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Distribution of Aeromonas bacterial population in water, sediment and Nile tilapia in fish culture pond, Guder, Ethiopia

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ABSTRACT

Assessing heterotrophic bacterial population in water, sediment and fish tissue assumes importance in predicting quality of the fish and water quality in culture system. The present study aimed to estimate the total heterotrophic bacterial population in water, sediment, and Nile tilapia (*Oreochromis niloticus)* cultured in an aquaculture farm at Guder Campus, Ambo University, Ethiopia. Water, sediment and fish body tissue were collected from the fish rearing pond, and were estimated for total heterotrophic bacterial population. Various physico-chemical characteristics were recorded following standard methods. The level of bacterial population in water, sediment and fish tissue were done by following standard methods and expressed as colony forming units (CFU) in water per milliliter (CFU ml^{-1}), sediment and fish tissues(CFU g^{-1}).The results revealed more bacterial population in sediment (3.43 $x10^6$ to 5.54 x 10^6 CFU g^{-1}) than in water (1.45$x10^6$ to 4.0$x10^6$ CFU ml^{-1}) and fish tissues (1.06 ± 1.10$x10^4$ to 1.74 ± 10.8$x10^4$ CFU g^{-1}in gill filaments; 1.62 ± 11.2$x10^4$ to 2.82 ± 13.0$x10^4$ CFU g^{-1} in intestine from; and 0.82 ± 5.9$x10^4$ to 1.60 ± 12.1.6$x10^4$ CFU g^{-1}; in kidney from 0.48 ± 5.0 to 0.77 ± 4.1$x10^4$ CFU g^{-1}. in skin). Among fish tissues; the heterotrophic bacterial population was more in the intestine than other organs of *Oreochromis niloticus*. In conclusion, the total heterotrophic bacterial population was dominated in sediment than the other samples. The present study concludes that physicochemical characters of water influence the growth and survival of total heterotrophic bacterial population in fish pond. The development of stress due to changes in physicochemical characters of water, and rich nutrient load in pond soil facilitate the growth of pathogenic bacteria which infect the culture fish *O. niloticus*. The detrivore feeding habits of *O. niloticus* is responsible for more number of bacterial populations in intestine than in other organs.

INTRODUCTION

Aquaculture is rapidly expanding worldwide, and among different fish species considered for culture, tilapia is favored most because of its suitable cultivable characteristics (Suresh and Lin, 1992).Although tilapia spp. are cultured under diversified aquaculture systems; pond

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culture of tilapia is widely practiced in several countries because earthen ponds used for fish culture producenatural fish food organisms due to soil-water interaction. To enhance fish production, fish ponds are excessively fertilized with organic and inorganic fertilizers, and fishes are fed with rich protein diets. This would lead to water quality deterioration when suitable pond management strategies arenot followed to control water quality deterioration. The increased in microbial load in unmanaged ponds reduces health and fish yield potential (Groff and Lapatra, 2000; Karunasagar and Otta, 2003). Therefore, evaluating microbial load in culture pond is fundamental and significantin aquaculture. Among microbes, bacterial pathogens assume importance as they produce great economic loss to aquaculture byproducing severe diseases, epizootics and mass mortality (Austin and Austin, 1999). Environmental changes accelerate bacterial infections (Ventura and Grizzle, 1987; Post, 1989; Zorrilla et al., 2003) and that, the rate of infection correspondingly increases with prolonged exposure of fish to stress (Sugita et al., 1985)

Extensive work has been done in certain countries in the field of finfish diseases (Ahmed et al., 2004; Islam et al., 2008; AlYahya et al., 2018). However, except for a very few studies on parasites and bacterial diseases of food fishes (Shibru and Tadesse,1979; Amare, 1986; Tefera, 1990; Eshetu, 2000), studies on bacterial diseases in fish culture in Ethiopia are scarce. As aquaculture has been identified as an important sector to ensure food security in Ethiopia, there is an imperative need to address factors that limit aquaculture production. This study focuses on heterotrophic bacterial population in water, sediment and Nile tilapia in Guder Aquaculture Farm of Ambo University. An assessment of bacterial population in fish and fish culture pond will provide an opportunity to prevent possible disease outbreaks in culture systems. This will also help to evolve suitable remedial measures to control bacterial infections in fish.

MATERIALS AND METHODS

Description of the study area

The study site, Guder Aquaculture Farm (Fig.1) is located in Guder Town at about 11 km away from the main campus of Ambo University. It is located between 1600 and 3192 meter above mean sea level. The rain fall ranges between 800 and 1000 mm and temperature between 10 $^{\circ}$C and 29 $^{\circ}$C. The soil characteristics are: 48% red soil, 27% black and 25% red and black soil (Anon, 2009). The soilis loamy clay, which is suitable for pond construction. Regarding weather condition, the Woreda has 27% arid, 55% semi-arid, and 18% desert. There are two distinct seasons; the dry season which occurs between October and March followed by a wet period between May and September. The main rainy season is from June to September. The area experiences moderately warm climate which is suitable for fish growth in ponds. The warmest months are from January to May with a peak in February whereas; the cold months extend from June to December. The area of the pond is 300m2 with a depth of 80 cm. The source water for the farm is from Endris River which is the tributary of Guder River.

Figure 1. Study site: Guder Aquaculture Farm of Ambo University

Enumeration of heterotrophic bacterial population from water, sediment and *O. niloticus*

Surface water samples were collected from the earthen pond by using sterilized glass bottles having 250ml capacity from about 20cm below the water surface from four locations of the pond for bacteriological investigation for a period of five months from November to March. Similarly the sediment samples were collected from the same location using submerging sterilized glass bottles and were centrifuged for decanting. For gills, intestine, kidney and skin sampling, fifty five Nile tilapia with a mean weight of 44.86g were randomly collected from the pond mentioned above. Individual fish was killed by a strong blow on the head and then surface disinfection was performed with 70% ethanol before gills, intestine, kidney and skin samples were taken aseptically. Later, 1ml of water and 1g of sediment and fish body samples were diluted (10^1-10^7 serialdilution factors) using 9ml normal saline solution. From each test tube of each sample, 100µL of sample was speared in duplicate on tryptic soy agree (TSA) plat and incubated at temperature of 37ºC for 48 hrs. The concentration of heterotrophic bacterial load at different samples was counted and expressed as colony forming unit per milliliter or gram (CFU/ml or CFU/g) (Cole et al., 1988; Austin and Austin, 1999; Pakingking et al., 2015). The plates with 30-300 colonies were used for the determination of bacterial population (Prakash and Karmagam, 2013). The bacterial colonies were observed according to shape, size, color and opacity (Garrity, 2001). All samples for the study were done in duplicate.

Pure culture of *Aeromonas* bacteria

Following morphological and colonial characteristics of bacterial on tryptone soy agar (TSA) plates, 3-5 representatives of each colony type were randomly picked from each plate and further sub-cultured to obtained pure cultures of bacterial following the method of Monghit-Camarin et al. (2020) and Pakingking et al. (2020). Later, bacterial cultures were stocked in TSA broth containing 15 % glycerol at 80 °C (Pakingking et al., 2015). Later, 1ml of sample were speared on tryptone soy agar (TSA) plat

and incubated at temperature of 37ºC for 48hrs for primary isolation and enumeration of total hetrotrophic bacterial following Bergey's Manual of Systemic Bacteriology (Holt et al., 1994) using Gram-staining and biochemical tests such as oxidase, catalase and indole tests etc.

Water quality parameters

To identify the relationship between water quality parameters and bacterial population during the study period, water temperature, pH and conductivity were measured around 10 a.m. and were determined by thermometer, digital pH meter and portable conductivity cell, respectively. Transparency was measured using Secchidisc (Trivedy and Goel, 1984). Dissolved oxygen of water sample was analyzed by following Winker's or titration-based on "drop count" method by fixing the samples using Alkaline iodide and Manganese sulfate and recorded as mg/L (APHA, 1998).Salinity was measured using portable conductivity meter. Ammonia, nitrate, total nitrogen and total phosphorous were measured using calibrated visible UV spectrophotometer in the Chemistry Laboratory of Ambo University.

Data analysis

Bacterial density data was transformed into Microsoft Excel spread sheet before statistical analysis. The means of bacterial load were compared using ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical Package for Social Sciences (SPSS) software version 16.0 windows were used to analyze the data with the level of significance at $p<0.05$.

RESULTS AND DISCUSSION

Water quality characteristics

The results of water quality characteristics are presented in Table 1. The results showed that DO content of the water varied between the months and it ranged between 6.3 mg/L in December and 7.25 mg/L in February. The water temperature ranged from a minimum of 18.1˚C in November and December to a maximum of 24.6˚C in February. There is an apparent difference in the water temperature between the months of the study. The lower temperature evidenced during November and December mainly coincided with the intermittent cloudy time which kept the area under cooler condition during this period. The temperature further showed a trend that it increased from January to February and a marginal decline from February to the end of March. Moreover, water pH also showed variation between the months. It was slightly acidic in November and December (6.60 - 6.65), and neutral in February and March (7.50 to 7.6). Total dissolved solid concentration was in the range of 186 - 191 mg/L. Chemical parameters like nitrate and phosphates showed marginal fluctuations between 27.1 and 33 and 1.9 and 2.11 respectively indicating the fact that it did not show much variation between the months. The salinity of pond was nil at all times. Conductivity values showed an increasing trend till February and showed a slight fall in March. February registered the highest value. Chien et al. (1999) reported that high conductivity has a direct bearing on the survival of microbes. Pond water transparency ranged from 27.3 to 34.9 cm which is within the desirable level for the culture of tilapia.

Table- 1: Physiochemical variables of pond water in different months

Water quality variables	Nov.	Dec.	Jan.	Feb.	Mar.
Water temperature (ºC)	18.1	18.1	22.2	24.6	24.2
pH	6.60	6.65	6.93	7.60	7.50
Dissolved oxygen (DO) (mg/L)	6.4	6.3	6.9	7.25	7.22
Ammonia (NH_3) (mg/l)	0.06	0.05	0.07	0.07	0.08
Nitrate (NO_3) (mg/l)	11.21	11.33	11.43	11.42	11.5
phosphate (mg/l)	1.91	1.9	1.91	2.11	2
Salinity (g/kg)	Nil	Nil	nil	nil	nil
Total dissolved solids (TDS) (ml/l)	186	186	187	191	189
Total hardness (as $Caco_3$) (ml/l)	79	79	80	79	79
Conductivity (µs/cm)	176	181	187	188	185
Secchi depth (cm)	27.3	33.1	34.9	34	34

Total heterotrophic bacterial (THB) population in water and sediment

The results of the quantitative estimation of heterotrophic bacteria in water and sediment of rearing pond in different months are given in Table 2. It is evident that the bacterial population varied between the months. Total heterotrophic bacterial (THB) population in the sediment ranged from 3.43 $x10^6$ to 5.54 x 10^6 CFU g^{-1} and in water it ranged between 1.45$x10^6$ in November and 4.0$x10^6$ CFU ml^{-1} in January. This finding revealed that bacterial populations were high in January and February and minimum in November in both water and sediment samples.

The results also showed that during January and February, there was maximum quantitative heterotrophic bacterial population in earthen pond when compared to November in both water and sediment samples. In the present study, it is clear that physico-chemical parameters alter the microbial environment leading to alteration in microbial community. The factors such as temperature, pH and DO when increased, the concentration of bacterial population also increased. This is because increased temperature in warmer months favors the growth of bacteria in the environment (Sugita et al., 1985; Markosova and Jezek, 1994).

Table- 2. Heterotrophic bacterial population in Water and Sediment samples

Months	Water (CFU ml^{-1})	Sediment (CFU g^{-1})
November	1.45$x10^6$	3.43$x10^6$
December	1.88$x10^6$	4.34$x10^6$
January	4.00$x10^6$	5.00$x10^6$
February	3.89$x10^6$	5.54$x10^6$
March	3.72$x10^6$	4.64$x10^6$
Mean	**2.98$x10^6$**	**4.59$x10^6$**

The mean population of bacterial in the sediment samples was 4.59 x 10^6 CFU g^{-1} and it was 2.98x10^6 CFU ml^{-1} in water, despite the fact that heterotrophic bacterial population was more in the sediment samples and enhances survival of aquatic bacteria than in water. Okpokwasili and Alapiki (1990) also observed higher bacterial population in fish pond sediment than in water. Anon (1997) also reported higher bacterial population density in the sediments than water in general due to the rich organic content of the former and lesser residence time of the microorganisms in the water column than the sediments.

Heterotrophic bacterial population in different organs of Nile tilapia (*O. niloticus*)

The distribution of heterotrophic bacteria in different organs (gills, intestine, kidney and skin) of *O. niloticus is* presented in Table 3. The bacterial population in gill filaments ranged from 1.06 ± 1.10x10^4 to 1.74 ± 10.8x10^4 CFU g^{-1}; in intestine from 1.62 ± 11.2x10^4 to 2.82 ± 13.0x10^4 CFU g^{-1}; in kidney from 0.82 ± 5.9x10^4 to 1.60±12.1.6x10^4 CFU g^{-1}; in skin from 0.48 ± 5.0 to 0.77 ± 4.1x10^4 CFU g^{-1}. Each count was the mean value of viable colonies grown on duplicate agar plates made per individual sample. There was no significant difference ($p>0.05$) during November and December of bacterial count in fish tissues, but significantly increase in February ($p<0.05$) was observed. This may be related to ambient water temperature. Similar observation was reported by Ferguson et al. (1996) who reported that change in water parameters have a positive correlation to total heterotrophic bacterial population. Pal and Das Gupta (1992) established that environment could influence the micro flora of the fish and pond system. When bacterial counts of different organs were compared, the count in the month of February was significantly increased ($p<0.05$) in intestine and gills. In this study, the presence of high bacterial population in the gills and intestine of fish might be due to the high metabolic activity of fish associated with increased feeding rates at higher water temperatures. The bacterial population observed in fish samples was highest in intestine. This may be due to the voracious feeding behavior of Tilapia which feeds on detritus, organic matter as reported by Beveridge et al. (1988). It is generally presumed that those bacteria which were consumed by fishes like tilapia are particle-bound (Bowen, 1976; Schroeder, 1978; Opuzynski, 1981). Next to intestine, higher bacterial load was found in the gills. This is mainly because of the role played by gills in filtering microscopic organisms (Hamplet al.,1983). Evidences from recent studies of feeding in tilapia suggest that small particles are entrapped among the gill apparatus in a mucous film (Drenner et al., 1987; Beveridge et al., 1988; Northcott and Beveridge, 1988).

Histological studies of the bucco-pharyngeal cavity showed that the mucous cells of the gill rakers produce a highly negatively charged mucous (Northcott and Beveridge, 1988) which may facilitate flocculation and retain very small particles. Next to intestine and gills, the bacterial count was more in kidney. Kidney being an excretory organ the bacterial population might have trapped inside the kidney in the process of excretion. Skin showed the minimum bacterial load. The reason for minimum load of bacteria in the skin may be due to its frequent contact with the

contaminated water and sediment in the aquatic media.

Table- 3: Monthly mean heterotrophic bacterial population in different organs of fish

Month	Gill ($x10^4$ CFU g^{-1})	Intestine ($x10^4$ CFU g^{-1})	Kidney ($x10^4$ CFU g^{-1})	Skin $x10^4$ CFU g^{-1}
November	1.06±1.10	1.62±1.12	1.06±1.5	0.76±0.26
December	1.13±1.06	2.03±3.40	1.07±1.3	0.68±0.49
January	1.14±2.10	1.67±1.98	0.82±5.9	0.48±0.50
February	1.49±1.28	2.82±1.30	1.60±1.21	0.77±0.41
March	1.74±1.08	2.36±1.45	1.27±2.26	0.75±0.40

Heterotrophic Bacterial population in fish, water and sediment

Based on morphological and biochemical characteristics, bacterial isolates namely *Escherichia coli, Aeromonas, Pseudomonas, Salmonella, Staphylococcus, and Streptococcus* from fish, water and sediment samples were identified.

***Aeromonas* in water and sediments**

The *Aeromonas* bacterial count in water and sediment is presented in Table 4. It ranged from 1.01 x 10^6 to 1.42 x 10^6 CFU $ml^{-1}$and 1.23 x 10^6 to 1.70 x10^6 CFUg^{-1} in water and sediment samples, respectively. The bacterial population was more in the sediment (1.47x10^6 g^{-1}) than in water samples (1.25x10^6 ml^{-1}). This observation is related to the fact that sediment contains more valuable nutrients for the growth of microorganism than the water column. This has to be ascribed to the sedimentation of the bulk of nutrients added in the form of fish feed or organic wastes for pond fertilization. Similar trend was noticed by Okpokwasili and Alapiki (1990) who related this with the decomposition of these organic adjuncts used for pond water fertilization. Furthermore, the favorable temperature and the permissible dissolved oxygen level of the sediment would have enhanced the survival of the bacteria (Ogbondeminu, 1993). The bacterial population in the month of January in both water (1.42x10^6 CFU ml^{-1}) and sediment (1.7x106 CFUg^{-1}) followed by February which registered the values as 1.39x10^6 CFU ml^{-1} for water and 1.54x10^6 CFU g^{-1} for sediments. The bacteria count in the months of November for both water (1.01x10^6 CFU ml^{-1}) and sediment (1.23x10^6 CFU g^{-1}) were less when compared to January and February. In general, the occurrences of bacterial population in fish in rearing ponds exhibited variation in relation to different months. It was reported that alteration in environmental parameters influences growth and survival of micro flora in aquatic environment.

Table – 4: *Aeromonas* bacterial population in water and sediment in different months

Months	Water(CFU $ml^{-1}10^6$)	Sediment (CFU $g^{-1}10^6$)
November	$1.01x10^6$	$1.23x10^6$
December	$1.08x10^6$	$1.44x10^6$
January	$1.42x10^6$	$1.70x10^6$
February	$1.39x10^6$	$1.54x10^6$
March	$1.33x10^6$	$1.42x10^6$
Mean	**$1.25x10^6$**	**$1.47x10^6$**

Relationship between water parameter and *Aeromonas* bacteria

The relationship between water quality parameters and *Aeromonas* bacteria in water and sediment in relation to water temperature is presented in Table 5. The relationship between physico-chemical parameters and bacterial count attracted much attention (Ogbondeminu and Adeniji, 1984; Ferguson et al., 1996). The results showed that bacterial counts were directly related to the various water variables examined. From November to December when DO, water temperature, pH and ammonia were found to be lower, the bacterial population was also lower, and again the bacterial population was higher in January and February.

Table -5: *Aeromonas* bacteria in relation with water parameters

Month	DO (mg L^{-1})	Temperature (^{0}c)	pH	NH_3 (mg L^{-1})	Mean *Aeromonas* population (CFU ml^{-1})	
					Water	**Sediment**
Nov.- Dec.	6.35	18.1	6.62	0.06	$1.05x10^6$	$1.34x10^6$
Jan. - Feb.	7.07	23.4	7.26	0.07	$1.41x10^6$	$1.62x10^6$

Morphological and biochemical characterization of *Aeromonas* bacteria

The characteristics recorded are Gram's negative, rod shape with round end and motile (Buller, 2004). Biochemical properties of *Aeromonas* bacterial isolates are described in Table 5. The isolates were positively reacted with cytochrome oxidase, catalase, gas production, and lactose, glucose, and sucrose fermentation and motile whereas the isolates were negative starch hydrolysis. H_2S production was positive on motility medium and negative on Triple sugar Iron Agar (TSIA). Microbiology Laboratory Guidebook (Bonnie et al., 1998) describes the biochemical characteristic of *Aeromonas* bacteria. Catalase and oxidase positive and, hydrogen sulfide production on Triple sugar Iron Agar (TSIA) negative, and growth temperature tests were conducted to demonstrate the biochemical characteristic of *Aeromonas* bacteria.

Table- 6. Cell morphology and biochemical characteristics of *Aeromonas* spp.

Test	Results
Shape	Straight, rod, pairs with round end
Motility test	Motile
Gram staining	-ve
Colony color	Yellowish with opaque
Hydrogen sulfide test	+ve/ -ve
Oxidase test	+ve
Catalase test	+ve
Starch hydrolysis test	-ve
Gas production	+ve
Lactose/sucrose/glucose fermented	+ve
Acid production	+ve
Triple sugar Iron Agar	Acid butt with gas

Note: Result from test conducted +ve indicate positive result

CONCLUSION

The present study revealed that physicochemical characteristics of water influence the growth and survival of heterotrophic bacterial population in fish culture pond. Increased water temperature, favorable dissolved oxygen content and pH facilitate the proliferation of heterotrophic bacterial population in water. Excess feed remains, rich nutrient content, organic matter and their longer resident time in pond soil profoundly favor the increase in bacterial count in sediment than water. The development of stress due to changes in physicochemical characteristics of water, and rich nutrient load in pond soil facilitate the growth of pathogenic bacteria which infect the culture fish *O. niloticus*. The detrivore feeding habits of *O. niloticus* is responsible for more number of bacterial populations in intestine than in other organs.

References

Ahmed G.U., Parveen R. and Sultana S. 2004. Disease investigation of small indigenous fishes from Kailla Beel in Mymensingh area. *J. Bangladesh Agril. Univ*. **2**(2):305-311.

AlYahya SA, Ameen F, Al-Niaeem KS, Al-Sa'adi BA, Hadi S, Mostafa AA. 2018. Histopathological studies of experimental Aeromonas hydrophila infection in blue tilapia, Oreochromis aureus. *Saudi J Biol Sci*. **25**(1):182-185.

Amare T. 1986. Parasites of fish from Lake Awassa and Chamo. DVM Thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit, Ethiopia.

Anon J. 1997. Ecological, toxicological and environmental impact assessment studies of the effluents discharge from MRLCHR in Marine environs of Nagapattinam, Tamil Nadu. Technology References, NIO.12/97, 86.

Anon J. 1997. Ecological, toxicological and environmental impacts assessment studies of the effluent discharge from MRLCHR in marine environs of Nagapattinam, Tamilnadu. Technology Ref. No: NIO 12/97,86

APHA 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC., USA., ISBN: 0-87553-235-7.

Austin B. and Austin D.A. 1999. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, 4thed

Barrow G.I. and Feltham R.K.A. 1993. Cowan and Steel's Manual for the identification of Medical Bacteria. Cambridge University, England.

Beveridge M.C.M., Briggs M.R.P., Northcott M.E. and Ross L.G. 1988. The occurrence, structure and development of microbranchiospines among the tilapias (Cichlidae: Tilapiini). *Canadian. J. Zool*. **66**: 2564 - 72.

Bonnie E., Rose A. and, Okrend J. G 1998. Isolation and identification of *Aeromonas* species from meat and poultry products. Microbiology Laboratory Guidebook 3rd Edition.

Bowen S.H. 1976. Mechanism for digestion of detrital bacteria by the cichlid fish *Sarotherodon mossambicus* (Peters). *Nature* **260**: 137–138.

Buller N. B. 2004. Bacteria from Fish and other Aquatic Animals: A practical identification Manual. Department of Agriculture, South Perth: Australia.

Chien Y., Laia H.T., Thi H. and Liub S.M. 1999. Modeling the effects of sodium chloride on degradation of chloramphenicol in aquaculture pond sediment. *Sci. Total Environ*. **239**: 81-87.

Cole J., Findlay S. and Pace M.L. 1988. Bacterial production in fresh and saltwater ecosystems: across review. *Mar. Ecol. Prog. Ser*. **43**:1-10.

Drenner R.W., Vinyard G.L., Hambright, K.D. and Gophen M. 1987. Particle ingestion by *Tilapia galilaea* is not affected by removal of gill rakers and microbranchiospines. *Trans. Am. Fish Soc.* **116**: 272-6.

Eshetu Y. 2000. Preliminarily survey of parasites and bacterial pathogen of fish. SINET: *Ethiopian J. Sci*. **23** (1): 25-33

Frguson C.M., Coote B.G., Ashbolt N.J. and Stevenson I.M. 1996. Relation between indicators, pathogen and water quality in an estuarine system. Water Res. 30:2045-50.

Garrity G.M. 2001. Bergy's Manual of systematic bacteriology, new York, Springer Verlag

Groff J. M. and Lapatra, S. R. 2000. Infectious diseases impacting the commercial salmonids. *J. Applied Aquaculture*. **10**(4): 17-90.

Hampl A., Jirasek J. and Sirotek D. 1983. Growth morphology of the filtering apparatus of silver carp *(Hypophthalmichthys molitrix). II.* Microscopic anatomy. Aquaculture **31**:153-8.

Holt J.G., Krieg N.R., Sneath P.H.A. and Williams S.T. 1994. Bergey's manual of determinative bacteriology. 9th Edition. Williams and Wilkins, Baltimore, USA. 787 pp

Islam M.T., Mostafa K. and Rashid, M.M. 2008. Histopathological studies of experimentally infected Heteropneustes fossilis with *Aeromonas hydrophila* Bacteria. Progress Agric., 1991)89-96.

Karunasagar I. and Otta S. K. 2003. Disease problems affecting fish in tropical environments. *J. Applied Aquaculture.* **13**(3/4): 231-249.

Mantoura R.F.C., Martin J.M. and Wollast R. (Eds.) Global Change, John Wiley and Sons, New York, 365-81.

Markosava R. and Jezek J. 1994. Indicator bacteria and limnological parameters in fish ponds. *Water Res*. **28**: 2477-85.

Monghit-Camarin M.A., Cruz-Lacierda E.R., Pakingking R.V., Cuvin-Aralar M.L., Traifalgar R.F., Añasco N.C., Austin F.W. and Lawrence M.L. 2020. Bacterial Microbiota of Hatchery-Reared Freshwater Prawn Macrobrachium rosenbergii (de Man, 1879). *Asian Fish. Sci.* **33**:241–248.

Northcott M.E. and Beveridge M.C.M. 1988. The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*). *J. Zool*. **215**: 133-49.

Ogbondeminu F.S. 1993. The occurrence and distribution of enteric bacteria in fish and water of tropical ponds in Nigeria. *J. Aquaculture. Trop*. **8**: 61-66.

Ogbondeminu F.S. and Adeniji H.A. 1984. Comparative study of bacterial flora in relation to water quality of fertilized and non-fertilized fish ponds in Nigeria. Kainji lake Research Institute, Annual Report, pp 32-39.

Okpokwasili G.C. and Alapiki A.M. 1990. Bacterial flora associated with a Nigerian freshwater fish culture. *J. Aqua. Trop*. **5**: 87–90.

Opuzynski K. 1981. The influence of the silver carp *(Hypophthalmichthys molitrix* Val.) on eutrophication of the environment of carp ponds. Part VII. Recapitulation. *Rocz. Nauk. Roln. Ser*. **94**: 127.-51.

Pakingking R., Palma P. and Usero R. 2020. *Aeromonas* load and species composition in tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. *Aqua Res* **51**: 4736–4747.

Pakingking R.J, Palma P. and Usero R. 2015. Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. *World J Microbiol. Biotechnol.* **31**:265–275.

Pal D. and Das-Gupta C.A.F. 1992. Microbial pollution in water and its effect on fish. *J. Aquat. Anim. Health*. **4**: 329.

Post G.W. 1989. Text book of fish health T. F. H. Publication, Inc Ltd. 211 West Syvania Avenue *Neptune city NJ 00753.*

Prakash M. and Karmagam N. 2013. A study on bacterial flora associated with freshwater prawn, *Macrobrachium rosenbergii. Int. J. Curr. Res. Acad. Rev.* **1**:01–16.

Schroeder G.L. 1978. Autotrophic and heterotrophic production of microorganisms in intensely manured fish ponds and related fish yields. *Aquaculture*. **14**: 303-25.

Shibru T. and Tadesse G.E. 1979. Observation on the parasites of *Tilapia niloticai*n Lake Ziway. *Ethiop. J. Sci.* **1**(2):126-130

Sugita H., Fushino T., Oshima K. and Deguchi Y. 1985. Microflora in the water and sediment of fresh water culture ponds. *Bull. Jap. Soc. Sci. Fish*. **51**:91-97.

Suresh A.V. and Lin C.K. 1992. Tilapia culture in saline water: a review. *Aquaculture*. **106**:201- 226.

Teferra W. 1990. Parasites of fish from Lake Tana: DVM Thesis; Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Toke Kutaye Woreda, R.D.O. 2009. Toke Kutaye Rural Development Office Annual Report.

Trivedy R.K. and Goel P.K. 1984. Chemical and biological method for water pollution studies. Environmental Publications, Karad, India, pp 1 - 22.

Ventura M.T. and Grizzle J.M. 1987. Evaluation of portal of entry of *Aeromonas hydropilla* in channel cat fish. *Aquaculture*. **65**: 205-214.

Zorrilla I., Chbrillon M., Arijo S., Diaz-Rosales P., Martinz-Manzanares E., Balebona M.C. and Marinigo M.A. 2003. Bacteria recovered from diseased cultured gillhead sea bream *(Sparusaurata L.)* in southeastern Spain. Aquaculture. **218**: 11 - 20.