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Effect of symbiotic administration on growth and intestinal microbiota variation of pacú (*Piaractus mesopotamicus)* in recirculating aquaculture systems

Efecto de la administración de simbióticos en el crecimiento y en la variación de la microbiota intestinal de pacú (*Piaractus mesopotamicus*) en sistemas acuícolas de recirculación

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ABSTRACT

Background. The application of symbiotics in aquaculture holds potential as a growth promoter and substitute for antibiotics, while also improving water quality and nutrient digestibility. **Goals.** This study aimed to assess the impact of symbiotics on the growth and bacterial populations dynamics of the intestinal contents of pacú (*Piaractus mesopotamicus*) cultured in recirculating aquaculture systems (RAS). **Methods.** 87-day cultures of *P. mesopotamicus* were conducted in RAS under three conditions: 1) food-mixed with symbiotic, 2) activated symbiotic directly added to the water and 3) control treatment (without symbiotic). The evaluation included growth parameters, water quality, and bacterial populations in both the water and intestinal content. **Results.** Under these conditions pacú showed isometric growth, and no significant differences were found between treatments for food conversion ratio and Fulton condition factor. Regarding the performance of the recirculating system, there were no differences in pH, dissolved oxygen and ammonia removal by the biofilter. However, pacú exhibited high ammonia tolerance (0.62 mg/L) in the 87-day cultures when the symbiotic was mixed with the food. Additionally, final weights and specific growth rate were significantly higher (5.4 g and 0.0321 days^{-1}, respectively) compared to the other conditions. Based on 16S rRNA gene sequence analysis, seven bacterial populations were identified in the intestinal content of pacú and in the culture water: *Microbacterium, Variovorax, Prosthecobacter, Bacillus, Asaccharospora, Turicibacter sanguinis,* and *Limnohabitans planktonicus*. **Conclusions:** symbiotics mixed with food, significantly promoted the growth of *P. mesopotamicus* and enhanced ammonia tolerance in RAS. These results set a benchmark in the study of the length-weight relationship of *P. mesopotamicus*, the biofilter´s capability to remove ammonia, and the relationship between symbiotics and bacterial dynamics in the water and intestinal content during pacú crops in RAS.

Keywords: growth, intestinal content, *Piaractus mesopotamicus*, symbiotics, RAS.

RESUMEN

Antecedentes. La aplicación de simbióticos en acuicultura tiene potencial como promotor del crecimiento, como sustituto a los antibióticos, para mejorar la calidad del agua y la digestibilidad de nutrientes. **Objetivos.** Evaluar el efecto de los simbióticos sobre el crecimiento y la dinámica bacteriana del contenido intestinal de pacú (*Piaractus mesopotamicus*) y del agua de cultivo, en sistemas acuícolas de recirculación (SAR). **Métodos.** Se realizaron cultivos de 87 días con *P. mesopotamicus* en SAR bajo tres condiciones:1) simbiótico mezclado con el alimento, 2) simbiótico activado agregado directamente al agua, y 3) tratamiento control (sin simbiótico). Se evaluaron la calidad del agua, las poblaciones bacterianas en el agua y en el contenido intestinal, y el crecimiento midiendo parámetros morfofisiológicos, **Resultados.** Pacú presentó crecimiento isométrico y no se encontraron diferencias significativas entre los tratamientos en la tasa de conversión alimenticia, en el factor de condición de Fulton, pH, oxígeno disuelto ni en la eliminación de amonio en el biofiltro. Sin embargo, cuando el simbiótico se mezcló con el alimento, pacú mostró una alta tolerancia al amonio (0.62 mg/L) en cultivos de 87 días. Además, los pesos finales y la tasa de crecimiento específico fueron sig-

nificativamente mayores (5.4 g y 0.0321 días-1, respectivamente) con probiótico en el alimento. Basado en análisis de secuencias del gen 16S rRNA, se identificaron siete poblaciones bacterianas en el contenido intestinal de pacú y en el agua de cultivo: *Microbacterium, Variovorax, Prosthecobacter, Bacillus, Asaccharospora, Turicibacter sanguinis* y *Limnohabitans planktonicus*. **Conclusiones.** Los simbióticos promueven un mayor crecimiento y tolerancia al amonio en *P. mesopotamicus*. Los resultados obtenidos marcan un referente en el estudio de la relación longitud-peso de pacú, en la capacidad del biofiltro para eliminar el amonio y en la relación de los probióticos con la dinámica bacteriana en el agua y en el contenido intestinal durante cultivos en SAR.

Palabras clave: contenido intestinal, crecimiento, *Piaractus mesopotamicus*, simbióticos, SAR.

INTRODUCTION

In recent years the global demand for food has had an exponential growth, and aquaculture has turned out an important ally for the production of animal protein. The development of new production processes and cultivation of new species has been postulated as alternatives to fill the increasing demand of fish and shellfish (Rise *et al*., 2019; FAO, 2020).

The genus *Piaractus* (Serrasalmidae) comprises fishes native to South America with three species: *P. brachypomus* (pirapatinga), *P. mesopotamicus* (pacú) and *P. orinoquensis* (cachama blanca) (ITIS, 2021). *Piaractus mesopotamicus* (Holmber, 1887) are naturally found in lakes and rivers with vegetation, provided that water temperature is between 20-30°C, dissolved oxygen concentration is 7-8 mg/L, and pH ranging from 7-8 (Soncini & Glass, 1997; dos Santos *et al*., 2020). Pacú is also of great importance in aquaculture and recreational fishing due to its robustness, ease of handling and adaptation to artificial feeding, as well as, its high growth rates, efficient food conversion and high fertility rates (Bacchetta *et al*., 2019). *Piaractus* spp. have been cultivated under varied aquacultural conditions, *i.e.*: in polyculture especially with carps (Kumar *et al.,* 2018), in saline or brackish waters, pacú has tolerated up to 4ppt NaCl (Jomori *et al.,* 2012). Assays at different water temperatures have shown that below 24°C, there is a delay in muscle growth in pacú juveniles (de Paula *et al*., 2014). In addition, pacú produced in aquaculture systems has great acceptance among consumers due to its pleasant taste (Pavon *et al*., 2018). Barrero *et al.* (2012), did not find significant differences in sensory appreciations (smell, flavor, texture and color), nor in bromatological parameters (protein, moisture and lipid contents), between *P. brachypomus* grown in recirculating aquaculture systems (RAS) and those obtained in extensive fish farms.

The advantages of RAS over conventional fish farms are: diminishing of water requirements and growing space, higher crop density (Piedrahita, 2003); production independent of seasonal variations, and reduction of environmental impacts, together with a better management of biosecurity measures (Aslam *et al*., 2019). However, the intensification of aquaculture practices increase among others, the concentration of ammonia (NH_3) in water, being a metabolic residue of major concern as it represents 70 to 90% of the total nitrogen input into aquaculture systems. NH3 concentrations larger than 0.0125 mg/L, can deteriorate gills' structures (Timmons *et al*., 2009), also it may accumulate in tissues and blood plasma causing morphological, physiological and behavioural alterations on most aquaculture species, affecting the immune system, growth and production (Medeiros *et al*., 2016).

Currently, the use of antibiotics in aquaculture has been banned due environmental and health issues, being a sustainable alternative the use of probiotics, prebiotics, and symbiotics (Lee *et al*., 2019). Probiotics are microbial supplements with either therapeutic, prophylactic, growth-promoting, stress tolerance-enhancing, and reproductive-improvement effects (Hoseinifar *et al*., 2016; Martínez-Cruz *et al*., 2012).

Prebiotics are no digestible substrate that are metabolized by specific health-promoting microorganism, therefore, probiotic microorganism use these compounds as a source of carbon for their developmet. A combination of pro-and prebiotics is referred to as a symbiotic product. It provide a competitive advantage over endogenous populations (Merrifield *et al*., 2010 Mugwanya *et al.,* 2022). This is done to increase the survival and implantation of probiotics in the host's gastrointestinal tract. So far, symbiotic studies have been conducted in salmonids, soft-shell turtles, shrimps and lobsters (Ringo *et al., 2010*; Hoseinifar *et al.,* 2016; Lee *et al*., 2019). Regarding the intestinal bacterial communities present in pacú; Castañeda-Monsalve *et al*. (2019) reported the presence of *Fusobacteria, Spirochaetes, Firmicutes* and *Proteobacteria* in *P. brachypomus;* while Rossi *et al*. (2020) found *Bacteroidetes, Firmicutes* and *Fusobacteria* in *P. mesopotamicus*. However, the effect of symbiotics on *Piaractus* intestinal bacterial communities has not yet been studied.

The objective of this study was to compare the effect of the administration mode of a commercial symbiotic on the growth of pacú (*P. mesopotamicus*), on the bacterial communities present in the intestinal microbiota and in the water of a RAS system.

MATERIAL AND METHODS

Experimental design and fish culturing conditions. The 87-day experiment consisted of a block design, using symbiotic administration mode as the only factor. After acclimatization, 234 *P. mesopotamicus* specimens (5.74 ± 0.51cm total length, 4.630 ± 0.369cm standard length, 2.607 ± 0.218cm height and 3.53 ± 0.89g weight) were randomly distributed in 9 aquariums/tanks of 70 L each, that were grouped into three vertical RAS. Therefore 26 organisms were placed in each aquarium, accounting for 78 fishes/treatment (n=78), at a density of 1 fish/2.7 L of water (Fig. 1a).

Three treatments were established: i) symbiotic in powder mixed with the food (MP), administered at 1g symbiotic powder/kg food; ii) activated symbiotic (AP); 1ml of the activated product was added to each tank during feeding; iii) control treatment (CT), with no symbiotic added. Activation of the product for AP treatment is described in "Symbiotic" subsection. The concentration of administered symbiotic was the same in the MP and AP treatments because the product was used according to the producer's instructions. The fishes were fed three times a day considering 3% of the biomass per day. The ration was adjusted weekly according to weight increases. The food was trout minipellet Silver Cup-El Pedregal (45% crude protein and 16% fat), and water temperature was maintained at 25 ± 2 °C, with 300W heater (Doplhin**®).** When needed, calcium carbonate ($CaCO_3$) was added to maintain the pH at alkaline values. The volume of water lost by evaporation was replenished daily. Every third day, the remaining food and accumulated faeces were collected, using a siphon and a net with a mesh size of 0.3 mm.

Biological material. Four hundred *P. mesopotamicus* juveniles (30 ± 1 days old, 4.35 ± 0.73 cm mean total length, 3.44 ± 0.626 cm mean standard length, 1.66 ± 0.381 cm mean height and 1.66 ± 0.90 g mean weight), were cultivated in recirculating aquaculture systems located at a greenhouse facility (19°18´32.8" N 99°06´17.0" W). The fishes were acclimatized for 20 days in an 80 L aquarium, with water parameters set at: 24 ± 1°C temperature, dissolved oxygen (DO) 6.86 ± 0.05 mg/L, and pH 8.5 ± 0.5. The fishes were fed daily with commercial flake food (Wardley, minimum 38% crude protein and 4% fat), at a 3% proportion of their body biomass, fed was divided into two servings. During the first 10 days of acclimatization Sulcoll (Collins Veterinary Division, Guadalajara, Mex), a sulpha-derived antibiotic was administered daily at 17 µL/L water.

Symbiotic. The commercial symbiotic Aqua-BOOSTER® (Smart Microbials, INC.) was used in the assay. It is a powder-formulated product containing viable non-disclosed microorganisms, among others *Saccharomyces cerevisiae*, and prebiotic oligosaccharides (mannans and β-glucanes). According to the manufacturer's instructions, this product is focused on improving the health, performance of aquaculture organisms in culture, and can be administered directly into the water as a powder, or after being activated by inoculating 0.5% (w/vol) of the powder, in 1% (w/vol) molasses in water, and incubating for 12 h at room temperature.

RAS. Water biofiltration was carried out in a three-phase cascade system, formed by 3 containers with 50 L each, operating in continuous flow at 2.5 L/min (Fig. 1b). The first phase contained 3cm-diameter plastic spheres aimed to generate nitrifying microbial biofilm, production of the biofilm was carried out during the fish acclimation; the second and third phases used a filtration system based on activated carbon and zeolite, respectively.

Growth analysis. Morphometric data were obtained weekly, by randomly selecting ten fishes from each (30 fishes per treatment). Total length (Lt), standard length (Lp) and height (H) were obtained with a vernier Scala 222b (Metromex), and the weight was determined in an Ohaus Scout STX balance. These morphometric data were used to calculate the following morphophysiological parameters:

S*pecific growth rate constant (SGR),* is obtained by integrating the growth equation dW/dt = **µ**W and plotting ln(Wt/Wo) as a function of culture time, where: Wo and Wt - Average weight of the fishes at time 0 and time t (Lugert *et al.*, 2016).

Food conversion ratio, FCR = **Σ**F/(Wt-Wo); where: **Σ**f - food administered during cultivation (Usaid-Harvest, 2011).

Protein efficiency ratio, PER = (Wt-Wo)/[N]$_f$ood; where [N]food - nitrogen concentration of the food (Rojas *et al.*, 2014).

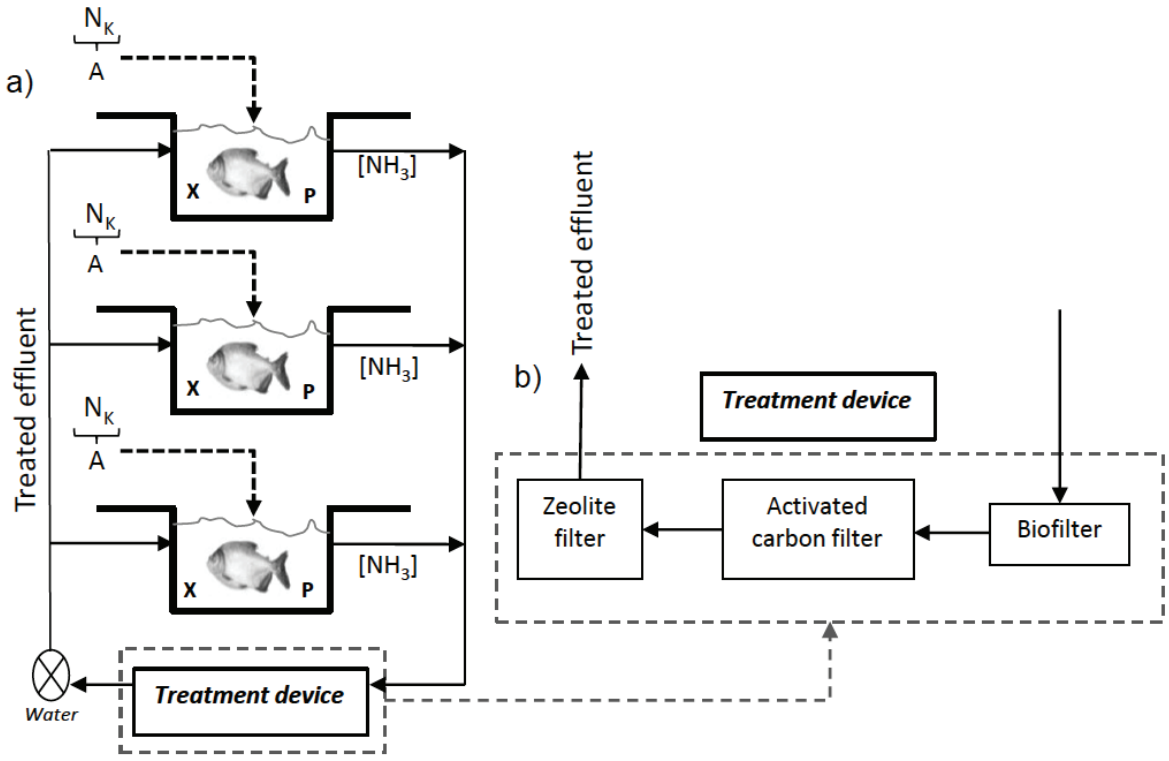

Figure 1. Scheme of the vertical Recirculating aquaculture system (RAS) used in the assays. Each system contained 78 *P. mesopotamicus* distributed in three 70 L-tanks (26 fishes/tank) The treatments included two ways of symbiotic administration, and a control non amended with symbiotic. Registered parameters included: A – amount of food (g); N_K - Kjeldahl nitrogen in the food (gN/gfood); X - recorded weight of fish (g); P – pacú protein content (%); [NH_3] - ammonia concentration (mg/L). Modified from Badiola *et al.* (2018); Timmons *et al.* (2009).

Crop density, CD = Wt/VT. Maximum mass of aquaculture product that can be maintained within the fish farm (Timmons *et al.*, 2009), where: VT - total water volume in the RAS.

Fulton condition factor, K = 100 (W/L^3); where: W - fish weight and L - Total fish length (Leyton F. *et al.*, 2015).

Length-weight relationship, WL = αL^{β}; where: α - intercept, L - standard length and β - ratio coefficient between length and weight (Rennie & Verdon, 2008). The experimental data was used to graphically represent each of the three treatments. Subsequently, the images were overlaid to facilitate comparison in a single figure.

Hypothesis of isometric growth, t_b. A Student's t test ($p < 0.05$), t_b = (b-3)/Sb; where b - coefficient of proportion between weight and length, Sb is the slope standard error (Ibáñez, 2015).

Water quality assessment. Water temperature, pH and dissolved oxygen concentration were monitored on a daily basis (YSI digital oximeter). The NH3 concentrations were quantified weekly with a modified Nessler method according to the Hanna C99 photometer and its reagents kit (Hanna Instruments Mexico). The ammonia removal capacity of the biofilter (ϒNH3) was calculated with a modified mass balance as proposed by Badiola *et al.* (2018), using the following equation:

ϒNH3 = W°N - VT(μX/Y) - V(dN/dt), where: W°N = nitrogen consumption by fish; VT(dX/Y) = ammonia nitrogen accumulation in fish; and V(dN/dt) = ammonia nitrogen accumulation in the tanks.

Statistical analysis. All values in tables are expressed as means ± SD. Normality of the data was assessed with the Shapiro-Wilk test, and the Levene test was used to assess equality of variances. Data meeting these premises were analysed by one-way ANOVA ($P < 0.05$), and further *post hoc* Tukey test for multiple comparisons, by using SAS JMP Software version 10. While data that didn't meet the premises, in despite of mathematical transformations, were subject to Tukey's nonparametric analysis (Montgomery, 2004).

DNA extraction and PCR amplification. Metagenomic DNA was extracted from the suspended bacteria in water (W), and from the symbiotic product, either as powder or activated, and from the fish gut content (FGC) from each treatment using the Wizard kit from Promega Inc., with modifications according to Aguirre-Garrido *et al.* (2016). A fragment of the 16S rRNA gene comprising the V6-V8 regions was amplified using the universal primers rL1401 (5´- GCGTGTGTACAAGACCC -3´) and f968 GC (5´-GCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGAACGCGAAGAACCTTACC- 3´, the underlined sequence corresponds to the GC-clamp), following the protocols described by Felske *et al.* (1996). The polymerase chain reaction (PCR) was carried out according to Aguirre-Garrido *et al.* (2016), and the amplification products were examined in agarose gel electrophoresis (1% agarose in TAE buffer) under previously described protocols (Ramírez-Saad *et al.*, 2004).

DGGE analyses. Metagenomic DNA was obtained from i) the fish gut contents (FGC), ii) the suspended bacteria in water (W) and in the symbiotic. The analysis was based on PCR-DGGE profiles of the V6-V8 region of the 16S rRNA. Obtained amplicons were electrophoresed in a DCode System (BioRad Labs, Hercules, CA, USA). PCR conditions and DGGE general methodology were performed according to Felske *et al.* (1996). The denaturing gels were silver-stained and preserved (Sanguinetti *et al.*, 1994). Predominant DGGE bands from each profile were excised, purified, and reamplified (Ramírez-Saad *et al.*, 2004). Image analysis of DGGE profiles was performed with Syngene software (Synoptics Ltd., UK), using Pearson matrix for pairwise similarity and UPGMA as clustering method. Furthermore, band surface and mean pixel intensities of every band within each pattern were integrated, these values were converted to percentage of relative abundance and used as surrogate abundance measure for each sequenced band within its respective profile.

Sequencing and bioinformatic analysis. Reamplified products from DGGE were sequenced (Macrogen, Seoul, Korea). Obtained sequences (<400 nt) were identified with the16S-based ID tool of the EZBioCloud (Yoon *et al.*, 2017). Furthermore, the taxonomic identification of sequences and their respective surrogated abundances were introduced in the PICRUSt2 (Douglas *et al.*, 2020) pipeline (https://github.com/picrust/picrust2).

Accession numbers. The sequences obtained in this study were deposited in GenBank databases under the accession numbers: MN845131 to MN845138.

RESULTS

Growth and morphophysiological parameters. The growth parameters of pacú were scored weekly (Table 1). ANOVA comparisons of weight, total length, standard length and height values (followed by *post hoc* Tukey test; $p < 0.05$) showed significantly higher values in the four parameters when using MP as compared to CT and AP treatments. AP and CT didn't show significant differences among them in any of the measured growth parameters. Also a significantly higher SGR ($p < 0.05$) was obtained when using MP, in relation to CT and AP. No mortality occurred during the experiment in any of the treatments.

On their side, PER values with both symbiotic treatments were significantly higher than the control. While after 87 days of culture, the obtained values for food conversion ratio (FCR), crop density (CD) and Fulton factor (K) did not present significant differences between treatments. However, in the latter factor values greater than 3.0 were obtained, indicating that the growth of the animals was not food limited (Leyton F. *et al.*, 2015). Likewise, the values obtained of *t*b, determined isometric growth in all conditions (table 2). The weight/length ratios (β) in pacú were close to 3.0 g/cm in all treatments so fishes deep their shape as they grow, however, a statistical significant difference ($p < 0.05$) was obtained when using MP, as seen in Figure 2.

Water quality. The average values of temperature, pH and DO did not show significant differences in any of the two experimental treatments and the control (Table 3). Although the ammonia nitrogen removal capacity of the biofilters (ΥNH_3) did not present significant differences, the ammonia concentration at the end of the culture was significantly higher with MP.

Analysis of microbial communities. Initial similarity clustering analysis pointed out that the DGGE patterns revealed that DGGE profiles clustered according to samples origin; fish gut content (FGC) and water from the tanks (W), regardless of the treatment. The FGC samples showed more homogeneous profiles forming a coherent cluster with 58% similarity (Fig. 3a). Eight DGGE bands considered representative of the different treatments were selected, for re-amplification, sequencing and identification (Fig. 3b, numbers 1-8). Six sequences were identified at the genus level, while the other two were identified at the species

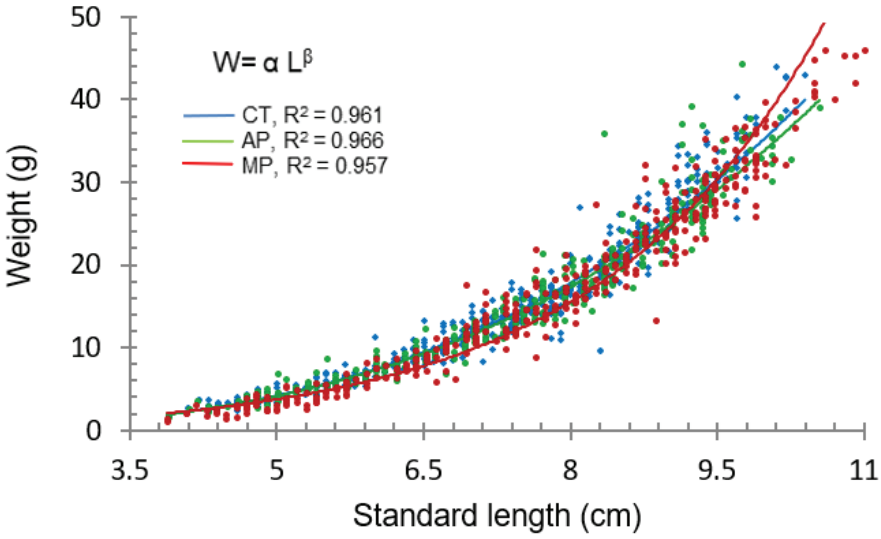

Figure 2. *P. mesopotamicus* length-weight relationship ($W= \alpha L^{\beta}$) at different symbiotic administration modes. α – intercept on X axis, β -ratio coefficient between length and weight, R^2 – determination coefficient; Control (CT), $\alpha = 0.029$, $\beta = 3.08^{a}$; Activated symbiotic (AP), $\alpha = 0.033$, $\beta = 3.0247$; Mixed symbiotic (MP), $\alpha = 0.026$, $\beta = 3.1374^{b}$. Levels not connected with the same letter are significantly different ($p<0.05$).

level (table 4). Although, bands 1 and 7 migrated to different position, they were identified as *Microbacterium aerolatum*, sequence comparison pointed several differences as they may be originating from different operons. The profiles of activated symbiotic and mixed symbiotic (Fig. 3b) showed some differences that could be related to activation in molasses than enriched certain populations, yielding different prominent bands. From the FGC profiles (Fig. 3b), the band identified as *Asaccharospora irregularis* (band 5) remained throughout the culture in the three experimental conditions (CT, AP and MP), and *Turicibacter sanguinis* (band 6), only in the FGC of CT fishes. While from the bands of the bacterial community in the water, *Prosthecobacter* sp (band 3) and *Limnohabitans planktonicus* (band 8) disappeared after 50 days. Regarding bands 1 and 7, both corresponded to *Microbacterium aerolatum* and they were found in the symbiotic powder, and in 3 experimental conditions at 50 days, but only prevailed in the CT tanks at the end of the culture. *Bacillus horti* (band 4), was detected in the MP water along the culture, but was not present in the FGC of any treatment.

For PICRUSt analysis, the presence and relative abundance of each sequenced band in the different DGGE profiles allowed to predict the functional potential of the respective bacterial communities on the basis of marker gene sequencing (Douglas *et al.*, 2020). In this regard, some DGGE profiles; *i.e.* MP-0-W, CT-0-W and CT-50-W (Fig. 3b) shared 4 out of the 8 sequenced (identified) bands, while other profiles only shared 3, 2 or 1, except for AP-87-FGC that did not presented any shared band. Under this approach, around 90% the predicted genes for the different profiles were grouped under 21 KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways. The pathways related to membrane transport and carbohydrates metabolism were the most represented in the majority of the community metabolic profiles (Supplementary Table 1). Based on these prediction values, several differences in gene numbers were detected among the different profiles, however, these differences were more related to the number of identified bands within the profiles, rather than to the administration of symbiotics.

DISCUSSION

Growth and morphophysiological parameters. Determining the gradual increase in length or weight of cultivated organisms is a basic parameter in aquaculture. In this study, dynamics of weight increase in pac**ú** presented an adaptation phase of 28 days in the three experimental conditions. Normally, fishes in their early stages of development grow a larger proportion in length, than in the other growth parameters (Nash *et al.*, 2006). Coincidentally, Mourad *et al.* (2018), also found an initial delay phase of 28 days in *P. mesopotamicus* juveniles. According to Jomori *et al.*, 2003, the adaptation phase delay could be shortened to a 6 to 9 day-period under intensive culturing of pacú larvae based on live food.

The statistically significant differences in size, weight, and growth rate of MP treatment as compared to CT and AP do not imply a random variability or small fluctuations, but a treatment-related variation in the fish growth parameters. Therefore, it is likely that with the use of larger tanks, such a biomass quantity will be produced with MP that will allow for greater gains for producer compared to the other experimental conditions. Guidoli *et al.,* 2018 reported similar values in the growth rate in culture of *P. mesopotamicus* larvae, by administering a mixture of probiotic bacteria. Considering the way that activated symbiotic was administered, by adding the fermented preparation directly into the water tanks (AP treatment). Under this condition, the symbiotic has been highly diluted in the water tanks (3ml AP/day in 50L water tanks), and the fishes did not take full advantage of it. While the administration of the symbiotic in powder mixed with food (MP treatment), allowed the fishes to consume it more efficiently. The water and in the FGC of the AP treatment were analyzed during culturing, in such low numbers that sensitivity of DGGE profiling was not enough to detect their presence. However, our results showed that amending with symbiotic as in MP treatment, significantly improved the development and growth of the fishes. Furthermore, our results exceeded those of Inoue *et al.* (2019),

that doubled the initial weight of *P. mesopotamicus* in RAS at 50 days, while in our trial the biomass increased three-fold in that time and became six-fold in 90 days. Although in this study the type of food used was the same in all treatments, the administration of symbiotic mixed with the food (MP) allowed a significantly higher SGR in relation to AP and CT treatments. Our SGR values with MP treatment contrast with those reported by Bicudo *et al.* (2010), between 0.0228 and 0.0264 days-1 in *P. mesopotamicus*, depending on the protein concentration in the diet.

Regarding the food conversion ratio (FCR), the values (1.18 – 1.67g food/g fish) obtained in the three experimental conditions indicated that the efficiency of *P. mesopotamicus* to use the food was practically the same, which may be due to the quality, nutritional composition and digestibility of the used feed. According to Bicudo *et al.* (2010), a low FCR value may indicate that the activity of fish proteolytic enzymes increase the availability of amino acids necessary for fish metabolism. Similarly, Gomez-Penaranda *et al.* (2016), reported a FCR of 1.28 in *P. brachypomus*, in contrast, Klein *et al.* (2014), observed mean FCR values of 1.7 when the protein content of food was 18.5%. The FCR values can vary from 1.2 to 4.0 depending on the aquaculture species, development stage, culture conditions, as well as the quality and frequency of the rations (Hickling, 1966), while the FCR values for *P. mesopotamicus* may range from 1.06 to 2.89 (Bicudo *et al.*, 2010).

Both symbiotic treatments produced statistically significant increases in protein efficiency ratio (PER) as compared to the CT treatment. Considering that protein is the most expensive part of the food (Rojas *et al.*, 2014), the use of symbiotic would allow a more profitable conversion ratio (g_{fish}/g_{prot}), that could contribute to the fish health and formation of body mass. The significantly higher levels of PER in MP and AP compared to the control could be attributed to the symbiotic effect that was exerted favoring the production of digestive enzymes and the absorption of dietary proteins, reflected in enhanced body growth. Similar studies with pacú reported a PER of 2.4 g_{fish}/g_{prot} when mixing soybean and wheat flour (Machado-Neto *et al.*, 2016), and 2.5 gfish/gprot when food was supplemented with 19.6 g of digestible lysine/kg of food (Abimorad *et al.*, 2010), however, this amino acid is more expensive than the probiotics.

After 87 days of culture our CD values ranged from 5.68 to 7.2kg fish/m^3, these values are higher than the RAS-reared pacú (control treatment; 5.5kg fish/m^3) obtained by Inoue *et al.* (2019). However, their fishes subjected to sustained swimming showed up to 51% growth increase, after 50 days rearing. While there are no significant differences in CD among treatments, our results showed logarithmic trends after the initial 28-day delay phase. In this regard, MP exhibits higher exponential growth (SGR) compared to AP and CT, suggesting that a longer cultivation time may be required to manifest even higher CD values. This is supported by Poleo *et al.* (2011), that reported a CD of 12 kg fish/m^3 after 192 days of culture with *P. brachypomus.* Our results showed logarithmic trends after the initial 28-day delay phase, making possible to obtain larger CD values with a longer culturing time.

The weight-length relationship and the K factor are relevant parameters for understanding the life cycle of a fish population, allowing individuals' growth estimations and determining their degree of robustness, respectively (Khanipour *et al.*, 2020; Leyton *et al.*, 2015). The K factor value can vary between 0.1 and 4.0, depending on fish maturity and the spawning periods (Barriga *et al.*, 2002; Ruiz & Marchant, 2006). In this study, K values greater than 3.0 were observed without significant differences between treatments, similar results were obtained by Bacchetta *et al.* (2019) also with *P. mesopotamicus.* Pointing to a tendency in pacú towards an isometric growth. However, other aspects of their performance may differ, as noted by Leyton *et al.*, 2015. So far, this is the first report regarding the length-weight relationship during the cultivation of *P. mesopotamicus*.

Table 1. Growth scores of *P. mesopotamicus* (Holmber, 1887) cultured 87 days in a Recirculating Aquaculture System (RAS). The treatments were: CT) Control, AP) Activated symbiotic, MP) Mixed symbiotic. The obtained growth parameters: W) weight, Lt) Total length, Lp) Standard length, H) Height, were used to calculate SGR) Specific Growth Rate constant. Values not connected with the same symbol or letter are significantly different ($P<0.05$)

		Days of culture										SGR
		0	28	35	42	50	56	63	70	77	87	(day-1)
CT	W	3.5±0.7	5.5±1.4	6.2±1.8	8.7±2.4	14.0±2.2	13.4±3.4	15.7±3.6	15.7±4.3	19.3±4.9	24.9^±7.4	
	Lt	5.8±0.4	6.6±0.6	7.2±0.7	8.0±0.7	8.8±0.4	9.1±0.8	9.7±0.7	9.6±0.9	10.4±0.9	11.0$^{\delta}$±1.0	0.0253^{a}
	Lp	4.6±0.3	5.3±0.5	5.7±0.5	6.3±0.5	7.22±0.4	7.3±0.6	7.7±0.6	7.8±0.6	8.2±0.6	8.7°±0.8	±0.008
	H	2.6±0.2	3.0±0.3	3.2±0.3	3.6±0.4	4.2±0.3	4.1±0.4	4.4±0.4	4.4±0.4	4.7±0.5	5.1*±0.6	
AP	W	3.5±0.7	5.6±1.0	6.6±1.3	9.1±2.0	13.9±2.3	13.1±2.8	14.8±3.3	17.7±4.0	21.2±4.6	26.3^±6.6	
	Lt	5.7±0.4	6.7±0.5	7.1±0.6	8.0±05	9.1±0.5	9.1±0.8	9.6±0.8	10.2±0.9	10.7±0.9	11.1$^{\delta}$±1.0	0.0258^{a}
	Lp	4.6±0.3	5.3±0.4	5.8±0.4	6.4±0.5	7.2±0.4	7.3±0.6	7.6±0.6	8.0±0.6	8.6±0.6	8.8°±0.8	±0.007
	H	2.6±0.2	3.1±0.3	3.3±0.2	3.7±0.3	4.2±0.3	4.2±0.4	4.2±0.4	4.5±0.4	4.7±0.4	5.1*±0.5	
MP	W	3.5±0.7	4.9±1.4	7.2±1.8	10.0±2.5	14.9±3.4	17.5±4.6	17.5±4.4	20.8±4.7	24.5±5.5	31.5^{x}±6.7	
	Lt	5.8±0.4	6.78±0.5	7.5±0.6	8.3±0.5	9.4±0.6	9.6±0.8	10.1±0.7	10.7±0.7	10.7±0.7	11.8~±0.7	0.0321^{b}
	Lp	4.6±0.3	5.48±0.5	6.0±0.5	6.6±0.5	7.3±0.4	7.6±0.7	8.0±0.6	8.5±0.7	8.9±0.6	9.6$^{\Phi}$±0.7	±0.008
	H	2.6±0.2	3.24±0.4	3.4±0.3	3.8±0.3	4.4±0.3	4.6±0.5	4.5±0.4	4.8±0.4	5.0±0.4	5.4^{Y}±0.3	

Supplementary Table 1. Metabolic functional predictions derived from PICRUSt of the experimental treatments control (CT), activated synbiotic (AP) and mixed synbiotic (MP) MP in the bacterial communities in the fish gut content and in the suspended bacteria in the water culture at days 0, 50 and 87. APP-Aquabooster activated product and MPP-Aquabooster mixed product. Values correspond to number of predicted genes in each

KEGG_Pathways; metabolic routes	Water Treatments									Fish gut content treatments							APP	MPP
	CT_0	CT_{50}	CT_{87}	AP_0	AP_{50}	AP_{87}	MP_0	MP_{50}	MP_{87}	CT_0	CT_{50}	CT_{87}	AP_{50}	AP_{87}	MP_{50}	MP_{87}		
Metabolism; Amino Acid Metabolism	87	97	63	63	67	0	97	62	87	65	65	65	43	43	43	42	94	53
Metabolism; Biosynthesis of Other Secondary Metabolites	7	7	5	6	7	0	7	6	7	7	7	7	6	6	5	5	6	5
Metabolism; Carbohydrate Metabolism	112	111	97	107	109	0	114	98	97	97	97	97	98	98	98	97	98	97
Cellular Processes; Cell Motility	51	43	13	34	45	0	61	34	38	43	36	36	30	30	14	30	28	30
Metabolism; Energy Metabolism	64	78	50	67	54	0	96	62	74	54	68	68	53	53	38	54	47	34
Metabolism; Enzyme Families	32	43	24	34	24	0	48	26	32	27	32	32	27	27	26	24	26	20
Genetic Information Processing; Folding, Sorting and Degradation	46	47	23	27	23	0	46	27	40	24	28	28	26	26	25	23	27	25
Unclassified; Genetic Information Processing	32	32	21	26	20	0	34	23	32	22	26	26	20	20	19	20	34	18
Metabolism; Glycan Biosynthesis and Metabolism	38	38	21	24	21	0	37	26	30	25	24	24	21	21	22	21	30	23
Metabolism; Lipid Metabolism	79	82	47	54	41	0	83	48	45	42	46	45	43	43	38	41	47	41
Environmental Information Processing; Membrane Transport	199	218	158	168	197	0	218	167	197	149	149	149	163	163	86	97	97	94
Unclassified; Metabolism	57	57	32	45	43	0	63	45	45	34	43	43	34	34	33	33	34	31
Metabolism; Metabolism of Cofactors and Vitamins	56	56	47	48	26	0	54	50	52	27	47	47	26	26	25	26	45	30

Supplementary Table 1. Metabolic functional predictions derived from PICRUSt of the experimental treatments control (CT), activated synbiotic (AP) and mixed synbiotic (MP) MP in the bacterial communities in the fish gut content and in the suspended bacteria in the water culture at days 0, 50 and 87. APP-Aquabooster activated product and MPP-Aquabooster mixed product. Values correspond to number of predicted genes in each profile.

KEGG_Pathways; metabolic routes	Water Treatments									Fish gut content treatments							APP	MPP
	CT_0	CT_{50}	CT_{87}	AP_0	AP_{50}	AP_{87}	MP_0	MP_{50}	MP_{87}	CT_0	CT_{50}	CT_{87}	AP_{50}	AP_{87}	MP_{50}	MP_{87}		
Metabolism; Metabolism of Terpenoids and Polyketides	43	39	16	22	16	0	43	23	41	17	23	23	16	16	16	16	20	16
Metabolism; Nucleotide Metabolis	48	56	32	35	34	0	52	35	47	39	31	31	34	34	32	34	39	24
Unclassified; Poorly Characterized	63	67	36	43	49	0	68	48	64	26	43	43	39	39	36	49	23	30
Genetic Information Processing; Replication and Repair	93	95	84	97	93	0	98	92	94	87	95	95	73	73	70	93	82	85
Environmental Information Processing; Signal Transduction	30	32	20	20	17	0	32	20	32	23	19	19	18	18	15	17	24	17
Genetic Information Processing; Transcription	37	39	32	36	32	0	47	36	38	28	33	33	31	31	20	32	28	19
Genetic Information Processing; Translation	77	78	41	64	54	0	74	63	68	68	54	54	57	57	50	54	41	53
Metabolism; Xenobiotics Biodegradation and Metabolism	71	60	37	45	39	0	72	52	53	40	41	41	39	39	32	39	62	30

Table 2. Morphophysiological growth parameters of *P. mesopotamicus* (Holmber, 1887) after 87 days culture in a RAS. FCR - Protein efficiency ratio, CD – Crop density, K - Fulton condition factor, tb - Hypothesis of isometric growth. Values not connected with the same letter are significantly different ($p<0.05$).

	FCR (g_{food}/g_{fish})	PER ($g_{fish}/g_{prot.}$)	CC (kg_{fish}/m^3)	K	t_b
Control	$1.67^a \pm 0.39$	$1.80^a \pm 0.78$	$5.68^a \pm 1.03$	$3.59^a \pm 0.36$	0.848
Activated symbiotic	$1.60^a \pm 0.55$	$2.31^b \pm 0.86$	$6.00^a \pm 1.29$	$3.67^a \pm 0.29$	0.851
Mixed symbiotic	$1.18^a \pm 0.36$	$2.57^b \pm 0.76$	$7.20^a \pm 0.43$	$3.56^a \pm 0.31$	0.744

Water quality. The pH, temperature and OD values obtained throughout the assay remained in the correct physiological range for *P. mesopotamicus* culturing (Soncini & Glass, 1997; dos Santos *et al*., 2020). Ammonia concentration is one of the most critical water quality parameters for the growth of aquatic animals. Its apparent toxicity is extremely variable and does not depend solely on its mean or maximum concentration in water (Timmons *et al*., 2009; Quaresma *et al*., 2020; Aissaoui *et al*., 2017). Although desirable levels of ammonia for the farming of tropical fish species should be less than 0.025 mg/L, *P. mesopotamicus* has proven highly tolerant to different ammonia concentrations; Barbieri & Bondioli (2015), reported LC_{50} = 0.023mg NH_3/L, in 96h; Nitz *et al*. (2019), found a higher value: LC_{50} = 0.5mg NH_3/L during 10 days. While Abreu de *et al*. (2012), reported LC_{50} values up to 3.0mg NH_3/L during 24 h, however, although this exposure to ammonia caused an elevation in total hemoglobin, and blood glucose increased to 2.0 mg/L after the trial period. Our results report pacú highest tolerance to ammonia concentration (average 0.62 mg NH_3/L) in the MP treatment, and also the longest with 87 days. Generally, those fishes like pacú, growing in temperate waters are more tolerant to ammonia, than those from cold or salty waters (Timmons *et al*., 2009). It does not seem to be a correlation between the use of symbiotics and the ammonia removal capacity in the biofilter. Instead, the biofilm was primarily composed of nitrifying bacteria developing during the fish acclimation process, prior to the assay. The observed increase in the final values of ammonia may be associated with the high biomass accumulated throughout the culture (reflected in the loading capacity), producing a larger amount of this compound that could not be efficiently removed by the filtration system. The ΥNH_3 values of these systems could be increased if the adaptation of the nitrifying biofilm in the biofilter is extended, as recommended by Wang *et al*., 2018; or even more, by inoculating the biofilter with selected nitrifying bacteria to have a mature biofilm at the beginning of the assays. Although the NH3 values in MP treatment were at least twice those of the other treatments, there were no detectable negative effects on the growth parameters of the fishes in the MP treatment.

Analysis of bacterial communities. Studies on the FGC of *P. mesopotamicus* have reported the presence of Bacteroidetes, Firmicutes and Fusobacteria (Rossi *et al*., 2020), as well as Fusobacteria, Spirochaetes, Firmicutes and Proteobacteria (Castañeda-Monsalve *et al*., 2019). Our results partially coincide with those, since Firmicutes were detected in the FGC of the fishes throughout the culture, standing out *Asaccharospora irregularis* (Firmicutes/Clostridiales) detected in all the experimental conditions, and *Turicibacter sanguinis* (Firmicutes) mainly in the control treatment; while another Firmicutes (*Bacillus horti*), was detected in MP treatment. The habitat of *Bacillus* is very wide and its application in aquaculture is widely documented (Mendoza *et al*., 2019; Soltani *et al*., 2019; Thurlow *et al*., 2019). *Microbacterium aerolatum* (Actinobacteria) isolated from marine environments has been detected in the activated symbiotic (Fig. 3a, bands 1 and 7), a probiotic potential has been suggested for this lactic acid bacteria (Orla-Jensen, 1943).

PICRUSt analysis requires both; identity and quantification of OTUs as provided by massive sequencing. We have implemented a surrogated quantification of the populations (bands) within our DGGE profiles, however, only few bands were identified, and our results are focused on those bands. Although, in this sense the approach is limited, it provided information on specific and prominent bacterial populations present in the water and the FGC under the experimental conditions.

Table 3. Water quality parameters (average values ± SD) of the RAS, after 87-day culturing of *P. mesopotamicus* (Holmber, 1887). T – Temperature; DO - Dissolved oxygen concentration; NH_3 Ammonia water concentration; ΥNH_3 - Ammonia removal capacity of the biofilter. Values not connected with the same letter are significantly different ($P<0.05$).

	T (°C)	DO (mg/L)	pH†	NH_3^* (mg/L)	NH_3^{**} (mg/L)	ΥNH_3 ($g_N día^{-1}$)
Control	$22.4^a \pm 1.9$	$6.1^a \pm 0.9$	$8.3^a \pm 0.7$	$0.19^a \pm 0.06$	$0.43^a \pm 0.01$	$51.7^a \pm 16.8$
Activated symbiotic	$22.4^a \pm 1.9$	$6.2^a \pm 1.1$	$8.3^a \pm 0.7$	$0.20^a \pm 0.12$	$0.44^a \pm 0.07$	$42.6^a \pm 13.5$
Mixed symbiotic	$23.0^a \pm 2.0$	$7.7^a \pm 1.4$	$8.4^a \pm 0.7$	$0.19^a \pm 0.03$	$0.62^b \pm 0.03$	$38.01^a \pm 7.4$

* Initial values (0 day-culturing, n=9) **Final values (87-day culturing, n=9)

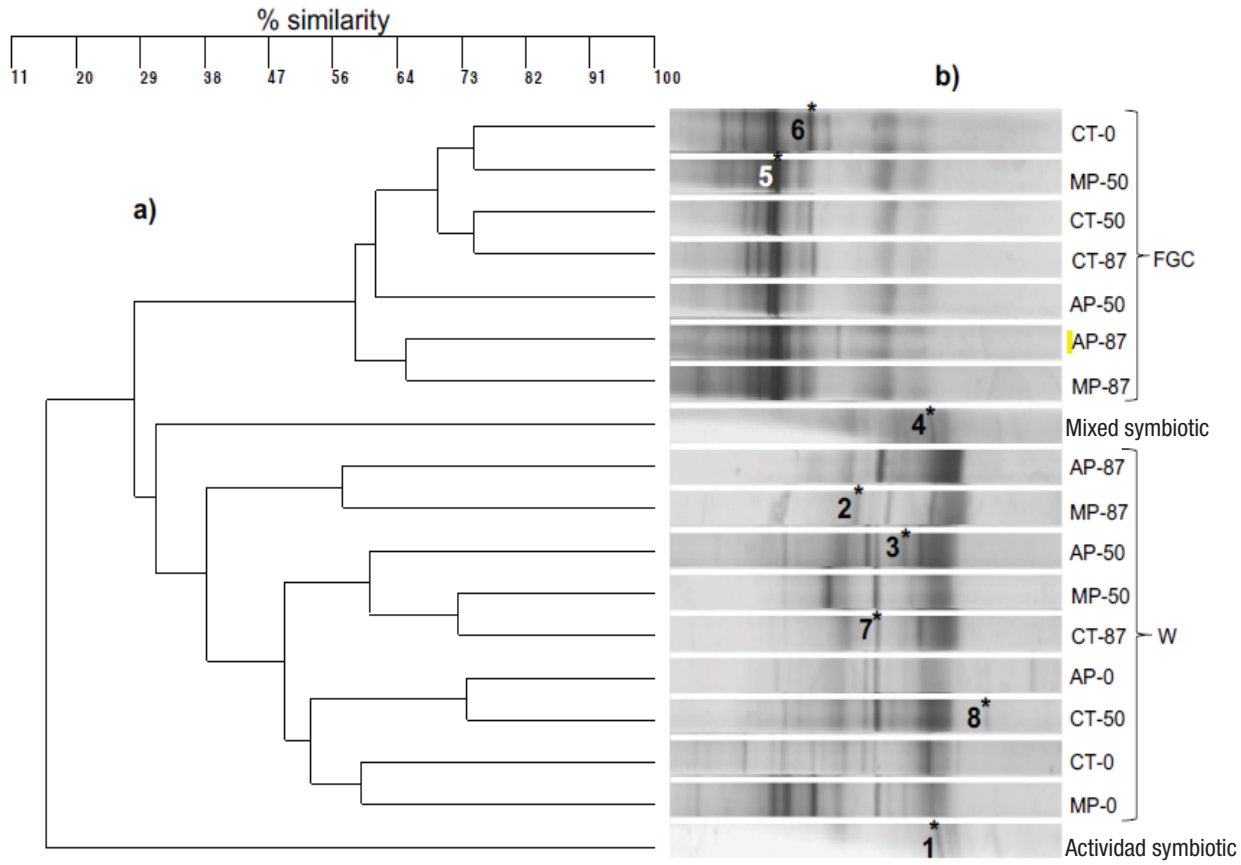

Figure 3. a) DGGE banding pattern of amplicons of the V6-V8 regions of the 16S rRNA from the experimental treatments CT, AP and MP, samples were obtained from the bacterial communities in the fish gut content (FGC) and in the suspended bacteria in the water (W) culture at days 0, 50 and 87. b) UPGMA dendrogram based on the DGGE profiles. * denotes the bands selected for excision and sequencing, and the 1-8 numbers the identification of each band.

Table 4. Phylogenetic identification and percentage of sequence similarity in the V6-V8 region of the gen 16S rRNA obtained from the excised DGGE bands (see Figure 4a). Labeling is as follows: first two letters are treatments; AP- activated symbiotic, MP- mixed symbiotic, CT- control. Letters after de hyphen correspond to sample origin; W- water in the RAS tank, FGC- fish gut content, and the numbers to the sampling time; 0, 50 and 87 days of *P. mesopotamicus* (Holmber, 1887) culturing. APP-Aquabooster activated product and MPP-Aquabooster mixed product.

DGGE band number (treatment-sample origin)	Accesion number	Phylum	Closest relative	Similarity (%)
1 (APP)	MN845131	Actinobacteria	*Microbacterium aerolatum* (BJUW01000027)	99.71
2 (MP-W_{87})	MN845132	Proteobacteria	*Variovorax* sp (BCUT01000013)	92.95
3 (AP-W_{50})	MN845133	Verrucomicrobiae	*Prosthecobacter* sp (AB305640)	95.02
4 (MPP)	MN845134	Firmicutes	*Bacillus horti* (D87035)	98.33
5 (MP-FGC_{50})	MN845135	Firmicutes	*Asaccharospora irregularis*^{T} (X73447)	98.53
6 (CT-FGC_0)	MN845136	Firmicutes	*Turicibacter sanguinis*^{T} (AF349724)	100
7 (CT-W_{87})	MN845137	Actinobacteria	*Microbacterium aerolatum* (BJUW01000027)	98.58
8 (CT-W_{50})	MN845138	Proteobacteria	*Limnohabitans planktonicus*^{T} (LFYT01000006)	99.72

CONCLUSION

This is the first report on the size-weight relationship of pacú at different times during a 87 days rearing, showing isometric growth. The administration of symbiotic mixed with the feed during the cultivation of *P. mesopotamicus*, significantly promotes the growth of the fishes in recirculating aquaculture systems. Furthermore, the use of activated symbiotic may have improved the capacity of the biofilter to partly remove high ammonia concentrations. In the mixed and activated symbiotic treatments were detected *Bacillus horti* and *Microbacterium aerolatum*, respectively, both species have the potential to be used as probiotics in aquaculture.

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REFERENCES

Abimorad, E. G., G. C. Favero, G. H. Squassoni & D. Carneiro. 2010. Dietary digestible lysine requirement and essential amino acid to lysine ratio for pacu *Piaractus mesopotamicus*. *Aquaculture Nutrition* 16**:** 370-377. DOI: 10.1111/j.1365-2095.2009.00674.x

Abreu de, J. S., F. R. Esteves & E. C. Urbinati. 2012. Stress in pacu exposed to ammonia in water. *Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science* 41**:** 1555-1560.

Aguirre-Garrido, J. F., H. C. Ramírez-Saad, N. Toro & F. Martínez-Abarca. 2016. Bacterial Diversity in the Soda Saline Crater Lake from Isabel Island, Mexico. *Microbial Ecology* 71: 68-77. DOI: 10.1007/s00248-015-0676-6

Aissaoui, S., H. Ouled-Haddar, M. Sifour, K. Harrouche & H. Sghaier. 2017. Metabolic and Co-Metabolic Transformation of Diclofenac by *Enterobacter hormaechei* D15 Isolated from Activated Sludge. *Current Microbiology* 74**:** 381-388. DOI: 10.15171/ijb.1530

Aslam, S. N., S. Navada, G. R. Bye, V. C. Mota, B.F. Terjesen & Ø. Mikkelsen. 2019. Effect of CO2 on elemental concentrations in recirculating aquaculture system tanks. *Aquaculture* 511: 734254. DOI: 10.1016/j.aquaculture.2019.734254

Bacchetta, C., A. S. Rossi, R. E. Cian, R. E., Drago, S. R. & Cazenave, J. 2019. Dietary beta-carotene improves growth performance and antioxidant status of juvenile *Piaractus mesopotamicus*. *Aquaculture Nutrition* 25**:** 761-769. DOI: 10.1111/anu.12893

Badiola, M., O. C. Basurko, R. Piedrahita, P. Hundley & D. Mendiola. 2018. Energy use in Recirculating Aquaculture Systems (RAS): A review. *Aquacultural Engineering* 81**:** 57-70. DOI: 10.1016/j.aquaeng.2018.03.003

Barbieri, E. & A. C. V. Bondioli. 2015. Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. *Aquaculture Research* 46**:** 565-571. DOI: 10.1111/are.12203

Barrero, M., A. Paredes, O. Romero & G. A. Poleo. 2012. Proximate composition and flsh quality of red bellied pacu, P*iaractus* b*rachypomus,* cultured in two different closed systems. *Zootecnia Tropical* 30 (3): 263-268.

Barriga, J. P., M. A. Battini, P. J. Macchi, D. Milano & V. E. Cussac. 2002. Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces:Galaxiidae) in a lake in the South American Andes. *New Zealand Journal of Marine and Freshwater Research* 36**:** 345-359. DOI: 10.1080/00288330.2002.9517092

Bicudo, A. J. A., R. Y. Sado & J. E. P. Cyrino. 2010. Growth performance and body composition of pacu *Piaractus mesopotamicus* (Holmberg 1887) in response to dietary protein and energy levels. *Aquaculture Nutrition* 16**:** 213-222. DOI: 10.1111/j.1365-2095.2009.00653.x

Castañeda-Monsalve, V. A., E. Junca, E. García-Bonilla, O. I Montoya-Campuzano & C. X. Moreno-Herrera. 2019. Characterization of the gastrointestinal bacterial microbiome of farmed juvenile and adult white Cachama (*Piaractus brachypomus*). *Aquaculture* 512: 734325. DOI: 10.1016/j.aquaculture.2019.734325

de Paula, T. G., F. L. A. de Almeida, F. R. Carani, I. J. Vechetti, C. R. Padovani, R. A. S. Salomao, E. A. Mareco, V. B. Dos Santos & M. Dal-Pai-Silva. 2014. Rearing temperature induces changes in muscle growth and gene expression in juvenile pacu (*Piaractus mesopotamicus*). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 169**:** 31-37. DOI: 10.1016/j.cbpb.2013.12.004

Douglas, G. M., V.J. Maffei, J.R. Zaneveld, S.N. Yurgel *et al.* 2020. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* 38: 685-688. DOI: 10.1038/s41587-020-0548-6

dos Santos, J. A., C.M. Soares & A. Bialetxki. 2020. Effects of pH on the incubation and early development of fish species with different reproductive strategies. *Aquatic Toxicology* 219: 105382. DOI: 10.1016/j.aquatox.2019.105382

FAO (Food and Agriculture Organization). 2020. *The State of World Fisheries and Aquaculture. Sustainability in action.* United Nations. Rome. 21-138 p. Also available at: htts://www.fao.org/3/ca9229en/CA9229EN.pdf

Felske, A., B. Engelen, U. Nubel & H. Backhaus. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. *Applied and Environmental Microbiology* 62: 4162-4167.

Gomez-Penaranda, J., L. Vasquez-Gamboa & D. Valencia. 2016. The effect of different feeding and starvation frequencies on growth utilization and nutrients, for *Piaractus brachypomus* (Cuvier, 1818). *Latin American Journal of Aquatic Research* 44: 569-575. DOI: 10.3856/vol44-issue3-fulltext-15

Guidoli, M. G., J. A. Mendoza, S. L. Falcón,S. I. Boehringer, S. Sánchez & M. E. F. N. Macías. 2018. Autochthonous probiotic mixture improves biometrical parameters of larvae of *Piaractus mesopotamicus* (Caracidae, Characiforme, Teleostei). *Ciencia Rural* 48 (7): e20170764. DOI: 10.1590/0103-8478cr20170764

Hickling, C. F. 1966. Socio-Economic Aspects Of Fish Farming. *Proceedings of the Nutrition Society* 25**:** 140-146. DOI: 10.1079/pns19660030

Hoseinifar, S.H., E. Ringo, A.S. Masouleh & M.Á. Esteban. 2016. Probiotic, prebiotic and synbioic supplements in sturgeon aquaculture: a review. 8 (1): 89-102. DOI: 10.1111/RAQ.12082

Ibáñez, A. L. 2015. Geographic differences and annual stability in length-weight relationships of fish mullets (Pisces: Mugilidae). *Hidrobiológica* 25**:** 146-150.

Inoue, L., A. Hackbarth, G. Arberlaez-Rojas & G. Moraes. 2019. Growth performance and metabolism of the Neotropical fish *Piaractus mesopotamicus* under sustained swimming. *Aquaculture* 511 (4): 734219. DOI: 10.1016/j.aquaculture.2019.734219

ITIS (Integrated Taxonomic Information System). 2021. *Piaractus.*Available on line at: https://www.itis.gov/advanced_search.html (downloaded May 23, 2021).

Jomori, R. K., D. J. Carneiro, E. B. Malheiros & M. C. Portella. 2003. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. *Aquaculture* 221**:** 277-287. DOI: 10.1016/s0044-8486(03)00069-3

Jomori, R. K., R. K. Luz & M. C. Portella. 2012. Effect of Salinity on Larval Rearing of Pacu, *Piaractus mesopotamicus*, a Freshwater Species. *Journal of the World Aquaculture Society* 43**:** 423-432. DOI: 10.1111/j.1749-7345.2012.00570.x

Khanipour, A. A., A. Noori, M. Amini & E. Kamrani. 2020. Length-weight relationship and Fulton's condition factor of *Macrobrachium nipponense* (De Haan, 1849) in Anzali lagoon of Iran. *Iranian Journal of Fisheries Sciences* 19**:** 496-500. DOI: 10.22092/ijfs.2019.118323

Klein, S., E. K. Lorenz, G.W. Bueno, A. Signor, A. Feiden & W. R. Boscolo. 2014. Levels of crude protein in diets for Pacu (*Piaractus mesopotamicus*) from 150 to 400 g reared in cages. *Archivos de zootecnia* 63: 599-611.

Kumar, A., P. K. Pradhan, P. C. Das, S. M. Srivastava, K. K. Lal & J. K. Jena. 2018. Growth performance and compatibility of pacu, *Piaractus brachypomus* with Indian major carps in polyculture system. *Aquaculture* 490**:** 236-239. DOI: 10.1016/j.aquaculture.2018.02.052

Lee, J.M., W.J. Jang, M.T. Hasan, B.-J. Lee, K.W. Kim, S.G. Lim, H.S. Han & I.-S- Kong. 2019 Characterization of a *Bacillus* sp. isolated from fermented food and its symbiotic effect with barley β-glucan as a biocontrol agent in the aquaculture industry. *Applied Microbiology and Biotechnology* 103: 1429-1439. DOI: 10.1007/s00253-018-9480-9

Leyton, F. S. A., E. Muñoz, S. M. Gordillo, G. G. C. Sánchez, L. A. Muñoz & D. A. Soto. 2015. Estimación del factor de condición de Fultron (*K*) y la relación longitud-peso en tres especies ícticas presentes en un sector sometido a factores de estrés ambiental en la cuenca alta del río Cauca. *Revista de la Asociación Colombiana de Ciencias* 27: 21-28.

Lugert, V., Thaller, G., Tetens, J., Schulz, C. & Krieter, J. 2016. A review on fish growth calculation: multiple functions in fish production and their specific application. *Reviews in Aquaculture* 8 30-42. DOI: 10.1111/raq.12071

Machado-Neto, R., D. B. Moretti, W. M. Nordi, T. M. P. Da Cruz, & J. E. P. Cyrino. 2016. Growth performance of juvenile pacu (*Piaractus mesopotamicus*) and dourado (*Salminus brasiliensis*) fed with lyophilized bovine colostrum. *Aquaculture Research* 47**:** 3551-3557. DOI: 10.1111/are.12805

Martínez Cruz, P., A. L. Ibáñez, O. A. Monroy Hermosillo & H. Ramírez-Saad. 2012. Use of Probiotics in Aquaculture. *International Scholarly Research Network ISRN Microbiology* 2012**:** 13. DOI: 10.5402/2012/916845

Medeiros, R. S., B. A. Lopez, L. A. Sampaio, L. A. Romano & R. V. Rodrigues. 2016. Ammonia and nitrite toxicity to false clownfish *Amphiprion ocellaris*. *Aquaculture International* 24**:** 985-993. DOI: 10.1007/s10499-015-9965-9

Mendoza, L. F. D., J. G. Q. Mujica, J. M. R. Cunayque, G. W. A. Lucana, J. J. I. Angulo, V. I. S. De La Cruz, V. A. C. Escobar & E. M. MatonnieR. 2019. Assessment of Heterotrophic Nitrification Capacity in *Bacillus* spp. and its Potential Application in the Removal of Nitrogen from Aquaculture Water. *Journal of Pure and Applied Microbiology* 13**:** 1893-1908. DOI: 10.22207/jpam.13.4.02

Merrifield, D., A. Dimitroglou, A. Foey, S.J. Davies, R.T.M, Baker, J. Bogwald, M. Castex & E. Ringo. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302 (1-2): 1-18. DOI: 10.1016/j.aquaculture.2010.02.007

Montgomery, D. C. 2004. *Diseño y Análisis de Experimentos,* USA, 2ª ed. Editorial Limusa S.A. de C.V. México. 60-125 p.

Mourad, N. M. N., A. C. Costa, R. T. F. Freitas, M. A. Serafini, R. V. R.M. Neto & V. O. Felizardo. 2018. Weight and morphometric growth of Pacu (*Piaractus mesopotamicus*), Tambaqui (*Colossoma macropumum*) and their hybrids from spring to winter. *Pesquisa Veterinaria Brasileira* 38**:** 544-550. DOI: 10.1590/1678-5150-pvb-4808

Mugwanya, M., M.A.O. Dawood, F. Kimera & H. Sewllam. 2022. Updating the Role of Probiotics, Prebiotics, and Symbiotics for Tilapia Aquacultures as Leading Candidates for Food Sustainability: A Review. *Probiotics and Antimicrobial Proteins* 14: 130-157. DOI: 10.1007/s12602-021-09852-x

Nash, R. D. M., A. H. Valencia & A. J. Geffen. 2006. The Origin of Fulton's Factor-Setting the Record Straight. *Fisheries* 31 (5): 236-238.

Nitz, L. F., L. C. Maltez, L. Pellegrin, L. D. Garcia, L. A. L. Barbas & C. Prentice-Hernandez. 2019. Flesh Quality And Stress Responses Of *Piaractus mesopotamicus* After Exposure To Sublethal Levels Of Ammonia And Subsequent Recovery. *Boletim Do Instituto De Pesca* 45 (1): e.325. DOI: 10.20950/1678-2305.2019.45.1.325

Orla-Jensen, S. 1943. *The Lactic Acid Bacteria*. Kobenhavn, 2nd ed. Assorted folio.

Pavon, Y., R. E. Cian, M. A. C. Soldini, D. R. Hernandez, S. Sanchez & S. R. Drago. 2018. Sensory and instrumental textural changes in fillets from Pacu (*Piaractus mesopotamicus*) fed different diets. *Journal of Texture Studies,* 49**,** 646-652.

Piedrahita, R. H. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* 226**:** 35-44. DOI: 10.1016/s0044-8486(03)00465-4

Poleo, G., J. V. Aranbarrio, L. Mendoza & O. Romero. 2011. High-density rearing of red-bellied pacu in two closed systems. *Pesquisa Agropecuaria Brasileira* 46**:** 429-437.

Quaresma, F. D., F. L. B. Dos Santos, P. F. Ribeiro, L. A. Leite, & Sampaio, A. H. 2020. Acute toxicity of non-ionized ammonia on tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*). *Revista Ciencia Agronomica* 51**:** 6. DOI: 10.5935/1806-6690.20200046

Ramírez-Saad H. W.L. Akkermans &. A.D.L. Akkermans. 2004. DNA Extraction from Actinorhizal Nodules. *In*: Kowalchuk, F., I. A. de Bruijn, L. Head, A. D. Akkermans, J. D. van Elsas (Eds). *Molecular Microbial Ecology. Manual II*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rennie, M. D. & R. Verdon. 2008. Development and evaluation of condition indices for the lake whitefish. *North American Journal of Fisheries Management* 28**:** 1270-1293. DOI: 10.1577/m06-258.1

Ringo E., R.E. Olsen, T.Q. Gifstad, R.A. Dalmo, H. Amlund, G.-I. Hemre & A.M. Bakke. 2010. Prebiotics in aquaculture: a review. *Aquaculture Nutrition.*16 (2): 117-136. DOI: 10.1111/j.1365-2095.2009.00731.x

Rise, M. L., C. J. Martyniuk & M. Y. Chen. 2019. Comparative physiology and aquaculture: Toward Omics-enabled improvement of aquatic animal health and sustainable production. *Comparative Biochemistry and Physiology D-Genomics & Proteomics* 31**:** 9. DOI: 10.1016/j.cbd.2019.100603

Rojas, P., J. E. Rosales, C. Espinoza & C. Ching. 2014. Protein Eficiency of feed in the Farming of *Litopenaeus vannamei*. Boletín Nicovita. Ecuardor. 1-4 p.

Rossi, L. T., A. R. Sharpen, J. A. Zimmermann, C. R. Olivero, M. V. Zbrun, L. S. Frizzo, M. L. Signorini, C. Bacchetta, R. E. Cian, J. Cazenave, L. P. Soto & S. R. Drago. 2020. Intestinal microbiota modulation in juvenile Pacú (*Piaractus mesopotamicus*) by supplementation with *Pyropia columbina* and beta-carotene. *Aquaculture International* 28**:** 1001-1016. DOI: 10.1007/s10499-020-00508-1

Ruiz, V. H. & M. Marchant. 2006. Ictiofauna de aguas continentales Chilenas. *Neotropical Ichthyology* 4 (4): 463.

Sanguinetti, C. J., E. D. Neto & A. J. G. Simpson. 1994. Rapid Silver Staining And Recovery Of PCR Products Separated On Polyacrylamide Gels. *Biotechniques* 17**:** 914-916.

Soltani, M., K. Ghosh, S. H. Hoseinifar, V. Kumar, A. J. Lymbery, S. Roye & E. Ringo. 2019. Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Reviews in Fisheries Science & Aquaculture* 27**:** 331-379. DOI: 10.1080/23308249.2019.1597010

Soncini, R & M. L. Glass. 1997. The effects of temperature and hyperoxia on arterial PO_2 and acid-base status in *Piaractus mesopotamicus. Journal of Fish Biology* 51(2): 225-233.

Thurlow, C. M., M. A. Williams, A. Carrias, C. Ran, M. Newman, J. Tweedie, E. Allison, L. N. Jescovitch, A. E. Wilson, J. S. Terhune & M. R. Liles. 2019. *Bacillus velezensis* AP193 exerts probiotic effects in channel catfish (*Ictalurus punctatus*) and reduces aquaculture pond eutrophication. *Aquaculture* 503**:** 347-356. DOI: 10.1016/j.aquaculture.2018.11.051

Timmons, M. B., J. M. Ebeling & R.H. Piedrahita. 2009. *Acuicultura en Sistemas de Recirculación.* 1° ed. Cayuga Aqua Ventures. USA. 50-57 p.

Usaid-Harvest (United States Agency International Development). 2011. Feed Conversion Ratio (FCR). Technical bulletin 7. Available online at: https://pdf.usaid.gov/pdf_docs/PA00K8MQ.pdf (downloaded February 6, 2021).

Wang, F., L. Zhou. & J. Q. Zhao. 2018. The performance of biocarrier containing zinc nanoparticles in biofilm reactor for treating textile wastewater. *Process Biochemistry* 74**:** 125-131. DOI: 10.1016/j.procbio.2018.08.022

Yoon, S. H., S. M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo. & J. Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *International Journal of Systematic and Evolutionary Microbiology* 67: 1613-1617. DOI: 10.1099/IJSEM.0.00175