E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(4): 428-434

www.fisheriesjournal.com
Received: 24-05-2019
Accepted: 28-06-2019

Reyes Alvin T
Soil and Water Quality Laboratory, College of Fisheries-Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

Correspondence
Reyes Alvin T
Soil and Water Quality Laboratory, College of Fisheries-Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

Outbreak investigation of *Pseudomonas aeruginosa* in tilapia grow-out farms in Minalin, Pampanga, Philippines

Reyes Alvin T

Abstract
This study was conducted in order to investigate the outbreak of *Pseudomonas aeruginosa* in tilapia grow-out farms in Minalin, Pampanga, Philippines and to identify the possible risk factors in the occurrence of the bacterium. Majority of the collected tilapia samples showed various signs of bacterial infection such as lesion, fin/skin rot, eye opacity and/or abnormal body coloration. The computed attack rate of *P. aeruginosa* in the whole municipality was 51.04%. Lower attack rate of *P. aeruginosa* was recorded in male tilapias (male = 50.70%; female = 52.00%). The temporal pattern of *P. aeruginosa* depicts that tilapias were continuously exposed over a long period of time to a common disease-causing factor. The top three identified risk factors for the occurrence of *P. aeruginosa* in grow-out tilapia were the pond preparation (relative risk = 1.53), source of water (relative risk = 1.45) and temperature (relative risk = 1.38). The occurrence of bacterial diseases in tilapia is caused by the interplay of various risk factors such as presence of pathogenic organism and source of contamination, and a susceptible host due to stressful environment.

Keywords: *Pseudomonas aeruginosa*, tilapia, attack rate, risk factors, relative risk

1. Introduction
Aquaculture is now a growing business around the world contributing to a large percentage of the total world fish production. In 2011, its world production of 62.7 million tons has increased by 6.2% from 2010 [1]. In the Philippines, one of the fastest growing aquaculture industries is the tilapia aquaculture which marked the country as one of the top tilapia-producing countries. In 2014, tilapia production from aquaculture sector has amounted to 259,198.16 MT [2]. The province of Pampanga, Philippines has become a major center of production with its polyculture system, associating the shrimp with milkfish and sometimes tilapia and/or crabs [3]. Forty per cent (40%) of tilapia production in Central Luzon has been supplied by Pampanga [4].

The success of aquaculture may be impeded by the incidence of diseases [5]. The impact of different bacteria on the tilapia industry is well-known among the population and health authorities because of their wide geographic distribution and the serious problems they cause [6]. Bacterial infection in fishes at present is growing fast with an approximately 12% annual increase[7]. Diseases in tilapia cause considerable economic losses in the world`s industry of aquaculture with an approximate cost of US $150 million annually[7]. Many cases of fish kill caused by different bacterial species were reported in various countries including the Philippines [6]. In 1992, a severe disease outbreak threatened the tilapia industry in the country and the causal organism was *Aeromonas hydrophila* [8].

Some of the most common species of bacteria present in pond-cultured tilapia are *Streptococcus*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Staphylococcus*, *Mycobacterium*, *Edwardsiella* and *Flexibacter* [6]. A huge number of bacteria in the gut of the fish have originated from water sediment. These natural gut bacteria can cause diseases when environmental and cultural conditions become favorable [9].

Pseudomonas spp. are opportunistic Gram-negative pathogens, naturally occurring in aquatic environment and as a part of normal gut flora of healthy fish [10, 11]. A number of *Pseudomonas* spp. are identified in various species of fish as causative agents of *Pseudomonas* septicemia and these include

P. fluorescens, *P. anguilliseptica*, *P. aeuroginosa* and *P. putida* [12-14]. The disease is characterized by petechial hemorrhage, darkness of the skin, detached scales, abdominal ascitis and exophthalmia [15]. Fish lesions caused by *Pseudomonas* are similar to *A. hydrophila*, being characterized by haemorrhage in fins and tail and ulceration of the skin [15]. Thus, this present study has investigated the outbreak of *P. aeruginosa* in tilapia grow-out farms in Minalin, Pampanga, Philippines and determined the possible risk factors in the occurrence of the bacterium.

2. Materials and Methods

2.1 Study area

Minalin, Pampanga is located southwest of the capital town of San Fernando (Figure 1). It is subdivided into 15 barangays namely Bulac, Dawe, Lourdes, Maniango, San Francisco de Asisi, San Isidro, San Nicolas (Poblacion), San Pedro, Sta. Catalina, Sta. Maria, Sto. Domingo, Sto. Rosario and Saplad. Aquaculture is one of the sources of livelihood in Minalin. In fact, it is the fourth leading municipality in Pampanga with respect to area of land allotted for tilapia farming (around 1,755 fishpond units with total area of 2,728.69 ha).

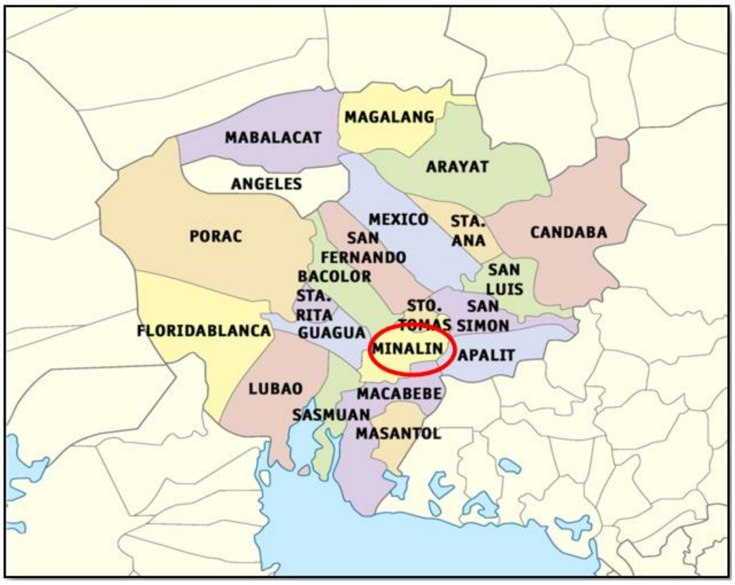

Fig 1: Political boundary of various municipalities in Pampanga

2.2 Collection of fish and water samples

Marketable size tilapia (Minimum of 100 g) that were grown in earthen ponds in Minalin, Pampanga, Philippines that manifested either one of the following signs such as lesion, fin or skin rot, eye opacity (Unclear eye) and body discoloration between November 2013 to March 2014 were considered in the investigation.

Tilapia samples were collected in 33 tilapia grow-out farms in 14 barangays (Figure 2; Table 1) of Minalin, Pampanga. For every farm, three pieces of tilapia were collected for bacteriological assessment. The samples were transported live back to the laboratory for analysis.

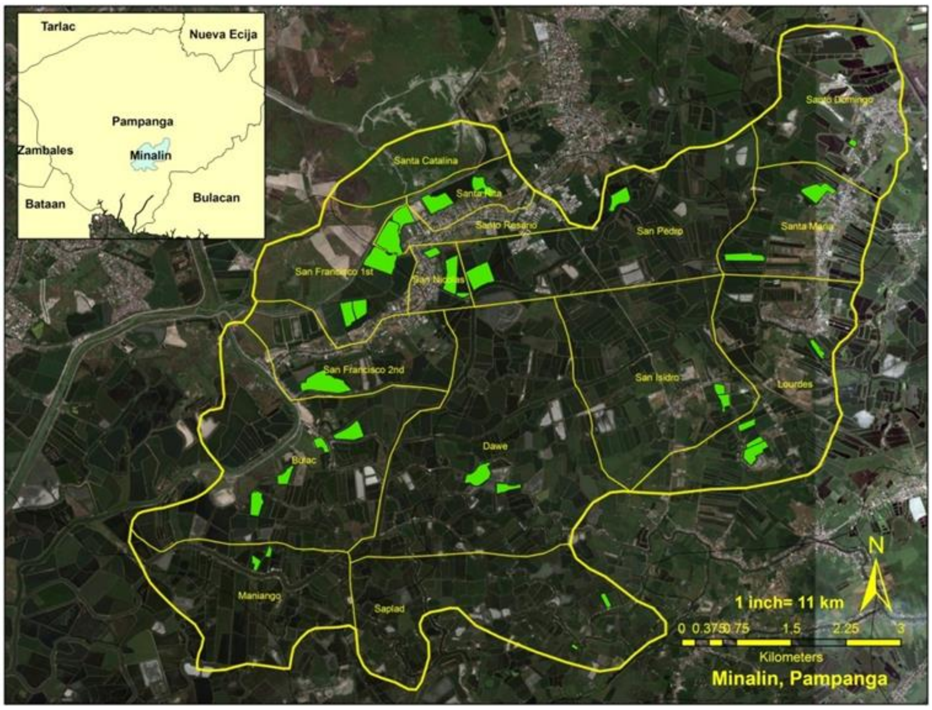

Fig 2: Digitized fishponds in Minalin, Pampanga, Philippines that served as collection sites

Table 1: Distribution of sampling sites per barangay of Minalin, Pampanga, Philippines.

Barangays	Number of Grow-out Farms
Bulac	3
Dawe	2
Lourdes	3
Maniango	3
San Francisco 1st	1
San Francisco 2nd	1
San Isidro	3
San Nicolas	2
San Pedro	3
Sta. Maria	3
Sta. Rita	3
Sto. Domingo	1
Sto. Rosario	3

2.3 Surveying of farm practices
A pre-tested survey form created by the Freshwater Aquaculture Center in Central Luzon State University was used in order to determine the farm practices being implemented in every collection site. The questionnaire also tries to assess the common problems encountered by the tilapia grow-out operators and their corresponding actions to solve the problems.

2.4 Isolation and identification of *P. aeruginosa*
Lateral and vertical incisions were made in the belly portion of the fish to reveal its internal organs. Smears from kidney were aseptically streaked in prepared Mueller Hinton Agar (MHA) plates. The plates were incubated at 37 °C for 18-24 hours. Bacterial colonies having different morphological growths in MHA were transferred in selective medium *Pseudomonas* selective Agar for the identification of *P. aeruginosa*.

2.5 Mapping
Digital mapping was performed using the ArcGIS software with ESRI basemaps and downloaded satellite images as references. Mapping of fishponds was done in a farm-level; each farm is represented by a digitized polygon. Collected data on the identified *P. aeruginosa* were incorporated in the digital maps.

2.6 Computation of attack rate and relative risk
Line listing of generated data on water quality and bacterial analysis were opened in EpiInfo for the computation of attack rate and relative risk (RR). RR value of >1 indicates positive association (The exposed group has higher incidence than the non-exposed group) while RR value of < 1 indicates negative association (The unexposed group has higher incidence).

3. Results and Discussions
3.1 Physical examination
Presented in Table 2 is the percentage of the tilapia samples collected in grow-out farms that manifested physical signs of bacterial infection. Around 85%, 53% and 15% of the tilapia samples had the presence of lesion, fin/skin rot, and eye opacity, respectively (Figure 3). Near 50% of the samples showed abnormal body coloration (e.g. whitening, blackening or reddening of a part or entire body). The presence of two and three physical signs of bacterial disease accounted to 44.79% and 21.88%, respectively. Only 3.13% of the samples had the appearance of the four signs.

A. B. C

Fig 3: Physical signs of bacterial diseases. (A) Eye opacity, discoloration of the body; (B) Lesion and haemorrhage; (C) Fin rot, discoloration of the body.

Table 2: Percentage of tilapia samples collected in grow-out farms in Minalin, Pampanga, Philippines that manifested physical signs of bacterial infection.

Physical Manifestations of Bacterial Infection	Proportion of the Sampled Tilapia (%)
Lesion	85.42
Fin or skin rot	53.13
Eye opacity	14.58
Body discoloration	44.79
Double combination of symptoms	44.79
Triple combination of symptoms	21.88
Presence of all symptoms	3.13

3.2 Computation of attack rate
The computed attack rate of *P. aeruginosa* in the whole municipality was 51.04%. Highest attack rate of *P. aeruginosa* (77.78%) was recorded in Barangay Sta. Maria and Sta. Rita (Table 3). By sex, female samples (52.00%) had higher attack rate than males (50.70%) (Table 4).

Table 3: Tilapia samples across barangay in Minalin, Pampanga, Philippines that were found positive to *P. aeruginosa*.

Barangays	Positive	Negative	Attack Rate (%)
Bulac	4	5	44.44
Dawe	2	4	33.33
Lourdes	6	3	66.67
Maniango	4	5	44.44
San Francisco 1st	2	1	66.67
San Francisco 2nd	0	3	0.00
San Isidro	4	5	44.44
San Nicolas	1	5	16.67
San Pedro	6	3	66.67
Sta. Maria	7	2	77.78
Sta. Rita	7	2	77.78
Sto. Rosario	3	6	33.33
Saplad	3	3	50.00
Minalin	49	47	51.04

Table 4: Proportion of male and female tilapia samples that were found positive to *P. aeruginosa*.

Sex	Positive	Negative	Attack Rate (%)
Male	36	35	50.70
Female	13	12	52.00

3.3 Temporal pattern

The temporal pattern of *P. aeruginosa* is presented in Figure 4. The epicurve depicted that tilapia were continuously exposed over a long period of time to a common disease-causing factor.

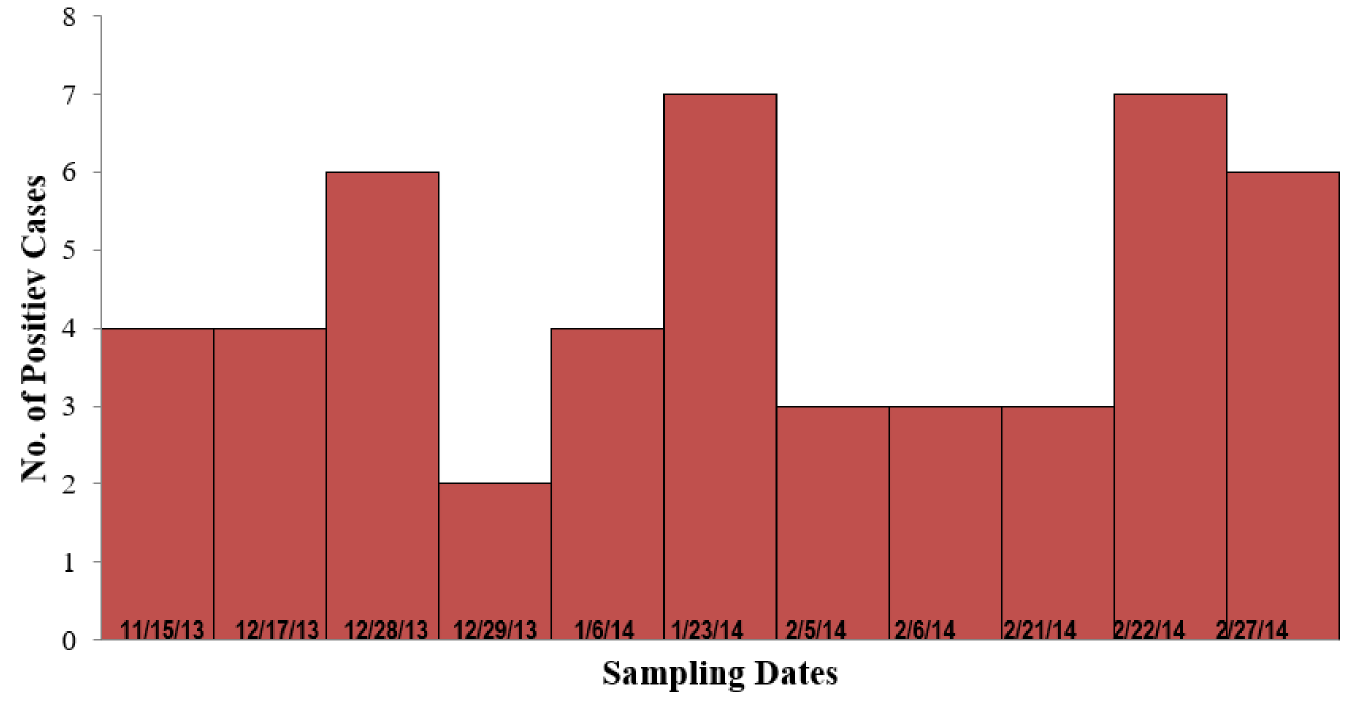

Fig 4: Occurrence of *P. aeruginosa* infection in grow-out tilapia in Minalin, Pampanga, Philippines

3.4 Possible identified risk factors

Two groups of possible risk factors in the occurrence of *P. aeruginosa* were considered, namely farm management practices and quality of the pond water. The computed relative risk (RR) in each considered risk factor is provided in Table 5. Relative risk having value of >1 indicates positive association (The exposed group has higher incidence than the non-exposed group) while RR value of < 1 indicates negative association (The unexposed group has higher incidence).

Under the farm management practices, exposure of the fish to insufficient/incomplete pond preparation (RR = 1.53) and unsafe source of water (RR = 1.45) yielded an RR >1. Meanwhile under the bracket of water quality, tilapia exposed to high level of phosphorous (RR = 1.09), not optimum temperature (RR = 1.38) and alkalinity (RR = 1.13) resulted also to RR>1.

Table 5: Possible risk factors in the occurrence of *P. aeruginosa* infection in grow-out tilapia in Minalin, Pampanga, Pampanga

Possible Risk Factors	Relative Risk (RR)
Management Practices	
Scale of operation	0.72 (0.48-1.08)
Pond preparation	1.53 (0.90-2.60)
Source of water	1.45 (0.90-2.33)
Rate of stocking	0.88 (0.60-1.31)
Feeding	0.91 (0.49-1.69)
Water Quality	
Temperature	1.38 (0.75-2.56)
Total dissolved solid (TDS)	0.98 (0.67-1.48)
Dissolved oxygen (DO)	0.79 (0.50-1.26)
pH	0.85 (0.48-1.48)
Alkalinity	1.13 (0.75-1.71)
Phosphorous	1.09 (0.66-1.80)

3.5 Transmission pathway: Source and spread

Provided in Figure 5 is the distribution of *P. aeruginosa* infection in tilapia grow-out farms in Minalin, Pampanga, Philippines. Around 76% of the visited tilapia farms were positive to *P. aeruginosa* infection. This bacterium could be transmitted only through direct contact with infected fish, water and/or equipment.

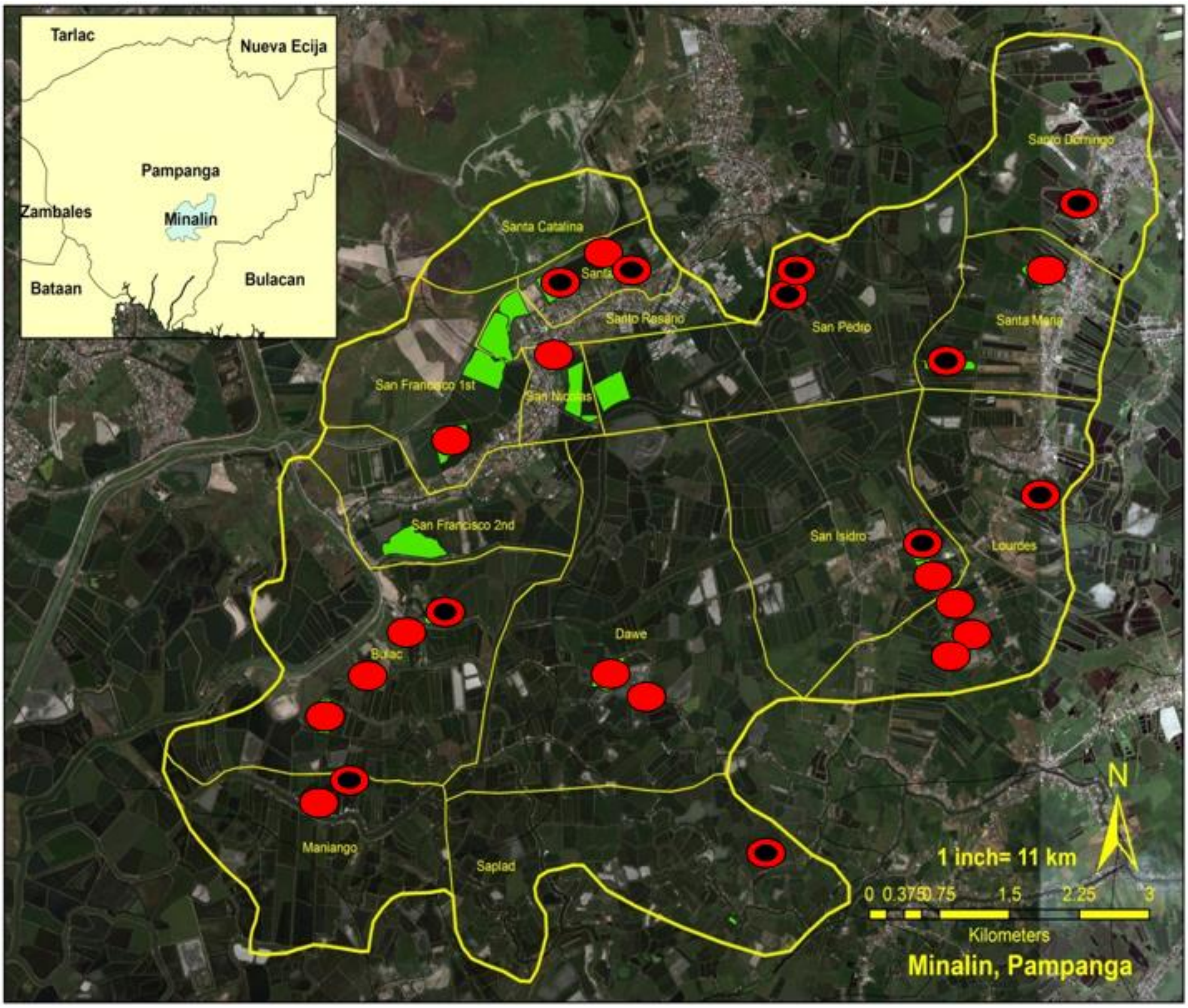

Fig 5: Distribution of *P. aeruginosa* in tilapia grow-out farms in Minalin, Pampanga, Philippines. (Note: Red circle = AR <50%; Red circle with black spot = AR >50%).

Tilapia aquaculture in Pampanga was dependent in Pampanga River, river tributaries and irrigation canals as main sources of water. However, the river was already highly polluted and contaminated based upon physical, chemical and biological analysis of its water and soil [16, 17]. The water had very low DO (2.66-3.43 mg/L) and very high phosphorous (0.30-0.60 mg/L). High level of phosphorous in water coupled with low DO is good indication of eutrophication [17]. A number of pathogenic bacteria in fishes (*A. hydrophila*, *P. aeruginosa*) and humans (*Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*) were also isolated from the water and soil of Pampanga River [16]. High level of total bacterial count (TBC) and total coliform count (TCC) was observed in the water (TBC = 1.93 x 10^7-2.72 x 10^8; TCC = 1.45 x 10^5-3.30 x 10^6) and soil (TBC = 3.62 x 10^9-4.38 x 10^9; TCC = 1.19 x 10^5-1.06 x 10^6) [16].

The water source could be considered unsafe to use for the tilapia aquaculture in the province. Basically, the tilapia operators are pumping river water to their pond system which is high in phosphorous, deficient in DO and with the presence pathogenic organisms such as bacteria. Based upon interview, their immediate remedy in case of fish mortality which was water exchange could only aggravate the problem. Contaminated water supply was the main identified risk factor for the occurrence and wide distribution of *P. aeruginosa*. This hypothesis was being supported by the type of epicurve that was generated which was common source epidemic (Continuous source) (Figure 4). Improper pond preparation practices and intensive stocking could be considered as additional risk factors for the deterioration of the quality of water in ponds. The interviewed farms failed to follow the complete sets of activities involved in pond preparation (Draining, elimination of predators and nuisance weeds, harrowing, drying, repairing of dikes, water inlet and outlet canals, screening of water canals, liming, initial filling of water and leveling of pond bottom, and fertilization). Pond preparation should be done properly in order to provide the fish an environment which is free of pests and predators, and a pond bottom suitable for growth of natural food. Most of tilapia growers in Pampanga had an intensified stocking rate in order to replenish loss of stocks due to mortality. Increase in stocking rate beyond the biological capacity of the pond system could necessitate the total dependency in commercial feeds. Excess feed input and accumulated waste in the system could deteriorate both the water and soil. Roughly, 25% of the phosphorus in aquafeeds is harvested in aquaculture biomass and the remainder is in uneaten feed and feces that microbially decompose and release phosphate, or metabolic excretions of culture animals [18]. However, almost 80% of the unrecovered phosphorus in harvested fish is found in the pond bottom. Another risk factor that might contribute to the faster multiplication and transmission of bacteria is elevated water temperature.

The municipality of Minalin was not only known for its tilapia production but also as egg granary in Luzon. A number of poultry farms were located within the municipality. Some of the interviewed farms are near to existing poultry farms. A number of flies and birds in the poultry farms move in the tilapia ponds during and after harvest. Flies and birds can carry pathogenic organisms to the pond water through direct and indirect means. Birds can also predate the tilapia fingerlings. In addition, flies can contaminate the food prepared by the laborers in the farm.

Majority of the farms in Minalin, Pampanga were not implementing any forms of biosecurity measures. Used equipment was not disinfected after use and there was no designated area for their safe keeping. Free-ranged animals such as poultry, cats and dogs were found inside the farm. They can contaminate the feeds that were improperly kept in a storage room made of light materials. Dead fishes were just thrown elsewhere thus produce a foul odor. The roaming animals can feed on tilapia carcasses. Flies that took their meal on rotting tilapia carcasses can contaminate the food of the farm laborers.

Listed below are the proposed strategies on a micro-and-macro environmental scales that would somehow preserve the biological integrities of the involved systems toward resilience on diseases and environmental degradation.

Under the micro-environmental scale, the following were recommended:

1. Tilapia operators should consider the put-up of water pump as their supplemental or main source of water since the quality of the water from the river, tributaries and irrigation canals were already deteriorated.
2. In order to help in improving the quality of water in the river system, wastewater from tilapia farms should be processed first in a siltation pond or a filter system before discharging the wastewater in the river. The water from the river should be filtered or treated first before pumping it towards the pond system.
3. Land preparation practices should be improved by following the standardized procedures being recommended by the Bureau of Fisheries and Aquatic Resources (BFAR) or any fisheries research institutions in the country such as Central Luzon State University (CLSU) and University of the Philippines (UP). The age of the fishponds should also be considered; fishponds with high organic matter and phosphorous should be rested for a while (at least one year). This only necessitates that analysis of pond bottom soil (e.g. organic matter, organic carbon, available nitrogen, phosphorous, sulfur, etc.) should be done at least in a yearly basis in order to understand better its condition. Most pond water quality variables are strongly influenced by pond bottom soil characteristics. However, less attention has been given to the management of bottom soil quality. More attention should be devoted on the study of pond bottom soil management.
4. Maintenance of moderate stocking density (4 pcs./m^2) in order to reduce dependency on commercial feeds is recommended. This practice could prevent further deterioration of the quality of water and pond bottom soil. The fish farmers should try other successful feeding strategies such as alternate day feeding and supplemental feeding. Moderate stocking density might also reduce the proliferation and transmission of diseases within the pond.
5. They should invest on important water quality equipment or kits such as pH meter/kit or DO meter/kit.
6. The farms should practice disinfection of equipment using salt solution or bleach for preventing the spread of pathogenic bacteria from one pond to another pond. A certain area of the farm should be allotted to cage/fence their farmed animals. The dead fishes should be buried to prevent foul odor and further spread of diseases. The farm laborers should practice proper washing of hands to reduce food contamination. Their foods should be kept in air-tight containers.
7. A clean and well kept storage room for feeds should be provided if ample funds permit to prevent adulteration and contamination of the feeds. Disinfected equipment such as nets should be stored in a separate area.
8. The poultry farms should be reported to the barangay officials for immediate action. To prevent entry of birds to the pond water area, a series of nylon strings should be constructed above the water area. This will reduce predation and possible transmission of diseases.
9. The farm owners with their technicians and laborers should update their knowledge and skills on current advances in tilapia farming. The provincial government in collaboration with some universities offer free training regarding this matter.

Under the macro-environmental scale, the following were recommended

1. The provincial government of Pampanga should continue their dredging program in the river to improve flow and circulation of nutrients. They should assist the fish farm operators in putting up a number of water pumps that will not cause depletion of ground water resource in a long run.
2. There is also a need to strengthen the skills and capabilities of the technical staff of every Municipal Agriculture Office in the province. More attentions should be given specifically in inland resources management such as fishponds and rivers. Interventions in the form of seminars, trainings and actual farm visits should be organized by the provincial government, BFAR, universities or any related private institutions/agencies.
3. The local and provincial government should form a technical team that is readily available to serve the fish farmers in case of water and soil quality problems and most especially during disease and fish kill outbreaks. There is also a need to improve the surveillance capacity of the local and provincial government. Data on fish farm operators should be updated annually in order to have a clearer understanding on the present situation of aquaculture industry in the province.
4. The local and provincial government should be strict in the implementation of existing ordinances and national laws concerning aquatic pollution. They should strategize ways on how to reduce the environmental impacts of aquaculture to the river system. A treatment pond should

be mandatory in all operating aquaculture farms in the province. The government should be strict in issuing permit to operate; they should see to it that the farms have the capacity to operate with fewer burdens to the environment.

4. Conclusion

To sum it up, the occurrence of bacterial diseases in tilapia is caused by the interplay of various risk factors such as presence of pathogenic organism and source of contamination, and a susceptible host due to stressful environment. Since wastewater from aquaculture and agriculture were dumped in the river system without any treatment, their main source of water could serve as reservoir of present and future problems on diseases. There is also a big possibility that a large percentage of the cultured tilapia from the unvisited farms during the time of investigation might be positive to *P. aeruginosa*. The presence of disease vectors and carriers inside and outside the farm could magnify the present problem of tilapia operators in Minalin, Pampanga, Philippines.

5. References

1. Food and Agriculture Organization of the United Nations. State of world fisheries and aquaculture. http://www.fao.org/3/a-i3720e.pdf. 13 June 2019.
2. Bureau of fisheries and aquatic resources. Philippine fisheries profile. https://www.bfar.da.gov.ph/files/img/photos/2014FisheriesProfile(Finalcopy).pdf. 15 June 2019.
3. Naylor R, Burke M. Aquaculture and ocean resources: Raising tigers of the sea. Annual Review of Environmental Resources. 2005; 30:185-218.
4. Bureau of Agricultural Statistics, Department of Agriculture. Fisheries statistics of the Philippines. https://psa.gov.ph/content/fisheries-statistics-philippines-2004-2006. 15 June 2019.
5. Suanyuk N, Kong F, Ko D, Gilbert G, Supamattaya K. Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand—Relationship to human isolates? Aquaculture. 2008; 284(1-4):35-40.
6. El-Sayed, AFM. Tilapia culture. CABI Publishing, Cambridge USA, 148-154.
7. Shoemaker CA, De-Hai X, Klesius PH, Evans J. Concurrent infections (Parasitism and bacterial disease) in tilapia. http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=228567. 8 May 2019.
8. Yambot AV, Inglis V. *Aeromonas hydrophila* isolated from Nile tilapia (*Oreochromis niloticus*) with "eye disease". In: M.K. Vidyadaran, M.T. Aziz and H. Sharif (eds.). International Congress of Quality Veterinary Services for 21st Century Kuala Lumpur. 1994; 87-88.
9. Sugita H, Tsunohara M, Ohkoshi T, Deguchi Y. The establishment of an intestinal microflora in developing goldfish (*Carassius auratus*) of culture ponds. Microbial Ecology. 1998; 15(3):333-344.
10. Angelini NM, Signer GN. Disease of the fins of *Rhamdia sapo*. Isolation of the etiological agents and experimental infection. Review of Argentinean microbiology. 1988; 20:37-48.
11. Roberts RJ. Fish Pathology. Edn 2, Bailliere Tindall, London, 1989, 100-102.
12. Domenech A, Garayzgbal JF, Lawson P, Garcia JA, Cutuli MT, Blanco M *et al*. Winter disease outbreak in sea-bream (*Sparus aurata*) associated with *Pseudomonas anguilliseptica* infection. Aquaculture. 1997; 156(3-4):317-326.
13. Akayl T, Timur G. Septicemia caused by pseudomonad in young rainbow trout. Veterinary Fakultesi Dergisi, Istanbul, Turkey. 2004; 30(1):121-131
14. Altinok I, Kayis S, Capkin E. *Pseudomonas putida* infection in rainbow trout. Aquaculture. 2006; 261(3):850-855.
15. Toranzo AE, Magarinos B, Romalde JL. A review of the main bacterial fish diseases in mariculture systems. Aquaculture. 2005; 246:37-61.
16. Atayde RD, Reyes AT. Total bacterial and total coliform count of water and sediment collected in Upper Eastern Pampanga river tributaries and irrigation canals. Unpublished Undergraduate Thesis, College of Fisheries-Central Luzon State University, Science City of Muñoz, Nueva Ecija.
17. Labasan RE, Reyes AT. Plankton as indicators of water quality in the Upper Eastern Pampanga river tributaries and irrigation canals. Unpublished Undergraduate Thesis, College of fisheries-central Luzon state university, science city of Muñoz, Nueva Ecija.
18. Boyd CE. Phosphorus fractions in soil and water of aquaculture ponds built on clayey ultisols at Auburn, Alabama. In: Journal of the World Aquaculture Society, 2007; 25(3):379-395.