

Import risk analysis:
Freshwater prawns
(Macrobrachium
rosenbergii) from Hawaii

22 March 2006

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**Biosecurity New Zealand
Ministry of Agriculture and Forestry
Wellington
New Zealand**



22 March 2006

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Biosecurity New Zealand

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22 March 2006

Approved for general release

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1. EXECUTIVE SUMMARY

This risk analysis examines the biosecurity risks associated with the importation of live freshwater prawns (*Macrobrachium rosenbergii*) into New Zealand.

A preliminary hazard list was compiled, comprising 76 viral, bacterial, fungal, protozoan, metazoan and other disease agents. The hazard identification concluded that six of these diseases should be considered potential hazards in the commodity: White Spot Disease, White Tail Disease, Lactococcosis, Rickettsial disease, *Aphanomyces* sp. infection and the nematode *Angiostrongylus cantonensis*.

Risk assessments were conducted for each of these organisms, and it was concluded that risk management measures were necessary for the viruses causing white spot disease and white tail disease the fungus *Aphanomyces astaci* and the nematode *Angiostrongylus cantonensis*.

The following risk management methods were recommended:

1. *M. rosenbergii* destined for export should be sourced only from an aquaculture facility which has a water supply free from known colonisation by *Procambarus clarkii*, a known carrier of *Aphanomyces astaci*.
2. The water supply for the facility must be treated by filtration and/or ultraviolet irradiation and/or ozonation to a level which can be shown to remove all known vectors of *A. cantonensis*.
3. The facility must have a verifiable history of testing for diseases in their stocks of *M. rosenbergii* which demonstrates that the animals are not infected with the organisms that cause White Spot Disease or White Tail Disease.
4. Within 1 month of the shipping date a sample of 150 individuals must be taken from the specific population of *M. rosenbergii* from which the animals are being sourced for export.
5. These samples must be submitted to the following diagnostic tests at a diagnostic facility approved by the competent authority in the exporting country :
 - a. Examination of haemolymph, gills or pleopods for the causative agent of White Spot Disease using the nested PCR method recommended by the World Organisation for Animal Health (OIE 2005a).
 - b. Examination of haemolymph, gills, tail muscle or pleopods for the organisms causing White Tail Disease using the PCR described by Yoganandhan et al. (2005).
6. The *M. rosenbergii* intended for export must be removed from the same population and at the same time as the animals being tested for viruses. The animals intended for

export must be placed in a tank which is physically isolated from other crustaceans held at the rearing facility and which has its own water supply filtered to permit entry of a particle size of no greater than 20µm. Any animals that die during the pre-export quarantine period must also be tested for the organisms that cause White Spot Disease and White Tail Disease at a diagnostic facility approved by the competent authority in the exporting country.

7. If any of the pre-export tests are positive for the organisms that cause White Spot Disease or White Tail Disease, the *M. rosenbergii* from that facility will not be permitted entry into quarantine in New Zealand.
8. The *M. rosenbergii* must be exported in water that is free from *Vibrio cholerae*, and is filtered to permit entry of a particle size of no greater than 20µm. Water must not be exchanged during transport.
9. On arrival in New Zealand, the *M. rosenbergii* must be transported directly to an approved transitional facility complying with MAF Standard 154.02.06: *Transitional Facilities for Ornamental Fish and Marine Invertebrates*. Here the *M. rosenbergii* can be released into quarantine tanks but the water used for transport must be treated before disposal as prescribed in the MAF Standard.
10. Any *M. rosenbergii* broodstock that are dead on arrival, and any dead or diseased animals that are detected during the quarantine period must be subjected to a thorough virological, bacteriological and histopathological examination by a laboratory approved by Biosecurity New Zealand.
11. The *M. rosenbergii* imported from Hawaii will not be permitted to leave quarantine at any time, but locally grown *M. rosenbergii* broodstock will be allowed within the quarantine facility to co-habit with imported broodstock, and resulting batches of larvae spawned from broodstock of imported and domestic origin will be released from the quarantine facility after 150 larvae have been tested and found free from the organism causing white spot disease, using the nested PCR method recommended by the OIE (2005a) for this organism, by a laboratory approved by Biosecurity New Zealand.
12. The above conditions will be followed for the first 3 batches of imported larvae, or a minimum of 12 months (whichever takes the greater period of time), after which if all batches of larvae test negative for the organism causing white spot disease, the broodstock will be considered free of that disease and will be permitted to leave the quarantine facility.

2. INTRODUCTION

2.1 BACKGROUND

Over the past decade the international trade of live and frozen crustacean products has been recognised to have spread many serious crustacean pathogens around the world (Lightner et al. 1997, OIE 2003, Briggs et al. 2004). For example, at least four virus pandemics have adversely affected the global penaeid shrimp farming industry since 1980 (Lightner 2003). One of these, Taura Syndrome Virus (TSV), was first identified from farms around the Taura River in Ecuador in 1992 and subsequently spread rapidly to the whole of Latin and North America within three years through the regional and international transfer of live post larvae (PL) and broodstock *Penaeus vannamei* (Lightner 1995, 1996, Brock et al. 1997, VanPatten et al. 2004). A few years later, this disease was introduced into Asia including mainland China and Taiwan (Tu et al. 1999, OIE 2003), and most recently Thailand (Neilsen et al. 2005) with movements of *P. vannamei*. It has been suggested that TSV caused direct losses (due to shrimp mortality) of up to US\$ 1.3 billion in the first three years in Latin America, and around \$2 billion dollars overall (Lightner 2003). However, indirect losses due to loss of sales, increased seed cost and restrictions on regional trade were considered to be much higher (Brock et al. 1997).

Some data suggest that TSV was introduced to Colombia and Brazil through contaminated broodstock from the Hawaiian Islands (Brock et al. 1997). These broodstock were untested for TSV since at the time it was not yet known that Taura syndrome had a viral cause. This situation is not unique. As a consequence of the rapid growth and development of the penaeid aquaculture industry, many of the most significant pathogens were moved from regions where they initially appeared to new regions even before the new pathogen had been recognised, named, proven to cause disease, and before reliable diagnostic methods were developed (Lightner 2003). Even when diagnostic methods are available, and the diseases are well known, the large volume of trade of commodities such as broodstock to some areas has resulted in the introduction of pathogens, even in the presence of disease certification programmes (Neilsen et al. 2005).

2.2 NEW ZEALAND'S CRUSTACEAN FAUNA

New Zealand's crustacean fauna is generally considered limited compared to other regions, given the extent of the country over 30° of latitude, the exceptionally large area of continental shelf and slope (fifth largest in the world), and the niches apparently available. It is generally felt that this limited diversity of species has resulted from New Zealand's isolation from centres of diversity that might have acted as sources of recruitment. However as shown in Table 1, the fauna is characterised by a high degree of endemism (Webber et al. in press).

No species within the order decapoda are categorised as threatened under the Department of Conservation's Species Priority Ranking System. One amphipod, *Chaetocorophium*

lucasi found in estuaries and lake edges, and one isopod, *Austridotea annectens*, which occurs in fresh water stream margins, are listed in threat category I, species about which little is known, but based on existing knowledge are considered to be under threat. *C. lucasi* is however a widespread species, and its threatened status is uncertain (McGuinness 2001). The marine crab, *Elamena momona* has been identified as of potential conservation concern because of its disjunct distribution, whilst the marine crab *Halimena aotearoa* is listed as of potential conservation concern because it is rare as it is seldom seen or collected. The marine crab, *Leptomithrax tuberculatus mortenseni* is listed as of potential conservation concern because it occurs in an intensively fished area, has a restricted distribution and is known from only 22 specimens collected in 1924 (McGuinness 2001).

Table 1. Crustacean fauna in New Zealand (Webber et al. in press)

Taxon	Described living species/subspecies	Known undescribed/undetermined species	Estimated unknown species/subspecies	Adventive species/subspecies	Endemic species/subspecies	Endemic Genera
Malacostraca	1186	291	3096	15	792	78
Nebaliacea	3	2	2	0	0	0
Stomatopoda	7	0	4	0	0	0
Anaspidacea	2	4	5	0	5	1
Bathynellacea	5	3	5	0	8	0
Lophogastrida	4	1	3	0	0	0
Mysida	16	2	2	0	11	0
Amphipoda	389	25	800	8	249	42
Isopoda	312	67	2000	6	318	20
Tanaidacea	40	41	200	0	6	0
Cumacea	47	27	25	1?	65	6
Euphausiacea	20	0	10	0	0	0
Decapoda	341	119	40	0	131	9

Due to their isolation, impacts on populations of these threatened and endemic species from the introduction of new diseases may be significant.

Freshwater prawns (*Macrobrachium rosenbergii*) were introduced into New Zealand from Malaysia in 1988 through a permit issued under the Animals Act 1967. On that occasion the introduced animals were quarantined for 15 months to assure that they were disease free (<http://geoheat.oit.edu/bulletin/bull19-3/art76.htm>). The likelihood of this species establishing in the wild in this country is considered to be negligible, as it is unable to survive at water temperatures below 15°C (Brock, 1988, Herrera et al. 1998).

Since that single importation, the passage of the Biosecurity Act 1993 means that further importations of these animals require an import health standard (IHS).

2.2 COMMODITY DEFINITION

The commodity considered in this risk analysis is the adult freshwater prawn species *Macrobrachium rosenbergii*, sourced from Hawaii, for use as brood stock.

Macrobrachium rosenbergii (De Mann 1879) is a decapod crustacean in the Family Palaemonidae (New 2002). Synonyms for *M. rosenbergii* have included *Palaemon rosenbergii*, *P. carcinus rosenbergii*, *P. (Eupalaemon) rosenbergii*, *P. whitei*, *P. spinipes*, *P. dacqueti*, *Cryphiops rosenbergii*, *C. (Macrobrachium) rosenbergii*, and *Macrobrachium rosenbergii dacqueti*. (source: Integrated Taxonomic Information System (ITIS)¹. Common names for this species include 'giant river prawn', 'giant Malaysian prawn', 'giant freshwater prawn', 'giant long-armed prawn', and 'cherabin'.

M. rosenbergii is indigenous to Southeast Asia as well as northern Oceania and the western Pacific islands. Thirty six specimens were imported into the Hawaiian Islands in 1965 from Malaysia (Fujimura and Okamoto 1972), and successful hybridization experiments were conducted with three of the four morphs in Hawaii (Malecha 1980). Some individuals were distributed in streams on all the major Hawaiian islands (Maciolek 1972), however, Davidson et al. (1992) indicated that the species had not become established (Eldridge 1994).

Some taxonomists recognise two sub-species of *M. rosenbergii* based on morphological differences: a western form, *M. rosenbergii dacqueti* (Sunier, 1935), which is distributed in western Asia (east coast of India, Bay of Bengal, Gulf of Thailand, Malaysia and the northern Indonesian islands of Sumatra, Java and Kalimantan), and an eastern form *M. rosenbergii rosenbergii* (De Man, 1879), which is native to the eastern Asia-Pacific, occurring in the Philippines, the Indonesian islands of Sulawesi and Irian Jaya, and in Papua New Guinea and northern Australia (Holthuis 2000). A recent study of the genetic diversity of this species has shown the presence of "eastern" and "western" clades (Mather and de Bruyn 2003).

The closely related *Macrobrachium lar* was brought to Honolulu, Hawaii, from Guam in 1956 (Brock 1960), and additional specimens were brought from Tahiti in 1961 (Maciolek 1972). After nine years, a large specimen was collected on the island of Hawaii (Kanayama 1967). At present *M. lar* is established on all the main Hawaiian Islands (Devick 1991).

¹ <http://www.itis.usda.gov/index.html>

2.3 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 Sanitary and Phytosanitary (SPS) agreement of the World Trade Organisation (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 S 22 (5) (a)] is the focus of the release assessment, and the possible effects of such organisms [BSA S 22 (5) (b)] is 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 follows the guidelines in section 1.3 of the *Terrestrial Animal Health Code* of the World Organisation for Animal Health ("the OIE") (OIE 2004). The risk analysis framework is applied by MAF as described in *Import Risk Analysis Animals and Animal Products* (Murray 2002).

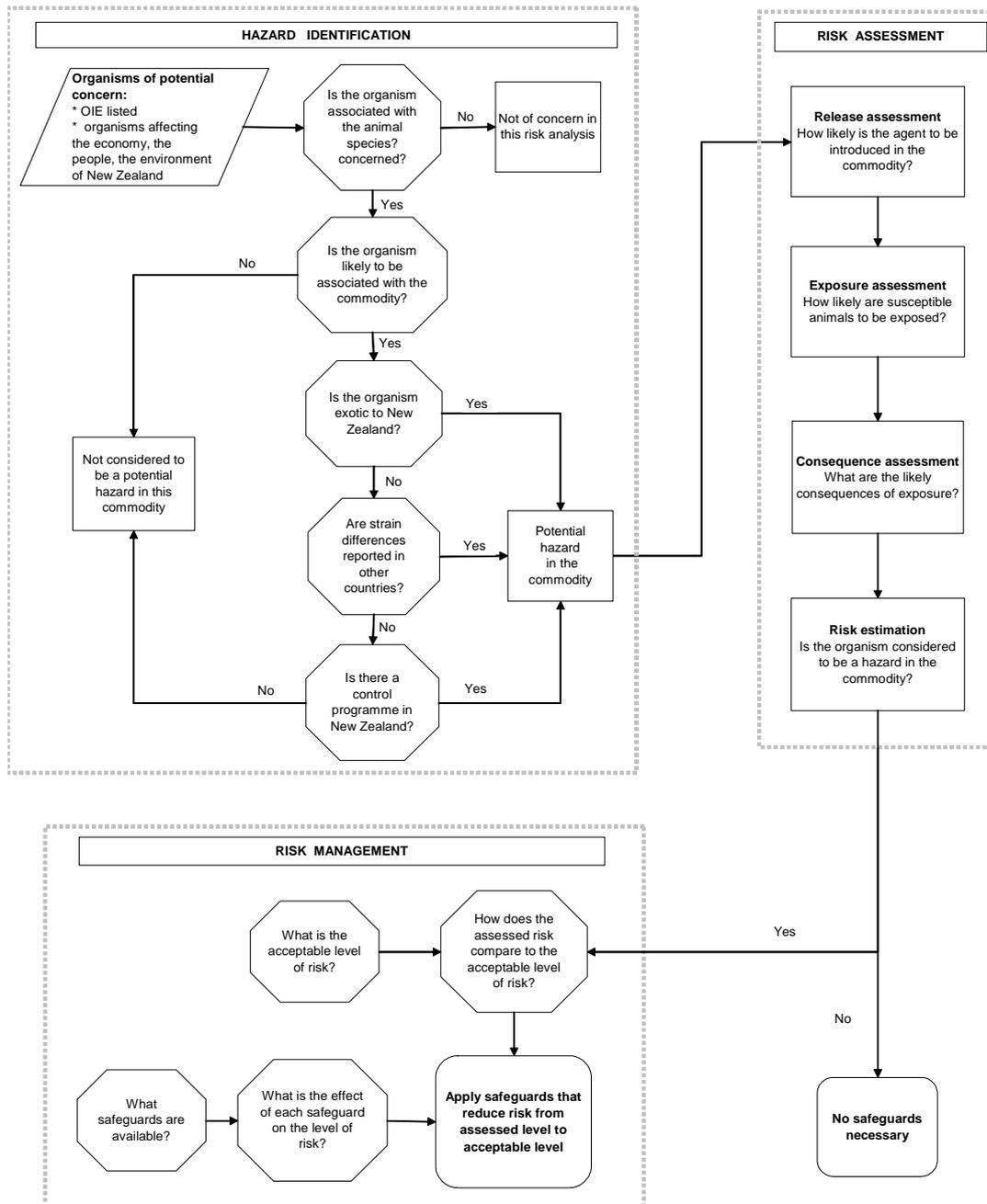
The risk analysis process used by MAF is shown in Figure 1.

2.3.1 Organisms of potential concern

The first risk analysis step is to compile a preliminary list of organisms of potential concern in the commodity under consideration. In this instance, because of the limited knowledge of the diseases of *Macrobrachium rosenbergii*, all of the organisms reported from this species throughout its world-wide distribution are considered as potential concern.

Each organism on this preliminary list is then examined in more detail, and any regarded as likely to cause significant harm in New Zealand are retained for further consideration in the risk analysis.

Figure 1. The risk analysis process



To be retained 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 was defined as either a native species that occurs in New Zealand waters naturally, or which was introduced into New Zealand, but which is now considered to be acclimatised); and/or
4. it is known to cause a threat to human health.

2.3.2 Hazard identification

Each of the organisms of potential concern is further considered against the following criteria:

1. whether the proposed commodity could potentially act as a vehicle for the introduction of the organism,
2. whether it is exotic to New Zealand but likely to be present in the exporting country,
3. For organisms that are present in New Zealand,
 - a) whether it is "under official control", which could be by government departments, by national or regional pest management strategies or by a small-scale programme, or
 - b) whether more virulent strains are known to exist in other countries.

For any organism, if the answers to questions one and either two or three are 'yes', it is classified as a potential hazard in the commodity and is subjected to an individual risk assessment.

2.3.3 Risk assessment

Section 22 (5) (a) of the Biosecurity Act 1993 requires consideration of the likelihood that organisms may be introduced in imported commodities. This is the focus of the release assessment. Section 22 (5) (b) of the Biosecurity Act 1993 requires consideration of the possible effects of such introduced organisms on the people, the environment and the economy of New Zealand. The exposure assessment is the first part of this consideration, comprising an assessment of the likelihood of spread and establishment of organisms introduced in specific commodities. 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.

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) Release assessment the likelihood of the organism being imported in the commodity.
- b) Exposure assessment the likelihood of animals or humans in New Zealand being exposed to the potential hazard.
- c) Consequence assessment the consequences of entry, establishment or spread 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 release, exposure 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 release 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 release is non-negligible but the exposure 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.

2.3.3.1 Release assessment

Release assessment consists of describing the biological pathways necessary for an importation activity to 'release' or introduce a *hazard* into a particular environment, and estimating the likelihood of that complete process occurring (OIE 2004).

The likelihood of a disease agent entering and becoming established 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 shrimp entering New Zealand,
- the likelihood of the disease agent being detected in quarantine.

The release assessment may require information on:

Biological factors

- the species, strain or genotype and age of the aquatic animal,
- the strain of the agent,
- epidemiology of the agent,
- tissue sites of infection or contamination,
- testing, treatment and quarantine.

Country factors

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

2.3.3.2 Exposure assessment

Exposure assessment involves the likelihood that the disease agent, having entered New Zealand's natural waters, will be exposed to susceptible species. This depends on the capacity of the disease agent to survive in its 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 for importations of *M. rosenbergii* include:

Biological factors

- presence of potential vectors or intermediate hosts,
- 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),
- geographical and environmental characteristics (current, temperature ranges, water courses).

Some of the disease agents of *M. rosenbergii* are likely to be parasites, which may have 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 products 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.

2.3.3.3 *Consequence assessment*

Consequence assessment consists of identifying the potential biological, environmental and economic consequences of disease introduction. A causal process must exist by which exposures to a hazard results in adverse health, or environmental, or socio-economic consequences (OIE 2004).

Examples of consequences relevant to *M. rosenbergii* 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. In general there is relatively little information on the parasites and diseases infecting *M. rosenbergii* and the epidemiology of those parasites or diseases. Therefore a qualitative approach was taken using the available information on similar pathogens if necessary, to determine a relative probability of an event occurring.
- 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.

2.3.3.4 *Risk estimation*

This final step involved with each assessment is to determine whether the level of risk presented by each disease agent to the New Zealand environment 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 a hazard in the commodity, for which risk management measures are necessary.

2.3.4 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 - a determination is made as to whether sanitary measures are necessary.
- b) Option evaluation - identify the options available for managing the risk, and consider risk reduction effects.
- 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.

3. HAZARD IDENTIFICATION

3.1 ORGANISMS OF POTENTIAL CONCERN

A number of significant OIE listed notifiable diseases have been reported from crustaceans in Hawaiian waters over the years, including Taura syndrome, Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), *Baculovirus penaei* (BP), White Spot Syndrome Virus (WSSV) and odon baculovirus (MBV) (Lightner et al. 1983, Brock et al. 1986b, Hasson et al. 1995, OIE 2003, USDA website²). Furthermore, a review of the literature and a previous risk assessment which investigated the risks associated with introduction of *M. rosenbergii* from Fiji to the Cook Islands (Arthur et al. 2004) found a number of organisms and disease agents associated with *Macrobrachium rosenbergii*. These are listed in Table 2, together with other information considered in this hazard identification. No significant diseases have been reported in the culture of *M. rosenbergii* in New Zealand to date, despite statistically significant numbers of larvae recently being examined (Diggles 2003).

Table 2. Organisms recorded from *M. rosenbergii* and other closely related species.

Common Name/ Disease	Scientific Name of disease agent	Reported in NZ?	OIE List?	Under official control or unwanted?	More virulent strains overseas?
VIRUSES					
White spot disease (WSD)	White spot syndrome virus (WSSV)	No	Yes	OIE listed	N/A
White Tail disease (WTD)	Macrobrachium rosenbergii nodavirus (MrNV)	No	No	Unwanted	N/A
White Tail disease	Extra small virus (XSV)	No	No	Unwanted	N/A
Hepatopancreatic parvo-like virus	HPV-Mac	No	No	No	N/A
BACTERIA*					
Normal flora	<i>Acinetobacter</i> sp.	Some spp.	No	No	No
Motile aeromonas septicaemia (MAS)	<i>Aeromonas caviae</i>	Yes	No	No	No
MAS and burn spot	<i>A. formicans</i>	Yes	No	No	No
MAS and burn spot	<i>A. hydrophila</i>	Yes	No	No	No
MAS and burn spot	<i>A. veronii</i>	Yes	No	No	No
Normal flora	<i>Agrobacterium</i> sp.	Some spp.	No	No	No
Normal flora	<i>Alcaligenes</i> sp.	No	No	No	N/A
Normal flora	<i>Arthrobacter</i> sp.	Some spp.	No	No	No
Normal flora	<i>Bacillus</i> sp.	Some spp.	No	No	No
Normal flora	<i>Benekea</i> sp.	Some spp.	No	No	N/A
Normal flora	<i>Chromobacter</i> sp.	Some spp.	No	No	No
Normal flora	<i>Chromobacterium</i> sp.	No	No	No	N/A

² http://www.aphis.usda.gov/vs/ceah/cei/IW_2004_files/wsd_us_0404_files/wsd_us_0404.htm

Common Name/ Disease	Scientific Name of disease agent	Reported in NZ?	OIE List?	Under official control or unwanted?	More virulent strains overseas?
BACTERIA* (cont)					
Normal flora	<i>Citrobacter freundii</i>	Yes	No	No	No
Epibiont fouling	<i>Cytophaga</i> sp.	Some spp.	No	No	No
Enterococcosis	<i>Enterococcus faecium</i>	Yes	No	No	No
None	<i>Enterobacter</i> sp.	Some spp.	No	No	No
None	<i>Flavobacterium</i> sp.	Some spp.	No	No	No
Lactococcosis	<i>Lactococcus garvieae</i>	No	No	Unwanted	N/A
Epibiont fouling	<i>Leucothrix</i> spp.	Some spp.	No	No	No
Normal flora	<i>Micrococcus</i> sp.	Some spp.	No	No	No
None	<i>Moraxella</i> sp.	Some spp.	No	No	No
Tuberculosis	<i>Mycobacterium</i> sp.	Some spp.	No	No	No
Normal flora	<i>Photobacterium</i> sp.	Some spp.	No	No	No
None	<i>Pseudomonas alcaligenes</i>	Yes	No	No	No
Rickettsial disease	Rickettsia-like organism	No	No	Unwanted	N/A
Normal flora	<i>Staphylococcus</i> sp.	Some spp.	No	No	N/A
Normal flora	<i>Streptococcus</i> sp.	Some spp.	No	No	Yes
Normal flora	<i>Vibrio alginolyticus</i>	Yes	No	No	No
Vibriosis	<i>Vibrio anguillarum</i>	Yes	No	No	No
Cholera	<i>V. cholerae</i>	Yes	No	Yes	No
Luminous vibriosis	<i>V. harveyi</i>	Yes	No	No	No
Vibriosis	<i>V. parahaemolyticus</i>	Yes	No	No	No
Vibriosis	<i>Vibrio</i> spp.	Yes	No	No	No
Normal flora	<i>Xanthomonas</i> sp.	Some spp.	No	No	No
FUNGI**					
Water mould	<i>Achyla</i> sp.	Yes	No	No	No
Phycomycete	<i>Aphanomyces</i> sp.	Some spp.	Yes	Unwanted	Yes
Yeast	<i>Candida sake</i>	Yes	No	No	No
Yeast	<i>Debaryomyces hansenii</i>	Yes	No	No	No
Yeast	<i>Endomyces fibuliger</i>	Yes	No	No	No
Yeast	<i>Metschnikowia bicuspidata</i>	Yes	No	No	No
Yeast	<i>Pichia anomala</i>	Yes	No	No	No
Phycomycete	<i>Lagenidium</i> sp.	Yes	No	No	No
Black spot	<i>Fusarium solani</i>	Yes	No	No	No
Water mould	<i>Saprolegnia</i> sp.	Yes	No	No	No
PROTOZOA					
Microsporidian infection	<i>Thelohania</i> sp.	Some spp.	No	No	Yes
Epibiont fouling	<i>Cothurnia</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Epistylis</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Lagenophrys</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Opercularia</i> sp.	No	No	No	N/A
Epibiont fouling	<i>Vaginicola</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Vorticella</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Zoothamnium</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Acineta</i> sp.	Some spp.	No	No	No

Common Name/ Disease	Scientific Name of disease agent	Reported in NZ?	OIE List?	Under official control or unwanted?	More virulent strains overseas?
PROTOZOA (cont)					
Epibiont fouling	<i>Acinetides</i> sp.	No	No	No	N/A
Epibiont fouling	<i>Ephelota</i> sp.	Some spp.	No	No	No
Epibiont fouling	<i>Podophrya</i> sp.	No	No	No	N/A
Epibiont fouling	<i>Tokophrya</i> sp.	No	No	No	N/A
Apicomplexan infection	<i>Nematopsis rosenbergii</i>	No	No	No	N/A
METAZOA					
Isopoda	<i>Augustogathoma</i> sp.	No	No	No	N/A
Isopoda	<i>Palaegyge bengalensis</i>	No	No	No	N/A
Isopoda	<i>Probopyrus buitendjiki</i>	No	No	No	N/A
Isopoda	<i>Tachaea spongillicola</i>	No	No	No	N/A
Digenea	Microphallid metacercaria	Some spp.	No	No	No
Digenea	Opecoelid metacercaria	Some spp.	No	No	No
Digenea	Digenean metacercariae	Some spp.	No	No	No
Nematode in <i>Macrobrachium</i> lar	<i>Angiostrongylus cantonensis</i>	No	No	Unwanted	N/A
OTHER					
Appendage deformity syndrome	Nutritional disease (carotenoid deficiency)	No	No	No	N/A
Branchiostegal blister disease	Idiopathic, probably due to poor water quality	No	No	No	N/A
Idiopathic muscle necrosis (IMN)	Idiopathic, probably due to poor water quality	No	No	No	N/A
Mid-cycle disease	Unknown aetiology	No	No	No	N/A
Moult-death syndrome	Various causes	Yes	No	No	No
Terminal growth	Senility	No	No	No	N/A

Notes:

N/A = the existence of more virulent strains in other countries is not applicable for organisms that are not present in New Zealand.

* Data on endemicity of some bacteria were obtained from NZ Reference Culture Collection
www.esr.cri.nz/NR/rdonlyres/D8C17243-95F7-4725-AF60-4E231AD722DF/0/culture_collection_catalogue_2004.pdf

** Data on endemicity of some fungi were obtained from the Landcare Research database
http://nzfungi.landcareresearch.co.nz/html/search_index.asp?ID=33-WNN-95

3.2 ORGANISMS REQUIRING FURTHER CONSIDERATION

3.2.1 Viruses

White spot disease (WSD), caused by white spot syndrome virus (WSSV), is an OIE listed notifiable disease which can affect all life stages of all decapod crustaceans and may result in significant mortalities (OIE 2005a). This virus has been recorded from *M. rosenbergii*, and has also been recorded from the waters of the Hawaiian Islands. Therefore this organism requires further consideration in this risk analysis.

White tail disease (WTD) is caused by *Macrobrachium rosenbergii* nodavirus (MrNV) and is also associated with a satellite virus called extra small virus (XSV) (Bonami et al. 2005). This disease was first reported causing mortalities in post larval *M. rosenbergii* in Taiwan as early as 1992 due to extensive necrosis of the tail muscle (Tung et al. 1999). It has since been recorded in several other parts of the world, including the French West Indies, China and India (Arcier et al. 1999, Quian et al. 2003, Sahul Hameed et al. 2004, Yoganandhan et al. 2005), where it has been reported to cause disease. Although it has not been recorded from the Hawaiian Islands, since these viruses are regionally significant and potentially could be translocated through movements of live adult *M. rosenbergii*, this disease will be considered further in this risk analysis.

A parvo-like virus has been reported to infect the hepatopancreas of *M. rosenbergii* in Malaysia (Anderson et al. 1990a). However, this was an incidental finding in a toxicity study, it was not associated with any pathology and it was demonstrated to be non-pathogenic (Anderson et al. 1990a). Because this virus does not cause disease (FAO 2002), and has not been recorded from *M. rosenbergii* in the Hawaiian Islands, it will not be considered further.

Other OIE-notifiable viral disease agents of crustaceans reported from the Hawaiian Islands include Taura Syndrome, Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), *Baculovirus penaei* (BP), and Monodon baculovirus (MBV) (Lightner et al. 1983, Brock et al. 1986b, Hasson et al. 1995, OIE 2003, 2004, 2005a). None of these disease agents have been reported to occur in *M. rosenbergii* anywhere in the world. Yellow Head Virus (YHV) has not been reported in Hawaiian waters, and in any case *M. rosenbergii* appears resistant to YHV (Longyant et al. 2005). Similarly, Spawner Isolated Mortality virus has not been recorded from *M. rosenbergii*, or the Hawaiian Islands (OIE 2005a). Therefore these organisms will not be considered in this risk analysis.

3.2.2 Bacteria

Bacteria of the genera *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Chromobacterium*, *Cytophaga*, *Micrococcus*, *Photobacterium*, *Staphylococcus*, *Streptococcus* and *Xanthomonas* have been isolated from egg, muscle and hepatopancreas of healthy *M. rosenbergii* (Anderson et al. 1990a, Phatarpekar et al. 2003).

Aeromonas caviae, *A. formicans*, *A. hydrophila* and *A. veronii* are motile aeromonads which are ubiquitous opportunistic species that can be associated with burn spot disease (El-Gamal et al. 1986) and septicaemia under adverse conditions (Sung et al. 2000). They have all been recorded from New Zealand where they are considered to be opportunistic pathogens of fish held under unsuitable conditions (Diggles et al. 2002a), including *M. rosenbergii* (see Diggles 2003). Therefore they will not be considered further in this risk analysis.

Arthrobacter sp., *Bacillus* sp., *Benekea* sp., *Chromobacter* sp., *Citrobacter freundii*, *Enterobacter* sp. and *Flavobacterium* sp. are also ubiquitous bacteria, various species of which have been recorded in New Zealand. None cause disease in *M. rosenbergii* following experimental challenge (Anderson et al. 1990a, Sung et al. 2000), and hence they are also likely to be members of normal microbial flora of *M. rosenbergii* or its rearing environment. Therefore these organisms will not be considered further.

Enterococcus faecium has been reported to be associated with a yeast infection disease mainly in adult prawns, causing a cumulative mortality of up to 25% in *M. rosenbergii* cultured in Taiwan (Chen et al. 2003). However, this bacterium has been reported in the New Zealand environment associated with raw sewage (Sinton and Donnison 1994) and occasionally, infections in humans (Kobayashi et al. 2000). This opportunistic bacterium only caused disease in *M. rosenbergii* reared in suboptimal conditions, and the yeast appeared the main pathogen in this infection (Chen et al. 2003). Since *E. faecium* is already present in New Zealand it will not be considered further in this risk analysis. The yeast *Metschnikowia bicuspidate*, which was associated with *E. faecium*, is discussed further in section 3.2.3.

Lactococcus garviae is a significant opportunistic pathogen of *M. rosenbergii* in Taiwan (Cheng and Chen 1998a, 1998b, 1999, 2002, Cheng et al. 2002). This bacterium causes disease in a wide variety of species, including finfish, and has not been recorded in New Zealand (Diggles et al. 2002a). This organism will be considered further.

Leucothrix spp. are ubiquitous filamentous bacteria commonly found as epibionts on crustaceans, including in New Zealand (Diggles 2000), hence they will not be considered further.

Moraxella sp., *Mycobacterium* sp. and *Pseudomonas alcaligenes* will not be considered further as they are ubiquitous bacteria in aquatic environments and they have been recorded in New Zealand (Diggles et al. 2002a, NZ Reference Culture Collection list).

A rickettsia-like organism (RLO) has been reported to cause disease in *M. rosenbergii* in Brazil (Cohen and Isaar 1989, Johnson and Bueno 2000), and a similar organism has been reported infecting 10% of wild juvenile *Melicertus marginatus* (syn. *Penaeus marginatus*, or aloha prawn) caught off the tidal flats of Manualua Bay, Hawaii (Brock et al. 1986a). Similar RLOs have not been reported from crustaceans in New Zealand and since these agents can sometimes be associated with disease, they will be considered further in this risk analysis.

Vibrio (Listonella) anguillarum, *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi* and other *Vibrio* species are ubiquitous opportunistic disease agents of fish and shellfish worldwide causing a variety of diseases including vibriosis and luminous vibriosis. These bacteria tend to cause disease in post larval *M. rosenbergii* reared in hatcheries (Tonguthai 1997), and not adults. In some cases the presence of these bacteria is not associated with disease (Colorni 1985), confirming their opportunistic nature. These species of *Vibrio* have already been reported from New Zealand, causing disease in fish and shellfish reared in suboptimal conditions (Diggles et al. 2000, 2002a). Because of this, and the fact that improvements in husbandry can significantly reduce or even eliminate disease caused by *Vibrio* spp, these bacteria will not be considered further.

Vibrio cholerae is the organism responsible for cholera in humans. Various serotypes of this disease agent are occasionally isolated from water used to rear *M. rosenbergii* in some parts of the world (Al-Harbi 2003). The presence of this bacterium in rearing water occurs where there is contamination of the water source by sewage or stormwater. Outbreaks of cholera in the Hawaiian Islands are sporadic (Mintz et al. 1994), as they are in New Zealand (Bennett 2005). *V. cholerae* is reported in returning travelers to New Zealand from time to time (Bennett 2005), but it is considered to be an exotic organism. Although it does not cause disease in crustaceans, a requirement of importation will be that waste water used to transport the *M. rosenbergii* to this country must be free from *V. cholerae* and it must be treated and disposed of according to New Zealand quarantine standards. Therefore, *V. cholerae* will not be considered further.

3.2.3 Fungi

Most of the fungi and yeasts listed in Table 2 are opportunistic pathogens of fish and shellfish which have been previously recorded in New Zealand. *Achyla* sp. and *Saprolegnia* sp. are ubiquitous water moulds that are found in New Zealand associated with disease in fish and shellfish that have been injured or immunocompromised (Hine and Diggles 2005). *Lagenidium* sp. have been recorded as saprophytes of eggs of lobsters in New Zealand (Diggles, personal obs.). *Fusarium solani* has been reported as causing black spots on the carapace of *M. rosenbergii* cultured in Florida, USA (Burns et al. 1979). In that case *F. solani* did not effect healthy prawns, but only those which had sustained previous cuticular damage (Burns et al. 1979). Moreover, *F. solani* is considered endemic in New Zealand (Dick and Dobbie 2002). Because *F. solani*, *Achyla*, *Saprolegnia* and *Lagenidium* are all opportunistic pathogens that occur ubiquitously worldwide including in New Zealand, they will not be considered further.

The yeasts *Pichia anomala*, *Endomyces fibuliger*, *Candida sake* and *Candida famata* (syn. *Debaryomyces hansenii*) have been recorded from diseased adult *M. rosenbergii* in Taiwan, mostly in winter (Lu et al. 1998). Another yeast, *Metschnikowia bicuspidata*, was reported to be associated with the bacterium *Enterococcus faecium* during mortalities of adult *M. rosenbergii* in Taiwan (Chen et al. 2003). *P. anomala* is considered a normal part of the intestinal flora of humans, although it has been associated with clinical disease in some circumstances where hosts are immunosuppressed (Reyes et al. 2004). The other

yeasts such as *E. fibuliger*, *C. sake* and *C. famata* are recorded worldwide as spoilage organisms, as opportunistic invaders of immunosuppressed hosts, including humans (Hoegl et al. 1998), as postharvest biocontrol agents on fruit (Abadias et al. 2002) and in uses such as fermentation of Chinese rice wine (Ngan et al. 2000). Their presence in diseased *M. rosenbergii* in Taiwan is therefore opportunistic, probably due to affected animals being immunosuppressed by low water temperatures. Since all of the yeasts mentioned above are ubiquitous and have been reported in New Zealand (e.g. Fell and Pitt 1969), and none have been reported to cause disease or mortalities in the culture of *M. rosenbergii* in the Hawaiian Islands, they will not be considered further.

Some species of *Aphanomyces* have previously been reported from *M. rosenbergii* (see Sindermann 1977, Brock 1988). Two species of *Aphanomyces* are listed by the OIE as notifiable diseases of aquatic animals. One is *Aphanomyces astaci*, the cause of crayfish plague in freshwater crayfish (Decapoda: Astacidae) and which is widespread in Europe as well as North America (OIE 2005a). The other is *Aphanomyces invadans*, the cause of Epizootic Ulcerative Syndrome (EUS) in fish (OIE 2005a). The Louisiana crayfish *Procambarus clarkii*, a known carrier of *A. astaci*, was introduced into Hawaii in 1934 (Eldredge 1994) and has established in the Hawaiian islands³. Because of this, it is considered possible that *A. astaci* was introduced into the Hawaiian Islands via the Louisiana crayfish, and this disease will be considered further. However, EUS has been recorded only from finfish (Lilley et al. 1998, Chinabut and Roberts 1999, OIE 2005a), it does not appear to infect crustaceans, and it has not been reported from Hawaii. Therefore EUS will not be considered further.

3.2.4 Protozoa

Infection by the microsporidian *Thelohania* sp. was recorded once in *M. rosenbergii* cultured in Thailand (Areerat 1988). Microsporidian infections are horizontally transmitted diseases with direct life cycles, and although they are common in marine shrimp, they are rarely found in freshwater shrimp (Tonguthai 1997). Species of *Thelohania* have already been recorded in native freshwater crayfish in New Zealand (*T. contejeani* in *Paranephrops planifrons* and *P. zealandicus*, see Quilter 1976, Jones 1980). Since infections by *Thelohania* sp. have never been recorded in *M. rosenbergii* in the Hawaiian Islands, and New Zealand already has endemic species of *Thelohania* in freshwater crayfishes, this disease agent will not be considered further.

A wide variety of epibiont ciliates have been reported as ectocommensals of *M. rosenbergii*, including *Acineta* sp., *Acinetides* sp., *Cothurnia* sp., *Ephelota* sp., *Epistylis* sp., *Lagenophrys* sp., *Opercularia* sp., *Podophrya* sp., *Tokophrya* sp., *Vaginicola* sp., *Vorticella* sp., and *Zoothamnium* sp. (see Sindermann 1977, Brock 1988, Camacho-Granados and Chinchilla-Carmona 1989, Tonguthai 1992, 1997, Johnson and Bueno 2000, Rodriguez et al. 2001, Jayasree et al. 2001). Most of these species have been recorded from various aquatic animals in New Zealand (Diggles, personal obs.), but there appear to be no records of some of them, such as *Opercularia* sp., *Acinetides* sp., *Podophrya* sp., and *Tokophrya* sp. These epibionts are transmitted horizontally through

³ http://iz.carnegiemnh.org/crayfish/country_pages/state_pages/hawaii.htm

the water and they colonise the carapace and other external surfaces of crustaceans, which they use as a substrate from which to feed on bacteria and other organic matter in the water. They are not obligate parasites, nor do they contribute to disease in farmed crustaceans or fish except under exceptional circumstances of poor water quality or suboptimal hygiene in aquaculture situations (Brock and Lightner 1990). They do not cause disease in wild fish or shellfish, hence all indications are that introduction of epibiont ciliates likely to occur on broodstock *M. rosenbergii* would have a negligible effect on the disease status of aquatic animals in New Zealand. Because of these reasons, none of these epibiont ciliates will be considered further.

The apicomplexan *Nematopsis rosenbergii* has been recorded from *M. rosenbergii* in Asia (Shanavas et al. 1989), but has not been recorded from *M. rosenbergii* in the Hawaiian Islands. Apicomplexan parasites infect the hindgut and have a two host life cycle which requires a molluscan intermediate host and a decapod crustacean definitive host (Lauckner 1983, Johnson 1995). Other species of *Nematopsis* have already been recorded in marine molluscs in New Zealand (Jones 1975, Diggles et al. 2002b) where they are benign and do not cause disease. In their crustacean host, these parasites are similarly benign, with the only indication of adverse consequences of infection recorded in hyperinfected individuals in culture situations, where large numbers of *Nematopsis* sp. trophozoites may cause damage to the gut, increasing the chances of bacterial infection (Johnson 1995). All data available suggest that these parasites are not present in *M. rosenbergii* from the Hawaiian Islands, and in any case they would have a negligible effect on the disease status of these crustaceans or indeed any other animals in New Zealand. For these reasons, apicomplexan parasites will not be considered further.

3.2.5 Metazoa

A number of epicaridean bopyrid isopods have been recorded from wild *M. rosenbergii* in various locations around the world, including *Augustogathoma* sp. in Australia (see Brock 1983), *Probopyrus buitendjiki* in Thailand (Tonguthai 1992, 1997), and *Palaegyge bengalensis* (see Jayasree et al. 2001) and *Tachaea spongillicola* in India (see Mariappan et al. 2003). None of these species have been recorded from *M. rosenbergii* in the Hawaiian Islands. Bopyrids are large ectoparasites which cause a prominent bulge on the side of the cephalothorax of affected shrimp (Montoya 2003). Normally, the host is infected by one large female and from one to several dwarf male copepods (O'Brien and Van Wyk, 1985). The effects of bopyrid parasites include castration of female hosts (by interruption of vitellogenesis) and morphological alteration of secondary sexual characters in male prawns (Beck 1980, Schuldt & Rodrigues-Capitulo 1985, Ordinetz-Collart 1990). However, infections do detract from the appearance of the final product. Numerous bopyrids have been recorded from decapod crustaceans in New Zealand (Page 1985), but none of the species found on *M. rosenbergii* have been recorded here. Given the large size of the female parasites, which makes them easy to detect, the risk of importing them into New Zealand with cultured broodstock *M. rosenbergii* would appear negligible as normal husbandry practices ensure these parasites are easily removed from cultured populations. They would also have a negligible effect

on the disease status of crustaceans in New Zealand. For these reasons, these parasites will not be considered further.

At least three species of digenean metacercariae have been recorded from *M. rosenbergii*, namely members of the Microphallidae and Opecoelidae (Jayasree et al. 2001) and unidentified digenean metacercariae (Nash 1989). Digeneans such as these have a minimum 2 host lifecycle involving a definitive host, a first intermediate host which is always a mollusc, and sometimes one or more additional intermediate hosts, which can include crustaceans (Rohde 1984). Despite the multihost lifecycle, metacercariae have been found in *M. rosenbergii* cultured in ponds (Nash 1989), suggesting in this case at least, they had access to molluscan intermediate hosts. However the lifecycle cannot be completed unless the infected prawn is eaten by a suitable definitive host, and as the proposed commodity would be imported as broodstock which will be held in an aquaculture facility, this process will effectively eliminate any risk of completion of the lifecycle of these parasites. These parasites have also not been recorded from *M. rosenbergii* in the Hawaiian Islands, and because of these reasons, they will not be considered further.

The neurotrophic, zoonotic nematode *Angiostrongylus cantonensis* has been reported from the Hawaiian Islands (Wallace and Rosen 1969, Duffy et al. 2004). The definitive host is the Norway rat, however dispersal of *P. cantonensis* has, in some instances, been linked to the introduction of intermediate hosts such as the African giant land snail, *Achatina fulica* (see Duffy et al. 2004). A survey of *A. fulica* in the Hawaiian Islands in the mid-1960s showed that *P. cantonensis* infection was both highly prevalent and intense (Wallace and Rosen 1969). Several papers have shown that freshwater prawns *Macrobrachium lar* can act as vectors for *A. cantonensis* (see Eldredge 1994). This parasite has not been recorded from *M. rosenbergii* in the Hawaiian Islands, however if *M. lar* is susceptible, there is a very high probability that *M. rosenbergii* can also carry infective stages of *A. cantonensis*. Due to the zoonotic potential of this parasite, the fact that it can cause severe eosinophilic meningoencephalitis and death in humans, and because it is present in the Hawaiian Islands and could infect the proposed commodity, it will be considered further in this risk analysis.

3.2.6 Other organisms

Appendage deformity syndrome was reported to be problematic in the culture of *M. rosenbergii* in the Nellore District, India (Kumar et al. 2004). The problem occurred in up to 50% of prawns after 4 or 5 months growout, and mainly in female prawns. Analysis for viral and bacterial pathogens was negative, however the syndrome was resolved by carotenoid supplementation of the diet, suggesting a nutritional aetiology (Kumar et al. 2004). Because this disease appears not to be caused by a transmissible agent, it will not be considered further in this risk analysis.

Branchiostegal blister disease characterised by abnormal swelling of the branchiostegal region and deformities of appendages was reported to be associated with significant mortalities of up to 80% in the grow out culture of *M. rosenbergii* in India (Salin and

Nair 2003). A recent investigation of this disease syndrome failed to find evidence of association by any disease agents, suggesting it is an idiopathic disease associated with poor water quality conditions associated with use of groundwater (Pillai et al. 2005). The disease is also reversible if affected prawns are held in clean water for a few months (Pillai et al. 2005). Because this disease appears not to be caused by a transmissible agent, it will not be considered further.

Idiopathic muscle necrosis (IMN) was reported by Akiyama et al. (1982), Nash et al. (1987) and Anderson et al. (1990b) in larval *M. rosenbergii* cultured in Asia. This disease is known by various names, white muscle disease, muscle necrosis, spontaneous muscle necrosis, muscle opacity or milky prawn disease (Tonguthai 1997). Nash et al. (1987) reported that IMN caused sudden mortality of up to 60% of 28 day old post larvae in intensive rearing systems in Thailand. The disease appears as multifocal diffuse opacity of striated muscle (Nash et al. 1987; Brock, 1988). IMN of *M. rosenbergii* is considered to be associated with environmental stressors including salinity and temperature fluctuation, hypoxia, hyperactivity and overcrowding. (Nash 1987, Brock 1988). It has been observed in various hatcheries that if necrosis has not progressed extensively, the disease process is reversible once the water is changed. Because this disease appears not to be caused by a transmissible agent, it will not be considered further.

Mid cycle disease is a poorly characterised syndrome of unknown aetiology reported from *M. rosenbergii* larvae from Hawaii, Mauritius, Thailand, Philippines, Malaysia and northern Australia (Bower 1998a). The main pathological feature of affected larvae is atrophy of the hepatopancreas epithelium (Brock 1988). The bacterium *Enterobacter aerogenes* has been associated with the syndrome, however toxic compounds and pesticides have also been suggested as causes (Arthur et al. 2004). The condition reportedly can be reversed by reducing stocking density, improving pond husbandry and sanitation, and providing adequate nutrition through good quality *Artemia* (see Bower 1998a), suggesting a non infectious cause. In Hawaii, routine disinfection practices between larval culture cycles has been attributed to be the main factor for a virtual elimination of cases of MCD (Brock 1993). Because it is highly unlikely that this syndrome is caused by a transmissible agent, it will not be considered further.

Moult death syndrome is a general term used to describe death of crustaceans which occurs during the process of ecdysis (moulting). In larval *M. rosenbergii* the syndrome is characterised by the appearance of numbers of moribund or dying larvae trapped in their exuvae, with mortalities sometimes reaching 80% (Brock 1993). This disease has been reported in Hawaii and Guam (Brock 1993), and has also been observed in larval and juvenile lobsters in New Zealand (Diggles, personal obs.). There appear to be various risk factors which may contribute to failure to complete the moult. Poor nutrition and/or the mineral composition of the water used for culture (high calcium and/or magnesium) are common hypotheses (Brock, 1993, Johnson and Bueno, 2000). While failure to complete the moult can be associated with disease, such as in penaeid shrimp infected with Taura syndrome virus (Lightner et al. 1995), the death of *M. rosenbergii* during the

moult is not a pathognomic sign of any disease. Because this syndrome can be due to a variety of factors, and is non-transmissible, it will not be considered further.

Terminal growth is a syndrome reported to affect large male *M. rosenbergii* (Brock 1983). This problem is more common where prawns have not been harvested, and the marked epibiotic fouling that occurs in animals with the condition (Brock 1983) suggests that terminal growth is a consequence of old age rather than a transmissible agent. Therefore it will not be considered further in this risk analysis.

3.2.7 Organisms of concern to be considered further

As a result of the above consideration, the list of parasites and disease agents that are classified as potential hazards and therefore require individual risk assessments is shown in Table 3.

Table 3. List of disease agents considered to be potential hazards in the commodity

Common Name/ Disease	Scientific Name of disease agent	Reported in NZ?	OIE List?	Under official control or unwanted?	More virulent strains overseas?
VIRUSES					
White spot disease (WSD)	White spot syndrome virus (WSSV)	No	Yes	OIE listed	N/A
White Tail disease (WTD)	<i>Macrobrachium rosenbergii</i> nodavirus (MrNV) and extra small virus (XSV)	No	No	Unwanted	N/A
BACTERIA					
Lactococcosis	<i>Lactococcus garvieae</i>	No	No	Unwanted	N/A
Rickettsial disease	Rickettsia-like organism	No	No	Unwanted	N/A
FUNGI					
Phycomycete	<i>Aphanomyces</i> sp.	Some spp.	Yes	Unwanted	Yes
METAZOA					
Nematode in <i>Macrobrachium</i> lar	<i>Angiostrongylus cantonensis</i>	No	No	Unwanted	N/A

4. RISK ASSESSMENT

For each organism of concern, the risk assessment begins with an examination the epidemiology of the organism, with particular emphasis on routes of transmission. The release assessment then considers the likelihood of the organism being introduced into New Zealand in the commodity, taking into account the initial prevalence of infection, the effects of stress from capture, transport and handling, the time course of the disease and how long the agent can survive outside the host.

If release assessment concludes that there is a non-negligible likelihood of release, then the likelihood of exposure of the organism to susceptible species in New Zealand is considered in the exposure assessment. However if the release assessment concludes that the infectious agent will not survive until the end of quarantine, then the exposure assessment step is not carried out. The exposure assessment process considers prevalence and intensity of infection, transmission to susceptible species, spread by vectors, possible treatment, and temperature tolerance ranges of *M. rosenbergii*.

If the conclusion of the exposure assessment is that the likelihood of exposure of New Zealands aquatic organisms and/or humans to the disease agent is not negligible, then the consequences of establishment of the organism in endemic populations will be examined in the consequence assessment step.

The final step of the risk assessment, risk estimation, comprises a summary of the likelihood of introduction and establishment and the expected consequences to give an overall assessment of the risk in the commodity. If the risk is estimated to be non-negligible, then risk management measures will be required.

The risk management options and recommended safeguards are discussed in section 5 of this document.

4.1 WHITE SPOT DISEASE

4.1.1 Aetiologic agent: White Spot Syndrome Virus (WSSV), a double-stranded DNA virus of the genus *Whispovirus* within the family *Nimaviridea* (Mayo 2002).

4.1.2 OIE List: Listed

4.1.3 New Zealand's status: Not recorded. Considered exotic.

4.1.4 Epidemiology

WSSV is one of four viruses of penaeid shrimp which have caused global pandemics, having been introduced virtually wherever penaeid shrimp are cultured (Lightner 2003). White Spot Disease (WSD) was first reported from Japan in 1993 (Flegel 1997, OIE 2003), apparently as a result of an importation of larvae from China (Nakano 1994). In 2003, WSD was reported to the OIE from Central America (Costa Rica, El Salvador, Honduras, Panama), Hong Kong and Taipei, China, Japan, Republic of Korea, Peninsular Malaysia, Thailand, Iran, Sri Lanka, Peru, Mexico and the continental USA. It has also been reported from India (Rajendran 1999), the People's Republic of China, Indonesia, Vietnam and Bangladesh (Lightner et al. 1997).

The most obvious sign of WSD is the appearance of white spots in the exoskeleton of diseased animals, but these can also be caused by environmental conditions and bacterial infections (Wang et al. 2000). Pathological changes associated with WSD include development of characteristic basophilic nuclei and necrosis of subcuticular epithelium and other tissues of ectodermal and mesodermal origin (Lo et al. 1997). WSD of farmed penaeid shrimp is characterised by high and rapid mortality but infection of prawns in the wild is usually sub-clinical and appears to be exacerbated by stress (Lo et al. 1996).

WSD is primarily a disease of crabs and penaeid shrimp, but other species have been shown to be infected both naturally and experimentally by injection and by feeding (Flegel 1997). These include freshwater crayfish, crabs, lobsters, penaeid shrimp and freshwater prawns (Peng et al. 1998, Wang et al. 1998, Rajendran et al. 1999, Edgerton 2004). In fact, all decapod crustaceans from marine, brackish water, or freshwater sources are considered to be potential hosts for WSD (OIE 2005a), hence once the disease agent is introduced, there are many species which can act as carriers and vectors (OIE 2005a). However, other crustacean species tend to be less susceptible than penaeid shrimps to WSD. Mortality rates when exposed to the virus also vary depending on environmental conditions and other variables. For example, Sahul Hameed et al. (2000) failed to induce mortality in *M. rosenbergii* exposed to WSSV by immersion challenge, oral route and injection. By contrast, Pramod Kiran et al. (2002) reported an overall mortality rate of up to 68% after experimental exposure of *M. rosenbergii* post larvae to WSSV, although mortality of adult prawns was only up to 26.7%. Rajendran et al. (1999) found mortality rates from feeding experiments of 100% in shrimps, 10% in freshwater prawns (including *M. rosenbergii*), 16-33% in lobsters and 10-100% in crabs. Freshwater prawns which survive disease outbreaks show no signs of the disease except

for tiny spots on the carapace (Rajendran et al. 1999), which are around half the size of those observed in penaeids (Peng et al. 1998). Tissue from sub clinically infected *M. rosenbergii* was fed to penaeid shrimp and disease resulted in all cases, confirming *M. rosenbergii* can act as asymptomatic carriers of WSSV (Rajendran et al. 1999). Carriers can have persistent, life long infections potentially undetectable by currently available diagnostic tests (OIE 2005a).

Transmission is vertical (trans-ovum), or horizontal via the water or contaminated equipment, while cannibalism is another significant method of transmission of WSSV. Transmission of infection can occur from apparently healthy animals in the absence of disease (OIE 2005a). Pramod Kiran et al. (2002) investigated vertical transmission and found that eggs of experimentally infected brooders were WSSV-negative by PCR. However, larvae hatched from them were positive. Contamination may have been via the water, however the OIE (2005a) considers all life stages of crustaceans to be potentially susceptible, from eggs to broodstock. The best life stages for detection of WSSV are late post-larval stages, juveniles and adults, while the probability of detection can be increased by exposure to stressful conditions (OIE 2005a). The virus remains viable for at least 30 days at 30°C in seawater under laboratory conditions (OIE 2005a).

4.1.5 Release assessment

WSD has been recorded in the Hawaiian Islands only recently in April 2004, when WSD was reported from a shrimp farm on Kaua'i – the northernmost island⁴. As *M. rosenbergii* is more tolerant of WSSV than penaeid shrimp (Peng et al. 1998, Sahul Hameed et al. 2000) it can act as an asymptomatic carrier and infected animals may not show signs of clinical disease. Hence the virus could be present yet unreported in *M. rosenbergii* cultured in brackish water in the Hawaiian Islands, but whether the virus is present in freshwater regions on the islands is not known. A precautionary approach is taken in this release assessment, and it is assumed that WSSV is endemic in all waters of the Hawaiian Islands, and thus could be present in the proposed commodity.

Given that *M. rosenbergii* can act an asymptomatic carrier of WSSV, and the carrier state may not be detectable by currently available diagnostic tests (OIE 2005a), it is possible that pre shipment testing may not detect the pathogen if it was present, and hence there is a chance that *M. rosenbergii* infected with WSSV could be transported into New Zealand with the proposed commodity.

4.1.6 Exposure assessment

If *M. rosenbergii* infected with WSSV were introduced into New Zealand, it is possible that the stress of transport and quarantine would disclose underlying infections. However because *M. rosenbergii* are more tolerant to the infection than other species (Sahul Hameed 2000), and adult *M. rosenbergii* are more tolerant than any other life stage (Pramod Kiran et al. 2002), disease may not necessarily be initiated and/or detected using the available diagnostic tests. If waste water from a quarantine facility containing

⁴ http://www.aphis.usda.gov/vs/ceah/cei/IW_2004_files/wsd_us_0404_files/wsd_us_0404.htm

infected individuals was not treated to deactivate infective viral particles, it is likely that crustaceans, including planktonic crustaceans, in water bodies receiving waste water from the quarantine or culture facility would become infected through horizontal transmission. This process could occur even in freshwater (Sahul Hameed et al. 2000, Edgerton 2004).

Although water temperatures below 30°C are known to be conducive to WSD outbreaks (OIE 2005a), the lower temperature threshold for transmission of WSSV is not known. In the absence of this information, a precautionary is taken, and it is assumed that transmission is likely to occur even if the temperature of the surrounding water was much cooler than maintained in the quarantine facility. If infective particles escaped from quarantine or a post-quarantine culture facility, their persistence in the environment and the wide host range of the virus would result in the exposure of many suitable hosts throughout New Zealand's freshwater, estuarine and marine environments (Table 1), including freshwater crayfish or Koura, (*Paranephrops planifrons*, *P. zealandicus*), shrimp (*Paratya curvirostris*), and possibly even estuarine shrimp (*Palaemon affinis*), which could present a route of exposure to commercially important lobsters *Jasus edwardsii* and *Jasus verreauxi*, and others. Under these conditions it is concluded that the likelihood of effective exposure and establishment would be moderate to high.

4.1.7 Consequence assessment

The establishment of WSSV into New Zealand would most likely be associated with significant impacts on local crustaceans. Infections of wild crustaceans are generally sub clinical (Lo et al. 1996), so the exposure of these species would most likely aid the spread of the organism in local crustacean populations. Since sub clinical infections can revert to the disease state in susceptible species after periods of stress (Lo et al. 1996), it is likely that any future attempts to culture endemic crustaceans would be adversely affected. The present practices of holding live rock lobsters at high densities in commercial holding facilities might also need to be revised if the disease became endemic in marine areas. It is likely that there would be significant economic losses to established industries such as the rock lobster industry, resulting in lost of export earnings through the imposition of trade barriers for New Zealand crustacean products to some parts of the world. The consequences of establishment of WSSV are therefore considered to be high.

4.1.8 Risk estimation

A moderate to high likelihood of introduction and establishment, combined with a high significance of the resulting consequences, mean that the risks to the New Zealand environment are considered to be non-negligible, and WSSV is classified as a hazard in the commodity.

4.2 WHITE TAIL DISEASE

4.2.1 Aetiologic agent: *Macrobrachium rosenbergii* nodavirus (MrNV), a non-enveloped icosahedral particle 26-27 nm in diameter (Bonami et al. 2005) with a 2 piece single stranded RNA genome, and its associated extra small virus (XSV), an icosahedral particle 15 nm in diameter with a single strand RNA genome.

4.2.2 OIE List: Not listed. Recommended for possible listing in the future (OIE 2005b)

4.2.3 New Zealand's status: Not recorded. Considered exotic.

4.2.4 Epidemiology

Since 1992, the post larvae of *M. rosenbergii* in Taiwan were affected by an epizootic disease characterised by white opaque areas in the abdominal muscle (Tung et al. 1999). Then in 1999 a similar disease was reported in *M. rosenbergii* reared in Guadeloupe, French West Indies (Arcier et al. 1999), followed by China (Qian et al. 2003) and India (Vijayan et al. 2003,2005, Sahul Hameed et al. 2004). In post-larvae, the clinical signs included lethargy, anorexia and opaqueness of the abdominal muscle, giving rise to the disease name White Tail Disease (WTD). The opaqueness gradually extends on both sides and leads to degeneration of telson and uropods, and mortalities can be as high as 99% within 2-3 days, reaching 100% within 10 days (Vijayan et al. 2005). Areas of necrotic muscle in WTD affected *M. rosenbergii* exhibit darkly basophilic cytoplasmic inclusion bodies (Arcier et al. 1999, Tung et al. 1999) associated with the presence of a nodavirus. Acute WTD disease can be tentatively diagnosed from gross signs of multifocal to generalized muscle necrosis visible as opaque muscles which give affected post-larvae white tails.

Two viruses, *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) have been found to be associated with WTD (Bonami and Widada, 2003, Bonami et al. 2005). MrNV is a non-enveloped icosahedral particle 26-27 nm in diameter with a genome consisting of two fragments of linear single stranded RNA (Bonami et al. 2005). Its associated extra small virus (XSV), which was first detected in the outbreaks of disease in China (Qian et al. 2003) is an icosahedral particle 15 nm in diameter with a single strand RNA genome. MrNV appears to have affinities with nodaviruses and is the first nodavirus isolated from an aquatic invertebrates (Bonami et al. 2005). XSV appears to be a true satellite virus, the first of its type reported in animals (Bonami et al. 2005). The relationship between the two viruses and how they interact during the disease process is unclear. Genome based detection methods for MrNV and XSV have been developed (Widada and Bonami, 2003, Widada et al. 2004, Yoganandhan et al. 2005).

Transmission of WTD appears to be horizontal through water, and introduction of the disease was thought to have occurred by the movement of infected postlarval *M. rosenbergii* from Guadeloupe to Puerto Rico (OIE 2005b). The sudden appearance of the disease in regions of China, Bangladesh and India suggests that it was introduced rather

than being endemic. However, the disease has not been reported from southeast Asia, where major industries are present that culture *M. rosenbergii* (see OIE 2005b).

4.2.5 Release assessment

To date neither *MrNV* nor *XSV* have been reported from *M. rosenbergii* in the Hawaiian Islands, but there has been no testing programme in place for this organism. When the novelty, pathogenicity and lack of knowledge of the epidemiology of these viruses are taken into consideration, it is not possible to be confident that *M. rosenbergii* from the Hawaiian Islands are free from these disease agents. Until evidence is presented showing that a statistically significant testing programme confirms that Hawaiian *M. rosenbergii* are free from the agents that cause WTD, the likelihood of their introduction into New Zealand from the Hawaiian Islands is considered to be low but non-negligible.

4.2.6 Exposure assessment

In the absence of knowledge of host specificity, likely host range, or potential for carriers other than *M. rosenbergii* to harbour the disease agents, it is difficult to determine the likelihood of effective exposure and establishment of the agents causing WTD in the event that infected *M. rosenbergii* were introduced into New Zealand. If the disease is specific only to *M. rosenbergii*, it is unlikely to become established in New Zealand even if waste water from the quarantine or post-quarantine culture facility was untreated. At present no other species have been reported to be infected by these viruses, however if the viruses could infect other members of the Family Palaemonidae and/or other closely related families, then the discharge of untreated waste water from the quarantine or post-quarantine culture facility might result in the disease becoming established in potential carrier species in New Zealand. Such species might include *Paratya curvirostris* (Family Atyidae) in freshwater regions down to brackish areas of estuaries, and *Palaemon affinis* (Family Palaemonidae), in estuaries and nearshore locations. However, given the lack of information, it is difficult to determine the chances of establishment of WTD in New Zealand, but even assuming that the above species may be susceptible, the likelihood of exposure is considered to be low. Therefore the exposure assessment is non-negligible.

4.2.7 Consequence assessment

Again, without knowledge of the host specificity, likely host range, or potential for carriers other than *M. rosenbergii* to harbour *MrNV* and *XSV*, it is difficult to determine the consequences of establishment of these disease agents in New Zealand. If the virus is specific only to *M. rosenbergii*, then since the culture of this species in New Zealand presently occurs only at one facility in the central North Island, then the only potential consequences could be on this facility. The annual production from this facility is approximately 30 tonnes, and this is mainly used in the on-site restaurant, and is also sold in selected low volume niche markets around New Zealand⁵. At \$40 per kg retail, the value of the existing industry is NZD 1.2 million. Besides the financial risk to the parties proposing the importation of the commodity, the existing producer would be the only

⁵ <http://geoheat.oit.edu/bulletin/bull19-3/art76.htm>

other party who could experience detrimental consequences if WTD became established. However if a larger industry was to be developed based on more than one or two operators, the consequences of introduction of WTD to New Zealand would also increase in proportion to the size of the industry. Also, the fact that the presence of this disease could represent a barrier to international trade should also be noted, especially if the disease becomes listed by the OIE in the future.

However, if the virus can also infect other members of the Family Palaemonidae and/or other closely related families, it is possible that the disease could detrimentally affect other species such as freshwater shrimp (*Paratya curvirostris*) and estuarine/marine shrimp (*Palaemon affinis*). In that case there could be unknown impacts on aquatic ecosystems which shrimp play an important role as scavengers and as a food source for economically important fishes such as trout. The significance of the consequences of establishment of WTD are considered to be very low at this point in time, but if an option for future expansion of the *M. rosenbergii* industry in New Zealand is to be retained, the significance of the consequences of introduction of WTD should be upgraded to moderate. Therefore the consequences of introduction are considered to be non-negligible.

4.2.8 Risk Estimation

A low likelihood of introduction and establishment, combined with a moderate significance of the resulting consequences, mean that the risks to the New Zealand environment due to the establishment of WTD are non-negligible, and the causative organisms are classified as hazards in the commodity.

4.3 LACTOCOCCOSIS

4.3.1 Aetiologic agent: *Lactococcus garviae*, (formerly *Enterococcus seriolicida*, see Eldar et al. 1996), a gram positive non-motile bacterium with spherical or ovoid cells with a diameter of 0.6 – 0.9 µm.

4.3.2 OIE List: Not listed

4.3.3 New Zealand's status: Not recorded. Considered exotic.

4.3.4 Epidemiology

Lactococcus garviae is an opportunistic pathogen of marine and freshwater fish worldwide, and has a wide host range which includes both aquatic and terrestrial vertebrates (including humans) (Eldar et al. 1999) and aquatic invertebrates (Cheng and Chen 1998a). Various strains exist in different geographic areas and there is evidence that some strains have been moved into different regions through importation of infected fish (Eldar et al. 1999). Under the right circumstances this bacterium causes disease and results in heavy mortalities in the culture of a very wide range of fish species, including *M. rosenbergii*. It causes typical bacterial haemorrhagic septicaemia in susceptible fishes, usually under conditions of environmental stress such as high temperatures in summer, especially if this occurs together with high stocking densities. Infections are frequently associated with stressors such as concurrent infections (Kumon et al. 2002), water quality (Cheng and Chen 1998b, Cheng and Chen 2002, Cheng et al. 2002), moult stage (Cheng and Chen 2003a) and chemotherapy (Cheng et al. 2003b), but sometimes primary outbreaks occur in fish without any predisposing factors (Eldar et al. 1999).

Lactococcosis caused by *L. garviae* is the major bacterial disease of yellowtail (*S. quinquerediata*) in Japan (Kusuda and Salati 1993, 1999). It has also been recorded to cause significant disease in the culture of eels, flatfish and trout (Muzquiz et al. 1999). One Australian strain was isolated from diseased rainbow trout (*Oncorhynchus mykiss*) in freshwater hatcheries in Tasmania and Victoria (Carson *et al.* 1993). Transmission is direct and horizontal, with entry via the water or the rectal-oral route.

4.3.5 Release assessment

L. garviae has been recorded from *M. rosenbergii* in Taiwan, but it has not been recorded from *M. rosenbergii* in the Hawaiian Islands, despite nearly 40 years of culture of the species there and the fact that the bacterium is easily detected using routine bacteriological methods. This bacterium has also not been recorded from cultured finfish in the Hawaiian Islands. For these reasons the likelihood of *M. rosenbergii* from the Hawaiian Islands harbouring *L. garviae* and introducing it into New Zealand is considered to be negligible.

4.4 RICKETTSIAL DISEASE

4.4.1 Aetiologic agent: Rickettsia-like organisms (RLOs), obligate intracellular microorganisms which infect the hepatopancreas and connective tissues of crustaceans.

4.4.2 OIE List: Not listed

4.4.3 New Zealand's status: Not recorded. Considered exotic.

4.4.4 Epidemiology

A rickettsia-like organism (RLO) was reported to cause significant disease in *M. rosenbergii* in Brazil (Cohen and Isaar 1989, Johnson and Bueno 2000). Affected larvae became white throughout their hepatopancreas and bodies and generally inactive before death. A similar organism has been reported infecting 10% of wild *Melicertus marginatus* (syn. *Penaeus marginatus*, or aloha prawn) juveniles caught off the tidal flats of Manuaia Bay, Hawaii (Brock et al. 1986a).

RLOs are generally benign agents in aquatic organisms, however these agents can proliferate when the host is stressed by poor environmental conditions. For example, branchial infections can be associated with disease and even mass mortalities in some molluscs (LeGall et al. 1998, Hine and Diggles 2002). In crustaceans, besides the cases listed above, RLOs have also been detected in wild-caught carideans in Canada (Bower et al. 1996), and during outbreaks of disease in cultured penaeids (Chong and Loh 1984, Krol et al. 1991). However in many cases their presence in crustaceans is asymptomatic. In some disease outbreaks associated with the presence of RLOs, other disease agents have also been implicated (Anderson et al. 1987), hence the role of RLOs in causing disease under these circumstances remains poorly understood.

For RLO infections of cultured crustaceans, it appears highly likely that natural crustacean reservoir hosts exist in the environment. For example, Bower et al. (1996) experimentally transmitted disease to *Pandalus platyceros* by feeding them on infected prawns and also via exposure to outflow water (screened to 1mm diameter) from a tank where RLO-infected prawns were held. Similarly, Brock et al. (1986a) demonstrated that *P. stylirostris* that were fed tissue from naturally infected *M. marginatus* developed severe RLO infections.

RLO infections in crustaceans are easily detected using histopathology, though the sensitivity of this technique for detecting RLOs remains undetermined. RLOs have not been recorded in *M. rosenbergii* in Hawaii, and do not appear to cause any problems if they do so. Even then, it is possible to treat farmed prawns infected with RLOs with antibiotics.

4.4.5 Release assessment

RLOs that infect crustaceans are endemic in Hawaiian marine waters (Brock et al. 1986a). Nevertheless, although *M. rosenbergii* are known to be susceptible to RLOs (Cohen and Isaar 1989, Johnson and Bueno 2000), RLO infection has not been reported from *M. rosenbergii* in Hawaii. This notwithstanding, it is considered that since *M. rosenbergii* in Hawaii may be reared in brackish water at certain stages during their development, it is likely that they are exposed to infective stages of RLOs endemic to that part of the world. Given that most RLO infections are asymptomatic, it is likely that infected but healthy *M. rosenbergii* could be exported to New Zealand. Therefore the likelihood of the commodity being infected by RLOs is considered to be non-negligible.

4.4.6 Exposure assessment

Since most infections with RLOs are asymptomatic, it is considered that if *M. rosenbergii* that were infected with RLOs were introduced into New Zealand the RLOs would not be detected without implementing an active surveillance programme. If waste water from the quarantine or post-quarantine culture facility was not treated to deactivate infective particles, it is possible that RLOs could become established in potential carrier species in New Zealand. Such species might include *Paratya curvirostris* (Family Atyidae) in freshwater regions down to brackish areas of estuaries, and *Palaemon affinis* (Family Palaemonidae), in estuaries and nearshore locations. Given the lack of information, it is difficult to determine the chances of establishment of RLOs in New Zealand, but it is assumed that the likelihood of this occurring is low. The release assessment is therefore non-negligible.

4.4.7 Consequence assessment

Expression of disease associated with RLO infection in crustaceans appears to occur only when they are stressed by adverse environmental conditions or concurrent infections. These conditions are likely to occur only in cultured crustaceans held in poor conditions. Since these disease agents seldom cause problems in the culture of penaeids or *M. rosenbergii* overseas, and are in any case treatable with antibiotics, there would be few if any long term consequences of their introduction to the crustacean aquaculture industry. There is also no evidence that RLO infection in wild prawns have any ecological or environmental impact, hence considering all of this information, the consequences of introduction of RLOs in *M. rosenbergii* are considered to be negligible.

4.4.8 Risk Estimation

The likelihood of introduction and establishment of RLOs is low, and the resulting consequences are considered to be negligible. Therefore the risk of RLOs in the commodity is considered to be negligible, and these organisms are not classified as hazards in the commodity.

4.5 APHANOMYCES SP. INFECTION

4.5.1 Aetiologic agent: Fungi of the genus *Aphanomyces* (Order Saprolegniales)

4.5.2 OIE List: Listed

4.5.3 New Zealand's status: Some species recorded (e.g. *A. ovidestruens*, see Burns 1980), however *A. astaci* is considered exotic.

4.5.4 Epidemiology

Some species of *Aphanomyces* have previously been reported from *M. rosenbergii* (see Sindermann 1977, Brock 1988). This observation is relevant in this risk assessment because the fungus *Aphanomyces astaci* causes crayfish plague in freshwater crayfish (Decapoda: Astacidae) in Europe and North America (OIE 2005a).

Crayfish plague is the most devastating disease of freshwater crayfish. Usually the first sign of this disease in a new geographic area is the appearance of numerous crayfish at large during the day, some with loss of co-ordination and righting reflex (OIE 2005a). In susceptible species, which include most crayfish species outside of North America (Unestam 1975, Alderman et al. 1987, 1990), if sufficient numbers of crayfish are present to allow infection to spread rapidly, the disease will spread quickly and stretches of over 50 km of river may lose all their crayfish within 21 days of the first observed mortality (OIE 2005a). Infected susceptible crayfish do not survive – 100% mortality is normal (OIE 2005a). Upstream spread has been recorded at up to 1000 m per week and 17 km in 10 months (Taugbol and Skurdal 1993). North American species such as *Pacifastacus leniusculus* (signal crayfish) and *Procambarus clarkii* (Louisiana swamp crayfish) from areas where the disease is endemic survive infection in many cases (Persson and Soderhall 1983) and then act as asymptomatic carriers, although under adverse conditions (stress, concurrent infections with other pathogens, etc.), mortality may occur (OIE 2005a). Infection is direct and horizontal through swimming zoospores, however *A. astaci* has few vectors, no known intermediate/secondary hosts, there are no resistant structures, and the spores have a limited viability outside the host (Bower 2005b).

4.5.5 Release assessment

Although *A. astaci* has not been reported in Hawaii, the Louisiana crayfish *Procambarus clarkii*, a known carrier of *A. astaci*, was introduced into Hawaii in 1934 (Eldredge 1994) and has since established in the Hawaiian islands⁶. *Procambarus clarkii* are carriers of the fungus and do not exhibit symptoms of the disease except in intensive culture (OIE 2005a), hence given the high prevalence of the disease agent in wild populations of *P. clarkii* (see Nylund and Westman 2000), it is possible that some of the *P. clarkii* introduced into Hawaii in 1934 were carrying *A. astaci*.

⁶ http://iz.carnegiemnh.org/crayfish/country_pages/state_pages/hawaii.htm

The key question in terms of assessing the likelihood of release is whether *M. rosenbergii* reared in the Hawaiian Islands are likely to come in contact with *A. astaci*, and if so, whether they can become infected and/or act as vectors for this pathogen. The known distribution of *P. clarkii* in the Hawaiian Islands is restricted to a few water courses in the southern parts of the islands of Hawaii and Kahului⁷.

Because of this restricted distribution of the host in the Hawaiian Islands, the obligate requirement of the disease agent for its host, and the fact that there are no resistant structures or spores in the lifecycle of the disease agent, it appears highly unlikely that cultured *M. rosenbergii* would be exposed to this disease agent if they were reared in water supplies which did not contain *P. clarkii*. Furthermore, besides freshwater crayfish, the only other crustacean known to be susceptible to *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*), which has been infected only under laboratory conditions (see OIE 2005a).

Because of the apparent inability of *A. astaci* to infect other reservoir hosts, the likelihood of *M. rosenbergii* harbouring *A. astaci* as an infection is considered to be negligible. However, if *M. rosenbergii* were taken for export from waters containing *P. clarkii*, in the absence of risk management measures the likelihood of the disease agent being inadvertently found free on the carapace or in the water used to transport the *M. rosenbergii* is considered to be non-negligible. This is because transmission of this disease agent has been linked to movement of zoospore-contaminated water (Bondad-Reantaso et al. 2001) and, theoretically, other potential vectors such as fish (Oditmann et al. 2002).

4.5.6 Exposure assessment

Aphanomyces astaci is lethal to virtually all crayfish hosts native to countries outside of North America (Alderman et al. 1987, 1990, Reynolds 1988). For example, 9 species of crayfish native to Australia and New Guinea were susceptible when experimentally exposed to zoospores of *A. astaci* (Unestam 1975). New Zealand has two species of native freshwater crayfish (Koura), namely *Paranephrops planifrons* and *P. zealandicus*, both of which would almost certainly be extremely susceptible to *A. astaci*. Such is the virulence of *A. astaci* for crayfish, even if a very low number of zoospores were transported with *M. rosenbergii* and released from quarantine, any native crayfish in the waters receiving the contaminated effluent would be extremely susceptible to infection. The likelihood of exposure and establishment of *A. astaci* in New Zealand is therefore considered to be moderate.

4.5.7 Consequence assessment

The introduction of this disease agent into New Zealand would have severe consequences for the health of populations of native crayfish. In other countries where *A. astaci* has been introduced, large scale extinctions of native crayfishes have been recorded. Hence the consequences of introduction of *A. astaci* into New Zealand are considered to be

⁷ http://iz.carnegiemnh.org/crayfish/country_pages/state_pages/hawaii.htm

catastrophic for populations of native crayfish. In view of this, the consequences of introduction are assessed as being extremely high.

4.5.8 Risk estimation

Given that the moderate likelihood of introduction and establishment is non-negligible, and considering the extremely high consequences that would result, the risk of *A. astaci* to the New Zealand environment is considered to be non-negligible and it is classified as a hazard in the commodity.

4.6 ANGIOSTRONGYLUS CANTONENSIS

4.6.1 Aetiologic agent: *Angiostrongylus cantonensis*, (syn. *Parastrongylus cantonensis*) a neurotrophic, zoonotic nematode

4.6.2 OIE List: Not listed

4.6.3 New Zealand's status: Not recorded. Considered exotic.

4.6.4 Epidemiology

The nematode *Angiostrongylus cantonensis* was initially considered to be a parasite of rodents. However, a few years after its discovery in rodents, this nematode was found in the brain of a teenager in Taiwan, and it has since been found in humans in Hawaii, Tahiti, the Marshall Islands, New Caledonia, Thailand, Vanuatu, the Caribbean, and even Australia and the USA (Kliks and Palumbo 1992, Prociv et al. 2000, Lindo et al. 2002, Senanayake et al. 2003). In rodents the adult worms live in the lungs⁸. Females produce eggs which hatch in the lungs, and the first stage larvae enter the respiratory tract, migrate up the trachea, and are then swallowed and passed in the faeces. The larvae is eaten by an invertebrate intermediate host, most often a terrestrial slug or snail, or an aquatic snail (Prociv et al. 2000), however other mollusc eating cold blooded animals such as crabs, reptiles (Radomyos et al. 1994) and freshwater prawns *Macrobrachium lar* can also act as paratenic hosts and vectors for *A. cantonensis* (see Eldredge 1994, Alto 2001, Lindo et al. 2004). The first stage larvae actively penetrate the intermediate host's body, and they molt twice into third stage larvae which infect the definitive host. Final hosts include a wide variety of warm blooded animals, including rodents, birds, dogs and humans, who become infected when they ingest an intermediate host containing infective stages without it being sufficiently cooked or frozen (Alicata 1967).

In humans, the parasites penetrate the gut and enter the circulation and end up in the tissues surrounding the brain, but usually do not develop further. The presence of larvae in the blood vessels, meninges, or tissue of the human brain can result in symptoms such as headache, fever, paralysis, and even coma followed in rare cases by death (Lindo et al. 2004). Because of the non-specific nature of these symptoms, angiostrongyliasis is difficult to differentiate from other infections (Senanayake et al. 2003, Lindo et al. 2004). The presence of worms is often associated with eosinophilia, and in areas where *A. cantonensis* is endemic, this parasite is a primary cause of eosinophilic meningoencephalitis (Punyagupta et al. 1975). *Angiostrongylus cantonensis* has been reported from the Hawaiian Islands (Wallace and Rosen 1969, Duffy et al. 2004), probably after introduction of both the Norway rat (*Rattus norvegicus*) definitive host, and intermediate hosts such as the African giant land snail, *Achatina fulica* (see Duffy et al. 2004). A survey of *A. fulica* in the Hawaiian Islands in the mid-1960s showed that *P. cantonensis* infection was both highly prevalent and intense (Wallace and Rosen 1969). This parasite has not been recorded from *M. rosenbergii* in the Hawaiian Islands, however *M. lar* is known to be susceptible (Eldredge 1994, Alto 2001).

⁸ <http://www.dpd.cdc.gov/dpdx/HTML/angiostrongyliasis.htm>

4.6.5 Release assessment

The parasite is established in the Hawaiian Islands and is known to utilise *M. lar* which occur in the wild there in freshwater streams. Given that *M. lar* and *M. rosenbergii* are very closely related, there is a very high probability that *M. rosenbergii* can also carry infective stages of *A. cantonensis*. Both terrestrial and aquatic snails are known hosts of this parasite, and its infective stages are present at high prevalence in suitable hosts in the wild (Wallace and Rosen 1969). The parasite has also infiltrated aquaculture establishments in the Hawaiian Islands, as cases of eosinophilic meningitis have been recorded in humans in Hawaii who have eaten uncooked aquaculture raised snails (Marsh 1998). There is a low likelihood of *M. rosenbergii* raised in aquaculture ponds being infected with 3rd stage larvae, and therefore the likelihood of *A. cantonensis* being introduced into New Zealand with the proposed commodity is low. The release assessment is non-negligible.

4.6.6 Exposure assessment

New Zealand has a number of species which could act as definitive and intermediate hosts for *A. cantonensis*, including Norway rats *Rattus norvegicus*, the ship rat (*Rattus rattus*) and Pacific rat (*Rattus exulans*), and many species of native birds. Potential intermediate hosts are also present, including various species of terrestrial and aquatic snails. However, for this parasite to become established in New Zealand, infected *M. rosenbergii* would have to be eaten by a suitable definitive host and eggs from adult worms would have to be passed into the environment and eaten by a suitable invertebrate intermediate host which is a normal part of the food chain of suitable definitive hosts. The likelihood of these multiple events occurring would appear to be remote, and hence the probability of establishment of *A. cantonensis* in New Zealand is considered to be very low. Nevertheless, the exposure assessment is non-negligible.

4.6.7 Consequence assessment

The introduction of this parasite into New Zealand would have negligible impact on the health of *M. rosenbergii* and other aquatic animals, but could most likely cause moderate to severe pathological damage to infected definitive hosts, which besides rats can also include native birds (Monks et al. 2005). Furthermore, due to the zoonotic potential of this parasite, and the fact that it can cause severe eosinophilic meningoencephalitis and even in rare cases death in humans (Lindo et al. 2004), the consequences of the introduction of *A. cantonensis* into New Zealand are considered to be moderate.

4.6.8 Risk estimation

Although there is a very low likelihood of establishment, because the consequences of introduction are considered to be moderate the risk is considered to be non-negligible. Therefore *A. cantonensis* is classified as a hazard in the commodity.

5. RISK MANAGEMENT

5.1 RISK EVALUATION

The risk assessment concluded that the following disease agents should be classified as hazards in the commodity :

- White Spot Syndrome Virus (WSSV),
- *Macrobrachium rosenbergii* nodavirus (MrNV), and its associated extra small virus (XSV),
- *Aphanomyces astaci*
- *Angiostrongylus cantonensis*.

Risk management measures are justified for these organisms in the commodity to reduce their risks to an acceptable level.

5.2 OPTION EVALUATION

5.2.1 Risk management objective

The objective is to effectively manage the risks of the above hazards in the commodity by ensuring that imported *M. rosenbergii* do not harbour the hazards when given a biosecurity clearance in New Zealand.

5.2.2 Options available

5.2.2.1 Pre-export measures

Given the potential presence of species harbouring *Angiostrongylus cantonensis* and *Aphanomyces astaci* in some of the freshwater areas of the Hawaiian Islands, adult *M. rosenbergii* destined for export could be obtained only from an aquaculture facility which has a water supply free from known colonisation by *Procambarus clarkii*, and treatment of the water supply by filtration and/or UV irradiation and/or ozonation to a level which proves exclusion of all known hosts or vectors of *A. cantonensis* from the facility.

For the viruses WSSV, MrNV, or XSV, the facility could be expected to have a demonstrable history of testing of their *M. rosenbergii* stocks. Each round of sampling of stock at the facility must be carried out on a statistically significant number of individuals. The number of samples often taken is 150, as this level of sampling gives (for a diagnostic test of perfect sensitivity and specificity) a confidence of 95% that at least one positive result will be seen in a population where the prevalence is at least 2%.

In addition, sampling for export certification should be carried out from the specific population of *M. rosenbergii* from which the animals are being taken for export, and the tests should be done within 1 month of the shipping date by a competent authority..

If any of the pre-export tests are positive for WSSV, MrNV, or XSV, the *M. rosenbergii* from that facility should not be permitted entry into quarantine in New Zealand.

5.2.2.2 Conditions of export

As part of a pre-export quarantine regime, at the time of carrying out the sampling for viruses, the *M. rosenbergii* destined for export could be removed from the tested population and placed in a separate tank. This tank should be physically isolated from other crustaceans held at the rearing facility, and it should have its own water supply filtered to permit entry of a particle size of no greater than 20µm. Holding these animals in isolation until the virus testing results are available would ensure that no cross-contamination of water is possible.

Biosecurity measures during export could include shipping under an approved seal and using water that has been filtered to permit entry of a particle size of no greater than 20µm. Preventing water exchange during transport is another possible measure to avoid cross-contamination.

In order to rule out the hazards any animals which die during the pre export quarantine period may be tested for WSSV, MrNV, and XSV, and if any of these tests provide a positive reaction for WSSV, MrNV, or XSV, the *M. rosenbergii* from that shipment could be denied entry into quarantine in New Zealand.

5.2.2.3 Post-arrival quarantine

Post-arrival quarantine in New Zealand allows the water used for transport to be disposed of and the *M. rosenbergii* can be released into quarantine tanks.

Given that adult *M. rosenbergii* are known carriers of WSSV and that this virus can persist in subclinical infections that may not be detected using current diagnostic techniques (OIE 2005a), the risk of introduction may be further reduced by not permitting the imported *M. rosenbergii* individuals to leave quarantine initially. Instead, locally grown *M. rosenbergii* broodstock could be allowed to co-habit with imported broodstock in the quarantine facility, and the resulting batches of larvae spawned from broodstock of imported and domestic origin could be allowed out of the quarantine facility after 150 larvae have been tested and found free from WSSV using the OIE method (OIE 2005a). For each shipment of adult broodstock, this arrangement could be followed for a minimum of three batches of larvae (or perhaps for a minimum of 12 months, whichever takes the greater period of time), and after this time if all batches of larvae have tested negative for WSSV, the broodstock could be considered free of WSSV and allowed to exit quarantine.

5.2.2.4 Mortalities during transport and quarantine

As a further safeguard, any imported *M. rosenbergii* broodstock that are dead on arrival, or dead, diseased or unhealthy animals that present over the quarantine period, may be

subjected to a thorough virological, bacteriological and histopathological examination by a competent authority in New Zealand to determine whether the disease is due to an exotic disease agent.

5.2.3 Recommended sanitary measures

1. *M. rosenbergii* destined for export should be sourced only from an aquaculture facility which has a water supply free from known colonisation by *Procambarus clarkii*, a known carrier of *Aphanomyces astaci*.
2. The water supply for the facility must be treated by filtration (to permit entry of a particle size of no greater than 20µm) and/or ultraviolet irradiation and/or ozonation to a level which can be shown to remove all known vectors of *A. cantonensis*.
3. The facility must have a verifiable history of testing for diseases in their stocks of *M. rosenbergii* which demonstrates that the animals are not infected with the organisms that cause white spot disease or white tail disease.
4. Within 1 month of the shipping date a sample of 150 individuals must be taken from the specific population of *M. rosenbergii* from which the animals are being sourced for export.
5. These samples must be submitted to the following diagnostic tests at a diagnostic facility approved by the competent authority in the exporting country :
 - a. Examination of haemolymph, gills or pleopods for the causative agent of white spot disease using the nested PCR method recommended by the World Organisation for Animal Health (OIE 2005a).
 - b. Examination of haemolymph, gills, tail muscle or pleopods for the organisms causing white tail disease using the PCR described by Yoganandhan et al. (2005).
6. The *M. rosenbergii* intended for export must be removed from the same population and at the same time as the animals being tested for viruses. The animals intended for export must be placed in a tank which is physically isolated from other crustaceans held at the rearing facility and which has its own water supply filtered to permit entry of a particle size of no greater than 20µm. Any animals that die during the pre-export quarantine period must also be tested for the organisms that cause white spot disease and white tail disease at a diagnostic facility approved by the competent authority in the exporting country.
7. If any of the pre-export tests are positive for the organisms that cause white spot disease or white tail disease, the *M. rosenbergii* from that facility will not be permitted entry into quarantine in New Zealand.

8. The *M. rosenbergii* must be exported in water that is free from *Vibrio cholerae*, and is filtered to permit entry of a particle size of no greater than 20µm. Water must not be exchanged during transport.
9. On arrival in New Zealand, the *M. rosenbergii* must be transported directly to an approved transitional facility complying with MAF Standard 154.02.06: *Transitional Facilities for Ornamental Fish and Marine Invertebrates*. Here the *M. rosenbergii* can be released into quarantine tanks but the water used for transport must be treated before disposal as prescribed in the MAF Standard.
10. Any *M. rosenbergii* broodstock that are dead on arrival, and any dead or diseased animals that are detected during the quarantine period must be subjected to a thorough virological, bacteriological and histopathological examination at a laboratory approved by Biosecurity New Zealand.
11. The *M. rosenbergii* imported from Hawaii will not be permitted to leave quarantine at any time, but locally grown *M. rosenbergii* broodstock will be allowed within the quarantine facility to co-habit with imported broodstock, and resulting batches of larvae spawned from broodstock of imported and domestic origin will be released from the quarantine facility after 150 larvae have been tested and found free from the organism causing white spot disease, using the nested PCR method recommended by the OIE (2005a) for this organism, at a laboratory approved by Biosecurity New Zealand.
12. The above conditions will be followed for the first 3 batches of imported larvae, or a minimum of 12 months (whichever takes the greater period of time), after which if all batches of larvae test negative for the organism causing White Spot Disease, the broodstock will be considered free of that disease and will be permitted to leave the quarantine facility.

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