Management of biofloc concentrations as an ecological strategy for microbial control in intensive shrimp culture

Fabrini Copetti[a], Gustavo Bueno Gregoracci[b], Olav Vadstein[c], Rodrigo Schveitzer[a,*]

[a] *Institute of Marine Science (IMar), Federal University of São Paulo (UNIFESP), Rua Dona Maria Máximo, 168, 11030-100, Santos, SP, Brazil*

[b] *IMar/UNIFESP, Rua Dr. Carvalho de Mendonça, 144, 11070-100 Santos, SP, Brazil*

[c] *Department of Biotechnology and Food Science, NTNU - Norwegian University of Science and Technology, N-7491, Trondheim, Norway*

[*] Corresponding author. E-mail addresses rschveitzer@hotmail.com, rodrigo.schveitzer@unifesp.br (R. Schveitzer).

ABSTRACT

This study investigated if the intensity of sedimentation through settling tanks modifies the bacterial community composition of biofloc systems and affects performance of shrimp. A 50-day trial, which was conducted with Pacific White Shrimp juveniles (2.6 g ± 0.0 g) stocked at a density of 906 shrimp m^{-3}, evaluated two different biofloc concentrations, i.e., total suspended solids: T500 (~ 500 mg L^{-1}) and T700 (~ 700 mg L^{-1}). To characterize the bacterial community, 40 water samples were taken for high throughput 16S-rDNA amplicon sequencing. Water quality variables were statistically similar among treatments and remained within the appropriate range for shrimp growth. No significant difference in the performance of animals was observed between treatments. Shrimp survival was high in both treatments (~ 90%), but the coefficient of the variation was 5.5 times higher in T500 (11.5%) than that observed in the T700 (2.1%). Bacterial richness was higher in T700, and composition of the

bacterial community was different from that of the T500. We argue that a higher intensity of sedimentation, which decreased the TSS level in the T500, promoted changes in the microenvironment of these tanks. This, in turn, affected the ecological state of the system by destabilizing the bacterial community and promoting proliferation of opportunistic species (e.g., *Vibrio*) in tanks with lower floc levels. The higher presence of opportunists is likely related to a reduction in microbial carrying capacity (CC) and an increase in substrate supply per microbial cell promoted by the higher floc removal. Under such conditions, fast-growing opportunistic r-strategists are favoured because they perform better in uncrowded environments with high resource availability per cell. Conversely, tanks with higher floc levels, closer to the CC, and thus with scarcer resources per cell, result in strong competition and selection of slow-growing competition specialists - the K-strategists. In conclusion, our results show that the control of floc levels can be an important strategy to obtain bacterial control and should be considered in management practices.

Keywords: BFT, Settling, Microbial Ecology, Metagenomic, Shrimp

1. Introduction

Biofloc Technology (BFT) represents one important strategy to achieve a sustainable aquaculture (Martínez-Cordova *et al*., 2015). In this system, bacteria play a fundamental role in nutrient cycling, which allows reduced water usage and makes BFT environmentally friendly and more biosecure (Avnimelech, 2012). Although many heterotrophic bacteria are beneficial, opportunistic pathogens may grow and impact production negatively (Johnson *et al*., 2008; Krummenauer *et al*., 2014; Moriarty, 1997; Skjermo & Vadstein, 1999; Toranzo *et al*., 2005; Tran *et al*., 2013). Thus, identifying which factors affect the dynamics of the microbiota in both bioflocs and water is necessary to have a better control and predictability of these systems. A common practice in BFT is the removal of excess microbial flocs because

they can clog the gills of shrimp (Schveitzer *et al*., 2013). It has been shown that floc removal through settling tanks modifies the structure of the bacterial community, probably due to bioflocculation that changes settling properties of flocs (Schveitzer *et al*., 2020). In addition to this short-term effect, this routine process may also act as a long-term selection pressure that reduces the microbial biomass in relation to the microbial carrying capacity (CC) and increases the resources supply per microbial cell. These changes in the ecological state of the culture systems may destabilise the microbial community and promote proliferation of pathogenic opportunists (Vadstein *et al*., 2018).

The differentiation between opportunistic and non-opportunistic species is probably a more adequate and successful strategy to manage microbial communities in aquaculture systems than using a species level approach (Vadstein *et al*., 1993; Vadstein *et al*., 2018). This division is theoretically formalized in the r/K-selection theory (MacArthur & Wilson, 1967), which states that selective pressure drives selection in one of two generalised directions by favouring species with distinct growth strategies. The r-selected organisms have high growth rates at the expense of competitive ability and specialisation, and they rapidly colonise unexploited environments with high resource availability per cell. In BFT tanks, biofloc levels below 800 mg L^{-1} are suggested as safe for *Litopenaeus vannamei* farming (Schveitzer *et al*., 2013). Below this threshold, however, systems can be operated in different ranges of minimal and maximal levels: e.g., 300-500, 400-600, 500-700 and 600-800 mg L^{-1}. A problem which might emerge in systems operated at the lower limits (e.g., 300-500) is that microbial biomass would be reduced in relation to the CC. Carrying capacity is the maximum number of cells that can be sustained in the system over time (Vadstein *et al*., 1993), and it has been shown that recirculating aquaculture systems operating below their CC are more susceptible for r-strategists proliferation (Attramadal *et al*., 2016). The r-selected species are often referred to as opportunistic, and most pathogens are characterised as r-strategists

(Andrews, 1984). In this sense, keeping floc levels in low levels may select for growth of undesirable microorganisms, as the opportunistic pathogenic bacteria within the *Vibrio* genus (Brown *et al*., 2012; Vadstein *et al*., 2013).

One alternative to restrict proliferation of potentially harmful bacteria (r-strategists) would be the establishment of communities with predominance of non-opportunistic K-strategists (Salvesen *et al*., 1999; Skjermo *et al*., 1997). These organisms have traits that make them successful in crowded environments, with microbial densities close to the CC, and they are competition specialists with high affinity for resources (Vadstein *et al*., 2018). In BFT, systems with higher floc levels and thus closest to the CC are hypothesized to select for a community with predominance of K-strategists. This is expected to occur because those systems have a more competitive environment with a low substrate supply per cell due to higher biomass, and they would reduce the probability of proliferation of opportunistic r-strategists by filling the niches of the rearing water and bioflocs with harmless K-strategists.

In the current study, we tested the above hypothesis by investigating if a higher intensity of the floc sedimentation, which reduces the TSS levels in culture tanks, modifies the structure of bacterial community and consequently affects the performance of shrimp.

2. Material and methods

2.1 Source of biological material and biofloc-rich water for the experimental phase

Ten-day-old specific pathogen free post-larvae (PL_{10}) of *Litopenaeus vannamei* (Speedline Aqua) were acquired from Aquatec LTDA, Canguaratema, RN, Brazil. Post-larvae were cultured in an intensive nursery tank at a stocking density of 18 animals L^{-1} for 16 days according to established procedures (Schveitzer *et al*., 2017). The survival rate in this phase was 98%.

Following the nursery phase, PLs were raised at a stocking density of 940 post-larvae m^{-2} in a 1 m^2 cylindrical tank (matrix tank) containing artificial substrates (Schveitzer *et al*., 2018). The final weight and survival of shrimp after 72 days were 2.7 g and 92%, respectively. The matrix tank was initially filled with natural seawater and no water was exchanged during the culture. The shrimp were fed a commercial feed with crude protein constituting 38-40% (Guabi Nutrição e Saúde Animal, Campinas, SP, Brazil) (Guabi Potimar). The temperature and the dissolved oxygen in the matrix tank were kept in ~ 28º C and ~ 6 mg L^{-1}, respectively. The maximum concentration of total suspended solids (TSS) was ~ 700 mg L^{-1}; salinity was ~ 20 g L^{-1}; pH 7.5-8.0; alkalinity 100-150 (mg $CaCO_3$ L^{-1}). Average ammonia nitrogen (mg TAN L^{-1}) and nitrite ($N\text{-}NO_2^-$) concentrations were 0.19 and 0.17 mg N L^{-1}, respectively.

Ammonia was controlled by different pathways during the culture. In the beginning, glycerol was added to the tank to stimulate the production of heterotrophic bacterial biomass from ammonia (Avnimelech, 1999). This action was initiated when total ammonia nitrogen (TAN) increased above 1 mg L^{-1}. The amount of glycerol was calculated based on Avnimelech (1999) and (Samocha, 2019), i.e., 20 g of carbohydrate (6 g of organic carbon) per 1 g of total ammonia nitrogen (TAN). After this initial period, the daily addition of calcium hydroxide was necessary to compensate for the acidification, which is an indicative of nitrification (Ebeling *et al*., 2006).

2.2. Experiment and sample collection

A 50-day (7 weeks) experiment was conducted to study the bacterial community composition in shrimp culture tanks submitted to two different biofloc concentrations, expressed as total suspended solids: T500 (~ 500 mg L^{-1}) and T700 (~ 700 mg L^{-1}). Each tank was randomly assigned to a one-factor experimental design with three replicates per

126 treatment. Each experimental unit was stocked with 29 juveniles of *L. vannamei* (average wet
127 weight of 2.6 ± 0.0 g) from the matrix tank, representing a stocking density of 906 shrimp m^{-3}.
128 Samples for bacterial analysis from each experimental unit were collected weekly. In total, 48
129 samples were collected, but only 40 were sequenced because eight samples were lost during
130 the DNA extraction process. Each sample of biofloc-rich water was collected at mid-depth of
131 the water column in the center of the tanks.

132 The water used in the experimental units was pumped from the matrix tank on the
133 same day as the shrimp were stocked. This water had a TSS concentration of ~ 700 mg L^{-1}
134 and no adjustments were required for the T700. For the T500 treatment, TSS reduction was
135 accomplished by decantation in settling tanks. The experimental units consisted of rectangular
136 plastic tanks (0.45 m length x 0.32 m width x 0.26 m height), with a useful volume of 32 L
137 (0.22 m height). Each tank contained a hose with micropores, which were used to oxygenate
138 the water and keep the solids in suspension. A 1.0-HP regenerative blower was used to aerate
139 all tanks. The tanks were kept in an isolated room and received only artificial lighting (LED
140 lamps) with 12/12 photoperiod. Water temperature was kept constant using 50-W submersible
141 heaters connected to thermostats (one thermostat for each three heaters). Conical bottom
142 settling tanks with a useful volume of 2.5 L were used to maintain the required TSS levels for
143 each treatment. In T500 and T700, the settling process occurred when TSS increased above
144 500 and 700 mg L^{-1}, respectively. After removal of solids from the settling tanks, the solids-
145 free water was returned to the respective tank.

146 A 35% protein commercial feed (Guabi Nutrição e Saúde Animal, Campinas, SP,
147 Brazil) (Guabi Potimar) was offered four times a day. About 5-10% of the feed in each
148 feeding was placed in checking trays to evaluate consumption and to adjust the amount
149 offered. Tray checking was performed 1 hr after offering feed. The addition of calcium
150 hydroxide $Ca(OH)_2$ (hydrated lime) was used to maintain alkalinity close to 150 mg L^{-1}.

Water was not exchanged during the experiment, but new freshwater was added to compensate for water loss due to evaporation (dechlorinated with sodium thiosulphate). Glycerol was not added to the tanks during the experimental phase.

2.3. Water quality variables

Dissolved oxygen and the temperature were measured twice a day using an YSI 550A probe (YSI Incorporated, Yellow Springs, OH, USA). The pH (pH meter PHB-500, IONLAB, Araucária, PR, Brazil) and total suspended solids (TSS) (APHA 2005—2540 D) were analysed daily. Salinity (refractometer RHS-10, YHequipment, Shenzen, China), alkalinity (APHA 2005 2320 B), ammonia (total ammonia nitrogen) and nitrite were analysed three times a week. TSS were measured using 0.6-µm fiberglass microfilters (GF-6, Macherey-Nagel, Duren, Germany). Total ammonia nitrogen (TAN) and nitrite ($N\text{-}NO_2$) analyses followed the spectrophotometric methods described by Koroleff (1970) and Bendschneider and Robison (1952), respectively.

2.4 Shrimp performance

To assess the performance of the shrimp we used final average wet weight (g), average wet weight gain (g $week^{-1}$), survival (%), final biomass (kg wet weight m^{-3}) and the feed conversion rate (FCR). The initial stocking biomass was subtracted from final biomass in the calculation of FCR. Seven shrimp per experimental unit were sampled and weighted individually every two weeks to monitor health, growth, and to adjust daily rations of feed. After weighing, the shrimp were returned to their respective tanks. In the end of the experiment, all shrimp from each experimental unit were weighed and counted.

176 *2.5. Bacterial community analyses*

177 The characterization of bacterial community composition was determined by high
178 performance sequencing and bioinformatics, with annotations up to genus level.
179 Approximately 50 ml of water from each tank were collected with a syringe, immediately
180 before the first feed ration that day, filtered with Sterivex filter (0.22 µm), and stored with 1.8
181 mL of TE buffer (50 mM Tris-50 mM EDTA) at -20 ºC until the extraction.

182 Samples were lysed with 0.5% SDS and 50 mg of proteinase-K (Machery-Nagel^{TM})
183 inside the *Sterivex* filter at 50 °C for 60 min, and then transferred to microtubes. DNA was
184 extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol.
185 After extraction, the concentration of DNA (ng uL^{-1}) and the purity (A260/280, A260/230)
186 were measured with a NanoDrop 2000 Spectrophotometer® (Thermo Scientific, Wilmington,
187 DE, USA).

188 The hypervariable V3-V4 region of the *16S ribosomal RNA* gene (*16S rDNA*) was
189 amplified with broad coverage primers (Klindworth, 2013) with commercial adapters
190 recommended in the 16S Sample Preparation Guide (Ilumina). PCR products with adaptors (~
191 460 bp) were indexed and quantified according to standard protocols. The amplification and
192 the sequencing were made at the *Centro de Metagenômica Funcional ESALQ/USP*
193 *Piracicaba*, employing a pair ended protocol with a 600 cycle kit for Illumina MiSeq
194 platform.

195 The raw sequences were dynamically trimmed using SolexaQA ++ (Cox *et al.*, 2010),
196 and barcodes and short sequences (< 75 bp) were removed. Paired ends were organized with
197 PAIRFQ (open license software) and joined with PANDASEQ (Masella *et al*., 2012).
198 Identical reads replicas were grouped using a *grep* command at the prompt system. Chimera
199 were checked through CATCh, in self reference mode (Mysara *et al.*, 2015), and no chimera
200 were found. To group the data into OTUs we used SWARM (Mahé, 2014). Mothur 1.36

(Schloss *et al.*, 2009) was then used for taxonomic assignment of the OTUs (classifiy.seqs) using the Silva SSU database (v1. 32) (Quast *et al*., 2012) made available by Mothur. OTUs representing eukaryotic and archaeal amplicons were removed before further statistical analysis. Data was normalized to the lowest read count (78,365) to avoid bias in diversity analyses due to variable sequencing depth.

2.6. Statistical analyses

Data on shrimp performance and water quality variables were analysed by one-way ANOVA. Prior to the analysis, normality (Shapiro-Wilk test) and homogeneity of variances (Levene test) were tested (Zar, 2010). Survival data were angular transformed before analysis.

Two-way ANOVA was applied to test the significance of the differences in the alpha-diversity indices (richness and evenness) of the bacterial communities, with treatments (solids level) and time (culture weeks) as the two factors. Richness was represented by the count of OTUs; and evenness was calculated as e^H/Richness, where H refers to the Shannon diversity Index (Hill, 1973). For ordination of samples we used non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity (Bray & Curtis, 1957). Two-way PERMANOVA based on Bray-Curtis similarities was used to test for differences in community structure due to treatment (solids level) and time (culture weeks) (Anderson, 2008).

Data analyses were conducted using the software STATISCA (Statsoft Inc., Tulsa, Oklahoma, USA), PAST v.4.03 (Hammer *et al*., 2001), and PRIMER v.6 (Clarke & Gorley, 2006) with the PERMANOVA1 package (Anderson *et al*., 2008).

3. Results

3.1. Water quality variables and performance of shrimp

Significant differences in water quality variables between treatments were observed only for alkalinity and nitrite, which were higher 9.5% and 87.5% higher, respectively in T500 (Table 1). TSS were controlled according to the experimental design (Figure 1), and the concentrations were kept at 514 ± 9 mg L^{-1} (T500) and 724 ± 43 mg L^{-1} (T700). There were no significant differences in the shrimp performance between treatments (Table 2). Importantly, the coefficient of the variation in the survival was 5.3 times higher in T500 (11.6%) than in the T700 (2.2%).

3.2. Bacterial community composition

The Illumina sequencing yielded a total of 1,768 OTUs, and the sequencing depth ranged between 78,365 and 135,429 reads per sample. To avoid sequencing depth bias, all samples were normalized to the lowest read for any sample (78,365). Nine OTUs occurred with abundance higher than 1% in both treatments, representing ~ 34% of all the reads (Table 3). Another six OTUs in the T500 and two in the T700 occurred with abundance > 1% (Table 3).

The OTU Richness was 3.7% lower in the T500, and evenness was statistically similar between treatments (Table 4). The variable time (culture weeks) also affected richness significantly and, in general, there was a reduction in the species number from the begging to the end of culture in both treatments (data not shown).

Visualization of similarities between samples with ordination by nMDS indicates separation of the samples based on differences in solids levels throughout the experiment (Figure 2). Moreover, for both treatments there is a shift in position with time along the first axis. The results of the two-way PERMANOVA reveal a significant effect on the bacterial

community structure by both the level of solids and the time (culture weeks) (Table 5), but without an interaction effect between these two variables. We conclude that the bacterial community composition was affected by the differences in selection pressure due to the solid level in the tanks, and that the community composition did not seem to stabilize during this 50 days experiment. (Table 5).

4. Discussion

4.1. Water quality variables and performance of shrimp

The water quality variables remained within the appropriate range for *L. vannamei* culture (Van Wyk & Scarpa, 1999). Alkalinity was lower in T700, and this is probably related to the higher nitrification activity in this treatment, which produces acidification. This is supported by the lower nitrite concentrations observed in these tanks, indicating a more complete oxidation of nitrite to nitrate (Cohen *et al*., 2005). In T500, however, higher nitrite concentrations may be associated with the higher intensity of floc removal. In such conditions, cell retention time can be reduced beyond the generation time of nitrifyers, lowering the biomass of these bacteria and promoting accumulation of nitrite (Schveitzer *et al*., 2013).

Although the average survival was high in both treatments, the coefficient of variation was higher in T500 with survivals of the 100.0, 93.1 and 79.3%. A higher variability in performance is a typical characteristic for r-selected communities (Vadstein et al., 2018) hypothesized for the microbial community structure in tanks with lower floc levels (discussed below). This argument is reinforced by the fact that mortality outbreaks in shrimp farming accompany the disruption of the core microbiota present in the shrimp intestine (Vadstein *et al*., 2018; Yao *et al*., 2018), which is induced by shifts in the environmental microbiota (Landsman *et al*., 2019).

4.2. Bacterial community composition

One of the hypotheses of this study was that tanks with higher floc levels would have higher richness and evenness of OTUs. Indeed, richness was higher in T700, and this may be related to a higher presence of rare species. These low abundant OTUs were probably associated with settled floc particles that were more frequently removed from the T500 treatment due to a higher sedimentation frequency. This is supported by our previous work (Schveitzer *et al*., 2020), which showed that sedimentation decreased the abundance of microorganisms strongly associated with settled flocs. Regarding the other alpha-diversity indices, no significant differences were observed between treatments. This is likely because the richness differences of rarer groups have less impact on the evenness.

Our data also showed that the bacterial community structure was altered in tanks subjected to different sedimentation intensities. We argue this change is related to a modification in the ecological condition of the culture environment due to the perturbations promoted by the process of sedimentation. The reduction of the microbial density (bioflocs) by settling increased the difference between the microbial biomass of the tanks and the microbial carrying capacity (CC) of the system. This enriched environment (high availability of nutrients per cell) with space for colonization (low competition) benefits fast-growing opportunistic r-strategists (Vadstein et al., 1993; Hess-Erga *et al.*, 2010). In the T500 treatment, floc levels were lower and the microbial biomass was more distant from the CC, a condition that supported the proliferation of opportunistic r-strategist bacteria such as *Vibrio* spp. (discussed below). Differently, tanks with higher floc levels (T700) in which the biomass of microorganisms was closer to the CC, the occurrence of K-strategist bacteria was encouraged (as discussed below). This occurs because K-selection takes place in stable environments (less disturbed, e.g., by sedimentation) where the ability to compete

301 successfully for limited resources (e.g., dissolved organic matter) is important, i.e., in a
302 community close to the CC (Vadstein *et al.*, 2018).
303 As mentioned above, the management of solids applied on the T700 tanks appeared to
304 have created an ecological state that was more suitable for K-strategic microbes. For example,
305 we observed a higher occurrence of bacteria of the family *Rhodobactereaceae* in these tanks.
306 They are colonizers of biofilm and predominantly K-strategists (Table 3, Fig. S1) (Bruhn *et*
307 *al.*, 2007; Cardona *et al.*, 2016; Xiong *et al.*, 2018). Organisms from this family have also
308 been suggested as probiotic species due to the antagonism towards members of the
309 *Vibrionaceae* family (Fjellheim *et al.*, 2010; Prol-García *et al.*, 2014). Additionally, they are
310 considered important organisms in water treatment (Zheng *et al.*, 2017) and in promoting the
311 health of cultivated shrimp (Cardona *et al.*, 2016; Wang *et al.*, 2014; Yao *et al.*, 2018).
312 Bacteria of the family *Rhodobactereaceae* are also found in higher abundance in the intestine
313 of healthy compared with sick shrimp (Chen *et al.*, 2017; Yao *et al.*, 2018; Zhu *et al.*, 2016).
314 In tanks of the T500, however, potentially pathogenic bacteria occurred in a higher
315 proportion, e.g., members of the genus *Vibrio* (Table 3, Fig. S1). *Vibrio* are opportunistic r-
316 strategists and some species are related with diseases in fish and shrimp (Fjellheim *et al.*,
317 2010; Sudheesh *et al.*, 2012). Representatives of this group can have a doubling time of 24
318 minutes under optimal conditions, and an even faster growth (10 minutes) is possible in
319 glucose-rich environments (Long *et al.*, 2017). Other organisms associated with diseases in
320 aquatic organisms were also more abundant in the T500. The genus *Tamlana* and
321 *Aurantivirga* are both members of the family *Flavobacteriaceae,* which includes fast-growing
322 pathogenic organisms (e.g., *Flavobacterium*) (Table 3, Fig. S1) (Farkas, 1985; Holt *et al.*,
323 1989; Nematollahi *et al.*, 2003; Starliper, 2011; Wakabayashi *et al.*, 1989). In addition, the
324 genus *Cyclobacterium* and the family *Pseudoalteromonadaceae* (genera *Pseudoalteromonas*

and uncultured) belong to a group of bacteria that often are pathogenic (Table 3, Fig. S1) (Huang *et al.*, 2020; Xiong *et al.*, 2018).

The selective effect of the sedimentation on particles with different settling properties may also have contributed to some extent to the change in the structure of the bacterial community. This has already been suggested in a previous study which observed that dense floc particles removed from the water column by sedimentation have a different set of species when compared to the unsettled particles (Schveitzer et al., 2020). These authors argue that settling process removes dense floc particles from water column and reduces the abundance of microorganisms affiliated with the settled flocs, possibly floc forming bacteria. As the tanks of the T500 treatment were submitted to more frequent settlements, the removal of dense bioflocs was more intense in these tanks. In fact, tanks of the T700 had a higher abundance of bacteria of the *Rhodobactereaceae* family, whose abundance is often highest in association with organic particles, suggesting that cell-surface interactions are a defining feature of lineage members (Slightom and Buchan, 2009). Similar results were observed by Schveitzer et al. (2020), which registered a higher abundance of *Rhodobactereaceae* in samples with TSS between 680 and 1030 mg L^{-1} than in samples in which TSS was lower than 200 mg L^{-1}.

Differences in the alkalinity and concentration of nitrite between treatments were observed, and we have suggested that this was a consequence of the reduction of the nitrifying bacterial biomass. Nevertheless, one may hypothesize that these water quality variables could have affected the composition of heterotrophic bacteria instead of, or additionally to, the solids level. Alkalinity is a variable that should affect autotrophic bacteria because it implies a source of inorganic carbon, or heterotrophic bacteria through pH. However, pH was equal between treatments and thus it is unlikely that alkalinity had affected the community of heterotrophic bacteria. A higher concentration of nitrite theoretically could inhibited specific groups, thereby conferring competitive advantages for some bacterial species. Few reports of

nitrite affecting bacteria are available, which impairs our discussion. For instance, Lin et al (2020) reported the inhibition of freshwater annamox bacteria in very high nitrite concentrations (above 450 mg L^{-1}), way above the values reported here. Nitrite could represent a substrate in processes such as aerobic nitrite oxidation or anaerobic ammonia oxidation. In this case, it should influence more autotrophic communities than heterotrophic ones such as in our study.

It is worth noting that the feed supply increased in both treatments during the culture period (Fig. 1). This, in turn, increased the CC of the tanks because parts of the feed that are not utilized by the shrimp are thus available for bacteria. As the floc levels remained constant, the difference between the microbial biomass in the tanks and the CC increased over time in both treatments. This change, which modified the availability of nutrients per microbial cell and affects the competition between microorganisms, may have been responsible for the temporal shift in the bacterial community in all tanks. Nevertheless, the microbial biomass in T700 treatment seemed to be high enough to assure selection against opportunists even when the feed load was increased.

The present results have practical implications for the management of biofloc system. It has been shown that some solids removal is essential to keep water quality stable and to prevent clogging of gills in cultured organism (Gaona *et al*., 2017; Ray *et al*., 2010; Ray *et al*., 2011, Schveitzer *et al*., 2013). However, our findings indicate that it would be microbiologically safer to keep higher floc concentrations in culture tanks as long as adequate concentrations are maintained for the cultured animal. This seems to be a better choice because a less perturbed system (i.e., lower number of settlings) is less prone to proliferation of opportunistic pathogenic bacteria. Thus, to increase the probability of better crop yields and healthier animals, the choice of a target floc concentration by the farm managers needs to consider the different variables affected by the solids management – physicochemical water

quality, microbial water quality, and animal welfare and viability. Target floc concentrations still need to be determined, as they can vary between species.

5. Conclusions

We have shown that the bacterial community composition and the probability of detrimental bacteria depends on the intensity of the floc sedimentation process applied in culture tanks. Culture tanks operated with higher TSS (close to 700 mg L^{-1}) have greater bacterial species richness and are less susceptible to the occurrence of opportunistic pathogenic bacteria (including vibrios), than tanks in which TSS were kept lower (close to 500 mg L^{-1}).

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq, Process 447160/2014-1, Rodrigo Schveitzer). The first author (Fabrini C.T.M) was funded by the grant #2017/24583-6 (master's degree scholarship), São Paulo Research Foundation (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES); and by the grant #2019/17521-0 (research internships abroad/BEPE), São Paulo Research Foundation (FAPESP). We thank the Guabi Nutrição e Saúde Animal S.A. for donating the experimental diets. We also thank the anonymous reviewers for their recommendations for improving the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at …

401 **References**

402 Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to
403 Software and Statistical Methods. Plymouth.

404 Andrews, J.H., 1984. Relevance of r- and K-theory to the ecology of plant pathogens. In:
405 Klug, M.J., Reddy, C.A., (Eds.), Current Perspectives in Microbial Ecology. American
406 Society for Microbiology, Washington, DC, pp. 1–7.

407 APHA (American Public Health Association), 2005. Standard Methods for the Examination
408 of Water & Wastewater**,** 21st ed. Byrd Prepress, Washington.

409 Attramadal, K.J.K., Minniti, G., Øie, G., Kjørsvik, E., Østensen, M-A., Bakke, I., Olav V.,
410 2016. Microbial maturation of intake water at different carrying capacities affects microbial
411 control in rearing tanks for marine fish larvae. Aquaculture 457, 68–72.
412 https://doi.org/10.1016/j.aquaculture.2016.02.015.

413 Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems.
414 Aquaculture 176, 227–35. https://doi.org/10.1016/S0044-8486(99)00085-X.

415 Avnimelech, Y., 2012. Biofloc Technology – A Practical Guide Book, Second ed. The
416 World Aquaculture Society, Baton Rouge.

417 Bendschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for the
418 determination of nitrite in sea water. J. Mar. Res. 11, 87-96.

419 Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern
420 Wisconsin. Ecol. Monogr. 27: 325–349.

421 Brown, S.P., Cornforth, D.M., Mideo, N., 2012. Evolution of virulence in opportunistic
422 pathogens: generalism, plasticity, and control. Trends Microbiol. 20, 336–42.
423 https://doi.org/10.1016/j.tim.2012.04.005.

Bruhn, J.B., Gram, L., Belas, R., 2007. Production of Antibacterial Compounds and Biofilm Formation by Roseobacter Species Are Influenced by Culture Conditions. AEM 73, 442–50. https://doi.org/10.1128/AEM.02238-06.

Cardona, E., Gueguen, Y., Magré, K., Lorgeoux, B., Piquemal, D., Pierrat, F., Florian N., Denis, S., 2016. Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. BMC Microbiol. 16, 157. https://doi.org/10.1186/s12866-016-0770-z.

Chen, W.-Y., Ng, T.H., Wu, J.-H., Chen, J.-W., Wang, H.-C., 2017. Microbiome Dynamics in a Shrimp Grow-out Pond with Possible Outbreak of Acute Hepatopancreatic Necrosis Disease. Sci Rep. 7, 9395. https://doi.org/10.1038/s41598-017-09923-6.

Clarke, K., Gorley, R.N., 2006. PRIMER v6: user manual/tutorial., Plymouth Routines in Multivariate Ecological Research. Plymouth.

Cohen, J., Samocha, T., Fox, J., Gandy, R., Lawrence, A., 2005. Characterization of water quality factors during intensive raceway production of juvenile using limited discharge and biosecure management tools. Aquacult, Eng. 32, 425–442. https://doi.org/10.1016/j.aq-uaeng.2004.09.005.

Cox, M.P., Peterson, D.A., Biggs, P.J., 2010. SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. BMC Bioinformatics. 11, 485. https://doi.org/10.1186/1471-2105-11-485.

Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture 257, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.

Farkas, J., 1985. Filamentous *Flavobacterium* sp. isolated from fish with gill diseases in cold water. Aquaculture 44, 1–10. https://doi.org/10.1016/0044-8486(85)90037-7.

Fjellheim, A.J., Klinkenberg, G., Skjermo, J., Aasen, I.M., Vadstein, O., 2010. Selection of candidate probionts by two different screening strategies from Atlantic cod (*Gadus morhua L.*) larvae. Vet. Microbiol. 144, 153–159. https://doi.org/10.1016/j.vetmic.2009.12.032.

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W., 2017. Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. Aquac. Res. 48, 1070–1079. https://doi.org/10.1111/are.12949.

Hammer Ø., Harper, D.A.T., Paul, D.R., 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4,1.

Hess-Erga, O.-K., Blomvågnes-Bakke, B., Vadstein, O., 2010. Recolonization by heterotrophic bacteria after UV irradiation or ozonation of seawater; a simulation of ballast water treatment. Water Res. 44, 5439–5449. https://doi.org/10.1016/j.watres.2010.06.059.

Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54, 427-432.

Holt, R.A., Amandi, A., Rohovec, J.S., Fryer, J.L., 1989. Relation of Water Temperature to Bacterial Cold-Water Disease in Coho Salmon, Chinook Salmon, and Rainbow Trout. J. Aquat. Anim. Health. 94, 101. https://doi.org/10.1577/1548-8667(1989)001<0094:ROWTTB>2.3.CO;2.

Huang, L., Guo, H., Chen, C., Huang, X., Chen, W., Bao, F., Liu, W., Wang, S., Zhang, D., 2020. The bacteria from large-sized bioflocs are more associated with the shrimp gut

469 microbiota in culture system. Aquaculture 523, 735159.
470 https://doi.org/10.1016/j.aquaculture.2020.735159.

471 Johnson, C.N., Barnes, S., Ogle, J., Grimes, D.J., Chang, Y.-J., Peacock, A.D., Kline, L.,
472 2008. Microbial Community Analysis of Water, Foregut, and Hindgut during Growth of
473 Pacific White Shrimp, *Litopenaeus vannamei*, in Closed-System Aquaculture. J. World.
474 Aquaculture Soc. 39, 251–258. https://doi.org/10.1111/j.1749-7345.2008.00155.x.

475 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O.,
476 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-
477 generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1–e1.
478 https://doi.org/10.1093/nar/gks808.

479 Koroleff, F., 1970. Direct determination of ammonia in natural waters as indophenol
480 blue. Information on techniques and methods for seawater analysis. 19-22.

481 Krummenauer, D., Poersch, L., Romano, L.A., Lara, G.R., Encarnação, P., Wasielesky, W.,
482 2014. The Effect of Probiotics in a *Litopenaeus vannamei* Biofloc Culture System Infected
483 with *Vibrio parahaemolyticus*. J. Appl. Aquaculture 26, 370–379.
484 https://doi.org/10.1080/10454438.2014.965575.

485 Landsman, A., St-Pierre, B., Rosales-Leija, M., Brown, M., Gibbons, W., 2019. Impact of
486 Aquaculture Practices on Intestinal Bacterial Profiles of Pacific Whiteleg Shrimp *Litopenaeus*
487 *vannamei*. Microorganisms. 7, 93. https://doi.org/10.3390/microorganisms7040093.

488 Lin, L., Pratt, S., Rattier, M., Ye, L., 2020. Individual and combined effect of salinity and
489 nitrite on freshwater Anammox bacteria (FAB). Water Research 169, 114931.
490 https://doi.org/10.1016/j.watres.2019.114931.

Long, C.P., Gonzalez, J.E., Cipolla, R.M., Antoniewicz, M.R., 2017. Metabolism of the fast-growing bacterium *Vibrio natriegens* elucidated by 13C metabolic flux analysis. Metab. Eng. 44, 191–197. https://doi.org/10.1016/j.ymben.2017.10.008.

MacArthur, R.H., Wilson, E.O., 2001. The theory of island biogeography. Princenton University Press, Princenton.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2014. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ. 2, e593. https://doi.org/10.7717/peerj.593.

Martínez-Córdova, L.R., Emerenciano, M., Miranda-Baeza, A., Martínez-Porchas, M., 2015. Microbial-based systems for aquaculture of fish and shrimp: an updated review. Rev. Aquacult. 7, 131–148. https://doi.org/10.1111/raq.12058.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., Neufeld, J.D., 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics 13, 31. https://doi.org/10.1186/1471-2105-13-31.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333–349. https://doi.org/10.1016/S0044-8486(96)01487-1.

Mysara, M., Saeys, Y., Leys, N., Raes, J., Monsieurs, P., 2015. CATCh, an Ensemble Classifier for Chimera Detection in 16S rRNA Sequencing Studies. Appl. Environ. Microbiol. 81, 1573–1584. https://doi.org/10.1128/AEM.02896-14.

Nematollahi, A., Decostere, A., Pasmans, F., Haesebrouck, F., 2003. *Flavobacterium psychrophilum* infections in salmonid fish. J. Fish Diseases 26, 563–574. https://doi.org/10.1046/j.1365-2761.2003.00488.x.

513 Prol-García, M.J., Gómez, M., Sánchez, L., Pintado, J., 2014. *Phaeobacter* grown in
514 biofilters: a new strategy for the control of Vibrionaceae in aquaculture. Aquac. Res. 45,
515 1012–1025. https://doi.org/10.1111/are.12046.

516 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner,
517 F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and
518 web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

519 Ray, A.J., Dillon, K.S., Lotz, J.M., 2011. Water quality dynamics and shrimp (*Litopenaeus*
520 *vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc
521 management. Aquacult. Eng. 45, 127–136. https://doi.org/10.1016/j.aquaeng.2011.09.001.

522 Ray, A.J., Lewis, B.L., Browdy, C.L., Leffler, J.W., 2010. Suspended solids removal to
523 improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed
524 in minimal-exchange, superintensive culture systems. Aquaculture 299, 89–98.
525 https://doi.org/10.1016/j.aquaculture.2009.11.021.

526 Salvesen, I., Skjermo, J., Vadstein, O., 1999. Growth of turbot (*Scophthalmus maximus L.*)
527 during first feeding in relation to the proportion of r/K-strategists in the bacterial community
528 of the rearing water. Aquaculture 175, 337–350.
529 https://doi.org/10.1016/S0044-8486(99)00110-6.

530 Samocha, T.M., 2019. Sustainable Biofloc Systems for Marine Shrimp, 1st edition. Academic
531 Press, London.

532 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,
533 Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger,
534 G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-
535 Independent, Community-Supported Software for Describing and Comparing Microbial
536 Communities. AEM 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Schveitzer, R., Arantes, R., Costódio, P.F.S., do Espírito Santo, C.M., Arana, L.V., Seiffert, W.Q., Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. Aquacult. Eng. 56, 59–70. https://doi.org/10.1016/j.aquaeng.2013.04.006.

Schveitzer, R., de Lorenzo, M.A., do Nascimento Vieira, F., Pereira, S.A., Mouriño, J.L.P., Seiffert, W.Q., Andreatta, E.R., 2017. Nursery of young Litopenaeus vannamei post-larvae reared in biofloc- and microalgae-based systems. Aquacult. Eng. 78, 140–145. https://doi.org/10.1016/j.aquaeng.2017.07.001.

Schveitzer, R., Leite, S.Z.L.T., Orteney, N.E., Menezes, F.C.T., Medeiros, I.D., 2018. Format and mode of artificial substrate fixation affect the performance of *Litopenaeus vannamei* in high-density rearing systems. Aquac. Res. 49, 1357–1362. https://doi.org/10.1111/are.13561.

Schveitzer, R., Fonseca, G., Orteney, N., Menezes, F.C.T., Thompson, F.L., Thompson, C.C., Gregoracci, G.B., 2020. The role of sedimentation in the structuring of microbial communities in biofloc-dominated aquaculture tanks. Aquaculture 514, 734493. https://doi.org/10.1016/j.aquaculture.2019.734493.

Skjermo, J., Salvesen, I., Øie, G., Olsen, Y., Vadstein, O., 1997. Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. Aquacult. Int. 5, 13–28. https://doi.org/10.1007/BF02764784.

Skjermo, J., Vadstein, O., 1999. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 177, 333–343. https://doi.org/10.1016/S0044-8486(99)00096-4.

Starliper, C.E., 2011. Bacterial coldwater disease of fishes caused by *Flavobacterium psychrophilum*. J. Adv. Res. 2, 97–108. https://doi.org/10.1016/j.jare.2010.04.001.

559 Slightom, R.N., Buchan, A., 2009. Surface colonization by marine roseobacters: integrating
560 genotype and phenotype. Appl. Environ. Microbiol. 75, 6027–6037.

561 Sudheesh, P.S., Al-Ghabshi, A., Al-Mazrooei, N., Al-Habsi, S., 2012. Comparative
562 Pathogenomics of Bacteria Causing Infectious Diseases in Fish. Int. J. Evol. Biol. 1–16.
563 https://doi.org/10.1155/2012/457264.

564 Toranzo, A.E., Magariños, B., Romalde, J.L., 2005. A review of the main bacterial fish
565 diseases in mariculture systems. Aquaculture 246, 37–61.
566 https://doi.org/10.1016/j.aquaculture.2005.01.002.

567 Tran, L., Nunan, L., Redman, R., Mohney, L., Pantoja, C., Fitzsimmons, K., Lightner, D.,
568 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis
569 syndrome affecting penaeid shrimp. Dis. Aquat. Org. 105, 45–55.
570 https://doi.org/10.3354/dao02621.

571 Vadstein, O., Øie, G., Olsen, I., Salvesen, I., Skjermo, J., Reinertsen, H., 1993. A strategy to
572 obtain microbial control during larval development of marine fish. International Conference
573 of Fish Farming Technology, orgs. Fish farming technology: proceedings of the First
574 International Conference on Fish Farming Technology, Trondheim, Norway, 9 - 12 August
575 1993. Rotterdam: Balkema.

576 Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galindo-Villegas, J., Mulero, V., Picchietti, S.,
577 Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver,
578 P., Bossier, P., 2013. Microbiology and immunology of fish larvae: Microbiology and
579 immunology of fish larvae. Rev. Aquacult. 5, S1–S25. https://doi.org/10.1111/j.1753-
580 5131.2012.01082.x.

581 Vadstein, O., Attramadal, K.J.K., Bakke, I., Olsen, Y., 2018. K-Selection as Microbial
582 Community Management Strategy: A Method for Improved Viability of Larvae in
583 Aquaculture. Front. Microbiol. 9, 2730. https://doi.org/10.3389/fmicb.2018.02730.

584 Van Wyk, P., Scarpa, J., 1999. Water quality requirements and management. In Van Wyk, P.,
585 Davis-Hodgkins, M., Laramore, R., Main, K.L., Scarpa, J. (Eds.), Farming Marine Shrimp in
586 Recirculating Freshwater Systems. Florida Department of Agriculture and Consumer
587 Services, Tallahassee, FL, pp. 141–162.

588 Wakabayashi, H., Huh, G.J., Kimura, N., 1989. *Flavobacterium branchiophila* sp. nov., a
589 Causative Agent of Bacterial Gill Disease of Freshwater Fishes. Int. J. Syst. Evol. Microbiol.
590 39, 213–216. https://doi.org/10.1099/00207713-39-3-213.

591 Wang, C., Lin, G., Yan, T., Zheng, Z., Chen, B., Sun, F., 2014. The cellular community in the
592 intestine of the shrimp *Penaeus penicillatus* and its culture environments. Fish. Sci. 80, 1001–
593 1007. https://doi.org/10.1007/s12562-014-0765-3.

594 Xiong, J., Dai, W., Qiu, Q., Zhu, J., Yang, W., Li, C., 2018. Response of host-bacterial
595 colonization in shrimp to developmental stage, environment and disease. Mol. Ecol. 27,
596 3686–3699. https://doi.org/10.1111/mec.14822.

597 Yao, Z., Yang, K., Huang, L., Huang, X., Qiuqian, L., Wang, K., Zhang, D., 2018. Disease
598 outbreak accompanies the dispersive structure of shrimp gut bacterial community with a
599 simple core microbiota. AMB. Expr. 8, 120. https://doi.org/10.1186/s13568-018-0644-x.

600 Zar, J. H., 2010. Biostatistical Analysis, 5th ed. Englewood Cliffs, NJ: Prentice Hall.

601 Zheng, Y., Yu, Min, Liu, J., Qiao, Y., Wang, L., Li, Z., Zhang, X.-H., Yu, Mingchao, 2017.
602 Bacterial Community Associated with Healthy and Diseased Pacific White Shrimp

(*Litopenaeus vannamei*) Larvae and Rearing Water across Different Growth Stages. Front. Microbiol. 8, 1362. https://doi.org/10.3389/fmicb.2017.01362.

Zhu, J., Dai, W., Qiu, Q., Dong, C., Zhang, J., Xiong, J., 2016. Contrasting Ecological Processes and Functional Compositions Between Intestinal Bacterial Community in Healthy and Diseased Shrimp. Microb. Ecol. 72, 975–985. https://doi.org/10.1007/s00248-016-0831-8.

Figure captions

Fig. 1. Total suspended solids (TSS) (mean ± SD) in T500 (○) and T700 (□) during 50 days of culture of *L. vannamei* at a stocking density of 906 shrimp m^{-3}. Numbers below and above the TSS is the number of times per week the settling process was performed: T500 (▲) and T700 (▼). The two lower lines [T500 (**…**) and T700 (**- -**)] represent feed consumption per week.

Fig. 2. Non-Metric Multidimensional Scaling (nMDS) ordination based on the Bray-Curtis similarities from abundance data of tanks of *L. vannamei* reared under two TSS concentrations during 50 days at a stocking density of 906 shrimp m^{-3}. Numbers above the symbols indicate the sampling date.

621 **Table 1.** Water quality variables in tanks of *L. vannamei* reared under two TSS concentrations

622 during 50 days at a stocking density of 906 shrimp m^{-3}.

Variables	T500	T700	p-value[a]
Temperature (°C)	29.3 ± 0.3	29.5 ± 0.2	0.2540
Dissolved oxygen (mg L^{-1})	6.2 ± 0.1	6.2 ± 0.1	0.7380
Salinity	20 ± 0	20 ± 0	0.3739
pH	7.9 ± 0	7.9 ± 0.1	0.3824
Alkalinity (mg $CaCO_3$ L^{-1})	153.6 ± 1.7	140.3 ± 3.6	**0.0042**
Ammonium nitrogen (mg TAN L^{-1})	0.4 ± 0.1	0.3 ± 0.1	0.1713
Nitrite (mg NO_2^- - N L^{-1})	1.5 ± 0.1	0.8 ± 0.1	**0.0004**

Values are means ± standard deviation.

[a] Bold values stand for significant effects by one-way ANOVA.

623

624 **Table 2.** Shrimp performance (means ± standard deviation) in tanks of *L. vannamei* reared

625 under two TSS concentrations during 50 days at a stocking density of 906 shrimp m^{-3}. Weights

626 given as wet weight.

Variable	T500	T700	p-value
Final average weight (g $individual^{-1}$)	6.3 ± 0.5	6.7 ± 0.1	0.1625
Avg. weight gain (g $week^{-1}$)	0.5 ± 0.1	0.6 ± 0.0	0.1625
Survival (%)	90.8 ± 10.5	90.8 ± 2.0	0.6821
Final biomass (kg m^{-3})	5.2 ± 0.9	5.5 ± 0.1	0.5450
Food conversion ratio (FCR)	3.1 ± 0.6	2.8 ± 0.1	0.1625

629 Initial average weight: 2.6 ± 0.0 g $individual^{-1}$.

630 **Table 3.** Relative abundance (percentage) of the most prevalent OTUs (abundance > 1%) present in tanks of *L. vannamei* reared

631 under two TSS concentrations during 50 days at a stocking density of 906 shrimp m^{-3}. All OTUs belong to the *Bacteria* domain.

Phylum	Class	Order	Family	Genus	T500	T700
Bacteroidetes	*Bacteroidia*	*Chitinophagales*	*Saprospiraceae*	*uncultured*	9.1 ± 5.4	10.1 ± 5.8
Chloroflexi	*Anaerolineae*	*Caldilineales*	*Caldilineaceae*	*uncultured*	5.5 ± 3.7	9.9 ± 6.0
Actinobacteria	*Actinobacteria*	PeM15	PeM15_fa	PeM15_ge	3.0 ± 4.1	< 1.0
Proteobacteria	*Gammaproteobacteria*	*Alteromonadales*	*Pseudoalteromonadaceae*	*Pseudoalteromonas*	2.1 ± 1.3	1.3 ± 0.6
Bacteroidetes	*Bacteroidia*	*Chitinophagales*	*Saprospiraceae*	*Phaeodactylibacter*	2.1 ± 2.5	2.4 ± 2.6
Proteobacteria	*Gammaproteobacteria*	*Alteromonadales*	*Psychromonadaceae*	*Motilimonas*	2.0 ± 0.7	2.0 ± 0.8
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Donghicola*	1.5 ± 2.8	2.0 ± 3.8
Proteobacteria	*Deltaproteobacteria*	PB19	PB19_fa	PB19_ge	1.4 ± 1.4	1.0 ± 1.0
Bacteroidetes	*Bacteroidia*	*Flavobacteriales*	*Flavobacteriaceae*	*Aurantivirga*	1.3 ± 1.5	< 1.0
Proteobacteria	*Gammaproteobacteria*	*Vibrionales*	*Vibrionaceae*	*Vibrio*	1.2 ± 1.5	< 1.0
Proteobacteria	*Gammaproteobacteria*	*Alteromonadales*	*Pseudoalteromonadaceae*	*uncultured*	1.2 ± 0.7	< 1.0
Bacteroidetes	*Bacteroidia*	*Flavobacteriales*	*Flavobacteriaceae*	*Tamlana*	1.1 ± 0.6	< 1.0
Bacteroidetes	*Bacteroidia*	*Cytophagales*	*Cyclobacteriaceae*	*Cyclobacterium*	1.1 ± 2.7	< 1.0
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Litorisediminivivens*	1.1 ± 1.4	1.1 ± 1.2
Chloroflexi	*Anaerolineae*	SBR1031	A4b	A4b_ge	1.0 ± 0.8	1.2 ± 0.9
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Maliponia*	< 1.0	1.6 ± 1.9
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Rhodobacteraceae_ge*	< 1.0	1.6 ± 2.7

632 Values are means ± standard deviation; n = 20 in T500 and n = 20 in T700.

633 <1.0: abundance lower than 1%.

634 **Table 4.** Alpha-diversity indices for bacterial communities in tanks of *L. vannamei* reared
635 under two TSS concentrations during 50 days at a stocking density of 906 shrimp m^{-3}, and
636 results from two-way ANOVA testing for differences between groups and sampling time.

Index	Treatment		ANOVA		
	T500	T700	TSS	Time (culture weeks)	TSS x Time
OTU-Richness (S)	994 ± 65	1033 ± 48	**0.0337**	**0.0009**	0.3732
Evenness	0.127 ± 0.025	0.124 ± 0.029	0.5409	0.9149	0.4867

Values are means ± standard deviation.

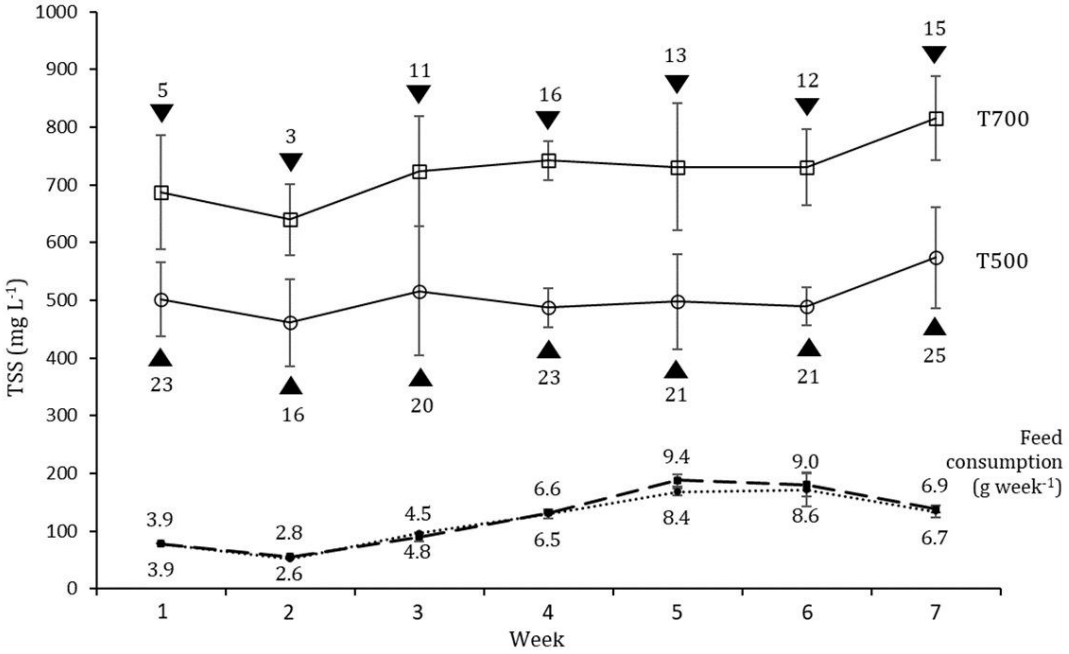
Bold values stand for significant effects by the two-way ANOVA.

Table 5. Results of the two-way permutational multivariate analysis of variance (PERMANOVA) of the microbiota from samples of tanks of *L. vannamei* reared under two TSS concentrations during 50 days at a stocking density of 906 shrimp m^{-3}.

Source	df	SS	MS	Pseudo-F	P (perm)	Perm.
TSS	1	3140	3140	5.0251	**0.0010**	997
Time (culture weeks)	7	23101	3300.1	5.2813	**0.0010**	997
TSS x Time	7	5710.1	815.72	1.3054	0.0670	997
Res	24	14997	624.86			
Total	39	48433				

Bold values stand for significant effects.

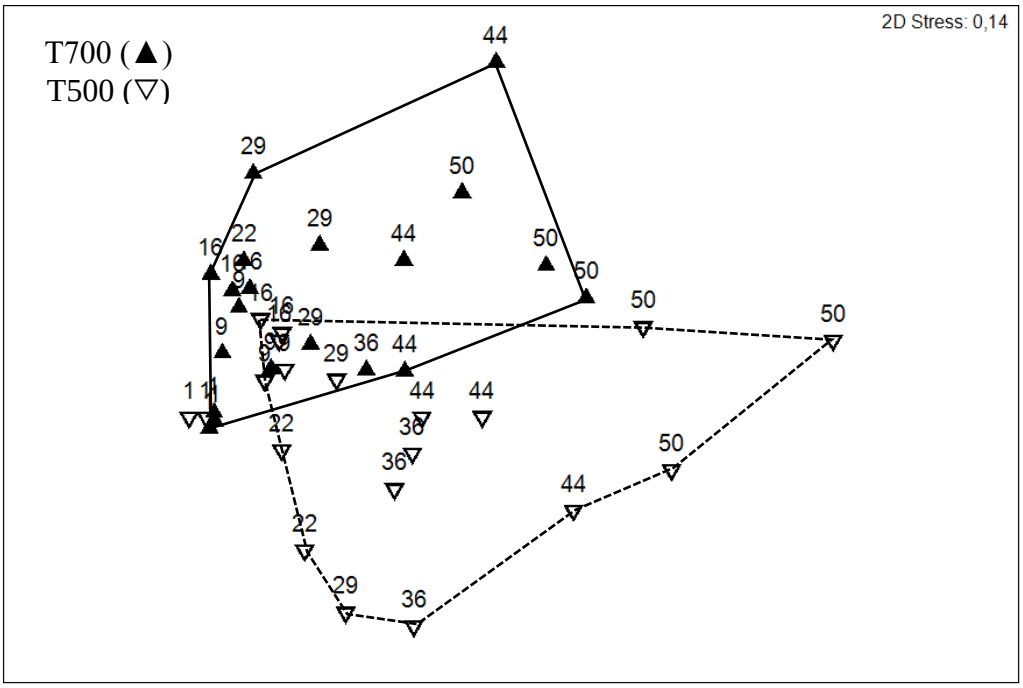
637
638
639
640
641



642

643 Fig.1.

644



645

646 Fig.2.