Int Aquat Res (2025) 17:149–164
https://doi.org/10.22034/iar.2025.2009291.1840

ORIGINAL RESEARCH

Bacterial microbiota of African catfish *Clarias gariepinus* during the hatchery and nursery phases of culture in the Philippines

Rolando Pakingking Jr . Faye Danielle Solis . Peter Palma . Leobert dela Peña

Received: 25 January 2025 / Accepted: 05 April 2025 / Published online: 16 April 2025

Abstract The bacterial microbiota of African catfish (*Clarias gariepinus*) during hatchery and nursery phases were examined across eight production cycles in two commercial farms in the Philippines. Bacterial identification was performed using conventional methods and the API 20E test, with further confirmation of selected isolates via 16S rRNA gene sequencing. Heterotrophic plate count (HPC), presumptive *Aeromonas* count (PAC), and presumptive *Vibrio* count (PVC) varied in hatchery rearing water (10^3–10^5 CFU/mL for HPC; 10^2–10^4 CFU/mL for PAC and PVC) and fry samples (10^4–10^7 CFU/g for HPC; undetectable–10^6 CFU/g for PAC; 10^2–10^4 CFU/g for PVC). In the nursery phase, similar fluctuations were observed in rearing water, fingerlings, and sediment. Microbial diversity analysis revealed 21 species (15 genera) in hatchery rearing water and 14 species (8 genera) in fry, with *Aeromonas hydrophila*, *A. sobria*, and *Vibrio cholerae* as predominant species (>15%). In the nursery phase, 29 species (16 genera), 28 species (15 genera), and 21 species (14 genera) were identified from rearing water, sediment, and fingerlings, respectively, with *A. hydrophila*, *A. sobria*, and *Bacillus subtilis* dominating (>14%). This study highlights the dynamic composition of bacterial communities in catfish culture systems, with rearing environments shaping the microbiota of juvenile fish. The presence of opportunistic and zoonotic pathogens underscores the risk of disease outbreaks, particularly under suboptimal conditions. These findings emphasize the need for stringent biosecurity measures and improved hatchery and nursery management practices to enhance fish health, prevent economic losses, and ensure the sustainability of aquaculture production. Educating hatchery and nursery workers on safe aquaculture practices and hygiene protocols is crucial to minimizing health risks to both fish and humans while ensuring efficient and responsible catfish production.

Keywords *Aeromonas* spp. . Bacterial load . Zoonotic potential . African catfish . Bacterial flora

Introduction

The African catfish *Clarias gariepinus* is an important food fish being farmed across Asia, Europe, and South America (Barasa and Ouma 2024). It has been increasingly recognized as an important food fish in the Philippines. Locally known as “hito” or “pantat”, African catfish is a large eel-like fish with dark gray or black coloration on the back and white belly. It is a hardy fish with an accessory air-breathing organ (labyrinth organ) and has been identified feasible for both small-scale and commercial aquacultures

Rolando Pakingking Jr. (✉)
College of Medical Laboratory Science, Central Philippine University, Jaro, Iloilo City 5000, Iloilo, Philippines
e-mail: rolando102969@gmail.com

Faye Danielle Solis . Peter Palma . Leobert dela Peña
Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC AQD), Tigbauan 5021, Iloilo, Philippines; Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom

since it has faster growth rate and apparently does not require extreme efforts and costs (Tan-Fermin et al. 2024). African catfish are usually grown in ponds or different types of tanks at different stocking densities (Barasa and Ouma 2024). Different culture systems are currently being practiced for African catfish including the traditional flooded ponds, earthen ponds, tanks, raceways, pits or ditches, and even in cages. Because of the suitability of African catfish for commercial aquaculture, i.e., it matures and is relatively easy to reproduce in captivity, grows fast, can be intensively cultured, hardy, and can tolerate adverse water conditions, hatchery production and grow-out culture of this species have markedly increased over the past several years in the Philippines, particularly in the municipalities of Leganes and Zarraga, Iloilo, Western Visayas (Region VI), with the latter popularly known as the catfish capital of Region VI. Because African catfish commands high price when sold in the Philippine market, its aquaculture production has continued to increase over the past few years as evidenced by production volumes ranging from 4,874.38 MT to 6,574.25 MT in 2019 and 2021, respectively (PSA 2022). Such increase in aquaculture production volume has contributed an annual value of about PHP 705 million in 2021 (PSA 2022).

Larval rearing has long been recognized as a major bottleneck in African catfish production. There have been unpublished cases of mass mortalities during the hatchery and nursery phases particularly among small scale African catfish growers in the Philippines. While several efforts have been tackled towards addressing several issues pertinent to African catfish larviculture over the past decade (Britz and Pienaar 1992; Kaiser et al. 1995), facts have remained obscured with regard to the occurrences of catfish mortalities at the hatchery and nursery phases of culture. To date, basic information including among others, the bacterial microbiota load and composition, and activities of these microorganisms during the early developmental stages of catfish in hatchery and nursery in the Philippines are scarce. Fish in their early developmental stages are highly vulnerable to bacterial and viral infections due to their immature immune system (Pakingking et al. 2011; Abraham et al. 2015; Pakingking and Nguyen 2022). Moreover, the unfavorable prevailing conditions of the rearing environments which may consequently induce stress on the fish coupled by the abrupt proliferation of the pathogenic microorganisms in the rearing water and concomitantly in cultured fish cannot be discounted. Undeniably, the high bacterial load on fish and in the rearing water at the early developmental stages of fish is deleterious as cultured fish could succumb to morbidity and eventual mortality. On the contrary, some published studies have shown that not all bacteria present in cultured fish or their rearing environments are pathogenic to fish. There are numerous bacteria present in cultured fish and their rearing environment that have been identified beneficial as they play a role in maintaining water quality thereby providing a conducive culture environment for the fish (Martínez Cruz et al. 2012; Pakingking et al. 2022). Additionally, these beneficial bacteria have been likewise proven to be involved in nutrient digestibility (Al-Dohail et al. 2009), pathogen inhibition (Al-Dohail et al. 2011), and as growth promoter (Queiroz and Boyd 1998) among other positive benefits (Fuller 1989; Martínez Cruz et al. 2012; Gao et al. 2022). The bacterial microbiota in fish and their rearing environment during early development play a crucial role in survival, as dominant bacteria can be either harmful or beneficial.

Considering the impact of bacterial microbiota on the survival of African catfish during early development, it is essential to establish baseline data on the microbiology of catfish and their rearing environments. This information is crucial for developing effective management protocols to enhance existing practices and prevent excessive mortalities and associated economic losses. Thus, to gain a comprehensive understanding of the quantitative and qualitative occurrence of bacterial populations in African catfish fry, fingerlings, and their rearing environments, this study examined bacterial microbiota load and species composition in land-based hatchery tanks and earthen nursery ponds during the hatchery and nursery phases of culture. The investigation was conducted at specific periods of each production run in two private catfish production facilities in Zarraga, Iloilo, Philippines. Not only the putative threshold levels to heterotrophic bacteria, *Aeromonas*, and *Vibrio* load were established, but unequivocally, data on both beneficial and opportunistic bacteria constituting the microbiota of catfish and their rearing environments, particularly those with zoonotic potential, were henceforth documented.

Materials and methods

Sampling sites and description of hatchery and nursery practices

Two commercial catfish production facilities, designated as Farm A (N 10°48′27.2412″, E 122°37′54.9258″) and Farm B (N 10°50′12.3714″, E 122°37′39.3384″) (Figure 1), respectively located in the municipality of Zarraga, Iloilo, Philippines, were selected in the current study. Farm A is approximately 7 km away from Farm B. It should be noted that while Farm B has been in operation for more than two decades, Farm A has been operating for around three years at the time of the implementation of this study. Since our goal was to document some baseline information on the microbiology of African catfish during their early developmental stages based on the practices being employed by the farm operators of these 2 commercial catfish production facilities, no modifications were carried out with regard to their technical operations and husbandry practices. As noted, these 2 catfish production facilities adhered to culture practices being recommended or prescribed by the competent authority, the Bureau of Fisheries and Aquatic Resources (BFAR) of the Philippines, including among others, proper disinfection of culture facilities, proper nursery pond preparation, and proper handling of catfish broodfish being subjected to induced spawning.

Farm A's hatchery larval rearing facility has an enclosed structure, i.e., with wooden walls, concrete floor, and corrugated galvanized iron roof. The hatchery facility has 10 concrete rectangular tanks measuring 1.2 × 2.7 × 0.3 m with the water level of about 10-15 cm. Moreover, Farm A conducts the nursery phase of culture in earthen ponds measuring 20 × 12 × 1.5 m. The water depth in these ponds is about 1 m. These ponds are equipped with aeration system. A total of 14 to 16 hapa nets measuring 1.5 × 3.0 × 1.5 m were installed in each pond. Because a flow-through aquaculture system is not practiced in these nursery ponds, water from deep wells was periodically added every 2 to 3 days to compensate for losses due to evaporation and seepage. Induced spawning is usually conducted every 2 weeks, referred to in this context as a "hatchery production run." The catfish breeders used in induced spawning were collected from nearby earthen ponds located in the same area where the hatchery and nursery ponds were located. As part of Farm A's protocol, each female fish was weighed, kept in separate fiber glass tank, and hormonally induced in the night using Ovaprim (Syndel Laboratories Ltd., USA) by injecting each fish with 0.5 mL of hormone per kilogram of fish. Prior to hormone injection, female broodfish were anesthetized with 2-phenoxyethanol (Sigma-Aldrich, Singapore) in a fiber glass tank, patted dry with a clean towel, and the site was wiped with cotton moistened with 70% ethyl alcohol before the hormone was intramuscularly injected. Similarly, prior to stripping, the male broodfish were anesthetized before they were sacrificed to collect the milts by aseptically dissecting the testes-seminal vesicles using sterile surgical scissors and forceps. The milt collected from male broodfish was pooled and used to fertilize eggs obtained from female broodfish. For injection, stripping, and fertilization, technicians at this facility follow a modified method described by Tan-Fermin et al. (2008; 2024). A total of 10 males and 18–20 females, with a mean body weight (MBW) of 1.2 kg, were used per production run, conducted every two weeks.

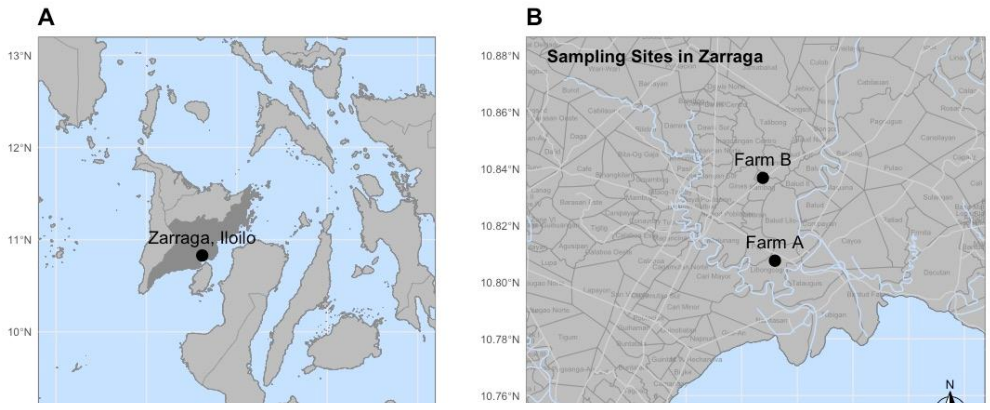

Fig. 1 Map showing the location of the municipality of Zarraga in the province of Iloilo (A), and the locations of the two commercial catfish production facilities (Farm A and Farm B) monitored in the current study (B).

Following artificial fertilization, fertilized eggs were transferred aseptically into the hatching net (framed screen nets) and allowed to incubate in the rectangular concrete tanks for 24-30 h after which, the hatching net was slowly moved allowing the newly hatched larvae to drop into the bottom of the tank, leaving only the dead eggs and unhatched larvae on it. A total of 5 incubation tanks were used. Incubation was done under a water flow-through system at a rate of approximately 2.5 L/min. The water flow was eventually increased to approximately 3.0 L/min and the larvae commenced feeding at Day 2 post-hatching. At this stage, each tank contained around 90,000 newly hatched larvae that were fed with decapsulated *Artemia* nauplii for 5 to 7 days. Thereafter, 5 to 7-day old fry were transferred into hapa nets (about 7,000 to 8,000 fry/ hapa net) in earthen ponds for nursery culture and fed with commercial floating feeds (0.25 mm particle size – powdered form) to satiation up to 4 times a day. After 10 days of rearing in hapa nets, catfish fingerlings (15 to 17-day old) were released into the earthen pond. The fingerlings were reared in these ponds for another 5 to 7 days before they were harvested to be sold to buyers for grow-out culture. At this stage, the ponds were completely drained and sun-dried. After the ponds were allowed to dry, water from the adjacent reservoir was pumped into the ponds up to a depth of approximately 1 m in preparation for the next production run. With these hatchery and nursery practices, Farm A has consistently achieved a hatching rate of 80–85% and survival rates of 80–85% for 5- to 7-day-old fry (hatchery) and 30–35% for 15- to 22-day-old fingerlings (nursery).

In the case of Farm B, the hatchery facility has no wall enclosure but has galvanized iron roofing and concrete floor. The hatchery has 6 concrete tanks measuring 1.7 × 1.45 × 0.3 m with water level of 10-15 cm. Additionally, the nursery phase of culture is also being done in earthen ponds measuring 20 × 12 × 1.5 m. The water depth in these ponds is also 1 m. Because flow-through aquaculture system is not also being practiced in these nursery ponds, water obtained from deep wells was likewise periodically added to the ponds every 2 to 3 days to compensate for the loss from evaporation and seepage. With regard to injection, stripping and fertilization, as well as larval and nursery rearing procedures, technicians in this facility employed the same protocol with Farm A, except that the number of broodfish subjected to induced spawning every two weeks were fewer in number, i.e., 2 males and 8 females. These broodfish were also reared or maintained in nearby ponds located in the same area where the hatchery and nursery ponds were located. However, it should be noted that in the case of Farm B, 2-day old larvae were fed with egg yolk instead of decapsulated *A. salina* nauplii. Additionally, after 10 days of rearing 5- to 7-day-old fry in hapa nets (7,000 to 8,000 fry/ hapa net) in nursery ponds, some 15-day old fingerlings were already sold by the hatchery owner to traders for nursery and subsequent grow-out culture. Moreover, those that could not be disposed were transferred to new hapa nets (1.5 ×1.3 × 1.5 m) with stocking density of about 3,000 to 4,000 individuals per hapa net and reared for another 5 days before they were completely harvested and the pond drained and prepared for the next production run. Fish reared in nursery ponds were also fed commercial floating feeds. Just like Farm A, Farm B has by far able to also consistently produced the same ranges of hatching rate, and as well as survival rates for 5 to 7-day old fry (hatchery) and 15- to 22-day-old fingerlings (nursery), respectively.

Thus, with the information gathered on hatchery and nursery practices at both Farm A and Farm B, we periodically assessed the bacterial load and species composition over eight production runs. Assessments were conducted on 5- to 7-day-old fry from hatchery tanks just before transfer to nursery ponds and on 15- to 22-day-old fingerlings from nursery ponds before they were harvested for sale to traders for grow-out culture in earthen ponds.

This study adhered to all relevant international, national, and SEAFDEC AQD institutional guidelines for animal care and use.

Physicochemical parameters

A multiparameter meter (YSI Pro Plus, USA) was used to determine the water physicochemical parameters including dissolved oxygen (DO) (mg/L), temperature (ºC), total dissolved solids (TDS) (g/L), salinity (ppt), and pH from three different points in the hatchery tank (water inlet, middle, and outlet) and nursery pond (middle and opposite edges) between 8:00 and 9:00 AM over a period of eight production runs. In addition, to determine the level of total hardness (ppm $CaCO_3$) (Baird et al. 2017), composite water samples were collected from the same three locations in the hatchery tank and nursery pond and brought to the

Laboratory Facilities for Advanced Aquaculture Technologies (LFAAT) of the Southeast Asian Fisheries Development Center/ Aquaculture Department (SEAFDEC/ AQD) in a chilled container within 1 h after collection.

Bacteriological sampling and analyses

The collection of samples for quantitative determination of heterotrophic plate count (HPC), presumptive *Aeromonas* count (PAC), and presumptive *Vibrio* count (PVC) in the tank water and fry (hatchery), and in pond water, sediment and fingerling (nursery) were respectively done for 8 production cycles, i.e., at 2-week interval per production run. Water, sediment, fry and fingerling samples for bacteriological quantification were likewise collected between 8:00 to 9:00 AM. Accordingly, since the number of broodfish subjected to induced spawning every production run, i.e. every two weeks, obtained from Farm A was higher in quantity compared with those obtained from Farm B, water and fry samples were randomly collected from 2 hatchery tanks per sampling. Similarly, water, sediment, and fry samples were randomly collected from 2 nursery ponds, respectively. On the other hand, similar specimen samples were respectively obtained from only one hatchery tank and one nursery pond in Farm B.

Tank and pond water

Composite water samples (600 mL), i.e., comprised of pooled water samples collected approximately 5 cm below the water surface from three different locations (200 mL/ location), water inlet, middle part and water outlet, in each of the tanks at every sampling, were collected using sterile bottles. Three composite water samples were collected from each of the tanks and individually processed for bacteriological examinations. The composite water samples were thoroughly mixed and diluted (10^{-1} – 10^{-5}) using normal saline solution (NSS) and 100 µL of each diluted sample was spread in triplicate on tryptic soy agar plates (TSA; Merck, Germany), glutamate starch phenol-red agar plates (GSP agar, Merck, Germany) plates containing 10 mg/L of penicillin, and thiosulfate-citrate-bile salts-sucrose agar (TCBS agar, Merck, Germany) plates to determine the HPC, PAC, and PVC, respectively. The plates were incubated upright at 30°C for 24 hours, after which the plates were examined for the presence of colonies. Plates containing 30 to 300 colonies, were accordingly counted and used for the calculation of HPC, PAC, and PVC, expressed as colony forming unit per mL (CFU/mL) (Bizani and Brandelli 2001; Al-Harbi and Uddin 2010; Pakingking et al. 2022). Moreover, aside from obtaining the PVCs, the proportion (percentage) of yellow (sucrose fermenter) and green (non-sucrose fermenter) colonies that grew on TCBS agar plates were also determined using the formula: Number of yellow or green colonies counted/total number colonies counted × 100% (Pakingking et al. 2022).

In the case of water samples obtained from nursery rearing ponds, composite water samples (600 mL), comprised of pooled water samples were collected approximately 20 cm below the water surface from three different locations (200 mL/location), i.e. middle part and opposite edges of the rectangular pond, in each pond at every sampling using sterile bottles (Pakingking et al. 2015; 2020; 2022). Three composite water samples were also collected and individually processed from each of the ponds per sampling. Water samples were likewise processed for bacteriological examination following the method described above to quantify the HPC, PAC, and PVC expressed as CFU/mL. The proportion (percentage) of yellow (sucrose fermenter) and green (non-sucrose fermenter) colonies that grew on TCBS agar plates were likewise determined using the formula described above (Pakingking et al. 2022).

Sediment

The pond bottom sediments were collected by submerging sterile glass bottles at the same three locations (approximately 200 g/ location) where water samples were collected in each pond per sampling (Pakingking et al. 2015). Three composite sediment samples were likewise collected and individually processed for bacteriological examinations from each of the ponds per sampling. The sediment samples were then thoroughly mixed by shaking to represent one composite sample, centrifuged at 5,000 rpm for 10 min before the supernatant was removed (Cai et al. 2019). Subsequently, 1 gm of the uniform sediment was suspended in

9 volumes of sterile NSS, serially diluted from 10^{-1} up to 10^{-5} and 100 µl of each diluted sample was spread in triplicate on TSA, GSP, and TCBS agar plates. After incubating plates at 30°C for 24 hours, the plates were retrieved and thereafter similarly processed for HPC, PAC, and PVC (CFU/g) following the protocol described above for water samples (Pakingking et al. 2015; 2020; 2022). The proportion (percentage) of yellow (sucrose fermenter) and green (non-sucrose fermenter) colonies on TCBS agar plates were also determined using the formula described for water samples.

Fry and fingerlings

Five to 7-day old fry and 15- to 22-day old fingerling samples were periodically collected from the hatchery tanks and nursery ponds, respectively. Three composite fry and fingerling samples were collected from two different locations, i.e., near the distal ends, of each of the hatchery tanks and nursery ponds per sampling. After the fry or fingerling samples were scooped out from the water using sterile fine mesh stainless steel (cup shaped) filter screen, they were first immersed in freshwater with 2-phenoxyethanol (Sigma-Aldrich, Singapore) for immobilization and thereafter, they were disinfected with 70% ethyl alcohol. The disinfected fish were then counted and transferred to sterile glass homogenizer using sterile forceps. The pooled fish samples, i.e., constituting a composite sample, was weighed and homogenized in 9 volumes of sterile NSS to make a 10^{-1} dilution (w/v). Each of the homogenates were serially diluted up to 10^{-7} and 100 µl of each diluted sample was spread in triplicate on TSA, GSP, and TCBS, incubated at 30°C for 24 hours, and thereafter processed for HPC, PAC, and PVC (CFU/g) quantification, respectively. Also, the proportion (percentage) of yellow (sucrose fermenter) and green (non-sucrose fermenter) colonies on TCBS agar plates were likewise determined using the formula described for water samples.

Bacterial isolation and identification

The bacterial isolates recovered from the hatchery and nursery were identified during each sampling. To establish the percentage composition of the bacterial microbiota in the water, sediment, fry, and fingerlings, 24-hour old bacteria that grew on TSA, GSP, and TCBS plates used for the determination of HPC, PAC, and PVC were accordingly divided into colony morphologies, i.e., shape, size, elevation, structure, color and opacity. The number of colonies of each type classified was accordingly counted. Three to five colonies representing each type of colony were then streaked onto fresh TSA plates and subsequently subcultured to obtain pure cultures. Pure cultures were stocked in tryptic soy broth (TSB) supplemented with 15% glycerol at -80°C (Pakingking et al. 2015; 2020).

The identification of selected bacterial isolates to genus or species level was conducted using the criteria specified in Bergey's Manual of Systemic Bacteriology (Holt et al. 1994) employing conventional method and further examined using API 20 E (bioMerieux, France) biochemical test system. It should be noted that representative isolates of the bacterial groups that could not be ascertained up to the species level by API 20E test were further subjected to 16s rRNA gene sequencing. After the genomic DNA of the representative isolates were isolated and purified using DNeasy® Tissue Kit (QIAGEN, Hilden, Germany), the purified bacterial genomic DNA samples were sent to Macrogen, Korea for 16s rRNA gene sequencing. The universal bacterial primers 27F and 1492R were used to amplify the 16S rRNA gene. The data obtained from Macrogen were compared with the sequence data in the GenBank using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI; http://blast.ncbi.nlm.nih.gov/).

Statistical analysis

The bacterial counts (CFU/mL or CFU/g of contents) were $\log_{10}$ transformed and data presented as mean ± SD. The HPC, PAC, PVC in the water, sediments, and in African catfish fry and fingerlings quantified among scheduled production runs were analyzed by ANOVA and compared by multiple comparison using Tukey test. The differences were considered significant at $P< 0.05$. Similarly, the data obtained for the water physicochemical parameters were likewise statistically analyzed using ANOVA and compared by multiple comparison using Tukey test. Also, differences were considered significant at $P< 0.05$.

Results

Physicochemical parameters

The physicochemical properties of rearing water from the hatchery and nursery facilities of Farms A and B are presented in Table 1. In the hatchery tanks, the levels of dissolved oxygen (DO), total dissolved solids (TDS), salinity, pH, and total hardness in Farm A's tanks 1 and 2 were comparable but significantly differed ($P< 0.05$) from those in Farm B's hatchery tank 1. However, water temperature showed no significant differences among all hatchery tanks. Similarly, the nursery rearing water, detailed in Table 1, revealed no significant variation in temperature, TDS, salinity, and pH among Farm A's nursery ponds 1 and 2 and Farm B's nursery pond 1. In contrast, the mean DO and total hardness were consistent between Farm A's nursery ponds but significantly varied ($P< 0.05$) compared to Farm B's nursery pond.

Despite some variations in the water physicochemical parameters in Farm A and Farm B's hatchery and nursery production facilities, we note that the observed values were within those required for catfish farming.

Table 1 Physicochemical parameters of catfish rearing water from hatchery tanks and nursery ponds of Farm A and Farm B. Each value indicates a mean (± SD) of physicochemical parameters quantified at different scheduled production runs (n=8). In each culture system, values with the same superscript are not significantly different at $P< 0.05$.

Culture system	Unit No.*	Dissolved oxygen (mg/L)	Temperature (°C)	Total dissolved solids (g/L)	Salinity (ppt)	pH	Total hardness (ppm CaCO3)
Hatchery	FA HT1	5.88 ± 0.54^{a}	27.8 ± 1.8^{a}	0.558 ± 0.136^{a}	0.43 ± 0.11^{a}	8.6 ± 0.2^{ab}	226.8 ± 43.3^{a}
	FA HT2	5.79 ± 0.54^{a}	27.4 ± 2.0^{a}	0.561 ± 0.130^{a}	0.43 ± 0.10^{a}	8.5 ± 0.2^{a}	226.6 ± 46.9^{a}
	FB HT1	4.95 ± 0.82^{b}	28.3 ± 1.5^{a}	0.710 ± 0.050^{b}	0.58 ± 0.08^{b}	8.8 ± 0.1^{b}	155.8 ± 13.1^{b}
Nursery	FA NP1	4.74 ± 0.54^{a}	28.8 ± 1.1^{a}	0.746 ± 0.216^{a}	0.58 ± 0.18^{a}	8.6 ± 0.4^{a}	196.8 ± 33.0^{a}
	FA NP2	5.02 ± 0.41^{a}	28.8 ± 1.7^{a}	0.671 ± 0.155^{a}	0.52 ± 0.13^{a}	8.5 ± 0.4^{a}	181.4 ± 36.1^{a}
	FB NP1	3.72 ± 0.53^{b}	28.8 ± 1.7^{a}	0.573 ± 0.167^{a}	0.45 ± 0.10^{a}	8.8 ± 0.1^{a}	119.7 ± 22.7^{b}

*FA HT1: Farm A hatchery tank 1; FA HT2: Farm A hatchery tank 2; FB HT1: Farm B hatchery tank 1; FA NP1: Farm A nursery pond 1; FA NP2: Farm A nursery pond 2; FB NP1: Farm B nursery pond 1

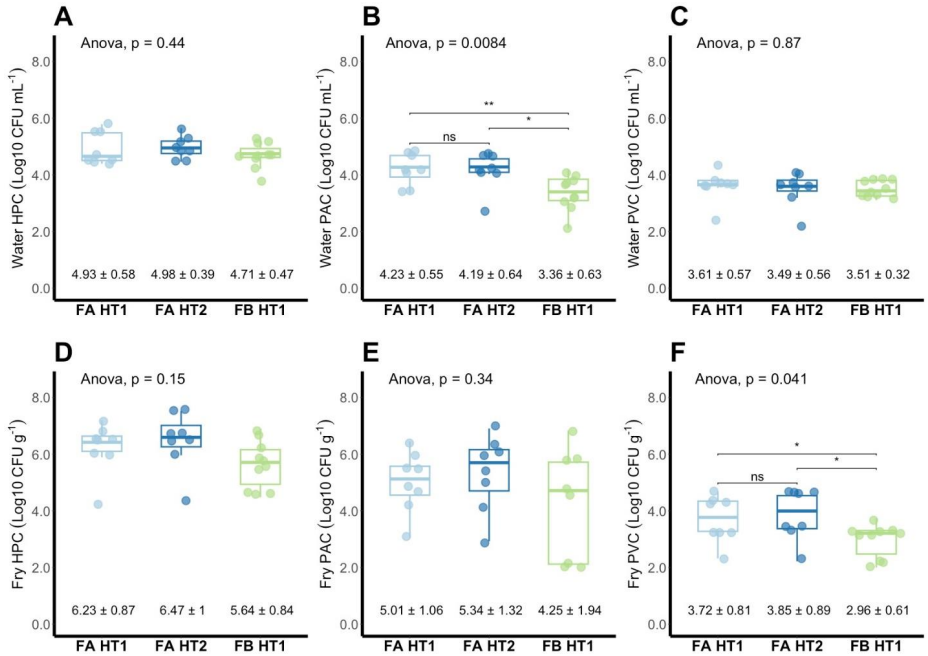

Fig. 2 Heterotrophic plate count (A, D), presumptive *Aeromonas* count (B, E), and presumptive *Vibrio* count (C, F) in the rearing water (A-C) and catfish fry (D-F) obtained from Farm A Hatchery Tanks 1 (FA HT1) and 2 (FA HT2) and Farm B Hatchery Tank 1 (FB HT1) quantified at different scheduled production runs. Each point in graphs A to F represents the value for each production cycle, determined by averaging the Log_{10} CFU/mL of water or Log_{10} CFU/g of fry from three composite samples collected from each tank per sampling. Mean value ± standard deviation is also indicated. In samples with significant variance between tanks (Anova, $P< 0.05$), pairwise comparisons were conducted and indicated by asterisk (*, $P< 0.05$; **, $P<0.01$) or n.s. (not significant).

Quantitative data

Hatchery water and fry

The mean levels of HPC, PAC, and PVC in the water samples obtained from the hatchery rearing tanks of Farm A and Farm B over the course of eight production cycles are shown in Figure 2A-C. HPC and PVC showed no significant differences among the hatchery tanks examined. HPC generally ranged from 5.62 x 10^3 to 6.17 x 10^5 CFU/mL while PVC ranged from 1.91 x 10^2 to 7.94 x 10^4 CFU/mL. Mean PAC levels were comparable between Tanks 1 and 2 of Hatchery 1 but were significantly higher ($P< 0.05$) than PAC in Tank 1 of Hatchery 2. The observed PAC in water ranged from 1.29 x 10^2 to 7.94 x 10^4 CFU/mL. It is also worth noting that except for the water sample collected in July wherein non-sucrose-fermenting vibrios constituted about 14% of the total colonies counted, only sucrose-fermenting colonies (100%) grew on the rest of the TCBS plates inoculated with water samples.

With regard to the levels of bacterial load in catfish fry samples, HPC, PAC, and PVC generally ranged from 2.04 x 10^4 to 3.39 x 10^7 CFU/g, undetectable (no growth at the lowest dilution) to 8.13 x 10^6 CFU/g, and 1.00 x 10^2 to 5.89 x 10^4 CFU/g, respectively (Figure 2D-F). The mean HPC and PAC were comparable among hatchery tanks examined. For PVC, mean levels were comparable between Tanks 1 and 2 of Hatchery 1 but were significantly higher ($P< 0.05$) than in Tank 1 of Hatchery 2. It is worth pointing out that in all of the samples examined during the different production runs, 100% of the *Vibrio* colonies that grew on TCBS plates were sucrose fermenters (yellow colonies on TCBS plates).

Nursery water, sediment, and fingerlings

The bacterial load quantified from the rearing water of Farm A's Ponds 1 and 2 and Farm B's Pond 1 showed no significant differences as shown in in Figure 3A-C. The levels of HPC, PAC, and PVC ranged from 2.57 x 10^3 to 8.71 x 10^4 CFU/mL, 3.31 x 10^2 to 3.63 x 10^4 CFU/mL, and undetectable to 7.76 x 10^2 CFU/mL, respectively. In catfish fingerlings, the HPC ranged from 6.03 x 10^4 to 6.31 x 10^6 CFU/g while PAC ranged from 3.16 x 10^3 to 1.86 x 10^6 CFU/g and PVC ranged from undetectable to 4.37 x 10^4 CFU/g (Figure 3D-F). Similarly, no significant differences were found in the mean values of bacterial load in catfish fingerlings among the ponds. The HPC, PAC, and PVC were also examined in the sediments of both farms and bacterial counts ranged from 3.55 x 10^5 to 6.17 x 10^6 CFU/g, undetectable to 4.37 x 10^5 CFU/g, and undetectable to 7.94 x 10^4 CFU/g, respectively (Figure 3G-I). Mean values of HPC, PAC, and PVC were comparable among the three ponds examined.

Notably, only sucrose-fermenting *Vibrio* were recovered from all TCBS plates inoculated with all of the samples collected from both Farms A and B nursery production facilities during the different production runs (Figure 4).

Taxonomic composition of the bacterial microbiota

Bacterial species from hatchery tank rearing water, fry, and nursery pond rearing water, sediment, and fingerlings from two catfish farms were identified. The data from both farms were similar, with Gram-negative rod-shaped bacteria dominating (86%) in hatchery water and fry samples. Fifteen genera and 21 species were identified in hatchery water, and 8 genera and 14 species in fry samples (Table 2). The most common species were *A. hydrophila, A. sobria,* and *V. cholerae*, with *A. hydrophila* comprising 31% of the total bacterial population. In the nursery, Gram-negative bacteria dominated in water (86%), sediment (75%), and fingerlings (90%), with *A. hydrophila* as the most dominant species. Sixteen genera and 29 species were recovered from water, 15 genera and 28 species from sediment, and 14 genera and 21 species from fingerlings. *A. hydrophila, A. sobria,* and *B. subtilis* were also prevalent in the nursery (Table 2). Due to incomplete identification by biochemical methods, selected isolates were subjected to 16S rRNA sequencing. The majority of these isolates were Gram-negative bacteria with zoonotic potential, including *A. caviae, A. veronii*, and *Pseudomonas* species, while Gram-positive bacilli such as *Bacillus cereus* were prevalent in the sediment. Sequence data have been deposited to the European Bioinformatics Institute (EBI) (Table 3).

Discussion

This is by far the first study on the bacteriology of African catfish during the hatchery and nursery phases of culture in the Philippines. Variations in bacterial quantities were observed across samples from two catfish production facilities. Although temperature did not vary significantly within the farms, it remains a critical factor influencing bacterial proliferation, particularly for pathogenic species. Warmer temperatures generally promote bacterial growth, which can impact microbial composition and potential disease outbreaks.

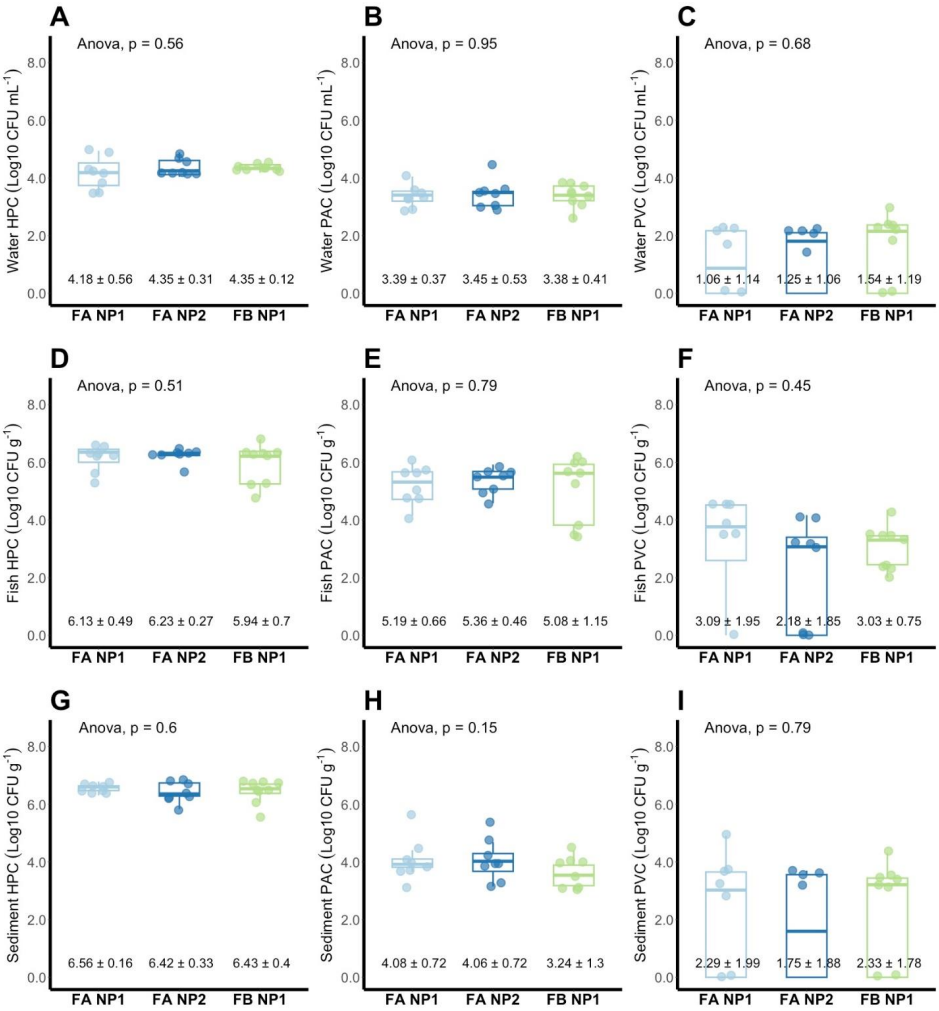

Fig. 3 Heterotrophic plate count (A, D, G), presumptive *Aeromonas* count (B, E, H), and presumptive *Vibrio* count (C, F, I) in the rearing water (A-C), catfish fingerling (D-F), and sediment (G-I) obtained from Farm A Nursery Ponds 1(FA NP1) and 2 (FA NP2) and Farm B Nursery Pond 1 (FB NP1) quantified at different scheduled production runs. Each point in graphs A to I represents the value for each production cycle, determined by averaging the Log_{10} CFU/mL of water, or Log_{10} CFU/g of sediment or fingerling from three composite samples collected from each pond per sampling. Mean value ± standard deviation is also indicated.

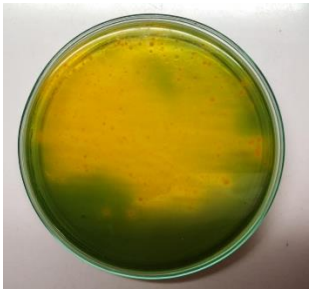

Fig. 4 Yellow colonies of sucrose-fermenting *Vibrio* spp. on TCBS agar

Therefore, temperature was considered in the analysis despite being within acceptable ranges. The study established threshold bacterial load tolerances for healthy catfish fry and fingerlings: up to 10^7, 10^6, and 10^4 CFU/g for HPC, PAC, and PVC in fry, and up to 10^6, 10^6, and 10^4 CFU/g in fingerlings. These findings align with Diyaolu (2015), though in the current study HPCs were 2 log units higher. However, the fact that catfish fry and fingerlings examined were apparently healthy, the maximum bacterial counts that we generated in the current study putatively reflect the threshold levels of hatchery-reared catfish fry and fingerlings not only to heterotrophic bacterial quantity, but also to *Aeromonas* and *Vibrio* loads in their rearing water. While bacterial loads in hatchery and nursery rearing water varied, fry and fingerlings tolerated up to 10^5 CFU/mL of heterotrophic bacteria.

Newly-hatched catfish larvae are exposed to bacteria in their rearing water, which colonize their gastrointestinal tract (GIT) as their mouth opens. Bacteria from the mother may also contribute to initial colonization (Sullam et al. 2012). In the hatchery, bacteria in the water are influenced by abiotic factors, such as water quality and organic waste buildup. These microbes colonize the larval gut, becoming part of the intestinal microbiota (Vadstein et al. 2018). Once beneficial bacteria dominate, they can protect against opportunistic pathogens through competitive exclusion. The composition of the intestinal microbiota is influenced by diet, microbial competition, host genetics, and GIT development, making the water microbiota species composition critical during the hatchery and nursery phases of culture (Vadstein et al. 2018).

Table 2. Number of bacterial isolates and percentage (%) composition recovered from samples of hatchery's tank water and fry, and nursery's pond water, sediment, and fingerlings.

Bacteria	Gram reaction	Hatchery						Nursery							
		Water		Fry		Total		Water		Sediment		Fingerling		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aeromonas caviae[*1]	−	5	3.6	6	5.0	11	4.3	7	4.8	6	4.3	6	4.8	19	4.6
Aeromonas diversa[*2]	−	2	1.4	0	0.0	2	0.8	1	0.7	2	1.4	0	0.0	3	0.7
Aeromonas hydrophila	−	44	31.9	36	30	80	31	34	23.1	25	18	42	33.6	101	24.6
Aeromonas salmonicida	−	0	0.0	1	0.8	1	0.4	0	0.0	2	1.4	0	0.0	2	0.5
Aeromonas sobria	−	28	20.3	26	21.7	54	20.9	17	11.6	12	8.6	37	29.6	66	16.1
Aeromonas veronii[*3]	−	1	0.7	0	0.0	1	0.4	2	1.4	0	0.0	1	0.8	3	0.7
Bacillus cereus[*4,5]	+	2	1.4	0	0.0	2	0.8	1	0.7	2	1.4	0	0.0	3	0.7
Bacillus haikouensis[*6]	+	0	0.0	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	2	0.5
Bacillus megaterium[*7,8]	+	0	0.0	0	0.0	0	0.0	0	0.0	3	2.2	0	0.0	3	0.7
Bacillus pseudomycoides[*9]	+	0	0.0	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	2	0.5
Bacillus subtilis	+	10	7.2	15	12.5	25	9.7	18	12.2	38	27.3	2	1.6	58	14.1
Bacillus thuringiensis[*10]	+	0	0.0	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	2	0.5
Bordetella spp.	−	0	0.0	0	0.0	0	0.0	0	0.0	3	2.2	0	0.0	3	0.7
Chromobacterium violaceum	−	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.8	2	0.5
Chryseobacterium indologenes	−	2	1.4	0	0.0	2	0.8	0	0.0	0	0.0	1	0.8	1	0.2
Citrobacter braaki	−	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	1	0.2
Citrobacter freundii[*11,12,13]	−	3	2.2	0	0.0	3	1.2	4	2.7	2	1.4	0	0.0	6	1.5
Citrobacter koseri	−	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.6	2	0.5
Citrobacter youngae	−	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	0	0.0	1	0.2
Comamonas aquatica[*14]	−	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	0	0.0	2	0.5
Edwardsiella hoshinae	−	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	0	0.0	1	0.2
Enterobacter cloacae[*15]	−	1	0.7	0	0.0	1	0.4	2	1.4	0	0.0	4	3.2	6	1.5
Escherichia coli	−	5	3.6	2	1.7	7	2.7	5	3.4	2	1.4	3	2.4	10	2.4
Exiguobacterium indicum[*16,17]	+	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	0	0.0	2	0.5
Grimontia hollisae	−	1	0.7	0	0.0	1	0.4	2	1.4	0	0.0	0	0.0	2	0.5
Klebsiella pneumoniae	−	1	0.7	0	0.0	1	0.4	0	0.0	0	0.0	0	0.0	0	0.0
Ochrobactrum ciceri[*18]	−	1	0.7	0	0.0	1	0.4	2	1.4	1	0.7	0	0.0	3	0.7
Pasteurella multocida	−	0	0.0	1	0.8	1	0.4	1	0.7	0	0.0	2	1.6	3	0.7
Pasteurella pneumotropica	−	1	0.7	7	5.8	8	3.1	3	2.0	0	0.0	2	1.6	5	1.2
Plesiomonas shigelloides	−	0	0.0	0	0.0	0	0.0	1	0.7	2	1.4	4	3.2	7	1.7
Pseudomonas aeruginosa	−	1	0.7	0	0.0	1	0.4	1	0.7	0	0.0	0	0.0	1	0.2
Pseudomonas fluorescens	−	0	0.0	0	0.0	0	0.0	1	0.7	0	0.0	0	0.0	1	0.2
Pseudomonas luteola	−	0	0.0	1	0.8	1	0.4	1	0.7	0	0.0	0	0.0	1	0.2
Pseudomonas mendocina[*19]	−	0	0.0	0	0.0	0	0.0	1	0.7	1	0.7	0	0.0	2	0.5
Pseudomonas pseudoalcaligenes[*20]	−	0	0.0	0	0.0	0	0.0	1	0.7	2	1.4	0	0.0	3	0.7
Pseudomonas putida	−	0	0.0	0	0.0	0	0.0	10	6.8	13	9.4	2	1.6	25	6.1
Raoultella terrigena	−	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	0	0.0	1	0.2
Serratia odorifera	−	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	1	0.2
Shewanella putrefaciens	−	2	1.4	1	0.8	3	1.2	3	2.0	2	1.4	1	0.8	6	1.5
Staphylococcus aureus	+	1	0.7	1	0.8	2	0.8	2	1.4	2	1.4	1	0.8	5	1.2
Stenophomonas maltophilia	−	3	2.2	0	0.0	3	1.2	4	2.7	6	4.3	0	0.0	10	2.4
Vibrio alginolyticus	−	3	2.2	2	1.7	5	1.9	3	2.0	2	1.4	2	1.6	7	1.7
Vibrio cholerae	−	21	15.2	19	15.8	40	15.5	12	8.2	1	0.7	7	5.6	20	4.9
Vibrio fluvialis	−	0	0.0	2	1.7	2	0.8	4	2.7	0	0.0	3	2.4	7	1.7
Total		138	100.0	120	100	258	100	147	100	139	100	125	100	411	100

*Identified by 16S rRNA sequencing. Isolated from [1]fry; [2]hatchery tank water; [3]fingerling; [4]hatchery tank water; [5,6,7,8,9,10]Nursery pond sediment; [11]hatchery tank water; [12]nursery pond water; [13]nursery pond sediment; [14]nursery pond water; [15]fingerling; [16,17,18,19,20]nursery pond water

In this study, bacterial counts were high in various samples, but this may not always be detrimental. If the majority of bacteria are non-pathogenic, their abundance could support organic matter recycling and re-mineralization in the rearing environments (Uddin and Al-Harbi 2012). However, as catfish fry mature and their metabolic activities increase with higher feeding rates, bacterial levels in the gills and gut may rise, especially if organic loads from uneaten feed accumulate (Al-Harbi and Uddin 2010). Therefore, monitoring bacterial loads in rearing environments is essential for predicting the survival of young catfish.

Seasonal and environmental variations significantly influence bacterial load dynamics in aquaculture systems. Temperature, rainfall, and organic matter accumulation can create favorable conditions for bacterial proliferation, particularly for opportunistic pathogens like *Aeromonas* spp. and *Vibrio* spp. Warmer temperatures, common in tropical and subtropical regions, promote bacterial growth and may increase disease risks in catfish farming. Conversely, heavy rainfall and flooding can introduce new microbial populations from surrounding environments, altering the existing microbiota composition (Marmen et al. 2021). Additionally, fluctuations in dissolved oxygen and pH levels impact bacterial survival and virulence expression (Abella et al. 2024). Understanding these seasonal patterns is essential for optimizing biosecurity measures, adjusting disinfection protocols, and mitigating pathogen outbreaks in hatchery and nursery settings.

This study reveals that both farms share a similar microbiota composition, dominated by Gram-negative bacteria such as *A. hydrophila*, *A. sobria*, *A. caviae, E. coli, P. pneumotropica, S. putrefaciens, V. cholera,* and *V. alginolyticus,* indicating that rearing water bacteria reflect the microbiota in young catfish. These species were also prevalent in nursery pond water, sediment, and fingerlings, along with *P. shigelloides* and *P. putida*. This finding aligns with Diyaolu (2015) regarding *Aeromonas, E. coli,* and *Bacillus* persistence.

Table 3. Identification of selected bacterial strains isolated from the African catfish hatchery and nursery production facilities based on 16s rRNA gene sequence analysis and their close relative published in National Center for Biotechnology Information (NCBI) databases.

Taxonomic identification/isolate code	Source of bacterial isolate	Number of nucleotides of 16S rRNA gene	Accession number	Closely related taxa	Reference accession number	Sequence similarity (%) with closely related taxa
Aeromonas caviae CF22F4	Fry	1269	ERS15900857	*Aeromonas caviae* strain ASH03 16S ribosomal RNA gene, partial sequence	KU725736.1	97
Aeromonas diversa CF2GP4S3	Hatchery Tank Water	1253	ERS15900847	*Aeromonas diversa* CDC 2478-85 strain CECT 4254 16S ribosomal RNA gene, partial sequence	GQ365710.1	98
Aeromonas veronii CF26NF188	Fingerling	1295	ERS15900861	*Aeromonas veronii* strain 126c 16S ribosomal RNA gene, partial sequence	EU488699.1	98
Bacillus cereus CF15HW1111210	Hatchery Tank Water	1292	ERS15900854	*Bacillus cereus* strain BD6 16S ribosomal RNA gene, partial sequence	KY773598.1	98
Bacillus cereus CF24HW1111209	Nursery Pond Sediment	1168	ERS15900859	*Bacillus cereus* strain E21 16S ribosomal RNA gene, partial sequence	JX627612.1	98
Bacillus haikouensis CF3N2S182009	Nursery Pond Sediment	1277	ERS15900848	*Bacillus haikouensis* strain Q2aS 16S ribosomal RNA gene, partial sequence	MF470197.1	96
Bacillus megaterium CF11N1S101518	Nursery Pond Sediment	1255	ERS15900852	*Bacillus megaterium* strain AVMB3 16S ribosomal RNA gene, partial sequence	KY476347.1	98
Bacillus megaterium CF25RS91722	Nursery Pond Sediment	1274	ERS15900860	*Bacillus megaterium* partial 16S rRNA gene, isolate OCP6	HG799979.1	96
Bacillus pseudomycoides CF12N1S90303	Nursery Pond Sediment	1262	ERS15900853	*Bacillus pseudomycoides* strain WZ002 16S ribosomal RNA gene, partial sequence	MF193911.1	96
Bacillus thuringiensis CF23NS1111202	Nursery Pond Sediment	1244	ERS15900858	*Bacillus thuringiensis* strain MSS-2 16S ribosomal RNA gene, partial sequence	KM280648.1	97
Citrobacter freundii CF6BNF101519	Hatchery Tank Water	1288	ERS15900849	*Citrobacter freundii* strain I-N-3-2-1 16S ribosomal RNA gene, partial sequence	KU570313.1	98
Citrobacter freundii CF9BNF101523	Nursery Pond Water	1305	ERS15900850	*Citrobacter freundii* strain I-T-1-3 16S ribosomal RNA gene, partial sequence	KU570303.1	97
Citrobacter freundii CF10BNF101524	Nursery Pond Sediment	1286	ERS15900851	*Citrobacter freundii* strain I-T-1-3 16S ribosomal RNA gene, partial sequence	KU570303.1	97
Comamonas aquatica CF1D4W3	Nursery Pond Water	1212	ERS15900846	*Comamonas aquatica* partial 16S rRNA gene, isolate RS6	LN558648.1	98
Enterobacter cloacae CF27NF108	Fingerling	1286	ERS15900862	*Enterobacter cloacae* strain RCB973 16S ribosomal RNA gene, partial sequence	KT261185.1	99
Exiguobacterium indicum CF4BNW90310	Nursery Pond Water	1312	ERS15900864	*Exiguobacterium indicum* strain IHB B 10090 16S ribosomal RNA gene, partial sequence	KR233792.1	96
Exiguobacterium indicum CF5BNW90307	Nursery Pond Water	1229	ERS15900865	*Exiguobacterium indicum* strain Lmb009 16S ribosomal RNA gene, partial sequence	KT986082.1	99
Ochrobactrum ciceri CF17N1S670801	Nursery Pond Water	1100	ERS15900855	*Ochrobactrum ciceri* strain HRJ1 16S ribosomal RNA gene, partial sequence	KP140839.1	89
Pseudomonas mendocina CF30NW	Nursery Pond Water	1315	ERS15900863	*Pseudomonas mendocina* strain W6 16S ribosomal RNA gene, partial sequence	KT380553.1	98
Pseudomonas pseudoalcaligenes CF21RS67806	Nursery Pond Water	1249	ERS15900856	*Pseudomonas pseudoalcaligenes* KF707 = NBRC 110670 DNA, complete genome	AP014862.1	97

A. hydrophila, a major pathogen for freshwater fish, poses a concern in the catfish industry, with emerging virulent strains affecting water systems in the USA (Cai et al. 2019). Further bioassays are needed to confirm the virulence of these strains. The dominance of *A. sobria* and *A. caviae* in the rearing environments should also be addressed, as they can cause disease outbreaks in fish (Li and Cai 2011; Thomas et al. 2013). *A. veronii*, identified in the hatchery and nursery, has caused mass mortality in farmed catfish (Hoai et al. 2019). Furthermore, *Aeromonas* spp. pose a zoonotic risk to hatchery and nursery technicians, causing gastroenteritis and other infections, particularly in immunocompromised individuals (Janda and Abbott 2010; Batra et al. 2016).

As shown in Table 2, Gram-positive *Bacillus* species, particularly *B. subtilis*, were abundant in all samples from the two hatcheries and nurseries. These bacteria are widespread in fish-rearing environments, commonly found in soil, freshwater, and seawater (Stein 2005). Recognized as a probiotic, *B. subtilis* is FDA-approved due to its safety for animals and humans. Its enzymes, secreted into the culture medium, facilitate purification and feed application (Olmos and Panigua-Michel 2014) and produce peptide antibiotics that inhibit fish pathogens (Stein 2005). *B. subtilis* can utilize diverse carbon and nitrogen sources by breaking down proteins, carbohydrates, and lipids (Cui et al. 2018). Its presence in hatchery and nursery environments suggests resilience under extreme conditions, benefiting young catfish by eliminating pathogens, promoting bioremediation, enhancing feed efficiency, and boosting immune responses (Olmos et al. 2020).

The recovery of other *Bacillus* spp., though in lower quantities, highlights their role in maintaining equilibrium in young catfish rearing environments. *B. cereus*, for instance, acts as a probiotic with antibacterial activity against pathogens like *V. harveyi, V. alginolyticus, Streptococcus iniae, S. agalactiae,* and *A. hydrophila* through competitive exclusion (Lalloo et al. 2010; Amenyogbe et al. 2021; Ke et al. 2022). *B. thuringiensis* has demonstrated anthelmintic properties against the gill trematode *Centrocestus formosanus* (Mendoza-Estrada et al. 2016). Additionally, *B. megaterium, B. pseudomycoides*, and *B. haikouensis* are potent bioremediation agents, capable of removing heavy metals like Pb, Ni, Cd, Cu, and Hg in polluted environments (Kumar et al. 2020; Njoku et al. 2020; Li et al., 2022). These *Bacillus* species, primarily found in nursery pond sediments, contribute to environmental balance by removing heavy metals and degrading organic matter from hatching remnants, mortality, defecation, microalgae, and live food (Vadstein et al. 2018). Their presence emphasizes the importance of maintaining a stable microbial load in hatchery tanks and nursery ponds through proper water management to prevent the dominance of opportunistic microbes (Vadstein et al. 2018).

A broader range of bacterial taxa was recovered from the hatchery rearing water compared to Diyaolu (2015) and from pond water and sediment compared to Al-Harbi and Uddin (2010). Notably, species such as *B. haikouensis*, *B. megaterium*, *B. pseudomycoides*, *B. thuringiensis*, *Bordetella* sp., *Chromobacterium violaceum, Chryseobacterium indologenes, Citrobacter braaki, C. koseri, C. youngae, Comamonas aquatica, Edwardsiella hoshinae, Enterobacter cloacae, Escherichia coli, Exiguobacterium indicum, Grimontia hollisae, Klebsiella pneumoniae, Ochrobactrum cicero, Pasteurella multocida, P. pneumotropica, Plesiomonas shigelloides, Pseudomonas aeruginosa, P. fluorescens, P. luteola, P. mendocina, P. pseudoalcaligenes, P. putida, Raoultella terrigena, Serratia odorifera, Stenotrophomonas maltophilia, Vibrio alginolyticus*, and *V. fluvialis* were identified (Table 2). These species had not been previously reported by Al-Harbi and Uddin (2010) or Uddin and Al-Harbi (2012) in water, sediment, gills, or intestines of African catfish grown in earthen ponds. Variations in results may be due to differences in sampling techniques, environmental factors, and developmental stages of catfish (Al-Harbi and Uddin 2010; Pakingking et al. 2015). Of the 44 bacterial species identified in the nursery samples, 17 were absent in hatchery water and fry samples, indicating greater bacterial diversity in earthen ponds. This study established baseline values for heterotrophic bacteria, *Aeromonas*, and *Vibrio* load in 21–22-day-old catfish fingerlings and their rearing environments at harvest, destined for grow-out culture.

Biotic factors, such as diverse bacteria and opportunistic pathogens like *Aeromonas* spp., along with abiotic factors, including handling, feeding practices, and water quality changes, likely contribute to lower survival rates during the nursery phase. Further study of these factors is crucial to improve survival rates and meet the increasing demand for catfish fingerlings. The presence of zoonotic bacteria, even those not typically linked to catfish diseases, poses risks to hatchery and nursery technicians, especially those with underlying health conditions. For instance, *P. aeruginosa* is known to cause pneumonia, endocarditis, and infections in various systems, including the urinary tract, central nervous system, and skin (Reynolds and

Kollef 2021). The isolation of *P. shigelloides* (1.7%) from water, sediment, and fingerlings is concerning, as it has been linked to gastroenteritis outbreaks from contaminated water and undercooked fish, with higher prevalence in Southeast Asia and Africa (Janda et al. 2016). Additionally, *P. shigelloides* has been associated with septicemia, central nervous system disease, and other infections (Janda et al. 2016).

V. cholerae and *V. alginolyticus* were dominant species recovered from hatchery and nursery samples (Table 2). While vibriosis primarily affects marine and estuarine species, the persistence of *V. cholerae* in pond water and sediment aligns with findings by Pakingking et al. (2015) and Al-Harbi and Uddin (2010) but contrasts with Diyaolu (2015), where it was absent in hatchery-reared African catfish. Although not a catfish pathogen, the zoonotic potential of *V. cholerae* (O1 and non-O1 strains) to hatchery and pond technicians through contact with fish or water warrants investigation, as non-O1 strains have been linked to wound infections (Bonner et al. 1983; Hlady and Klontz 1996). Similarly, the persistence of *V. alginolyticus* in catfish culture environments is notable due to its association with human infections, including ear, soft tissue, and wound infections, often complicated by antibiotic resistance (Horii et al. 2005). Notably, *Vibrio* spp. have also been persistently documented in shrimp farms, further highlighting their ability to thrive in aquaculture environments (Choudhary et al. 2025).

S. putrefaciens was isolated from water and sediment samples, consistent with prior findings (Al-Harbi and Uddin 2010; Uddin and Al-Harbi 2012; Pakingking et al. 2015). Though not a catfish pathogen, *S. putrefaciens* is a significant fish spoilage organism (Gram et al. 1987) and an opportunistic human pathogen linked to abscesses, cellulitis, peritonitis, and ear infections (Vignier et al. 2013), underscoring its zoonotic risk. The recovery of *Citrobacter* spp., including *C. freundii* and *C. aquatica*, also warrants attention. While *C. freundii* is typically part of fish and human gut microbiota, it can cause pneumonia, meningitis, sepsis, and urinary tract infections in immunocompromised individuals (Anderson et al. 2018; Liu et al. 2018). It has also been associated with mass mortalities in aquatic species like grass carp, red swamp crayfish, and crucian carp (Liu et al. 2020; Xiong et al. 2020; Pan et al. 2021). Meanwhile, *C. aquatica*, though rarely linked to human infections, was recently implicated in a case of bacteremia with septic shock (Kaeuffer et al. 2018).

Conclusion

In summary, this study demonstrates that the microbiota of rearing environments significantly influences the bacterial load and composition of developing African catfish. Opportunistic pathogens present in these environments can colonize fish and, under stress conditions common in aquaculture, contribute to disease outbreaks. Factors such as high organic load, elevated temperatures, and high stocking densities favor the proliferation of these microbes, particularly *Aeromonas* spp., underscoring the need for effective disinfection in production facilities. Monitoring opportunistic pathogens in nursery-produced fingerlings is crucial, as purchasing infected fish from poorly managed farms can facilitate disease transmission. The baseline threshold values for heterotrophic bacteria, *Aeromonas*, and *Vibrio* provided in this study can serve as reference points for microbial regulation through disinfection and improved husbandry practices.

The detection of *Aeromonas*, *P. shigelloides*, *Pseudomonas*, *S. putrefaciens*, and *Vibrio* species with zoonotic potential highlights the importance of stringent safety measures to mitigate health risks. Educating hatchery and nursery technicians on proper hygiene and biosecurity protocols is essential to reducing contamination risks. While this study provides valuable insights into the bacterial microbiota of African catfish, it is limited by its reliance on culture-based bacterial identification, which may not capture the full microbial diversity. Future research incorporating high-throughput sequencing and broader environmental assessments would provide a more comprehensive understanding of microbial dynamics in catfish aquaculture. Additionally, investigating the presence of virulence genes in *Aeromonas* spp. and exploring probiotic applications could help mitigate pathogen proliferation.

To improve aquaculture practices and reduce disease risks, catfish farmers should implement regular disinfection of hatchery and nursery facilities, maintain optimal water quality, and monitor stocking densities to limit bacterial growth. Routine microbial assessments of rearing water and fish stock are vital for early disease detection. Worker education on hygiene and safety practices can further minimize zoonotic risks, while probiotic applications may help suppress harmful bacteria and enhance fish health. Taken together, these measures can work in concert to improve fish survival, reduce disease outbreaks, and promote more sustainable aquaculture production.

Conflict of interest The authors have no relevant conflict of interest to declare.

Authors' contributions RPJr and PAP conceptualized the study and developed the experimental design. RPJr, FDS, PAP, and LDP contributed to the data acquisition, analyses, and interpretation. RPJr and PAP prepared the figures and tables. RPJr wrote the manuscript with input from all authors. The final manuscript was approved by all authors.

Acknowledgements We would like to thank the owners of IJY Catfish Farm and Bambie Pantat Hatchery for giving us access to their catfish production facilities. We gratefully acknowledge the invaluable assistance of the personnel and staff of IJY Catfish Farm and Bambie Pantat Hatchery, especially Mr. Larry Paňoso during the conduct of our sampling. We would like to thank Dr. Evelyn Grace de Jesus-Ayson for the critical review of the manuscript. This study was funded by SEAFDEC AQD (Study Code: FH-02-F2020T).

References

Abdella B, Shokrak NM, Abozahra NA, Elshamy Y, Kadira HI, Mohamed RA (2024) Aquaculture and *Aeromonas hydrophila*: a complex interplay of environmental factors and virulence. Aquac Int 32:7671−7681. https://doi.org/10.1007/s10499-024-01535-y

Abraham TJ, Mallick PK, Adikesavalu H, Banerjee S (2015) Pathology of *Edwardsiella tarda* infection in African catfish, *Clarias gariepinus* (Burchell 1822), fingerlings. Fish Aquat Life 23:141–148. https://doi.org/10.1515/aopf-2015-0016

Al-Dohail MA, Hashim R, Aliyu-Paiko M (2009) Effects of the probiotic, *Lactobacillus acidophilus,* on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquac Res 40:1642–1652. https://doi.org/10.1111/j.1365-2109.2009.02265.x

Al-Dohail MA, Hashim R, Aliyu-Paiko M (2011) Evaluating the use of *Lactobacillus acidophilus* as a biocontrol agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African catfish *Clarias gariepinus* juveniles. Aquac Res 42:196−209. https://doi.org/10.1111/j.1365-2109.2010.02606.x

Al-Harbi AH, Uddin MN (2010) Bacterial populations of African catfish, *Clarias gariepinus* (Burchell 1822) cultured in earthen ponds. J Appl Aquac 22:187–193. https://doi.org/10.1080/10454438.2010.497736

Amenyogbe E, Huang J, Chen G, Wang W (2021) Probiotic potential of indigenous (*Bacillus* sp. RCS1, *Pantoea agglomerans* RCS2 and *Bacillus cereus* strain RCS3) isolated from cobia fish (*Rachycentron canadum*) and their antagonistic effects on the growth of pathogenic *Vibrio alginolyticus, Vibrio harveyi, Streptococcus iniae* and *Streptococcus agalactiae*. Front Mar Sci 8:672213. https://doi.org/10.3389/fmars.2021.672213

Anderson MT, Mitchell LA, Zhao L, Mobley HLT (2018) *Citrobacter freundii* fitness during bloodstream infection. Sci Rep 8:11792. https://doi.org/10.1038/s41598-018-30196-0

Baird RB, Eaton AD, Rice EW (2017) Standard methods for the examination of water and wastewater. 23rd edn. American Public Health Association, American Water Works Association, Water Environment Federation, Washington D.C.

Barasa JE, Ouma DF (2024) Towards sustainability in seed supply for African catfish, *Clarias gariepinus* (Burchell 1822) culture in Kenya: lessons from Asian catfishes industry. Aquac Res 341858. https://doi.org/10.1155/2024/1341858

Batra P, Mathur P, Misra MC (2016) *Aeromonas* spp.: an emerging nosocomial pathogen. J Lab Physicians 8:1–4. https://doi.org/10.4103/0974-2727.176234

Bizani D, Brandelli A (2001) Antimicrobial susceptibility, hemolysis, and hemagglutination among *Aeromonas* spp. isolated from water of a bovine abattoir. Braz J Microbiol 32:334–339. https://doi.org/10.1590/S1517-83822001000400016

Bonner JR, Coker AS, Berryman CR, Pollock HM (1983) Spectrum of *Vibrio* infections in a Gulf Coast community. Ann Intern Med 99:464−469

Britz PJ, Pienaar AG (1992) Laboratory experiments on the effect of light and cover on the behaviour and growth of African catfish, *Clarias gariepinus* (Pisces: Clariidae). J Zool 227:43–62. https://doi.org/10.1111/j.1469-7998.1992.tb04343.x

Cai W, Willmon E, Burgos FA, Ray CL, Hanson T, Arias CR (2019) Biofilm and sediment are major reservoirs of virulent *Aeromonas hydrophila* (vAh) in catfish production ponds. J Aquat Anim Health 31:112–120. https://doi.org/10.1002/aah.10056

Choudhary B, Pawase AS, Ghode G S, Tibile RM, Bhatkar VR, Choudhary D, Choudhary U (2025) A study of bacterial community structure of shrimp farms along the Ratnagiri coast, Maharashtra. Heliyon 11:1. https://doi.org/10.1016/j.heliyon.2025.e41712

Cui W, Han L, Suo F, Liu Z, Zhou L, Zhou Z (2018) Exploitation of *Bacillus subtilis* as a robust workhorse for production of heterologous proteins and beyond. World J Microbiol Biotechnol 34:145. https://doi.org/10.1007/s11274-018-2531-7

Diyaolu DO (2015) Aerobic bacterial flora and survival of African catfish *Clarias gariepinus* during early life stages in hatchery. Int J Fish Aquat Stud 3:380–384

Fuller R (1989) Probiotics in man and animals. J App Bacteriol 66:365–378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x

Gao Z, Zhang Y, Wang Q, Ren H, Gao G, Jin X, Fang H (2022) Preliminary screening, identification and biological characteristic analysis of *Bacillus* probiotics isolated from *Cynoglossus semilaevis*. Isr J Aquac-Bamidgeh 74:1–12. https://doi.org/10.46989/001c.57752

Gram L, Trolle G, Huss HH (1987) Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int J Food Microbiol 4:65–72. https://doi.org/10.1016/0168-1605(87)90060-2

Hlady WG, Klontz KC (1996) The epidemiology of *Vibrio* infections in Florida, 1981–1993. J Infect Dis 173:1176–1183. https://doi.org/10.1093/infdis/173.5.1176

Hoai TD, Trang TT, Van Tuyen N, Giang NTH, Van Van K (2019) *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. Aquaculture 513:734425. https://doi.org/10.1016/j.aquaculture.2019.734425

Holt JG, Krieg NR, Sneath PHA, Williams ST (1994) Bergey's manual of determinative bacteriology, 9th ed. Williams and Wilkins, Baltimore, U.S.A.

Horii T, Morita M, Muramatsu H, Monji A, Miyagishima D, Kanno T, Maekawa M (2005) Antibiotic resistance in *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from a wound infection: a case report. J Trauma Acute Care Surg 58:196. https://doi.org/10.1097/01.TA.0000066381.33339.C0

Janda JM, Abbott SL (2010) The genus *Aeromonas*: taxonomy, pathogenicity and infection. Clin Microbiol Rev 23:35–73. https://doi.org/10.1128/CMR.00039-09

Janda JM, Abbott SL, McIver CJ (2016) *Plesiomonas shigelloides* revisited. Clin Microbiol Rev 29:349–374. https://doi.org/10.1128/CMR.00103-15

Kaeuffer C, Schramm F, Meyer A, Hansmann Y, Guffroy A, Argemi X (2018) First case of *Comamonas aquatica* bacteremia complicated by septic shock. Méd Mal Infect 48:540–542. https://doi.org/10.1016/j.medmal.2018.08.004

Kaiser H, Weyl O, Hecht T (1995) The effect of stocking density on growth, survival and agonistic behaviour of African catfish. Aquac Int 3:217–225. https://doi.org/10.1007/BF00118103

Ke X, Liu Z, Zhang M, Zhu W, Yi M, Cao J, Gao F, Wang M, Lu M (2022) A *Bacillus cereus* NY5 strain from tilapia intestine antagonizes pathogenic *Streptococcus agalactiae* growth and adhesion in vitro and in vivo. Aquaculture 561:738729. https://doi.org/10.1016/j.aquaculture.2022.738729

Kumar P, Fulekar MH, Hiranmai RY, Kumar R, Kumar R (2020) 16S rRNA molecular profiling of heavy metal tolerant bacterial communities isolated from soil contaminated by electronic waste. Folia Microbiol 65:995–1007. https://doi.org/10.1007/s12223-020-00808-2

Lalloo R, Moonsamy G, Ramchuran S, Görgens J, Gardiner N (2010) Competitive exclusion as a mode of action of a novel *Bacillus cereus* aquaculture biological agent. Lett Appl Microbiol 50:563–570. https://doi.org/10.1111/j.1472-765X.2010.02829.x

Li H, Ai C, Zhao, X, Pang B, Xu X, Wu W, Liu G, Jiang C, Pan Z, Shi J (2022) The capability of *Bacillus pseudomycoides* from soil to remove Cu (II) in water and prevent it from entering plants. J App Microbiol 132:1914–1925. https://doi.org/10.1111/jam.15343

Li Y, Cai S-H (2011) Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in juvenile tilapia *Oreochromis niloticus*. Curr Microbiol 62:623–627. https://doi.org/10.1007/s00284-010-9753-8

Liu L-H, Wang N-Y, Wu AY-J, Lin C-C, Lee C-M, Liu C-P (2018) *Citrobacter freundii* bacteremia: Risk factors of mortality and prevalence of resistance genes. J Microbiol Immunol Infect 51:565–572. https://doi.org/10.1016/j.jmii.2016.08.016

Liu X, He X, An Z, Sun W, Chen N, Gao X, Li X, Zhang X (2020) *Citrobacter freundii* infection in red swamp crayfish (*Procambarus clarkii*) and host immune-related gene expression profiles. Aquaculture 515:734499. https://doi.org/10.1016/j.aquaculture.2019.734499

Marmen S, Fadeev E, Al Ashhab A, Benet-Perelberg A, Naor A, Patil H J, Cytryn E, Viner-Mozzini Y, Sukenik A, Lalzar M, Sher D (2021) Seasonal dynamics are the major driver of microbial diversity and composition in intensive freshwater aquaculture. Front Microbiol 12:679743. https://doi.org/10.3389/FMICB.2021.679743

Martínez Cruz P, Ibáñez AL, Monroy HOA, Ramírez SHC (2012) Use of probiotics in aquaculture. Int Sch Res Notices 916845. https://doi.org/10.5402/2012/916845

Mendoza-Estrada LJ, Hernández-Velázquez VM, Arenas-Sosa I, Flores-Pérez FI, Morales-Montor J, Peña-Chora G (2016) Anthelmintic effect of *Bacillus thuringiensis* strains against the gill fish trematode *Centrocestus formosanus*. Biomed Res Int 8272407. https://doi.org/10.1155/2016/8272407

Morris JG (2003) Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin Infect Dis 37:272–280

Njoku KL, Akinyede OR, Obidi OF (2020) Microbial remediation of heavy metals contaminated media by *Bacillus megaterium* and *Rhizopus stolonifer*. Sci African 10:00545. https://doi.org/10.1016/j.sciaf.2020.e00545

Olmos J, Acosta M, Mendoza G, Pitones V (2020) *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases and avoid water pollution. Arch Microbiol 202:427–425. https://doi.org/10.1007/s00203-019-01757-2

Olmos J, Panigua-Michel J (2014) *Bacillus subtilis* a potential probiotic bacterium to formulate functional feeds for aquaculture. J Microb Biochem Technol 6:361–365. https://doi.org/10.4172/1948-5948.1000169

Pakingking R Jr, Mori K-I, Bautista NB, de Jesus-Ayson EG, Reyes O (2011) Susceptibility of hatchery-reared snubnose pompano *Trachinotus blochii* to natural betanodavirus infection and their immune responses to the inactivated causative virus. Aquaculture 311:80–86. https://doi.org/10.1016/j.aquaculture.2010.11.035

Pakingking R Jr, Palma P, de Jesus-Ayson EG, Usero R (2022) *Vibrio* load and percentage composition of sucrose-fermenting vibrios in Nile tilapia (*Oreochromis niloticus*) cultured in brackish water earthen ponds and biocontrol potential of recycled tilapia-conditioned greenwater against population growth of *V. parahaemolyticus* and non-sucrose-fermenting vibrios in the grow-out culture of white leg shrimp (*Litopenaeus vannamei*). Aquac Res 53:6816–6831. https://doi.org/10.1111/are.16148

Pakingking R Jr, Palma P, Usero R (2015) Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. World J Microbiol Biotechnol 31:265–275. https://doi.org/10.1007/s11274-014-1758-1

Pakingking R Jr, Palma P, Usero R (2020) *Aeromonas* load and species composition in tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. Aquac Res 51:4736–4747. https://doi.org/10.1111/are.14820

Pakingking RV Jr, Nguyen VV (2022) Chapter 26 – Edwardsiellosis. In: Kibenge FSB, Baldisserotto B, Chong RS-M (eds.), Aquaculture Pathophysiology. Academic Press, pp. 345–356. https://doi.org/10.1016/B978-0-12-812211-2.00026-3

Pan L, Yang Y, Peng Y, Li D, Khan TA, Chen P, Yan L, Hu S, Ding X, Sun Y, Xia L, Yi G (2021) The novel pathogenic *Citrobacter freundii* (CFC202) isolated from diseased crucian carp (*Carassius auratus*) and its ghost vaccine as a new prophylactic strategy against infection. Aquaculture 533:736190. https://doi.org/10.1016/j.aquaculture.2020.736190

PSA (Philippine Statistics Authority) (2022) Aquaculture: volume of production by species, geolocation, year and quarter. OpenSTAT Database. https://openstat.psa.gov.ph/PXWeb/pxweb/en/DB/DB__2E__FS/0072E4GVAP0.px/?rxid=bdf9d-8da-96f1-4100-ae09-18cb3eaeb313t. Accessed on 22 May 2023

Queiroz JF, Boyd CE (1998) Effects of a bacterial inoculum in channel catfish ponds. J World Aquac Soc 29:67–73. https://doi.org/10.1111/j.1749-7345.1998.tb00300.x

Reynolds D, Kollef M (2021) The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: an update. Drugs 81:2117–2131. https://doi.org/10.1007/s40265-021-01635-6

Stein T (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 56: 845–857. https://doi.org/10.1111/j.1365-2958.2005.04587.x

Sullam KE, Essinger SD, Lozupone CA, O'connor MP, Rosen GL, Knight R, Kilham SS, Russell JA (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol Ecol 21:3363–3378. https://doi.org/10.1111/

j.1365-294X.2012.05552.x

Tan-Fermin JD, Fermin AC, Bombeo RF, Evangelista MAD, Catacutan MR, Santiago CB (2008) Breeding and seed production of the Asian catfish *Clarias macrocephalus* (Gunther). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center

Tan-Fermin JD, Navarro J, Arnaldo GN, Dayrit R (2024) Breeding, seed production, and culture of the African catfish *Clarias gariepinus*. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center

Thomas J, Madan N, Nambi KSN, Abdul Majeed S, Nazeer Basha A, Sahul Hameed AS (2013) Studies on ulcerative disease caused by *Aeromonas caviae*-like bacterium in Indian catfish, *Clarias batrachus* (Linn). Aquaculture 376–379:146–150. https://doi.org/10.1016/j.aquaculture.2012.11.015

Uddin N, Al-Harbi AH (2012) Bacterial flora of polycultured common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*). Int Aquat Res 4:10. https://doi.org/10.1186/2008-6970-4-10

Vadstein O, Attramadal KJK, Bakke I, Forberg T, Olsen Y, Verdegem M, Giatsis C, Skjermo J, Aasen IM, Gatesoupe F-J, Dierckens K, Sorgeloos P, Bossier P (2018) Managing the microbial community of marine fish larvae: a holistic perspective for larviculture. Front Microbiol 9:1820. https://doi.org/10.3389/fmicb.2018.01820

Vignier N, Barreau M, Olive C, Baubion E, Théodose R, Hochedez P, Cabié A (2013) Human infection with *Shewanella putrefaciens* and *S. algae*: report of 16 cases in Martinique and review of the literature. Am J Trop Med Hyg 89:151–156. https://doi.org/10.4269/ajtmh.13-0055

Xiong F, Qin L, Hao Y, Zhao D, Li W, Zou H, Li M, Wu S, Wang G (2020) Gut microbiota modulation and immunity response induced by *Citrobacter freundii* strain GC01 in grass carp (*Ctenopharyngodon idellus*). Aquaculture 521:735015. https://doi.org/10.1016/j.aquaculture.2020.735015