The application of progressive systems in high density vannamei shrimp culture

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Abstract. The increase of super-intensive pond waste and the rearing period of high-density shrimps, has been reported to be a problem of environmental quality deterioration, which is accompanied by the chance of disease outbreak. Therefore, a progressive system with two phases of cultural activities, namely nursery and grow-out, is known to be an alternative in the process of avoiding the critical period between Day of Culture (DOC) 30-50, as well as increasing the chances of a successful culture. This research aims to examine the application of a progressive system, in the super-intensive culture of vannamei shrimps. The two methods used were Progressive & Non-Progressive treatments (PG & NPG). The NPG was used by directly stocking PL-10 to a grow-out pond of 1,000 m\textsuperscript{2}, with a density of 500 PL/m\textsuperscript{2}. However, PG (nursed for 21 days) was used by stocking PL-10 to PL-31 in 100 m\textsuperscript{2} pond with a density of 5,000 PL/m\textsuperscript{2}, which were then transferred to a grow-out pond of 1,000 m\textsuperscript{2}. The results showed that the PG treatment had better water quality parameters compared to the NPG. The survival rate, production, and feed conversion ratio in the PG treatment produced better performance than the NPG, with differences in the value of feeds used and yield of shrimp sales at 25.64\% and 64.33\%, respectively.

1. Introduction
The culture of high-density shrimp is known to be confronted with the problem of increasing waste loads and the rearing period. The resulting waste load leads to a decrease in the aquaculture quality, as well as threaten the habitat feasibility for shrimps' life and growth [1–3]. The critical period for high density vannamei shrimp culture is in the DOC 30-50, due to the increased waste load accumulation, decreased cultural environmental quality, and the emergence of various pathogenic diseases, which in turn causes livestock mortality. This risk factor is then reduced by applying the stocking of fry (PL-31), which is produced in controlled culture facilities, where space and feed utilization is more efficient. Even though shrimp development is slightly hampered during the nursery period, the vannamei species are still able to take advantage of the compensatory growth during the next grow-out stage, which in then shortens the rearing period.

Generally, the shrimp culture production consists of two phases, namely the single & two-phase systems. The single-phase system involves the rearing of fry or post larvae in a grow-out pond, till consumption size is attained (selling). However, the two-phase system involves the initial rearing of fry or post larvae in a transition tank (nursery), before being transferred into grow-out ponds [4]. Several
studies on tiger prawn culture via the use of fry, have been reported on extensive [5], semi-intensive [6–8], and intensive technologies of vannamei shrimps [9–11]. It was also reported that the fry stocking in tiger prawn culture resulted in higher growth performances, compared to the direct stocking of PL-12 [5]. Furthermore, it was also reported that multi-phase penaeid culture have considerable profits, compared to conventional single-phase systems. Moreover, optimal efficiency was achieved in two-phase culture systems [12]. The stocking of vannamei fry for 10 and 20 days resulted in higher survival rate and increased uniformity of harvested shrimp size, compared to that of PL-10 post larvae [11]. Mangampa [13], reported that using fry for 15 days in semi-intensive vannamei shrimp culture, resulted in better survival rate, production, and feed conversion ratios, compared to the use of direct post larvae (PL-12) [13]. Also, the direct stocking and nursery (14 and 21 days) do not have significant effects on the characteristics of vannamei shrimp production [14]. The application of a two-stage cultural production (nursery & grow-out), also has the potential to produce higher quality shrimp fry, which are to be stocked in grow-out pond that applies a limited water exchange system [15,16]. However, Van-Wyk [10], stated that a three-stage strategy of growing vannamei shrimps has the potential to increase the chances of successful culture, in a very sustainable way.

Additionally, the application of business segmentation was carried out as effort to reduce the risk of critical DOC, in high density vannamei shrimp culture. This application started from the nursery (DOC 1-21), which was then accompanied by the grow-out (DOC 22 to harvest), with different culture facilities, especially in terms of the culture pond size and its supporting equipment. This segmentation culture is known as a progressive system, where the rearing pond is provided in an excellent way, when the environmental quality conditions of the shrimp begin to decline. This interpreted that the carrying capacity of the ponds was increased according to the shrimp biomass density, which was produced in the previous segmentation. During the nursery stage, the progressive system also applies a high stocking density, as the shrimp population and size decreases and increases in the rearing period, respectively. However, information on the application of progressive systems to super-intensive technology is still limited. Based on these considerations, it is important to study the development of high density (super-intensive) vannamei shrimp culture with a progressive system.

2. Methodology

2.1. Research Location
The research was conducted at the Experimental Pond Installation of Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) in Punaga Village, Mangarabombang Sub-District, Takalar Regency, from June–October 2020.

2.2. Facilities and Infrastructure for Research Containers
The facilities used were one and two units of nursery and grow-out ponds, with capacities of 100 m$^3$ and 1000 m$^3$, as well as water levels of 1 m & 1.5-2.0 m, respectively. The pond facilities were also equipped with a central drain, water distribution and basic aeration installation, as well as a paddle wheel. The number of aeration system used was adjusted to the target tonnage of shrimp produced, where one unit of 1 HP capacity mill was allocated to support 400-500 kg of the shrimps biomass [17].

2.3. Nursery of Shrimp Fry
The nursery of vannamei shrimp fry with a production target of PL-30 (rearing period of 21 days), was carried out in a concrete tank, which had a volume of 100 m$^3$ and a water level of 1 m. This pond was equipped with a central drain system, as well as water distribution and aeration facility. Moreover, the main supply of dissolved oxygen came from the basic aeration system, which was in the root blower.

This preparation included water sterilization via the use of 60 ppm chlorine, fertilization, and growth of natural food, for 7-10 days. Furthermore, PL-10 fry was obtained from commercial shrimp hatcheries with pathogen-free certification (WSSV, IHHNV, TSV, IMNV, EHP, and EMS) [18] as the stocking density in the initial nursery segmentation was 5,000 PL-10/m$^3$ [19]. According to the blind feeding method, a commercial feed of 38% crude protein content was provided 6 times a day, at 07:00, 10:00,
13:00, 16:00, 19:00, and 22:00. Sludge disposal at the bottom of the nursery pond was also observed to have started from DOC-8, through the central drain. Also, this water addition was carried out as much as the estimated volume that was reduced, due to disposal and evaporation.

2.4. Grow-out

There were two concrete grow-out ponds with an area of 1,000 m² each, and a water level of 1.5-2.0 m. The preparations included installation of basic aeration systems and laying of paddle wheel, cleaning, washing, filling & sterilizing water, fertilizing, and natural food growth. This preparation stage was conducted between 10–14 days.

In non-progressive (NPG) or control treatments, PL-10 fry from the hatchery was stocked directly into a 1000 m² grow-out pond, without nursery stages. However, in the progressive treatment (PG), the fry (PL-30) from the nursery stage was transferred to a 1000 m² pond. During the rearing process, feed with a crude protein content of 38-37% was carried out six times daily according to the standard feeding program, at 07:00, 10:00, 13:00, 16:00, 19:00, and 22:00. Observation of shrimp appetite through the feeding tray approach, was also carried out by checking the remaining feed, every 1-2 hours after feeding. The shrimp appetite information was collected and used as a reference, in determining additional or reduced feed doses at the next feeding schedule.

Moreover, the disposal of sludge settling at the bottom of the pond via the central drain was carried out thrice daily (morning, afternoon, and evening), before feeding. The water addition as much as the volume was also reduced every morning and evening. According to the existing standard procedures, the provision of additives in the form of probiotics, minerals, and more, was also carried out as needed and applied.

2.5. The Measured Parameter

During the stages of nursery and grow-out, measurements of the water quality were carried out twice daily (morning and evening). These measured parameters were temperature, salinity, pH, and dissolved oxygen, which was collected via the YSI Professional-Pro DO metre. However, the measurements of other parameters, such as Total Organic Matter, Suspended Solid, and Ammonia Nitrogen (TOM, TSS, & TAN), nitrite, nitrate, phosphate, and alkalinity, were also conducted weekly. Also, the measurement on Total Plate Count and Bacteria Vibrio (TPC & TBV) data was carried out twice weekly, and analyzed in the laboratory. Moreover, observation of shrimp disease was carried out once in a month.

At the end of the nursery, the weighing process of individual shrimp was conducted, in order to calculate the average final weight and diversity of the resulting fry. In the grow-out stage, the measurement on shrimp growth was carried out weekly, by randomly weighing 100 individuals per pond. A digital weighing tool with a capacity and accuracy level of 500 & 0.1 g, was often used for the measurements, respectively. As the basis for calculating the feed dose for the next rearing period, the results of weighing individual shrimp were used.

At the end of the research, individual weight measurements (shrimp size per kilogram) were carried out, by sampling (20 kg) and counting the number of shrimps, in order to determine the shrimp size. Weighing shrimp production was also carried out by using a digital scale, with a capacity and accuracy level of 100 & 0.01 kg, respectively. As an approach in calculating survival rate (SR), the shrimp size data was also used. In order to determine the diversity level in the shrimp weight, a total of 300 pcs were randomly weighed at the beginning (100 pcs), mid (100 pcs), and end (100 pcs) of the harvest. This weighing process was performed via the use of a digital scale, with a capacity and accuracy level of 500 & 0.1 g, respectively. However, samplings of the Total Haemocyte Count (THC) and Prophenoloxidase (ProPO) was carried out at the end of the research, in order to determine the character of shrimp immunity.

2.6. Data analysis

The data of nursery fry in stage one included survival rate and final weight, with grow-out in level two consisting of production, SR, water quality, total bacteria, immune response (THC, ProPO), and unit
cost of production. These were further presented in a table and figure format, as they were also descriptively analyzed.

3. Results

3.1. Growth, Survival Rate, Production, and Feed Conversion Ratio
The direct stocking of fry (PL-10) to the pond (NPG) resulted in higher relative weight growths, compared to that of the progressive system (PG) (PL-31). At DOC-21, the fry stocked directly in the NPG pond reached a weight of 0.38 ± 0.16 g/ind. This was higher than the fry produced in the initial stage of rearing at the nursery pond (0.17 ± 0.08 g/ind), with stocking to the PG grow-out pond further carried out. This meant that the fry with stocking density of 5000 PL-10/m³ in the nursery pond (first stage) was experiencing stunting, as this condition had an impact on shrimp growth. At the end of the DOC-92 study, the directly stocked shrimps (NPG) reached an average weight of 12.82 ± 3.34 g/ind. This was much higher than the shrimp weight in the progressive system (PG), which was at 11.36 ± 2.70 g/ind (Figure 1). However, the shrimps in PG pond began to exhibit the ability to take advantage of the compensatory growth, in order to catch up with development lags during rearing.

![Figure 1. The development of shrimp weight during rearing process in a progressive system](image-url)

During the 92 days rearing period, the development of ADG values in the NPG were higher than that of the PG pond. The growth of average daily weight (ADG) in the direct stocking system (NPG) also had a value range of 0.09-0.28 (0.18 ± 0.07) g/day, which was higher than the PG, at 0.05-0.29 (0.16 ± 0.09) g/day (Figure 2). Only in the last month did the ADG value in PG pond began to increase quite sharply since DOC-63, as the use of compensatory growth was displayed, due to stunting during the nursery process.
During rearing, the shrimp showed varying size values, as there was a declination tendency towards the end of the culture (Figure 3). The weight diversity value of shrimps in the NPG pond was between 18.56-37.94 (25.68 ± 6.69)\%, which was lower than that of the PG, at 19.78-45.46 (30.57 ± 7.73)\%. However, during the period of harvest, the weight diversity value of shrimps in the PG pond was 19.78\%, which was lower than the 22.13\% of the NPG.

Based on the harvest data, the survival rate in the NPG plot was 58.20\%, which was lower than that of the PG, at 78.14\% (Table 1). The fry stocking that resulted from the first phase of rearing in the grow-out pond, had an effect on the survival rate of the shrimp. This was due to the fry conditions that have adapted to the pond environment, before being stocked in the grow-out pond. The shrimp survival rate in the progressive system (PG) contributed to the production value of 4,070 kg, which was higher
than that of the NPG pond, at 3,731 kg. Moreover, the PG also resulted in a smaller shrimp size per kilogram (96 ind/kg), compared to the non-progressive system at 78 ind/kg. This indicated that the direct stocking system still produced a larger weight of shrimp. However, the higher survival rate in progressive systems resulted in greater shrimp production, compared to the NPG. Additionally, the low survival rate was due to the attack of AHPND and EHP, during the third month of rearing.

The feed conversion ratio (FCR) value in the PG pond was also observed to be 1.45, which was lower than the direct stocking system, at 1.95. However, the applied progressive system was able to streamline feed use by 25.64%. This efficiency value was sufficient to provide a significant contribution to the cost of shrimp production, considering the fact that feed contributed more than 65% of the cost. In previous studies with a stocking density of 500 individuals/m², the FCR value was 1.52 [20].

| Variables                           | Progressive (PG) | Non-Progressive (NPG) |
|-------------------------------------|------------------|-----------------------|
| Survival rate (%)                   | 78.14            | 58.20                 |
| Production (kg)                     | 4,070            | 3,731                 |
| Size (pcs/kg)                       | 96               | 78                    |
| Feed Conversion Ratio               | 1.41             | 1.94                  |
| Feed efficiency (%)                 | 68.96            | 51.28                 |
| Water use (m³/kg shrimp produced)   | 3.36             | 3.67                  |
| Electrical uses (kW/kg shrimp produced) | 4.62           | 6.00                  |
| Cost of production (IDR/kg shrimp produced) | 38,972   | 50,500                |
| Profit (IDR)                        | 74,188,000       | 45,144,000            |

3.2. Water and Electrical Uses
The water used in producing super-intensive vannamei shrimp was observed to be 3.36 m³/kg and 3.67 m³/kg, for PG & NPG pond, respectively (Table 1). Water used in PG were lesser than in NPG, due to the fact that the productivity level of the progressive system was higher than that of the non-progressive. Furthermore, the high water used in this culture system was due to the sludge disposal carried out 1-3 times daily. The water used in this research was higher, compared to the reports made by Tahe et al. (2014) and Rachmansyah et al. (2017), with usages at 2.12-2.73 m³/kg and 1.6-2.24 m³/kg, at stocking densities of 500-600 PL/m² and 750-1,250 PL/m², respectively [3,20]. Also, Boyd and Clay, (2002) obtained water uses of 2.26 m³/kg shrimp produced [21]. A similar case was also reported by Samocha et al. (2010) through the implementation of super-intensive shrimp culture in raceway, and zero water exchange ponds [22]. A water use value varied from 98—126 L/kg shrimp produced with a production rate varied 9.34—9.75 kg/m³, was further obtained in the study. The application of zero water exchange also had the ability to minimize water use, during the culture process.

Furthermore, the main energy consumption used in this research, was for the needs of aeration operation. The maximum number of paddle wheel used was 12 HP, with the addition of a 5.5 kW root blower, which was used for three ponds assuming the same usage. During the culture period, the amount of electrical energy for the aeration system and pumps reached 22,379.9 and 18,798.7 kW, for both PG & NPG ponds, respectively. Therefore, the electrical energy used to produce one kilogram of shrimp was 4.62 and 6.00 kW/kg, for both PG & NPG ponds, respectively (Table 1). This result was observed to be higher, compared to previous studies, between 2.37-2.82 and 2.4-3.2 kW/kg, at stocking densities of 500-600 & 750-1,250 PL/m², for both Tahe et al. (2014) and Rachmansyah et al. (2017), respectively [3,20]. Also, the electrical energy used in this super-intensive vannamei shrimp culture was dominated by the use of the aeration system, which ranged from 98.6-98.8%. This result was relatively similar and higher to the research outcomes of Tahe et al. (2014) and Rachmansyah et al. (2017), at 95.75-96.17%
& 85.29-89.87%, respectively [3,20]. Therefore, this data indicated that the higher the shrimp production, the lower the electrical energy used.

Also, the cost of vannamei shrimp production with a progressive system was IDR 38,972/kg shrimp produced, which was lower than that of the non-progressive at IDR 50,500/kg shrimp produced, with each profit for both PG & NPG ponds at IDR 74,188,000 & IDR 45,144,000, respectively (Table 1). There was also a difference in profit of IDR 29,044,108 or 64.33%, when compared to the NPG pond.

3.3. Water quality

During the super-intensive vannamei shrimp culture period, water quality variables including temperature, salinity, pH, and dissolved oxygen measured in situ, had relatively similar values (Table 2). The water quality conditions in the experimental pond water column were also relatively homogeneous. Also, the operation of the paddle wheel used was sufficient in moving the water flow beside the basic aeration role, which had the ability to ensure a more homogeneous water mass mixing process.

Even though this variation does not affect shrimp growth, the salinity of pond water slightly increased higher from the beginning of stocking to the end, at 33 to 37 ppt, respectively. This condition was triggered by a dry season, which was accompanied by strong winds during the culture period. During the dry season in the experimental pond area (June to September 2020), there was also a "bediding" period, where the air temperature experienced large fluctuations between 35 & 23°C, during the period of the day and night, respectively. However, fluctuations in the air were observed to have affected the variations in pond water temperature. As a result of this, the water temperature varied between 23.0-29.7°C, during the culture period (Table 2).

Table 2. Water quality variables were temperature, salinity, pH, and dissolved oxygen (range values, mean ± sd) during rearing

| Parameters          | Nursery | PG     | NPG    |
|---------------------|---------|--------|--------|
| Temperature (°C)    | 26.0—29.9 | 22.9—28.0 | 23.0—29.7 |
| Salinity (ppt)      | 34.1—36.3 | 34.6—38.7 | 33.4—38.4 |
| pH                  | 8.0—9.0  | 7.7—8.9 | 7.5—9.0 |
| Dissolved oxygen (ppm) | 4.1—6.2 | 3.7—6.2 | 3.7—6.1 |

The pH value of pond water was also relatively stable, with a range of 7.5-9.0 and a deviation between 0.4-0.5. This meant that the daily pH fluctuation was still below 0.5. Also, the efforts to maintain daily water pH in super-intensive vannamei shrimp culture were imperative, as the stability of water quality was maintained properly. When the pH of the water was maintained in the range of variations < 1, the other variables did not experience shocks, and were in a suitable condition for shrimp life.

Dissolved oxygen content was also in a range of 3.7-6.2 ppm, with an average value between 4.8-4.9 ppm. However, this range was still considered feasible for the life of vannamei shrimps. Dissolved oxygen is one of the water quality variables, which is very important to sustain the shrimp life. Therefore, the aeration system was essential in the shrimp production system, as a supplier of dissolved oxygen in pond water. Moreover, the system also supported the aerobic decomposition of organic matter and bacteria nitrification. When the aeration stopped, dissolved oxygen dropped below 3 ppm in one hour. Aeration also produced flow and stirring processes of pond water mass, in order to maintain bacteria and other microorganisms in a suspended condition. At night, when there was no photosynthetic process to produce oxygen, operating the paddle wheel at full capacity was necessary, in order to maintain dissolved oxygen level till the period of day, as it does not decrease below 3-4 ppm.
In super-intensive culture, there was high total ammonium nitrogen (TAN) excretion, due to the fact that shrimps used a lot of protein as an energy source. The organic N in the feed and faeces formed TAN, after undergoing the decomposition process by bacteria. However, this TAN is toxic when accumulated to a certain level. Therefore, in intensive aquaculture, sludge disposal should often be carried out, in order to avoid the accumulation of organic matter at the bottom of the pond, as a TAN source. Also, the feed used had a protein content of 38-36%, making it sufficient to provide a significant contribution to the TAN concentration, which had attained the range of 0.0856-2.9667 ppm and 0.0789-3.6830 ppm in both NPG & PG ponds, respectively (Table 3). Furthermore, the nitrification process became the main factor, as nitrifying bacteria quickly converted ammonia nitrogen to nitrate. Moreover, ammonia concentrations above 4 or 5 ppm were toxic to shrimps [21]. In this research, the nitrate concentration was between 0.1038-4.0976 and 0.0807-3.6208 ppm, for both NPG and PG ponds, respectively. According to Boyd and Clay (2002), nitrates were not toxic to shrimps at concentrations below 50 ppm [21].

Table 3. Average progressive and non-progressive system pond water quality parameters during rearing

| Parameters   | PG          | NPG         |
|--------------|-------------|-------------|
| TAN (ppm)    | 0.0789-3.6830 (1.9744±1.4501) | 0.0856-2.9667 (1.4076±1.0426) |
| Nitrite (ppm)| 0.0063-3.9856 (1.1952±1.5626) | 0.0063-3.8399 (2.0776±1.7342) |
| Nitrate (ppm)| 0.0807-3.6208 (1.2547±1.4671) | 0.1038-4.0976 (2.2087±1.7089) |
| Phosphate (ppm)| 0.1144-1.5366 (0.8214±0.3813) | 0.0926-1.3628 (0.8454±0.4585) |
| TOM (ppm)    | 25.02-63.81 (49.48±9.87) | 30.03-68.19 (51.98±11.63) |
| Alkalinity (ppm)| 118.59-186.93 (137.06±15.17) | 90.45-150.75 (124.14±19.91) |
| TSS (ppm)    | 66-250 (158±58) | 45-240 (151±53) |

The TAN development during the rearing period was observed to have had a relatively different pattern between Non-progressive (NPG) and Progressive (PG) ponds. The TAN in NPG pond experienced a sharp increase at DOC-8 to 29, which remained stagnant till DOC-43, began to decline until DOC-89, and increased again at harvest (Figure 4). However, in the PG pond, TAN experienced a relatively rapid increase from the beginning of stocking to DOC-21, during the nursery period (Figure 5). This period was carried out in a 100 m² pond, as the volume of rearing water was far different from the NPG system, which was directly stocked in the 1,000 m² pond. In the second stage of grow-out at the PG pond, TAN relatively fluctuated below 1.5 ppm, increased sharply at DOC-61-82, and decreased again at the end of rearing.
Figure 4. Dynamics of TAN, nitrite, nitrate, and phosphate in non-progressive ponds (NPG)

Moreover, nitrite and nitrate dynamics relatively had the same pattern in both the NPG and PG ponds. Both dynamics experienced a sharp increase in DOC-43 & 57 (NPG & PG), and remained stagnant until the end of rearing at DOC-92. In the PG system, these two parameters experienced a 14-day increase in slowdown compared to NPG, due to differences in the stocking time at the grow-out pond. This proved that the progressive system was used as an attempt to avoid a critical period in the DOC-40-50, after stocking.

The dynamics of phosphate in NPG pond also experienced a sharp increase from DOC-26, stagnated till DOC-75, and decreased until harvest time at DOC-92. However, in the PG pond, the phosphate increased at DOC-8-12, and decreased till DOC-21 in the nursery pond. In the second stage of grow-out in the PG pond, the phosphate increased at DOC-40, stagnated till DOC-75, and decreased until
DOC-92. Also, the sludge disposal before feeding was able to maintain the phosphate concentration, which was in the range of 0.8454 ± 0.4585 and 0.8214 ± 0.3813 ppm in both NPG & PG ponds, respectively.

The alkaline water in the NPG pond also ranged from 90.45-150.75 (124.14 ± 19.91) ppm. However, in the PG pond, it ranged between 118.59-186.93 (137.06 ± 15.17) ppm, as it was more relatively stable than the NPG (Table 3). At DOC-64-75, the alkalinity value in the NPG pond decreased below 100 ppm, which was further accompanied by a decrease in the range of pH value, at 7.5-7.6. These daily pH and alkalinity changes triggered water quality shocks, which led to disease infection.

Also, TOM levels fluctuated between 30.03-68.19 (51.98 ± 11.63) and 25.02-63.81 (49.48 ± 9.87) ppm in the NPG & PG ponds, during the rearing period, respectively (Table 3). The dynamics of this variable was also observed to have a relatively similar pattern between NPG and PG ponds (Figure 6). The sludge disposal before feeding, which was carried out daily, was able to keep the TOM content fluctuation at < 70 ppm during rearing, as that was the required limit for vannamei shrimp culture media [23].

![Figure 6. BOT and Alkalinity Dynamics of non-Progressive and Progressive systems of vannamei shrimp ponds](image)

### 3.4. TPC and TVB bacteria

The observation results on total plate count and vibrio bacteria (TPC & TBV) during the research, are presented in Figure 7. The average TPC in the PG and NPG ponds were 1.78x10^5±1.84x10^5 and 2.74x10^5±4.63x10^5 cfu/mL, respectively. The TPC in the NPG pond was observed to have increased at DOC-14, reaching 106 cfu/mL, which decreased with relatively stable movement in the range of 10^4-105 cfu/mL, until harvest. A relatively similar pattern was also shown by TPC on PG pond.

*Vibrio sp.* is an opportunistic bacteria in shrimp culture. Based on the fact that these microorganisms remained in the aquaculture environment and bodies of cultured shrimp, periodic monitoring was reported to be subsequently required. Furthermore, the monitoring results showed that the average TBV was 1.36x10^4±6.45x10^3 and 9.57x10^3±9.16x10^3 for both PG & NPG, respectively. The highest population was observed during the period of early July and mid-August, and was within the range of 10^4 cfu/mL (Figure 7).
The TPC observed in pond water in this research was only slightly higher than TBV. However, the TBV/TPC ratio was still within the range of 4-26%. The average TBV/TPC ratio was also observed to be 10.5 ± 5.9% (PG) and 9.0 ± 9.3% (NPG), which was within the recommended limits of not more than 10%.

3.5. Shrimp Disease
The results of monitoring shrimp disease in ponds also discovered the presence of protozoa, which caused hemocytosis since the first month of rearing. Afterwards, the population decreased and was not observed in the second and end of the third rearing months (Table 4). The widespread threat of AHPND in aquaculture had also proved that the presence of PirA and PirB caused the disease symptoms (AHPND) in progressive ponds (PG) during the first week of July 2020. However, this symptoms were not further detected in the third month. Also, monitoring in early August led to the discovery of EHP disease in non-progressive pond (NPG). This continued until rearing in September 2020, as WSSV was also detected earlier in the culture month. The simultaneous emergence of EHP with WSSV in the NPG pond triggered a mortality rate, as survival level reached 58.20%.

Table 4. Development of shrimp disease during rearing period in non-progressive and progressive ponds

| Culture system   | Month-01                                      | Month-02       | Month-03                                      |
|------------------|-----------------------------------------------|----------------|-----------------------------------------------|
| Non Progressive  | Protozoa cause hemocytosis                    | Not detected   | EHP was positive in Week-1, August, and lasted until September 2020. WSSV was positive at Week-1, September 2020 |
| Progressive      | Protozoa cause hemocytosis                    | PirA and PirB, the cause (AHPND / EMS) to be positive at Week-1, July 2020 | Not detected |

Figure 7. TPC and TBV in a non-progressive and progressive system of shrimp pond water media
3.6. Shrimp Immunity
At the end of the research, Shrimp Total Hemocyte Count (THC) content showed a significant difference in the amount. The shrimp THC in the PG was 10,550 ± 4,449 million cells/mL, which appeared to be higher than that of the NPG, at 9,250 ± 10,434 million cells/mL (Figure 8A). A relatively similar pattern was also shown in the ProPO value of shrimp in the PG, which was at 0.12 Abs, and was higher than that of the NPG at 0.05 Abs (Figure 8B).

![Figure 8](image.png)

**Figure 8.** THC (A) and ProPO (B) content in shrimp at the end of the research in non-progressive and progressive ponds

4. Discussion
Nursery for 21 days in the first phase of rearing was able to produce a relatively uniform size. The increase in ADG value on the PG pond was also accompanied by a decrease in the weight diversity rate of shrimps. Based on the growth data obtained, it was indicated that the shrimp in the PG pond was able to catch up with the weight development that started from DOC-63, leading to relatively similar body weights of both livestocks at the end of the research. The value of shrimp diversity in the PG pond at the time of total harvest, was also observed to be lower, compared to that in the NPG. This showed that the shrimp weight in the PG pond was relatively more uniform than that in the NPG system. At the end of the research, about 78-80% of the harvested shrimp size had relatively uniform weights. This uniformity level was an indicator of the fry and shrimp qualities, which was produced at harvest. The use of fry produced relatively uniform shrimp at harvest [24], as the time to reach market size was also shortened. Furthermore, this uniformity increased pond productivity, was efficient in feed use, and ensured the availability of fry in the application of cropping patterns, at the grow-out. Moreover, it also shortened the rearing period at the grow-out, therefore, reducing the chance of disease attack [6].

The relatively stagnant development of ADG values after DOC-63 & 77 in the NPG and PG pond, occurred after the shrimp weight reached 8 g/ind, respectively. However, in the NPG pond, the ADG value decreased again till the end period of rearing, at DOC-92. In high density vannamei shrimp culture, this pattern was used as a reference in determining partial harvest, even though dissolved oxygen conditions were still above 4-5 ppm. Also, In the NPG & PG ponds, partial harvesting should be carried out in DOC-77 & 85, respectively, as there was no decrease in ADG at the next weighing. This meant that the carrying capacity of the ponds at that time, had exceeded the size to accommodate the shrimp biomass.

Yta et al. (2004), reported that the survival rate of vannamei shrimp in grow-out ponds was higher within the treatment of 10 and 20 days, compared to direct stocking (PL-10) [11]. The use of fry for at least 10 days also improved and increased the survival rate and uniformity of harvested shrimp size. However, using fry for 20 days showed an increase in survival rate and uniformity of shrimp size at harvest, compared to being used for 10 days. Mangampa (2007), reported that using fry for 15 days in semi-intensive vannamei shrimp culture, resulted in better survival rate, production, and FCR,
compared to using direct post larvae (PL-12) [13]. According to Yta et al. (2004), the length of nursery time affected the survival rate and growth of vannamei shrimp, until market size (consumption) had been attained [11].

The observation results on the shrimp immunity also showed that the prophenoloxidase activity of reared vannamei shrimp, was quite good. A good quality feed and sufficiently diverse microorganisms in the pond as an additional form of nutrition, increased the shrimp immunity. Ponds containing a variety of bacteria, microalgae, and microbial-detritus, also increased the growth rate of shrimp [25]. They supply food and contain enzymes, which helps in increasing the activity of digestive catalysts, in order to aid digestion [26]. The probiotic bacteria used were also eaten by shrimps, as their immunity system was stimulated, due to the presence of lipopolysaccharides contained in the microorganisms. Based on the results of routine monitoring as a reference in providing feed doses, good water quality management also played a role in supporting the success of shrimp culture.

Also, the high THC and ProPO values in the PG pond were related to the health condition of the shrimp at the end of rearing, as they were supported by the relatively stable conditions of water quality, secchi disk transparancy and colour, during culture period. The conditions of the nitrite, nitrate, and phosphate variables were also relatively low in the PG, compared to the NPG pond. Also, the alkalinity content in the PG was observed to be higher and more stable, compared to the NPG pond. In August, the alkalinity value in the NPG pond was in the range below 100 ppm, accompanied by a decrease in water pH. The high THC and ProPO values in the PG pond also indicated that the shrimp immune system was better, as AHPND disease marked by the discovery of PirA and PirB in July was able to be controlled, leading to a negative result in the third month. Conversely, the THC and ProPO contents in the shrimp on the NPG pond were low, making it unable to withstand the positive EHP attack, which lasted from the first week of August till September (DOC-92). Therefore, the survival rate obtained was 58.2%, which was much lower than the rate of PG at 78.14%.

Furthermore, water quality management applied in this research was quite effective in preventing shrimp disease attacks. It was suspected that the rotating application of RICA probiotics, periodic agricultural lime, and additives in shrimp feed, reduced disease development, as the attack was controlled, with AHPND symptoms in PG pond also turning out negative in August. The progressive system was considered to also be quite effective, as an effort to anticipate a critical period in shrimp rearing. Also, the shrimps used were also observed to have better vitality than PL-10, as they have adapted to the pond environment, and were more resistant to fluctuations in environmental changes. This observation was confirmed by the survival rate of shrimp in the PG pond (78.14%), with production reaching 4,070 kg, which was higher than the NPG (58.20%) at 3,731 kg. In the NPG pond, the simultaneous attack of the EHP and WSSV in the first week of September, had an impact on the mortality rate of shrimp, causing the survival rate to be lower than on the PG.

It has also been reported that shrimp culture with a two-phase system had started to be commonly carried out. The first phase involved the stocking of PL-10, in order to produce shrimp fry in less than a month (PL-30). Afterwards, the second phase involved stocking the results of the first stage into the grow-out (production) pond, until the period of harvest (marketable size). Also, the nursery system was one of the culture management strategies, with a significant impact in regulating the cropping pattern of shrimp, which had adapted to the environmental conditions, with the availability and preparation of quality fry supply from the hatchery. The use of fry is often recommended in intensive and super-intensive shrimp culture. This was because it had good vitality, in terms of being stocked in production/grow-out ponds. They also have adaptability attributes to the aquatic pond environment, as they reduce mortality during rearing, accurately estimate the fry stocked, and conduct better management of feed input. Moreover, they also reduce predation, due to the fry size being more uniform. The fry also take advantage of its compensatory growth properties, in order to shorten the culture cycle, while consequently maximizing the utilization of the grow-out pond. Also, the application of the progressive system needs to consider the stressful conditions of the shrimp, when they are being transferred to the grow-out pond. Therefore, the transfer of fry to grow-out pond by flowing water through a hose is considered quite effective, as it reduces the stress levels of shrimp seeds.
5. Conclusion

Progressive system culture resulted in better and more stable water quality conditions, as it was used as a strategy to avoid critical periods at DOC-30-50, while also possessing the ability to reduce the level of failure risk. Progressive system culture also resulted in higher production performance and income, compared to direct stocking systems.

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