Water quality and bacterial community management in shrimp ponds in Rembang, Indonesia: Towards sustainable shrimp aquaculture

SUMMARY

Shrimp pond aquaculture, including the Pacific white-leg shrimp *Litopenaeus vannamei* farming has to deal with water deterioration and bacterial diseases. To tackle these problems, water is exchanged and probiotics are applied. However, these approaches may lead to environmental problems and an increase of rearing operational cost.

We investigated water quality and aquatic bacterial communities in two farming systems, semi-intensive and intensive, of which no water was exchanged during shrimp rearing. We found diverse halophilic ('salt-loving') bacteria in pond waters, which may become candidates of beneficial bacteria for shrimp farming. Disease events in both systems coincided with sudden decrease of pH and shift of dominant bacterial communities. The disease events ceased when water pH was adjusted to above pH 8. Therefore, we counselled to add limestones to discard sludge regularly into sludge reservoir and to add treated fresh-seawater to maintain shrimp pond water quality, especially pH and salinity. Finally, we promoted a molasses addition into pond water to increase natural food availability and to maintain beneficial bacterial communities.

We conclude that maintenance of water quality and bacterial community dynamics allow avoiding rearing failure, reducing operational costs and minimizing pollution caused by shrimp pond effluents.

KEY RESULTS

- Intensive ponds have greater salinity, phytoplankton, suspended particles, fluctuated pH and dissolved oxygen. They also yield better harvest compared to semi-intensive systems.
- Halophilic heterotrophic bacteria such as *Halomonas*, *Psychrobacter*, *Salegentibacter*, and *Sulfitobacter* are the dominant bacteria in both farming systems. These bacteria, along with pH above 8, may suppress the outbreak of opportunistic pathogenic bacteria, such as *Alteromonas*, *Pseudoalteromonas* and *Vibrio*, which may contribute to disease events.
- Molasses may improve natural food (bio-flocs) quality and halophilic bacterial communities. The bio-flocs serve as an alternative food source, which may substitute pellets and reduce feed cost.

RECOMMENDATIONS

- Regulation should include standard operating procedures (SOP) for pond water quality maintenance and allowed water exchange rate.
- Regulation should include standardization of probiotics considering bacterial content and application procedures.
- Rearing can be improved by generating bio-flocs, removing sludge and maintaining water quality.

THE CONTEXT

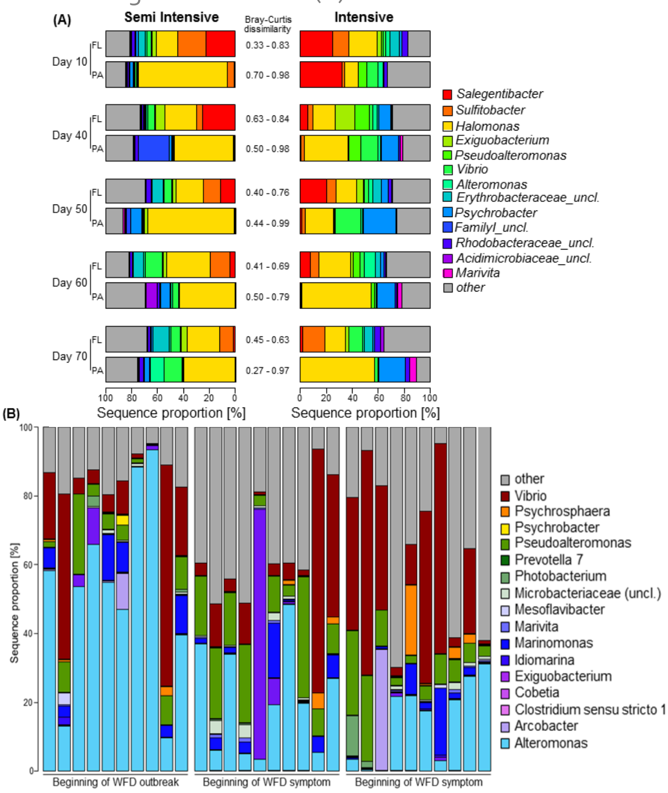
Rembang Regency is one of the main shrimp producers in Central Java (Figure 1). Shrimp farming has been massively operated since 1990, owned by individuals or communities. Since 2005, monoculture shrimp farming has been operated by several small and medium sized enterprises (SME). Recently, shrimp harvests are below expectation due to rearing failure or shrimp diseases. To reduce rearing failure, SMEs apply various farming systems, such as extensive, semi-intensive, and intensive farming. They have also added probiotics to rearing ponds to enhance shrimp growth, to maintain inorganic nutrient concentration and to suppress growth of *Vibrio*. However, shrimp production remains suboptimal, and even bacterial disease outbreaks occur frequently.

Shrimp pond management, specifically rearing process management, is a crucial factor for shrimp farming. It is well known that continuous feed supply and accumulation of organic matter during shrimp rearing affect water quality, bacterial community and cultured shrimps, which has become a concern for pond owners and operators. Furthermore, common probiotic application, which is currently ineffective to prevent shrimps from bacterial disease, has to be evaluated. The cost for probiotics, along with feed pellets, contributes to more than 50% of shrimp rearing operational costs, resulting in an even more severe economic loss if the rearing process fails. Another negative side effect of pond farming is potential eutrophication. Regular water exchange, a common practice in shrimp pond aquaculture, may pollute adjacent ecosystems such as mangrove, sea grass or other coastal ecosystems.

As a eutrophic environment, a shrimp pond contains various microorganisms including bacteria that interact with cultured shrimps in different ecological niches. Bacteria thrive shrimp farming by being involved in some vital roles, for example inorganic nutrient cycles, organic matter decompositions and source of nutrient. In contrast, they may provoke shrimp diseases in various lethal levels. Therefore it is necessary to understand bacterial community composition in shrimp ponds as well as water parameters that significantly affect or shape bacterial communities. In addition, it is essential to establish a precise method for pathogenic bacteria identification and quantification. This comprehensive approach can lead to an improved management of the rearing process in terms of preventing bacterial disease events, achieving higher efficiency in operational costs, specifically for expenses of feed pellets and probiotics, and reducing the negative ecological impact of shrimp pond aquaculture.

Figure 1: Map of Rembang Regency, Central Java Indonesia. Yellow boxes indicates sampling sites.

Figure 2: Bacterial community compositions in pond water at non-white faeces disease (WFD) event (A) and in shrimp faecal strings at WFD event (B).



RESEARCH & RESULTS

Our study offers rearing management approaches based on a time-series water quality measurement (biogeochemical parameters) and bacterial community profiles, including pathogenic *Vibrio parahaemolyticus*, a common pathogen in shrimp aquaculture. We compared water quality and bacterial community compositions in two different pond-farming systems in Rembang Regency, Central Java, Indonesia, which were semi-intensive and intensive systems. They were characterized by different shrimp stocking densities (post-larvae (PL)/fries) in amount of 40 PL m^{-3} and 90 PL m^{-3} for semi-intensive and intensive ponds, respectively. In addition, we generated aggregates of suspended particulate matter, so called bio-flocs, to provide natural shrimp food during shrimp rearing in order to reduce feed pellet usage and to maintain beneficial bacterial compositions.

Physico-chemical parameter of pond water in semi-intensive and intensive ponds

Biogeochemical parameters, such as pH, salinity, suspended particulate matter, chlorophyll a, and inorganic nutrients varied between systems due to different rearing strategies and daily operational practices. These differences include for example provision of different amounts of feed as well as additional treatments, such as addition of limestones, commercial probiotics, fermented rice brans and molasses. These different treatments also cause temporal changes in ponds.

Abundances of phytoplankton increased with rearing time. Evaporation rates and addition of water to maintain pond water level that decrease due to evaporation may affect salinity in shrimp pond. To avoid drastic change in salinity, sufficient amounts of 'sterile' seawater is needed. Despite different amounts of provided feed pellets and cultured shrimps in semi-intensive and intensive systems, the inorganic nutrient concentrations in the investigated ponds, particularly ammonium and nitrite, were always far from lethal concentrations for *L. vannamei* shrimps.

pH values decreased gradually from their initial value which was around pH 8.4 to 7.8. During disease event (white faeces disease/WFD), pH values were considerably low (pH 7.71-7.84). This suggests that pH may play a role in disease occurrence and highlights the importance of monitoring/ managing pH values during shrimp rearing. Since constantly low pH values can threaten shrimps, limestone or similar structures (i.e. dolomite) were added to the ponds to increase alkalinity whenever necessary.

Feed conversion ratio (FCR) and final harvest

Feed conversion ratio (FCR) varied in range 1.4 to 2.0. The FCR is depended on the density of the shrimps and the amount of provided pellet. The intensive ponds obtained final harvests 2-4 folds higher than those of semi-intensive ponds, as observed in our study with 3,950 ± 284 kg and 1,990 ± 151 kg, for intensive and semi-intensive ponds, respectively. In addition, the intensive ponds can be harvested 4-5 times from a complete rearing cycle by doing 3 to 4 partial harvests, while the semi-intensive ponds only allow for 2 or 3 harvests.

Bacterial community composition: candidates of beneficial bacteria

Heterotrophic halophilic bacteria, such as *Exiguobacterium*, *Halomonas*, *Psychrobacter*, *Salegentibacter* and *Sulfitobacter* dominated bacterial community composition in pond water of both systems. In our study, these bacteria were always present at non-disease events. *Exiguobacterium*, *Psychrobacter* and *Salegentibacter* may contribute to organic matter decomposition, while *Sulfitobacter* may contribute to sulfur oxidation processes in the water column. *Halomonas*, the most abundant bacteria (Figure 2), may play a role in denitrification processes. In addition, they may produce bio-molecules so called polyhidroxyalkanoate (PHA) or polyhidroxybutirate (PHB) which may ameliorate shrimp growth.

Bacterial community composition: pathogenic bacteria and *Vibrio parahaemolyticus'* virulence genes

Alteromonas, *Arcobacter*, *Marinomonas*, *Photobacterium*, *Pseudoalteromonas* and *Vibrio* became the dominant bacteria at disease event, in shrimp pond water or in diseased shrimp samples. Moreover, higher concentration of virulence genes, such as transmembrane regulatory protein (*toxR*) and termolabile haemolysin (*tlh*) genes were found in suspended particulate matter (SPM) rather than in the SPM-free pond water, suggesting that particles (SPM) represent a hotspot for pathogenic bacteria. The high occurrence of contaminated aggregates in shrimp pond waters may increase intoxication among cultured shrimps since shrimps may eat the contaminated particles.

Impact of additional treatment: probiotics, limestones and molasses

Decrease of water pH below 8 resulted in a shift of bacterial communities where opportunistic pathogenic bacteria became the dominant ones. Concomitantly, it may lead to a shift of intestinal bacteria in shrimp where the abundance of beneficially bacteria (i.e. *Pseudomonas* and *Acinetobacter*) decreased significantly, which may then lead shrimps to be more vulnerable to pathogen infection. To maintain water pH, it is necessary to add pH buffer such as limestones or dolomite regularly. In addition, regular sludge discharge in the pond bottoms may favor pH level stability.

Commercial probiotic bacteria were absent in pond water. Therefore, application of these probiotics needs to be changed for example by augmentation of the dose of probiotics. However, it will increase operational cost. Alternatively, pond operators can spread the probiotics into feed pellets upon feeding time. With this approach, beneficial bacteria will still be alive and can colonize shrimp intestine.

The addition of molasses into pond water increase pH. They also sustain dominance of non-pathogenic heterotrophic bacteria, such as *Exiguobacterium, Halomonas, Psychrobacter, Salegentibacter and Sulfitobacter*. Furthermore, the addition of molasses can regulate inorganic nutrient concentrations in shrimp pond waters. Molasses may facilitate bacterial growth, which uptake inorganic nutrients, such as ammonium, nitrate and nitrite. Finally, molasses may improve macromolecules contents, such as carbohydrates, proteins, and lipids in particles (bio-flocs), which allow shrimps to get their natural food source.

POLICY RECOMMENDATIONS

The Indonesian Ministry of Marine Affairs and Fisheries issued a regulation guiding shrimp pond aquaculture activities (No. 75/PERMEN-KP/2016). We offer the following recommendations to the existing regulation, local government and practitioners in order to improve shrimp aquaculture and protect surrounding ecosystems in Indonesia:

- Existing guidance on water quality maintenance is not sufficiently described. There is excessive inorganic nutrients in shrimp ponds, which may harm shrimps and lead to potential eutrophication due to effluent discard. To minimize pollution, local government should regulate the allowed water exchange rate and provide incentives to SMEs, e.g. subsidizing electrical costs or supporting regular water monitoring for pathogens.
- In shrimp farming, the usage of commercial probiotics is not specifically regulated, leading to high production costs and ineffective probiotic application. We recommend to standardize probiotics application in terms of bacterial composition, dosage and application procedures.
- Existing policies specify the usage of large sizes of sludge reservoirs considering daily sludge discard and evaporation rate. Large reservoirs, however, lead to an excessive sludge accumulation, which may harm shrimp farming. Therefore, we recommend reducing sludge reservoir size and regulating size based on pond size. A pond of size 100 x 40 m requires a sludge reservoir size of 20 x 5 x 2 m. A sludge reservoir can reserve sludge from a maximum of three ponds.
- Our study indicates that shrimp disease events coincided with pH below 7. Therefore, water pH shall be maintained above pH 8, e.g. through regular addition of limestone or dolomite and regular sludge discard.
- Bacterial measurements shall consider culture independent methods to obtain precise estimations of pathogenic bacteria. Pathogenic bacteria can be monitored through investigation of virulence genes in suspended particulate matter fraction.
- Feed pellets and probiotics (if necessary) can be reduced by using molasses to form natural feed (bio-flocs). If probiotics are incorporated into feed pellets instead of introduced solely into the pond water, operational costs of rearing can be reduced.

References

1. Alfiansah, Y.R., Hassenrück, C., Kunzmann, A., Taslihan, A., Harder, J., Gärdes, A., 2018. Bacterial abundance and community composition in pond water from shrimp aquaculture systems with different stocking densities. *Frontiers in Microbiology* 9:2457. doi:10.3389/fmicb.2018.02457.
2. Ministry of Marine Affairs and Fisheries of the Republic of Indonesia. 2017. Regulation of the minister of Marine Affairs and Fisheries No. 75/PERMEN-KP / 2016 concerning General guidelines for Windu shrimp (*Penaeus monodon*) and Vaname shrimp (*Litopenaeus vannamei*).43 pp. (Indonesian language).
3. Avnimelech, Y. (2015). *Biofloc technology- a practical guide book, 3rd edition*. Baton Rouge, Louissiana, United States. 285pp. doi:10.13140/2.1.4575.0402.

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