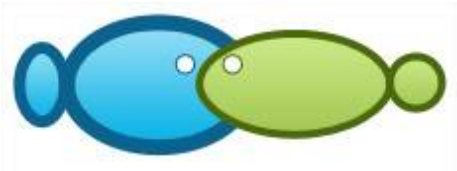

# Assessment of *Enterococcus* in fish and its environment: a study in tilapia farms operating in the BUDAMASA areas of Minalin, Pampanga, Philippines

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**Abstract**. The study aimed to examine the presence and abundance of *Enterococcus* from Nile tilapia (*Oreochromis niloticus*) and its environment in selected farms operating in the BUDAMASA areas of Minalin, Pampanga. Moreover, the relationship of environmental quality to the abundance of the bacteria was investigated. Results revealed that there 100% prevalence rate of enterococcus in tilapia farms. Bacterial count revealed a mean of 1.89 x $10^7$ CFU $g^{-1}$, 2.83 x $10^3$ CFU $mL^{-1}$ and 8.01 x $10^7$ CFU $g^{-1}$ in fish, water and sediment, respectively. The abundance of *Enterococcus* in water and sediment is significantly correlated ($p < 0.05$) with temperature, salinity and phosphorous. However, only temperature and phosphorous has direct relationship, implying the preponderance of these variables to the increase of bacterial abundance. Despite the presence and high abundance of *Enterococcus* population in tilapia farms, fish are still in healthy condition. However, appropriate cultural practices must be followed to maintain conducive environment and control the proliferation of *Enterococcus* in the production system.
**Key Words**: colony count, environmental quality, *Enterococcus*, prevalence, tilapia farms.

**Introduction**. Pampanga is a coastal province located in the Central Luzon Region of the Philippines. It is endowed with vast agriculture and aquaculture resources. Aquaculture production accounts for almost 99% of its fish production while only 1% is contributed by its inland capture fisheries (Bureau of Fisheries and Aquatic Resources 2015). Among the cultured species in the province, Nile tilapia (*Oreochromis niloticus*) has the most significant contribution. Based on the recent statistical report of the Philippine Statistics Authority (2021), there is an increasing trend on the tilapia production of Pampanga in the past three years, from 113,767.82 metric tons in 2019 to 131,895.78 metric tons in 2021. Moreover, the province remained a major producer contributing 42.92% of the total tilapia production across the country (PSA 2021). The impressive performance of the province can be linked to its wide area of fishponds and the Pampanga River that supplies ample amount of water that allows year-round production. The municipality of Minalin is considered as one of the major centers of tilapia production in the province. It has 15 component villages/barangays with a total land area of 4,827 ha. Almost 57% of its land area is used for aquaculture activities with total functional fishponds of 1,755 units for tilapia farming (Vera Cruz & Reyes 2014). The BUDAMASA areas refer to the villages/barangays of Bulac, Dawe, Maniango and Saplad in the southwestern portion of the town. These villages are traversed by Maniango River, a major water body supporting the capture and culture fisheries of the town.

In spite the current state of tilapia production in the province, several production factors may still challenge its sustainability. Sustainable growth should be secured to meet the demand of the growing population for cheap and reliable protein source. Worldwide, fish farmers are confronted by various constraints including inadequate supply of quality seeds, water quality degradation, proliferation of invasive and nuisance

species, disease outbreaks, and increasing production costs mainly contributed by the spike in feed prices (Liao & Chao 2009; Ateweberhan et al 2018; Haji & Workagegn 2021; Naylor et al 2021; Uddin et al 2021). As a result, farmers tend to adopt alternative methods in the production process. However, as in any other aquaculture operations further intensification could increase the threat of pathogenic organisms. Recently, a study showed that one of the main risks to sustain tilapia aquaculture is the outbreak of infectious diseases (Tahiluddin & Terzi 2021). Fish and its environment harboring disease-causing organisms may not only adversely affect production but also threatens human health. Despite the claims that tilapia is a hardy fish, several studies also showed its susceptibility to various pathogens including parasites, viruses, fungi, and bacteria in farm settings (Morales-Serna et al 2018; Machimbirike et al 2019; Mahboub & Shaheen 2021; Tahiluddin & Terzi 2021). Co-infections among these pathogens in farmed tilapia were also reported to trigger mass mortality (Khotob et al 2016; Abdel-Latif & Khafaga 2020; Basri et al 2020; Assane et al 2021; Swaminathan et al 2021). Bacterial pathogens are major threat to culture fisheries because of their ability to survive and proliferate in aquatic systems without requiring a host (Pridgeon & Klesius 2013). Ismail et al (2016) revealed the association of environmental quality to the occurrence of bacteria in tilapia. Among the studied parameters, they found that temperature and ammonia exhibit strong association with bacterial presence. The abrupt change of water temperature is a direct effect of changing climate whilst spike in ammonia is an indication of water pollution. Several studies have already given emphasis on the role of water pollution on the emergence of infectious bacterial diseases in tilapia (El-Shafai et al 2004; Osman & El-Khateeb 2016; Iyiola et al 2019; Ibrahim 2020). Although tilapia has high resistance to various pathogens, the presence of species that can cause zoonotic diseases is of special concern. Among the species found in tilapia and other aquatic food products with associated human health risks are enterococci (Daniel et al 2015; Osman et al 2016). These species are commensal to many animals and typically harmless in healthy individuals (Arumugam et al 2017; Ramos et al 2020). However, acquisition of antimicrobial resistance by many *Enterococcus* species became a major clinical problem nowadays (Osman et al 2016; Said et al 2017; Igbinosa & Beshiru 2019).

In the past decades, enterococci have emerged as an important causative agent for nosocomial infections (Hunt 1998; Prieto et al 2016; Bhardwaj 2019). Infections include bacteremia, endocarditis, meningitis, periodontitis, urinary tract infections, neonatal sepsis and wounds (Fisher & Phillips 2009). It has been considered that intestinal colonization may incur high mortality rate, particularly in patients with compromised immunity (Vydra et al 2012). In the United States alone, enterococcal pathogens are accounted for 14% of hospital-acquired infections (Weiner et al 2016). Statistical reports indicated that 80 to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis* (Karmakar et al 2004). In addition, previous reports indicated that the species is responsible for an estimated 5-20% of human endocarditis cases (Higuita & Hycke 2014; Ferede et al 2018; Khan et al 2018; Seby et al 2022).

Despite such efforts to prevent and control zoonotic pathogens, information is still dearth to substantiate the requirements for the development of preventive and control measures. Hence, an assessment was conducted to provide baseline information that could be used for future undertakings that may protect fish and human health against enterococci infections specifically in the municipality of Minalin, Pampanga. Specifically, this undertaking aimed to determine the: (1) cultural practices of the farms; (2) physical characteristics and health condition of fish samples; (3) water and sediment quality; (4) prevalence and abundance of enterococci in tilapia stocks and their environment (water and sediment); and (5) relationship of water and sediment quality to abundance of *Enterococcus*.

## Material and Method

***Sampling sites.*** The assessment of *Enterococcus* prevalence was carried out by collecting fish, water and sediment samples from the tilapia grow-out farms operating in the BUDAMASA (stands for the barangays of Bulac, Dawe, Maniango and Saplad) areas of

Minalin, Pampanga (Figure 1). These barangays are among the areas of Minalin where tilapia aquaculture is the major livelihood activity. Farms in these areas are fed with water from the traversing Maniango River, a major water body that supports both fishing and culture fisheries of the town. Based on the latest statistical data of the Municipal Agriculture's Office, there is a total of 144 farm operators (Bulac - 44; Dawe - 100; Maniango - 153; and Saplad - 124) in the BUDAMASA. In this study, two (2) farms were designated as sampling and collection areas for fish, water and sediments in each village/barangay. The selection of farms is purposive, and was based on the utilization of chicken manure as fertilizer or direct feed for the stocks. Identification will be made with the aid of a peer contact. The geographic positions of each farm were determined using an installed GPS application in phone.


| Barangay | Farm no. | Geographic position | |
|---|---|---|---|
| Maniango | F1 | 14 56 20.148 N | 120 39 29.484 E |
| | F2 | 14 56 16.000 N | 120 39 05.990 E |
| Saplad | F3 | 14 56 13.020 N | 120 41 05.856 E |
| | F4 | 14 56 31.128 N | 120 41 11.369 E |
| Dawe | F5 | 14 56 51.373 N | 120 41 17.320 E |
| | F6 | 14 56 51.820 N | 120 41 04.610 E |
| Bulac | F7 | 14 57 14.370 N | 120 40 19.770 E |
| | F8 | 14 57 25.493 N | 120 40 27.480 E |

Figure 1. Map showing the collection sites ( tilapia farms) (exported from Google Earth 2022).

***Survey of cultural practices***. A survey questionnaire was developed to determine the production practices of each farm participated in the study. The questionnaire was divided into 7 major sections which include: 1) basic farm profile; (2) pond preparation; (3) stock information and stocking; (4) feeds and feeding management; (6) water quality management; (6) disease prevention and control strategies; and (7) harvesting and marketing. The questionnaire was given to the farm caretaker or farm owner during the period of sample collection. The questionnaire was retrieved from the respondents after they provided the necessary information.

***Collection of samples***. Sediments were collected from each farm site using a steel trowel garden scoop. Approximately, 5 g of sub-surface sediment were taken from the pond floor (specifically, one sample from four sides near the dikes and central portion of the pond) of a representative unit. The sediments were mixed thoroughly to obtain a

homogenous sample and were placed in sterile sealed plastic packs. Water samples on the other hand were taken from the surface (the same positions where the sediments were taken) using a syringe with 5.0 mL capacity. Collection of water was made five times and samples were stored in sterile polyethylene (PE) plastic bottles. The sediment and water samples were kept in insulated box with ice and transported to the laboratory within 6 hours. In terms of fish samples, a cast net was used to capture the target fish. A total of 5 fish (at least 10 g) was randomly selected from the catch in each farm. Live samples were transported to the laboratory by keeping them in oxygenated plastic bags using the same pond water. Microbiological analyses were initiated on the same day the samples were obtained.

***Assessment of samples***. Composite sediment sample (1,000 g) was collected from farm. The samples were kept in chilling temperature and air-dried for 7 days. After drying, the samples were brought to the Department of Agriculture regional Field Office – Soils Laboratory located at the City of San Fernando, Pampanga for the analysis of pH, organic matter, nitrogen and phosphorous. The pH was measured using potentiometric method. The organic matter, nitrogen and phosphorous on the other hand were quantified using the methods Walkley-Black, Kjeldahl, and Olsen, respectively. Water quality variables were measured in the field using digital equipment and in the laboratory. The dissolved oxygen (DO) and temperature of the water was measured using a handheld digital dissolved oxygen meter (Extech Instruments, New Hampshire). Salinity level was assessed using RCYAGO portable refractometer (Shenzen Yage Technology Limited, China). The total dissolved solids (TDS) and electrical conductivity (EC) were also measured *in-situ* using RCYAGO pen type monitoring devices. Meanwhile, composite water samples (1,000 mL) were kept in insulated box with ice and the concentration of total ammonia nitrogen (TAN) was evaluated using positive electrometry at the Bureau of Fisheries and Aquatic Resources Regional Field Office 3 – Water Quality Testing Laboratory.

Prior to bacterial isolation, morphometric and gravimetric measurements were undertaken in fish samples. Samples were segregated also based on sex and health condition. The total length and weight of individual fish were measured using digital caliper with 0.01 cm sensitivity and digital weighing balance with 0.01 g sensitivity, respectively. The body condition of fish was computed using Fulton's condition factor (Datta et al 2013):

$$K = \frac{W \times 100}{L^3}$$

where: $K$ is the condition factor, $W$ is the weight of fish and $L$ is the total length of fish.

The body condition of fish is an important parameter that is widely used to understand fish biology and pathology (Ridanovic et al 2015). Assessment of body condition provides an alternative for expensive gross evaluation of tissues (Datta et al 2013).

As to health status, samples not manifesting any clinical signs of bacterial infection such as scale erosion, skin hemorrhages, unilateral or bilateral exophthalmia, fin rot, abnormal body coloration and among others were tagged as healthy individuals (Baums et al 2013; Austin 2019; Reyes & Madrid 2020; Rizkiantino et al 2021).

***Bacterial isolation and quantification***. Microbiological analysis was conducted at the Fish Pathology Laboratory of the Freshwater Aquaculture Center (FAC) of Central Luzon State University (CLSU) in the Science City of Munoz, Nueva Ecija, Philippines. Isolation of enterococci from the sediments was made in accordance with the method applied by Guan et al (2020) with some modifications. One (1) gram of sediment was diluted in 9.0 mL sterilized physiological saline (0.9% NaCl). As to water samples, one (1) mL was also diluted to the same amount of physiological saline to prepare an aliquot that is used for plating in selective culture medium (Adamu et al 2018). Both water and sediment samples passed through series of 10-folds dilution ($10^{-1}$ - $10^{-6}$). The prepared dilutions were incubated at room temperature for 6 hours.

Meanwhile, isolation of bacteria from fish is commonly made by taking samples from live, moribund and dead fish. However, this study only used live samples as sources of possible enterococci isolates. Most studies have isolated the bacterium from the skin and internal organs of fish such as brain, kidney, gut and liver (Osman et al 2017; Elgohary et al 2020; Reyes & Madrid 2020; Rizkiantino et al 2020). Hence, isolation was made by taking tissues from the external (skin and eyes) and internal organs (intestine, kidney and liver) to prepare a homogenate sample. The fish was euthanized by chilling in ice and dissected to obtain the target organs. Then, portions of the organs were aseptically removed using a sterile scissor, then pooled and homogenized through dilution in physiological saline at 10% concentration. The dilutions were also incubated for 6 hours.

After the dilution of samples, 100 µLl aliquots from serially diluted ($10^{-1}$ - $10^{-6}$) samples were transferred to bile-esculin azide agar (Himedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India) plates and incubated for 18-24 hours at room temperature (37$^{o}$C) (Adeniji et al 2021). Presumption on the presence of *Enterococcus* was based on the morphology of the colonies emerged from the selective medium. Colonies exhibiting black to dark brown coloration were presumed as *Enterococcus*. *Enterococcus* hydrolyses esculin present in the bile resulting to dark/brown coloration (Wanger et al 2017). Bile-esculin test was regarded to have 100% sensitivity and 97% specificity in detecting *Enterococcus* species (Facklam 1973).

The prevalence rate of *Enterococcus* in fish, water and sediment samples was determined using the formula (Yang et al 2019):

$$\text{Prevalence rate (\%)} = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100$$

The number of colonies was counted and the average colony count was used to establish the colony forming units following the procedure of Collins & Lyne (1984). Counting was made manually and with the aid of an installable application for bacterial colony counting (BactLAB, Nissui Pharmaceutical Co., LTD., Japan). Counts within 30-300 colony forming units was regarded as total viable count (TVC). Bacterial count (in terms of colony forming units) was expressed as CFU $g^{-1}$ for fish and sediment and CFU $mL^{-1}$ for water using the formula (Reyes et al 2019):

$$\text{CFU } g^{-1} \text{ or } mL^{-1} = \frac{\text{number of colonies x dilution factor}}{\text{volume plated}}$$

where: the dilution factor is calculated by dividing the volume of aliquot and diluent to the volume of aliquot.

For consecutive dilutions showing between 30 and 300 colonies, the count in each dilution was taken as too few and too many colonies may not be accurate (O'Toole 2016). When the count of one dilution is not more than double the other, the average of dilutions was taken. When the count of one dilution is twice greater than the other, the lower value was taken for the computation of colony forming units. However, when all dilutions show more than 300 colonies, dilutions where the colonies are countable were considered.

***Statistical analysis.*** The data obtained for fish characteristics, water and sediment variables, and *Enterococcus* abundance in each farm were analyzed using descriptive statistics such as mean and standard deviation. Meanwhile, the relationship between the level of environmental quality and bacterial count in sediment and water was determined using Pearson Moment Correlation in SPSS ver. 22.

## Results and Discussion

***Operational practices.*** The study included a total of 8 operational tilapia grow-out farms in the BUDAMASA areas of Minalin, Pampanga. Of these farms, 87.50% are rented

while the remaining fraction (12.50%) is privately owned. These farms allotted 1.4 to 12.5 ha comprising 1 to 3 pond units for fish production. Majority (75.00%) have two production cycles per year while the rest (25.00%) can only operate a single cycle. All farms are dependent on Maniango River as source of water for each production cycle. The maintained water depth of ponds ranges from 1.2 to 3.6 m. Most (75.00%) of these farms are devoted for polyculture system as this system maximized production and profit. In terms of stocks, the farmers usually cultured Nile tilapia and white shrimp (*Penaeus vannamei*). This culture practice was also reported in previous studies conducted in the province (Mialhe et al 2016; Mialhe et al 2018; Reyes & Reyes 2019). Tilapia was stocked at a rate of 5 to 12 pieces $m^{-2}$, indicating that these farms are operating under semi-intensive (4 to 8 pcs $m^{-2}$) to intensive (> 8 pcs $m^{-2}$) level of management (Reyes et al 2021). Majority (75.00%) of farms practice full feeding with different brands of commercial feed are used. However, it is surprising that one participating farm used chicken manure not only as fertilizer but also as diet for tilapia. These farms seldom practice water change as the river is the main source of water. Half (50.00%) of the assessed farms do not conduct water quality monitoring due to the absence of equipment while the remaining half (50.00%) assess their pond water for at least once a month. According to the respondents, some of the problems that they are commonly encountered include flooding, poor water quality and fish mortality. It was suspected that the occurrence of mortality is caused by water quality degradation and disease. Most (75.00%) of the farms linked the disease to bacterial and fungal infections. Some of the clinical signs that they commonly observed were skin lesions, fin erosions, scale loss, exophthalmia, presence of cotton-like materials in the skin or gills, color changes and loss of appetite. These clinical signs are commonly observed in cultured fish during dry season as claimed by majority (62.50%) of the farmers. Meanwhile, some of the remedies identified include water change, reduction of feeding, application of salt and lime, and disinfection of culture unit. None of these farms have history of using antibiotics. Moreover, these farms are not applying probiotics during the incidence of poor water quality or disease outbreak.

***Characteristics and health condition of fish.*** The sex ratio, morphometric characteristics and health condition of fish samples are indicated in Table 1.

Table 1

Characteristics of fish samples collected from participating farms

| *Variable* | *Characteristics of fish in each farm* | | | | | | | | *Mean±SD* |
|---|---|---|---|---|---|---|---|---|---|
| | *F1* | *F2* | *F3* | *F4* | *F5* | *F6* | *F7* | *F8* | |
| Sex ratio (M:F) | 5:0 | 4:1 | 4:1 | 2:3 | 2:3 | 2:3 | 1:4 | 5:0 | 3:2 |
| Standard length (cm) | 17.00 | 16.20 | 13.30 | 17.70 | 14.00 | 15.40 | 14.30 | 15.00 | 15.36±1.52 |
| Total length (cm) | 20.60 | 20.04 | 16.20 | 21.50 | 16.70 | 18.90 | 17.50 | 18.10 | 18.69±1.90 |
| Weight (g) | 168.56 | 143.22 | 79.44 | 210.20 | 94.84 | 132.34 | 93.02 | 118.12 | 129.97±43.75 |
| Condition factor | 1.88 | 1.74 | 1.86 | 2.00 | 2.01 | 1.92 | 1.69 | 1.99 | 1.89±0.12 |
| % of healthy fish | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Based on the result, male to female sex ratio is 3:2. Fish samples are having mean standard length and total length of 15.36 and 18.69, respectively. Moreover, fish samples are having a mean weight of 129.97 g. The obtained value for weight suggests that the samples are already in adult stage (> 20.0 g) (Food and Agriculture Organization 2022).

The condition factor of fish samples ranges from 1.69 to 2.01 with a mean of 1.89. These values were close to the reported condition factor of Nile tilapia (*Oreochromis niloticus*) fed with farm-made and commercial diet (Anani & Nunoo 2016) but higher than the values obtained from different stages (Olurin & Aderibigbe 2006; Migiro et al 2014). According to Ayode (2011), a condition factor greater than 1.0 suggests better health condition of fish and its desirability for farming. The observed health condition of fish also conforms to the condition factor values. All fish samples from each farm did not exhibit any signs and symptoms of a disease, suggesting that the samples are healthy.

***Water and sediment quality***. The mean level of water and sediment quality variables of the farms during the period of assessment is shown in Table 2. Comparing the level of these variables to the range provided by various authors, it can be noted that DO, pH, TDS, EC and P are not within the optimum level for tilapia grow-out. This condition could be linked to the degree of management employed by each farm in the area.

Table 2

Level of water and sediment quality variables of participating farms

| *Variable* | *Concentration/level in each farm* | | | | | | | | *Mean ±SD* | *Ideal range for grow-out* |
|---|---|---|---|---|---|---|---|---|---|---|
| | *F1* | *F2* | *F3* | *F4* | *F5* | *F6* | *F7* | *F8* | | |
| Water temp. (℃) | 29.88 | 30.22 | 32.04 | 31.58 | 32.54 | 33.00 | 33.56 | 33.58 | 32.05 ±1.42 | 24.0 -32.0 (El-Sayed & Kawanna 2008) |
| DO (mg $L^{-1}$) | 4.61 | 4.72 | 4.19 | 4.63 | 4.66 | 4.73 | 4.52 | 4.50 | 4.57 ±0.17 | 5.0-23.0 (Makori et al 2017) |
| pH | 7.57 | 7.78 | 8.13 | 8.73 | 8.99 | 8.23 | 8.35 | 8.66 | 8.30 ±0.48 | 7.0-8.0 (El-Sherif & El-Feky 2009) |
| SDV (cm) | 18.24 | 43.00 | 26.6 | 26.5 | 24.8 | 29.05 | 23.95 | 23.40 | 26.94 ±7.22 | 15.0-40.0 (Ali & Cagauan 2007) |
| Sal. (ppt) | 2.4 | 4.0 | 0.0 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 ±1.51 | 0.0-8.0 (De Alvarenga et al 2018) |
| TDS (mg $L^{-1}$) | 2080 | 2734 | 1188 | 2222 | 1256 | 2522 | 2656 | 3442 | 2262.5 ±759.4 | < 400 (Boyd et al 2016) |
| EC (µS $cm^{-1}$) | 7832 | 10529 | 4194 | 8202 | 4498 | 9270 | 27836 | 12178 | 10567.7 ±7493.2 | 100-2000 (Stone et al 2013) |
| TAN (mg $L^{-1}$) | 0.084 | 0.115 | 0.040 | 0.014 | 0.022 | 0.355 | 0.036 | 0.013 | 0.085 ±0.115 | < 1.50 (Zhou & Boyd 2015) |
| *Sediment* | | | | | | | | | | |
| pH | 7.40 | 7.04 | 4.68 | 6.21 | 6.31 | 7.09 | 6.54 | 6.26 | 6.44 ±0.84 | 6.5-7.5 (Boyd & Tucker 1998) |
| OM (%) | 1.32 | 1.27 | 3.61 | 5.80 | 6.76 | 1.50 | 1.08 | 2.61 | 2.99 ±2.21 | 1.0-3.0 (Sipauba-Tavares et al 2013) |
| N (%) | 0.07 | 0.07 | 0.13 | 0.20 | 0.19 | 0.03 | 0.02 | 0.08 | 0.10 ±0.07 | - |
| P (mg $L^{-1}$) | 27.16 | 25.63 | 14.60 | 31.62 | 64.66 | 35.31 | 9.69 | 68.57 | 34.65 ±21.46 | 80-100 (Reyes et al 2021) |

***Prevalence rate***. Based on the result in Table 3, presumptive *Enterococcus* was found on fish, water and sediment of all farms assessed in this study. Hence, the computed prevalence rate is 100.00%. This result could be linked to the interconnection of tilapia farms as these farms are dependent on Maniango River for water. Although fish samples are all in healthy condition, they can still harbor *Enterococcus*. This corresponds with the report of Mayada et al (2019) that the bacterium can be isolated from healthy and diseased tilapia. Horizontal transmission is the common mechanism of *Enterococcus* and the presumed main point of entry in fish is via gastrointestinal tract (Rizkiantino et al 2021). Enterococcal infections could occur through wounds and abrasions due to high stocking densities and between different species of wild and cultured fish within the same environment (Evans et al 2004; Xu et al 2007). Acquisition could also be due to the intrusion of polluted waters leading to the degradation of fish environment (Osman & El-Khateeb 2016). Byappanahalli et al (2012) claimed that freshwater habitats in general do not support the growth of enterococci; their presence is an evidence of point or non-point source pollution or an indication of re-suspension from other reservoirs. The presence of enterococci has been used as parameter for water quality changes (Lebreton et al 2014). Harvested fish and other foods from areas contaminated with domestic and agriculture/ aquaculture wastewater has the potential to carry enterococci. In the aquatic environment, water degradation played an important role for their successful invasion (Araujo et al 2021; Akter et al 2021). Hence, it was emphasized that the existence of *Enterococcus* in ponds must be taken seriously (Said et al 2017; Rizkiantino et al 2021). Transmission into aquatic animals from polluted waters has been reported (Araujo et al 2021). In this study, low DO and high P was recorded, suggesting a certain level of pollution. Maniango River is part of the Pampanga River System and it was reported that this lotic system is highly polluted and contaminated based on the studied environmental parameters (Reyes & Labasan 2018). As cited by Reyes & Reyes (2019), *E. faecalis* is among the bacterial species isolated from the water and sediment of Pampanga River. Organic fertilizers such as chicken droppings may also serve as vector in the occurrence of *Enterococcus*. Some participating farms are using chicken droppings as fertilizer during pond preparation. Also, a number of poultry farms are operating within the municipality which could transmit bacteria through direct and indirect means (Reyes 2018). Fecal materials from local and migratory birds feeding on tilapia ponds might also be involved (Radhouani et al 2011). The persistence of bacteria in manures is one of the important factors of transmission (Elsaidy et al 2015). Petersen & Daalsgard (2003) observed that the presence of various *Enterococcus* species in ponds is due to fecal inputs from the integrated fish-poultry systems. Igbenosa et al (2016) suggested proper use of manure products in pond preparation to prevent enterococci infection. Kuhn et al (2003) reported that a 30% reduction on *Enterococcus* incidence was observed in crops that were not applied with animal fertilizers.

Table 3

Presence and prevalence rate of *Enterococcus* in participating farms

| *Sample* | *Presence in each farm* | | | | | | | | *Prevalence rate (%)* |
|---|---|---|---|---|---|---|---|---|---|
| | *F1* | *F2* | *F3* | *F4* | *F5* | *F6* | *F7* | *F8* | |
| Fish | + | + | + | + | + | + | + | + | 100.00 |
| Water | + | + | + | + | + | + | + | + | 100.00 |
| Sediment | + | + | + | + | + | + | + | + | 100.00 |

"+" = present.

***Colony count***. In terms of bacterial abundance, the farm level count revealed a mean of 1.89 x $10^7$ CFU $g^{-1}$, 2.83 x $10^3$ CFU $mL^{-1}$ and 8.01 x $10^7$ CFU $g^{-1}$ in fish, water and sediment, respectively (Table 4). In fish, the highest number was observed in F3. In water and sediment, the highest number was recorded in F8. At present, the current legislation in the country does not determine the permissible limit for enterococci in fish and its environment.

Table 4

Colony count of *Enterococcus* in participating farms

| *Sample* | *Total viable count in each farm* | | | | | | | | *Mean±SD* |
|---|---|---|---|---|---|---|---|---|---|
| | *F1* | *F2* | *F3* | *F4* | *F5* | *F6* | *F7* | *F8* | |
| Fish (CFU $g^{-1}$) * $10^7$ | 1.72 | 1.64 | 2.42 | 1.93 | 1.73 | 2.38 | 1.64 | 1.65 | 1.89±0.33 |
| Water (CFU $mL^{-1}$) * $10^3$ | 3.80 | 1.03 | 3.20 | 4.13 | 8.20 | 5.08 | 8.40 | 8.70 | 2.83±2.83 |
| Sediment (CFU $mL^{-1}$)* $10^7$ | 7.58 | 3.40 | 4.80 | 3.55 | 9.37 | 6.27 | 5.00 | 14.12 | 6.76±3.59 |

***Relationship of environmental variables and abundance of Enterococcus***. The correlation coefficients between environmental variables and colony count of enterococcus in water and sediment samples are given in Tables 5 and 6.

Table 5

Correlation coefficients (r) between water quality variables and colony count

| | | *Colony count* | *Temp* | *DO* | *pH* | *SDV* | *Sal* | *TDS* | *EC* | *$NH_3$* |
|---|---|---|---|---|---|---|---|---|---|---|
| Colony count | R | 1 | 0.85** | -0.07 | 0.68 | -0.58 | -0.78* | 0.13 | 0.39 | -0.26 |
| Colony count | Sig. | | 0.01 | 0.86 | 0.06 | 0.13 | 0.02 | 0.75 | 0.33 | 0.54 |

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Table 6

Correlation coefficients (r) between sediment quality variables and colony count

| | | *Colony count* | *pH* | *OM* | *N* | *P* |
|---|---|---|---|---|---|---|
| Colony count | r | 1 | 0.038 | 0.095 | -0.024 | 0.824* |
| Colony count | Sig. | | 0.929 | 0.822 | 0.955 | 0.012 |

* Correlation is significant at the 0.05 level (2-tailed).

In this study, the correlation coefficients were used to measure the strength of linear association between the two variables. As revealed, colony count is significantly associated with temperature ($p < 0.01$) and salinity ($p < 0.05$). This suggests that these variables have significant influence on the abundance of *Enterococcus*. However, these two variables may have different effects on abundance. It can be noted that the relationship of temperature and abundance has strong positive value, indicating that an increase in the abundance of the bacterium could be a function of temperature. This is in conformity with the findings of Ismail et al (2016) and Reyes et al (2021) that bacterial count and water temperature are significantly correlated with one another. This is in contrast with the relationship exhibited by salinity and abundance. The result revealed a strong negative value, indicating that an increase in the level of salinity could negatively affect the abundance of the bacterium. Enterococci are free-living and opportunistic bacteria, either in water or fish (David et al 2010). It can thrive in environmental conditions that destroy other microorganisms and on the contrary to other bacteria, *E. faecalis* and related species can survive for a longer time outside its host. Ecologically, *Enterococcus* grow in a wide range of temperature, usually 10 to 45°C (Ramsay et al 2014); however, optimal temperature was recorded at 42.7°C under aerobic condition (Van den Berghe et al 2006). *E. faecalis* and *E. faecium* can survive a 60°C heating temperature for a short period (Hardie & Whiley 1997; Moreno et al 2006). As to salinity, the pathogen is known to be euryhaline and growth was still observed even at 6.5% NaCl (Byappanahalli et al 2012). Growth can still be observed in both hypertonic and hypotonic conditions (Van Tyne & Gilmore 2014). *E. faecalis* also exhibits tolerance to wide pH changes, of which optimum was observed at pH 7.5 (Van den Berghe et al 2006). Nakajo et al (2006) suggested that the resistance of *E. faecalis* to acidic and alkaline conditions can be associated with membrane-bound $H^+$-ATPase activity. This could explain that despite of high pH level in water and low pH level in the sediment of

tilapia farms, abundance did not widely vary. In terms of sediment quality and abundance, P concentration also showed certain level of significance ($p < 0.05$). The obtained value 0.824 suggests that P has strong positive effect on the abundance of *Enterococcus* in the sediment. Toothman et al (2009) cited that P limits bacterial production in freshwater and *Enterococcus* is among the types of fecal bacteria that can be enhanced by increasing P concentrations. Most of the farms in the area are heavily dependent on commercial feed to support the culture of tilapia. According to Sun & Boyd (2013) feed is the major source of P (estimate of 98.9%) in feed-based aquaculture ponds. Provision of excessive feeds and accumulation of fish wastes in the pond could deteriorate both water and sediment leading to high P (Reyes et al 2021). Highest abundance of *Enterococcus* in sediment was observed in farm with the highest P concentration, indicating its significance. Moreover, the observed mean abundance of *Enterococcus* in the sediment is relatively higher compared with the report of Di Cesare et al (2013) in central Italy and Araujo et al (2021) in southern Brazil.

Noteworthy, the ability of the bacteria to survive adverse environments allows multiple routes of cross contamination (Fisher & Phillips 2009). The abundance of the species is also commonly correlated with the human wastes disposal and widely used as a tool for the recreational quality of fresh and marine waters (Byappanahalli et al 2012; Gin & Goh 2013). It was reported earlier that *Enterococcus* species can be present in the water and substrates of coastal farms throughout the year, with seasonal changes in their distribution and abundance (Yasunaga 1982). The primary factor responsible for the spread of enterococci in freshwater is human activity (Wade et al 2008). However, previous researches have shown that the distribution of this species in a variety of environments is not a total function of human and animal fecal inputs. Hence, this supports the results of previous studies that the species can be isolated from aquatic habitats, life forms and food products (Signoretto et al 2004; Noordiana et al 2013; Alipour et al 2014; Braiek & Smaoui 2019; Araujo et al 2021). Aquaculture farms can serve as reservoirs and influence the abundance and distribution of enterococci which may constitute an underestimated health risk (Di Cesare et al 2013).

Despite having 100% prevalence rate and high abundance, the fish remained healthy. Fish might have activated its adaptive immunity. According to Reyes et al (2021), the adaptive systems of the fish tend to be activated at high temperature. In this process, innate responses including lysosome and phagocytic cells are involved (Zahran et al 2019). The immunity of the fish against the pathogen was not compromised as the mean temperature in the period of assessment is still in the optimum range. However, the fish is at risk when environmental quality has been degraded. Plumb & Hanson (2010) mentioned that tilapia is highly susceptible to the attack of enterococcal pathogen. At present, different strains of *E. faecalis* are playing crucial role in the development of streptococcosis-like disease in tilapia (Akter et al 2021). Previously, it was reported that *E. faecalis* has brought mass mortality in the integrated farm system in Thailand (Petersen & Daalsgard 2003). In Egypt, enterococcal infection (enterococcosis) is associated with other known diseases such as lactococcosis and streptococcosis of which the causative agents are claimed to be synonymous. Prevalence can reach up to 85% in extensive, semi-intensive and intensive farms (Abdel-Aziz et al 2003). An assessment of Nile tilapia septicemia from aquaculture and wild sites in Egypt showed the dominance of *E. faecalis* in the isolates (Osman et al 2017). Recently, Hassan et al (2022) reported a 50.5% total prevalence of *E. faecalis* in cultured Nile tilapia and *Mugil cephalus*. Abu-Elala et al (2019) mentioned that the Egyptian strain of the pathogen can cause 30% mortality in tilapia. To add, *E. faecalis* and related species have been isolated and causing infections in some tropical tilapia farms (David et al 2010; Arumugam et al 2017; Reyes & Madrid 2020; Rizkiantino et al 2020; Akter et al 2021). The presence of the pathogen in other freshwater fishes indicates the probability of its virulence (Rahman et al 2017). *E. faecalis* infections were also observed in some marine fishes. Barros et al (2011) isolated 73 enterococci from the fecal samples of gilthead sea bream (*Sparus aurata*) of which 8% of these are *E. faecalis*. Furthermore, the study of Mahmoud et al (2021) showed the opportunistic activity of *E. faecalis* in flathead grey mullet (*M. cephalus*) infested with the ectoparasite *Ergasilus extensus*. A 7% prevalence rate was

also detected in cultured Indian prawn (*Fenneropenaeus indicus*) implying that crustaceans are also vulnerable to infection (El-far et al 2015).

**Conclusions**. In conclusion, the study was able to generate an information on the prevalence and abundance of enterococcus bacteria as well as some important farm, stock and environmental characteristics that could be used to identify appropriate actions that could prevent the occurrence of such infection and public health risk in the tilapia production system of BUDAMASA areas in Minalin, Pampanga. *Enterococcus* has high prevalence and abundance. The abundance in the environment is a direct influence of water temperature and sediment phosphorous. Although samples of fish were in healthy condition, appropriate cultural practices must be followed to maintain conducive environment and control the proliferation of enterococcus in the production system. However, the study has an inherent limitation. Therefore, follow up studies must be conducted particularly on the seasonal occurrence of enterococcal infections, risk factors associated with the disease, and presence of multi-drug resistant isolates.

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## References

Abdel-Aziz E. S., Dardiri M. A., Ali M. N., 2003 Clinical and pathological investigations on enterococcosis in *Oreochromis niloticus* cultured under different fish culture systems. Journal of the Egyptian Veterinary Medical Association 62:217-239.

Abdel-Latif H. M. R., Khafaga A. F., 2020 Natural co-infection of cultured Nile tilapia *Oreochromis niloticus* with *Aeromonas hydrophila* and *Gyrodactylus cichlidarum* experiencing high mortality during summer. Aquaculture Research 51(5):1880-1892.

Abu-Elala N. M., Samir A., Wasfy M., Elsayed M., 2019 Efficacy of injectable and immersion polyvalent vaccine against streptococcal infections in broodstock and offspring of Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish Immunology 88: 293-300.

Adamu K. M., Zhigun I. I., Iloba I. K., Babadoko A. M., Ikomi R. B., Shuaibu R., Ayodapo K. O., Ibrahim A. A., 2018 Prevalence of bacteria isolates in water and some biota of Lapai-Agaie Dam, Nigeria. Science World Journal 13(4):75-80.

Adeniji O. O., Sibanda T., Okoh A. I., 2021 Molecular detection of antibiotic resistance and virulence gene determinants of *Enterococcus* species isolated from coastal water in the Eastern Cape Province, South Africa. International Journal of Environmental Studies 78(2):208-227.

Akter T., Foysal M. J., Alam M., Ehsan R., Paul S. I., Momtaz F., Siddik M. A. B., Tay A. C. Y., Fotedar R., Gupta S. K., Islam T., Rahman, M. M., 2021 Involvement of *Enterococcus* species in streptococcosis of Nile tilapia in Bangladesh. Aquaculture 531:735790.

Ali M. H., Cagauan A. G., 2007 Relationships between secchi disk visibility, water temperature and dissolved oxygen in freshwater fishpond. Bangladesh Journal of Fisheries Research 11(1):45-50.
Alipour M., Hajiesmaili R., Talebjannat M., Yahyapour Y., 2014 Identification and antimicrobial resistance of *Enterococcus* spp. isolated from the river and coastal waters in northern Iran. The Scientific World Journal 2014:287458.
Anani F. A., Nunoo F. K. E., 2016 Length-weight relationship and condition factor of Nile tilapia, *Oreochromis niloticus* fed farm-made and commercial tilapia diet. International Journal of Fisheries and Aquatic Studies 4(5):647-650.
Araujo A. J. G., Grassotti T. T., Frazzon A. P. G., 2021 Characterization of *Enterococcus* spp. isolated from a fish farming environment in southern Brazil. Brazilian Journal of Biology 81(4):954-961.
Arumugam U., Stalin N., Rebecca G. P., 2017 Isolation, molecular identification and antibiotic resistance of *Enterococcus faecalis* from diseased Tilapia. International Journal of Current Microbiology and Applied Sciences 6(6):136-146.
Assane I. M., Prada-Mejia K. D., Gallani S. U., Weiser N. F., Valladao G. M. R., Pilarski F., 2021 Mass mortality of Nile tilapia caused by co-infection with *Enterogyrus* spp. (Monogenea: Ancyrocephalinae) and *Aeromonas jandaei* following transport stress. Authorea. doi: 10.22541/au.161837724.41123668/v1.
Ateweberhan M., Hudson J., Rougier A., Jiddawi N. S., Msuya F., Stead S. M., Harris A., 2018 Community based aquaculture in the western Indian Ocean: challenges and opportunities for developing sustainable coastal livelihoods. Ecology and Society 23(4):17.
Austin B., 2019 Methods for the diagnosis of bacterial fish diseases. Marine Life Science and Technology 1:41-49.
Ayode A. A., 2011 Length-weight relationship and diet of African carp *Labeo ogunensis* (Boulenger, 1910) in Asejire Lake Southwestern Nigeria. Journal Fisheries and Aquatic Sciences 6(4):472-478.
Barros J., Igrejas G., Andrade M., Radhouani H., Lopez M., Torres C., Poeta P., 2011 Gilthead seabream (*Sparus aurata*) carrying antibiotic resistant enterococci. A potential bioindicator of marine contamination? Marine Pollution Bulletin 62(6): 1245-1248.
Basri L., Nor R., Salleh A., Yasin I. S. M., Saad M. Z., Rahaman N. Y. A., Barkham T., Amal M. N. A., 2020 Co-infections of tilapia lake virus, *Aeromonas hydrophila* and *Streptococcus agalactiae* in farmed red hybrid tilapia. Animals 10(11):2141.
Baums C. G., Hermeyer K., Leimbach S., Adamek M., Czerny C. P., Hörstgen-Schwark G., Valentin-Weigand P., Baumgärtner W., Steinhagen D., 2013 Establishment of a model of *Streptococcus iniae meningoencephalitis* in Nile tilapia *(Oreochromis niloticus*). Journal of Comparative Pathology 149(1):94-102.
Bhardwaj S. B., 2019 Enterococci: an important nosocomial pathogen. In: Pathogenic bacteria. Kırmusaoğlu S., Bhardwaj S. B. (eds), IntechOpen. Available at: https://doi.org/10.5772/intechopen.90550. Accessed: December, 2022.
Boyd C. E., Tucker C. S., 1998 Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, USA, 700 pp.
Boyd C. E., Tucker C. S., Somridhivej B., 2016 Alkalinity and hardness: critical but elusive concepts in aquaculture. Journal of the World Aquaculture Society 47(1):6-41.
Braiek O. B., Smaoui S., 2019 Enterococci: between emerging pathogens and potential probiotics. Hindawi BioMed Research International 2019:5938210.
Byappanahalli M. N., Nevers M. B., Korajkic A., Staley Z. R., Harwood V. J., 2012 Enterococci in the environment. Microbiology and Molecular Biology Reviews 76(4): 685-706.
Collins C. H., Lyne P. M., 1984 Microbiological methods. 5th edition. Butterworths Co. Ltd., London, pp. 56-113.
Daniel D. S., Lee S. M., Dykes G. A., Rahman S., 2015 Public health risks of multiple-drug-resistant *Enterococcus* spp. in Southeast Asia. Applied Environmental Microbiology 81(18):6090-6097.

Datta S. N., Kaur V. I., Dhawan A., Jassal G., 2013 Estimation of length-weight relationship and condition factor of spotted snakehead *Channa haracte* (Bloch) under different feeding regimes. SpringerPlus 2:436.
David O. M., Falegan C. R., Ogunlade J. T., 2010 Levels of heavy and other metals in tilapia species raised in different ponds and resistant pattern of associated *Enterococcus* species. Advances in Environmental Biology 4(1):47-52.
de Alvarenga É. R., de Oliveira Alves G. F., Fernandes A. F. A., Costa G. R., Da Silva M. A., de Alencar Teixeira E., Turra E. M., 2018 Moderate salinities enhance growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in the biofloc system. Aquaculture Research 49(9):2919-2926.
Di Cesare A., Luna G. M., Vignaroli C., Pasquaroli S., Tota S., Paroncini P., Biavasco F., 2013 Aquaculture can promote the presence and spread of antibiotic-resistant enterococci in marine sediments. PLoS ONE 8 (4):e62838.
Elgohary I., Eissa A. E., Fadel N. G., Abd Elatief J. I., Mahmoud M. A., 2020 Bacteriological, molecular, and pathological studies on the Gram-positive bacteria *Aerococcus viridans* and *Enterococcus faecalis* and their effects on *Oreochromis niloticus* in Egyptian fish farms. Aquaculture Research 52(5):2220-2232.
El-Far S. A. H., Khalil R. H., Saad T. T., El-Tanekhy M., Abdel-Latif H. M. R., 2015 Occurrence, characterization and antibiotic resistance patterns of bacterial communities encountered in mass kills of pond cultured Indian prawn (*Fenneropenaeus indicus*) at Damietta governorate, Egypt. International Journal of Fisheries and Aquatic Studies 2(4):271-276.
El-Sayed A. M., Kawanna M., 2008 Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquaculture Research 39:670-672.
El-Shafai S. A., Gijzen H. J., Nasr F. A., El-Gohary F. A., 2004 Microbial quality of tilapia reared in fecal-contaminated ponds. Environmental Research 95(2):231-238.
El-Sherif M. S., El-Feky A. M. I., 2009 Performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. I. Effect of pH. International Journal of Agriculture and Biology 11:297-300.
Elsaidy N., Abouelenien F., Kirrella G. A. K., 2015 Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. The Egyptian Journal of Aquatic Research 41(1):93-100.
Evans J. J., Klesius P. H., Shoemaker C. A., 2004 Starch hydrolysis testing of multiples isolates for rapid differentiation of *S. iniae*. Bulletin of European Association of Fish Pathology 24(5):231-239.
Facklam R. R., 1973 Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Applied Microbiology 26(2): 138-145.
Ferede Z. T., Tullu K. D., Derese S. G., Yeshanew A. G., 2018 Prevalence and antimicrobial susceptibility pattern of *Enterococcus* species isolated from different clinical samples at Black Lion Specialized Teaching Hospital, Addis Ababa, Ethiopia. BMC Research Notes 11(1):793.
Fisher K., Phillips C., 2009 The ecology, epidemiology and virulence of *Enterococcus*. Microbiology (Reading) 155(Pt 6):1749-1757.
Gin K. Y., Goh S. G., 2013 Modeling the effect of light and salinity on viable but non-culturable (VBNC) *Enterococcus*. Water Research 47(10):3315-3328.
Guan T. W., Lin Y. J., Ou M. Y., Chen K. B., 2020 Isolation and diversity of sediment bacteria in the hypersaline aiding lake, China. PLoS ONE 15(7):e0236006.
Haji B., Workagegn K. B., 2021 Constraints hindering small scale aquaculture production in southern Ethiopia. Aquaculture International 29(2):565-574.
Hardie J. M., Whiley R. A., 1997 Classification and overview of the genera *Streptococcus* and *Enterococcus*. Journal of Applied Microbiology 83(S1):1S-11S.
Hassan M. A., Abdel-Naeim N. S., Mabrok M., Dessouki A. A., Hassan A. M., 2022 Isolation and identification of *Enterococcus faecalis* from cultured *Oreochromis niloticus* and *Mugil cephalus* with a special emphasis on a possible integrated control strategy. Aquaculture Research 53(16):5521-5535.

Higuita N. I. A., Hycke M. M., 2014 Enterococcal disease, epidemiology, and implications for treatment. In: Enterococci: from commensals to leading causes of drug resistant infection. Gilmore M. S., Clewell D. B., Ike Y., Shankar N. (eds), Boston: Massachusetts Eye and Ear Infirmary. Available at: https://www.ncbi.nlm.nih.gov/books/NBK190429/. Accessed: December, 2022
Hunt C. P., 1998 The emergence of enterococci as a cause of nosocomial infection. British Journal of Biomedical Science 55(2):149-156.
Ibrahim T., 2020 Diseases of Nile tilapia with special emphasis on water pollution. Journal of Environmental Science and Technology 13(1):29-56.
Igbenosa E. O., Onuoha T., Udochukwu U., 2016 Antimicrobial resistance profile characterization of *Enterococcus* species isolated from aquaculture environment. Journal of Microbiology and Biotechnology 1(2):000107.
Igbinosa E. O., Beshiru A., 2019 Antimicrobial resistance, virulence determinants, and biofilm formation of *Enterococcus* species from ready-to-eat seafood. Frontiers of Microbiology 10:728.
Ismail N. I. A., Amal M. N. A., Shohaimi S., Saad M. Z., Abdullah S. Z., 2016 Associations of water quality and bacteria presence in cage cultured red hybrid tilapia*, Oreochromis niloticus* × *O. mossambicus*. Aquaculture Reports 4:57-65.
Iyiola A. O., Asiedu B., Kolawole A. S., Failler P., 2019 Assessment of water quality and bacteriological levels in Nile tilapia (*Oreochromis niloticus*) of Aiba reservoir, Nigeria, west Africa. Tropicultura 37(3):367.
Karmakar M. G., Gershom E. S., Mehta P. R., 2004 Enterococcal infections with special reference to phenotypic characterization and drug resistance. Indian Journal of Medical Research 119:22-25.
Khan Z., Siddiqui N., Saif M. W., 2018 *Enterococcus faecalis* infective endocarditis and colorectal carcinoma: case of new association gaining ground. Gastroenterology Research 11(3):238-240.
Khotob M. H., Menanteau-Ledouble S., Kumar G., Abdelzaher M., El-Matbouli M., 2017 The impact of co-infections on fish: a review. Veterinary Research 47:98.
Kuhn I., Iversen A., Burman L. G., Olsson-Liljequist B., Franklin A., Finn M., Aarestrup F., Seyfarth A. M., Blanch A. R., Vilanova X., Taylor H., Caplin J., Moreno M. A., Dominguez L., Herrero I. A., Mollby R., 2003 Comparison of enterococcal populations in animals, humans, and the environment - a European study. International Journal of Food Microbiology 88(2-3):133-145.
Lebreton F., Willems R. J. L., Gilmore M. S., 2014 *Enterococcus* diversity, origins in nature, and gut colonization. In: Enterococci: from commensals to leading causes of drug resistant infection. Gilmore M. S., Clewell D. B., Ike Y., Shankar N. (eds), New York: Eye and Ear Infirmary, pp. 1-82.
Liao I. C., Chao N. H., 2009 Aquaculture and food crisis: opportunities and constraints. Asia Pacific Journal of Clinical Nutrition 18(4):564-569.
Mahboub H., Shaheen A. A., 2021 Mycological and histopathological identification of potential fish pathogens in Nile tilapia. Aquaculture 530:735849.
Machimbirike V. I., Jansen M. D., Senapin S., Khunrae P., Rhattanarojpong T., Dong H. T., 2019 Viral infections in tilapines: more than just tilapia lake virus. Aquaculture 503:508-518.
Mahmoud M. A., Attia M. M., Abdelsalam M., Abdel-Moneam D. A., Zaki Ewiss M. A., 2021 *Ergasilus extensus* and bacterial co-infection in flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758), are associated with pathological changes and immunological gene expression alterations. Aquaculture Research 52(12):6143-6151.
Makori A. J., Abuom P. O., Kapiyo R., Anyona D. N., Dida G. O., 2017 Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. Fisheries and Aquatic Sciences 20:30.
Mayada A. M. A. Z., Hala A. M. A. E., Doaa M. G., 2019 Molecular detection of virulence and antibiotic resistance genes in *Enterococcus faecalis* isolated from diseased tilapia fish. Animal Health Research Journal 7(5):81-95.

Mialhe F., Gunnel Y., Mering C., Gaillard J., Coloma J. G., Dabbadie L., 2016 The development of aquaculture on the northern coast of Manila Bay (Philippines): an analysis of long-term land-use changes and their causes. Journal of Land Use Science 11(2):236-256.
Mialhe F., Morales E., Dubuisson-Quellier S., Vagneron I., Dabbadie L., Little D. C., 2018 Global standardization and local complexity. A case study of an aquaculture system in Pampanga Delta, Philippines. Aquaculture 493:365-375.
Migiro K. E., Ogello E. O., Munguti J. M., 2014 The length-weight relationship and condition factor of Nile tilapia (*Oreochromis niloticus* L.) broodstock at Kegati Aquaculture Research Station, Kisii, Kenya. International Journal of Advanced Research 2(5):777-782.
Morales-Serna F. N., Medina-Guerrero R. M., Pimentel-Acosta C., Ramirez-Tirado J. H., Fajer-Avila E. J., 2018 Parasite infections in farmed Nile tilapia *Oreochromis niloticus* in Sinaloa, Mexico. Comparative Parasitology 85(2):212-216.
Moreno M. R. F., Sarantinopoulos P., Tsakalidou E., De Vuyst L., 2006 The role and application of enterococci in food and health. International Journal of Food Microbiology 106(1):1-24.
Nakajo K., Komori R., Ishikawa S., Ueno T., Suzuki Y., Iwami Y., Takahashi N., 2006 Resistance to acidic and alkaline environments in the endodontic pathogen *Enterococcus faecalis*. Oral Microbiology and Immunology 21(5):283-288.
Naylor R. L., Hardy R. W., Buschmann A. H., Bush S. R., Cao L., Klinger D. H., Little D. C., Lubchenco J., Shumway S. E., Troell M., 2021 A 20-year retrospective review of global aquaculture. Nature 591:551-563.
Noordiana N., Fatimah A., Mun A. S., 2013 Antibacterial agents produced by lactic acid bacteria isolated from threadfin salmon and grass shrimps. International Food Research Journal 20(1):117-124
Olurin K. B., Aderibigbe O. A., 2006 Length-weight relationship and condition factor of pond reared juvenile *Oreochromis niloticus*. World Journal of Zoology 1(2):82-85.
Osman G. A., El-Khateeb M. A., 2016 Impact of water contamination on tilapia (*Oreochromis niloticus*) fish yield. International Journal of ChemTech Research 9(12):166-181.
Osman K. M., Ali M. N., Radwan I., ElHofy F., Abed A. H., Orabi A., Fawzy N. M., 2016 Dispersion of the vancomycin resistance genes vanA and vanC of *Enterococcus* isolated from Nile tilapia on retail sale: a public health hazard. Frontiers in Microbiology 7:1354.
Osman K. M., Al-Maary K. S., Mubarak A. S., Dawoud T. M., Moussa I. M. I., Ibrahim M. D. S., Hessain A. M., Orabi A., Fawzy N. M., 2017 Characterisation and susceptibility of streptococci and enterococci isolated from Nile tilapia (*Oreochromis nicotilus*) showing septicaemia in aquaculture and wild sites in Egypt. BMC Veterinary Research 13(1):357.
O'Toole G. A., 2016 Classic spotlight: plate counting you can count on. Journal of Bacteriology 198(23):3127.
Petersen A., Daalsgard A., 2003 Species composition and antimicrobial resistance genes of *Enterococcus* spp., isolated from integrated and traditional fish farms in Thailand. Environmental Microbiology 5(5):395-402.
Plumb J. A., Hanson L. A., 2010 Health maintenance and principal microbial diseases of cultured fish. 3rd edition. Wiley-Blackwell, Iowa, USA, 504 pp.
Pridgeon J. W., Klesius P. H., 2012 Major bacterial diseases in aquaculture and their vaccine development. In: Animal science review 2012. Hemming D. (ed), UK: CAB International, pp. 141-156.
Prieto A. M. G., van Schaik W., Rogers M. R. C., Coque T. M., Baquero F., Corander J., Willems R. J. L., 2016 Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? Frontiers of Microbiology 7:788.
Radhouani H., Igrejas G., Pinto L., Goncalves A., Coelho C., Rodrigues J., Poeta P., 2011 Molecular characterization of antibiotic resistance in enterococci recovered from seagulls (*Larus cachinnans*) representing an environmental health problem. Journal of Environmental Monitoring 13:2227-2233.

Rahman M., Rahman M. M., Deb S. C., Alam M. S., Alam M. J., Islam M. T., 2017 Molecular identification of multiple antibiotic resistant fish pathogenic *Enterococcus faecalis* and their control by medicinal herbs. Scientific Reports 7(1):3747.
Ramos S., Silva V., Dapkevicius M. L. E., Igrejas G., Poeta P., 2020 Enterococci, from harmless bacteria to a pathogen. Microorganisms 8(8):1118.
Ramsay M., Hartke A., Huycke M., 2014 The physiology and metabolism of enterococci. In: Enterococci: from commensals to leading causes of drug resistant infection. Gilmore M. S., Clewell D. B., Ike Y., Shankar N. (eds), Boston: Massachusetts Eye and Ear Infirmary; 2014.
Reyes A. T., 2018 Outbreak investigation of *Aeromonas hydrophila* in tilapia grow-out farms in Minalin, Pampanga, Philippines. International Journal of Biology, Pharmacy, and Allied Sciences 7(8):1464-1473.
Reyes A. T., Labasan R. E. B., 2018 Plankton as biomonitoring agent of water quality in upper eastern Pampanga River, Philippines. International Journal of Biology, Pharmacy and Allied Sciences 7(9):1717-1730.
Reyes A. T., Reyes A. T., 2019 Profiling of the cultural and management practices of tilapia grow-out operators in Lubao, Pampanga, Philippines using geographic information system. International Journal of Biology, Pharmacy and Allied Sciences 8(3):594-616.i
Reyes A. T., Madrid F. G. N., 2020 Isolation and biochemical identification of *Enterococcus faecalis* on moribund Nile tilapia (*Oreochromis Niloticus* L). Advances in Pharmacology and Clinical Trials 5(2):000177.
Reyes A. T., Raymundo A. K., Baldrias L. R., Paller V. G., Dalmacio I. F., 2021 Occurrence of *Streptococcus* spp. on farmed Nile tilapia (*Oreochromis niloticus* L.) in Lubao, Pampanga, Philippines. International Journal of Agriculture Technology 17(3):1041-1060.
Ridanovic S., Nedic Z., Ridanovic L., 2015 First observation of fish condition from Sava river in Bosnia and Herzegovina. Journal of Survey in Fisheries Sciences 1(2):27-32.
Rizkiantino R., Wibawan I. W. T., Pasaribu F. H., Soejoedono R. D., Arnafia W., Ulyama V., Wibowo D. B., 2020 Isolation and characterization of the *Enterococcus faecalis* strain isolated from red tilapia (*Oreochromis* hybrid) in Indonesia: a preliminary report. Journal of Survey in Fisheries Sciences 7(1):27-42.
Rizkiantino R., Pasaribu F. H., Soejoedono R. D., Purnama S., Wibowo D. B., Wibawan W. T., 2021 Experimental infection of *Enterococcus faecalis* in red tilapia (*Oreochromis* hybrid) revealed low pathogenicity to cause streptococcosis. Open Veterinary Journal 11(2):309-318.
Said L. B., Hamdaoui M., Klibi A., Slama K. B., Torres C., Klibi N., 2017 Diversity of species and antibiotic resistance in enterococci isolated from seafood in Tunisia. Annals of Microbiology 67:135-141.
Seby R., Kim C., Khreis M., Khreis K., 2022 *Enterococcus faecalis*-induced infective endocarditis: an unusual source of infection and a rare clinical presentation. Journal of International Medical Research 50(7):3000605221112019.
Signoretto C., Burlacchini G., Lleo`M. M., Pruzzo C., Zampini M., Pane L., Franzini G., Canepari P., 2004 Adhesion of *Enterococcus faecalis* in the nonculturable state to plankton is the main mechanism responsible for persistence of this bacterium in both lake and seawater. Applied Environmental Microbiology 70(11):6892-6896.
Sipauba-Tavares L. H., Millan R. N., do Amaral A. A., 2013 Influence of management on the water quality and sediment in tropical fish farm. Journal of Water Resource and Protection 5(5):495-501.
Stone N. M., Shelton J. L., Haggard B. E., Thomforde H. K., 2013 Interpretation of water analysis reports for fish culture. Southern Regional Aquaculture Center; Stoneville, Mississippi. SRAC Publication No. 4606, pp. 1-12.
Sun W., Boyd C. E., 2013 Phosphorus and nitrogen budgets for inland, saline water shrimp ponds in Alabama. Fisheries and Aquaculture Journal 4(1):080.
Swaminathan T. R., Nityanantham S. R., Narendrakumar L., Dharmaratnam A., Sood N., Pradhan P. K., Ramachandra K. S. S., Lal K. K., 2021 Co-infection of *Lactococcus garvieae* and tilapia lake virus (TiLV) in Nile tilapia *Oreochromis niloticus* cultured in India. Diseases of Aquatic Organisms 147:127-140.

Tahiluddin A. B., Terzi E., 2021 A review of reported bacterial diseases and antibiotic use in tilapia culture in the Philippines. Acta Natura et Scientia 2(2):141-147.
Toothman B. R., Cahoon L. B., Mallin M. A., 2009 Phosphorus and carbohydrate limitation of fecal coliform and fecal enterococcus within tidal creek sediments. Hydrobiologia 636:401-412.
Uddin M. N., Kabir K. H., Roy D., Hassan M. T., Sarker M. A., Dunn E. S., 2021 Understanding the constraints and its related factors in tilapia (*Oreochromis* sp.) fish culture at farm level: a case from Bangladesh. Aquaculture 530:735927.
Van den Berghe E., De Winter T., De Vuyst L., 2006 Enterocin A production by *Enterococcus faecium* FAIR-E 406 is characterized by a temperature- and pH-dependent switch-off mechanism when growth is limited due to nutrient depletion. International Journal of Food and Microbiology 107(2):159-170.
Van Tyne D., Gilmore M. S., 2014 Friend turned foe: evolution of enterococcal virulence and antibiotic resistance. Annual Review of Microbiology 68:337-356.
Vera Cruz E. M., Reyes A. T., 2014 Development of comprehensive geo-referenced database for ecological risk analyses of tilapia pond culture in Pampanga. Central Luzon State University, Science City of Muñoz, Nueva Ecija.
Vydra J., Shanley R. M., George I., Ustun C., Smith A. R., Weisdorf D. J., Young J. H., 2012 Enterococcal bacteremia is associated with increased risk of mortality in recipients of allogeneic hematopoietic stem cell transplantation. Clinical Infectious Diseases 55(6):764-770.
Wade T. J., Calderon R. L., Brenner K. P., Sams E., Beach M., Haugland R., Wymer L., Dufour A. P., 2008 High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. Epidemiology 19(3):375-383.
Wanger A., Chavez V., Huang R. S. P., Wahed A., Actor J. K., Dasgupta A., 2017 Microbiology and molecular diagnosis in pathology: a comprehensive review for board preparation, certification and clinical practice. Elsevier, 304 pp.
Weiner L. M., Webb A. K., Limbago B., Dudeck M. A., Patel J., Kallen A. J., Edwards J. R., Sievert D. M., 2016 Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the centers for disease control and prevention, 2011-2014. Infection, Control and Hospital Epidemiology 37(11):1288-1301.
Xu D. H., Shoemaker C. A., Klesius P. H., 2007 Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia (*O. niloticus*) L. Journal of Fish Diseases 30(4):233-238.
Yang X., Wu Q., Zhang J., Huang J., Chen L., Wu S., Zeng H., Wang J., Chen M., Wu H., Gu Q., Wei X., 2019 Prevalence, bacterial load, and antimicrobial resistance of *Salmonella* serovars isolated from retail meat and meat products in China. Frontiers in Microbiology 10:2121.
Yasunaga N., 1982 [Occurrence of *Streptococcus* sp., a pathogen of cultured yellowtail, (*Seriola quinqueradiata*) in muscle of sardine (*Sardinops melanosticta*) for diets]. Fish Pathology 17(3):195-198. [in Japanese]
Zahran E., Mahgoub H. A., Abdelhamid F., Sadeyen J., Risha E., 2019 Experimental pathogenesis and host immune responses of *Enterococcus faecalis* infection in Nile tilapia (*Oreochromis niloticus*). Aquaculture 512:734319.
Zhou L., Boyd C. E., 2015 Ammonia nitrogen management in aquaculture ponds. Aquaculture Magazine (October-November) 41:18-21.
*** Bureau of Fisheries and Aquatic Resources (BFAR), 2015 Pampanga fisheries profile. Available at: https://region3.bfar.da.gov.ph/cmsFiles/region3/fisheriesprofile/pdf/7f141b9c-54ef-472f-bb26-fa1efe739274(01-24-2017).pdf. Accessed: December, 2022.
*** Food and Agriculture Organization (FAO-UN), 2022 Nile tilapia - nutritional requirements. Aquaculture Feed and Fertilizer Resources Information System. Available at: https://www.fao.org/fishery/affris/species-profiles/nile-tilapia/ nutritional-requirements/en/. Accessed: December, 2022.
*** Philippine Statistics Authority [PSA], 2021 Fisheries statistics of the Philippines. PSA CVEA Building, East Avenue, Diliman Quezon City, Philippines. Available at: https://psa.gov.ph/content/fisheries-statistics-philippines. Accessed: December, 2022.

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