



# International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2021; 9(1): 385-391

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[www.fisheriesjournal.com](http://www.fisheriesjournal.com)

Received: 10-11-2020

Accepted: 12-12-2020

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## Managing infectious myonecrosis virus (IMNV) in *Vannamei* shrimp culture: Learning by doing

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DOI: <https://doi.org/10.22271/fish.2021.v9.i1e.2424>

### Abstract

Infectious Myonecrosis Virus (IMNV) was first reported in Indonesia in 2006. By 2009, it spread all over the significant shrimp farming areas in Indonesia. There was a significant drop in shrimp production due to IMNV outbreak. Several efforts were made to understand the nature, mode of disease progression, and minimize the virus load. The IMNV progression was divided into four stages. Chemicals, especially of chlorine origin, showed protection against IMNV. Tilapia came up with an effective biological remedy against IMNV. The blend of essential oils, pondguard showed significant protection against IMNV in the laboratory and farms.

**Keywords:** infectious myonecrosis virus (IMNV), shrimp production, mixed farming, *Vannamei* cum-tilapia culture, NOBF

### Introduction

Infectious Myonecrosis Virus (IMNV) was first emerged and reported in 2002-3 in Brazil. It was first reported in 2006 in Indonesia in East Java Islands slowly spread over Indonesia [1, 2, 3, 4]. It brought devastating downfall in Indonesia's shrimp production as *Vannamei* id cultured in at least 17 provinces, which are, East Java, Central Java, West Java, Yogyakarta, Banten, Bali, West Nusa Tenggara, Lampung, South Sumatra, Riau, Bengkulu, West Sumatra, North Sumatra, South Sulawesi, South Kalimantan, East Kalimantan, and West Kalimantan) [5, 6]. Farmers recognized the typical clinical or gross sign with white muscle initially and red coloured finally [1, 6], which was quite similar to the infected shrimp in Brazil [7, 8, 9, 10, 11, 12].

IMN Virus is a non-enveloped, icosahedral virion of 40 nm in diameter and a genome of dsRNA comprising 7560 nucleotides, containing two ORFs. The ORF 1 encodes a putative RNA-binding protein and a capsid protein and ORF 2 codes for putative RNA-dependent RNA Polymerase (RdRp) [1, 2, 3, 10]. IMNV was classified in Family Totiviridae, which was a similar group with Protozoa and Fungal virus. Natural hosts *Penaeus vannamei* [1, 2, 3, 10].

The typical symptoms of IMNV, like reddening of the last two segments, mortality at a slow rate in normal conditions and heavy mortality in stressed conditions, were reported [5, 13, 14, 15, 16]. It was a new pathogen for the Asian region with little information. The characteristics and behaviour of IMNV were studied.

The changes in cultural practices, including higher biosecurity in hatchery and farm by disinfection of water using chlorine or other effective agents, helped reduce the free-IMNV load in the environment's incoming water. The reduction in stocking density of Postlarvae, and other efforts to reduce the stress in the culture environment and shrimp were performed. Several commercially available chemicals or disinfectants, culture water and parameters, and shrimp species were tested against IMNV. Natural products were successfully tested and tried to minimize the risk of IMNV in culture ponds. The shrimp production brings bought to a sustainable level. This article will discuss the characteristics of IMNV, and efforts made to reduce the incidence of IMNV for sustainable shrimp farming.

### Characteristics of Infectious Myonecrosis Virus (IMNV)

The Infectious Myonecrosis Virus (IMNV) have some peculiar and specific characteristics. These specific characteristics and behaviours made this double-stranded RNA virus one of the most dangerous shrimp industry viruses. It is a stress-dependent virus.

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It became lethal when there is a sudden change in critical water quality parameters, like, pH, temperature, plankton crash, dissolved oxygen. The typical white and red colouration in the muscle first appears in the second and third segments and later on fifth and sixth segments, with the most active metabolism rate. The requirement of oxygen is the most in these tissues and so the stress. The IMNV infection causes less oxygen supply, and finally, the tissue dies and turned in white and finally to red (Figure 1).



**Fig 1a:** Live *L. vannamei* collected from Ponds showing typical red tail symptoms of IMNV



**Fig 1b:** Live *L. vannamei* in a Pond showing typical white muscle symptoms of IMNV.



**Fig 2.a:** Progression of IMNV in shrimp, grade 1 stage.



**Fig 2.b:** Progression of IMNV in shrimp, grade 2 stage.



**Fig 2.c:** Progression of IMNV in shrimp as Grade 3.



**Fig 2.d:** Progression of IMNV in shrimp as Grade 4.

Figure 2 (a-d) describes the progression of disease in terms of typical visual clinical and gross signs of IMNV infection. The figure 2a, describing grade 1, which is indicated with a minor, light whitish to pink colouration, opacity or grey discoloration in focal areas, expanded slightly along with the abdominal segments' muscle from the underparts to the upper parts. The behaviour would be expected, feeding regular and no mortality at this stage. Grade 2 of infection is shown in figure 2b. During this phase of the disease, the opacity increases in size, reaching other areas of the abdominal regions and becoming more whitish than the colouration

found in the initial phase. Here, the muscular necrosis extends more into the abdominal area. Behaviour and feeding are normal. The focal whitish opacities are more evident and concentrated mainly on the upperparts and sides of the abdominal segments, including the base of pleopods at grade 3 stage (figure 2c). The extensive white colouration can appear in the cephalothorax. The behaviour would start becoming abnormal, less feeding (score one or above) and still no mortality at this stage. Nested PCR check will be light to medium and rarely severe. The grade 4, is characterized by complete necrosis of the abdominal striated muscles of shrimp. A diffuse milky white opacity can be observed in the entire abdominal area. A reddish-pink necrosis colouration was evident on the tail fan and in the last segment. The behaviour will be abnormal and easily seen floating on the surface near to the dike, feeding reduced, and mortality starts at this stage (figure 2 d). Some particular parts/segments of the body become opaque/milky/reddish – because these abdominal regions have the highest metabolic activities and most obviously in oxygen-deficient conditions and during hyperactive stress.

The second typical symptom, enlargement of lymphoid organs are described in figure 3.



**Fig 3:** Enlarged lymphoid organ is the second clinical symptoms to observe for IMNV.

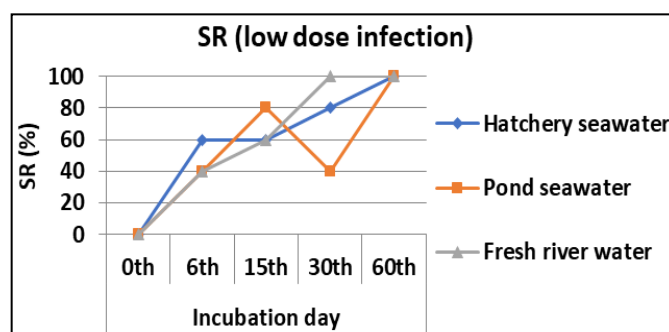


**Fig 4:** IMNV infected shrimp in the pond. The last segment is detached whereas the gut is full which indicates that shrimp doesn't stop eating and growing in IMNV infection.

The stress caused by mass moulting and the lunar cycle, full moon and new moon was also the farmers' challenges. Unlike, WSSV infection, shrimp does not stop eating during IMNV infection. An image of shrimp taken from severely infected IMNV ponds is the best example to demonstrate. The last two shrimp segments turned white and detached from the body, but the gut is full of food (Figure 4).

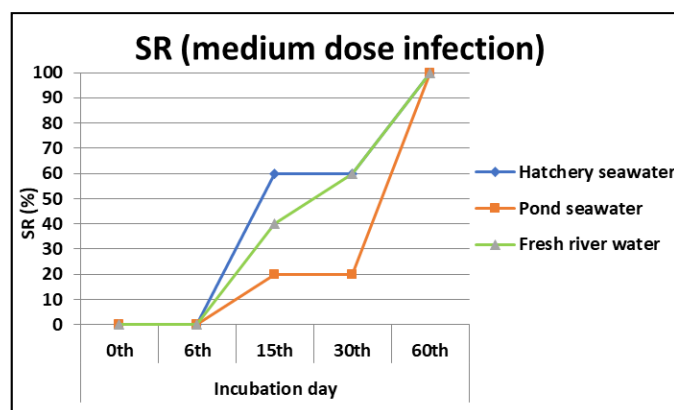
### Impact of water salinity IMNV

IMNV can stay active and viable in its free form in saline water (> 20 ppt) for over 60 days. It can stay alive and viable in freshwater (salinity < 5 ppt) for less than two weeks. A lab trial was conducted to determine the efficacy of free-IMNV by mixing at different salinity water (32 ppt) from a hatchery reservoir, saline water (17 ppt) from a culture pond reservoir and water (0 ppt) from the river. Free-IMNV was procured from the Disease Research Centre, Central Proteina Prima and diluted in the collected waters in two doses, log 3 (low dose) and log 5 (medium dose). The IMNV-enriched mixture was stored at room temperature. The samples were collected from the tubes at a different time interval, day zero (0), day 6, day 15, day 30 and day 60 of storage for challenge test and PCR test.



**Fig 5:** Survival rate of shrimp infected with low dose (log 3) of free-IMNV in different water source.

The lethality of low dose and high doses of IMNV were tested on shrimp of 1.5 g with a density five pieces a density of five pieces per 5 litre of water in glass aquaria. The shrimp were divided into two groups, one group to challenge with a low dose and another with a medium dose of IMNV.



**Fig 6:** Survival rate of shrimp infected with medium dose (log 5) of free-IMNV in different water source.

The chart in figure 5 showed that lethality of low dose (log 3) of free-IMNV on shrimp after different days of incubation. The survival of shrimp in IMNV stored at 32 ppt (hatchery water) was 0% on day 0, 60% on day six and day 15, 80% on day 30 and 100% on day 60 incubation. The survival of shrimp in IMNV stored at 17 ppt (pond water) was 0% on day 0, 40% on day 6, 80% on day 15, 40% on day 30 and 100% on day 60 incubation. The survival of shrimp in IMNV stored at 0 ppt (river water) was 0% on day 0, 40% on day 6, 60% on day 15, 80% on day 30 and 100% on day 60 incubation. The chart in figure 6, showed that lethality of low dose (log 5) of free-IMNV on shrimp after different days of incubation. The



survival of shrimp in IMNV stored at 32 ppt (hatchery water) was 0% on day 0, 0% on day 6, 60% on day 15 and 30, and 100% on day 60 incubation. The survival of shrimp in IMNV stored at 17 ppt (pond water) was 0% on day 0, 0% on day 6, 20% on day 15 and 100% on day 30 and day 60 of incubation. The infection rate was almost the same from 17 ppt to 32 ppt. The highest rate of IMN Virus deactivation was recorded in river water at zero ppt.

The characteristics of the free form of IMNV in salinity water made it possible to stay and become part of the culture environment. It can travel miles to contaminate the culture area. Farmers in countries like Vietnam, China, and some parts of Thailand culture shrimp at lower salinity (<10 ppt), potentially making the virus not established in those countries.

### Impact of water turbidity on IMNV

The turbid water contains globules or small particles. The virus can stick on the particles which are more than 5-micron using anionic forces. The higher turbid water which would contain a high number of particles behave like a vector for the virus. These particles can carry the virus in the water body to the culture water.

The CP ponds started the strategies like sedimentation of water, slow water flow in the long canal, and application of chemicals that reduced water turbidity. It helped to reduce the number of free-IMNV in the water.

### Potential carriers of IMNV

Several known living species, which are potential carriers of WSSV, like crab, artemia, and wild shrimp, were discovered as IMNV carrier. The other wild species tested were bird, clam, mussel, snail, water insects, copepods, rotifers, fungus, algae, protozoan.

The bird could be a vector and not the carrier. An experiment was conducted by collected faeces of birds which were fed on IMNV infected tissue. Those faeces were negative to IMNV by PCR and did not infect the shrimp *in vivo* trials.

### IMNV infected shrimp carcass as potential carrier

IMNV is heat sensitive. It deactivates when heated at or above 80°C. The shrimp carcass, including tail part, detected potential carrier of IMNV. It is highly recommended to use the IMNV infected shrimp carcass heated at or above 80°C before using for any food purposes.

### Efficacy of chemicals and disinfectant against IMNV

Several commercially available chemicals and disinfectants were tested against IMNV. The commercially available chemicals with chlorine origin, like, Sodium hypochlorite (30 ppm), Kaporit (20 ppm), TCCA (30 ppm), Hi-Clon, Calcium hypochlorite (20 ppm) and Chloramine T (40 ppm) showed anti-IMNV properties.

The non-chlorine chemicals, like, Biocid (not effective up to 400 ppm) BKC (not effective up to 200 ppm), Potassium permanganate (not effective up to 10 ppm), Iodine or Povidone not effective up to 200 ppm.

### Efficacy of Tilapia on IMNV

Tilapia were tested to reduce the load of free IMNV in culture water. It was observed that IMNV deactivation was faster in the presence of tilapia compared to without tilapia. The presence of Toll-Like Receptor (TLR-3) on the outer skin of tilapia might be the factor to recognize IMNV as an enemy. The role of mucus present on the skin of tilapia cannot be ignored [16]. The stocking ratio of tilapia to shrimp was optimized. The different methods and stocking density of tilapia were optimized and established based on the infrastructure and threat of IMNV, as described in table 2. The polyculture of vannamei shrimp with tilapia was successfully practised in the CPP farms (Figure 7a and 7b).



**Fig 7a:** Polyculture Vannamei and Tilapia culture to reduce the load of free-IMNV in water.



**Fig 7b:** Polyculture Vannamei and Tilapia culture to reduce the load of free-IMNV in water to achieve successful crop.

The polyculture of vannamei with different fish species, like tilapia, milkfish, and pomfret, is expected in China <sup>[16]</sup>.

Time Interval of PCR Check after inoculation in virus (days)	Fish density/tank (pcs)					
	1	2	3	4	5	6
Day 0	S	S	S	S	S	S
	S	S	S	S	S	S
	S	S	S	S	S	S
Control (+)	S	S	S	S	S	S
Day 3	M	S	S	S	S	S
	S	M	S	S	S	M
	S	S	S	S	S	S
control (+)	S	S	S	S	S	S
Day 6	S	S	S	S	S	S
	S	S	S	S	L	S
	S	S	S	S	S	S
control (+)	S	S	S	S	S	S
Day 9	S	S	M	S	L	S
	L	S	L	S	S	M
	S	S	S	S	M	M
control (+)	S	S	S	S	S	S
Day 15	S	M	M	L	L	L
	M	M	M	M	L	L
	S	M	M	L	L	L
control (+)	M	M	S	S	M	M
Day 30	M	M	(-)	(-)	(-)	(-)
	M	(-)	L	(-)	(-)	(-)
	M	M	(-)	(-)	(-)	(-)
control (+)	M	M	M	S	M	M

**Fig 8:** The nested PCR data showed that IMNV start deactivating in presence of tilapia.

The trial result explained in figure 8 showed that free-IMNV degenerated faster in the presence of tilapia. The nested PCR data showed that IMNV starts deactivating in the presence of tilapia. The severity reduces with time progression, i.e., day nine onwards and becomes negative at day 30. In contrast, control, free-IMNV without tilapia showed strong positive until day 30 in the experiment. Keeping and rearing tilapia in the culture reservoir and ponds will reduce the load of free-IMNV from the environment.

#### Effect of acidic and basic medium

The viability of IMNV was tested in highly acidic and basic medium. It deactivates in an acidic medium at pH 1.5 but did not deactivate at basic medium up to pH 12.

#### Alternative culture species than *Penaeus vannamei* – *Penaeus monodon* and *Penaeus stylirostris* resistant to IMNV

Efficacy and hardiness of *Penaeus monodon* and *Penaeus stylirostris* were tested against IMNV and cultured in the IMNV contaminated water. The most encouraging results obtained were from Monodon. Monodon did not get diseased, and no mortality occurred due to IMNV infection. Monodon could carry the IMNV in its viable form, i.e., cohabitation of Monodon and vannamei, Monodon carrying IMNV could transmit the virus horizontally. The results obtained on Monodon are contradictory to Tang *et al.* <sup>[5]</sup>. A set up of trial was conducting using Monodon and vannamei. Vannamei was raised in the tank. After acclimation of vannamei, Monodon carrying IMNV put in a cage and kept in the tank. After a week, all the vannamei detected positive PCR to IMNV and started showing IMNV symptoms in stressed

conditions, whereas Monodon remained healthy.

*Penaeus stylirostris* could carry and get infected by IMNV. Comparatively, the rate of infection in Stylirostris was slower than in Vannamei. The interesting thing found in severity with Stylirostris was the size or age. The bigger size, 15-20 g were more resistant to IMNV than the smaller size (<5 g). The primary issue in farm trial came up with stylirostris was black gill disease which mainly caused by *Fusarium* species. *Fusarium* was isolated from the infected gills of *P. stylirostris* and challenged using log 3 spore per ml to both *P. stylirostris* and *P. vannamei*. It was found that Stylirostris developed the black gill symptoms in 7 days of challenge, whereas Vannamei did not show any symptoms.



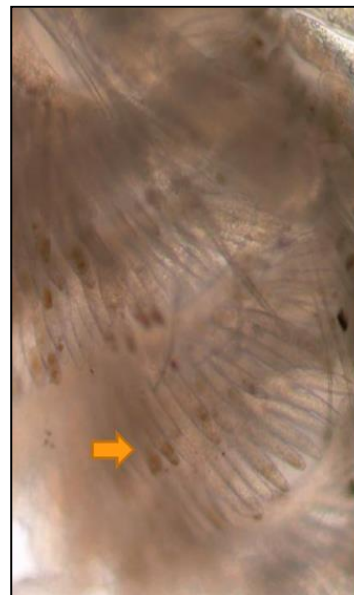
**Fig 9a:** *Fusarium* species under 100 X compound microscope. It was isolated from *P. stylirostris* with black gill symptoms.



**Fig 9b:** Vannamei challenged intramuscularly using log 3 of *Fusarium* species. No symptoms appeared after 7 days of challenge.



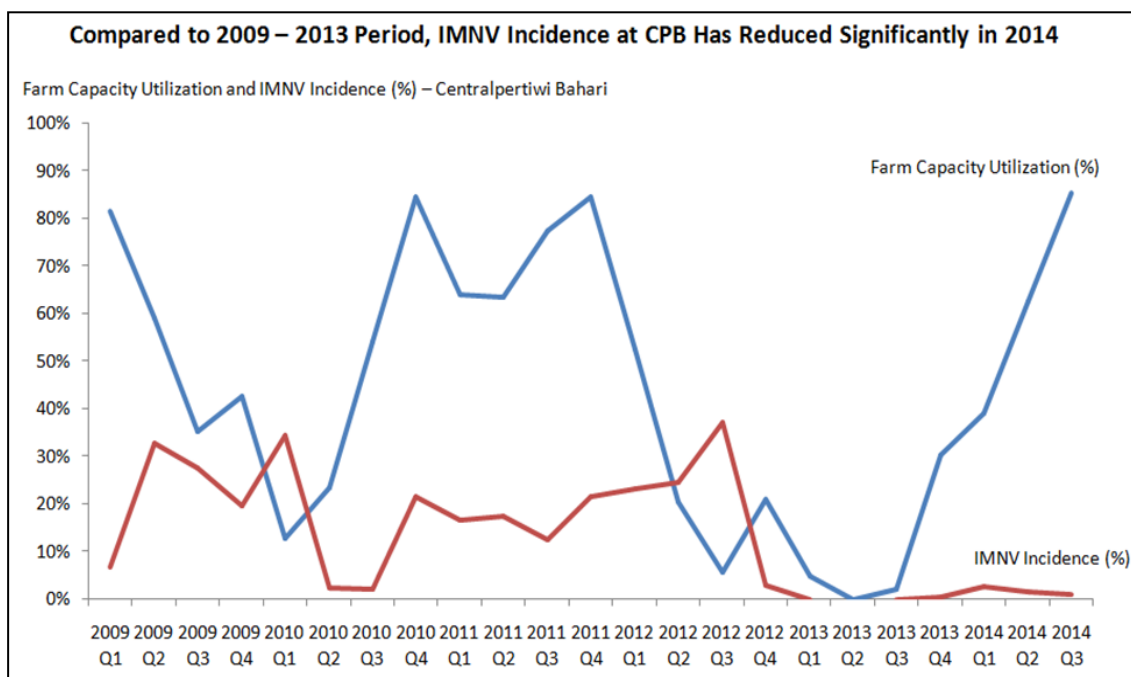
**Fig 9c:** Stylostris challenged intramuscularly using log 3 of *Fusarium* species. Black gill symptoms appeared after 3 days of challenge.



**Fig 9d:** *Fusarium* species infection in the gills of Stylostris.

#### Efficacy of Natural Oil Blend Formulations against IMNV

A blend of natural oils with anti-viral and immunomodulating properties was tried against IMNV successfully. The in-vivo level trials showed its efficacy against IMNV [17]. The formulation, commercially known as Pondguard, was applied in one of CPP's biggest farms and showed a significant drop in IMNV cases [18, 19]. As described in figure 10, the IMNV incidence and cases significantly reduced from 30% to less than 1% after applying pondguard in the Central Pertiwi Bahari ponds. Simultaneously, the number and percentage of culture ponds increased (Figure 10).



**Fig 10:** The figure showed decrease in the IMNV incidence and simultaneous increase in percentage of culture ponds after application of natural herbal supplement, Pondguard.

#### Conclusion

Several improvements and modifications were required to bring the culture environment virus free of virus or prevent the virus entry. Since IMNV entered Indonesia, lots of efforts have been made to understand the virus's nature and

behaviour. Efforts were made to find ways to minimize the load of the virus and to deactivate the virus. Efforts were also made to find the best species for culture. Based on outcomes, the clean intake water, rearing tilapia in culture water to deactivate the free-IMNV, and deactivate the virus using



chemicals or natural supplements. Discipline is the key to success. Maintaining biosecurity of hatchery and farm with zero tolerance is the essential factor.

### Acknowledgements

The authors are thankful for PT. Central Proteina Prima, Indonesia, PT. Central Pertiwi Bahari and PT. Rhea Natural Sciences, Indonesia for necessary funding for the research and assistance.

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