Applied Science Reports
www.pscipub.com/ASR
E-ISSN: 2310-9440 / P-ISSN: 2311-0139
DOI: 10.15192/PSCP.ASR.2014.1.3.118121

App. Sci. Report.
5 (3), 2014: 118-121

Studies on Water-media Characteristics and Bacterial Population Associated with Laboratory-reared Clarias gariepinus Fingerlings

[1]Ayanwale, A.V., [1]Ihindan, P.T., [1]Chukwuemeka, V. I. and [2]Erhabor, O. F.

1. Department of Biological Sciences, Federal University of Technology, Minna, Nigeria
2. Department of Biology, College of Education, Warri, Delta State, Nigeria
[*]**Corresponding Author email:** kaylatiyemi@yahoo.com

Paper Information

Received: 26 January 2014

Accepted: 28 February 2014

Published: 20 March 2014

A B S T R A C T

This study was aimed at elucidating prevailing physic-chemical properties of culture media of the fish species, Heteroclarias, as well as the associated-bacterial population under Laboratory conditions. Adequately replicated fingerling specimens of the species were raised under artificial conditions in the Laboratory, following recommended procedures for fish culture. Physico-chemical analysis of the culture media, as well as, bacteriological studies of swab samples from external surfaces of the skin, fins and gills of the fishes, were carried out following standard protocols. The results showed that with the exception of Ammonia (range = 0.05±0.01 to 0.54±0.01mg/l), the physic-chemical properties investigated namely, pH (mean = 8.58±0.01), temperature (26.50±0.50^{o}C), Dissolved Oxygen (6.29±0.10mg/l) and Biochemical Oxygen Demand (0.21±0.03mg/l), did not differ significantly (P>0.05); even with increasing age and/or size of the fingerlings. Six bacterial species: S. faecalis, K. pneumonea, P. aeruginosa, E. coli, B. subtilis and S. mutans, were encountered on the external parts of the fishes. However, while three of the species namely, S. faecalis, K. pneumonea and E. coli, were recovered from all three body parts of the fishes, the remaining three species were less distributed. The number of associated-bacterial species was similar for the fin and gills, and was higher than that of the skin. The bacterial load was significantly highest on the fins (mean = 12.24±2.21cfu/g), followed by the gills (9.31±2.45cfu/g) and least on the skin (6.04±1.49cfu/g).

Key words: *Bacterial population, water characteristics survival, laboratory reared Clarias gariepinus.*

Introduction

Bacterial infection is an important economic and limiting factor in intensive fish production (Robert, 1989). Microbial quality of culture fish is largely determined by the quality of water in which they were reared (Buras, 1993). Bacterial diseases are more acute in cold than warm water aquaculture, and may be aggravated by unfavorable conditions such as crowding, malnutrition and unstable water temperature (Fafioye, 2011). Fish dependency on water is crucial; hence the source, volume and quality of physico-chemical parameters such as dissolved oxygen, total hardness, pH, alkalinity, carbonate, ammonia are salient factors to consider in relation to fish health as documented by Fafioye (2011). This observation underscores the influence of bacterial load and species composition in fish culture water bodies (Liu et al., 1996). Similarly, fish feeds given to cultured fishes either in ponds or aquaria, have been reported to have considerable impacts on bacterial load associated with the fishes (Buras, 2008; Azad et al., 1997). Also, water of poor physico-chemical quality may have adverse effects on fish and fish consumers, thereby, resulting in serious economic and human losses (Amadi, 2009).

Clarias gariepinus is widely cultivated in Africa where it derived its name, the 'African Cat Fish' (Ita, 1980). It is an important commercial fish species in the Nigerian fishing industry (Fafioye, 2011); and enjoys wide-spread acceptability in all parts of the country. However, productivity of the fish species, particular, under artificial culture is grossly below optimum potential, due to challenges ranging from poor culturing environment and spoilage resulting from microbial infections (Ayanwale et al., 2009). Though, the relationship between water quality and microbial biota of cultured-fish environment has reported (Guo et al., 1998; Zhang and Tang, 1989), there is a dearth of information on how such relationships influence the

bacterial load of the fishes reared in such water media. This study was, therefore, carried out to evaluate the composition and relative abundance of bacterial species associated with Clarias gariepinus, in relation to water quality under laboratory conditions.

Materials And Methods

Source of Fish Specimens

Four weeks old of C. gariepinus fingerlings were obtained from a commercial fish farm in Minna (Long. 6°33´E and Lat. 9°37´N), Niger state, Nigeria. The fingerlings were transported to Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria, where they acclimatized to laboratory conditions for one week. The fishes were confirmed to be void of infection prior to the commencement of the investigation (Adewolu et al., 2008).

Experimental Set-up and Laboratory Maintenance of Fish Culture

Nine plastic indoor aquaria tanks (35 liters capacity) were individually filled with 25 litres of borehole water and stocked with 10 specimens of C. gariepinus fingerlings each. The open end of the aquaria tanks were screened with net (2mm mesh size), to prevent the fishes from trashing out (Olufayo, 2009). The fishes were fed to satiation with a commercial diet (Coppens®), during the hours of 0800 and 1800 daily (Dang Han et al., 2005). The water in the tanks was changed regularly at weekly intervals, and the experiment was monitored for physic-chemical properties and bacterial growth, between the months of June to August, 2011.

Physico-chemical and Bacteriological Analyses

Water samples were collected weekly from the experimental tanks (i.e., just before replacement of culture media), for the analysis of physico-chemical parameters namely, pH, Ammonia, dissolved oxygen, temperature and biochemical oxygen demand, using the standard methods of APHA (1992).

For bacteriological studies, swabs were taken from different external parts of the body of the fishes (i.e., skin, gills and fins); and cultured in appropriate media (i.e., Nutrient Agar and SDA for bacteria and fungi species, respectively). The culturing was done in three replicates, using a 3x4x3 factorial experimental design. The culture media were then incubated at 30°C for 72 hours. The colonies that appeared on the media were counted and characterized. Thereafter, gram-staining of the microbes was carried out to the cellular morphology of the microbes. The isolates were identified further by series of biochemical tests such as catalase, coagulase, hydrogen sulphide production, indole, methyl red, citrate utilization and voges proskauer tests (Olutiola et al., 1991).

Data Analysis

All replicate data collected were processed as mean±SD. Differences in mean values of weekly distribution of the physic-chemical parameters, as well as, those of bacterial counts among the species or body parts, were compared for statistical significance using Ch-square tests, at P = 0.05 level of significance.

Results

Table 1 highlights weekly distributon of physico-chemical properties of the water media in which the Clarias gariepinus fingerlings were reared during the study period. Except for Ammonia, which reduced significantly ($p<0.05$) after the first week (range = 0.05±0.01 mg/l in week 4, to 0.54±0.01mg/l in week 1), all other parameters analysed varied insignificantly ($P>0.05$) within narrow limits while the study lasted. While, mean pH and temperature of the fish-rearing water were 8.58±0.01 and 26.50±0.50°C, respectively, those of dissolved oxygen and biochemical oxygen demand were 6.29±0.10 and 0.21±0.03mg/l, respectively.

The relative distribution of bacterial microbes in the three parts (i.e., gill, skin and fin) of the fingerlings examined is presented in Table 2. On the whole, six bacterial species namely, S. faecalis, K. pneumonea, P. aeruginosa, E. coli, B. subtilis and S. mutans were encountered on the fingerlings. However, only three of the isolated bacterial species (i.e., S. faecalis, K. pneumonea and E. coli) were found in the three parts of the fingerlings from which swabs were taken. While P. aeruginosa was not isolated from the fins of the fingerlings, B. subtilis was missing from species inhabiting the skin; and S. mutans was only encountered on the fin.

Five of the six bacterial species encountered in the study were found on the gill and fin, while only four species inhabited the skin. Except for B. subtilis, the densities of the bacterial species were not significantly ($P>0.05$) different between the gill and skin; but such densities were significantly ($P<0.05$) higher in the fin than the other two sites of bacterial swab sampling though, P. aeruginosa was not encountered on the fins. Aggregate-wise, bacterial load was significantly highest on the fins (mean = 12.24±2.21cfu/g), followed by the gills (mean = 9.31±2.45cfu/g) and least on the skin (6.04±1.49cfu/g). The results of the aggregate relative abundance of the bacterial species show that the densities of P. aeruginosa, B. subtilis and

S. mutans (range = 2.40±0.00 t0 3.98±0.46cfu/g), were significantly lower than those of the remaining three species namely, S. faecalis, K. pneumonea and E. coli (range = 5.36±0.60 to 6.82±0.48cfu/g).

Discussion

The results of physico-chemical analysis of the water in which the Clarias gariepinus fingerlings were cultured indicated that with the exception of Ammonia, all the parameters investigated were not significantly different in all the weeks. This finding indicates that the physico-chemical properties that varied within narrow limits were not affected by the activities and age of the fingerlings. Studies have shown that the intensity or concentration of the physico-chemical parameters involved namely, pH, Temperature, Dissolved Oxygen and Biochemical Oxygen Demand, are mainly influenced by environmental factors such as photosynthetic activities, mineral nutrient concentrations, edaphic factors, degree of exposure to sunlight, etc (Ayanwale et al., 2009; Fafioye, 2011); factors that are clearly not operational in indoor laboratory studies such as this one. However, it is not clear why the concentration of Ammonia in fish-rearing water reduced significantly with weeks, as ordinarily, fishes excrete increasing amounts of nitrogenous wastes (mainly Ammonia) with growing age and size. Interestingly, mean weekly values of the physico-chemical parameters investigated either fall within the range recommended for optimum fish growth, or were out-rightly much better. For example, while pH range of 6.50 – 9.00 has been recommended (Bryan, 2004), mean pH obtained in this study was 8.58±0.01, while the obtained dissolved oxygen of 6.29±0.10mg/l was much better than the recommended 5.00mg/l (Svobodova et al., 1993). The mean water temperature of 26.50±0.50^{o}C obtained in this laboratory-based study was, however, close to the upper optimum limits of 27.00^{o}C. The Mean Ammonia concentration of 0.21±0.20mg/l and Biochemical oxygen demand of 0.21±0.03, however, were increasingly favourable for good fish growth performance when compared with the recommended ranges of 0.01 - 1.55mg/l (Kohinoor et al., 2001) and 1.00 - 5.00 mg/l (CIESE, 2009), respectively.

The relatively favourable physico-chemical conditions of the fish rearing-water under laboratory conditions, as obtained in this study thus, indicates that the fingerlings may attain better growth performance than those grown outside the controlled conditions of the laboratory. However, such clement water quality conditions may also promote the proliferation of microbial biota thus, pre-disposing the fingerlings to infestation. Another finding of this study which indicate that the fingerlings could have performed better under the laboratory conditions, is the temporal decrease in the concentrations of Ammonia recorded, which otherwise is a mitigating factor in the wild. High concentration of un-ionized Ammonia is known to cause serious physiological deficiencies in fishes (Fang et al., 1993; Fafioye, 2011).

Six bacterial species were isolated from the fingerlings including, those that are pathogenic and of faecal origin. This finding agrees with those of Liu et al. (1992a) and Fafioye (2011) who encountered similar bacterial species in fish ponds. Pelezar (1993) attributed the occurrence of some of these bacterial species, especially, E. coli and S. faecalis, in fish ponds to faecal contamination. The isolation of pathogenic bacterial species, such as K. pneumonea, B. subtilis and S. mutans, is worrisome despite the relatively high level of hygiene maintained in the laboratory during the rearing of the Heteroclarias fingerlings. These bacterial species have been incriminated in acute septicemia asymptomatic latent infections and high mortalities in fishes (Banjo et al., 2004). The fingerlings examined in this study were maintained under hygienic laboratory conditions but, yet, they harboured faecal-based bacteria. Such bacteria probably could have come from the fish feed and tells a lot on the sources of raw material for fish feed production and/or level of hygiene surrounding feed preparation. Therefore, the isolation of pathogenic faecal-prone bacteria from the fingerlings in this study suggest that fish supply from commercial and subsistence farms, where feeds similar to that used in this study are used, may be infested with such potentially harmful bacteria and thus, pose serious threat to public health in areas where the fishes are consumed.

More bacterial species were encountered on the gills and fins than the skin. This may be due to increased vulnerability of the former fish body parts to microbial attack than the latter. Also, the gills and fins, being organs that create and wafts through water currents, are likely to come more frequently and intensely in contact with the water borne bacteria. According to Sun and He (1997), the external body surface is a major source of nutrients for aquatic microbes.

Conclusion

Age and/or body size of Heteroclarias fingerlings had no significant effect on critical physic-chemical properties of culture media; and their concentrations were effectively within ranges reported for good fish performance. Even under the relatively hygienic Laboratory environment, the Heteroclarias fingerlings were still at risk of microbial (including pathogenic species) infestation. Not surprisingly, the fish organs swabbed for bacteriological analysis were differentially vulnerable to bacterial infestation. The findings of this study should provide baseline information for improving productivity of Heteroclarias fish species in particular, as well as, suggest primary fish organs to be targeted for microbial deterioration infection by fish consumers.

Table 1. Physico-chemical parameters of the water culture media of laboratory-reared Clarias gariepinus fingerlings

Parameters	Weekly Variation				
	1	2	3	4	Mean
pH	8.58±0.10[a*]	8.58±0.09[a]	8.59±0.97[a]	8.57±0.11[a]	8.58±0.01
Temperature (^oC)	26.00±0.30[a]	27.00±0.20[a]	26.00±0.20[a]	27.00±0.00[a]	26.50±0.50
Ammonia (mg/l)	0.54±0.01[b]	0.18±0.23[a]	0.06±0.01[a]	0.05±0.01[a]	0.21±0.20
Dissolved Oxygen (mg/l)	6.38±0.92[a]	6.25±0.71[a]	6.13±0.83[a]	6.38± 0.74[a]	6.29±0.10
Biochemical Oxygen Demand (mg/l)	0.17±0.80[a]	0.21±0.80[a]	0.21±0.09[a]	0.26±0.5345[a]	0.21±0.03

[*]Values followed by same superscript alphabets, in a row, are not significantly different at P = 0.05.

Table 2. Relative densities (x10^4 cfu/g) of bacterial species encountered on external parts of Clarias gariepinus fingerlings, reared under laboratory conditions

Isolated Bacterial Species	Gill	Skin	Fin	Aggregate
S. faecalis	1.88±0.52[a*]	1.08±0.08[a]	2.40±0.00[b]	5.36±0.60$_{b**}$
K. pneumonia	1.87±0.42[a]	1.60±0.6[a]	2.52±0.64[b]	5.99±1.66$_b$
P. aeruginosa	1.88±0.52[a]	1.16±0.01[a]	0.00±0.00[a]	3.04±0.53$_a$
E. coli	1.84±0.10[a]	2.20±0.00[a]	2.78±0.38[b]	6.82±0.48$_b$
B. subtilis	1.84±0.20[b]	0.00±0.00[a]	2.14±0.26[b]	3.98±0.46$_a$
S. mutans	0.00±0.00[a]	0.00±0.00[a]	2.40±0.00[b]	2.40±0.00$_a$
Aggregate	9.31±2.45[b]	6.04±1.49[a]	12.24±2.21[c]	27.59±6.15

[*]Values followed by same superscript alphabets, in a row, are not significantly different at P = 0.05.
[**]Values followed by same subscript alphabets, in the column, are not significantly different at P = 0.05.

References

Adewolu MA, Ogunsanmi AO, Yunusa A. 2008. Studies on growth performance and feed utilization of two Clariid catfish and their hybrid reared under different culture system. European Journal of Scientific Research 23(2): 252-260.

Amadi AN. 2009. Physico-chemical and bacteriological evaluation of groundwater in parts of Aba, Abia State, Southeastern Nigeria. International Journal of Applied Biological Research 1(1): 63-71.

APHA. 1992. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C., USA., 1134pp.

Ayanwale AV, Olayemi IK, Zubairu AA. 2009. Mycoflora survey of three commercially important fish species from Tagwai Lake, Minna, Nigeria. International Journal of Applied Biological Research 1(1): 45-51.

Azad IS, Shankar KM, Mohan CV. 1997. Evaluation of Aeromonas hydrophila for Oral vaccination of carp. In: T.W. Flagel and I.H. Macrae (eds.), Disease in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila, pp181-185.

Banjo TT, Axtell Q, Frerichs DC, Gay SA, Murray C, Mills E. 2000. Zoosporic fungi growing on the carapace of dead zooplankton organisms. Limnologica 30: 37-43.

Bryan R. 2004. Technical Memorandum; pH requirements of fresh water aquatic life. Robertson-Bryan Inc.9766. Waterman Road, suite L2.E/K Grove, CA 95624(916) pp. 714-1802

Buras NL. 1993. Microbiological aspects of fish grown in treated wastewater. Water Resources 21(10): 1-10.

CIESE (Centre for Innovation in Engineering and Science Education). 2009. Biological Oxygen demand. Stevens Institute of Technology.

Dong H, Shouqi X, Wu L, Xiaoming Z, Yunxia Y. 2005. Effects of light intensity on growth, survival and skin colour of juvenile Chinese long snout catfish (Leiocassis longirostris Gunther). State key laboratory of fresh water ecology and biotechnology. Institute of hydrobiology, The Chinese Academic Journal of Sciences Wuhan, Hubei 430072, PR, china.

Fafioye OO. 2011. Preliminary studies on water characteristics and bacteria population in high yield Kajola fish ponds. Journal of Agricultural Extension and Rural Development 3(3): 66-71.

Fang X, Guo X, Yu T. 1993. The bacteria in nitrogen cycling and mud activation in the pond mud. Fish J (19)2: 137-145.

Guo X, Fang Y, Wang J. 1988. The preliminary studies on bacteria types in the fish pond applied with four kinds of animal manure and the affect upon ecosystem and yield. J Chin Acad Fish Sci (Li): 18-28.

Ita EO. 1980. A review of recent advance in warm water aquaculture resources and a proposed experimental design for maximizing fish production in Nigeria. K. L.R.I. Tech. Rep. Ser. 51: 30.

Kohinoor AHM, Wahab MA, Islam ML, Thilsted SH. 2001. Culture potentials of Mola (Amblypharyngodon mola), Chela (Chela cachicus) and punti (Punti sophore) under monoculture system. Bangladesh J Fish Res 5(2): 123-124.

Liu G, Bao W, Liu Z. 1992a. The growth and seasonal changes of bacteria biomass in fish ponds. Fish J 16(1): 24-31.

Liu G, Bao W, Liu Z. 1992b. Preliminary studies on the bacteria in pond mud. J Fish Biol 16(3): 248-286.

Mims SD. 2001. Aquaculture of paddle fish in the United State. Aquaculture Research Centre, Kentucky State University, Frankfort, KY 40601, USAAquat. Living Res 14: 391-398

Olutiola TO, Famurewa O, Sontage HG. 1991. An introduction to General Microbiology. A Practical Approach. Cad Heideberg Virlag Sanstaltund Druckerei Gmbh Helderberg, Germany, pp. 38-51.

Pelezar MJ. 1993. Microbilogy of natural waste water. Microbial Concepts Appl 29: 821-823.

Robert RJ. 1989. The pathophysiology and systematic pathology of teleosts. In: Fish pathology (Ed. By R.J Roberts) Ballure Tindal, London, pp. 56-134.

Sivakami R, Premkishore G, Chandra MR. 1999. Occurrence and Distribution of potential pathogenic enterobacteriaceae in Clarias gariepinus fingerlings in Laboratory. Wade Publications, India. p. 163.

Sun Y, He Z. 1997. The study of fish pond settlement and nutrient dispersion between water and pond mud contacting surfaces disease in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society manila, pp. 135-142

Svobodova Z, Richard L, Jana M, Blanka V. 1993. Water quality and fish health. EIFAC Technical paper 54.

Wedemeyer GA. 1996. Interaction with water quality conditions in physiology of fish in Intensive culture systems. Chapman and Hall, New York, USA.

Zhang Z, Tang Y. 1989. Pond fish culture in China. Beijing Science Pulishing House, China. pp. 45-47.