White spot disease in Australian crustaceans

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Global epidemics have been in the news recently. But for aquatic animal health specialists, this story is all too familiar, albeit with the aquatic diseases spreading silently and more slowly, underwater. The aquatic panzootic case study with most relevance to Australia in recent times is white spot disease (WSD) of crustaceans in Queensland. WSD is caused by white spot syndrome virus (WSSV), a very large (up to 150 × 380 nm) and very virulent double-stranded, rod-shaped DNA virus, which is currently the only member of the genus *Whispovirus* within the family *Nimaviridae.*[14] This unique viral disease agent of crustaceans should not be confused with white spot disease of fish, which is caused by the now ubiquitous ciliate protozoan *Ichthyophthirius multifiliis* (itself a foreign invader of Australia) in freshwater fishes, and *Cryptocaryon irritans*, which is endemic in Australian marine fishes. WSSV first emerged in 1992–1993 in shrimp (known as prawns in Australia) farmed in several locations in Asia, including Taiwan and Japan, although epidemiological evidence suggests it originated from China (possibly from wild crabs fed as trash feed to prawn broodstock). The new virus was lethal to cultured prawns and caused devastating epizootics (up to 100% mortality within 3–10 days) on prawn farms throughout Asia, with many affected prawns displaying characteristic white spots on the carapace (Figure 10). The white spots are due to disruption of calcification of the cuticle, as the virus infects tissues of ectodermal and mesodermal origin, with virions replicating inside hypertrophied nuclei of infected cells. White spots on the carapace are, however, not pathognomonic, as they can also be caused by environmental insults, bacterial infections and mineral mobilisation in moulting crustaceans (Figure 14).

By 1995, the new disease had been quickly dispersed, with infected prawn post-larvae and broodstock across the Asian continent to India and, by 1998, despite attempts at control, into Europe and the Americas. The emergence of WSSV in the absence of adequate biosecurity measures had caused a major aquatic panzootic even before WSD was listed by the World Organisation for Animal Health (OIE) and declared a notifiable disease in 1999. From 2000, the WSD panzootic spread even further, to South America, Africa and the Middle East, resulting in economic losses to prawn aquaculture industries worldwide exceeding US$20 billion, increasing at a rate of around US$1 billion per year. Studies conducted in affected countries found that WSSV infected not only prawns, but many other crustaceans, including crabs, freshwater crayfish, lobsters, copepods and various types of plankton. It is now known that WSSV can infect all decapod crustaceans, with mortality rates varying depending on host species, environmental conditions

Figure 10 White spot disease (WSD) in a cultured black tiger prawn (*Penaeus monodon*) showing classic white spots on the carapace. Photo: Ben Diggles.

14 Department of Agriculture, Water and the Environment 2019. *Aquatic animal diseases of significance to Australia: identification field guide*, 5th edition (including web links to the department's Aquatic Disease Field Guide phone application). www.agriculture.gov.au/animal/aquatic/guidelines-and-resources/aquatic_animal_diseases_significant_to_australia_identification_field_guide

Figure 11 Dead prawns scattered near the edges of the index pond on the Logan River. Photo: Ben Diggles.

and other variables such as initial dose and age of the host. Transmission of WSSV is horizontal through the water and pseudo-vertically to post-larvae spawned from infected broodstock, but most effective transmission is by the oral route, through consumption of dead or moribund affected hosts. Non-decapod crustaceans and non-crustaceans which can act as vectors include rotifers, marine molluscs, polychaete worms, *Artemia salina*, copepods, aquatic insect larvae and other arthropods. Movements of birds can also spread the virus from affected aquaculture ponds.

Analysis conducted by Professor Don Lightner's group in the OIE reference laboratory in the late 1990s deduced that the spread of WSD from Asia into North America was most likely the result of importation of frozen prawn products from WSSV-affected areas of Asia into coastal processing plants in the US. This theory was subsequently proven plausible when WSSV also reached Spain and Australia (Darwin Harbour) in 2000–2001. In both cases, successful containment and eradication was reported, and for both events the use of imported WSSV-infected frozen prawns as feed for broodstock prawns or other crustaceans was implicated as the route of introduction. This is not surprising, as the scientific community has known for over two decades that WSSV (along with several other viruses of prawns and crabs) is well preserved in frozen tissues and remains viable and infective when thawed.

The 'Darwin incident' found WSSV in broodstock black tiger prawns (*Penaeus monodon*) and mud crabs (*Scylla serrata*) accidentally fed frozen imported prawns at an

Figure 12 Tanker truck dispensing liquid chlorine into an infected pond. Photo: Ben Diggles.

aquaculture hatchery in Darwin Harbour in December 2000. In that case, healthy wild mud crabs and prawns adjacent to the hatchery outlet were also transiently infected but, over time, this infection was apparently self-limiting. Subsequent molecular testing showed WSSV did not become established in Darwin Harbour. The incident stimulated interest in tightening border controls for frozen commodity prawns imported into Australia. After a prolonged and sometimes controversial process, this culminated in the publication of the *Generic import risk analysis report for prawns and prawn products*.[15]

The 2009 risk analysis proposed several new biosecurity conditions for imported commodity prawns, based mainly on increased processing as well as testing for WSSV and yellowhead virus. After enhanced quarantine conditions were implemented by the Australian Government, Australia had, and continues to have, the most stringent quarantine measures for prawn commodities in the world. However, import volumes increased, new prawn diseases continued to emerge overseas, and as the panzootic rolled on and overseas supplies of WSSV-free prawns became scarce, unscrupulous importers found ways around the testing procedures, dumping large quantities of WSSV-infected prawns into Australian supermarkets.[16] In late November 2016 WSD was detected in black tiger prawns farmed on the Logan River in southeast Queensland.

Biosecurity Queensland responded to Australia's first clinical outbreaks of WSD by beginning destruction and decontamination activities at the affected farm to try to eradicate the infection (Figures 11 and 12). Restrictions were also put in place on commercial and recreational fishers, making it illegal for them to capture or move crustaceans from the Logan and Albert river systems.[17] Despite these precautions, as was the case in the Darwin Harbour incursion, a small number of prawns (six) and a mud crab from the Logan River tested positive for the virus. Eventually, over the next two months, all seven active prawn farms in the area became infected, usually a few days after they took water in from the Logan River to avoid mass mortalities due to oxygen depletion. In all but one case, the spread of the virus on each farm was non-random, with the ponds downwind from the water intake becoming infected first. This suggested the virus was being concentrated by wind forcing in intake canals on particles or planktonic vectors before entering the index ponds.[18]

No sooner had destruction on all the prawn farms been completed (using over 6.8 million litres of chlorine), delimiting surveillance undertaken by Biosecurity Queensland in March 2017 detected WSSV in wild-caught crustaceans in the northwestern parts of Moreton Bay, over 70 km to the north of the Logan River.[19] WSSV infections were not detected during the winter months, but follow-up results from surveys in March 2018 found that a variety of wild prawns (greasyback, brown tiger prawns, banana prawns) and crabs (mangrove crab *Thalamita* spp.) were infected with WSSV, with quantitative polymerase chain reaction (qPCR) cycle threshold (CT) values as low as 13.8 in some infected wild prawns. For comparison, the CT values obtained from *P. monodon* dying from WSD on the first infected farm on the Logan River in November 2016 ranged from 14 to 21.[18] There is an inverse relationship between viral load and CT values (i.e. a high amount of virus gives a low CT value). Therefore, the fact that some wild prawns in northern Moreton Bay had CT values as low as 13.8 strongly suggests that the WSSV disease incursion was causing mortalities in wild crustaceans in Moreton Bay. This was not necessarily unexpected, given that WSSV is a highly pathogenic disease agent that was introduced into a naïve population of crustaceans which had no natural resistance to the disease.

Because WSSV is a notifiable disease agent, there were significant trade and economic implications following its introduction into Australia. Prawn farmers on the Logan River lost around $20 million worth of production during the destruction phase, while the cost of the government eradication response and clean-up also exceeded $20 million.[20] Furthermore, helped by emergency funding from the Australian Government, the prawn farms chose to fallow for an additional year and did not stock any ponds in the 2017–2018 growing season. Instead, they used that time and money to

15 Biosecurity Australia 2009. *Generic import risk analysis report for prawns and prawn products*. Final report. Biosecurity Australia. Canberra: Australia, 7 October 2009. www.agriculture.gov.au/biosecurity/risk-analysis/animal/prawns/final_generic_import_risk_analysis_ira_report_for_prawns_and_prawn_products

16 Scott-Orr H, Jones JB, Bhatia N 2017. Uncooked prawn imports: effectiveness of biosecurity controls. Australian Government Inspector-General of Biosecurity Review report No. 2017–18/01. www.igb.gov.au/uncooked-prawn-imports-effectiveness-biosecurity-controls

17 Biosecurity Act 2014 (Qld). The Biosecurity (white spot syndrome virus) Amendment Regulation 2017. cabinet.qld.gov.au/documents/2017/May/WhiteSpotReg/Attachments/Reg.pdf

18 Diggles BK 2017. Field observations and assessment of the response to an outbreak of white spot disease (WSD) in black tiger prawns (*Penaeus monodon*) farmed on the Logan River in November 2016. FRDC Project Number 2016-064. February 2017. http://www.frdc.com.au/project/2016-064

19 Oakey J, Smith C, Underwood D, Afsharnasab M, Alday Sanz V, Dhar A et al. 2019. Global distribution of white spot syndrome virus genotypes determined using a novel genotyping assay. *Archives of Virology*; 164: 2061–2082.

20 Scott-Orr H, Jones JB, Bhatia N 2017. Uncooked prawn imports: effectiveness of biosecurity controls. Australian Government Inspector-General of Biosecurity Review report No. 2017–18/01. www.igb.gov.au/uncooked-prawn-imports-effectiveness-biosecurity-controls

reinforce on-farm biosecurity arrangements, which included installation of crab fencing and water treatment equipment (Figure 13).

The Logan River prawn farms restarted limited production in September 2018, and after successful completion of the 2018–2019 season, production capacity this year was rebuilt to around 60% of that before the WSD outbreak. Unfortunately, outbreaks of WSD were again reported on some Logan River prawn farms in April 2020 at the very end of the 2019–2020 growing season, suggesting that the disease may be an ongoing problem for the region.

Major economic impacts were also experienced by the commercial fishing industry in Moreton Bay, due to the movement controls placed on at-risk products taken from the WSSV biosecurity control zone between Caloundra and the NSW border. The losses of the commercial wild fisheries for baitworms, yabbies, crabs, and prawns were estimated at $20.5 million in the first six months alone.[20] Furthermore, the fisheries in Moreton Bay were Australia's largest suppliers of commercially gathered bait prawns. Disruption of the bait prawn supply continues to affect recreational fisheries nationwide due to reduced availability and increased cost of domestic bait prawns. This has led to a perverse economic incentive for recreational fishers to use more imported prawns from supermarkets as bait. As long as the WSSV biosecurity zone remains in place, the ongoing economic impact on the commercial fishery in Moreton Bay will exceed that experienced by the aquaculture industry. This is because aquaculturists can improve biosecurity (given enough financial investment) to try to prevent the virus from entering their farm but commercial fishers cannot do this. Ironically, even though WSSV did not kill all of their wild catch outright, commercial fishers in Moreton Bay found that domestic biosecurity arrangements meant they could not sell their products into their usual markets. This was equivalent to having all of the animals 'dying economically' from the virus anyway, as they were no longer saleable.

Figure 13 Drum filters operating at a prawn farm on the Logan River in February 2020. Photo: Ben Diggles.

Because of the high risk of spreading WSSV via domestic bait prawns from Moreton Bay, state governments throughout Australia demonstrated that Australia's domestic acceptable level of protection (ALOP) for prawn products originating from regions where WSSV occurs is one requiring sanitary measures equivalent to cooking or exposing risky products to high levels of gamma irradiation (50 kilogray [kGy]). This relatively high domestic ALOP contrasted markedly with the ALOP applied at the international border for uncooked prawn products, signalling an urgent need to thoroughly review the 2009 risk analysis for prawn products imported into Australia.

The increased surveillance of crustaceans in Moreton Bay after the WSSV incursion revealed other interesting findings. A freshly dead mud crab (*S. serrata*) exhibiting multiple white spots

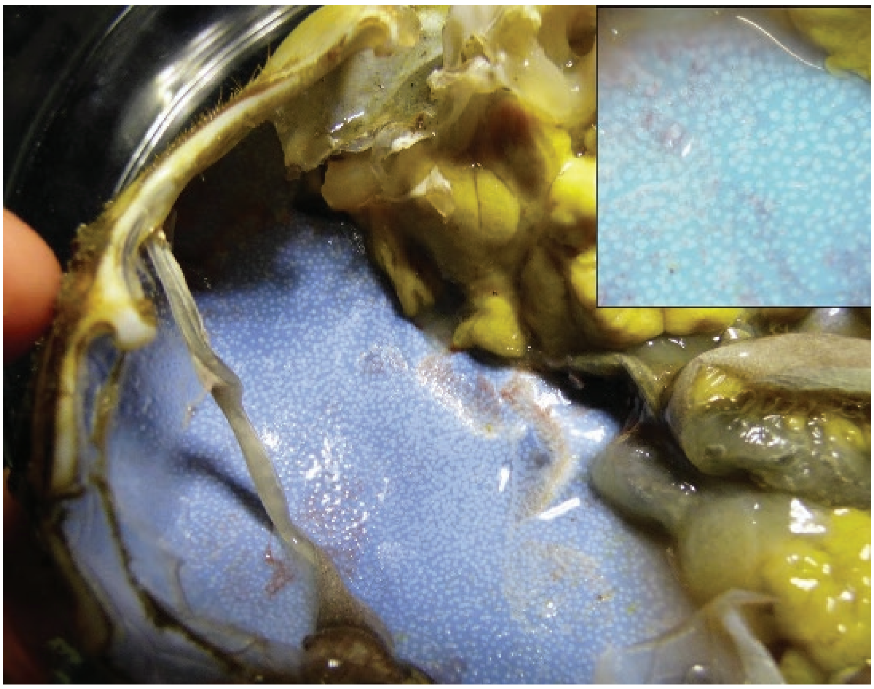

Figure 14 Grossly visible white spots (0.5–1 mm in diameter) due to mineral mobilisation under the carapace of a freshly dead mud crab. This crab tested negative for WSSV. Photo: Ben Diggles.

under the carapace was found in Pumicestone Passage, in northern Moreton Bay (Figure 14). Grossly visible white spots had been previously reported inside the carapace of moribund mud crabs with WSD in India; however, the crab from Pumicestone Passage was negative for WSSV when gill samples were tested using qPCR. Instead, histology found evidence the spots were due to mineral mobilisation within the carapace during the pre-moult (D1 or D2) stage of the moult cycle. These observations confirmed that formation of white spots under the carapace of mud crabs are not pathognomonic for infection with WSSV, suggesting that similar observations in previous studies from India where WSSV was detected in this host may have been incidental findings. Jelly prawns (*Acetes sibogae australis*) from Pumicestone Passage exhibiting opacity of the hepatopancreas were also negative for WSSV using qPCR, but histopathology revealed infection by a novel haplosporidian parasite which destroyed most of the epithelial cells of the hepatopancreas (Figures 15a–15b). This was the first report of a haplosporidian disease in wild sergestid shrimp, and the parasite may play a role in regulating the population dynamics of its host. These discoveries confirm our incomplete understanding of the disease status of wild crustaceans in Australia, and reinforce the importance of biosecurity on prawn farms, the need to develop pathogen-free broodstock and the ongoing need for passive and active disease surveillance.

While the exact route of entry of WSSV remains unclear,[21] evidence of recreational fishing activity using imported, WSSV-positive prawns as bait within northern Moreton Bay, the Logan River and even near the intake canals of the affected prawn farms themselves, have again highlighted the dangers of using uncooked imported prawns as bait. Unfortunately, recent surveys in Queensland have shown there is still a significant percentage of recreational anglers (27%) who specifically purchase raw supermarket prawns for bait.[22] The survey researchers observed that the key drivers of purchase (convenience and price) are challenging for government to control, hence the vital importance of getting the risk analysis for imported prawn products right the second time around.

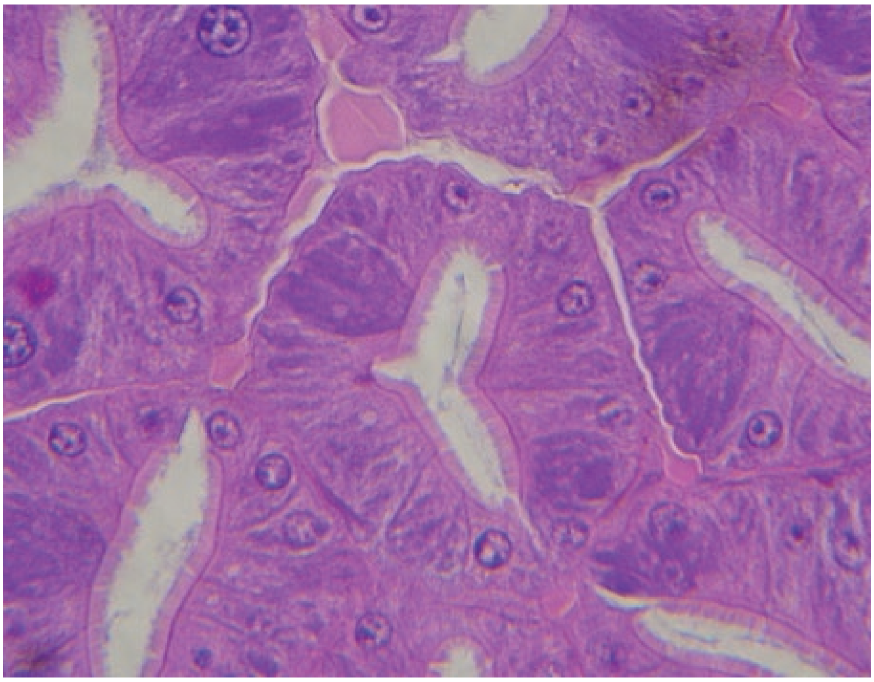

Figure 15a The tubule epithelium of a normal hepatopancreas in a jelly prawn from northern Moreton Bay.

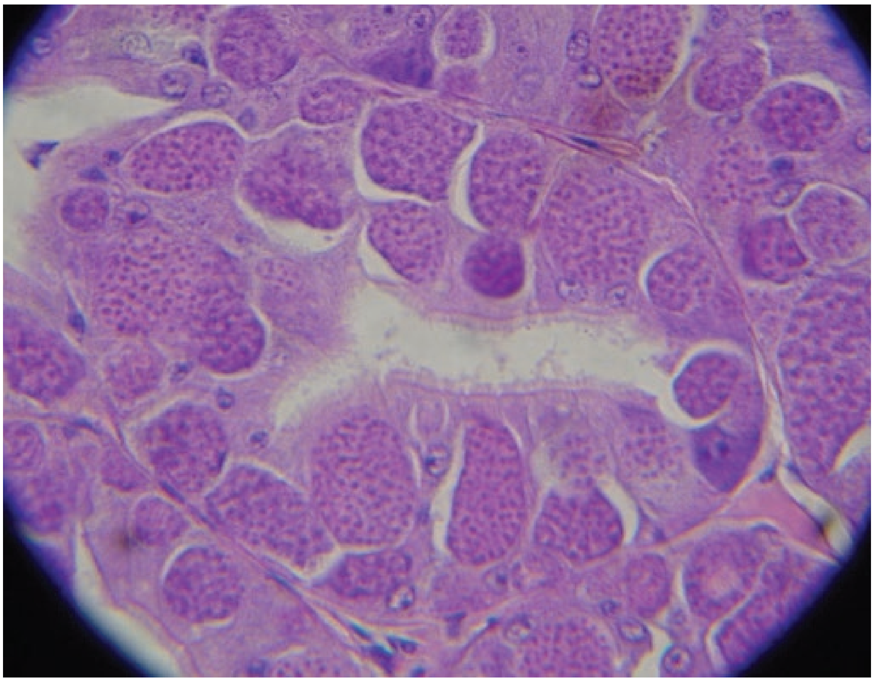

Figure 15b The tubule epithelium of the hepatopancreas of a jelly prawn from northern Moreton Bay heavily infected with a novel haplosporidian parasite. Photos: Ben Diggles.

21 Oakey J, Smith C, Underwood D, Afsharnasab M, Alday Sanz V, Dhar A et al. 2019. Global distribution of white spot syndrome virus genotypes determined using a novel genotyping assay. *Archives of Virology*; 164: 2061–2082.

22 Kantar Public 2019. White spot disease market research. Report prepared for Biosecurity QLD, May 2019.