



**DETECTION OF WHITE SPOT SYNDROME VIRUS (WSSV)
IN VANNAME SHRIMP (*Litopenaeus vannamei*)
BASED ON SHRIMP LIFE STAGE**

Isnaeni Faizah¹, Rosidah², Sri Astuty³, Walim Lili²

¹Mahasiswa Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran

²Departemen Perikanan Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran

³Departemen Ilmu Kelautan Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran
Universitas Padjadjaran

Email: isnaenifaizah2@gmail.com

ABSTRACT

The White Spot Syndrome Virus (WSSV) has a high virulence, can attacks shrimp at post larval stage (PL) to *adult*, with mortality above 60% up to 100%. Shrimp resistance to pathogens can vary based on the life cycle of shrimp. Research has been conducted in April-July 2018, which aims to determine the presence of WSSV in Vanname shrimp (*Litopenaeus vannamei*) based on shrimp live stages or different age of shrimp. The vanname shrimp samples examined were vanname shrimp which were maintained at BPBAPLWS Pangandaran. Observation of the presence of WSSV using molecular analysis carried out at the virus laboratory of SKIPM Bandung, which included detection of WSSV virus in vanname shrimp on juvenile stage (PL 8-PL12), *sub adult* (14 days -30 days), and *adult* (> 30 <75 days). The number of shrimp samples was 15 individu for each stage, divided into 3 replications. Detection was carried out by the PCR method on the target organ of the swimming feet and gills. Water quality parameters measured including temperature, salinity, pH, dissolved oxygen (DO), and ammonia, The results of molecular analysis of vanname shrimp in juvenile, sub-*adult* and *adult*, show negative results. This shows that the vaname shrimp where is maintained at BPBAPLWS is not infected with the WSS virus. This healthy condition of vanname shrimp is due to the vanname shrimp cultivation technique that is it applies according to SNI 8037.1:2014 and CBIB for Vannamei shrimp and the quality of maintenance water in the *hatchery* and brackish water ponds, still meets water quality quality standards for vanname shrimp cultivation based on PERMEN KP No 75 Tahun 2016 SNI no 8037.1:2014, and SNI 7311:2009.

Keyword : *Live stage* ,*PCR*, *Shrimp*, *Vaname*, *WSSV*

INTRODUCTION

Pangandaran coastal area, West Java Province, is a mainstay area for the marine tourism sector and capture fisheries is also a place for developing potential fish and shrimp cultivation. The tourism and fisheries sector contributes greatly to the regional economy and the people in the Pangandaran region. Many local and foreign tourists visit Pangandaran beach to enjoy beach tourism, and enjoy various processed seafood products, in addition to fish, namely shrimp, squid, crab and shellfish.

Marine products are very popular with tourists, especially shrimp, both tiger shrimp and vanname shrimp. Shrimp contain active compounds such as omega-3, minerals, fats, cytins, carotenoids and vitamins (Ngginak et al 2012). Therefore, in the Pangandaran region there are many shrimp farms. Shrimp is also a mainstay export commodity for Indonesia, contributing considerable foreign exchange. Indonesia as a world shrimp producing and exporting country Shrimp commodities are produced from capture fisheries and mainly from traditional and intensive pond cultivation.

The glory of Indonesia as a producer and exporter of world shrimp has decreased from 300,000 tons in 1995 to 150,000 tons in 1996 to 1999 due to attacks from diseases caused by Monodon Baculo Virus (MBV), White Spot Syndrome Virus (WSSV) and others (Dahuri 2013). At least 20 types of disease-causing viruses in shrimp farming have been reported (Zhang et al 2004).

Seeing the production of tiger shrimp which continues to decline due to disease attacks and decreasing water quality, finally on July 12, 2001 vanname shrimp was officially allowed into Indonesia through the Indonesian Minister of Maritime Affairs and Fisheries Decree. No. 41/2001. This vanname shrimp has more resistance to disease and has a high tolerance for poor water quality. However, vanname shrimp also apparently experienced WSSV attacks (Arafani 2016). It was reported that around 60% of the 410,000 traditional ponds of tiger shrimp were destroyed due to the import of pathogenic and undetectable shrimp (Aliah et al 2015). The prevalence of ponds affected by WSSV (White Spot Syndrome Virus) is reported to be quite high, even reaching 100%. The location of shrimp farms close to the coastline can be a cause of WSSV infection (Mohan et al 2008).

WSSV is a virus that has a double-stranded DNA (dsDNA) genome that is very dangerous and is known to infect shrimp and other crustaceans in East Asia in 1992. The virus has spread rapidly to shrimp in the pond area. Based on the genome structure and phylogenetic analysis, this virus is a member of the genus Whispovirus in the new virus family, Nimaviridae (Vlak et al 2002).

Coelho et al (2009) stated that viral infections cause mortality rates above 60% in shrimp and can attack post-larvae (PL), juvenile, and adult shrimp. Shrimp resistance to pathogens also varies based on the life cycle of shrimp (Manoppo et al 2011). Transmission of WSSV is very fast and causes 100% death within 3-10 days of clinical symptoms. This virus can infect shrimp in post larvae (PL) to size 40 g. The spread of WSSV can be vertically through the parent transmitting to its larvae and waterborne transmission horizontally, infected shrimp feces, cannibalism, natural / fresh food crustacean species and crustacean type of pond pests (Kono et al 2004).

Based on the high virulence of WSSV, it can cause shrimp mortality above 60% to 100%, and can infect shrimp in the post larval stage to adulthood, with a very short transmission time, it is necessary to monitor the presence of this virus during the maintenance of shrimp. For this reason, it is necessary to conduct a study on the detection of WSSV in vanname shrimp with different life stages, with the hope that the results of the study can be known that shrimp stages are susceptible to WSSV viruses. The shrimp samples used as test biota are vanname shrimp which are maintained at the Southern Region Brackish and Sea Aquaculture Development Center (BPBAPLWS), Pangandaran. This center produces shrimp seed (shrimp) which is distributed to farmers who are in the working area of the hall.

The purpose of this research is to determine the presence of the White Spot Syndrome Virus in Vaname shrimp (*Litopenaeus vannamei*) based on shrimp live stages or different age of shrimp.

METHOD

This research was conducted from April to September 2018 at the Virus Laboratory at the Fish Quarantine Station, Fisheries Product Quality and Safety Control (SKIPM), Bandung Class II, Shrimp sampling was carried out at the Southern Region of Brackish and Sea Aquaculture Development Center (BPBAPLWS) Pangandaran, West Java. Analysis of NH₃ levels in water samples taken from the location of vanname shrimp samples was carried out at the Bandung Regency Environmental Service Laboratory

The research method used is non-experimental with observation techniques in the laboratory. This research includes detection of WSSV virus in vanname shrimp, vanname shrimp samples examined were vanname shrimp which were maintained at BPBAPLWS Pangandaran, shrimp were taken at the post larva / seed stage (PL8-PL12), juvenile / sub adult (14-30 days), and adult (> 30 <75 days). The number of shrimp samples was 15 tails for each stage, divided into 3 (three) replications. Detection of WSSV is carried out by the PCR method on the target organ of the swimming pool and gills. Supporting data are parameters of water quality (maintenance media) in the sample pool including: temperature, salinity, ammonia, pH and dissolved oxygen (DO).

Sampling method

Shrimp sampling is carried out randomly from hatcheries for seed size shrimp and maintenance ponds or ponds for sub-adult and adult shrimp, which are in BPBAPLWS Pangandaran. Each stage is taken 15 shrimps, for 3 replications, meaning that each replication is 5 sample shrimp. In each shrimp sample the target organ is taken, namely the entire swimming leg and gills, then homogenized. All swimming legs and separated gills are put in a 90% alcohol solution. Each sample contains 5 shrimps and then is compiled into one sample which is then given the name and code according to the live stage of the shrimp.

Molecular analysis

Molecular analysis carried out in the form of Extraction, Amplification and Electrophoresis processes which are then visualized in agar media.

Data Analysis

PCR data obtained in the form of bands or DNA bands, a number of 9 bands, consisting of 3 stages of live vanname shrimp, each stage there are three PCR results. Each PCR result was compared descriptively quantitatively with WSSV DNA results detected (positive controls) and negative using conventional PCR methods that referred to IQ 2000 MultiVirTM. The discussion was conducted in a comparative descriptive manner between the three stages of live vanname shrimp (seeds, sub-adult and adult). The water quality parameters of each maintenance stage are compared with the PERMEN KP Standard Quality Standard No. 75 of 2016 concerning Water Quality for Vanname Shrimp Cultivation, SNI 8037.1-2014 and SNI SNI 7311: 2009.

RESULT AND DISCUSSION

Water Parameter Quality

When compared with water quality quality standards according to SNI 7311: 2009 for the production of scattered seed vanname shrimp, the water quality parameters for the pond maintenance of vaname shrimp seeds in BPBAPL WS Pangandaran, are still within the range of quality standards (Table 1), although there are several parameters slightly different, but still within tolerance. This is reinforced by the WWF statement (2014), that the dissolved oxygen value for vaname shrimp cultivation is optimal > 4 ppm, while the tolerance limit is > 3 ppm.

Table 1. Result of Water Parameter Quality in Hatchery

Parameter Kualitas Air	Tambak B	Tambak C	Permen KP No 75 th 2016	SNI 8037.1- 2014
Suhu (°C)	28	27	28-31.5	28-33
Salinitas (ppt)	24	28	10-35	30-34
pH	7,8	7,6	7,5-8,5	7,0-8,5
DO (ppm)	4,2	4,4	> 3,0	> 4,0
NH ₃	0,004	0,02	< 0,1	< 0,1

Tabel 2. Result of Water Parameter Quality in pond

Water Parameter	BPBALPWS Pangandaran	SNI 7311:2009
Suhu (°C)	30	29-32
Salinitas (ppt)	35	29-34
pH	7,3	7,5-7,8
DO (ppm)	4,0	5
NH ₃ (ppm)	0,01	< 0,1

The results of measurements of water quality parameters carried out in the shrimp pond maintenance sub stage (14 days-30 days) and adult stage shrimp (> 30 <75 days) are listed in Table 2.

In Table 2 it can be seen that the water quality parameter values obtained in the vanname shrimp enlargement ponds in the sub adult and adult stages when compared with the water quality standard for vanname shrimp enlargement according to Permen KP No. 75 of 2016 and SNI 8037.1-2014, are within the appropriate range for the maintenance of vanname shrimp at the enlargement stage. Water quality measurements are carried out during the sampling of vanname shrimp, which is in the morning at 08.00 p.m. at 8:30 in the dry season. When viewed from each parameter, both hatchery and vanname shrimp enlargement ponds.

Visually Observation of WSSV Virus in Shrimp

When viewed visually or in plain view, vanname shrimp are taken from hatcheries and enlargement ponds in BPBALPWS Pangandaran. included in the category of healthy shrimp. This can be seen from the morphology of the shrimp itself which is that there are no white spots on the carapace and also the color of the shrimp has not changed. When taking shrimp samples in farms and hatcheries, it shows the health of shrimp, seen from its active movements, response to good feed, and active swimming.

The characteristics of WSSV-affected shrimp according to Corteel (2013) are reddish color on the head and tail end. Clinical symptoms that arise in WSSV-infected shrimp include appetite decreases dramatically, weak, shrimp clustered at the waist, swimming with unstable conditions is not sensitive to stimulation. Meanwhile, if seen physically, the body is milky yellow, the redness of the abdomen and white spots on the carapace, loose cuticles, pale hepatopancreas and anorexia (Corteel 2013). The difference in appearance between healthy shrimp and shrimp infected with WSSV is shown in Figure 1.



(a) (b)
Figure 1. Healthy shrimp and shrimp infected with WSSV
(a). Health Shrimp (b) Shrimp who Infect WSSV

Molecular Analysis

The process of molecular analysis of vaname shrimp was carried out in several stages, namely DNA extraction, amplification and electrophoresis. The vaname shrimp used is vaname shrimp originating from a hatchery for PL 12 shrimp and shrimp from ponds for shrimp aged 14-30 days and > 30 days. The target organ used in this molecular analysis is the swimming leg and gills. After the amplification process is complete, the electrophoresis stage is then carried out. Positive control used is with the 914 band bp.

This can be seen by not matching the band band which shows 914 bp or according to positive control (+) on the 9 wells found in Figure 8. Based on the results of amplification and electrophoresis, that shrimp from all stages did not show WSSV virus infection, either from shrimp aged PL 12 (A1-A3), 14-30 days (B1-B3) and shrimp that are > 30 days old (C1-C3).

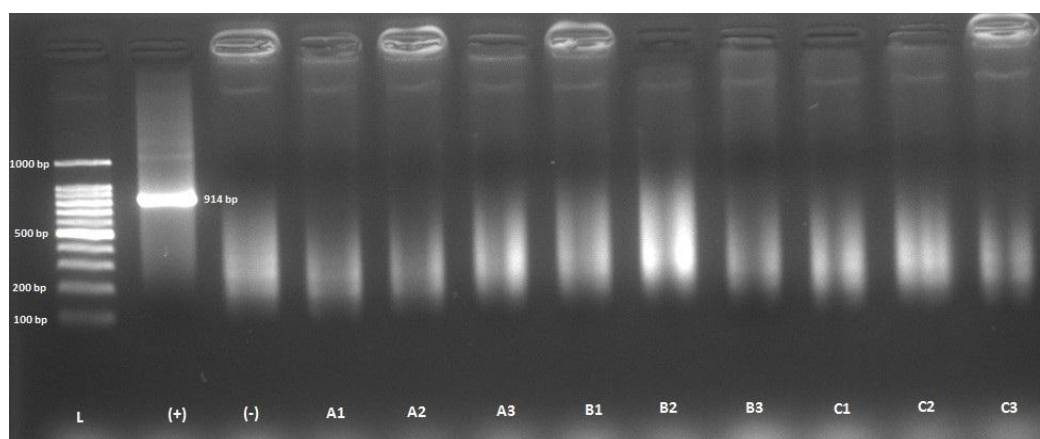


Figure 2. Reasult of Molecular Analysis

Information :

- L = Leader
- (+) = Kontrol positif
- (-) = Kontrol negative
- A1-A3 = Treatment (Udang umur PL 12)
- B1-B3 = Treatment (Udang umur 14-30 hari)
- C1-C3 = Treatment (Udang umur > 30 hari)

Factors that can influence the results of WSSV testing

Based on observations, the factors that are expected to influence the results of WSSV testing on vanname shrimp in this research include technical testing factors, shrimp maintenance environment conditions, shrimp conditions, and cultivation techniques

a. Technical testing factor

Technically, the success of testing the sample using the PCR method is influenced by several factors including, cross contamination, age of reagents or enzymes, number of enzymes, accuracy at amplification and extraction, and conditions of buffer solutions used.

Based on the technical factors that have been described, this is not an influential factor, because the tools used before testing are sterilized equipment, the age of the reagent or enzyme is not in an expired condition, the amount of enzyme used is in accordance with the dose in standard operating procedures. If the reagents used are reagents that have expired, the extraction process will not be maximized. If the equipment used is not sterile, it is feared that cross contamination will occur which will affect the results of the PCR.

b. Shrimp Environmental Conditions

Environment has a very large contribution to the condition of the biota. The polluted environment will cause the condition of the biota to decline so that it is susceptible to disease. In principle, diseases that attack shrimp farming do not come just like that, but through a process of relationships between three factors namely environmental conditions (water quality), host conditions (shrimp), and the presence of pathogenic bodies (diseases) (Kilawati and Maimunah 2015). One of the environmental factors that trigger the emergence of WSSV is the high organic matter that can cause phytoplankton blooms, which results in low dissolved oxygen levels, large daily pH fluctuations, low water temperatures, sudden rainfall and poor management. These environmental factors resulted in the body resistance of vannamei shrimp to decreased disease attacks, which then vanname shrimp themselves can experience sudden death (Soetomo 2000). This is reinforced by a statement (Kordi 2007) that unbalanced interactions between environment, host, shrimp and the bodies of these disease organisms cause stress on shrimp, so that the self-defense mechanisms they have become weak eventually become susceptible to disease.

The time for taking water samples and shrimp samples from hatcheries and ponds was carried out in August, including in the dry season, where the water quality was quite stable. Based on the results of measurements of water quality parameters both in hatcheries and ponds, the measured water quality was within the threshold according to PERMEN KP No. 75 Year 2016, SNI 8037.1: 2014 and SNI 7311: 2009 (Table 1 and Table 2). This stable water quality causes the shrimp maintained in the Pangandaran WSB BPLAPL to grow healthy, so the shrimp samples examined show negative results of WSSV.

Kokarkin (2004), states that the rainy season is the most proven factor influencing the explosion of disease in shrimp, which from the beginning has been infected with low-level WSSV (2-step PCR), especially those with high levels of infection (1step PCR). The rainy season will cause stress on aquatic biota due to very high fluctuations in salinity, temperature, oxygen and pH (Boyd 1989).

c. Condition of the host (shrimp)

Another factor is the condition of the host (shrimp), when sampling in the field showed that the morphology of the shrimp did not show a match with the clinical symptoms of WSSV-infected shrimp. Shrimp samples taken randomly from hatcheries and ponds showed the results that the shrimp moved akif and the response to the feed was good. The brood used in the spawning process is the brood whose quality is good and certified.

Larvae cultivated in BPBAPLWS Pangandaran are also larvae that have passed selection from 3 stages, namely the stages of Nauplius, zoea and mysis. Selection is done based on the absence of abnormalities in shrimp, their health, and their growth.

If the seed or shrimp in the larval stage that is attacked by WSSV, the usual thing to do is by seed destruction, and disinfection of tools and hatchery ponds. In ponds that were attacked by the WSSV virus, the steps taken were emergency harvesting. The general steps taken are as follows:

- If there is an illness in one pond, isolate the farm to do so that the disease from one pond does not spread to the other ponds.
- Use dosing with KMNO₄ by hanging it on the drain pipe at a dose of 10 ppm
- Give KMNO₄ approximately 5 ppm in the area around the sub road. after the harvest process is complete, give the disinfectant to the sub-outlet channel so that the seeds of the disease do not pollute the environment.
- Burn and bury the shrimp carcass so as not to cause disease.

d. Cultivation Technique

The cultivation techniques carried out in BPBAPL in Pangandaran WS are intensive systems seen from stocking densities, feeding and management, provision of probiotics, and use of water flow.

- Maintenance of larvae in the indoor hatchery; stocking larvae density in each maintenance tank is 1,000,000 naupli per 10 tons of water; feeding is done 12 times 1 day or every 2 hours. The diet given is 2 times to feed shrimp pellets and 1 time for natural feed. The feed given for each age level is different, in the PL stage the feed given is pellet with a gold coin trademark (protein content in feed is 50%, and 3% HUFA content). Artemia or natural feed given in the post larval phase 1-12 is 40-60 gr / 1,000,000 head naupli.

- Enlargement of shrimp:

Shrimp with the age of 14-30 days and > 30 days, the maintenance process is carried out in the pond. The stocking density used is 150,000 for 800m² pond area. The frequency of feeding is 5 times 1 day, with an interval of 4 hours 1 time. The feed used is a gold coin trademark with 42% protein content. According to SNI 8037.1-2014 artificial feed in vanname shrimp parent production is in the form of pellets with a minimum protein content of 36% and a frequency of 4 times - 6 times a day. This shows that the feeding procedure is in accordance with the provisions that have been issued by SNI. The following is a standard table for maintenance of vaname shrimp based on SNI No. 8037.1-2014

The feed given at the Pangandaran WS BPBAPL was first fermented using yeast. Feed fermentation using this yeast serves to improve the immune system in fish or shrimp. Healthy fish determines good fish growth and high success rates (Lumenta et al 2017).

To maintain water quality on farms and hatcheries every 2 days, probiotics are added to BPBAPLWS Pangandaran. The probiotic dose used in 1 hatchery tank is 10 ml / day, while for ponds it is a concentration of 2 ppm. This is in accordance with SNI No 8037.1-2014 which states that the addition of probiotics for the maintenance of vaname shrimp is carried out once every 3 days with the dosage according to the packaging and registered in the Ministry of Maritime Affairs and Fisheries.

Probiotics function to decompose food waste, organic compounds from dissolved feed, and fish feces. The rest of the metabolism and the rest of the feed can pollute the water and become a place to grow pathogenic bacteria or microbes, and fungi that cause fish to become susceptible to

disease. Probiotic properties of beneficial bacteria for fish can also suppress the growth of bad bacteria (pathogens). With probiotics, fish become healthier, can help the healing process of sick fish, and maintain water quality so that it is not cloudy or frothy (Diskanlaut Jogja 2015).

The results of research conducted by Yanti (2017), regarding the molecular identification of WSSV in shrimp originating from PT. Hasfam Inti Sentosa shows that there is no WSSV or negative (-) virus. The healthy condition of shrimp is supported by optimal overall water quality parameters for vannamee shrimp cultivation, except for ammonia and nitrite parameters, but ammonia parameters are still within tolerance limits for vannamee shrimp so that the pond water quality still does not cause WSSV-infected shrimp. While,

In the research conducted by Latrati (2017), the detection of WSSV using Polymerase Chain Reaction (PCR) and histopathological observations in vannamee shrimp from Pekalongan area, showed that three shrimp from eight ponds were positively infected with WSSV, with prevalence values of 37.5% . The results of nested PCR show a band 333 bp. Histopathological observations carried out on the gill and hepatopancreas organs of the vannamee shrimp, it appears that there are several inclusion and hypertrophy bodies in the nucleus. The existence of the WSSV virus in shrimp in three ponds in the study was caused by the condition of the pond environment. The design and layout of inlets and outlets that are not equipped with filters makes it easy for polychaeta worms, shrimp, crabs and wild fish to easily enter the pond. According to Chang et al. (1996), transmission of WSSV can go through infection horizontally, such as through water and exposure to dead shrimp, or carriers.

Horizontal transmission often occurs through the food chain with animal species crustaceans and other aquatic animals in the pond environment. All cultivated sea water shrimp are easily infected by White Spot Syndrome Virus (WSSV). Other aquatic and benthic organisms are carriers of the virus and include polychaeta worms (Desrina et al. 2013), microalgae (Liu et al 2007) and rotifer eggs (Yan et al 2004). Another potential source of transmission of the virus is human activity, birds and contamination of aquatic waste (Sanchez-Martinez et al 2007). Ponds that are in the BPBAPL in Pangandaran WS have been equipped with filters at both the inlet and outlet so as to minimize the entry of pests that can cause WSSV.

According to Afrianto and Liviawaty (2010), if a positive (+) shrimp farm is infected with WSSV, the farm must be closed for two cycles to repair or sterilize the tools used for shrimp farming. The success of shrimp farming is also determined by the quality of the parent and seed, early detection of disease and screening of broodstock and fries before stocking. Only healthy mothers are free of viruses that can produce fries that are of high quality and more resistant to disease.

Close supervision of these procedures (early detection, parent screening and fries before being stocked), as well as the prevention of the entry of animals that become "natural carriers" have been shown to reduce the rate of WSSV infections as successfully carried out in Japan. According to Sritunyalucksana et al. (2006), the most effective method of preventing losses due to disease in shrimp is to prevent the entry of pathogens into the cultivation system. At present, the method of early detection and parent screening, as well as the most widely used seeds are PCR (Lo et al. 1998).

This molecular based method is an option because it is very sensitive and specific for detecting viruses that cause disease in shrimp. The cause of WSSV is the SEMBV virus (Systemic Ectodermal and Mesodermal Baculo Virus). This virus is a virus made from DNA (Dioxyribonucleic Acid), rod-shaped (Baciliform). Organs infected with the virus are swimming feet, road legs, gills, stomach, abdominal muscles, gonads, intestines, carapace, heart, causing a complete infection.

Efforts made to tackle the problem of the disease have been carried out from screening formaldehyde to the use of antibiotics, which are carried out in hatchery activities to enlargement. One of the hatchery prevention procedures suggested by Wang et al. (1998) is the provision of

immunostimulants to increase the resistance or natural immunity of the seed against disease. Efforts to use excessive chemicals can have an impact on the aquatic environment, the health of consumers and cause pathogenic resistance (Alifuddin 1998). A natural ingredient that has a function to improve environmental conditions and as an immunostimulant for the shrimp body is highly desirable, so that there is no use of some types of drugs that are excessive and cause resistance to pathogens.

CONCLUSION

Observations on vanname shrimp samples taken from the BP Pangandaran BPBAPL, at three stages of shrimp life, namely seed stage (PL8 - PL12), sub adult (14 days - 30 days), and adult (> 30 <75 days) showed negative WSSV results. , means not infected with the WSSV virus or including healthy shrimp. It can be concluded that the condition of healthy vanname shrimp due to the BPBAPLWS Pangandaran vanname has implemented a good method of fish / shrimp cultivation (CBIB) and water quality management that meets hatchery quality standards (SNI 7311: 2009) and vanname shrimp enlargement (KP PERMEN No 75 of 2016 and SNI 8037.1: 2014).

ACKNOWLEDGMENT

I would like to express my deep gratitude to Dra Rosidah, M.Si, Dra. Sri Astuty, M.Sc, and Drs. Walim Lili, M.Si, my parents, my friends enthusiastic encouragement and useful critiques of this research work.

References

- Afarani L, M. Ghazali, dan M. Ali. 2016. Pelacakan Virus Bercak Putih pada Udang Vaname di Lombok dengan *Real Time Polymerase Chain Reaction*. *Jurnal Veteriner*. Vol 17 No. 1:88-95.
- Afrianto E. dan E. Liviawati. 2010. *Pembuatan Tambak Udang* , Kansinus : Jakarta
- Coelho, M.G.L., A.C.G. Silva, C.M.V. Vila Nova, J.M.O. Neto, A.C.N. Lima, R.G. Feijó, D.F. Apolinário, R. Maggioni dan T.C.V. Gesteira. 2009. *Susceptibility of the Wild Southern Brown Shrimp (Farfantepenaeus subtilis) to Infectious Hypodermal and Hematopoietic Necrosis (IHHN) and Infectious Myonecrosis (IMN)*. *Aquaculture*., 294: 1-4
- Corteel, M. 2013. *White Spot Syndrome Virus Infection in P. vannamei and M. rosenbergii: Experimental Studies on Susceptibility to Infection and Disease*. Dissertation, Ghent University, Belgium: 7-34.
- IQ2000TM. IQ2000 TM WSSV Instruction Manual. 2009. GeneResearch Biotechnology Corp. Taiwan. 19 hlm
- Kilawati, Yuni dan Y. Maimunah. 2015. Kualitas Lingkungan Tambak Intensif Litopenaeus vannamei Dalam Kaitannya Dengan Prevalensi Penyakit White Spot Syndrome Virus. *Research Journal of Life Science* Vol 02 No. 01
- Kokarkin, C.S. 2004. Mensiasati Penyakit WSSV di Tambak Udang. *Jurnal Aquatica Indonesiana*. 5 (1) : 19-31
- Kono, T. (2004) *Birth of parthenogenetic mice that can develop to adulthood*. *Nature*, 428, 860-864. doi:10.1038/nature02402

- Kordi, M.G.H. dan A.B.Tancung. 2007. *Pengelolaan Kualitas Air Dalam Budi Daya Perairan*. Rineka Cipta. Jakarta.
- Lo CF, Ho CH, Peng SE, Chen CH, Chiu YL, Chang CF, Hsu HC, Liu KF, Su MS, Wang CH, Kou GH. 1996. *White Spot Syndrome Baculovirus (WSBV) Detected in Cultured and Captured Shrimp, Crabs and Other Arthropods*. Dis Aquat Org 27: 215–225
- Manoppo, H. 2011. Peran Nukleotida Sebagai Immunostimulan Terhadap Respon Imun Nonspesifik Dan Resistensi Udang Vannamei (*Litopenaeus vannamei*). *Disertasi Pascasarjana*. IPB.
- Ngginak, J, H. Semangun, Jubhar C. Mangimbulude, Ferdy S. Rondonuwu. 2012. Komponen Senyawa Aktif pada Udang Serta Aplikasinya dalam Pangan. *Jurnal*. Universitas Kristen Satya Wacana.
- OIE. 2012. *Manual diagnostik test for aquatik animal. White Spot Disease. Chapter 2.2.6*: 179-190
- Peraturan Menteri Kelautan dan Perikanan Republik Indonesia Nomor 75 Tahun 2016 Tentang Pedoman Umum Pembesaran Udang Windu (*Penaeus monodon*) dan Udang Vaname (*Litopenaeus vannamei*).
- SNI 7311:2009. 2009. Produksi Benih Udang Vannamei (*Litopenaeus vannamei*) Kelas Benih Sebar.
- SNI 8037.1-2014. Udang vaname (*Litopenaeus vannamei*, Boone, 1931).
- Soetomo, M. H. A. 2000. *Teknik Budidaya Udang Windu*. Sinar Baru Algensindo. Bandung
- Sritunyalucksana, K., J. Srisala, K. McColl, L. Nielson dan T.W. Flegel. 2006. *Comparison of PCR testing methods for white spot syndrome virus (WSSV) infections in penaeid shrimp*. Aquaculture, 255: 95-104.
- Vlak, J. M., J.R. Bonami., T.W. Flegel., G.H. Kou., D.V. Lighner., C.F. Lo., P.C. Loh dan P.J. Walker. 2002. *A New Virus Family Infecting Aquatic Invertebrates. 12th International Congress of Virology*. Paris.
- WWF. 2014. *Budidaya Udang Vannamei*. WWF Indonesia. Jakarta