

Occurrence of presumptive Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) and analysis of water quality parameters of river-dependent and deepwell-dependent tilapia grow-out farms in Pampanga, Philippines

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Abstract. This study aimed to quantify the abundance of Gammaproteobacteria (*Aeromonas* and *Vibrio* spp.) in Nile tilapia (*Oreochromis niloticus*) grow-out farms, emphasizing the possible influence of water source (i.e., deep well and river). Moreover, the occurrence of the target bacteria is correlated to the existing water quality condition of the evaluated farms. Findings revealed that temperature, dissolved oxygen, and pH in both Minalin and Magalang were within the permissible limits for tilapia aquaculture. Significant variations to the levels of total dissolved solids, salinity, phosphorous, organic matter and total nitrogen content were observed, with Minalin farms presenting the highest values ($p < 0.05$). High prevalence rate of the viable bacteria was obtained in the two municipalities ranging from 80 to 100%. Bacterial counts in fish and water samples were significantly higher ($p < 0.05$) in deep well-dependent farms. In contrast, significant abundance of bacterial load in sediment ($p < 0.05$) was recorded in river-dependent farms. In addition, yellow colony prevalently grew in thiosulfate-citrate-bile salts-sucrose (TCBS) agar in all collected samples in both sampling sites. Despite the presence of presumed *Aeromonas* spp. and *Vibrio* spp. in Minalin and Magalang farms, all the fish were classified as healthy and biologically active based on the computed mean condition factor and the absence of clinical signs.

Key Words: bacterial count, condition factor, correlation, farm management, prevalence.

Introduction. Tilapia farming has expanded to more than 140 countries driven by ease of production, ability to thrive in diverse environmental conditions, rapid growth and high nutritional value of the species (Debnath et al 2023). Based on FAO (2022), Nile tilapia (*Oreochromis niloticus*) ranks third in the major aquaculture species produced in global inland aquaculture with 4.4 million tonnes in 2020. This fish plays a vital role not only as a staple fish, but also as source of livelihood of local communities across continents (El-Sayed & Fitzsimmons 2023). However, the current intensification of aquaculture activities is concurrent with a rise in disease incidences, posing potential adverse impacts on the industry's production, profitability and sustainability (Novriadi 2016). Bacterial infection is one of the main problems that affect the sector's production, development and expansion (Alapide-Tendencia & De la Peña 2001). In the recent years, several bacterial disease outbreaks in tilapia farming have been reported in the Philippines (Yambot 1998; Reyes 2018; Limbauan 2018; Reyes 2019; Legario et al 2020), despite it being considered a hardy fish (Romana-Eguia et al 2020). Concomitant to disease incidences is the presence of various stressors, since bacteria are considered as opportunistic pathogens, and rarely act as primary pathogens (Haenen et al 2023). Tilapia and its rearing environment harbor diverse and large numbers of bacteria (Pakingking et al 2015). Hence, episodes of disease epizootics may associate with the disturbance of environmental quality suitable for farming, farm management and practices (Reyes 2018; Ali et al 2020).

Haenen et al (2023) compiled the common and emerging bacterial diseases of tilapia, providing their zoonotic potentials, and the status and risk of antimicrobial resistance. Among the described bacterial infections of significant importance are aeromoniasis and vibriosis. These diseases are caused by *Aeromonas* and *Vibrio*, respectively, both Gram-negative bacteria that belong to class Gammaproteobacteria (Bhowmick & Bhattacharjee 2018; Sampaio et al 2022). Initially placed in the Vibrionaceae family based on phenotypic traits, *Aeromonas* underwent reclassification after molecular identification revealed its unique evolutionary history, which led to its placement in a newly designated family known as Aeromonadaceae (Colwell et al 1986). Based on the FAO (2009), the same clinical signs are exhibited by affected Nile tilapia with vibriosis and motile *Aeromonas* septicaemia (MAS). These include loss of equilibrium, lethargic swimming, gasping at surface, hemorrhages, bulging eyes, opacity of corneas, swollen abdomen containing cloudy or bloody fluid, and chronic with low daily mortality. In the Philippines, severe disease outbreaks caused by *Aeromonas hydrophila* noted in both low-volume and high-density during rainy season and cold months were first reported by Yambot (1998). *V. cholerae* on the other hand, was among the commonly isolated bacteria in diseased tilapia farmed in Taal Lake (Limbauan 2018). According to other reports, bacterial species in these two genera may also demonstrate co-infection (Abdelsalem et al 2021).

In addition, zoonotic potentials have been documented in these two groups. Zoonosis may be acquired through ingestion of contaminated seafood and water and/or direct exposure to water via open wounds, which pose higher risks in the development of human illnesses (Baker-Austin et al 2018; Haenen et al 2023). Clinical manifestations of *Vibrio* infections include gastroenteritis, septicemia, sepsis, wound, eye, and ear infections, and cholera (Baker-Austin et al 2018). The likely mode of *Aeromonas* infection for humans is through drinking untreated water, which causes human enteritis (Haenen et al 2023). Horizontal transmissions via water, food and feces were identified as possible transmission routes of *Aeromonas* (Haenen et al 2023).

Presently, there is limited published research in the quantification and identification of bacteria in tilapia aquaculture in the Philippines as seen in the works of Tahiluddin & Terzi (2021) and Pakingking et al (2022). Monitoring bacterial diversity and quantity in aquaculture is important in developing protocols to prevent disease occurrences (Al-Harbi & Uddin 2005). Hence, this study was conducted to substantiate the knowledge gap about the occurrences of Gammaproteobacteria in association with the water quality parameters and the influence of farm management practices.

Material and Method

The study site. This study was conducted in the municipalities of Minalin and Magalang, Pampanga, the Philippines from October to November 2023. Minalin is situated in the southwest capital town of San Fernando and known to comprised a huge land area allotted for tilapia farming (2728.69 ha total area) (Reyes 2018). The town of Magalang is located 20.79 km south of San Fernando, Pampanga (PhilAtlas 2024), and is known to produce tilapia through pond production. Tilapia grow-out operators in Minalin typically utilize water from the river for fish farming, while those in Magalang use water from creeks and deep wells. Selection of farms intended for this study was based on their dependence on river water and deep well as water sources for tilapia grow-out operations. A total of 5 farms for each of the said municipalities were identified (Figure 1A and 1B). The geographic location of each sampling site was identified using GPS coordinates recorded in the GeoCam application installed in an android phone.

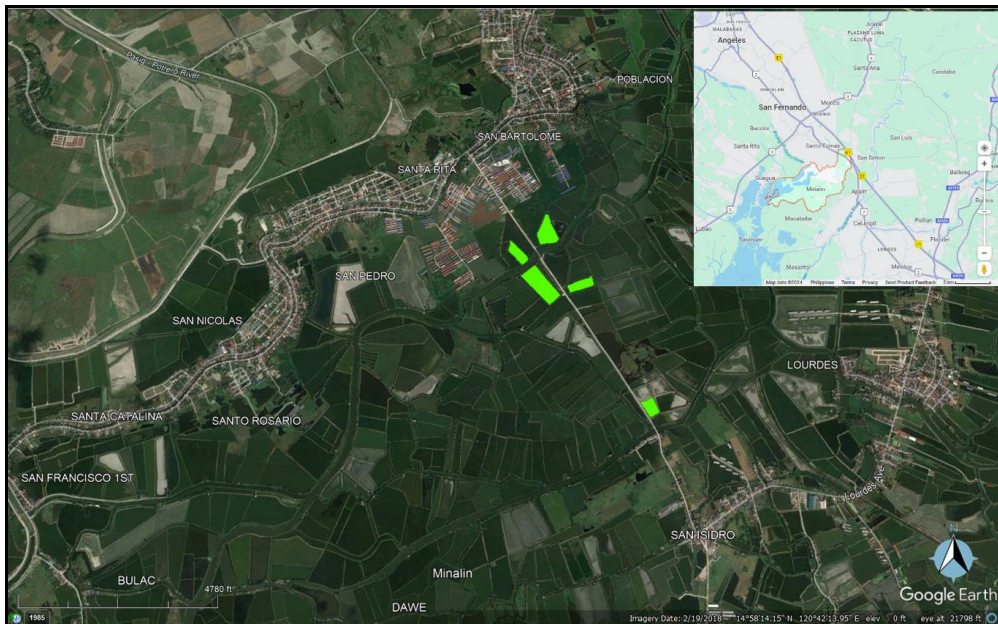


Figure 1A. Sampling sites in Minalin, Pampanga (exported from Google Earth 2024).



Figure 1B. Sampling sites in Magalang, Pampanga (exported from Google Earth 2024).

Survey of farm management and practices. Purposive sampling was employed to attain the target respondents in this study. A face-to-face interview guided with a structured questionnaire was administered to gather data regarding the farm management and practices of tilapia operators. Identified respondents were oriented with the objectives of the study and given confidentiality assurances. An informed consent document was provided and signed by the respondents (N=10). The survey questionnaire includes personal information, farm profile, pond preparation, water management, feeds and feeding management, fish health information, antibiotic administration and other supporting medication, farm biosecurity measures, and farm economics data. The obtained information was used to relate with the findings of the study.

Analysis of water and sediment quality. *In situ* assessment of water parameters such as temperature, dissolved oxygen (DO), salinity, pH and total dissolved solids (TDS) was

conducted using a DO meter Proi10 model YellowSpring, a portable handheld refractometer and pen-type pH and TDS monitoring device. 3 L of water sample was collected in three different locations in each farm and submitted to the Bureau of Fisheries and Aquatic Resources III, City of San Fernando, Pampanga, for ammonia (NH₃-N) analysis. Spectrophotometry and electrometry were carried out to determine the ammonia content of the water samples. Approximately 2 kg of composite sediment samples were also collected in each farm and air-dried prior to submission for phosphorous, organic matter, and total nitrogen analysis at the Department of Agriculture Regional Field Office III Soils Laboratory, City of San Fernando, Pampanga. Methods employed for the analysis of sediment parameters were the Olsen method, Walkley-Black colorimetric method, and Kjeldahl method, respectively.

Collection and analysis of samples. Fish, water and sediment samples were collected in each farm. 30 fish specimens (n=3 per farm) were randomly caught using a cast net. The total length and weight of the fish were measured and observations for any clinical signs of disease were conducted prior to aseptic dissection. The Fulton's condition factor was estimated using the following formula (Pauly 1984):

$$\text{Condition factor} = W*100/L^3$$

Where: W - weight of the fish (g); L - length of the fish (cm).

Individual fish was euthanized by means of immobilization and inactivation through pithing, and placing it in basin containing water and ice (Bautista et al 2022). Composite samples of fish brain, skin, kidney, liver, and intestine were obtained and placed in sterile centrifuge tubes. 100 mL of water sample and approximately 100 g of sediment sample were likewise collected in the same sampling point in each farm. Water samples were obtained approximately within 25-30 cm below the water column using sterile polyethylene (PE) bottles (Abioye et al 2021). In addition, samples from the river water in Minalin, Pampanga, were also collected. The pond bottom sediment samples were taken using a steel trowel garden scoop from a depth of 5 cm (Boyd et al 2002) and placed in sterile PE bags. All samples were kept in ice boxes and transported under chilled condition. Bacteriological analysis was performed at the Central Luzon State University – Freshwater Aquaculture Center Pathology Laboratory, Science City of Munoz, Nueva Ecija.

Bacterial isolation and quantification. Preparation of fish and environmental samples for bacterial isolation and quantification followed the protocol of Pakingking et al (2022) with minor modifications. 1 g of composite parts of fish samples (brain, skin, kidney, liver and intestine), 1 g of sediment samples, and 1 mL of water samples were separately prepared and homogenized in a 9 mL of 0.85 sterile normal physiological saline to make a 10⁻¹ dilution. Each of the samples were individually serially diluted up to 10⁻⁶. A 100 µL of aliquot of each diluted sample was spread in TCBS agar (Himedia, India) plates and incubated upright at room temperature for 24 h. In addition to serially diluted water samples, 1 mL of pond water sample was directly spread-plated in the TCBS agar. Thereafter, plates with 30-300 colonies were used for the quantification of presumptive Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) (Al-Harbi & Uddin 2005; Pakingking et al 2022). Bacterial counts were calculated using the following formula:

For one dilution with valid count (Rabadon et al 2022):

$$\text{CFU g}^{-1} \text{ or CFU mL}^{-1} = (\text{number of colonies} \times \text{dilution factor})/\text{final volume plated}$$

For consecutive dilutions with valid count:

$$N = \Sigma C / [(1 \times n_1) + (0.1 \times n_2) \times (d \times V_p)]$$

Where: N - number of colonies per mL or g of a product; ΣC - sum of all colonies on all plates counted; n1 - number of plates in first dilution counted; n2 - number of plates in second dilution counted; d - dilution from which the first counts were obtained; Vp - volume plated.

The proportion of green and yellow colonies in each TCBS agar plates was determined following the formula described by Pakingking et al (2022):

Number of yellow or green colonies / total number colonies counted x 100%

Meanwhile, the prevalence rate (%) of presumed Gammaproteobacteria in each sample was identified as follows (Reyes et al 2021):

Prevalence rate of Gammaproteobacteria = Number of positive samples / total number of samples x 100.

Data treatment and statistical analyses. Descriptive statistics, such as mean, standard error, and the minimum and maximum values of the obtained data were presented. Differences regarding the condition factor, water quality parameters, and bacterial count among the study sites were evaluated by employing independent T-test. Values in colony forming unit per g or mL (CFU g⁻¹ or CFU mL⁻¹) were log10 transformed prior to analysis to satisfy the criteria for a normal distribution. Pearson product-moment correlation coefficient (R) was performed to determine the relationship of bacterial count in water and sediment to the obtained water parameters. Data treatment and statistical analyses were carried out using Microsoft Excel 2021 and Statistical Tool for Agricultural Research (STAR) version 2.0.1.

Results and Discussion. The morphometric characteristics of fish samples from Minalin and Magalang farms is presented in Table 1. The obtained values for mean body weight indicate that the fish were in adult stage (>25 g) (FAO 2024). Based on minimum values, however, some of the collected samples in both sites were in sub-adult stage (10-25 g) (FAO 2024).

Table 1
Morphometric analyses (mean±standard error) of collected Nile tilapia (*Oreochromis niloticus*) in Minalin and Magalang, Pampanga farms

Characteristics	Minalin Farms			Magalang Farms		
	Mean	Min	Max	Mean	Min	Max
Total length (cm)	20.43±1.81	8	27	15.47±1.18	9	26
Body weight (g)	208.8±33.50	10	389	97.6±23.06	15	347

Table 2 presents the mean condition factor of collected tilapia samples in Minalin and Magalang farms with K values of 2.00±0.07 and 2.04±0.08, respectively. In this study, the computed K values, which were greater than 1, denote that the fish were healthy and biologically active (Ngodhe & Owuor 2019).

Table 2
Fulton's condition factor (FCF) (mean±standard error) of Nile tilapia (N=30) collected in Minalin and Magalang, Pampanga

Municipality	FCF*	Remarks
Minalin, Pampanga	2.00±0.07	Healthy
Magalang, Pampanga	2.04±0.08	Healthy

Note: comparison of means (±SE) between rows are not statistically significant (p>0.05).

Aside from the calculated condition factor, the fish samples from both municipalities exhibit no clinical signs, being classified as presumably healthy. The result corresponds with the suitability of the obtained key physicochemical parameters of the aquaculture pond water such as temperature, dissolved oxygen and pH.

Temperature is the most important parameter among other variables, as it has pronounced effect on both chemical and biological processes (Boyd & Litchkoppler 1979). This parameter influences the metabolic rate of fish, affecting their energy balance, locomotor and feeding behaviour (Volkoff & Rønnestad 2020). The best growth and feed utilization of Nile tilapia were observed at 26° and 30°C (Azaza et al 2008). DO is also a significant factor that influences the condition of the fish. Low levels of DO in aquaculture may lead to stress, poor appetite, slow growth, disease susceptibility and even mortality (Boyd & Hanson 2010). In general, the recommended minimum DO requirement for tropical freshwater fish is 5 mg⁻¹ (80% saturation) (Mallya 2007). Tilapia, however, could tolerate DO concentration down to 0.1 mg L⁻¹, but maximum growth can be attained at DO levels greater than 3 mg L⁻¹ (El-Hack et al 2022). In terms of pH, the ideal level for most freshwater species ranges from 6.5 to 9 (PHILMINAQ 2004). Consequently, the pH values obtained in this study are within the optimum levels considered best for fish production (Boyd & Litchkoppler 1979).

Analysis of water quality parameters of tilapia grow-out farms. The computed mean values of the physicochemical parameters, such as water temperature, DO, and pH, and organic matter in sediment in both municipalities were within the recommended levels. Additionally, TDS in Magalang farms also displayed suitability for tilapia farming. The other parameters lie beyond permissible limits according to the cited references (Table 3). The levels of total nitrogen in sediment, however, posed indeterminate suitability for tilapia aquaculture due to the unavailability of references for comparison. Results obtained may associate with the farm management and practices of the tilapia farmers, and may also possibly link with the quality of the water source.

Analysis of water parameters between farms in Minalin and Magalang, Pampanga revealed significant variations in the computed mean values for TDS, salinity, phosphorous, organic matter and total nitrogen (Table 3). All of the aforementioned parameters exhibited higher readings in Minalin compared to Magalang. Significant differences may relate to the use of different water sources between the two study sites. Notably, farms in the municipality of Minalin rely on the river as their water source for fish production, while farms located in Magalang are deepwell-dependent. The river traversing the Minalin farms is a tributary of the Pampanga River system. According to analysis using Palmer's pollution index, the Upper Eastern Pampanga River has high organic pollution (Reyes et al 2018). Generally, the TDS include various elements, compounds, organic ions and other ions present in the water. Whereas, the levels of these materials in the water are influenced by several inputs such as urban and fertilizer run-off, wastewater and septic effluent, soil erosion, decomposition of dead plants and animals, and geological features in the area (PHILMINAQ 2004). The total concentration of all dissolved ions in a natural water is referred to as the salinity (Boyd & Litchkoppler 1979). The salinity levels obtained in this study classify water from Minalin farms as oligohaline (0.5-4.0 g L⁻¹) and from Magalang farms as freshwater (<0.5 g L⁻¹) (Cañedo-Argüelles et al 2013). Salinization in the river is caused by primary and secondary factors as defined by Cañedo-Argüelles et al (2013). Primary salinization includes the complex and dynamic function of the past and existing climate, weathering of the catchment, distance from the sea, topography and vegetation. Secondary salinization involves anthropogenic activities like irrigation, mining, deicer for roadways, and discharges from sewage and industrial activities. In terms of higher phosphorous, organic matter and total nitrogen content in Minalin farms, this may also be exacerbated by the influx of water from the river carrying accumulated organic and inorganic sources discharged by neighboring aquaculture and agricultural farms adjacent to the same river system.

Table 3

Water and sediment quality parameters (mean±standard error) of tilapia (*Oreochromis niloticus*) grow-out farms (N=10) in Minalin and Magalang, Pampanga, Philippines

Parameters	Minalin farms			Magalang farms			Recommended level
	Mean	Min	Max	Mean	Min	Max	
Water parameters							
Temperature (°C)	30.21±0.23 ^a	28.20	31.10	30.11±0.29 ^a	28.90	32.10	20-35 ¹
Dissolved oxygen (mg L ⁻¹)	5.35±0.31 ^a	3.21	6.71	4.74±0.67 ^a	1.49	7.92	3.0 ²
pH	7.77±0.11 ^a	7.28	8.34	7.68±0.17 ^a	6.95	8.49	6.5-9.0 ^{1,2,3}
Total dissolved solids (ppm)	1578.67±142.58 ^a	1010.00	2540.00	154.13±9.03 ^b	112.00	213.00	≤400 ¹
Salinity (ppt)	1.59±0.14 ^a	1.01	2.54	0.16±0.01 ^b	0.11	0.21	10-15 ²
NH3-N (mg/L)	0.42±0.11 ^a	0.01	1.09	0.53±0.17 ^a	0.07	1.91	<0.300 ⁴
Sediment parameters							
Phosphorous (ppm)	31.70±1.61 ^a	21.87	40.88	16.15±2.26 ^b	5.44	33.47	80 – 100 ¹
Organic matter (%)	1.85±0.21 ^a	1.01	3.76	1.14±0.23 ^b	0.25	3.89	≤5.8 ¹
Total nitrogen (%)	0.09±0.01 ^a	0.05	0.14	0.05±0.01 ^b	0.01	0.14	-

Note: different letters show significant differences ($p < 0.05$); numerical superscripts in the final column denote the corresponding references, as follows: ¹Reyes et al (2021); ²BFAR-NFFTC; ³Boyd & Pillai (1984); ⁴BFAR Regional Office III (2023).

Prevalence and load of presumptive Gammaproteobacteria isolated in tilapia grow-out farms. Table 4 shows that fish, water and sediment samples from Magalang, Pampanga yielded 100% prevalence rate of presumed Gammaproteobacteria. Only the sediment samples from Minalin, Pampanga displayed 100% positivity for these viable bacteria. 14 fish and 12 water samples contained detectable viable bacteria resulting to 80% and 93.33% prevalence rate, respectively.

The genus *Aeromonas* and *Vibrio* are Gram-negative bacteria both classified under class Gammaproteobacteria (Bhowmick & Bhattacharjee 2018; Sampaio et al 2022). *Aeromonas* was initially designated under the family Vibrionaceae based on its phenotypic characteristics. But with subsequent analyses using molecular identification, its distinct evolutionary history was defined, prompting a reclassification into a new family now referred to as the Aeromonadaceae (Colwell et al 1986). The high prevalence rate (80-100%) reflects the ubiquity of these genera in soil and aquatic environments (Igbiosa et al 2012; Percival & Williams 2014; Bhowmick & Bhattacharjee 2018; Sampaio et al 2022). Suitability of the existing water condition or the adaptability to a broad range of environments are the possible factors that influence the omnipresence of these bacteria. In particular, temperature and salinity are considered vital factors affecting the distribution and existence of bacterial populations. Most of the species under these two genera thrive in varying temperature spectra and could be isolated from freshwater to marine waters (Wright et al 1996; Louis et al 2003; Chen et al 2006; Deeb et al 2018; Sampaio et al 2022; Majeed et al 2023). Moreover, these bacteria were also reportedly isolated in healthy and diseased tilapia fish (Yambot 1998; Pauzi et al 2020; Zaher et al 2021; Fauzi et al 2021; Elgendy et al 2022; Pakingking et al 2022). Furthermore, growth of these bacteria using TCBS agar was observed in several other studies. Growth of *Aeromonas* spp. occurs in TCBS agar, thus, concluding the culture medium's non-selectivity (Esteve et al 2003; Aboyadak et al 2017; Al-shalah 2017).

Table 4
Prevalence of presumptive Gammaproteobacteria (*Aeromonas* and *Vibrio* spp.) in fish and environmental samples obtained from tilapia (*Oreochromis niloticus*) grow-out farms in Minalin and Magalang, Pampanga, Philippines

Sample	Number of analyzed samples	Number of positive samples	Percentage (%)
Minalin, Pampanga			
Fish	15	14	93.33
Water	15	12	80
Sediment	15	15	100
Magalang, Pampanga			
Fish	15	15	100
Water	15	15	100
Sediment	15	15	100

Bacterial concentration in fish, water and sediment vary significantly ($p < 0.05$) between farms in Minalin and Magalang, Pampanga (Figure 2). The \log_{10} transformed of presumptively identified Gammaproteobacteria counts in both fish (5.18 ± 0.41 CFU g^{-1}) and water (3.54 ± 0.07 CFU mL^{-1}) from Magalang was higher compared to Minalin with 3.4 ± 0.28 CFU g^{-1} and 2.32 ± 0.34 CFU mL^{-1} in fish and water, respectively. As for the sediment, bacterial count in Magalang (3.62 ± 0.12 CFU g^{-1}) was lower than in Minalin (4.4 ± 0.25 CFU g^{-1}). In addition, comparison of mean bacterial count in water between the farms and river (3.03 ± 0.07 CFU mL^{-1}) in Minalin showed non-statistical difference ($p > 0.05$). Yellow colonies (55.97-94.37%) prevalently grew in TCBS agar plates than green colonies (5.63-44.03%) among samples in both municipalities.

Possible reasons for the significantly higher bacterial counts in fish and water in Magalang farms may pertain to the following aspects: smaller farm size averaging 3.3 ha; the volume of water in the ponds as is computed based on the maintaining mean

water depth (1.12 m) and area; no to seldom water change in tilapia farms; and the average stocking density (4.4 fish m⁻²) with no significant difference to Minalin farms (5.75 fish m⁻²). In terms of bacterial abundance in sediment, Minalin had higher loads compared to Magalang. This may due to the longer duration of culture (>6 months) and the intrusion of river water that carries other organic and inorganic sediments. The direct relationship in the increase of bacterial loads towards the rearing time was given emphasis by Alfiansah et al (2018). Elevation of organic matter in the pond may associate to the excreted materials of the cultured fish, uneaten feed, as well as the decaying of macro- and microphytes. In the present study, the phosphorous, organic matter, and total nitrogen level in sediment were significantly higher in Minalin compared to Magalang. The bacteria may find the sediment as a favorable environment for reproduction due to its nutrient content (Sherer et al 1988; Davies et al 1995; Reyes 2020).

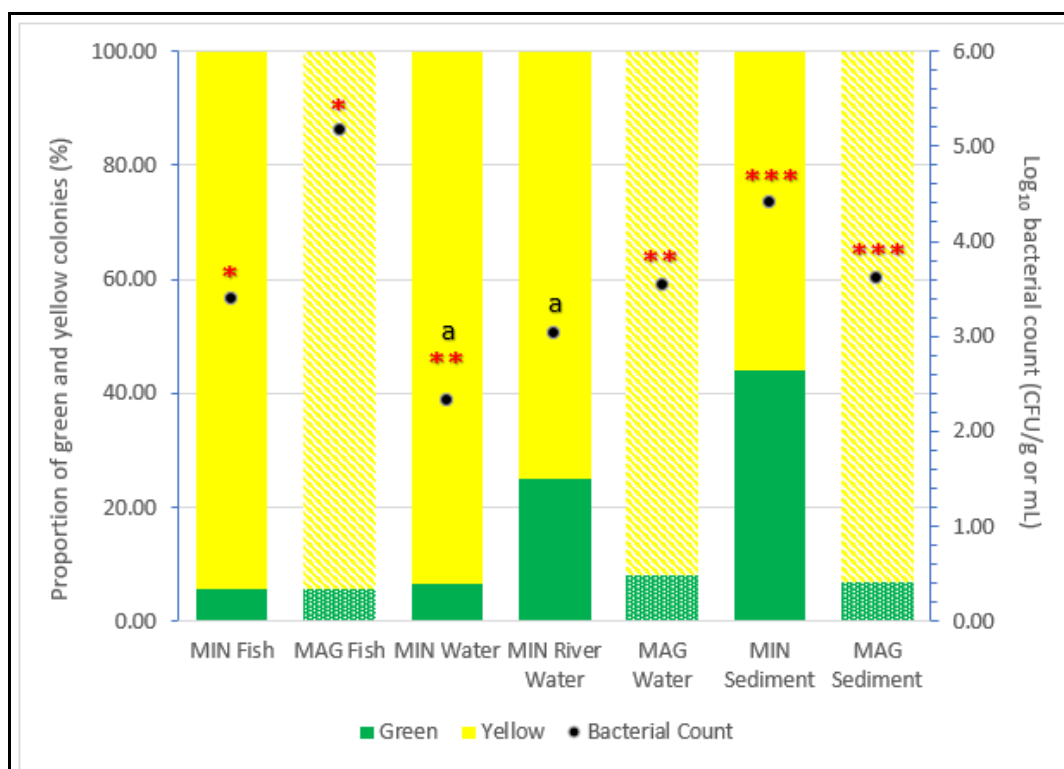


Figure 2. Bacterial count of presumptive Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) in fish, water and sediment collected in the municipality of Minalin (MIN) and Magalang (MAG), Pampanga, Philippines; * in fish samples, ** in water samples, and *** in sediment samples denote significant differences of mean bacterial counts between the two municipalities in each parameter ($p < 0.05$); the same superscripts indicate no statistical difference ($p > 0.05$).

The composition percentage of yellow and green colonies is consistent with the recently published works of several authors (Pakingking et al 2022; Aquino et al 2023). Higher percentage of yellow colonies over green colonies may indicate dominance of isolated sucrose-fermenting *Vibrio* species (Pakingking et al 2022). In TCBS agar, *V. cholerae* and *V. alginolyticus* appear yellow because of the fermentation of sucrose, while non-sucrose fermenting Vibrionaceae, which include *V. parahaemolyticus* and *V. vulnificus* have green colonies (Kaysner et al 2004). Despite of the considerably high selectivity of TCBS agar for *Vibrio* isolation, several studies shown the growth of *Aeromonas* spp. in the said media (Shikongo-Nambabi et al 2012; Wejdan et al 2014; Lijon et al 2015; Al-shalah 2017). *Aeromonas* spp. was described to have a shiny yellow appearance and 1-3 mm in diameter in TCBS (Wejdan et al 2014; Lijon et al 2015; Al-shalah 2017). *A. veronii* isolated in shrimp and tilapia hatchery in Bangladesh exhibits varying coloration in TCBS

(i.e., yellow, blue, blue green and dark green) (Hasan et al 2017). In relation to salinity tolerance, the yellow and green colonies possibly dictate the presence of *V. cholerae* and *Aeromonas* spp. *V. cholerae* stands out among *Vibrio* species due to its unique ability to tolerate the absence of salt despite of its typical occurrence in estuarine and coastal environments (Daboul et al 2020). Furthermore, studies show that *V. cholerae* could tolerate different concentrations of salt (Fakruddin et al 2011). A comprehensive review conducted by Majeed et al (2023) notes that diverse *Aeromonas* spp. were prevalent in freshwater to seawater environments.

Correlational analyses between environmental parameters and presumptive Gammaproteobacteria counts. The relationship between environmental parameters and bacterial counts in Minalin and Magalang farms is presented in Tables 5 and 6. The present study showed that there was a significant moderate relationship of DO, TDS, salinity and total nitrogen to the abundance of presumed Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) in Minalin ($p < 0.05$). In Magalang, not a single parameter displayed any significant effect to the occurrence of viable bacteria.

Table 5

Correlation between water quality parameters and bacterial count of presumptively identified Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) in tilapia (*Oreochromis niloticus*) grow-out farms in Minalin and Magalang, Pampanga

Parameters	Minalin	Magalang
Temperature	- 0.466	- 0.017
Dissolved oxygen	- 0.525*	0.116
pH	0.196	0.440
Total dissolved solids	0.602*	- 0.274
Salinity	0.611*	- 0.265
NH ₃ -N	0.474	- 0.467

Note: * - correlation is significant at the 0.05 level (2-tailed).

Table 6

Correlation between sediment quality parameters and bacterial count of presumptively identified Gammaproteobacteria (*Aeromonas* spp. and *Vibrio* spp.) in tilapia (*Oreochromis niloticus*) grow-out farms in Minalin and Magalang, Pampanga

Parameters	Minalin	Magalang
Phosphorous	- 0.272	- 0.252
Organic matter	- 0.388	0.005
Total nitrogen	- 0.516*	0.091

Note: * - correlation is significant at the 0.05 level (2-tailed).

The resulting inverse relationship of bacterial counts to DO corroborates with the conducted microbiological survey in *A. hydrophila* in Chesapeake Bay and its tributaries by Kaper et al (1981), and in *Vibrio* spp. as studied by Froelich et al (2019). These microorganisms were described to be facultative anaerobes (Youngren-Grimes et al 1988; Tomás 2012). A reciprocal correlation of the total nitrogen content of sediment to *Aeromonas* spp. in the present study was also observed in accordance to the findings of Pinto et al (1998), but the nitrogen level was measured in river water. Limited works were also conducted on the relationship of total nitrogen levels to *Vibrio* spp. counts. Findings of the current study show inconsistency with the observations of Rosales et al (2022), whereas, *V. parahaemolyticus* has a significant positive association to total dissolved nitrogen. Consequently, the resulting inverse association of DO and total nitrogen to colony counts in both bacterial genera provide limited evidence to support the causal links.

Direct correlation of total dissolved solids and salinity to the abundance of bacteria in the pond water in Minalin expressed indiscernible results due to the nature of

habituation of *Aeromonas* spp. and *Vibrio* spp. These bacterial genera are recognized for their ubiquity and resilience to different environments (Vezzulli et al 2013; Canellas & Laport 2022; Canellas et al 2023). As stated earlier, TDS level is influenced by the intrusion of wastewater effluents (PHILMINAQ 2004). As such, isolation of *Aeromonas* spp. in treated municipal and urban wastewater effluents validates the existing relationship (Harnisz & Korzeniewska 2018; Skwor et al 2020). However, *Aeromonas* bacteria were also isolated in both untreated and treated drinking waters (Sen & Rodgers 2004; Ghenghesh et al 2014). As to salinity levels, *Aeromonas* spp. is prevalently isolated from surface water, groundwater, well water, pond water, freshwater, river water and seawater (Chacón et al 2003; Khajanchi et al 2010; Carvalho et al 2012; Hu et al 2012; Li et al 2015; Majeed et al 2023). Several studies showed that *Vibrio* spp. could thrive to a salinity from 0.05-27.6 ppt (Louis et al 2003; Deeb et al 2018). As an example, the first ever recorded isolation of *V. vulnificus* in freshwater and low-salinity ponds in Taiwan indicates that this bacterium has the ability to adapt in freshwater environments (Chen et al 2006). Notably, *V. vulnificus* is a halophilic marine bacterium (Li et al 2018), and occurrence is highest at salinity levels between 5 and 20 ppt (Deeb et al 2018). In addition, increment in the detectable *V. cholerae* was observed at salinity levels ranging from 2 to 14 ppt (Louis et al 2003).

Conclusions. Water parameters such as temperature, DO, water pH and organic matter in sediment were within the suitable threshold for tilapia aquaculture in both municipalities. Significantly higher TDS, salinity, phosphorous, organic matter and total nitrogen levels was recorded in Minalin, Pampanga, which may relate to the influence of the river as a water source. The river carries organic and inorganic sources from the terrestrial environment that lies across the region of the river system. Further findings revealed a direct relationship between gammaproteobacterial counts and TDS and salinity in river-dependent farms. A reciprocal relationship to the bacterial abundance was noted to DO and total nitrogen. Significantly higher bacterial counts in fish and water in deep-well dependent farms from Magalang, Pampanga may relate to the basic farm features and the frequency of water change. In terms of sediment's mean bacterial abundance, Minalin farms acquired higher counts, associated with the influence of river water quality. Despite of the abundance of Gammaproteobacteria in both fish and water samples, all of the collected fish were healthy and possessed no clinical signs of disease. Although there was no disease outbreak during the study, the routine monitoring of bacteria and assessment of environmental parameters in tilapia farms is vital for sustainable production. It can be noted that prior to sampling, some of the respondents had experienced fish kill incidences in their farms.

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