Environmental impact of the establishment of exotic prawn pathogens in Australia

a consultancy report to AQIS

Prepared for: Australian Quarantine and Inspection Service
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Abbreviations and Acronyms

AEP	Aquatic Ecosystem Protection
ANZECC	Australia and New Zealand Environment and Conservation Council
AQIS	Australian Quarantine and Inspection Service
ASS	acid sulfate soil
ca	approximately
CIA	Cowdry type A inclusion body
CV	coefficient of variation
DNA	deoxyribonucleic acid
gm	gram(s)
ha	hectare(s)
IB	inclusion body
IHHNV	infectious hypodermal and hematopoietic necrosis virus
kg	kilogram(s)
km	kilometre(s)
l	litre(s)
min	minute(s)
mg	milligram(s)
nm	nanometre(s)
m	metre(s)
OB	occlusion body
OIE	Office International des Epizooties
PCR	polymerase chain reaction
PL	postlarvae
ppt	parts per thousand
ppm	parts pre million
RDS	runt deformity syndrome
RNA	ribonucleic acid
TOR	terms of reference
TPWD	Texas Parks and Wildlife Department
TS	Taura syndrome
TSV	Taura syndrome virus
UV	ultraviolet
WSSV	white spot syndrome virus
WSS	white spot syndrome
YHD	yellow-head disease
YHV	yellow-head virus

Taxonomic Nomenclature

Throughout this report the taxonomic nomenclature used is that of Holthuis LB. 1980. *Shrimps and prawns of the world*. FAO Species Catalog. FAO Fisheries Synopsis No. 125, Vol 1. Rome. 271 pp.

Executive Summary

This study was commissioned by the Australian Quarantine and Inspection Service (AQIS) to evaluate the potential impact on the natural environment of the establishment of exotic prawn diseases, using White Spot Syndrome Virus (WSSV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) as examples.

The introduction describes the terms of reference, the consultants who undertook the study and the methods used.

The second section deals with the distribution, habitats, biology, ecology and commercial importance of Australian prawn species. Most of the commercial species of marine prawns caught in Australia belong to the family Penaedae. Table 2.1 shows the primary prawn species and indicates which have had reports of WSSV or IHHNV as natural or experimental infections in other countries.

Section three summarises the Australian prawn industries which are located mainly in northern Australia. In 1996-97, prawns comprised 19.5% of the total Australian fisheries production of $1.76 billion and 14% of total fisheries exports of $1.3 billion. Australian prawn production is primarily through trawling in marine waters with lesser amounts produced from river trawling and aquaculture. In 1996-97, the trawling catch was 26,230 tonnes while 1,626 tonnes was produced from culture.

Section four reviews relevant information on the two viruses of interest. WSSV infection has been found in a number of species of penaeid prawns in both wild and farmed situations. It can produce high mortality rates in farmed prawns, usually in young juveniles and major epidemics have occurred with devastating consequences. WSSV also infects a wide range of other crustacean species and aquatic arthropods, causing mortality in some but not others. Copepods are potentially important hosts for the virus. The level of environmental stress probably plays a significant role in determining the mortality rate due to WSSV infections. Transmission is both vertical (probably during spawning) and horizontal by ingestion of infected tissues. Spread of infection between regions can be by infected prawns or vectors and carriers such as other crustaceans, seabirds and aquatic arthropods. Although WSSV infection is common in wild prawn populations in Asia, the weight of evidence suggests that the virus has not caused measurable reductions in catches based on information from China, Thailand and Japan, where WSS has occurred in farmed prawns and adjacent wild catch data are available. A monitoring program for WSSV in selected US wild fisheries commenced in 1997 but there is insufficient data to assess impacts to this point in time.

IHHNV has a wide geographic range and has caused mortalities in some species of prawns while others appear refractory to infection. Although Australia is free of classical IHHNV, an IHHNV-like infection has been detected in hybrid prawns. The virus can cause a variety of syndromes depending on the species of prawn infected. The host range of IHHVV appears to be much more restricted than that for WSSV with no evidence for infection in other crustacean species nor arthropods. Like WSSV, environmental stress may play a role

in determining the consequences of infection. Both vertical and horizontal transmission occur and brood stock can carry inapparent infections for life, resulting in high infection rates in progeny. Most information on the impact of IHHNV on wild prawn populations comes from the US and Mexico with less from China. The introduction of IHHNV into prawn farms adjacent to the Gulf of California in Mexico in 1987 was followed by serious epizootics of IHHNV in farmed *P. stylirostris* 1989 and 1990. A high prevalence of IHHNV in wild prawns in the Gulf of California was subsequently detected in 1990 and this coincided with a marked decline in the catch of wild *P. stylirostris* followed by a recovery, despite the persistence of very high infection rates in the population. The catch of *P. californiensis*, a less susceptible species, also declined and recovered during this period. In evaluating the available information, the consultants could not be sure if the decline in catch of *P. stylirostris* was caused by IHHNV or whether some other factor was responsible for the decline in both *P. stylirostris* and *P. californiensis* catches, coinciding with the introduction of IHHNV. Both possibilities were therefore considered in assessing potential impacts in Australia. In other situations where IHHNV has affected farmed prawns, adjacent wild commercial species appear to have remained unaffected. This may result from many prawn species being refractory which could also be the case for Australian species, though this is by no means certain.

The fifth section assesses the likelihood that WSSV and IHHNV would establish and spread in Australia if introduced as well as the likely consequences based on the experience in other countries. If Australian prawns are to be exposed to exotic viruses, it is most likely to be through imported prawn feed, waste from processing imported products or imported bait prawns. Wild prawn populations could thus be infected directly from contaminated processing waste and infected bait or indirectly through spillover from prawn farms originally infected via imported prawn feed. Overseas experience suggests that once farms are infected, this provides the greatest virus load for adjacent wild fisheries and may play a significant role in sustaining infection rates in wild prawns. However, the density of prawn farms in Australia is much lower than in many countries where WSSV and IHHNV have been a problem and it is reasonable to assume that, if farms in Australia were infected, virus loads on adjacent natural systems would be very much lower than, say, in Asia.

A wide range of factors attributable to the host, agent and environment will determine the impact of a virus incursion. Strain variation probably occurs in both WSSV and IHHNV and this may result in variations in infectivity, pathogenicity and virulence. Wild penaeid prawns may be more susceptible to infection at certain critical times such as when population densities are high during immigration of postlarvae and emigration of juveniles. On the other hand, depleted prawn populations have the capacity to be rapidly replenished through several different mechanisms. Environmental stressors are thought to be important determinants of the level of impact of virus infections in prawn populations. In Australia, such stressors include acidification through run-off from acid sulfate soils, low dissolved oxygen concentrations and habitat degradation.

Although WSSV infection is relatively common in wild prawn populations in Asia, the weight of evidence suggest that this virus has not caused measurable reductions in catches. This is in contrast to farmed prawns where epizootics have been initially devastating. Many factors

probably contribute to this difference. For example, farmed prawns are at a much higher stocking density, are under considerably more environmental stress than those in the wild and there is a lack of predators and scavengers to remove moribund and dead hosts which may be infectious.

The episode with IHHNV in the Gulf of California in the late 1980s and early 1990s is the best evidence available for an impact of this virus on susceptible wild prawn populations. Nevertheless, the reduced catch of *P. stylirostris* can not be conclusively attributed to viral infection. Species of prawns presently farmed in Australia are unlikely to be significantly impacted by IHHNV infections but may provide a source of infection for adjacent wild populations. Although not known with certainty, it is feasible that some Australian species of prawns in wild populations may be impacted, depending on their level of susceptibility and conditions of stress prevailing at the time. Predators and scavengers as well as natural prawn replenishment mechanisms are likely to play a significant role in limiting the impact of an incursion. Overseas experience also suggests that any impacts will abate with time despite persistence of high infection rates in the population.

Section six incorporates the findings from section five into two case studies based on the Clarence River region in northern New South Wales and the Townsville region of north Queensland. Each region is described in terms of its potentially susceptible populations, prawn industries, human alterations and water quality. Likely sources of infection and spread are discussed. Infections could originate from improperly handled waste from processing imported product (though at present, only domestic product is processed), material from infected prawn farms or infected bait. The likelihood of a WSS outbreak in wild prawns and other crustaceans in either case study area is low for all three possible sources of infection. For IHHNV, if wild prawns were mainly refractory species, then the likelihood of an outbreak is low. However, if some or all wild species of wild prawns were susceptible, then the chances of an outbreak of IHHN are higher. Other crustacean species would not be directly affected by IHHNV as they are refractory to infection.

It is concluded that WSSV is unlikely to have any measurable impact on wild prawn populations should it become established in Australia. The situation with IHHNV could be similar but there is also a chance that it may be different. In some situations overseas there is no evidence to show that IHHNV has affected wild populations. However, if some Australian wild prawn species are susceptible to IHHNV under stressful environmental conditions, and recognising that this virus may have substantially reduced *P. stylirostris* populations in the Gulf of California, a similar scenario is possible in Australia. However, the flow on effect of reduced prawn populations on the wider food web would be pure speculation at present, as there is insufficient information on which an assessment can be based.

1 Introduction

This assessment has been undertaken by AusVet Animal Health Services for the Australian Quarantine and Inspection Service (AQIS). It follows from the Scientific Review of Prawn Diseases which identified a number of exotic prawn pathogens and assessed their risks to Australian prawn populations. The present study is one of a series being undertaken. The others are: An Economic Study of the Establishment of Exotic Prawn Pathogens; Routes for Exposure of Aquatic Animals to Aquatic Animal Products Intended for Human Consumption; and Consultancy on Crustacean Feeds.

The present project overlaps to some extent with all others in the series, but there is considerable overlap with the economics study. These two projects were therefore undertaken collaboratively to ensure consistency. Some people were members of both project teams and consultation was maintained throughout the period of the two studies.

1.1 Terms of reference

1. To report on the impact on the natural environment of the establishment of exotic disease, using WSSV and IHHNV as examples of exotic disease agents. The consultant will use scientific information provided by AQIS and other relevant sources of information as a basis for his/her conclusions. Such impact might arise for harmful effects on (*inter alia*):

- the survival, growth and reproduction of wild prawns and other crustaceans in Australia
- the ecological structure and function of aquatic populations which depend on prawns; for example, species for which prawns are the major food source.

2. To document which components of the aquatic environment may be affected by the establishment of WSSV and IHHNV and to estimate the impact these viruses would have if they became established at the following sites:

- the Clarence River, an environment which has populations of estuarine and near-shore prawns
- marine and freshwater environments in and around Townsville, which share their crustacean populations with those of the Great Barrier Reef.

1.2 Personnel and roles

The contact person in AQIS throughout the project was Dr Judith Bourne.
People who undertook the project and their roles were:

Dr Chris Baldock BVSc(Hons) MPVM PhD FACVSc

- Team leader
- Client liaison

- Combine information on WSSV and IHHNV infections, prawn populations, other crustacean populations and associated aquatic communities to develop an impact assessment model
- Apply the model to assess the impacts of WSSV and IHHNV on the above populations in Australia generally and in the Clarence River and the Townsville areas specifically
- Coordinate preparation of consultants' report

Dr Richard Callinan BVSc(Hons) MVSc PhD

- Obtain and review published and unpublished information on pathogenesis and epidemiology of WSSV and IHHNV infections in prawns and other crustaceans
- Obtain and review published and unpublished information on the consequences, for populations of wild prawns and other crustaceans, of exposure to WSSV and IHHNV infections
- Coordinate and assist with analyses to assess the impacts of WSSV and IHHNV on populations of wild prawns, other wild crustaceans and associated aquatic communities in Australia generally and in the Clarence River and the Townsville areas specifically
- Assist in preparation of consultants' report

Dr Neil Loneragan BSc(Hons) PhD

- Obtain and review information on biology of wild prawn populations, other crustacean populations and associated aquatic communities in Australia and the Asia-Pacific region generally, and in the Clarence River and the Townsville areas specifically
- Assist in analyses to assess the impacts of WSSV and IHHNV on populations of wild prawns, wild crustaceans and associated aquatic communities at the nominated sites
- Assist in preparation of consultants' report

1.3 Methodology

TOR 1

The consultants had ready access to extensive collections of information on the biology of prawns and associated aquatic communities both in Australia and the wider Asia-Pacific region, together with published and unpublished information on WSSV and IHHNV infections in farmed and wild prawn populations. They also had ready access to an extensive network of researchers both in Australia (Departments of Agriculture and Fisheries, Universities and CSIRO) and internationally (Asia, the Americas and Europe including the unit at Stirling University) who are active in these fields. In addition to undertaking a formal literature search, the consultants used this network to :

- obtain unpublished information on relevant comparative biological issues;
- obtain unpublished information on putative impacts, or lack of impacts, of WSSV and IHHNV infections on wild prawn populations and on associated aquatic communities.

Information was specifically sought on putatively affected or unaffected prawn populations and associated aquatic communities in endemic areas overseas which share important biological characteristics with identified key populations in Australia.

The information collected was critically evaluated and key issues synthesised. The following were then linked:

- information on the biological characteristics of the major prawn populations and associated aquatic communities in Australia and elsewhere;
- information on pathogenesis and epidemiology of WSSV and IHHNV infections (or where this is lacking, reasonable assumptions were applied, derived from first principles);
- available information on the reported effects on similar aquatic animal populations elsewhere.

This information was then used to develop an impact assessment model, which in turn was used to assess the impact on the Australian environment of establishment of WSSV and IHHNV.

TOR 2

A team member visited the Townsville and Clarence River sites to collect, from appropriate sources, necessary information on the nature of the areas' prawn and other crustacean populations, and their associated aquatic communities. As part of the general information gathering described under TOR 1, specific published and unpublished information was gathered on putative effects, or lack of effects, of WSSV and IHHNV infections on prawn populations, other crustacean populations and associated aquatic animal communities in specific overseas locations which are biologically and environmentally similar to those of the Clarence River and Townsville areas.

The collected information was critically evaluated and then key issues synthesised. As for TOR 1, the impact assessment model was then applied to assess the likely impacts of establishment of WSSV or IHHNV infections in the Townsville and Clarence River areas.

2 Australian prawns

Prawns are decapod crustaceans, a group which also includes lobsters and crabs. They belong to the suborder Natantia, which includes the infraorders Penaeidea, Caridea and Stenopodidae. In Australia, almost all prawns caught in commercial quantities are classified in the infraorder Penaeidea; relatively small quantities of caridean shrimp are caught off the northwest coast of Western Australia.

The commercial species of marine prawns belong to the family, Penaedae. Over 50 different species of penaeid prawns have been recorded from Australian waters, six of which are uniquely Australian. Of these, 10 species are considered to be of major economic importance, all belonging to two genera, *Penaeus* and *Metapenaeus*. Worldwide, the cultured prawn industry is based on *Penaeus* spp, the most desirable species being *P. vannamei*, and *P. stylirostris* which are indigenous to the Pacific west coast of the Americas; and *P. monodon* and *P. japonicus* which are Indo-Pacific species. Of the six uniquely Australian species, four have been cultured commercially: *P. esculentus, M. bennettae, M. macleayi* and *P. plebejus*.

The freshwater prawns belong to the genus, *Macrobrachium* which is a large genus comprising over 150 species of which several occur in Australia. The genus is widely distributed, mainly throughout the tropics but to a lesser degree within 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 *M. rosenbergii* which has consequently been transplanted to many places outside of its natural range.

A list of primary Australian prawn species indicating which have had reports of WSSV or IHHNV either as natural or experimental infections in other countries is shown in Table 2.1. The 12 major prawn species are also noted in the table.

Table 2.1: Summary of primary Australian prawn species indicating which have had reports of WSSV or IHHNV in other countries but not Australia

	Species		Major species	Virus report[1]
	Scientific name	**Common name**		
Cultured penaeids	*Penaeus esculentus*	brown tiger or tiger prawn		
	P. japoncius	Japanese king, Kuruma or tiger prawn	✓	**W, I**
	P. merguiensis	banana prawn		**W, I-**
	P. monodon	jumbo tiger, giant tiger, blue tiger, leader or panda prawn	✓	**W, I**
	P. plebejus	eastern king prawn		Aus
	Metapenaeus bennettae	greentail, inshore greasy-back or bay prawn		Aus
	M. macleayi	school or New South Wales school prawn		Aus
Wild penaeids	*Penaeus canaliculatus*	striped or witch prawn		Aus
	P. esculentus	brown tiger or tiger prawn	✓	
	P. indicus	banana, Indian banana or red legged banana prawn	✓	**W, I-**
	P. japonicus	Japanese king, Kuruma or tiger prawn		**W, I**
	P. latisculatus	western king prawn	✓	
	P. longistylus	red spotted or red spot king prawn		
	P. marginatus	aloha prawn		
	P. merguiensis	banana prawn	✓	**W, I-**
	P. monodon	jumbo tiger, giant tiger, blue tiger, leader or panda prawn		**W, I**
	P. plebejus	eastern king prawn	✓	Aus
	P. semisulcatus	grooved tiger prawn	✓	**W**
	Metapenaeus bennettae	greentail or greasy-back prawn	✓	Aus
	M. dalli	western school prawn		**W?**
	M. eboracensis	york prawn		**W?**
	M. endeavouri	endeavour or blue tail endeavour prawn	✓	Aus
	M. ensis	endeavour, offshore greasy-back or red endeavour prawn	✓	**W?**
	M. insolitus	greasy-back prawn		Aus
	M. macleayi	school or New South Wales school prawn	✓	Aus
Freshwater prawns	*Macrobrachium rosenbergii*	cherabin prawn		**W**
	Macrobrachium australiense			**W?**
	Macrobrachium intermedium			**W?**
	Macrobrachium novaehollandiae	long-armed prawn		**W?**
Common estuary shrimps	*Palaemonetes* spp			
Rock pool shrimps	*Palaemon* spp			

[1] Information obtained from Section 4 of this report.
W: WSSV; W?: Uncertain regarding WSSV infection as only name of infected genus provided; I: IHHNV; I-: Reported to be refractory to infection with IHHNV; Aus: Species found in Australia only, therefore there will be no reports of WSSV or IHHNV from other countries
Sources: A Guide to the Australian Penaeid Prawns, 1983; A Field Guide to Crustaceans of Australian Waters, 1994; Crustacean Farming, 1992; Production of Aquatic Animals, 1991 (see reference list)

2.1 Distribution, habitats and commercial importance

All Australian primary species of prawns are largely limited to the Indo-West Pacific region to a greater or lesser extent except where transplantations have occurred. Penaeid prawns in the genera *Penaeus* and *Metapenaeus* contribute to valuable commercial fisheries in all states of Australia, except Victoria and Tasmania (Dall *et al*. 1990, Kailola *et al*. 1993, Table). They are also caught by recreational fishers in the estuaries and nearshore waters of Australia.

Most species of Australian prawns are found in waters north of latitude 26ºS, which is in the region of Exmouth Gulf on the west coast and Moreton Bay on the east coast (Kailola *et al*. 1993). Species that extend into southern waters include:

- The western king prawn, *Penaeus latisculatus*, which is also found in south-western Australia and South Australia;
- The eastern king prawn *P. plebejus*, which is found only from the Swain Reefs (north of Fraser Island in Queensland) south to Port Phillip Bay in Victoria and northern Tasmania;
- The river and school prawns, *Metapenaeus macleayi* (Fraser Island to Corner Inlet in Victoria), *M. bennettae* (Rockhampton in Queensland to Gippsland Lakes in Victoria), and *M. dalli* (southwestern Australia).

A brief summary of the geographical distribution and habitat type for Australian prawn species is given below. Further discussion of habitat preferences for different life cycle stages of important commercial species is given in the next section.

2.1.1 *P. canaliculatus*

East and south-east Africa, Red Sea, Indian subcontinent, south-east Asia and Papua New Guinea to the Torres Straight in northern Australia. Reported in sea waters from depths of 33 to 46 metres. Only reported from the Torres Straight and considered rare and not of commercial importance in Australia.

2.1.2 *P. esculentus*

Restricted to tropical and subtropical Australia from central New South Wales through Queensland, Gulf of Carpentaria, Northern Territory to Shark Bay in Western Australia. Juveniles occupy shallow waters in estuaries. Adults found in coastal sea waters to depth of 200 metres over mud or sandy mud bottom. This species is fished commercially throughout its range.

2.1.3 *P. indicus*

East and south-east Africa, Red Sea, Persian Gulf, Indian subcontinent, south-east Asia and Papua New Guinea to northern Australia. In northern Australia from northern Western Australia through the Northern Territory to the Gulf of Carpentaria and north-east Queensland. Juveniles inhabit shallow estuarine waters. Adults found to depth of 90 metres over muddy or sandy bottom. Previously considered rare south of Kalimantan but now known to exist in commercial quantities in north western Australia. Forms the basis of the major commercial catches in east Africa, India, Malaysia, Thailand and Indonesia.

2.1.4 *P. japonicus*

East and south-east Africa, Red Sea, Persian Gulf, Indian subcontinent, south-east and north-east Asia, Papua New Guinea, northern Australia and Fiji. In northern Australia from the Northern Territory to the Gulf of Carpentaria and north-east Queensland. Transplanted to Mediterranean, Brazil and France. Inhabits inshore sea waters to depth of 90 metres over sand or mud and sand bottom. This is the major species in Japanese prawn fisheries and throughout the South China Sea. It supports small commercial fisheries in east Africa, Philippines and is a component of commercial catches in Papua New Guinea. Relatively uncommon in Australian waters. Along with *P. monodon*, it forms the basis of the cultured prawn industry in much of Asia as well as Australia.

2.1.5 *P. latisculatus*

East and south-east Africa, Red Sea, Persian Gulf, Indian subcontinent, south-east and north-east Asia, Papua New Guinea, Australia. In Australia from South Australia, Western Australia, Northern Territory, Gulf of Carpentaria and down the east coast to northern New South Wales. In Western Australia, juveniles occupy nursery areas in shallow waters of high salinity. Adults found to depth of 90 metres over hard, sand, sandy mud or gravel bottom. This species is the basis of the South Australian prawn fishing industry and provides a major component of the Western Australian catch in Shark Bay and Exmouth Gulf. It forms a minor component of the northern prawn fishery, generally appearing in isolated pockets.

2.1.6 *P. longistylus*

South-east Asia, Papua New Guinea to northern Australia. In northern Australia from central Queensland, Gulf of Carpentaria, Northern Territory to Shark Bay in Western Australia. Inhabits hard bottoms near reefs in depths from 35 to 55 metres. This species is included in commercial landings throughout its range but not in significant quantities.

2.1.7 *P. marginatus*

East and south-east Africa, Indian subcontinent, south-east and north-east Asia, Papua New Guinea to northern Australia. In northern Australia in north east Queensland and north west Western Australia. Inhabits inshore sea waters to a depth of 300 metres over sand or muddy sand bottom. This is an uncommon species in Australian waters.

2.1.8 *P. merguiensis*

Persian Gulf, Indian subcontinent, south-east Asia, Papua New Guinea to northern Australia. In Australia, in tropical and subtropical areas from mid New South Wales through Queensland, Gulf of Carpentaria, Northern Territory to Shark Bay in Western Australia. Postlarvae and juveniles enter shallow estuaries and rivers as part of their life cycle. Adults found in coastal sea waters to depths of 10 to 45 metres over muddy bottom. This is one of the most important commercial species in the Indo-Pacific region, being the basis of extensive prawn fisheries in Australia, Papua New Guinea, Indonesia, Philippines, and to a lesser extent in Malaysia, India, Pakistan and the Persian Gulf.

2.1.9 *P. monodon*

East and south-east Africa, Red Sea, Persian Gulf, Indian subcontinent, south-east and north-east Asia, Papua New Guinea and northern Australia. In northern Australia from Exmouth Gulf in Western Australia, Northern Territory, Gulf of Carpentaria and down the east coast to Moreton Bay in Queensland. Transplanted to Middle East, west Africa, central America and Italy. Juveniles occupy shallow estuarine waters occasionally entering rivers. Adults found in coastal sea waters to a depth of 110 metres over mud or sandy bottom. Generally uncommon in Australian waters but taken regularly in northern prawn fisheries. Taken in commercial quantities in India, Bangladesh and Malaysia. Forms the basis of the cultured prawn industry in both south east Asia and Australia.

1.1.10 *P. plebejus*

Restricted to the east coast of Australia form Lakes Entrance in Victoria to North Reef in Queensland. Inhabits estuarine and coastal sea waters to depth of 220 metres over sandy bottom. This species forms the basis of important prawn fisheries in New South Wales and Queensland and is fished in both the oceanic and estuarine phases of its life cycle.

2.1.11 *P. semisulcatus*

East and south-east Africa, Red Sea, eastern Mediterranean, Persian Gulf, Indian subcontinent, south-east and north-east Asia, Papua New Guinea and northern Australia. In northern Australia from northern Western Australia, Northern Territory, Gulf of Carpentaria and north east coast Queensland to Bowen. Juveniles inhabit shallow waters generally associated with seagrass beds. Adults found in coastal sea waters to a depth of 130 metres over sandy or muddy bottom. Previously considered rare in Australia but comprises a significant component of tiger prawn catches for Groote Eylandt, Melville Island and Port Essington grounds. Replaces *P. esculentus* as the most abundant species in northern prawn fisheries but the two species are not distinguished commercially.

2.1.12 *M. bennettae*

Restricted to the east coast of Australia from eastern Victoria to Hervey Bay in Queensland. Adults found in coastal rivers up to 15 km from the mouth and in coastal sea waters to depth of 14 metres, but predominantly in embayments in estuaries and coastal lakes. Generally found over soft, muddy bottom rich in organic detritus. This is an important species in New South Wales and southern Queensland where in forms the basis of extensive inshore prawn fisheries both amateur and commercial.

2.1.13 *M. dalli*

Restricted to south western Java to northern Australia from the west coast of the Northern Territory to southern Western Australia. Inhabits inshore waters in estuaries and rivers out to sea waters to a depth of 33 metres generally over sand or sandy mud bottom. This species in abundant in Western Australia where it forms the basis of an extensive amateur fishery in the Swan River, Peel Inlet and some other areas. It is common in inshore areas around Darwin and is occasionally reported from Indonesian waters near Java.

2.1.14 *M. eboracensis*

Restricted to the eastern Timor Sea, Arafura Sea, New Guinea and northern Australia. In northern Australia from Darwin through the Gulf of Carpentaria to Townsville on the east coast of Queensland. Inhabits inshore waters in estuaries and rivers out to sea waters to a depth of 33 metres generally over mud or sandy mud bottom. This is an abundant inshore species in the Northern Territory, especially during the monsoon period. It supports a small amateur fishery near Darwin but is not considered a commercial species.

2.1.15 *M. endeavouri*

Restricted to tropical and subtropical Australia from northern New South Wales through Queensland, Gulf of Carpentaria, Northern Territory to Shark Bay in Western Australia. Juveniles found in shallow estuarine areas less than 10 metres in depth with adults down to 50 metres off shore usually over mud or sandy mud bottoms. This species forms an important component of catches in the northern prawn fishery where it is often taken in association with tiger prawns (*P. esculentus, P. semisulcatus*) as well as *M. ensis*. The two species are not distinguished commercially.

2.1.16 *M. ensis*

East coast of Indian subcontinent, south-east and north-east Asia, Papua New Guinea to northern Australia. In Australia, in tropical and subtropical areas from central New South Wales through Queensland, Gulf of Carpentaria, Northern Territory to Shark Bay in Western Australia. Juveniles found in estuaries and occasionally in rivers. Adults in inshore sea waters at depths of less than three metres to offshore waters to a depth of 65 metres usually over mud or sandy mud bottom. This species is of major commercial importance in Singapore, Malaysia, Indonesia and north to Japan. It forms a significant component of endeavour prawn catches in northern Australia where it is generally found in association with *M. endeavouri*. The two species are not distinguished commercially.

2.1.17 *M. insolitus*

Restricted to northern Australia from Darwin eastward to the Gulf of Carpentaria. Inhabits inshore waters including creeks and shallow estuaries to a depth of 33 metres generally over mud or sandy mud or sandy bottom. This is an abundant inshore species around Darwin but not commonly reported in commercial catches in deeper waters.

2.1.18 *M. macleayi*

Restricted to the east coast of Australia from north eastern Victoria to Moreton Bay in Queensland. Abundant in New South Wales. Occupies estuarine to offshore waters to a

depth of 60 metres showing a preference for turbid water and soft muddy bottoms. An important prawn species for the Australian domestic market especially along the east coast.

2.1.19 *Macrobrachium rosenbergii*

North west India, south-east Asia, Papua New Guinea to northern Australia. Transplanted to Caribbean Islands, USA, Hawaii, Israel and southern Africa. The largest freshwater species in Australia and inhabits streams across northern Australia. It is an important aquaculture species in south east Asia.

2.1.20 *Macrobrachium australiense*

Occurs in freshwater streams throughout eastern Australia from Townsville to central New South Wales and South Australia.

2.1.21 *Macrobrachium intermedium*

The commonest freshwater species in southern Australia. Inhabits estuaries and shallow inshore waters often in seagrass beds.

2.1.22 *Macrobrachium novaehollandiae*

Common in estuaries in eastern States of Australia.

2.1.23 *Palaemonetes* spp

Small, translucent shrimps found in inshore marine waters and estuaries as well as rivers and lakes with low salinities.

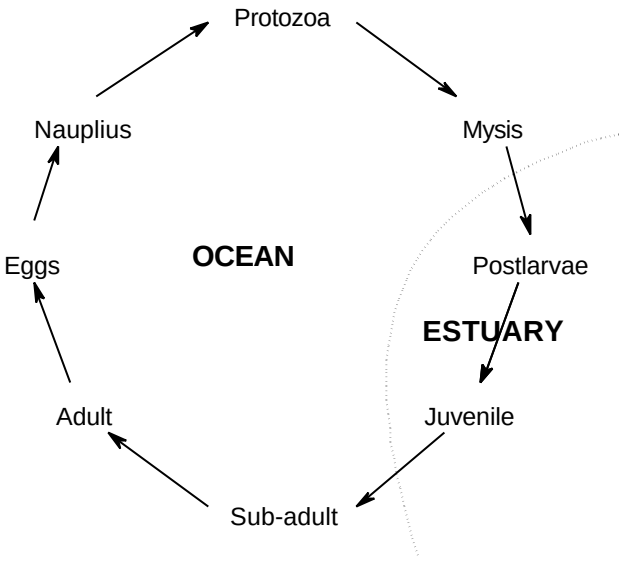
2.1.24 *Palaemon* spp

Small shrimps occurring Australia wide in shallow subtidal and intertidal marine and estuarine waters, often rock pools. Species of this genus occur elsewhere in the world.

2.2 Biology and ecology of prawns

The basic environmental requirements for penaeid prawns are: temperature 23-32^0 C (73-90^0 F); dissolved oxygen above three ppm; pH 8.0; and salinity 10-30 parts per thousand. They reproduce sexually. Maturation and spawning generally occurs in offshore waters with eggs hatching in 18-24 hours at 28^0 C. Figure 2.1 illustrates the life cycle.

Figure 2.1: Schematic illustration of life cycle of penaeid prawns



The first stage (nauplius) has five sub-stages. During this period, lasting approximately 2-3 days, the larvae change from totally planktonic organisms subsisting on their own egg yolk, to having rudimentary feeding appendages.

The protozoal stage which follows lasts 3.5-5 days and consists of three sub-stages. During this period of development, the larvae are capable of feeding on planktonic plant material and occasionally planktonic animals in addition to their egg yolk. They are unable to swim for food and therefore rely upon encountering food as they float within the water column. During this development stage, the body becomes more elongated and a carapace, two compound eyes, and uropods are present.

The mysis stage is about the same duration as the previous stage and also consists of three sub-stages. Development is characterised by further elongation of the body, telson and pleopods. Swimming and food seeking occurs and the preferred diet changes from phytoplankton to zooplankton.

Generally it takes 12-15 days from egg to postlarval stage depending on temperature and food availability. During the initial 5-6 days, postlarvae change from pelagic to benthic organisms. The postlarval prawns migrate from the open ocean into nearshore areas and estuaries which serve as nurseries where suitable food is usually plentiful.

Prawns typically feed on a wide range of food items and their diet changes as they increase in size. In the early larval stages, microalgae can be a major part of the diet, which typically diminishes in importance at later stages of development. Small crustaceans such as amphipods and copepods are important components of the juvenile diet (Dall *et al.* 1990). Subadult and adult prawns also feed on polychaete worms and molluscs.

Densities of prawns in different environments are dependent on a wide range of factors. High densities of tiger and endeavour prawns in seagrass are from about two to four prawns per square metre, compared with five to 60 prawns per square metre for banana prawns in mangroves (Haywood and Staples 1993).

2.2.1 Larvae, postlarvae and juveniles

For species with an offshore spawning, the planktonic larvae migrate inshore within two to three weeks and settle in coastal and estuarine nursery grounds as postlarvae, the smallest juvenile stage. The planktonic phase in the life cycle involves a transition in stages from egg to nauplii, protozoae, mysis and postlarvae. In the north-eastern and north-western Gulf of Carpentaria, benthic postlarvae recruit to their estuarine and inshore nursery habitats in most months, except winter (June to August) (Loneragan *et al.* 1994, Vance *et al.* 1996, 1998).

The juvenile nursery habitat differs between species of prawns. For example, juvenile tiger prawns (*Penaeus esculentus* and *P. semisulcatus*) and blue endeavour prawns (*Metapenaeus endeavouri*) are found in beds of seagrass and algae (Staples *et al.* 1985, Coles and Lee Long 1985, Coles *et al.* 1993, Haywood *et al.* 1995); western king prawns (*P. latisculatus*) in sandy substrates (Potter *et al.* 1991) and banana prawns (*P. merguiensis*) in mangrove-lined creeks and rivers (Staples *et al.* 1985, Vance *et al.* 1990). Juvenile grooved tiger, banana and greasy back prawns (*Metapenaeus* spp) extend into estuarine waters where salinities decline below that of seawater.

Tropical postlarval and juvenile prawns are usually prolific in their nursery habitats between September and May with low numbers from July to August (Vance *et al.* 1996, 1998). An exception to this is the eastern king prawn *Penaeus plebejus* in Moreton Bay, which is found in high numbers from about May until October (Young and Carpenter 1977).

Juvenile prawns emigrate from their nursery habitats in tropical waters at a size of about 10 mm carapace length and one gm wet weight (Loneragan *et al.* 1994, Vance *et al.* 1996, 1998). This emigration is related to both attaining a certain size, and for some species, an environmental stimulus. For example, banana prawns (*P. merguiensis*) and school prawns (*Metapenaeus macleayi*) emigrate from their nursery habitats in response to factors associated with rainfall (eg decreased salinity, increase in river flow, increase in the disruption of the substratum) (Ruello 1973, Glaister 1978, Staples and Vance 1986, Vance *et al.* 1998). The size at emigration may be slightly higher (1 to two g, or 15 mm carapace length) in sub-tropical waters such a those of Moreton Bay (O'Brien 1994).

Mortality rates of early prawn stages are variable and difficult to estimate. For tiger prawns (*P. esculentus*) in Moreton Bay, O'Brien (1994) estimated the weekly at 0.16 million (range = 0.06 to 0.29) or as a percentage at 14% per week (range = 5.8 to 25%). For banana prawns (*P. merguiensis*) in the Embley River estuary (Haywood & Staples 1993) natural mortality rates are higher than those of tiger prawns with weekly mortalities of 0.46 million (range = 0.23 to 0.94) or as a percentage at 36% per week (range = 21 to 61%).

Natural stressors on juvenile prawns in the wild are likely to be prolonged periods of exposure to high temperatures and/or low dissolved oxygen concentrations and, possibly,

run-off from acid sulfate soils. Low dissolved oxygen concentrations can be induced by die-off or respiration of algal blooms.

2.2.2 Sub-adults and adults

Prawns reach sexual maturity at an age of about six months in tropical waters and may live to between 18 and 24 months of age (Dall *et al.* 1990). The offshore habitats of the sub-adult and adult prawns also differ between species, mostly in relation to substrate type and depth (Somers 1994). The white banana prawn *P. merguiensis* is most abundant in shallow less than 20 m deep, independent of substrate type, while the red-legged banana prawn *P. indicus* is caught in deeper waters (55 to 70 m). The brown tiger prawn *P. esculentus* and blue endeavour prawn *M. endeavouri* are most abundant in relatively shallow water (< 35 m deep), where the substrate is sand or muddy sand. In contrast, the grooved tiger prawn *P. semisulcatus* is most abundant in deeper water (> 35 m deep) on substrates of mud or sandy mud. The red endeavour prawn *M. ensis* has a more limited distribution than the blue endeavour and is most abundant between water depths of 35 to 45 m, where the substrate contains more than 60% mud. The red-spotted king prawn *P. longistylus* is found on sandy sediments.

Tagging experiments involving adult *M. endeavouri* and *P. esculentus* in the Gulf of Carpentaria, indicate prawns generally do not move large distances, with a mean distance moved from three releases for *M. endeavouri* of about 15 km over a period of 41 days (Somers and Kirkwood 1984, Buckworth, 1992). The grooved tiger prawn *P. semisulcatus* migrates further offshore than *P. esculentus*. The largest recorded distances moved by tagged prawns in Australia are for eastern king prawns *Penaeus plebejus*, with a maximum recorded distance of over 700 km (Ruello, 1975).

2.2.3 Spawning times and habitats

Mating and spawning of penaeids are separated in time. Spermatophores are formed during the passage of spermatozoa down the vasa deferentia. The spermatophore contains the spermatozooans and is implanted on the female during mating. Most penaeids have open thelyca and they mate towards the end of the moult cycle after the ovary has matured. In species with a closed thelyca, the spermatophore is deposited when the female has just moulted. In the closed-thelycum species, spermatophores are implanted just after the female moults, and spawning occurs when the cuticle has hardened and is in early premoult. The spermatophore for some species must remain viable for 10 to 20 days. In open-thelycum species, the female must be impregnated less than three days before spawning if fertilisation is to be successful. Fertilisation is external and occurs when the eggs are extruded and pass the spermatophore and spermatozooa are released (Dall *et al.* 1990).

In general, prawns in the genus *Penaeus* mate and spawn in offshore waters. However, some species of prawns, mainly in the genus *Metapenaeus* (the greasy back or school prawns), are able to complete their life cycles within estuaries or nearshore waters (eg. Grey *et al.* 1983, Potter *et al.* 1989, Dall *et al.* 1990).

Prawns in tropical waters can spawn throughout much of the year although two peaks spawning have been identified for grooved tiger prawns *P. semisulcatus*: spring – August to

November and summer – February to April (Crocos and van der Velde 1995). For more temperate regions, there may be only one peak of spawning (eg *Metapenaeus dalli*, Potter *et al.* 1989).

Prawn farmers buy mature, mated female *P. monodon* and *P. esculentus* caught in the wild to provide their broodstock. However, they are able to mate and spawn *P. japonicus* in captivity. Most of the broodstock for *P. monodon* comes from the Cairns region in northern Queensland.

The habitat of the sub-adult and adult prawns, and hence probable spawning location, varies with the type of substrate and depth. The habitats and spawning depths are summarised in Table 2.2. Little is known about the life history of *P. monodon* or *P. japonicus* in the wild in Australia (Kailola *et al.* 1993).

Table 2.2: Preferred habitats of sub-adult and adult prawns (based on Somers 1994)

Species	Depth (m) at which abundant	Spawning depth (m)	Preferred substrate
White banana prawn *P. merguiensis**	10 to 20	<20 m	mud
Red-legged banana prawn *P. indicus*	55 to 70	unknown	mud
Brown tiger prawn *P. esculentus**	<35 m	<35 m	sand to muddy sand
Blue endeavour prawn *M. endeavouri**	<35 m	<35 m	sand to muddy sand
Grooved tiger prawn *P. semisulcatus**	> 35 m	> 35	mud or sandy mud
Red endeavour prawn *M. ensis*	35 to 45 m		> 60% mud
Red-spotted king prawn *P. longistylus**			sand
Kuruma prawn *P. japonicus*			sandy mud, sand
Giant tiger prawn *P. monodon*			mud or sand

* Abundant in the Townsville region.

2.2.4 Prawns in the food web

Prawns typically feed on a wide range of food items and their diet changes as they increase in size. In the early larval stages, microalgae can be a major part of the diet, which typically diminishes in importance at later stages of development.

Penaeid prawns are eaten by many species of fish and elasmobranchs such as sharks, rays, sawfishes (Derbyshire and Dennis 1990.). In a study of predation on juvenile tiger prawns in the Embley River estuary (north-eastern Gulf of Carpentaria), 37 of the 132 species of fish and elasmobranchs were found with prawns in their guts (Haywood *et al.* 1998). This included five species caught by commercial and recreational fishers (queenfish *Scomberomoides commersonianus*, giant threadfin *Eleutheronema tetradctylum*, estuary

cod *Epinephelus suillus*, barramundi *Lates calcarifer*, moses perch *Lutjanus russelli*). Previous work in the Embley River estuary has shown that another commercially and recreationally important fish, the threadfin *Polydactylus sheridani*, is also a major predator of prawns (Salini *et al.* 1990). Sharks and rays were also important predators on prawns in the Embley River estuary (Salini *et al.* 1990, Haywood *et al.* 1998) and the western Gulf of Carpentaria (Brewer *et al.* 1995). Several small species of fish that are eaten by larger fish, such as glassfish (Ambassidae, *Ambassis nalua*), also ate prawns in the Embley River (Haywood *et al.* 1998).

Laboratory studies have shown that when the movement of prawns is restricted by tethering them to short lengths of tubing, other species such as blue swimmer crabs (*Portunus pelagicus*) and pufferfish (family Tetradontidae) will successfully capture and eat prawns (M Haywood, CSIRO Marine Research, unpublished data). Moribund or diseased prawns, may therefore be eaten by a wider suite of species than healthy prawns.

3 Australian prawn industries

The prawn industries are discussed, mainly with a view to identifying the different components of the industries likely to be affected in the event of establishment of exotic viruses.

In 1996-97, prawns comprised 19.5% of the total Australian fisheries production of $1.76 billion and 14% of total fisheries exports of $1.3 billion. Domestic demand in 1996-97 (also referred to as "disappearance") was 29,853 tonnes (domestic production of 27,856 tonnes plus imports of 12,887 tonnes less exports of 10,890 tonnes) which includes all product types (ABARE 1997). Australian prawn production is primarily through trawling in marine waters with lesser amounts produced from river trawling and aquaculture. Recent production levels are shown in Table 3.1.

Table 3.1: Australian prawn production - capture and culture fisheries

		Production (tonnes)		
Location and fishery type		**1994-95**	**1995-96**	**1996-97**
New South Wales	Capture	1 662	1 822	1 849
	Culture	248	271	271
Victoria	Capture	32	12	2
Queensland	Capture	7 436	8 804	8 270
	Culture	1 424	1 294	1 355
Western Australia	Capture	3 989	3 940	3 995
South Australia	Capture	2 059	2 271	2 024
Northern Prawn[1]	Capture	9 097	8 860	8 279
Torres Strait[1]	Capture	1 821	1 584	1 624
Other C'wealth	Capture	197	329	187
Total	**Capture**	**26 293**	**27 622**	**26 230**
	Culture	**1 672**	**1 565**	**1 626**

[1] Commonwealth Fishery areas: Northern Prawn - Gulf of Carpentaria, Cape York to Cape Londonderry; Torres Strait - Cape York to south coast Papua New Guinea; Other Commonwealth - South East and other Commonwealth fisheries.
Source: ABARE - Australian Fisheries Statistics1997, Tables 2, 3, 4, 6, 8, 13.

The major commercial wild and cultured species as well as the geographical distribution and habitats for wild prawns were described in Section 3 of this report.

The Northern Prawn Fishery is located in Commonwealth waters bordered by Cape Londonderry in the west and Cape York in the east. It is the largest fishery by area at over one million square kilometres and an annual catch over 8,000 tonnes. The three main types of prawn caught are banana (*P. merguiensis*), tiger (*P. esculentus*, *P. semisulcatus*) and endeavour (*M. endeavouri*, *M. ensis*) with banana and tiger comprising almost 85% of the catch. There are smaller catches of king prawns (*P. latisculatus*). In 1996-97, the total catch declined for the third consecutive year mainly due to decreasing catches of tiger prawns. The sustainable, long term average annual yield is considered to be 4,000 tonnes of

banana prawns and 3,785 tonnes of tiger prawns (1,866 tonnes of *P. esculentus* and 1919 of *P. semisulcatus*). There was evidence that tiger prawn stocks were overfished in 1996 (ABARE 1997).

The Torres Strait Fishery is located between the tip of Cape York Peninsula and the south coast of Papua New Guinea, bordered on the west by the Arafura Sea and the east by the Coral Sea. The two main types of prawns caught are brown tiger (*P. esculentus*) and endeavour with small amounts of red spotted king (*P. longistylus*). Tiger and endeavour comprise 95% of the annual catch of around 1,500 tonnes. Estimated, sustainable yields from the fishery range from 1,370 to 2,850 tonnes annually (ABARE 1997).

The cultured prawn industry is located on the coast in northern New South Wales, southern and northern Queensland and contributed approximately 5.8% of Australia's total prawn production in 1996-97. Most of the industry is in Queensland. The main cultured species in Australia is the giant tiger prawn *Penaeus monodon*, with much smaller quantities of kuruma prawns *P. japonicus* and brown tiger prawns *P. esculentus*. In 1996-97 *P. monodon* comprised approximately 82% of the harvest. There were approximately 35 farms in production covering approximately 480 hectares in total with an average pond size of nearly one hectare. Of these 35 farms, 27 produced *P. monodon*. In 1993/94 there were nine hatcheries which produced approximately 146 million *P. monodon* and 17.5 million *P. japonicus* postlarvae requiring 2,600 and 550 spawners respectively (Anon 1995). Prawn farmers buy female *P. monodon* and *P. esculentus* caught in the wild to provide their broodstock. However, they are able to mate and spawn *P. japonicus* in captivity. Most of the broodstock for *P. monodon* comes from the Cairns region in northern Queensland. In 1996 there was a shortage of spawners on the east coast and consideration was given to bringing some from the north-west of Australia.

The size of the cultured prawn industry in Australia is quite small by international standards, but it is an emerging industry with potential for growth and export income generation. It has been estimated that growth to 11,000 tonnes per year is achievable by the year 2005 (Anon 1995). However, given the relative stability in production over the last three years at around 1,600 tonnes, it appears unlikely that this estimate will be realised.

While world production of prawns obtained from capture fisheries has been fairly stable in recent years, that of cultured prawns has increased dramatically from around 90,000 tonnes in 1980 to 762,000 tonnes in 1995 and now represents almost a third of global supply (Briggs 1994). Approximately 50% of cultured prawn production is based on *P. monodon* while more than 80% of total world production of cultured prawns is in Asia. Thailand is the dominant cultured prawn producer with more than 80,000 hectares under production in 20,000 farms and 2,000 hatcheries in 1994 (Anon 1995).

The increase in the production of cultured prawns has some significance with respect to international spread of disease as pathogens are likely to be more prevalent in culture systems compared with wild prawns. Disease is one of the predominant technical issues faced by the cultured prawn industry in many countries, especially in Asia (Patmasiriwat *et al.* 1996), a phenomenon common to all intensive animal systems when management and

hygiene are not practised at the highest level. For example, production of cultured prawns in Taiwan collapsed over three years from approximately 80,000 tonnes in 1988 to 9,000 tonnes in 1990 mainly because of disease (Funge-Smith and Stewart 1996). In Sri Lanka, an epidemic of WSSV in 1996 was estimated to have resulted in 85% of total farm area becoming nonfunctional during that year (Jayasinhe 1996). Most countries with large cultured prawn industries have substantial problems with disease with many of the viruses showing epidemic and sometimes pandemic behaviour. Recent rapid growth in production in countries such as Thailand is likely to mean that infectious diseases will be a major problem for some time.

4 Viruses of interest

This section reviews information about the two viruses of interest which is relevant to disease spread and impact on populations. Additional information contained in the previous Scientific Review of Prawn Diseases has also been updated and is attached as Appendix 1.

4.1 White spot syndrome virus (WSSV)

Five baculoviruses have been reported to cause white spot syndrome (WSS) in prawns. These are: hypodermal and haematopoietic necrosis baculovirus (HHNBV; Huang *et al.* 1994, cited by Lightner 1996b) in China; rod-shaped nuclear virus of *P. japonicus* (RV-PJ; Inouye *et al.* 1994) in Japan, China and Korea; systemic ectodermal and mesodermal baculovirus (SEMBV; Wongteerasupaya *et al.* 1995) in Thailand; white spot baculovirus (WSBV; Wang *et al.* 1995) in Indonesia, Vietnam, Malaysia, India, South Carolina and Texas; and *Penaeus monodon* non-occluded baculovirus (PMNOB; Lo *et al.* 1995) in Taiwan. In this document these viruses will be referred to collectively as white spot syndrome virus (WSSV; Lightner 1996b). It will be assumed that they are closely related and that the pathogenicity of each for Australian prawns is essentially similar.

Given that a wide range of penaeid prawn species, other prawns, crabs, lobsters and miscellaneous arthropods have been reported, or suspected, to be infected (Flegel 1997, Wang *et al.* 1998), it is likely that many Australian prawn, other crustacean and arthropod species, in addition to those included in the lists below, will be susceptible to WSSV infection.

4.1.1 Infections in wild prawns

Infections have been observed in wild populations of *P. monodon, P. japonicus, P. semisulcatus, P. penicillatus, P. duorarum* and *Metapenaeus ensis* (Lo *et al.* 1996; Kou *et al.* 1997b; Wang *et al.* 1997c; Chanratchakool unpublished; Flegel unpublished; Ray unpublished). It is important to note, however, that the levels of infection in wild prawns may be much lower than in farmed stocks. Lo *et al.* (1997b) used *in situ* hybridisation methods to examine a wide range of tissues from wild-caught *P. monodon* naturally infected with WSSV. They found that the number of positive cells in each tissue was relatively limited, in contrast to findings in farmed or experimentally infected prawns, which showed serious levels of infection in a similar range of tissues. Similarly, Kou *et al.* (1997b) reported consistently large amounts of WSSV, as detected in farmed *P. monodon, P. japonicus, P. penicillatus* and *Metapenaeus ensis* by one-step PCR. However, of 30 wild *P. monodon* which tested positive for WSSV on capture, only six were positive with one-step PCR. Infection in the remaining 24 could be detected only via two-step PCR, which is 10^3-10^4 times more sensitive than one-step PCR (Lo *et al.* 1996).

WSSV prevalence in wild stocks may also vary between species. Kou *et al.* (1997b) noted the prevalence of WSSV in wild-caught *P. monodon* populations from Taiwanese coastal waters was relatively high compared with *P. japonicus, P. semisulcatus* and *P. penicillatus*.

4.1.2 Infections in farmed prawns

Infections have been observed in farmed *Penaeus monodon, P. japonicus, P. chinensis, P. indicus, P. merguiensis, P. penicillatus, P. setiferus* and *Metapenaeus ensis* (Lightner 1996b; Lo *et al.* 1996; Kou *et al.* 1997b; Momoyama *et al.* 1997).

4.1.3 Experimentally induced infections in prawns

Experimental infections have been reported in *P. vannamei, P. stylirostris, P. aztecus, P. duorarum, P. semisulcatus, P. setiferus, Metapenaeus ensis, Metapenaeus* spp and *Parapenaeopsis* spp (Chang *et al.* 1996; Lightner, 1996b; Momoyama *et al.* 1997). Lo *et al.* (1997b) noted significant differences in susceptibility to experimental WSSV infection amongst postlarvae of *P. japonicus, P. monodon* and *P. semisulcatus*.

4.1.4 Age/size susceptibility

While most mortalities in farmed prawns occur in young juvenile prawns weighing 3-5 gm (Takahashi *et al.* 1994), WSSV outbreaks can occur in ponds at any time during the postlarval to adult stages (Chanratchakool *et al.* 1998). There is no information regarding disease outbreaks in wild stocks.

4.1.5 Other Australian crustacea and arthropods potentially at risk

4.1.5.1 WSSV in wild non-penaeid populations

Lo *et al.* (1996) detected WSSV in wild populations of crabs *(Charybdis feriatus, Portunus pelagicus* and *P. sanguinolentus)* from Taiwanese coastal waters. In addition, copepods, the pest crab *Helice tridens*, small pest prawn (Family Palaemonidae) and insect larvae (Family Ephydridae) collected from WSSV-affected prawn farms were shown to be carrying the virus. Yu and Wang (1997) showed that, of 36 copepod samples collected from Chinese growout ponds and coastal waters, 16 were positive, 10 doubtful and 10 negative. They suggested that the 'viruliferous rate' of copepods was higher than that of prawns and that viral infection was detected earlier in these animals than in prawns. In a survey of infection in small crustaceans, also collected from growout ponds and coastal waters, these authors suggested a viral load sequence ie Artemia > mysis > *Acetes* > *Palaemon* > *Carinicauda* > *Alpheu* > crab > gammarid. In a study of non-crustaceans, they found 'very high' P/N values for sea anemones and a goby (*Synechogobius hasta*), while values for clam worms, shellfish, insects (mainly from prawn ponds) and other small fish were low. They suggested that filter feeders such as sea anemones, or fish, may reduce viral loads if they co-exist with other infected populations. They further suggested that small crustaceans such as copepods and artemias may be vectors of WSSV. However, it is important to recognise that it is not known if WSSV replicates in insects or copepods nor whether it causes disease in them Wang *et al.* (1997a).

4.1.5.2 Infections in farmed non-penaeid populations

Natural infections have been observed in farmed *Scylla serrata* and farmed *Macrobrachium rosenbergii* (Lo *et al.* 1996; Kou *et al.* 1997b).

4.1.5.3 Experimental infections in non-penaeid animals

Experimental infections have been induced in larval *Macrobrachium rosenbergii*, resulting in some mortalities and carrier status amongst adult survivors (Chang *et al.* 1996).

Table 4.1 shows a summary of the results obtained by Jiang *et al.* (1996) who examined the susceptibility of some non-penaeid crustacean species to experimental WSSV infection, and the infectivity of clinically normal survivors to transmit infection when fed to *P. monodon*.

Table 4.1: Summary of experimental findings of Jiang *et al.* (1996)

Species	Route of exposure	Effects	Consequences for *P. monodon* which were fed injected survivors
P. pelagicus	Injection	100% mortality within 10 days	NA
M. rosenbergii	Injection	20% mortality after 10 days	All survived
Palaemon styliferus	Injection	35% mortality after 10 days	All died within 14 days

The authors concluded that (1) *P. styliferus* are carriers of WSSV or are susceptible to infection with a relatively long incubation period; (2) *P. pelagicus* are susceptible to WSSV; and (3) *M. rosenbergii* are resistant to WSSV.

Supamattaya *et al.* (1998) demonstrated the consequences for experimental infection of non-penaeid species and these are shown in Table 4.2.

Table 4.2: Consequences of experimental infections of WSSV on various crustacea demonstrated by Supamattaya *et al.* (1998)

Species	Route of exposure	Consequence
Sand crab (*P. pelagicus*)	Ingestion Injection	Infection; no mortalities 100% mortalities in 8 days
Mud crab (*S. serrata*)	Ingestion Injection	Infection; no mortalities 20% mortalities in 9 days
Krill (*Acetes* sp.)	Ingestion Injection Immersion	20% mortalities in 9 days 100% mortalities in 3 days 100% mortalities in 5 days

4.1.6 Role of stress in WSSV infections

Limited research has shown that natural mortality can be as high as 93% for sub-adult and adult prawns of *Penaeus* spp (Glaister *et al.* 1993 - cited by Ludescher 1997).

It is likely that environmental conditions largely determine mortality rates following experimental or natural WSSV infection. In an experimental infection trial, Wang *et al.* (1997b) showed that cumulative mortality was 40% within 14 days under stress conditions. No mortality was observed in controls or in nonstressed infected prawns. The results suggested that environmental stressors such as high levels of unionised ammonia may increase mortality rates associated with WSSV infections in prawns.

Other evidence suggests that morbidity and mortality rates in WSSV infected prawns increase when the prawns are stressed. This is the basis for the pond-side pre-stocking postlarval stress test involving 30 minutes exposure to 100 ppm formalin, and removal of weak (putatively WSSV-infected) animals. It is also the basis for the recommendation that 25-30 ppm formalin be added to WSSV-positive ponds in the period up to 60 days post stocking. The formalin selectively kills or debilitates infected prawns, and they can be removed before they transmit infection to other prawns in the pond (Limsuwan 1997).

Similarly, Chanratchakool (unpublished; AAHRI shrimp health management course 1998) suggests losses in farmed prawns infected with WSSV can be minimised by good management. He suggests that cuticular epidermal cells are responsible for osmoregulation and, if the pond's osmotic environment is optimal, many infected prawns will survive. In a farm trial, approx 40% survival (versus the standard healthy crop expectation of 60%) was achieved by good management and progressive removal of infected prawns using pre and post-stocking formalin exposures.

4.1.7 Transmission of WSSV

Mohan *et al.* (1997) suggested that, since WSSV targets the prawn's ectodermal and mesodermal tissues, and is not seen in endodermal tissues such as hepatopancreas and midgut epithelium, faecal contamination is unlikely to be important in transmission of WSSV. They concluded that most infection is transmitted either vertically, or horizontally via ingestion of infected tissue.

4.1.7.1 Vertical transmission

Postlarvae are thought to be infected during spawning. It is not known whether infection is transmitted via gametes, but current evidence suggests connective tissues in gonads may be a source of viral contamination. Lo *et al.* (1997a) were first to detect evidence of WSSV in reproductive organs of black tiger prawns. In testes, WSSV-positive cells were located in the connective tissue layer surrounding the seminiferous tubules but no germ cells were found to be infected. In the spermatophore, only muscle and connective tissue cells were WSSV positive. In the ovary, follicle cells, oogonia, oocytes and connective tissue cells were WSSV positive. However, the authors were unable to find infected mature eggs, and suggested that infected ova were killed by the virus before maturation.

Kou *et al.* (1997a) reported WSSV infection rates of 16-75%, depending on capture location, in wild caught adult *P. monodon* from Taiwanese waters. The authors examined ovaries of WSSV positive animals and found that most of the WSSV positive cells were follicle cells, oogonia and connective tissue cells. Many oogonia and a few developing egg cells gave strong positive signals of WSSV DNA in the nucleus .

In a related study, Kou *et al.* (1997b) reported 30 of 48 (62.5%) wild-caught *P. monodon* broodstock from Taiwanese waters were WSSV PCR positive. Of these, six were positive in one-step WSSV PCR, while 24 others were positive only in two-step WSSV PCR. Not all of these broodstock specimens spawned successfully during the study period (several days to several months). Broodstock which were one-step PCR positive invariably died before spawning. Among remaining prawns that did spawn successfully, none were heavily infected (ie none tested positive with the one-step PCR) prior to spawning. After spawning, however, most of these specimens became one-step PCR positive, which the authors attributed to the stress inherent in spawning. They also noted that, on rare occasions, the offspring of two-step PCR positive female spawners were two-step PCR negative, while the offspring of two-step PCR negative female spawners were two-step PCR positive.

Any increase in probability of vertical transmission of infection is unlikely under natural conditions, given that carriers are probably to some extent debilitated by infection, and therefore less likely to spawn successfully. However, wild stock enhancement programs, such as that in Thailand, may increase the proportion of infected adults in a wild population and hence increase the proportion of infected postlarvae subsequently produced by that population. Under the Thai program, some pond-reared stock (presumably on average having a higher WSSV infection rate than wild stocks) are released into the wild as part of a government sanctioned wild stock enhancement program (Tookwinas 1996).

Similarly, the Chinese wild stock enhancement program (Ren and Deng 1996), under which hatchery-reared *P. chinensis* postlarvae derived from wild-caught broodstock are released back into the wild, may have increased the proportion of infected animals in wild populations. Kou *et al.* (1997b), in a study of WSSV PCR positive wild-caught *P. monodon* broodstock from Taiwanese waters, showed that broodstock which were one-step PCR positive invariably died before spawning. Of the remaining broodstock which spawned successfully, none were heavily infected (ie they tested negative with the one-step PCR) prior to spawning. Following spawning, however, most of these broodstock were one-step PCR positive, which the authors attributed to the stress inherent in spawning. If a similar mechanism operated in the *P. chinensis* broodstock used in the stock enhancement program, it seems likely that the progeny of these spawnings would have a higher prevalence of WSSV infection than would the progeny of less stressful natural spawnings.

4.1.7.2 Horizontal transmission

WSSV is thought to be transmitted primarily via ingestion of infected clinically normal, moribund or dead prawns, or other crustacean carriers, perhaps including copepods, which may be a major source of virus (Chang *et al.* 1996; Flegel *et al.* 1997; Lo *et al.* 1996; Mohan *et al.* 1997; Yu and Wang 1997).

Results of transmission experiments conducted in aquaria indicate WSSV can be transmitted via water (Flegel unpublished) and that WSSV remains viable in seawater for 3-4 days (Flegel *et al.* 1997).

Chanratchakool (unpublished) failed to induce infection in prawns in aquaria exposed to crabs which had been injected with WSSV. Prawns became infected only when the crabs died and were eaten by the prawns. When infected crabs were placed inside cages in aquaria, prawns became infected and died only after the crabs died. By contrast, Maeda *et al.* (1998) showed that WSSV was transmitted from infected shore crabs to healthy kuruma prawns in a cohabitation experiment. The crabs used in the experiment also died during the cohabitation period without showing white spots but showing positive reaction by 1-step PCR. This result suggested that the virus carried in shore crabs was released into the tank water, and infected the healthy prawns.

Prawn-eating gulls, other seabirds and aquatic insects may also be factors in the spread of viruses such as WSSV between and within regions (Lightner 1996a; Garza *et al.* 1997).

If we assume that ingestion of infected material is the most likely initial exposure route for individual wild prawns or other animals in naïve populations, the replicating virus must either kill or debilitate any new host in order to facilitate the subsequent horizontal transmission of infection via ingestion by other susceptible host animals. At the individual animal level, several factors will influence this process :

- infectivity, pathogenicity and virulence of the virus;
- size of the infective dose;
- susceptibility of the exposed species;
- level of stress in the exposed species.

The probability that infection will be transmitted to other susceptible hosts, rather than to non-susceptible hosts such as finfish and other predators/scavengers, will be increased if the following occur:

- there is a high probability that infective material will be ingested by susceptible hosts; presumably this will occur if susceptible individuals are concentrated at a contaminated site or if distances between animals in an infected population remain low;
- animals are debilitated by infection, thereby increasing the probability of predation;
- infected populations remain physically concentrated or;
- the populations are stressed.

Wild prawns may be most susceptible to such exposures during certain critical time periods. Wild penaeid populations are most dense during immigration of postlarvae and emigration of juveniles. In addition to these spatial and temporal relationships, other important factors in assessing potential exposures to native prawn species include the volume of effluent discharges from prawn farms and processors, as well as disinfection and quarantine procedures used in these facilities.

4.2 Impacts of WSSV

4.2.1 Wild prawn populations

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 effects of infection on wild stocks in China, Thailand and Japan, where WSS has occurred in adjacent populations of farmed prawns and useful wild catch data are available, are discussed below. However, in considering this information, it is essential the impacts of WSSV on wild populations in each these countries are assessed in the light of official stock enhancement programs undertaken during recent decades.

The earliest recorded reseeding of prawns was in Japan (Seto Inland Sea) beginning in 1964 (Kurata 1981). China has been releasing juvenile prawns into the wild on a commercial scale since 1984. The Chinese seed intensively in semi-enclosed bays and monitor the stocks and catches (Liu 1990). Reseeding was working very successfully in China, increasing catches as much as five-fold and at the same time dampening the interannual variation in stock size. However, in at least one region, no reseeding has been carried out since 1995 because of disease in the aquaculture ponds (Prof. Liu, Institute of Oceanology, pers. comm.). In China most restocking is carried out in the north (Yellow Sea) on one species (*P. chinensis*, Chinese white prawn). About 2.5 billion eight to 10 mm carapace length (CL) juveniles are released each year (at 10% recapture and 25/kg = 10,000 t) at a cost of AUS$2.5 million for seedstock (AUS 0.1 c each) (CSIRO unpublished information).

Reseeding works in China because its prawn aquaculture industry was approaching production close to 200,000 tonnes annually (cf 1,600 tonnes for Australia). In China, reseeding is very much a by-product of the aquaculture industry, where excess postlarvae are produced and then raised at little marginal cost to a pre-supplementary-feeding stage. The Government pays the farmer for the seedstock, and the fishers repay the Government in order to fish for it.

The effectiveness of the Chinese reseeding efforts is obvious because the natural fishery has been depleted to such an extent that it is virtually dependent on reseeding. It is successful, however, because the releases are carefully planned for places and times that minimise the mortality of released animals. The reseeding is also cost-effective because of enormous economies of scale associated with the size of their aquaculture industry, giving a very low cost of production and release of juveniles at about 8-10 mm CL. The Chinese estimate a 7:1 return on investment.

4.2.2 Country examples

4.2.2.1 China

WSSV caused major losses in farmed prawns in China in 1993 and in subsequent years. Consequently, *P. chinensis* production fell from 14% of world farmed prawn production in 1992 to 6% in 1993.

Table 4.3: Chinese farmed prawn production 1991-93.

Head-on production of farmed shrimp (t)			% change
1991	1992	1993	1992 to 1993
145,000	140,000	30,000	-79

From the mid-1980s an official *P. chinensis* stock-enhancement program, using hatchery-derived prawns, was implemented along the coasts of the Bohai Sea and the central and northern Yellow Sea. For example, 1.2 billion individuals on average were released annually in the northern Yellow Sea. It is important to recognise that the released prawns made up more than 90% of the total subsequent catch (Ren and Deng 1996). Survey results confirmed that the transplanted prawns were able to spawn normally in the receiving waters.

Table 4.4: Chinese commercial wild prawn catch (live weight; thousands of metric tonnes)

	Chinese live weight catch in '000 tonnes in different years					
Species	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**
Acetes	228	263	326	330	300	310
Other	101	122	169	154	140	130
Total	**329**	**385**	**495**	**484**	**440**	**440**

(Sources : FAO and LMR shrimp market report : 1995 preliminary; 1996 preliminary with estimates; 1997 estimated)

WSS and WSSV were demonstrated in 1993 in wild *P. chinensis* from the Bohai Sea (Jiang *et al.* 1996) but, based on the data in Table 4.4, did not appear to have any serious commercial impacts. In support of this evidence, Rohana Subasinghe (FAO fish disease officer - personal communication), despite regular consultations with Chinese aquaculture and fisheries experts in recent years, has heard of no reports of declines in Chinese wild prawn catches.

On the other hand Professor Huang (Yellow Sea Fisheries Research Institute, Qingdao, China) suggested that the production of wild prawns from the Bohai or Yellow Sea areas has became 'unstable' since 1993, and that some of this decline may be attributed to virus infection. However, he was unable to provide catch volumes or WSSV prevalence data. Lundin (1997), citing a personal communication from R.Zweig, states that prawn catches in the Yellow Sea decreased by 90% at the time of the WSSV outbreaks in farms. There are several possible explanations for this reported reduction. It is possible that the catch decline was a direct consequence of disease outbreaks on *P. chinensis* wild stocks or that a high proportion of the hatchery-reared postlarvae used for restocking were infected, and experienced very high mortality rates (D. Lightner - personal communication). It is also possible that the catch decline followed the suspension of the restocking program in 1995 (N Loneragan - personal communication), given that 90% of the catch in the Bohai Sea is derived from artificially stocked prawns.

4.2.2.2 Thailand

Despite the presence of WSSV infection in a high proportion of *P. monodon* from the Gulf of Thailand, possibly as a result of a government-sponsored stock enhancement program involving release of farmed prawns to the wild (Tookwinas 1996), there are no reports of reductions in wild fishery catches (M. Phillips, pers comm; Immink, pers comm.).

In winter (Nov-Feb) in Thailand, when broodstock are taken from the Gulf of Thailand, approximately 50% of PL batches test positive for WSSV. In summer (March-Oct), when broodstock are taken from the Andaman Sea, approximately 15% of broodstock test positive for WSSV (Chanratchakool, unpublished). Moreover, Chanratchakool (unpublished) stated that, under a broodstock testing program at Banjong hatchery, Chachoengsao Province, where 10-20 broodstock per month are tested for WSSV infection, 15% of Gulf of Thailand animals and 3-5% of Andaman Sea animals were positive. He speculated that differences in infection rates between the two areas were due to the influences of aquaculture (escapees, restocking) on wild populations. The differences in infection rates between broodstock and postlarvae may be due to a combination of factors in the hatchery, including expression of latent infection in captive broodstock caused by spawning stress (Kou *et al.* 1997b).

Flegel (unpublished) states that up to 70% of Thai wild-caught *P. monodon* broodstock test positive for WSSV infection.

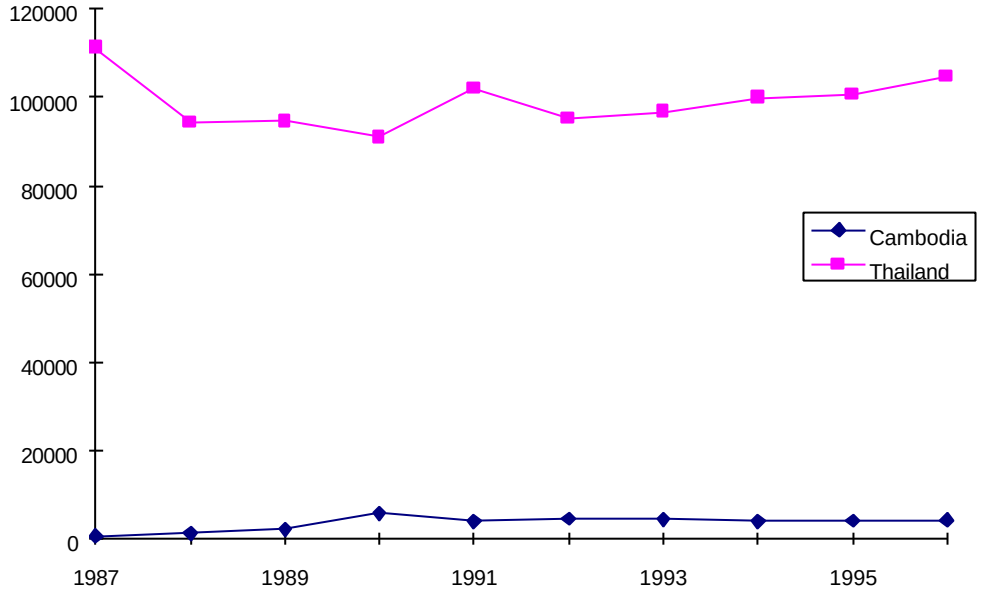
Of the 25,000 farms in Thailand in 1997, almost all were on the coast of the Gulf of Thailand (Anon. 1997a). A survey by CP Shrimp Feed Marketing Department (Jan - Sept 1994) showed that, of the 19,700 intensive *P. monodon* farms in Thailand in September 1994, 66% were in the Southern Region, 31% in the Eastern Region and 3% in the Central Region. Compared to 1992, the total number of farms had increased by 57%, mainly in the south (up 78%) and east (up 52%) , with a decline of 52% in the number of farms in the Central Region (Anon. 1998a)

There is no evidence that the Gulf of Thailand commercial prawn catch has declined since the appearance of WSSV in Thailand in 1994. Table 4.5 and Figure 4.2 shows prawn catch volumes taken by the Thai and Cambodian fleets from the Gulf in the decade 1987-1996 (FAO; A. Immink, pers. comm.). No data on variations in effort nor on what species make up this fishery and catch are available.

Table 4.5: Prawn catches (tonnes) taken by Cambodia and Thailand from the Gulf of Thailand in the decade 1987-1996

Year	Cambodia	Thailand	Total	Year	Cambodia	Thailand	Total
1987	640	111,175	**111,815**	**1992**	4,593	95,311	**99,904**
1988	1,222	94,276	**95,418**	**1993**	4,500	96,639	**101,139**
1989	2,245	94,532	**96,777**	**1994**	4,000	99,921	**103,921**
1990	5,881	91,037	**96,918**	**1995**	4,200	100,629	**104,829**
1991	3,955	101,998	**105,953**	**1996**	4,300	104,710	**109,010**

Figure 4.1 Prawn Catch Volumes in The Gulf of Thailand for 1987-1996

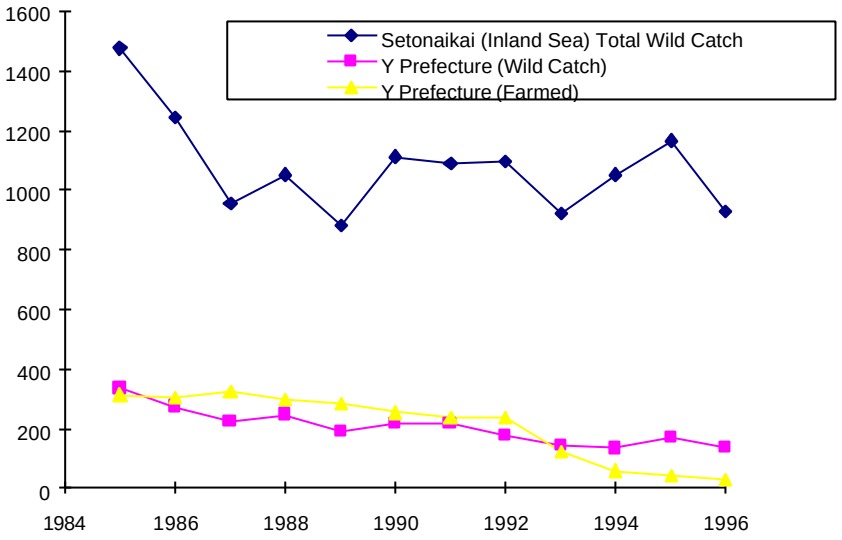


4.2.2.3 Japan

Every year about 700 million juvenile crustaceans belonging to approximately 20 species are produced at public sea farming centres for stocking into coastal waters (Japan Sea Farming Association 1990; cited by Momoyama 1992). The main species are *P. japonicus* (84%), *Metapenaeus ensis* (7%) and *Portunus trituberculatus* (7%).
Three other penaeid prawns, *P. semisulcatus*, *P. chinensis* and *P. latisulcatus* are also produced for sea farming in certain regions (Momoyama 1992).

Changes in volumes of *P. japonicus* over time in Japan are shown in Figure 4.2. The catch from the Inland Sea (Setonaikai) dropped from approximately 1,500 to 1,000 tonnes between 1985 and 1987 and then remained relatively stable around this figure through to 1996. The catch from Prefecture "Y" which abuts the Inland Sea has shown a gradual decline from approximately 350 tonnes to a little under 200 tonnes between 1985 and 1996. These changes can not be attributed to the occurrence of WSSV which was first reported in Japan in 1993. Off-take of farmed prawns in Prefecture "Y" was also showing a gradual decline from 350 tonnes in 1985 to 250 tonnes in 1992 with a sharper decline to 120 tonnes in 1993 which continued through 1994 when it began to level off. WSSV is likely to have contributed to the accelerated decrease in off-take in farmed prawns which was seen from 1992.

Figure 4.2 Changes in volumes of *P.japonicus* over time



Despite there being no evidence for sudden changes in wild catch volumes in Japan, there is evidence that WSSV infects wild prawns at reasonably high rates. Table 4.6 shows that the prevalence in female spawners and immature prawns was fairly stable over the two year period 1996-97. Also, the higher prevalence in older animals is noteworthy. An increase in prevalence with age is consistent with reduced virulence all other factors being equal. If reliable age data were also available, the approximate incidence could be estimated based on the assumption that it is constant over time and that all age groups are equally susceptible if previously unexposed. Mathematical modelling could then be used to ascertain the approximate age-specific mortality rates in wild prawn populations. Unfortunately, the absence of age data precludes these determinations.

Table 4.6: Proportions of wild-caught *P. japonicus* from Japanese coastal waters which tested positive using 2-step WSSV PCR (Meada *et al.*, in press; Itami, unpublished).

	1996	1997	Average
Female spawners[1]	51/202[3] (25.2%)	40/172 (23.3%)	91/374 (24.3%)
Immature shrimp[2]	45/272 (16.5%)	14/83 (16.9%)	59/355 (16.7%)

Notes -

1 From June through December in 1996 and 1997, immature kuruma prawns (body weight: 13.3 to 48.0 g) were collected from coastal waters of western Japan.

2 From April through September in 1996 and 1997, adult kuruma prawns (body weight: 71 to 140 g) were obtained from four ports in Kyushu.

3 WSSV prevalence: No. positive/No. tested with % prevalence in brackets.

4.2.2.4 United States of America

In 1995, outbreaks of WSS and Yellow Head (although retrospective studies of the presumptive YHV positive samples from both the 1995 disease outbreak in Texas and 1997 outbreak in Carolina with specific gene probes were negative for YHV - D Lightner - personal communication) occurred in pond-reared *P. setiferus* on a farm in Cameron County (close to Brownsville - see below), while outbreaks of Taura Syndrome occurred in ponds at a Cameron County hatchery and at two coastal farms further north. Given that studies indicated that WSSV (as well as YHV and TSV) can infect native prawns experimentally and cause mortalities in aquaria, Texas Parks and Wildlife Department (TPWD) began a prawn virus monitoring program in October 1997 to determine the presence or absence of exotic viruses in native stocks. The prevalence of these viruses in Texas bays and their possible effect on native prawn populations is not yet known, but the information generated from this monitoring program is expected to eventually provide baseline data. Prawns are collected monthly from each Texas bay system, identified to species, preserved, labelled, packaged and shipped to a laboratory for testing. To date, approximately 76 bay samples (450 individual prawns) have been examined histologically. Specimens with suspicious lesions are subjected to in situ hybridisation (gene probe) testing in an effort to detect the viruses.

One pink shrimp (*P. duorarum*) collected from the Brownsville ship channel in late December 1997 tested positive for WSSV under this program. In response, an extensive sampling program of prawns and crabs was conducted in the area from which the positive animal was collected. One hundred and eight grouped lots (5 to 10 specimens per lot) were collected and examined by EM. Some of the specimens were also examined by PCR for WSSV, all with negative results.

On the basis of these results, TPWD conclude that WSSV is probably present in the Texas Bay environment but is not widespread in the Brownsville shipping channel. They suggest that the program must run for at least one year before it will be known if viruses are more prevalent during a particular season, in specific bay systems, in certain species, in proximity to environmental stressors or in association with other factors. TPWD express cautious optimism that viral diseases are rare in native stocks and the effect of WSSV on wild crustacean populations can not yet be determined.

Ray (pers comm) states that from June 1998, blue crabs and lesser blue crabs will also be tested for viruses.

4.2.2.5 Philippines

WSS and WSSV have not been reported from the Philippines (Regidor, unpublished). Since 1993, the Philippine Government has prohibited importation of prawn postlarvae (Fisheries Administrative Order No. 189, Series of 1993 : "Prohibiting the importation of live shrimp of all stages"), but allows importation of prawn feed for use in aquaculture (Regidor, pers. comm.).

4.2.3 Wild non-penaeid populations

No information could be found on the consequences of WSSV infection for wild non-penaeid populations. However, the following are relevant:

- Chinese studies indicate that copepods may be important hosts for WSSV;
- Experimental infections in which mortality rates were higher in some species than in others, suggests that for these and other species, mortalities may occur in naturally infected populations, particularly those which are stressed.

4.2.4 Farmed prawns

Some information on the impact of WSSV on farmed prawn populations was provided in Section 4.2.1 to contrast with impacts on wild prawns. Additional information is provided in this section.

Cumulative mortalities in infected farmed prawn populations may reach 100% within two to 10 days of the onset of clinical signs (Chou *et al.*, 1995; Lightner, 1996b). Mohan (unpublished data) recorded the following mortality rates and duration of WSS outbreaks in ponds in India :

- April-May 1995 outbreak: 100% mortality in 4-5 days.
- January 1996 outbreak: slow mortality in 10-12 days.
- May-June 1996 outbreak: severe mortality in 3-5 days.

However, limited evidence suggests that prawns, within the lifetime of a single batch of larvae, may develop some tolerance to WSSV (and YHV) infection, although the mechanism for such a change remains obscure (Flegel 1997). In Thailand when WSS first appeared in 1994, farms reported 100% mortality in three days in affected ponds. By 1998, however, in some cases 1 -2 weeks elapsed before 100% mortalities occurred. Moreover, it now appears that significant losses may be avoided in infected ponds if ideal environmental conditions are maintained (Flegel *et al.* 1997; Chanratchakool, unpublished).

4.2.5 Farmed non-penaeids

Natural WSSV infections have been observed in farmed *Scylla serrata* and farmed *Macrobrachium rosenbergii* (Lo *et al.* 1996; Kou *et al.* 1997b). Clinical effects of these infections were not reported, although Flegel (1997; citing Kou and Lo, unpublished data), stated that *M. rosenbergii* becomes infected with WSSV as larvae (Peng *et al.* 1998), but not as adults, and that adults eventually show signs of WSS.

4.3 Infectious hypodermal and hematopoietic necrosis virus (IHHNV)

IHHNV is widely distributed in farmed penaeid prawns and is currently presumed to be enzootic in wild penaeids in the Indo-West Pacific and the Eastern Pacific. However, its original distribution in wild penaeids remains unknown. Recent studies of wild penaeid prawn populations from numerous locations on the western coasts of Mexico, Guatemala, Costa Rica, Honduras, Panama and Ecuador indicate that IHHNV is widely distributed in Pacific American penaeids (Pantoja *et al.* 1999; Larramore 1992; Lotz 1992). The occurrence in South-East Asia (Singapore, Malaysia, Indonesia, and the Philippines) and

India of IHHNV in prawn culture facilities using only captive-wild *P. monodon* broodstock suggests that the region is within the virus' geographic range and that *P. monodon* may be among its natural host species (Lightner 1993; Panchayuthapani 1997).

Owens *et al.* (1992) described an IHHNV-like infection in hybrid (*P. monodon* x *P. esculentus*) prawns in Australia. Infected tissues from these animals, when examined using a monoclonal antibody to IHHNV in a developmental ELISA system (Poulos *et al.* 1994) gave values of 38% to 78% intensity compared with positive controls (Lightner, unpublished). It has subsequently been shown with a commercial IHHNV probe that there is limited genetic similarity between the Australian isolate and IHHNV (Owens, 1997). These results, together with the clinical and pathological findings in the affected prawns, indicate that the Australian IHHNV strain is a variant of the classical IHHNV strain present in Asia and the Americas (Owens, pers. comm.). It remains possible, though perhaps less likely, that this putative Australian IHHNV strain is, in fact, LOPV, not IHHNV (Lightner1996b). In either case, available evidence suggests that Australian prawn populations are probably free of classical IHHNV.

4.3.1 Infections in wild prawns

Infections have been observed in wild populations of *P. stylirostris*, *P. californiensis*, *P. vannamei* (Larramore 1992; Pantoja *et al.* 1999) and *P. occidentalis* (Lotz 1992). In addition, the occurrence in Southeast Asia (Singapore, Malaysia, Indonesia, the Philippines) of IHHNV in prawn culture facilities using only wild-caught broodstock, and where American penaeids had not been introduced, suggests that the region is within the virus' geographic range and that *P. monodon* and *P. japonicus* may be among its natural host species (Lightner *et al.* 1997).

4.3.2 Infections in farmed prawns

Infections have been observed in farmed *P. stylirostris*, *P.vannamei*, *P. monodon*, *P. semisulcatus* and *P. japonicus* (Turnbull *et al.* 1994; Lightner, 1996b; Lightner *et al.* 1997; Panchayuthapani 1997).

Available evidence suggests *P. monodon* is susceptible to IHHNV infection, but IHHN outbreaks in farmed populations occur only if they are exposed to relatively large quantities of virus. Lightner *et al.* (1983a) identified IHHNV as the cause of death in numerous *P. monodon* samples at an experimental facility in Hawaii. While these authors did not see highly acute epizootics in this species, they speculated that cumulative mortalities recorded during several months in an IHHNV-exposed sub-adult *P. monodon* population indicated that IHHN may be potentially as serious a disease in this species as in *P. stylirostris*. Importantly, however, Lightner *et al.* (1983b) stated that IHHN outbreaks occurred in *P. monodon* populations only when they had been concurrently cultured with stocks of *P. stylirostris* or *P. vannamei*.

Natividad and Lightner (1992) reported that, although they had previously recognised IHHNV infection in a *P. monodon* population in the Philippines, in a subsequent survey of diseases of *P. monodon* in Philippine hatcheries and grow-out ponds, IHHNV was not found. The authors therefore suggested that either IHHNV is not widely distributed in the

Philippines or that routine histological diagnostic procedures are not sensitive enough to detect infection in subacute cases. There is a single report from the Philippines which implicated IHHNV as the cause of a serious disease outbreak in *P. monodon* (Rosenberry 1992). Flegel (1997) concluded that *P. monodon* has a high tolerance to infection by IHHNV, given that he observed heavy infection in a clinically normal group during a laboratory trial.

As for *P. monodon*, *P. japonicus* is listed by Lightner *et al.* (1992) as showing "significant disease sometimes due to IHHNV reported in one or more life stages", but whether this refers to natural or experimentally induced infection is not stated. However, Loh *et al.* (1997), in their equivalent tabulation, record no entry for susceptibility of *P. japonicus* to IHHNV infection, indicating scant available information on the topic.

Momoyama (1992) warned of the danger of introducing IHHNV infection to Japan via the large numbers of unscreened seed *P. japonicus* imported annually from Taiwan, given that the virus had been detected there in farmed *P. vannamei* imported from Panama (Lightner *et al.* 1987). However, there are no subsequent reports of infection or disease associated with this agent in Japan (Humphrey *et al.* 1997; Inouye 1996), suggesting that *P. japonicus* is not highly susceptible to infection.

Zhang and Sun (1997) suggested IHHNV may have been involved in the severe viral disease outbreaks in farmed *P. chinensis* in northern China in 1993, but, as discussed above, there is some doubt regarding the accuracy of this diagnosis.

In summarising the current view on the significance of IHHNV in Asia, Lightner *et al.* (1997) states that the virus is increasingly viewed as a generally insignificant pathogen in the region.

4.3.3 Experimentally induced infections in prawns

P. setiferus, P. duorarum and *P. aztecus* have been infected experimentally with IHHNV, while *P. indicus* and *P. merguiensis* appear to be refractory to infection (Lightner, 1996b).

4.3.4 Age/size susceptibility

IHHN is an acute disease causing very high mortalities in juvenile *P. stylirostris*. Vertically infected larvae and early postlarvae do not become diseased, but at approximately PL35 or older, gross signs of the disease may be observed, followed by mass mortalities. In horizontally infected juveniles, the incubation period and severity of disease is somewhat size and/or age dependent, with young juveniles always being the most severely affected. Infected adults seldom show signs of disease or suffer mortality (Lightner 1996b).

In contrast to *P. stylirostris*, IHHNV in *P. vannamei* is typically a chronic disease. Runt deformity syndrome (RDS) in this species has been linked to IHHNV infection. Juvenile prawns with RDS display bent or deformed rostrums, wrinkled antennal flagella, cuticular roughness, and other cuticular deformities. Populations of juvenile prawns with RDS display a relatively wide distribution of sizes with many smaller than expected (runted) prawns. The coefficient of variation (CV) for populations with RDS is typically greater than 30% and may

approach 50%, while IHHNV-free (and thus without RDS) populations of juvenile *P. vannamei* usually show much less variation in size with CVs of 10-30% (Lightner 1996b).

Given the variability in expression of IHHN between prawn species, it is likely that some wild Australian prawn species which have not yet, been exposed to 'classical' IHHNV may, like *P. stylirostris*, be highly susceptible to infection. Others, like *P. vannamei* in the Americas, may be less susceptible, while others may be refractory.

4.3.5 Other Australian crustacea and arthropods potentially at risk

4.3.5.1 Infections in wild non-penaeid populations

In a survey of farmed penaeid prawns in Taiwan for viral and other diseases, Lightner *et al.* (1987) examined adult grass shrimp (tentatively identified as *Palaemon japonicus*) collected from the same tank as one of the test *P. vannamei* populations. Adults of both species were free of IHHNV infection. However, histological lesions (ie Cowdry type A inclusion bodies, but not abundant) suggestive of IHHNV infection were present in developing embryos on the pleopods of the female *Pal. japonicus*. The authors suggested that if infection in grass shrimp is confirmed, it would imply that non-penaeids could serve as alternate or reservoir hosts for the virus, complicating measures aimed at controlling the disease.

4.3.5.2 Infections in farmed non-penaeid populations

Not reported.

4.3.5.3 Experimental infections in non-penaeid animals

Not reported.

4.3.6 Role of stress in IHHNV infections

Browdy *et al.* (1993), in a study of IHHNV-infected *P. vannamei*, showed that low dissolved oxygen concentrations resulted in poor survival rates (43.5% and 53.6%) in tanks which were stocked at 100/m^2 and limited to 10% water exchange per day. Density and water exchange regimes had little effect on water quality, survival, or growth in other experimental groups.

4.3.7 Transmission of IHHNV

4.3.7.1 Vertical transmission

It is believed that IHHNV may be transmitted vertically from broodstock to their progeny (Lightner *et al*. 1983b). However, no studies confirming this have, to our knowledge, been published. Lightner (personal communication) has stated "it appears that IHHNV is very efficient at vertical transmission, with 100% transmission/infection rates typically measured in batches of PLs introduced from infected broodstock. There is no reason to presume that a vertically transmitted virus like IHHNV would be any less successful in being transmitted from spawner to progeny in the wild." In addition, Lightner (personal communication) states

that the *P. stylirostris* fishery in the northern Gulf of California collapsed in 1990 due to a year-class recruitment failure due to IHHNV. Pantoja. *et al.* (1999) suggested vertical transmission may have contributed significantly to the rapid spread of IHHNV in aquaculture operations in Sonora and Sinaloa, Mexico and could have played an important role in the apparent IHHNV epizootic in wild prawns. They suggested, however, that to confirm the epidemiological significance of vertical transmission of IHHNV in *P. stylirostris*, the effect of IHHNV infection on the reproductive organs of both male and female broodstock must be determined. The presence of IHHNV Cowdry Type A inclusion bodies (CIAs) in apparently healthy, fully functional gonads of both sexes in their study suggested the potential for vertical transmission. By contrast, severe liquefactive necrosis and CIAs in the ovaries of one female specimen and an idiopathic lesion in the vas deferens of one male specimen were thought to indicate gonad impairment perhaps due to IHHNV infection, which could hinder vertical transmission.

Many captive *P. stylirostris* which survive the acute phase of IHHNV infection become carriers of the virus for life. These animals appear clinically normal, but they pass the virus on to their progeny. A 1997-98 Mexican survey of wild adult *P. stylirostris* in the Gulf of California (using in situ hybridisation with gene probes) detected IHHNV in close to 100% of animals tested. It is therefore likely that these wild stocks similarly pass virus on to their progeny (Lightner - personal communication).

Wild stock enhancement programs may also increase the proportion of infected adults in a wild population and hence increase the proportion of infected postlarvae subsequently produced by that population. Under the Thai program, some pond-reared stock (presumably on average having a higher IHHNV infection rate than wild stocks) are released into the wild as part of a government sanctioned wild stock enhancement program (Tookwinas 1996). Given the very low apparent prevalence of IHHNV infection in farmed *P. monodon* in southeast Asia (Turnbull *et al.* 1994; Flegel *et al.* 1992; Natividad and Lightner 1992), the absence of reports of IHHNV infection in wild caught broodstock probably reflects the high innate resistance of this species to infection.

4.3.7.2 Horizontal transmission

Horizontal transmission of IHHNV is known to occur by cannibalism of infected carcasses, by direct contact between prawns, and by indirect contact via water. Cannibalism is known to be the most rapid and effective mechanism of infection and is the basis of the bioassay test for asymptomatic carriers in prawn populations. IHHNV-resistant penaeid species and early life stages may carry the virus latently and transfer it to more susceptible species and life stages (Lightner *et al.* 1983b; Bell and Lightner, 1984).

4.4 Impacts of IHHNV

4.4.1 Wild prawns populations

Most information is available from US and Mexican studies with less from China.

4.4.1.1 USA and Mexico

Historical data from the Gulf of California support the contention that IHHNV was not present in that region of Mexico prior to 1987 (Pantoja *et al.* 1999), when IHHN was first diagnosed in commercial shipments of postlarval *P. vannamei*. The introduction of IHHNV into the Gulf of California in Mexico in 1987 was followed by serious epizootics of IHHNV in *P. stylirostris* stocks in prawn farms in the Mexican states of Sonora and Sinaloa in 1989 and 1990 (Lightner 1996a).

However, the relationship between aquaculture, IHHNV, and the decrease in prawn populations in the Gulf of California is not clear. Although Lightner *et al.* (1992; cited by Lightner 1996a) suggested that cross-contamination among prawn farms was the means by which the virus was transmitted, a 1990 survey (see Pantoja *et al.* 1999, below) of wild prawn stocks in the commercial fishery of the northern Gulf of California revealed that IHHNV infections were present at high prevalences. The broodstock used by the affected farms had been collected from IHHNV-infected wild stock.

A histopathological survey of wild Pacific Blue shrimp *Penaeus stylirostris*, from the Gulf of California (Sonora, Mexico), revealed the presence of IHHNV (Pantoja *et al.* 1999). The survey performed at 39 sampling stations during August-September 1990, showed the prevalence of IHHNV infection to be 46% in the Upper Gulf zone and 26 % in the Central-Lower Gulf zone. The presence of IHHNV was confirmed with an IHHNV-specific gene probe by means of in situ hybridisation. Specimens of the Pacific Brown shrimp (*P. californiensis*) and Pacific White shrimp (*P. vannamei*), incidentally captured during the survey, were also found to be infected by IHHNV. The survey demonstrated that IHHNV was widely distributed in a region where it had not been previously detected and, presumably, was not present before 1987. These findings suggest that IHHNV has become established in wild populations of *P. stylirostris*, and perhaps in *P. californiensis* and *P. vannamei*, in the Gulf of California.

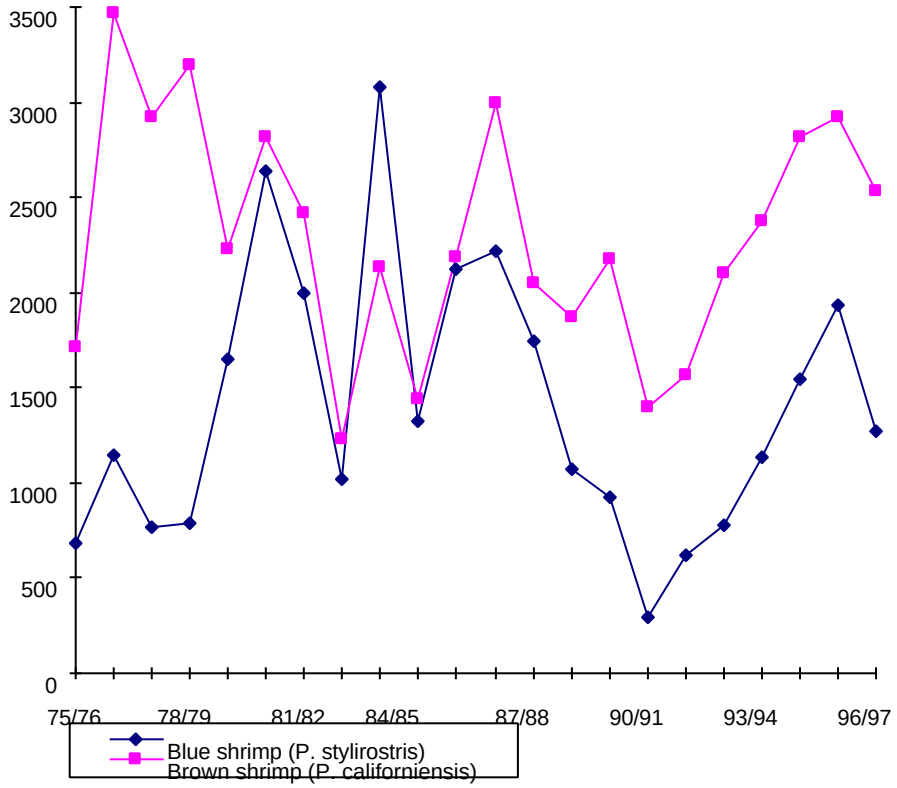
Although not fully supported by statistical analysis, Pantoja *et al.* (1999) proposed that the characteristic oceanographic conditions of the Upper Gulf zone, may have been a factor responsible for the high prevalence observed in that region. The Upper Gulf zone, from the mouth of the Colorado River to Angel de la Guarda and Tiburón islands, has been considered a semi-isolated oceanographic cell. Furthermore, the shallow depth, the high evaporation rates and the Coriolis effect are responsible for a rotatory pattern of the deep and superficial currents in that zone. These currents may prevent a major proportion of prawn larvae and postlarvae from leaving that zone. Conceptually, prawn populations in the Upper Gulf zone are restricted by these oceanographic barriers for at least part of their life cycle. Thus, restricted out-migration may have facilitated both horizontal and vertical transmission and the accumulation of infected animals may have contributed to the higher prevalence of IHHNV in the Upper Gulf zone (46%) compared to the prevalence in the Central-Lower Gulf zone (26%), where oceanographic conditions resemble more those of the open ocean. Further, genetic differences among prawn populations in each zone may have contributed to regional differences in prevalence of IHHNV infection. Pantoja *et al.* (1999; citing Aubert 1997) suggest that six genetically distinct populations of *P. stylirostris* are distributed along the coast of Sonora. Since one of these genetically distinct populations is confined to the Upper Gulf zone, the authors speculated that there is the possibility that it

may have been more susceptible to IHHNV infection than those in the Central-Lower Gulf zone.

Results (Pantoja *et al.* 1999) also suggested that transmission of the disease in *P. californiensis* may have occurred mostly through the horizontal route. In this species, exposure to the virus may have been facilitated in the Upper Gulf, where the infection was found to be highly prevalent, and where the only IHHNV positive *P. californiensis* were found.

Concurrent with the high prevalence of IHHNV infections in the Gulf of California in 1990 was a marked decline in prawn landings at the major ports of the region from 1989 until 1994. However, in late 1994, landings of *P. stylirostris* returned to pre-1989 levels (Figure 4.3), suggesting that the fishery was recovering (Holtschmit, unpublished; Anonymous 1997c).

Figure 4.3 Catches of Blue (*P. stylirostris*) and Brown (*P. californiensis*) shrimp in the Gulf of California



High IHHNV infection rates, combined with high levels of infection severity, in wild *P. stylirostris* in the Gulf of California were associated with a marked decline, and subsequent recovery, in wild catches in the late 1980s and early 1990s. However, catch data for *P. californiensis*, a less susceptible species having much lower infection rates and levels of infection severity (see Table 4.7), show a concomitant decline and recovery during the same period. Furthermore, data for both species in the Gulf of California as a whole show parallel fluctuations in catch from 1979/80. No useful data for fishing effort are available for the

period. Having examined available data and discussed the issues in detail with US and Mexican scientists, we believe more data are required if the effects of IHHNV infection on wild *P. stylirostris* stocks are to be determined beyond reasonable doubt. However, until conclusive data are available, we suggest the following two scenarios are consistent with currently available data.

1. IHHNV infection did not cause significant losses in *P. stylirostris* populations in the Gulf of California. As stated above, available data show that catches of *P. californiensis*, a less susceptible species having much lower infection rates and levels of infection severity, declined and recovered in the late 1980s and early 1990s, in a similar fashion to changes in *P. stylirostris* catches. Moreover, data for both species show similar fluctuations in catch from 1979/80, and it has been established that IHHNV infection was not present in these populations prior to 1987 (Pantoja *et al*. in press). It therefore seems reasonable to suggest that the observed fluctuations in catches of both species were caused by factors unrelated to disease, such as fishing pressure and/or environmental changes. In fact, Mexican modelling studies indicated changes in surface water temperature or over-fishing may have been factors in the decline in catches for both species. These studies suggested recovery of the prawn populations in the Gulf may be attributed more reasonably to the stabilisation of factors unrelated to disease rather than to adaptation to the virus. It was concluded that additional studies are needed to determine whether the declines were due to a combination of different factors, or to IHHNV alone (Anonymous 1997b).

2. An alternative explanation for the catch variations has been suggested by Lightner (personal communication). He proposed that IHHN probably caused the decline in *P. stylirostris* stocks, while changes in *P. californiensis* catches were due to remaining fishers subsequently targeting this alternative population. He states that "Prior to the collapse of the fishery in 1990, the fishing fleet had grown to more than 1,000 trawlers and these were over-fishing the resource in the northern Gulf of California, a fishery dominated by the blue shrimp, *P. stylirostris*. When this *P. stylirostris* fishery collapsed in 1990 in the northern Gulf of California due to a year-class recruitment failure due to IHHNV, many trawlers were withdrawn from the fishery and many packing plants were closed for several years. What remained of the commercial fleet of the region shifted to fishing the smaller and generally less valuable brown shrimp (*P. californiensis*). Even with government assistance, it was not profitable for trawlers to fish the northern Gulf from 1990 to 94/95. Low prices for shrimp on the international market also contributed to less fishing pressure in the northern Gulf. With far fewer vessels fishing from 1990 (when the IHHN epizootic peaked in the Gulf) through to 94/95 (when *P. stylirostris* stocks began to rebound), landings for all penaeids from the upper Gulf declined." However, this suggestion seems to be at odds with the available Gulf catch data. These data show that landings for both shrimp species in the Gulf as a whole increased between 90/91 and 94/95, suggesting that *P. stylirostris* (and *P. californiensis*) stocks had began to rebound in 91/92, rather than in 94/95.

Table 4.7: Prevalence of IHHNV infection (as determined by H&E histology) in wild *P. stylirostris, P. californiensis* and *P. vannamei* from the Gulf of

California (August-September 1990).

Sampling station	Species	Total number analysed	Total IHHNV positive	Prevalence (%)	Maximum severity grade
Upper Gulf zone Total	*P. stylirostris*	146	67	46	G4*
	P. californiensis	39	7	18	G1
Central/Lower Gulf zone Total	*P. stylirostris*	73	19	26	G2
	P. californiensis	35	0	0	-
	P. vannamei	24	3	12	G1

* Severity of infection was graded according to the degree of target tissue and organ damage as follows: G0 = no signs of IHHNV infection were detected; G1 = characteristic IHHNV inclusions were observed but were uncommon; G2 = IHHNV inclusions were moderately common and some focal tissue damage was evident; G3 = IHHNV inclusions and tissue destruction were widespread; G4 = abundant IHHNV inclusions and severe tissue destruction occurred in one or more target organs or tissues

The situation in the Gulf of California should be contrasted with that in South Carolina and the Gulf of Mexico. For example, prawns have been farmed in South Carolina since 1987. IHHNV has been documented in aquaculture ponds there, but to date, no IHHNV has been detected in wild stocks. A possible explanation for this is that the three indigenous commercial (Gulf of Mexico) prawn species (*P. aztecus*, *P. duorarum*, and *P. setiferus*) are relatively resistant to infection; they are listed by Lightner (1993) as capable of being infected experimentally, but with insignificant effects. Notably, also, there are no reports from Asia of IHHNV infection in wild prawn stocks. This apparent general resistance of wild stocks to infection and disease suggests that most indigenous Australian prawn species, whose susceptibility to IHHNV is currently unknown, will more probably be resistant than sensitive.

4.4.1.2 China

There are no reports of the consequences, with respect to prevalence of IHHNV infection in wild penaeid stocks, of the Chinese wild stock enhancement program (Ren and Deng 1996). Under this program, hatchery-reared postlarvae derived from wild-caught broodstock are released back into the wild. Zhang and Sun (1997; abstract only, 4th Asian Fisheries Forum), in a study of the pathogenesis of viral diseases of *P. chinensis*, reported, presumably on the basis of histopathological evidence only (methods not described in the available abstract), that three viruses (WSSV, IHHNV and HPV) were involved in the 1993 northern region epidemic, with WSSV and IHHNV being the main pathogens involved. However, it seems more likely that, for the following reasons, the IHHNV diagnosis was erroneous:

- no other investigators of the 1993-94 Chinese outbreaks mention involvement of IHHNV;
- *P. chinensis* is reported to be only slightly susceptible to IHHNV infection (Lightner *et al.* 1992);
- Cowdry Type A inclusions are commonly caused by WSSV (Flegel *et al*. 1997); and
- in some circumstances at least, similar lesions may be induced by rather general types of cell injury unrelated to viruses (Loh *et al.* 1997).

4.4.2 Wild non-penaeid populations

There is no evidence IHHNV infects aquatic animals other than penaeid prawns, given that retrospective studies of possible IHHNV infection in developing *Palaemon japonicus* embryos in Taiwan (Lightner *et al*. 1987) showed that the lesions were not associated with IHHNV (Lightner - personal communication).

We have found no other data on consequences of IHHNV infection for wild non-penaeid populations. There are no reports of disease/mortalities in freshwater crustacean populations from countries where IHHNV is endemic.

4.4.3 Farmed prawns

IHHNV is distributed widely in prawn culture facilities in the Americas and Asia. Countries which have reported serious production losses caused by IHHN in *P. stylirostris* and/or *P. vannamei* include south-east USA, Mexico, Ecuador, Peru, Brazil, Caribbean and Central American countries, Hawaii, Guam, Tahiti and New Caledonia.

Lightner *et al.* (1997) note that IHHNV is increasingly viewed as an insignificant pathogen in Asia. Available evidence suggests *P. monodon* is susceptible to IHHNV infection, but IHHN outbreaks in farmed populations occur only if they are exposed to relatively large quantities of virus. I n practice, this usually means outbreaks occur only when *P. monodon* populations are concurrently cultured with stocks of *P. stylirostris* or *P. vannamei* (Lightner *et al.* 1983b). We have found no reports of IHHN outbreaks in farmed *P. japonicus*.

P. merguiensis, which is now being farmed in Australia, is reported to be refractory to IHHNV infection (Lightner 1996b).

4.4.4 Farmed non-penaeids

No infections or production losses caused by IHHNV have been reported.

5 Potential Impacts in Australia

This section explores the likelihood that WSSV and IHHNV would establish and spread in Australia as well as the likely consequences based on the experience in other countries which was described in Section 4. The discussion assumes that virus will be introduced to a population of Australian prawns and a number of scenarios are outlined. Introduction may be into farmed prawns with subsequent spill-over to wild stocks or vice versa. However, this report is only concerned with the impacts of viruses on populations of wild prawns and the associated food web and environment in Australia.

Among other things, the degree of impact of an exotic disease will depend on the amount of spread which occurs following the initial introduction and establishment of the exotic pathogen of interest. It is therefore worthwhile to briefly consider issues related to the introduction, establishment and spread of WSSV and IHHNV in Australia.

5.1 Introduction of WSSV and IHHNV to wild populations

In considering the potential impacts of pathogenic, exotic prawn viruses on wild prawns and associated wild aquatic animal populations, the likely routes of agent introduction must be considered. Exotic viruses may first be introduced into prawn farms with subsequent spillover to wild populations. In this case, farmed prawns may act as a reservoir where virus loads are quite heavy because of the stress of intensified production. Alternatively, infection could be introduced directly into wild populations and then transferred into aquaculture premises via capture of spawners.

Currently available information presented in Section 4 suggests that there is a low probability of exotic prawn viruses entering Australia via the following routes :

a) *Migration of infected wild stocks.* Studies of *P. monodon* population genetics indicate little mixing between Australian populations in northern and eastern waters (Benzie and Ballment 1997). Similarly, it is likely that there is little mixing with potentially infected wild *P. monodon* populations from Indonesian waters (J. Benzie, personal communication);

b) *Importation of live prawns*. Such importations are prohibited under current quarantine legislation. This practise is also prohibited under the Wildlife Protection (Regulation of Export and Import) Act.

c) *Wild prawn stock enhancement programs.* Consideration is being given to the consequences of implementing these programs in Australia, and it is possible that they may aid the spread of endemic agents (eg the Australian IHHNV strain). However, as long as broodstock and imported feed used in hatcheries remain free of exotic pathogens, there is little danger such programs could spread exotic agents to wild aquatic animal populations.

The entry of exotic prawn viruses into Australia is more likely to occur via one or more of the following routes :

a) *Infected feed*. Given that feed for use in hatcheries is not subjected to heat treatment during manufacture, it probably poses the highest feed-related risk. However, the fact that WSS and WSSV have not yet been recorded in either the Philippines or Australia, despite importation of large quantities of feed from countries where infection is endemic, suggests this is a relatively low risk activity.

b) *Processing plant waste.* Evidence suggests that WSSV and YHV infections were spread from inadequately treated processing plant waste to farmed prawns in the U.S. by means of contaminated runoff or scavenging birds (Lightner *et al*. 1997). In relation to this episode, only a single wild prawn has so far (surveillance is continuing) been found infected with WSSV in the adjacent marine environment, and it is not known whether this infection was derived from the processing plant waste or the infected farm.

c) *Infected bait prawns*. WSSV infections in captive crayfish at a US zoo were attributed to feeding infected, imported frozen prawns (Lightner *et al*. 1997). It is likely that use of such prawns as bait in Australia could spread infection to prawn populations.

5.2 Establishment and spread in wild populations

In order for an exotic disease to become established in a population, the following must occur:

1. introduction of an exotic pathogen via importation or migration of an animal or product infected or contaminated with the pathogen;
2. a lack or failure of any processing or treatment that might inactivate the pathogen (eg chemotherapy, cooking or pasteurisation);
3. access of the pathogen to a suitable natural aquatic environment;
4. the presence of one or more susceptible host species in appropriate concentrations in the natural aquatic environment to which the pathogen gains access;
5. persistence of the pathogen in sufficient numbers to infect a susceptible host by ingestion, immersion, contact or inoculation via a vector; and
6. replication or multiplication of the pathogen in the infected host resulting in the release of a sufficient number of pathogens to infect other susceptible host animals.

Although reliable data are limited and there is a moderate level of uncertainty surrounding most assumptions, on the balance of available evidence, the following conclusions regarding establishment of infection in wild prawn stocks appear warranted. These are discussed in the following sections.

5.2.1 WSSV

There are at least three possible explanations for the high prevalences of WSSV infection in wild *P. monodon* , *P. japonicus* and *P. chinensis* stocks in the Gulf of Thailand, Inland

Sea of Japan and Bohai Sea in China, respectively. Using *P. monodon* in Thailand as an example, the scenarios are :

I. Spread of infection from farmed stocks to wild stocks via frequent releases, by farmers in response to outbreaks, of large quantities of infected material, infected prawns and contaminated water, over large sections of coastline, leading to:
    a) infection of (mainly) juvenile *P. monodon* and other susceptible crustaceans in contaminated estuarine and in-shore coastal areas;
    b) exposure of these infected individuals to stress caused by adverse water quality conditions arising from urban, industrial, agricultural and aquacultural pollution in these areas;
    c) local propagation of outbreaks in these coastal areas, with low to moderate morbidity and mortality rates in susceptible species, including *P. monodon*;
    d) low morbidity and mortality rates in infected *P. monodon* and in the population as a whole following emigration to the wider marine environment because of low stress levels and possibly the development of 'tolerance' within the lifetime of the individual.

II. Spread of infection from farmed stocks to wild stocks via government-sponsored stock enhancement programs under which wild-caught, latently infected *P. monodon* broodstock are spawned in hatcheries. Prawn hatcheries offer particular opportunities for horizontal transmission of infection. When infectious agents are present, even at very low infection rates, in wild-caught broodstock used in hatcheries, the agent may rapidly spread to all other broodstock and all resultant progeny. The propensity of WSSV (and IHHNV, as well as other viruses) to infect, but not cause disease, in larvae or early PL stages further complicates this situation, because signs of infection or disease may not be apparent until farms or fisheries stocked with infected PLs observe disease outbreaks or stock deficits. Features of this pathway include :
    a) stress-related expression of latent infection in broodstock following capture and confinement in the hatchery;
    b) vertical transmission, probably as a result of contamination of eggs during spawning, from these spawners to their progeny;
    c) increased proportions of infected individuals in wild stocks following release of these infected juveniles to the wild;
    d) minimal consequences for most of these infected individuals and for the *P. monodon* population as a whole because of low stress levels in the wider marine environment and the possible development of 'tolerance' within the lifetime of the individual.

III. Spread of infection from farmed to wild stocks via frequent releases of large quantities of infected material, infected prawns and contaminated water, over large sections of coastline, leading to :
    a) infection of (mainly) juvenile *P. monodon* and other susceptible crustaceans in contaminated coastal areas;

b) exposure of these infected individuals to stress caused by adverse water quality conditions arising from urban, industrial, agricultural and aquacultural pollution in these areas;
c) propagation of outbreaks, with high morbidity and mortality rates in *P. monodon*, and other highly susceptible crustacean populations these coastal areas, with extension to, and major losses in, these highly susceptible species amongst wild stocks in the wider marine environment;
d) possible development of 'tolerance' in infected survivors.

Although data on effort and species composition of catches are not available, data from Thailand and Japan indicate that wild prawn stocks as a whole have not been adversely affected by spread of disease from farmed stocks. The unconfirmed reports of major declines in catches of wild prawns in the Yellow Sea area (but not the national catch as a whole) may have been due to spread of WSSV infection from farmed stocks on the adjacent coastline as in scenario III above. However, even if confirmed, such declines could equally have been due to the discontinuation, in the face of increased disease risk, of the restocking programs on which the Yellow Sea fisheries depend rather than the disease itself.

5.2.2 IHHNV

There are at least two possible explanations for the high prevalences of IHHNV infection in wild *P. stylirostris* and *P. californiensis* stocks in the Gulf of California :

I. Spread of infection from farmed stocks to wild stocks via frequent releases, by farmers in response to outbreaks, of large quantities of infected material, infected prawns and contaminated water, over large sections of coastline, leading to:
    a) infection of mainly juvenile *P. stylirostris* and other susceptible prawns in contaminated coastal areas;
    b) exposure of infected individuals to stress caused by adverse water quality conditions arising from urban, agricultural and aquacultural pollution in these areas;
    c) local propagation of outbreaks in these coastal areas, with low to moderate morbidity and mortality rates in susceptible species, including *P. stylirostris*;
    d) low morbidity and mortality rates in infected *P. stylirostris* and in the population as a whole following emigration to the wider marine environment because of low stress levels and the possible development of 'tolerance' within the lifetime of the individual.

II. Mixing, via migration, of infected wild prawn stocks with stocks in the Gulf of California, leading to:
    a) vertical and horizontal transmission of infection;
    b) exposure of infected individuals to stress caused by adverse water quality conditions arising from urban, agricultural and aquacultural pollution in these areas;
    c) local propagation of outbreaks in these coastal areas, with low to moderate morbidity and mortality rates in *P. stylirostris* ;

d) low morbidity and mortality rates in infected *P. stylirostris* and in the population as a whole following emigration to the wider marine environment because of low stress levels and the (postulated) development of 'tolerance' within the lifetime of the individual.

5.3 Factors determining the impact of disease on a population

The severity and duration of a disease outbreak in a particular population will be influenced by the following :

- The incubation period of the disease;
- The infectivity, pathogenicity and virulence of the agent for the different susceptible species in the ecosystem;
- The proportion of susceptible animals in the population;
- The level of stress to which the susceptible animals are subjected;
- The distance between susceptible animals; a minimum density, the threshold level, of susceptible animals is required to allow a contact-transmitted epidemic to commence. Above a certain density of susceptible animals, one infected animal can, on average, infect more than one susceptible animal and an epidemic can occur. The greater the density, the steeper the slope of the progressive stage of the epidemic curve. Few threshold values relating to animal diseases are known.

Before a specific consideration of WSSV and IHHNV was undertaken, two general conceptual model of factors driving disease impacts were developed. These are shown in Figure 5.1. Figure 5.1a shows what might occur, for example, in those areas of Asia where intensive prawn farming abuts coastal waters while Figure 5.1b shows what might happen where there is less interaction between farms and the adjacent seawaters, the more likely situation in Australia.

Figure 5.1a: Conceptual model of the factors relevant to the impact of disease impact under the assumption that ongoing virus load from sources external to a particular natural ecosystem contributes to the level of impact of the disease once established.

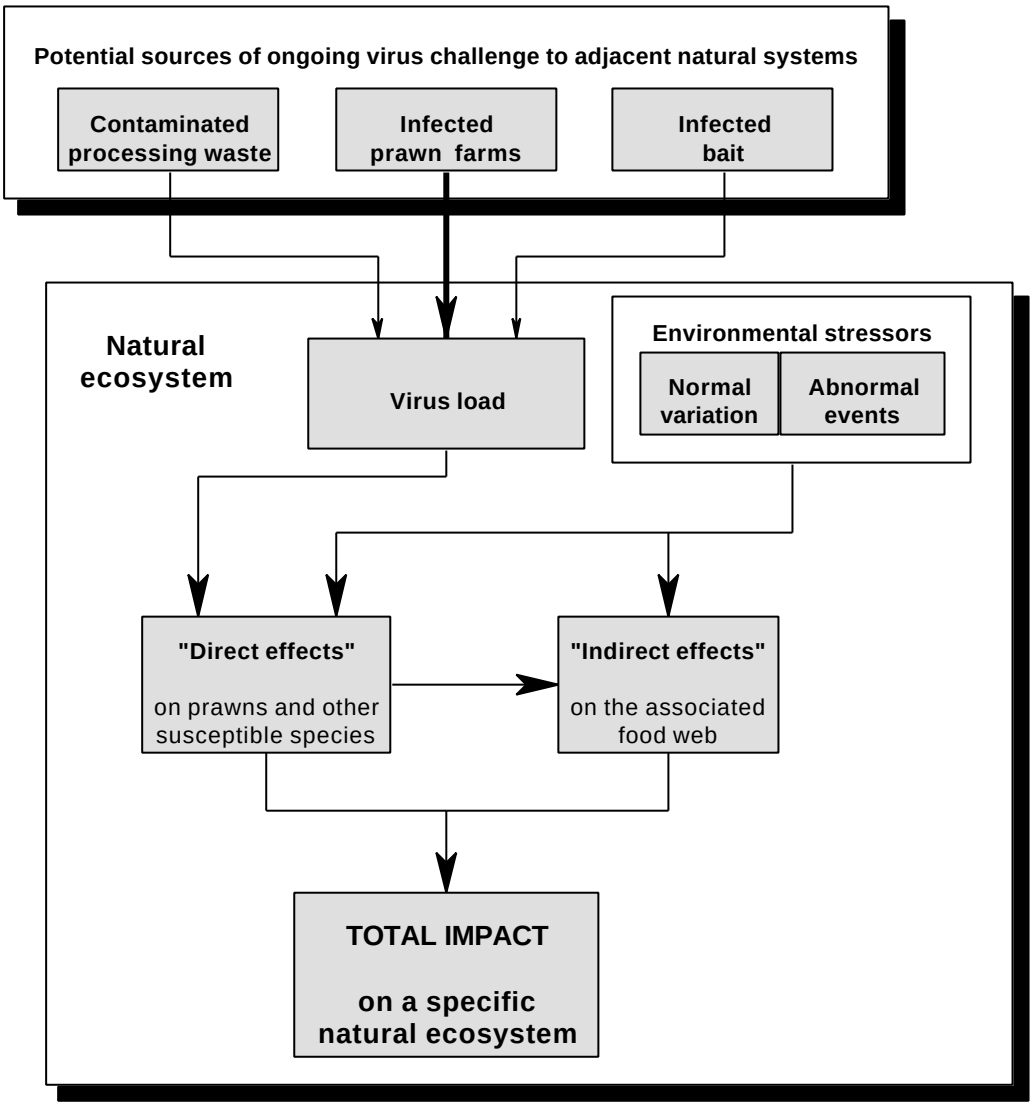
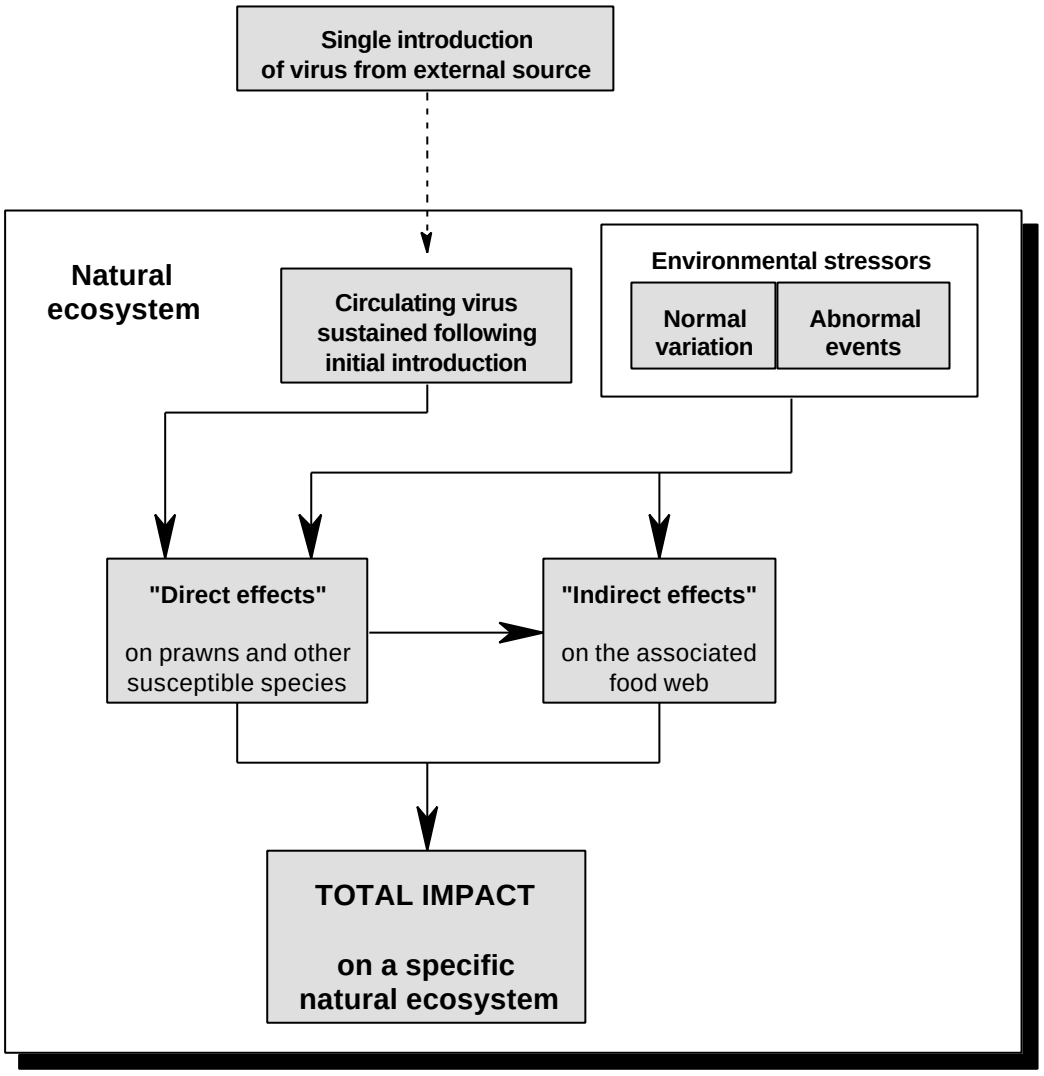


Figure 5.1b: Conceptual model of the main factors driving total disease impact under the assumption that a virus is introduced on a single occasion and becomes established in a particular natural ecosystem but there is no ongoing external source of virus load.



5.3.1 Agent factors

Strain variation appears to occur in both WSSV and IHHNV and this may result in variations in infectivity, pathogenicity and virulence.

5.3.2 Host factors

Wild penaeid prawns may be most susceptible to infection during certain critical time periods, and populations are most dense during immigration of postlarvae and emigration of juveniles. For example, densities of juvenile banana prawns in mangrove areas may reach 60 prawns m^{-2} (Haywood & Staples 1993). This compares with the density of prawns in aquaculture ponds which usually does not exceed 40 prawns m^{-2}.

Limited research has indicated that natural mortality can be as high as 93% for sub-adult and adult prawns of *Penaeus* spp. (Glaister *et al.* 1993; cited by Ludescher 1997**)**.

If prawn populations were depleted through disease in one area, prawns have the biological characteristics that would make it highly probable that the depleted population is replenished quickly through natural recruitment. These characteristics (from Dall *et al*. 1990) include:

- the adults are distributed over large areas, generally at low densities (except for banana prawns at certain times). The sub-adults and adults also migrate over varying distances. Prawns are therefore likely to naturally move back into an area. The possible exception to this is the smaller species of prawns that are able to complete their life-cycle within an estuary eg the greasyback prawn, *Metapenaeus bennettae*.
- prawns produce many eggs (100,000 to 1,000,000 eggs per female, depending on the size and species of prawn) and females are able to spawn several times during one season. As the eggs develop into larvae and postlarvae, they migrate to the inshore nursery areas by the ocean currents (larval advection). There is great potential for the postlarvae to be distributed widely across a large area of the inshore nursery habitats. Hence spawning from outside the affected region is likely to provide new juveniles to the system.
- spawning for many species extends over many months. It is likely that some parts of the population are spawning at some time of the year and able to provide recruits for replenishing depleted populations.

Experience with freshwater crayfish (Alderman 1996) and finfish (Lilley *et al.* 1997) indicates that disease organisms may be highly pathogenic to new host species, and that severe and debilitating epizootics may follow translocation of pathogens to areas with new hosts.

5.3.3 Environmental stressors

Poor water quality in coastal waters caused by pollution from urban, industrial, agricultural and aquacultural sources is believed to have contributed to the severity of disease outbreaks in farmed prawns (Cai and Wang 1996; Phillips *et al.* 1993) and possibly, wild prawn stocks (Lundin 1997).

There are at least three main environmental stressors likely to affect susceptible wild populations in Australian estuaries where prawns are farmed, namely run-off from acid sulfate soils, low dissolved oxygen concentration and habitat degradation. These are discussed below.

5.3.3.1 Run-off from acid sulfate soils

Episodic acidification (pH <5) of estuarine tributaries caused by the oxidation of sulfidic floodplain sediments is widespread in eastern Australia. Drainage and flood mitigation works promote oxidation and the export of sulfuric acid and dissolved aluminium and iron into streams. Sammut *et al.* (1996) examined the acidification of a tidal reach on the Richmond River, New South Wales. Acid discharge was controlled by the floodplain water balance, drainage of shallow acidic groundwater, and tidal floodgate operation. Floodgates stored acid water for more than six months. Acid discharges ranged from short pulses

during light rains to ~950 tonnes of sulfuric acid in a major flood that acidified the reach for over seven weeks. Extensive iron flocs accompanied acidification and coated the benthos. The chemistry of the reach reflected mixing of the acid groundwater with upland waters and showed pH-dependent enhancement or depletion of species relative to chloride. Concentrations of monomeric aluminium were over 300 times larger than local (ANZECC) guidelines and 90 km of the river were acidified after the floods. The estimated rate of sulfuric acid production from the floodplain is ~300kg ha $^{-1}$ year^{-1} and discharge may occur for over 1000 years. Management options have been devised but the long-term consequences for tidal reaches are unknown.

It is likely that exposure to runoff from acid sulfate soil areas would have adverse effects on wild prawns; Nash *et al*. (1988) reported abnormal gill discolouration, soft shells and decreased survival in *P. monodon* in growout ponds in acid sulfate soil areas in Malaysia.

5.3.3.2 Low dissolved oxygen concentrations

Callinan (1998) conducted water quality monitoring studies at representative mainstream and tributary sites after major rainfall events on the Richmond and Clarence Rivers in May-June 1987 and April-May 1988. Dissolved oxygen concentrations fell from normal levels of >6 mg/L to approximately one mg/L within 7-10 days of major lower-catchment rainfall events in some tributaries, and at some mainstream sites, of the lower Clarence and Richmond Rivers and remained at these very low concentrations for a number of days as flood waters receded. It is likely that these declines in dissolved oxygen concentrations were caused by microbial decomposition of suspended organic matter in receding flood waters or by inorganic chemical processes in inundated acid sulfate soil (ASS) (J. Sammut, personal communication), or a combination of both. On both rivers, results suggested that most tributaries draining the lower floodplain contributed water with low dissolved oxygen concentrations to the main channel in the immediate post-flood periods. By contrast, dissolved oxygen concentrations in the main channels upstream of tributaries draining ASS areas (ie as measured at Coraki on the Richmond and at Grafton on the Clarence) remained within normal limits. Factors relating to soil types, land use (particularly the failure of some 'improved' pastures to survive periods of inundation) and drainage patterns between the upper and lower catchments probably account for this difference in water quality.

5.3.3.3 Habitat degradation

Trawling, mangrove removal, nursery area damage, urban development/runoff may cause nutritional stress and crowding in smaller, partly degraded remnant areas.

It is likely that prawns and other susceptible crustaceans escaping such unsuitable water quality conditions would concentrate at sites where water quality was more favourable. This generally would be estuarine sites close to the river mouth where, as a consequence of buffering and mixing, acidification and deoxygenation are usually less severe. Since most shrimp farms are located in such areas, the displaced populations may be stressed and concentrated at sites where infected material from farms would be most common. Deterioration of water quality as described is therefore likely to increase the probability of outbreak initiation by concentrating stressed populations at sites where exposure to infectious material is more likely. However, because these water quality deteriorations

generally follow significant rainfall events, they are typically associated with high volumes and flow rates. Under these circumstances, significant dilution and flushing of infectious discharges from shrimp farms may occur, thereby decreasing the opportunities for infection from this source.

5.4 WSSV

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.

Available evidence also suggests the following:

- WSSV infection is likely to cause significant losses in farmed *P. monodon*, *P. japonicus* and *P. merguiensis*;
- WSSV infects a wide range of aquatic animals other than prawns.

Figure 5.2 below illustrates possible patterns of spread of infection in the range of predators/scavengers at a WSSV release site. The scenarios are based on the following assumptions :

- most transmission of infection in the wild occurs by ingestion of subclinically infected prawns, infected moribund prawns or infected prawn tissue;
- fish are the major predators for clinically normal infected prawns;
- a wide range of predators/scavengers, dominated by fish (50% of consumption) but including prawns (25% of consumption) and crabs (25% of consumption) ingests material from moribund or dead prawns;
- up to four animals may ingest material from any infected prawn or piece of prawn tissue;
- All predator/scavenger prawn species have high susceptibility to infection, crab species have low to moderate susceptibility and fish species are not susceptible;
- There are moderate levels of environmental stress at the release site, sufficient to induce 50% morbidity rate in infected prawns and 10% morbidity rate in infected crabs.

Figure 5.2a Subclinically infected prawn(s) released with predation by fish resulting in failure of establishment and spread

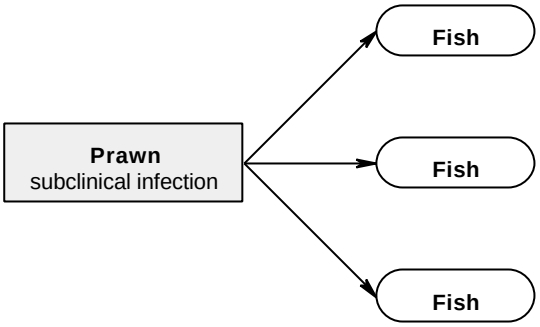
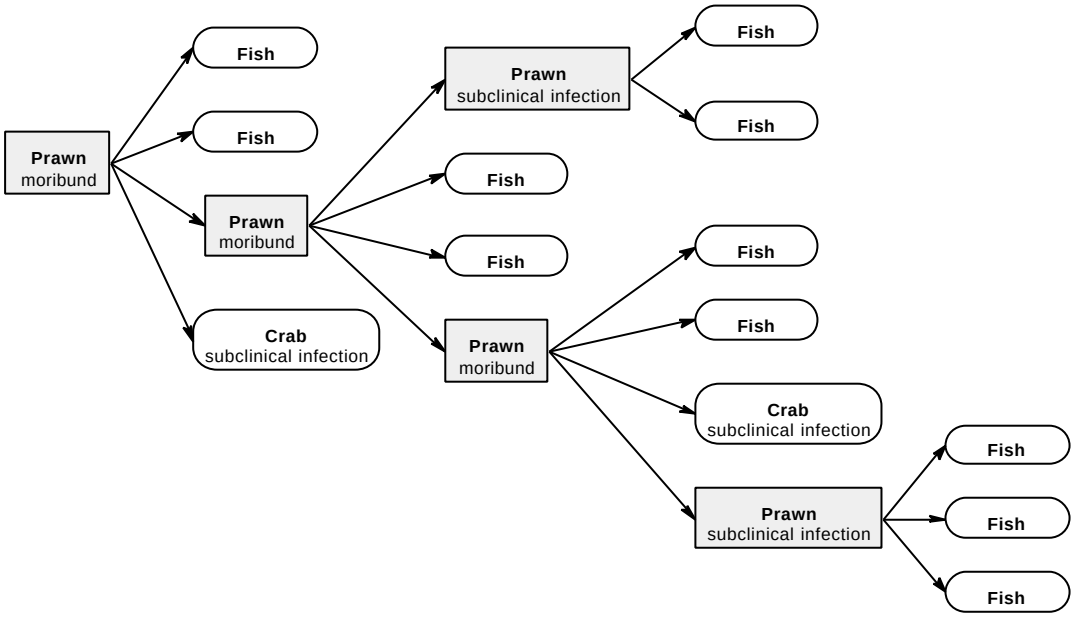


Figure 5.2b Infected moribund or dead (eg bait) prawn(s) released with predation by a wider range of predators but with the outbreak rapidly dying out due to dominance of non-susceptible and non-infectious predators



Considering the scenarios shown in Figure 5.2 and acknowledging moderate uncertainty, the weight of evidence supports the following conclusions regarding the potential impacts of WSSV following introduction of infection into Australia via specified routes:

Contaminated processing waste -

Event	Probability	Comment
Exposure of prawns in Australia	High	Depends on import sources and volumes
Significant impact on farmed prawns	Low to moderate	Depends on waste disposal practices and relative locations (see Section 6 - case studies)
Significant impact on wild prawns	Low	Based on : • relatively lower prawn densities • lower stress levels in wild • greater competition from non-susceptible scavengers compared with pond populations • evidence of minimal spread

Prawn farm waste -

Event	Probability	Comment
Significant impact on wild prawns	Low	Based on : • lower prawn densities relative to farms • lower stress levels relative to farms • greater competition from non-susceptible predators/scavengers compared with pond populations • evidence of minimal spread

Bait prawns (single or multiple releases) -

Event	Probability	Comment
Exposure of prawns in Australia	High	Depends on import sources and volumes as well as usage patterns
Significant impact on farmed prawns	Minimal	Depends on level of biosecurity
Significant impact on wild prawns	Low	Based on : • relatively low prawn densities • lower stress levels • low probability of outbreak propagation, given high level of competition from non-susceptible scavengers (see Figure 5.2)

5.5 IHHNV

High IHHNV infection rates, combined with high levels of morbidity in wild *P. stylirostris* in the Gulf of California were associated with a marked decline, and subsequent recovery, in wild catches in the late 1980s and early 1990s. However, catch data for *P. californiensis*, a less susceptible species having much lower infection rates and morbidity, showed a similar

decline and subsequent recovery during the same period. Furthermore, catch data for both species show matching fluctuations in catch from 1979/80. These observations are consistent with at least two possibilities:

- IHHNV infection resulted in a decline in the population of *P. stylirostris* with consequent reduced catches. Fishing effort was diverted to *P. californiensis* which resulted in a concomitant decline in that species.

- The catches for both prawn species fluctuate in parallel over time as shown in Figure 4.3 for factors not associated with disease. A decline in the catch for both *P. stylirostris* and *P. californiensis* coincided with the introduction of IHHNV.

Available evidence also suggests the following :

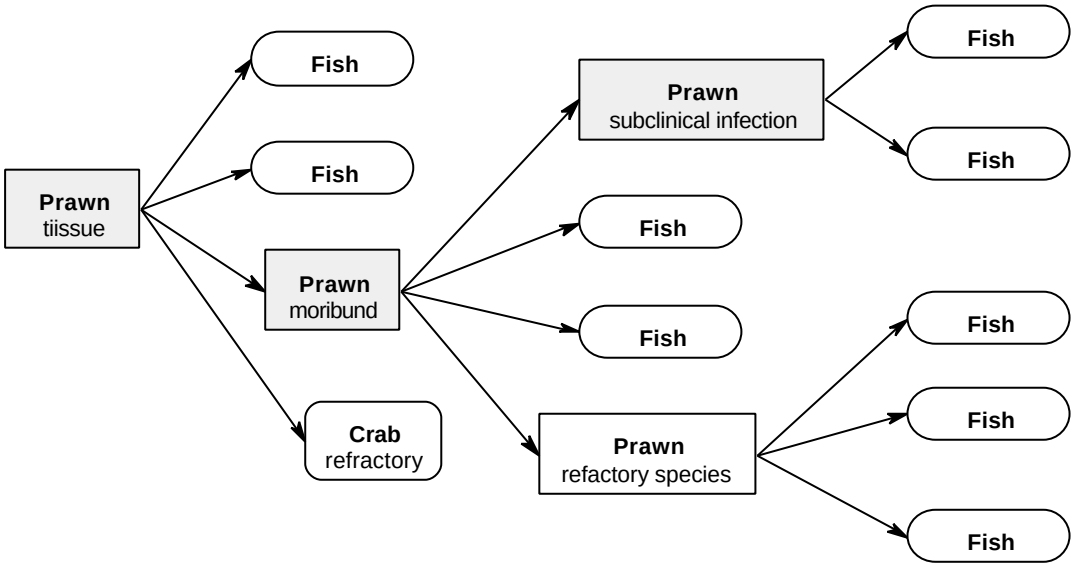
- IHHNV infection is unlikely to cause significant losses in farmed *P. monodon*, *P. japonicus* and *P. merguiensis*;
- IHHNV does not cause significant infections in aquatic animals other than prawns;
- given that IHHNV infection causes serious losses in farmed *P. stylirostris* and *P. vannamei*, it may cause similar losses in some species of Australian prawns, other than those mentioned above, if they are farmed at some time in the future. In particular, it is possible that IHHNV, released from farmed *P. monodon*, *P. japonicus* or *P. merguiensis* in Australia, may infect one or more wild, putatively susceptible populations. Although there is no published information confirming that IHHNV is transmitted vertically, Lightner (unpublished) states "it appears that IHHNV is very efficient in vertical transmission, with 100% transmission/infection rates typically measured in batches of PLs produced from infected (presumably *P. stylirostris*) broodstock." If such a route is confirmed, it is therefore possible, but of low probability, that IHHNV, released from an infected farm, may infect, via ingestion, one or more susceptible wild Australian species and subsequently be more widely transmitted vertically (and horizontally) within those populations. However, given the low prevalence rate of IHHNV infection in prawn species currently farmed in Australia, only relatively very small amounts of infectious material are likely to be released from prawn farms.

Figure 5.3 below illustrates possible patterns of spread of infection in the suite of predators/scavengers at a IHHNV release site. They are based on the following assumptions :

- most horizontal transmission of infection in the wild occurs by ingestion of subclinically infected prawns, infected moribund prawns or infected prawn tissue;
- outbreaks of IHHN are unlikely to occur in farmed *P. monodon* or *P. japonicus* in Australia and, consequently, any exposure of wild populations will be via infected bait prawns or via infected processing plant waste and not from spillover from farmed prawns;
- although fish are the major predators for clinically normal infected prawns, a wider suite of predators/scavengers, dominated by fish (50% of consumption) but including prawns (25% of consumption) and crabs (25% of consumption) ingests material from moribund or dead prawns;
- up to four animals may ingest material from any infected piece of prawn tissue;

- approximately 20% of predator/scavenger prawn species have high to moderate susceptibility to infection, approximately 80% of prawn species have low susceptibility to infection and other crustacean and fish species are not susceptible;
- there are moderate levels of environmental stress at the initial release site and at some PL nursery sites, sufficient to induce 50% morbidity rate in highly susceptible infected prawn species and 20% morbidity rate in moderately susceptible prawn species.
- For (currently unknown) highly susceptible wild prawn species, only a small proportion of individuals infected at the initial release site will survive to spawn, and most of the progeny of these individuals will be vertically infected. None of these infected PLs which subsequently migrate to stress-free nursery sites will develop clinical IHHN, but most infected PLs which subsequently migrate to moderate stress nursery sites will die from IHHN. Since most nursery sites will be stress-free, most infected PLs will survive to pass infection to progeny.

Figure 5.3a Infected prawn material derived from processing plant (single pulse release) or bait prawn released, followed by consumption by the wide suite of predators at a moderate stress release site. Consequences for most susceptible prawn species scavenging infected tissue at the site are :



However, if the release occurs over a prolonged period at a moderate stress site or is a single pulse release at a high stress site, some individuals of the susceptible species may consume infected tissue to become subclinically infected. Figures 5.3b and 5.3c illustrate the possible consequences depending on whether such individuals are debilitated by infection (b) or remain unaffected and sustain the infection through vertical transmission (c). It is likely that there will be a range of possibilities combining elements of b and c with some prawns debilitated and consumed by predators and some surviving to reproduce. However, for simplicity, the two limits are illustrated.

Figure 5.3b **Infected prawn material released over a prolonged period at a moderate stress site or as a single pulse at a high stress site with some infected prawns becoming debilitated and consumed by a range of predators**

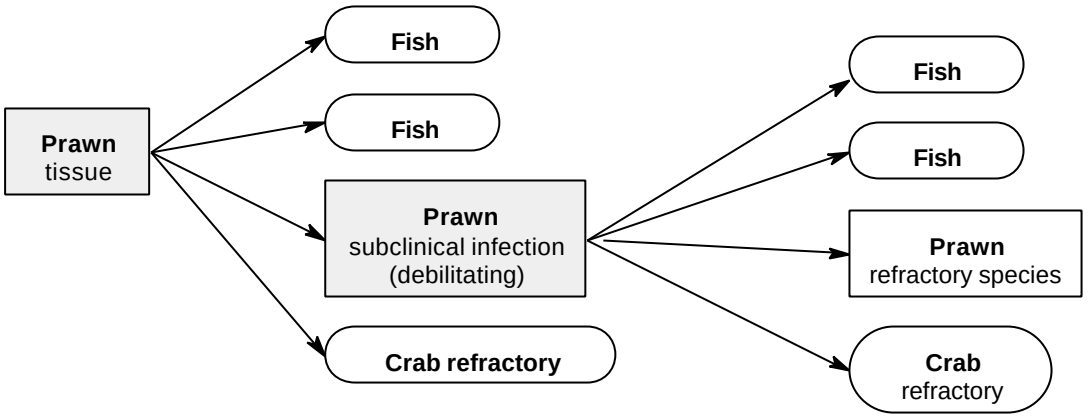
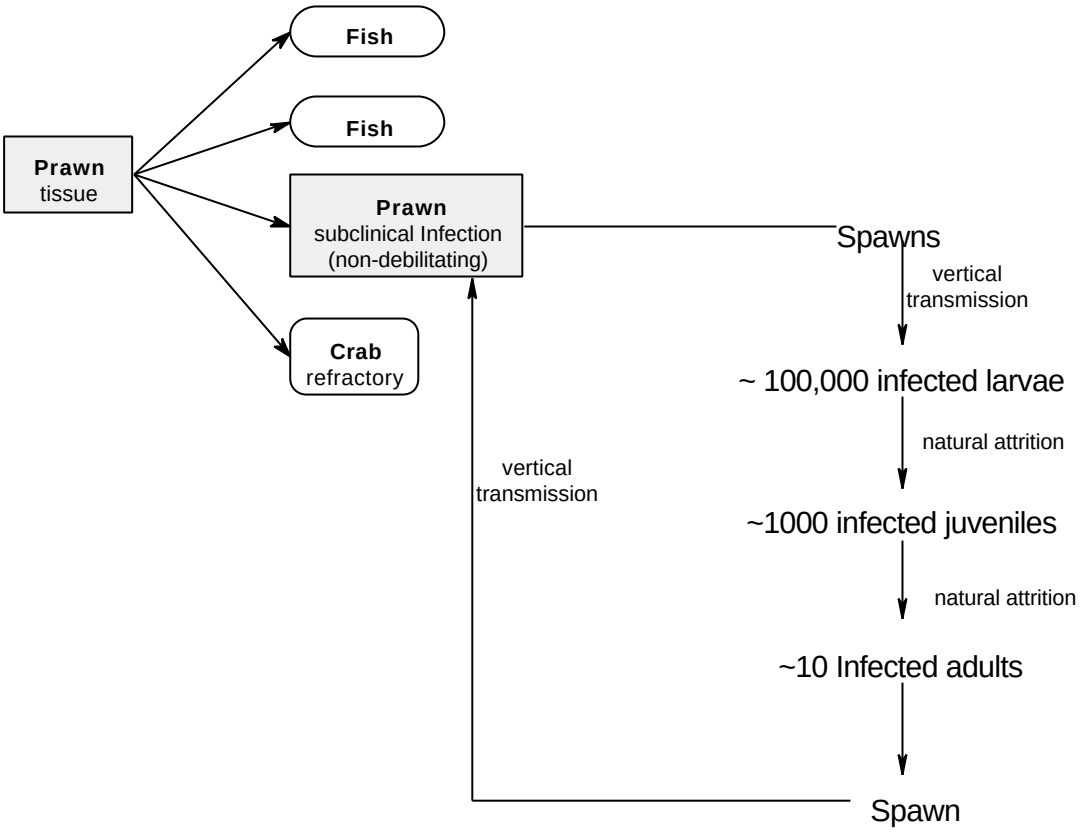


Figure 5.3c Infected prawn material released over a prolonged period at a moderate stress site or as a single pulse at a high stress site with infected prawns not debilitated and therefore reproducing and sustaining the infection



Considering the scenarios shown in Figure 5.3 and acknowledging moderate uncertainty, the weight of evidence supports the following conclusions regarding potential impacts of IHHNV following introduction of infection into Australia via the most likely routes:

Contaminated processing waste -

Event	Probability	Comment
Exposure of prawns in Australia	High	Depends on import sources and volumes
Significant impact on farmed prawns	Low to moderate	Depends on waste disposal practices and relative locations (see Section 6 - case studies)
Significant impact on wild prawns	Low	Based on : • low susceptibility of farmed species • relatively lower wild prawn densities • lower stress levels • greater competition from non-susceptible scavengers compared with pond populations • evidence of minimal spread

Prawn farm waste -

Factor	Probability	Comment
Significant impact on wild prawns	Minimal	Based on : • low infection rates in farmed stocks • relatively lower prawn densities • lower stress levels • greater competition from non-susceptible predators/scavengers compared with pond populations • evidence of minimal spread

Bait prawns (single or multiple releases) -

Factor	Probability	Comment
Exposure of prawns in Australia	Low	Depends on import volumes from endemic countries as well as usage patterns
Significant impact on farmed prawns	Minimal	Depends on level of biosecurity
Significant impact on wild prawns	Low	Based on : • relatively low prawn densities • lower stress levels • low probability of outbreak propagation, given high level of competition from non-susceptible scavengers

6 Case studies

The two case study areas were the Clarence River of northern New South Wales and the Townsville region of north Queensland. Both have significant prawn industries and relatively complex, but very different ecosystems. This section examines the potential impacts of WSSV and IHHNV in each geographic area. Elements common to both areas are dealt with first, followed by separate discussions on each specific area.

6.1 Issues common to both regions

A number of factors will influence the impacts of WSSV or IHHNV infection on prawn and other aquatic animal communities independent of the specific attributes of the location. These are briefly reiterated here, although some are discussed more fully in other sections of this report.

6.1.1 Exotic virus introduction and spread

Evidence presented in Section 5 suggests the most likely sources of exotic prawn viruses for Australian prawns (farmed or wild) are :

- Imported feed
- Viruses present in prawn-derived material incorporated into prawn feeds may survive the manufacturing processes;
- Processing plant waste
- Improper disposal of untreated waste from plants processing imported, uncooked prawns may allow spread of infection to farmed or wild stocks;
- Prawn farm effluent
- Effluent from infected farms may contain virus in moribund or dead prawns, prawn tissue or water. The suspended solids component of prawn farm effluent is usually detectable only within one km of the discharge point. However, under high discharge conditions, or abnormal flow conditions in the receiving estuary, suspended solids may be detectable within five km of the discharge point. For purposes of this study, it is assumed that inanimate infected material discharged from a prawn farm is similarly distributed.
- Bait prawns
- Wild prawns and other aquatic animals may become infected following ingestion of infected prawns used for bait.

6.1.2 Host factors

Available evidence suggests that most new WSSV or IHHNV infections in ponds or in the wild are acquired by ingestion of subclinically infected prawns, infected moribund prawns or infected prawn tissue. It is therefore likely that infection rates in the wild for each exposed species will depend, at least in part, on that species' ability to successfully compete with other predatory/cannibalistic species for infectious material.

6.1.3 Patterns of spread

Possible patterns of dissemination of infection amongst the suite of predators/scavengers at a WSSV or IHHNV release site were discussed in Section 5. These possible spread patterns were based on the following assumptions:

- Most transmission of infection in the wild occurs by ingestion of tissue from subclinically infected prawns, infected moribund prawns or infected prawn tissue;
- Fish are the major predators of clinically normal infected prawns;
- A wider suite of predators/scavengers, dominated by fish (50% of consumption) but including prawns (25% of consumption) and crabs (25% of consumption) ingests material from moribund or dead prawns;
- Up to four animals may ingest material from any infected prawn or piece of prawn tissue.

6.1.4 Environmental factors

As discussed in Section 5, morbidity and mortality rates are likely to increase when prawns infected with WSSV (Wang *et al*. 1997b) or IHHNV (Browdy *et al*. 1993) are also stressed by factors such as adverse environmental conditions. It is assumed in the case studies that the effects of stressors are compounded (eg a wild prawn population towards the limits of its natural range would experience higher levels of stress when exposed to an environmental stressor such as low dissolved oxygen concentrations caused by eutrophication than would a similar population exposed in its preferred habitat).

Environmental stressors causing low to moderate stress levels may occur at any hypothetical infection release site. It is assumed that wild prawns, but probably not crabs or small crustaceans such as copepods, are highly mobile and, if exposed to stressors, will migrate to more suitable environments.

6.1.5 Virus/host interactions

It is assumed for the case studies that all strains of WSSV are closely related and that the pathogenicity of each for Australian penaeid prawn species is essentially similar.

Given that a wide range of penaeid prawn species, other prawns, crabs, lobsters and miscellaneous arthropods have been reported, or suspected, to be infected by WSSV elsewhere (Flegel 1997), it is likely that many Australian prawn species, other crustacean and arthropod species will be similarly susceptible to infection. Finfish are considered not to be susceptible.

WSS affects prawns of all sizes, and outbreaks have been observed in postlarvae through to 40g animals. Outbreaks have been reported in all types of farms, from high yield (>30 prawns per sq m) to lower yield traditional systems (~ five prawns per sq m) (Chanratchakool *et al*. 1998). As discussed in Section 5, WSSV is likely to cause significant losses in Australian farmed prawns, including *P. monodon*, *P. japonicus* and *P. merguiensis*. The evidence also suggests that WSSV infects, but does not cause significant disease or mortality in wild prawns or other aquatic animal populations.

IHHNV commonly causes disease in farmed *P. stylirostris* and *P.vannamei,* but only rarely in farmed *P. monodon, P. semisulcatus* and *P. japonicus* (Turnbull *et al*. 1994;

Lightner, 1996b; Lightner *et al*.1997; Panchayuthapani 1997). Farmed juvenile *P. stylirostris* are particularly susceptible, experiencing acute disease and high mortality rates. By contrast, IHHNV infection in farmed *P. vannamei* causes a chronic disease, RDS. Therefore, given the variability in expression of IHHNV infection between prawn species, it is likely that some Australian prawn species which have not yet been exposed to 'classical' IHHNV may, like *P. stylirostris,* be highly susceptible to infection if held under crowded, stressful conditions. Others, like *P. vannamei*, may be less susceptible, and yet others may be refractory. However, IHHNV is increasingly viewed as a generally insignificant pathogen of farmed prawns in the Asia-West Pacific region (Lightner 1997). IHHNV infection has been observed in wild populations of *P. stylirostris*, *P. californiensis*, *P. vannamei* (Larramore 1992; Pantoja *et al.* 1999) and *P. occidentalis* (Lotz 1992). However, as discussed above, evidence that such infection causes significant losses in wild populations is at present inconclusive.

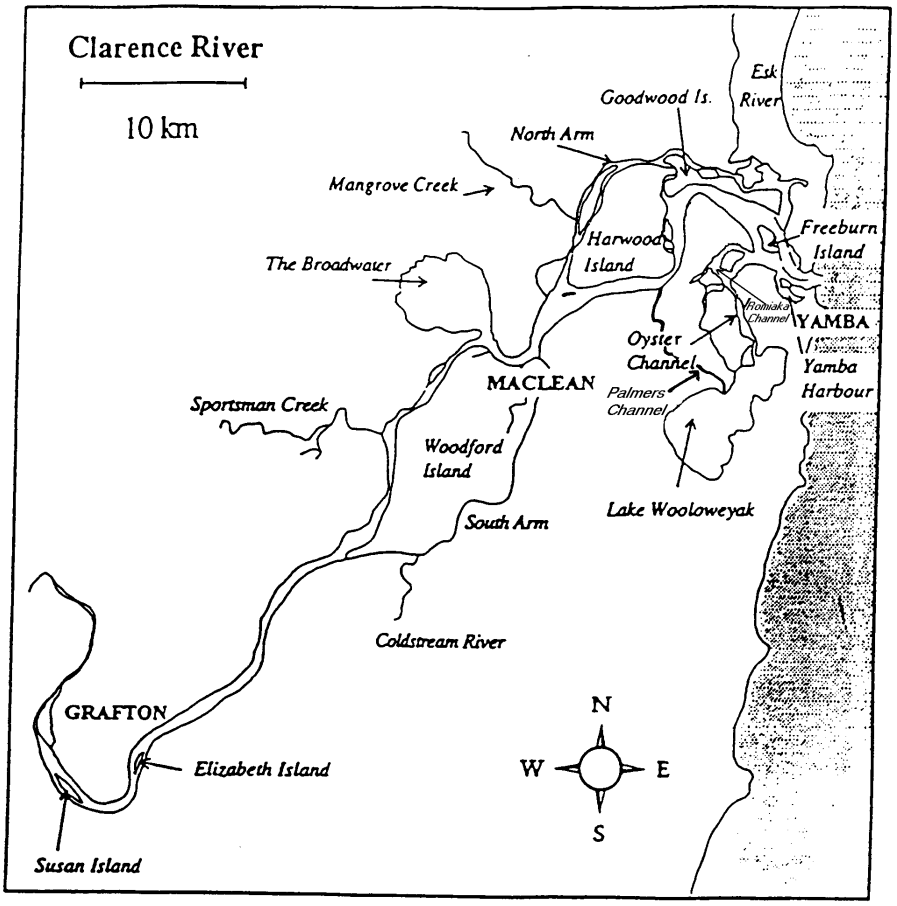
On the basis of this evidence, it will be assumed in these case studies that IHHNV infection is unlikely to cause disease in prawn species currently farmed in Australia. It will also be assumed that infection is likely to cause disease in currently unidentified wild prawn species in Australia. Furthermore, it will be assumed that other Australian aquatic animals exposed to IHHNV will in most cases be refractory to infection, but if infected, will not become diseased.

6.2 Clarence River region

6.2.1 General description

In terms of both catchment area and discharge, the Clarence River is the largest coastal river in southeastern Australia. The catchment covers an area of some 22,000 km^2 and includes most of the coastal zone east of the Great Dividing Range from the Queensland/NSW border in the north to Dorrigo in the south. The total water area of the river is approximately 103 km^2. There are several large tributaries in the upper catchment, with about 19,800 km^2 (90%) of the total catchment area lying above the floodplain, which begins around the city of Grafton, approximately 70 km upstream from the river mouth. There are four major sub-catchments in the lower floodplain, where the river divides into natural sections forming an extensive estuarine area, before flowing past Maclean and out to the ocean between Iluka and Yamba. The lower estuary consists of an extensive network of major and minor channels, islands and bays, making the area an important aquatic animal habitat (McVerry, undated). The Lower Clarence region is shown in Figure 6.1.

Figure 6.1: Lower Clarence region of northern New South Wales



Climate in the catchment is predominantly of a sub-tropical maritime type characterised by an annual rainfall exceeding 1400 mm. Approximately 60% of this rainfall occurs between December and April. Summers are usually warm to hot, while winters are drier and mild. Rainfall is highest in the upper reaches of the catchment and varies with topography. Periodical flooding of the lower catchment occurs after heavy rainfall events and is considered a normal occurrence affecting river and estuarine ecosystems. The estuarine area of the river is affected by semi-diurnal tides with a range of about two m in water levels. Saline intrusion into the river reaches approximately 65 km upstream in dry weather. However, these upstream salinity levels are typically very low, indicating freshwater dominance in the majority of the river.

6.2.2 Crustacean populations

The school prawn, *M. macleayi* and the eastern king prawn, *P. plebejus* breed offshore, but the larvae enter the estuary to feed and grow. The inshore greasyback, *M bennettae* is the only resident penaeid species to complete its entire life cycle in estuarine waters.

Penaeid prawns vary in their feeding habits, but are commonly classed as 'opportunistic omnivores'. The main part of the prawn diet is made up of bacteria, algae and microscopic animals which grow on the surface of fine silt, sand and mud particles on the sediment

surface. Detritus is an important part of the natural diet of penaeids, particularly juvenile school prawns (Lancaster 1990).

As well as the natural migrations of the prawns there are various irregular movements. Many commercial species are distinguished as either 'consistent' or 'inconsistent' forms. The school prawn is considered an inconsistent species, forming pronounced age groups and dense schools. The species shows a preference for turbid waters and sites with soft, muddy sediment. It is essentially associated with rivers and its abundance may be linked with the occurrence of river floods (Racek 1959; cited by Lancaster 1990). Wooloweyah Lagoon, together with the adjacent Oyster, Micalo and Romiaka Channels, is the most ecologically important habitat area of the lower Clarence River (Soros-Longworth and McKenzie 1978, cited by Lancaster 1990). The Lagoon and channels are important nursery grounds for juvenile prawns and fish (Clarke and Geary 1988, cited by Lancaster 1990). However, the Lagoon is under increasing pressure as a recreational and tourism resource, and recreational fishing in the Lagoon for blue swimmer and mud crabs, as well as various finfish, is an important activity (Lancaster 1990).

The dominant prawn species in the Lagoon is the school prawn *Metapenaeus macleayi*, but other species such as the eastern king and greasyback prawns are also commercially important. School prawns migrate out of estuaries following disturbance of the bottom sediments associated with floods, although juveniles can tolerate large falls in salinity (as much as 30 ppt in 30 hours) without ill-effects (Ruello 1973).

Lancaster (1990) suggested the dominance and abundance of school prawns in the Lagoon may be a consequence of the favourable sediment conditions, shallow depth and turbid waters. He further suggested that prawns in the Lagoon would respond to adverse, stressful, environmental conditions (such as changes in dissolved oxygen concentrations associated with algal blooms or floods) by migrating to another estuarine site or out to sea.

6.2.3 Prawn-related industries

The Clarence River supports both offshore and riverine prawn capture fisheries, as well as farmed prawn production.

6.2.3.1 Capture fisheries

The Clarence capture fishery operates out of the Iluka and Yamba ports and is the largest on the east coast of Australia. The offshore prawn fishery represents the most valuable commercial fishery in both the lower Clarence and northern NSW area. While offshore school prawns make up a proportion of the offshore prawn catch, king prawns provide the best returns to fishers. There is also an important riverine prawn fishery, with school prawns being the primary target species. The Clarence River region is the largest production area for school prawns, *Metapenaeus macleayi* in NSW, contributing about 30% of the State's total annual catch. Of this, Wooloweyah Lagoon provides approximately one quarter to one third of the total Clarence catch (Lancaster 1990).

Fishing for prawns is usually undertaken at night. However trawlers also operate during the day in times of flood. Offshore school prawns are caught in water depths of 3-12 fathoms

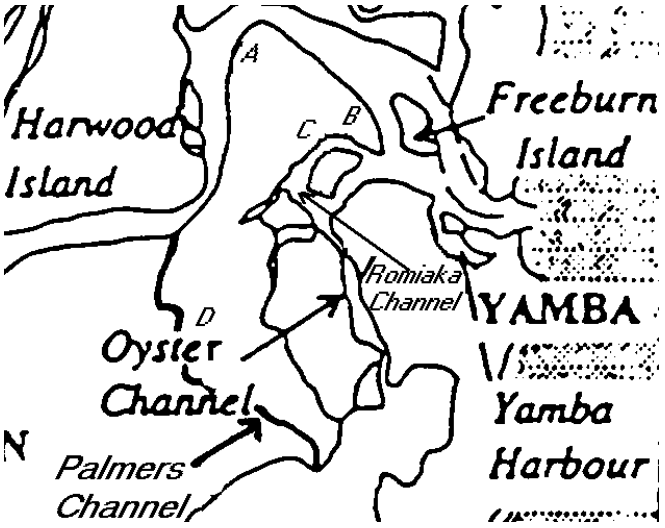
(5.5-22 m), while king prawns are caught in depths ranging from 12-100 fathoms (22-180 m) (McVerry, undated).

In 1996-97, 900 tonnes of prawns were delivered to the Clarence River Fishermen's Cooperative. The proportion of school prawns to king prawns was particularly high in 1996-97 at 70:30, while the annual average for the previous five years was 50:50. Fluctuations in the prawn catch have been attributed to changes in the distribution and abundance of prawns associated with the influence of rainfall (Ruello 1973).

6.2.3.2 Prawn farming

Currently, four prawn farms are operating on the Clarence estuary with their approximate locations shown in Figure 6.2. Together they occupy about 106 ha with 80% of this area devoted to production of *P. monodon*. Effleunt from Farms A and B is released into the mainstream of the lower river, while effluent from farms C and D is released into Romiaka Channel and Palmers Channel, respectively.

Figure 6.2: Location of Clarence River prawn farms



6.2.4 Human alterations

The Clarence River has undergone many major changes over the last 150 years. The majority of the river's catchment has been cleared for agricultural use, resulting in sedimentation problems associated with upstream erosion, particularly in times of high river flow (State Pollution Control Commission 1987; cited by Lancaster 1990). There are many sugar cane farms on the river floodplain, resulting in inputs of fertilisers and pesticides to the river which have impacted on water quality.

Major alterations to the lower floodplain of the Clarence River have included structural flood mitigation and drainage works completed in the 1960s and 1970s. These have significantly altered the hydrology and wetland systems of the river. In particular, the drainage works have lowered the water table in many of the estuary's extensive acid sulfate soil areas and

have increased the rate and volume of acidified runoff water after rain events (Lancaster 1990; Tulau 1998).

Lancaster (1990) lists the following as having potentially negative impacts on the Wooloweyah Lagoon environment :

- Flood mitigation works
  These structures cause increased quantity and rate of runoff. The direct effect of draining swampland is conversion of brackish estuarine wetlands to freshwater wetlands. The water table of the drainage basin is lowered as a result of the sub-surface drainage.
- Sugar cane farming
  Runoff from cane farms increases the concentrations of nutrients, and possibly pesticides, in drainage waters.
- Grazing
  Grazing in the Lagoon basin has increased the concentrations of nutrients derived from fertilisers and cattle faeces in drainage waters.
- Prawn farming
  Prawn farms release effluent (water quality of such effluent must meet NSW Environment Protection Authority standards) to adjacent waterways.
- Acid sulfate soils
  Acidified runoff water may be released from drains into the lagoon after rain events.
- Tourism
  Developments such as lakeside resorts and marina developments in the Lagoon have diverse impacts on the Lagoon ecosystems.
- Urban development
  Expanding urban development adjacent to the Lagoon may result in increased discharge of nutrients to the Lagoon.
- Trawling
  Operation of trawlers and nets in the shallow depths of the Lagoon causes disturbance and mixing of the Lagoon bottom.

6.2.5 Water quality

Water quality at 49 representative sites in the Clarence River catchment has been assessed by the NSW Environment Protection Authority. Eight sampling runs (7 during low flow conditions and one during high flow conditions) were carried out during 1994 and 1995. Findings at each of the sites were assessed in relation to seven broad classes of environmental values, including 'Aquatic Ecosystem Protection'(AEP). This environmental value specified water quality conditions under which native fauna and flora are most likely to survive, and was based on indicators and criteria developed by the Australia and New Zealand Environment and Conservation Council (ANZECC 1992). These indicators and criteria were used to assess the potential long term threats to the aquatic ecosystem, rather than dramatic, 'overnight' losses of habitat and resource.

Of the Clarence River estuarine sites studied during low flow periods, most were ranked 'Poor' or 'Very poor' in the AEP category. The suspended solids indicator was responsible for the majority of fails, with 45% of observations not meeting the criterion. Dissolved oxygen (percent saturation) and pH failed between 10% and 15% of

observations while the remaining indicators failed less than 10% of observations. Samples collected in the main channel of the Clarence River generally ranked better than sites located on tributaries, indicating that tidal exchange and marine water dilution processes may be active in the main channel of the estuary. Notably, AEP was assessed as 'Fair' at a mainstream site adjacent to the effluent discharge points for farms A and B, while it was assessed as 'Poor' and 'Very Poor' at channel sites close to the discharge points for farms C and D, respectively. Furthermore, AEP at a site in the centre of Wooloweyah Lagoon under low flow conditions was assessed as 'Very Poor'.

Although only four freshwater (non-estuarine) sites were examined during the study under high flow conditions, results showed 'massive' loads of total nitrogen, total phosphorus and suspended solids in the catchment. Consequently, none of the variables used in assessing AEP were within acceptable ANZECC limits for any of the four Clarence sites under high flow conditions (NSW Environment Protection Authority 1996). In a separate study, Lancaster (1990) found that Wooloweyah Lagoon was periodically eutrophic during periods of freshwater input and that, consequently, dissolved oxygen concentrations may reach critically low levels.

Given that prawns, particularly school prawns can tolerate conditions of high turbidity/high suspended solids, the following conclusions seem reasonable:

- Under low flow conditions, it is likely that prawns and other aquatic animals in the lower Clarence River mainstream will generally be exposed only to low levels of environmental stress.

- Under low flow conditions, it is likely that prawns and other crustacean species in Wooloweyah Lagoon and adjacent channels will also be exposed to low levels of environmental stress.

- Under high flow conditions, it is likely that most prawns in the mainstream of the lower Clarence River will be exposed to moderate levels of environmental stress (associated with high concentrations of nutrients and suspended solids, together with increased flow rates), and will tend to migrate to more favourable environments. However, crabs and less mobile crustacean species may be unable to migrate elsewhere.

- Following major rain events, prawns in Wooloweyah Lagoon and adjacent channels may be exposed to moderate to high levels of environmental stress (eg low dissolved oxygen concentrations associated with eutrophication) and will tend to migrate to more favourable environments. However, crabs and less mobile crustacean species may be unable to migrate elsewhere.

- However, during potential periods of moderate and high environmental stress, there will be concurrent high dilution levels for any infectious material because of the high flow conditions in both the mainstream channels and lagoon. This reduction in the concentration of infectious organisms, may thus counterbalance the environmental stressors to some extent.

6.2.6 Sources and spread of WSSV infection

The most likely sources of WSSV infection for wild stocks on the Clarence River are:

1. Improperly handled waste from infected, imported prawns following processing at a local plant (although Clarence River Fishermen's Cooperative currently and historically processes only domestic product);

2. Effluent from a prawn farm infected via postlarvae or feed used during growout;

3. Infected bait prawns released into the environment.

Each of these is considered in Tables 6.1 to 6.3 below in terms of risks of outbreak occurrence in farmed prawns, wild prawns and non-prawn crustaceans according to WSSV infection source.

Table 6.1: Improperly handled processing waste:

Event	Probability	Comments
Virus entry to the Clarence River system	Very low	* Depends on import sources and volumes. * Risk will increase to moderate if imported product processed.
Spread of infection among farms following entry to Clarence	Low to moderate	Depends on waste disposal practices and relative locations.
Spread of infection among local wild prawn populations which may or may not involve infected farms	Low to moderate	Based on : * high level of competition for infected tissue from non-susceptible scavengers compared with pond populations; * high probability of farm outbreak reporting compliance and stamping out policy implementation on farms; * evidence of minimal spread of infection in Texas.

Table 6.1 (continued): Improperly handled processing waste:

Event	Probability	Comments
Spread of infection from wild or farmed prawns, to local, wild (non-prawn) crustacean populations	Low to moderate	Based on : * high level competition for infected tissue from non-susceptible scavengers compared with pond populations; * high probability of farm outbreak reporting compliance and stamping out policy implementation on farms; * evidence of minimal spread of infection in Texas.
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks	Low	Based on : * wild prawn population densities are low relative to farms; * prawns may avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Low	Based on : * evidence suggests virus is less pathogenic to other crustaceans than to prawns; * wild crustacean population densities are low relative to prawn population densities on farms; * relatively sessile crustacean populations may not be able to avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.
Re-introduction into local prawn farms by infected crabs or other wild	Moderate	Based on: * Anecdotal evidence from Thailand if not excluded by piscicides and fences.

crustaceans		

Table 6.2: Prawn farm effluent

Event	Probability	Comments
Spread of infection from farmed to local wild prawn populations	Low to moderate	Based on : * high probability of farm outbreak reporting compliance and stamping out policy implementation on farms; * high level of competition for infected tissue from non-susceptible scavengers compared with pond populations; * evidence of minimal spread of infection in Texas and probably South Carolina.
Spread of infection to local wild (non-prawn) crustacean populations	Low to moderate	Based on : * high probability of farm outbreak reporting compliance and stamping out policy implementation on farms; * high level competition for infected tissue from non-susceptible scavengers compared with pond populations; * evidence of minimal spread of infection in Texas.
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks	Low	Based on : * wild prawn population densities are low relative to farms; * prawns may avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.

Table 6.2 (continued): Prawn farm effluent

Event	Probability	Comments
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Low	Based on : * evidence suggests virus is more pathogenic to prawns than to other crustaceans; * wild crustacean population densities are low relative to prawn population densities on farms; * relatively sessile crustacean populations may not be able to avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.
Re-introduction into local prawn farms by infected crabs or other wild crustaceans	Moderate	Based on: * Anecdotal evidence from Thailand, if not excluded by piscicides, fences

Table 6.3: Bait prawns (single or multiple species)

Event	Probability	Comments
Virus entry to the Clarence River system	High	Depends on import sources and volumes as well as usage patterns.
Spread of infection to local farms following entry to Clarence	Minimal	Based on: * farms usually have good quarantine measures.
Spread among local wild prawn populations	Low	Based on : * high level of competition for infected tissue from non-susceptible scavengers compared with pond populations; * low prawn densities relative to farms.
Spread of infection to local wild (non-prawn), crustacean populations	Low	Based on : * high level competition for infected tissue from non-susceptible scavengers compared with pond populations.
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks	Low	Based on : * wild prawn population densities are low relative to farms; * prawns may avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.

Table 6.3 (continued): Bait prawns (single or multiple species)

Event	Probability	Comments
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Low	Based on : * evidence suggests virus is more pathogenic to prawns than to other crustaceans * wild crustacean population densities are low relative to prawn population densities on farms * relatively sessile crustacean populations may not be able to avoid stress by migrating elsewhere * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions * no convincing evidence of overseas disease occurrence
Probability of infected crabs or other wild crustaceans subsequently re-introducing infection into adjacent prawn farm	Moderate	Based on: * Anecdotal evidence from Thailand, if not excluded by piscicides, fences

6.2.7 Sources and spread of IHHNV infection

Possible sources of IHHNV infection for wild stocks on the Clarence River are :

1. Improperly handled waste from infected, imported prawns following processing at a local plant (Clarence River Fishermen's Cooperative processes only domestic product);

2. Effluent from a prawn farm infected via postlarvae or feed used during growout;

3. Infected bait prawns released into the environment.

Each of these is considered in Tables 6.4 to 6.6 below in terms of risks of outbreak occurrence in farmed prawns, wild prawns and non-prawn crustaceans according to IHHNV infection source.

Table 6.4: Improperly handled processing waste

Event	Probability	Comments
Virus entry to the Clarence River system	Very low	* Depends on import sources and volumes. * Risk will increase to moderate if imported product processed.
Spread of infection to local farms	Very low	Depends on waste disposal practices, relative locations; and based on : * very low susceptibility to infection of farmed prawn species in Australia.
Spread of infection among local wild prawn populations which may or may not involve infected farms	Low to moderate	Based on : * frequency, duration and extent of release; * moderate to high dilution of infectious material under low and high rainfall conditions, respectively; * high level of competition for infected tissue from non-susceptible scavengers; * unknown, but probably generally low, susceptibilities of most exposed Australian prawn species to infection; * most release sites are likely to be stress-free, resulting in non-debilitating infections in at least some prawns of susceptible species.
Spread of infection directly, or from infected farms, to local, wild (non-prawn) crustacean populations,	Very low	Based on : * high level competition for infected tissue from non-susceptible scavengers compared with pond populations; * no confirmed evidence of infection in non-prawn aquatic animals.
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks (susceptible species only)	Moderate	Based on : * probability of vertical transmission at spawning by surviving, infected, non-debilitated prawns of susceptible species.
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Very low	Based on : * no confirmed evidence of infection in non-prawn aquatic animals.

Table 6.5: Prawn farm effluent

Event	Probability	Comments
Probability of infection initially spreading to local wild prawn populations	Low	Based on : * low prevalence and pathogenicity of IHHNV in currently farmed species; * moderate to high dilution of infectious material under low and high rainfall conditions, respectively; * high level of competition for infected tissue from non-susceptible scavengers; * unknown, but probably generally low, susceptibility to infection of most exposed Australian prawn species.
Spread of infection to local wild (non-prawn) crustacean populations	Very low	Based on : * high level competition for infected tissue from non-susceptible scavengers compared with pond populations * no confirmed evidence of infection in non-prawn aquatic animals
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks	Low	Based on : * prawns may avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Very low	Based on : * no confirmed evidence of infection in non-prawn aquatic animals.

Table 6.6: Bait prawns (single or multiple species)

Event	Probability	Comments
Virus entry to the Clarence River system	High	Depends on import sources and volumes as well as usage patterns
Spread of infection to local farms	Minimal	Based on: farms usually have good quarantine measures.
Spread of infection to local wild prawn populations	Low to moderate	Based on : * frequency and extent of use; * high level of competition for infected tissue from non-susceptible scavengers; * unknown, but probably generally low, susceptibility to infection of most exposed Australian prawn species; * most release sites are likely to be stress-free, resulting in non-debilitating infections in at least some prawns of susceptible species.
Spread of infection to local wild (non-prawn), crustacean populations	Very low	Based on : * high level competition for infected tissue from non-susceptible scavengers compared with pond populations; * no confirmed evidence of infection in non-prawn aquatic animals.
Spread to other areas from initial focus in local wild prawns with subsequent disease outbreaks	Low	Based on : * prawns may avoid stress by migrating elsewhere; * low-moderate environmental stress levels combined with moderate dilution of infectious material under low rainfall conditions; * moderate stress levels combined with high levels of dilution of infectious material under high rainfall conditions; * no convincing evidence of overseas disease occurrence.
Spread to other areas from initial focus in local, wild (non-prawn) crustaceans with subsequent disease outbreaks	Very low	Based on : * no confirmed evidence of infection in non-prawn aquatic animals.

6.3 Townsville region

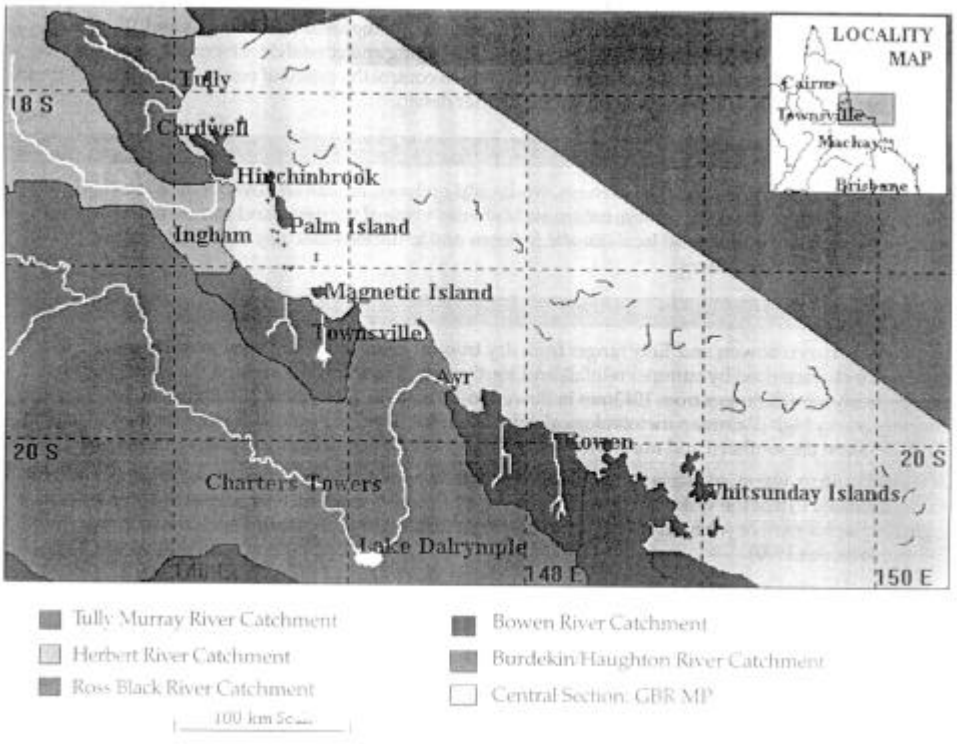
6.3.1 General description

For the purposes of this report, the Townsville area is taken to comprise most of the Central Section of the Great Barrier Reef Marine Park and 400 kms of the coastal zone, from Bowen in the south (ca 20° S) to Tully in the north (ca 18°S). Major river catchments include (from south to north) the Don River, the Burdekin and Haughton Rivers, the Ross and Black Rivers, the Herbert River and the Murray and Tully Rivers. This is seen in figure 6.3. Given the presence of two large prawn farms and concerns about environmental stressors (Cappo, unpublished) in the Tully and Murray River sub-catchments, this area and adjacent waters will be used as a focus location for the case study.

The following description of the area is taken directly from Ludescher (1997).

The Great Barrier Reef , which stretches from the Torres Strait in the north to Bundaberg in south east Queensland is the largest reef complex in the world and is second only to the Philippine reefs in species diversity (Weber 1993; cited by Ludescher 1997). Thus it has high conservation, tourism and scientific value. The varied deep-water, coral reef, lagoon and estuarine habitats of the region also support many species of value to commercial, recreational and indigenous fishing and hunting communities (Qld Government 1993).

Figure 6.3 Major Catchment areas in the Townsville region



Major surface currents, tides and tidal currents, winds and cyclones, rainfall and evaporation rates and geomorphology shape the composition, nature and fisheries value of all coastal and marine habitats. The Tully and Murray River sub-catchments together drain an area of 2,825 sq km. Both rivers rise in the Cardwell Range and discharge into Rockingham Bay.

Their small channel capacities and the very high rainfall in the region (2970 mm/yr) make both these rivers prone to frequent flooding. Smaller rivers and creeks in the catchment area include the Hull River to the north and the many creeks to the south which discharge into Rockingham Bay and the Hinchinbrook Channel, which separates Hinchinbrook Island from the mainland.

Extensive mangrove forests line both sides of the Hinchinbrook Channel, Missionary Bay on Hinchinbrook Island, the coastal fringe of Edmund Kennedy wetlands and the mouths of the Murray, Tully and Hull Rivers. There are rocky headlands at Clump Point and Tam O'Shanter Point and on Dunk, Eva and Hinchinbrook Islands, while sandy shores and sand/mud flats are found at Mission and Googarra beaches, Cardwell and on the Islands. There are significant freshwater wetlands, including deep lagoons and melaleuca swamps on the Tully/Murray flood plain, in the licula palm forest at Mission beach and in Edmund Kennedy National Park. The upper reaches of both rivers comprise fast flowing rainforest stream environments. Fringing reefs line the Brook Islands situated further out in Rockingham Bay (Saenger 1986; cited by Ludescher 1997). Significant seagrass beds are found in the Hinchinbrook Channel and Rockingham Bay (Coles *et al*. 1989).

The main land use in this catchment is grazing, followed by sugarcane and banana cropping. Assigned cane land covered 24,000 ha of the catchment in 1996, with 20,400 ha of cane being harvested in that year. The main population centres are Ingham, Tully and Cardwell. Mission Beach, Dunk Island and Hinchinbrook Island are important tourist destinations (Saenger 1986; cited by Ludescher 1997). A large tourist resort/marina complex is planned for Oyster Point, at the northern end of the Hinchinbrook Channel, and this development has potential adverse implications for the adjacent marine ecosystem. Major industrial developments include the Tully sugar and timber mills.

6.3.2 Crustacean populations

The area supports a diverse range of marine species. The major crustacean species in the Townsville area are described.

6.3.2.1 Banana prawns

Research on the biology and ecology of banana prawns (*Penaeus merguiensis* and *P. indicus*) in the Gulf of Carpentaria shows that they spawn in waters between 10-30 m deep and have two spawning events which peak in early spring and late summer. It is not clear whether one or two spawning peaks occur at more southern latitudes. Postlarvae then enter estuarine mangrove nursery habitats. Recruitment to the relatively shallow adult habitat and trawling grounds takes place after the wet season when young prawns are flushed out of the estuaries with the flood waters. The annual wet season flows are thought to prevent many larvae getting in to the estuaries form the late summer spawning event. Hence only one major recruitment to the fishery occurs at the beginning of each year. Mangrove-lined estuaries are a critical habitat for these prawns (Staples and Vance 1987).

6.3.2.2 Tiger prawns

The two species of tiger prawns (brown - *Penaeus esculentus*, and grooved - *P. semisulcatus*) vary slightly with respect to preferred adult habitat as well as in the timing and extent of spawning events. Tiger prawns spawn in water 20-50 m deep. The grooved tiger prawn has two main seasonal spawning events, leading to two pulses of recruitment to the fishery during the following spring and late summer. The brown tiger prawn has a single spawning event which is less consistently seasonal, and during the course of which less eggs per female are released (Crocos 1987). This spawning behaviour leads to a prolonged recruitment to the fishery which usually peaks in late summer (Gribble and Dredge 1991; cited by Ludescher 1997). Adult grooved tiger prawns have been found to prefer habitats having >70% fine mud sediment, whereas brown tiger prawns are more common on slightly less muddy substrates. Adults of both species of tiger prawn are most commonly found at depths between 13-22 m. Endeavour prawns (*Metapenaeus endeavouri* and *M. ensis*) are also found in these habitats. However greatest abundances of these species occur over coarser sediments closer to the reef (Somers 1987).

The juvenile phase of the life cycle of tiger prawns is spent in shallow water seagrass beds along much of the coast between Bowen and Tully, and especially in Cleveland Bay and Upstart Bay (Coles *et al*. 1989). Juvenile endeavour prawns also use seagrass nurseries although their timing is different from that of the tiger prawn species. In the Gulf of Carpentaria, catches of endeavour and tiger prawns species peak together as they are all washed out to the fishing grounds after the monsoon (Somers 1987). However, in the Bowen area where the wet season is less prominent, movement onto the fishing grounds is through slow dispersal rather than mass migration (Gribble and Dredge 1991; cited by Ludescher 1997).

6.3.2.3 King prawns

King prawns, comprising approximately 80% red spot (*Penaeus longistylus*) and 10% blue leg (*P. latisulcatus*) are most abundant in inter-reefal waters between Lucinda and Mackay. Spawning takes place in waters as deep as 60 m and adults are most commonly found on substrates comprising not more than 20% fine mud sediment, at depths greater than 25 m (Somers 1987). Unlike most other prawn species, king prawns use coral reef flats as nursery habitats and are thus not often influenced by wet season flooding events. However, juvenile populations in all shallow water environments cannot escape the impacts of natural disasters such as cyclones.

6.3.2.4 Bugs

Two species of bug belonging to the genus *Thenus* are distributed throughout coastal waters of northern Australia, from northern NSW to Shark Bay in Western Australia. *T. orientalis* is commonly known as the mud bug or inshore bug. It can be distinguished from the offshore or reef bug (*Thenus* sp). Their preferred habitat is the flat seabed of open waters, with mud bugs being found in tiger and endeavour prawn habitat and reef bugs being found in the same habitat as king prawns. While their preferred food source is bivalve molluscs, they are also known to prey on bottom dwelling fish and prawns. Bugs have a planktonic larval life of about three months, after which they moult into a tiny adult-like bug called a nisto that drops down onto the sea floor and moults to become an adult bug. Tagging

studies in the Townsville region have found that bugs swim as far as 50 nautical miles, although not migrating in any one direction. It is thought that these movements are undertaken in search of food (Jones 1991; cited by Ludescher 1997).

6.3.2.5 Mud crab

The mud crab (*Scylla serrata*) is distributed throughout the estuarine and inshore waters of the tropical and sub-tropical Indo-West Pacific region. They live in protective burrows, from which they venture out into the mangrove channels or into the tidal flats to feed on slow moving or sedentary prey such as small crabs, oysters and clams. Juveniles remain in the intertidal mangrove habitat at all times; sub-adults migrate to intertidal flats to feed and retreat to subtidal waters at low tide; and adults generally remain in subtidal waters, with a few venturing into the intertidal zone during high tide (Hill 1982; cited by Ludescher 1997).

Mud crab zoea larvae spend three weeks in the plankton before moving back inshore as megalopa larvae. These larvae moult again to become juvenile crabs which then live and grow in a shallow estuarine habitat.

Apart from the female spawning migration, mud crabs remain mostly within a three km range of their juvenile habitat (Hill 1982; cited by Ludescher 1997).

6.3.3 Crustacean-related industries

The Townsville area supports trawl fisheries for prawns and bugs, an inshore crab fishery, as well as farmed prawn production.

6.3.3.1 Capture Fisheries

In terms of tonnage landed, prawns are the most important fishery between Bowen and Tully, with an average yearly catch close to 1000 tonnes. This is a multispecies fishery with variable catches. Since 1988, average prawn landings have comprised approximately 45% king prawns (*Penaeus longistylus* and *P. latisulcatus*), 35% tiger prawns (*P. esculentus* and *P. semisulcatus*), 20% banana prawns (*P. merguiensis* and *P. indicus*) and 10% endeavour prawns (*Metapenaeus endeavouri* and *M. ensis*). Coral prawns (various species) contribute around 10 tonnes to the local prawn catch, and bay and school prawns are caught in amongst the inshore reefs between Lucinda and Cape Bowling Green. Other species taken include bugs, mostly *Thenus* sp. taken from king prawn grounds, and sand crabs (*Portunus pelagicus*).

In terms of tonnage of both finfish and crustaceans, the black mud crab (*Scylla serrata*) is the most important commercial species caught in the estuaries between Bowen and Tully in most years. The main fishing grounds are located within and adjacent to major mangrove systems in Missionary Bay and Hinchinbrook Channel, Bowling Green Bay and Upstart Bay.

6.3.3.2 Prawn farming

The Townsville region is ideally suited to prawn farming due to an abundance of coastal plains, year round water temperatures which are high and excellent transport and communications infrastructure. Currently there are five prawn grow-out farms and one

hatchery operating in the region with a sixth grow-out farm being constructed. These farms, including two farms producing *Penaeus monodon* and *P. merguiensis* in the southern part of Hinchinbrook Channel, are geographically dispersed from Innisfail to Bowen and range in size from three to 90 ha. The total area presently under production is about 220 ha. Annual production is estimated at 400 tonnes.

6.3.4 Human alterations

Ludescher (1997) cited draft results of a survey conducted within the Townsville Zonal Advisory Committee Fisheries Habitat Working Group which nominated major threats to fisheries habitats, including:

1. filling of swamps, acid sulfate soil drainage and obstruction to fish movement by flood gates;
2. land clearing, deep drainage canals, mangrove and river bank vegetation destruction, farm run-off, dumping and chemical pollution;
3. herbicides, pesticides, bund wall construction, siltation, heavy metal build-up;
4. reduced freshwater flow and lack of flushing, prawn farm effluent, algal blooms, silting of reefs.

6.3.5 Water quality

Ludescher (1997) listed a number of water quality-related threats to fisheries habitats with special reference to the Townsville region. These are discussed below.

6.3.5.1 Siltation

High sediment loading of fish habitats can have such negative impacts as smothering incubating eggs, corals and marine and freshwater plants; compromising the respiratory functions of crustacean and fish gills, reducing food visibility and availability; and burying microhabitats. High sediment loading can also affect temperature, light and oxygen concentrations in the water column which, in turn, can cause fish kills or sub-lethal stress.

6.3.5.2 Nutrient loading

Excessive nutrient levels in the water column (eutrophication) can have several impacts on marine and freshwater ecosystems. Elevated nutrient levels can promote the growth of algal blooms, which may subsequently collapse and decompose, thereby consuming dissolved oxygen and stressing aquatic organisms, including crustaceans. Elevated nutrient concentrations are reported in the Tully/Murray catchment (Mitchell *et al*. 1990) and adjacent coastal waters. However, there is evidence that the overall nutrient levels remain within ecologically acceptable limits set by ANZECC, except during major rainfall events (Ludescher 1997).

6.3.5.3 Acidity

High levels of acidity affect egg and larval survival as well as affecting aquatic animal respiration and increasing susceptibility to disease. Runoff from acid sulfate soil areas is proposed as a major source of acidity for water bodies in the Townsville area (Ludescher 1997), notably in the waters adjacent to the Oyster Point development at the northern end of Hinchinbrook Channel (Cappo, unpublished).

6.3.5.3 Toxic pollutants

High levels of toxic chemicals entering the aquatic environment via stormwater runoff or accidental discharge can cause fish kills, as reported at a nickel refinery north of Townsville (Brodie 1990; cited by Ludescher 1997).

Taken together, the following conclusions therefore seem reasonable:

1. Under low flow conditions, it is likely that prawns and other aquatic animals in the Hinchinbrook Channel will generally be exposed only to low levels of environmental stress;

2. Under high flow conditions, it is likely that prawns in Hinchinbrook Channel will generally be exposed to moderate levels of environmental stress (associated with high concentrations of nutrients and suspended solids), and will tend to migrate to more favourable environments. However, crabs and less mobile crustacean species may be unable to migrate elsewhere.

6.3.6 Sources and spread of WSSV infection in the Hinchinbrook Channel area

Possible sources of WSSV infection for wild stocks in the Hinchinbrook Channel area are :

1. improperly handled waste from infected, imported prawns following processing at a local plant;
2. effluent from a prawn farm infected via postlarvae or feed used during growout;
3. infected bait prawns released into the environment.

Assessments relating to probability of infection and disease occurring in farmed and wild prawn and wild (non-prawn) crustacean populations are similar to those for the Clarence River area.

6.3.7 Sources of IHHNV infection in the Hinchinbrook Channel area

Possible sources of IHHNV infection for wild stocks in the Hinchinbrook Channel area are :

1. improperly handled waste from infected, imported prawns following processing at a local plant;
2. effluent from a prawn farm infected via postlarvae or feed used during growout;
3. infected bait prawns released into the environment.

Assessments relating to probability of infection and disease occurring in farmed and wild prawn and wild (non-prawn) crustacean populations are similar to those for the Clarence River area.

6.4 Conclusions from case studies

Risks of WSS and IHHN outbreak occurrence for various crustacean categories in both the Clarence and Townsville areas are summarised in Tables 6.7 and 6.8 below.

Table 6.7 Summary of risks of WSS outbreak

	Wild prawns	Non-prawn crustaceans
Plant waste	Low	Low
Farm effluent	Low	Low
Bait prawns	Low	Low

Table 6.8 Summary of risks of IHHN outbreak

	Wild prawns (low susceptibility)	Wild prawns (high susceptibility)	Non-prawn crustaceans
Plant waste	Low	Moderate	Low
Farm effluent	Low	Moderate	Low
Bait prawns	Low	Moderate	Low

Based on the available information, assessing the consequences of the risks is more problematic. Provided environmental stress levels remain low to moderate, available evidence described in Section 5 combined with an understanding of the systems in the Clarence River and Townsville regions suggests that WSSV and IHHNV outbreaks in wild prawn or other aquatic animal populations may not progress to any extent. However, it is recognised that there is evidence that IHHNV had a substantial impact on *P. stylirostris* stocks in the Gulf of California and recovery of populations took several years. If some Australian prawn species were just as susceptible to IHHNV, a similar scenario could ensue in either the Clarence of Townsville regions.

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Appendix 1
Review of WSSV and IHHNV updated from previous scientific review

This appendix is a review of each of the two viruses chosen for inclusion in the present study. For completeness, the text updates that from Section 4 of the original *Scientific Review of Prawn Diseases* undertaken by AusVet Animal Health Services and incorporates the additional information provided by the Risk Analysis Panel in the paper, *Review of Pathogens of Prawns*.

1 White Spot Syndrome Virus Complex (WSSV)

Five baculoviruses have been reported to cause white spot syndrome in cultured *Penaeus monodon, P. japonicus, P. chinensis, P. indicus, P. merguiensis* and *P. setiferus* stocks world-wide. These are: hypodermal and haematopoietic necrosis baculovirus (HHNBV; Huang *et al.*, 1994) in China; rod-shaped nuclear virus of *P. japonicus* (RV-PJ; Inouye *et al.*, 1994) in Japan, China and Korea; systemic ectodermal and mesodermal baculovirus (SEMBV; Wongteerasupaya *et al.*, 1995) in Thailand; white spot baculovirus (WSBV; Wang *et al.*, 1995) in Indonesia, Vietnam, Malaysia, India, South Carolina and Texas; and *Penaeus monodon* non-occluded baculovirus (PMNOB; Lo *et al.*, 1995) in Taiwan. SEMBV has recently been identified in cultured *P. monodon* in Bangladesh (Ahmed, 1996).

All viruses in this group are reported to be very similar in morphology and replicate in the nuclei of infected cells. Lightner *et al.* (1997) consider them to be similar, if not the same virus. White Spot Syndrome Virus is not a baculovirus (Volkmann *et al.*, 1995) so it is preferable to refer to it as "White Spot Syndrome Virus or WSSV (Dr Don Lightner, personal communication).

White spot syndrome was first recognised in 1992-1993 in North East Asia (Takahashi *et al.*, 1994; Chou *et al.*, 1995), and has spread throughout most prawn culture areas of the Indo-Pacific. SEMBV first appeared in Thailand in 1994 where it surpassed yellow-head virus (YHV) as the primary cause of stock losses. In 1995 WSSV was observed in pond-reared *P. setiferus* in Texas. The virus was apparently introduced with raw and frozen prawns from Thailand which had been processed at nearby plants (Lightner, *et al.*, 1997). Most mortalities occur in young juvenile prawns weighing 3-5 gm (Takahashi *et al.*, 1994).

WSSV causes mortalities in *P. vannamei, P. stylirostris, P. aztecus, P. duorarum* and *P. setiferus* when experimentally infected (Lightner, 1996). The wild penaeids *Parapenaeopsis* spp, *P. semisulcatus, Metapenaeus* spp and *Macrobrachium* spp (*a caridean not a penaeid)* from Taiwan developed disease following experimental infection with WSSV (Chang *et al.*, 1996). Larvae of the freshwater shrimp, *Macrobrachium rosenbergii* may be infected experimentally and suffer some mortality, however, survivors can carry an infection without mortality as adults. Resistance to WSSV has not been

reported for any penaeid species. (Lightner, 1996). WSSV has not been reported in Australia.

1.1 Clinical signs

Infected juvenile and adult prawns become lethargic, cease feeding and have a loose cuticle with white calcium deposits embedded in the cuticle (Takahashi *et al.*, 1994). Infected prawns may display pink to red colouration of the body surface and appendages (Takahashi *et al.*, 1994; Wang *et al.*, 1995). Cumulative mortalities in infected populations may reach 100% within 2 to 10 days of the onset of clinical signs (Chou *et al.*, 1995; Lightner, 1996).

1.2 Gross Pathology

There is very little gross pathology associated with WSSV. Abnormal deposits of calcium, the accumulation of vacuoles and lysed debris and the necrosis of cuticular pore canals produce white spots, 0.5 to 2.0 mm in diameter, on the cuticular epidermis (Lightner, 1996; Wang *et al.*, 1995). Not all prawns infected with WSSV display white spots on the carapace (Lightner, 1996). Red body discolouration is also common (Inouye *et al.*, 1996). The lymphoid organ of diseased prawns may be swollen or shrunken (Takahashi *et al.*, 1994). Infiltration of haemolymph in the enlarged hemal sinuses and interstitial spaces may cause the hepatopancreas to become swollen, fragile and pale yellow in colour (Wang *et al.*, 1995).

1.3 Histopathology

WSSV infects cells of mesodermal and ectodermal origin, such as the subcuticular epithelium, lymphoid organ, haemocytes, haematopoietic tissue, stomach cuticular epidermis and connective tissue (Momoyama *et al.*, 1995; Lightner, 1996). Infected tissues display widespread focal necrosis (Wongteerasupaya *et al.*, 1995). Degenerate cells are characterised by hypertrophied nuclei with marginated chromatin and eosinophilic to basophilic intranuclear inclusions (Chou *et al.*, 1995; Wongteerasupaya *et al.*, 1995). Haemocytic encapsulation of necrotic cells as small brown masses in the stomach may be associated with infection (Momoyama *et al.*, 1995).

The average virion size for baculoviruses from the WSSV complex is 70-150 nm x 250-380 nm (Wongteerasupaya *et al.*, 1995). Replication appears to occur in the nucleus and protective occlusion bodies are not formed.

1.4 Diagnosis

Diagnosis of white spot syndrome depends mainly on the demonstration of eosinophilic to basophilic Inclusion bodies in stained fresh squashes or impression smears of ectodermal and mesodermal tissues. Feulgen-positive intranuclear Inclusion bodies may be identified in cuticular epithelial cells and connective tissue cells. A rapid field test for WSSV has been developed. The gills and epithelium under the carapace are excised, stained with haemotoxylin and eosin, mounted and then viewed as squash preparations (Flegel and Sriuriairatana, 1993; K. Supamattaya, personal communication). WSSV infection may be confirmed by the demonstration of rod-shaped, non-occluded virions in the intranuclear Inclusion bodies of affected cells using electron microscopy. The history of disease within

the cultured facility, region and species, and the presence of clinical signs are also considered (Lightner, 1996).

Diagnostic DNA probes have been developed and published primers are available for PCR assays from Japan (Kimura *et al.*, 1996) and Taiwan (Lo *et al.*, 1996a and b). Probes have also been developed in Thailand (Wongteerasypaya *et al.*, 1996) and through cooperation between France and the USA (Durand *et al.*, 1996) from prawn tissues infected with SEMBV from Thailand. The Thai probe is being marketed by DiagXotics Co. Ltd and has positively identified WSSV in six penaeids from China, India, Indonesia, Malaysia and Thailand (Wongteerasupata *et al.*, 1996). Diagnostic PCR is used routinely in Thailand to screen postlarvae, broodstock and potential carrier animals (Flegel *et al.*, 1997). PCR primers from Thailand, Taiwan and Japan are being used successfully for the diagnosis of WSSV in penaeids and other crustacea throughout Asia.

Asymptomatic infection of wild-caught *Metapenaeus ensis* with WSSV has been detected using in situ hybridisation and PCR (Wang *et al.*, 1997).

1.5 Transmission and potential carriers

Recent experiments and surveys using diagnostic PCR have shown that approximately forty arthropods, including penaeids, crabs, lobsters, *Macrobrachium* spp, and possibly copepods and insects can act as carriers (Chou *et al.*, 1996; Lo *et al.*, 1996b; Flegel, 1997; Maeda *et al.*, 1997). Many of these arthropods, such as the wild crab, *Portunus pelagicus*, and wild krill, *Acetes* sp., are common in prawn culture areas and may transmit the virus to penaeid culture systems with the in-take water (Supamattaya *et al.*, 1996).

Within the culture system WSSV is transmitted by cannibalisation of moribund prawns and carcasses or via contaminated water (Chang *et al.*, 1996). Crustacean carriers which enter prawn ponds may transmit WSSV when they die and are eaten by prawns. Birds may mechanically transmit the virus between ponds by releasing captured prawns over neighbouring ponds.

Unpublished work done by Charoen Pokphand (CP) and Aquastar Co. Ltd. in Thailand (Tim Flegel, personal communication) showed that there was a clear correlation between some batches of postlarvae used to stock ponds and subsequent WSSV outbreaks. This has led to the general practice of testing batches of postlarvae for WSSV by PCR assay before stocking. It suggests that unrestricted transportation of live postlarvae from areas known to be infected by WSSV to uninfected areas is likely to be very hazardous.

Kou *et al.*, (1997) detected WSSV by in situ hybridisation in oogonia and follicle cells in *P. monodon* ovarian tissues. Mohan *et al.* (1997) observed intranuclear viral inclusions in the gonads of *P. monodon* and concluded that WSSV could be transmitted vertically. However Lo *et al.* (1997) in their studies of WSSV tissue tropisms were unable to find any infected mature eggs and suggested that infected eggs cells were killed by the virus before maturation.

1.6 Viability

Experiments indicate that WSSV can remain viable in seawater for four to 7 days, although data is not yet available (Flegel, 1997). No published data is available on the effect of heat and desiccation on the viability of WSSV. However, unpublished work by CP and Aquastar Co Ltd in Thailand showed that there was no correlation between feed batches and pond outbreaks of WSSV. The viability of WSSV in frozen prawns is uncertain, although Chou *et al.* (1995) observed 100% mortality in prawns fed frozen tissues infected with WSSV.

WSSV (PRDV in the paper) is inactivated after 50 min at 50 ºC, but after only 1 min at 60 ºC. The virus is sensitive to low levels of exposure to UV and is inactivated by desiccation (to 3.7% water remaining) after only three hours (Nakano *et al.*, 1998).

1.7 Prevention

As for Yellow Head Virus, SEMBV is being controlled in Thailand by the use of closed and semi-closed systems (Limsuwan, 1996) involving the pre-treatment of water with formalin or chlorine and storage of any water to be exchanged. Unpublished aquarium trials from CP (Boonsirm Withayachumnarnkul, personal communication) indicated that 70 ppm formalin treatment at 6 hour intervals could prevent the transmission of WSSV from infected to non-infected shrimp. By contrast, similar unpublished work from Aquastar Co. Ltd. (Vithaya Thammavit, personal communication) and field experience (Chalore Limsuwan, personal communication) indicate that formalin administered at 20 to 40 ppm at five to 7 day intervals is sufficient to prevent the spread of infection. It is likely that the effectiveness of this treatment would depend upon the quantity of virus present (ie, concentration in the water, number of infected shrimp and severity of infection).

The elimination of fresh feed from the diet, the exclusion of potential carriers from prawn culture ponds and PCR screening of postlarvae prior to stocking are also recommended as control measures (Flegel *et al.*, 1996; Boonsirm Withayachumnarnkul, personal communication).

1.8 Present status of white spot syndrome

SEMBV infection in *P. monodon* from Thailand alone, resulted in a US$ 600 million loss in 1996 (Dr. Lin, CP, personal communication). The epidemic of WSSV in Thailand, China and India appears to be abating due to the widespread use of the recommended preventative measures and better farming practices. WSSV continues to cause massive stock losses in other affected prawn culture countries.

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2 Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) is thought to be indigenous to the Indo-Pacific region and has been reported from numerous geographic regions many countries including the southeastern USA, Mexico, Ecuador, Peru, Brazil, various Carribean countries, Central America, Hawaii, Guam, Tahiti, New Caledonia, Singapore, Malaysia, Thailand, Indonesia and the Philippines (Lightner, 1996) and China (Zhang & Sun 1997).

Natural infections have been reported from *Penaeus stylirostris, P. vannamei, P. occidentalis, P. californiensis, P. monodon, P. semisulcatus* (Lightner 1990) and *P. chinensis* (Zhang & Sun 1997). IHHNV has been infectious for all species of penaeid shrimp tested thus far in experimental challenges, including *P. japonicus, P. setiferus, P. dourarum* and *P. aztecus* (Lightner 1990). *P. indicus* and *P. merguiensis* may be infected with the virus but appear to be refractory to disease (Brock and Lightner, 1990; Lightner, 1996). IHHNV is believed to be enzootic in wild reservoir hosts such as *P. monodon* (Brock and Lightner, 1990).

An IHHNV-like virus has been reported from a hybrid penaeid, *P. monodon* x *P. esculentus,* bred in Australia (Owens *et al.*, 1992).

Bonami *et al.* (1990) described the IHHNV genome and the basis for classification of the virus. It is not known if distinct geographic strains of IHHNV exist.

2.1 Clinical signs

IHHNV disease has been studied closely in *P. vannamei* and *P. stylirostris* in the Americas (Lightner *et al.*, 1983b; Bell and Lightner, 1984). The clinical signs of IHHNV disease in *P. stylirostris* are nonspecific and include anorexia, lethargy, weakness and erratic swimming. Early larvae and postlarvae, which have been vertically infected do not become diseased until they are older and within the size range 0.05 to 1 gm (Lightner *et al.*, 1983). Infected juvenile prawns have been observed to rise to the water surface, remain motionless for a few moments then roll over and sink to the bottom with a slowed righting response (Brock and Lightner, 1990). This behaviour may be repeated until mortality occurs. Mortality may exceed 90% within several weeks of onset of infection in juvenile *P. stylirostris* (Bell and Lightner, 1987).

In *P. vannamei*, IHHNV is typically a chronic disease linked to runt deformity syndrome and infected populations of juvenile shrimp typically display a wide distribution of sizes (Kalagayan *et al.*, 1991).

Australian hybrid prawns infected with an IHHN-like virus became weak and lethargic with mortality occurring when they reached three to four gm (Owens *et al.*, 1992).

P. monodon can appear clinically normal even when heavily infected with IHHNV (Flegel

1997).

2.2 Gross Pathology

Gross signs of infection include white to buff mottling of the cuticle, opacity of striated muscle and melanised foci within the hypodermis (Bell and Lightner, 1987). In the later stages of infection *P. stylirostris* and *P. monodon* may appear bluish in colour. Infected *P. vannamei* display deformed rostrums, cuticle and antennal flagella (Lightner *et al.*, 1983b; Lightner, 1996). Australian hybrids infected with an IHHN-like virus had no noticeable changes in colouration (Owens, *et al.*, 1992).

2.3 Histopathology

IHHNV forms Cowdry Type A intranuclear inclusion bodies associated with widespread cytopathological changes including hypertrophy of the nucleus and margination of the chromatin (Lightner *et al.*, 1983b).

Cowdry Type A inclusion bodies were observed in cells of ectodermal and mesodermal origin in Australian hybrid prawns infected with an IHHN-like virus. The hearts of some prawns investigated had focal haemocyte infiltrations and in some prawns melanised nodules were observed in the connective tissues (Owens *et al.*, 1992).

2.4 Diagnosis

IHHNV may be diagnosed by the demonstration of Cowdry Type A inclusion bodies using direct histochemical techniques for light microscopy and electron microscopy. Bioassays may be used to detect asymptomatic carriers of the virus, using *P. stylirostris* as the indicator host. IHHNV-specific gene probes have been developed from naturally infected *P. stylirostris* juveniles to use for *in situ* and dot blot hybridisation (Lightner *et al.*, 1992; Mari *et al.*, 1993). These probes are commercially available and severe to low grade infections may be detected. Non-lethal screening of broodstock may be carried out by removing an appendage such as a pleopod or gill process, or by taking a sample of haemolymph and processing for routine histology to test with the probe by *in situ* hybridisation (Bell *et al.*, 1990). Polymerase chain reaction (PCR) primers have also been developed which allow IHHNV to be detected in fresh, frozen or fixed (90% ethanol but not Davidson's AFA, formalin or gluteraldehyde) samples of tissue or haemolymph (Lightner, 1996). Murine monoclonal antibodies to IHHNV have been developed for an ELISA detection system (Poulos *et al.*, 1994). However, further work is required before this system can be used for reliable routine diagnosis.

Tissue samples from prawns infected with the Australian IHHN-like virus which were sent to the USA and tested with a monoclonal antibody to IHHNV in a developmental ELISA system (Poulos *et al.*, 1994) gave values of 38% to 78% intensity when compared to the known positive control (Dr Don Lightner, personal communication).
The Australian virus initially gave negative results when tested with a probe for IHHNV developed in the USA (Leigh Owens, personal communication) and it is diagnosed using routine histochemical techniques. It has subsequently been shown with a commercial IHHNV probe that there is limited genetic similarity between the Australian isolate and IHHNV (Owens, 1997).

2.5 Transmission

It is believed that IHHNV may be transmitted vertically from broodstock to their progeny (Lightner *et al.*, 1983a). However, this has not been proven. IHHNV-resistant penaeid species and early life stages carry the virus latently and transfer it to more susceptible species and life stages. It appears that the virus is transmitted either directly from prawn-to-prawn in water or is ingested by such mechanisms as cannabilism of infected prawns (Bell and Lightner, 1984).

2.6 Viability

IHHNV will survive storage at -5°C to -10°C (Bell and Lightner, 1984). The survival of IHHNV after exposure to high temperatures is not known.

2.7 Prevention

Effective control measures for IHHNV disease are not known. Avoidance of the virus through quarantine is strongly recommended (Brock and Lightner, 1990). The impact of IHHNV outbreaks may be reduced by improving farm management practices, such as lowering stocking densities, using nutritionally balanced feeds and stocking ponds with more resistant prawn species.

2.8 Present status of IHHNV

Disease caused by IHHNV infection continues to be a chronic problem of cultured prawns in a number of countries. However, reports of serious epidemics have been rare in recent years. IHHNV has occurred in multiple infections with other, more pathogenic viruses and is considered, in most cases, to be a chronic infection which suppresses the prawn's defence system, allowing infection by other disease-causing agents. No further outbreaks of IHHN-like virus infections have been recorded in Australia since the one described by Owens *et al.* (1992).

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