



E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2021; 9(1): 235-239

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[www.fisheriesjournal.com](http://www.fisheriesjournal.com)

Received: 25-11-2020

Accepted: 27-12-2020

**Anifowose Olayinka Remilekun**

Department of Veterinary  
Medicine, Faculty of Veterinary  
Medicine, University of Ibadan,  
Nigeria

**Oladosu Gbolahanmi Akinola**

Department of Veterinary  
Medicine, Faculty of Veterinary  
Medicine, University of Ibadan,  
Nigeria

**Oladele Oludotun Olubusola**

Department of Veterinary  
Medicine, Surgery and  
Radiology, Faculty of Veterinary  
Medicine, University of Jos,  
Nigeria

**Corresponding Author:**

**Anifowose Olayinka Remilekun**

Department of Veterinary  
Medicine, Faculty of Veterinary  
Medicine, University of Ibadan,  
Nigeria

# International Journal of Fisheries and Aquatic Studies

## Causal factors of mass mortality of hatchery reared *Clarias gariepinus* fry during exogenous feeding

**Anifowose Olayinka Remilekun, Oladosu Gbolahanmi Akinola and  
Oladele Oludotun Olubusola**

DOI: <https://doi.org/10.22271/fish.2021.v9.i1c.2409>

### Abstract

The study investigated the causal factors associated with mass mortality of hatchery-reared *Clarias gariepinus* Burchell 1822 fry during exogenous feeding. A feeding trial and fry re-infection trial were conducted using four different starter diets. The food samples were fed to 480 post-yolk-absorbed fries for 21 days in two replicates. Samples of dead fry were cultured for bacteria using standard methods. The total aerobic and coliform were significantly higher in all food samples, meanwhile, alkaline pH ( $9.25 \pm 0.34$ ) and low dissolved oxygen ( $4.5 \pm 1.08$ ) were not significantly different ( $P > 0.05$ ) in all water tanks. The bacterial pathogens isolated from dead fries were *Aeromonas hydrophila* Stainer, 1943 and *Pseudomonas aeruginosa* Migula, 1990, whereas significantly higher mortality in experimentally infected fry was observed ( $\alpha < 0.05$ ).

Mass mortality of *C. gariepinus* fry observed in this study was associated with bacterial pathogens, bacterial contamination of food, alkaline pH, and low dissolved oxygen.

**Keywords:** fry, mortality, food samples, *Clarias gariepinus*, pH

### 1. Introduction

Aquaculture, which is dominated by African catfish farming, has in recent decades increased at a phenomenal rate in many parts of the country, including Southwest Nigeria<sup>[19]</sup>. Fertilization, hatching, and early survival of larvae are vital for successful aquaculture of the African catfishes and this has been investigated earlier<sup>[5]</sup>. It has been estimated that 10% of all cultured aquatic animals are lost as a result of infectious disease<sup>[18]</sup>, meanwhile, environmental circumstances such as poor water quality, fluctuations in temperature, poor nutrition, overcrowding, poor handling, and transportation are common in intensive fish farming could pose stressful conditions to fish and make them more susceptible to a wide variety of pathogens. Hatchery propagation of fish seeds is sometimes hampered by mass mortality during fry nursing, with environmental factors, first feed sources, and infectious agents exerting great influence in this regard<sup>[9]</sup>. Mass mortality of fry would result in poor fingerling harvest and hence inadequate supply of fish seed which will, in turn, result in inadequate fish production. The suitability of first feed sources for fry rearing is very important in the light of the need for good growth and high survival rates<sup>[3]</sup> and the high cost of importing encapsulated artemia has necessitated the search for other first feed sources with fish culturists using several materials to rear the larvae of *Clarias gariepinus*<sup>[2]</sup>. The studies on the causal factors of *Clarias gariepinus* fry mortality following exogenous feeding in Nigeria have not been reported. Therefore, this study sets out to investigate the causal factors of mass mortality of hatchery-reared *C. gariepinus* fry during exogenous feeding.

### 2. Materials and Methods

#### 2.1 Handling of experimental Fish

Ethical clearance and valid approval were obtained from the University of Ibadan, Ethical Committee for Medical and Scientific Research before the commencement of the experiment. Fry used in this study were handled according to the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals<sup>[10]</sup>.

## 2.2 Collection of Samples

The feed samples collected were identified as follows: commercial Skretting gemma wean feed (Group 1 Durante<sup>R</sup>), Group 2 (Artemia Inve<sup>R</sup>; decapsulated or Shell Free Artemia), Group 3 (Dried-ground Shrimp), and Group 4 (Artemia Lush<sup>R</sup>; decapsulated or Shell Free Artemia). The microbial analysis of all the feed samples was carried out. Four hundred and eighty pieces of 3 days old African catfish fries were collected from the University of Ibadan fish farm, transported under hygienic conditions to the Department of Veterinary Medicine (Fish Medicine Unit). The fries were divided into four groups of forty fish each in three subgroups. Group 1 had subgroups 1a, 1b, and 1c, fed with commercial Skretting gemma wean diet, group 2 had subgroups 2a, 2b, and 2c, fed with shell-free artemia Inve<sup>R</sup>, group 3 had subgroups 3a, 3b, and 3c, fed with Dried-ground Shrimp, while group 4 had subgroups 4a, 4b, and 4c, fed with shell-free Artemia Lush<sup>R</sup>. The experimental fries were stocked in a 6L experimental plastic tank containing 4 liters of water (100ml of water per fry). The feeding started third-day post-hatch and they were fed 0.03g of feed per fry daily for 21 days

The fifty percentage of water in each experimental fish tank was changed every 48 hours to avoid the death of fries due to poor water quality (<sup>25</sup>). Water quality parameters namely pH, Dissolved Oxygen, Ammonia, Nitrite, and Total Hardness was measured on days 11 and 21 using digital pH meter, Dissolved Oxygen meter, chemical titration for total hardness, and ELISA reader (ELX 800 from Biotek) for the optical density of color development for ammonia and nitrite.

Every forty-eight hours, mortality rates were monitored and recorded. Clinical Signs of infectious diseases were also observed.

Dead fries were daily collected from each experimental tank and bacteriology carried out on freshly dead fries from each group at the Veterinary Diagnostic Laboratory of Animal Care Consult Ibadan.

## 2.3 Bacterial cultures and preparation of inoculum

The swabs from homogenized tissues of topically sterilized fries carcasses were streaked on nutrient agar, MacConkey agar, Thiosulfate-citrate-bile-salts-sucrose agar and incubated at 37°C for 24 hours and 48 hours respectively while their identities were confirmed using standard techniques as described by Olutiola *et al.*, <sup>[20]</sup> as outlined. The bacteria isolates were further identified based on the results of various biochemical tests using Bergey's Manual of Systemic Bacteriology <sup>[27]</sup> and Global Infectious Diseases and Epidemiology Network (GIDEON) Online microbiology database <sup>[14]</sup>

The fries re-infection trail was done by homogenizing the surface of identified bacteria isolates in sterile phosphate-buffered saline (PBS) and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard equivalent to 1 X 10<sup>8</sup> cfu/ml <sup>[4]</sup>

## 2.4 Experimental fry re-infection

Two hundred and forty pieces of fries were divided into three groups and 6 subgroups of forty fries, and were infected by immersing fish in water containing 1 X 10<sup>8</sup>cfu/ml of the isolates at the rate of 1ml/L as follows: subgroups 1a and 1b were infected with isolates of *Aeromonas hydrophila*, subgroups 2a and 2b were infected with isolates of

*Pseudomonas aeruginosa*, subgroups 3a and 3b were the control group and they were not infected. The fries were fed the same amount of feed, 0.03g of feed per fry making 1.2g per fish tank for 16 days and half of the water was replaced at 24 hour-interval to further ensure good water quality <sup>[25]</sup>. The Total Hardness, Ammonia, Nitrite, pH and Dissolved oxygen concentration of the water was monitored and recorded at 8 days interval during the period of this study <sup>[12]</sup>.

The experimentally-infected fries were observed daily for 16 days. Clinical signs, skin lesions, mortalities, and the quality parameters of water were noted and recorded. Dead fries from the experimental infection trials were processed for re-isolation of Infective bacterial isolates. A positive culture was confirmed if the morphology and biochemical characteristics of the re-isolated strain were identical with those of the isolates used in the experimental infection

## 2.5 Statistical analysis

The water quality parameters in different experimental tanks during the feeding trial and fry re-infection trial with bacteria isolates were determined and the analysis of variance (ANOVA) was used to determine the level of statistical significance in all four water tanks. The analysis of variance (ANOVA) was also used to determine the level of significance in mortality rate during fries re-infection trial with bacterial isolates, a p-value of less than 0.05 ( $p < 0.05$ ) was considered as significant.

## 3. Results

The physicochemical findings such as pH, Total hardness, Nitrite, Ammonia, and Dissolved oxygen in all fish tanks during the feeding trial were not significantly different at 95% confidence limit ( $p > 0.05$ ) as indicated in Table 1, meanwhile, the physicochemical findings such as pH, Total hardness, Nitrite, Ammonia, and Dissolved oxygen in all fish tanks during the re-infection trial were significantly different at 95% confidence limit ( $p < 0.05$ ) as indicated in Table 1. All the feed samples had high total aerobic bacteria counts and were not significantly different at 95% confidence limit ( $p > 0.05$ ) as indicated in Table 2.

The mortality rate was increasing daily during the feeding trial in which the fries group fed with dried shrimp (group 3) had the highest (98.3%), followed by the fries group fed with Artemia Lush<sup>®</sup> (group 4) 97.5% and 79.1% in fish tanks fed with Artemia Inve<sup>®</sup> (group 2) respectively while the mortality rate of fries group fed with commercial Skretting gemma wean diet (group 1) was 35.8% as indicated in Table 3. The values for the total mortality rate of all the fry groups were significantly different at 95% confidence limit ( $p < 0.05$ ) as indicated in Table 3.

There was an increase in the mortality rate daily during the fry re-infection trial period, fry group infected with *Pseudomonas aeruginosa* had the highest mortality rate 78%, while, 74% mortality rate was observed in the group infected with *Aeromonas hydrophila* while the control group had the lowest mortality rate of 6.3% as indicated in Table 3.

The values for the total mortality rate of all the infected groups were significantly different at 95% confidence limit ( $p < 0.05$ ) as indicated in Table 3.

The bacterial isolates from dead fries during the feeding trial and re-infection trial were *Aeromonas hydrophila* and *Pseudomonas aeruginosa* as indicated in Table 4.

**Table 1:** Physicochemical findings of experimental water tanks during feeding trial and re-infection trial

Physicochemical Parameters	Day 11 post-feeding trial	Day 21 post-feeding trial	Day 8 post-re-infection trial	Day 16 post-re-infection trial	Standard Values for Fish Farming APHA 1989, Boyd and Tucker 1992
pH	9.25±0.34 <sup>b</sup>	8.98±0.38 <sup>b</sup>	8.36 ± 1.15 <sup>a</sup>	8.4 ± 1.23 <sup>a</sup>	6.5 – 8.5
Total Hardness (ppm)	121.3±2.5 <sup>b</sup>	165±12.9 <sup>b</sup>	143 ± 40.4 <sup>a</sup>	130 ± 17.3 <sup>a</sup>	50 – 150
Nitrite (ppm)	0.13±0.008 <sup>b</sup>	0.18±0.006 <sup>b</sup>	0.023 ± 0.006 <sup>a</sup>	0.02 ± 0.001 <sup>a</sup>	0.05 max
Ammonia (ppm)	0.57±0.22 <sup>b</sup>	1.58±0.23 <sup>b</sup>	0.84 ± 0.68 <sup>a</sup>	0.88 ± 0.70 <sup>a</sup>	2.0 max
Dissolved Oxygen (ppm)	4.5±1.1 <sup>b</sup>	4.5±1.08 <sup>b</sup>	4.53 ± 1.01 <sup>a</sup>	4.50 ± 0.87 <sup>b</sup>	5.0 min

Values with (<sup>b</sup>) across row are not significantly different at 95% confidence limit ( $p>0.05$ ) – ANOVA, min = minimum, max = maximum  
 Values with (<sup>a</sup>) across row are significantly different at 95% confidence limit ( $p<0.05$ ) – ANOVA

**Table 2:** Microbial Analysis of Feed Samples

Parameters	Group 1 Feed cfu/g x 10 <sup>5</sup>	Group 2 Feed cfu/g x10 <sup>5</sup>	Group 3 Feed cfu/g x 10 <sup>5</sup>	Group 4 Feed cfu/g x 10 <sup>5</sup>
Total Aerobic Counts	1.18 <sup>a</sup>	7.68 <sup>a</sup>	2.18 <sup>a</sup>	4.39 <sup>a</sup>
Total Coliform Counts	1.19 <sup>a</sup>	3.06 <sup>a</sup>	1.10 <sup>a</sup>	3.75 <sup>a</sup>

Values with (<sup>a</sup>) across the column are not significantly different at 95% confidence limit ( $p>0.05$ ) – ANOVA

**Table 3:** Percentage mortality of fry during the feeding trial and re-infection trial

DAYS	Percentage mortality of fry during the feeding trial			
	Group 1 Tanks (%)	Group 2 Tanks (%)	Group 3 Tanks (%)	Group 4 Tanks (%)
DAY 7	3.3	38.3	45.8	40.8
DAY 14	23.5	54.1	75.8	65.8
DAY 21	35.8	79.1	78.3	97.5
Total Mortality	35.8 <sup>a</sup>	79.1 <sup>a</sup>	98.3 <sup>a</sup>	97.5 <sup>a</sup>
Survival Rate	64.2	21.9	1.7	2.5
Mean ± S.D	14.3 ± 8.17 <sup>a</sup>	31.7 ± 11.1 <sup>a</sup>	39.3 ± 11.7 <sup>a</sup>	39 ± 7.8 <sup>a</sup>
DAYS	Percentage mortality of fry during re-infection trial			
	A Tanks (%)	P Tanks (%)	C Tanks (%)	
DAY 8	45	43	4	
DAY 16	74	78	6.3	
Total Mortality	74 <sup>a</sup>	78 <sup>a</sup>	6.3 <sup>a</sup>	
Survival Rate	26	22	93.7	
Mean ± S.D	29.5 ± 6.5 <sup>a</sup>	31 ± 3 <sup>a</sup>	2.5 ± 0.5 <sup>a</sup>	

**Table 4:** Bacteria Associated with fry mortality during the feeding trial

Bacterial isolates from each group	Gram-Staining	Mo	Mc	C	O	SC	I	MR	VP	H	E	T	U	CH	GH	SH	MS	G	M	L	S
Group 1 ( <i>Pseudomonas aeruginosa</i> )	Gram-negative rod -shaped	+	NLF	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Group 2 ( <i>Pseudomonas aeruginosa</i> )	Gram -negative rod-shaped	+	NLF	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Group 3 ( <i>Aeromonas hydrophila</i> )	Gram-negative rod -shaped	+	LF	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+
Group 4 ( <i>Pseudomonas aeruginosa</i> )	Gram-negative rod -shaped	+	NLF	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Group 4 ( <i>Aeromonas hydrophila</i> )	Gram-negative rod-shaped	+	LF	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+

Mo = Motility, Mc = Macconkey agar, C = Catalase, O = Oxidase, Sc= Simon citrate, I = indole, MR = Methyl red, VP = Voges Proskauer, H = H<sub>2</sub>S production, E = Eosine methylene blue, T = Thiosulfate-citrate-bile salts-sucrose agar, U = Urease, CH = Casein Hydrolysis, GH = Gelatin hydrolysis, SH = Starch hydrolysis, MS = Manitol salt agar, G = Glucose, M = Maltose, L = Lactose, S = Sucrose, LF = Lactose Fermenter NLF = Non Lactose Fermenter.



**Fig 1:** Dead fry



**Fig 2:** TSA agar of bacterial Isolates



**Fig 3:** Macconkey Agar of Bacteria Isolates

#### 4. Discussion

In the feeding trial, the pH values of water observed in this study on day 12 in the water tanks fed with four first feed sources were alkaline as indicated in Table 1. The alkaline pH values persisted for the whole 21 days in this study. The fishes can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 [13]. The pH observed in this study was higher than 8.5 and this high alkaline pH may contribute to mass mortality of fry observed in this study during the feeding trial as indicated in Table 3.

Dissolved Oxygen affects the growth, survival, distribution, behavior, and physiology of fish [22]. Oxygen depletion in water leads to poor feeding of fish, starvation, reduced growth, and more fish mortality, either directly or indirectly [7]. According to Banerjee [6], dissolved oxygen for average fish production should be above 5ppm, and dissolved oxygen 1 – 3ppm has a sublethal effect on growth and feed utilization [8].

Low dissolved oxygen was observed in this study within the first 21 days in fry groups fed with artemia (group 2 & 4) and shrimp (group 3) as indicated in Table 1 and this may be as a result of environmental temperature which decreases oxygen solubility and increase the metabolism of tank organisms, subsequently increasing the biological oxygen demand. Chronic environmental hypoxia as observed in this study caused stress, reduced feed consumption, slow growth, and high mortalities [25] (Table 3, Figure 1). Ubiebi [26] reported higher bacteria loads in fish feeds; Durante<sup>R</sup> ( $3.3 \times 10^5$ cfu/g), Aqua<sup>R</sup> ( $1.75 \times 10^5$ cfu/g), Multi<sup>R</sup> ( $3.3 \times 10^5$ cfu/g), and these were similar to the high level of bacteria contamination recorded in all four first food sources in this study as indicated in Table 2, meanwhile, Kings *et al.*, [16] had earlier reported that ingestion of contaminated feed may be a source of *Aeromonas Hydrophila* infection in catfish.

The fry in group two during the feeding trial had high mortality of 70.8% as indicated in Table 3, which may be attributed to the high level of bacterial count ( $7.68 \times 10^5$  cfu/g) in the feed sample as indicated in Table 2.

The high percentage mortality 70.8% and 97.5% observed during feeding trial for fry fed with Artemia in group 2 and group 4 as indicated in Table 3 is similar to the observation of Adewolu *et al.* [1] who recorded mortality of 66.7% for fry fed with Artemia. The highest percentage mortality (98.3%)

Table 3, observed in the fry group fed with shell-free Artemia (group 4) in this study may be attributed to bacterial contamination of water through feed since the highest bacteria load ( $7.8 \times 10^5$ cfu/g) was observed in the food source (Table 2).

The bacteria isolated and identified from freshly dead fry from all the four groups based on microbial and biochemical characterization were *Pseudomonas aeruginosa* and *Aeromonas hydrophila* (Table 4, Figure 2, and 3).

The high mortality observed in the re-infection trial with the bacterial isolates, and the re-isolation of these pathogens from freshly dead fry tend to lend credence to the culpability of the bacterial pathogens.

The most important fish pathogen was *Aeromonas hydrophila* [11] and the highest prevalence is in polluted waters [15]. The mortality rate of 73.7% (Table 3) was observed in the fry group infected with *Aeromonas hydrophila* in this study. This is similar to the report of Anyanwu *et al.*, [4] who observed a cumulative mortality rate of 30 – 90% in catfish infected with *Aeromonas hydrophila* at the rate of  $1 \times 10^8$ cfu/ml of the pond water. In Bangladesh, Sarkar *et al.*, [21] also reported 60-80% mortality at day 15 post-infection in catfish with *Aeromonas hydrophila*. The variation in the pattern and mortality rates may be related to the species of fish, the strain of *Aeromonas* species, experimental conditions, the dose of the infective pathogen given, route of administration of the pathogen, and duration of the experiment [4].

Catfish are reported to be susceptible to *Pseudomonas spp.*, with moderate to high losses [23]. The percentage mortality of fry re-infected with *Pseudomonas aeruginosa* in this study was 77.5 (Table 3). In the fry re-infection trial, fry re-infected with *Pseudomonas aeruginosa* had the lowest mortality (Table 3), and this may be due to the incrimination of *Pseudomonas aeruginosa* as the most common bacterial infection among fish and appear to be the stress-related disease of freshwater fish under culture conditions [17]. The results of the feeding trial and fry re-infection trial in this study are similar to each other concerning poor water quality, high percentage mortality, and presence of bacteria isolates. Therefore, causal factors of mass mortality of hatchery-reared fry during exogenous feeding include poor water quality, feed contamination, and the presence of bacterial pathogens.

#### 5. Conclusion

Factors that were observed to predispose the African catfish fry to mass mortality are bacterial contamination of feed sources, extremes of water quality parameters like pH (alkaline), and low dissolved oxygen.

Bacterial pathogens associated with mass mortality in the African Catfish nursing during exogenous feeding are *Aeromonas hydrophila* and *Pseudomonas aeruginosa*.

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