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Effect of Fish Farming Practices on the Biodiversity of Bacterial Intestinal Flora of the Common Carp (*Cyprinus carpio*) in Ponds

Sylvia Winifred Dembeng Yemelong[1*], Berlin - Léclair Songmo[1], Georges Fonkwa[3], Algrient Towa Nana[2], Thomas Ewoukem Efole[2]

[1]Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, BP 222, Dschang Cameroon
[2]Department of Forestry, Faculty of Agronomy and Agricultural Sciences, University of Dschang, BP 222, Dschang Cameroon
[3]Department of aquaculture, Institute of Fisheries and Aquatic Sciences, University of Douala, Cameroon
[*]**Corresponding author: sylviayemelong@gmail.com**

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ABSTRACT

Therapeutic failures and persistent bacterial diseases in fish farming are linked to the complex intestinal microbiota in fish. Understanding both beneficial and pathogenic bacteria can aid in diagnostics and inform farming practices. This study, conducted in East Cameroon's bimodal forest agro-ecological zone, examined the intestinal bacterial diversity in *Cyprinus carpio* from 15 ponds practicing unfertilized, composting, and piscirice culture. Intestinal contents were analyzed using microbial cultures and biochemical tests. Fifteen bacterial species were identified, including *Salmonella arizonae*, *Escherichia coli*, *Proteus mirabilis*, and *Shigella* spp., with pathogenic species more common in compost ponds. Beneficial species were more abundant in piscirice culture ponds. A positive correlation between bacterial abundance and ammonium levels in water was observed. These findings suggest that farming practices influence bacterial diversity in common carp. To reduce contamination risks, minimizing in-pond composting is recommended.

INTRODUCTION

Bacteria are unicellular organisms that possess elements necessary for cellular life. The comfort or discomfort of fish results from the dynamic equilibrium between the different populations of commensal or transient bacteria in its intestinal microbiota. Several studies highlight the important role of the commensal intestinal bacterial flora in fish physiology. It aids in enzymatic digestion, particularly of fibers, modulates the digestive epithelium's nutrient absorption capacity, supplies vitamins K and B12, and has the ability to degrade certain anti-nutritional factors in the diet **(Ray *et al.*, 2012; Buffie & Pamer, 2013; Borey, 2017)**.

However, despite the zoo-sanitary control carried out on fish farms for several years, fish develop several bacterial diseases during their life cycle, some of which have

little or no known etiology **(Kara *et al.*, 2016; Maatouk *et al.*, 2023)**. Some of these diseases are transmissible to fish consumers and can thus constitute a public health problem **(OMS, 2013)**. Means of control such as vaccination by immersion and injection, antibiotic therapy and annual updating of the zoo-sanitary code for aquatic animals through epidemiological disease surveillance have been deployed to fight and prevent bacterial infections in farming **(Kinkelin *et al.*, 2014)**. However, their impact remains a cause for concern since they account for a significant proportion of production costs, alongside the supply of fry and feed **(Kara *et al.*, 2016)**. Strains of *Flavobacterium columnare*, a Gram-negative bacterium, are responsible for huge economic losses in trout aquaculture **(Evenhuis *et al.*, 2021)**. Furunculosis is also a freshwater bacterial disease found in all salmonids, with lesions and symptoms comparable to those of vibriosis, but only recognition and identification of the germ make diagnosis possible **(Hountcheme, 2021)**. This problem seems to be intensified by the complexity of the richness and specific diversity of the intestinal bacterial flora, which reflects the fish's living environment.

However, changes in the composition of the intestinal bacterial flora evolve according to parameters such as water quality, diet, overuse of antibiotics, **(Buffie & Pamer, 2013)** and bacterial bio-aggressors, which alone or in combination can infect fish **(Sylvain *et al.*, 2022)**.

Several authors have demonstrated the modification of bacterial flora composition, blaming exogenous factors. This is the case of **Borey (2017)**, who has shown that modification of the microbiota associated with the digestive mucosa of the rainbow trout following a plant-based diet reduces digestion performance and consequently its growth performance. Similarly, **Yemelong *et al*. (2020)** also found that feed type influences the composition of the bacterial gut flora and growth performance in common carp (*Cyprinus carpio*). Like other farmed fish species that have shown changes in gut bacterial composition as a function of water characteristics and feed type, common carp is one of the three most farmed species worldwide **(FAO, 2009)** under different farming systems and polyculture.

However, few studies have examined the composition of its intestinal bacterial flora. The common carp is an omnivorous species that feeds on plant debrits, the digestion of which is facilitated by bacterial enzymes. It has no stomach cavity and contains neither hydrochloric acid nor the enzymatic equipment (pepsin) required for food digestion. Thus, entero-pancreatic digestion remains the only option for common carp. This work was therefore initiated to help optimize fish production by gaining a better understanding of the effects of fish farming practices on the diversity of intestinal bacterial flora in *Cyprinus carpio*.

MATERIALS AND METHODS

1. Period and geo-climatic characteristics of the study area

The study was carried out from January to December 2023 in the bimodal rainfall forest agro-ecological zone, East Cameroon region and more precisely in the Mandjou (13°62'-13°05'N; 14°08'-14°05'E), Bertoua 1er (04°34'-04°30'N; 13°41'-13°04'E) and Diang (04°35'-04°00'N; 13°21'-04°00'E) subdivisions. This zone covers an area of 109,002 km² with an average annual rainfall of 2,149mm. It has a humid tropical climate with four seasons: a long dry season (November to March), followed by a short rainy season (March to June), a short dry season (June to August), and a long rainy season (August to November). The average temperature is 24.5°C **(INC, 2015)**. The soils are ferralitic, acidic, and clayey.

2. Animal material

A total of 18000 common carp fingerlings (unsexed) with mean weight 40 ± 2.22g and mean total length 13.04 ± 1.08cm were used in this study. These fish were obtained from semi-artificial reproduction in fish farmers' ponds. Sample size was calculated using Lorenz's formula.

$$N = \frac{t^2 \times p \times (1-p)}{m^2}$$

Where, N is the sample size; P the assumed prevalence; t the confidence level (the standard value of the 95% confidence level considered was 1.96); and m is the margin of error set at 5%. This gave us a predicted sample size N equal to 24 fish per farming practice. For a considered prevalence of 20% **(D'amico *et al*., 2018)**.

3. Farming structure

The study was carried out in fifteen (15) fish ponds with an average surface area of 1200 ± 150.25m², an average depth of 1.27 ± 0.26m and fed by streams running through each locality. These ponds were selected on the basis of their morphological and functional characteristics, in agreement with the fish farmers for production monitoring. The different treatments used in this study were: Unfertilization of the ponds (NF), fertilization through the intra-pond compost plant (CP), which occupied 10% of the total pond surface area, and the integration of rice cultivation (PR).

4. Conducting the study

An agreement with the fish farmers made it possible to carry out the sanitary clean-up, which involved refitting the selected ponds prior to the start of the study. This involved drying out the ponds for a week in order to disinfect them, followed by impounding the various ponds for fish production. The composter consisted of fresh kitchen waste (cocoyam and banana peelings), fresh plants (*Chromolena odorata* and *Tithonia diversifolia* leaves) and chiken manure at a rate of 1500kg/ha/month **(Coche, 1997)**. An input of 1500kg/ha/month of kitchen waste (50%) and plants (50%) was applied to each compost pond. The waste was stirred once a week to facilitate mineral release. The pisci-riceculture ponds received rice seedlings (NERICA 56 variety)

previously produced in the growers' nurseries and occupying 1/3 of the total pond area. The spacing between the rice plants was 40cm to facilitate fish circulation. Once the ponds had been rehabilitated, stocking was carried out with common carp fingerlins averaging 40 ± 2.22g, at a density of 1 individual/m².

5. Data collection and characteristics studied

The main data collected in this study were the bacteriological characteristics of the intestinal contents and the physicochemical characteristics of the water.

The physico-chemical characteristics of the water in the various ponds were measured *in situ* between 06:00 and 08:00 each month using the methodology recommended by **Rodier *et al.* (2009)**. These included transparency, temperature, dissolved oxygen, hydrogen potential and nitrite, nitrate, ammonium and phosphate, measured respectively using a Secchi disk, a pHep multi-parameter, an Oxy-thermometer and VISCOLORECO® test kits.

The bacteriological characteristics of the intestinal contents were obtained on one hundred and twenty (120) common carp collected from the ponds using fishing nets during the study, i.e. 30 fish per quarter at a rate of 10 fish per fishing practice. The common carp samples were placed in sterile plastic bags on which were written: the day of collection, the sample number and the name of the fish farming practice. They were packed in a cool box at a temperature of 4°C and were transported to the Laboratoire National Vétérinaire annexe in Yaoundé. Once at the laboratory, the common carp collected and conditioned were examined using **Damergi's (2007)** methodology. The fish were dissected using a pair of scissors and a sterile knife, taking care to highlight the digestive system. Sampling and microbiological analysis of gut contents were carried out according to the methodology proposed by **Shoemark *et al.* (2001)** and **Damergi (2007)**, as follows:

6. Collection of intestinal contents

Intestinal contents were collected after dissection and opening of the common carp intestine using a sterilized scalpel. Intestinal contents were placed in sterile 5ml Ependorfs tubes containing 50% diluted glycerol and stored at -20°C.

Microbiological analysis of intestinal content samples

Bacterial cultures were cultured for bacterial research and identification following the methodology recommended by **Guillot *et al.* (1998)**:

❖ **Preparation and casting of culture media**

EMB agar, MAC CONKEY agar, SS agar, nutrient agar, Chapman agar and fresh blood agar were prepared.

❖ **Sample plating and incubation**

Samples were streaked onto the various culture media and were autoclaved at 37°C for 24 hours.

❖ **Identification of bacterial colonies**

Bacterial colonies grown on the culture media were identified on the basis of their morphological and color aspects. Each colony was then transferred to nutrient agar and was incubated at 37°C for 24 hours. Non-isolated colonies were subcultured again until complete isolation. Gram staining was then performed.

❖ **Gram staining**

The Gram stain developed by Hans Christian Gram in 1884 was used to categorize bacteria as Gram-positive or Gram-negative **(Li *et al.*, 2020)**. In principle, after observation under the microscope, Gram-positive bacteria retain their violet stain, while Gram-negative bacteria are bleached by alcohol and stained more or less bright red or pink by fuchsin. Biochemical tests were then carried out to identify the different bacterial species. The Api 20 E macro and micro galleries were used for enterobacteria **(Camille, 2007)**.

❖ **Biochemical tests on macro gallery**

Biochemical tests namely catalaze, oxidaze, glucose, lactose, H2S; gas and urea／indole were carried out.

❖ **Tests on the Api 20 E microgallery**

Inocula of 5ml of each colony were obtained after introduction of a few colonies into 5ml of physiological water at a density of between 45 and 50. The dishes in which the Api 20 E plates were placed were pre-filled with distilled water, taking care to fill all the wells. The Api 20 E plates were placed in each dish, and the inocula were introduced and incubated for 24h at 37℃. Reading was done by observing color changes and also after addition of Covax; TDA; VP1 and VP2 in the corresponding tubes. A specific code for a bacterium was obtained after addition of the values mentioned according to the color variation on the reading sheet **(BioMérieux, 2010; Kari & Laifaoui, 2013)**.

The characteristics studied related to bacterial diversity (relative abundance of different bacterial species) and the growth of common carp (weight).

7. Characteristics of bacterial diversity

Diversity indices were calculated using the following formulas:

❖ **Relative abundance of different bacterial species**

This is the number of individuals of a given species per unit of surface area or volume, relative to the total number of individuals of all species combined. It was evaluated as follows:

$$A = Ni/Nt \times 100$$

Where, *Ni* = number of individuals of the given species and *N* = total number of individuals.

The classification of infection prevalence used by **Fonkwa *et al.* (2020)** was adapted to relative abundance, which was classified as very low (A< 10%), low (10% ≤ A ≤ 50%) and high (A > 50%).

❖ **Simpson index**

Measures the probability that two randomly selected individuals in a stand belong to the same species. It was calculated using the following formula:

$$C = \sum Ni(Ni - 1)/N\ (N - 1)]$$

Where, *Ni*= number of individuals of a given species and *N*= total number of individuals

❖ **Shannon & Weaver diversity index (H')**

H' was used to estimate the taxonomic diversity of species making up the bacterial population in intestinal contents. It was determined as follows:

$$H' = \sum[(ni / N) \times \log 2\ (ni / N)]$$

Where, *H'* = specific diversity, in bits/individual; ∑= sum of results obtained for each species present; *ni* = number of species I; *N*= total number of individuals considering all species; and *log2*= logarithm to base 2.

❖ **Equitability index or Pielou's J index**

Equitability index was used to measure the distribution of species in the intestinal contents in relation to a theoretical equal distribution for all species **(Barbault, 1995)**. Equitability was obtained by the following formula:

$$J = H'/\log 2S$$

Where, *H'*= Shannon and Weaver diversity index, *log2* = logarithm to base 2, and *S* = number of species present.

Statistical analysis

The characteristics studied were related to bacterial diversity (diversity index and relative abundance), farm water physico-chemistry and carp weight. They were subjected to descriptive statistical analysis. Analysis of variance (ANOVA) was used to compare the values of these characteristics as a function of fish farming practice. In the event of a significant difference, i.e. a probability of error (*P*) of less than 0.5, the means were separated using Duncan's test. Pearson's coefficient was used to determine the correlations between the various characteristics. Statistical Packages for Social Sciences (version 18) was used for these analyses.

RESULTS

1. **Variation in water physic-chemical characteristics as a function of fish farming practices**

The effect of fish farming practices on the physico-chemical characteristics of the water is summarized in Table (1). It shows that the values of physic-chemical characteristics varied according to fish farming practices. Temperature, pH and nitrite values were significantly ($P<0.05$) higher in ponds with a composter compared to other pond types. On the other hand, dissolved oxygen values were significantly ($P<0.05$) higher in unfertilized ponds compared to other pond types. However, no significant

differences (P>0.05) were recorded for transparency, nitrates and ammonium regardless of the fish farming practice used.

Table 1. Mean and standard deviations of water physic-chemical characteristics according to fish farming practices

Water physico-chemical characteristics	Fish farming practices			P
	Unfertilized ponds (n=5)	Compost ponds (n=5)	Pisciriceculture ponds (n=5)	
Transparency (cm)	38.00 ±2.73[a]	35.00 ±6.12 [a]	36.60 ±6.69[a]	0,69
Temperature (°C)	25.98 ±1.13[b]	27.70 ±0.44 [a]	26.54 ±1.26 [ab]	0.05
pH (UI)	7.06 ±0.35[ab]	7.40 ±0.38[a]	6.69 ±0.43[b]	0.04
Dissolved oxygen (mg/l)	6.85 ±1.32 [a]	4.54 ±0.41[b]	6.04 ±1.63 [ab]	0.03
Nitrites (mg/l)	0.02 ±0.00 [b]	0.04 ±0.01 [a]	0.02 ±0.01 [b]	0.05
Nitrates mg/l)	3.40 ±0.89[a]	3.80 ±1.30 [a]	2.20 ±1.09 [a]	0.10
Ammonium (mg/l)	0.24 ±0.54 [a]	0.30 ±0.07 [a]	0.24 ±0.54 [a]	0.23
Phosphates (mg/l)	0.22 ±0.44 [a]	0.20 ±0.44 [a]	0.20 ±0.00 [a]	0.39

a, b: means marked with the same letter on the same line do not differ significantly at the 5% threshold; n = number of ponds according to fish farming practices.

2. Biodiversity of intestinal bacterial flora in common carp as a function of fish farming practices

Bacterial species classified by phylum according to different fish farming practices are summarized in Table (2). A total of 15 bacterial species were identified, of which 92.30% (13 species) belong to the Proteobacteria *phylum* and 15.38% (02 species) to the Firmicutes *phylum*.

Table 2. Bacterial species classified by phylum according to fish farming practices

Phyum	Bacterium	Fish farming practices		
		Unfertilized ponds (n=40)	Compost ponds (n=40)	Pisciriceculture ponds (n=40)
Protéobactéria	***Salmonella arizonae***	+	+	+
	***Shigela* spp.**	+	+	+
	Escherichia coli	+	+	+
	Proteus Mirabilis	+	+	+
	Klebsiella pneumonae	+	+	+
	Klebsiella Ozaenae	+	+	+
	Citrobacter Freundii	+	+	+
	Enterobacter cloacae	+	+	+
	Enterobacter agglumerans	+	+	+
	Enterobacter sakazaki	+	+	+
	Pseudomonas cipacia	+	+	+
	Pseudomonas luteola	+	+	+
	Serratia ordifera	+	+	+
Firmicute	***Staphylococcus aureus***	+	+	+
	Streptococcus inae	+	+	+

+ = Present; - = Absent, n = number of samples.

3. Nature and relative abundance of bacteria in the intestinal flora of common carp as a function of fish farming practices

The distribution and relative abundance of bacteria in the intestinal flora of common carp varied according to fish farming practices. Table (3) shows that 60% of the identified bacteria were beneficial, while 40% were pathogenic, regardless of the farming practice.

The relative abundances of gut bacterial species in common carp also varied depending on the farming practice (Table 3). In general, pathogenic bacteria were more abundant (65.11%) in the gut of the common carp reared in compost ponds, while beneficial bacteria were more abundant (52.40%) in carp reared in unfertilized ponds (control).

Regarding beneficial species, *Escherichia coli* was not only the most abundant in the gut of the common carp, regardless of farming practice, but it was also more abundant in carp reared in unfertilized ponds.

Pathogenic species such as *Salmonella arizonae*, *Shigella* spp., *Klebsiella pneumoniae*, *Klebsiella ozaenae*, and *Streptococcus inae* were more abundant in the gut flora of common carp reared in compost ponds. In contrast, *Citrobacter freundii*, *Pseudomonas cepacia*, and *Pseudomonas luteola* were more abundant in the common carp raised in piscirice culture ponds.

Table 3. Distribution of the nature and relative abundance of intestinal bacteria species according to fish farming practices

Bacterium		Fish farming practices		
		Unfertilized (n=40)	**Compost (n=40)**	**Pisciriceculture (n=40)**
Pathogens	***Salmonella arizonae***	2.7±2.21	10.9±1.93	9.9±3.90
	***Shigela* spp.**	1.3±1.08	4.04±1.15	3.45±1.91
	Streptococcus inae	1.22±0.25	2.63±1.03	0.93±0.03
	Staphylococcus aureus	17.97±6.08	13.77±6.08	14.05±5.32
	Klebsiella pneumonae	7.18±2.56	14.10±3.31	6.12±1.06
	Klebsiella Ozaenae	6.11±2.77	17.00±3.30	1.84±0.10
	Citrobacter Freundii	5.05±0.94	2.53±0.45	2.67±1.16
	Pseudomonas cipacia	1.43±0.63	0.11±0.03	7.15±1.34
	Pseudomonas luteola	4.63±0.95	0.03±0.01	4.94±1.15
Useful	***Serratia ordifera***	0.28±0.03	0.05±0.01	0.52±0.02
	Proteus Mirabilis	11.98±4.58	7.53±1.37	7.89±1.94
	Enterobacter agglumerans	8.45±1.69	1.81±0.37	11.31±4.41
	Enterobacter cloacae	2.39±1.70	11.08±3.31	3.96±1.37
	Enterobacter sakazaki	0.10±0.03	2.64±0.29	3.93±2.22
	Escherichia coli	29.20±5.61	13.07±3.08	20.04±6.91

n = Number of samples.

4. Characteristics of specific bacterial diversity in common carp (*Cyprinus carpio*) as a function of fish farming practices

Bacterial diversity characteristics were influenced by fish farming practices (Table 4). Simpson's and Piélou's equitability index values were significantly higher ($P< 0.05$) in composting ponds and lowest in unfertilized ponds. However, for the Shannon index, no significant difference ($P> 0.05$) was observed, regardless of the fish farming practice considered.

Table 4. Characteristics of gut bacterial species diversity as a function of fish farming practices

Diversity index	Fish farming practices			*P*
	Unfertilized ponds (n=5)	**Compost ponds (n=5)**	**Pisciriceculture ponds (n=5)**	
Simpson_1-D	0.84 ± 0.02^{b}	0.90 ± 0.02^{a}	0.88 ± 0.01^{a}	0.00
Shannon_H	2.15 ± 0.11^{a}	2.41 ± 0.14^{a}	1.78 ± 0.83^{a}	0.17
Equitability_J	0.80 ± 0.04^{b}	0.89 ± 0.05^{a}	0.88 ± 0.03^{a}	0.01

a, b: means marked with the same letter on the same line do not differ significantly at the 5% threshold according to the different breeding practices.

5. Correlation between bacterial abundance and physico-chemical water characteristics as a function of fish farming practices

Table (5) summarizes the correlations between bacterial abundance and water physico-chemical characteristics. It shows that water transparency in unfertilized ponds and pisciriziculture ponds was negatively correlated ($P< 0.05$) with the development of *Staphylococcus aureus*. While *Enterobacter sakazaki, Citrobacter Feundii* were negatively correlated ($P< 0.05$) to water transparency only in pisciriceculture ponds. Temperature was negatively correlated ($P< 0.05$) with *Salmonella arizonae* and *Staphylococcus aureus* in unfertilized ponds and with *Streptococcus inae* in pisciriceculture ponds. pH was positively correlated ($P< 0.05$) to *Salmonella arizonae*, *Enterobacter agglumerans*, *Shigella* spp. in pisciriceculture ponds while *Enterobacter cloacae* was negatively correlated ($P< 0.01$) in unfertilized ponds. Oxygen was negatively correlated ($P< 0.05$) with *Citrobacter feundii*, *klebsiella pneumonia* and *Staphylococcus aureus* in pisciriceculture ponds. *Streptococcus inae* was negatively correlated ($P< 0.05$) with nitrates, while with phosphates, it was positively correlated in unfertilized ponds. Positive correlations were recorded in *Pseudomonas cipacia, Klebsiella pneumonia, Serratia ordifera, Klebsiella ozaenae* ($P< 0.01$) and *Enterobacter sakazaki* ($P< 0.05$) with ammonium in pisciriceculture ponds.

Table 5. Correlation between bacterial abundance and water physico-chemical characteristics as a function of fish farming practices

		Physico-chemical water characteristics							
Bacteria species		**Transp (cm)**	**T (°C)**	**pH (UI)**	**O_2 (mg/l)**	**NO_2^- (mg/l)**	**NO_3^- (mg/l)**	NH_4^+ (mg/l)	**PO_4^{3-} (mg/l)**
Unfertilized ponds	*Shigella* spp.	-0.78	-0.81	-0.08	-0.58	-0.15	+0.06	-0.32	+0.21
	Salmonella arizonae	-0.87	**-0.89***	-0.21	-0.54	-0.23	-0.01	-0.44	+0.26
	Enterobacter cloacae	-0.70	-0.59	**-0.97****	-0.11	+0.22	-0.87	-0.56	**+0.97***
	Escherichia coli	+0.45	+0.43	-0.14	+0.59	-0.22	-0.08	-0.03	-0.23
	Protéus Mirabilis	+0.24	+0.25	-0.45	+0.54	-0.11	-0.35	-0.18	+0.09
	Citrobacter Feundii	+0.16	+0.21	-0.59	+0.49	+0.05	-0.54	-0.21	+0.32
	Pseudomonas cipacia	-0.82	-0.73	-0.64	-0.46	+0.29	-0.60	-0.41	+0.85
	Enterobacter agglumerans	+0.40	+0.35	-0.09	+0.57	-0.35	+0.04	-0.08	-0.33
	Pseudomonas lutéola	-0.33	-0.37	-0.57	+0.29	-0.48	-0.25	-0.57	+0.10
	Klebsiella pneumonia	+0.70	+0.62	**+0.99****	+0.03	-0.07	+0.82	+0.64	-0.86
	Serratia ordifera	-0.76	-0.69	-0.46	-0.53	+0.29	-0.46	-0.31	+0.74
	Klebsiella ozaenae	+0.65	+0.66	-0.03	+0.61	-0.02	-0.08	+0.16	-0.22
	Enterobacter sakazaki	+0.21	+0.34	+0.04	-0.17	+0.74	-0.31	+0.39	+0.42
	Streptococcus inae	-0.51	-0.37	**-0.89***	-0.08	+0.43	**-0.91***	-0.38	**+0.99****
	Staphylococcus aureus	**-0.98****	**-0.96****	-0.78	-0.30	-0.18	-0.50	-0.72	+0.66
Compost ponds	*Shigella* spp.	-0.60	-0.49	+0.24	+0.43	+0.25	+0.16	-0.42	-0.15
	Salmonella arizonae	-0.82	-0.44	-0.09	+0.38	+0.05	+0.05	-0.59	-0.39
	Enterobacter cloacae	-0.50	-0.60	+0.37	+0.57	+0.05	-0.07	-0.16	-0.38
	Escherichia coli	-0.50	-0.38	+0.25	+0.32	+0.41	+0.31	-0.45	+0.08
	Protéus Mirabilis	-0.65	-0.13	-0.23	+0.04	+0.34	+0.36	-0.74	+0.10
	Citrobacter Feundii	-0.25	-0.58	+0.63	+0.56	+0.24	+0.05	+0.00	-0.11
	Pseudomonas cipacia	+0.45	+0.58	-0.26	-0.58	+0.24	+0.32	+0.00	+0.65
	Enterobacter agglumerans	-0.14	+0.50	-0.72	-0.54	+0.07	+0.26	-0.49	+0.28
	Pseudomonas luteola	+0.45	+0.58	-0.26	-0.58	+0.24	+0.32	+0.00	+0.65
	Klebsiella pneumonia	+0.15	-0.52	+0.80	+0.55	+0.05	-0.16	+0.46	-0.17
	Serratia ordifera	+0.26	+0.55	-0.36	-0.57	+0.27	+0.37	-0.19	+0.62
	Klebsiella ozaenae	-0.07	-0.55	+0.77	+0.54	+0.28	+0.06	+0.15	-0.01
	Enterobacter sakazaki	+0.45	+0.38	+0.12	-0.40	+0.55	+0.51	-0.01	+0.87
	Streptococcus inae	+0.17	+0.46	-0.26	-0.50	+0.41	+0.47	-0.28	+0.69
	Staphylococcus aureus	-0.52	-0.57	+0.18	+0.56	-0.26	-0.32	-0.07	-0.69
Pisciriceculture	*Shigella* spp.	-0.04	+0.54	**+0.97****	+0.12	-0.07	+0.07	+0.13	-0.13
	Salmonella arizonae	+0.07	+0.63	**+0.97****	+0.24	-0.11	+0.11	+0.01	-0.07
	Enterobacter cloacae	-0.10	-0.31	-0.78	-0.16	+0.42	-0.42	-0.26	+0.04
	Escherichia coli	-0.29	-0.43	+0.27	-0.39	-0.35	+0.35	+0.80	-0.18
	Protéus Mirabilis	-0.77	-0.29	+0.51	-0.72	+0.34	-+0.34	+0.84	-0.43
	Citrobacter Feundii	**-0.96****	-0.60	-0.18	**-0.95***	+0.80	-0.80	+0.67	-0.44

ponds	*Pseudomonas cipacia*	-0.72	-0.59	+0.20	-0.78	+0.12	-0.12	**+0.97****	-0.37
	Enterobacter agglumerans	+0.23	+0.81	**+0.95***	+0.43	-0.10	+0.10	-0.24	+0.00
	Pseudomonas luteola	-0.58	-0.18	+0.61	-0.54	+0.11	-+0.11	+0.78	-0.35
	Klebsiella pneumonia	-0.82	-0.86	-0.22	**-0.93***	+0.28	-0.28	**+0.96****	-0.37
	Serratia ordifera	-0.62	-0.86	-0.20	-0.78	-0.02	+0.02	**+0.96****	-0.29
	Klebsiella ozaenae	-0.77	-0.78	-0.06	-0.87	+0.18	-0.18	**+0.99****	-0.37
	Enterobacter sakazaki	**-0.88***	-0.45	+0.32	-0.86	+0.45	-0.45	**+0.90***	-0.47
	Streptococcus inae	-0.38	**-0.96****	-0.57	-0.61	-0.20	+0.20	+0.78	-0.13
	Staphylococcus aureus	**-0.97****	-0.50	+0.19	**-0.94***	+0.64	-0.64	+0.85	-0.49

Trans: transparency, T: temperature, pH: hydrogen potential, O_2: dissolved oxygen, NO_3-: nitrate, NH_4^+: ammonium, PO_4^{3-}: total phosphate. * : significant correlation $P < 0.05$ (two-tailed),
*. Correlation is significant at the 0.05 level (two-tailed). **. Correlation is significant at the 0.01 level (two-tailed).

6. Evolution of bacterial abundance as a function of common carp weight classes and fish farming practices.

The results in Fig. (1) show the evolution of bacterial load based on common carp weight classes and fish farming practices. The findings are as follows:

- At 40g, the load of beneficial bacteria is higher than that of pathogenic bacteria in all fish farming practices.
- Between 40 and 240g, the load of beneficial bacteria remained stable in unfertilized ponds, while the load of pathogenic bacteria increased. Ponds with composting and piscirice culture facilities exhibited identical and higher loads of both beneficial and pathogenic bacteria compared to unfertilized ponds.
- Between 240 and 440g, the load of both beneficial and pathogenic bacteria increased, with a higher load of pathogenic bacteria in unfertilized ponds. Ponds with composting and piscirice culture facilities maintained identical and stable loads of both beneficial and pathogenic bacteria.
- Between 440 and 640g, the loads of beneficial and pathogenic bacteria remained stable in ponds with composting and piscirice culture facilities.
- Between 640 and 840g, the loads of beneficial and pathogenic bacteria increased and were identical in piscirice culture ponds. In contrast, the loads of beneficial and pathogenic bacteria remained stable in compost ponds.
- From 840 to 1040g, the load of pathogenic bacteria decreased and the load of beneficial bacteria increased in piscirice culture ponds. In compost ponds, both pathogenic and beneficial bacteria loads increased, with a higher load of pathogens.
- Between 1040 and 1240g, the load of pathogenic bacteria increased, while the load of beneficial bacteria decreased in compost ponds.

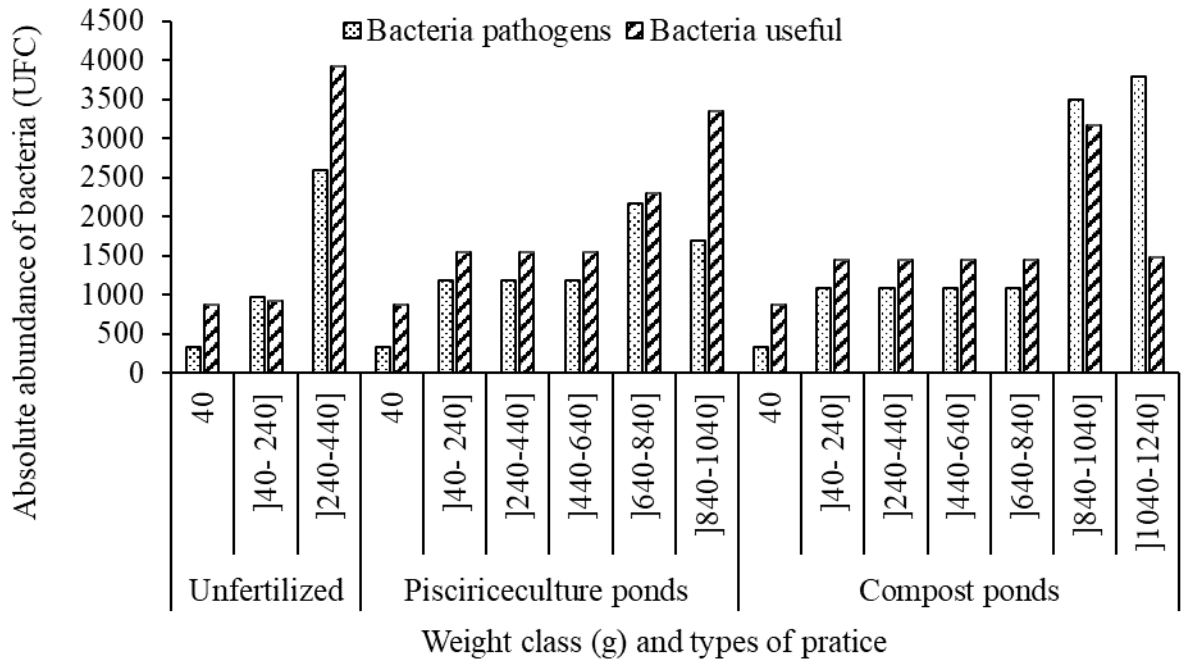

Fig. 1. Evolution of bacterial abundances as a function of common carp weight classes and fish farming practices

DISCUSSION

The intestinal microbiota of common carp is mainly composed of bacteria belonging to the Firmicute and Proteobacteria phyla. Its composition evolves according to parameters such as water quality or temperature and feeding **(Buffie & Pamer, 2013)**. A better understanding of the different intestinal bacterial populations as a function of fish farming practices helps maintain digestive comfort and health for fish and consumers alike.

Values of water physic-chemical characteristics varied within the tolerance limit of common carp depending on fish farming practices **(Boyd, 2012)**. Temperature, pH and nitrite values were significantly ($P< 0.05$) higher in ponds with composting compared to other fish farming practices with reduced dissolved oxygen levels. This could be explained by the solar heating of open ponds and the decomposition of organic matter of plant and animal origin present in the composter favoring the multiplication of thermophilic bacteria **(Knud-Hansenap & Batterson, 1994; Baccarin & Camargo, 2005)**. On the other hand, dissolved oxygen values were lowest in these ponds compared with other pond types, with a value (4.54mg/ l) below the norm of ≥ 5mg/ l. This could be explained by the rise in temperature, which would have increased the metabolic rate in common carp, and consequently increased tissue oxygen demand **(Franklin *et al.*, 1995; El-Sayed, 2006)**. **Daskalov *et al.* (2014)** demonstrated in their trial that carp subjected to a reduced concentration of dissolved oxygen (2.41 ± 0.33mg/ l) for four days showed

higher levels of cortisol than unstressed fish, which favored the development of *Salmonella*.

A total of fifteen (15) bacterial species were identified, irrespective of fish farming practices, of which 92.30% (13 species) belong to the Proteobacteria phylum and 15.38% (02 species) to the Firmicutes phylum. Their presence is explained by their role in maintaining the balance of intestinal homeostasis (Firmicutes) and acting as a barrier against pathogens for commensal ones (Proteobacteria). This result is similar to that obtained by **Desai *et al.* (2012)** who demonstrated that Firmicutes and Proteobacteria were most frequently detected in all samples of the rainbow trout (*Oncorhynchus mykiss*) gut microbiota. Furthermore, **Ghanbari *et al.* (2015)** demonstrated that Proteobacteria in addition to Firmicutes make up over 90% of the fish gut microbiota in different species studied. **Lynch *et al.* (2016)** supported these results by demonstrating that the microbiota of fish distal gut contents and mucosa is composed of bacteria belonging predominantly to these phyla. **Vernocchi *et al.* (2016)** demonstrated that Firmicutes are the main bacteria involved in the metabolism of short-chain fatty acids important in fish digestion.

In general, pathogenic bacterial species were more abundant (65.11%) in the gut of common carp reared in compost ponds, while beneficial bacteria were more abundant (52.40%) in carp reared in unfertilized ponds (control). This could be explained by the fact that chicken manures are composed of both mineral elements (carbon, nitrogen, phosphorus, etc.) and organic matter and bacteria **(Wohlfarth & Schroeder, 1979; Schlumberger, 2002)**. As far as useful species are concerned, *Escherichia coli* was not only the most abundant in the gut of the common carp, regardless of fish farming practice, but was also more abundant in carp reared in unfertilized ponds. This could be justified because it represents an important part of the indigenous microbiota in fish, and is also a major reservoir of resistance genes that can be responsible for treatment failures in veterinary medicine. Bioaccumulations of *Escherichia coli* (log10 CFU/ml) in fish from their growing waters in earthen ponds ranged from 0.94 to 2.65 **(Olalemi *et al.*, 2023)**.

Pathogenic species such as *Salmonella arizonae, Shigella* spp., *Klebsiella pneumonae, Klebsiella Ozaenae* and *Streptococcus inae* were more abundant in the intestinal flora of common carp raised in ponds with a compost bin. This could be explained by the fertilization of these ponds with chicken manures, which generally contain these bacteria and would have contaminated the water **(Elgroud *et al.*, 2008)**. *Salmonella* has been detected in carp due to water contamination **(Daskalova *et al.*, 2014)**. *Salmonella* spp. were isolated from six pools of chicken droppings collected from 24% of farms by **Abdellah Chaiba *et al.* (2016)**. **Hudecová *et al.* (2010)** demonstrated that *Salmonella enteritidis* has shown the ability to penetrate and persist in the tissues of live common carp, which may reinforce our interpretations. Our results corroborate those of **Tshinyama *et al.* (2018)**, who obtained a predominance of *Salmonella* spp. (8.3%) followed by *Shigella* spp. (6.5%) and *Streptococcus* spp. (0.9%) in the gut of the Nile

tilapia reared in ponds fertilized with poultry droppings. **Budiati *et al.* (2011)** isolated *Salmonella* (20%) from the intestines of catfish. In contrast, *Citrobacter freundii, Pseudomonas cepacia* and *Pseudomonas luteola* were more abundant in common carp raised in pisciriziculture ponds. This could be explained by the relatively lower water temperature in pisciriceculture ponds than in compost ponds, which favored the development of these bacteria **(Diabaté *et al.*, 2019)**. The organic matter and suspended particles present in ponds also account for the abundance of these bacteria, since they promote the proliferation of cyanobacteria such as Pseudomonas, which are pyocyanobacteria **(Pierre-Alain, 2009)**. Infections with *Pseudomonas fluorescens*, the cause of haemorrhage, have been described in various fish species such as the silver carp, Chinese carp and grass carp (**Hamdan *et al.*, 1991**; **Mavrodi *et al.*, 2005)**. *Pseudomonas aeroginosa* (49.25%) and *Citrobacter freundii* have been observed in fish **(Samake *et al.*, 2022)**.

Escherichia coli, Proteus Mirabilis, Enterobacter agglumerans, Enterobacter sakazaki and *Serratia ordifera* were positively correlated with ammonium concentrations in pisciriziculture ponds. This result could be explained by the low concentration of ammonia in the water of the pisciriziculture ponds, as a rise in temperature and pH of the water in the compost ponds would have increased the amount of ammonia (NH_4) from protein catabolism, transforming it into the non-ionized form (NH_3) that is toxic to the common carp. The indirect consequence would be the stress and weakening of these reducing the development of these commensal barrier bacteria **(Kinkelin *et al.*, 2018)**. The work of **Smart (1978)** describes the clinical picture of acute NH_3 intoxication in rainbow trout, leading to a tripling of oxygen consumption, an increase in heart rate, followed by increased ammonia (brown blood disease) and excretion, then a convulsive phase and the animal falls into a fatal coma. *Staphylococcus aureus* are more abundant in pisciriziculture ponds and less abundant in compost ponds. This result could be justified by the fact that the various rice cultivation operations (ploughing and transplanting) and for the maintenance of plants spilled by burrowing carp in pisciriziculture ponds; farmers enter unprotected **(Foucard *et al.*, 2019)**, hence the possible contamination of water is by *staphylococci* and *streptococci* from skin and nasal sputum **(Braun, 2022)**. Our results are similar to those of **Tshinyama *et al.* (2018)**, who identified *Staphylococcus aureus* (1.2%) and *Streptococcus* spp. (0.9%) in fish intestine.

Bacterial diversity characteristics were affected by the type of fish farming practice. Simpson's and Piélou's Equitability index values were significantly ($P<0.05$) higher in ponds with composting and lowest in unfertilized ponds. This result shows low species diversity and equi-partitioning in the different fish farming practices.

Specific bacterial loads increased equitably and irregularly according to weight classes and fish farming practices. This could be explained by the increase in gut epithelial mucus cells with the growth of common carp, which secrete mucin (glycosylated protein) used by bacteria as fermentable substrates, thus favoring the

establishment of certain bacteria to the detriment of others **(Rokhsefat *et al.*, 2016)**. As explained by **Stirling and Wilsey (2001)**, variations in feed intake associated with temperature variations would have resulted in a metabolic resting state of certain intestinal bacteria in trout, which could also justify our results. At adulthood or sexual maturity, bacterial diseases include mycobacteriosis caused by a bacterium of the genus mycobacterium, furunculosis caused by *Aeromonas Salmonicida*, and vibriosis which develops at temperatures above 10°C **(Evenhuis *et al.*, 2016)**. It has been shown that in juvenile salmonids, bacterial diseases appear with the onset of heat, with an incidence that increases with temperature **(Murphy *et al.*, 2011; Hountcheme, 2021)**. The work of **Borey (2017)** demonstrates a change in the bacterial community reflected by the high total biodiversity values observed between the rainbow trout fed with marine feed and those fed with vegetable feed, with a consequent drop in their growth performance. **Désai *et al.* (2012)** obtained an abundance of the Firmicute: Protoebacteria ratio in trout after inclusion of soybean meal in their diet. **Estrush *et al.* (2015)** showed a variation in the abundance of Pseudomonas and Firmicutes in the sea bream following a mixture of pea and sunflower soybean meal.

CONCLUSION

For the effect of fish farming practices on the biodiversity of the intestinal bacterial flora in common carp, the main conclusions are as follows:

The bacterial species identified in the intestines of the common carp belong to the Firmicutes and Proteobacteria phyla, regardless of the type of fish farming practiced. The type of fish farming did influence the intestinal bacterial flora. Of the fifteen bacterial species identified, nine were pathogenic, and six were beneficial to the common carp. The relative abundance of intestinal bacterial species in the common carp varied according to the type of fish farming practice. *Salmonella arizonae* and *Shigella* spp. were more abundant in the gut of the common carp raised in ponds with composting facilities, and less abundant in piscirice culture ponds. On the other hand, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter agglomerans*, *Enterobacter sakazakii*, and *Serratia odorifera* were the most abundant species in the common carp reared in piscirice culture ponds compared with those raised in ponds with composting facilities.

Based on these results, it can be concluded that these piscicultural practices promote the development of several pathogenic bacteria responsible for carp mortality. The abundance of pathogenic bacteria causes an imbalance in the intestinal flora, which can affect both carp growth and human health, as these bacteria are linked to food poisoning.

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