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Study of an economical shrimp farming protocol aiming at improving control over water quality

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List of abbreviations

- AHPND: Acute hepatopancreatic necrosis disease
- BOD: Biological oxygen demand
- DO: Dissolved oxygen concentration
- DOC: Days of culture
- EMS: Early mortality syndrome, similar to AHPND
- EHP: *Enterocytozoon hepatopenai* parasite
- FCR: Feed conversion ratio
- ha: Hectare
- hp: Horsepower
- PL: Post-larvae
- SGR: Specific growth rate
- TAN: Total ammonia nitrogen, which is the concentrations in NH_4^+-NH and NH_3-NH combined
- USD: United States dollars
- VND: Vietnamese dongs
- WSSV: White spot syndrome virus

General introduction

Penaeid shrimps and especially *Penaeus vannamei* and *Penaeus monodon* are species of major importance for worldwide aquaculture, their total production exceeding 5 million tons in 2017, which represents more than 60% of all crustaceans produced from aquaculture (FIGIS, 2019). Vietnam was in 2017 the second largest producer of peneaeid shrimps behind China, with more than 700 000 tons produced, 63 % of which being *P. vannamei*. This production represented a total of more than 4 billion USD in 2017 or 42 % of its total aquaculture value (FIGIS, 2019).

Shrimp farming in Vietnam really began in the mid-1990s along with a growing international demand and a strong support from the government (Anh et al., 2010). Shrimps are mostly raised in the South part of the country which features more suitable environmental conditions than the North with higher temperatures in winter and less fluctuations (Lan, 2013). Shrimp farms are located for the most part in the southeast region near Ho Chi Min City and in the Mekong Delta, in a dense river network. Over the years, shrimp production has intensified, farms shifting from extensive and rather traditional systems to semi-intensive or intensive aquaculture (Anh et al., 2010; FAO, 2019a). Today, pond production intensity averages 9.3 tons per hectare and per year for *P. vannamei*, and 1.5 t/ha/y for *P. monodon* farms in the Mekong Delta (Boyd et al., 2017). Production is performed into small ponds compared to India and Ecuador, with 0.3 ha per pond on average for both species (Boyd et al., 2017).

However this production, still growing, faces major challenges. It is held responsible for major loss of mangrove areas, as well as pollution of coastal water and salinization and erosion of lands (Anh et al., 2011; Ha et al., 2013; FAO, 2019b). Also, major disease outbreaks such as the white spot syndrome virus (WSSV) and since 2010, the so-called early mortality syndrome (EMS or AHPND) were and still are responsible for major economic losses for the all industry (OIE, 2019). In addition, Vietnamese farmers have to face a competition always fiercer with price fluctuations as high as 15 % throughout the same year, which sometimes makes them switch from a shrimp species to another and constrain them to further lower their production costs and increase their efficiency (Ha et al., 2013; ShrimpTails, 2019).

It is within this context that Skretting, subsidiary of Nutreco International, works towards being one of the leading players in shrimp and fish feed production in Asia. With 2 plants and almost 400 employees in Vietnam, Skretting produces feed for 14 shrimp and fish species in Southeast Asia, among which are *P. vannamei* (whiteleg shrimp) and *P. monodon* (giant tiger prawn). After more than 7 years of operation in Vietnam, the company now aims at offering more services to its customers, such as providing advice on farming techniques. The firm also seeks to strengthen its local R&D to develop its products in conditions similar to their customers. To achieve that goal, Skretting runs trials on some of its clients farms and sets up a first new experimental shrimp farm in the suburbs of Ho Chi Minh City (Appendix I). This 2 ha validation farm is meant to become a local research and development center, as well as a training facility for Skretting technicians and customers.

For a first cycle at the new validation farm with *P. vannamei*, several bioremediation techniques were tried and assessed to improve control over water quality. When gathered and detailed, these techniques form a protocol, which encompasses both nursery and grow-out phases of shrimps.

The following report is therefore divided into two distinct parts. The first part is focused on the nursery phase where a protocol enhancing nitrogen assimilation from heterotrophic bacteria was tried. The

second part of this report is focused on the grow-out phase and compares two different strategies to balance heterotrophic and photosynthetic activities, either based on the regular addition of sugar cane molasses or liquid fermented rice bran.

I. Set up of an heterotrophic system as a bioremediation tool during the nursery phase of *P. vannamei*

I.1 Introduction

After receiving post-larvae from a nearby hatchery, farmers can decide either to stock shrimps directly into grow-out ponds, or choose to run a nursery phase, during which shrimps are closely monitored and kept at high densities (from 500 to 10 000 post-larvae per square meter) until they are judged strong and large enough to be stocked into larger ponds. This phase enables farmers to shorten the production cycle, potentially resulting in more crops per year (Louis, 2006). The nursery phase also improves biosecurity in the farm by being a quarantine measure for new post-larvae, whose health can be assessed before being stocked in the rest of the facility (Louis, 2006). Such a system generally gives better results in terms of survival than direct stocking (Louis, 2006). It is also useful to acclimate post-larvae to grow-out conditions, and represents a precision tool to closely manage water quality, feeding and survival during a highly sensitive period for *P. vannamei* (Louis, 2006). As nursery ponds are generally smaller and less numerous in the farm than grow-out ponds, it is also easier for the farmer to exclude pathogens and predators by applying strict biosecurity measures.

A way to further increase biosecurity inside the nursery pond is to practice water-exchange rates as low as possible, thus avoiding as much as possible the introduction of pathogens and reducing pumping costs for the farmer. But if nothing is done, low water exchanges at high feeding rates often result in an accumulation of nitrogenous wastes into the pond, such as ammonia. Ammonia is an end product of protein catabolism and is excreted under its ionized form (NH_4^+) through the gills of shrimps (Ebeling et al., 2006). Its accumulation also results from the mineralization of unconsumed feed and feces (Lin and Chen, 2001). In water, both the ionized and unionized (NH_3) forms of ammonia exist and the proportion of each one is a function of pH, temperature and salinity (Ebeling et al., 2006). Accumulation of ammonia in the rearing water can lead to a reduction in growth, a higher oxygen consumption and even the death of the shrimps (Lin and Chen, 2001).

According to Ebeling et al. (2006) and Crab et al. (2007), in low water-exchange systems, there are 3 major ways to control ammonia levels (Fig. 1):

- By photo-autotrophy with micro-algae
- By nitrification with chemo-autotrophic bacteria, converting ammonia into nitrite and nitrate
- By assimilation within heterotrophic bacteria

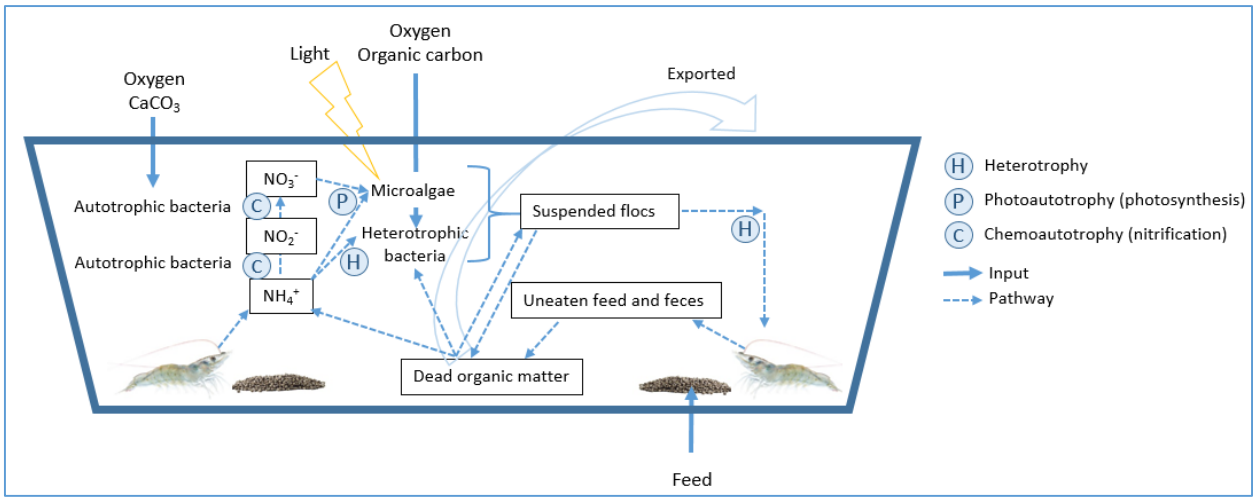

Figure 1: Nitrogen (and carbon) cycles into a shrimp pond (adapted from Moriarty, 1997 and Crab. et al., 2007).

Serra et al. (2015) define biofloc technology for aquaculture as the conversion of nitrogen supplied by feed into microbial protein available as an additional source of feed for the cultured organism. To be run successfully, this system requires regular additions of organic carbon into the pond. Such a technique is characterized by reduced needs in clean water, down to 0.5 % per day (Hargreaves, 2013), as well as nutritional advantages for shrimps (Avnimelech, 1999; Crab et al., 2007; Serra et al., 2015; Xu et al., 2016). Biofloc systems for shrimp farming have been the subject of numerous publications which gave interesting results in terms of growth, survival and water quality (Samocha et al., 2007; Serra et al., 2015; Xu et al., 2015; Panigrahi et al., 2017).

Biofloc technology relies on the regular addition of simple carbohydrates on which heteretrophic bacteria can thrive, such as molasses which are highly digestible (Serra et al., 2015). This carbon addition in the pond is meant to enhance a bacterial bloom which consumes NH_4^+ for the synthesis of bacterial proteins, thus leaving less room for photosynthesis in the pond and making water parameters stable (Ebeling et al., 2006). Such a system is supposed to ensure more stable DO between days and nights, and to avoid algae crashes which are associated to low DO and poor water quality events (Ebeling et al., 2006; Samocha et al., 2007). Furthermore, the dense bacterial population is suspected to have a controlling effect over pathogenic bacteria for shrimps, such as *Vibrio parahaemolyticus* (Crab et al., 2007; Panigrahi et al., 2017).

Another claimed advantage of biofloc-run ponds is the production of large amounts of bacterial flocs, which are described by Crab et al. (2007) as a dense culture of bacteria, algae, fungi, protozoa and zooplankton around a core of dead organic matter. Those flocs can then be consumed by shrimps, enabling a better use of the feed and reducing the feed conversion ratio (Avnimelech, 1999; Crab et al., 2007; Serra et al., 2015).

Inside the pond, the carbon:nitrogen (C:N) ratio needs to be manipulated through the addition of organic carbon. Required amounts of carbon need to be calculated, either on the actual levels of ammonia in the pond, or based on the daily amount of proteins from the feed (Avnimelech, 1999; Ebeling et al., 2006; Samocha et al., 2007; Serra et al., 2015; Xu et al., 2016). The chosen C:N ratio then drives carbohydrates addition in the pond, a ratio of 12:1 or above inducing a shift from a photoautotrophy to an heterotrophy-dominated system (Avnimelech, 1999; Ebeling et al., 2006; Xu et al., 2016).

Therefore, the purpose of the following trial was to assess the efficiency of the biofloc technology on *P. vannamei* juveniles in terms of water quality and production performances. One nursery pond was run using this technique, and its water parameters, as well as its zootechnical and economic results were analyzed.

I.2 Materials and methods

The study took place at Skretting Vietnam validation farm, based in Nhà Bè, in the suburbs of Ho Chi Minh City, from May to June 2019 and lasted 36 days. The trial was performed in a 1000 m^2 square pond covered with a plastic liner, for a total volume of 1200 m^3. *P. vannamei* post-larvae (PL) at a PL10 stage were used (from ShrimpVet hatchery, Vietnam). Upon arrival, the number of shrimps was estimated (using XpertCount from XpertSea, Canada) and PLs stocked at a density of 1000 post-larvae per square meter. The pond was shaded with green plastic straps to lower sun exposure and curb the development of algae. The pond also featured a sludge concentration area at the middle, which is a depression made of concrete aiming at concentrating organic matter (Appendix II).

Intense aeration was applied, with paddlewheels and air blowers being used at 120 and 500 hp/ha, respectively (Appendix III). Four paddlewheels were installed in the corners of the pond as described in Boyd (1998) to increase dissolved oxygen levels and concentrate sludge in the middle, which was regularly syphoned out by gravity. Air blowers alimented by an air pump were evenly disposed on the bottom to increase dissolved oxygen levels and partly ensure suspension of biological flocs.

Shrimps were fed a commercial diet (Tomboy, Skretting Vietnam) containing 42% crude protein, 5 times a day during the first 18 days of culture, and 4 times a day afterwards. Feed was given by hand around the edges of the pond, and paddlewheels were stopped during feeding time to avoid concentrating the feed into the sludge area. At first, feed rations were based on a theoretical growth rate, but were later adjusted using a feeding tray. The feeding tray was used to assess apparent consumption after 1 hour and apply corrections to quantities fed (Appendix IV).

I.2.1 Water preparation and biofloc management

One month before stocking, water was pumped into the nursery pond from the reservoir pond of the farm. A 30 ppm chlorination was performed under full aeration, followed by an application of a commercial probiotic (AOcare™, Skretting Vietnam) at a concentration of 0.1 ppm. 8 kilograms of sugar cane molasses previously fermented for 24 h with the same probiotic were then applied on a daily basis for 2 weeks under full aeration until water color turned from green to brown. Pond bottom was raked manually during several days prior to stocking in order avoid the formation of potentially harmful biofilms for shrimps.

In order to increase alkalinity, dolomite ($CaMg(CO_3)_2$) and baking soda ($NaHCO_3$) were spread into the pond to reach an alkalinity of 80 mg $CaCO_3$/L. Alkalinity was then maintained above 80 mg $CaCO_3$/L by a daily additions of 4 ppm of calcite ($CaCO_3$) and dolomite. One week before stocking, 30 ppm of teaseed cake powder (14 % saponin) were mixed into the pond to kill tadpoles, following recommendations of Terazaki et al. (1979). After stocking, Azomite® was applied every 3 days at an average concentration of 4 ppm to supply some trace minerals (suspected to be involved the molting process) and silica to favor diatoms. To curb the development of pathogenic bacteria and promote

nitrogen assimilation, probiotics were applied into the pond in the morning every 3 days at an average concentration of 0.1 ppm.

One week after stocking, the biofloc was maintained at a C:N ratio of 12:1 during 17 days, which is meant to be the most suitable ratio for *P. vannamei* in intensive zero exchange systems according to Xu et al. (2016). Sugar cane molasses (28 % carbon, wet weight) was used to target the desired C:N ratio. Carbon content of molasses was determined using calculations described in Romano et al. (2018), and detailed results are shown in Appendix V. Daily amounts of molasses (Appendix VII) were calculated according to Samocha et al. (2007) as follows:

$$Q_m = {}^{1}/_{CC_m} * Q_f * \left(\frac{C:N * CP_f}{6.25} - CC_f\right)$$

With:

- Q_m and Q_f the amounts of molasses and feed, respectively (in kilograms)
- CC_m and CC_f the carbon contents of molasses and feed, respectively (as a percentage of wet weight)
- CP_f the protein content of the feed
- $C:N$ the targeted carbon:nitrogen ratio

Thus, for each kg of feed, assuming a carbon content of 50 % (Avnimelech, 1999) and a protein level of 42 %, 1.1 kg of molasses was added into the pond. Molasses was applied on a daily basis directly into the pond around 11 am, previously mixed with pond water and spread in the flow of a running paddlewheel. The C:N ratio was then progressively reduced from 24 days of culture (DOC), and small water exchanges regularly performed from 20 to 31 DOC as high TAN levels and low dissolved oxygen issues started to occur. Then, from 32 to 34 DOC, a continuous water exchange was carried out. The total volume of water exchanged during the trial was estimated to be close to 200 % of the pond volume.

I.2.2 Water quality monitoring

Dissolved oxygen, temperature and pH were measured 3 times a day at 8:30 am, 1 pm and 5 pm with an oximeter and a pH-meter, respectively (HANNA Instruments, USA). Turbidity, salinity and suspended flocs (ie settleable solids) were monitored once a day, using a Secchi disk, a refractometer and an Imhoff cone, respectively. TAN (as TAN-NH), nitrite (NO_2^--NH), nitrate (NO_3^--NH), phosphate (PO_4^{3-}) concentrations as well as alkalinity (as mg $CaCO_3$/L) were measured once a day using colorimetric test kits (sera GmbH, Germany).

Monitoring of presumptive *Vibrio* spp. populations was performed every 3 days using the spread plate method on CHROMagar™ media for enumeration of colonies from *V. parahaemolyticus, V. vulnificus, V. cholerae* and *V. alginolyticus*. Petri dishes were seeded with 0.1 mL of pure or diluted samples and kept at room temperature for 24 hours before counting. Only readings between 30 and 300 colonies were used to quantify bacterial concentration, expressed in CFU/mL. Densities in phytoplankton were assessed every 2 or 3 days using samples fixed with lugol (1 %) on an hemacytometer under microscope (Neubauer improved, Paul Marienfeld GmbH, Germany). Phytoplankton concentrations were expressed as a number of cells per milliliter of sample using the methodology described in FAO (2019c). Groups of phytoplankton species were identified following the methodology described by Van Vuuren et al. (2006).

I.2.3 Shrimp performance assessment

During this nursery phase, growth was assessed at least 2 times a week by sampling an average of 10 shrimps in the feeding tray to adjust feed rations. Shrimps were weighted individually using a digital scale (accuracy of 10 mg). These samplings also enabled health monitoring of the shrimps, as well as gut fullness. At harvest after 36 days of culture, final mean weight was determined by weighing individually 30 shrimps several times. Final harvested biomass was assessed by catching all shrimps with a net, and weighing landing nets full of shrimps before transfer to grow-out ponds. For all calculations described below, the initial weight of post-larvae was considered negligible, as they were stocked at a PL11 stage.

Apparent feed conversion ratio (%) was obtained using the following formula:

$$\text{Apparent FCR} = \frac{\text{Total amount of feed}}{\text{Biomass at harvest}}$$

Average weekly growth rate (g/week) was calculated at harvest as follows:

$$\text{AWG} = \frac{\text{Mean weight at harvest}}{\text{Number of culture days}/7}$$

Productivity per hectare (t/ha) was calculated according to the following formula:

$$\text{Productivity} = \frac{\text{Biomass at harvest}}{\text{Surface of the pond}}$$

Finally, survival rate (%) was estimated as follows:

$$\text{Survival} = \frac{\text{Biomass at harvest}}{\text{Number of stocked shrimps} \times \text{Mean weight at harvest}}$$

I.2.4 Economic analysis

A costs analysis was performed using the enterprise budget format from Ullman et al. (2019). As data over fixed costs (farm rental, depreciations...) were not available, only variable costs were taken into account for the analysis of production costs. Laboratory consumables were not considered either.

I.2.5 Data analysis

All data were represented using Microsoft Excel 2013.

I.3 Results

I.3.1 Water quality

Table 1: Summary of the main water parameters during the nursery phase. Values are represented as means ± SD. Minimums and maximums are shown in brackets.

Dissolved oxygen (mg/L)	5,8 ± 0,6 (4,4/7,6)
pH	7,5 ± 0,2 (7,1/8,2)
Temperature (°C)	30,8 ± 1,2 (28,3/33,4)
Turbidity (cm)	43 ± 28 (20/100)
Salinity (g/L)	9,2 ± 1,2 (7/10,5)
TAN (mg/L)	1,3 ± 1,7 (0/5)
NO_2^- (mg/L)	-
NO_3^- (mg/L)	-
PO_4^{3-} (mg/L)	1 ± 0,6 (0,1/2)
Suspended flocs (mL/L)	0,3 ± 0,4 (0/1,5)
Alkalinity (as mg $CaCO_3$/L)	97 ± 15 (71/142)
Total *Vibrio spp.* (log CFU/mL)	3,1 ± 0,2 (2,9/3,4)
Total phytoplankton (log cells/mL)	5,9 ± 0,4 (4,9/6,4)

Results for the main water quality parameters are summarized in Table 1. The minimum recorded dissolved oxygen (DO) concentration was 4.4 mg/L at 5 pm. However, several extra DO measures performed at night during feeding time showed concentrations as low as 2.7 mg/L, period during which all paddlewheels were turned off, but not the air blowers. Average dissolved oxygen concentrations showed no particular pattern during the first 17 days of culture (Fig. 2). Mean concentrations then decreased from 17 to 27 days of culture, and featured less variations between morning and late afternoon as well. At 27 days of culture, mean DO reached its recorded minimum at 4.6 ± 0.1 mg/L. DO values increased again from 30 days of culture to the end of the nursery phase, and showed larger daily fluctuations, along with large water exchanges a reduction in the C:N ratio. pH showed a similar pattern (Fig. 3), with high values and large fluctuations at the beginning of the trial (8.2 and 0.5 units at most, respectively). Daily fluctuations and values were then greatly reduced from 18 to 30 days of culture, with a period of 7 days during which pH featured fluctuations of only 0.1 log at most. Similar to DO, pH values and fluctuations increased again from 30 days of culture to the end of the trial.

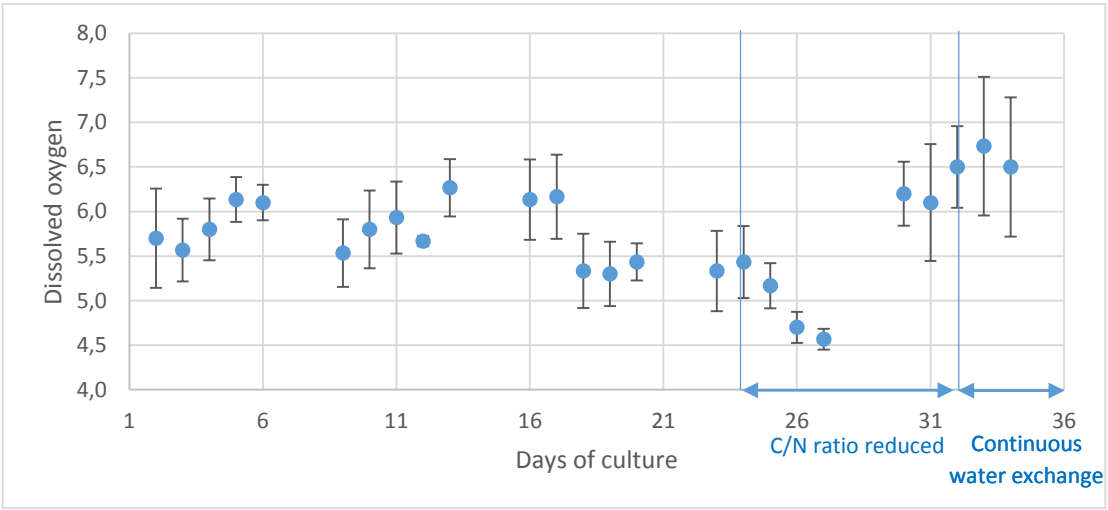

Figure 2: Dissolved oxygen (mg/L) fluctuations throughout the nursery experiment. Values are represented as means ± SD (n=3).

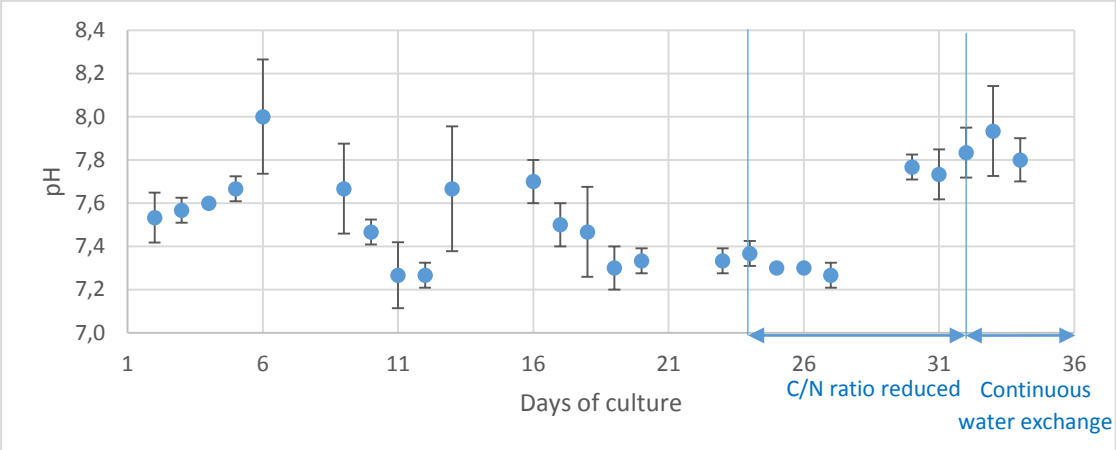

Figure 3: pH fluctuations throughout the nursery experiment. Values are represented as means ± SD (n=3).

TAN concentrations were kept below 1 mg/L for the first 3 weeks following stocking (Fig. 4). However, ammonia concentrations increased quickly from 27 to 29 days of culture, climbing from 2 to 5 mg/L in 2 days. This high level triggered the application of emergency measures, consisting in continuous water exchanges and a reduction of feeding rates. TAN concentrations then decreased again from 33 days of culture along with the application of these measures. During the whole trial, no nitrite or nitrate were found in the water. Phosphate increased overtime, from 0.1 mg/L at 13 DOC to 2 mg/L at 34 DOC.

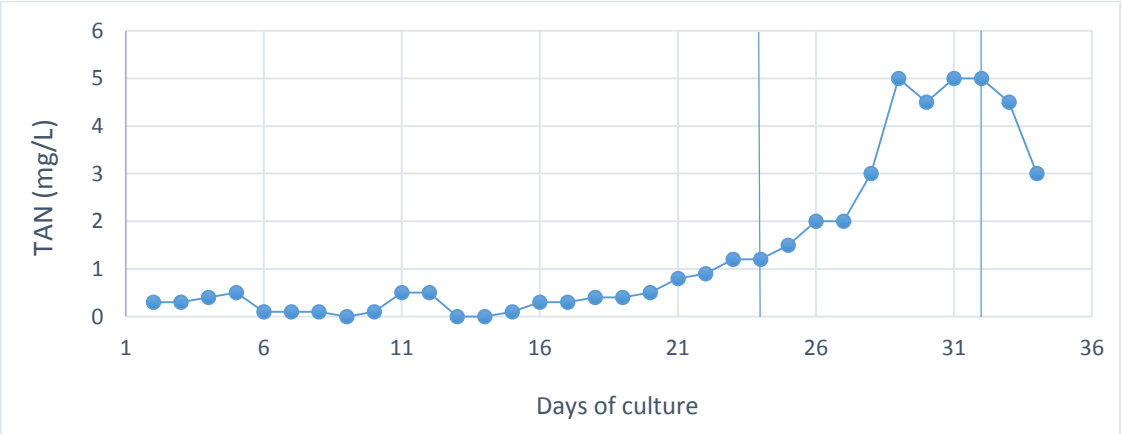

Figure 4: TAN (mg/L) concentration fluctuations throughout the nursery experiment.

Total phytoplankton showed no particular trend from 2 to 13 days of culture (Fig. 5), with a population dominated by Chlorophyta (green algae). However, at 13 days of culture, a cyanobacteria bloom occurred (blue-green algae), with concentrations reaching 2 million cells/mL within a few hours. Phytoplankton populations remained dominated by cyanobacteria and chlorophytes afterwards until 29 days of culture, showing no particular trend and remaining between 6 x 10^5 and 1.1 x 10^6 cells/mL. Phytoplankton counts then increased until the end to reach a maximum of 2.6 x 10^6 cells/mL, mainly composed of chlorophytes. Following water preparation, water color remained brown from the stocking day until 13 days of culture. At 13 DOC, coinciding with the previously described blue-green algae bloom, water turned from dark-brown to glowing green after a few hours. 48 hours after the beginning of the bloom, water took a brown color again and remainded so until the end of the nursery phase.

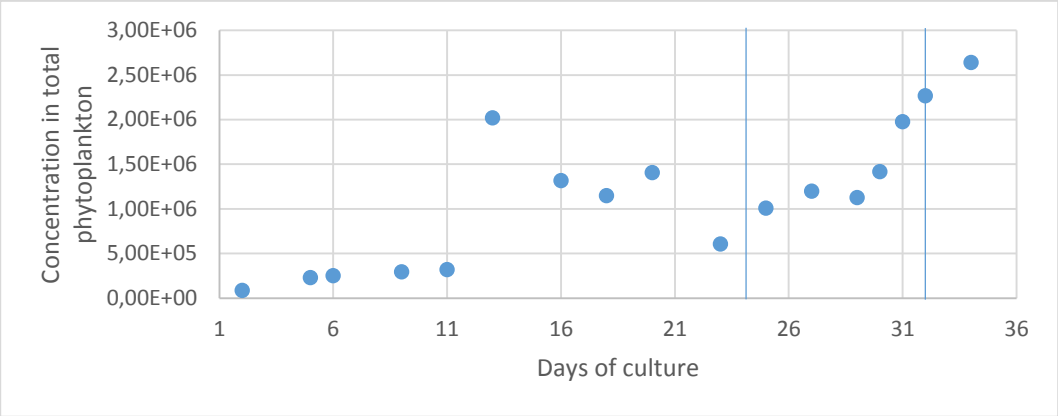

Figure 5: Fluctuations in total phytoplankton concentrations during the nursery experiment (cells/mL).

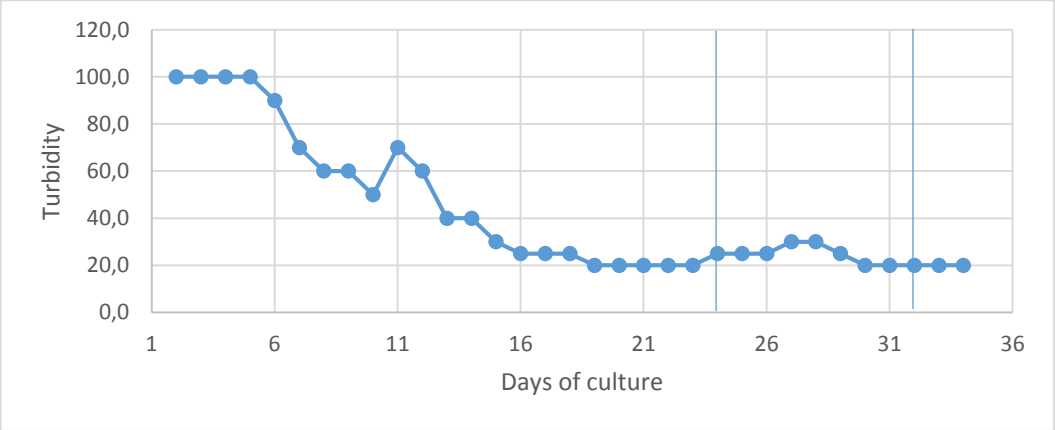

Figure 6: Fluctuations in Secchi disk readings during the nursery trial (cm).

Turbidity remained very low during the first week after stocking (Fig. 6), Secchi readings being higher than 60 cm. It then showed a clear downward trend, readings reaching a minimum of 20 cm after 19 days of culture. Turbidity then remained between 20 and 30 cm. Although not shown here, concentration in suspended flocs showed an upward trend from 17 to 24 days of culture, reaching a maximum at 24 days of culture with 1.5 mL/L. Flocs then took a downward trend until the end of the experiment, with no suspended flocs being recorded 3 days before transferring the shrimps.

Vibrio spp. counts averaged 1300 CFU/mL, with a spike at 20 days of culture of 2800 CFU/mL. *V. parahaemolyticus* remained below 600 CFU/mL during the whole trial.

I.3.2 Shrimp performance

Table 2: Production results for the nursery phase. Mean weight is represented as mean ± SD (n=30).

Mean weight (g)	1,43 ± 0,52
Yield (kg)	1438
Productivity (t/ha)	14,38
Survival (%)	100
Total feed input (t/ha)	10,41
Apparent FCR	0,72
Average weekly growth rate (g/week)	0,28

After 36 days of culture, shrimps showed a mean weight of 1.43 ± 0.52 g, with a variation coefficient of 36.2% (Table 2). Total yield was 1.44 ton, which gives a survival rate of virtually 100% as 1 million post-larvae were stocked. The apparent (or economical) feed conversion ratio was 0.72 for this first stage of culture.

I.3.3 Economic performance

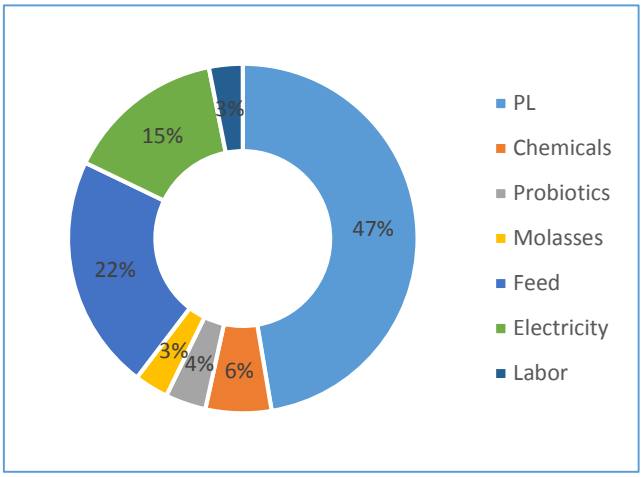

Figure 7: Summary of variable costs involved during the nursery phase.

Variable costs in VND and USD are detailed in Appendix VII, and summarized in Fig. 7. The purchase of post-larvae represented nearly half of all variable costs for this nursery phase, followed by feed (22 %) and electricity (15 %). The 5 hp air pump alone for the airblowers represented almost 10 % of all variable costs, which is twice the operating cost of the paddlewheels. Molasses, despite being applied in large quantities, represented only 3 % of variable costs, the same as labor. Variable costs represented a total amount of 6 876 USD, which corresponds to 0.007 USD/PL.

I.4 Discussion

I.4.1 Choice of method

The choice of the carbohydrates source to adjust the C:N ratio during this first trial is debatable. During this nursery phase, sugar cane molasses was chosen as the source of carbohydrates to adjust the C:N ratio, but other carbon sources could have been used, such as rice bran or wheat flour. On the one hand, Serra et al. (2015) found that rice bran was associated to better shrimp performance results than molasses. On the other hand, the same authors also reported that molasses were more effective in reducing ammonia during the nursery and grow-out phases of *P. vannamei* than rice bran, at similar C:N levels. They explain this better efficiency by the fact that simple sugars such as those contained in molasses are degraded faster by bacteria than the more complex polysaccharides contained in rice bran. More complex carbohydrates therefore induce slower response times (Khanjani et al., 2016). During this trial when a 12:1 C:N ratio was applied, morning TAN samplings often showed concentrations around 0.5 mg/L TAN-NH, but then decreased to close to 0 mg/L in late afternoon once molasses had been applied. The same concentration of 0.5 mg/L was again recovered the following day in the morning, which is supportive of a fast action of molasses for decreasing ammonia levels.

Calculations performed to adjust the C:N ratio were based on the publication from Samocha et al. (2007), in which carbon addition was based on the feed addition. However other ways do exist, such as in Xu et al. (2016) where carbon addition in one of the treatments was based on the actual TAN levels in the water. In this scenario 6 g of organic carbon are used to remove 1 g of TAN, and could be performed in the future as an emergency measure in case of high TAN levels in the pond, as done by Serra et al. (2015). The choice of a 12:1 C:N ratio is also arguable, but was found to be the best one in terms of shrimp performance for *P. vannamei* (Xu et al., 2016).

I.4.2 Water quality

During the trial, before large water exchanges were performed, dissolved oxygen and pH appeared very stable throughout the day, with daily fluctuations at 26 DOC as low as 0.2 mg/L and 0.0 log for oxygen and pH, respectively. This is consistent with the observations from Becerril-Cortés et al. (2018), who found significantly lower but more stable DO in biofloc tanks than conventional ones. However, they did not report a significant difference in pH values, which may be due to the experimental design.

Dissolved oxygen issues appeared to be one of the major drawbacks of the application of the biofloc technology. Oxygen concentrations during feeding time sometimes dropped below 3 mg/L, stressing the animals which therefore grow slower and poorly metabolize the feed. These low DO episodes made it necessary to lower the C:N ratio in order to decrease the biological oxygen demand (BOD) of the water. Similar oxygen depletions were observed by Maia et al. (2016), who systematically reported values below 3 mg/L early in the morning, which in that case even led to mortalities. However, aeration rates performed in the same paper were much smaller than those applied during this trial, with 800 kg of shrimps per hp in Maia et al. (2016) against 290 kg of shrimps per hp in this nursery for the air pump only, paddlewheels not included.

Hargreaves (2013) claimed that biofloc systems have a higher biological oxygen demand compared to recirculating systems because of a high concentration in suspended solids and of the resulting enhanced bacterial activity. The same author reports that in such a system, respiration rate of water alone can reach 6 mg/L per hour, which is greater than the consumption from the cultured species inside. Few papers raise awareness over the high BOD of a biofloc system and the need for high aeration inputs, however Hargreaves (2013) mentions the use of 150 hp/ha for the most intensive tilapia tanks reared in biofloc. In this nursery trial, airblowers and paddlewheels combined represented an aeration rate of 620 hp/ha. However, the efficiency of paddlewheels in maintaining suitable DO concentrations compared to airblowers is arguable (Boyd, 1998). This high BOD means that the response time in case of a power cut, for instance, is very short. To be run safely, the nursery pond would have required real time monitoring of DO with an alarm in case of an oxygen depletion, as well as backup systems such as power generators and spare air pumps. None of these solutions were in place at the farm during this first trial. Also, removal of microbial flocs with a skimmer or by recirculating the water into a mechanical filter are solutions which could be experimented for a second cycle to further enhance DO levels.

Over the course of this trial, TAN concentration slowly increased to 1.2 mg/L at 24 DOC, but then increased faster reaching 5 mg/L at 29 DOC before decreasing again following large water exchanges and a reduction in feeding rates. It is difficult to say whether TAN increased fast after 24 DOC due to a decrease in the performed C:N ratio resulting in lower uptakes from bacteria, or if the concentration would have increased anyway even if the 12:1 ratio had been maintained. In that case, a daily addition of 60 kg of feed (42 % proteins) should theoretically result in an increase in TAN of 1.69 mg/L in the 1200 m^3 nursery pond (based on the calculations of Avnimelech, 1999 and Samocha et al., 2007). It is interesting to notice that between 28 and 29 DOC, TAN showed a 2 mg/L increase, which means that virtually no ammonia was assimilated by bacteria or algae.

At 27 DOC, TAN had already reached 3 mg/L, but pH was still around 7.3 and very stable likely because of low photosynthetic activity on the one hand, and bacterial respiration which produces CO_2 on the other hand. This TAN value at such a pH corresponds to a concentration in NH_3-N of 0.048 mg/L, well below the safe level defined by Lin and Chen (2001) of 0.16 mg/L at low salinities. However, following small water exchanges TAN levels kept increasing in the first place, reaching 5 mg/L, as well as pH,

which made an already bad situation even worse. For instance, at 32 DOC, TAN were still at 4.5 mg/L but pH reached 8.1 at noon, which gave a concentration in NH_3-N of 0.42 mg/L, the highest level of this trial. This is well above the previously cited safe limit for *P. vannamei*, and must have had detrimental sub-lethal effects on shrimps. TAN levels decreased again with continuous water exchanges. Therefore, one can argue over the usefulness of small daily water exchanges (20 to 35 %) in such a case, not great enough to significantly decrease ammonia levels but large enough to disrupt the whole biofloc system and induce larger and therefore more dangerous pH variations.

No apparent nitrification was observed, as nitrite and nitrate remained undetected. This observation has to be contrasted with others made by Brito et al. (2015) and Serra et al. (2015), which saw ammonia spikes between the third and fourth weeks of culture (as high as 3 mg/L in Serra et al.), before decreasing again as nitrite and then nitrate rose. Therefore, it is possible that this trial was not performed long enough to see the nitrification process starting. This hypothesis is supported by the fact that a grow-out pond half-filled with this "mature" nursery water showed high levels of nitrite and nitrate only two weeks after the shrimps were stocked, unlike the other grow-out ponds which took longer to have a working nitrification process.

Several authors argue over the fact that an organic carbon supplementation in the pond has a negative effect over the development of nitrifying bacteria (Burford et al., 2003; Ebeling et al., 2006; Crab et al., 2007). Ebeling et al. (2006) reported that autotrophic bacteria had a growth rate 5 times slower than heterotrophic bacteria, and were therefore unable to outcompete them. Crab et al. (2007) also explain that nitrifying bacteria are very sensitive organisms and can be negatively affected by many water parameters, such as high concentrations in ammonia or low pH (below 7.5). Furthermore, water was first chlorinated before being immediately seeded with molasses and heterotrophic bacteria (as probiotics) as soon as chlorine had disappeared, which may have further delayed the start of a hypothetical nitrification process. A solution to this outcome may lies in the protocol described by Xu et al. (2016), who seeded their nursery water with water from a previous biofloc system.

In the long run in a so-called "mature" biofloc, the nitrification pathway is responsible for most of the ammonia consumption in the pond (Hargreaves, 2013). In such a case, no organic carbon supplementation is even necessary. However, the advantages from a system dominated by chemoautotrophy are debatable, firstly because of the dangerousness of nitrification if transformation of ammonia to nitrate was to be incomplete, nitrite being very toxic to shrimps. Similar to TAN, nitrites are toxic to *P. vannamei* at young stages and even more at low salinities (Lin and Chen, 2003). Furthermore, as the nitrification process consumes inorganic carbon (Ebeling et al., 2006), baking soda needs to be added on a daily basis to maintain alkalinity in the pond which comes at a cost for the farmer.

During the trial, phosphate accumulated in the pond, which is consistent with the findings of Maia et al. (2016) and Xu et al. (2016). However these authors found much higher concentrations, with a maximum of 40 mg/L in PO_4^{3-} reported by Xu et al. (2016) in a biofloc managed at a C:N ratio of 12:1 after 4 weeks. An accumulation of PO_4^{3-} indicates that it is not used by phytoplankton (Maia et al., 2016). During this nursery phase, the relatively low phosphate concentrations (2 mg/L or below) could be explained by an increased photosynthesis from microalgae compared to the previously cited findings, or by water exchanges which prevented reaching high levels.

A biofloc system promotes bacterial domination over algae (de Souza et al., 2014), but could also favor pathogenic bacteria for shrimps. *Vibrio* spp. can cause severe diseases in penaid aquaculture, including retards in growth and mortalities (Brito et al., 2015). Some strains of *V. parahaemolyticus* can for example cause acute hepatopancreatic necrosis disease (AHPND), often leading to mass mortalities of post-larvae (OIE, 2019). Whether or not adding carbohydrates increases *Vibrio* spp. loads in such a

complex bacterial community is debatable. Brito et al. (2015) found significantly higher *Vibrio* spp. counts in biofloc waters compared to a conventional system, up to 4.5 log CFU/mL. On the contrary, Panigrahi et al. (2017) reported significantly lower *Vibrio* spp. loads in bioflocs. Furthermore, bioflocs seem to stimulate the non-specific immune system of shrimps, which could therefore lead to an increase in resistance when exposed to pathogens (Panigrahi et al., 2017). As a matter of fact, the previous authors observed significantly lower mortalities in shrimps reared in bioflocs when exposed to high loads of *V. parahaemolyticus* compared to shrimps grown in a conventional system. Finally, the role of bioflocs in enabling or not *V. parahaemolyticus* to express its pathogenicity is also yet to be understood (Hargreaves, 2013).

During the water preparation phase of this trial, in which water was regularly seeded with molasses and probiotics, water color turned from blue following chlorination to green and to brown. Except from two days which saw a cyanobacteria bloom, water color remained stable and brown afterwards, which is consistent with the observations from Xu et al., 2016. According to various authors, this color transition from green to brown is not due to a change in algal populations, but to a shift from a photoautotrophy-dominated to an heterotrophy-dominated system, with the water color being darker as the C:N ratio increases (Hargreaves, 2013; Xu et al., 2016).

Once shrimps were stocked, turbidity gradually increased before reaching a plateau at 20 cm after 19 DOC. This observation is consistent with Brito et al. (2015), who saw a gradual increase in turbidity before reaching stabilizing at 20 cm, but only after 4 weeks of culture. Suspended flocs reached a maximum of 1.5 mL/L, which is low compared to the findings of Panigrahi et al. (2017), who reached 6 mL/L after 30 days of culture. The choice to monitor settleable solids is debatable, and is far from being the best way to manage a biofloc system. Measurement of settleable solids involves taking 1 liter of pond water, which is then settled in an Imhoff cone for one hour. This technique is first extremely inaccurate as the final value greatly depends on where the sample was taken in the pond. Readings made the same day greatly differed whether the sample was taken close to a running paddlewheel, or in a corner with standing water. Total suspended solids or volatile suspended solids appear to be more consistent parameters to better follow the development of a biofloc system (Serra et al., 2015; Xu et al., 2016). Unfortunately, no filtration and drying equipment was available on the farm to perform these readings.

I.4.3 Production performances

In terms of shrimp performance, the survival rate of virtually 100 % was similar to those observed by Serra et al. (2015), who found survival rates ranging from 98 to 99.5 % in biofloc-run nurseries after 35 days of culture. The apparent FCR of 0.72 was better than the one observed in the same publication, which ranged from 0.89 to 1.12 after 35 DOC. Weight gain in this nursery trial was also better than in Serra et al. (2015). However, the variation coefficient over final weight of 36.2 % was far worse than in the same publication, in which the largest one was 19.7%. It is possible that low DO events and high TAN levels had detrimental effects on the growth of the whole population, but do not explain this high variability in weights. The reduction of feeding rates during the last week of trial due to high TAN concentrations is very likely to have increase variability in final weights, as the shrimps were not fed ad libitum but competed for the same feed.

II. Liquid fermented rice bran as a carbon source to improve water quality and natural productivity during the grow-out phase of *P. vannamei*

II.1 Introduction

In order to reduce needs for water exchanges and therefore save costs over water treatment and pumping, several farming techniques have been developed to recycle inorganic nutrients within the pond. A first way is to promote algal growth, thus the assimilation of inorganic nitrogen through photosynthesis. The main drawbacks of this technique are a poor control over phytoplankton composition and the risk to get harmful algal blooms for the shrimps, large dissolved oxygen and pH fluctuations, as well as ammonia spikes when the weather stays cloudy for several days (Hargreaves, 2013; Romano, 2017). Furthermore, microalgae can crash causing oxygen depletion if sludge is not quickly removed from the pond bottom. Another technique, described in the previous chapter, is the biofloc technology. Although effective in reducing inorganic wastes, aeration needs are very high compared to a standard protocol, and such a system requires strong skills and experience to be operated successfully over a whole rearing cycle. Another way is to try to provide a more natural environment to the shrimps, which can be named as biomimicry.

The concept of biomimicry in shrimp farming was first used and developed in Thailand during the 1990s, in extensive ponds during severe disease episodes. At a time of large economic losses due to disease outbreaks, farmers tried to grow shrimps extensively without giving artificial feed. The only organic input was rice bran, and shrimps performed well despite being close to diseased ponds (Romano, 2017). Although being described recently in some publications (Romano, 2017; Chakravarty et al., 2018 ; Santhanam et al., 2019), very few results have yet been published over this farming technique (Campos et al., 2009).

The concept of biomimicry in shrimp farming is based on the regular application in the pond of a carbon source such as rice bran, previously fermented with probiotics, in order to mimic the natural composition of an estuary (Romano, 2017). Biomimicry in shrimp farming is based on 3 main notions (Romano, 2017):

- Providing a more natural environment for shrimps with high turbidity and natural productivity
- Recycling nitrogenous wastes within the pond through bacteria and algae
- Stimulating natural production through zooplankton blooms, which can then feed shrimps

To be efficient, this protocol needs to be performed at reduced water exchanges, close to zero during a whole cycle, which reduces pumping costs and improves biosecurity (Becerril-Cortés et al., 2018; Chakravarty et al., 2018).

Underlying mechanisms of such a protocol are yet to be understood, but the main claimed advantages of the application of this concept are a better feed conversion ratio, a faster growth, an improved survival, reduced water exchanges and an overall enhanced biosecurity (Campos et al., 2009; Romano, 2017; Chakravarty et al., 2018; Santhanam et al., 2019). The major suspected modes of action of liquid fermented rice bran are reviewed below.

II.1.1 Liquid fermented rice bran as a food source for live preys

Rice bran represents 5 to 10% of the total weight of a rice grain (Ribeiro et al., 2017; FAO, 2019d), and comes from the pericarp of rice. It contains at least 11 % crude proteins (Supriyati et al., 2014; FAO, 2019d) and is high in lipids. Rice bran is commonly used as an organic fertilizer, as well as a raw material in feed (Doi et al., 2013; Ranjan et al., 2018) as it contains high levels of non-starch polysaccharides (Ranjan et al., 2018).

The application of rice bran as an only source of feed for copepods has been proven to increase their concentration. Lemus et al. (2004) found that rice bran could be used as a unique source of feed to grow copepod in tanks for catfish hatcheries, either by being a direct food source for copepods or for their preys (protozoa). *P. vannamei* has already shown in several studies an efficient predatory behavior on copepods, which can represent a non-negligible part of its daily feed ration (Campos et al., 2009; Porchas-Cornejo et al., 2012). Furthermore, a regular application of rice bran in the pond seems to enhance the creation of colloids made of rice bran particles and various organisms such as bacteria, algae, protozoans, nematodes and copepods. Those colloids are suspected to be a part of the diet of shrimps (Becerril-Cortés et al., 2018). Furthermore, according to Chakravarty et al. (2018), rice bran in itself could also be used as a direct food source by shrimps.

II.1.2 The application of liquid fermented rice bran as a way to improve water quality

Another claimed advantage of a regular application of liquid fermented rice bran is an enhanced recycling of both inorganic and organic nitrogen wastes within the pond (Romano et al., 2018). Fermented with probiotics, rice bran becomes a synbiotic, which is defined by Huynh et al. (2017) as the joint application of prebiotics (rice bran) and probiotics (mostly *Bacillus* spp.). Probiotics are micro-organisms beneficial for the health of the host (Cerezuela, 2011). On the other hand, prebiotics constitute a carbon source on which probiotics can grow (Huynh et al., 2017).

Probiotics are meant to produce inhibitory molecules against pathogens, compete with other bacteria for nutrients, and enhance immune response of shrimps (Van Hai and Fotedar, 2010). Some probiotics also enhance decomposition of organic matter at the bottom of the pond through its mineralization (Van Hai and Fotedar, 2010). Bacteria of the *Bacillus* genera are the most commonly used probiotics in aquaculture along with yeasts (Cerezuela, 2011). Some strains even have a proven inhibitory role against the formation of biofilm from *Vibrio parahaemolyticus*, pathogenic bacteria involved in the AHPND syndrome for shrimps (Chakravarty et al., 2018).

As heterotrophic organisms, probiotics also assimilate inorganic nitrogen under the form of proteins. Rice bran constitutes a source of carbon which increases the carbon:nitrogen ratio in the pond, enhancing the dominance of heterotrophic bacteria in water (Serra et al., 2015; Panigrahi et al., 2017). A shift from a full autotrophic to a mixed autotrophic-heterotrophic system would therefore take place, along with a better stability of water parameters (mainly pH and dissolved oxygen) due to a reduced photosynthesis (Panigrahi et al., 2017).

The aim of this chapter is therefore to assess the impact of such a protocol over water quality, but also on zootechnical and economic performances in an intensive rearing system of *P. vannamei* compared to a standard protocol using molasses as a carbon source.

The following study is divided in 3 distinct parts. The first phase of the study is focused on the evaluation of different strategies for the fermentation of rice bran. The impact of different probiotics on *Bacillus* spp. counts and the digestion of rice bran was assessed.

The second part of this study is a small-scale trial over the incidence of liquid fermented rice bran on major water quality parameters and concentrations in copepods.

The third and last part of this study is a live trial carried out in shrimp ponds during the grow-out phase, during which two protocols involving the use of either rice bran or molasses were compared in terms of water quality, production and economical performances.

II.2 Materials and methods

II.2.1 Impact of different fermentation strategies on rice bran characteristics

To ferment rice bran, two probiotics at different concentrations were tried. White cap™ (Engest® from Baxel Co, Thailand) and AOcare™ (Skretting Vietnam) were compared for their respective capacity to grow on rice bran and digest it.

4 different treatments were performed over liquid rice bran. For each treatment, 4 x 250 mL glass beakers received 10 grams of finely grinded rice bran along with 50 mL of sterilized water. The solution was then thoroughly mixed and inoculated or not with one of the two probiotics. In the control group, no probiotic inoculation was performed. In the White cap group, the mix received an initial inoculum of 0.9 gram of White cap per kilogram rice bran (dry weight), which is approximately twice the recommended dosage from the manufacturer. According to the concentrations given by the manufacturer, this dosage corresponds to an inoculation in *Bacillus* spp. of 9×10^9 CFU/kg rice bran, or 1.3×10^6 CFU/mL solution, assuming a density of 0.5 for rice bran. In the AOcare treatment group, the mix received a similar amount of bacterial spores with an inoculum with 6,6 grams of AOcare™ per kilogram rice bran (dry weight), which according to the concentrations given by the manufacturer corresponds to 1×10^{10} CFU/kg rice bran in *Bacillus* spp. (AOcare™ also contains *Pediococcus* spp.). In the AOcare/10 treatment group, an inoculum 10 times lower than the previous concentration was added. All beakers were then kept for 24 hours at room temperature (22 °C) in a rotary shaker for fermentation, without extra aeration.

White cap™ was stored under a liquid state and contained a combination of 3 different species of Bacillus bacteria, with spores of *B. subtilis, B. megaterium* and *B. licheniformis*, as well as enzymes. AOcare™ was under a solid form (powder) and contained a combination 3 different Bacillus species, with spores of *B. amyloquefaciens, B. pumilis* and *B. subtilis*. AOcare™ also contained spores of *Pediococcus acidilactici*.

After fermentation and for each treatment, 4 subsamples were taken from 4 beakers and gathered into a 4 mL sample. Each sample (one per treatment) was then diluted and seeded on a media selective for *Bacillus* spp. after several dilutions, using the drop-plating technique. Petri dishes were kept at 37°C for 24 h, and results were expressed as colony forming units per mL (CFU/mL).

To assess the efficiency of the fermentation from each probiotics, a crude fiber analysis after acid and alkaline digestion (ISO 6865:2000) was performed over a sample taken from each treatment.

II.2.2 Comparison of different fermentation strategies over water quality and natural productivity

In order to compare the impact of different fermentation strategies over water parameters and natural productivity in the pond, 4 different protocols were tried at a small scale over a period of 9 days, which is theoretically the time needed for pond water preparation (Chakravarty et al., 2018). 16 x 20 liters bottles were first filled with 9 liters of pond water using the protocol that follows. Water was taken with a bucket at the surface of a pond which had not received any chemical treatment. The operation was performed at night, around 7 pm, to maximize the density in copepods at the surface of water, according to prior observations (data not shown) and Fulton (1984). Once collected, water was transferred into a large 45 liters bucket kept under vigorous aeration to ensure an even distribution of zooplankton throughout water column. Water was then poured into the bottles following a random pattern. Bottles were later kept under strong aeration during the duration of the experiment to maintain a concentration in dissolved oxygen above 5 mg/L through the use of an air pump and airstones. Bottles were kept outside the laboratory, in the open air, but shaded with a tarpaulin to avoid too high temperatures and direct sunlight, but not totally opaque to allow photosynthesis to occur. In all treatments, Azomite® was applied daily at 3 ppm as a supply of silica to promote growth of diatoms. 3 days after the beginning of the experiment, 6 liters of pond water were again added to all bottles using the previously described protocol to rise the water volume and compensate evaporation (as described in Bioshrimp, 2019).

Each treatment group consisted in 4 bottles, randomly chosen. The first group of bottles was a control and received no fertilization and no probiotics were added. For the remaining 3 treatments, bottles received an initial fertilization of liquid fermented rice bran of 50 ppm based on dry weight, and a daily inoculation of liquid fermented rice bran of 10 ppm from day 4 after the beginning of the experiment (following recommendations from Romano, 2017). Among these 3 groups, two received liquid rice bran previously fermented with an inoculum of AOcare™ (1.6 g/kg of rice bran) with (aerated AOcare) or without aeration (AOcare). Finally, a third group of bottles received liquid rice bran fermented with White cap™ (0.4 g/kg of rice bran) under aeration (White cap aerated). White cap™ and AOcare™ were added to the liquid rice bran mixture at the same theoretical concentrations in bacterial spores per kg of rice bran (dry weight), ensuring an inoculation of 4×10^9 CFU/kg. To obtain the fermented mixtures, finely grinded rice bran was blended with previously chlorinated water (30 ppm) at a weight ratio of 1:5 (Romano, 2017), along with baking soda for pH buffering. Fermentation was performed during 24 hours in 5 liters bottles, and aeration ensured by an air pump and airstones for aerated treatments.

Temperature, dissolved oxygen and pH were recorded 3 times a day, at 9 am, 1 pm and 5 pm with an oximeter and a pH-meter, respectively (HANNA Instruments, USA).

Zooplankton densities were assessed at the first day of experiment (T0), and at T0+3 and T0+8 days afterwards from a 500 mL sample from each bottle, filtered through a 150 µm mesh to keep only large specimen (as described in Schipp et al., 1999; Cardozo et al., 2007; Gao et al., 2012 and FAO, 2019e). Once concentrated on the mesh, organisms were killed and preserved using lugol (1%), and counted under a binocular using a glass plate and grid paper. Only copepods were counted as they represented the large bulk of all organisms present in the samples, and seemed to be the most interesting organisms as live preys for shrimps (Romano, 2017; Chakravarty et al., 2018). Results were expressed as a number of individuals per liter, adults and nauplii combined.

Alkalinity, TAN (total ammonia nitrogen), nitrite and nitrate were also recorded at T0, T0+3 and T0+8 days as described in chapter I. Vibrio counts and algal density estimations were also performed at T0, T0+3 and T0+8 days as described in chapter I for each bottle.

II.2.3 Field trials

The experiment took place over 41 days at Skretting Vietnam validation farm, Nhà Bè, Vietnam, from July 1st to August 9th, 2019. The trial ended early at 76 DOC following the termination of two ponds, which had to be harvested in emergency due to sudden mortalities.

Shrimps came from the nursery pond previously described in chapter I at a mean weight of 1.43 g (± 0.52 g) and were transferred to the grow-out ponds on motorbikes over a period of 2 days. No acclimation was performed during transfer operations, and shrimps post-larvae were stocked at a density of 132.8 ± 1.7 PL/m^2 into 6 square-shaped ponds, ranging from 1000 to 1400 m^2. Each pond was covered with a polyethylene liner and featured 4 paddlewheels (3 hp each), one at each corner and disposed as described in Boyd (1998) to increase dissolved oxygen levels and concentrate organic matter at the center of the pond. During daytime, only one paddlewheel was run per pond to avoid stratification of water, but full aeration was performed during heavy rains or nighttime (from 7 pm to 7 am), which represented between 86 and 120 hp/ha at full aeration.

Ponds featured a central sludge concentration area made of concrete, from which sludge and dead shrimps were pumped out every day after 17 days of culture. 4 ponds were filled with previously settled brackish water (6-7.5 g/L) which was chlorinated 4 days before stocking at 10 ppm. Due to a clean water shortage, one pond was filled with highly turbid water, also chlorinated at 10 ppm and another pond half-filled with water from the nursery pond, the other half of it being filled with settled and chlorinated water. The choice was made to perform a chlorination to avoid pathogens as much as possible and secure the production.

II.2.3.1 Fertilization of water and treatments

The two protocols used to assess the potential of liquid fermented rice bran for bioremediation were a standard protocol using sugar cane molasses and probiotics, and another one using liquid fermented rice bran and probiotics. The protocol with molasses (control) was internal to Skretting Vietnam, whereas the rice bran protocol (synbiotic) was based on Romano (2017). Each treatment was assigned to 3 ponds, but not randomly in order to have the same total pond area for each treatment.

In the control group, water was first inoculated with a total of 30 to 45 ppm of sugar cane molasses (28 % carbon) to get a brown water color, along with 0.15 ppm of probiotics (AOcare™) at 2.5 x 10^9 CFU/g. This initial fertilization was performed over a week overlapping the stocking date. Ponds were filled at full capacity since the beginning of the experiment, representing between 1500 and 2000 m^3 of water per pond. One week after stocking until the end of the trial, these 3 ponds were regularly seeded with sugar cane molasses and probiotics, with amounts of molasses based on water color. In order to curb algal blooms, more molasses was added when water color turned into green. When a cyanobacteria bloom occurred, 80 kg molasses were applied per pond along with probiotics as an emergency measure to increase ammonia uptake and shade the water. During normal operations, the amounts of probiotics were based on the manufacturer's recommendations. In total, each control

pond received between 128 and 149 ppm of sugar cane molasses over the trial, as well as 1 to 1.4 ppm of probiotics.

The remaining 3 ponds receiving the rice bran treatment were first let half-filled with water for the first 12 days after stocking to maximize the effect of fermented rice bran (Bioshrimp, 2019), before being full filled with previously settled and chlorinated water. Final volumes ranged from 1500 to 2000 m^3 per pond. Water was first fertilized with a total of 100 to 120 ppm of liquid fermented rice bran over a week overlapping the stocking date of shrimps. Ponds were then seeded on a daily basis with liquid fermented rice bran at a maximum rate of 10 ppm a day (based on dry weight), until turbidity reached 30 cm and water color turned to brown-yellow, as described in Romano (2017). Each pond was then fertilized with an average of 5 ppm rice bran per day to maintain water color and turbidity (Romano, 2017). Similar to the previous protocol, molasses and probiotics were applied when a cyanobacteria bloom occurred, which happened once. Probiotics were also directly added into the pond on a weekly basis. In total, each treated pond received from 157 to 173 ppm of rice bran, as well as 0.6 to 1 ppm of probiotics (in direct application or used for fermentation). Unfortunately due to a raw material shortage, no rice bran was added from 69 DOC until the end of the trial one week later (76 DOC).

During the trial, rice bran was fermented daily into 500 liters tanks under strong aeration during 24 hours (Appendix IX), at a rice bran/fresh water ratio of 1:5 in weight along with 10% of baking soda for pH buffering. 1.6 g of probiotic (AOcare™) per kg of rice bran was used for the fermentation, following the results from previous experiments. Only the liquid phase was used to seed ponds, and the largest particles were left at the bottom of the tank as much as possible.

For all treatments, fermented rice bran, molasses and probiotics were preferably applied in the morning, in the water flow of a running paddlewheel.

Alkalinity was maintained as much as possible with daily additions of dolomite ($CaMg(CO_3)_2$), calcite ($CaCO_3$) and baking soda ($NaHCO_3$). Regular additions of Azomite® were also performed at a rate of 1 ppm/d.

II.2.3.2 Feeding strategies

Shrimps were fed a commercial diet (Lorica and Xpand from Skretting Vietnam) containing between 40 and 42 % proteins. Two different feeding strategies were performed during the trial. During the first three weeks of trial, shrimps were fed by hand, at 7:30 am, 10 am, 2 pm and 5 pm by manually spreading the feed from the edges of the pond. Shrimps were fed ad libitum using one feeding tray per pond to assess apparent consumption as described in chapter I. Later, shrimps in 4 out of 6 ponds were fed using acoustic automatic feeders (Eruvaka Technologies, India), with amounts of feed driven by the feeding activity of shrimps. The two remaining ponds were still fed by hand because their small size did not allow the installation of acoustic feeders. Therefore in each group, control and synbiotic, two ponds were fed automatically and one still fed by hand after the third week of trial.

In ponds supplied with automatic feeders, shrimps were fed ad libitum from 7 am to 7 pm, the feeders being run in automatic mode, which means that feed amounts were only based on shrimp feeding activity. The quantity of feed was controlled by a controller for each feeder, which contains an algorithm analyzing the feeding response of shrimps and adapting feeding frequency and rate in return. One feeder was installed per pond, tied to the dikes with ropes (Appendix VIII). One hydrophone was disposed at the bottom of each pond in the feeding area to record noises from

shrimps while they eat. One feeding tray was disposed into the feeding area and checked every day to make sure no pellets remained at the bottom. Feeding was automatically paused during heavy rains due to noise interferences. No feeding was performed at night because of sound interference with the paddlewheels and increased oxygen consumption related to feeding. Daily feed amounts were checked on a smartphone through the PondLogs application from Eruvaka.

II.2.3.3 Monitoring of water quality

For each pond and during the first 3 weeks, dissolved oxygen, temperature and pH were measured 2 times a day at 7 am and 4 pm with an oximeter and a pH-meter, respectively (HANNA Instruments, USA). Later, a real time and remote monitoring of temperature and dissolved oxygen in all ponds was ensured by constantly immersed sensors (PondGuard from Eruvaka Technologies, India). However pH was still read manually twice a day.

Salinity was recorded on a daily basis and turbidity once every 3 days following the same methods as described in chapter I. TAN, nitrite, nitrate and alkalinity were measured daily, and phosphate concentration every 3 days as described in chapter I.

Monitoring of presumptive *Vibrio* spp. populations as well as phytoplankton was performed every 3 days as in chapter I.

In order to avoid pathogens and parasites as much as possible in grow-out ponds, the choice was made to treat all ponds with chlorine at a concentration of 10 ppm, one week prior to stocking. This concentration is equivalent to the LC50 (after 1 minute) of *A. tonsa* according to Heinle and Beaven (1977). Thus, densities in copepods and zooplankton in general were not taken into account during the trial, and some samplings performed at night did not reveal any zooplankton over 150 microns once shrimps were stocked.

II.2.3.4 Assessment of zootechnical performances

To assess shrimp performance, a sampling over 30 shrimps per pond was performed once a week by casting a net in ponds. Individual weights were recorded using a digital scale (accuracy of 0.01 g). At the time of harvest for 2 ponds, mean weight was assessed by counting the number of animals in 1 kilogram. For these 2 ponds, survival rate (%) was calculated as follows:

$$\text{Survival} = \frac{\text{Biomass at harvest}}{\text{Number of shrimps stocked} \times \text{Mean weight at harvest}}$$

Apparent or economical feed conversion ratio (%) was estimated as follows:

$$\text{Apparent FCR} = \frac{\text{Amount of feed}}{\text{Biomass at harvest} - \text{Biomass at stocking}}$$

Average weekly growth rate (g/week) was calculated according to the following calculations:

$$\text{AWG} = \frac{\text{Mean weight at harvest} - \text{Mean weight at stocking}}{\text{Number of culture days}/7}$$

Finally, the specific growth rate (days^{-1}) was estimated as follows:

$$\text{SGR} = \frac{\ln(\text{Mean weight at harvest}) - \ln(\text{Mean weight at stocking})}{\text{Number of culture days}}$$

II.2.3.5 Economic performance

Economic analyses were performed using an enterprise budget as in chapter I. Similar to the previous chapter, fixed costs were not taken into account, as well as laboratory consumables. For the two harvested ponds, the analysis was complete as shrimps were sold. A variable cost ratio (USD/kg) for each one of those ponds was calculated as follows:

$$\text{Variable cost ratio} = \frac{\text{Variable costs}}{\text{Harvested biomass}}$$

For the four remaining ponds, only variable costs engaged until the harvest date of the two previous ponds were taken into account.

II.2.4 Statistical analysis

All data were represented using Microsoft Excel 2013 and R statistical software with the package ggplot2.

Statistics were run using R statistical software. One-way analyses of variance were performed over phytoplankton, zooplankton and Vibrio counts at T0+8 days in the in-lab trial over different fermentation strategies. Normality of the distribution of data and homogeneity of variances assumptions were verified by plotting the residuals. When normality assumptions were not met, data were log-transformed and normality verified again.

Comparison of means tests were used to identify significant differences between treatments for all water quality parameters during the field trial. Normality and homogeneity of variances were verified using the Shapiro-Wilk and Fisher-Snedecor tests, respectively. When normality assumptions were met, a student's t-test was performed, and a non-parametric Kruskal-Wallis test otherwise.

A two-way analysis of variance was carried out over shrimp weight data gathered during the field trials. Normality and homogeneity of variances assumptions were verified by plotting the residuals, and data were log-transformed. As time and treatment effects were found to be significant, a Tukey test was applied to compare means.

II.3 Results

II.3.1 Impact of different fermentation strategies on rice bran characteristics

Results from the 24 hours fermentation of rice bran with different inoculums are summarized in Table 3. No difference in *Bacillus* spp. concentrations could be found among the 3 probiotics treatments, which showed concentrations ranging from 8.7 to 9.6 ± 0.5 log CFU/mL. The control sample showed a high *Bacillus* spp. concentration of 8.4 ± 0.5 log CFU/mL, although not inoculated with any kind of probiotics. However, given the uncertainty of measurement set at 0.5 log CFU/mL, a difference can be seen between the control sample and AOcare and White cap treatments after 24 hours of fermentation.

Regarding crude fiber contents after 24 hours, the control sample showed the lowest crude fiber content of all, with 0.26 g/kg. No clear differences in crude fiber could be found between treatments.

Table 3: Microbiological and crude fiber content results after 24 hours of fermentation. Bacterial counts are shown in log CFU/mL ± 0.5.

Treatment	Inoculum (log CFU/mL)	*Bacillus* spp. after fermentation (log CFU/mL)	CF content after fermentation (g/kg)
CONTROL		8,4 ± 0,5	0,26
AOCARE	6,1	9,6 ± 0,5	0,29
AOCARE/10	5,1	8,7 ± 0,5	0,32
WHITE CAP	6,1	9,6 ± 0,5	0,32

II.3.2 Comparison of different fermentation strategies over water quality and natural productivity

The results from this experiment are shown in Table 4, which summarizes the values at the end of the trial (T0+8 days).

Table 4: Summary of the main water parameters at the end of the trial (T0+8 days). Values are represented as means ± SD (n=4). Minimums and maximums are shown in brackets. Values within the same line but with different letters are significantly different (p<0.05).

Parameter	Control	White cap aerated	AOcare aerated	AOcare
Dissolved oxygen (mg/L)	5,8 ± 0,3 (5,3/6,1)	5,5 ± 0,3 (5,1/6)	5,6 ± 0,4 (5/6)	5,7 ± 0,3 (6,2/5,4)
pH	8,2 ± 0,1 (8/8,3)	8,2 ± 0,1 (8,1/8,3)	8,2 ± 0,0 (8,1/8,2)	8,2 ± 0,1 (8,1/8,3)
Temperature (°C)	36,1 ± 2,8 (33,3/39)	36,8 ± 2,6 (33,1/39,8)	35,9 ± 2,9 (33/39,4)	36,3 ± 3 (33,1/39,3)
Salinity	16 ± 0 (16/16)	16 ± 0 (16/16)	16 ± 0 (16/16)	16 ± 0 (16/16)
TAN (mg/L)	-	-	-	0,1 ± 0,1 (0/0,2)
Nitrite (mg/L)	-	-	-	-
Nitrate (mg/L)	-	-	-	-
Copepods (ind/L)	37 ± 6 (30/42)	66 ± 18 (56/92)	111 ± 87 (32/210)	73 ± 41 (26/102)
Phytoplankton (log cells/mL)	5,4 ± 0,1 (5,2/5,5)	5,1 ± 0,4 (4,8/5,6)	5,1 ± 0,1 (5/5,1)	5,2 ± 0,1 (5,1/5,3)
Total *Vibrio* spp. (log CFU/mL)	3,1 ± 0,2 (2,9/3,3)	3,2 ± 0 (3,2/3,3)	3,3 ± 0,2 (3,2/3,4)	3,3 ± 0 (3,3/3,4)

No significant differences were found among treatments for all monitored parameters ($p>0.05$). Dissolved oxygen was similar among treatments, between 5 and 6 mg/L, which indicates that aeration was homogeneous. Temperature rose very high during the experiment, especially during the last day (during which no wind or clouds were present) with a recorded maximum of 39.8 °C in the White cap aerated group. Those high temperatures undoubtedly impacted final concentrations in copepods.

Unsurprisingly, NO_2^- and NO_3^- were not detected in any of the treatments, and some ammonia was only found during the last day of experiment in the non-aerated AOcare treatment, although at a low concentration of 0.1 ± 0.1 mg/L.

Densities in copepods had to be log-transformed to ensure their normal distribution. All observed copepods belonged to the cyclopoid group of species. Densities were similar among treatments at the beginning of the trial (Fig. 8), which means that the protocol used to mix and allocate pond water into the different bottles actually worked. However, due to large variability in densities within each group, no significant difference could be found in log-transformed copepod densities at the end of the trial ($p>0.05$), which were supposed to be heavily impacted by high temperatures. Nevertheless, although not significant, all groups receiving fermented rice bran featured higher averages in copepod densities than the control group at T0+8days. The maximum density was found in the AOcare aerated group, with 210 individuals per liter.

Final phytoplankton counts were similar at the end of the experiment between groups ($p>0.05$), with an average density of 2.7 ± 0.9 x 10^5 cells/mL, the majority of which being cyanobacteria. A small bloom appeared to have occurred in the 3 fertilized groups around T0+3 days with densities exceeding 4 x 10^5 cells/mL (Fig. 8), but then crashed to reach lower values than at the beginning of the trial. Although not shown here, the control group also experienced a bloom with concentrations higher than 4 x 10^5 cells/mL after T0+3 days, but were also reduced at the end.

Vibrio counts gave similar results among treatments at the end of the trial ($p>0.05$), with an average count of 3.3 ± 0.2 log CFU/mL.

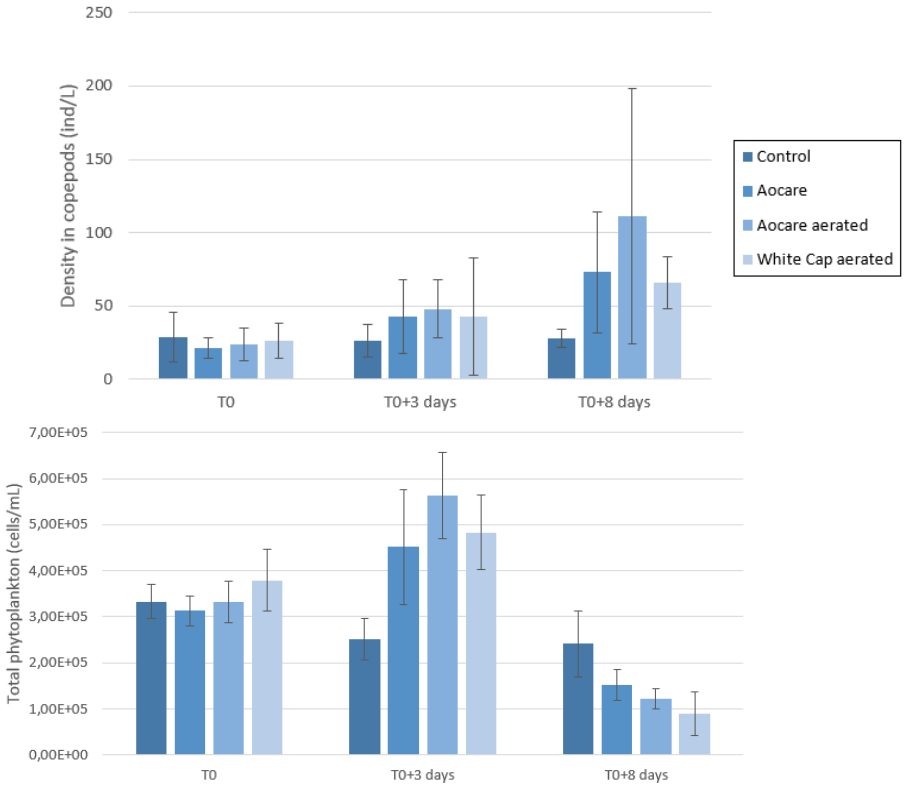

Figure 8: Evolution of phytoplankton and copepod concentrations during the trial. Values are represented as means ± SD (n=4).

II.3.3 Field trials

In total over the trial (until 69 days of culture), 745 kg of molasses and 900 kg of rice bran were added in control and synbiotic ponds, respectively. Appendix V shows the carbon and nitrogen contents of rice bran and sugar cane molasses. Calculations made with the protein content of the feed (as described in chapter I) gave a similar C:N ratio for both groups over the trial of 8.9 ± 0.1. However, the regularity of applications was not the same between the two groups. Synbiotic ponds received regular rice bran applications of 5 ppm per day on average, whereas control groups received daily molasses applications ranging from 1 to 40 ppm.

II.3.3.1 Water quality

Table 5: Summary of the main water parameters during the grow-out trial until 69 DOC. Values are represented as means ± SD. Minimums and maximums are shown in brackets. Values within the same line but with different letters are significantly different (p<0.05).

Parameter	Control	Synbiotics
Morning DO (mg/L)	5,7 ± 1,1 (2,4/7,8)	5,7 ± 0,9 (2,9/7,5)
Afternoon DO (mg/L)	8,4 ± 2,3[a] (3,2/13,5)	8 ± 2,3[b] (2,5/15)
Morning pH	7,6 ± 0,2 (7,2/8,2)	7,6 ± 0,2 (6,9/8)
Afternoon pH	8,3 ± 0,5 (7/9,1)	8,2 ± 0,5 (7,3/9,3)
Temperature (°C)	30,3 ± 1,5 (28,3/32,6)	30,3 ± 1,1 (28,3/32,2)
Salinity (g/L)	6,3 ± 0,8 (5/8)	5,9 ± 0,7 (5/7,5)
Turbidity (cm)	38 ± 20 (20/90)	31 ± 15 (15/100)
TAN (mg/L)	0,7 ± 0,6 (0/2)	0,8 ± 0,8 (0/4)
Nitrite (mg/L)	6,2 ± 12[a] (0/40)	0,7 ± 2,5[b] (0/15)
Nitrate (mg/L)	54 ± 100[a] (0/400)	19 ± 66[b] (0/300)
Phosphate (mg/L)	0,9 ± 0,7[a] (0/2,5)	1,3 ± 0,7[b] (0,2/4)
Alkalinity (mg $CaCO_3$/L)	92 ± 11 (71/116)	94 ± 12 (71/116)
Total *Vibrio* spp. (log CFU/mL)	2,9 ± 0,2 (2,5/3,3)	3 ± 0,3 (2,6/3,7)
Total phytoplankton (log cells/mL)	5,3 ± 0,3[a] (4,6/5,9)	5,7 ± 0,4[b] (5,3/7,3)

Table 5 summarizes all main water parameters during the grow-out trial, from the beginning at 36 days of culture to the last day liquid fermented rice bran was added, at 69 DOC. Regarding dissolved oxygen, morning values did not significantly differ among groups ($p>0.05$). However, afternoon DO was found to be significantly higher in control ponds than in synbiotic ponds ($p<0.05$). This indicates a better dissolved oxygen stability inside synbiotic ponds between early morning and afternoon. Morning and afternoon pH readings did not significantly differ between groups ($p>0.05$), although ponds fertilized with rice bran had the tendency to have lower afternoon pH values than control ponds ($p=0.07$). Temperature and salinity were similar in both groups, as well as turbidity ($p>0.05$). TAN concentrations did not significantly differ among treatments ($p>0.05$), with an average of 0.7 ± 0.6 and 0.8 ± 0.8 mg/L for control and synbiotic groups, respectively. However, the highest values (4 mg/L) were recorded in 2 ponds fertilized with rice bran. TAN levels increased for both groups during the first 3 weeks of trial (Fig. 9), and decreased again as nitrite increased, reaching values below 0.5 mg/L at 76 days of culture, except from one synbiotic ponds which still showed concentrations as high as 4 mg/L.

Nitrite and nitrate concentrations were significantly different between both groups of ponds ($p<0.05$) during the time rice bran was applied (until 69 DOC), with control ponds featuring higher NO_2^- and NO_3^- values than synbiotic ponds. Nitrite started rising from 51 DOC in one control pond which had been previously half-filled with mature nursery water. The other ponds from both groups took longer to show an increase in nitrite, which started to rise only after 61 DOC. Nitrate concentrations started to increase after 50 DOC in a control pond, started to build up in more ponds from both groups after 62 DOC, and kept rising afterwards, reaching concentrations of 500 mg/L for some ponds. Unsurprisingly, as a necessary component for the nitrification process to occur, alkalinity decreased overtime in both groups, from an average of 100 mg/L at 38 DOC, to 65 mg/L at 76 DOC.

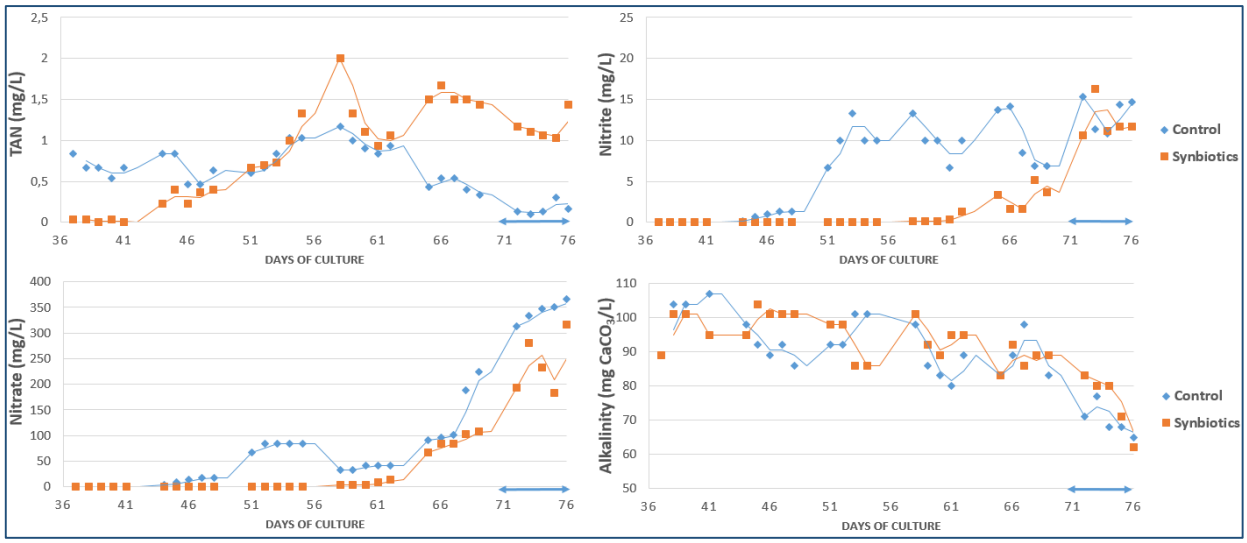

Figure 9: Daily fluctuations in TAN, nitrite, nitrate and alkalinity (mg/L) for both groups of ponds. Values are represented as means for each group. Lines represent the moving average over 2 values. No error bars were added for clarity reasons. Values are shown from the beginning of the trial (36 DOC) to the emergency harvest of 2 ponds (76 DOC). Arrows represent the period during which no rice bran could be added.

PO_4^{3-} concentrations were significantly higher in ponds with rice bran, with some values reaching 4 mg/L. Treatments did not have a significant impact over Vibrio counts, although the highest values were found in ponds with rice bran, with a maximum of 3.7 log CFU/mL.

Total phytoplankton counts were significantly higher in synbiotic ponds ($p<0.05$), with one of them experiencing a cyanobacteria bloom during the trial, showing densities as high as 20 million cells/mL at the bloom peak. This concentration then decreased but remained very high for several days compared to other ponds. Algal populations were made of Cyanophyta (cyanobacteria), Chrysophyta (golden-brown algae), Bacilliariophyta (diatoms), Euglenophyta and Chlorophyta (green algae) genera. Euglena algae were only found in a pond fertilized with rice bran. From observation, apart from one pond which showed high cyanobacteria counts, proportions of diatoms and chrysophytes were higher in synbiotic ponds than in control ones. Control ponds also showed larger proportions of cyanobacteria and chlorophytes, but no clear comparison of data could be made because of identification issues when lugol was used.

Regarding water color, water remained colorless for a few days after chlorination, and then turned brown for all ponds following initial fertilization. Later, water in control ponds quickly took a green color between 10 days and 2 weeks after stocking, and remained green until the end of the trial. In synbiotic ponds, water color remained brown to yellow during most of the experiment unlike control ponds. In one pond fertilized with rice bran, water turned green for 3 days during a strong cyanobacteria bloom, but turned brown again afterwards following the application of more rice bran and some molasses. In another synbiotic pond, water turned red for 2 days during an apparent euglena bloom. In short, the total number of days during which water color was brown to yellow in control ponds was 46, against a total of 86 days for synbiotic ponds.

II.3.3.2 Shrimp performance

Figure 10 represents the individual weight results over the course of the trial. Individual weights values are summarized in Appendix X. Boxplots of the results for each group and pond are shown in

Appendices XI and XII, respectively. Data had to be log-transformed to ensure their normal distribution. Treatment, time and interaction effects were found to be significant ($p<0.05$). It can be assumed that shrimps in both groups grew from sampling to sampling ($p<0.05$). According to these results, shrimps in ponds with rice bran grew faster than in the control group. A significant difference in weights between groups was found from 69 DOC, and average weights stayed significantly different at 76 DOC ($p<0.05$).

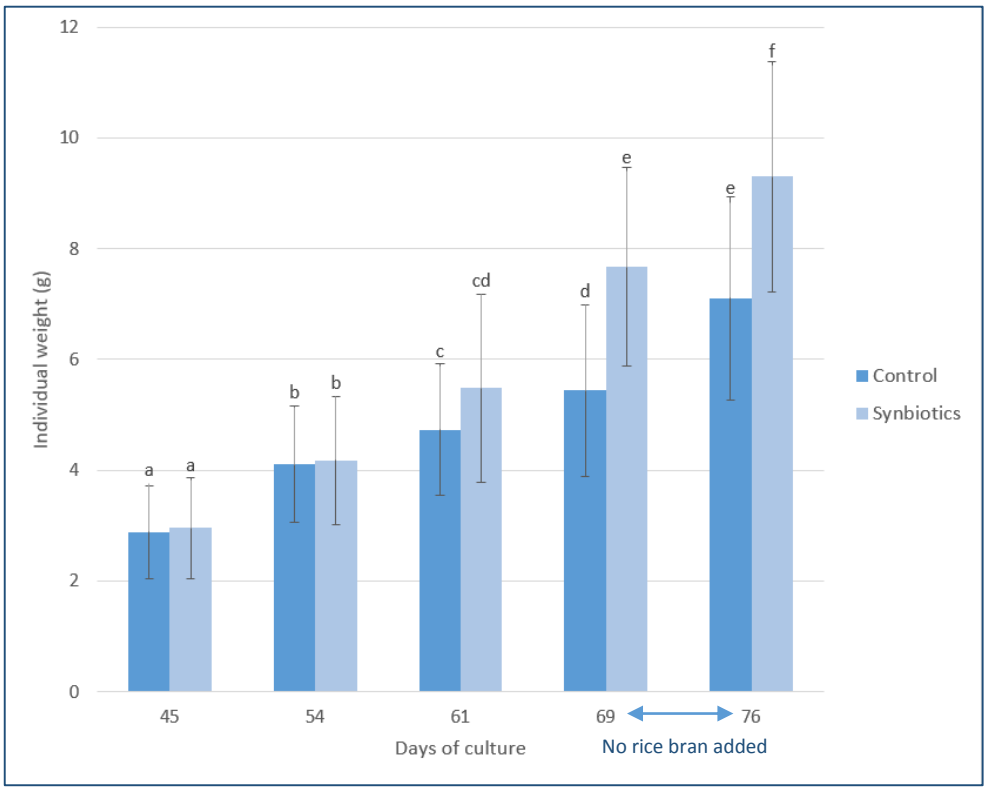

Figure 10: Weight results for both groups of ponds during the grow-out trial. Values are represented as means ± SD (n=90). Different letters indicate significant differences ($p<0.05$). No rice bran was applied in synbiotic ponds after 69 days of culture.

The harvest results following the emergency harvest of two synbiotic ponds are shown in Table 6. The mean weight at harvest was calculated by counting the number of individuals in 1 kilogram of shrimps. They showed clearly different results than the weights presented in figure 10, with only 7.35 and 6.49 grams as mean weights at 76 DOC, when samplings the same day using a cast net gave mean weights of 11 and 9 grams, respectively.

Parameter	S1	S2
Mean weight at harvest (g)	7,35	6,49
Yield (kg)	983,0	699,7
Survival (%)	72,4	58,6
Total amount fed (kg, grow-out)	1205,3	952,4
Days of culture (grow-out)	41	41
Total days of culture	76	76
Apparent FCR (grow-out)	1,68	2,18
Apparent FCR (whole culture period)	1,42	1,63
Average weekly growth (g/week, grow-out)	1,01	0,86
Average specific growth rate (%/day, grow-out)	4,0	3,7

Table 6: Summary of the harvest results for 2 synbiotic ponds.

II.3.3.3 Economic performance

Variable costs in VND and USD for both groups of ponds at harvest time are detailed in Appendix XIV, and summarized in figure 11. The main variable costs were feed and electricity for both groups, feed representing 36 % of these expenses. In second position comes the cost of the nursery phase, presented in chapter I. In third position comes electricity for aeration, which is higher in ponds fertilized with rice bran than control ponds (2 230 USD and 1 689 USD, respectively) due to the use of a powerful air pump for the fermentation of rice bran. Chemicals come fourth and represent a non-negligible cost with more than 690 USD spent per group of ponds, mainly on chlorine, Azomite® and baking soda. In total, total variable costs reached 10 673 USD for the 3 control ponds, and 11 527 USD for synbiotic ponds. Costs were higher in ponds fertilized with rice bran because of increased electricity and feed costs, as shrimps ate more feed in these ponds.

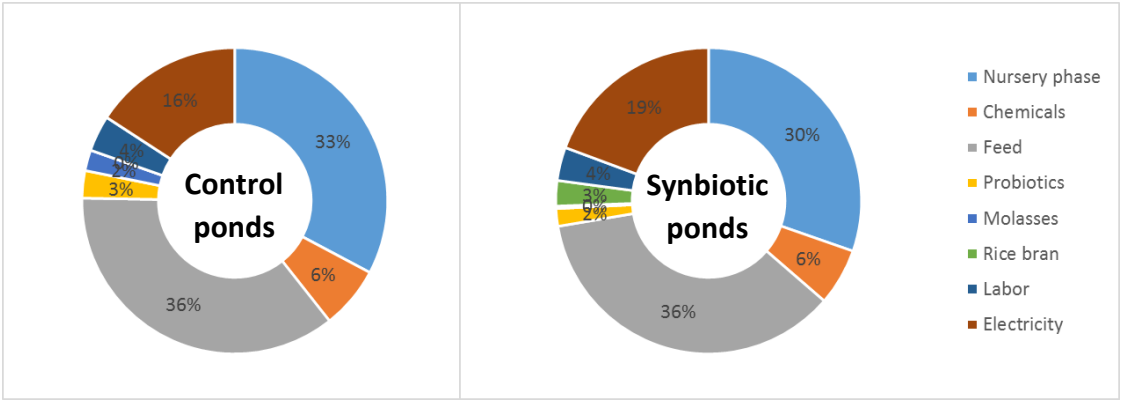

Figure 11: Production costs for control and synbiotic ponds (at the time of emergency harvest for 2 ponds). For each group, sums of costs for all ponds are represented.

A partial budget for the 2 harvested ponds can be found in Appendix XIII. Variable cost ratio was 5.7 and 4.4 USD for these ponds, which means that each kilogram of shrimps costs 5.7 and 4.4 USD to produce. As shrimps had to be harvested early in the cycle, sale prices were low, with 2.6 and 2.3 USD/kg shrimps. Based only on variable costs, losses of 1 826 and 2 378 USD were reported for harvested ponds.

II.4 Discussions

II.4.1 Impact of different fermentation strategies on rice bran characteristics

Unfortunately, hardly anything can be concluded from the fermentation results, given that the three treatments gave similar *Bacillus* spp. counts. Surprisingly, the control sample showed a very high concentration in *Bacillus* spp., with more than 8 log CFU/mL although no inoculation was performed. Raw rice bran must contain Bacillus bacteria, but certainly not at this level. If it was the case, the three treatment groups which received inoculums should have given much higher counts. Hence, an obvious problem of method appears in that experiment. Due to a lack of materials, Bacillus counts were not performed in aseptic conditions ie with sterile rakes, pipettes and a benzene burner for instance. Some contamination may have occurred on the Petri dishes, giving false results. As a matter of fact, some colonies could be counted on a Petri dish seeded with only sterilized water kept at 100 °C for 20

minutes. Furthermore, the agar plate technique in itself may not be the right method to assess the development of bacteria during fermentation, and other techniques such as real-time PCR would have been more appropriate to properly benchmark the different probiotics. Bacteria counts obtained here cannot be directly compared with any publication at this date, but Supriyati et al. (2014) who performed solid-state fermentations of rice bran with *B. amyloliquefaciens* at similar inoculation rates only found a 10 times increase in Bacillus counts 3 days after inoculation.

Similar to the previous results, no conclusion can be drawn from the crude fiber contents. Supriyati et al. (2014) and Romano et al. (2018) both found a reduction in fiber content following fermentation of rice bran with similar inoculums of *Bacillus* spp. They explain this decrease by the action of enzymes during fermentation. For instance, *B. amyloliquefaciens* which is present in the AOcare™ probiotic produces α-amylase which can hydrolyse complex carbohydrates such as starch and cellulose into simpler and more available molecules (Wizna et al., 2012; Supriyati et al., 2014; Romano et al., 2018). Romano et al. (2018) then concluded that the quality of rice bran as a carbon source for aquaculture ponds was enhanced by fermentation, being more soluble in water. Heterotrophic bacteria in the pond would then better grow on rice bran, potentially converting more ammonia into microbial proteins. Other than fiber content, water solubility could have been assessed, but would have required the use of a centrifuge. Romano et al. (2018) found a significant increase in water solubility of rice bran after 24 hours of fermentation with *B. megaterium* and *B. licheniformis*. Protein content was also found to increase following fermentation of rice bran with *Bacillus* spp., but with *Rhizopus oryzae* (Supriyati et al., 2014; Ranjan et al., 2018 and Romano et al., 2018).

Besides the choice of probiotics, another parameter which was not assessed here is the use of aeration or not to ferment rice bran. In this trial, as rice bran was fermented in small beakers inside a rotary shaker, no extra aeration was applied. But the manufacturer of White cap™, as well as Romano (2017) and Santhanam et al. (2019) advise the use of aerators during fermentation of liquid rice bran. It is known that the genus *Bacillus* spp. include both aerobic and facultative anaerobic bacteria (O'Leary, 1989). A trial was made over different conditions for the fermentation of rice bran, with both White cap™ and AOcare™ used to ferment rice bran under strong or no aeration. Results showed a sharp decrease in pH for all groups, but even sharper for the unaerated groups during the first 24 hours ($p<0.05$). Dissolved oxygen decreased significantly for all groups from the beginning of the trial to 24 hours ($p<0.05$), and then stabilized with no significant differences after 48 hours among groups. Those findings were difficult to interpret, but a sharp decrease in dissolved oxygen for aerated groups indicates that some kind of fermentation process does occur. The stronger decrease in pH for unaerated groups seems to indicate that the action of *Lactobacillus* spp. was favored, which is a genus of bacteria naturally occurring in rice bran (Zubaidah et al., 2012). Aero-tolerant or anaerobic, they produce lactic and acetic acids (pKa of 3.86 and 4.76, respectively). Romano et al. (2018) stated that production of α-amylase from *Bacillus* spp. was greater in anaerobic conditions. However, aerobic conditions seemed more suitable for the production of proteases, making more amino acids available. Haq et al. (2002) also insist on the need to buffer the pH during fermentation, the production of α-amylase being maximized at a pH ranging from 7.5 to 8 with *B. subtilis*.

II.4.2 Comparison of different fermentation strategies over water quality and natural productivity

Very high temperatures, as high as 39.8 °C, as well as large temperature fluctuations between day and night must have largely impacted copepod densities. However all densities higher than 100 individuals

per liter were seen in bottles fertilized with rice bran, with a maximum of 210 individuals per liter. This maximum could be put into perspective with previous findings in shrimp ponds all over the world, although a clear comparison would be futile, as densities largely depend on the species involved and the pond condition. Furthermore, the concentrations found in this study were assessed using a 150 microns mesh, which only traps the largest zooplankton. To give an order of magnitude, Campos et al. (2009) assessed the influence of wheat bran on the availability of plankton during the grow-out of *P. vannamei* in Brazil, and found total zooplankton densities over 1000 individuals/mL upon stocking the shrimps, the vast majority of which being rotifers. Cardozo et al. (2007) observed inside shrimp ponds in Mexico copepod densities of over 120 ind/L prior to stocking. Gálvez et al. (2015) reported into an intensive biofloc system a zooplankton density of 1700 ind/L, mainly copepods and rotifers. Chakravarty et al. (2018) argued that cyclopoid copepods, the group of species involved in this trial, featured promising nutritional values and could reached densities as high as 5000 ind/L in controlled conditions (Chakravarty et al., 2018). The dominance of cyclopoids during the experiment is consistent with low salinities, as this group is more abundant in freshwater (Støttrup, 2003).

Santhanam et al. (2019) pointed out that ciliates and dinoflagellates represented the bulk of copepods' diet. As no significant differences were found between control and treated groups in terms of final phytoplankton densities, and as the vast majority of observed algae consisted only of cyanobacteria, it is legitimate to think that rice bran did indeed increase densities in copepods. However, according to Lemus et al. (2004) and Chakravatry et al. (2018), the densities reached during this experiment could have been far higher. These enhanced densities could provide an interesting supplemental food source for newly stocked shrimps, which is one of the cores of this concept as described by Romano (2017).

Following these findings, the previous ones over the effectiveness of fermentation, as well as the advice given by Romano (2017) and Santhanam et al. (2019), the choice was made to ferment rice bran under strong aeration, with 1.6 g of AOcare™ per kilogram of rice bran (ie 4 x 10^9 CFU/kg rice bran) for the pond trial with shrimps.

II.4.3 Field trials

II.4.3.1 Water quality

Regarding water quality, dissolved oxygen was not always kept within the optimal range for *P. vannamei* (Louis, 2006; Prapaiwong and Boyd, 2012), which should always be above 3 mg/L. Low DO events (below 3 but above 2 mg/L) were reported 4 times during the first 3 weeks of trial, following algal crashes in 3 ponds. Although not lethal, a concentration below 3 mg/L is known to induce stress which can cause increased susceptibility to diseases, and lead to slow growth (Louis, 2006; Prapaiwong and Boyd, 2012). Oxygen levels were later better managed following removal of sludge on a daily basis which decreased organic contents in the ponds. Also, the installation during the experiment of sensors from Eruvaka which provide real-time monitoring over dissolved oxygen and temperature helped decide when to apply full aeration or not. Such a system can help save costs over electricity, aeration being performed only when necessary.

A significant difference in afternoon dissolved oxygen values was observed between control and synbiotic groups, with synbiotic group featuring significant lower oxygen values than control ponds. This observation leads to think that concentrations in dissolved oxygen were better regulated in synbiotic ponds. Both groups of ponds did not feature significant differences in morning or afternoon

pH values, although the p-value for afternoon pH is close to 0.05. However, pH was significantly more stable during the day in synbiotic ponds, the comparison of means test giving a p-value of 0.02 on absolute differences between morning and afternoon pH values. These observations on both pH and oxygen are linked and indicative of a better control over photosynthesis activity in synbiotic ponds, potentially leading to fewer stress for shrimps as variations of the main water parameters are minimized. This is coherent with the claimed advantages of the application of fermented rice bran from Romano (2017). This suspected enhanced control over photo autotrophy could have different origins, such as a better shading of water due to rice bran particles and an enhanced heterotrophic pathway which leaves less room for algal activity.

The evolution of nitrogenous parameters was similar for both control and synbiotic ponds. Significant differences ($p<0.05$) in nitrite and nitrate concentrations and summarized in Table 5 have to be interpreted carefully, as they do not take into account the last week of experiment from 70 to 76 DOC as rice bran was not added. Furthermore, these significant differences ($p<0.05$) were probably due to one control pond which featured high levels of nitrite and nitrate early in the trial, as it was half-filled with mature nursery water. Mean concentrations in nitrite and nitrate would probably have been similar otherwise.

However, the highest ammonia concentrations were seen in synbiotic ponds, with values as high as 4 mg/L for one pond. The maximum NH_3-N value was 0.59 mg/L, given the pH, temperature and salinity when it occurred. This is well above the safety limit of 0.1 mg/L recommended by Prapaiwong and Boyd (2012), and must have impacted general health of the shrimps. This result could indicate that less ammonia was assimilated by algae in ponds fertilized with rice bran compared to control ones. It is also consistent with concentrations in phosphate, which were significantly higher in synbiotic ponds. According to Maia et al. (2016), an accumulation of PO_4^{3-} means it is not used for photosynthesis.

Nitrite reached a maximum of 40 mg/L in 2 ponds corresponding to a concentration in $N0_2^-$-N of 12 mg/L, which is twice the safety limit recommended by Lin and Chen (2003) for *P. vannamei* juveniles (at 15 ppt salinity). Vinatea et al. (2010) reported that concentrations as low as 9.49 mg $N0_2^-$-N/L were enough to significantly impact growth of *P. vannamei.* As low water exchange rates were performed, nitrate accumulated in all ponds, reaching 500 mg/L in 2 ponds. Although often not considered, high concentrations in nitrate can have detrimental effects on shrimps. Furtado et al. (2014) advise a maximum concentration in NO_3^--N of 177 mg/L (or 784 mg/L NO_3^--NH) at a salinity of 23 ppt, beyond which a decrease in performance, as well as damages to the gills and the hepatopancreas and even mortalities can be observed in the long run. Similar to ammonia and nitrite, nitrate toxicity is known to increase with lower salinity values, and was not studied at such low salinities (5.4 ± 0.4 g/L at the end of this experiment). However, due to the small volume of the treatment pond for sedimentation and chlorination of incoming water (equivalent to 13% of the total grow-out area), few water exchanges were performed during the trial. On average, 30% and 27% of ponds' volume was exchanged over the course of the trial in control and synbiotic groups, respectively.

It is interesting to notice that alkalinity fell quickly in all ponds from 67 to 76 DOC, dropping from an average of 92 ± 15 mg $CaCO_3$/L to 63.5 ± 9 mg $CaCO_3$/L. As nitrification consumes inorganic carbon, ie 3.13 mg/L for each gram of NH_4^+-N consumed, it is not surprising to see a fall in alkalinity (Ebeling et al., 2006). Correction of alkalinity was done using either baking soda ($NaHCO_3$), dolomite ($CaMg(CO_3)_2$) and calcite ($CaCO_3$), 100 mg $CaCO_3$/L being the lower limit for an ideal development of *P. vannamei* (Furtado et al., 2011; Serra et al., 2015). However, apart from baking soda which was effective in raising alkalinity, dolomite and calcite did not seem to have an impact over alkalinity, although 12 kilograms of either one of the other were applied every day in each pond. According to Wurts (2002) and Boyd

et al. (2017), liming materials hardly dissolve in ponds when alkalinity is over 50 mg/L, and solubility of dolomite and calcite is very low compared to baking soda (0.01 g/L, 0.1 g/L and 87 g/L, respectively) (Sherman and Barak, 2000).

Surprisingly, ponds fertilized with rice bran showed significantly higher concentrations in phytoplankton than control ponds, although this result is due to a cyanobacteria bloom episode which occurred in a synbiotic pond. Concentrations in phytoplankton would have been pretty similar otherwise. Rather than algae counts, determination of chlorophyll a using a spectrophotometer would have been more useful for the purpose of this experiment, being directly linked to the activity of photosynthesis. Water color in all ponds with rice bran appeared brown to yellow during almost all the time rice bran was added, which is not the case of control ponds which appeared green most of the time. This difference in color can have various origins, such as an increased concentration in suspended organic matter with higher heterotrophic activity, or a difference in algal populations (Hargreaves, 2013; Xu et al., 2016). A brown to yellow color would be consistent with higher proportions of diatoms and chrysophytes, and a green color with a dominance of cyanobacteria and chlorella (Van Vuuren et al., 2006).

Another interesting consequence of the application of liquid fermented rice bran is the quantity of sludge produced at the bottom of the ponds, which was present in far smaller amounts in synbiotic ones. Unfortunately, no accurate data could be gathered over the amounts of sludge from each pond, which would have required close observation of the exit pipes and the use of a chronometer. But from these findings it is legitimate to think that liquid fermented rice bran with *Bacillus* spp. does indeed have a beneficial impact over mineralization of organic matter in the pond. This is coherent with the claimed advantages of this technique from Romano (2017).

Vibrio counts did not differ significantly between the 2 groups. However, synbiotic ponds showed high *Vibrio* spp. loads at the beginning of the trial, above 3000 CFU/mL, probably due to the establishment of a biofilm from settled rice bran at the bottom. Unlike the recommendations of Romano (2017), no bottom raking was performed for practical reasons and lack of time, and this decision may have favored bacterial biofilms. High *Vibrio* spp. loads (above 2000 CFU/mL) were also seen in the 2 synbiotic ponds harvested in emergency at 76 DOC, and may be related to the fact that shrimps were controlled positive in these ponds to acute hepatopancreatic necrosis disease (AHPND). These results are commented later in this chapter.

II.4.3.2 Shrimp performance

Regarding shrimp performance from samplings performed on a weekly basis, growth appeared to be significantly faster in ponds fertilized with rice bran. This difference in growth rates could be partially explained by the fact that shrimps can feed on rice bran particles as explained by Chakravarty et al. (2018). Furthermore, the concept of liquid fermented rice bran as a synbiotic for shrimp farming is described by Romano (2017) as the mime of the natural composition of an estuary, thus the establishment of a more natural and less stressful environment for shrimps. Moreover, in the same line of reasoning, the best mean weights and highest feed consumptions before the emergency harvest were seen in a highly turbid pond fertilized with rice bran, which showed a high turbidity (30 cm) prior to stocking (but after chlorination). This pond was indeed filled with water which did not have time to properly settle, and showed high concentrations (0.5 mg/L) of ionic iron which resulted in a strong red to brown color after chlorination as iron precipitated. Water in the other ponds appeared crystal clear after chlorination, with Secchi disk readings higher than 1 meter. This could lead to think the more

natural the water, the better the performances. Basically two schools of thought collide here. The first one is based on the idea that the environment of shrimps should be closely controlled and other organisms kept outside the pond as much as possible. The other one is described in Romano (2017), Chakravarty et al. (2018) and Santhanam et al. (2019) and is based on the "copefloc" concept, where water is not treated with chemicals and, as opposed to the previous way, the natural fauna is favored. Risks and gains have to be balanced, but there is a high probability better performances could have been reached if water had not been treated in the first place.

However, at 76 DOC, 2 ponds previously fertilized with fermented rice bran had to be harvested in emergency, as some large mortalities began to occur. Surprisingly, the mean weights obtained from samplings during harvest gave far smaller values than the ones obtained from cast nets. These large differences in mean weights (3.65 g for the largest) are difficult to understand, especially since very small shrimps were also caught in the cast net. It nevertheless appears that this technique induces a strong selection pressure on large shrimps, and this method will have to be reviewed for the next cycle. However, as the same method was used for all ponds, these sampling results were kept for interpretation to compare ponds' performances. Samplings with a cast net but from different locations in the pond and over more shrimps per pond (60 instead of 30) as described in Ullman et al. (2019) could have improved the accuracy of the results. However the amount of time needed for this operation was already very long, with more than 2 hours for 3 people to sample all 6 ponds. For production purposes, the number of shrimps in 1 kilogram could be counted, which the fastest way of doing. However, even with 3 ponds per treatment, statistics which can be run with this counting method are far less powerful than the ones performed in this report.

Survival rates calculated over both harvested ponds can be compared with the results from Boyd et al. (2017), who recently conducted a survey over 30 *P. vannamei* farms in the Mekong Delta. They reported an average survival of 70.8 ± 4.4 %, which is comparable to the 72.4 % survival obtained in a pond fertilized with rice bran, but far higher than the other pond which featured a 58.6 % survival. However, it is worth mentioning that the average crop duration from the farms in the survey was 90 days, against only 76 days in this trial, which makes a straight comparison difficult. The average apparent FCR reported by Boyd et al. (2017) was 1.33 ± 0.40 over the whole culture period. The apparent FCRs calculated in this trial from the beginning of the nursery phase are rather high compared to the previous findings, with 1.42 and 1.63 for both harvested ponds.

The reason why shrimps started to die in great numbers in 2 ponds before the emergency harvest is still unclear. As AHPND is characterized by fast and mass mortalities (OIE, 2019), this disease was being looked for. Bacterial counts performed the same day from samples taken in these ponds revealed rather high numbers of total *Vibrio* spp. (1.73 and 1.95 x 10^3 CFU/g), but did not reveal the presence of *V. parahaemolyticus*, species involved in the AHPND syndrome (OIE, 2019). However, samplings performed in the gut and hepatopancreas of moribund shrimps revealed high loads of *V. parahaemolyticus*, ranging from 5.45 to 5.65 x 10^5 CFU/g. Furthermore, shrimps in these ponds tested positive for AHPND using real-time PCR methods. It is then highly probable that AHPND was the main cause of this event. The reason why only two ponds experienced sudden mortalities is unclear, especially since these very ponds showed the best performances. It is possible that a biofilm highly loaded in pathogenic bacteria developed at the bottom of the ponds. But since similar outbreaks occurred at the same time in neighboring farms, the question remains. At the time of writing all four remaining ponds got a similar outbreak afterwards and shrimps had to be sold at an early stage. In addition, the rather poor growth performances revealed during harvest may also be linked to the occurrence of the *Enterocytozoon hepatopenai* parasite (EHP), to which all ponds were tested positive using real-time PCR methods. This microsporidian parasite is confined to the hepatopancreas and is involved in slow growth performances but not mass mortalities as opposed to AHPND (Santhoshkumar et al., 2017). Thus performance data should be interpreted with caution, as the infection sequence

among ponds is not known. Biosecurity therefore appears to be of even greater importance, and basic measures such as the use of boot baths or farm fencing were unfortunately not put in place at that time. Furthermore, in order to pump sludge out of the ponds on a daily basis, workers had to dive into the ponds, and went from pond to pond without taking any particular care.

II.4.3.3 Economic performance

Regarding economic performances for harvested ponds, as shrimps had to be sold at an early stage, net losses were reported. It is worth mentioning that electricity represents the third expense after feed and the nursery phase, and as mentioned earlier a real-time monitoring of dissolved oxygen could help reduce these costs. For ponds fertilized with rice bran, only a powerful air pump was available to ferment rice bran, resulting in even higher electricity costs. However such a pump was not necessary, therefore electricity costs from fermentation could be easily mitigated. Unfortunately, as data over fixed costs was not available, only variable costs were taken into account in this study. It is nevertheless worth comparing production costs to those from Engle et al. (2017), who conducted a survey on 43 shrimp farms in Vietnam. The authors found a total cost per metric ton of *P. vannamei* of 5 387 USD for the most intensive farms, which is close to the variable costs involved during this trial (Appendix XIII).

General conclusion

During this study, the application of a biofloc technology was assessed during the nursery phase of *P. vannamei* as a way to assimilate ammonia inside the pond. Sugar cane molasses were chosen as the source of carbohydrates, at a carbon/nitrogen ratio of 12:1 based on the amounts of feed. The resulting heterotrophic system appeared to be efficient in controlling ammonia levels, and featured very stable pH and oxygen values. However, low oxygen issues quickly appeared inside the pond as the bacterial biomass was not removed. Therefore, the carbon/nitrogen ratio was reduced and water exchanges performed to decrease the biological oxygen demand, resulting in larger pH variations and in an increase in oxygen levels which can be explained by the return of photosynthesis. However during this transition time ammonia levels increased fast and NH_3-N reached dangerous concentrations for the shrimps. Large and continuous water exchanges were finally successful in reducing these levels again before shrimps were harvested and transferred. Although often close to the safe biological limits of the cultured species, this trial gave good results in terms of survival and feed conversion ratio, but not on growth because of a high variation coefficient among shrimps at harvest. Unfortunately, as only one pond was available, this protocol was not compared to a standard one which would had left more room for photosynthesis for example. Furthermore, the follow-up of the system was not performed properly, as measurement of total suspended solids instead of settleable solids would have been much more appropriate. Also, as the biological oxygen demand is very high, a spare air pump and an emergency generator seem to be compulsory to run such a system safely. Mechanical filtration of water to remove bacterial flocs seems also very important to keep optimal oxygen levels.

The second part of this study was focused on the application of liquid fermented rice bran as a way to balance heterotrophy and photosynthesis inside grow-out ponds of *P. vannamei*. Different fermentation strategies were tried, but as the methods used were probably not suitable, very few conclusions could be drawn. A real-time PCR to assess the concentration in *Bacillus* spp. after fermentation would probably have given exploitable results. The amount of probiotics and the

aeration conditions for fermenting rice bran were therefore based on recommendations from previous publications. The application of liquid fermented rice bran seemed however to have an impact over copepod densities, although the effect was not found to be significant, probably because of high temperatures during the trial. The maximum copepod densities reached were certainly far below to what could be done inside a pond.

During the field trial with shrimps, the application of fermented rice bran was compared to another source of carbon. The choice was made to chlorinate the water in order to keep shrimps as safe as possible from diseases, therefore annihilating most of the natural fauna of the ponds. Nevertheless, the application of liquid fermented rice bran seemed to have a significant impact over photosynthesis as ponds fertilized with it featured less pH and dissolved oxygen variations and had their phosphate levels rising. Measurements of concentrations in chlorophyll a would have been very useful to confirm these findings. Rice bran application also had a beneficial impact over the mineralization of dead organic matter at the bottom of the ponds, which is consistent with the claimed advantages of this technique from various authors. Based on weekly samplings, shrimps also performed better in the ponds supplied with rice bran, with a faster growth compared to control ponds. However, the sampling method needs to be revised as it was found to select mostly large shrimps. After 41 days of trial a disease outbreak occurred into two ponds, probably due to the AHPND syndrome, resulting in economic losses. Also, the poor growth performances for all ponds may be due to the occurrence of the *Enterocytozoon hepatopenai* parasite, which made growth performance results hardly interpretable. The application of strict biosecurity measures on the farm could help prevent similar episodes in the future. A trial with liquid fermented rice bran in a nursery pond would be interesting to carry out with water not treated with chemicals to let zooplankton densities rise, as they could provide an extra food source to newly stocked post-larvae (as suggested in Cardozo et al., 2007 and Santhanam et al., 2019).

More generally, a life cycle analysis of the next production cycles on the farm would be useful to assess environmental impacts of this production, in particular on the eutrophication of coastal waters as large volumes of water highly loaded in nitrate are unfortunately dumped into the rivers by all shrimp farms. This analysis would therefore complete this study, as environmental costs were not taken into account here.

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Appendices

Appendix I: Satellite view of the facilities. Arrows represent water flows (brown: untreated turbid brackish water, white: treated water, grey: discharge water) (Google, 2019, Nhà Bè, Vietnam).

Appendix II: Picture of a sludge collector in the middle of a pond. Pipes are used to pump sludge out of the pond (Jean-Benoît Darodes de Tailly, 2019, Nhà Bè, Vietnam).

Appendix III: Picture of part of the nursery pond after stocking. Full aeration is applied, water color is brown, and foam is seen at the surface due to the regular addition of organic carbon (Jean-Benoît Darodes de Tailly, 2019, Nhà Bè, Vietnam).

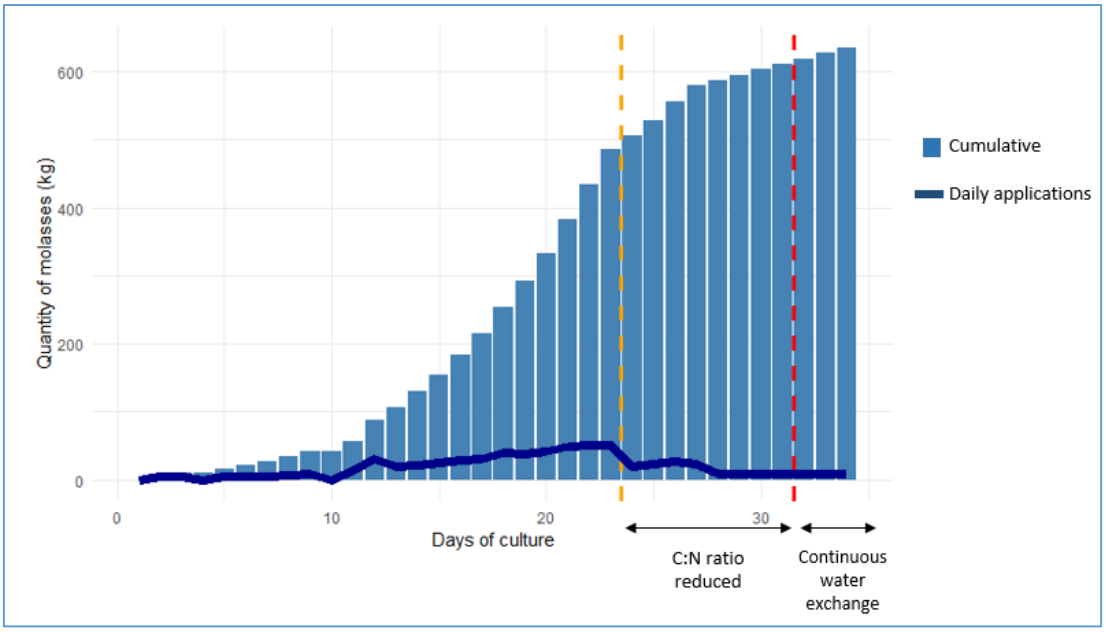
Appendix IV: A feeding tray is being positionned into the pond with feed. It will be checked an hour later to assess feed consumption (Jean-Benoît Darodes de Tailly, 2019, Nhà Bè, Vietnam).

Appendix V: Summary of a comparative study of molasses and rice bran to determine organic carbon and nitrogen contents (adapted from Skretting, 2019, unpublished results).

	Molasses				
Proximate analysis	**Total (g/kg wet weight)**	**Carbon content (%)**	**Carbon content (g/kg wet weight)**	**Nitrogen content (%)**	**Nitrogen content (g/kg wet weight)**
Dry matter	780				
Crude protein	30	53	15,9	16	4,8
Crude fat	5	80	4	1,2	0,1
Crude fiber	0,1	42	0		
Crude ash	120				
Carbohydrate	625	42	262,5		
Total (g/kg wet weight)			282,4		4,9

	Rice bran				
Proximate analysis	**Total (g/kg wet weight)**	**Carbon content (%)**	**Carbon content (g/kg wet weight)**	**Nitrogen content (%)**	**Nitrogen content (g/kg wet weight)**
Dry matter	900				
Crude protein	124	53	65,7	16	20
Crude fat	120	80	96	1,2	1
Crude fiber	95	42	39,9		
Crude ash	85				
Carbohydrate	476	42	199,9		
Total (g/kg wet weight)			401,5		21

Appendix VI: Daily applications and cumulative amounts of molasses added into the nursery pond.



Appendix VII: Partial budget describing the production costs during the nursery phase.

Category	Unit	VND/Unit	Quantity	Cost (VND)	Cost (USD)
Variable costs					
PL cost	Individual	110	700 000	77 000 000	3 311
Chemicals					
Chlorine	Kg	50 000	100	5 000 000	215
Saponin	kg	6 000	30	180 000	8
Azomite	Kg	22 000	75	1 650 000	71
Baking soda	Kg	12 000	115	1 380 000	59
Calcite	Kg	5 500	200	1 100 000	47
Dolomite	Kg	3 500	170	595 000	26
Feed	Kg	34 000	1 041	35 407 600	1 523
Other organic inputs					
Probiotics	kg	1 200 000	5,1	6 120 000	263
Molasses	kg	7 000	732,2	5 125 400	220
Labor	Week	975 000	5,2	5 070 000	218
Electricity					
Paddlewheels	kWh	3 000	2 600	7 798 658	335
Air pump	kWh	3 000	5 153	15 458 613	665
Water pumps (filling and water exchanges)	kWh	3 000	239	715 884	31
Total variable costs				162 601 154	6 992

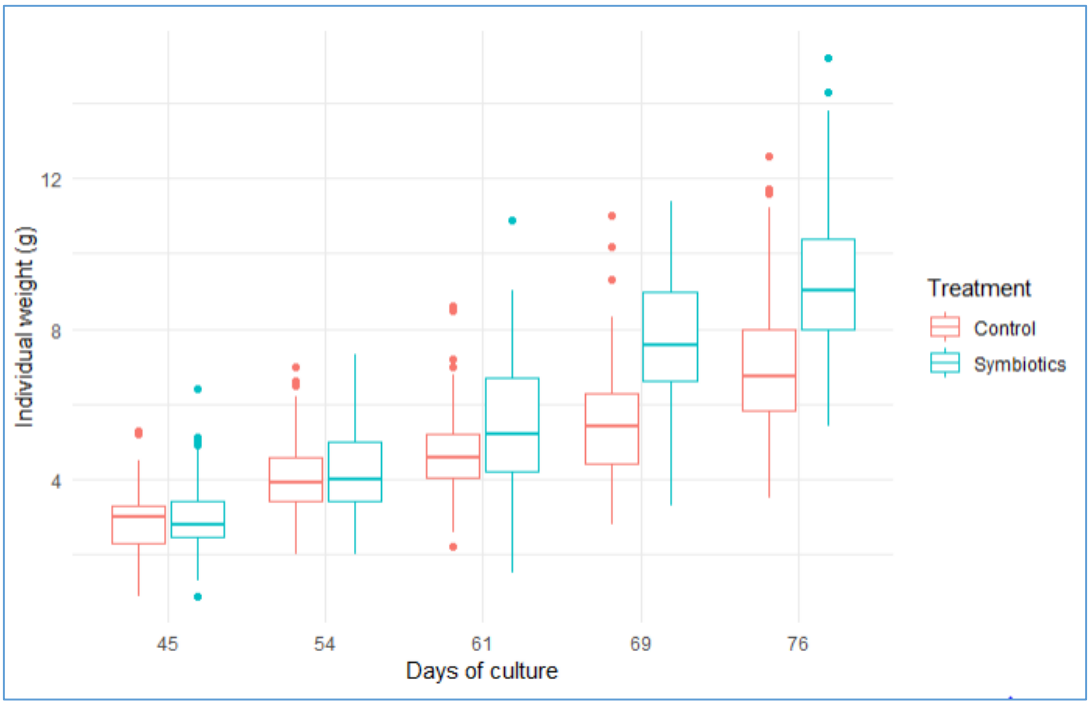
Appendix VIII: Picture of a grow-out pond at daytime. An automatic feeder can be seen in the center, and one paddlewheel per pond is working in the background (Jean-Benoît Darodes de Tailly, 2019, Nhà Bè, Vietnam).

Appendix IX: Picture of a fermentation tank. Rice bran is fermenting under full aeration, which along with baking soda produces foam (Jean-Benoît Darodes de Tailly, 2019, Nhà Bè, Vietnam).

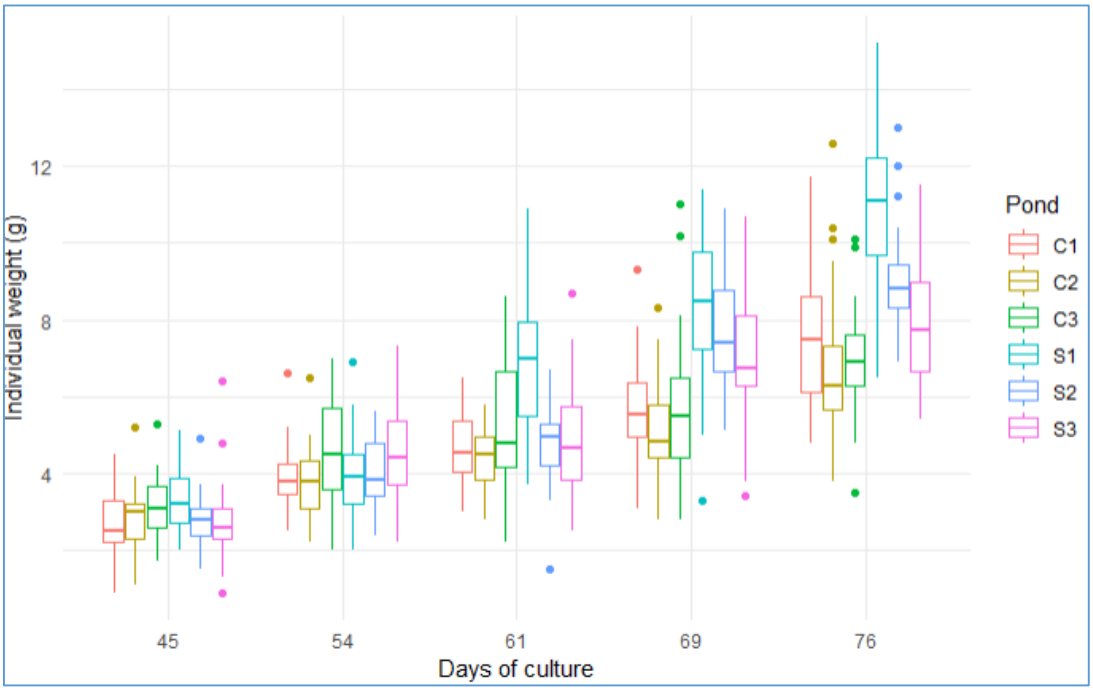
Appendix X: Summary of the mean weights recorded for the two groups of ponds during the trial. Values are represented as means ± SD (n=9). Minimums and maximums are shown in brackets. Values with different letters are significantly different (p<0.05).

DOC	Control	Synbiotic
45	2,9 ± 0,8[a] (0,9/5,3)	3 ± 0,9[a] (0,9/6,4)
54	4,1 ± 1[b] (2/7)	4,2 ± 1,2[b] (2/7,3)
61	4,7 ± 1,2[c] (2,2/8,6)	5,5 ± 1,7[cd] (1,5/10,9)
69	5,4 ± 1,5[d] (2,8/11)	7,7 ± 1,8[e] (3,3/11,4)
76	7,1 ± 1,8[e] (3,5/12,6)	9,3 ± 2,1[f] (5,4/15,2)

Appendix XI: Boxplots of the individual weights for both groups of ponds during the grow-out trial. No rice bran was applied in synbiotic ponds after 69 days of culture.



Appendix XII: Detail boxplots of individual weights for each pond during the grow-out trial. C-ponds stand for control ponds, S-ponds for synbiotic ponds. No rice bran was applied in synbiotic ponds after 69 days of culture.



Appendix XIII: Partial budget describing the benefits and variable costs for 2 synbiotic ponds which had to be harvested in emergency.

Category	Unit	VND/Unit	Quantity	Value/Cost (VND)	Value/Cost (USD)
1. Gross receipts					
136 counts/kg	kg	60 000	983	58 980 000	2 536
Total receipts				58 980 000	2 536
2. Variable costs					
Nursery phase		162 601 154	0,2	29 795 135	1 281
Chemicals				0	0
Chlorine	kg	50 000	37	1 850 000	80
Azomite	kg	22 000	83	1 826 000	79
Baking soda	kg	12 000	138	1 650 000	71
Calcite	kg	5 500	109	599 500	26
Dolomite	kg	3 500	97	339 500	15
Feed	kg	34 000	1 205	40 980 200	1 762
Other organic inputs					
Probiotics	kg	1 200 000	1	1 584 000	68
Rice bran	kg	8 000	345	2 760 000	119
Labor					
Stocking	Total	2 800 000	0	513 074	22
Daily operations	Week	455 000	6	2 684 500	115
Electricity				0	0
Paddlewheels	kWh	3 000	4 430	13 288 591	571
Water pumps for sludge removal	kWh	3 000	22	65 623	3
Water pumps for water exchange	kWh	3 000	64	192 394	8
Air pump for fermentations	kWh	3 000	1 104	3 310 962	142
Total variable costs				101 439 478	4 362
3. Income above variable costs				-42 459 478	-1 826
4. Variable cost ratio				103 194	4,4

Category	Unit	VND/Unit	Quantity	Value/Cost (VND)	Value/Cost (USD)
1. Gross receipts					
154 counts/kg	kg	53 000	700	37 084 100	1 595
Total receipts				37 084 100	1 595
2. Variable costs					
Nursery phase		162 601 154	0,2	29 806 442	1 282
Chemicals					
Chlorine	kg	50 000	37	1 850 000	80
Azomite	kg	22 000	83	1 826 000	79
Baking soda	kg	12 000	100	1 200 000	52
Calcite	kg	5 500	109	599 500	26
Dolomite	kg	3 500	97	339 500	15
Feed	kg	34 000	952	32 381 600	1 392
Other organic inputs					
Probiotics	kg	1 200 000	1	1 536 000	66
Rice bran	kg	8 000	320	2 561 600	110
Labor					
Stocking	Total	2 800 000	0	513 268	22
Daily operations	Week	455 000	6	2 684 500	115
Electricity					
Paddlewheels	kWh	3 000	4 510	13 530 201	582
Water pumps for sludge removal	kWh	3 000	21	62 640	3
Water pumps for water exchange	kWh	3 000	63	187 919	8
Air pump for fermentations	kWh	3 000	1 104	3 310 962	142
Total variable costs				92 390 134	3 973
3. Income above variable costs				-55 306 034	-2 378
4. Variable cost ratio				132 042	5,7

Appendix XIV: Partial budget describing variable costs involved for both groups of ponds during the grow-out trial. Values represent the sum of costs for all 3 ponds within the same group (at the time of emergency harvest for 2 ponds).

			Control ponds			Symbiotic ponds		
Category	Unit	VND/Unit	Total quantities	Total costs (VND)	Total costs (USD)	Total quantities	Total costs (VND)	Total costs (USD)
Variable costs								
Nursery phase		162 601 154						
Chemicals								
Chlorine	kg	50 000	0,5	81 300 577	3 496	0,5	81 300 577	3 496
Azomite	kg	22 000	99	4 950 000	213	90	4 500 000	194
Baking soda	kg	12 000	249	5 478 000	236	244	5 368 000	231
Calcite	kg	5 500	263	3 150 000	135	288	3 450 000	148
Dolomite	kg	3 500	327	1 798 500	77	327	1 798 500	77
Feed	kg	34 000	291	1 018 500	44	291	1 018 500	44
Other organic inputs			2 622	89 161 600	3 834	2 837	96 464 800	4 148
Probiotics	kg	1 200 000	6,0	7 171 200	308	4,1	4 964 400	213
Molasses	kg	7 000	778	5 448 800	234	100	700 000	30
Rice bran	kg	8 000	0			900	7 201 600	310
Labor								
Stocking	Total	2 800 000	0,5	1 392 930	60	0,5	1 392 930	60
Daily operations	Week	455 000	17,7	8 053 500	346	17,7	8 053 500	346
Electricity								
Paddlewheels	kWh	3 000	12 832	38 496 644	1 655	13718	41 154 362	1 770
Water pumps for sludge removal	kWh	3 000	64	190 902	8	65	193 885	8
Water pumps for water exchange	kWh	3 000	198	595 078	26	190	570 000	25
Air pump for fermentations	kWh	3 000	0			3 311	9 932 886	427
Total variable costs				248 206 232	10 673		268 063 940	11 527

	Diplôme : Ingénieur agronome Spécialité : Sciences halieutiques et aquacoles Spécialisation / option : Aquaculture Enseignant référent : Pr. Hervé Le Bris

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Nb pages : 35 Annexe(s) : 14	
Année de soutenance : 2019	

Titre français : Etude d'un protocole économique d'élevage de crevettes visant à améliorer le contrôle de la qualité de l'eau.

Titre anglais : Study of an economical shrimp farming protocol aiming at improving control over water quality.

Résumé (1600 caractères maximum) :

Pour un premier cycle d'élevage de *Penaeus vannamei* sur la nouvelle ferme expérimentale de Skretting Vietnam, plusieurs techniques de bioremédiation visant à améliorer la qualité de l'eau ont été testées. Pour la phase de nurserie, un protocole visant à améliorer l'assimilation de l'azote par bactéries hétérotrophes fut évalué. Un système biofloc fut mis en place avec un ratio carbone/azote de 12:1. Le système hétérotrophe qui en découle s'est montré efficace pour contrôler les niveaux d'ammoniaque, et présenta de faibles variations journalières d'oxygène et de pH. Cependant, des problèmes de faibles concentrations en oxygène sont rapidement apparus, la biomasse bactérienne du bassin n'étant pas extraite. Cet essai donna néanmoins de bons résultats en termes de survie et d'indice de conversion des crevettes. La deuxième partie de cette étude est centrée sur la phase de grossissement, et évalue le potentiel du son de riz fermenté pour équilibrer l'activité bactérienne et photosynthétique dans les bassins. Un essai à grande échelle dans des bassins de crevettes fut réalisé en comparant deux protocoles basés sur l'ajout régulier de mélasse d'une part, et de son de riz fermenté d'autre part. L'ajout de son de riz fermenté a montré une réduction significative des variations d'oxygène et de pH. De plus, son application a amélioré la minéralisation de la matière organique dans les bassins, et les crevettes ont montré de meilleures performances par rapport aux bassins témoins. Cependant, du fait de l'apparition d'une maladie, l'expérience dut être terminée de manière prématurée.

Abstract (1600 caractères maximum) :

For a first cycle at Skretting Vietnam new validation farm with *Penaeus vannamei*, several bioremediation techniques were assessed to improve control over water quality. For the nursery phase, a protocol meant to enhance nitrogen assimilation from heterotrophic bacteria was tried. A biofloc system was set up with a carbon/nitrogen ratio of 12:1. The resulting heterotrophic system appeared to be efficient in controlling ammonia levels, and featured very stable pH and oxygen values. However, low oxygen issues quickly appeared inside the pond as the bacterial biomass was not removed. Nevertheless, this trial gave good results in terms of survival and feed conversion ratio. The second part of this study is focused on the grow-out phase and investigates the potential of fermented rice bran to balance heterotrophic and photosynthetic activities. A large scale trial in shrimp ponds was performed, where two protocols either based on the regular addition of sugar cane molasses or fermented rice bran were compared. The application of fermented rice bran appeared to have significantly reduced oxygen and pH variations. Furthermore, rice bran also appeared to have a beneficial impact over the mineralization of organic matter, and shrimps in ponds fertilized with fermented rice bran showed better performance results. However due to a disease outbreak, the experiment had to be terminated early and was therefore incomplete.

Mots-clés : Aquaculture, *Penaeus vannamei*, bioremédiation, biofloc, mélasse, son de riz, fermentation

Key Words: Aquaculture, *Penaeus vannamei*, bioremediation, biofloc, molasses, rice bran, fermentation