HATCHING SUCCESS OF *Clarias gariepinus* EGG USING DIFFERENT INCUBATING SUBSTRATES AND VARYING EGG AREA DENSITY

SOLOMON S.G., *OKOMODA V.T., ATAGUBA G.A. & Q. O. IKWUTA
Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Nigeria
*Correspondence: okomodavictor@yahoo.com, +234 80333 19959

ABSTRACT
This study evaluated the efficiency of different substrates and best egg area density for hatching Clarias gariepinus egg. The different substrates used were Polyvinyl chloride (PVC) frame with fine mesh (1 mm x 1mm) net, fine mesh net, sack thread and the control was without a substrate. Result obtained shows that eggs hatched on PVC frame with fine mesh net and ordinary fine mesh net had higher hatchability (79.40% and 77.55% respectively) compared to eggs hatched on sack (64.75%) and the control bowl without a substrate (20.70%). However, screening efficiency of un-hatched eggs from the hatched eggs were better using PVC frame with fine mesh net compared to other forms of substrate used. Based on the efficacy of PVC frame with fine mesh net substrate, this substrate was then used for the next study which was designed to determine the best area density for optimum hatching; increasing egg-mesh ratio (estimated in gcm^{-2} i.e. mass of egg to the mesh area) namely, 0.58, 1.16, 1.74, 2.31, 2.85, 3.47,4.05, 4.64 and 5.52 was used for this study.The result revealed that beyond 4.05 area density, hatchability was significantly reduced (below 50%). Hence, for higher hatching success of African catfish, PVC frame with fine mesh net accompanied with egg area density not greater than 4.05gcm^{-2} is recommended.
Keywords: Artificial breeding, African catfish, area density, hatchability, fertilization.

INTRODUCTION

Aquaculture production is becoming more and more intensive; this is because production from capture fisheries has reached its maximum possible potential and catch per unit effort is dwindling with each passing day (Gabriel *et al.,* 2007). Artificial propagation of fish is the most promising and reliable way of ensuring availability of good quality fish seeds all year round hence ensuring sustainability of the aquaculture industry. It involves the use of natural (hypophysation) or synthetic hormones to induce ovulation and spawning in fined fishes (Olumuji and Mustapha 2012), hence, enabling the hatchery operator to set their time table for fish spawning (Kamthorn and Jim *et al*., 2006).

One of the most important aquaculture candidates in Africa is *Clarias gariepinus.* The family Clariidae is endemic to Africa and ranges from North Africa, through the mid-Sahara and covering both East and West down to the South (Teugels, 1986). *C. gariepinus* adapt well to artificial environments, and has rapidly gained status as premier aquaculture species (Britz and Hetch 1989; Hetch, *et al*., 1996). *Clarias* spp have a rapid growth, a high reproductive potential and sturdy resistance to environmental variations, hence, their widespread distribution. The potential for culture of Clarias is enormous. The African catfish naturally spawns in floodplains during wet season in response to changes in water levels (Pillay, 1990). Seed collection from the wild is however unreliable and limited only to rainy seasons. However, under culture conditions, ovulation of the species is induced either by environmental manipulation and/or hormonal stimulation. Catfish seed production has varying degree of success due to the high mortality of eggs and larvae (Clay, 1977; Msiska, 1981; and Hogendoorn, 1990). The hatching rates are usually as low as between 50-70% in well-managed hatcheries of developed countries (de Graaf *et al*., 1995). However breeding failure has been attributed to biological (broodstock size and age, strain and species) and environmental (dissolved oxygen, pH, temperature, stocking density, photoperiod etc.) factors (Ataguba *et al.,* 2012, Ataguba *et al.,* 2013). To mitigate these low hatching problems, research has focused on the suitability of the environment and biological factors as it affect breeding success. Oyelese (2006) demonstrated hatching success in relation to temperature, while Silva *et al*. (2003) reported effect of water hardness on breeding parameters of fish. Handling stress and health status of female broodfish as biological indices have been reported to be of great importance in the reproductive performance of fish (Muchlisin *et al*., 2006 and Aiyelari *et al*., 2007). Preliminary studies by Ataguba *et al.* (2012) and Ataguba *et al*. (2013) revealed that increasing broodstock size cause significant increase in hatching success, however, there is paucity of information on the effect of different substrate technology and varying egg area density on breeding performance of the African catfish. These factors are under the direct control of the fish breeder with potential effect on breeding successes. Fish farmers

sometimes use unsuitable incubation substrates, such as mud, sand or concrete despite the poor results. Many hardly take into consideration egg area density during incubation of eggs for reasons ranging from lack of suitable and adequate facility to variation in fish's fecundity and lack of knowledge of potential effect of egg area density on hatchability. This study was therefore designed to evaluate best artificial substrate technology for catfish hatching and to recommend appropriate egg area density that will ensure higher hatching of this extensively important aquaculture species of Africa.

MATERIALS AND METHODS

Twenty broodstock (mean weight of 850 g) of the same breeding history reared for approximately fifteen months were obtained from the fisheries research farm of the University of Agriculture, Makurdi Benue state Nigeria. The broodstocks where separated by sex and acclimatized for two weeks in concrete tanks at the Hatchery unit of the Department of Fisheries and Aquaculture, Makurdi. Broodstocks were fed with 35% Crude protein in pelleted feed administered daily at 5% of their body weight.

Breeding was performed according to the method described by Ataguba *et al.,* 2012, and Ataguba *et al.,* 2013. Males were used to fertilize three females. The milt was poured on the eggs in a dry bowl and stirred using feather The feather was then used to spread the eggs on the substrate as determined by the different research outline.

Experiment 1: Hatching success of *Clarias gariepinus* egg using different substrate

This experiment was designed to investigate different hatching substrate as it affect hatchability of eggs and screening efficiency of un-hatched eggs, PVC frame with fine mesh (1 mm x 1mm) net, sack thread and fine mesh net were the substrate used for this study while the control of the study was without a substrate, the substrate were evaluated for hatching successes and suitability in screening hatched from un-hatched egg.

Experiment 2: Effects of egg density on hatching success of African catfish

This experiment was carried out using the best substrate in the first experiment to evaluate the effect of varying egg-mesh ratio on hatching success of African catfish i.e. the number of meshes on the substrate were counted to establish an increasing egg-mesh ratio for the experiment, the following egg-mesh ratio were used; 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1 and 4.5:1.

Determination of water quality parameters

Dissolved oxygen in each experimental bowl was monitored using the dissolved oxygen test kit. Temperature was determined using mercury in glass thermometer, pH, conductivity and total dissolved solids (TDS) were determined using the multi-parameter water checker.

STATISTICAL ANALYSIS

Descriptive statistics were obtained using Minitab® edition 14. Mean data were subjected to analysis of variance and where significant differences were occurred ($P < 0.05$) means were separated using least significant difference (LSD).

Parameters measured

$$fertilization = \frac{spawn\ egg - dead\ egg}{spawn\ egg} x100$$

$$hatchability = \frac{hatched\ egg}{fertilized\ egg} x100$$

Screening efficiency: this was estimated using two parameters designed for the study

$$\%hatchability\ on\ substrate = \frac{Wt\ of\ hatchling\ on\ substrate}{Wt\ of\ all\ content\ on\ substrate} x100$$

Where weight (*Wt*) of all content on substrate indicates both hatched and un-hatched egg that are retained on substrate.

$$\%hatchability\ in\ bowls = \frac{Wt\ of\ hatchling\ in\ bowl}{Wt\ of\ all\ content\ in\ bowl} x100$$

Where weight (Wt) of all content in bowl indicates both hatched and un-hatched egg that passed through the substrate into the bowl.

$$\%Dead\ eggs\ in\ bowls\ or\ chamber = (100\% - hatchlings\ in\ bowl\ or\ chamber)$$

RESULTS

Tables 1 and 2 shows the nature of eggs on substrate as observed during incubation period and some water quality parameters of the medium used to incubate the eggs. Panelist who assessed the egg during incubation concluded that egg spawn on PVC frame with fine mesh net gave an excellent view of eggs as compare to other substrates. Acidic conditions were observed for water in all incubation substrates with the least acidic being the fine mesh net (pH 6.10) followed by the sack substrate (pH 5.60) while the control was most acidic (pH 4.90). Dissolved oxygen

was highest in water with the fine mesh (4.80 $mg.l^{-1}$), 3.50 $mg.l^{-1}$ for water with sack substrate and 2.10 $mg.l^{-1}$ for water with control substrate.

Screening efficiency of substrate and hatchability of egg spawn on the different substrates is given in Table 3. Result reveals Hatchability to be higher in eggs incubated on PVC frame with fine mesh Net (79.40%) and lowest in eggs in the control treatment (20.70%), while efficiency of screening un-hatched eggs was also highest using the PVC frame with fine mesh Net (90% hatchling in bowls) compared to any other substrate (80%, 44.2% and 14.48% for fine mesh net, sack substrate and control respectively).The second study as presented in Table 4 reveals hatchability of eggs to significantly reduce beyond egg area density of 3.5:1 (Below 50% hatchability). More so water quality parameter in hatching bowls with different egg area density reveals (Table 5) lower level of dissolved oxygen and pH as egg density increase while conductivity however increases with increase in egg density.

Table 1: Assessment of hatching substrate during incubation

	Control	Fine mesh net	PVC frame with fine mesh Net	Sack strands
Ease of observation	Good	Good	Excellent	Poor
Nature of eggs on strands	Clustered	Scattered	Scattered	Un-observed

Table 2: Physico-chemical parameters of water in hatching bowls with different hatching substrate

	Control	**Fine mesh net**	**PVC frame with fine mesh Net**	**Sack strands**	**P- Value**
pH	4.90 ± 0.01^{c}	6.1 ± 0.04^{a}	6.0 ± 0.2^{a}	5.6 ± 0.01^{b}	0.01
Dissolved oxygen	2.1 ± 0.12^{c}	4.8 ± 2.01^{a}	4.5 ± 0.6^{a}	3.5 ± 1.5^{b}	0.001
Temperature	27.2±0.42	27.1±2.1	27.3±1.1	27.0±0.15	0.12
Conductivity	$250.2\pm0.2.21^{a}$	244.3 ± 0.21^{c}	243.3 ± 1.3^{c}	249.0 ± 1.6^{b}	0.012
Total dissolved solids	129.6 ± 0.11^{b}	114.1 ± 1.21^{c}	118.4 ± 0.5^{d}	131.1 ± 2.2^{a}	0.012

Mean in the same row with different superscripts differ significantly ($P < 0.05$)

Table 3: Hatching performance of egg spawn on different substrate

	No substrate (Control)	**Fine mesh net**	**PVC frame with fine mesh (size: 1 mm x 1 mm) net**	**Sack strands substrate**	**P- Value**
Fertilization rate	96.5±0.5	96.5±1.5	97.5±0.5	97.0±2.0	0.934
% dead eggs in bowls	87.95 ± 5.65^{a}	19.45 ± 1.05^{c}	8.70 ± 26^{d}	55.8 ± 10.2^{b}	0.002
% hatchlings in bowls	14.48 ± 1.02^{d}	80.55 ± 1.05^{b}	91.30 ± 2.60^{a}	$44.2\pm10.1\pm^{c}$	0.002
% hatchlings on substrate	-	15.15 ± 3.25^{b}	12.10 ± 3.70^{c}	28.61 ± 2.81^{a}	0.009
% hatchability	20.70 ± 1.2^{c}	77.55 ± 1.6^{a}	79.40 ± 2.10^{a}	64.75 ± 5.35^{b}	0.001

Mean in the same row with different superscripts differ significantly ($P < 0.05$)

Table 4: Hatching performance of *Clarias gariepinus* egg using varying egg area density

Treatment (Trt)	Egg area density ($g\ m^{-2}$)	Egg mesh ratio	Egg mass (g)	Fertilization (%)	Hatchability (%)
Trt 1	0.58	0.5:1	7.5 ± 0.001^{i}	84.5±0.5	52.00 ± 4.00^{a}
Trt 2	1.16	1:1	15 ± 0.003^{h}	87.0±2.0	54.9 ± 0.34^{a}
Trt 3	1.74	1.5:1	22.5 ± 0.001^{g}	86.0±1.0	54.89 ± 0.67^{a}
Trt 4	2.31	2:1	30 ± 0.001^{f}	86.5±0.5	52.3 ± 13.7^{a}
Trt 5	2.85	2.5:1	37 ± 0.002^{e}	86.5±1.5	54.0 ± 5.74^{a}
Trt 6	3.47	3:1	45 ± 0.01^{d}	83.0±1.0	52.34 ± 5.23^{a}
Trt 7	4.05	3.5:1	52.5 ± 0.01^{c}	85.5±0.0	51.33 ± 0.61^{a}
Trt 8	4.64	4:1	60 ± 0.01^{b}	80.1±0.6	40.15 ± 0.12^{b}
Trt 9	5.20	4.5:1	67.5 ± 0.01^{a}	83.0±0.2	30.73 ± 1.00^{c}
P-value	-	-	0.001	0.06	0.001

Mean in the same row with different superscripts differ significantly ($P < 0.05$)

Table 5: Water quality parameter of culture medium with different egg area density

Treatment (Egg area density)	pH	Dissolved Oxygen	Temperature	NH_3	Conductivity	TDS
Trt 1 (0.58)	5.55 ± 0.12^{a}	4.2 ± 0.21^{a}	27.02±0.2	0.20 ± 0.12^{e}	232±0.11	117±0.11
Trt 2 (1.16)	5.57 ± 0.20^{a}	4.0 ± 015^{b}	27.1±0.3	0.19 ± 011^{e}	247±0.13	212±0.2
Trt 3 (1.74)	5.50 ± 0.01^{a}	4.1 ± 0.41^{b}	26.9±0.2	0.19 ± 0.01^{e}	256±0.01	119±1.3
Trt 4 (2.31)	5.49 ± 0.12^{a}	3.8 ± 2.1^{c}	27.01±0.6	0.28 ± 1.0^{e}	258±1.5	123±0.41
Trt 5 (2.85)	5.48 ± 042^{a}	3.5 ± 2.1^{d}	27.3±1.1	0.25 ± 0.1^{e}	250±0.15	122±0.20
Trt 6 (3.47)	5.45 ± 2.21^{a}	3.3 ± 0.21^{e}	27.13±1.3	0.33 ± 0.91^{d}	259.0±1.6	119±0.15
Trt 7 (4.05)	5.40 ± 0.11^{a}	3.3 ± 1.00^{e}	27.10±0.2	0.44 ± 0.00^{c}	259.1±2.2	123±0.10
Trt 8 (4.64)	5.00 ± 0.11^{b}	2.4 ± 0.92^{f}	27.01±0.1	0.54 ± 0.12^{b}	261.4±0.2	131±0.60
Trt 9 (5.52)	4.10 ± 0.11^{c}	1.7 ± 2.1^{d}	27.01±0.1	0.68 ± 0.2^{a}	270.0±0.1	139±0.12
P- value	0.05	0.05	0.992	0.05	0.231	0.09

Mean in the same row with different superscripts differ significantly ($P < 0.05$)

DISCUSSION

Research on suitability of substrate for hatchability is very scarce and to our knowledge this studies represent the first attempt to investigate the effect of varying egg area density on the hatching success of *Clarias gariepinus* egg. Fertilization rate of eggs in both study were statistically same across the different treatments applied in both study ($P>0.05$); this observation may be due to the fact that egg for the treatments in each study came from same source of broodstock and werefertilized with the sperm from same milt, hence suggesting that fertilization is not affected by egg density or differences in substrate but largely due to the biological factor such as broodstock viability and gamete quality (McAndrew *et al.* 1993). This position is also supported by Ataguba *et al*. (2013) who reported significant difference in fertilization of eggs from different broodstock combinations by weight. Tiamiyu *et al*. (2015) also reported significant differences in fertilization of eggs from broodstocks administered serially diluted ovaprim® hormone with saline water and coconut water. It can therefore be concluded that differences in fertilization are largely a result of biological characteristics of fish used to spawn hence the wide differences in the fertilization recorded in the first study (96-97%) compared to the second study (84-87%) as they were from different broodstocks and spawn at different time. Despite same fertilization rate recorded across the treatments hatchability of eggs incubated on PVC with fine mesh net substrate were significantly higher in the first study, this may largely be due to higher screening efficiency recorded for the substrate compared to other substrates, it is assumed that hatched eggs were suffocated amiss surrounding bad

egg in substrate with poor screening efficiencies, this was evident in observed flagellates noticeable on some of the dead egg, the observation of flagellates on dead eggs were more pronounced in the second experiment beyond 2.31gm^{-2} density despite using substrate of better screening efficiency. However this did not affect hatchability percentage until 4.64 gm^{-2} density and beyond where hatchability significantly reduced. Hatchability recorded in this study is similar to the finding of de-Graaf *et al*. (1995) for the breeding of *C. gariepinus* in the republic of Congo (59%) and higher than Macharia *et al*. (2005) report of 4% hatchability as worst case scenario of *C. gariepinus* eggs on a nylon substrate. The mean water quality parameters obtained in the studies indicated fouling in treatment with low hatchability as elevated level of ammonia were recorded. The level of NH_3 observed in this study was at a range of 0.19 mgL^{-1}to 0.68mgL^{-1} which is not detrimental to tropical fish as reported by Stone and Thomforde (2004). However, significant reduction in oxygen is likely the cause of mortality and lower hatchability recorded in this study. Generally under the same experimental conditions (i.e. atmospheric temperature, humidity, artificial aeration and same water source) water quality of experimental units are likely to have similar value for parameters such as temperature and dissolved oxygen, however, increased fouling caused by increase in the number of dead eggs must have led to increased biological oxygen demand, hence, reducing the dissolved oxygen significantly despite aeration. It is concluded that PVC with fine mesh net substrate is efficient for better hatching of African catfish egg. More so, egg area density should not be beyond 4.05gm^{-2} for optimum spawning performance.

REFERENCES

Aiyelari, T. A., Adebayo, I. A., and Osiyemi, A.S. (2007); reporting fitness of stressed and steroids. Fish Physiol. Biochem. 14, 237-246. *Animal Biology*, 1, 5, 78-81.

Ataguba G.A., Okomoda V.T. and Onwuka M. C. (2013): Relationship between broodstock weight combination and spawning success in African catfish (*Clarias gariepinus*). *Croatian Journal of Fisheries* Vol.71 (4) Pp 176-181. Retrieved December 15th 2013, from

Britz P.J., and Hecht T., (1989), Effect of salinity on growth and survival of African sharptooth catfish (*Clarias gariepinus*) larvae, *Journal of Applied Ichthyology*, 5: 1994-2002 http://dx.doi.org/10.1111/j.1439-0426.1989.tb00492.x

deGraaf G.J., Galemoni F., and Banzoussi B., (1995), The artificial reproduction and fingerling production of the African catfish *Clariasgariepinus* (Burchell 1822) in protected and unprotected ponds, Aquaculture Research, 26: 233-242 http://dx.doi.org/10.1111/j.1365-2109.1995.tb00908.x

Ataguba G. A. Solomon S. G. and Onwuka M. C. (2012). Broodstock Size Combination in artificial spawning of cultured *Clarias gariepinus*. *Livestock Research for Rural Development*. Vol 24(12).www.Irrd.org/Irrd24/12atag24223

Gabriel, U. U., A. O. Akinrotimi, P. E. Anyanwu, D. O., Bekibele and D. N. Onunkwo (2007). Locally produced fish feed; potential for aquaculture development in Africa. *Journal of Agriculture.* 20(10):536-540.

Hetch, T.; Oellermann, L.; Verheust, L. (1996). Perspectives and clariid catfish culture in Africa. In: Legendre, M. and Proteau, J–P. (eds.). The Biology and Culture of Catfishes. *Aquatic Living Resources*. Paris, , 9, Hors Series. 197–206.

Hogendoorn, H. (1990) Controlled propagation of the African catfish *Clarias lazera* (C&V). III. Feeding and growth of fry. *Aquaculture*, 21: 233-241.

Kamthorn P. and Jim M. (2006) manual on catfish Hatchery and production 163 (125 moo 6f. Ramallrd.Samae – dumbanglehoontien Bangkok 10150. Thailand. Aquaculture and inland fisheries project.

Macharia S.K., Ngugi C.C., and Rasowo J., (2005), Comparative study of hatching rates of African cat fish (*Clarias gariepinus* Burchell 1822) Eggs on different substrates, NAGA, World fish Center Quarterly, 28(3/4): 23-26

McAndrew, B.J, Rana, K.J. and Penman, D.J. (1993).Conservation and preservation of genetic variation in aquatic organisms. In: Muir, J.F. and Roberts R.J. (Eds.), *Recent advances in Aquaculture* Vol. 4. Blackwell Science, Oxford, UK pp. 295-336.

Olumuji O. K. and Mustafa, J (2012). Induced breeding of African mud catfish, *Clariasgariepinus*(Burchell) (1822) using different Doses of Normal saline Diluted ovaprin. *Journal of Aquaculture Research and development* Vol 3 (4): 1-4.

Oyelese, O. A. (2006): Water Temperature a determinant of fertilization and Hatchability rates in artificially induced Breeding and *Clarias gariepinus* (Teleosstei: Clariidae). *Research Journal of Biological Sciences*, 1, 1-4, 83-87.

Pillay, T.V.R. (1990). Aquaculture Principles and Practices. Fishing News Books, London. Statgraphics Ver.2.1 (1994) Statistical Graphics *Corporation*, Maryland, USA

Silva, L. V. F., Golombieski, J. I., Baldisserotto, B. (2003). Incubation of silver catfish, *Rhamdia quelen* (Pimelodidae), eggs at different calcium and magnesium concentrations. *Aquaculture*, 172, 3-4, 279-287.

Stone, N.M. and Thomforde H.K.. (2004). Understanding your fish pond water analysis report. FSA9090-PD-7-04N. University of Arkansas Cooperative Extension Service, Little Rock, Arkansas. 4pp

Teugels, G.G., (1986). A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). Ann. Mus. R. Afr. Centr., Sci. Zool., 247:199 p.

Tiamiyu, L. O., Okomoda V. T., Oyeniyi M. E. and Aperegh J.K (2015). Spawning performance of *Clarias gariepinus* administered serially diluted doses of ovaprim. *Banat's Journal of Biotechnology*. Vol. 11 (1) pp 30-35.