# Characterization of water quality using IoT (Internet of Things), plankton and expression of virus-like particles in vannamei shrimp ponds of different constructions

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**Abstract**. This study aimed to analyze the effect of pond construction on physical and chemical water quality, plankton community, bacterial density and virus-like particles (VLPs) intensity. The approach for measuring water quality was done by using Internet of Things (IoT), while for identifying the presence of VLPs a confocal laser scanning microscope was used. Both methods were expected to accelerate the detection of the physical, chemical and biological water quality. This study was conducted in six ponds (three concrete ponds and three plastic liner ponds) in Situbondo Regency, East Java, Indonesia, from July to September 2020. The water was sampled in the middle of the pond and replicated four times. Four water quality parameters, consisting of temperature, DO, pH and salinity, were measured using IoT. Other quality parameters consisted of water level, hardness, alkalinity, $NH_4+$, $NO_2$, total organic matter (TOM) and total bacteria were measured manually using a standard equipment. Plankton collection was performed eight times by sampling pond water. The pond built with concrete had higher water levels, $NH_4+$, TOM, total bacteria and VPLs, but lower plankton abundance and taxa richness. In contrast, the plastic liner ponds had a higher temperature, brightness, DO, pH, salinity, hardness, $NO_2$, and alkalinity. The TOM and total bacteria had a strong correlation with temperature and water levels. Chlorophyceae dominated the plankton sample, followed by Cyanobacteria and Chryotophyceae. The yields of shrimp production in concrete ponds were higher than those in the plastic liner pond. The application of IoT to measure water quality and of the confocal laser scanning microscopy to identify the presence of VLPs can characterize the aquatic environments faster.

**Key Words**: plankton, water quality, VLPs, TOM, monitoring, vannamei shrimp.

**Introduction**. The demand for fishery commodities, followed by the development of more intensive aquaculture, has increased rapidly in the last two decades. One of the economically important fishery commodities is the vannamei shrimp (*Litopenaeus vannamei* Boone, 1931). This shrimp is mainly cultured in South African and Asian countries, especially Brazil, Ecuador, Mexico, China, Thailand, Indonesia, and Vietnam (Quyen et al 2020). Shrimp export is considered a major sector providing considerable economic benefits to these countries. Approximately 3.8 million tons of shrimps were produced in 2018 globally. Water quality is a very important natural indicator for evaluating the feasibility of shrimp farming (Ariadi et al 2019a). The intensive vannamei shrimp culture system is characterized by a high stocking density, intensive and large quantities of supplementary feed and the construction of modern ponds. This system may cause water quality to decrease, due to the organic matter from shrimp artificial feed and feces. Deteriorating water quality can cause negative effects both for shrimp and the environment. The impact on shrimp can occur directly or indirectly through the effect on microorganisms. It can cause disease, slow shrimp growth and increased mortality, due to fluctuations in the ecosystem dynamics (Anand et al 2019; Thitamadee et al 2016), becoming one of the main obstacles to increase the production (Didar-Ul Islam & Bhuiyan 2016). The impact of disposal of pond effluents, which are usually enriched with nutrients and antimicrobial agents, can severely affect the ecosystems adjacent to the shrimp ponds (Didar-Ul Islam & Bhuiyan 2016). Therefore, efforts to carry out proper waste management practices, including monitoring the water quality, are vital to determine the success of this system. A study in Vietnam showed that the management of shrimp pond aquaculture, particularly in relation to the reservoir construction, water monitoring and use of chemicals, had a significant impact on reducing disease reports and safety rejection (Quyen et al 2020).

The high organic matter content in pond waters may affect water microorganisms such as plankton, bacteria, VLPs abundance (Alfiansah et al 2018; Travaini-Lima et al 2016). Those play a critical role in maintaining the pond ecosystem balance and its complex food webs, both beneficial (nutrients recycling, organic matter degrading, etc.) and harmful role (parasites) (Ahmad et al 2017). The growth and development of phytoplankton and bacteria in pond waters require energy resulted from nutrients supplied from the decomposition of shrimp feed organic matter. The beneficial microorganism, mostly phytoplankton, acts as a producer of oxygen through the photosynthesis process. The process effectively prevents the environment from degradation of organic waste and ensures the oxygen availability for shrimp respiration. The high diversity and abundance of phytoplankton maintains ecological balance, absorbs toxic compounds in water, spurs growth and reduces the mortality rate of cultured shrimp (Atmomarsono & Nurbaya 2014). In contrast, some harmful microorganisms such as blue-green algae plankton or Cyanobacteria such as *Oscillatoria* sp. and *Microcystis* sp. may cause a toxic environment and affect the freshwater food web structures when blooming abundently (Ait et al 2018; Ariadi et al 2019b). The other microorganisms such as vibrio, a type of pathogenic bacteria and VLPs may trigger various serious problems in aquaculture (Otta & Hoque 2020). The presence of VLPs can cause primary and secondary infection by bacteria which commonly occur on a large scale, due to virus-infected organisms. Detection of the quantity of VLPs quickly in the aquatic environment is unknown. Therefore, the approach to identify the presence of VLPs, by using confocal laser scanning microscopy (CLSM), in the aquatic environment is expected to help solving this problem. Previous research indicated a strong influence of high concentrations of organic matter on the abundance of bacteria in the waters (Dai et al 2018). Therefore, monitoring of water quality in intensive ponds is vital to support the success of shrimp culture.

Monitoring of water quality in intensive ponds is commonly done manually, using field or laboratory standard processing. Such processes tend to be impractical, labored and have high risks of human error. With advances in Information Technology, data can now be collected at locations and transmitted across a wide area using wireless sensor networks and the Internet of Things (IoT) (Zainuddin et al 2018). Several studies have reported the advantage of this system (Rosaline & Sathyalakshimi 2019; Zainuddin et al 2018). However, the application of this system to measure data of water quality, while integrating it with a manual system, has never been done. A future use of the results for supporting a complex system with automatic aeration, water level control, heat protection, etc. may improve the water quality in intensive ponds. The development of an intensive vannamei shrimp culture system is usually carried out with concrete construction. In recent years in Indonesia, several shrimp farming ponds have adopted plastic liner ponds, because it is considered cheaper and easier. However, research on the most influential water quality parameters for both the shrimp survival rate and the microorganism abundance and composition in plastic liner ponds is very rare. This study aimed to analyze the effect of pond construction on physical and chemical water quality parameters, bacterial density, plankton community structure and VLPs intensity.

## Material and Method

***Study area and sample***. This study was conducted in six intensive ponds consisting of three concrete ponds (C1, C2, and C3) with the dimension of 2,700; 2,000 and 2,000 $m^2$

respectively, and three polyethylene plastic liner ponds (P1, P2, and P3) with the dimension of 3,200; 2,700 and 2,800 $m^2$ respectively, situated in Situbondo Regency, East Java Province, Indonesia. Water samples were collected from the center of the ponds from July 2020 to September 2020. The ponds were filled with vannamei shrimp with a stocking density of 100-130 shrimps $m^2$. The stocking period of vannamei shrimp was conducted in July 2020, at 9 or 10 day old post-larvae. Shrimp are harvested after about 128-130 days of age. It was observed that there was no partial harvest. The harvest for each pond was considered good with an average survival rate of 80%. Pond control was carried out intensively, the dead shrimps were immediately removed from the pond. Data were captured from the first day of stocking and the water quality parameters were analyzed in situ and ex-situ. The average of survival rate of vannamei shrimp rearing in all ponds was 83.11%. The total production of vannamei shrimp in both types of pond construction ranged from 10.078 to 23.184 tons, with an average of 15.10 tons (Table 1).

Table 1

Survival rate and harvesting data (means, with ranges in parenthesis) of *Litopenaeus vannamei* cultured in concrete and plastic liner ponds

| *Parameters* | *Concrete* | *Plastic* |
|---|---|---|
| Pond width (ton $ha^{-1}$) | 2,233.3 (2,000–2,700) | 2,750 (2,700–3,200) |
| Shrimp survival rate (%) | 80.5 (65.8–91.7) | 84.1 (76.1–93) |
| Shrimp harvest (ton $ha^{-1}$) | 16.13 (10.08–23.18) | 14.07 (10.75–18.62) |

This study adopted a dual system of water quality measurements. Four parameters, the temperature, DO, pH and salinity were measured using the IoT. This tool is equipped with sensor node 1 using a TTGO LoRa microcontroller board with a frequency of 915 Mhz. Each node is able to acquire data from sensors. The data from the sensor is sent to the gateway by using the LoRa communication module. All sensor nodes are also assigned to receive data from the gateway when the user sends a message to turn on the actuator (Figure 1). Other variables were measured manually using laboratory standards. Water samples for manual analysis were taken using the Kmerer water sampler and preserved by following the APHA guidelines. Samples were collected in sterile reagent bottles and screw cap tubes and immediately taken to the laboratory for analysis. The sample was stored in the refrigerator until the analysis process. Measurement of total bacteria and total organic matter was carried out in the Laboratory of Fish Diseases and Health, Faculty of Fisheries and Marine Sciences, Brawijaya University.

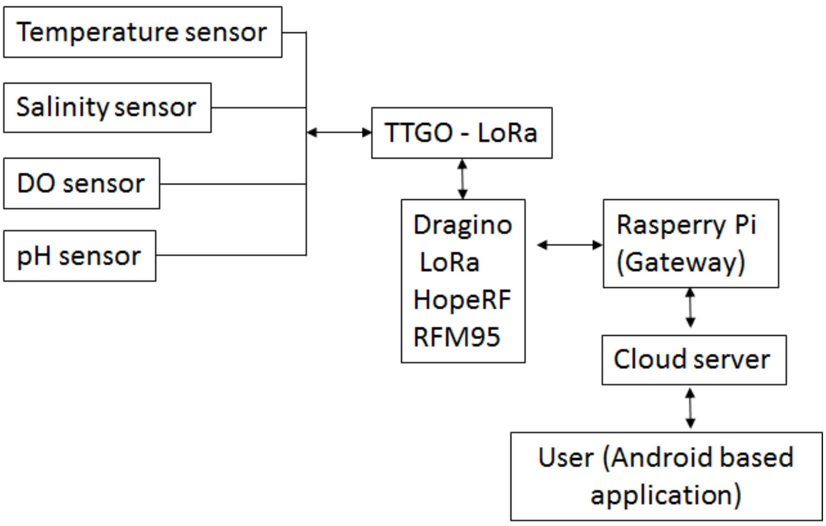


Figure 1. Scheme of the IoT application.

The amount of organic matter was measured by using a titration method (Indonesia National Standard number 06-6989.22-2004). The content of total bacteria and Vibrio was measured by using the Prescott method (Schrader et al 2011). A total 100 ml of water was extracted, then diluted gradually until a $10^{-3}$ dilution was achieved for the total bacteria, while the Vibrio content reached a $10^{-1}$ dilution, then the result was successively planted on thiosulfate citrate bile salts medium. Both media were incubated at room temperature for 24 hours and the number of colonies was counted following the morphological characters based on shape, color and size using the Total Plate Count method. Bacterial density is calculated based on the number of colonies per unit or colony-forming unit (CFU) in every 1 mL. The abundance of bacteria can be calculated by the formula (Damongilala 2009):

$$N = n/df$$

Where:
N - bacterial density;
n - number of colonies;
df - dilution factor.

The number of colonies counted at the first dilution have the dilution factor of $10^{-1}$ and at the second dilution, the dilution factor is $10^{-2}$ and so on.

The intensity of Virus-Like Particles (VLPs) expression was observed from a total of 100 mL of the water sample, filtered by an Anodisc filter (Whatman 0.02 μm pore size). VLPs were determined after staining with SYBR™ Gold by conventional epifluorescence microscopy and compared to enumerations performed by CLSM.

***Plankton****.* Pond water samples were taken eight times during the study, namely at the beginning and end of the cultivation period. The time of collection is between 11.00 and 14.00 WIB. Water samples from each pond were taken at four points at a depth of 30 cm below the water surface. Sampling was done using a water bottle volume of 1.5 L. A total of 50 mL of water sample was preserved with 1% Lugol's iodine solution and stored at 4°C in the dark until further processing. Identification and counting of the microalgae were carried out by taking 1 mL of water and placing it in the Sedgewick-Rafter counting chamber for microscope observation, at a 400 × magnification. Natural units (single cell and colony) are counted on five randomly selected fields of view. Identification of microalgae was carried out based on a reference (Castellani & Edwards 2017; Patel et al 2007; Schrader et al 2011). Phytoplankton densities are reported as individuals $mL^{-1}$.

***Data analysis***. Prior to the data collection, the calibration and validation of the IoT were done by using a data comparison with the Pearson's correlation test. Data from daily and weekly measurements were analyzed separately by analysis of variance (ANOVA). The results of daily measurements were analyzed using general linear multivariate ANOVA models, while weekly measurements were analyzed using general linear univariate ANOVA models.

The Principal Component Analysis (PCA) was performed to reduce environmental factors and determine correlations between factors. The differences in the abundance and diversity of plankton were analyzed by using general linear model (multi-factor ANOVA) with the pond type as between-subject factor and the sampling date as a within-subject factor. The number of individuals, taxa richness and diversity were considered normal because the number of observations was large. The canonical correspondence analysis (CCA) was applied to analyze the relationship between the abundance of plankton genera and the environmental variables (pH, Salinity, $NH_4$+, $NO_2$, alkalinity, TOM, total hardness, total bacteria, DO and temperature), using the SPSS software. All factors were coded as categorical variables. Genera with low density (less than 25,000 cells $mL^{-1}$) were excluded from the analysis. Genera and environmental factors are grouped by the Morishita similarity index. The results of the observation of VLPs levels were analyzed using the unpaired samples of t-test analysis.

## Results

***Pond water quality***. The validity and calibration test indicated that the data from IoT were valid. The salinity test showed that the level of similarity of the results was 98.9%, with a correlation of 0.93. The DO test showed a 99% similarity level, with a correlation of 0.92. The pH test showed the level of similarity of results of 98.8% with a correlation of 0.96. While the temperature test showed the similarity level of the results of 97.4% with a correlation of 0.73. Among 12 water quality parameters, 6 were measured daily. The water level data in concrete ponds ranged from 116.67 to 140 cm, with an average value in the morning of 128.68 while in the afternoon the average water level was 131.18. In plastic liner ponds it ranged from 96.33 to 119 cm. The average in the morning was 108, while in the afternoon it was 110.62 (Figure 2a). Statistically, the difference of the mean water level in the two ponds was significant, but there was no difference between the morning and afternoon water levels (Table 2). Brightness data on concrete ponds ranged from 28.33 to 117.67 cm, with an average value in the morning of 45.72, while in the afternoon the average brightness was 44.25. In plastic liner ponds, brightness ranged from 40 to 138.33 cm; the average brightness in the morning was 63.27 while in the afternoon it was 63 (Figure 2b). Statistically, the difference in brightness in the two ponds was significant, but there was no difference between morning and afternoon brightness (Table 2). Temperature data in concrete ponds ranged from 21.47 to 32.67$^{o}$C; the average temperature in the morning was 28.29$^{o}$C while in the afternoon, the average temperature was 30.21$^{o}$C. In plastic ponds, it ranged from 28 to 34°C; the average temperature in the morning was 29.37$^{o}$C while in the afternoon it was 31.64$^{o}$C (Figure 2c). Statistically, the means of temperature in the two ponds, as well as between morning and afternoon differed significantly (Table 2). Dissolved oxygen values in both ponds ranged from 6.5 to 8 ppm, with morning averages of 6.65 ppm (concrete ponds) and 7.71 ppm (plastic ponds) and during the afternoon they were of 6.67 and 7.72 ppm, respectively (Figure 2d). Statistically, the difference in DO in the two ponds was significant, but there was no difference between morning and afternoon DO (Table 2). Salinity data in concrete ponds ranged from 25 to 32 ppt, with an average value in the morning of 28.85, while in the afternoon it was 28.92. In plastic liner ponds it ranged from 30.33 to 35.66 ppt. The average salinity in the morning was 33.5 ppt while in the afternoon it was 33.58 ppt (Figure 2e).

Statistically, the difference in salinity in the two ponds was significant, but there was no difference between morning and afternoon salinity (Table 2). The pH data in concrete ponds ranged from 7.4 to 8.2, with an average value in the morning of 7.60 while in the afternoon the average pH was of 7.82. In plastic liner ponds it ranged from 7.33 to 8.23, with an average of 7.55 in the morning and 7.79 in the afternoon (Figure 2f). Statistically, the difference in pH in the two ponds was significant, but there was no difference between morning and afternoon pH (Table 2).

The hardness values ranged from 4083.33 to 6583.33, with an average value in concrete ponds of 5343.98, while in plastic ponds it was 5703.71 (Figure 3a). The alkalinity data ranged from 117.67 to 141.33, with an average value in concrete ponds of 130.63, while in the plastic liner ponds it was of 126.31 (Figure 3b). The $NH_4$+ data ranged from 0.023 to 0.84 ppm, with an average value for concrete ponds of 0.16, while in plastic ponds it was 0.14 (Figure 3c). The $NO_2$ data ranged from 0.01 to 0.07 ppm, with an average value for concrete ponds of 5343.98, while in the plastic liner ponds it was 5703.71 (Figure 3d). The TOM data ranged from 82.33 to 203 ppm, with an average value for concrete ponds of 142.02, while in plastic ponds it was 109.54 (Figure 3e). The total bacteria data ranged from 8633.33 to 14666.67, with the average value in concrete ponds of 26249.71, while in plastic ponds it was 9086.25 (Figure 3f). Statistically, the average alkalinity, TOM and total bacteria in the concrete pond were significantly higher than in plastic liner ponds (Table 2).

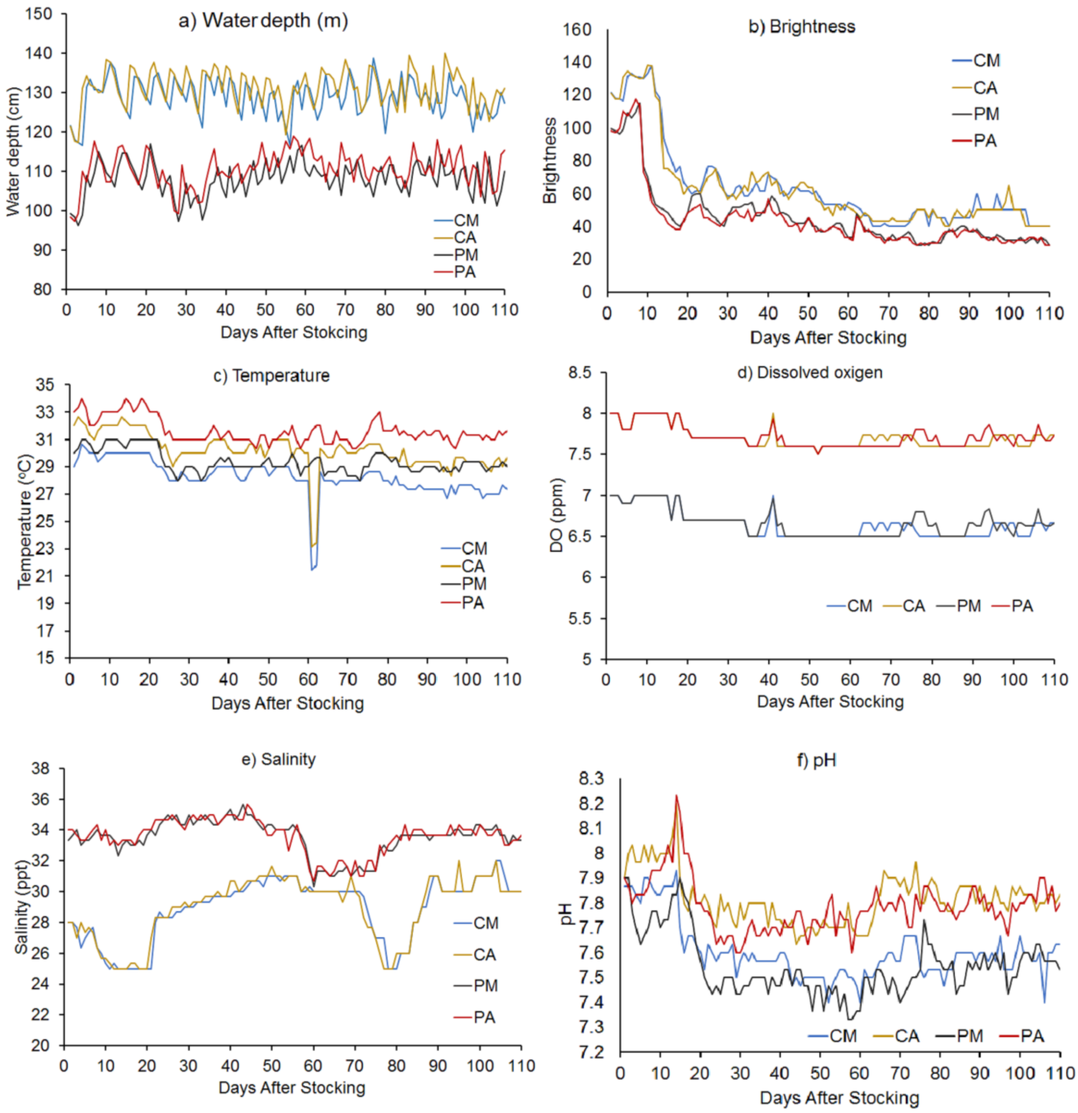


Figure 2. Daily water quality fluctuations between concrete and plastic liner shrimp ponds.

Table 2

Summary of F statistic and significance of the effect of pond construction (concrete and plastic) and time (morning and afternoon) on water quality parameter values

| *Factors* | *Treatment (p)* | *Time (t)* | *(p*t)* |
|---|---|---|---|
| Water level | 306.57*** | 46.41*** | 0.02 ns |
| Brightness | 15.36*** | 0.43 ns | 0.21 ns |
| Salinity | 206.61*** | 0.53 ns | 0.00 ns |
| DO | 0.97 ns | 11135.26*** | 0.28 ns |
| pH | 8.34** | 682.82*** | 1.99 ns |
| Temperature | 5.63* | 465.78*** | 3.28 ns |
| Hardness | 4.10ns | | |
| $NH_4+$ | 0.15 ns | | |
| $NO_2$ | 0.08 ns | | |
| Alkalinity | 5.70* | | |
| TOM | 11.47** | | |
| Total bacteria | 52.32*** | | |

Asterisk indicate of significant level at * = p<0.05, **= p<0.01, ***= p<0.001.

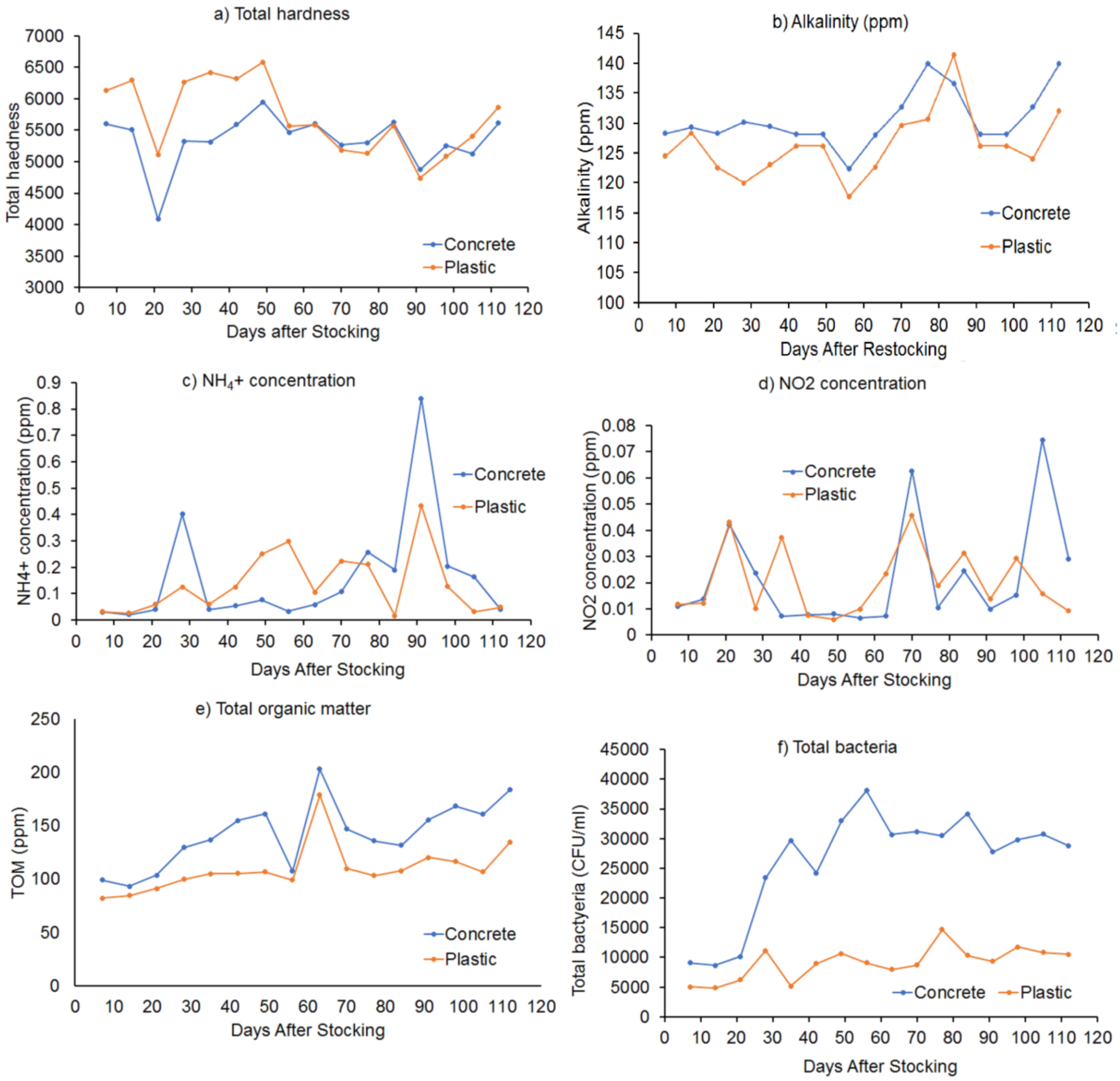


Figure 3. Weekly water quality fluctuations between concrete and plastic liner shrimp ponds.

Of the 12 water quality parameters, 11 of them have a role in determining the differences in the characteristics of the two pond constructions. The first component factor consisted of temperature, brightness, pH, DO and TOM, having an eigenvalue of 3.77, and accounted for 34.3% of the data variability. The second consisted of water level, salinity, alkalinity and total bacteria, having an eigenvalue of 3.33, and contributed to 30.3% of the data variability. The last component consisted of hardness and $NO_2$, having an eigenvalue of 1.56 and contributed to 14.1% of the data variability (Figure 4).

The temperature had a negative relationship with the water level, TOM and total bacteria. The temperature had a positive correlation with DO. The water level had a positive correlation with the total bacteria, but it had a negative correlation with the salinity. The brightness had a negative correlation with the DO and pH. The DO was positively correlated with the pH. The TOM was positively correlated with the total bacteria (Table 3).

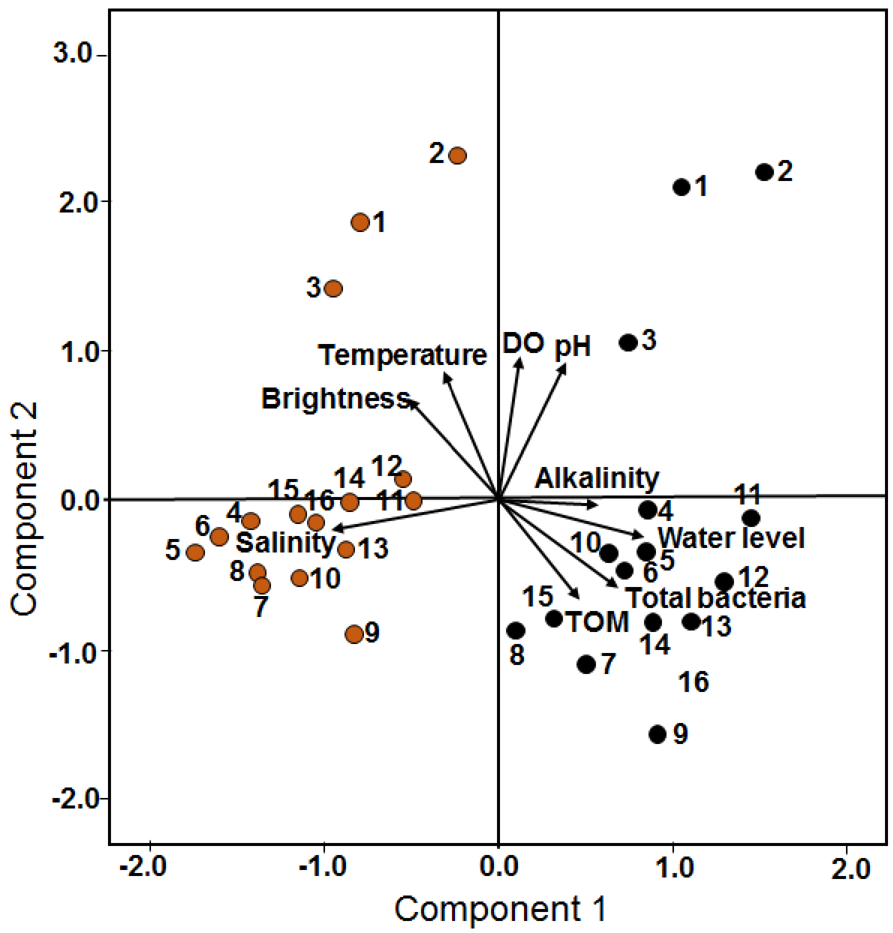


Figure 4. PCA result of water quality between concrete and plastic liner shrimp ponds (circle accompanied with a number indicate the week of sampling: sandy brown = plastic, black = concrete).

Table 3

Correlation matrix among the water quality parameters

| | *Water level* | *Brightness* | *Salinity* | *DO* | *pH* | *Hardness* | *Alkalinity* | *$NO_2$* | *TOM* | *Total bacteria* |
|---|---|---|---|---|---|---|---|---|---|---|
| Temperature | *-0.56* | 0.73 | 0.28 | *0.66* | 0.44 | 0.18 | -0.34 | 0.08 | *-0.83* | *-0.78* |
| Water level | | 0.43 | *-0.85* | -0.08 | 0.21 | -0.42 | 0.40 | 0.05 | *0.55* | *0.77* |
| Brightness | | | 0.27 | *0.74* | 0.49 | 0.47 | -0.37 | -0.26 | 0.27 | 0.11 |
| Salinity | | | | -0.16 | -0.40 | 0.48 | -0.45 | -0.08 | -0.27 | -0.48 |
| DO | | | | | *0.83* | 0.07 | -0.02 | 0.01 | *-0.54* | -0.49 |
| pH | | | | | | -0.17 | 0.27 | 0.09 | -0.34 | -0.21 |
| Hardness | | | | | | | -0.18 | -0.38 | -0.17 | -0.21 |
| Alkalinity | | | | | | | | 0.24 | 0.32 | 0.40 |
| TOM | | | | | | | | | | *0.67* |

Numbers in italics indicate a significant correlation between the water quality variables.

***Plankton and VLPs communities***. The ponds were categorized by the high stocking density of phytoplankton, consisting of 32 genera. The abundance of plankton was greater in the plastic pond (10,255.0±973.2 cells $mL^{-1}$) than in concrete ponds (1,942.5±40.1 cells $mL^{-1}$). Chlorophyceae dominated the sample (40.1%), followed by Cyanobacteria (27.5%) and Chryotophyceae (25%) (Table 4). At the genera level, *Chlorella*, *Oscillatoria* and *Chlamydomonas* were co-dominated the sample, with a proportion of 89%. The abundance and richness of taxa in plastic liner ponds were higher than in concrete ponds, while the diversity of taxa was higher in concrete ponds. The abundance, taxa richness and diversity of the two types of ponds differ significantly. The results of the repeated measures analysis showed that the fluctuation in the abundance was not significantly different from the first to the eighth sampling, while taxa richness and diversity markedly fluctuated over time.

Table 4

Abundance of plankton genera (x 1,000 cells)

| *Genera* | *Concrete pond* | *Plastic pond* |
|---|---|---|
| *Chlorella* | 270.0±94.5 | 4536.7±774.9 |
| *Oscillatoria* | 839.2±131.5 | 2280.0±156.2 |
| *Chlamydomonas* | 103.3±61.9 | 2834.2±994.7 |
| *Amphora* | 225.0±36.2 | 143.3±53.3 |
| *Chaetoceros* | 197.5±41.8 | 140.0±125.3 |
| *Cryptomonas* | 141.7±5.1 | 25.0±12.6 |
| *Anabaena* | 30.8±15.8 | 115.8±8.2 |
| *Treubaria* | 0.0±0.0 | 43.3±14.5 |
| *Navicula* | 30.0±5.2 | 10.8±0.8 |
| *Microcystis* | 14.2±4.2 | 24.2±13.4 |
| Miscellanies | 90.8±5.1 | 156.0±25.9 |
| Total abundance*** | 1942.5±40.1 | 10255.0±973.2 |
| Taxa richness*** | 5.46±0.23 | 5.79±0.36 |
| Diversity*** | 1.07±0.08 | 0.81±0.08 |

*** significance level $p<0.001$.

Based on the calculations in Table 4, the most influential factor that determines the total bacteria is the type of pond. The higher density of bacteria in concrete ponds may associate with enormous content of total organic matter. Naturally, total organic materials come from the waters themselves through processes of decomposition of food waste and feces. Organic matter and essential nutrients are materials needed in the metabolic process of microorganisms as components that function as growth media, so organic matter also directly affects the development and growth of microbes. We assume that the type of concrete pond that directly promotes high organic matter can also increase the total bacterial density.

The canonical correspondence analysis reveals that environmental variables were significant for explaining variance in species abundance patterns. The sum of the first two canonical eigenvalues is 11.39. The first axis, with a correlation of 0.94 between taxa and environmental factors, explains 39.9% of the species-environment relationship and 35.8% of the variation of taxa. The second axis shows a species-environment correlation of 0.9, and the first axis explains 62.9% of the taxa-environment relationship and 42.1% of taxa variation. $NO_2$ ($F=3.23$, $p<0.01$), alkalinity ($F=3.63$, $p<0.01$), TOM ($F=2.96$, $p<0.01$) and total hardness ($F=2.09$, $p<0.05$) significantly explain the composition variation of species while the other six factors were not significant.

The CCA scores are plotted for taxa in Figure 5. This figure shows the grouping of the genera into four groups. Group I have consisted of the more abundant genera including *Chlorella*, *Oscillatoria*, *Chlamydomonas*, *Chaetoceros*, *Anabaena*, *Treubaria*, *Microcystis* and *Streptotheca*. Group II was very abundant in the early of the shrimp culture cycle including *Amphora*, *Amphiphora* and *Cryptomonas*. Group III consisted of a group that responded negatively to the total bacterial abundance and TOM. This group included *Navicula* and *Apanochapsa*. Group IV includes many genera that are abundant at the end of the shrimp culture cycle namely *Dictyosphaerium*, *Gymnodinium* and *Gyrodinium*.

The intensity of Virus-Like Particles (VLPs) expression ranged from 0 to 1238 Arbitrary Unit (AU). The VLPs were higher in concrete (103.67 AU) than in plastic liner ponds (82.78 AU) (Table 5). These were found in slightly higher concentration in water surfaces than at the bottom of ponds and consistent in both pond constructions (Table 5).

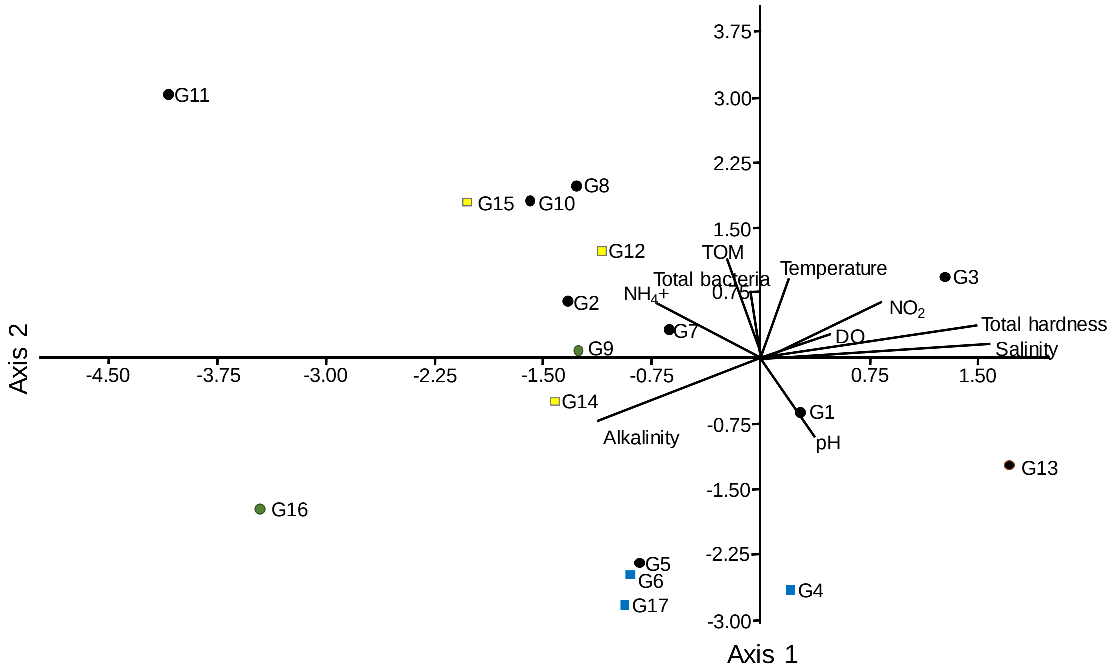


Figure 5. CCA result for the water quality parameters between concrete and plastic liner shrimp ponds. G1=*Chlorella*, G2=*Oscillatoria*, G3=*Chlamydomonas*, G4=*Amphora*, G5=*Chaetoceros*, G6=Cryptomonas, G7=*Anabaena*, G8=*Treubaria*, G9=*Navicula*, G10=*Microcystis*, G11=*Streptotheca*, G12=*Gymnodinium*, G13=*Prymnesium*, G14=*Dictyosphaerium*, G15=*Gyrodinium*, G16=*Apanochapsa*, G17=*Amphiphora.* Genera with same symbol of shape and color are in the same group.

Table 5

Super Imposed Imaging and VLPs expression intensity (arbitrary unit)

| *Position* | *Concrete* | *Plastic* |
|---|---|---|
| Surface | 129.99 | 105.83 |
| Bottom | 77.35 | 59.73 |
| Means | 103.67 | 82.78 |

**Discussion**. The results of daily water quality measurements by considering six factors showed that the average water level and pH in the concrete ponds were higher than those in plastic liner ponds. Conversely, the average level of brightness, temperature, DO and salinity in the concrete ponds were lower than those in the plastic liner ponds. The results of weekly water quality measurements for another six factors showed that the average alkalinity, $NH_4$+, TOM and total bacteria in the concrete ponds are higher than those in plastic liner ponds. The average hardness and $NO_2$ in concrete ponds were lower than those in plastic liner ponds.

The high average pH in concrete ponds might be due to the high activity of bacteria in those ponds. The activity of bacteria in decomposing organic matter produces higher $NH_4$+, This situation leads to an increase in alkalinity. The high activity of bacteria may associate with a high level of TOM. The high concentration of this organic matter reduced water brightness and DO level. The decomposition process occurred aerobically, hence it required oxygen. On the contrary, the temperature in the concrete pond was lower than that in the plastic liner pond. This contradicts the original assumption that decomposition activity by bacteria generates heat. In previous research, it was stated that the temperature in plastic ponds is generally less stable and tends to be warmer than that in concrete ponds.

The result of the factor analysis showed that 11 environmental factors determine the water quality, including: temperature, water level, salinity, hardness, alkalinity, $NO_2$, TOM, total bacteria, brightness, pH and DO. Temperature is one of the most important external factors for aquatic life in regulating life processes and the distribution of organisms. Water temperature is influenced by season, latitude, time of day, air circulation, cloud cover, water flow and water depth (Hamuna et al 2018). Increased temperature may affect the decomposition of organic matter by microbes and stratification of water coating, hence becoming a limiting factor for several biological functions of aquatic organisms. Physicochemical parameters of polluted water (dissolved oxygen, pH, total nitrogen, total phosphorus, nitrite, nitrate, ammonia and COD, etc.) and poor management practices can cause a series of physiological alterations on the growth and even survival of aquatic animals (Ni et al 2018; Xu et al 2017).

In this study, the water quality measured by IoT includes merely 4 parameters, because those had a high sensitivity of the proper sensor. So far, the development of IoT technology is limited to a few water quality parameters such as temperature, salinity, pH, DO, turbidity (Uddin et al 2020; Zainuddin et al 2018).

Organic matter and essential nutrients are source materials for the metabolic process of microorganisms, as pivotal components of the growth media. They also directly affect the development and growth of microbes, namely as a source of energy, hormones, vitamins and other growth stimulating compounds. Another study stated that the concentration of organic matter is closely related to the total density of bacteria in the waters of the Babon River: the higher the concentration of organic matter, the higher the total density of bacteria contained in the waters. This result is supported by other parameters such as physical and chemical parameters. The oxidation of organic matter in waters is influenced by temperature, degree of acidity, dissolved oxygen, types of organic matter and nitrogen. The decomposition process continues along with the availability of organic matter and heterotrophic bacteria.

This study showed a high density of plankton in the plastic pond, while a high density of bacteria occurred in the concrete ponds. Chlorophyceae and Cyanophyceae dominated the sample. This supports the results of earlier studies that indicated the dominance of Chlorophyta in shrimp farming ponds (Arifin et al 2018). *Oocystis*, *Chlorella*, *Nannochloropsis*, *Chaetoceros*, *Stephanodiscus*, *Nitzschia*, *Coscinodiscus*, *Cyclotella* and *Ulothrix* were found to dominate the plankton composition in shrimp ponds. The abundance of Cyanophyceae was consistent with another study conducted in Brazil. Cyanophyta dominated the sample and contributed to almost 70% of the species number and high densities from blooms, mainly *Pseudanabaena cf limnetica* (Casé et al 2008).

The increase in nutrients that enter the pond affects the composition and density of phytoplankton in the waters (Travaini-Lima et al 2016). In this study $NO_2$, alkalinity, TOM and total hardness are factors that have a significant effect on determining the structure of the plankton community. Total organic matter has a negative correlation with the plankton productivity. Plastic ponds have a higher plankton productivity but lower TOM levels. There is a possibility that low levels of TOM occur due to the plankton utilized and converted it to biomass.

Unlike previous research which states that the productivity of ponds dominated by phytoplankton from the Diatom group is lower than ponds dominated by the Chlorophyta group (Arifin et al 2018), in this study the opposite occurred. Chlorophyceae is dominant in plastic ponds, whereas diatoms (Bacillarophyceae) are more commonly found in concrete ponds. Diatom dominance has also been found in other studies (Fernandes et al 2019). The low productivity of shrimp in plastic ponds may be due to the high density of several dangerous cyanobacteria such as *Oscilatoria* sp., *Anabaena* sp. and *Mycrosistis* sp. in the pond. Several previous studies have stated that this type of Cyanobacteria can excrete toxin compounds that can harm other organisms (Ait et al 2018; Ariadi et al 2019b) and can impact the productivity of shrimps.

In this study, the zooplankton composition in both pond types was very small. This is likely to cause phytoplankton blooming which partially has a negative role in the response to the pond eutrophication. Grazing by the herbivorous-bacterivorous ciliate communities may have controlled the blooms of undesirable groups of phytoplankton, ensuring better shrimp growth, higher survival and a lower food conversion ratio.

Effective uptake of ammonium and nitrate by the blooming diatoms and phytoflagellates possibly prevented nutrient concentrations from reaching toxic levels, thereby generating an eco-friendly aquaculture water discharge into the adjacent ecosystem (Fernandes et al 2019).

The abundance of VLPs in the concrete pond was slightly higher than that of those in plastic liner ponds, but this difference was not statistically significant. The high abundance of VLPs in the concrete pond was probably due to the high levels of TOM and total bacteria in this pond. Viruses are the most abundant biological entities in the aquatic environment and may exceed the abundance of bacteria. Various types of viruses are known to be infectious against vannamei shrimp. More than 20 types of viruses have been reported as shrimp pathogens. Among those, white spot syndrome virus, infectious hypodermal and hematopoietic necrosis virus, and infectious myonecrosis virus are considered perilous pathogens because of their strong dissemination and infectious abilities (Peruzza et al 2019; Trang et al 2019). In 2007, outbreaks of infectious myonecrosis virus in *L. vannamei* farmed in Indonesia were confirmed (Senapin et al 2007). The outbreak of viruses generally occurs when the water quality fluctuates dramatically and exceeds the normal conditions (for example temperature, dissolved oxygen, ammonia and nitrite). This abnormal fluctuation stressed the shrimp, enabling the disease attack. Fluctuations in pH, oxygen levels, temperature, salinity, ammonia and sulfate levels, as well as other organic materials, may cause stress on shrimp and trigger disease outbreaks (Xu et al 2017).

Current knowledge indicates that pond temperature and salinity are major factors determining the outbreak severity. White spot syndrome virus appears to be the most virulent in water temperatures between 25 and 28°C and salinity values situated far from the isoosmotic point of shrimp (Millard et al 2020). In this study, the hardness, alkalinity, $NH_4$+, $NO_2$ were among the factors which fluctuate frequently. These four factors are important to take into account in monitoring water quality. Dramatic changes that exceed the tolerance thresholds can lead to an increase in bacteria and VLPs. One type of infectious bacteria is *Vibrio* sp., a major cause of disease in pond-reared shrimp. Finally, though TOM and total bacteria were more abundant in the concrete pond, our study recommends the use of concrete ponds, with a more thorough monitoring of the water quality.

Research on the effect of the type of pond construction on the water quality is rare. In previous studies, a comparison of the plastic-lined and soil substrates for shrimp ponds was carried out. Previous studies have shown that cultivating vannamei in plastic-liner ponds is more profitable than in soil ponds. However, certain results showed that substrate differences do not affect shrimp growth. This shows that algal-clay flocculation is not an important mechanism in eliciting the growth-enhancing effect of shrimp pond water and that particles suspended in pond water did not interact with the substrate.

**Conclusions**. The concrete pond had higher water levels, $NH_4$+, TOM, total bacteria and VPLs, but lower plankton abundance and taxa richness. In contrast, plastic liner ponds had a higher temperature, brightness, DO, pH, salinity, hardness, $NO_2$ and alkalinity. The TOM and total bacteria had a strong correlation with temperature and water levels. The ponds were categorized by the high stocking density of phytoplankton, consisting of 32 genera. Chlorophyceae dominated the sample, followed by Cyanobacteria and Chryotophyceae. The shrimp production in concrete ponds was higher than that in the plastic liner pond. This study recommends the construction of concrete ponds with more attention on the quality management of levels of organic matter and total hardness generated from the food wastes. The TOM and total bacteria had a strong correlation with temperature and water levels. Chlorophyceae dominated the plankton sample, followed by Cyanobacteria and Chryotophyceae. The yields of shrimp production in concrete ponds were higher than those in the plastic liner pond. The application of IoT was used to measure water quality and the confocal laser scanning microscopy was used to identify the presence of VLPs, which can quickly characterize the aquatic environments. This study merely measured four water quality parameters: temperature, DO, pH and salinity, using IoT. Given the important role of other parameters, using sensors that measure

$NO_2$, alkalinity, TOM and total hardness is a priority for improving the IoT system in the future. Further study is needed to measure more water variables using IoT and to identify in more detail the species composing the plankton group and VLPs.

**Acknowledgements**. The authors would like to thank the Rector of Brawijaya University and the Head of the Institute for Research and Community Service for supporting this research. The research was funded by the Institute for Research and Community Service, through the Non-Tax State Revenue Fund of the Universitas Brawijaya, in accordance with the Budget Implementation List Number: DIPA-042.01.2.400919/2020.

**Conflicts of interest**. The authors declare no conflict of interest.

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Received: 13 January 2022. Accepted: 08 April 2022. Published online: 18 April 2022.
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How to cite this article:
Kilawati Y., Maimunah Y., Amrillah A. M., Kartikasari D. P., Bhawiyuga A., 2022 Characterization of water qualitiesy using IoT (Internet of Things), plankton and expression of virus-like particles in vannamei shrimp ponds of different constructions. AACL Bioflux 15(2):912-926.