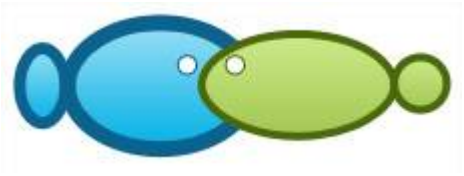

Quantitative analysis of *Vibrio* spp. and environmental quality assessment of tilapia grow-out ponds in Minalin, Pampanga, Philippines

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Abstract. Generally, *Vibrio* are well-known pathogen causing high fish mortalities and economic losses in aquaculture industry. This bacterial genus thrives ubiquitously in the aquatic environment. Bacterial disease outbreak in the aquatic environment occurs when environmental parameters are outside the suitable range causing stress to the fish alongside with the attack of opportunistic pathogens like *Vibrio* spp. In the previous studies, this bacterial flora caused disease and recorded mortalities in tilapia. Therefore, this study quantified the presumptive *Vibrio* spp. in Nile tilapia (*Oreochromis niloticus*) and environmental samples obtained in the grow-out earthen ponds in Minalin, Pampanga. Majority of the environmental water quality parameters were within the recommended levels for aquaculture except for salinity, Secchi disc visibility, total dissolved solids, electrical conductivity and sediment pH. The observed prevalence rate of presumptive *Vibrio* spp. was 100% in both fish and water samples while only 65% in sediment sample. Further analysis however shown that the highest count was obtained from sediment with 10^8 CFU g^{-1}. Presumptive *Vibrio* spp. count in both fish and water ranged from 10^6 to 10^7 CFU g^{-1} and CFU mL^{-1}, respectively.. Moreso, yellow colonies were dominant (≥ 86.00%) in all collected samples. Correlation analysis showed no significant relationships between bacterial counts and the environmental quality parameters. Despite of the high load of presumptive *Vibrio* spp., all of the collected fish were apparently healthy.

Key Words: count, environmental quality, Nile tilapia, prevalence, *Vibrio* spp.

Introduction. Aquaculture is the fastest growing sector for food production (Azra et al 2021; Nagappan et al 2021) and a sustainable option to attain food security (Azra et al 2021) in response to continuously overgrowing global population. This also remains potential for expansion as productive agricultural lands beset with rampant conversion into residential, commercial, and industrial purposes (Moya et al 1994), whilst the marine fisheries resources are faced with continuous decline (Eluriaga et al 2019).

Tilapia is the second-most important aquaculture finfish commodity in the world (Miao & Wang 2020). Also, it is ranked second most preferred cultured aquatic animal next to milkfish (*Chanos chanos*) in the Philippines despite of being an introduced species (Tahiluddin & Terzi 2021). Central Luzon is the leading tilapia producer contributing 45.71% to the country's total tilapia production in 2020 (BFAR 2022). Among its provinces, Pampanga is the largest tilapia contributor in terms of volume of production from 2018 to 2020 (PSA 2020). The province also has the largest area of tilapia fishponds in the region, thus dubbed as "Tilapia Country" (Guerrero 2018). Further, intensification of tilapia culture practices and the massive Pampanga River as the principal source of water for aquaculture is also accounted in its remarkable tilapia production (Reyes 2020). Despite of these notable performances however, tilapia industry is confronted with challenges that could have adverse impact on its production and profitability. Among these were high water temperature, disease outbreaks, inadequacy of water supply, and natural calamities like flooding (Guerrero 2019; BFAR 2022). Disease occurrences and fish kill incidence has been reported in the province, and this is found associated with several factors like unsuitable water quality for aquaculture (i.e., elevated temperature, low dissolved oxygen), high level of phosphorous, high total

bacterial load, poor land preparation practices, poor responses on problems related to water quality and diseases, and water pollution (Vera Cruz & Reyes 2015 in Reyes 2020; Asian Development Bank [ADB] 2005). According to Reyes (2020), there were 14 associated bacteria in the collected tilapia fish in the different municipalities in the province relative to the fish kill incidence and that includes *Vibrio vulnificus* and *Vibrio parahaemolyticus.* Further assessment had shown that these bacterial microbiota were congruently present in Pampanga River and irrigation canals (Esteban 2018).

Vibrio spp. are a well-known prevalent cause of high fish mortalities and considerable economic losses worldwide (Abdelaziz et al 2017). Although tilapia is known as a hardy fish, disease outbreaks caused by this bacterial genus in aquaculture farms has been reported (Al-Sunaiher et al 2010; El-Sayed et al 2019; Sumithra et al 2019; Elgendy et al 2022). In the Philippines, *V. cholerae* was one of the commonly isolated bacteria from diseased tilapia cultured in intensive cage farming systems in Taal Lake (Limbauan 2018). Further, these bacteria are documented with zoonotic potential. *Vibrio* infection to human is attributed to direct contact with environmental strains through wound exposure and in ingestion of raw, undercooked, or mishandled seafood (Letchumanan et al 2014; Kokashvili et al 2015; Baker-Austin et al 2018). Clinical reports include gastroenteritis (McLaughlin et al 2005), sepsis, severe skin and soft-tissue infection including fasciitis and gangrene (Horseman & Surani 2011), skin and ear infections (Jacobs Slifka et al 2017), and acute otitis (Chen et al 2012). Increasing reports on the emergence of resistant strains to several antimicrobial drugs pose public health and aquaculture threat in *Vibrio*-related infection worldwide, thus, warrants ongoing surveillance (Mandal et al 2012; Letchumanan et al 2015; Loo et al 2020; Tan et al 2020).

Based on the recent reviews, there is still limited information on the surveillance and quantification of bacterial load in tilapia aquaculture ponds in the country wherein such information could serve as baseline in the formulation of disease management measures in aquaculture. Therefore, this study was conducted to quantify the prevalence and count of *Vibrio* spp. in tilapia growout ponds in Minalin, Pampanga and correlated with the present environmental conditions.

Material and Method

The study site. A total of eight tilapia growout farms situated along the Maniango river was selected as the study site. Two earthen ponds was chosen in each Barangays of Maniango, Saplad, Dawe and Bulac (Figure 1). Water sources of these ponds were derived from the river. Based on the survey of farm management practices, most (75%) of the farms practiced polyculture of tilapia with either milkfish or *vannamei* shrimp or both. Feeding operation in these farms was mostly in full feeding or given with commercial feeds but some also still applies either organic or chemical fertilizers for the production of natural foods in ponds. Currently, majority of the cultured tilapia is approximately ≥ 4 months old.

Water and sediment quality analyses. The determination of physico-chemical water parameters was conducted *in-situ* using Smart Sensor digital dissolved oxygen meter in measuring temperature and dissolved oxygen, Yieryi LCD digital pH meter for water pH, RCYAGO refractometer for salinity and Secchi disk for Secchi disc visibility. For the analysis of ammonia, five liters of pond water sample were collected in five different locations in each pond and submitted to the Bureau of Fisheries and Aquatic Resources Regional Office III. Ammonia (mg L^{-1}) was analysed through titrimetry, spectrophotometry and electrometry. Simultaneous collection of composite water sample was also done for the analysis of total dissolved solids (TDS) and electrical conductivity (EC) using TDS and EC pen type monitoring devices. In terms of sediment quality analysis, approximately 2 kg of composite sediment samples in each pond were collected and air dried. The dried soil samples (1 kg) were sent to the Department of Agriculture Regional Field Office III Soils Laboratory. The parameters measured were soil pH, organic matter, phosphorous and total nitrogen through potentiometric method (1:1 soil:water), Walkley-Black method, Olsen method and Kjeldahl method, respectively.

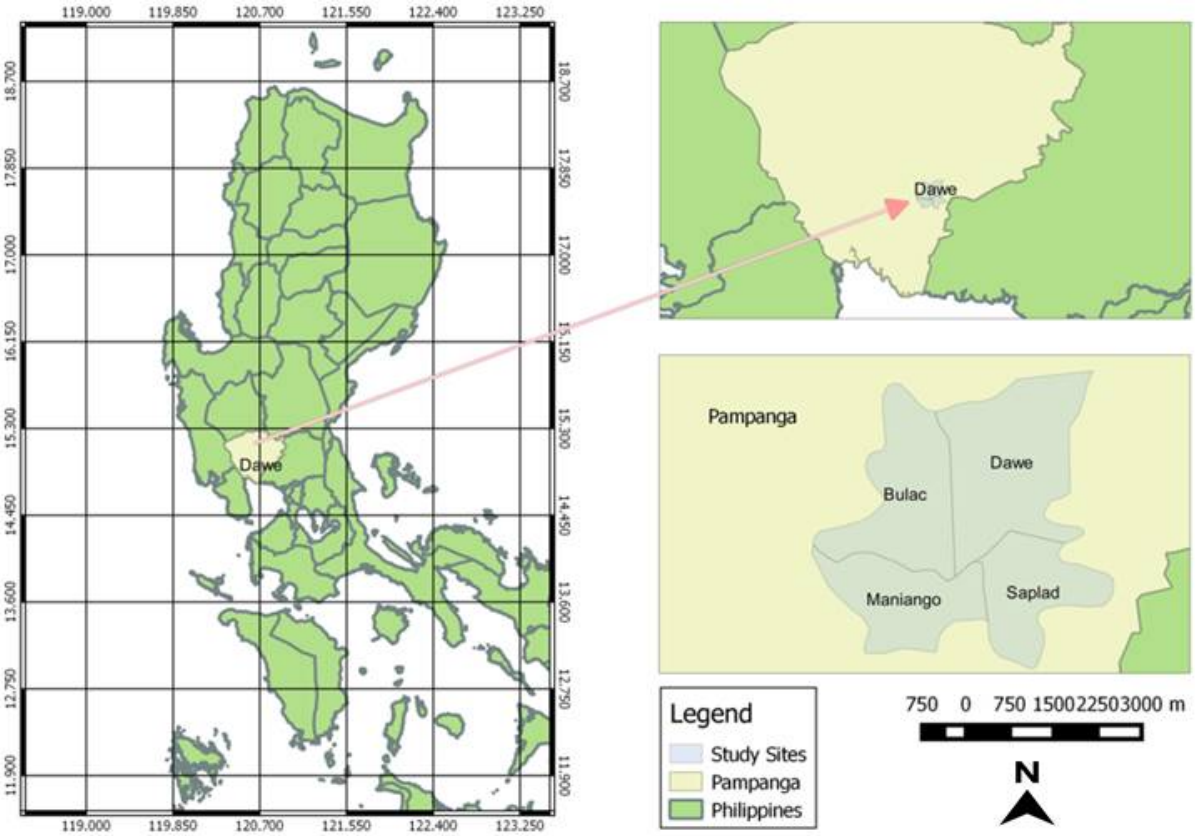

Figure 1. Map showing the study sites (Barangays Bulac, Dawe, Maniango and Saplad).

Collection of samples for bacteriological analyses. Fish, water and sediment samples for quantification of presumptive *Vibrio* spp. were collected in the same ponds where environmental quality analysis was conducted. A total of 40 fish was randomly collected from the eight farms (n = 5) using cast net. Collected samples were placed separately in a labelled and aerated plastic bag containing pond water. A total of 500 mL water sample were collected in five different locations of each pond. Water samples were obtained by dipping the 100 mL capacity sterile bottle within 25-30 cm of the water column (Abioye et al 2021). The pond bottom sediments were taken from a depth of 5 cm using steel trovel garden scoop (Boyd et al 2002) and were kept in Ziploc bags. Both of the environmental samples were temporarily stored in an insulated box filled with ice. All the samples were transported to the Central Luzon State University – Freshwater Aquaculture Center for immediate analysis.

Quantitative determination of Vibrio spp. Morphometric measurements such as standard length (cm) and body weight (g) of the fish were obtained prior to bacteriological analysis. Collected fish samples were also observed for any clinical signs of bacterial infections such as exophthalmia, corneal opacity, distended abdomen, hemorrhages and darkening in the body, necrotic fins, pale gills, and the presence of granulating lesions (Alapide-Tendencia & dela Peña 2001; El-Sayed et al 2019). Then, fish were identified as presumed healthy for the absence of any external abnormalities and presumed unhealthy for those exhibiting the aforementioned clinical signs. Prior to dissection, fish were euthanized by chilling in ice. Eye, skin, kidney, liver and intestine of the fish were aseptically obtained using sterile scissors and forceps. One gram of homogenate mixture of tilapia organs was diluted in a 9 mL of alkaline peptone water (APW) (Conda Pronadisa, Spain) (Traore et al 2014). The prepared 10^{-1} dilution was serially diluted up to 10^{-4} and incubated at room temperature for 6 hours. Thereafter, a 100 µL of each diluted samples were spread plated onto the thiosulphate citrate bile salt sucrose (TCBS) agar plates (HiMedia, India) and incubated upright at room temperature for 24 hours. Plates with ≥ 30 to 300 colonies were used for quantification of

presumptive *Vibrio* spp. and was expressed as colony forming unit per gram (CFU g^{-1}) (Al-Harbi & Uddin 2005; Pakingking et al 2022). The percentage composition of green and yellow colonies in each TCBS agar plates were calculated using the formula: number of yellow or green colonies counted/total number colonies counted x 100 (Pakingking et al 2022). The prevalence rate of presumptive *Vibrio* spp. in fish samples was determine following the formula: number of positive samples/total number of samples x 100 (Reyes et al 2021).

The presumptive *Vibrio* spp. from the water samples was isolated by direct inoculation of one mL of sample into 9 mL of APW (Traore et al 2014) and serially diluted up to 10^{-4}. The prepared dilutions were incubated at room temperature for 6 hours, and 100 µL was spread onto TCBS agar plates. A 24-hour incubation of the TCBS agar plates was done at room temperature. The calculation for the presumptive *Vibrio* spp. count, expressed in CFU mL^{-1}, the proportion of green to yellow colonies in each plates, and the prevalence rate was the same with the process applied for the fish samples.

As for the isolation of *Vibrio* spp. in sediments, one gram of sample was homogenized with 9 mL of APW making a 10^{-1} dilution (De Menezes et al 2017) and then serially diluted up to 10^{-6}. Prepared serial dilutions were incubated at room temperature for 6 hours. Afterwards, 100 µL of each diluted sample were pipetted and spread onto the TCBS agar plates and incubated for 24 hours at room temperature. Thereafter, similar procedure as described in the fish and water samples was employed for the quantitative determination of presumptive *Vibrio* spp. (CFU g^{-1}), in the computation of the proportion of green to yellow colonies in each plates, as well as with the calculation of prevalence rate.

Statistical analysis. Descriptive statistics of the collected data was determined and presented in tabular form. The presumptive *Vibrio* spp. counts in CFU g^{-1} or CFU L^{1} was transformed into Log_{10} values prior to analysis. Pearson's Product Moment Correlation was employed to identify the environmental variables influencing the presumptive *Vibrio* spp. count. Data treatment and statistical analyses were performed using Microsoft Excel 2016 and Statistical Tool for Agricultural Research version 2.0.1.

Results. The mean values±SE of the environmental quality parameters in the tilapia grow-out farms and the recommended levels are presented in Table 1. All of the environmental parameters were within the optimum range for pond aquaculture except for salinity, Secchi disc visibility, TDS, EC and sediment pH. Notably, reference for comparison of the determined sediment total nitrogen (TN) is unavailable. Therefore, the current TN content could not identify whether it is suitable for pond aquaculture.

Table 1

The environmental parameters (mean±standard error) of tilapia grow-out farms (N = 8) in Minalin, Pampanga, Philippines and the corresponding recommended level for aquaculture

Parameters	*Mean*	*Minimum*	*Maximum*	*Recommended level*
Water parameters				
Temperature (°C)	32.05±1.42	29.88	33.58	20-35[a]
Dissolved oxygen (mg L^{-1})	4.57±0.17	4.19	4.73	3.0[b]
Salinity (ppt)	0.90±1.51	0.00	4.00	10-15[b]
pH	8.30±0.48	7.57	8.99	6.5-9.0[a,b,c,d]
Secchi disc visibility (cm)	26.94±7.22	18.24	43.00	30-60[e]
TDS (ppm)	2,262.55±759.45	1,118.40	3,442.00	≤ 400[a]
EC (µs cm^{-1})	8,283.75±2779.73	4,194.00	12,178.40	100-2,000[f]
Total ammonia (mg L^{-1})	0.09±0.12	0.01	0.36	0.02-0.5[b]
Sediment parameters				
pH	6.44±0.84	4.68	7.40	7-8[a]
Organic matter (%)	2.99±2.21	1.08	6.76	≤ 5.8[a]
Phosphorous (ppm)	34.65±21.46	9.69	68.57	80-100[a]
Total nitrogen (%)	0.10±0.07	0.02	0.20	-

[a] Reyes et al (2021); [b] BFAR – NFFTC; [c] Boyd & Pillai (1984); [d] PHILMINAQ; [e] Boyd & Litchkoppler (1979); [f] Stone & Thomforde (undated).

Morphometric measurement and condition of collected Nile tilapia samples. The mean total length and body weight (mean±SE) of the Nile tilapia collected in eight grow-out ponds are presented in Table 2. Based on the body weights of the collected samples, fish were already in adult stage (> 25.0 g) (FAO 2023). Moreover, collected fish in all farms were apparently healthy due to the absence of any clinical signs.

Table 2

Morphometric measurement (mean±standard error) of Nile tilapia samples collected in Minalin, Pampanga, Philippines

	Mean	*Minimum*	*Maximum*
Total length (cm)	18.69±0.43	13.5	26.0
Body weight (g)	132.49±9.42	53.4	344

Quantitative data of presumptive Vibrio spp. Table 3 and Figure 2 respectively present the prevalence rate and the counts of *Vibrio* spp. in fish, water and sediment. The prevalence rate in fish and water were both 100% while only 65% in sediment samples. Despite of lower prevalence rate in sediment, the presumptive *Vibrio* spp. count is approximately > 1 Log10 CFU unit^{-1} higher (8.53) compared to fish (6.98) and water (7.11). In equivalence, sediment has a *Vibrio* spp. load of 10^8 CFU g^{-1} and a range of 10^6 to 10^7 in both fish (CFU g^{-1}) and water (CFU mL^{-1}). Yellow colonies that grew on TCBS agar plates was generally dominant (86.92-93.93%) over green colonies (6.07-13.08%) in both fish, water and sediment samples.

Table 3

Prevalence of presumptive *Vibrio* spp. in fish, sediment and water collected in tilapia grow-out ponds in Minalin, Pampanga, Philippines

Sample	*Number of analysed samples*	*Number of positive samples*	*Percentage (%)*
Fish	40	40	100.00
Water	40	40	100.00
Sediment	40	26	65.00

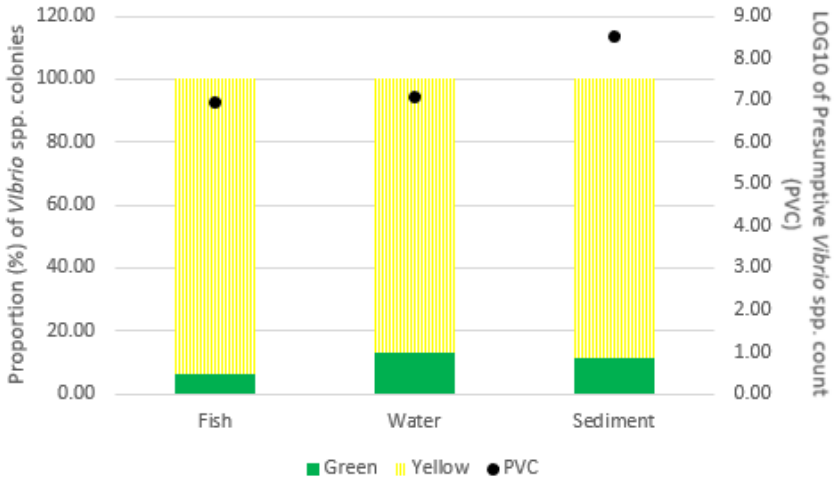

Figure 2. Presumptive *Vibrio* spp. counts (PVC) in fish, sediment and water collected in tilapia grow-out ponds in Minalin, Pampanga, Philippines. Data is presented with the percentage composition of green and yellow colonies that grew in the TCBS agar plates and the mean Log10 values (CFU mL^{-1} in water and CFU g^{-1} in fish and sediment) of the total viable count (TVC, 30 – 300).

Correlation of environmental parameters with presumptive Vibrio spp. counts. The present study showed that there is no significant relationship between environmental parameters and *Vibrio* spp. counts (Table 4). The dissolved oxygen, salinity and Secchi disc visibility positively correlated to bacterial counts in water and sediment. In addition, ammonia is also positively correlated to sediment *Vibrio* spp. count.

The sediment pH in relation to *Vibrio* spp. counts in water exhibits positive correlation. Among sediment parameters, only the phosphorous was found to have negative correlation with bacterial counts in the sediment.

Table 4

Relationship of presumptive *Vibrio* spp. counts from sediment (log CFU g^{-1}) and water (log CFU mL^{-1}) to the correlated environmental parameters in tilapia grow-out ponds in Minalin, Pampanga, Philippines

Parameters	*Water$_{PVC}$*	*Sediment$_{PVC}$*
Water parameters		
Temperature	-0.3858	-0.6646
DO	0.3604	0.2520
Salinity	0.5078	0.3962
Water pH	-0.2912	-0.2648
SDV	0.1028	0.3250
TDS	-0.1166	-0.6102
EC	-0.0695	-0.5428
Ammonia	-0.1794	0.3444
Sediment parameters		
pH	0.4857	0.0642
Organic matter	-0.1782	0.2976
Phosphorous	-0.1328	-0.1201
Total nitrogen	-0.1663	0.4159

PVC = presumptive *Vibrio* spp. counts; DO = dissolved oxygen; SDV = Secchi disc visibility; TDS = total dissolved solids; EC = electrical conductivity.

Discussion. Maintaining good water quality in aquaculture provides healthier fish, better production and profit. Poor water condition stresses the cultured aquatic animal making them susceptible to disease, and in worst case, causes fish mortality (Boyd 2017). Current water quality readings such as temperature (32.05±1.42°C), dissolved oxygen (4.57±0.17 mg L^{-1}), pH (8.30±0.48) and ammonia (0.09±0.12 mg L^{-1}) are within the optimum levels for tilapia culture specifically for growth and reproduction (BFAR-NFFTC; PHILMINAQ; Boyd & Pillai 1984; Reyes et al 2021). The salinity, SDV, TDS and EC were outside the recommended levels. Salinity reading in the present study however may not pose any problem to the cultured stock despite of its value falls below the suitable range. Nile tilapia is widely cultured in freshwater and can tolerate certain salinity levels due to its euryhaline characteristics (De Azevedo et al 2015). The SDV beyond the recommended level may cause problem in DO concentrations in ponds, specifically when plankton is the primary source of turbidity (Boyd & Litchkoppler 1979). This reflects on the importance of phytoplankton as the primary source of oxygen in water. Gains and losses of DO in pond water is primarily influenced by the photosynthetic activity and respiration of the phytoplankton (Boyd & Litchkoppler 1979). Dissolved solids in water contain ions necessary in sustaining aquatic life, but higher concentrations have negative consequence to other physico-chemical water parameters (e.g., increase water temperature, decrease DO concentration due to reduction of photosynthetic activity of autotrophic organisms, affects water turbidity) and can be also damaging to organism's cells (PHILMINAQ). Higher reading of electrical conductivity in the sampling site may relate to the high TDS level. According to EPA (2022), an increase in dissolved solids concentration in the water elevates conductivity. An increase of conductivity in the water could lead to osmotic stress to aquatic biota (Zhang et al 2019).

Vibrio spp. has been reportedly present in apparently healthy or diseased fish, water and sediments of tilapia farms (Thanh et al 2014; Abd El-Tawab et al 2021; Pakingking et al 2022). The maximum count of presumptive *Vibrio* spp. in fish samples (10^7 CFU g^{-1}) is higher than the recent report of Pakingking et al (2022). However, fish examined were all apparently healthy. This suggest the nature of *Vibrio* spp. as opportunistic pathogens which may propel the ascendance of bacterial disease outbreaks when fish are subjected to environmental stress (Pakingking et al 2022). Higher recoveries of presumptive *Vibrio* spp. in fish may attribute to the presence of this bacterial genus in higher amount in both rearing water (10^6 to 10^7 CFU mL^{-1}) and bottom sediment (10^8 CFU g^{-1}). The external organs of the fish such as skin and eye come in contact with the external environment (Mukwabi et al 2019). However, skin is covered with mucus in which it was identified to have antibactericidal property against *Vibrio* bacteria (Garcia-Marciano et al 2019). The kidney, liver and intestine were among the commonly identified internal organs for the isolation of *Vibrio* spp. in tilapia as noted from several studies (Traore et al 2014; Langaoen et al 2018; Sumithra et al 2019; Hassan et al 2020; Pakingking et al 2022). Selection of these internal organs as specimens may reflect on their physiological functions in the fish body. The liver is a frontline immune tissue designed for the detection, capturing and clearing of bacteria, viruses and macromolecules (Kubes & Jenne 2018). The kidneys function mainly as a water excretory device (Hickman & Trump 1969). And, the intestine is responsible for digestion and absorption of feed, functions for the stability of water and electrolytes, in endocrine regulation of digestion and metabolism, and also with immune functions (Buddington et al 1997). However, there are limited studies in the quantification of *Vibiro* spp. load among these internal organs in tilapia. Mostly, prevalence and incidence rate between organs of tilapia was studied. Prevalence of *V. alginolyticus* from the naturally infected tilapia was higher in the liver ranging from 38.5 to 40.0% while from 29.2 to 30.6% in the kidney (El-Sayed et al 2019). In comparison, *Vibrio* sp. incidence rate in Nile tilapia in Egypt showed lower percentage with 23.3% in liver and 20.0% in kidney (Hassan et al 2020). These implies that prevalence of *Vibrio* spp. in tilapia varies with geographical location and environmental quality. The intestine of tilapia is host to numerous bacterial species of which *Vibrio cholerae* was identified among the dominant intestinal microbiota (Pakingking et al 2015). Genera-wise, presumptive *Vibrio* load in intestinal gut of tilapia ranged from 10^2 to 10^6 CFU g^{-1} (Pakingking et al 2022). Bacterial route to the intestine of the fish may relate through the ingestion of microbial loads present in the surrounding environment during feeding and grazing.

The *Vibrio* spp. count in the environmental samples of the current study were much higher than the findings of Pakingking et al (2022). This bacterial genus is autochthonous in fresh- and saline environments (Uchiyama 2000; Gurbanov et al 2011). Proliferation of *Vibrio* spp. occurs when environmental conditions are at optimum level necessary for their growth (Esteves et al 2015). Higher counts of bacteria in sediment compared to the overlying water is well-established in literatures (Hassard et al 2017; Luo et al 2019). The *Vibrio* load in sediment of the present study averaging 10^8 CFU g^{-1} is two-fold higher than the findings of Pakingking et al (2022) in the semi-intensive tilapia earthen ponds with a maximum count of 10^4 CFU g^{-1}. In other studies, *Vibrio* species count in sediment peaked at 10^7 CFU g^{-1}, and bacterial loads have increasing trend towards the rearing time (Anand Ganesh et al 2010; Alfiansah et al 2018). Hence, higher values of *Vibrio* in the environmental samples of the studied earthen ponds may associate to the duration of culture as majority of the farms had approximately ≥ 4 months of culture at the time of assessment. The culture period may be further linked to the possible elevation of organic matter in the pond bottom which is mainly sourced from uneaten feeds and other particulate organic matter. Water from the river may also serve as vector of other organic wastes and microbial contamination in farms as wastes derived from agricultural farms, household areas, sewage overflows and wildlife activity may flushed during rainy season and surface run-off.

The present study is in congruence with the findings of Pakingking et al (2022) with the high and low abundance of yellow and green colonies, respectively in both fish and environmental samples. The cited authors further suggest the importance of

detecting low level of green colonies since this is the morphological colouration of prominent infection-causing *Vibrio* species in tilapia aquaculture. The *V. parahaemolyticus* and *V. vulnificus* were described to bear green colouration in TCBS agar (Kaysner et al 2004). These two species were prevalently isolated from diseased reared tilapia (Sumithra et al 2019; Abd El-Tawab et al 2021). Low proportion of these green colonies may be also linked to the salinity level of examined farms (0.90±1.51 ppt) as the two aforementioned *Vibrio* species thrive in higher salinity zones (Salomon et al 2013; Li et al 2018). But, first isolation of *V. vulnificus* in infected hybrid tilapia raised in freshwater and low-salinity environments was reported by Chen et al (2006). Abundance in proportion of yellow colonies however does not warrant the absence of risk in the development of bacterial disease outbreaks in aquaculture. *V. cholerae* and *V. alginolyticus* characterized with yellow colony in the TCBS medium (Kaysner et al 2004) were also isolated to diseased tilapia in aquaculture farms (Limbauan 2018; El-Sayed et al 2019; Abd El-Tawab et al 2021; Elgendy et al 2022). *V. mimicus* in addition, was recently reported to associate with mass mortality in farmed Nile tilapia in Egypt (Elgendy et al 2022). Further, the presence of these species particularly *V. vulnificus* and *V. cholerae* posed threat not only to aquaculture but also to humans due to their zoonotic potential. *V. vulnificus* is an aetiological agent of primary sepsis, and severe skin and tissue infection (Horseman & Surani 2011). Wound infection caused by the biotype 3 of this species was reported after handling of fresh, whole fish obtained in inland fishponds (Bisharat et al 1999). Other strains of *V. cholerae* also cause wound and ear infection and rarely primary septicaemia when wounds are also exposed to contaminated water (Baker-Austin et al 2018).

Positive correlation of dissolved oxygen to bacterial counts may have a contradicting idea with the classification of Vibrionaceae as facultative anaerobes (Youngren-Grimes et al 1988). Facultative anaerobic organisms were capable of growing in the presence or absence of oxygen (Andre et al 2021). And, *Vibrio* spp. can survive, grow, and exhibit active metabolism under prolonged anaerobic conditions (Youngren-Grimes et al 1988). Salinity was also found playing a role on the growth of the *Vibrio.* Field studies suggests that *Vibrio* spp. could thrive from near fresh (0.05 ppt) to brackishwater environment (27.6 ppt) (Louis et al 2003; Deeb et al 2018). Highest counts in *V. vulnificus* occurs between salinity 5 and 20 ppt (Deeb et al 2018), while increase in number of detectable *V. cholerae* yields at salinities ranging from 2 to 14 ppt (Louis et al 2003). In terms of pH, *Vibrios* tend to have better growth under alkaline condition (8.5) (Huq et al 1984; Percival & Williams 2014), but most species can grow from pH 6.5 to 9 (Percival & Williams 2014). The measured soil pH in the present study however has slightly lower value (6.44±0.84) compared to the minimum value for optimal growth of *Vibrio.* Organic carbon, nitrogen and phosphorous also influences bacterial growth (Vrede et al 2002). Dissolved organic matter was considered as a driving factor in increasing *Vibrio* concentrations in ponds (Greenfield et al 2017). Sources of organic matter in ponds may derive from accumulation of uneaten feeds, animal feces, and decomposing materials from plant litters and phytoplankton during die-off. Furthermore, activities that may contribute in the increase of organic matter that leads to the enhancement of bacterial population were the application of artificial feed and fertilizers, high stocking density and the entry of microbial contaminated water discharge from other farms located in the same coastal belt (Anand Ganesh et al 2010).

Conclusions. Presumptive *Vibrio* spp. counts in the tilapia grow-out farms is high compared to the available published reference. Despite of the high bacterial count, tilapia collected in the sampling site did not exhibit any clinical signs or external abnormalities. This may reflect to the opportunistic nature of the isolated bacteria that can cause disease outbreaks when the cultured fish are subjected to stress brought by unfavorable environmental condition. Moreso, most of the environmental quality parameters were within the optimum level. However, current absence of infection to fish does not guarantee the absence of risk in both aquaculture and to human. Some *Vibrio* spp. have zoonotic potential that may cause serious disease outbreak to mankind. Therefore, good aquaculture practices is necessary. Furthermore, hygienic preparation and adequate

cooking temperature of tilapia and its polycultured species is recommended given the presence of presumptive *Vibrio* spp. in fish.

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Conflict of interest. The authors declare that there is no conflict of interest.

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