
**ISSN: 2347-5129**
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(1): 380-384

www.fisheriesjournal.com
Received: 15-07-2015
Accepted: 14-08-2015

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# Aerobic bacterial flora and survival of African catfish *Clarias gariepinus* during early life stages in the hatchery

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**Abstract**
This study estimated quantitatively and qualitatively the aerobic bacterial flora associated with early developmental stages of African catfish *Clarias gariepinus* during artificial propagation in a hatchery in Nigeria. Eggs hatchability and larval survival during the propagation were also monitored. The results obtained showed that the total number of colony-forming units per gram (cfu/g) for ova, fertilized eggs, hatchlings, fry and advanced fry ranged from $2.83\pm0.38x10^3$ to $4.23\pm0.49x10^3$, $6.73\pm0.10x10^3$ to $1.27\pm0.3x10^4$, $6.00\pm0.40x10^4$ to $8.57\pm1.62x10^4$, $9.00\pm2.17x10^4$to $3.27\pm1.46x10^5$ and $9.00\pm2.17x10^5$ to $1.6\pm0.56x10^7$, respectively. The numbers of bacteria on the fish eggs, hatchlings, fry, and advanced fry were higher than those found in their respective culture water. In all, eight bacteria genera, consisting of *Aeromonas*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Escherichia coli*, *Klebsiella*, *Proteus* and *Salmonella*, were identified. Mortalities were observed in all stages of growth, with the highest losses occurring as the fish grew from hatchlings to fry.

**Keywords:** African catfish, eggs hatchability, hatchery, bacteria, fry, culture water.

## 1. Introduction
Larval rearing has long been identified as a major bottleneck in African catfish production There are several unpublished cases of high loses in the hatcheries especially in Nigeria, where African catfish is commercially valued. While several efforts had been geared toward addressing numerous issues relating to African catfish larval culture during the last decade [5, 7, 10, 11, 3], many facts remain obscure regarding mass mortalities of catfish larvae in the hatchery. Till date little is known about the composition and activities of microorganisms during early developmental stages of these economically important fish species.
Generally, early developmental stages of fishes are believed to be more susceptible to infections due to the immature nature of the various components of their immune systems, as well as the prevailing environmental conditions at this period, which may stress the fish and favour the proliferation of pathogenic organisms [13, 6]. Consequently, high bacterial loads on the fish and in the cultured water at this stage are considered deleterious. In contrast to this general view, several studies in recent years have proven that it is not all bacteria found on fish or in rearing water that are pathogenic to fish. There are numerous bacteria that are beneficial to fish by restoring water quality and creating a conducive cultured environments[16], and by having nutritional and other positive benefits [22, 25]. The microflora of fish during early developmental stages of growth, therefore plays a key role in their survival, as they can be either deleterious or beneficial to fish [20].
Given the impacts of microflora on the survival of young fish, it is very essential to understand the microbiology of fish and their cultured environment in order to design approved management practices that can prevent possible disease outbreak and mortalities [2]. This paper reports the bacteriology and survival of early life stages of African catfish *Clarias gariepinus* under water flow-through hatchery conditions

## 2. Materials and methods
### 2.1. Experimental procedure
African catfish *Clarias gariepinus* brooders (1.21±0.07 kg) were collected from three reputable farms (designated as Farm 1, 2 and 3) in Akure metropolis (South-western Nigeria)

and transported in plastic containers with well-oxygenated fresh water to the hatchery unit of the Federal University of Technology Akure for artificial propagation. Each female fish was weighed and kept in a separate container, and were all hormonally induced in the night, using Ovaprim (Aquatic Sciences Companies, Holland. In total, 18 female brooders (6 from each farm) were injected at the rate of 0.5 ml of hormone per kilogram of fish. Prior to stripping, the males were sacrificed and the milt collected from each male was used to fertilize the eggs obtained from two females of the same farm. Samples of milt from each male fish were also taken for bacteriological analyses. The injection, stripping and fertilization was done following the methods described by Viveen *et al.* (1985) [27].

Following fertilization, 50 g of fertilized eggs from each female were transferred aseptically into the hatching nets and incubated separately in 35 litres rectangular tanks. A total of nine (9) incubation tanks (3 tanks per farm) were set up. The incubation was done under a water flow-through system at flow rate of approximately 1.9 litre per minute. 1 g of the fertilized eggs was also removed and counted. Water quality parameters – temperature, dissolved oxygen, pH, salinity, turbidity, conductivity – for each tank were measured using Hanna Multiparameter HI 9282 model. The hatching net was removed after 30 hours of incubation and the whitish eggs that did not hatch were counted. The percentage hatchability of the fertilized eggs was determined as;

$$\% \text{ hatchability} = 100 - \left( \frac{\text{Number of whitish eggs}}{\text{Number of incubated (fertilized) eggs}} \times 100 \right)$$

Water flow was increased to 2.4 litres per minute and the larvae commenced feeding two days after hatching. Newly hatched larvae were fed with decapsulated *Artemia* nauplii for 14 days, after which they were offered imported floating feeds (Coppens Inc., Holland) of 0.3 mm and 0.5 mm sizes respectively. The fish at these stages were fed to satiation up to 4 times a day. The percentage survivals of larvae at 2, 14 and 25 days which were termed hatchlings, fry and advanced fry respectively, were determined as;

$$\% \text{ larval survival} = \frac{\text{Number of larvae remaining}}{\text{Initial number of larvae at the start}} \times 100$$

### 2.2. Bacteriological analyses

Prior to fertilization, samples of unfertilized eggs and sperm sac from female and male brooders respectively were collected in sterile nylon materials. Samples of fertilized eggs and their respective spawning water were collected in sterile containers at about 5 hours after fertilization. Furthermore, the two day old hatchlings, 14 day old fry, as well as 25 days old advanced fry with their respective culture water was also collected.

In the laboratory, each of the collected milt samples was drawn into sterile 2ml syringe. 0.5ml of milt was added to 4.5ml of sterile physiological saline solution (0.85% w/v NaCl per litre of sterile distilled water). Further dilutions were made serially in order to obtain $10^{-2}$ dilutions of the original sample. 2g of unfertilized eggs were weighed aseptically and homogenized in a mortar and pestle. The homogenate was serially diluted to $10^{-2}$. The samples of the fertilized eggs, hatchlings, fry and advanced fry were separated from the sterile water using filter paper (0.25µm), and water adhering to the filtered samples was removed by means of sterile blotting paper. The samples were then treated as described for ova. 1 ml of all culture water samples were also taken and diluted serially to $10^{-2}$.

Tryptone soya agar (TSA, BIOTEC Laboratory Ltd, United Kingdom) was used for the primary isolation of bacteria isolates. Aliquot of 0.1ml of all serial dilutions were inoculated into TSA plates by the pour plating method. The plates were incubated at 37 $^0$C for 48 hours, and colony forming units (cfu) of each sample were counted to determine the bacterial population. The percentage composition of the isolated bacteria was estimated following the methods described by Al-Harbbi and Uddin [2]. The isolated bacteria were characterized using standard morphological and biochemical tests, such as Gram staining, motility, catalase, oxidase, methyl red, Voges Proskaeur, sugar (glucose, galactose, arabinose, lactose, mannitol, inositol and dulcitol) fermentations, growth on eosin methylene blue and nitrate reduction.

### 2.3. Statistical analysis

Computer software (SPSS 12.0) was employed for the statistical analysis. The data obtained from water analysis and bacteriological enumerations were recorded as means±standard deviation, while the hatchability/survival of the fish and the distribution of the isolated bacteria were recorded in percentages. One-way analysis of variance (ANOVA) was used to compare the means differences between the number of bacteria isolated from brooders obtained from different farms. The values were first log-transformed before they were subjected to analyses. Where there were significant differences among the means, Duncan multiple range tests were used to separate them.

## 3. Results

The results of the analysis of the rearing water used for incubation and larval rearing of *C. gariepinus* during this experiment are presented in Table 1. The water quality parameters were within those required for catfish farming

**Table 1:** Water quality parameters (means ± standard deviation) during hatchery propagation of *C. gariepinus*

| Sources | Temperature ($^0$C) | Dissolved oxygen (ppm) | pH | Salinity (ppt) | Turbidity (ppm) | Conductivity (mΩ) |
|---|---|---|---|---|---|---|
| Spawning tanks | 27.59±0.27 | 8.60±0.25 | 6.78±o.06 | 0.10±0.02 | 121.00±7.54 | 0.0042±0.00015 |
| Hatchlings tanks | 27.82±0.26 | 8.44±0.10 | 6.57±0.04 | 0.12±0.03 | 148.66±4.16 | 0.0033±0.00005 |
| Fry tanks | 23.45±0.30 | 8.24±0.21 | 6.77±0.04 | 0.15±0.04 | 144.33±6.50 | 0.0034±0.00032 |
| Advanced fry tanks | 23.96±0.05 | 6.46±0.07 | 6.53±0.07 | 0.23±0.02 | 166.00±10.53 | 0.0031±0.00025 |

The numbers of bacteria isolated from *C. gariepinus* culture were found to increase gradually as the fish grow from one developmental stage to another, with similar trends observed in the three farms (Table 2). No bacteria were detected in the milt samples. The total number of colony-forming units per gram (cfu/g) for ova, fertilized eggs, hatchlings, fry and

advanced fry ranged from $2.83\pm0.38x10^3$ to $4.23\pm0.49x10^3$, $6.73\pm0.10x10^3$ to $1.27\pm0.3x10^4$, $6.00\pm0.40x10^4$ to $8.57\pm1.62x10^4$, $9.00\pm2.17x10^4$to $3.27\pm1.46x10^5$ and $9.00\pm2.17x10^5$ to $1.6\pm0.56x10^7$, respectively. The total numbers of bacteria recovered from the culture water were found to be lowest in spawning tanks ($9.73\pm1.11x10^2$ to $3.53\pm1.19x10^3$cfu/ml) and highest in hatchling tanks ($6.30\pm2.43x10^3$ to $1.56\pm0.74x10^4$cfu/ml). Except for spawning water, the values obtained from the three sources are not significantly higher ($P<0.05$) than each other.

Qualitative analysis of bacterial flora associated with different developmental stages of *C. gariepinus* revealed gradual increase in the types of bacteria as fish grow. *Aeromonas sp.* (42.42%), *Citrobacter sp.* (22.21%), and *Escherichia coli* (36.37%) were detected in ova, and these bacteria were among the most frequently isolated organisms from fertilized eggs, hatchlings, fry, advanced fry and in their respective rearing water (Table 3). In addition, *Klebsiella*, *Proteus* and gram-positive *Bacillus* were detected in the hatchlings, fry, advanced fry and their respective culture water. *Chromobacterium* was isolated in the hatchlings (4.35%), spawning water (16.13%), and larval water (12.22%), while *Salmonella* was found only in advanced fry (6.67%) and advanced fry water (10.71%).

Mortalities occurred at every stage of fish growth during this experiment. It was observed that highest losses occurred as the fish grew from hatchlings to fry

**Table 2:** Number (means ± standard deviation, cfu/g) of aerobic bacteria isolated during early developmental stages of *C. gariepinus*

| Sources | Milt | Ova | Fertilized eggs | Hatchlings | Fry | Advanced fry |
|---|---|---|---|---|---|---|
| Farm 1 | - | $2.8\pm30.36x10^{3b}$ | $1.27\pm0.31x10^{4b}$ | $7.87\pm0.71x10^{4a,b}$ | $1.25\pm0.33x10^{5b}$ | $5.17\pm1.44x10^{6b}$ |
| Farm 2 | - | $3.60\pm0.35x10^{3a,b}$ | $6.73\pm0.01x10^{3a,b}$ | $6.00\pm0.04x10^{4b}$ | $3.27\pm1.46x10^{5a}$ | $1.60\pm0.56x10^{7a}$ |
| Farm 3 | - | $4.23\pm0.49x10^{3a}$ | $9.63\pm1.35x10^{3a}$ | $8.57\pm1.62x10^{4a}$ | $9.00\pm0.40x10^{4b}$ | $9.00\pm2.17x10^{5b}$ |

Means (n=3) with different superscripts on the same row are significantly different at $P< 0.05$

**Table 3:** Number (means ± standard deviation, cfu/mL) of aerobic bacteria isolated from rearing water during early developmental stages of *C. gariepinus*

| Sources | Spawning water | Hatchling water | Fry water | Advanced fry water |
|---|---|---|---|---|
| Farm 1 | $9.73\pm1.11x10^{2b}$ | $6.30\pm2.43x10^{3a}$ | $6.07\pm1.53x10^{3a}$ | $6.07\pm1.53x10^{3a}$ |
| Farm 2 | $3.53\pm1.19x10^{3a}$ | $8.17\pm1.86x10^{3a}$ | $4.00\pm1.35x10^{3a}$ | $4.00\pm1.35x10^{3a}$ |
| Farm 3 | $3.58\pm0.35x10^{3a}$ | $1.56\pm0.74x10^{4a}$ | $6.37\pm1.42x10^{3a}$ | $6.37\pm1.44x10^{3a}$ |

Means (n=3) with different superscripts on the same row are significantly different at $P< 0.05$

**Table 4:** Percentage distribution of aerobic bacteria isolated during early developmental stages of *C. gariepinus*

| Bacteria | Ov | Fe | Sw | H | Lw | F | Fw | AF | AFw |
|---|---|---|---|---|---|---|---|---|---|
| *Aeromonas* | 41.93 | 30.95 | 32.26 | 23.91 | 23.08 | 17.78 | 16.13 | 18.89 | 16.07 |
| *Bacillus* | - | - | 25.81 | 10.87 | 17.95 | 13.33 | 25.81 | 17.78 | 17.85 |
| *Chromobacterium* | - | 7.14 | 16.13 | 4.35 | 12.82 | - | - | - | - |
| *Citrobacter* | 22.58 | 23.81 | - | 17.39 | 10.26 | 26.67 | 6.45 | 22.22 | 12,50 |
| *Escherichia coli* | 35.48 | 26.19 | - | 28.26 | 7.69 | 26.67 | 9.68 | 15.56 | 8.93 |
| *Klebsiella* | - | 11.90 | 19.35 | 6.52 | 12.82 | 8.89 | 19.35 | 7.78 | 16.07 |
| *Proteus* | - | - | 6.45 | 8.69 | 15.38 | 6.67 | 22.58 | 11.11 | 17.85 |
| *Salmonella* | - | - | - | - | - | - | - | 6.67 | 10.71 |

Ov – ova; Fe – fertilized eggs; Sw – spawning water; H – hatchlings; Lw – larval water; F – fry; Fw – fry water; AF – advanced fry; AFw – advanced fry water

**Table 5:** Percentage hatchability/survival (Mean±standard deviation) of *C. gariepinus* during early developmental stages

| Stages of growth | % hatchability/survival |
|---|---|
| Fertilized eggs | 83.91±31 |
| Hatchlings | 97.42±11 |
| Fry | 64.15±07 |
| Advanced fry | 67.25±13 |

## 4. Discussion

In this study, a gradual increase in the number and type of bacteria was observed during early developmental stages of *Clariasgariepinus*. No bacteria were found in the milt, whereas ova indicated the presence of bacteria. Although bacteria have been isolated in the reproductive organs of other aquatic organisms, such as*Ostrea edulis* [12], *Argopecten purpuratus* [22] and penaeid prawn *Penaeus indicus* [8], it is unclear from this study whether *Aeromonas sp.*, *Citrobacter sp.* and *Escherichia coli* found in catfish ova are associated with female gonads or are only transmitted from the brooders' gut through the urogenital papillae during artificial spawning (stripping). Nevertheless, this observation supports the occurrence of bacterial transmission from parent brood stocks to their offspring during *C. gariepinus* propagation under artificial reproduction.

Bacterial colonization may have adverse effects or protective effects on eggs and developing larvae of fish [20]. In the present study, the high bacterial population was observed in incubating fertilized eggs than in ova, irrespective of the source of brooders. The numbers of bacteria estimated in fertilized eggs and larvae {hatchlings fry and advanced fry) were also observed to be relatively higher than in their respective rearing water. These observations are in agreement with the reports of other fish larvae in the hatchery [24, 22, 1].

Many factors might have influenced bacterial increase during larval culture. The bacterial flora of fish at the time of hatching originates from the flora of eggs and spawning water. The presence of dead organic materials (dead eggs, hatchlings and fry) in rearing tanks, possibly due to poor egg quality,

environmental conditions or husbandry practises, may influence the release of considerable amount of nutrients, which in turn enhance bacterial multiplication [8]. The newly hatched larvae normally live in close contact with eggshells and debris and thus acquire bacteria from them [1]. During larval rearing, feed given to fish may contain bacteria, and the remaining uneaten feed further constitute an excellent medium for bacterial growth if left in tanks, so fish hatchlings and larvae may ingest bacteria during feeding and drinking [14, 15].

In terms of composition, bacteria belonging to eight genera were recovered from this study. Earlier studies by Ogbondeminu *et al*, (1991) [18] and Ogbondeminu (1993) [17] revealed a diverse type of bacteria flora, including *Aeromonas sp, Pseudomonas sp,* members of enterobacteriacae and some gram-positive bacteria, in the hatchery of related species *Clarias anguillaris*. While the ubiquitous and facultative motile *Aeromonas sp.* has been generally recognized as fish pathogens [19], reports of infections caused by other bacteria isolated in this study on *C. gariepinus* larvae are rare.

Although the bacterial flora that develop on eggs and larvae and their diversities are the reflection of the bacteria composition of rearing water, other factors may be responsible for their attachments on the fish. Hansen and Olafsen (1999) [9] reported that species-specific adhesions of bacteria to surface receptors on the fish may affect the composition of the epiflora. The high percentages of motile *Aeromonas sp.* in fish larvae during culture may have been enhanced by the presence of adhesin in these bacterial species. These adhesins appear to be selective, recognizing D-mannose and L-fructose side chains on polymers located on the surface of eukaryotic cells [4].

Other major finding of this study was the survival of hatchery-bred *C gariepinus* during their early developmental stages. Using the mean values plotted in Table 5, it was estimated that only 35% of the total eggs incubated were able to reach advanced fry stage, thus implying that more than half of the procured eggs died before they reached advanced fry stage. Similar unpublished cases were continuously experienced by majority of Nigerian hatchery operators. From this study, the reasons for these losses cannot be attributed to bacterial prevalence only; hence the urgent need for extensive research to address mass mortalities of catfish eggs and larvae in the hatchery in order to meet the ever-increasing demand for catfish seeds for stocking.

Moreover, it was observed from this study that highest mortalities occurred as the fish grew from newly hatched larvae into fry i.e. between the 3rd and 14th day of rearing. This period appears to be the most crucial stage during African catfish propagation in the hatchery. Similar results of low larval survival during this period had been observed earlier in the work of Olurin *et al* 2012) [21]. Beside appropriate water management and adequate feeding regimes it is essential to maintain a clean environment during culture in order to achieve higher larval survival.

## 5. Conclusion

This study only targeted aerobic bacteria that are easily cultured on tryptone soya agar, thus excluding isolation of those that required specialized growth requirements. Furthermore, the impact of the isolated bacteria on early stages of catfish propagation is still unknown, as this finding did not reflect any direct relationship between bacterial number or type and egg hatchability/larval mortality. Nevertheless, this study provides some useful information in understanding prevalent aerobic bacteria organisms during early developmental stages of this commercially important fish species under hatchery conditions.

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