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Research Article

# Epidemiological and Zootechnical Consequences Associated with the Selective and Differential Implementation of Biosecurity Measures in *Clarias gariepinus* (Burchell, 1822) Farming

**Georges Fonkwa[1,2*], Georgette Judith Makombu[3], Nasser Abdel Njikam[1], Amidou Nsangou Kpoumie[1,2], Junior Franck Djamou Kametieu[1], Minette Eyango Tomedi[1] and Joseph Tchoumboue[2]**

*[1]Laboratory of Aquaculture and Demography of Aquatic Resources, Department of Aquaculture, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O. Box 7236 Douala-Cameroon*

*[2]Applied Hydrobiology and Ichthyology Research Unit, Department of Animal Science, Faculty of Agronomy and Agricultural Science, University of Dschang, P.O. Box 222, Dschang-Cameroon*

*[3]Department of Fisheries and Aquatic Resources Management, Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon, P.O. Box 63 Buea- Cameroon*

## Abstract

Biosecurity practices limit the spread of diseases in a production system. The present study aims to evaluate the effect of selective and differential implementation of the Sets (groups) of Biosecurity Measures (SBM) on the Epidemiological and Zootechnical Characteristics (EZC) of *Clarias gariepinus* fry. A total of 270 specimens with an average weight of 10±1g and disease free were divided into triplicates in three (03) treatments T1, T2 and T3 of 90 fish each, corresponding respectively to the sets of biosecurity measures less adopted, moderately adopted and most adopted by fish farmers, and then reared for 90 days. Evaluation of the EZC showed that of a total of five clinical signs observed, 60% were anatomical (trauma, dropsy, cranial hypertrophy) while 20% were physiological (skin discoloration) and 20% ethological (anorexia). Overall, the highest prevalence ($p = 0.787$) of affections was observed at T2 (14.30%), followed by T3 (11.01%) and T1(10.70%). Skin trauma and skin discoloration were more represented ($p < 0.05$) at T2, followed by T1 and T3. The condition factor varied ($K < 1$) non-significantly between the SBM. Mortality rates ranged ($p = 0.42$) from very low to low for T2, T3 and T1 respectively. Set 3 resulted in a higher average daily weight gain (2,705±1.08 g/day) and productivity (3,685±1.451 Kg/m3/day) ($p > 0.05$) and a lower biosecurity production cost per kg of fish (0.837±0.445$/kg) ($p = 0.003$) and fitted better with the socio-economic realities of fish farmers and the geoclimatic characteristics of the study area.

**Keywords:** Biosecurity; *Clarias gariepinus*; Epidemiology; Pathologies; Zootechnics

## Introduction

The fish farming industry in Cameroon is confronted with diseases, the result of which is massive fish mortality leading to reduced productivity and economic losses [1]. Diseases should then be taken into account for healthy and sustainable fish farming. The absence or poor implementation of biosecurity measures by fish farmers has been reported as a major cause of the onset and spread of diseases in fish farms [2,3]. According to Alarcon *et al*. [4], biosecurity is the application of measures to reduce the level of risk or probability of introduction (external biosecurity) and spread of pathogens within the farm (internal biosecurity). The key concept of biosecurity is therefore to prevent the transmission of disease, either between farms or within the farm. Biosecurity is also a strategic approach that integrates and encompasses both policy and regulatory frameworks to analyze and manage risks in order to prevent the exposure, introduction, transmission and spread of diseases in aquaculture operations [5].

Preliminary work on mapping biosecurity practices has been carried out in the Wouri Division [3,6] and in the Centre Region of Cameroon [7-9]. This work helped to determine the adoption rates for each of the 24 biosecurity measures recommended by Arthur *et al*. [10]. The adoption rates of a biosecurity measure which according to Racicot and Vaillancourt [2] is the proportion of farms implementing the measure, have been classified as low, moderate and high in Cameroon by Fonkwa *et al*. [3]. Moreover, none of the fish farms studied practiced all the prescribed biosecurity measures. Furthermore, fish farmers were applying biosecurity measures selectively and differentially, due to tehnico-economic constraints, level of education, negligence and ignorance of these measures. It becomes essential to select and group or combine all the biosecurity measures implemented or not by fish farmers according to adoption rates, and to assess the

***Corresponding author:** Fonkwa G, Laboratory of Aquaculture and Demography of Aquatic Resources, Department of Aquaculture, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O Box 7236 Douala-Cameroon, Applied Hydrobiology and Ichthyology Research Unit, Department of Animal Science, Faculty of Agronomy and Agricultural Science, University of Dschang, P.O. Box 222, Dschang-Cameroon, Email: fonkwageorges@gmail.com

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epidemio-zootehnical efficiency of the three sets (packages) of measures obtained, in order to propose to producers a range of measures that better matches their socio-economic and geoclimatic reality.

In Cameroon, there are no data on the epidemiological, zootechnical and economic effects of implementing the three groups/sets of biosecurity measures. In other words, correlations between the application of sets of biosecurity measures and the epidemiological, zootechnical and economic characteristics have not yet been established. Do the measures most adopted by fish farmers, when applied together; generate the most relevant epidemio-zootechnical and economic characteristics? Wouldn't the biosecurity measures least adopted by fish farmers be the most effective in epidemio-zootechnical respects? The aim of the present study was to evaluate the effect of selective and differential implementation of biosecurity measures by fish farmers in the Wouri Division on epidemiological (prevalence of clinical signs of pathologies or abnormalities, condition factor, mortality rate) and zootechnical (average daily weight gain, productivity, biosecurity production cost per kg of fish) characteristics.

## Materials and Methods

### Geoclimatic characteristics of the study area

The study was carried out from February to June 2024 on a pilot farm located in the Douala 3 District (4°02'36" - 4°05'43"N; 9°44'33" - 9°47'06" E) belonging to the Wouri administrative Division, Littoral Region of Cameroon. The climate is of the equatorial type with a rainy season (March to November) and a dry season (December to February).

### Experimental set-up and animal material

The experimental set-up consisted of three hatcheries, each containing three 20l basins supplied with groundwater. The basins of the first two hatcheries (T1 and T2) were set up in series, and those of the third hatchery (T3) in parallel. Based on previous work carried out in the Wouri Division of Cameroon [3,6], biosecurity measures were regrouped into 3 sets or bundles i.e. the measures less adopted, moderately adopted and most adopted by fish farmers. These sets corresponded respectively to low, moderate and high adoption rates of biosecurity measures. Each set of measures was implemented at a single hatchery to obtain three treatments, the characteristics of which are summarized in (Table 1).

As for the animal material, a total of 500 *Clarias gariepinus* fry of average weight 10 ±1g were transferred to 40l basins in order to be disinfected according to the protocol used by Kone *et al*. [11]. The fish were disinfected for one hour in a solution (250mg/l) of antiobiotic (Oxytetracycline) and antiparasitic (Potassium Permanganate) at a concentration of 500mg/l to eliminate potential pathogens, then acclimatized for 10h. Each fry was then examined minutely with the naked eye and then with a hand-held magnifying glass at 10x magnification to detect any signs of pathology or abnormalities. Among the previously disinfected fry, a total of 270 showing no sign of pathology were selected and distributed among the 9 basins. They were fed with Aller Aqua brand feed three times a day by hand at a ration rate of 6.5% of itchyobiomass. Water physico-chemical values were assessed twice daily using an analysis kit.

### Evaluation of the epidemiological characteristics and zootechnical performances

Harvest controls were carried out every 14 days [12]. Fish behavior was firstly observed to determine any behavioral affection (anorexia, abnormal swimming…). Thereafter, 100% fish specimens per basin were captured using a fish net. Thereafter, the fish total lengths and weight were measured using an ichthyometer and sensitive weighing balance of Scaletec brand respectively. Each fish sample was first examined with naked eyed before using a hand lens to diagnose any clinical sign of diseases or abnormalities. These signs included dropsy, external hemorrhage, deep necrosis, pale gills, hypertrophy of scales etc. In this study, pathology is defined as any abnormality or affection occurring in the fish no matter their origin. The number of dead fish per treatment was also recorded. An infected fish sample was coded as 1 and uninfected as 0. The pH electrode and multimeter were used to determine the water hydrogen potential and temperature respectively after each harvest.

### Data processing

The characteristics studied were both epidemiological and zootechnical and defined as follows:

- Prevalence of clinical signs of pathologies or abnormalities (Pr): number of fish showing a clinical sign of pathology/abnormality divided by the number of fish examined. The prevalence (Pr) was classified after Fonkwa *et al*. [13] i.e. very low (Pr < 10 %), low (10 % ≤ Pr ≤ 50 %) or high (Pr > 50 %). The prevalence of the clinical signs of pathology was evaluated in this study instead of the prevalence of infection/disease or the prevalence of infection usually evaluated because the etiology of these abnormalities was not determined. Also, the ethological, anatomical and some physiological signs are the first ones to draw the attention of the farmers.
- Condition factor (K): ratio of the weight of the fish to the cube of the total length
- Mortality rate: percentage of deaths fishes in a population within a period of time in relation to the initial total number of the population. The mortality rate was classified as very low [0-2]%, low] 2-5]% or high i.e. > 5%.
- Daily average weight gain: mass gained or lost by the fish over the number of days.
- Productivity: total weight of fishes produced divided by the volume of infrastructures multiplied by the period of production.
- Biosecurity production cost of a kg of fish: ratio of the implementation cost of a set of biosecurity measures by the total weight gain.

The epidemiological and zootechnical characteristics were subjected to descriptive statistics (mean ± standard deviation). The analysis of variance (F) was used to assess the effect of the sets of biosecurity measures on the epidemiological characteristics and zootechnical performances of fish. In the case of a significant difference, that is the error probability $p < 0.05$, the means were separated using Tukey's multiple comparison tests (q). Graph Pad Prism 8.0 software was used for analysis.

| Hatcheries/treatments | Characteristics of des treatments | | | | |
|---|---|---|---|---|---|
| | Repetitions | Number of fry | Sets/bundles of biosecurity measures | Adoption rates (%) | Adoption levels of biosecurity measures |
| T1 (Set 1) | T1' | 30 | - Water supply tracks protected to trap debris and unwanted aquatic animals<br>- Use of footbaths<br>- Special outfit for staff<br>- Special outfit for visitors<br>- hand disinfection on entering and exit from the hatchery | [0-25] = low | Less adopted measures |
| | T1" | 30 | | | |
| | T1"' | 30 | | | |
| T2 (Set 2) | T2' | 30 | - New fish are quarantined before rearing<br>- Visitors not allowed to have contact with water<br>- Disinfection of breeding tools before use<br>- Disinfection of breeding tools after use<br>- Incineration of dead fish | ]25-75] = moderate | Moderately adopted measures |
| | T2" | 30 | | | |
| | T2"' | 30 | | | |
| T3 (Set 3) | T3' | 30 | - Rearing facilities are layout in derivation<br>- No exchange of breeding tools between hatcheries<br>- Hatchery is fenced<br>- well-preserved feed<br>- Water flow is continuous | ]75-100] = high | Most adopted measures |
| | T3" | 30 | | | |
| | T3"' | 30 | | | |

**Table 1:** Synopsis of the characteristics of established hatcheries.

## Results

### Effect of selective and differential implementation of sets of biosecurity measures on the prevalence of clinical signs of pathologies

The effect of the selective and differential implementation of sets of biosecurity measures on the prevalence of clinical signs of pathologies is illustrated in (Figure 1). It appears that out of six clinical signs observed, 60% were anatomical (trauma, dropsy, cranial hypertrophy) while 20% were physiological (skin discoloration) and 20% ethological (anorexia). Regardless of the Sets of Biosecurity Measures (SBM), skin discoloration recorded a prevalence approximately 16.53 times higher than cranial hypertrophy, but with no significant difference ($F = 0.967$; $p = 0.475$). Physiological and ethological abnormalities were common to the three SBM. When comparing the occurrence of clinical signs between the SBM, it follows that skin trauma, dropsy, skin discoloration and anorexia were common to all three sets while cranial hypertrophy was specific to set 2. Regardless of the SBM, the proportion of fish showing at least one pathology was low (< 10%). The highest prevalence was observed at T2, followed by T3 and T1 ($F = 0.24$; $p = 0.787$). The prevalence of skin trauma and skin discoloration was higher at T2 followed in order by T1 and T3, however without significant difference.

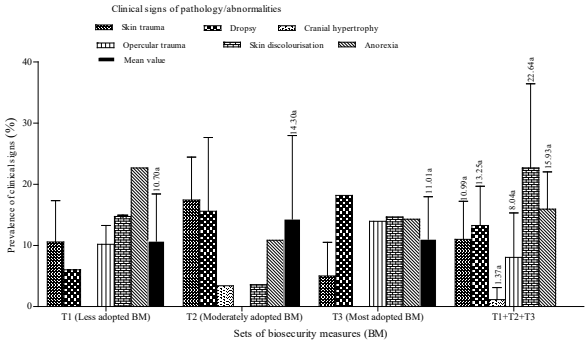


**Figure 1:** Effect of selective and differential implementation of sets of biosecurity measures on the prevalence of clinical signs of pathologies.

(a,b) : For a clinical sign, bars with the same letter do not significantly differ ($p > 0.05$)

### Effect of selective and differential implementation of sets of biosecurity measures on the condition factor and mortality rate of *Clarias gariepinius* fry

The condition factor (Figure 2) varied insignificantly ($k<1$) between the SMB ($F = 0.24$; $p = 0.79$). Mortality rates (Figure 2B)

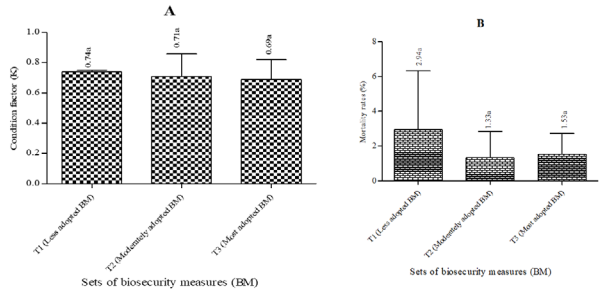


**Figure 2:** Effect of selective and differential implementation of sets of biosecurity measures on the condition factor (A) and mortality rate (B) of *Clarias gariepinius* fry.

a: Bars with the same letter do not significantly differ (p> 0.05)

| Zootechnical Caractéristiques | Sets/Bundles of biosecurity measures | | | F | p |
|---|---|---|---|---|---|
| | T1 (Less adopted measures) | T2 (Moderately adopted measures) | T3 (Most adopted measures) | | |
| DAWG (g/day) | 2.332a±1.11 | 2.507a±1.17 | 2.705a±1.08 | 0.16 | 0.840 |
| Productivity (Kg/m3/day) | 2.885a±1.318 | 3.462a±1.552 | 3.685a±1.451 | 0.48 | 0.623 |
| BPC ($/kg of fish ) | 6.932a±6.383 | 2.314a±1.713 | 0.837b±0.445 | 4.15 | 0.03* |

**Table 2:** Effect of selective and differential implementation of sets of biosecurity measures on zootechnical characteristics.

DAWG :daily average weight gain ;BPC : biosecurity production cost of a kg of fish ; ( a,b,c) : values with the same letter on a given row do not significantly differ (p> 0.05); F: Anova test value; p: Error probability;*: significant

ranged non-significantly from very low to low for T2, T3 and T1 respectively (F = 0.91; p = 0.42).

### Effect of selective and differential implementation of sets of biosecurity measures on zootechnical characteristics

The effect of selective and differential implementation of sets of biosecurity measures on zootechnical characteristics, as summarized in (Table 2), indicates that the SBM most adopted generated a higher average daily weight gain (F=0.16; p = 0.84). The highest productivity was observed in set 3, followed by set 2 and set 1(F=0.48; p=0.623). The biosecurity production cost per kg of fish was 8.28 times significantly more expensive for T1 than T3 (F=4.15; p=0.03).

## Discussion

Although the least, moderately and most adopted biosecurity measures have been determined [3,6-9], there is no literature on the effect of these three sets of biosecurity measures on the epidemiological and zootechnical characteristics of fish farming. Most of the clinical signs observed (60%) were anatomical because they were the most perceptible compared to other signs. Some abnormalities of haematological profiles (abnormal serum protein levels, eosinophilia, etc.), were likely to have occurred but were not diagnosed, as they required a more in-depth diagnosis. WOAH (World Organization for Animal Health 2023) noted that the presence of virus in a farming environment increases the chance of anatomical clinical signs. It would be useful to determine the etiology of these pathologies.

Irrespective of the sets/bundles of biosecurity measures, the skin was the target organ that harbored more abnormalities (skin trauma and skin discoloration) because of its direct contact with farm water, which is thought to contain more skin-specific pathogens. This observation calls for systematic analysis of the microbiological quality of the water in order to establish correlations with the clinical signs observed in the fish Physiological and ethological clinical signs were common to all three SBM. In fact, selective and differential implementation of the three SBM produced clinical signs common to all treatments. This result is contradictory to the one expected, since in epidemiological terms, some biosecurity measures are more effective than others. Indeed, biosecurity measures do not have the same weight in disease transmission, as direct contact poses a greater risk of contamination compared to indirect contact [14,15]. Whatever the set of biosecurity measures, the proportion of fish showing at least one pathology was low, probably because the physico-chemical characteristics of the farm water did not favor the development and proliferation of virulent pathogens likely to induce disease in fish. A similar observation was made by Fonkwa *et al*. [16], who showed that 24.50% of fish *Oreochromis niloticus* were infested by *Myxobolus tilapiiae.* This low prevalence would also reflect disease resistance by the strain of *Clarias gariepinus* fry used in the present study. Although this prevalence is low, it is nonetheless likely to result in economic losses. Set 2 recorded the highest prevalence, followed in order by T3 and T1, probably because Set 2 lacks effective biosecurity measures against pathogens. For the same clinical signs, the prevalence of skin trauma and skin discoloration was higher in T2. This result could be explained by the fact that T2 treatment water contains more skin-specific pathogens.

With regard to the effect of selective and differential implementation of the sets of biosecurity measures on the condition factor (k) of *Clarias gariepinus* fry, it appears that the fish did not do well (k <1). In other words, none of the three SBM proposed in the present study resulted in fish or well-being healthy fish. Although the k value recorded at set 1 was higher, this set should generate the most relevant condition factor (k>1). This higher value of k value with set 1 would be justified by the fact that it includes more effective biosecurity measures against pathogens (water supply tracks protected to trap debris and unwanted aquatic animals + special outfit for staff + special outfit for visitors + hand disinfection on entering and exit from the hatchery) compared with sets 2 and 3. In fact, biosecurity measures do not have the same contribution (same weight) to disease transmission, because direct contact poses a greater risk of contamination compared with indirect contact [14,15]. Fish endogenous (strains) and exogenous (water physico-chemical characteristics) factors would have disfavored the optimal expression of fish embonpoint or well-being, making them more susceptible to disease. These results are similar to those recorded by Diop *et al*. [17] and Da *et al*. [18], who also obtained a condition factor k <1 in *Clarias gariepinus* and *Clarias anguilarus* species respectively probably because of the close farming practices. The condition factor varies according to fish age, sex, season, reproductive organ development stage, diet, amount of food in the gut, amount of lipid reserve and degree of muscle development. Estensoro *et al.* [19] reported that a high-fat diet (17.00-22.00%) increases the prevalence and intensity of the *Entemyxum leei*, a myxosporean parasite in fish.

Although the mortality rate fluctuated non-significantly between very low and low, it was nevertheless higher than the 0% value predicted by the standard. These mortalities are believed to be caused by diseases. The difference in mortalities recorded between the sets of biosecurity measures would be due to the disparity of the biosecurity measures which constituted each range. The higher mortality rate at set 1, contrary to the expected value (0%), cannot be attributed solely to the infectious pathologies because the average prevalence of clinical signs was not high with set 1 as it was the case in set 2. This result is in disagreement with those obtained by Fonkwa *et al*. [3] and Fonkwa *et al.* [8] who recorded a high mortality rate, probably because the works have been carried out in different geographical areas with different farming practices.

As far as the effect of selective and differential implementation of sets of biosecurity measures on zootechnical characteristics is concerned, it appears that average daily weight gain oscillated between 2.33 and 2.70g/d. These values were below the 5.6g/d predicted by the "Skretting" feed technical data sheet and 4.03g/d obtained by Limbu [20]. Given that the "Skretting" feed specification sheet was respected, this result would depend on the diseases that led in some cases to anorexia, the cause of growth retardation in the fish. Compared with the work of Limbu [20], the difference in findings is thought to be dependent on farming practices. The higher value of average daily weight gain at set 3 would be justified by the fact that this bundle of biosecurity measures generated the lower prevalence of clinical signs and mortality rates.

Productivity was highest in set 3, which also recorded the lowest biosecurity production cost per kg of fish. Productivity is correlated with mortality rate. This would explain why sets 1 and 3, which recorded the highest and lowest mortality rates respectively, showed productivities varying in the same order. With regard to the biosecurity cost of production per kg of fish, set 3 was less expensive due to the lower cost of implementing its biosecurity measures. Set 3 being the less expensive is the one to be recommended to fish farmers, as it fits better with their socio-economic and geoclimatic reality in order to optimize aquaculture production in a healthy, efficient and sustainable way.

## Conclusion

The study on the epidemiological and zootechnical consequences associated with the Selective and Differential Implementation of Biosecurity Measures (SDIBM) in *Clarias gariepinus* farming revealed that the clinical signs of abnormalities were anatomical, physiological and ethological. Skin trauma and skin discoloration were significantly the most represented. Overall, the prevalence of clinical signs was not significantly affected by the SDIBM. The condition factor varied non-significantly between the 3 sets of biosecurity measures. Mortality rate ranged no significantly from very low to low. Set 3 resulted in a non-significantly higher average daily weight gain and productivity, and a significantly lower biosecurity cost of production per kg of fish. It is recommended to fish farmers to implement set 3 biosecurity measures (Rearing facilities are layout in derivation + no exchange of breeding tools with other hatcheries + hatchery is fenced + well-preserved feed + continuous water flow), as it fits better with their socio-economic and geoclimatic reality.

## Credit Authorship Contribution Statement

Fonkwa Georges: Conceptualization, writing original draft. Makombu Judith Georgette: Draft editing. Njikam Abdel Nasser: Writing original draft. Kpoumie Nsangou Amidou: Conceptualization. Kametieu Djamou Franck Junior: Conceptualization. Tomedi Eyango Minette: Coordination. Tchoumboue Joseph: Coordination.

## Conflits of Interest

The authors declare that they have no conflicts of interest.

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