AQUAEXCEL

Aquaculture Infrastructures for Excellence in European Fish Research

Project number: 262336

Combination of CP & CSA
Seventh Framework Programme
Capacities

Deliverable 3.1

Sanitary prescriptions and procedures for transfer, and safety standards

Due date of deliverable: M12

Actual submission date: M13

Start date of the project: March 1st, 2011 **Duration**: 48 months

Organisation name of lead contractor: ULPGC

Revision: Gunnar Senneset

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

Table of contents

Glossary 5

Summary 6

Introduction 7

CHAPTER I

I.- Fish diseases: Diagnosis and surveillance 9

I.1.- Exotic diseases according to Directive 2006/88 EC 12

I.1.1.- Epizootic haematopoietic necrosis 12

I.1.2.- Epizootic ulcerative syndrome 13

I.2.- Non-exotic diseases according to Directive 2006/88 EC 14

I.2.1.- Infectious haematopoietic necrosis 14

I.2.2.- Infectious salmon anaemia 16

I.2.3.- Koi herpes virus disease 18

I.2.4.- Viral haemorrhagic septicaemia 20

I.3.- Other important diseases of interest for the AQUAEXCEL Project 22

I.3.1.- Other viral diseases of interest for AQUAEXCEL 22

I.3.1.1.- Nodavirosis 22

I.3.1.2.- Red sea bream iridoviral disease 23

I.3.1.3.- Infectious pancreatic necrosis 24

I.3.1.4.- Spring viraemia of carp 25

I.3.2.- Other bacterial diseases of interest for AQUAEXCEL 26

I.3.2.1. *Vibrio anguillarum* and other vibrios 26

I.3.2.2.- Winter ulcers (*Moritella viscosa*) 27

I.3.2.3.- *Cytophaga* – *Flavobacterium* – *Bacteroides* group 28

I.3.2.4.-*Photobacterium damselae* subsp. *piscicida* (Pasteurellosis) 29

I.3.2.5.- *Aeromonas salmonicida* (Furunculosis) and other *Aeromonas* spp. 30

I.3.2.6.- *Yersinia ruckeri* (Enteric redmouth disease) 31

I.3.2.7.- *Streptococcal* disease 32

I.3.2.8.- *Renibacterium salmoninarum* 33

I.3.2.9.- *Piscirickettsia salmonis* 34

I.3.3.- Parasitic diseases of interest for AQUAEXCEL 35

I.3.3.1.- *Phylum Amoebozoa* 35

I.3.3.2.- *Phylum Parabasalia* 36

I.3.3.3.- *Phylum Euglenozoa* 37

I.3.3.4.- *Phylum Oflagellata* 39

I.3.3.5.- *Phylum Ciliophora* 40

I.3.3.6.- *Phylum Apicomplexa* 43

I.3.3.7.- *Phylum Microspora* 44
I.3.3.8.- *Phylum Myxozoa* 45
I.3.3.9.- *Phylum Plathyelminthes: Monogenea Class* 48
I.3.3.10.- *Phylum Platyhelmintes: Trematoda Class, Digenea Sub-class* 52
I.3.3.11.-*Phylum Platyhelminthes: Cestoidea Class* 53
I.3.3.12.- *Phylum Nematoda* 54
I.3.3.13.-*Phylum Acanthocephala* 55
I.3.3.14.- *Phylum Arthropoda* 56
I.3.4.- Fungal diseases of interest for AQUAEXCEL 58
I.3.4.1.-Saprolegnia 58
I.3.5.-Other diseases of interest for AQUAEXCEL 58
I.3.5.1.- Nephrocalcinosis 58
I.4.- Diagnosis of fish diseases 60

CHAPTER II

II. Transport of fish and/or germplasm in the framework of the AQUAEXCEL Project 72
II.1. Requisites for the import of fish and/or germplasm from third countries to the European Union 72
II.1.1.- List of authorized countries and areas 74
II.1.2.- Animal health control of fish, aquaculture and psiciculture products 74
II.2.- Movement of fish and germplasm between States of the AQUAEXCEL Project 76
II.2.1.- Health status of the Member States 78
II.2.2.- Means of transport 87
II.2.3.- Health Certification during transport 87
II.2.4.- Notification of movement by TRACES 93
II.3- Transport of fish and/or germplasm 94
II.3.1.- General recommendations during transport 94
II.3.2.- Welfare of farmed fishes during transport 95
II.3.3.- Water quality during transport 96
II.3.4.- Responsibilities during transport 96
II.3.5.- Documentation 97
II.4.- Disease prevention and control: general recommendations 97
II.4.1.- Disinfection of installations, equipment, means of transport and eggs 98
II.5.- Handling, elimination and treatment of waste of aquatic animals during transport 101
II.6.- Killing of fish for disease control purposes 102
II.6.1.- Killing by an overdose of an anaesthetic agent 104
II.6.2.- Mechanical killing methods 105

ANNEXES

Annex I.- Protocol of Action in the transport of fish and/or germplasm 106

Annex II.- List of species susceptible to and vectors of different diseases in Directive2006/88 EC and Commission Decision 2010/221 EU requiring health certification 107

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook 108

TABLES

Table 1.- Diseases subject to movement restrictions between States of the AQUAEXCEL Project 10

Table 2.- Diseases subject to national measures according to Commission Decision 2010/221 EU 11

Table 3.- Methods of diagnosis: viral diseases 61

Table 4.- Taking samples for viral diseases 62

Table 5.- Methods of diagnosis: bacterial diseases 63

Table 6.- Taking samples for bacterial diseases 66

Table 7.- Methods of diagnosis: parasitic and fungal diseases 67

Table 8.- Taking samples for parasitic and fungal diseases 71

Table 9.- Vertical transmission of diseases listed in Directive 2006/88 EC (exotic and non-exotic Diseases) and Commission Decision 2010/221 EU (national measures) 77

Table 10.- States declared free from Infectious haematopoietic necrosis 80

Table 11.- States declared free from Viral haemorrhagic septicaemia 81

Table 12.- States, zones and compartments declared free from Infectious salmon anaemia 82

Table 13- States, zones and compartments subject to authorised surveillance programmes 83

Table 14.- Member States, zones and compartments subject to authorised eradication Programmes 84

Table 15.- Health status of States belonging to the AQUAEXCEL Project 85

Table 16.- Outbreaks of disease during 2011 in States belonging to the AQUAEXCEL Project 86

Table 17.- Sanitary certification needs according to disease status 87

FIGURES

Figure 1.- Movement of fishand/or germplasm from a State declared disease-free (Category I) 88

Figure 2 Movement of fish and/or germplasm from a State with a surveillance programme (Category II) 89

Figure 3.- Movement of fish and/or germplasm from a State with undetermined health status (Category III) 90

Figure 4.- Movement of fish and/or germplasm from a State with an eradication programme (Category IV) 91

Figure 5.- Movement of fish and/or germplasm from a State declared infected (Category V) 92

Glossary

AGD: Amoebic Gill Disease

ANIMO: Animal Movement System

AQUAEXCEL: Aquaculture Infrastructures for Excellence in European Fish Research

BKD: bacterial Kidney Disease

DNA: Deoxyribonucleic acid

EC: European commission

EFSA: The European Food Safety Authority

EFTA: European free trade Association

EHN: Epizootic haematopoietic necrosis

EHNV: Epizootic haematopoietic necrosis virus

ELISA: Enzyme-Linked ImmunoSorbent Assay

EU: European Union

EEA: European Economic Area

FIP: Frontier Inspection Posts

IF: Immunofluorescence

IHN: Infectious haematopoietic necrosis

IHNV: Infectious haematopoietic necrosis virus

LMS: List Management System

OIE: World Organisation for Animal Health

PCR: Polymerase chain reaction

PKD Proliferative Kidney Disease

Rcs: Rejected Consignments System

RFLP: restriction fragment length polymorphism

RT-PCR: Reverse transcription polymerase chain reaction

SHIFT: System to assist with the Health controls of Import of items of veterinary concern at Frontier inspection posts from Third countries

SPS: Sanitary and Phytosanitary Measures

SVC: Spring viraemia of carp

TRACES: TRAde Control and Expert System

VHS: Viral haemorrhagic septicaemia

WTO: World Trade Organisation

Summary

The sanitary prescriptions and procedures for transfer, and safety standards in the framework of the AQUAEXCEL Project aims to define the optimum conditions in terms of health and welfare for the transport of fish and/or germplasm by means of regulations for the safe transport between the different research centres of the project members. The sanitary measures featured in this manual are based on the standards officially approved by European Legislation, with specific reference to European Directive 2006/88 EC of 24th October, 2006 and the World Assembly of Delegates of the OIE. This Handbook of Best Practices includes a description of the main diseases that affect the fish species included in the AQUAEXCEL Project and corresponding methods of diagnosis, paying particular attention to the diseases identified by the research centres in the initial survey, as well as criteria to evaluate harmlessness in the transport of fish and germplasm, the welfare of the farmed fish during transport and the disinfection of eggs.

Objectives: The purpose of this manual is to make it easier for the different researchers of the AQUAEXCEL project to draw up sanitary measures applicable to the movement of fish and/or germplasm between the different research centres belonging to the AQUAEXCEL Project. The recommendations put forward in this Handbook of Best Practices take into consideration the fish species to be transported and the health status of the country of origin and the research centre in order to avoid the disease to which they are applied being introduced into the research centre or country to which the fish and/or germplasm are transported. This means that the recommendations, if correctly applied, confers an optimum level of sanitary security to the transport of fish or germplasm between the different research centres participating in the AQUAEXCEL Project. The recommendations of this manual refer only to the sanitary conditions that should be met by the participating research centre and are governed by the principle that the disease is not present in the research centre to which the animals are transported or that the centre is subject to control or eradication programmes. A research centre can authorize the transport of fish or germplasm in conditions that are either more or less strict than those recommended in this manual, but it must base its decision on a scientific risk analysis and abide by the obligations imposed by the provisions of the WTO SPS Agreement.

Teams involved: The team involved in the development on this deliverable has been the University of Las Palmas de Gran Canaria (ULPGC).

Geographical areas covered: The geographical areas covered has been all Europe.

Introduction

The guide for "sanitary prescriptions and procedures for transfer, and safety standards" is redacted as a Handbook of Best Practices for the Transport of Fish and Germplasm, in the framework of the AQUAEXCEL Project, and aims to define the optimum conditions in terms of health and welfare for the transport of fish and/or germplasm by means of regulations for the safe transport between the different research centres of the project members. The sanitary measures featured in this Handbook of Best Practices are based on the standards officially approved by European Legislation, with specific reference to European Directive 2006/88 EC of 24th October, 2006 and the World Assembly of Delegates of the OIE. This Handbook of Best Practices includes a description of the main diseases that affect the fish species included in the AQUAEXCEL Project and corresponding methods of diagnosis, paying particular attention to the diseases identified by the research centres in the initial survey, as well as criteria to evaluate harmlessness in the transport of fish and germplasm, the welfare of the farmed fish during transport and the disinfection of eggs.

The purpose of this "Handbook of Best Practices for the Transport of Fish and Germplasm" is to make it easier for the different researchers of the AQUAEXCEL project to draw up sanitary measures applicable to the movement of fish and/or germplasm between the different research centres belonging to the AQUAEXCEL Project. The recommendations put forward in this Handbook of Best Practices take into consideration the fish species to be transported and the health status of the country of origin and the research centre in order to avoid the disease to which they are applied being introduced into the research centre or country to which the fish and/or germplasm are transported. This means that the recommendations, if correctly applied, confers an optimum level of sanitary security to the transport of fish or germplasm between the different research centres participating in the AQUAEXCEL Project. The recommendations of the Handbook of Best Practices for the Transport of Fish and Germplasm refer only to the sanitary conditions that should be met by the participating research centre and are governed by the principle that the disease is not present in the research centre to which the animals are transported or that the centre is subject to control or eradication programmes. A research centre can authorize the transport of fish or germplasm in conditions that are either more or less strict than those recommended in the Handbook of Best Practices, but it must base its decision on a scientific risk analysis and abide by the obligations imposed by the provisions of the WTO SPS Agreement.

However, three basic recommendations that may have limited the effectiveness of the drawing up of this Handbook of Best Practices should be born in mind:

1) That not all those research centres participating in the AQUAEXCEL Project have given the same amount of detail regarding the diseases they habitually come across and may represent a risk on exchange. More details are being collected after the writing of this handbook, however this will not change the general rules set up in the present document, which remains fully valid in its principles.

2) That the sanitary status may change in time for different reasons.

3) That although the specific species detailed on this Handbook are those included in the Project AQUAEXCEL, the conditions set out here are not exclusive to only these species and, therefore, can be

applied to any other species of interest in the AQUAEXCEL Project.

These three considerations have been taken into account in the drawing up of this Handbook of Best Practices. At the same time, the OIE provides data to the Veterinary Authorities or any Authority Responsible regarding the worldwide animal health status. Thanks to these data, the importer can find out about the sanitary situation, frequency of diseases and control programmes of the exporter, regardless of what the research centres participating in the AQUAEXCEL Project have declared. If the importer considers the data declared by the research centre from which the fish and/or germplasm originate to be insufficient, he should contact the exporting country directly through the OIE's Central Office in order to obtain complementary data.

For all purposes, Norway follows the same rules that the rest of member states of the European Union. The European Economic Area (EEA) unites the 27 EU Member States and the three EEA EFTA States (Iceland, Liechtenstein, and Norway) into an Internal Market governed by the same basic rules. These rules aim to enable goods, services, capital, and persons to move freely about the EEA in an open and competitive environment, a concept referred to as the four freedoms.

Chapter I

I.- Fish diseases: diagnosis and surveillance

European Directive 2006/88EC classifies notifiable diseases that should be monitored and subject to restrictions during the transport of fish and/or germplasm, into two different groups: exotic diseases and non-exotic diseases. Table 1 shows diseases subject to movement restrictions listed in Directive 2006/88 EC. In the group of exotic diseases, we can find a viral disease, epizootic haematopoietic necrosis as well as epizootic ulcerative syndrome caused by the fungus *Aphanomyces invadans.* To be considered exotic, a disease must comply with the criteria established in point 1, and in point 2 or 3.

1) The disease is exotic in the EU, *i.e*. it is not established in the European Union aquaculture, and it is not known whether or not the pathogen is present in EU waters.

2) If it is introduced into the EU, it may have important economic repercussions in the EU, either because of production losses in the aquaculture sector, or because the commercial potential of aquaculture animals and product may suffer.

3) If it is introduced in the EU, it may have a negative environmental impact on populations of wild aquatic animal species, which constitute an asset that is worth protecting by means of provisions of EU or international Law.

In the group of non-exotic diseases, according to European Directive 2006/88 EC, we can find the following viral diseases: infectious haematopoietic necrosis, infectious salmon anaemia, Koi herpes virus disease and viral haemorrhagic septicaemia. In order to be considered non-exotic, a disease must comply with the critera set out in points 1, 4, 5, 6, 7, and 2 or 3.

1) Various Member States or regions of various Member States are free of the specific disease.

2) The introduction into a Member State that is free of it may cause important economic repercussions, either due to production losses and annual disease-related and disease control-related costs that can rise above 5% of the production value of the aquaculture species susceptible in the region, or by limiting the opportunities for international trade of aquaculture animals and products.

3) If the disease is introduced into a Member State free of it, it has been demonstrated that, when it appears, it has a negative environmental impact for the populations of wild aquatic animals, which constitute an asset that is worth protecting by means of provisions of EU or international Law.

4) The disease is difficult to control and confine in the area of the farms or breeding areas of molluscs if strict control measures and trade restrictions are not adopted.

5) The disease may be controlled at Member State level and experience has shown that disease-free zones and compartments can be established, and that this type of maintenance offers good value for money.

6) During the placement in the market of aquaculture animals, there is a high risk that the disease become established in an area that was not previously infected.

7) Reliable, simple tests are available for infected aquatic animals. Said tests must be specific and sensitive, and the test method must be harmonized across the whole of the European Union.

Table 1.- Diseases subject to movement restrictions between States of the AQUAEXCEL Project

Exotic diseases (Directive 2006/88 EC)	epizootic ulcerative syndrome
	epizootic haematopoietic necrosis
Non exotic diseases (Directive 2006/88 EC)	Viral haemorrhagic septicaemia
	Infectious salmon anaemia
	Koi herpes virus disease
	Infectious haematopoietic necrosis

Apart from these diseases (exotic and non-exotic) described in Directive 2006/88 EC, article 43 of the Directive sets out national measures for some diseases in order to limit their impact on aquaculture and wild aquatic animals. Thus, according to Commission Decision 2010/221 EU, Denmark, Finland, Sweden, Ireland and parts of the United Kingdom (Northern Ireland, Isle of Man, Jersey and Guernsey) have national measures for Spring viraemia of carp, Ireland, Northern Ireland, Isle of Man and Jersey for Bacterial Kidney Disease, Finland, Sweden and Isle of Man for Infectious pancreatic necrosis, and Finland (water catchment areas of the Tenojoki and the Näätämönjoki; the water catchment areas of the Paatsjoki, Luttojoki and Uutuanjoki), Ireland and parts of The United Kingdom (Territories of Great Britain, Northern Ireland, Isle of Man, Jersey and Guernsey) for *Gyrodactylus salaris*, so movement of fish and/or germplasm to these areas are subject to health certificates where susceptible and/or vector species are concerned. Table 2 shows diseases subject to national measures listed in Commission Decision 2010/221 EU.

Table 2.- Diseases subject to national measures according to Commission Decision 2010/221 EU

Disease	Member State	Geographical demarcation of the area with approved national measures
Spring viraemia of carp	Denmark	Whole territory
	Ireland	Whole territory
	Finland	Whole territory
	Sweden	Whole territory
	United Kingdom	The whole territory of the United Kingdom; the territories of Guernsey, Jersey and the Isle of Man
Renibacterium salmoninarum	Ireland	Whole territory
	United Kingdom	The territory of Northern Ireland; the territories of Jersey and the Isle of Man
Infectious pancreatic necrosis	Finland	The continental parts of the territory
	Sweden	The continental parts of the territory
	United Kingdom	The territory of the Isle of Man
Gyrodactylus salaris	Ireland	The whole territory
	Finland	The water catchment areas of the Tenojoki and Näätämönjoki; the water catchment areas of the Paatsjoki, Luttojoki, and Uutuanjoki are considered as buffer zones
	United Kingdom	The whole territory of the United Kingdom; the territories of Guernsey, Jersey and the Isle of Man

We will now go on to describe the diseases present on these two lists of European Directive 2006/88 EC, those of Commission Decision 2010/221 EU subject to national measures, as well as other fish diseases that the different research centres participating in the AQUAEXCEL Project have requested be included based on their experience and interest shown in the initial questionnaire. Moreover, the legislation in force allows for the surveillance of diseases that do not figure on the official lists, such as emerging diseases with the potential to cause significant mortality. In the text, we will mark in red those susceptible or vector fish species which are part of the fish species we will be working with in the AQUAEXCEL Project.

I.1.- Exotic diseases according to Directive 2006/88 EC

I.1.1.- Epizootic haematopoietic necrosis

Definition: A clinical or sub-clinical systemic fish infection produced by the epizootic haematopoietic necrosis virus (EHNV). It is an iridovirus with unwound double spiral DNA. The virus shares an antigen with the iridovirus that infect *Silurus glanis* and *Ictalurus melas* in Europe, and with the amphibious iridovirus of North America (Frog virus 3) and Australia (Bohle iridovirus). Fish are susceptible to this virus at any age. The clinical symptoms are usually more evident in fish of under a year and in juvenile *Oncorhynchus mykiss* and *Perca fluvialis.* Many other species are experimentally prone to the EHNV. The virus spreads rapidly in water, but infection can be transmitted in aquaculture establishments as a result of fish movements. As the virus is very resistant, it can spread by fishing gear and other inanimate objects, as well as by means of fish-eating birds. The target organs and infected tissues are the liver, kidney, spleen and other parenchymal tissue. In *Oncorhynchus mykiss*, the high mortality of cases and the low prevalence of infection by this virus implies that the rate of incorporation of vectors is probably very low (< 2%). *Perca fluvialis* is very susceptible to this virus and it is unlikely to constitute a reservoir as an appropriate host. The virus reproduces at a very high percentage in infected fish and is propagated through bodily fluids and fish corpses as they decompose in the water

Susceptible species: *Oncorhynchus mykiss and Perca fluvialis*

Vector species: *Cyprinus carpio, Aristichthys nobilis*, *Carassius auratus*, *Carassius carassius*, *Hypophthalmichthys molitrix*, *Leuciscus* spp, *Rutilus rutilus*, *Scardinius erythrophthalmus* and *Tinca tinca*.

Geographic distribution: Australia and Namibia, although there may be other areas where the presence of the disease is not well documented.

N.B.: As this disease is listed under Directive 2006/88 EC as an exotic disease, when fish and/or germplasm are imported from a non-EU country, it will be necessary to request an international health certificate issued by the authority responsible for the centre from which the fish and/or germplasm originate, or an official certificate approved by a centre importing said animals that attests the fact that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from epizootic haematopoietic necrosis. The importation of fish and/or germplasm from a centre or country declared NOT free from epizootic haematopoietic necrosis is forbidden, unless exceptional authorization is given further to justification of import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.
- Treatment of all effluent and waste material in such a way as to guarantee that the epizootic haematopoietic necrosis virus is deactivated.

I.1.2.- Epizootic ulcerative syndrome

Definition: Epizootic ulcerative syndrome is an infection produced by an Oomycete known as *Aphanomyces invadans* or *A. piscicida*. It is an epizootic condition of wild, river- or estuary-farmed fish. The disease affects a very wide range of fresh- and saltwater fish in Asia, Australia, the USA and the African continent. The fact that it is so widespread is due to the migration and transfer of species such as mullet or commercial movements of ornamental fish. Although the infection has not yet been described in Europe, many European species are susceptible to the disease (salmonids, mullet and eels). However, it may be that environmental conditions in Europe do not favour its propagation, although the possibility that climate change plays a fundamental role in the factors required for it to appear, such as the increase in water temperature and the frequency of drought periods followed by copious rain, cannot be ruled out. Mortality is very variable but could reach pandemic levels and the disease is contagious, although these factors depend largely on both environmental factors and stress levels.

Susceptible species: *Acantopagrus australis*, *Anabas testudineus*, *Fluta alba*, *Ictaluridae*, *Bagridae*, *Bidyanus bidyanus*, *Brevoortia tyrannus*, *Catla catla*, *Channa striatus*, *Cirrhinus mrigala*, *Clarius batrachus*, *Esomus* sp., *Glossogobius giuris*, *Oxyeleotris marmoratus*, *Glossogobius* sp., *Labeo rohita*, *Lates calcarifer*, *Mugil cephalus*, *Mugilidae*, *Plecoglossus altivelis*, *Puntius sophore*, *Sillago ciliata*, *Siluridae*, *Trichogaster pectoralis*, *Colisa lalia*, *Osphonemus goramy*, *Trichogaster trichopterus*, *Puntius gonionotus*, *Scatophagus argus*, *Platycephalus fuscus*, *Psettodes sp*., *Rohtee sp*., *Terapon sp*. and *Toxotes chatareus*, *Carassius auratus.*

Vector species: *Cyprinus carpio, Aristichthys nobilis*, *Carassius auratus*, *Carassius carassius*, *Hypophthalmichthys molitrix*, *Leuciscus* spp, *Rutilus rutilus*, *Scardinius erythrophthalmus* and *Tinca tinca*.

Geographic distribution: Australia, Botswana, Namibia, South Africa, Bangladesh, Bhutan, Cambodia, USA, Filipinas, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Singapore, Sri Lanka, Thailand and Vietnam, although there may be other areas where its presence is not well-documented.

N.B.: As this disease is listed under Directive 2006/88 EC as an exotic disease, when fish and/or germplasm are imported from a non-EU country, it will be necessary to request an international health certificate issued by the authority responsible for the centre from which the fish and/or germplasm originate, or an official certificate approved by a centre importing said animals that attests the fact that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from epizootic ulcerative syndrome. The import of fish and/or germplasm from a centre or country declared NOT free from epizootic ulcerative syndrome is forbidden, unless exceptional authorization is given further to justification of import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.
- Treatment of all effluent and waste material in such a way as to guarantee that *Aphanomyces Invadans* is deactivated.

I.2.- Non-exotic diseases according to Directive 2006/88 EC

I.2.1.- Infectious haematopoietic necrosis

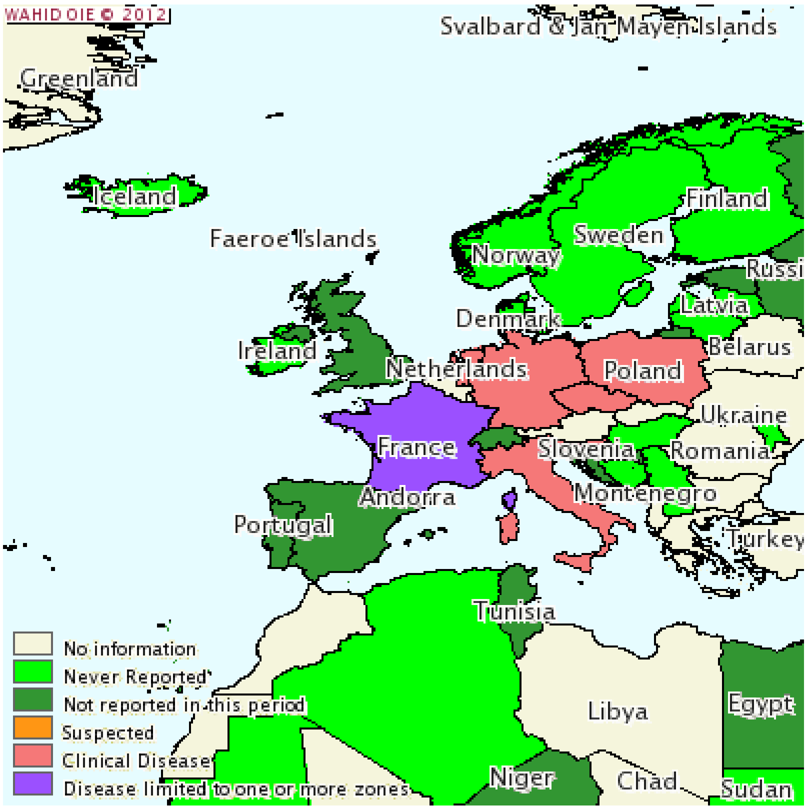
Definition: Infectious haematopoietic necrosis (IHN) is a viral disease that affects various salmonid species. The most important clinical and economic consequences of IHN are produced in nurseries of *Oncorhynchus mykiss* fry or juveniles in fresh water where acute outbreaks can lead to high mortality. However, both *Oncorhynchus tshawytscha* and *Salmo salar* farmed in fresh or salt water can be seriously affected.

The disease, caused by a rhabdovirus, the infectious haematopoietic necrosis virus (IHNV), is characterised by striking symptoms such as lethargy interspersed with outbreaks of frenetic, abnormal behaviour, a darkening of the skin, pale gills, ascites, a swollen abdomen, exophthalmia, and internal and external petechial haemorrhages. Internally, the fish appears anaemic and there is a lack of food in the intestine. The liver, kidney and spleen are pale. Histopathological findings reveal necrosis in the tissue of the kidney, spleen, liver, pancreas and digestive tract. The blood of affected fry shows a reduced hematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large quantities of cellular remains.

Susceptible species: *Oncorhynchus mykiss*, *Salmo salar, Salmo trutta*, *Oncorhynchus tshawytscha, Oncorhynchus nerka, Oncorhynchus keta, Oncorhynchus masou, Oncorhynchus rhodurus, Esox luciusand and Oncorhynchus kisutch.*

Vector species: *Cyprinus carpio*, *Gadus morhua*, *Hippoglossus hippoglossus*, *Huso huso*, *Acipenser gueldenstaedtii*, *Acipenser ruthenus*, *Acipenser stellatus*, *Acipenser sturio*, *Acipenser Baerii*, *Aristichthys nobilis*, *Carassius auratus*, *Carassius carassius*, *Hypophthalmichthys molitrix*, *Leuciscus* spp, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Clarias gariepinus*, *Ictalurus* spp., *Ameiurus melas*, *Ictalurus punctatus*, *Pangasius pangasius*, *Sander lucioperca*, *Silurus glanis*, *Platichthys flesus*, and *Melanogrammus aeglefinus*.

Geographic distribution: USA, Asia and Europe, except Denmark, Ireland, Cyprus, Finland, Norway, Sweden and United Kingdom, although there may be other areas where its presence is not well-documented. The following map shows the notifications of outbreaks of the disease during the last final months of 2011 in Europe (OIE, 2012).



N.B.: As this disease is listed in Directive 2006/88 EC as a non-exotic disease, when fish and/or germplasm are imported between European Union countries, a Sanitary Certificate is needed in the following cases:

- When the fish and/or germplasm are going to a country declared free of this disease or that has surveillance or eradication programmes.
- When the fish and/or germplasm originate from areas with an eradication programme or that are infected. In this case, the transport of fish and/or germplasm is only authorised to a destination with the same health status, i.e., that also has eradication programmes or is infected.

In the case of germplasm, it must be disinfected before import, in accordance with the methods described in this Handbook of Best Practices which is based on the norms established in the OIE's Aquatic Manual or those specified by the Authority Responsible in the importing country. Between disinfection and import, germplasm must not come into contact with anything that might affect their health status. The import of fish and/or germplasm to a country declared disease free from one that is NOT free from infectious haematopoietic necrosis is forbidden, unless exceptional authorization is given further to justification of their import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.
- Treatment of all effluent and waste material in such a way as to guarantee that the infectious haematopoietic necrosis virus is inactivated.

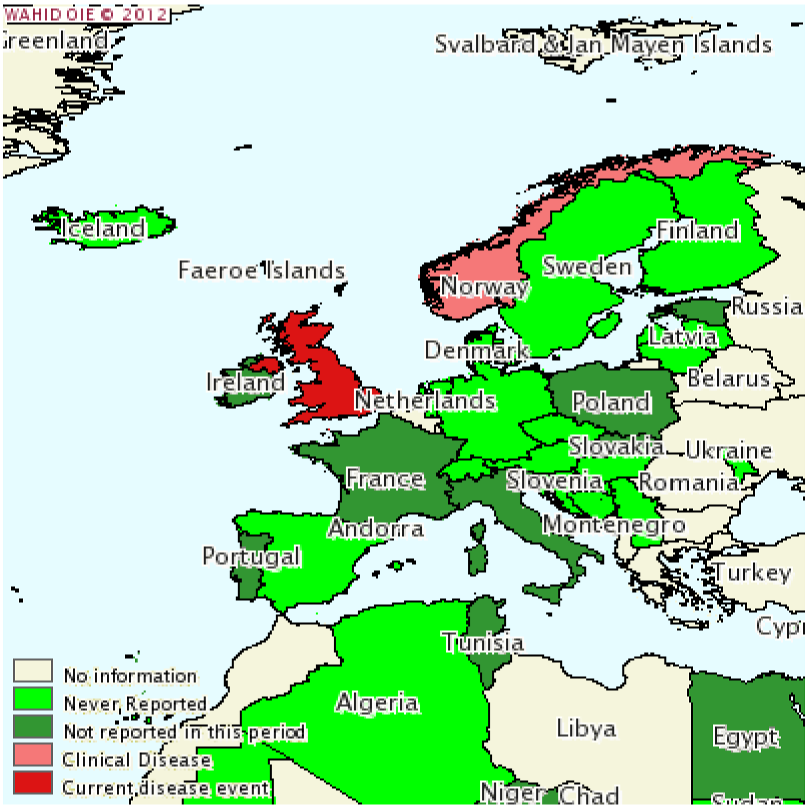
When fish and/or germplasm are imported from a non-EU country, an international health certificate issued by the authority responsible in the centre from which the fish and/or germplasm originate is required, or an official certificate approved by the importing centre that attests that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from Infectious haematopoietic necrosis

I.2.2.- Infectious salmon anaemia

Definition: Infectious salmon anaemia is a disease that affects *Salmo salar* caused by an orthomyxovirus, the infectious salmon anaemia virus. Infectious salmon anaemia basically affects fish maintained in or exposed to seawater. However, outbreaks have also been described in fish kept in fresh water. The disease may appear as systemic and lethal, characterised by serious anaemia and haemorrhages in various organs. The most striking symptoms that can be observed are pale gills, exophthalmos, a swollen abdomen and petechia in the eye chamber and abdomen, as well as oedemas on the scales. The main findings in a post-mortem examination are circulation anomalies in various organs due to endothelial damage in the periphery blood vessels. Clinical signs affect four organs: the liver, kidney, intestine and gills. Hepatic manifestation is characterised by a dark liver caused by haemorrhagic necrosis. The kidney shows moderate inflammation with interstitial bleeding and tubular necrosis. The intestine is dark red due to haemorrhages within the intestinal wall, not in the lumen. Apart from being pale, the gills sometimes reveal an accumulation of blood, above all in the central venous sinus of gill filaments. In some outbreaks, one of the manifestations in these organs may be predominant, while in other outbreaks, all four may be found. Outbreaks in which manifestations in the liver or kidney dominate appear to be the most common. A hematocrit of <10% can be seen in the final stages of the disease. Initially, daily mortality can vary between 0.5 and 1%, but it may rise over time, whereas the accumulated mortality varies between moderate and high, and may stand at over 90% in serious cases.

Susceptible species: *Salmo salar*, but they have been identified by RT-PCR in *Salmo trutta* and *Onchorynchus mykiss* with sub-clinical infection. The infectious salmon anaemia virus has also been detected in two marine species, *Pollachius virens* and *Gadus morhua*, but only in fish captured near to cages with *Salmo salar* which had this disease. Experimental infection has shown the replication of this virus in *Salvelinus alpinus* and *Clupea harengus*.

Geographic distribution: Norway, United Kingdom, Northern Ireland, Canada, Faeroe Islands, USA and Chile, although there may be other areas where its presence is not well-documented. The following map shows the notifications of outbreaks of the disease during the final months of 2011 in Europe (OIE, 2012).



N.B.: As this disease is listed in Directive 2006/88 EC as a non-exotic disease, when fish and/or germplasm are imported between European Union countries, a Sanitary Certificate is needed in the following cases:

- When the fish and/or germplasm are going to a country declared free from this disease or that has surveillance or eradication programmes.
- When the fish and/or germplasm originate from areas with an eradication programme or that are infected. In this case, the transport of fish and/or germplasm is only authorised to a destination with the same health status, i.e., that also has eradication programmes or is infected.

In the case of germplasm, it must be disinfected before import, in accordance with the methods described in this Handbook of Best Practices which is based on the norms established in the OIE's Aquatic Manual or those specified by the Authority Responsible in the importing country. Between disinfection and import, germplasm must not come into contact with anything that might affect their health status. The import of fish and/or germplasm to a country declared disease free from one that is NOT free from Infectious salmon anaemia is forbidden, unless exceptional authorization is given further to justification of its import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.
- Treatment of all effluent and waste material in such a way as to guarantee that the Infectious salmon anaemia virus is inactivated.

When fish and/or germplasm are imported from a non-EU country, an international health certificate issued by the authority responsible in the centre from which the fish and/or germplasm originate is required, or an official certificate approved by the importing centre that attests that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from Infectious salmon anaemia.

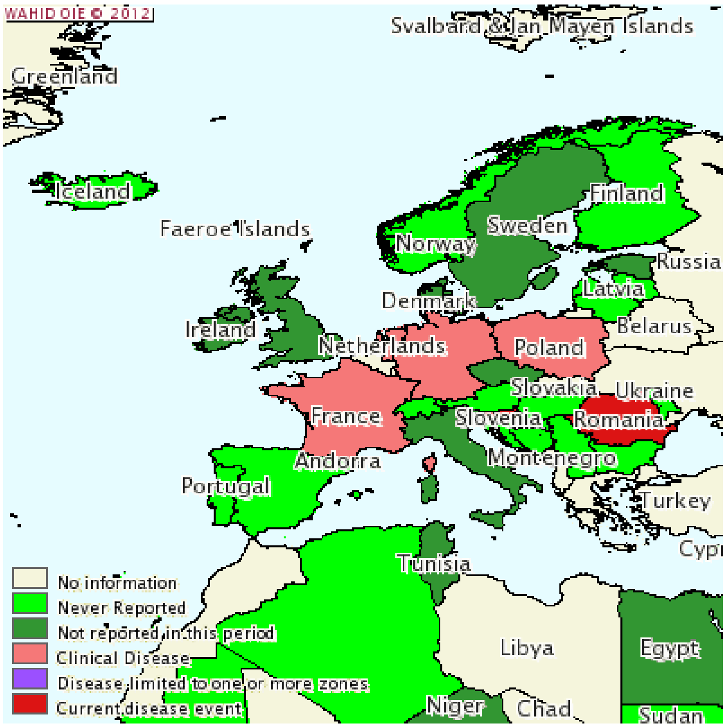
I.2.3.- Koi herpes virus disease

Definition: Natural infections caused by this virus have only been recorded in *Cyprinus carpio carpio*, *Cyprinus carpio goi* and in hybrids of these varieties. Fish are susceptible at any age, but fry and juveniles are much more so than older fish. The morbidity of affected populations can reach 100%, and mortality, 70%-80%, or even 90% or 100%. Carp are often farmed in mixed farms together with other fish species, but no signs of disease have been observed in these species during outbreaks of this pathogen in normal mixed farming conditions. The disease is temperature-dependent, occurring between 16-25ºC. In experimental conditions, the disease causes a high mortality at 28ºC but not at 29ºC or 30ºC or at 13ºC. However, viral DNA was detected in fish by means of PCR at 13ºC, and infected fish surviving at low temperatures may constitute reservoirs for the virus. The Disease may develop fast and signs may appear 3 days after infection, but it is more usual for -21 days to go past before the disease is observed. The virus is disseminated by the faeces, urine, gills and the skin mucus. However, the gills, kidney and spleen are the organs with the highest level of the virus during the course of the infection. Virus transmission is horizontal but egg-related transmission cannot be ruled out. Horizontal transmission may be direct (from fish to fish) or vectorial, with water being the major abiotic vector. However, animate vectors (e.g. invertebrate parasites and fish-eating birds and mammals) and fomites can also be involved in transmission. During the outbreak of the disease, a notable increase in the population's mortality will take place. The fish become lethargic, break off from the shoal, swim to the water inlet or the pond edges and open their mouths at the water's surface. Some fish may lose their balance and become disoriented, but they may also show signs of hyperactivity. On close examination, the clinical signs observed are loss of colour a pale or reddened skin, which may appear rough, localised or total loss of the epidermis and over- or under-production of mucus in the skin and gills. Other macroscopic injuries are anophthalmia (sunken eyes), haemorrhaging on the skin and at the base of the fins, and erosion of the fins. The histopathology of the disease may be unspecific and variable, but the inflammation and necrosis of gill tissue are always observed. The gills also present hyperplasia and hypertrophy of the gill epithelium and the fusion of the secondary lamellae and sticking together of gill filaments can be observed. Necrosis can be seen, ranging from small areas of necrotic epithelial cells of the secondary lamellae to the complete disappearance of the lamellae. The epithelial cells and leucocytes of the gills may present a notable swelling of the nucleus, together with a chromatin margination in the shape of a "seal ring", and pale, diffuse eosinophilic intranuclear inclusions have been observed. Inflammation, necrosis and nuclear inclusions (either separately or together) have been observed in other organs, above all in the kidneys, but also in the spleen, pancreas, liver, brain, intestine and oral epithelium.

Susceptible species: *Cyprinus carpio carpio*, *Cyprinus carpio goi*, *Cyprinus carpio koi* and hybrid species of *Cyprinus carpio*.

Vector species: Not described

Geographic distribution: USA, Germany, Austria, Rumania, Belgium, Denmark, Poland, France, Holland, Israel, United Kingdom, Switzerland, Japan and Taiwan, although there may be other areas where its presence is not well-documented. The following map shows the notifications of outbreaks of the disease during the last final months of 2011 in Europe (OIE, 2012).



N.B.: As this disease is listed in Directive 2006/88 EC as a non-exotic disease, when fish and/or germplasm are imported between European Union countries, a Sanitary Certificate is needed in the following cases:

- When the fish and/or germplasm are going to a country declared free from this disease or that has surveillance or eradication programmes.
- When the fish and/or germplasm originate from areas with an eradication programme or that are infected. In this case, the transport of fish and/or germplasm is only authorised to a destination with the same health status, i.e., that also has eradication programmes or is infected.

In the case of germplasm, it must be disinfected before import, in accordance with the methods described in this Handbook of Best Practices which is based on the norms established in the OIE's Aquatic Manual or those specified by the Authority Responsible in the importing country. Between disinfection and import, germplasm must not come into contact with anything that might affect its health status. The import of fish and/or germplasm from a centre or country declared NOT free from Koi herpes virus disease is forbidden, unless exceptional authorization is given further to justification of import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.
- Treatment of all effluent and waste material in such a way as to guarantee that the epizootic Koi herpes virus is inactivated.

When fish and/or germplasm are imported from a non- EU country, it will be necessary to request an international health certificate issued by a responsible authority from the centre from which the fish and/or germplasm originate, or an official certificate approved by the importing centre that attests that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment declared free from Koi herpes virus disease. When fish and/or germplasm are imported from a non-EU country, an international health certificate issued by the authority responsible in the centre from which the fish and/or germplasm originate is required, or an official certificate approved by the importing centre that attests that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from Koi herpes virus disease.

I.2.4.- Viral haemorrhagic septicaemia

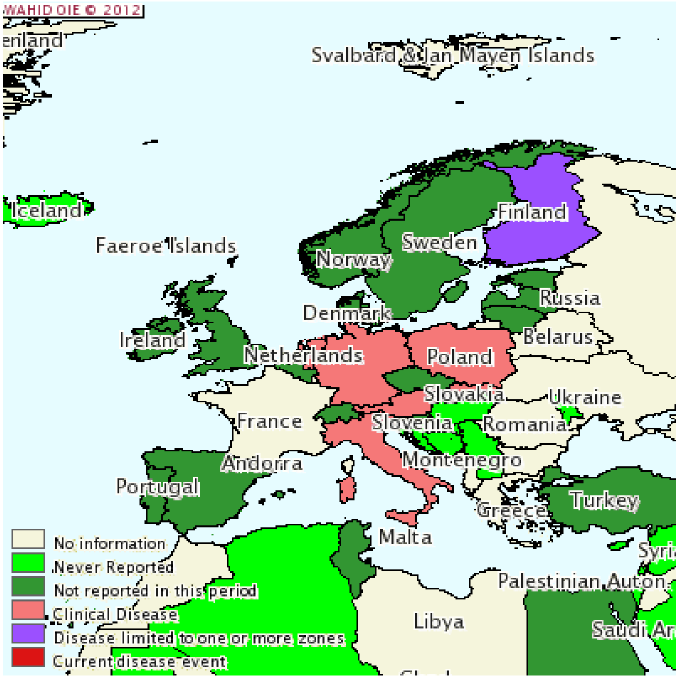
Definition: Viral haemorrhagic septicaemia (VHS) is an infectious disease caused by the viral haemorrhagic septicaemia (a synonym for the Egtved virus), belonging to the *Novirhabdovirus* genus of the *Rhabdoviridae* family. The acute phase of the disease occurs during the early stages of infection (up to 30 days after infection), a period during which diseased fish show clear clinical symptoms: a rapid onset of mortality (that may reach 100% in fry), lethargy, a darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins, the gills, eyes and skin, erratic intermittent swimming or swimming around in circles, a swollen abdomen due to an oedema in la peritoneal cavity. Moreover, after the acute phase, the disease may occur in a chronic subclinical form, during which the affected fish shows no external signs of disease. Due to the tropism of the virus by the brain, VHS may appear in the nerves, characterised by seriously abnormal or intermittent swimming or swimming round in circles.

Susceptible species: *Gadus morhua*, *Oncorhynchus mykiss*, *Scophthalmus maximus,* *Clupea* spp., *Coregonus* sp., *Esox lucius*, *Gadus aeglefinus*, *Gadus macrocephalus*, *Oncorhynchus* spp., *Onos mustelus*, *Sprattus sprattus*, *Thymallus thymallus* and *Salmo trutta*

Vecor species: *Sparus aurata, Dicentrarchus labrax, Solea senegalensis*, *Cyprinus carpio*, *Argyrosomus regius*, *Thunnus thynnus,* *Aristichthys nobilis*, *Carassius auratus*, *Carassius carassius*, *Hypophthalmichthys molitrix*, *Leuciscus* spp, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Clarias gariepinus*, *Esox lucius*, *Ictalurus* spp., *Ameiurus melas*, *Ictalurus punctatus*, *Pangasius pangasius*, *Sander lucioperca*, *Silurus glanis*, *Morone chrysops x M. saxatilis*, *Mugil cephalus*, *Sciaenops ocellatus*, *Umbrina cirrosa*, *Thunnus* spp., *Epinephelus marginatus*, *Solea solea*, *Pagellus erythrinus*, *Dentex dentex*, *Diplodus sargus*, *Pagellus bogaraveo*,

Pagrus major, *Diplodus puntazzo*, *Diplodus vulgaris*, *Pagrus pagrus* and *Oreochromis*.

Geographic distribution: Japan, USA, United Kingdom, Italy, Holland, Germany, Poland, Finland, Baltic Sea and the Skagerrak and Kattegat straights, although there may be other areas where its presence is not well-documented. The following map shows the notifications of outbreaks of the disease during the last final months of 2011 in Europe (OIE, 2012).



N.B.: As it is classified in Directive 2006/88 EC as a non-exotic disease, when fish and/or germplasm are imported between EU countries, health certificates will be necessary in the following cases:

- When the fish and/or germplasm are travelling to a country declared free of the disease or that have surveillance or eradication programmes.
- When the fish and/or germplasm come from zones which either have eradication programmes or are infected. In this case, the transport of fish and/or germplasm is only authorised to a destination with the same health status, i.e., that also have either eradication programmes or are infected.

In the case of germplasm, it should be disinfected before import, in accordance with the methods described in this Handbook of Best Practices which is based on the norms established in the OIE's Aquatic Manual or those specified by the Authority Responsible in the importing country. Between disinfection and import, germplasm must not come into contact with anything that might affect its health status. The import of fish and/or germplasm to a country declared disease free from one that is NOT free from viral haemorrhagic septicaemia is forbidden, unless exceptional authorization is given further to justification of import, a risk assessment and provided a set of measures are taken to reduce the risk, such as:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.

- Treatment of all effluent and waste material in such a way as to guarantee that viral haemorrhagic septicaemia virus is inactivated

When fish and/or germplasm are imported from a non-EU country, an international health certificate issued by the authority responsible for the centre from which the fish and/or germplasm originate is required, or an official certificate approved by the importing centre that attests that the place of origin of the fish and/or germplasm belongs to a country, zone or compartment that has been declared free from the viral haemorrhagic septicaemia virus.

I.3.- Other important diseases of interest for the AQUAEXCEL Project

I.3.1.- Other viral diseases of interest for AQUAEXCEL

I.3.1.1.- Nodavirosis

Definition: This is an infectious, very contagious disease produced by a *Nodavirus*, that selectively affects some seawater fish species with the appearance of a process, which is generally fatal, characterized by a lack of locomotor coordination and a heavily swollen abdomen due to hyperinflation of the swimbladder. The disease is characterized by the appearance of neurological alterations, such as erratic swimming (round in circles, in ever-decreasing circles or belly-up, as if resting), as a consequence of the vacuolization of central nervous tissue and retina. In general, younger fish suffer more serious injuries than adult fish. The virus general multiplies in the digestive tissue and gonads of healthy fish and, in these fish, the diseases remains symptom-free. Stress is what makes the virus multiply also in the nervous tissue (brain, spinal cord and retina), turning the animal into a diseased fish, the seriousness of which will basically depend on the temperature of the fish, as this is the main environmental factor that governs the level of fish deaths produced. Thus, in sea bass, it has been proven that temperatures of 25ºC cause an acute onset of the disease with a death rate of 60% while at 20ºC, a sub-acute picture of some 15% of losses is observed.

Susceptible species: *Dicentrarchus labrax, Scophthalmus maximus*, *Solea senegalensis, Hippoglossus hippoglossus, Lates calcarifer, Epinephelus akaara, Epinephelus fuscogutatus, Epinephelus malabaricus, Epinephelus moara, Epinephelus septemfasciatus, Epinephelus tauvina, Epinephelus coioides, Cromileptes altivelis, Pseudocaranx dentex, Oplegnathus fasciatus, Takifugu rubripes, Verasper moseri, Paralichthys olivaceus* and less frequently, other species not specified.

Vector species: *Sparus aurata*

Geographic distribution: Asia, Australia, Canada, China, Korea, USA, Spain, France, Greece, Italy, Malta, Norway, Portugal, United Kingdom, the Philippines, Hong Kong, Indonesia, Japan, Malaysia, Martinique,

Singapore, Tahiti, Thailand, Taiwan and Tunisia, although there may be other areas where its presence is not well-documented.

N.B.: As this disease is not listed in Directive 2006/88 EC, no measures are established for the movement of fish susceptible to this disease. However, given the seriousness of the process in marine aquaculture, it is recommended that diagnostic studies be carried out to check that the virus is not present in fish and/or germplasm to be transported belonging to the following species: *Dicentrarchus labrax, Scophthalmus maximus, Solea senegalensis, Hippoglossus hippoglossus,* and *Sparus aurata,* as a vector species.

I.3.1.2.- Red sea bream iridoviral disease

Definition: Infection caused by the iridovirus of the red sea bream (*Pagrus major*) and another 30 more farmed species belonging basically to the Perciformes and Pleuronectiformes orders. Affected fish become lethargic, show serious anaemia, petechia in the gills, and a swollen spleen. The disease is characterised by the appearance of enlarged cells that are heavily stained with Giemsa solution in microscopic observations of tissue taken from the spleen, heart, kidney, intestine and gills of infected fish. The distribution of the disease is not restricted to Japan, and it is widespread throughout countries in the east and southeast of Asia.

Susceptible species: *Thunnus thynnus, Pagrus major*, *Acanthopagrus schlegeli*, *Acanthopagrus latus*, *Evynnis japonica*, *Seriola quinqueradiata*, *Seriola dumerili*, *Seriola lalandi*, *Seriola lalandi* × *Seriola quinqueradiata*, *Pseudocaranx dentex*, *Scomberomorus niphonius*, *Scomber japonicus*, *Trachurus japonicus*, *Oplegnathus fasciatus*, *Oplegnathus punctatus*, *Rachycentron canadum*, *Trachinotus blochii*, *Parapristipoma trilineatum*, *Plectorhynchus cinctus*, *Lethrinus haematopterus*, *Lethrinus nebulosus*, *Girella punctata*, *Sebastes schlegeli*, *Pseudosciaena crocea*, *Epinephelus akaara*, *Epinephelus septemfasciatus*, *Epinephelus malabaricus*, *Epinephelus bruneus*, *Epinephelus coioides*, *Epinephelus awoara*, *Epinephelus tauvina*, *Epinephelus fuscoguttatus*, *Epinephelus lanceolatus*, *Lateolabrax japonicas*, *Lateolabrax* sp., *Lates calcarifer*, *Morone saxatilis* × *Morone chrysops*, *Micropterus salmoides*, *Paralichthys olivaceus*, *Verasper variegatus*, *Takifugu rubripes* and, less frequently, other unspecified species.

Vector species: Not described

Geographic distribution: Japan, eastern and south-western countries in Asia such as China, Hong Kong, Korea, Malaysia, The Philippines, Singapore and Thailand, although there may be other areas where its presence is not well-documented.

N.B.: As this disease is not listed in Directive 2006/88 EC, no measures are established for the movement of fish susceptible to this disease. However, it is recommended that diagnostic studies be carried out to check that the virus is not present in fish and/or germplasm to be transported belonging to the *Thunnus thynnus* species.

I.3.1.3.- Infectious pancreatic necrosis

Definition: Infectious pancreatic necrosis (IPN) is a very contagious viral disease that affects young salmonid fish living in conditions of intensive production. The disease is typical of *Oncorhynchus mykiss*, *Salmo salar*, *Salvelinus fontinalis*, *Salmo trutta*, and *Oncorhynchus* spp. Susceptibility diminishes with age, with resistence to the clinical disease in salmonids generally reaching approximately 1,500 degree-days, except in young salmon (between 1 and 3 years) *Salmo salar*, which may be affected on moving from fresh water to salty water. The first symptom of the epidemic in salmonid fry is often a progressive increase in daily mortality, particularly in those individuals that grow most quickly. The clinical symptoms include dark pigmentation, a bloated, sticking out abdomen and swimming around in circles. Accumulated mortalities may vary from less than 10% to more than 90%, depending on the combination of various factors such as the viral strain and the quantity, host and surroundings.

Susceptible species: *Oncorhynchus mykiss*, *Salmo salar*, *Scophthalmus maximus*, *Gadus morhua*, *Hippoglossus hippoglossus,* *Salvelinus fontinalis*, *Salmo trutta*, *Oncorhynchus* spp., *Seriola quinqueradiata*, *Misgurnus anguillicaudatus*, *Esox lucius*, *Astacus astacus*, *Anguillidae, Atherinidae, Bothidae, Carangidae, Catostomidae, Cichlidae, Clupeidae, Cobitidae, Coregonidae, Cyprinidae, Esocidae, Moronidae, Paralichthydae, Percidae, Poecilidae, Sciaenidae, Soleidae*, *Thymallidae* and, less frequently, other unspecified species.

Vector species: Not described

Geographic distribution: North and South America,Asia and Europe (Germany, Denmark, Spain, Finland, France, Greece, Holland, Italy, Norway, Poland, United Kingdom, Sweden, Switzerland, Turkey and the area of the Balkan states, although there may be other areas where its presence is not well-documented.

N.B.: Despite the fact that this disease is not listed in Directive 2006/88 EC, it is subject to national measures in order to limit its impact on aquaculture and wild aquatic animals in accordance with article 43 of Directive 2006/88 EC in Finland, Sweden and Isle of Man. At the same time, the same Directive indicates that Sweden has eradication programmes for this disease, and it is authorised to take national measures to combat it. In both cases, health certification is necessary. In case of movements of *Oncorhynchus mykiss*, *Salmo salar*, *Scophthalmus maximus*, *Gadus morhua*, *Hippoglossus hippoglossus*, the carrying out of diagnosis to verify that that the fish and/or germplasm to be transported are free from virus is recommended.

I.3.1.4.- Spring viraemia of carp

Definition: **S**pring viraemia of carp (SVC) is an infection by rhabdovirus capable of inducing an acute haemorrhagic viraemia and contagious in several carp species and in some other species of Cyprinids and Ictalurids. Up until a few years ago this disease was classified as a non-exotic disease, but when Directive 2008/53 EC of the Commission, of 30th of April 2008 came out, Annex IV of IV Directive 2006/88 EC of the Council was modified and this pathogen was excluded from the list of non-exotic diseases where viral haemorrhagic septicaemia, infectious haematopoietic necrosis, infectious salmon anaemia and the Koi herpes virus disease still remain. Generally, young fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected. Moreover, there is a high variability in the degree of susceptibility to SVC among individuals of the same fish species. Apart from the physiological state of the fish, the role of which is poorly understood, age or the age-related status of innate immunity appears to be extremely important: the younger the fish, the higher the susceptibility to overt disease, although even adult broodfish can be susceptible to infection. Apart from the above-cited cyprinid and ictalurid species, it seems that very young fish of various pond fish species are susceptible to SVC under experimental conditions over a wide temperature range. High titres of virus occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain. The mode of transmission is horizontal, but 'egg-associated' transmission (vertical transmission) cannot be ruled out following the report of isolation of this virus from carp ovarian fluid. Horizontal transmission may be direct or vectorial, water being the major abiotic vector. Animate vectors and fomites may also be involved in the transmission. Once SVC virus is established in pond stock or pond farm stock, it may be very difficult to eradicate without destroying all types of life at the fish production site. Disease patterns are influenced by water temperature, age and condition of the fish, population density and stress factors. Poor physiological condition of over-wintered fish is a contributory factor to disease susceptibility. In European aquaculture, losses can be up to 70% in young carp, but are usually from 1 to 40%. Approximately 20% of the carp population in a lake in the USA died from SVC during a disease outbreak. Disease outbreaks in carp generally occur between 11 and 17°C. They rarely occur below 10°C, and mortalities, particularly in older fish, decline as the temperature exceeds 22°C. Secondary and concomitant bacterial and/or parasitic infections can affect the mortality rate and display of signs. In carp, the disease is often observed in spring time (hence the common name for the disease), particularly in countries having cold winters. It is believed that the poor condition of the over-wintered fish may be a contributory factor in disease occurrence. The disease can occur in fish in quarantine following the stress of transportation, even though there has been no evidence of virus in the fish prior to transportation.

Susceptible species: *Cyprinus carpio carpio, Cyprinus carpio koi*, *Carassius carassius*, *Silurus glanis*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Ctenopharyngodon idella*, *Carassius auratus*, *Leuciscus idus*, *Tinca tinca* and, less frequently, other unspecified species. It has been demonstrated that other Cyprinids, such as *Rutilus rutilus* and *Danio rerio*, are susceptible to this virus by means of experimental infection by immersion, so it may reasonably be assumed that other warm water Cyprinids species are susceptible to infection, Species such as *Esox lucius*, *Lebistes reticulatus* and *Lepomis gibbosus* have been seen to be susceptible following experimental infection.

Geographic distribution: Bolivia, Brazil, Canada, China, USA, Germany, Austria, Bosnia, Croatia, Denmark, Slovenia, Spain, France, Holland, Hungary, Italy, Kuwait, Lithuania, Macedonia, Moldavia, Poland, United Kingdom, Czech Republic, Rumania, Russia, Serbia, Slovakia, Switzerland, the Ukraine, the area of the Balkan states and Laos, although there may be other areas where its presence is not well-documented.

N.B.: Despite the fact that this disease is not listed in Directive 2006/88 EC, it is subject to national measures in order to limit its impact on aquaculture and wild aquatic animals in accordance with article 43 of Directive 2006/88 EC in Denmark, Ireland, Finland, Sweden, Northern Ireland, Isle of Man, Jersey and Guernsey. At the same time, the same Directive indicates that Great Britain has eradication programmes for this disease, and it is authorised to take national measures to combat it. In both cases, health certification is necessary. In case of movements of *Cyprinus carpio carpio*, the carrying out of diagnosis to verify that that the fish and/or germplasm to be transported are free from virus is recommended. The virus is not considered to be transmitted via the egg, but if thought necessary, eggs could be disinfected by iodophor treatment

I.3.2.- Other bacterial diseases of interest for AQUAEXCEL

I.3.2.1.- *Vibrio anguillarum* and other vibrio

Definition: Vibrioses are infections produced by different species of the Genus *Vibrio* that affect sea water fish or fish in estuaries. The disease appears linked to a situation of stress and/or serious deterioration of the environment in which the fish live. There are more than 40 species of vibrios recognised as causes of infection in the marine environment. Of all these species, *Vibrio anguillarum* is responsible for the most serious outbreaks. Diseased fish are characterized by starting with a degree of anorexia that they maintain throughout the whole course of the disease and erratic swimming. After a short period of time, scaly areas are often found that quickly become ulcers. The areas worst affected tend to be the dorsal surface and the flanks near the caudal edge. All these affected areas end up suffering some sort of haemorrhage. Petechial haemorrhages are also observed in the corner of mouth and at the base of the fins. Indices of mortality may reach 30% of the group. The most disease-resistant fish often have exophthalmia and corneal opacity that leads to reversible blindness. The most representative injuries look like a real “haemorrhagic septicaemia” where we see haemorrhaging ulcers all over the body, ranging from small petechia and groove-shaped injuries to large areas with ecchymosis. The haemorrhage also affects various areas of subcutaneous tissue and muscles, in the form of ecchymosis. Vibriosis caused by other *Vibrio* sp. presents the same clinical symptoms and injuries, but they are much less serious.

Susceptible species: *Salmonidae, Dicentrarchus labrax, Scophthalmus maximus, Gadus morhua*, *Oncorhynchus mykiss, Hippoglossus hippoglossus, Cyprinus carpio, Anguillidae, Plecoglossus altivelis*, *Epinephelus* spp. *Oncorhynchus kisutch, Gadus callaris, Salvelinus alpinus*, *Anguilla japonica*, *Plecoglossus altivelis*, *Mugil cephalus, Seriola dumerelli, Lates calcarifer, Oncorhynchus tshawytscha, Pseudopleuronectes americanus*, and, less frequently, other unspecified species. In the case of vibriosis caused by species other than *V. anguillarum*, any species of fish is susceptible to suffering this process, basically in the wake of adverse environmental

conditions and stress, so it is a disease to be taken into consideration when fish are moved between the different centres belonging to the AQUAEXCEL Project.

Geographic distribution: Worldwide

N.B.: No special measures for the movement of fish exist, but the movement of fish with injuries compatible with this disease must be restricted.

I.3.2.2.- Winter ulcers (*Moritella viscosa*)

Definition: *Moritella viscosa* is the aetiological agent of winter ulcers in farmed salmonids. The disease has caused important losses in the salmonid industry in northern Europe, despite the fact that commercial vaccinations exist. Outbreaks of disease occur mainly among young fish and adults during the winter, at temperatures below 8°C. The disease was originally identified in Norway during the 1980s but quickly spread to countries such as Iceland, Scotland, Faeroe Islands, Canada and Ireland. The most serious outbreaks occur most often in *Salmo Salar* and *Oncorhynchus mykiss*, but outbreaks have also been described in *Gadus morhua*, *Salmo trutta* and *Pleuronectes platessa*. This pathogen has been isolated using healthy wild fish such as *Cyclopterus lumpus*. It has been demonstrated in experiments that *Scophthalmus maximus* and *Hippoglossus hippoglossus* are susceptible to the disease. The mortality rate for this disease normally ranges between 0 and 10%, although higher rates have been described. Infected fish can survive for a long time despite having large ulcers all over their body. Ulcers appear initially as small skin injuries that gradually become bigger, eventually covering huge areas of the body's surface. External signs of the disease include pale gills, haemorrhages in the eye, exophthalmia, rotting fins, and petechial haemorrhages on the skin. When the water temperature rises above 8°C in spring, the surviving fish recover completely from the infection.

Susceptible species: *Salmo salar*, *Gadus morhua, Oncorhynchus mykiss, Hippoglossus hippoglossus, Scophthalmus maximus*, *Salmo trutta, Pleuronectes platessa* and and, less frequently, other unspecified species.

Geographic distribution: Norway, Iceland, Scotland, Faeroe Islands, Canada and Ireland, although there may be other areas where its presence is not well-documented.

N.B.: No special measures for the movement of fish exist, but the movement of fish with ulcerous injuries compatible with this disease must be restricted.

I.3.2.3.- *Cytophaga – Flavobacterium – Bacteroides* group

Definition: The terms "*Cytophaga– Flavobacterium – Bacteroides* group" cover different bacterial species that were, until recently, placed in the Genera *Mixobacterium* and *Flexibacter*. After their latest reclassification a few years ago, they have been placed in different genera but their microbiology and various pathogenic aspects are very similar: they are both filamentous Gram negative bacilli (large in size, between 10-40 μ long), and make the most of specific environmental conditions. *Tenacibaculum maritimum* is a marine pathogen, while the pathogens *Flavobacterium columnare*, *F. psychrophilum* and *F. Branquiophilum* are important pathogens in continental aquaculture. The disease is found all over the world, and young fish are the most susceptible. The main modulator of the consequences produced by the disease in fish is water temperature and higher temperatures lead to higher mortality rates. For example, in salmonids infected with *F. columnare* no mortality is observed at a water temperature of 9.4ºC, a mortality of 4-20% at 12.5 ºC and 100% mortality at 20.5 ºC. *Flavobacterium psychrophilum* affect salmonids causing a darkening of the skin. The disease usually causes bacteraemia and the appearance of granulomatous lesions on the skin and internal tissues, and a high mortality rate, accompanied by splenomegaly, a pale liver, kidney and gills, together with inflammation of the intestine and an accumulation of ascetic fluid in the peritoneum. *Flavobacterium columnare* and *F. Branquiophilum* affects various species in continental aquaculture, causing congestion in gill vessels, large haemorrhages on any part of the body surface, ulcerative erosive injuries and systemic infections that lead to the appearance of granuloma, causing high death rates. *Tenacibaculum maritimum*, a pathogen of marine aquaculture, causes variable sized erosions in the mouth and putrefaction of the tail, often causing ulcerative erosive injuries in the head, fins and trunk.

Susceptible species for *Flavobacterium psychrophilum*, *F. columnare* and *F. branquiophilum*: *Salmonidae* and fresh water non-salmonids such as *Plecoglossus altivelis*, *Micropterus salmoides floridanus*, *Perca fluviatilis*, and, less frequently, other unspecified species.

Geographic distribution of *Flavobacterium psychrophilum, F. columnare* and *F. branquiophilum*: Europe, Japan and North America, although there may be other areas where its presence is not well-documented.

Susceptible species for Tenacibaculum maritimum: *Sparus aurata, Solea* spp. *Scophthalmus maximus, Dicentrarchus labrax, Salmonidae, Pleuronectidae, Dicologoglossa cuneata*, and, less frequently, other unspecified species.

Geographic distribution of *Tenacibaculum maritimum*: Europe, Japan and North America, although there may be other areas where its presence is not well-documented.

N.B.: No special measures for the movement of fish exist, but the movement of fish with injuries compatible with this disease must be restricted.

I.3.2.4.- *Photobacterium damselae* subsp. *Piscicida* (Pasteurellosis)

Definition: This infectious contagious disease is produced by *Photobacterium damselae* subsp. *piscicida*, basically affects marine fish, and can range from per-acute to chronic, causing considerable financial losses and a high mortality in these species, above all in warm-water areas. The disease is serious in fry and juveniles. Adult fish are more resistant to the disease and yet may transmit the infection to younger animals. There are three different clinical forms, per-acute, acute and chronic. The per-acute form tends to occur in fry with little or no immunity to the agent that causes it, or where the environment is very favourable to its appearance. In these conditions, fish tend to die showing hardly any signs of the disease; the most one might observe is a degree of anorexia and melanosis, with sudden death affecting some 60-80% of the group affected. In this clinical form, diseased fish present no external or internal injuries, and they die suddenly from endotoxic shock. The acute form presents melanosis, anorexia, and inflammation of the gills with a large amount of mucus present. This form occurs in juvenile fish with little or no immunity. The necropsy reveals a congested liver and splenomegy. The chronic form tends to occur in fish that have a degree of natural or artificial immunity to the agent in question, in fish that have survived the outbreak of the disease, or where environmental conditions are not ideal for the disease to prosper. Under these circumstances, diseased fish are very weak and tend to suffer from delayed growth and anorexia. Moreover, there is a continuous trickle of dead fish, which can reach 20-30% of the total, and that may have swollen abdomens. Necropsy reveals the presence of small whitish nodules in the liver, spleen and kidney that have destroyed the tissue's structure. Under the microscope, these nodules are seen to be granulomas.

Susceptible species: *Sparus aurata, Dicentrarchus labrax, Salmo salar, Scophthalmus maximus*, *Solea senegalensis*, *Anguilla reinhardtii*, *Chromis punctipinnis*, *Seriola quinqueradiata*, *Pagrus pagrus*, *Morone americanus*, *Morone saxatilis*, *Morone saxatilis* x *M. chrysops*, *Mugil cephalus*,*Atherinopsidae*, *Cynoglossus semilaevis*, *Rachycentron canadum* and, less frequently, other unspecified species.

Geographic distribution: USA, Japan, China, France, Spain, Italy, Greece, Turkey, Portugal and Malta, although there may be other areas where its presence is not well-documented.

N.B.: There are no special measures for the movement of fish but, given the seriousness of this process, particularly during the summer months, the carrying out of some diagnosis procedure is recommended to rule out the presence of fish that are vectors of the pathogen. As the vertical transmission of the pathogen has been demonstrated, a diagnostic procedure to rule out the presence of the pathogen in the germplasm to be transported is recommended, as is the disinfection of the germplasm using one of the procedures described in this Handbook.

I.3.2.5.- *Aeromonas salmonicida* (Furunculosis) and other *Aeromonas* spp.

Definition: This is a very contagious infectious disease produced by *Aeromonas salmonicida*, which mainly affects salmonids and develops in different ways, from per-acute processes with high mortality to chronic varieties with low mortality but which cause considerable financial losses in affected groups and which gives the disease its name due to the typical injuries(forunculo) produced in the fish affected. Non-Salmonids may also be affected, as may some marine species such as Sparus aurata and Scophthalmus maximus. The per-acute form affects above all fry and fish lacking immune protection against the pathogen, and is characterised by the fact that fishes stop eating suddenly, becoming darker in colour and dying a few hours later. The mortality rate can reach between 80 and 95 % of the total number of fish, and occurs within a week. The disease progresses so fast that there is no time to establish specific injuries, and it is perfectly normal for dead fish to be seen in necropsy not to have any injuries. Some animals may present ascites, as a result of the strong, fast proteolisis suffered by the tissues. The disease may also evolve in acute form. In these cases, fish present signs typical of septicaemia. The fish affected suffer intense anorexia and lack of appetite and many fish may die having shown signs of a darkening of the skin. Sometimes, moderate haemorrhagic injuries may be present, from petechia to ecchymosis, to be found at the base of the fins, in subcutaneous tissue, musculature, mesentery and intestines. There is normally a marked splenomegaly. Fish suffering this form are normally juvenile or semi-adult, or fish of any age with a specific degree of immunity against the pathogen and may present a mortality of 20-40 %. Finally, we can also find the chronic variety. The fish affected in this case are adults or juveniles that have previously come into repeated contact with the pathogen or have acquired a degree of immunity artificially. The diseased fish sometimes show exophthalmia or a certain thinning. But the most characteristic trait of this variety of the disease is that, coinciding with states of certain immunodepression, (for example, the spawning period) boils of varied size appear, and are located at any point of the body’s surface and affect both the skin and subcutaneous tissue. Said boils often burst in the normal course of their evolution, exuding a wine-coloured dense-looking fluid. These boils generally form scar tissue when they evolve, normally in a period of 15-21 days, and the injuries disappear completely, but the bacteria that tend to remain end up in the subcutaneous tissue do not, as they wait for the next opportunity when the fish’s immunity is not strong enough to mobilise once again in its tissues and cause new boils. Sometimes, small haemorrhagic injuries may occur in the same places we indicated in the previous variety. Mortality in this form is normally between 0-10%. From an epidemiological point of view, it is important to know that some fish affected in this way show no symptoms, and as such may transmit the bacteria to their descendants. Infections from other *Aeromonas* such as *A. sobria* and *A. hydrophila* affect both marine and continental species, producing the same clinical symptoms and injuries, but which are less serious than those caused by *A. salmonicida*.

Susceptible species: *Salmonidae, Cyprinus carpio*, *Gadus morhua*, *Scophthalmus maximus*, *Hippoglossus hippoglossus, Sparus aurata, Labridae*, *Ciprínidae*, *Pollachius virens*, *Cottus gobio, Esox* spp, , *Galaxiidae* spp and, less frequently, other unspecified species. In those infections caused by species of Aeromonas other than *A. salmonicida*, all those species of continental and marine waters are susceptible, particularly after adverse environmental conditions and/or stress, so this disease should be born in mind when fish are moved between the different centres of the AQUAEXCEL Project.

Geographic distribution: Australia, Canada, USA, Denmark, Spain, Finland, France, Iceland, Norway, United Kingdom, Sweden and Japan, although there may be other areas where its presence is not well-documented.

N.B.: There are no special measures for the movement of fish, but given the seriousness of this process, and especially the existence of asymptomatic vectors, the carrying out of a diagnosis procedure is recommended to rule out the presence of these vector fish, particularly in the case of spawning fish. Although vertical transmission of the pathogen has not been demonstrated, as a precautionary measure the disinfection of germplasm is recommended.

I.3.2.6.- *Yersinia ruckeri* (**Enteric** redmouth disease)

Definition: This is an infectious, contagious disease produced by *Yersinia ruckeri*, which mainly affects salmonids and is characterised by the appearance of the onset of septicaemia in which fish present haemorrhages of different degrees (from petachia to ecchymosis) that affect above all the ventral part of the head, the corner and inside of the oral cavity and base of the fins. There are 2 biotypes, biotype 1 (traditional) and biotype II, which has led to this disease no longer being considered a controlled disease but rather an emerging disease, as the traditional vaccinations developed to combat biotype I are not effective against biotype II. This disease occurs worldwide, although the species affected tend to be salmonids. The appearance of the disease is closely linked to the existence of stress factors. These include excessive handling of fish that have not previously been sedated, an increase in water temperature to levels of 15-18ºC, or an excess of ammoniac or metabolic waste products in the water. The disease is characterised by melanosis, exophthalmia and the reddening of the mouth and neck of affected fish, especially in ventral zone of the neck. Injuries correspond to haemorrhages of these tissues, and may vary considerably, from small petachia to ecchymosis of the area affected. The oral cavity suffers alterations both on the inside and the outside. There are also haemorrhages at the base of the fins. The control of Redmouth disease is carried out, on the one hand, by applying "basic" sanitary measures such as the reduction of stress levels in the affected fish (careful handling, use of sedatives when we transfer them from one tank to another, etc.); enhancing the water quality, reducing possible faecal contamination where appropriate or ensuring there is not an excess of organic material in suspension.

Susceptible species: *Salmonidae*, *Cyprinidae* and, less frequently other unspecified species.

Geographic distribution: Worldwide

N.B.: No special measures for the movement of fish exist, but the movement of fish with clinical symptoms and injuries compatible with this disease must be restricted.

I.3.2.7.- Streptococcal disease

Definition: Over the last decade, gram-positive cocci have become important aquatic species pathogens. Epidemic and sporadic disease outbreaks have been recorded in different parts of the world, mainly in Japan, Singapore, Australia, Israel, Italy, Spain, France, South Africa and the United States. The modified taxonomy revealed that at least 4 gram-positive bacterial Genera, such as *Streptococcus*, *Lactococcus*, *Vagococcus* and *Enterococcus* were responsible for these conditions. Despite the progress in taxonomy and diagnosis, the pathological basis of this group of diseases has not been tackled in depth, because infection from catalase-negative gram-positive cocci produce non specific injuries, such as haemorrhages, congestion and ophthalmitis, and that it is difficult to differentiate between these four bacterial genera by means of conventional traditional biochemical tests. For this reason, the term streptococcis is currently considered as a complex of similar diseases causes by different genera and species capable of inducing damage to the central nervous system, with suppurative exophthalmia "pop-eye" and meningoencephalitis, so it includes the diseases produced by the genera *Streptococcus, Lactococcus*, *Vagococcus* and *Enterococcus*. *Lactococcus garvieae* is an important pathogen in aquaculture that is capable of infecting both saltwater and freshwater species, causing important disease outbreaks in Italy, Spain and France, and, to a lesser extent, United Kingdom and Australia. On the other hand, *Vagococcus salmoninarum* is another important pathogen that mainly affects salmonids. The aquaculture industry, both marine and continental, has suffered enormous financial losses as a result of various outbreaks of disease caused by different species of *Streptococcus*, such as *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus parauberis, Streptococcus phocae* and *Streptococcus iniae*. The distribution areas of these diseases in modern aquaculture tend to affect coastal zones where there is not a great deal of sea movement, or little renewal of the water of the affected region. They tend to be areas with scant or no difference between high tide and low tide, in which there is a high level of colonization and human activity nearby that generates a lot of organic contamination. This is why the bibliography describes severe outbreaks of the disease in regions of the United States, Japan, Greece, Italy, Israel or Spain. Reference is also made to seasonal factors in the presentation of the disease, often coinciding with the warmest periods. The water temperature is considered to be a predisposing factor in the evolution of the clinical process. Thus, outbreaks associated with infections of *Lactococcus piscium* and *Vagococcus salmoninarum* appear at water temperatures of less than 15ºC, and this is why it is known as cold water streptococcosis. The outbreaks that occur at water temperatures of more than 15ºC, or warm water streptococcosis, are produced by *Lactococcus garvieae*, *Streptococcus iniae*, *Streptococcus parauberis* and *Streptococcus difficilis*. In streptococci, exophthalmia, a distended abdomen and erratic swimming appear, as do injuries such as different types of haemorrhages on the tissue affect, in the eye, operculum and base of the fins, the presence of ascetic fluid in the abdominal cavity that may be bloody, and finally, catarrhal –haemorrhagic enteritis and degeneration of the liver. Moreover, we can observe cases of encephalitis with areas of necrosis. To control this group of diseases, one of the initial measures is treatment. In this case, penicillin and derived products such as oxytetracicline or quinolones have shown good results. The treatment must last for a minimum of ten days. On the other hand, over recent years, various commercial vaccinations have emerged that can be used in areas where repeated outbreaks occur, such as the entire Mediterranean basin.

Susceptible species: *Oncorhynchus mykiss, Sparus aurata, Scophthalmus maximus, Cyprinus carpio, Salmo salar, Dicentrarchus labrax, Pagrus pagrus, Oncorhynchus kisutch, Paralichthys olivaceus, Crysoleucas Notemigonus, Brevoortia patronus, Arius felis, Mugil cephalus, Lagodon romboides, Micropogonias undulatus, Leiostomus xanthurus, Dasyatis* sp., *Cynoscion nothus, Fundulus grandis, Morone saxatilis, Pomatomus saltatrix, Cynoscion regalis, Oreochromis aureus* x *Oreochromis niloticus, Liza klunzingeri, Leponis macrochirus, Lepomis cyanellus, Ictalurus punctatus, Seriola dumerili, Seriola quinqueradiata, Oncorhynchus rhodurus, Oreochromis niloticus, Lates calcarifer, Sciaenops ocellatus, Siganus rivulatus* and, less frequently, other unspecified species.

Geographic distribution: Italy, Greece, Spain, USA, France, United Kingdom, Japan, Taiwan, Singapore, Australia, Israel, China, South Africa, Chile, Caribbean Sea, Barbados and the Island of Grenada, although there may be other areas where its presence is not well-documented.

N.B.: No special measures for the movement of fish exist, but the movement of fish with clinical symptoms and injuries compatible with this disease must be restricted.

I.3.2.8.- *Renibacterium salmoninarum* (bacterial Kidney Disease)

Definition: This pathogen produces bacterial Kidney Disease (BKD), which has been known since the last century and is located almost exclusively in Central Europe. It affects salmonids and tends to be chronic with the appearance of abscesses mainly in the liver, spleen and kidney. Contagion takes place when the fish are young, although the symptoms do not appear until several months later. The reservoirs are represented by vector fish (infected but that have not yet fallen ill) that eliminate the bacteria into the environment and by water it self, as the bacteria survive for more than 21 days in continental water and up to 1 week in seawater. The pathogen acts as a real “parasite” on fish and the disease appears linked to some environmental changes related with the hardness of the water, salinity, temperature or diet. Soft water and abrupt changes in salinity favour the appearance of the disease. The temperature of the water modulates the consequences when the disease is already present, as with an increase in temperature the number of dead fish also increases, while a fall in temperature favours the spreading of the pathogen and the appearance of epidemic outbreaks. As far as diet is concerned, the role played by deficiencies in vitamin A, zinc and iron as causes that favour the increase in morbidity has been demonstrated. The sources of infection remain unclear today. However, they appear to be represented by diseased fish (whether or not they show symptoms) that eliminate bacteria into the water. It has been demonstrated that contagion can occur by all possible means, and an interesting fact is that, considering that the egg can transmit the infection to descendents, yodophors, habitually used to disinfect the pathogen’s eggs, do not eliminate the infection in this case. The disease is characterized by a gradual production of injuries in infected fish. It normally starts with astriking abdominal distension and exophthalmia. Subsequently, ulcers can be observed on the body surface, growth is delayed and the fish eventually die. Necropsy of the diseased fish reveals a notable increase in the size of the liver, spleen and kidney. In these organs, but especially in the kidney, the presence of different-sized abscesses can be seen all over the organ. Sometimes, the musculature and heart

can also be affected. Occasionally, a false membrane can be seen surrounding the organs affected, as well as petechia haemorrhages. Disease control is currently carried out using general antitiotherapy. The use of aminoglycosides and quinolones gives good results as, although the pathogen is not eliminated, the general state of the diseased animals improves. As there are no effective commercial vaccinations, the improvement of environmental conditions of the fish, particularly in terms of the provision of a balanced diet (vitamin A, zinc and iron), and avoidance of abrupt changes in the hardness and salinity of the water is recommended.

Susceptible species: *Salmonidae,* and, less frequently, other unspecified species

Geographic distribution: Central Europe, Iceland, Japan, USA and Chile, although there may be other areas where its presence is not well-documented.

N.B.: Despite not being listed as a disease in Directive 2006/88 EC, it is subject to national measures to limit its impact on aquaculture and wild aquatic animals in accordance with article 43 of Directive 2006/88 EC, in Ireland, Northern Ireland, Isle of Man and Jersey. At the same time, this Directive indicates that Great Britain and the continental territories of Finland and Sweden have eradication programmes for this disease, and are authorised to take national measures to combat it. As the vertical transmission of the pathogen has been demonstrated, a diagnosis procedure to rule out the presence of the pathogen in the germplasm to be transported is recommended, as is the disinfection of the germplasm by one of the procedures described in this Handbook.

I.3.2.9.- *Piscirickettsia salmonis*

Definition: The first outbreaks of this disease appeared at the end of 1989 and caused financial losses because of its high mortality rate. Initially, it was only described in *Oncorhynchus kisutch*, but all salmonid species farmed in Chile were rapidly affected; in some centres a mortality of up to 90% was recorded. The disease has been described mainly in sea water and estuaries, and very occasionally in fresh water. The first outbreaks were related to a period following water temperature fluctuations, the presence of a bloom of non-toxic algae and/or stress related to severe storms. The epizootics appear particularly between autumn and spring when temperatures fluctuate between 9 and 16ºC, and it has been suggested that the process of smoltification may increase the susceptibility of infected salmon. The clinical signs are characterized by slow, erratic swimming on or near the surface of the water. Other symptoms described include lethargy, anorexia, bumping into walls and nets and melanosis. The most relevant macroscopic injuries include scaly, pale gills, ecchymotic and petechial haemorrhages at the base of the fins, nodules and ulcers on the skin of up to 2cm in diameter. Hematocrit levels reflect severe anaemia. In the necropsy analysis of the abdominal cavity, ascites and splenomegaly are often found, as are creamy-to-yellow coloured subcapsular nodules in the liver, the presence of a pseudomembrane over the heart and petechial haemorrhages in the stomach, pyloric caeca, intestine, swimbladder, muscular and visceral fat. In most cases the intestine is full of a yellow mucus substance and the stomach of a transparent seromucous fluid. The natural disease tends to appear some 6 to 12 weeks after the smolts enter the sea.

Susceptible species: *Salmo salar, Dicentrarchus labrax, Oncorhynchus mykiss, Tetrodon fahaka*, *Oreochromis niloticus, Oncorhynchus gorbuscha, Paneque suttoni, Oncorhynchus kisutch, Oncorhynchus tshawytscha, Oncorhynchus masou*, *Callionymus lyra* and, less frequently, other unspecified species

Vector species: *Paralabrax humeralis, Trachurus murphyi, Basilichthys australis, Eliginops maclovinus* and *Merluccius* sp.

Geographic distribution: Egypt, Germany, Scotland, France, Norway, Gales, Canada, Colombia, Ireland, Japan, Taiwan and Chile, although there may be other areas where its presence is not well-documented.

N.B.: Nospecial measures are in place for the movement of fish, but as the vertical transmission of the pathogen has been demonstrated, some diagnosis procedure is recommended to rule out the presence of the pathogen in the germplasm to be transported, together with disinfection of the germplasm using one of the procedures described in this Handbook.

I.3.3.- Other parasitic diseases of interest for AQUAEXCEL

I.3.3.1.- *Phylum Amoebozoa*

Definition: Amoeba are unicellular organisms characterised by having pseudopods for locomotion and catching prey (bacteria). The only species that will be of interest to us as a pathogen in aquaculture is *Neoparamoeba pemaquidensis*, the agent that causes Amoebic Gill Disease (AGD) in *Salmo salar* and *Scophthalmus maximus*. This process may take place as of 12-14 ºC, and it becomes more serious as the temperature goes up. The development of the disease in *Salmo salar* takes place at a salinity of over 32 ‰ and in *Scophthalmus maximus* at 22 ‰. The disease is transmitted between fish by water. Significant presence of *N. pemaquidensis* has also been described in the nets of floating cages with a large amount of fouling, probably feeding off the bacteria present. The most typical injury (although it is not always observed) is the appearance of whitish or grey-ish injuries on the gill filaments, normally accompanied by excess mucus. Heavily parasited *Scophthalmus maximus* may show alterations in its behaviour: a reduction in feeding rate, hyperventilation and, sometimes, inverted swimming. Histologically, hyperplasia and hypertrophy of gill epithelial are observed. The amoeba are normally seen in a kind of bladder inside the hypertrophied epithelial tissue. The most effective and most commonly-used treatment consists of fresh-water baths. The advantage of this treatment is two-fold: it eliminates most of the amoebae and also reduces the gill injuries (it relieves the physiological disturbances and eliminates the excess mucus). The dead fish will act as a concentrated of amoebae for at least 3 days after death, so it is very important to remove dead fish on a daily basis. It is important to clean and disinfect the nets properly in order to limit the quantity of *N. Pemaquidensis* present in them. It is important to use nets with antifouling treatment.

Susceptible species: *Salmo salar, Scophthalmus maximus, Dicentrarchus labrax, Oncorhynchus kisutch, Oncorhynchus tshawytscha, Diplodus puntazzo* and, less frequently, other unspecified species

Distribution: France, Spain, Scotland, Norway, Ireland, Australia, New Zeland, Chile and USA, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.2.- *Phylum Parabasalia*

Definition: Hexamitosis is a freshwater fish disease produced by the flagellate protozoan *Hexamita* spp. *Hexamita* is related to gastrointestinal diseases in fish. Stress appears as an important factor that favours the disease, as *Hexamita* and similar flagellates (*Spironucleus* type) are sometimes found living as flora in the gastrointestinal system of clinically normal fish, waiting for the moment when the fish's immunological defences are low to cause the disease. In the parasite's cycle there are stages of encystment during which it is not always possible to combat the disease. Transmission is produced by the ingestion of infected cysts. Fish normally carry small quantities of parasitic protozoa in their intestines that have been taken in with food. Stress situations such as an inappropriate population, unsuitable water quality, abrupt changes in temperature, imbalanced diet and a long list of other factors may trigger the multiplication of said flagellates. Those hexamites that are latent in an intestinal infection spread via the blood affecting the peritoneal cavity, spleen and liver and are related to problems of vascularisation. In subsequent stages, typical hole-like wounds in the fish's head appear and later on, these spread and crater-like injuries appear, that may suffer secondary infections from bacteria and fungi, although the hexamite-disease relationship of the holes in the head are not currently 100% accepted, despite the fact that the last research indicates that a high concentration of flagellates in the digestive systems of the fish leads to nutrients being insufficiently absorbed and thus favouring an unstable balance of minerals, which causes damage to the fish's skeleton, leading to erosive lesions (dimple-like holes) along the lateral line of the fish's head. The first external symptoms are not different from those presented by other diseases: a darkening of the body, loss of appetite, isolation and lack of reaction. The ultimate cause of death may be due to a secondary microbial infection. The invading protozoa invaders cause infections in the intestinal organs. The intestines become inflamed and are covered with mucus and blood. It also attacks the bladder, making it inflamed and hardened. Once the external conditions wear this protection system down, the protozoan is free to propagate. The first symptoms tend to be an overall darkening in colour and a thinning of the animal's ventral area. Fish become withdrawn and apathetic, and may present tearing at the base of the fins and along the lateral line. Affected fish lose their appetite, and subsequently weight, are darker in colour than usual and their faeces are filamentary, whitish in colour and long. In advanced stages of the disease, the fish will become acutely thin and a series of orifices appear on their head through which oozes a gelatinous substance that comes from the destruction of the muscles. Hexamitiasis is a serious disease that can lead to death if measures are not taken in time. The orifices in the head are only visible in the advanced stage of the disease. Previously, numerous symptoms that may leave us in doubt as to the origin of the disease can be seen.

Susceptible species: *Salmonidae* and, less frequently, other unspecified species

Distribution: Europe, Asia and Americas, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.3.-*Phylum Euglenozoa*

Criptobia Genus

Definition: The Cryptobia Genus is the one that interests us the most. Species of the Cryptobia Genus are like trypanosomes, but they have two anterior flagellates, one of which is free-moving and another that forms an undulating membrane. Most of the members of this genus are parasites on invertebrates, but some species are important fish parasites. Their size can make them difficult to identify and study. In fact, 52 species of *Cryptobia* have been described in fish: 5 are classified as ectoparasites that infect the skin; 7, as enteric parasites that infect the gastrointestinal system; and 40 have been classified as hemoflagellates found in the blood stream. The hemoflagellates have an indirect biological cycle and the gastrointestinal and ectoparasitic forms have direct biological cycles. They live in the stomach of marine fish and some fresh water fish, damaging the digestive tract. They are also the cause of a disease of the gills that leads to death. *Cryptobia* spp. found enterically and externally has a direct biological cycle. The contagious forms are eliminated with the faeces, and the ingestion of these forms gives rise to infection. It can live in the water for at least a few hours. Wild fish are vectors of the disease but the disease only appears under conditions of stress. Diseased fish eliminate white elongated faeces and die in a few days with a bloated abdomen. *Cryptobia* spp. is typically associated with the presence of granuloma in the stomach, but systemic infections have been reported that place the organism in the blood and other organs (liver, kidney, ovary, brain, and eye). Mortality rates associated with the systemic form may exceed 50% of the infected population. Diseases fish stop eating in one or two days and then fall back gradually and break away from the rest. Before dying, they move to the surface and their breathing becomes dramatically quicker, suggesting hypoxia (low level of oxygen in the blood). Death normally occurs some 24 hours after the development of severe anaemia. The clinical signs of cryptobiosis include anorexia, exophthalmia, splenomegaly, enlarged liver, general oedema and a bloated abdomen with ascites and anaemia. Infected fish are very susceptible to environmental hypoxia, and their metabolism falls. Anaemia and anorexia contribute to immunodepression. The parasite causes a granulomatous gastritis and can generally be linked to low rates of chronic mortality. A systemic form of the disease has been seen in some cases. This form was associated with mortality and acute losses of 50% of affected animals.

Susceptible species: *Salmonidae, Cyprinus* spp.and, less frequently, other unspecified species.

Distribution: Worldwide

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Ichthyobodo (*Costia*) Genus

Definition: Costiasis is a disease that generally spreads in places where there is a large concentration of fish. For this reason, it tends to appear most often in trout and carp nurseries and ornamental fish factories after hibernation. It is almost always linked to other processes, and can therefore be considered an opportunist parasite. It is a small cosmopolitan pear-shaped parasite that measures some 6-20 micra long. They have from 2 to 4 flagella, at least one of which sticks out backwards. When fixed to the fish, it penetrates the epithelial cells thanks to a kind of hook and it reproduces over the fish's body surface. These are free-living species that reproduce by binary fission, are incapable of living for more than an hour on their own outside the host. Occasionally, *Costia*, live in the skin and gills of health fish and the fish's defences are thought to maintain the parasite population under control. As is the case with many fish parasites, a small number of Costia individuals are not unusual and to not appear to be harmful for the fish's health. In small numbers, these parasites appear to remain in the cells in a comensal relationship with their host. This parasite becomes a serious threat when, for various reasons, an excessive multiplication occurs and produces serious damage to the fish's skin and gill tissues, as well as secondary effects such as hyperplasia or secondary infections, particularly of the gills. The triggers that can lead to an increase in its population include stress or fish disease, as well as poor water quality and overpopulation. Under these conditions, they reproduce quickly by binary fission and attack living cells with disastrous consequences. Infected fish normally rub against objects, may stop eating and open their mouths at the water's surface. After a period of time, they may show signs of starvation. A characteristic sign of costiasis is the production of an excess of mucus. Sometimes the epithelium may have come completely away and the skin becomes strangely pigmented. *Costia* infections cause a typical fish response of irritation. Breathing becomes heavy and agitated (judging by the movements of the operculum), due to congestion of the gills. The skin becomes cloudy because of excess mucus. In a more advanced stage (that may arrive too late for treatment) fish often become isolated, sometimes closeto the surface. They may also be lethargic, closer to the bottom. It must be pointed out that these clinical signs are not exclusive to infestations of this parasite and that they may be caused by other serious factors including poor water quality. This parasite moves into the fish's skin and uses an anchoring system so as not to fall off, and this is when the wound in the epidermis is produced, perforating the scales, producing small wounds and haemorrhages that constitute the point of entry for parasites, bacteria or fungi. Initially they will affect the skin, and later, the gills. Other symptoms are common to a variety of diseases, such as folded fins, a rocking movement and a lack of reactions in more advanced stages.

Susceptible species: *Salmonidae, Cyprinus* spp.and, less frequently, other unspecified species.

Distribution: Worldwide

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.4.- *Phylum Oflagellata*

Definition: Within this *Phylum*, the *Amyloodinium* Genus is of most interest. Individuals characterised by the presence of 2 flagella, of which one transverse one disappears when the parasites latch on by pseudopods to the fish's epidermis. Amyloodiniosis (marine velvet) is produced by the dinoflagellate parasite *Amyloodinium ocellatum* (in freshwater fish we find the *Oodinium* Genus, but it is only important in fishkeeping). It is commonly confused with the whitespot disease produced by *Criptocaryon*, but an easy way of telling the difference is clear to the naked eye: *Amyloodinium* is only seen when light it shed on the fish's skin and only clearly on the most transparent fins. This parasite was named velvet because of the coloured appearance of the skin produced in highly infected fish. It affects a wide range of fish species and can be deadly for small fish. *Amyloodinium ocellatum* goes through different stages during its life cycle, with three clearly differentiated phases, the parasitic stage (trophont), the reproductive stage (tomites), dispersal stage (dinospore) and parasitic stage (trophont). The trophonts of *A. ocellatum*, constitute the vegetative or immobile feeding stage. They are spherical, ovoid or elliptic, with an average size of 100 μm long and 61 μm wide, whitish or gold in colour, covered in a pseudochitinous cover or capsule. An anchoring organ emerges through a polar opening, made up of a short peduncle, endowed with numerous rhizoids, which it uses as "roots" to penetrate and fix onto the host's cells. Once stuck onto the fish, the trophont breaks off between the third and fourth day (at an ideal temperature of between 18°C and 30°C). During the reproductive stage, the parasite reproduced by binary fission. In the sixth division, 64 tomites are produced (where the trophonts are larger, a 7th division may be reached). The ideal temperature for the development of this stage is between 23°C and 27°C. At 15°C, the division process stops after the fourth cellular division of the tomite. The dinospore constitutes the active stage of the parasite, during which it swims in search of its next host and adopts the typical morphology of a dynoflagellate. It is 4.5 to 5.0 μm long. It has two flagella for swimming that emerge close to one another in the ventral region. This stage is reached when release occurs as a result of the capsule wall bursting, once the 6^{th} cellular division is culminated. In less than 24 hours, these dinospores have to find a fish to infest, and 4 days later the developed trophonts are ready to break away and start a new reproductive cycle. At temperatures below 17ºC infestations are not produced. In farm tanks the disease is characterised by the fish scratching vigorously against the walls and bottom of the tanks. As the disease advances, the breathing and opercula open and close quickly. The clinical signs of amyloodiniosis include anorexia, depression, breathing disorders or irritation of the gills. The fish may also suffer irritation, as a consequence of the high number of parasites per fish, which gives the fish a velvety appearance, leads to excessive production of mucus and changes in behaviour (irregular swimming and scales that glimmer). In massive infestations, the affected fish die. The excess in mucus production complicates the process, as it produces a malfunction of the epithelium of the gill filaments, which leads to difficulties in the free exchange of gases, which may lead the fish to die from suffocation in less than 12 hours. In large ingestations (more than 200 trophonts for each gill filament), apart from serious hyperplasia, inflammation and haemorrhagic injuries that lead to necrosis can be seen. This dusty appearance may be of a different hue depending on the species: greyish-white or even reddish. In any case, the onset of the disease may not be noticed, so any change in behaviour by the fish should be taken seriously. On the other hand, when the process starts, the diagnosis may be confused with the onset of a fungosis, given the initial opacity of the area affected. Predisposing factors include abrupt changes in temperature, transfer of fish without acclimatisation, stressed fish and insufficient water renewal. In terms of treatment, solutions of formol at 300 ppm have not yielded very good results. Hydrogen

peroxide has also been tried out, but with differing results. Fresh water baths for one minute have been used to make the parasites fall off in some cases. Copper compounds are very effective but extremely toxic, so their use is strictly controlled. The best measure is to empty to tanks affects and given them a thorough clean, as well as renewing the tank water more often and cleaning the tank bottoms more thoroughly and more often, thereby shortening the parasite's cycle.

Susceptible species: All marine species

Distribution: Tropical and subtropical, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.5.- *Phylum Ciliophora*

Definition: The *Phylum Ciliophora* contains some 7200 species, although many more unknown species are suspected to exist. They can be found in almost any water mass, from small puddles to the open ocean. Most will be free-living protozoa, although they may also be comensal or symbiotic, and, in some cases, they may be parasites. The ciliates can measure between 10 µm and 4500 µm and their morphology is normally spherical or ovoid. The life cycles are very varied in this group. In some species, the cycle consists only of permanent divisions with conjugation or autogamy intervening alternately in random fashion and with no formation of cysts (*Trichodina*, *Tetrahymena*). In other species, cysts normally form under adverse conditions. These forms of resistance have walls of one or two layers of gelatinous material. The function of these cysts is survival, but they also act as a way of distributing the species (for example, by aquatic birds). Apart from these basic life cycles, there are differences between the different groups that we will describe in each specific case. Most of the ciliated ectoparasites tend to respond well to treatments with external disinfectants such as formol, copper sulphate, salt baths or freshwater baths.

Cryptocarion irritans and *Ichthyopththirius multifiliis*

Definition: This is a process that can affect numerous fish species (to a greater or lesser extent), including farmed species. In marine fish, the aetiological agent is *Cryptocarion irritans*, while the ciliate that attacks fresh water species is *Ichthyopththirius multifiliis*. These two species are very similar in terms of morphology, life cycle, pathogenicity and treatment, so we will describe them at the same time. The disease owes its name to the appearance of small white dots, visible to the naked eye, on the skin and gills of the host. The life cycle of this pathogen goes through 4 different phases. The first, or *trophont*, is the stage in which the parasite is found on the fish's skin, feeding and growing in size. During this phase, the ciliate is surrounded by a gallbladder of tissue, inside which it moves around, forming the characteristic white dots on the host's surface. The next phase is when the trophont has "fattened" enough; then it falls to the bottom of the tank, where it creates a cyst and starts to multiply (*tomont*). The tomont starts to divide up, and may form up to 2,000 *tomites* (200 in the case of en *C.*

irritans). The tomites, once the cyst at the bottom has burst, break free and swim actively until they find a new host (*teront*). The teronts have to find a new host within 24 hours or they will die. The really dangerous thing about this parasite is its high capacity for multiplication and that, moreover, the cycle is asynchronous, i.e., not all the parasites are in the same phase at the same time. This last characteristic makes treatment much more difficult as only the free stages will be sensitive to the different disinfectants. Both the life cycle of *I. multifiliis* and that of *C. irritans* are highly dependent on temperature, and thus the cycle of *I. multifiliis* can last from 3 months at 3ºC and then close down in only 3-6 days at 23-24 ºC. Although the life cycle of *C. irritans* is very similar, it takes a little longer to come to an end (8 days at 25 ºC and up to 28 days at low temperatures). Above 37 ºC and below 7 ºC this parasite loses the capacity to form cysts. Salinity is also a factor that can affect the development of these ciliates considerably: *C. irritans* undergoes cellular destruction below salinity levels of 20 ‰, while *I. multifiliis* dies at salinity levels of over 20 ‰ (although there are references to successful treatments with salinity levels as low as 7 ‰). This is a process that will appear in tanks in land-based installations, recirculation systems and farming systems with slow renewal. As the parasite needs a substrate where it can turn into a tomont and start replication, it is very rare for it to appear in farm systems in the open sea. The first symptoms of a severe infestation are restlessness, with fish often scratching against the walls of the bottom or jumping out of the water. Some days later, a thickening of the surface mucus can be seen and white dots of up to 1mm in size start to appear. Subsequently, there is a loss of scales and the skin may develop ulcers. Finally, fish die, mainly due to the osmotic alterations produced due to the extensive damage to the skin. Formol is the treatment most commonly chosen to control whitespot. As we have commented above, this treatment will only be effective to eliminate the free forms of the parasite, but it will not affect the cysts on the bottom, where the parasite divides, or the forms that are protected in the fish's skin. For this reason, it is a treatment that we will have to carry out regularly to gradually eliminate the free forms that appear. The most usual pattern consists of two to three cycles of three consecutive days of treatment, resting one day between each cycle, at a dose of 250 ppm (ml/m3). Common salt baths constitute a more powerful treatment, as, apart from eliminating the free forms swimming in the water, they allow the forms that are stuck on the fish's skin to fall off, will also help the wound produced on the fish's skin to heal and will relieve the osmotic imbalance. It is advisable to use them in massive infestations of *I. multifiliis*. Copper sulphate is a highly toxic product for aquatic animals in general, so it must be used at very low doses (between 1 and 3 g/m^3, depending on the hardness of the water, for 45-60 minutes). It is a good idea to bath the fish with this product in the open air. It is a very useful product in terms of the prevention of the disease: two or three weekly baths are sufficient to eliminate the possible teronts swimming freely in the water, which will prevent them from infecting the fish. It will have practically no effect in other stages of the parasite. It is a very useful product for treating large volumes of water, given that the amount of product to be used is very small. Baths in water with a low saline content (<20 ‰) or even freshwater baths are very effective for controlling infestation for *Cryptocarion irritans*. A duration of 45 minutes twice or three times in a row is recommended. This is an appropriate treatment in fish species from estuary areas of diadromous areas. *Scophthalmus maximus* tolerates hyposalinity quite well for an hour or more.

Susceptible species: All marine and fresh water species

Distribution: Tropical and subtropical, although there may be other areas where its presence is not well-

documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Philasterides dicentrarchi

Definition: This ciliate of the *Scuticociliata* Order can act as an opportunist parasite and cause a systemic disease in *Dicentrarchus labrax* and *Scophthalmus maximus*. Clinical outbreaks in *Scophthalmus maximus* are the most serious, and mortality rates of up to 100% in fry have been described, as have rates of up to 30% in fattening stages. The infestation occurs mainly at temperatures of >20ºC, and mortality can be high in fish affected during fattening. In situations of stress, ciliates may colonize the inside of the fish. Transmission is horizontal by water and superficial wounds. The parasite can enter through the eye and the nasal-olfactory circuits, and travel to the brain along the optic nerve. Wounds in the eye caused by supersaturation of oxygen in combination with low water exchanges could represent risk factors, as may injuries in the gills or skin caused by other factors. Clinical signs may change from some outbreaks to others, but we commonly observe ulcers in the skin and subcutaneous tissue around the eye causing periorbital inflammation, together with alterations in behaviour when the ciliate reaches the central nervous system. In experimental infections, numerous organs have been affected, especially the pancreas and the digestive tract. Free-living ciliates or those in external injuries respond well to most treatments with disinfectants (formol, hydrogen peroxide), but are not sensitive once they penetrate the inside of the fish. Once the parasite penetrates, the only option is to apply diets with supplementary vitamins or stimulants for the immune system to try to minimize the damage the parasite might do, although the results are very variable. Prevention is based on general measures such as cleaning the tanks more thoroughly, selection of stock and vaccinations (under study).

Susceptible species: *Scophthalmus maximus*, *Dicentrarchus labrax*, *Paralichthys olivaceus* and, less frequently, other unspecified species

Geographic distribution: Worldwide

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Uronema marinum and *Uronema nigricans*

Definition: These histiophagous ciliates of the *Scuticociliata* Order mainly affect *Sparus aurata*, *Dicentrarchus labrax* and *Scophthalmus maximus*. These ciliates are free-living species that live in marine sediment, but can act as facultative parasites, and can cause serious systemic infestations. Infestations by these parasites produce intermittent agitated breathing, loss of weight and dehydration. The disease only appears in weakened fish, so low immunity is a key factor in the development of the process, and stress factors such as inappropriate handling, poor water quality, variations in temperature, etc. also play a role. The possible means of entry of the

ciliate into the fish may be external wounds, gills, nostrils, lateral line and mouth and gill cavity. Once inside, the ciliate may cause a systemic infestation, spreading through all organs and presenting a special tropism in the connective tissue of the organs. Thus, in the histological preparations, we may find parasites in numerous locations such as gill, skin, muscle, spleen, kidney, intestine, heart, pancreas and gonads. Given the seriousness of the process, early diagnosis is essential, before the systemic infestation occurs. We can scrape the skin and carry out and gill preparations to observe the ciliate.

Susceptible species: *Sparus aurata, Dicentrarchus labrax, Scophthalmus maximus, Salmo salar*, *Paralichthys olivaceus*, *Polyprion oxygeneios*, *Thunnus maccoyii* and, less frequently, other unspecified species

Geographic distribution: Worldwide

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.6.-*Phylum Apicomplexa*

Definition: The *Cryptosporiidae* Family is the one of most interest in aquaculture. Their life cycle is direct and is divided into three phases, merogony, gametogony and sporogony. The merogony phase starts with the host cell by the parasite. Once the sporozoite penetrates the cell of the host, it is wrapped in a parasitophorous gallbladder in the cytoplasm or nucleus of the cell and starts to grow and to adopt a rounded shape, becoming a meront. Inside the meront merozoitos start to be produced, and once they have been released will invade other cells and form second and third generation meronts. The Gametogony starts when the merozoites form macro and microgermplasm, which will join together to form zygotes. After fertilization, the macrogamont will evolve to form anoocyst. During the sporogony phase, the zygote inside the oocyst will divide and form sporocysts, where new sporozoites are formed. The Sporogony phase can take place inside the organism of the fish (endogenous sporulation), or outside (exogenous sporulation). Not much is known about the prevention of losses produced by coccidiosis of fish. This is largely due to the fact that most species only affect fish farmed in tanks on land (different species of carp, eels, tilapia).

Cryptosporidium molnari

Definition: *Cryptosporidium molnari* is an intracellular protozoan that infects gastrointestinal epithelial cells of fish species such as *Sparus aurata*, *Dicentrarchus labrax.* In *Sparus aurata* the infestation may be very intense, with the appearance of whitish faeces, a swollen abdomen and ascites on rare occasions, despite the intense damage observed on a histopathological level, where necrosis and flaking off of epithelial cells are observed, with abundant cellular remains.

Susceptible species: *Sparus aurata*, *Dicentrarchus labrax, Poecilia reticulata* and, less frequently, other unspecified species

Geographic distribution: Atlantic, Cantabrian and Mediterranean Coasts, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.7.- *Phylum Microspora*

Definition: These are strict intracellular parasites with unicellular spores with an imperforated cell wall that contain sproroplasm and a complex eclosion apparatus. One of the main parts of this apparatus, an extractible polar tube, serves to inject sporoplasm into the host cell. Some 1300 species have been identified, most of which are parasites on invertebrates, although some are considered important fish pathogens. Apart from some exceptions, the genera that affect fish do not parasite other animals. Although they have traditionally been studied in the field of animal parasitology, recent studies based on several molecular markers place the *Phylum Microspora* wihin the *Fungi*. The life cycle of the microsporidios of the fish is direct, i.e. no intermediate host is needed. The development cycle is divided into two stages, the Merogony, during which a large number of parasites are produced, and the Sporogony, during which mature spores with the capacity to infect are produced. Many genera do not cause major morphological changes in the affected cells, although in some cases, a tremendous hypertrophy of the cells is produced with markedly enlarged nuclei and modifications to the cell's membrane: the so-called "xenomas". These xenomas may measure as much as 14 mm. There is no effective treatment to combat the microsporidios. The only thing that can be applied is a diet with vitamins and stimulants for the immune system supplements to try to prevent infection.

Tetramicra brevifilum

Definition: *Tetramicra brevifilum* is a microsporidium that produces xenoma that causes systemic microsporidiosis in *Scophthalmus maximus* from 20 to 500 g of live weight. The spore of *T. brevifilum* has a characteristic inclusion body inside the posterior vacuole. *T. brevifilum* is a microsporidium that has a special tropism thanks to the cells of its connective tissue, so it can appear practically anywhere in the fish's organism. Moreover, the xenomas produced by *T. brevifilum* have a series of microvilli, in the external covering of the xenoma. It is a disease that occurs at low temperatures, mainly in winter and spring, although it may appear throughout the year. Mortality tends not to be too high, although cases of massive infection with significant mortality havebeen reported. Initial infection takes place in the digestive tract. Once the sporoplasm has been extruded, the infecting cells disperse the disease through the defence cells to other organs. The xenomas are initially found in the digestive tract and liver, although in more advanced infections, they are typically prolific in the muscles and skin. They may also be found in the gills, although this is less usual.

Susceptible species: *Scophthalmus maximus* and, less frequently, other unspecified species

Geographic distribution: Growing areas of susceptible species

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Pleistophora senegalensis and *Glugea* sp

Definition: These two parasites cause microsporidiosis in *Sparus aurata*. In Malta and Greece, chronic mortality has been described in juveniles in floating cages, although there is normally no mortality. If there are a large number of xenomas, problems may arise in the selling of the fish as a result of depreciation of the filets. Seriously affected fish look emaciated and lethargic. The musculature shows whitish cysts of between1 and 5 mm long.

Susceptible species: *Sparus aurata* and, less frequently, other unspecified species

Geographic distribution: Mediterranean and African Atlantic coast, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.8.- *Phylum Myxozoa*

Definition: The myxozoa are fish parasites that are becoming more and more important from a financial point of view given the serious problems that they cause in modern fish-farming. More than 2,700 species distributed in 61 genera are currently known. Unlike Microsporidia, Myxozoa spore are multicelular and consist of various highly specialized cells. The Phylum Myxozoa has 61 genera divided into two clases, the Malacosporea Class (with one family and two genera *Buddenbrokia* and *Tetracapsuloides*), and the Myxosporea Class, with the Bivalvulida Orders (Sphaeromyxina, Variisporina and Platysporina) and Multivalvulida Orders (*Trilospora*, *Kudoa*, *Hexacapsula*). Normally, the Myxozoa's life cycle requires that they alternate between a vertebrate and invertebrate host. For prevention, as in the case of any other parasitic disease, general measures will be applied, such as removing dead fish every day (as they are a major source of spores), changing the habitat where the intermediate hosts live, preventing actinospora from entering the installation (UV Rays, filerting and ozone), selection of fish strains that are increasingly resistant to parasitosis and the administration of diets that stimulate the immune system in periods of risk.

Enteromyxum (Myxidium) leei and *Enteromyxum scophthalmi*

Definition: *Enteromyxum leei* acts as a parasite on more than 45 marine species and produces a severe pathological process in some farmed fish of great commercial interest , such as *Sparus aurata*, *Diplodus puntazzo*, *Pagrus* spp. and *Pagellus* spp. Moreover, the susceptibility of various freshwater species has been shown, such as *Puntius tetrazona*, *Danio rerio, Astronotus ocellatus* and *Oreochromis mossambicus* by means of experimental inoculation. The fact that it is so easy to transmit makes the disease even more important in the temperature range of between 15 and 20ºC. The turbot becomes infested with –*Enteromyxum scophthalmi***,** in a very serious process that may lead to the destruction of 100% of the farmed fish. Both parasitations produce

desquamative enteritis that provokes ematiation and abdominal distension. *E.leei* and *E. scophtalmi* are directly transmitted from fish to fish by cohabitation, contact with water and the ingestion of material with parasites. The infectious stages are not the spores, but the cells present in the development phases in the intestine. The parasites are liberated together with the rest of the intestinal epithelium in the faeces and remain viable for short periods of time (24 hours). These infectious cells can easily pass the parasites on to other individuals nearby given the intensive conditions of the farm. *Enteromyxum leei* and *E. scophthalmi* are thought to have a life cycle that is similar to other mixosporidios, i.e. through an invertebrate in which the infectious spores develop. In bream, it mainly affects adult fish (>100 gr), causing chronic mortality, although the accumulated loss can reach important levels if the temperature is high. The incidence of the disease will depend on various factors, the most important of which are high temperature (19-20 ºC and above) and the recirculation of water (in land-based systems). The latent period is approximately 3 months at 20 ºC. This process is considered serious in bream, so the elimination of the cage affected is recommended as soon as the problema is detected, as the problema may spread to other production units. There is no cure, although the symptoms can be treated with stimulants for the immune system. Given the seriousness of the process (particularly in turbot), the application of effective preventative measures is imperative. These measures include early diagnosis and the rigorous elimination of the affected batches, analysis before introducing new fish from areas of risk and the search for stock that is resistant to the parasitosis.

Susceptible species for *Enteromyxum leei*: *Sparus aurata, Diplodus puntazo,* and other marine and continental fish as *Sparidae, Mugilidae, Labridae, Centracanthidae, Mullidae, Pomacentridae, Scorpaenidae, Molidae, Blenniidae, Gobiidae, Tetraodontidae, Oplegnathidae, Paralichthyidae, Danio rerio, Puntius tetrazona, Astronotus ocellatus, Oreochromis mossambicus* and, less frequently, other unspecified species.

Vector species for *Enteromyxum leei*: *Dicentrarchus labrax* and, less frequently, other unspecified species.

Geographic distribution of *Enteromyxum leei*: Cyprus, Croacia, Spain, France, Greece, Italy, Malta, Turkey, Israel and Japan, although there may be other areas where its presence is not well-documented.

Susceptible species for *Enteromyxum scophthalmi*: *Scophthalmus maximus*, *Solea senegalensis* and, less frequently, other unspecified species

Geographic distribution of *Enteromyxum scophthalmi*: Atlantic Europe, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Tetracapsuloides byosalmonae

Definition: The agent that causes Proliferative Kidney Disease (PKD) is *Tetracapsuloides byosalmonae*. PKD is a serious disease that affects salmonids across Europe and North America. The infection starts when the spores enter the fish through the epithelium of the skin and gills. The extra- sporogonic phase proliferates rapidly in the

fish, and reaches almost all organs by means of the blood. In the kidney and spleen, both the proliferation of this phase and the host's response are much more intense, causing typical injuries: an intense inflammation of the kidney and spleen, with granulomatous injuries. On occasions, the parasite manges to reach of the kidney tubule, and this is where the sporagonic phase starts, where the spores are produced. PKD occurs mainly at temperatures of above 15 ºC. The infection occurs in spring and the parasites and injuries can normally be seen some 4 weeks after infection. Topical external injuries are melanosis (darkening), bilateral exophthalmia and anaemia. Internally, an intense inflammation of the kidney and spleen is observed, which may sometimes present granuloma.

Susceptible species: *Salmonidae* and, less frequently, other unspecified species

Geographic distribution: Europe, USA and Canada, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Myxosoma (Myxobolus) cerebralis

Definition: This is the agent that causes whirling disease, a serious process that affects salmonids in Europe and America. The intermediate host where actinospores will develop are worms belonging to the *Tubificidae* family, mainly *Tubifex tubifex*. This type of oligoquetos live buried in the earth and mud at the bottom of the tanks. *M. cerebralis* is a myxosporidian that has a tropism in the cartilage of salmonids, and deformities are produced once the cartilage ossifies. Sometimes, a darker colouring of the fish tail can be seen, due to the pressure of the deformed bone on the nerves that control the pigmentation in this area. Currently, this problem has practically disappeared in modern fish farming, as the populations of tubificids are being eliminated in industrial installations (there are hardly any installations with tanks that have earth at the bottom any more).

Susceptible species: *Salmonidae* and, less frequently, other unspecified species

Geographic distribution: Europe and USA, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Sphaerospora testicularis

Definition: This is a parasitosis that only affects the testicle of the sea bass producing testicular esferosporiasis. Sphaerospora testicularis is a parasite that develops in the seminiferous tubules and absorbs spermatozoids. It will cause granulomatous lesions and necrosis, which produces a parasitic castration of the males. Prevalence is high, up to 80%, in cages. It appears not to cause mortality, although it may increase the susceptibility to different bacterial infections. The life cycle is unknown, and may include an intermediate host like other mixosporidios. Direct transmission cannot be ruled out.

Susceptible species: *Dicentrarchus labrax*

Geographic distribution: Mediterranean Europe, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Sphaerospora dicentrarchi

Definition: *Sphaerospora dicentrarchi* affects the connective tissue of the sea bass, so can be found in various places. Prevalence is very high (80-100%) and the intensity of the parasitosis increases with the age of the individual. High rates of mortality have been described in sea bass of 2g. Fish shows signs of nerves, anorexia and delayed growth.

Susceptible species: *Dicentrarchus labrax*

Geographic distribution: Mediterranean Europe, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.9-*Phylum Plathyelminthes*: Monogenea Class

Definition: The Monogeneas are flat worms found in fresh and salt water. They all have a direct life cycle. Although the vast majority are ectoparasites (skin, gills, fins, oral cavidad and nostrils), some Monogeneas have adopted an endoparasitic life (bladder and urine tracts, stomach and even cloacae). Generally speaking, Monogeneas are very species specific. At least one Monogenea species is suspected to exist for each fish species. Different chemical products have been used to control monogenea, both in fresh water and in salt water. The most frequently used products in bath therapies are copper sulphate copper sulphate, formol, sodium chloride, hydrogen peroxide and potassium permanganate. The biological cycle of monogenea can be interfered with by

filetering the water in recirculation systems, thereby avoiding the reintroduction of eggs. Given that the eggs of many monogeneas have filaments to enable them to adhere to the substrates, they often become anchored in the nets and ropes used in floating cages. For this reason, thorough cleaning and disinfection of this material helps to limit reinfection in cages.

The most common genera of this *Phylum* in farmed species are:

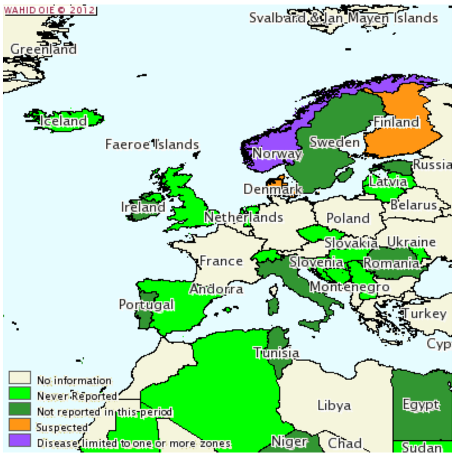
- *Continental aquaculture*
  - *Gyrodactylus*: important in the farming of rainbow trout
  - *Dactylogyrus*: most frequent in Cyprinids
- *Marine aquaculture*
  - *Diplectanum aequans and Diplectanum laubieri*
  - *Furnestinia echeneis*: in bream.
  - *Entobdella soleae*: in common sole
  - *Serranicotyle labracis*: in sea bass.
  - *Sparycotyle chrisophrii*: in bream.
  - *Zeuxapta seriolae*: in seriola.

Gyrodactylus salaris

Definition: Girodactilosis is the infestation by the viviparous fresh water ectoparasite *Gyrodactylus salaris* (*Phylum Platyhelminthes; Class Monogenea*). Gyrodactilosis mainly affects *Salmo salar* in all stages of the salmon's life in fresh water, including spawning adults, although the disease and mortality have only been observed in juvenile stages prior to the development of scales and before migration to marine water. In the initial stages of the disease, the fish will often scratch against the substrate. Subsequently, it turns a greyish colour due to an increase in the production of mucus and the fins may become eroded. Diseased fish are lethargic and are generally found in slow-moving water. The parasite is mainly found in the fins, although it may spread to the rest of the body, including cornea and nostrils. The mortality rate in farmed species can reach 100% if the disease is not treated, and in rivers, decreases in the population of up to 98% have been observed. It is a Disease of Obligatory Declaration for the OIE, but not for the European Union. There are various chemical products for treatment by immersion, such as formaldehyde in seawater, although this does not generally eliminate all the parasites. Recent results have shown that oral treatment with triclabendazole in food (40 g per kg. of food for 10 days) and with nitroscanate (>0.6 g per kg. of food per day) can eliminate an infection. In terms of the preventative measures, each production unit should be equipped for the clearing and handling of fish independently of other units. The equipment used to transport the fish or that has been in contact with infected fish must be dried, frozen or disinfected (for example using 1 % Chloramine -T [Halamid]). In farming conditions, it is properly controlled using formol baths.

Susceptible species: *Salmo salar*, *Oncorhynchus mykiss*, *Salvelinus alpinus*, *Salvelinus fontinalis*, *Thymallus thymallus*, *Salvelinus namaycush*, *Salmo trutta* and, less frequently, other unspecified species Other fish species living in waters where the parasite survives are also potentially dangers as these species may transmit the parasite and act as potential vectors, so they must be taken into consideration.

Geographic distribution: The distribution of *Gyrodactylus salaris* is very limited to Europe, although it has recently been described in Costa Rica. It has mainly been found in farms of *Salmo salar* and rainbow *Oncorhynchus mykiss* in northern European countries such as Norway, Sweden, Finland, Denmark, Russia, Germany, Italy and the Czech Republic, although there may be other areas where its presence is not well-documented. Great Britain and Ireland appear to be free from this parasite. The following map shows the notifications of outbreaks of the disease during the last final months of 2011 in Europe (OIE, 2012).



N.B.: Although this disease is not listed in Directive 2006/88 EC, it is subject to national measures to limit its impact on aquaculture and wild aquatic animals, in accordance with article 43 of Directive 2006/88 EC in Ireland, Finland (water catchment areas of the Teneyeki and the Näätämönjoki, the water catchment areas of the Paatsjoki, the Lutteyeki and the Uutuanjoki), Great Britain, Northern Ireland, Jersey and Guernsey. Close external viewing of the fish is recommended to rule out parasitation by this agent.

<u>*Diplectanum aequans* and *Diplectanum laubieri*</u>

Definition: These are gill parasites of the sea bass. They measure between approximately 0.53-1.45 mm in length. In massive infestations several hundred parasites may appear in one host. They will provoke a very intense inflammatory response in the gills that may jeopardise the fish's breathing. They are oviparous monogenea that lay pyramid-shaped eggs, with a short filament that they use to hook onto different objects. They respond well to treatments using disinfectants, although it may be unwieldy to apply them in floating cages and there is considerable risk involved. As far as prevention is concerned, it is important to clean and disinfect the nets thoroughly, as the eggs normally get stuck to them.

Susceptible species: *Dicentrarchus labrax* and, less frequently, other unspecified species

Geographic distribution: Mediterranean and Atlantic coast, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Furnestinia echeneis

This is a gill parasite that is very common in bream. It appears in the gills and, in intense infestations may also be found in the internal face of the opercula. It is similar in size to *Diplectanum* sp. Eggs are very similar to those of *Diplectanum sp*. Control and prevention is similar to those of other monogenea. Infestations may be serious mainly in spring and summer, and a high level of chronic mortality is reached. The predisposing factors and environmental stress play a decisive role in outbreaks (high densities, infrequent water renewal in cages that are blocked up with algae, high temperature, previous infections, etc.)

Susceptible species: *Sparus aurata* and, less frequently, other unspecified species

Geographic distribution: Mediterranean coast, Atlantic coast and Red Sea, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Entobdella soleae

This parasite affects *Solea solea*. The adult parasite measures between 2 and 5 mm long. It is found in the skin: the initial part of the body of the sole is used in early developmental stages and subsequently the parasite migrates towards ventral areas in order to reproduce. The eggs are tetrahedral in shape, and have stick glands to adhere to grains of sand and other substrates. Parasitosis is controlled well by formol baths.

Susceptible species: *Solea solea*

Geographic distribution: Atlantic Europe, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Sparycotyle chrisophrii / Serranicotyle labracis / Microcotyle sp.

These parasites are commonly found in *Sparus aurata* (*Sparycotyle chrisophrii and Microcotyle* sp.) and very rarely in sea bass (*Serranicotyle labracis*). Adult parasites are visible to the naked eye, and measure up to 5 mm. They tend to appear in large numbers and may cause serious anaemia, as well as irritation in the gills and breathing problems. The parasitosis responds well to treatments with formol, although this treatment is risky in floating cages. Thorough cleaning and disinfection not nets can help to limit the dispersion of parasites in the cage. The histopathological effects of this *Sparycotyle chrisophrii* centre on the gills, where it produces a shortening and fusion of the laminae, and proliferation of the epithelial tissue can be seen, leading to hypochromic anaemia.

Susceptible species: *Sparus aurata, Dicentrarchus labrax*, *Diplodus puntazzo* and, less frequently, other unspecified species

Geographic distribution: Atlantic, Cantabrian and Mediterranean coasts, although there may be other areas where its presence is not well-documented.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

I.3.3.10.-*Phylum Platyhelmintes*: Trematoda Class,Digenea sub-Class

Platyhelminthes of the digenea sub-class include some 70 families with 1700 parasite species in their adult state. They are heteroxenous parasites, i.e. they need more than one intermediate host to complete their biological cycle. With just one exception, Digenea complete one or all the larval stages in a mollusc. The definitive host will always be a vertebrate. The fish can also act as intermediate hosts or paratenic hosts for metacercariae. Digenea are basically parasites of the digestive tract, although they may be found in very varied places (skin, gills, muscle, gallbladder, swimbladder, body cavity, bladder and even the circulatory system). The size of digenea varies from 250 µm to 10 cm in length, and there is a species that reaches 12 m in length in the muscle of the *Mola mola* fish. However, the vast majority of digenea tend to measure between 0.5 and 5 mm long. The life cycle of digenea is complex (involving at least two hosts) and has free-living as well as parasitic stages. The most effective way of preventing parasitosis by digeneas is the elimination of intermediate hosts, mainly molluscs, using molluscicide substances such as copper sulphate copper sulphate. As far as treating the fish is concerned, praziquantel, ivermectin and masoten have been tried, although checks are required to see if the product is authorised in each country.

Sanguinicolidae Family

These are digenea trematodes that cause the white gill syndrome. Adult parasites live in the heart and the kidney of the bream. They liberate the eggs that will then circulate in the blood until they get caught up in the fine branquial blood vessels. When the eggs break and the larva forms break free, they provoke severe haemorrhages. The full biological cycle is not known, although a mollusc is suspected of being an intermediate host. It mainly affects juvenile bream, and is particularly serious in on land installations and floating cages in closed bays. Fish normally suffer from serious anaemia, and the gill appears a whitish colour. Moreover, as a result of the damage to the gills, breathing symptoms may appear. There is not cure. The only thing one can do is to treat the symptoms by administrating food with vitamin supplements and stimulants for the immune system.

Susceptible species: *Sparus aurata* and, less frequently, other unspecified species

Geographic distribution: Presence is not well-documented

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Diplostomum Genus

This is a process that can affect species of continental and marine fish, and is produced by different species of the *Diplostomum* genus, producing ocular displostomiasis. Larval stages of the parasite penetrate the eye of the secondary host (fish), where they form cysts, making the fish go blind, making them easier to catch by fish-eating birds, the definitive hosts.

I.3.3.11.-*Phylum Platyhelminthes*: *Cestoidea* Class

There are some 5000 species of cestodes, 1400 of which occur in fish. Adults live in the gastrointestinal tract, although on occasions they can be found in organs associated with the digestive tract. Cestodes have no digestive system and absorb nutrients through a highly specialized external cover (neodermis). The most important species are the *Diphyllobotrium latum* and plerocercoids. The first is only a problem in man if contaminated fish are consumed.

Diphyllobothrium latum

Two intermediate hosts are described in its cycle, freshwater crustaceans (copepod) and freshwater fish that feed on the crustaceans, although salt water fish that develop part of their cycle in fresh water like salmonids may also intervene.

Geographic distribution: Worldwide, although they are more prevalent in the northern Hemisphere, especially where food that is only slightly cooked (marinaded, etc.) is consumed.

N.B.: No measures for movement of fish exist, but the carrying out of random sampling of fish to rule out parasitation by this agent is recommended.

Other Plerocercoids

Effects on fish depend on the intensity of the parasitation and the organs affected. Plerocercoids in muscles, the liver and other viscera of the fish can survive for a long time, and pass on from prey to predator and form cysts again. If they are found in the gonads, they can cause sterility, but the major importance of these larval phases is that, through raw or only slightly cooked infected fish, they can reach humans, where they cause diphyllobothriasis.

I.3.3.12.-*Phylum Nematoda*

Nematodes are divided into 256 families and more than 40,000 species. Despite the fact that most of them are free living, some of them are parasites on plants and animals.

Anisakidae Family

In marine fish, infections caused by nematod larvae are mainly produced by Anisakis species (Ascaridida, Anisakidae) whose definitive hosts are fish-eating vertebrates. Half way through the twentieth century, the financial losses produced by the presence of these larvae in the muscle of the cod and their presence detected in the gastrointestinal tract of man determined the onset of the study of the biology of these parasites. The genera responsible for infection in man are *Anisakis*, *Pseudoterranova* and *Contracaecum*. Anisakidosis is a parasitic zoonosis produced by larvae III of nematods of the Anisakidae Family whose definitive hosts are marine mammals. These larvae, encapsulated in the viscera and in muscle tissue of fish and cephalopods, can be ingested by man on accidentally consuming raw or insufficiently cooked fish carrying parasites. In marine fish, infections for larvae III of nomatods most often found in the fish that are commonly consumed belong to the species *A, simplex, P.decipiens* and *C. osculatum* of 17-50 mm in length and 1-2 mm in diameter. In the biological cycle of these nematods, crustaceans participate as intermediate hosts and fish-eating vertebrates as definitive ones. The fish and occasionally cephalopods and other invertebrates, can enter the cycle as paratenic hosts or transport for the larvae. From these hosts, the larvae may reach humans when they eat raw fish containing parasites, and they may develop and even partially mature (L4), and produce alterations in the gastrointestinal wall. The definitive hosts are marine mammals, such as dolphins, whales, seals and sea lions, where adult Anisakis live in their stomachs. The eggs of these nematods are released at sea, where a second stage larva emerges. When these larvae are ingested by intermediate hosts, small crustaceans, they develop intolarvae III and remain encysted there until the crustacean is eaten by a fish of cephalopod like a squid. In these new hosts, the larva III develops still further in the muscles or in the body cavity. When these larvae move along the food chain from one fish to another, the number of larvae increases but there are no morphological changes (these hosts therefore constitute transport). The life cycle comes to an end when the fish or cephalopod is eaten by a marine mammal, in which the nematod develops. Humans are accidental or non-susceptible hosts, hence no maturation takes place in their tissues. They are typical nematods, from 1 to 6 cm long, and if eaten live by humans, can penetrate the gastrointestinal tract and cause acute inflammation. After ingestion, the larvae migrate from the stomach to different normally subcutaneous parts of the thorax, arms, head and neck, where nematods provoke a sensation of tingling and oedemas. The larvae infective can be destroyed quickly at 60 ºC and they are known not to be able to survive at - 20 ºC for 24 hours. Therefore, infection can be prevented either by heating it up enough or keeping it at -20 ºC before consumption. Recently it has been proved that ingestion of infected fish with larvae of *A. simplex*, that have died either from the heat or from freezing, may produce acute hives or even anaphylaxis. The allergic reaction appears in the first hours after eating the infected cooked fish and is mediated by IgE antibodies through temperature-resistant antigens (that resist the heat and freezing) of larvae of the parasite. Hake and anchovy are the main types of food associated with allergy for anisakis.The European Food Safety Authority (EFSA) said that Atlantic salmon when kept in cages or in tanks on land and are fed with feed compounds free of parasites, risk of contaminating the fish with Anisakis is negligible. However, for other

aquaculture species as gilthead seabream, sea bass, etc, the risk is unknown.

Anisakis Genus

Hosts: *Gadus morhua*, *Merlangius merlangus*, *Gadus poutassou*, *Sardina pilchardus*, *Engraulis encrasicolus*, *Trachurus* sp., *Scomber scombrus*, *Micromesistius poutassou*, *Pollachius virens*,*Trisopterus luscus*,*Trisopterus luscus*, *Percophis brasiliensis*, *Merluccius* sp. *Albula vulpes*, *Brama brama*, *Pagellus* sp. *Scomber scombrus*, *Trichiurus lepturus*, *Pleuronectes platessa*, *Chelidonichthys cuculus*, *Conger* sp. and, less frequently, other unspecified species.

Pseudoterranova and *Contracaecum Genus*

Hosts: *Gadus morhua* and *Conger conger*, and, less frequently, other unspecified species

Other nematods of interest

Those of interest for public health, such as *Capillaria philippinensis*, adults of which are intestinal parasites of fish-eating birds and as intermediate hosts, are small freshwater fish. Infestation in humans causes serious diarrheal and even death from dehydration. *Angiostrongylus cantonensis* is well-known and common in Asia; adult individuals are found in the lungs of rats and intermediate hosts include snails, fresh water king size prawns and giant freshwater prawns and land crabs. They can cause meningitis in humans.

I.3.3.13.-*Phylum Acanthocephala*

Definition: These are a small *phylum* of highly specialised parasites of the digestive tract of vertebrates. Many cases of serious damage in fish carrying acanthocephalan parasites have been reported. They have been responsible for mass mortality both in nature and in farms. *Acanthocephalae* can cause severe damage to the intestine of their hosts. They can completely perforate the intestine, which gives rise to serious problems, including secondary bacterial infection that can lead to peritonitis, haemorrhages, pericarditis, myocarditis, arteritis and other problems. Although this perforation may occur, normally a small inflammatory response to the penetration of the proboscis is produced. Currently, the most effective measures aim to eliminate the crustaceans that act as intermediate hosts using organophosphate, although the adoption of these measures is not very safe for the environment. Given the biological cycle of these parasites, that need the collaboration of smaller fish, parasitosis by acanthocephalae is fairly infrequent in today's fish farms, as food is based on compound fodder and, therefore, there are no intermediate hosts to closet the cycle. The Acanthocephalae are common fish parasites, and the Phylum Acantocephala comprises four Classes:

- *Archiacanthocephala*
- *Eoacanthocephala*: *Neoechinorhynchus*
- *Palaeacanthocephala*: *Echinorhynchus Pomphorhynchus* and *Acanthocephalus*
- *Polyacanthocephala*

The *Echinorhynchus and Acanthocephalus* genera are perhaps those with the largest geographic distribution. They represent a serious threat for the development of fish both in the wild and in industrial farms. This is because these parasites get deep into the intestinal epithelium of fish, producing a injury in the enteric wall that is much more serious than that caused by other intestinal parasites such as the cestodes. The number of these parasites tends to be very high (up to 300 or more), and the intestine often becomes completely blocked.

The most common freshwater species are:
- *Pomphorhynchus laevis*
- *Neoechinorhynchus rutili*
- *Acanthocephalus lucii* and *Metechinorhynchus truttae*

I.3.3.14.- *Phylum Arthropoda*

These are parasites with bilateral segmented symetry whose tegument is covered with chitin, and sometimes calcified, which implies strong, grooved articulations and muscles, articulated appendages and a discontinuous growth as a result of molts. There are some 900,000 species and those that affect fish are found as crustaceans, the most important of which are the following:

Lepeophtheirus salmonis

Definition: The sea louse (*Lepeophtheirus salmonis*) is a marine copepod that, although it does not constitute a disease per se, promotes the contagion of other diseases, as it produces wounds in the fish's skin and depresses their immune system. This plague can produce a serious loss of biomass in the farming of salmon given the susceptibility it generates in the fish. Although *Lepeophtheirus salmonis* is a parasite commonly found in wild salmonids, the concentration and/or number of individuals in which it is found in wild fish is much less than the levels recorded at increased fish density, as occurs in farms. Despite the fact that it is not actually confirmed, *Lepeophtheirus salmonis* is suspected to be a vector for contagion of the infectious salmon anaemia virus. Moreover, as these parasites weaken the immune system of the fish, they favour the spreading of any other infectious disease.

Susceptible species: *Salmo salar*, *Oncorhynchus mykiss*, *Oncorhynchus kisutch*, *Oncorhynchus gorbuscha*, *Oncorhynchus keta*, *Odonthestes regia*, *Paralichthys microps*, *Eleginops maclovinus* and, less frequently, other unspecified species

Geographic distribution: Chile, Norway, Scotland, Faeroe Islands, Ireland, Japan and Canada, although there may be other areas where its presence is not well-documented.

N.B.: No measures for the movement of fish, but close external viewing of the fish is recommended to rule out parasitation by this agent.

Lernaea sp.

Popularly known as the "anchor worm", it receives this name because of the instruments it uses to hook onto its hosts. The parasite invades the skin of the fish affected, normally at the base of one of its fins. They anchor onto the epidermis by means of hooks that grow on their head, creating a perfectly visible ulcer around the crustacean. Only the female is a parasite, in the form of a worm with two long sacks with eggs. The sores and wounds take a long time to heal. The virulence of the infestation depends on the number of parasite and extent of the secondary invasion of wounds by bacteria and fungi. Initial fixation of the parasite often causes swelling and a reddening, even when the organisms are not visible. Diseased fish rub up against the nets or tank walls. Scales may be raised, inflamed or cloudy. The parasite can be seen by the naked eye or with a magnifyng glass, in the form of whitish worms hanging from the fish's body.

Argulus sp

The typical species is *Argulus foliaceus*, mainly found in ciprinids. The females are bigger than the males, and both are parasites. Their preferred place are the most irrigated parts of the skin (mouth, fin base, etc). The parasite fish shows red dots of 1mm in diameter on the skin where the crustacean has been stung. If there are lots of them, reddened and cloudy areas can be seen due to an excessive secretion of mucus. They are clearly visible to the naked eye. Wounds caused by their legs, which constantly move during fixation cause the mucus and epithelia tissue to fall away which leaves the fish exposed to secondary diseases, (bacteria and fungi).

Ceratothoa ostreoideszed

This is an isopod that causes serious losses in Greek fish farming, basically in the farming of *Dicentrarchus labrax*, although it may also affect *Sparus aurata*. Adult isopods are blood-sucking and can provoke serious anaemia. Moreover, the fact that they live in the mouth cavity of the host can seriously jeopardize the fish's capacity to feed, leading them to lose weight gradually. In small fish, the larvae may lodge in the head, skin and gills, where they provoke serious ulcers that may then be colonized by opportunist bacterial agents (*Vibrio spp.*, *Tenacibaculum spp.*, *Aeromonas spp*.). The fish affected tend to be dark in colour, thin and can suffer breathing problems. The simplest treatment is to eliminate the larvae one by one of young fish while they are being vaccinated, although bath in hydrogen peroxide baths at a strength of 1500 ppm for 20 minutes and formol at 150 ppm for 60 minutes. The best way of preventing massive infestations from isopods is by reducing the farming density and putting the cages that contain juvenile fish as far as possible from cages with older fish, where adult forms of the parasite are found and where the larvae are liberated from.

I.3.4.- Fungal diseases of interest for AQUAEXCEL

I.3.4.1.-Saprolegnia

Definition: Fungal infection of the fish and gills produced by fungi that belong to the Saprolegnia Orden. Once the fungus has invaded the skin, it is established in a localised form invading the stratum spongiosum of the dermis spreading laterally over the epidermis. This determines an imbalance in the organic fluids and a peripheral circulatory failure (shock), given the impossibility of maintaining the volume of blood in circulation. The disease normally presents a chronic process and bacterial complications may appear. Infection in salmonids normally has a more acute process, and may lead to death in thirty days. It is characterized by the appearance of whitish-grey in the skin and gills of the fish. Under the water they look cottony, initially circular, and in latter stages the stains take on a dark colour and grow radially until they take shape in the periphery. Some cases of infections affecting internal organs arising from earlier wounds in the epidermis (intestine, peritonea) in which ulcers are observed.

Susceptible species: *Salmo salar*, *Orconectes propinquus*, *Carassius auratus*, *Danio rerio*, *Salmo trutta*, *Ictalurus punctatus* and, less frequently, other unspecified species

Geographic distribution: Worldwide

N.B.: No special measures for the movement of fish exist, but no fish with injuries compatible with this disease may be moved

I.3.5.-Other diseases of interest for AQUAEXCEL

I.3.5.1.-Nephrocalcinosis

Definition: Nephrocalcinosis is a non-infectiuos kidney pathology characterised by the deposit of calcium and magnesium salts in the kidney and other tissues, associated with a degeneration and chronic inflammatory response. In the symptom-free form of the disease, no external clinical signs are observed, and often only kidney histopathological injuries or the presence of localized deposits in the kidneys are observed. As the diseases progresses, the external clinical signs are characterized by abdominal distention, exophthalmia and death. Internally, affected fish have pale livers, ascitis, pale kidneys and variable calcification of the kidney. Exposure to high levels of CO_2 initially produces a reduction of pH in the blood, which in turn diminishes the affinity of the haemoglobin To transport oxygen. Chronic exposure to high levels of CO_2 produces mineral deposits in all organs, mainly the kidney, made up mainly of calcium phosphate. Adverse effects due to dissolved CO_2 between

20 mg/L and 45 mg/L, but the nephrocalcinosis starts to be expressed when CO_2 levels exceed 12 mg/L. Moreover, the CO_2 tends to be an important handicap for the transport of live fish, as it accumulates progressively as the fish breath and it is a factor to be taken into account in these cases.

This affection is common in salmonids farmed in open and closed flow systems, and the *O. Mykiss* is the most susceptible species. The appearance of the clinical process depends on the level of CO_2 and exposure time. In subclinical cases, the only obvious sings are delayed growth and an increase in conversion rate. In the clinical presentation of the disease, the fish affected are lethargic, swim near to the surface, bilateral exophthalmia are observed externally, distension of the abdomen and in some cases anal protrusion, with severe cases leading to death. Internally, the fish present ascites, a pale liver, pale kidneys, enlarged kidneys, and sometimes calcium deposits in kidney tubules, ureters and interstitial tissue. The main consequences of this affection include increased predisposition to infections such as Flavobacteriosis or Infectious pancreatic necrosis.

The clinical disease can be diagnosed by the presence of different shaped calcareous deposits in the kidney. The subclinical affection is diagnosed using histology or by measuring blood parameters such as creatinine, an indicator of how the kidneys are working

Susceptible species: *Salmonidae* and, less frequently, other species as sea bass.

I.4.- Diagnosis of fish diseases

Directive 2006/88 EC includes a list of pathogens to be watched, based on the scant possibility of their responding appropriately to therapy, a restricted geographic distribution, the high social and economic importance and that they occur in species transferred for commercial reasons. However, the legislation in force allow disease that do not appear in the official lists to be surveyed, such as emerging diseases with the potential to cause significant mortality. Therefore, this chapter contemplates diagnosis methods for a wide range of pathogens, based both on the official lists of the European Directive 2006/88 EC, and on the questionnaire handed in by the research centres participating in AQUAEXCEL Project.

The diagnosis of fish diseases included in the list of Directive 2006/88 EC is carried out using standardised protocols following the recommendations of European legislation, the OIE or the national Benchmark Laboratories in fish diseases. In terms of (exotic and non-exotic) fish diseases not listed in Directive 2006/88EC we have followed the protocols specified in different scientific publications of reference.

Table 3.- Methods of diagnosis for viral diseases

Virus	Diagnosis	Reference
Epizootic haematopoietic necrosis	Isolation of virus Neutralization Immunofluorescence ELISA, Sequencing, PCR	OIE 2010
Spring viraemia of carp	Isolation of virus Neutralization Immunofluorescence (IF) ELISA, PCR Sequencing	OIE 2010
Koi herpes virus disease	Isolation of virus Neutralization ELISA, PCR Isothermal DNA Amplification Reverse mRNA Transcription Indirect immunofluorescence Histopathology, Sequencing Hibridization *in situ* Electron microscopy	OIE 2010
Infectious haematopoietic necrosis	Isolation of virus Neutralization Immunofluorescence ELISA, Sequencing RT-PCR	OIE 2010 CE 2001
Nodavirus	Isolation of virus Neutralization Histopathology Electron microscopy RT-PCR, Sequencing Tissue inmunohistochemistry Indirect immunofluorescence ELISA	OIE 2010
Infectious salmon anaemia	Immunofluorescence Immunohistochemical tests Isolation of virus RT-PCR, ELISA	OIE 2010
Infectious pancreatic necrosis	Isolation of virus Neutralization Immunofluorescence ELISA, Sequencing RLFP, RT-PCR	OIE 2010
Viral haemorrhagic septicaemia	Isolation of virus Neutralization Immunofluorescence ELISA, Sequencing RLFP, RT-PCR	OIE 2010 CE, 2001
Red sea bream Iridovirus	Isolation of virus Neutralization, Immunofluorescence Electron microscopy , Sequencing PCR	OIE 2010

References

OIE (Office International des Epizooties), 2010. Manual of Diagnostic Tests for Aquatic Animals 2010
http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online/

CE (2001/183/CE), 2001. Decisión de la Comisión, de 22 de febrero de 2001, por la que se establecen los planes de muestreo and los métodos de diagnóstico para la detección and confirmación de determinadas enfermedades de los peces and se deroga la Decisión 92/532/CEE.

Table4.- Taking of samples for viral diseases

Organ/tissue to be sampled for viral diseases	Epizootic haematopoietic necrosis	Viral haemorrhagic septicaemia	Infectious salmon anaemia	Spring viraemia of carp	Infectious haematopoietic necrosis	Red sea bream Iridovirus	Koi herpes virus disease	Nodavirosis	Infectious pancreatic necrosis
Spleen	X	X		X	X	X	X		X
Gill			X	X			X		
Brain		X		X	X		X	X	
Heart		X	X		X	X			
Seminal fluids		X			X			X	
Liver	X			X		X			
Intestine				X			X	X	
Musculature affected									
Anterior kidney	X	X		X	X	X	X		X
Middle kidney			X						

Table 5.- Diagnosis methods for bacterial diseases

Bacteria	Diagnosis	Reference
Aeromonas spp	Culture ELISA Latex agglutination PCR RAPD-PCR	McCarthy, 1975 Adams and Thompson, 1990 Gustafson *et al.*, 1992 Oakey *et al.*, 1998 Byers *et al.*, 2002 Austin and Austin, 2007
Vibrio spp	Culture PCR DNA microarray	Dorsch *et al.*, 1992 González *et al.*,2004 Gonzalez *et al.*,2003 Holt, 1970 Maugeri *et al.*, 1983 Rehnstam *et al.*,1989 Alsina *et al.*,1994 Martínez-Picado *et al.*,1994 Tiainen *et al.*,1994
Yersinia ruckeri	Culture ELISA PCR	Altinok I *et al.*, 2008 Onuk *et al.*, 2011 Onuk *et al.*, 2010 Tobback *et al.*, 2007 Welch *et al.*, 2011
Moritella viscosa	Culture PCR Immunohistochemical tests	Grove *et al.*, 2010 Grove *et al.*, 2008 Benediktsdóttir *et al.*, 2007 Benediktsdóttir *et al.*, 2000
Photobacterium damselae subsp. *piscicida*	Culture PCR Sequencing	Osorio *et al.*,2000 Romalde *et al.*,2002 Thyssen *et al.*,1998 Wang *et al.*,2007 Zappulli *et al.*,2005
Renibacterium salmoninarum	Culture in KDM-2 PCR ELISA	OIE, 2003
Streptococcus spp	Culture PCR Sequencing LAMP	Geng et al. 2011 El Aamri et al., 2010
Piscirickettsia salmonis	Cellular culture Smear Indirect Immunofluorescence ELISA PCR	Fryer *et al.*, 1990 Lannan & Fryer, 1991 Mauel et al., 1996
Cytophaga – Flavobacterium – Bacteroides group	Culture PCR Smear	Orieux *et al.*,2011 Touchon *et al.*,2011 Högforsy Wiklund, 2010 Fujiwara *et al.*,2009 Valdebenito *et al.*,2009 Cain and Lafrentz, 2007 Pilarski *et al.*,2008 Altinok *et al.*,2008 Arai *et al.*,2007

References

Dorsch M, Lane D. Stackebrandt E.cTowards a phylogeny of the genus Vibrio based on 16S rRNA sequences. Int J Syst Bacteriol. 1992;42(1):58-63.

González SF, Krug MJ, Nielsen ME, Santos Y, Call DR. Simultaneous detection of marine fish pathogens by using multiplex PCR and a DNA microarray. J Clin Microbiol. 2004;42(4):1414-9.

Gonzalez SF, Osorio CR, Santos Y.Development of a PCR-based method for the detection of Listonella anguillarum in fish tissues and blood samples. Dis Aquat Organ. 2003. 8;55(2):109-15.

Holt G. Vibriosis (Vibrio anguillarum) as an epizootic disease in rainbow trout (Salmo gairdneri).Acta Vet Scand. 1970;11(4):600-3.

Maugeri TL, Crisafi E, Genovese L, Scoglio ME.Identification of Vibrio anguillarum with the API 20 E system. Microbiologica. 1983;6(1):73-9.

Rehnstam AS, Norqvist A, Wolf-Watz H, Hagström A.Identification of Vibrio anguillarum in fish by using partial 16S rRNA sequences and a specific 16S rRNA oligonucleotide probe.Appl Environ Microbiol. 1989;55(8):1907-10.

Alsina M, Martínez-Picado J, Jofre J, Blanch AR. A medium for presumptive identification of Vibrio anguillarum.Appl Environ Microbiol. 1994 ;60(5):1681-3.

Martínez-Picado J, Blanch AR, Jofre J. Rapid detection and identification of Vibrio anguillarum by using a specific oligonucleotide probe complementary to 16S rRNA. Appl Environ Microbiol. 1994;60(2):732-7.

Tiainen T, Larsen JL, Pelkonen S.Characterization of Vibrio anguillarum strains isolated from diseased fish in Finland. Acta Vet Scand. 1994;35(4):355-62.

Grove S, Wiik-Nielsen CR, Lunder T, Tunsjø HS, Tandstad NM, Reitan LJ, Marthinussen A, Sørgaard M, Olsen AB, Colquhoun DJ. Previously unrecognised division within Moritella viscosa isolated from fish farmed in the North Atlantic. Dis Aquat Organ. 2010. 7;93(1):51-61.

Grove S, Reitan LJ, Lunder T, Colquhoun D.Real-time PCR detection of Moritella viscosa, the likely causal agent of winter-ulcer in Atlantic salmon Salmo salar and rainbow trout Oncorhynchus mykiss. Dis Aquat Organ. 2008. 20;82(2):105-9.

Benediktsdóttir E, Heidarsdóttir KJ.Growth and lysis of the fish pathogen Moritella viscosa. Lett Appl Microbiol. 2007;45(2):115-20.

Benediktsdóttir E, Verdonck L, Spröer C, Helgason S, Swings J.Characterization of Vibrio viscosus and Vibrio wodanis isolated at different geographical locations: a proposal for reclassification of Vibrio viscosus as Moritella viscosa comb. nov. Int J Syst Evol Microbiol. 2000;50 Pt 2:479-88.

Orieux N, Bourdineaud JP, Douet DG, Daniel P, Le Hénaff M. Quantification of Flavobacterium psychrophilum in rainbow trout, Oncorhynchus mykiss (Walbaum), tissues by qPCR. J Fish Dis. 2011;34(11):811-21. .

Touchon M, Barbier P, Bernardet JF, Loux V, Vacherie B, Barbe V, Rocha EP, Duchaud E. Complete genome sequence of the fish pathogen Flavobacterium branchiophilum. Appl Environ Microbiol. 2011;77(21):7656-62.

Högfors-Rönnholm E, Wiklund T. Phase variation in Flavobacterium psychrophilum: characterization of two distinct colony phenotypes. Dis Aquat Organ. 2010. 18;90(1):43-53.

Fujiwara-Nagata E, Eguchi M.Development and evaluation of a loop-mediated isothermal amplification assay for rapid and simple detection of Flavobacterium psychrophilum. J Fish Dis. 2009;32(10):873-81.

Valdebenito S, Avendaño-Herrera R. Phenotypic, serological and genetic characterization of Flavobacterium psychrophilum strains isolated from salmonids in Chile. J Fish Dis. 2009;32(4):321-33.

Cain KD, Lafrentz BR. Laboratory maintenance of Flavobacterium psychrophilum and Flavobacterium columnare. Curr Protoc Microbiol. 2007;Chapter 13:Unit 13B.1.

Pilarski F, Rossini AJ, Ceccarelli PS. Isolation and characterization of Flavobacterium columnare (Bernardet et al. 2002) from four tropical fish species in Brazil. Braz J Biol. 2008;68(2):409-14.

Altinok I, Capkin E, Kayis S.Development of multiplex PCR assay for simultaneous detection of five bacterial fish pathogens. Vet Microbiol. 2008.15;131(3-4):332-8.

Arai H, Morita Y, Izumi S, Katagiri T, Kimura H. Molecular typing by pulsed-field gel electrophoresis of Flavobacterium psychrophilum isolates derived from Japanese fish.J Fish Dis. 2007;30(6):345-55.

Adams, A. and K. Thompson., 1990. Development of an enzyme-linked immunosorbent assay (ELISA) for the detection of *Aeromonas salmonicida* in fish tissue. *J. Aquat. Anim. Health*, 2, 281-288.

Austin, B. and Austin, D.A. 2007. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Springer Praxis Books, UK, 4th ed., 552 p.; ISBN: 978-1-4020-6068-7.

Byers, H.K., Cipriano, R.C., Gudkovs, N. and Crane, M. St.J., 2002. PCR-based assays for the fish pathogen *Aeromonas salmonicida*. II. Further evaluation and validation of three PCR primer sets with infected fish. *Dis. Aquat. Organ.*, 49, 139-144.

Gustafson, C.E., Thomas, C.J. and Trust, T.J., 1992. Detection of *Aeromonas salmonicida* from fish by using polymerase chain reaction amplification of the virulence surface array protein gene. *Appl. Environ. Microbiol*., 58, 3816-3825.

McCarthy, D.H., 1975. Detection of *Aeromonas salmonicida* antigen in diseased fish tissue. *J. Gen. Micro*., 88, 384 - 386.

Oakey, H.J, Ellis, J.T. and Gibson, L.F., 1998. The development of random DNA probes specific for *Aeromonas salmonicida*. *J. Appl. Microbiol*., 84, 37-46.

Altinok I, Capkin E, Kayis S.Development of multiplex PCR assay for simultaneous detection of five bacterial fish pathogens. Vet Microbiol. 2008. 15;131(3-4):332-8.

Onuk EE, Ciftci A, Findik A, Ciftci G, Altun S, Balta F, Ozer S, Coban AY. Phenotypic and molecular characterization of Yersinia ruckeri isolates from rainbow trout (Oncorhynchus mykiss, Walbaum, 1792) in Turkey. Berl Munch Tierarztl Wochenschr. 2011; 124(7-8):320-8.

Onuk EE, Ciftci A, Findik A, Durmaz Y. Development and evaluation of a multiplex PCR assay for simultaneous detection of Flavobacterium psychrophilum, Yersinia ruckeri and Aeromonas salmonicida subsp. salmonicida in culture fisheries. J Vet Sci. 2010;11(3):235-41

Tobback E, Decostere A, Hermans K, Haesebrouck F, Chiers K.Yersinia ruckeri infections in salmonid fish. J Fish Dis. 2007;30(5):257-68. Review.

Welch TJ.Rapid genotyping assays for the identification and differentiation of Yersinia ruckeri biotype 2 strains.Lett Appl Microbiol. 2011;53(3):383-5.

Osorio CR, Toranzo AE, Romalde JL, Barja JL.Multiplex PCR assay for ureC and 16S rRNA genes clearly discriminates between both subspecies of Photobacterium damselae. Dis Aquat Organ. 2000. 20;40(3):177-83.

Romalde JL.Photobacterium damselae subsp. piscicida: an integrated view of a bacterial fish pathogen. Int Microbiol. 2002;5(1):3-9. Review

Thyssen A, Grisez L, van Houdt R, Ollevier F.Phenotypic characterization of the marine pathogen Photobacterium damselae subsp. piscicida. Int J Syst Bacteriol. 1998;48 Pt 4:1145-51.

Wang Y, Han Y, Li Y, Chen JX, Zhang XH Isolation of Photobacterium damselae subsp. piscicida from diseased tongue sole (Cynoglossus semilaevis Gunther) in China. Wei Sheng Wu Xue Bao. 2007 ;47(5):763-8.

Zappulli V, Patarnello T, Patarnello P, Frassineti F, Franch R, Manfrin A, Castagnaro M, Bargelloni L.Direct identification of Photobacterium damselae subspecies piscicida by PCR-RFLP analysis. Dis Aquat Organ. 2005 14;65(1):53-61

Fryer JL, Lannan C, Garcés L, Larenas J, Smith, P. Isolation of a rickettsiales-like organism from diseased coho salmon (*Oncorhynchus kisutch*) in Chile. Fish Pathology 1990. 25: 107-114.

Lannan C and Fryer J. Recommended method for inspection of fish for the salmonid rickettsia. *Bulletin of European Association Fish Pathologists* 1991. 11: 135-136.

Mauel M.J, Giovannoni SJ, Fryer JL**.** Development of polymerase reaction assays for detection, identification, and differentiation of *Piscirickettsia salmonis*. Diseases of Aquatic Organisms 1996. 26: 189 - 195.

Geng Y, Wang KY, Huang XL, Chen DF, Li CW, Ren SY, Liao YT, Zhou ZY, Liu QF, Du ZJ, Lai WM.*Streptococcus agalactiae*, an Emerging Pathogen for Cultured Ya-Fish, Schizothorax prenanti, in China. *Transbound Emerg Dis*. 2011 doi: 10.1111/j.1865-1682.2011.01280.x.

El Aamri F, Padilla D, Acosta F, Caballero MJ, Roo J, Bravo J, Vivas J, Real F.First report of Streptococcus iniae in red porgy (*Pagrus pagrus*, L.). J Fish Dis. 2010 33(11):901-905.

Han HJ, Jung SJ, Oh MJ, Kim DH.Rapid and sensitive detection of *Streptococcus iniae* by loop-mediated isothermal amplification (LAMP).J Fish Dis. 2011. 34(5):395-8. DOI: 10.1111/j.1365-2761.2011.01242.x.

Evans JJ, Klesius PH, Shoemaker CA. First isolation and characterization of *Lactococcus garvieae* from Brazilian Nile tilapia, *Oreochromis niloticus* (L.), and pintado, *Pseudoplathystoma corruscans* (Spix & Agassiz).J Fish Dis. 2009. 32(11):943-51.

Table 6.- Taking of samples for bacterial diseases

Organ/tissue to be sampled for bacterial diseases	***Vibrio* spp**	***Moritella viscosa***	***Renibacterium salmoninarum***	***Piscirikettsia salmonis***	***Yersinia ruckeri***	***Streptococcus* spp**	***Cytophaga – Flavobacterium – Bacteroides***	***Photobacterium damselae* subsp. *piscicida***	***Aeromonas* spp**
Spleen	X	X	X		X	X		X	X
Gill		X					X		
Liver	X	X		X	X	X		X	X
Musculature affected									X
Anterior kidney	X	X	X	X	X	X		X	X
Nervous system						X			
Middle kidney	X							X	
Seminal fluids			X						
Skin		X					X		X

Table 7.- Diagnosis methods for parasitic and fungal diseases

Parasites and fungi	Diagnosis	Reference
Aphanomyces invadens	Histology Isolation of *Aphanomyces invadans* Epifluorescence PCR	OIE 2010
Saprolegnia	Culture Histology Electron microscopy PASS Stain Grocott Stain	Ke *et al*., 2009 Fregened *et al*., 2009 Stueland *et al*.,2005 Paul and Steciow, 2004 Bangyeekhun *et al*., 2001
Clase Monogenea	Smear Sequencing Histology Electron microscopy	OIE, 2010 Collins & Cunningham, 2000 Collins et al., 2010 Harris et al.,2011 Paladini et al.,2009 Antonelli *et al*.,2010 Sitjà-Bobadilla *et al*.,2009
Phylum Amoebozoa	Smear Histology PCR	Kent et al, 1998 Zilberg et al.,1999 Young et al., 2008
Phylum Parabasalia	Smear Histology	Alvarez-Pellitero et al., 2008
Phylum Euglenozoa	Smear Giemsa stained smears Histology of gills	Alvarez-Pellitero et al., 2008
Phylum Oflagellata	Observation of skin in dark background Histology ELISA PCR	Noga and Levy, 1999 Smith et al., 1992 Levy et al., 2007
Phylum Ciliophora	Histology Smear Sequencing ELISA Culture *in vitro* PCR Electron microscopy	Iglesias *et al*., 2001 Palenzuela *et al*., 2005 Paramá *et al*., 2006 Sitjà-Bobadilla *et al*., 2008 Dyková *et al*.,2010 Smith *et al*.,2009 Jee *et al*.,2001
Phylum Myxozoa	Smear Histology PCR Immunohistochemical tests ELISA	Padrós *et al*., 2001 Palenzuela *et al*., 2004 Yasuda *et al*., 2005 Diamant *et al*., 2006 Cuadrado *et al*., 2007 Sitjà-Bobadilla *et al*., 2007a Yanagida *et al*., 2008 Sitjà-Bobadilla *et al*., 2004 Vázquez *et al*., 2005 Quiroga *et al*., 2006 Palenzuela *et al*., 2007 Sitjà-Bobadilla *et al*., 2007b
Clase Trematoda	Histology PCR Morphological identification	Bullard and Overstreet, 2008
Phylum Microspora	PCR Sequencing Electron microscopy Histology	Lom, 2008
Phylum Apicomplexa	Immunofluorescence Hystology PCR Sequencing Electron microscopy	Barugahare *et al*.,2011 Alvarez-Pellitero *et al*.,2002 Palenzuela *et al*.,2010 Ryan *et al*.,2004 Sitjà-Bobadilla *et al*.,2005

Table 7.- Diagnosis methods for parasitic and fungal diseases (cont.)		
Parasites and fungi	**Diagnosis**	**Reference**
Clase Cestoidea	Morphological identification Hystology	Borucinska, 2008
Phylum Nematoda	Morphological identification PCR Histological sections of tissues ELISA	Choudhury and Cole, 2008
Phylum Acanthocephala	Morphological identification Histological sections of tissues	Taraschewski, 2008
Phylum Arthropoda	Direct Observation	Tully *et al*.,1999 Wagner *et al*.,2008

References

Tully O, Gargan P, Poole WR, Whelan KF. Spatial and temporal variation in the infestation of sea trout (Salmo trutta L.) by the caligid copepod Lepeophtheirus salmonis (Krøyer) in relation to sources of infection in Ireland.Parasitology. 1999;119 (Pt 1):41-51.

Wagner GN, Fast MD, Johnson SC. Physiology and immunology of Lepeophtheirus salmonis infections of salmonids.Trends Parasitol. 2008;24(4):176-83.

Collins CM, Kerr R, McIntosh R, Snow M. Development of a real-time PCR assay for the identification of Gyrodactylus parasites infecting salmonids in northern Europe.Dis Aquat Organ. 2010. 11;90(2):135-42.

Harris PD, Bachmann L, Bakke TA. Freshwater charr (Salvelinus alpinus) as hosts for Gyrodactylus salaris: implications for management.Vet Rec. 2011. 12;168(6):161.

Paladini G, Gustinelli A, Fioravanti ML, Hansen H, Shinn AP. The first report of Gyrodactylus salaris Malmberg, 1957 (Platyhelminthes, Monogenea) on Italyn cultured stocks of rainbow trout (Oncorhynchus mykiss Walbaum).Vet Parasitol. 2009. 12;165(3-4):290-7.

Collins CM. & Cunningham CO. Characterization of the *Gyrodactylus salaris* Malmberg,1957 (Platyhelminthes: Monogenea) ribosomal intergenic spacer (IGS) DNA. 2000. *Parasitology*, 121, 555–563.

Dyková I, Tyml T, Kostka M, Pecková H.Strains of *Uronema marinum* (Scuticociliatia) co-isolated with amoebae of the genus Neoparamoeba. Dis Aquat Organ. 2010. 24;89(1):71-7.

Smith PJ, McVeagh SM, Hulston D, Anderson SA, Gublin Y.DNA identification of ciliates associated with disease outbreaks in a New Zealand marine fish hatchery. Dis Aquat Organ. 2009. 23;86(2):163-7.

Jee BY, Kim YC, Park MS.Morphology and biology of parasite responsible for scuticociliatosis of cultured olive flounder Paralichthys olivaceus. Dis Aquat Organ. 2001. 29;47(1):49-55.

Antonelli L, Quilichini Y, Marchand B. *Sparicotylechrysophrii* (Monogenea: Polyopisthocotylea) parasite of cultured Gilthead sea bream *Sparus aurata* (Linnaeus 1758) (Pisces: Teleostei) from Corsica: ecological and morphological study. Parasitol Res. 2010;107(2):389-98.

Sitjà-Bobadilla A, Alvarez-Pellitero P. Experimental transmission of *Sparicotylechrysophrii* (Monogenea: Polyopisthocotylea) to gilthead seabream (Sparus aurata) and histopathology of the infection. Folia Parasitol. 2009;56(2):143-51.

Barugahare R, Dennis MM, Becker JA, Slapeta J.Detection of *Cryptosporidium molnari* oocysts from fish by fluorescent-antibody staining assays for cryptosporidium spp. affecting humans.Appl Environ Microbiol. 2011;77(5):1878-80.

Alvarez-Pellitero P, Sitjà-Bobadilla A. *Cryptosporidium molnari n. sp. (Apicomplexa: Cryptosporidiidae*) infecting two marine fish species, *Sparus aurata* L. and Dicentrarchus labrax L.Int J Parasitol. 2002;32(8):1007-21.

Palenzuela O, Alvarez-Pellitero P, Sitjà-Bobadilla A. Molecular characterization of *Cryptosporidium molnari* reveals a distinct piscine clade. Appl Environ Microbiol. 2010;76(22):7646-9.

Ryan U, O'Hara A, Xiao L. Molecular and biological characterization of a *Cryptosporidium molnari*-like isolate from a guppy (*Poecilia reticulata*).Appl Environ Microbiol. 2004;70(6):3761-5.

A. Sitjà-Bobadilla, F. Padrós, C. Aguilera, and P. Alvarez-Pellitero. Epidemiology of *Cryptosporidium molnari* in Spanish Gilthead Sea Bream (*Sparus aurata* L.) and European Sea Bass (*Dicentrarchus labrax* L.) Cultures: from Hatchery to Market Size. Appl Environ Microbiol. 2005; 71(1): 131–139.

Bangyeekhun E, Quiniou SMA, Bly JE, Cerenius L Characterisation of Saprolegnia sp. isolates from channel catfish. Dis Aquat Organ. 2001. 4;45(1):53-9.

Cuadrado, M., Albinyana, G., Padrós, F., Redondo, M.J., Sitjà-Bobadilla, A., Alvarez-Pellitero, P., Palenzuela, O., Diamant, A. and Crespo,

S., 2007. An unidentified epi-epithelial myxosporean in the intestine of gilthead sea bream (*Sparus aurata* L.). *Parasitol. Res*., 101, 403-411.

Diamant, A., Ram, S. and Paperna, I., 2006. Experimental transmission of *Enteromyxum leei* to freshwater fish. *Dis. Aquat. Organ*., 72, 171-178.

Fregeneda-Grandes JM, Carbajal-González MT, Aller-Gancedo JM. Iglesias, R., Paramá, A., Alvarez, M.F., Leiro, J., Fernández, J. and Sanmartín, M.L., 2001. *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). *Dis. Aquat. Organ*., 46, 47-55.

Ke XL, Wang JG, Gu ZM, Li M, Gong XN. Morphological and molecular phylogenetic analysis of two Saprolegnia sp. (Oomycetes) isolated from silver crucian carp and zebra fish. Mycol Res. 2009;113(5):637-44.

OIE (Office International des Epizooties), 2010. Manual of Diagnostic Tests for Aquatic Animals 2010[online, en ingles]; http://www.oie.int/eng/normes/fmanual/A_summry.htm.

Padrós, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Crespo, S. and Alvarez-Pellitero, P., 2001. *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Dis. Aquat. Organ*., 47, 57-62.

Palenzuela, O., Agnetti, F., Albiñana, G., Alvarez-Pellitero, P., Athanassopoulou, F., Crespo, S., Diamant, A., Ghittino, C., Golomazou, H., Le Breton, A., Lipshitz, A., Marques, A., Padrós, F., Ram, S. and Raymond, J.C., 2004. Applicability of PCR screening for the monitoring of *Enteromyxum leei* (Myxozoa) infection in Mediterranean aquaculture: an epidemiological survey in sparid facilities. *In:* Aquaculture Europe International Conference. Biotechnology for Quality. Adams and Olafsen (eds.). EAS Special publication 2004: extended abstracts. Barcelona, August 2004.

Palenzuela, O., Redondo, M.J., López, E. and Alvarez-Pellitero, P., 2007. Cultured sole, *Solea senegalensis* is susceptible to *Enteromyxum scophthalmi*, the myxozoan parasite causing turbot emaciative enteritis. *Parasitologia*, 49, 73.

Palenzuela, O., Yoshinaga, T. and Alvarez-Pellitero, P., 2005. Conserved rDNA gene sequences among parasitic scuticociliates of flatfish. What, if anything, is *Philasterides dicentrarchi*? *In:* Abstracts of the EAFP 12th International Conference, Diseases of Fish and Shellfish, Copenhagen, 11-16 Sept, 2005, p 70.

Paramá, A., Arranz, J.A., Alvarez, M.F., Sanmartín, M.L. and Leiro, J., 2006. Ultrastructure and phylogeny of *Philasterides dicentrarchi* (Ciliophora, Scuticociliatia) from farmed turbot in NW Spain. *Parasitol*., 3, 1-10.

Paul B, Steciow MM. Prevalence of serum antibodies against Saprolegnia parasitica in wild and farmed brown trout Salmo trutta.Dis Aquat Organ. 2009. 28;83(1):17-22.

Quiroga, M.I., Redondo, M.J., Sitjà-Bobadilla, A., Palenzuela, O., Riaza, A., Macías, A., Vázquez, S., Pérez, A., Nieto, J.M., and Alvarez-Pellitero, P., 2006.Risk factors associated with *Enteromyxum scophthalmi* (Myxozoa) infection in cultured turbot (*Scophthalmus maximus* L.). *Parasitol*., 133, 433-442.

Paul B, Steciow MM. Saprolegnia multispora, a new oomycete isolated from water samples taken in a river in the Burgundian region of France.FEMS Microbiol Lett. 2004. 15;237(2):393-8.

Sitjà-Bobadilla, A., Diamant, A., Palenzuela, O. and Alvarez-Pellitero, P., 2007a. Host factors and experimental conditions on the horizontal transmission of *Enteromyxum leei* (Myxozoa) to gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). *J. Fish Dis*., 29, 1-8.

Sitjà-Bobadilla, A., Palenzuela, O. and Alvarez-Pellitero, P., 2008. Immune response of turbot, *Scophthalmus maximus* (L.) (Pisces: Teleostei), to formalin-killed scuticociliates (Ciliophora) and adjuvanted formulations. *Fish Shellfish Immunol*., 24, 1-10.

Sitjà-Bobadilla, A., Palenzuela, O., Macías, M.C., Riaza, A. and Alvarez-Pellitero, P., 2007b. Acquired protective immunity to *Enteromyxum scophthalmi* (Myxozoa) is related to specific antibodies in turbot, *Psetta maxima* (Pisces: Teleostei). *Scand. J. Immunol*., 66, 26-34.

Kent ML, Sawyer TK, Hedrick RP (1988) *Paramoeba pemaquidensis*(Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in seawater. Dis Aquat Org 5:163–169

Zilberg, D., Nowak, B.F., Carson, J. and Wagner, T. (1999). Simple gill smear staining for diagnosis of amoebic gill disease. *Bull. Eur. Ass. Fish Pathol*., 19: 186-189.

Young, ND and Dykova, I and Nowak, BF and Morrison, RN (2008) *Development of a diagnostic PCR to detect Neoparamoeba perurans, agent of amoebic gill disease.* Journal of Fish Diseases, 31 (4). pp. 285-295.

Noga E.J. and Levy M.G. 1995. Dinoflagellida (Phylum Sarcomastigophora) In: P T K Woo (ed.) Fish Diseases and Disorders. Volume 1: Protozoan and Metazoan Infections. CAB International, Wallingford, Oxon. pp 1-25.

Levy, M.G., Poore, M.F., **Colorni, A.**, Noga, E.J., Vandersea, M.W. and Litaker, R.W. (2007). A highly specific PCR assay for detecting the fish ectoparasite *Amyloodinium ocellatum*. *Dis. Aquat. Org.* 73:219-226.

Smith S, Levy M, Noga E (1992) Development of an enzymelinked immunosorbent assay (ELISA) for the detection of antibody to the parasitic dinoflagellate *Amyloodinium ocellatum* in *Oreochromis aureus*. Vet Parasitol 42:145–155

Alvarez-Pellitero, P. 2008. Diseases caused by flagellates. In: Fish Diseases (Vol. I). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Bullard, S and Overstreet, R. 2008. Digeneans as enemies of fishes. In: Fish Diseases (Vol. II). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Lom, J. 2008. Microsporidia. In: Fish Diseases (Vol. I). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Borucinska, JD. 2008. Diseases caused by Cestoda. In: Fish Diseases (Vol. II). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Choudhury, A. and Cole, R. 2008. Phylum Nematoda. In: Fish Diseases (Vol. II). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Taraschewski, H. 2008. Acanthocephala. In: Fish Diseases (Vol. II). Eiras, Segner, Hahli, Kappor Eds. Science Publishers, Enfield, USA.

Sitjà-Bobadilla, A., Redondo, M.J., Macias, M.A., Ferreiro, I., Riaza, A. and Alvarez-Pellitero, P., 2004. Development of immunohistochemistry and enzyme-linked immunosorbent assays for the detection of circulating antibodies against *Enteromyxum scophthalmi* (Myxozoa) in turbot (*Scophthalmus maximus* L.). *Fish Shellfish Immunol*., 17, 335-345.

Stueland S, Hatai K, Skaar I. Morphological and physiological characteristics of Saprolegnia spp. strains pathogenic to Atlantic salmon, Salmo salar L.J Fish Dis. 2005;28(8):445-53.

Vázquez, S., Redondo, M.J., Palenzuela, O., Quiroga, M.I., Alvarez-Pellitero, P. and Nieto, J.M., 2005. Detección de ácidos nucléicos de *Enteromyxum scophthalmi* en intestino de turbots (*Scophthalmus maximus*) mediante la técnica de hibridación in situ (HIS). XVII Reunión de SEAPV, Jarandilla de la Vera (Cáceres), Junio 2005.

Yanagida, T., Palenzuela, O., Hirae, T., Yokoyama, H. and Ogawa, K., 2008. Myxosporean emaciation disease of cultured red sea bream *Pagrus major* and spotted knifejaw *Oplegnathus punctatus*. *Fish Pathol.*, 43, 45-48.

Yasuda, H., Ooyama, K., Nakamura, A., Iwata, K., Palenzuela, O. and Yokoyama H., 2005. Occurrence of the myxosporean emaciation disease caused by *Enteromyxum leei* in cultured Japanese flounder *Paralichthys olivaceus*. *Fish Pathol*., 40, 175-180.

Table 8.- Organ/tissue to be sampled for parasitic and fungal diseases

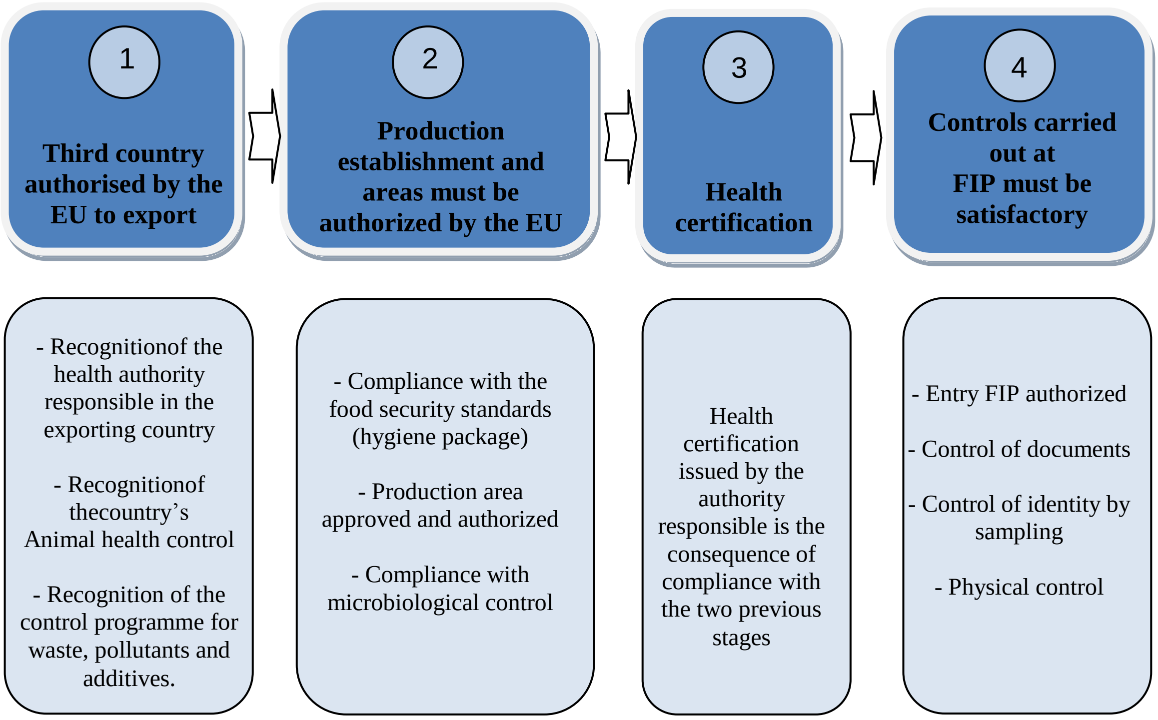
Organ/tissue to be sampled for parasitic and fungal diseases	***Aphanomyces invadans***	***Saprolegnia***	***Phylum Amoebozoa***	***Phylum Parabasalia***	***Phylum Euglenozoa***	***Phylum Oflagellata***	***Phylum Ciliophora***	***Phylum Myxozoa***	***Clase trematoda***	***Phylum Microspora***	***Clase monogenea***	***Phylum Apicomplexa***	***Phylum nematoda***	***Phylum acanthocephala***	***Clase cestoidea***	***Phylum arthropoda***
Spleen				X			X	X								
Gill		X	X			X	X		X	X	X					
Brain							X									
Heart																
Gonads								X							X	
Liver				X			X			X					X	
Stomach					X						X	X				
Intestine				X				X	X	X		X	X	X	X	
Musculature	X								X	X			X		X	
Anterior kidney							X	X								
Posterior kidney																
Skin		X			X	X	X		X	X	X					X
Blood					X											

Chapter II

II. Transport of fish and/or germplasm in the framework of the AQUAEXCEL Project

II.1. Requisites for the import of fish and/or germplasm from third countries to the European Union

Imports from third countries to the European Union (EU) of fish and aquaculture products are subject to official certification, based on the recognition of the authority responsible for disease control in the non-European exporting country. This formal recognition of the trustworthiness of the authority responsible is a prerequisite for the country to be eligible and authorized to export to the EU. In accordance with the European procedures, for a producer of a country that does not belong to the EU to be able to export and sell animals and/or products of aquaculture origin to the European Union, it must go through the following steps:



FIP: Frontier Inspection Posts

Stage 1: Authorization

Countries of origin must feature on the list of authorized countries for transporting fish and/or germplasm to the EU. The EU criteria for allowing authorization are based on:

- Countries must have a designated authority responsible for official inspections and controls. The health control authority must be structured and have resources to carry out effective inspections and ensure credible public health guarantees and animal health checks that make up part of the animal health certificate that accompanies the products sent to the EU.

- The fish, their eggs and germplasm must comply with all the relevant animal health standards. For this reason, the veterinary services must ensure strict and effective compliance (control and supervision) of all programmes based on the health requisites.

Stage 2: Register of production establishments

- The farms of the research centres of aquaculture businesses where the fish are travelling from must be registered by the responsible authority in the third country (including their georeferenced location).

- The farms of the research centres of aquaculture businesses where the fish are travelling from must be under an animal health surveillance and hygiene practices programme put in place by the authority responsible.

- Imports will only be authorized from approved centres that have been inspected by the authority responsible in the exporting country and that met EU requisites. The authority will provide sufficient guarantees and be responsible for carrying out the regulation inspections and corrective action, where necessary. Inspections by the authorities are necessary to confirm compliance with the above-mentioned requisites. Therefore, an inspection mission is established in by agreement between the exporting country's authority responsible and the European Commission.

Stage 3: Animal health certification

As a general rule, imports of fish and/or germplasm in the EU must be accompanied by the health certificates established by EU legislation. This determines the conditions that must be met and the checks that must have been carried out in order for the imports to be authorised. Certificates must be signed by an official veterinary scientist or an official inspector registered by the Authority Responsible and must be in line with Directive 96/93 EC regarding certification of animals and animal products. On entering the EU, each shipment must be accompanied by the original certificate. Normally, certificates must be made out in the language of the issuing country, as well as in that of the destination Member State and that of the Member State where the inspection is carried out, although these Member States may accept, is they so desire, that the certificates are made out in an official EU language that is different from their own. For each animal and product category, there are a series of public health or animal health requisites, which may include animal welfare requisites (regarding, for example, stunning and sacrifice). It is important to ensure that appropriate certificates are used and that all necessary provisions are met, so Decision 79/542/EEC (list of third countries or parts of third countries, and veterinary, sanitary and animal health conditions for the import of specific life animals), Directive 96/93 EC (animal and animal product certificates) and Regulation 2074/2005 EC (measures to be applied for specific products in accordance with provisions of other norms) should be consulted.

Stage 4: controls

The controls carried out by official inspectors at the Frontier Inspection Posts (FIP) must verify compliance with what appears on the certificates, for example that the products come from an authorized

country, an authorized centre and have been elaborated in accordance with the animal hygiene and health requisites regulated by the European Commission and the Member State to which they are travelling. Fish and aquaculture products imported from non-EU countries must enter via an approved frontier inspection post, under the authority of an official veterinary scientist. All embarkations are subject to a systematic control of documents, of identity and, if needs be, to a physical check. The frequency of the physical checks depends on the level of risk presented by the product and the results of previous inspections. Any shipment that does not comply with the requisites of EU legislation may be destroyed or, under certain circumstances, sent back to the exporting country within 60 days. The exporter must verify the specific FIPs for specific product, depending on the EU country in question. The list of CIP characteristics can be found in Decision 2008/807 EC, modified by Decision 2009/38 EC for some countries. The language of the animal health certificate must be chosen depending on the language of the country of origin, the country of entry to the EU (FIP for entry into EU territory) and the country of destination (any of the 23 official EU languages). This implies, for example, that if an exporter sends a product from a Spanish-speaking third country to Germany, entering the EU through a Dutch port, the certificate should comprise three parts: a) in Spanish (language of the country of origin), which will be signed and sealed b the authority responsible issuing the certificate; b) a copy of the certificate in Dutch (as the shipment enters the EU in Holland; this certificate will only bear the seal of the authority responsible), and a copy in German (country of destination, which will only bea the seal of the authority responsible). Where the TRACES system is used, multi-language certificates are automatically issued when the FIP and destination countries are entered into the form in Spanish. Labels on the boxes of product must at least be in English or in the language of the destination country. Consumer labels must at least be in the language of the destination country.

II.1.1.- List of authorized countries and areas

In order to obtain authorization for import, the third countries or parts of third countries in question must figure on a list drawn up by the European Commission. These countries or parts of countries are included in the list once they have been assessed by the European Commission in terms of the disease status of the aquaculture animals of the third country, its legislation, the organization of the local authority responsible and the inspection services, etc. Where necessary, European Commission experts may carry out an inspection in situ in order to complete their assessment. The list of authorized countries can be consulted at the following link:

http://ec.europa.eu/food/food/biosafety/establishments/third_country/index_es.htm

II. 1.2.- Animal disease control of fish, aquaculture and fish-farming products

We will now describe the most important legislation on animal health controls for fish, aquaculture and fish-farming products when fish and/or germplasm are imported from third countries. Directive 2006/88 EC establishes the animal health requisites and those for aquaculture products, and the prevention and control of diseases specific to aquatic animals. The Directive lays down:

- Animal health requisites applicable to the placing in the market, import and transit of aquaculture animals and products.

- Preventative measures relating to diseases suffered by aquaculture animals.

- Minimum control measures to be applied when specific diseases affecting aquaculture animals either appear of are suspected.

Regulation 1251/2008 EC lays down the conditions governing the certification for trading and importing aquaculture animals and derived products in the European Union. The territory of a State or part of a State may be declared free from a non-exotic disease if no species susceptible to said disease are present, or if the State has adopted disease surveillance and detection measures for a sufficiently long period of time. At the same time, the State must establish security areas between its territory and that of its neighbour States that have not been declared free of the disease in question. The Commission draws up, updates and publishes the list of the States and areas declared disease-free. For further information, consult Directive 2006/88 EC. Where suspicions arise regarding a disease listed under Directive 2006/88 EC or where there is a rise in the mortality of aquatic animals, the authority responsible in the country affected must notify the European Commission of the presence of the listed disease in a maximum period of 24 hours, when the area in question has been declared free of that particular disease. The Directive establishes the measures that European countries have to adopt if suspicions exist as to the possible presence of a listed disease. Measures for Third Countries can be the same or equivalent. If the presence of a listed exotic disease is confirmed, a confinement area surrounding the farm must be established, in combination with a ban on moving the animals concerned. Moreover, all dead animals, those with symptoms of the disease and those animals below marketable size that do not present any symptoms must beemoved and eliminated as soon as possible (see II.6). At the same time, the capture and complementary transformation of animals are possible as long as these activities are carried out in conditions that ensure that the pathogen is not propagated. Where appropriate, the farm affected must be subjected to a suitable quarantine period. If a listed non-exotic disease is confirmed as present in an area that has been declared free from that disease, the country concerned can apply identical measures to those regarding contamination by an exotic diseases or minimum confinement measures, restriction of movement and withdrawal and elimination of dead animals. If wild animals are contaminated by a listed disease, or any such contamination is suspected, the country concerned must monitor the situation and adopt the necessary measures to ensure that the disease does not spread. If an emerging disease appears, the country affected must adopt appropriate measures to avoid it spreading and must also inform the Commission.

II.2.- Movement of fish and germplasm between States of the AQUAEXCEL Project

Movement of fish and/or germplasm between European Union countries and between EU and Norway must comply with the following golden rule: “if they are travelling to a free territory, they must come from a free country”. Member States of the AQUAEXCEL Project may allow aquaculture animals and products that do not comply with this golden rule to be placed on the market for scientific purposes under the strict supervision of the body responsible. No movements of this type will take place unless the responsible bodies of the Member States in question have been notified in advance. This exception must be authorized by the authority responsible in the EU country to which the fish and/or germplasm are travelling, with a justification for moving the fish and/or germplasm, a risk evaluation, the establishment of a quarantine period and the taking of a series of measures to reduce the risks, for example:

- The direct handing over of the shipment in question to biologically secure installations where it will remain for the rest of its life in continuous isolation from the local environment.

- Treatment of all effluent and waste material in such a way as to guarantee that the pathogen is inactivated.

To introduce fish and/or germplasm into a Member State, area or compartment that has been declared free from a specific disease, aquaculture animals of species that are susceptible to said disease must come from another Member State, area or compartment that has also been declared free from said disease. Where it can be scientifically justified that species that are susceptible to the particular disease in question do not transmit that disease at specific stages of their life, for example through the germplasm by vertical contagion, this ban will not apply to those life states. Table 8 shows a list of diseases subject to restriction and whether or not vertical contagion takes place.

If a disease that is not listed as exotic or non-exotic under Directive 2006/88 EC represents a serious risk for the health status of aquaculture animals, of wild aquatic animals or the environment of a member State, the Member State in question may adopt measures to prevent the introduction of said disease or to control it. Thus, according to Commission Decision 2010/221 EU, Denmark, Ireland, Finland, Sweden and The United Kingdom have national authorised measures for the restriction of the entry of Spring Viraemia of Carp, Ireland and The United Kingdom for Renibacterium salmoninarum, Finland, Sweden and The United Kingdom for Infectious pancreatic necrosis, and Finland, Ireland and The United Kingdom for Gyrodactylus.

Table 9.- Vertical transmission of the diseases listed in Directive 2006/88 EC (Exotic and non-exotic Diseases) and Commission Decision 2010/221 EU (subject to National measures)

Disease	Vertical transmission
Epizootic haematopoietic necrosis (Exotic disease: Directive 2006/88 EC)	UNKNOWN
Epizootic ulcerative syndrome (Exotic disease: Directive 2006/88 EC)	RULED OUT
Viral haemorrhagic septicaemia (Non-exotic disease: Directive 2006/88 EC)	UNKNOWN
Infectious salmon anaemia (Non-exotic disease: Directive 2006/88 EC)	UNKNOWN
Koi herpesvirus disease (Non-exotic disease: Directive 2006/88 EC)	CONFIRMED
Infectious haematopoietic necrosis (Non-exotic disease: Directive 2006/88 EC)	CONFIRMED
Infection by *Gyrodactylus salaris* (National measures Commission Decision 2010/221 EU)	RULED OUT
Renibacterium salmoninarum (National measures Commission Decision 2010/221 EU)	CONFIRMED
Infectious pancreatic necrosis (National measures Commission Decision 2010/221 EU)	CONFIRMED
Spring viraemia of carp (National measures Commission Decision 2010/221 EU)	UNKNOWN

CONFIRMED | **UNKNOWN** | **RULED OUT**

In order to improve animal health in general and help to prevent and control animal diseases by means of enhanced traceability, movements of fish and/or germplasm must be registered. Where appropriate, said movements must also be subject to an animal health certificate. The basic points regarding the movement of fish and/or germplasm between EU countries are listed below:

- The use of registered, authorized means of transport.
- Animal health certificates.
- Movement of species susceptible to or vectors of diseases to disease-free destinations or with surveillance or eradication programmes.
- Movement of species susceptible to or vectors of diseases to areas with eradication programmes or that are already infected.
- Notification of the movement using TRACES

II.2.1.- Health Status of the Member States

Directive 2006/88 EC sets out five health statuses for member countries: category I (disease-free), category II (State subject to a surveillance programme), category III (Undetermined, but with no known infection), category IV (with an eradication programme) and category V (infected). The health status may include Member States, geographic areas or compartments, and thus, a Member State that has not been declared free of some of the diseases listed in the above-mentioned Directive, may have areas or compartments that are declared disease-free, where a compartment is understood to be one or more farms that make up a common bio security system with a population of aquatic animals in a specific health situation vis-à-vis a particular disease. In a common bio security system, the same health surveillance measures of disease prevention and control for aquatic animals are applied.

A Member State will be declared free from one or more non-exotic diseases listed in Directive 2006/88 EC if none of the species susceptible to that disease is present in its territory, or if the pathogen is known to be unable to survive in a Member State and its water sources. It can also be declared disease-free for historic reasons, when in a Member State in which susceptible species exist, the disease has not been observed for at least ten years before the Member State is declared disease-free, even though the conditions are favourable to the clinical manifestation of the disease, provided that the basic conditions of the bio security measures have been fulfilled uninterruptedly for at least ten years before the date the Member State is declared disease-free, where no infection has been observed in wild populations and commercial and import conditions are applied in order to prevent the disease being introduced into the Member State.

If the neighbouring Member States, or the water catchment areas shared with neighbouring Member States, have not been declared disease-free, the Member State will set up appropriate buffer zones in its territory. The delimitation of buffer zones will be carried out in such a way as to ensure that they protect the disease-free Member State from the passive introduction of the disease in question. A member State may declare an area or compartment of its territory free from one or more of the non-exotic diseases listed under Directive 2006/88 EC if none of the species that are susceptible to the disease in question are present in the area or compartment, or, where appropriate, in its water sources, or if the pathogen is known not to be able to survive in the area or compartment or, where appropriate, in its water sources, or where the area or compartment fulfils all the conditions established in part II of annex V to Directive 2006/88 EC, which are summarised below:

- The last clinical manifestation took place ten years before the date the disease-free status was declared or the date when the state of infection before the specific surveillance was unknown, for example, due to the lack of favourable conditions for any such clinical manifestation.

- There are susceptible species in the area, but the illness has not been observed during a minimum period of ten years before it was declared disease-free, despite the existence of conditions favourable to its clinical manifestation.

Surveillance Programmes aim to obtain or maintain the status of "free from non-exotic diseases", as well as moving from Category III (Undetermined, but with no known infection) to Category I (free). Eradication programmes aim to obtain the status of "free" from non-exotic diseases, as well as moving form category V (infected) to Category I (free).

The Member States, zones and compartments subject to authorized surveillance programmes are represented in the Commission Decision of 31st of October, 2008, which establishes the provisions under which Directive 2006/88 EC is to be applied in terms of surveillance, eradication programmes and the disease-free status of Member States, zones and compartments.

For more information see the following link:
http://ec.europa.eu/food/animal/liveanimals/aquaculture/heath_status_en.htm

Table 10.- Member States declared free from Infectious haematopoietic necrosis (Commission Decision 2010/171 EU)

Disease	Member State	Geographical demarcation of the "disease-free" area (Member State, zone or compartment)
Infectious haematopoietic necrosis	Denmark	Whole Territory
	Ireland	Whole Territory
	Cyprus	All continental areas
	Finland	Whole Territory
	Sweden	Whole Territory
	United Kingdom	All continental areas and coastal areas of Great Britain, Northern Ireland, Guernsey, the Isle of Man and Jersey

The following link provides detailed information as to the areas and compartments of the European Union Member States declared free from Infectious haematopoietic necrosis, as well as those fish farms that have been authorized, included in the Commission Decision 202/308 EU and its subsequent modifications.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002D0308:20080618:EN:PDF

Table 11.- Member States declared free from Viral Haemorrhagic Septicaemia (Commission Decision 2010/171 EU and Norwegian authority)

Disease	Member State	Geographical demarcation of the "disease-free" area (Member State, zone or compartment)
Viral Haemorrhagic Septicaemia	Denmark	The water catchment and the coastal areas of: Hansted, Hovmølle, Grenå,Treå,Alling, Kastbjerg, Villestrup, Korup, Sæby, Elling, Uggerby, Lindenborg, Øster, Hasseris, Binderup, Vidkær, Dybvad, Bjørnsholm, Trend, Lerkenfeld, Vester, Lønnerup med tilløb, Fiskbæk, Slette, Bredkær Bæk, Vandløb til Kilen, Resenkær, Klostermølle, Hvidbjerg, Knidals, Spang, Simested, Skals, Jordbro, Fåremølle, Flynder, Damhus, Karup, Gudenåen, Halkær, Storåen, Århus, Bygholm, Grejs, Ørum
	Ireland	Whole Territory
	Cyprus	All continental areas
	Finland	All continental and coastal areas, except the region of Åland and the municipalities of Uusikaupunki, Pyhäranta and Rauma
	Sweden	Whole Territory
	United Kingdom	All continental and coastal areas of Great Britain, Northern Ireland, Guernsey, the Isle of Man and Jersey
	Norway	Whole Territory (*)

The following link provides detailed information as to the areas and compartments of the European Union member States declared free from Viral Haemorrhagic Septicaemia, as well as those fish farms that have been authorized, included in the Commission Decision 2002/308 EU and its subsequent modifications.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002D0308:20080618:EN:PDF

(*) For more information see the following link:
http://www.mattilsynet.no/english/animal_disease_control/vhs_confirmed_in_rainbow_trout_53830

Table 12.- Member States, zones and compartments declared free from Infectious salmon anaemia (Commission Decision 2010/171 EU and Norwegian authorities)

Disease	Member State	Geographical demarcation of the "disease-free" area (Member State, zone or compartment)
Infectious salmon anaemia	Belgium	Whole Territory
	Norway	See: http://www.mattilsynet.no/english/animal_disease_control/isa/isa_free_zones
	Bulgaria	Whole Territory
	Czech Republic	Whole Territory
	Denmark	Whole Territory
	Germany	Whole Territory
	Estonia	Whole Territory
	Ireland	Whole Territory
	Greece	Whole Territory
	Spain	Whole Territory
	France	Whole Territory
	Italia	Whole Territory
	Cyprus	Whole Territory
	Latvia	Whole Territory
	Lithuania	Whole Territory
	Luxembourg	Whole Territory
	Hungary	Whole Territory
	Malta	Whole Territory
	Netherlands	Whole Territory
	Austria	Whole Territory
	Poland	Whole Territory
	Portugal	Whole Territory
	Rumania	Whole Territory
	Slovenia	Whole Territory
	Slovakia	Whole Territory
	Finland	Whole Territory
	Sweden	Whole Territory
	United Kingdom	All continental and coastal areas of Great Britain, Northern Ireland, Guernsey, the Isle of Man and Jersey, except South West Shetland Islands

Table 13- Member States, zones and compartments subject to authorized surveillance programmes

Disease	Member State	Geographical demarcation of the "disease-free" area (Member State, zone or compartment)
Viral Haemorrhagic Septicaemia		
Infectious haematopoietic necrosis		
Koi herpesvirus disease	Ireland	Whole Territory
	Hungary	Whole Territory
Infectious salmon anaemia		

The Member States, zones and compartments subject to authorized surveillance programmes are included in Commission Decision of 31st of October, 2008 which establishes provisions for the application of Directive 2006/88 EC regarding surveillance, eradication programmes and disease-free status of Member States, zones and compartments.

Table 14.- Member States, zones and compartments subject to authorized eradication programmes

Disease	Member State	Geographical demarcation of the "disease-free" area (Member State, area or compartment)
Viral Haemorrhagic Septicaemia	Denmark	The water catchment and the coastal areas of: Tim Å, Hover Å, Heager Å, Velling Å, Skjern Å, Hemmet Mølle Bæk, Lydum Å, Kongeå, Kolding Å, Vejle Å and Holmsland Klit
	Finland	The region of Åland; the municipalities of Uusikaupunki, Pyhäranta and Rauma
Infectious haematopoietic necrosis		
Koi herpesvirus disease	Germany	The Federal State of Saxony
Infectious salmon anaemia	United Kingdom	South West Shetland Islands

The Member States, zones and compartments subject to authorized surveillance programmes are represented in the Commission Decision of 31st of October, 2008, which establishes the provisions of application of Council Directive 2006/88 EC regarding the surveillance, eradication programmes and the disease-free» status of Member States, zones and compartments. At the same time, Commission Decision 2009/975EU of 14th December, 2009 modifies Commission Decision 2009/177EU regarding the eradication programmes and the disease-free status of Member States, zones and compartments regarding specific diseases of aquatic animals.

Table 15. Health status of States belonging to the AQUAEXCEL Project

	Spain	Holland	France	Czech Republic	United Kingdom	Norway	Greece	Hungary	Belgium	Ireland
Viral Haemorrhagic Septicaemia										
Infectious salmon anaemia										
Koi herpesvirus disease										
Infectious haematopoietic necrosis										

CATEGORY I (DISEASE-FREE)

CATEGORY II (SURVEILLANCE) PROGRAME)

CATEGORY III (UNDETERMINED)

CATEGORY IV (ERADICATIONPROGRAMME)

CATEGORY V (INFECTED)

ZONES APPROVED COMMISSION DECISION 2002/308 EC AND AMENDMENTS

3 ZONES AND 19 COMPARTMENTS FREE

Table 16.- Outbreaks of Disease during 2011 in States belonging to the AQUAEXCEL Project (OIE, 2012)

	Spain	Holland	France	Czech Republic	United Kingdom	Norway	Greece	Hungary	Belgium	Ireland
Viral Haemorrhagic Septicaemia (Non-exotic disease: Directive 2006/88 EC)	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	NO INFORMATION	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	NO INFORMATION	INFECTION-FREE	OUTBREAK OF DISEASE	NO INFORMATION
Infectious salmon anaemia (Non-exotic disease: Directive 2006/88 EC)	INFECTION-FREE	INFECTION-FREE	NO INFORMATION	INFECTION-FREE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	NO INFORMATION	INFECTION-FREE	NO INFORMATION	OUTBREAK OF DISEASE
Koi herpesvirus disease (Non-exotic disease: Directive 2006/88 EC)	INFECTION-FREE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	INFECTION-FREE	NO INFORMATION	INFECTION-FREE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE
Infectious haematopoietic necrosis (Non-exotic disease: Directive 2006/88 EC)	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	OUTBREAK OF DISEASE	INFECTION-FREE	INFECTION-FREE	NO INFORMATION	INFECTION-FREE	OUTBREAK OF DISEASE	INFECTION-FREE

OUTBREAK OF DISEASE | **NO INFORMATION** | **INFECTION-FREE**

II.2.2.- Means of Transport

Carriers using any type of transport (road, air, maritime or rail), must be duly authorized by the authority responsible in the country of origin of the fish and/or germplasm. The TRACES system demands these authorizations and checks their validity before issuing the corresponding certificate.

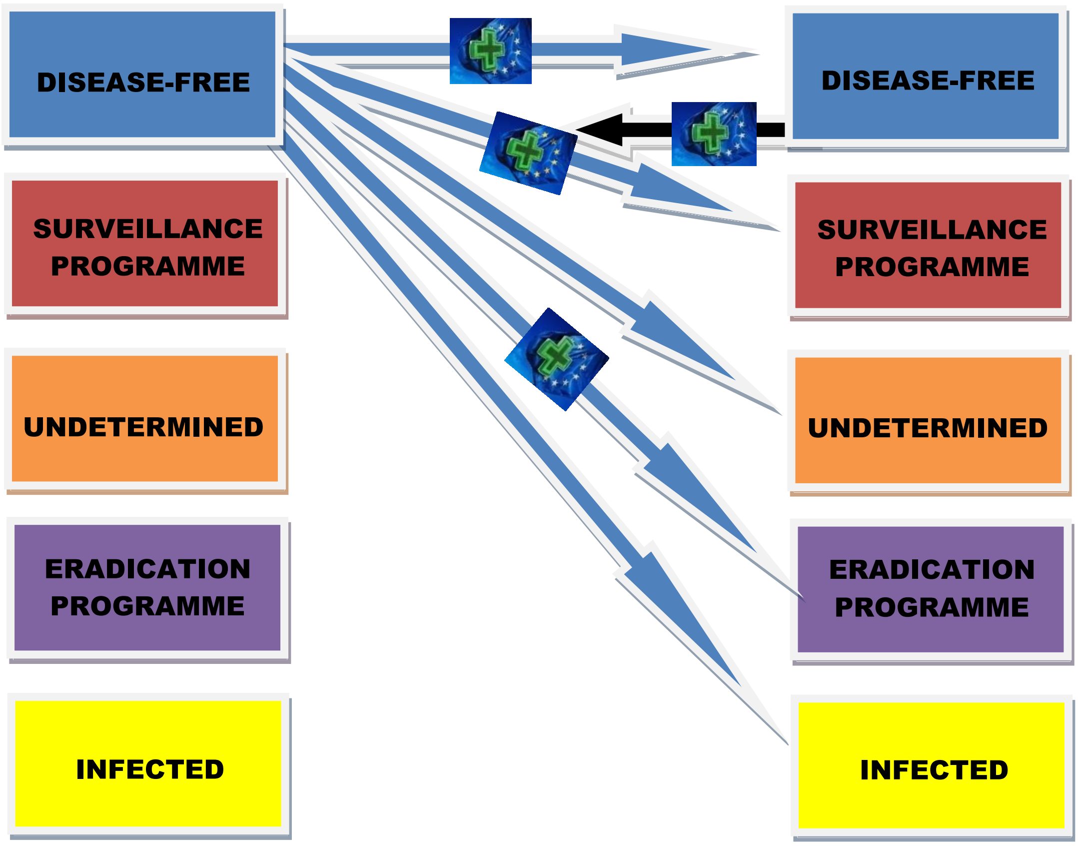
II.2.3.- Health certification during transport

Directive 2006/88 EC establishes that health certificates are required when fish and/or germplasm are introduced into a Member State, zone or compartment declared disease-free in accordance with said Directive or subject to a surveillance or eradication programme. The following Table shows the health certificates required depending on the area of origin and/or destination of the fish and/or germplasm. We will then go on to detail the certification needs and the movements allowed depending on the health status of the State, territory or zone of the country of origin/destination of the fish and/or germplasm. An important point to remember is that although the legislation in force does not require health certificates to be issued in some cases, we can find ourselves confronted by customs officials who demand said documentation even though it is not legally imperative, so it is advisable to process said certificates in all cases, to facilitate proceedings and prevent fish and/or germplasm being held up until their legal situation is resolved.

Table 17.-Health certificates needed depending on health status

Category	**Health status**	**Animals can be introduced from:**	**Health Certificate**		**Can send animals to:**
			Introduction	Shipment	
I	Disease-free (art. 49 or art. 50)	Only category I	YES	NO for shipments to categories III or V YES for shipments to categories I, II or IV	All categories
II	Surveillance programme (art. 44.1)	Only category I	YES	NO	Categories III and V
III	Undetermined (without any known infection but not subject to a programme for the disease-free category)	Categories I, II and III	NO	NO	Categories III and V
IV	Eradication Programme (art. 44.2)	Only category I	YES	YES	Only category V
V	Infected (art. 39)	All categories	NO	YES	Only category V

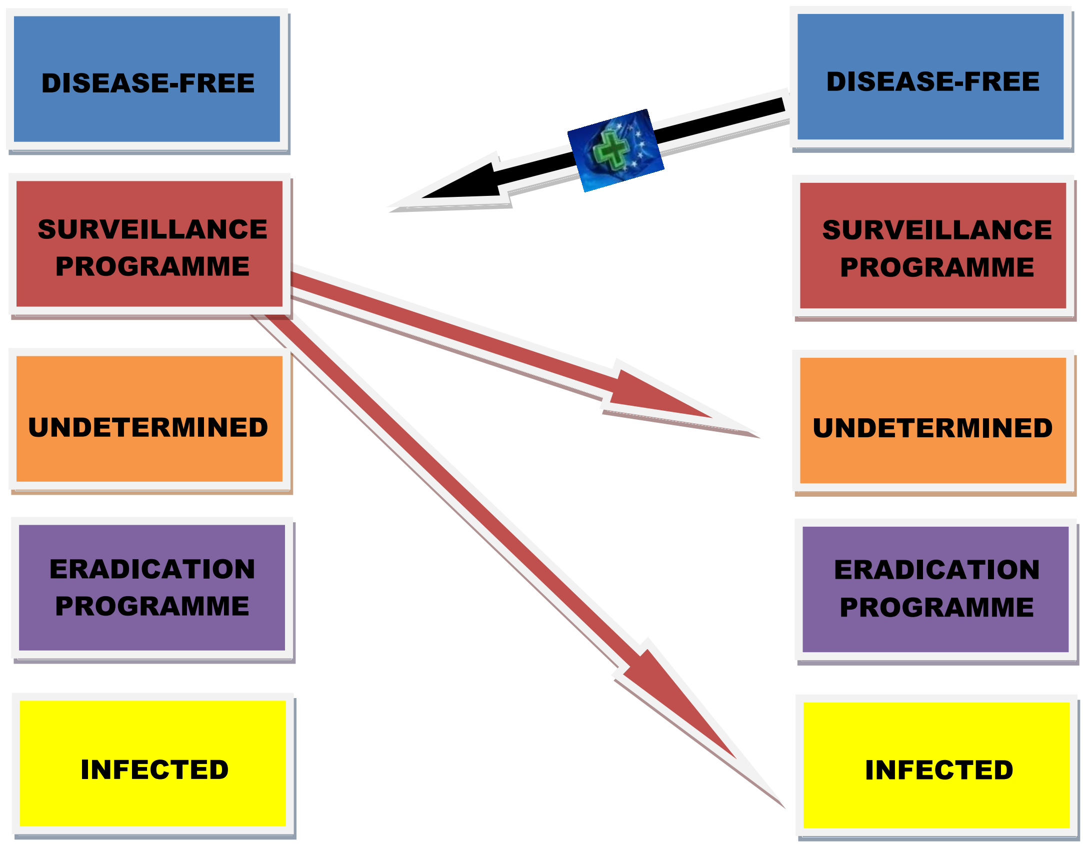
Figure 1.- Movement of fish and/or germplasm from a state declared disease-free (Category I)



As this chart shows, if a State, territory or zone is declared disease-free, it can transport fish and/or germplasm to any other EU State, territory or zone without restrictions, and health certificates will not be necessary if the destination is either category III (undetermined) or V (infected). By contrast, if the health status of the EU State from which the fish originate is category I (disease-free), II (surveillance programme) or IV (eradication programme), health certificates are necessary, and must indicate that the state from which the animals originated is disease-free. Similarly, the disease-free State can only receive fish and/or germplasm from another disease-free State, thereby complying with the golden rule "***if they are travelling to a free territory, they must come from a free country***".

 Health certifícate needed

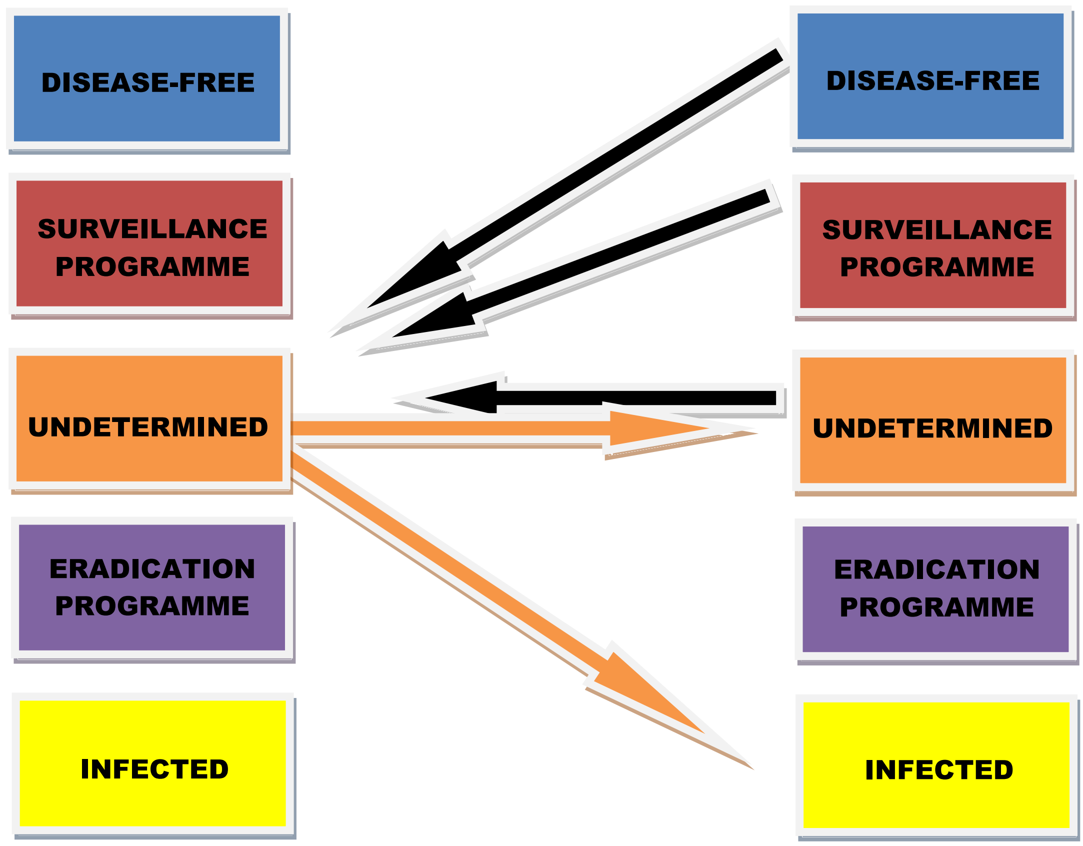
Figure 2.- Movement of fish and/or germplasm from a state with a surveillance programme (Category II)



As this chart shows, if a State, territory or zone has a surveillance programme in place, it can only transport fish and/or germplasm to an EU State, territory or zone of status III (Undetermined, not known to be infected) or category V (infected), and no health certificates will be needed. Similarly, it can only receive fish and/or germplasm from a State, territory or zone that has been declared disease-free, always with a health certificate.

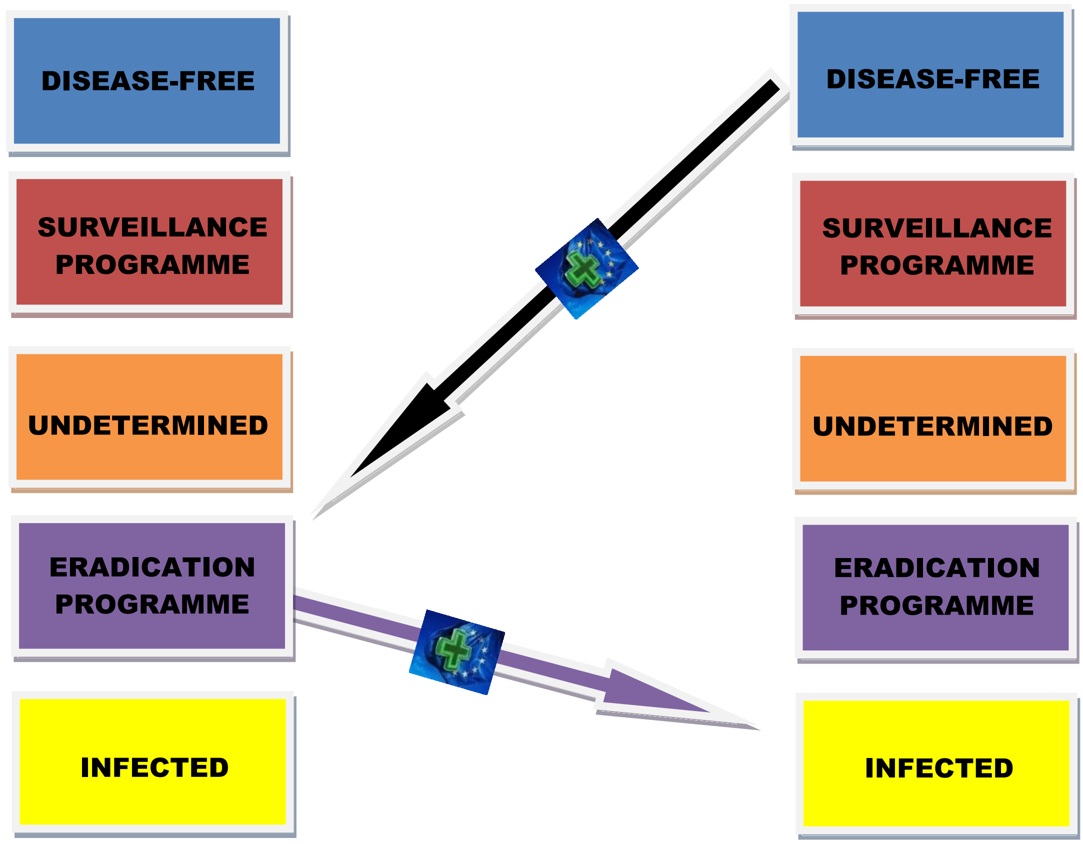
Health certifícate needed

Figure 3.- Movement of fish and/or germplasm from a State with health status Undetermined (Category III)



As this chart shows, if the health status of a State, territory or zone is Undetermined (category III), it can only transport fish and/or germplasm to an EU State, territory or zone of the same status (category III) or category V (infected), with no health certificates required. At the same time, it can only receive fish and/or germplasm from a State, territory or zone of categories I (disease-free), II (surveillance programme) and III (Undetermined); no health certificate is required for any of the cases described.

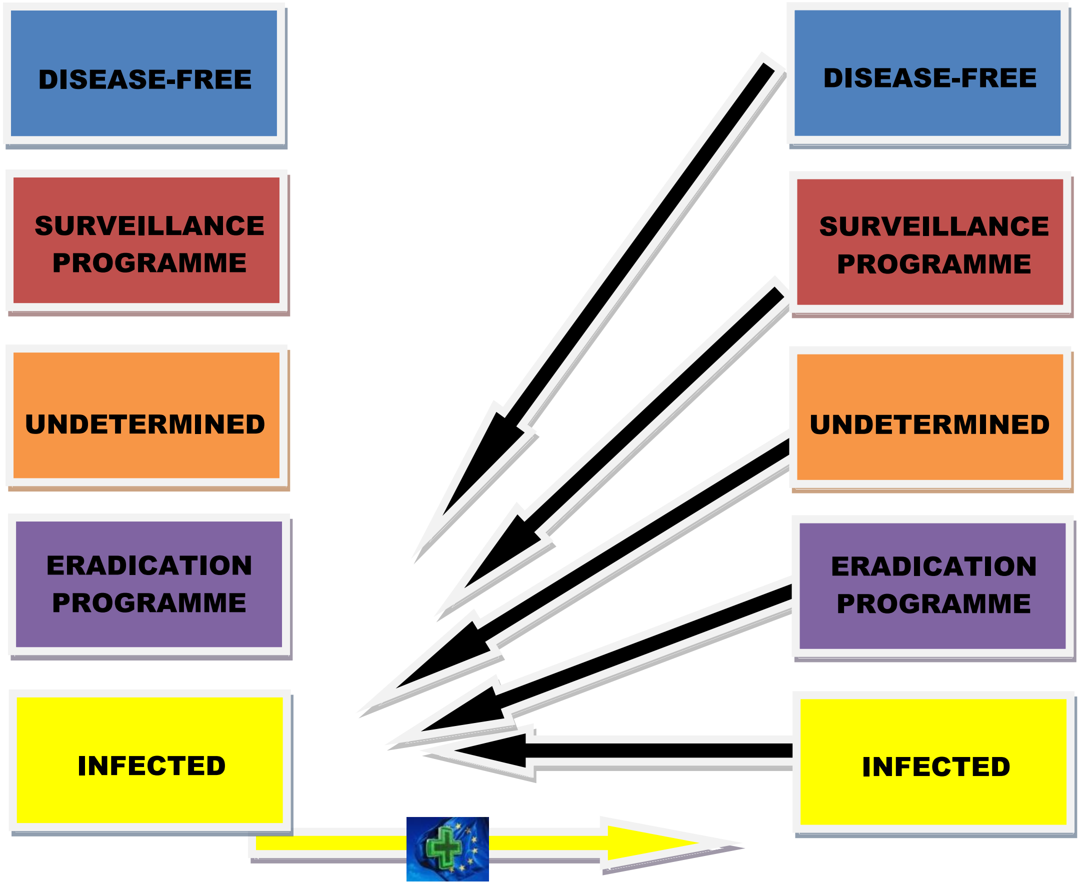
Figure 4.- Movement of fish and/or germplasm from a State with an eradication programme (Category IV)



As this chart shows, if a State, territory or zone has an authorized eradication programme in place (category IV), it can only transport fish and/or germplasm to an EU State, territory of zone in category V (infected), with no health certificates being necessary. At the same time, it can only receive fish and/or germplasm from a State, territory or zone belonging to category I, and health certificates will be required.

 Health certifícate needed

Figure 5.- Movement of fish and/or germplasm from an infected state (Category V)



As this chart shows, if the health status of a State, territory or zone is infected (category V), it can only transport fish and/or germplasm to an EU State, territory or zone with the same health status (category V), and the corresponding health certificate will be required By contrast, an EU State, territory or zone can receive fish and/or germplasm from other States, territories or zones, regardless of their health status, with no health certificate requirements.

Health certifícate needed

II.2.4.- Notification of movement by TRACES

The TRACES system is a management system for the movement of animals and products of animal origin coming from outside the European Union or its territory. This system consolidates and simplifies the existing systems. It represents an important innovation in the improvement of the management of epizootic diseases and reduces the administrative proceedings that affect the economic operators and authorities responsible. The TRACES system (***TRA**de **C**ontrol and **E**xpert **S**ystem*) creates one single central database to monitor the movements of animals and some products of animal origin within the European Union (EU) and those that come from outside the EU.

Its main characteristics are to:

- Electronic transmission of information;
- Centralized management of the statutory reference data;
- Interoperability with other information systems;
- Multilinguism.

Its objectives are to:

- improve the quantity and quality of information on animal movements;
- improve the exchange of information between the national and European authorities;
- improve the exchange of information between the national and European authorities;
- provide a system of electronic veterinary certificates which enables commercial operations to obtain information on-line;
- produce lists of establishments from countries outside of the EU which are authorised to export products of animal origin to the EU;
- manage consignments rejected at EU borders;
- target controls on public and animal health and animal welfare (particularly during their transport, etc.);
- centralise the evaluation of potential risks of an epidemic;
- overcome linguistic difficulties by making information from other countries more accessible;
- integrate all the operators concerned by putting in place a system for implementing operations related to exchanging documents between economic operators and competent authorities.

The research centres belonging to the AQUAEXCEL Project can be incorporated into TRACES when the authority responsible to which they are linked registers them. When they wish to proceed to transport animals, they will fill in an electronic form (one sole format) with all the necessary information about the animal or product of animal origin, the destination and possible stages. Should there be intraeuropean exchanges of animals or products of animal origin, this information will be transmitted to

the authority responsible of the Member State of origin. Once the contents of the form have been checked, said authority will either authorize the transport or not. Where necessary, it will issue the health certificate and the journey plan corresponding to the animals' welfare in the official languages of the Member States of origin and destination. The research centre member of the AQUAEXCEL Project may only proceed to carry out the transport if authorisation has been approved. All this information is sent to the veterinary authority of the destination Member State, to the central veterinary authority of the transit Member State(s) and all pertinent check points. Said check points may, therefore, be consulted regarding the motives behind controls carried out during the transport of at the destination point.

The TRACES system replaces other earlier systems, mainly ANIMO and SHIFT. ANIMO was created to monitor the movements of live animals and exchange information between national and European Community authorities. In order to improve the health security of the import of animals and products of animal origin coming from outside the EU, the SHIFT information programme was developed. This programme also included two further systems: the LMS system for managing the lists of establishments authorized to export to the EU and the RcS system for managing the shipment rejected at EU frontiers. The replacement of all these systems by one sole one, TRACES, ensures that work is not duplicated and simplifies the monitoring of movements of animals, making it more efficient. Generally speaking, the requisites for intracommunity trade are harmonized between the Member States. In order to guarantee that harmonization is maintained, the fish and/or germplasm have to come from authorized centres, normally under the supervision of an official veterinary scientist. Random controls may also be carried out on the products at their final destination. Apart from the general health requisites, specific requisites may be applied to animal products relating to questions such as the evolution of the situation of a disease in the Member States. In these cases, specific veterinary certificates may be demanded.

II.3- Transport of fish and/or germplasm

II.3.1.- General recommendations during transport

The following basic recommendations will apply for the transport of fish and/or germplasm:

1) Only those fish in a state such that they will not be injured or suffer unnecessarily may be transported.

2) Those fish with injuries, physiological problems or pathological processes will not be considered fit for transport. However, ill or injured fish may be considered fit to travel if:

- they have slight injuries or minor diseases and transporting them will not give rise to additional suffering.
- the disease or injury is part of a research programme.
- they are transported under veterinary supervision or following a veterinary treatment or diagnosis. However, any such transport shall only be authorized if it does not cause any unnecessary suffering.

3) Fish that fall ill or become injured during transport should be separated from the rest and receive appropriate veterinary attention. If necessary, they will be sacrificed quickly, to avoid any unnecessary suffering.

4) The centre from which the fish and/or germplasm originate will be responsible for ensuring that all necessary disease prevention measures are applied during the transport of fish and/or germplasm so as not to modify the sanitary situation of said animals while they are being transported and to reduce the risk of diseases propagation.

5) Fish and/or germplasm will not be transported in conditions that might affect their health status. In particular, they will not be transported in the same water or micro container as other aquatic animals of lesser health status. Aquaculture animals are to be transported in conditions that neither modify their health status nor jeopardise the health status of the point of destination or, where appropriate, places of transit.

6) During transport, the fish will not be unloaded from the micro container, and the water in which they are transported will not be changed in any country whose health status is lower than that of the destination point. The Member States will make sure that, during transport, all water changes will take place in places and conditions that do not jeopardise the health status of the aquaculture animals transported, the aquatic animals in the places where the water is changed, or the aquatic animals at the point of destination.

II.3.2.- Welfare of farmed fish during transport

The use of fish for research purposes entails the ethical responsibility of safeguarding their welfare as far as possible. In order to guarantee the welfare of fish during transport, it is necessary to resort to handling methods that are appropriate for the biological characteristics of the animal, as well as an environment adapted to their needs. The objective is to reduce as much as possible the effects of the air, maritime or land transport on the welfare of the fish, in the research centres of all the countries concerned.

In terms of preparing the fish for transport, the basic rules are as follows:

- Before transport, the fishes will be deprived of food, bearing in mind the species in question and the stage of development of the individual fish that are going to be transported.
- The capacity of the fish to bear the stress of the transport must be evaluated, bearing in mind their state of health and the preparatory handling and recent transport operations that they have undergone. Generally speaking, only those fish fit to be transported should be loaded.

- Fish will be considered unfit to be transported in the following cases:
    - when they show clinical signs of disease
    - when they suffer major physical injuries or behave abnormally, such as rapid breathing or unusual swimming movements.
    - when they have recently been exposed to stress factors, such as extreme temperatures and chemical products, that affect their behaviour or physiological state negatively.
    - insufficient or excessive length of fasting.

An emergency plan is needed to foresee significant incidents that might occur during the transport and affect the welfare of the fish negatively, as well as the management procedures and measures to be taken in each case. For each type of incident the plan will detail the measures to be adopted and the responsibilities of all the parties, which includes notification and registration of the facts.

II.3.3.- Water quality during transport

Periodical inspections will be carried out during transport to check that acceptable welfare conditions are maintained. Water quality will be controlled, taking whatever steps are necessary and the appropriate adjustments will be made to avoid extreme conditions. Water quality (such as O_2, CO_2 and NH_3, pH, temperature and salinity) must be appropriate for the species in transport and the means of transport used. Depending on how long the transport lasts, material may be needed to monitor and maintain water quality.

II.3.4.- Responsibilities during transport

The research centre from which the fish and/or germplasm originate is responsible for the following aspects:

- ensuring that the fish and/or germplasm are in a good general state of health and fit to travel at the beginning of the journey, and safeguarding their overall welfare during the period of transport, even if these tasks are contracted out to third parties;
- ensuring that the operations that take place in their installations are supervised by qualified competent personnel, so that the loading and unloading of the fish produce the least possible stress and injuries to the animals;
- drawing up an emergency plan stipulating that, where necessary, fish may be killed humanely at the beginning or end of the journey, or en route;
- ensuring that the fish are introduced into a suitable environment that guarantees their welfare when they arrive at their destination.

The person in charge of supervising the transport is responsible for all the corresponding documentation as well as the practical application of the recommendations for fish welfare during

transport.

The journey should take place in such as way as to reduce as much as possible any uncontrolled movements of fish that could cause them stress or injury. Should the fish suffer a health emergency during the period of transport, the expert in charge of the vehicle will put the emergency plan into action. If it becomes necessary to sacrifice the fish during transport, the killing should be humane, in accordance with the legislation in force.

II.3.5.- Documentation

Fish will not be loaded until the required documentation is available. The documentation accompanying the shipment must include:

- Notification of movement via TRACES

- Health certificate (where necessary)

- Certificate of authorised carriers.

II.4.- Disease prevention and control: General recommendations

Disinfection is a very habitual tool in disease management in aquaculture. Disinfection can be used in bio security programmes to eradicate or exclude specific diseases, and as a systematic health measure to reduce the incidence of a disease in aquaculture farms. The disinfection of installations, material and means of transport must be carried out following procedures that avoid contaminating other water and populations of aquatic animals with infectious material. There is a very large range of products and procedures with which to wash and disinfect the installations, equipment and means of transport, or to treat waste products generated during transport. The final choice will depend on effectiveness as a microbicide, and degree of harmlessness for aquatic animals and the environment.

The manufacturer's instructions must be followed to ensure that the use of disinfectants in aquaculture is effective. The disinfectants to be used must have proved to be effective regarding the aquatic pathogens and the conditions in question. The disinfection may be altered by a number of factors, such as temperature, pH or the presence of organic material. At high temperatures, a disinfectant works faster, provided the product does not suffer any degree of decomposition. At low temperatures, the biocide effectiveness of most disinfectants diminishes. Many have an optimum pH level, and so should be chosen depending on the pH of the diluent (water). For example, quaternary ammonium is more effective at alkaline pH levels, while iodine and iodophors are more effective at neutral or acidic pH levels. The presence of organic material and greasy substances may considerably reduce the disinfectant's

effectiveness. Surfaces must therefore be thoroughly cleaned before disinfectants are applied.

The general principles relating to the disinfection of aquaculture farms consists of the application of sufficiently concentrated chemical treatments for sufficiently long periods of time to kill all pathogenic organisms. As the toxicity inherent in disinfectants makes them unsafe to use in open waters or open water systems, disinfectants can only be applied in storage tanks. This chapter offers an overall perspective of disinfection methods. It is important to remember that there are other alternative methods in use. The choice of disinfection method depends on the size, type and nature of the material and installations to be disinfected, and on the products that are legally available in the country at the time. The surfaces to be disinfected may include fabrics or material (clothes, nets), hard surfaces (plastic, cement) or permeable material (earth, gravel). Disinfection is more difficult on permeable surfaces and takes longer. As the presence of organic material reduces the capacity of disinfection of most disinfectants, filtering the incoming water is highly recommended. Moreover, all surfaces should be cleaned before disinfection takes place. The detergent used must be compatible with the disinfectant and both must be compatible with the surface in question. Thus, for example, iodophor solutions tend to be acidic and should not therefore be used on concrete, which is alkaline. The waste material produced during the washing process must be disinfected before being eliminated.

Disinfection procedures must be established and used in accordance with the disinfection objectives, and taking any possible risks into consideration. Ill fish, animal liquids and tissue, and their contact with equipment and workers constitute a risk of transmission of pathogens that might end up infecting healthy fish. This process must be supervised at all times by a technical expert who has the necessary protection systems (protective clothing, mask, eye protection, etc) against contact with any dangerous substance. Disinfectants must be stored in such a way that they represent no direct or indirect danger to human or animal health or to the environment.

Washing and disinfection procedures must include at least the following steps:

- The elimination of solid waste, etc, followed by a prewash
- In-depth cleaning and washing
- Disinfection
- Rinsing

II.4.1.- Disinfection of installations, equipment, means of transport and eggs

Disinfection of fish farms and equipment

All the material used in the transport of the fish and/or germplasm must be used exclusively for said transport, and must be cleaned and disinfected before setting out and at destination once the transport has concluded, following the established protocols, as required, with authorized products. Machinery used in the research centre must not be transported to a different research centre, unless the latter has no fish.

The transport of machinery will only be allowed in exceptional cases, and only once it has been washed and disinfected with authorised products before entering and leaving the centre.

Disinfection of means of transport

As far as vehicles are concerned, the person in charge of transport from the research centre of origin must ensure that the following requisites are met:

- Any vehicle without exception entering the production area or transit zones must be disinfected with authorized products on entry and on leaving the fish farm, and must spend the shortest possible time in the area.

- The person in charge of the research centre (or person designated by the person in charge) must request a “Disinfection certificate” or similar document before a transport vehicle enters the fish farm. The certificate must confirm that the vehicle’s tanks in which the fish and/or germplasm are to be transported have been disinfected using authorized products.

- All those people who enter with a vehicle must follow the disinfection protocols established by the research centre.

- Vehicles and containers must be disinfected at the end of each transport carried out, and a “disinfection certificate” or similar to accredit said procedure must be requested periodically.

- There must be a register of all those vehicles that have entered the fish farm. The register must contain at least the date, hour, location, motive of visit, driver’s name and the last centre visited.

Disinfection of eggs with iodine

Generally speaking, the recommended concentration of iodine will be 100 mg/litre for 10 minutes. The pH of the solutions of iodophor products should range between 6 and 8. With a pH reading of 6 or less, toxicity for the eggs increases and at pH readings of 8 or over, the antiseptic effectiveness diminishes. Thus, pH surveillance is absolutely vital and, therefore, 100 mg/litre of $NaHC0_3$ to low alkaline waters. It is recommended to rinse the eggs with clean water before and after the disinfection or neutralize the iodine with sodium thiosulphate and use water that is free of organic material to prepare the iodophor solution. It is also recommended to use an abundant amount of solution and to renew it once it takes on a light yellow colour and before the colour disappears. One litre of solution concentrated at 100 mg/litre of disinfectant for 10 minutes will permit the disinfection of 2000 salmonid eggs.

Finally, when eggs are to be transported, the packaging must also be disinfected or, better still, destroyed by means of a procedure that prevents the water and/or other fish from the place of destination

from running any contamination or health risk. The use of iodophors demands certain prior precautions, as the products on the market contain a variable amount of detergents and may lead to toxic effects. It is therefore advisable to test the products before they are placed in the market and to choose the most satisfactory one, bearing in mind its expiry date. Iodine can be used to disinfect the eggs of any fish species, but it is mainly used for salmonids. For other species it will be necessary to test the products to determine the time and the concentration that can be used without causing any danger. The disinfection of eggs with iodine can be carried out for various fish species, but it is most commonly used for salmonid eggs. Although it is generally effective for the decontamination of the surfaces of embryonated and recently fertilized eggs, the use of disinfectants such as iodophors does not prevent the vertical transmission of some bacterial pathogens such as *Renibacterium salmoninarum*, the Infectious haematopoietic necrosis virus and the infectious pancreatic necrosis virus. Iodine is highly toxic for fish, and, to avoid serious accidents as a result of handling mistakes, it is advisable to neutralize them with sodium thiosulphate, using an amount of thiosulphate equivalent to 0.78 times the quantity of iodine administered.

Disinfection of embryonated salmonid eggs with iodophors

It is recommended that the eggs be washed with clean, clear water, or saline solution at 0.9%, before and after the disinfection. The disinfectant solution will be a solution of iodophors of 100 ppm of free iodine in saline solution at 0.9% free of organic material. The contact time for solutions of iodophors in a 100 ppm solution must not be less than 10 minutes, and the solution must be used once only.

Disinfection of recently fertilized salmonid eggs through a process of water hardening

To disinfect the recently fertilized eggs of salmonids by means of a process of water hardening with iodophors, the concentration of active iodine must be 100 ppm. One of the most commonly used procedures is the following:

- Eggs must be stripped and separated of ovarian fluid with a saline solution at 0.9% (30-60 seconds), the sperm added and the fertilization carried out for 5-15 minutes.
- Eggs must be washed with saline solution at 0.9% for 30-60 seconds to eliminate excess sperm and other organic material.
- Eggs must be washed with a solution of iodophor at 100 ppm for 1 minute. The solution must be thrown away and replaced with a fresh solution at the same concentration of iodophors and incubated for a further 30 minutes. This solution, and the solutions used for washing, must be used only once. The proportion of eggs and iodophor solution must be of a minimum of 1:4.
- Eggs must be rinsed in fresh water or sterile incubation water for 30-60 seconds. It is important that the eggs are not fertilized in the presence of iodophor solution, as this kills the spermatozoids.

Disinfection of eggs of other species

For species other than salmonids, preliminary tests must be carried out in order to determine the optimum concentration of iodophor and incubation time. In the disinfection of the eggs of marine species such as plaice, cod and Atlantic halibut, adverse effects have been described with the use of iodophors, and in these cases a solution of 400-600 mg/litre of glutaraldehyde should be used with a contact time of 5-10 minutes. However, this is not effective against nodavirus, whereas ozone at a concentrate of 1 mg O_3mg/litre for 30 seconds, is. As a general rule, a concentration of 0.1-0.2mg O_3/litre of 3 minutes inactivates most of the bacterial pathogens of fish.

Precaution with the use of iodophors

Certain precautions must be taken before using iodophors, as there are certain products in the market that contain a variable amount of detergents that may cause toxic effects on the eggs. For this reason, it is always advisable to carry out preliminary tests if we change product and/or if we use a new one for the first time. It is also important that any chemical product used to disinfect the eggs be used following the standards of each country in security and environmental protection matters. Finally, in the case of those eggs that have been transported, the container must also be disinfected or, better still, destroyed in a way that does not represent any contamination risk for the water and/or risk for the health of other fish.

II.5.- Handling, elimination and treatment of aquatic animals during transport

The water used in the transport of fish and/or germplasm will be treated appropriately before transport and while it is being discharged to reduce the risk of propagating pathogens as much as possible. During the transport of fish and/or germplasm, the carrier will not be authorised to discharge and replace the water of the transport tanks anywhere that is not especially reserved for this purpose. The waste and rinse water must not be emptied into a discharge system that is directly connected to an aquatic environment populated with fish. Consequently, the water in the tanks must be disinfected by an authorized procedure or be discharged in land that does not drain into water populated with fish (each country will designate in its national territory the places in which these operations may be carried out).

The chlorine administered as sodium hypochlorite at a chlorine concentration of 25 mg/litre is effective against certain protozoa. However, 50 mg of chlorine per litre is recommended for a complete microbial sterilization.

Ozone has been used successfully in the control of the microbial contents of waste water. Waste compounds formed as a result of the interaction of ozone with water at levels of 0.08-1.0 mg/litre are considered sufficient to significantly reduce the pathogens (mainly bacteria). The ensuing waste is toxic for early-stage fish, so needs to be neutralised before being liberated into the environment. An additional

treatment with ultraviolet radiation after ozonisation may be necessary to ensure complete sterilisation when fish die during transport possibly as a result of the existence of an infectious-contagious disease.

Iodophors are not as effective as the two previous treatments in the killing of protozoa, but they are effective for the main bacterial pathogens. Chlorine and iodine are very toxic for aquatic animals and in order to avoid accidents as a result of mistakes in their handling, these products are recommended to be neutralized with sodium thiosulphate. In order to inactivate the chlorine, the amount of thiosulphate must be 2.85 times the amount of chlorine (in grams). For iodine, the amount of thiosulphate must be 0.78 times the amount of iodine in grammes.

II.6.- Killing of fish for disease control purposes

These guidelines are based on the report of the meeting of the OIE Aquatic Animal Health Standards Commission Paris, October 2011). These recommendations are based on the premise that a decision to kill the farmed fish for disease control purposes has been made, and address the need to ensure the welfare of the farmed fish until they are dead. The culling of individual farmed fish, in the course of farming operations (i.e. sorting, grading, or background morbidity), is out of the scope of this chapter.

General principles

- Fish welfare considerations should be addressed within contingency plans for disease control.
- The killing method should be selected taking into consideration fish welfare and biosecurity requirements as well as safety of the personnel.
- When fish are killed for disease control purposes, methods used should result in immediate death or immediate loss of consciousness lasting until death; when loss of consciousness is not immediate, induction of unconsciousness should be non-aversive or the least aversive possible and should not cause avoidable anxiety, pain, distress or suffering in fish.
- Depending on the situation, emergency killing of fish may be carried out on site or after fish are transported to an approved killing facility.

Operational guidelines for affected premises

The following should apply when killing fish:

- Operational procedures should be adapted to the specific circumstances on the premises and should address biosecurity and fish welfare specific to the *disease* of concern.
- Killing of fish should be carried out without delay by appropriately qualified personnel with all due consideration made to increased biosecurity protocols.
- Handling of fish should be kept to a minimum to avoid stress and to prevent spread of *disease*. This should be done in accordance with the articles described below.
- Methods used to kill the fish should render them unconscious or kill them in the shortest time possible in the circumstances, and should not cause avoidable pain or distress.

- There should be continuous monitoring of the procedures to ensure they are consistently effective with regard to biosecurity and fish welfare.
- Standard operating procedures (SOP's) should be available and followed at the premises.

A protocol for the killing of fish on affected premises for disease control purposes should be developed by the operator and approved by the *Competent Authority*, taking into consideration fish welfare and biosecurity requirements as well as safety of the personnel and should include consideration of:

- handling and movement of fish;
- species, number, age, size of fish to be killed;
- methods for killing the fish;
- availability of anaesthetic agents suitable to kill the fish;
- equipment needed to kill the fish;
- any legal issues (e.g. the use of anaesthetic agents suitable for killing fish);
- presence of other nearby aquaculture premises;

Competencies and responsibilites of the operational team

The operational team is responsible for planning, implementation of, and reporting on the killing of the fish.

<u>Team leader</u>

a) Competencies

- Ability to assess fish welfare, especially relating to the effectiveness of the stunning and killing techniques selected and utilised in the fish killing operations, to detect and correct any deficiencies;
- ability to assess biosecurity risks and mitigation measures being applied to prevent spread of *disease*;
- skills to manage all activities on premises and deliver outcome on time;
- awareness of the emotional impact on fish farmers, team members and general public;
- effective communication skills.

b) Responsibilities

- Determine most appropriate killing method(s) to ensure that the fish are killed without avoidable pain and distress while balancing biosecurity considerations;
- plan overall operations on the affected premises;
- determine and address requirements for fish welfare, operator safety and biosecurity;
- organise, brief and manage a team of people to facilitate killing of the relevant fish in accordance with national contingency plans for disease control;
- determine logistics required;
- monitor operations to ensure that fish welfare, operator safety and biosecurity

requirements are met;

- report upwards on progress and problems;
- provide a written report summarising the killing, practices utilised in the operation and their effect on fish welfare and subsequent biosecurity outcomes. The report should be archived and be accessible for a period of time defined by the *Competent Authority*;
- review on-site facilities in terms of their appropriateness for mass destruction.

On-site personnel responsible for killing of fish

a) Competencies

- Specific knowledge of fish, their behaviour and environment;
- trained and competent in fish handling, stunning and killing procedures;
- trained and competent in the operation and maintenance of equipment.

b) Responsibilities

- Ensure killing of fish through effective stunning and killing techniques;
- assist team leader as required;
- design and construct temporary fish handling facilities, when required.

II.6.1.- Killing by an overdose of an anaesthetic agent

Anaesthetic agents used for killing fish should kill the fish effectively, not merely have an anaesthetic effect. When using anaesthetic agents, the operating personnel should ensure that the solution has the correct concentration, and that sea water is used for marine fish species and freshwater for freshwater species. Fish should be kept in the anaesthetic solution until they are dead.

Advantages

- Large numbers of fish may be killed in one batch
- handling is not required until fish are anaesthetised
- use of anaesthetic agents is a non-invasive technique and thus reduces biosecurity risks.

Disadvantages

- May need to be followed by killing if fish are only anaesthetised
- some anaesthetic agents may induce a transient aversive reaction in the fish
- care is essential in the preparation and provision of treated water, and in the disposal of water and/or fish carcasses that have been treated with anaesthetic agents.

II.6.2.- Mechanical killing methods

Decapitation

- Decapitation, using a sharp device such as a guillotine or knife, may be used
- the required equipment should be kept in good working order
- contamination of the working area by blood, body fluids and other organic material may present a biosecurity risk and is the major disadvantage of this method.

Maceration

- Maceration by a mechanical device with rotating blades or projections causes immediate fragmentation and death in newly hatched *fish* and embryonated eggs, as well as fertilised/unfertilised eggs of *fish*. It is a suitable method for the processing of such material. The procedure results in rapid death and a large number of eggs/newly hatched fry can be killed quickly.
- maceration requires specialised equipment which should be kept in good working order. The rate of introducing material into the device should be such that the cutting blades continue to rotate at their fully functional rate and that they do not fall below the defined critical speed defined by the manufacturer.
- contamination of the working area by blood, body fluids and other organic material may present a biosecurity risk and is the major disadvantage of this method.

Annex I. Protocol of Action in the transport of fish and/or germplasm

STEP I

Notification of movement to the responsible authority via TRACES
See point II.2.4

STEP II

Issue health certificate

Said certificate will only be made out by Official State Veterinaries. See point II.2.3, Table 1, Table 17 and Figures 1-5

NB: As stated in point II.2.3, although the current law in force does not require a health certificate in all cases, we may come up against Customs Officials who demand said documentations despite the fact that it is not required by law. It is therefore advisable to obtain certificates to avoid unnecessary delays and to avoid the fish and/or germplasm being held up until their legal situation is clarified.

STEP III

Certificate of authorised carriers

See point II.3.5.

Annex II List of species susceptible to and vectors of different diseases in Directive 2006/88 EC and Commission Decision 2010/221EU requiring health certification

	Oncorhynchus mykiss	*Salmo salar*	*Cyprinus carpio*	*Gadus morhua*	*Scophthalmus maximus*	*Sparus aurata*	*Dicentrarchus labrax*	*Solea senegalensis*	*Hippoglossus hippoglossus*	*Thunnus thynnus*	*Argyrosomus regius*
Epizootic haematopoietic necrosis											
Epizootic ulcerative syndrome											
Viral haemorrhagic septicaemia											
Infectious salmon anaemia											
Koi herpes virus disease											
Infectious haematopoietic necrosis											
Gyrodactylus salaris											
Renibacterium salmoninarum											
Infectious pancreatic necrosis											
Spring viraemia of carp											

SUSCEPTIBLE SPECIE

VECTOR SPECIE

EXOTIC DISEASE DIRECTIVE 2006/88

NON EXOTIC DISEASE DIRECTIVE 2006/88

NATIONAL MEASURES COMMISSION DECISION 2010/221 EU

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook

Disease/pathogen	Susceptible species	Vector species	Geographical distribution	Diagnosis
Epizootic haematopoietic necrosis (Exotic disease)	*Perca fluvialis* *Oncorhynchus mykiss*	*Aristichthys nobilis* *Carassius auratus* *Carassius carassius* *Cyprinus carpio* *Hypophthalmichthys molitrix* *Leuciscus* spp *Rutilus rutilus* *Scardinius erythrophthalmus* *Tinca tinca*	Australia and Namibia	Virus isolation Neutralization Immunofluorescence ELISA PCR
Spring viraemia of carp (Disease with approved national measures by Commission Decision 2010/221 EU)	*Cyprinus carpio carpio* *Cyprinus carpio koi* *Carassius carassius* *Silurus glanis* *Hypophthalmichthys molitrix* *Aristichthys nobilis* *Ctenopharyngodon idella* *Carassius auratus* *Leuciscus idus* *Tinca tinca* *Rutilus rutilus* *Danio rerio* *Esox lucius* *Lebistes reticulatus* *Lepomis gibbosus*	Not described	Bolivia, Brazil, Canada, China, USA, Germany, Austria, Bosnia-Herzegovina, Croatia, Denmark, Slovenia, Spain, France, Holland, Hungary, Italy, Kuwait, Lithuania, Macedonia, Moldova, Poland, United Kingdom, Czech Republic, Romania, Russia, Serbia, Slovakia, Switzerland, Ukraine, and Laos	Virus isolation Neutralization Immunofluorescence ELISA PCR Sequencing
Koi herpes virus disease (Non Exotic disease)	*Cyprinus carpio carpio* *Cyprinus carpio goi* *Cyprinus carpio koi* Hybrids of *Cyprinus carpio*	Not described	USA, Europe, Germany, Austria, Belgium, Denmark, France, Holland, Israel, United Kingdom, Switzerland, Japan and Taiwan	Virus isolation Neutralization ELISA PCR DNA amplification Reverse transcription mRNA Indirect Immunofluorescence Histopathology study Hybridization *in situ* Electron microscopy

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook

Infectious haematopoietic necrosis (Non Exotic disease)	*Oncorhynchus mykiss* *Salmo trutta* *Oncorhynchus tshawytscha* *Oncorhynchus nerka* *Oncorhynchus keta* *Oncorhynchus masou* *Oncorhynchus rhodurus* *Oncorhynchus kisutch* *Salmo salar*	*Acipenser gueldenstaedtii* *Acipenser ruthenus* *Acipenser stellatus* *Acipenser sturio, Acipenser Baerii* *Aristichthys nobilis* *Carassius auratus* *Carassius carassius* *Cyprinus carpio* *Hypophthalmichthys molitrix* *Leuciscus spp, Rutilus rutilus* *Scardinius erythrophthalmus* *Tinca tinca, Huso huso* *Clarias gariepinus* *Ictalurus* spp., *Ameiurus melas* *Ictalurus punctatus* *Pangasius pangasius* *Sander lucioperca, Silurus glanis* *Hippoglossus hippoglossus* *Platichthys flesus, Gadus morhua* *Melanogrammus aeglefinus*	USA, Asia and Continental Europe	Virus isolation Neutralization Immunofluorescence ELISA RT-PCR
Nodavirus	*Lates calcarifer* *Dicentrarchus labrax* *Epinephelus akaara* *Epinephelus fuscogutatus* *Epinephelus malabaricus* *Epinephelus moara* *Epinephelus septemfasciatus* *Epinephelus tauvina* *Epinephelus coioides* *Cromileptes altivelis* *Pseudocaranx dentex* *Oplegnathus fasciatus* *Takifugu rubripes* *Verasper moseri* *Hippoglossus hippoglossus* *Paralichthys olivaceus* *Scophthalmus maximus* *Solea senegalensis*	*Sparus aurata*	Asia, Australia, Canada, China, Korea, USA, Spain, France, Greece, Italy, Malta, Norway, Portugal, United Kingdom, Philippines, Hong Kong, Indonesia, Japan, Malasia, Martinique, Singapore, Tahiti, Thailand, Taiwan and Tunisia	Virus isolation Neutralization Histopathology study Electron microscopy RT-PCR Immunohistochemistry Indirect Immunofluorescence ELISA

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Viral haemorrhagic septicaemia (Non Exotic disease)	*Clupea* spp. *Coregonus* sp. *Esox lucius* *Gadus aeglefinus* *Gadus macrocephalus* *Gadus morhua* *Oncorhynchus* spp. *Onos mustelus* *Sprattus sprattus* *Thymallus thymallus* *Oncorhynchus mykiss* *Salmo trutta* *Scophthalmus maximus*	*Aristichthys nobilis* *Carassius auratus* *Carassius carassius* *Cyprinus carpio* *Hypophthalmichthys molitrix* *Leuciscus* spp, *Rutilus rutilus* *Scardinius erythrophthalmus* *Tinca tinca* *Clarias gariepinus* *Esox lucius* *Ictalurus* spp. *Ameiurus melas* *Ictalurus punctatus* *Pangasius pangasius* *Sander lucioperca* *Silurus glanis* *Dicentrarchus labrax* *Morone chrysops x M. saxatilis* *Mugil cephalus* *Sciaenops ocellatus* *Argyrosomus regius* *Umbrina cirrosa* *Thunnus* spp. *Thunnus thynnus* *Epinephelus marginatus* *Solea senegalensis* *Solea solea* *Pagellus erythrinus* *Dentex dentex* *Sparus aurata* *Diplodus sargus* *Pagellus bogaraveo* *Pagrus major* *Diplodus puntazzo* *Diplodus vulgaris* *Pagrus pagrus* *Oreochromis*	Japan, USA, United Kingdom, Baltic Sea and Strait of Skagerrak and Kattegat	Virus isolation Neutralization Immunofluorescence ELISA PCR

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Infectious salmon anaemia (Non Exotic disease)	*Salmo salar*	*Salmo trutta* *Onchorynchus mykiss* *Pollachius virens* *Gadus morhua* *Salvelinus alpinus* *Clupea harengus*	Norway, Canada, Scotland, Faroe Islands, USA and Chile	Immunofluorescence Immunohistochemistry Virus isolation RT-PCR ELISA
Infectious pancreatic necrosis (Disease with approved national measures by Commission Decision 2010/221 EU)	*Oncorhynchus mykiss* *Salvelinus fontinalis* *Salmo trutta* *Salmo salar* *Oncorhynchus* spp. *Seriola quinqueradiata* *Scophthalmus maximus* *Hippoglossus hippoglossus* *Gadus morhua* *Misgurnus anguillicaudatus* *Esox lucius* *Astacus astacus* *Anguillidae* *Atherinidae* *Bothidae* *Carangidae* *Catostomidae* *Cichlidae* *Clupeidae* *Cobitidae* *Coregonidae* *Cyprinidae* *Esocidae* *Moronidae* *Paralichthydae* *Percidae* *Poecilidae* *Sciaenidae* *Soleidae* *Thymallidae*	Not described	North America, South America, Asia and Europe (Germany, Denmark, Spain, Finland, France, Greece, Holland, Italy, Norway, Poland, United Kingdom, Sweden, Switzerland, Turquía and Balkan region)	Virus isolation Neutralization Immunofluorescence ELISA RLFP RT-PCR

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Red sea bream iridoviral disease	*Acanthopagrus schlegeli* *Acanthopagrus latus* *Evynnis japonica, Pagrus major* *Seriola quinqueradiata* *Seriola dumerili, Seriola lalandi* *Seriola lalandi* × *S.quinqueradiata* *Pseudocaranx dentex* *Thunnus thynnus* *Scomberomorus niphonius* *Scomber japonicus* *Trachurus japonicus* *Oplegnathus fasciatus* *Oplegnathus punctatus* *Rachycentron canadum* *Trachinotus blochii* *Parapristipoma trilineatum* *Plectorhynchus cinctus* *Lethrinus haematopterus* *Lethrinus nebulosus* *Girella punctata* *Sebastes schlegeli* *Pseudosciaena crocea* *Epinephelus akaara* *Epinephelus septemfasciatus* *Epinephelus malabaricus* *Epinephelus bruneus* *Epinephelus coioides* *Epinephelus awoara* *Epinephelus tauvina* *Epinephelus fuscoguttatus* *Epinephelus lanceolatus* *Lateolabrax japonicas* *Lateolabrax* sp. *Lates calcarifer* *Morone saxatilis* × *M. chrysops* *Micropterus salmoides* *Paralichthys olivaceus* *Verasper variegatus* *Takifugu rubripes*	Not described	Japan, China, Hong Kong, Korea, Malasia, Philippines, Singapore and Thailand.	Virus isolation Neutralization Immunofluorescence PCR Sequencing

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook

Aeromonas spp	*Salmonidae* *Labridae* *Ciprínidae* *Gadus morhua* *Pollachius virens* *Scophthalmus maximus* *Cottus gobio* *Cyprinus carpio* *Esox* spp., *Gadus morhua* *Galaxiidae* spp. *Hippoglossus hippoglossus* *Sparus aurata*	Not described	Australia, Canada, USA, Denmark, Spain, Finland, France, Iceland, Norway, United Kingdom, Sweden and Japan	Culture ELISA latex agglutination PCR RAPD-PCR
Vibrio spp	*Salmónidae* *Dicentrarchus labrax* *Scophthalmus maximus* *Gadus morhua* *Oncorhynchus mykiss* *Anguillidae* *Plecoglossus altivelis* *Epinephelus* spp. *Oncorhynchus kisutch* *Gadus callaris* *Salvelinus alpinus* *Hippoglossus hippoglossus* *Anguilla japonica* *Plecoglossus altivelis* *Mugil cephalus* *Seriola dumerelli* *Lates calcarifer* *Oncorhynchus tshawytscha* *Pseudopleuronectes americanus.* *Cyprinus carpio*	Not described	Worldwide	Culture PCR DNA microarray
Winter ulcer disease (*Moritella viscosa*)	*Salmo salar* *Gadus morhua* *Oncorhynchus mykiss* *Scophthalmus maximus* *Salmo trutta* *Pleuronectes platessa* *Hippoglossus hippoglossus*	Not described	Norway, Iceland, Scotland, Faroe Islands, Canada and Ireland	Culture PCR Immunohistochemistry

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook

Yersinia ruckeri	*Salmonidae* *Cyprinidae*	Not described	Worldwide	Culture ELISA PCR
Renibacterium salmoninarum (Disease with approved national measures by Commission Decision 2010/221 EU)	*Salmonidae*	Not described	Central Europe	Culture in KDM-2 PCR ELISA
Piscirickettsia salmonis	*Salmo salar* *Dicentrarchus labrax* *Tetrodon fahaka* *Oncorhynchus mykiss* *Oreochromis niloticus* *Oncorhynchus gorbuscha* *Paneque suttoni* *Oncorhynchus kisutch* *Oncorhynchus tshawytscha* *Callionymus lyra*	*Paralabrax humeralis* *Trachurus murphyi* *Basilichthys australis* *Eliginops maclovinus* *Merluccius* sp.	Egypt, Germany, Scotland, France, Norway, Wales, Canada, Colombia, Ireland, Japan, Taiwan and Chile	Cellular culture Smear Indirect Immunofluorescence ELISA PCR
Photobacterium damselae subsp. piscicida	*Anguilla reinhardtii* *Chromis punctipinnis* *Seriola quinqueradiata* *Dicentrarchus labrax* *Solea senegalensis* *Scophthalmus maximus* *Sparus aurata* *Pagrus Pagrus*	Not described	USA, Japan, Portugal, Malta, China, France, Spain, Italy, Greece and Turkey.	Culture PCR Sequencing
Cytophaga – Flavobacterium – Bacteroides group	*Salmonidae* *Plecoglossus altivelis* *Micropterus salmoides floridanus* *Perca fluviatilis* *Ictalurus punctatus* *Sparus aurata, Solea* spp. *Scophthalmus maximus* *Dicentrarchus labrax* *Pleuronectidae* *Dicologoglossa cuneata*	Not described	Europe, Japan, North America	Culture PCR Smear

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Streptococcus spp	*Oncorhynchus mykiss* *Sparus aurata* *Scophthalmus maximus* *Dicentrarchus labrax* *Cyprinus carpio* *Salmo salar* *Pagrus pagrus* *Oncorhynchus kisutch* *Paralichthys olivaceus* *Crysoleucas Notemigonus* *Brevoortia patronus* *Arius felis* *Mugil cephalus* *Lagodon romboides* *Micropogonias undulatus* *Leiostomus xanthurus* *Dasyatis sp.,* *Cynoscion nothus* *Fundulus grandis* *Morone saxatilis* *Pomatomus saltatrix* *Cynoscion regalis* *Oreochromis aureus x O. niloticus* *Liza klunzingeri* *Leponis macrochirus* *Lepomis cyanellus* *Ictalurus punctatus,* *Seriola dumerili* *Seriola quinqueradiata* *Oncorhynchus rhodurus* *Oreochromis niloticus* *Lates calcarifer* *Sciaenops ocellatus* *Siganus rivulatus*	Not described	USA, Japan, Taiwan, Singapore, Australia, Israel, Italy, Greece, Spain, China, France, South Africa. United Kingdom, Chile, Caribbean Sea, Barbados and Grenada	Culture PCR Sequencing LAMP Immunohistochemistry

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Epizootic ulcerative syndrome(*Aphanomyces invadens*) (Exotic disease)	*Acantopagrus australis* *Anabas testudineus* *Fluta alba, Icraluridae* *Bagridae* *Bidyanus bidyanus* *Brevoortia tyrannus* *Catla catla* *Channa striatus* *Cirrhinus mrigala* *Clarius batrachus* *Esomus* sp. *Glossogobius giuris* *Oxyeleotris marmoratus* *Glossogobius* sp. *Labeo rohita* *Lates calcarifer* *Mugil cephalus* *Mugilidae* *Plecoglossus altivelis* *Puntius sophore* *Sillago ciliata* *Siluridae* *Trichogaster pectoralis* *Colisa lalia* *Osphonemus goramy* *Trichogaster trichopterus* *Puntius gonionotus* *Scatophagus argus* *Platycephalus fuscus* *Psettodes sp.* *Rohtee sp.* *Terapon sp.* *Toxotes chatareus* *Carassius auratus*	*Aristichthys nobilis* *Carassius auratus* *Carassius carassius* *Cyprinus carpio* *Hypophthalmichthys molitrix* *Leuciscus spp* *Rutilus rutilus* *Scardinius erythrophthalmus* *Tinca tinca*	Australia, Bangladesh, Bhutan, Cambodia, USA, Philippines, India, Indonesia, Japan, Laos, Malasia, Myanmar, Nepal, Pakistan, PapuaNewGuinea, Singapore, Sri Lanka, Thailand and Vietnam.	Histopathology study Isolation (*Aphanomyces invadans*) Epifluorescence PCR
Saprolegnia	*Salmo salar, Orconectes propinquus, Carassius auratus, Danio rerio, Salmo trutta, Ictalurus punctatus*	Not described	Worldwide	Culture Histopathology study Electron microscopy PASS staining Grocott´s staining

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Phylum Myxozoa *Enteromyxum leei*	*Diplodus puntazo* *Sparus aurata* *Sparidae* *Mugilidae* *Labridae* *Centracanthidae* *Mullidae* *Pomacentridae* *Scorpaenidae* *Molidae* *Blenniidae* *Gobiidae* *Tetraodontidae* *Oplegnathidae* *Paralichthyidae* *Danio rerio* *Puntius tetrazona* *Astronotus ocellatus* *Oreochromis mossambicus*	Not described	Cyprus, Croatia, Spain, France, Greece, Italy, Malta, Turkey, Israel and Japan.	Smear Histology PCR Immunohistochemical tests ELISA
Phylum Myxozoa *Enteromyxum scophthalmi*	*Scophthalmus maximus* *Solea senegalensis*	Not described	Atlantic Europe	Smears Histopathology study PCR Immunohistochemistry ELISA PCR
Class Monogenea *Dactylogyrus*	*Ciprinidae*	Not described	Growing areas of susceptible species	Scrapingof the skin andgills Smear Sequencing Histology Electron microscopy
Class Monogenea *Diplectanum aequans* *Diplectanum laubieri*	*Dicentrarchus labrax*	Not described	Mediterranean and Atlantic coasts	Scrapingof the skin andgills Smear Sequencing Histology Electron microscopy
Class Monogenea *Furnestinia echenei*	*Sparus aurata*	Not described	Mediterranean and Atlantic coasts and Red Sea	Scrapingof the skin andgills Smear Histology Electron microscopy

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Class Monogenea *Serranicotyle labracis*	*Sparus aurata, Dicentrarchus labrax, Diplodus puntazzo*	Not described	Atlantic, Cantabrian and Mediterranean coasts	Scrapingof the skin andgills Smear Sequencing Histology Electron microscopy
Class Monogenea *Entobdella soleae*	*Solea solea*	Not described	Atlantic Europe	Scrapingof the skin andgills Smear Sequencing Histology Electron microscopy
Class Monogenea *Sparycotyle chrisophrii*	*Sparus aurata, Dicentrarchus labrax, Diplodus puntazzo*	Not described	Growing areas of susceptible species	Histopathology study Sequencing Electron microscopy Scrapingof the skin andgills
Class Monogenea *Zeuxapta seriolae*	*Seriola quinqueradiata* *Seriola dumerili*	Not described	Growing areas of susceptible species	Scrapingof the skin andgills Smear Sequencing Histology Electron microscopy
Phylum Nematoda *Anisakis*	*Gadus morhua, Merlangius merlangus, Gadus poutassou, Sardina pilchardus, Engraulis encrasicolus, Trachurus* sp.*Scomber scombrus, Micromesistius poutassou, Pollachius virens,Trisopterus luscus, Trisopterus luscus, Percophis brasiliensis, Merluccius* sp.*Albula vulpes, Brama brama, Pagellus* sp.*Scomber scombrus, Trichiurus lepturus,Pleuronectes platessa, Chelidonichthys cuculus, Conger* sp.	Not described	Growing areas of susceptible species	Morphological identification PCR Histological sections of tissues ELISA
Phylum Myxozoa *Sphaerospora testicularis* *Sphaerospora dicentrarchi*	*Dicentrarchus labrax*	Not described	Mediterranean Europe	Smear Histology PCR Immunohistochemical tests ELISA

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Phylum Ciliophora *Cryptocarion irritans* *Ichthyopththirius multifiliis*	All marine species	Not described	Tropical and subtropical	Histology Smear Sequencing ELISA Culture *in vitro* PCR Electron microscopy
Phylum Myxozoa *Sphaerospora dicentrarchi*	*Dicentrarchus labrax*	Not described	Mediterranean Europe	Smear Histology PCR Immunohistochemical tests ELISA
Class Trematoda *Familia Sanguinicolidae*	*Sparus aurata*	Not described	Presence is not well-documented	Histology PCR Morphological identification
Phylum Myxozoa *Tetracapsuloides byosalmonae*	*Salmonidae*	Not described	Europe USA Canada	Smear Histology PCR Immunohistochemical tests ELISA
Phylum Myxozoa *Myxosoma cerebralis*	*Salmonidae*	Not described	Europe USA	Smear Histology PCR Immunohistochemical tests ELISA
Phylum Ciliophora *Philasterides dicentrarchi*	*Scophthalmus maximus* *Paralichthys olivaceus* *Dicentrarchus labrax*	Not described	Growing areas of susceptible species	Histology Smear Sequencing ELISA Culture *in vitro* PCR Electron microscopy
Class Cestoidea *Diphyllobotrium latum*	Large number of species	Not described	Worldwide	Morphological identification Hystology

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Phylum Acanthocephala	Large number of species	Not described	Worldwide	Morphological identification Histological sections of tissues
Phylum Apicomplexa *Cryptosporidium molnari*	*Sparus aurata* *Dicentrarchus labrax* *Poecilia reticulata*	Not described	Mediterranean, Atlantic and Cantabrian coast.	Immunofluorescence Hystology PCR Sequencing Electron microscopy
Phylum Arthropoda *Ceratothoa ostreoides*	*Dicentrarchus labrax* *Sparus aurata*	Not described	Greece	Direct Observation Morphological identification
Class Monogenea *Gyrodactylus salaris* (Disease with approved national measures by Commission Decision 2010/221 EU)	*Salmo salar* *Oncorhynchus mykiss* *Salvelinus alpinus* *Salvelinus fontinalis* *Thymallus thymallus* *Salvelinus namaycush* *Salmo trutta*	Not described	Russia, Sweden, Denmark, Finland, Norway, Italy and Czech Republic	Direct Observation Morphological identification Smear Sequencing Histology Electron microscopy
Phylum Ciliophora *Uronema marinum* *Uronema nigricans*	*Sparus aurata* *Dicentrarchus labrax* *Scophthalmus maximus* *Salmo salar* *Paralichthys olivaceus* *Polyprion oxygeneios* *Thunnus maccoyii*	Not described	Growing areas of susceptible species	Histology Smear Sequencing Electron microscopy
Phylum Euglenozoa *Criptobia*	*Salmonidae* *Cyprinus* sp	Not described	Growing areas of susceptible species	Smear Giemsa stained smears Histology of gills
Phylum Euglenozoa *Costia*	*Salmonidae* *Cyprinus* sp	Not described	Growing areas of susceptible species	Smear Giemsa stained smears Histology of gills
Phylum Parabasalia *Hexamita*	*Salmonidae*	Not described	Europe, Asia and America	Smear Histology
Phylum Oflagellata *Amyloodinium ocellatum*	All marine species	Not described	Tropical and subtropical	Observation of skin in dark background Histology ELISA PCR

Annex III.- Table summarizing vector species and susceptible species, geographical distribution and methods of diagnosis of the diseases included in the Handbook				
Phylum Microspora *Tetramicra brevifilum*	*Scophthalmus maximus*	Not described	Growing areas of susceptible species	PCR Sequencing Electron microscopy Histology
Phylum Microspora *Pleistophora senegalensis* *Glugea* sp	*Sparus aurata*	Not described	Mediterranean and AfricanAtlantic coast	PCR Sequencing Electron microscopy Histology
Phylum Amoebozoa *Neoparamoeba pemaquidensis*	*Salmo salar* *Scophthalmus maximus* *Oncorhynchus kisutch* *Oncorhynchus tshawytscha* *Dicentrarchus labrax* *Diplodus puntazzo*	Not described	Spain, Scotland, Norway, Ireland, Australia, New Zeland, Chile and USA,	Smear Histology PCR
Phylum Arthropoda *Lepeophtheirus salmonis*	*Salmo salar* *Oncorhynchus kisutch* *Oncorhynchus mykiss* *Oncorhynchus gorbuscha* *Oncorhynchus keta* *Odonthestes regia* *Paralichthys microps* *Eleginops maclovinus*	Not described	Chile, Norway, Scotland, Faroe Islands, Ireland, Japan and Canada	Direct Observation

Annex IV

Deliverable Check list (to be completed by Deliverable leader)

	Check list		Comments
BEFORE	I have checked the due date and have planned completion in due time		*Please inform Management Team of any foreseen delays*
	The title corresponds to the title in the DOW		*If not please inform the Management Team with justification*
	The dissemination level corresponds to that indicated in the DOW		
	The contributors (authors) correspond to those indicated in the DOW		
	The Table of Contents has been validated with the Activity Leader		*Please validate the Table of Content with your Activity Leader before drafting the deliverable*
	I am using the AQUAEXCEL deliverable template (title page, styles etc)		*Available in "Useful Documents" on the collaborative workspace*
The draft is ready			
AFTER	I have written a good summary at the beginning of the Deliverable		*A 1-2 pages maximum summary is mandatory (not formal but really informative on the content of the Deliverable)*
	The deliverable has been reviewed by all contributors (authors)		*Make sure all contributors have reviewed and approved the final version of the deliverable. You should leave sufficient time for this validation.*
	I have done a spell check and had the English verified		*Ask a colleague with a good level of English to review the language of the text and do a spell-check too.*
	I have sent the final version to the Activity Leader and to the 2nd Reviewer for approval		*Send the final draft to your Activity Leader and the 2nd Reviewer and leave 2 weeks for feedback and final changes before the due date. Once validated by the 2 reviewers, the draft is ready to be sent to the Management Team that will ask for the Coordinator validation and then transfer it to the EC.*