

EFFECT OF SPAWNING METHODS ON FERTILIZATION, HATCHABILITY AND FRY SIZE VARIATION IN *Clarias gariepinus*

*¹Oyebola, O.O. and ²Awodiran, M.O.

Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria¹.

Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria²

Correspondence emails: olusegun.oyebola@yahoo.com

Telephone: 234-803-3950-321

(Received: 12th December, 2014; Accepted: 15th December, 2014)

ABSTRACT

Investigation on the effects of separating and pooling gametes on fertilization, hatchability and size variation in hatched fry of *Clarias gariepinus* was carried out. Four matured females and four males of *C. gariepinus* 1.2±0.2 kg) was used to generate the experimental lines. Three replicates of 10 g each of pooled and un-pooled spawned eggs (Fp and Fs-maternal) obtained from 3 females were fertilized with (1 ml each) milt from a male donor. Similarly, three replicates of 1 ml each of pooled and un-pooled milt (Mp and Ms -paternal) obtained from 3 males were used to fertilize 10 g each of spawned eggs from a female donor. Data were obtained on fertilization, hatchability and frequency of shooters at 3 weeks of fry rearing. Data on coefficient of variability was compared among treatments. Fertilization, hatchability and shooters frequency ranged between 13.5±5.90% (Mp) and 56.6±11.63% (Fs); 2.3±1.11% (Mp) and 26.0±6.23% (Fs) and 5.9±0.38% (Ms) and 19.5±3.92% (Fp) respectively. The rate of fertilization and hatchability were significantly higher ($p < 0.05$) while shooters frequency was low in all treatments of un-pooled gametes to the pooled treatments. Coefficient of variability showed that paternal and maternal treatments significantly affected variability of hatchability and frequency of shooters in the pooled treatments. Un-pooling egg masses in spawns of *C. gariepinus* enhanced fry production as it facilitated comparative higher fertilization, hatchability; reduction in shooters frequency and size variation in the spawned fry population.

Keywords: *Clarias gariepinus*, spawn, shooters frequency, fry production

INTRODUCTION

Aquaculture has been accepted the world over as a means for increasing fish production. Aquaculture production has been growing at a rate that exceeded that of capture fisheries and other animal food production systems (FAO, 2003). In Nigeria, catfish production accounts for 70 percent of the fish production from aquaculture (Williams *et al.*, 2008). The catfish (family Clariidae) is very popular in Nigeria due to its adaptable culture characteristic which has endeared it to many fish farmers. *Clarias gariepinus* is the most popular fish of culture in Nigeria and most fish farmers undertake its production.

The popularity of culture has paved way for an increased demand for high quality fish seed culminating in sporadic increase in fish hatchery in Nigeria. Despite increased demand, production in fish hatcheries is still low. Possible reasons for low fry production include poor broodstock management, inappropriate spawning techniques, poor brood-stock nutrition and high fry mortality

(Salama, 1996). Poor broodstock productivity and optimization of spawning techniques are outstanding constraints upon commercial fry production and its future expansion. The menace of growth variants (shooters) in bred population of cultured species is an outstanding problem in fish hatcheries as this limits yield (Baras and d'Almedia, 2001). Growth heterogeneity has been associated with cannibalism and therefore with mortality (Baras and d'Almedia, 2001; Baras and Jobling, 2002). Cannibalism which is a major problem under aquaculture conditions (Portz *et al.*, 2006; Fessehaye *et al.*, 2006; Ashley, 2007) has been reported in the larvae and juvenile of many important culture species such as the Nile tilapia (Fessehaye *et al.*, 2006); Arctic charr, *Salvelinus alpinus* (Svenning and Borgström, 2005), and sharptooth catfish (Baras and d'Almeida, 2001). The cannibalistic tendency of the faster growing individuals on the slower growing ones has attendant negative effect on yield and final remuneration from aquaculture industry (Uka *et al.*, 2005). Management of growth variants by sorting out of the shooters is laborious, increases

cost of production, and induce stress on the population which often results in fry mortality and may not result in compensatory growth in the remaining small size fish (Baardvik and Jobbling, 1990; Kamstra, 1993 and Sunde *et al.*, 1998)

Pooling spawned eggs or milt from broodstocks is often used in generating a batch of fish seeds. Pooling of breeding materials from different females or males could generate heterogeneous progeny population with variable results in production units. There is need to have understanding of the effect of pooling genetic resources on the general variability in fertilization, hatchability and frequency of production of fast growers in the population. The information obtained could be used in the programming of reproductive development in the fish to produce reliable numbers of fry required in grow-out farms. The study therefore is aimed at assessing fertilization, hatchability and fry size variation in spawned *C. gariepinus* under conditions of pooled and un-pooled gametes from the broodstocks.

MATERIALS AND METHODS

Description of the Experimental Site

The experiment was carried out in the Fish Laboratory, Department of Animal Production, College of Agricultural Sciences, Yewa Campus, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

The Experimental Fish

Four matured females and males each of *Clarias gariepinus* (1.2 ± 0.2 kg) obtained from a commercial fish farm in Abeokuta, Ogun State, Nigeria were utilized for the study. The stocks were selected following FAO (1996) description for matured *C. gariepinus* brooder.

Experimental Design

Spawning was induced in the females through intramuscular injection of the synthetic hormone, Ovaprim (Western Chemical Inc. Femdale, WA) at the rate of 0.5 ml/kg of fish. The brooders were subsequently stripped of the egg mass after latency period of 12 ± 0.15 hours. The spawned eggs were fertilized with milt from the male donors to generate four experimental lines:

paternal pooled (pooled milt from different males), paternal un-pooled (separate milt from the same different male), maternal pooled (pooled eggs from different female) and maternal un-pooled (separate egg from the same different females) treatments.

Paternal pooled and Un-pooled (single) Lines

Males (3) were sacrificed to generate milts. Two (2) ml of the milt was collected from each of the 3 males and separated to two equal half (1 ml each) in different Petri dishes. Three out of the 6 milt portions were mixed together to produce 3 ml of the pooled milt which was later divided to 3 equal parts as replicate of the pooled milt in separate Petri-dishes. The remaining 3 Petri dishes represent replicates of un-pooled milt. Sixty (60) g of eggs obtained from a female donor was divided to 6 equal portions (10 g each of egg mass) and were then fertilized by the pooled and unpooled milt.

Maternal pooled and Un-pooled (single) Lines

Twenty (20) g of stripped eggs were generated from each of the 3 female broodstock to produce the female reproductive materials needed for the experiment. Each of the stripped eggs from each of the females was separated to two equal halves (10 g each) in different Petri dishes. Ten (10) g of eggs obtained from each of the female broodstock was mixed together to produce 30 g of pooled egg which was later subdivided into 3 equal parts. The remaining 3 Petri dishes contained 10 g each of un-pooled eggs. Six (6) ml of the milt which was obtained from a sacrificed male donor was divided to 6 equal portions and were used to fertilize each of the 3 pooled and 3 unpooled egg batches. Figures 1 and 2 shows a schematic drawing of how the respective paternal and maternal lines were generated.

Incubation and Hatching Procedures

The fertilized eggs were incubated in twelve (12) plastic incubation tanks (56.5 x 40 x 24.5 cm) each containing a batch of fertilized eggs representing the treatments and the replicates. Eggs were incubated at room temperature (25°C) while a water flow-through system was maintained throughout the incubation period. Hatching was

concluded at 30 hours post-fertilization while the hatched fry were separated after clean-up.

Determination of Fertilization and Hatchability

Total number of incubated eggs was estimated following FAO (1996). The greenish egg mass at the 12th hour of incubation which was taken as the fertilized eggs. This was estimated following Omitoyin *et al.* (2011). Percentage fertilization and hatchability were determined for all the treatments. Percentage of fertilization in eggs was calculated as follows:

$$\% \text{ Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{No of incubated eggs}} \times 100 \text{ percentage}$$

Number of hatchlings was obtained by direct counting of the fry in each tank after clean up. Percentage hatchability was also calculated using the formula:

$$\% \text{ Hatchability} = \frac{\text{No. of hatchlings}}{\text{No of fertilized eggs}} \times 100 \text{ percentage}$$

Determination of Frequency of Shooters

The incubation tanks were cleaned and restocked with 72 hours post hatchlings with completed yolk sac absorption per treatment and reared for 21 days. During the rearing period, decapsulated *Artemia nauplis* was fed to the fry for 7 days followed by Coppens ® *ad libitum*. Pellet sizes were changed every 7 days during the experimental feeding which lasted for 21 days. A water flow through system was maintained throughout the rearing period and water quality of the rearing environment was maintained at a pH of 6.5, temperature at 25.0 - 26.1 °C and dissolved oxygen at 4.0 – 4.5 mg/L. At the expiration of the rearing period, the fry in each treatment were sorted into different size categories designated as: extra large size growers (shooters-S), average sized growers (A) and slow growers (runt-R) as described by Nwadukwe and Nana (2000). Size grading at 21 days rearing period was picked to coincide with the reported greatest size variation in *Clarias gariepinus* fry (Martins, 2005). Number of fry and the weight of individuals in each size group was determined. Weights were measured using digital weight balance (Falcon, Bl 3002). Percentage of shooters was calculated as

$$\% \text{ Shooters} = \frac{\text{No. of shooters}}{\text{No of stocked fry}} \times 100 \text{ percentage}$$

Statistical Analysis

Fertilization, hatchability and frequency of shooters were presented as percentages while One-way ANOVA (Analysis of Variance) was used to compare the means between the treatments. The values obtained were further analyzed for coefficient of variability. SPSS windows Evaluation version 15.2 software was employed for the analysis.

RESULTS AND DISCUSSION

Spawning techniques have significant influence on the parameters studied. Mean values for fertilization, hatchability and shooters frequency ranged between 13.5±5.90% (Mp) and 56.6±11.63% (Fs); 2.3±1.11%(Mp) and 26.0±6.23%(Fs), and 5.9±0.38% (Ms)-19.5±3.92% (Fp) (Table 1). The values recorded implied a generally low percentage fertilization and hatchability in all the groups. Fertilization and hatchability were significantly higher (P<0.05) with un-pooled cases of paternal and maternal spawns and in the pooled and unspooled female spawns. The generally low values of the indices in all the groups as well as higher values for female spawns compared to the male spawns is probably indicating differential quality of the genetic materials of the utilized broodstocks.

Reproductive success in fish species has been shown to be influenced by factors such as: the broodstock sex ratio, stocking density, age, size, nutrition and feeding regime (Ridha and Cruz, 1989; Smith *et al.*, 1991; Salama, 1996; Izquierdo *et al.*, 2001; Chong *et al.*, 2004; Tahoun, 2007; Hammouda *et al.*, 2008 and Ibrahim *et al.*, 2008). The brood-stock quality is affected by the nutritional status (Kondowe and Eiriksson, 2003) which in turn affects the quality of eggs, which ultimately determines the hatchability of the eggs and larvae survival (Silverstein and Hershberger, 1992; De Silva and Anderson, 1995). The significantly higher fertilization and hatchability in the un-pooled treatments in both maternal and paternal spawns probably indicated superiority of the un-pooled gametes over pooling, either male or female gametes in *C. gariepinus*. Also,

differences in gamete quality of the parents broodstock used for generating the paternal and maternal groups could have significant influence on fertilization and hatchability in the maternal groups (Fs and Fp) compared to paternal groups (Ms and Mp).

The major problem in catfish production schemes is growth heterogeneity and cannibalistic tendency of faster growing individuals (Shooters-S) and its attendant negative effect on yield and final remuneration from the industry (Uka *et al.*, 2005). Gamete variation significantly affected the shooters heterogeneity, frequencies and the weight. Lower frequency of shooters was recorded in the fry population with un-pooled spawn. Coefficient of variability of fertilization, hatchability and frequency of shooters is shown in Table 2. Apart from percentage fertilization, the

other parameters in both paternal and maternal spawns showed greater variability for pooled gametes compared to the un-pooled. The coefficient of variation also showed comparatively low size heterogeneity occurred under un-pooled spawns. Apart from increasing fertilization and hatchability and reducing shooters frequency, un-pooling the gametes for spawning however reduced variability and induced production of relatively uniform sized fry especially within the shooter's grades. Growth heterogeneity has been associated with cannibalism and therefore with mortality (Baras and Almedia, 2001; Baras and Jobling, 2002). Un-pooling gametes for spawning therefore is expected to assist in reducing degree of heterogeneity and cannibalism and ultimately mortality in *C. gariepinus* fry populations.

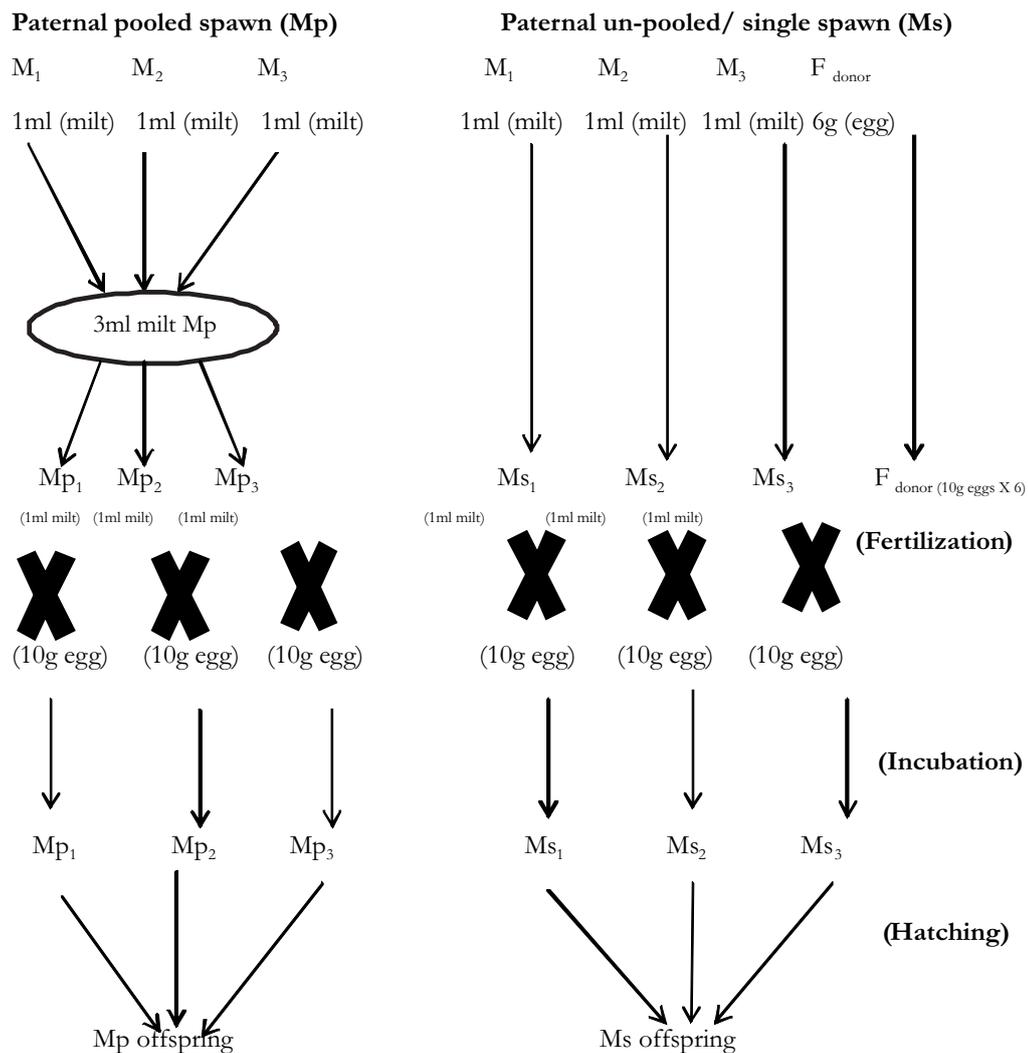


Figure 1: A schematic diagram showing paternal pooled and un-pooled spawns
 * Mp = pooled milt, Ms = single milt, M= male F= female

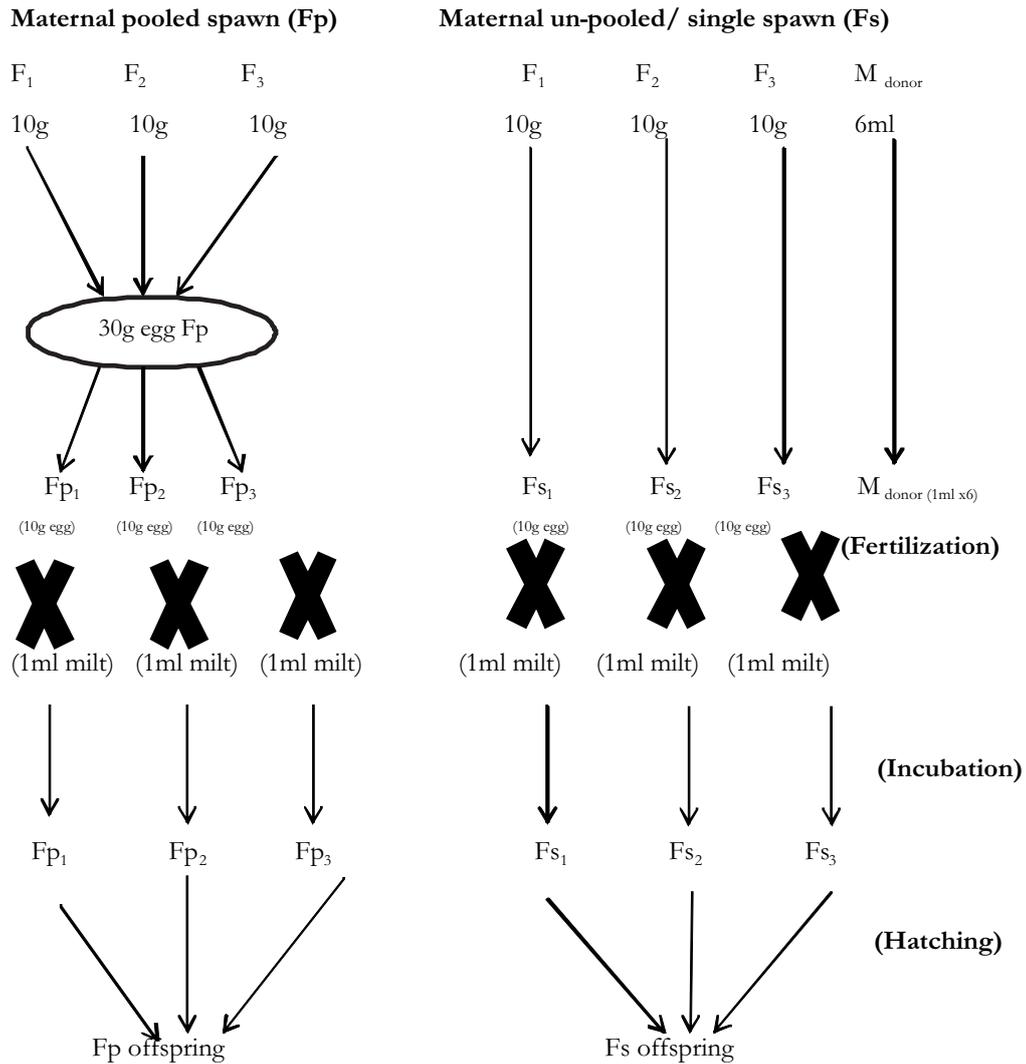


Figure 2: A schematic diagram showing maternal pool and single spawns
 * Fp = pooled eggs, Fs = un-pooled/single eggs, M= male F= female

Table 1: Fertilization, Hatchability and Shooters Frequency in the Groups Studied

Groups	Fertilization (%)	Hatchability (%)	Shooter's frequency (%)	Shooter's Weight(g)
Fs	56.6±11.63 ^a	26.0±6.23 ^a	13.1±2.32 ^b	3.20±0.25
Fp	46.9±5.58 ^b	19.6±12.80 ^b	19.5±3.92 ^c	3.48±1.02
Ms	23.2±14.87 ^c	8.3±11.61 ^c	5.9±0.38 ^a	4.21±0.03
Mp	13.5±5.90 ^d	2.3±1.11 ^d	6.8±0.49 ^a	3.52±0.81

*Means with the same superscript along columns are not significantly different (p>0.05).

* Fs=Egg from single female fertilized by milt from single male; Fp=Pooled egg from females fertilized by milt from single male; Ms= milt from single male fertilized egg from single female; Mp= pooled milt fertilized egg from single female

Table 2: Coefficient of Variability (CV measured in %) Mean Values of Percentage Fertilization, Hatchability and Shooters Frequency in the Groups Studied

Groups	Fertilization	Hatchability	Shooters frequency
Fs	20.5	23.9	17.8
Fp	11.9	65.3	20.2
Ms	64.1	39.9	6.4
Mp	43.6	48.9	7.2

* Fs=Egg from single female fertilized by milt from single male; Fp=Pooled egg from females fertilized by milt from single male; Ms= milt from single male fertilized egg from single female; Mp= pooled milt fertilized egg from single female

REFERENCES

- Ashley, P.J., 2007. Fish welfare: current issues in aquaculture. *Applied Animal Behavioural Science* 104: 199-235.
- Baardvick, B.M. and Jobbling M. 1990. Effect of size sorting on biomass gain and individual growth rates in Arctic, *Salvelinus alpinus* L. *Aquaculture* 90: 11-16.
- Baras, E. and d'Almeida, A.F. 2001. Size heterogeneity prevails over kinship in shaping cannibalism among larvae of sharptooth catfish *Clarias gariepinus*. *Aquatic Living Resources* 14: 251-256.
- Baras, E. and Jobling, M. 2002 Dynamics of intracohort cannibalism in cultured fish. *Aquaculture Research* 33: 7: 461-479.
- Chong, A. S. C; Ishak, S. D; Osman. Z. and Hashim. R. 2004. Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture* 234: 381-392.
- Fessehaye, Y; Kabir, A; Bovenhuis H. and Komen, H. 2006. Prediction of cannibalism in juvenile *Oreochromis niloticus* based on predator to prey weight ratio, and effects of age and stocking density. *Aquaculture* 255: 314-322.
- Food and Agriculture Organization (FAO). 1996. Artificial production and pond rearing of the African catfish *Clarias gariepinus* in sub-Saharan Africa: a handbook FAO Fish Technical Paper 362. Rome.
- FAO, 2003. Aquaculture Production Statistics 1988-1997, Rome, Food and Agriculture Organization (FAO).
- Hammouda, Y. A. F; Ibrahim, M. A-R; Zaki El-Din, M. M. A; Eid, A. M. S; Magouz, F. I. and Tahoun, A. M. 2008. Effect of dietary protein levels and sources on reproductive performance and seed quality of Nile tilapia *Oreochromis niloticus* (L.) broodstock. *Abbassa International Journal for Aquaculture* 1(A): 55-78.
- Ibrahim, M.A-R; Hammouda, Y. A. F; Zaki, M. M. El-Din, A. Eid, . A. M. S, Magouz, F. I and Tahoun, A. M. 2008. Effect of dietary protein levels and sources on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.) broodstock. *Abbassa International Journal for Aquaculture* 1(B): 251-274.
- Izquierdo, M. S; Fernandez-Palacios, H. and Tacon, A. G. J. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197: 25-42.
- Kamstra A. 1993. The effect of size grading on individual growth in eel, *Anguilla anguilla* Measured by individual marking. *Aquaculture* 112: 67-77.
- Kondowe, M. and Eiriksson, G.M. 2003. Effect of protein level in broodstock diet on egg size of *Oreochromis shiranus*. *Aquaculture Fish Technical Paper*. 2: 26-29.
- Martins, C. I. M. 2005. Individual variation in

- growth of African catfish *Clarias gariepinus*: a search for explanatory factors (2005), PhD thesis, Wageningen University, The Netherlands, ISBN: 90-8504-306-9.
- Nwadukwe, F. O. and Nana, I.G. 2000. Effect of rearing periods on the production of hybrid catfish (*Clarias gariepinus* × *Heterobranchus longifilis*) fingerling. *Journal of Aquatic Sciences* 15: 23-26.
- Omitoyin, B.O; Ajani, E.K. and Oyebola, O.O. 2011. Cold shocking at 5°C and 7°C in triploid production: effects on embryonic development, hatchability and fry performance in *Clarias gariepinus*. *African Journal of Livestock Extension* 9: 13-18.
- Portz, D.E; Woodley, C.M., and Cech Jr., J.J. 2006. Stress associated impacts of short-term holding on fishes. *Reviews Fish Biology* 16: 125-170.
- Ridha, M. and Cruz. E. M. 1989. Effect of age on the fecundity of the tilapia *Oreochromis spilurus*. *Asian Fishery Science* 2 (2): 239-247.
- Salama, M. E. 1996. Effects of sex ratio and feed quality on mass production of Nile tilapia, *Oreochromis niloticus* (L.), fry. *Aquaculture Resources* 27 (8): 581-585.
- Smith, S. J; Watanabe, W.O; Chan J.R; Ernst, D.H, Wicklund, R. I. and Olla, B. L. 1991. Hatchery production of Florida red tilapia seed in brackish-water tanks: the influence of broodstock age. *Aquaculture Fishery Management* 22 (2): 141-147
- Sunde, L.M., Inslund A.K., Folkvord A., Setfansson S.O. (1998). Effect of size grading on growth and survival of juvenile turbot at two temperatures. *Aquaculture International* 6: 19-32.
- Svenning, M.A. and Borgström, R. 2003. Cannibalism in Arctic charr: do all individuals have the same propensity to be cannibals? *Journal of Fish Biology* 66: 957-965.
- Tahoun, A. M. A. 2007. Studies on some factors affecting the production and reproduction of Nile tilapia. Ph. D. Thesis, University of Kafr El-sheikh, Egypt. 325pp.
- Tahoun, A.M, Ibrahim, A.R, Hammouda, Y.F, Eid, M.S, Zaki E.1 and Magouz, F.I. 2008. Effects of age and stocking density on spawning performance of Nile tilapia, *Oreochromis niloticus* broodstock reared in hapas. *8th International Symposium on Tilapia in Aquaculture* at the Central Laboratory for Aquaculture Research, Cairo Egypt pp 329-343.
- Uka, A, Bashir, R. M. and Akogun, S. O. 2005. Size variations at different ages in *Clarias gariepinus* and *Clarias gariepinus* × *Heterobranchus longifilis* hybrid. African Regional Aquaculture Centre, Nigerian Institute for Oceanography and Marine Research, Port Harcourt, Rivers State, Nigeria. *Journal of Aquatic Sciences* 20 (2): 85-88.
- Williams, S.B; Olaosebikan, B.D; Adeleke, O.A. and Fagbenro, O.A. 2008. Status of African catfish farming in Nigeria. In: Ponzoni, R. W. and N. H. Nguyen (eds) 2008. *Proceedings of a Workshop on the Development of a Genetic Improvement Program for African Catfish Clarias gariepinus*. World Fish Centre Conference Proceedings Number 1889. The World Fish Centre, Penang, Malaysia, pp 130.