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Bacteriological status and the water quality in ornamental aquaculture farms in Morelos, Mexico

Estatus bacteriológico y calidad del agua de cultivo en granjas acuícolas ornamentales de Morelos, México

Orozco-Rojas Dafne*[1] ID, Monroy-Dosta María[2] ID, Bustos-Martínez Jaime[3] ID, Ocampo-Cervantes José[4] ID, Barajas-Galván Emilio[2] ID, Ramírez-Torrez José[1] ID

[1]Doctorado en Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana. CDMX. México. [2]Laboratorio de Análisis Químico de Alimento Vivo. Departamento El Hombre y su Ambiente. Universidad Autónoma Metropolitana. Unidad Xochimilco, CDMX. México. [3]Laboratorio de Microbiología y Biología Molecular. Departamento de Atención a la Salud. Universidad Autónoma Metropolitana. Unidad Xochimilco, CDMX. México. [4]Laboratorio de Producción de Alimento Vivo. Departamento El Hombre y su Ambiente. Universidad Autónoma Metropolitana. Unidad Xochimilco, CDMX. México. *Responsible author and for correspondence: Orozco-Rojas Dafne. Calzada del Hueso 1100, Coapa, Villa Quietud, Coyoacán, C.P. 04960, CDMX. México. E-mail: d.orozco508@gmail.com, monroydosta@hotmail.com, jbustos@correo.xoc.uam.mx, jaocampoc@hotmail.com, bioem_bg91@outlook.com, ramirez-torrez@hotmail.com.

Abstract

This work aimed to determine the bacteriological status and the water quality during the culture process of four aquaculture farms in Morelos, Mexico. The bacteriological analyses were done from fishes (*Poecillia sphenops*), feed and culture water samples, and culture media BHI, TCBS and EMB; the isolated bacteria were identified by biochemical tests. Samples from ponds and supply channels were analyzed to determine the water quality according to the official Mexican standards. Microbiological results of water indicated that supply ditches presented a more considerable bacterial diversity and load than the analyzed farms, identifying ten bacterial species belonging to Proteobacteria and Firmicutes; regarding feed and fish samples, species like *Bacillus megaterium*, *Vibrio alginolyticus*, and *Enterococcus faecalis* were identified. According to the official Mexican standards stipulations, all production units have good water quality; however, Las Estacas ditch exceeds LMP of faecal coliforms (2400 NMP 100 mL^{-1}) and phosphorus (0.83 mg L^{-1}). In conclusion, the sanitary quality of the organisms produced in the farms is influenced by contamination of the supply ditches.

Keywords: ornamental fishes, *Poecillia sphenops*, water quality, Morelos.

Resumen

El objetivo del presente estudio fue determinar el estado bacteriológico y la calidad del agua empleada durante los procesos de cultivo de cuatro granjas acuícolas del estado de Morelos, México. El análisis bacteriológico se realizó a partir de muestras de peces (*Poecillia sphenops*), alimento y agua de cultivo, empleando los medios BHI, TCBS y EMB; las bacterias aisladas fueron identificadas mediante pruebas bioquímicas. Para determinar la calidad del agua, se analizaron muestras provenientes de estanques y canales de abastecimiento según normas oficiales mexicanas. Los resultados bacteriológicos del agua indicaron que los canales presentaron la mayor diversidad y carga bacteriana, identificándose 10 especies pertenecientes al grupo de las Proteobacterias y Firmicutes; para el caso de alimento y peces se

identificaron especies como *Bacillus megaterium*, *Vibrio alginolyticus* y *Enterococcus faecalis*. Según las estipulaciones de las normas oficiales mexicanas, todas las unidades de producción presentan una buena calidad de agua; sin embargo, el canal de Las Estacas supera los LMP de coliformes fecales (2400 NMP 100 mL^{-1}) y fósforo (0.83 mg L^{-1}). Con lo anterior se concluye que la calidad sanitaria de los organismos producidos en las granjas se encuentra comprometida por los contaminantes presentes en los canales de abastecimiento.
Palabras clave: peces ornamentales, *Poecillia sphenops*, calidad de agua, Morelos.

INTRODUCTION

Worldwide, ornamental aquaculture is an activity that has gained importance due to the economic contribution it represents for the aquaculture sector, which is estimated to generate about 500 million dollars annually (Opiyo *et al*., 2016; Sicuro, 2018; Velasco-Santamaría & Corredor-Santamaría, 2011; Ghosh *et al*., 2003). Currently, member countries of the European Union, the United States and Japan represent the largest markets for ornamental fish worldwide, with species such as discus, guppy, sword, molly, molly sailfish and goldfish standing out, in addition to marine species (Ghosh *et al*., 2003; Velasco-Santamaría & Corredor-Santamaría, 2011).

Mexico has not been the exception, since ornamental fish production has allowed regional development through the creation of jobs and income, especially in rural areas (Martínez *et al*., 2010; CONAPESCA, 2018; Sivakumar *et al*., 2015); however, the productive success of the activity is compromised by low technical efficiency within the aquaculture production units (APU). Its development in an empirical manner conflicts the operation of the units, especially when seeking to move from an extensive to an intensive production system; so it is common for aquaculture farmers to face a great diversity of problems, where those associated with water quality represent the most common (Njoku *et al*., 2015; Martínez *et al*., 2010; Rajeshwari & Devasree, 2017). In this sense, it has been determined that the physical and chemical parameters of the culture water influence the health status of the organisms and in turn the composition of the pond microbiota, which determines the incidence of diseases during the productive cycles (Chidambaram *et al*., 2013; Ginson *et al*., 2017; Ajayi & Okoh, 2014). In addition to the above, it is important to mention that at the national level there is a need to update and/or modify legislation regarding the production of aquatic species for ornamental purposes. In particular, the issues to be worked on are related to health, including issues of culture water quality, as well as management practices applied in the APUs. With the above, the aim is to guarantee a product free of pathogens, since the lack of regulation in these aspects could have consequences not only at the productive level, but also on human health and the surrounding environments (Valenzuela-Armenta *et al*., 2018; Vasile *et al*., 2017; Saremi *et al*., 2013).

Due to the above, the objective of the present study was focused on determining the bacteriological status of ornamental fish farms in Morelos and the quality of the water used during the culture processes, considering the current national standards that apply.

MATERIAL AND METHODS

Study area

The study was carried out in four ornamental fish farms located in the Apatlaco River sub-basin in the Morelos State, Mexico. The coordinates of each farm and how they were identified are as follows:

Farm A: 18° 38' 53.9" N and 99° 13' 13.2" W
Farm B: 18° 38' 52.3" N and 99° 13' 10.4" W
Farm C: 18° 38' 55.9" N and 99° 13' 09.4" W
Farm D: 18° 39' 20.4" N and 99° 12' 00.9" W

The study comprised two seasons in 2017, corresponding to the dry season during the months of February to March and the rainy season during June and July.

Sampling

Samples of water, feed and organisms of the species *Poecillia sphenops*, commonly known as molly, were collected in each of the units; this species was considered because it represents one of the most demanded and produced in the region. To evaluate the health status of the fish and determine the sample size, the tables proposed by the Manual of Diagnostic Tests for Aquatic Animals were used, considering fish with normal clinical appearance and the volume of production in each unit (around 2000 organisms); in addition to a prevalence of 10% and a reliability of 95%, so 27 organisms were included in each unit per visit (OIE, 2008).

The organisms were collected randomly from each of the ponds and transported alive with continuous aeration, in plastic bags with water from the same ponds.

Water was collected from the *P. sphenops* production ponds at each of the farms; water samples were also collected from the Las Estacas ditch (supplying water to farms A, B and C) and from the Apatlaco River ditch (supplying water to farm D). Sampling followed the stipulations of the Mexican Official Standards NMX-AA-003-1980 and NOM-001-ECOL-1996. Three single samples were obtained from the same point, collected aseptically using sterile bottles; they were transported and kept at 4 ºC (FAO, 2011).

Finally, the collection of feed samples in each of the production units was done in triplicate and aseptically, using sterile bags for transportation (NOM-109-SSA1-1994).

All samples were collected weekly during the seasons studied, and were transported to the Live Food Chemistry Laboratory of the Autonomous Metropolitan University, Xochimilco Unit, for analysis.

Microbiological analysis

The analysis of the organisms was approved by the Ethics Committee of the Biological and Health Sciences Division of the Autonomous Metropolitan University. Organisms were euthanized with an overdose of benzocaine, using the immersion method (AVMA, 2013); subsequently, under aseptic conditions, they were dissected to expose the internal organs and extract the viscera, which were homogenized in sterile 0.89% saline solution (OIE, 2008). For the case of feed and water samples, 1 g^{-1} and 1 mL^{-1} of each was analyzed, which were homogenized in 9 mL of sterile saline solution (0.89%) (Prado *et al*., 2013).

In all cases, three serial dilutions were performed, which were inoculated in triplicate on BD Bioxon® brand brain heart infusion (BHI) agar (214700), BD Difco™ brand thiosulfate citrate bile sucrose (TCBS) (265020) and BD Bioxon ® brand eosin and methylene blue (EMB) (210600) plates and incubated at 35 ± 2º C for 48 hours under aerobic conditions. After this period, the colonial morphology was characterized and the colony forming unit (CFU) count was performed using a colony counter and selecting those plates with between 30 and 300 colonies (Velasco & Tapia, 2014).

The following formula was used to determine the number of CFU mL^{-1} or CFU g^{-1} (NMX-AA-003-1980 and NOM-001-ECOL-1996):

$$CFU\ mL\ ó\ g\ = \frac{N\ \times ID}{V}$$

Where:
N: average number of colonies
ID: inverse of dilution
V: volume of inoculated sample

For bacterial identification, a differential selection of colonies was made according to morphology, so that they would be representative of the different types of colonies recovered. By successive reseeding, the bacteria were isolated and their purity was verified by Gram staining (Velasco & Tapia, 2014). Bacteria were identified by biochemical tests and using a commercial bacterial identification system using API® galleries (20 E; 20 NE; 50 CH and 50 CHB) (BioMerieux, Mexico).

Coliform analysis

Coliform counts were determined following the methodology proposed by NMX-AA-042-SCFI-2015, using Bioxon® lactose broth (211700) and Difco™ brilliant green bile broth (273000) as culture medium. The incubation temperature was 35º C for 48 hours for the presumptive test; while the confirmatory test for total coliforms was incubated at 37º C for 48 hours, and for fecal coliforms at 44º C for 24 hours. The analyses of each sampled point were performed in triplicate and the results were expressed as MPN per 100 mL^{-1} of water.

Physicochemical parameters

Parameters such as pH, temperature, dissolved oxygen (D.O.), salinity and total dissolved solids (TDS) were measured in situ (NMX-AA-008-SCFI-2011), using a multiparameter probe model HI 98194 (HANNA®, Mexico). Chemical parameters such as nitrogen (N) and phosphorus (P) were analyzed with a multiparameter photometer model HI 83203 (HANNA®, Mexico), following the manufacturer's instructions for each case.

Biological oxygen demand analysis

The determination of biological oxygen demand at five days (BOD5) was carried out using the Winkler method determined by NMX-AA-028-SCFI-2001, performing the tests in triplicate. The initial and final dissolved oxygen concentration was measured with a HI 9146 model oximeter (HANNA®, Mexico).

Statistical analysis

The data were subjected to a descriptive analysis; subsequently, to determine differences between sampling sites and monitored seasons, multiple mean comparison tests were applied by analysis of variance and Student's t-test for minimum significant difference ($P<0.05$), after verifying that the data complied with the assumptions of normality. In the case of microbiological results (CFU mL^{-1}), the data were transformed to Log 10 to meet the assumptions of normality. All statistical tests were performed using the SYSTAT 13.0® statistical program.

RESULTS

Microbiological water analysis

The microbiological analysis of water allowed the isolation and identification of a total of 10 bacterial species belonging to the group of Proteobacteria and Firmicutes, where both water tributaries presented the greatest bacterial diversity and abundance during the seasons analyzed, including significant differences for most of the bacteria identified in Las Estacas ditch, with respect to the farms that are supplied by it, as can be seen in Table 1. Likewise, a change in the composition of the microbiota was observed during the rainy season in Las Estacas ditch and the farms supplied by it, displacing species such as *Burkholderia gladiolii* and *Bacillus thuringiensis*; on the contrary, the species *Vibrio parahaemolyticus* and *Aeromonas hydrophila* were recorded. It should be noted that *B. gladiolii* was only recorded in Las Estacas ditch during the dry season, while *Pseudomonas luteola* was reported only in farm A during the rainy season. In relation to the group of *Enterobacteriaceae*, *Klebsiella* sp. and *Escherichia coli* showed a slight increase in the CFU mL^{-1} count during the rainy season in Las Estacas ditch; however, no statistical difference was determined for E. coli ($p>0.05$) from one season to the other. Similar behavior was observed in the Apatlaco River ditch, where high bacterial counts were recorded; however, no statistical difference was determined with respect to farm D ($p >0.05$). Nevertheless, it could be seen that as bacterial counts such as *Klebsiella* sp. and *Bacillus cereus* increased, they could be recorded in farm D during the rainy season. In contrast, *E*. *coli* was only detected in the Apatlaco river ditch, while *A*. *hydrophila* was only present in farm D (Table 1).

Microbiological analysis of fish

From the microbiological analysis of the organisms, a total of seven bacterial species were identified for the dry season and five for the rainy season, with the greatest diversity in farm B during both seasons (Table 2). Of the total species recorded, only *Enterococcus faecalis* and *Vibrio alginolyticus* were found to be different from those reported in the water analysis.

The highest bacterial counts were recorded in farms A and D, with significant differences as shown in Table 2. These data suggest that there is a direct influence of the microbiota of the water on the bacteria present in the organisms, since during the rainy season, it was observed that as bacterial concentrations of species such as *Bacillus cereus* and *Burkholderia cepacea* were reduced in the water, these were no longer recorded in the fish, and on the contrary, the species *Pseudomonas luteola* and *A. hydrophila* were reported (Table 2).

In relation to *E. coli* and *Klebsiella* sp. species, the microbiological analysis of the fish revealed a reduction in their concentration during the rainy season; the highest concentrations were reported in the organisms collected in farm D, with a statistical difference (p=0.0212 and p=0.0009) with respect to the rest of the units (Table 2).

Table 1. Average values of bacterial counts in water samples

Species	Season	Counts of CFU mL^{-1}					
		Las Estacas ditch	Farm A	Farm B	Farm C	Apatlaco ditch	Farm D
Escherichia coli	D	2.6 ± 0.2	2.4* ±0.2	2.6 ± 0.0	2.8* ± 0.1	3.0 ± 0.3	N/D
	R	2.8 ± 0.0	N/D	2.7 ± 0.5	N/D	2.2 ± 0.2	N/D
Klebsiella sp.	D	3.5* ± 0.1	3.2 ± 0.5	2.6 ± 0.0	2.7 ± 0.2	3.7 ± 0.5	N/D
	R	4.5* ± 0.1	N/D	3.2 ± 0.2	2.7 ± 0.1	4.4 ± 0.1	2.4 ± 0.1
Bacillus thuringiensis	D	3.5* ± 0.3	3.3 ± 0.6	2.5 ± 0.3	2.0* ± 0.0	N/D	N/D
	R	N/D	N/D	N/D	N/D	N/D	N/D
Burkholderia cepacian	D	2.6 ± 0.3	2.5 ± 0.1	2.3 ± 0.3	N/D	3.2 ± 0.5	2.4 ± 0.1
	R	3.0* ± 0.2	N/D	N/D	2.0 ± 0.0	4.4 ± 0.2	2.4 ± 0.1
Bacillus cereus	D	3.2 ± 0.5	2.7 ± 0.0	N/D	3.3 ± 0.4	2.6 ± 0.6	N/D
	R	3.2* ± 0.1	2.3 ± 0.0	2.5 ± 0.0	2.4 ± 0.5	4.2 ± 0.2	4.7 ± 0.1
Burkholderia gladioli	D	2.5 ± 0.2	N/D	N/D	N/D	N/D	N/D
	R	N/D	N/D	N/D	N/D	N/D	N/D
Aeromonas hydrophila	D	N/D	N/D	N/D	N/D	N/D	N/D
	R	3.1* ± 0.5	2.0 ± 0.0	2.3 ± 0.2	2.8 ± 0.1	N/D	2.4 ± 0.1
Vibrio parahaemolyticus	D	N/D	N/D	N/D	N/D	N/D	N/D
	R	2.2 ± 0.3	N/D	N/D	N/D	N/D	N/D
Pseudomonas luteola	D	N/D	N/D	N/D	N/D	2.1 ± 0.2	2.5 ± 0.2
	R	N/D	2.2 ± 0.2	N/D	N/D	3.4 ± 0.3	N/D
Bacillus firmus	D	N/D	N/D	N/D	N/D	2.0 ± 0.0	N/D
	R	N/D	N/D	N/D	N/D	N/D	N/D

D: Dry; R: Rainfall; N/D: Not detected; ± Standard deviation; * Significant difference ($p < 0.05$).

Microbiological analysis of feed

The data obtained from the microbiological analysis of feed are presented in Table 3, which shows the isolation of six bacterial species. Of the total bacteria recovered, *Bacillus megaterium* was only recorded from feed samples, where the highest counts were found in farms C and D, for which a statistical difference (p=0.0272) was determined with respect to the rest of the farms; likewise, *P. luteola* and *B. cereus* were only isolated from samples from farm B during the rainy season.

In general, the analysis of feed from farm D showed the highest CFU mL^{-1} counts, including significant differences with the rest of the production units, as shown in Table 3.

Table 2. Average values of bacterial counts in *Poecillia sphenops* during the analyzed seasons

Species	Season	CFU mL^{-1} counts			
		Farm A	Farm B	Farm C	Farm D
Vibrio alginolyticus	D	2.5 ± 0.2	2.6 ± 0.0	3.2 ± 0.0	4.6* ± 0.2
	R	2.7 ± 0.4	2.7 ± 0.3	N/D	3.5* ± 0.4
Enterococcus faecalis	D	3.4* ± 0.1	2.0 ± 0.0	N/D	N/D
	R	N/D	N/D	N/D	N/D
Escherichia coli	D	4.4 ± 0.0	4.4 ± 0.0	4.8 ± 0.1	4.7 ± 0.5
	R	4.3 ± 0.1	4.2 ± 0.0	4.3 ± 0.0	4.6* ± 0.1
Klebsiella sp.	D	4.4 ± 0.0	4.8 ± 0.0	4.4 ± 0.1	5.0* ± 0.2
	R	4.1 ± 0.0	4.4 ± 0.3	4.2 ± 0.0	4.4 ± 0.0
Bacillus cereus	D	4.4 ± 0.0	N/D	4.4 ± 0.0	4.6 ± 0.2
	R	N/D	N/D	N/D	N/D
Vibrio parahaemolyticus	D	N/D	2.5 ± 0.6	N/D	2.8 ± 0.3
	R	2.3 0.0	3.2* ± 0.2	N/D	2.6 ± 0.0
Burkholderia cepacian	D	4.4 ± 0.0	5.3 ± 0.2	5.7* ± 0.3	5.0 ± 0.1
	R	N/D	N/D	N/D	N/D
Brevibacillus sp.	D	N/D	4.2 ± 0.3	N/D	N/D
	R	N/D	N/D	N/D	N/D
Aeromonas hydrophila	D	N/D	N/D	N/D	N/D
	R	4.9 ± 0.1	4.4 ± 0.0	4.9 ± 0.1	4.9 ± 0.0

D: Dry; R: Rainfall; N/D: Not detected; ± Standard deviation; * Significant difference ($p < 0.05$).

Table 3. Average values of bacterial counts in feed samples during the analyzed seasons

Species	Season	CFU mL^{-1} counts			
		Farm A	Farm B	Farm C	Farm D
Klebsiella sp.	D	2.4 ± 0.6	N/D	2.6 ± 0.2	3.9* ± 0.6
	R	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	2.8 ± 0.2
Bacillus cereus	D	2.3 ± 0.0	2.8 ± 0.3	2.5 ± 0.2	4.4* ± 0.2
	R	N/D	2.8 ± 0.2	N/D	N/D
Burkholderia cepacia	D	2.2 ± 0.2	2.5 ± 0.3	N/D	5.1* ± 0.6
	R	2.8 ± 0.1	N/D	4.0 ± 0.1	4.7* ± 0.2
Bacillus megaterium	D	2.7 ± 0.1	2.6 ± 0.0	3.8* ± 0.3	5.4* ± 0.2
	R	N/D	N/D	N/D	N/D
Aeromonas hydrophila	D	N/D	N/D	N/D	N/D
	R	4.0 ± 0.0	3.2* ± 0.2	3.9 ± 0.2	N/D
Pseudomonas luteola	D	N/D	N/D	N/D	N/D
	R	N/D	3.1 ± 0.2	N/D	N/D

D: Dry; R: Rainfall; N/D: Not detected; ± Standard deviation; * Significant difference ($p < 0.05$).

Determination of coliforms

Figure 1, section a, shows that in the case of the farms supplied with water from Las Estacas ditch, the highest concentration of total coliforms is reported in farm C during the dry season, while during the rainy season there is a reduction in their concentration; in contrast, Apatlaco ditch and farm D show an increasing trend.

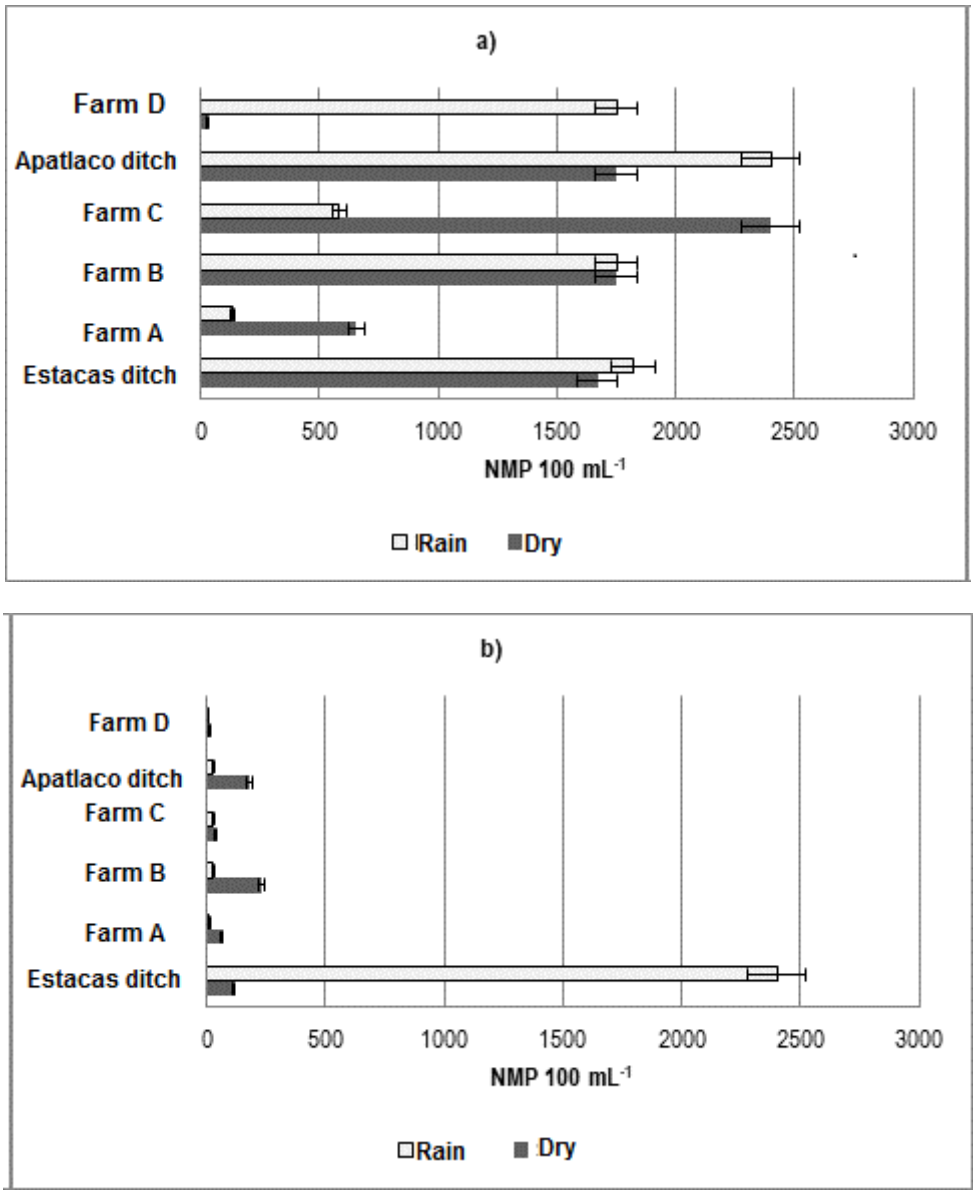
With respect to fecal coliforms, the highest densities were recorded at farm B during the dry season, while Las Estacas canal showed a significant increase (p=0.0482) during the rainy season, which even exceeds the maximum permissible limit (1000 NMP per 100 mL^{-1}) according to NOM-001-ECOL-1996. On the other hand, farm D and Apatlaco river ditch showed a reduction in the bacterial load of this group during the rainy season, with a significant difference for the Apatlaco river ditch (p=0004) (Figure 1, section b).

Physicochemical parameters

The data obtained for the physicochemical parameters are presented in Table 4, which shows that the water temperature remained between 21.9 ºC and 25.7 ºC during the dry season and between 23.2 ºC and 29.5 ºC during the rainy season, with the lowest temperatures recorded in both canals with respect to the farms; a statistical difference was found for Las Estacas ditch during the dry season (p=0.0360). In contrast, the highest temperatures were recorded in farms B and D. In the case of pH, once again the tributaries had the lowest values, while farms D and A had the highest alkalinity with values of 9.8 and 9.6 units during the dry and rainy seasons, respectively.

In general, the concentration of O.D showed a decrease from the dry to the rainy season (Table 4), with the lowest concentration recorded in farm C; while the highest concentration was recorded in farm B during the dry season (10.2 mg L^{-1}) and in farm A during the rainy season (7.9 mg L^{-1}). In no case was there a statistical difference.

Regarding STD, the highest values were reported in farm A during both seasons, even determining a statistical difference in the rainy season (p=0.0007); in contrast, the lowest values were recorded in farm D (0.43 ppt) and in Apatlaco river ditch (0.37 ppt). On the other hand, the nitrogenous forms showed an increasing trend from the dry season to the rainy season in both canals, with the exception of nitrates (NO_3) in Apatlaco ditch. As for phosphorus concentrations, a decreasing trend was observed during the rainy season compared to the dry season; however, the opposite occurred in Las Estacas ditch (Table 4).



* Significant difference ($p < 0.05$)

Figure 1. Mean coliform bacteria count values. a) Total coliforms; b) Faecal coliforms

Table 4. Average values of physico-chemical parameters recorded in water during the stations analysed

Parameter	Season	Las Estacas ditch	Farm A	Farm B	Farm C	Apatlaco ditch	Farm D
Temperature (°C)	D	21.9* ± 1.6	22.6 ± 3.5	24.7 ± 4.5	23.1 ± 0.2	23.4 ± 1.6	25.7 ± 0.2
	R	24.2 ± 1.4	29 ± 0.3	29.2 ± 0.4	28.3 ± 0.3	23.2 ± 1.2	29.5 ± 0.5
pH	D	7.9 ± 0.2	8.16 ± 0.2	8.8 ± 0.4	7.9 ± 0.3	8.4 ± 0.003	9.8* ± 0.4
	R	8.3 ± 0.5	9.6* ± 0.2	8.6 ± 0.1	8.8 ± 0.4	8.2 ± 0.4	9.2 ± 0.001
OD (mg L^{-1})	D	9.1 ± 0.8	9.7 ± 2.7	10.2 ± 1.6	6.6 ± 2.9	8.2 ± 0.01	7.5 ± 1.9
	R	7.1 ± 2.9	7.9 ± 0.4	7.7 ± 1.2	5.5 ± 2.3	7.7 ± 1.1	7.6 ± 1.1
STD (ppt)	D	0.61 ± 0.01	0.64 ± 0.01	0.57 ± 0.01	0.58 ± 0.01	0.51 ± 0.01	0.43 ± 0.0
	R	0.59 ± 0.04	0.78* ± 0.08	0.62 ± 0.04	0.52 ± 0.03	0.37 ± 0.2	0.44 ± 0.06
NH_4 (mg L^{-1})	D	0.26 ± 0.0	0.17 ± 0.02	1.3 ± 0.4	0.21 ± 0.03	0.29 ± 0.2	0.19 ± 0.08
	R	3.21* ± 0.9	0.39 ± 0.1	0.28 ± 0.1	0.62 ± 0.2	0.37 ± 0.09	0.19 ± 0.09
NO_2 (mg L^{-1})	D	0.07 ± 0.03	0.19 ± 0.1	0.07 ± 0.04	0.03 ± 0.01	0.13* ± 0.06	0.04 ± 0.01
	R	0.2 ± 0.1	0.07 ± 0.0	0.12 ± 0.0	0.07 ± 0.1	0.63* ± 0.2	0.02 ± 0.02
NO_3 (mg L^{-1})	D	2.2 ± 1.0	3.55 ± 0.4	0.73* ± 0.9	3.27 ± 0.5	6.85 ± 0.4	2.65 ± 1.2
	R	3.45 ± 1.1	2.57 ± 0.6	0.95 ± 0.2	1.9 ± 0.6	5.3 ± 0.7	0.46* ± 0.8
P (mg L^{-1})	D	0.51 ± 0.25	0.39 ± 0.3	0.15 ± 0.07	0.26 ± 0.07	0.82 ± 0.0	0.56 ± 0.4
	R	0.83 ± 0.5	0.32 ± 0.3	0.05* ± 0.0	0.15 ± 0.1	0.75 ± 0.2	0.4* ± 0.06
DBO_5 (mg L^{-1})	D	3.4 ± 1.8	34.5* ± 0.42	16.7 ± 2.8	6.82 ± 3.1	10 ± 3.1	11.8 ± 3.4
	R	4.5 ± 2.6	17.3 ± 1.8	17 ± 3.7	13.6 ± 2.6	3.8* ± 0.4	8.8 ± 4.7

D: Dry; R: Rainfall; ± Standard deviation; * Significant difference ($p < 0.05$)

Finally, DBO_5 values are presented (Table 4), where the highest concentration was reported in farm A with 34.5 mg L^{-1} during the dry season, showing a significant difference (p=0.001), with respect to the rest of the farms supplied by Las Estacas ditch and the same ditch; as well as a reduction in its concentration (17.3 mg L^{-1}) during the rainy season. On the contrary, the lowest concentration was registered in Las Estacas ditch and in Apatlaco ditch during the rainy season, determining statistical differences for the latter (p=0.0279), with respect to farm D which it supplies.

DISCUSSION

The microbiological results of the present study are similar to those reported by Smith *et al*. (2012), who, based on a metagenomic analysis of aquarium water from ornamental fish. They report the Proteobacteria group as the most representative bacterial species, including the species *A. hydrophila*, *V. alginolyticus* and *V. parahaemolyticus*; bacteria that could be identified in the present study from water samples and from the organisms. These bacteria are considered part of the normal microbiota of aquatic organisms; however, they are classified as opportunistic bacteria, so an increase in their

concentration associated with stress in organisms can cause infectious outbreaks in fish (All-Sunaiher *et al*., 2010, Sahoo *et al*., 2016, Younes *et al*., 2016, Rameshkumar *et al*., 2017). It is worth mentioning that during the rainy season *A. hydrophila* presented the highest frequency of isolation in the organisms, an event that is consistent with what has been reported in other studies when monitoring bacteria present in *Poecillia sphenops* (Carnevia *et al*., 2013; Rajeshwari & Devasree, 2017); however, in this case no damage or lesions were recorded in the organisms.

It is important to keep in mind that during culture cycle's fish are prone to be colonized by bacteria present in their environment, so the microbiological characteristics of the water, pond and feed play an important role on their microbiota (Sivakumar *et al*., 2015; Vasile *et al*., 2017). Results show the impact that the water from the tributaries has on the fish microbiota, which could be attributed to the fact that it does not receive any treatment prior to being used in the units, so the presence of bacteria such as *E. coli* and *Klebsiella* sp. indicated faecal contamination from homeothermic organisms (Tenaillon *et al*., 2010). Such results are in agreement with those obtained by Valenzuela-Armenta *et al*. (2018), who report high concentrations of *E. coli* in water samples obtained from tilapia production ponds in farms in Sonora, Mexico. On the other hand, Njoku *et al*. (2015), from bacteriological analysis of fish production ponds, report *E. coli* bacteria as dominant, which was attributed to the use of organic fertilizers based on animal manure. In the present study this practice could not be confirmed; however, the lack of good sanitary measures in the units is evident, as even the microbiological results of the feed could be caused by cross-contamination due to improper handling.

On the other hand, the *B. firmus* and *B. thuringiensis* species detected in the ponds could be attributed to the fact that the region studied is an agricultural region, and both bacteria are commonly used as biological pathogen control of agricultural importance, so their presence may be the cause of runoff into the ditches (Terefe *et al*., 2009).

In relation to the presence of coliforms and according to the Mexican standards in charge of regulating the quality of effluents from aquaculture activities, all the farms comply with the maximum permitted limits for total and faecal coliforms (NOM-001-ECOL-1996). Although the organisms produced in the farms studied are not for human consumption, it is important to bear in mind that this type of microorganisms could affect the health of the personnel of the units.

Parameters such as temperature and organic matter evaluated in Las Estacas ditch agree with those reported by Gómez-Márquez *et al*. (2013) for the reservoir located in Tepalcingo municipality, Morelos. They report an increase in temperature from the dry season (25 ºC) to the rainy season (28 ºC), as well as an increase in the presence of organic matter in June and July (rainy season). It is due to the transport of organic matter caused by the rains; In addition, they point out that the increase in the water level of the reservoir during the rainy season generates a dilution in the concentration of chemical

parameters; such was the case of the concentrations of ammonium and phosphorus in Apatlaco ditch. In contrast, these parameters increased in Las Estacas ditch, exceeding even the limits allowed by the official standard NOM-001-ECOL-1996, so it is important to mention that during this season, the canal was cleared and although there were rains in the area, the water level was strongly reduced, affecting in turn the farms that are supplied by it.

With respect to the physicochemical parameters reported in the ponds, all were found to be within the stipulations of the standards in charge of regulating the quality of effluents from aquaculture activities (NOM-001-ECOL-1996; Proyecto NOM-089-ECOL-1994). Parameters such as phosphorus, considered a trophic indicator and essential for phytoplankton production, remained below the maximum supported by aquatic organisms (2 mg L^{-1}); as well as below what is considered a process of eutrophication (> 0. 5 mg L^{-1}) (Coldebella *et al*., 2018). Likewise, the concentration of nitrites, which are toxic to fish at concentrations above 1 mg L^{-1} (Ginson *et al*., 2017) remained below the above-mentioned, despite the fact that during the rainy season the farms that depend on Las Estacas ditch did not carry out water exchanges. Finally, the considerable increase in DBO_5 during the dry season in farm A may be a reflection of the management practices employed, since as mentioned by Sipaúba-Tavares *et al*. (2013). Water quality in farms is a function of fish density and feed management, where high feeding rates increase the amount of organic matter, reduce O.D, produce an excess of compounds such as NH_3 and NO_2 and even lead to an increase in bacteria due to the availability of nutrients (Ajayi & Okoh, 2014). In addition, it should be noted that according to the study by Coldebella *et al*. (2018), as the production cycle progresses, the organic matter present is less biodegradable by the bacteria responsible for nutrient removal, so biological decomposition slows down. Considering the above, it is important to consider that if the effluents from aquaculture farms are not managed correctly. There is a significant contribution of organic matter and chemical substances to the receiving water bodies, implying a constant source of pollution; therefore, it is essential to have works that provide prior treatment and constant monitoring of the quality of the discharge water (Boyd, 2003; Velasco-Amaro *et al.*, 2015).

CONCLUSIONS

The results of the present study determined that the water quality of Las Estacas ditch and Apatlaco river ditch is altered, which has a significant impact on the sanitary quality of the organisms produced in the APU's; nevertheless, the farms comply with the official Mexican standards in charge of regulating the effluents derived from the aquaculture activity. However, it is important to implement sanitary measures in the units in order to reduce production risks, guarantee the quality of the product and thus increase its value, as well as to take care of the health of the personnel and the surrounding environment.

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Errata Erratum

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