

# Effects of different latency periods on fingerlings production using *Clarias gariepinus* (African mud catfish)

Audu, S. I.<sup>1</sup>, Onyia, E. C.<sup>3</sup> and Onyia, L.U.<sup>2\*</sup>

<sup>1</sup>Department of Fisheries and Aquaculture, Adamawa State University, Mubi, Nigeria.

<sup>2</sup>Department of Fisheries, Modibbo Adama University, Yola, Adamawa State, Nigeria.

<sup>3</sup>Department of Zoology, Modibbo Adama University, Yola, Adamawa State, Nigeria.

\*Correspondence author. Email: [luconyia@gmail.com](mailto:luconyia@gmail.com); Tel: +2348058047800.

Copyright © 2023 Audu et al. This article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 23rd July 2023; Accepted 4th August 2023

**ABSTRACT:** The research was carried out in the Fish Hatchery of the Department of Fisheries, Teaching and Research Farm, Modibbo Adama University. *Clarias gariepinus* belongs to the family Clariidae. The latency period had been demonstrated to affect fingerling production in *C. gariepinus*. Fifteen (15) gravid broodstock females weighing 367 to 1000 g and males of 800 to 1200 g were used at different latency periods of 9, 11, 13, 15 and 17 hours designated D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub>. Three replicates of the female broodstock were injected with 0.5 ml Ovaprim for each latency period. The number of 1 g of eggs from each treatment was D<sub>1</sub> (847), D<sub>2</sub> (705), D<sub>3</sub> (847), D<sub>4</sub> (692) and D<sub>5</sub> (847). Percentage fertilization was highest in 11, 13 and 15 hours latency periods (99.19%, 97.66% and 99.33%) respectively; and were significantly different ( $p < 0.05$ ). Percentage hatchability was highest in 13 hours latency period 63.73% and the least was in 17 hours latency period (1.99%). Unhatched eggs were higher in a latency period of 17 hours (5612). Number of hatchlings was highest in latency period of 13 hours. The highest percentage survival was in a latency period of 11 hours. Any attempt to improve fingerling production in *C. gariepinus* should consider the effect of the latency period in relation to weight. After three weeks of indoor incubation, a latency period of 15 hours showed better weight gain than others. The best latency period achieved in this study was 11 hours.

**Keywords:** *Clarias gariepinus*, eggs, fertilization, fingerling production, latency period, survival.

## INTRODUCTION

Over the past years, aquaculture has grown in leaps and bounds in response to an increased demand for fish as a source of protein globally (Akinrotimi *et al.*, 2007). To ensure fish food in Africa, increased production of fry and fingerlings with attributes of faster growth rates, high food conversion ratio and better environmental tolerance is highly required (Adah *et al.* 2014). African catfish is one of the most suitable species in aquaculture. It has been considered to hold great promise for fish farming. *C. gariepinus* has a high growth rate at high stocking densities under culture conditions, high fecundity rate, resistance to diseases, ability to tolerate a wide range of natural and artificial food and adapts to a variety of feeding made in expanded niches. It has good meat quality and smoking characteristics as well as year round production

(Dauda *et al.* 2018).

Artificial propagation involves human intervention in the process of natural propagation. Artificial propagation is the most promising and reliable way of ensuring the availability of good quality fish seed all year round and the sustainability of the aquaculture industry (Olumuji and Mustapha 2012). The success of artificial propagation of *C. gariepinus* through induced breeding under controlled environmental conditions is mostly dependent on the latency period, humidity and temperature. It gives better rates of fertilization, hatchability and survival (Agbebi *et al.*, 2013). According to Agbebi *et al.* (2013), the latency period is described as the time interval between injections of the female fish and the stripping of eggs.

There are some other factors to be considered

simultaneously with the latency period. They include water temperature, types of hormones and dosage of hormones. Water temperature; the optimum temperature to keep the brood stock of *C. gariepinus* after injection of hormones is 29.5°C and the fish was ready in about 14 hours (Agbebi *et al.*, 2013). Akankali *et al.* (2011) stated that a number of physiochemical factors play a decisive role in ovulation and that temperature is of vital importance.

There is a decrease in the viability of eggs during hatching due to over ripening, poor hatchability, fertilization and survival as a result of wrong stripping periods and these results in loss of quality in gonadal products resulting in deformed fry and fingerlings. Observing latency has been a problem for breeders due to haste and demand for income from sellers of fingerlings (Esa *et al.* 2023).

Since aquaculture is a fast growing sector in Nigeria, to sustain a high rate of increased production of fish fingerlings for stocking, the latency period of post-ovulation of fish has to be put into consideration. By so doing the fish farmers would be able to prevent loss of eggs during fish breeding exercise which could be as a result of over-ripening and under-ripening of eggs. Hence there was availability of quality and quantity of fingerlings of *C. gariepinus* for stocking aquaculture system. The aim of the work is to evaluate the effects of the latency period on the fertilization, hatchability, survival and growth of *C. gariepinus*.

## MATERIALS AND METHODS

The experiment was carried out in the Department of Fisheries, Adamawa State University, Mubi, Adamawa State. Adamawa State University, Mubi, is situated at latitude 10.27°N, and longitude 13.26°E. The hatchery unit of the department has a well-organized set-up with quality and sufficient water. The experiment was carried out during the rainy season that was in September 2018. Rainy season lasts from April to September.

### Sources of broodstock

A complete randomized design was used for this experiment. Gravid females as well as matured males of *Clarias gariepinus* were purchased from a private fish farm in Mubi, Adamawa State. The broodstock was approximately fourteen (14) months old; the female weighed 900 g each while the male weighed 1 kg. The age of the broodstock was determined because they were purchased from a private fish farm in Mubi. The broodstock was transported from the fish farm to the experimental site with the use of modified Jerry cans. A total of fifteen (15) females and three (3) males were used for the experiment. The matured female broodstock was determined through the swollen reddish genital opening, while the males had projected genital papillae that were reddish at the tip with a gentle massage. The broodstock was acclimatized in

large bowls containing 20 litres of water for two (2) days. Each of the broodstock was weighed and recorded before the commencement of the experiment.

### Hormone injection

Broodstock was injected with Ovaprim hormone to induce ovulation. Only the female was injected. The dosage of Ovaprim for each female was 0.5 ml per kilogram of fish and was injected during early morning hours; that is, 6 am, 8 am, and 10 am, (Table 1). This was to make the counting of eggs and the removal of unfertilized eggs easier.

### Fertilization

Injected female brooders were removed from their respective troughs after two (2) hours intervals starting from a latency period of 9 hours, 11 hours, 13 hours, and 15 hours respectively. The eggs were then stripped into dry bowls and eggs were collected from each sample into labelled bowls for easy identification. Testes of the male brooders were removed by sacrificing the male through dissection. The milt was then collected by laceration of the testes with a clean (sterile) razor blade into 25 ml of normal saline in a Petri dish. The milt was then used to fertilize each treatment by mixing both the egg and milt with a plastic spoon after adding the equivalent volume of distilled water.

### Incubation

Immediately after fertilization, each of the replicates was incubated in a well aerated 7 cm x 49 cm x 49 cm trough containing 20 litres of water. To maintain oxygen, water was flowing in and out of the trough.

The observed eggs were removed from each trough after eight hours. This was done by siphoning out the dead (unfertilized) eggs which appeared whitish. The percentage (%) fertilization was estimated (Agbebi *et al.*, 2013) as;

$$\% \text{ Fertilization} = \frac{\text{number of fertilized eggs}}{\text{Total number of eggs incubated}} \times 100$$

### Feeding rates and methods

#### Percentage hatchability

Several hours (9,11,13,15 and 17 hours) after the incubation processes, hatching began. The time interval for the hatching of eggs varied with replicates. The hatched larvae were counted while the unhatched eggs were discarded. The percentage hatchability was estimated as;

**Table 1.** The experimental design for the injection of the female breeders and their time.

Replicates	Treatment (hours)				
	09	11	13	15	17
1	6 am	8 am	10 am	12 pm	2 pm
2	6 am	8 am	10 am	12 pm	2 pm
3	6 am	8 am	10 am	12 pm	2 pm

**Table 2.** Reproductive and survival performance of *C. gariepinus* under different latency periods.

Indices	Latency Periods				
	9 hours	11 hours	13 hours	15 hours	17 hours
Weight of Male brooders* (g)	1200 <sup>a</sup>	1000 <sup>ab</sup>	900 <sup>b</sup>	900 <sup>b</sup>	800 <sup>b</sup>
Weight of Female brooders (g)*	1000 <sup>a</sup>	800 <sup>ab</sup>	766.67 <sup>bc</sup>	516.67 <sup>cd</sup>	366.67 <sup>d</sup>
Weight of Testes (g)	1.5 <sup>b</sup>	2.0 <sup>b</sup>	1.0 <sup>b</sup>	6.0 <sup>a</sup>	2.0 <sup>b</sup>
No. of eggs	8470 <sup>a</sup>	7058.33 <sup>b</sup>	8470 <sup>a</sup>	6917.33 <sup>b</sup>	8470 <sup>a</sup>
No. of fertilized eggs	574.33 <sup>c</sup>	8401.5 <sup>a</sup>	8279 <sup>a</sup>	6873 <sup>a</sup>	2856.33 <sup>b</sup>
% Fertilization	5.05 <sup>c</sup>	99.19 <sup>a</sup>	97.66 <sup>a</sup>	99.33 <sup>a</sup>	33.72 <sup>b</sup>
No. of Unhatched eggs	0.0 <sup>d</sup>	69 <sup>c</sup>	193.33 <sup>b</sup>	40.67 <sup>c</sup>	5612.67 <sup>a</sup>
No. of Hatchlings	0.0 <sup>d</sup>	4483.5 <sup>a</sup>	5397.67 <sup>a</sup>	2840 <sup>b</sup>	169.67 <sup>c</sup>
% Hatchability	0.0 <sup>c</sup>	53.36 <sup>b</sup>	65.19 <sup>a</sup>	47.85 <sup>b</sup>	1.99 <sup>c</sup>
No. of Survival	0.0 <sup>e</sup>	4252.5 <sup>a</sup>	1086.3 <sup>b</sup>	634 <sup>c</sup>	59.67 <sup>d</sup>
% Survival	0.0 <sup>d</sup>	77.61 <sup>a</sup>	13.07 <sup>c</sup>	11.34 <sup>c</sup>	58.63 <sup>b</sup>

Means with different superscript are significantly different ( $p < 0.05$ ).

$$\% \text{ Hatchability} = \frac{\text{number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

The fry that hatched was weighed at 1 mg. Feeding commenced seventy two (72) hours after hatching, Artemia was used to feed the fry for fourteen (14) days and later with 0.2 mm of commercial feed (Coppens).

#### Daily percentage survival value

The duration of this experiment was twenty-one (21) days (three weeks). Daily survival was achieved through the visual counting of the fry using a magnifier (hand lens) on a daily basis. At the end of three weeks, the remaining fry was counted and their percentage was estimated as;

$$\% \text{ Survival} = \frac{\text{No. of fry at the end of the study}}{\text{No. of fry at the beginning of the study}} \times 100$$

#### Growth monitoring

The bulk weight and the length of the fish were recorded after 3 days, 7 days and 21 days to determine the growth performance. This was carried out in the laboratory using the meter rule and analytical balance.

#### Water parameters

The following water parameters were monitored during the experiment. These were pH, temperature, dissolved oxygen and ammonia.

#### Data analysis

Data obtained from the experiment was subjected to line graphs and one-way analysis of variance (ANOVA) using statistical software SPSS version 20. The differences among means were determined using least significant differences (LSD) at a 95% confidence level ( $p < 0.05$ ).

## RESULTS

The reproductive performance of male and female *C. gariepinus* brood stock is shown in Table 2. The best weight of testes (6.0 g) was in 15 hours latency period while the least was in 13 hours (1.0 g). The highest quantity (fecundity) was recorded in 11 hours latency period (8,467) and the least was in 9 hours latency period. There was a significant difference ( $p < 0.05$ ) in the weight of testes and number of eggs respectively. The percentage of fertilization was highest in 11 hours and 15 hours latency periods (99.19% and 99.33%) respectively. The percentage

**Table 3.** Correlation indices  $r^2$  of the latency periods of *C. gariepinus* breeding.

	9 hours	11 hours	13 hours	15 hours	17 hours
9 hours	1				
11 hours	0.50699	1			
13 hours	0.619451	0.942349	1		
15 hours	0.664411	0.932176	0.98474	1	
17 hours	0.78031	0.474422	0.582826	0.629408	1

**Table 4.** Correlation of each of the reproductive indices of *Clarias gariepinus* bred at different latency periods.

	Weight of Male brooders	Weight of Female brooders	Weight of Milt sac	No. of eggs	No. of fertilized eggs	% Fertilization	No. of Unhatched eggs	No. of Hatchlings	% Hatchability	No. of Survival	% Survival
Weight of Male brooders	1										
Weight of Female brooders	0.901361	1									
Weight of Milt sac	-0.24727	-0.46374	1								
No. of eggs	0.074368	0.140348	-0.72671	1							
No. of fertilized eggs	-0.41927	-0.10802	0.193619	-0.57247	1						
% Fertilization	-0.44454	-0.18325	0.363165	-0.65891	0.983846	1					
No. of Unhatched eggs	-0.60509	-0.72832	-0.1508	0.415248	-0.38648	-0.40086	1				
No. of Hatchlings	-0.22974	0.147021	-0.0246	-0.38775	0.952075	0.904076	-0.52758	1			
% Hatchability	-0.26875	0.05892	0.184924	-0.51423	0.968177	0.958147	-0.56383	0.977044	1		
No. of Survival	0.053698	0.240333	-0.11126	-0.5957	0.675892	0.600795	-0.35969	0.647451	0.585027	1	
% Survival	-0.37635	-0.33664	-0.17709	-0.28939	0.291425	0.217425	0.438555	0.139639	0.06092	0.67488	1

hatchability was highest in 13 hours latency period (63.73%) while the least was in 17 hours latency period (1.99%).

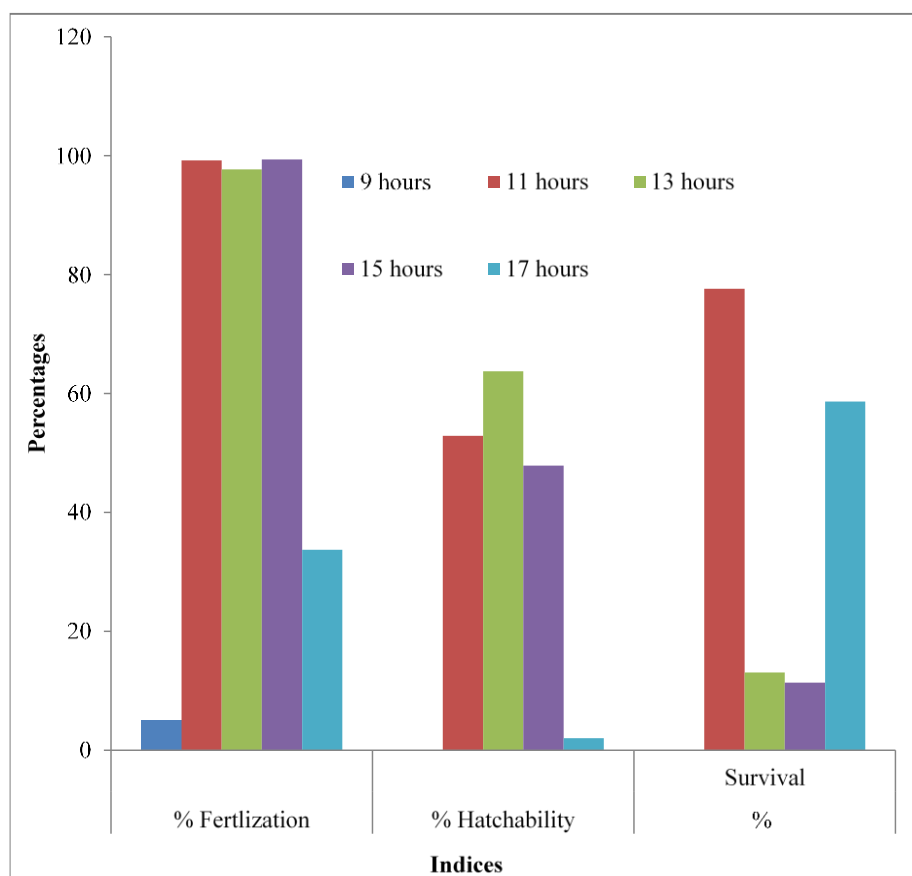
Unhatched eggs were highest in 17 hours latency period (5,612) and the least was in 11 hours (69). There was a significant difference ( $p < 0.05$ ) in percentage hatchability.

The correlation indices of the latency period of reproductive performance of *C. gariepinus* were presented in Table 3. Thirteen (13) hours latency

period having  $r = 0.9423$  ( $p < 0.05$ ) indicated a significant association between latency period and reproductive performance of *C. gariepinus* while a latency period of 17 hours indicated a low association  $r = 0.474422$ ; ( $p < 0.05$ ) which is not significantly different ( $p > 0.05$ ).

Table 4 shows correlations between the different reproductive indices. The association between the weight of male and female brooders showed  $r^2 = 0.901301$ ; which indicated a significant

association between the weight of the male and female brooders. Similarly, the association between percentage fertilization and hatchability  $r^2 = 0.95814$ ; this indicated that there was a significant association between percentage fertilization and hatchability. The association between percentage hatchability and number of survivals  $r^2 = 0.585027$  and indicated a significant association between hatchability and number of survivals.



**Figure 1.** Fertilization, hatchability and survival percentages of *Clarias gariepinus* bred at different latency periods.

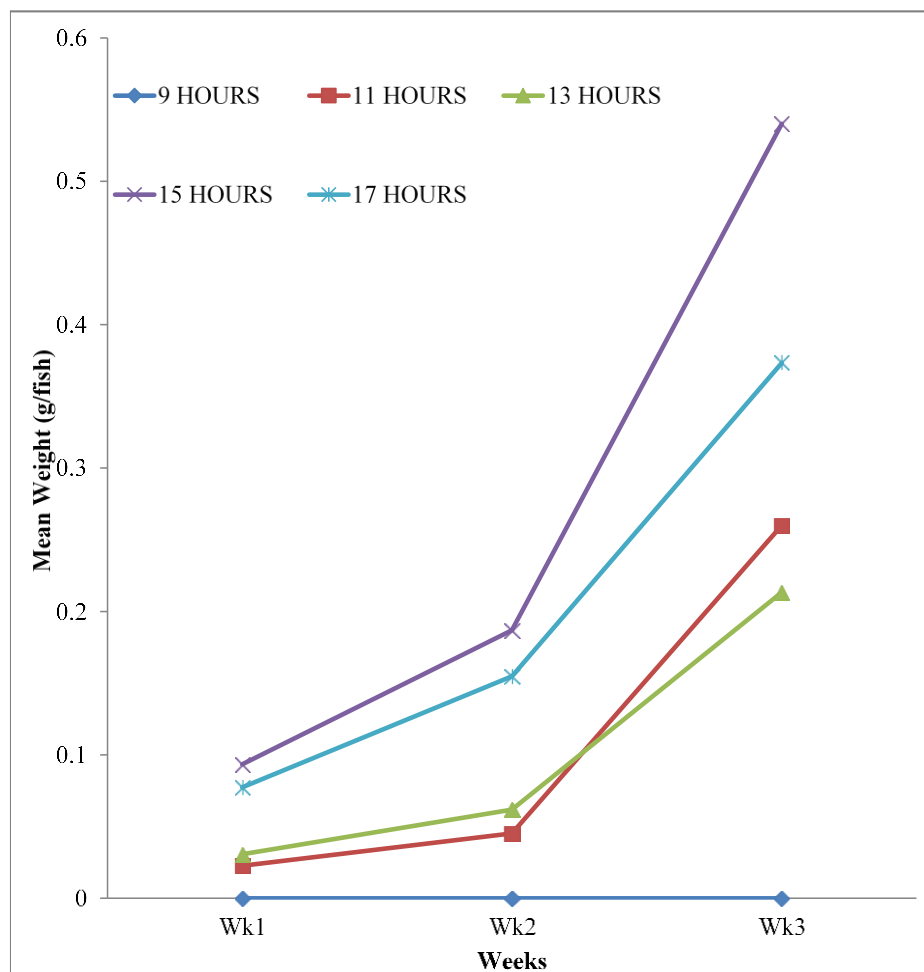
Percentage fertilization, hatchability and survival are also presented in Figure 1. The weight of *C. gariepinus* after three weeks of indoor culture (Figure 2), showed that 15 hours latency period had the highest weight gain, than 17 hours and 11 hours respectively while 13 hours had the lowest weight.

## DISCUSSION

The weight of the broodstock in this study was in agreement with the findings of Olumuji and Mustapha (2012), who reported that *C. gariepinus* became mature from 200 g body weight. The highest number of hatchlings (539.67) recorded in the latency period of 13 hours may be due to the weight of the female (766.67 g) in line with Viveen *et al.* (1985) who stated that larger females contain more eggs. Agbebi *et al.* (2013) recorded 7050 hatchlings for 14 hours latency period and 3880 hatchlings for a latency period of 12 hours. The number of eggs obtained in this study 1 g (847) was higher than the report of Shinkafi and Ilesami (2014) who reported 657 for the same species but were injected with different hormones. The hormone used in this study was Ovaprim (0.5 ml) but in the

case of Sahoo *et al.* (2007) Ovotide of more than 0.5 ml (0.10 - 0.25 ml) was used at a latency period of 8 hours. Ndeham *et al.* (2018) had 1,204 and 1,021 in 1 g of *C. gariepinus* eggs, which was higher than the result obtained in this study.

The 97.66% fertilization recorded for a latency period of 13 hours in this study might be due to a delay of 13 hours post-injection and was higher than the one reported by Shinkafi and Ilesami (2014), which was 85% for a latency period of 8 hours. Agbebi *et al.* (2013) also reported 70% fertilization at 14 hours. At 11 hours latency period, 99.19% fertilization was also recorded which was higher than that of Agbebi *et al.* (2013) who reported 45% fertilization for 10 hours latency period. The difference between the two results above might be due to one hour's interval between the latency periods and the weight of the fish. At 15 hours latency period, the percentage of fertilization (99.33%) was higher than that of Shinkafi and Ilesami (2014). The lowest percentage of fertilization recorded in a latency period of 17 hours might be due to over-ripening of eggs as in high latency periods that could result in poor fertilization (Oyelese, 2006), who had 5.51% fertilization in 9 hours latency period. This might be due to early stripping. Eggs stripped early will result in loss of eggs or yield poor



**Figure 2.** Weekly weight of *Clarias gariepinus* fry from different latency periods.

fertilization (FAO, 2018).

The higher percentage hatchability of 63.73% at a latency period of 13 hour obtained from this study is slightly in agreement with Shinkafi and Ilesami (2014) who reported 62% at a latency period of 18 hours, 52.44% at the same hour by Agbebi *et al.* (2013). However, zero hatchability was obtained in 9 hours latency period. This might be due to under ripening of eggs that resulted in low fertilization (5.05%) and is in agreement with FAO (2018). Ataguba *et al.* (2023) recorded 77% and 62% fertilization and hatching in *C. gariepinus*. while Mosha and Mling (2018) had the highest percentage of fertilization and hatchability using 4 mg/kg pituitary extract.

Survival of 77.61% was obtained in a latency period of 11 hours. This might be due to the weight of the female given yield to better fertilization, hatchability and survival. This is in agreement with Abdulraheem *et al.* (2012) and Tiogue *et al.* (2020) recommended a latency period of 10 hours for *C. gariepinus*. Agbebi *et al.* (2013) reported that 10 – 14 hours will give better fertilization, hatchability and survival. From this study, the latency period of 11 hours was unarguably the best latency period for *C. gariepinus*.

At any period lower than that, there will be insufficient action of the hormonal treatment leading to failed ovulation (Tan-Fermin *et al.*, 1997).

The weekly weight of *C. gariepinus* fry from different latency periods in this study indicated a post-injection period of 15 hours to have a better weight compared to other latency periods. The post-injection period of 17 hours also gave a better weight after three weeks of indoor culture. This is in line with FAO (2018) which reported 0.52 g weight of hatchlings after two weeks.

## Conclusion

From the results of this research, *C. gariepinus* response after 11 hours post-injection gave the best results with reference to the number of eggs, percentage fertilization, hatchability and survival. Therefore, the present study showed that *C. gariepinus* of mean body weight 367 to 1000 g can successfully be induced with 0.5 ml of Ovaprim to obtain eggs and hatchability of good quality at a latency period of 11 hours.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## ACKNOWLEDGEMENT

We thank the Adamawa State University, Mubi for sponsoring this research and Dr Emmanuel Ngadina for his assistance during the period of preparation for this research.

## REFERENCES

- Abdulaheem, I., Otubusin, S. O., Agbebi, O. T., Olowofeso, O., Alegbeleye, W. O., Abdul, W. O., Adeyemi, K., Ashley-Dejo, S. S. & Nathaniel, B. (2012). The growth response of *Clarias gariepinus* hatchlings to different dry feeds. *Journal of Agricultural Science*, 4(10), 75-80.
- Agbebi, O.T., Faseluka, O and Idowu, A.A. (2013). Effects of various latency periods on the fertilization, hatchability and survival of *Clarias gariepinus*. *Journal of Fisheries and Aquatic Science*, 8(1), 178-183.
- Adah, P. M., Onyia, L. U., & Obande, R. A. (2013). Fish hybridization in some catfishes: A review. *28th Annual Conference of the Fisheries Society of Nigeria (FISON)*. Pp. 220-223.
- Akankali, J. A., Seiyaboh, E. I., & Abowei, J. F. N. (2011). Fish Breeding in Nigeria. *International Journal of Animal and Veterinary Advances*, 3(3), 144-155.
- Akinrotimi, O. A., Gabriel, U. U., Owhonda, N. K., Onunkwo, D. N., Opara, J. Y., Anyanwu, P. E., & Cliffe, P. T. (2007). Formulating an environmentally friendly fish feed for sustainable aquaculture development in Nigeria. *Agricultural Journal*, 2(5), 606-612.
- Ataguba, G. A., & Angela, A. (2023). Hybridization and Growth Performance of Progeny from Crosses between *Clarias gariepinus* and *Heterobranchus* sp. *Aquaculture Studies*, 24(1), Article number 1154.
- Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S., & Bichi, A. H. (2018). African catfish aquaculture in Malaysia and Nigeria: status, trends and prospects. *Fisheries and Aquaculture Journal*, 9(1), 1-5.
- Esa, Y. B., Dadile, A. M., Syukri, F., Christianus, A., & Diyaware, M. Y. (2023). Evaluation of fecundity, fertilization, hatching, and gonadosomatic index of exotic *Clarias gariepinus* (Burchell, 1822) and native *Clarias macromystax* (Gunther, 1864) under semi-arid conditions of Nigeria. *Animals*, 13(11), Article number 1723.
- FAO (2018). The State of Food Security and Nutrition in the World, Rome.
- Mosha, S. S., & Mlingi, F. T. (2018). Effects of different catfish pituitary gland extract dosages on eggs and hatchlings quantity of African catfish, *Clarias gariepinus* at a constant latency period. *Journal of Aquaculture Research and Development*, 9(3), Article number 526.
- Ndeham, V. R., Ladu, B. M. B., & Onyia, L. U. (2018): Comparison of fertilization, hatchability, growth, and survival of *Clarias gariepinus* from Adamawa and Katsina States. *Advances in Life Science and Technology*, 53,1-7.
- Olumuji, O. K., & Mustapha, M. K. (2012). Induced breeding of African mud catfish, *Clarias gariepinus* (Burchell 1822), using different doses of normal saline diluted Ovaprim. *Journal of Aquaculture Research and Development*, 3(4), 133.
- Oyelese, O. A. (2006). Fry survival rate under different anoxic conditions in *Clarias gariepinus*. *Research Journal of Biological Sciences*, 1(1-4), 88-92.
- Sahoo, S. K., Giri, S. S., Chandra, S., & Sahu, A. K. (2007). Spawning performance and egg quality of Asian catfish *Clarias batrachus* (Linn.) at various doses of human chorionic gonadotropin (HCG) injection and latency periods during spawning induction. *Aquaculture*, 266(1-4), 289-292.
- Shinkafi, B. A., & Ilesanmi, B. D. (2014). Effect of varying doses of ovotide on the breeding performance of African catfish (*Clarias gariepinus* Burchell, 1822) in Sokoto, North-western Nigeria. *Asian Journal of Animal Sciences*, 8(2), 56-64.
- Tan-Fermin, J. D., Pagador, R. R., & Chavez, R. Z. (1997). LHRA and Pimozoid-induced spawning of ancient catfish: *Clarias macrocephalus* (Gunther) at different times during an annual reproductive cycle. *Aquaculture*, 148, 323-331.
- Tiogué, C. T., Nyadjeu, P., Mouokeu, S. R., Tekou, G., & Tchoupou, H. (2020). Evaluation of hybridization in two African catfishes (Siluriformes, Clariidae): Exotic (*Clarias gariepinus* Burchell, 1822) and Native (*Clarias jaensis* Boulenger, 1909) species under controlled hatchery conditions in Cameroon. *Advances in Agriculture*, Volume 2020, Article ID 8985424, 11 pages.
- Viveen, W. J. A. R., Richter, C. J. J., Van-odt, P. G., Jansen, J. A. L., & Huisman, E. A. (1985): Practical manual for the culture of the African Cat Fish (*Clarias gariepinus*). Section for Research and Technology, Box 20061, 5600EB. The Hague, The Netherlands, Page 121.