

Import risk analysis:
Frozen, skinless and
boneless fillet meat of
Pangasius spp. fish from
Vietnam for human
consumption

March 2008

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Import risk analysis: Frozen, skinless and boneless fillet meat of Pangasius spp. fish from Vietnam for human consumption

March 2008

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1. Executive Summary

This risk analysis examined the biosecurity risks associated with the importation into New Zealand of frozen, skinless and boneless fillet meat of pangasid catfish (*Pangasius* spp.) from Vietnam.

General sanitary measures were considered necessary:

a) to ensure that the likelihood of clinically or subclinically diseased fish being harvested for processing is minimised:

- both the farm of origin and the processing facility must be registered with the competent authority of the country in question; and
- fish processed must be derived from broodstock resident in the exporting country; and
- fish showing clinical signs of disease, septicaemia or skin ulceration must not be harvested for processing into this commodity; and
- fish harvested must not be subject to emergency slaughter for disease reasons, regardless of whether or not they display clinical signs themselves.

b) to avoid contamination of the commodity with exotic foodborne pathogens:

- only potable water should be used during the processing of the fish into fillet meat

c) to ensure compliance with freezing and transport regime included in the commodity definition:

- to ensure that the inactivation of pathogenic and parasitic organisms, caused by the freezing process, does occur it must be determined that the commodity was frozen and held at -18°C, or lower, for at least 7 days (168 hours) before a biosecurity clearance is issued.

An initial list of organisms of potential concern was developed from published literature, scientific texts, the OIE (World Organisation for Animal Health) list of notifiable fish diseases and official disease reporting statistics. This list was critically examined using a number of criteria including the status of the organism in New Zealand and the exporting region, the presence of more virulent strains in the region of origin, restricted geographical range of organisms in New Zealand if applicable, different host associations in different areas and the official control status in New Zealand.

Eight potential hazards were identified from the list of organisms of potential concern and subjected to further risk assessment. These were iridoviruses, atypical *A. salmonicida*, *Flavobacterium* spp., *Edwardsiella ictaluri*, *Kabatana arthuri*, digenean metacercaria, larval nematodes, and *Aphanomyces invadans*. Waterborne contaminants were also considered as a ninth hazard.

None of the eight primary potential hazards were identified as requiring specific risk management measures. The separation of the fillets from the rest of the carcass effectively removes the majority of organisms that might be present in the live animal. Titres of pathogenic organisms in muscle are usually many times lower than those found in the viscera. Quantities of waste in New Zealand are likely to be small and it was apparent that the likelihood of product entering the aquatic environment in sufficient quantities to represent an infectious dose is so low as to be negligible. In addition, the period of time frozen effectively

reduces any parasitic burdens to levels where the likelihood of entry to New Zealand is negligible. To mitigate any residual risk to human health, water quality standards were specified to prevent entry of foodborne hazards.

2. INTRODUCTION

2.1 BACKGROUND

There has been a request for the development of an Import Health Standard (IHS) to permit the entry of frozen skinless, boneless fish fillets (or mince derived from fillets) into New Zealand for further processing prior to sale. The imports are said to be required to meet the gap in supply of white fish portions brought about by local quota cuts. The proponent has requested the entry of frozen fillet meat (whole or minced) derived from farmed catfish (also known commonly as “basa”, “tra” and “sutchi” catfish) from Vietnam. Catfish (*Pangasius* spp.) are teleost Siluriforme fish and members of the Pangasiidae family. A risk analysis is required to identify actual hazards and recommend risk management measures for incorporation into an IHS for this commodity.

The New Zealand Food Safety Authority (NZFSA) has made a preliminary evaluation of the food safety risks associated with the importation of skinless, boneless fillet meat of basa from Vietnam. While it has no specific food safety concerns associated with import of these products, it does have general concerns about hazards that may be present in the commodity, particularly chemical hazards such as antimicrobial drugs, residues of agricultural compounds, and heavy metals. While there are currently no specific food safety standards or import requirements that would apply to basa from Vietnam, if imported they would need to meet the requirements of all relevant food legislation, including the Food Act 1981 and the Australia New Zealand Food Standards Code. In future, additional requirements may apply to these products as NZFSA is in the process of implementing the outcome of a major review of its imported food programme. Implementation will occur over the next few years and will involve grouping imported foods into one of three categories of regulatory interest with different requirements and clearance options applying to each category. Foods may also be put on a “scanning list” and subjected to additional monitoring (including sampling and testing) should this be warranted. Further information on NZFSA's import requirements and the new imported food programme is available on NZFSA's website at <http://www.nzfsa.govt.nz/imported-food/index.htm>.

2.2 COMMODITY DEFINITION

The commodity considered in this risk analysis is frozen, skinless, boneless fish fillets (or mince derived from fillets) from non-CITES listed *Pangasius* spp. farmed in Vietnam. Fish are harvested from the farm, bled, scaled, eviscerated, filleted, skinned, trimmed, washed and graded. Potable water is used in the manufacturing process. Product may contain sodium tripolyphosphate as additive. Fillets and fillet mince are then packaged/wrapped and plate or tunnel frozen to a core temperature of -18°C or colder and then stored and transported at those temperatures. Deliveries are expected to be made by sea with a time of at least 3 weeks at -18°C between completion of the order assembly and arrival at the New Zealand border.

2.3 RISK MANAGEMENT OBJECTIVE AND DEFINITIONS

The risk management objective is *to effectively manage any risk from importing the defined commodity by ensuring that there is a negligible likelihood of pests or pathogens in, or on, the commodity being exposed to and establishing in native, resident aquatic animals or the environment resulting in adverse consequences; or causing disease in humans.*

For the purposes of this risk analysis, likelihood is defined as *“The quality or fact of being likely or probable; probability”* and negligible is defined as *“Of a thing, quantity, etc.: able to be neglected or disregarded; unworthy of notice or regard; specifically so small or*

insignificant as not to be worth considering". These definitions are derived from the Oxford English Dictionary.

In the context of these definitions, when assessing the likelihood of exposure and establishment, it is the local population level that is being considered. Thus an individual animal, or restricted number of individual animals, may be affected but at levels below that required to establish the infection in the local population. When considering the consequence, it is necessary to estimate both the likelihood of the consequence occurring and the effect of the consequence (Anonymous 2006). Negligible may therefore refer to a likelihood of a consequence occurring being so remote as to be ignored, or to the level of the consequence itself. In this case a negligible consequence would be one where there is no overall adverse effect on the local population, even though there could be an adverse consequence for one individual, or a small number of individuals, when the disease is unlikely to spread or when treatment or control would be highly effective.

2.4 RISK ANALYSIS METHODOLOGY

In developing Import Health Standards, MAF is required under Section 22 (5) of the Biosecurity Act 1993 (BSA) to consider the likelihood that the imported commodities may harbour organisms and the effect that these organisms may have on the people, the environment and the economy of New Zealand. MAF is also obliged to have regard to New Zealand's international obligations, foremost among which is Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures of the World Trade Organization (WTO). A key requirement under the SPS agreement is that members cannot impose measures on imported goods that are more restrictive than those placed on domestically-produced goods, which in effect means that measures may be considered only for exotic organisms or for endemic organisms that are under official control in this country.

The likelihood of imported goods harbouring exotic organisms [BSA Section 22 (5) (a)] is the focus of the release assessment, and the possible effects of such organisms [BSA Section 22 (5) (b)] are considered in the exposure and consequence assessments. The exposure assessment considers the likelihood of spread and establishment of organisms introduced in the commodity, and the consequence assessment follows on from the exposure assessment in considering the impacts of such organisms if they were to be introduced, to spread and to become established.

MAF's risk analysis methodology (Anonymous 2006) follows the guidelines in section 1.4 of the Aquatic Animal Health Code of the World Organisation for Animal Health ("the OIE") (OIE 2006a) and consists of hazard identification, risk assessment and risk management.

2.4.1 Hazard Identification

The first risk analysis step is to compile a preliminary list of organisms that are of potential concern and that Pangasiidae are, or may be, susceptible to. To be considered as being of potential concern, an organism must satisfy one or more of the following criteria:

1. it would be expected to cause a distinct pathological effect in a significant proportion of an infected population; and/or
2. it would be expected to cause significant economic harm (e.g. increased mortality, reduced growth rates, decreased product quality, loss of market access, increased management costs; and/or

3. it would be expected to cause significant damage to the environment and/or endemic species (an endemic species is defined as either a native species that occurs in New Zealand waters naturally, or which was introduced, but which is now considered to be acclimatised); and/or
4. it is known to cause a threat to human health.

This preliminary list may be generated from scientific and literature searches, overseas and New Zealand experience of pathway/commodity and organism associations, interception databases, expert consultation, targeted surveillance and information from other countries or regions.

Each organism on this list is then examined in more detail to determine which could be associated with the commodity under consideration, i.e. organisms that may be present in the muscle tissue.

Each of the organisms is further considered against the following criteria to develop a list of potential hazards:

1. whether it is exotic to New Zealand but likely to be present in the exporting country;
2. For organisms that are present in New Zealand and likely to be present also in the exporting country the following are also considered: -
 - a) Whether the organisms are vectors of pathogens or parasites that are not present in New Zealand;
 - b) Whether more virulent strains are known to exist in other countries;
 - c) Whether the organisms differ genetically from those that occur in New Zealand in a way that may present a potential for greater consequences here, either from the organism itself or through interactions with organisms already here;
 - d) The organisms are already in New Zealand, however the nature of the imports would significantly increase the existing hazard, if for instance the commodity were to bring the organism into contact with a susceptible animal or human host that was not normally exposed;
 - e) The organism or disease is present in New Zealand but is restricted to specific areas;
 - f) Whether the organism or disease has host associations different from those currently found in New Zealand;
 - g) Whether it is "under official control", which could be by government departments, by national or regional pest management strategies or by a small-scale program, or
 - h) The organism or disease is listed on the unwanted organism register.

In some instances it may be necessary to group hazards according to a higher classification if there is insufficient information about individual organisms to allow an adequate assessment of risk to be carried out.

If no hazards are identified, the risk analysis can be concluded; otherwise the risk assessment is carried out.

2.4.2 Risk assessment

For each organism considered to be a potential hazard in the commodity, a risk assessment is carried out. Under the OIE methodology, risk assessment is comprised of the following sub-steps:

- a) **Assessment of likelihood of entry** - the likelihood of the organism being imported in the commodity.
- b) **Assessment of likelihood of exposure and establishment** - the likelihood of the potential hazard, having entered a risk analysis area, becoming established in it and/or having the potential to cause an adverse consequence.
- c) **Assessment of consequences** - the consequences associated with entry, exposure and establishment of the organism, and the nature and possible effect of the organism on people, the New Zealand environment and the New Zealand economy
- d) **Risk estimation** - a conclusion on the risk posed by the organism based on the entry, exposure and establishment, and consequence assessments. If the risk estimate is non-negligible, then the organism is classified as a hazard.

Not all of the above steps may be necessary in every risk assessment. The OIE methodology makes it clear that if the likelihood of entry is negligible for a certain potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out.

The same situation arises where the likelihood of entry is non-negligible but the exposure and establishment assessment concludes that the probability of establishment in the importing country is negligible, or where both release and exposure are non-negligible but the consequences of introduction are concluded to be negligible.

2.4.2.1 Entry assessment

Entry assessment consists of describing the biological pathways necessary for an importation activity to introduce a hazard into a particular environment, and estimating the likelihood of that complete process occurring.

The likelihood of a disease agent entering depends on:

- the likelihood of the disease agent being present in the source country/region, and if present, its prevalence,
- the likelihood of the disease agent being present in an infective form in the commodity entering New Zealand,
- the likelihood of the disease agent being detected in quarantine (if any).

The entry assessment may require information on:

Biological factors such as: -

- the species, strain or genotype and age of any whole animal,
- the strain of the agent,
- epidemiology of the agent,

- tissue sites of infection or contamination,

Country factors such as: -

- prevalence of infection,
- the certifying authority, surveillance and control programs of the exporting country.

Commodity and pathway factors: -

- ease of contamination
- effect of processes and conditions during production, transport and storage
- vulnerability of life-stages during transport and storage.

The term “entry assessment” used in this risk analysis is equivalent to the release assessment detailed in the OIE risk analysis guidelines (OIE 2006a).

2.4.2.2 Exposure and establishment assessment

Exposure and establishment assessment involves an examination of the likelihood that the disease agent, having entered New Zealand, will be exposed to susceptible species, resulting in a consequence directly, or becoming established in the environment. This depends on the capacity of the disease agent to survive in the environment in an infective form, and the ease of infection of susceptible hosts and subsequent transmission of infection to others within a population.

Factors that may need to be considered include:

Biological factors

- the means of transmission and presence of potential vectors or intermediate hosts;
- routes of infection; and
- properties of the agent (e.g. virulence, pathogenicity, and survival parameters).

Country factors

- aquatic animals (presence of known susceptible and carrier species, and their distribution),
- terrestrial animals (scavengers, birds) that may act synergistically or antagonistically to the establishment and spread of the agent,
- geographical and environmental characteristics (current, temperature ranges, water courses).

Commodity and pathway factors

- the intended and unintended use of the commodity;
- the volume of the commodity to be imported;
- waste disposal; and
- time factors (e.g. seasonality).

Some disease agents may be parasites with complex life-cycles. The more complicated the life cycle, the less likely it is that a parasite may become established, as each stage in the life cycle has a probability attached to it. For example, for a parasite with a 3-host life-cycle, the overall probability of the parasite being transmitted between the definitive hosts is the product of the probability that it will establish in the first intermediate host, the probability that it will

establish in the second intermediate host, and the probability that it will establish in the definitive host.

2.4.2.3 Consequence assessment

Consequence assessment consists of identifying the potential biological, environmental and economic consequences of disease introduction and their likelihood. A causal process must exist by which exposures to a hazard results in adverse health, or environmental, or socio-economic consequences (Anonymous. 2006). Speed of spread may be important when considering risk management. A detailed analysis of estimated consequences is not necessary if there is sufficient evidence or it is widely agreed that the introduction would have unacceptable consequences. However, impact assessment is required if the consequences are in question or to assess the appropriateness and efficacy of the risk management measures.

Examples of consequences are:

Direct consequences:

- aquatic animal infection, disease, production losses and facility closures,
- adverse, and possibly irreversible, consequences to fisheries, the environment and/or human health.

Indirect consequences:

- surveillance and control costs,
- potential trade losses.

Where insufficient data are available on a parasite or disease agent, a precautionary approach is adopted, and evidence from similar disease agents is taken into account.

The key factors in classifying the significance of consequences of disease establishment are:

- The biological effects on aquatic species. The establishment of a new disease agent may have a biological effect and consequential effects on industry and the environment. The biological effect on establishment of disease is normally evaluated in terms of morbidity and mortality data reflecting epidemiological features of the disease.
- The availability, cost and effectiveness of methods for control/eradication.
- The economic effects at an establishment/industry/national level, including effects on commerce and marketing.
- The biological effects on endemic species of aquatic animals, terrestrial and avian fauna, the environment (including any loss of social amenity) and human health.

2.4.2.4 Risk estimation

The final step involved with each assessment is to determine whether the level of risk presented by each disease agent is sufficient to require risk management. This is done by summarising the likelihood of introduction and establishment and the significance of the consequences of an introduction. Any organism for which the risk is summarised as non-negligible is considered to be an actual hazard in the commodity, for which risk management measures are necessary.

2.4.3 Risk management

The risk management process has three main components, namely risk evaluation, option evaluation and recommended measures used to achieve a negligible likelihood of introduction.

- a) Risk evaluation – if the risk estimate, determined in the risk assessment, is non-negligible measures are justified.
- b) Option evaluation - identify the options available for managing the risk, and consider risk reduction effects. The measures recommended by international standard setting bodies should be considered, where available. Measures must be specific in their objective. Where appropriate the likelihood of exposure, establishment and spread may be re-evaluated in the light of the risk management measures.
- c) Recommended measures -the recommendation of the appropriate option or combination of options that achieve a negligible likelihood of entry, spread or establishment, while minimising negative trade effects.

2.4.4 Risk communication

Risk communication is the process by which information and opinions regarding hazards and risks are gathered during a risk analysis, and by which the results of the risk assessment and proposed risk management measures are communicated to the decision makers and interested parties in the importing and exporting countries. It is a multidimensional and iterative process and should continue throughout the risk analysis process.

The principal participants in risk communication include the authorities in the exporting country and other stakeholders such as domestic aquaculturists, recreational and commercial fishermen, conservation and wildlife groups, consumer groups, and domestic and foreign industry groups.

The assumptions and uncertainty in the model, model inputs and the risk estimates of the risk assessment should be communicated.

Peer review of risk analyses is an essential component of risk communication for obtaining a scientific critique aimed at ensuring that the data, information, methods and assumptions are the best available.

3. HAZARD IDENTIFICATION

3.1 ORGANISMS OF POTENTIAL CONCERN

Peer-reviewed journal articles, published reports, published reference texts, health databases and information freely provided by the governments of other countries were examined and a list of pathogenic organisms, reported to be associated with Pangasiidae, or to which Pangasiidae are known or believed to be susceptible, was developed, including a description of the causative agent, host, effects on pangasid fish or other organisms or the environment, reported locations and any relevant epidemiological information (Appendix 1).

In addition, the OIE list of fish diseases (OIE 2006a) was included in the list of pathogens of potential concern as it contains many diseases to which high value New Zealand fish species are susceptible. This list consists of the following pathogens: -

- Epizootic haematopoietic necrosis virus (EHNV)
- Infectious haematopoietic necrosis virus (IHNV)
- Spring viraemia of carp virus (SVCV)
- Viral haemorrhagic septicaemia virus (VHSV)
- Infectious salmon anaemia virus (ISAV)
- Epizootic ulcerative syndrome (EUS) caused by *Aphanomyces invadans*
- *Gyrodactylus salaris*
- Red Sea bream iridovirus (RSIV)
- Koi herpes virus (KHV)

3.2 IDENTIFICATION OF POTENTIAL HAZARDS

Each organism or agent listed was assessed individually against a range of criteria (detailed in Section 2.3.1) to determine if it qualified as a potential hazard (Appendix 2). Every organism listed as being of potential concern was also considered for its zoonotic potential. For those organisms identified as having zoonotic potential, the human risks were considered in this section or in the individual risk assessments, where a more detailed examination was considered necessary.

3.2.1 Viruses

There are no published reports of viral diseases of pangasid catfish in the usual fisheries literature databases. Of the virus diseases listed by the OIE as being of concern in fish none lists pangasid catfish as susceptible species.

Whilst EHNV is regarded as a natural disease of only redbfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (OIE 2006b), it is known that a number of other teleost species are susceptible to it (Langdon 1989). Related viruses have also been identified from black catfish (*Ictalurus melas* now known as *Ameiurus melas*) and sheatfish/European catfish (*Siluris glanis*). However there have been no reports of infection of Pangasiidae over a considerable period of time. Iridoviruses, as a family, will be considered further in this risk analysis, however, because of the current uncertainty regarding the host specificity and occurrence of iridoviruses. The number of susceptible species being identified is increasing, as are the number of countries infected with a range of iridoviruses.

Infectious haematopoietic necrosis (IHN) is solely a disease of salmonids, particularly *Salmo* spp. and *Oncorhynchus* spp. (OIE 2006b).

Spring viraemia of carp virus (SVCV) infects mainly carp, as the name suggests. However, it has been isolated from sheatfish (*Siluris glanis*), orfe (*Leuciscus idus*) and tench (*Tinca tinca*) (OIE 2006b). There have been no reports of its isolation, anywhere, from pangasid catfish. In addition, Vietnam has reported no cases of SVCV infection to the OIE (OIE2006).

In addition to pangasid catfish not being considered susceptible to VHSV, the water temperatures of the Mekong delta in Vietnam exceed 20°C (Isobe *et al.* 2004), the temperature above which viral haemorrhagic septicaemia virus (VHSV) could not classically be isolated (Smail 1999). Recent isolations of VHSV strain IVb in the Great Lakes, USA have demonstrated a widening host range (Grocock *et al.* 2007, Lumsden *et al.* 2007) potentially in warmer water conditions. However, Vietnam has never reported VHSV infection (NACA2007).

Vietnam is a member of the Network of Aquaculture Centres in Asia-Pacific (NACA). As such they have a statutory reporting and investigative programme for aquatic animal diseases considered to be of significance to the region. Both SVCV and VHSV are listed diseases; neither of which have ever been reported by Vietnam.

Infectious salmon anaemia virus (ISAV) only causes natural disease in salmonids. It has been isolated from pollock (*Pollachius virens*) and cod (*Gadus morhua*) in the vicinity of infected salmon farms and from experimentally infected Arctic char (*Salvelinus alpinus*) and herring (*Clupea harengus*) (OIE 2006b). There is no evidence that it would infect Siluriformes and the disease appears restricted to Europe and North America.

Red Sea bream iridovirus (RSIV) infects a range of marine fish only.

Koi herpes virus (KHV) appears restricted to common carp (*Cyprinus carpio*), koi carp (*Cyprinus carpio koi*) and ghost carp (*Cyprinus carpio goi*).

It is, therefore, not necessary to consider EHNV, IHNV, SVCV, VHSV, ISAV, RSIV or KHV further.

3.2.2 Bacteria

Under conditions of stress, pangasid catfish are susceptible to a wide range of facultative pathogenic bacteria. These secondary invaders tend to be ubiquitous in the freshwater environment and thus, where identified, will be excluded from this risk analysis.

Edwardsiella ictaluri has been reported from *Pangasius hypophthalmus* in both Vietnam and Indonesia (Crumlish *et al.* 2002, Yuasa *et al.* 2003). The outbreaks presented as septicaemia accompanied by necrosis and inflammation of the internal organs. Whilst the majority of the bacteria will be located in the internal organs it is apparent that muscle tissue may also carry *E. ictaluri* bacteria and therefore *E. ictaluri* will require further consideration.

Aeromonas spp. are generally ubiquitous opportunist pathogens and *A. hydrophila* and *A. sobria* already occur in New Zealand (Diggles *et al.* 2002). *A. salmonicida*, both typical and atypical strains, are considered exotic to New Zealand. However, neither have been reported from pangasid catfish. Nevertheless, the potential, particularly for atypical strains, to be present in the freshwater environment and infect *Pangasius* spp. warrants further consideration.

Pseudomonas spp., especially the common *P. aeruginosa* and *P. fluorescens*, are found throughout the aquatic environment and are associated with both healthy and diseased fish (Daly 1999). In addition, they have been reported in New Zealand. Whilst pseudomonads can cause disease in humans, the principal species causing opportunist infections in fish are not the same as those causing disease in humans (Buller 2004). The exception to this would be *P. fluorescens*, which does cause disease in both humans and fish, but is commonly found in the environment and tends to contaminate wounds. The isolation of *P. aeruginosa* from fish is rare. Thus, there is negligible likelihood of increased exposure to disease-causing pseudomonads via the importation of fillet meat. For this reason and due to their ubiquitous and opportunist nature these bacteria do not require further consideration in this risk analysis.

There was a report of bacillary necrosis caused by a *Bacillus* sp. in farmed *P. hypophthalmus* in Vietnam (Ferguson *et al.* 2001). This organism was said to have caused a septicemic condition in *Pangasius* sp. resulting in economically significant disease in the Mekong Delta. Since that initial report the condition has been attributed to *Edwardsiella ictaluri* (Crumlish *et al.* 2002) and thus *Bacillus* sp. need not be considered further.

Flavobacterium spp. are generally ubiquitous freshwater organisms causing surface lesions under conditions of stress or poor water quality and could be excluded from the risk analysis for these reasons. However, there have been reports of a more virulent genomovar from Asia that causes muscle lesions in addition to the usual skin and fin lesions (Michel *et al.* 2002). Since *Flavobacterium* spp. have been reported from *Pangasius* spp. it is necessary to consider this organism further.

Micrococcus spp. and *Staphylococcus* spp. are normal flora found on the surface of fish. Staphylococcal contamination of food tends to occur during processing and highlights a requirement to address the quality of the water used in the processing of the commodity, however, given the cosmopolitan distribution of these organisms there is no reason to believe that the importation of the commodity would increase the risk of exposure to the public as the use of potable water during processing and general sanitary measures would mitigate against contamination regardless. This, together with the ubiquitous and opportunistic nature of the organism means that there is no need to consider it further in this risk analysis.

Corynebacterium spp. are reported to cause clinical disease, but are found worldwide in fish and the environment (Buller 2004). Whilst *Corynebacterium* spp. may cause disease in humans, the species responsible (*C. paratuberculosis* (=ovis), *C. equi*, *C. bovis*, *C. pyogenes*, *C. ulcerans*) (Acha and Szyfres 1994) are not the species reported from aquatic animals (Buller 2004). In addition, the majority of human infections occur via wound contamination (Acha and Szyfres 1994), thus the import of processed fillet meat does not represent a significant pathway of risk to humans. This organism will not be considered further.

3.2.3 Parasites

The protozoans, *Balantidium* spp., *Protoopalina* spp. and *Hexamita* spp., are unlikely to be associated with the commodity, being restricted to the digestive tract and internal organs (Basson and Van As 2006). These structures would be removed during evisceration, prior to filleting. They are also very common and likely to be present in New Zealand and are unlikely to produce clinical disease. *H. salmonis* is known to cause disease, but only in salmonids. The protozoans *Ichthyophthirius multifiliis*, *Apiosoma* spp., *Chilodonella* spp., *Cyrtobia* spp.,

Epistylis spp., *Trichodina* spp. and *Tripartiella* spp. are found on the skin and/or gills and would be removed during processing. These organisms do not require further consideration.

The microsporidian, *Kabatana arthuri*, has been reported to infect the musculature of *Pangasius sutchii* and as such could be present in the commodity. It is present in Thailand (South East Asia) and could therefore be present in Vietnam (Lom *et al.* 1990, Lom *et al.* 1999, Dykova and Lom 2000, Dykova 2006). It is considered to be exotic to New Zealand and will therefore require further consideration.

Myxobolus spp. may be present in the connective tissue of muscle (Feist and Longshaw 2006) and therefore could be associated with the commodity. However, they are common in the aquatic environment (Feist and Longshaw 2006) and are reported in New Zealand, including the species pathogenic to salmonids, *M. cerebralis* (Hewitt and Hine 1972). In addition, they tend to be host specific and require specific oligochaete intermediate hosts making it highly unlikely that they could establish in New Zealand. Neither of the *Myxobolus* spp. reported from *Pangasius hypophthalmus* caused significant pathology (Molnar *et al.* 2005). The myxosporeans *Ceratomyxa* spp. and *Zschokkella* spp. reported from *Pangasius* spp. in Vietnam are coelozoic species. They are found in the gall bladder and urinary bladder and would, therefore, be removed during processing. Furthermore, despite the findings of *Myxobolus* spp. in human stools, there is no evidence that these organisms are pathogenic to humans (Boreham *et al.* 1998, Moncada *et al.* 2001). Therefore, *Myxobolus* spp. will not be considered further.

The *Hennegoides* spp. and *Henneguya shariffi* have only been reported from gills (Molnar *et al.* 2005) and would not, therefore, be associated with the commodity and will not be considered further.

The myxosporean, *Sphaerospora* spp., is found in the kidney of *Pangasius* spp. in South East Asia (Dykova and Lom 1997). The removal of the fillets from the carcass means that this organism would not be associated with the commodity and thus does not require further consideration.

The trematode platyhelminths, *Protocladorchis* spp., are found in the intestine (Jones and Seng 1986, Jones 1987). They would thus not be associated with the commodity as the intestines are removed during processing.

Gyrodactylus salaris, an OIE listed parasite, does not affect *Pangasius* spp. (OIE 2006a) and therefore does not require further consideration. The monogeneans, *Silurodiscooides* spp., *Bifurcohaptor* spp., *Pangasitrema* spp., *Thaparocleidus* spp. and other *Gyrodactylus* spp. and *Dactylogyrus* spp., are ectoparasites (Buchmann and Bresciani 2006). An undescribed *Gyrodactylus* sp. has been reported from wild flounder in New Zealand (Diggles *et al.* 2002). As the fish are both filleted and skinned none of these parasites is likely to be associated with the commodity and will not be considered further in this risk analysis.

Whilst the digenean trematodes reported from Vietnam (Arthur and Te 2006, Nguyen *et al.* 2007) were found in the liver and intestines and would not be associated with the commodity, some *Haplorchis* spp. may encyst subcutaneously and may not be removed with the skin. In addition *Haplorchis* spp. utilise the New Zealand native snail, *Melanooides tuberculata*, as an intermediate host (Paperna and Dzikowski 2006), a species that has been introduced here (Duggan 2002). Some digenean species are zoonotic, including *Haplorchis pumilio* (Ko

2006). Encysted digenean metacercaria will therefore be considered further in this risk analysis.

The cestodes, *Lytocestus* spp. and *Proteocephalus* spp. are found in the intestines and would not, therefore, be associated with the commodity. They will not be considered further in this risk analysis.

The nematodes *Cucullanus* spp., *Procamallanus* spp. and *Spectatus* spp. are found in the intestine and would be removed during processing. In addition, both *Cucullanus* spp. and *Procamallanus* spp. have been reported here (Hine *et al.* 2000). They do not require further consideration. *Philometra* spp. could be present in the commodity if the larvae were encysted deeply enough in the subcutaneous tissues to escape removal with the skin. However, *Philometra* spp. are reported here (Hine *et al.* 2000) and so this organism does not require further consideration. *Hysterothylacium fluvatile* is an anisakid nematode reported from Vietnam (Moravec and Sey 1988). Its larvae may be found in the coelomic cavity (Moravec and Sey 1988). They would not normally be associated with the commodity. However, it is possible for nematode larvae to migrate from the coelomic cavity into the musculature if there is a delay between slaughter and evisceration (Smith 1984) although it is generally accepted that such migration is unusual (Karl *et al.* 2002, Lymbery *et al.* 2002). A number of nematode species are zoonotic, but the more serious *Anisakis simplex* and *Pseudoterranova decipiens* have not been found in freshwater fish and do not therefore pose a risk in this commodity. *Hysterothylacium* spp. are not regarded as zoonotic. Nematode species and their larvae will be considered further as some, such as *Hysterothylacium* spp., have reasonably wide host ranges (Molnar *et al.* 2006) and could potentially establish in endemic fish here.

The acanthocephalans are intestinal parasites and would not be associated with the commodity. *Pseudorhadinorhynchus vietnamensis* does not, therefore, require further consideration.

The external copepod parasite *Ergasilus* sp. would also be removed during processing and thus requires no further consideration.

3.2.4 Fungi

Aphanomyces invadans, the causative agent of epizootic ulcerative syndrome (EUS), which is characterised by congested skin lesions and ulceration, affects over 100 freshwater and estuarine fish species (Bondad-Reantaso *et al.* 2001, Diggles *et al.* 2002). Whilst *Pangasius* spp. are not listed specifically, some catfish are included as hosts and there are susceptible species in New Zealand. As a precautionary measure this organism will be considered further.

3.2.5 Waterborne food poisoning

Contamination of fillets with water not of a suitable purity could result in the presence of exotic strains of *Corynebacterium diphtheriae*, *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae* and *Cryptosporidia* spp. These may survive the freezing process and therefore require further consideration.

3.2.6 Summary

An initial list of organisms of potential concern was developed from scientific literature and texts. It consisted of approximately 35 genera of potential pathogens linked with Pangasiidae. Following consideration of a range of factors, including the likelihood of association with the commodity and presence or absence from New Zealand and Vietnam, it was concluded that

eight organisms required further consideration, namely iridoviruses, *Edwardsiella ictaluri*, atypical *Aeromonas* spp., *Flavobacterium* spp., *Kabatana arthuri*, digenean metacercaria, larval nematodes and *Aphanomyces invadans*.

In addition it was considered necessary to consider the water used in processing and freezing in terms of waterborne contamination with potentially harmful organisms.

4. RISK ASSESSMENT

For each organism of concern, the risk assessment begins with an examination of the epidemiology of the organism, with particular emphasis on routes of transmission. The entry assessment then considers the likelihood of the organism entering New Zealand in the commodity, taking into account such factors as the initial prevalence of infection, the effects of handling, transporting and storing the commodity and the environmental susceptibility of the organism.

If the entry assessment concludes there is a non-negligible likelihood of entry, then an exposure and establishment assessment is carried out. There may be consequences associated with exposure alone, or it may be determined that the organism needs to establish to have consequences. If the assessment determines there is a non-negligible likelihood of either of the above then a consequence assessment is carried out. All the above steps are summarised in the risk estimation statement.

Where a consequence is determined to be non-negligible, risk management measures will be suggested and evaluated.

4.1 IRIDOVIRUSES

4.1.1 Aetiological agent: Iridoviruses are non-enveloped double stranded DNA viruses in the family Iridoviridae. Iridoviruses of concern in fish include: -

- Epizootic haematopoietic necrosis virus (EHNV) and the related European catfish virus (ECV) and European sheatfish virus (ESV)
- Red Sea bream iridovirus
- Exotic unclassified strains of iridovirus

4.1.2 OIE List: EHNV and RSIV are listed

4.1.3 New Zealand status: Both EHNV and RSIV are considered exotic (Diggles *et al.* 2002). Exotic pathogenic iridoviruses are listed as “unwanted organisms”. Lymphocystivirus is present here (Stone 2003).

4.1.4 Epidemiology: European catfish virus (ECV) infects *Ameiurus melas* (OIE 2006b), which is related to *A. nebulosus*. The latter has been introduced, and is now established in New Zealand. European sheatfish virus (ESV) infects another Siluriforme, *Siluris glanis*.

There are three major types of piscine iridovirus based on pathology, morphology and antigenicity (Ahne 1994): -

- Lymphocystiviruses – associated with hypertrophy of connective tissue cells. This form affects the skin, fins and internal organs of more than 140 species of teleosts;
- Erythrocytic necrosis viruses – replicate in and cause destruction of red blood cells. These viruses have been described from a number of marine species, but are of most clinical significance in salmonids;
- Systemic iridoviruses – inducing septicaemia, endothelial necrosis (Fijan 1999) and haematopoietic necrosis. This group contains a considerable list of hosts.

Natural outbreaks of EHNV appear restricted to *Perca fluviatilis* and *Oncorhynchus mykiss*, although a number of Australian native fish species have been shown to be experimentally susceptible (Langdon 1989). ECV has been reported from *A. melas* in France and Italy only (Ahne 1994, OIE 2006b) and ESV from *S. glanis* in Germany (OIE 2006b). Other species of fish were cohabiting with the *A. melas* at the times of the ECV outbreaks and remained unaffected (Fijan 1999). Whilst this might suggest a high host specificity, iridoviruses are capable of infecting a broad range of hosts. There has been a recent emergence of many new iridoviruses, some linked to fish kills and declines in amphibian numbers (Goldberg *et al.* 2003).

RSIV is restricted to marine fish (OIE 2006b).

Other exotic systemic iridoviruses are recognised from Asia as well as Australia, Europe and the USA (Ahne *et al.* 1997). These systemic iridoviruses appear to have similar physicochemical properties, are antigenically related and can be of high virulence to a number of teleost species (Ahne *et al.* 1997).

In summary these agents represent a range of closely related viruses infecting a broad range of fish species, with the potential to impact on amphibian populations.

4.1.5 Entry assessment: Despite the wide range of iridoviruses and hosts reported, none has been found in *Pangasius* spp. even though iridoviruses have been detected in Asia (Ahne *et al.* 1997).

Lymphocystiviruses are not expected to be present in the commodity as the processing involves skinning of the fish. Erythrocytic necrosis virus titres would be significantly reduced by bleeding of the fish during harvest. The muscle tissue of septicaemic fish could contain high levels of virus as a result of infection of endothelial tissue, but the highest titres would be in internal organs such as kidney and spleen. It is unlikely, however, that septicaemic fish would be harvested for export processing as their generalised haemorrhagic condition would result in their rejection at inspection. That is, normal commercial quality assurance measures would mitigate septicaemic fish being exported.

Iridoviruses can occur in a carrier state in some fish species. However, in both subclinical and carrier states the virus titre in the muscle is probably undetectable by virus isolation and is expected to be many times lower than in the viscera, given the tropism of the virus for endothelium, haematopoietic tissue and the reticuloendothelial system of the spleen and liver (McGrogan *et al.* 1998, Fijan 1999).

The likelihood of fillet meat from Vietnam containing an iridovirus is thus considered to be low.

Iridoviruses appear to be reasonably stable to freezing (Plumb and Zilberg 1999b), surviving for more than two years in frozen tissue (OIE 2006b) and could therefore be expected to survive freezing and transport to New Zealand.

Iridoviruses thus represent a pathogenic agent, recognised as being of significance, but associated with a great deal of uncertainty. Whilst the likelihood of the commodity containing an iridovirus is low, it is non-negligible and it is necessary to carry out an exposure and establishment assessment.

4.1.6 Exposure and establishment assessment: Exposure of susceptible native fish to an exotic iridovirus would require imported fillets, or scraps derived from them, infected with an iridovirus, entering the aquatic environment in sufficient quantities to produce an infectious dose. As previously discussed, viral titres of muscle would be much lower than titres found in the internal organs, even in diseased fish. Subclinically infected fish would have much lower titres overall.

There are no reports of the spread of iridoviruses via the movement of dead fish for human consumption. The commodity itself is highly processed and it is likely that the volume of scraps generated at end use will be small. The likelihood of exposure is further reduced by the necessity for scraps to enter the environment in sufficient quantities to actually constitute an infectious dose. Largemouth bass virus (LMBV) is closely related to iridoviruses found in ornamental fish from South East Asia and it can infect multiple species (Goldberg *et al.* 2003), making it a useful model virus to consider. Fish infected with LMBV were found to have viral titres in the order of $10^{3.2}$ TCID₅₀/g of spleen to $10^{5.2}$ TCID₅₀/g of kidney. Although muscle was not assayed the virus was undetectable in the blood, and muscle tissue could be taken to have similar or lesser titres. In one study a LMBV titre of 10^3 PFU induced a cytopathic effect in 100% of tissue culture plates inoculated (McClenahan *et al.* 2005), thus it

seems likely that the limit of detection for LMBV by cell culture would be less than 10^2 PFU. In addition, it is known that in systemically infected fish the virus is concentrated in the viscera (Woodland *et al.* 2002). Immersion challenge of the highly susceptible largemouth bass, *Micropterus salmoides*, with LMBV at a dose of $10^{6.5}$ TCID₅₀/mL for 1 hour resulted in a mortality rate of less than 17%, although 40% became infected (Plumb and Zilberg 1999a). Per os dosing of $10^{5.6}$ TCID₅₀ per fish resulted in 21% infection but no clinical disease (Woodland *et al.* 2002). It is apparent, therefore, that muscle tissue with a presumed titre well below 10^2 PFU is unlikely to represent a source of an infectious dose of iridovirus by immersion or per os.

In addition, the OIE regards evisceration of fish as significantly decreasing the likelihood of transmission of a number of serious pathogens (such as VHSV, ISA) and has indicated that additional sanitary measures may not be required (OIE 2006a).

Taking all these factors into consideration, the likelihood that an exotic iridovirus would be exposed to, and establish in, native fish is so remote as to be negligible. There is also no indication that iridoviruses are of any significance to human health. Thus, no further assessment is required and no specific sanitary measures are warranted.

4.2 EDWARDSIELLA ICTALURI

4.2.1 Aetiological agent: *Edwardsiella ictaluri* is a pleomorphic, gram negative, rod shaped member of the Enterobacteriaceae. It was first reported to cause enteric septicaemia of catfish (ESC) in 1979 (Plumb 1999), but is now known to infect a wider host range.

4.2.2 OIE List: Not listed

4.2.3 New Zealand status: Not reported, considered exotic

4.2.4 Epidemiology: *E. ictaluri* has been reported from the following Siluriformes: *Ictalurus punctatus*, *I. furcatus*, *Clarias batrachus* and *Ameiurus catus*, with *Siluris glanis* being slightly susceptible to experimental infection (Plumb 1999). It has also been recently isolated from *Pangasius hypophthalmus* in Vietnam (Crumlish *et al.* 2002). The introduced *Ameiurus nebulosus* is considered susceptible (OIE 2006b) and *Oncorhynchus* spp. can be experimentally infected (Plumb 1999).

Transmission is direct horizontal via the water column, with an incubation period of 5-7 days in *I. punctatus* (Ahne 1994). The bacterium is thought to be shed in the faeces (OIE 2006b) with gills and nares the main uptake sites (Plumb 1999, OIE 2006b). Uptake via the gills tends to produce a systemic infection whereas infection via the nares leads to chronic encephalitis, the so called “hole in the head disease” (Plumb 1999, OIE 2006b).

Outbreaks mainly occur at water temperatures of between 18 and 28°C (OIE 2006b), although there are increasing numbers of outbreaks at temperatures outside this range, indicating the bacterium may be adapting to a broader temperature range (Plumb 1999).

Systemic infection, accompanied by septicaemia, causes petechial haemorrhages on jaw, operculum, ventral abdomen and fin bases. There may be exophthalmia, ascites, skin ulceration and granulomatous lesions in the internal organs, brain and gills (Plumb 1999, Buller 2004). Infection and inflammation extends into the skeletal muscle under skin ulcers and in cases of septicaemia. Mortality rates can reach 50%. Survivors are generally carriers of the bacteria, with bacteria possibly located in the intestines (Plumb 1999), although they have been isolated from blood and internal organs, including the kidney for up to 4 months post infection (Klesius 1992, Mgalomba and Plumb 1992).

E. ictaluri does not tolerate salinities above 15 ppt (Buller 2004, OIE 2006b) so is of concern only in freshwater and brackish environments. The bacteria will survive for up to 30 days in fish tissue frozen to -20 °C (Brady and Vinitnantharat 1990).

4.2.5 Entry assessment: *E. ictaluri* has been isolated from *Pangasius hypophthalmus* in Vietnam, although not from co-habitant *P. bocourti* (Crumlish *et al.* 2002). A previous report of bacillary muscle necrosis reported from Vietnam (Ferguson *et al.* 2001) has now been identified as being attributable to *E. ictaluri* (Crumlish *et al.* 2002). In both instances the disease was characterised by multiple bacterial granulomata notable in the spleen, liver and kidney with some multifocal lesions in the muscles (Ferguson *et al.* 2001). The bacteria isolated were biochemically and genetically identified as *E. ictaluri*, although morphologically the rods were longer than previously reported (Crumlish *et al.* 2002).

Whilst infection of *Pangasius hypophthalmus* does result in clinically apparent disease and septicaemic fish are extremely unlikely to be harvested for human consumption, there remains the possibility that some fish could be carriers of *E. ictaluri* without displaying clinical signs. These fish could be harvested and processed for export.

If storage and transportation to New Zealand of the resultant frozen fillets took less than 30 days then viable *E. ictaluri* could remain in the commodity.

Whilst heading, gilling, eviscerating and skinning greatly reduces any bacterial burden in the commodity there is a low, but non-negligible, likelihood of *E. ictaluri* entering New Zealand in the commodity.

4.2.6 Exposure and establishment assessment: Exposure of susceptible native fish to *E. ictaluri* would require imported fillets, or scraps derived from them, infected with *E. ictaluri*, to enter the aquatic environment in sufficient quantities to produce an infectious dose. Evidence suggests that the concentration of the bacterium in muscle tissue is expected to be significantly lower than that found in the internal organs, particularly in the case of inapparent infections.

There are no reports of the spread of *E. ictaluri* via the movement of dead fish for human consumption. The most likely route of spread is the movement of live, infected carrier fish (Plumb 1999). The commodity itself is highly processed and it is likely that the volume of scraps generated at end use will be small. The likelihood of exposure is further reduced by the necessity for scraps to enter the environment in sufficient quantities to actually constitute an infectious dose.

The LD₅₀, by injection, for *I. punctatus* is 7.1×10^4 CFU, whilst that of *Siluris glanis* is 5.4×10^6 CFU (Plumb and Hilge 1987). *Ictalurus* spp. are recognised as being the most susceptible; *Pangasius* spp. are likely to require higher doses. Doses of 2×10^7 CFU were used in *P. hypophthalmus* to replicate the natural infections (Ferguson *et al.* 2001). Immersion challenge would require an even greater dose to successfully result in infection. We know that channel catfish of 2 to 24 weeks of age can be exposed to 2.7×10^5 CFU/mL in an immersion challenge without developing the disease (Petrie-Hanson and Ainsworth 1999) and that it took 5×10^8 CFU/mL by immersion to successfully and consistently produce serious disease in another challenge trial (Newton *et al.* 1989). Salmonids also appear relatively resistant to infection, requiring doses of 4 to 7.9×10^8 CFU/mL to induce disease (Baxa *et al.* 1990).

Given that harvesting and processing of clinically affected fish is unlikely, the greatest risk is from carrier, or covertly infected, fish. The bacterial titre in their muscle tissue is expected to be low. A filtration/ELISA test was developed in 1996 to assist in the detection of carriers. This assay was sensitive down to less than 10 CFU/g tissue (Earlix *et al.* 1996), indicating just how low expected bacterial titres would be. It is evident that vast quantities of covertly infected fillet tissue would be required to pose any risk to fish through immersion challenge.

In addition, the OIE regards evisceration of fish as significantly decreasing the likelihood of transmission of a number of serious pathogens and has indicated that additional sanitary measures may not be required (OIE 2006a). Large quantities of catfish product is moved annually from southeastern states of the USA, where *E. ictaluri* is enzootic, to other states without any reports of the movement of the disease.

Taking all these factors into consideration, the likelihood that *E. ictaluri* would be exposed to, and establish in, native fish is so remote as to be negligible. *E. ictaluri* is not generally regarded as zoonotic, and although there are rare reports of opportunist infection (Hou *et al.* 1999), huge quantities of catfish meat are consumed in North America where *E. ictaluri* is enzootic without adverse human health effects. Thus, no further assessment is required and no specific sanitary measures are warranted.

4.3 AEROMONAS SALMONICIDA.

4.3.1 Aetiological agent: Atypical *Aeromonas salmonicida* bacteria are gram negative, coccoid to rod shaped members of the Aeromonadaceae family. There are at least 7 different recorded subspecies of *A. salmonicida* regarded as being “atypical” despite having phenotypical characteristics similar to the “typical” strain of *A. salmonicida* that causes classical furunculosis (Hiney and Olivier 1999).

The following represent the current classification of subspecies of atypical *A. salmonicida* (Buller 2004):

- *A. salmonicida* ssp. *achromogenes* – widespread globally, causing skin lesions in cod, silver bream, perch, roach, goldfish (goldfish ulcer disease, GUD) and flounder.
- *A. salmonicida* ssp. *masoucida* – reported from salmonids in Japan.
- *A. salmonicida* ssp. *nova* – reported from goldfish (GUD), eels, carp and marine fish from UK, Japan, USA and Australia.
- *A. salmonicida* ssp. *smithia* – caused superficial skin lesions in UK, an presumptive identification in China (Wang and Huang 2006).
- *A. salmonicida* ssp. “Atypical strains” – wide range of fish species and global distribution
- *A. salmonicida* ssp. “Atypical strains; oxidase negative” – Isolated from skin ulcers of turbot and flounder in the Baltic, Denmark and USA.
- *A. salmonicida* ssp. “Atypical strains; growth at 37°C” – isolated from skin ulcers in UK.

4.3.2 OIE List: Not listed

4.3.3 New Zealand status: Not reported, considered exotic. A study of 624 farmed fish and 253 wild fish failed to isolate any *A. salmonicida* (Anderson *et al.* 1994). Repeated surveys have similarly not detected the bacteria here (Anonymous 2000, Anonymous 2001, Duignan *et al.* 2003) and thus all strains of *A. salmonicida* are considered exotic.

4.3.4 Epidemiology: The most common clinical sign of infection with atypical *A. salmonicida* is skin ulceration, although this can progress to mortalities. Often the organism is only isolated from the lesion, i.e. it is not systemic, in the early stages of clinical signs (Hiney and Olivier 1999).

In an earlier MAF risk analysis (MacDiarmid 1994), Dr Trevor Evelyn was reported as discussing the host range of *A. salmonicida*. Evelyn noted that whilst non-salmonids may be clinically affected by atypical *A. salmonicida* subspecies, they may also be covertly infected with typical *A. salmonicida*. Typical *A. salmonicida* is primarily a disease of salmonids and maintained in salmonid reservoirs. Salmonids are largely absent from Vietnam and there is, therefore, little opportunity for development of non-clinical typical *A. salmonicida* infections. In addition, it is understood that covert *A. salmonicida* infection exists in the mucus of the skin and gills and within the intestine (Hiney and Olivier 1999). As these portions of the fish are removed during processing, typical *A. salmonicida* would not be associated with the commodity and requires no further consideration in this risk analysis.

Whilst there have been no reports of *Pangasius* spp. being infected with atypical *A. salmonicida*, the number of freshwater species from which the bacterium is being isolated

is rapidly increasing (Hiney and Olivier 1999), and as a precautionary measure this risk analysis will assume that *Pangasius* spp. are susceptible.

4.3.5 Entry assessment: Despite a sizeable global trade in salmonid products, there is no indication that there has been spread of typical *A. salmonicida* via the movement of non-viable fish for human consumption (MacDiarmid 1994). The OIE Code clearly indicates that eviscerated fish pose negligible risk as regards the transmission of typical *A. salmonicida* (OIE 2006a). The movement of live fish is the primary suspect in the translocation of *A. salmonicida* (Hiney and Olivier 1999).

Data specific to the atypical subspecies of *A. salmonicida* are lacking. However, there are plenty of data regarding typical *A. salmonicida*. It is widely accepted that atypical *A. salmonicida* subspecies are less invasive than typical *A. salmonicida*. Thus an analysis based on data relating to typical *A. salmonicida* will, in all likelihood, be conservative with respect to the risks from atypical *A. salmonicida* subspecies.

As atypical forms of the disease tend to produce visible skin lesions, it is unlikely that obviously clinically diseased animals will be harvested for export processing. Trimming of lesions would also reduce bacterial loads even if fish were displaying ulcerative skin lesions (Hiney and Olivier 1999). Even if fish subclinically infected with an atypical *A. salmonicida* subspecies, or showing very early signs of skin lesions, were harvested it is unlikely that the bacterium would be present systemically to any significant degree.

Studies of typical *A. salmonicida* have indicated that the viscera of clinically affected animals contain, in the order of, 10^3 times the bacterial load of the muscle tissue (Evelyn 2001). As carriers of typical *A. salmonicida* are known to have bacterial loads of less than 10^4 CFU/g in their viscera, the muscle load is likely to be less than 10 CFU/g (Evelyn 2001). The earlier MAF risk analysis (MacDiarmid 1994) detailed a personal communication from Drs. Menzies and McLoughlin of the Department of Agriculture for Northern Ireland which indicated that the *A. salmonicida* bacterium is not recoverable from the muscle tissue of carrier fish. Clinical infection with *A. salmonicida* mainly occurs in temperate fishes subjected to warm water. This is due to stress on the fish, and whilst the most efficient replication of the bacterium is between 22 and 25°C (Munro and Hastings 1993), it must be remembered that the basa raised in the elevated water temperatures of Vietnam are adapted to warmer water and any effects of heat stress on infection are likely to be negated.

Even assuming a load of 10 CFU/g muscle tissue, this represents less than an infectious dose if immersion in doses of 3×10^3 CFU/mL/day for 3 days failed to induce disease (Rose *et al.* 1989). In addition, as previously stated, atypical strains of *A. salmonicida* are recognised as being less invasive and thus infectious doses would be expected to be higher. Furthermore this bacterium shows poor survival outside the host or within mammals and birds potentially feeding on scraps of the discarded product (Evelyn 2001).

Finally, the commodity will be frozen. Whilst *A. hydrophila* can survive for 20 days at -20°C (Brady and Vinitnantharat 1990), it is more environmentally adapted than atypical subspecies of *A. salmonicida*. In addition, studies in Canada have indicated that *A. salmonicida* undergoes a 100-fold decrease in titre when flesh is frozen to -20°C for 5-7 days (Evelyn 2001). Combined with an expected muscle titre of less than 10 CFU/g the likelihood of viable atypical strains of *A. salmonicida* being present in the commodity is negligible.

4.4 FLAVOBACTERIUM COLUMNARE.

4.4.1 Aetiological agent: *Flavobacterium columnare* is a filamentous rod shaped motile gram negative bacterium, in the Flavobacteriaceae family. *Flavobacterium columnare* exists in at least 3 genomovars (Buller 2004). Genomovars are phenotypically similar but genetically distinct.

4.4.2 OIE List: Not listed

4.4.3 New Zealand status: *F. columnare* is considered to be present in New Zealand (Diggles *et al.* 2002), but there are more virulent genomovars (Michel *et al.* 2002) which are considered to be exotic (Duignan *et al.* 2003).

4.4.4 Epidemiology: It is estimated that all fish species are susceptible to infection by some member of the Flavobacteriaceae. They are ubiquitous bacteria in the environment but, in stressed fish, can cause fin and gill lesions and mortalities of up to 70% (Shotts and Starliper 1999). They are therefore considered an opportunistic pathogen.

Flavobacteria may also cause disease in humans but the species involved are different and thus the likelihood that this commodity would increase exposure and disease rates in the human population is negligible.

Transmission is direct horizontal via the water column and tends to occur in warmer waters (>14°C), and especially in waters of 25-30°C (Buller 2004).

There have been reports of a highly virulent *F. columnare* that causes muscle lesions in *Paracheirodon innesi* (neon tetras) in Asia (Michel *et al.* 2002). Whilst it has not been isolated from catfish, the ubiquitous nature of this family of bacteria means that the possibility of it infecting *Pangasius* spp. cannot be discounted.

4.4.5 Entry assessment: The primary site of attachment and action on the fish is the gills and skin (Shotts and Starliper 1999). The process of heading, gilling, eviscerating and skinning would effectively remove the bacteria from the commodity. In addition, washing of the product in potable water would also be expected to lower any levels of contamination.

If a highly virulent genomovar were present, the muscle lesions would result in rejection of the fillets for processing.

The likelihood, therefore, that an exotic *Flavobacterium* spp. would be present in the commodity is low, but non-negligible.

4.4.6 Exposure and establishment assessment: Exposure of susceptible native fish to an exotic *Flavobacterium* spp. would require imported fillets, or scraps derived from them, infected with an exotic *Flavobacterium* spp. to enter the aquatic environment in sufficient quantities to produce an infectious dose.

The commodity itself is highly processed and it is likely that the volume of scraps generated at end use will be small. The likelihood of exposure is further reduced by the necessity for

scraps to enter the environment in sufficient quantities to actually constitute an infectious dose.

Taking these factors into account, the likelihood that an exotic *Flavobacterium* spp. would be exposed to, and establish in, native fish is so remote as to be negligible. In addition, it poses no risk to human health. No further assessment is required and no specific sanitary measures are warranted.

4.5 KABATANA ARTHURI

4.5.1 Aetiological agent: *Kabatana arthuri* is a microsporidian parasite (Phylum Microspora).

4.5.2 OIE List: Not listed

4.5.3 New Zealand status: Not reported, considered exotic

4.5.4 Epidemiology: *Kabatana takedai* is found to infect the musculature of at least eight salmonids (*Oncorhynchus* spp. and *Salvelinus* spp.), *K. seriolae* infects *Seriola lalandi* in Japan (Dykova 2006), and presumably could infect *S. lalandi*, which is found in New Zealand waters. *K. arthuri* was detected in cultured *Pangasius sutchi* from Thailand in 2000 (Dykova and Lom 2000).

Kabatana spp. are similar to other microsporidians such as *Pleistophora* spp., *Heterosporis* spp. and *Microsporidium* spp. in that transmission is presumed to be direct and horizontal with infection by the ingestion of spores. *Kabatana* spp., like *Pleistophora* spp., do not induce xenoma formation in infected hosts. Instead, the multinucleate meronts develop through sporogonial plasmodia into uninucleate spores within muscle fibres themselves. This results in necrosis of adjacent muscle fibres (Dykova and Lom 2000, Dykova 2006). The fish responds with an inflammatory reaction principally involving macrophages, which encapsulate replete muscle fibres and phagocytose the spores. The pathology can extend into the overlying epidermis, causing epidermal lesions. Macrophages may be observed to migrate into the epidermis where they burst and release spores in a process that may allow more efficient transmission of the disease (Dykova and Lom 2000).

Affected fish may be emaciated, display anomalous behaviour and have whitish areas visible through the skin or, infrequently, skin lesions (Dykova and Lom 2000).

4.5.5 Entry assessment: Heavily infected fish would display clinical signs as described above and would not be harvested for human consumption. In addition, white lesions in the muscles would be recognised during processing and fillets containing lesions are unlikely to be further processed. It is possible that low grade infections could occur without clinical signs or macroscopic muscle lesions and, therefore, infected fillets could be prepared for export.

Specific information on the survival of *Kabatana* spp. spores following freezing is lacking. However, studies have been carried out on *Loma salmonae* (Shaw *et al.* 2000) and *Glugea plecoglossi* (Takahashi 1978). Both were reported to be completely inactivated by freezing to -20°C (for 24 hours in the case of *L. salmonae*). An earlier study had indicated that infectivity of *L. salmonae* was greatly reduced by freezing to -20°C, such that, when used to challenge fish, infection rates were reduced from 59% to 4.7% (Speare *et al.* 1998). *Pleistophora* spp., also a non-xenoma forming microsporidian, is a recognised problem in Alaskan pollock (*Theragra chalcogramma*). However, it is generally not regarded as an infection risk when fish have been frozen for several weeks (Priebe 1986).

Taking all the above into consideration, the likelihood of the commodity on entry to New Zealand containing viable spores of a microsporidian is low, but non-negligible. For completeness an exposure and establishment assessment will be carried out.

4.5.6 Exposure and establishment assessment: Exposure of susceptible native fish to *Kabatana arthuri* would require infected imported fillets, or scraps derived from them, to enter the aquatic environment in sufficient quantities to produce an infectious dose.

The commodity itself is highly processed and it is likely that the volume of scraps generated at end use will be small. The likelihood of exposure is further reduced by the necessity for scraps to enter the environment in sufficient quantities to actually constitute an infectious dose.

In addition, *Kabatana arthuri* appears to be host specific (Dykova 2006) and would be unlikely to infect salmonids, kingfish or other species of fish in New Zealand.

Taking these factors into account, and the low likelihood that viable spores would be imported, the likelihood that *Kabatana arthuri* would be exposed to, and establish in, native fish is so remote as to be negligible. There are no reports of adverse effects on human health. No further assessment is required and no specific sanitary measures are warranted.

4.6 DIGENEANS (INCLUDING MUSCLE ENCYSTING METACERCARIA)

4.6.1 Aetiological agent: Digeneans are members of the phylum Platyhelminthes and are generally endoparasitic in fish. Those reaching the adult life stage in fish, i.e. where fish are the definitive hosts, tend not to cause overt harm to the host. The exception to this would be the blood flukes, e.g. *Sanguinicola* spp., whose adults block blood vessels and whose eggs can cause embolic disease. As a result of processing, those digeneans found as the adult form in fish are unlikely to be associated with this commodity and can be discounted.

Digeneans of interest are those that encyst as metacercaria in the fish, utilising them as intermediate hosts. In these cases the definitive host is usually a piscivorous bird or a mammal. Some can affect humans (zoonotic) with varying degrees of severity.

4.6.2 OIE List: Not listed

4.6.3 New Zealand status: There have been no reports of *Exorchis* or *Haplorchis* spp. in this country (Hine *et al.* 2000).

4.6.4 Epidemiology: *Haplorchis* spp. can use *Gambusia affinis* and *Oncorhynchus* spp. as second intermediate hosts, with mammals, including humans, and birds as definitive hosts. They can use the endemic snail, *M. tuberculata*, as first intermediate host. However, they are usually found encysted in gills, skin and fins (Paperna and Dzikowski 2006) and are more likely to be found in those locations than in muscle tissue.

Exorchis oviformis is a member of the family Cryptogonimidae. It is found encysted in flesh, scales and fins (Lee 1968). There is one report of *E. oviformis* utilising the mollusc *Stenothyra recondita* as first intermediate host (Besprozvannykh *et al.* 2000). This mollusc is exotic to New Zealand (Spencer *et al.* 2002). Another Cryptogonimidae utilises *Hydrobia* spp. snails (Pampoulie *et al.* 2000) which are also not found here, other than on sub-Antarctic islands (Spencer *et al.* 2002).

Freezing to -10 °C for 3 days or -20 °C for 2 days is reported to be sufficient to inactivate all encysted metacercaria of trematodes in tilapia muscle, including the Cyathocotylidae and the more resistant Heterophyidae (Elnawawi *et al.* 2000). Metacercaria of *Metagonimus* spp. were demonstrated to be inactivated by temperatures of -26 °C for 3 days in the case of whole fish (Racz and Zemankovics 2002). Another study indicated that Heterophyid metacercaria in prepared fish flesh were inactivated by freezing to -20 °C for 8 hours (Wiwanitkit *et al.* 2001). *Opisthorchis* sp. metacercaria died after 20 hours at -28 °C (Fattakhov 1989); another experiment indicated 96% inactivation following freezing at -22 °C for 92 hours (Fattakhov 1985). *Pygidiopsis* sp. metacercaria were inactivated by temperatures of -4 °C for 12 days (Youssef *et al.* 1981). *Clonorchis sinensis* was reported to be inactivated by 20 days at -12°C (Fan 1998) and *Heterophyes heterophyes* infectivity was reported to be eliminated after 30 hours at either -10°C or -20°C (Hamed and Elias 1970).

4.6.5 Entry assessment: The likelihood of metacercaria being present in the commodity is low; *H. pumilio* favours integumentary connective tissue, whereas *E. oviformis* is more likely to be found in the flesh than the skin.

The freezing process will, however, inactivate metacercaria present in the commodity and further reduce the likelihood of viable digenean metacercaria entering New Zealand. An

appropriate freezing regime will reduce the likelihood of entry of viable digenean metacercaria to negligible. The available data suggest that being held for sufficient periods of time at freezing temperatures will result in complete inactivation, as in the case of *Pygidiopsis* spp. metacercaria inactivated by a 12-day period at -4°C (Youssef *et al.* 1981). *Pygidiopsis* spp. are members of the Heterophyidae, recognised as being harder to inactivate by freezing than other families (Elnawawi *et al.* 2000).

The time and temperature data detailed above are complex and it is difficult to definitively establish a time to complete inactivation of digenean metacercaria at -18°C or lower. For example, whilst one report indicates that 20 days at -12°C and more than 7 days at -20°C is required to inactivate *Clonorchis sinensis*, another (Fang *et al.* 2003) indicates that 3 days at either -18°C or -20°C is sufficient to remove *C. sinensis* infectivity. The United States food and drug administration (US FDA) recommends storage of raw fish for human consumption at -20°C for 7 days (USFDA 2001). This is a reasonably conservative figure and it seems appropriate to use a similar regime at this time. The data above suggest that this time frame is likely to be as effective at -18°C as at -20°C . As the commodity will be frozen at -18°C or lower for at least 3 weeks, the likelihood of entry of viable digenean metacercaria is negligible.

4.7 NEMATODIASIS

4.7.1 Aetiological agent: Adults and larvae of the Phylum Nematoda

4.7.2 OIE List: Not listed

4.7.3 New Zealand status: A number of nematodes have been reported from fish in New Zealand including *Ascarophis* sp., *Anguilicola australiensis*, *Contracaecum* sp., *Eustrongylides* sp., *Spirocamallanus* sp. (Boustead 1982), *Anisakis simplex*, *Capillaria* sp., *Cucullanus* sp., *Philometra* sp. and *Terranova* sp. (Hewitt and Hine 1972) amongst others.

4.7.4 Epidemiology: Nematodes infecting fish tend to have a complicated lifecycle, having final, intermediate and paratenic hosts. Fish may be involved as intermediate or final hosts. Where fish act as intermediate or paratenic hosts the final host is usually a bird or a mammal. The requirement for multiple hosts greatly reduces the chances of successful establishment following translocation of the host.

Nematodes may be found throughout the body of the fish, in adult or larval form.

4.7.5 Entry assessment: The removal of head, gills, guts and skin greatly reduces the chances of nematodes and/or their larvae being present in the commodity. It is possible however for larvae to be present in the musculature and there have been reports of nematode larvae penetrating the musculature post mortem, although this is most likely in fish with a greater fat content than *Pangasius* spp. (Smith 1984).

Potentially zoonotic nematodes such as *Anisakis* spp., *Capillaria* spp. and *Terranova* spp. have been reported from more than 70 species of fish of New Zealand origin. Even unfrozen, this commodity would not necessarily represent a greater risk to the public than unfrozen fish caught off the coasts of New Zealand.

Even so, freezing of the fillets is an effective method of killing nematode larvae. All larvae of *Anisakis simplex* in flounder were killed by freezing to -15°C for 96 hours, -20°C for 60 hours, -30°C for 12 hours and -40°C for 9 hours (Adams *et al.* 2005). Similarly freezing of whole fish to -35°C for 24 hours was also effective in the case of sockeye salmon (Deardorff and Throm 1988). Freezing is the treatment of choice as inspection of fillets individually in a process referred to as candling is ineffective (Levsen *et al.* 2005). Freezing at -20 °C for at least 48 hours is also effective against encysted plerocercoids of cestodea (Dovgalev 1988, Pronin *et al.* 1989). The likelihood of the commodity containing viable nematodes, larval nematodes (and, incidentally) plerocercoids of cestodes on entry to New Zealand is negligible as long as the commodity has been frozen to -18°C, or lower, for at least 96 hours (4 days). As the commodity will be frozen at -18°C for at least 3 weeks the likelihood of entry of viable nematode or cestode parasites is negligible.

4.8 APHANOMYCES INVADANS

4.8.1 Aetiological agent: *Aphanomyces invadans* is an oomycete fungus of the family Saprolegniaceae. It has broad (12-30µm), non-septate hyphae.

4.8.2 OIE List: Not listed

4.8.3 New Zealand status: Not reported, considered exotic

4.8.4 Epidemiology: Whilst *A. invadans* is recognised to be the causative agent of epizootic ulcerative syndrome (EUS), it requires an initial skin lesion to attach to and invade the underlying tissue. The precipitating event may be physical damage (e.g. handling), environmental stressors (e.g. acid sulphate soil runoff) or another disease agent (e.g. *Aeromonas* sp. or rhabdoviruses) (Bondad-Reantaso *et al.* 2001, Diggles *et al.* 2002). EUS was first reported from Japan, but has now spread through Asia, into India and, recently, Pakistan (Bondad-Reantaso *et al.* 2001). It has low host specificity, affecting more than 100 species of fish. The New Zealand grey mullet (*Mugil cephalus*) is particularly susceptible to infection (Fraser *et al.* 1992, Shaheen *et al.* 1999).

The life cycle is similar to other oomycete fungi with infectious spores transmitting infection directly and horizontally to cohabiting fish. The movement of live affected, or carrier, fish is recognised as the primary means of translocation (Bondad-Reantaso *et al.* 2001). As the syndrome name suggests, infection with *A. invadans* results in congested skin lesions and ulceration, with fungal hyphae penetrating into the underlying musculature. Mortality rates tend to be high (Bruno and Wood 1999).

Both hyphae and spores are considered infectious and need to be considered in the risk analysis.

4.8.5 Entry assessment: EUS is recognised in Vietnam (Bondad-Reantaso *et al.* 2001) and suspected outbreaks have been reported by the Competent Authority there. *Pangasius* spp. are not listed specifically in either the outbreak reports or on the current list of susceptible species, but given the prevalence of the agent through South East Asia it is reasonable to assume that the agent could be present on the farms from which the fish are harvested.

Clinical disease has never been reported in *Pangasius* spp. but, were it to occur, the signs would be apparent and would tend to preclude harvesting of the fish for human consumption. Subclinical infections may occur but fungal hyphae are unlikely to penetrate to the musculature in the absence of obvious skin ulceration.

Infective spores can survive in the environment and could act as external contaminants of the harvested fish. In this case the process of filleting and washing would greatly reduce any contamination of the fillets. Even if spores or hyphae were to be present on or in the fillets, it has been shown that the infective stages of a related fungus, *Aphanomyces astaci*, is inactivated by freezing to -20°C for 72 hours. Evidence also suggests that temperatures as high as -5°C would also be effective after 72 hours (Oidtmann *et al.* 2002).

Given that the time period between freezing and entry to New Zealand is at least 3 weeks, the likelihood of viable, infective stages of *A. invadans* being present in the commodity is negligible and no specific sanitary measures are warranted.

4.9 WATERBORNE CONTAMINANTS

4.9.1 Aetiological agent: Waterborne contaminant organisms that could be a risk to human health e.g. *Salmonella* spp., *Vibrio cholerae*.

4.9.2 OIE List: N/A

4.9.3 New Zealand status: Only exotic strains considered

4.9.4 Discussion: Zoonotic agents are unlikely to be present in the commodity given that septicaemic fish are extremely unlikely to be harvested for human consumption and the freezing process will inactivate other agents such as larval nematodes.

However, there is still the possibility that waterborne food poisoning agents may contaminate the water from which the fish are harvested, or the water supply used in the processing plant. The process of filleting the fish should remove the external contamination via the removal of head and skin and the washing of the carcasses, although there is opportunity for cross contamination if equipment is not kept clean. If contaminated water was used in the processing plant it would represent a risk.

While bacterial contaminants would be expected to survive the freezing process, the protozoans, such as *Cryptosporidium* spp. and *Giardia* spp., would be inactivated by freezing. *Cryptosporidium* spp. have been reported to be inactivated by freezing to -20 °C for 24 hours or -15 °C for 7 days (Fayer and Nerad 1996) and *Giardia* spp. were inactivated by freezing to -4 °C for 7 days (Olson *et al.* 1999). Being frozen to, and maintained at, a temperature no warmer than -18 °C for at least 3 weeks should be sufficient to inactivate protozoans in the commodity.

The New Zealand drinking water standards indicate that to consistently eliminate *Escherichia coli* and other coliforms from a water supply requires a range of treatments and/or the sourcing of a microbiologically secure supply (Ministry of Health 2005). The use of such water should mitigate against the presence of human pathogenic bacteria being carried on the commodity.

4.9.5 Risk management measure: The use of potable water is specified in the commodity definition. It is recommended that the water used to wash the fish in the processing plant and any water used in the filleting process and the freezing process must be of potable standard.

5. CONCLUSION

The commodity assessed in this risk analysis consists of skinless, boneless fillets (and mince derived from fillets) from farmed *Pangasius* spp. from Vietnam. The fish are harvested, bled, scaled, eviscerated, filleted, skinned, trimmed, washed and graded. Treated water (chlorinated with or without UV irradiation) is used during processing of the fish. The fillets are frozen to -18 °C or colder following processing and are maintained at those temperatures for transport to New Zealand, a process expected to take at least 3 weeks.

Eight potential hazards were identified from the list of organisms of potential concern and subjected to further risk assessment. These were iridoviruses, atypical *A. salmonicida*, *Flavobacterium* spp., *Edwardsiella ictaluri*, *Kabatana arthuri*, digenean metacercaria, larval nematodes, and *Aphanomyces invadans*. Waterborne contaminants were also considered as a ninth hazard.

The degree of processing involved in the production of the commodity greatly reduces the likelihood of pests or pathogenic organisms entering New Zealand and resulting in harm. The separation of the fillets from the rest of the carcass effectively removes the majority of organisms that might be present in the live animal. Titres of pathogens in muscle tend to be many times lower than those found in the viscera.

None of the eight primary potential hazards were identified as requiring specific risk management measures; the process of filleting and the period of time frozen effectively reducing any pathogenic burden to levels where the likelihood of exposure and establishment in New Zealand is negligible.

It was considered necessary to specify some general sanitary measures covering the health of harvested fish, compliance with freezing schedules and the use of potable water for processing.

5.1 GENERAL SANITARY MEASURES

5.1.1 To ensure that the likelihood of clinically or subclinically diseased fish being harvested for processing is minimised:

- 5.1.1.1 Both the farm of origin and the processing facility must be registered with the Competent Authority of the country in question; and
- 5.1.1.2 Fish processed must be derived from broodstock resident in the exporting country; and
- 5.1.1.3 Fish showing clinical signs of disease, septicaemia or skin ulceration must not be harvested for processing into this commodity; and
- 5.1.1.4 Fish harvested must not be subject to emergency slaughter for disease reasons, regardless of whether or not they display clinical signs themselves.

5.1.2 To avoid contamination of the commodity with exotic foodborne pathogens it is necessary to use potable quality water in the processing plant

5.1.3 The commodity definition includes freezing and maintenance at temperatures of -18°C or lower. The product is expected to remain at these temperatures for at least 3 weeks during

transport to New Zealand. As a function of the extended period in cold storage, a number of potential hazards were determined to have a negligible likelihood of entry into New Zealand. These were digenean metacercaria, larval nematodes (and incidentally cestodes), *Aphanomyces invadans* and waterborne protozoans. As the likelihood of entry was determined to be negligible it was not necessary to consider exposure and establishment or consequence. However, for this assessment to remain valid the length of time that the commodity remains at -18°C or lower must exceed a minimum period during which inactivation of the organisms occur. Inactivation efficiency is a function of the size of the product being frozen, the length of time the product is frozen for before entry into New Zealand and the temperature achieved in the freezing process. Generally, however, these minimum periods may be summarised in the table below:

Organism	Core temperature -18 °C
Digenean metacercaria	168 hours (7 days)
Encysted nematode larvae (and cestode plerocercoids)	96 hours (4 days)
<i>Aphanomyces invadans</i>	72 hours (3 days)
Water borne contaminants (protozoans)	168 hours (7 days)

It is evident that any period in excess of 7 days (168 hours) at -18°C, or colder, will result in a negligible likelihood of entry for all of the organisms listed. Therefore, a general sanitary measure to ensure this is warranted.

To meet the commodity definition the imported product must be shown, at minimum, to have been frozen to and maintained at a core temperature of -18 °C , or colder, for 7 days (168 hours).

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♣ Abstract only

APPENDIX 1. – Pathogenic organisms reported to be associated with Pangasid fish and fish diseases listed by the OIE

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
Viruses					
EHNV	Ranavirus; Iridoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
IHNV	Novirhabdovirus; Rhabdoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
SVCV	Vesiculovirus; Rhabdoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
VHSV	Novirhabdovirus; Rhabdoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
ISAV	Isavirus; Orthomyxoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
RSIV	Iridoviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
KHV	Cyprinid herpesvirus 3; herpesviridae	No pangasiidae listed	Septicaemia, mortality in susceptible hosts		OIE list
Bacteria					
<i>Edwardsiella ictaluri</i>	gram –ve, Enterobacteriaceae	<i>P. hypophthalmus</i>	Septicaemia	Vietnam	(Crumlish <i>et al.</i> 2002)
<i>Edwardsiella ictaluri</i>	gram –ve, Enterobacteriaceae	<i>P. hypophthalmus</i>	Septicaemia	Indonesia	(Yuasa <i>et al.</i> 2003)
<i>Aeromonas</i> spp.	Aeromonadaceae	<i>P. sutchi</i>		Bangladesh	(Chowdhury 1998)
<i>Aeromonas</i> spp.	Aeromonadaceae	<i>P. sutchi</i>	Kidney culture	Bangladesh	(Mirdha <i>et al.</i> 2000)
<i>Pseudomonas</i> spp.	Pseudomonadaceae	<i>P. sutchi</i>		Bangladesh	(Chowdhury 1998)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Pseudomonas</i> spp.	Pseudomonadaceae	<i>P. sutchi</i>		Bangladesh	(Mirdha <i>et al.</i> 2000)
<i>Bacillus</i> sp.	Bacillaceae	<i>P. hypophthalmus</i>	Granulomata in spleen, liver and kidney	Vietnam	(Ferguson <i>et al.</i> 2001)
<i>Flavobacterium</i> spp.	Flavobacteriaceae	<i>P. sutchi</i>	Surface mucus culture	Bangladesh	(Mirdha <i>et al.</i> 2000)
<i>Micrococcus</i> spp.	Micrococcaceae	<i>P. sutchi</i>	Surface mucus culture	Bangladesh	(Mirdha <i>et al.</i> 2000)
<i>Staphylococcus</i> spp.	Staphylococcaceae	<i>P. sutchi</i>	Surface mucus culture	Bangladesh	(Mirdha <i>et al.</i> 2000)
Corynebacterineae	Actinobacteria	<i>P. sutchi</i>	Surface mucus culture	Bangladesh	(Mirdha <i>et al.</i> 2000)
Parasites					
<i>Balantidium</i> sp.	Ciliophora; Protozoa	<i>P. pangasius</i>	Intestinal facultative parasite	India	(Mukherjee and Haldar 1980)
<i>Balantidium</i> sp.	Ciliophora; Protozoa	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i> , <i>P. larnaudii</i>	Intestinal facultative parasite	Vietnam	(Arthur and Te 2006)
<i>Ichthyophthirius multifiliis</i>	Ciliophora; Protozoa	<i>P. bocourti</i> , <i>P. hypophthalmus</i> , <i>P. micronemus</i>	External epithelial parasite	Vietnam	(Arthur and Te 2006)
<i>Apiosoma</i> spp.	Ciliophora; Protozoa	<i>P. hypophthalmus</i>	External epithelial parasite	Vietnam	(Arthur and Te 2006)
<i>Chilodonella</i> spp.	Ciliophora; Protozoa	<i>P. hypophthalmus</i>	External epithelial parasite	Vietnam	(Arthur and Te 2006)
<i>Cryptobia branchialis</i>	Ciliophora; Protozoa	<i>P. hypophthalmus</i> , <i>P. micronemus</i>	Gill parasite	Vietnam	(Arthur and Te 2006)
<i>Epistylis kronwerci</i>	Ciliophora; Protozoa	<i>P. hypophthalmus</i> , <i>P. micronemus</i>	Sessile external parasite	Vietnam	(Arthur and Te 2006)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Protoopalina</i> sp.	Opalinidae; Protozoa	<i>P. bocourti</i>	Intestinal lumen parasite	Vietnam	(Arthur and Te 2006)
<i>Trichodina</i> spp.	Ciliophora: Protozoa	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i> , <i>P. larnaudi</i> , <i>P. micronemusi</i>	External parasite	Vietnam	(Arthur and Te 2006)
<i>Tripartiella</i> spp.	Ciliophora: Protozoa	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i> , <i>P. larnaudii</i> , <i>P. micronemus</i>	External parasite	Vietnam	(Arthur and Te 2006)
<i>Hexamita</i> sp.	Diplomonadida; Protozoa	<i>P. sutchi</i>		South East Asia	(Lom <i>et al.</i> 1990)
<i>Kabatana arthuri</i>	Microsporea; Microsporidia	<i>P. sutchi</i>	Large masses of spores form in musculature	Thailand	(Lom <i>et al.</i> 1990, Lom <i>et al.</i> 1999, Dykova and Lom 2000, Dykova 2006)
<i>Myxobolus</i> sp.	Myxosporea; Myxozoa	<i>P. sutchi</i>		South East Asia (Malaysia, Thailand)	(Lom <i>et al.</i> 1990)
<i>Myxobolus baskai</i> , <i>M. pangasii</i>	Myxosporea; Myxozoa	<i>P. hypophthalmus</i>	Gill capillaries and splenic serosa respectively	Malaysia	(Molnar <i>et al.</i> 2005)
<i>Myxobolus</i> spp.	Myxosporea; Myxozoa	<i>P. hypophthalmus</i> , <i>P. micronemus</i>		Vietnam	(Arthur and Te 2006)
<i>Ceratomyxa</i> sp.	Myxosporea; Myxozoa	<i>P. hypophthalmus</i>	Gall and urinary bladder	Vietnam	(Arthur and Te 2006)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Hennegoides berlandi</i> , <i>H. malayensis</i> , <i>H. pangasii</i>	Myxosporea; Myxozoa	<i>P. hypophthalmus</i>	Found on gills and within gill arteries and cartilage	Malaysia	(Molnar <i>et al.</i> 2005)
<i>Henneguya shariffi</i>	Myxosporea; Myxozoa	<i>P. hypophthalmus</i>	Found on gills	Malaysia	(Molnar <i>et al.</i> 2005)
<i>Henneguya</i> sp.	Myxosporea; Myxozoa	<i>P. hypophthalmus</i> , <i>P. larnaudii</i>	Found on gills	Vietnam	(Arthur and Te 2006)
<i>Sphaerospora ojiroveci</i> sp. nov.	Myxosporea; Myxozoa	<i>P. sutchi</i>	Found in kidney	South East Asia	(Dykova and Lom 1997)
<i>Zschokkella parasiluri</i>	Myxosporea; Myxozoa	<i>P. bocourti</i>	Gall bladder	Vietnam	(Arthur and Te 2006)
<i>Protocladorchis chinabutae</i> sp. nov.	Trematoda; Platyhelminthes	<i>P. nasutus</i>	Found in intestine	Thailand	(Jones 1987)
<i>Protocladorchis pangasii</i>	Trematoda; Platyhelminthes	<i>P. pangasius</i>	Found in intestine	Malaysia	(Jones and Seng 1986)
<i>Gyrodactylus salaris</i>	Monogenea; Platyhelminthes	No pangasiidae listed	Ectoparasitism, skin and fin lesions and death in susceptible salmonids		OIE list
<i>Silurodiscoides siamensis</i>	Monogenea; Platyhelminthes	<i>P. hypophthalmus</i>	Found on gills	Bangladesh	(Das <i>et al.</i> 2006)
<i>Bifurcohaptor indicus</i>	Monogenea; Platyhelminthes	<i>P. hypophthalmus</i>	Found on gills	Bangladesh	(Das <i>et al.</i> 2006)
<i>Pangasitrema camillae</i> gen. nov. sp. nov.	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. polyuranodon</i>	Found on gills	Indonesia	(Pariselle <i>et al.</i> 2004)
<i>Thaparocleidus caecus</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. mahakamensis</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2005a)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Thaparocleidus caecus</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. hypophthalmus</i> , <i>P. djambal</i>	Found on gills	Indonesia, Vietnam	(Pariselle <i>et al.</i> 2002a, Arthur and Te 2006)
<i>Thaparocleidus caecus</i> , <i>T. pouyaudi</i> sp. nov., <i>T. tengelsi</i> sp. nov.	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. mahakamensis</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2005a)
<i>Thaparocleidus furcus</i> , <i>T. infundibulus</i> , <i>T. sudartoi</i> , <i>T. turbinatio</i> (all sp. nov.)	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. polyuradon</i> , <i>P. elongatus</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2005b)
<i>Thaparocleidus caestus</i> , <i>T. crassipenis</i> , <i>T. legendrei</i> , <i>T. levangi</i> , <i>T. slembroncki</i> , <i>T. virgula</i> (all sp. nov.)	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. polyuradon</i>	Found on gills	Indonesia, Malaysia	(Pariselle <i>et al.</i> 2004)
<i>Thaparocleidus serpens</i> , <i>T. ocrea</i> , <i>T. megagripis</i> , <i>T. eitream</i> , <i>T. alatus</i> (all sp. nov.)	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. nasutus</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2003)
<i>Thaparocleidus vietnamensis</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. kempfi</i> , <i>P. kunyit</i> , <i>P. mekongensis</i> , <i>P. sabahensis</i>	Found on gills	Indonesia, Malaysia, Vietnam	(Pariselle <i>et al.</i> 2002b)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Thaparocleidus vietnamensis</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. bocourti</i> , <i>P. hypophthalmus</i>	Found on gills	Indonesia, Vietnam	(Pariselle <i>et al.</i> 2002a)
<i>Thaparocleidus humerus</i> sp. nov., <i>T. culter</i> sp. nov.	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. kunyit</i>	Found on gills	Indonesia, Malaysia, Vietnam	(Pariselle <i>et al.</i> 2002b)
<i>Thaparocleidus mehurus</i> sp. nov., <i>T. culteroides</i> sp. nov.	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. sabahensis</i>	Found on gills	Indonesia, Malaysia, Vietnam	(Pariselle <i>et al.</i> 2002b)
<i>Thaparocleidus phuongi</i> sp. nov.	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. kempfi</i> , <i>P. kunyit</i> , <i>P. mekongensis</i> , <i>P. sabahensis</i>	Found on gills	Indonesia, Malaysia, Vietnam	(Pariselle <i>et al.</i> 2002b)
<i>Thaparocleidus siamensis</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. hypophthalmus</i>	Found on gills	Indonesia, Vietnam	(Pariselle <i>et al.</i> 2002a)
<i>Thaparocleidus combesii</i> , <i>T. komarudini</i> , <i>T. euzeti</i> , <i>T. sadilii</i> (all sp. nov.)	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. boucorti</i> , <i>P. djambal</i>	Found on gills	Vietnam, Indonesia	(Pariselle <i>et al.</i> 2002a)
<i>Thaparocleidus sinespinae</i> , <i>T. brevicochleus</i> , <i>T. kapuaensis</i> , <i>T. gustiano</i> i (all sp. nov.)	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. humeralis</i>	Found on gills	Indonesia	(Pariselle <i>et al.</i> 2001b)
<i>Thaparocleidus pangasi</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. pangasius</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2001a)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Thaparocleidus pangasi</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. bocourti</i> , <i>P. conchophilus</i>	Found on gills	Vietnam	(Arthur and Te 2006)
<i>Thaparocleidus chandpuri</i> , <i>T. bahari</i> , <i>T. sabanensis</i> , <i>T. redebensis</i> , <i>T. mahakamensis</i>	Monogenea (Ancylo-discoididae); Platyhelminthes	<i>P. kinabatanganensis</i> , <i>P. rheophilus</i> , <i>P. nieuwenhuisii</i>	Found on gills	South East Asia	(Pariselle <i>et al.</i> 2001a)
<i>Dactylogyrus</i> spp., <i>Gyrodactylus</i> spp., <i>Silurodiscoides</i> spp.	Monogenea, Platyhelminthes	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i>	Found on skin and gills	Vietnam	(Tran <i>et al.</i> 2002)
<i>Haplorchis pumilio</i> , <i>Exorchis oviformis</i>	Digenea; Platyhelminthes	<i>P. hypophthalmus</i>	Metacercaria in liver and intestines	Vietnam	(Nguyen <i>et al.</i> 2007)
<i>Prosorhynchoides gracilescens</i>	Digenea; Platyhelminthes	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i> , <i>P. micronemus</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Lytocestus parvulus</i>	Caryophyllidea; Cestoidea	<i>P. conchophilus</i> , <i>P. hypophthalmus</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Proteocephalus</i> sp.	Cestoda; Platyhelminthes	<i>P. pangasius</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Cucullanus cyprini</i>	Ascaridida; Nematoda	<i>P. bocourti</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Procamallanus</i> sp.	Ascaridida; Nematoda	<i>P. conchophilus</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Philometra</i> sp.	Spirurida; Nematoda	<i>P. bocourti</i> , <i>P. hypophthalmus</i> , <i>P. larnaudii</i>	Internal organs and/or subcutaneous sites	Vietnam	(Arthur and Te 2006)
<i>Hysterothylacium fluvatile</i> sp. nov.	Anisakidae; Ascaridoidea; Nematoda	<i>P. pangasius</i>	Larvae found in coelomic cavity	Vietnam	(Moravec and Sey 1988)

AGENT	DESCRIPTION	PANGASID HOST	EFFECTS	REPORTED LOCATIONS (IF APPLICABLE)	REFERENCES
<i>Spectatus</i> sp.	Kathlaniidae; Nematoda	<i>P. bocourti</i> , <i>P. conchophilus</i> , <i>P. hypophthalmus</i> , <i>P. larnaudii</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Pseudorhadinorhynchus vietnamensis</i>	Acanthocephala: Micracanthorhynchinae	<i>P. bocourti</i>	Intestinal parasite	Vietnam	(Arthur and Te 2006)
<i>Ergasilus</i> sp.	Maxillopoda; Copepoda	<i>P. hypophthalmus</i> , <i>P. larnaudii</i>	External parasite	Vietnam	(Arthur and Te 2006)
Fungi					
EUS	<i>Aphanomyces invadans</i>	No Pangasiidae or Siluriformes listed by OIE, but infection known from >100 spp. of freshwater fish & reports of <i>Clarias</i> and <i>Ictalurus</i> spp. infected.	Congested skin lesions in susceptible hosts		OIE list

APPENDIX 2.

Agent	Likely to be associated with commodity ?	OIE listed?	In NZ?	Likely to be in exporting country?	More virulent strain o/s?	Genetic difference → greater consequence?	In New Zealand but proposed import increases hazard?	In New Zealand but geographically bounded?	In New Zealand but different host associations ?	Little or no information on the organism?	Under official control or notifiable?	Potential hazard?
VIRUSES												
EHNV	No pangasid hosts listed	Yes	No	No ¹	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable unwanted	No
IHNV	No pangasid hosts listed	Yes	No	No ¹	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable unwanted	No
SVCV	No pangasid hosts listed	Yes	No	No ¹	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable unwanted	No
VHSV	No pangasid hosts listed	Yes	No	No ¹	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable unwanted	No
ISAV	No pangasid hosts listed	Yes	No	No	n/a	n/a	n/a	n/a	n/a	n/a	Other exotic unwanted	No
RSIV	No pangasid hosts listed	Yes	No	No ²	n/a	n/a	n/a	n/a	n/a	n/a	No	No
KHV	No pangasid hosts listed	Yes	No	No ²	n/a	n/a	n/a	n/a	n/a	n/a	No	No
BACTERIA												
<i>Edwardsiella ictaluri</i>	Possible	No	No	Yes ³	n/a	n/a	n/a	n/a	n/a	n/a	Other exotic, unwanted	Yes
<i>Aeromonas</i> spp.	Possible	No	Ubiquitous spp.	Likely (world wide)	Yes (<i>A. salmonicida</i>)	No	No	No	No	No	Notifiable unwanted (<i>A. salmonicida</i>)	Yes
<i>Pseudomonas</i> spp.	Possible	No	Ubiquitous spp.	Likely (world wide)	No	No	No	No	No	No	No	No
<i>Bacillus</i> spp.	Possible	No	Unknown but strain unlikely	Yes	n/a	n/a	n/a	n/a	n/a	Yes	No	Yes

Agent	Likely to be associated with commodity ?	OIE listed?	In NZ?	Likely to be in exporting country?	More virulent strain o/s?	Genetic difference → greater consequence?	In New Zealand but proposed import increases hazard?	In New Zealand but geographically bounded?	In New Zealand but different host associations ?	Little or no information on the organism?	Under official control or notifiable?	Potential hazard?
<i>Flavobacterium</i> spp.	Generally not, but possible ⁴	No	Yes	Likely (world wide)	Yes, Asian genomovar ⁴	No	No	No	No	No	No	Yes
<i>Micrococcus</i> spp.	No, surface mucus only ⁵	No	Likely, normal flora	Likely, normal flora	No	No	No	No	No	No	No	No
<i>Staphylococcus</i> spp.	No, surface mucus only ⁵	No	Likely, normal flora	Likely, Normal flora	No	No	No	No	No	No	No	No
Corynebacteria	Generally surface mucus only	No	Yes, worldwide	Yes, worldwide	No	No	No	No	No	No	No	No
PARASITES												
<i>Balantidium</i> spp.	No, found in intestine	No	Likely to be present	Likely to be present ⁶	not reported	No	No	No	No	No	No	No
<i>Ichthyophthirius multifiliis</i>	No, external parasite	No	Yes ¹³	Yes ¹⁴	No	No	No	No	No	No	No	No
<i>Apiosoma</i> spp.	No, external parasite	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Chilodonella</i> spp.	No, external parasite	No	Yes ¹³	Yes ¹⁴	No	No	No	No	No	No	No	No
<i>Cryptobia branchialis</i>	No, gills only	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Epistylis kronwerci</i>	No, external parasite	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Protoopalina</i> sp.	No, intestinal lumen	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Trichodina</i> spp.	No, external parasite	No	Yes ¹³	Yes ¹⁴	No	No	No	No	No	No	No	No
<i>Tripartiella</i> spp.	No, external parasite	No	Yes ¹³	Yes ¹⁴	No	No	No	No	No	No	No	No
<i>Hexamita</i> spp.	No, digestive	No	Not reported,	Reported from	<i>H. salmonis</i> but	No	No	No	No	No	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In NZ?	Likely to be in exporting country?	More virulent strain o/s?	Genetic difference → greater consequence?	In New Zealand but proposed import increases hazard?	In New Zealand but geographically bounded?	In New Zealand but different host associations ?	Little or no information on the organism?	Under official control or notifiable?	Potential hazard?
<i>Philometra</i> sp.	Possible – fins and s/cut tissues	No	<i>Philometra</i> spp. in NZ ¹³	Yes ¹⁴	No	No	No	No	No	No	No	No
<i>Hysterothylacium fluvatile</i>	Possible, larvae found in coelom	No	Not reported	Yes	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes
<i>Spectatus</i> sp.	No, intestinal parasite	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Pseudorhadinorhynchus vietnamensis</i>	No, intestinal parasite	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
<i>Ergasilus</i> sp.	No, external parasite	No	Not reported	Yes ¹⁴	n/a	n/a	n/a	n/a	n/a	n/a	No	No
FUNGI												
<i>Aphanomyces invadans</i> (EUS)	Potential exists ¹⁵	Yes	No	Suspected but not confirmed	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes

TABLE REFERENCES

¹ OIE disease reports via Handistatus

² Network of Aquaculture Centres in Asia Pacific (NACA) – Quarterly Aquatic Animal Disease Report

³ (Crumlish *et al.* 2002)

⁴ (Michel *et al.* 2002)

⁵ (Mirdha *et al.* 2000)

⁶ Reported from central Asian rivers (Basson and Van As 2006)

⁷ *Myxobolus* spp. common in eels in New Zealand, most have high host specificity and require specific oligochaete intermediate hosts

⁸ (Hine 1978, Hine *et al.* 2000)

⁹ *Sphaerospora* spp. reported from New Zealand (Hine *et al.* 2000)

¹⁰ *G. salaris* restricted to salmonids

¹¹ Undescribed *Gyrodactylus* sp. from wild flounder in NZ (Diggles *et al.* 2002)

¹² Vietnam (Nguyen *et al.* 2007)

¹³ (Hine *et al.* 2000)

¹⁴ (Arthur and Te 2006)

¹⁵ With over 100 spp. of freshwater fish, including Siluriformes (*Clarias* spp. and *Ictalurus* spp.), affected a precautionary approach has been adopted