PRELIMINARY STUDIES ON ASSOCIATED BACTERIA AND FUNGAL LOAD OF ARTIFICIALLY CULTURED *Clarias gariepinus* BURCHELL 1822 FINGERLINGS

Oni, T. A.[1], Olaleye, V.F.[1*] and Omafuvbe, B.O.[2]

[1]Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria

**Corresponding Author:*
Email: olafoluwa_59@yahoo.com
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ABSTRACT

Bacteria and fungi associated with cultured *Clarias gariepinus* Burchell fingerlings were studied. One thousand (1000) 4-week old fingerlings (5.5 ± 1.1 g) collected from NIFAGOL fish farm, Ile-Ife, were cultured for 6 months in three large concrete tanks (6.0m × 5.0m × 1.2m) and three small concrete tanks (1.5 m × 1.5 m × 1.2 m) at the rate of 150 fingerlings per tank. During the study, the fish were fed 40% crude protein based diet at the rate of 3% of the fish body weight per day, 6 days a week. Physico-chemical parameters of water as well as the bacterial and fungal flora were determined on the fish body parts and the culture water. Analyses showed that mean values for temperature, pH and total alkalinity in smaller culture tanks (31.4±0.4^{0}C; 7.7±0.1 and 122.8±10.4 mgL^{-1} respectively) were higher than values recorded in larger culture tanks (29.4±0.5^{0}C; 7.6±0.1 and 114.8±9.0 mgL^{-1} respectively), though not significantly (P>0.05).

Conversely, the mean values for dissolved oxygen, biological oxygen demand and organic matter contents were higher in the large culture tanks (5.7±1.0 mgL^{-1} ; 3.1±0.5 mgL^{-1} and 11.4±1.6 mgL^{-1} respectively) than in smaller culture tanks (4.8±0.8 mgL^{-1} ; 2.4±0.3 mgL^{-1} and 10.2±1.5 mgL^{-1} respectively). The mean total mesophilic bacteria count (TMB) of water in the large culture tanks (5.20±0.78 cfu/ml) was significantly lower (P<0.05) than those of small culture tanks (6.08±0.70 cfu/ml). Also, the TMB of the stomach contents of fingerlings cultured in small tanks (10.95±0.99 cfu/g) was significantly higher (P<0.05) than those of fingerlings cultured in large tanks (9.72±0.13 cfu/g). The total fungal count (TFC) on the skin of the fingerlings cultured in small tanks (3.04±1.05 cfu/g) was significantly higher (P<0.05) than those on the skin of fingerlings cultured in large concrete tanks (1.76±0.87 cfu/g).

Generally, 12 bacterial and 10 fungal species were found either in the culture water, on the skin, gills of fish and the stomach contents. *Pseudomonas sp., P. aeruginosa, Aeromonas hydrophila, Shigella sp., Proteus vulgaris* and *Staphylococcus aureus* were found in the water column as well as the fish parts examined. The dominant fungal isolates identified were *Cladosporium sp., Microsporium sp., Trichophyton sp., Aspergillus sp., Botrytis sp.,* and *Mucor sp.*

The low mortality rate during the period of study showed that *Clarias gariepinus* is hardy and uniquely suited to cope with the high microbial load present in the culture environment.

Keywords: Bacteria, Fungal, *Clarias gariepinus,* Culture media

INTRODUCTION

Epidemic spread of aquatic animal diseases is an ever present danger to various degrees in artificial culture systems. Diseases caused by bacteria, fungi, viruses and parasites are serious threat to sustainability of aquaculture industry (DAS, 2002). Serious epizootics of fish occur when fish are cultured under stressful environmental and inadequate husbandry conditions (Subasinghe and Bartley, 2004).

Reported cases of serious diseases outbreak include the epizootic ulcerative syndrome (EUS) in freshwater fish (DAS, 1994), koi herpes virus (KHV) among common carp *Cyprinus carpio* populations (Iida *et al.*, 2005). High stocking densities employed in intensive culture systems put fish in close proximity to each other, subjecting them to increased physiological stress (Nedoluha and Westhoff, 1997). Crowding and stress enhances disease transmission (Subasinghe and Bartley, 2004), culminating in overall increase in microbial load which ultimately compromise the fish microbial integrity for human consumption.

Information on bacteria and fungal diseases of Nigerian cultured species is essential to prevent loss of the crop during production activities. The Nigerian aquaculture industry dominated by *Clarias* culture (Adewumi, 2005) is characterized by poor documentation of fish associated microbial and fungal diseases (Oni, 2007).

Appropriate documentation of the bacterial and

fungal flora of a culture system will minimize the risk of major disease incursions, improve diagnostic capacity and the control measures needed to tackle the threat of newly emerging diseases. The present study is aimed at identifying and documenting the types and population of the microbial flora of artificially cultured *C. gariepinus* fingerlings.

MATERIALS AND METHODS

Fish Collection and Acclimatisation

One thousand (1000) 4-weeks old *C. gariepinus* fingerlings (5.5 ± 1.1 g) collected from NIFAGOL Fish Farm, Ile-Ife, were acclimatised and cultured in 6 open concrete tanks located in the Aquaculture Centre, Obafemi Awolowo University, Ile-Ife, Nigeria. The fish which were stocked at the rate of 150 fingerlings per tank were acclimatised for 2 weeks during which the fish were fed Coppens© feed (45% crude protein content) at the rate of 5% of the fish body weight per day (Adewumi, 2005). Mortalities in the tanks were replaced with fish of comparable weight during the period of acclimatisation.

Experimental Design

C. gariepinus fingerlings were cultured for 6 months in 3 large (6.0 m x 5.0 m x 1.2 m) and 3 small (1.5 m x 1.5 m x 1.2 m) concrete tanks at the rate of 150 fingerlings per tank. During the period, the fish were fed soybean-based formulated diet containing 40% crude protein (Adewumi, 2005) for 6 days a week, at the rate of 3% body weight, in two equal installments between 07.00 - 08.00 G.M.T. and 17.00 - 18.00 G.M.T. Fish ration adjustments during the period of study was based on weight differential obtained from random forthnightly weighing of 20 fish per tank. Evaporation in each of the culture tanks was compensated for through periodic replenishment with untreated reservoir water from an overhead water tank.

Water Quality Determination

Some physico-chemical parameters of water determined daily *in situ* using Orion Water Quality Laboratory System, Model 1260 were: pH, dissolved oxygen and water temperature. In the laboratory, the biological oxygen demand, organic matter and total alkalinity were determined weekly on subsurface water samples collected from the culture tanks (APHA *et al*., 1992).

Microbial Analysis

Bacterial and fungal flora of water in the fish culture media were determined monthly following standard methods (Soper, 1998). For each monthly sampling for the microbial load on the fish body parts (gill filament, skin and the stomach contents), 6 fish fingerlings were randomly selected and sacrificed for analysis. Sterilized scalpel was used to collect the fish skin scrapings which was then pooled and transferred into a pre-weighed McCartney bottle. The weight of the skin scrapings was then taken using a Mettler Balance after which 1 g of the fish skin scrapings was removed and then aseptically suspended in 10 mL of sterile 0.10% peptone water diluent.

Five grammes each of the gill filaments and stomach contents were also aseptically suspended in 45 mL 0.1% peptone water diluents in stomacher bags and homogenized by stomaching for 2 minutes. Subsequent serial dilutions from the concentrated sample suspensions were made with 0.1% sterile peptone water and 1.0 mL of appropriately diluted sample suspension was then mixed with molten (45°C) media (nutrient agar and malt extract agar) and poured into sterile plates.

Total mesophilic bacteria (TMB) were enumerated in nutrient agar (NA) incubated at 35 °C for 48 hours. Fungi were enumerated on malt extract agar (MA) incubated at 30°C for 5-7 days. Colonies appearing at the end of incubation periods were counted (and the results expressed as colony forming units (CFU) per millilitre or gram of the original sample). Representative colonies of the different bacteria and fungi (yeast and moulds) were isolated from their respective plates and purified by repeated streaking on fresh nutrient agar and malt extract agar plates respectively. The pure bacteria isolates were identified following standard methods (Buchanan and Gibbons, 1974; Harrigan and McCance, 1976). The mould and yeast isolates were identified according to Barnett and Hunter (1972) and Harrigan and McCance (1976).

Statistical Analysis

Statistical analysis was carried out on the data collected using SPSS software package (Version II). Correlation analysis was done to establish the

relationship between the tank sizes and water physico-chemical parameter; and to determine the relationship between fungal and bacterial count on the fish part (skin, gills and stomach contents) and the size of the culture tanks.

RESULTS AND DISCUSSION

The levels of some physico-chemical parameters of water in the culture tanks are shown in Table 1. The result showed that the temperature of water in the fish culture tanks ranged between 27 °C and 31 °C in large tanks, and 29.1 °C and 32.5 °C in small tanks, while the pH values was between 7.3 and 8.1 for the large tanks and between 7.2 and 8.2 for the small tanks. The temperature and pH value obtained was within the range reported by Boyd and Lickoppler (1979) and Okoye *et al.* (1994) as suitable for fish pond culture. The mean total alkalinity values were between 64 and 131.0 mg/L for the large culture tanks and 62 and 142.0 mg/L

Table 1: Monthly Mean* Values of Some Physico-Chemical Parameters of Water in the Culture Media of During the Period of Study

Month	Temperature (°C)		pH		Total Alkalinity (mg/L)		DO (mg/L)		BOD5 (mg/L)		Organic Matter (mg/L)	
	Large Tank	Small Tank	Large Tank	Small Tank	Large Tank	Small Tank	Large Tank	Small Tank	Large Tank	Small Tank	Large Tank	Small Tank
Jan.	27.0	29.1	8.1	8.2	64.0	62.0	12.6	8.4	6.7	4.0	10.5	11.6
Feb.	30.0	32.0	7.7	8.0	131.0	142.0	4.7	4.0	2.6	2.0	18.8	15.7
March	31.0	32.5	7.3	7.2	131.0	133.0	4.8	4.4	2.4	2.4	15.5	15.1
April	30.5	32.0	7.7	7.8	121.0	131.0	5.8	5.6	3.3	3.1	2.0	7.0
May	30.0	31.0	7.3	7.4	118.0	140.0	3.8	3.1	2.1	1.8	8.5	3.1
June	28.0	29.5	7.5	7.5	124.0	129.0	2.7	3.1	1.5	1.5	13.0	7.7
*Mean	29.4±0.5	31.4±0.4	7.6±0.1	7.7±0.1	114.8±9.0	122.8±10.4	5.7±1.0	4.8±0.8	3.1±0.5	2.4±0.3	11.4±1.6	10.2±1.5

DO - Dissolved Oxygen; BOD - Biological Oxygen Demand.

*Mean ± Standard error

Table 2: Total Mesophilic Bacteria Load of Water Media and Fish Parts during the Culture Period

Month	Water Media (cfu/ml)		Fish Part (cfu/g)					
			Skin		Gill Filament		Stomach Content	
	A	B	A	B	A	B	A	B
Initial Load	3.00±1.16	2.95±0.93	5.60±1.30	4.60±1.15	2.94±0.90	2.93±0.93	8.97±.1.46	9.78±1.37
January	6.10±1.73	5.97±1.24	9.18±1.24	9.03±1.31	8.94±0.96	10.53±0.99	11.32±1.34	10.18±1.17
February	3.31±1.10	4.28±1.17	6.83±1.17	8.54±1.18	6.98±1.34	6.52±1.20	10.98±1.07	9.33±0.90
March	3.37±1.06	4.28±1.17	7.44±1.44	6.72±1.24	9.16±1.50	8.77±1.45	11.03.112	12.31±0.93
April	3.41±1.10	6.71±1.18	4.70±1.19	5.20±1.18	6.50±1.15	6.69±1.35	6.29±0.20	5.17±1.03
May	3.79±1.11	6.15±1.20	5.89±1.28	6.81±1.18	7.13±1.16	7.08±1.22	8.30±0.85	5.98±1.13
June	7.09±1.34	8.99±1.19	8.49±1.35	5.42±1.12	10.36±1.08	10.99±9.05	9,05±0.93	8.99±0.91

A - Large Culture Tanks B - Small Culture Tanks
Values are means ± S.E.

for the small culture tanks. The value obtained were however, higher than the values reported by Wedemeyer (1996).

The dissolved oxygen (DO) content of the water in the large tanks ranged between 2.7 mg/L and 12.6 mg/L while in the small tanks it was between 3.1 mg/L and 8.4. mg/L with a mean of 5.7 mg/L ± 1.0 and 4.8 mg/L ± 0.8 mg/L respectively. The variable values of DO throughout the period of study could be attributed to the oxygen demand by algal respiratory activities and to bacterial decomposition of organic matter in the uneaten food and faecal matters (Srisuwantech *et al.,* 1982). The relatively low level of dissolved oxygen in the culture tanks was not lethal to the fish because of the presence of accessory respiratory organ (Lowe-McConnell, 1975) which ensure that the fish survive poor water quality. However, such low

level of DO has been reported to promote microbial growth and the spread of bacterial infection in fish culture system (Kiplaget Kotut *et al*., 2011).

Algal and bacterial respiration was probably responsible for high BOD_5 values which ranged from 1.5 mg/L to 6.7mg/L in the large culture tanks and between 1.5 mg/L and 5.0 mg/L in small culture tanks (Schroeder, 1980 and Srisuwantach *et al.,* 1982). The high values of organic matter which ranged between 2.0 mg/L and 18.8 mg/L in the large culture tanks and between 3.1 mg/L and 15.7 mg/L in the small culture tanks with means of 11.4 mg/L ± 1.6 mg/L and 10.2 mg/L ± 1.5 mg/L respectively, could be attributed to accumulation of uneaten food, faecal matter and algal decomposition.

The results of the microbial load in the fish culture media and fish parts are shown in Tables 2 and 3. Analysis of the result showed comparatively that the total mesophilic bacteria count of water in the large culture tank was significantly lower ($P<0.05$) than that of the small culture tanks (Table 4). The result was similar to the report of Kumar *et al.* (2012). Analysis also showed that the TMB of the stomach contents of fish cultured in the smaller tanks was significantly higher than thoseof the large tanks. The total fungi count (TFC) on the skin of fish cultured in the smaller tanks was also significantly higher ($P<0.05$) than those cultured in the large tanks (Table 4). Analysis showed very low relationship between water physico-chemical parameters and the TMB and TFC counts on the body parts of the fish (Table 5). Similar result was reported by Ogbuagu *et al.* (2011). This result is an indication that the mean total bacteria and fungal counts were not affected by the physical and chemical quality of water in the culture media.

Table 3: Total Fungal Count of Water Media and Fish Parts during the Culture Period

Month	Water Media		Fish Part					
			Skin		Gill Filament		Stomach Content	
	A	B	A	B	A	B	A	B
Initial Load	2.30±0.98	2.054±0.92	2.70±0.60	3.65±1.10	2.28±0.85	3.24±0.62	2.58±.0.95	2.57±0.87
January	3.29±0.58	2.30±0.81	5.22±0.86	5.60±0.82	3.70±0.56	4.13±0.76	3.40±0.74	3.53±0.70
February	2.48±0.89	2.48±0.84	2.65±0.70	3.78±0.98	3.30±0.70	3.65±1.12	3.74±0.56	3.78±1.14
March	2.48±0.72	2.18±0.76	2.93±0.96	4.04±0.58	5.30±0.76	5.40±0.70	5.17±0.71	5.40±1.13
April	2.79±1.02	2.79±0.70	3.98±0.87	1.54±0.95	5.18±0.72	4.54±0.78	5.00±0.90	5.30±0.74
May	2.54±0.93	2.30±1.07	3.48±0.58	3.54±0.70	6.10±0.83	4.30±0.71	4.38±0.72	3.60±1.09
June	2.60±0.91	3.88±0.58	4.46±0.82	3.81±0.71	3.65±0.60	3.88±0.55	6.70±1.18	3.88±0.56

A - Large Culture Tanks B-Small Culture Tank

Table 4: Relationship between the size of the culture media and the mean total bacteria count (TMB) (cfu/g) and TFC (cfu/g) during the period of study.

	Big tank	Small tank	F calculated	Level of significance
TMB	± S.E	± S.E		
Water*	5.20±0.78	6.08±0.70	5.427**	
Skin	8.38±0.92	8.26±0.75	0.098	N.S
Gill	9.52±0.92	9.65±0.81	0.725	N.S
Stomach content	9.72±0.13	10.95±0.99	6.157**	
TFC				
Water*	1.76+0.87	3.04±1.05	2.546	N.S
Skin	3.90+0.56	4.87±0.75	12.904**	
Gill	6.41+0.82	5.00±0.86	2.282	N.S
Stomach content	5.83±0.70	4.78±0.76	3.960	N.S

F tab 0. 05 = 4.60

N.S. = Not significant ($P>0.05$).

**Significantly Different ($P<0.05$)

*cfu/ml

Table 5: Relationship* between the Physico-chemical Parameters of Water and Mean Total Bacteria Count and the Mean Total Fungal Count Water and on the Skin, Gill and Stomach Content of *Clarias gariepinus* Fingerlings during the Period of Study.

		Fish Part		
Parameters	Water Media	Skin	Gill	Stomach Content
TMB				
Temperature	-0.504	-0.544	-0.178	-0.453
pH	-0.427	-0.500	-0.352	-0.339
Alkalinity	0.338	0.308	-0.176	0.537
Dissolved oxygen	-0.397	-0.477	-0.375	-0.489
Biochemical oxygen demand	-0.408	-0.486	-0.364	-0.485
Organic matter	0.135	0.052	-0.451	0.348
TFC				
Temperature	-0.155	-0.331	-0.368	-0.177
pH	-0.55	-0.675	0.149	-0.384
Alkalinity	0.098	0.031	0.406	-0.152
Dissolved oxygen	-0.133	-0.599	-0.065	-0.404
Biochemical oxygen demand	-0.142	-0.602	-0.002	-0.396
Organic matter	-0.023	-0.299	-0.651	-0.471

F tab 0. 05 = 4.60
*= value not significant

Table 6: Occurrence of Bacteria Isolates in the Water Media and Various Parts of *C. gariepinus* Fingerlings during the Period of Study

Bacteria species	Water	Skin	Gills	Stomach Content
Pseudomonas sp.	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Aeromonas hydrophila	+	+	+	+
Achromobacterium sp.	+	+	-	-
Alcaligenes faecalis	+	-	-	-
Escherichia intermedium	+	-	+	-
Salmonella sp.	+	-	+	-
Shigella sp.	+	+	+	+
Klebsiella pneumoniae	+	-	+	+
Proteus vulgaris	+	+	+	+
Proteus mirabilis	+	+	+	-
Staphyloccocus aureus	+	+	+	+
Micrococcus luteus	-	+	+	+
Bacillus subtilis	-	+	-	-
Enterobacter aerogenes	-	-	-	+
Actinomyces sp.	+	+	-	-
Acinetobacter sp.	+	-	-	+
Number of Species	12	11	11	9

- = Absent
+ = Present

Table 7: Occurrence of Fungi Isolates in the Water Media and Some Organs of Fish Fingerlings during the Six Months Culture Period

Fungi	Water	Skin	Gills	Stomach Content
Cladosporium sp.	+	+	+	+
Microsporum sp.	+	+	+	+
Trichophyton sp.	+	+	+	+
Candida sp.	-	-	-	-
Fusarium sp.	+	-	-	-
Penicillium sp.	+	+	-	+
Aspergillus sp.	+	+	+	+
Botrytis. sp.	+	+	+	+
Mucor sp.	+	+	+	+
Rhizopus sp.	+	+	+	-
Absida sp.	+	+	+	-
	10	9	8	7

\- = Absent
\+ = Present

The occurrence of bacterial isolates in the fish water media and some parts of *C. gariepinus* fingerlings during the culture periods is shown in Table 6. A total of 12, 11 and 8 bacterial species were identified in the fish water media, skin and gills and stomach content of the fingerlings respectively. *Pseudomonas* sp., *Pseudomonas aeruginosa, Aeromonas hydrophila, Shigella* sp., *Proteus vulgaris, Staphylococcus aureus*, were identified in the water and the fish parts sampled. *Pseudomonas, Proteus* and *Staphylococcus* species have been implicated in food poisoning (Hobb *et al.,* 1987). *Shigella* species causes dysentery in human and are usually transmitted through faecal route (Akinde and Obire, 2011). Other bacterial species identified either in the fish culture media, skin, gills and stomach content included *Achromobacter, Alcaligenes, Escherichia, Salmonella, Micrococcus, Bacillus, Actinomyces* and *Acinetobacter*. The fungal species associated with culture water and fish parts include *Candida, Microsporium, Trichophyton*, *Mucor, Absida, Rhizopus*, *Fusarium, Aspergillus* and *Penicillium* (Table 7).

In conclusion, the mortality rate of the culture fish was very low despite the high microbial load and presence of pathogenic species in the fish water media sampled. This is an indication that *C. gariepinus* is a hardy fish and is suitably adapted to living in polluted waters due to the presence of mucous lectins on the skin of the fish which protects the fish against most aquatic bacteriophages (Tsutsui *et al.,* 2011).

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