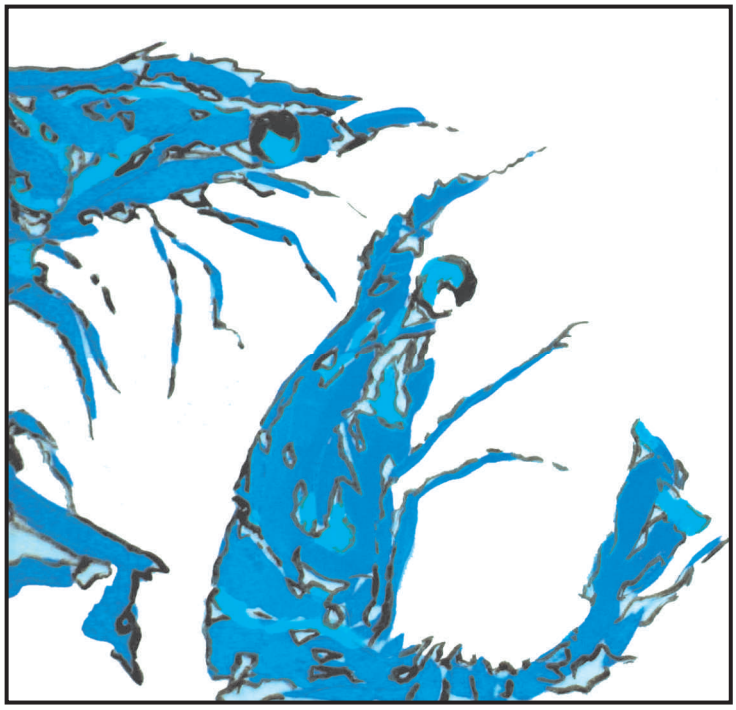
Australian Government

Biosecurity Australia

Generic Import Risk Analysis Report for Prawns and Prawn Products

Final Report



October 2009

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Glossary of terms and abbreviations

ABARE	Australian Bureau of Agricultural and Resource Economics
ABPM	Animal Biosecurity Policy Memorandum
ACIAR	Australian Centre for International Agricultural Research
ADVS	Aquaculture Development and Veterinary Services
AIMS	AQIS Import Management System
ALOP	appropriate level of protection
AQIS	Australian Quarantine and Inspection Service
AQPM	Animal Quarantine Policy Memorandum
BAPM	Biosecurity Australia Policy Memorandum
Biosecurity Australia	A unit within the Biosecurity Services Group, in the Australian Government Department of Agriculture, Fisheries and Forestry, responsible for recommendations for the development of Australia's biosecurity policy
BP	*Baculovirus penaei*
BRS	Bureau of Rural Sciences
CCEAD	Consultative Committee on Emergency Animal Disease
CFU	colony forming units
Codex	Codex Alimentarius Commission
COMPILE	Customs Online Method of Preparing from Invoices. Lodgeable Entries
CRC	Cooperative Research Centres
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
DEWHA	Australian Government Department of the Environment, Water, Heritage and the Arts
DNA	deoxyribonucleic acid
DoHA	Australian Government Department of Health and Ageing
ELISA	enzyme linked immunosorbent assay
EMS	Environmental Management Services
EXDOC	Export Documentation
FAO	Food and Agriculture Organisation of the United Nations
FRDC	Fisheries Research and Development Corporation
FSANZ	Food Standards Australia New Zealand
FSC	Australia New Zealand Food Standards Code

Generic IRA	An import risk analysis relevant to all exporting countries. The generic IRA does not consider the disease status or data of individual countries, but is based on estimates of the most likely situation in a hypothetical infected country. Country specific data may be considered at a later date should appropriate data from prospective exporting countries be supplied
HACCP	Hazard Analysis Critical Control Point
HPV	hepatopancreatic parvovirus
ICES	International Council for the Exploration of the Sea
ICON	AQIS Import Conditions Database
ICS	Integrated Cargo System
ICTV	International Committee on Taxonomy of Viruses
IFAT	immunofluorescent antibody test
IHHNV	infectious hypodermal and haematopoietic necrosis virus
IMNV	infectious myonecrosis virus
IPPC	International Plant Protection Convention
IQF	individually quick frozen
IRA	import risk analysis
IUCN	International Union for Conservation of Nature and Natural Resources
JSA	Joint Subcommittee on Aquaculture
LD_{50}	The amount of agent that causes an average 50% mortality of exposed animals
LR	likelihood of release
MOU	Memorandum of Understanding
MSGS	monodon slow growth syndrome
NACA	Network of Aquatic Centres in Asia-Pacific
NHPB	necrotising hepatopancreatitis bacterium
NSW	New South Wales
NT	Northern Territory
NTF	National Task Force on Imported Fish and Fish Products
OIE	World Organization for Animal Health (the Office International des Epizooties)
OIE Aquatic Code	OIE International Aquatic Animal Health Code
OIE Code	OIE International Terrestrial Animal Health Code
PALEE	partial annual likelihood of entry and exposure
PAR	partial annual risk
PCR	polymerase chain reaction
PIMC	Primary Industries Ministerial Council

PL (number)	Postlarvae (age in days as a postlarva)
PLE	partial likelihood of exposure
PLES	partial likelihood of establishment or spread
QAAD	Quarterly Aquatic Animal Disease Report
QLD	Queensland
RDS	runt-deformity syndrome
Restricted risk	risk estimates derived when 'risk management' is applied
RLO	rickettsia-like organism
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SA	South Australia
SCARM	Standing Committee on Agriculture and Resource Management
SPF	specific pathogen free
SPR	specific pathogen resistant
SPS	sanitary and phytosanitary
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures
TAS	Tasmania
TEM	transmission electron microscopy
TSV	Taura syndrome virus
Unrestricted risk	risk estimates derived in the absence of specific risk management measures
US	United States of America
USFDA	United States Food and Drug Administration
VIC	Victoria
WA	Western Australia
WSD	white spot disease
WSSV	white spot syndrome virus
WTD	white tail disease
WTO	World Trade Organization
YHV	yellowhead virus

Summary

Prawns and prawn products[1] imported into Australia for human consumption have been subject to interim quarantine conditions, introduced progressively since 2000, while a comprehensive import risk analysis (IRA) was being completed.

Biosecurity Australia undertook an IRA, with the assistance of a specialist scientific team, to assess pathogenic agents that could potentially be introduced to Australia through the importation of uncooked prawns and prawn products intended for human consumption, and examined a range of risk management options for pathogenic agents considered to pose an unacceptable biosecurity risk.

This IRA report recommends that the importation of prawns and prawn products could be permitted subject to compliance with risk management measures to manage the quarantine risks of a range of significant pathogenic agents to a very low level, in line with Australia's conservative approach to quarantine. These pathogenic agents include white spot syndrome virus (WSSV), yellowhead virus (YHV), Taura syndrome virus (TSV) and necrotising hepatopancreatitis bacterium (NHPB) (in the case of the last disease agent, for unfrozen product only).

Details of risk management measures which have been deemed acceptable are as follows[2]:

- sourcing all uncooked prawn product from a country or zone determined to the satisfaction of Australian government authorities to be free of WSSV, YHV and TSV, and in addition, NHPB if the product is not frozen (i.e. the product is chilled);

  OR

- having the head and shell removed (the last shell segment and tail fans permitted), and each imported batch held on arrival in Australia under quarantine control, tested and found to be free of WSSV and YHV. Testing is based on the polymerase chain reaction tests in the current version of the World Organization for Animal Health (OIE) *Manual of Diagnostic Tests for Aquatic Animals*, or equivalent, and a sampling regimen that would provide 95% confidence of detecting the agent if present at 5% prevalence;

  OR

- being highly processed[3], that is with the head and shell removed (the last shell segment and tail fans permitted) and;
  - coated for human consumption by being breaded (crumbed) or battered, or
  - coated for human consumption by being marinated in a wet marinade (the marinade must be no less than 12% of the total weight of the product), or
  - coated for human consumption by being marinated in a dry marinade (the marinade must be clearly seen to cover the product), or
  - coated for human consumption by being marinated and placed on skewers (the marinade must be clearly seen to cover the product), or

[1] Not including live prawns.

[2] Shelf-stable food products containing prawns, such as dried prawns, canned prawns or condiments containing prawns as an ingredient (e.g. prawn balachan, shrimp paste), would not be subject to these requirements.

[3] Uncooked prawns that are considered to be highly processed would be subject to random inspection by AQIS to ensure the imported commodity complies with the product description on the import permit and health certificate.

- the raw prawn meat processed into dumpling, spring roll, samosa roll, ball or dim sum-type product;

OR

- being cooked in premises approved by and under the control of an appropriate Competent Authority in the exporting country to a minimum time and temperature standard where all the protein in the prawn meat is coagulated and no uncooked meat remains.

The IRA report recommends that, for uncooked prawns and prawn products, health certification (to accompany each shipment of imported prawns) be issued by the relevant Competent Authority in the exporting country, attesting that the prawns had been inspected, processed and graded in premises approved by and under the control of the Competent Authority, were free from visible lesions associated with infectious disease and are fit for human consumption. Uncooked prawns imported for human consumption that are not considered to be highly processed be marked with the words 'for human consumption only' and 'not to be used as bait or feed for aquatic animals'.

For cooked prawns, the IRA report recommends that health certification accompanies each shipment which attests that the prawns have been cooked in premises approved by and under the control of the Competent Authority, all the protein in the prawn is coagulated and no raw meat remains, and that the prawns are fit for human consumption.

The IRA report recognises that there might be other treatments, new technologies, or other combinations of measures that may provide an equivalent level of quarantine protection for the pathogenic agents identified as requiring risk management. Submissions supporting equivalence measures will be evaluated on a case-by-case basis.

Full details of the analysis and the conclusions reached, in addition to a summary of the main changes to the IRA report since release of the 2006 draft, are provided in this IRA report.

1 Introduction

This document is Biosecurity Australia's import risk analysis (IRA) report on non-viable[4], uncooked (green) prawns and prawn products intended for human consumption.

This IRA report was written in consultation with, and under the supervision of, an IRA team, the membership of which is detailed in section 1.4 of this document. The information in the report is based on the scientific literature and expert opinion as cited in the text. Information that is not specifically referenced is based on the knowledge, expert views/opinions of the IRA team.

In undertaking the risk assessment documented in this report, Biosecurity Australia and the IRA team have taken into consideration the various stakeholder submissions relating to this matter that have been received by Biosecurity Australia since the release of the 2000 draft IRA report on prawns and prawn products (Animal Quarantine Policy Memorandum — AQPM 2000/41) and the 2006 revised draft generic IRA report for prawns and prawn products (Biosecurity Australia Policy Memorandum — BAPM 2006/35b). The main changes in this IRA report compared to the 2006 draft report are described in Appendix 1.

1.1 Background

Prior to 1992, there was no animal health related policy in Australia for the importation of prawns or prawn products — the only restriction related to insect contamination of dried prawn imports. In 1992, permits were required for the importation of manufactured prawn meals and prawn feeds (AQPM 1992/57 Requirements for the importation of fish feed and fish meal), which included heat treatment as a condition of the permit.

In 1992, AQIS commissioned the then Bureau of Rural Resources, later Bureau of Resource Sciences (BRS), to conduct a major review of aquatic animal health and quarantine. The report, released in 1995, was a comprehensive examination of Australia's quarantine policies and practices regarding aquatic animals and their products (Nunn 1995). It considered the review of a consultant, Dr J. D. Humphrey, and identified concerns in relation to quarantine policy on importation of several aquatic animal species (Humphrey 1995).

In 1995, the National Task Force on Imported Fish and Fish Products (NTF) was established to examine the BRS report and related issues. The NTF included representatives of relevant Commonwealth, State and Territory government agencies, commercial and recreational fishing groups, importers, aquaculturists, research organisations and environmental groups. It recommended that AQIS review aquatic animal quarantine policies and practices, including that quarantine requirements for imported bait prawns, prawn feeds and prawns for human consumption, be revised as a high priority (Higgins 1996).

In its response to the NTF report, the Australian Government supported most of the recommendations made by the NTF.

In September 1996, the policy arm of AQIS announced the commencement of a review of its aquatic animal quarantine policies, including on importation of bait prawns, prawn feeds and prawns for human consumption (AQPM 1996/59), based on the NTF's recommendations. At the same time, restrictions (i.e. banning) were imposed on the entry of uncooked prawns and prawn-based products containing uncooked prawns for bait use, to address concerns relating to use of imported prawns as recreational fishing bait.

In May 1997, AQIS formally announced the commencement of the import risk analysis (IRA)

[4] That is, not including live prawns.

for prawns and prawn based products (AQPM 1997/28).

In 2000, Biosecurity Australia announced the release of a draft report of the IRA for prawns and prawn products (AQIS 2000 — AQPM 2000/41). This draft report identified white spot syndrome virus (WSSV) and yellowhead virus (YHV) as pathogenic agents posing an unacceptable risk, and proposed risk management measures, including health certification and minimum size requirements.

The 2000 draft report proposed that commercial processing of imported uncooked prawns in Australia must be conducted at a premises approved by AQIS. The draft IRA report also noted that the implementation of measures required for WSSV and YHV would reduce the risks associated with emerging but as yet unknown pathogenic agents, and would effectively manage risks associated with pathogenic agents such as Taura syndrome virus (TSV) in (as judged at the time) the unlikely event that such agents enter Australia and Australian crustacean species prove susceptible.

In December 2000, interim measures including size limitation, Competent Authority health certification and post-arrival AQIS inspection were introduced to manage WSSV and YHV risks, based on findings of the 2000 draft report (Animal Biosecurity Policy Memorandum — ABPM 2000/57).

In February and May 2001, specific post-arrival testing requirements for WSSV were added to the interim measures in response to a December 2000 incident in Darwin where imported prawns were used as aquaculture feed (announced in ABPM 2001/06 and 2001/11). These interim measures were fully implemented in June 2001 and included:

- minimum size limits (a ban on whole uncooked prawns less than 15 grams to minimise their use as bait);
- health certification from the relevant government authority in the exporting country, attesting that prawns had not been emergency harvested[5], have been inspected, processed and graded in a premises approved by and under the control of the Competent Authority, were free from visible lesions associated with infectious disease and have been found fit for human consumption;
- post-arrival inspection in Australia by AQIS; and
- WSSV testing of all imported batches of uncooked whole prawns or unpeeled headless prawns.

A summary of the December 2000 Darwin WSSV incident is provded in Appendix 2.

Uncooked, highly processed prawns were permitted importation under conditions introduced in 2003, which include requirement of health certification issued by the overseas Competent Authority attesting that the prawns:

- have been processed, inspected and graded in a premises approved by and under the control of the Competent Authority;
- are free from visible signs of infectious disease and are fit for human consumption;
- have been peeled to at least the last tail segment;
- are breaded or battered;
- have a finished product grade size count of, at least, 21/25 per pound (or 55 per kilogram); and
- are packaged in lots of no more than 3 kg in weight.

In May 2002, following a public workshop on the IRA, the IRA team recommended that a revised draft IRA report be produced (announced in ABPM 2002/32).

In August 2004, the IRA team decided on the need for trials on the susceptibility of selected

[5] Aborted early to minimise disease related losses.

species of Australian crustaceans to TSV. Following a call for tender, this research was undertaken by the Aquaculture Pathology Laboratory at the University of Arizona.

After a period of ongoing monitoring and evaluation of risks (including consideration of the preliminary outcomes of the University of Arizona research), Biosecurity Australia advised AQIS in late 2005 of its view that given the increased availability of imported farmed vannamei prawns (*Litopenaeus vannamei*) from Asia and the spread of TSV to Asia from the Americas, importation of vannamei prawns presented an unacceptable quarantine risk, but that the interim measures targeting WSSV and YHV introduced in 2000–01 would address this TSV risk, pending completion of this IRA.

1.2 Australia's current quarantine policy

In November 2006, Biosecurity Australia released the revised draft generic IRA report for prawns and prawn products. Strengthened interim measures were announced in July 2007 following consideration of stakeholder comments on the revised draft IRA report (Biosecurity Australia Policy Memorandum — BAPM 2007/16). The stronger measures were considered necessary to manage the altered quarantine risks associated with the importation of prawns and prawn products. AQIS implemented these measures in October 2007. The measures were further revised in September 2008 (BAA 2008/30) to remove the conditions associated with IHHNV. The current conditions are those risk management measures recommended in Chapter 16 of this report.

1.3 Biosecurity framework

1.3.1 Australian legislation

The *Quarantine Act 1908* and its subordinate legislation, including the *Quarantine Proclamation 1998*, administered by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), provide the legislative basis of human, animal and plant biosecurity in Australia.

Some key provisions are set out below.

Quarantine Act: Scope

Subsection 4 (1) of the *Quarantine Act 1908* defines the scope of quarantine as follows.

In this Act, quarantine includes, but is not limited to, measures:

(a) for, or in relation to:

(i) the examination, exclusion, detention, observation, segregation, isolation, protection, treatment and regulation of vessels, installations, human beings, animals, plants or other goods or things; or

(ii) the seizure and destruction of animals, plants, or other goods or things; or

(iii) the destruction of premises comprising buildings or other structures when treatment of these premises is not practicable; and

(b) having as their object the prevention or control of the introduction, establishment or spread of diseases or pests that will or could cause significant damage to human beings, animals, plants, other aspects of the environment or economic activities.

Section 5D of the *Quarantine Act 1908* covers the level of quarantine risk.

A reference in this Act to a level of quarantine risk is a reference to:

(a) the probability of:

(i) a disease or pest being introduced, established or spread in Australia or the Cocos Islands; and

(ii) the disease or pest causing harm to human beings, animals, plants, other aspects of the environment, or economic activities; and

(b) the probable extent of the harm.

Section 5D of the *Quarantine Act 1908* includes harm to the environment as a component of the level of quarantine risk.

Environment is defined in Section 5 of the *Quarantine Act 1908,* in that it:

> *includes all aspects of the surroundings of human beings, whether natural surroundings or surroundings created by human beings themselves, and whether affecting them as individuals or in social groupings.*

Quarantine Proclamation

The *Quarantine Proclamation* 1998 is made under the *Quarantine Act 1908*. It is the principal legal instrument used to control the importation into Australia of goods of quarantine (or biosecurity) interest. The Proclamation empowers a Director of Quarantine to grant a permit to import.

Section 70 of the *Quarantine Proclamation* 1998 sets out the matters to be considered when deciding whether to grant a permit to import:

> *Things a Director of Quarantine must take into account when deciding whether to grant a permit for importation into Australia*
>
> *(1) In deciding whether to grant a permit to import a thing into Australia or the Cocos Islands, or for the removal of a thing from the Protected Zone or the Torres Strait Special Quarantine Zone to the rest of Australia, a Director of Quarantine:*
>
> *(a) must consider the level of quarantine risk if the permit were granted; and*
>
> *(b) must consider whether, if the permit were granted, the imposition of conditions on it would be necessary to limit the level of quarantine risk to one that is acceptably low; and*
>
> *(c) for a permit to import a seed of a kind of plant that was produced by genetic manipulation – must take into account any risk assessment prepared, and any decision made, in relation to the seed under the Gene Technology Act; and*
>
> *(d) may take into account anything else that he or she knows that is relevant.*

1.3.2 Development of biosecurity policy

As can be seen from the above extracts, the legislation establishes the concept of the level of biosecurity (quarantine) risk as the basis of decision-making under Australian quarantine legislation.

Import risk analyses are a significant contribution to the information available to the Director of Animal and Plant Quarantine – a decision maker for the purposes of the Quarantine Proclamation. This import risk analysis is conducted within an administrative process – known as the IRA process; described in the *IRA Handbook* (Biosecurity Australia 2003).

The purpose of the IRA process is to deliver a policy recommendation to the Director of Animal and Plant Quarantine that is consistent with Government policy and which is characterised by sound science and by transparency, fairness and consistency.

What is Import Risk Analysis?

For the purposes of animal and plant biosecurity, an IRA identifies the pests and diseases relevant to an import proposal, assesses the risks posed by them and, if those risks are unacceptable, specifies the measures that could be taken to reduce those risks to an acceptable level. These analyses are conducted via an administrative process (described in the *IRA Handbook*) that involves, among other things, notification to the WTO, consultation and appeal.

When are IRAs undertaken?

Biosecurity Australia may undertake an IRA if:

- there is no relevant existing biosecurity measure for the commodity and pest/disease combination; or
- a variation in established policy is desirable because pests or diseases, or the likelihood and consequences of entry, establishment or spread of the pests or diseases could differ significantly from those previously assessed.

Environment and human health

When undertaking an IRA, the *Quarantine Act* requires the Director of Animal and Plant Quarantine to ensure that environmental factors are considered in the decision-making process. A memorandum of understanding (MOU) is in place between Biosecurity Australia and the Department of the Environment, Water, Heritage and the Arts (DEWHA), formerly Department of Environment and Heritage to facilitate input of advice on environmental matters in IRAs.

Where appropriate, Biosecurity Australia consults with the Australian Government Department of Health and Ageing (DoHA), and with Food Standards Australia New Zealand (FSANZ), on the assessments for zoonotic pests or diseases that may establish in Australia's animal population through the importation of prawns and prawn products.

The IRA process in summary

The process consists of the following major steps:

- *Initiation*: This is the stage where the identified need for an IRA originates.
- *Scheduling and Scoping*: At this stage, Biosecurity Australia considers all the factors that affect scheduling. Consultation with States, Territories and other Commonwealth agencies is involved. There is opportunity for appeal by stakeholders at this stage.
- *Risk Assessment and Risk Management*: Here, the major scientific and technical work relating to risk assessment is performed. There is detailed consultation with stakeholders.
- *Reporting*: Here, the results of the IRA are communicated formally. There is consultation with States and Territories, and the report is referred to the Eminent Scientists Group. The Chief Executive of Biosecurity Australia then delivers the biosecurity policy recommendation arising from the IRA to the Director of Animal and Plant Quarantine. There is opportunity for appeal by stakeholders at this stage.

Policy determination

The Director of Animal and Plant Quarantine makes the policy determination, which is notified publicly.

1.4 Australia's international rights and obligations

Biosecurity restrictions on imports must conform to Australia's rights and obligations as a World Trade Organization (WTO) Member country. These rights and obligations derive principally from the WTO *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement), although other WTO agreements may also be relevant. Specific international guidelines on risk analysis developed under the International Plant Protection Convention (IPPC) and by the World Organization for Animal Health (OIE) are also relevant.

The SPS Agreement recognises the right of WTO Member countries to determine the level of sanitary and phytosanitary protection they deem appropriate, and to take the necessary measures to achieve that protection. Sanitary (human and animal health) and phytosanitary (plant health) measures typically apply to trade in or movement of animal and plant based goods within or between countries. The SPS Agreement applies to all SPS measures that may directly or indirectly affect international trade. An SPS measure is any measure applied to protect human, animal or plant life or health within the Territory of a Member from risks arising from the entry of pests/diseases or from contaminants in food. An SPS measure may also be applied to limit other damage within the Territory of a Member from the entry of a pest.

The SPS Agreement provides, *inter alia*, for the following:

- A WTO Member country has the right to determine the level of sanitary and phytosanitary protection it deems appropriate (designated the appropriate level of protection, or ALOP).
- An importing Member has the sovereign right to take measures to achieve the level of protection it deems appropriate to protect human, animal or plant life or health within its territory.
- An SPS measure must be based on scientific principles and must not be maintained without sufficient scientific evidence.
- An importing Member must avoid arbitrary or unjustifiable distinctions in the levels of protection it considers to be appropriate in different situations, if such distinctions result in discrimination or a disguised restriction on international trade.
- An SPS measure must not be more trade restrictive than required to achieve an importing Member's ALOP, taking into account technical and economic feasibility.
- An SPS measure should be based on relevant international standards, guidelines or recommendations where these exist, unless there is a scientific justification for a stricter measure, or unless a stricter measure is required in order to achieve the importing Member's ALOP.
- An SPS measure conforming to an international standard, guideline or recommendation is deemed to be necessary to protect human, animal or plant life or health, and to be consistent with the SPS Agreement.
- Where a relevant international standard, guideline or recommendation does not exist or where, in order to achieve an importing Member's ALOP, a measure needs to provide a higher level of protection than accorded by the relevant international standard, such a measure must be based on a risk assessment; the risk assessment must take into account available scientific evidence and relevant economic factors.
- Where risk assessment is required, risk assessment techniques developed by the relevant international organisations must be taken into account.
- Where the relevant scientific evidence is insufficient, an importing Member may provisionally adopt SPS measures on the basis of available pertinent information; but in such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the SPS measure accordingly within a reasonable period of time.

- An importing Member shall accept the measures of other countries as equivalent, if it is objectively demonstrated that the measures achieve the importing Member's ALOP.
- An importing member shall recognise the concepts of pest- or disease-free areas and areas of low pest or disease prevalence, and shall take into account, *inter alia*, the level of prevalence of specific diseases or pests, the existence of eradication or control programs, and appropriate criteria and guidelines which may be developed by the relevant international organisations.

Australia's appropriate level of protection (ALOP)

The SPS Agreement defines the concept of an 'appropriate level of sanitary or phytosanitary protection (ALOP)' as the level of protection deemed appropriate by the WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Like many other countries, Australia expresses its ALOP in qualitative terms. Australia's ALOP, which reflects community expectations through government policy, is expressed as providing a high level of sanitary or phytosanitary protection whereby risk is reduced to a very low level, but not to zero. This definition of ALOP, and its illustration by way of a risk estimation matrix is shown below in Table 1.1. The State and Territory governments have indicated their support through the Primary Industries Ministerial Council (PIMC), which agreed in 2002 that Australia's needs are met by this definition of the ALOP (PIMC 2002).

The cells of the risk estimation matrix contain the qualitative descriptors which apply to the product of different degrees of likelihood[6] and different levels of consequences — termed 'risk'. When interpreting the risk estimation matrix, it should be remembered that, although the descriptors for each axis are similar ('low', 'moderate', 'high' etc), the vertical axis refers to *likelihood* and the horizontal axis refers to *consequences*.

Risk Management and SPS Measures

Australia's animal and plant health status is maintained through the implementation of measures to facilitate the importation of products while protecting the health of people, animals and plants.

Australia bases its national measures on international standards where they exist and where they deliver the appropriate level of protection from pests and diseases. However, where such standards do not achieve Australia's appropriate level of protection, or relevant standards do not exist, Australia exercises its right under the SPS Agreement to take appropriate measures, justified on scientific grounds and supported by risk analysis.

[6] The terms "likelihood" and "probability" are synonymous. "Probability" is used in the *Quarantine Act 1908* while "likelihood" is used in the WTO SPS Agreement. These terms are used interchangeably in this IRA Report.

Table 1.1 Risk estimation matrix: estimation of the partial annual risk of exposure

Likelihood of entry and exposure							
	High likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
	Moderate Likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
	Low likelihood	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
	Very low likelihood	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
	Extremely low likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
	Negligible likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk
		Negligible impact	*Very low impact*	*Low impact*	*Moderate impact*	*High impact*	*Extreme impact*

Consequences of entry and exposure

NOTE: The band of cells in Table 1.1 marked 'very low risk' represents Australia's ALOP.

1.5 Import risk analysis team

The current membership of the IRA team is as follows:

Name	Organisation/Position	Expertise
Dr Mike Nunn (Chair) *2006 – 2009*	Biosecurity Australia – Principal Scientist, Animal Biosecurity	Animal health policy advice and scientific analysis
Assoc. Prof Leigh Owens *1998 – 2009*	James Cook University, Townsville	Aquatic animal health management
Dr Brian Jones *2004 – 2009*	Department of Fisheries, WA	Aquatic animal health management
Dr Robyn Martin *2004 – 2009*	Biosecurity Australia – General Manager, Animal Biosecurity	Animal quarantine policy/ practice

Drs. David Banks, Robyn Martin, David Wilson and Sarah Kahn of Biosecurity Australia have each served as the Chair of the IRA team in the past. Mr Glenn Hurry, Drs. Richard Callinan (formerly NSW Department of Fisheries), Steve Percival (private consultant) and Biosecurity Australia staff Drs. Peter Beers, Vanessa Findlay, Brett Edgerton and Judith Bourne have also served as members of the IRA team.

1.6 Scope of IRA

Prawns, commonly known as 'shrimp' outside Australia, are decapod crustaceans, belonging to the suborder Dendrobranchiata, which includes the superfamilies Penaeoidea and Caridea.

The risk assessment detailed in this report is confined to an assessment of quarantine risks associated with importation of non-viable[7], uncooked prawns and prawn products intended for human consumption, and includes consideration of potential post-import misdirection of prawns or prawn products for use as bait or aquaculture feed.

This scope is narrower than that indicated in the original public announcement initiating the IRA on prawn and prawn products (AQPM 1996/59) or that of the 2000 draft IRA (AQPM 2000/41). The 2000 draft IRA's scope covered all 'non-viable prawns and prawn products' for all intended end-uses, including for use as recreational fishing bait — the term 'prawn product' was used to collectively refer to 'non-viable whole prawns, processed prawns and other products containing materials derived from prawns'. The IRA team and Biosecurity Australia deemed this change in scope necessary to ensure timely completion in light of the potential for increased risks associated with rapidly changing trade patterns and the emergence of new prawn pathogenic agents.

Further, for the purposes of risk assessment, the 'unrestricted commodity' given consideration in this report is non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption — the IRA team, based on first principles, considered farmed, whole, uncooked prawns to represent the highest risk commodity type.

Disease outbreaks have mostly been reported in farmed prawns. Although there are occasional reports of disease in wild populations, mainly associated with easily seen, obvious clinical signs (e.g. microsporidiosis), in general disease outbreaks are rarely observed in wild prawn populations. Most affected prawns in the wild are likely to be eaten by non-susceptible predators and high recruitment rates tend to mask the expression of disease at a population level.

Farmed prawns are often held at high stocking rates, with minimum water exchange being an industry norm. Thus, the environmental conditions to which farmed prawns are subject can facilitate the multiplication and spread of pathogenic agents that are transmitted horizontally; although the health status of farmed prawns is monitored more closely and managed more effectively than is the case with wild prawn stocks. Disease management, including practices such as emergency harvest, may further increase quarantine risks — because they are likely to be clinically diseased, the prevalence and amount of pathogenic agent in emergency harvested prawns would be higher than in normal farmed prawns. Prawns sourced from wild fisheries, part-prawn products (including head-off prawns), processed prawns (including cooked prawns) are given consideration in the context of potential risk management measures. The outcome of the risk assessment is an estimation of the quarantine risk associated with unrestricted importation of the commodity.

Frozen product, in preference to chilled product, makes up the vast majority of imports. However, chilled prawns are included in the range of products covered in developing quarantine requirements (see Chapter 16). This inclusion was based on consideration of the determined risks for frozen product and whether the measures developed for frozen product would also address any risks posed by importation of chilled product.

This assessment is 'generic' in nature, in that its scope included importation from all countries. The release assessment component of this IRA assumed, for the purposes of risk assessment, that the pathogenic agents of concern are present in all source countries. Country

[7] That is, not including live prawns.

or zone freedom from particular pathogenic agents is considered in the context of potential risk management measures.

Finally, this risk assessment is based on current estimates import volumes and trends. As such, it may be necessary to review import policies based on this assessment in light of any significant changes to prawn importation patterns.

The IRA team notes that there may be other potential pathways by which disease agents associated with imported prawns may be introduced into Australia, such as via ballast water discharge or hull fouling or import of other aquatic animal commodities such as crabs. Consideration of such pathways is outside the scope of this risk assessment.

Human health and environment

In accordance with the SPS Agreement, IRAs assess risks to human, animal and plant life or health. However, under Australian administrative arrangements, Biosecurity Australia provides advice to the Director of Animal and Plant Quarantine in relation to the life or health of animals and plants, while risks to human health are the responsibility of DoHA. Risks to human health associated with the consumption of imported prawns and prawn products are assessed by FSANZ. Biosecurity Australia consulted FSANZ on public health issues, and with the DEWHA on environmental issues associated with the importation of prawns and prawn products, during the preparation of this IRA.

Products intended for human consumption may undergo a separate risk assessment by FSANZ and/or DoHA to determine the public health risks.

Imported prawns and prawn products must comply with the *Imported Food Control Act 1992* and the Australia New Zealand Food Standards Code (FSC) in its entirety. Under the *Imported Food Control Act 1992*, AQIS may inspect, or inspect and conduct an analysis of imported prawns and prawn products to determine its compliance with the FSC.

2 Crustaceans in Australia

2.1 Prawn fisheries

The commercial species of marine prawns belong to the family Penaeidae. Over 50 different species of penaeid prawns have been recorded from Australian waters (Grey et al. 1983), six of which are uniquely Australian. The following species of penaeid prawns are of economic importance in Australia:

black tiger prawn (*Penaeus monodon*)
brown tiger prawn (*Penaeus esculentus*)
grooved tiger prawn (*Penaeus semisulcatus*)
banana prawn (*Fenneropenaeus merguiensis*)
red-legged banana prawn (*Fenneropenaeus indicus*)
Kuruma prawn (*Marsupenaeus japonicus*)
blue Endeavour prawn (*Metapenaeus endeavouri*)
red Endeavour prawn (*Metapenaeus ensis*)
school prawn (*Metapenaeus macleayi*)
greasyback prawn (*Metapenaeus bennettae*)
western king prawn (*Melicertus latisulcatus*)
eastern king prawn (*Melicertus plebejus*)
red-spot king prawn (*Melicertus longistylus*).

Worldwide, the prawn aquaculture industry is based on *Penaeus* species, the most desirable being *Litopenaeus vannamei* and *Litopenaeus stylirostris*, which are indigenous to the Pacific west coast of the Americas, and *P. monodon* and *M. japonicus*, which are Indo-Pacific species. *Litopenaeus vannamei* and *L. stylirostris* are not present in Australia. *Penaeus monodon* and *M. japonicus*, together with *P. esculentus* and *F. merguiensis* are present in Australia and dominate the prawn aquaculture sector. Of the six uniquely Australian species, four have been cultured commercially: *P. esculentus*, *M. bennettae*, *M. macleayi* and *M. plebejus*.

The freshwater prawns belong to the genus *Macrobrachium*, a large genus comprising over 150 species of which several occur in Australia. The genus is widely distributed, mainly throughout the tropics and to a lesser extent in the subtropical and temperate zones. Many of these species provide significant local fisheries where they occur. By far the most popular species for aquaculture is *Macrobrachium rosenbergii*, which has been translocated globally to many places outside of its natural range. *Macrobrachium rosenbergii* is present in Australia although it is yet to be farmed commercially.

Capture fisheries

The majority of prawn capture fisheries in Australia are found in waters north of latitude 26ºS, in the region of Exmouth Gulf on the west coast, and Moreton Bay on the east coast (Kailola et al. 1993). Commercial prawn trawling operations use multi- or single-rig otter trawl setups, ranging from 5 to 32 metres in length (Jones et al. 2002). Penaeid prawns form the basis of valuable commercial capture fisheries in all States of Australia (except Tasmania) and are the basis of Australia's prawn aquaculture industry.

Other commercially important marine species include deepwater prawns belonging to the families Aristeidae, Solenoceridae and Pandalidae. These are trawled in waters off the north-west coast of Western Australia and to a lesser extent, off the coast of NSW (Jones et al. 1994).

In addition to commercial prawn species, there are many other prawn species of minor or no

commercial value throughout Australia's marine and freshwater aquatic environments. These include freshwater prawns (such as *Macrobrachium* species), common estuary prawns, commensal prawns and rock pool prawns. These species are likely to be important in the food chain of various aquatic animals. They contribute to the sustainability of commercial and recreational fisheries as well as in maintaining the balance of aquatic ecosystems.

Australia's prawn capture fisheries currently employ approximately 1000 people (ABARE 2006). Australia's prawn capture fisheries are valued at over $300 million and are expected to remain relatively static in the medium term (ABARE 2005, ABARE 2006).

Aquaculture

Four prawn species are farmed in Australia: black tiger prawns (*P. monodon*), Kuruma prawns (*M. japonicus*), brown tiger prawns (*P. esculentus*) and banana prawns (*F. merguiensis*). The black tiger prawn accounts for the majority of production. The Kuruma prawn is also of particular commercial importance as a live product and can fetch prices of up to three times that of the other species when offered to the Japanese market (Love and Langenkamp 2003). Aquaculture contributes 16% of the total Australian prawn production volume (ABARE 2006).

Recreational and traditional fisheries

The National Recreational and Indigenous Fishing Survey revealed there to be significant recreational fisheries for prawns, including freshwater caridean prawns and saltwater penaeid prawns (Henry and Lyle 2003). Nationally, an estimated 194 tonnes were harvested by recreational fishers over a 12-month monitoring period, with over half (59%) reported from NSW. Catches in Queensland approximated 29%, while harvests in Victoria and Western Australia were 5–6%. Marine penaeid prawns were primarily harvested from estuarine waters, whereas most freshwater prawns were fished from rivers. Net-catching was the dominant fishing method for marine prawns, while freshwater prawns were mainly caught using traps.

2.1.1 Regional importance of prawn aquaculture and capture industries

Table 2.1 shows tonnage and dollar value of Australia's prawn aquaculture and capture fisheries production.

Commonwealth fisheries

The Australian Government is constitutionally responsible for managing offshore fisheries between three and 200 nautical miles from Australia's mainland and territories coastlines (Love et al. 2004), covering almost 9 million square kilometres[8].

Commonwealth fisheries that include prawn harvesting are comprised of:

- Northern Prawn Fishery (83 vessels), which covers the Gulf of Carpentaria from Cape Londonderry to Cape York. The main species targeted in this range are the banana prawn (*F. merguiensis*), black tiger prawn (*P. monodon*), Endeavour prawn (*M. endeavouri*) and the king prawn (*M. latisulcatus*) (ABARE 2006).
- Torres Strait Fishery (70 vessels), which encompasses the Torres Strait waters located between the tip of the Cape York Peninsula, the south coast of Papua New Guinea (PNG) and the Arafura (west) and Coral (east) Seas (Love et al. 2004). The species targeted are blue Endeavour prawns (*M. endeavouri*), brown tiger prawns

[8] Available at: http://www.daffa.gov.au/fisheries/domestic/zone

(*P. esculentus*) and king prawns (ABARE 2006).

- South East Trawl Fishery (118 vessels), of which prawns only account for a small percentage of the total aquatic animal species targeted. This fishery therefore contributes only a small percentage of the total Commonwealth prawn catch. It covers the area from Barrenjoey Point, NSW around Tasmania to Cape Jervis, South Australia (ABARE 2006).

Northern Territory

Capture fisheries

See Commonwealth fisheries.

Aquaculture

In 2003–04, of the nine farms licensed to grow prawns, only five were productive, covering an area of 66 hectares and producing 78 tonnes of prawns in total (QDPIF 2005b). As broodstock and postlarvae are currently imported from Queensland, projects to access Northern Territory waters to capture broodstock and to develop hatchery production are currently underway (DPIFM 2004).

Queensland

Capture fisheries

Extending from Cape York to the NSW border, Queensland's East Coast Trawl Fishery dominates prawn landings. Most of the spawners for aquaculture are trawled from Queensland waters and shipped to hatcheries around Australia.

Queensland fisheries that include prawn harvesting are:

- East Coast Trawl Fishery (478 licence holders), which targets eastern king prawns (*M. plebejus*), black tiger prawns, Endeavour prawns, banana prawns, red spot king prawns (*M. longistylus*) and bay prawns (predominately *M. bennettae*) (ABARE 2006).
- River and Estuary Trawl Fishery (144 licence holders), which targets banana, bay and black tiger prawns (ABARE 2006).

Aquaculture

Queensland has the largest portion of Australian prawn aquaculture by volume and value. The State had approximately 14 hatcheries (Lobegeiger and Wingfield 2005) and 34 grow-out farms in operation during 2003–04 (QDPIF 2005b) situated along the coast between Brisbane and Cooktown. Most farms produce black tiger prawns, the remainder producing Kuruma, brown tiger and banana prawns (Love and Langenkamp 2003).

Queensland hatcheries supply both banana and black tiger prawn postlarvae for grow-out farms within the State and interstate — the broodstock for these hatcheries are collected from wild populations. Research to develop techniques to produce broodstock in captivity has led to the successful closing of the banana prawn life-cycle (Love and Langenkamp 2003). One banana prawn hatchery in Queensland now develops its own broodstock, removing reliance on wild broodstock (Lobegeiger and Wingfield 2005). There are also hatcheries producing Kuruma prawn postlarvae from pond-reared spawners or wild broodstock (Lobegeiger 2003). Approximately 150 tonnes of Kuruma prawns are produced annually in Queensland, the majority of which are exported live to Japan (Love and Langenkamp 2003). Promising results have also been reported from pond-reared *P. monodon* spawners, making Queensland prawn farmers world leaders in successfully closing the *P. monodon* life-cycle and producing

commercial quantities of quality prawns from domesticated broodstock (Austasia Aquaculture Fish eNews, May 2006[9]).

Queensland currently has 62 prawn farming licence holders (ABARE 2006) with a ponded area of 785 hectares (Lobegeiger and Wingfield 2005).

Western Australia

Capture fisheries

The two main Western Australian prawn fisheries are the Shark Bay Prawn Fishery (27 licence holders) and Exmouth Prawn Fishery (12 licence holders).

Western Australian prawn fisheries target predominantly the western king prawn (*M. latisulcatus*), brown tiger prawn and Endeavour prawn (ABARE 2006)[10].

Aquaculture

Three farms hold licenses and are currently in the developmental phase. The development and diversification of hatchery facilities in Exmouth and Broome is reported to be in progress (Love and Langenkamp 2003).

New South Wales

Capture fisheries

There are two main prawn fisheries in NSW: the Ocean Prawn Trawl Fishery (306 licence holders operating north of Barrenjoey Headland) targeting mainly eastern king prawns, and the Estuary Prawn Trawl Fishery (216 licences operate in the Clarence, Hunter and Hawkesbury Rivers) targeting mainly school prawns (*M. macleayi*) (ABARE 2006). In December 2005, the NSW Department of Fisheries placed a total ban on commercial harvesting of prawns from Sydney Harbour as a precautionary measure following test results that revealed elevated levels of dioxin in prawns from the harbour.

Aquaculture

The NSW prawn farming industry is located on the banks of the Clarence and Richmond Rivers. The industry currently produces black tiger prawns, although there is also some banana prawn and brown tiger prawn production. There has also been Kuruma prawn production in the past.

As only one NSW hatchery produces *P. monodon*, most postlarvae are sourced from Queensland hatcheries (Allan and Callinan 2001). NSW currently has 15 licence holders for pond-rearing prawns (ABARE 2006), although only six farms (136 hectares) were productive in 2003–04 (QDPIF 2005b).

South Australia

Capture fisheries

There are three State prawn fisheries in South Australia: Spencer Gulf (39 licence holders), Gulf St Vincent (10 licence holders) and West Coast (3 licence holders). The western king

[9] Available at http://www.austasiaaquaculture.com.au

[10] Details of the fishery and catches can be found in the annual "State of the Fisheries Report" available at http://www.fish.gov.au/

prawn is the main species caught in all three fisheries (ABARE 2006).

Victoria

Capture fisheries

The two main species taken are the eastern king prawn and the school prawn, caught mostly during offshore trawling. Catches of eastern king prawns have declined from 144 tonnes in 2001–2002 to a total prawn catch of 23 tonnes in 2004–2005 (McCormack and Morison 2004, ABARE 2006).

Table 2.1 Australian fisheries production statistics 2004–2005*

Fishery Area		Production Type	Prawn Species	Volume (t)	Value ($'000)
Commonwealth fisheries	Northern Prawn	Wild-caught[s]	Banana	2764	30634
			Tiger	1785	29152
			Endeavour	412	3948
			King	2	30
			Other	73	499
			TOTAL	5036	64263
	Torres Strait	Wild-caught[s]	Endeavour	663	4908
			Tiger	706	9525
			King	59	587
			Other	12	123
			TOTAL	1440	15143
	Other	Wild-caught[s]	TOTAL	173	328
			Commonwealth TOTAL	6649	79734
State fisheries	QLD	Wild-caught[s]	King	2956	35210
			Tiger	1841	27622
			Endeavour	811	9728
			Other	964	7118
			TOTAL	6572	79678
		Aquaculture[s]	TOTAL	2940	45400
			QLD TOTAL	9512	125078
	WA	Wild-caught[s]	TOTAL	3585	42557
	SA	Wild-caught[p]	TOTAL	2173	35805
	NSW	Wild-caught[p]	King	629	13092
			School	547	2892
			Other	157	541
			TOTAL	1333	16525
		Aquaculture[p]	TOTAL	294	4464
			NSW TOTAL	1627	20989
	VIC	Wild-caught[s]	TOTAL	23	303
Australia wide		Wild-caught	TOTAL	20335	254602
		Aquaculture	TOTAL	3234	49864
			Overall TOTAL	23569	304466

*All data sourced from ABARE Australian Fisheries Statistics 2005. Data on the emerging prawn aquaculture industries in the Northern Territory and Western Australian were not quoted in ABARE Australian Fisheries Statistics 2005.
a excludes hatchery production. p preliminary data. s estimates.

2.1.2 Australia's trade in prawns and prawn products

Table 2.2 shows tonnage and dollar value of Australian prawn imports and exports, and domestic production between 2001 and 2005.

Exports

The majority of Australian prawn exports (both in value and weight) from 2001 to 2005 have been to Japan, followed by China. Most of the prawns are exported whole and unprocessed. The majority of Kuruma prawns produced in Australia (approximately 150 tonnes per year) are sold live on the Japanese market. Live Kuruma prawns are a high value export, typically sold for $55 per kg, with a total value of $8.5 million per year (Love and Langenkamp 2003).

Imports

The majority of prawn imports into Australia between 2001 and 2005 originated from Thailand, Vietnam, China and India. Most of the imported prawns fall into the category of fresh, frozen or chilled product, followed by a smaller percentage of canned prawns and other further processed prawn products. AQIS AIMS data indicate that approximately half of all prawns imported into Australia over the last five years have been uncooked.

Table 2.2 ABARE Commodity statistics for all prawn products 2001–2005*

	Australian production[i]		Total exports		Total imports	
Year	Volume (t)[a]	Value ($m)	Volume (t)[b]	Value ($m)	Volume (t)[b]	Value ($m)
2001—02	29400	428.7	11900	262.8	17600	221.4
2002—03	26300	360	9500	208.2	18100	220.2
2003—04	27200	355.3	9400	160.6	24400	227.6
2004—05[p]	23500	326	10300	163.1	29900	257.8

*All data sourced from ABARE Commodity Statistics 2005
a live weight. b product weight. i includes estimates of farmed and wild-caught prawns. p preliminary data.

Table 2.3 Prawn imports based on preparation category (raw versus cooked/highly processed) 2001-2005*

	2001		2002		2003		2004		2005	
Preparation Category	Weight (t)	%	Weight (t)	%	Weight (t)	%	Weight (t)	%	Weight (t)	%
Raw	6715	49.5	8270	55.6	11808	50.1	13940	50.9	13874	46.4
Processed	6850	50.5	6596	44.4	11775	49.9	13449	49.1	15997	53.6
Total	13565	100	14866	100	23583	100	27389	100	29871	100

*Data are based on prawn import statistics provided by AQIS-COMPILE system from 2001-2004 and 2005. Biosecurity Australia cannot guarantee the validity of this raw data as it was not involved in its production, and some anomalies were noted during the analysis.

2.2 Other crustacean fisheries

Table 2.4 shows tonnage and dollar value of Australia's non-prawn crustacean aquaculture and capture fisheries production.

Freshwater crayfish

Australia has over a hundred freshwater crayfish species, several of which are of commercial and/or environmental importance (Crandall et al. 1995). Three species are of particular commercial importance; namely, *Cherax quadricarinatus* (redclaw), *Cherax destructor* (yabby) and *Cherax tenuimanus* (marron)[11]. Redclaw is native to tropical Queensland and the Northern Territory. It occurs predominantly around the Gulf of Carpentaria and on Cape York Peninsula. The yabby is native to central and south-eastern Australia and is present in waterways over a large area of the Australian continent. There are three subspecies, being *C. destructor destructor*, *C. destructor albidus* and *C. destructor rotundus*. Marron is one of the largest freshwater crayfish in the world, and is native to the high-rainfall areas in the south-west of WA.

The vast majority of commercial Australian freshwater crayfish production that is sold for human consumption is derived from trapping wild animals from water storage dams (Lawrence 1998, Lawrence et al 2000). Aquaculture produces the remainder of the commercial harvest and small amounts of both wild and aquaculture product is sold for broodstock, restocking of recreational fisheries or for ornamental use in the aquarium trade (Lawrence 1998). Over half of Australia's yabby production is exported, mostly to markets in Europe and Asia; smaller export markets exist for both marron and redclaw (Love and Langenkamp 2003).

Cherax destructor is the only freshwater crayfish commercially fished on a large scale in Australia. Commercial fishing takes place in NSW, Victoria and South Australia, principally in the Murray-Darling River system and throughout north-west NSW (Kailola et al. 1993). Although there is no official combined production data for the freshwater crayfish capture fisheries, State catches are thought to be less than 20 tonnes per annum (NSW Fisheries, The Freshwater Yabby 2006[12]). In addition to the commercial yabby fisheries, broodstock of farmed freshwater crayfish species are sourced from the wild.

Catches of *C. destructor albidus* in Western Australia from harvesting of farm dams produced 71 tonnes in 2004–05 with an estimated value to producers of $1 million. Production in non-drought years has been as high as 200 tonnes (WA Fisheries, State of the Fishery Report 2004–2005[13]).

Recreational fisheries exist for a variety of freshwater crayfish throughout their natural range, including gilgie (*Cherax quinquecarinatus*), koonac (*C. preissii*), marron (*C. tenuimanus*), redclaw (*C. quadricarinatus*) and yabbies (*C. destructor*). The recreational fishery in Western Australia was estimated to have produced approximately 14 tonnes of marron in 2004 (WA Fisheries, State of the Fishery Report 2004–2005[10]). Redclaw populations have been established in a number of dams throughout Queensland to enhance recreational fisheries (Lobegeiger 2003).

Marine lobsters

Many species of marine lobsters are found throughout Australian coastal waters. Species of

[11] The nomenclature of marron is currently under review by the International Committee for Zoological Nomenclature (case number 3269).

[12] Available at http://www.fisheries.nsw.gov.au

[13] Available at http://www.fish.wa.gov.au

commercial importance include southern rock lobster (*Jasus edwardsii*), the eastern rock lobster (*Jasus verreauxi*), the western rock lobster (*Panulirus cygnus*) and the ornate rock lobster (*Panulirus ornatus*). The southern rock lobster is found on coastal reefs from northern NSW, around Tasmania, across southern Australia to Dongara in Western Australia (Kailola et al. 1993). The eastern rock lobster inhabits the continental shelf along the east coast of Australia from northern NSW, through Bass Strait, around Tasmania and as far west as Port MacDonnell in South Australia (Kailola et al. 1993). The western rock lobster lives only in Western Australian waters, from Albany to the North West Cape and also off Geraldton (Kailola et al. 1993). Recreational fishing of western rock lobster is concentrated inshore (in depths of less than 20 metres) between North West Cape and Augusta. Ornate rock lobster, also known as tropical rock lobster, is normally found from the North West Cape through northern Australia to Sydney (Kailola et al. 1993). Other commercially important lobster species in Australia include the painted rock lobster (*Panulirus longipes*) in NSW and a number of slipper lobsters. The term slipper lobster encompasses a variety of species including species of *Ibacus*, *Thenus* and *Scyllaroides* — these species include Balmain, Moreton Bay, reef and mud bugs (Kailola et al. 1993). Marine lobsters also contribute to the stability of aquatic ecosystems in Australia's coastal waters.

The Fisheries Research and Development Corporation (FRDC) provides funding for the Rock Lobster Enhancement and Aquaculture Subprogram: Propagation of southern rock lobster (*J. edwardsii*) in Tasmania. The research program, based at the Marine Research Laboratories in Taroona, Tasmania, has had success in closing the life-cycle of the southern rock lobster; however, the production of commercial quantities of juveniles to supply aquaculture and restocking programs is yet to be realised.

Lobster production in Australia (Table 2.4) is dominated by the wild-caught sector. The western rock lobster makes up the bulk of the Australian lobster capture fishery and is landed in Western Australia. Most of the southern rock lobster catch is landed in South Australia and Tasmania. The Southern Rock Lobster Fishery in Victoria has a current total allowable commercial catch of 510 tonnes. NSW annually produces less than 1 tonne of southern rock lobster (Liggins et al. 2003). Despite its distribution, significant landings of eastern rock lobster are reported only from NSW. The State's total allowable commercial catch has been set at 102 tonnes for the 2005–06 season. Ornate rock lobster production is largely from the Commonwealth fisheries (Torres Strait Fishery), with 330 tonnes landed in 2001–02. Of the State fisheries, NSW landed less than 1 tonne of ornate rock lobster as by-catch of the eastern rock lobster fishery. No other States report commercial landings of this species (Liggins et al. 2003). Approximately 460 tonnes of slipper lobsters, including reef bugs, mud bugs and Balmain bugs, were landed in Queensland in 2000–01 (Sumpton and Williams 2002). The only other State to report slipper lobster catches is NSW, which produces about 50 tonnes per year of *Ibacus* spp. as by-catch from the State's Ocean Prawn Trawl Fishery.

About three-quarters of Australia's lobster production is exported (Table 2.5). In 2004–05, rock lobster continued to be the most valuable Australian fisheries export product, followed by pearls and abalone (Newton et al. 2006). Australia imported 469 tonnes of lobster[14] in 2004–05, valued at approximately $8.1 million (Table 2.5). Thailand, Papua New Guinea and the US were the major countries exporting to Australia.

Australian lobsters are taken by recreational fishers from their natural habitats with restrictions such as pot numbers and gear limits. Recreational lobster fishing is very popular in South Australia, Western Australia, Victoria and Tasmania; there are minor catches in NSW and Queensland (Henry and Lyle 2003). It has been projected that 900–1200 tonnes of lobster were caught in Western Australia in 2004–05 by the 40000 recreational rock lobster fishing licence holders. In Tasmania, 15500 licence holders took an estimated 148 tonnes of

[14] Products include fresh, chilled or frozen lobster products including dried and salted, but excluding canned, extracts, pastes and others.

southern rock lobster in 2002–03 (Lyle et al. 2005). South Australian recreational fishers took 118 tonnes in 2000–01 and 10–20 tonnes are taken annually from Victoria.

Crabs

Crabs, as bottom-feeding carnivores or omnivores, play an important role in aquatic ecosystems. Many crabs are adept scavengers and will utilise any available food source (Kailola et al. 1993). Adults as well as juvenile crabs are a significant part of the diet of other predators such as rock cod, shark and octopus. Crocodiles, turtles and herons also prey on crabs (Kailola et al. 1993).

Crabs are found throughout Australia's freshwater and marine environments. The most important commercial species are the blue swimmer crab (*Portunus pelagicus*), the mud crab (*Scylla serrata*), the spanner crab (*Ranina ranina*) and the giant crab (*Pseudocarcinus gigas*).

The blue swimmer crab, also known as the sand crab, inhabits coastal and estuarine waters from Cape Naturaliste in Western Australia, around the north of Australia to the south coast of NSW. This species is also present around Lord Howe Island and in the warmer waters of South Australia's gulfs, as far south as Barker Inlet and Gulf St Vincent (Kailola et al. 1993, Sumpton and Williams 2002). The mud crab, or mangrove crab, inhabits tropical to warm temperate waters from Exmouth Gulf in Western Australia to the Bega River in NSW. They are present only in isolated populations south of Broome in Western Australia and Sydney in NSW, associated with pockets of mangrove habitat (Kailola et al. 1993). Spanner crabs live from Yeppoon in Queensland to Nowra in NSW along the east coast of Australia, and from Quinns Rocks north of Perth to the Houtman Abrolhos and Geraldton along the west coast (Kailola et al. 1993). The giant crab (giant southern crab or giant Tasmanian crab) is found across southern Australia, from the Perth in Western Australia, east to Tasmania and Victoria. The distribution of giant crabs is similar to southern rock lobsters. Giant crabs are most abundant on soft sediments in deeper water between 150 and 350m (DPI 2003).

Mud crab aquaculture in Australia is an emerging industry. The life-cycle of the mud crab has been closed and current research is focussed on developing the species commercially. Existing operational farms either focus solely on crab production, or produce crabs as a secondary crop in association with other industries (for example, prawn aquaculture). The soft shelled crab industry is also in the early stages of development in Australia — the industry relying on the harvesting of mainly wild blue swimmer crabs. The crabs are held until they undergo moulting, at which point they are harvested and frozen while the new shell is still soft.

As with lobster industries, the production of crabs in Australia is dominated by the wild-caught sector. The main mud crab fisheries in Australia are in the Northern Territory, Queensland, and NSW. The Northern Territory and Queensland commercial landings of mud crab are each approximately 1000 tonnes per year (DEH 2003, DEH 2004a). In 2000–01, NSW produced 128 tonnes, mostly from the Estuary General Fishery (NSW Fisheries 2003). Blue swimmer crabs are commercially landed in Queensland, Western Australia, South Australia and NSW. Queensland's combined mud and blue swimmer crab fishery statistics indicate that approximately 2500 tonnes per year were landed commercially in 2003 and 2004 — 1376 tonnes of blue swimmer crab in 2003 (DEH 2004b). The Western Australia catch is approximately 1000 tonnes per year (Bellchambers et al. 2005). The blue swimmer crab fishery in South Australia operates an approximate 600-tonne annual quota (Currie and Hooper 2006). The blue swimmer crab harvest in NSW is small with around 150 tonnes taken commercially in 2000–01 (DEH 2004b).

Approximately 2000 tonnes of spanner crabs are commercially fished each year with Queensland accounting for 85% and 15% for NSW (QDPIF 2005a). The Giant Crab Fishery is a limited-entry fishery based in western Victoria, Tasmania, South Australia and Western Australia. Tasmanian total allowable catch (TAC) is 62.1 tonnes, Victorian TAC, 25 tonnes, and South Australian TAC, 22.1 tonnes. The Western Australian Deep Sea Crab Fishery

landed 226 tonnes of crystal (snow) crabs (*Chaceon bicolor*) for 2004, with other deep sea crabs such as the giant crab and the champagne crab (*Hypothalassia acerba*) caught in very small quantities (Melville–Smith and Gould 2005). In general, Australia exports approximately one-quarter of its crab production volume (Table 2.5). The majority of these exports are to Taiwan, China, Hong Kong and Japan. Australia imports around 500 tonnes of crab products every year (Table 2.5), including canned product. About three-quarters of these imports originate in Vietnam (Newton et al. 2006).

There is a substantial recreational fishery for all crab species in the same areas as the commercial fishery, but concentrated in the more accessible inshore locations (Kailola et al. 1993). The national recreational and indigenous fishing survey estimated that 1085 tonnes and 816 tonnes per year of blue swimmer crabs and mud crabs, respectively, were taken by recreational fishers (Henry and Lyle 2003). Mud crabs are also believed to be a significant food source for coastal indigenous Australians (Hay and Souter 2004). The estimated annual mud crab harvests by recreational and indigenous fishers in the Northern Territory were about 66 and 69 tonnes, respectively (Hay and Souter 2004). In Queensland, the recreational mud crab harvest was estimated at approximately 1000 tonnes in 2002 (DEH 2004a). Recreational blue swimmer and sand crab fishing is a popular activity throughout Australia from south-eastern Queensland extending southwards to Western Australia. Baited line and scoop-net, baited witches hats, rakes and drop-nets are some of the more common methods used for catching crabs.

Table 2.4 Australian fisheries production of non-prawn crustaceans for 2004–2005*

Fishery Area		Production Type	Target Species	Volume (t)	Value ($'000)
Commonwealth fisheries		Wild-caught	Rock lobster	686	12297
			Crab	15	160
			Commonwealth TOTAL	701	12457
State fisheries	QLD	Wild-caught[s]	Lobster (mainly bugs)	600	6957
			Crabs	3574	23813
			TOTAL	4174	30770
		Aquaculture[se]	Redclaw	100	1400
			QLD TOTAL	4274	32170
	WA	Wild-caught[s]	Rock lobster	12303	264659
			Crab	1224	7451
			TOTAL	13527	272110
		Aquaculture[sae]	Yabby	73	1120
			Marron	55	1485
			TOTAL	128	2605
			WA TOTAL	13655	274715
	SA	Wild-caught[p]	Rock lobster	2343	66041
			Crab	780	4125
			TOTAL	3123	70166
		Aquaculture[pe]	Yabby	20	306
			Marron	22	587
			TOTAL	48	893
			SA TOTAL	3171	71059
	NSW	Wild-caught[p]	Rock lobster	99	3767
			Crab	411	4279
			TOTAL	510	8046
		Aquaculture[pe]	Yabby	23	362
			NSW TOTAL	533	8408
	VIC	Wild-caught[s]	Rock lobster	467	13697
			Giant crab	33	669
			TOTAL	500	14366
		Aquaculture[se]	Yabby	4	78
			VIC TOTAL	504	14444
	TAS	Wild-caught[s]	Rock lobster	1602	47630
			Giant crab	57	1600
			TAS TOTAL	1659	49230
	NT	Wild-caught[s]	Crab	437	4473
Australia wide		Wild-caught	TOTAL	24631	461618
		Aquaculture	TOTAL	303	5338
			Overall TOTAL	24934	466956

*All data sourced from ABARE Australian Fisheries Statistics 2005.
a aquaculture excludes algae production for betacarotene — some data not available due to confidentiality restrictions. e excludes hatchery production. p preliminary data. s estimates.

Live crustacean exports

Examination of AQIS Export Documentation (EXDOC) database revealed that in the thirteen months from April 2005 to May 2006, live exports of crustaceans including lobsters, crabs and crayfish totalled 6925 tonnes. Exports were dominated by lobster (89%), followed by crab (11%) and crayfish (0.4%). Live western rock lobster represented 67% of all lobster exports followed by southern rock lobster (31%), with the remaining species including eastern rock lobster and ornate rock lobster. Live crab exports include spanner crabs, giant crabs, mud crabs and crystal crabs, with spanner crabs representing 98% of total live crab exports. Two species of crayfish were exported live in small quantities (24.4 tonnes), with yabbies dominating sales (81%) over marron. Hong Kong, Taiwan, Japan and China purchased 99% of the total volume of live crustacean exports.

Table 2.5 ABARE commodity statistics for other non-prawn crustacean products 2001–2005*

Year	Species	Production		Total exports		Total imports	
		Volume (t)	Value ($m)	Volume (t)	Value ($m)	Volume (t)	Value ($m)
2001—02	Lobster	14300	501.8	10900	492.68	320	8.1
	Crabs	na	na	2300	29.9	450	4.1
2002—03	Lobster	16700	455.5	11500	463.1	480	11.1
	Crabs	na	na	1700	21.1	560	4.6
2003—04	Lobster	19300	402.2	13500	426.8	440	7.1
	Crabs	na	na	1600	17.5	540	3.9
2004—05	Lobster	17500	408.9	12600	439.6	470	8.1
	Crabs	na	na	1600	18.2	na	na

* Data sourced from ABARE Australian Fisheries Statistics 2005 (data for total export and import year 2003–04 are preliminary) and from ABARE Commodity Statistics 2005. All lobster production figures refer specifically to rock lobster. All production data includes capture fisheries and where relevant, aquaculture production.

2.3 Seafood trade in Australia

Seafood is a popular food type enjoyed by many Australians. Seafood in Australia is typically sold in wholesale markets, supermarkets and seafood retail outlets. Australians consume seafood both in their own home and increasingly whilst eating out (Ruello and Associates Pty Ltd 2002). Shark and flathead are the leading products by volume in-home, with cooked prawns the most popular in-home seafood product. Atlantic salmon and prawns are the preferred species for people eating out.

In the last 20 years, it has become apparent that many fisheries have reached, or in some cases have exceeded, their maximum sustainable yield. Kailola et al. (1993) foreshadowed little future expansion in Australia's fisheries catch, instead cautioning that a high proportion of fisheries were fully exploited. This stabilisation of global wild fisheries production has resulted in a rapid expansion in aquaculture to meet the increasing demand for seafood.

Although Australia produces considerable quantities of seafood, significant imports are required to satisfy domestic consumption (Kailo et al. 1993). Australia imported $1.2 billion worth of fisheries products in 2004–2005, comprising 82% of edible seafood — 40% of which comprised fish and 21% prawns ($201 million) (Newton et al. 2006). The major sources of edible seafood products by value were Thailand and New Zealand; 25% and 16%, respectively (Newton et al. 2006).

The popularity of seafood as a food product in the home and the increasing trend of seafood

consumption when eating out provides employment in production, processing and retail industries throughout Australia.

2.4 Australian crustaceans and the environment

Australia has a diverse marine, brackish water and freshwater crustacean fauna. Crustaceans occupy significant niches in freshwater and marine ecosystems in Australia. Therefore, reduction in the number of a particular crustacean from an ecosystem may cause significant changes in the ecosystem as a whole.

Of significance in this IRA, and specifically in relation to pathogenic agents that infect a wide range of hosts, are the Australian freshwater crayfish species that are threatened with extinction. Over one quarter of Australia's freshwater crayfish species are considered to be worthy of concern from a conservation viewpoint (Horwitz 1995). Twenty-three Australian Parastacidae species were listed as endangered, and 11 were listed as vulnerable in the IUCN (The World Conservation Union) Red List of Threatened Animals. The DEWHA list of *critically endangered*, *endangered* or *vulnerable* species include the following:

Critically Endangered	*Cherax tenuimanus*	hairy marron, Margaret River hairy marron, Margaret River marron
	Engaewa pseudoreducta	Margaret River burrowing crayfish
	Engaewa reducta	Dunsborough burrowing crayfish
Endangered	*Engaeus granulatus*	Central North burrowing crayfish
	Engaeus martigener	Furneaux burrowing crayfish
	Engaeus spinicaudatus	Scottsdale burrowing crayfish
Vulnerable	*Astacopsis gouldi*	Tasmanian giant freshwater lobster, giant lobster, giant freshwater crayfish
	Engaeus orramakunna	Mount Arthur burrowing crayfish

2.5 Prawn health in Australia

Australia has several Federal and State based facilities, and associated expertise, that are involved in the investigation, diagnosis and research of prawn disease and health. These facilities are in the main used to monitor disease episodes in farmed prawns. A number of both passive surveillance programs and periodic active surveillance programs operate throughout Australia with respect to aquatic animal diseases. Changes in Australia's aquatic animal health status detected through these surveillance programs are reported to the OIE and NACA by the Australian Chief Veterinary Officer in the Quarterly Aquatic Animal Disease Report.

Significant disease events affecting prawns in Australia are investigated. To date, such episodes have only been observed in experimental and aquaculture situations where high stocking density and sub-optimal environmental conditions may contribute to disease outbreaks, with close monitoring of farmed populations resulting in early recognition and diagnosis.

The following information on prawn health in Australia is based on:

- published scientific literature;
- reports provided by the Commonwealth and State/Territory government agencies, including official notifications to regional and international organisations; and
- published and unpublished material held by Commonwealth and State/Territory government agencies, universities, industry and research organisations.

Prawn diseases/pathogenic agents reported in Australia

Many pathogenic agents have been reported from Australian prawns. These include:

Bennettae baculovirus (BBV)
Gill-associated virus (GAV)[15]
Gut and nerve syndrome (GNS)
Hepatopancreatic parvovirus (HPV)
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
Lymphoid organ vacuolization virus (LOVV)
Lymphoid organ virus (LOV)
Lymphoidal parvo-like virus (LPV)
Mourilyan virus (MoV)
Parvo-like virus of *Marsupenaeus japonicus* (P-PJ)
Penaeid haemocytic rod-shaped virus (PHRV)
Plebejus baculovirus (PBV)
Spawner-isolated mortality virus (SMV)
Spherical baculovirosis (*Penaeus monodon*-type Baculovirus, MBV).

The mandatory reporting to authorities of the occurrence of specified ('listed') diseases, including diseases exotic to Australia, provides information to fulfil Australia's international reporting requirements. Within states and territories, legislative requirements associated with specific diseases of concern assist in the prevention and management of disease outbreaks. Such reporting information can be used in combination with official controls on the movement of live animals and, where appropriate, their products to establish disease-free areas within infected countries or zones.

Nationally reportable pathogenic agents

The following crustacean pathogenic agents are included in the National List of Reportable Diseases of Aquatic Animals, November 2008:

Crayfish plague (*Aphanomyces astaci*) (exotic)
Gill-associated virus (GAV)
Infectious hypodermal and haematopoietic necrosis virus (IHHNV) (exotic strains)
Infectious myonecrosis virus (IMNV) (exotic)
Spherical baculovirosis (*Penaeus monodon*-type Baculovirus, MBV)
Taura syndrome virus (TSV) (exotic)
Tetrahedral baculovirosis (*Baculovirus penaei*, BP) (exotic)
White spot disease virus (WSSV) (exotic)
Yellowhead virus (YHV) (exotic).

[15] GAV is the junior synonym of LOV.

Domestic restrictions on movement of prawns and prawn products

During the preparation of this document, the appropriate agency within each State and Territory Government was asked to provide information on prawn disease control zones and details of intra- or inter-state movement controls on prawns and prawn products within their jurisdiction. The following is a summary of their responses.

New South Wales

All postlarvae entering NSW must test negative for MBV prior to stocking into ponds. Similarly, postlarvae produced at NSW hatcheries which were derived from spawners imported from interstate must test negative for MBV prior to stocking into ponds.

Queensland

Under Queensland State legislation, it is an offence to unlawfully sell fisheries resources, or a product derived from fisheries resources, knowing the fisheries resources or product is infected with or contains a declared disease; to unlawfully leave fisheries resources, or a product derived from fisheries resources, in a place knowing the fisheries resources or product is infected with or contains a declared disease; or to unlawfully bring fisheries resources, or a product derived from fisheries resources, into the State knowing the fisheries resources or product is infected with or contains a declared disease. Diseases and pathogenic agents of concern on Queensland's Declared Disease List for prawns are BMNV, BP, IHHNV, TSV, WSSV, YHV and NHP.

Northern Territory

The *Fisheries Act 1988*, administered by the Northern Territory Department of Primary Industries and Fisheries prohibits the importation of live prawns into the Northern Territory unless under and in accordance with a permit. Strict protocols are in place for the issue of the permit. The movement of prawns from one designated zone within the Territory to another zone within the Territory is also controlled. Current controls apply to the sale of overseas imported crustaceans from being utilised as bait or fish food.

Western Australia

The importation of prawn postlarvae into the State is restricted. The power to restrict prawns entering the State is an instrument of the *Fish Resources Management Act 1994* and *Fish Resources Management Regulations 1995* — a person must not bring into the State, or a particular area of the State, a live fish not endemic to the State or to that area of the State other than in accordance with the written approval or written authority of the Executive Director of Fisheries WA. Any application to import prawn postlarvae is assessed on a case-by-case basis. Additionally, prawns are gazetted as "stock" under the *Stock Diseases (Regulations) Act 1968* and the *Enzootic Diseases Regulations 1970*[16]. The Biosecurity and Agricultural Management (BAM) bill currently before parliament is expected to replace current legislation concerning the importation and movement of animals in Western Australia. The IRA team is aware that under the BAM bill, it will be illegal to bring any unlisted organism into the State without a permit — live prawns will be unlisted organisms. Furthermore, prawns will be listed as a potential "carrier" of a prohibited organism (including all non-endemic disease agents on the nationally reportable list and including GAV).

South Australia

Under the Livestock Notice 2005 (Restrictions on Entry of Aquaculture Stock), aquaculture

[16] Gill-associated virus is listed as a notifiable stock disease in WA.

stock that do not have specific translocation protocols described in that notice can enter South Australia from interstate legally only under a Ministerial approval.

Tasmania

Importation of live prawns into Tasmania requires a general or special authority issued under the *Animal Health Act 1995*. These authorities may set conditions on the import. Some live prawns are allowed for ornamental aquaria and special authorities have been issued for live prawns for research purposes. There are no authorities issued for the import of prawns for commercial production, as there is no commercial prawn production in Tasmania. There are no internal restrictions on the movement of live or dead prawns within the State. Fresh and frozen prawns for human consumption may be imported.

Victoria

Entry into Victoria of live prawns for placing into water is regulated via the Victorian *Fisheries Act 1995*. 'Waters of Victoria' cannot be stocked without authority under this Act. Those wishing to relocate prawns to or within Victoria require authorisation from Fisheries Victoria. This is only an issue for aquaculture and has been applied for on several occasions, where authorisation has required that the prawns are certified disease free prior to being brought into the State. There is no regulation on food prawns.

There are currently no restrictions on prawns or prawn products entering Victoria under the *Livestock Disease Control Act 1994*.

Australian Capital Territory

No measures were reported.

3 Method

Under the World Organization for Animal Health (OIE) *Terrestrial Animal Health Code* (OIE 2005a) (*OIE Code*), *import risk analysis* for animals and animal products are based on the following procedures:

- hazard identification;
- risk assessment (incorporating: release assessment, exposure assessment, consequence assessment and risk estimation);
- risk management; and
- risk communication.

While hazard identification, risk assessment and risk management tend to occur consecutively within the context of a particular IRA, risk communication occurs in an ongoing and iterative manner throughout the process, and includes both formal and informal consultation with stakeholders. The release of this *IRA Report* forms part of the risk communication process.

The method adopted by Biosecurity Australia for performing import risk analysis conforms to that recommended by the OIE, as described in summary above. The methods for *hazard identification*, *risk assessment* (consisting of release assessment, exposure assessment, consequence assessment and risk estimation) and *risk management* are described below. Results of the hazard identification, including hazard refinement are detailed in Chapter 4. Individual disease risk assessments and risk estimates are reported separately, in Chapters 6 to 14. Proposals for risk management, for those pathogenic agents for which the risk estimate exceeds Australia's ALOP, are described in Chapter 15.

3.1 Hazard identification

Hazard identification is described in the *OIE Code* as a classification step that is undertaken to identify pathogenic agents, or clearly defined strains of pathogenic agents, that could be associated with the importation of a commodity.

The *OIE Code* states that, to be identified as a potential hazard, a pathogenic agent should comply with *all* the following criteria:

- The pathogenic agent should be 'appropriate' to the species to be imported, or from which the commodity is derived.
- The pathogenic agent may be present in the exporting country[17].
- The pathogenic agent could potentially produce adverse consequences in the importing country.
- The pathogenic agent should not be present in the importing country. If present, the pathogenic agent should be associated with a notifiable disease, or should be subject to control or eradication measures.

For this IRA, hazard identification was initiated by generating a comprehensive list of pathogenic agents likely to be relevant to the importation of uncooked prawns and prawn products for human consumption. The list includes those pathogenic agents associated with the diseases listed by the OIE and known to be carried by prawns, and any other diseases or pathogenic agents considered relevant to prawns. The list was subsequently refined by applying the four criteria stated above to each pathogenic agent. If reasons for the inclusion or

[17] The *OIE Code* also states that ' *... the evaluation of the veterinary services, surveillance and control programs and zoning and regionalisation systems are important inputs for assessing the likelihood of hazards being present in the animal population of the exporting country ...*'.

exclusion of particular pathogenic agents were not clear cut, these agents were retained on the list and were examined in the formal risk assessment.

3.2 Risk assessment

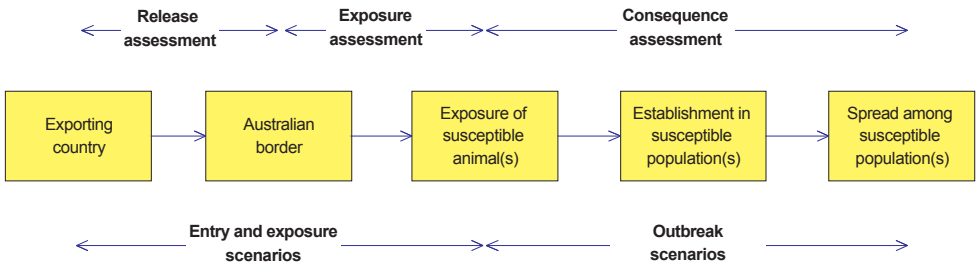
Risk assessment is defined in the OIE Code as:

> *... evaluation of the likelihood and the biological and economic consequences of entry, establishment, or spread of a pathogenic agent within the territory of an importing country.*

The likelihood that a pathogenic agent would enter an importing country, and the likelihood that susceptible animals would be exposed to that agent, were determined through a 'release assessment' and an 'exposure assessment', respectively. The 'likelihood of establishment or spread', and the 'biological and economic consequences of introducing a pathogenic agent', are determined through a 'consequence assessment'. The risk assessment for an identified agent concludes with 'risk estimation' — the combination of the likelihoods and consequences — and yields the 'unrestricted risk estimate'.

These steps are shown in Figure 3.1. Details of the components of the release, exposure and consequence assessments are illustrated in Figure 3.2.

Figure 3.1 Components of risk assessment



3.2.2 Evaluating and reporting likelihood

Likelihood estimations made in this assessment are based on information available in the scientific literature, unpublished data, as well as the expert judgment of IRA team members.

This assessment was conducted using a qualitative approach — the likelihood (or probability) that an event would occur was evaluated and reported using qualitative likelihood descriptors as described in the Biosecurity Australia *Guidelines for Import Risk Analysis*[18] (Table 3.1).

[18] Available at: http://www.biosecurityaustralia.gov.au/ — from Biosecurity Australia Documents follow links to 'Import risk analyses (IRAs)', 'Review of the IRA Process' and 'Draft Import Risk Analysis Guidelines'.

Table 3.1 Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Likelihoods were combined using the matrix of 'rules' for combining descriptive likelihoods shown in Table 3.2.

Table 3.2 Matrix of 'rules' for combining descriptive likelihoods

	High	Moderate	Low	V. low	E. low	Negligible
High	High	Moderate	Low	V. Low	E. Low	Negligible
Moderate		Low	Low	V. Low	E. Low	Negligible
Low			V. low	V. Low	E. Low	Negligible
V. low				E. Low	E. Low	Negligible
E. low					Negligible	Negligible
Negligible						Negligible

3.2.3 Risk assessment framework

An evaluation of disease risks resulting from non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption involved determining for each pathogenic agent of concern, the likelihood of a susceptible host crustacean in Australia becoming exposed to the pathogenic agent and the likely consequences of such exposure.

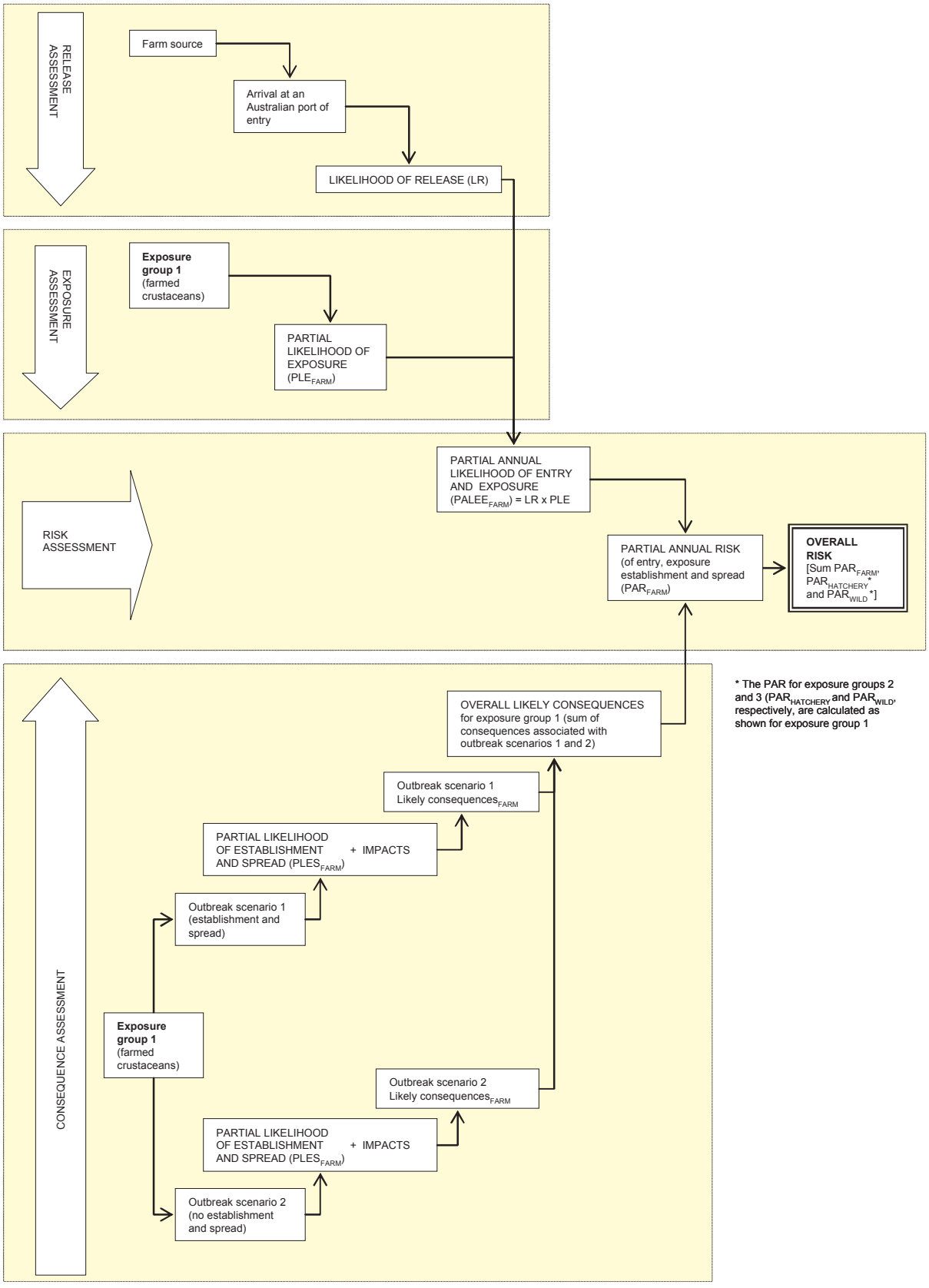
In evaluating the probability of a susceptible host animal in Australia becoming exposed to a pathogenic agent of concern, the following factors were considered for each agent:

- the likelihood of the agent entering Australia via the commodity being imported (release assessment); and
- the likelihood of a susceptible host crustacean (representing one or more exposure groups) becoming exposed to the agent (exposure assessment).

Determination of the 'likely consequences' (consequence assessment) required:

- identification of the potential scenarios (outbreak scenarios) that could follow the exposure of a susceptible host (ranging from no infection occurring to the agent establishing in a local population and spreading to its natural limits);
- determination of the likelihood of each outbreak scenario occurring; and
- evaluation of the impacts (economic, social and environmental) associated with each outbreak scenario.

Figure 3.2 Elements of the risk assessment



* The PAR for exposure groups 2 and 3 (PAR$_{HATCHERY}$ and PAR$_{WILD}$, respectively, are calculated as shown for exposure group 1

Estimating the likelihoods associated with release, exposure or outbreak scenarios involved examination of the various factors that influence the likelihood that a particular scenario would occur. For example, the ability of the pathogenic agent to remain infectious in frozen product is a key factor in determining the likelihood of the agent entering Australia in a shipment of prawns, i.e. in the release assessment. Evaluation of these factors therefore, formed the basis of the overall likelihood assigned to each of the release and exposure assessments and each outbreak scenario.

This risk assessment looked at the likelihood of entry and exposure of a pathogenic agent over a period of a year. As such, the release and exposure assessments for each agent were based on expected annual volume of trade in the commodity.

3.2.4 Release assessment

The release assessment considered a single release scenario — the importation into Australia of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption.

The final outcome of the release assessment was the annual likelihood of release (LR) of the pathogenic agent into Australia.

3.2.5 Exposure assessment

The exposure assessment considered the key distribution pathways and end-uses that could lead to each of three exposure groups coming into contact with each pathogenic agent of concern. The three exposure groups were:

- farmed crustaceans;
- hatchery crustaceans; and
- wild crustaceans.

The hatchery crustacean exposure group is considered to include crustaceans in research facilities and public aquaria, as well as crustacean hatchery broodstock.

For each pathogenic agent of concern, the final outcome of the exposure assessment was an estimation of the partial likelihood of exposure (PLE) for each exposure group (PLE_{FARM}, $PLE_{HATCHERY}$ and PLE_{WILD}). The PLE represents the likelihood that a domestic population of a susceptible host species is exposed to a pathogenic agent of concern through contact with imported prawns (or associated wastes) that are infected or contaminated. Estimation of PLE took into consideration, *inter alia*, the relative volumes of potentially infected or contaminated prawns (or associated wastes) likely to be directed toward each exposure group.

The next consideration following exposure of local crustacean species to any potentially contaminated material, i.e. the likelihood that an agent might establish in a local crustacean population (including the index case of infection), was dealt with in the consequence assessment (see section 3.2.7).

3.2.6 Calculation of the partial annual likelihood of entry and exposure (PALEE)

The partial annual likelihood of entry and exposure (PALEE) is the exposure group-specific likelihood that there would be one or more host exposure events over a period of one year. This likelihood was determined for each of the three exposure groups ($PALEE_{FARM}$, $PALEE_{HATCHERY}$ and $PALEE_{WILD}$).

The PALEE for each exposure group was calculated by determining the product of the likelihood of release (LR) and the corresponding partial likelihood of exposure (PLE) using

the matrix of 'rules' for combining descriptive likelihoods (Table 3.2).

For example, for the hatchery crustacean exposure group:

$PALEE_{HATCHERY} = LR \times PLE_{HATCHERY}$

Release and exposure likelihood estimations were based on the likelihood of the event occurring over a one-year period. This was considered a sufficient period to enable evaluation of seasonal effects, but not so long as to incorporate effects that may be associated with significant changes in disease factors, host factors or factors associated with trade.

3.2.7 Consequence assessment

According to the *OIE Code*, a consequence assessment should 'describe the potential consequences of a given exposure, and estimate the probability of them occurring'.

Consequence assessment describes the process which was used to analyse the likelihood and impacts of establishment or spread of disease for each of the identified pathogenic agents of concern.

Plausible 'outbreak scenarios' were considered for each identified exposure group. The likelihood of each outbreak scenario occurring was estimated, based on species and management or behaviour of each exposure group, and the characteristics of the pathogenic agent. The impact for each outbreak scenario was also estimated.

The likelihood of establishment or spread associated with each outbreak scenario was then combined with the corresponding estimation of impacts to determine the 'likely consequences' of exposure.

Steps in assessing the 'likely consequences' associated with entry and exposure for each pathogenic agent of concern were:

- Identification of the major outbreak scenarios that might occur as a result of host exposure to the pathogenic agent of concern.
- Determination of the likelihood of each outbreak scenario occurring — to obtain a partial likelihood of establishment or spread (PLES) for each outbreak scenario.
- Determination of the nature and magnitude of adverse effects (economic, social and environmental) for each outbreak scenario.
- Combining the likelihood of occurrence of each outbreak scenario with the corresponding estimation of adverse effects (impacts) to obtain an estimate of the 'likely consequences' associated with each outbreak scenario.
- Combining these outbreak scenario-specific consequences to obtain an overall estimation of 'likely consequences' resulting from the exposure of one or more susceptible host crustaceans in Australia, for each of the three exposure groups.

Identification of outbreak scenarios

For each pathogenic agent of concern, the consequence assessment determined the likelihood of occurrence and the associated impact for each of two complementary outbreak scenarios:

Outbreak scenario 1	the agent establishes and spreads to wild and farmed populations of susceptible species in Australia — it is assumed that if an agent were to establish in a local population it would eventually spread to its natural geographical limits.
Outbreak scenario 2	the agent does not establish — an index case may occur and infection may spread to co-habiting animals, but the agent does not persist sufficiently long to be detected.

Likelihood associated with outbreak scenarios

When determining the likelihood associated with each of the two outbreak scenarios, qualitative descriptors such as *negligible*, *low*, *moderate* etc, were used as detailed previously.

Adverse (economic, social and environmental) impacts

The potential impacts of an 'exposure' may be direct or indirect and may occur over an extended period. Consideration of impacts is not limited to what might occur during one year, but covers a period as long as impacts are discernable. Adverse impacts are evaluated in terms of seven (two direct and five indirect) impact criteria, as follows.

Direct impacts are those on:

- the life or health (including production effects) of production, domestic or feral animals[19]; and
- the environment, including life and health of native wild animals and direct effects on the non-living environment[20].

Indirect impacts are those on:

- new or modified eradication, control, surveillance or monitoring and compensation strategies or programs;
- domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries;
- international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand;
- indirect effects on the natural environment, including biodiversity, endangered species, and the integrity of ecosystems; and
- indirect effects on communities, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

Consideration of *the indirect impacts on the environment* includes harm arising from the impact of the pathogenic agent itself, as well as from any treatments or procedures used to control it. The extent of harm was evaluated taking into account:

- all on-site and off-site impacts;
- the geographical scope and magnitude of the impact;
- the frequency and duration of the action causing the harm;
- the total impact which can be attributed to that action over the entire geographic area affected, and over time (i.e. cumulative impact);
- reversibility of the impact; the sensitivity of the receiving environment (recognised environmental features of high sensitivity); and
- the degree of confidence with which the impacts of the action are known and understood.

The direct and indirect impacts described above collectively cover the economic, environmental and social effects of a disease — the so-called 'triple bottom line'. In assessing direct and indirect impacts, it was important to ensure that particular impacts were not accounted for more than once. In particular, the direct impacts of a disease on native, non-commercial, wild populations were assessed under the criterion describing the

[19] Impacts on crustacean aquaculture industries and wild capture fisheries were considered under this direct criterion.

[20] Aside from farmed salmonids and ornamental fish, all aquatic animal species commercially farmed in Australia are native to Australia.

'environment, including the life or health of native animals', whereas the indirect or 'flow-on' effects on the environment were assessed under the last two indirect criteria.

Describing impacts

Estimating the overall impact associated with an outbreak scenario involved a 2-step process where first, a qualitative descriptor of the impact of a pest or disease was assigned to each of the identified direct and indirect criteria in terms of the *level of impact* and the *magnitude of impact*. The second step involved combining the impacts for each of the seven criteria to obtain an overall impact estimation.

Step 1: Assessing direct and indirect impacts

Each direct and indirect impact was estimated at four levels — national, State or Territory, district or regional, and local — and the values derived subsequently translated into a single qualitative score (A to G). In this context, the terms 'national', 'State or Territory', 'regional' and 'local', were defined as follows:

National	Australia-wide
State/ Territory	an Australian 'State' (NSW, Victoria, Queensland, Tasmania, South Australia or Western *Territory* Australia) or 'Territory' (the ACT, the Northern Territory, the Australian Antarctic Territory and other Australian Territories covered under the Quarantine Act)[21].
District/ Region	a geographically or geopolitically associated collection of aggregates — generally a recognised section of a State or Territory, such as the 'North West Slopes and Plains' or 'Far North Queensland'.
Local	an aggregate of households or enterprises — e.g. a rural community, a town or a local government area.

At each level, the magnitude of impact was described as 'unlikely to be discernible', of 'minor significance', 'significant' or 'highly significant':

- An 'unlikely to be discernible' impact is not usually distinguishable from normal day-to-day variation in the criterion.
- An impact of 'minor significance' is recognisable, but minor and reversible.
- A 'significant' impact is serious and substantive, but reversible and unlikely to disturb either economic viability or the intrinsic value of the criterion.
- A 'highly significant' impact is extremely serious and irreversible and likely to disturb either economic viability or the intrinsic value of the criterion.

When assessing impacts, the frame of reference was the impact of each disease agent on the community as a whole, rather than on the directly affected parties. A related consideration is the persistence of an effect. In general, the consequences were considered greater if the effect is prolonged, as would be the case if the agent was expected to persist for several production cycles or if restocking following eradication programs was expected to take several generations. If an effect is not prolonged, consequences are likely to be less serious.

Step 2: Combining direct and indirect impacts

To estimate the overall impacts of a disease outbreak on a national scale, it was necessary to combine the effects of the direct and indirect impacts on the national economy or the Australian community. The impacts were combined by first translating each individual direct

[21] This excludes the Cocos (Keeling) Islands.

or indirect impact to an overall score (A–G) using the schema outlined in Table 3.3. This was done by determining which of the shaded cells with bold font in the table corresponded to the level and magnitude of the particular impact. At each of the lower geographic levels, an impact more serious than 'minor' was understood to be discernible at the level above (e.g. a 'significant' impact at the State/Territory level would be considered to be equivalent to at least a 'minor' impact at national level). In addition, the impact of a disease at a given level in more than one State/Territory, district/region or local area was considered to represent at least the same magnitude of impact at the next highest geographic level.

Once the appropriate shaded cell had been selected, the appropriate overall score for the outbreak scenario was assessed by reading the alphabetic (A–G) score from Table 3.3, starting at the national level and working down until the highest applicable combination of level and magnitude was reached. It is important to note that 'impact' at the national level is a different issue from 'spread of disease'. A disease may have serious consequences at the national level, despite only occurring in a small area.

Table 3.3 Assessment of direct or indirect impacts on a national scale

National Impact Score	national	State or Territory	district or region	local
G	**Highly significant**			
F	**Significant**			
E	**Minor** ←	*Greater than 'minor' at State level equals at least 'minor' at National level*		
D	Unlikely to be discernible	**Minor** ←	*Greater than 'minor' at district/region level equals at least 'minor' at State level*	
C	-	Unlikely to be discernible	**Minor** ←	*Greater than 'minor' at Local level equals at least 'minor' at district/region level*
B	-	-	Unlikely to be discernible	**Minor**
A	-	-	-	**Unlikely to be discernible**

Geographical Level

[1]Shaded cells with bold font are those that dictate national impact scores. Impacts greater than 'minor' at local, district/regional or State/Territory level are considered to represent at least 'minor' impacts at the next higher geographic level.

The measure of impact (A-G) obtained for each direct and indirect criterion was combined to give the overall impacts of a pathogenic agent. The following rules were used for the combination of direct and indirect impacts.

These rules are mutually exclusive, and should be addressed in the order that they appear in the list. For example, if the first set of conditions does not apply, the second set should be considered. If the second set does not apply, the third set should be considered, and so forth until one of the rules applies.

1. Where the impact of a disease with respect to any direct or indirect criterion is G, the overall impact is '*extreme*'
2. Where the impact of a disease with respect to more than one criterion is F, the overall impact is '*extreme*'
3. Where the impact of a disease with respect to a single criterion is F and the impact with respect to each remaining criterion is E, the overall impact is '*extreme*'
4. Where the impact of a disease with respect to a single criterion is F and the impact with respect to remaining criteria is not unanimously E, the overall impact is '*high*'
5. Where the impact of a disease with respect to all criteria is E, the overall impact is '*high*'
6. Where the impact of a disease with respect to one or more criteria is E, the overall impact is '*moderate*'
7. Where the impact of a disease with respect to all criteria is D, the overall impact is '*moderate*'
8. Where the impact of a disease with respect to one or more criteria is D, the overall impact is '*low*'
9. Where the impact of a disease with respect to all criteria is C, the overall impact is '*low*'
10. Where the impact of a disease with respect to one or more criteria is C, the overall impact is '*very low*'
11. Where the impact of a disease with respect to all criteria is B, the overall impact is '*very low*'
12. Where the impact of a disease with respect to one or more criteria is B, the overall impact is '*negligible*'
13. Where the impact of a disease with respect to all criteria is A, the overall impact is '*negligible*'.

Combination of the partial likelihood of occurrence of each potential outbreak scenario with the estimated adverse impacts

The overall impact associated with each outbreak scenario was combined with the likelihood that the scenario would occur using the matrix in Table 3.4, so that a scenario-specific measure of 'likely consequences' was derived for each outbreak scenario.

Table 3.4 Matrix for estimating the 'likely consequences' for each outbreak scenario

Likelihood of establishment and/or spread	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
V. Low	Negligible	Negligible	Negligible	Very low	Low	Moderate
E. Low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

Consequences of establishment or spread

Combination of outbreak scenario-specific consequences

Because the 'likely consequences' associated with each of the outbreak scenarios were derived qualitatively, they cannot be 'summed' in the usual sense. Instead, a system of eleven rules has been developed to provide a conservative approximation. These rules are mutually exclusive, and should be addressed in the order that they appear in the list. For example, if the first set of conditions does not apply, the second set should be considered. If the second set does not apply, the third set should be considered, and so forth until one of the rules applies.

1. Where the 'likely consequences' for any outbreak scenario are '*extreme*', the overall 'likely consequences' are also considered to be '*extreme*'.
2. Where the 'likely consequences' for more than one outbreak scenario are '*high*', the overall 'likely consequences' are considered to be '*extreme*'.
3. Where the 'likely consequences' for a single outbreak scenario are '*high*' and the 'likely consequences' for each remaining scenario are '*moderate*', the overall 'likely consequences' are considered to be '*extreme*'.
4. Where the 'likely consequences' for a single outbreak scenario are '*high*' and the 'likely consequences' for remaining criteria are not unanimously '*moderate*', the overall 'likely consequences' are considered to be '*high*'.
5. Where the 'likely consequences' for all outbreak scenarios are '*moderate*', the overall 'likely consequences' are considered to be '*high*'.
6. Where the 'likely consequences' for one or more outbreak scenarios are '*moderate*', the overall 'likely consequences' are considered to be '*moderate*'.
7. Where the 'likely consequences' for all outbreak scenarios are '*low*', the overall 'likely consequences' are considered to be '*moderate*'.
8. Where the 'likely consequences' for one or more outbreak scenarios are '*low*', the overall 'likely consequences' are considered to be '*low*'.
9. Where the 'likely consequences' for all outbreak scenarios are '*very low*', the overall 'likely consequences' are considered to be '*low*'.
10. Where the 'likely consequences' for one or more outbreak scenarios '*very low*', the overall 'likely consequences' are considered to be '*very low*'.
11. Where the 'likely consequences' for all outbreak scenarios are '*negligible*', the overall 'likely consequences' are considered to be '*negligible*'.

The result of the complete process was an estimate of the 'likely consequences' associated with the introduction of a pathogenic agent of concern into Australia.

3.2.8 Risk estimation

'Risk estimation' is the integration of 'likelihood of entry and exposure' and 'likely consequences' to derive the overall risk associated with introduction, establishment or spread of a pathogenic agent of concern from importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption.

Risk estimation was undertaken in two stages:

- estimation of the partial annual risk (of entry, exposure, establishment or spread) for each of the three exposure groups; and
- combination of these three partial annual risks to give an estimate of 'overall annual risk'.

Estimation of partial annual risks

The annual risk associated with each *exposure group* was obtained by:

- determining the 'partial annual likelihood of entry and exposure' (PALEE) associated with each of the three exposure groups, and
- combining the PALEE with the estimate of 'likely consequences' obtained from the consequence assessment.

This was termed the partial annual risk (PAR).

Combination of likelihood and consequences was undertaken using the 'rules' shown in the risk estimation matrix in Table 3.5 below. The principle underlying this matrix is that the cells are expressed in the units, and represent the 'expected loss' associated with a particular combination of likelihood and consequences.

It was assumed that likelihoods greater than or equal to Biosecurity Australia's definition of 'moderate' were not sufficiently small to reduce consequences *within the limits of measurement*. This means that two rows of the matrix corresponding to 'high' and 'moderate' likelihoods of entry and exposure are the same as the consequence scale on the horizontal axis. The remaining levels of probability (i.e. 'low', 'very low', 'extremely low' and 'negligible') reduced the consequences by one, two, three and four categories, respectively, or to 'negligible'.

Table 3.5 Risk estimation matrix: estimation of the partial annual risk of exposure

Likelihood of entry and exposure	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequences of entry and exposure

Estimation of overall annual risk

The partial annual risk (of entry, establishment or spread) (PAR) obtained for each of the three exposure groups were combined to give an overall estimate of annual risk. This was undertaken using the 11 rules outlined below.

These rules are mutually exclusive, and should be addressed in the order that they appear in the list. For example, if the first set of conditions does not apply, the second set should be considered. If the second set does not apply, the third set should be considered, and so forth until one of the rules applies:

1. Where any one partial annual risk is *extreme*, the overall annual risk is also considered *extreme*
2. Where more than one partial annual risk is *high*, the overall annual risk is considered *extreme*
3. Where any one partial annual risk high and each remaining partial annual risk is *moderate*, the overall annual risk is considered *extreme*
4. Where a single partial annual risk is high and the remaining partial annual risks are not unanimously *moderate*, the overall annual risk is considered *high*
5. Where all partial annual risks are *moderate*, the overall annual risk is considered *high*
6. Where one or more partial annual risks are *moderate*, the overall annual risk is considered *moderate*
7. Where all partial annual risks are *low*, the overall annual risk is considered *moderate*
8. Where one or more partial annual risks are considered *low,* the overall annual risk is considered *low*
9. Where all partial annual risks are *very low*, the overall annual risk is considered *low*
10. Where one or more partial annual risks are *very low*, the overall annual risk is considered *very low*
11. Where all partial annual risks are *negligible*, the overall annual risk is considered *negligible*.

The result of this process was an estimate of the annual risk of introducing a pathogenic agent of concern into Australia as a result of importing non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. This was considered the final output of the risk assessment. The key steps in calculating the overall annual disease risks are summarised in Figure 3.2 and Table 3.6.

Table 3.6 Calculation of overall annual risk

Criterion	Calculation / description
Release and exposure assessment	
Likelihood of release (LR)	Likelihood of *release*
Partial likelihood of exposure ($PLE_{EXPOSURE\ GROUP}$)	Partial likelihood of *exposure* for each exposure group
Partial likelihood of entry and exposure ($PALEE_{EXPOSURE\ GROUP}$)	Partial annual likelihood of *entry and exposure* — using: $PALEE_{EXPOSURE\ GROUP} = LR \times PLE_{EXPOSURE\ GROUP}$
Consequences assessment	
Partial likelihood of establishment or spread (PLES)	Partial likelihood of *establishment or spread* associated with each *outbreak scenario*
Impacts	Consequences of establishment or spread — outbreak scenario-specific impact (economic, social and environmental) of establishment or spread
'Likely consequences'	Combining the PLES with the estimated impact (economic, social and environmental) impact for each outbreak scenario using the matrix shown in Table 3.4 to obtain 'likely consequences' for each outbreak scenario. For each exposure group, the 'likely consequences' for each outbreak scenario were combined using the decision tool shown on page 37 to obtain an overall likely consequence rating
Risk assessment	
Partial annual risk (PAR)	Partial annual risk of entry, exposure, establishment or spread associated with each exposure group — combining the PALEE with the overall 'likely consequences' rating for each exposure group using matrix in Table 3.5
Annual risk	Annual risk of entry, establishment or spread — "summing" of PARs for each exposure group using the decision tool shown on page 39 to obtain an overall annual risk of entry, establishment or spread for all exposure groups, combined

3.3 Risk management

Risk evaluation is described in the *OIE Code* as the process of comparing the estimated risk with a country's appropriate level of protection (ALOP). ALOP was defined previously in this document as '… *the level of protection deemed appropriate by the WTO member country establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory* …'.

Australia has traditionally maintained a 'very conservative' attitude to quarantine risk. Given this, a risk that was either 'very low' or 'negligible', was considered sufficiently conservative to achieve Australia's ALOP. Australia's ALOP is shown in the risk estimation matrix (Table 3.5) as the band of cells associated with 'very low' risk. This provides a benchmark for evaluating risk and determining whether risk management is required.

The use of a benchmark for evaluating risk is illustrated in the process outlined below:

- for each potential hazard, the level of risk, or expected loss, associated with the unrestricted importation of uncooked prawns and prawn products was estimated;
- the unrestricted risk was then evaluated using the risk estimation matrix (Table 3.5) to determine where it fell in relation to Australia's ALOP;
- if the unrestricted risk was 'negligible' or 'very low', then it was considered acceptable and further risk management was not required;
- if the unrestricted risk was 'low', 'moderate', 'high' or 'extreme', then risk management strategies were identified and, for each pathogenic agent of concern, the risk was recalculated; and
- where the subsequently restricted risk derived using a particular risk management strategy was 'very low' or 'negligible', that strategy was considered acceptable.

3.3.1 Biosecurity measures

Biosecurity measures considered in this report are aimed at reducing the likelihood that the importation of prawns from any country would lead to the entry, exposure, establishment or spread of exotic pathogenic agents in Australia. There are two means by which this may be achieved:

- reducing the likelihood of pathogenic agents entering Australia in imported commodities by imposing conditions that would reduce the likelihood of release (LR) — i.e. 'pre-import measures'; and
- reducing the likelihood that susceptible host species in Australia would be exposed to the pathogenic agent in a contaminated imported commodity, or in other products or waste derived from that commodity, by imposing conditions that would reduce one or more of the partial likelihoods of exposure (PLE) — i.e. 'post-import measures'.

4 Hazard identification

The list of potential pathogenic agents (hazards) outlined below was compiled based on the diseases listed by the OIE, and a list of the causative agents for other diseases considered as being of importance with respect to the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption.

The method of hazard identification and refinement was previously described in Chapter 3 (section 3.1). The preliminary index of agents/diseases, and subsequent hazard refinement, is shown in Table 4.1.

There have been some changes to the base hazard list as presented in the 2000 *Draft IRA Report.* Infectious myonecrosis virus (IMNV), monodon slow growth syndrome (MSGS), Mourilyan virus (MoV) and white tail disease (WTD) have been included in the hazard identification list — they were not considered in the 2000 draft. The 2000 draft also considered several microsporidian species separately. This revised draft considered microsporidians as a group due to their similar biology. Finally, *Caledoniella montrouzieri* and *Enteromorpha* spp. are no longer listed for the following reasons.

The mollusc*, Caledoniella montrouzieri,* affects stomatopods, including *Gonodactylus viridis* and *Pseudosquillana richeri* (mantis shrimp). It is not considered to cause significant disease (Meyers 1990). This agent has been removed from the hazard identification table as prawn species are not susceptible to *C. montrouzieri*.

The algal *Enteromorpha* spp. are typically associated with various sessile and motile substrates, including the hulls of sea craft. They may also be incidentally associated with prawn species. With a worldwide distribution, *Enteromorpha* species are known to be present in Australia (Owens et al. 1988), and are not the subject of official control programs. The IRA team considered the likelihood of viable *Enteromorpha* species entering Australia with imports of non-viable prawns and prawn products to be rare.

Where the conclusions from the hazard refinement process for a particular agent/disease represents a substantial change from its listing in the 2000 *Draft IRA Report*, explanatory notes have been provided in Section 4.1.

Table 4.1 Hazard Identification

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Viruses[25]						
Aqua-birnaviruses (including infectious pancreatic necrosis virus, IPNV)	Farmed and wild finfish species. Also isolated from molluscs	Australia: exotic/present Worldwide: Americas, Asia and Europe	No	Yes (IPNV only) / No	No	An aquatic birnavirus was isolated from fish off Tasmania (Crane et al. 1999) — it is not considered to be IPNV. No longer retained for risk assessment — refer to explanation in section 4.1.
Bacilliform virus of *Crangon crangon*	*Crangon crangon*	Australia: not reported Worldwide: Scotland	No	No	No	Not considered to cause significant disease
Baculovirus midgut gland necrosis virus (BMNV) and BMNV-like viral infections	Various penaeid species	Australia: exotic Worldwide: Japan and Korea (BMNV); East and South-East Asia (BMNV-like viral infections)	No	No	No	No longer retained for risk assessment — refer to explanation in section 4.1.

[22] Entries in this column focus on the range of penaeid and caridean prawn species that may be affected by the agent/disease listed.

[23] The distributions listed refer only to species detected in penaeid and caridean prawns, as included in the scope of this IRA.

[24] Comments or information that is not specifically referenced is based on the knowledge or views of the IRA team.

[25] For many of the viruses affecting prawn species, the precise relationship between a named virus and its various geographical isolates may remain unclear. The IRA team drew on available scientific information in determining whether any known variations in geographical isolates were sufficient to warrant their consideration as separate strains of the named virus.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Baculovirus penaei (BP)	Various penaeid species	Australia: exotic Worldwide: Americas and Hawaii	Yes	Yes	Yes	BP has been associated with significant disease in overseas countries when general farming practices (such as washing eggs or nauplii in clean seawater) are not implemented. Although BP is readily controllable it will be retained for risk assessment due to its impact on hatchery production overseas and its international significance with respect to OIE listing. This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.
Bay of Piran shrimp virus	*Palaemon elegans*	Australia: exotic Worldwide: Mediterranean Sea	No	No	No	Not considered to cause significant disease.
Gill-associated virus (GAV)	*Penaeus monodon* (natural infections) and various penaeid species (experimentally)	Australia: present Worldwide: not reported	No	Uncertain	No	Yellowhead virus (genotype 1) is one of six known genotypes in the yellowhead complex of viruses and is the only known agent of yellowhead disease. GAV is designated as genotype 2. The 2008 OIE Code lists only yellowhead disease, ie genotype 1. GAV is no longer OIE-listed.
Hepatopancreatic parvovirus (HPV) (includes HPV-like viruses and *Fenneropenaeus chinensis* parvovirus)	Various penaeid species, *Macrobrachium rosenbergii*	Australia: endemic strain present, other strains exotic Worldwide: Asia, Africa, Americas, Middle-East	No	Yes	Yes	Exotic strains of this pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Various penaeid species	Australia: present Worldwide: Americas, Asia, Africa, Australia	Yes	Yes	No	A strain of IHHNV closely related to the Indian Ocean strain is endemic in Australia. More pathogenic exotic strains were considered exotic and considered a potential hazard in the 2006 Revised draft IRA report. In 2008 Australia notified the detection of a strain very similar to those strains found in Asia. As a result IHHNV is no longer considered a potential hazard.
Infectious myonecrosis virus (IMNV)	*Litopenaeus vannamei*	Australia: exotic Worldwide: north-eastern Brazil Indonesia, Thailand, China	Yes	Yes	Yes	IMN is an emerging disease in Brazil Indonesia, Thailand and China. IMNV is listed as a disease notifiable to the OIE. Although IMNV is restricted in its known host range, IMN has been responsible for considerable losses in the Brazilian prawn farming industry. The IRA team considered there is sufficient information to further assess this disease. This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.
Irido-like virus	*Protrachypene precipua* (penaeid)	Australia: exotic Worldwide: Ecuador	No	No	No	Not considered to cause significant disease.
Lymphoid organ vacuolization virus (LOVV)	*Litopenaeus vannamei* and *Litopenaeus stylirostris*	Australia: exotic Worldwide: Americas and Hawaii	No	No	No	Not considered to cause significant disease.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Lymphoid organ virus (LOV)	*Penaeus monodon*	Australia: present Worldwide: not reported	No	No	No	Work by Cowley et al. (2000a) indicates that LOV is a non-pathogenic variant of GAV. LOV is not exotic and not subject to an official control program.
Lymphoidal parvo-like virus (LPV)	Various penaeid species	Australia: reported Worldwide: not reported	No	No	No	Not exotic and not subject to an official control program.
Monodon baculovirus (MBV) (includes *Plebejus* Baculovirus and *Bennettae* Baculovirus)	Various penaeid species	Australia: endemic strains present (Lester et al. 1987a, Spann and Lester 1996, Vickers et al. 2000); other strains exotic Worldwide: Most areas of the Indo-Pacific region, including Asia and the Middle East	Yes	No	No	The relationship between endemic and exotic strains of MBV remains unclear. MBV will not be retained for risk assessment as it is readily controllable and because exotic strains and endemic strains cannot be differentiated based on the available information.
Monodon Slow Growth Syndrome (MSGS) Aetiology is unknown, but viral agent suspected – the putative agent has been termed monodon slow growth agent (MSGA)	*Penaeus monodon*	Australia: exotic Worldwide: Thailand, India, Malaysia, Indonesia and Vietnam	No	Yes	Yes	The IRA team recognised that although there is limited information regarding MSGS, the syndrome is considered a serious emerging disease in *P. monodon* aquaculture in Thailand and appears to be spreading throughout Asia — large volumes of imported prawns are sourced from countries that may be affected by MSGS. The IRA team considered that there is insufficient information on which to base a risk assessment.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Mourilyan virus (MoV)	Various penaeid species including *Penaeus monodon* and *Marsupenaeus japonicus*	Australia: present Worldwide: not officially reported	No	Uncertain	No	Mortalities may be seen in MoV-infected prawns with concurrent infections of GAV and/or SMV. As such, the IRA team considered it difficult to determine which, if any, of the viruses present are specifically associated with disease. Not exotic and not subject to an official control program.
Penaeid haemocytic rod-shaped virus (PHRV)	Hybrid *Penaeus esculentus* x *Penaeus monodon*	Australia: present Worldwide: not reported	No	No	No	Not exotic and not subject to an official control program.
Reo-III and IV (including Reo-like virus and Palaemon B-cell reo-like virus)	Various penaeid species	Australia: not reported Worldwide: parts of Asia, Europe and the Americas	No	No	No	Not considered to cause significant disease.
Rhabdovirus of penaeid shrimp (RPS)	*Litopenaeus stylirostris* and *Litopenaeus vannamei*	Australia: exotic Worldwide: Hawaii and Ecuador	No	No	No	No longer retained for risk assessment — refer to explanation in section 4.1.
Spawner-isolated mortality virus (SMV)	*Penaeus monodon*	Australia: reported Worldwide: not reported	No	Uncertain	No	Mortalities may be seen in SMV-infected prawns with concurrent infections of gill-associated virus (GAV) and/or Mourilyan virus (MoV). As such, the IRA team considered it difficult to determine which, if any, of the viruses present are specifically associated with disease. SMV is not exotic and not subject to an official control program.
Taura syndrome virus (TSV)	Various penaeid species	Australia: exotic Worldwide: Americas, Hawaii, parts of Asia	Yes	Yes	Yes	This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
White spot syndrome virus (WSSV)	Various decapod crustaceans	Australia: exotic Worldwide: Asia, Americas and Middle-East	Yes	Yes	Yes	This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.
White tail disease (WTD) (*Mr*NV and XSV)	*Macrobrachium rosenbergii*	Australia: not reported Worldwide: parts of Asia and Central America	Yes	No	No	Not considered to cause significant disease. However, the IRA team recognised that this situation may be changing. The IRA team considered that there is insufficient information to undertake a risk assessment.
Yellowhead virus (YHV)	Various penaeid species *Parapenaeopsis stylifera, Euphasia superba, Acetes* species	Australia: exotic (see GAV) Worldwide: Asia and Pacific	Yes	Yes	Yes	Because YHV has been found in many commercially important wild and cultured species throughout the world at relatively high prevalence and are increasingly being associated with multiple infection and stunting (Chayaburakul et al. 2004, Flegel et al. 2004), the IRA team considered that there is sufficient evidence for further assessment of this disease. This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.
Rickettsia, Chlamydia, Mycoplasma						
Rickettsia-like organisms (RLOs) from *Macrobrachium rosenbergii,* penaeid and pandalid prawns (stained prawn disease)	Various penaeid and caridean species	Australia: some present Worldwide: South-East Asia, Mexico, Hawaii, Canada, Madagascar	No	Yes	Yes	Some RLOs have been reported in Australia. Some RLOs are not reported in Australia and have been associated with significant disease episodes in other countries. No longer retained for risk assessment — refer to explanation in section 4.1.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Necrotising hepatopancreatitis bacterium (NHPB) — Alpha proteobacterium species	Various penaeid species	Australia: exotic Worldwide: US and several countries in Central and South America, Indonesia, Thailand and (possibly) Eritrea.	No	Yes	Yes	This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment. Necrotising hepatopancreatitis is currently OIE listed as under study.
Mycoplasma species	Various aquatic animals, including penaeid species	Australia: present Worldwide: China	No	No	No	Not exotic and not subject to an official control program.
Chlamydia species	Various aquatic animals, including penaeid species	Australia: present (Owens and Hall–Mendelin 1989) Worldwide: Ecuador (Jimenez et al. 2001)	No	No	No	Not exotic and not subject to an official control program.
Planctomycete bacteria	*Penaeus monodon*	Australia: present Worldwide: not reported	No	No	No	Not exotic and not subject to an official control program.
Bacteria						
Aerococcus viridans var *homari* (gaffkemia)	Homarid lobsters, *Farfantepenaeus aztecus*, *Pandalus platyceros* and several crab species	Australia: exotic Worldwide: North America and Europe	No	Yes	No	*Aerococcus viridans* var *homari* was reported as an incidental finding in healthy *Farfantepenaeus aztecus* from the Gulf of Mexico (Liuzzo et al. 1965). It has also reportedly been experimentally transmitted to certain crab species (Bell and Hoskins 1966). No longer retained for risk assessment — refer to explanation in section 4.1.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Aeromonas species	Various aquatic animals, including penaeid and caridean species	Australia: generally present (note that *Aeromonas salmonicida* subsp. *salmonicida* is exotic) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Aquatic epicommensal bacteria (*Leucothrix mucor*, *Thiothrix* species, *Flavobacterium* species, *Cytophaga* species, *Leucothrix* species)	Various aquatic species, including penaeid and caridean species	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Diplococcus species	Various aquatic species, including penaeid species	Australia: present Worldwide: presumed to be widely distributed	No	No	No	A *Diplococcus*-like bacterium was isolated in Australia by Owens and Hall–Mendelin (1989). Not exotic and not subject to an official control program.
Flexibacter species	Various aquatic animals, including penaeid and caridean species	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Hepato-pancreatic brush border lysis (HBL) bacterium	*Palaemon elegans*	Australia: exotic Worldwide: Adriatic Sea	No	No	No	Not considered to cause significant disease.
Micrococcus species	Various species, including penaeids	Australia: present Worldwide: China, India, Pakistan, Singapore, Sri Lanka	No	No	No	Seen in association with microsporidial disease; also found as microflora in haemolymph of crabs. Not exotic and not subject to an official control program.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Mycobacterium species	Various aquatic animals, including penaeid and caridean species	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Pseudomonas species	Various aquatic animals, including penaeid and caridean species	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Vibrio penaeicida	*Marsupenaeus japonicus, Litopenaeus stylirostris*	Australia: exotic Worldwide: Japan, New Caledonia	No	Yes	Yes	This pathogenic agent complies with the criteria described in the method Section 3.1 Hazard Identification and will be retained for risk assessment.
Vibrio species	Various aquatic animals, including penaeid and caridean species	Australia: exotic/some species present (Oxley et al. 2002) Worldwide: widely distributed	No	No / Yes	No	Other than *V. penaeicida* (see above), *Vibrio* species affecting penaeid and caridean species are either not considered to cause significant disease, or are present in Australia and not subject to an official control program.
Fungi						
Achlya species	Various aquatic animals including freshwater and marine crustaceans	Australia: present (Humphrey 1995) Worldwide: widely distributed	No	No	No	Considered rare pathogens of marine crustaceans (Brock and Lightner 1990).
Atkinsiella dubia	Marine crustaceans	Australia: present Worldwide: US	No	No	No	Not exotic and not subject to an official control program.
Cladosporium species	Various species including octopus and penaeid species	Australia: present (Owens and Hall–Mendelin 1989) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Fusarium solani, *Fusarium* species (burn spot disease, black gill disease, fusariosis)	Finfish, decapod crustaceans, carp, and sea turtles	Australia: present (Humphrey 1995) Worldwide: United Kingdom, France, America, Mexico, Malaysia, Japan, Philippines	No	No	No	Not exotic and not subject to an official control program.
Lagenidium species (larval mycosis)	Various aquatic animals including marine crustaceans	Australia: present (Owens and Hall–Mendelin 1989) Worldwide: US, Central America, South America, Philippines	No	No	No	*Lagenidium* species have been associated with significant disease in overseas countries when general farming practices (such as washing eggs or nauplii in clean seawater) are not followed. Not exotic, not subject to an official control program and readily controllable.
Leptolegnia species (=*Leptolegniella* species)	Crustaceans	Australia: present in *Cherax* species (Humphrey 1995) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Leptomitus species	Various aquatic animals including freshwater and marine crustaceans	Australia: exotic Worldwide: United Kingdom, France, US, China, India	No	No	No	Not considered to be significant pathogens.
Pythium species	Various aquatic animals including freshwater and marine crustaceans	Australia: present (Owens et al. 1988) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Saprolegnia species	Various aquatic animals including freshwater and marine crustaceans	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Sirolpidium species (= *Haliphthoros* species) (Larval mycosis, Brown spot disease)	Various mollusc and crustacean species including penaeids	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Yeasts						
Several yeast species including *Candida sake, Debaryomyces hansenii, Metschnikowia bicuspidate, Metschnikowia pulcherrima*	*Macrobrachium rosenbergii*	Australia: not reported Worldwide: Taiwan	No	No	No	Not considered to cause significant disease.
Protozoa						
Apostome ciliates (*Ascophrys* species, S*ynophrya* species, *Gymnodinoides* species)	Penaeid species, other benthic decapods serve as hosts	Australia: present (Owens et al. 1988; Paynter 1989) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Bodo-like flagellates *Chrysidella* species	Octopods and decapods	Australia: exotic Worldwide: widely distributed	No	No	No	Not considered to be pathogenic (Humphrey 1995).

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Gregarines (including *Nematopsis* species, *Cephalolobus* species, *Paraophioidina* species)	Various mollusc and penaeid species (including *Fenneropenaeus merguiensis*)	Australia: present Worldwide: widely distributed	No	No	No	Specific taxonomic identification of gregarines remains difficult and is not undertaken in all published findings. The IRA team considered it inappropriate to list the geographical range of individual species. Although some gregarines have been reported in Australia (Owens 1986) their relationship to overseas species remains unclear. Further, gregarines are not considered to cause significant disease and will not be retained for risk assessment.
Haplosporidan species	Various penaeid species (including *Litopenaeus vannamei, Penaeus monodon* and *Farfantepenaeus duorarum)*	Australia: present Worldwide: Cuba, Nicaragua, Mexico, US, Indonesia, Philippines, Thailand	No	No	No	Haplosporidans have been identified in wild-caught *Metapenaeus endeavouri* and *Penaeus esculentus* in Western Australia (Jones 1998). Not considered to cause significant disease (Lightner 1996a).
Hematodinium-like species (spot prawn parasite)	*Pandalus platyceros* and *Pandalus borealis*	Australia: exotic Worldwide: Canada (British Columbia) and the US	No	No	No	The taxonomy of this agent remains uncertain. Latest reports refer to the organism as “spot prawn parasite”, a protistan pathogen of pandalid shrimp (Bower and Meyer 2002). No longer retained for risk assessment — refer to explanation in section 4.1.
Leptomonas species	Decapods	Australia: exotic Worldwide: US	No	No	No	Not considered to be pathogenic (Humphrey 1995, Lightner 1996a).
Microsporidian species (including *Ameson*, *Agmasoma*, *Pleistophora*, amongst others)	Various decapod crustaceans	Australia: present Worldwide: widely distributed; some species thought to have limited distribution	No	No	No	No longer retained for risk assessment — refer to explanation in section 4.1.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Parauronema species	Various marine molluscs and crustaceans including *Penaeus aztecus*	Australia: exotic Worldwide: US	No	No	No	No longer retained for risk assessment — refer to explanation in section 4.1.
Peritrichous and loricate ciliates (*Epistylis* species, *Vorticella* species, *Zoothamnium* species, *Lagenophrys* species, *Cothurnia* species)	Marine and freshwater crustacea including penaeid species, *Macrobranchium* species, *Jasus edwardsii* and *Callinectes sapidus*	Australia: present (Paynter 1989) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Rhabdostyla species, *Ciliophora* species, *Stylohedra* species	Marine and freshwater crustacea including *Penaeus* species, *Alpheus* species, and *Mesamphisopus capensis*	Australia: present Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Suctorian ciliates (*Ephalota* species, *Acineta* species, *Terebrospira* species)	Marine and freshwater crustacea including penaeid species, *Palaemon* species, and *Palaemonetes* species	Australia: exotic Worldwide: widely distributed	No	No	No	Not considered to be significant pathogens (Humphrey 1995).
Thalassomyces species	Decapods	Australia: exotic Worldwide: widely distributed	No	No	No	Not considered to be pathogenic (Humphrey 1995).

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Metazoa						
Anisarthus species	Caridean species including *Athanas* species	Australia: exotic Worldwide: Japan	No	No	No	Not considered to cause significant disease (Nakashima 1995).
Anisorbione species	Penaeid species	Australia: present (Paynter 1989) Worldwide: Philippines	No	No	No	Not exotic, not subject to an official control program and not considered to cause significant disease (Paynter 1989).
Ascarophis species	*Fenneropenaeus merguiensis*, *Homarus americanus* (American lobster)	Australia: present (Owens 1987) Worldwide: US, Barents Sea (Northern Scandinavia/Russia)	No	No	No	Not exotic and not subject to an official control program.
Austogathona species	*Macrobrachium* species	Australia: present (Brock 1983 cited in Paynter 1986) Worldwide: not reported	No	No	No	This parasite was referred to as *Augustothoma* species in the 2000 draft IRA report. Not exotic and not subject to an official control program.
Bopyrella species, *Bopyrinella albida*	Various crustacea (caridean species and palaemonid species) and mollusc species (including *Pinna* species)	Australia: present (Humphrey 1995) Worldwide: Japan	No	No	No	Not exotic and not subject to an official control program.
Bulbocephalus inglissi	*Fenneropenaeus merguiensis*	Australia: present (Owens 1987) Worldwide: Indo-west Pacific, west Africa	No	No	No	Not exotic and not subject to an official control program.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Cabirops orbionei	Hyperparasitic on various penaeid species (causing sterilisation of several bopyrid parasites)	Australia: present (Owens 1993) Worldwide: Indo-West Pacific, South Africa, Red Sea	No	No	No	Not exotic and not subject to an official control program.
Diceratocephala species	Decapod crustaceans	Australia: present (Langdon 1990) Worldwide: New Guinea	No	No	No	Not exotic and not subject to an official control program.
Epipenaeon species	Various penaeid species, primarily *Penaeus semisulcatus*	Australia: present (Owens and Glazebrook 1985) Worldwide: Indo-West Pacific, Turkey, Israel, Persian Gulf, Red Sea, Suez Canal, South Africa	No	No	No	Not exotic and not subject to an official control program.
Eutetrarhynchus ruficollis	Various penaeid species	Australia: present (Owens 1987) Worldwide: India, Tunisia	No	No	No	Not exotic and not subject to an official control program.
Hemiarthus species	Various *Pandalus* species, *Spirontocaris* species	Australia: exotic Worldwide: north-eastern US, Greenland, Japan	No	No	No	Not considered to cause significant disease (Nakashima 1995).
Ionella maculate	*Callianassa* species	Australia: exotic Worldwide: tropical Indo-Pacific, New Caledonia	No	No	No	Not considered to cause significant disease (Markham 1994).
Kronborgia caridicola	Various crustaceans including caridean species and ampeliscid amphipods	Australia: exotic Worldwide: Greenland	No	No	No	Not considered to cause significant disease (Meyers 1990).

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Metaphrixus species	Various palaemonid species	Australia: present (Humphrey 1995) Worldwide: New Caledonia	No	No	No	Not exotic and not subject to an official control program.
Microphallus breviacaeca	Various marine crustaceans	Australia: exotic Worldwide: south-eastern US	No	No	No	Not considered to cause significant disease (Meyers 1990).
Microphallus species	Various marine crustaceans including penaeid species	Australia: exotic Worldwide: south-eastern US	No	No	No	Not considered to cause significant disease.
Nectonema species	Various decapod crustaceans including caridean and brachyuran species	Australia: exotic Worldwide: North America (Eastern coast), North Norway, New Zealand	No	No	No	Not considered to cause significant disease (Meyers 1990).
Opecoeloides fimbriatis	Various marine crustaceans including penaeid species	Australia: exotic Worldwide: south-eastern US	No	No	No	Not considered to cause significant disease.
Opecoeloides variabilis	*Macrobranchium australiensis* and penaeid species including *Litopenaeus vannamei*	Australia: present (Owens 1987) Worldwide: south-eastern US, Mexican Pacific	No	No	No	Not exotic and not considered to cause significant disease.
Orbione halipori	Various penaeid species	Australia: present (Humphrey 1995) Worldwide: Indo-west Pacific	No	No	No	Not exotic and not subject to an official control program.
Parachristianella dimegacantha	Various penaeid species	Australia: exotic Worldwide: Gulf of Mexico	No	No	No	Not considered to cause significant disease.

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Parachristianella monomegacantha	Various bivalve mollusc and penaeid species	Australia: present (Owens 1987) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Parapenaeon species	Various penaeid species	Australia: present (Owens and Glazebrook 1985) Worldwide: Indo-west Pacific, Pakistan	No	No	No	Not exotic and not subject to an official control program.
Parapenaeonella lamellate	Various penaeid species	Australia: present (Owens and Glazebrook 1985) Worldwide: Hong Kong	No	No	No	Not exotic and not subject to an official control program.
Polypocephalus species	Various penaeid species including *Fenneropenaeus merguiensis*	Australia: present (Humphrey 1995, Owens and Glazebrook 1985, Owens 1987) Worldwide: widely distributed	No	No	No	Not exotic and not subject to an official control program.
Probopyrus species	Various palaemonid and caridean species	Australia: present (Glazebrook et al. 1986) Worldwide: US (Biscayne Bay, Florida), North America (Atlantic seaboard)	No	No	No	Not exotic and not subject to an official control program.
Prochristianella penaei	Various penaeid species and *Dasyatis Sabina* (Atlantic stingray)	Australia: present (Owens 1987) Worldwide: Gulf of Mexico	No	No	No	Not considered to cause significant disease (Meyers 1990), not exotic and not subject to an official control program.
Pseudophyllodistomum johnstoni	Freshwater finfish species and decapod crustaceans including *Macrobrachium* species	Australia: present (Humphrey 1995) Worldwide: Asia	No	No	No	It is probable that *P. johnstoni* is present in Asia— see Cribb (1987) for details. Not exotic and not considered to cause significant disease (Humphrey 1995).

Agent / Disease	Susceptible species[22]	Occurrence[23] (Australia and worldwide)	OIE-listed (Yes / No)	Expected to cause significant disease in Australia? (Yes / No)	Potential hazard (Yes /No)	Comments[24]
Rhadinorhynchids	*Fenneropenaeus merguiensis* and the cephalopod *Ommastrephes bartrami* (red flying squid)	Australia: present (Owens 1987) Worldwide: Northwest Pacific Ocean	No	No	No	Not exotic, not subject to an official control program and not considered to cause significant disease.
Sacculina species	Various brachyuran species	Australia: present (Langdon 1990) Worldwide: Turkish Mediterranean coast, Sweden, United Kingdom, Ireland, Japan, Taiwan	No	No	No	Not exotic and not subject to an official control program.
Sylon hippolytes	Caridean species (*Spirontocaris lilljeborgi, Pandalus platyceros*)	Australia: exotic Worldwide: Faroe Islands, Norway, Canada (British Columbia)	No	No	No	Not considered to cause significant disease (Meyers 1990).
Temnocephala carpentariae	*Macrobrachium* species and the mollusc *Pomacea canaliculata*	Australia: present (Humphrey 1995) Worldwide: Argentina	No	No	No	*T. carpentariae* has only been detected in Australia, however several other *Temnocephala* species have been detected in Argentina (Damborenea 1996). Not exotic and not subject to an official control program.
Tetrarhynchus rubromaculatus	*Penaeopsis* species	Australia: not reported Worldwide: Japan	No	No	No	Not considered to cause significant disease.
Thynnascaris species (= C*ontracaecum* species = *Hysterothylacium*)	Various penaeid prawns and caridean species	Australia: present (Owens 1987) Worldwide: US, Mexican Pacific, El Salvador, Canada (British Columbia), Barents Sea (Northern Scandinavia/Russia)	No	No	No	Not exotic and not subject to an official control program.

Conclusions

The following pathogenic agents were retained for risk assessment according to the method described in Section 3.1 Hazard Identification and on the basis of the information in Table 4.1.

OIE-listed pathogenic agents

- White spot disease – White spot syndrome virus (WSSV)
- Taura syndrome – Taura syndrome virus (TSV)
- Yellowhead disease – Yellowhead virus (YHV)
- Tetrahedral baculovirosis – *Baculovirus penaei* (BP)
- Infectious myonecrosis – Infectious myonecrosis virus (IMNV)

Other pathogenic agents

- *Vibrio* sp. infection – *Vibrio penaeicida*
- Hepatopancreatic parvovirus (HPV exotic strains)
- Necrotising hepatopancreatitis (NHP) – Necrotising hepatopancreatitis bacterium (NHPB)

The IRA team noted the emergence of monodon slow growth syndrome (MSGS) and white tail disease (WTD). Currently the cause of MSGS has not been determined. Similarly, the relationship between the two viruses associated with WTD and their role in pathogenicity remains unclear. Biosecurity Australia will continue to monitor developments in relation to the scientific knowledge and understanding of MSGS and WTD and review import requirements as appropriate.

4.1 Hazards no longer retained for risk assessment

The following hazards were retained for risk assessment in the 2000 draft IRA report, but are not considered for risk assessment in this report based on the information below.

Baculoviral midgut gland necrosis virus (BMNV)

BMNV, first reported in Japan in 1971, has in the past been associated with severe mortalities of up to 100% in larval and early postlarval stages of Kuruma prawns (*M. japonicus*) (Sano et al. 1981). BMNV has also been reported in Korea (Park 1992). BMNV is currently regarded as an unclassified virus by the International Committee on Taxonomy of Viruses (ICTV) (Murphy et al. 1995). Non-occluded, bacilliform, BMNV-like viruses have been reported in Australia (Lightner 1996a). The relationship between these viruses and BMNV is unclear.

Natural infections have only been reported from *M. japonicus*, although several other penaeid species are susceptible to experimental infection with BMNV (Momoyama and Sano 1996). Infection, but not disease, due to BMNV has also been reported in wild *M. japonicus* (Sano et al. 1981, Momoyama 1988, Momoyama and Sano 1989). Infection with BMNV (whether natural or experimental) has not been reported in non-penaeid species (Momoyama and Sano 1996).

Epidemics due to BMNV occurred in Japan almost every year from 1977 until early 1990s (Momoyama 1992). However, outbreaks have been less frequent since the introduction of control measures (such as the routine washing of eggs and nauplii in clean seawater) in the mid-1980s (Momoyama 1992). According to OIE reports, BMNV has not been observed in Japan since 1992, or in Korea since 2000 (OIE 2004).

BMNV is no longer of international significance in prawn health and is no longer OIE-listed. It is considered that BMNV-associated clinical disease in prawns would be rare and that significant disease would not result, as BMNV is readily controllable (for example, by the routine washing of eggs and nauplii in clean seawater). BMNV is not retained for risk assessment.

Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

In July 2008 Australia notified the detection of a strain very similar to those strains found in Asia. As a result IHHNV is no longer considered a potential hazard.

Infectious pancreatic necrosis virus (IPNV) and aquatic birnaviruses

Aquatic birnaviruses are commonly isolated from a wide range of aquatic animal species (Reno 1999). Infectious pancreatic necrosis (IPN) is an acute disease of juvenile salmonids caused by the aquatic birnavirus, IPNV, and is an OIE-listed disease (OIE 2009a).

Scientific literature often refers to aquatic birnaviruses as IPNV without evidence of their pathogenicity in salmonids. Many aquatic birnavirus isolates that are serologically related to IPNV are not pathogenic to salmonids and therefore should not be referred to as IPNV (Reno 1999). The term IPNV will only be used for virus isolates pathogenic to salmonids; prawn isolates of birnaviruses that are serologically related to IPNV, but that have not been shown to cause disease in salmonids, will be referred to as IPNV-related aquatic birnaviruses.

Despite the numerous reports of aquatic birnavirus infections in aquatic animal species, there have been only three reports of isolation from prawns (Bovo et al. 1984, Giorgetti 1989, Mortensen 1993).

An aquatic birnavirus was isolated from adult, laboratory-bred *M. japonicus* experiencing high mortality (Bovo et al. 1984). Serological testing determined that the virus was antigenically related to IPNV (Bovo et al. 1984); however, the pathogenicity of the virus to salmonids was not investigated. There was insufficient evidence to conclude that the isolated virus was responsible for the observed mortality in *M. japonicus*, or that the virus had caused any pathological effects in the prawns.

An IPNV-related aquatic birnavirus was isolated from healthy farmed spawner *M. japonicus* in Italy (Giorgetti 1989). Experimental bath infection using this virus isolate was conducted on postlarval *M. japonicus*. Mortalities were not observed, although some locomotory abnormalities and slight histological changes in the hepatopancreas compared to controls were noted. Attempts to detect the presence of the virus in the challenged postlarvae were unsuccessful (Giorgetti 1989).

Mortensen (1993) used an IPNV serotype[26] previously isolated from scallops (Mortensen et al. 1990) to contaminate scallops (*Pecten maximus*). Prawns (*Pandalus borealis*) were subsequently exposed to the virus either by the ingestion of the contaminated scallops or by ingestion of the faeces and pseudofaeces from contaminated scallops. The virus could be re-isolated from the visceral mass (which included the gastrointestinal tract) of experimental prawns, however, viral titres in prawns decreased dramatically after the contaminated feed source had been removed. The study did not demonstrate whether the experimental prawns were infected; recovered virus may have been passaging through the prawn gastrointestinal tract. Further experiments in the same study involved feeding IPNV-injected scallops to prawns (*Palaemon elegans* and *P. borealis*) and subsequently feeding exposed prawns to brown trout (*Salmo trutta*). The virus could be re-isolated from experimental prawns in these experiments (either whole prawns or visceral masses, including the gastrointestinal tract, were

[26] Mortensen (1993) concluded that the virus isolated from scallops was IPNV serotype N1. In a subsequent study by the same author and using the same isolate, the virus is referred to as both 'IPNV' and an 'IPNV-like aquatic birnavirus'.

sampled), but not from experimental fish.

In the same report, Mortensen (1993) refers briefly to an unpublished report of the isolation of an IPNV-related aquatic birnavirus from *P. elegans*. The prawns had most likely encountered the virus via ingestion of deteriorating turbot fry during an IPN epidemic in a commercial farm.

There have been no recent reports of the detection of IPNV-related aquatic birnavirus in prawns, which suggests that infection is rare. There is also no conclusive evidence that prawn associated IPNV-related aquatic birnaviruses cause disease in prawns or any other aquatic animals.

There is no conclusive evidence that prawns may be infected (even experimentally) with IPNV or IPNV-related aquatic birnaviruses. Whilst it may be hypothesised that prawns could act as mechanical vectors of the virus as suggested by the work of Mortensen (1993), the failure of the experimental feeding trials to infect brown trout suggests that this is unlikely. IPNV-related aquatic birnaviruses and IPNV are not retained for risk assessment.

Rhabdovirus of penaeid shrimp (RPS)

RPS was first isolated from *L. stylirostris* and *L. vannamei* collected from three different farms in Hawaii. RPS was isolated from both healthy prawns and those showing clinical signs of infectious hypodermal and haematopoietic necrosis (IHHN) (Lu et al. 1991). RPS has also been isolated from farmed *Penaeus* species originating in Ecuador (Nadala et al. 1992).

Experimental infection of *L. stylirostris* with RPS resulted in cumulative mortalities of approximately 12% (3/25), 20–21% (2/10 and 3/14) and 43–50% (6/14 and 5/10) by waterborne transmission (experimental animals 0.2 grams), *per os* and intramuscular injection (experimental animals 2.2–3.0 grams), respectively (Lu and Loh 1994). In contrast, older *L. stylirostris* (sub-adults, 5–6 grams; and adults 15–16 grams), experimentally infected with RPS by intramuscular injection, showed neither clinical signs of disease nor mortality (Nadala et al. 1992).

Results from serological studies have shown that RPS is related to, but serologically distinct from finfish rhabdoviruses (Lu et al. 1994), a significant pathogen of cyprinids listed by the OIE (OIE 2009a). Genetic studies, using the first isolate of RPS from Hawaii, examined the nucleotide sequence of the glycoprotein gene (1 of the 5 genes on the genome[27]) from both RPS and SVCV. Results showed that the nucleotide sequence of RPS is 99% identical to that of SVCV (Johnson et al. 1999). However, the pathogenicity of RPS for carp and other finfish has not been investigated.

No further isolations of RPS have been reported since that of Lu et al. (1991), and its role as a pathogen remains inconclusive. Despite the experimental work of Lu and Loh (1994), RPS is generally considered not to cause disease in prawns (Lightner 1996a) and there is no evidence that it causes disease in finfish or other aquatic animals. RPS will no longer be retained for risk assessment.

Rickettsia-like organisms (RLOs)

Some RLOs are not reported in Australia and have been associated with significant disease episodes in other countries. However, the IRA team considered that given the range and number of organism types covered by the term ‘RLO’, the general confusion in the taxonomy of RLOs and that NHPB (an RLO-like organism) was deemed to require risk assessment (see Table 4.1), the NHPB risk assessment would also cover other RLOs. Hence, a specific risk assessment of exotic RLOs as a group is not considered necessary.

[27] As reviewed by Ahne et al. (2002).

Aerococcus viridans var homari (gaffkemia)

Aerococcus viridans var. *homari* is the causative agent for gaffkemia, a disease that has caused significant losses to the live homarid lobster trade in North America and Europe (Stewart 1980). The disease affects marine lobsters (*Homarus* spp.) and some species of crabs, including *Carcinus* spp. and *Cancer* spp. (Brock and Lightner 1990).

Liuzzo et al. (1965) reported the isolation of *Gaffkya homari* (a previous name for *A. viridans* var *homari*) from healthy wild *F. aztecus* caught in the Gulf of Mexico. This isolation was considered an incidental finding as it was not associated with any clinical signs of disease.

Bell and Hoskins (1966) experimentally infected *Pandalus platyceros* with *G. homari* via intramuscular injection. Mortalities resulted and the bacterium was re-isolated from these prawns.

Based on the lack of reports, infection and disease in prawns due to this bacterium are considered to be rare events. *A. viridans* var *homari* will no longer be retained for risk assessment.

Hematodinium-like species

A *Hematodinium*-like[28] protozoan (now referred to as spot prawn parasite or SPP) was identified in wild populations of *P. platyceros* near British Columbia (south-west Canada) (Bower et al. 1994) and *P. platyceros* and *P. borealis* in Alaska in 1994 (Meyers et al. 1994). In Canada, animals with overt infection were an abnormal colour (orange-pink), lethargic and oozed milky fluid on removal of the cephalothorax (Bower et al. 1994). The parasite has not been reported from other species or locations, has now virtually disappeared from British Columbia and has not been reported from Alaska since its first reported detection in 1994 (Bower and Meyer 2002).

Experimental attempts to transmit the parasite between *P. platyceros* via inoculation, feeding and cohabitation were unsuccessful, suggesting that other host(s) may be involved, and/or that other developmental stages may occur in the life-cycle (Bower and Meyer 2002). It is also speculated that *Pandalus* species may be an abnormal host for the parasite (Bower and Meyer 2002).

Hematodinium species have been reported in a number of crab species in Australia, including *Portunus pelagicus*, *Scylla serrata* and *Trapezia areolate* (Shields 1992, Hudson unpublished work cited in Hudson et al. 1993, Hudson and Adlard 1994). However, there are no reports of significant disease in association with *Hematodinium* species in Australia.

After consideration of the parasite's limited distribution (both geographical and species) and the lack of recent reports of the parasite's occurrence, the IRA team concluded that SPP would rarely be associated with imports of non-viable prawns and prawn products. Further, after consideration of reports to date, and the failure of experimental transmission studies, the IRA team considered that SPP was not expected to cause significant disease in Australia. *Hematodinium*-like species will no longer be retained for risk assessment.

Microsporidia

Microsporidiosis, also known as milk or cotton shrimp disease, has been reported from prawn populations in parts of the Americas (Sprague 1950), Europe (Sprague 1970), Africa (ClotildeBa and Toguebaye 1994, Toubiana et al. 2004), Asia (Flegel et al. 1992a, Anderson et al. 1989) and Australia (Owens and Glazebrook 1988, Hudson et al. 1994). The taxonomy of Microsporidia is poorly understood, with species currently categorised by the size and

[28] More recent studies suggest that the parasite may be more closely related to the phylum Haplosporidia, however, its precise taxonomy remains unclear (Bower and Meyer 2002).

number of spores per sporont, and on the host species and organs affected. As molecular biological techniques advance, current taxonomical categorisations are likely to change significantly. To date, species reported from prawns include those from the genera *Ameson* (also known as *Nosema*) (Sprague 1950, Anderson 1989, Owens and Glazebrook 1988), *Thelohania* (also known as *Agmasoma)* (Sprague 1950, Owens and Glazebrook 1988, ClotildeBa and Toguebaye 1994)*, Pleistophora* (also known as *Plistophora*) (Sprague 1970), *Perezia* and *Tuzetia* (Canning et al. 2002). It remains taxonomically unclear which species exist in Australia and overseas.

Microsporidiosis has most often been reported at very low prevalence (typically less than 5%) from wild populations (Olson and Lannan 1984, Clotilde–Ba and Toguebaye 1994, Ramasamy et al. 2000, Clotilde–Ba and Toguebaye 2001, Canning et al. 2002), and has rarely been reported in prawn aquaculture populations (Anderson et al. 1989, Flegel et al. 1992a, Felix and Devaraj 1996, Hudson et al. 2001, Vidal–Martinez et al. 2002). Affected animals have opaque musculature and may be unmarketable (Sprague 1950), however, the condition has rarely been associated with significant disease (Lightner 1996a).

It has been suggested that the life-cycles of microsporidia affecting prawns may be indirect, as attempts at direct transmission (by the feeding of infected tissues, or spores, to prawns) have been unsuccessful (Roth and Iversen 1971 cited in Couch 1978); (Iversen and Kelly 1976, Breed and Olson 1977, Flegel et al. 1992a).

Given the current lack of taxonomical clarity, the probable indirect life-cycle of microsporidia (i.e. involving at least two host species life-cycle stages), their typically low prevalence in affected populations and, most importantly, their rare association with significant disease, microsporidia will no longer be retained for risk assessment.

Parauronema species

There has been one reported isolation of *Parauronema* species from prawns. In 1974, a scuticociliate identified as belonging to the genus *Parauronema* was detected during a mortality event involving *F. aztecus* in a commercial hatchery in the Gulf of Mexico (Bower et al. 1994, Couch 1978). *Parauronema* species were detected in the haemolymph and, in severe infections, the ciliates were numerous enough to fill the entire haemocoel and abdomen. Other pathogenic agents, including *Baculovirus penaei*, were associated with this mortality event. It was concluded that *Parauronema* species contributed to the pathogenesis and mass mortality only as an opportunistic invader (Couch 1978).

The scarcity of reports of *Parauronema* species in prawns suggests that infection and disease caused by the organism are rare and may be associated with co-infection. *Parauronema* species will no longer be retained for risk assessment.

5 General considerations in assessing risks

The following chapter provides details of the general considerations that were taken into account by the IRA team undertaking the risk assessments for the identified pathogenic agents in Chapters 6 to 14.

5.1 Release assessment

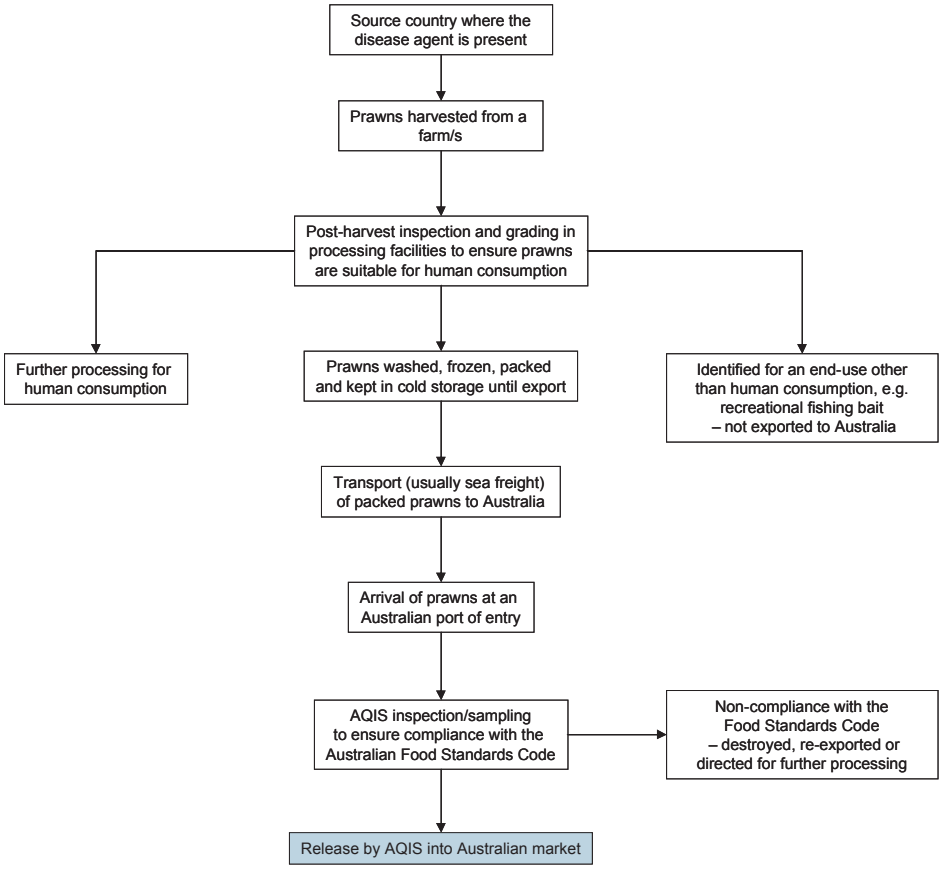
The release assessment component of the risk analysis determines the annual likelihood of release (LR) into Australia of each pathogenic agent of concern. In this assessment, consideration was given to a single release scenario, being importation into Australia from any country of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The key pathway steps that make up the release scenario, from sourcing prawns from farms in the exporting country, through to the point of release into Australia at a port of entry, are shown in Figure 5.1.

Almost all prawn products arrive in Australia in frozen form via sea container in large consignments (i.e. approximately 1500 cartons per consignment, with some consignments weighing up to 20 tonnes). AQIS officers responsible for inspecting and testing imported prawn consignments report that most of the product is individually quick frozen (IQF) and there is also a large proportion in the form of 2 kg frozen blocks.

In determining the likelihood of viable and infective pathogenic agents occurring in prawn products imported into Australia, the following key factors were considered relevant:

- The prevalence of the pathogenic agent in harvested prawns.
- The efficacy of post-harvest inspection and grading in detection and removal of infected or contaminated prawns.
- The capacity of the pathogenic agent to remain infectious through processing, storage and transport to Australia.

Figure 5.1 Release pathway



Prevalence of the pathogenic agent in harvested prawns

For most prawn pathogens there are few data on the epidemiology of disease in wild or farmed prawns, including on the prevalence of infection, the effect of infection on spawning, the probability of postlarvae being infected via infected spawners, or the likelihood that infected postlarvae will survive grow-out to harvest. Even for white spot syndrome virus (WSSV), the most-studied prawn pathogen, there are only limited data on these aspects and mostly in relation to farmed prawns.

The absence of a disease from a region can be an important consideration in risk assessment. However, this assessment is generic in nature, in that its scope includes importation of prawns from all countries. To accommodate this generic scope, the release assessment assumed that the pathogenic agents of concern are present in all source countries. Country or zone freedom from particular pathogenic agents of concern was considered in the context of potential risk management measures.

The prevalence (and expression) of infection in aquatic animal populations may vary markedly from one country or region to another. The significant effect of disease on prawn aquaculture, and the development of rapid and effective diagnostic tools, has led to the implementation of screening programs by some governments and companies. However, not all prawn-producing countries have established active surveillance programs for prawn disease. Passive surveillance systems can be effective in detecting disease and can support the implementation of response and control programs, although the lack of trained personnel and appropriate facilities limits the effectiveness of passive surveillance in some countries. General factors that affect the prevalence of disease in harvested prawns are considered below.

Species of prawn

Some pathogenic agents infect a wide range of species. For example, WSSV can infect many prawn and other crustacean species. Other pathogenic agents are generally more restricted in host range.

Life-cycle stage

The prevalence of infection and/or the expression of disease may vary with the life-cycle stage of the host. For example, the prawn baculoviruses primarily affect larvae and early stage postlarvae (Lightner et al. 1997a). Survivors of disease outbreaks may carry persistent subclinical infection.

Aquaculture system

The production system, husbandry techniques and health management employed can have a profound influence on the health status of prawns. Prawns produced in extensive systems with low stocking densities typically have a lower prevalence of disease, presumably due to less efficient transmission of pathogenic agents and greater resistance to infection as a result of lower stress levels; intensive culture systems require a much higher level of management to maintain productivity.

Local dispersal of pathogenic agent

The dispersal of pathogenic agents can occur via several pathways. In wild prawns, pathogenic agents are typically dispersed by the movement of live hosts, including during natural migration. The movement of infected broodstock to hatcheries, and of infected larvae from hatcheries to grow-out ponds, has also facilitated national and international spread of pathogenic agents.

Seasonality

Season, or time of year, can affect the prevalence of disease. For example, outbreaks of white spot disease (WSD) occur more frequently in the monsoon season, almost certainly due to stressors such as fluctuations in salinity, temperature and pH (Karunasagar et al. 1997, Limsuwan 1999).

Efficacy of post-harvest inspection and grading in detection and removal of infected or contaminated prawns

Prawns for human consumption are normally inspected by industry or government employees to verify that they are fit for human consumption. Inspectors conduct an organoleptic (touch, smell, visual) assessment, that allows abnormal prawns (e.g. those with visible lesions or physical damage) to be identified and rejected, or diverted for further processing. Prawns that are downgraded for aesthetic reasons may be further processed, often by cooking, to ensure consumer acceptance.

Prawns for human consumption are also graded according to quality factors such as size and appearance. Prawns are typically graded to fill a particular buyer's order of specified size, weight or count. Prawns with a loose, limp cephalothorax, discolouration or visible lesions are usually removed from the production line and discarded or directed for further processing, bait or pet food.

Prawn processing lines usually operate at high speed, allowing little time for detailed inspection. However, under normal commercial arrangements, inspection and grading decisions are made at multiple points along the processing line. Employees are trained to detect prawns that do not meet specified criteria, which are usually simple and clear-cut (e.g. no visible lesions and normal clean colour). Inspection and grading can provide for the removal of a majority of animals of abnormal appearance and thereby contribute to the reduction of quarantine risk.

Prawn processing plants in some countries are required by government to use quality

assurance (QA) or hazard analysis critical control point (HACCP) systems to ensure compliance with food quality and safety requirements. HACCP systems are based on the monitoring of key (critical control) points in the production process to verify that the system is operating within defined standards and that action is taken to detect and correct deficiencies, including in the management of 'failed' product. Such systems have largely replaced the traditional approach, which relied on inspection of the end-product for compliance with product safety and quality parameters. HACCP systems provide a structured approach to the control of key processes, such as operational hygiene and refrigeration that minimise potential problems with food safety and quality failures. HACCP systems emphasise early detection and prevention of undesirable practices (such as cross-contamination between cooked and raw product) that are important to food safety and that may also be relevant to quarantine risk.

Inspection agencies usually supervise the implementation of HACCP-based food processing systems by conducting periodic random audits, based on maintenance by the operator of complete and accurate records. If required, such systems may support trace-back of product or the provision of official attestations to meet importing country requirements.

Inspection and grading procedures typically focus on human health concerns and the aesthetics, or acceptability, of the commodity to the consumer. As such, they represent imperfect tests for addressing any quarantine risks associated with imported prawns. Not all infected animals would be removed as infection may not result in visible disease signs; and where obvious signs of clinical disease are present, not all such prawns would be detected and removed. Even those animals detected with lesions indicative of a pest or an infectious disease may not be rejected if the pest or disease is not of concern to human health and does not result in visible lesions that affect marketability.

Notwithstanding these limitations, inspection and grading can still contribute to the reduction of quarantine risk by removing many abnormal animals from the import chain.

As there is little pathogenic agent-specific information on the efficacy of inspection and grading for human consumption at removing infected prawns from a production line, the following risk assessments involved consideration of available scientific data on the likelihood that infected animals would show clinical signs and the likelihood that these clinical signs would result in the rejection of harvested prawns for human consumption.

Capacity of the pathogenic agent to remain infectious through processing, transport or storage

The factors relevant to the persistence of pathogenic agents through processing, transport and storage include those intrinsic to the agent (that allow it to persist in an infectious form) and the actual conditions of processing, storage and transport of prawns.

Prawns for human consumption are frequently packaged whole, after sorting, washing and freezing. It is also common for whole prawns to be cooked and then frozen (although this assessment assumes imported prawns are uncooked). Whether cooked or uncooked, rapid freezing is important to maintain quality and wholesomeness.

Washing

Although actual processing procedures can vary considerably, both within and between exporting countries, all prawns are expected to undergo washing in some form. Prawns are most commonly washed in a water bath, rather than in a pressurised washing system.

Washing would be expected to reduce the amount of organisms located on the shell. Further, HACCP procedures usually specify that water used in food-processing plants contain levels of residual chlorine that would contribute to the inactivation of any bacterial pathogens on the product. In most developed countries, human health authorities require the use of potable water in land-based food-processing plants, so that the water would usually contain a

minimum residual level of 0.2 to 0.5 mg/L of free chlorine. However, some prawn pathogens would be unaffected by this concentration of chlorine.

Washing may also facilitate contamination, i.e. the transfer of an agent within and between processing runs (or batches). The significance of such transfer will vary with the agent under consideration. For example, pathogenic agents for which the expected prevalence between and within batches is already high, the transfer of the agent in water baths is not likely to significantly alter any evaluations made.

Cold storage

The vast majority of whole, uncooked prawns imported into Australia are frozen. Frozen prawns intended for human consumption are normally transported at a temperature of less than –18°C, and may be held in frozen storage for many months (ADVS 1999).

Freezing will generally reduce the rate of inactivation of microorganisms (ADVS 1999). However, storage at freezing temperatures kills many food-borne pathogenic protozoa, cestodes and nematodes (Kim 1997). Most viruses are stable at freezing temperatures, but bacteria that are pathogenic or potentially pathogenic to aquatic species are inactivated to some degree by freezing (ADVS 1999).

Diagnostic and research laboratories commonly freeze prawn samples to ensure the preservation of viruses. Under laboratory conditions, maximum preservation of viral infectivity is achieved when samples are held at very low temperatures (–70°C or lower).

Multiplication during storage

In considering the effect of storage on microorganisms in or on food, it is important to note that viral, metazoan and most protozoal pathogens do not multiply in the tissues of a dead host.

Prawn tissues rapidly deteriorate if they suffer temperature abuse, developing volatile spoilage compounds that would render the prawns unacceptable for human consumption. The quarantine significance of bacterial replication in prawn products is unclear, as commensal organisms and environmental bacteria are likely to multiply much more rapidly, and would effectively overgrow any aquatic pathogens present in the tissues. Although some pathogenic agents, such as *Vibrio* spp., can multiply in dead prawn tissue under certain conditions, none of the pathogenic agents of concern identified in this analysis would be expected to multiply during normal processing, storage and transport of prawns for human consumption.

Post-arrival food inspection

Food sold in Australia (whether domestically produced or imported) must comply with the Australian Food Standards Code (FSC), developed by Food Standards Australia New Zealand[29] (FSANZ). The Imported Food Inspection Scheme has been developed to ensure that imported foods comply with the FSC. AQIS has operational responsibility for the inspection and sampling of imported foods under this scheme, which is conducted under the *Imported Food Control Act 1992*. AQIS officers sample imported foods at the border, assessing their ability to meet the requirements of the code. At present, FSANZ have categorised imported cooked prawns as *risk* foods and imported uncooked prawns as *random surveillance* foods, as defined in the *Imported Food Control Regulations*. *Risk* category foods are subject to "risk" and "random" tests, whilst *random surveillance* category foods are only subject to "random" tests.

[29] FSANZ is a bi-national, independent statutory authority that develops food standards for composition, labelling and contaminants (including microbiological limits) that apply to all foods produced or imported for sale in Australia and New Zealand. Further information on FSANZ is available on its website at www.foodstandards.gov.au

The products under these categories are tested as follows:

COOKED PRAWNS		UNCOOKED PRAWNS
"risk tests"	"random tests"	"random tests"
• Coagulase positive *Staphylococcus* • *Salmonella* spp. • *Vibrio cholerae* • Standard Plate Count	• Chloramphenicol (farmed prawns only) • Nitrofurans (farmed prawns only)	• Sulphur dioxide • Nitrofurans (farmed prawns only) • Chloramphenicol (farmed prawns only)

Briefly, 100% of *risk* categorised foods are referred to AQIS by the Australian Customs Service for inspection. Five percent of all consignments in the random surveillance category are referred to AQIS for inspection. These products are released upon sampling. Neither AQIS nor the importer has the ability to predict which shipment or which foods will be held for inspection.

Conclusions

Whether prawns harvested for export to Australia contain infectious pathogenic agents of concern will depend on many factors, including the species of prawn, the method of production, and the size of the prawn.

Prawns for human consumption are inspected and graded. Prawns with visible lesions and blemishes, including those resulting from infection, are likely to be rejected. Although prawns are inspected at a high speed, normal procedures include inspection at several points along the processing line and most prawns with detectable lesions or blemishes will be rejected. Infected prawns that are free of externally visible lesions or that have subtle lesions are likely to pass inspection and grading.

Processing, including washing, will also reduce risk through the physical removal of pathogenic agents on the surface of prawns, although there is a risk of cross-contamination. Prawns are usually stored and transported frozen. Once frozen, the amount of most pathogenic agents that might be present is relatively stable. However, freezing and thawing will reduce the number of most pathogenic agents present, depending on the agent and the physical conditions.

Post-arrival inspection by AQIS at the point of entry would be expected to reduce the risk associated with pathogenic agents that cause external disease signs likely to render the prawns unsuitable for human consumption on aesthetic grounds. AQIS inspection for purposes of implementing the FSC also represents an added layer of verification of the intended end-use of imported prawns as being for human consumption.

5.2 Exposure assessment

The exposure assessment determines, for each pathogenic agent of concern, the likelihood that a susceptible host crustacean population in Australia would be exposed to the agent via potentially infected imported prawns or associated wastes. The likelihood of subsequent establishment or spread of each pathogenic agent are considered in the consequence assessment (see section 5.3). All estimates of the likelihood of exposure in this risk analysis were based on an assumption that the agent would be present in the imported commodity at the time of arrival in Australia.

Prawns imported for human consumption may be sold to consumers, become waste or be diverted to other uses. Exposure pathways that are direct and that have a high probability of completion contribute substantially to the total likelihood of exposure occurring (e.g. the use of prawns as bait for recreational fishing). Some pathways may lead to the accumulation of biologically significant numbers of a pathogenic agent in the aquatic environment (e.g. commercial processing of imported prawns for human consumption) and thereby contribute significantly to risk.

Major exposure pathways

The following scenarios were considered to represent the most likely pathways that could lead to exposure of susceptible host animals in Australia (belonging to one of the three identified exposure groups[30]) to potentially infected non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption (or associated wastes) (see Figure 5.2):

- use of imported prawns as fresh feed for crustacean broodstock and as feed for crustaceans in research facilities and public aquaria;
- disposal of solids and liquid waste from commercial processing of imported prawns; and
- the use of imported prawns as bait for recreational fishing.

The majority of prawns imported for human consumption and purchased as seafood would be 'used' in one of three ways; namely, consumption by humans, disposal to a municipal garbage disposal system or diversion to use as bait or berley. Prawns purchased as seafood might be used, or discarded, in other ways such as the deliberate feeding of seabirds, the 'disposal' of uncooked prawn waste from picnics and other outdoor events to open areas where they might be accessible to scavengers such as seabirds, and direct use (whether deliberate or inadvertent) in aquaculture ponds. However, it was considered that a comparatively low volume of commodity would be used or discarded in this manner. These potential pathways were therefore incorporated into the evaluation of the pathway for prawns purchased as seafood but used as bait or berley.

Estimation of the likelihood of an exposure group encountering an infectious pathogenic agent via the above pathways took into account the following factors from the point of release into Australia by AQIS at a port of entry (i.e. following AQIS food inspection procedures), through storage, transport, end-use and any associated waste disposal:

- the amount of imported commodity or associated wastes that enters the general environment of the exposure groups;
- the amount of viable pathogenic agent in the commodity or associated wastes; and
- the amount of contact between susceptible host animals and the imported commodity or associated wastes.

Amount of imported commodity or associated wastes that enters the general environment of the exposure groups

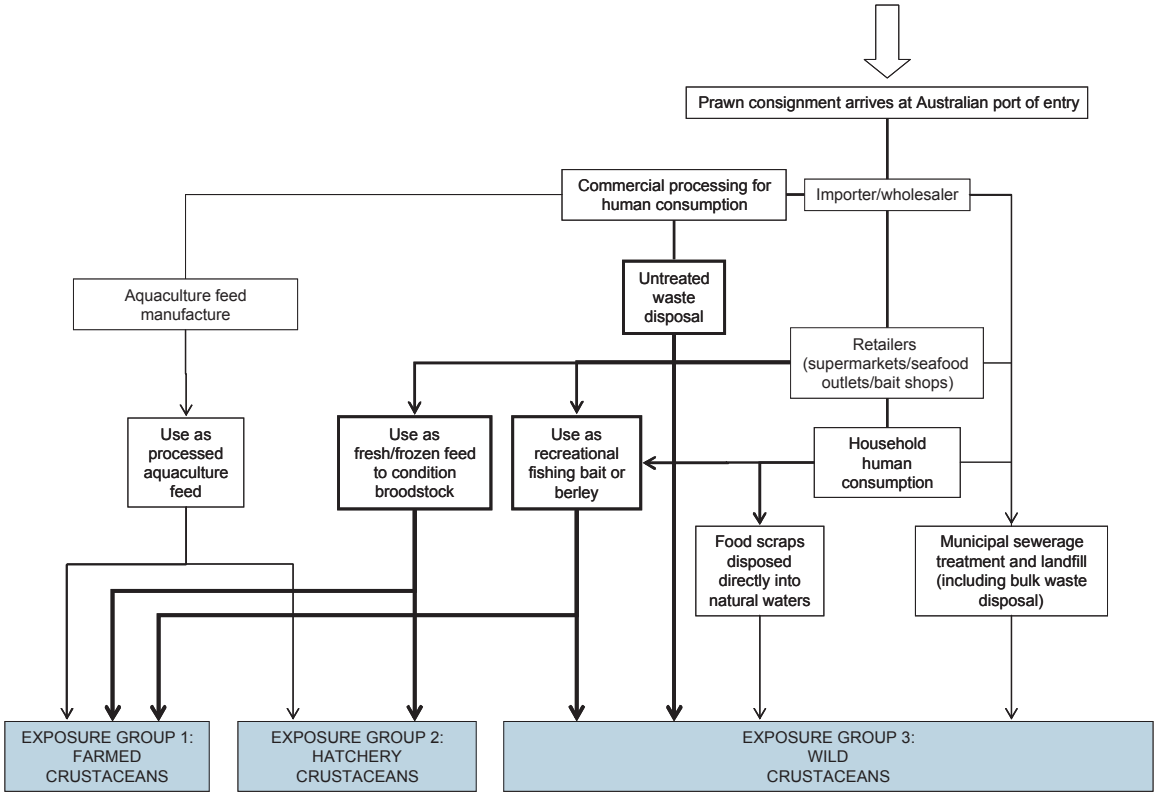
The use of imported prawns and disposal of associated wastes were considered in terms of the probability of their entry into the environment of each of the three exposure groups; namely, farm, hatchery and wild crustaceans (exposure groups 1, 2 and 3, respectively) including those species of importance to the Australian prawn aquaculture industry — *P. monodon*, *M. japonicus*, *P. esculentus*, *F. indicus* and *F. merguiensis*. There are many potential pathways by which the key exposure groups could become exposed to such products (and the

[30] Exposure groups 1, 2 and 3 are farmed crustaceans; crustaceans in hatcheries, research facilities and public aquaria; and wild crustaceans, respectively.

pathogenic agents might contain).

Those pathways that substantially contribute to the total risk, as depicted in Figure 5.2 are considered further in the exposure assessment.

Figure 5.2 Major exposure pathways



Exposure pathways considered to be potentially significant are highlighted in bold lines

Use of imported prawns as fresh feed for crustacean broodstock and as feed for crustaceans in research facilities and public aquaria

Imported prawns may be used as feed for live crustaceans, including to condition hatchery broodstock and as feed for aquatic animals kept in research facilities or public aquaria.

Uncooked prawns are known to form significant components of broodstock conditioning diets (Crocos et al. 1997, Wouters et al. 2001, Coman et al. 2007). Although this practice is no longer widespread due to disease risks (Wouters et al. 2001), imported uncooked prawns may still be being used as feed in crustacean hatcheries. Conditioning and feeding of crustaceans is not limited to the hatchery setting as fresh seafood is a primary dietary component for feed used in research facilities, teaching institutions and public aquaria throughout Australia. Uncooked frozen seafood is often used as feed to condition broodstock, with head-on prawns being the preferred form from a nutritional perspective. Evidence of this is further supported by studies that show prawn head meal (SHM) contains growth promoting factors (Fox et al. 1994, Sudaryono et al. 1995, Williams et al. 2005). In 2000, imported prawns were fed to broodstock in a Northern Territory crustacean hatchery, resulting in a national emergency animal disease response to the suspected establishment of WSSV in Australia. In that instance, the prawns, imported for human consumption, were considered to be of poor quality (based on smell), and were subsequently repackaged, unlabelled and diverted into the bait market — after which the prawns were purchased and used to feed hatchery broodstock. The broodstock from the 2000 Darwin incident were destroyed after the source of the feed prawns

was realised.

Although the volume of imported prawns used in these ways would be very small, it represents a direct and potentially significant pathway by which crustaceans in hatcheries, research facilities and public aquaria (exposure group 2) could become exposed to a pathogenic agent of concern.

There is also potential for whole prawns imported for human consumption to be used as feed for large adult prawns held in farm grow-out ponds until maturation. Such practices represent a significant pathway for the potential exposure of farmed prawns (exposure group 1).

There is no legislation in Australia that refers specifically to the use of *imported* seafood as bait or fish food, although holders of licences issued in accordance with Section 11 of the *Northern Territory Fisheries Act 1988* can have restrictions placed on the licence that prohibit the use of uncooked imported prawns as bait or as aquaculture feed. Under Queensland State legislation, it is an offence to unlawfully sell, leave or bring fisheries resources, or a product derived from fisheries resources, knowing the fisheries resources or product is infected with or contains a declared disease — Queensland's Declared Disease List includes BP, IHHNV, TSV, WSSV, YHV and NHP.

Disposal of solids and liquid waste from commercial processing of imported prawns

Waste from commercial processing in the Americas has been suspected to be associated with the introduction of prawn diseases (Lightner et al. 1997b, Lightner et al. 1998).

For economic reasons, commercial processing of imported prawns in Australia is not commonly practised. ADVS (1999) stated that:

> *currently there is little processing of imported prawns after arrival in Australia, therefore exotic prawn viruses are less likely to be present in commercial prawn waste.*

There is currently one AQIS-approved prawn processing plant in Australia.

Types of processing could include removal of head (cephalothorax), removal of head and shell (the shell covering the last tail segment may remain) and further processing such as coating, marinading or crumbing.

Waste prawn material from commercial processing may be disposed of as either solid waste or in wastewater. The majority of such waste is likely to be solid waste (including that screened from the liquid waste before its disposal as wastewater) and would be disposed of at municipal landfill sites. Solid waste derived from imported product would constitute a minuscule proportion of the solid waste processed through such controlled waste disposal systems. Further, pathogenic agents are unlikely to survive the environment at waste disposal sites, due to desiccation, ultraviolet radiation, low oxygen potential, daily variations in temperature, and competition from other microorganisms for nutrients. In combination with dilution, unfavourable physical conditions would significantly reduce the importance of uncontrolled run-off, leachate or the dispersal of waste by scavenging seabirds at landfill operations as possible exposure pathways.

Only a small proportion of the waste from the commercial processing of prawns in Australia[31] would be discharged in wastewater. With increased awareness of the detrimental effects that such discharge may have on receiving waters, most States and Territories in Australia now require that wastewater from commercial prawn processing facilities is either discharged to a municipal sewer or undergoes treatment before discharge into natural waterways. Commercial prawn processing facilities are often situated near waterways in urban areas and may have access to both municipal sewers and natural waterways. In the absence of specific data, this

[31] It is acknowledged that, in some cases, any hazard present in solid waste might leach and so be present in the wastewater from the facility. However, it is considered that this possibility would add little to the overall risk.

assessment assumes that wastewater from commercial processing may be directly discharged to a waterway without treatment. The IRA team is aware of an example of a processing plant in Western Australia that processes prawns from the North-West Shelf fishery and that has no effluent treatment, with effluent being discharged straight to sea. Thus a significant proportion of wastes from the commercial processing of the domestic catch, in this instance, is not subject to effluent controls.

Fish processors in some Australian States and Territories are required to disinfect effluent to prevent the incursion of pests and diseases. Currently, fish processors in the Northern Territory are required to disinfect (by chlorination) all wastewater leaving the processing plant. Tasmanian legislation allows wide-ranging rules to be made for fish-processing and the release of fish into Tasmanian waters to ensure, as far as practicable, fish disease outbreaks are prevented. Guidelines may be issued relating to the control and regulation of disease affecting fish. Regulations may also be made detailing measures to prevent or control disease in fish and importation or possession of fish that may be affected by disease. West Australian and South Australian legislation has in place the provision to regulate the processing of fish, however, at present there are no legislative, regulatory or State protocols or licence conditions imposed on industries that would limit the end-uses or waste disposal practices of imported prawns within these States. Australian laws pertaining to bulk solid and liquid waste disposal from commercial processing exist under various State and Territory legislation. Generally, States and councils can issue a trade waste disposal permit containing requirements for the disposal of bulk, highly biodegradable waste and effluent in the interest of public health and the protection of the environment.

The trend towards water re-use in some Australian jurisdictions is noted, although current re-use practices would not be expected to contribute significantly to the biosecurity risk. It is, nonetheless, an area that may require future monitoring.

Although commercial prawn processing plants are typically distant from crustacean aquaculture areas, and processing of prawns on-site at prawn farms is typically restricted to the farm's own produce, there are some processing plants in proximity to prawn farming areas such as those along the Brisbane River. Under such circumstances, prawn material (and any associated pathogens), discharged from these plants may enter crustacean aquaculture facilities, albeit at very dilute concentrations.

Over the last few years there has been an increased demand for prawn head and shell processing waste to supply chitin for the manufacture of glucosamine, thereby providing greater incentive for harvested prawns to have the head and shell removed prior to export. Currently, chitin demand exceeds supply; resulting in higher global glucosamine prices — raw material prices are as high as US$4 per kilogram. The global glucosamine market relies heavily on the Chinese prawn farming industry.

Use of imported prawns as bait for recreational fishing

Recreational fishers throughout Australia commonly use prawns as bait or berley when fishing (ADVS 1999). The ADVS report noted:

> *It is also evident that … prawns … are favoured bait used in very significant quantities by recreational fishers*
>
> and:
>
> *cooked or uncooked prawns and prawn heads or shells left over after the preparation of food for humans are highly valued by many anglers as an attractive berley additive.*

In Australia, bait prawns have had a commercial value comparable to that of similar-sized prawns for human consumption. Thus, there may be redirection of product intended for human consumption into the bait market. ADVS (1999) stated:

> *There is considerable scope for the redirection of prawns intended primarily for the human consumption market onto the bait market, and vice versa. The major motivations driving this redirection of prawns from one end-use to another are price, quality, demand and relative availability. Imported and domestic frozen or fresh frozen or fresh prawns intended specifically for human consumption are frequently purchased by fishers seeking larger, higher quality or, on occasion, cheaper products than those offered by bait suppliers. In addition, commercial fishers, co-operatives and wholesalers regularly redirect smaller or less uniform-sized prawns toward the bait market, which often tends to have a more stable pricing structure than the seafood sector. The practice of redirecting prawns intended for human consumption into the bait market was clearly identified during the case study investigating prawn products in the Moreton Bay region. It is evident that market forces identify prawns (along with squid and octopi) as one of the most dynamic of all aquatic products in terms of their ability to be quickly and readily switched from one end-use to another at any stage in the process; from harvester or farmer to consumer. Importation of prawns to Australia as bait was suspended in November 1996 following recommendations of the National Taskforce Force on Imported Fish and Fish Products (Higgins 1996). This might be expected to have further encouraged diversion of prawns imported for human consumption to supply the demand for recreational fishing bait created by the suspension of imported bait prawns.*

In a 2002 national survey commissioned by Biosecurity Australia on bait-use by recreational fishers, Kewagama Research (2002) estimated that each year, between 35 tonnes and 117 tonnes (with a most likely value of 76 tonnes) of prawns purchased from seafood outlets were used as bait or berley in Australia[32]. Over recent years, the total volume of prawns purchased as seafood in Australia each year has been increasing. For 2002–03 and 2004–05, this volume was estimated at 33900 tonnes and 42100 tonnes, respectively[33].

Kewagama Research (2002) found that there was a strong preference for prawns sold as bait, and that (in order of importance) quality, convenience, price and size were the key determinants in choice of bait type used by recreational fishers. Previous Biosecurity Australia inquiries (in 2000) revealed that, based on the species and retail price of imported prawns at the time, uncooked prawns more than 15 grams were less likely to be used as bait. These findings were subsequently confirmed in the national survey by Kewagama Research (2002).

However, since 2002 there has been a significant decrease in the retail price of prawns imported for human consumption (ABARE 2006). This has largely been attributed to the increase in *L. vannamei* farming in Asia, from where the vast majority of Australian imports of whole uncooked prawns are now sourced (ABARE 2006). Information on imported prawns and prawn products is collected by the Australian Customs COMPILE[34] system and the AQIS

[32] The study involved a random sample of 8000 households across Australia with 1123 fishers surveyed in detail, and provides useful information on the use of aquatic animals as bait or berley at the time.

[33] This figure was derived by adding reported domestic production (26300 and 23500 tonnes in 2002-03 and 2004-05, ABARE 2006) and imports of prawns (18100 and 29900 tonnes in 2002-03 and 2004-05, ABARE 2006) then deducting both the amount reported to be exported (9500 and 10300 tonnes in 2002-03 and 2004-05, ABARE 2006) and the amount estimated to be purchased as bait and berley annually (1008 tonnes, Kewagama Research 2002).

[34] Customs Online Method of Preparing from Invoices. Lodgeable Entries. System decommissioned February 2006. Replaced by the Integrated Cargo System (ICS) in October 2005.

AIMS[35] system. These systems do not differentiate between species for prawn product imports. Although some imported prawn products are labelled with the species, such identification is not a labelling requirement and is usually provided only as packaging and marketing information for the distributor, retailer and consumer. As such, although it is generally accepted that vannamei prawns contribute substantially to the increased volume and lower price, the available data do not allow for a meaningful estimation of the amount of vannamei prawns imported into Australia. Australian commodity statistics produced by ABARE from 2001–02 to 2004–05 show an increase in volume of imports for all imported prawn products (cooked and uncooked) from 17600 tonnes to 29900 tonnes, and a decrease in value per kilogram from $12.58 to $8.62, respectively (ABARE 2005, ABARE 2006).

There is anecdotal evidence that the low retail price of vannamei prawns and increased availability at supermarkets and seafood outlets has resulted in them being purchased more frequently by recreational fishers for use as bait. This may include uncooked, peeled, head-off product preparations, as well as whole uncooked prawns of all sizes. As such, use of prawns imported for human consumption as recreational fishing bait represents a potentially significant pathway by which wild crustacean populations might become exposed to disease agents of concern associated with imported prawns.

The IRA team sought verification of this anecdotal evidence through the 2007 National Bait and Berley Follow-up Survey, a follow-up survey to the National Bait and Berley Survey 2002 focussing particularly on prawns.

The sample fishers questioned in the 2007 survey were made up of the 'repeat' fisher group of the 2002 survey, representing 33% of fishers in the 2002 survey, 54% of 'sold as seafood' prawn users and 67% of estimated quantities used at that time.

A major objective of the 2007 survey was to determine if 'sold as seafood' prawns were still being used as bait or berley in recreational fishing. The survey confirmed this to be the case.

Among the 'repeat' fisher group, a significant increase was detected in the numbers using 'sold as seafood' prawns between the two surveys (7.9% up from 6.0%, a 33.5% increase). In terms of quantities used, there was a lesser increase of 18% for the 'repeat' fisher group between the 2002 and 2007 surveys (59.6 tonnes and 50.5 tonnes, respectively). However, the apparent 9 tonne increase should be regarded with some caution given that the 95% confidence intervals for these tonnage estimates were 29.8–89.4 tonnes (2007) and 12.2–88.8 tonnes (2002).

More specifically, the 2007 survey looked at the use of potentially imported prawns 'sold as seafood' (i.e. prawns/products other than whole uncooked prawns below the 13cm size limit for importation of prawns in place at the time). Such use was reported by 7 respondents representing an estimated 6.3 tonnes (or 11% of the 59.6 tonnes) - all but one of these respondents reported the purchase of *shelled* prawns. In the 2002 survey, 1 'repeat' fisher reported such use (purchase form *head off*), corresponding to an estimated 1.8 tonnes. Of the total sample for 2002 survey, 4 respondents reported use of prawns purchased in the *shelled* form, representing 9.5 tonnes, with 2 reporting *head off*, 1 *shelled* and 1 using *heads/shells* only (but purchasing whole prawns – size not assessed). The small sub-samples preclude any comparative analysis or relative standard error assessment.

The 2007 survey confirmed that the use of potentially imported prawns 'sold as seafood' still occurs, albeit at low levels when compared to total bait usage. Further, among the 7 respondents that reported using potentially imported prawns in the 2007 survey, 1 cited knowingly using imported product (marinated prawns on skewers in Tasmania), 2 reported using local product only and 4 were 'unsure'.

[35] AQIS Import Management System.

Neither survey detected the use of large whole uncooked prawns (>13cm size limit), such usage from any respondent or any purchase source (including 'sold as bait'), meaning that if it occurs, it is at levels below the detection capabilities of the surveys. In both the 2007 and 2002 surveys, small quantities of 'sold as seafood' prawns were reported for the 9–13cm group (2.6 tonnes and 3.5 tonnes, respectively). Although it is expected that reporting errors where the prawns were actually >13cm may have occurred, these errors would have been relatively minor and related quantities therefore quite small.

The 2007 survey identified a significant increase in reporting of *convenience/access* as the main reason for purchasing 'sold as seafood' prawns (47% up from 36%). In 2002, the predominant reason was *freshness/quality* (now down to 34% from 42%). *Price* did not alter as the third ranked reason, with around 15% of users identifying price as the main reason for purchasing 'sold as seafood' prawns. This convenience shift tends to support anecdotal information that increased availability (not just lower prices) may have led to increased numbers of fishers using 'sold as seafood' prawns.

The 2007 survey also found increased reporting of *peeled* prawns being used to bait recreational fishing hooks (17% up from 8%, 46 and 17 respondents respectively). However, the results suggest that many of these might actually prefer to peel the prawn and use the head/shell as berley as opposed to buying them in that form. In fact, just 6 respondents reported using *shelled* prawns purchased from a seafood supplier in the 2007 survey, with none in 2002 survey's 'repeat' fisher group (and only 1 in the remainder of the sample). Whilst this latter change is ineligible for significance testing, it represents low level evidence that potentially imported prawns 'sold as seafood' are being used as bait and berley and cannot be discounted in an assessment based on a conservative ALOP.

In summary, the 2007 survey establishes consistent increases across the 'sold as seafood' issue among the 'repeat' fisher group, where some are statistically significant and some are not. But nowhere has an aggregate decrease been observed, notwithstanding the theoretical potential for this in the confidence intervals reported above.

The potential also exists for recreational bait-use to lead to direct exposure of farmed crustaceans through fishing in farm inlet channels. Although a potentially significant exposure pathway, the IRA team considered the risks associated with such practices would be limited as much of this bait is likely to be taken by non-susceptible finfish species.

In 2006, in response to the potential risks associated with using imported prawns as recreational fishing bait, the Queensland Department of Primary Industries and Fisheries began an awareness campaign aimed at educating fishers not to use uncooked imported prawns as bait. The campaign provides information about exotic prawn viruses that may be carried in uncooked imported prawns, their potential impacts on the Australian prawn industry, marine and fishing industries and the environment, and steps that fishers can take to prevent the use of imported prawns as bait.

The only State with specific legislation on the use of crustaceans as bait is Tasmania, which currently has specified restrictions on the use of southern rock lobster as bait. Under Queensland State legislation, it is an offence to sell unlawfully, leave or bring fisheries resources, or a product derived from fisheries resources, knowing the fisheries resources or product is infected with or contains a declared disease — diseases and pathogenic agents of concern on Queensland's Declared Disease List for prawns are BMNV, BP, IHHNV, TSV, WSSV, YHV and NHP.

Presence/amount of viable pathogenic agent in the commodity and/or associated wastes at point of exposure

Tissue tropism

Uncooked prawn heads and shell wastes from commercial or domestic processing of imported prawns represent a high-risk commodity in terms of agent titre for those pathogenic agents that have a predilection for endodermal enteric tissues (e.g. *Baculovirus penaei*), and the subcutis (e.g. WSSV).

Pathogenic agents that cause systemic infections, such as *Vibrio penaeicida*, may persist for a longer period in prawn tails, which are less prone to enzymatic degradation than are head-on prawns. Removal of the shell would be expected to reduce the number of organisms that are preferentially located on it or in the sub-cuticular tissues. Pathogenic agents such as microsporidians that are preferentially located in muscular tissues would not be significantly reduced as a consequence of shell-removal or removal of the head.

Infection by many bacterial or viral pathogenic agents may result in a bacteraemia or viraemia, so that the agent will be present throughout the body. In such cases, the removal of haemolymph-rich organs by removing the head would reduce the amount of agent. However, an amount of agent sufficient to cause infection in a susceptible host could still remain.

In subclinically infected or chronically infected and recovered prawns, many pathogenic agents would not be expected to occur in high concentrations throughout the body. They would be concentrated in particular tissues, such as the lymphoid organ in the prawn cephalothorax (as is the case with TSV and YHV).

Agent stability

The ability of any pathogenic agent present in prawns or associated wastes to persist and remain infectious at the point of exposure to a susceptible host animal depends to a great extent on the stability of the agent through transport, storage or processing.

Prawns are usually cooked whole, and the cephalothorax and shell are removed before consumption of tail meat (abdominal muscle) (ADVS 1999). The pathogens of prawns are generally susceptible to inactivation by heating, thus cooking would be expected to inactivate or significantly reduce the titre of most pathogenic agents of potential quarantine concern, including viruses, bacteria, protozoa and metazoa. Cooking before using scraps as bait or berley would significantly reduce the amount of any pathogenic agents present. Similarly, pathogenic agents present in imported uncooked prawn waste from commercial processing plants that is used in the manufacture of aquaculture feed would be subject to a degree of heat treatment (based on current prawn feed manufacturing practices in Australia), generally resulting in the reduction of any pathogenic agents.

The use of imported uncooked prawns as a fresh feed for crustaceans in hatcheries, research facilities and public aquaria, including for conditioning broodstock, or as recreational fishing bait represent potentially high risk exposure pathways, as any pathogenic agents present would be subject only to the minimal inactivation associated with freezing and thawing of prawns. Freezing and thawing decreases the titre of some agents such as YHV (Wongteerasupaya et al. 1995b). Other pathogenic agents, such as IHHNV, can persist and maintain infectivity in frozen prawns for extended periods. Pathogenic agents present in uncooked solid and liquid wastes from the commercial processing of whole uncooked prawns would not necessarily be expected to experience significant inactivation before the release of waste from such plants.

Contact between susceptible host animals and the imported commodity and/or associated wastes

Farmed crustaceans are generally stocked at relatively high densities and not usually subject

to competition from non-aquaculture species. As such, in the case of exposure group 1 (farmed crustaceans) and exposure group 2 (hatchery crustaceans), it is almost certain that any imported prawns directed to these exposure groups in the form of processed or unprocessed feed would make contact with, and be consumed by, potentially susceptible animals.

The probability of susceptible wild crustaceans (exposure group 3) having contact with imported product or associated wastes depends on the volume of product released into the natural environment, the dispersal and dilution of that material, the presence and concentration of host animals in the area, and the proportion of material that might be consumed by other non-susceptible species in the vicinity. Wild prawns would be less abundant than those in aquaculture facilities and may encounter greater competition from other animals for any prawn material present in their environment. Wild prawns may be moderately likely to come into contact with prawn material introduced into their environment. However, as the result of greater competition from other aquatic animals (especially fish, crabs and other crustaceans), only a small proportion of such material may end up being ingested by wild prawns (Baldock 1999). Wild finfish are highly likely to access any prawn material entering their environment and are likely to ingest a moderate to high proportion of any such material. Wild crabs are also likely to access any prawn material entering their environment but would be expected to ingest only a small proportion of such material. Other (non-crab and non-prawn) wild crustaceans may also be likely to access prawn material in estuarine environments, but are unlikely to access prawn material in open ocean environments. Regardless, non-crab, non-prawn crustaceans are likely to ingest only a small proportion of any such material (Baldock 1999).

The key end-uses or disposal methods that present a potentially significant risk to wild crustaceans are the use of imported prawns as recreational fishing bait or berley, and the disposal of untreated solid or liquid wastes from commercial processing plants. Prawn species are widely distributed in fresh and marine waters in Australia and thus it is expected that all waters in which recreational fishing is carried out would be home to many crustacean species. Crustaceans must compete with predatory finfish and other scavengers (including other invertebrates and birds) for bait scraps and berley. Competition with non-susceptible aquatic species would reduce the likelihood of susceptible crustaceans consuming scraps potentially containing infectious organisms — a high proportion of prawns introduced into aquatic environments as bait or berley will be consumed by finfish species. The nature of many popular fishing spots is such that fishing bait, which may include imported prawns, often enters a circumscribed body of water. This would increase the probability of any susceptible species present being exposed to imported product, compared to bait-use in open waters.

The amount of infectious pathogenic agent, if present in the product, will depend on the initial titre, storage conditions, environmental conditions, and length of time in the water. Many prawn viruses have a relatively short half-life and the amount of viable virus would, once thawed, decline, but nonetheless may still be retained at an infectious dose.

Crustaceans are generally expected to be present in the aquatic environment where any commercial processing wastes would be discharged. Commercial processing of prawns will result in substantial volumes of waste. If untreated or ineffectively treated wastes were to enter the aquatic environment in a circumscribed area, it is likely that local crustaceans would come in direct contact with that waste.

Minor pathways

Many pathways have a much lower probability of completion as they are not common, involve only indirect exposure of the aquatic environment or inactivation of the pathogens of concern occurs before potential exposure. These pathways include such things as disposal of wastes at municipal landfill sites and processing of by-products for aquaculture feeds.

Human consumption is the primary purpose for which prawns are imported. It is expected that of the pathogenic agents of concern identified in this risk analysis, the amount of infectious agent present would be dramatically reduced, if not entirely eliminated, in the human gastrointestinal tract. Additionally, in Australia, human faecal wastes are normally disposed of via domestic sewerage systems. The physico-chemical environment of such systems, combined with the effect of dilution with other wastes, is expected to reduce substantially both the level and concentration of any remaining aquatic animal pathogens. As such, prawns that are eaten by humans would not contribute significantly to the biosecurity risk to aquatic animals assessed in this risk analysis.

Use of prawn by-product from commercial processing of imported prawns in the manufacture of pelletised feed for crustacean aquaculture

Historically, pelletised prawn feed used for grow-out have contained prawn or other crustacean meal. Feeding farmed and hatchery crustaceans with commercial feeds (manufactured in Australia) that use prawn-based ingredients (specifically prawn head and shell by-product from commercial processing in Australia of prawns for human consumption) represents a direct pathway by which farmed crustaceans (exposure group 1) or hatchery crustaceans (exposure group 2) could become exposed to prawns imported for human consumption.

At present, there is a single commercial prawn feed manufacturer in Australia. This company uses the steam-pelleting process. Krill meal is the only crustacean meal currently used in manufacturing commercial prawn aquaculture feeds in Australia (R. Smullen, Ridleys AgriProducts Australia, pers. comm. March 2006). Many prawn farmers use imported processed aquaculture feeds. Imported prawn feeds made up 83% of the total prawn aquaculture feed used in Queensland 2003–04 (Lobegeiger and Wingfield 2005).

Of the pathogenic agents considered in this risk analysis, the parvoviruses IHHNV and HPV are considered the most heat stable. Most parvoviruses can survive heat treatment at 56°C for at least 60 minutes (Fauquet et al. 2005). Some animal parvoviruses such as bovine parvovirus show significant resistance to dry heat treatment and require treatments of up to 100°C for 30 minutes for effective inactivation (Dichtelmuller et al. 1996).

The manufacture of prawn feed pellets involves either steam pelleting or the more popular extrusion method which is used for over 80% of prawn feed products sold in Australia (Lobegeiger and Wingfield 2005). During the manufacturing process the temperature can often exceed 100°C, especially in the extrusion process where temperatures can reach 140°C. Using the steam pelleting process as an example, ingredients are conditioned over three stages at 30 seconds per stage at a temperature of 85–90°C, the mix is then passed through a die in excess of 100°C and then dried for 30–40 minutes at 90°C (Edgerton and Owens 1999a).

Further, the manufacture of fish and prawn meals used as ingredients in pelletised prawn feeds includes dry heating to desiccate the raw tissue so that the product can be pulverised into a meal that can be more efficiently mixed in the pelleting process (AquaTactics 1999).

The risk relating to the ability of prawn feeds to carry viable pathogenic agents comes less from the agent surviving the pelleting process and mainly from the possibility of contaminating the pellets with infected raw ingredients due to poor hazard control during manufacture. A report by Edgerton and Owens (1999a), commissioned by Biosecurity Australia, that included a discussion of processing procedures at a large feed mill in Thailand[36], noted that quality control measures to prevent contamination with potentially infected raw ingredients were in practice and operated to required standards set by the Government of Thailand. Similar quality control measures would be expected in Australian feed mills. Flegel and Fegan (2002) note that disease outbreaks in farmed prawns have never

[36] Thailand is the main source of imported prawn feed preferred by Australian prawn farmers (AquaTactics 1999).

been causally linked with use of pelleted feeds.

Given the above, in the event prawns imported for human consumption or associated wastes were to be used in Australia for manufacturing pelleted aquaculture feeds, the IRA team was of the view that the heat treatments associated with feed manufacture would substantially, if not completely, inactivate any prawn pathogens present.

Prawn waste disposed at controlled landfill sites

As discussed in the release assessment, the vast majority of uncooked imported prawns for human consumption are transported frozen (at or below –18°C). Some prawns in the fresh frozen form may be discarded in small quantities from retail outlets (e.g. after defrosting for sale). However, should for example refrigeration equipment fail or freezer burn occur, large quantities of prawns could spoil and become waste. Nonetheless this is considered to be a relatively infrequent event. For example, of imported prawn products purchased by one major Australian prawn retailer in the financial year 2003–04, 0.26% was rejected and returned to the vendor due to poor quality after the prawns had arrived in stores and had been thawed for sale (B. Hillen, seafood business manager, pers. comm. July 2004). In addition to inspection on arrival in Australia by AQIS, prawns are inspected at the processing facility in the exporting country. The overseas vendor provides an 18-month 'assurance guarantee' so that faulty products can be returned (e.g. due to freezer burn). If the product is deemed unsuitable for sale as a result of post-thaw product failure at the point of sale (e.g. due to soft-shell and drop-head), the product may be returned to the overseas vendor or, if deemed still fit for human consumption, the prawns may be sold at other local markets for human consumption at a cheaper price.

The disposal of bulk aquatic animal waste is subject to State or Territory and local authority environmental protection legislation and subject to licence or permit requirements. Bulk waste is not usually disposed into a natural waterway, but rather, disposed at controlled landfill sites via commercial waste disposal operators. In the absence of quarantine controls, the disposal of aquatic animal waste by deep burial or incineration at such sites is not considered to be a normal occurrence; in which case, prawn waste at open landfill sites might enter the aquatic environment via mechanical transfer by scavenging birds (and insects) or via leachate. It is expected that if any particles of prawn waste were to accompany leachate, they would be very small. Particles of such size are not expected to represent a significant proportion of any bulk prawn waste at open landfill sites. Further, landfill facilities frequently have mechanisms to control leachate. This exposure pathway is not expected to contribute significantly to the overall risk.

ADVS (1999) considered the processes used for waste disposal in Australia, including solid wastes generated by seafood processing (e.g. cephalothorax, shell and pleopods). Such waste represents an insignificant exposure pathway if disposed of in properly designed and controlled landfills. In combination with dilution, physical conditions at such sites would significantly reduce the importance of uncontrolled run-off, leachate or the dispersal of waste by scavenging seabirds at landfill operations as a possible exposure pathway.

The majority of prawn wastes discarded by consumers and retail outlets such as restaurants and hotels will also be disposed of at landfill sites. The management of Australian refuse dumps was reviewed by Environmental Management Services Pty Ltd (EMS) (*Report on Factors Affecting the Exposure of Australian Animals to Imported Pig Meat*, submitted 1999). Although this report considered specifically the potential for feral pigs throughout Australia to gain access to refuse, some of its findings on the management of refuse dumps are relevant to scavenging (wild) birds. A part of this review is paraphrased below:

The management of refuse disposal in Australia is undergoing a systematic process of improvement as State Governments dictate, and local authorities implement, modern procedures. The EMS consultants found the *NSW Landfill Guidelines* produced by the NSW Environment Protection Authority (EPA) to be the most comprehensive and advanced. This

document describes several issues that may influence the ability of wild birds to gain access to human refuse:

- compaction of waste;
- the regular covering of waste; and
- site capping — the final coverage of waste as a dumping area is sealed.

Despite these measures, all users of refuse dumps will have noted large areas of recently dumped uncovered waste and, commonly, large flocks of scavenging bird life (such as gulls). Prawn material might enter the aquatic environment via a number of routes, including being passed in bird faeces, being regurgitated, or by being dropped by birds before ingestion.

There have been several experimental studies which have demonstrated that some aquatic pathogens can remain viable after passage through the gastrointestinal tract of birds. Work in the US investigated the infectivity of four prawn viruses after passage through the gull gastrointestinal tract (Vanpatten et al. 2004). Gulls were fed prawns that had tested PCR/RT-PCR positive for IHHNV, TSV, WSSV or YHV. IHHNV, TSV and WSSV were detected in gull faeces by PCR/RT-PCR. Bioassays (by injection of dilute faecal homogenate into specific pathogen free [SPF] *L. vannamei*) were positive (by both histology and PCR/RT-PCR) for IHHNV and TSV, but not WSSV or YHV. These results suggest that IHHNV and TSV may remain infective after passage through the gull gastrointestinal tract, but that WSSV and YHV do not. TSV and IHHNV virions are non-enveloped and are simple and inherently more stable than the lipid-containing enveloped virions such as WSSV and YHV. The stable and simple structure of TSV and IHHNV, and the added stability of IHHNV DNA (compared to RNA), have been proposed as factors contributing to stability and resistance to digestion (Vanpatten et al. 2004).

Garza et al. (1997) also reported that seagulls (*Larus atricilla*) could serve as vectors of TSV and that gulls and other prawn-eating seabirds could transmit TSV to prawn farms within their flight path. However, these authors noted that it is not known how long TSV remains infective in the gut contents of gulls or other seabirds and, therefore, how important these birds might be in spreading this disease. Alternatively, seabirds may mechanically move waste prawn material from inappropriately dumped areas to the aquatic environment.

Aquatic insects, particularly the water boatman *Trichocorixa reticulata*, have also been suggested as possible vectors of TSV (Hasson et al. 1995), as TSV in their gut contents was shown to be infectious (Lightner 1995, Lightner and Redman 1998). However, aquatic insects would only be able to move pathogenic agents over short distances and are unlikely to play a significant role in exposure of susceptible animals.

The role of seabirds and other mechanical vectors in disseminating pathogenic agents such as TSV may be significant if a large amount of the infectious agent is present (e.g. in animals dead or dying as a consequence of disease or in high concentrations of infected wastes) (Garza et al. 1997). If pathogenic agents are present at low concentration, as would be the likely case with waste from apparently healthy prawns imported for human consumption discarded at rubbish tips, seabirds and other mechanical vectors would be much less likely to transmit disease. In the case of many pathogenic agents, any agent present in infected uncooked prawn waste at landfill sites that is ingested by scavenging birds would be reduced or eliminated in the avian gastrointestinal tract, although some viruses such as IHHNV and HPV may be more resistant.

Moreover, the IRA team considered that the environmental conditions at landfill sites would be likely to result in the exposure of any aquatic animal pathogens present to desiccation, ultraviolet radiation, low oxygen potential, daily variations in temperature, or competition from other microorganisms for nutrients. Such exposure would be expected to reduce the amount of any pathogenic agent present or, in some cases, may eliminate the agent entirely.

Food scraps discarded directly into the aquatic environment

Waste from imported prawns could be discarded as food scraps directly into the aquatic environment. Susceptible prawns or other crustaceans would be unlikely to become infected in this way because such scraps would not be expected to contain pathogenic agents in infective form or in high concentrations (as most would be cooked). Moreover, discarded scraps would more likely be consumed by non-susceptible than susceptible species.

Prawn wastes disposed through municipal sewerage systems

The processing of effluent in a domestic sewerage system would, even if it were limited to primary level processing, significantly reduce, if not eliminate, the concentration of any prawn pathogens that might be present. The physical conditions in sewerage systems, including the presence of chlorine and other chemicals inimical to the survival of microorganisms, and competition from other microorganisms for nutrients, would be expected to limit the survival of many of the pathogenic agents considered in this IRA. The dilution of effluent with wastewater from other sources may also significantly decrease the concentration of any pathogenic agents present. At a minimum, physical and biological treatment, disinfected secondary treatment (chlorination) and dilution of effluent in most Australian sewerage systems is capable of eliminating pathogenic agents prior to discharge.

Discharge of effluent into freshwater

The discharge of effluent into freshwater is usually controlled by local authorities that normally require processing to a secondary or tertiary level to protect public health and the environment. Such processing would reduce the concentration of pathogenic agents entering freshwater systems by several orders of magnitude.

Other minor pathways

Other possible but unlikely exposure pathways that are not given further consideration in this assessment include diversion of prawns for human consumption for use as agricultural fertiliser, disposal of packaging materials used in importation of whole uncooked prawns, use of imported prawns as an ingredient in animal feed manufacture (other than use in manufacture of crustacean aquaculture feeds), and use of imported prawns as feed for display animals kept in home aquaria.

Conclusions

It is recognised that there are other potential pathways by which susceptible host animals in Australia might become exposed to imported, whole uncooked prawns or associated wastes. However, these pathways are considered to be minor in nature, in that they are collectively not expected to add appreciably to the overall risk. Furthermore, any risk management measures that may be considered necessary to mitigate the major risk pathways, would also likely be sufficient to manage the minor risk pathways.

Although it is recognised that farmed prawns (exposure group 1) represent the exposure group least likely to be exposed to imported prawns tissues or associated wastes, there is potential for infected prawn tissues to be introduced direct into crustacean grow-out systems via whole uncooked prawns used as feed in broodstock maturation ponds and, to a lesser extent, use of imported prawns as bait for recreational fishing in farm inlet channels. It is unlikely that infected imported prawn tissue would be carried to aquaculture ponds by natural means such as wild birds or via locally produced aquaculture feed containing imported prawn material as an ingredient.

The use of imported prawns as feed to condition broodstock in crustacean hatcheries and as feed for crustaceans in research institutions and public aquaria (exposure group 2) represents the most significant pathway by which crustaceans in aquaculture and holding systems could become exposed to imported prawns potentially infected at the time of harvest. Head-on uncooked prawns are considered to be the preferred product form for feeding crustacean

broodstock in hatcheries and research institutions and imported uncooked prawn meat is commonly used as a feed for aquatic animals in research institutions and public aquaria.

The regular introduction of relatively small amounts of prawn material into the aquatic environment through use as bait (particularly at popular fishing spots) would present a significant pathway whereby wild crustaceans (exposure group 3) could become exposed to imported prawns potentially infected at the time of their harvest. Disposal of untreated waste from commercial prawn processing could result in periodic introduction of large amounts of prawn tissue into the aquatic environment, again presenting a significant pathway by which wild crustaceans could become exposed to pathogenic agents of concern.

5.3 Consequence assessment

'Likely consequences' were assessed separately for each exposure group. Assessment of likely consequences required determination of the partial likelihood of establishment or spread (in the assumed event of exposure occurring) and the magnitude of any resulting biological, economic and social impacts associated with each of two outbreak scenarios. The following two outbreak scenarios were identified in this assessment:

Outbreak scenario 1	the agent establishes and spreads to wild and farmed populations of susceptible species in Australia — it is assumed that if an agent were to establish in a local population it would eventually spread to its natural geographical limits.
Outbreak scenario 2	the agent does not establish — an index case may occur and infection may spread to co-habiting animals, but the agent does not persist sufficiently long to be detected.

The IRA team considers the above to be the two most likely scenarios, i.e. that the disease does not establish, or it establishes and spreads. The 'no-establishment' scenario (outbreak scenario 2) is one that captures a range of eventualities – for example, instances where there may be an index case of infection but no spread to other animals, or spread to a local population (be it farmed or wild) but the agent does not spread or persist in that population.

The outbreak scenario that captures the events that lead to disease spread (including to wild crustaceans populations), i.e. the 'establishment and spread' scenario (outbreak scenario 1), would be the risk determinant. That is, it is under this scenario that significant impacts might occur. The IRA team took the approach of focusing on the 'establishment and spread scenario', and grouped a range of other eventualities into the 'no-establishment' scenario, given that detailed consideration of these other eventualities would not change the overall risk determination.

Such an approach is suited to the unique situation in aquatic environments where the number of meaningful outbreak scenarios is generally limited, compared to terrestrial environments. In the terrestrial situation, there may be a wider range of likely outbreak scenarios depending on such things as livestock management practices, the epidemiology of the pathogenic agent, and established control and eradication programs. In the aquatic environment, if a disease does establish in a population following exposure, it is generally not possible to prevent its spread by natural means. Based on the IRA team's understanding of the effectiveness of control and eradication programs for aquatic animal diseases, and the speed at which authorities would be able to detect outbreaks, control and eradication are generally not viable.

5.3.1 Partial likelihoods of establishment or spread

Outbreak scenarios 1 and 2 are mutually exclusive and were assumed to represent the most plausible outbreak scenarios that could occur as a result of a local crustacean population being exposed to a pathogenic agent of concern. The two 'partial likelihoods of establishment or

spread' (PLES) associated with each outbreak scenario are thus complementary.

The interaction between host, environment and agent-related factors is critical to the likelihood of establishment or spread of a pathogenic agent. Information on these factors in relation to the pathogenic agents of concern is limited. Where available, such information was considered in the likelihood estimations — conservative assumptions were made where information was lacking.

The IRA team considered the following factors relevant to determining the partial likelihood of establishment or spread (PLES) for each of the two outbreak scenarios associated with each of the three exposure groups: infectious dose, transmission, susceptibility of Australian crustaceans to infection, and predation of infected tissues and animals.

Infectious dose

ADVS (1999) noted that it could be misleading to recommend a minimum infective dose of any pathogen, and that such a recommendation would need to take into account the capability of a laboratory to determine accurately the number of cells or viruses present when they might be in a cryptic state. Moreover, studies on the infectious dose of crustacean viruses are further complicated by the lack of continuous crustacean cell lines for titration of the virus. The environmental conditions at the time of infection or release from a carrier, and the health and immunological status of the recipient host animal, would also have to be taken into account.

For most pathogenic agents considered in this IRA, data are not available to provide a meaningful quantification of infectious dose, and at best, it would only be possible to conclude that the minimum infective dose is likely to be high or low, relative to the range of pathogenic agents under consideration.

With respect to the amount of agent that might be presented to a potential host animal, the effect of dilution is an important consideration, particularly in the case of waterborne transmission. For example, prawn farm effluent in Australia may be treated through settlement and dilution before it is released into natural waters, effectively reducing the amount of agent (or dose) encountered by a susceptible animal. Similarly, commercial processing waste released into natural waters would be expected to encounter a high degree of dilution. Tissue-bound pathogenic agents are more likely to be transmitted orally by host animals feeding on infected material. The effect of dilution under these circumstances would be less, due to the ability of potentially susceptible animals to detect and capture food material, notwithstanding competition from non-susceptible species that may be more adept at retrieving such material.

Transmission

For most prawn pathogenic agents with a direct life-cycle, infection usually occurs as a result of the introduction of a live, infected host into a naive (and susceptible) population, either from waterborne transmission through shedding of infectious agent into the water or orally, via ingestion of infected host tissues. Some agents may cause subclinical infection, so apparently normal, infected prawns (i.e. carriers) may still be a source of infection. The greater the population density of host animals susceptible to disease, the more readily disease may be transmitted, resulting in higher morbidity and increased likelihood of pathogenic agent establishment.

In addition to the density of susceptible species, other factors that affect the susceptibility of the host to infection (e.g. life-cycle stage, environmental conditions, and intercurrent stress) may also affect transmission.

Susceptibility of Australian crustaceans to infection

Most reports of prawn pathogens are from species that are important in commercial aquaculture. In Australia the main aquaculture species are *P. monodon*, *F. merguiensis* and

M. japonicus. Other commercially cultured crustacean species include freshwater crayfish and to a lesser extent, mud crabs and sand crabs. Some pathogenic agents may be host-specific and infect only one or several prawn species, possibly from more than one genus. Other pathogenic agents have a much wider host range and can infect other groups of crustaceans and even other arthropod groups. Pathogenic agents such as WSSV that have a very wide host range would have a higher likelihood of establishing in Australia.

Australian prawn species are likely to be at least as susceptible to infection as the same species found in other regions. However, environmental conditions that might favour the expression of disease in prawn populations in other regions may not be present in Australia. The effects of some pathogenic agents (e.g. WSSV and MBV) in prawn aquaculture throughout Asia are considered to have been exacerbated by environmental pollution and other stressors (Flegel and Sriurairatana 1993).

In this risk analysis, conservative judgments have been used for the susceptibility of Australian prawns to infection with exotic pathogenic agents. The range of species infected overseas, their relatedness to Australian species, and other relevant epidemiological information about each pathogenic agent was considered when specific information on the susceptibility of an Australian species to infection was unavailable.

Predation of infected tissues and animals

The IRA team noted that prawn pathogens such as WSSV had failed to establish in wild prawn populations in Australia despite the presence of the agent in frozen prawn products (McColl et al. 2004) during times of unrestricted importation (including for use as recreational fishing bait). In addition, the prevalence of disease in some exporting countries may have been higher than now — although import volumes were significantly lower than at present. The IRA team considered that the establishment or spread of disease may be limited due to the high predation rate by finfish of wild prawns (exposure group 3).

The IRA team was also of the view that in the event that a limited number of index cases of infection did result from the exposure of wild prawns to an exotic pathogenic agent (as might be the case where wild crustaceans are exposed via the use of imported prawns as recreational fishing bait), the infected animals are most likely to be consumed by predatory finfish, thereby limiting the likelihood of the agent spreading more widely within the population. The likelihood of predation would be expected to increase many-fold if infection resulted in some level of morbidity. Equally, the infected animals might die of other causes and be removed by scavenging finfish, crabs or other animals — non-prawn crustaceans, particularly brachyurans (crabs) in marine environments, are also a major prey for fish (Salini et al. 1994).

The appearance of WSSV in 2001 in wild Gulf of California *L. vannamei* inhabiting a coastal zone with high prawn aquaculture activity and its absence by 2003 as reported by Mijangos–Alquisires et al. (2006) supports this view.

The scientific literature indicates that natural mortality of crustaceans and in particular wild prawn populations (including as a result of predation), is high. Prawns are r-selected[37] species and as such, are small, exhibit very high fecundity, high mortality rates throughout their life-cycle, and respond rapidly to change. Instantaneous rates of natural mortality in prawns of up to 94% have been reported (Haywood and Staples 1993, Wang and Haywood 1999) with mortality in general in many prawn nurseries being highly variable (Minello et al. 1989, Glaister et al. 1993).

Predation is a major contributor to the high mortality of juvenile, sub-adult and adult prawns in the wild, with predation being the greatest cause of mortality in some prawn species

[37] r-selected: a reproductive strategy commonly characteristic of species associated with situations that select for a high reproductive rate (r), tending to result in species that produce many offspring, with minimal resource investment on individual offspring by the parents.

(Minello et al. 1989). Brewer et al. (1991), estimated that predation on commercially important penaeid prawn species in Albatross Bay in the Gulf of Carpentaria was at least three-fold greater than the commercial prawn catch.

Commonly farmed omnivorous prawns species such *P. monodon* and *L. vannamei* are considered an important component of the diet of many teleost predators (Salini et al. 1990, Brewer et al. 1995, Vance et al. 1998, Haywood et al. 2003, BondadReantaso et al. 2005). Many studies in Australia have confirmed the importance of teleosts as predators of prawns, with prawns found at a high frequency in the gut of many species (Brewer et al. 1991, Salini et al. 1994, Brewer et al. 1995, Salini et al. 1998). One study showed that prawns made up 22% of the diet (by volume) of young barramundi (Robertson 1988). Knowledge of prey interactions has also resulted in some farms overseas incorporating biological controls to prevent the establishment and spread of WSSV by introducing mildly carnivorous fish such as tilapia. These farm pond cohabitants prey on lethargic and moribund infected prawns and thereby slow the spread of clinical disease to healthy prawns (Briggs et al. 2004).

The IRA team also identified contact with farmed prawns that had escaped from aquaculture ponds as a means by which a pathogenic agent could spread from a farmed to a wild prawn population. Although similar mortality factors to those discussed above would limit the likelihood of agent establishment, the IRA team considered that escape *en masse* of infected prawns (or continuous escape of small numbers over extended periods), especially into an environment affording increased protection from predators, would generally pose a greater risk to wild prawn populations than that associated with limited numbers of index cases likely to result from the exposure of wild prawns to recreational fishing bait.

Predation of commercially important penaeid prawns by fish predators is influenced by environmental factors and habitat types that have an effect on the type of predator and prey species present (Salini et al. 1998). Although predation of penaeid prawns in tropical inshore areas can be high if prawn densities are also high (Salini et al. 1998), some inshore habitats can provide greater protection for prawns from predatory finfish. Haywood et al. (2003) reported that in a predation experiment conducted with tethered prawns, prawns within a natural protective environment such as seagrass survived longer than prawns exposed to predators on bare substrate, depending on the suite of predators present at the time. The mangrove habitats associated with many prawn farming areas in Australia are considered ideal in the context of providing protection of escaped farm prawns from predatory finfish.

Disease spread from prawn farms to wild populations has been reported. For example, the IRA team is aware that gill-associated virus (GAV) is considered to have spread into the Joseph Bonaparte Gulf through escapes from Northern Territory prawn farms. Wild *L. vannamei* from the Gulf of California inhabiting a coastal zone with high prawn aquaculture activity were shown to be infected with WSSV — these wild prawn populations had previously tested WSSV-negative (Mijangos–Alquisires et al. 2006). The authors suggest that extreme weather conditions in 2001 that caused farm damage and escape of prawns may have resulted in the spread of WSSV to wild *L. vannamei*. Indeed, all reported detections of WSSV and TSV in wild prawns in Asia may have been as a result of aquaculture escapes and repeated restocking by the culture industry (Withyachumnarnkul et al. 2003, Chang et al. 2004). Similarly, susceptible wild crustacean stocks in the Gulf of California had been monitored for disease mortalities since 1974 without detection, until the introduction in 1987 of IHHNV into aquaculture facilities on the western coast of Mexico after the importation of subclinically infected *L. vannamei* postlarvae. Pantoja et al. (1999) showed that by 1990, IHHNV was common in wild stocks of *L. stylirostris*, and present in Gulf of California stocks of wild *Farfantepenaeus californiensis* and *L. vannamei*.

Predation of prawns and other crustaceans would thus be expected to reduce significantly the likelihood of establishment of an exotic pathogenic agent in wild prawn populations (exposure group 3). If the density of susceptible crustaceans in the wild is high relative to fish and other predators, the probability of disease spreading in a wild crustacean population

would be greater. In this context, there would be no predator density associated with farmed (exposure group 1) and hatchery (exposure group 2) crustaceans. Overall, it is considered that the likelihood of disease establishment or spread from an index case of infection would be greatest in the case of farmed (exposure group 1) and hatchery crustaceans (exposure group 2) (although possibly to a lesser extent), than would be the case for wild crustaceans (exposure group 3).

Conclusions

Compared to wild crustaceans (exposure group 3), disease establishment and spread is more likely in the case of farmed and hatchery populations (exposure groups 1 and 2) because of the high density of susceptible host animals, the environmental conditions associated with intensive husbandry practices, and the absence of predators to remove diseased animals — in natural waters, it is considered more likely that diseased prawns would be consumed by fish and other predators, rather than crustaceans susceptible to infection. Pathogenic agents are highly likely to spread from hatcheries to (several) farms, and from farms to hatcheries where broodstock are sourced from grow-out, with a lower yet significant likelihood of spread to wild populations.

The high predation rate of wild prawns by non-susceptible finfish species may limit the opportunity of establishment or spread of diseases from the limited numbers of index cases of infection that might result from the exposure of wild prawns to recreational fishing bait. However, the escape of large numbers of infected prawns from aquaculture ponds (or prolonged low level escape), especially into habitats where they would be protected from finfish predators, is considered to pose a greater risk.

The dilution of effluent water from aquaculture ponds would be expected to reduce the amount of pathogenic agent, and therefore, the likelihood that this effluent would cause infection in susceptible hosts in the surrounding natural environment — the release of effluent water and the density of prawn farms in Australia are regulated. Any animals that were to become infected (such as crabs) would move between ponds and the surrounding environment, and some infected prawns may occasionally escape. Many of these animals would more likely be eaten by non-susceptible species than by susceptible prawns or other crustaceans.

In the event of exposure, the likelihood of disease establishment in wild crustaceans (exposure group 3) is less than that associated with farmed crustaceans (exposure group 1) and hatchery crustaceans (exposure group 2), due in main to the better condition of susceptible animals in the wild compared to farm or hatchery conditions and the presence of predators that remove diseased animals. However, should a pathogenic agent establish in a wild population, then the likelihood of spread to other populations (farmed, hatchery and wild) is expected to be higher than compared to pathogenic agent spread from exposure groups 1 and 2. It is considered that if an exotic disease agent were to establish in a wild aquatic animal population, it would be near-impossible to eradicate.

5.3.2 Impacts

This section considers the biological, economic and social impacts associated with the two outbreak scenarios:

Outbreak scenario 1	the agent establishes and spreads to wild and farmed populations of susceptible species in Australia — it is assumed that if an agent were to establish in a local population it would eventually spread to its natural geographical limits.
Outbreak scenario 2	the agent does not establish — an index case may occur and infection may spread to co-habiting animals, but the agent does not persist sufficiently long to be detected.

Only the first scenario was considered in detail because the occurrence of the second scenario would not be expected to have a measurable impact.

Although the likelihood of the first outbreak scenario occurring may depend on the exposure group, the impact is the same regardless of the exposure group being considered, because it is assumed that the disease would eventually spread to all susceptible populations.

The IRA team considered the following general points to be relevant in determining impacts of each pathogenic agent of concern.

Direct effects

Animal health (production losses in aquaculture and commercial fisheries)

The biological effect of disease depends on the interaction of the environment, pathogenic agent and host. The nature of this interaction reflects factors intrinsic to the pathogenic agent (such as virulence and infectivity), the host (such as susceptibility, immune competence and population density), and the environment (such as quality and availability of habitat for susceptible hosts).

The biological effect of disease is normally evaluated in terms of morbidity and mortality. Morbidity can be evaluated in terms of reduced production and described by parameters such as food conversion efficiency and fecundity of a population under study. Diseases that reduce the efficiency of production without causing large increases in mortality are more likely to be significant in farmed prawns than wild-caught prawns.

Generally, the epidemiology of disease in aquatic animal populations is poorly understood. In farmed prawns 'normal' or baseline values for production and mortality are often highly variable, reflecting husbandry practices, stocking rates and stress. Many economically significant diseases of farmed prawns are caused by commensal organisms that are opportunistic pathogens (i.e. they cause disease only when environmental or other conditions predispose prawns to infection). The generally higher prevalence of disease and the frequent emergence of new disease problems in farmed prawns support the view that farmed prawns are subject to more environmental stresses and higher disease transmission rates due to high population density. It also reflects closer monitoring of farmed prawns.

Further, the IRA team was of the view that the impact of a new virus in Australia may not be the same as that seen overseas because the impact would depend on the overall effect of the new virus acting in combination with the suite of viruses already endemic in Australian crustacean populations.

The underlying 'baseline' or 'normal' rate of mortality in wild populations can be estimated from data collected in studies of population density, age/size structure and catch rates. Population fluctuations can be linked quite closely to other factors, such as fishing pressure, using these sorts of data. However, only major epidemics involving significant mortalities or grossly visible clinical signs are likely to be detected in wild crustacean populations.

Disease is seen as a component of natural mortality that is difficult or impossible to estimate, except in general terms. Prawn populations may fluctuate by orders of magnitude for a variety of reasons, including environmental changes. In addition, the IRA team is of the view that stock assessment of wild fisheries is an imprecise science — population estimates of prawn stocks have high coefficients of variation. As a result, a disease event may kill a large proportion of the population without being detected.

Perhaps the best known epidemic of wild crustaceans is crayfish plague, caused by the fungus *Aphanomyces astaci*, which has eliminated native freshwater crayfish from many river systems in Europe. Other examples are microsporidians, which are commonly found in wild crustacean populations, including prawns. Whitening of the musculature, an obvious clinical sign of microsporidiosis, is frequently reported in wild crustacean populations. Prevalence of

microsporidiosis in wild populations of up to 90% have been reported (Viosca 1945, Miglarese and Shealy 1974). However, there are few data relating disease occurrence in wild populations of prawns to a decline in fishery catches.

Baldock (1999) examined the impact of WSSV and IHHNV in wild prawn populations. For WSSV, the report concluded that:

> *Although WSSV infection is common in several wild prawn populations in Asia, the weight of evidence suggests that the virus has not caused measurable reductions in catches.*

The situation with respect to the impact of IHHNV on wild prawn populations is less clear. Lightner et al. (1992a) reported the introduction in 1987 of IHHNV into aquaculture facilities on the western coast of Mexico, with the importation of subclinically infected *L. vannamei* postlarvae. Wild stocks of species susceptible to IHHNV in the Gulf of California had been monitored since 1974, without a case of IHHNV infection being detected until 1987. Pantoja et al. (1999) showed that by 1990, IHHNV was common in wild stocks of *L. stylirostris* and present in wild stocks of *F. californiensis* and *L. vannamei* from the Gulf of California. The Joint Subcommittee on Aquaculture (1997) stated:

> *Beginning with the 1987–88 season, landings of blue shrimp (Saulnier et al. 2000) decreased by about 1000 tons per year for four consecutive years. Stocks began to recover only after about six years. This is the best chronological association of a disease and wild population effects currently known.*

However, in a subsequent workshop, the Eastern Research Group (1998) noted that:

> *it would be very difficult to diagnose the cause of a decline in a population of shrimp because many factors interact to cause natural population fluctuations of up to 25 percent per year*
>
> and concluded that:
>
> *identification of virus in the shrimp would indicate that the virus may have played a part in the change, but it would not establish a cause-and-effect relationship.*

The contribution of the introduction of IHHNV to the population decline remains unresolved.

There is a body of evidence emerging that prawn populations may rapidly develop tolerance or resistance to pathogenic agents that initially cause very serious disease in aquaculture. Although this may be the case, relatively minor stress events may predispose latently infected prawns to clinical disease.

Initially, yellowhead disease (YHD) and later, WSD, were associated with widespread epidemics in prawn aquaculture in South-East Asia in the early to mid-1990s. The prevalence of WSSV in wild prawns also reached high levels in the mid-1990s (Lo and Kou 1998).

In the latter 1990s, techniques to manage serious diseases in prawn aquaculture in the region combined with improved diagnostic techniques have lessened the impact of disease. The epidemiology of WSSV in severely affected regions has also altered. Flegel (1997a) stated:

> *The most intriguing feature of the WSBV [white spot baculovirus = WSSV] epizootic in Thailand is that many farmers now obtain good to excellent harvests (4–7 tons per hectare) in spite of finding a few specimens with gross signs of WSBV infection present in their ponds during the early stages of cultivation and then later throughout the cultivation cycle… . This contrasts with the situation at the height of the epizootic, where massive mortalities and essentially total crop losses were experienced. The shrimp appear to have rapidly developed a kind of tolerance or resistance to the new virus within a period of 1.5 years since it first caused heavy losses.*

Notably, by the end of the 1990s prawn aquaculture production for Thailand was approaching pre-WSD levels (Flegel 1997a).

The phenomenon of increased tolerance of prawns to initially serious pathogenic agents has now been observed by many researchers and prawn aquaculturists. That is not to say that WSD and YHD are no longer serious problems in prawn aquaculture in Asia. However, evidence would suggest that the worst of the YHD and then WSD epidemics in South-East Asia had passed by the mid to late 1990s. Consequently, the proportion of aquaculture ponds now emergency harvested (aborted early) would be lower than at the height of the epidemics.

The immune regulation of this putative tolerance or resistance is not understood, nor is the current epidemiology of WSSV in populations that are known to be infected. At this stage, there are few data on the prevalence of infection by pathogenic agents, such as WSSV, in 'tolerant' or 'resistant' populations or the typical level of infection (i.e. titre of pathogen).

The consequences of establishment of an exotic disease in Australian prawn aquaculture must be assessed in relation to characteristics of the local industry that may differentiate it from counterpart industries overseas. Prawn farming in Australia is a relatively small industry. In Queensland, the main prawn aquaculture state, there are about 14 hatcheries and 34 grow-out farms in operation. The farms are situated singly or in small groups along the State's approximately 2000 km eastern coastline. The Queensland Department of Primary Industries and Fisheries has developed a policy for the sustainability of land-based aquaculture. Through a coordinated whole-of-government assessment and decision-making process, applications for an aquaculture licence within 5 km of an existing operation require additional assessments to ensure that disease and environmental risks are minimised as the industry expands (Robertson 1999). Many Australian prawn farmers practise minimal water exchange policies in the interests of improving environmental management practices and sustainable aquaculture. The dispersed nature of the prawn aquaculture industry in Australia, and the trend of reducing water exchange rates, may help to prevent rapid spread of prawn pathogenic agents between farms and spread from farms to wild crustaceans. However, the spread of disease between farms might be exacerbated by the limited extent of structured surveillance and disease control policies in some States or Territories (or jurisdictions), as well as the generally limited biosecurity measures applied to the translocation of locally caught broodstock and their postlarvae between farms.

The complex interaction between host, environment and agent makes it difficult to forecast the potential effect of the establishment of exotic disease. The IRA team has assumed that farmed and wild prawns (including native species) in Australia would be at least as susceptible to infection as prawns of the same, or closely related, species reported as susceptible under similar conditions in other countries. In the case of pathogenic agents shown by overseas experience to be highly pathogenic (e.g. WSSV and YHV), it has been assumed that, where susceptible species exist in Australia, rates of morbidity and mortality would be comparable to those reported overseas.

The environment (native animals/plants, and non living environment)

In considering the significance of the establishment of disease, Biosecurity Australia also takes into account effects on the environment. The establishment of a new disease could affect the survival of native species that are not farmed or otherwise commercially exploited. For example, the ecological balance of aquatic systems and the quality of the environment could be disturbed if the normal proportions of different native species were significantly altered by the selective loss of one or more particularly disease-sensitive species.

In determining the likely effect of exotic pathogenic agents on Australian native species, consideration was given to evidence that the agents could infect a wide range of species or families, including any that are related to Australian native species. In the case of pathogenic

agents that infect a narrow or specific range of hosts that are unrelated to Australian species, it was assumed that effects on native species would be negligible. However, for exotic pathogenic agents that have a wide or non-specific host range, including prawn species that are related or similar to Australian species, it was assumed that native species would be susceptible to infection and that the establishment of disease would have consequences at least as severe as those reported overseas.

Indirect effects

Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)

Australia has a highly developed animal health system that can thoroughly investigate disease problems and a high priority is placed, both at national and State/Territory levels, on preventing exotic animal disease incursions. Contingency planning for aquaculture disease emergencies is well advanced at the national level. AQUAPLAN is Australia's national strategic aquatic animal health plan, jointly developed by governments and private industry sectors. Since the inception of AQUAPLAN in 1998, significant progress has been made on preparedness and response plans to deal with aquatic animal disease emergencies through development of AQUAVETPLAN manuals, including a specific strategy manual for WSD.

In addition, an emergency response infrastructure, Aquatic CCEAD[38], is well established and has been used on several occasions, including in response to the suspected outbreak of WSSV in 2000. The ensuing emergency response included a national surveillance exercise to confirm Australia's health status with respect to WSSV freedom, at an estimated cost of $172,000, which does not include in-kind contributions of State/Territory laboratory and field staff (I. East, Dept Agriculture Fisheries and Forestry, pers. comm. June 2006).

On that occasion, infection had not established. However, if it had established, then zoning of the affected area and introduction of interstate movement controls on live crustaceans and crustacean products, pending eradication of the agent, would have been expected.

In this regard, a conservative approach was taken in this risk analysis, in the light of the high cost associated with attempts to eradicate new aquatic animal diseases and the low likelihood of success. It was assumed that diseases that have been shown by overseas experience to be difficult or impossible to eradicate once established (e.g. WSSV and YHV) would present similar difficulties in Australia. It was considered that the size of Australia and the difficulty of managing remote areas, and the sparsity of population centres outside of the major capitals, as well as the problems of wet-season impassability of roads would further compound problems.

Environmental conditions (including husbandry) clearly influence the expression of clinical disease and the amenability of introduced disease to prevention and control. Thus, methods used with success overseas may not be feasible or similarly effective in Australia. For the diseases that are routinely controlled overseas by husbandry measures (e.g. reduced stocking rate), it was assumed that a similar approach would be applicable in Australia.

For some diseases there are clear parallels. For example, *P. monodon* nauplii can be washed to manage monodon baculovirus (MBV) in Australian hatcheries (Spann et al. 1993). These same techniques are used in aquaculture to manage other intranuclear bacilliform viruses such as *Baculovirus penaei* (BP) in the Americas. Thus, in the event that BMNV or BP were to become established in Australia, it is likely that these pathogenic agents could be controlled by similar means and the consequences of establishment on farmed prawns significantly mitigated.

[38] CCEAD – Consultative Committee on Emergency Animal Diseases.

There would be a need for regulatory approval of any drug that is not currently registered for use with prawns in Australia if such drugs were to be used to control a newly established disease. The costs of registration are significant. The implementation of a control strategy in aquaculture would introduce new costs and have adverse implications for product quality and image. For some pathogenic agents, the cost of implementation of measures for control or eradication would be so high as to be unfeasible in practice.

The costs of disease eradication or containment measures, including movement controls, would be expected to undermine farm profitability.

Economic (domestic trade effects and impact on other associated industries)

The establishment of a disease can harm economic performance of Australian prawn farms indirectly (e.g. through the costs of implementing additional farm management procedures, disease screening of broodstock, treatment of water and exclusion of pests). There may also be economic effects due to the loss of domestic (and international) markets, market oversupply and resulting reduction of prices received for product. Equally, significant production losses due to disease may (in addition to impacting farm profitability) adversely impact associated industries including processors and retailers. Farm insurance premiums may be increased or it may be necessary to increase stocking rates to offset the effects of mortality. Indirect impacts would also be expected to affect farms that are free of infection and would be most felt in those parts of Australia where crustacean farming (particularly prawn farming) makes a significant contribution the overall local economy, such as Cardwell, Innisfail and Ingham on the coast of north Queensland.

There is no clear documented evidence that the pathogenic agents under consideration in this IRA have adversely affected wild prawn fisheries. The impact of WSSV and IHHNV on wild fisheries was previously discussed. Similar mechanisms for the development of tolerance or resistance by farmed prawns to newly recognised pathogenic agents may occur in wild prawns. Predation of clinically ill prawns may limit spread of pathogenic agents in wild populations and favour the selection of highly tolerant or resistant strains of prawns.

However, any reduction in the commercial wild catch would be expected to decrease the capacity of a fishery to support the same number of fishers. A reduction the size of the fishery would also be expected to have commensurate impact on associated industries.

Recreational fisheries present a special case in that ‘production’ is not easily quantified. Prawns are most commonly caught with dip-nets or two-person hand-hauled nets in local estuaries, for human consumption or use as bait. Recreational fishing for prawns is a widespread fishing activity, particularly in Queensland and NSW. In NSW, there has been an increased focus on recreational fishing through the introduction of recreational fishing havens in 2002 by the NSW Department of Primary Industries-Fisheries. Recreational fishers in NSW harvested over 100 tonnes of prawns (Henry and Lyle 2003), which is approximately 4.5% of the total commercial prawn catch in NSW. Although spending by recreational fishers is likely to provide economic and social benefits to rural and regional areas, recreational prawn fishers represent only a few percent of total fishers, so that (in the event of the introduction, establishment and spread of an exotic pathogenic agent) economic losses associated with recreational prawn fishing would make a limited contribution to the total loss.

Economic (international trade effects)

The concept of zoning (regionalisation) is recognised in the *OIE Aquatic Code*. However, there are currently few animal health related restrictions on international trade in prawns or prawn products for human consumption. Thus, in practice, zoning of prawn diseases normally only applies to trade in sperm, fertilised eggs, nauplii, larvae, postlarvae, juveniles and broodstock.

However, Briggs et al. (2004) reported that several Central and South American countries

closed their borders to the importation of live, fresh and frozen prawns after the introduction of WSSV to the region in 1999 from unknown sources. Most of those countries imposed new regulations in late 1999 (e.g. Mexico) or 2000 (e.g. Ecuador), which typically included specifying imports of only SPF stocks from certified, tested and enclosed facilities to certified and controlled facilities with quarantine in the respective countries. They also insisted on PCR testing of all imported prawns for WSSV and YHV. Brazil had banned frozen prawn imports in 1999 to prevent the introduction of prawn diseases. Similarly, Mexico, Colombia and Nicaragua implemented mandatory PCR screening of frozen prawn imports as a precautionary measure to prevent the introduction of prawn diseases.

Brazil began importing non-indigenous prawns in 1980 and *L. vannamei* and *L. stylirostris* in 1983 from all parts of Latin America. This resulted in the introduction of various viral and bacterial diseases including IHHN, Taura syndrome and necrotising hepatopancreatitis. By 1998, Brazil began to invest more in captive breeding programs for *L. vannamei*. After WSSV arrived in Latin America in 1999, Brazil immediately closed its borders to imports of live, fresh or frozen crustaceans (including artemia) and polychaete worms (De Barros Guerrelhas 2003). To date, the restriction has been successful and Brazil remains free of WSSV and YHV. Because of this and successful genetic selection programs (i.e. for TSV-resistant strains), Brazil has increased its production more than 12-fold since 1998 to 60,000 metric tonnes in 2002 and an estimated 90,000 metric tonnes in 2003 (de Paiva Rocha 2004). Ecuador is reported to currently require frozen prawn imports to be tested for WSSV and TSV, and testing of imported postlarvae (for aquaculture) for WSSV, YHV and IMNV.

On the basis of the above, the establishment of WSSV, YHV, TSV or IMNV in Australia might have an adverse impact on export markets for Australian prawns.

If an exotic disease were to become established, Australia could use zoning to maintain access to international markets for live crustaceans including prawns and, if required, non-viable product, noting that importing countries may not necessarily accept zoning arrangements. Zoning would require additional specific regulatory measures such as movement controls, testing and certification, with attendant costs. In 2004–05, Australia exported more than 10000 tonnes of prawns valued at $163 million, 150 tonnes of which were live Kuruma prawns to Japan valued at $8.5 million. Export of live non-prawn crustaceans from April 2005 to May 2006 totalled approximately 6900 tonnes, comprising mainly lobsters (88%), crabs (11%) and crayfish (0.4%). Hong Kong, Japan, Taiwan and China were the major importers of live non-prawn crustaceans from Australia, taking 99% of the total product with Hong Kong importing 66%.

Environment (biodiversity, endangered species and the integrity of ecosystems)

The potential loss of biodiversity if an exotic pathogenic agent were to be introduced, establish and spread, would be of concern to the Australian community. A conservative approach was taken when considering the susceptibility of native species, particularly those that are endangered or threatened, to infection with exotic pathogens.

The DEWHA has the following crustacean species listed as *critically endangered*, *endangered* or *vulnerable*[39]:

Critically Endangered	*Cherax tenuimanus*	hairy marron, Margaret River hairy marron, Margaret River marron
	Engaewa pseudoreducta	Margaret River burrowing crayfish
	Engaewa reducta	Dunsborough burrowing crayfish
Endangered	*Engaeus granulatus*	Central North burrowing crayfish
	Engaeus martigener	Furneaux burrowing crayfish
	Engaeus spinicaudatus	Scottsdale burrowing crayfish
Vulnerable	*Astacopsis gouldi*	Tasmanian giant freshwater lobster, giant lobster, giant freshwater crayfish
	Engaeus orramakunna	Mount Arthur burrowing crayfish

In considering the consequences of establishment of an exotic disease, the establishment of any disease that is likely to result in the extinction of a species (which equates to having a serious, irreversible effect on the environment) would be classified as 'extreme'. In most cases, there is only limited information on the potential effect of exotic pathogenic agents in Australian conditions. However, in drawing conclusions on the likely impact of exotic disease on the environment, overseas data were considered on the species of prawns and other crustaceans that are susceptible to infection, the effect of infection on those prawn populations, and the influence of the physical environment on the outcome of infection.

Social (changes in tourism, side effects from control measures, and loss of social amenity)

Communities where prawn farming is a significant employer would be expected to experience social impacts such as increased management inputs, owner stress associated with loss of livelihood and welfare concerns (including family disruptions, loss of employment and decreased living standard), impacts on businesses and industries supporting rural centres and impacts of movement restrictions on social amenity. Such impacts would be most significant in areas where crustacean aquaculture, particularly prawn farming plays a major role in the local economy, for example Cardwell, Ingham and Innisfail in Northern Queensland.

Public perception of risks can significantly affect the markets for product for human consumption. This public reaction may occur whether the problem is effectively managed or not. The use of chemical treatments or the occurrence of lesions or blemishes on the product could also affect any price premiums paid for high quality products. This may occur regardless of whether the effect on quality was real or perceived. Further, pathogenic agents such as WSSV and microsporidians can cause visible lesions in crustacean tissues, and affected product would be unacceptable to the consumer for reasons of quality and aesthetic appeal.

[39] See DEWHA website at: http://www.environment.gov.au/

6 White spot syndrome virus

The following provides an estimation of the white spot syndrome virus (WSSV) risk associated with the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the pathogenic agent-specific information presented in this chapter, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

6.1 Release assessment

A wide range of decapod crustaceans, including marine and freshwater prawns, crabs and crayfish are reported to be susceptible to natural infection with WSSV. Feed for crustacean broodstock such as polychaete worms have been identified as mechanical carriers of WSSV. All prawn species imported into Australia are susceptible to WSSV; most decapod crustaceans in Australia would be either susceptible to clinical disease or be asymptomatic carriers of WSSV.

Little information is available on the prevalence of WSSV or white spot disease (WSD) in prawn aquaculture. Serious outbreaks of WSD were common throughout Asia in the mid-1990s (Lightner 1999) and in South America in the late 1990s. Ponds that are seriously affected by WSD may be emergency harvested if the prawns are of commercial value (Jory and Dixon 1999). In a study conducted on one farm in Thailand in 1996, Withyachumnarnkul (1999) found that all ponds in which WSSV-infected prawns were detected, at some stage in the production cycle, experienced an outbreak of WSD. About half of these severe outbreaks resulted in crop failure (i.e. the crop was terminated before the prawns were big enough for commercial harvest) while 50% of crops were harvested. Severe WSD outbreaks in farmed prawns are stress-induced and are more common during the monsoon season (Karunasagar et al. 1997, Limsuwan 1999, Peinado–Guevara and Lopez–Meyer 2006).

In contrast, Flegel (1997a) reported a trend in the Asian region of excellent farm harvests from ponds in which there were a few prawns displaying gross clinical signs of WSSV infection. *Penaeus monodon* embryos and very early stage larvae that were infected with WSSV and held in tanks for their entire lives, showed a consistently high prevalence of WSSV infection but mortalities occurred only once the prawns were 13 months old (Tsai et al. 1999).

Clinical signs observed in farmed prawns may include rapid onset of high mortality and the presence of white spots in the cuticle. The presence of clinical signs is variable — in some animals the only sign noted may be mortality. Infection may be patent, latent or transitional. In patent infections, clinical signs (including mortality) are evident within 2–10 days. Latent infections may continue for extended periods. It is possible that overseas processors may remove prawns displaying obvious clinical signs of WSD and divert the inferior product to more highly processed procedures to be transformed into saleable product forms e.g. by removing the head and shell for sale as prawn meat.

In laboratories, prawn viruses are typically stored frozen in tissue or in various states of purity and in media. Wang et al. (1999a) propagated WSSV that was frozen at –70°C in prawn tissue for two years. Wang et al. (1997b) transmitted WSSV by feeding prawns with infected prawn tissue that had been frozen at –20°C for an unspecified period. Nunan et al. (1998) transmitted WSSV to susceptible prawns by injecting a homogenate made from the pleopods of frozen prawn tails purchased from a supermarket in the US. The prawn tails had white spots typical of WSD.

Infectious WSSV (verified through PCR testing and bioassay) has been detected in Australia in imported frozen uncooked prawns intended for human consumption (McColl et al. 2004). In one incident, WSSV DNA was detected in imported frozen uncooked prawns, intended for human consumption, that were inadvertently fed to crustacean hatchery broodstock (all exposed hatchery animals were promptly destroyed) — the incident did not result in an outbreak of clinical disease (East et al. 2004). WA and QLD State Government PCR testing in 2006 of raw peeled imported supermarket prawns found 20-100% WSSV prevalence in the 14 batches tested (5 prawns tested per batch).

Conclusions

- WSSV has a wide decapod host range and all prawn species would be susceptible to infection. Serious disease occurs when WSSV is first introduced into an area, although cases of serious disease and the practice of emergency harvest following the entry of WSSV into new areas occurs less frequently over time. Emergency harvest of prawns from ponds in the early stage of a WSD outbreak is not uncommon, even when prawns are relatively small. There is evidence that WSSV infection occurs at a high prevalence in commercial prawns produced in areas that were severely affected several years before — so there is a high likelihood that prawns harvested for the export market from such areas are infected with WSSV.
- WSD is typically associated with obvious white spots in the cuticle of infected prawns. Crops harvested in these circumstances would contain many prawns that are smaller than usual and many prawns with white spots on the cuticle. However, the presence of clinical signs is variable, and clinically and subclinically infected prawns would be expected to routinely pass inspection procedures.
- It is expected that the amount of virus in such prawns would generally be lower than from crops affected by serious disease, although individual infected prawns from apparently healthy crops may contain a high virus concentration — prawns that pass post-harvest inspection may therefore have significant amounts of virus.
- WSSV (verified through PCR testing) has been detected in Australia in imported frozen uncooked prawns. WSSV in the tissues of harvested prawns would be expected to survive commercial freezing, storage and transport to Australia.

WSSV Liklihood of resease (LR)

The LR of WSSV via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be high.

6.2 Exposure assessment

WSSV infects ectodermal and mesodermal tissues, and infections are most severe in the epidermis, and the gut and gill epithelia. In an infected prawn, the cephalothorax contains about half of the total amount of WSSV and the tail shell, about one third (Durand et al. 2003). Head and shell wastes would therefore be expected to contain significant amounts of virus.

WSSV in tissues of prawns is known to retain infectivity through storage at sub-zero temperatures. Purified WSSV remained viable for at least 28 days in sterile seawater kept in dark conditions at temperatures of up to 30°C (Momoyama et al. 1998) — under normal pond conditions WSSV is infectious for about three days after removal of infected prawns. Given this, WSSV in uncooked prawns or associated wastes is likely to remain viable at the point of exposure, be it to susceptible animals in hatcheries, farms or in the wild.

WSSV has a very wide host range and can infect many life stages of crustaceans. Prawns, freshwater crayfish and other crustaceans are reported to be susceptible to WSSV infection, and are common in freshwater and marine environments throughout Australia. However, the

diagnostic PCR test recommended in the *Manual of Diagnostic Tests for Aquatic Animals 2006* (OIE 2006b) is known to give false positive reactions due to a cross-reaction with crustacean DNA (Claydon et al. 2004). Thus, the list of species reported as susceptible to WSSV should be interpreted with some caution.

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and, to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels (resulting in infected prawn tissues entering ponds through intake water), are potentially significant WSSV exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to WSSV. An incident of WSSV DNA detection in Australia was attributed to inadvertent feeding of hatchery prawns and crabs on infected, imported, frozen, uncooked prawns. Both clinically and subclinically infected prawns would be expected to contain significant amounts of virus. All species of crustaceans cultured in Australia, including prawns, are susceptible to WSSV.
- In the absence of any import controls, it is expected that significant volumes of prawns or associated wastes potentially infected with WSSV would be directed for use as recreational fishing bait or berley. As such, bait-use represents a significant pathway by which wild crustaceans (exposure group 3) could become exposed to WSSV. Disposal of untreated waste from commercial processing premises into natural waters also represents a potentially significant pathway for exposure of wild crustaceans to WSSV. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.
- Uncooked prawns, or parts thereof, used for bait, and prawn tissues in untreated liquid waste from commercial processing of imported prawns disposed into natural waters could contain significant amounts of virus, particularly if the prawns had been emergency harvested due to WSD — much of this virus would be expected to remain infectious at the point of exposure.
- Most bait and commercial processing waste material is expected to be taken by non-susceptible finfish species. However, prawns, freshwater crayfish and other crustaceans are common in freshwater and marine environments throughout Australia, and are likely to encounter some of this material — WSSV has a wide decapod host range and all prawn species and most other decapod species in Australia are expected to be susceptible to infection.

WSSV Partial likelihood of exposure (PLE)

The PLE to WSSV from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*low*
Exposure group 2	$PLE_{HATCHERY}$	*high*
Exposure group 3	PLE_{WILD}	*high*

6.3 Consequence assessment

6.3.1 Partial likelihood of establishment or spread

WSSV has a very wide host range and can infect many life stages of crustaceans. Prawns, freshwater crayfish and other crustaceans known to be susceptible to WSSV infection are common in freshwater and marine environments throughout Australia.

WSSV is effectively transmitted via water or when a susceptible crustacean consumes infected tissues. Infections have been found in all life stages of penaeids (Chang et al. 1998a, Wang et al. 1999a). In trials with penaeids, a single feed of 5% bodyweight of heavily infected tissue resulted in WSSV transmission (Wang et al. 1999a). The introduction of a single dead infected prawn into a pond can cause several prawns to become infected as several prawns may feed on one dead prawn. For infected broodstock, it has been postulated that vertical transmission of WSSV within the egg may occur but this has not been proven (Mohan et al. 1997).

The IRA team noted that non-susceptible finfish species have a high predation rate for prawns. This may limit the opportunity of establishment or spread of diseases in the wild, from the limited numbers of index cases of infection that might result from the exposure of wild prawns to recreational fishing bait. The IRA team was of the view that the escape of large numbers of infected prawns from aquaculture ponds (or prolonged low level escape), especially into habitats where they would be protected from finfish predators, would pose a greater risk. The appearance of WSSV in 2001 in wild Gulf of California *L. vannamei* inhabiting a coastal zone with high prawn aquaculture activity (possibly due to infected prawns escaping into the wild following hurricane Julliete that year) and its absence in 2003 as reported by Mijangos–Alquisires et al. (2006) supports this view.

In 2000, imported prawns were fed to broodstock in a Northern Territory crustacean hatchery, resulting in a national emergency animal disease response to suspected establishment of WSSV in Australia. In that instance, five of 12 shore crabs collected adjacent to the hatchery outfall tested PCR-positive for WSSV (East et al. 2004). However, follow-up testing at the same site and a subsequent national survey showed that WSSV was not present in Australia.

Conclusions

- WSSV can be transmitted via waterborne virus or *per os* by ingestion of infected tissues. It is expected that susceptible host animals feeding on tissues of imported prawns that had been heavily infected with WSSV would receive an infectious dose of the virus.
- For a given quantity of infected material entering the environment of an exposure group, the likelihood of WSSV establishment is higher for farmed and hatchery crustaceans (exposure groups 1 and 2, respectively) than for wild crustaceans (exposure group 3) because of the greater density of susceptible animals that are more prone to infection under the environmental conditions associated with aquaculture.
- In the event that one or more index cases of WSSV infection were to occur, virus establishment or spread to other susceptible animals would be unlikely in the case of wild crustaceans (exposure group 3) because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish.
- Spread of WSSV from hatcheries to farms is likely. The likelihood of the spread of WSSV from farms to neighbouring farms or wild prawn populations through waterborne virus in effluent water would be moderated by dilution effects. The likelihood of WSSV spread from farms to wild populations or neighbouring farms via escaped prawns may be higher, especially if large numbers of prawns escape *en masse*.

- The likelihood of spread from farms and hatcheries would be reduced by farm and hatchery level control measures that are likely to be implemented on detection of WSSV — the likelihood of WSSV detection is high given that the agent would cause clinical disease in the key species farmed in Australia.
- In the unlikely event that WSSV did establish in a localised wild prawn population for an extended period, the virus may eventually spread to other wild populations and subsequently to farm and hatchery populations.

WSSV Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*very low*
Outbreak scenario 2 (no establishment):	*high*

6.3.2 Impacts

Many crustacean species that occur in Australia are susceptible to WSSV infection and many of these are economically or environmentally important.

Farmed prawns would be most severely affected by WSD, particularly in the period immediately after first establishment of disease. When WSD first enters a region, susceptible species (such as *P. monodon*, *F. merguiensis* and *M. japonicus*) in aquaculture conditions can suffer 100% mortality within 3–10 days of the first signs of clinical disease (Lightner 1996a).

Should WSSV establish in populations from which broodstock prawns are sourced for aquaculture, the movement of broodstock and postlarvae would be expected to result in rapid dispersal of WSSV throughout the prawn aquaculture industry. Losses due to WSD would be significant over several crops. The severity of WSD outbreaks in an area tends to decrease after about 1.5 years, postulated to be due mainly to the development of tolerance or resistance in local prawns (Flegel 1997a). This effect, combined with management activities, would be expected to result in reduced losses to WSD after a few years.

Alliance Resource Economics 1999 determined that losses due to establishment of WSSV would probably be small relative to the industry as a whole (farmed and capture fisheries). Maximum national cost during the initial incursion was an estimated $17 million per year and $7 million during the epidemic phase. The 'seriousness' of WSSV incursion as determined in relation to the total value of the industry at the time was determined to be a 2% loss, attributed mainly to increased production costs. The report concluded that while disease impact on the industry as whole would be small, the economic impact on the farm sector would be significant especially during the initial incursion.

Based on recent studies on the epidemiology of WSD in farmed prawns in Asia, farmers are being encouraged to test postlarvae for WSSV and to accept only those that test negative (using one-step PCR, but that may test positive using two-step PCR). The researchers have found that by this method, prawns can be harvested before the WSSV in the population rises to the point at which ponds 'crash' (Callinan, ACIAR Project ID: FIS/2000/061: Development and delivery of practical disease control programs for small-scale shrimp

farmers in Indonesia, Thailand and Australia[40]).

The implementation of systems to manage WSD, including testing and decreased stocking, would add significantly to the cost of prawn production.

If WSSV were to become established in wild crustacean populations in the vicinity of prawn aquaculture facilities, WSSV could be introduced to farmed populations via movements of wild crustaceans. The establishment of WSSV in a prawn hatchery could be followed by rapid spread (via postlarvae) and serious losses.

The establishment of WSSV in an individual prawn farm could be followed by spread to adjacent farms, then general dispersal within a region and interstate spread. The initial general effects on affected farms, regions and, potentially, entire States would be significant.

The wide host range for WSSV suggests that many crustacean species in the wild, particularly penaeid and parastacid species, in Australia would be susceptible to WSSV infection. The potential consequences of WSSV infection of these species are difficult to forecast. North American freshwater crayfish in captivity are highly susceptible to WSD (Lightner et al. 1997a; Richman et al. 1997; Wang et al. 1998). Of the *Cherax* species in Australia, *C. quadricarinatus* has been shown experimentally to be susceptible to infection with WSSV via injection (Shi et al. 2000). *Cherax destructor albidus* has also been shown to be susceptible via injection and ingestion of infected tissue, although results suggested that *C. destructor albidus* would be less susceptible to infection than *P. monodon* via natural routes of infection, both in farms and in the wild (Edgerton 2004). *Panulirus* species and *Scylla serrata* are reported to have a low susceptibility to clinical WSD (Supamattaya et al. 1998, Wang et al. 1998, Rajendran et al. 1999).

Throughout Asia there has been no reported decline in catch rates from wild prawn populations with a high prevalence of WSSV (Baldock 1999). The absence of an observable effect on wild prawn populations may be due to lower stress levels in wild prawns and lower levels of infection. However, given uncertainties surrounding the ability of current methods to detect significant impacts to wild crustacean populations, there is considered to be insufficient evidence to make a clear determination on the effect of WSSV on wild prawn fisheries in Australia.

WSD is on the *National List of Reportable Diseases of Aquatic Animals* and would also be reportable under State/Territory government legislation. Also, WSD is specifically covered by an AQUAVETPLAN manual and thus, there exists a pre-agreed strategy for control and eradication in the event of a reported outbreak. AQUAVETPLAN (Edition 2) states:

> *the choice of response option will be decided by the director of fisheries and/or the chief veterinary officer (CVO) of the State or Territory in which the outbreak occurs, following initial epidemiological investigations. There are three possible response options for WSD control in Australia:*
>
> *Option 1 — eradication with the aim of having Australia return to being free from WSV;*
>
> *Option 2 — containment, control and zoning of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas; and*
>
> *Option 3 — control and mitigation of disease if it is accepted that the virus will remain endemic in Australia.*
>
> *All these response options involve the use of a combination of strategies, which may include:*

[40] Available at: http://www.aciar.gov.au/

- *quarantine and movement controls on crustaceans and things in declared areas to prevent spread of infection;*
- *destruction of all clinically diseased or dead prawns as soon as possible, to prevent further virus shedding;*
- *decontamination of facilities, products and things to eliminate the virus from infected premises and to prevent spread of infection;*
- *surveillance to determine the source and extent of infection and to provide proof of freedom;*
- *zoning to define and maintain infected and disease-free zones; and*
- *hygiene and biosecurity measures aimed at mitigating the on-farm effects of WSD.*

Eradication may not be feasible if epidemiological investigations determine that WSV infection is widespread across most or all Australian prawn producing zones, has no controllable source or is otherwise unable to be contained. Similarly, the feasibility of containment and zoning will depend on farm management practices, the extent to which infection has already spread, and the location, distribution and migratory behaviour of infected species. If infection is widespread, and there is evidence of widespread infection in wild broodstock populations, control and mitigation of the disease is likely to be the most appropriate option.

Brazil, Mexico and Ecuador introduced restrictions on the importation of live, fresh or frozen prawns, following introduction of WSSV into the region in 1999 (Briggs et al. 2004). Mexico and Ecuador currently require PCR testing of all frozen prawn imports for WSSV and TSV. Some potential markets for Australian exports, particularly of live crustacean, may be affected if WSSV were to establish in Australia. The major export markets for Australian crustaceans do not currently have animal health related restrictions.

Conclusions

- WSSV would be expected to cause serious prawn aquaculture production losses, causing significant impacts to multiple regional prawn farming areas in multiple States/Territories — although these impacts would ameliorate over a few years as has occurred overseas, including through adoption of new husbandry practices.
- The potential impact on native animals and any associated ecological ramifications are unknown and in view of this uncertainty and in the context of the wide host-rage of WSSV, an appropriately cautious approach is warranted. Once established in wild crustacean populations, eradication is unlikely.
- WSD is an OIE-listed disease. It is on the Australian *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments would report on the agent. WSD is specifically covered by an AQUAVETPLAN manual and thus, there exist pre-agreed strategies for control and eradication in the event of a reported outbreak. However, because of the difficulties inherent to the eradication of aquatic animal diseases from wild populations, a campaign aimed at eradicating WSSV from wild crustacean populations is unlikely to be initiated. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely — associated economic impacts, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- Multiple regions (where prawn farming makes a significant contribution to the local economies) would be expected to experience significant flow-on economic impacts as an indirect result of WSSV establishment and spread.
- WSSV establishment and spread may potentially result in loss of live crustacean export markets, e.g. as a result of possible loss of the Chilean market for Australian

live yabbies. These international trade impacts would be minor, local level impacts.

- DEWHA lists three crustacean species as *critically endangered*, three as *endangered* and four as *vulnerable*. All of these are decapod crustaceans and are expected to be susceptible to WSSV infection; it is not known whether they would be prone to clinical disease — a conservative approach has been adopted in light of this uncertainty.
- The social impacts of WSSV establishment and spread would mostly be on communities where prawn farming and possibly, capture fisheries play a major role in the local economy (e.g. Cardwell, Ingham and Innisfail in northern Queensland); such impacts are expected to be minor at multiple local levels.

The impacts of WSSV establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	State/ Territory (multiple)	Significant	F
The environment (native animals/plants, and non-living environment)	Regional (multiple)	Significant	E
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Regional (multiple)	Significant	E
Economic (international trade effects)	Local	Minor	B
Environment (biodiversity, endangered species and the integrity of ecosystems)	Regional (multiple)	Significant	E
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local (multiple)	Minor	C
Overall impact (negligible – extreme)		*High*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

6.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'.

The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Moderate*	*Moderate*	*Very low*
Impact	*High*	*High*	*High*
'likely consequences'	*High*	*High*	*Low*
Outbreak scenario 2			
PLES	*Moderate*	*Moderate*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*High*	*High*	*Low*

6.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*High*	*High*	*Low*	*High*	*High*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*High*	*High*
'Likely consequences'	*High*	*High*	*Low*
Partial annual risk	*Moderate*	*High*	*Low*

Conclusion

The unrestricted risk associated with WSSV is determined (by combining the three partial annual risks associated with each exposure group) to be *high*. The unrestricted risk exceeds Australia's ALOP and, therefore, risk management is deemed necessary.

7 Infectious hypodermal and haematopoietic necrosis virus

A strain of IHHNV closely related to the Indian Ocean strain is endemic in Australia. The 2006 revised draft IRA report concluded that more pathogenic strains were exotic and therefore a potential hazard that warranted risk assessment. The risk assessment found the importation of uncooked whole prawns exceeded Australia's ALOP with respect to IHHNV risk and determined the need for risk management.

In July 2008 Australia notified the detection of a strain of IHHNV in farmed Queensland prawns very similar to those strains found in Asia. As a result, IHHNV is no longer considered a potential hazard (see Table 4.1).

Information gathered on IHHNV and presented in the 2006 Revised Draft IRA Report has been retained and included in Appendix 3.

8 Taura syndrome virus

The following provides an estimation of the Taura syndrome virus (TSV) risk associated with the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the pathogenic agent-specific information presented in this chapter, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

8.1 Release assessment

Natural infections of TSV occur in penaeid prawns of commercial importance including *L. vannamei* (Lightner et al. 1995), *Litopenaeus stylirostris*, *Litopenaeus setiferus* (Overstreet et al. unpublished data cited from Bonami et al. 1997), *Penaeus monodon* and *Metapenaeus ensis* (Chang et al. 2004). Outbreaks of Taura syndrome have been mostly associated with farmed *L. vannamei*, although an outbreak in farmed *L. stylirostris* in the Americas is reported to have resulted in up to 90% mortality (Erickson and Lightner 2001). Experimental infection has been reported in several other prawn species including *Farfantepenaeus aztecus*, *Farfantepenaeus duorarum*, *Fenneropenaeus chinensis*, *L. setiferus* (Overstreet et al. 1997), *Palaemon styliferus*, *Litopenaeus schmitti* (Brock 1997), *P. monodon* (Srisuvan et al. 2005), *Fenneropenaeus merguiensis*, *Marsupenaeus japonicus* (Brock et al. 1997), *M. ensis* and *Metapenaeus monoceros* (Ruangsri et al. 2004).

Prevalence of TSV in affected prawn farming regions around the world varies greatly between countries, within countries, within regions and between individual farms. The high variation is attributed to many factors including the species farmed, seasonal variation and the diagnostic tests employed. Current prevalence data from Thailand indicates TSV in *L. vannamei* hatcheries and grow-out ponds is typically less than 7% (Ruangsri et al. 2004, NACA 2004a, NACA 2005b, NACA 2005c, NACA 2005d). In one case, 10/10 headed and shelled vannamei prawns imported to Australia from China from a single 500-gram pack were found to be PCR-positive for TSV, and 6/10 were positive from a 1 kg pack (Ueda et al. 2008).

Taura syndrome in *L. vannamei* aquaculture exhibits as acute onset mortality 2–7 weeks after stocking postlarvae, when animals are less than 5 grams in size (Brock 1995, Lightner et al. 1995). Cumulative mortalities in juveniles may exceed 90% within three weeks of displaying clinical signs (Brock 1995). Disease may occur at any stage of the grow-out cycle (Brock 1995, Brock 1997, Lotz 1997b), although cumulative mortalities in adult populations are usually less than 50% (Brock 1995). Environmental stressors such as temperature and salinity changes following heavy rain can result in recurrence of disease outbreaks in chronically infected aquaculture stock (Edwards 1998, Lotz et al. 2005).

Clinical signs associated with Taura syndrome are non-specific and include lethargy, anorexia, ataxia, red or blue colouration, and soft-shell (Chamberlain 1994, Brock 1995, Lightner et al. 1995, Yu and Song 2000). Most prawns with Taura syndrome die within one week, although some prawns may survive and become chronic carriers showing multiple melanised cuticular lesions that diminish following further moulting cycles (Brock 1995, Lightner et al. 1995).

Viral load in apparently healthy TSV carrier prawns can remain stable in the first 40 weeks post-infection and then decreases by several orders of magnitude in chronically infected animals (Tang et al. 2004).

TSV remains infectious after freezing at 0°C (Brock 1995), –70°C (Bonami et al. 1997,

Overstreet et al. 1997, Tang et al. 2004) and –80°C (Hasson et al 1995). TSV can remain infectious in water for up to 48 hours, in prawn head tissues for at least 14 days, and in prawn tail tissues for at least 21 days at 27°C (Prior and Browdy 2002). TSV has been shown to maintain infectivity following passage through the gastrointestinal tract of seagulls and chickens that have been experimentally fed infected prawns (Garza et al. 1997, Vanpatten et al. 2004), whereas YHV and WSSV do not (Vanpatten et al. 2004). Australian imports of frozen, headed and shelled uncooked vannamei prawns originating from China have been shown to be PCR-positive for TSV (Ueda et al. 2008). WA and QLD State Government PCR testing in 2006 of raw peeled prawns available in supermarkets from China, Indonesia, Thailand, and Vietnem detected TSV in half of the 14 batches tested, with prevalence in those TSV positive batches ranging from 20% to 80% (5 prawns tested per batch). It is highly likely that imported frozen uncooked prawns that are infected with TSV at the time of harvest would contain viable virus.

The introduction of TSV into new areas is primarily accredited to the movement of live prawns, particularly broodstock and postlarvae (Brock 1995, Lightner et al. 1995, Tu et al. 1999, Yu and Song 2000, Limsuwan 2003a, Nielsen et al. 2005).

Conclusions

- The natural host range of TSV is limited and infections in aquaculture are primarily associated with juvenile *L. vannamei*. Serious disease, and associated high prevalence of infection, occurs mainly when TSV is first introduced into an area. Emergency harvest of prawns from ponds during a Taura syndrome outbreak is known to occur, but is not common due to the very small size of the prawns that are typically affected (less than 5 grams).
- Taura syndrome is a serious disease of juvenile vannamei prawns which usually results in very high mortality. The surviving adult prawns that carry TSV can have an unmarketable appearance and are generally of very poor quality. Most juvenile prawns with Taura syndrome would have melanised cuticles. These prawns would be detected during inspection and rejected for human consumption. However, clinical signs are not always apparent in adult prawns, especially following further moulting cycles — these prawns may pass inspection procedures.
- The amount of virus in apparently healthy carrier prawns can remain stable in the first 40 weeks post-infection (although grow-out periods are unlikely to exceed 3–4 months).
- TSV in the tissues of harvested prawns would be expected to survive commercial freezing, storage and transport to Australia. Frozen uncooked vannamei prawns imported into Australia have tested PCR-positive for TSV.

TSV Likelihood or release (LR)

The LR of TSV via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be *high*.

8.2 Exposure assessment

TSV infects cells of the cuticular epithelium of the general body surface, appendages, mouth, oesophagus, stomach and hindgut (Hasson et al. 1995, Lightner et al. 1995). The antennal gland tubule epithelium is rarely affected (Lightner et al. 1995). However, the sub-cuticular connective tissue and adjacent striated muscle fibres basal to cuticular epithelial cells are also involved (Lightner et al. 1995). In chronic infections, TSV is concentrated in the lymphoid organ (located in the cephalothorax or 'head' of a prawn), but may also be detected in other tissues. In chronically infected adults that reach market size, the highest viral titres would be in the lymphoid organ (Tang et al. 2004).

TSV is stable in frozen tissues and can remain infectious in water for up to 21 days at 27°C (Prior and Browdy 2002). TSV can maintain infectivity following passage through the gastrointestinal tract of seagulls and chickens (Garza et al. 1997, Vanpatten et al. 2004). Head and shell wastes would be expected to contain high virus titres, particularly in prawns emergency harvested due to Taura syndrome.

TSV has a limited natural host range with infections reported from a range of marine prawn species including; *L. vannamei* (Lightner et al. 1995), *Litopenaeus stylirostris*, *Litopenaeus setiferus* (Overstreet et al. unpublished data cited from Bonami et al. 1997), *Penaeus monodon* and *Metapenaeus ensis* (Chang et al. 2004). However, a number of prawn and other freshwater and marine crustacean species are known to be susceptible to experimental infection, at least by injection challenge, e.g. krill (*Acetes* sp.) and crabs (*Scylla serrata* and *Sesarma* sp.) (Ruangsri et al. 2004).

The Biosecurity Australia-commissioned infection trials (conducted by Professor Donald Lightner) at the University of Arizona, using a Thai and a Belize isolate of TSV, on five species of Australian crustaceans, showed that *F. merguiensis* and *P. monodon* could be infected with TSV (although the virus did not cause severe disease or significant mortality)[41]. *Fenneropenaeus merguiensis* became infected with the Thai isolate of TSV by injection but not following *per os* challenge. *Penaeus monodon* did not become infected with the Thai isolate of TSV; some were infected with the Belize isolate of TSV, although only after injection. *Cherax quadricarinatus*, *Cherax tenuimanus* and *M. rosenbergii* were seen to retain or sequester the virus (or the RNA of the virus) — the virus did not form an active (replicative) infection. A repeated trial on *P. monodon* found that prawns challenged *per os* with the Belize isolate tested RT-PCR-positive, but that the virus did not produce an infection that could be demonstrated by routine histology or by *in situ* hybridization. The researchers considered the RT-PCR finding was likely due to remnants of circulating injected TSV, rather than due to an active infection. None of the five species was successfully infected *per os*, using either of the TSV isolates.

Penaeus monodon is the most important commercial aquaculture species in Australia. Brock (1997) considered *P. monodon* to be susceptible to infection with TSV, but largely resistant to Taura syndrome, as supported by the University of Arizona findings. Chang et al. (2004) reported natural and experimental infection of *P. monodon* and *M. ensis* using two atypical Taiwanese TSV isolates, although clinical signs were only observed in *M. ensis.* Phalitakul et al. (2006) found that a Thai TSV isolate (ThMay04PmPL-TSV) naturally infected and caused mortality in cultured *P. monodon* and suggested good adaption of TSV to this species.

Metapenaeus ensis (the red Endeavour prawn) and the closely related *M. endeavouri* (the blue Endeavour prawn) are abundant and widely distributed in the tropical and sub-tropical waters around Australia (Kailola et al. 1993).

[41] Researchers reporting on the trial defined 'infection' to mean the presence of an actively replicating agent within the host and not its mere presence — experimental animals were not considered to be infected with the virus unless the virus was detected by histological methods or by *in situ* hybridisation.

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels, are potentially significant TSV exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to TSV. The highest TSV concentrations would be expected in clinically diseased juvenile vannamei prawns. Most of any virus present would be expected to remain infectious at the point of exposure. *Penaeus monodon*, the most important commercial aquaculture species in Australia, is considered to be susceptible to infection with TSV (although largely resistant to significant clinical disease). However, Biosecurity Australia-commissioned infection trials found that monodon prawns injected with a Thai isolate of TSV did not become infected, but did become infected via injection with a Belize isolate — a follow-up study confirmed that *per os* challenge with the Belize isolate could not produce an active infection.
- In the absence of any import controls, it is expected that significant volumes of imported *L. vannamei* or associated wastes potentially infected with TSV would be directed for use as recreational fishing bait or berley — the volume of uncooked vannamei prawns imported into Australia from Asia for human consumption has increased significantly over the past 3–4 years. As such, bait and berley use represents a significant pathway for exposure of wild crustaceans (exposure group 3) to TSV. Disposal of untreated wastes from commercial processing premises into natural waters also represents a significant pathway for exposure of wild crustaceans to TSV. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.
- Uncooked prawns, or parts thereof, used for bait, and prawn tissues in untreated liquid waste from commercial processing of imported prawns disposed into natural waters, could contain significant amounts of virus, particularly if the prawns had been emergency harvested due to outbreak of Taura syndrome — much of this virus would be expected to remain infectious at the point of exposure. The simple, single-stranded, non-enveloped virion structure confers on TSV a greater stability compared to some other lipid-containing enveloped viruses such as WSSV and YHV.
- Much of any bait and commercial processing waste material is expected to be taken by non-susceptible finfish and crustacean species. The number of crustacean species in Australia that would be susceptible to TSV infection under these circumstances is considered to be few — the natural host range of TSV is narrow (notably in comparison WSSV). However, *M. ensis*, which is abundant and widely distributed in the tropical and sub-tropical waters around Australia, is known to be susceptible to TSV infection.

TSV Partial likelihood of exposure (PLE)

The PLE to TSV from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*low*
Exposure group 2	$PLE_{HATCHERY}$	*low*
Exposure group 3	PLE_{WILD}	*moderate*

8.3 Consequence assessment

8.3.1 Partial likelihood of establishment or spread

TSV would have a limited natural host range in Australia, compared to WSSV and IHHNV. Some potential host species in Australia such as *P. monodon* and *M. ensis* have been reported to carry species-specific TSV isolates (Chang et al. 2004, Phalitakul et al. 2006) — these most likely originate from, but are different to the *L. vannamei* TSV strains (TSV strains A, B and C) from the Americas (Flegel et al. 2003, Chang et al. 2004, Nielsen et al. 2005, Tang and Lightner 2005, Phalitakul et al. 2006). The Asian (Taiwanese) TSV isolates were reported from wild *P. monodon* broodstock (PmTSV) and farmed adult *M. ensis* (MeTSV). The MeTSV isolate reportedly caused clinical disease in *M. ensis*, whilst *P. monodon* broodstock infected with the PmTSV isolate showed no disease signs (Chang et al. 2004). Phalitakul et al. (2006) found that a Thai TSV isolate (ThMay04PmPL-TSV) naturally infected and caused mortality in cultured *P. monodon* and suggested good adaption of TSV to this species. Many other species of crustacean have been infected with TSV under experimental conditions, but are characteristically refractory to clinical disease. TSV infection trials commissioned by Biosecurity Australia found that *P. monodon* did not become infected with a Thai isolate of TSV but some were infected with the Belize isolate, but only after injection — *per os* challenge did not result in an active (replicative) infection, as would be evidenced by histopathological changes or positive reaction to *in situ* hybridisation tests.

Given the species of prawns farmed in Australia (*P. monodon*, *M. japonicus*, *P. esculentus* and *F. merguiensis*), TSV is unlikely to readily establish in exposed farm populations. The natural host range of TSV in Australia is expected to much narrower than compared to WSSV or IHHNV.

Natural spread of TSV in *L. vannamei* occurs by both waterborne transmission and ingestion of infected tissues, causing acute mortality 2–7 weeks after stocking of postlarvae into grow-out ponds.

Spread of TSV via mechanical vectors such as seagulls is a possibility; however, the primary means by which TSV has spread overseas is considered to be the movement of live prawns, as demonstrated by the spread of TSV throughout Asia and the Americas.

Conclusions

- Susceptible species of crustaceans feeding on prawns that had been heavily infected with TSV may receive an infectious dose of the virus — TSV can be transmitted via waterborne virus or *per os*, by ingestion of infected tissues.
- For a given quantity of infected material entering the environment of an exposure group, the likelihood of TSV establishment is higher for farmed and hatchery crustaceans (exposure groups 1 and 2, respectively) than for wild crustaceans (exposure group 3) because of the greater density of susceptible animals that are more prone to infection under the environmental conditions associated with aquaculture.
- In the event that one or more index cases of TSV infection were to occur, virus establishment and spread to other susceptible animals would be unlikely in the case of wild crustaceans (exposure group 3) because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish. Further, this likelihood would be less than that for WSSV or IHHNV, due to the narrower host range of TSV. However, compared to WSSV, TSV is less likely to cause clinical disease and infected prawns may therefore, be less prone to predation. Also, TSV would not be inactivated by passage through the bird digestive system and as such, spread via mechanical vectors such as seagulls is possible.
- Spread of TSV from hatcheries to farms is likely where susceptible species are involved. The likelihood of the spread of TSV from farms to neighbouring farms or

wild prawn populations through waterborne virus in effluent water would be moderated by dilution effects. The likelihood of TSV spread from farms to wild populations or neighbouring farms via escaped prawns may be higher, especially if large numbers of prawns escaped *en masse*, although the likelihood is expected to be less than that for WSSV because of the narrower host range of TSV.

- The likelihood of spread from farms and hatcheries would be reduced by farm and hatchery level control measures that are likely to be implemented on detection of TSV — although the IRA team notes that the likelihood of TSV detection is minimal given that clinical disease is not expected.
- If in the unlikely event that TSV did establish in a localised wild prawn population, the likelihood of eventual spread to other wild populations and subsequently to farm and hatchery populations would be comparable to an agent with a broader host range such as WSSV, albeit at a much slower rate — recent research conducted on susceptibility of five Australian crustacean species (of commercial importance) to TSV, showed them unlikely to become infected under the conditions of exposure described in this assessment.

TSV Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*low*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*low*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*very low*
Outbreak scenario 2 (no establishment):	*high*

8.3.2 Impacts

With the possible exception of *M. ensis*, there are no crustacean species in Australia known to be susceptible to significant disease caused by pathogenic strains of TSV such as that reported for *L. vannamei*. Clinical disease was reported from farmed adult *M. ensis* infected with an Asian (Taiwanese) TSV isolate (Chang et al. 2004), but a causal relationship was not demonstrated. Phalitakul et al. (2006) found that a Thai TSV isolate (ThMay04PmPL-TSV) naturally infected and caused mortality in cultured *P. monodon* and suggested good adaption of TSV to this species. Experimental infection trials commissioned by Biosecurity Australia demonstrated that *per os* challenge of five species of crustaceans cultured in Australia (*F. merguiensis*, *P. monodon*, *C. quadricarinatus*, *C. tenuimanus* and *M. rosenbergii*) did not result in an active (replicative) infection in any of the species, although *F. merguiensis* and *P. monodon* did become infected following injection.

It is recognised that genotypic TSV strains exist in both Asia and the Americas and these strains can be found overseas in species of commercial importance in Australia, e.g. *P. monodon* and *M. ensis*. Although TSV strains found in *P. monodon* and *M. ensis* do not cause serious disease outbreaks, there is a real, albeit a very small likelihood that TSV may emerge as an important pathogen in *P. monodon* or *M. ensis* culture in the future.

Experimental studies have demonstrated the potential for TSV to mutate. Filtered tissue homogenates from TSV-positive animals were injected into *P. monodon* (weighing approximately 40 grams), resulting in higher levels of virus in *P. monodon* injected with a *M. ensis* TSV isolate than in those injected with a TSV isolate from *P. monodon* (Chang et al. 2004). RT-PCR revealed the *M. ensis* isolate replicated more freely in *P. monodon* than did

the *P. monodon* isolate, although development of clinical disease in *P. monodon* was not reported (Chang et al. 2004). The authors concluded that the apparently high mutation rate, coupled with other evolutionary pressures, had caused adaptive local sub-strains of TSV in new hosts. In support of this conclusion, TSV sub-strains have been detected in *P. monodon* cultured in Thailand (Srisuvan et al. 2005, Phaliltakul et al. 2006). Further, naturally infected *L. stylirostris* were originally thought to suffer only mild disease (Chamberlain 1994, Lightner 1996a). However, mortalities of up to 90% have been reported in this species during TSV outbreaks in the Americas (Erickson and Lightner 2001). Similarly, *L. vannamei* populations in Belize, bred for resistance to TSV-A, experienced 90–100% mortality when infected with TSV-C (Moss 2004, Erickson et al. 2005).

In the event that a strain of TSV capable of causing serious disease in *P. monodon* or other important aquaculture species in Australia did emerge, overseas experience indicates that prawn aquaculture operations would be significantly impacted for at least a few years, after which production levels would be expected to recover and exceed pre-TSV levels (Lightner and Redman 1998, Melendez 1998 cited in Jimenez et al. 2000a), both as a result of tolerance or resistance in local prawns (Flegel 1997a) and adaptive management practices. It is expected that any activity related to TSV management or eradication would add to the cost of prawn production.

Other crustacean aquaculture industries in Australia include freshwater production of *Cherax* species and the emerging mud crab and soft-shelled sand crab industries. Australian *Cherax* species of commercial significance have been experimentally infected with TSV and appear to be refractory to disease. Although TSV has been experimentally transmitted to crabs via injection, it is not known if commercially important Australian crab species would be susceptible to TSV under natural conditions.

Whether TSV would have a deleterious effect on wild prawn populations in Australia is unknown. However, as far as available techniques of detection allow, no declines in wild prawn populations have been attributed to TSV overseas, despite TSV being prevalent in wild asymptomatic carrier prawns in most areas where TSV is present.

Clinical disease has been observed in adult wild *M. ensis* found to be infected with TSV — no causal relationship was reported (Chang et al. 2004). *Metapenaeus ensis* is abundant and widely distributed in the tropical and subtropical waters around Australia and forms an integral part of the ecosystem in these waters (Kailola et al. 1993).

Conclusions

- The likelihood that strains more virulent to wild or farmed crustacean species of importance in Australia (e.g. *P. monodon*) emerging in the future may be greater for TSV than for other less mutable viruses such as WSSV or YHV. Impact estimations need therefore, to take an appropriately cautious approach, particularly with respect to potential environmental impacts, which are more likely to go undetected for a significant period of time.
- Minor impacts to multiple regional prawn farming areas would be expected as a result of production losses, should a strain of TSV capable of causing disease in monodon prawns emerge. However, these impacts would be expected to ameliorate over a few years, as has occurred overseas with vannamei prawn aquaculture, including through adoption of new husbandry practices. The potential impacts of TSV on monodon aquaculture are considered to be less than that associated with WSSV or IHHNV due to the demonstrated impact of WSSV on monodon farming, and the reported potential for IHHNV to cause RDS in farmed monodon and its demonstrated propensity to mutate to cause serious disease in new host species.
- The establishment and spread of TSV may have minor impact on native animals at multiple local levels over at least a period of several years. Once established in wild crustacean populations, successful eradication is unlikely.

- Taura syndrome is an OIE-listed disease. It is on the Australian *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments would report on the agent. Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating TSV from wild crustacean populations is unlikely to be initiated. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. The indirect economic impacts associated with such a control program, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- Multiple regions (where prawn farming makes a significant contribution to the local economies) may experience significant flow-on economic impacts as an indirect result of virus establishment and spread.
- TSV establishment and spread may potentially result in loss of live crustacean export markets. These international trade impacts would be minor, local level impacts.
- Given the abundance and widespread distribution of *M. ensis* (and other metapenaeids) in Australian waters, some local level impacts to ecosystems integrity might be expected should TSV prove (in light of the mutability of the virus) to have a significant impact on the survival of this species in the wild.

The impacts of TSV establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Local (multiple)	Minor	C
The environment (native animals/plants, and non-living environment)	Local (multiple)	Minor	C
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Regional (multiple)	Significant	E
Economic (international trade effects)	Local	Minor	B
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local (multiple)	Minor	C
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*Moderate*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

8.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'. The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Low*	*Low*	*Very low*
Impact	*Moderate*	*Moderate*	*Moderate*
'likely consequences'	*Low*	*Low*	*Very low*
Outbreak scenario 2			
PLES	*High*	*High*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Low*	*Low*	*Very low*

8.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the annual partial likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*Low*	*Moderate*	*Low*	*Low*	*Moderate*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*Low*	*Moderate*
'Likely consequences'	*Low*	*Low*	*Very low*
Partial annual risk	*Very low*	*Very low*	*Very low*

Conclusion

The unrestricted risk associated with TSV is determined (by combining the three partial annual risks associated with each exposure group) to be *low*. The unrestricted risk exceeds Australia's ALOP and, therefore, risk management is deemed necessary.

9 Yellowhead virus

The following provides an estimation of the yellowhead virus (YHV) risk associated with the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the pathogenic agent-specific information presented in this chapter, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

9.1 Release assessment

Although natural infections of YHV have only been found in prawns, many other species of crustacean are susceptible to experimental infection with YHV. Penaeid species reported as being naturally susceptible to YHV infection include *M. japonicus* (Wang et al. 1996), *F. merguiensis* (Chantanachookin et al. 1993), *P. monodon* (Boonyaratpalin et al. 1993), *L. setiferus* and *L. stylirostris* (Lightner 1996a).

Yellowhead disease (YHD) is primarily a disease of farmed prawns, particularly *P. monodon*. YHV was endemic in *P. monodon* farms throughout Asia possibly as early as the late 1980s (Lightner 1996a). Initially, clinical disease and high mortality were widespread in infected countries but soon became less common (Flegel et al. 1997). Although not quantified, the prevalence of YHV infection in *P. monodon* seed stock in Thailand is now thought to be much higher than previously suspected (Walker et al. 2001). Whilst little data exists, YHV prevalence in wild-caught prawns is thought to be very low (Flegel et al. 1995a). However, since the mid-1990s, researchers have routinely detected YHV in apparently healthy *P. monodon* stocks of wild prawns (Flegel 1997a). YHV is also prevalent in wild adult carrier prawns resident in and around prawn farm ponds in areas in which YHV is endemic (Longyant et al. 2005).

Typically, severe YHD in *P. monodon* aquaculture ponds occurs up to 10 weeks after stocking when prawns are 5–15 grams in size (Limsuwan 1991 cited in Chantanachookin et al. 1993, Lotz 1997a). Affected prawns are pale bodied with reddening of the appendages and have a yellow cephalothorax due to a yellow hepatopancreas visible through the translucent carapace (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). Yellowing of the cephalothorax is not necessarily present in all affected animals (Chantanachookin et al. 1993) and is not typical for all species (Lu et al. 1994, Tang and Lightner 1999). Emergency harvest of prawns affected by YHD is reportedly less common now than in previous years due to the presumed development of tolerance or resistance in local prawns (Flegel et al. 1997). Emergency harvested crops would be expected to contain many prawns that are smaller than usual, prawns showing yellowing of the cephalothorax or general signs of ill thrift such as loose cephalothorax and shell-fouling — signs that would likely be detected during post-harvest inspection.

Most infections observed now are subclinical and YHV infection is increasingly reported as a co-infection with WSSV and other viruses (Mohan et al. 1998, Durand et al. 2000, Chang and Wang 2001, Madhavi et al. 2002It is expected that large adult *P. monodon* subclinically infected with YHV would pass post-harvest inspection and be supplied to the export market.

The amount of virus in subclinically infected prawns would generally be less than in prawns affected by serious disease, although individual infected prawns (including from apparently healthy crops) may contain a high titre of virus.

YHV-infected tissues stored at –70°C (Lu et al. 1995b) and –80°C (Direkbusarakom et al. 1998) can maintain infectivity (Lu et al. 1995b, Direkbusarakom et al. 1998).

Introduction of YHV into new areas has primarily been attributed to the movement of live animals, particularly broodstock and postlarvae (Briggs et al. 2004).

Conclusions

- YHV is known to infect a number of commercially important prawn species, but infections in aquaculture are mainly associated with farmed *P. monodon*. Emergency harvest of prawns from ponds is known to occur but is less common now due to development of tolerance to YHV in endemic areas. YHV in Thai prawn farms is believed to be more prevalent in *P. monodon* postlarvae seed stock than previously thought.
- YHV causes serious disease and high mortality in naïve stocks of farmed prawns. Most surviving adult prawns that carry YHV have a distinctive and unmarketable appearance and would be detected during post-harvest inspection for human consumption. However, clinical signs are not always apparent in adult prawns, and these prawns would pass post-harvest inspection.
- The viral load in some apparently healthy carrier prawns may be high. Any YHV in the tissues of harvested prawns would be expected to survive commercial freezing, storage and transport to Australia.

YHV Likelihood of release (LR)

The LR of YHV via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be *high*.

9.2 Exposure assessment

YHV infects mesodermal and ectodermal tissues (Tang and Lightner 1999). Uncooked prawn heads used as bait or prawn head waste would be expected to have high viral loads — the highest virus concentrations would be expected to occur in the cephalothorax (Chantanachookin et al. 1993, Lightner 1996a).

YHV maintains infectivity in frozen tissues (Lu et al. 1995b, Direkbusarakom et al. 1998), although it is thought that freeze-thaw cycles may damage virions (Wongteerasupaya et al. 1995b). YHV is inactivated when held at 60°C for 15 minutes (Flegel et al. 1995a). Unlike TSV, YHV cannot maintain infectivity following passage through the gastrointestinal tract of seagulls (Vanpatten et al. 2004).

Penaeus monodon is the most important commercial aquaculture species in Australia and is susceptible to infection with YHV. *Penaeus monodon* is susceptible to infection with gill-associated virus (GAV), a closely related virus within the same group of viruses — GAV has been associated with a complex of viruses linked to monodon mid-crop mortality syndrome in Australia. Chronic GAV infection is prevalent in wild and farmed Australian *P. monodon*. Although YHV is infectious to other commercially important Australian prawn species, the pathogenicity of YHV to these species is unknown. YHV can be experimentally transmitted to many marine crustaceans of commercial importance, including crabs (by injection) — infected crabs can transmit the virus to *P. monodon* under experimental cohabitation (Boonsaeng et al. 2000). YHV has not been reported in freshwater crustaceans.

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels, are potentially significant YHV exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to YHV — much of this virus would be expected to remain infectious at the point of exposure. *P. monodon* is the most important commercial aquaculture species in Australia and is susceptible to infection.
- In the absence of any import controls, it is expected that significant volumes of imported *P. monodon* or associated wastes potentially infected with YHV would be directed for use as recreational fishing bait or berley. As such, bait and berley use represents a significant pathway for exposure of wild crustaceans (exposure group 3) to YHV. Disposal of untreated wastes from commercial processing premises into natural waters also represents a significant pathway for exposure of wild crustaceans to YHV. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.
- Uncooked prawns, or parts thereof, used for bait, and prawn tissues in untreated liquid waste from commercial processing of imported prawns disposed into natural waters, could contain significant amounts of virus, particularly if the prawns had been emergency harvested due to outbreak of YHD (although emergency harvested prawns may be detected during post-harvest inspection) — much of this virus would be expected to remain infectious at the point of exposure, although the enveloped nature of the virion would generally make YHV inherently less stable than simple, single-stranded, non-enveloped viruses such as TSV or IHHNV.
- Much of any bait and commercial processing waste material is expected to be taken by non-susceptible finfish and crustacean species. The number of crustacean species in Australia that would be susceptible to YHV infection under these circumstances is considered to be less in comparison to pathogenic agents with a broader natural host range such as WSSV.

YHV Partial likelihood of exposure (PLE)

The PLE to YHV from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*low*
Exposure group 2	$PLE_{HATCHERY}$	*high*
Exposure group 3	PLE_{WILD}	*moderate*

9.3 Consequence assessment

9.3.1 Partial likelihood of establishment or spread

Many prawns of economic and/or environmental significance are within the natural host range of YHV. The related GAV is found in both wild and cultured *P. monodon* in Australia (Spann et al. 1997b). Freshwater crustaceans have not been reported to carry YHV.

Non-prawn marine crustaceans have been experimentally infected with YHV and are capable of transmitting YHV to prawns via water (Boonsaeng et al. 2000).

Natural spread of YHV in cultured *P. monodon* is via the water and *per os* by ingestion of infected tissues (Flegel et al. 1995a). Unlike other serious viral prawn pathogens, YHV remains viable for a comparatively short time in water. Where pathogenic agents such as TSV and WSSV may remain viable for periods of 21 and 28 days, respectively (Prior and Browdy 2002), YHV remains infectious for up to 3 days at 27°C (Flegel et al. 1995a).

Australian aquaculture of *P. monodon* currently relies on wild-caught broodstock. Although infected broodstock are not considered the major route of YHV infection (Flegel et al. 1997), the establishment of YHV in Australia in wild populations from which broodstock *P. monodon* are sourced, may result in the dispersal of YHV throughout prawn aquaculture via the unrestricted movement of broodstock and postlarvae.

The spread of YHV overseas is mostly attributed to the introduction of live prawn stocks and subsequent unrestricted movement of live broodstock and postlarvae. The IRA team is of the view that GAV has spread into the Joseph Bonaparte Gulf through escapes from Northern Territory prawn farms.

Conclusions

- YHV can be transmitted via waterborne virus or *per os*, by ingestion of infected tissues. It is expected that susceptible host animals feeding on tissues of imported prawns that had been heavily infected with would receive an infectious viral dose.
- For a given quantity of infected material entering the environment of an exposure group, the likelihood of YHV establishment is higher for farmed and hatchery crustaceans (exposure groups 1 and 2, respectively) than for wild crustaceans (exposure group 3) because of the greater density of susceptible animals that are more prone to infection under the environmental conditions associated with aquaculture.
- In the event that one or more index cases of YHV infection were to occur, virus establishment and spread to other susceptible animals would be unlikely in the case of wild crustaceans (exposure group 3) because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish. Further, this likelihood would be less than that for WSSV, due to YHV having a narrower host range compared to WSSV — non-penaeid crustaceans are not reported as being susceptible to natural YHV infection.
- Spread of YHV from hatcheries to farms is likely. The likelihood of the spread of YHV from farms to neighbouring farms or wild prawn populations through waterborne virus in effluent water would be moderated by dilution effects — YHV has been shown to survive free in water for much shorter durations than WSSV or TSV. The likelihood of YHV spread from farms to wild populations or neighbouring farms via escaped prawns may be higher, especially if large numbers of prawns escaped *en masse*, although the likelihood is expected to be less than that for WSSV because of the narrower host range of YHV.
- The likelihood of spread from farms and hatcheries would be reduced by farm and hatchery level control measures that are likely to be implemented on detection of YHV — the IRA team notes that the YHV is less likely to be detected than WSSV, given the presence of GAV in Australia.
- If in the unlikely event that YHV did establish in a localised wild prawn population, the likelihood of eventual spread to other wild populations and subsequently to farm and hatchery populations would be comparable to an agent with a broader host range such as WSSV, albeit at a much slower rate.

YHV Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*very low*
Outbreak scenario 2 (no establishment):	*high*

9.3.2 Impacts

YHV was the first major virulent disease to impact *P. monodon* aquaculture in Asia (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). The total production loss attributed to YHV during the initial outbreak in Thailand in the early 1990s has been estimated at approximately 3% of total production volume (Flegel et al. 1995a). During early outbreaks, mass mortalities and entire crop loss occurred over a period of 3–5 days (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993).

Production volume following an outbreak of YHD returns to pre-outbreak levels over a relatively short period due to the apparent development of tolerance in *P. monodon* populations (Flegel et al. 1997). YHV-infected stocks can survive to normal harvest time and achieve weights of 20–25 grams (Coelen and Walker 1996).

In the event that YHD were to establish in Australia, farmed prawns would be most at risk. Australian *P. monodon* have a high prevalence of GAV, the strain of YHV present in Australia (Walker et al. 2001). The impact of YHD on Australian prawn farms and in particular *P. monodon* would be expected to be similar to the current impact of disease caused by GAV.

The development of disease tolerance or resistance in local prawns combined with management activities would be expected to result in reduced losses due to YHD after a few years. The implementation of systems to manage YHD overseas include legislation to restrict the movement of live broodstock and postlarvae, use of SPF broodstock, enforcement of codes of conduct and management practices, improving husbandry technology in intensive culture and active surveillance (Briggs et al. 2004).

There is no evidence that YHV has had an impact on wild prawn fisheries overseas. GAV is found in wild and cultured Australian *P. monodon*, but impacts of disease have only been reported in cultured *P. monodon* (Spann et al. 1997b). The absence of an observable impact on wild prawn populations may be due to lower environmental and crowding stress levels in wild prawns, or the absence of tools that allow detection of impacts on wild populations.

YHV is not known to infect freshwater crustaceans and is therefore not considered a risk to Australia's freshwater crayfish industry or wild freshwater crayfish population. As YHV is a disease of marine prawns and is not known to naturally infect non-prawn crustaceans, emerging marine aquaculture, e.g. mud crab and soft-shelled sand crab, would not be expected to be at risk.

Conclusions

- The establishment and spread of YHV may most significantly impact farmed monodon prawns at multiple State/Territory levels over at least a period of several years — however impacts would not be expected to be as significant after a few years

with effective health management through new husbandry measures, and the potential for host species to develop tolerance to YHD over time.

- Based on the absence of serious effects on wild prawn populations overseas, and the absence of any known impact of GAV on Australian wild populations, the environmental effects of YHV establishment and spread in Australia are expected to be limited.
- YHD is an OIE-listed disease. It is on the Australian *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments would report on the agent. Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating YHV from wild crustacean populations is unlikely to be initiated. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. However, YHV may be mistaken for endemic GAV, leading to establishment and extensive spread before detection, further reducing the likelihood of attempted control or eradication. The indirect economic impacts associated with such a control program, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- Multiple regions (where prawn farming makes a significant contribution to the local economies) would be expected to experience significant flow-on economic impacts as an indirect result of YHV establishment and spread.
- YHV establishment and spread may potentially result in loss of live crustacean export markets. These international trade impacts would be minor, local level impacts.
- Indirect environmental impacts of YHV establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of YHV establishment and spread are not expected to be discernible at any level.

The impacts of YHV establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	State (multiple)	Significant	F
The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Regional (multiple)	Significant	E
Economic (international trade effects)	Local	Minor	B
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*High*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

9.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'.

The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Moderate*	*Moderate*	*Very low*
Impact	*High*	*High*	*High*
'likely consequences'	*High*	*High*	*Low*
Outbreak scenario 2			
PLES	*Moderate*	*Moderate*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*High*	*High*	*Low*

9.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*High*	*Moderate*	*Low*	*High*	*Moderate*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*High*	*Moderate*
'Likely consequences'	*High*	*High*	*Low*
Partial annual risk	*Moderate*	*High*	*Low*

Conclusion

The unrestricted risk associated with YHV is determined (by combining the three partial annual risks associated with each exposure group) to be *high*. The unrestricted risk exceeds Australia's ALOP and, therefore, risk management is deemed necessary.

10 Hepatopancreatic parvovirus

The following provides an estimation of the risk associated with exotic strains of hepatopancreatic parvovirus (HPV) from the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the following pathogenic agent-specific information, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

10.1 Release assessment

HPV has been reported in postlarvae and juveniles of wild and farmed penaeid prawns. HPV is often found in healthy animals and is not known to cause severe disease. However, HPV infection and in particular, multiple infections with other pathogenic agents such as monodon baculovirus (MBV), has been reported in association with stunted growth (Flegel et al. 1999, Flegel et al. 2004).

HPV was initially reported from Asia and Indo-Pacific regions including Australia (Chong and Loh 1984, Lightner et al. 1985, Paynter et al. 1985, Roubal et al. 1989, Lightner et al. 1989a, Flegel et al. 1992b, Lightner et al. 1993, Mohan et al. 1998). HPV has spread to many parts of the world via the movement of live animals (Colorni et al. 1987, Lightner et al. 1989a, Lightner and Redman 1992, Turnbull et al. 1994, Sukhumsirichart et al. 2002, Manjanaik et al. 2005). Natural infections have been reported from a wide range of penaeid species and viruses morphologically similar to HPV have also been found in diseased *Carcinus mediterraneus* (Mari and Bonami 1988) and *M. rosenbergii* (Anderson et al. 1990).

Using molecular techniques, three geographic strains of HPV have been identified from prawns: HPV*mon* from Thai *P. monodon* (Phromjai et al. 2001), HPV*chin* from Korean *F. chinensis* (Lightner et al. 1994a) and HPV*merg* from *F. merguiensis* (La Fauce et al. 2007). Other HPV strains have also been reported in *P. semisulcatus* from India (Manjanaik et al. 2005), as well as wild and cultured Australian prawns (Paynter et al. 1985, Roubal et al. 1989, Spann et al. 1997a, Jones 1998).

HPV strains have been found in many commercially important wild and cultured prawn species throughout the world at relatively high prevalence.

Gross signs are not specific to HPV and include poor growth, anorexia, reduced preening activity and increased surface fouling (Lightner and Redman 1985). Lesions associated with infection are hepatopancreas atrophy and infrequently, abdominal muscle opacity (Lightner and Redman 1985).

HPV has been detected from apparently healthy animals (Chong and Loh 1984, Lightner et al. 1985, Flegel et al. 1992b, Jones 1998). All infected animals appear to pass at least the normal larval and postlarval stages without clinical signs (Lightner and Redman 1985).

Postlarvae infected with HPV and stored whole at –80°C have been used to successfully infect prawns in experimental trials (Catap et al. 2003).

Conclusions

- Various strains of HPV are known to naturally infect a wide range of penaeid prawns including species of economic and environmental importance to Australia such as *P. monodon*.
- Gross signs are not specific to HPV and include increased surface fouling and infrequently, abdominal muscle opacity. However, HPV is often found in healthy

animals at high prevalence — these would not be detected during post-harvest inspection.
- Whole, uncooked, frozen, prawns infected with HPV would be expected to contain significant amounts of virus.
- HPV in the tissues of prawns would be expected to survive commercial freezing, storage and transport to Australia.

HPV Likelihood of release (LR)

The LR of exotic strains of HPV via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be *high*.

10.2 Exposure assessment

HPV occurs in cells of the hepatopancreatic tubule epithelia (hepatopancreatocytes) (Lightner and Redman 1985, Paynter et al. 1985, Lightner et al. 1989b, Anderson et al. 1990, Owens and HallMendelin 1989, Flegel et al. 1992b, Lightner et al. 1993) and rarely in cells of the anterior midgut or caecum epithelia (Lightner and Redman 1985, Mohan et al. 1998a).

HPV infections are usually observed in aquaculture grow-out ponds, but have also been reported in hatcheries (Manivannan et al. 2002, Umesha et al. 2003) and wild-sourced adult broodstock prawns (Colorni et al. 1987, Turnbull et al. 1994, Morales–Covarrubias and Chavez–Sanchez 1999). Stressful conditions are known to induce morbidity in already infected prawns (Paynter et al. 1985).

Natural infections have been reported from a wide range of penaeid species. HPV was initially found in *F. indicus*, *F. merguiensis* (Chong and Loh 1984), *F. chinensis*, *P. semisulcatus*, *P. monodon*, *P. esculentus* (Lightner et al. 1985) and *Fenneropenaeus penicillatus* (Lightner et al. 1989a), from Asia and the Indo-Pacific. Subsequently, HPV has been detected in *L. vannamei* (Lightner et al. 1989a) and *L. stylirostris* (Lightner 1993). Manjanaik et al. (2005) reported HPV from India in wild *Parapenaeopsis stylifera*, *M. japonicus*, *M. monoceros*, *M. affinis*, *M. elegans*, *M. dobsoni*, *M. ensis* and *Solenocera choprai*. Parvo-like viruses, morphologically similar to HPV, have also been found in diseased *C. mediterraneus* (Mari and Bonami 1988) and *Machrobrachium rosenbergii* (Anderson et al. 1990). The relationship between these agents is not known.

Exotic HPV would be expected to infect many farmed and wild crustacean species in Australia.

HPV can remain infectious following storage at –80°C (Catap et al. 2003).

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels, are potentially significant HPV exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to HPV. Most of any virus present would be expected to remain infectious at the point of exposure.
- It is expected that in the event of unrestricted importation of whole uncooked prawns, significant volumes of prawns or associated wastes potentially infected with HPV

would be directed for use as recreational fishing bait or berley. As such, bait-use represents a significant pathway by which wild crustaceans (exposure group 3) could become exposed to HPV. Disposal of untreated wastes from commercial processing premises into natural waters also represents a significant pathway for exposure of wild crustaceans to HPV. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.

- Uncooked prawns, or parts thereof, used for bait, and prawn tissues in untreated liquid waste from commercial processing of imported prawns disposed into natural waters could contain significant amounts of virus — much of this virus would be expected to remain infectious at the point of exposure. The simple, single-stranded, non-enveloped virion structure confers on HPV a greater resilience compared to some other lipid-containing enveloped viruses such as WSSV and YHV — added stability is also conferred by having a DNA-based genome compared to RNA viruses such as YHV or TSV.
- Although any bait and commercial processing waste material is expected to be rapidly diluted or taken by non-susceptible finfish species, prawns and other crustaceans that may be susceptible to HPV infection are common in freshwater and marine environments throughout Australia and are therefore likely to encounter a small but significant amount of this material. A significant proportion of virus in bait, berley and commercial processing waste effluent is expected to remain viable in infected prawn tissues at the point of exposure.

HPV Partial likelihood of exposure (PLE)

The PLE to exotic strains of HPV from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*low*
Exposure group 2	$PLE_{HATCHERY}$	*high*
Exposure group 3	PLE_{WILD}	*moderate*

10.3 Consequence assessment

10.3.1 Partial likelihood of establishment or spread

HPV strains have a wide host range and can often be found at high prevalence in farmed and wild crustaceans in both marine and freshwater environments.

Experimental transmission of HPV *per os* by ingestion of infected tissues has been demonstrated (Catap et al. 2003). Vertical transmission has been suggested in *F. chinensis* (Lightner et al. 1992b), however, this has not been verified experimentally. Transmission of HPV resulting from co-habitation is considered unlikely (Paynter et al. 1985, Flegel et al. 1995b). Susceptible species would need to ingest the viable virus to become infected.

Stressful conditions such as crowding stress, poor water quality and poor husbandry techniques are known to induce morbidity in prawns (Paynter et al. 1985).

In the event of exposure, HPV would be expected to readily establish in hatchery and farm crustaceans. Because disease would only be expected to occur under stressful husbandry conditions and/or in the presence of at least one other viral pathogen, there is a high likelihood that initial establishment and spread would go undetected.

The spread of HPV throughout the world has been attributed to the movement of live animals for aquaculture (Colorni et al. 1987, Lightner et al. 1992b, Turnbull et al. 1994, Morales-Covarrubias and ChavezSanchez 1999, Sukhumsirichart et al. 2002, Manjanaik et al. 2005).

Conclusions

- HPV can be transmitted *per os* (by ingestion of infected tissues). Susceptible species of crustaceans feeding on prawns that had been heavily infected with HPV may receive an infectious dose of the virus. HPV strains have wide host range and can often be found at high prevalence in farmed and wild crustaceans in both marine and freshwater environments.
- For a given quantity of infected material entering the environment of an exposure group, the likelihood of HPV establishment is higher for farmed and hatchery crustaceans (exposure groups 1 and 2, respectively) than for wild crustaceans (exposure group 3) because of the greater density of susceptible animals that are more prone to infection under the environmental conditions associated with aquaculture.
- In the event that one or more index cases of HPV infection were to occur, virus establishment and spread to other susceptible animals would be unlikely in the case of wild crustaceans (exposure group 3) because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish. Compared to WSSV, HPV is less likely to cause clinical disease and infected prawns may therefore, be less prone to predation. Also, HPV may not be inactivated by passage through the bird digestive system and as such, spread via mechanical vectors such as seagulls is possible.
- Spread of HPV from hatcheries to farms is likely. The likelihood of the spread of HPV from farms to neighbouring farms or wild prawn populations through waterborne virus is unlikely given dilution effects. Nonetheless, HPV may spread from farms to wild populations or neighbouring farms via escaped prawns, especially if large numbers of prawns escape *en masse*.
- If in the unlikely event HPV were to establish in a localised wild prawn population, it is expected that the agent would eventually spread to other wild populations and subsequently to farm and hatchery populations.

HPV Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*very low*
Outbreak scenario 2 (no establishment):	*high*

10.3.2 Impacts

Dual infection with other pathogenic agents may result in mortality and stunting (Flegel et al. 1999, MoralesCovarrubias and ChavezSanchez 1999, Manivannan et al. 2002, Umesha et al. 2003, Flegel et al. 2004). Diseased prawns infected with HPV as the solitary detectable viral agent have rarely been found (Chong and Loh 1984, Lester et al. 1987b, Flegel et al. 1992b, Flegel et al. 1999, Flegel et al. 2004). HPV infection is frequently observed in dual infections with MBV in Thailand (Flegel et al. 1992b, Flegel et al. 1995b, Flegel et al. 1999,

Chayaburakul et al. 2004, Flegel et al. 2004). A strain of HPV is present in Australia (Lester et al. 1987b). The IRA team is of the view that HPV infection concurrent with stress tends to predispose crayfish to infection with (perhaps more virulent) pathogenic agents.

HPV appears to have little impact on mysis and early postlarval stages, but may exhibit as disease in animals from approximately PL10 onwards (Lu 1997, Manivannan et al. 2002). Juveniles can suffer moderate to severe mortalities of up to 100% within 4–8 weeks of disease onset (Lightner et al. 1985).

Although HPV is not likely to cause a severe epidemic, it may cause economic loss. Anecdotal information by farmers in Thailand reported that 20% of the production profit was lost in one season due to stunted prawns possibly associated with a dual infection involving HPV (Flegel et al. 1999).

The presence of HPV in healthy wild broodstock suggests some naturally occurring viruses are tolerated and are a normal occurrence (Flegel et al. 2004). Although HPV is present in wild prawns there is little or no evidence of a discernible impact.

HPV is known to infect freshwater and non-penaeid marine crustacean species. The potential impact to Australia's freshwater crayfish industry or wild freshwater crayfish population and the emerging mud crab and soft-shelled sand crab aquaculture industries is generally unknown, although the IRA team is aware of James Cook University studies showing that in Australia, mud crab larvae, mud crab adults and adult sand crabs have been found heavily infected with an endemic strain of HPV. However, HPV has not been reported to have any impact on similar crustacean populations or industries in countries where the virus is found.

Conclusions

- Farmed *P. monodon* in Australia carrying other viral pathogens may develop a dual infection following exposure to an exotic strain of HPV. Dual infections have been associated with stunting and economic loss in *P. monodon* aquaculture. There is no evidence that exotic HPV strains cause significant impact on farming of non-penaeid species or freshwater crustaceans.
- If exotic strains of HPV were to establish in wild crustacean populations, successful eradication is unlikely. If established, exotic strains of HPV would not be expected to cause discernible impact to wild crustacean populations.
- HPV is not listed by the OIE. It is not on the *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments may not report on the agent. The likelihood of a control eradication campaign being launched if exotic HPV is detected in Australia is much less than that for other pathogenic agents such as WSSV, TSV, YHV and IHHNV.
- Establishment and spread of HPV may have a minor impact at multiple local levels (where prawn farming makes a significant contribution to the local economies).
- Given the absence of HPV-specific controls on Australian crustacean products exported overseas, the international trade impacts resulting from HPV establishment and spread are not expected to be discernable at any level.
- Indirect environmental impacts of HPV establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of HPV establishment and spread are not expected to be discernible at any level.

The impacts of establishment and spread (outbreak scenario 1) of an exotic strain of HPV are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Local (multiple)	Minor	C
The environment (native animals/plants, and non-living environment)	Local (multiple)	Minor	C
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Local	Minor	B
Economic (domestic trade effects and impact on other associated industries)	Local (multiple)	Minor	C
Economic (international trade effects)	Local	Unlikely to be discernable	A
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*Very low*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

10.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'. The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Moderate*	*Moderate*	*Very low*
Impact	*Very low*	*Very low*	*Very low*
'likely consequences'	*Very low*	*Very low*	*Negligible*
Outbreak scenario 2			
PLES	*Moderate*	*Moderate*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Very low*	*Very low*	*Negligible*

10.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*High*	*Moderate*	*Low*	*High*	*Moderate*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*High*	*Moderate*
'Likely consequences'	*Very low*	*Very low*	*Negligible*
Partial annual risk	*Negligible*	*Very low*	*Negligible*

Conclusion

The unrestricted risk risk associated with exotic strains of HPV is determined (by combining the three partial annual risks associated with each exposure group) to be *very low*. As the unrestricted risk estimate achieves Australia's ALOP, no risk management is considered necessary.

11 Infectious myonecrosis virus

The following provides an estimation of the infectious myonecrosis virus (IMNV) risk associated with importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the following pathogenic agent-specific information, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

11.1 Release assessment

Infectious myonecrosis virus (IMNV) is the causative agent of infectious myonecrosis (IMN) (Poulos et al. 2006). Natural infections and disease due to IMNV have been reported in farmed *L. vannamei* in Brazil and Indonesia (Lightner et al. 2004). (T. Flegel, Mahidol University Thailand, pers. comm. August 2006), and suspected in Thailand and China (Briggs 2006) — the IRA team considers IMNV likely to be widespread in Asia. IMN can affect postlarvae, juveniles, sub-adults and adults; apparently healthy chronically infected animals have also been reported (Lightner et al. 2004, OIE 2004, Tang et al. 2005a). *Litopenaeus stylirostris* and *Penaeus monodon* have been experimentally infected by injection of purified virions (Tang et al. 2005a).

The prevalence of IMNV in farmed or cultured *L. vannamei* is unknown.

The clinical signs associated with IMNV infection include focal to extensive areas of muscle necrosis, particularly of the distal abdominal segments and tail fan (Lightner et al. 2004). Affected muscles typically have white with opaque lesions (Tang et al. 2005a). In some affected animals, the tail fan may be necrotic and reddened, taking on a cooked appearance (Lightner et al. 2004). Apparently healthy, chronically infected animals have been detected (OIE 2004). The IRA team notes that the presence of muscle necrosis is not indicative of viral infection — Australian *P. monodon* with muscle necrosis proved negative for Brazilian IMNV using *in situ* hybridisation (Tang et al. 2005a).

IMNV has been shown to remain infectious following freezing at –70°C (Tang et al. 2005a).

International spread of IMNV is considered to have occurred via the unrestricted movement of live prawns (T. Flegel, Mahidol University Thailand, pers. comm. August 2006).

Conclusions

- IMNV is likely to be widespread in Asia — the agent has been reported from Brazil and Indonesia and suspected in Thailand and China.
- Most clinically infected prawns would be expected to be detected and removed during post-harvest inspection. Infected prawns may be subclinical carriers — these are likely to pass post-harvest procedures.
- Any virus in harvested prawns would be expected to survive commercial freezing, storage and transport to Australia.

IMNV Likelihood of release (LR)

The LR of IMNV via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be *high*.

11.2 Exposure assessment

Studies on infection of *L. vannamei*, *L. stylirostris* and *P. monodon* showed IMNV infected tissues to include skeletal muscles, lymphoid organs (both tubules and spheroids), hindgut, and phagocytic cells of the hepatopancreas and heart (Tang et al. 2005a). Additionally, gill tissue taken from infected *P. monodon* tested positive using *in situ* hybridisation (Tang et al. 2005a). Tail muscle is the primary target tissue for IMNV, this being proposed as a factor relating to the reduced mortality seen with IMNV when compared to WSSV, TSV and YHV, which attack more vital organs and cause higher mortality within a shorter period (Tang et al. 2005a).

Environmental and physical stressors such as extremes of salinity and temperature, cast net collection and possibly the feeding of low quality diets have been associated with IMNV outbreaks (Lightner 2004, Lightner et al. 2004).

Although *L. stylirostris* and *P. monodon* have been experimentally infected via injection, no mortalities were recorded (Tang et al. 2005a), suggesting that these commercially important prawn species, if naturally susceptible, are refractory to significant clinical disease. IMNV infections in non-penaeid marine crustaceans and freshwater crustaceans have not been reported.

Conclusions

- Of the three exposure groups, wild crustaceans (exposure group 3) were considered more likely to be exposed to imported prawns or associated wastes than hatchery crustaceans (exposure group 2) and farm crustaceans (exposure group 1). In all instances, any virus present in the prawns or associated wastes is expected to be viable at the point of exposure. However, the IRA team considers that for each of the three exposure groups, the overall likelihood of exposure would be *very low*, because the known natural host range of IMNV is narrow. IMNV has not been reported overseas from crustacean species that are endemic to Australia.

IMNV Partial likelihood of exposure (PLE)

The PLE to IMNV from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*very low*
Exposure group 2	$PLE_{HATCHERY}$	*very low*
Exposure group 3	PLE_{WILD}	*very low*

11.3 Consequence assessment

11.3.1 Partial likelihood of establishment or spread

The host range for IMNV appears to be limited to cultured *L. vannamei*. *Litopenaeus vannamei* is not cultured in Australia and is not found in Australian waters. IMNV can cause disease in postlarvae, juveniles, sub-adults and adult *L. vannamei* late in the production cycle (Lightner et al. 2004, OIE 2004, Tang et al. 2005a). Although *L. stylirostris* and *P. monodon* have been experimentally infected via injection, no mortalities were recorded (Tang et al. 2005a), suggesting these commercially important prawn species are refractory to disease. IMNV infections in non-penaeid marine crustaceans and freshwater crustaceans have not been reported.

IMNV is considered to be less virulent compared to WSSV, TSV and YHV (Tang et al. 2005a). The most likely mode of transmission in prawn grow-out ponds is cannibalism (Tang et al. 2005a).

Chronically diseased carrier prawns have been found in affected farm ponds (Lightner et al. 2004). Environmental and physical stressors such as extremes of salinity and temperature, cast net collection and possibly the feeding of low quality diets have been associated with IMNV outbreaks (Lightner 2004, Lightner et al. 2004).

Conclusions

- Horizontal transmission of IMNV *per os*, via ingestion of infected prawn tissues has been demonstrated. Whether IMNV can be transmitted via waterborne virus is not known. IMN outbreaks appear typically to be stress-induced.
- Due to the greater density of animals in farms and hatcheries (exposure groups 1 and 2), infection should it occur would spread more easily than in the case of wild crustaceans (exposure group 3). Also, in the case of wild crustaceans establishment and spread to other susceptible animals would be unlikely because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish.
- Notwithstanding the above differences, the overall likelihood of establishment and spread for each exposure group is considered to be *negligible*, because the known natural host range of IMNV is very narrow. IMNV has not been reported overseas from crustacean species that are endemic to Australia. Commercially important prawn species in Australia are likely to be refractory to IMN disease.

IMNV Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*

11.3.2 Impacts

IMNV is the causative agent of infectious myonecrosis (IMN) in *L. vannamei* (Poulos et al. 2006). To date, natural infections and disease due to IMNV have been reported in farmed *L. vannamei* from the north-eastern States of Brazil (Lightner et al. 2004) and Indonesia (T. Flegel, Mahidol University Thailand, pers. comm. August 2006), and suspected in Thailand and China (Briggs 2006). IMNV can affect postlarvae, juveniles, sub-adults and adults; apparently healthy chronically infected animals have also been reported (Lightner et al. 2004, OIE 2004, Tang et al. 2005a). IMNV associated mortality, reduced growth and poor product quality from north-eastern Brazil prawn farms caused an estimated financial loss of approximately 8.8% of national prawn production in 2003 (de Paiva Rocha 2004). Chronically diseased carrier prawns exhibit low-level persistent mortality, poor feed conversion ratios and associated production losses (Lightner et al. 2004).

Litopenaeus stylirostris and *P. monodon* have been experimentally infected by injection of purified virions (Tang et al. 2005a). Two other IMN-like infections displaying similar gross

signs have been reported in *L. vannamei* from Belize and *P. monodon* from Australia, however, *in situ* hybridisation using Brazilian IMNV-specific primers proved negative (Tang et al. 2005a). The IRA team notes that the presence of muscle necrosis is not indicative of viral infection. IMNV would not be expected to cause significant disease in crustacean species of commercial or environmental importance in Australia.

As there are no known natural hosts among non-penaeid crustacean species, IMNV is not considered a risk to farmed or wild freshwater crayfish populations in Australia, nor to the emerging mud crab and soft-shelled sand crab aquaculture industries.

IMN is listed as a notifiable disease by the OIE.

Conclusions

- The establishment and spread of IMNV would be expected to have little if any impact on farmed prawns in Australia — IMNV is not pathogenic to commercially important penaeid species farmed in Australia. IMNV is not known to be pathogenic to other wild or farmed prawns, non-penaeid crustaceans or freshwater crustaceans.
- There is no evidence that IMNV causes serious disease in non-penaeid species or freshwater crustaceans. IMNV would not be expected to infect Australian wild crustacean species. *P. monodon* can be infected experimentally but is refractory to clinical disease. However, if the virus were to establish in wild crustacean populations, successful eradication is unlikely. The limited host range among penaeid prawns and the absence of natural hosts among non-penaeid crustaceans would suggest the environmental effects of the introduction of IMNV in Australia would be minimal.
- IMNV is listed as a notifiable disease by the OIE. Following OIE listing, IMNV is now included in the Australian *National List of Reportable Diseases of Aquatic Animals* and State/Territory Governments would be expected to report on the agent. Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating IMNV from wild crustacean populations is unlikely to be launched. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. The indirect economic impacts associated with such a control program, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- Little if any flow-on economic impacts would be expected to result if IMNV were to establish in Australian farmed prawns.
- IMNV establishment and spread may potentially result in loss of live crustacean export markets. These international trade impacts would be minor, local level impacts.
- Indirect environmental impacts of IMNV establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of HPV establishment and spread are not expected to be discernible at any level.

The impacts of IMNV establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Local (multiple)	Unlikely to be discernable	B
The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernable	A
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Local	Unlikely to be discernable	A
Economic (international trade effects)	Local	Minor	B
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*Moderate*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

11.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'. The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Negligible*	*Negligible*	*Negligible*
Impact	*Moderate*	*Moderate*	*Moderate*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Outbreak scenario 2			
PLES	*High*	*High*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Negligible*	*Negligible*	*Negligible*

11.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Very low*	*Very low*	*Very low*	*Very low*	*Very low*	*Very low*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Very low*	*Very low*	*Very low*
'Likely consequences'	*Negligible*	*Negligible*	*Negligible*
Partial annual risk	*Negligible*	*Negligible*	*Negligible*

Conclusion

The unrestricted risk associated with IMNV is determined (by combining the three partial annual risks associated with each exposure group) to be *negligible*. As the unrestricted risk estimate achieves Australia's ALOP, no risk management is considered necessary.

12 *Baculovirus penaei*

The following provides an estimation of the *Baculovirus penaei* (BP) risk associated with the importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the following pathogenic agent-specific information, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

12.1 Release assessment

BP infects wild and farmed penaeid prawn species in the Americas and can cause significant mortality in larval and postlarval stages in commercial hatcheries (Overstreet 1994). Significant mortalities up to 100% resulting from BP infection have been reported from *F. aztecus*, *F. duorarum*, *M. marginatus*, *L. vannamei* and *L. stylirostris* but BP is primarily a disease associated with cultured *L. vannamei* (Overstreet 1994).

BP was first reported in wild *F. duorarum* from the northern Gulf of Mexico (Couch 1974). BP can affect larval to adult stages in grow-out ponds, but the prevalence and severity of both infection and disease are less with older prawns (Lightner 1988, Lightner et al. 1989b, Le Blanc and Overstreet 1990, Overstreet 1994, Stuck and Overstreet 1994).

The prevalence of BP in wild penaeid prawns in the Americas from 1976 to 1993 range from 3% to 40%, depending on species infected, geographical location and season (Couch 1976, Overstreet 1994, Fajer et al. 1998).

There is evidence that experimentally infected animals may be able to eliminate the virus over time (Le Blanc and Overstreet 1990, Stuck and Overstreet 1994, Stuck and Wang 1996) however, asymptomatically infected carrier animals may exist (Stuck and Wang 1996). Older *L. vannamei* (PL63–157) have proved difficult to infect with BP, infection in these prawns being less extensive and persistent (LeBlanc and Overstreet 1990).

The virus has been experimentally transmitted to prawns after storage of infected samples at temperatures of –40 to –70°C (Overstreet et al. 1988). BP virulence does not appear to be affected by frozen storage for up to 3.5 years (Overstreet 1994, Stuck and Wang 1996, Hammer et al. 1998).

Conclusions

- The natural host range of BP is limited to a few species of penaeid prawns, and is primarily a disease of importance in *L. vannamei* farming in the Americas.
- Harvest-size prawns are unlikely to be infected with BP. However, if infected, they are highly likely to pass post-harvest procedures.
- BP in the tissues of uncooked prawns would be expected to survive commercial freezing, storage and transport to Australia.

BP Likelihood of release (LR)

The LR of BP via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption is estimated to be *high*.

12.2 Exposure assessment

BP infects the epithelial cells of the hepatopancreatic tubules and the midgut (Overstreet et al. 1988, Stuck and Overstreet 1994). As such, imported prawns, if infected, would harbour virus mainly within the cephalothorax.

BP in the tissues of uncooked prawns has been shown to remain infectious for extended periods in frozen products (Overstreet et al. 1988). BP is inactivated by heating for 10 minutes 60–90°C, but remains infective after heating for the same period at 50°C (Le Blanc and Overstreet 1991a). The virus is inactivated when allowed to dry out at 22°C for between 24–48 hours (Le Blanc and Overstreet 1991a).

The host range for BP is limited to penaeid prawns. *Melicertus marginatus*, the only Australian prawn species known to be susceptible to BP, is restricted to a population in the Torres Strait. BP has not been reported in non-penaeid crustaceans or freshwater crustacean species.

Conclusions

- Of the three exposure groups, wild crustaceans (exposure group 3) were considered more likely to be exposed to imported prawns or associated wastes than hatchery crustaceans (exposure group 2) and farm crustaceans (exposure group 1). In all instances, any virus present in the prawns or associated wastes is expected to be viable at the point of exposure. However, the IRA team considers that for each of the three exposure groups, the overall likelihood of exposure would be *very low*, because the only Australian prawn species known to be susceptible to BP is *M. marginatus*, restricted to a population in the Torres Strait.

BP Partial likelihood of exposure (PLE)

The PLE to BP from imported non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*very low*
Exposure group 2	$PLE_{HATCHERY}$	*very low*
Exposure group 3	PLE_{WILD}	*very low*

12.3 Consequence assessment

12.3.1 Partial likelihood of establishment or spread

BP can cause significant mortality in larval and postlarval stages in commercial *L. vannamei* hatcheries (Overstreet 1994). BP has not been reported to cause disease problems in post-juvenile prawns in grow-out facilities. BP has not been reported in non-prawn species. The susceptibility of farmed or wild crustacean in Australia to BP is unknown, although non-penaeid crustaceans from affected countries have not been reported to be susceptible to BP infection.

BP may be transmitted orally, either by the uptake of virus from contaminated water containing faeces shed from infected prawns or by cannibalism on dead and dying prawns (Overstreet et al. 1988, Overstreet 1990, Overstreet 1994, Stuck and Overstreet 1994, Hammer et al. 1998). Overstreet et al. (1988) reported polyhedra in free and attached faecal strings from infected larvae through to adult prawns. Virions and tetrahedral occlusion bodies released into the midgut lumen may be consumed by non-infected prawns when excreted with

faeces into the environment (Bruce et al. 1994a). Broodstock releasing BP-contaminated faeces whilst spawning can transmit infection to eggs and newly hatched nauplii (OIE 2006b). There are conflicting reports regarding vertical transmission of BP — attempts to detect BP in female reproductive tissues including developing ova have been unsuccessful (Bruce et al. 1994a). Conversely, virions have been observed within an egg by transmission electron microscopy (Overstreet 1990, Overstreet 1994). BP inoculates from patently infected juveniles appear to be more infectious than inoculate from adult broodstock (Overstreet et al. 1988).

BP can survive in seawater between 7–14 days, depending on temperature (Le Blanc and Overstreet 1991a). Ultraviolet irradiation has been shown to be effective in inactivating the virus (Le Blanc and Overstreet 1991a).

Conclusions

- BP may be transmitted *per os*, either by the uptake of virus from contaminated water containing faeces shed from infected prawns or by cannibalism on dead and dying prawns. Susceptible species of crustaceans feeding on prawns that had been heavily infected with BP may receive an infectious dose of the virus.
- Due to the greater density of animals in farms and hatcheries (exposure groups 1 and 2), infection should it occur would spread more easily than in the case of wild crustaceans (exposure group 3). Also, in the case of wild crustaceans establishment and spread to other susceptible animals would be unlikely because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish.
- Notwithstanding the above differences, the overall likelihood of establishment and spread for each exposure group is considered to be *negligible*, because the only Australian prawn species know to be susceptible to BP is *M. marginatus*, restricted to a population in the Torres Strait. BP is unlikely to readily establish in exposed farm populations given the species of prawns farmed in Australia. BP has not been reported in non penaeid crustaceans or freshwater crustacean species.

BP Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*negligible*
Outbreak scenario 2 (no establishment):	*high*

12.3.2 Impacts

There is no evidence of clinical BP in *P. monodon* overseas. Non-penaeid crustaceans from affected countries have not been reported to be susceptible to BP infection.

Restricting the movement of live animals and screening postlarvae and potential broodstock via faecal examination for the presence of tetrahedral inclusion bodies prior to their introduction into facilities may limit the spread of the disease (OIE 2006b).

BP impacts at the hatchery level and these impacts are readily controllable — current best practice in controlling monodon baculovirus (MBV) in Australia that involves a double separation technique, would also manage BP associated risks.

Despite being reported since 1974, there are no reports of mortalities in wild prawn populations.

Conclusions

- The establishment and spread of BP would be expected to have little if any impact on farmed prawns in Australia — the main farmed species are not known to be susceptible to clinical disease from BP infection.
- There is no evidence that BP causes serious disease in non-penaeid species or freshwater crustaceans. BP would not be expected to infect Australian wild crustacean species. If BP were to establish in wild crustacean populations, successful eradication is unlikely.
- Tetrahedral baculovirosis is an OIE-listed disease. It is on the *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments would report on the agent. Given the difficulties inherent to the eradication of aquatic animal diseases from wild populations, a campaign aimed at eradicating BP from wild crustacean populations is unlikely to be initiated. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. The indirect economic impacts associated with such a control program, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- Minor flow-on economic impacts at multiple local levels may potentially result if BP were to establish in Australian farmed prawns.
- Given the absence of BP-specific controls on Australian crustacean products exported overseas, international trade impacts resulting from BP establishment and spread are not expected to be discernable at any level.
- Indirect environmental impacts of BP establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of BP establishment and spread are not expected to be discernible at any level.

The impacts of BP establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Local (multiple)	Unlikely to be discernable	B
The environment (native animals/plants, and non-living environment)	Local (multiple)	Unlikely to be discernable	B
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Local (multiple)	Minor	C
Economic (international trade effects)	Local	Unlikely to be discernable	A
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*Moderate*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

12.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'. The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Negligible*	*Negligible*	*Negligible*
Impact	*Moderate*	*Moderate*	*Moderate*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Outbreak scenario 2			
PLES	*High*	*High*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Negligible*	*Negligible*	*Negligible*

12.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Very low*	*Very low*	*Very low*	*Very low*	*Very low*	*Very low*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Very low*	*Very low*	*Very low*
'Likely consequences'	*Negligible*	*Negligible*	*Negligible*
Partial annual risk	*Negligible*	*Negligible*	*Negligible*

Conclusion

The unrestricted risk associated with BP is determined (by combining the three partial annual risks associated with each exposure group) to be *negligible*. As the unrestricted risk estimate achieves Australia's ALOP, no risk management is considered necessary.

13 Necrotising hepatopancreatitis bacterium

The following provides an estimation of the necrotising hepatopancreatitis bacterium (NHPB) risk associated with the importation of non-viable, farm-sourced, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the following pathogenic agent-specific information, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

13.1 Release assessment

The aetiological agent of necrotising hepatopancreatitis (NHP) is a pleomorphic, Gram-negative, intracytoplasmic bacterium (Krol et al. 1991, Frelier et al. 1992, Lightner et al. 1992c, Lightner and Redman 1994). The NHP-bacterium (NHPB) is a member of the α-subclass of proteobacteria and remains unclassified (Loy et al. 1996b). Genetic analysis of the NHPBs associated with North and South American outbreaks of NHP suggest that the isolates are either identical or very closely related sub-species (Loy et al. 1996a).

NHP has only been reported in farmed penaeid prawns, from postlarvae to adults. Outbreaks of disease have been reported in *L. vannamei* (Krol et al. 1991), *L. stylirostris* (Lightner and Redman 1994) and *F. aztecus* (Frelier et al. 1994). NHP has also been seen in *F. californiensis* and *L. setiferus* (Frelier et al. 1994, Lightner 1996a). *Litopenaeus setiferus* is reportedly less susceptible to disease than *L. vannamei* (Frelier et al. 1994).

NHP was first reported in the US State of Texas in 1985 (Johnson 1989 cited in Frelier et al. 1992). The disease has since been reported from many prawn farming countries in Central and South America (Lightner and Redman 1994, Lightner 1996a, Loy et al. 1996b, Briñez et al. 2003). NHP has been reported in farmed *L. vannamei* and *L. stylirostris* prawns from the Pacific coast of Mexico since 1999. An outbreak of NHP in *L. vannamei* was recently confirmed to be present in Campeche, southern Mexico (del Rio–Rodriguez et al. 2006). Occasional outbreaks of NHP continue to be reported, particularly from South American countries (del Rio–Rodriguez et al. 2006). In Eritrea, within a year of introduction, NHP resulted in severe losses in an importing facility (OIE 2004). NHPB has recently been reported from farmed *L. vannamei* in Thailand and Indonesia (Briggs 2006).

Research shows that most farms in an affected area suffer from the disease, with between 30–100% prevalence in affected farms (Frelier et al. 1992, Frelier et al. 1993, Briñez et al. 2003).

A NHP survey of Gulf of Mexico *L. setiferus* and *F. duorarum* in the vicinity of coastal prawn farms along the Yucatan and Campeche coast revealed no histological evidence of NHP (del Rio–Rodriguez et al. 2006).

Heavily infected prawns exhibit blackened gills, softening of the shell, chromatophore expansion causing pleopod edge darkening, an increase in secondary bacterial infections and epicommensal fouling (Frelier et al.1992, Lightner and Redman 1994, Vincent et al. 2004). Such prawns can be easily detected and removed during post-harvest inspection procedures.

Vincent et al. (2004) reports a pre-patent infection period lasting approximately 15 days post-exposure and an acute stage, typified by disease and mortality, from approximately 16 to 35 days post-exposure. After 60 days post-exposure, surviving animals showed no sign of infection. They also concluded that a carrier state does not exist in NHPB infections, and that all infected animals eventually die.

NHPB can remain infectious in chilled prawns for up to 2 days (D. Lightner, Arizona University, pers. comm. March 2007). NHPB frozen at –70°C and –80°C have been shown to retain infectivity in experimental transmission trials with *L. vannamei* (Frelier et al. 1993,

Crabtree et al. 2006, D. Lightner, Arizona University, pers. comm. March 2007). However, NHPB is highly sensitive to freezing (D. Lightner, Arizona University, pers. comm. March 2007) and requires specially developed fast freezing techniques to –80°C to maintain infectivity (Crabtree et al. 2006). Lightner (Arizona University, pers. comm. March 2007) states; that with NHPB and its apparent high sensitivity to freezing in mind, it would be expected that NHPB would not pose a significant risk for introduction in frozen unprocessed prawns.

Conclusions

- The known natural host range of NHPB is limited to farmed penaeid prawn species in the Americas. Some species are less susceptible to clinical disease caused by NHPB.
- Clinically infected penaeid prawns show significant clinical signs and most such prawns would be detected and removed during post-harvest inspection — prawns that have just become infected would not show clinical signs for approximately two weeks and thus would pass inspection.
- NHPB is only found in the hepatopancreas, therefore, only whole prawns would carry the bacterium.
- NHPB in the tissues of harvested prawns would not be expected to survive commercial freezing, storage and transport to Australia. NHPB in the tissue of chilled prawns would be expected to survive transport to Australia.

NHPB Likelihood of release (LR)

The LR of NHPB via the unrestricted importation of non-viable, farm-sourced, *frozen*, uncooked, whole prawns intended for human consumption is estimated to be *very low*.

The LR of NHPB via the unrestricted importation of non-viable, farm-sourced, *chilled*, uncooked, whole prawns intended for human consumption is estimated to be *high*.

13.2 Exposure assessment

NHPB infects the epithelial cells of the hepatopancreatic tubules (Krol et al. 1991, Frelier et al. 1992). Head and shell wastes would be expected to contain high concentrations of the agent.

Cultured penaeid prawn species from the Americas are susceptible to NHP — the bacterium has not been reported from any non-penaeid marine crustaceans or freshwater crustaceans. Whether Australian penaeid species are susceptible to NHPB infection is unknown, although this is likely to be the case, given the known multispecies host-range of NHPB. Australian non-penaeid marine crustaceans and freshwater crustaceans are not likely to be susceptible.

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels, are potentially significant NHPB exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to NHPB. It is expected that NHPB present in the tissues of prawns that have

been frozen would not remain infective at the point of such exposure. Most penaeid prawns, including *P. monodon* may be susceptible to NHPB infection — *P. monodon* is the most important commercial aquaculture species in Australia.

- It is expected that in the event of unrestricted importation of whole uncooked prawns, significant volumes of prawns or associated wastes potentially infected with NHPB would be directed for use as recreational fishing bait or berley. As such, bait-use represents a significant pathway by which wild crustaceans (exposure group 3) could become exposed to NHPB. Disposal of untreated wastes from commercial processing premises into natural waters also represents a significant pathway for exposure of wild crustaceans to the agent. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.
- Uncooked prawns, or parts thereof, used for bait, and prawn tissues in untreated liquid waste from commercial processing of imported prawns disposed into natural waters would not be expected to contain high NHPB concentrations — much of the agent would be expected to not be infectious at the point of exposure.
- Although most bait and commercial processing waste material entering natural waters is expected to be taken by non-susceptible finfish and crustacean species, susceptible prawn species may be present in Australian marine waters, and are likely to encounter some of this material.
- NHPB is sensitive to freezing.

NHPB Partial likelihood of exposure (PLE)

The PLE to NHPB from imported non-viable, farm-sourced, *frozen*, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*Extremely low*
Exposure group 2	$PLE_{HATCHERY}$	*Extremely low*
Exposure group 3	PLE_{WILD}	*Very low*

The PLE to NHPB from imported non-viable, farm-sourced, *chilled*, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*Low*
Exposure group 2	$PLE_{HATCHERY}$	*High*
Exposure group 3	PLE_{WILD}	*Moderate*

13.3 Consequence assessment

13.3.1 Partial likelihood of establishment or spread

The known host range of NHPB is limited to farmed penaeid prawns (postlarvae to adults). NHPB has not been found in non-penaeid marine or freshwater crustaceans. Whether Australian penaeid species are susceptible to NHPB infection is unknown, although this is likely to be the case.

Natural transmission of NHPB is thought to occur *per os*, by cannibalism (Frelier et al. 1994, Vincent et al. 2004), although cohabitation and dissemination of NHPB via the water column may also play a role (Frelier et al. 1994). NHPB in faeces shed into pond water has also been suggested as a possible means of transmission (Vincent and Lotz 2005). Outbreaks of disease are often preceded by prolonged periods of high water temperature (approximately 30°C) and

salinity (up to 40 ppt) (Frelier et al. 1992, Frelier et al. 1993, Lightner and Redman 1994). Prawns infected with NHPB are either dying or dead — a chronic, carrier state of infection is not known to exist (Vincent et al. 2004). However, waterborne NHPB would be expected to survive as a free-living bacterium.

NHP has not spread to wild penaeid population close to areas where NHP outbreaks have occurred in aquaculture populations (del Rio–Rodriguez et al. 2006).

Conclusions

- Susceptible species of crustaceans feeding on prawns that had been infected with NHPB may receive an infectious dose of the agent — natural transmission of NHPB is thought to occur *per os*, by cannibalism.
- For a given quantity of infected material entering the environment of an exposure group, the likelihood of NHPB establishment is higher for farmed and hatchery crustaceans (exposure groups 1 and 2, respectively) than for wild crustaceans (exposure group 3) because of the greater density of susceptible animals that are more prone to infection under the environmental conditions associated with aquaculture — all species of farmed penaeid prawns in Australia may be susceptible to NHPB infection.
- In the event that one or more index cases of NHPB infection were to occur, agent establishment and spread to other susceptible animals would be unlikely in the case of wild crustaceans (exposure group 3) because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish. However, unlike the viral pathogens considered in this risk assessment NHPB could survive as a free-living bacterium. In this respect, the likelihood of establishment and spread in wild prawn populations would be higher than for NHPB than for the viral pathogens considered in this risk assessment.
- Spread of NHPB from hatcheries to farms is likely. The likelihood of the spread of NHPB from farms to neighbouring farms or wild prawn populations through waterborne agent in effluent water would be moderated by dilution effects, although NHPB would be expected to survive as a free-living bacterium in the environment. NHPB spread from farms to wild populations or neighbouring farms via escaped prawns is also a potential means of spread, especially if large numbers of prawns escape *en masse*.
- The likelihood of spread from farms and hatcheries would be reduced by farm and hatchery level control measures that are likely to be implemented on detection of NHPB.
- In the event that NHPB did establish in a localised wild prawn population, the agent would be expected to eventually spread to other wild populations and subsequently to farm and hatchery populations.

NHPB Partial likelihood of establishment or spread (PLES)

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*moderate*
Outbreak scenario 2 (no establishment):	*moderate*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*low*
Outbreak scenario 2 (no establishment):	*high*

13.3.2 Impacts

NHP, also reported as Texas NHP, Texas pond mortality syndrome, Peru NHP and granulomatous hepatopancreatitis (Frelier et al. 1992, Lightner and Redman 1994), has caused mortalities of up to 95% in prawn grow-out farms in the Americas (Lightner 1996a).

A farm in Texas that reported NHP for the first time during late 1980s reportedly discontinued prawn farming as a result (Frelier et al. 1992). Lightner and Redman (1994) reported that about half of Peru's active prawn farms closed following NHP outbreaks in 1993. In Eritrea, within a year of introduction, NHP resulted in severe losses in an importing facility (OIE 2004). The Central American epidemic of NHP in 1993 was attributed to drought (Lightner and Redman 1994). The pond water salinity and temperature in affected farms had been elevated several weeks before the outbreak; the most affected farms had temperatures of 29–35°C and salinities of 30–38 ppt (Lightner and Redman 1994).

Penaeus monodon, the main farmed species in Australia, is not considered to be susceptible to clinical disease caused by NHPB (Briggs 2006). NHPB is not known to infect non-penaeid freshwater and marine crustacean species. Impact to Australia's freshwater crayfish industry or wild freshwater crayfish populations and the emerging mud crab and soft-shelled sand crab aquaculture industries is not expected.

NHPB has not been reported from wild crustaceans.

In the event NHP did establish and spread in Australian farmed penaeids, a number of measures are reportedly useful for controlling disease outbreaks, including culling of infected animals such as broodstock (Briñez et al. 2003), avoidance of conditions such as high water temperature and salinity, use of medicated feed early in the disease course (Frelier et al. 1992, Frelier et al. 1994, Lightner and Redman 1994), prophylactic application of medicated feeds (Lightner and Redman 1994), and disinfection of pond water used in hatcheries (Frelier et al. 1994) — use of chemotherapeutants in Australia for NHPB is unlikely to gain approval. Any prevention and control measures for NHP would add to the cost of production.

NHP is under study for listing by the OIE.

Conclusions

- The establishment and spread of NHPB may have a minor impact on some farmed prawns in multiple regions in Australia — the main farmed species, *P. monodon*, is unlikely to be susceptible to clinical disease.
- There is no evidence that NHPB causes serious disease in non-penaeid species or freshwater crustaceans. NHPB would not be expected to significantly impact wild crustaceans in Australia. If NHPB were to establish in wild crustacean populations, eradication is highly unlikely.
- NHPB is under study for listing by the OIE — this assessment assumes that NHPB will be listed. If listed by the OIE, NHPB would be included in the Australian *National List of Reportable Diseases of Aquatic Animals* and State/Territory Governments would be expected to report on the agent. Difficulties inherent to the eradication of aquatic animal diseases from wild populations would mean that a campaign aimed at eradicating NHPB from wild crustacean populations is unlikely to be launched. If infected animals were considered likely to be confined to an aquaculture facility (farm or hatchery), then an attempt at eradication is more likely. The indirect economic impacts associated with such a control program, including those associated with zoning and movement controls, are not expected to be discernable at a State/Territory level, but would be significant for affected regions.
- As an indirect result of NHPB establishment and spread, multiple regions (where prawn farming makes a significant contribution to the local economies) would be expected to experience significant flow-on economic impacts.

- NHPB establishment and spread would potentially result in loss of live crustacean export markets. These international trade impacts would be minor, local level impacts.
- Indirect environmental impacts of NHPB establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of NHPB establishment and spread are not expected to be discernible at any level.

The impacts of NHPB establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Regional (multiple)	Minor	D
The environment (native animals/plants, and non-living environment)	Local	Minor	B
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Regional (multiple)	Significant	E
Economic (domestic trade effects and impact on other associated industries)	Regional (multiple)	Significant	E
Economic (international trade effects)	Local	Minor	B
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Minor	A
Overall impact (negligible – extreme)		*Moderate*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

13.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'. The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Moderate*	*Moderate*	*Low*
Impact	*Moderate*	*Moderate*	*Moderate*
'likely consequences'	*Moderate*	*Moderate*	*Low*
Outbreak scenario 2			
PLES	*Moderate*	*Moderate*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Moderate*	*Moderate*	*Low*

13.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Frozen product

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
Very low	*Extremely low*	*Extremely low*	*Very low*	*Extremely low*	*Extremely low*	*Extremely low*

Chilled product

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*High*	*Moderate*	*Low*	*High*	*Moderate*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

Frozen product

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Extremely low*	*Extremely low*	*Extremely low*
'Likely consequences'	*Moderate*	*Moderate*	*Low*
Partial annual risk	*Negligible*	*Negligible*	*Negligible*

Chilled product

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*High*	*Moderate*
'Likely consequences'	*Moderate*	*Moderate*	*Low*
Partial annual risk	*Low*	*Moderate*	*Low*

Conclusion

The unrestricted risk associated with NHPB is determined (by combining the three partial annual risks associated with each exposure group) to be *negligible* for *frozen* product. As the unrestricted risk estimate for frozen product achieves Australia's ALOP, no risk management is considered necessary.

The unrestricted risk associated with NHPB is determined (by combining the three partial annual risks associated with each exposure group) to be *moderate* for *chilled* product. The unrestricted risk for chilled product exceeds Australia's ALOP and, therefore, risk management is deemed necessary.

14 *Vibrio penaeicida*

The following provides an estimation of the *Vibrio penaeicida* risk associated with importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption. The likelihood and impact estimations that constitute the final risk estimation are based on the pathogenic agent-specific information presented in this chapter, as well as the general considerations detailed in Chapter 5 and the agent-specific information presented in Appendix 3.

14.1 Release assessment

Vibrio penaeicida is a highly pathogenic bacterium causing high mortalities in *M. japonicus* aquaculture in Japan (Takahashi et al. 1985a) and *L. stylirostris* aquaculture in New Caledonia (Costa et al. 1996, Costa et al. 1998b, Goarant et al. 1998). Two geographically different clusters of strains have been identified to date (Goarant et al. 1999); one from Japan (Ishimaru et al. 1995) and the other from New Caledonia (Costa et al. 1998b). Both strains display different pathogenicity but it is unclear whether the differences result from differences in dose, bacterial strain or species-susceptibility (Aguirre–Guzmán et al. 2005).

V. penaeicida is considered to be common in the marine environment around affected areas (de la Peña et al. 1992) and asymptomatic carrier prawns may also act as reservoirs of infection (de la Peña et al. 1992, de la Peña et al. 1997, Nakai et al. 1997). Prevalence data from Japan and New Caledonia are not available. However, since initial outbreaks in the early 1990s, health management and improved husbandry methods have combined to reduce *V. penaeicida* associated losses to the extent where they have not recently been reported. Prevalence of *V. penaeicida* in wild crustacean populations has not been reported.

Heavily infected prawns exhibit localised lesions on the cuticle and cloudy musculature (Takahashi et al. 1985a, de la Peña et al. 1993, Costa et al. 1998b, Mermoud et al. 1998), and can be easily detected and removed during processing, whereas carrier prawns with very low titres of *V. penaeicida*, have a healthy appearance and would pass inspection. Ñ

Very little is known about the stability of *V. penaeicida*. *Vibrio* species in general do not tolerate heating or freezing. Freezing will kill vibrios but is ineffective at killing the bacteria completely (Vanderzant and Nickelson 1972, Cook and Ruple 1992, Nascumento et al. 1998).

Conclusions

- *V. penaeicida* is reported to cause mortalities in prawn aquaculture in Japan and New Caledonia in *M. japonicus* and *L. stylirostris*, respectively. The agent is considered to be common in the marine environment around affected areas.
- The likely removal of most clinically diseased prawns due to gross signs (including localised lesions on the cuticle and cloudy musculature), the low titre of bacteria in apparently healthy prawns and the general intolerance of vibrio species to freezing, would be expected to reduce significantly the likelihood of *V. penaeicida* being present in imported prawns in high concentrations.

***Vibrio penaeicida* Likelihood of release (LR)**

The LR of *V. penaeicida* via the unrestricted importation of non-viable, farm-sourced, *frozen*, uncooked, whole prawns intended for human consumption is estimated to be *very low*.

The LR of *V. penaeicida* via the unrestricted importation of non-viable, farm-sourced, *chilled*, uncooked, whole prawns intended for human consumption is estimated to be *high*.

14.2 Exposure assessment

Experimental infection with *V. penaeicida* results in systemic bacterial infection (de la Peña et al. 1995, Costa et al. 1998b, Goarant et al. 1998, Avarre et al. 2003). Imported uncooked frozen *L. stylirostris* and *M. japonicus* infected with *V. penaeicida* would most likely carry a low bacterial titre, at a very low prevalence. Any additional treatments or sub-optimal environmental conditions post-arrival would further inactivate any *V. penaeicida* that survived the freezing process. Unpublished work by Saulnier (cited in Saulnier et al. 2000a) found that the number of viable *V. penaeicida* declined after 2 days of incubation at 25–30°C in artificial seawater with no organic matter.

As an opportunistic pathogen commonly found in seawater in affected areas, *V. penaeicida* has been shown to be able to survive under experimental conditions for more than a year in normal seawater (at 10°C and 20°C), but less than 1 hour in freshwater (de la Peña et al. 1993). *V. penaeicida* is typically a "winter disease" associated with water temperatures below 25°C and does not grow above this temperature — the water in most Australian prawn farms and hatcheries would be at a temperature above 25°C.

Natural hosts of *V. penaeicida* include cultured *L. vannamei* and *M. japonicus*. No other fresh or saltwater crustaceans have been identified with disease caused by *V. penaeicida*. *V. penaeicida* is ever-present in the prawn culture environment in affected areas in Japan — it has been detected in water samples, from affected prawns and in rare instances, from pond sediments (de la Peña et al. 1992, Saulnier et al. 2000b). *Marsupenaeus japonicus*, commonly known as the Kuruma prawn, is of commercial importance to the Australian prawn aquaculture industry. A small population of wild *M. japonicus* can be found near Mackay in Queensland; otherwise the southern limit of distribution from the Indo-West Pacific extends to the tropical coast of Queensland.

Conclusions

- Farmed crustaceans (exposure group 1) were considered very unlikely to be exposed to imported prawns or associated wastes. However, feeding imported head-on uncooked prawns to adult prawns in maturation ponds and to a lesser extent, use of imported prawns as bait for recreational fishing in prawn farm inlet channels, are potentially significant *V. penaeicida* exposure pathways.
- It is expected that a very small, yet significant volume of whole uncooked prawns could be used as feed to condition broodstock in crustacean hatcheries, presenting a direct pathway by which hatchery crustaceans (exposure group 2) could become exposed to *V. penaeicida*. However, very little if any *V. penaeicida* would be expected to remain infectious at the point of exposure. *M. japonicus* is susceptible to *V. penaeicida* infection and is a commercially produced aquaculture prawn species in Australia — it is not known if *P. monodon*, the most important commercial aquaculture species in Australia, is susceptible to *V. penaeicida* infection. However, prawns are not likely to be susceptible to infection at the warm water temperatures typical of Australian prawn farms and hatcheries.
- It is expected that in the event of unrestricted importation of whole uncooked prawns, significant volumes of prawns or associated wastes potentially infected with *V. penaeicida* would be directed for use as recreational fishing bait or berley (including in water below 25°C. As such, bait-use represents a significant pathway by which wild crustaceans (exposure group 3) could become exposed to *V. penaeicida*. Disposal of untreated wastes from commercial processing premises into natural waters also represents a significant pathway for exposure of wild crustaceans to the agent. Although value adding through further commercial processing of imported prawns is not commonly practised in Australia, effluent from such facilities entering natural waters would represent a significant exposure pathway.

- Most bait and commercial processing waste material entering natural waters is expected to be taken by non-susceptible finfish and crustacean species. Susceptible prawn species may to encounter some of this material at temperatures below 25°C, but given the relatively low stability of *V. penaeicida* under sub-optimal environmental conditions, bait-use and disposal of untreated wastes from such premises into natural waters is not considered to represent a significant pathway for exposure of wild crustaceans to *V. penaeicida* — the bacterium is not expected to remain infectious at the point of exposure.

***Vibrio penaeicida* Partial likelihood of exposure (PLE)**

The PLE to *V. penaeicida* from imported non-viable, farm-sourced, *frozen*, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*Extremely low*
Exposure group 2	$PLE_{HATCHERY}$	*Extremely low*
Exposure group 3	PLE_{WILD}	*Very low*

The PLE to *V. penaeicida* from imported non-viable, farm-sourced, *chilled*, uncooked, whole prawns intended for human consumption, with respect to each of the three exposure groups, is estimated to be:

Exposure group 1	PLE_{FARM}	*Low*
Exposure group 2	$PLE_{HATCHERY}$	*High*
Exposure group 3	PLE_{WILD}	*Moderate*

14.3 Consequence assessment

14.3.1 Partial likelihood of establishment or spread

V. penaeicida has a very limited geographical distribution and host range, which includes *M. japonicus* (Takahashi et al. 1985a, Costa et al. 1998b). *M. japonicus*, commonly known as the Kuruma prawn, is of commercial importance to the Australian prawn aquaculture industry. A small population of wild *M. japonicus* can be found near Mackay in Queensland; otherwise the southern limit of distribution from the Indo-West Pacific extends to the northern coast of Queensland. Although the life-cycle of the Kuruma prawn has been closed and a selective breeding program conducted by CSIRO has delivered impressive results, Australian prawn hatcheries rely on both domesticated and wild caught broodstock to supply domestic demand.

The bacterium is considered an opportunistic pathogen ubiquitous in the marine environment in affected areas and has only been known to cause serious disease in aquaculture when the temperature drops and environmental conditions deteriorate (de la Peña et al. 1992). *V. penaeicida* does not grow above 25°C — most prawn farm and hatchery water in Australia would be warmer than 25°C.

Generally, vibriosis is a common secondary systemic infection associated with injury, stress or disease caused by other pathogenic agents. *Vibrio* species are opportunistic in aquaculture, causing mass mortalities in larvae, juveniles and adult prawns already suffering from stress or any deficiency (de la Peña et al. 1992, de la Peña et al. 1993, de la Peña et al. 1997, Costa et al. 1998b, Costa et al. 1998a).

Natural transmission of *V. penaeicida* is thought to occur either *per os* by ingestion of infected material (de la Peña et al. 1998) or by exposure to waterborne bacteria (Aguirre-Guzman et al. 2001). Although experimental prawns can be infected *per os*, transmission via

the water is the most effective means of infecting naïve experimental animals (Le Moullac et al. 1997, de la Peña et al. 1998, Aguirre–Guzmán et al. 2001). Vertical transmission has not been demonstrated.

If established, *V. penaeicida* is unlikely to become widespread due to limited potential host range, the small number and geographic dispersal of prawn farms culturing susceptible species, high dilution of effluent water and predation of clinically infected prawns by non-susceptible species. However, asymptomatic carrier prawns may ac t as reservoirs of infection (de la Peña et al. 1992, de la Peña et al. 1997, Nakai et al. 1997), and the bacterium could, if conditions are favourable, remain viable in water and soil for prolonged periods.

Conclusions

- Susceptible species of crustaceans feeding on prawns that had been infected with *V. penaeicida* may receive an infectious dose — natural transmission of *V. penaeicida* is thought to occur either *per os* by ingestion of infected material or by exposure to waterborne bacteria. *V. penaeicida* is an opportunistic pathogen that may remain viable in the marine environment as a free-living bacterium for prolonged periods, and cause infection and disease when temperatures fall during winter and environmental conditions deteriorate.
- Due to the greater density of animals in farms and hatcheries (exposure groups 1 and 2), infection should it occur would spread more easily than in the case of wild crustaceans (exposure group 3). Also, in the case of wild crustaceans establishment and spread to other susceptible animals would be unlikely because infected animals (particularly those clinically affected) are likely to be predated by non-susceptible finfish.
- Notwithstanding the above differences, the overall likelihood of establishment and spread for each exposure group is considered to be *extremely low*, because the water temperatures of most prawn aquaculture facilities in Australia would be too high for infection to occur. Further, of the species known to be susceptible to *V. penaeicida* infection, only *M. japonicus* is present in Australia — *M. japonicus* is present in the wild and there is a small aquaculture industry. *V. penaeicida* is not known to naturally cause disease in any marine non-penaeid crustaceans or freshwater crustaceans.

***Vibrio penaeicida* Partial likelihood of establishment or spread (PLES)**

The PLES for the three exposure groups is estimated to be:

Exposure group 1 ($PLES_{FARM}$)	
Outbreak scenario 1 (establishment and spread):	*extremely low*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 2 ($PLES_{HATCHERY}$)	
Outbreak scenario 1 (establishment and spread):	*extremely low*
Outbreak scenario 2 (no establishment):	*high*
Exposure group 3 ($PLES_{WILD}$)	
Outbreak scenario 1 (establishment and spread):	*extremely low*
Outbreak scenario 2 (no establishment):	*high*

14.3.2 Impacts

Experimental infection has shown that there is considerable variation in the pathogenicity of different *V. penaeicida* strains (KH-1 and AM101). Juvenile *L. vannamei* (1–1.2 gams) exposed to *V. penaeicida* AM101-strain (10^4 CFU/ml) displayed higher mortality than prawns exposed to KH-1-strain, 120 hours post-exposure. This greater virulence is associated with an exotoxin (protease) secreted by *V. penaeicida* AM101 (Aguirre–Guzmán et al. 2005).

There are few data on production losses attributed to *V. penaeicida*. Between 1988 and 1994, Japanese production of *M. japonicus* reportedly fell by 50% (Takahashi et al. 1998). Approximately 60% of the losses up to 1992 have been attributed to *V. penaeicida* (Takahashi et al. 1998).

Assuming these losses occurred evenly over this time (although this is unlikely due to the onset of WSSV in the early 1990s), it could be estimated that *V. penaeicida* may have caused production losses in *M. japonicus* aquaculture of about 30% per annum.

Total New Caledonian farmed prawn production volume decreased by 15% from 1992 to 1993 (Costa et al. 1998b). Stocking densities and growth cycle durations also changed during this period, making it difficult to determine what proportion of the loss could be attributed to *V. penaeicida* (Mermoud et al. 1998).

In the event that *V. penaeicida* did establish and spread in Australian prawn aquaculture, *M. japonicus* would likely be the only species affected. Although *M. japonicus* culture is important to the prawn farming industry, the production volume of this species is limited to supplying a high-value niche live export market. Since initial outbreaks in the early 1990s in Japan and New Caledonia, health management and improved husbandry methods have combined to reduce *V. penaeicida* associated losses to the extent where they have not recently been reported. A similar trend would be expected following *V. penaeicida* establishment in Australia, as production losses are brought under control with improved health management practices at the farm level.

If *V. penaeicida* were to establish in Australia, eradication would be near impossible as the bacterium is able to survive in seawater, provided conditions are suitable (de la Peña et al. 1992).

There are no known human health impacts associated with *V. penaeicida*.

Conclusions

- The establishment and spread of *V. penaeicida* is expected to have a minor regional impact on farmed prawns in Australia — the only commercial Australian species known to be susceptible is *M. japonicus*.
- There is no evidence that *V. penaeicida* causes serious disease in non-penaeid species or freshwater crustaceans. *V. penaeicida* would not be expected to significantly impact wild crustaceans in Australia.
- *V. penaeicida* is not listed by the OIE. It is not on the *National List of Reportable Diseases of Aquatic Animals* and as such, State/Territory Governments may not report on the agent. If *V. penaeicida* were to establish in Australia, eradication would be near impossible as the agent is able to survive in seawater, provided there are suitable conditions, and therefore eradication is unlikely to be attempted. The likelihood of a control eradication campaign being launched if *V. penaeicida* is detected in Australia is much less than that for other pathogenic agents such as WSSV, TSV, YHV and IHHNV.
- As an indirect result of *V. penaeicida* establishment and spread, an affected locality (where prawn farming makes a significant contribution to the economy) would be expected to experience minor flow-on economic impacts.
- Given the absence of *V. penaeicida*-specific controls on Australian crustacean

products exported overseas, international trade impacts resulting from *V. penaeicida* establishment and spread are not expected to be discernable at any level.
- Indirect environmental impacts of *V. penaeicida* establishment and spread (e.g. on biodiversity or endangered species) are not expected to be discernable at any level.
- The social impacts of *V. penaeicida* establishment and spread are not expected to be discernible at any level.

The impacts of *V. penaeicida* establishment and spread (outbreak scenario 1) are estimated to be as follows:

Impacts of establishment or spread for outbreak scenario 1			
	Level	*Impact*	*Score*
Direct effects			
Animal health (production losses in aquaculture and commercial fisheries)	Regional	Minor	C
The environment (native animals/plants, and non-living environment)	Local	Unlikely to be discernable	A
Indirect effects			
Economic (costs associated with eradication, control, surveillance and monitoring, and compensation)	Local	Minor	B
Economic (domestic trade effects and impact on other associated industries)	Local	Minor	B
Economic (international trade effects)	Local	Unlikely to be discernable	A
Environment (biodiversity, endangered species and the integrity of ecosystems)	Local	Unlikely to be discernable	A
Social (changes in tourism, side effects from control measures, and loss of social amenity)	Local	Unlikely to be discernable	A
Overall impact (negligible – extreme)		*Very low*	

The overall impact associated with the occurrence of outbreak scenario 2 (i.e. that the agent does not establish) would be *negligible*.

14.3.3 Determination of 'likely consequences'

For each of the two outbreak scenarios, the impact estimation made in the previous section is combined with the partial likelihood of establishment or spread (PLES) to obtain an estimation of the 'likely consequences'.

The likely consequences for the two outbreak scenarios are then combined to determine the likely consequences for each exposure group, as follows:

	Farm crustaceans (Exposure group 1)	Hatchery crustaceans (Exposure group 2)	Wild crustaceans (Exposure group 3)
Outbreak scenario 1			
PLES	*Extremely low*	*Extremely low*	*Extremely low*
Impact	*Very low*	*Very low*	*Very low*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Outbreak scenario 2			
PLES	*High*	*High*	*High*
Impact	*Negligible*	*Negligible*	*Negligible*
'likely consequences'	*Negligible*	*Negligible*	*Negligible*
Combined 'likely consequences' for the two outbreak scenarios	*Negligible*	*Negligible*	*Negligible*

14.4 Overall risk determination

Determination of the partial annual likelihood of entry and exposure

For each exposure group, the partial annual likelihood of entry and exposure (PALEE) is determined by 'multiplying' the likelihood of release (LR) with the partial likelihood of exposure (PLE), as follows:

Frozen product

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
Very low	*Extremely low*	*Extremely low*	*Very low*	*Extremely low*	*Extremely low*	*Extremely low*

Chilled product

Likelihood of Release	Partial likelihood of exposure			Partial annual likelihood of entry and exposure (PALEE = LR x PLE)		
LR	PLE_{FARM}	$PLE_{HATCHERY}$	PLE_{WILD}	$PALEE_{FARM}$	$PALEE_{HATCHERY}$	$PALEE_{WILD}$
High	*Low*	*High*	*Moderate*	*Low*	*High*	*Moderate*

Determination of the partial annual risk

The partial annual risk for each of the three exposure groups is determined by combining the partial annual likelihood of entry and exposure (PALEE) with the corresponding 'likely consequences' (using Table 3.5), as follows:

Frozen product

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Extremely low*	*Extremely low*	*Extremely low*
'Likely consequences'	*Negligible*	*Negligible*	*Negligible*
Partial annual risk	*Negligible*	*Negligible*	*Negligible*

Chilled product

	Exposure group		
	Farm	Hatchery	Wild
PALEE	*Low*	*High*	*Moderate*
'Likely consequences'	*Negligible*	*Negligible*	*Negligible*
Partial annual risk	*Negligible*	*Negligible*	*Negligible*

Conclusion

The unrestricted risk associated with *V. penaeicida* is determined (by combining the three partial annual risks associated with each exposure group) to be *negligible* for *frozen* or *chilled* product. As the unrestricted risk estimate achieves Australia's ALOP, no risk management is considered necessary.

15 Risk management

The method adopted by Biosecurity Australia for performing import risk analysis conforms to that recommended by the OIE as described earlier in this report. The method for risk management described here is consistent with that described by the OIE, and is applied in turn to each of the pathogenic agents identified as posing an unrestricted risk that exceeds Australia's ALOP.

Because of the generic nature of this risk analysis, the IRA team has based its evaluation on an assumption that the pathogenic agents of concern are present in the exporting country. Where exporting countries can provide specific data on their own disease status, including evidence to support disease freedom, Biosecurity Australia will reconsider the risk assessment based on that data.

For the following pathogenic agents, the unrestricted risk estimate was deemed not to achieve Australia's ALOP and as such, risk management measures would be necessary to reduce the risk associated with each to an acceptable level:

Pathogenic agent	**Unrestricted annual risk**
White spot syndrome virus (WSSV)	*High*
Yellowhead virus (YHV)	*High*
Necrotising hepatopancreatitis bacterium (NHPB)	*Moderate**
Taura syndrome virus (TSV)	*Low*

* For chilled product only.

In this chapter, having identified and evaluated the pathogenic agents that would require risk management before importation could be permitted, the least trade restrictive risk management measures that could be applied to achieve Australia's ALOP are evaluated. These options were selected from a range of measures considered practicable by the IRA team and form the basis for the recommendations for the importation of prawns and prawn products for human consumption. However, alternative risk management measures that are demonstrated, to the satisfaction of Australian government authorities, to provide equivalent quarantine protection would be considered. Those seeking to propose alternative risk management measures should provide a submission for consideration. Such proposals are welcome and should include supporting scientific data that explain the extent to which the alternative measures would achieve Australia's ALOP.

The method for risk management was outlined in Chapter 4. The approach to risk management was to consider a range of pre-import and post-import measures that might be applied including, where available, the recommendations in the international standard for trade in aquatic animal products (OIE 2006a). Each measure and combination of measures was evaluated to determine the effect on the likelihood of the pathogenic agent entering Australia and/or the likelihood of susceptible host animals becoming exposed. Where the effect was to reduce the overall annual risk to 'very low' or lower, the measure was deemed acceptable.

An important consideration when evaluating the effectiveness of risk management measures was the ability to confirm that the measure would be properly implemented and would deliver the desired effect.

Australia has a long history of implementing measures to reduce the likelihood of susceptible host exposure (e.g. farmer awareness and biosecurity practices). Other programs help to limit the impact of disease establishment, for example, emergency control plans to limit spread and stamp-out disease. These programs were taken into consideration in making the unrestricted risk estimate, in particular in the consequence assessment. The existence of these programs need, however, to be balanced against the generally accepted understanding that aquatic animal diseases would be very difficult to eradicate, particularly if they were to become established in wild host populations.

Australia is committed to exotic disease preparedness and will continue to investigate and develop emergency programs for the rapid identification, containment and stamping-out of exotic diseases.

15.1 Risk management options

The following risk management options were considered by the IRA team.

Sourcing from free stocks (option 1)

Importation of prawns could be permitted from countries or zones determined to be free of the pathogenic agent of concern. Determination of agent freedom would need to be to a standard consistent with that recommended by the OIE, or equivalent. For Australian government authorities to be satisfied that a country or zone is free of a given disease, they must have a knowledge of the Competent Authority (e.g. the veterinary services or equivalent) of that country and be satisfied that the Competent Authority has the capacity for disease control, monitoring and surveillance as appropriate for the disease. In some cases, it might be necessary for the disease to be subject to compulsory reporting or be the subject of consideration in disease investigation. Australia's 'Guidelines for the approval of countries to export animals (including fish and shellfish) and their products to Australia' have been published (ABPM 1999/41 – see Appendix 4). The OIE's international health standard on zoning and compartmentalisation for aquatic animal diseases is provided in Appendix 5 as a guide. Biosecurity Australia recognises that some exporting countries may wish to make a claim for access based on equivalent risk management measures, such as prawn stock accreditation schemes or the concept of compartmentalisation[42]. These would need to be assessed on a case-by-case basis.

A rigorous assessment of any application for approval of compartmentalisation or stock accreditation schemes would be undertaken to ensure that effective biosecurity measures are implemented and maintained throughout the complete chain from source population to point of export. A detailed submission would need to be provided by the competent authority of the exporting country and Australia would conduct an on-ground assessment of the proposed compartment or stock accreditation scheme.

Importation from free countries or zones is expected to reduce the overall risk associated with each pathogenic agent so as to achieve Australia's ALOP, subject to a satisfactory assessment of the country's Competent Authority and its capacity to determine and maintain disease freedom.

[42] A compartment is defined by the OIE as 'one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. Such compartments must be clearly documented by the Competent Authority(ies)' (OIE 2006a).

Pathogen inactivation — cooking or freezing (option 2)

Importation of prawns could be permitted subject to cooking off-shore in a premises approved by, and under the control of, the Competent Authority to ensure inactivation of the pathogenic agent of concern, and subject to verification by the overseas Competent Authority. Alternatively, prawns could be cooked post-arrival, at a quarantine approved premise.

Typically, cooking raw prawns involves placing the product into potable boiling water (100°C) for short periods (e.g. 3–5 minutes) until the product is cooked through (protein is coagulated), not raw on the inside, but not overcooked.

In Australia, in the case of wild-caught prawns, these are usually cooked on board the trawler following the recommended guidelines in the Australian Prawn Industry Association (APIA), *Handling Prawns at Sea, A learning guide for prawn trawler crew at level 1*, viz:

> *The prawn to water ratio is 6:1 (6 litres of water to 1 kg of prawns). Make sure cooker is clean. Fill to required level. Turn cooker on. Add salt to flavour. Allow water to come to the boil before adding prawns. Place required amount of prawns into basket that is to be dipped into water. Lower basket and stir evenly. Visually assess best method for cooking. Once prawns float (caused by the cooked meat shrinking and air bubbles forming under the shell), cook for a further minute. Remove prawns and chill in clean seawater for 2 minutes to stop the cooking process. Drain for 1 minute prior to storage or packaging. Note, even cooking depends on grading. Cooking times are critical. 10–15 seconds can make a difference.*

The UK Department of Trade and Industry, Torry Research Station. Handling and Processing Shrimp. Torry Advisory Note No. 54 (2001) states:

> *When boiling prawns at a ratio of 1 kg raw prawns to 20 litres of water (with 3–5 per cent salt), the temperature of the water will fall to approximately 95°C when the prawns are added and then return to the boil in 1–2 minutes. At least 3 minutes total cooking time is usually sufficient.*

The *Codex Alimentarius* Code of Practice for Fish and Fishery Products (CAC/RCP 52–2003, Rev. 2–2005) incorporates Hazard Analysis Critical Control Point (HACCP) which is an internationally recognised food safety management system that is commonly understood by the food production and manufacturing industry. The seafood industry food standards code of practice used in Australia is the Food Standards Australia New Zealand Standard 4.2.1 (Australia New Zealand Food Standards Code 2005, Chapter 4 Primary Production and Processing Standard for Seafood). When seafood products such as prawns are processed by cooking as a critical step of the HACCP process, minimum standards must be met to eliminate pathogenic agents or reduce them to an acceptable level, prevent contamination and ensure the product is safe for human consumption.

The amount of heat applied during a cooking process will determine which of the identified hazards will be eliminated at that point (FOA Fisheries Technical Paper 334, Assurance of Seafood Quality, 1994). *Listeria monocytogenes* is often identified as the target pathogen as it is regarded as the most heat tolerant, food-borne pathogen that does not form spores (U.S. Food and Drug Administration [USFDA] Centre for Food Safety and Applied Nutrition, Fish and Fisheries Products Hazards and Controls Guidance, Third Edition, June 2001). When seafood processors in the US implement HACCP systems to eliminate *L. monocytogenes* contamination, the USFDA guideline recommends minimum internal product temperature/time treatments that include 63°C for 17 minutes and 72°C for 1 minute.

For a whole prawn to become completely cooked (i.e. for all protein to be coagulated) under commercial conditions depends on the size and quality of the prawn, and is normally determined through experimentation by the seafood processor. Winkel (1998) in an evaluation of the cooking process for Australian farmed *P. monodon*, recommended that

prawns be cooked to a core temperature of 85°C so that the product is marketable (i.e. completely cooked, not chewy, no black spot and aesthetically acceptable). Prawn grades from 11–28 grams (at 20°C) placed into boiling water were reported to reach a core temperature of 85°C at 2.40–4.55 minutes, respectively. It is the opinion of the IRA team that cooking prawns in boiling water for short periods such as those used by seafood processors and recommended by guidelines and advisory notes cited above would be sufficient to kill many prawn pathogens (including WSSV) or substantially reduce more robust pathogenic agents — the IRA team acknowledges that standard commercial cooking practices may not inactivate or only partially inactivate some viruses of concern such as TSV.

Cooking is also expected to significantly reduce the likelihood of imported prawns being diverted to use as bait, as crustacean broodstock feed, or being further processed in Australia (given the limited value-adding processing options following cooking).

Taking into account the varying degrees of pathogen inactivation by commercial cooking, in combination with the reduced likelihood of inappropriate end-use, this option is expected to reduce the partial likelihoods of release and exposure to at least *very low* and therefore the overall risk to an acceptable level. Cooking could be done either off-shore or on-shore in Australia under quarantine control, where all waste is treated as quarantinable.

Freezing is expected to inactivate some agents such as NHPB.

Testing (option 3)

Testing of imported prawns on arrival in Australia at a laboratory approved by AQIS is another option to manage disease risk. Only those batches that test negative would be released for retail sale. The testing would need to be to a standard consistent with that recommended in the OIE *Manual of Diagnostic Tests for Aquatic Animals*, or equivalent. Testing should be at a standard which provides 95% confidence of detecting the agent if it is present at a prevalence of 5%. The level of protection provided by testing would depend on the availability of effective tests (including with respect to their sensitivity and commercial availability, as well as sampling and other operational procedures). The option of testing off-shore would need to be considered on a case-by-case basis, taking into account the ability and capacity of the overseas Competent Authority to audit and provide assurance that the required testing standards and the validity of the test itself have been met to the satisfaction of Australian government authorities and that product integrity is maintained throughout the chain of custody.

Given uncertainty about the sensitivity of available tests for prawn pathogens, this option alone is not expected to reduce the likelihoods of entry and exposure sufficiently to reduce the overall risk to an acceptable level, but may be effective in combination with other measures.

Note that imported food, including prawns and prawn products must comply with the *Imported Food Control Act 1992* and the *Australia New Zealand Food Standards Code* (FSC) in its entirety. Under the *Imported Food Control Act 1992*, AQIS may inspect, or inspect and analyse imported prawns and prawn products after arrival in Australia to determine compliance with the FSC. These food safety and labelling requirements are separate from, and additional to, Australian quarantine requirements. Information on the FSC may be obtained from Food Standards Australia New Zealand.

Highly processed prawns (option 4)

Imported uncooked prawns that have been highly processed, i.e. head-off, shelled and coated for human consumption (e.g. breaded prawns) could be permitted. Such measures would reduce the likelihood of exposure in terms of those pathways associated with unintended end-use such as for recreational fishing bait, both as a result of the preference of recreational fishers for head-on prawns for use as bait (Kewagama Research 2002) as well as the generally higher cost of highly processed prawns. This option is expected to reduce the likelihood of

exposure to at least *very low* and thereby achieve Australia's ALOP. Such processing could occur either off-shore or on-shore in Australia under quarantine control, where all waste is treated as quarantinable.

Uncooked prawn product types that the IRA team considers to be highly processed are:

- breaded (crumbed) or battered prawns, that have had the head and shell removed (except for the last shell segment and tail fans), or
- prawns, that have had the head and shell removed (the last shell segment and tail fans permitted) and have been marinated to a minimum standard, or
- dumpling, spring roll, samosa, roll, ball or dimsum-type products containing uncooked prawn ingredients.

The IRA team does not consider uncooked prawns with the head and shell removed (either in the round, deveined or butterflied forms) as being highly processed — prawns on a skewer or in a 'ring' packaging arrangement are also not considered highly processed.

Note: "round" refers to whole prawn meat that is not deveined or butterflied.

Minimum size (option 5)

Permitting only imported prawns that are above a minimum size is another risk management measure that could be considered. Such measures would reduce the likelihood of exposure by reducing the likelihood that imported prawns are used as recreational fishing bait, given fisher preference for small whole prawns. The Biosecurity Australia-commissioned 2002 National Bait and Berley Survey and a recent follow-up survey indicates that the practice of purchasing prawns for use as bait from seafood outlets still exists at relatively low levels. Whole uncooked prawns dominate the purchase forms reported, with none reported in the large size category (>13 cm). The effectiveness of minimum size restrictions would, however, be significantly reduced if fishers purchase larger prawns and then cut into smaller pieces for use as bait, although the follow-up survey did not find this to be a common practice. The survey also confirmed anecdotal evidence that there is use of peeled prawns purchased from seafood outlets as bait, albeit at a relatively low level.

Minimum size restrictions would not be expected to reduce the exposure of farmed or hatchery crustaceans via the use of imported whole prawns as feed for broodstock. Further, minimum size limits are not expected to significantly reduce the likelihood that imported prawns are emergency harvested.

Therefore, this option by itself is not considered likely to reduce the overall risk to an acceptable level.

Labelling for human consumption (option 6)

Labelling of imported prawns "for human consumption only" and "not to be used as bait or feed for aquatic animals" may reduce the likelihood of exposure by making clear the intended end-use as being for human consumption and prevent diversion at wholesale. Nonetheless, as this labelling would not necessarily apply at retail sale, the general public may be unaware of this requirement. This option by itself is not considered likely to reduce the overall risk to an acceptable level.

Post-harvest inspection to ensure absence of clinical signs of disease (option 7)

Import of prawns could be permitted subject to verification by the overseas Competent Authority that the prawns showed no signs of clinical disease on post-harvest inspection. This measure is expected to reduce the number of clinically infected prawns and in general terms, reduce the number of prawns containing significant amounts of pathogenic agent.

Nonetheless, as many of the diseases of concern can result in sub-clinical infection, the IRA team considers the level of risk reduction provided would be minimal.

Head/shell removal (option 8)

Another option is to allow imported uncooked prawns that have had their head and shell removed (except for the last shell segment and tail fans), subject to offshore inspection and attestation by overseas Competent Authority and post-arrival verification by AQIS. For those pathogenic agents deemed to require risk management, this measure will reduce the amount of agent present in prawns by at least half, and thereby the likelihood of release, although head and shell removal would not be expected to completely eliminate any of the pathogenic agents in most cases. Head and shell removal would also reduce the likelihood of exposure in terms of those pathways associated with head/shell disposal or unintended end-use such as for recreational bait/berley or feed for hatchery broodstock, both as a result of the expected higher cost of such product and the reported preference for head-on prawns for use as recreational fishing bait or hatchery broodstock feed. The extent to which this option would reduce the likelihood of release and/or exposure would depend on the specific pathogenic agent of concern. Head and/or shell removal could occur either off-shore or on-shore in Australia under quarantine control, where all waste is treated as quarantinable.

Sourcing from wild stocks (option 9)

Allowing imported prawns that are wild-caught from populations tested and found free of pathogenic agents of concern (subject to verification by the overseas Competent Authority), may reduce the amount of hazard present in prawns, and thereby the likelihood of release.

The IRA team considers this option to be generally unfeasible, in that existing audit procedures in most exporting countries would not facilitate Competent Authority attestation to this effect. However, the IRA team recognises that it may be possible to introduce species restrictions, such that only species that are known not to be farmed are permitted entry. The effectiveness of such measures would depend on the pathogenic agent of concern, as well as the practicality of ensuring compliance with respect to prawn species identification/confirmation — identification based on DNA analysis may be possible. It may also be possible to ensure prawns are wild-caught by restricting imports to prawns that have been caught, processed and packed on-ship, again contingent on the practicality of ensuring compliance. This option is not considered further, but could be examined on a case-by-case basis subject to a detailed submission being provided.

Sourcing from non-emergency harvested stock (option 10)

Allowing only importation of farmed prawns that have not been emergency harvested (subject to verification by the overseas Competent Authority), may reduce the amount of hazard present in prawns, and thereby the likelihood of release and exposure. The extent to which this option would reduce the likelihood of release and exposure would depend on the specific pathogenic agent of concern. However, the IRA team considers this option would be very difficult to certify accurately and is not considered further.

15.2 Pathogenic agent-specific risk management measures

White spot syndrome virus

The overall unrestricted risk associated with WSSV was estimated as *high*. Of the pathogenic agents covered in this risk analysis, the highest likelihoods of entry and exposure were

associated with WSSV — the likelihood of WSSV entry and exposure for hatchery and wild crustaceans (exposure groups 2 and 3, respectively) was estimated to be *high*, and that for farm crustaceans (exposure group 1) *low*.

The IRA team considers that the following risk management measures would each reduce the overall WSSV risk from *high* to at least *very low*, thereby achieving Australia's ALOP.

- Option 1 (country or zone freedom).
- Option 2 (cooking) would be expected to reduce the likelihoods of WSSV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *extremely low* (Table 15.1).
- Option 4 (high level of processing) would be expected to reduce the likelihood of WSSV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *very low* (Table 15.1).
- Options 3 (testing) and 8 (head/shell removal) would in combination be expected to reduce the likelihoods of WSSV entry and exposure for exposure groups 1 and 2 to *extremely low* and that of exposure group 3 to at least *very low* (Table 15.1).

Yellowhead virus

The overall unrestricted risk associated with YHV was estimated as *high*. The likelihood of YHV entry and exposure for hatchery crustaceans (exposure group 2) was estimated to be *high*, *moderate* for wild crustaceans (exposure group 3), and *low* for farm crustaceans (exposure group 1).

The IRA team considers that the following risk management measures would each reduce the overall YHV risk from *high* to at least *very low*, thereby achieving Australia's ALOP.

- Option 1 (country or zone freedom).
- Option 2 (cooking) would be expected to reduce the likelihoods of YHV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *extremely low* (Table 15.1).
- Options 3 (testing) and 8 (head/shell removal) would in combination be expected to reduce the likelihoods of YHV entry and exposure for exposure groups 1 and 2 to *extremely low* and that of exposure group 3 to at least *very low* (Table 15.1).
- Option 4 (high level of processing) would be expected to reduce the likelihood of YHV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *very low* (Table 15.1).

Necrotising hepatopancreatitis bacterium

The overall unrestricted risk for uncooked *chilled* product associated with NHPB was estimated as *moderate*[43]. The likelihood of NHPB entry and exposure for hatchery crustaceans (exposure group 2) was estimated to be *high*, *moderate* for wild crustaceans (exposure group 3), and *low* for farm crustaceans (exposure group 1).

The IRA team considers that the following risk management measures would each reduce the overall NHPB risk for uncooked *chilled* product from *moderate* to at least *very low*, thereby achieving Australia's ALOP.

- Option 1 (country or zone freedom).
- Option 2 (cooking) would be expected to reduce the likelihoods of NHPB entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *extremely low* (Table 15.1).

[43] The overall risk for uncooked *frozen* product is determined to be acceptable – see page 156.

- Option 4 (high level of processing) would be expected to reduce the likelihood of NHPB entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *very low* (Table 15.1).
- Option 8 (head/shell removal) would be expected to reduce the likelihoods of NHPB entry and exposure for exposure groups 1, 2 and 3 to *negligible* (Table 15.1) — because the bacterium is concentrated in the cephalothorax and the gut of prawns, removal of the head would result in significant risk reduction.

Taura syndrome virus

The overall unrestricted risk associated with TSV was estimated as *low*. The likelihood of TSV entry and exposure for farm (exposure group 1) and hatchery (exposure group 2) crustaceans was estimated to be *low*; that of wild crustaceans (exposure group 3) was estimated to be *moderate*.

The IRA team considers that the following risk management measures would each reduce the overall TSV risk from *low* to *negligible*, thereby achieving Australia's ALOP.

- Option 1 (country or zone freedom).
- Option 2 (cooking) would be expected to reduce the likelihoods of TSV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *very low* (Table 15.1).
- Option 4 (high level of processing would be expected to reduce the likelihood of TSV entry and exposure for exposure groups 1 and 2 to *negligible* and that of exposure group 3 to at least *very low* (Table 15.1).
- Option 8 (head/shell removal) would be expected to reduce the likelihoods of TSV entry and exposure for exposure groups 1 and 2 to *very low* and that of exposure group 3 to at least *low* (Table 15.1).

Table 15.1 Details of risk assessment values for quarantine measures

Measure	WSSV				YHV				TSV				NHPB*			
	LR	PLE(F)	PLE(H)	PLE(W)	LR	PLE(F)	PLE(H)	PLE(W)	LR	PLE(F)	PLE(H)	PLE(W)	LR	PLE(F)	PLE(H)	PLE(W)
Unrestricted likelihood value	H	L	H	H	H	L	H	M	H	L	L	M	H	L	H	M
Cooking	VL	N	N	VL	VL	N	N	VL	L	N	N	VL	VL	N	N	VL
PALEE		N	N	EL		N	N	EL		N	N	VL		N	N	EL
Consequence		H	H	L		H	H	L		L	L	VL		M	M	L
Risk		N	N	N		N	N	N		N	N	N		N	N	N
Overall risk	NEGLIGIBLE				NEGLIGIBLE				NEGLIGIBLE				NEGLIGIBLE			
Testing	VL	L	H	H	VL	L	H	M	VL	L	L	M	VL	L	H	M
PALEE		VL	VL	VL		VL	VL	VL		VL	VL	VL		VL	VL	VL
Consequence		H	H	L		H	H	L		L	L	VL		M	M	L
Risk		L	L	N		L	L	N		N	N	N		VL	VL	N
Overall risk	LOW				LOW				NEGLIGIBLE				VERY LOW			
Highly processed	H	N	N	VL	H	N	N	VL	H	N	N	VL	H	N	N	VL
PALEE		N	N	VL		N	N	VL		N	N	VL		N	N	VL
Consequence		H	H	L		H	H	L		L	L	VL		M	M	L
Risk		N	N	N		N	N	N		N	N	N		N	N	N
Overall risk	NEGLIGIBLE				NEGLIGIBLE				NEGLIGIBLE				NEGLIGIBLE			
Head/Shell removal	H	VL	VL	M	H	VL	VL	L	H	VL	VL	L	N	VL	VL	L
PALEE		VL	VL	M		VL	VL	L		VL	VL	L		N	N	N
Consequence		H	H	L		H	H	L		L	L	VL		M	M	L
Risk		L	L	L		L	L	VL		N	N	N		N	N	N
Overall risk	MODERATE				LOW				NEGLIGIBLE				NEGLIGIBLE			
Head/Shell removal + testing	VL	VL	VL	M	VL	VL	VL	L								
PALEE		EL	EL	VL		EL	EL	VL								
Consequence		H	H	L		H	H	L								
Risk		VL	VL	N		VL	VL	N								
Overall risk	VERY LOW				VERY LOW											

LR – likelihood of release, PLE – partial likelihood of exposure (F) farm; (H) hatchery; (W) wild, PALEE – partial annual likelihood of entry and exposure, Consequence – likely consequences, Risk – annual risk for each exposure group, Overall risk – overall risk determination (overall risk must be very low or less to meet Australia's ALOP).

*NHPB likelihood estimations are based on importation of prawns that are not frozen (i.e. chilled prawns). The overall risk for uncooked frozen product is determined to be acceptable.

15.3 Risk management conclusions

To achieve Australia's ALOP with respect to the pathogenic agents identified in this risk analysis, all imported prawns or prawn products would need to be:

- sourced from countries or zones determined to the satisfaction of Australian government authorities to be free of white spot syndrome virus (WSSV), yellowhead virus (YHV), and Taura syndrome virus (TSV), and in addition, necrotising hepatopancreatitis bacterium (NHPB) if the product is not frozen (i.e. the product is chilled);

OR

- cooked in premises approved by and under the control of an appropriate Competent Authority to a minimum time and temperature standard where all the protein in the prawn meat is coagulated and no uncooked meat remains;

OR

- highly processed, that is with the head and shell removed (the last shell segment and tail fans permitted) and coated for human consumption as follows:
  - breaded (crumbed) or battered, or
  - marinated to a minimum standard, or
  - processed into dumpling, spring roll, samosa, roll, ball or dimsum-type product,

OR

- have had the head and shell removed (the last shell segment and tail fans permitted) and each batch tested on arrival in Australia and found to be free of WSSV and YHV: testing is based on the polymerase chain reaction (PCR) tests in the current version of the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests for Aquatic Animals* or equivalent, and a sampling regimen that would provide 95% confidence of detecting the agent if present at 5% prevalence.

The IRA team recommends that for uncooked prawns and prawn products, the above measures aimed at specific pathogenic agent-associated risks be underpinned by general health certification (to accompany each shipment of imported prawns) issued by the relevant Competent Authority in the exporting country, attesting that the prawns had been inspected, processed and graded in premises approved by and under the control of the Competent Authority, were free from visible lesions associated with infectious disease and are fit for human consumption. The IRA team also recommends that uncooked prawns imported for human consumption that are not considered to be highly processed be marked with the words 'for human consumption only' and 'not to be used as bait or feed for aquatic animals'. For cooked prawns, the IRA team recommends that health certification accompanies each shipment which attests to the application of the minimum standard of heat treatment and that the prawns are fit for human consumption.

Where it is intended that imported prawns be processed (removal of head and/or shell or further processing into 'highly processed' product) or cooked in Australia, rather than off-shore, this must (if required) be undertaken under quarantine control where all waste is treated as quarantinable.

Marinated highly processed prawn products must be marinated to a minimum standard as defined in the quarantine measures. Products imported under the highly processed category are reviewed on a case-by-case basis and assessed as to whether they meet the definition of a highly processed product. For example, a wet marinade must be no less than 12% of the total weight of the product and consist of a genuine marinade. Non-flavoured ingredients such as oil, maltodextrin or a sprinkling of lemon/lime juice are not considered to contribute to the 12% weight requirement. A dry marinade must be clearly seen to coat the product with items

such as herbs and/or chopped/crushed garlic and/or spices.

The IRA team recognises that there may be other treatments such as high hydrostatic pressure, new technologies, or other combinations of measures that may provide an equivalent level of risk reduction for the pathogenic agents identified as requiring risk management. These would need to be evaluated on a case-by case basis. A rigorous assessment of any application for alternative treatments would be undertaken to ensure the effectiveness of the treatment. A detailed submission would need to be provided by the competent authority of the exporting country to Australia for consideration.

The IRA team notes the emergence of monodon slow growth syndrome (MSGS) and white tail disease (WTD). Currently the cause of MSGS has not been determined. Similarly, the relationship between the two viruses associated with WTD and their role in pathogenicity remains unclear. Biosecurity Australia will continue to monitor developments in relation to the scientific knowledge and understanding of MSGS and WTD and review import requirements as appropriate.

The IRA team recognises that there are a range of shelf-stable food products (for human consumption) that are not specifically covered in this risk analysis report. Shelf-stable food products containing prawns such as dried prawns, canned prawns or condiments containing prawns as an ingredient (e.g. prawn balachan, shrimp paste) are considered to pose a negligible risk because they are highly unlikely to come into contact with live crustaceans in Australia. Such products would not be subject to the risk management measures recommended in this report.

16 Quarantine measures for the importation of prawns and prawn products for human consumption

The following quarantine requirements apply to the importation of prawns and prawn products for human consumption (other than shelf-stable prawn-based food products[44]), and are issued under the authority of *Quarantine Proclamation 1998*.

NOTE: Imported food, including prawns and prawn products must comply with the *Imported Food Control Act 1992* and the *Australia New Zealand Food Standards Code* (FSC) in its entirety. Under the *Imported Food Control Act 1992*, the Australian Quarantine and Inspection Service (AQIS) may inspect, or inspect and analyse imported prawns and prawn products to determine compliance with the FSC. These food safety and labelling requirements are separate from, and additional to, Australian quarantine requirements. Information on the FSC may be obtained from Food Standards Australia New Zealand[45].

1 Import Permit

The importer must obtain a permit to import all *uncooked* prawns and prawn products into Australia for human consumption from AQIS, before the goods are imported.

The application to import must include:

- the name and address of the importer and exporter; and
- a description of the commodity to be imported.

The application will be assessed on the above information as well as any other criteria deemed relevant by the Australian Director of Animal and Plant Quarantine.

Cooked prawns and prawn products do not require an import permit but will be required to meet conditions that are specified in the *Quarantine Proclamation 1998* (see point 3).

2 Uncooked prawns

2.1 All imported *uncooked* prawns must:

- be sourced from a country or zone that is recognised by Australia to be free of ALL the following pathogenic agents:
  - White spot syndrome virus (WSSV),
  - Yellowhead virus (YHV), and
  - Taura syndrome virus (TSV),

  and in addition

[44] Shelf-stable prawn-based food products include dried prawns, canned prawns or condiments containing prawns as an ingredient (e.g. prawn balachan, shrimp paste).

[45] Available at: www.foodstandards.gov.au

- Necrotising hepatopancreatitis bacterium (NHPB) if the product is not frozen (i.e. the product is chilled);

OR

- be highly processed[46], that is with the head and shell removed (the last shell segment and tail fans permitted) and;
  - coated for human consumption by being breaded (crumbed) or battered, or
  - coated for human consumption by being marinated in a wet marinade (the marinade must be no less than 12% of the total weight of the product), or
  - coated for human consumption by being marinated in a dry marinade (the marinade must be clearly seen to cover the product), or
  - coated for human consumption by being marinated and placed on skewers (the marinade must be clearly seen to cover the product), or
  - the raw prawn meat processed into dumpling, spring roll, samosa, roll, ball or dim sum-type product;

OR

- have had the head and shell removed (the last shell segment and tail fans permitted), be frozen and each batch tested on arrival in Australia and found to be free of WSSV and YHV.

Batch testing for a pathogenic agent does not occur if sourced from a country or zone recognised by Australia to be free of that agent.

Testing is based on the polymerase chain reaction (PCR) tests in the current version of the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests for Aquatic Animals* or equivalent, and a sampling regimen that would provide 95% confidence of detecting the agent if present at 5% prevalence.

All consignments of prawns to be tested will be held under quarantine control in Australia where they will be sampled for testing. Prawns will remain under quarantine control until the results of the tests are available. Batches that return positive results must be re-exported, destroyed or further processed (i.e. cooked) in a facility approved by AQIS for that purpose.

For the purpose of this testing, a batch is defined as a population from a different pond population or fishing period population. Documentation from the exporter, supplier or the Competent Authority verifying the number of batches in the consignment must be provided to AQIS. This documentation must clearly detail the labelling of each batch in the consignment. If the number of batches cannot be determined from documentation, full unpacking and inspection may be required in order to determine the number of batches.

2.2 For all *uncooked* prawns and prawn products (including those that are considered to be highly processed, as defined in Section 2.1), the Competent Authority in the exporting country must certify that the prawns or prawn products:

- are fit for human consumption, and
- have been processed, inspected and graded in premises approved by and under the control of the Competent Authority, and
- are free from visible signs of infectious disease.

[46] An AQIS fact sheet that provides definitions and descriptions of highly processed wet and dry marinated prawn products as relevant to AQIS import permit applications is at Appendix 6.

In addition, for *uncooked* prawns that are not considered to be highly processed (as defined in Section 2.1), the Competent Authority must certify that:

- each package is marked with the words '*for human consumption only'* and *'not to be used as bait or feed for aquatic animals*'.

2.3 *Uncooked* prawns and prawn products that are considered to be highly processed (as defined in Section 2.1) will be randomly inspected by AQIS to ensure the imported commodity complies with the product description on the import permit and health certificate.

3 Cooked prawns

For *cooked* prawns and prawn products, the Competent Authority in the exporting country must certify that the prawns or prawn products:

- are fit for human consumption, and
- have been cooked in premises approved by and under the control of the Competent Authority to a minimum time and temperature standard where all the protein in the prawn is coagulated and no uncooked meat remains [47].

4 Review

Conditions for importation may be reviewed if there are any changes in the source country's import policy or its animal disease status, or at any time at the discretion of the Australian Director of Animal and Plant Quarantine.

[47] For example, cooking to a minimum 70°C core temperature for at least 11 seconds is considered to achieve coagulation of all proteins in prawns and prawn products.

APPENDIX 1 Changes to the IRA final report since the 2006 draft report

The following details the main changes to the final IRA report (this report) since the 2006 revised draft IRA report was released for stakeholder comment in November 2006. It includes changes as a result of stakeholder comments and comments from the Eminent Scientists Group (ESG).

Several stakeholders queried the inclusion of infectious hypodermal and haematopoietic necrosis virus (IHHNV) as a hazard and the recommended risk management measures for this pathogenic agent. IHHNV was included as a hazard in the 2006 draft report because:

- there are susceptible species in Australia,
- there were strains exotic to Australia, and
- it is listed by the World Organisation for Animal Health (OIE) and expected to cause significant disease in Australia.

In July 2008, Australia notified the detection of a strain of IHHNV in Australia very similar to those strains found in Asia. The Australian Chief Veterinary Officer, as chair of the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD) advised Biosecurity Australia of Australia's changed aquatic animal health status with regard to IHHNV and that it was not feasible to undertake an eradication or control program.

Following discussion of this advice with the IRA team, Biosecurity Australia recommended the interim quarantine measures for IHHNV (announced in July 2007 and implemented in October 2007) be removed.

As a result, IHHNV has been ruled out of the IRA report as a hazard requiring further consideration (see page 46).

Several stakeholders also queried the risk estimation for necrotising hepatopancreatitis bacterium (NHPB) and associated risk management measures. New information on NHPB since release of the revised draft IRA report suggests that freezing at commercial storage temperatures would be a suitable risk management measure. The IRA team has re-evaluated the new information and, in its judgement, considers frozen product would meet Australia's appropriate level of protection (ALOP). The IRA report has been amended (see page 156).

In the 2006 draft report, the IRA team estimated the likelihood of release (LR) of *Vibrio penaeicida* to be low. The IRA team has reviewed the literature on the effect of freezing on the vibrios and concluded that the LR for *V. penaeicida* via the unrestricted importation of non-viable, farm-sourced, frozen, uncooked, whole prawns intended for human consumption should be very low. This has not changed the overall conclusion that the agent does not pose an unacceptable risk. The report has been amended on page 163.

The 2006 draft report accepted that imported uncooked prawns that have been highly processed, i.e. head-off, shelled and coated for human consumption (e.g. breaded prawns) could be permitted. Uncooked prawn product types that the IRA team considered to be highly processed included breaded (crumbed) or battered prawns, that have had the head and shell removed (except for the last shell segment and tail fans), and dumpling, spring roll, samosa, roll, ball or dimsum-type products containing uncooked prawn ingredients. Following consideration of an industry request for alternative risk management measures, the IRA team accepted that prawns, that have had the head and shell removed (the last shell segment and tail fans permitted) and have been marinated to a minimum standard could also be considered

highly processed. Thus, marinated prawn products have been added to this report as an acceptable risk management option (see page 168). Marinated prawns as highly processed prawns were included in the interim quarantine measures announced in July 2007. AQIS is in the process of introducing a process of inspection of marinated prawns to verify the meeting of import permit conditions.

As requested by several stakeholders, this final report includes information on the follow-up bait and berley survey concluded in 2007, which confirmed the findings of the 2002 National Bait and Berley Survey (see page 78).

Following ESG recommendation, a table of data on prawn imports based on preparation category (raw versus cooked/highly processed) 2001-2005 has been added to this IRA report (see Table 2.3, page 16). The data are based on prawn import statistics provided by AQIS-COMPILE system from 2001-2004 and 2005. However, Biosecurity Australia cannot guarantee the validity of this raw data as it was not involved in its production, and some anomalies were noted during the analysis.

A stakeholder considered that in the interest of transparency, the effect of the risk management measures on the likelihoods of entry and exposure should be fully documented. In light of this comment a table that details the determination of restricted risk resulting from a range of risk management options has been included in the IRA report (see page 173).

As recommended by the ESG, an appendix has been added to the report which summarises the 2000 Darwin WSSV incident where imported prawns were inadvertently fed to hatchery crustaceans, resulting in activation of the Aquatic CCEAD and a subsequent national WSSV survey to confirm Australia's WSSV free status (see page 183).

Finally, this IRA report differs in format to the 2006 draft in that it is contained in a single document, rather than being separated into Parts A and B.

APPENDIX 2 2000 Darwin WSSV incident: a summary

On 15 November 2000, a box of frozen prawns labelled 'Cocktail Prawns' and 'Product of Indonesia' was identified at a government run aquaculture facility in the Northern Territory (NT). The prawns had been purchased from a Darwin wholesaler on the understanding they were of Australian origin, in accordance with the facility's policy of feeding locally caught rather than imported prawns to reduce disease risks.

As a consequence, an immediate audit of the origin of earlier batches of prawns was undertaken. These included a batch of 'River Prawns' purchased from the same wholesaler also on the understanding they were of Australian origin. These 'River Prawns' had no labelling to indicate their origin. The wholesaler, on questioning, indicated that these 'River Prawns', as well as the 'Cocktail Prawns', may have been imported from Indonesia via Perth, Western Australia (WA). This was subsequently confirmed. Further, it was established they were part of a large consignment imported in 1999 and that prawns from this consignment had been distributed for sale in three states as well as the NT. A significant amount had been distributed for sale as bait.

As a precautionary measure against transmission of exotic diseases, all crustaceans at the aquaculture facility were destroyed, feeding of prawns discontinued and holding tanks and all associated equipment disinfected. Representative samples of crab tissues and the imported prawns were collected for testing for white spot syndrome virus (WSSV).

On 20 November 2000, the NT Department of Primary Industry and Fisheries informed the Australian Chief Veterinary Officer (ACVO) of this incident and of the actions taken. The ACVO then informed State and Territory Chief Veterinary Officers and Directors of Fisheries. It was later reported that a Northern Territory university had been using uncooked 'River Prawns' from the same consignment to feed Black Tiger Prawns (Penaeus monodon) at the University. The cultured prawns were killed, after collection of samples for testing, and the facility disinfected.

By 5 December 2000, polymerase chain reaction (PCR) test results had confirmed the presence of DNA consistent with WSSV in:

- 'River Prawns' and 'Cocktail Prawns' imported from Indonesia and used to feed mud crabs at the government aquaculture facility and monodon prawns at the university (3 of 6 tested);
- cultured monodon prawns at the university (11 of 11 tested);
- cultured mud crabs at the government aquaculture facility (2 of 15 tested); and
- wild shore crabs (5 of 12 tested) and prawns (2 of 4 tested) collected at the outfall of the aquaculture facility (in Darwin Harbour).

Subsequent testing of wild crustaceans indicated that WSSV has not become established in Darwin Harbour.

A national survey to confirm Australia's WSSV-free health status was carried out and its findings reported to the OIE in 2002. WSSV was not confirmed in any of 3,051 crustacean samples collected from 64 locations throughout Australia. In addition, throughout the course of the survey, neither clinical signs of WSSV infection nor raised mortality were observed in any crustacean population. Sites included those identified as carrying a high risk that green prawns imported for human consumption may have been used as feed or bait (e.g. popular

fishing sites) as well as low-risk sites as controls (e.g. sites with no proximity to aquaculture facilities and recreational fishing). Although focused on wild crustaceans, the survey also included farmed crustaceans where appropriate.

APPENDIX 3 Background information: pathogenic agents of concern

White spot syndrome virus

White spot syndrome virus (WSSV) infects a wide range of decapod crustaceans and is known to cause white spot disease (WSD) in farmed prawns. The virus is especially pathogenic to penaeid prawn species. Serious losses in farmed prawns due to WSD were first reported from Taiwan in 1992 (Chou et al. 1995). WSSV has since spread throughout most prawn culture areas of the Indo-Pacific and the Americas — WSD continues to cause stock losses in affected countries. WSD is listed as a disease notifiable to the OIE.

Agent taxonomy

WSSV is a rod-shaped, enveloped, double-stranded DNA virus (Inouye et al. 1996) and is the sole species in the newly created genus *Whispovirus*, family Nimaviridae (Mayo 2002a, Mayo 2002b).

Differences in tandem repeat sequences between isolates from different geographical locations has been noted (Wongteerasupaya et al. 2003). Differences in PCR profiles have been employed to identify different strains of WSSV in epidemiological studies (Galaviz–Silva et al. 2004); however, the full significance of these differences is uncertain and remains the topic of ongoing research (He et al. 2005, Leu et al. 2005, Liu and Yang 2005, Wu et al. 2005).

Agent stability

Several studies have demonstrated that WSSV can remain infectious following exposure to freezing temperatures (–20°C and –70°C) for prolonged periods (Wang et al. 1997b, Nunan et al. 1998, Wang et al. 1999a).

Heat treatment has been shown to inactivate WSSV suspended in sterile water at 70°C within 5 minutes (Chang et al. 1998b) and at 60°C for 20 minutes for homogenised viral preparations (Balasubramanian et al. 2006). Treatment with ultraviolet light, ozone, low and high pH, sodium hypochlorite, povidone iodine, benzalkonium chloride (Chang et al. 1998b) and formalin (Flegel 1996) have also been shown to deactivate WSSV to varying degrees. Irradiation (from a Cobalt-60 source) applied at a dose rate of 0.8 kGy/h for 12 to 36 hours has been shown to decrease the infectivity of a 30 ml virus preparation of WSSV, but can only partly decrease the infectivity of WSSV in infected whole prawns (Liu et al. 2004).

WSSV infectivity trials conducted using infectious material obtained from prawn carcasses has revealed that WSSV can retain infectivity for six days at 25.5 – 28.8°C (Wang et al. 2002). Further trials indicate prawn heads from WSSV-infected animals retained infectivity for at least 14 days, and prawn tails from WSSV-infected animals can remain infectious for at least 28 days, at 27°C (Prior and Browdy 2002).

Speculation existed on the role of seabirds in the spread of WSSV to prawn farms, until Vanpatten et al. (2004) demonstrated there to be no detectable infection (using histopathology and PCR) as a result of *Litopenaeus vannamei* prawns being injected with PCR-positive seagull faeces.

Epidemiology

Host range

A wide range of decapod crustaceans, including marine and freshwater prawns, crabs and crayfish are reported to be susceptible to natural infection with WSSV (Wang et al. 1995, Lo et al. 1996a, Lo et al. 1996b, Flegel 1997a, Lo et al. 1997a, Richman et al. 1997, Peng et al 1998, Chang et al. 2001. Many other decapod crustaceans including lobsters are reportedly susceptible to experimental WSSV infection (Chang et al. 1998a, Maeda et al 1998a, Supamattaya et al. 1998, Wang et al. 1998, Maeda et al. 2000, Sahul Hameed et al. 2003, Edgerton 2004). However, the list of species reported as susceptible to WSSV should be regarded with some caution as the diagnostic test recommended by the Manual of Diagnostic Tests for Aquatic Animals (OIE 2006b) is known to give false positive reactions due to a cross-reaction with crustacean DNA (Claydon et al. 2004).

It is generally accepted that farmed prawns would carry a natural multiple virus load due to the nature of their immune system (Flegel 2001, Chayaburakul et al. 2004, Flegel et al. 2004). The ability of many crustacean species to act as carriers for multiple viable viruses greatly extends the potential host range for WSSV and some carrier crustacean species have been shown to act as vectors for the virus in laboratory experiments (Kanchanaphum et al. 1998).

Galaviz-Silva et al. (2004) reported WSSV in farmed *L. vannamei* and wild crustacean species found at farm sites including *Litopenaeus stylirostris* and the crab *Callinectes arcuatus* on the west coast of Mexico. Although WSSV infections can be detected in wild prawn populations (Hossain et al. 2001a, Hossain et al. 2001b). WSD outbreaks have only been reported in farmed prawns, including one report of WSD in zoo-kept freshwater crayfish (*Procambarus* spp. and *Orconectes punctimanus*) (Richman et al. 1997).

It has also been demonstrated that the polychaete worm, *Marphysa gravelyi*, used as live feed for broodstock can mechanically carry WSSV (Vijayan et al. 2005). Yan et al. (2004) proposed that rotifer eggs may act as reservoirs of WSSV in prawn ponds.

Geographical distribution

WSSV is widespread in Asia and many parts of Central and South America. It has been reported in mainland US (Chang et al. 2001), Saudi Arabia (Yap 2001b) and Iran (NACA 2003). WSSV is exotic to Australia (East et al. 2004).

Transmission

Natural transmission of WSSV may be horizontal by ingestion of infected tissue (*per os*) (Chang et al. 1996) or by immersion in infected water (Chou et al. 1995). Water can be infected with faecal pellets from WSSV-positive animals (Rajan et al. 2000). Infection *per os* has been shown to be a more effective inoculation route for *L. vannamei* than immersion in viral extract (Perez et al. 2005).

It is unclear whether transovarial transmission of WSSV takes place, or whether the spread of virus from broodstock to offspring occurs via contamination of the external surface of the egg (Chang et al. 1998a, Lo and Kou 1998).

In bioassays with *P. monodon* fed WSSV infected crab and lobster tissues, clinical signs of WSD and mortality occurred in the prawns within two to four days (Rajendran et al. 1999). In cohabitation studies of *P. monodon* with WSSV infected crabs prawn mortalities and infection as confirmed by histology and *in situ* hybridisation began 3 days after exposure. Cumulative prawn mortalities reached 100% by day 8 of the trial (Kanchanaphum et al. 1998).

Rotifers, commonly used as a prey feed source in an aquaculture setting have been experimentally exposed as a potential vector of WSSV via the virus-phytoplankton adhesion route whereby water-bourne WSSV adheres to phytoplankton, which in turn is ingested by zooplankton such as rotifers, which in turn provide a food source for prawns in aquaculture ponds and hatcheries (Zhang et al. 2006).

Tissue titres

The WSSV load in infected prawns can be highly variable when calculated using real time PCR. Many factors have been suggested that may influence variability, including host species and the route and stage of infection (Durand and Lightner 2002, Durand et al. 2003).

Although highly variable (depending on species, route of transmission, tissue tested and stage of infection), the mean WSSV viral load found in naturally infected, moribund prawns (*P. monodon*, *L. stylirostris* and *L. vannamei* juveniles) was $1.0x10^{10}$ genome copies per microgram DNA extracted (Durand and Lightner 2002). When juvenile *P. stylirostris* were tested they were found to have a mean viral load of $5.7x10^{11}$ genome copies per gram of whole animal tissue (Durand and Lightner 2002).

Juvenile *L. vannamei* (mean weight 3g) in the acute phase of WSSV infection (experimentally infected *per os*), have been shown to contain 10^5 to 10^9 WSSV copies per microgram of total DNA extracted and 10^5 to 10^{11} copies per gram of host tissue with means in the range of 10^{10} for all tissues sampled (Durand et al. 2003). The study found that, on a per weight basis, whole heads and whole tails of WSSV-infected *L. vannamei* contain similar viral loads, estimating that approximately 49% of the viral load in WSSV-infected *L. vannamei* is within the cephalothorax, 28% within the tail shell and 23% within the tail meat (Durand et al. 2003). However, because PCR inhibitors in some tissues of the cephalothorax such as the eye (Lo et al. 1997a) were not considered, the WSSV load in the cephalothorax may be higher than reported.

Qualitative analysis performed using *in situ* hybridisation techniques indicates that the number of WSSV-positive cells in wild-caught carrier prawns was relatively low compared to the number of WSSV-positive cells in cultured and experimentally infected prawns (Lo et al. 1997b).

Infectious dose

WSSV was transmitted to juvenile *Farfantepenaeus duorarum* (mean weight 6.4g) and *L. vannamei* (mean weight 5.0g), by one feeding (5% body weight w/w) of WSSV-infected tissue (Wang et al. 1999a). *L. vannamei* (mean weight 3g) became infected with WSSV after two feeds of WSSV-infected tissue at a rate of 2.5% bodyweight for 2 days (Durand et al. 2003). It has been estimated that a minimum of 10^5 WSSV genome copies are required for transmission of the virus to *L. vannamei* (weight 5g) via immersion, but it took 10^4 WSSV copies (or less) to transmit the virus by intramuscular injection (Durand and Lightner 2002).

The WSSV LD_{50} by intramuscular injection for *Marsupenaeus japonicus* is reported to be 950 genome copies of WSSV per gram of prawn tissue (Wu et al. 2002).

Cohabitation is considered to be associated with a lower rate of viral transmission than ingestion of infected animals (Soto and Lotz 2001). Similarly, horizontal transmission of WSSV increases at higher stocking densities of experimental animals; surmised to be due to an increased opportunity for cannibalism rather than an increase in water-borne transmission (Wu et al. 2001). Furthermore, the susceptibility of *L. vannamei* to WSSV has been shown to increase after postlarvae day 30 (PL30), possibly due to genetic, physiological or ethological characteristics (Perez et al. 2005).

Less work has been published on WSSV in non-prawn crustaceans than that published on WSSV in prawns. However, it has been demonstrated that the average yield of WSSV in experimentally infected freshwater crayfish is approximately 10^9 virions per 5 ml of crayfish

haemolymph (Huang et al. 2001). It was estimated that it took in the order of 10^6 WSSV virions to initiate infection within 5–7 days in freshwater crayfish (using a previously frozen inoculum delivered by intramuscular injection) (Huang et al. 2001). The same study showed that lower doses (10^4 to 10^5 virions) could initiate infection in the crayfish when the inoculum was prepared from freshly extracted haemolymph rather than from previously frozen material (Huang et al. 2001). Other non-prawn crustacean studies have demonstrated that crabs may have high levels of WSSV viraemia yet remain asymptomatic (Kanchanaphum et al. 1998).

Prevalence

Most studies into WSSV in prawn aquaculture populations have relied on PCR techniques for the detection of WSSV DNA; investigation into the prevalence of WSSV infection throughout areas where the disease is either endemic or ecdemic is ongoing but by no means comprehensive.

In the Philippines, 16/16 (100%) of *P. monodon* ponds sampled with unexplained prawn mortality, and 35/55 (64%) ponds sampled for monitoring purposes only, were positive for WSSV DNA using either PCR or Western blot analysis (Magbanu et al. 2000). WSSV DNA was detected by PCR in 15/24 of grow-out *P. monodon* ponds monitored in Vietnam (Corsin et al. 2001). In a Taiwanese study of *P. monodon* postlarvae in culture ponds, WSSV DNA was detected by PCR in samples from 20/27 ponds (approximately 74%) (Peng et al. 2001). In an epidemiological study in India, 29/44 (approximately 66%) of monitored *P. monodon* ponds were positive for WSSV by PCR (Mohan et al. 2002). In a similar study, hatchery-sourced *P. monodon* postlarvae in India were positive for WSSV by PCR in 36/73 stocking events (Corsin et al. 2003). In a study of the performance of WSSV-infected and WSSV-negative *P. monodon* postlarvae in culture ponds in Taiwan, samples of postlarvae were tested by a single-step nested PCR within one month of stocking. Samples from 7/27 (approximately 26%) ponds tested negative for WSSV; 14/27 (approximately 52%) had a WSSV prevalence of between 1 and 49%; and, 6/27 ponds (approximately 22%) had a WSSV prevalence of over 50% (Peng et al. 2001). PCR testing of *P. monodon* postlarvae (PL15–18) from four hatcheries in India found prevalence of WSSV to range from approximately 50 to 84% (24/30, 26/31, 20/25 and 15/30) between the hatcheries (Otta et al. 2003). Surveys of farmed *L. vannamei* in Ecuador (sampling multiple ponds from multiple farms) found approximately 9.8% (20/205), 41.8% (97/232) and 12.5% (19/152) of animals were positive for WSSV on PCR analysis (Rodriguez et al. 2003). Monitoring of WSSV in commercial prawn ponds in Mexico have reported WSD causing mortalities between 48–80% at selected sites (Peinada-Guevara and Lopez-Meyer 2006). A more comprehensive research effort showed 62% of Mexican prawn farms sampled were positive for WSSV by PCR testing (Galaviz-Silva et al. 2004). Detection of WSSV in cultured prawns from Bangladesh confirmed the presence of the virus, but variation in actual prevalence amongst *P. monodon* broodstock and postlarvae was found to be dependent upon the primer used in the PCR analysis (Hossain et al. 2004).

WSSV DNA has been detected in wild prawn populations using PCR analysis. Most studies have presented information collated from multiple sample collection times and/or multiple sampling sites. Results from the larger studies are summarised below.

- Studies of wild *P. monodon* caught off southern Taiwan detected WSSV DNA by PCR in 50/66 (approximately 76%) (Lo et al. 1996b), 63/88 (approximately 72%) (Lo et al. 1997a), and 30/45 (approximately 67%) (Hsu et al. 1999) of the animals sampled.
- In samples from wild-caught *P. monodon* broodstock presented at Thai laboratories for screening for WSSV by PCR, the three-year mean monthly prevalence of WSSV detection between 1998 and 2000 was reported to be approximately 5% (Withyachumnarnkul et al. 2003).
- In studies of wild-caught *M. japonicus*, WSSV DNA was detected by PCR in 14/23

(approximately 61%) of animals sampled in southern Taiwan (Lo et al. 1996b). Of the animals sampled in Japan 20/134 (approximately 15%), 25/138 (approximately 18%) and 51/202 (approximately 25%) were positive for WSSV DNA (Maeda et al. 1998a).

- WSSV DNA was detected by PCR in 2/32 (approximately 6%) (Lo et al. 1996b) and 4/15 (approximately 27%) (Wang et al. 1998) of *Penaeus semisulcatus* sampled in southern Taiwan. *Fenneropenaeus penicillatus* sampled in southern Taiwan were PCR-positive for WSSV DNA, 3/27 (approximately 11%) (Lo et al. 1996b). Approximately 33% (10/30) of *Metapenaeus ensis* sampled in southern Taiwan were PCR-positive for WSSV DNA (Wang et al. 1997a). Less than 2% (2/104) of *Litopenaeus vannamei* sampled off Panama were PCR-positive for WSSV DNA (Nunan et al. 2001).
- Commercially important Atlantic prawn stocks off south-eastern US were PCR tested for WSSV resulting in 2.8% of all prawns testing positive. Most of the positive prawns were *Litopenaeus setiferus* (Chapman et al. 2004). Conversely, a three year survey of wild crustaceans from Texas coastal waters in the Gulf of Mexico did not detect WSSV (Dorf et al. 2005).
- Wild *L. vannamei* from the Gulf of California inhabiting a coastal zone with high prawn aquaculture activity were shown to be infected with WSSV at a prevalence of 9% in 2001 following a hurricane. Subsequent testing in 2003 did not detect WSSV in these populations (Mijangos-Alquisires et al. 2006). It was suggested that the hurricane caused dispersal of WSSV from nearby infected-farms to the marine environment.

Pathogenesis

WSSV infects cells in a wide range of tissues of mesodermal and ectodermal origin; it is commonly seen in the stomach, gills, cuticular epithelium, haemocytes, nervous tissue, lymphoid organ, muscle, midgut, hindgut and connective tissue (Wang et al. 1995, Chang et al. 1996, Durand et al. 1996, Lo et al. 1997a, Wang et al. 1999b, Di Leonardo et al. 2005).

After ingestion, the virus is thought to first infect epithelial cells of the digestive system. Following replication of the virus and host cell lysis, the virus circulates via the haemolymph to other body tissues (Huang et al. 2000). Shedding via faeces is likely as inoculation of tank water with faecal pellets from WSSV-positive animals resulted in WSSV infection in previously uninfected prawns (Rajan et al. 2000).

Infection may be patent, latent or transitional. In patent infections, clinical signs (including mortality) are evident within 2–7 days. Latent infections may continue for extended periods; however, the transition period to patent infection, or WSD, is generally short, perhaps only lasting a few hours (Lo and Kou 1998). The transition to patent infection may be triggered by stressors, such as environmental stress during the monsoon season (Karunasagar et al. 1997), rainy periods in combination with temperature and salinity fluctuations (Peinado-Guevara and Lopez-Meyer 2006), temperature changes (Vidal et al. 2001), and spawning stress (Hsu et al. 1999). The culture of prawns at higher water temperatures may provide protection against WSD. Increased survival has been reported when WSSV-infected prawns are held at a minimum temperature of 32°C (Vidal et al. 2001, Sonnenholzner and Calderon 2004).

Clinical signs

In patent WSSV infections, affected animals are said to have WSD. WSD in penaeid prawns may result in pink to red discolouration of the body (Takahashi et al. 1994, Momoyama et al. 1994, Wang et al. 1995), the appearance of white spots (0.5 to 2.0 mm in diameter) on the cuticle (Takahashi et al. 1994, Chou et al. 1995, Wang et al. 1995, Lightner 1996b), anorexia

(Durand et al. 1997) and high mortality (Momoyama et al. 1994, Chou et al. 1995). The presence of clinical signs is variable, in some animals the only sign noted may be mortality.

Whether by natural or experimental infection, asymptomatic carrier crustaceans (including copepods) have been reported (Flegel 1996, Lo et al. 1996b, Kanchanaphum et al. 1998, Supamattaya et al. 1998, Otta et al. 1999, Rajendran et al. 1999, Hossain et al. 2001a, Sahul Hameed et al. 2002). The presence of white spots on the carapace of otherwise asymptomatic WSSV-infected crustaceans has also been reported (Otta et al. 2003). Richman et al. (1997) reported clinical signs of discolouration and mottling of the exoskeleton and mortality in association with outbreaks of WSD in freshwater crayfish. Clinical signs similar to those reported in WSD in penaeid prawns have been reported in some experimental infections of non-prawn crustaceans (Supamattaya et al. 1998, Sahul Hameed et al. 2002).

Outbreaks in naïve farmed prawn populations frequently result in cumulative mortalities of up to 100% within 2–12 days of the onset of clinical signs (Chou et al. 1995, Lightner 1996a). Alternatively, infection may persist throughout the crop cycle, with only occasional mortality (Tsai et al. 1999).

Diagnosis

Diagnosis of WSD is required for confirmation of disease outbreaks, certification of broodstock and postlarvae for aquaculture, and research purposes. According to the OIE, a presumptive diagnosis of WSD may be made after observation of eosinophilic to pale basophilic intranuclear inclusion bodies in affected tissues under light microscopy of haematoxylin and eosin stained tissues. Definitive diagnosis may be accomplished by transmission electron microscopy, PCR analysis, *in situ* hybridisation or Western blot assay (OIE 2009b).

Introduction of WSSV into new countries or areas

Mechanism of disease spread

The introduction of WSSV into new areas has most often been attributed to the movement of live animals, particularly broodstock and post-larval prawns (Nakano et al. 1994 cited in Momoyamo et al. 1995). Humphrey (1995) considered the importation and reprocessing of frozen food products to be a potential route of viral introduction; it has been suggested that this route may be responsible for the introduction of WSSV into the USA (Durand et al. 2000). Other studies suggest WSSV first entered the Americas in Mexico via the arrival (either occasional or accidental) of infected Asian *P. monodon* and spread by natural dispersion of the carrier hosts (Córdova–Murueta et al. 1994 cited in Galaviz-Silva et al. 2004).

Other speculated routes of introduction of prawn viruses that were highlighted in the *Draft Prawn Import Risk Analysis* (2000) include the use of infected bait prawns by fishermen and the use of infected prawn material as feed for aquaculture crustaceans. In 2000 WSSV DNA was detected in imported frozen raw prawns, intended for human consumption, but were fed to crustacean hatchery broodstock (all exposed animals were promptly destroyed). The incident is not known to have resulted in an outbreak of clinical disease although WSSV was briefly detected in mud crabs near the hatchery outlet channel (East et al. 2004).

While it has been speculated that some prawn viruses may be spread locally via mechanical transfer by seabirds (Garza et al. 1997), a recent study suggests that the protective envelope of the WSSV is degraded during digestion, making it unlikely that WSSV is spread by bird faeces (Vanpatten et al. 2004).

Rotifer eggs have been suggested to act as reservoirs of WSSV in prawn ponds (Yan et al. 2004); however, PCR-positive artemia cysts failed to infect *P. monodon* when hatched and fed to postlarvae (Chang et al. 2002).

Wild *L. vannamei* from the Gulf of California inhabiting a coastal zone with high prawn aquaculture activity were shown to be infected with WSSV at a prevalence of 9% in 2001 — these wild prawn populations had previously tested WSSV-negative (Mijangos–Alquisires et al. 2006). The authors suggested that a hurricane in 2001 which caused farm damage and escape of prawns may have resulted in the spread of WSSV to wild *L. vannamei*. The study highlighted the risk of otherwise WSSV-free farms becoming infected via WSSV-positive prawns that escape in to the wild from nearby farms holding WSSV-positive prawns.

Impacts of introduction

Substantial production losses have been reported in the 2–3 years following the introduction of WSSV into many prawn production areas. Losses are estimated at over 50% in the main prawn producing state in India (Yap 2001a), between 70–75% in Ecuador (Hill 2001, Yap 2001a) , approximately 45% (in 1996) in Bangladesh (Mazid and Banu 2002), approximately 95% in the USA during 1995 (Lightner 1999), approximately 80% along the Pacific coast of Colombia (Gitterle et al. 2005)and approximately 80% in Peru (Yap 2001a). Losses in Mexico from 1999 affected 6500 hectares of prawn farms causing substantial economic losses (GalavizSilva et al. 2004). It is likely that similar production losses have been experienced in other prawn producing countries such as Thailand and China. Emergency harvesting of animals with WSD showing discolouration of the carapace results in a reduced market price for the product (Wongteerasupaya et al. 1996).

It has also been reported that good prawn aquaculture harvests can be obtained within one and a half years of the introduction of WSSV, despite the continued presence of the virus (Flegel 1997a). It is now accepted that the presence of WSSV alone does not necessarily result in dramatic production losses due to WSD, once the disease is established and recommended management techniques are adopted.

Control measures

A number of measures to control WSSV and WSD have been proposed. They are primarily aimed at preventing the introduction of WSSV into susceptible populations by purchasing and stocking only specific pathogen free (WSSV PCR-negative) broodstock and postlarvae, screening and disinfecting water intake, constructing physical barriers to prevent access by wild crustaceans, preventing unrestricted movement of stock, avoiding cohabitation of different species, and avoiding the use of fresh feed, amongst others (Flegel 1996, Corsin et al. 2002, Corsin et al. 2003, Limsuwan 1997, Lo and Kou 1998, Maeda et al. 1998b, Peng et al. 2001). Where populations may already be WSSV-infected control measures focus on reducing the spread of the virus to neighbouring populations by treating and delaying the discharge of water from infected ponds, fallowing, optimising environmental conditions, reducing stress levels, and generally improving husbandry methods (Flegel 1996, Limsuwan 1997, Lo and Kou 1998, Peng et al. 2001). Some authors have advocated the use of immunostimulants to reduce the likelihood of disease (Maeda et al. 1997, Chang et al. 1999, Takahashi et al. 2000) and probiotics (Farzanfar 2006) and vitamins (Merchie et al. 1998) to enhance the immune response of crustaceans.

Crustaceans do not have an adaptive immune system as antibodies targeting specific pathogens have not been found — they possess a non-specific, innate immune system involving humoral and cellular features. However, 'vaccines'[48] consisting of inactivated viral preparations (Singh et al. 2005, Namikoshi et al. 2004), egg yolk antibodies (Kim et al. 2004) and viral subunits (Witteveldt et al. 2004a, Witteveldt et al. 2004b) have been developed that offer protection against diseases, including WSD. Protection against specific pathogens following "vaccination" thus demonstrates an immune memory which has been referred to as an adaptive secondary immune response (Singh et al. 2005) with the ability to prevent disease

[48] These are not considered true vaccines as they do not stimulate antibody production.

but not infection. Commercial production of a vaccine to prevent WSD in aquaculture is the focus of ongoing research.

A specific strategy for the control and eradication of WSD is an integral part of the Australian Aquatic Veterinary Emergency Plan, or AQUAVETPLAN, which outlines national emergency preparedness and response and control strategies for aquatic animal disease emergencies in Australia [49].

[49] AQUAVETPLAN (edition 2) sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of WSD in Australia. It comprises a series of manuals; available at: http://www.daff.gov.au

Taura syndrome virus

Taura syndrome virus (TSV) is the aetiological agent of Taura syndrome, an epidemic disease of farmed penaeid prawns. Taura syndrome was first reported in farmed *Litopenaeus vannamei* in Ecuador in 1992 (Jimenez et al. 2000b) and has since spread to many parts of the Americas and throughout the prawn growing regions of Asia. Taura syndrome is listed as a disease notifiable to the OIE.

Taxonomy

TSV is an icosahedral, non-enveloped, single-stranded, positive-sense RNA virus 31-32nm in diameter (Bonami et al. 1997, Mari et al. 2002). It is an unassigned member of the family Dicistroviridae (Mayo 2005). A single isolate of TSV had originally been shown to be responsible for outbreaks of Taura syndrome in Ecuador (Hasson et al. 1995, Bonami et al. 1997, Mari et al. 1998) and Hawaii (Bonami et al. 1997, Mari et al. 1998).It now appears that at least three different strains of TSV exist. Hawaiian TSV-A, which is identical to the Ecuadorian strain, Mexican TSV-B, from Sinaloa (Erickson et al. 2002a, Erickson et al. 2002b) and the virulent Belize strain (TSV-C) (Erickson et al. 2005, Tang and Lightner 2005). Phylogenetic analyses of TSV isolates from other areas such as Thailand and China form distinct monophyletic clades based on geographic origin and are very closely related to these strains (Nielsen et al. 2005). It has been suggested that Asian TSV isolates originate from infected *L. vannamei* introduced into Asia from America for aquaculture (Tu et al. 1999, Flegel et al. 2003, Chang et al. 2004, Nielsen et al. 2005, Tang and Lightner 2005).

Agent stability

There are few reported investigations into the stability of TSV. Infectivity is retained after freezing at 0°C (Brock 1995), –70°C (Bonami et al. 1997, Overstreet et al. 1997, Tang et al. 2004) and –80°C (Hasson et al. 1995). TSV reportedly survives multiple freeze-thaw cycles in prawn tissues (Hasson et al. 1995) and can remain infectious in water for up to 48 hours, in prawn head tissues for at least 14 days, and in prawn tail tissues for at least 21 days at 27°C (Prior and Browdy 2002). TSV has been shown to maintain infectivity following passage through the gastro-intestinal tract of seagulls and chickens that have been experimentally fed infected prawns (Garza et al. 1997, Vanpatten et al. 2004), whereas YHV and WSSV do not (Vanpatten et al. 2004).

Epidemiology

Host range

The susceptibility of various host species and the virulence of the virus appear to vary with TSV strain (Erickson et al. 2002a, Erickson et al. 2002b, Erickson et al. 2005, Jiang et al. 2004, Srisuvan et al. 2005, Tang and Lightner 2005).

A range of penaeid species are known to be susceptible to TSV. Natural infections of TSV have been reported in *L. vannamei* (Lightner et al. 1995), *Litopenaeus stylirostris*, *Litopenaeus setiferus* (Overstreet et al. unpublished data cited from Bonami et al. 1997), *Penaeus monodon* and *Metapenaeus ensis* (Chang et al. 2004).

Experimental infections have been reported in *L. vannamei*, *Farfantepenaeus aztecus*, *Farfantepenaeus duorarum*, *Fenneropenaeus chinensis*, *L. setiferus* (Overstreet et al. 1997), *Palaemon styliferus*, *Litopenaeus schmitti* (Brock 1997), *P. monodon* (Srisuvan et al. 2005), *Fenneropenaeus merguiensis*, *Marsupenaeus japonicus* (Brock et al. 1997), *M. ensis* and *Metapenaeus monoceros* (Ruangsri et al. 2004).

While outbreaks of Taura syndrome have been most frequently associated with *L. vannamei* (Lightner et al. 1995, Tu et al. 1999, Limsuwan 2003a), the susceptibility of host species to

disease changes as the virus mutates and so may vary with viral strain. For example, naturally infected *L. stylirostris* were originally thought to suffer only mild disease (Chamberlain 1994, Lightner 1996a). However, mortality rates of up to 90% have been reported in this species during TSV outbreaks in the Americas (Erickson and Lightner 2001). Similarly, *L. vannamei* populations in Belize, bred for resistance to TSV-A, experience 90–100% mortality when infected with TSV-C (Moss 2004, Erickson et al. 2005).

It has been noted that there is an increased possibility that TSV strains could emerge that are more virulent to species previously considered largely resistant to Taura Syndrome (Chang et al. 2004, Nielsen et al. 2005, Srisuvan et al. 2005). Cultured *P. monodon* have been considered susceptible to infection with TSV, but largely resistant to Taura syndrome (Brock 1997). However, natural and experimental infections of *P. monodon* and *M. ensis* with new Taiwanese TSV isolates have been reported, although clinical signs were present only in *M. ensis* (Chang et al. 2004). Filtered tissue homogenates from these TSV-positive animals were then injected into *P. monodon* (weight approximately 40g). The researchers found higher levels of TSV in *P. monodon* injected with the *M. ensis* TSV isolate (MeTSV) than in those injected with the TSV isolate from *P. monodon* (PmTSV). RT-PCR revealed MeTSV replicated more freely in *P. monodon* than did PmTSV (Chang et al. 2004). It was concluded that TSV's apparent high mutation rate, coupled with other evolutionary pressure factors, had caused adaptive local sub-strains in new hosts. Further TSV sub-strains have been detected in *P. monodon* cultured in Thailand (Srisuvan et al. 2005, Phalitakul et al. 2006). Although conclusive evidence in one Thai study did not show TSV to be highly pathogenic to *P. monodon*, it was recognised that TSV may emerge in the future as an important pathogen in *P. monodon* culture (Srisuvan et al. 2005). Phalitakul et al. (2006) found that a Thai TSV isolate (ThMay04PmPL-TSV) naturally infected and caused mortality in cultured *P. monodon* and suggested good adaption of TSV to this species.

The susceptibility of host species to Taura syndrome may also vary with the life stage of the animal. In *L. vannamei*, infection with TSV appears to have no impact on nauplii, mysis and early post-larval stages, but may exhibit as disease in animals from approximately PL12 onwards (Lightner 1996a, Brock 1997).

Hatchery reared *Macrobrachium rosenbergii* postlarvae from Thailand showing clinical signs of disease were reportedly lightly PCR-positive for TSV (Nash et al. 2003), but results were inconclusive as to whether the animals were infected with TSV. *Sciaenops ocellatus*, *Cynoscion nebulosus*, *Palaemonetes* species and *Callinectes sapidus*, fed with TSV-infected prawn tissue did not become infected (Erickson et al. 1997a). *Macrobrachium lanchesteri*, *Macrobrachium equideus*, *Metapenaeus monoceros, Chloridopsis immaculatus*, krill (*Acetes* species) and crabs (*Sesarma* species) became infected when injected with viral extracts (Ruangsri et al. 2004).

It has been reported that mammalian cell lines can be infected with TSV (Audelo–del–Valle et al. 2003), however two other research groups could not replicate these results and concluded that there is no evidence that TSV has zoonotic potential (Luo et al. 2004, Pantoja et al. 2004).

University of Arizona research (commissioned by Biosecurity Australia) on the susceptibility of several Australian crustacean species to TSV (Thai and Belize isolates), revealed that although some prawn species may be susceptible to infection, serious clinical disease is unlikely. *Fenneropenaeus merguiensis* and *P. monodon* were found to be susceptible to infection but the virus did not cause severe infections associated with significant mortality — *F. merguiensis* became infected with the Thai isolate of TSV by injection but did not become infected by *per os* challenge, and *P. monodon* did not become infected with the Thai isolate of TSV but some were infected with the Belize isolate of TSV by injection challenge only[50]. The

[50] Infection was defined for the purposes of this report as the presence of an actively replicating agent within the host and not its mere presence.

study also showed that *Cherax quadricarinatus, Cherax tenuimanus* and *Macrobrachium rosenbergii* could not be infected with either the Thai or Belize isolates of TSV — the virus was sequestered in the tissues of challenged animals but the virus did not form an active (replicative) infection.

Geographical distribution

TSV was first reported in Ecuador (Jimenez et al. 1992 cited in Jimenez et al. 2000b) in 1992 however it is now widespread in the Americas (Jimenez et al. 2000b). More recently, TSV has been reported from a number of Asian countries, including Taiwan (Te et al. 1999), China (NACA 2003), Thailand (Limsuwan 2003b), Indonesia (NACA 2004b) and Korea (Do et al. 2006). TSV has also been detected by RT-PCR in *P. monodon* in Myanmar (NACA 2006, Nielsen et al. 2005) and is reportedly known to occur in Malaysia and Vietnam (NACA 2004b). TSV was detected in a quarantine facility in Tahiti and subsequently eradicated (Le Moullac et al. 2003). TSV is exotic to Australia.

Transmission

Natural spread of the virus is thought to occur by both water-borne transmission and ingestion or injection of infected tissues (Brock et al. 1995). Although vertical transmission is thought to occur, transmission from broodstock to offspring has not been shown to occur via oocytes (Lotz and Ogle 1997). In a single incident, female *L. stylirostris* inseminated with refrigerated Ecuadorian spermatophores imported to Tahiti produced offspring positive for TSV (Le Moullac et al. 2003). Animals that survive infection during outbreaks of TSV can become chronic, asymptomatic carriers (Krol et al. 1997, Hasson et al. 1999a, Lotz et al. 2003, Lotz et al. 2005).

Both aquatic insects and seabirds may play a role in the mechanical spread of TSV over short distances. Injection of homogenised aquatic insects such as water boatman, *Trichocorixa reticulata*, collected near ponds undergoing an outbreak of Taura syndrome, or of homogenised faeces from seabirds feeding on prawns in affected ponds, has resulted in Taura syndrome in experimental *L. vannamei* (Hungerford 1977 cited in Lightner 1995). TSV also remains infectious to prawns following passage through the gut of chickens (*Gallus domesticus*) and seagulls (*Larus atricilla*) (Garza et al. 1997, Vanpatten et al. 2004).

Experimental infections have also been induced by ingestion of infected prawns (Brock et al. 1995, Overstreet et al. 1997, Nunan et al. 2004), water-borne transmission (Prior et al. 2003), intramuscular injection of viral preparations (Hasson et al. 1995, Overstreet et al. 1997, Prior et al. 2003, Nunan et al. 2004) and incorporation of infected material into dietary brine shrimp (Overstreet et al. 1997) and co-habitation with infected prawns (Prior and Browdy 2002).

Tissue titre

Few studies have examined the titre of TSV in infected prawn tissues. Nunan et al. (2004) fed juvenile *L. vannamei* (mean weight approximately 3g) on minced TSV-positive prawn tissues at a rate of 5% bodyweight per day for 2 days. Subsequently, various body parts were examined by real time RT-PCR to determine the mean number of viral copies per gram of host tissue. Body parts examined included whole tails, tail muscles, tail fans, gills, pleopods and heads. For the body parts examined, the number of TSV genome copies per gram of host tissue ranged from 10^6 to 10^{10} (Nunan et al. 2004).

Tang et al. (2004) reported on the comparative levels of TSV in various tissues, in both acutely and chronically infected *L. vannamei*. Prawns were experimentally infected *per os* with TSV-positive tissues. No significant difference in the numbers of viral copies per microgram of total RNA extracted from the gills and pleopods of acutely infected animals were found (Tang et al. 2004). The TSV levels in these tissues did not differ significantly between acutely and chronically infected animals. In chronically infected *L. vannamei*, the number of viral copies per microgram of total RNA extracted was one to two orders of

magnitude higher in the lymphoid organ than in the gills and pleopods (Tang et al. 2004). However, TSV RNA levels remain reasonably stable in experimentally infected *L. vannamei* for approximately the first 40 weeks post-infection, viral load then decreases by several orders of magnitude in chronically infected animals (Poulos and Lightner 2003, cited in Tang et al. 2004).

Infectious dose

TSV was transmitted by feeding infected tissues, at a rate of 7.5% bodyweight twice daily for four days to *L. vannamei* (approximate mean weight 0.35g) (Erickson et al. 1997b). In a similar trial, successful transmission of the virus was achieved by feeding *L. vannamei* (weighing approximately 0.5 to 3.0g) on infected tissues at a rate of approximately 10% bodyweight daily for three days (White et al. 2002). In contrast, *L. vannamei* (weighing approximately 3.0 to 5.0g) were infected by being fed once with 3% bodyweight of TSV-infected tissues (Argue et al. 2002).

In transmission trials using water-borne exposure, the LD_{50} of the TSV-B strain was $1:9.92\times10^3$ and $1:15.7\times10^3$ at 20°C, and $1:1.27\times10^3$ at 27°C (Prior et al. 2003). It was suggested that the changes may indicate a relationship between decreasing temperature and increasing pathogenicity of the TSV-B strain. However, further work is required before any relationship can be confirmed.

Prevalence

- The prevalence of TSV in aquaculture, the wild and between or within populations is not widely reported. A histological study of farmed *L. vannamei* populations in one Mexican state reported the prevalence of TSV between farms as approximately 87% (13/15), 82% (27/33), 48% (11/23) and 34% (8/23) in 1995, 1996, 1997 and1998 respectively (ZarainHerzeberg and AscencioValle 2001). This decrease in between-farm prevalence coincides with a change from culturing *L. vannamei* to *L. stylirostris*. It was hypothesised that the reduction in the number of susceptible hosts within the state may have been responsible for the reduction seen in between-farm prevalence over this period (ZarainHerzeberg and AscencioValle 2001).
- In Taiwan, one study found TSV in farmed *L. vannamei* (as detected by RT-PCR) to be approximately 30% (Wang and Chang 2001).
- Prawn aquaculture populations in Thailand surveyed for the presence of TSV using RT-PCR revealed 0 to 6.1% TSV prevalence in surveyed hatchery populations and 0 and 11.29% in grow-out pond populations (Ruangsri et al. 2004). Under Thailand's general health management program 6.1% (64/1050) (NACA 2004a), 2.6% (9/352) (NACA 2005a), 0.6% (8/1265) (NACA 2005b), 1.9% (9/352) (NACA 2005c) and 1.0% (NACA 2005d) of postlarvae samples were PCR-positive for TSV.
- A survey conducted in East Java, Indonesia reported TSV prevalence to be 51.6%, 35.7% and 23.5% in 2003, 2004 and 2005 (up until February) respectively (Hanggono et al. 2005).
- A histological investigation into the health status of wild, broodstock *L. vannamei* caught off the Mexican Pacific coast, reported prevalence of TSV to be approximately 27% (15/56) and 32% (18/56) respectively, in the first and second months of the study (MoralesCovarrubias and ChavezSanchez 1999).
- On the east coast of the Gulf of Mexico, in Texas coastal waters a three year survey (largest to date), revealed that wild penaeid prawns and Callinectid crabs were PCR negative to TSV from 1997 to 2000 (Dorf et al. 2005).
- TSV has been detected with nested RT-PCR in wild *P. monodon* broodstock caught off Taiwan in 2000. The prevalence was approximately 8% (2/24) (Chang et al. 2004).

Clinical signs

Most clinical signs associated with Taura syndrome are not specific. Individuals in the acute stage of Taura syndrome are lethargic, anorexic, ataxic, and, as they are typically in late stages of the moult cycle, soft-shelled (Brock 1995, Lightner et al. 1995, Yu and Song, 2000). Prawns with Taura syndrome may appear either red or blue due to the expansion of chromatophores (Chamberlain 1994, Lightner et al. 1995). Most prawns with Taura syndrome die within one week, although some prawns may survive and become chronic carriers (Brock 1995, Lightner et al. 1995). Gross pathology of surviving prawns initially show multiple melanised cuticular lesions, giving them a peppered appearance, but exhibit normal feeding behaviour and activity (Brock 1995, Lightner et al. 1995). Following their next moult, the cuticular lesions may either disappear, or pale, de-pigmented foci may remain (Brock 1995). Recurrence of disease outbreaks in chronically infected animals is usually precipitated by environmental stressors such as temperature and salinity changes following heavy rain or drought (Edwards 1998, Lotz et al. 2005).

In populations, Taura syndrome typically exhibits as acute onset mortality between 2–40 days after postlarvae have been stocked into grow-out ponds and when animals are between 0.1–5 grams in weight (Brock 1995, Lightner et al. 1995). Cumulative mortalities in juveniles may exceed 90% within three weeks of the onset of clinical signs (Brock 1995). Disease may occur at any stage of the grow-out cycle, with adults also being considered highly susceptible (Brock 1995, Brock 1997, Lotz 1997b). Cumulative mortalities in adult populations are typically less than 50% (Brock 1995).

Pathogenesis

TSV is reported to infect cells of the cuticular epithelium of the general body surface, appendages, mouth, oesophagus, stomach and hindgut (Hasson et al. 1995, Lightner et al. 1995). The antennal gland tubule epithelium is rarely affected (Lightner et al. 1995). However, the sub-cuticular connective tissue and adjacent striated muscle fibres basal to cuticular epithelial cells are also involved (Lightner et al. 1995). In chronic infections, TSV is concentrated in the lymphoid organ, but may also be detected in other tissues (Tang et al. 2004).

There are three distinct but overlapping phases of TSV infection in penaeid prawns (Hasson et al. 1999b). Following *per os* exposure TSV is detectable in cells of the foregut, gills and general cuticle within 24 hours. The acute phase of infection is characterized by severe multifocal to diffuse necrosis of the cuticular epithelium and sub-cutis of the foregut, gills, appendages, general body surface, and, to a lesser extent, the hindgut (Hasson et al. 1999b). This phase lasts up to 7 days. The transition phase begins on about the fourth day post-exposure, and lasts for approximately 5 days. Multiple, multifocal, melanised cuticular lesions are present. Some acute phase lesions are evident, but there is also the beginning of lymphoid organ spheroid formation, with the uptake of TSV in the cells of the lymphoid organ tubules. If prawns survive this stage, they moult, shedding their melanised cuticle to enter the chronic phase of infection. The chronic phase starts at about 6 days post-exposure, overlapping with the previous two phases. Chronically infected animals display no clinical signs; however, there is marked lymphoid organ hypertrophy due to spheroid formation (Hasson et al. 1999b). As determined by bioassay, the virus can remain infectious in survivors of Taura syndrome for at least 8 months after an outbreak (Krol et al. 1997).

Diagnosis

Currently available methods used for surveillance, detection and diagnosis of TSV include light microscopy, transmission electron microscopy, molecular genetic methods (such as in situ hybridisation, RT-PCR and dot blot assay), antibody-based tests and bioassay (OIE 2006c). It has been reported that, depending upon the antibodies used, immunological-based tests may not detect all strains of TSV (Erickson et al. 2002b, Robles–Sikisaka et al. 2002,

Erickson et al. 2005).

Introduction of TSV into new countries or areas

Mechanism of disease spread

The introduction of TSV into new areas has primarily been accredited to the movement of live animals, particularly broodstock, postlarvae (Brock 1995, Lightner 1995, Tu et al. 1999, Yu and Song 2000, Nielsen et al. 2005) and genetic material such as sperm (Le Moullac et al. 2003). Phylogenetic analysis has been used in conjunction with the chronology of outbreaks to propose how the international movement of live stock has resulted in the spread of TSV (Nielsen et al. 2005, Tang and Lightner 2005).

It has been speculated that the international trade of frozen raw prawns may facilitate the introduction of prawn viruses into new areas through the inappropriate disposal of processing and retail wastes, the use of imported prawns for bait, and inadequately processed prawn feeds resulting in possible pathways of TSV exposure to farmed and wild crustaceans (Humphrey 1995, Lightner 1995, Durand et al. 2000).

Mechanical transmission by flying aquatic insects and seabirds may possibly play a role in the local spread of TSV due to short term virus stability following passage through the gastro-intestinal tract of insects and birds that have fed on infected prawns (Lightner 1995, Garza et al. 1997, Vanpatten et al. 2004).

Impacts of introduction

While substantial production losses have been reported from individual farms following the introduction of TSV overseas, there are few data on national prawn aquaculture production losses following outbreaks of TSV.

- Farmed prawn production in Ecuador was reportedly around 100000 tonnes in 1991 (Lightner 1995), and fell by approximately 16% in the first year, and cumulatively 30% in the first two years (to around 70000 tonnes in 1993) following the first outbreaks of Taura syndrome (Shrimp News International 1994). Within four years of the initial outbreaks, Ecuadorian prawn aquaculture production had returned to pre-TSV levels, with production reportedly over 100000 tonnes in 1996 (Lightner and Redman 1998) and 1997 (Melendez 1998 cited in Jimenez et al. 2000a).
- The national prawn aquaculture production loss in Honduras was approximately 68% in the first year following the introduction of TSV, with production dropping from 7200 tonnes in 1993 to 2300 tonnes in 1994 (Lightner 1995). Production in Honduras reportedly exceeded pre-TSV levels within two to three years, with 1996 production estimated at 10000 tonnes (Lightner and Redman 1998).
- There are no data to suggest that TSV has any impact on wild prawn populations. Although TSV was found in wild prawns in Ecuador (Brock 1995), Mexican Pacific coast (Morales–Covarrrubias and Chavez–Sanchez 1999) and Taiwan (Chang et al. 2004) there was reportedly no decline in wild broodstock levels. Similarly, good wild prawn catches were reported following the outbreak of TSV in Texan prawn farms in 1995 (Campbell 1996).

Control measures

Control measures for Taura syndrome and other viral diseases are primarily aimed at preventing the introduction of the virus into susceptible populations by stocking only specific pathogen free animals, screening and disinfecting water intake, avoiding the use of raw feed, avoiding cohabitation of species, and generally following recommended husbandry practices (Brock 1997, Brock et al. 1997, Fegan and Clifford 2001). Where populations may already be infected, control measures focus on reducing the spread of the virus to neighbouring populations by treating and delaying the discharge of water from infected ponds, fallowing and disinfecting empty ponds, increased pond aeration and stock thinning during periods of heavy rain, and reducing the likelihood of an outbreak of disease in the infected population by optimising environmental conditions and reducing stress levels (Brock 1997, Brock et al. 1997, Fegan and Clifford 2001). Further, some authors have advocated the use of selective breeding to reduce the susceptibility of animals to infection and disease (Argue et al. 2002, Jiang et al. 2004) . TSV-resistant (TSR) prawns have been selectively bred (Xu et al. 2003, Srisuvan et al. 2006). When experimentally challenged with pathogenic TSV, TSR prawns were found to be susceptible to infection, but had a higher survival rate than prawns selected as non-resistant (Xu et al. 2003, Srisuvan et al. 2006).

Strategies to deliver acquired immunity against specific pathogens such as TSV in cultured prawns are being researched, although the most promising strategy against Taura syndrome appears to be the development of a transgenic, TSV-resistant prawn strain (Lu and Sun 2005).

Yellowhead virus

Yellowhead virus (YHV) causes severe disease (yellowhead disease or YHD) and mortality in cultured *Penaeus monodon*. YHV is widespread in Asia (Walker et al. 2001) and was first reported from Thailand in the early 1990s (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). Severe outbreaks of YHD most commonly occur in *P. monodon* aquaculture ponds 50–70 days after stocking, when prawns are in the juvenile to sub-adult stage (5–15g) (Limsuwan 1991 cited in Chantanachookin et al. 1993, Lotz 1997a). Early outbreaks were associated with mass mortalities and entire crop loss by the third day post-infection (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). YHV-infected stocks have been known to survive infection to normal harvest time (Walker et al. 2001). YHD is listed as a disease notifiable to the OIE.

Agent taxonomy

YHV is a positive-sense, single-stranded RNA virus (Tang and Lightner 1999). YHV was originally classified as a coronavirus or rhabdovirus based on morphology and genome type (Wongteerasupaya et al. 1995b), but is now thought to be a member of the *Okavirus* genus, family Roniviridae, order Nidovirales (Cowley et al. 2000b, Mayo 2002a, Sitilidilokratana et al. 2001, Jitrapakdee et al. 2003). The rod-shaped, enveloped virions are typically 150–200nm in length and 40–50nm wide (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993).

Different genotypes of YHV have been found in cultured *P. monodon* in Australia (Spann et al. 1997b) and in Taiwan (Wang et al. 1996, Wang and Chang 2000). Gill-associated virus (GAV), a closely related virus within the same group of viruses, was first isolated from prawn farms in 1996 as lymphoid organ virus (LOV) (Spann et al. 1997b). GAV infection, previously considered to be infection with lymphoid organ virus (Walker et al. 2001), is thought to approach 100% prevalence in wild *P. monodon* in eastern Australia. Comparison of YHV and GAV sequences at the nucleotide and amino acid levels indicated differences of between 15–20% (Cowley et al. 1999). Diagnostic probes are able to differentiate between YHV and GAV, and the virus is considered to be a distinctive genotype of YHV (Cowley et al. 1999, OIE 2006b).

YHV genotypes have also been reported from Taiwan where yellowhead-like virions were noted through transmission electron microscopy (TEM) of WSSV-infected *Marsupenaeus japonicus* (Wang et al. 1996) and TEM and RT-PCR of WSSV-infected *P. monodon* (Wang and Chang 2000) . The pathogenicity of the viruses and their relationship to YHV are yet to be fully determined.

Agent stability

Research at Charoen Pokphand Shrimp Culture Research Centre (CPSCRC) in Thailand showed that tissue extracts containing YHV remained infectious for more than 72 hours in seawater (Flegel et al. 1995b). The survival of YHV in frozen prawn tissue has not been thoroughly studied. However YHV-infected tissues or extracts stored at –70°C (Lu et al. 1995b) and –80°C (Direkbusarakom et al. 1998) remain infective. Infectious YHV has also been detected in frozen prawns sourced from retail outlets in the USA (Nunan et al. 1998). YHV extracts may be inactivated by heating to 60°C for 15 minutes (Flegel et al. 1995b). Additionally, freeze-thaw cycles (Wongteerasupaya et al. 1995b) and digestion in bird gut (Vanpatten et al. 2004) may damage virions.

Epidemiology

Host range

The following host species have been reported for YHV:

Penaeids:
Farfantepenaeus aztecus [E] (Lightner et al. 1998)
Farfantepenaeus duorarum [E] (Lightner et al. 1998)
Marsupenaeus japonicus [C] (Wang et al. 1996)
Fenneropenaeus merguiensis [CE] (Chantanachookin et al. 1993, Flegel et al. 1997b)
Penaeus monodon [CE] (Boonyaratpalin et al. 1993)
Litopenaeus setiferus [CE] (Coelen and Walker 1996, Lightner 1996a)
Litopenaeus stylirostris [CE] (Lu et al. 1994, Lightner 1996b)
Litopenaeus vannamei [E] (Lu et al. 1994, Lightner 1996b)

Other prawn species:
Metapenaeus ensis [C] (Chantanachookin et al. 1993, Flegel et al. 1997b)
Euphasia superha [C] (Lightner et al. 1998)
Macrobrachium rosenbergii [C] (Lightner et al. 1997a)
Acetes sp. [C] (Chantanachookin et al. 1993)
Palaemon styliferus [C] (Flegel et al. 1995b)

Other crustacean species:
Sesarma sp. [E] (Boonsaeng et al. 2000)
Uca spina [E] (Boonsaeng et al. 2000)
Scylla serrata [E] (Boonsaeng et al. 2000)
Portunus pelagicus [E] (Boonsaeng et al. 2000)

C= cultured (non-cultured species sourced from prawn aquaculture ponds included), E= experimental infection. Flegel (1997a) included data from personal communications with B. Withyachumnarnkul and V. Boonsaeng. It should be noted that not all of the species reported as naturally infected with YHV showed clinical signs of YHD. Natural infections of YHV have only been found in prawns.

Fenneropenaeus merguiensis, *Acetes* species (Chantanachookin et al. 1993), *M. ensis* (OIE 2009b) and *P. styliferus* (unpublished report cited in Flegel et al. 1995b) are considered to be asymptomatic carriers of YHV. *Fenneropenaeus indicus* co-cultured with YHV infected *P. monodon* in India did not get YHD (Mohan et al. 1998). *Euphasia* species may eventually die from YHV infection (unpublished report cited in Flegel et al. 1997). There are unconfirmed reports of YHV infection in mantis shrimp and rock head shrimp (Asian Shrimp News 1992). Other studies failed to find natural YHV infection in *Artemia* spp, *Sesarma* spp, *Portunus pelagicus* and *Somanniathelapusa* spp (Flegel et al. 1997).

Geographical distribution

YHV was first reported in Thailand in the early 1990s (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). It is now widespread in Asia (Walker et al. 2001) and is also known to occur in Sri Lanka (Fish Farming International 1999), India (Shankar and Mohan 1998). YHD has been reported in Taiwan (Wang and Chang 2000) and after review of particle morphology, morphogenesis and histopathology (Walker et al. 2001) is thought to have been associated with the *P. monodon* industry crash in Taiwan in 1986–1987 (Chen and Kou 1987, Chantanachookin et al. 1993). YHD has also been associated with disease epidemics in Indonesia, Malaysia, China, and the Philippines (Lightner 1996a). Presumptive reports of YHV involvement in epidemics in prawn aquaculture in USA (Lightner 1996a) and South America have proven incorrect (OIE Fish Diseases Commission 1999, Jory and Dixon 1999). However, de la Rosa-Vélez et al. (2006) provide presumptive evidence of the presence of YHV in Mexican shrimp farms along the Pacific Coast during 1999 and 2000. The YHV

genotype 1, the only known agent of yellowhead disease, is exotic to Australia, although one of other genotypes in the yellowhead complex of viruses known as GAV is present (Spann et al. 1997b).

Transmission

Few published transmission trials have involved natural routes of infection. However unpublished studies indicate that YHV can be transmitted *per os* (ingesting infected prawns) and via seawater to juvenile and sub-adult *P. monodon* (Flegel et al. 1995b). The susceptibility of postlarvae to infection with YHV appears to be age-dependant (Khongpradit et al. 1993. YHV has been transmitted to juvenile prawns by feeding infected tissues, but postlarvae were found to be resistant to infection (Lightner et al. 1998). *Penaeus monodon* exposed to YHV by co-habitation with experimentally infected crabs contracted YHD despite physical separation of the animals (Boonsaeng et al. 2000). Evidence suggests that YHV may be transmitted vertically, similar to GAV in wild east coast Australian *P. monodon* (Cowley et al. 2002). Surviving experimentally infected prawns have been shown to be asymptomatic carriers of YHV (Longyant et al. 2005). It has been suggested birds may act as mechanical transmission agents of some prawn viruses by feeding on infected prawns and spreading infectious virions in their faeces (Garza et al. 1997). While some virus species, such as TSV and IHHNV, can remain infectious following passage through a bird gut, it has been shown that YHV virions do not (Vanpatten et al. 2004).

Prevalence

Mortalities due to YHD in Thailand were initially serious and widespread. However, high level mortality of *P. monodon* attributed to YHD declined within 1.5 years (Flegel 1997a). The prevalence of subclinical infection with YHV and the titre of virus in subclinically infected prawns are unknown. Little is known of the prevalence of YHV in wild prawn populations however, evidence of YHV virions in a single wild-caught broodstock specimen has been reported (Flegel et al. 1995b). The study reported prevalence of YHV in wild-caught *P. monodon* spawners to approximately 3% (Flegel et al. 1995b). Prevalence of YHV in wild collected *P. monodon* broodstock using 2-step RT-PCR was 78.9% (Wongteerasupaya et al.1997). Although not quantified, the prevalence of YHV infection in *P. monodon* seed stock in Thailand is now thought to be much higher than previously suspected (Walker et al. 2001). The prevalence of GAV in *P. monodon* broodstock captured off the east coast of Australia is almost 100% (Cowley et al. 2000a, Walker et al. 2001).

During severe outbreaks, high prevalence of YHD is most common in *P. monodon* aquaculture 50–70 days after stocking, when prawns are in the juvenile to sub-adult stage (5–15g) (Lightner 1996a, Lotz 1997a). A pond undergoing a serious YHD outbreak may be emergency harvested as a disease control measure. The practice of emergency harvest of prawns affected by YHD is considered less common now than in previous years due to the presumed development of tolerance or resistance in local prawns (Flegel 1997a).

The OIE Manual of Diagnostic Tests for Aquatic Animals 2006 states: the overall prevalence of yellowhead complex viruses in healthy *P. monodon* (as detected by nested polymerase chain reaction [PCR]) is very high (50-100%) in most sampled farmed and wild populations in Australia, Asia, and East Africa. The prevalence of individual genotypes varies according to the geographic origin of the shrimp. The prevalence of YHV (genotype 1) may be low (>1%) in healthy wild or farmed *P. monodon*, but would be very high (approaching 100%) in disease outbreak ponds. By other less sensitive detection methods (e.g. histology, immunoblot, dot-blot, in-situ hybridisation), the prevalence of infection in healthy shrimp would be lower. Economic and/or production impact of the disease: The economic impact of YHV in Thailand in 1996 has been estimated to be USD 30 million in 1992 and USD 40 million in 1993. Although this represents only 3-4% of the gross value of production, total crop loss during grow-out has a very significant impact on individual farmers.

Pathogenesis

YHV affects tissues of ectodermal and mesodermal origin (Wongteerasupaya et al. 1995a), intensely infecting the lymphoid organ, cuticular epithelium and gill. Epicardium, connective tissues, and glial cells in nerve tracts are also infected (Tang and Lightner 1999). Systemic infection causes extensive necrosis in all tissues with intense basophilic, cytoplasmic and spherical inclusions (Flegel et al. 1997). Haemocytes from smears display pyknotic and karyorrhectic nuclei, and an absence of bacteraemia (Lu et al. 1994, Nash et al. 1995).

Clinical signs

When first described, YHD was characterised by cessation of feeding, swimming slowly near the surface at the edges of ponds, quickly followed by high mortality of up to 100% over a period of 3–5 days (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). Affected prawns are pale bodied (bleached appearance) with reddening of the appendages, and have a yellow cephalothorax due to a yellow hepatopancreas visible through the translucent carapace (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). Yellowing of the cephalothorax does not always occur in affected animals (Chantanachookin et al. 1993), and is not typical for all species (Lu et al. 1994, Tang and Lightner 1999). Many infections are subclinical and YHV infection is increasingly reported as a co-infection with WSSV and other viruses (Mohan et al. 1998, Durand et al. 2000, Wang and Chang 2000, Madhavi et al. 2002).

Diagnosis

Histopathology

Changes are most pronounced in the lymphoid organ, gill and haemocytes and include degeneration and severe necrosis that is multifocal to diffuse (Chantanachookin et al. 1993). Necrotic cells with pyknotic or karyorrhectic nuclei and dense, spherical, basophilic, perinuclear inclusions occur in the lymphoid organ, haemolymph, haematopoietic tissue, gill epithelium and spongy connective tissues (Lightner 1996a). Flegel and Sriurairatana (1993) have developed a rapid processing and staining technique which can provide a definitive diagnosis for acute YHV infection within 3 hours.

Electron microscopy

Flexuous, cylindrical virions with an envelope with knob-like projections are observed in the cytoplasm of infected cells (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993), and in purified suspensions prepared from haemolymph (Wongteerasupaya et al. 1995b, Nadala et al. 1997).

Cell culture

Lu et al. (1995a) developed a quantal assay in primary prawn cell culture for YHV.

Genetic and immunoassay techniques

RT-PCR (Wongteerasupaya et al. 1997), *in situ* hybridisation with gene probes (Tang and Lightner 1999), nitrocellulose-enzyme immunoassay (Lu et al. 1996, Nadal and Loh 2000) and monoclonal antibody techniques (Sithigorngul et al. 2000, Sithigorngul et al. 2002, Soowannayan et al. 2003) have been developed for the detection of YHV.

For definitive diagnosis, the most effective method of YHV detection is by a sensitive nested RT-PCR that will detect and distinguish YHV from closely related genotypes such as GAV (OIE 2006b, Cowley et al. 2004).

Introduction of YHV into new countries or areas

Mechanism of disease spread

Although YHV has been detected in frozen product from Asia (Nunan et al. 1998), the virus has not been reported in cultured prawns in the Americas. The spread of YHV is mostly attributed to the uncontrolled introduction of live prawn stocks and subsequent unrestricted movement of live broodstock and postlarvae (Briggs et al. 2004). The IRA team is of the view that GAV has spread from Northern Territory aquaculture facilities to wild crustaceans in the Joseph Bonaparte Gulf.

Impacts of introduction

YHV was widely reported as the first major virulent disease threat to *P. monodon* aquaculture (Boonyaratpalin et al. 1993, Chantanachookin et al. 1993). Total production loss attributed to YHV during the initial outbreak in Thailand in the early 1990s was estimated at US$30–200 million or approximately 3% of total production volume (Flegel et al. 1995b). A major setback to *P. monodon* culture due to YHV was reported in India in 1994 (Mohan et al. 1998) and Sri Lanka in 1999 (Fish Farming International 1999). Outbreaks have also been reported for China, Philippines, Malaysia, Indonesia, Mozambique, Vietnam and Taiwan, although there is little detailed quantitative data on losses (Flegel et al. 1995b, Lightner 1996a, Wang et al. 1996, Mohan et al. 1998, Shankar and Mohan 1998, Wang and Chang 2000). It has been reported that production volumes following an outbreak of YHD return to pre-outbreak levels over a relatively short period (Flegel 1997a, Flegel 1997b).

Control measures

General control measures have been suggested by the Network of Aquatic Centres in Asia-Pacific (Briggs et al. 2004), including legislation to restrict the movement of live broodstock and postlarvae, use of SPF broodstock, enforcement of codes of conduct and management practices, improving husbandry technology in intensive culture, and active surveillance.

YHV-host interactions and host immune system response are being investigated at the genetic and molecular level (Assavalapsakul et al. 2005, Assavalapsakul et al. 2006). Inactivated viral preparations and ribonucleic acid interference (RNAi) proteomic research currently being conducted may lead to a YHV-specific vaccine equivalent, capable of eliciting an adaptive secondary immune response.

Infectious hypodermal and haematopoietic necrosis virus

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) is the causative agent of Infectious hypodermal and haematopoietic necrosis (IHHN) (Bonami et al. 1990). IHHNV was initially reported in juveniles of *Litopenaeus stylirostris* in Hawaii (Lightner et al. 1983), however, the virus is thought to originate from South-East Asia as a naturally occurring virus of wild *Penaeus monodon* (Lightner 1996a, Lightner 1999, Tang et al. 2003a). IHHN is a serious disease resulting in mass mortality of cultured juvenile *L. stylirostris* and an economically important disease of cultured *Litopenaeus vannamei*. IHHNV is identified as the causative agent of a chronic disease in *L. vannamei* known as runt deformity syndrome (RDS), resulting in growth retardation and associated aquaculture production losses (Kalagayan et al. 1991). IHHNV and WSSV are the two most prevalent and widespread prawn viruses in aquaculture (Tang et al. 2003b). IHHN is listed as a disease notifiable to the OIE.

Agent taxonomy

IHHNV is a small, linear single-stranded DNA virus with an estimated size of 4.1kb by molecule length measurement. The non-enveloped icosahedron virions are 22nm in length (Bonami et al. 1990). IHHNV has been classified as a member of the family Parvoviridae (Bonami et al. 1990, Shike et al. 2000).

Genome organisation has been utilised to determine the taxonomic position of IHHNV. The virus is similar to mosquito viruses (*Aedes aegypti densovirus* and *Aedes albopictus densovirus*). Based on these relationships, IHHNV is known as *Penaeus stylirostris densovirus*, in the genus *Brevidensovirus* (Fauquet et al. 2005).

Phylogenetic analysis of the IHHNV genome from various geographical isolates has shown the virus genome to be very stable among isolates derived from *L. vannamei* (Nunan et al. 2000, Tang and Lightner 2002) which cluster into three groups corresponding to their geographical origins (Tang and Lightner 2002, Tang et al. 2003a).

A strain of IHHNV infecting hybrid *P. monodon* x *Penaeus esculentus* prawns has been recorded from Australia (Owens et al. 1992a).

There are two recognised lineages of IHHNV, the Philippines strain considered to be the most virulent and associated with RDS in *P. monodon* (Primavera and Quinitio 2000) and *P. vannamei* (Kalagayan et al. 1991, Browdy et al. 1993) and the Indian Ocean strain which is considered to be less virulent to penaeids (Tang et al. 2003a). The strain of IHHNV known for some time to be in Australia is likely related to the Indian Ocean strain (Krabsetsve et al. 2004). In 2008, Australia notified the detection of a strain of IHHNV in Australia very similar to those strains found in Asia.

Agent stability

In general, because parvoviruses have very simple and stable virions when compared to many other viruses they are stable in the presence of lipid solvents, lipases, exposure to pH 3–9 and stable for at least 60 minutes at an incubation temperature of 56°C (Fauquet et al. 2005).

The lack of a lipid containing envelope and absence of essential lipids ensures lipid solvents (including acidic and alkaline solvents pH 3–9) and lipases do not adversely affect the virions. The parvovirus genome which consists of a molecule of single-stranded DNA rather than a genome made up of RNA provides additional stability. This is due to RNA having a hydroxyl group attached to the pentose ring in the two-prime position, whereas DNA has a hydrogen atom (Fauquet et al. 2005). The hydroxyl group makes RNA less stable than DNA because it is more prone to hydrolysis, especially under alkaline conditions. Furthermore, single-stranded DNA viruses are more heat stable than either double-stranded RNA or DNA viruses

as they are less prone to heat denaturation. When heated, the hydrogen bonds that join the double strands together are disrupted causing the double strand to break apart, thus destroying the functionality of the genetic material; whereas the stronger bonds within the single DNA strand can remain intact following similar heat exposure (Fauquet et al. 2005).

Various studies have reported on the infectivity of IHHNV following freezing. IHHNV can remain infectious in seawater and can survive storage at –5°C to –10°C (Bell and Lightner 1984). IHHNV infected tissues have been maintained at –70°C prior to being used in experimental infection trials (Tang et al. 2003a, Tang et al. 2003b). Prawns infected with IHHNV frozen and stored between –20°C and –80°C have remained infectious for over five years (Tang et al. 2003b, OIE 2006b). The IRA team is aware of James Cook University studies showing the Australian strain of IHHNV can remain infectious and maintains infectivity for up to 15 years frozen storage.

IHHNV has been shown to maintain infectivity for up to one day following passage through the gastro-intestinal tract of seagulls and chickens experimentally fed infected prawns, whereas YHV and WSSV do not (Garza et al. 1997, Vanpatten et al. 2004). The simple non-enveloped virion particle structure and the general stability of DNA (compared to RNA viruses) have been proposed as factors that contribute to the ability of IHHNV to survive the digestive tract of birds (Vanpatten et al. 2004).

Epidemiology

Host range

Natural IHHNV infections have been reported from *L. stylirostris*, *L. vannamei*, *P. monodon* (Lightner et al. 1983), *Macrobrachium rosenbergii* (Hsieh et al. 2006), *Litopenaeus occidentalis, Farfantepenaeus californiensis, Penaeus semisulcatus*, *Marsupenaeus japonicus* and *P. esculentus* (Lightner 1996a). Other species such as *Litopenaeus setiferus*, *Farfantepenaeus aztecus* and *Farfantepenaeus duorarum* (Lightner 1996a) have been infected experimentally with IHHNV. IHHNV infection has also been reported in *Fenneropenaeus chinensis* (Zhang and Sun 1997). *Fenneropenaeus indicus* and *Fenneropenaeus merguiensis* appear to be refractory to infection (Brock and Lightner 1990, Lightner 1996a). The susceptibility to IHHNV of other crustacean species is unknown.

Geographical distribution

Aquaculture populations

IHHNV is distributed widely throughout prawn aquaculture in Asia and the Americas. Countries which have reported IHHNV include the USA (South-East, Texas), Mexico, Ecuador, Peru, Brazil, Caribbean, Panama, Hawaii, Guam, Columbia, Tahiti, New Caledonia, Singapore, Malaysia, Thailand, Vietnam, Taiwan, China, Indonesia, the Middle East and the Philippines (Lightner 1996a).

An IHHNV strain was reported from *P. monodon* and the hybrid *P. monodon* x *P. esculentus* from Australia (Owens et al. 1992a, Krabsetsve et al. 2004). Histopathological characteristics of the Australian strain of IHHNV present similarly to classical American IHHNV, causing chronic low-grade mortalities when the hybrid prawns reached 3–4 grams in weight (Owens et al. 1992a). The small perinuclear, cytoplasmic particles did not react with a gene probe to American IHHNV, suggesting a genomic difference greater than 10% (Owens 1997).

Parvoviruses are known to be able to integrate into the host genome (Fauquet et al. 2005). Previous reports of IHHNV from Tanzania, Madagascar and Australia (Tang et al. 2003a, Krabsetsve et al. 2004) have also been reported as having an IHHNV-related sequence integrated into the prawn genome (Tang and Lightner 2006).

Wild populations

Epizootiology and phylogenetic analysis suggest that IHHNV originated in the Philippines and is endemic in South-East Asia in wild reservoir hosts such as *P. monodon* (Brock and Lightner 1990, Lightner 1996b, Lightner 1999, Tang et al. 2003a). A nucleotide sequence difference of 14% in *P. monodon* isolated strains compared to 0.5% in *L. stylirostris* and *L. vannamei* isolated strains (Tang and Lightner 2002) suggests IHHNV is a relatively recent disease in *L. stylirostris* and *L. vannamei*, over the last 2–3 decades (Tang et al. 2003a).

IHHNV has been reported throughout wild prawn populations of *L. stylirostris* (Unzueta–Bustamante et al. 1998, Morales–Covarraubias et al. 1999, Pantoja et al. 1999), *L. vannamei* and *F. californiensis* (Pantoja et al. 1999)in the region of the Gulf of California IHHNV has also been detected further south of the Gulf of California off the coast of Panama in wild *L. vannamei* (Nunan et al. 2001). IHHNV has not been reported along the Atlantic coast but is widespread in wild *P. monodon* populations throughout the Asian region (Lightner 1996a, Flegel 1997a, Alcivar–Warren et al. 2003).

Transmission

Horizontal transmission of IHHNV to susceptible prawns occurs as a result of feeding on infected carcasses, direct contact between prawns and indirectly through contaminated water (Lotz 1997a). Experimental infection methods commonly used include ingestion of infected carcasses or injection of viral suspension (Bell and Lightner 1984).

Vertical transmission of IHHNV in *L. vannamei* from infected females has been confirmed by laboratory experiments (Motte et al. 2003). There is speculation, based on the high prevalence of IHHNV positive eggs and sperm in healthy breeding populations of wild *L. stylirostris* that vertical transmission of IHHNV may have contributed to the spread of infection in cultured and wild prawns in Mexico (Morales–Covarrubias et al. 1999, Pantoja et al. 1999).

Tissue titre

Surviving *L. stylirostris* pre-infected with IHHNV and then challenged with WSSV had 10^9 copies of the targeted IHHNV PCR DNA fragment per microgram of DNA when quantified using real-time PCR, whilst moribund prawns had only 10^4–10^6 copies. The molecular test in the study targeted the PCR DNA fragment and did not specifically determine the titre of IHHN virus. Interestingly, the study concluded that surviving prawns with a high level of the IHHNV PCR DNA fragment were more resistant to WSSV infection (Tang et al. 2003b).

An investigation into IHHNV in prawn aquaculture in Thailand found similar levels of IHHNV in *P. monodon* and *L. vannamei* tissues. However, *P. monodon* had no apparent disease while infected *L. vannamei* displayed RDS (Chayaburakaul et al. 2005).

Wild *L. stylirostris* from the Gulf of California infected with IHHNV contained $6x10^8$ single-stranded genome equivalents per gram of tissue and were of normal appearance (Shike et al. 2000).

Experimentally infected *L. stylirostris* bred for resistance to IHHNV typically showed very low levels of virus DNA (Tang et al. 2000) in the range of 48-339 copies per nanogram of prawn DNA (Dhar et al. 2001).

Infectious dose

L. stylirostris (1g) and *L. vannamei* (mean weight 2g) became infected with IHHNV following were feeding of infected prawn tissue at a rate of 20% body weight twice daily for two days (Tang et al. 2000). *L. stylirostris* (1g) became infected with IHHNV following injection with a viral inoculum containing approximately 10^6 copies of IHHNV (Dhar et al. 2001). *L. stylirostris* (1–3 g) and *L. vannamei* (1g) were infected with IHHNV by being fed minced IHHNV-tissue for 3 days at 5% of their body weight daily (Tang et al. 2003b).

Prevalence

Penaeus monodon, *M. japonicus* and *P. semisulcatus*, species common in Australia in the wild and/or in aquaculture, are known to be susceptible to IHHNV infection (exotic strains) in overseas situations. The prevalence of the Australian strain of IHHNV in cultured *P. monodon* is estimated to be 80% in Australia (Krabsetsve et al. 2004).

IHHNV prevalence in wild *L. vannamei* collected for broodstock development off the west coast of Central America is reported to be 28% (Nunan et al. 2001). The reported prevalence of IHHNV in wild *L. stylirostris* in the Gulf of California ranges between 44–100% (Unzueta–Bustamante et al. 1998, Morales–Covarrubias et al. 1999). A generally higher prevalence is reported in the upper Gulf when compared to the central–lower Gulf (Pantoja et al. 1999). Lightner et al. (1983) reported that although *P. monodon* is susceptible to IHHNV infection, disease outbreaks in aquaculture facilities appear to require prolonged exposure to high doses of the virus as occurs during co-culture with the highly susceptible *L. stylirostris*. Previously, IHHNV epidemics in *L. stylirostris* aquaculture facilities typically caused mortalities in excess of 80–90%, indicating a high prevalence of the virus in susceptible stock (Lightner et al. 1983); however, IHHNV has not been associated with mass mortalities or production volume loss in *L. stylirostris* aquaculture in the Americas in recent years (Tang et al. 2003b).

In South-East Asia, the current status of IHHNV prevalence in farmed *P. monodon* (where postlarvae have been sourced from wild-caught South-East Asian broodstock) is as high as 94% (Baldock 1999, Flegel et al. 2004). The high prevalence of IHHNV in cultured *P. monodon* throughout Asia is reported to cause no apparent disease and appears to have no effect on production volume (Flegel et al. 2004, Chayaburakul et al. 2004, Chayaburakul et al 2005). Isolated IHHN outbreaks causing RDS in *P. monodon* have been reported in the central Philippines, although no data was provided on associated production losses (Primavera and Quinitio 2000).

Pathogenesis

In susceptible species, the virus replicates in the cytoplasm of cells of ectodermal origin (epidermis, gills, fore and hind gut, antennal gland and neurons) and mesodermal origin (haematopoietic tissue, haemocytes, striated muscle, heart, lymphoid organ and connective tissues) (Lightner et al. 1983, Lightner 1996a). Infection of the midgut epithelium is rare. Cells infected by IHHNV display eosinophilic staining Cowdry Type A intranuclear inclusion

bodies (CAIs) (Lightner et al. 1983, Lightner 1996a).

It has been suggested that IHHNV is more likely to infect juvenile prawns, which have relatively fast cell generation, as parvoviruses depend on the host cell DNA replication mechanism to multiply (Kalagayan et al. 1991, Lightner 1996a, Tang et al. 2000).

Despite a genetic difference of 3.8% between IHHNV in Thailand and the Philippines, the histopathology of disease is alike (Tang et al. 2003a). Reduced virulence of IHHNV in wild and cultured prawn stocks over time has not been attributed to genetically variant strains (Tang and Lightner 2002), but rather to an increase in host tolerance (Morales–Covarrubias et al. 1999, Tang and Lightner 2002).

Clinical signs

Cultured *L. stylirostris* displays the most acute form of IHHN, causing very high mortalities in juveniles older than 35 days. Clinical signs of IHHN in *L. stylirostris* are non-specific and include anorexia, lethargy, blue colouration, opaque abdominal musculature, reduced feeding and erratic swimming. Infected prawns have been observed to rise to the water surface, remain motionless for a few moments, then roll over and sink, a behaviour that may be repeated until death (Lightner et al. 1983). Mortality may exceed 90% within several weeks of onset of infection in juvenile *L. stylirostris* (Lightner et al. 1983, Bell and Lightner 1987). Infected adult *L. stylirostris* do not always show clinical signs (Bell and Lightner 1987) of IHHN or mortalities (Bell and Lightner 1984, Bell and Lightner 1987).

RDS is primarily a chronic disease of cultured *L. vannamei* infected with IHHNV. *L. vannamei* displaying gross morphology associated with RDS are believed to be initially infected during the larval or early post-larval stages (Kalagayan et al. 1991). Juvenile prawns with RDS display reduced growth rates of up to 30% and deformities of the cuticle, rostrums, appendages and other parts (Kalagayan et al. 1991).

Based on a 3-year study, Flegel et al. (2004) reported that *P. monodon* in Asia were not adversely affected by IHHNV. Despite a high prevalence IHHNV is reported to be well tolerated in farmed *P. monodon* in Thailand (Flegel et al. 2004, Chayaburakul et al. 2005).

It has been suggested that the Philippines strain of IHHNV may cause RDS in *P. monodon*, although no clear causal relationship has been demonstrated between IHHNV and the morphological abnormalities observed (Primavera and Quinitio 2000). However, laboratory infection trials have not demonstrated any significant impact of IHHNV infection on *P. monodon* growth, fecundity or hatching rate and it was suggested that problems associated with IHHNV infection of monodon prawns are rare (Withyachumnarnkul et al. 2006). The authors further suggest that it is unlikely that the strain of IHHNV used in their infection trials differed significantly from that which causes RDS in *L. vannamei*, given that very little difference in the DNA sequences of IHHNV isolates from various regions in Thailand has been found (Charyaburakul et al. 2004).

The Australian strain of IHHNV has only been reported to cause mortality in 3–4g size (90–100 day old) hybrid prawns (Owens et al. 1992a).

Diagnosis

Current diagnostic methods listed by the OIE (OIE 2009b) include molecular tests such as PCR, dot-blot hybridisation and *in situ* hybridisation. Routine histology and antibody assays such as ELISA and Western-blot are available. *In situ* hybridisation is considered the most reliable method of accurate diagnosis when used in combination with other available methods to provide diagnostic certainty. Presumptive diagnosis using routine histology requires demonstration in specific target tissue of intranuclear eosinophilic inclusion bodies (CAIs) (OIE 2009b). Rapid and highly sensitive molecular diagnostics incorporating loop-mediated isothermal amplification (LAMP) method have been developed as a IHHNV test capable of

rapidly screening high volumes of samples for use as a health management tool for the prawn industry and epidemiological research (Sun et al. 2006).

The three OIE recommended tests associated with high volume screening (e.g. PCR) do not distinguish between strains of IHHNV (OIE 2006b) and only one of the tests reacts with the Australian strain of IHHNV (Krabsetsve et al. 2004).

Introduction of IHHNV into new countries or areas

Mechanism of disease spread

Phylogenetic relationships suggest IHHNV was introduced from the Philippines to Hawaii two to three decades ago in live asymptomatic *P. monodon*, which later resulted in the more susceptible and commercially important *L. stylirostris* becoming infected, and subsequently spreading throughout the western hemisphere with live prawn movements (Lightner 1999, Tang et al. 2003a). Introduction of IHHNV into prawn farming in Mexico in 1987 occurred as a result of sourcing infected post-larval *L. vannamei* from infected hatcheries located in the US and Central America (Lightner et al. 1992a, Lightner 1996b). Genetic similarity also suggests IHHNV was introduced into Taiwan from Thailand potentially via trade in live or frozen prawns (Tang et al. 2003a). Exposure to infected frozen, uncooked prawn products and subsequent transmission of infection has been suggested, as IHHNV remains infectious after freezing for several years (Bell and Lightner 1984, Lotz 1997a, Tang and Lightner 2002, Tang et al. 2003b, OIE 2006b).

Hatchery inputs such as feed source are also considered to be a possible route of IHHNV introduction into farmed prawn populations. Shi et al. (2003) found, however, that samples of artemia shrimp (used as a food source for juvenile hatchery prawns) to be free from IHHNV.

Transmission of IHHNV in an intensive aquaculture situation is exacerbated by high stocking densities leading to crowding stress, poor water quality and poor husbandry techniques (Browdy et al. 1993).

Impacts of introduction

Initial establishment and spread of IHHNV in cultured naïve *L. stylirostris* populations throughout the Americas resulted in significant losses estimated at 80–90% (Lightner et al. 1983). Prawn aquaculture in the Americas has since recovered and IHHNV has not been associated with mass mortalities and associated economic loss in recent years (Tang et al. 2003b).

RDS in *L. vannamei* does not cause mass mortality however, disease outbreaks have caused decreased production, reduced product quality and increased costs of production associated with health management (Lightner 1999). While it is difficult to estimate economic loss throughout entire regions, individual prawn farm profitability has been estimated based on a typical outbreak, representing a 20% prevalence of RDS. The resulting reduction in crop value translates to an 18% economic loss, which would usually represent total farm profit (Kalagayan et al. 1991).

Although widespread and highly prevalent in Asian farmed prawns, IHHNV is not considered to be a cause of serious disease in traditional culture species (Lightner et al. 1997a, Chayaburakul et al. 2004, Flegel et al. 2004, Chayaburakul et al. 2005) — noting that *L. vannamei* is now widely farmed in S.E. Asia. In contrast, IHHNV has been reported to cause RDS and production volume loss in farmed *P. monodon* in the Philippines (Primavera and Quinitio 2000).

Based on the information available at the time, a report on the potential economic impact of establishment of IHHNV in Australia concluded that IHHNV infection would cause very low rates of infection and minor production losses in farmed *P. monodon* and *M. japonicus* under

Australian conditions (Alliance Resource Economics 1999).

The impact of IHHNV on wild prawn populations is not yet fully understood. High rates of IHHNV infection were associated with a marked pre-1990 decline, and subsequent recovery, in the wild population of *L. stylirostris* in the Gulf of California (JSA 1997). However, there has been no cause-and-effect relationship established between IHHNV and the decline in catch rates (Eastern Research Group Inc. 1998).

High prevalence of IHHNV in wild *L stylirostris* in the Gulf of California was still apparent ten years following initial epidemics; however, authors noted that prawns did not show clinical signs of disease and suggested an equilibrium in the host-pathogen relationship (Morales–Covarrubias et al. 1999, Tang and Lightner 2002).

Control measures

Prevention of IHHNV in prawn aquaculture relies primarily on husbandry techniques such as eradication of infected stock, continued screening, within hatchery quarantine and biosecurity to prevent re-introduction of the virus, disinfection of ponds and facilities and re-stocking of specific pathogen free (SPF) broodstock and postlarvae (Lightner 1999). The use of SPF *L. vannamei* broodstock and verified IHHNV-freedom of broodstock and postlarvae have been shown to significantly increase nauplii production from domesticated IHHNV-free females in South America, and has been proposed as a routine hatchery procedure (Motte et al. 2003).

The greatest benefit to the prawn farming industry to date has been the success of selective breeding programs. This has resulted in the development of a line of *L. stylirostris*, known as Super Shrimp®, resistant to IHHNV infection (Tang et al. 2000). Further research is being conducted to elucidate the mechanisms of resistance (Hizer et al. 2002).

Baculovirus penaei

Baculovirus penaei (BP) infects wild and farmed penaeid prawn species in the Americas, causing tetrahedral baculovirosis, including significant mortality in larval and post-larval stages in commercial hatcheries (Overstreet 1994). Tetrahedral baculovirosis is listed as a disease notifiable to the OIE and exotic to Australia.

Agent taxonomy

BP is a rod-shaped, 269nm long, 50nm diameter, enveloped, occluded baculo-like, tetrahedral, double-stranded DNA virus (Couch 1974). Although previously considered a possible member of the genus containing single nuclear polyhedrosis viruses from the family Baculoviridae (Couch 1974, Wilson 1991), the International Committee on Virus Taxonomy lists the related virus MBV (spherical baculovirosis) as a tentative species in the genus Nucleopolyherdovirus (Fauquet et al. 2005, OIE 2006b). Therefore, BP should also be considered as a tentative species in this genus (OIE 2006b).it has since been removed from Baculoviridae by the International Committee on Taxonomy of Viruses - ICTV (Murphy et al. 1995, Van Regenmortel et al. 2000). At least two strains of the virus are thought to exist (Durand et al. 1998). The most characterised geographical strain of BP has been designated PvSNPV or singly enveloped nuclear polyhedrosis virus from *Litopenaeus vannamei* (Bonami et al. 1995a).

Agent stability

BP can be inactivated by heating for 10 minutes in water baths at 60°C to 90°C but remains infectious after heating for the same period in water baths at 50°C (LeBlanc and Overstreet 1991a).

The virus has been experimentally transmitted to prawns after storage of infected samples at temperatures of –40°C to –70°C (Overstreet et al. 1988). BP virulence does not appear to be affected by frozen storage for up to 3.5 years (Overstreet 1994, Stuck et al. 1996, Hammer et al. 1998).

BP remained infectious after seven days at 22°C in seawater, but was inactive by day 14 (LeBlanc and Overstreet 1991a). In the same study, the virus was infectious after 14 days at 5°C but was not infectious by day 59. When homogenates of the virus were held in Petri dishes at 22°C and allowed to desiccate, BP was completely inactivated within 48 hours (LeBlanc and Overstreet 1991a). Ultraviolet irradiation (LeBlanc and Overstreet 1991a) and chemical disinfection using chlorine have also effectively inactivated the virus (LeBlanc and Overstreet 1991b).

Epidemiology

Host range

Severe disease is typically associated with mysis, post-larval and juvenile stages in hatcheries (Overstreet 1994). Infections have been reported from many penaeid species, significant mortalities have been reported from *Farfantepenaeus aztecus*, *Farfantepenaeus duorarum*, *Melicertus marginatus*, *L. vannamei* and *L. stylirostris* (Overstreet 1994). Although infection is reported from larval to adult stages, increase in host age is associated with reduction in the likelihood and severity of infection and disease (Lightner 1988, Lightner et al. 1989b, LeBlanc and Overstreet 1990, Overstreet 1994). BP has not been reported in non-prawn species.

Geographic distribution

BP was first reported in wild *F. duorarum* from the northern Gulf of Mexico (Couch 1974) and is now widely distributed in wild and cultured prawns throughout the Americas, including Hawaii. BP has not been reported outside these regions (Lightner 1996a).

Transmission

BP may be transmitted orally either by the uptake of virus from water contaminated with faeces shed from infected prawns or by cannibalism on dead and dying prawns (Overstreet et al. 1988, Overstreet 1990, Overstreet 1994, Stuck and Overstreet 1994, Hammer et al. 1998). Overstreet et al. (1988) reported polyhedra in free and attached faecal strings from infected larvae through to adult prawns. Virions and tetrahedral occlusion bodies released into the mid-gut lumen may be consumed by non-infected prawns when excreted with faeces into the environment (Bruce et al. 1994a). BP is transmitted horizontally and not by *true* vertical transmission (Bruce et al. 1994a) however, broodstock releasing BP-contaminated faeces whilst spawning can transmit infection to eggs and newly hatched nauplii (OIE 2006b). Vertical transmission of BP may occur, although attempts to infect eggs with BP by immersing in a homogenised viral slurry were not successful (Overstreet 1994). BP has not been detected in femaile reproductive tissues including developing ova as assessed using *in situ* hybridisation (Bruce et al. 1994a). However, virus has been detected in eggs using transmission electron microscopy (Overstreet 1990, Overstreet 1994). BP has been experimentally transmitted to *L. vannamei* mysis larvae via feeding rotifers and brine shrimp fed on BP infected prawn tissue (Overstreet et al. 1988, LeBlanc and Overstreet 1990) and by feeding specific pathogen-free (SPF) *L. vannamei* of various ages with homogenised BP infected postlarvae (Stuck and Overstreet 1994, Stuck and Wang 1996, Hammer et al. 1998). There is evidence that experimentally infected animals may be able to eliminate the virus over time (LeBlanc and Overstreet 1990, Stuck and Wang 1996), however, asymptomatically infected carrier animals may exist (Stuck and Wang 1996). Older *L. vannamei* (PL63-157) have proved difficult to infect with BP— infection in these prawns being less extensive and less persistent (LeBlanc and Overstreet 1990).

Tissue titre

There are no data available on the titre of BP in infected prawn tissues.

Infectious dose

One infectivity study suggested that the stage of infection may influence the infectivity of the tissue. Inocula prepared from patently infected animals consistently produced infections whereas inocula prepared from pre-patently infected animals caused infections only in some experimental animals (Hammer et al. 1998). In a similar experiment an inoculate prepared using infected broodstock resulted in longer pre-patent periods and fewer mortalities in experimental animals than a similar inoculate prepared from infected juveniles (Overstreet et al. 1988).

Prevalence

There are few reported data on the prevalence of BP infection in aquaculture populations. BP is primarily a disease of economic importance associated with prawn hatcheries and nurseries, where mortality rates of up to 100% in mysis larvae and early postlarvae have been reported (Lightner 1988, Lightner et al. 1989b, Stuck and Overstreet 1994).

There are few published reports on the prevalence of BP in wild prawn populations. A survey of BP in wild adult *F. duorarum* in Florida, conducted over a four year period, reported an average prevalence of 20% (Couch 1976). Monthly sampling of wild populations of *F. aztecus* in Mississippi from 1989 to 1993 showed a seasonal variation in the detection of BP. The virus was detected in up to 40% of samples collected during March to September

each year, peaking during May to August, but was not detected at other times (Overstreet 1994). The prevalence of BP infection in *Litopenaeus schmitti* and *Farfantepenaeus notialis* sourced from Cuban waters in 1991 was reported as 31% and 21% respectively and in and 1993 it was reported as 3% and 7% respectively (Fajer et al. 1998).

Clinical signs

Infection with BP may result in non-specific signs of disease such as reduced feeding, poor growth rate, increased exoskeletal and gill fouling, morbidity and mortality (Lightner 1988, Stuck and Overstreet 1994).

BP outbreaks, mostly affecting mysis larval and early post-larval stages, are characterised by the sudden onset of acute disease, typically causing cumulative losses of up to 100% of the affected population within 24–48 hours of onset (Lightner 1988, Lightner 1996a). In older postlarvae and juveniles in high-density culture, the disease is often sub-acute to chronic, however, cumulative mortality rates may exceed 50% within 4–8 weeks of onset (Lightner 1988). Surviving postlarvae exhibit markedly reduced growth in the first four weeks following infection, but the long-term effects on the growth of survivors are thought to be minimal (Stuck and Overstreet 1994). LeBlanc and Overstreet (1990) demonstrated that in commercially produced *L. vannamei* older than PL63, virus-induced mortalities ceased and the infection rate decreased. Additionally, the virus was undetectable in exposed prawns older than 325 days of age. Adult prawns show an increase in the time until infection can be detected (pre-patent period) and an increase in the prawn's ability to eradicate infection (LeBlanc and Overstreet 1990).

Pathogenesis

BP infects the epithelial cells of the hepatopancreatic tubules and midgut. Viral replication results in nuclear hypertrophy, followed by nuclear and cellular disruption (Overstreet et al. 1988, Stuck and Overstreet 1994). Free virions and occlusion bodies are released into the lumen of the intestinal tract and then shed in the faeces (Adams et al. 1991). Up to 90% of hepatopancreatic cells can be infected without causing death (Overstreet 1994).

Diagnosis

BP infection can be diagnosed using light microscopy. Tetrahedral occlusion bodies can be readily seen in tissue wet mounts from postlarvae, or using a non-lethal wet mount technique to examine occlusion bodies in faeces. Wet mounts may be most useful as a non–lethal screening method for valuable broodstock (OIE 2006b). Histology can demonstrate tetrahedral occlusion bodies in hepatopancreatocytes, gut epithelial cells, or gut lumen. Cells show hypertrophied nuclei with single or multiple occlusion bodies, chromatin diminution and margination (Brock et al. 1986a, Brock and Lightner 1990, Lightner 1996a). A diagnostic PCR test is available (Wang et al. 1996), and provides means of diagnosis during the early stages of disease when tetrahedral occlusion bodies are not visible in wet mounts (Bruce et al. 1994b), although the IRA team is of the view that the test is prone to false-positives and may require confirmation using transmission electron microscopy.

Introduction of BP into new countries or areas

Impacts of introduction

Quantitative information on aquaculture production losses due to BP infection is not available. Despite being reported since 1974, there are no reports of mortalities in wild prawn populations. This suggests younger and highly susceptible life stages inhabit offshore environments distant from BP-contaminated estuaries (Overstreet 1994).

Control measures

Disinfection with calcium hypochlorite and drying of tanks and equipment may assist with the elimination of BP infection from culture and research facilities (LeBlanc and Overstreet 1991b, Overstreet 1994). Similarly, washing eggs or nauplii with formalin, iodophores or clean seawater may reduce transmission of infection caused by faecal contamination (Bruce et al. 1994a, OIE 2006b). Restricting the movement of live animals, quarantine isolation/observation, and improved prawn farming health and husbandry management techniques have also been suggested (Overstreet 1994). Screening postlarvae and potential broodstock via faecal examination for the presence of tetrahedral inclusion bodies prior to their introduction into facilities may also limit the spread of the disease (OIE 2006b). Providing disease-free prawns is considered the key management tool in limiting the impact of BP in hatcheries (Overstreet 1994).

Hepatopancreatic parvovirus

Hepatopancreatic parvovirus (HPV) has been reported in postlarvae and juveniles of wild and farmed penaeid prawns. HPV is often found in healthy animals and considered not to cause significant disease. However, HPV infection and in particular, multiple infections with other pathogens such as monodon baculovirus (MBV), has been reported in association with stunted growth, resulting in production losses in Thai prawn farms (Flegel et al. 1999, Flegel et al. 2004).

Agent taxonomy

HPV is an icosahedral, non-enveloped, negative-sense, single-stranded DNA virus, with a mean diameter of 22nm, belonging to the family Parvoviridae (Bonami et al. 1995b). Molecular phylogenetic analysis shows that HPV is closely related to Brevidensoviruses (prawn parvovirus IHHNV and mosquito densoviruses, *Aae*DNV and *Aal*DNV). Genome differences suggest HPV is a new type of Parvoviridae and the name *Penaeus monodon* densovirus (PmDNV) has been suggested (Sukhumsirichart et al. 2006). To date, three geographic strains of HPV have been identified from prawns using molecular techniques; HPV*mon* from Thai *P. monodon* (Phromjai et al. 2001), HPV*chin* from Korean *Fenneropenaeus chinensis* (Lightner et al. 1994a) and HPV*merg* from *Fenneropenaeus merguiensis* (La Fauce et al. 2007). Homology of sequence for the part of DNA between HPV*mon* and HPV*chin* was 77% (Phromjai et al. 2001, Roekring et al. 2002). A potential HPV strain has been reported from wild *Penaeus semisulcatus* in India (Manjanaik et al. 2005). Possible HPV-strains have also been identified in wild and cultured Australian prawn species based on morphology and pathogenicity (Roubal et al. 1989, Spann et al. 1997a, Jones 1998). DNA probes based on HPV*chin* did not react with HPV found in Malaysian *Macrobrachium rosenbergii*, suggesting these two are not closely related (Lightner et al. 1994a, Lightner et al. 1994b).

Agent stability

There are no inactivation data specific to HPV. Postlarvae infected with HPV and stored whole at –80°C have been used to successfully infect prawns in experimental trials (Catap et al. 2003).

In general, parvoviruses have a very simple virion structure compared to many other viruses, making them able to tolerate a range of adverse environments, including pH ranges of 3–9, a temperature of 56°C for at least 60 minutes, and the presence of lipid solvents and lipases (Fauquet et al. 2005).

Epidemiology

Host range

Natural infections have been reported from a wide range of penaeid species. HPV has been found in *Fenneropenaeus indicus*, *F. merguiensis* (Chong and Loh 1984), *F. chinensis, P. semisulcatus*, *P. monodon*, *Penaeus esculentus* (Lightner et al. 1985), *F. penicillatus* (Lightner et al. 1989a), *Litopenaeus vannamei* (Lightner et al. 1989a) and *Litopenaeus stylirostris* (Lightner 1993) from Asia and the Indo-Pacific region. The level of disease and mortality varies amongst species (Lightner et al. 1989, Lightner et al. 1992b, Lightner et al. 1994a). Most recently, HPV has been detected in wild *Parapenaeopsis stylifera, Marsupenaeus japonicus*, *Metapenaeus monoceros*, *Metapenaeus affinis*, *Metapenaeus elegans*, *Metapenaeus dobsoni*, *Metapenaeus ensis* and *Solenocera choprai* from India (Manjanaik et al. 2005). Parvo-like viruses, morphologically similar to HPV, have also been found in diseased *Carcinus mediterraneus* (Mari and Bonami 1988) and *M. rosenbergii*

(Anderson et al. 1990).

Geographical distribution

HPV was initially reported from Asia and Indo-Pacific regions including Australia (Chong and Loh 1984, Lightner et al. 1985, Roubal et al. 1989, Lightner et al. 1989a, Flegel et al. 1992b, Lightner 1993, Mohan et al. 1998). HPV has subsequently been found in the Middle East (Colorni et al. 1987) and Africa (Lightner et al. 1985) and is reported to have spread to the Americas including Hawaii via live introduced *F. penicillatus* (Lightner et al. 1989a). HPV is now widely distributed in many parts of the world.

Transmission

Juvenile *P. esculentus* susceptible to natural HPV infection were placed with HPV-positive prawns in a confined environment for 5 weeks with no horizontal pathogen transmission observed (Paynter et al. 1985). Flegel et al. (1995a) observed that HPV infected postlarvae from the nursery were still infected in the rearing pond, but the prevalence of HPV infection in the pond remained the same level, further suggesting that horizontal transmission did not occur. *Penaeus monodon* postlarvae were experimentally infected by feeding frozen HPV infected postlarvae (Catap et al. 2003).

Possible vertical transmission was considered when postlarvae from HPV-infected adult *F. chinensis* broodstock at a quarantine facility in Hawaii were reported to be infected with HPV (Lightner et al. 1992b).

Tissue titre

There are no quantitative data available on the titre of HPV in infected prawn tissues.

Infectious dose

There are no data available on the infectious dose of HPV.

Prevalence

Prevalence data on HPV have primarily been based on histology (i.e. finding typical intranuclear inclusion bodies) and conducted as part of general monitoring of prawn health. Because molecular diagnostic tests that could confirm HPV presence were not used in many studies, histology based prevalence data should be regarded with caution.

In an early histological study on hatchery reared *F. indicus* and *F. merguiensis* in Singapore, HPV was monitored monthly and detected in up to 100% of prawns sampled, although the monthly sample numbers in this study were very low (n=3) (Chong and Loh 1984). The prevalence of HPV infection in *F. chinensis* postlarvae sourced from Korea and shipped to the US was up to 100% at six weeks using a simple smear diagnostic test (Lightner et al. 1993). In Thailand, the prevalence in *P. monodon* postlarvae that had been overdosed with vitamin C was over 90%, compared to 43% in the non-overdosed group (Flegel et al. 1995a). Cultured healthy *P. monodon* collected randomly from separate ponds in Thailand were 45% HPV-positive (Flegel et al. 1992b). HPV was found in 49% of *P. monodon* in four rearing ponds and further analysis was conducted to investigate the relationship between HPV infection and stunted growth (Flegel et al. 1999). Another histological monitoring program of Thai *P. monodon* ponds with known high prevalence of HPV infection reported HPV at 30% (Sukhumsirichart et al. 1999). Research on hatchery-reared *P. monodon* postlarvae in the Philippines showed overall prevalence of HPV between 1993 and 1996 ranged from 1.5–9.4% (Gomez 1998 cited in Catap et al. 2003). More recent histological monitoring in the Philippines determined the prevalence of HPV in healthy *P. monodon* postlarvae (PL13 to PL26) was 20–100% in one hatchery, and 70, 89 and 99% in other hatcheries (Catap et al. 2003). HPV was detected by *in situ* hybridisation as well as histology in 70% of juvenile

F. chinensis sampled from a known HPV-positive population in China (Pantoja and Lightner 2000).

Molecular HPV detection using PCR-ELISA with Thai HPV*mon* probes detected HPV DNA in 62% of histologically negative *P. monodon* and in 87% of stunted animals collected from ponds in Thailand (Sukhumsirichart et al. 2002). Other studies utilising molecular detection techniques to investigate stunted growth in *P. monodon* in Thailand, found HPV present in 60% of specimens (Flegel et al. 2004) and in 72% of grow-out ponds (Chayaburakul et al. 2004) suffering from stunted growth, but only in combination with other viruses. In an Indian study of *P. monodon* (PL10 to PL20) in hatcheries, HPV DNA was detected by PCR using HPV*mon* primers in 34% of samples taken from 22 hatcheries, and 31% of samples submitted by farmers. Of those samples, 27% and 29% respectively, were PCR positive to three viruses (HPV, MBV and WSSV) (Umesha et al. 2003). None of the HPV*mon* PCR positive samples reacted with the Korean HPV*chin* primers, suggesting that the Indian HPV found in *P. monodon* was more closely related to Thai HPV (Umesha et al. 2003).

One out of seven *P. monodon* broodstock (14%) captured from the north-east coast of Sumatra, Indonesia was infected with HPV and MBV without clinical signs (Turnbull et al. 1994). In India, approximately 5% of wild *P. monodon* and *F. indicus* proved HPV-positive on histological examination, and co-infected with WSSV (Mohan et al. 1998). A total of 11 species of wild prawns from India were demonstrated to be PCR positive for HPV (Thai strain), with an overall prevalence of 58% (Manjanaik et al. 2005).

In a study of wild broodstock *L. vannamei* from the Pacific coast of Mexico, HPV was detected in 21% and 50% of two separately sampled groups of animals. Some prawns had dual or multiple infections with HPV, IHHNV and/or TSV, as well as non-viral pathogens (Morales–Covarrubias and Chavez–Sanchez 1999).

An Australian investigation into the health status of wild *P. esculentus* populations caught off Moreton Bay and kept under stressful conditions to induce disease reported 0.2% HPV prevalence, based on histology (Paynter et al. 1985). The prevalence of HPV in wild healthy adult *F. merguiensis* from north Queensland ranged from 6.7 to 22% (Roubal et al. 1989, Owens and Hall–Mendelin 1990), while the prevalence for healthy juveniles was 3% (Owens and Hall–Mendelin 1990). During health monitoring of wild prawn stocks in Western Australia, HPV prevalence in *F. merguiensis*, *Melicertus latisulcatus* and *P. esculentus* was 28%, 0.5% and 5%, respectively (Jones 1998). Positive results were confirmed by *in situ* hybridisation (Jones 1998).

Clinical signs

HPV infections are usually observed in aquaculture grow-out ponds, but have also been reported in hatcheries (Manivannan et al. 2002, Umesha et al. 2003). Gross signs are non-specific and include poor growth, anorexia, reduced preening activity and increased surface fouling (Lightner and Redman 1985). Lesions associated with infection are hepatopancreas atrophy and infrequently, abdominal muscle opacity (Lightner and Redman 1985). Infected animals appear to pass the normal larval and post-larval stages without clinical signs, although juveniles can suffer moderate to severe mortalities up to 100% within 4–8 weeks of disease onset (Lightner and Redman 1985). HPV appears to have little impact on mysis and early post-larval stages, but may exhibit as disease in animals from approximately PL8PL10 onwards (Lu 1997, Manivannan et al. 2002). HPV also infects wild adult broodstock (Colorni et al. 1987, Turnball et al. 1994, Morales–Covarrubias and Chavez-Sanchez 1999) and has been detected from apparently healthy animals (Chong and Loh 1984, Lightner et al. 1985, Flegel et al. 1992b, Jones 1998).

Dual infection with other pathogens may result in mortality and stunting (Flegel et al. 1999, MoralesCovarrubias and ChavezSanchez 1999, Manivannan et al. 2002, Umesha et al. 2003, Flegel et al. 2004). Diseased prawns infected with HPV as the solitary detectable viral agent

have rarely been found (Chong and Loh 1984, Lester et al. 1987b, Flegel et al. 1992b, Flegel et al. 1999, Flegel et al. 2004). HPV infection is frequently observed in dual infections with MBV in Thailand (Flegel et al. 1992b, Flegel et al. 1995a, Flegel et al. 1999, Chayaburakul et al. 2004, Flegel et al. 2004). Stressful conditions are known to induce morbidity in moderately infected prawns (Paynter et al. 1985).

Pathogenesis

HPV occurs in cells of the hepatopancreatic tubule epithelia (hepatopancreatocytes) (Lightner and Redman 1985, Paynter et al. 1985, Lightner et al. 1989, Anderson et al. 1990, Owens and HallMendelin 1989, Flegel et al. 1992b, Lightner et al. 1993) and rarely in cells of the anterior midgut or caecum epithelia (Lightner and Redman 1985, Mohan et al. 1998). Acute infection results in atrophy of the hepatopancreas, reduced lipid storage and reduced secretory vacuoles in hepatopancreatocytes (Lightner and Redman 1985). Other than atrophy, hepatopancreas cells show no obvious signs of necrosis or associated inflammatory response (Lightner and Redman 1985, Anderson et al. 1990).

Diagnosis

HPV is typically diagnosed histologically, with affected cells of the hepatopancreatic tubule epithelia showing characteristic single, large basophilic intranuclear inclusion bodies. Transmission electron microscopy reveals electron-dense, granular inclusion bodies centrally located in the karyoplasm with aggregations of viral particles (Lightner and Redman 1985). Although less sensitive than histopathology, a rapid diagnostic technique such as Giemsa-stained impression smears of hepatopancreas can be used in the field and this has been demonstrated to compare favourably in diagnostic sensitivity to histology (Lightner et al. 1993). Molecular techniques such as *in situ* hybridisation and PCR, based on the HPV*chin* strain are commercially available, including a non-invasive PCR method for detecting virus DNA in faecal samples (Mari et al. 1995, Pantoja and Lightner 2000, Pantoja and Lightner 2001. *In situ* hybridisation, PCR and PCR-ELISA diagnostics have also been developed based on the Thai HPV*mon* strain (Flegel et al. 1999, Sukhumsirichart et al. 1999, Phromjai et al. 2002, Sukhumsirichart et al. 2002). Monoclonal antibodies from HPV*mon* have been developed, however, when tested, some high density inclusion bodies were seen not to have reacted with the antibodies (Rukpratanporn et al. 2005). A sensitive nested-PCR test has been developed for screening wild prawns in India, with potential as a health management tool for screening wild broodstock (Manjanaik et al. 2005).

Introduction of HPV into new countries or areas

Mechanism of disease spread

Since HPV was first reported from Asia, the Indo-pacific and Australia (Chong and Loh 1984, Lightner and Redman 1985, Paynter et al. 1985), the virus has spread to the Middle East and the Americas, including Hawaii, via the importation of live animals, especially broodstock (Colorni et al. 1987, Lightner et al. 1989). The reported occurrence of natural infections in wild prawn populations suggests that infection in aquaculture is spread by wild infected broodstock (Colorni et al. 1987, Lightner et al. 1992b, Turnbull et al. 1994, Morales-Covarrubias and ChavezSanchez 1999, Sukhumsirichart et al. 2002, Manjanaik et al. 2005).

Impacts of introduction

Although HPV is not likely to cause severe epidemics, it may cause economic loss. Anecdotal information by farmers in Thailand reported that 20% of the production profit was lost in one season due to stunted prawns possibly associated with HPV dual infection (Flegel et al. 1999). The presence of HPV in healthy broodstock suggests some naturally occurring viruses are tolerated and are normal in healthy prawns (Flegel et al. 2004). Although HPV is present in

adult wild prawns, there is no evidence of serious losses.

Control measures

As with other viral pathogens of prawns, control measures for HPV are primarily aimed at preventing the introduction of the virus into susceptible populations. As HPV has typically been found in postlarvae and young juveniles for stocking, a testing scheme for hatchery broodstock has been suggested as a means of preventing virus introduction (Manivannan et al. 2002, Manjanaik et al. 2005). It may be possible to control disease and mortality by optimising environmental conditions such as reduced stocking densities in post-larval rearing tanks, since HPV can occur at high prevalence in prawns without exhibiting clinical signs or mortality.

Infectious myonecrosis virus

Infectious myonecrosis virus (IMNV) is the causative agent of infectious myonecrosis (IMN) (Poulos et al. 2006). IMN emerged in farmed *Litopenaeus vannamei* populations in north-eastern Brazil in 2002 and causes mortality and production losses in affected populations. IMN is listed as an OIE notifiable crustacean disease.

Agent taxonomy

IMNV is a non-enveloped, icosahedral shaped, double-stranded RNA virus, 40nm in diameter with a 7.5 kilobase genome and tentatively assigned to the Totiviridae (Lightner et al. 2004, Tang et al. 2005a, Poulos et al. 2006). Further, phylogenetic analysis clustered IMNV with another member of the family Totiviridae, *Giardia lamblia* virus (Poulos et al. 2006). These findings suggest IMNV may be a new dsRNA virus family that infects invertebrate hosts or a unique member of the Totiviridae (Poulos et al. 2006).

Agent stability

IMNV sourced from infected Brazilian *L. vannamei* maintained at –70°C have been successfully used in experimental infection trials (Tang et al. 2005a).

Epidemiology

Host range

The disease can affect postlarvae, juveniles, sub-adults and adults late in the production cycle, although apparently healthy chronically infected animals have also been reported (Lightner et al. 2004, OIE 2004, Tang et al. 2005a). Experimental infection by injection of purified virions has been achieved in *Litopenaeus stylirostris* and *Penaeus monodon*; however, no mortality ensued (Tang et al. 2005a, Tang et al. 2007).

Geographical distribution

IMNV has been reported from the north-eastern states of Brazil (Lightner et al. 2004) and Indonesia (T. Flegel, Mahidol University Thailand, pers. comm. August 2006), and suspected in Thailand and China (Briggs 2006). An infection displaying muscle necrosis has been reported in farmed *L. vannamei* from Belize and *P. monodon* from Australia. *In situ* hybridisation tests were performed on these infected prawns using Brazilian IMNV-specific primers with negative results (Tang et al. 2005a).

Transmission

IMNV has been transmitted to *L. vannamei* via ingestion of infected prawn tissues and by injecting *L. vannamei*, *L. stylirostris* and *P. monodon* with purified virions sourced from infected frozen prawn tissue (Tang et al. 2005a). *L. vannamei* mortality increased to 35% (over the 5% mortality seen in the control group) following ingestion of infected prawn material (Graf et al. 2004). Various other attempted methods of experimental horizontal transmission have proven ineffective; including, contamination of the water with liquefied, centrifuged and filtered infected prawn tissue extract, ingestion of faecal material from ducks fed infected prawn tissue, exposure of prawns to excrement and remaining food portions from infected prawns, and ingestion of live adult artemia previously fed on infected prawn tissue (Graf et al. 2004). Due to successful transmission using infected prawn tissues it has been suggested that IMNV is most likely spread in prawn grow-out ponds by cannibalism (Tang et al. 2005a).

Infectious dose

It was noted that when increasing doses (0.2, 0.4, 0.8, 1.6g) of infected prawn tissue were fed to disease-free prawns, the average mortality rate increased in a linear fashion (Graf et al. 2004). However, the authors concede they had not confirmed, through laboratory analysis, the different viral loads from the various doses of infected prawn tissue fed to the experimental prawns. In other studies, when IMNV infected tissue was fed at 6% body weight for 3 days, 20% infection resulted (Tang et al. 2005a). Further research relating to titre in infected prawn tissues and inoculum will lead to a recommended infectious dose for challenge models used in research (de Andrade et al. 2006).

Prevalence

In a 2004 study of farmed *L. vannamei* located in the Pernambuco State of Northern Brazil, 9 out of 11 farms sampled had at least one pond test positive for IMNV (Pinheiro et al. 2007). No published reports of the prevalence of IMNV in wild prawns were found.

Clinical signs

IMNV infected prawns show focal to extensive areas of muscle necrosis, particularly of the distal abdominal segments and tail fan (Lightner et al. 2004). Affected muscles typically have whitish opaque lesions (Tang et al. 2005a), although white opaque lesions in muscle fibres can be due to other disease agents including non-viral causes, as seen in Australian *P. monodon* (Tang et al. 2005a). In some affected animals, the tail fan may be necrotic and reddened, taking on a cooked appearance (Lightner et al. 2004). Infected animals in time become lethargic (Tang et al. 2005a). Experimentally infected animals develop whitish lesions in the tail muscle after 6 days (Tang et al. 2005a). The onset of gross signs occurs at 6 to 13 days in experimentally infected animals following exposure to IMNV (Tang et al. 2005a). Apparently healthy, chronically infected animals have been detected (OIE 2004).

IMN may present as elevated mortalities during the acute onset phase of disease, but progresses to a chronic disease with low-level persistent mortality, increased feed conversion ratios and associated production losses (Lightner et al. 2004). Mortalities of 40–60% have been reported from infected ponds (Nunes et al. 2004 cited in Tang et al. 2005a). Environmental and physical stressors such as extremes of salinity and temperature, cast net collection and possibly, the feeding of low quality diets have been associated with IMNV outbreaks in vannamei prawns (Lightner et al. 2004).

Pathogenesis

Cytoplasmic inclusion bodies are often formed in IMNV infection (Tang et al. 2005a). IMN-affected *L. vannamei* had coagulative muscle necrosis with oedema, with the disease progressing to liquefactive necrosis with accompanying haemocytic infiltration and fibrosis (Lightner et al. 2004). Significant spheroid formation accompanies lymphoid organ hypertrophy in chronically infected animals in addition ectopic spheroids are not uncommon (Lightner et al. 2004).

Studies on infection of *L. vannamei*, *L. stylirostris* and *P. monodon* showed IMNV infected tissues to include abdominal muscles, lymphoid organs (both tubules and spheroids), hindgut, and phagocytic cells of the hepatopancreas and heart (Tang et al. 2005a, Tang et al. 2005b, Tang et al. 2007). Additionally, gill tissue taken from infected *P. monodon* tested IMNV positive using *in situ* hybridisation (Tang et al. 2005a, Tang et al. 2005b, Tang et al. 2007). Skeletal muscle is the primary target tissue for IMNV, this being proposed as a factor relating to the reduced mortality seen with IMN when compared to WSSV, TSV and YHV, which attack more vital organs of prawns and cause higher mortality within a shorter period (Tang et al. 2005a, Tang et al. 2005b, Tang et al. 2007).

Diagnosis

IMN can be tentatively diagnosed based on gross signs and histological findings. Histopathology of acute phase disease can show perinuclear basophilic inclusion bodies in muscle cells, connective tissue cells, lymphoid organ cells and haemocytes (Lightner 2004). An RT-PCR molecular diagnostic technique has been developed (Lightner 2004, de Andrade et al. 2006) and an RT-PCR kit is commercially available, although the technique has been standardised, it has not been formally validated (OIE 2004). IMNV has been demonstrated in infected tissues using *in situ* hybridisation. The *in situ* hybridisation assay is specific to Brazilian IMNV. Muscle necrosis in farmed prawns from Belize and Australia were negative to Brazilian IMNV based on *in situ* hybridisation (Tang et al. 2005a).

Introduction of IMNV into new countries or areas

Impacts of introduction

Losses due to IMNV infection of vannamei prawns result from both mortality and reduced growth (Lightner et al. 2004). Production losses due to IMNV in north-eastern Brazil in 2003 have been estimated at US$20 million, with 40–60% mortality reported in affected grow-out ponds (Lightner 2004, Lightner et al. 2004, Nunes et al. 2004 cited in Tang et al. 2005a, OIE 2004). Mortality of 40% has also been reported to occur late in the production cycle, causing additional economic losses in surviving prawns due to necrotic tails and associated loss of product quality (Tang et al. 2005a). On the basis of the above estimated losses and an estimated Brazilian prawn production of US$226 million in 2003 (de Paiva Rocha 2004), it can be extrapolated that IMNV caused a financial loss of approximately 9% of national prawn production in 2003. However, as losses are focussed in north-eastern Brazil, the percentage production losses experienced in endemic areas may be somewhat greater. IMNV is not considered to be particularly virulent compared to WSSV, TSV and YHV (Tang et al. 2005a, Tang et al. 2005b, Tang et al. 2007) and only appears to be of significance to *L. vannamei* farmers in north-eastern Brazil and Indonesia. IMNV is of growing concern to vannamei farmers in Thailand and China (Briggs 2006).

Control measures

With the availability of commercial diagnostic kits, the identification and culling of infected animals, such as broodstock, may prove a useful health management tool. The avoidance of environmental and physical stressors may reduce the occurrence of outbreaks of disease in infected animals (Lightner 2004, Lightner et al. 2004). The Regional Advisory Group on Aquatic Animal Health of the Network of Aquaculture Centres in the Asia-Pacific recommend that relevant authorities prevent the use of non-SPF prawns, restrict trans-boundary movement of broodstock, and avoid holding multi-species broodstock together, as positive initiatives in stopping the spread of prawn pathogens (Lightner 2004) such as IMNV.

Necrotising hepatopancreatitis (alpha proteobacterium)

Necrotising hepatopancreatitis (NHP) is a bacterial disease that has caused mortalities in prawn grow-out farms in the Americas. NHP has also been known as Texas NHP, Texas pond mortality syndrome, Peru NHP and granulomatous hepatopancreatitis (Frelier et al. 1992, Lightner and Redman 1994). NHP has been recommended to be added to the OIE list of diseases (OIE 2006c).

Agent taxonomy

The aetiological agent of NHP is a pleomorphic, Gram-negative, intracytoplasmic bacterium (Krol et al. 1991, Frelier et al. 1992, Lightner et al. 1992c, Lightner and Redman 1994). The NHP-bacterium (NHPB) is a member of the α-subclass of proteobacteria and remains unclassified (Loy et al. 1996b). It is closely related to, but distinct from, the rickettsia and rickettsial-like organisms known to affect penaeid prawns (Loy et al. 1996b). The two main morphological forms of the NHPB are a small pleomorphic, round to rod shaped form without flagella, and a longer helical rod with several flagella (Krol et al. 1991, Frelier et al. 1992, Lightner et al. 1992c, Lightner and Redman 1994).

Genetic analysis of the NHPBs associated with North and South American outbreaks of NHP suggest that the isolates are either identical or very closely related sub-species (Loy et al. 1996a).

Agent stability

NHP bacterium in tissues frozen at –70°C was shown to retain infectivity in experimental transmission trials with *Litopenaeus vannamei* (Frelier et al. 1993). Experimental *per os* transmission with NHPB relies on *in vivo* propagation of the organism (Crabtree et al. 2006) and would benefit from an *in vitro* culture source of the agent, however, successful attempts to culture NHPB *in vitro* have not been reported (Crabtree et al. 2006). Another problem associated with experimental transmission is the collection of large amounts of material required for success in *per os* challenge studies (Crabtree at el. 2006, D. Lightner, Arizona University, pers. comm. March 2007). This has led to the development of a suitable freezing technique to store viable pathogenic NHPB. Lightner (Arizona University, pers. comm. March 2007) has found infectivity with NHPB to be highly variable following freezing at –80°C, possibly due to the freeze/thaw method adopted. Crabtree et al. (2006) have presented a flash freezing method to –80°C that is capable of maintaining infectivity of NHPB no different to fresh tissue. It is clear that NHPB is highly sensitive to freezing (D. Lightner, Arizona University, pers. comm. March 2007) and requires specially developed fast freezing techniques to –80°C to maintain infectivity. NHPB can remain infectious in chilled prawns for up to 2 days (D. Lightner, Arizona University, pers. comm. March 2007). Although distinct from this NHPB, a Canadian rickettsia-like organism retained infectivity in prawn tissues after freezing at –10°C for at least 10 days (Bower et al. 1996).

Epidemiology

Host range

NHP has only been reported in farmed penaeid prawn populations (from postlarvae to adults). Based on histopathological and ultrastructural examinations, natural outbreaks of disease have been reported in *L. vannamei* (Krol et al. 1991), *Litopenaeus stylirostris* (Lightner and Redman 1994) and *Farfantepenaeus aztecus* (Frelier et al. 1994). NHP has also been seen in *Farfantepenaeus californiensis* and *Litopenaeus setiferus* (Lightner and Redman 1994, Lightner 1996a).

NHP has been experimentally induced in both *L. vannamei* (Frelier et al. 1993, Frelier et al. 1994, Vincent et al. 2004) and *L. setiferus* (Frelier et al. 1994). *Litopenaeus setiferus* is reportedly less susceptible to disease than *L. vannamei* (Frelier et al. 1994). Vincent et al. (2004) confirmed NHPB infection in *L. vannamei* by PCR and histology.

Geographical distribution

NHP was first reported in Texas in 1985 (Johnson 1989 cited in Frelier et al. 1992). The disease has since been reported from many prawn-producing countries in Central and South America (Lightner and Redman 1994, Lightner 1996a, Loy et al. 1996b, Brinez et al. 2003). Prawn aquaculture on the Pacific coast of Mexico has reported NHP since 1999 in *L. vannamei* and *L. stylirostris*. An outbreak of NHP in *L. vannamei* has also been confirmed in Campeche, southern Mexico (del Rio-Rodriguez et al. 2006). NHPB was also observed in an importing facility in Eritrea (northeast Africa) where the infection was found in *L. vannamei* imported from Mexico (OIE 2005b, OIE 2006c). Thailand and Indonesia are also reported to have NHPB in farmed prawns (Briggs 2006). NHPB is exotic to Australia.

Transmission

Natural transmission of NHP is thought to occur by cannibalism (Frelier et al. 1994, Vincent et al. 2004), although cohabitation and dissemination of NHPB via the water column may also play a role (Frelier et al. 1994). NHPB from faeces shed into the pond environment has also been suggested as a possible source of transmission (Vincent and Lotz 2005). Outbreaks of disease are often preceded by prolonged periods of high water temperature (approximately 30ºC) and water salinity greater than 20 ppt (normal seawater is 35 ppt salt) (Frelier et al. 1992, Frelier et al. 1993, Lightner and Redman 1994).

The results of experimental transmission trials appear consistent with reports of naturally occurring disease. Studies attempting to transmit NHP to non-SPF (specific pathogen-free) animals by ingestion or cohabitation conducted at temperatures below 30°C were unsuccessful (Frelier et al. 1993). Similarly, NHP infection could not be demonstrated when farm prawns were held at low salinity (below 15 ppt) (Frelier et al. 1994). However, when the animals were placed under higher temperature (30ºC) and marine salinity greater than 20 ppt, NHPB was successfully transmitted to non-SPF *L. vannamei* (Frelier et al. 1994) and SPF *L. vannamei* (Vincent et al. 2004). It is not known whether water temperature and salinity affect the transmission of NHPB, the expression of NHP, or both. NHPB DNA has been detected by PCR in samples of water but not in samples of sediment, plankton or invertebrates collected from an affected pond (Varner and Frelier 1998). A subclinical carrier state of NHPB infection is not known to exist — all prawns that become infected with the agent are seen to be either dying or dead (Vincent et al. 2004).

Tissue titre

Prior to the advent of molecular quantification techniques, NHBP titre in infected prawns was difficult to determine due to problems associated with the successful *in vitro* culture of alpha-proteobacteria. Real-time PCR has since been employed to quantify the unculturable bacteria from infected prawn tissues. Following experimental *per os* infection, NHPB numbers in live infected prawns were extrapolated as 10^3–10^7 copies/mg of hepatopancreas and 10^1–10^5 copies/mg of faeces. Lethal amounts of NHBP were extrapolated as 10^6–10^7 copies/mg of hepatopancreas and 10^3–10^6 copies/mg of faeces (Vincent and Lotz 2005).

Infectious dose

Little data are available on the infectious dose of the NHPB under a range of natural 7g respectively) with 0.05g of hepatopancreas taken from diseased prawns (Vincent et al. 2004) conditions. NHP has been induced after feeding susceptible *L. vannamei* (mean weight 5.6 g and 5.al. 2004, Vincent and Lotz 2005). Sixty percent of animals in one trial (n=20), and 20%

of animals in another (n=120), were infected at 60 days post-exposure, as determined by PCR (Vincent et al. 2004). Failure to infect 100% of animals may be attributed to dose.

Prevalence

Few studies have investigated the prevalence of NHP in farmed prawns, however, NHP is known to cause mortalities of up to 90% in affected grow-out ponds (Lightner 1993). Research shows that most farms in an affected area suffer from the disease, and on one farm, 13 out of 30 ponds were affected. Histological changes consistent with NHP were also found in 46% of animals from one farm experiencing mortality and in 50% of animals from another (Frelier et al. 1992). Histological studies, including electron microscopic examination, on prawns from another farm found evidence of the NHPB in approximately 27% of the animals examined (Frelier et al. 1993).

During an outbreak in Columbia, 100% of animals sampled had histological lesions consistent with NHP. Of these, NHPB DNA was detected in 56% of the animals using both *in situ* hybridisation and PCR (Brinez et al. 2003). It should be noted that the estimated prevalence of infection may vary with the diagnostic method used.

Wild *L. setiferus* and *Farfantepenaeus duorarum* from the Gulf of Mexico in the vicinity of coastal prawn farms along the Yucatan and Campeche coast have been surveyed with no histological evidence of NHP (del RioRodriguez et al. 2006).

Clinical signs

Infection and disease have been reported in life stages from postlarvae to adults, in both grow–out ponds and broodstock rearing facilities (Krol et al. 1991, Frelier et al. 1992, Lightner and Redman 1994, Brinez et al. 2003).

Individuals with NHP may show many non–specific signs of disease including lethargy, blackened gills, inappetence, poor growth, softening of the shell, chromatophore expansion causing pleopod edge darkening, an increase in secondary bacterial infections and epicommensal fouling, and mortality (Frelier et al. 1992, Lightner and Redman 1994, Vincent et al. 2004). Affected animals may also show whitening and atrophy of the hepatopancreas (Krol et al. 1991, Frelier et al. 1992, Lightner and Redman 1994).

NHP typically occurs within the first three months after stocking of postlarvae in grow-out ponds, but has also been reported from broodstock populations (Krol et al. 1991, Frelier et al. 1992, Lightner and Redman 1994, Brinez et al. 2003). Cumulative mortalities vary from about 20% to nearly 100% in affected populations, typically within 30 days of the onset of clinical signs (Krol et al. 1991, Frelier et al. 1992, Frelier et al. 1993). Outbreaks have been associated with prolonged periods of elevated water temperature (approximately 30ºC) and high salinity (20 – 40 ppt) (Frelier et al. 1992, Frelier et al. 1993, Lightner and Redman 1994).

Pathogenesis

The mechanisms of pathogenesis of NHPB are not fully understood (Vincent and Lotz 2005). NHPB infects the epithelial cells of the hepatopancreatic tubules (Krol et al. 1991, Frelier et al. 1992). As infection progresses, cellular hypertrophy, necrosis and sloughing may be seen (Frelier et al. 1992). During the course of infection there is an increase in the number of mature hepatopancreatic cells containing NHPB. Bacterial multiplication and spread may be associated with increased tissue damage (Vincent and Lotz 2005). There are two stages of infection following experimental exposure of juvenile *L. vannamei* to infected prawn tissues.Firstly, the pre-patent period which lasted approximately 15 days post-exposure and was characterised by a period of asymptomatic disease and mortality rates similar to uninfected control prawns. Secondly, the acute stage which was typified by disease and mortality, commencing approximately 16 days post-exposure and continuing for approximately 35 days (Vincent et al. 2004). At the end of the trials at 60 days post-exposure,

no surviving animals were found to have been infected as determined by histological examination of hepatopancreatic tissues and by PCR. The authors concluded that a carrier state does not exist in NHPB infections, and that all infected animals die (Vincent et al. 2004).

Diagnosis

A presumptive diagnosis of NHP may be made by examination of wet mounts of the hepatopancreas of affected animals, or by histopathology (Lightner 1996a). The hepatopancreas may appear pale with black streaks and/or soft and watery. Diagnosis can be confirmed by *in situ* hybridisation, transmission electron microscopy and PCR (Krol et al. 1991, Frelier et al. 1992, Lightner and Redman 1994, Loy et al. 1996a, Loy et al. 1996b, Brinez et al. 2003). A monoclonal antibody has been produced and used in an immunohistochemistry technique for the rapid detection of NHPB (BradleyDunlop et al. 2004). Because NHPB is difficult to culture, standard bacterial culture confirmation is not used for diagnostic purposes (Lightner 1996a).

Introduction of NHP into new countries or areas

Mechanism of disease spread

Under appropriate environmental conditions, transmission of NHP has been achieved by feeding (*per os*) infected hepatopancreatic tissue to susceptible animals (Frelier et al. 1994). The most effective technique of experimental infection is by feeding infected prawn tissue from prawns that have had NHPB passaged through generations of live SPF animals (Vincent et al. 2004).

Impacts of introduction

Data on the impacts attributable to NHP are scarce; however, a farm in Texas which recorded NHP for the first time during late 1980s reportedly discontinued prawn farming as a result (Frelier et al. 1992). Following outbreaks of NHP in Peru in 1993, it was reported that about half of the country's active prawn farms (44 in 1993) closed (Lightner and Redman 1994). The Central American epidemic of NHP in 1993 was attributed to drought as pond water pond water temperatures were 29–35°C and salinities 30–38 ppt (Lightner and Redman 1994). In Eritrea, within a year of NHP introduction to the country, severe losses had destroyed all animals in the importing facility (OIE 2004).

Control measures

A number of measures are reportedly useful in the control of NHP. The identification and culling of infected animals such as broodstock may aid in preventing the spread of NHP to the hatchery (Brinez et al. 2003). Conditions such as high water temperature and salinity which may predispose animals to the disease should be avoided. The use of medicated feed early in the disease course has also proven beneficial (Frelier et al. 1992, Frelier et al. 1994, Lightner and Redman 1994). When infected prawns were given medicated feed, production volume increased by approximately five times compared to the production volumes in the previous two years when medicated feeds were not used (Frelier et al. 1994). Oxytetracycline added to the feed during processing at a rate of 2–4 kg per metric tonne and fed for 14 days to animals in infected grow-out ponds is reportedly effective provided medication commences before the prawns cease feeding (Lightner and Redman 1994). Medicated feeds have been used prophylactically to maintain low prevalence on severely affected farms (Lightner and Redman 1994). However, control with medicated feeds is not always effective (OIE 2005b). Standard disinfection treatments for water used in hatcheries appears to eliminate the bacteria, as only animals in untreated water pumped from the NHP affected area resulted in disease (Frelier et al. 1994).

Rickettsia-like organisms

A number of rickettsia-like organisms (RLOs) have been associated with disease in aquatic crustaceans, with at least two found infecting prawns. However, as prawns infected with RLOs often have concurrent infections with other agents (be it viral or bacterial), the significance of RLOs as causative bacterial agents of disease is not always clear.

Agent taxonomy

RLOs reported from prawns are Gram-negative bacteria and are obligate intracellular intracytoplasmic microorganisms (Brock et al. 1986b, Anderson et al. 1987, Bower et al. 1996, Nunan et al. 2003a). As with the majority of crustacean RLOs, the prawn RLOs have yet to be isolated and characterised and as such, their taxonomic relationships are unclear. There are at least two RLOs capable of infecting prawns, one infects the epithelial cells of the hepatopancreatic tubules (Chong and Loh 1984, Brock et al. 1986b, Lightner 1996a, Vogt and Štrus 1998), the other results in a systemic infection involving tissues of mesodermal and ectodermal origin (Anderson et al. 1987, Lightner et al. 1992b, Bower et al. 1996, Nunan et al. 2003b). Most prawn RLOs are rod to comma-shaped, 0.2–0.7µm in diameter and 0.9–1.6µm in length (Brock et al. 1986b, Anderson et al. 1987, Nunan et al. 2003b). In contrast, a prawn RLO reported from Canada was monomorphic and spherical (Bower et al. 1996).

Agent stability

Through its use in a successful bioassay (by ingestion), Bower et al. (1996) found the Canadian RLO retained infectivity after freezing in prawn tissues at –10°C for at least 10 days. Hepatopancreatic RLO was infectious via *per* os challenge following storage of the agent in prawn tissues at –70°C (Brock et al. 1986b).

No quantitative data on the survival of prawn RLOs free in seawater are available, although Bower et al. (1996) found the Canadian RLO could be transmitted to *Pandalus platyceros* by immersion in effluent water from tanks containing infected *P. platyceros.* Similarly, RLOs infecting crayfish (Tan and Owens 2000) and fish (Lannan and Fryer 1994) have been reported to survive extracellularly. Survival of the crayfish RLO in water was not quantified (Tan and Owens 2000), however, in a semi-purified suspension, *Piscirickettsia salmonis*, a salmon pathogen, reportedly survived for up to 15 days in seawater held at 5°C (Lannan and Fryer 1994).

Epidemiology

Host range

Natural infections with hepatopancreatic prawn RLOs have been reported from farmed *Fenneropenaeus merguiensis*[51] (Chong and Loh 1984, Lightner et al. 1992b), *Litopenaeus vannamei* (Lightner 1996a), wild-caught *Melicertus marginatus* (Brock et al. 1986b) and *Palaemon elegans* (Vogt and Štrus 1998). Natural infection with a hepatopancreatic RLO in farmed *Macrobrachium rosenbergii* larvae has also been reported (Cohen and Isaar 1990).

Natural infections with systemic prawn RLOs have been detected in farmed *Penaeus monodon* (Anderson et al. 1987, Lightner et al. 1992b, Nunan et al. 2003b). In one case, cohabiting *Fenneropenaeus indicus* and *F. merguiensis* were unaffected. The study did not investigate whether these species were infected but reported that they were not overtly diseased (Anderson et al. 1987). A naturally occurring, systemic RLO-infection in wild

[51] The initial report did not specify whether *F. indicus* or *F. merguiensis* had been sampled in the study; however, subsequent literature uniformly refers to the RLO as having been detected in *F. merguiensis*.

P. platyceros has been reported (Bower et al. 1996).

Litopenaeus stylirostris have been infected by a hepatopancreatic RLO from *M. marginatus* (Brock et al. 1986a).

Primary lymphoid cell cultures from *Fenneropenaeus chinensis* were reportedly infected with RLO, however, the source of infection was unclear (Wang et al. 2001).

Natural outbreaks of disease have been reported in *Macrobrachium rosenbergii* larvae (Cohen and Isaar 1990), *P. monodon* juveniles (Anderson et al. 1987) and *P. monodon* adults (Nunan et al. 2003b).

RLOs have also been reported from a variety of other aquatic crustacean species including crabs and crayfish (Ketterer et al. 1992, Owens et al. 1992b, Jimenez and Romero 1997, Edgerton and Owens 1999b, Wang and Gu 2002). The relationship between the RLOs in prawns and those in other aquatic crustacean species is unknown.

Geographical distribution

Hepatopancreatic prawn RLOs have been reported in Singapore (Chong and Loh 1984), Hawaii (Lightner 1996a), Brazil (Cohen and Isaar 1990), Mexico (Lightner 1996a), Mediterranean sea (Vogt 1994) and Adriatic Sea (Vogt and Štrus 1998). Systemic prawn RLOs have been reported in Malaysia (Anderson et al. 1987), Indonesia (Lightner et al. 1992b), Madagascar (Nunan et al. 2003b) and Canada (Bower et al. 1996).

RLO-infected *F. chinensis* lymphoid cell culture was reported from China (Wang et al. 2001). RLOs have not been reported in wild or cultured prawns from Australia, however they have been isolated from farmed Australian freshwater redclaw crayfish, *Cherax quadricarinatus* (Owens et al. 1992b, Edgerton et al. 1995 cited in Edgerton and Owens 1999b).

Transmission

Experimental transmission of prawn RLOs has been achieved by feeding infected tissues to susceptible animals (Brock et al. 1986b, Bower et al. 1996). However, in one experiment, juvenile *L. vannamei* fed naturally infected adult *P. monodon* tissues did not become infected (Nunan et al. 2003a). Transmission may have failed due to the age of the experimental animals, dose received, environmental conditions during the trial or possibly due to the lack of an intermediate host or vector (Nunan et al. 2003a). Experimental transmission of a prawn RLO has also been achieved by exposing susceptible animals to effluent water (screened to 1mm) from infected populations (Bower et al. 1996), cannibalism (Bower et al. 1996) and by intramuscular injection of infected tissue homogenate (Nunan et al. 2003a). Bower et al. (1996) It has been suggested that natural reservoir hosts are likely to exist (Bower et al. 1996).

Tissue titre

There is little information available on the number of the RLOs present in infected prawns. Qualitatively, RLO microcolonies were evident in approximately 20–30% of the hepatopancreatic epithelial cells of infected wild-caught prawns. In contrast, microcolonies were seen in almost all hepatopancreatic epithelial cells of animals experimentally infected with the same RLO (Brock et al. 1986b).

Infectious dose for prawns

No quantitative data is available on the dose used in successful experimental transmission trials using prawn RLOs. A RLO reported from Australian redclaw (*C. quadricarinatus*) has been experimentally transmitted to other redclaw by ingestion of infected tissues, immersion in a suspension of infected tissue homogenate and injection of an infected tissue homogenate (Tan and Owens 2000). Data on the titre of RLO in infected redclaw and on its infectious dose was not reported.

Prevalence

In Malaysia, RLO infections were detected in prawns from 2 out of 3 ponds sampled for testing after the farm reported recurring mortalities. RLO microcolonies were observed in 80% and 66.6% of prawns sampled from the two infected ponds (Anderson et al. 1987).

The prevalence of RLO infections in wild penaeid populations is largely unknown. Ten percent of wild *M. marginatus* juveniles caught off Hawaii and maintained in captivity for 30 days tested positive for the presence of RLO infection (Brock et al. 1986b). The prevalence of RLO related disease (stained prawn disease-SPD) in *P. platyceros* in Canada was reported to range from 4–15% during 1990 and 1991 (Bower et al. 1996).In approximately 90% of the samples the prevalence of RLO was 5% or less. The prevalence of RLO in *P. elegans* sampled from the Adriatic Sea was 29% (Vogt and Štrus 1998).

Clinical signs

Individuals

Clinical signs associated with RLO infections include lethargy, lack of appetite, poor growth, erratic swimming, congregation near the pond edge, delayed escape response, whitening with or without atrophy of the hepatopancreas and death (Brock et al. 1986b, Anderson et al. 1987, Cohen and Isaar 1990, Nunan et al. 2003b). However, many RLO infections are asymptomatic (Chong and Loh 1984, Vogt and Štrus 1998). Wild prawns infected with the Canadian RLO (SPD) show black stippling of the hepatopancreas and dark staining of the cuticle (Bower et al. 1996).

Populations

As with infected individuals, infected populations may remain asymptomatic. However, significant mortality has been reported from farmed *P. monodon* populations approximately 2–3 months after stocking in grow-out ponds (Anderson et al. 1987, Nunan et al. 2003b). In one case, subsequent production in the affected population was markedly reduced (Anderson et al. 1987). In experimental infections, Canadian RLOs were not detected in *P. platyceros* until 38 days post-exposure (Bower et al. 1996). Mortalities ranging from 40% to 95% have been reported from *M. rosenbergii* larvae in a commercial hatchery (Cohen and Isaar 1990). A RLO has also been associated with a mortality event in farmed *L. vannamei* (Lightner et al. 1992b).

Pathogenesis

In hepatopancreatic RLO infections, the cells lining hepatopancreatic tubules are infected. RLO microcolonies expand in the cytoplasm of host cells resulting in cellular hypertrophy and rupture (Brock et al. 1986b, Vogt 1994). It is thought that the RLOs are subsequently discharged via the digestive tract of the host animal (Vogt 1994).

In systemic RLO infections, the principal target tissues are of mesodermal and ectodermal origin such as the lymphoid organ, connective tissues, haemocytes, fixed phagocytes and cuticular epithelial cells (Anderson et al. 1987, Nunan et al. 2003b). In stained prawn disease (SPD) the RLO has an affinity for fixed phagocytes and haemocytes of the host (Bower et al. 1996).

Diagnosis

Diagnosis of RLO-infection is normally by standard histology, with confirmation by transmission electron microscopy (Brock et al. 1986b, Anderson et al. 1987, Bower et al. 1996, Nunan et al. 2003b). A PCR diagnostic assay and an *in situ* hybridisation technique have been developed for a systemic RLO from *P. monodon* (Nunan et al. 2003b).

Introduction of RLOs into new countries or areas

Mechanism of disease spread

The success of experimental infection trials where infected tissues were fed to susceptible animals and the ability of some RLOs to survive freezing suggests that transmission of RLO via non-viable prawns and prawn products is possible (Brock et al. 1986a, Bower et al. 1996).

Impacts of introduction

Published data on the effect of RLOs on national production figures are unavailable. As RLOs are frequently seen in association with multiple concurrent infections in prawns, it may be difficult to ascribe production losses to these agents individually.

The impacts of RLOs in wild prawn populations remain unknown. It has been estimated that survival rates were reduced in one wild population with a higher prevalence of the Canadian RLO. While the authors considered the reduced survival of the local *P. platyceros* population could be ascribed to the increased presence of the RLO, a causal association could not be proven (Bower et al. 1996).

Control measures

Some prawn RLOs may be species-specific. In one outbreak of systemic RLO infection in Malaysian *P. monodon*, cohabiting *F. indicus* and *F. merguiensis* were apparently unaffected (Anderson et al. 1987). Although, this paper does not report whether the *F. indicus* or *F. merguiensis* were infected with the RLO, just that they were not apparently diseased. The farm subsequently switched to production of *F. merguiensis* and reportedly had no recurrence of the disease (Anderson et al. 1987). The production of different prawn species may therefore be a control option following an outbreak of RLO-associated mortality. However, it should be noted that although the RLO was considered to be significantly associated with the disease, affected *P. monodon* had multiple concurrent infections (Anderson et al. 1987). As such, it may be that the lack of disease in *F. indicus* and *F. merguiensis* reflected a different set of agent interactions in these animals rather than a resistance to the RLO.

The use of medicated feed has also been reported to reduce both clinical signs and mortality rates due to RLOs in *M. rosenbergii* (Cohen and Isaar 1990). The addition of lime to the culture water was also associated with a decrease in mortalities (Cohen and Isaar 1990). Preventing the introduction of RLO through quarantine and screening of potential carriers, the destruction of infected stock and disinfection of contaminated areas are the preferred means of controlling prawn RLOs (Lightner 1996a). It has been suggested that pathogens such as RLOs are always present in the environment, but are only a potential disease threat when poor nutritional and environmental conditions conspire to increase pathogenicity (Vogt and Štrus 1998).

Vibrio penaeicida

Vibrio penaeicida is a highly pathogenic bacterium associated with significant disease and mortality in *Marsupenaeus japonicus* in grow-out ponds in Japan (Takahashi et al. 1985a), and *Litopenaeus stylirostris* in grow-out ponds and broodstock tanks in New Caledonia (Costa et al. 1996, Costa et al. 1998b, Goarant et al. 1998), *V. penaeicida* is exotic to Australia.

Agent taxonomy

Previously referred to as *Vibrio* sp. PJ (PJ: *Penaeus japonicus*) (de la Peña et al. 1993), *V. penaeicida*, is a Gram-negative, facultatively anaerobic, motile, slightly curved rod bacterium (Ishimaru et al. 1995, Takahashi et al. 1985a). There are two known clusters of strains (Goarant et al. 1999) based upon geographic isolation. The first group from Japan (Ishimaru et al. 1995) and the second from New Caledonia (Costa et al. 1998b).

Agent stability

There is little species-specific information on the stability of *V. penaeicida*.

At both 10°C and 20°C, free *V. penaeicida* survived for more than a year in seawater, but less than 1 hour in freshwater (de la Peña et al. 1993). Unpublished data indicated that the number of viable *V. penaeicida* grown in artificial seawater free of organic matter, declined after 2 days of incubation at 25–30°C (unpublished results, Saulnier cited in Saulnier et al. 2000a).

Information on the stability of other *Vibrio* species may also be applicable to *V. penaeicida*. Vanderzant and Nickelson (1972) found that heating at 100°C for one minute was sufficient to kill *V. parahaemolyticus* in prawn homogenates. Heating at 65°C for 10 minutes was sufficient to kill *V. cholerae* (Nascumento et al. 1998). Furthermore, *V. cholerae* could not be recovered from crayfish homogenate (originally containing 10^7 colony-forming units) after heating at 66°C for 9.5 minutes (Grodner and Hinton 1985).

A number of studies have also investigated the heat stability of *Vibrio* species in whole animals or meat. Cook and Ruple (1992) reported a 4-log reduction in the levels of *V. vulnificus* in oyster meats, to below the level of detection, after heating at 50°C for 10 minutes. *V cholerae* (10^6 cells) injected into whole crayfish (weighing 18–25g) was totally inactivated when the crayfish were cooked in boiling water (100°C) for 5 minutes (Grodner and Hinton 1985). For larger specimens such as crabs (weighing 294–347g), boiling at 100°C for 15 minutes was required to achieve a 7-log reduction of *V. cholerae* (Schultz et al. 1984). It was concluded that the core temperature of the crabs needed to reach 71°C for one minute for the accumulated lethality over the entire cooking process to adequately pasteurize and therefore inactivate the *V. cholerae* (Schultz et al. 1984).

Freezing may result in a reduction in the number of viable *Vibrio* cells, but is ineffective at killing the bacteria completely. Freezing at –18°C for 8 days resulted in a 2 log reduction in the number of *V. parahaemolyticus* present in whole prawns, but did not eliminate the bacteria completely (Vanderzant and Nickelson 1972). Freezing at –20°C resulted in approximately a 6-log reduction in the number of *V. cholerae* present in *Litopenaeus schmitti* (Nascumento et al. 1998). Reduction in bacterial numbers took longer in prawns with their carapace still attached (approximately 36 days) than in prawns whose carapace had been removed (approximately 26 days) (Nascumento et al. 1998).

Similar results have been found in studies investigating the effect of chilling on the viability of *Vibrio* species. Storage between 3°C and 10°C for 8 days resulted in a 1–2-log reduction in the numbers of *V. parahaemolyticus* in whole prawns (Vanderzant and Nickelson 1972). Storage at between –1.9°C and 0°C for 6 days resulted in a 3 to 4-log reduction in the numbers of *V. vulnificus* in oyster meats (Cook and Ruple 1992). *V. cholerae* in crabmeat remained viable at 5°C for 35 days (Guthrie et al. 1985 cited in Nascumento et al. 1998).

Epidemiology

Host range

Natural infections with *V. penaeicida* have been reported in cultured juvenile and adult *M. japonicus* in Japan (Takahashi et al. 1985a) and in *L. stylirostris* in grow-out ponds and broodstock in New Caledonia (Costa et al. 1998b, Goarant et al. 1998).

Litopenaeus vannamei (AguirreGuzman et al. 2005,)and *Fenneropenaeus indicus* (Avarre et al. 2003) are reportedly susceptible to experimental infection with *V. penaeicida*.

Two studies have examined the susceptibility of early life-stages to experimental infection with *V. penaeicida* by immersion in a bacterial suspension. One study found that susceptibility of *L. stylirostris* to the New Caledonian strain of *V. penaeicida* did not begin until the ninth post-larval stage (Goarant et al. 1998). In contrast, a Japanese strain of *V. penaeicida* produced significant mortality in larval life stages of *L. vannamei* (Aguirre-Guzman et al. 2001). It is unclear whether these contrasting results were due to differences in dose, bacterial strain or host species-susceptibility.

V. penaeicida is not known to infect humans.

Geographical distribution

V. penaeicida has been reported in Japan (Takahashi et al. 1985a) and New Caledonia (Costa et al. 1998b).

Transmission

Natural transmission of *V. penaeicida* is thought to occur either by ingestion of infected material (cannibalism) (de la Peña et al. 1998) or by exposure to water-borne bacteria (AguirreGuzman et al. 2001). Vertical transmission has not been demonstrated.

V. penaeicida is considered ubiquitous in the prawn culture environment in affected areas in Japan. It has been detected in water samples from ponds containing infected prawns (Ishimaru et al. 1995) and in rare instances from pond sediments (de la Peña et al. 1992). Asymptomatic carrier prawns may also act as reservoirs of infection (de la Peña et al. 1992, de la Peña et al. 1997, Nakai et al. 1997).

Experimental transmission of *V. penaeicida* has been achieved by immersion (Egusa et al. 1988, Le Moullac et al. 1997, de la Peña et al. 1998), oral intubation (de la Peña et al. 1998), ingestion (de la Peña et al. 1998) and intramuscular injection (de la Peña et al. 1992, Costa et al. 1998a).

Transmission studies suggest the gills and the lymphoid organ are natural routes of entry for *Vibrio* sp. (Muñoz et al. 2004). Furthermore, it has been reported that the gastro-intestinal tract, the epidermis or wounds may also be portals of entry for *V. penaeicida* (de la Peña et al. 1998).

Tissue titre

In *M. japonicus* (mean weight 17 grams) challenged with 10^4 CFU of *V. penaeicida* via oral intubation (10^4 CFU/prawn) the numbers of bacteria in tissues increased 24–48 hours post-inoculation (de la Peña et al. 1995). 48 hours post-inoculation the concentration of *V. penaeicida* ranged from 10^2 to 10^7 CFU/g of hepatopancreas, stomach, haemolymph, lymphoid organ, muscles and gills (de la Peña et al. 1995). Following challenge of *M. japonicus* (mean weight 14g) via intramuscular injection with 10^6 CFU/animal of *V. penaeicida* bacteria were detected 36 hours post-inoculation in the hemolymph (10^5 CFU/ml), lymphoid organ (10^6 CFU/g) and muscle (10^6 CFU/g) (de la Peña et al. 1998).

Infectious dose

In a study reporting on *per os* experimental challenge with *V. penaeicida*, experimental animals (*M. japonicus* 10–12g) were fed material (either prawn meat or commercial pellets) inoculated with *V. penaeicida* (10^7 CFU/g) twice daily for 3 days at a rate of 4–5% bodyweight per day (de la Peña et al. 1998). Mortality resulted in trial animals but not in the control groups, although confirmation of infection in trial animals was not reported (de la Peña et al. 1998).

Experimental infections have been induced by exposure of *L. stylirostris* juveniles to suspensions of *V. penaeicida* containing 10^5 CFU/ml for 1 hour (Le Moullac et al. 1997) and *L. vannamei* larvae to 10^3, 10^5 or 10^7 CFU/ml for 30 minutes (AguirreGuzman et al. 2001). However, confirmation of infection in the experimental animals was not reported. Furthermore, *L. stylirostris* juveniles (Saulnier et al. 2000b) and *F. indicus* (mean weight) (Avarre et al. 2003) have been experimentally infected with *V. penaeicida* by exposure to 10^4 CFU/ml for 2 hours, infection was confirmed by PCR.

The LD_{50} of *M. japonicus* (13–22g) challenged with a Japanese strain of *V. penaeicida* by intramuscular injection was 10^2–10^3 CFU/prawn (de la Peña et al. 1993). The infection was confirmed by re-isolation of *V. penaeicida* from infected prawns. In contrast, the LD_{50} following intramuscular injection of *L. stylirostris* (10–14g) with a New Caledonian strain of *V. penaeicida* was estimated at less than 5 CFU/prawn (Saulnier et al. 2000b). Positive PCR signal confirmed infection in this study. It is unclear whether the differences in LD_{50} between these two studies results from differences in strain of *V. penaeicida*, size and/or species of experimental animal, or the method used to confirm infection.

Prevalence

Few studies have reported the prevalence of *V. penaeicida* in farmed prawn populations. Four ponds of healthy *M. japonicus* from three farms in Japan were sampled monthly from May to December during 1990. The prevalence of *V. penaeicida* (detected by bacterial culture) in these samples ranged from 0–80%, but was typically 10–30% (de la Peña et al. 1992). In the same study prawns from two ponds were sampled monthly for 3 months in 1991, and examined using both bacterial culture and IFAT. On the basis of culture results, the prevalence of *V. penaeicida* prevalence ranged from 0–10% (typically 3–10%), and 0–7% (typically 3–7%) using IFAT (de la Peña et al. 1992).

In a 1996 study of farmed, healthy *M. japonicus* (10–20 grams) *V. penaeicida* was detected in 0–20% of animals by bacterial culture and 3–20% by RT-PCR (Naki et al. 1997).

Prevalence data for *V. penaeicida* in wild prawn populations has not been reported.

Clinical signs

The clinical signs of disease due to infection with *V. penaeicida* are similar whether infection is natural or experimental (Costa et al. 1998a). Diseased prawns present with soft, dark shells and may have opaque musculature and brown spots in the gills and lymphoid organ (Takahashi et al. 1985a, de la Peña et al. 1993, Costa et al. 1998b, Mermoud et al. 1998). Moribund animals may swim erratically near the surface and close to the pond edge and appear lethargic and weak (Costa et al. 1998b, Mermoud et al. 1998). Affected animals are often moulting (Mermoud et al. 1998). *L. vannamei* larvae surviving experimental challenge were variably malformed (AguirreGuzman et al. 2001). Infected animals may also be asymptomatic (de la Peña et al. 1992, de la Peña et al. 1997, Nakai et al. 1997, Saulnier et al. 2000b).

Early signs of disease in populations affected by *V. penaeicida* may include reduced growth rates and increased food conversion rates (Mermoud et al. 1998). Disease may be either chronic or acute and as such, mortality rates may vary considerably between affected ponds (Costa et al. 1998a, Mermoud et al. 1998). Acute episodes are typically preceded by stress,

such as water temperature fluctuations, deterioration of the pond environment or transport stress. The disease is more common in summer and fall (de la Peña et al. 1992, de la Peña et al. 1993, de la Peña et al. 1997, Costa et al. 1998a, Costa et al. 1998b).

Pathogenesis

Experimental infection with *V. penaeicida* results in systemic bacterial infection(de la Peña et al. 1995, Costa et al. 1998b, Avarre et al. 2003). Mortalities commence approximately 48 hours following the introduction of *V. penaeicida* into the stomach of *M. japonicus* via oral intubation (de la Peña et al. 1995).

The ability of prawns to resist *V. penaeicida* infection is closely related to the health status of the prawn haematopoietic process, as response to infection involves substantial activation and haemocyte recruitment, and subsequent isolation of the bacteria by the hemocytes (Munoz et al. 2004).

Experimental infection has also shown that there is considerable variation in the pathogenicity of different *V. penaeicida* strains (KH-1 and AM101). Juvenile *L. vannamei* (1–1.2g) exposed to *V. penaeicida* AM101-strain (10^4 CFU/ml) displayed higher mortality than prawns exposed to the KH-1-strain, 120 hours post-exposure. An exotoxin (protease) secreted by *V. penaeicida* AM101 has been suggested as a primary virulence factor (AguirreGuzman et al. 2005).

Diagnosis

A presumptive diagnosis of *V. penaeicida* can be made on the basis of clinical signs and light microscopy (Takahashi et al. 1985a, Costa et al. 1998b, Mermoud et al. 1998). A confirmatory diagnosis should be made using conventional culture methods and physiological and biochemical characterisation (de la Peña et al. 1993, Ishimaru et al. 1995); however, it may be difficult to isolate *V. penaeicida* if large numbers of other bacteria are present (Nakai et al. 1997). PCR and RT-PCR assays have been developed to detect *V. penaeicida* in prawns and environmental samples (Genmoto et al. 1996, Nakai et al. 1997, Saulnier et al. 2000b). Antibody tests such as IFAT have also been developed (de la Peña et al. 1992).

Introduction of *V. penaeicida* into new countries or areas

Mechanism of disease spread

The route of *V. penaeicida* introduction into Japan and New Caledonia is unknown. In Japan, *V. penaeicida* is considered to be ubiquitous in the aquaculture environment in infected areas (de la Peña et al. 1992).

Impacts of introduction

There are few data on production losses attributed to *V. penaeicida*. Between 1988 and 1994, Japanese production of *M. japonicus* reportedly fell by 50% (Takahashi et al. 1998). Approximately 60% of the losses up to 1992 have been attributed to *V. penaeicida* (Takahashi et al. 1998). Assuming these losses occurred evenly over this time (although this is unlikely due to the onset of WSSV in the early 1990s), it could be estimated that *V. penaeicida* may have caused production losses in *M. japonicus* aquaculture of approximately 30% per annum.

Total New Caledonian farmed prawn production volume dropped by 15% from 1992 to 1993 (Costa et al. 1998b). Stocking densities and growth cycle durations also changed during this period, making it difficult to determine what proportion of the loss can be attributed to the outbreak termed “Syndrome 93” at the time (Mermoud et al. 1998).

Control measures

Generally, vibriosis is a common secondary infection associated with injury, stress or disease associated with other pathogens. *Vibrio* species are opportunistic, causing mass mortalities in larvae, juveniles and adult prawns already suffering from stress or nutrient deficiency, whilst post-larval stages remain unaffected. Once *V. penaeicida* has established in a prawn farm, it is reportedly difficult to eradicate from the environment (de la Peña et al. 1992). Disease prevention techniques include stress reduction (de la Peña et al. 1992, de la Peña et al. 1997, Costa et al. 1998b), and the use of immunostimulants such as 'vaccination' with formalin-killed *V. penaeicida* (Itami et al. 1989, Itami et al. 1992) or the use of β-1, 3 glucans (CampaCordova et al. 2005) and peptidoglycans (Itami et al. 1998) in feed. In outbreaks of disease, mortality may also be reduced by the use of in-feed antibiotics such as oxytetracycline (Takahashi et al. 1985b, Takahashi et al. 1998), although *Vibrio* sp. resistance to many antibiotics used in prawn aquaculture has been reported (Vaseeharan et al. 2005). There is little on-going research aimed at delivering acquired immunity in prawns. However, molecular immunological research has identified genes involved in the transcription of products used directly in the immune and hematopoietic processes of prawns infected with *V. penaeicida* (de Lorgeril et al. 2005).

Monodon slow growth syndrome/agent

Since 2001, farmed *Penaeus monodon* crops throughout Thailand have experienced severe growth retardation (Chayaburakul et al. 2004). The phenomenon has tentatively been named monodon slow growth syndrome (MSGS) and has been associated with significant production losses, but not mortality (Chayaburakul et al. 2004, Flegel and Mohan 2004).

The primary concern is that occurrence of the putative agent, monodon slow growth agent (MSGA), may have been introduced as a result of the wide-spread culture and unrestricted translocation of live *Litopenaeus vannamei* (Flegel and Mohan 2004).

Working case definition

The disease has been termed a syndrome as it presents as a pattern of symptoms indicative of an infectious disease, but of unknown aetiology. A working case definition for MSGS has been developed for surveillance and epidemiology. The affected population must have the following pattern of symptoms (Flegel and Mohan 2004):

- a co-efficient of variation (= standard deviation/mean) of more than 35% by weight and the absence of hepatopancreatic parvovirus or other severe hepatopancreatic infections by known agents, and
- any 3 of the following 5 signs: (1) unusually dark colour, (2) average daily weight gain of less than 0.1 gram / day at 4 months, (3) unusually bright yellow markings, (4) 'bamboo-shaped' abdominal segments, and (5) brittle antennae .

Aetiology

Although the aetiology of MSGS is unknown, a putative agent has been acknowledged as monodon slow growth agent (MSGA) (Chayaburakul et al. 2004). Research efforts have concluded that MSGS is most likely to be caused by an unknown pathogen(s). Other known pathogens and non-pathogenic agents such as, uniform environmental changes in affected areas, uniform changes in husbandry practice or uniform genetic changes in *P. monodon* stock, are considered less likely to cause or contribute to the syndrome, but have not yet been fully explored (Chayaburakul et al. 2004).

Recently, two previously unrecognised small viral-like particles (25–30nm) have been detected in *P. monodon* with MSGS symptoms, suggesting a viral infection may be associated with the syndrome (Panphut et al. 2004, Anantasomboon et al. 2005, Flegel and Withyachumnarnkul 2005). Research is underway to identify the viral-like particles and preliminary molecular results have shown the possible involvement of an RNA virus (Apisawetakan et al. 2005, Flegel and Withyachumnarnkul 2005). Although a recent first round of results has determined that one of the possible viral-like particles is a new RNA prawn virus with similarities to a Luteoviridae-like virus, it does not appear to be a primary pathogen causing MSGS (Apisawetakan et al. 2005).

Epidemiology

Fundamental qualitative data from the literature reports MSGS as a sudden and nation-wide phenomenon causing a 36% downturn in *P. monodon* production, resulting in the majority of Thai prawn farmers to turn future efforts toward culture of *L. vannamei* (Flegel and Mohan 2004). Otherwise, there exists little or no epidemiological research based evidence for either MSGS or the putative causative agent, MSGA. A specific surveillance system to determine the level and location of MSGS throughout the prawn growing regions of affected areas has been suggested (Flegel and Mohan 2004, NACA 2005c). To date, MSGS is not listed in the regional Quarterly Aquatic Animal Disease (QAAD Asia-Pacific) reporting list and basic data

has been collected in a passive manner as anecdotal evidence from producers, distributors and suppliers.

Host range

MSGS has only been associated with *P. monodon*. An initial experimental transmission trial has shown *L. vannamei* does not develop symptoms of MSGS when injected with a bacteria-free filtrate from affected *P. monodon* (Flegel and Withyachumnarnkul 2005). However, the injection of lymphoid organ filtrates derived from *L. vannamei* co-habited with affected *P. monodon* resulted in MSGS in healthy *P. monodon*, suggesting MSGA may originate from *L. vannamei* (Briggs et al. 2004, Flegel and Mohan 2004) and that *L. vannamei* could act as a carrier (Anantasomboon et al. 2005).

Geographical distribution

Since the emergence of MSGS in *P. monodon* in 2001, as reported by Thai prawn farmers, the disease has now been reported by Network of Aquaculture Centres in Asia-Pacific (NACA) staff, delegates and trainees via passive surveillance in five countries including Thailand, India, Malaysia, Indonesia and Vietnam (C.V. Mohan, NACA, pers. comm. January 2006; NACA 2005c, NACA 2005d).

Transmission

Experimental transmission of MSGS to *P. monodon* via injection of filtered lymphoid organ extracts (LOE) has been demonstrated (Anantasomboon et al. 2005, Flegel and Withyachumnarnkul 2005), although results have been presented, the experimental design was not reported and the research has not yet been published in peer reviewed journals. Injection of LOE from *P. monodon* displaying symptoms for MSGS produced MSGS in *P. monodon*, but failed to reproduce MSGS in *L. vannamei* (Flegel and Withyachumnarnkul 2005). Further, *L. vannamei* co-habiting with MSGS affected *P. monodon* grew normally. LOE from the same co-cultured *L. vannamei* was then injected into healthy *P. monodon* causing MSGS, suggesting the presence of an infectious pathogen (Anantasomboon et al. 2005).

Prevalence

The syndrome is reported as widespread in Thailand (Chayaburakul et al. 2004), although the prevalence of MSGA (in both aquaculture and wild populations) is unknown.

Pathogenesis

The tissue tropism of MSGA has not been reported. Histopathology performed on *P. monodon* suspected of having MSGS under the working case definition, shows that MSGA infection does not cause recognisable pathological lesions (Chayaburakul et al. 2004). Recent studies suggested that MSGA could be located in the lymphoid organ and gills, as unknown viral-like particles were observed in those tissues, and because filtered LOE causes MSGS when injected into healthy *P. monodon* (Panphut et al. 2004, Anantasomboon et al. 2005).

Impacts/Control

National production volume losses in Thailand for 2002 are frequently reported at 36% (Flegel and Mohan 2004) associated with significant financial losses (Chayaburakul et al. 2004), although it is unlikely that these losses are solely attributable to MSGS (Chayaburakul et al. 2004). No management measures have yet been developed for controlling MSGS in Thailand.

In the absence of a known causative agent or a standard diagnostic test, Flegel and Mohan (2004) recommended implementation of the following mitigation measures:

- *L. vannamei* and *P. monodon* be reared separately, particularly at the hatchery,
- increased surveillance for MSGS be conducted,
- care be taken during the introduction of new species into previously unaffected areas. Interim measures such as improved quarantine husbandry and adherence to International Council for the Exploration of the Sea (ICES) transfer protocol (ICES 2005), and
- co-habitation tests using important endemic crustacean species to determine the risk of importing exotic pathogens into local environments, aquaculture and wild fisheries.

APPENDIX 4 Guidelines for the approval of countries to export animals (including fish) and their products to Australia (ABPM 1999/41)

1. Introduction

Where generic conditions for the importation of animals or animal products are developed as a result of a generic risk analysis, it will generally be appropriate to specify as part of the conditions that permits will only be issued for importations from countries that have been specifically approved by AQIS. Approval would normally be based on an assessment of the ability of the certifying authority of the country to provide informed and reliable certification that Australia's quarantine requirements have been met. The 'approved country' approach provides a mechanism for rapid introduction of new controls on importations from a particular country in the event of a change in the animal health status of that country or where AQIS detects breaches of quarantine requirements, such as fraudulent certification.

AQIS takes into account the following criteria when considering the approval of countries to export animals/products to Australia:

- the effectiveness of veterinary services and other relevant certifying authorities
- the animal health status of the country
- legislative controls over animal health, including quarantine policies and practices
- the standard of reporting to the Office International des Epizooties (OIE) of major contagious disease outbreaks
- effectiveness of veterinary laboratory services, including compliance with relevant international standards
- effectiveness of systems for control over certification/documentation of products intended for export to Australia.

The import conditions will identify the key risk management issues that should be considered in the approval of countries.

This paper provides a framework, based on guidelines as specified in section 1.4.3 of the OIE International Health Code for the assessment of a country for approval to export to Australia. Although some countries may be able to provide quantitative data, in most cases AQIS's assessment will be based on qualitative information.

Where import requirements include pre-export processing as part of the risk management measures, AQIS may restrict the issue of permits to product prepared in plants that have been formally approved by the exporting country authority and/or AQIS. Guidelines for the approval of plants for the processing of animal products for export to Australia are also included in this paper.

These guidelines refer to terrestrial, aquatic and avian species and their products.

2. Criteria for the approval of exporting countries

AQIS considers that exporting countries are responsible for the sanitary standard of goods exported to Australia. Where product is sourced in one country and exported from another, AQIS holds the exporting country responsible for the health certification that accompanies those goods. In this context, it is the exporting country and its official certifying authority that must be approved.

In some exporting countries, AQIS may assess several competent authorities, including the relevant authority for animal health, fish health and human health. These authorities may operate at a Federal, State or provincial level.

2.1 Countries with an established export trade in animals/products to Australia.

This section deals with countries that regularly export to Australia items such as live animals, genetic material and animal products in commercial volume. It does not include countries that export items such as laboratory specimens, artefacts and samples for evaluation, i.e. non-commercial exports or countries that export products that are exempt from quarantine control.

AQIS would normally approve without formal assessment those countries that have a history of exporting animals/products in compliance with Australia's sanitary requirements. All approvals remain under review and can be suspended on an emergency basis at any time. Such action may be taken, for example, if AQIS were to detect serious non-compliance, such as the provision of false certification by a regulatory authority.

AQIS monitors the performance of approved countries in reporting OIE-listed diseases, and notifying Australia of changes in disease status, including any incursions of disease that might affect bilateral trade in animals/products. On the basis of formal bilateral agreement, exporting countries may undertake to directly notify Australia of changes in status for diseases other than those listed by the OIE.

AQIS will monitor the performance of approved countries via routine collection of intelligence on disease, including from scientific literature and internet postings, through the conduct of visits and inspections and by liaison with other veterinary authorities (including chief veterinary officers of Australian states/territories). If AQIS becomes aware that unreported serious disease is present in the country of export, approval may be suspended pending clarification of the situation.

2.2 Countries with no established export trade in animals/ products to Australia

AQIS's formal assessment of a country for approval to export to Australia, may include:

- examination of information supplied by the country
- consideration of the results of an assessment by Australia's major trading partners to the country as an exporter of like commodities (such assessment will take into account the extent to which the regulatory requirements of trading partners are consistent with those of Australia)
- formal evaluation of the country's veterinary services and/or certifying authority (this may involve country visits by AQIS or AQIS authorised officers).

a) An effective veterinary/fish health service

An approved country should have national veterinary and fish health authorities, which are responsible for animal health, quarantine, export certification and international reporting of the country's animal disease status.

- Where non-government veterinarians provide export services, they should be Official Veterinarians as defined in the OIE Code. The national veterinary authority must be responsible for the overall system of control of the export-related activities of private veterinarians, including arrangements for training, auditing and compliance.
- The performance of the certifying authority should be subject to independent audit and a satisfactory level of competency must be maintained.

b) Animal health status of the country of origin/export

The country should be free from or have effective zoning of diseases as appropriate to AQIS's quarantine requirements. This should be supported by legislative controls such as mandatory notification of disease outbreaks and official control programs.

c) Quarantine measures

AQIS will consider the disease status of neighbouring countries and the effectiveness of border measures and buffer zones in preventing disease incursions in assessing countries for approval to export to Australia.

d) Animal health controls

An approved country should be able to demonstrate mechanisms for official notification and control or eradication of diseases identified in the import risk analysis as important in relation to the animal species/product in question. Animal health controls should include arrangements for animal health surveillance, regulatory controls for specified diseases and a formal system of response to animal disease events. AQIS will take into account the country's policies with respect to outbreaks of diseases of concern.

Border controls should be effective in preventing the entry and establishment of significant exotic disease agents relevant to the animal species/product in question.

There should be legislative provisions covering movement controls and inspection procedures in relation to the prevention, control and eradication of disease.

e) Performance in reporting disease

AQIS will take into account the performance of approved countries in reporting OIE-listed diseases and significant new or emerging diseases and of notification to Australia of incursions of disease relevant to the bilateral trade in animals/products. If AQIS becomes aware that serious disease is present, unreported, in the country of export, the country's approved status may be suspended, pending clarification, or withdrawn.

f) Access to laboratories that can conduct recognised diagnostic tests to an international standard of competence.

It is accepted that not all countries are able to perform all the necessary tests to definitively diagnose all diseases. Countries should, however, have access to laboratories that meet the OIE Standard for the diagnosis of diseases that AQIS identifies (in an import risk analysis) as being of concern. They should also have competence in the collection, preservation and transport of specimens to these laboratories.

g) Appropriate arrangements for certification/documentation.

Countries should be able to demonstrate:

- legislative controls over the process of export of animals and animal products, to provide for enforcement of Australia's import requirements. This includes supervision by the official veterinary (or other competent) authority of the export certification process;
- legislative arrangements that provide for the approval/registration of export premises and provide powers to deny or withdraw registration for premises or certification for commodities as the case may be;
- arrangements to ensure that certifying officers performing official duties have no conflict of interest;
- a system of control that provides for reliable correlation of the results of inspections with the documentation provided for export consignments and
- a system of audit and review of official and private certifying procedures.

3. Criteria for approval of exporting facilities

Where there is an appropriate Australian standard (for example, relating to inspection requirements) the exporting country would be expected to follow a standard that would provide an equivalent outcome to that provided by the Australian standard.

Where the certifying and/or veterinary services in the exporting country have previously been

assessed and approved, AQIS will normally base approval of processing plants on advice from the certifying authority that the plant meets AQIS's requirements.

In cases where the certifying authority in the exporting country has not previously been assessed, AQIS may conduct an on-site assessment of a plant.

The processing plant will normally be required to demonstrate, as appropriate:

- suitable separation of raw and processed product;
- reliable compliance with minimum processing requirements for the product;
- auditable records of information required by AQIS, for example on the source of raw materials and ingredients, processing records and test results;
- controls to prevent post-processing contamination; and
- standards of hygienic construction and operation that provide equivalent public health safeguards to those provided by relevant Australian standards.

APPENDIX 5 OIE Aquatic Animal Health Code: Zoning and Compartmentalisation

The following is taken from the the OIE Aquatic Animal Health Code (OIE 2009a).

Chapter 4.1 Zoning and Compartmentalisation

Article 1.4.4.1

Introduction

Given the difficulty of establishing and maintaining freedom from a particular disease for an entire country especially for diseases whose entry is difficult to control, there may be benefits to one or more Members in establishing and maintaining a subpopulation with a distinct aquatic animal health status. Subpopulations may be separated by natural or artificial geographical barriers or, in certain situations, by the application of appropriate management practices.

Zoning and compartmentalisation are procedures implemented by a country under the provisions of this chapter to define subpopulations of distinct aquatic animal health status for the purpose of disease control or international trade. Compartmentalisation applies to a subpopulation when management practices related to biosecurity are the defining factors, while zoning applies when a subpopulation is defined on a geographical basis. In practice, spatial considerations and good management play important roles in the application of both concepts.

This chapter is to assist OIE Members wishing to establish and maintain different subpopulations, using the principles of compartmentalisation and zoning. These principles should be applied in accordance with the measures recommended in the relevant disease chapter(s). This chapter also outlines a process through which trading partners may recognise such subpopulations. This process is best implemented by trading partners through establishing parameters and gaining agreement on the necessary measures prior to outbreaks of disease.

Before trade in aquatic animals or aquatic animal products may occur, an importing country needs to be satisfied that its aquatic animal health status will be appropriately protected. In most cases, the import regulations developed will rely in part on judgements made about the effectiveness of sanitary procedures undertaken by the exporting country, both at its borders and within its territory.

In addition to contributing to the safety of international trade, zoning and compartmentalisation may assist disease control or eradication within Members. Zoning may encourage the more efficient use of resources, and compartmentalisation may allow the functional separation of a subpopulation from other domestic or wild aquatic animals through biosecurity measures, which a zone (through geographical separation) would not achieve. Following an outbreak of disease, compartmentalisation may allow a Member be able to take advantage of epidemiological links among subpopulations or common practices relating to biosecurity, despite diverse geographical locations, to facilitate disease control and/or the resumption of trade.

Zoning and compartmentalisation may not be applicable to all diseases, but separate requirements will be developed for each disease for which the application of zoning or compartmentalisation is considered appropriate.

To regain the status of a free zone or free compartment following an outbreak of disease, Members should follow the recommendations in the relevant disease chapter in the Aquatic Code.

Article 1.4.4.2

General considerations

The Competent Authority of an exporting country that is establishing a zone or compartment for international trade purposes should clearly define the subpopulation in accordance with the recommendations in the relevant chapters in the Aquatic Code, including those on surveillance, and the identification and traceability of aquatic animals. The Competent Authority of an exporting country should be able to explain to the Competent Authority of an importing country the basis for its claim of a distinct aquatic animal health status for the zone or compartment in such terms.

The procedures used to establish and maintain the distinct aquatic animal health status of a zone or compartment should be appropriate to the particular circumstances and will depend on the epidemiology of the disease, environmental factors, risk of introduction and establishment of disease, and applicable biosecurity measures. The exporting country should be able to demonstrate, through detailed documentation supplied to the importing country, published through official channels, that it has implemented the recommendations in the Aquatic Code for establishing and maintaining such a zone or compartment.

An importing country should recognise the existence of this zone or compartment when the appropriate measures recommended in the Aquatic Code are applied, and the Competent Authority of the exporting country certifies that this is the case. Note that an importing country may adopt a higher level of protection where it is scientifically justified and the obligations referred to in Article 2.1.2. are met. Article 4.1.4. is also relevant.

Where countries share a zone or compartment, the Competent Authority of each country should collaborate to define and fulfil their respective responsibilities.

The exporting country should conduct an assessment of the resources needed and available to establish and maintain a zone or compartment for international trade purposes. These include the human and financial resources and the technical capability of the Competent Authority (and of the relevant industry, in the case of a compartment) including on disease surveillance and diagnosis.

Article 1.4.4.3.

Principles for defining a zone or compartment

In conjunction with the above considerations and the definitions of zone and compartment, the following principles should apply when Members define a zone or compartment:

1. The extent of a zone should be established by the Competent Authority on the basis of the definition of zone and made public through official channels.
2. The factors defining a compartment should be established by the Competent Authority on the basis of relevant criteria such as management and husbandry practices related to biosecurity, and made public through official channels.
3. Aquatic animals belonging to such subpopulations need to be recognizable as such through a clear epidemiological separation from other aquatic animals and all things presenting a disease risk.
4. For a zone or compartment, the Competent Authority should document in detail the measures taken to ensure the identification of the subpopulation, for example by means of registration of all the aquaculture establishments located in such a zone or compartment and the establishment and maintenance of its aquatic animal health status through a biosecurity plan. The measures used to establish and maintain the distinct aquatic animal health status of a zone or compartment should be appropriate to the particular circumstances and will depend on the epidemiology of the disease, environmental factors, the aquatic animal health status in adjacent areas, applicable biosecurity measures (including movement controls, use of natural and artificial

boundaries, the spatial separation of aquatic animals, and commercial management and husbandry practices), and surveillance.

5. For a compartment, the biosecurity plan should describe the partnership between the relevant enterprise/industry and the Competent Authority, and their respective responsibilities, including the procedures for oversight of the operation of the compartment by the Competent Authority.
6. For a compartment, the biosecurity plan should also describe the routine operating procedures to provide clear evidence that the surveillance conducted and the management practices are adequate to meet the definition of the compartment. In addition to information on aquatic animal movements, the biosecurity plan should include production and stock records, feed sources, traceability, surveillance results, visitor logbook, morbidity and mortality history, medications, vaccinations, documentation of training and any other criteria necessary for evaluation of risk mitigation. The information required may vary according to the aquatic animal species and disease(s) under consideration. The biosecurity plan should also describe how the measures will be audited to ensure that the risks are regularly re-assessed and the measures adjusted accordingly.
7. Thus defined, the zones and compartments constitute the relevant subpopulations for the application of the recommendations in Section 8 to Section 11 of the Aquatic Code.

Article 1.4.4.4.

Sequence of steps to be taken in establishing a zone or a compartment and having it recognised for international trade purposes

There is no single sequence of steps which should be followed in establishing a zone or a compartment. The steps that the Competent Authority of the importing country and the exporting country choose and implement will generally depend on the circumstances existing within the countries and at their borders, and their trading history. The recommended steps are:

- For zoning
    - The exporting country identifies a geographical area, which it considers to contain an aquatic animal subpopulation with a distinct aquatic animal health status with respect to a specific disease/specific diseases, based on surveillance.
    - The exporting country describes in the biosecurity plan for the zone the measures which are being, or will be, applied to distinguish such an area epidemiologically from other parts of its territory, in accordance with the recommendations in the Aquatic Code.
    - The exporting country provides the above information to the importing country, with an explanation of why the area can be treated as an epidemiologically separated zone for international trade purposes.
    - The importing country determines whether it accepts such an area as a zone for the importation of aquatic animals and aquatic animal products, taking into account:
        - an evaluation of the exporting country's Competent Authority;
        - the result of a risk assessment based on the information provided by the exporting country and its own research;
        - its own aquatic animal health situation with respect to the disease(s) concerned; and

        - other relevant OIE standards.
    - The importing country notifies the exporting country of the result of its determination and the underlying reasons, within a reasonable period of time, being either:
        - recognition of the zone;
        - request for further information; or
        - rejection of the area as a zone for international trade purposes.
    - An attempt should be made to resolve any differences over the recognition of the zone, either in the interim or finally, by using an agreed mechanism to reach consensus (such as the OIE dispute settlement mechanism).
    - The importing country and the exporting country should enter into a formal agreement recognising the zone.
- For compartmentalisation
    - Based on discussions with the relevant enterprise/industry, the exporting country identifies a compartment of one or more aquaculture establishments or other premises that operate under common management practices related to biosecurity, and which contains an identifiable aquatic animal subpopulation with a distinct aquatic animal health status with respect to a specific disease/specific diseases; the exporting country describes how this status is maintained through a partnership between the relevant enterprise/industry and the Competent Authority of the exporting country.
    - The exporting country examines the compartment's biosecurity plan and confirms through an audit that:
        - the compartment is epidemiologically closed throughout its routine operating procedures as a result of effective implementation of its biosecurity plan; and
        - the surveillance programme in place is appropriate to verify the status of such aquaculture establishment(s) with respect to such disease(s).
    - The exporting country describes the compartment, in accordance with the recommendations in the Aquatic Code.
    - The exporting country provides the above information to the importing country, with an explanation of why such an enterprise can be treated as an epidemiologically separated compartment for international trade purposes.
    - The importing country determines whether it accepts such an enterprise as a compartment for the importation of aquatic animals and aquatic animal products, taking into account:
        - an evaluation of the exporting country's Competent Authority;
        - the result of a risk assessment based on the information provided by the exporting country and its own research;
        - its own aquatic animal health situation with respect to the disease(s) concerned; and
        - other relevant OIE standards.

- The importing country notifies the exporting country of the result of its examination and the underlying reasons, within a reasonable period of time, being either:
  - recognition of the compartment;
  - request for further information; or
  - rejection of such an enterprise as a compartment for international trade purposes.
- An attempt should be made to resolve any differences over the recognition of the compartment, either in the interim or finally, by using an agreed mechanism to reach consensus (such as the OIE dispute settlement mechanism).
- The importing country and the exporting country should enter into a formal agreement recognising the compartment.

APPENDIX 6 AQIS Fact Sheet: Highly processed wet and dry marinated prawn products

The purpose of this fact sheet is to provide definitions and descriptions of highly processed wet and dry marinated prawn products relevant to the importation of these products under import permits issued by the Australian Quarantine and Inspection Service (AQIS).

Highly processed prawn products for human consumption can be imported into Australia provided the head and shell have been removed (last shell segment and tail fans are permitted) and the product falls into one of the following categories:

- breaded (crumbed) or battered
- marinated in a wet marinade
- marinated in a dry marinade
- marinated and placed on skewers
- dumpling, spring roll, samosa, roll, ball or dim sum-type product.

Importers seeking an import permit for highly processed prawn products must, as part of their AQIS import permit application, provide photographs, details of manufacturing steps and a complete ingredients list (totalling 100% of product weight).

The requirements and the general criteria currently used by AQIS to assess import permit applications for marinated and skewered products are set out below.

Wet Marinated Prawn Products

The prawn meat must be coated for human consumption by being marinated in a wet marinade (the marinade must be no less than 12% of the total weight of the product).

- colourless flavourings are not visible and are not considered to contribute to the 12% marinade requirement.

Dry Marinated Prawn Products

The prawn meat must be coated with a dry marinade that is clearly seen to coat the product.

Marinated and Skewered Prawn Products

The prawn meat must be coated for human consumption by being marinated and placed on skewers.

- the flavour component of the marinade must be clearly seen to coat the product for both wet and dry marinades.

For All Marinated Prawn Products

Unflavoured components of marinades such as water, maltodextrin, oil, starch, rice flour, tapioca, wheat flour, thickeners and the like, are not considered a flavour component when assessing these products and do not contribute to the 12% marinade requirement.

- in most cases, powdered and/or dehydrated garlic alone is not considered to be part of the flavour component and such products are not considered to be highly processed.

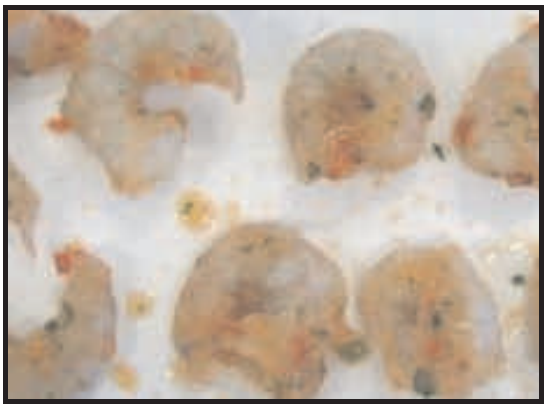
Photographs of frozen and thawed product accompanying import permit applications must be:

- clear and of high resolution (ie not blurred or glared due to flash photography);
- labelled; and
- genuinely represent the product to be imported and presented for sale.

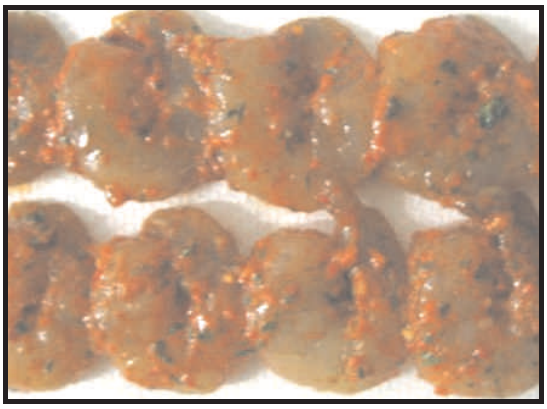
Photographs of some products that are considered highly processed are shown overleaf.

NOTE: As of October 2009 AQIS will introduce a program of inspections to ensure consignments comply with permit conditions.

For further information regarding import requirements for prawn products please refer to AQIS import conditions database ICON (www.aqis.gov.au/ICON). Further information on the Import Risk Analysis Report for Prawns and Prawn Products is available from the Biosecurity Australia website: www.biosecurity.gov.au



Wet marinated products. Prawns coated in a 12% sweet chilli marinade (L), a 12% garlic, herb marinade (R) and 12% wet garlic marinade (C).



Skewered/Dry marinated products. Prawns coated with sweet chilli (L) and a herb and garlic marinade (R).

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