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PANDA

Permanent network to strengthen expertise on infectious diseases of aquaculture species and scientific advice to EU policy

Coordination Action

Scientific support to policies

Work Package 2
Risk analysis of exotic, emerging and re-emerging disease hazards

Deliverable 2: Creation of a platform of experts for risk analysis of aquatic animal diseases

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Deliverable 3: Identification of the most significant exotic, emerging and re-emerging disease hazards for European aquatic animals

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Executive overview

1. **Task force:** a total of five experts were selected to form a task force to identify the most significant exotic/emerging and re-emerging disease hazards. The task force initially drew up a dialogue document to address the deliverables and milestones of the work package. This document formed the basis of a series of discussions and four group meetings based on the available data. Subsequently, the task force used the information for the identification of most aquatic disease hazards (i.e. fish, shellfish, crustacean and amphibian) from a European perspective. Each hazard was assessed by susceptible host species, geographical distribution, its disease listing (EC/91/67 and OIE) and exotic status (relevant to the EU). In addition, the hazards were subjected to a pre-filter related to whether they satisfied the OIE disease listing/notification criteria for consequences and spread. In order to prioritise the hazards that satisfied the criteria, a scoring system based on 29 questions divided into five sections was developed. This system considered each hazard by presence or absence in the EU and its regulatory status, the potential pathways of introduction, establishment, consequences, and risk mitigation measures. The results were used to produce a hazard listing, which was then made available to the other work packages.

2. **Network:** a network of experts associated with work package 2 was established in order to provide a risk analysis platform. The network was initially formed from the database of registered PANDA members, but was subsequently augmented by a proactive invitational approach. The platform was used to provide specialised input to the hazard scoring exercise and to provide background data on specific disease hazards.

3. **Outcomes:** A full picture of the most significant exotic, emerging and re-emerging disease hazards for aquatic animal health in the EU has been obtained and has proved valuable for assessing their potential impact.

   However, the work package has shown that, currently, there is not enough expertise for actually conducting risk analyses and this makes their interpretation difficult within the context of the providing scientific information in support of aquatic animal health programmes. As a result, there is a broad need to provide basic training for understanding the risk analysis (RA) concepts and the process of risk assessment.

   A flexible platform of experts for risk analysis associated with aquatic animal health should become a permanent feature in order to support policy decision making.

   More information is needed in areas such as the status of certain emerging and exotic diseases, as well as the current situation regarding shrimp farming in Europe in relation to the existence of crustacean disease hazards.

   The work package has led to the improved consideration of the prevention, vigilance and contingency plans of the identified diseases, as well as the availability of adequate diagnostic methods. Nevertheless, the listed hazards should be submitted to full risk analysis in order to be refined, particularly with regard to pathways of introduction (e.g. trade movements).

Introduction

The first step in import risk analysis is hazard analysis, which is otherwise known as risk identification. The whole process is a step-wise progression that subsequently combines the additional components of risk assessment, risk management and risk communication. However, all potential adverse outcomes, including marginal entries, must be listed at the outset because if any diseases caused by specific pathogens are missing the risk analysis could omit aspects of great importance for regulatory decision-making (North, 1995). This "all inclusive" approach can naturally involve a filtration step designed to remove the hazards that can be classed as having low probability for consequences (e.g. impact) and establishment. Only such a comprehensive scheme designed to gather all the available relevant data can support transparency and provide assistance for any subsequent decision-making process related to the risk of pathogen transfer through the movement of live aquatic animals and/or their products.

The work package (WP 2) objective was to identify the exotic, emerging and re-emerging disease hazards of potential risk to Europe, and subsequently make an assessment of their potential impact on aquaculture and aquatic wildlife in the EU. As such, in consideration of the available time and resources, it was not intended to provide a full risk analysis but rather recommend those hazards which should receive priority attention in other work packages by considering pathways of introduction, consequences of introduction, establishment and the feasibility of possible risk mitigation as the final goal. The work approach therefore centred on the following two main areas:

Description of work

1. **Task force:** leading experts in risk analysis in aquatic animal diseases would be selected by project participant 8 in consultation with the Project Steering Group, to form a task force to identify the most significant exotic/emerging and re-emerging disease hazards. It was anticipated that close liaison would be maintained with pathologists and epidemiologists identified in WP 3 (epidemiology) in order to adopt a relevant list of diseases through a consideration of such hazards, which had to provide the direction for all work packages.
2. **Network:** a network of experts in risk analysis would be established to form a risk analysis platform. The task force, chaired by participant 8, would co-ordinate the activities within the network. The task force would determine the necessities for conducting an assessment(s) of the likelihood of disease entry and establishment in the event that this should happen, and the likely consequences for European aquaculture and aquatic wildlife. The network would also identify knowledge and skill gaps.

Deliverables

The deliverables concerned the creation of a platform of experts for risk analysis of aquatic animal diseases (D2) and the identification of the most significant exotic, emerging and re-emerging disease hazards for European aquatic animals (D3).

Milestones and expected result

The milestones consisted in the creation of a network of epidemiologists, aetiologists and pathologists identified from the project database for conducting a preliminary feasibility exercise for risk assessment(s) for the identified disease hazards (M2.1), as well as the assessment of exotic, emerging and re-emerging diseases and their potential impact and identification of those posing the greatest threat to European aquaculture and aquatic wildlife

(M2.2). This information would then be used to form recommendations and input to WP 3 for prevention, vigilance and contingency plans of the identified diseases (M2.3).

Materials and Methods

1. Task force

A small task force was formed in order to identify the most significant exotic/emerging and re-emerging disease hazards of potential risk to Europe. The task force also assessed their potential impact on aquaculture and aquatic wildlife in the EU and recommended those which should receive priority attention in the other work packages. A total of five experts were selected to form the task force. The members of the task force included:

Chris Rodgers (CA-IRTA, Spain) – WP 2 Leader
Giuseppe Bovo (IZSV, Italy)
Edgar Brun (NVI, Norway)
Laurence Miossec (IFREMER, France)
Larry Paisley (DVI, Denmark)
Ed Peeler (CEFAS, UK)

The task force initially drew up a dialogue document to address the deliverables and milestones of the work package. The task force maintained contact by email and through regular task force meetings.

2. Network platform

A network of experts was established in order to provide a risk analysis platform. The network was initially formed from the database of registered PANDA members, but was subsequently augmented by a proactive invitational approach. The platform was used to provide input to the hazard scoring exercise and to provide background data on specific disease hazards. The members of the platform are detailed in Acknowledgements – Network platform members.

3. Disease definitions

Definitions of the terms exotic, emerging and re-emerging were necessary and they were adapted for WP 2 from three main published sources: the OIE, the field of veterinary sciences and the medical field. Although, in the first place, WP 2 was dealing with diseases exotic to the EU, a case was made for also considering the situation where a disease may already be present in a particular European region (or entire country) but was exotic to the remainder. Consequently, the definitions were standardised as:

a. Exotic to the entire EU

A disease that is currently absent or unknown within the EU but could be introduced from another (third) country. This definition implies regional and zonal freedom.

b. Exotic to EU regions

A disease that is currently absent or unknown outside a limited distribution zone within the EU but could be introduced or transferred to another, currently uninfected, area. This may be the case for a disease which is confined to one particular region because of containment (i.e. movement) restrictions, where stamping out procedures are not possible, but that has potential for further spread if controls are removed. This definition embodies the potential for spread.

c. Emerging

A disease that has already appeared but is increasing in incidence and becoming more geographically widespread (i.e. reported in new areas or populations). This could be due to a new strain of an organism and increased recognition or changes related to husbandry practices

or environmental conditions. This definition considers a time factor and possible transfer to a new host.

d. Re-emerging

A disease that is present or has declined in incidence but has begun to reassert itself or reappear, possibly with a more widespread distribution. This could be due to the genetic variation of an existing pathogen (e.g. drug resistant strains) or changes related to husbandry practices or environmental conditions or trade patterns. This definition implies increasing incidence of an already known and characterized disease.

4. Identification of hazards

The World Organisation for Animal Health (OIE) defines a hazard as any pathogen that could produce adverse consequences on the importation of a commodity. More specifically, hazard identification is the process of identifying the pathogenic agents that could potentially be introduced in the commodity considered for importation (OIE Aquatic Animal Health Code, 2007; http://www.oie.int/eng/normes/fcode/A_summry.htm; OIE, 2007). Consequently, although the term hazard may also additionally include transport and movements, fishmeal, recycling of animal by-products, origin of supplies and waste water it was only necessary to consider the diseases themselves for the WP 2 exercise. Additionally, the ability to produce zoonoses was also an important consideration for certain specific hazards, although the terms of reference for the work package only concerned the potential impact on aquaculture and aquatic wildlife in the EU.

A large and comprehensive number of potential disease hazards (bacterial, viral, parasitic and fungal pathogens), as well as their associated fish, shellfish, crustacean and amphibian host species, from a wide range of data sources, were considered. All hazards considered are shown in the tables detailed in Annex 1 (1.1-1.4) and the main external listings consulted initially are specified in Annex 2 (2.1-2.5).

In order to identify and prioritise the diseases and disease causal agents considered certain criteria were established. These criteria were related to the infectious nature of the disease causal agent, whether it was exotic to the EU or not, its listed status (e.g. the OIE and 91/67/EC), the presence of the host species in the EU and the expected potential impact or significance of establishment within the EU.

As a guide, the OIE indicates that identified hazards would be those appropriate to the species being imported, or from which the commodity is derived, and which may be present in the exporting country (OIE, 2007). It was also necessary to identify whether each hazard was already present in the importing country (in this case the EU as a whole), and whether it was subjected to control or eradication. Nevertheless, for the purposes of the work package it was not necessary to consider individual EU countries as part of the scope of the exercise, since no single import was examined. However, existing published risk analyses were consulted and preventing the introduction of *Gyrodactylus salaris* with fish imports via intra-Community trade was used as a more detailed potential model for other diseases.

Application of a prefilter

The OIE criteria for listing an aquatic animal disease and an emerging aquatic animal disease (see Annex 3.1) were used as a pre-filter for hazard determination for diseases not already listed by Directive 91/67/EC or the OIE itself. The criteria were also compared with those specified for the EU by Directive EC/2006/88 (Anon, 2006), as shown in Annex 3.2.

The three OIE criteria that support the listing of an aquatic animal disease include criteria A (consequences) and B (spread). The additional criterion C (diagnosis) was not considered as part of the pre-filter since it was incorporated into the hazard scoring system developed (see below). The identification of the hazard also took into account the presence of the host species in the EU and this was an essential prerequisite for scoring,

5. Hazard scoring and classification for risk assessment

In order to help identify and prioritise the disease hazards, a simple hazard scoring system was used, which was divided into five categories concerning:

i) presence or absence (i.e. exotic) of the disease agent in the EU and its regulatory status (i.e. OIE notifiable and Directive EC/91/67 listed)

ii) pathways of introduction (e.g. existence of commodity (live or processed product) trade into the EU - including illegal trade - from a known positive country and any known spread at source)

iii) establishment (e.g. favourable climatic conditions, presence of natural and alternative host(s) in the EU, host species density, survival and potential for pathogen spread in the EU)

iv) consequences (e.g. importance of environmental, social and cultural damage, as well as economic loss and damage to export markets).

v) risk mitigation (e.g. effectiveness of husbandry measures, pathogen eradication, surveillance systems and diagnostic test availability).

For scoring, each of the five categories was given equal weighting (25%) and each of the 29 questions had a weighting within its category (5, 10, 15, 20, 25 or 50%). Although the maximum score for each category was 100, the total score was also standardised automatically to 100. The system format also allowed the responder to be unsure of an answer and this was considered as a degree of uncertainty. Consequently, this was an additional input required for all the questions, with 1 = very certain, 2 = reasonably certain, 3 = reasonably uncertain, 4 = very uncertain and 5 = no available data. This score itself gave an indication of the uncertainty about the information on which the assessment was made and was considered separately to the hazard score. Definitions of the uncertainty categories are shown below.

Uncertainty estimate (UE):	% equivalence	Description
1= very certain	100	sure and indisputable
2= reasonably certain	80-55	fairly or moderately confident
3= reasonably uncertain	45-25	fairly questionable or dubious
4= very uncertain	20-5	highly doubtful
5= no data	0	no available information

The overall assumption for using the scoring system was that susceptible species existed within the EU and the system was developed using an Excel spreadsheet (see Annex 4).

6. Hazard scoring method

Once the OIE disease listing criteria prefilter had been applied to the complete hazard listing a separate more specific list of disease hazards was compiled, and these were subjected to scoring by either the Task Force or the platform of experts (or both), as appropriate. Scoring was undertaken by entering the appropriate values in only the Score and Uncertainty Estimate

columns (see Annex 4) and the final scores were automatically generated. Drop down lists were provided for ease of use.

7. Analysis of data

i) Base analysis

The mean, median and range were calculated for each hazard group scored (e.g. fish viral, mollusc parasitic, etc.), and the mean was additionally calculated for each individual hazard within the groups, both for the risk and uncertainty scores. The results were represented graphically by plotting risk against uncertainty for each hazard. The resultant figures were divided into four quadrants using the mean as a central point. The quadrants corresponded to:

I – high risk/high uncertainty

II – high risk/low uncertainty

III – low risk/high uncertainty

IV – low risk/low uncertainty.

This descriptive approach was also tabulated for ease of interpretation and specified the number of responses (n) received from the hazard scoring exercise. All calculations were performed by a summary Excel spreadsheet using the data extracted from the hazard scoring exercise for each separate pathogen.

ii) Statistical analysis

The benefit of using Bayesian statistics to normalize the ranking of the disease hazards was considered, since the number of responders was variable between hazards scored. Consequently, the following formula was applied to the risk and uncertainty means for each hazard scored:

$$b(r) = [(W(a)*a+(W(r) *r))/(W(a)+W(r))]$$

where

r is the original mean rating (risk or uncertainty) of the disease hazard scored;

W(r) is the weight of the rating r (i.e. the number of ratings/responses);

a is the mean rating for the whole group;

W(a) is the weight of the mean rating a (i.e. a higher arbitrary number based on the expected number of ratings/responders);

b(r) is the new Bayesian risk or uncertainty rating.

In addition, the distribution of the scores (e.g. normal, etc.), the differences between ranges for the pathogens (both risk and uncertainty scores), non-parametric statistics to compare scores (i.e. ranking of hazards within a group through the use of the Kruskal-Wallis test), and bootstrapping followed by ANOVA were all considered due to the difficulty of applying more classical methods to subjective opinion.

Results

Two specific interest areas were created for work package 2 (WP 2) at the beginning of the project. These were the creation of a task force and a network of experts. These led to the exercise of hazard characterization, hazard scoring and ranking, which was followed by a descriptive assessment of the exotic, emerging or re-emerging disease hazards identified as posing the greatest threat to European aquaculture and aquatic wildlife.

1. Task force

Two task force (TF) meetings were organised during the first year of the project, one in San Carlos de la Ràpita (Spain; April 2004) and one in Barcelona (Spain; October 2004). The first meeting was also arranged in conjunction with a work package 3 TF meeting on epidemiology. In addition, the occasion provided an opportunity to organise a seminar concerning the work of the EU Reference Laboratories for fish and shellfish diseases, as well as that of the OIE CEFAS Collaborating Centre. Two further WP 2 TF meetings were held in Lelystad, Netherlands (April 2006) and Weymouth, UK (March 2007) in order to discuss progress, present the results and define the input for the final report.

2. Network platform

The establishment of a network of experts associated with WP 2 was a continuous process throughout the project and formed the basis of a risk analysis platform, which proved essential for data gathering and hazard scoring. Unfortunately, the network relied initially on the database of registered PANDA members, which was slow at start-up and, consequently, a proactive approach had to be adopted to form the network. In addition, many experts approached were unable to contribute because of time constraints or unfamiliarity with some of the hazards identified, particularly diseases exotic to Europe. However, final input to WP 2 for hazard characterization was from a total of 29 specifically invited experts targeted both from within Europe and other countries (see Acknowledgements for a full list of experts by country and contribution).

3. Hazard characterization and classification

A discussion document containing background information for use by task force members was produced and additionally contained details of hazard identification methodology and the means for classifying hazards for risk assessment purposes. In addition, a listing of most known diseases, their distribution and their causal agents was provided in tabular form (see Annex 1.1). This information was further considered from a European perspective (exotic status) and from the point of view of susceptible host species. The detailed tables included:

a) disease agents (hazards) listed by the OIE and EU Directive EC/91/67

b) disease agents not listed by the OIE and 91/67

c) host ranges and geographical distribution for the disease agents.

This data was further broken down into additional tables concerning whether the disease agents were considered exotic to the EU (Annex 1.2), present in the EU but with limited distribution (Annex 1.3), and present in the EU but with widespread or unknown distribution (Annex 1.4).

The number of disease hazards considered is shown in Table 1.

	Viral	Bacterial	Parasitic	Fungal	Misc.	Total
Fish	49	24	81	4	0	158
Molluscs	12	12	29	2	3	58
Crustaceans	21	8	8	3	0	40
Amphibians	1	1	0	1	0	3
Total	83	45	118	10	3	259

Table 1. Number of disease hazards considered

A total of 259 potential disease hazards were considered, although this was complicated by the existence of data gaps and the difficult interpretation of some of the available information.

It was decided that in order to reduce the number of hazards and make them more relevant all the diseases had to be submitted to a pre-filter defined by the OIE disease listing and notification criteria, which normally considers three concepts (consequences, spread and diagnosis), as indicated in Materials and Methods (Application of a pre-filter). For the pre-filter, only the parameters related to consequences and spread were considered since diagnosis was already a requirement of hazard scoring. Consequently, the disease hazards proposed for listing had to meet the relevant parameters set for each of these two criteria (e.g. be the cause of significant production losses, have negative effects on valuable aquatic animal populations, have any public health concern, have proven or suspected infectious aetiology, have potential for international spread, etc.; see Annex 3 for further detail).

4. Hazard scoring

In order to help identify and prioritise the disease hazards a simple hazard scoring system was devised. The scoring system consisted of information on presence or absence of a disease agent in the EU and its regulatory status, as well as pathways of introduction, establishment, consequences and risk mitigation. These fields were represented by five categories and 29 questions. An uncertainty score was also included as a requirement, since it was felt it would be useful to identify data gaps that could point out the need for aquatic animal disease research projects in specific targeted areas. The overall assumption for using the scoring system was that susceptible species existed within the EU for each specific hazard.

Once the design of the hazard scoring template had been agreed and modified the process of hazard characterisation was largely carried out in the first part of the project. This was initially only undertaken by the work package task force while the network to create the platform was still being developed. However, the subsequent creation of the network facilitated the completion of scoring.

The feedback from users of the hazard scoring system, although generally positive, indicated some problems and minor modifications were made to the developed template. The main problems were related to the high degree of subjectivity required and the fact that it was a time consuming exercise for individuals to complete. In some cases, it was found more beneficial to have group sessions to complete the exercise.

The number of disease hazards considered after application of the pre-filter is shown in Table 2.

	Viral	Bacterial	Parasitic	Fungal	Misc.	Total
Fish	11	14	12	1	0	38
Molluscs	6	4	12	0	0	22
Crustaceans	5	3	0	1	0	9
Amphibians	1	1	0	1	0	3
Total	23	22	24	3	0	72

Table 2. Number of disease hazards for hazard scoring

Ranking of the identified exotic, emerging and re-emerging disease hazards to the EU was then completed after collating and analysing the data for each hazard. In general, scoring was consistent between experts for all diseases and due to time constraints, related to the fact that the other work packages were reliant on receiving a final listing, it was therefore decided that additional input was not necessary, even though, in some cases, the number of responders was low. This was particularly evident for specific exotic parasitic disease hazards and the amphibian diseases. Also, information was scarce on the current situation regarding shrimp farming in southern Europe and the present disease status, some of which appears to be anecdotal. In addition, the large time commitment required by experts to complete the scoring exercise occasionally resulted in delays in the return of the hazard scoring questionnaires.

As a result of the hazard scoring and ranking exercise, an initial list of disease hazards was produced, and a final modified version was made available in month 22 (October 2005) after input discussions from the PANDA Steering Group and the work package co-ordinators for WPs 3 and 4. Subsequently, all data gathered on the diseases listed was passed to the leader of work package 3 for inclusion in the epidemiology database, together with input to strategies for prevention, vigilance and contingency plans in the event of an outbreak of the identified diseases, as well as to work package 4 concerning diagnostic methods. The complete list is shown below (section 4.1). In addition, the diseases listed by PANDA are shown compared to those listed by the OIE and EU Directive 2006/88 in Annex 5 (5.1-5.3). It should be noted that the list of the most significant hazards could be modified as new information arises and the disease status in Europe changes. The new EU Directive2006/88/EC (Anon, 2006) is listed separately (Annex 2.3)

4.1 Diseases listed by PANDA

Exotic Diseases		
	Disease agent	**Susceptible species**
Fish	**Epizootic haematopoietic necrosis virus**	***Perca fluviatilis*** **and** ***Oncorhynchus mykiss*** **(EHNV)**
	Red sea bream iridovirus	***Pagrus major***, ***Seriola quinqueradiata***, ***Seriola*** **spp.**, ***Lateolabrax*** **sp.**, ***Oplegnathus fasciatus***, ***Epinephelus malabaricus***, ***Epinephelus*** **spp.**, ***Lates calcarifer*** **and** ***Thunnus thynnus*****, as well as possibly Perciformes, Pleuronectiformes and Tetradontiformes**
	Streptococcus agalactiae	***Sparus aurata, Liza klunzingeri, Pampus argenteus, Oreochromis*** **spp.**
	Trypanoplasma salmositica	**Salmonids and other freshwater fish**
	Ceratomyxa shasta	**Salmonidae**
	Parvicapsula pseudobranchicola	***Salmo salar***
	Neoparamoeba pemaquidensis	***Salmo salar***

	Aphanomyces invadans	***Anguillidae* spp., *Caranx* spp., *Plecoglossus altivelis*, *Clarius* spp., *Channa striatus*, Cichlidae, Cyprinidae, *Lates calcarifer*, *Mugil cephalus*, Siluridae and many other different species (incl. possibly *Brevoortia tyrannus*)**
Mollusc	***Perkinsus marinus***	***Crassostrea virginica*, *C. gigas* and *C. ariakensis***
	***Marteilioides* spp. (*M. chungmuensis* : Marteilioidosis)**	***Crassostrea gigas***
Crustacean	**Yellowhead**	***Penaeus monodon*, *P. japonicus*, *P. vannamei*, *P. setiferus*, *P. aztecus*, *P. duorarum*, *P. stylirostris*, *Palaemon styliferus*, *Fenneropenaeus merguiensis*, *Metapenaeus ensis*, *Euphausia* spp. and *Acetes* spp.**
	Taura	***Penaeus vannamei*, *P. stylirostris*, *P. setiferus*, *P. schmitti*, *P. aztecus*, *P. duorarum*, *P. chinensis*, *P. monodon* and *P. japonicus***
	Infectious hypodermal and haematopoietic necrosis	***Penaeus vannamei*, *P. stylirostris*, *P. occidentalis*, *P. monodon*, *P. semisulcatus*, *P. californiensis*, *P. japonicus*, *P. setiferus*, *P. aztecus* and *P. duorarum***
	***Coxiella cheraxi* (crayfish systemic rickettsiosis)**	***Cherax quadricarinatus***
Amphibian	**Ranavirus**	**Amphibians**

Non-Exotic Diseases		
	Disease agent	**Susceptible species**
Fish	**KHV**	***Cyprinus carpio***
	ISAV	***Oncorhynchus kisutch*, *Salmo salar*, *Salmo trutta*, *Oncorhynchus mykiss*, *Clupea harengus* and *Lepeophtheirus salmonis***
	Streptococcus iniae	***Oncorhynchus mykiss*, *Paralichthys olivaceous*, *Sardinops melanostictus*, *Brevoortia patronus*, *Morone saxatilis*, Cichlidae and *Lates calcarifer***
	Lactococcus garviae	***Oncorhynchus mykiss*, *Seriola quinqueradiata* and *Coris aygula***
	Gyrodactylus salaris	***Salmo salar*, *Oncorhynchus mykiss*, *Salvelinus alpinus*, *S. fontinalis*, *Thymallus thymallus*, *Salvelinus namaycush* and *Salmo trutta***
Mollusc	***Candidatus Xenohaliotis californiensis***	***Haliotis* spp. (e.g. black abalone *H. cracherodii*, red abalone *H. rufescens*, pink abalone *H. corrugata*, green abalone *H. fulgens* and white abalone *H. sorenseni*)**
	***Nocardia* spp. (Pacific oyster nocardiosis)**	***Crassostrea gigas* and possibly *Mytilus edulis***
	Perkinsus olseni/atlanticus	***Haliotis ruber*, *H. cyclobates*, *H. scalaris*, *H. laevigata*, *Anadara trapezia*, *Ruditapes philippinarum* and *Austrovenus stutchburyi* / *Ruditapes decussatus***
Crustacean	**White spot**	***Penaeus japonicus*, *P. chinensis*, *P. indicus*, *P. merguiensis*, *P. monodon*, *P. setiferus*, *P. stylirostris*, *P. vannamei*, *P. aztecus* and *P. duodarum***
Amphibian	**Ranavirus**	**Amphibians**
	***Batrachochytrium dendrobatidis* (amphibian chytridiomycosis)**	**Amphibians**

The WP 2 task force explored the possibility of putting the hazard scoring template on the PANDA website and inviting experts to complete it "on-line" for scoring specific disease hazards/pathogens. However, time constraints and the modifications required to the web site meant that this was not undertaken, although this could still be a valuable longer-term exercise which may be used as the basis for an expert opinion workshop or for assessing newly emerging diseases. Its use in this way would provide a rapid response for assimilating new disease developments or supplying valuable background opinion for risk managers faced with a changing disease status.

It was also observed by some responders that the PANDA accredited hazard scoring method can also be used for educational purposes, as well as for its originally intended purpose and is useful as a training tool for promoting opinion in academic situations (e.g. post-doc groups). It was also requested by the coordinator of a Nordic risk analysis project and it will used, possibly with some modifications, in another ranking exercise.

It was recognised by the task force that certain exotic diseases do not currently pose an immediate threat because of the lack of trade with Europe. However, the potential future threat should trade start will need to be taken into account for diseases such as Herpes virus salmonis type 1, New Japan virus, sturgeon herpesviruses, oyster velar iridovirus or *Oncorhynchus masou* virus, if trade originates from countries or regions where these diseases have been described. The hazards in question are indicated in Annex 1.2.

5. Uncertainty estimate

The uncertainty estimate was used to assist in prioritising the list (i.e. high risk in combination with high or low uncertainty) and for identification of data gaps, and therefore the potential to determine research priorities.

In many cases, the highest uncertainty was associated with the consequences category for areas concerning the affect of the hazard (i.e. pathogen) on export markets, the ability to cause environmental harm and the extent of the region in the EU likely to suffer damage from the hazard. The existence of trade in products or gametes from known positive areas also produced uncertainty for *Lactococcus garviae* and *Streptococcus agalactiae*, *Coxiella cheraxi*, *Nocardia crassostrea*, *Candidatus* Xenohaliotis californiensis, *Marteilioides chungmuensis*, *Perkinsus marinus*, *Perkinsus olseni/atlanticus*, Red seabream iridovirus, as did the possibility of international spread via product for *Ceratomyxa shasta*, *Neoparamoeba pemaquidensis*, *Parvicapsula pseudobranchicola* and *Trypanoplasma samositica*, as well as EHNV. The single question that led to most uncertainty was the length of time a pathogen can live in the environment without a host. However, the questions driving the total risk score appeared to be those representing the possibility of risk mitigation and the means of establishment or, occasionally, the consequences of introduction. There was general agreement that diseases would be easily spread by human assistance but the speed of spread was uncertain once established in the EU. Conversely, it was not always certain if natural spread would occur in the case of all hazards, although it was agreed that this would be much slower if it did occur than spread aided by human assistance. The highest single uncertainty was for *Coxiella cheraxi* (62.8) that was directly related to the lack of available data with which to make an assessment, whereas the lowest single uncertainty was for *Gyrodactylus salaris* (26.3), which was related to the availability of good characterization information.

On the other hand, the highest levels of certainty where responders were sure of their response (i.e. very to reasonably certain) were consistently related to certain risk factors concerning establishment in the EU (e.g. the similarity of climatic conditions compared to the area of current distribution, number of different host species present, extension/density of host species and farmed/wild status), consequences of introduction (e.g. potential for economic loss) and

risk mitigation (e.g. prevention of establishment with existing controls, possibility of eradication).

6. Data analysis

Where more than one independent individual or group was involved in scoring, it was difficult to find a single method for combining different hazard scores. Group scores were effectively consensus scores compiled in a single session from individual experts and were used for fish viral hazards and the shellfish hazards. Nevertheless, individual experts were also used in conjunction with group scores were necessary, which led to the need for a simple analytical approach. Therefore, the mean and median were used successfully to define cut-off points and then a straight forward visual plot of risk against uncertainty was employed to provide initial interpretation for ranking, using the four (I-IV) risk quadrants outlined in the Materials and Methods.

The benefit of using Bayesian statistical treatment to normalize the ranking of the disease hazards was considered, particularly because there was inconsistency in the total number of responses between individual hazards. This technique places more emphasis on the number of responses (i.e. a larger number of responses provides greater confidence in the mean response) and uses an arbitrary weighting for the number of expected responses, which is not necessarily the number of requests initially sent out. The cases where there were few responders related mainly to hazards where there were few experts to respond (e.g. exotic diseases), particularly in a European context. Consequently, it was impossible to statistically assess the value of being an expert in these cases and therefore arbitrary non-numeric weighing had to be placed instead on the ability of an expert to provide accurate scientific opinion. This was the case particularly for certain exotic fish parasites, exotic crustacean diseases and hazards related to amphibians. In addition, the ranking of the hazards was also subject to further scrutiny by both the work package task force and the Project Steering Group prior to obtaining a definitive listing, which resulted in some modifications due to the positioning and subsequent removal of certain endemic disease hazards. Although some of these hazards had been scored in order to provide useful comparative background data, it was not possible to apply any meaningful statistical analysis to such subjective decisions, since the final listing was designed to represent exotic and emerging hazards, not endemic diseases.

In addition, a determination of the perceived relevance of the 29 potential individual risk factors using Bayesian likelihood-ratios (Gustafson *et al*., 2005) was not undertaken, since it was not considered necessary to provide such detailed analysis for more global categories such as establishment, consequences and risk mitigation. These factors contributed to the overall score used for ranking rather than being employed to determine any within-category influence. The assessment of the hazards following numerical ranking was therefore approached from a purely descriptive point of view and there was no need to apply additional analytical methods, such as the Kruskal-Wallis test or bootstrapping followed by ANOVA. Nevertheless, further detailed analysis would not be ruled out for considerations of single disease hazards, although it is recognised that this would be outside the scope of the current exercise, since only preliminary feasibility (descriptive) assessments were envisaged by the project.

6.1 Fish

The hazards identified for fish with their risk (hazard), uncertainty scores and ranges are shown in Table 3.

Table 3. Fish disease hazards.

Exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Fish	**Epizootic haematopoietic necrosis virus**	**45.08**	**63.0-25.5**	**49.8**	**57.0-41.0**	**13****
	Red sea bream iridovirus	**53.0**	**54.0-52.0**	**44.5**	**47.0-42.0**	**2**
	Streptococcus agalactiae	**51**	**53.0-49.0**	**32.8**	**35.2-30.4**	**2**
	Trypanoplasma salmositica	**61.25**	**62.5-60**	**39.7**	**42.0-37.4**	**2**
	Ceratomyxa shasta	**55.0**	**55.5-54.5**	**43.9**	**45.2-42.6**	**2**
	Parvicapsula pseudobranchicola	**58.0**	**61.0-55.0**	**50.2**	**55.4-45.0**	**2**
	***Neoparamoeba pemaquidensis*[1]**	**56.5**	**58.0-55.0**	**39.9**	**42.6-37.2**	**2**
	Aphanomyces invadans	**60.5**	**N/A**	**26.6**	**N/A**	**1**

Non-exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Fish	**KHV**	**57.8**	**68.5-42.5**	**32.3**	**53.6-21.0**	**15****
	ISAV	**45.7**	**60.5-27.0**	**32.29**	**42.4-24.6**	**15****
	Streptococcus iniae	**58.75**	**66.0-51.5**	**35.2**	**37.2-33.2**	**2**
	Lactococcus garviae	**50.33**	**70.5-35.5**	**35.87**	**42.6-26.6**	**3**
	Gyrodactylus salaris	**60.9**	**69.0-54.5**	**26.3**	**28.2-24.2**	**5**

*Single group score; **Including two group scores

6.1.1 Fish bacterial hazard analysis

Risk and uncertainty for fish bacterial hazards scored (listed diseases in bold)

	Aeromonas salmonicida	*Edwardsiella tarda*	*Edwardsiella ictaluri*	*Flavobacterium psychrophilum*	***Lactococcus garvieae***	*Mycobacterium marinum*	*Piscirickettsia salmonis*	*Photobacterium piscicida*	*Pseudomonas anguilliseptica*	*Renibacterium salmoninarum*	***Streptococcus agalactiae***	***Streptococcus iniae***	*Vibrio* spp.	*Yersinia ruckeri*	**Mean**	**Median**
mean risk	49,50	45,75	33,75	46,75	**50,33**	58,75	44,00	50,25	44,25	46,75	**51,00**	**58,75**	62,25	40,75	**48,77**	**48,13**
risk range max	51,50	51,00	38,00	54,00	**70,50**	59,00	45,50	50,50	45,50	48,50	**53,00**	**66,00**	66,00	44,50	53,11	51,25
risk range min	47,50	40,50	29,50	39,50	**35,50**	58,50	42,50	50,00	43,00	45,00	**49,00**	**51,50**	58,50	37,00	44,82	44,00
mean uncertainty	26,00	34,80	36,50	32,60	**35,87**	29,10	39,30	30,30	32,00	30,10	**32,80**	**35,20**	25,70	27,70	**32,00**	**32,30**
uncertainty range max	31,60	35,00	39,60	33,60	**42,60**	32,00	47,80	33,80	34,00	30,80	**35,20**	**37,20**	30,60	31,20	35,36	33,90
uncertainty range min	20,40	34,60	33,40	31,60	**26,60**	26,20	30,80	26,80	30,00	29,40	**30,40**	**33,20**	20,80	24,20	28,46	29,70

Figure 1. Ranking by risk score against uncertainty for fish bacterial hazards considered

[1] Since the completion of the hazard identification exercise AMD has been described in turbot from Spain (http://www.fao.org/fi/website/FIRetrieveAction.do?dom=culturespecies&xml=Psetta_maxima_es.xml).

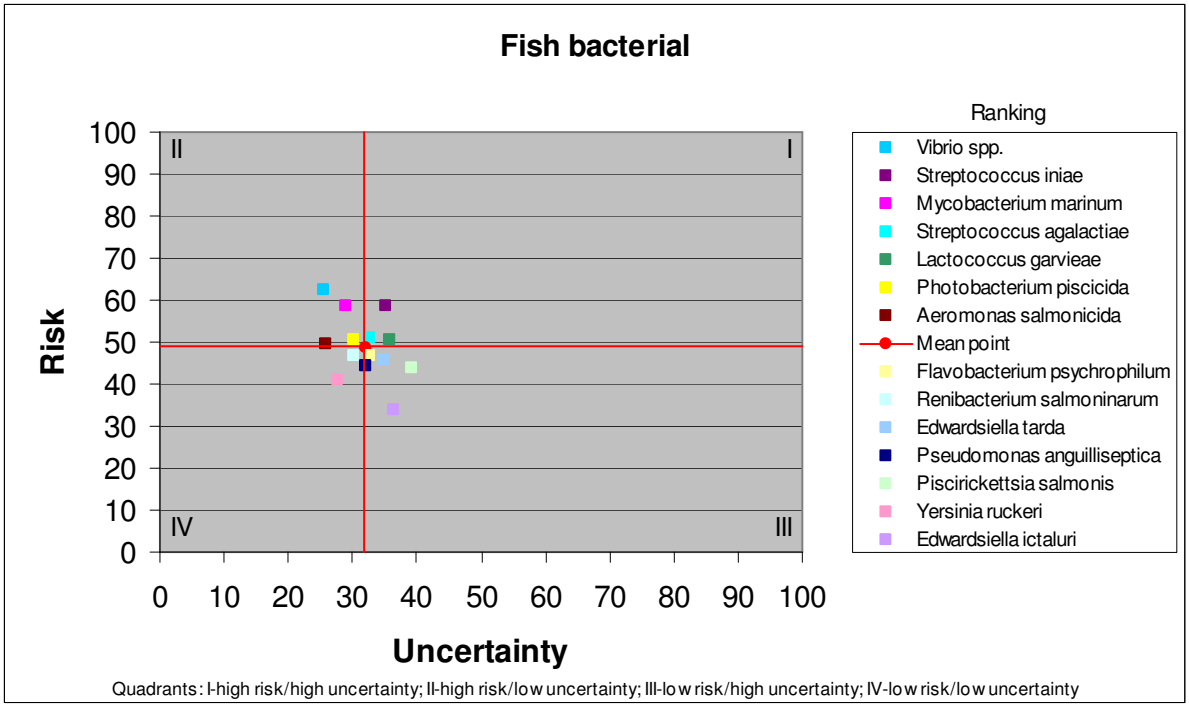

Table 4. Fish bacterial hazards by risk quadrant

Quadrant	**Fish bacterial hazard** (underlining means considered further)
I. High risk/high uncertainty	*Streptococcus iniae*; *Streptococcus agalactiae*; *Lactococcus garviae*
II. High risk/low uncertainty	*Vibrio* spp.; *Mycobacterium marinum*; *Photobacterium piscicida*; *Aeromonas salmonicida*
III. Low risk/high uncertainty	*Flavobacterium psychrophilum*; *Edwardsiella tarda*; *Piscirickettsia salmonis*; *Edwardsiella ictaluri*
IV. Low risk/low uncertainty	*Renibacterium salmoninarum*; *Pseudomonas anguilliseptica*; *Yersinia ruckeri*

6.1.1.1 Description of listed fish bacterial diseases

i) *Lactococcus garviae*

Disease:

Lactococcosis

Description of disease:

The disease is increasing in importance and geographic range as a fish disease, due to the fact that it is a highly virulent streptococcal infection of the aquatic environment. It is also

potentially zoonotic, since it is associated with bovine mastitis and very occasionally with bacterial endocarditis or septicemia in immunosuppressed individuals. Lactococcosis can cause significant economic problems for trout farms in Mediterranean countries during the summer months (Vela *et al*., 2000; Padros, pers comm.), and is associated with high mortality, with larger fish appearing to be most susceptible. Affected fish undergo bacteraemia with widespread haemorrhage, and inflammation.

Geographical distribution:
Australia (Tasmania, Victoria), Europe (Italy, Spain, Turkey), Israel, Japan, South Africa, and Taiwan

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Non-exotic

Transmission:
Horizontal (water, faecal-oral), particularly when fish are stressed.

Host range (susceptible species):
Natural: *Seriola quinqueradiata*, *Seriola dumerili*, *Seriola lalandi*, *Anguilla anguilla / japonica*, *Oncorhynchus mykiss*, *Oreochromis* sp., *Paralichthys olivaceous*, *Scopthalmus maximus*, *Sebastes schlegali*, *Mugil cephalus*, *Coris aygula*, *Macrobranchium rosenbergii*
Experimental: none described

Assessment:
See Annex 6 (6.1.1, i)

Useful links:
None

ii) *Streptococcus agalactiae*
Disease:
Streptococcosis, caused by *Streptococcus agalactiae*, is an important pathogen of some fish species, which can lead to serious economic losses.

Description of disease:
Streptococcosis can cause large fish mortalities, which are economically important in species such as tilapia. Massive mortalities of large sized fish can occur, which lead to significant economic losses. It is also zoonotic, since it is associated with endocarditis, as well as septicaemia and meningitis in neonates. Symptoms can include skin lesions, petechaeal haemorrhages in the fins and exophthalmia, as well as internal haemorrhaging. Hyperaemia of the eye can occur and the liver is pale with focal necrosis. Epizootics occur when water temperatures exceed 20 ºC.

Geographical distribution:
Israel, Kuwait, USA

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Horizontal transmission (from fish to fish) through cannibalism or skin injuries. There have also been reports of transmission from wild to aquacultured fish. Larger fish are usually more susceptible. In the EU, host species are present in both aquaculture and the wild.

Host range (susceptible species):
Natural: *Sparus aurata, Liza klunzingeri, Pampus argenteus, Oreochromis* spp.
Experimental: none described

Assessment:
See Annex 6 (6.1.1, ii)

Useful links:
None

iii) *Streptococcus iniae*
Disease:
Streptococcosis, caused by *Streptococcus iniae*, can affect various freshwater and marine fish species, from both cultured and wild fish populations. *S. iniae* has become one the most serious aquatic pathogens in the last decade causing high losses in farmed finfish in warmer regions (Agnew and Barnes, 2007). It is also zoonotic, since it has been identified as an emerging human pathogen producing fulminant soft tissue infection and, in some cases, transmission to humans resulting from handling infected fish (Miller and Neely, 2004).

Description of disease:
Disease progression in fish is somewhat variable and has been shown to be dependent on the virulence of the isolate, the host species affected, route of infection, fish age and other environmental and water quality factors (Agnew and Barnes, 2007). Infected fish do not feed, and are lethargic and thin with skin petechaeal haemorrhages. Liquid also occurs characteristically in the cranial cavity, with abdominal ascites and associated splenomegaly and pale liver. Epizootics occur when water temperatures exceed 20 ºC with mortality rates in infected fishponds ranging from 5 to 50%. Associated conditions include panopthalmitis, meningitis, necrotizing dermatitis and spleen and kidney destruction (Miller and Neely, 2004). *S. iniae* tends to cause different disease status depending on the type of hosts it infects and it can result in high levels of morbidity and mortality (Agnew and Barnes, 2007).

Geographical distribution:
Australia, China, Europe (Italy, Spain) Israel and USA

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Non-exotic

Transmission:
Horizontal

Host range (susceptible species):
Natural: *Oncorhynchus mykiss*, *Paralichthys olivaceous*, *Sardinops melanostictus*, *Brevoortia patronus*, *Morone saxatilis*, Cichlidae, *Lates calcarifer* and *Rana castesbeiana*
Experimental: *Danio rerio*

Assessment:
See Annex 6 (6.1.1, iii)

Useful links:
See Agnew and Barnes (2007) for a more extensive host range.

6.1.2 Fish parasitic hazard analysis

Risk and uncertainty for fish parasitic hazards scored (listed diseases in bold)

	Caligus elongatus	***Ceratomyxa shasta***	*Enteromyxum leei**	*Enteromyxum scophthalmi**	***Gyrodactylus salaris***	*Lepeophtheirus salmonis*	*Myxobolus cerebralis*	***Neoparamoeba pemaquidensis***	***Parvicapsula pseudobranchicola***	*Spironucleus barkhanus*	*Tetracapsuloides bryosalmonae**	***Trypanoplasma samositica***	**Mean**	**Median**
mean risk	41,00	**55,00**	35,50	29,50	**60,90**	53,13	51,50	**56,50**	**58,00**	47,25	58,75	**61,25**	**53,84**	**55,00**
risk range max	43,00	**55,50**	N/A	N/A	**69,00**	60,00	62,50	**58,00**	**61,00**	48,00	N/A	**62,50**	57,72	60,00
risk range min	39,00	**54,50**	N/A	N/A	**54,50**	45,00	40,50	**55,00**	**55,00**	46,50	N/A	**60,00**	50,00	54,50
mean uncertainty	32,40	**43,90**	N/A	N/A	**26,30**	23,87	32,20	**39,90**	**50,20**	39,90	N/A	**39,70**	**36,49**	**39,70**
uncertainty range max	39,00	**45,20**	N/A	N/A	**28,20**	26,20	32,20	**42,60**	**55,40**	44,40	N/A	**42,00**	39,47	42,00
uncertainty range min	24,00	**42,60**	N/A	N/A	**24,20**	22,00	32,20	**37,20**	**45,00**	35,40	N/A	**37,40**	33,33	35,40

Figure 2. Ranking by risk score against uncertainty for fish parasitic hazards considered

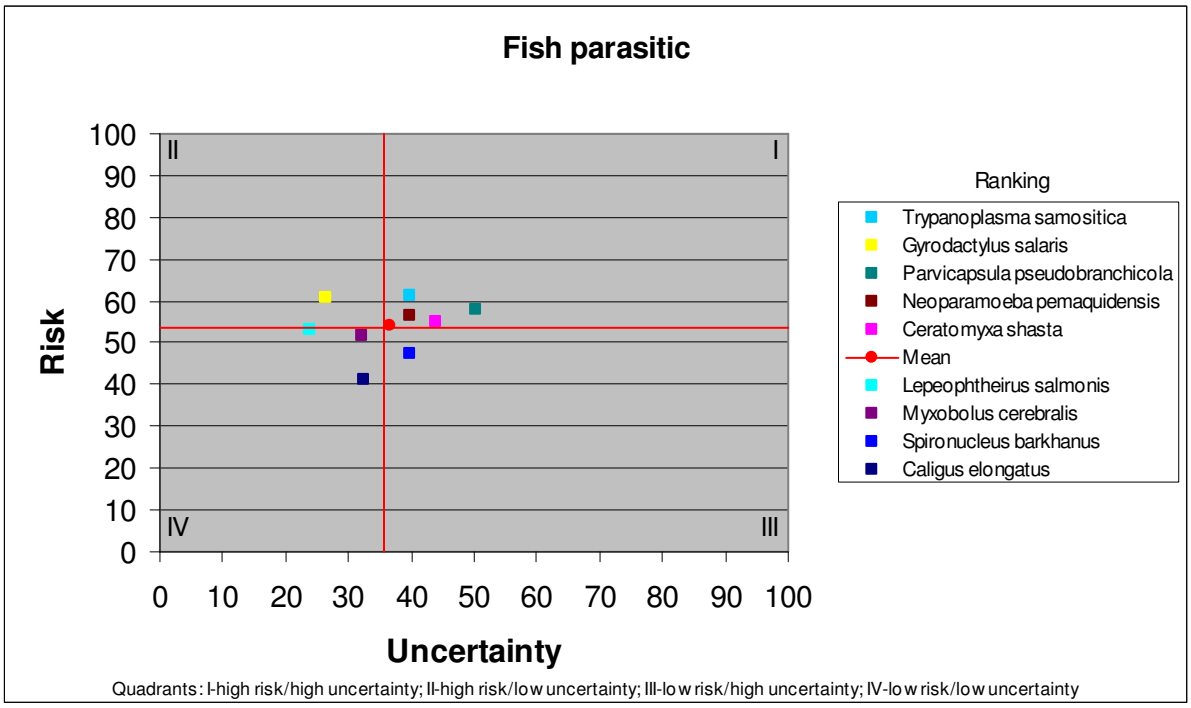

Table 5. Fish parasitic hazards by risk quadrant

Quadrant	**Fish parasitic hazard** (underlining means considered further)
I. High risk/high uncertainty	*Trypanoplasma salmositica*; *Parvicapsula pseudobranchicola*; *Neoparamoeba pemaquidensis*; *Ceratomyxa shasta*
II. High risk/low uncertainty	*Gyrodactylus salaris*
III. Low risk/high uncertainty	*Spironucleus barkhanus*
IV. Low risk/low uncertainty	*Lepeophtheirus salmonis*, *Myxobolus cerebralis*, *Caligus elongatus*

6.1.2.1 Description of listed fish parasitic diseases

i) *Ceratomyxa shasta*

Disease:

Ceratomyxosis is caused by a myxosporean, *Ceratomyxa shasta*, and it can lead to high mortalities in salmonids. It causes losses in juvenile fish, both hatchery-reared and wild, as well as pre-spawning adults.

Description of disease:

Ceratomyxa shasta causes an initial enteric infection in susceptible salmonids that proceeds to an often fatal systemic condition at permissive temperatures (Lom and Dyková, 1995). It shows tropism for the intestinal tissue of the fish with variable clinical signs, depending on the salmonid species affected, that include anorexia, lethargy, abdominal ascites, swollen haemorrhaged vent, and/or exophthalmia. The intestinal tract can be swollen and contain mucous. Older fish can have nodular lesions in the intestine leading to perforation and death. Infection occurs at low water temperatures of <10 °C, but the disease is temperature dependent and, therefore, seasonal, which can be manifest at up to 23 °C (Bartholomew *et al*., 1989).

Geographical distribution:
Canada (NW Pacific) and USA (NW Pacific)

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Transmission occurs on exposure to water or sediments containing the infective actinospore stage (Bartholomew *et al*., 1989) and an intermediate host is necessary (e.g. freshwater polychaete *Manayunkia speciosa*; Bartholomew *et al*., 1997). Experimental horizontal and vertical transmission have not been demonstrated.

Host range (susceptible species):
Natural: Salmonidae
Experimental: none described

Assessment:
See Annex 6 (6.1.2, i)

Useful links:
1. U.S. Department of the Interior, Fish and Wildlife Service, Leetown Science Center: J.L. Bartholomew, J.L., J.S. Rohovec and J.L. Fryer. Fish Disease Leaflet 80. *Ceratomyxa shasta*, a myxosporean parasite of salmonids.
http://www.lsc.usgs.gov/FHB/leaflets/80.asp
2. PacifiCorp, Portland, Oregon, USA. *Ceratomyxa shasta* fact sheet - 2002
http://www.pacificorp.com/File/File19355.pdf

ii) *Gyrodactylus salaris*
Disease:
Gyrodactylosis of salmonids

Description of disease:
Gyrodactylosis is a disease of Atlantic salmon (*Salmo salar*) caused by the freshwater parasite *Gyrodactylus salaris*. All stages of salmon, including adult spawners, in freshwater, can be infected, but disease and mortality has only been observed in pre-smolt

stages (Peeler *et al*., 2006). Diseased fish are lethargic and are usually found in slower-moving water. Mortalities in farmed fish may be 100% if not treated while population reductions as high as 98% of salmon have been observed in rivers (OIE, 2006a).

Gyrodactylosis as probably been spread widely within Europe with the movement of live rainbow trout and is therefore likely to be present in more countries than currently known, although reports in some countries (e.g. France, Portugal, Spain) are probably erroneous and refer to other *Gyrodactylus* spp. (OIE, 2006a)

Geographical distribution:
Bosnia, Denmark, Finland, France, Germany, Norway, Portugal, Russian Federation, Spain and Sweden, as well as possibly the Czech Republic, Georgia and Ukraine.

EU listed (Dir. 2006/88):
No

OIE listed:
Yes

EU status:
Non-exotic

Transmission:
Transmission and spread through transport/restocking of live fish.

Host range (susceptible species):
Natural: *Salmo salar, Oncorhynchus mykiss* and *Salvelinus alpinus*
Experimental: *Salvelinus fontinalis, S. namaycush, Thymallus thymallus* and *Salmo trutta*

Assessment:
See Annex 6 (6.1.2, ii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00031.htm
2. FRS Marine Laboratory, Aberdeen, UK: *Gyrodactylus salaris*
http://www.marlab.ac.uk/FRS.Web/Delivery/display_standalone.aspx?contentid=861

iii) *Neoparamoeba pemaquidensis*
Disease:
Amoebic gill disease in Atlantic salmon is caused by *Neoparamoeba pemaquidensis*, which is a ubiquitous amphizoic marine protozoan, although a recent study indicates that another *Neoparamoeba* species (*N. perurans* n. sp.) may be predominant aetiological agent for the disease (Young *et al*., 2007).

Description of disease:

Neoparamoeba pemaquidensis contains parasomes in association with characteristic histological changes in gill tissue (e.g. including severe hyperplasia of lamellar epithelium and inflammatory response) caused by epithelial hyperplasia and, in heavy infections, fusion of the secondary lamellae with subsequent gill dysfunction (Rohde, 2005). Fish become lethargic and thin.

Geographical distribution:
Australia (Tasmania), Europe (Spain), USA (West coast)

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic[2]

Transmission:
Unknown

Host range (susceptible species):
Natural: *Salmo salar*, *Scophthalmus maximus*, *Oncorhynchus kisutch*
Experimental: none described

Assessment:
See Annex 6 (6.1.2, iii)

Useful links:
None

iv) *Parvicapsula pseudobranchicola*
Disease:
Parvicapsulosis, results from pseudobranch infections associated with low-grade to significant mortalities in Atlantic salmon.

Description of disease:
The causal agent is the *Parvicapsula pseudobranchicola* parasite which is found as mature spores in pseudobranchs although it has also been detected in the gills, liver and kidney (Nylund *et al*., 2005). Pseudobranchs are normally the same healthy colour as gills but with the presence of the disease they gradually become more bloody before they change to become more greyish and slimy due to the dead tissue. Finally they disappear and are replaced by scar tissue. Fish are often blinded by the disease. The final host occurs within the EU where salmon are produced, but at present there is no idea whether there is an invertebrate final host for this parasite. It is a problem in Norwegian salmon farming that appears to be greatest in Northern Norway (NVO, 2006).

Geographical distribution:

[2] Since the completion of the hazard identification exercise AMD has been described in turbot from Spain (http://www.fao.org/fi/website/FIRetrieveAction.do?dom=culturespecies&xml=Psetta_maxima_es.xml).

Norway

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Unknown but may require an invertebrate intermediate host.

Host range (susceptible species):
Natural: *Salmo salar*
Experimental: none described

Assessment:
See Annex 6 (6.1.2, iv)

Useful links:
None

v) *Trypanoplasma (Cryptobia) salmositica*
Disease:
Salmonid cryptobiosis caused by *Trypanoplasma* (*Cryptobia*) *salmositica*.

Description of disease:
The parasite divides rapidly by binary fission in the blood to cause disease, the severity of which is directly related to parasitaemia. An important virulent factor in cryptobiosis is a secretory metalloprotease. The protective mechanism involves production of complement fixing antibodies, phagocytosis by macrophages, and cell-mediated cytotoxicity. Recovered fish are protected, probably for life as the immunity is non-sterile. Clinical signs of the disease include anaemia, anorexia, splenomegaly, general oedema and abdominal distension with ascites. Fish are susceptible to hypoxia and their immune system is depressed during acute cryptobiosis. Severity of the disease and mortality rates vary significantly between species and stocks of salmon (Woo, 2003).

Geographical distribution:
North America

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Horizontal in aquaculture facilities but normally transmitted by the freshwater leech, *Piscicola salmositica*, in streams and rivers, and sculpins, *Cottus* spp., are considered important reservoir hosts (Woo, 2003).

Host range (susceptible species):
Natural: Salmonids and other freshwater fish
Experimental: none described

Assessment:
See Annex 6 (6.1.2, v)

Useful links:
None

6.1.3 Fish viral hazard analysis

Risk and uncertainty for fish viral hazards scored

	Channel catfish virus (Ictaluridae herpesvirus type 1)	**Epizootic haematopoietic necrosis virus**	Infectious haematopoietic necrosis virus	Infectious pancreatic necrosis virus	**Infectious salmon anaemia virus**	**Koi herpes virus**	*Oncorhynchus masou* virus (salmonid herpesvirus type 2)	**Red sea bream iridovirus**	Spring viraemia of carp virus	Viral haemorrhagic septicaemia virus	White Sturgeon Iridovirus	**Mean**	**Median**
mean risk	35,88	**45,08**	50,80	46,67	**45,67**	**57,80**	41,67	**53,00**	43,70	40,67	30,50	**44,67**	**45,08**
risk range max	43,50	**63,00**	58,50	56,00	**60,50**	**68,50**	50,00	**54,00**	53,50	47,50	N/A	55,50	55,00
risk range min	26,50	**25,50**	32,00	35,00	**27,00**	**42,50**	34,50	**52,00**	31,00	36,50	N/A	34,25	33,25
mean uncertainty	37,55	**36,75**	31,08	33,57	**32,29**	**32,31**	35,20	**44,50**	34,20	29,00	42,60	**35,37**	**34,20**
uncertainty range max	45,80	**46,00**	39,60	51,00	**42,40**	**53,60**	38,60	**47,00**	42,80	37,20	N/A	44,40	44,30
uncertainty range min	30,60	**30,20**	22,20	22,00	**24,60**	**21,00**	33,00	**42,00**	27,60	20,20	N/A	27,34	26,10

Figure 3. Ranking by risk score against uncertainty for fish viral hazards considered

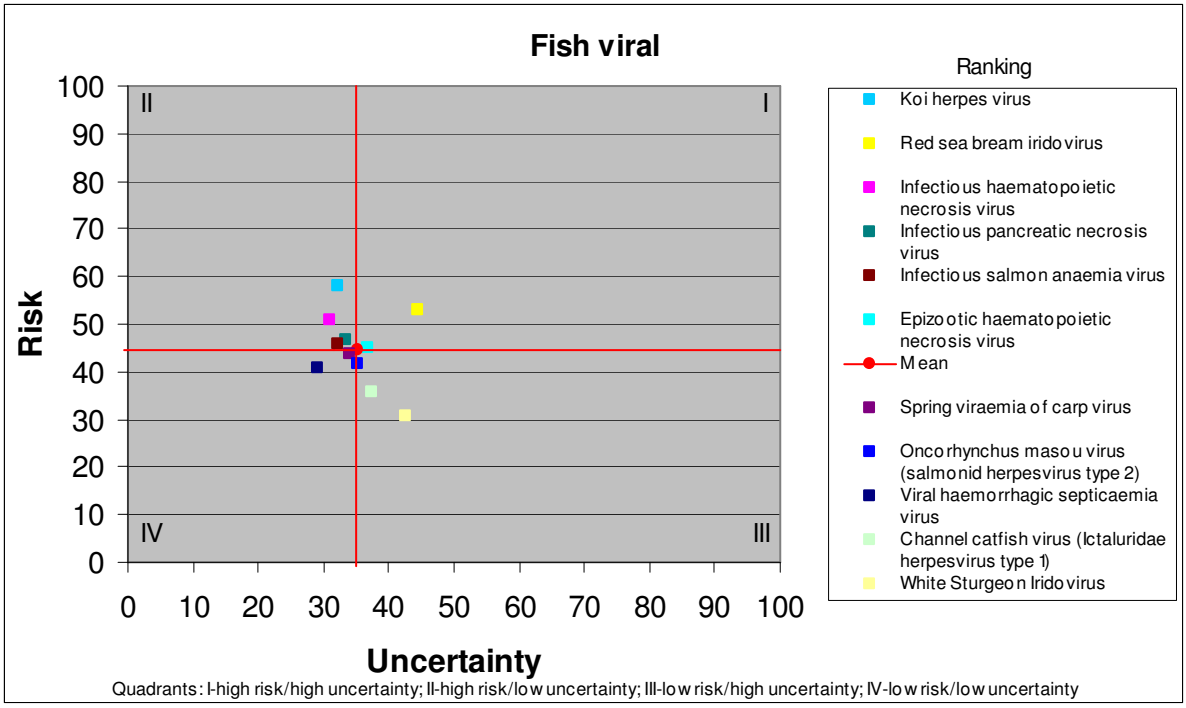

Table 6. Fish viral hazards by risk quadrant

Quadrant	**Fish viral hazard** (underlining means considered further)
I. High risk/high uncertainty	Red sea bream iridovirus, Epizootic haematopoietic necrosis virus
II. High risk/low uncertainty	Koi herpes virus, Infectious haematopoietic necrosis virus, Infectious pancreatic necrosis virus, Infectious salmon anaemia
III. Low risk/high uncertainty	*Oncorhynchus masou* virus (salmonid herpesvirus type 2), Channel catfish virus (Ictaluridae herpesvirus type 1), White sturgeon iridovirus
IV. Low risk/low uncertainty	Spring viraemia of carp, Viral haemorrhagic septicaemia

6.1.3.1 Description of listed fish viral diseases[3]

i) Epizootic haematopoietic necrosis

Disease:

Epizootic haematopoietic necrosis (EHN)

[3] Fish viral disease profiles compiled by G. Bovo

Description of disease:
EHN is a serious disease causing significant losses in redfin perch (*Perca fluviatilis*) and moderate-low mortalities in rainbow trout (*Oncorhynchus mykiss*). No specific clinical signs are associated to EHNV infection. Affected fish may display loss of equilibrium, flared opercula and increase skin pigmentation. Gross signs include skin, gills and fins lesions. Focal necrosis is a common finding in liver and kidney haematopoietic portion while heart, pancreas, gastrointestinal tract, gill and pseudobranch are less frequently interested.

Geographical distribution:
Australia

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:
During epizootics the disease is likely horizontally transmitted from diseased to healthy fish through contaminated water. Due to the high resistance of the virus outside the host, the involvement of mechanical and biological vectors have been proposed.

Host range (susceptible species):
Natural: *Perca fluviatilis* and *Oncorhynchus mykiss*
Experimental: *Macquaria australasica, Maccullochella peeli, Gambusia affinis, Bidyanus bidyanus* and *Galaxias olidus*

Assessment:
See Annex 6 (6.1.3, i)

Useful links:
1. OIE Manual (OIE, 2006) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00018.htm
2. Aquatic Animal Diseases Significant to Australia (2004) for an overview and additional susceptible species.
http://www.disease-watch.com/documents/CD/index/html/fv001ehn.htm

ii) Infectious salmon anaemia
Disease:
Infectious salmon anaemia (ISA)

Description of disease:

Infectious salmon anaemia (ISA) is a systemic viral infection of reared Atlantic salmon mainly in the marine environment. Affected fish show ascites, petechiae on internal organs and haemorrhagic liver necrosis. Dark liver, swollen haemorrhagic kidney, haemorrhagic intestine, gill congestion and low haematocrit values (<10) are common findings during ISA outbreaks. Mortality may exceed 90% in severe cases.

Geographical distribution:
Norway, Canada (New Brunswick and Nova Scotia), Chile, the Faeroe Islands and USA (Maine).

Outside Norway, the disease has been reported from Faeroes islands, Atlantic coast of Canada and USA. In addition ISA virus has been reported in Chile, from Pacific Coho salmon (*Oncorhynchus kisutch*) and in Ireland in clinically healthy rainbow trout

Agent description:
The causal agent of ISA is a pleiomorphic enveloped virus belonging to the Ortomixoviridae family, genus ISAvirus. The virus has a single stranded RNA genome and it has surface projections associated with haemagglutination receptor-destroying and fusion activity.

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:
During outbreaks the disease is likely horizontally transmitted from diseased to healthy fish through contaminated water. Contaminated water and contact with infected population represent the most common origin of new outbreaks. Vertical transmission has not been definitively excluded.

Host range (susceptible species):
Natural ISA outbreaks have been observed only in *Salmo salar* while subclinical infection has been detected in brown and sea trout (*Salmo trutta*). Furthermore the virus has been detected in Pollock (*Pollachius virens*) cod (*Gadus morhua*) and in the Pacific coho salmon (*Oncorhynchus kisutch*). Replication of ISAV has been demonstrated following experimental infection in several species including rainbow trout (*Oncorhynchus mykiss*), brown and seatrout (*Salmo trutta*) herring (*Clupea harengus*) and Arctic char (*Salvelinus alpinus*). Sea lice (*Lepeophtheirus salmonis*), an ectoparasite often found in salmon farms, may be of importance as carriers of virus and as reservoirs.

Assessment:
See Annex 6 (6.1.3, ii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and

prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00026.htm

iii) Koi herpes virus
Disease:
Koi herpes virus disease (KHVD) or KHV infection

Description of disease:
KHVD is a lethal infection of carps (*Cyprinus carpio*) particularly koi carps the ornamental variety. Affected fish show lethargy, anorexia, erratic swimming behaviour and increase ventilation. Gills are severely damaged showing focal discoloration areas associated to necrotic lesions which, in some cases, may be very extensive. In addition, exophthalmia, haemorrhages at the fin base and irregular patches of skin decolouration have been reported. Usually the disease appears at relative high temperature (17-26 °C) but severe outbreaks have been reported at lower temperatures. Morbidity is often 100% and mortality may reach 90%.

Geographical distribution:
KHVD has been observed in more than 20 Countries. In Europe, Austria, Belgium, Denmark, France, Italy, Luxembourg, The Netherlands, Poland, Switzerland and the United Kingdom. In Asia, China, Hong Kong, Indonesia, Japan, Malaysia, Singapore, Taiwan and Thailand. Furthermore, South Africa and the United States of America have reported the occurrence of KHVD.

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Non-exotic

Transmission:
During outbreaks the disease is likely horizontally transmitted from diseased to healthy fish through contaminated water. Contaminated water and contact with infected population represent the most common origin of new outbreaks. Vertical transmission has not been definitively excluded.

Host range (susceptible species):
Cyprinus carpio represents the only susceptible species but other different species like gold fish may harbour and spread the virus in the environment

Assessment:
See Annex 6 (6.1.3, iii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and

prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00034.htm

iv) Red sea bream iridovirus
Disease:
Red sea bream iridoviral disease (RSIVD)

Description of disease:
Red seabream iridovirus disease (RSIVD) is a serious disease firstly observed in Japan causing significant losses mainly in cultured red seabream (*Pagrus major*). Juvenile red seabream reared in cages are highly susceptible but significant mortalities have also been observed in market-size fish. Overt infections have been reported from further cultured marine fish including yellowtail (*Seriola quinqueradiata*), Japanese seabass (*Lateolabrax* sp.) and Japanese parrotfish (*Oplegnatus fasciatus*). Affected fish are lethargic and exhibit severe anaemia. Haemorrhagic petechiae are found in the gills and the spleen is very often enlarged. Hyperthrophied cells staining deeply basophilic with Giemsa are a common finding in spleen, heart, kidney, liver and gills of infected fish. A similar disease, affecting brown-spotted grouper (*Epinephelus malabaricus*) has been observed in Thailand by Danayadol *et al*. (1996) but, nevertheless, experimental infection with the same isolate failed to induce the disease in red seabream. According to the recent report of the International Committee of Virus Taxonomy (Chinchar *et al*., 2005), most systemic iridoviruses isolated during the last decade in Asian countries from different hosts appear to be strains of the same viral species namely *Infectious spleen and kidney necrosis virus.*

Geographical distribution:
Heavy losses associated to RSIV and RSIV-like have been reported in Japan and several Asian countries including China, Hong Kong, Korea, Malaysia, Philippines, Taiwan, Thailand, Singapore (OIE, 2006a).

EU listed (Dir. 2006/88):
No

OIE listed:
Yes

EU status:
Exotic

Transmission:
During outbreaks the disease is likely horizontally transmitted from diseased to healthy fish through contaminated water. Vertical transmission has not been investigated yet.

Host range (susceptible species):
RSIV has been reported in more than 30 marine species including *Pagrus major*, *Seriola quinqueradiata*, *Seriola* spp., *Lateolabrax* sp., *Oplegnathus fasciatus*, *Epinephelus malabaricus*, *Epinephelus* spp., *Lates calcarifer*, *Thunnus thynnus*, etc. (OIE, 2006a).

Assessment:
See Annex 6 (6.1.3, iv)

Useful links:
1. OIE Manual (OIE, 2006) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00032.htm
2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/fv045rsb.htm

6.1.4 Fish fungal hazard analysis

Aphanomyces invadans, the causal agent of EUS, was the only fish fungal hazard that was considered further after the application of the prefilter and, as a result, it is not represented by comparative graphical representation. *A. invadans* had a risk score of 60.5 and an uncertainty score of 26.6.

6.1.4.1 Description of listed fish fungal diseases

i) *Aphanomyces invadans*

Disease:
Epizootic ulcerative syndrome (EUS) is caused by *Aphanomyces invadans* and is an economically devastating fish disease in southern, south-eastern and western Asia, occurring as a seasonal epizootic condition of wild and farmed freshwater and estuarine fish. Outbreaks of ulcerative disease in the USA have been shown to be very similar to EUS in Asia (OIE, 2006a).

Description of disease:
EUS is characterized by necrotising ulcerative lesions that usually result in a granulomatous response. EUS occurs mostly during periods of low temperatures or 18-22 ºC, after periods of heavy rainfall, which favour sporulation, and from freshwater up to a salinity of 4 ppt (OIE, 2006a). It has potential to cause severe problems but the environmental conditions are possibly unfavourable in Europe (particularly in northern latitudes), although increasing mean annual temperatures as a result of climate change may lead to more favourable conditions.

Geographical distribution:
Australia, Bangladesh, Bhutan, Cambodia, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Singapore, Sri Lanka, Thailand, USA and Vietnam.

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:

Horizontal transmission through *Aphanomyces* zoospores.

Host range (susceptible species):
Natural: *Anguillidae* spp., *Anabas testudineus, Bidyanus bidyanus*, *Caranx* spp., *Plecoglossus altivelis*, *Clarius* spp., *Channa striatus*, Cichlidae, Cyprinidae, *Lates calcarifer*, *Mugil cephalus,* Bagridae, Siluridae and many other different species (including possibly *Brevoortia tyrannus*)
Experimental: *Cinetodes froggatti*, *Kurtus gulliveri*, *Platycephalus fuscus*, *Scatophagus argus* and *Toxotes chartareus*

Assessment:
See Annex 6 (6.1.4, i)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00027.htm
2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/ff001eus.htm
3. Bondad-Reantaso *et al*. (2001); eNACA Disease Library
http://library.enaca.org/Health/DiseaseLibrary/EpizooticUlcerativeSyndrome.pdf
Asian Diagnostic Guide to Aquatic Animal Diseases.
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

6.2 Shellfish

The hazards identified for mollusc shellfish with their hazard uncertainty scores and ranges are shown in Table 7.

Table 7. Mollusc disease hazards

Exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Mollusc	***Perkinsus marinus***	**44.0**	**N/A**	**43.8**	**N/A**	**1***
	***Marteilioides* spp. (*M. chungmuensis* : Marteilioidosis)**	**40.0**	**N/A**	**52.6**	**N/A**	**1***

Non-exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Mollusc	***Candidatus Xenohaliotis californiensis***	**53.5**	**N/A**	**37.8**	**N/A**	**1***
	***Nocardia crassostrea* (Pacific oyster nocardiosis)**	**49.0**	**N/A**	**47.2**	**N/A**	**1***
	Perkinsus olseni/atlanticus	**51.0**	**N/A**	**44.0**	**N/A**	**1***

*Single concensus group score

6.2.1 Shellfish bacterial hazard analysis

Risk and uncertainty for shellfish bacterial hazards scored (listed diseases in bold)

	***Candidatus* Xenohaliotis californiensis**	***Nocardia crassostrea***	*Vibrio splendidus*-like (*lentus*)	*Vibrio tapetis*	**Mean**	**Median**
mean risk*	**53,5**	**49**	48,5	59	**52,5**	**51,25**
mean uncertainty*	**37,8**	**47,2**	35,6	30,8	**37,85**	**36,7**

*no range values are shown due to single group consensus score

Figure 4. Ranking by risk score against uncertainty for shellfish bacterial hazards considered

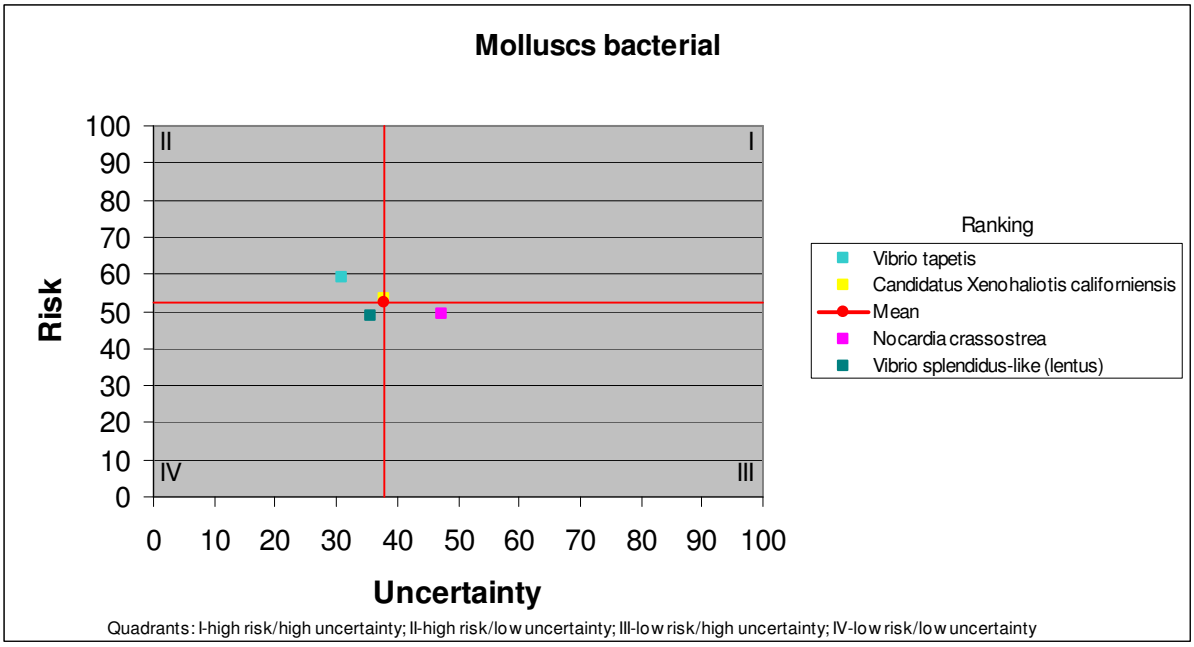

Table 8. Shellfish bacterial hazards by risk quadrant

Quadrant	**Shellfish bacterial hazard** (underlining means considered further)
I. High risk/high uncertainty	*Candidatus Xenohaliotis californiensis**
II. High risk/low uncertainty	*Vibrio tapetis*
III. Low risk/high uncertainty	*Nocardia crassostrea*
IV. Low risk/low uncertainty	*Vibrio splendidus*-like (*lentus*)

*The uncertainty was almost the same as the mean value; therefore, this hazard could equally be placed in quadrant II

6.2.1.1 Description of listed shellfish bacterial diseases[4]

i) *Candidatus* Xenohaliotis californiensis

Disease:
Withering syndrome of abalone

Description of disease:
Candidatus Xenohaliotis californiensis causes lethargy, retracted visceral tissues, atrophy of the foot muscle (thereby adversely affects the ability of the abalone to adhere to the substrate) and is lethal.

Geographical distribution:
USA (California), Mexico (Baja California), Europe (Ireland, Spain), Iceland

EU listed (Dir. 2006/88):
No

OIE listed:
Yes

EU status:
Non-exotic

Transmission:
Candidatus Xenohaliotis californiensis is transmissible between abalones by cohabitation but close and direct contact is not required. The movements of infected animals can spread the disease to new areas. Some studies suggest that *Candidatus* Xenohaliotis californiensis can be transmitted via a waterborne, faecal-oral route and by ingestion of contaminated food. No studies have demonstrated the vertical transmission of *Candidatus* Xenohaliotis californiensis.

Host range (susceptible species):
Natural: *Haliotis* spp. (e.g. black abalone *H. cracherodii*, red abalone *H. rufescens*, pink abalone *H. corrugata*, green abalone *H. fulgens* and white abalone *H. sorenseni,* European abalone *H. tuberculata*)
Experimental: no data

Assessment:
See Annex 6 (6.2.1, i)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00044.htm

[4] Shellfish bacterial disease profiles compiled by L. Miossec

2. Fisheries and Oceans Canada: Bower, S.M. (2006e): Synopsis of infectious diseases and parasites of commercially exploited shellfish: Withering syndrome of abalone. http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/fwsab_e.htm

ii) ***Nocardia crassostrea***
Disease:
Pacific oyster nocardiosis

Description of disease:
Pacific oyster nocardiosis is characterized by round yellow-to-green pustules up to 1 cm in diameter on the surface of the mantle, gill, adductor muscle and heart, with tissue lesions.

Geographical distribution:
Canada (British Columbia), Europe (Netherlands), Japan and the USA

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Non-exotic

Transmission:
Horizontal transmission is suspected

Host range (susceptible species):
Natural: *Crassostrea gigas* and *Ostrea edulis* cultivated near infected *C. gigas*.
Experimental: no data

Assessment:
See Annex 6 (6.2.1, ii)

Useful links:
1. Fisheries and Oceans Canada: Bower, S.M. (2006b). Synopsis of infectious diseases and parasites of commercially exploited shellfish. Nocardiosis of oysters. http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/nocardoy_e.htm

6.2.2 Shellfish parasitic hazard analysis

Risk and uncertainty for shellfish parasitic hazards scored (listed diseases in bold)

	Bonamia exitiosa	*Bonamia ostrea*	*Haplosporidium nelsoni*	*Marteilia* sp.	*Marteilia christenseni*	*Marteilia maurini*	*Marteilia refringens*	***Marteilioides chungmuensis***	*Mykrocytos mackini*	***Perkinsus marinus***	***Perkinsus olseni/atlanticus***	Quahuag parasite X	**Mean**	**Median**
mean risk	33	44	38	26	27,5	26,5	40	**40**	31,5	**44**	**51**	28	**35,79**	**35,5**
mean uncertainty	35,8	31,4	43,6	47,8	40,4	38,6	32,6	**52,6**	46	**43,8**	**44**	58,8	**42,95**	**43,7**

*no range values are shown due to single group consensus score

Figure 5. Ranking by risk score against uncertainty for shellfish parasitic hazards considered

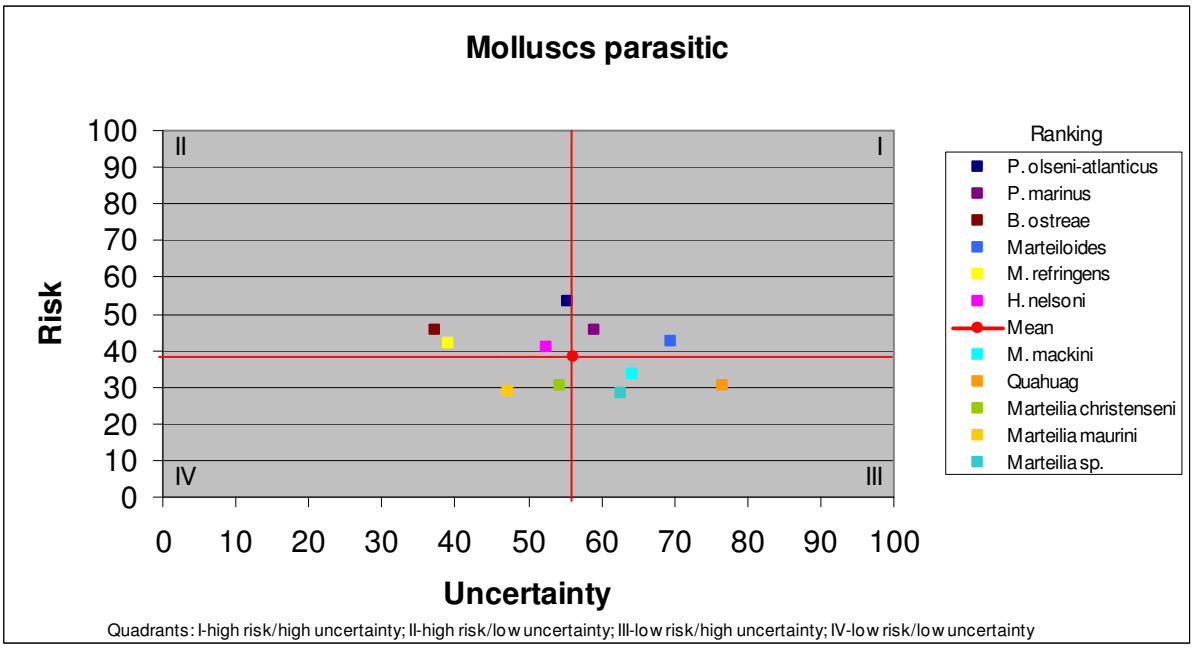

Table 9. Shellfish parasitic hazards by risk quadrant

Quadrant	**Shellfish bacterial hazard** (underlining means considered further)
I. High risk/high uncertainty	*Perkinsus marinus*; *Marteilioides* spp. (*M. chungmuensis*);
II. High risk/low uncertainty	*Perkinsus olseni-atlanticus*; *Bonamia ostreae*; *Marteilia refringens*; *Haplosporidium nelsoni*
III. Low risk/high uncertainty	*M. mackini*; *Quahaug*; *Marteilia* sp.
IV. Low risk/low uncertainty	*Marteilia christenseni*; *Marteilia maurini*

6.2.2.1 Description of listed shellfish parasitic diseases[5]

i) *Marteilioides* spp. *(M. chungmuensis)*

Disease:

Marteilioides spp. (*M. chungmuensis* : Marteilioidosis)

Description of disease:

[5] Shellfish parasitic disease profiles compiled by L. Miossec

Marteilioides chungmuensis infects the cytoplasm of oocytes and can affect large areas of the reproductive follicles causing irregular enlargement of the infected gonadal tissues. Histological observations have suggested that *M. chungmuensis* invades immature ova which move to the center of the follicle. Growth of the parasite was been shown to be highly correlated with the growth and maturation of host gonadal cells. Infected eggs may be liberated via the genital canal or retained in the ovarian follicle and this parasite can have a significant effect on the reproductive output of an infected female oyster. Infection can also cause spawning failure by delaying spawning and destroying ripe oyster oocytes. Infection also significantly reduces glycogen levels and serum protein concentrations, thereby affecting metabolic recovery after spawning. Infected oysters lose their marketability due to the poor aesthetic appearance, which thus causes a serious economical impact.

Geographical distribution:
China, Japan, Korea

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Unknown

Host range (susceptible species):
Natural: *Crassostrea gigas* and *Crassostrea nippona*
Experimental: no data

Assessment:
See Annex 6 (6.2.2, i)

Useful links:
1. Fisheries and Oceans Canada: Bower, S.M., Itoh, N., Choi, D.-L., Park, M.S. (2006). Synopsis of infectious diseases and parasites of commercially exploited shellfish: *Marteilioides chungmuensis* of oysters.
http://www-sci.pac.dfo-mpo.gc.ca/shelldis/pages/mcoy_e.htm
2. Bondad-Reantaso *et al*. (2001): NACA/FAO: Asian Diagnostic Guide to Aquatic Animal Diseases.
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

ii) *Perkinsus marinus*
Disease:
Perkinsus marinus

Description of disease:
The symptoms of infection in *Crassostrea virginica* range from pale appearance of the digestive gland, and reductions in condition index, haemolymph protein concentrations

and lysozyme activity, to severe emaciation, gaping, shrinkage of the mantle away from the outer edge of the shell, retarded growth and occasionally the presence of pus-like pockets. Proliferation of the parasite causes systemic disruption of connective tissue and epithelial cells and is correlated with warm summer water temperatures (higher than 20 °C) when pathogenicity and associated mortalities are highest.

Geographical distribution:
USA (East Coast and Gulf of Mexico, introduced in Hawaii), Venezuela, Puerto Rico, Cuba and Brazil

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:
No intermediate host is reported. The life cycle of *P. marinus* includes non-motile vegetative stages (trophozoites) and free-living stages (zoospores). Trophozoites are phagocytosed by oyster hemocytes, where they proliferate by palintomy (merogony or schizogony). Trophozites from infected oysters are released into the water where they undergo palintomic zoosporulation. In the environment, transmission of *P. marinus* between oysters likely occurs by transfer of the trophozoite stage released from dying oysters, and filtered from the water by adjacent oysters. The primary portal of entrance is gut epithelium of oyster. Moreover, experimental observations demonstrate that other tissues, such as gill epithelia, labial palps and mantle are infection routes too.

Infected *C. virginica* can eliminate viable *P. marinus* with the feces and pseudofeces at a rate correlated to both *P. marinus* body burden and subsequent survival time. However, in an epizootic, shedding of *P. marinus* via feces is relatively small compared to the potential number released by heavily infected oysters but this may be important in transmission before infections become lethal.

Host range (susceptible species):
Natural: *Crassostrea virginica*
Experimental: *Crassostrea gigas* and *C. ariakensis*

Assessment:
See Annex 6 (6.2.2, ii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00042.htm

2. Aquatic Animal Diseases Significant to Australia (2004) for an overview and additional susceptible species.
http://www.disease-watch.com/documents/CD/index/html/mp045per.htm

3. Fisheries and Oceans Canada: Bower, S.M. (2006d): Synopsis of infectious diseases and parasites of commercially exploited shellfish: *Perkinsus marinus* ("Dermo" disease) of oysters.
http://www-sci.pac.dfo-mpo.gc.ca/shelldis/pages/pmdoy_e.htm.

iii) *Perkinsus olseni/atlanticus*
Disease:
Perkinsus olseni/atlanticus

Description of disease:
In most clam species, the parasite frequently induces the formation of visible milky white cysts or nodule on the gills, foot, gut, digestive gland, kidney, gonad and mantle of heavily infected clams.

Geographical distribution:
Eastern and Southern Australia, New Zealand, Korea, Japan and Europe (France, Italy, Portugal and Spain)

EU listed (Dir. 2006/88):
No

OIE listed:
Yes

EU status:
Non-exotic

Transmission:
Transmission is direct from host to host. The life cycle includes different stages, all infective, one is a vegetative stage (trophozoites) and the zoospores are the free living stage.

Host range (susceptible species):
Natural: *Haliotis ruber*, *H. cyclobates, H. scalaris*, *H. laevigata*, *Anadara trapezia*, *Ruditapes philippinarum*, *Austrovenus stutchburyi* and *Ruditapes decussatus*
Experimental: no data

Assessment:
See Annex 6 (6.2.2, iii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00043.htm

2. Aquatic Animal Diseases Significant to Australia (2004) for an overview and additional susceptible species.
http://www.disease-watch.com/documents/CD/index/html/mp050per.htm

3. Fisheries and Oceans Canada: Bower, S.M. (2006c): Synopsis of infectious diseases and parasites of commercially exploited shellfish: *Perkinsus* of clams and cockles.
http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/perkincc_e.htm

6.3 Crustaceans

The hazards identified for crustaceans with their hazard uncertainty scores and ranges are shown in Table 10.

Table 10. Crustacean disease hazards

Exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Crustacean	**Yellowhead**	**58.5**	**69.0-49.0**	**37.93**	**39.2-36.8**	**3**
	Taura	**50.83**	**57.5-40.5**	**29.07**	**31.6-27.0**	**3**
	Infectious hypodermal and haematopoietic necrosis	**36.95**	**39.2-34.7**	**34.4**	**39.0-29.8**	**2**
	***Coxiella cheraxi* (crayfish systemic rickettsiosis)**	**53.5**	**N/A**	**62.8**	**N/A**	**1**

Non-exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Crustacean	**White spot**	**55.5**	**71.0-43.0**	**37.04**	**42.2-25.6**	**5**

6.3.1 Crustacean bacterial hazard analysis

Risk and uncertainty for crustacean bacterial hazards scored

Coxiella cheraxi was the only crustacean bacterial hazard that was considered further after the application of the prefilter and, as a result, it is not represented by comparative graphical representation. *C. cheraxi* had a risk score of 53.5 and a high uncertainty score of 62.8.

6.3.1.1 Description of listed crustacean bacterial diseases

i) *Coxiella cheraxi*

Disease:

Crayfish systemic rickettsiosis

Description of disease:

Associated with serious mortality in Australian redclaw freshwater crayfish where it has been shown to occur in the cytoplasm of hepatopancreatic cells and in the connective tissues throughout moribund crayfish. Experimental infection led to crayfish becoming reddened and putrefied with the eyes of highly infected crayfish totally necrotised and the hepatopancreas liquefied. Experimental injection has shown mortality of 100% at 28 °C and 80% at 24 °C. Transmission by food and the waterborne route gave lower mortalities at 30% and 10%, respectively, over a 4 wk period (Tan and Owens, 2000).

Geographical distribution:

Australia, Ecuador

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Exotic

Transmission:
Horizontal through the water, although a secondary arthropod host may be necessary.

Host range (susceptible species):
Natural: *Cherax quadricarinatus*
Experimental: none described

Assessment:
See Annex 6 (6.3.1, i)

Useful links:
None

6.3.2 Crustacean viral hazard analysis

Risk and uncertainty for crustacean viral hazards scored (listed diseases in bold)

	AaBV	ApBV	**IHHNV**	**Taura syndrome**	**White spot**	**Yellowhead**	**Mean**	**Median**
mean risk	43,00	43,00	**36,95**	**50,83**	**55,50**	**58,50**	**47,96**	**46,92**
risk range max	N/A	N/A	**39,20**	**57,50**	**71,00**	**69,00**	59,18	63,25
risk range min	N/A	N/A	**34,70**	**40,50**	**43,00**	**49,00**	41,80	41,75
mean uncertainty	45,60	45,60	**34,40**	**29,07**	**38,16**	**39,87**	**38,78**	**39,02**
uncertainty range max	N/A	N/A	**39,00**	**31,60**	**42,20**	**39,20**	38,00	39,10
uncertainty range min	N/A	N/A	**29,80**	**27,00**	**25,60**	**36,80**	29,80	28,40

Figure 6. Ranking by risk score against uncertainty for crustacean viral hazards considered

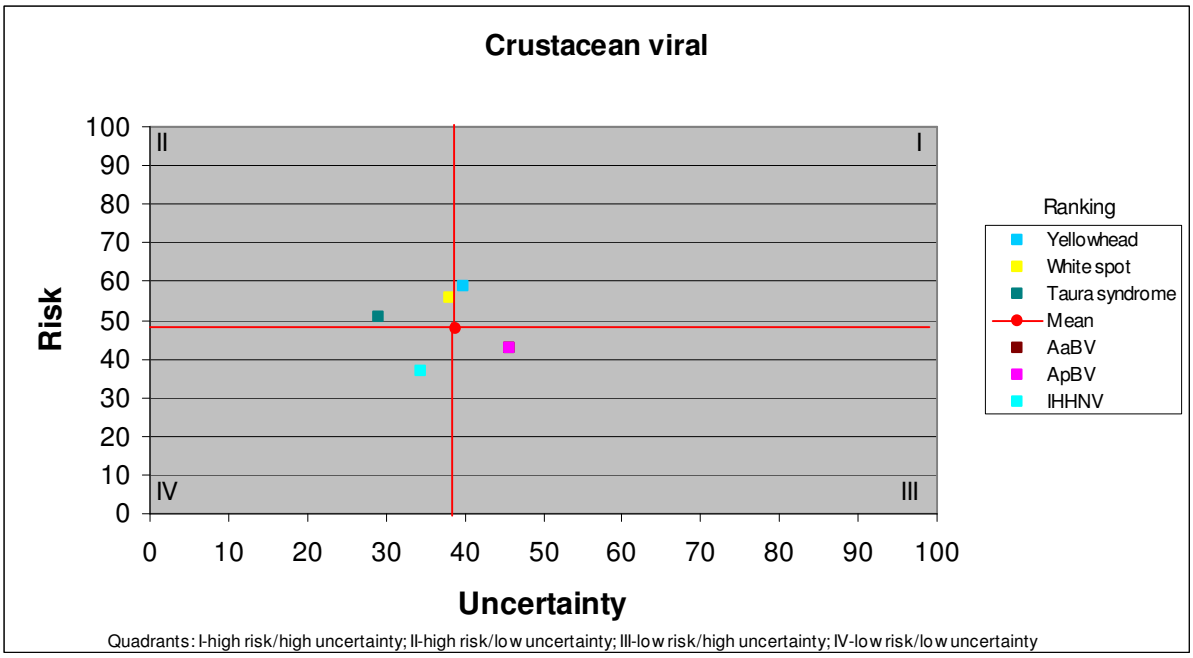

Table 11. Crustacean viral hazards by risk quadrant

Quadrant	**Crustacean viral hazard** (underlining means considered further)
I. High risk/high uncertainty	Yellowhead
II. High risk/low uncertainty	White spot, Taura
III. Low risk/high uncertainty	AaBV, ApBV (same scores)
IV. Low risk/low uncertainty	IHHNV

6.3.2.1 Description of listed crustacean viral diseases

i) IHHNV

Disease:

Infectious hypodermal and haematopoietic necrosis

Description of disease:

IHHNV infections are most severe in the Pacific Blue Shrimp, *L. stylirostris,* where the virus can cause acute epizootics and mass mortality (> 90%). In *L. stylirostris* the juvenile and subadult life stages are the most severely affected (OIE, 2006a). IHHNV causes the chronic disease "runt-deformity syndrome" (RDS) in *L. vannamei*, which produces reduced, irregular growth and cuticular deformities, rather than mortalities. IHHNV infection in *P. monodon* is usually subclinical, but RDS, reduced growth rates and reduced culture performance has been reported in infected stocks (OIE, 2006a). Some penaeid shrimps that survive infections and/or epizootics may carry the virus for life and pass the

virus on to their progeny and other populations by vertical and horizontal transmission. Prevalence can range from 0-100% in wild stocks from enzootic areas.

Geographical distribution:
Australia, China, Costa Rica, Ecuador, French Polynesia, Guam, Guatemala, Honduras, India, Indonesia, Iran, Malaysia, Mexico, Myanmar, New Caledonia, Panama, Philippines, Peru, Singapore, Taiwan, Thailand and USA

EU listed (Dir. 2006/88):
No

OIE listed:
Yes

EU status:
Exotic

Transmission:
Horizontal (e.g. cannibalism and water) and vertical (e.g. infected eggs)

Host range (susceptible species):
Natural: *Penaeus vannamei*, *P. stylirostris*, *P. occidentalis*, *P. monodon*, *P. semisulcatus*, *P. californiensis*, *P. schmitti* and *P. japonicus*
Experimental: *Penaeus setiferus*, *P. aztecus, P. chinensis*, *P. merguiensis*, *P. indicus* and *P. duorarum*

Assessment:
See Annex 6 (6.3.2, i)

Useful links:
1. OIE Manual (OIE, 2006) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00048.htm
2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/cv005ihh.htm
3. Bondad-Reantaso *et al*., (2001): Asia Diagnostic Guide to Aquatic Animal Diseases.
ftp://ftp.fao.org/docrep/fao/005/y1679e/y1679e00.pdf
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

ii) Taura syndrome
Disease:
Taura syndrome

Description of disease:
In on-farm epizootics of TS involving unselected stocks of *L. vannamei,* the principal host species for TSV, typical cumulative mortalities range from 40 to >90% in cultured populations of postlarval (PL), juvenile, and subadult life stages, whereas survivors of TSV infections may carry the virus for life (OIE, 2006a). TSV infects and has been shown

to replicate in principally the cuticular epithelium (or hypodermis) of the general exoskeleton, foregut, hindgut, gills and appendages, and often in the connective tissues, the haematopoietic tissues, the lymphoid organ, and antennal gland. The enteric organs (endoderm-derived hepatopancreas, midgut and midgut caeca mucosal epithelia) and smooth, cardiac, striated muscle, and the ventral nerve cord, its branches and its ganglia typically show no histological signs of infection by TSV (OIE, 2006a). TS is a disease of nursery- or grow-out-phase *L. vannamei* that occurs within ~14-40 days of stocking PLs into grow-out ponds or tanks. Larger shrimp may also be affected, especially if they are not exposed to the virus until they are larger juveniles or adults (OIE, 2006a). The prevalence of TSV can range from 0 to 100% enzootic in regions where the virus is in farmed stocks.

Geographical distribution:
Latin America, Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Indonesia, Korea R., Malaysia, Mexico, Myanmar, Nicaragua, Panama, Peru, Taiwan, Thailand, Venezuela and USA (Florida, South Carolina and Texas)

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:
Horizontal (e.g. cannibalism and water) and probably vertical (e.g. infected eggs)

Host range (susceptible species):
Natural: *Penaeus vannamei*, *P. stylirostris* and *P. setiferus*
Experimental: *Penaeus schmitti*, *P. aztecus*, *P. duorarum*, *P. chinensis*, *P. monodon* and *P. japonicus*, *Metapenaeus ensis* and *Penaeus aztecus*

Assessment:
See Annex 6 (6.3.2, ii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00048.htm

2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/cv025tau.htm

3. Bondad-Reantaso *et al*., (2001): Asia Diagnostic Guide to Aquatic Animal Diseases.
ftp://ftp.fao.org/docrep/fao/005/y1679e/y1679e00.pdf
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

iii) White spot
Disease:
White spot

Description of disease:
White spot syndrome virus (WSSV) is a pathogen of major economic importance in cultured penaeid shrimp and infection can reach a cumulative mortality of up to 100% within 3-10 days (Marks *et al*., 2005). The acute phase of the disease is characterised by the presence of white spots on the inner surface of the exoskeleton, whereas other clinical signs include anorexia, lethargy and reddish discoloration of the body
(Wang *et al.*, 1999).

Geographical distribution:
Bangladesh, Brazil, China PR, Colombia, Costa Rica, Ecuador, possibly Europe, Guatemala, Honduras, Hong Kong, India, Indonesia, Iran, Japan, Korea RO, Malaysia, Mexico, Myanmar, Nicaragua, Panama, Peru, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Togo, USA, and Vietnam

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Non-exotic[6]

Transmission:
Horizontal and vertical

Host range (susceptible species):
Natural: *Penaeus japonicus*, *P. chinensis*, *P. indicus*, *P. merguiensis*, *P. monodon*, *P. setiferus*, *P. stylirostris*, and *P. vannamei*
Experimental: *Penaeus aztecus* and *P. duodarum*

Assessment:
See Annex 6 (6.3.2, iii)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00048.htm

2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/cv020wsd.htm

[6] Listed as non-exotic by EU Directive 2006/88

3. Bondad-Reantaso et al. (2001): Asia Diagnostic Guide to Aquatic Animal Diseases.
ftp://ftp.fao.org/docrep/fao/005/y1679e/y1679e00.pdf
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

4. OIE International database on aquatic animal diseases for more susceptible species
http://www.collabcen.net/toWeb/aq2.asp

iv) Yellowhead
Disease:
Yellowhead

Description of disease:
There are variations in the susceptibility of different penaeid species to disease. YHV targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antenal gland, gonads, nerve tracts and ganglia. The high prevalence (50-100%) of infection of yellowhead complex viruses in healthy farmed and wild *P. monodon* by PCR suggests that lifelong chronic infections occur commonly, although the prevalence of individual genotypes varies according to the geographic origin of the shrimp and may be low (>1%) for genotype 1 (OIE, 2006a). Yellowhead disease can cause up to 100% mortality in infected *P. monodon* ponds within 3 days of the first appearance of clinical signs (OIE, 2006a).

Geographical distribution:
Asia, Australia, Bangladesh, China PR, India, Indonesia, Malaysia, Philippines, Sri Lanka, Taiwan, Thailand, USA (Texas) and Vietnam

EU listed (Dir. 2006/88):
Yes

OIE listed:
Yes

EU status:
Exotic

Transmission:
Horizontally by injection, ingestion of infected tissue, immersion in membrane-filtered tissue extracts, or by co-habitation with infected shrimp. For GAV, vertical transmission has been shown to occur from both male and female parents, probably by surface contamination or infection of tissue surrounding the fertilised egg (OIE, 2006a).

Host range (susceptible species):
Natural: *Penaeus* spp., *Penaeus monodon*, *Metapenaeus ensis*, *Palaemon styliferus*, *P. aztecus*, *P. duorarum*, *P. japonicus*, *P. indicus*, (*Fennero)penaeus merguiensis*, *P. setiferus*, *P. stylirostris* and *P. vannamei*
Experimental: *Acetes* spp. and *Euphausia* spp.

Assessment:
See Annex 6 (6.3.2, iv)

Useful links:
1. OIE Manual (OIE, 2006a) detailing information for the design of surveillance programmes (agent factors, host factors and disease pattern, as well as control and prevention), diagnostic methods (field, clinical, agent detection and identification), rating of tests against purpose of use, corroborative diagnostic criteria (suspect and confirmed cases) and diagnostic/detection methods to declare freedom.
http://www.oie.int/eng/normes/fmanual/A_00050.htm

2. Aquatic Animal Diseases Significant to Australia (2004) for an overview.
http://www.disease-watch.com/documents/CD/index/html/cv010yhd.htm

3. Bondad-Reantaso *et al*., (2001): Asia Diagnostic Guide to Aquatic Animal Diseases.
ftp://ftp.fao.org/docrep/fao/005/y1679e/y1679e00.pdf
http://library.enaca.org/NACA-Publications/ADG-complete.pdf

6.4 Amphibians

The hazards identified for amphibians with their hazard and uncertainty scores are shown in Table 12.

Table 12. Amphibian disease hazards

Exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Amphibian	**Ranavirus**[1]	**56.5**	**N/A**	**35.2**	**N/A**	**1**

Non-exotic Diseases						
	Disease agent	**Risk score**	**Range**	**Uncertainty score**	**Range**	**n**
Amphibian	**Ranavirus**[1]	**56.5**	**N/A**	**35.2**	**N/A**	**1**
	Batrachochytrium dendrobatidis **(amphibian chytridiomycosis)**	**70.0**	**N/A**	**31.6**	**N/A**	**1**

[1]It is thought that several amphibian Iridoviridae are exotic but others are already present in the EU and appear to be emerging

6.4.1 Amphibian viral hazard analysis

Ranavirus was the only amphibian viral hazard that was considered further after the application of the prefilter and, as a result, it is not represented by comparative graphical representation. It had a risk score of 56.5 and an uncertainty score of 35.2.

6.4.1.1 Description of listed amphibian viral diseases

i) Ranavirus

Disease:
Ranaviral disease

Description of disease:
Ranaviral disease has been seen in captive amphibians and in epizootics in wild amphibians in North America and the United Kingdom. Apart from causing high rates of mortality in amphibians, some members of this genus can also infect fish and reptiles, resulting in morbidity and mortality (OIE, 2006b). The syndrome manifests itself as skin ulceration or systemic haemorrhages.

Geographical distribution:
?Canada, UK and USA

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Non-exotic

Transmission:
Horizontal

Host range (susceptible species):
Natural: Amphibians
Experimental: no data

Assessment:
See Annex 6 (6.4.1, i)

Useful links:
1. Amphibian Disease Home Page
http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

6.4.2 Amphibian fungal hazard analysis

Batrachochytrium dendrobatidis (chytridiomycosis) was the only amphibian fungal hazard that was considered further after the application of the prefilter and, as a result, it is not represented by comparative graphical representation. It had a risk score of 70.0 and an uncertainty score of 31.6.

6.4.2.1 Description of listed amphibian fungal diseases

i) *Batrachochytrium dendrobatidis*

Disease:
Amphibian chytridiomycosis

Description of disease:
Chytridiomycosis has become pandemic in wild amphibians, resulting in loss of amphibian populations across 5 continents. This is due to its low host specificity, since it has infected at least 200 species of amphibians from 43 genera, up to 19 families and 2 orders, and is responsible for at least 1 species extinction, indicating that globally probably most or all species of amphibians could be infected (OIE, 2006b; Hyatt *et al*., 2007). Morbidity and mortality caused varies with the species of amphibian and the environmental conditions, and mortality increases with lower temperatures. Nevertheless, mortality rates of up to 100% have occurred during natural outbreaks in captivity and in transmission experiments in captive amphibians of susceptible species (Berger *et al*., 2005). Death in susceptible experimental animals usually occurs from between 18 and 70 d post exposure and incubation time varies with dose, fungal strain, temperature and amphibian species (Berger *et al*., 2005). Fungal sporangia infect cells in the stratum

granulosum and stratum corneum in the superficial epidermis. Immature sporangia occur within the deeper, more viable cells while mature zoosporangia and empty sporangia are more prevalent in the outer keratinized layers (Berger *et al*., 2005). Mortality results by either the release of proteolytic enzymes or other active compounds that are absorbed through the permeable skin of the frog or, possibly, damage to skin function results in disturbance of oxygen, water or electrolyte balance (Berger *et al*., 2005). Higher temperatures (i.e. >25 °C) increase the rate of epidermal turnover and reduce the growth of amphibian chytrid.

Geographical distribution:
Africa, Asia, Australia, Central America, Europe (exact distribution unknown), Japan, New Zealand, South America and USA[7]

EU listed (Dir. 2006/88):
No

OIE listed:
No

EU status:
Non-exotic

Transmission:
Horizontal

Host range (susceptible species):
Natural: Amphibians
Experimental:

Assessment:
See Annex 6 (6.4.2, i)

Useful links:
1. Amphibian Disease Home Page
http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

[7] See: Speare R, Berger L. Global distribution of chytridiomycosis in amphibians.
http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm

General Discussion and Assessment of Listed Hazards

A full picture of the most significant exotic, emerging and re-emerging disease hazards for aquatic animal health in the EU has been obtained and has proved valuable for assessing their potential impact. Information on presence or absence of the considered disease agents in the EU and their regulatory status, as well as pathways of introduction, establishment, consequences and risk mitigation has been gathered, and now forms the basis for improved understanding because of the provision of such data. Assessments of the risks associated with live fish movements and aquaculture must be securely based on scientific evidence (Gozlan *et al*., 2006). Nevertheless, it was not originally envisaged that full risk analysis would be conducted on each hazard identified, due to resource constraints, or on the hazards listed by the PANDA exercise. Consequently, the results simply represent a descriptive assessment, in this case hazard identification in conjunction with expert opinion related to pathways of introduction, consequences, establishment and risk mitigation. The hazards identified therefore concern pathogens that need further consideration for purposes of risk estimation. This would be best achieved by the formation of specialist working groups conceived to consider the listed fish, shellfish, crustacean and amphibian hazards separately or by group (fish viral, shellfish parasitic, etc.).

The process of risk analysis is step-wise and need not necessarily lead to full hazard characterization. For instance, at any point in an assessment the analysis may stop if the risk is shown to be at such a low level that the continuation of the pathway would not be necessary, even if subsequent steps are considered a higher risk (see detailed hazard identification below). For instance, if pathways of introduction do not exist then the risk analysis can stop, despite the fact that the consequences of introduction and subsequent establishment in a non-infected area may be considered to be high risk. This could be the situation with some of the hazards listed by PANDA, since detailed consideration of trade movements, including volumes of trade, may have reduced the risk to such an extent that would have led to their reconsideration for listing purposes. This level of detail was impossible to achieve in the PANDA exercise, although the known existence of simple trade links were considered. However, greater detail in this area is recommended for any continuation project, since no specific consignment sources or destinations (other than the EU as a whole) were considered. Consequently, the hazard scoring exercise inevitably introduced a greater degree of uncertainty into the assessment than usual. In addition, the acceptable level of protection for the EU was not established and this is an important factor that determines the risk analysis process and the need to apply any risk mitigation measures.

Hazard identification

In general, there are two types of hazard identification that need to be considered; i) a preliminary identification, for an initial assessment as to whether importation (or intra-Community movement) is likely to involve a significant hazard, and, ii) a detailed identification, for an in-depth appraisal of the hazards involved.

Preliminary hazard identification

Preliminary hazard identification is for making an initial decision on the necessity for a full risk analysis and to determine the probable level of unmitigated risk (i.e. the estimated risk before any potential risk management measures have been considered). Such an approach can quickly identify potentially serious hazards and in the PANDA exercise (WP 2) this was achieved through the compilation of a series of disease tables (Annex 1; 1.1-1.4) that considered virtually all known diseases or conditions. The subsequent use of a pre-filter based

on the OIE disease listing criteria proved very valuable for reducing the number of hazards by removing the less important ones, although spread (OIE criteria B) was difficult to determine in some cases (e.g. the fish parasites). In addition, the current EU status of a disease (or pathogen) was not always apparent for non-statutory diseases.

Detailed hazard identification

Detailed hazard identification is a more exhaustive and comprehensive search for information on the potential hazards carried by a particular commodity (e.g. live fish or product). Information is required on the potential pathogens for the commodity in the country (or Community region) of origin, as well as on a world-wide basis. The health status of the commodity in the exporting country (or region) will often be poorly known, and specific diseases are often difficult to detect due to their low prevalence in a population or the absence of a reliable validated diagnostic test.

It has to be remembered though that "evidence of absence" of a hazard does not necessarily equate with "absence of evidence (hazard)". It may be that the aquatic animal species of concern has been poorly studied, and, consequently, there is little or no information available on the pathogens that it might carry. In addition, an evaluation of the Competent Authority in the exporting country, any disease surveillance and control programs that are in place, and any zoning systems are important in assessing the likelihood of hazards being present in the aquatic animals present in the exporting country (or European region).

Following hazard identification, a list of hazards (pathogens) of concern was generated. This list was then used in the next step in the risk analysis process, risk assessment, to determine the level of risk that each hazard represented to the importing region (in this case European as a whole or between regions within Europe). The logical step-wise process considers that a disease has a risk of introduction and a likelihood of becoming established in a country, followed by the consequences of such a disease being established. Consequently, risk assessment is comprised of four components designated as release assessment, exposure assessment, consequence assessment and risk estimation.

The OIE definitions (OIE, 2007) of these components are:

i) release assessment – describing the biological pathway(s) necessary for an importation activity to 'release' (that is, introduce) a hazard into a particular environment, and estimating the likelihood of that complete process occurring

ii) exposure assessment – describing the biological pathway(s) necessary for exposure of humans and aquatic and terrestrial animals in the importing country to the hazards and estimating the likelihood of the exposure(s) occurring, and of the spread or establishment of the hazard

iii) consequence assessment – identifying the potential biological, environmental and economic consequences. A causal process must exist by which exposures to a hazard result in adverse, health, environmental or socio-economic consequences.

iv) risk estimation – integrating the results of the release assessment, exposure assessment and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset.

An example of how to classify an agent as a potential hazard would use the following criteria (modified from Murray, 2004; as detailed in the Manual on risk analysis for the safe movement of aquatic animals – Arthur *et al*., 2004):

- The agent must be appropriate to the species being imported (or transhipped), or from which the commodity is derived
- It may be present in the exporting country (or Community region)
- If present in the importing country (or Community region), it should be a notifiable disease or subject to control or eradication.

The overall process, including hazard identification and a consideration of the commodity itself, in the case of a specific import or intra-European movement, can be represented as a stepwise process, as follows (modified from Murray, 2002):

1) Is the commodity a potential vehicle for the organism?

- If Yes, proceed to step 2;
- If No, the organism is not a potential hazard.

2) Is the organism exotic to the importing country (or importing Community region) but likely to be present in the exporting country (or exporting Community region)?

- If Yes, it is classified as a potential hazard;
- If No, proceed to step 3

At this point, an exporting country's Competent Authority, surveillance and control programs and zoning and regionalization systems are important factors to consider when assessing the likelihood of hazards being present in the animal population of the exporting country. They enable the exporting country to substantiate claims of disease status and the importing country to establish and maintain confidence in such claims.

3) For an organism reported in both the exporting and importing countries (or Community regions), either if:

a) there are free zones or zones of low prevalence in the importing country (or Community region) that are established under a national or regional pest management strategy or small-scale program and where the movement of animals and/or animal products into the zone is under statutory control; or if

b) it is listed on the unwanted organisms register (e.g. Directives 91/67/EC and 2006/88) as a notifiable organism; or if

c) there is a more virulent strain in the exporting country (or Community region).

Then the organism is classified as a potential hazard.

For the WP 2 exercise the starting point was the disease hazards themselves followed by identification of potential routes of introduction for the commodities by considering the existence of trade as a more general concept through the hazard scoring exercise, but without

specifying exact pathways or quantities of commodity traded, as mentioned above. This was completed by collecting data and opinion on establishment, consequences and risk mitigation. The decision not to include volume of trade was based on the fact that hazard identification was already a broad enough concept and to consider quantity did not contribute to framing the right general question. However, it was generally considered that the higher the volume of trade, the higher the disease risk, although other factors such as surveillance and monitoring by a competent authority would also be important considerations.

Hazard scoring method

The method used was a combination of subjective opinion and objective fact based on the scoring method developed. The technique considered the presence of host species (this was a prerequisite), the European status of the hazard, the potential pathways of introduction, the consequences of introduction, establishment and risk mitigation factors. Although part of the exercise was subjective, this was inevitable for areas where data was scarce for exotic or emerging diseases. In fact, the template was designed with this in mind and it largely used expert opinion to help gauge the importance of the hazard, since it was recognised in advance that extensive documented data would not be available in all cases. This, in fact, proved a highly valuable approach and for some of the lesser well characterised diseases the use of experts or specialists was especially necessary, even though the number of responders was very low in some cases. Nevertheless, an assessment of "expertise" did not form a detailed part of the hazard scoring exercise, although the number of years working in the field and the recognised prestige or position of the expert was arbitrarily considered as an important contributory factor. Experts were difficult to find though for such a short-term exercise (e.g. because of time constraints and the specific nature of the subject), particularly for exotic diseases within a European context. However, subjective probability-estimation methods have been shown to provide an alternative approach to risk factor evaluation in the absence of empirical data (Gustafson *et al*., 2005). In cases where the data was more extensive and readily available (e.g. for non-exotic diseases) an overview had to be provided because all the other work packages depended on WP 2 producing a hazard list and therefore the time factor was very important to the success of the whole project.

In general, there was a long response time from many of the experts who were consulted and there was difficulty in finding expertise related to some of the diseases which required assessing. Consequently, there was a need to seek expertise from outside the network for some diseases, in particular, exotic parasites. In addition, some diseases were scored which were not strictly exotic, emerging or re-emerging and these provided good comparative data. Some of these were exotic to certain areas of Europe, and others, which were non-exotic European diseases, provided a useful baseline with which to assess the risk score of the true exotics.

It is possible that pathogenicity could have been considered in greater detail, such as whether a pathogenic agent (i.e. hazard) was capable of inducing disease in natural infections, under experimental conditions or was not capable of inducing disease at all. Nevertheless, pathogenicity proved to be a difficult category to score and it was shown that the scoring system, as proposed, provided the levels of priority required for the exercise.

The group scoring approach was considered the best and quickest way to arrive at a consensus (e.g. as used by the EU References laboratories for both fish and shellfish at Arhus and La Tremblade, respectively). However, the use of individual experts for some areas was valuable and unavoidable (e.g. the crustacean and amphibian diseases), although the difficulty of comparing group and individual scores was recognised. Nevertheless, as mentioned above,

weight was given to expertise in these cases, although the hazard score from a particular group was interpreted as a single consensus score, as opposed to the variance score from an individual.

For purposes of epidemiology and diagnostic methods, it was shown that more evidence is required for certain hazards in order to support their status of "emerging", such as *Perkinsus olseni/atlanticus*, oyster herpesvirus and *V. lentus* in molluscs and the amphibian Iridoviridae, the latter of which are thought to be both exotic and already present, and appear to be emerging. The current status of crustacean diseases in Europe was also difficult to ascertain. Apart from *Aphanomyces astaci*, the causative agent of crayfish plague, which has received considerable research attention over the last century, there is a paucity of knowledge of other pathogens affecting freshwater crayfish. In Europe, this may be due to diagnostic and research bias toward *A. astaci*, and consequent lack of capacity for general disease diagnosis in crustaceans (Edgerton *et al*., 2004). However, there remains a very real lack of data on the pathological consequence of these pathogens in the wild and in aquaculture, related to their geographical and potential host ranges, transmissions routes, species/strain genetic and virulence variation. It is likely that more crayfish pathogens will satisfy the OIE criteria in the future when more data is available following cotranslocation of pathogens, and their consequent negative impacts on wild and farmed populations (B. Edgerton, pers. com.). A similar situation currently exists for the penaeid shrimp diseases from a European perspective.

The feedback information on the hazard scoring methodology was favourable, and it would also be useful to receive comments on the hazard list itself. This sort of information could be provided possibly using a questionnaire through the NRLs, aquatic animal farmers, additional PANDA experts, researchers, etc. These comments could be useful for more in-depth considerations of the hazards.

Further data manipulation (now outside the scope of the project) will allow the generation of reports concerning individual species and their reported diseases by country, as well as individual country disease profiles. This would be additional complementary information to that available in the current OIE International Database on Aquatic Animal Diseases (http://www.collabcen.net/toWeb/aq2.asp). Examples of data extraction for a species profile detailing species by reported disease (Annex 7.1), as well as for a country profile showing incidences of disease (Annex 7.2) are included for illustrative purposes.

The provision and availability of data was a problem for WP 2, and it is expected that the identification of data gaps will lead to the need for a listing of the type of product imported into the EU, or moved within the EU, in conjunction with pathogen survival parameters. Product should be considered as live product or non-viable processed product (eviscerated, whole or consumer ready) and more accurate data will indicate whether such product could act as a disease vector. In this respect, the PANDA web site could act as an indispensible source of information related to all aspects of hazard identification.

There is also a need for the organisation of further targeted workshops, expert sessions or working groups and training exercises concerning risk analysis and parallel concepts related to epidemiology, which could be addressed by the PANDA project providing additional funding was available.

Fish hazards

There are many factors that can influence the effect of microbial pathogens on fish populations. However, climate change seems to be a recent phenomenon that has not

previously been considered in too much detail. It is thought that increasing water temperatures will change the geographic distribution of marine species, with potential disease consequences. In freshwater, increasing water temperature will increase the ability of exotic fish species, introduced into Europe as a result of international trade, to establish and spread, thereby increasing the probability that pathogens spread to native fish populations. In addition, some introduced pathogens, such as koi herpes virus, may survive better and exert greater impact at higher water temperatures (Gozlan *et al*., 2006). EUS, a PANDA-listed exotic disease, is a case in point since it has the potential to cause severe problems, as already demonstrated in many Asian countries. However, although current environmental conditions, such as a mean temperature range of 18-22 ºC after periods of heavy rainfall may be unfavourable in Europe, increasing temperatures as a result of climate change may lead to the existance of more favourable conditions. Another PANDA-listed disease (although non-exotic), caused by *Lactococcus garviae*, has increased its incidence and geographic range within Europe in recent years as the mean water temperature has risen (Gozlan *et al*., 2006).

The assessment of EUS depended to a certain extent on potential pathways of introduction related primarily to the existence of trade in live host species and/or their products from known positive countries. It is thought that some fish species, such as certain carps, mullet, catfish or ornamentals are traded (EFSA, 2007) from Asian regions but their final destination and use is unclear. Hence, there was uncertainty in this area during the assessment, particularly for live fish movements, since the host range of *Aphanomyces invadans* is very large. Apart from the effect of climatic conditions, as mentioned above, other risk factors considered important for establishment of EUS were the potential number of different host species present in Europe, their fairly widespread extension, the length of time the pathogen can live in the environment without a host, and the rapidity of spread by both natural means and human assistance, as well as the historical evidence that this pathogen has often shown the ability to successfully establish in new areas outside its original range. The consequences of introduction were thought to be very important because of the possibility to cause environmental harm (e.g. potential reduction of native species and changes in biodiversity), the potential for economic loss, any related additional costs and the disruption of existing biological systems if control/eradication measures were attempted. Nevertheless, it was also recognised that if EUS were to become established it would not in fact be possible to eradicate it from the EU and attempts to do so would not be worthwhile, except perhaps in specific, highly contained outbreaks where there was no possibility of environmental release. However, existing control or husbandry measures (in cultured/farmed populations) were not thought likely to prevent establishment of the pathogen, unless there was a rapid response to such a specific incidence, although a response of this type would rely on the use of an active surveillance system, which is not currently implemented in the EU.

Although *Streptococcus agalactiae* and *S. iniae* were considered together and they both have the potential to cause zoonotic problems, as does *L. garviae*, *S. iniae* is associated with fish disease problems in a wider range of species, including wild fish, whereas *S. agalactiae* was the only exotic bacterial fish hazard listed. Nevertheless, all three hazards were considered important by the assessment. The potential for further spread of *S. iniae* was quite likely, whereas the consequences of introduction into other regions, such as potential economic loss, were high. The introduction of *L. garviae* was less likely to cause the same problems. The inability to eradicate these hazards, in conjunction with the lack of an active surveillance system, were additionally important risk factors. The potential pathways of introduction for *S. agalactiae* were unclear but there is no documented evidence that the pathogen has been spread by international trade in the products of susceptible species. For this reason, its establishment, although possible, was uncertain and the consequences of introduction were not

thought to be particularly important, although risk mitigation measures were not likely to either prevent establishment or eradicate the hazard if it were to establish itself. Consequently, although it was retained on the PANDA list it could generally be considered as less of a risk than either *S. iniae* or *L. galactiae*, which are already established in southern Europe.

Reservoir hosts for fish viral pathogens cannot be excluded and therefore these represent a potential infection pathway between infected areas or regions, and disease-free zones. One example is ISAV, since transmission has been shown from wild reservoirs to farmed populations on at least three occasions and it is widely distributed in the North Atlantic area (Dipnet, 2007). This is significant for the EU (currently free of ISA) because, in this case, it is bordered by Norway that is one of the countries where it is detected annually in cultured salmonids. Consequently, the PANDA exercise indicated that the risk of establishment was thought to be high, since the environmental conditions are very similar and both the number of cultured species and their widespread distribution (farmed and wild), as well as the ability to be rapidly spread by human assistance were identified as important risk factors. This is suppported by a study, also using expert opinion, for identifying risk factors important to ISA outbreaks that found some of the strongest independent predictors of ISA infection included a site's proximity to other farms with clinically infected fish and whether a site employs harvest vessels practicing full containment of blood and stun water (Gustafson *et al*., 2005), which is particularly relevant for aquaculture companies that have facilities in more than one region or that operate from a logistical base in a neighbouring country. In addition, the PANDA assessment indicated that the consequences of ISA establishment would be economically important and an extensive region could also be affected. As a result, additional costs related to areas such as control, research and certification schemes would be very important, as has been shown in the UK when the only known historical incidence of ISA was stamped out in 1998. This supports the fact that existing risk mitigation controls or current husbandry measures for cultured populations, in conjunction with the existence of an active surveillance system, were thought likely to prevent establishment.

The case of viral hazards such as EHNV or RSBIV is different, since, although the potential consequences of introduction are high, the pathways of introduction represent a much lower risk. This is related to the fact that there is no trade in live fish species from Australia or Japan (or probably other Asian countries), respectively, were the diseases occur. Nevertheless, they are members of the Ranavirus group and ranaviruses are highly infective to a range of animal orders and species, and they have the potential to persist in the environment (Daszak *et al*., 2003). In addition, it is not certain whether amphibians, or even reptiles, are the natural reservoirs of these viruses (EFSA, 2007). The isolation of ranavirus from aquarium fish has also suggested that these viruses could possibly be spread by trade in ornamental fish (Hedrick and McDowell, 1995) or movement of amphibians and reptiles (EFSA, 2007), and exchange of virus between amphibians and fish can occur in nature (Mao *et al*., 1999). Consequently, the possibility that existing control or husbandry measures (in cultured/farmed populations) would prevent establishment of such hazards or that the pathogens could be eradicated from the EU were considered unlikely, particularly since this depends to a large part on the implementation of an active surveillance system. A similar situation could have occurred historically with KHV, since the disease has been spread predominantly through the trade in koi carp and it is known to occur in, or has been recorded in, fish imported into many countries (EFSA, 2007). Consequently, it is not surprising that the pathways of introduction for KHV were still thought to be of high risk and, since the disease could still possibly be considered as emerging, its further spread and establishment, related to the extent and existence of host species, the history of successful establishment outside its original range and the continuing potential for

economic loss were all assessed as very likely. On the other hand, there was uncertainty concerning the length of time the pathogen could live in the environment without a host.

The history of *Gyrodactylus salaris* in Norway indicates that it was spread to numerous rivers through introductions of infected fish for stock enhancement purposes (Johnsen *et al*., 1999). In addition, a recent Dipnet report states that there is good scientific evidence for the transmission of *G. salaris* between farmed and wild Atlantic salmon, which can result in massive reductions in population size (Dipnet, 2007). As a result, the PANDA listing retains this parasite, despite the fact that it does not occur as a listed disease for EU Directive 2006/88, since it was recognised that the principal means of preventing introductions is through the regulation of live fish movements from affected areas on to farms or for enhancing wild populations (Dipnet, 2007), except in specific circumstances, such as those related to salinity in coastal areas that would reduce the risk of transmission to an acceptable level (Peeler *et al*., 2006). Therefore, as with ISAV, the risk of establishment was still considered high when related to additional factors and the consequences of establishment over an extensive area could still be very important. Current risk mitigation measures would also be unlikely to prevent establishment, despite the presence of an active surveillance system, and subsequent eradication once established would not be possible. Other PANDA-listed parasites, *Parvicapsula pseudobranchicola* and *Trypanoplasma* (*Cryptobia*) *salmositica*, reported from Norway and North America, respectively, have very little known concerning their epizootiology and natural life cycles, although in the case of the former an invertebrate intermediate (or primary) host is possibly involved and the latter is transmitted by a leech. Faced with a great deal of uncertainty regarding these hazards and the possibility that *P. pseudobranchicola* infections may be an important indirect cause of mortalities in wild salmon (Dipnet, 2007) they were included on the PANDA list. Again, the most important risk factors were related to the consequences of introduction, the potential for establishment and the inability to apply subsequent risk mitigation measures, as well as possible trade links with infected areas in Norway where *P. pseudobranchicola* has been reported. However, there is more data available for *Ceratomyxa shasta* and it can lead to high mortalities in salmonids, although it appears historically limited to north-western Pacific areas (Canada and USA) where it has a complex life cycle probably involving an intermediate host. As a result, although the pathways of introduction were not considered too important, despite some uncertainty, the likelihood of establishment in the EU, given similar conditions, the widespread existence of host species, the longevity of the parasite and the potential for rapid spread were important risk factors. In addition, the consequences of introduction were considered important and existing risk mitigation was not likely to prevent establishment. In general, a similar situation existed for *Neoparamoeba pemaquidensis* although the current status of this marine protozoan as the single causal agent of amoebic gill disease in Atlantic salmon has recently been questioned (Young *et al*., 2007). Consequently, additional data regarding another *Neoparamoeba* species (*N. perurans* n. sp.) thought by these authors to be the predominant aetiological agent for the disease may now be required to support any assessment.

All myxosporeans are most likely to have a two host lifecycle (fish is the intermediate host and an invertebrate -mostly Oligochaetes- is the final host based on where sexual reproduction occurs). This makes risk assessment difficult since only the fish host species was considered (e.g. for the hazard scores). For example, potential fish hosts for the North-American Myxosporean *Ceratomyxa shasta* are present within the EU, but if the other needed host(s) in the life cycle is not present, the risk for establishment will be very low (however, a host switch for this parasite could occur) (Tor Atle Mo, pers comm.).

Shellfish hazards

When the PANDA exercise started in 2004 the PANDA-listed shellfish bacterial hazards, *Candidatus* Xenohaliotis californiensis and *Nocardia crassostrea*, were regarded as exotic to Europe. However, more recently they have been detected (L. Miossec, pers comm.), which indicates the point in time nature of data gathering and the value of having dynamic accurate information. Nevertheless, even before they were confirmed in Europe they were considered as a sufficient risk to be listed, even though the potential pathways of introduction were unclear, particularly for *Nocardia Crassostrea*, although trade in host species for *Candidatus* Xenohaliotis californiensis is reasonably certain to exist. The most important risk factors though were related to establishment (ease of spread) and consequences of introduction, which were thought to be important, although there was a high degree of uncertainty attached to their assessment. Naturally, being marine organisms it was not thought likely that these hazards could be eradicated from the EU and there is no active surveillance system for the pathogens.

Perkinsus marinus is still considered to be exotic and it is thought that *Crassostrea gigas* is probably more resistant than *C. virginica* (the natural host) following the results of experimental infection by this parasite (Bower, 2006b). In addition, in the 1960s and 1970s, large quantities of *C. gigas* were introduced into Europe from Japan and from Canada without apparently introducing the disease. Those introduced into France were checked first for diseases and none were recorded (L. Miossec, pers. comm.). Nevertheless, there is evidence for impact on wild and aquacultured shellfish, since this endoparasite is one of the primary risk factors that adversely affects the abundance and productivity of *C. virginica*. Mortalities of up to 95% have occurred in *C. virginica* during the second summer following transfer to disease enzootic areas (Dipnet, 2007). *P. olseni/atlanticus*, on the other hand, is already present in European coastal waters and its transfer should be possible between wild and farmed animals, although this has not been formally determined even though there are reports of extensive mortalities and even population declines (Dipnet, 2007). Similarly to the bacterial hazards, the potential pathways of introduction were unclear, particularly for *P. marinus*, although trade in *P. olensi/atlanticus* host species may exist, and there was a high degree of uncertainty associated with this information. Despite this, important potential risk factors were establishment and consequences of introduction but again there was great uncertainty related to the potential for natural spread of *P. marinus*, and even no data with which to base an opinion on for areas such as the frequency of successfully establishing in new areas outside the original prevalence range and the extent of the region in the EU likely to suffer damage from the pathogen. Nevertheless, it was agreed that the potential economic loss related to introduction, or further spread in the case of *P. olseni/atlanticus*, would be very important, as would the additional costs arising from control and certification schemes. As for the bacterial hazards, eradication was considered unlikely and this is supported by the evidence from the USA where it has proven impossible (L. Miossec, pers. comm.).

Marteiloides chungmuensis has shown evidence for impact on wild and aquacultured shellfish (Dipnet, 2007) but there is little data available on whether pathways of introduction into Europe actually exist or whether the parasite has actually been known to be spread by trade practices and, as a result, there was a high degree of uncertainty attached to these assessments. Greater uncertainty (e.g. no available data) was shown for an estimate of how long the pathogen would live in the environment without its host species, as well as how quickly it could spread by both natural means and human assistance. Nevertheless, the potential economic losses in conjunction with additional costs related to its presence were considered important, and the region of the EU likely to suffer damage from the pathogen was thought to be extensive. Interestingly, similarly to the other shellfish hazards, it was considered likely

that existing control or husbandry measures (in cultured/farmed populations) could prevent establishment of the pathogen although eradication would not be likely.

Bonamia exitiosa, although listed by EU Directive 88/2006, was not considered sufficiently important as a hazard during the PANDA exercise, due to the fact that the potential pathways of introduction (e.g. from known positive countries) and subsequent establishment in the EU were considered to be a very low risk. Additionally, the apparent host species *Ostrea chilensis*, the dredge oyster, (Cranfield *et al*., 2005) is not present in the EU. However, the disease hazard was scored to provide useful baseline information, since it is thought to be similar to *B. ostreae* and it has been reported in other *Ostrea* spp. (Bower, 2006a).

Oyster velar iridovirus disease (OVVD) was actually the highest rated hazard but it also had a high uncertainty attached to the estimate. Although there is a possibility that Iridoviruses could be more widespread in all temperate waters of the world where the *C. gigas* oyster is found, reports of the occurrence of this disease in the Pacific North West have not been confirmed now for many years (Bower, 2001). This seems to indicate that it is not as prevalent a disease hazard as originally thought and was possibly confined to a small specific area. For this reason, it was not listed in the PANDA exercise, since it is not clear whether it remains a problem in the original distribution area.

Crustacean hazards

The levels of risk posed by most individual pathogens of freshwater crayfish are difficult, if not impossible, to accurately assess with the currently available data (Edgerton, 2002). The single bacterial hazard on the WP 2 PANDA list, *Coxiella cheraxi*, was a good example and, in fact, it could be argued that the possible consequences following establishment in the EU would not be very high. However, there was no consensus on this issue during hazard scoring. Nevertheless, the potential pathway of entry for this hazard is linked to the fact that *Cherax quadricarinatus*, or redclaw, is the only live non-European crayfish permitted importation to the UK, and only for ornamental purposes. There is also interest in farming the species in southern Europe, particularly Italy, Spain, Cyprus and possibly others (B. Edgerton, pers. com.). Consequently, direct live importation in conjunction with the difficulty to contain, at least farmed, crayfish were considered important factors when the hazard was evaluated and, furthermore, there is no data on the presence of rickettsiae in European freshwater crayfish. In addition, several acute cases resulting in losses in redclaw aquaculture in Australia and Ecuador have already been documented. The lack of data on *C. quadricarinatus* was reflected in a high uncertainty score, which is often equated with high risk as a result of the absence of information. The specific areas with a paucity of information were related to spread of the pathogen by trade practices, the presence of susceptible crustacean species in Europe and their density, pathogen survival parameters outside the host, the ability to spread by natural means or by human assistance, successful establishment outside the original range, the extent of a European region likely to be affected, additional costs resulting from introduction, the possibility that existing control or husbandry measures could prevent establishment of the pathogen and the likelihood of eradication. Faced with this uncertainty, in conjunction with a high risk score, related partly to the potential pathways of introduction, the possibility of economic loss for any potential aquaculture venture and the lack of an active surveillance system, it was decided to retain the hazard on the list as a precautionary measure. Nevertheless, it was also recognised that the status of this hazard should be reviewed further, since such a cautious approach was adopted in consideration of its potential for aquaculture in Europe.

The penaeid crustaceans were also difficult to assess from a European point of view, since it is arguable whether the host species for disease hazards such as IHHNV, Taura, White spot and Yellowhead actually exist. Nevertheless, there is anecdotal evidence that experimental farming has taken or is taking place in, at least, southern Europe and that some establishments have suffered disease outbreaks, and even complete closures as a result. This, coupled with the fact that EU Directive 2006/88/EC (Anon, 2007) on aquatic animal health, due to be implemented in 2008, lists white spot as non-exotic, and Taura and Yellowhead as exotic indicates the potential seriousness of these diseases. Although IHHNV was ranked much lower than these other diseases in the WP 2 exercise, and a case could be made for not including it on the list, it was difficult not to consider them all as a group, particularly bearing in mind the serious historical problems caused in Asia and Central America in penaeid shrimp culture. For instance, frozen commodity shrimp have been implicated as the route by which WSSV was moved from Asia to the Americas, while TSV was moved in the opposite direction with infected live broodstock from Central America (Lightner, 2004). There are wild native penaeid shrimps in coastal European waters that may be susceptible to this group of hazards but there is a lack of data to support this. Consequently, faced with such uncertainty and considering the history of penaeid diseases it was decided to include all these viral hazards on the list, particularly since at least three of the four had a high risk score anyway. This is especially important for epidemiological and diagnostic purposes because, since the European experience related to host susceptibility of native species is negligible, that data on potential transmission pathways, consequences of introduction and establishment is extremely limited. However, given the wide host range for these hazards in other parts of the world and the ease of their spread or transmission through uncontrolled movements, it is highly likely that European species will be susceptible to disease, particularly if they are regarded as naïve species, although there are reported differences between some species (Lightner, 2004). It has also been documented that WSSV can be passaged to wild shrimps, crabs, crayfish and lobsters from a wide range of environments and the natural presence of WSSV has been demonstrated in wild stocks in non-European coastal regions (Dipnet, 2007). Consequently, the establishment of any shrimp production facilities in, particularly southern, Europe should start with the stocking of high quality, disease free post-larvae, since this is considered to be a key element in avoiding possible epidemics (Withyachumnarnkul, 1999).

The assessment for IHHNV indicated that the pathways of introduction were a lower risk than for the other penaeid viruses considered, which was related to movements of the host species from known positive countries and the fact that this particular hazard has probably not been spread by international trade in the products of the susceptible species. In addition, the climatic conditions that would affect pathogen establishment in conjunction with the low density (probably rare) of host species were favourable factors leading to a lower risk estimate in this particular case. Nevertheless, there was a good deal of uncertainty attached to other factors, such as the length of time the pathogen could live in the environment without a host and the effect of the reproductive strategy of the pathogen, as well as the duration of its life cycle, when they were considered in the context of whether these factors could aid establishment. Potential spread was also considered to be slow to moderate, both by human assistance and naturally, although this was contrasted with the fact that the pathogen has often successfully established new areas outside its original range. There was much more certainty about the consequences of IHHNV introduction but interestingly this was related to the fact that potential economic loss was not considered to be particularly important, the region affected would be limited, its presence would be unlikely to affect export markets, additional costs related to introduction would not be very important and possible control/eradication measures wre unlikely to disrupt existing biological systems. On the other hand, existing

control or husbandry measures (in cultured/farmed populations) were unlikely to prevent establishment of the pathogen and eradication would not be possible.

Amphibian hazards

Declines of amphibian populations in their natural habitats, with no identifiable direct causes, have become a subject of increasing concern for the scientific community in recent years (Bosch *et al*., 2001). Explaining the reasons for this situation is not possible without additional data. The first hypothesis is a recent introduction of a pathogen to an area, which could have happened through an uncontrolled introduction of infected non-native species (Bosch *et al*., 2001). Investigations in Australia, the United Kingdom, and North and Central America have repeatedly found two diseases, classed as emerging, as the causes of amphibian mass deaths globally, chytridiomycosis and *Ranavirus* iridoviral infections (Daszak *et al*., 1999). In the case of chytridiomycosis, only one factor has been implicated so far in forcing emergence, that is, the anthropogenic introduction of this disease to new regions and host species (Daszak *et al*., 2003). The pattern of amphibian deaths and population declines associated with chytridiomycosis is characteristic of an introduced virulent pathogen dispersing through a naïve population, and population declines outside Europe have been reported to be catastrophic, which is further evidence for such an introduction (Daszak *et al*., 1999).

The epizootiology of ranaviral disease in amphibians is poorly understood and the genus *Ranavirus* also contains pathogens of fish and reptiles. Although ranaviruses are associated currently with amphibian mortalities, but not declines, the potential for ranaviruses to impact amphibian populations significantly cannot be dismissed (Daszak *et al*., 2003).

The available information leading to an assessment of these hazards for the PANDA project recognised the risk associated with the pathways of introduction which would support their continued potential for spread. The trade in live host species and their products from known positive countries were considered to be continuingly important contributory factors, despite the fact that both pathogens can be regarded as non-exotic in Europe. In fact, there was a high level of certainty attached to the possibility of further spread and establishment of *Ranavirus* and *Batrachochytrium dendrobatidis*, particularly for the potential number of host species at risk, their widespread extension and the ability of the pathogens to survive in the environment without a host. The ability of both hazards to cause environmental harm as a consequence of further introduction was also rated highly. This was associated with a possible reduction of native species and the effects on designated environmentally sensitive areas, which would lead to changes in ecological processes and structures, such as biodiversity. Other factors considered important or very important were the potential economic loss to cultivated amphibians caused by the pathogens, the continued extension of affected areas, and other costs related to further introduction, such as those destined for control, the need for additional research, advice and publicity. Also, interestingly, it was thought that the continued presence and/or spread of the hazards would be likely to affect the potential export markets for the aquaculture sector. Naturally, it was not thought possible to eradicate the hazards from the EU and neither, in the case of *Batrachochytrium dendrobatidis*, would the existing control or husbandry measures (in cultured/farmed populations) prevent further establishment of the pathogen. The lack of an active surveillance system for the hazards within the EU was also an important risk mitigation factor linked to the provision of data that could improve the understanding of their spread and control.

General comment

A greater understanding of potential disease hazards and their relevance to Europe within the framework of aquatic animal health will benefit the decision making process. As a result, the

listing of fish, shellfish and crustacean species relevant to the EU (including new species with potential for aquaculture) in conjunction with their pathogens of concern will become a valuable tool for scientists and managers. However, there were many uncertainties and data gaps which affected the assessment of the listed hazards, and wide-ranging European expertise related particularly to exotic diseases was lacking, although this was also a concern for some emerging hazards, such as the amphibian pathogens. The most important area revealed by the assessment as requiring priority attention was the lack of information concerning pathogen survival parameters, such as the length of time a pathogen can live in the environment without its host and survival in traded product or commodity.

In addition, the work package has shown that, currently, there is not enough expertise for actually conducting risk analyses and this makes their interpretation difficult within the context of providing scientific information in support of aquatic animal health programmes. As a result, there is a broad need to provide basic training for understanding the risk analysis (RA) concepts and the process of risk assessment. Training support, through WP 6, could be related to capacity building and promotion of workshops for RA issues, especially in newer Member States and/or non-EU countries that export to Europe. Additional potential themes include optimal strategies for aquatic animal disease RAs, the likelihood and consequences of exotic disease entry, the assimilation of current opinion and the identification of knowledge gaps, the latter of which will benefit to a certain extent from the uncertainty score built into the hazard scoring exercise. This type of support initiative would help to make risk analysis interpretation more consistent in, for instance, an organization, by dealing more fully with the limits of current knowledge. This would also include the relationship of epidemiology to risk assessment and an introduction to the principles, terminologies, tools and techniques used to provide an awareness of the current hazards and disease situation.

Conclusions

- A full picture of the most significant exotic, emerging and re-emerging disease hazards for aquatic animal health in the EU has been obtained and has proved valuable for assessing their potential impact.
- Information on presence or absence of the considered disease agents in the EU and their regulatory status, as well as pathways of introduction, establishment, consequences and risk mitigation has been gathered, and forms the basis for improved understanding.
- Data generated by the work package has been assimilated by the epidemiology database (WP 3), which provides a useful tool for scientists and managers.
- The hazard listing has been the basis for the completion of other work packages related to epidemiology (WP 3), diagnostic methods (WP 4) and alternative treatments (WP 5).
- The work package has had a major input into a consideration of the prevention, vigilance and contingency plans of the identified diseases, as well as the availability of adequate diagnostic methods.
- For purposes of epidemiology and diagnostic methods, more evidence is required for certain hazards to support their status of "emerging" (e.g. *Perkinsus olseni/atlanticus* in molluscs and the amphibian Iridoviridae).
- The ranking of the exotic, emerging and re-emerging disease hazards to the EU should be a post-project ongoing flexible process to reflect the emergence of additional hazards.
- The listed hazards and their ranking should be submitted to risk analysis in order to be refined, particularly with regard to pathways of introduction (e.g. trade movements).
- Feedback information, possibly through a questionnaire format, on how the hazard list is perceived should be obtained by consulting with the NRLs, aquatic animal farmers, PANDA experts and researchers.
- The level of risk associated with each disease should be regionalised within Europe through comparative risk ranking.
- More information is needed on the current situation regarding shrimp farming in southern Europe and the present crustacean disease status.
- A flexible platform of experts for risk analysis associated with aquatic animal health should become a permanent feature in order to support policy decision making.

Training needs

- There is a lack of training for the basic concepts of risk analysis applied to aquatic animal health.
- Training could be related to capacity building and promotion of workshops for RA issues.
- Optimal strategies for aquatic animal disease risk analyses.
- The interrelationship of epidemiology and risk assessment, as well as an introduction to the principles, terminologies, tools and techniques used.

Likely Outcomes

- A greater understanding of potential disease hazards and their relevance to Europe within the framework of aquatic animal health will benefit the decision making process.
- Further data manipulation will allow the generation of reports concerning individual species and their reported diseases by country, as well as individual country disease profiles. This would be additional complementary information to that available in the current OIE International Database on Aquatic Animal Diseases.
- Data input to the epidemiology database (WP 3) will form the basis of the eJournal Aquatic Disease Risk Review.
- The listing of fish, shellfish and crustacean species relevant to the EU (including new species with potential for aquaculture) and their pathogens of concern will become a valuable tool for scientists and managers.
- The identification of data gaps will lead to the need for a listing of the type of product imported into the EU, or moved within the EU. Product should be considered as live product or non-viable processed product (eviscerated, whole or consumer ready) and more accurate data (e.g. pathogen survival parameters) will indicate whether such product could act as a disease vector.
- The information from the work package will lead to more detailed assessments of each individual disease hazard in relation to their relevance for Europe.
- The establishment of a platform of experts for risk analysis associated with aquatic animal health will become an essential part of future considerations.
- Optimal strategies related to the necessities for conducting risk analyses will benefit from the information generated by the work package.
- An awareness of the hazards and the disease situation for candidate EU members, or third country trading partners, will provide more accurate information for future risk analyses.
- Identified training needs will improve the current situation and lead to greater consistency in response times, as well as leading to more even interpretation across Member States.

Recommendations for future research

- Pathogen survival parameters in:

  a) traded product

  b) the environment, outside the host

  c) vector species

- Exact host ranges for the identified disease hazards (natural, experimental and carrier/vector)
- Validated, diagnostic tests for listed diseases

Acknowledgements - Network platform members[8]

Fish disease hazards		
Expert	**Country**	**Contribution**
P. Álvarez Pelletero	Spain	Parasitic hazards
K. Amos	USA	Viral hazards
E. Ariel[9]	Denmark	Viral hazards
B. Austin	UK	Bacterial hazards
J. Barja	Spain	Viral hazards
O. Bendik Dale	Norway	Viral hazards
S. Bergmann	Germany	Viral hazards
K. Buchmann	Denmark	Parasitic hazards
J. Castric	France	Viral hazards
K. Davenport	UK	Viral hazards
P. Dixon	UK	Viral hazards
J. Finlay	UK	Viral hazards
O. Haenen	Netherlands	Viral hazards
B. Hill	UK	Viral hazards
T. Hastings	UK	Bacterial and viral hazards
E. Hudson	UK	Viral hazards
S. LaPatra	USA	Viral hazards
K. MacKenzie	UK	Parasitic hazards
T.A. Mo	Norway	Parasitic hazards
N. Olesen	Denmark	Viral hazards
F. Padros	Spain	Bacterial hazards
T. Taksdal	Norway	Viral hazards
H. Tapiovaara	Finland	Viral hazards
K. Way[10]	UK	Viral hazards

Mollusc disease hazards		
Expert	**Country**	**Contribution**
L. Miossec[11]	France	All hazards identified

Crustacean disease hazards		
Expert	**Country**	**Contribution**
J.-R. Bonami	France	All hazards identified
B. Edgerton	Australia	All hazards identified
A. Roque	Spain	Viral hazards

Amphibian disease hazards		
Expert	**Country**	**Contribution**
A. Hyatt	Australia	All hazards identified

[8] Additional input was provided by members of the Task Force and included G. Bovo (fish viral hazards), E. Peeler (fish parasitic hazards) and C. Rodgers (fish bacterial, fish fungal, fish parasitic, crustacean and amphibian disease hazards).
[9] Consensus hazard group leader for European Community Reference Laboratory for Fish, Århus.
[10] Consensus hazard group leader for CEFAS, UK.
[11] Consensus hazard group leader for European Community Reference Laboratory for Molluscs, La Tremblade.

Useful terminology

(modified or taken from OIE, 2007)

Acceptable risk: Risk level judged by an importing country (or Community region) to be compatible with the protection of public health, aquatic animal health and terrestrial animal health within the country (or region).

Aquatic animal products: Products from aquatic animals (fish, molluscs and crustaceans) whether they are intended for farming (e.g., eggs, gametes, larvae, etc.), for human consumption, for use in animal feeding or for pharmaceutical, biological, or industrial uses.

Aquatic animals: Live fish (including eggs and gametes), molluscs and crustaceans from aquaculture establishments or aquatic animals removed from the wild, for farming purposes or for release into the aquatic environment.

Commodity: Aquatic animals, aquatic animal products, aquatic animal genetic material, feedstuffs, biological products and pathological material.

Competent Authority: The National Veterinary Services, or other Authority of a country, having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health measures recommended in the OIE Aquatic Code.

Consequence assessment: The process of identifying the potential biological, environmental and economic consequences.

Diseases listed by the OIE: Diseases that fulfil the criteria outlined in Chapter 1.1.2 of the OIE Aquatic Code.

Emerging (disease): A disease that has already appeared but is increasing in incidence and becoming more geographically widespread (i.e. reported in new areas or populations). This could be due to a new organism and increased recognition or changes related to husbandry practices or environmental conditions.

Exotic (disease) – entire EU: A disease that is currently absent or unknown but could be introduced from another (third) country.

Exotic (disease) – EU regions: A disease that is currently absent or unknown outside a limited distribution zone within the EU but could be introduced or transferred to another, currently uninfected, area. This may be the case for a disease which is confined to one particular region because of containment (i.e. movement) restrictions, where stamping out procedures are not possible, but that has potential for further spread if controls are removed or circumvented.

Exporting country: A country (or Community region) from which aquatic animals or aquatic animal products, biological products or pathological material are sent to a destination in another country (or Community region).

Exposure assessment: The process of describing the biological pathway(s) necessary for exposure of humans and aquatic and terrestrial animals in the importing country (or Community region) to the hazards and estimating the likelihood of the exposure(s) occurring, and of the spread or establishment of the hazard.

Hazard: Any pathogen that could produce adverse consequences on the importation of a commodity.

Hazard identification: The process of identifying the pathogenic agents that could potentially be introduced in the commodity considered for importation.

Importing country: A country (or Community region) that is the final destination to which aquatic animals, aquatic animal products, biological products or pathological material are sent.

Qualitative risk assessment: An assessment where the conclusions on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as high, medium, low or negligible.

Quantitative risk assessment: An assessment where the outputs of the risk assessment are expressed numerically, as probabilities or distributions of probabilities.

Re-emerging (disease): A disease that is present or has declined in incidence but has begun to reassert itself or reappear possibly with a more widespread distribution. This could be due to the genetic variation of an existing pathogen (e.g. drug resistant strains) or changes related to husbandry practices or environmental conditions.

Release assessment: The process of describing the biological pathway(s) necessary for an importation activity to 'release' (that is, introduce) a hazard into a particular environment, and estimating the likelihood of that complete process occurring.

Risk: The probability of an adverse event of aquatic animal health, public health or economic importance, such as a disease outbreak, and the magnitude of that event.

Risk analysis (also termed Import risk analysis): The complete process composed of hazard identification, risk assessment, risk management and risk communication.

Risk assessment: The processes of identifying and estimating the risks associated with the importation of a commodity and evaluating the consequences of taking those risks.

Risk communication: The processes of communicating the risk assessment results to the regulators of the import programmes, and to other interested parties, such as industry and the public.

Risk estimation: The process of integrating the results of the release assessment, exposure assessment, and consequence assessment to produce an overall measure of risks associated with the hazards identified at the outset.

Risk evaluation: The process of comparing the risk estimated in the risk assessment with the importing country's appropriate level of protection.

Risk management: The identification, documentation and implementation of the measures that can be applied to reduce risks and their consequences.

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