

## THE OCCURRENCE OF BACULOVIRUS IN MUD CRABS AT DARWIN AQUACULTURE CENTRE AND IN REGIONAL AREAS OF THE NORTHERN TERRITORY: Characterisation, Pathological Significance and Impact on Proposed Mud Crab Aquaculture

J. D. Humphrey, G. Williams and E. Needham, Fisheries, Darwin

---

### SUMMARY

This Technote describes the occurrence of, and attempts to establish the pathogenic significance of, inclusion bodies caused by baculoviral infection of the hepato-pancreas in juvenile and mature mud crabs in the Northern Territory (NT).

Individual populations of juvenile mud crabs spawned at the Darwin Aquaculture Centre (DAC) showed a prevalence of infection of up to 100%. Individual crabs showed high numbers of viral inclusions in the hepato-pancreas. Low numbers of viral inclusions were seen in broodstock and non-captive or wild-caught crabs. Although the source of infection remains uncertain, likely sources include cannibalism and faecal contamination. The possibility of vertical transmission from infected broodstock cannot be excluded. Sequential sampling of crabs from a single cohort indicated rapid spread resulting in 100% prevalence of infection. The pathogenic significance of the infection remains uncertain. No clinical disease was evident in infected juvenile or mature crabs. The possibility of covert disease, resulting in weakened mud crabs or mud crabs more susceptible to stress and inter-current bacterial infection cannot be excluded. Examination of crabs from diverse geographic regions separated by large distances indicates the virus is present in the NT. From a zoogeographic perspective, it can be concluded that the Maningrida region, DAC and Bynoe Harbour are areas of equivalent disease status with respect to this particular baculovirus and movement of infected crab-lets from DAC to Maningrida will not change the overall *status quo* of the distribution of the virus. Translocation between zones of equivalent disease status notwithstanding, infected juvenile crabs destined for semi-intensive grow-out may be subject to greater stress than their non-captive counterparts and may be more susceptible to inter-current disease or cannibalism. It is possible that the virus may be pathogenic under conditions of stress and high stocking densities; in such situations, losses or disease may occur in translocated stocks. Therefore, translocation of infected stocks should be avoided. Transmission studies in penaeid prawns failed to induce viral inclusions or disease in those species.

## INTRODUCTION

Following long-term research in the reproductive behaviour and larval rearing of the species, spawning and rearing of juvenile mud crabs (*Scylla serrata*) in numbers sufficient to support commercial farming have been successful at DAC (Shelley 2008). There is considerable potential to exploit this success by establishing grow-out facilities for commercial farming of mud crabs throughout the NT.

To prevent the spread of disease through movements of living aquatic animals for aquaculture, juvenile mud crabs destined for grow-out on commercial farms or in regional centres remote from Darwin were tested for disease freedom prior to transport. In July 2005, a cohort of 31-day-old crabs spawned and reared at DAC (June 2005) was examined prior to transport to Maningrida for grow-out purposes. Histopathological examination showed abnormal, enlarged cells in the hepato-pancreas. This was subsequently confirmed by electron microscopy to be due to a baculovirus infection. Based on these findings, the shipment was stopped pending further investigations into occurrence and distribution of baculoviral infections in mud crabs in the NT, and their pathogenic significance and potential for spread to other crustacean species.

The study aimed to:

- Characterise the nature and occurrence of the inclusions in the hepato-pancreas of mud crabs at DAC.
- Determine the pathogenic significance of the inclusions in infected crabs.
- Monitor the spread of the baculovirus in infected cohorts of mud crabs spawned and reared at DAC.
- Determine the geographic distribution of crabs with baculoviral inclusions in the NT in order to establish zones of equivalent health status for translocation of mud crabs within or between zones.
- Determine the infectivity of the virus to penaeid prawn species.

## METHOD

### CHARACTERISATION OF HEPATO-PANCREATIC INCLUSIONS

#### *Histopathological examination*

Crab-lets from the June 2005 cohort were sectioned longitudinally along the midline and preserved in Davidson's fixative for histopathological examination. Tissues were retained in the preservative for sufficient time to allow demineralisation of the carapace, approximately 48 hours. Tissues were embedded in paraffin; sections were cut at 5 microns and stained with haematoxylin and eosin for histopathological examination.

Samples of hepato-pancreas from four broodstock crabs at DAC were also histopathologically examined. Crabs were euthanized by injection of preservative into the ventral nerve cord and samples of hepato-pancreas were collected in 10% buffered formalin for histological examination.

#### *Electron microscopic examination*

Eleven crab-lets from the June 2005 cohort known to have a high prevalence of hepato-pancreatic inclusions through earlier histopathological examinations were sectioned longitudinally in the midline. From one half, the hepato-pancreatic tissue was gently excised and placed in 2.5% glutaraldehyde. The other half was preserved in Davidson's fixative. Following confirmation of the presence of hepato-pancreatic inclusions through histological examination, tissues from the affected hepato-pancreas of three crabs were processed for transmission electron microscopic examination.

## **PREVALENCE AND INTENSITY OF INFECTION**

Samples were taken from three cohorts of crabs spawned and reared at DAC. They were identified as the March 2005 cohort, the June 2005 cohort and the July 2005 cohort. Samples were preserved in Davidson's fixative, processed for histopathological examination as described above and the sections of hepato-pancreas were examined for the presence of inclusions.

The intensity of infection in any one individual was estimated as the percent of cells affected in the histological section of the tissue.

## **PATHOGENIC SIGNIFICANCE OF INCLUSIONS**

The pathogenic significance of the presence of hepato-pancreatic inclusions was evaluated in reference to the history of disease in affected cohorts, the intensity or numbers of the inclusions and gross pathological examination of infected crabs. The infected July 2005 cohort was kept under on-going surveillance to monitor any progress of disease in the cohort.

## **SPREAD OF BACULOVIRUS WITHIN COHORTS OF JUVENILE MUD CRABS**

Following confirmation of the viral nature of the hepato-pancreatic inclusions, the spread of infection within individual cohorts of juvenile mud crabs at DAC was monitored by sequential histological examination of cohorts of crabs spawned and reared at DAC in which viral inclusions had been confirmed. In addition, broodstock crabs were examined for evidence of viral hepato-pancreatic inclusions.

## **GEOGRAPHIC DISTRIBUTION OF MUD CRABS WITH INCLUSIONS**

The geographic distribution of mud-crabs infected with baculoviral inclusions was determined by histological examination of juvenile and mature mud crabs from Bynoe Harbour, DAC and the Maningrida region (Table 1).

## **INFECTIVITY FOR PENAEID PRAWNS**

Following confirmation of the viral nature of the hepato-pancreatic inclusions in the mud crabs, transmission trials were conducted to evaluate the infectivity of the baculovirus in the prawn species *Penaeus monodon* and *Metapenaeus insolitus*. Prawns were maintained in separated groups and were fed the viscera of mud crabs from the June 2005 cohort, which had previously been determined to have a prevalence of 100% infection with baculovirus. Prior to being used as a feed source, the crabs had been stored frozen at -30°C. Prawns were allowed to feed on infected crab tissues for five days, following which they were monitored for disease. Samples were taken for histopathological examination at seven and 14 days after the commencement of feeding. The control group of prawns, collected before the feeding commenced, and from a non-fed group, were also examined. The prawns were euthanized by inoculation with Davidson's fixative into the thorax; the cephalothorax was transected longitudinally and the cephalothorax was placed in Davidson's fixative for histopathological examination.

## **RESULTS**

### **CHARACTERISATION OF HEPATO-PANCREATIC INCLUSIONS**

#### ***Histopathological examination***

The hepato-pancreatic inclusions in the July 2005 cohort were characterised by the presence of dense amphiphilic staining bodies showing a fine granularity present within the nucleus and occurring primarily in the distal cells of the hepato-pancreatic epithelium. Affected cells were enlarged, with nuclear hypertrophy and loss of nuclear and cytoplasmic detail. In many cases, an indistinct enlarged nucleus appeared to be compressing

adjacent cytoplasm (Figures 1 and 2). Low numbers of inclusions were identified in the hepato-pancreas of one of the four broodstock crabs at DAC (BVL Accession No 05/1056).

### ***Electron microscopic examination***

All three samples subjected to electron microscopic examination showed large numbers of viral particles, approximately 100-150 nm in diameter by 250-300 nm in length (Figure 3). Viral particles were present in huge arrays in the cytosol. The size, shape and ultra-structural changes were consistent with baculovirus.

### **PREVALENCE AND INTENSITY OF INFECTION**

Hepato-pancreatic inclusions were detected in the June 2005 cohort examined at 31, 46, 68 and 74 days of age at a prevalence of up to 100% (Figure 4), (BVL Accession Nos 05/0977, 05/1056, 05/1187, 05/1238). Similarly, hepato-pancreatic inclusions were present in the July 2005 cohort at 34 and 43 days of age at a prevalence of up to 36% (05/1186, 05/1260, 05/1317). Hepato-pancreatic inclusions were not identified in the March 2005 cohort examined at 115 days of age (05/1056).

Numbers of hepato-pancreatic cells with inclusions varied between individuals from a few to many and appeared in small irregular aggregations, clearly contiguous with normal epithelium. In heavily infected crabs, extensive sheets of cells were affected, estimated to involve approximately 50% of the cellular component of the tissue (Figure 2). Affected cells appeared to be the more mature apical cells of the gland with sparing of the more basalar cells. There was no apparent inflammatory response or fibrosis associated with the abnormal cells. Abnormal cells appear to dissociate from the surrounding epithelium into the lumen of the gland without undergoing overt necrosis.

### **PATHOGENIC SIGNIFICANCE OF INCLUSIONS**

There was no clear history of disease in either the infected June 2005 and July 2005 cohorts, or the infected broodstock crab. Gross pathological examination showed no abnormalities that could be directly attributed to infection. A small proportion of crabs in the June 2005 cohort had minor claw abnormalities characterised by misshapen claws. The carapace of one crab was grey-white in appearance. A single crab that had been found dead, revealed, on histological examination, massive bacterial colonisation and degeneration of the hepato-pancreas.

### **SPREAD OF BACULOVIRUS WITHIN COHORTS OF JUVENILE MUD CRABS**

Sequential histopathological examination of crabs from the June 2005 cohort at 31, 46, 68 and 74 days of age (05/0977, 05/1056, 05/1187, 05/1238) showed a progressive increase in incidence of hepato-pancreatic inclusions from 32% to 100%. Similarly, an increasing incidence of infection was found in the July 2005 cohort, increasing from 0% at 21 days of age to 36% at 43 days of age. (Lab. Nos. 05/1317, 05/1186). The sequential prevalence of these infections is presented in Figure 4.

### **GEOGRAPHIC DISTRIBUTION OF MUD CRABS WITH INCLUSIONS**

The results of examination of mud crabs from different localities in the NT for baculoviral inclusions in the hepato-pancreas are show in Table 1.

A high prevalence, up to 39%, of baculoviral hepato-pancreatic inclusions was found in mature and sub-mature mud crabs from river systems from the Maningrida region. In contrast, no inclusions were found in juvenile mud crabs from the immediate Maningrida area. A single mature mud crab from Bynoe Harbour was found to have hepato-pancreatic inclusions. Examination of the hepato-pancreas of one broodstock crab, which was the mother of the infected June 2005 cohort, and originally derived from Maningrida, failed to demonstrate inclusions.

## INFECTIVITY FOR PENAEID PRAWNS

There was no mortality or disease in the prawns and no inclusions consistent with baculovirus were seen on histological examination of the hepato-pancreas.

## DISCUSSION

The baculoviral hepato-pancreatic inclusions described in this Technote are similar to those recorded earlier from mud crabs obtained from the Darwin region as *Scylla* baculovirus (Anderson and Prior 1992). The demonstration of viral arrays consistent with baculovirus in the present study confirmed that the inclusions are due to baculovirus.

With the exception of one dead crab from the June 2005 Cohort, there was no clinical evidence of disease in the juvenile mud crabs from the two infected cohorts examined at DAC. The one dead crab had a massive bacterial infection of the hepato-pancreas. It is possible that the bacterial infection was pre-disposed by viral infection in this animal. There was no evidence of disease in either captive broodstock or wild-caught mature crabs.

Given the high numbers of inclusions in some of the juvenile crabs and the likely dysfunction of the hepato-pancreas that such a level of infection might be expected to cause, it was surprising that there was no clinical evidence of disease. The possibility exists, however, that the virus may increase the susceptibility of infected crabs to inter-current disease or weaken the crabs as a result of digestive dysfunction, rendering them more susceptible to predation. The true pathogenic significance of the virus at present cannot be accurately determined.

Based on the increasing incidence of inclusions in infected cohorts, the virus shows a high propensity to spread laterally within infected populations (Figure 4). The spread is possibly a result of cannibalism and/or ingestion of contaminated faecal material. The mechanism whereby infection initially establishes in the populations of crabs is uncertain. Infection via the egg, zoeal and megalopa stages, or infection from outside sources on nets or equipment or introduction in water, cannot be excluded. The absence of inclusions in the mother of the infected June 2005 cohort, while not conclusive, suggests that infection may not necessarily be transmitted vertically from mother to progeny through the egg. Further, examination of 21-day-old crabs in the July 2005 cohort for inclusions was negative (Figure 4), with inclusions appearing at 34 days of age. This also suggests that infection was acquired from the environment.

The virus appears to be widespread in the NT; infection was demonstrated in diverse river systems in the Maningrida region. Infection was also evident in crabs from Bynoe Harbour. It is likely that the distribution of the virus may occur throughout the range of mud crabs in the NT.

The feeding of hepato-pancreatic material confirmed as positive for hepato-pancreatic baculovirus from infected mud crabs to juvenile *P. monodon* and *M. insolitus* failed to induce disease in the prawns, suggesting that the mud crab baculovirus is at least not pathogenic to these species. Given the host specificity of baculoviruses, the mud-crab baculovirus is unlikely to be pathogenic to crustaceans taxonomically diverse from mud crabs.

By defining the nature and distribution of the hepato-pancreatic baculovirus in regions of the NT, this study provides supporting evidence for permitting the translocation of mud crabs with latent baculoviral infections between zones of similar health status; that is, between DAC and the Maningrida region. Caution is, however, warranted as infected juvenile crabs may be more susceptible to inter-current disease. It would therefore appear prudent to maintain a policy that favours the translocation of juvenile mud crabs that have been confirmed free of baculovirus.

## REFERENCES

Anderson, I. G. and Prior, H. G. (1992). Baculovirus infections in the mud crab, *Scylla serrata*, and a freshwater crayfish, *Cherax quadricarinatus*, from Australia. *Journal of Invertebrate Pathology* **60**:265-273

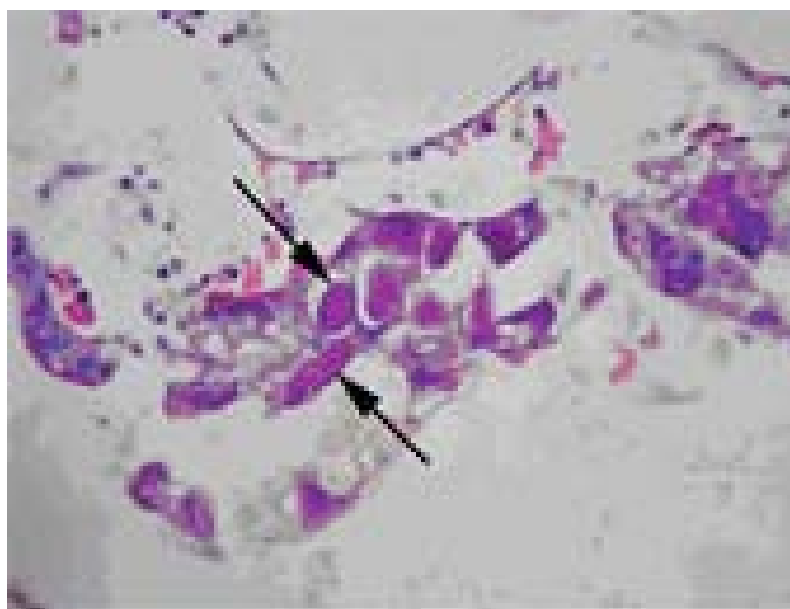
Shelley, C.C. (2008). Development of commercial production systems for mud crab (*Scylla serrata*) aquaculture in Australia: hatchery and nursery. FRDC Project 2000/210 Final Report

## ACKNOWLEDGEMENTS

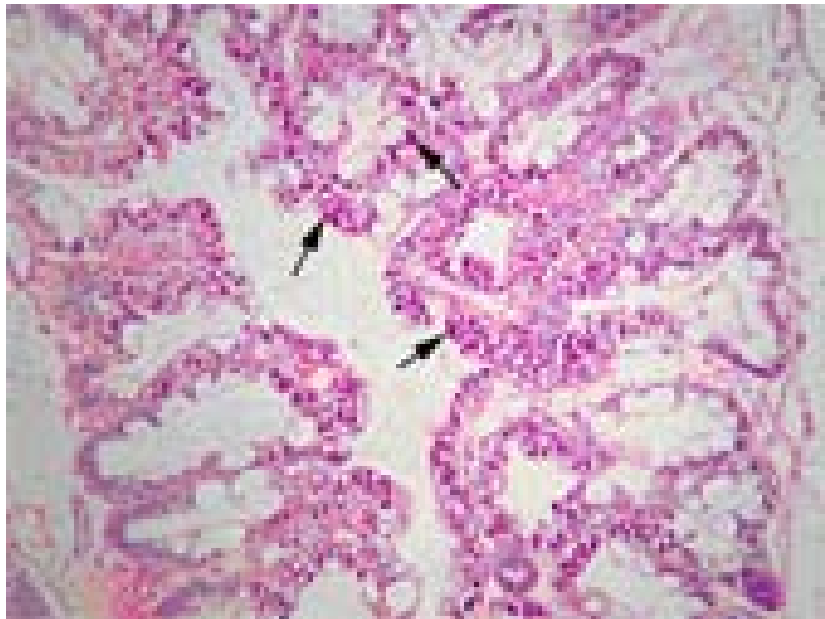
The authors thank Dr Ian Anderson, Oonoonba Veterinary Laboratories, QDPI&F for examining the histological sections and for his interpretation and opinion on the nature of the inclusions. The authors also thank Dr Richard Weir for conducting and interpreting the electron microscopic examinations and Mr Glenn Schipp for critically reviewing the manuscript.

**Table 1.** Occurrence and distribution of inclusions consistent with baculovirus in the hepato-pancreas of mud crabs from different regions and localities of the NT

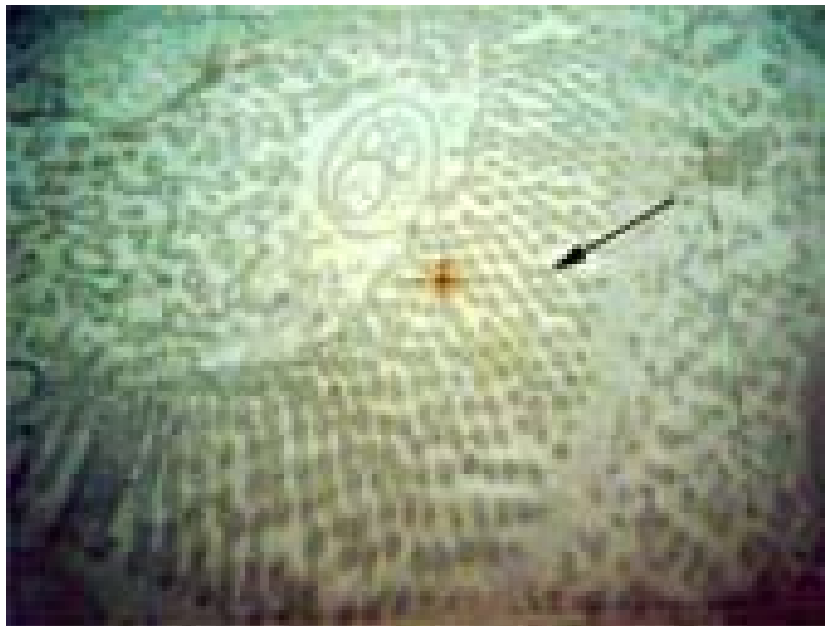
Region	Locality	Age / size	Prevalence	Lab No.
Maningrida	Liverpool River	>8 cm	5/28 (18%)	05/1326
	Blyth River	>8 cm	6/24 (25%)	05/1326
	Crab Creek	>8 cm	14/36 (39%)	05/1326
	Maningrida	<2 cm	0/19 (0%)	05/1070
	Maningrida	Mature	0/3 (0%)	05/1036
Darwin	Bynoe Harbour	Mature	1/1 (100%)	05/1036



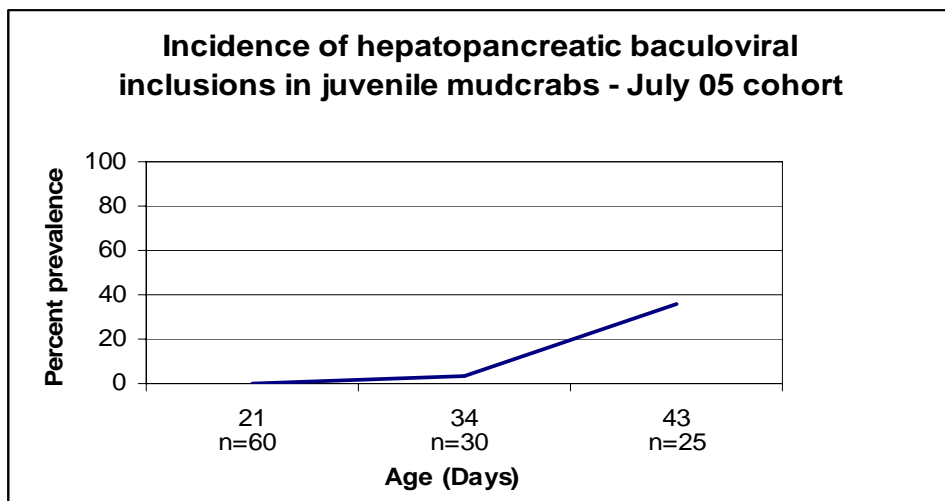
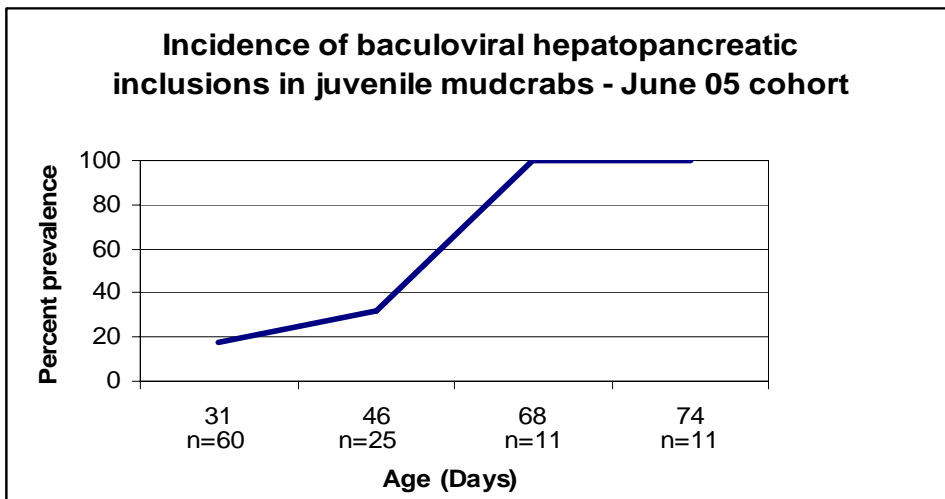
**Figure 1.** Large, amorphous ovoid intra-nuclear baculoviral inclusions (arrows) in hepatopancreatic epithelium of 31-day-old juvenile mud crab



**Figure 2.** Hepatopancreas of 43-day-old juvenile mudcrab showing high numbers of baculoviral inclusions within the epithelium (arrows)



**Figure 3.** Transmission electron micrograph of hepatopancreatic inclusion showing array of large viral particles (arrow) morphologically consistent with baculovirus



**Figure 4.** Incidence of hepatopancreatic inclusions in June 05 cohort and July 05 cohort crablets spawned and reared at DAC

Note progressive increase in incidence in both cohorts.

Please visit us at our website:

**[www.nt.gov.au/d](http://www.nt.gov.au/d)**

---

Department of Regional Development, Primary Industry, Fisheries and Resources

© Northern Territory Government

ISSN 0158-2755

Serial No.

Agdex No.

**Disclaimer:** While all care has been taken to ensure that information contained in this document is true and correct at the time of publication, the Northern Territory of Australia gives no warranty or assurance, and makes no representation as to the accuracy of any information or advice contained in this publication, or that it is suitable for your intended use. No serious, business or investment decisions should be made in reliance on this information without obtaining independent and/or professional advice in relation to your particular situation.