FINAL REPORT

Diseases of prawns in aquaculture: to develop procedures to detect pathogens of prawns shipped interstate

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The University of Queensland

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Summary

We found and described a baculovirus similar to Monodon Baculovirus (MBV) that we called Plebejus Baculovirus (PBV) as it was in eastern king prawns (*Penaeus plebejus*). We later found it in Australian cultured *Penaeus monodon*. We investigated other conditions, particularly Hepatopancreatic Parvolike Virus (HPV) and Shann Bodies. Results were published in the scientific literature and presented at industry meetings. We recomended that prawns be screened for the known virus infections in Australian prawns by subsamples of 25 prawns examined in H. and E. sections without stressing prawns for viral enhancement. We screened prawns to be moved interstate for the governments of Queensland, New South Wales, Victoria, Western Australia and the Northern Territory, carried out many examinations for individual prawn farmers, and published 4 papers and 7 reviews on prawn diseases.

Background

Catastrophic losses had occurred in prawn farms overseas because of the accidental introduction of disease, particularly IHHN virus from prawns imported from Ecuador. Nevertheless, Queensland prawn farmers were pressing the Government for permits to import live prawns from interstate and overseas.

Objectives of the project

To establish procedures to screen prawns moved interstate so that the emerging prawn farm industry was not jeopardised by disease.

The application contained a provisional protocol based on the only virus known in Australian prawns at that time, HPV (Paynter et al., 1985), and on information on viruses found overseas. We aimed to screen cultured prawns for disease and modify the protocol as necessary.

Introductory technical information

Diagnosis of crustacean viruses was, and still is, based on histological and ultrastructural examination of tissues because no cell lines for Crustacea have yet been developed.

Methods

We screened samples of all live prawns shipped into Queensland and also took routine samples from four cooperating prawn farms (two in Queensland and two in NSW) to determine the presence of indigenous diseases.

Prawns were examined immediately using wet smears to detect parasites (*Synophrya*), bacteria (*Leucothrix*) and fungi (*Fusarium*, *Lagenidium*). Prawns were divided into two groups, one group was fixed in Davidson's fixative for histology for routine detection of virus disease, and the second group fixed in glutaraldehyde for electron microscopy should a new condition be noticed in the histology.

Initially, we also held some of each batch under controlled conditions for 4 weeks in case viral replication within the prawns would be enhanced and thus make detection of the viruses easier.

We attempted to develop a prawn cell line using techniques of insect cell culture.

Results

Ciliate epicommensal protozoans and *Leucothrix* bacteria were common on prawns but did not cause significant problems. Viruses appeared to be potentially much more serious. We found and described a baculovirus similar to Mondon Baculovirus (MBV) that we called Plebejus Baculovirus (PBV). This virus subsequently proved to be common in Australian cultured *Penaeus monodon* (see Doubrovsky et al., 1988). We found inclusion bodies identified as Hepatopancreatic Parvo-like Virus (HPV) in *P. merguiensis* from Mackay and used electron microscopy to confirm the diagnosis (Roubal et al., 1989).

We investigated other abnormalities, particularly inclusion bodies of irregular shape that occurred in the hepatopancreatic nuclei of farmed *Penaeus esculentus*. No evidence of virus could be seen under the electron microscope; the cause of the bodies, which we refer to as Shann Bodies, remains unknown (Lester et al., 1987). *Synophrya* sp. was found in the school prawn *Metapenaeus macleayi*. No cases of *Fusarium* or *Lagenidium* were detected.

We screened prawns to be moved interstate and overseas for the fisheries departments of Queensland, New South Wales, Victoria, Western Australia and the Northern Territory, and carried out many examinations for individual prawn farmers.

Implications and recommendations

We found 2 viruses in Australian prawns, an MBV-like virus and HPV. We recomended that prawns be screened for these and other infections by histological examination of 25 prawns per batch, stained with haematoxylin and eosin. Holding the prawns under stress for 4 weeks prior to examination did not increase our virus detection rate and was therefore not recommended.

The results were communicated to industry through meetings of the Australian Mariculture Association (AMA) and industry workshops. The recommended method for screening was adopted by the Queensland DPI.

Ms Jan Paynter, the Research Assistant employed on the project, was Honorary Secretary of the Australian Mariculture Association (AMA) during the period of the grant. She gave talks about prawn disease at the following meetings:

AMA Conference, Cairns, Qld., June, 1987.
Prawn Farming Workshop, Port Stephens, NSW, August, 1987.
Prawn Farming Workshop, Wollongbar, NSW, December, 1987.
AMA Conference, Lismore (informal presentation only), June, 1988.
1st Australian Shellfish Aquaculture Conference, Curtin University, W.A., October, 1988.
Prawn Growers Workshop, Griffith University, Qld., November, 1988.

During the project two student scholarships, one for electron microscopy and one for culture of prawn viruses, were provided by a grant from the Reserve Bank to R.J.G. Lester and J.G. Atherton. The work of these students, Sharan Sambhi and Anna Doubrovsky, contributed to the FIRTA project.

Intellectual property

The results have been published in scientific journals and hence are in the public domain. Publications arising from FIRTA 86/96 are:

Doubrovsky, A., J.L. Paynter, S.K. Sambhi, J.G. Atherton and R.J.G. Lester 1988. Ultrastructural observations on baculovirus in Australian *Penaeus monodon* and *P. merguiensis*. Australian Journal of Marine and Freshwater Research **39**: 743- 9.

Lester, R.J.G. 1986. Parasites and parasitic diseases of aquatic animals. In Humphrey, J.D. and J.S. Langdon eds. Proceedings of the Workshop on Diseases of Australian Fish and Shellfish. Australian Fish Health Reference Laboratory, Victoria, pp. 87-101.

Lester, R.J.G., A. Doubrovsky, J.L. Paynter, S.K. Sambhi and J.G. Atherton 1987. Light and electron microscope evidence of baculovirus infection in the prawn *Penaeus plebejus*. Diseases of Aquatic Organisms **3**: 217-219.

Lester, R.J.G., P. Ketterer and J.L. Paynter 1986. Intra- nuclear inclusion bodies in the hepatopancreas of the brown tiger prawn *Penaeus esculentus*. In Azevedo, C. ed. Proceedings of the Second International Colloquium on Pathology in Marine Aquaculture. University of Oporto, Portugal, pp. 111-112.

Lester, R.J.G., P.J. Ketterer and J.L. Paynter 1987. Intranuclear inclusion bodies in the hepatopancreas of the brown tiger prawn *Penaeus esculentus*. Aquaculture **67**: 238-239.

Lester, R.J.G. and J.L. Paynter. 1989. Diseases of cultured prawns in Australia. Advances in Tropical Aquaculture, IFREMER, Tahiti. P. 19. (Abstract).

Paynter, J.L. 1989. Penaeid Prawn Diseases, with case studies and practical notes. In Invertebrates in Aquaculture, Refresher Course for Veterinarians, D.I. Bryden ed.. University of Sydney, pp.145-210.

Paynter, J.L. and R.J.G. Lester. 1987. Diseases of prawns in aquaculture. Proceedings of Prawn Farming Workshop, Wollongbar, August 1987. NSW Dept. Agriculture, pp. 49-50.

Paynter, J.L. and R.J.G. Lester. 1988. Diseases of cultured prawns in Australia. Proceedings of the 1st Australian Shellfish Aquaculture Conference, Curtin University, pp. 161-179.

Paynter, J.L., R.J.G. Lester and P.J. Ketterer 1987. A review of the diseases of penaeid prawns in Australian aquaculture. In Jones, T. ed. Proceedings of the Australian Mariculture Association Annual Meeting 1987. Pp. 46-59.

Paynter, J.L. and R.J.G. Lester. 1988. Diseases of cultured prawns in Australia. In Evans, L.H. and D. O'Sullivan, eds. Proceedings of First Australian Shellfish Aquaculture Conference. Curtin University, pp. 161-179.

Roubal, F.R., J.L Paynter and R.J.G. Lester 1989. Electron microscopic observation of parvo-like virus (HPV) in the penaeid prawn *Penaeus merguiensis* from Australia. Journal of Fish Diseases **12**: 199-201.

Sambhi, S.K. 1987. The cultivation of viruses from prawns. B.Sc. Honours Report. Department of Microbiology, University of Queensland.

Technical advances

We used standard techniques for histology and electron microscopy.