FINAL REPORT

Pathogenic viruses of cultured prawns

FIRDC 89/77

The University of Queensland

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FISHING INDUSTRY RESEARCH & DEVELOPMENT COUNCIL

Summary

We showed that the MBV-type virus in Australian *Penaeus monodon* was transmitted through contaminated water, that infections were recognisable with the light microscope within 2 to 6 days of exposure in early post larval prawns. Within 20 days of infection most post larvae had recovered or were recovering from the infection. High mortality was not necessarily associated with the infection. MBV was encountered in most Australian hatcheries and survived normal chlorination procedures.

Background

Large losses of juvenile prawns in a hatchery in Hawaii were found by Lightner et al. (1983) to be caused by a new prawn virus, Infectious haemocytic and hematopoetic necrosis virus (IHHNV). As a result, we examined Australian prawns for IHHNV and other viruses that could hinder the development of prawn aquaculture (FIRC Project 86/96).

We found in cultured king prawns *Penaeus plebejus* a baculovirus which we called Plebejus baculovirus (PBV). Later, we found what appeared to be the same virus in the black tiger prawn, *P. monodon*. Infections of this virus were rumoured to be associated with high mortalities in hatcheries and consequently quarantine requirements were introduced for prawns shipped into Queensland, New South Wales or Western Australia.

It was not known if the viruses themselves were pathogens or whether they merely multiplied when the hosts were compromised by some other factor. The crash in prawn production in Taiwan in 1987/8 was in part attributed to another baculovirus, Monodon Baculovirus (MBV). There was a need to determine the pathogenicity of the viruses present in Australia to help fight disease of Australian prawns in culture.

Objectives of the project

We aimed to differentiate the baculoviruses found in Australian cultured prawns; to determine their pathogenicity to the main cultured species; and to provide guidelines to avoid and to minimise losses caused by virus infections.

Introductory technical information

Most research in virology requires growing the virus in cultured host cells. For Crustacea there were, and still are, no cell lines. Thus research on crustacean viruses depends heavily on histology and electron microscopy. The large quantities of virus required for molecular studies have to be grown in susceptible intact prawns.

Baculoviruses are classified as occluded or unoccluded. Occluded baculoviruses produce a mass of protein (polyhedrin) that encloses virions. The protein masses can be large enough to be seen in host cells viewed under the light microscope, when they are referred to as 'occlusion' bodies. Unoccluded baculoviruses do not produce these masses.

Penaeid prawns moult 11 times and develop through three larval stages before they resemble adults. The stages are nauplius (5), zoea (3) and mysis (3). After the mysis stage they are 'post larvae' or 'PL's. These moult about every 24 hr and are described according to how many post-larval moults they have undergone (e.g. PL1, PL2, PL3, etc.).

MBV occurs in the epithelium of the digestive gland of the prawn. The digestive gland is also referred to as the hepatopancreas and as the mid-gut gland.

Methods

We used histology and electron microscopy to compare the baculoviruses. To determine pathogenicity and the course of the infection in different host species, post larvae were purchased from hatcheries and exposed to virus-infected tissue. Most experiments were done with *P. monodon* and *P. japonicus*. Commercial production of postlarval *P. plebejus* and *P. esculentus* was much reduced after the project started and larvae were not readily available.

Results

Species of baculovirus

The occluded baculoviruses we found in *P. plebejus* and *P. monodon* both appeared to be of the species we found first in *P. plebejus* and called PBV. We distinguished PBV from MBV on the small number of occlusion bodies per nucleus, on its staining characteristics, and on the 3 layers visible in the virion envelope. However, with further study we found the number and staining properties of occlusion bodies to be variable, and learnt that the 2 layers illustrated by Lightner et al. (1983) was a result of processing technique and that 3 layers could be demonstrated by other methods (Lightner, personal communication, Chen et al., 1989). We concluded, therefore, that our baculovirus was morphologically indistinguishable from MBV and in subsequent papers refer to it as *Penaeus monodon*-type baculovirus (MBV) (Paynter et al, 1992; Vickers et al., 1993). Lightner et al. (1986) suggested that viruses reported as MBV probably consist of a complex of strains.

Recently we found a second baculovirus in *P. monodon* from Queensland. It was unoccluded and occurred in the lymphoid organ. It showed some characteristics of yellowhead baculovirus, though its presence in Queensland prawns has not been associated with any mortality (Spann et al., 1993, and submitted).

Transmission

The means of transmission of the virus had to be known before we could attempt pathogenicity trials or recommend effective methods of control. The route of transmission of MBV was unknown. Bonami et al. (1986) was unable to infect prawns through feed and suggested transmission may be transovarian, though Lightner et al. (1900) and Overstreet et al. (1988) had shown that transmission of IHHN and BP respectively could be accomplished through contact of postlarvae with infected tissue.

A preliminary experiment to transmit the MBV-type virus to juvenile prawns was unsuccessful. We successfully transmitted it to post larvae aged PL 4 to PL 25 by feeding infected tissue to them (Paynter et al., 1992). This was the first experimental transmission of MBV.

Pathogenicity

We followed the course of 4 epizootics at 28 C over 5 weeks by histological examination of subsamples of the postlarvae. The prawns were fed daily and the water changed every 2 days.

Occlusion bodies were evident within 5 days in all infections and were found within 2 days in one. The infections peaked 12 to 16 days after exposure and then declined to close to zero (Paynter and Lester, 1992; Paynter et al., in press). Infections were assessed both as numbers of occlusion bodies seen in sections and percentage of prawns infected. At a lower temperature (22) the peaks were lower and the infection was maintained at a higher level that at 28 C.

The recovery of the post larvae was apparently related to age as recovering prawns when challenged with a second dose of the virus carried the same level of infection as prawns not challenged. Naive prawns exposed at the same age as challenged prawns developed infections that were similar to the prawns that were recovering (Fig. 1).

Histological sections revealed major destruction of the epithelium of the digestive gland at the height of infection, generally 8 to 12 days after infection. However, this did not cause significant mortality, possibly because an individual prawn experienced heavy infection for only 1 or 2 days after which they began to recover.

Mortality in the different batches of prawns was very variable and we were unable to show a link between mortality and heavily baculovirus infection. By not changing the water we were able to produce very heavy infections of baculovirus and the prawns died. However, the immediate cause of death may not have been the baculovirus. We concluded that the strain of MBV with which we were working was unlikely to cause major mortality, though it could be a contributory factor in prawns stressed in other ways.

Host susceptibility

We tested *Penaeus monodon* and *P. japonicus*. In the original application we proposed using *Penaeus monodon*, *P. esculentus* and *P. plebejus* as these were the main species of prawn cultured in Queensland and NSW. By the time the project started, culture of *P. japonicus* was being developed, and postlarvae of *P. esculentus* and *P. plebejus* were no longer available.

Postlarvae of *P. japonicus* of three ages, PL1, PL5 and PL18, were exposed to viral infected tissue under the same conditions as PL5 *P. monodon*. What resembled occlusion bodies were seen in sections of only one of the 396 *P. japonicus* and there was no other sign of baculovirus infection such as swollen nuclei, margination of nucleolus or cell necrosis (Paynter et al., in press). Most of the *P. monodon* developed typical infections of MBV. We concluded that *P. japonicus* was much less susceptible than *P. monodon*, was possibly totally refractory, and unlikely to be affected by the virus.

We had no *P. esculentus* to experimentally expose as none were produced at hatcheries. However, we have looked at 737 sections of *P. esculentus* from both farms and the wild and have never seen occlusion bodies resembling MBV-type virus. Angular inclusion bodies are occasionally present in cells from the hepatopancreas but electron microscopy has consistently failed to detect any sign of virions (Lester et al., 1986 and recent observations).

Control

A summary of our data collected from 1986 to 1990 showed that most hatcheries in eastern Australia had contracted the virus and then eliminated it, in some cases several times (Paynter & Lester, 1991; Fig. 2).

In 1992 we carried out a survey of hatcheries to determine the chlorine levels they used for disinfection. We then exposed virions to those levels and tested to see if the virions were still infective by exposing prawns to them. Only chlorine at very high concentrations was effective (1000 mg/L for 24 hr). This is a much more intense treatment than is normally used in hatcheries (100mg/L for 60 min).

Transfer of results to industry

Ms J.L. Paynter, Senior Research Assistant employed on the project, was Honorary Secretary to the Australian Mariculture Association from 1989 to 1992. She gave formal presentations on disease at the following meetings:

Prawn Growers Workshop, Townsville, November, 1990.
Asian Fisheries Society, Bali, November, 1990
Australian Society for Microbiology, Gold Coast, July 1991.
Australian Mariculture Association, Ann. Meeting, July, 1991.

Discussion

The objectives were met. We showed that with the techniques available the MBV-like baculovirus in *P. plebejus* was identical to that in *P. monodon*. We have since found a second baculovirus provisionally named lymphoid organ baculovirus (LOBV). We showed that *Penaeus japonicus* was refractory to Australian MBV under normal hatchery conditions and that infected post larvae of *P. monodon* could survive and recover from infection.

The virus was not easily inactivated by chlorine. Drying out the tanks appears to be the method of choice to eradicate the disease (LeBlanc & Overstreet, 1991).

Implications and recommendations

Disease is an ongoing risk to aquaculturalists. Our research showed that the relatively common and widespread baculovirus MBV was not particularly pathogenic, though nevertheless, steps should be taken to minimise its prevalence.

Detection of the virus is time consuming and can be inconclusive using current methods. The next step was to develop a DNA probe for the virus. For this we obtained a grant from the Australian Research Council to fund Dr Joan Vickers as Research Associate to carry out the molecular studies. She concentrated the virus, extracted and purified the DNA, and developed a DNA probe which can detect MBV in sections of infected prawns (Vickers et al., 1993). To refine this so that it can be used in industry applications, and to develop a probe to a second virus, we have a new grant from the CRC Aquaculture. This will fund a new Ph.D. student to be co-supervised by me and Dr Paul Young, Senior Lecturer in the Department of Microbiology, University of Queensland.

DNA probes for MBV, and for other viruses, are essential to determine whether infection can be passed through eggs (i.e. vertical transmission), to detect low levels of infection particularly in the development of SPF (Specific Pathogen Free) shrimp, to determine whether species such as *P. japonicus* that show no outward sign of MBV infection can carry the virus, and to screen broodstock for infection.

In a separate project we work to transform prawns genetically using prawn parvovirus as a vector. Professor Bergoin, University of Montpellier, showed that parvovirus in insects integrates with host DNA and he used the parvovirus to introduce a foreign gene into an insect cell line. It is possible prawn parvovirus could be used to introduce desirable traits such as enhanced disease resistance into prawns. Ms Kirsten Spann, Ph.D. student in the Department of Parasitology, University of Queensland, is investigating this aspect. She collaborates with Dr Nigel Preston (CSIRO Fisheries), Dr Peter Atkinson (CSIRO Entomology), and has been awarded a French Government scholarship to work with Professor Bergoin, University of Montpellier, for 3 months in 1994.

Thus the support from FIRDC for this project allowed us to develop our research on prawn viruses such that we were able to attract substantial funding from other sources. Our work is now at the forefront of prawn virus research. The University of Queensland has been invited to become a partner in a proposed 6 year, 10 million dollar, international project for the control of prawn diseases. The project is expected to be established by the World Bank in early 1994. Through this international

network, and through our training of Ph.D. students, we plan that the Australian prawn culture industry will have access to the latest information and technology in disease control.

Intellectual property

Results have been published in scientific journals and hence are in the public domain. References are given below.

Technical summary of information

Heavy infections of MBV were produced by keeping early postlarvae without water changes. A smear method was developed to speed diagnosis (Vickers et al., 1993).

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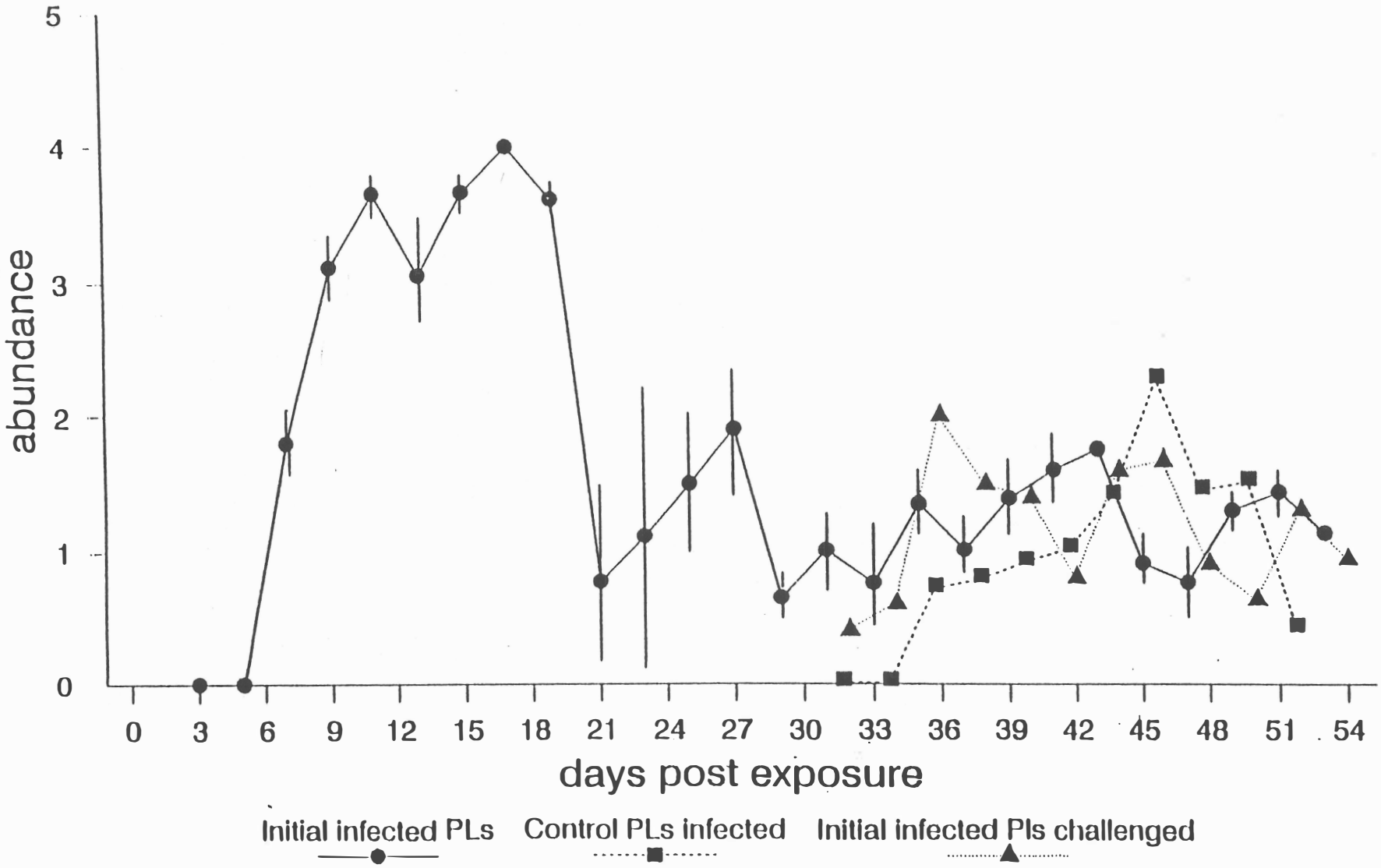
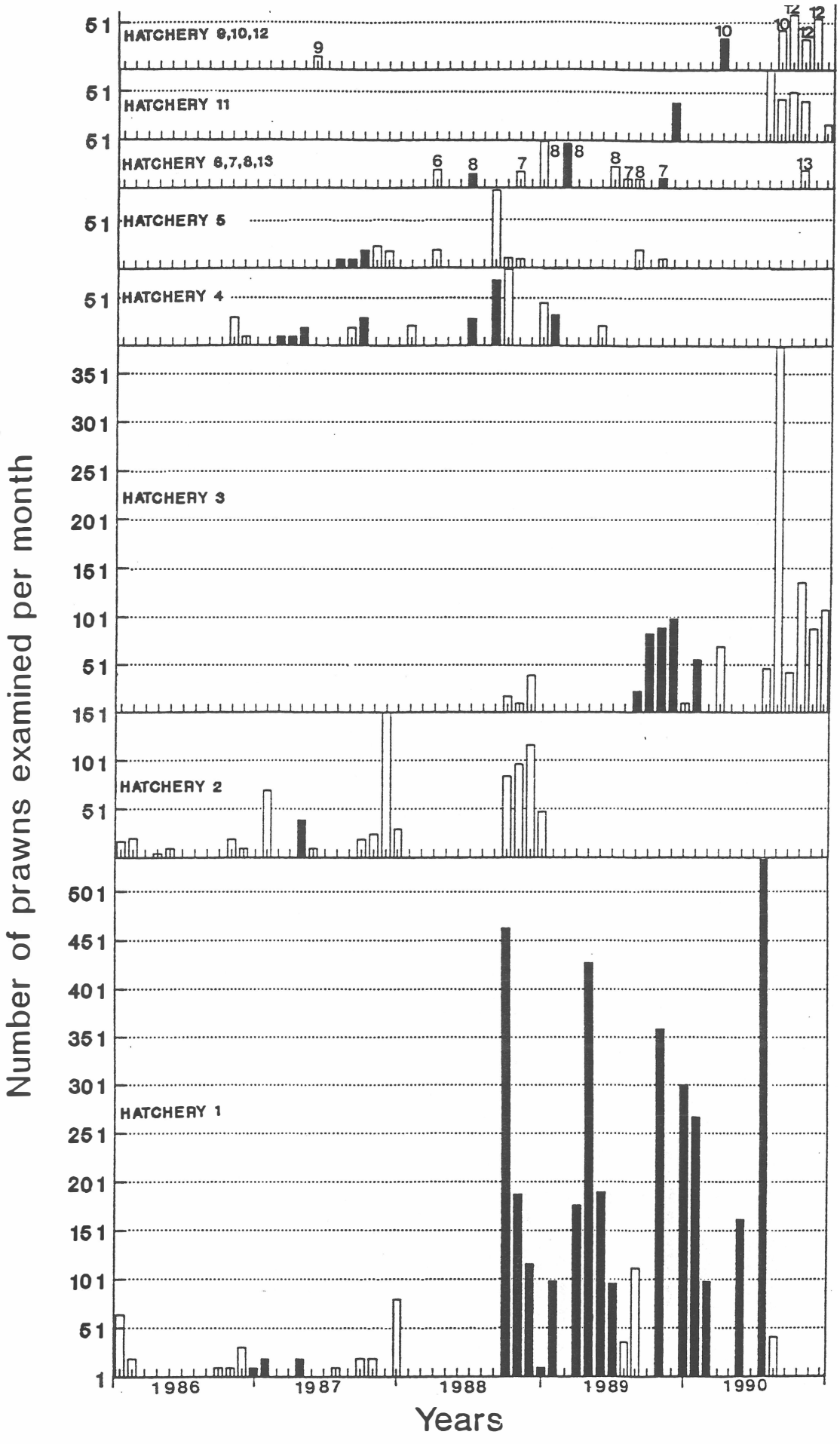
Figure captions

Fig. 1. Mean abundance of MBV occlusion bodies in postlarvae of *Penaeus monodon* experimentally exposed to homogenate of infected prawns at 28oC. Solid line: prawns exposed at age PL5; dashed line, exposed at age PL35; dotted line, part of group exposed as PL5 that was re-exposed at age PL35. (From Paynter *et al.*, in press).

Fig. 2. Occurrence of MBV in *Penaeus monodon* postlarvae from Australian hatcheries showing that MBV was widespread and frequently detected in routine samples. Diagnosis from histological sections.


abundance
5
4
3
2
1
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54
days post exposure
Initial infected PLs
Control PLs infected
Initial infected Pls challenged



Key:
Empty column = hatchery not infected with MBV-like virus.
Solid column = hatchery infected with MBV-like virus.