


Profiling of Microbial Community in Rearing Water of White Shrimp (*Litopenaeus vannamei*) Infected with White Feces Disease Syndrome

Karina Kristanti Ekarani Rahardjo¹, Woro Hastuti Satyantini¹  and Muhamad Amin^{1*} 

¹Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Mulyorejo, Surabaya, East Java 60115, Indonesia

*Correspondence :
muhamad.amin@fpk.unair.ac.id

Received : 2022-04-14
Accepted : 2023-01-09

Keywords :
Next-generation sequencing,
Vannamei, *Vibrio*, White feces
syndrome

Abstract

Shrimp farmers have reported mass mortality of white shrimp (*Litopenaeus vannamei*), reaching 2-3 kg/day in Gerongan Village, Kraton District, Pasuruan Regency in mid-2021. Preliminary analysis suggests that mass mortality was caused by bacterial diseases. Thus, to find out the main pathogen causing the mass mortality, the present study investigated microbial composition in rearing media of white shrimp (*L. vannamei*) infected by the white feces disease (WFD) and healthy white shrimp using next-generation sequencing (NGS) technology. The research was conducted by collecting normal water samples and infected shrimp pond water samples. The results of the NGS assay showed that *Vibrio vulnificus* was found dominant in WFD-infected shrimp pond water, therefore was suspected to be the main cause of WFD.

INTRODUCTION

White shrimp (*Litopenaeus vannamei*) is an economically important shrimp in Indonesia and become one of the leading fishery commodities (Juarno, 2012). The production of white shrimp continues to increase by 15.7% per year (2013–2017). It was recorded that in 2020, Indonesia's total shrimp exports reached 881,559.16 tons (KKP, 2022). Based on these data, Indonesia become the 5th largest shrimp exporting country in the world (Annisa *et al.*, 2022). Intensive farming systems are widely applied by shrimp farmers. However, the implementation of intensive farming which is not appropriate and wise can have negative impacts, such as the emergence of disease.

The disease can be caused by bacterial or viral infections (Arafani *et al.*, 2016).

White shrimp farmer in Gerongan Village, Kraton District, Pasuruan Regency in August 2021, reported mass mortality of white shrimp. Clinical symptoms began to appear when the shrimp entered the age of culture 22 days (DOC 22), with clinical signs including empty intestines, pale hepatopancreas, decrease in feed consumption, and delayed growth. At day 23 after post larvae (PL) stocking, a string of white feces floating on the water surface was accompanied by continuous shrimp mortality of approximately 2-3 kg per day. The survival rate value obtained is 30%.

Cite this document as Rahardjo, K.K.E., Satyantini, W.H. and Amin, M., 2023. Profiling of Microbial Community in Rearing Water of White Shrimp (*Litopenaeus vannamei*) Infected with White Feces Disease Syndrome. *Journal of Aquaculture and Fish Health*, 12(2), pp.216-225.

This article is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

Hamzah *et al.* (2019) stated that the common source of pathogens is aquaculture water. Therefore, this study aims to determine the main pathogens that cause mass mortality and also microbial composition in rearing media infected with certain diseases and healthy ones. This study observed clinical symptoms in infected shrimp, as well as the profile of infected pond water through the composition of the microbes in it using next-generation sequencing (NGS) technology. The advantages of analyzing variance using NGS are the sensitivity of single molecules and also being able to detect rare and low-frequency alleles even in very heterogeneous samples. The main principle of NGS is to identify all types of microbes that exist in a particular environment such as rearing media which are sources of pathogens (Nkili-Meyong *et al.*, 2016). This allows researchers to compare the microbial composition of infected rearing media and those that are healthy. The results of this comparison will be able to detect the main pathogens that cause disease (Gu *et al.*, 2019).

METHODOLOGY

Place and Time

The research was carried out for 6 (six) months starting from August 2021-February 2022. Water samples were obtained from intensive shrimp ponds belonging to the Raja Vaname Group which are located in Gerongan Village, Kraton District, Pasuruan Regency, East Java Province. The process of DNA extraction from rearing water samples was carried out aseptically at the Microbiology Laboratory of the Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga Surabaya. DNA concentration test was carried out at the Biomolecular Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The DNA sequencing process was carried out at Novogene AIT Genomics Singapore PTE LTD Singapore.

Research Materials

This study used several materials including QIAamp DNA Mini Kit, absolute ethanol, ATL Buffer, Proteinase K, AW1 Buffer, AW2 Buffer, AE Buffer, NanoDrop Spectrophotometer, Benchmark MC-12 microcentrifuge, Rocker 300 vacuum pump, 0.22 μm millipore membrane filter, and 0.45 μm millipore filter membrane.

Research Design

This research is structured survey research, with a sampling procedure using a purposive sampling method where the collection process is following the requirements needed in the research. The samples used in this study were rearing medium water from healthy intensive shrimp ponds and intensive ponds with WFD symptoms in Gerongan Village. The total samples used in this study were 2 samples. Parameters of the rearing medium's water bacterial composition were analyzed using next-generation sequencing.

Work Procedure

Sampling

Water samples were collected from Gerongan Village, Pasuruan Regency according to a protocol of Amin (2018) with some modifications. In brief, the samples were taken from two ponds (a healthy pond and a WFD-infected pond). Water samples in each pond were collected from 3 different sampling points (@60ml) and stored in 100 sterile bottles was previously filled with 90 ml of ethanol. The water samples were then transported within 8 hours to the Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga.

Bacterial DNA Extraction and Purification

Bacterial DNA from the water samples was extracted according to a protocol previously described by Amin *et al.* (2022). First, the water sample was filtered using a vacuum pump. The filtered

rearing medium water sample (pond water) was then extracted using the QIAamp DNA Mini Kit (Qiagen), according to the manufacturer's instructions. The concentration and quality of the extracted DNA were measured using a DNA-RNA protein Quantification Spectrophotometer (MN-913A MaestroNano Pro). Sterile microtubes containing extracted DNA were then stored at -20 °C until further use.

16S rRNA Gene Amplification

The 16S rRNA gene amplification step and microbial community analysis were carried out at the Novogene Biological Information Technology Co. (Singapore). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using one set of coded fusion reverse primer and the same forward primers. The 16S rRNA genes of the different 16S V3–V4 regions were amplified using a pair of specific primers (515F: CCTAYGGGRBG-CASCAG, and 806R: GGACTACNGGG-TATCTAAT) with barcodes. PCR amplification was performed according to the protocol of Amin *et al.* (2022). Then, the PCR products were adjusted to the same concentration and then sent to the Genome Sequencing Company (Novogene, China) for microbial analysis. Paired-end libraries were created with the NEBNext® Ultra™ DNA Library Preparation Kit for Illumina and quantified via Qubit and Q-

PCR and further analyzed by Illumina HiSeq.

Data Analysis

The bacterial sequencing results that were obtained were analyzed using the UPARSE software (Gao *et al.*, 2019). Sequences that have a similarity of $\geq 97\%$ are designated as the same OUT. The taxonomic classification of each OTU representative sequence was carried out using the MOTHUR program through the SILVA database with a confidence level of 97%. The approach used in this research is descriptive qualitative.

RESULTS AND DISCUSSION

When the rearing age entered 23 days after the cultured period, one of the rearing ponds was attacked by disease with accompanying symptoms such as growth retardation, pale hepatopancreas, decreased appetite, and shrimps swimming on the surface of the pond. An indication that the shrimp were infected by the disease, can also be seen from the continuous mortality, with the number of deaths as much as 2-3 kg per day. The survival value obtained in this farming activity is only 30%. Based on the clinical symptoms that appear, the incidence of this death is associated with the presence of WFD infection, which is related to the conditions of maintaining water quality.

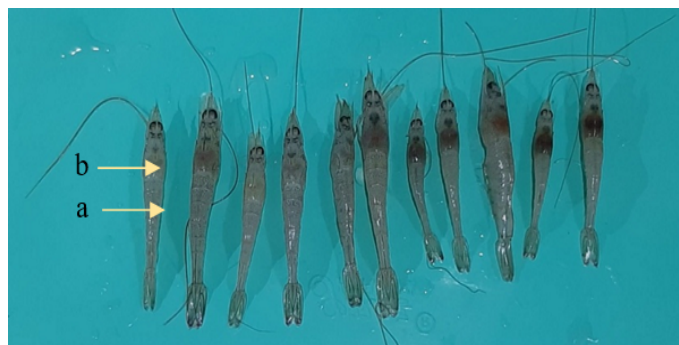


Figure 1. Intestine (a) and hepatopancreas of shrimp infected with WFD (b).



Figure 2. Normal shrimp intestine (a) and hepatopancreas (b).

WFD conditions also have an impact on the condition of the shrimp hepatopancreas, where the infected shrimp hepatopancreas has a pale color and is smaller in size than healthy shrimp. The hepatopancreas is the main organ for food absorption, transportation, and secretion of digestive enzymes as well as lipid, glycogen, and some mineral deposition. Shrimp infected with WFD get interference with hepatopancreatic epithelial tubular cells (hepatopancreatic tubular cell), so the main processes of absorption and digestion will be disrupted. Disorders of the hepatopancreas disrupt nutrient absorption and delayed shrimp growth (Sumini and Kusdarwati, 2020). This condition causes a decrease in the ADG value.

In addition to affecting the condition of the hepatopancreas, WFD symptoms can also be observed through the condition of the shrimp intestine. In healthy

shrimp, the intestine looks full and has a brown color. This indicates that the shrimp absorbs the nutrients well. While the intestines of infected shrimp appear empty and white. This condition indicates that there is damage to the shrimp intestine (Aldama-Cano *et al.*, 2018) and a lack of food intake because the shrimp didn't eat for a certain time.

Water Profile of Pond Water

Based on biometric analysis results, WFD-infected rearing media water samples were dominated by Bacteroidota (31.55%), followed by Cyanobacteria (22.73%) and Actinobacteria (4.24%). Meanwhile, the phylum that dominated the normal rearing water was Proteobacteria (97.991%), followed by Firmicutes (1.347%) and Cyanobacteria (0.451%) (Figure 3).

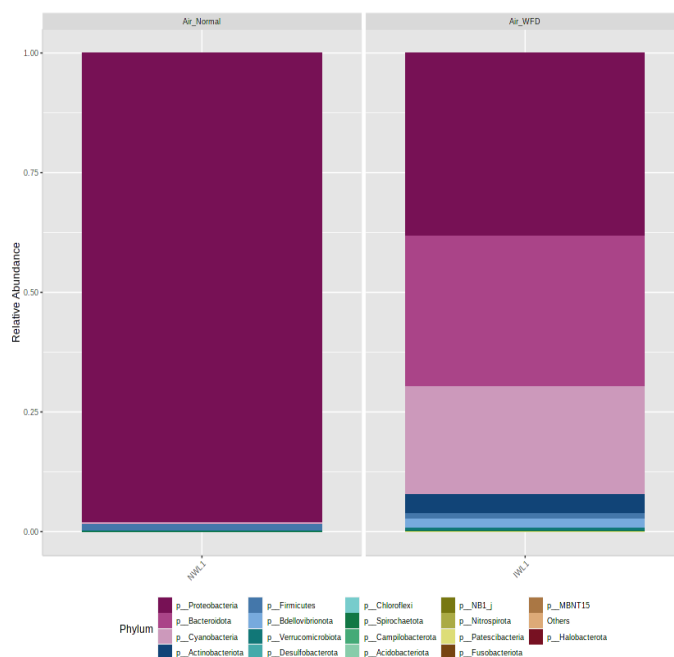


Figure 3. The composition of bacteria at the phylum level in white shrimp rearing media: (a) normal rearing media (healthy) and (b) WFD-infected rearing media.

The Bacteroidota phylum that dominates in WFD-infected rearing media water is the dominant member of marine heterotrophic bacterioplankton and is often found scattered in macroscopic organic matter particles. Bacteroidota is gram-negative bacteria and does not have spores. Bacteroidota tends to be a major contributor to the formation of propionate in the gut. In addition, Bacteroidota is also the main producer of vitamin B12 in the intestine (Zeng *et al.*, 2017).

Proteobacteria was the predominant phylum in normal water samples. The Proteobacteria phylum belongs to gram-negative bacteria, which consist of aerobic and facultative bacteria. The abundance of Proteobacteria phylum in shrimp gut is

quite high, i.e., by 40.83% (Fan *et al.*, 2019). As reported by Rungrasamee *et al.* (2014; 2016), Proteobacteria are found in more than 80% of shrimp intestines of white shrimp (*L. vannamei*) or Tiger shrimp (*Penaeus monodon*).

Bacterial composition at the species level (Figure 4), from the results of the biometric analysis in normal rearing media water, 2 types of predominant bacteria were found based on their relative abundance, namely *Acinetobacter schindleri* (20.35%) and followed by *Comamonas denitrificans* (13.67%). Meanwhile, based on the results of biometric analysis on WFD-infected media water samples, *Vibrio* group, especially *Vibrio vulnificus* was found to be the most dominant.

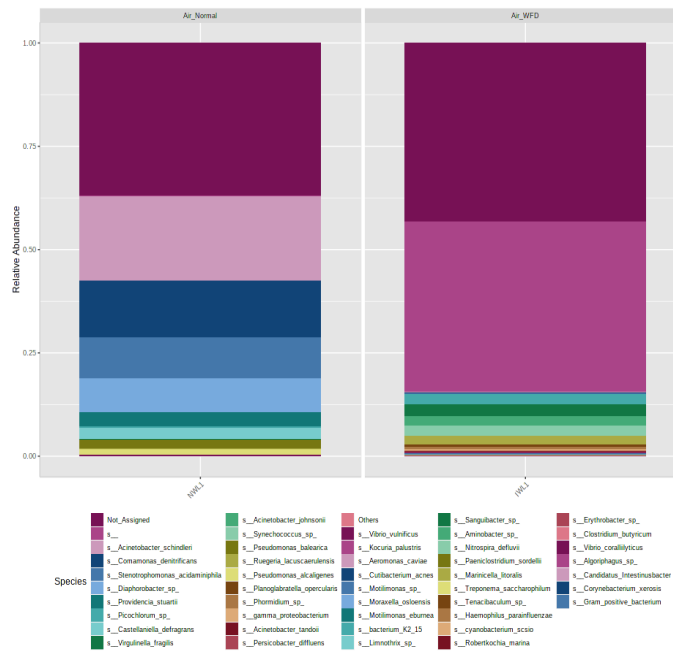


Figure 4. Survival rate of saline tilapia fry.

Species belonging to the genus *Acinetobacter* are widespread because they are often found in water, soil, and dry environments. The characteristics of *Acinetobacter* are gram-negative, rod-shaped, non-fermentative, and aerobic bacteria (Falagas *et al.*, 2006). Kozińska *et al.* (2014), stated that some *Acinetobacter sp.* identified as opportunistic bacterial pathogens for rainbow trout and common carp in Poland, including *A. baumannii*, *A. lwoffii*, *A. johnsonii*, and *A. calcoaceticus*. Other species such as *Acinetobacter schindleri* have been identified as involved in the red eye infection of *Pangasius* in India (Reddy and Mastan, 2013).

Vibrio bacteria are very commonly found in the shrimp intestine related to their habitat. Kent (2000) reported that *vibrio* bacteria are potentially coming up as opportunistic pathogens. *Vibrio* in general can reproduce well at warm temperatures, these conditions may support the transition of the bacterial behavior from commensal to pathogenic (Haenen *et al.*, 2014; El-Bouhy *et al.*, 2016; El-Sayed *et al.*, 2019). Another thing that encourages microflora to develop into opportunistic pathogens is that there is an increase in the amount of organic matter sourced

from feed and fecal inputs (Otta *et al.*, 2001). Pariakan and Rahim (2021) state that a salinity between 20-30 ppt affects the presence of *Vibrio sp.* bacteria. and becomes stronger at salinity >28 ppt. Several species of the *vibrio* genus that are often reported can cause vibriosis include *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. harveyi* and *V. vulnificus* (Chatterjee and Haldar, 2012).

Vibrio vulnificus is a gram-negative, halophilic, and mesophilic bacteria that naturally exist in estuarine and sea waters throughout the world (Drake *et al.*, 2007; Linkous and Oliver, 1999). Besides infecting shrimp, *Vibrio spp.* can also infect giant prawns (Mishra *et al.*, 2010), rainbow trout (Tanrikul, 2007), and also grouper fish and cause acute death within 17-46 hours (Nitimulyo *et al.*, 2005). The growth and existence of *V. vulnificus* in its natural habitat are strongly influenced by the salinity and temperature of the waters. The suitable salinity range for the growth of *V. vulnificus* is between 5-25 ppt and >20 °C for the temperature parameter, where these conditions are very favorable for its growth (Motes *et al.*, 1998). The GXFL1-3 strain of *V. vulnificus* was found to be path-

ogenic to the zoea and post-larvae of *Macrobrachium rosenbergii* (Li *et al.*, 2019). The LD₅₀ of this strain was 1.16×10⁶ CFU/ml for zoea and 1.45×10⁶ CFU/ml for post larvae of *M. rosenbergii*. *V. vulnificus* produces protease, amylase, urase, lecithin, and hemolysin activities which can support the strong virulence of this bacterium against *M. rosenbergii*. In *V. vulnificus* also found a TCP gene associated with virulence regulation (Li *et al.*, 2019).

In this study, the results showed that the *Vibrio* bacteria causing the disease in this case was mainly *V. vulnificus*. Rearing water which contains high organic matter makes the shrimp more susceptible to opportunistic pathogenic bacterial infections. Based on previous studies, white feces syndrome is caused by different pathogens, such as *V. parahaemolyticus* (Zhang *et al.*, 2021), *V. anguillarum*, *V. fluvialis*, *V. alginolyticus*, and *V. mimicus* (Supono *et al.*, 2019; Jayadi *et al.*, 2016). However, some state that WFD is caused by the *Enterocytozoan hepatopenei* parasite.

The existence and dominance of the *Vibrio* cause these bacteria are easy to penetrate the shrimp's immune system and reach the hepatopancreatic tubular cells and then interfere with the epithelial cells. This causes the tubular epithelial cells to suffer detachment, making the intestine and feces have white color (Sriurairatana *et al.*, 2014; Somboon *et al.*, 2012).

CONCLUSION

Clinical symptoms of infected shrimp in Gerongan village show similarities with clinical symptoms of white shrimp that are infected with WFD. The bacterial profile of pond water shows that the predominant bacteria in WFD-infected rearing media is *Bacteroidota*. Meanwhile, healthy shrimp pond water was dominated by *Proteobacteria*. At a lower taxa level, *Vibrio vulnificus* bacteria were found in WFD-infected rearing media water. However, to confirm the level of pathogenicity of these bacteria further tests are needed.

ACKNOWLEDGEMENT

The authors thank all parties who have aided in the completion of this research.

REFERENCES

- Aldama-Cano, D.J., Sanguanrut, P., Munkongwongsiri, N., Ibarra-Gómez, J.C., Itsathitphaisarn, O., Vanichviriyakit, R., Flegel, T.W., Sritunyalucksana, K. and Thitamadee, S., 2018. Bioassay for spore polar tube extrusion of shrimp *Enterocytozoon hepatopenaei* (EHP). *Aquaculture*, 490, pp.156–161. <https://doi.org/10.1016/j.aquaculture.2018.02.039>
- Amin, M., 2018. *Isolation and selection of probiotic candidates from gastrointestinal tracts of teleosts and molluscs*. Institute for Marine and Antarctic Studies, University of Tasmania.
- Amin, M., Kumala, R.R.C., Mukti, A.T., Lamid, M. and Nindarwi, D.D., 2022. Metagenomic profiles of core and signature bacteria in the guts of white shrimp, *Litopenaeus vannamei*, with different growth rates. *Aquaculture*, 550, 737849. <https://doi.org/10.1016/j.aquaculture.2021.737849>
- Annisa, K., Sutinah and Jusni, 2022. Vaname shrimp (*Litopenaeus vannamei*) export marketing strategy in South Sulawesi. *International Journal of Social Science Research and Review*, 5(1), pp.134-143. <https://doi.org/10.47814/ijssrr.v5i1.168>
- Arafani, L., Ghazali, M. and Ali, M., 2016. Detection of white spot syndrome virus in *Litopenaeus vannamei* in Lombok Island using real-time polymerase chain reaction. *Jurnal Veteriner*, 17(1), pp.88-95. <https://ojs.unud.ac.id/index.php/jvet/article/view/19171>
- Chatterjee, S. and Haldar, S., 2012. *Vibrio* related diseases in aquaculture and development of rapid and accurate identification methods. *Journal of*

- Marine Science: Research & Development*, pp.1-7.
- Drake, S.L., DePaola, A. and Jaykus, L.A., 2007. An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Comprehensive Reviews in Food Science and Food Safety*, 6(4), pp.120-144. <https://doi.org/10.1111/j.1541-4337.2007.00022.x>
- El-Bouhy, Z., El-Nobi, G., El-Murr, A.E. and El-Hakim, S.A., 2016. Study on vibriosis in *Mugil capito* in El-Dakahlia and Damitta Governorates, Egypt. *Abbassa International Journal for Aquaculture*, 9(1), pp.19-35. <https://abbassajournal.com/magazine/study-on-vibriosis-in-mugil-capito-in-el-dakahlia-and-damitta-governorates-egypt/>
- El-Sayed, M.E., Algammal, A.M., Abouel-Atta, M.E., Mabrok, M. and Emam, A.M., 2019. Pathogenicity, genetic typing, and antibiotic sensitivity of *Vibrio alginolyticus* isolated from *Oreochromis niloticus* and *Tilapia zillii*. *Revue de Medicine Veterinaire*, 170(4-6), pp.80-86. <https://www.rvmedvet.com>
- Falagas, M.E., Bliziotis, I.A. and Siempos, I.I., 2006. Attributable mortality of *Acinetobacter baumannii* infections in critically ill patients: a systematic review of matched cohort and case-control studies. *Critical Care*, 10, R48. <https://doi.org/10.1186/cc4869>
- Fan, J., Chen, L., Mai, G., Zhang, H., Yang, J., Deng, D. and Ma, Y., 2019. Dynamics of the gut microbiota in developmental stages of *Litopenaeus vannamei* reveal its association with body weight. *Scientific Reports*, 9, 734. <https://doi.org/10.1038/s41598-018-37042-3>
- Gao, S., Pan, L., Huang, F., Song, M., Tian, C. and Zhang, M., 2019. Metagenomic insights into the structure and function of intestinal microbiota of the farmed Pacific White Shrimp (*Litopenaeus vannamei*). *Aquaculture*, 499, pp.109-118. <https://doi.org/10.1016/j.aquaculture.2018.09.026>
- Gu, W., Miller, S. and Chiu, C.Y., 2019. Clinical metagenomic next-generation sequencing for pathogen detection. *Annual Review of Pathology: Mechanisms of Disease*, 14, pp.319-338. <https://doi.org/10.1146/annurev-pathmechdis-012418-012751>
- Haenen, O.L.M., van Zanten, E., Jansen, R., Roozenburg, I., Engelsma, M.Y., Dijkstra, A., Boers, S.A., Voorbergen-Laarman, M. and Moller, A.V.M., 2014. *Vibrio vulnificus* outbreaks in Dutch eel farms since 1996: strain diversity and impact. *Diseases of Aquatic Organisms*, 108, pp.201-209. <https://doi.org/10.3354/dao02703>
- Hamzah, Srinawati, Jumriadi, Afandi, A.A., Asa'at, M., Herawaty and Hartanto, N., 2019. Penilaian kesehatan udang melalui kaki renang. *Prosiding Simposium Nasional. Kelautan dan Perikanan*, 6. <http://journal.unhas.ac.id/index.php/proceeding-simnaskp/article/view/7727>
- Jayadi, M., Prajitno, A. and Maftuch, 2016. The identification of *Vibrio* spp. bacteria from *Litopenaeus vannamei* infected by white feces syndrome. *International Journal of ChemTech Research*, 9(7), pp.448-452. [https://sphinxsai.com/2016/ch_vol9_no7/2/\(448-452\)V9N7CT.pdf](https://sphinxsai.com/2016/ch_vol9_no7/2/(448-452)V9N7CT.pdf)
- Juarno, O., 2012. *Daya saing dan strategi peningkatan ekspor udang Indonesia di pasar internasional*. Dissertation, Institut Pertanian Bogor, Bogor.
- KKP : Kementerian Kelautan dan Perikanan, 2022. *Produksi perikanan budidaya udang*. Kementerian Perikanan dan Kelautan Republik Indonesia.
- Kent, M.L. 2000. Marine netpen farming and infections with some unusual parasites. *International Journal for*

- Parasitology*, 30(3), pp.321-26. [https://doi.org/10.1016/s0020-7519\(00\)00018-7](https://doi.org/10.1016/s0020-7519(00)00018-7)
- Kozińska, A., Paździor, E., Pekala, A. and Niemczuk, W., 2014. *Acinetobacter johnsonii* and *Acinetobacter lwoffii* - the emerging fish pathogens. *Journal of Veterinary Research*, 58(2), pp.193-199. <https://doi.org/10.2478/bvip-2014-0029>
- Li, X., Zhou, Y., Jiang, Q., Yang, H., Pi, D., Liu, X., Gao, X., Chen, N. and Zhang, X., 2019. Virulence properties of *Vibrio vulnificus* isolated from diseased zoea of freshwater shrimp *Macrobrachium rosenbergii*. *Microbial Pathogenesis*, 127, pp.166-171. <https://doi.org/10.1016/j.micpath.2018.12.002>
- Linkous, D.A. and Oliver, J.D., 1999. Pathogenesis of *Vibrio vulnificus*. *FEMS Microbiology Letters*, 174(2), pp.207-214. <https://doi.org/10.1111/1/j.1574-6968.1999.tb13570.x>
- Nitimulyo, K.H., Isnansetyo, A., Triyanto, Istiqomah, I. and Murdjani, M., 2005. Isolasi, identifikasi dan karakterisasi *Vibrio* spp. patogen penyebab Vibriosis pada kerapu di Balai Budidaya Air Payau Situbondo. *Journal Perikanan Universitas Gadjah Mada*, 7(1), pp.80-94. <https://doi.org/10.22146/jfs.9053>
- Mishra, P., Samanta, M., Mohanty, S. and Maity, N.K., 2010. Characterization of *Vibrio* species isolated from fresh water fishes by ribotyping. *Indian Journal of Microbiology*, 50, pp.101-103. <https://doi.org/10.1007/s12088-010-0010-4>
- Motes, M.L., DePaola, A., Cook, D.W., Veazey, J.E., Hunsucker, J.C., Garthright, W.E., Blodgett, R.J. and Chirtel, S.J., 1998. Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology*, 64(4), pp.1459-1465. <https://doi.org/10.1128/aem.64.4.1459-1465.1998>
- Nkili-Meyong, A.A., Bigarré, L., Labouba, I., Vallaey, T., Avarre, J.C. and Berthet, N., 2016. Contribution of next generation sequencing to aquatic and fish virology. *Intervirology*, 59(5-6), pp.285-300. <https://doi.org/10.1159/000477808>
- Otta, S.K. and Karunasagar, I. and Karunasagar, I., 2001. Bacteriological study of shrimp *Penaeus monodon* Fabricius, Hatcheries in India. *Journal of Applied Ichthyology*, 17(2), pp.59-63. <https://doi.org/10.1046/j.1439-0426.2001.00249.x>
- Pariakan, A. and Rahim, 2021. Karakteristik kualitas air dan keberadaan bakteri *Vibrio* sp. pada wilayah tambak udang tradisional di Pesisir Wundulako dan Pomalaa Kolaka. *Journal of Fisheries and Marine Research*, 5(3), pp.547-556. <https://doi.org/10.21776/ub.jfmr.2021.005.03.5>
- Reddy, M.R.K. and Mastan, S.A., 2013. Emerging *Acinetobacter schindleri* in red eye infection of *Pangasius sutchi*. *African Journal of Biotechnology*, 12(50), pp.6992-6996. <https://www.ajol.info/index.php/ajb/article/view/139666>
- Rungrassamee, W., Klanchui, A., Maibunkaew, S., Chaiyapechara, S., Jiravanichpaisal, P. and Karoonuthaisiri, N., 2014. Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*). *PLoS ONE*, 9(3), e91853. <https://doi.org/10.1371/journal.pone.0091853>
- Rungrassamee, W., Klanchui, A., Maibunkaew, S. and Karoonuthaisiri, N., 2016. Bacterial dynamics in intestines of the black tiger shrimp and the Pacific White Shrimp during *Vibrio harveyi* exposure. *Journal of Invertebrate Pathology*, 133, pp.12-19. <https://doi.org/10.1016/j.jip.2015.11.004>

- Somboon, M., Purivirojkul, W., Limsuwan, C. and Churchird, N., 2012. Effect of *Vibrio* spp. in white feces infected shrimp in Chanthaburi, Thailand. *Fisheries Research Bulletin*, 36(1), pp.7-15. <https://li01.tci-thaijo.org/index.php/JFE/article/view/80769>
- Sriurairatana, S., Boonyawiwat, V., Gangnonngiw, W., Laosutthipong, C., Hiranchan, J. and Flegel, T.W., 2014. White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. *PLoS ONE*, 9(6), e99170. <http://doi.org/10.1371/journal.pone.0099170>
- Sumini and Kusdarwati, R., 2020. The discovery of *Vibrio harveyi* on *Litopenaeus vannamei* infected white feces disease in Situbondo, East Java. *Jurnal Perikanan Universitas Gadjah Mada*, 22(1), pp.9-18. <https://doi.org/10.22146/jfs.47791>
- Supono, Wardiyanto, Harpeni, E., Khotimah, A.H. and Ningtyas, A., 2019. Identification of *Vibrio* sp. as a cause of white feces diseases in white shrimp *Penaeus vannamei* and handling with herbal ingredients in East Lampung Regency, Indonesia. *AACL Bioflux*, 12(2), pp.417-425. <http://bioflux.com.ro/docs/2019.417-425.pdf>
- Tanrikul., T.T., 2007. Vibriosis as an a epizootic diseases of rainbow trout (*Onchorynchuss mykiss*) in Turkey. *Pakistan Journal of Biological Sciences*, 10(10), pp.1733–1737. <https://doi.org/10.3923/pjbs.2007.1733.1737>
- Zeng, S., Huang, Z., Hou, D., Liu, J., Weng, S. and He, J., 2017. Composition, diversity and function of intestinal microbiota in Pacific White Shrimp (*Litopenaeus vannamei*) at different culture stages. *PeerJ*, 5, e3986. <https://doi.org/10.7717/peerj.3986>
- Zhang, X., Sun, J., Han, Z., Chen, F., Lv, A., Hu, X., Sun, X., Qi, H. and Guo, Y., 2021. *Vibrio parahaemolyticus* alters the community composition and function of intestinal microbiota in Pacific white shrimp, *Penaeus vannamei*. *Aquaculture*, 544, 737061. <https://doi.org/10.1016/j.aquaculture.2021.737061>