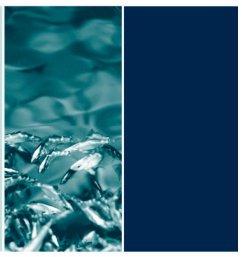
Future Fisheries
VETERINARY SERVICE Pty Ltd
ABN: 520 830 961 17
PO Box 7142 East Ballina NSW 2478
Phone **0437 492 863**
matty.landos@gmail.com



Exotic Disease Avoidance and Response Strategies for Farms Free from White Spot Syndrome Virus (WSSV)

Document prepared for the Australian Prawn Farmers Association

Background

WSSV is a disease which affects all species of farmed prawns and can infect all decapod crustaceans. It can progress very fast in a prawn pond leading to high mortality within 1-10 days of the first signs of sick prawns, or reduced feed rate. All stages of prawns are potentially susceptible to infection by the virus. The first reported outbreak of disease in a prawn farm was in late November 2016. It has since quickly spread/occurred at two closely adjoining farms. These farms are under Government Movement Control orders and are under-going disinfection in an effort to eradicate the disease and maintain Australia's freedom from this disease.

Currently there are no effective treatments or vaccinations available for this disease.

The responses to a WWSV outbreak detailed in this brief, are based on the assumption that eradication is the overall aim, to re-instate Australia's freedom from WSSV.

Should in the future WSSV become endemic across Australia then different farm management strategies will need to be developed.

How can WSSV virus get into a prawn farm pond

1) Through pumping in viable virus from the water source
2) Through stocking PL's which are sub-clinically infected from the hatchery
3) Through infected crustaceans being dropped into ponds by birds
4) Through infected crustaceans crawling into ponds, or be pumped into ponds
5) Through infected feed sources
6) Through people/clothing/vehicles entering with viable virus on them
7) Through other, vectors including rotifers, marine molluscs, polychaete worms and non-decapodal crustaceans including *Artemia salina*, the copepods, non-crustacean arthropods and insect larvae.

Principles of disease control

1) The earlier an infection is detected, the better the likelihood of successful eradication (so high levels of surveillance for early signs of loss of appetite, elevated mortality are warranted and should trigger immediate further laboratory investigation)
2) The risk of escapement of virus from an infected pond is related to:
    a. The amount of virus present- as it amplifies, risk escalates
    b. The vectors available to move virus out of pond include:
        i. Infected crustaceans/carriers (eg crabs) crawl out to another water body

ii. Birds capture infected prawns and spread them to another water body
iii. Free virus in water discharged or pumped in, or in aerosol from paddlewheels
iv. Virus contaminated equipment (nets/boots/clothing)

c. Contact between a crustacean and viable virus. Highest risk of transfer is through eating infected tissue. Transfer via water alone is a lower risk.
d. Cooking prawns kills the virus in them.

What to do before the signs of any disease on farm

1) Maintain diagnostic fixatives for histopathology (Davidson's solution) and molecular testing (RNA later), dissection kit, compound light microscope with dark field capacity, Trypan blue Eosin stain
2) Consider erecting crab fence to control movement of crustaceans into and out of the farm.
3) Keep a stock of chemical to rapidly disinfect a pond – eg calcium hypochlorite to deliver >10ppm active chlorine residual. Chlorine test kits to measure achieved level.
4) Put in place a protocol to test PL's at source hatchery immediately pre-stocking using a highly sensitive PCR method. Only release PCR negative PL's to growout. Stressing PL's prior to test can improve test sensitivity
5) Ensure sufficient water holding capacity on farm, to be able to drop a couple of ponds for emergency harvest, without needing to discharge any water to the environment.
6) Engineer ability to be able to rapidly close off effluent channels
7) Engineer ponds to minimise seepage, such that water requirements for pumping can minimised should an outbreak occur in your farming area.
8) Source a harvest net capable of harvesting small sized prawns eg 10g.
9) Keep grass levels around ponds well mowed to reduce habitat for frogs/grasshoppers and other potential mechanical vectors that may crawl from pond to pond
10) Capacity for rapid cooking for stock salvage
11) Support APFA to develop Minor Use Permit through Australian Pesticides and Veterinary Medicines Authority for Trichlorfon for emergency eradication of stock in a pond.

Figure 1: Individual pond crab fence containment. Recommend use of UV stabilise HDPE sheet ~ 400-500mm high, buried into ~ 300-400mm trench, and staked.

What to do to contain the virus if suspicious signs occur

1) Immediately initiate disease investigation with sampling at least 5 sick (but not yet dead) prawns to confirm diagnosis at a NATA certified diagnostic laboratory. Ensure gill and pleopod samples into RNA later, and Davidson's fixed whole prawns are sent. Both are required for diagnosis. Contact your farm veterinarian for guidance on sampling.
2) Undertake on-farm diagnostics:
    a. **4.2.3.1. Wet mounts**

    Demonstration of hypertrophied nuclei in squash preparations of the gills and/or cuticular epithelium, which may be stained or unstained.

        i. *4.2.3.1.1. T-E staining*

        A. T-E staining solution may be prepared from Trypan blue 0.6%, Eosin Y 0.2%, NaCl 0.5%, phenol 0.5%, and glycerol 20% (Huang & Yu , 1995), and used as follows:

        B. Place a piece of lesion tissue (e.g. a piece of gill or stomach epithelium without the cuticle) on a slide and mince with a scalpel.

        C. Add 1–2 drops of the T-E staining solution to the minced tissue, mix and allow to stain for 3–5 minutes.

        D. Lay a cover glass over the stained tissue and cover with several pieces of absorbent paper. Use a thumb to squash the mince into a single layer of cells. If the sample was taken from a heavily infected shrimp, it should be easy to see the hypertrophied nuclei and intranuclear eosinophilic or vacuolation-like inclusion bodies under a 400-1,000 × light microscope.

    b. **4.2.3.2. Smears**

    Demonstration of aggregates of WSSV virions in unstained smear preparations of haemolymph by dark-field microscopy.

    NOTE: This is the simplest of the microscopic techniques and is recommended for people with limited expertise in WSSV. The aggregates appear as small reflective spots of 0.5 µm in diameter (Momoyama et al., 1995).
3) Advise Q DAF management (Tim Lucas) & APFA executive (Helen Jenkins)
4) If farm level diagnostics are highly suspicious then urgent disinfection of the pond should be considered with chlorine (ensuring permits in place) to achieve >10ppm for >30 minutes. If chlorine unavailable, then the use of alternative crusticides such as trichlorfon can be considered (ensuring permits in place) to halt the amplification of virus in the suspect pond.
5) Shut-down vectors from the suspect pond:
    a. Eliminate all bird interactions through scaring (ensure bird mitigation permits in place)
    b. Turn off influent flow to farm (as you may be pumping in the virus)
    c. Shut off effluent flow from suspect pond- to avoid discharging suspect virus
    d. Shut off effluent flow from farm to avoid discharge to environment
    e. Turn off aeration on suspect pond- to minimise aerosol from suspect pond
    f. Crab fence individual pond and ensure entire farm crab fence is intact- to minimise cross contamination between ponds, and escape to environment.
    g. Consider emergency harvest to remove suspect stock from the water to avoid risk of viral amplification

h. Minimise staff movements around suspect pond
i. Avoid any movement of equipment from the suspect pond (cast nets/ feed trays/ paddlewheels etc) to any other areas of the farm

6) Advise neighbouring prawn farms of suspect pond and risk of simultaneous outbreak or spreading outbreak

Images: Digsfish

Clinical signs: see white patches on carapace, slow swimming at surface and red colour