

Running Mortality Syndrome (RMS) in Farmed *Litopenaeus vannamei* Culture System

Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, is now the species of choice of shrimp farming industry in India. This is because of its inherent advantage viz., high tolerance for salinity, perennial high health, seed availability; low protein requirement and high yield in a short grow out period. Considering these advantages, commercial farming of *Litopenaeus vannamei* was permitted in India in the year 2009 by coastal Aquaculture Authority (CAA) and in order to prevent entry of exotic pathogens into the India aqua culture system, the imported specific pathogen free (SPC) brood stock from approved suppliers are screened for all OIE (World Organization for Animal Health) listed shrimp viruses at Rajiv Gandhi Centre for Aquaculture (RGCA) quarantine facility and then supplied to CAA registered hatcheries. Although, the imported bloodstock has been certified as specific pathogen free (SPC) by bloodstock supplier and ratified by Quarantine facility in Tamil Nadu that the bloodstock is Hi-health, infection by endemic viral pathogens like white spot syndrome virus (WSSV) and Infectious hypodermal hematopoietic necrosis virus (IHHNV) have increased as a result of continuous expansion and intensification of farming operations without implementing proper management practices.

However, so far there are no reported incidents of listed exotic viral infections of *L. vannamei* such as Infectious Myonecrosis virus (IMNV), Taura syndrome virus (TSV) and Yellow head virus (YHV). Since March 2011; a new syndrome has struck the industry. The syndrome was such that, the farmed shrimp in the affected ponds show different mortality patterns which are the result of unusual symptoms with no co-relation to any other reported diseases. Some farmers have lost upto four crops, with mortality percentage reaching 50-70% in the most cases. As a result of this several farmers have closed down their operations. This condition is called as running mortality Syndrome (RMS).

The symptoms are as follows:

- In early stages, *Litopenaeus vannamei* shows certain gross symptoms.

These include:

- Antennae are cut and the uropods turn red colour. Later on, it is found that the hepatopancreas begins to turn reddish yellow; finally entire body turns dark red in colour.
- Continuous internal mortality was noticed.

An article titled "Running Mortality Syndrome (RMS) in farmed reared *Litopenaeus vannamei* culture system". Dr S.A. Mastan Vali has discussed various aspects of Running Mortality Syndrome in *L. vannamei* culture system in Andhra Pradesh. Since March 2011, a new syndrome has struck the Aquaculture industry, particularly in *L. vannamei* culture system. The syndrome was such that, the farmed shrimp in the affected ponds show different mortality patterns which are the result of unusual symptoms with no co-relation to any other previously reported diseases. Some farmers have lost upto four crops, with mortality percentage reaching 50-70% in most of the cases. As a result of this several farmers have closed down their operations. This condition is known as running mortality Syndrome (RMS).

- The dead shrimp settle at the bottom of the pond and do not come to sides or surface.
- Mortality noticed only during inter-moult stage. Mortality rate is relatively more in low saline ponds.
- White or yellow fecal matter noticed in the gut.

Diagnosis

- 1) Microscopic observation of wet mounts of pleopod shows high external fouling with filamentous bacteria and protozoans.
- 2) The infected shrimp shows delayed hemolymph clotting time.
- 3) High mortality is however reported in shrimps associated with high *Vibrio* loads

Histopathological studies

In the early stages there are relatively more B-cells in the tubules of hepatopancreas. Later stages of disease show sloughing of hepatocytes and light hemocytic inflammation with no symptoms of known enteric viruses. Few cells are seen exhibiting karyomegaly. High B cells in the hepatopancreas in the early stage of disease indicate the over feeding of shrimp.

Management

In the early days, farmers used to manage this condition by regularly removing the dead shrimp from the pond or by reducing feed for few days were also found to reduce mortality. Some farmers opted for extensive culture and were able to reach the production up to 30 counts without RMS.

Dr S.A. Mastan Vali

M.Sc., M.Sc., Ph.D, F.S.E.Sc

(Aquatic Animal Health Management and Biosecurity Consultant)

Guntur-532 004, Mobile No: +19 9490398073



The culture practices like good pond preparation, selection of good quality seed, low stocking density, nursery pond management, maintaining good water quality parameters like optimum DO levels (above 4 ppm), pH (7.8-8.4), alkalinity (80-150 ppm), salinity (above 10 ppm), frequent use of good quality water and soil probiotics, partial harvest, strict feed management, use of organic acids in feed and polyculture which has reduced the incidence.

Treatment

There is no treatment methods are available at present. ■

Role of Monogenean Group of Parasites in Aquaculture Ponds and their Management

Introduction

Monogeneans (flukes) are a group of parasites best described as flatworms. Monogeneans are commonly found on the gills, skin or fins of fishes and lower aquatic invertebrates. A few may invade the rectal cavity, urethra, body cavity and even the blood vascular system. There are more than 100 families of monogeneans found on fishes in worldwide, in both fresh and salt water ecosystem and at a variety of temperatures.

Most monogeneans are browsers, moving about the body surface and feeding on dermal (skin) mucus and gill debris. Monogeneans have a series of hooks that enable them to attach while feeding. Most species are host- and site-specific, requiring only one host to complete an entire life cycle. In fact, some adult monogeneans will remain permanently attached to a single site on the host.

Morbidity and mortality epidemics in cultured fish caused by excessive parasite loads are associated with crowding, inadequate sanitation and deterioration of water quality. Although monogeneans are commonly found on wild fish, they are rarely a direct cause of disease or death in free-ranging populations.

Dactylogyrus is usually attached to the gills of freshwater fish. It reproduces by laying eggs, which are often resistant to chemical treatment, therefore weekly treatment over a period of 3-4 weeks is recommended. *Gyrodactylus* is usually found on the skin and fins of freshwater fish and produces live young, so one treatment may be adequate to control an infestation. *Benedeniella* is a large monogene that can cause chronic problems in marine systems and is difficult to eliminate from a system once established.

Clinical Signs and Effects

Freshwater fish infested with skin-inhabiting flukes become lethargic, swim near the surface, seek the sides of the pond and their appetite dwindles. They may be seen rubbing the bottom or sides of the holding facility (flashing). The skin,

where the flukes are attached, shows areas of scale loss and may ooze a pinkish serous fluid. Heavy gill infestations result in respiratory disease. Gills may be swollen and pale, respiration rate may be increased, and fish will be less tolerant of low oxygen conditions. "Piping," gulping air at the water surface, may be observed in fish with severe respiratory distress. Large numbers of monogeneans on either the skin or gills may result in significant damage and mortality. Secondary infection by bacteria and fungus is common on tissue that has been damaged by monogeneans.

In salt water fish, sharks, skates and rays the monogenean, *Neobenedinia* spp., may infest the skin and gills, resulting in extreme irritation to the host. Sharks with heavy infestations swim erratically, and exhibit behavior such as flashing and rubbing on the bottom of the tank. Gray patches and open wounds may appear on the skin. Ulcerated skin lesions are susceptible to secondary bacterial infections, which may result in mortality. Affected gills may become irritated, hemorrhaged and swollen. Sand grains may stick to the gills as infested sharks suck in sand in an attempt to rub off the parasites.

- Classification: Monopisthocotylean & Polypisthocotylean
- Polyonchoinea, Polyostomatoinea & Oligonchoinea
- Monopisthocotylean: Opisthaptor with anchors & hooklets, Gill, skin, fins ingesting epithelial cells, mucus. Move over the host surface Polyopisthocotylean
- Opisthaptor with clamps & suckers
- Generally gills
- Blood feeder less motile Both- preference for attachment to particular sites on the host

Abhay Kumar¹ Paras Nath Jha¹ Saurav Kumar²

¹Central Institute of Fisheries Technology,
Cochin, India. 682029

²Central Institute of Fisheries Education, Mumbai-400061

*Corresponding Author: Abhay.kumar.abhay@gmail.com

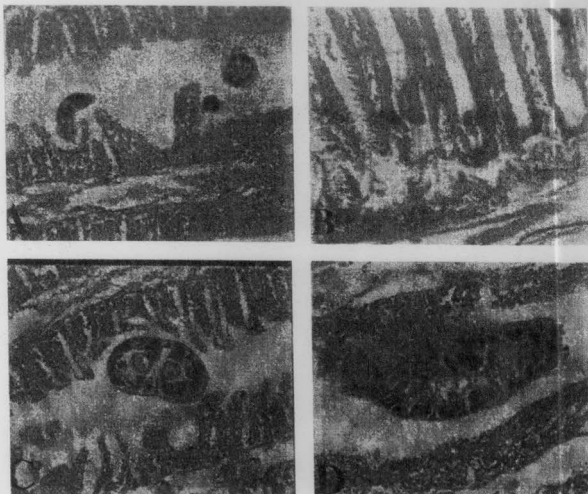


FIG-A-D: Histological section of gills showing monogenean infection

Transmission

Transmission of monogenean flukes from fish to fish is primarily by direct contact. Monogeneans tend to have direct life cycles, which mean that no intermediate host is required for the parasite to reproduce. Adults are hermaphroditic, which means that each organism has both male and female reproductive structures. Oviparous monogenes (i.e., Dactylogyridae) release eggs into the water column that hatch and mature prior to seeking a new host. Viviparous monogenes (i.e., Gyrodactylidae) release live larvae that are immediately able to attach to the same host as the parent or be carried by the water to another host. The direct life cycle can contribute to population explosions in aquaculture systems, resulting in clinical disease.

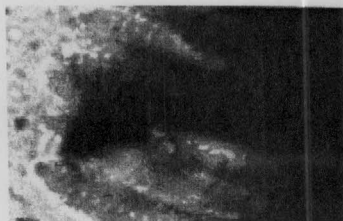


Fig 2 E:- Wet mount preparation of the gill showing monogenean infection

Host Range

Spiny Sturgeon, Atlantic Salmon, Japanese Anchovy, Japanese Flounder, Fresh Water fishes like –Common Carp, Grass Carp, Bighead Carp, Tench, Silver Carp, European Eel, Japanese Eel, Rainbow trout, brown trout, European Catfish, African Catfish, Guppy, Marine Water fishes like –Seabass, Grouper, Gillhead Seabream, Japanese Yellow Tail, Red Sea Bream, Sharks & Rays, Tilapia, Mullet.

Opisthaptor

The most important adhesion apparatus is opisthaptor which is located posteriorly. It is often equipped with characteristic sclerotized structures used for attachment to the host. The attachment sclerites can be formed as large hooks or anchors (hamuli). Some may be able to penetrate host tissue.

Tegument

Outer lining of the monogenean is a syncytial tegument composed of an outer anucleated layer and a basal nucleated layer in the parenchyma. The tegument seems to be quite active and may serve. Osmoregulatory and Excretory function.

Muscular System

Muscles in monogeneans are generally arranged Below worm surface as inner layer of longitudinal & outer tranverse fibres

- **Alimentary canal-** Gastrodermis for absorption, Monopisthocotylean- epithelial feeder, Polyopisthocotylean - sanguinivorous
- **Excretory system-** Protonephridial system (flame cells, capillaries, ducts), osmoregulation.
- **Reproduction & life cycle-** Hermaphrodites Some oviparous-simple life cycle egg oncomiracidium (free swimming larvae) adult Free swimming phase<24h, Oviposition rate, embryonation, hatching time, free living phase, PL, adult life span-temp dependent.
- **Most gyrodactylids** – viviparous, Reproductive rate (temp dep) Offspring directly on host.

Host-Parasite Relationship

- **Egg hatching-** Both abiotic factors & host factors, Change of light intensity – egg hatching. In *E.solea* egg hatching stimulated by illumination. *D. sagittata* egg hatching is induced by darkness, Mucus – hatching stimuli, *E.soleae* from sole.
- **Host finding-** *G.salaris*, *G.derjivani* select the specific host – atlantic salmon, rainbow trout, involvement of lectin in trout skin & carbohydrate in *G.derjivani* to be part of communication., PH of mucus plays an important role for host identification (hirazawa et al., H.okamotoi sensitive to the pH of tiger puffer, Sugar/lectin association responsible for communication.
- **Pathogenicity-** Polyopisthocotylean- Direct blood feeding– results in anaemia ,Associated with lethargy, anorexia, dark skin, paleness of muscle , kidney, liver, Enzyme activity lowered, Mortality increased by VHS

Table 1. Some important species of the Monogenea

Species	Size (mm)	Host	Habitat
Monopisthocotylea			
<i>Gyrodactylus elegans</i>	0.9 × 0.2	Carp	Gills, fins
<i>Paragyrodactylus iliensis</i>	0.4–0.08	Spotted stone loach	Gills
<i>Dactylogyrus vastator</i>	1.3 × 0.3	Carp	Gills
<i>Falciunguis parabranchis</i>	0.6 × 0.16	Bream	Gills
<i>Ancyrocephalus paradoxus</i>	4.0–0.8	Perch	Gills
<i>Alcopenferon nephriticum</i>	0.9 × 0.15	Gray loach	Ureter
<i>Nitzschia sturionis</i>	20 × 5	Sturgeon	Gills, oral cavity
<i>Calicocotyle kroyeri</i>	3 × 2.5	Rays	Cloaca
<i>Entobdella soleae</i>	5 × 2.2	Common sole	Skin
<i>Capsala martinieri</i>	22 × 23	Ocean sunfish	Gills
<i>Pseudodactylus anguillae</i>	0.6–1.1	Eels	Gills
Polyopisthocotylea			
<i>Diplozoon paradoxum</i>	7 × 1.8	Bream	Gills
<i>Polystomum^a integerrimum</i>	10 × 2	Frogs	Urinary bladder
<i>Oculotrema hippopotami</i>	12 × 2	Hippopotamuses	Eyes
<i>Rajonchocotyle prenanti</i>	8 × 0.4	Rays	Gills
<i>Dididaphora merlangi</i>	9 × 3	Whiting	Gills
<i>Axine belones</i>	6 × 1.3	Garfish	Gills
<i>Discocotyle sagittata</i>	7 × 2	Trout	Gills
<i>Mazocraes allosae</i>	11 × 2.2	Herrings	Gills
<i>Kuhnia scombri</i>	2.8 × 0.6	Mackerel	Gills
<i>Hexastoma lintoni</i>	9 × 3.2	Tunny	Gills

^a Some authors prefer *Polystoma*.

- **Monopisthocotylean-** Epithelial feeder (size & no), Hyperplasia, Gills- club shaped filaments, Mortality – hypoxia.

Mechanical effects- Hamuli, marginal hooklets, suckers and clamps cause mechanical damage, Each worm – 16 minute holes in epithelial cells at each attachment site. Probably- devastating impact in osmoregulation. Effect of Gland secretions – the enzymes such as proteases, phosphatases and other in the pharynx, oesophagus and intestine caeca, associated with digestion may affect host epithelia and provoke reactions.

Management

The best way to manage monogeneans is to avoid introducing parasites to a new system. This can be done by following a quarantine protocol whenever feasible. If quarantine is not possible, a simple way to minimize the introduction of monogeneans, as well as other external parasites, is to dip fish in fresh or salt water, depending on the fish species. Salt water fish can be dipped in freshwater which will eliminate many single-celled external parasites and freshwater fish can be dipped in sea water to accomplish the same goal. This practice will not completely eliminate the risk of introducing parasites to an established tank or system, but will help minimize the numbers brought in.

Ideally, fish should be quarantined for at least three weeks prior to being placed into a new system. While in quarantine, gill and skin biopsies can be performed to determine whether monogeneans or other gill or skin pathogens are present. Any parasites identified using biopsy techniques can then be specifically treated and eliminated. If biopsies cannot be done, prophylactic treatment with a broad spectrum parasiticide, such as formalin or potassium permanganate, should be carried out. A quarantine system should be very simple so that fish are readily accessible for observation and handling, water can be easily changed, and treatments readily administered.

Treatment of monogeneans is usually not satisfactory unless the primary cause of increased fluke populations is found and alleviated. The treatment of choice for freshwater fishes is formalin, administered as a prolonged bath at 25 mg/L or a short-term bath at 150–250 mg/L for 30 minutes. Sick fish do not tolerate formalin well and all fish should be carefully watched during chemical administration. If adverse reaction is observed, fish should be removed from the treatment tank at once and placed in clean water.

Potassium permanganate is also effective against monogeneans, and is the treatment of choice if columnaris bacteria or fungi are invading damaged tissues. Potassium permanganate can be administered as a prolonged bath at a concentration of 2 mg/L or as a short-term bath (30 minutes) at a concentration of 10 mg/L. Again, fish must be observed carefully while they are in contact with the chemical and they should be removed at once if adverse effects.

Conclusion

Monogeneans are found on fresh and salt water fishes throughout the world. They have a direct life cycle and can reproduce in a wide range of temperatures. Monogeneans

have a series of hooks that attach to the fish causing irritation, excessive mucus production, and which create an open window for bacterial invasion. A few flukes on a healthy mature fish are not usually significant; however, moderate numbers on a young fish can cause significant mortalities. When fish are crowded or sanitation practices are poor, the potential damage from monogeneans is greater. Prevention of monogenean infestations by following appropriate quarantine practices is preferable to treating the parasites after they have become established in a system. Formalin and potassium permanganate baths are effective for controlling monogenean infestations in freshwater fish, and copper is often used to control infestations of marine fish.

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NIHAL TRADERS PVT LTD

3-3-66, Flat no. 103, Sikhara Heights, Besides Manjira Hotel, Chappal Bazar, Hyderabad - 27 (A.P)
Ph: 040-24656968, 24746534, 24650253
Tele Fax: 040-24658097; Mobile: 9848040025
Email : nihaltraders@yahoo.com; www.nihaltraders.com