Import risk analysis: Frozen, skinless and boneless fillet meat of *Oreochromis* spp. from China and Brazil for human consumption

FINAL

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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 fillets (or mince derived from fillets) of tilapia (*Oreochromis* spp.) from Brazil and China.

General sanitary measures were considered necessary:

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.

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..

To ensure compliance with the freezing and transport time regime included in the commodity definition.

- To ensure that the inactivation of organisms that is inherent in this 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.

Thirteen potential hazards were identified from the list of organisms of potential concern and subjected to further risk assessment. These were iridoviruses, aquatic birnaviruses, *Aeromonas salmonicida, Flavobacterium* spp., *Streptococcus iniae, Edwardsiella* spp., intracellular bacteria, *Yersinia ruckeri, Henneguya* spp., digenean metacercaria, larval nematodes, *Ichthyophonus hoferi* and *Aphanomyces invadans*. Waterborne contaminants were considered as a fourteenth hazard.

None of the thirteen 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 extremely 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. 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 tilapia from Brazil and China. Tilapia (*Oreochromis* spp.) are teleost fish and members of the Cichlidae 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 tilapia from China and Brazil. 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 tilapia from China and Brazil, 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 *Oreochromis* spp. farmed in Brazil and China. 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 at a rate of 300 – 350g per 7.1 – 7.2 kg of fish. Fillets are then packaged/wrapped and block/individually frozen to a core temperature of -18 °C or -20 °C and then stored and transported at those temperatures. Deliveries are expected to be made by sea with a time of approximately 30 days between freezing 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, 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 the Agreement on the Application of the Sanitary and Phytosanitary (SPS) Agreement 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, being those that *Oreochromis* spp. can carry, 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 New Zealand, becoming established in it and/or having the potential to cause an adverse consequence.
c) **Assessment of consequences** – the consequences associated the 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 (Anonymous 2006).

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's natural waters, 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 *Oreochromis* spp. or to which *Oreochromis* spp. are known, or believed, to be susceptible was developed. This included a description of the causative agent, host, effects on tilapia 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 2.4.1) 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

Of the virus diseases listed by the OIE as being of concern in fish, none lists tilapia as susceptible species.

EHNV is regarded as a natural disease of redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (OIE 2006b), related viruses have been identified from black catfish (*Ictalurus melas* now known as *Ameiurus melas*) and sheatfish (*Siluris glanis*). However there have been no reports of infection of *Oreochromis* species or other Cichlidae since the initial reports on EHNV in 1986.

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 *Siluris glanis* (a silurid catfish), orfe (*Leuciscus idus*) and tench (*Tinca tinca*) (OIE 2006b). Whilst there are no reports that tilapia have been deliberately exposed to SVCV, the virus is reported from China, an extensive producer of tilapia. It is likely that

tilapia have been naturally exposed to the virus. Were they susceptible to SVCV, it is likely that there would be reports of disease.

Tilapia are not considered as susceptible to infection with VHSV (OIE 2006a, OIE 2006b), so whilst this virus has been reported from Brazil, it is of no concern to tilapia and will not be considered further.

Infectious salmon anaemia virus (ISAV) only causes natural disease in salmonids (OIE 2006a, OIE 2006b). It has been isolated from pollock (*Pollachius virens*) and cod (*Gadus morhua*) in the vicinity of infected salmonid farms and from experimentally infected Artic char (*Salvelinus alpinus*) and herring (*Clupea harengus*) (OIE 2006b). There is no evidence that it would infect fish from the family Cichlidae.

Red Sea bream iridovirus (RSIV) infects a range of marine fish only (OIE 2006a, OIE 2006b).

Koi herpes virus (KHV) appears restricted to *Cyprinus carpio* (OIE 2006a, OIE 2006b).

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

Bohle iridovirus, which affects both fish and amphibians, is restricted to Australia. Reports of infection are limited to an Australian aquatic laboratory (Ariel and Owens 1997) and as such this virus will not be considered further. An iridovirus-like agent identified in Canada was determined to be the cause of elevated mortalities of *Oreochromis niloticus* moved from Florida, USA (McGrogan *et al.* 1998). Iridoviruses, however, represent an area of considerable uncertainty. The number of susceptible species being identified is increasing, as are the number of countries infected with a range of iridoviruses. Iridoviruses are known from freshwater ornamentals in Asia (Ahne *et al.* 1997) and therefore, in a precautionary measure, iridoviruses will be considered further.

Tilapia larval encephalitis virus (TLEV), a herpes-like virus causing neurological signs and increased mortality, has only been reported from Israel (Avtalion and Shlapobersky 1994). There have been no reports of similar neurological conditions elsewhere and no identification of this virus outside Israel. TLEV will not, therefore, be considered further.

Aquatic birnaviruses are widespread in the aquatic environment and are known to infect Cichlidae (OIE 2006a, OIE 2006b). Tilapia have been shown to be experimentally susceptible to birnaviruses (Tu *et al.* 2003) and an aquabirnavirus related to the Ab serotype of IPNV was isolated from *O. mossambicus* in Taiwan (Hedrick *et al.* 1983). IPNV has been isolated in China (Jiang and Li 1987, Niu and Zhao 1988, Jiang *et al.* 1989, Liu *et al.* 1991) and therefore aquatic birnaviruses will be considered further.

3.2.2 Bacteria

Tilapia, under conditions of stress, 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.

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 from 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.

Aeromonas spp. are generally ubiquitous opportunist pathogens and *A. hydrophila* (Diggles *et al.* 2002) and *A.sobria* already occur in New Zealand. *A. salmonicida*, both typical and atypical strains, are considered exotic to New Zealand. Atypical *A. salmonicida* has been isolated from *O. niloticus* (Castro-Escarpulli *et al.* 2003), although not in the exporting countries. Atypical strains of *A. salmonicida* have been reported from China (Yang and Chen 1996, Wang and Huang 2006) and there has been at least one report from Brazil (Pavanelli *et al.* 2000). The potential for tilapia to be carriers of *A. salmonicida* warrants further consideration in this risk analysis.

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 *Oreochromis* spp. (Amin *et al.* 1988, Badran *et al.* 1994, Figueiredo *et al.* 2005) it is necessary to consider these organisms further.

Streptococcus spp., gram positive cocci, are common in the environment and some species are found worldwide (Buller 2004). They tend to be opportunistic pathogens of stressed fish and they respond to treatment and changes in the environment. As such they will be excluded from this risk analysis. However, one *Streptococcus* sp., namely *S. iniae*, is considered exotic to New Zealand and can cause serious outbreaks. It also has zoonotic potential and given its identification in *O. niloticus* (Bowser *et al.* 1998) it will be considered further.

Edwardsiella tarda is widespread globally, although it is considered exotic to New Zealand. It is an opportunistic pathogen and has been reported from *O. niloticus* in Brazil (Muratori *et al.* 2000). The outbreaks presented as septicaemia and whilst the majority of the bacteria will be located in the internal organs it is apparent from the behaviour of the related *E. ictaluri*, that muscle tissue will also carry *Edwardsiella* bacteria (Ferguson *et al.* 2001). As *E. tarda* can have serious consequences, is exotic and has zoonotic potential it will require further consideration. *E. ictaluri* itself has been reported once in experimentally infected *Sarotherodon aureus* (= *O. aureus*) (Plumb and Sanchez 1983). Its geographical distribution is, however, more restricted than *E. tarda,* being reported from Australia, Taiwan, Thailand, the USA (Plumb 1999) and Vietnam (Buller 2004). *E.ictaluri* has also been identified in Brazil (Segabinazi *et al.* 2005) and, presumably, China given the reports of a vaccine trial in Guangxi province (Yu *et al.* 2005). Since infection is possible in tilapia, albeit experimentally, and it has been isolated in Brazil and has a presumed presence in China, *E. ictaluri* will be considered alongside *E. tarda*.

Vibrio vulnificus is associated with severe foodborne illness in humans (Buller 2004) and has been reported from New Zealand, China and Brazil. It may also cause disease amongst fish populations, resulting in septicaemia. *V. vulnificus* is primarily a marine organism. It requires ≥ 0.5 percent NaCl to grow (Buller 2004). It is primarily linked with the consumption of raw, marine filter feeders and may be associated with contamination of wounds by direct contamination from the marine environment. Given this, its isolation in the freshwater environment is a rare occurrence. As a ubiquitous marine organism, there is negligible likelihood that importation of the commodity would increase the risk of exposure for people

here. In addition, given its cosmopolitan distribution and its clear picture of septicaemia and ulceration in infected fish it will not be considered further in this risk analysis.

Vibrio ordalii has a predilection for muscle tissue and therefore is more likely than other *Vibrio* spp. to be present in the commodity. It is considered to be present in the aquatic environment worldwide (Buller 2004) and has been isolated in New Zealand (Diggles *et al.* 2002). Therefore, *V. ordalii* will not be considered further in this risk analysis.

Both *Photobacterium damselae damselae (Vibrio damselae)* and *Listonella anguillarum* are ubiquitous opportunists with a cosmopolitan distribution. They have been isolated in New Zealand and will not be considered further. Human infections with *P. damselae damselae* have been reported following filleting injuries (Novotny *et al.* 2004). As the product is highly processed on import there is negligible likelihood that the importation of the commodity would result in increased risk of exposure to the public.

Rickettsia-like organisms are well known in the environment and have been linked to epithelial cell hypertrophy in a number of aquatic animal species in New Zealand, for example oysters, scallops and clams, causing hypertrophy of gill and digestive tract epithelium but with little consequence (Hine 2002). These epitheliotropic rickettsial organisms appear ubiquitous and will not be considered further. However, there is a report of a systemic disease associated with rickettsia-like organisms in *O. niloticus* from Taiwan (Chen *et al.* 1994). The clinical signs included skin ulceration and multiple granulomata in the internal organs, a clinical picture more reminiscent of piscirickettsiosis, a serious rickettsial disease of salmonids caused by the notifiable organism *Piscirickettsia salmonis.* These organisms will therefore be considered further.

Epitheliocystis is an epithelial disease, characterised by massive hypertrophy of individual epithelial cells on the integument or in the gills. Generally the condition is benign, but heavy infections may compromise gill or skin function. It is considered to be caused by organisms in the order Chlamydiales (Lannan *et al.* 1999, Nowak and LaPatra 2006) and has worldwide distribution but high host specificity (Meijer *et al.* 2006, Nowak and LaPatra 2006). Because of their ubiquitous nature and high host specificity, the causative agents of epitheliocystis will not be considered further.

The enterobacterium *Yersinia ruckeri* is the cause of enteric redmouth (ERM) disease in a range of fish species including salmonids (Horne and Barnes 1999). It is known that a carrier status can exist. This organism has been isolated from New Zealand, at this time from two East coast hatcheries only (Diggles *et al.* 2002), and is known to occur in China (Raidal *et al.* 2004). There are a number of strains causing the more serious ERM and the milder yersiniosis (Buller 2004). Given the geographic localisation within New Zealand and the range of strains, *Y. ruckeri* will be considered further.

Staphylococcus spp. are normal flora found on the surface of fish, however in stressed fish they may cause systemic infections (Ahmed *et al.* 1990, El-Khatib 1998, Huang *et al.* 1999). *S. epidermidis* is common in marine and estuarine environments (Buller 2004) and can be expected to be present in both the exporting countries and New Zealand. 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. The ubiquitous and opportunistic nature of the organism means that there is no need to consider it further in this risk analysis.

Plesiomonas shigelloides has been reported from New Zealand and is considered to be ubiquitous in the environment (Buller 2004). Tilapia fry in Taiwan were reported to have suffered mortalities as a result of *P. shigelloides* infection (Faisal *et al.* 1989). *P. shigelloides* can cause diarrhoea, fever, vomiting and abdominal pain (Novotny *et al.* 2004), however the public is more likely to be exposed to the organism from sources here, then from the importation of the commodity. As this organism is rarely reported, and only from fry, and is already present in New Zealand it will not be considered further.

The enterobacterium, *Providencia rettgeri,* was isolated from the kidney of *O. niloticus* in Egypt (Faisal *et al.* 1987). A related *Providencia* sp. is present in New Zealand. There have been no other reports and it is highly unlikely to be associated with fillets and will not, therefore, be considered further in this risk analysis.

A *Francisella* sp. bacterium was determined to be the cause of visceral granulomatosis of *Oreochromis* spp. in Taiwan (Hsieh *et al.* 2006). This condition has never been reported in New Zealand. *Francisella* spp. are facultatively intracellular bacteria (Olsen *et al.* 2006). This organism will be considered further, along with the rickettsia-like organisms highlighted earlier.

Pasteurella multocida infection of fish is extremely rare, with only one reported case in hybrid tilapia in Israel (Nizan and Hammerschlag 1993). As this organism has not been reported widely in fish, or from the exporting countries, and as it is endemic in other animals in New Zealand, it will not be considered further.

Actinomyces spp. are found in the environment, are recorded in New Zealand and rarely cause disease in aquatic animals. Given their ubiquitous and opportunistic nature, and whilst they may cause disease in humans, their presence in tilapia is extremely rare, they will not be considered further in this risk analysis.

3.2.3 Parasites

Ichthyobodo necatrix is solely an ectoparasite affecting the skin and gills (Woo 2006). Both these organs are removed in the production of fillets. Even if there was some cross contamination of the fillet meat the parasites would be washed off with the chlorinated water used during processing. This organism is ubiquitous, not associated with the commodity, and therefore does not require further consideration.

Similarly, *Piscinoodinium* spp. are obligate ectoparasites and would not be associated with the commodity despite being reported from tilapia in Brazil (Martins *et al.* 2001, Tavares-Dias *et al.* 2001a). These organisms will not be considered further.

The *Trypanosoma* spp. are blood parasites and would be expected to be mostly removed from the fish during bleeding. Some *Trypanosoma* spp. have been reported from New Zealand, and it is likely that any found in tilapia would be reasonably host specific. They are unlikely to be found in significant numbers in fillet meat following bleeding, and any that are present can be expected to be inactivated by the freezing process. As such these organisms will not be considered further.

The amoebae, *Hartmanella* spp., *Mayorella*-like spp., *Platyamoeba*-like spp. and *Rosculus ithacus,* are found in the internal organs of the fish (Dykova *et al.* 1997) and have not been reported from the exporting countries. Whilst this does not preclude their presence, the

removal of the viscera during processing would prevent their entry in the commodity and they do not require further consideration.

The ciliophorans, *Cryptocaryon irritans, Trichodinella* spp., *Trichodina* spp., *Ichthyophthirius multifiliis, Chilodonella* spp. and *Tripartiella* spp. are all ectoparasites that are common in the aquatic environment and would not be associated with the commodity. They do not, therefore, require further consideration.

The apicomplexans, *Eimeria vanasi* and *Goussia cichlidarum*, are found in the intestines and swim bladder respectively (Landsberg and Paperna 1987). Both these organs are removed during processing therefore neither organism is associated with the commodity and need no further consideration.

Tilapia are known to be susceptible to *Enteromyxum leei*, a member of the family Myxidiidae, with, unusually for a myxosprean, a direct life cycle (Diamant *et al.* 2006). However, the infection is limited to the intestinal tract and thus would not be associated with the commodity. *E. leei* will not, therefore, be considered further.

Henneguya piaractus and other *Henneguya* spp. are myxosporeans. *H. piaractus* has been reported from tilapia in Brazil (Tavares-Dias *et al.* 2001b), however it has a predilection for the gills and would not be associated with the commodity. Tilapia could however be infected with other *Henneguya* spp., some of which may cause infection of the musculature and thus be associated with the commodity. Whilst *Henneguya* spp. have been reported in New Zealand, they were found in eels (different host range) and geographically restricted to one lake (Hine 1978). It is therefore necessary to consider *Henneguya* spp. further.

Myxobolus spp., also myxosporeans, 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 from New Zealand, including *M. cerebralis*, the species pathogenic to salmonids (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. The *Myxobolus* spp. reported in literature from tilapia infects the eyes, spleen, kidney, skin, gill, liver and ovary. All these organs would be removed during processing. These organisms will not therefore be associated with the commodity and do not need to be considered further.

The monogenean parasites *Dactylogyrus* spp., *Enterogyrus* spp., *Gyrodactylus* spp., *Cichlidogyrus* spp. and *Neobenedenia melleni* will not be associated with the commodity being ectoparasites of the skin and gills, with the exception of the *Enterogyrus* spp. which are found in the stomach and anterior intestine. Whilst there is one report of *E. cichlidarum* being found in peritoneal cavity, liver, heart and swim bladder (Noga and Flowers 1995), these organs are removed during processing. These monogeneans will not be considered further in this risk analysis.

The digenean parasites *Diplostomum* spp., *Posthodiplostomum cuticula, Carassotrema tilapiae, Amirthalingamia* sp. and *Cyclustera* sp. will not be considered further as they have a predilection for the eyes, skin, intestine and liver respectively and would not therefore be associated with the commodity. However, the digenean parasites *Clinostomum* spp., *Euclinostomum* spp., *Pygidiopsis* spp., *Centrocestus* spp., *Heterophyes* spp., *Ascocotyle ascolonga, Clonorchis sinensis, Haplorchis* spp., *Metagonimus* spp., *Phagicola ornata, Pharyngostomum flapi, Prosostephanus industrius, Procerovum calderoni, Moedlingeria amphoraeformis, Bolbophorus levantinus, Echinochasmus perfoliatus* and *Prohemistomum*

vivax may be found encysted in muscle tissue and could be associated with the commodity. In addition a number are zoonotic (Paperna and Dzikowski 2006) and require further consideration.

Both *Paradilepsis* sp. and *Wenyonia* sp. are cestodes (tapeworms). *Paradilepsis* sp. was reported from the skin, underneath the scales (Ezeri 2002), whilst *Wenyonia* sp. was reported from the intestine (Nmor *et al.* 2003). Neither would be associated with the commodity due to the processing involved, therefore, they will not be considered further in this risk analysis.

The acanthocephalans, *Acanthogyrus* sp., *Octospiniferoides* sp., *Polyacanthorhynchus kenyensis* and *Acanthocephalus lucii* are all found in the intestines and thus would not be associated with the commodity and do not require further consideration.

The nematodes, *Contracaecum* spp., are found as larvae in fish (Molnar *et al.* 2006). Whilst the report of infection in tilapia in Egypt indicates the larvae were found in the mesentery (Aloo 2002), it is possible for nematode larvae to infect the musculature and thus pose a risk to consumers of the end product (Ko 2006). Nematodes infecting fish have a cosmopolitan distribution and the likelihood remains that fish could have variable burdens of nematodes, thus the presence of larval nematodes in the commodity will be considered further in this risk analysis. The presence of both nematode larvae and plerocercoids of cestodes can be considered together.

Camallanus spp. and *Cucullanus* spp. develop into adults in the intestine of fish (Molnar *et al.* 2006) and thus would not be associated with the commodity and therefore do not require further consideration.

Piscicola geometra, an annelid worm, is purely ectoparasitic and would be removed during processing. It requires no further consideration. Similarly the arthropod parasites, *Argulus* spp., *Lamproglena* spp., *Lernaea cyprinacea, L. tilapiae, Caligus epidemicus* and *C. orientalis* are all ectoparasites and would be removed during processing. They will not be considered further.

3.2.4 Fungi

Saprolegnia spp. are ubiquitous oomycete fungi that can cause skin lesions on a wide range of fish species. Due to their ubiquitous and opportunistic nature they will not be considered further.

Ichthyophonus hoferi, which may be considered to be a protozoan, can infect musculature as hard to detect, resting spore stages (McVicar 1999). It is considered, by some authors, to have a global distribution (McVicar 1999), but it is not recorded from New Zealand. Given that it is possible that it is present in exporting countries it is necessary to consider it further for completeness.

Branchiomyces sp. infect the gills of fish and as such would be removed during processing so will not be associated with the commodity and therefore will not be considered further.

Rhizomucor spp. are present in New Zealand and are considered as ubiquitous in the environment, affecting only stressed fish. *Rhizomucor* spp. are commonly associated with compost heaps and are a rare human pathogen. As disease in fish is rare and produces obvious clinical signs the commodity does not represent any increased risk to human health. This fungus will not, therefore, be considered further.

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 tilapia are not listed specifically, it is known that other cichlids can be infected (Pathiratne and Rajapakshe 1998) and tilapia may be carriers or become contaminated by spores. In addition there are susceptible species in New Zealand and as a precautionary measure this organism will be considered further.

3.2.5 Water-borne food poisoning

Contamination of fillets with water not of a suitable purity could result in the presence of exotic strains of *Escherichia coli, Salmonella* spp., *Vibrio cholerae* and *Cryptosporida* 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 89 genera of potential pathogen that are, or could be, linked with tilapia. Following consideration of a range of factors, including the likelihood of association with the commodity and presence or absence from New Zealand and the exporting countries, it was concluded that 13 organisms, or groups of organisms, required further consideration, namely:

- iridoviruses
- aquatic birnaviruses (including IPNV)
- *Aeromonas* spp.
- *Flavobacterium* spp.
- *Streptococcus iniae*
- *Edwardsiella* spp.
- *Rickettsia*-like organisms (including *Francisella* spp.)
- *Yersinia ruckeri*
- *Henneguya* spp.
- digenean parasites encysting in muscle tissue
- larval nematodes
- *Ichthyophonus hoferi*
- *Aphanomyces invadans*

In addition it was decided that it was necessary to consider any water used in processing, in terms of water-borne 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 have been 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 (RSIV)
- 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: 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, tilapia were not tested (Langdon 1989).

The iridovirus, European catfish virus (ECV), which is related to EHNV, infects *Ameiurus melas* (OIE 2006b). This species is related to *A. nebulosus,* a fish that has been introduced, and has established in New Zealand. ECV has been reported from France and Italy only (Ahne 1994, 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, Australia, Europe and the USA (Ahne *et al.* 1997). A number of ornamental cichlids are known to be susceptible to iridovirus infection including *Apistogramma* spp., *Etroplus* spp. (Armstrong and Ferguson 1989) and *Pterophyllum* spp. (Ahne *et al.* 1997). Iridoviruses have also been reported from a number of other ornamental Cichlidae from South East Asia (Hetrick and Hedrick 1993). Of greater interest is the isolation of a putative iridovirus in *Oreochromis* spp. (McGrogan *et al.* 1998), which demonstrates their susceptibility to these agents. The 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: *Oreochromis* spp. are almost certainly susceptible to iridovirus infection and it is likely that some infections would be subclinical as has been found in other fish species (Anderson *et al.* 1993), thus a variable viral titre is possible in the tilapia population. Whilst iridoviruses have not been reported from diseased, or healthy, tilapia in China or Brazil the global distribution of iridoviruses suggests that farmed tilapia may well be exposed to exotic strains of iridoviruses.

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.

Oreochromis spp. infected with a putative systemic iridovirus displayed classic septicaemic signs, including dark colouration, lethargy, erythema, gill pallor, exophthalmia and ascites (McGrogan *et al.* 1998); findings very similar to iridovirus infection in another cichlid, *Etroplus maculatus* (Armstrong and Ferguson 1989). Given this clinical picture it is highly unlikely that tilapia with clinical iridovirus septicaemia would be harvested for export processing. That is, normal commercial quality assurance measures would mitigate septicaemic fish being exported. However, subclinically infected animals may be harvested.

Iridoviruses can occur in a carrier state in some fish species. It is not known if tilapia can act as true carriers, but it must remain a possibility. 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 Brazil or China 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 extremely 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 $10^{3.2}$ $TCID_{50}$/g of spleen to $10^{5.2}$ $TCID_{50}$/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 cytopathic effect in 100 percent 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 $10^{6.5}$ $TCID_{50}$/mL for 1 hour resulted in a mortality rate of less than 17 percent, although 40 percent became infected (Plumb and Zilberg 1999a). Per os dosing of $10^{5.6}$ $TCID_{50}$ per fish resulted in 21 percent 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. Thus, no further assessment is required and no specific sanitary measures are warranted.

4.2. AQUATIC BIRNAVIRUSES (INCLUDING IPNV)

4.2.1 Aetiological agent: Aquabirnaviruses are non-enveloped, icosahedral, double-stranded RNA viruses of the Birnaviridae family. This family includes infectious pancreatic necrosis virus (IPNV), the cause of infectious pancreatic necrosis (IPN). These viruses are also responsible for diseases such as turbot haematopoietic necrosis, yellowtail ascites disease, eel nephritis, kumura shrimp disease and clam gill necrosis (Reno 1999).

4.2.2 OIE List: Not listed

4.2.3 New Zealand status: An aquatic birnavirus has been recorded from returning, sea run Quinnat salmon (*Oncorhynchus tshawytscha*) caught in the South Island of New Zealand on a number of occasions (Tisdall and Phipps 1987, Anderson 1996, Anderson 1998). The fish showed no clinical signs of infection. Aquatic birnaviruses have also been isolated from turbot and flounder following mass mortalities in aquaculture facilities (Hine, P M; Pers. Comm.), although these isolates were non-pathogenic to salmon (Horner 2003).

Exotic strains of IPNV are notifiable organisms in New Zealand.

4.2.4 Epidemiology: Aquatic birnaviruses have been isolated from at least 70 species of aquatic animals including two cichlids (*Symphysodon discus* and *Oreochromis mossambicus*), molluscs and crustaceans (Reno 1999). Aquatic birnaviruses have been isolated from China and Taiwan, amongst other countries. Asian isolates tend to be of the Ab serotype, although Wb and Sp serotypes have been isolated, but are restricted to farmed salmonids (Ahne 1994).

Transmission of aquatic birnavirus in fish is direct, horizontal via faeces and urine as well as vertical (Mortensen *et al.* 1992, Reno 1999). The birnavirus that causes IPNV affects young fish and smolts moved to seawater, but tends to be carried subclinically in older fish (Reno 1999). Birnaviruses may be isolated from water (Mortensen *et al.* 1992, McAllister and Bebak 1997), indicating the relative ease of horizontal transmission. Birnaviruses taken up by other animals, including crayfish, birds and mammals, can be viable when excreted and can infect fish experimentally. However, the principal method of translocation remains live fish and eggs (Reno 1999). Infection of tilapia with IPNV results in a classical septicaemic clinical picture with ventral skin haemorrhages, especially of fin bases and around the vent. Internally the virus markedly affects the liver, intestine and swim bladder with high virus titres in the liver and intestines, although the virus can also be detected in the kidney and brain (Mangunwiryo and Agius 1987). *In vitro* infection of various tilapia tissues resulted in highest titres in skin and muscle, however when repeated *in vivo*, a more natural model, the highest titres were recorded in the intestine, kidney and spleen, with the intestine harbouring virus for up to 35 days (Tu *et al.* 2003).

The virus is relatively stable and can survive for nearly a year at 4 °C in buffer and for long periods in sea water, brackish water and freshwater (Reno 1999). It is reasonably stable at -20°C, although each freeze/thaw cycle reduces viable virus titre (Mortensen *et al.* 1998).

4.2.5 Entry assessment: For aquabirnaviruses to enter New Zealand in the commodity, fish infected with an aquabirnavirus would have to be harvested and processed. The virus would have to be present in the muscle tissues prepared into the fillets and survive the freezing process and storage prior to import.

It has been shown that tilapia infected with IPNV display classical septicaemic signs and develop pathological changes that would render the fish unfit for human consumption (Mangunwiryo and Agius 1987). These not only include the haemorrhagic and septicaemic changes but could also include muscle necrosis (Eleouet *et al.* 2001) that would further render the fillets unmarketable. It is unlikely therefore that clinically diseased fish would be harvested for export processing. However, it is possible that subclinically infected fish could be harvested, however the viral titre in these fish is expected to be much lower or undetectable (Bowden *et al.* 2002). Given that *in vivo* the highest titres are present in intestine, kidney and spleen, it is likely that muscle titres in subclinical fish would be undetectable, but could still be present.

Since the virus is reasonably stable at -20°C, it would be reasonable to conclude that, if there was virus present in the fillet meat, it would survive the freezing process and transport to New Zealand.

Given the factors discussed above, the likelihood of entry is assessed to be extremely low but non-negligible.

4.2.6 Exposure and establishment assessment: Exposure of susceptible native fish to an exotic aquabirnavirus would require imported fillets or scraps derived from them, infected with an aquabirnavirus, entering the aquatic environment in sufficient quantities to produce an infectious dose. Evidence suggests that viral titres of muscle tissue are significantly lower than titres found in the internal organs.

There are no literature reports of the spread of IPN or iridoviral diseases 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 would be extremely 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

IPNV challenge trials with highly virulent Sp serotypes of the virus routinely use 10^5 PFU/mL to achieve infection (McAllister and Bebak 1997, Gibson *et al.* 1998). This dose is equivalent to approximately 1.4 x 10^5 $TCID_{50}$/mL. Fillet tissue, even of carrier fish, is expected to have virtually undetectable levels. Carrier salmonids had a muscle titre of $10^{0.3}$ (= 2) $TCID_{50}$/g. The infectious dose of IPNV by immersion varies according to the strain of virus used and the species challenged. However, some useful information can be obtained from recent challenge experiments with virulent Sp, N1 and VR-299 serotypes of IPNV:

a) challenge titres of 10^5 pfu/mL (approximately 1.4 10^5 $TCID_{50}$/mL) induced mortalities of up to 95 percent in brook trout (*Salvelinus fontinalis*) (McAllister and Owens 1995, McAllister *et al.* 2000) and 20 percent in Arctic char (*S. alpinus*) (McAllister *et al.* 2000);
b) challenge titres of 10^4 $TCID_{50}$/mL induced mortalities of 20 percent to 76 percent in Atlantic salmon (*Salmo salar*) and 43 percent in *Salvelinus fontinalis* (Taksdal *et al.* 1997, Kjoglum *et al.* 2005); and
c) challenge titres of 10^3 pfu/mL (approximately 1.4 10^3 $TCID_{50}$/mL) failed to induce mortalities in *S. alpinus*, but did result in ≤ 10 percent carrier status (McAllister *et al.* 2000).

It is apparent that the viral titre required to induce infection by immersion challenge is less than 10^3 $TCID_{50}$/mL. Even to reach a highly conservative value of 10 $TCID_{50}$/mL it would be necessary to suspend the viral content of 5000g of muscle in every litre of water. The likelihood of discarded material from the proposed commodity accumulating to this level is remote in the extreme.

Oral challenge of brown trout (*Salmo trutta*) with IPNV (N1 serotype) at doses of $10^{2.5}$ to $10^{3.2}$ $TCID_{50}$/g food failed to transmit infection to the experimental salmonids, whereas doses of 10^{6} $TCID_{50}$/g food resulted in detectable infection in the trout (Mortensen 1993). The viral titre in the flesh of imported carrier fish is expected to be, conservatively, 150 times less than the dose that failed to transmit IPNV orally.

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).

The likelihood, therefore, of potentially harmful aquabirnaviruses from the commodity causing disease in a susceptible host in New Zealand, or becoming established in the environment is so low as to be negligible. No further risk assessment is required and no specific sanitary measures are warranted.

4.3. AEROMONAS SALMONICIDA

4.3.1 Aetiological agent: *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: In an earlier MAF risk analysis (MacDiarmid 1994), Dr Trevor Evelyn was reported as discussing the host range of *A. samonicida*. 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. Farmed salmonids are present in China and Brazil, with one report of isolation of typical *A. salmonicida* in Brazil (Pavanelli *et al.* 2000). 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.

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).

Whilst there has been one report of an atypical subspecies of *A. salmonicida* being isolated from tilapia flesh (Castro-Escarpulli *et al.* 2003), the number of freshwater species from which the bacteria are being isolated from is rapidly increasing (Hiney and Olivier 1999) and *Aeromonas* spp. are commonly found in the environment. As a precautionary measure this risk analysis will assume that infection could be present, but unreported, in China or Brazil.

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. 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 generally accepted as the primary means of spread in the translocation of *A. salmonicida* (Hiney and Olivier 1999).

Data specific to the atypical subspecies of *A. salmonicida* are lacking, however there is 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.

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 SPP.

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. *F. johnsoniae* has been shown experimentally to affect tilapia (Flemming *et al.* 2007).

4.4.2 OIE List: Not listed

4.4.3 New Zealand status: *F. columnare* is considered to be present in New Zealand (Boustead 1982, Diggles *et al.* 2002), but more virulent genomovars (Michel *et al.* 2002) are considered to be exotic (Duignan *et al.* 2003). *F. johnsoniae* is also considered to be exotic.

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

Flavobacteria may also cause disease in humans but the species involved appear 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).

Variable levels of virulence and pathogenicity, of different *F. johnsoniae*-like isolates, to tilapia have been reported. Where virulence and pathogenicity were high, this was associated with gill necrosis, fin rot and skin ulceration (Flemming *et al.* 2007). 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 tilapia, the ubiquitous nature of this family of bacteria means that the possibility of it infecting *Oreochromis* spp. cannot be discounted.

4.4.5 Entry assessment: The primary site of attachment and action on the fish is the gills and skin. 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 skin ulceration and 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., entering 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 would be extremely 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. No further assessment is required and no specific sanitary measures are warranted.

4.5. *STREPTOCOCCUS* SPP.

4.5.1 Aetiological agent: *Streptococcus iniae* is a gram positive, facultatively anaerobic, coccoid bacterium that frequently forms chains (Kusuda and Salati 1999). It is a member of the Streptococcaceae. Other significant members of this group, as regards disease in fish, are *S. agalactiae* Group B (β-haemolytic) and *S. agalactiae* Group B type 1b (non-haemolytic) (formerly *S. difficile*) (Buller 2004).

4.5.2 OIE List: Not listed

4.5.3 New Zealand status: *S. iniae* not reported and considered exotic. *S. agalactiae* is found in New Zealand.

4.5.4 Epidemiology: *Streptococcus* spp. have been reported from diseased tilapia from as early as 1970, with the first identification of *S. iniae* in 1976 (Salvador *et al.* 2005). *Streptococcus* infection is now widespread in aquaculture and reported from wild fish mortality events. *S. iniae* has been reported from China (Buller 2004) and a group B *Streptococcus* sp. from tilapia in Brazil (Salvador *et al.* 2003). *S. iniae* was thought to have entered the USA in 1994 (Perera *et al.* 1994) demonstrating its propensity to translocate.

Streptococcosis is primarily a disease of stressed fish, especially those stressed by poor water quality. *Streptococcus* spp. are known to survive well in the environment around infected farms (Kusuda and Salati 1999), where infections tend to recur. Transmission is direct and horizontal via the water column with uptake of the agent via ingestion, across the gills, via skin lesions or across the nares, although experimentally nares inoculation is most effective (McNulty *et al.* 2003b). In general, the disease course is short with an incubation period in the order of two days (Evans *et al.* 2000, McNulty *et al.* 2003a). Fish display lethargy and dark colouration with variable degrees of other cardinal signs of septicaemia (ascites, eye haemorrhage, skin haemorrhage, behavioural changes etc.) (Perera *et al.* 1998, Chen *et al.* 2004). Survivors tend to develop a strong cellular immunity (Kusuda and Salati 1999).

Histopathology demonstrates cocci in the blood, cellular infiltration and inflammation in the eyes and kidney, granulomatous meningitis, epicarditis, myocarditis and bacterial colonies in the subcapsular capillaries of the liver and in splenic sinusoids (Perera *et al.* 1998). Somatic muscle does not seem to be a primary target of the bacteria and plasma levels of creatine kinase in infected tilapia remain normal, indicating no significant muscle pathology (Chen *et al.* 2004). The viscera, blood and brain are expected to contain the highest level of bacteria.

In one challenge study, *S. iniae*, was administered into the nares of *Oreochromis niloticus*. A dose of 4.8×10^5 PFU/fish was required to induce mortality of 20 percent. Fish inoculated with doses 10-fold and 100-fold less did display transient lethargy but recovered fully (Evans *et al.* 2000), indicating that low doses do not lead to a self sustaining infection in populations.

S. agalactiae can be recovered from frozen fish tissue after 180 days at -70 °C, and after 9 months at -20 °C. However, to effect recovery of the bacterium it was necessary to sample brain, eye, kidney and intestine, rather than fillet meat. The organs sampled representing those primarily affected by the bacterium (Evans *et al.* 2004).

S. iniae may also be associated with disease in humans, although this is linked with people handling and processing freshly killed fish (Novotny *et al.* 2004). As this commodity is highly

processed, and the muscle tissue is not a primary site of infection, the likelihood of this commodity resulting in disease in humans in New Zealand is negligible.

4.5.5 Entry assessment: Tilapia are susceptible to pathogenic *Streptococcus* spp., including *S. iniae*. These potentially serious pathogens have been reported from China (Buller 2004) and from tilapia in Brazil (Salvador *et al.* 2003, Salvador *et al.* 2005). However, infection is followed by a reasonably short incubation period before the onset of clinical signs of septicaemia. It is highly unlikely that these fish would be harvested for human consumption. Even if they were, the histopathology and clinical pathology suggests that the muscle tissue would contain considerably fewer bacteria than the viscera, which would be discarded (Perera *et al.* 1998, Chen *et al.* 2004). There remains the potential for incubating fish or subclinical carriers, perhaps from a non-naive population in an endemically located area, to be harvested. These fish may contain bacteria; however it is likely that they would be concentrated in the gills, eyes, liver, spleen and kidney. All are organs that would be removed during processing.

The likelihood is therefore that fillet meat derived from clinically normal fish would not contain significant numbers of exotic pathogenic *Streptococcus* spp. bacteria. The small number that might be contained in the tissue would, however, be likely to survive the freezing process and subsequent transport to New Zealand. So whilst the likelihood of viable *S. iniae* being present in the commodity is low it is non-negligible and further assessment is required.

4.5.6 Exposure and establishment assessment: Exposure of susceptible native fish to *S. iniae* (or any other exotic pathogenic *Streptococcus* spp. for that matter) would require infected fillet meat or scraps derived from them entering the aquatic environment in sufficient quantities to produce an infectious dose. Evidence suggests that the concentration of the bacterium in muscle tissue is significantly lower than in the internal organs, particularly in the case of inapparent infections.

There are no literature reports of the spread of piscine streptococcosis via the movement of dead fish for human consumption. Zoonotic infections tend to result from handling and processing infected, whole fish. The commodity itself is highly processed and it is likely that the volume of scraps generated would 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, 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).

Taking all these factors into consideration, the likelihood that exotic pathogenic *Streptococcus* spp. (including *S. iniae*) would be exposed to, and establish in, native fish is so remote as to be negligible. Thus, no further assessment is required and no specific sanitary measures are warranted.

4.6. EDWARDSIELLOSIS

4.6.1 Aetiological agent: *Edwardsiella tarda* is a facultatively aerobic, gram negative, usually motile, rod shaped member of the enterobacteriaceae (Plumb 1999, Buller 2004, Panangala *et al.* 2006). *E. ictaluri* is a gram negative, pleiomorphic, generally rod shaped member of the Enterobacteriaceae (Plumb 1999).

4.6.2 OIE List: Not listed

4.6.3 New Zealand status: Not reported, considered exotic

4.6.4 Epidemiology: *E. tarda* is related to *E. ictaluri* but has a much lower degree of homogeneity between strains when compared with *E. ictaluri* (30 percent homogeneity compared with ≥ 70 percent homogeneity respectively) and has a broader host range of both marine and freshwater fish (Panangala *et al.* 2006) as well as infecting reptiles, birds, cattle, pigs and marine mammals (Plumb 1999). Of significance is the susceptibility of *Salmo salar, Oncorhynchus mykiss, Mugil cephalus, Paralichthys olivaceus* and *Seriola* spp. (Benli and Yildiz 2004) to infection; all these fish being present in the waters of New Zealand.

The *E. tarda* bacterium causes a generalised enterohaemorrhagic septicaemia, characterised by haemorrhages on the skin and mouth and cutaneous ulceration (Benli and Yildiz 2004); this is accompanied by septicaemic signs such as reddening of fin bases and the development of exophthalmia and cataracts in some cases in tilapia (Plumb 1999). Cutaneous lesions can extend, resulting in deeper muscle necrosis. Internally the kidney and liver appear swollen and haemorrhagic and tilapia develop abscesses in their internal organs (Plumb 1999). In a study of naturally infected *O. niloticus*, 25 percent of the study population displayed clinical signs of *E. tarda* infection with the bacterium being isolated from 29 percent each of livers, kidneys and spleens, 14 percent of intestines and, significantly, there were no isolations from deeper muscle (Saleh 2005).

Transmission is direct and horizontal in the water column (Plumb 1999, Wiedenmayer *et al.* 2006), with entry of the pathogen via skin lesions or by ingestion (Plumb 1999). Disease usually only occurs in stressed or injured fish and is linked with poor water quality and increased water temperatures (Plumb 1999, Benli and Yildiz 2004, Wiedenmayer *et al.* 2006). Incubation periods vary between 2 and 7 days (Plumb 1999, Wiedenmayer *et al.* 2006).

E. tarda may be found in the intestinal tract of a number of aquatic organisms, including carrier fish (Plumb 1999), and is ubiquitous in the environment in endemic areas (Buller 2004, Wiedenmayer *et al.* 2006). *E. tarda* has been reported in turtles, eels, bullfrogs and humans from China (Plumb 1999) and from *O. niloticus* from Brazil (Muratori *et al.* 2000).

The *E. tarda* bacterium will survive for up to 50 days in whole fish frozen to -20 °C (Brady and Vinitnantharat 1990).

E. tarda is zoonotic, causing gastroenteritis and diarrhoea in humans (Plumb 1999, Benli and Yildiz 2004). On occasion, possibly because of the route of infection, concurrent disease or an immunocompromised patient, the bacterium will cause a typhoid-like illness, septicaemia, cellulitis and meningitis (Plumb 1999, Buller 2004). Infections are, however, considered to be rare and are associated with the consumption of raw fish or the contamination of wounds. Whilst there are no controls to prevent the consumption of raw product following import, clinically infected fish are unlikely to be processed and there is evidence that the muscle titre

is so low as to prevent isolation of the bacterium (Saleh 2005). The likelihood, therefore, that the commodity would result in increased exposure and disease in the public is negligible.

The only report of *E. ictaluri* in tilapia is an experimental infection to determine the susceptibility of *Sarotherodon aureus* (= *O. aureus*) to *E. ictaluri* (Plumb and Sanchez 1983). Intraperitoneal injection of 1.5 x 10^8 CFU of *E. ictaluri* resulted in 70 percent mortality, with a 5 day incubation period. Those tilapia injected with 1.5 x 10^7 CFU and 10-fold dilutions down to 1.5 x 10^3 PFU did not display clinical signs, nor suffered any mortality. The bacterium could be isolated from the peritoneal cavity of all fish and the liver and kidney of most fish for up to 10 days post inoculation. In subclinically infected fish the bacteria multiply in the liver, reaching a maximum titre at 72 hours post inoculation. The titre then declines (Plumb and Sanchez 1983).

4.6.5 Entry assessment: Exposure rates in natural *E. ictaluri* infections are likely to be in the order of 10^3 CFU (Plumb and Sanchez 1983). In addition, natural infections do not occur via the intraperitoneal route, an artificially direct entry route to the animal. It can be concluded that, as regards *E. ictaluri,* tilapia have a low susceptibility, and when combined with the low natural dose rate and no natural infections ever being reported, it is evident that the likelihood of tilapia becoming naturally infected with *E. ictaluri*, even transiently, is negligible and need not be considered further.

The *E. tarda* bacterium has been reported from both exporting countries and, unlike *E. ictaluri*, is known to naturally infect tilapia and become resident in the environment in infected areas.

It would be unlikely that clinically diseased fish would be harvested for export processing. Muscle lesions would result in rejection of fillets at inspection. That is, normal commercial quality assurance would mitigate the risk of clinically affected fish being exported. However, it is possible that subclinically infected animals, enteric carriers and healthy animals environmentally exposed to *E. tarda* could be harvested. These fish could harbour the bacterium, albeit at lower levels than clinically affected animals. In addition it is known that undetected muscle lesions in catfish suffering from edwardsiella septicaemia can contaminate processing equipment necessitating a halt in processing and cleaning of the equipment. The study of natural infection in *O. niloticus* (Saleh 2005) indicates that, even in clinically diseased animals, muscle tissue carries an extremely low burden of the bacterium. The majority of infection would be found in the internal organs.

If transportation to New Zealand and storage of the resultant frozen fillets took less than 50 days then viable *E. ictaluri* could remain in the commodity.

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

4.6.6 Exposure and establishment assessment: Exposure of susceptible native fish to *E. tarda* would require imported fillets or scraps derived from them, infected with *E. tarda*, entering the aquatic environment in sufficient quantities to produce an infectious dose. The concentration of the bacterium in muscle tissue is expected to be extremely low unless the fish is demonstrating necrotic muscle lesions, which would preclude the fish from being processed.

There are no reports of the spread of piscine *E. tarda* 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 would be extremely 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. Immersion challenges suggest that a relatively large dose of 10^6 – 10^7 CFU/mL would be required to induce disease (Wiedenmayer *et al.* 2006).

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 likely to be extremely low. 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).

Taking all these factors into consideration, the likelihood that *E. tarda* would be exposed to, and establish in, native fish is considered to be so remote as to be negligible. Thus, no further assessment is required and no specific sanitary measures are warranted.

4.7. INTRACELLULAR BACTERIA (RICKETTSIAE, *PISCIRICKETTSIA SALMONIS*, *FRANCISELLA* SPP. AND RICKETTSIA-LIKE ORGANISMS)

4.7.1 Aetiological agent: Rickettsiae are members of the α-proteobacteria class. They have a gram negative cell wall and replicate by binary fission (Lannan *et al.* 1999). *Piscirickettsia salmonis* and *Francisella* spp. are members of the thiotrichales order in the γ-proteobacteria class (Nylund *et al.* 2006, OIE 2006b). Rickettsia-like organisms (RLOs) may fall into the rickettsiales or be related to the thiotrichales, as the identification is usually morphological.

4.7.2 OIE List: Not listed

4.7.3 New Zealand status: Systemic *rickettsia*-like bacterium not reported and, thus, considered exotic. *Rickettsia*-like infections of epithelial tissues have been reported from molluscs here (Hine 2002) and are probably common in the environment.

4.7.4 Epidemiology: *P. salmonis* is a useful type-species to examine initially, followed by a consideration of *Francisella* spp. and RLOs in other species as the behaviours and risks appear similar.

P. salmonis infection was first reported in 1989 from *Oncorhynchus kisutch* (Lannan *et al.* 1999, OIE 2006b). The bacterium can be transmitted horizontally, directly between fish. This horizontal transmission is more effective when fish are in direct contact (Almendras *et al.* 1997), but can also occur via the water column without direct contact (Lannan *et al.* 1999, Mauel *et al.* 2003). Transmission via the water column is less likely for *P. salmonis* in freshwater as compared with seawater, as the agent is rapidly inactivated in freshwater but remains infective for up to 2 weeks in the marine environment (Lannan and Fryer 1994). Whether this is applicable to all strains of RLO or intracellular bacterium is unknown. *P. salmonis* seems to have the ability to penetrate intact skin and gills, although skin damage will assist entry (Smith *et al.* 1999).

In salmon, *P. salmonis* has an incubation period of approximately 12 days (Almendras *et al.* 1997) and infections result in lethargy, anorexia, gill pallor and dark body colouration. Affected fish can develop shallow haemorrhagic skin lesions. Internally the disease is marked by swollen spleen and kidney, liver lesions (may be characteristic grey rings), petechial haemorrhages on the visceral organs and internal fat. Histopathologically there is evidence of haematopoietic necrosis, necrosis of the intestinal epithelium, hyperplasia of gill epithelium, lamellar fusion, chronic inflammatory cell infiltration in the kidney and spleen and vascular and perivascular necrosis in the liver (Lannan *et al.* 1999). Experimental infection across the gill or by mouth results in a similar picture with vasculitis, fibrin thrombi, presence of the replicating agent in vacuoles in hepatocytes (Lannan *et al.* 1999) and focal necrosis. The agent targets endothelial cells giving rise to vasculitis and thrombosis, followed by perivascular ischaemic necrosis (Almendras *et al.* 2000). Reticuloendothelial cells in the kidney and liver are also affected, and the agent may be shed in the urine of infected fish (Almendras *et al.* 1997). Studies on *P. salmonis* indicate the gill and skin route of infection to bear a significantly greater chance of mortality than oral transmission (Almendras *et al.* 1997, Smith *et al.* 1999). In an immersion challenge, exposure to 10^5 $TCID_{50}$/mL for 1 hour resulted in only 10 percent mortality (Birkbeck *et al.* 2004). *P. salmonis* is sensitive to freezing; a single freez/thaw cycle at -70 °C reduced titres of the agent by 99 percent (Lannan *et al.* 1999).

Since the first cases of piscirickettsiosis were reported, other fish species have suffered mortalities as a result of similar organisms. In 2004, Sea bass displaying lethargy, inappetence, discolouration and up to 80 percent mortalities (Athanassopoulou *et al.* 2004) were found to be infected with a RLO. The pathological picture was remarkably similar to that seen in cases of piscrickettsiosis in salmonids. The kidneys were swollen and discoloured, the spleen was enlarged, there were internal haemorrhages in the fat, intestine and swim bladder, ascites was noted as was neurological involvement (Athanassopoulou and Karagouni 2004, Athanassopoulou *et al.* 2004, OIE 2006b). There was classical vascular and perivascular necrosis in the liver, accompanied by meningitis, endocarditis, peritonitis, pancreatitis and bronchitis (OIE 2006b). The RLO involved was found to be closely related to *P.salmonis* (Athanassopoulou *et al.* 2004), sharing antigens and identical DNA sequences of 16S rDNA (McCarthy *et al.* 2005).

A piscirikettsiosis-like disease was also reported for the first time in Hawaiian tilapia in 1994 (Mauel *et al.* 2003). In 2002 a study reported the granulomatous disease to be due to a rickettsia-like organism that did not react to *P. salmonis* specific PCR or IFAT (Mauel and Miller 2002).

In 1992 an outbreak of granulomatous disease was reported in tilapia in Taiwan (Hsieh *et al.* 2006). By 1994 there were mass mortalities in Taiwanese tilapia farms, with fish displaying clinical signs similar to *P. salmonis* infection in salmon. The *O. niloticus* were noted to be swimming erratically, pale, lethargic, off feed, displaying exophthalmia and developing haemorrhages and ulcers on their skin. Internally ascitic fluid was seen, as were enlarged spleens, kidneys and livers with marked white nodules. Histopathology revealed gill epithelial hyperplasia, RLO laden cells, fibrin thrombi, perivascular necrosis and chronic inflammatory cell infiltration (Chen *et al.* 1994). These outbreaks were reported as RLO infections (Chen *et al.* 1994, Chern and Chao 1994). Outbreaks continued through 1996 in both freshwater and seawater farms (Fryer and Lannan 1996), but no relationship with *P. salmonis* was ever reported. In 2000, groupers (*Epinephalus* spp.) in Taiwan were reported to be suffering a similar disease with virtually identical clinical signs and pathology. RLOs were identified in macrophages. They were shown to be positive to *P. salmonis* polyclonal antibodies (Chen *et al.* 2000). From 2001 to 2003 a similar granulomatous disease, tentatively diagnosed as a *Piscirickettsia*-like organism, was reported from tilapia in continental USA (Mauel *et al.* 2005) .

Ongoing studies of the granulomatous diseases in tilapia in Taiwan from 2001 to 2006 meanwhile showed the condition becoming widespread in fresh, salt and brackish water, infecting *O. mossambicus, O. aureus, Tilapia zillii* and *T. honorum* with mortality rates up to 95 percent (Mauel and Miller 2002). The organism involved in these cases was identified, by sequencing of 16s rDNA, to be a *Francisella* sp. (Hsieh *et al.* 2006). Similarly, from 2004, *O. niloticus* in a number of aquaculture facilities in Central America have displayed clinical and pathological signs of mortality, skin haemorrhage and erosion, splenomegaly and renomegaly with white granulomata and gill lesions, including necrosis and nodule development. In these outbreaks granulomata were present in the skeletal muscle of up to 14 percent of fish. Sequencing of the 16S rDNA indicated that the causative agent was a *Francisella* sp. (Mauel *et al.* 2007).

In 1999 and 2000, three-line grunts (*Parapristipoma trilineatum*) imported into Japan, from China, were found to be suffering from a granulomatous disease of their kidney and spleen. Intracellular bacteria were identified as *Francisella* sp. (Kamaishi *et al.* 2005).

Recently Atlantic cod (*Gadus morhua*) in Norway were found to be suffering from a granulomatous condition caused by a *Francisella* species. The 16s rDNA had related sequences to the *Francisella* sp. isolated from the three-line grunts in Japan and tilapia in Taiwan. DNA sequencing also indicated that the cod *Francisella* sp. probably represented a novel species as it was not identical to either *F. philomiragia* or *F. tularensis* (Nylund *et al.* 2006, Olsen *et al.* 2006). In an intraperitoneal injection challenge trial of *G. morhua*, a dose of 2×10^7 cultured infectious units resulted in 77 percent mortality over 122 days, with maximum mortalities between days 7 and 12. Fish introduced to the challenge tank at day 19 suffered 50 percent mortalities over the following 103 days. However, when cod were injected intraperitoneally with filtered splenic homogenate from *Francisella* sp. infected fish there was only a 13 percent mortality rate (Nylund *et al.* 2006). *Francisella* spp., like *P. salmonis*, can penetrate intact skin, resulting in local papular ulcers prior to lymphohaematogenous spread to parenchymal tissues (Hsieh *et al.* 2006).

It is unknown whether the RLO detected in Hawaii, and not reacting to *P. salmonis* PCR or IFAT could be a *Francisella* sp. bacterium.

4.7.5 Entry assessment: The diseases caused by intracellular bacteria appear clinically and pathologically similar and have demonstrated a widening host and geographic range. They have been found in fish exported from China (Kamaishi *et al.* 2005), although not specifically in tilapia, and there have been no reports of intracellular bacterium-linked disease in Chinese tilapia. As previously indicated a *Francisella* sp. bacterium has been reported from tilapia in Central America (Mauel *et al.* 2007), although not from South America at this time.

Whilst infection of fish tends to result in quite obvious clinical signs that would preclude the fish from being harvested for human consumption, the likelihood is that, were infection to be present in the areas the tilapia are farmed in, the fish could be subclinically affected, either during the incubation period or in more resistant individuals (mortality rates vary from 30-95%). In the case of the outbreaks in Central America, the majority of market size tilapia had splenic granulomata, despite a mortality rate of ≤ 50 percent (Mauel *et al.* 2007), clearly indicating the potential for a large proportion of the population to be infected with the organism without necessarily showing clinical signs.

The tropism of the organism for endothelial cells, reticuloendothelial cells, haematopoietic tissue and mononuclear phagocytes, the gross and histopathological patterns and clinical signs indicate that the highest infective burden would be in gills, liver, spleen, kidney and intestine. Muscle lesions have only been reported from the *Francisella* sp. outbreaks in Central America (Mauel *et al.* 2007) and it is possible that in very early infections there could be bacteria present in the muscle tissue without visible granulomata. In a sample of fish from these outbreaks, muscle granulomata were present in 11 percent of fish, whilst splenic and renal granulomata were present in 82 percent and 93 percent of cases respectively (Mauel *et al.* 2007). So, whilst this species of *Francisella* can affect muscle, it apparently preferentially targets kidney and spleen. The possibility still remains that a *Francisella* sp. bacterium could be present in muscle tissue without obvious granulomata, but logically at much lower levels than in the viscera, which would be removed, or in clinically affected muscle. Obvious muscle lesions would result in fillet rejection. It is considered that, overall, the likelihood of RLOs being present in the fillet meat is low but non-negligible.

Whilst a single freeze/thaw cycle at -70 °C was shown to reduce infective titre of *P. salmonis* by 99 percent (Lannan *et al.* 1999), it is likely that a temperature of -20 °C would be less efficient at inactivating any *Piscirickettsia* spp. present in the muscle tissue. *Francisella tularensis*, closely related to the *Francisella* spp. isolated from fish, is relatively stable during

freezing, with survival for up to 75 days in sheep muscle at -20°C (Airapetyan *et al.* 1957) and 94.1 percent survival in spleen and liver tissues of prairie dogs following freezing to -20°C for 3 weeks. Freezing of fillet tissue is unlikely to significantly reduce the muscle titre.

4.7.6 Exposure and establishment assessment: Exposure of susceptible native fish to these organisms would require imported fillets or scraps derived from them, infected with viable intracellular bacteria, entering the aquatic environment in sufficient quantities to produce an infectious dose. Evidence suggests that the concentration of the organisms in muscle tissue would be low unless the fish were demonstrating frank clinical signs of skin ulceration and muscular involvement (haemorrhage or granulomata), which would preclude the fish from being processed.

There are no literature reports of the spread of piscirickettsiosis between salmon producing areas 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 would be extremely 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. Experimental challenges with *Piscirickettsia salmonis* suggest that relatively large doses of between 10^2 and 10^5 $TCID_{50}$/mL would be required to induce disease (Almendras *et al.* 1997, Birkbeck *et al.* 2004) and there is no evidence to suggest that *Francisella* sp., also an intracellular bacterium, would require a lesser infectious dose. On the contrary, in Atlantic cod (*Gadus morhua*), intraperitoneal doses of 10^7 $TCID_{50}$ produced only 77 percent mortality over an extended 122 days despite being directly inoculated into the fish (Nylund *et al.* 2006), suggesting relatively high doses are also required for *Francisella* species.

Given that harvesting and processing of clinically affected fish is unlikely, the greatest risk is from subclinically infected fish. The rickettsial titre in their muscle tissue is, however, likely to be low. Large 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).

Taking all these factors into consideration the likelihood that RLOs would be exposed to, and establish in, native fish is so remote as to be negligible, no further assessment is required and no specific sanitary measures are warranted.

4.8. YERSINIA RUCKERI

4.8.1 Aetiological agent: *Yersinia ruckeri* is a gram negative, non-haemolytic, rod shaped bacterium, which is motile between 9 °C and 35 °C. It is a member of the Enterobacteriaceae family (Buller 2004). There are five serovars, namely I, II, III, V and VI; serovar IV has been discounted by DNA homology (Horne and Barnes 1999).

4.8.2 OIE List: Not listed

4.8.3 New Zealand status: *Y. ruckeri* has been isolated here. It was first isolated from a salmonid hatchery in the South Island in 1989; a second South Island farm was discovered to be infected in 1990 (Anderson *et al.* 1994). The disease, however, appears restricted to these two locations (Anderson 1996), as evidenced by subsequent isolations (Poland 2005). Despite no internal controls on the movement of eviscerated fish there is no evidence to suggest the dispersion of the disease beyond these two locations and the disease remains highly localised. An earlier MAF risk analysis (Stone *et al.* 1997) detailed a personal communication from Dr. C. Anderson (MQM Animal Health Laboratory) indicating that the New Zealand isolate is most closely related to serovar I, also known as the Hagerman strain, the strain most associated with disease in fish (Austin and Austin 1993).

Exotic strains are listed as unwanted organisms in New Zealand.

4.8.4 Epidemiology: *Yersinia ruckeri* was first described from the Hagerman Valley of Idaho in the early 1950s. It is mostly a disease of young salmonids, affecting fingerlings (Anderson *et al.* 1994) and small fry. The course is usually acute in fry and there may be a lack of clinical signs before death. Larger fish demonstrate lethargy and darkening of the skin before developing septicaemic signs (Horne and Barnes 1999). Serovar I tends to produce erythema in the throat and mouth with haemorrhage of the gills and fins, whereas Serovar III can result in haemorrhage in the eyes and musculature (Buller 2004). Internally there is congestion of blood vessels in the peritoneum and haemorrhage in the liver, pancreas, swim bladder and muscles (Horne and Barnes 1999).

Yersiniosis is a disease of fish stressed by poor water quality, low dissolved oxygen or high water temperature (Buller 2004) and it can be controlled through good husbandry (Horne and Barnes 1999). Transmission is direct and horizontal with the primary source being large numbers of bacteria shed in the faeces of infected or carrier fish (Horne and Barnes 1999). A carrier state is recognised in salmonids with very low numbers of the bacterium in kidney, spleen and posterior intestinal tract (Anderson *et al.* 1994). Some gram negative bacteria may also be found in lymphoid tissue (Horne and Barnes 1999). The immersion infectious dose is not clear, but appears to be high; in the order of 10^5 cells/mL for Serovar I and 10^7 cells/mL for Serovar III (Stone *et al.* 1997).

Y. ruckeri has been isolated from a number of other fish species including *Acipenser* spp., *Anguilla anguilla, Coregonus* spp. and *Cyprinus carpio* (Horne and Barnes 1999). There has been one report of *Y. ruckeri* from *O. niloticus* in Egypt (El-Khatib 1998). In this case it was an incidental finding in 4 percent of tilapia that had been sampled to investigate eye infections.

The bacterium will remain viable in frozen fish for over six months (Anderson *et al.* 1994).

4.8.5 Entry assessment: *Y. ruckeri* has been reported from China (Raidal *et al.* 2004), and tilapia have been shown to be susceptible to it (El-Khatib 1998); although the bacterium was only found at low prevalence in the studied population. It must be concluded therefore that fish used to produce the commodity could have been exposed to *Y. ruckeri*.

As previously discussed, it is unlikely that muscle titres would be significant unless the fish were suffering clinical disease with muscle involvement. This would render the fish unsuitable for processing. The clinical signs of septicaemia would be obvious and the infected population could be avoided. The risk therefore arises from carrier fish. These fish carry the bacterium mainly in the posterior intestinal tract but also in kidney and spleen, and inconsistently in lymphoid tissue. Whilst carrier fish, if they occur on the farm of origin, are likely to be harvested, the processing would remove those portions of the fish with detectable bacterial titres.

Any *Y. ruckeri* present in fillet meat is likely to survive freezing and transport to New Zealand. However, overall, the likelihood of viable *Y. ruckeri* being present in fillet meat on entry to New Zealand is low.

4.8.6 Exposure and establishment assessment: Exposure of susceptible native fish to *Y. ruckeri* would require imported fillets or scraps derived from them, infected with *Y. ruckeri*, entering the aquatic environment in sufficient quantities to produce an infectious dose. Evidence suggests that the concentration of the bacterium in muscle tissue is extremely low unless the fish is demonstrating muscle haemorrhages, which would preclude the fish from being processed.

Given that harvesting and processing of clinically affected fish is unlikely, the greatest risk is from carrier fish. The bacterial titre in the muscle tissue of carrier fish is likely to be extremely low.

There are no reports of the spread of *Y. ruckeri* 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 would be extremely 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. Immersion challenges suggest that a relatively large dose of $10^5 - 10^7$ cells/mL would be required to induce disease (Austin and Austin 1993). The primary source of infection in outbreaks is live, infected fish shedding large numbers of the bacterium in their faeces (Horne and Barnes 1999). This route is not replicated when tissue scraps are discarded into an aquatic environment.

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

Taking all these factors into consideration the likelihood that *Y. ruckeri* would be exposed to, and establish in, native fish is so remote as to be negligible, no further assessment is required and no specific sanitary measures are warranted.

4.9. HENNEGUYA SPP.

4.9.1 Aetiological agent: *Henneguya* spp., a member of the Myxobolidae family in the phylum Myxozoa.

4.9.2 OIE List: Not listed

4.9.3 New Zealand status: *Henneguya* spp. are rarely encountered in New Zealand and are confined to Lake Ellesmere (Hine 1978).

4.9.4 Epidemiology: The life cycle of the *Henneguya* spp. is indirect. They require an oligochaete intermediate host to take up the myxospores and release the actinomyxon stages that are infectious to other fish. These intermediate hosts are generally of the Tubificidae and Naididae families, such as *Tubifex tubifex* or *Dero digitata* (Feist and Longshaw 2006). In the case of *H. exilis*, the intermediate host was identified as *D. digitata* (Lin DanJuan *et al.* 1999). Evidence suggests that *D. digitata* is absent from New Zealand, however the identification of *Henneguya* spp. in a lake in New Zealand indicates that there must be an oligochaete worm in New Zealand capable of acting as an intermediate host, although it may be restricted to this lake.

There are over 21 different species of *Henneguya* identified in a number of fish species in the Amazon basin alone. They demonstrate a range of tissue tropisms including gill and ovary (Tavares-Dias *et al.* 2001b). The *Henneguya* sp. identified from Tilapia in Brazil was *H. piaractus*, which has a predilection for the gills, as does *H. exilis* and *H. ictaluri* (Feist and Longshaw 2006). All available literature reports indicate that the *Henneguya* spp. identified on tilapia are restricted to the gills.

There are some *Henneguya* spp. that are found in musculature (*H. zschokkei* and *H. salminicola*) but they appear to be specific to the Salmonidae (Feist and Longshaw 2006). Infections in the muscle are recognisable from surface irregularities or during filleting when the fillets are rejected due to the presence of large white cysts, containing milky fluid.

There are no studies to indicate the susceptibility of *Henneguya* spp. spores to freezing, although the myxozoan, *Myxobolus cerebralis* can tolerate freezing to -20 °C for more than 3 months (El-Matbouli and Hoffmann 1991). Freezing is effective against the actinomyxon stages (Wagner *et al.* 2003).

4.9.5 Entry assessment: It is possible that farmed tilapia would be exposed to *Henneguya* spp., however the likelihood is that infection would be restricted to the gills. Infection of any portion of the carcase, with the exception of the muscles, would be insignificant as those parts would be discarded during processing. Heavy infections of the muscle would be detected at processing and the fillets discarded. The risk, therefore, arises from low grade muscle infections that pass fillet inspection and grading. Any myxospores present are likely to survive freezing and transport to New Zealand, however given the very infrequent reports of infection in tilapia, the apparent predilection for the gills and the ability to detect and reject heavy muscle infections the likelihood of the commodity containing viable *Henneguya* spp. on entry to New Zealand is low.

4.9.6 Exposure and establishment assessment: For exotic *Henneguya* spp. to establish and cause disease in New Zealand fish would require viable spores, in sufficient quantities, to be released from muscle tissue in an aquatic environment where susceptible oligochaete worms

were found. A susceptible fish host would also need to be present in the vicinity of the oligochaete worms to become infected with the actinomyxon spore. Given an apparent family specificity of the *Henneguya* spp. parasite it is unlikely that New Zealand fish would be susceptible to the same species of *Henneguya* as tilapia. The likelihood of exposure and establishment is, considering all factors, negligible. Risk management measures are not required.

4.10. *DIGENEANS* (INCLUDING MUSCLE ENCYSTING METACERCARIA)

4.10.1 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.

The digeneans of interest are:

Clinostomum spp. (zoonotic)	[Clinostomidae]
Euclinostomum spp.	[Clinostomidae]
Clonorchis spp. (zoonotic)	[Opisthorchiidae]
Pygidiopsis spp. (zoonotic)	[Heterophyidae]
Centrocestus spp. (zoonotic)	[Heterophyidae]
Heterophyes spp. (zoonotic)	[Heterophyidae]
Haplorchis spp. (zoonotic)	[Heterophyidae]
Metagonimus spp. (zoonotic)	[Heterophyidae]
Phagicola spp. (=*Ascocotyle* spp.)	[Heterophyidae]
Procerovum spp.	[Heterophyidae]
Bolbophorus spp.	[Bolbophoridae]
Prosostephanus spp.	[Cyathocotylidae]
Prohemistomum spp.	[Cyathocotylidae]
Pharyngostomum spp.	[unclassified digenea]
Echinochasmus spp.	[Echinostomatidae]
Moedlingeria spp. (=*Allassogonoporus*)	[Allassogonoporidae]

4.10.2 OIE List: Not listed

4.10.3 New Zealand status: One *Clinostomum* sp. has been identified in a marine fish here (Boustead 1982). Otherwise all are considered as being exotic.

4.10.4 Epidemiology: The Clinostomidae have birds as definitive hosts; this includes herons, which are found in New Zealand. They use Lymnaeid snails, present in New Zealand (Spencer *et al.* 2002) as their first intermediate host and freshwater fish as secondary intermediate host. *Clinostomum tilapiae* and *Euclinostomum heterostomum* have been reported from tilapia, although they infect the kidney and branchial cavity and are thus removed during processing. *C. complanatum* utilises *Lymnaea auricularia* as its first intermediate host, but *L. auricularia* has been virtually eliminated from New Zealand (Spencer *et al.* 2002) and thus it is highly unlikely that it could complete its life cycle in this country. Some *Clinostomum* spp. encyst in muscle tissue and can cause laryngitis in humans if ingested. The metacercaria are, however, large (roughly 10 x 3 x 5mm), yellow coloured and can move around after death of the host, thus they are relatively easy to identify and it is likely that fillets would be rejected.

Clonorchis sinensis has mammals and birds as definitive hosts and can cause liver disease in humans as a result of the flukes infecting the bile ducts. Clonorchiasis is recognised as a risk

factor in the development of cholangiocarcinoma (cancer of the biliary system). Molluscan hosts of this parasite include *Parafossarulus manchouricus, Bithynia (=Alocima) longicornis, Semisulcospira libertina* and *Thiara granifera*, all of which are absent from New Zealand. However, the parasite can also utilise *Bulimus* spp. (present in the Kermadec Islands), *Assiminea* spp. and *Melanoides tuberculata* (both present in New Zealand) (Spencer *et al.* 2002, Ko 2006).

The Heterophyidae utilise birds, including herons, and mammals as definitive hosts (Paperna and Dzikowski 2006). Some can be zoonotic with adults developing in the intestine following ingestion of fish tissue containing metacercaria (Ko 2006); diarrhoea, colic and abdominal tenderness may result, although in small numbers the infection is likely to be sub-clinical. As a generalisation the Heterophyidae are likely to be able to use the New Zealand resident *M. tuberculata* snail as intermediate host, in addition to the exotic *Pirenella conica*.

Pygidiopsis spp. are reported to use the New Zealand endemic *Cerithidea* spp. snail (Koie 1990), in addition to the *Tympanotonus* spp. which are exotic. The New Zealand waters resident, *Mugil cephalus,* can act as a secondary intermediate host and herons can act as definitive host (Paperna and Dzikowski 2006).

The *Centrocestus* spp. are known to use *M. tuberculata* as first intermediate host (Paperna and Dzikowski 2006). The *Heterophyes* spp. can infect the molluscs *Cerithidea* spp. (endemic) and *Pirinella conica* (exotic) as first intermediate hosts, with herons as definitive host. Both *Centrocestus* spp. and *Heterophyes* spp. cause zoonotic enteric infections in humans.

The *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 *M. tuberculata* as first intermediate host, however they are usually found encysted in gills, skin and fins and are therefore much less likely to be found in fillet tissue.

Metagonimus spp. are reported to use the exotic *Semisulcospira* spp. and *Koreanomelania* spp. of snails as first intermediate host, however, they are members of the Heterophyidae and it is likely that they could also utilise the endemic *M. tuberculata* as part of their life cycle.

Phagicola (=Ascocotyle) spp. can infect herons as their defintive host and *Poecilia* spp. and *Gambusia affinis* (Stein 1968) as their second intermediate host. They are recognised as utilising *M. tuberculata* (Scholz *et al.* 1997) and lymnaeid snails, which can be found in New Zealand. Their metacercaria, however, display a predilection for the heart and truncus arteriosus (Paperna and Dzikowski 2006) and thus are much less likely to be present in the commodity than other Heterophyidae.

Procerovum spp. can infect endemic New Zealand herons as a definitive host and have been reported to utilise *Thiara* spp. molluscs as first intermediate host (Velasquez 1973). *Thiara* spp. are regarded as exotic to New Zealand, however it is possible that they could utilise the endemic *M. tuberculata* snail as first intermediate host.

Bolbophorus spp. use the exotic *Bulinus truncatus* and *Helisoma* spp. as intermediate mollusc hosts, but can also utilise the endemic *Planorbis* sp. snail. It is known to infect *Perca fluviatilis* as a second intermediate host, with pelicans as the definitive host, although presumably the heron would also be susceptible.

Prosostephanus spp. and *Prohemistomum* spp. are members of the Cyathocotylidae, with mammals as the definitive host. The mollusc intermediate host has been reported as

Pleurocera acuta, *Cleopatra bulimoides* and *Lioplax subcarinata* (Vernberg 1952), none of which is recorded from New Zealand. They are not considered zoonotic.

Pharyngostomum spp. can utilise freshwater fish and amphibians as second intermediate hosts, and are reported to utilise *Segmentia* spp. as first intermediate host (Wallace 1939). *Segmentia complanata* is endemic to New Zealand (Spencer *et al.* 2002). *Echinochasmus* spp. are reported to use the exotic *Oxytrema* spp. and *Amnicola* spp. as mollusc hosts and are thus unlikely to establish. *Moedlingeria* spp. are likely to use Hydrobiid snails (Bogea 2004) as first intermediate hosts. The Hydrobiid snails are found only on subantarctic islands, thus, there is negligible likelihood that *Moedlingeria* spp. would establish here.

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 percent 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).

4.10.5 Entry assessment: The likelihood of metacercaria being present in the commodity is much lower for those digeneans that exhibit a predilection for tissues other than the somatic musculature, e.g. *Clinostomum tilapiae, Euclinostomum heterostomum, Haplorchis* spp. and *Phagicola* spp. (=*Ascocotyle* spp.), which have predilections for the kidney, branchial cavity, integument and heart respectively. Metacercaria of the other digeneans considered are likely to be associated with the commodity as they encyst in the somatic musculature. There are reports of *Centrocestus* spp., *Heterophyes* spp., *Clonorchis sinensis, Haplorchis* spp. and *Metagonimus* spp. in tilapia from China; the Heterophyidae are well recognised in Brazil. Whilst heavy infections, especially of Clinostomidae, are likely to lead to rejection of the fillets, low grade infections could pass inspection and thus there is a non-negligible likelihood that digenean metacercaria would be present in the fillet tissue prior to freezing.

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 -20°C. 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 utilise this standard at this time. This time frame is likely to be effective at both -18°C and -20°C. As the commodity will be frozen at -18°C or -20°C for approximately 30 days, the likelihood of entry of viable digenean metacercaria is negligible.

4.11. NEMATODIASIS

4.11.1 Aetiological agent: Adults and larvae of the Phylum Nematoda

4.11.2 OIE List: Not listed

4.11.3 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.

4.11.4 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.

4.11.5 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 *Oreochromis* spp. (Smith 1984).

Potentially zoonotic nematodes such as *Anisakis* spp., *Capillaria* spp. and *Terranova* spp. have been reported from more than 70 species of marine fish caught in the waters of New Zealand. Even unfrozen, therefore, this commodity would not necessarily represent a greater risk than muscle tissue derived from fish caught here.

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 cestoidea (Dovgalev 1988, Pronin *et al.* 1989).

The likelihood of the commodity containing viable nematodes, larval nematodes (and, incidentally) plerocercoids on entry to New Zealand is therefore negligible as long as the commodity has been frozen to -20 °C for at least 60 hours or -18°C for at least 4 days (96 hours). As the commodity will be frozen for approximately 30 days, the likelihood of entry of viable nematode (or cestode) parasites is negligible.

4.12. ICHTHYOPHONUS HOFERI

4.12.1 Aetiological agent: *Ichthyophonus hoferi* is classified in the clade Mesomycetozoa and the order Ichthyophonida. It is related to Dermocystidia and is now considered a protistan rather than a fungus (McVicar 1999). The Ichthyophonida contains both fish pathogens and saprotrophic microbes (Mendoza *et al.* 2002).

4.12.2 OIE List: Not listed

4.12.3 New Zealand status: Not reported, considered exotic

4.12.4 Epidemiology: *I. hoferi* has been reported from at least 35 marine fish species and 48 freshwater fish species, and from many temperate and some tropical waters (McVicar 1999). Despite its recognition in freshwater species it is considered to be primarily of marine origin, with many of the freshwater isolations linked to the use of marine fish as feed (McVicar 1999).

Transmission is direct and horizontal, either by the ingestion of infectious spores from the water column (Athanassopoulou 1992) or the ingestion of spores in infected fish tissues (McVicar 1999). The spores germinate in the fish tissues after the death of the host (McVicar 1999). The discovery of infected copepods suggests that these organisms could act as paratenic hosts for *I. hoferi* (McVicar 1999).

Natural infections result in the presence of the organism in the internal organs and muscle tissue. The spore or granuloma location is dependent on the species of fish infected (Rahimian 1998, McVicar 1999). Infections may be active or passive. Passive infections consist of a number of resting spores in the tissues, whereas active infection results in granuloma development in the internal organs, necrotic skin lesions, emaciation, loss of internal fat stores and invariable results in poor flesh quality and fillet rejection (Rahimian 1998, McVicar 1999).

I. hoferi survives well at 4 °C (Athanassopoulou 1992), up to 2 days at -8 °C (McVicar 1999) and is rapidly killed by freezing to -20 °C (Athanassopoulou 1992)

4.12.5 Entry assessment: Whilst it is possible that tilapia could be exposed to *I. hoferi* it is unlikely as it is primarily a disease of marine fish. Clinical disease resulting in skin lesions, emaciation and fillet quality issues (softness and malodour) would preclude these fish from being used for human consumption. If tilapia were infected with a passive, or resting-spore, infection then it is possible that *I. hoferi* could be contained in the fillets; however the act of freezing the fillets would rapidly inactivate the organism.

As the commodity wil be frozen at -18°C or -20°C for approximately 30 days, and it is known that -8°C for 48 hours is sufficient to inactivate spores, the likelihood that viable *I. hoferi* would be present in the commodity on entry to New Zealand is negligible.

4.13. APHANOMYCES INVADANS

4.13.1 Aetiological agent: *Aphanomyces invadans* is an oomycete fungus of the family Saprolegniaceae. It has broad, non-septate hyphae.

4.13.2 OIE List: Not listed

4.13.3 New Zealand status: Not reported, considered exotic

4.13.4 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 co-habiting 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.

Whilst tilapia have been described as resistant to infection with *A. invadans* (OIE 2006b), it is apparent that they are not refractory, but are, rather, of low susceptibility. *Aphanomyces* sp. was identified from the gills, skin and fins of diseased tilapia, but not isolated internally (El-Sharouny and Badran 1995). Experimental challenge via scale removal and inoculation or intramuscular injection with the same strain demonstrated that tilapia can become infected, with deaths over an 11 day course. *T. nilotica* (= *O. niloticus*) was less susceptible than *T. galileae* and overall tilapia were most resistant to infection, but they were not refractory (El-Sharouny and Badran 1995). Another transmission trial involving intramuscular inoculation with $10^3 – 10^4$ *A. invadans* spores resulted in the majority of tilapia (*O. niloticus*) used remaining unaffected, with classical disease development in only two fish (Khan *et al.* 1998).

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

4.13.5 Entry assessment: Whilst *A. invadans* has not been reported from China, it has been isolated from Taiwan (Chien 1981) and as a precautionary measure it will be assumed that the agent could be present on the farms from which the fish are harvested. There is no evidence to suggest the agent is present in Brazil.

It is most likely that if tilapia were exposed to *A. invadans* naturally, that is by immersion, the organism would be restricted to the gills, skin and fins (El-Sharouny and Badran 1995). Processing of the fish would therefore be expected to remove these tissues. Injuries to a fish might make the animal more susceptible to developing clinical disease, but given their apparent resistance to infection (El-Sharouny and Badran 1995, Khan *et al.* 1998) the likelihood is low.

Clinical disease thus appears highly unlikely but, were it to occur, the clinical signs would be apparent and would tend to preclude harvesting of the fish for human consumption.

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*, are 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).

In the absence of alternative data, taking into consideration that the hyphae of *A. invadans* are thicker than those of *A. astaci*, thus potentially being more resistant to freezing, and that temperatures of -5°C appear effective within 72 hours, it is likely that temperatures of -18°C or -20°C would inactivate all *A. invadans* hyphae or spores present in, or on, the commodity within 72 hours. Given that the commodity will be frozen for approximately 30 days at -18°C or -20°C, the likelihood of viable *A. invadans* lifestages being present in the commodity on entry to New Zealand is negligible.

4.14. WATER-BORNE CONTAMINANTS

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

4.14.2 OIE List: N/A

4.14.3 New Zealand status: Only exotic strains considered

4.14.4 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, temperatures of -18°C or -20°C for 7 days should be sufficient to inactivate protozoans. As the commodity will be frozen at -18°C or lower for approximately 30 days, the likelihood of viable protozoa being present on netry to New Zealand is negligible.

The New Zealand drinking water standards require the maintenance of 0.2 mg/L (ppm) of free available chlorine (FAC) at pH 8.0 to consistently eliminate *Escherischia coli* and other coliforms from a water supply. To reduce protozoal contamination to safe levels the standards require chemical coagulation and filtration to less than 0.1 NTU (nephelometric turbidity units) for 95 percent of tests, with no tests giving a reading of greater than 0.5 NTU at any time. Alternatively if filtration without coagulation is employed the filter must remove 99.99 percent of particles in the 3 to 15 μm size range. For water not exceeding 1.0 NTU and lying between pH 6 and pH 8 inclusive, ozonation to achieve 14 mg.min/L at 15°C (or equivalent at other temperatures) would be sufficient to eliminate protozoa. Alternatively, for water with a measured light transmission of ≥ 80 percent per cm at 254nm and either filtered to 5μm or not exceeding 1.0 NTU, ultraviolet radiation can be used with a dose rate of ≥ 40 MJ/cm^2 (Ministry of Health 2005).

4.14.5 Risk management measure: The use of treated water is specified in the commodity definition. However, a range of treatment conditions may be employed. It is therefore necessary to expand the definition of treated water at this time. 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 should be of potable standard, being treated to a standard equivalent to those prescribed in the Drinking Water Standards for New Zealand (Ministry of Health 2005).

5. CONCLUSION

The commodity assessed in this risk analysis consists of skinless, boneless fillets (and mince derived from fillets) from farmed *Oreochromis* spp. from Brazil and China. The fish are harvested, bled, scaled, eviscerated, filleted, skinned, trimmed, washed and graded. Treated water (chlorinated) is used during processing of the fish. The fillets are frozen to either -18 °C or -20 °C following processing and are maintained at those temperatures for approximately 30 days during transport to New Zealand.

Thirteen potential hazards were identified from the list of organisms of potential concern and subjected to further risk assessment. These were iridoviruses, aquatic birnaviruses, *A. salmonicida, Flavobacterium* spp., *Streptococcus iniae, Edwardsiella* spp., intracellular bacteria, *Yersinia ruckeri, Henneguya* spp., digenean metacercaria, larval nematodes, *Ichthyophonus hoferi* and *Aphanomyces invadans.* Waterborne contaminants were also considered as a fourteenth 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 pathogenic organisms in muscle tend to be many times lower than those found in the viscera.

None of the thirteen 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 (based on assumptions made in the risk assessments) and specific sanitary measures related to the quality of water used in the processing plant.

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 water at all times in the processing plant.

5.1.3 The commodity definition includes freezing and maintenance at temperatures of -18°C or -20°C. The product is expected to remain at these temperatures for approximately 30 days 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), *Ichthyophonus hoferi, 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 -20°C 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	Core temperature -20 °C
Digenean metacercaria	168 hours (7 days)	168 hours (7 days)
Encysted nematode larvae (and cestode plerocercoids)	96 hours (4 days)	60 hours (2.5 days)
Ichthyophonus hoferi	48 hours (2 days)	48 hours (2 days)
Aphanomyces invadans	72 hours (3 days)	72 hours (3 days)
Water borne contaminants (protozoans)	168 hours (7 days)	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 168 hours (7 days).

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♣ abstract only

Appendix 1: Organisms Reported to be Associated with Oreochromis spp. & Fish Diseases Listed by the OIE

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Viruses					
EHNV	Ranavirus; Iridoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
IHNV	Novirhabdovirus; Rhabdoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
SVCV	Vesiculovirus; Rhabdoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
VHSV	Novirhabdovirus; Rhabdoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
ISAV	Isavirus; Orthomyxoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
RSIV	Iridoviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
KHV	Cyprinid herpesvirus 3; herpesviridae	No *Oreochromis* spp. listed (nor cichlidae)	Septicaemia, mortality in susceptible hosts		OIE list
Bohle iridovirus	Ranavirus; Iridoviridae	*O. mossambicus*	Corkscrew swimming, mortality	Australia (aquatic animal laboratory)	(Ariel and Owens 1997)
Iridovirus-like agent	?Iridoviridae	*O. niloticus*	Elevated mortalities	Canada, ex-Florida (USA)	(McGrogan *et al.* 1998)
Tilapia Larvae Encephalitis (TLEV)	herpes-like virus	*O. aureus, O. niloticus*	Neurological signs and increased mortality	Israel	(Avtalion and Shlapobersky 1994)
Aquatic birnavirus	Aquabirnavirus; Birnaviridae	*O. aureus*	No observable effect (experimental infection)	Taiwan	(Tu *et al.* 2003)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Infectious Pancreatic Necrosis Virus	Aquabirnavirus; Birnaviridae	*Tilapia* spp.			(Mangunwiryo and Agius 1987)
Aquatic birnavirus (related to Ab strain of IPNV)	Aquabirnavirus; Birnaviridae	*O. mossambicus*		Taiwan	(Hedrick *et al.* 1983)
Bacteria					
Pseudomonas fluorescens	Pseudomonadaceae	*O. niloticus*	Chronic infection to acute septicaemia	Egypt	(Abdel-Aziz 1999, Ahmed and Shoreit 2001)
Aeromonas hydrophila	Aeromonadaceae	*O. niloticus*	Septicaemia	Egypt	(Faisal *et al.* 1989, El-Khatib 1998, Ahmed and Shoreit 2001)
Aeromonas hydrophila	Aeromonadaceae	*O. niloticus*	Septicaemia	China	(Shi 2000)
Aeromonas spp. (including *A. salmonicida)*	Aeromonadaceae	*O. niloticus*	Non-clinical – isolated from sampled food fish	Mexico	(Castro-Escarpulli *et al.* 2003)
Flavobacterium columnare	Flavobacteriaceae	*O. niloticus*	Skin and fin lesions	Egypt	(Amin *et al.* 1988, Badran *et al.* 1994)
Flavobacterium columnare	Flavobacteriaceae	*O. niloticus*	Skin and fin lesions	Brazil	(Figueiredo *et al.* 2005)
Streptococcus sp. (α haemolytic)	Streptococcaceae	*O. niloticus x O. aureus*	Septicaemia	Saudi Arabia	(Al-Habi 1994)
Streptococcus iniae	Streptococcaceae	*O. niloticus*	Septicaemia		(Bowser *et al.* 1998)
Streptococcus spp.	Streptococcaceae	*O. niloticus*	Septicaemia	Brazil	(Salvador *et al.* 2003, Salvador *et al.* 2005)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Streptococcus spp.	Streptococcaceae	*O. niloticus*	Septicaemia	Egypt	(El-Khatib 1998)
Streptococcus sp. (β haemolytic)	Streptococcaceae	*O. mossambicus*	Septicaemia	India	(Mukhi *et al.* 2001)
Streptococcus groupB	Streptococcaceae	*O. niloticus*	Incidental finding	Brazil	(Salvador *et al.* 2005)
Edwardsiella tarda	Enterobacteriaceae	*O. niloticus*	Septicaemia	Ethiopia	(Yimer 2000)
Edwardsiella tarda	Enterobacteriaceae	*O. niloticus*	Septicaemia	Brazil	(Muratori *et al.* 2000)
Edwardsiella tarda	Enterobacteriaceae	*O. niloticus*	Septicaemia	Egypt	(Saleh 2005)
Edwardsiella tarda	Enterobacteriaceae	*O. mossambicus*	Septicaemia	South Africa	(Bragg 1988)
Vibrio vulnificus	Vibrionaceae	*Oreochromis* spp.	Septicaemia and ulceration	Taiwan	(Chen *et al.* 2006)
Vibrio spp. (*V. anguillarum, V. ordalii, V. damsela, V. vulnificus*)	Vibrionaceae	*O. niloticus*	Isolated from randomly sampled fish from freshwater lakes	Egypt	(El-Gaber *et al.* 1997)
Rickettsia-like organism	potentially Rickettsiaceae	*O. niloticus x O. honorum*	Skin petechiae, necrotic gills, white nodules in internal organs	USA	(Mauel *et al.* 2005)
Rickettsia-like organism	potentially Rickettsiaceae	*O. niloticus*	skin ulcers and multiple granulomas	Taiwan	(Chen *et al.* 1994)
Rickettsia-like organism	potentially Rickettsiaceae	*O. mossambicus*	multiple granulomas	Hawaii	(Mauel *et al.* 2003)
Epitheliocystis	Chlamydiales	*O. niloticus*	Gill lesions	Brazil	(Lima *et al.* 2001)
Yersinia ruckeri	Enterobacteriaceae	*O. niloticus*	Bacterial septicaemia	Egypt	(El-Khatib 1998)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Staphylococcus spp.	Staphylococcaceae	*O. niloticus*	Bacterial septicaemia	Egypt	(El-Khatib 1998)
Staphylococcus epidermidis	Staphylococcaceae	*Oreochromis* spp.	Septicaemia, mass mortality	Taiwan	(Huang *et al.* 1999)
Staphylococcus epidermidis	Staphylococcaceae	*O. niloticus*.	Septicaemia	Egypt	(Ahmed *et al.* 1990)
Plesiomonas shigelloides	Enterobacteriaceae	*Oreochromis* spp.	Mortality in fry	Taiwan	(Faisal *et al.* 1989)
Providencia rettgeri	Enterobacteriaceae	*O. niloticus*	Isolated from kidney	Egypt	(Faisal *et al.* 1987)
Francisella spp.	Francisellaceae	*Oreochromis* spp.	Visceral granulomas	Taiwan	(Hsieh *et al.* 2006)
Francisella spp.	Francisellaceae	*O. niloticus*	Skin haemorrhage and ulcers, splenomegaly, renomegaly, hepatomegaly, white nodules in internal organs	Taiwan	(Hsieh *et al.* 2006)
Francisella spp.	Francisellaceae	*O. mossambicus, O. aureus, T. zillii, T. honorum*	Skin haemorrhage and ulcers, splenomegaly, renomegaly, hepatomegaly, white nodules in internal organs	Taiwan	(Mauel and Miller 2002)
Francisella spp.	Francisellaceae	*O. niloticus*	Skin haemorrhage and ulcers, splenomegaly, renomegaly, hepatomegaly, white nodules in internal organs	Central America	(Mauel *et al.* 2007)
Pasteurella multocida	Pasteurellaceae	*O. aureus x O. niloticus*	Septicaemia	Israel	(Nizan and Hammerschlag 1993)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Actinomyces sp.	Actinomycetaceae	*O. niloticus, Tilapia zillii*	Rust-yellow skin discolouration		(Oladosu *et al.* 1994)
Parasites					
Ichthyobodo necatrix	Protozoa; Kinetoplastida	*O. niloticus*	Recovered from gills	Saudi Arabia	(Afifi *et al.* 2000)
Trypanosoma choudhuryi sp. nov., *T. mukasai*	Protozoa; Kinetoplastida	*O. mossambicus*	Infects blood cells	India	(Mandal 1977, Sinha 1986)
Piscinoodinium pillulare	Protozoa; Dinoflagellata	*O. niloticus*	Found on gills and skin	Brazil	(Martins *et al.* 2001)
P. pillulare	Protozoa; Dinoflagellata	*Tilapia rendalli*	Found on gills and skin	Brazil	(Tavares-Dias *et al.* 2001a)
Piscinoodinium sp.	Protozoa; Dinoflagellata	*O. mossambicus*	Found on gills and skin	India	(Ramesh *et al.* 2000)
Hartmanella vermiformis	Lobosea; euamoebida	*O. niloticus*	Infection in kidney	Czech Republic	(Dykova *et al.* 1997)
Mayorella-like & *Platyamoeba*-like organisms	Lobosea; euamoebida	*O. niloticus*	Infection in liver	Czech Republic	(Dykova *et al.* 1997)
Rosculus ithacus	Heterolobosea; schizopyrenida	*O. niloticus*	Infection in kidney	Czech Republic	(Dykova *et al.* 1997)
Cryptocaryon sp.	Protozoa; Ciliophora	*O. niloticus*	Found on gills	Saudi Arabia	(Afifi *et al.* 2000)
C. irritans	Protozoa; Ciliophora	*O. mossambicus*	Found on gills	South Africa	(Britz *et al.* 1985)
Trichodinella sp.	Protozoa; Ciliophora	*O. mossambicus*	Found on gills	South Africa	(Basson and Van As 1987)
Trichodina spp.	Protozoa; Ciliophora	*O. niloticus*	Found on gills	Brazil	(Vargas *et al.* 2003, Azevedo *et al.* 2006)
Trichodina spp.	Protozoa; Ciliophora	*T. rendalli*	Found on gills	Brazil	(Tavares-Dias *et al.* 2001a)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Trichodina spp.	Protozoa; Ciliophora	*O. niloticus*	Found on gills	Saudi Arabia	(Afifi *et al.* 2000)
Trichodina spp.	Protozoa; Ciliophora	*O. mossambicus*	Found on gills	South Africa	(Basson and Van As 1987)
T. truttae, T. fultoni	Protozoa; Ciliophora	*O. niloticus*	Found on gills	Egypt	(Aly *et al.* 1995, Badran *et al.* 1996)
T. oreochromisi sp. nov.	Protozoa; Ciliophora	*O. mossambicus*	Found on gills	Bangladesh	(Asmat and Nazma 2005)
Trichophrya sp.	Protozoa; Ciliophora	*O. niloticus*	Found on gills	Saudi Arabia	(Afifi *et al.* 2000)
Ichthyophthirius multifiliis	Protozoa; Ciliophora	*O. niloticus*	Found on skin	Egypt	(Ahmed and Shoreit 2001)
Ichthyophthirius multifiliis	Protozoa; Ciliophora	*T. rendalli, O. niloticus*	Found on skin	Brazil	(Tavares-Dias *et al.* 2001a)
Chilodonella sp.	Protozoa; Ciliophora	*O. niloticus*	Found on skin	Brazil	(Alexandrino *et al.* 2001)
C. hexasticha	Protozoa; Ciliophora	*O. niloticus*	Found on skin	Egypt	(Badran *et al.* 1996)
Tripartiella clavodonta, T. leptospina, T. nana	Protozoa; Ciliophora	*O. mossambicus*	Found on gills	South Africa	(Basson and Van As 1987)
Eimeria vanasi	Protozoa; Apicomplexa	*O. mossambicus*	Intestinal infection	South Africa	(Landsberg and Paperna 1987)
Eimeria vanasi	Protozoa; Apicomplexa	*O. niloticus*	Intestinal infection	Israel	(Landsberg and Paperna 1987)
Goussia cichlidarum	Protozoa; Apicomplexa	O. aureus x O. niloticus	Swim bladder infection	Israel	(Paperna et al. 1986)
Enteromyxum leei	Myxosporea; Myxidiidae	O. mossambicus	Experimental infection (intestine)	Israel (laboratory location)	(Diamant et al. 2006)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Henneguya sp.	Myxosporea; Myxobolidae	O. niloticus	Found on gills	Saudi Arabia	(Afifi et al. 2000)
H. piaractus	Myxosporea; Myxobolidae	Tilapia rendelli, O. niloticus	Found on gills	Brazil	(Tavares-Dias et al. 2001b)
Myxobolus sp.	Myxosporea; Myxobolidae	O. niloticus	Eye infection	Egypt	(El-Khatib 1998)
Myxosoma spp., Myxobolus spp.	Myxosporea; Myxobolidae	Oreochromis spp.	Spleen and kidney infections	Israel	(Landsberg 1985)
Myxobolus dermatobia	Myxosporea; Myxobolidae	O. niloticus	Skin infection	Egypt	(Aly et al. 1995, Badran et al. 1996)
Myxosoma tilapiae	Myxosporea; Myxobolidae	O. niloticus	skin, gill, eye, spleen, liver and kidney infected	Egypt	(Faisal and Shalaby 1987)
Myxobolus colossomatis	Myxosporea; Myxobolidae	T. rendelli, O. niloticus	Incidental finding	Brazil	(Tavares-Dias et al. 2001b)
Myxobolus dahomeyensis	Myxosporea; Myxobolidae	Oreochromis spp.	Ovarian infection	Benin	(Gbankoto et al. 2001)
Dactylogyrus spp.	Platyhelminthes; Monogenea	O. niloticus	Skin and gill infestation	Brazil	(Vargas et al. 2003)
Dactylogyrus spp.	Platyhelminthes; Monogenea	O. niloticus	Skin and gill infestation	Egypt	(Ahmed and Shoreit 2001)
Enterogyrus cichlidarum	Platyhelminthes; Monogenea	O. niloticus, T. zillii	Found in stomach	Egypt	(Khidr 1990)
Enterogyrus cichlidarum	Platyhelminthes; Monogenea	O. mossambicus	Found in anterior intestine, peritoneal cavity, internal organs	USA	(Noga and Flowers 1995)
E. niloticus	Platyhelminthes; Monogenea	O. niloticus	Found in stomach and anterior intestine	Egypt	(Eid and Negm 1987)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Gyrodactylus elegans	Platyhelminthes; Monogenea	O. niloticus	External parasite	Egypt	(Aly et al. 1995, Badran et al. 1996)
G. shariffi, G. niloticus	Platyhelminthes; Monogenea	O. niloticus	External parasite	Philippines	(Cone et al. 1995)
Cichlidogyrus sp.	Platyhelminthes; Monogenea	O. mossambicus	Found on gills	Venezuela	(Aragort F. et al. 1997)
C. sclerosus	Platyhelminthes; Monogenea	O. niloticus	Found on gills	Brazil	(Azevedo et al. 2006)
C. tubiicrrus magnus	Platyhelminthes; Monogenea	O. niloticus	Caused gill lesions	Egypt	(Shalaby and Ibrahim 1988)
C. digitialis, C. albareti, C. hemi, C. nuniezi, C. bonhommei, C. Slembroucki	Platyhelminthes; Monogenea	T. brevimanus, T. buttikoferi, T. cessiana	Found on gills	Guinea, Ivory Coast, Sierra Leone	(Pariselle and Euzet 1998)
Neobenedenia melleni	Platyhelminthes; Monogenea	O. mossambicus	Skin infestation	Hawaii	(Kaneko et al. 1988)
Clinostomum sp.	Platyhelminthes; Digenea	O. leucostictus, T. zillii	Found during parasite survey	Kenya	(Aloo 2002)
C. tilapiae	Platyhelminthes; Digenea	O. mossambicus	Found during parasite survey	South Africa	(Britz et al. 1985)
Clinostomum sp.	Platyhelminthes; Digenea	O. niloticus	Found in branchial cavity and skin	Ethiopia	(Yimer 2000, Eshetu and Enyew 2003)
C. phacrocoracis, Clinostomum sp.	Platyhelminthes; Digenea	O. niloticus	Gill, kidney, mandibular muscle	Egypt	(El-Khatib 1998, Arafa et al. 2005)
C. tilapiae, C. complanatum	Platyhelminthes; Digenea	O. niloticus	Gill, kidney, mandibular muscle	Egypt	(Arafa et al. 2005)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Euclinostomum ardeola	Platyhelminthes; Digenea	O. niloticus	Gill, kidney, mandibular muscle	Egypt	(Arafa et al. 2005)
Euclinostomum spp.	Platyhelminthes; Digenea	O. niloticus	Found in kidney	Ethiopia	(Yimer 2000, Eshetu and Enyew 2003)
E. heterostomum	Platyhelminthes; Digenea	O. mossambicus	Found in muscles	South Africa	(Britz et al. 1985)
Diplostomum compactum	Platyhelminthes; Digenea	O. mossambicus	Eye infection	Venezuela	(Aragort F. et al. 1997)
D. paracaudam, D. spathaceum	Platyhelminthes; Digenea	O. niloticus	Eye infection	Egypt	(El-Khatib 1998)
Posthodiplostomum cuticula	Platyhelminthes; Digenea	O. niloticus	Metacercaria encysted in skin	Egypt	(Badran et al. 1996)
Pygidiopsis genata, P. summa	Platyhelminthes; Digenea	O. niloticus, T. zillii, T. ouria	encysted in muscles and gills	Egypt	(Mahdy et al. 1995, El-Ezz et al. 2000)
Centrocestus armatus	Platyhelminthes; Digenea	O. niloticus	encysted in muscles and gills	Egypt	(El-Ezz et al. 2000)
C. formosanus	Platyhelminthes; Digenea	O. niloticus	encysted in gills	Saudi Arabia, China	(Kalantan et al. 1999, Zhang et al. 2006)
Heterophyes equalis, H. dispar, H. heroni	Platyhelminthes; Digenea	O. niloticus	encysted in muscles and gills	Egypt	(Mahdy et al. 1995, El-Ezz et al. 2000)
H. heterophyes, H. aequalis	Platyhelminthes; Digenea	O. niloticus, T. zillii, T. ouria	encysted in muscles	Egypt	(Mahdy et al. 1995)
Heterophyes sp.	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	China	(Zhang et al. 2006)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Ascocotyle ascolonga	Platyhelminthes; Digenea	O. niloticus, T. zillii, T. ouria	encysted in muscles	Egypt	(Mahdy et al. 1995)
Clonorchis sinensis	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	China	(Zhang JinHui and Zhang Ping 2003)
Haplorchis pumilio	Platyhelminthes; Digenea	O. niloticus, T. zillii, T. ouria	encysted in muscles	Egypt	(Mahdy et al. 1995)
Haplorchis pumilio, H. taichui	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	China	(Zhang et al. 2006)
Phagicola ornata, Pharyngostomum flapi sp. nov.	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	Egypt	(Shalaby et al. 1993)
Prosostephanus industrius, Procerovum calderoni, Moedlingeria amphoraeformis	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	Egypt	(Shalaby et al. 1993)
Metagonimus spp.	Platyhelminthes; Digenea	O. niloticus	encysted in muscles	China	(Zhang et al. 2006)
Carassotrema tilapiae	Platyhelminthes; Digenea	O. mossambicus	Found in intestine	Venezuela	(Nasir and Gomez 1976)
Bolbophorus levantinus	Platyhelminthes; Digenea	T. zillii, O. aureus	encysted in muscles	Israel	(Dzikowski et al. 2003)
Echinochasmus perfoliatus, Prohemistomum vivax	Platyhelminthes; Digenea	Tilapia spp.	encysted in muscles	Egypt	(Tawfik et al. 2000)
Amirthalingamia sp., Cyclustera sp.	Platyhelminthes; Cestoidea	O. leucostictus, T. zillii	Encysted in intestines and liver	Kenya	(Aloo 2002)
Paradilepsis sp.	Platyhelminthes; Cestoidea	O. niloticus	Larvae and eggs encysted under scales	Nigeria	(Ezeri 2002)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Wenyonia sp.	Platyhelminthes; Cestoidea	T. mariae, T. zillii, O. aureus	Found in intestines	Nigeria	(Nmor et al. 2003)
Acanthogyrus sp.	Acanthocephala; Eoacanthocephala	T. mariae, T. zillii, O. aureus	Found in intestines	Nigeria	(Nmor et al. 2003)
Octospiniferoides sp.	Acanthocephala; Eoacanthocephala	T. mariae, T. zillii, O. aureus	Found in intestines	Nigeria	(Nmor et al. 2003)
Polyacanthorhynchus kenyensis	Acanthocephala; Polyacanthocephala	O. leucostictus, T. zillii	Found in intestines	Kenya	(Aloo 2002)
Acanthocephalus lucii	Acanthocephala; Palaeacanthocephala	O. niloticus	Found in intestine	Egypt	(Aly et al. 1995)
Contracaecum spp.	Nematoda; Ascaridida	O. leucostictus, T. zillii	Found in mesentery	Kenya	(Aloo 2002)
Contracaecum sp.	Nematoda; Ascaridida	O. niloticus	Found in pericardium	Ethiopia	(Yimer 2000, Eshetu and Enyew 2003)
Camallanus sp.	Nematoda; Camallanida	T. mariae, T. zillii, O. aureus	Found in intestines	Nigeria	(Nmor et al. 2003)
Cucullanus sp.	Nematoda; Ascaridida	T. mariae, T. zillii, O. aureus	Found in intestines	Nigeria	(Nmor et al. 2003)
Piscicola geometra	Annelida; Hirudinida	O. niloticus	Found on skin	Nigeria	(Opara 2002)
Argulus spp.	Arthropoda; Branchiuria	O. niloticus	Found on skin	Egypt	(Ahmed and Shoreit 2001)
Argulus spp.	Arthropoda; Branchiuria	O. niloticus, T. rendelli	Found on skin	Brazil	(Tavares-Dias et al. 2001b)

Agent	Description	Tilapia Host	Effects	Reported locations (if applicable)	References
Lamproglena sp.	Arthropoda; Copepoda; Lernaea	O. niloticus	Found on skin	Brazil	(Azevedo et al. 2006)
L. monodi	Arthropoda; Copepoda; Lernaea	O. niloticus	Found on skin	Egypt	(Badran et al. 1996)
Lernaea cyprinacea	Arthropoda; Copepoda; Cyclopoida	O. niloticus, T. rendelli	Found on skin	Brazil	(Fortes et al. 1998, Tavares-Dias et al. 2001b)
L. tilapiae	Arthropoda; Copepoda; Cyclopoida	O. mossambicus	Found on skin	Thailand	(Ho and Kim 1997)
Caligus epidemicus	Arthropoda; Copepoda; Siphonostomatoida	O. urolepis, O. mossambicus	Found on skin	Philippines, Taiwan	(Ho et al. 2004)
C. orientalis	Arthropoda; Copepoda; Siphonostomatoida	O. mossambicus	Found on skin	China	(Nagasawa 2004)
Fungi					
Saprolegnia spp.	Saprolegniaceae; Oomycetes	O. niloticus; O aureus	Skin lesions	Egypt	(Menendez et al. 1990, El-Khatib 1998)
Ichthyophonus hoferi	Ichthyosporea	O. niloticus	Visceral lesions and skin roughening	Egypt	(El-Khatib 1998)
Branchiomyces sp.	Taxonomy uncertain; probable oomycete	O. aureus x O. niloticus	Gill lesions	Israel	(Paperna and Smirnova 1997)
Rhizomucor sp.	Mucoraceae; Zygomycetes	O. niloticus x O. aureus x O. mossambicus	Skin haemorrhage, ascites, swollen and friable liver	USA	(Wolf and Smith 1999)

Appendix 2: Assessment of Organisms against Potential Hazard Criteria

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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 tilapia hosts listed	Yes	No	No	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable	No
IHNV	No tilapia hosts listed	Yes	No	Reported in China	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable	No
SVCV	No tilapia hosts listed	Yes	No	China has isolated SVCV	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable	No
VHSV	No tilapia hosts listed	Yes	No	Reported in Brazil	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable	No
ISAV	No tilapia hosts listed	Yes	No	No	n/a	n/a	n/a	n/a	n/a	n/a	Other exotic	No
RSIV	No tilapia hosts listed	Yes	No	2 cases June 2006 [1]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
KHV	No tilapia hosts listed	Yes	No	No	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Bohle iridovirus	Possible when viraemic [2]	No	Not reported	Negligible likelihood [4]	n/a	n/a	n/a	n/a	n/a	n/a	Other exotic [5]	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
iridovirus-like agent	Possible when viraemic [2]	No	Not reported	Possible – iridoviruses widespread and increasing in significance.	n/a	n/a	n/a	n/a	n/a	n/a	Other exotic [5]	Yes
Tilapia larval encephalitis	Possible when viraemic [2]	No	No	No – Israel only	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Aquabirnavirus (includes IPNV)	Possible when viraemic [2]	No	Yes [3]	Probable (China)	Yes [6]	n/a	n/a	n/a	n/a	n/a	Notifiable (exotic strains)	Yes
BACTERIA												
Pseudomonas fluorescens & spp.	Possible	No	Ubiquitous spp.	Likely (world wide)	No	No	No	No	No	No	No	No
Aeromonas hydrophila & spp.	Possible	No	Ubiquitous spp. Common in NZ freshwater [13]	Yes (world wide) [7]	Yes (*A. salmonicida*) [8]	No	No	No	No	No	Notifiable (*A. salmonicida*)	Yes
Flavobacterium columnare & spp.	Generally not, but possible [9]	No	Yes	Likely (world wide)	Yes, Asian genomovar [9]	No	No	No	No	No	No	Yes
Streptococcus iniae & spp.	Possible	No	Yes – but not *S. iniae*	Yes [10]	Yes	No	No	No	No	No	No	Yes (*S. iniae*)

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Edwardsiella tarda	Possible	No	No	Yes [11]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes
Vibrio vulnificus	Possible	No	Likely to be present	Yes [12]	No	No	No	No	Yes	No	No	No
Vibrio ordalii	Yes – muscle predeliction	No	Yes [13]	Likely – ubiquitous [14]	No	No	No	No	No	No	No	No
Vibrio damselae (Photobacterium damselae damselae)	Possible	No	Likely – ubiquitous opportunist	Likely – ubiquitous opportunist	No	No	No	No	No	No	No	No
Listonella anguillarum	Possible	No	Yes [13]	Likely	No	No	No	No	No	No	Unwanted (exotic strains)	No
Rickettsia-like organism	Possible	No	Not reported	Yes [15]	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable (*P. salmonis*)	Yes
Epitheliocystis	No	No	Yes	Yes [16]	No	No	No	No	No	No	No	No
Yersinia ruckeri	Possible	No	Yes [13]	Yes [17]	Virulent and non-virulent strains	No	No	East coast hatcheries	No	No	Notifiable (exotic strains)	Yes
Staphylococcus spp.	Possible	No	Likely,(*S. epidermidis*) [18]	Yes [19]	Possible	No	No	No	No	No	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Plesiomonas shigelloides	Possible	No	Present in NZ and considered ubiquitous [14]	Yes [20]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Providencia rettgeri	Unlikely – kidney incidental [21]	No	Not reported	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Francisella spp.	Possible	No	Not reported	Yes [22]	n/a	n/a	n/a	n/a	n/a	n/a	Unwanted (other exotic) *F. tularensis*	Yes
Pasteurella multocida	Possible	Not for fish	Not in fish	Not in fish – Israel only [23]	n/a	n/a	n/a	n/a	n/a	n/a	Notifiable (B:2 and E:2 only)	No
Actinomyces sp.	No	No	Likely – common organism	Likely – common organism	No	No	No	No	No	No	No	No – opportunist
PARASITES												
Ichthyobodo necatrix	No – skin & gills	No	Likely	Likely	No	No	No	No	No	No	No	No
Trypanosoma choudhuryi, T. mukasai	No – infects blood cells	No	7 spp. listed [24]	Some spp. likely	No	No	No	No	No	No	No	No
Piscinoodinium pillulare & spp.	No – ectoparasite	No	Likely	Yes [25]	No	No	No	No	No	No	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Hartmanella vermiformis	Unlikely – kidney infection	No	Not reported [26]	Not reported [27]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Mayorella-like & *Platyamoeba*-like	Unlikely – liver infection	No	Not reported [26]	Not reported [27]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Rosculus ithacus	Unlikely – kidney infection	No	Not reported [26]	Not reported [27]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Cryptocaryon irritans	No – ectoparasite	No	Yes [28]	Likely	No	No	No	No	No	No	No	No
Trichodinella sp.	No – ectoparasite	No	Likely	Likely	No	No	No	No	No	No	No	No
Trichodina spp.	No – ectoparasite	No	Yes [28]	Yes [29]	No	No	No	No	No	No	No	No
Ichthyophthirius multifiliis	No – ectoparasite	No	Yes [28]	Yes [30]	No	No	No	No	No	No	No	No
Chilodonella spp.	No – ectoparasite	No	Yes [31]	Yes [32]	No	No	No	No	No	No	No	No
Tripartiella spp.	No – ectoparasite	No	No – eradicated in quarantine	Likely [33]	No	No	No	No	No	No	No	No
Eimeria vanasi	No – intestine	No	Not reported	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Goussia cichlidarum	No – swim bladder	No	Not reported	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Enteromyxum leei	No – intestine	No	Not reported	No – Mediterra-nean & Japan	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Henneguya sp. & *H. piaractus*	Possible, some spp. affect musculature	No	*Henneguya* spp. reported from eels [34]	Yes [35]	Possible	No	No	Yes [34]	Yes	No	No	Yes
Myxobolus spp. & *Myxosoma* spp. (including: *M. dermatobia, M. tilapiae*	Some spp. may be associated with muscle [36]	No	Yes – widespread & common. NZ also has *M. cerebralis*[34]	Yes [35]	No	No	No	No	No	No	Notifiable (*M. cerebralis*)	No [37]
Dactylogyrus spp.	No – ectoparasite	No	Yes [38]	Yes [39]	No	No	No	No	No	No	No	No
Enterogyrus cichlidarum, E. niloticus	No – stomach, anterior intestine	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Gyrodactylus elegans, G. shariffi, G. niloticus	No – ectoparasite	No	*Gyrodactylus* sp. reported from NZ [40]	Some spp. likely	Yes (*G. salaris*)	No	No	No	Yes – but tend to be host specific	No	Exotic unwanted (*G. salaris*)	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Cichlidogyrus spp.	No – gill parasites	No	Unlikely	Yes [41]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Neobenedenia melleni	No - ectoparasite	No	Not reported	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Clinostomum spp. (including: *C. tilapiae*, *C. phacrocoracis*, *C. complanatum*)	Possible – although predominant in gills [42]	No	Yes [43]	Likely – wide distribution	Possible	No	No	No	Possible	No	No	Yes [49,] mildly zoonotic
Euclinostomum ardeola, *E. heterostomum*, *Euclinostomum* sp.	Possible [44]	No	Not reported	Likely	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes [49]
Diplostomum spp.	No – eye infection	No	Not reported	Likely	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Posthodiplosto--mum cuticula	No- skin infection	No	Not reported	Likely	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Pygidiopsis genata, P. summa	Yes – encysts in muscle	No	No	Unknown	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]
Centrocestus armatus, *C. formosanus*	Yes- encysts in muscles	No	No	Yes [45]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Heterophyes spp. (including: *H. equalis*, *H. dispar*, *H. heroni*, *H. aequalis*, *H. heterophyes*)	Yes- encysts in muscles and gills	No	No	Yes [45]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]
Ascocotyle ascolonga	Possible – reported from muscle [46]	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes [49]
Clonorchis sinensis	Yes – encysts in muslce	No	No	Yes [47]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]
Haplorchis pumilio, H. taichui	Yes – encysts in muscle	No	No	Yes [45]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]
Phagicola ornata, Pharyngostomum flapi, Prosostephanus industrius, Procerovum calderoni, Moedlingeria amphoraeformis, Bolbophorus levantinus, Echinochasmus perfoliatus, Prohemistomum vivax	Possible – have been reported from muscle	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes [49]

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Metagonimus spp.	Yes – encysts in muscle	No	No	Yes [45]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes – Zoonosis [49]
Carassotrema tilapiae	No – found in intestine	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Amirthalingamia sp., *Cyclustera* sp.	No – found in intestine and liver	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Paradilepsis sp.	No – encysts in dermis	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Wenyonia sp.	No – found in intestines	No	No	Not reported	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Acanthogyrus sp., *Octospiniferoides* sp., *Polyacanthorhynchus kenyensis, Acanthocephalus lucii*	No – found in intestines	No	Yes [48]	Not reported, but some spp. likely	Possible	No	No	No	Possible	No	No	No
Contracaecum spp.	Possible in some spp.	No	5 spp. recorded in NZ [31]	Likely [50]	Pathology related to species	No	No	No	Probable	No	No	Yes (larval infection)
Camallanus spp.	No – found in intestines	No	1 sp. reported [51]	Likely	Pathology related to species	No	No	No	Probable	No	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Cucullanus spp.	No – found in intestines	No	11 spp. recorded in NZ [24]	Yes [35]	Pathology related to species	No	No	No	Probable	No	No	No
Piscicola geometra	No – ectoparasite	No	Piscicolid leech reported in NZ [51]	Unknown – generally palaearctic distribution	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Argulus spp.	No – ectoparasite	No	*A. japonicus* in NZ	Yes [35]	*A. foliaceus*	No	No	No	Yes	No	No	No
Lamproglena monodi & sp.	No – ectoparasite	No	Not reported	Yes [35]	n/a	n/a	n/a	n/a	n/a	n/a	No	No
Lernaea cyprinacea, L. tilapiae	No – ectoparasite	No	Yes [31]	Yes [52]	No	No	No	No	No	No	No	No
Caligus epidemicus, C. orientalis	No – ectoparasite	No	14 spp. recorded in NZ [24]	Yes [53]	No	No	No	No	Probable	No	No	No
FUNGI												
Saprolegnia spp.	No – surface infection	No	Yes	Yes	No	No	No	No	No	No	No	No

Agent	Likely to be associated with commodity ?	OIE listed?	In New Zealand?	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?
Ichthyophonus hoferi	Possible	No	Considered global distribution [54] although not reported from NZ	Considered global distribution [54]	No	No	No	No	No	No	No	Yes
Branchiomyces sp.	No – limited to gills	No	Likely	Likely	No	No	No	No	No	No	No	No
Rhizomucor sp.	Possible	No	Yes	Likely	No	No	No	No	No	No	No	No
Aphanomyces invadans	Potential exists [55]	Yes	No	Possible, spread noted through Asia [55]	n/a	n/a	n/a	n/a	n/a	n/a	No	Yes

Table References

[1] Hong Kong China; Network of Aquaculture Centres in Asia Pacific (NACA) – Quarterly Aquatic Animal Disease Report Q2
[2] All have been reported from tilapia
[3] Returning, sea-run quinnat salmon (*Oncorhynchus tshawtscha*); South Island (Diggles *et al.* 2002) & turbot and flounder from aquaculture facility (Hine PM, Pers comm.)
[4] Restricted to Australia (Ahne *et al.* 1997)
[5] Exotic pathogenic iridoviruses
[6] NZ isolate related to less pathogenic Ab serotype (Tisdall and Phipps 1987)
[7] China (Shi 2000)
[8] *A. salmonicida* isolated from *Oreochromis niloticus* in Mexico (Castro-Escarpulli *et al.* 2003)
[9] (Michel *et al.* 2002)
[10] Brazil (Salvador *et al.* 2003, Salvador *et al.* 2005)
[11] Brazil (Muratori *et al.* 2000)
[12] Taiwan (Chen *et al.* 2006)
[13] (Diggles *et al.* 2002)
[14] (Buller 2004)
[15] Taiwan (Chen *et al.* 1994)
[16] Brazil (Lima *et al.* 2001)
[17] China (Raidal *et al.* 2004)
[18] Common in marine and estuarine environments (Buller 2004)
[19] Taiwan (Huang *et al.* 1999)
[20] Taiwan (Faisal *et al.* 1989)
[21] (Faisal *et al.* 1987)
[22] Taiwan (Hsieh *et al.* 2006)
[23] Only reported from Israel (Nizan and Hammerschlag 1993)
[24] (Hewitt and Hine 1972, Hine *et al.* 2000)
[25] Brazil (Martins *et al.* 2001, Tavares-Dias *et al.* 2001a)
[26] But various amoeba spp. widespread and reported from NZ
[27] Only reported from Czech Republic (Dykova *et al.* 1997)
[28] (Boustead 1982, Hine *et al.* 2000, Diggles *et al.* 2002)
[29] Brazil (Tavares-Dias *et al.* 2001a, Vargas *et al.* 2003, Azevedo *et al.* 2006)
[30] Brazil (Tavares-Dias *et al.* 2001a)
[31] (Boustead 1982, Hine *et al.* 2000)
[32] Brazil (Alexandrino *et al.* 2001)
[33] Eurasia-wide (Basson and Van As 2006)
[34] (Hine 1978, Hine *et al.* 2000)
[35] Brazil (Tavares-Dias *et al.* 2001b)
[36] Quoted papers indicate infection in skin, gill, eye, ovary and internal organs
[37] *Myxobolus* spp. widespread and common, most have high host specificity & require specific oligochaete intermediate hosts.
[38] (Boustead 1982)
[39] Brazil (Vargas *et al.* 2003)
[40] (Boustead 1982, Hine *et al.* 2000, Diggles *et al.* 2002)
[41] Brazil (Azevedo *et al.* 2006)
[42] Some metacercaria may be found in muscle tissue (El-Khatib 1998, Arafa *et al.* 2005)
[43] *Clinostomum* sp. found in marine fish in NZ (Boustead 1982)
[44] Some metacercaria may be found in muscle tissue (Britz *et al.* 1985, Arafa *et al.* 2005)
[45] China (Zhang *et al.* 2006)

[46] Although predilection for heart and truncus arteriosus (Paperna and Dzikowski 2006)
[47] China (Zhang JinHui and Zhang Ping 2003)
[48] *Acanthocephalus* sp. reported in NZ (Hewitt and Hine 1972)
[49] Will be considered together.
[50] Worldwide distribution (Molnar *et al.* 2006)
[51] (Hine *et al.* 2000)
[52] Brazil (Fortes *et al.* 1998, Tavares-Dias *et al.* 2001b)
[53] China, Taiwan (Ho *et al.* 2004, Nagasawa 2004)
[54] (McVicar 1999)
[55] With over 100 spp. of freshwater fish, including cichlids, affected a precautionary approach has been adopted