APPENDIX I

APPROVED ZONES FOR INFECTIOUS HAEMATOPOIETIC NECROSIS (IHN) AND VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS)

1. The United Kingdom, in accordance with Article 5 of Directive 91/67/EEC, requests that Great Britain be recognised from 1 January 1993 as an approved zone free from IHN and VHS on the basis of her historical record of freedom from these diseases.

2. IHN and VHS have been notifiable diseases in Great Britain since January 1974. There have been no recorded cases of IHN or VHS nor have the disease agents been isolated during salmonid virus investigations undertaken by the Fish Diseases Laboratory, Weymouth or the Marine Laboratory, Aberdeen. A summary of the viral investigations conducted over the last 10 years is given at Appendix V. The production of farmed rainbow trout and Atlantic salmon, the species most susceptible to IHN or VHS in Great Britain, has increased from less than 500 tonnes in the early 1970s to more than 55,000 tonnes in 1991. During this period, the growth of the fish farming industry and its activities have been closely monitored by the Fisheries Departments in conjunction with both regional and national water authorities. All farms must be registered; maintain records of stock movements and submit annual returns of those movements to the Fisheries Departments.

3. The absence of IHN and VHS has again been confirmed by the epidemiological survey conducted by the UK Fisheries Departments between October 1991 and April 1992 (Appendix VI), in response to Council Decision 90/495/EEC, and by ongoing monitoring. Great Britain's continuing freedom

from IHN and VHS has been aided by her island status and long-standing controls applied to the importation of live fish and ova. In particular, the introduction of live salmon and trout, other than from Northern Ireland (which enjoys equivalent fish health status), has been prohibited since 1937. Imports of ova have been allowed under licence only from sources outside Great Britain which have been inspected, tested and certified as free of IHN and VHS.

4. From 1 January 1993 all fish farms in Great Britain holding species susceptible to VHS and IHN will be inspected twice yearly and sampled at least once every two years. The samples will be taken and tested in accordance with agreed procedures awaiting confirmation by the Standing Veterinary Committee (Appendix XII). Both diseases will continue to be notifiable. As previously mentioned, existing legislation already provides for powers of inspection and sampling; controls over introductions and the maintenance of movement records by fish farmers. Other controls, including the power to designate infected farms, to order the removal and disposal of dead and dying fish and to regulate movements, will be maintained.

5. It is intended that the inland and coastal waters of Great Britain should constitute a single zone, reflecting the absence of disease and the anadromic nature of salmon production. In the event of a suspected outbreak of IHN or VHS, the Commission and other Member States will be informed and immediate action taken to isolate the infected area. The movement of both live and dead fish will be placed under official control to protect stocks in and to safeguard the disease free status of the remainder

of Great Britain. An isolated area will normally comprise a complete river catchment and the adjoining coastal region. Previous movements into and out of that area over a period of at least 6 months will be investigated and this period may be extended to 24 months depending on the species involved and the date of their transfer. Where the disease is confirmed, all fish on infected farms in the area will be slaughtered, the infected fish destroyed and disinfection undertaken. An application would then be made to the Commission to undertake a 4 year programme of checks and tests to regain approved zone status for the isolated area.

## History of salmonid virus testing in Great Britain 1982-1991

| Year | Total farms tested | Total fish tested | Tests showing positive results for | | |
|---|---|---|---|---|---|
| | | | IPN | VHS | IHN |
| 1982 | 173 | 19593 | 50 | 0 | 0 |
| 1983 | 150 | 15749 | 51 | 0 | 0 |
| 1984 | 214 | 19500 | 33 | 0 | 0 |
| 1985 | 251 | 19498 | 19 | 0 | 0 |
| 1986 | 299 | 24361 | 22 | 0 | 0 |
| 1987 | 442 | 34178 | 47 | 0 | 0 |
| 1988 | 480 | 33500 | 72 | 0 | 0 |
| 1989 | 549 | 38385 | 98 | 0 | 0 |
| 1990 | 460 | 34912 | 142 | 0 | 0 |
| 1991 | 620 | 45605 | 183 | 0 | 0 |

APPENDIX VI

# EPIDEMIOLOGICAL SURVEY FOR INFECTIOUS HAEMATOPOIETIC NECROSIS AND VIRAL HAEMORRHAGIC SEPTICAEMIA IN THE UNITED KINGDOM

An epidemiological survey for IHN and VHS was conducted in the United Kingdom between October 1991 and May 1992 in accordance with the plan submitted by the UK authorities in March 1991 and approved by the Commission and the Standing Veterinary Committee on 16 October 1991 (Commission Decision 92/46/EEC).

Salmon or trout from a total of 288 sites were sampled and tested for the presence of IHN and VHS. No evidence of clinical or sub-clinical infection was found. Details of the farms surveyed in England and Wales, Scotland and Northern Ireland are appended.

Ministry of Agriculture, Fisheries and Food
Scottish Office Agriculture and Fisheries Department
Welsh Office Agriculture Department
Department of Agriculture for Northern Ireland

May 1992



DRD/APR/92/SWIFA.DFT

IHN/VHS SURVEY 1992 (ENGLAND AND WALES)

| CATCHMENT | NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 043 | ALLENBROOK | F/019A | DORSET | TRB | Y | N | N | NEG |
| 042 | AVINGTON TROUT FISHERIES | F/114A | HAMPSHIRE | TRB | Y | N | N | NEG |
| 072 | BENTHAM TROUT FARM | F/235A | LANCASHIRE | TRB | Y | N | N | NEG |
| 075 | BORROWDALE FISHERIES | F/195A | CUMBRIA | TRB | N | N | Y | NEG |
| 062 | BRONGEST FISH FARM | F/W024 | DYFED | TRB | N | N | Y | NEG |
| 074 | BURLINGTON LAKES TROUT | F/139A | CUMBRIA | TRB | N | N | Y | NEG |
| 054 | BURWARTON | F/215A | SHROPSHIRE | TRB | Y | N | N | NEG |
| 037 | CENTRAL ESSEX FISH FARM | F/005A | ESSEX | TRB | N | N | Y | NEG |
| 067 | CHARK FISHERY COMPANY | F/W009 | CLWYD | TRB | Y | N | N | NEG |
| 052 | CLAWWORTHY RESERVOIR | F/277B | SOMERSET | TRB | Y | Y | Y | NEG |
| 068 | DANABRIDGE FISH FARM | F/101A | CHESHIRE | TRB | N | N | Y | NEG |
| 046 | DART VALE TROUT | P/086A | DEVON | TRB | N | N | Y | NEG |
| 057 | DRAETHAN TROUT FARM | F/W019 | GWENT | TRB | N | N | Y | NEG |
| 041 | DUNCTON MILL | F/008A | SUSSEX | TRB | Y | Y | N | NEG |
| 071 | DUNSOP TROUT FARM | F/157A | LANCASHIRE | TRB | N | Y | N | NEG |
| 064 | DYFI VALLEY TROUT FARM | F/W01 | POWYS | TRB | N | Y | N | NEG |
| 045 | EXE VALLEY | F/008A | SOMERSET | TRB | Y | Y | Y | NEG |
| 050 | EXMOOR | F/099A | DEVON | TRB | Y | Y | Y | NEG |
| 066 | FELIN-Y-GORU FISHERY | F/W022 | CLWYD | TRB | N | N | Y | NEG |
| 073 | HAWKSHEAD TROUT FARM | F/247A | CUMBRIA | TRB | N | N | Y | NEG |
| 060 | HOME FARM LLANLLAWDDOG | F/W003 | DYFED | TRB | N | N | Y | NEG |
| 044 | HOOKE SPRINGS | F/148A | DORSET | TRB | Y | N | N | NEG |
| 031 | HORN MILL TROUT FARM | F/248B | LEICS | TRB | N | Y | N | NEG |
| 101 | ISLAND FISH FARM | F/227A | HAMPSHIRE | TRB | N | N | Y | NEG |
| 022 | KIELDER | F/428A | NORTH/LAND | SAL | Y | Y | Y | NEG |

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IHN/VHS SURVEY 1992 (ENGLAND AND WALES) continued:

| CATCHMENT | NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 023 | KNARSDALE | F/409A | NORTH/LAND | TRB | N | Y | Y | NEG |
| 056 | LANDLOCKED SALMON EUROPE | F/W018 | GWENT | SAL | Y | N | N | NEG |
| 069 | LITTLE HOLLINS | F/522A | DERBYSHIRE | TRB | N | N | Y | NEG |
| 048 | LITTLE ROSEMORRAN | F/367A | CORNWALL | TRB | N | Y | N | NEG |
| 059 | LLIW MILL TROUT FARM | F/W045 | W.GLAM | TRB | N | N | Y | NEG |
| 040 | LUDDENHAM TROUT FARM | F/075A | KENT | TRB | N | N | Y | NEG |
| 049 | MELLINGEY TROUT FARM | F/097A | CORNWALL | TRB | N | N | Y | NEG |
| 030 | MAPLE HOUSE TROUT FARM | F/202A | LINCS | TRB | N | N | Y | NEG |
| 034 | MENDHAM MILL TROUT FARM | F/295A | NORFOLK | TRB | N | N | Y | NEG |
| 027 | MOORLAND TROUT | F/073A | YORKSHIRE | TRB | Y | N | N | NEG |
| 039 | PADWORTH FISHERIES | F/006A | BERKSHIRE | TRB | Y | Y | Y | NEG |
| 021 | PAXTON | F/291A | NORTH/LAND | TRB | N | N | Y | NEG |
| 058 | PENSCYNOR WILDLIFE PARK | F/W044 | W.GLAM | TRB | N | N | Y | NEG |
| 063 | RHEIDOL POWER STATION | F/W008 | DYFED | TRB | Y | Y | Y | NEG |
| 051 | ROADWATER | F/012A | SOMERSET | TRB | Y | Y | Y | NEG |
| 065 | SEA STREAM | F/W050 | GWYNEDD | TRB | N | N | Y | NEG |
| 076 | SOUTHWAITE GREEN | F/078A | CUMBRIA | TRB | N | N | Y | NEG |
| 028 | TRENT TROUT FARM | F/191B | DERBYSHIRE | TRB | Y | N | N | NEG |
| 047 | TAVISTOCK TROUT FARM | F/169A | DEVON | TRB | N | Y | Y | NEG |
| 024 | THE LIDO, WITTON-LE-WEAR | F/518A | DURHAM | TRB | N | N | Y | NEG |
| 025 | THORNDALE | F/475A | YORKSHIRE | TRB | N | Y | N | NEG |
| 053 | UBLEY HATCHERY | F/066C | AVON | TRB | Y | N | Y | NEG |
| 061 | VICAR'S MILL TROUT FARM | F/W026 | DYFED | TRB | Y | N | Y | NEG |
| 026 | WANSFORD TROUT FARM | F/085A | HUMBERSIDE | TRB | Y | N | N | NEG |

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IHN/VHS SURVEY 1992 (ENGLAND AND WALES) continued:

| CATCHMENT | NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 033 | WESTACRE TROUT FARM | F/028A | NORFOLK | TRB | N | Y | N | NEG |
| 029 | WITHERN MILL TROUT FARM | F/071A | LINCS | TRB | Y | Y | N | NEG |
| 055 | WYE VALLEY FISHERIES | F/W010 | POWYS | TRB | N | N | Y | NEG |

NUMBER OF SITES SAMPLED: 52

Key:

REC OVA/REC LIVE: FARM RECEIVING OVA/LIVE FISH

SPECIES EXAMINED: TRB = RAINBOW TROUT
TRS = BROWN TROUT
SAL = SALMON

BROOD: Brood stock examined

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## IHN/VHS SURVEY 1992 (SCOTLAND)

| | CATCHMENT NAME | REC CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 081 | Torhouse Trout | 0013 | Kircudbright | TRB | N | Y | Y | Neg |
| 082 | River Doon Trout | 0144 | Ayr | TRB | N | N | Y | Neg |
| 083 | Fencefoot Farm | 0271 | Ayr | TRB | N | N | Y | Neg |
| 107 | A A Banks | 0129 | Orkney | SAL | N | N | Y | Neg |
| 087 | Castle Fisheries | 0100 | Argyll | TRB | N | N | Y | Neg |
| 089 | Lorne Fisheries | 0037 | Argyil | TRB | N | N | Y | Neg |
| 106 | Siddinish Salmon | 0135 | Western Isles | SAL | N | N | Y | Neg |
| 021 | Muirhouse Trout Farm | 0112 | Selkirk | TRB | N | Y | Y | Neg |
| 021 | Kendal Trout Farm | 0156 | Selkirk | TRB | N | N | Y | Neg |
| 021 | Galahaugh | 0092 | Selkirk | TRB | N | Y | Y | Neg |
| 021 | Yarrow Fisheries | 0110 | Selkirk | TRB | N | N | Y | Neg |
| 078 | Selcoth Fisheries | 0067 | Dumfries | TRB | N | Y | Y | Neg |
| 086 | Newmill Trout | 0054 | Lanark | TRB | N | N | Y | Neg |
| 013 | Kinnaird Mill | 0290 | Angus | TRB | N | N | Y | Neg |
| 020 | South Belton | 0096 | East Lothian | TRB | N | N | Y | Neg |
| 020 | Maltings | 0096 | East Lothian | TRB | N | Y | N | Neg |
| 083 | GSP (Hunterston) | 0125 | Ayr | TUR | N | Y | N | Neg |
| 081 | Glenkens | 0085 | Galloway | TRB | N | N | Y | Neg |
| 078 | Terregles | 0254 | Dumfries | SAL | N | Y | Y | Neg |
| 078 | Barony College | 0065 | Dumfries | TRB | N | Y | N | Neg |
| 019 | Pennicuik Fish | 0099 | Mid Lothian | TRB | N | Y | Y | Neg |
| 004 | Cromarty Salmon | 0139 | Ross & Cromarty | TRB | N | N | Y | Neg |
| 003 | Fearn | 0150 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 007 | Springburn Trout Farm | 0038 | Moray | TRB | N | N | Y | Neg |
| 008 | Rothiemurchus | 0018 | Inverness | TRB | N | N | Y | Neg |
| 091 | Glencoe Salmon | 0072 | Argyll | SAL | N | N | Y | Neg |
| 089 | Kames Loch Awe | 0134 | Argyll | TRB | N | N | Y | Neg |
| 091 | MH Arkaig | 0119 | Inverness | SAL | N | N | Y | Neg |
| 092 | MH Shiel | 0119 | Inverness | SAL | N | N | Y | Neg |
| 006 | MH Inchmore | 0119 | Inverness | SAL | N | N | Y | Neg |
| 091 | MH Invergarry | 0119 | Inverness | SAL | N | N | Y | Neg |

| CATCHMENT | NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 091 | MH Loch Garry | 0119 | Inverness | SAL | N | N | Y | Neg |
| 105 | Aros Loch | 0140 | Mull | TRB | N | N | Y | Neg |
| 105 | Tobermory Bay | 0140 | Mull | SAL | N | N | Y | Neg |
| 105 | MH Scailacastle | [illegible] | Mull | SAL | N | N | Y | Neg |
| 004 | Lovat-Cruives | 0075 | Inverness | SAL | N | Y | N | Neg |
| 004 | Lovat-Eskadale | 0075 | Inverness | SAL | N | Y | N | Neg |
| 106 | McConnell L Clachan | 0094 | Western Isles | SAL | N | N | Y | Neg |
| 106 | McConnell Mingarry | 0094 | Western Isles | SAL | N | Y | Y | Neg |
| 106 | McConnell L A'Laio | 0094 | Western Isles | SAL | N | N | Y | Neg |
| 095 | J Johnston Fannagmore | 0046 | Sutherland | SAL | N | N | Y | Neg |
| 095 | J Johnston Calva | 0046 | Sutherland | SAL | N | N | Y | Neg |
| 107 | Uyeasound Salmon | 0230 | Shetland | SAL | N | N | Y | Neg |
| 107 | Skaw Smolts | 0300 | Shetland | SAL | N | N | Y | Neg |
| 107 | Balta Island Seafare | 0220 | Shetland | SAL | N | N | Y | Neg |
| 107 | Quoys Hatchery | 0257 | Shetland | SAL | N | N | Y | Neg |
| 107 | Shetland Norse-Basta | 0048 | Shetland | SAL | N | N | Y | Neg |
| 107 | Orkney Fish Farms | 0031 | Orkney | SAL | N | N | Y | Neg |
| 107 | Norsea - Ore Bay | 0213 | Orkney | SAL | N | N | Y | Neg |
| 016 | Kames Loch Tay | 0134 | Orkney | TRB | N | N | Y | Neg |
| 016 | Aberuchill | 0103 | Perthshire | TRB | N | N | Y | Neg |
| 105 | Rothesay Sea Foods | 0027 | Argyll | TRB | N | N | Y | Neg |
| [illegible] | Landcatch Jura | 0061 | Argyll | SAL | N | N | Y | Neg |
| 105 | Strathaird L Na Bleste | 0021 | Skye | SAL | N | N | Y | Neg |
| 105 | Raasay | 0041 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 105 | Balmeanach | 0011 | Skye | SAL | N | N | Y | Neg |
| 105 | Glendale Salmon | 0277 | Skye | SAL | N | N | Y | Neg |
| 086 | Kyles of Bute Salmon | 0116 | Argyll | SAL | N | N | Y | Neg |
| 106 | McConnell Creed | 0094 | Western Isles | SAL | Y | Y | Y | Neg |
| 106 | Amhuinnsuidh | 0094 | Western Isles | SAL | N | Y | N | Neg |
| 087 | Otter Ferry Salmon | 0012 | Argyll | SAL | Y | Y | Y | Neg |
| 107 | Weddell Fish Farms | 0289 | Orkney | SAL | Y | N | Y | Neg |
| 107 | Mainland Salmon | 0077 | Orkney | SAL | Y | N | Y | Neg |

| | CATCHMENT NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| | R Conon DSFB | . | Ross & Cromarty | SAL | Y | Y | N | Neg |
| 095 | Finfish Ltd | 0132 | Ross & Cromarty | SAL | Y | N | Y | Neg |
| 091 | MH Leven | 0119 | Argyll | SAL | N | N | Y | Neg |
| 093 | MH Duich | 0119 | Ross & Cromarty | SAL | Y | N | Y | Neg |
| 092 | MH Ardnish | 0119 | Inverness | SAL | Y | N | Y | Neg |
| 016 | Ochil Foods | 0091 | Perthshire | TRB | N | N | Y | Neg |
| . | . | . | . | . | . | . | . | . |
| 093 | HFF Fada | 0022 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 093 | HFF Tuilich | 0022 | Ross & Cromarty | SAL | N | Y | N | Neg |
| 093 | HFF Sgamhain | 0022 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 092 | GSP Ardtornish | 0125 | Argyll | SAL | N | Y | N | Neg |
| 105 | Penmore Mill | 0279 | Mull | SAL | N | N | Y | Neg |
| 105 | GSP Frisa | 0125 | Mull | SAL | N | N | Y | Neg |
| 105 | GSP Loch Ba | 0125 | Mull | SAL | N | N | Y | Neg |
| 092 | GSP Arienas | 0125 | Mull | SAL | N | N | Y | Neg |
| 093 | MH Nostie | 0119 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 093 | MH Duich | 0119 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 093 | MH Ardentoul | 0119 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 088 | Landcatch Ormsary | 0061 | Argyll | SAL | N | Y | N | Neg |
| 104 | Landcatch Islay | 0061 | Argyll | SAL | N | N | Y | Neg |
| 006 | Foyers Salmon | 0123 | Inverness | SAL | N | Y | N | Neg |
| 094 | Landcatch Gairloch | 0061 | Ross & Cromarty | SAL | N | Y | N | Neg |
| 094 | Torridon Smolts | 0024 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 088 | Tornaturk | 0016 | Argyll | SAL | N | Y | N | Neg |
| 088 | Meall Mhor | 0025 | Argyll | SAL | N | Y | N | Neg |
| 104 | Killean | . | Argyll | SAL | N | Y | N | Neg |
| 087 | Loch Glashan | 0012 | Argyll | SAL | N | N | Y | Neg |
| 095 | Culnacraig Hatchery | 0189 | Ross & Cromarty | SAL | N | Y | N | Neg |
| 095 | J Johnson Duartmore | 0046 | Sutherland | SAL | N | Y | N | Neg |
| 095 | J Johnson L Nam Brac | 0046 | Sutherland | SAL | N | N | Y | Neg |
| 096 | Inverpolly | 0132 | Ross & Cromarty | SAL | N | Y | Y | Neg |
| 093 | Loch Lundie | 0152 | Ross & Cromarty | SAL | N | N | Y | Neg |

| CATCHMENT NAME | | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 094 | Loch Damph | 0152 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 093 | Corrie Mhor Salmon | 0281 | Ross & Cromarty | SAL | N | Y | N | Neg |
| 105 | Storr | 0021 | Skye | SAL | N | N | Y | Neg |
| 105 | Achnacloich | 0021 | Skye | SAL | N | Y | N | Neg |
| 105 | Kilmarie | 0021 | Skye | SAL | N | Y | N | Neg |
| 095 | Elphin | 0044 | Sutherland | SAL | N | Y | N | Neg |
| 095 | Assynt | 0044 | Sutherland | SAL | N | Y | N | Neg |
| 095 | Glenleraig | 0080 | Sutherland | SAL | N | Y | N | Neg |
| 003 | Merkland | 0044 | Sutherland | SAL | N | N | Y | Neg |
| 003 | Loch Migdale | 0341 | Sutherland | SAL | N | N | Y | Neg |
| 107 | Snarravoe - Loch | 0235 | Shetland | SAL | N | N | Y | Neg |
| 107 | Snarravoe - Hatchery | 0235 | Shetland | SAL | N | Y | N | Neg |
| 107 | Quoys - Loch of Cliff | 0257 | Shetland | SAL | N | Y | N | Neg |
| 107 | Fetlar Community | 0196 | Shetland | SAL | N | N | Y | Neg |
| 107 | Millbrook Fisheries | 0285 | Shetland | SAL | N | Y | N | Neg |
| 107 | Shetland Salmon Co-Kergord | 0052 | Shetland | SAL | N | Y | N | Neg |
| 107 | Hallmark Hatchery - Houl | 0296 | Shetland | SAL | N | Y | N | Neg |
| 106 | Red River | 0094 | Western Isles | SAL | N | Y | N | Neg |
| 106 | Scaslavat | 0094 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Eishken | 0094 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Ahmuinnsuidh | 0094 | Western Islands | SAL | N | Y | N | Neg |
| 106 | Lewis Smolts - L Soval | 0243 | Western Islands | SAL | N | N | Y | Neg |
| 106 | Co-Chomun na Pairc | 0145 | Western Islands | SAL | N | N | Y | Neg |
| 106 | Hebridean Fishery | 0040 | Western Islands | SAL | N | N | Y | Neg |
| 106 | Eilan Glas - Barvas Hatch | 0283 | Western Islands | SAL | N | Y | N | Neg |
| 106 | Western Isles Salmon | 0105 | Western Islands | SAL | N | N | Y | Neg |
| 106 | Atlantic West Ltd | 0111 | Western Islands | SAL | N | Y | N | Neg |
| 089 | Caledonian Trout | 0129 | Argyll | TRB | N | N | Y | Neg |
| 088 | Teq Aqua Salmon | 0219 | Argyll | SAL | N | N | Y | Neg |
| 089 | Kinloch Awe | 0078 | Argyll | TRB | N | Y | Y | Neg |
| 088 | Cleighmill | 0171 | Argyll | SAL | N | Y | N | Neg |
| 104 | J & S Salmon | 0071 | Argyll | SAL | N | Y | N | Neg |

| CATCHMENT NAME | | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 016 | Westhill | 0134 | Perthshire | SAL | N | Y | Y | Neg |
| 021 | Hopewood Fish Farm | 0087 | Peebles | TRB | N | N | Y | Neg |
| 019 | Beecraigs | 0003 | West Lothian | TRB | N | N | Y | Neg |
| 018 | Loch Fitty | 0066 | Fife | TRB | N | N | Y | Neg |
| 016 | Strathmore Angling Assoc | | Angus | TRB | N | Y | Y | Neg |
| 018 | Frandy | 0001 | Perthshire | TRB | N | N | Y | Neg |
| 018 | Kinross Estate | | Fife | TRB | N | Y | N | Neg |
| 106 | Clachan Hatchery | 0234 | Western Isles | SAL | N | Y | N | Neg |
| 106 | S & S Smolts | 0247 | Western Isles | SAL | N | N | Y | Neg |
| 106 | North Scadavaig | [illegible] | Western Isles | SAL | N | N | Y | Neg |
| 106 | Loch Gierman | 0013 | Western Isles | SAL | N | N | Y | Neg |
| 106 | South Scadavaig | 0013 | Western Isles | SAL | N | N | Y | Neg |
| 107 | Stenness Fish Farm | | Orkney | SAL | N | N | Y | Neg |
| 107 | Millburn Salmon | 0137 | Orkney | SAL | N | Y | N | Neg |
| 107 | Lyrawa | 0031 | Orkney | SAL | N | N | Y | Neg |
| 107 | Rackwick | 0206 | Orkney | SAL | N | Y | N | Neg |
| 107 | Flotta Fish Farms | 0340 | Orkney | SAL | N | N | Y | Neg |
| 107 | Weddell Fish Farms | 0239 | Orkney | SAL | N | N | Y | Neg |
| 107 | Orkney Seafoods | 0102 | Orkney | SAL | N | N | Y | Neg |
| 107 | Mainland Salmon | 0077 | Orkney | SAL | N | N | Y | Neg |
| 107 | Woodwick Fish Farms | 0312 | Orkney | SAL | N | Y | N | Neg |
| 018 | Swans Water Fishery | 0053 | Stirling | TRB | N | N | Y | Neg |
| 018 | Lake of Menteith | 0208 | Stirling | TRB | N | N | Y | Neg |
| 018 | Trossachs Trout | 0308 | Stirling | TRB | N | N | Y | Neg |
| 018 | Howietoun - Buckieburn | 0151 | Stirling | SAL | N | Y | N | Neg |
| 018 | Howietoun - Dunblane | 0151 | Stirling | SAL | N | N | Y | Neg |
| 016 | Kindrochet Fish Farm | 0070 | Perth | TRB | N | Y | Y | Neg |
| 016 | Loch Earn | 0103 | Perth | TRB | N | N | Y | Neg |
| 009 | Forgue | 0297 | Aberdeen | TRB | N | N | Y | Neg |
| 009 | Stoneyhill | 0130 | Aberdeen | TRB | N | N | Y | Neg |
| 009 | Tynet Valley | 0005 | Banff | TRB | N | N | Y | Neg |
| 011 | Mill of Elrick | 0214 | Aberdeen | TRB | N | N | Y | Neg |

| CATCHMENT NAME | | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 010 | Bishops Bridge | 0123 | Aberdeen | TRB | N | N | Y | Neg |
| 079 | Ae Fishery | 0336 | Dumfries | TRB | N | Y | Y | Neg |
| 079 | Cargenglen | 0159 | Dumfries | TRB | N | N | Y | Neg |
| 080 | Kenmure | 0081 | Galloway | TRB | N | Y | Y | Neg |
| 079 | Invicta Trout | 0007 | Dumfries | TRB | N | Y | Y | Neg |
| 016 | Drumore | 0069 | Perthshire | TRB | N | N | Y | Neg |
| 107 | Laxfirth | 0173 | Shetland | SAL | N | N | Y | Neg |
| 107 | Dury Salmon | 0032 | Shetland | SAL | N | N | Y | Neg |
| 107 | North Atlantic | 0190 | Shetland | SAL | N | N | Y | Neg |
| 107 | Streamsound | 0066 | Shetland | SAL | N | N | Y | Neg |
| 107 | Olnafirth | 0286 | Shetland | SAL | N | N | Y | Neg |
| 107 | Bressay | 0253 | Shetland | SAL | N | N | Y | Neg |
| 107 | Colla Firth | 0249 | Shetland | SAL | N | N | Y | Neg |
| 107 | Olna Marine | 0298 | Shetland | SAL | N | N | Y | Neg |
| 107 | Shetland Salmon Comp | 0052 | Shetland | SAL | N | N | Y | Neg |
| 087 | Lighthouse Quarry | 0169 | Argyll | SAL | N | N | Y | Neg |
| 088 | Loch Sween | 0010 | Argyll | TRB | N | N | Y | Neg |
| [illegible] | Lamlash | 0119 | Bute | SAL | N | N | Y | Neg |
| [illegible] | Machrihanish | | Argyll | SAL | N | N | Y | Neg |
| [illegible] | Isle of Gigha | 0179 | Argyll | SAL | N | N | Y | Neg |
| 104 | West Coast Salmon | 0256 | Argyll | SAL | N | N | Y | Neg |
| 105 | Fishnish B | 0125 | Mull | SAL | N | N | Y | Neg |
| 105 | Soeive B | 0125 | Mull | SAL | N | N | Y | Neg |
| 105 | Ulva | 0130 | Mull | SAL | N | N | Y | Neg |
| 106 | Southside | 0303 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Meanenvagh | 0274 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Uiskevagh | 0274 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Barra Sea Products | 0266 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Bruernish | 0162 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Barra Salmon | 0266 | Western Isles | SAL | N | N | Y | Neg |
| 106 | Salar | 0047 | Western Isles | SAL | N | N | Y | Neg |
| 091 | Ardchattan | 0114 | Argyll | SAL | N | N | Y | Neg |
| 091 | Creran | 0125 | Argyll | SAL | N | N | Y | Neg |

| CATCHMENT NAME | | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 088 | Loch Feochan | 0335 | Argyll | SAL | N | N | Y | Neg |
| 088 | Sailean Mor | 0169 | Argyll | SAL | N | N | Y | Neg |
| 095 | Ardessie | 0157 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 095 | Summer Isles | 0002 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 094 | L Ewe | 0119 | Ross & Cromarty | SAL | N | N | Y | Neg |
| 095 | Ardvar | 0080 | Sutherland | SAL | N | N | Y | Neg |
| 096 | Rispond | 0202 | Sutherland | SAL | N | N | Y | Neg |
| 107 | Papil | 0229 | Shetland | SAL | N | N | Y | Neg |
| 107 | Viking | 0223 | Shetland | SAL | N | N | Y | Neg |
| 107 | Ocean Reaper | 0058 | Shetland | SAL | N | N | Y | Neg |
| 107 | G Johnson | 0064 | Shetland | SAL | N | N | Y | Neg |
| 107 | Gonfirth | 0226 | Shetland | SAL | N | N | Y | Neg |
| 107 | Thompson Bros | 0098 | Shetland | SAL | N | N | Y | Neg |
| 107 | Shetland Sea Farms | 0026 | Shetland | SAL | N | N | Y | Neg |
| 006 | Dores | 0146 | Inverness | SAL | N | N | Y | Neg |
| 088 | Landcatch | 0061 | Argyll | SAL | Y | Y | Y | Neg |
| 095 | Joseph Johnston | 0046 | Sutherland | SAL | Y | N | Y | Neg |

RESULTS OF IHN/VHS SURVEY 1992 (NORTHERN IRELAND)

| CATCHMENT | NAME | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 3 | 7 SPRINGS | F-1 | ANTRIM | TRB | Y | N | N | NEG |
| 3 | SPERRIN MOUNTAIN | F-3 | TYRONE | TRB | Y | N | [illegible] | NEG |
| 3 | INVER TROUT FARM | F-2 | ANTRIM | TRB | Y | N | Y | NEG |
| 3 | NORTHERN SALMON | F-26 | ANTRIM | SAL | Y | Y | N | NEG |
| 3 | ROCKHILL FISH FARM | F-21 | TYRONE | TRB | Y | N | [illegible] | NEG |
| 3 | GLENGONNA BRIDGE | F-33 | TYRONE | TRB | Y | N | N | NEG |
| 3 | GLENOAK FISHERIES | [illegible] | ANTRIM | TRB | Y | N | Y | NEG |
| 1 | BALLYARTEN HATCHERY | F-19 | L'DERRY | TRB | N | Y | Y | NEG |
| 1 | R. WHITESIDE | F-24 | L'DERRY | TRB | N | N | Y | NEG |
| 8 | SILVERSTREAM | F-22 | ANTRIM | TRB | Y | Y | N | NEG |
| 2 | BUSH SALMON STN | | ANTRIM | SAL | Y | N | Y | NEG |
| 1 | GLASAVISK BURN | F-25 | TYRONE | TRB | N | N | Y | NEG |
| 1 | CORGARY TROUT FARM | F-12 | TYRONE | TRB | N | N | Y | NEG |
| 3 | MOVANAGHER | F-28 | ANTRIM | TRB-TRS | Y | Y | Y | NEG |
| 3 | LOUGHINSHOLIN | F-18 | ANTRIM | TRB | N | N | Y | NEG |
| 3 | JUDESTREAM | F-9 | TYRONE | TRS | N | N | Y | NEG |
| 3 | D McALLISTER | F-13 | TYRONE | TRB | N | N | Y | NEG |
| 3 | RIVERSDALE FARM | F-8 | ANTRIM | TRB | N | N | Y | NEG |
| 2 | BUSH VALLEY FARM | F-7 | ANTRIM | TRB | N | N | Y | NEG |
| 3 | ARDALINIS HATCHERY | F-16 | ANTRIM | TRB | N | Y | Y | NEG |
| 3 | GLENARIFFE FISH FARM | F-15 | ANTRIM | TRB | Y | Y | Y | NEG |
| 1 | ROCKS LODGE | F-6 | TYRONE | TRB | N | N | Y | NEG |
| 3 | FLOWING RIVERS FARM | F-10 | DOWN | TRB | N | N | Y | NEG |
| 8 | JUBILEE MILL | F-14 | L'DERRY | TRB | N | N | Y | NEG |
| 8 | OTTERBURN TROUT FARM | F-5 | ANTRIM | TRB | N | N | Y | NEG |

rg1/may92/survey.lst/1

RESULTS OF IHN/VHS SURVEY 1992 (NORTHERN IRELAND) continued

| CATCHMENT NAME | | REG CODE | COUNTY | SPECIES | BROOD | REC OVA | REC LIVE | RESULT |
|---|---|---|---|---|---|---|---|---|
| 8 | STRAID LAKE | F-36 | ANTRIM | TRS | Y | N | Y | NEG |
| 7 | ERRINGTON TROUT FARM | F-23 | FERMANAGH | TRB | N | N | Y | NEG |
| 5 | HERON FISHERIES | [illegible] | DOWN | TRB | N | N | Y | NEG |

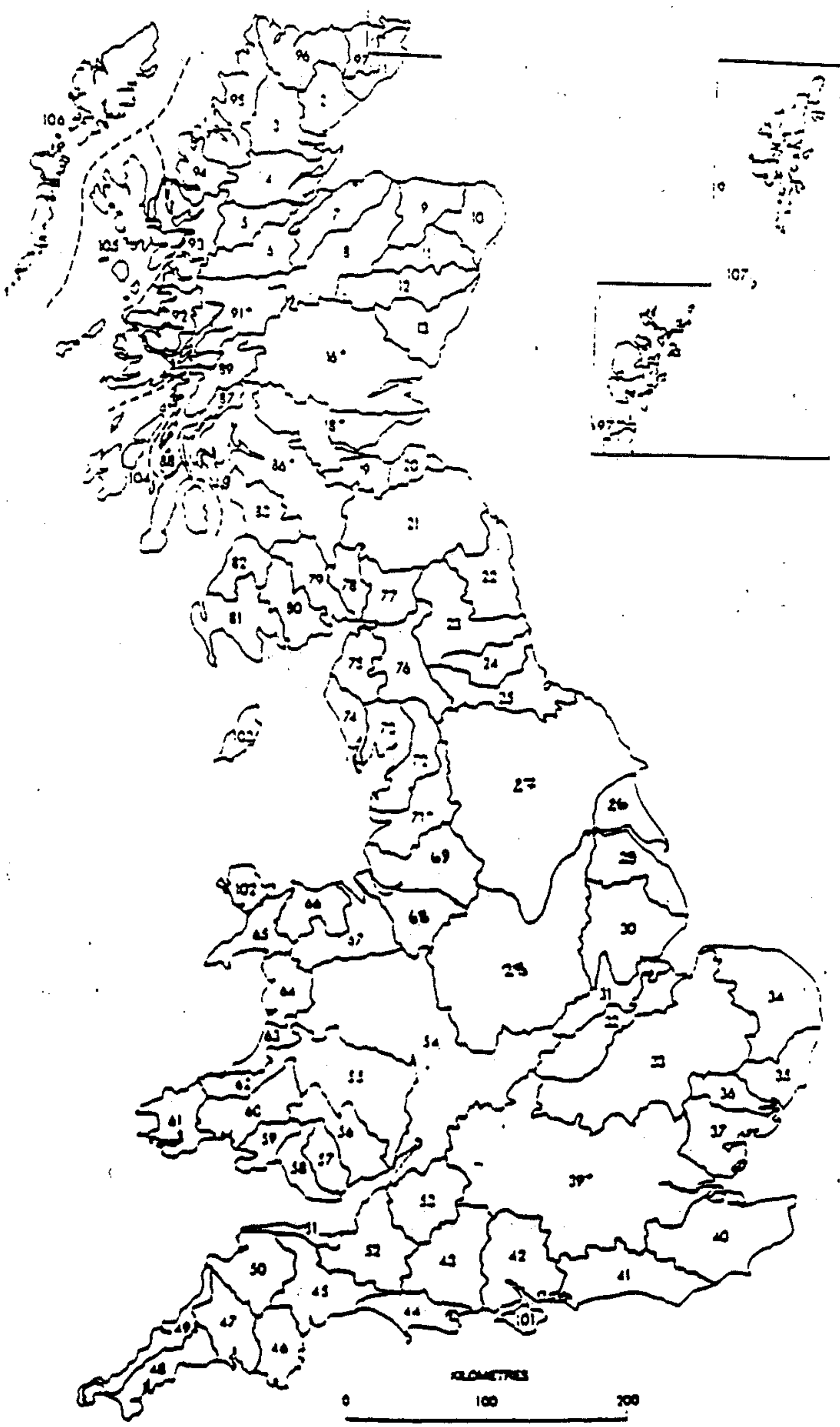
Key:

REC OVA/REC LIVE: FARM RECEIVING OVA/LIVE FISH

SPECIES EXAMINED: TRB = RAINBOW TROUT
TRS = BROWN TROUT
SAL = SALMON

BROOD: Brood stock examined

rgl/may92/survey.lst/1


KILOMETRES
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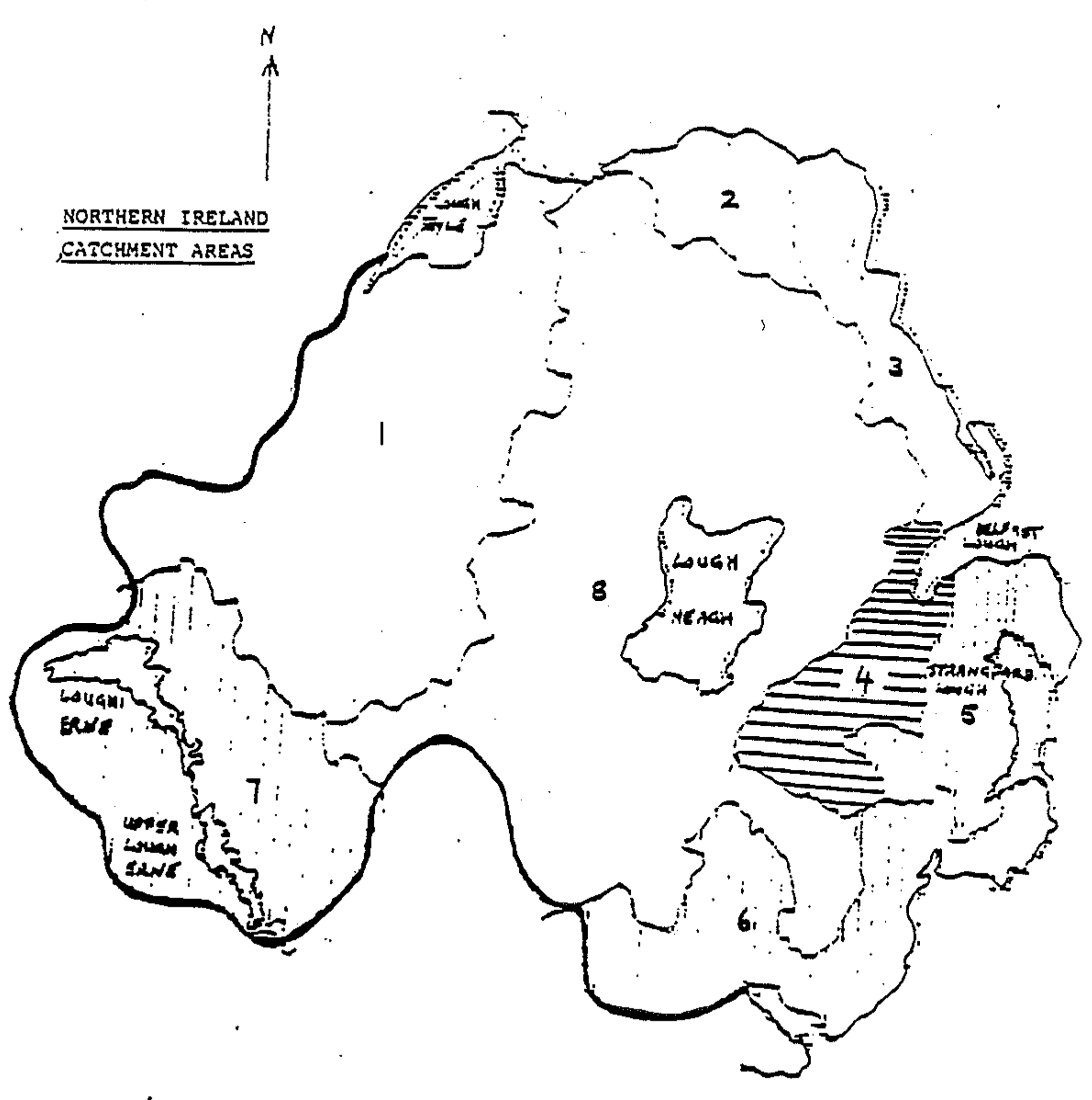
100
200




INTERNATIONAL LAND BOUNDARY

NO SALMONID FARMS

WITH BROODSTOCK

WITHOUT BROODSTOCK

## PART I : SAMPLING AND TESTING PROCEDURES FOR VHS - AND IHN MONITORING

### I. SAMPLING

#### 1. Sampling time

Farms are inspected clinically at least twice per year during the period October to June or whenever the water temperature is below 14°C. Intervals between inspections must be at least 4 months. All production units (ponds, tanks, aquaria, net-cages etc.) are inspected for the presence of dead, weak or abnormally behaving fish. Particular attention has to be paid to the water outlet area (if feasible) where weak fish tend to accumulate because of the water current.

#### 2. Selection and collection of samples

Thirty to 150 fish and/or ovarian fluid samples are collected for examination in connection with the inspections according to Table 1. If rainbow trout are present fish of that species should make up the whole sample. If rainbow trout are not present the sample has to contain fish of all other species present whenever these species are susceptible to VHS and/or IHN as listed in Annex A of Council Directive 91/67/EEC concerning the animal health conditions governing the placing on the market of aquaculture animals and products. The species have to be equally represented in the sample. During the initial four-year control period which precedes achievement of approved status the sample size is 150 in order to ensure detection at a 95 % confidence level of virus carriers at a carrier prevalence of 2 % (4). During the subsequent years (maintenance of approved status) the sample size can be reduced to 30 to ensure detection at a 95 % confidence level of virus at a prevalence of 10 %.

In farms which have a documented history of freedom from VHS and IHN (based on a regular official health inspection programme) the small sample size can be used also during the initial four-year control.

If more than one water source is utilized for fish production, fish representing all water sources may be included in 150 or 30 fish-sample. If weak, abnormally behaving or freshly dead (not decomposed) fish are present, these must primarily be included in the sample. If such fish are not present the sample must be composed of normally appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are represented in the sample.

### 3. Preparation and shipment of samples from fish

Before shipment or transfer to the laboratory pieces of the organs to be examined are removed from the fish with sterile scissors and forceps and transferred to plastic tubes containing transportation medium i.e. cell culture medium with 10 % calf serum and antibiotics. The combination of 200 i.u. penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml can be recommended but other antibiotics of proven efficiency may be used as well. The tissue material to be examined is spleen, anterior kidney, encephalon and in some cases ovarian fluid (Table 1).

Organ pieces from 5 to 10 fish (Table 1) may be collected in one tube and represent one pooled sample. The tissue in each sample should weigh a minimum of 1 g and such that the final dilution is 1:10.

The tubes are placed in insulated containers (for instance thick-walled polystyrene boxes) together with sufficient ice of "frost blocks" to ensure chilling of the samples to between 0 and 5°C during transportation to the laboratory. Freezing must be avoided.

The virological examination must be started as soon as possible and not later than 48 hours after the collection of the samples. If the fish to be examined are less that 6 cm in length, the whole fish may be shipped to the laboratory in plastic bags chilled as mentioned above.

### 4. Collection of supplementary diagnostic material

According to agreement with the involved diagnostic laboratory, other fish tissues may be collected as well and prepared for supplementary examinations.

## II. PREPARATION OF SAMPLES FOR VIROLOGICAL EXAMINATION

### 1. Homogenization of organs

In the laboratory the tissue in the tubes must be completely homogenized (either by stomacher, blender or mortar and pestle) and subsequently suspended in the original transport medium. If a sample consisted of whole fish, i.e. fish less than 6 cm long, these are minced with sterile scissors after removal of the body behind the gut opening, homogenized as described above and suspended 1:10 in transport medium.

### 2. Centrifugation of homogenate

The homogenate is centrifuged in a refrigerated centrifuge at 2°C - 5°C at 2000 to 4000 x g for 15 minutes and the supernatant collected for examination.

If shipment of the sample has been made in transport medium (i.e. with exposure to antibiotics) the supernatant needs no further treatment with antibiotics.

If the sample was shipped as whole fish the homogenate following centrifugation has to be exposed to the antibiotics in the transport medium used for resuspension for either 4 hours at room temperature or over night at 4°C.

The anitibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary.

Immediately after the centrifugation a volume of the supernatant is mixed with equal parts of a suitably diluted divalent antiserum to IPN virus (reference strains Sp and Ab) and incubated with this for a minimum of one hour at 15°C or a maximum of 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50 % plaque neutralization test.

Treatment of all inocula with antiserum to IPN virus (a virus which is some parts of Europe occurs in 50 % of fish samples) aims at preventing CPE due to IPN virus from developing in inoculated cell cultures. This will reduce duration of the virological examinations as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHS or IHN.

When samples come from production units which are considered free from IPN, treatment of inocula with antiserum to IPN virus may be omitted.

## III. VIROLOGICAL EXAMINATION

### 1. Cell cultures and media

BF-2 and either EPC or FHM cells are grown at 20° to 25°C in Eagle's MEM (or modifications thereof) with a supplement of 10 % foetal bovine serum and antibiotics in standard concentrations.

When the cells are cultivated in closed vials, medium is buffered with bicarbonate. Medium used for cultivation of cells in open units has to be buffered with Tris-HCl (23 mM) and Na-bicarbonate (6 mM) at a pH as close as possible to 7.6, a pH which is optimal for virus multiplication.

Cell cultures to be used for inoculation with tissue material should be young (4 to 48 hours old) and actively growing (not confluent) at inoculation.

### 2. Inoculation of cell cultures

Antibiotic-treated organ suspension is inoculated into cell cultures in two dilutions, i.e. the primary dilution and in addition a 1:100 dilution thereof (in order to prevent homologous interference). At least two cell lines have to be inoculated (see III.1.). The ratio between inoculum size and volume of cell culture medium should be about 1:10.

For each dilution and each cell line a minimum of about 2 $cm^2$ cell area, corresponding to 1 well in a 24-well cell culture tray, has to be utilized. Use of cell culture trays is recommended, but other units of similar or bigger growth area are acceptable as well.

3. Incubation of cell cultures

Inoculated cell cultures are incubated at 15°C for 7 days. If the colour of the cell culture medium changes from red to yellow indicating medium acidification, pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to ensure cell susceptibility to virus infection.

Titration every six months of frozen stocks of VHSV and IHNV is performed to verify the susceptibility of the cell cultures to infection.

4. Microscopy

Inoculated cell cultures are inspected daily for the occurence of CPE at about 40 x magnification. If obvious CPE is observed, virus identification procedures according to Section IV have to be initiated immediately.

At the same time appropriate steps are taken to suspend the approved status of the production unit from where the virus positive sample originated as well as from any production units situated downstream.

Suspension of approved status has to be maintained until laboratory tests have proved the virus in question not to be VHSV or IHNV. A maximum of four weeks are allowed for identification of the virus, including the time needed for examination in reference laboratories.

5. Subcultivation

If no CPE has developed after incubation for 7 days, subcultivation is performed to fresh cell cultures utilizing a cell area similar to that of the primary culture.

Aliquots of medium from all cultures/wells constituting the primary culture are pooled according to cell line following one freeze-thaw cycle of the cultures and 0.5 ml of the medium mixed with equal parts of antiserum to IPN as described in Section II.3. and the mixture incubated at 15°C for 60 minutes. The mixture is then inoculated into homologous cell cultures at dilutions 1:1 and 1:100 as described in Section III.2. The inoculation may be preceded by preincubation of the dilutions with divalent antiserum to IPN virus at appropriate dilution.

The inoculated cultures are then incubated for 7 days at 15°C with observation as in Section III.4.

## IV. VIRUS IDENTIFICATION

### 1. Neutralization

If evidence of CPE has been observed in a cell culture, medium is collected, cells removed by low speed centrifugation or membrane filtration (0.45 um) and medium then diluted 1:100 and 1:10000 in cell culture medium.

Aliquots of the dilutions are mixed and incubated for 60 minutes at 15°C with equal parts of the following reagents separately :

| | |
|---|---|
| group specific antibody to VHSV (Egtved virus) | 1:50 * |
| group specific antibody to infectious hematopoietic necrosis virus (IHNV) | 1:50 * |
| specific divalent antiserum to infectious pancreatic necrosis virus (IPNV) (reference strains SP and Ab) | 1:50 * |
| medium | 1:1 |

* or as specified by the reference laboratory with regard to the possible cytotoxicity of the antisera.

The reagents used have to be of reference quality with regard to titre and specificity.

From each virus-serum mixture at least two cell cultures are inoculated with 50 ul each and then incubated at 15°C. Development of CPE is checked as described in Section III.4.

If the test has not allowed safe identification of the virus within one week, one of the following steps has to be taken :

a. Transfer of the virus to a national fish virus reference laboratory for immediate identification.

b. Application of IFAT (Section IV.2.), ELISA (Section IV.3.) or other virus identification techniques with reagents of reference quality.

### 2. Immunofluorescence (IFAT)

For each virus isolate to be identified, at least 8 coverglasses or equivalent are seeded with EPC cells at a density leading to about 60 to 90 % confluency after 24 hours of cultivation. EPC cells are chosen for this purpose because of their strong adherence to glass surfaces.

When the cells have sedimented onto the glass surface (about one hour after seeding), or when the cultures have been incubated for up to 24 hours, the virus to be identified is inoculated. Four cultures are inoculated at a volume to volume ratio to 1:10, and four cultures at a ratio of 1:100.

Between 20 and 30 hours post inoculation, the cultures are rinsed twice in Eagle's MEM without serum, fixed in acetone and then stained by means of a two-layer IFAT. The first reagent layer consists of approved antiserum (as in Section IV.1.). The second reagent layer is an FITC-conjugated antiserum to the immunoglobulin used in the first layer. For each of the antisera tested at least one high-dose and one low-dose inoculated culture have to be stained. Proper negative and positive controls have to be included in the test.

Mount stained cultures using glycerol saline. Examine under incident U-V light. Use 10X or 12X eyepieces and X25 or X40 objective lens with numerical apertures >0.7 and >1.3 respectively. Dilutions of primary antiserum and FITC conjugated anti-species immunoglobulin will depend on the microscope set-up chosen or available.

Some Egtved virus strains react strongly with antiserum to reference strain F 1 in IFAT although not reacting in neutralization tests.

3. Enzyme linked immunosorbent assay (ELISA)

Wells in microtiter plates (for instance Nunc-immunoplates, Maxisorp, Nunc, Denmark) are coated overnight with recommended dilutions of Protein-A purified immunoglobulin fractions of the antisera mentioned in Section IV.1.

After rinsing of wells with PBS-Tween-20 buffer, the virus to be identified is added to the wells in two or four-fold dilution steps and allowed to react with the coating antibody for 60 minutes at 37°C. Following rinsing with PBS-Tween-20 buffer, biotinylated antibodies of a specificity corresponding to that of the coating anitbodies are added and allowed to react for 60 minutes at 20°C. Following another rinse as above, HRP conjugated streptavidin is added and allowed to react for one hour at 20°C. After a last rinse, bound enzyme is visualized using appropriate ELISA substrates (OPD, TMB or others).

The above biotin-avidin based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

TABLE 1 A

ACHIEVEMENT OF STATUS

| | No. of clinical insp. per year | Examination of organs from no. of fish | Examination of ovarian fluid from no. of fish |
|---|---|---|---|
| **Continental zones:** | | | |
| a Farms with broodstock | 2 | 120 (1st)<br>150 (2nd) | 30 (1st)<br>0 |
| b Farms with broodstock only | 2 | 0 | 150 (1st or 2nd) |
| c Farms without broodstock | 2 | 150 (1st & 2nd) | 0 |
| **Coastal zones:** | | | |
| a Farms without broodstock | 2 | 30 (1st & 2nd) | 0 |
| b Farms with broodstock | 2 | 120 (1st)<br>150 (2nd) | 30 (1st)<br>0 |

Maximum number of fish per pool: 5

## TABLE 1 B

## MAINTENANCE OF STATUS

| | No. of clinical insp. per year | Examination of organs from no. of fish | Examination of ovarian fluid from no. of fish |
|---|---|---|---|
| **Continental zones:** | | | |
| a Farms with broodstock | 2 | 15 (1st or 2nd) | 15 (1st or 2nd) |
| b Farms with broodstock only | 2 | 0 | 30 (1st or 2nd) |
| c Farms without broodstock | 2 | 30 (1st or 2nd) | 0 |
| **Coastal zones:** | | | |
| a Farms without broodstock | 1 | 30[1] (1st or 2nd) | 0 |
| b Farms with broodstock | 2 | 0 | 30 (1st or 2nd) |

Maximum number of fish per pool: 10

(1) The samples have to be collected during the months 2 to 6 after transfer of fish from fresh to salt-water.

## PART II : DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION OF IHN AND VHS IN CASE OF SUSPECTED OUTBREAKS

Diagnosis of IHN and VHS can be achieved by one of the following techniques :

A. Conventional virus isolation with subsequent serological virus identification.
B. Virus isolation with simultaneous serological virus identification.
C. Rapid diagnostic techniques (IFAT, ELISA).

The first diagnosis of IHN and VHS in farms in approved zones, must not be based on method C alone. Either method A or B must also be used..

The tissue material meant for virological examination in some cases may have to be accompanied by supplementary material for bacteriological, parasitological, histological or other examination to allow for a differential diagnosis. Such material is collected according to procedures outlined by OIE.

### A. CONVENTIONAL VIRUS ISOLATION WITH SUBSEQUENT SEROLOGICAL VIRUS IDENTIFICATION

#### A.I.1-2. Selection of samples

At least 10 fish showing typical signs of IHN or VHS must be selected for examination.

#### A.I.3. Preparation and shipment of samples from fish

Before shipment or transfer to the laboratory, pieces of the organs to be examined are removed from the fish with sterile scissors and forceps and transferred to plastic tubes containing transportation medium, i.e. cell culture medium with 10 % calf serum and antibiotics. The combination of 200 I.u. penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml can be recommended but other antibiotics of proven efficiency can be used as well. The organs to be examined are spleen, anterior kidney, and encephalon.

Organ pieces from 5 to 10 fish may be collected in one tube and represent one pooled sample. The tissue in each sample should weigh a minimun of 1g and such that the final dilution is about 1:10..

The tubes are placed in insulated containers (for instance thick-walled polystyrene boxes) together with sufficient ice or "frost blocks" to ensure chilling of the samples to between 0 and 5°C during transportation to the laboratory. Freezing must be avoided.

The virological examination must be started as soon as possible and not later than 48 hours after the collection of the samples. If the fish to be examined are less than 6 cm in length, the whole fish may be shipped to the laboratory in plastic bags chilled as mentioned above.

A.I.4. Collection of supplementary diagnostic material

According to agreement with the involved diagnostic laboratory, other fish tissues may be collected as well and prepared for supplementary examinations.

A.II.1. Homogenization of organs

In the laboratory the tissue in the tubes is completely homogenized (stomacher, blender or mortar and pestle) and subsequently the homogenate is suspended in the original transport medium. If a sample consisted of whole fish, i.e. fish less than 6 cm long, these are minced with sterile scissors after removal of the body behind the gut opening, homogenized as described above and suspended 1:10 in transport medium.

A.II.2. Centrifugation of homogenate

The homogenate is centrifuged in a refrigerated centrifuge at 2-5°C at 2000 to 4000 x g for 15 minutes and the supernatant collected for examination.

If shipment of the sample has been made in transport medium (i.e. with exposure to antibiotics) the supernatant needs no further treatment with antibiotics.

If the sample was shipped as whole fish, the homogenate following centrifugation has to be exposed to the antibiotics in the transport medium used for resuspension for either four hours at room temperature or over night at 4°C.

The antibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary. Immediately after the centrifugation a volume of the supernatant is mixed with equal parts of a suitably diluted divalent antiserum to IPN virus (reference strains Sp and Ab) and incubated with this for a minimum of one hour at 15°C or a maximum of 18 hours at 4°C. The titre of the antiserum must be at least 1:2000 in a 50 % plaque neutralization test.

Treatment of all inocula with antiserum to IPN virus (a virus which in some parts of Europe occurs in up to 50 % of fish samples) aims at preventing CPE due to IPN virus from developing in inoculated cell cultures. This will reduce the duration of the virological examinations as well as the number of cases in which occurrence of CPE would have to be considered potentially indicative of VHS or IHN.

When samples come from production units which are considered free from IPN, treatment of inocula with antiserum to IPN virus may be omitted.

A.III.1. Cell cultures and media

BF-2 and either EPC or FHM cells are grown at 20° to 25°C in Eagle's MEM (or modifications thereof) with a supplement of 10 % foetal bovine serum and antibiotics in standard concentrations.

When the cells are cultivated in closed vials, medium is buffered with bicarbonate. Medium used for cultivation of cells in open units has to be buffered with Tris-Hcl (23 mM) and bicarbonate (6 mM) at a pH as close as possible to 7.6, a pH which is optimal for virus multiplication.

Cell cultures to be used for inoculation with tissue material should be young (four to 48 hours old) and actively growing (not confluent) at inoculation.

<u>A.III.2. Inoculation of cell cultures</u>

Antibiotic-treated organ suspension is inoculated into cell cultures in two dilutions, i.e. the primary dilution and in addition a 1:100 dilution thereof (in order to prevent homologous interference). At least two cell lines have to be inoculated (see A.III.1.).
The ratio between inoculum size and volume of cell culture medium should be about 1:10.

For each dilution and each cell line, a minimum of about 2 cm2 cell area, corresponding to 1 well in a 24-well cell culture tray, has to be utilized. Use of cell culture trays is recommended, but other units of similar or bigger growth area are acceptable as well.

<u>A.III.3. Incubation of cell cultures</u>

Inoculated cell cultures are incubated at 15°C for 7 days. If the colour of the cell culture medium changes from red to yellow, indicating medium acidification pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to ensure cell susceptibility to virus infection.
Titration every six months of frozen stocks of VHSV and IHNV is performed to verify the susceptibility of the cell cultures to infection.

<u>A.III.4. Microscopy</u>

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 x magnification. If obvious CPE is observed, virus identification procedures according to Section AIV have to be initiated immediately.

At the same time appropriate steps are taken to suspend the approved status of the production unit from where the virus positive sample originated as well as from any production units situated downstream.

Suspension of approved status has to be maintained until laboratory tests have proved the virus in question not to be VHSV or IHNV. A maximum of four weeks are allowed for identification of the virus, including the time needed for examination in reference laboratories.

<u>A.III.5. Subcultivation</u>

If no CPE has developed after incubation for 7 days, subcultivation is performed to fresh cell cultures utilizing a cell area similar to that of the primary culture.

Aliquots of medium from all cultures/wells constituting the primary culture are pooled according to cell line following one freeze-thaw cycle of the cultures and 0.5 ml of the medium mixed with equal parts of antiserum to IPN as described in Section A.II.2 and the mixture incubated at 15°C for 60 minutes. The mixture is then inoculated into cell cultures at dilutions 1:1 and 1:100 as described in Section A.III.2. The inoculation may be preceded by preincubation of the dilutions with divalent antiserum to IPN virus at appropriate dilution.

The inoculated cultures are then incubated for 7 days at 15°C with observation as in Section A.III.4.

A.IV.1. Neutralization

If evidence of CPE has been observed in a cell culture, medium is collected, cells removed by low speed centrifugation or membrane filtration (0.45 um) and medium then diluted 1:100 and 1:10000 in cell culture medium.

Aliquots of the dilutions are mixed and incubated for 60 minutes at 15°C with equal parts of the following reagents separately :

| | |
|---|---|
| group specific antibody to VHSV (Egtved virus) | 1:50 * |
| group specific antibody to infectious hematopoietic necrosis virus (IHNV) | 1:50 * |
| specific divalent antiserum to infectious pancreatic necrosis virus (IPNV) (reference strains Sp and Ab) | 1:50 * |
| medium | 1:1 |

* or as specified by the reference laboratory with regard to the possible cytotoxicity of the antisera.

The reagents used have to be of reference quality with regard to titre and specificity.

From each virus-serum mixture at least two cell cultures are inoculated with 50 µl each and then incubated at 15°C. Development of CPE is checked as described in Section A.III.4.

If the test has not allowed safe identification of the virus within one week, one of the following steps has to be taken :

a. Transfer of the virus to a national fish virus reference laboratory for immediate identification.

b. Application of IFAT (Section A.IV.2.), ELISA (Section A.IV.3.) or other virus identification techniques with reagents of reference quality.

## A.IV.2. Immunofluorescence (IFAT)

For each virus isolate to be identified at least 8 coverglasses or equivalent are seeded with EPC cells at a density leading to about 60 to 90 % confluency after 24 hours of cultivation. EPC cells are chosen for this purpose because [illegible]r strong adherence to glass surfaces.

When the cells have sedimented onto the glass surface (about one hour after seeding), or when the cultures have been incubated for up to 24 hours, the virus to be identified is inoculated. Four cultures are inoculated at a volume to volume ratio of 1:10, and four cultures at a ratio of 1:100.

Between 20 and 30 hours post inoculation the cultures are rinsed twice in Eagle's MEM without serum, fixed in acetone and then stained by means of a two-layer IFAT. The first reagent layer consists of approved antiserum (as in Section IV.1.). The second reagent layer is an FITC-conjugated antiserum to the immunoglobulin used in the first layer. For each of the antisera tested at least one high-dose and one low-dose inoculated culture have to be stained. Proper negative and positive controls have to be included in the test.

Mount stained cultures using glycerol saline. Examine under incident U-V light. Use 10X or 12X eyepieces and X25 or X40 objective lens with numerical apertures >0.7 and >1.3 respectively. Dilutions of primary antiserum and FITC conjugated anti-species immunoglobulin will depend on the microscope set-up chosen or available.

Some Egtved virus strains react strongly with antiserum to reference strain F 1 in IFAT although not reacting in neutralization tests.

## A.IV.3. Enzyme linked immunosorbent assay (ELISA)

Wells in microtiter plates (for instance Nunc-immunoplates, Maxisorp, Nunc, Denmark) are coated over night with recommended dilutions of Protein-A purified immunoglobulin fractions of the antisera mentioned in Section A.IV.1.

After rinsing of wells with PBS-Tween-20 buffer, the virus to be identified is added to the wells in two- or four fold dilution steps and allowed to react with the coating antibody for 60 minutes at 37°C. Following rinsing with PBS-Tween-20 buffer biotinylated antibodies of a specificity corresponding to that of the coating antibodies are added and allowed to react for 60 minutes at 20°C. Following another rise as above HRP conjugated streptavidin is added and allowed to react for one hour at 20°C. After a last rinse bound enzyme is visualized using appropriate ELISA substrates (OPD, TMB or others).

The above biotin-avidin based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

## B. VIRUS ISOLATION WITH SIMULTANEOUS VIRUS IDENTIFICATION

### B.I.1-2. Selection of samples

As A.I.1-2.

### B.I.3. Preparation and shipment of samples from fish

As A.I.3.

### B.II.1. Homogenization of organs

As A.II.1.

### B.II.2. Centrifugation of homogenate

As A.II.2.

### B.II.3. Treatment of supernatant with diagnostic antisera

The antibiotic and anti-IPN treated organ suspension is diluted 1:10 and 1:10000 in cell culture medium and aliquots mixed and incubated for 60 minutes at 15°C with equal parts of the reagents listed in Section A.IV.1.

### B.III.1. Cell cultures and media

As A.III.1.

### B.III.2. Inoculation of cell cultures

From each virus serum mixture (prepared according to B.II.3.) at least two cell cultures per cell line are inoculated with 50 µl each.

### B.III.3. Incubation of cell cultures

As A.III.3.

### B.III.4. Microscopy

Inoculated cell cultures are inspected daily for the occurrence of CPE at about 40 x magnification. If CPE is prevented by one of the antisera used, the virus can be considered to be identified accordingly.
If CPE is not prevented by any of the antisera, virus identification procedures according to A.IV. have to be performed.

### B.III.5. Subcultivation

If no CPE has occurred after 7 days, subcultivation has to be performed from cultures inoculated with supernatant plus medium (B.II.3.).

## C. RAPID DIAGNOSTIC TECHNIQUES (IFAT, ELISA)

Supernatant prepared as described under A.II.2. is submitted to IFAT or ELISA according to A.IV.2. or A.IV.3., respectively. These rapid techniques have to be supplemented with a virological examination according to either A or B within 48 hours after sampling if:

a) a negative result is obtained, or

b) a positive result is obtained with material representing the first case of IHN or VHS in approved zones.

COUNCIL DIRECTIVE 91/67/EEC : APPROVED ZONES AND OTHER MEASURES TO PREVENT THE INTRODUCTION AND SPREAD OF FISH AND SHELLFISH DISEASE IN GREAT BRITAIN

1. This document forms the application by the United Kingdom authorities to the Commission seeking approval to establish in Great Britain from 1 January 1993:

   (i) approved zones for the fish diseases infectious haematopoietic necrosis and viral haemorrhagic septicaemia;

   (ii) control programmes to prevent the spread of the fish diseases bacterial kidney disease, infectious pancreatic necrosis, furunculosis in salmon and spring viraemia of carp;

   (iii) measures to prevent the introduction of the fish disease gyrodactyliasis caused by *Gyrodactylus salaris*;

   The application also sets out, for approval, plans to undertake a two year programme to achieve approved zone status in respect of *Bonamia*, *Marteilia*, and other List II diseases of shellfish. This application is submitted in accordance with Articles 5, 10, 12 and 13 of Council Directive 91/67/EEC concerning the animal health conditions for placing on the market of aquaculture animals and products. Separate applications are to be presented for Northern Ireland, the Isle of Man, Guernsey and Jersey.

2. The measures proposed by the United Kingdom are designed to safeguard the health of Great Britain's farmed and natural stocks of fish and shellfish by preventing the introduction and spread of infectious diseases, and are set out in detail at Appendices I to IV. They are

consistent with the provisions of Directive 91/67/EEC and allow for the free movement of live fish and shellfish between farms and zones of equivalent health status. Existing restrictions on trade with other parts of the European Community, in particular the prohibition on the importation of live salmonids which has operated for more than 50 years and licensing controls for the importation of other species of fish and shellfish, are to be lifted.

3. Great Britain's fish and shellfish stocks have an estimated annual value approaching £1000 million. In 1991 the output of her fish and shellfish farming industry was close to £200 million, with 55,000 tonnes of salmon and trout being harvested for table consumption at a first sale value of £150 million. The economic value of Great Britain's natural salmon and trout fisheries is put at £500 million and that of her coarse fisheries at £300 million : together they are fished by some 3 million anglers.

Responsible authorities

4. The authorities responsible for ensuring that the measures to be adopted in Great Britain comply with the provisions of Directive 91/67/EEC will be:-

in England : Ministry of Agriculture,
Fisheries and Food (MAFF)
Nobel House
17 Smith Square
London SW1P 3JR

In Wales : Welsh Office Agriculture
Department (WOAD)
Crown Offices
Cathays Park
Cardiff CF1 3NQ

in Scotland : Scottish Office Agriculture
and Fisheries Department (SOAFD)
Pentland House
47 Robbs Loan
Edinburgh EH14 1TW

5. The responsible authorities will be assisted by:-

in England and Wales : Fish Diseases Laboratory
Ministry of Agriculture,
Fisheries and Food
33 Albany Road
Granby Industrial Estate
Weymouth
Dorset DT4 9TH

in Scotland : The Marine Laboratory
Scottish Office Agriculture and
Fisheries Department
PO Box 101
Victoria Street
Aberdeen AB9 8DB

Staff at the two laboratories, as at present, will be responsible for the inspection and surveillance of all fish and shellfish farms in Great Britain, the collection of samples, diagnostic testing and for the supervision of measures required to eradicate disease. They will also be responsible for the completion of health certification for fish and shellfish movements. Close liaison will be

maintained with the veterinary authorities for Great Britain (the State Veterinary Service of the Ministry of Agriculture, Fisheries and Food). Both laboratories are to be connected to the Community's computerised network for the notification of animal movements, ANIMO.

6. The Fish Diseases Laboratory (FDL) is part of MAFF's Directorate of Fisheries Research and has a present complement of 54 posts. It is equipped to undertake bacterial, virological, histopathological and serological testing and will shortly be expanded to include a molecular biology section. Corresponding activity in Scotland is undertaken by the Fish Cultivation Section of the Marine Laboratory, Aberdeen, the research arm of the Scottish Office Agriculture and Fisheries Department. The Fish Cultivation Section has a similar complement to FDL and is an integrated, multidisciplinary fish disease diagnostic and research group with full capability in all aspects of infectious disease functions. The combined budget of the two laboratories for the control of fish disease amounts to some £2.3 million a year. This is supported by an active and on-going research programme, mostly conducted in-house but partly in association with universities, polytechnics and colleges specialising in fish cultivation and disease. A summary of the research programme for 1991-92, wit a budget totalling £1.1 million, is given at Appendix XX.

**Legislative framework**

7. Regulations to apply the provisions of Directive 91/67/EEC within Great Britain are being prepared and will be presented to Parliament for approval during the Autumn. These regulations will supplement existing measures for the control of fish and shellfish disease in the United Kingdom.

8. Under the Diseases of Fish Act 1937, powers already exist to control the introduction of live fish and ova; to inspect fish farms and other waters and to take samples; to require the notification of specified fish diseases; to designate areas of water and land, including fish farms, as infected with notifiable disease; to order the removal and disposal of dead or dying fish from designated areas; and to regulate the movement of fish and other materials into and out or such areas. Corresponding powers to control shellfish disease reside in the Sea Fisheries (Shellfish) Act 1967. Action may be taken to regulate the introduction, deposit and taking of specified molluscs and crustacea from coastal waters and adjoining land and to destroy stocks affected by shellfish pests and diseases. Finally the Diseases of Fish Act 1983 provides for the registration of both fish and shellfish farming businesses, the maintenance of movement records and the submission of annual movement returns to the Fisheries Departments. Copies of these measures and relevant subordinate legislation may be found at Appendix XXI.

<u>Contact</u>

9. [redacted]

Salmon, Whaling and Inland Fisheries Division "A"
Ministry of Agriculture, Fisheries and Food
Nobel House
17 Smith Square
London SW1P 3JR
Tel: [redacted]
Fax: [redacted]

Ministry of Agriculture, Fisheries and Food
Scottish Office Agriculture and Fisheries Department
Welsh Office Agriculture Department

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