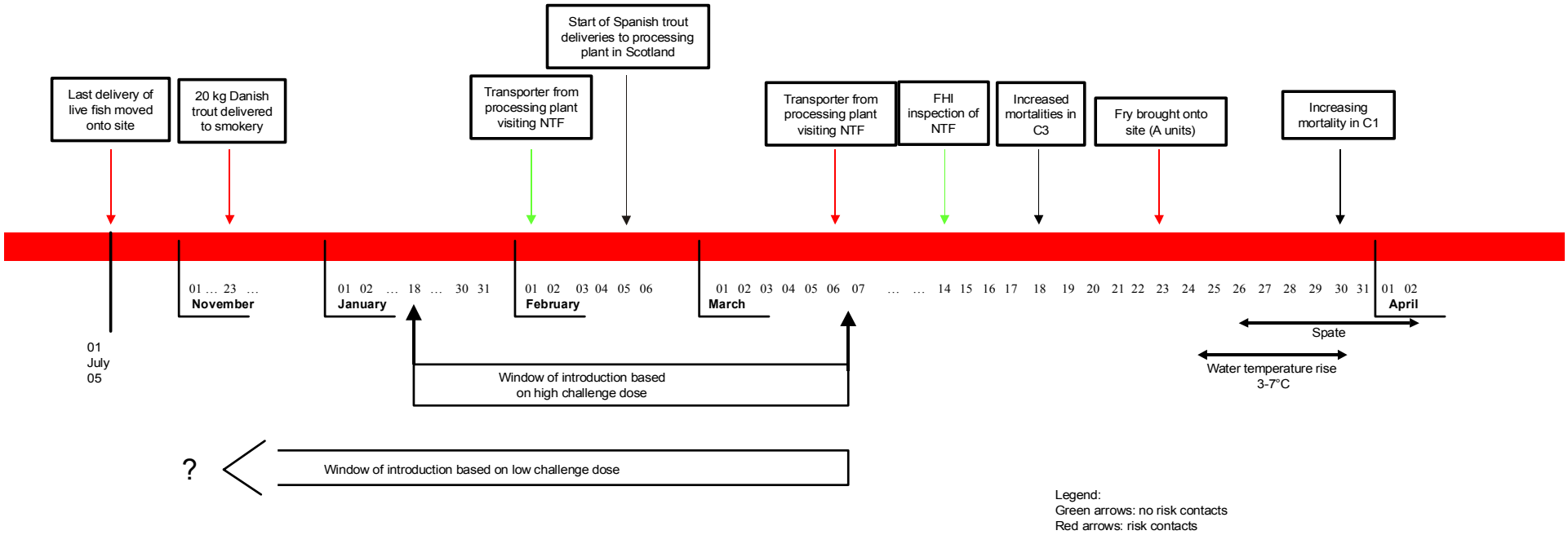
A. Time-line of events from increase in mortalities to completion of the cull



B. Timeline of control measures imposed and surveillance conducted on River Nidd



C. Chronology of events leading up to increase in mortalities at Nidderdale Trout Farm



Appendix 3. Samples of wild fish from the River Nidd and VHSV test results

Table 1: Sample sizes and test results for wild fish sampled from the River Nidd. Samples were processed as pools of 5 fish unless otherwise stated.

Sampling location	Date	Water Temp °C	Grayling +ve/no. tested		Brown trout +ve/no. tested		Rainbow trout +ve/no. tested		Pike +ve/no. tested	
			Virus isolation	RT-PCR*	Virus isolation	RT-PCR	Virus isolation	RT-PCR	Virus isolation	RT-PCR
Pateley Bridge 4 miles upriver of Nidderdale	31.05.06	10.6	0/4	0/4	0/26	0/26				
River Nidd 2 miles upriver of Nidderdale	31.05.06	10.7	0/5	0/5	0/22	0/22	0/1	0/1		
Smokery outlet 1.5 miles upriver of Nidderdale	31.05.06	11.2	0/3	0/3	0/30	0/30				
Immediately upriver of Nidderdale inlet	13.06.06	13.6	0/9 [b]	0/4 [a]	0/141	0/44 [a]				
Immediately downriver of Nidderdale outlet	31.05.06	11.4	**1/7**	0 /7	0/21	0/21				
Up to 1 mile downriver of Nidderdale	14.06.06	14.9	0/52 [b]	**1/52 [a]**	0/98	0/67 [a]				
7 miles downriver of Nidderdale	11.07.06	15.6	0/31	0/31 [c]	0/107	0/107 [c]				
18 miles downriver of Nidderdale	12.07.06	16.3	0/75	0/75 [c]	0/19	0/19 [c]			0/19	0/19 [c]
35 miles downriver of Nidderdale	13.07.06	16.7	0/1	0/1 [c]	0/1	0/1 [c]			0/55	0/55 [c]
Totals			**1/187**	**1 /182**	**0/465**	**0 /337**	**0/1**	**0 /1**	**0/74**	**0 /74**

[a] Individual organs tested /brain, heart, kidney/spleen for individual fish
[b] visceral tissues tested separately from brain tissues for individual fish
[c] Individual fish tested
* polymerase chain reaction