Biosecurity and disease status of prawn nurseries in South Australia

Report to PIRSA Biosecurity and PIRSA Fisheries

Roberts, S.D., Deveney, M. and Sierp, M.

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SARDI Aquatic Sciences
PO Box 120 Henley Beach SA 5022

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South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

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Author(s): Dr Shane Roberts, Dr Marty Deveney and Dr Michael Sierp

Reviewer(s): Dr Adrian Linnane (SARDI) and Dr Nathan Bott (SARDI)

Approved by: Tim Ward
Assoc Prof – Wild Fisheries

Signed:

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The report was reviewed by Dr Adrian Linnane and Dr Nathan Bott of SARDI Aquatic Sciences. Cameron Dixon (SARDI) also provided comment on the report. Associate Professor Tim Ward formally approved the report for release.

EXECUTIVE SUMMARY

1. This report provides information from surveys conducted in Spencer Gulf and Gulf St Vincent, within intertidal prawn nursery habitat, during 2009.

2. The aim of this study was to provide assessment of the current biosecurity and disease status of prawn nurseries by: a) comparing current and historical juvenile prawn densities; b) documenting any marine pest incursions, and c) assessing current (2009) disease status of juvenile prawns, with a focus on viral fauna.

3. Relative abundance (density) was comparable to historical data. In Spencer Gulf, mean annual relative abundance during 2009 (3.22 ± 0.36 prawns.m^2) was similar to that during 2001 (3.62 ± 1.0 prawns.m^2). Relative abundances were well distributed among the five key nursery sites surveyed, with the greatest densities again observed at False Bay.

4. In Gulf St Vincent, mean annual relative abundance during 2009 (1.74 ± 0.19 prawns.m^2) was greater than 1994 and 1996 levels. Of note, relative abundances were significantly highest during 1992 (fishery closure).

5. No marine pest species were observed during this study.

6. Juvenile prawn populations in South Australia (SA) are free (not greater than 4–5% prevalence) of the key pathogenic (and notifiable) viruses found both internationally and nationally. These include: IHHNV, WSSV, HPV and GAV. This highlights the risks associated with prawn and crustacean products sourced from outside of the State.

7. A number of pathologies and parasites were observed using histology, providing baseline data of current disease status. Of note was the observation of an MBV-like virus, occurring at relatively high prevalence in both Spencer Gulf (61%) and Gulf St Vincent (60%). MBV-like viruses are common in penaeid prawns, and are known to occur throughout Australia. They pose no health risk for human consumption. It is suggested that this endemic virus be studied further to determine age / environment related prevalence and mortality, and assess strain variability between States.

8. The occurrence of endemic MBV-like virus at high prevalence in juvenile *P.latisulcatus* may hold implications for the culture of this species and in understanding stock-recruitment relationships.

9. Continuing juvenile prawn surveys on a regular basis over the next few years would provide consistent and adequate data, in conjunction with current structured adult biomass surveys, to assess whether relative abundances can be used as a predictive index of future stock status.

10. Furthermore, surveys such as this provide the opportunity to assess the health of the population and their habitat (pest incursions), and subsequently may provide the forewarning to respond to disease and pest incursions.

1. INTRODUCTION

1.1 Background

South Australia has three prawn fisheries that target the western king prawn, *Penaeus (Melicertus) latisulcatus*, two of which are situated within South Australia's gulf systems: Spencer Gulf (39 licence holders: ~1,800 t harvested in 2008/09), Gulf St. Vincent (10 licence holders: ~290 t harvested in 2008/09) and the West Coast (3 licence holders: ~112 t harvested in 2009).

As with other penaeids (Gulland and Rothschild, 1984), *P. latisulcatus* has an offshore adult life phase and an inshore juvenile phase. *P. latisulcatus* becomes sexually mature at approximately 1.5 years of age, and lives up to 4 years (Carrick, 2003). Adult prawns aggregate and spawn in deep water (>10 metres) with the subsequent larvae living a planktonic phase. The length of the larval period depends on water temperature, with faster development in warmer water (Hudinaga, 1942). Unpublished data from a current FRDC project 2008/011 (SARDI) demonstrated that the larval period varies from 15 days (25° C) to 34 days (17° C) (Roberts *et al*, 2009). Subsequently, post-larvae settle in inshore nursery areas at approximately 1–3 mm carapace length (CL) (Carrick, 1996; Kangas 1999).

In South Australia, juvenile *P. latisulcatus* occur predominately on intertidal sand- and mud-flats, generally located between shallow subtidal / intertidal seagrass beds and mangroves higher on the shoreline (Kangas and Jackson 1998; Tanner and Deakin 2001). In Spencer Gulf, relative abundance was significantly greater in the mid intertidal zone compared to lower and upper zones (Roberts *et al.,* 2005) while in GSV relative abundance was similar within intertidal zones (Kangas and Jackson 1998). Nursery habitat primarily occurs in the northern regions of both gulfs (Figure 1.1), with the extent of available mangrove habitat associated with production from each fishery (Roberts *et al*., 2009).

Kangas (1999) showed that juveniles of 2–7 mm CL comprised 90% of the surveyed population in GSV. Mean growth rates in nurseries varied from 0.53–0.65 mm CL per week in winter to 0.71–1.28 mm CL per week in summer (Kangas, 1999). Furthermore, Kangas (1999) found mean annual relative abundances positively correlated to mean annual temperature. In both gulfs, the post-larvae produced from early spawning events settled in nursery areas during December or January, when growth was high, and then emigrated to deeper water in May or June (at ~20 mm CL). Alternatively, post-larvae produced from late spawning settled in nurseries from March and due to slow growth they "over-wintered" in the nursery areas, recruiting to the trawl grounds in the following summer (Kangas, 1999; Carrick, 2003).

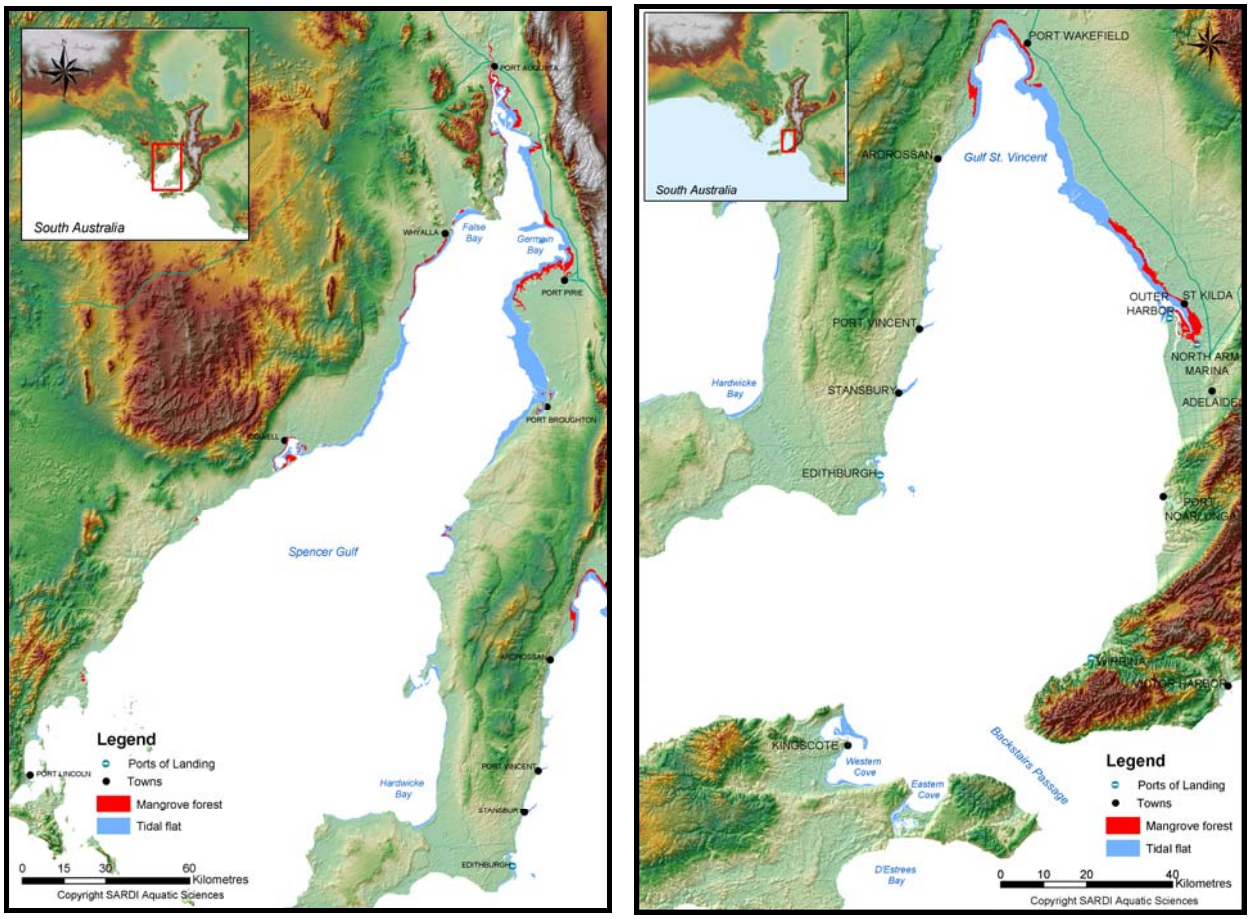

Figure 1.1 Important juvenile nursery habitat, mangrove forest and tidal flats, around coastal Spencer Gulf and Gulf St. Vincent. Reproduced from Bryars (2003).

1.2 Historical juvenile prawn surveys

Juvenile prawn surveys were historically conducted in both Spencer Gulf (1992–2003; Carrick, 1996 and Roberts *et al*, 2005) and Gulf St Vincent (1990–1996: Kangas, 1999). Methodologies were similar in that they utilised a modified "jet net", or epibenthic sledge fitted with a water pump, which was towed behind a small vessel within the intertidal zone during high tide. Historical surveys identified key nursery areas in both gulfs and collected information on juvenile prawn size and relative abundances at various spatial and temporal scales. In GSV, key nursery sites were Port Wakefield, Port Arthur and Webb Beach, while highest relative abundances were observed between late February and May. A correlation was found between juvenile densities and recruits as well as commercial catch rates, although it was suggested that further data was required to provide confidence in the relationships. Such relationships may identify juvenile density estimates as predictive indices, thus increasing confidence in the current assessment (based on adult prawn surveys and catches) and management of the fisheries.

In Spencer Gulf, key nursery sites were False Bay, Shoalwater Pt, Plank Pt, Mt Young, South Cowleds, the Spit and 5th Creek, with peak relative abundances occurring in April (1.81 prawns.m^2) and significant variation observed among years (2000=2001>2003=1993>1994). Carrick (1996) identified Port Pirie as an important nursery area in 1993 and 1994, although this site was not sampled on an ongoing consistent basis in subsequent years. Inter-annual patterns were generally consistent across sites except for 5th Creek during 1993, which was attributed to a localised oil spill at this site during 1992.

1.3 Biosecurity

Invasive species are a major threat to coastal ecosystems and are second only to habitat destruction as a cause for environmental decline (Crookes & Soulé 1999). Marine pests can be translocated and introduced by numerous vectors including the aquarium trade, ship ballast, hull fouling, floating debris and man-made structures such as drilling platforms and canals (Bax *et al*., 2003). They consequently have a negative impact on endemic species by predation and competition. Invasive species can also decrease public amenity, foul commercial infrastructure and impact directly on marine industries.

The combined costs of invasive species outbreaks can undermine the viability of coastal communities and increased maintenance and pest control activities are particularly costly for governments (Pimentel *et al*. 2000). The South Australian Gulf and coastal environments have the potential to be severely impacted by marine pests. Information is emerging that some marine pests, particularly the invasive alga *Caulerpa taxifolia* can modify inshore environments in ways that may decrease prawn recruitment (Fernandes *et al.* submitted for publication).

Australia's National System for the Prevention and Management of Marine Pest Incursions forms a policy and management framework for minimising the risk of spread and the long-term impacts of marine pest incursions.

The National System has three major aspects:

1. Prevention - systems to reduce the risk of introduction and spread of marine pests, including management arrangements for ballast water and biofouling;
2. Emergency Management - national response mechanism to control or eradicate pests that do get in;
3. Ongoing Management and Control - management of marine pests already here, where eradication is not feasible.

There are also four supporting components:

1. Monitoring - ongoing national program to provide early detection of new pests
2. Communication - industry and community awareness and education
3. Research and Development - targeted research to assist with development of policy and management measures
4. Evaluation and Review - evaluating the effectiveness of the National System.

The current project concerns three of the four supporting components under the National System (see www.marinepests.gov.au).

1.4 Prawn health

Disease status and parasite loads are limiting factors in marine animal populations, although generally overlooked in fisheries management (Harvell *et al.,* 2004). Climate change can substantially increase the risks associated with spread of disease, and push species towards their physiological thresholds (Harvell *et al.,* 1999; Harvell, 2002; Ignacio Vilchis *et al.,* 2005; Portner and Knust, 2007). Furthermore, environmental pollution from coastal industries can increase the susceptibility of aquatic animals to disease and reduce reproductive output (Nash *et al.*, 1988). Stock collapse of the Australian Bass (*Macquaria novemaculeata*) in NSW, due to recruitment failure, has been partially attributed to acid sulphate discharge (Harris, 1989). Common coastal marine pollutants in South Australia include heavy metals, high nutrient loads from coastal industries and petroleum (hydrocarbon) discharges (Edyvane 1999). These issues are of particular relevance to the sustainability of fisheries located within South Australia's semi-enclosed and isolated gulf systems, although this area of research is limited.

Disease and parasite loads can affect growth, reproductive output and ultimately survival. Parasites, such as bopyrid isopods, have been shown to affect the reproductive output of penaeid prawns (*P. plebejus*; Courtney *et al.*, 1995). Bopyrid isopods have been observed to parasitise adult *P. latisulcatus* in South Australia (S. Roberts & M. Deveney, personal observation). In *Fenneropenaeus indicus* (a penaeid species), it was shown that viral infections affected moulting and reproduction (Vijayan *et al.,* 2003).

Penaeid prawns may be host to a number of endemic ("native") viruses which may or may not cause disease. However, exotic ("introduced") viral pathogens may be considered one of the highest health risks for a naïve prawn population due to their 1) potential virulence, 2) rapid proliferation and infection, 3) general host non-specific nature and 4) resistance and durability, which increases their chances of spread through national and international

movements of prawns, prawn products (ie. bait prawns) and other crustacean products. The ability for viral pathogens to survive the freezing process enabled one of the most virulent and economically damaging penaeid viruses (White Spot Syndrome Virus, WSSV) to spread from Asia into the USA (Lightner *et al.*, 1997). Viral disease has been associated with dramatic reduction of stock in prawn fisheries. In 1987, infectious hypodermal and haematopoietic necrosis virus (IHHNV) was linked to a decline in the Gulf of California shrimp fishery to levels that could not support commercial harvest until 1994 (Anon, 1997). In southern Australian wild fisheries, two examples of catastrophic disease outbreaks were caused by viral pathogens: the pilchard herpes virus (Ward *et al.*, 2001; Whittington *et al.*, 2008) and abalone viral ganglioneuritis (AVG) (Hooper *et al.,* 2007; http://www.dpi.vic.gov.au). Both outbreaks caused considerable economic loss, with high mortalities (60%–90%) which are characteristic of viral outbreaks. Knowledge of current disease status and endemic pathogens (particularly viruses) underpins risk assessment and greatly aids disease mitigation and control strategies (ie. national and international border security). All prawn species harbour their own viral fauna, which if documented, provides 1) information to assess risk to the population, and 2) baseline information that would be pivotal to understanding causes of future disease outbreaks. This is particularly relevant to the uniquely isolated prawn populations in SA's gulf systems.

Disease status of Western Australian (WA) commercial prawn species, including *P. latisulcatus*, was documented by Jones (2003). It was determined that WA prawn populations in general (six penaeid species) were free of Gill-Associated Virus (GAV) (a virus found in eastern states), although exposed to Monodon baculovirus-like virus (MBV-like virus) and Hepatopancreatic parvo-virus (HPV) (both known to occur in Australia). However, of 514 *P. latisulcatus* collected, from Exmouth Gulf and Shark Bay, none showed MBV-like virus, while 1 animal had HPV (in the hepatopancreas). Eosinophilic-like inclusions observed using histology are typically suggestive of viral infection, and while they were documented in most species in the WA study, they were not observed in *P. latisuclatus* (Jones 2003). The finding of GAV-free prawns in WA was of importance since this virus is known to occur in Queensland, NSW and introduced into the Northern Territory. This highlighted the risks associated with prawn translocation and prawn products (including bait prawns) into WA from the eastern States. To date, no assessment of disease status has been documented for South Australian prawn populations.

1.5 Aims

This report provides information collected from both Spencer Gulf and Gulf St. Vincent to assess the current (2009) biosecurity and disease status of intertidal prawn nurseries. To achieve this, the aims were to:

1. Compare current and historical relative abundances (density) of juvenile prawns in key nursery sites;
2. Document marine pest incursions, if any, within key nursery sites; and
3. Provide a preliminary assessment of the disease status of juvenile prawns, focussing on presence of viral fauna.

Prawn samples were tested for the presence of four key viruses that are notifiable under the import risk assessments documented by the World Organisation for Animal Health (OIE 2005) and Biosecurity Australia. These viruses were to represent economically and ecologically important viruses that may pose a threat to prawn populations in SA. The viruses specifically tested for in this study were: IHHNV, WSSV, GAV and HPV. At the start of this study IHHNV and WSSV were considered to not occur within Australia, while GAV and HPV were known to occur in other States and Territories of Australia. However, IHHNV was recently detected in Queensland, and while considered to be no longer an import threat for Australia (Biosecurity Australia, 2009), it now poses a threat to other States and Territories.

2. METHODOLOGY

2.1 Study sites

Five sites in Spencer Gulf and 3 sites in Gulf St Vincent (GSV) (Table 2.1, Figure 2.1) were chosen based on: 1) historical importance and 2) proximity to major ports. Key prawn nursery sites have previously been documented for each gulf (Spencer Gulf: Roberts *et al*., 2005; GSV: Kangas, 1999), while major northern gulf ports are Whyalla, Port Pirie and Port Adelaide. Historical survey sites were chosen both near and away from these major ports (Figure 2.1). Sites in Spencer Gulf were sampled 10–13 March 2009, and in GSV 7–8 April 2009. In Spencer Gulf, evidence of historical sampling was observed as marked buoys attached to star droppers. GPS coordinates are provided for future reference (Table 2.1).

Table 2.1 Juvenile prawn nursery sites and GPS coordinates as recorded during the 2009 survey

Gulf	Site	Latitude	Longitude
Spencer Gulf	Spit	33°22.280	137°23.440
Spencer Gulf	Mt. Young	33°07.188	137°30.151
Spencer Gulf	False Bay	32°58.023	137°38.430
Spencer Gulf	Port Pirie	33°08.069	137°59.557
Spencer Gulf	5th Creek	33°10.356	137°52.784
GSV	Pt. Arthur	34°09.073	138°03.993
GSV	Pt. Wakefield	34°11.780	138°08.569
GSV	Webb Beach	34°27.000	138°15.800

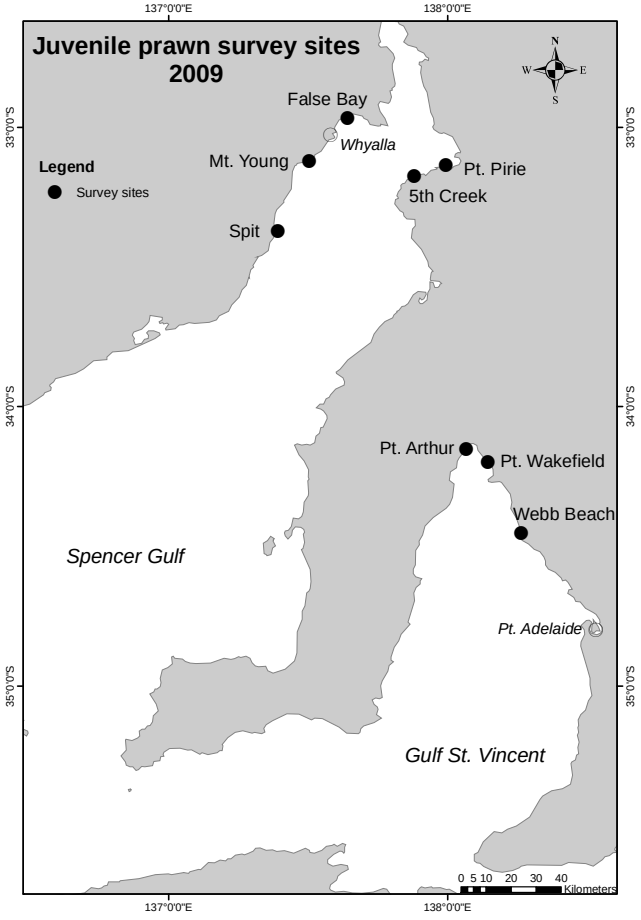

Figure 2.1 Juvenile prawn nursery sites sampled during 2009 in Spencer Gulf and GSV.

2.2 Sampling gear and methodology

A “jet net”, or epibenthic sledge fitted with a water pump, was modified from that described by Penn and Stalker (1975) as outlined in Roberts *et al*. (2005). The modified “jet net” used the same water-jet principle but the frame and net were significantly larger. The “jet net” has been described by Carrick (1996) and Kangas and Jackson (1998). In this study, the frame was 1.1 m wide (with a path width of 0.85–0.9 m, the internal distance between the frame) X 1.05 m long X 0.30 m high. It was constructed from galvanised steel tubing (25 mm in diameter), with galvanised iron skis (100 mm wide X 1200 mm long X 5 mm thick) lined with marine plywood or plastic, and a plastic roller (70 mm diameter) at the back of the frame for ease of towing. A central galvanised iron T-bar (35 mm diameter), 0.9 m wide, mounted on the base of the frame, 0.3 m from the front bar, had holes (3 mm diameter) every 50 mm, through which water was pumped. This was achieved with a Finsbury centrifugal pump (500 L/min capacity), set at an optimum pump rate of 300 L/min and powered by a 5.5 HP petrol engine, which was connected to the “jet net” via a rubber hose (30 mm internal diameter). The water jet penetrated the sand-mud substrate to a depth of approximately 5 cm. The net was 2 mm nylon (Nytal) square mesh fitted to cover the entire frame, with a tail length from the frame of 3.2 m long and with the cod-end tied off at approximately 2.85 m (Figure 2.2).

The towing vessels used were either SARDI R.V. Odyssey (twin 150 HP outboard engines) or R.V. Karrawa (twin 140 HP outboard engines). The net was towed from the stern. The optimum towing speed was approximately 1 knot (Nm/h), using 1 engine at approximately 1200 rpm, depending on tide, wind, and sediment load. The “jet net” was previously shown to have high catch efficiency (catchability estimated at 95-98%) with no difference between sampling season (summer, winter) or water depth (0.6 versus 2 metres) (see Carrick, 1996).

Figure 2.2 Profile view of the “jet net” used to sample juvenile prawns

At each site, four random 100 m transects were conducted midshore (halfway between the high water mark and seagrass line) on sandy bottom parallel to the coastline at approximately 1–1.2 m depth. Each transect was conducted greater than 3 metres apart in order to maintain independence. The trawl distance (100 m) was determined using marked buoys attached to weights as well as navigation equipment on board the vessel. Sampling was conducted during daylight hours and during the highest tides of the month (>2.0 m high tide).

2.3 Biological samples, processing and data analyses

2.3.1 Prawn samples

Because the “jet net” is essentially an epibenthic sledge, substantial effort in sorting prawns from sediment matter was required. The juvenile prawns in each sample were separated from the remainder of the sample (dead seagrass fronds, substrate, epibenthic organisms) and 50 animals were immediately preserved in ~70% ethanol (for later PCR analyses) while remaining prawns were fixed in sea-water buffered formalin (~20%) for later histological processing.

Laboratory identification and separation of *P. latisulcatus* from strawberry (also known as coral) prawns (*Metapenaeopsis* spp.) was based on *P. latisulcatus* having: 1) mandibles and rostrum of similar length, and 2) three moveable spines on the telson.

2.3.2 Marine pests

The National System for the Prevention and Management of Marine Pest Incursions (www.marinepests.gov.au) suggests an Australian priority list of marine pests covering 50 species in 10 groups. Based on the sampling method used in this study, habitat and substrate type sampled (soft sediment), 37 species identified in Table 2.2 would likely be detectable. Dr Mike Sierp of PIRSA Biosecurity was present during field sampling, and visually assessed samples during collection as well as in the laboratory using light microscopy.

Table 2.2. Potential detection for 50 marine pest species, outlined in the National priority list, based on the sampling method used in this study. Adapted from O'Loughlin *et al.* (2006).

Group	Species	Possible detection (Y= yes/N=no)
Microalgae	*Alexandrium catenella*	N
	Alexandrium minutum	N
	Alexandrium tamarense	N
	Gymnodinium catenatum	N
	Dinophysis norvegica	N
	Pfiesteria piscicida	N
	Pseudo-nitzschia seriata	N
Macroalgae	*Caluerpa taxifolia*	Y
	Codium fragile ssp. *tomentosoides*	Y
	Polysiphonia brodiaei	Y
	Undaria pinnatifida	Y
Echinoderms	*Asterias amurensis*	Y
Crustaceans	*Carcinus maenas*	Y
	Charybdis japonica	Y
	Eriocheir sinesis	Y
	Hemigrapsus sanguineus	Y
	Pseudodiaptomus marinus	Y
	Balanus eburneus	N
	Balanus reticulates	N
	Megabalanus rosa	N
	Megabalanus tintinnabulum	N
Molluscs	*Varicorbula gibba*	Y
	Crassostrea gigas	Y
	Petricolaria pholadiformis	Y
	Potamocorbula amurensis	Y
	Musculista senhousia	Y
	Perna perna	Y
	Limnoperna fortunei	Y
	Mytilopsis sallei	Y
	Perna viridis	Y
	Crepidula fornicate	Y
Polychaetes	*Hydroides ezoensis*	Y
	Hydroides elegans	Y
	Hydroides sanctaecrucis	Y
	Polydora cornuta	Y
	Polydora websteri	Y
	Pseudopolydora paucibranchiata	Y
	Sabella spallanzanii	Y
Tunicates	*Ciona intestinalis*	Y
	Styela clava	Y
Jellyfish	*Blackfordia virginica*	Y
	Mnemiopsis leidyi	Y
Bryozoans	*Bugula flabellate*	Y
	Bugula neritina	Y
	Schizoporella errata	Y
	Tricellaria occidentalis	Y
	Watersipora arcuata	Y
	Watersipora subtorquata	Y
Fish	*Neogobius melanostomus*	N
	Tridentiger bifasciatus	N

2.3.3 PCR

Ethanol preserved juvenile prawns were sent to the Fish Health Laboratory at the Department of Fisheries, Western Australia, to test for the presence of four key notifiable viruses: 1) infectious hypodermal and haematopoietic necrosis virus (IHHNV), 2) white spot syndrome virus (WSSV), 3) hepatopancreatic parvovirus (HPV) and 4) gill-associated virus (GAV) using specific Polymerase Chain Reaction (PCR) tests.

At least 12 prawns from each site (excluding 5th Creek due to low numbers caught) were tested. A total of 6 replicates, each containing ~2 prawns (to provide enough nucleic acid material) from each site were used for DNA and RNA extraction. DNA was extracted from all samples using DNAzol (Invitrogen), while RNA was extracted from all samples using Trizol (Invitrogen). Extracted DNA and RNA were quantified by spectrophotometry. Modified methods from Tang *et al*. (2007) and Cowley *et al.* (2000) were used to test all samples for the presence of IHHNV and GAV, respectively. The method for the detection of WSSV was based on the ANZSDP (http://www.scahls.org.au/NAAH-TWG/procedures/anzsdp.htm), while the method from La Fauce *et al*. (2007) was used to test all samples for the presence of HPV.

2.3.4 Histology

Thirty prawns per site were sent to Adelaide University for routine histological processing. In short, samples were processed, embedded in paraffin wax, and serial sectioned at 5 µm and stained with haematoxylin and eosin (H&E). Each slide contained approximately four sections from one prawn. Histopathology was conducted at SARDI Aquatic Sciences primarily by Dr Shane Roberts, while slides of interest and pathological descriptions were validated or suggested by Dr Marty Deveney (SARDI) and Dr Brian Jones (Department of Fisheries, WA). Ten prawns from each site were assessed using compound microscope and image analyses software (OPTIMAS 6.5). Two sections per slide were assessed targeting the hepatopancreas, gills, and heart (see Figure 2.3), with pictures taken of each organ at 20x magnification, and higher magnification for sections of interest. The primary interest was viral-like infected cells, while immune reactions, pathologies and parasites were also noted and photographed if observed. The remaining 20 prawns per site were assessed using compound microscope at 20x magnification to quantify the prevalence of viral-like cells in the hepatopancreas.

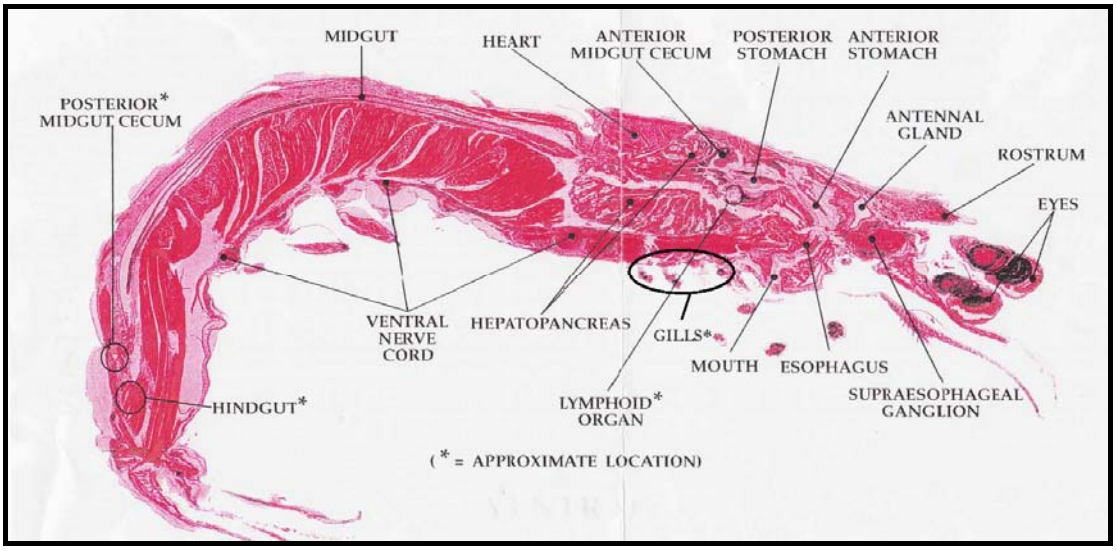

Figure 2.3 Internal anatomy of a cross sectioned post-larval penaeid prawn prepared using histology (stained with Haematoxylin and Eosin) (diagram modified and sourced from University of Arizona, pathology group, D. Lightner).

2.3.5 Electron Microscopy

Due to a large proportion of prawn samples being observed with viral-like eosinophilic inclusions within the hepatopancreas (using histology), a small number of these samples were sent to Adelaide University to confirm the presence of virus in these cells using high powered electron microscopy (EM). Routine EM was performed using formalin fixed samples.

2.3.6 Data acquisition and analyses

Relative abundance was estimated as number of prawns.m^{-2} as determined from the distance trawled and width of the net (0.9m). Historical data were used for comparative purposes and sourced from 1) Dr Mervi Kangas (Department of Fisheries, WA) for GSV and 2) SARDI Aquatic Sciences database for Spencer Gulf. Relative abundances during 2009 were compared to historical data for the same time of year (historical April data for both gulfs). Note for Spencer Gulf, while sampling was conducted during March 2009 for logistical reasons, comparisons were made with historical April data due to greater consistency in the database. While Roberts *et al*. (2005) found no statistically significant difference in mean relative abundance between March (1.51 prawns.m^2) and April (1.81 prawns.m^2), data for 2009 (March) was corrected (+20%) for comparison with historical data (April). Juvenile prawn relative abundance was assessed for significant differences among sites and years.

Freedom of disease was calculated by the number of samples tested, the sensitivity and specificity of the tests used and the minimum accepted prevalence using the freedom of

disease calculator on the Ausvet website (http://epitools.ausvet.com.au). Test sensitivity was assumed as 95%, while specificity as 100%.

Where statistical analyses were appropriate, analysis of variance (ANOVA) and Tukey's post-hoc tests were used to determine significant differences at spatial and temporal scales. Homoscedasticity and normality of the data were determined by Levene's test and visually assessing residual plots. Where necessary, $\log_{10}$ (n + 10) transformations of data were employed to increase homogeneity of variances. Spearman's rank correlations were used to test pair-wise differences between two datasets. In all cases, significance was accepted at $P<0.05$. Data analyses were performed using SPSS software (SPSS, version 18.0) and values are presented as mean ± standard error (SE).

3. RESULTS

3.1 Relative abundance

3.1.1 Spencer Gulf

Relative abundance (density) of juvenile prawns significantly differed among sites and years (statistical interaction: $F_{8,60}$=21.6, P<0.001). Relative abundances were greatest at False Bay during all years, while the remaining sites varied among years. Remaining sites on the western side of Spencer Gulf (Mt Young and the Spit) were observed with greater relative abundances during 1993 and 2009 compared to sites on the eastern coast (Port Pirie and/or 5th Creek), which had greater relative abundances during 1994 and 2001 (Figure 3.1a). Mean annual relative abundance during 2009 (3.22 ± 0.36 prawns.m^2) was similar to that during 2001 (3.62 ± 1.0 prawns.m^2) (Figure 3.1b).

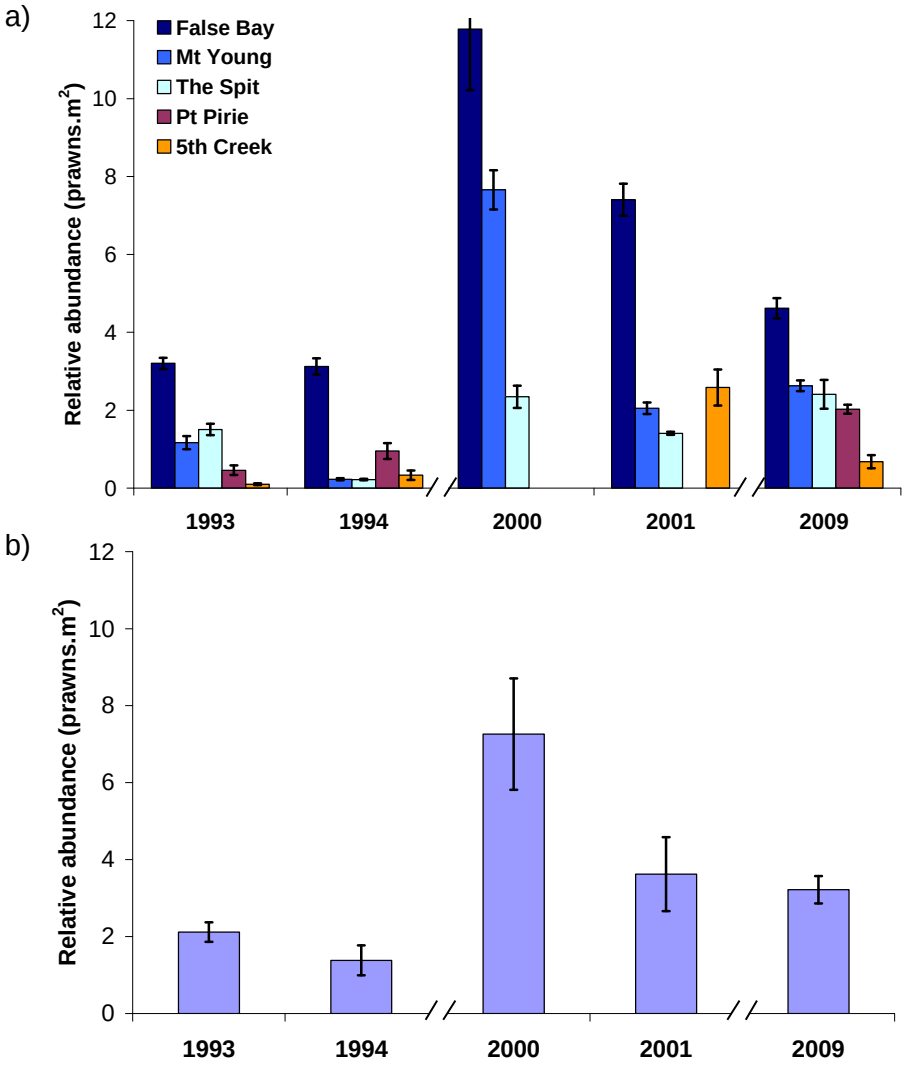

Figure 3.1 Historical mean (±se) relative abundance (prawns.m^2) of juvenile prawns in Spencer Gulf (April) for: a) five key nursery sites and b) annual measures utilising consistently sampled sites (False Bay, Mt Young and the Spit) across years.

3.1.2 Gulf St Vincent

Relative abundance of juvenile prawns significantly differed among sites and years (statistical interaction: $F_{11,94}$=3.76, P<0.001). Relative abundances were generally greatest at Pt Wakefield in most years except 1990 and 2009, while the remaining sites varied among years (Figure 3.2a). Mean annual relative abundance during 2009 (1.74 ± 0.19 prawns.m^2) was greater than 1994 and 1996 levels, although lower than 1992 levels. Mean annual relative abundance was significantly greater during the closure of the fishery in 1992 (3.2 ± 0.60 prawns.m^2) compared to all other years sampled (Figure 3.2b).

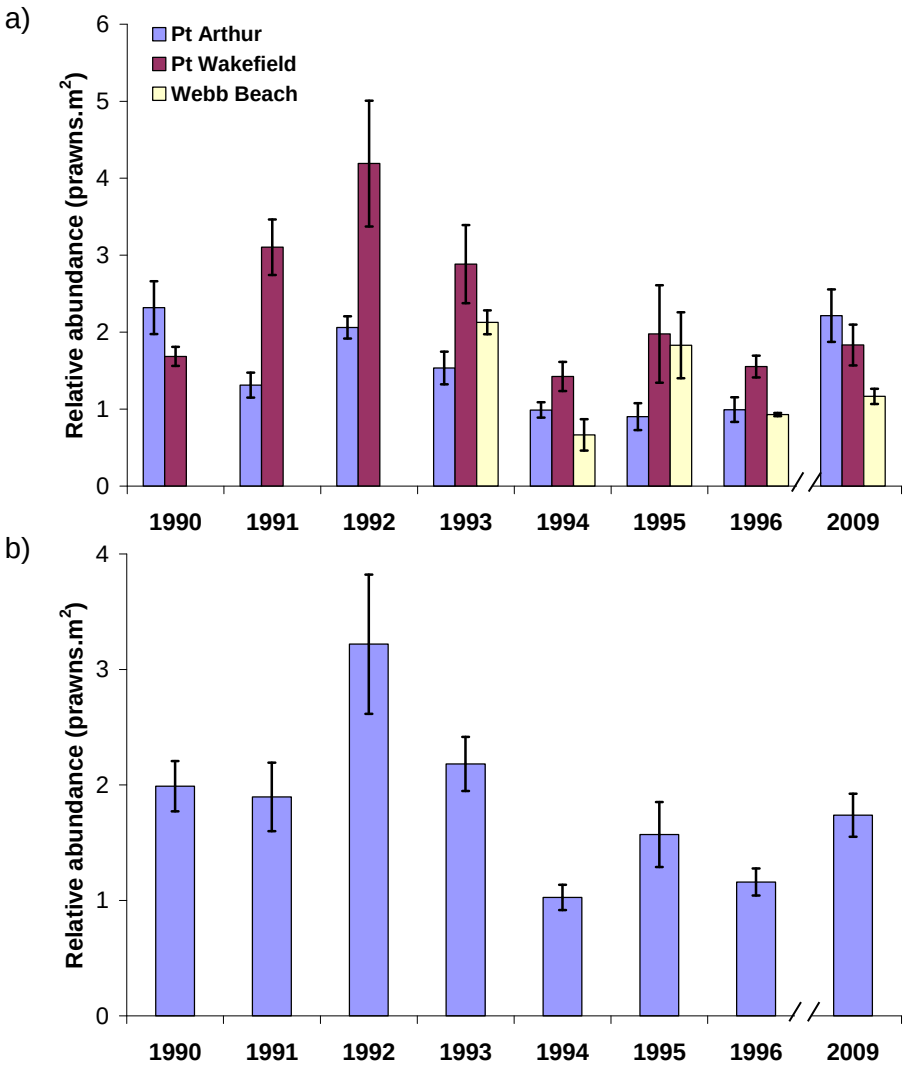

Figure 3.2 Historical mean (±se) relative abundance (prawns.m^2) of juvenile prawns in Gulf St. Vincent for: a) three key nursery sites and b) annual measures across years.

3.2 Marine pests

No marine pest species were observed during field sampling, or during visual examination of samples using light microscopy (organisms of approximately 5–20 mm). These examinations targeted 37 of the 50 marine pest species outlined in O'Loughlin *et al*. 2006 (see Table 2.2).

3.3 Current disease status (2009)

3.3.1 PCR tests for notifiable viruses

IHHNV:
A minimum of 48 prawns from Spencer Gulf (4 sites) and 36 prawns from GSV (3 sites) were tested for the presence of IHHNV, with all samples returning a negative result (Table 3.1). The positive control consisted of DNA extracted from an infected raw prawn from Indonesia, which was consistently PCR-positive and highly suitable for use as a positive control. The negative control was water substituted for DNA and this was consistently PCR-negative.

The number of pooled samples tested for IHHNV provides 95% confidence of detecting this virus at greater than 10% prevalence in Spencer Gulf (24 pooled samples) and GSV (18 pooled samples). When samples are pooled between gulfs (42 pooled samples), this provides 95% confidence of detecting this virus at greater than 4% prevalence in South Australia. Thus, these populations can be considered free of IHHNV (with 95% confidence), assuming the above prevalence.

GAV:
A minimum of 48 prawns from Spencer Gulf (4 sites) and 36 prawns from GSV (3 sites) were tested for the presence of GAV, with all samples returning a negative result (Table 3.1). The positive control consisted of purified plasmid containing a 650 base pair fragment of GAV, which was used for PCR, and this was consistently PCR-positive. The negative control was water substituted for DNA and this was consistently PCR-negative.

The number of pooled samples tested for GAV provides 95% confidence of detecting this virus at greater than 10% prevalence in Spencer Gulf (24 pooled samples) and GSV (18 pooled samples). When samples are pooled between gulfs (42 pooled samples), this provides 95% confidence of detecting this virus at greater than 4% prevalence in South Australia. Thus, these populations can be considered free of GAV (with 95% confidence), assuming the above prevalence.

WSSV:

A minimum of 30 prawns from Spencer Gulf (3 sites) and 36 prawns from GSV (3 sites) were suitable for testing for the presence of WSSV, with all samples returning a negative result (Table 3.1). The positive control consisted of purified plasmid containing an insert of WSSV DNA, which was used for PCR, and this was consistently PCR-positive. The negative control was water substituted for DNA and this was consistently PCR-negative.

The number of pooled samples tested for WSSV provides 95% confidence of detecting this virus at greater than 20% prevalence in Spencer Gulf (15 pooled samples) and 10% prevalence in GSV (18 pooled samples). When samples are pooled between gulfs (33 pooled samples), this provides 95% confidence of detecting this virus at greater than 5% prevalence in South Australia. Thus, these populations can be considered free of WSSV (with 95% confidence), assuming the above prevalence.

HPV:

A minimum of 48 prawns from Spencer Gulf (4 sites) and 36 prawns from GSV (3 sites) were suitable for testing for the presence of HPV, with all samples returning a negative result (Table 3.1). All controls worked well. The positive control was provided by Prof. Leigh Owens of James Cook University, QLD, while the negative control was water substituted for DNA.

The number of pooled samples tested for HPV provides 95% confidence of detecting this virus at greater than 10% prevalence in Spencer Gulf (24 pooled samples) and GSV (18 pooled samples). When samples are pooled between gulfs (42 pooled samples), this provides 95% confidence of detecting this virus at greater than 4% prevalence in South Australia. Thus, these populations can be considered free of HPV (with 95% confidence), assuming the above prevalence.

Table 3.1. PCR test results for four key notifiable viruses: IHHNV, GSV, WSSV and HPV using 6 pooled samples per site, with each sample containing 2 prawns (n=12 per site). N/A indicates partial degradation DNA, which renders some samples unsuitable for WSSV testing.

Gulf	Site	IHHNV	GAV	WSSV	HPV
SG	Spit	negative	negative	negative	negative
SG	Mt. Young	negative	negative	3/6 negative 3/6 N/A	negative
SG	False Bay	negative	negative	negative	negative
SG	Port Pirie	negative	negative	N/A	negative
GSV	Pt. Arthur	negative	negative	negative	negative
GSV	Pt. Wakefield	negative	negative	negative	negative
GSV	Webb Beach	negative	negative	negative	negative

3.3.2 Histopathology and electron microscopy

Three target organs (hepatopancreas, gills, and heart) were assessed for abnormalities and pathologies, with a focus on viral-like cells. Other pathologies and parasites were also noted if observed.

The primary pathology associated with the hepatopancreas was the presence of viral-like eosinophilic inclusions (Figure 3.3), which were observed at a similar prevalence between gulfs (SG: 61%; GSV: 60%; Table 3.2). It is worth noting that the highest prevalence for both gulfs occurred at the most northern sites (SG: False Bay, 70%; GSV: Pt Arthur, 63%), while prevalence generally decreased with latitude in both gulfs, and was lowest on the eastern coast in Spencer Gulf. Due to the relatively high prevalence of viral-like eosinophilic inclusions, it was decided to confirm the presence of virus in these cells using high powered electron microscopy (EM). Using EM, a similar cell was observed with an occlusion body and a marginated nucleus, resembling a late stage MBV-like infected cell. Furthermore, within the cytoplasm, and adjacent to the occlusion body, were rod-shaped virions of approximately 250nm in length, characteristic of MBV-like virus (Figure 3.4).

Heart abnormalities occurred at 6% and 5% prevalence in Spencer Gulf and GSV respectively (Table 3.2). Abnormalities were associated with myocardial atrophy (degeneration of heart cells) and focal myocarditis (lesions) (see Figures 3.5 – 3.7). Both types of pathologies were of unknown aetiology.

Gill abnormalities occurred at 6% and 15% prevalence in Spencer Gulf and GSV respectively (Table 3.2; see Figures 3.8 – 3.10). In Spencer Gulf, two prawns collected from 'the Spit' were observed with small enigmatic eosinophilic spheroids of unknown aetiology (Figure 3.9). In GSV, four prawns (3 from Webb beach, 1 from Pt Wakefield) were observed with gill fouling of fibrous appearance, possibly algal or bacterial in origin.

Other observations included two types of parasites: 1) a helminth worm (possibly an acanthocephalan) generally positioned amongst muscle tissue under the carapace of the cephalothorax (Figure 3.11) and observed in a small number of prawns, and 2) an unknown parasite (possibly a Metazoan) positioned between the hepatopancreas and heart of a single prawn (Figure 3.12). A small number of prawns were also observed with granuloma's (indicative of an immune response), generally positioned within or amongst muscle tissue (Figure 3.13). Eosinophilic inclusions were also infrequently observed within midgut epithelium (Figure 3.14) and within the midgut cecum, while cells with eosinophilic occclusion bodies (atypical MBV-like) were occasionally observed witihin tissue adjacent to nerve ganglion (Figure 3.15). These 'other' observations occurred infrequently, or in non targeted organs, and as such were not consistently recorded to provide an accurate prevalence.

Table 3.2 Summary of key histological abnormalities and associated pathologies observed in three targeted organs: hepatopancreas, heart and gills. Number of slides represents those that were in plane for adequate assessment. EI = eosinophilic inclusions (viral-like).

		Hepatopancreas			Heart				Gills			
Gulf	Site	# Slides	EI +ve	% occurrence	# Slides	Abnorm.	% occurrence	pathology	# Slides	Abnorm.	% occurrence	pathology
SG	Spit	30	19	63%	7	0	0%		5	2	40%	eosinophilic spheroids
SG	Mt. Young	30	19	63%	3	1	50%	myocardial atrophy	6	0	0%	
SG	False Bay	30	21	70%	9	1	13%	focal myocarditis	6	0	0%	
SG	Port Pirie	30	17	57%	9	0	0%		9	0	0%	
SG	5th Creek	24	12	50%	6	0	0%		7	0	0%	
SG	**Total**	**144**	**88**	**61%**	**34**	**2**	**6%**		**33**	**2**	**6%**	
GSV	Pt. Arthur	30	19	63%	8	0	0%		9	0	0%	
GSV	Pt. Wakefield	29	18	62%	7	0	0%		9	1	11%	fouling
GSV	Webb Beach	30	16	53%	7	1	17%	myocardial atrophy	9	3	33%	fouling
GSV	**Total**	**89**	**53**	**60%**	**22**	**1**	**5%**		**27**	**4**	**15%**	
ALL	**Total**	**233**	**141**	**61%**	**57**	**4**	**8%**		**60**	**6**	**10%**	

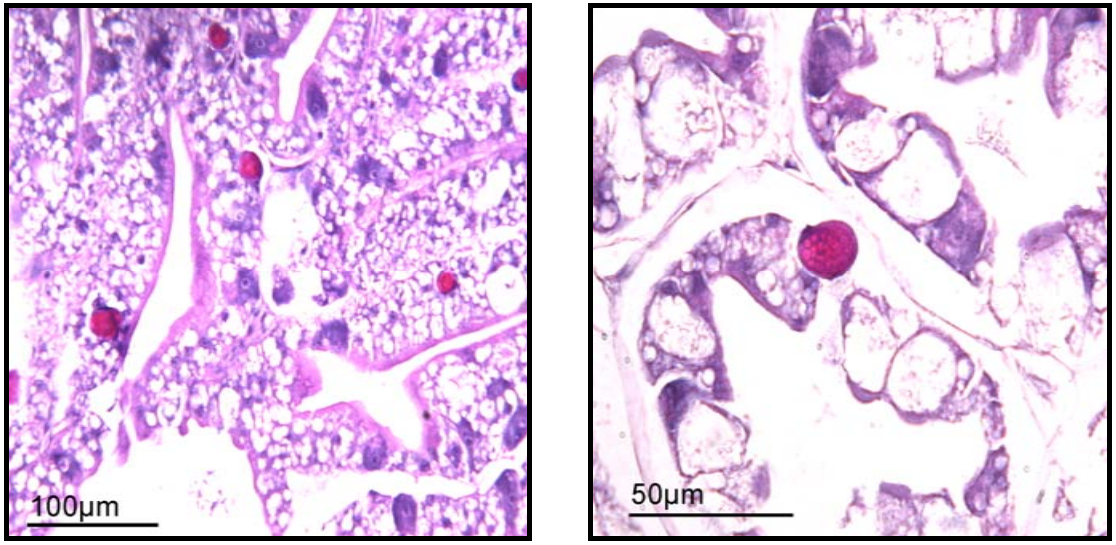

Figure 3.3 Viral-like eosinophilic inclusions (stained magenta) observed within the hepatopancreas.

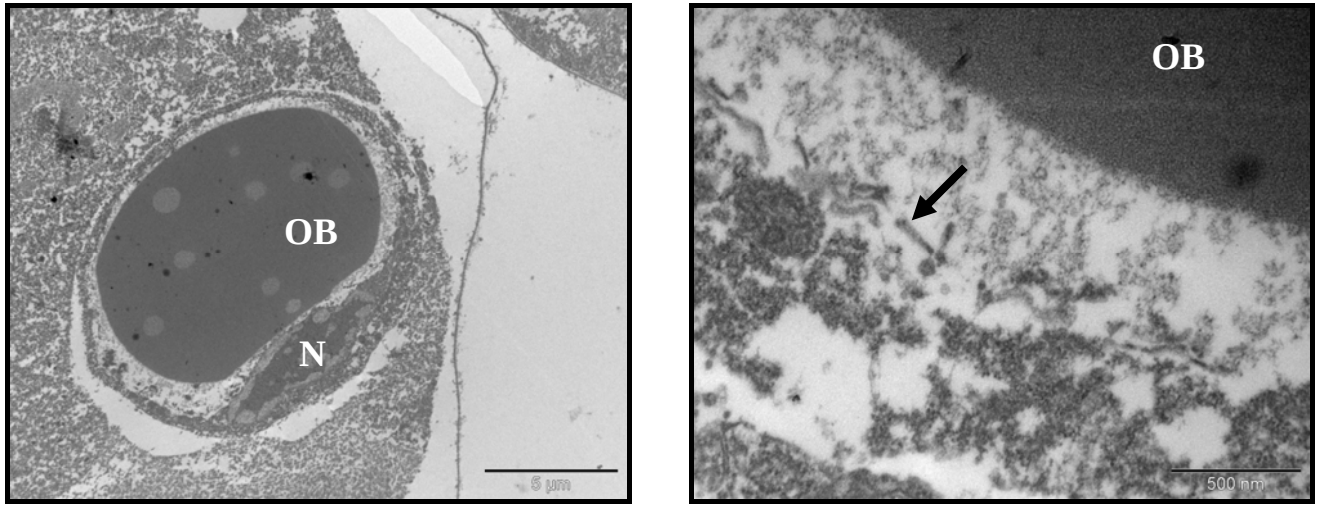

Figure 3.4 Electron microscopic images of an hepatopancreatic cell, resembling a late stage MBV-like infected cell, with marginated nucleus (N), occlusion body (OB) and cytoplasmic rod-shaped virions (arrow) at ~250nm in length, characteristic of MBV-like virus.

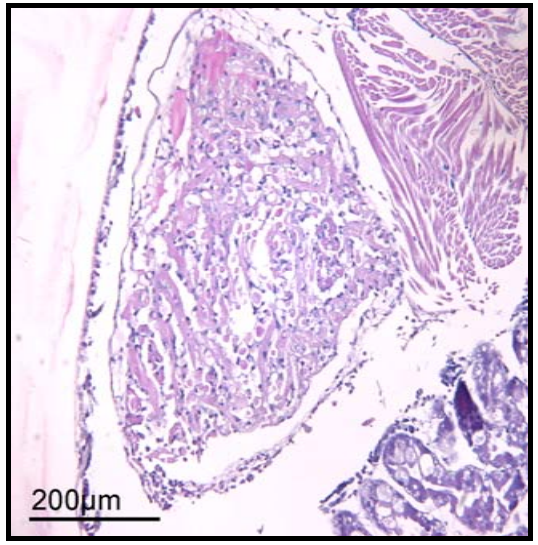

Figure 3.5 Heart of normal appearance

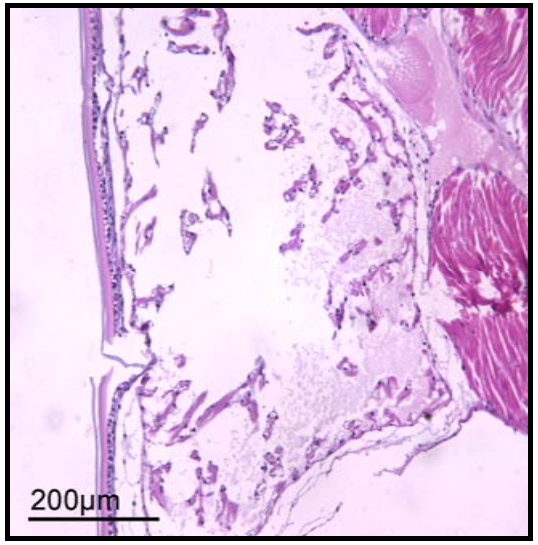

Figure 3.6 Heart pathology: myocardial atrophy

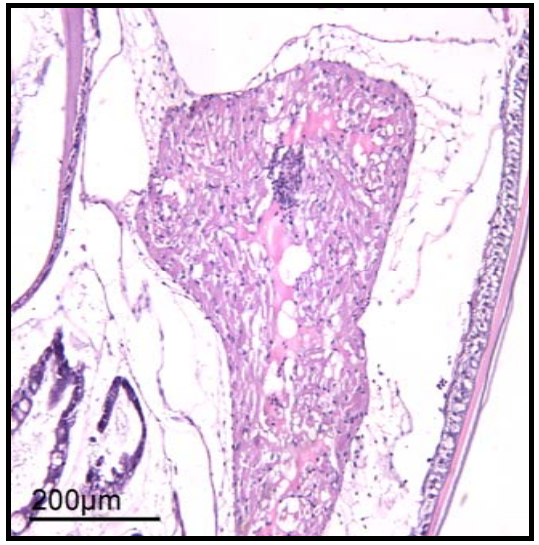

Figure 3.7 Heart pathology: focal myocarditis

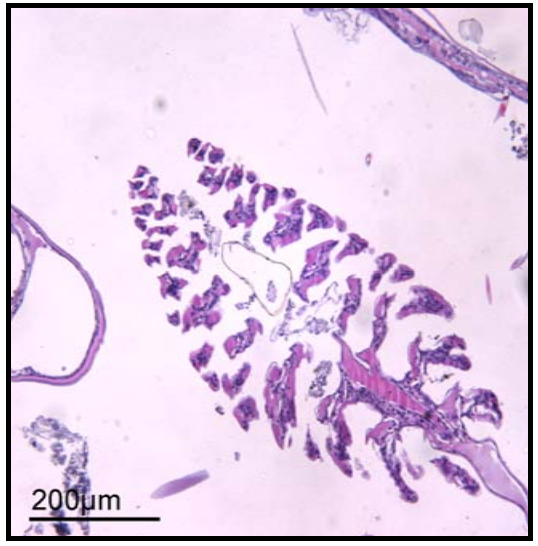

Figure 3.8 Gill filament of normal appearance

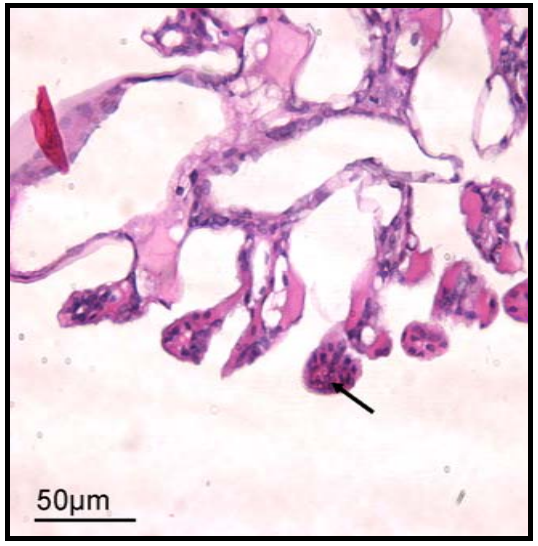

Figure 3.9 Gill pathology: small enigmatic eosinophilic spheroids (arrow).

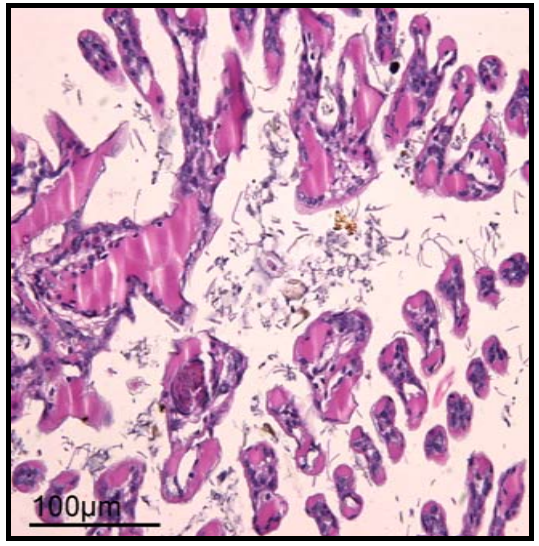

Figure 3.10 Gill fouling.

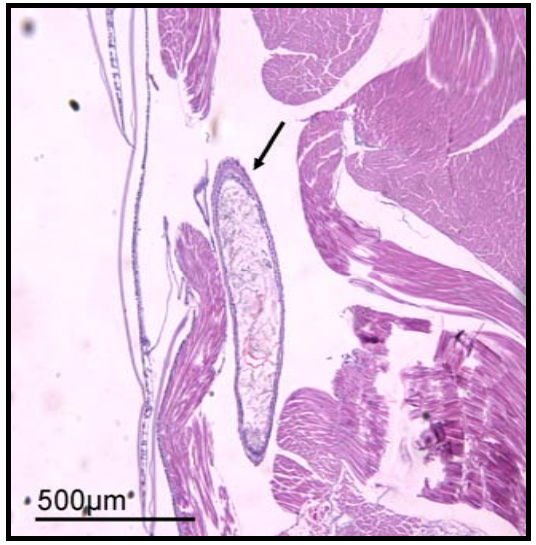

Figure 3.11 Helminth parasite (possibly an acanthocephalan, arrow) amongst muscle tissue under the carapace of the cephalothorax.

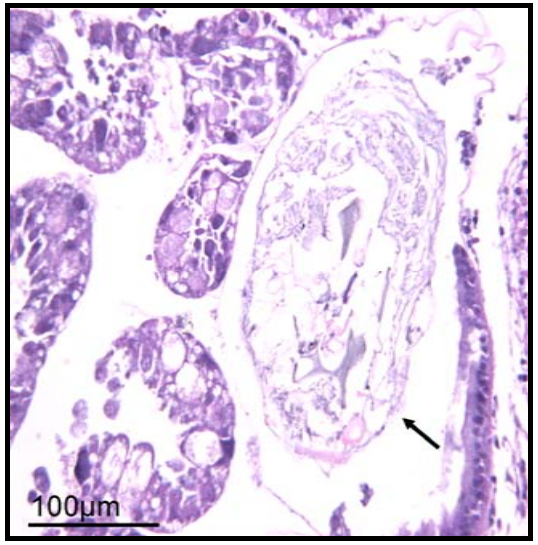

Figure 3.12 Unknown parasite (Metazoan?, arrow) observed between the hepatopancreas and heart.

Figure 3.13 Small granuloma (arrow) positioned adjacent to the heart.

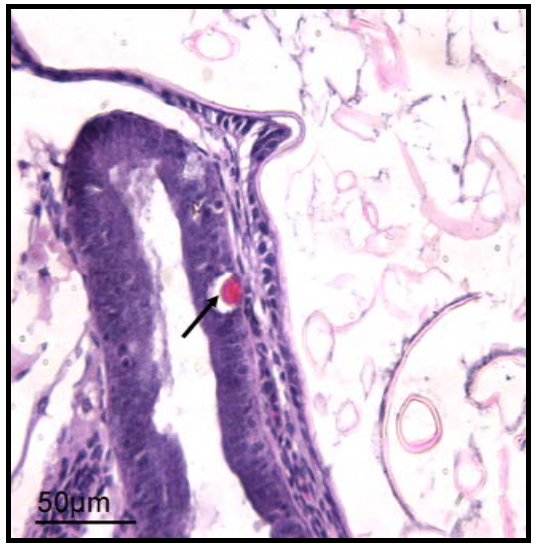

Figure 3.14 Eosinophilic inclusion (arrow) observed within the midgut epithelium.

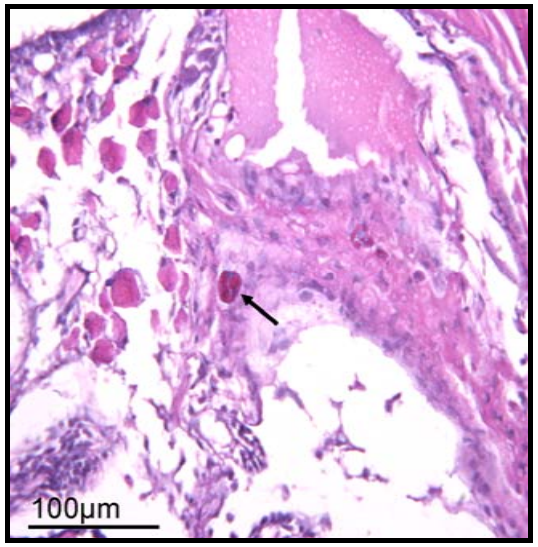

Figure 3.15 Eosinophilic occlusion bodies within a cell (arrow) adjacent to nerve ganglion.

4. SUMMARY AND CONCLUSION

4.1 Relative abundance

Relative abundance (density) of juvenile prawns were comparable (within similar ranges) to historical data for both gulfs. In Spencer Gulf, while False Bay was again observed with the highest relative abundances, overall abundances were well distributed among sites in contrast to previous years. Mean annual relative abundance during 2009 was similar to that observed during 2001. In GSV, relative abundance was highest in the north (Pt Arthur) and lowest in the south (Webb beach). Similarly, Kangas and Jackson (1998) found greater abundances of juveniles in northern GSV. Mean annual relative abundance during 2009 was higher than 1994 and 1996 levels, while abundances significantly peaked during the closure of the fishery in 1992.

Determining indices of future biomass for prawn fisheries using post-larval and juvenile abundance have been problematic (Lee, 2004). However, the semi-enclosed gulf systems of South Australia provide a good opportunity to explore stock-recruitment relationships in penaeids. Kangas (1999) explored a number of adult indices to correlate with juvenile prawn densities, and found a weak relationship (with few data points) with commercial recruitment to the GSV fishery. Furthermore, Roberts *et al.* (2005) concluded that the paucity of relative abundance data for juvenile prawns and associated adult biomass estimates for Spencer Gulf prohibited adequate assessment of the stock-recruitment relationship. This conclusion still stands in the absence of historical adult biomass estimates (fishery-independent surveys). However, now that structured and consistent surveys of adult biomass are routinely conducted in both gulfs as part of harvest strategy development and stock assessment, continuing juvenile prawn surveys for the next few years will provide the robust dataset needed to adequately explore the stock-recruitment relationship for *P. latisulcatus* in South Australia. Furthermore, environmental data is concurrently collected during adult surveys, while a current FRDC project (2008/011) will provide temperature and oceanographic related larval development and settlement models, which will greatly assist with understanding stock-recruitment relationships. If juvenile prawn densities are found to be a good predictive index of future adult stock, it will provide substantial benefits to the sustainability and economics of the prawn fisheries.

4.2 Marine pests and current disease status

No marine pest species were observed during this study. This survey targeted 37 of the 50 marine pest species outlined in O'Loughlin *et al*. 2006 that may occur in shallow intertidal habitats. Future marine pest surveys should be conducted in these habitats to provide the forewarning of any future incursions required to adequately respond to potential outbreaks.

Disease status is a broad overview of the health and parasite load of an organism. This report aimed to provide a preliminary assessment of disease status, focussing on viral fauna with comment on pathologies and parasites if observed. Disease status of WA commercial prawn species, including *P. latisulcatus*, was documented by Jones (2003), which provides a thorough background to many known penaeid parasites, diseases and viruses, and equally provides a good comparative study to this report.

Juvenile prawn samples were tested for the presence of four key notifiable viruses: WSSV (currently not found in Australia), and IHHNV, HPV and GAV (known to occur within Australia). All samples tested were negative. The sampling design provided 95% confidence of detecting these viruses at greater than 4% prevalence (and 5% for WSSV) in South Australia (SA). The absence, or undetectable low prevalence, of these viruses in SA is of significance. Specifically, it reflects the geographically isolated location of these penaeid populations in SA and highlights the risks associated with prawn and crustacean products sourced from outside of the State, particularly the use of bait prawns. These results should be considered by policy makers in regard to the entry of prawn products into the State. Currently, under the Livestock Notice 2005 (Restrictions on Entry of Aquaculture Stock), aquaculture stock that do not have specific translocation protocols described in that notice can enter South Australia from interstate legally only under a Ministerial approval. The use of imported prawns as bait by recreational fishers was considered one of three major exposure pathways under an import risk assessment conducted by the Commonwealth government (Biosecurity Australia, 2009).

Histological analyses of juvenile prawns targeted the digestive gland (hepatopancreas), heart and gills. A number of pathologies and parasites were observed at low prevalence, providing baseline data of current disease status. It is noteworthy that in GSV, gill fouling increased in prevalence from north to south and in proximity to the major port. Furthermore, an MBV-like virus (confirmed using electron microscopy), was observed at relatively high prevalence in both Spencer Gulf (61%) and Gulf St Vincent (60%), with prevalence highest in the north of the gulfs (up to 70%).

Lester *et al*. (1987) described an MBV-like virus in *P. latisulcatus* in New South Wales (NSW). While MBV-like virus was not detected in adult *P. latisulcatus* from Western Australia (WA), it was found to be prevalent in other wild-caught penaeids in WA (Jones, 2003). Susceptibility to MBV-like virus is believed to be age-related, with high prevalence (72%) found in post-larval and juvenile *P. esculentus*, while no evidence of infection was found in adults residing in close proximity (Jones 2003). This may be a result of adults acquiring a resistance to infection, and possibly becoming carriers (Ramasamy *et al*., 2000). Ramasamy *et al.* (2000) noted that infected larval *P. monodon* exhibited lethargy and reduced feeding activities, while infected juveniles, sub-adults and brooders exhibited normal behaviour. Under culture conditions, infection rates in zoea, mysis and post-larvae were 28%, 57% and 91% respectively, while mortality rates were similarly greater with higher levels of infection. It was unclear whether similar infection levels and mortality rates occurred under wild conditions. MBV infection may result in non-specific signs of disease such as reduced feeding, poor growth rate, increased exoskeletal and gill fouling, morbidity and mortality (Stuck and Overstreet 1994). The pattern of age-related infection and mortality resultant from MBV-like viruses may also be apparent in *P. latisulcatus*, although infection and mortality levels in larval and adult populations in SA have not been documented. While not all strains of MBV cause disease, the age-related and environment-related prevalence, taxonomy, and virulence of MBV-like virus in SA should be studied further. Results may hold implications for the culture of this species and in understanding stock-recruitment relationships in fisheries.

4.3 Conclusion

Relative abundance data collected during 2009 were comparable (within similar ranges) to historical data for both gulfs. Continuing juvenile prawn surveys over the next few years, in parallel with the current surveys of adult biomass, would provide a good opportunity to explore the stock-recruitment relationship. Subsequently, juvenile prawn density may provide a good predictive index of future stock status, which would substantially augment management of the prawn fisheries in SA.

While no marine pests within prawn nursery habitat were observed during this study, future routine surveys would provide the forewarning of any future incursions required to adequately respond to potential outbreaks.

Juvenile prawn populations in South Australia are free of the key pathogenic viral fauna found both internationally and nationally. These include: IHHNV, WSSV, HPV and GAV. This highlights the risks associated with prawn and crustacean products sourced from

outside of the State, particularly the use of bait prawns. These results may be considered by policy makers to restrict where necessary the entry of prawn products into the State.

An MBV-like virus was observed at high prevalence (~60%) in juvenile *P. latisulcatus* in SA. While not all strains of MBV cause disease in prawns, the age-related and environment-related prevalence and virulence of MBV-like virus in SA should be studied further, while strain variation between States should be determined. Results may hold implications for the culture of this species and in understanding stock-recruitment relationships in these fisheries. MBV-like viruses are common in penaeid prawns where they can occur in high prevalence. As such they are not a notifiable pathogen (Biosecurity Australia, 2009), and are unlikely to pose a health risk to humans on consumption.

5. REFERENCES

Anon (1997). An evaluation of potential shrimp virus impacts on cultured and wild shrimp populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters. A report to the Joint Subcommittee on Aquaculture. JSA Shrimp Virus Work Group, National Marine Fisheries Service, USA.

Bax N., Williamson A., Aguero M., Gonzalez E. and Geeves W. (2003) Marine invasive alien species: a threat to global biodiversity. Mar Pol 27: 313-23.

Biosecurity Australia (2009) Generic Import Risk Analysis Report for Prawns and Prawn Products. Biosecurity Australia, Canberra, Australia.

Bryars S. (2003) An inventory of important coastal fisheries habitats in South Australia. Fish Habitat Program, Primary Industries and Resources South Australia.

Carrick N.A. (1996) Key factors which affect prawn recruitment and implications to harvesting prawn stocks. FRDC report 91/3, Canberra, Australia.

Carrick N.A. (2003) Spencer Gulf Prawn (*Melicertus latisulcatus*) Fishery. Fishery Assessment report to PIRSA. SARDI Aquatic Sciences RD03/0079–2.

Courtney A.J., Montgomery S.S., Die D.J., Andrew N.L., Cosgrove M.G. and Blount C. (1995) Maturation in the female eastern king prawn *Penaeus plebejus* from coastal waters of eastern Australia, and consideration for quantifying egg production in penaeid prawns. Mar. Biol. 122(4): 547–556.

Cowley J.A., Dimmock C.M., Spann K.M. and Walker P.J. (2000). Detection of Australian gill-associated virus (GAV) and lymphoid organ virus (LOV) of *Penaeus monodon* by RT-nested PCR. Dis Aquat Org 36:153-157

Crookes, J.A. and Soulé, M.E. (1999) Lag times in the population explosions of invasive species: Causes and implications. In. Sandlund O.T., Schei P.J. & Viken Á. (eds). Invasive species and biodiversity management. (Kluwer Academic Publishers. Netherlands).

Dixon C.D., Hooper G.E. & Roberts S.D. (2009) Spencer Gulf Prawn, *Penaeus (Melicertus) latisulcatus*, Fishery 2007/08. Fishery Assessment report to PIRSA Fisheries. SARDI Aquatic Sciences Publication No. F2007/000770-3, Research Report Series No 387.

Edyvane K. (1999) Coastal and marine wetlands in Gulf St Vincent, South Australia: understanding their loss and degradation. Wetlands Ecol. Manag. 7: 83–104

Fernandes M.B., Wiltshire K.H. and Deveney, M.R. (submitted for publication) Macroalgae (*Caulerpa taxifolia*) and seagrasses (*Zostera muelleri*) as benthic ecosystem engineers and their effects on sediment biogeochemistry. Estuarine, Coastal and Shelf Science.

Gulland, J.A. and Rothschild, B.J. (1984) Penaeid shrimps – their biology and management. Fishing News Books, Surrey, England.

Harris J.H. (1989) In L Hicks M, Eiser P (eds). The conservation of threatened species and their habitats. Australian Committee for IUCN (International Union for Conservation of Nature), Canberra, p 190-191.

Harvell D., Aronson R., Baron N., Connell J., Dobson A., Ellner S., Gerber L., Kim K., Kuris A., McCallum H., Lafferty K., McKay B., Porter J., Pascual M., Smith G., Sutherland K., and Ward J. (2004) The rising tide of ocean diseases: unsolved problems and research priorities. Front. Ecol. Environ. 2(7): 375–382.

Harvell C.D., Kim K., Burkholder J. M., Colwell R. R., Epstein P. R., Grimes D. J., Hofmann E.E. and Lipp E. K. (1999) Emerging Marine Diseases--Climate Links and Anthropogenic Factors. Science Magazine 285(5433,3):1505–1510.

Harvell C.D., Mitchell C.E., Ward J.R., Altizer S., Dobson A., Ostfeld R.S. and Samuel M.D. (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158-2162.

Hooper C., Hardy-Smith P. and Handlinger J. (2007) Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). Aust. Vet. J. 85(5): 188-193.

Hudinaga M. (1942) Reproduction, development and rearing of *Penaeus japonicus* Bate. Jap. J. Zool. 10: 305–393

Ignacio Vilchis L., Tegner M.J., Moore J.D., Friedman C.S., Riser K.L., Robbins T.T. and Dayton P.K. (2005) Ocean warming effects on growth, preproduction and survivorship of southern California abalone. Ecological Applications, 15(2): 469–480.

Jones B. (2003) Determination of the disease status of Western Australian commercial prawn stocks. FRDC project 1998/212, Canberra, Australia.

Kangas M. (1999) Postlarval and juvenile western king prawns *Penaeus latisulcatus* Kishinouye studies in Gulf St Vincent, South Australia with reference to the commercial fishery. PhD thesis, University of Adelaide.

Kangas M. and Jackson B. (1998) Sampling juvenile *Penaeus latisulcatus* Kishinouye with a water-jet net compared with a beam-trawl: spatial and temporal variation and nursery area in Gulf St Vincent, South Australia. Mar. Freshwater Res. 49: 517–523.

La Fauce K.A., Elliman J. and Owens L. (2007). Molecular characterisation of hepatopancreatic parvovirus (PmergDNV) from Australian *Penaeus merguiensis*. Virology 362 (2):397-403.

Lee S.Y. (2004) Relationship between mangrove abundance and tropical prawn production: a re-evaluation. Mar. Biol. 145:943–949.

Lester R.J.G, Doubrovsky A., Paynter J.L., Sambhi S.K. and Atherton J.G. (1987) Light and electron microscope evidence of baculovirus infection in the prawn *Penaeus merguiensis*. Dis. Aquat. Org. 3: 217-219.

Lightner D.V., Redman R.M., Nunan L.N., Mohney L.L., Mari J.L. and Poulos B.T. (1997) Occurrence of WSSV, YHV and TSV in Texas shrimp farms in 1995: possible mechanisms for introduction. World Aquaculture 97 Book of abstracts. World Aquaculture Society Baton Rouge, LA. p 288.

Nash G., Anderson I.G. and Shariff M. (1988) Pathological changes in the tiger prawn, *Penaeus monodon Fabricus*, associated with culture in brackishwater ponds developed from potentially acid sulphate soils. J. Fish Dis. 11: 113–123.

O'Loughlin, E., McCloud, C., Sierp, M. and Westphalen, G. (2006) Temperature and Salinity Tolerances of Priority Marine Pests. Prepared for PIRSA Biosecurity. South Australian Research and Development Institute (Aquatic Sciences), Adelaide 45 pp. SARDI Publication Number RD 06/0751.

OIE (2005) Terrestrial Animal Health Code, Edition 14. World Organisation for Animal Health, Paris, France, ISBN 92-9044-635-8.

Penn, J.W. and Stalker, R.W. (1975). A daylight sampling net for juvenile penaeid prawns. Aust. J. Mar. Fresh. Res. 26: 287-291.

Pimental D., Lach L., Zuniga R. and Morrison D. (2000) The environmental and economic cost of non-indigenous species in the United States. Bioscience 50: 53-65.

Portner H.O. and Knust R. (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315: 95-97.

Ramasamy P., Rajan P.R., Purushothaman V. and Brennan G.P. (2000) Ultrastructure and pathogenesis of *Monodon baculoĺirus* (Pm SNPV) in cultured larvae and natural brooders of *Peneaus monodon*. Aquaculture 184: 45-66.

Roberts S.D., Dixon C.D. and Hooper G.E. (2009) Gulf St Vincent Prawn, *Penaeus (Melicertus) latisulcatus*, Fishery 2007/08. Fishery Assessment report to PIRSA Fisheries. SARDI Aquatic Sciences Publication No. F2007/000782-3, Research Report Series No 382.

Roberts, S.D, Dixon C.D. and Ward T.M. (2005) Assessment of juvenile prawn (*Melicertus latisulcatus*) surveys in Spencer Gulf, South Australia. Report to PIRSA Fisheries. SARDI Aquatic Sciences RD04/0211–2, SARDI Research Report Series No 95.

Stuck K.C. and Overstreet R.M. (1994) Effect of *Baculovirus penaei* on growth and survival of experimentally infected postlarvae of the Pacific white shrimp, *Penaeus vannamei*. J. Invert. Path. 64(1): 18-25.

Tang K.F.J., Navarro S.A. and Lightner D.V. (2007). PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and virus-related sequences in the genome of *Penaeus monodon*. Dis Aquat Org 74:165-170.

Tanner J.E. and Deakin S. (2001) Active habitat selection for sand by juvenile western king prawns, *Melicertus latisulcatus* (Kishinouye). J. Exp. Mar. Biol. Ecol. 261: 199–209.

Vijayan K.K., Balasubramanian C.P., Jithendran K.P., Alavandi S.V. and Santiago T.C. (2003) Histopathology of Y-organ in Indian white shrimp *Fenneropenaeus indicus*, experimentally infected with white spot syndrome virus. Aquaculture 221: 97–106.

Ward T.M., Hoedt F.E., McLeay L.J., Dimmlich W.F., Kinloch M.W., Jackson G., McGarvey R., Rogers P.J., and Jones K. (2001). Effects of the 1995 and 1998 mass mortalities on the spawning biomass of *Sardinops sagax* in South Australia. ICES. J. Mar. Sci. 58(4). 830-841.

Whittington R.J., Crockford M., Jordan D. and Jones B. (2008) Herpesvirus that caused epizootic mortality in 1995 and 1998 in pilchard, *Sardinops sagax neopilchardus* (Steindachner), in Australia is now endemic. J. Fish Dis. 31 (2) 97–105.