Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.16, 2014

The Effect of Environmental and Nutritional Manipulation On Year-Round Gonadal Development, Spawning And Recrudescence Of Female *Clarias Gariepinus* Broodfish.

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ABSTRACT

A study was carried out to determine the influence of environmental and nutritional manipulation on cyclical gonadal development, spawning and recrudescence of female pond- bred *Clarias gariepinus* broodfish. This was aimed at proposing a substitute to chemical/hormonal stimulation for year- round gonadal development and proffering a simple adaptive, cost efficient and effective method of year-round fingerling production, for sustainable Catfish production. A total of 400 hundred adult catfish, weighing between180 and 250grams, measuring between17cm and 20cm were stocked in replicate experimental and control earthen ponds. In the experimental treatment, fishes were fed 40% Crude protein diet, the pond water continually pumped in and the level maintained at 30 cm, whereas water introduction and level in the control pond was dependent on nature (rainfall) and the fishes fed on natural food organisms from the pond waters. Fish were sampled randomly for the 12 months duration, for gonadal development, spawning and recrudescence using standard qualitative and quantitative methods. Qualitative, macro-morphologic assessment showed that, from June to November, both simulated and non-simulated fish groups exhibited a gradual improvement in gonadal development, from Immature Stage I to Fully matured stage IV, and then running ripe Stage V. By November, they all had Spent/Resting Stage VI. This was supported by the quantitative assessment-Gonadosomatic index (GSI), which rose gradually from May, peaking twice followed by a drop in October/November. This trend of gonadal development is natural as fish gonadal development commences and reproduction occurs during the rainy season, and ends as the dry season approaches (Clay, 1979; Legendre, 1986 and Fruend *et al*, 1995). Subsequently, from November up until January, the non-simulated fish group exhibited mainly the Spent/Resting Stage VI gonads, followed by a gradual improvement from Immature Stage I in February to Maturing Stage II in May. Hyder (1970) and Sikoki (1978) reported that reproductive activity is poor when favorable conditions do not persist. Contrarily, from November, up until May, the simulated fish groups were observed to continually exhibit a steady and stable supply of ready-to-spawn Fully mature Stage IV, and Running ripe Stage V. This was supported by high GSI values of the simulated fish groups as opposed to a drastic drop in GSI of non-simulated fish groups. Subsequently, GSI values of both fish groups showed a gradual rise from February to May, however, the simulated fish groups exhibited higher GSI. In conclusion, the simulated fishes had improved gonadal development as also supported by statistical data (probability of 0.01%). Thus, it is recommended that, further research be carried out on this method, to standardize it for year-round fingerling production and supply, as other forms of stimulants are either in short supply, complex to use for some, too expensive; and even the world at large is going GREEN, and avoiding the use of chemical or biological manipulation/ genetic multiplication of Food organisms.

Key Words: Environmental and Nutritional Manipulation, *Clarias gariepinus* brood-fish, Gonadal development, Simulated fish, Non-simulated fish.

1. INTRODUCTION

Increasing population in most countries especially Africa is not accompanied by increasing food production. While quantity of food is already creating considerable concern, the quality of food consumed is even more critical. The protein-calorie balance is inadequate. The African catfish *Clarias gariepinus,* like other fishes, are known to be highly nutritious, having a high protein content of 69.9-70.4% (Hecht *et al*, 1996), and a high proportion of poly-unsaturated fatty acid (Guha, 1991), trace elements (Kuhnan, 1991 and vitamins A and D) (Combs, 1991) and B (Guha, 1991). They are also highly acceptable to consumers as they possess a palatable fish with excellent meat quality, no intra-muscular bones.

Also the African catfish have high economic potentials as they are widely accepted by consumers in many countries, they are good aquacultures species due to their unique attributes (hardiness (Hecht, 1972), opportunistic feeders (Clay, 1979), tolerate high environmental extremes (Hecht *et al,* 1996) as they possess a unique air breathing structures (Goos and Richter, 1996)) and also, they could be sold in various forms (smoked, fresh-frozen or filleted) thus attracting good prizes.

However, the fingerlings of *Clarias gariepinus* are not available all year round, as the fishes are naturally seasonal spawners, spawning only during the wet season. A central problem for continuous unrestricted, year-round fingerling production of *C. gariepinus* naturally, is the non-progression or seasonal character of gondal activity of the fish (Richter and Vanden Hurk, 1982; Freund *et al*, 1985). This is due to the absence of a pre-ovulatory gonadotropin surge (Sundararaj, 1981; Janssen *et al*, 1995) in the non-breeding dry season.This has made regular and large scale production of *Clarias gariepinus* a mirage

Secondly, controlled propagation techniques that are involved in the mass propagation of this species to bridge the gap in seed supply by providing chemical and biological stimulants/hormones (Janssen, 1985; Huisman, 1986; van Oordt and Goos 1987) is yet to achieve success because of issues such as, cost and availability of hormones, technology transfer, acceptance and adaptation by the artisanal/poor subsistence fish farmers, accessibility to fish farmers and most importantly, the need to produce food organically as inorganic-compounds used will inevitably find its way back into the bodies of the consumers. This has led to a global move towards "Green Food Production", a global thrust towards natural simulation of reproduction for year-round seed production.

Natural spawning technique by environmental manipulation was initiated by Woynarovich and Hovarth (1986) and Davy and Chouinard (1989)have been renewed by knowledge gained in more intensive systems (deGraaf and Jansen, 1996) and modified severally to successfully reproduce *Ictalurus punctatus* (Legendre *et al*, 1996); *Clarias batrachus, C. hurruchus, and C. macrocephalus (*Areerat *1987; and* Khan *et al, 1990); Silurusglanis (*Legendre *et al,*1996);*Chrysichthys nigrodigitatus (*Hem1986,Hem *et al,* 1994 Oteme 1993) among others. Also, environmental manipulation technique has been used to reproduce *C. gariepinus* in Congo (de Graaf and Jansen, 1996), in South Africa (De Kimpe and Micha, 1974) and in Israel-(Richter, 1976). Areerat *et al (*1987) carried out environmental and nutritional manipulation on *C. macrocephalus, C. hurruclus and C. batrachus* and obtained high success rates in Asia. There is paucity of information on natural reproduction stimulated by a combination of environmental and nutritional stimulation on the African catfish, *Clarias gariepinus.*

2. MATERIALS and METHODS

2.1 The Study Area

The study was carried out at the African Regional Aquaculture (A.R.A.C), Aluu, near Port Harcourt in Rivers State (fig.1). The area is a low land area, with a major dry (October to April) and wet (May to September) seasons, followed by a brief drier Harmathan period (December to January). The area has minimal vegetation cover. Facilities in the centre were utilized for the work.

2.2 Experimental Design

The experiment was carried out in earthen ponds for a period of twelve months, from June to May of the next year. The study was designed to compare the simulated fish group A, fed 40% crude protein diet and the environment manipulated; with the non-simulated control fish group B, raised under natural environmental condition, and without supplemental diet. All treatments were carried out in duplicates using four 110m^2 earthen ponds, randomly assigned to the two treatments and their replicates.

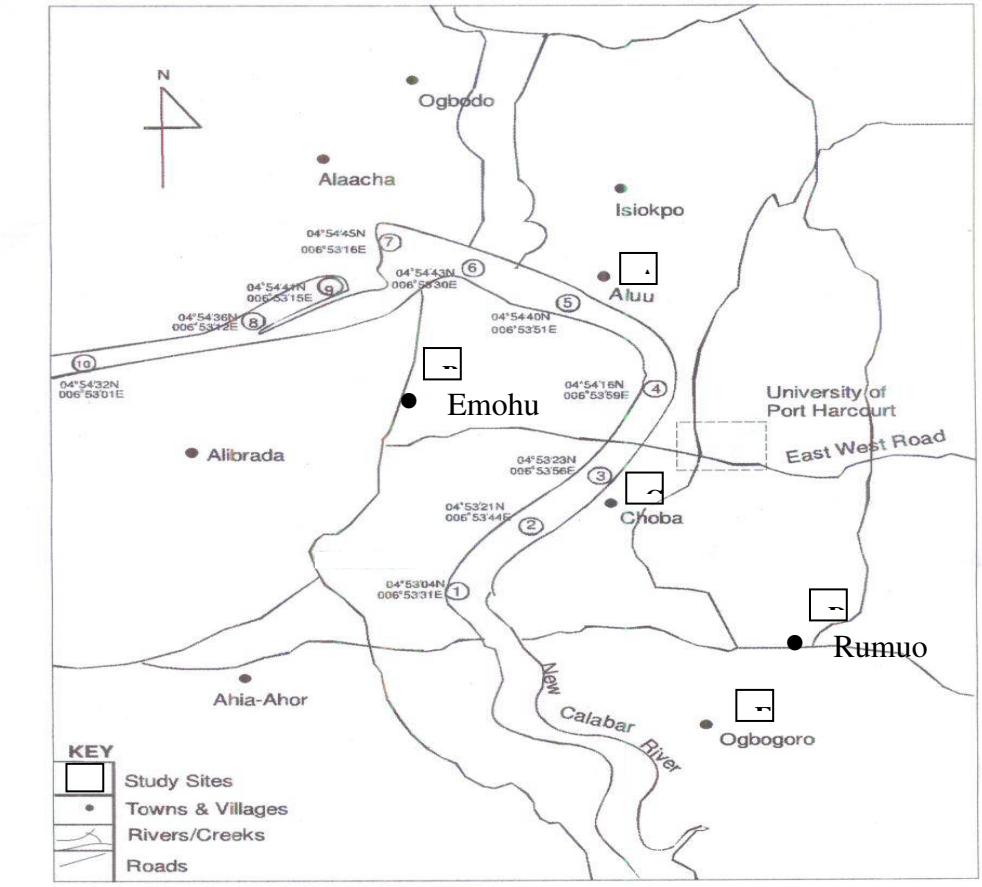

Figure 1.Map of the New Calabar River showing the study area.

2.3 **Experimental Procedure**

Fish Collection and Stocking: A total of four hundred (400) healthy and mature adult male and female *Clarias gariepinus* brood-fish, weighing between 180gms and 250gms, with lengths ranging between 17cm and 20cm in length were collected form grow-out ponds in the study area. A hundred (100) fish were randomly stocked into each of the four ponds at a ratio of 1male to 1female (Simtherman *et al*, 1993).

2.4 **Experimental Fish and Pond Preparation.**

Experimental fish were divided into Experimental Fish Group A- Treated Fishes and B for the Control Fishes. Experimental ponds were prepared by de-silting, clearing and fertilizing for the experiment and titled Experimental ponds- A for the treated fish group A and B for Control fish group-B.

2.5 **Environmental Manipulation**

I. Fish Group A:

Pond water manipulation

-Pond water level was maintained at 30cm throughout the experimental period by –daily monitoring using a fixed meter-rule to measure the water level.

-Replenishing lost water by regular pumping in of water from a bore hole using a water pump (Yamaha series)

-Once Weekly spray pumping of water into pond to mimic rain fall.

Nest Provision – The pond bottom and was organized to induce reproduction by introducing logs of wood, pipes and kakaban serve as spawn nests. Aquatic macrophytes were also introduced at the shoreline of the earthen ponds.

Mating Partners: All broodfish which were stocked were healthy and ready to breed. The fish were stocked at the rate of 1 male: 1 female (Smitherman *et al,* 1993).

II Fish Group B:

The environment of the experimental fish group B were not manipulated rather the fishes were reared under normal environmental conditions in the pond.

Nutritional Manipulation of Fishes

Fish Group A:

The experimental fish group - A were fed 40% crude protein diets (table 1.) prepared from local ingredients blended with added vitamin and minerals. Fish were fed thrice daily at 3% of the total fish biomass, five (5) days in a week for the duration of the experiment.

Table – 1: GROSS COMPOSITION OF EXPERIMENTAL DIET

NOS	*FEED INGREDIENTS*	*DIET – (40% PROTEIN)*
1	YELLOW MAIZE	29.39
2	GROUNDNUT CAKE	23.38
3	BLOOD MEAL	23.38
4	FISH MEAL	10.00
5	PALM OIL	5.00
6	COAGULATED GARRI OR STARCH	5.00
7	BONE MEAL	2.50
8	OYSTER SHELL	0.50
9	VITAMIN/MINERAL PREMIX	0.60
10	SALT	0.25
	TOTAL	100%

Experiment al Fish Group B:

These fishes were maintained on the natural feed organisms found in the fertilized ponds all year round, and no supplemental diet was administered to them.

Fish Sampling: During the experimental period, monthly fish samples were collected randomly from each pond using a dragnet after water reduction. Sexes were separated by manual sexing (Legendre *et al*, 1991 and 1992)

Fresh fish weights were measured to the nearest 1gm and lengths to the nearest 1cm and recorded. The Fishes were then killed, degutted and the gonads extracted and weighed to the nearest 0.1cm and recorded.

2.6 **Data Collection**

Determination of gonadal development/maturation, spawning and recrudescence were carried out by quantitative and qualitative methods (Mackie and Lewis, 2001).

A) Qualitative Assessment of Gonadal Maturation, Spawning and Recrudescence was carried out by macro-morphological method -assessing the gonads with the naked eye to determine the stage based on the observed features and classifying the gonad into developmental stages as adapted from Clay (1977 and 1979) Legendre *et al* (1991 and 1992); Arockiaraj *et al* (2004); Garcia- Diaz and Gonzalez (2006); and Cek and Yilmaz (2007).

B) Quantitative Assessment of Gonadal Maturation Spawning and Recrudescence was estimated using the Gonadosomatic index (GSI) with the formula;

% GSI = (Wg) 100/Wb

Where Wg= weight of fish gonad,

Wb = body weight of fish.

Thus % GSI = gonad weight (g) x 100/Fish body weight (g)

(Garcia –Diaz, 2006; and Cek and Yilmaz, 2007)

Monthly variations in the GSI were analyzed to determine the cyclic changes in the annual gonadal development.

2.7 **Statistical Analysis**

Data were subjected to a one-way analysis of variance (ANOVA) using the SAS, 1999 package. Data were derived were used to determine the effects of treatments on gonadal development. Significant values were tested using the Duncan t-test to confirm difference at $P<0.05$.

3. RESULTS

3.1 Qualitative Gonadal Maturation, Spawning and Recrudescence pattern

The macro-morphological assessment of the simulated (A) and non-simulated (B) female fish ovaries were staged (table 2), showing their cyclical gonadal maturation, spawning and recrudescence, corresponding to different levels of gametogenic activities of the ovaries. It could be seen from June that, the ovaries of all fish groups increased in size and volume, changing in color and texture, from maturing stage II. The ovaries progressed, peaked and dropped continually from maturing stage II in June, to Running ripe, showing (tab. 2) visible morphological changes associated with increased, peaking and dropping gametogenic activities from June to November. This was a period of high gametogenic activity.

From November, there was a distinct difference observed between the two fish groups. The B fish groups exhibited continually numerous Spent/Resting Stage VI gonads from November up until January, followed by a gradual improvement from Immature Stage I in February to Maturing Stage II in May2004. Meanwhile the A group interestingly exhibited some gonads in the spent/resting stage VI, alongside some mature stage III ovaries. From December up until January a majority of the ovaries were fully matured stage IV to running ripe stage V gonads showing that gametogenic activities were maintained. Fingerlings were actually collected from ponds.

Table – 2: MONTHLY GONADAL MATURATION STAGES FOR SIMULATED (A) AND NON-SIMULATED (B) FEMALE CLARIAS GARIEPINUS BETWEEN JUNE 2003 AND MAY 2004

MONTH	*SIMULATED (A)*	*NON-SIMULATED (B)*
June	II	II
July	IV	IV
August	V	V
September	VI	VI
October	V	V
November	VI	VI
December	V	VI
January	IV	VI
February	V	I
March	IV	II
April	V	II
May	V	III

I – Immature. II - Maturing. III – Mature. IV - Fully Mature. V - Running Ripe. VI - Spent/Resting.

Quantitative (Gonadosomatic index (GSI)) Gonadal Maturation, Spawning and Recrudescence patterns: The Cyclic changes in female gonadosomatic index (GSI) for the fish group A (simulated)and the fish group B (non-

simulated)revealed a sharp rise from June, peaking in August, followed by a fall in September, after which was another peak in October, followed again by a sharp drop in November. The sharp rise indicates increase in maturation, and the peak in August was as a result of increased gametogenic activities and finally gonads becoming fully matured. The sharp drop is synonymous with spawning of fishes and gonads undergo atresia, as observed in September. The second rise in the mean GSI indicated recrudescence and maturation of ova to a peak at full maturity as observed in October for both fish groups A and B. The later drop in mean GSI observed in November indicated spawning and ova atrophy. Though the pattern of gonadal activity was similar for both fish groups A and B from June to November, there was a slight difference observed in their cyclical GSI as shown in fig.2. The A group performed better.

However, it was observed that, from November 2003 to May 2004 there was a distinct difference between both fish groups A and B. The non-simulated (group B) fish showed a continuous sharp drop in mean GSI from November, followed by a gradual rise from January to May 2004 (fig. 2). The simulated (group A) fishes on the other hand, showed a distinct rise in mean GSI from November 2003, and followed by a small peak in December 2003 and then a drop when fish fingerlings were actually observed in the ponds. This period was followed by a rise in mean GSI to a higher peak in April in before a sharp drop in May 2004.

Fig.2 Cyclic Mean Gonadosomatic Index (GSI) for Simulated(A) and Non-Simulated (B) Fish Groups

3.2 **Statistical data**

Table 3 revealed that, the differences between the two fish groups were significant (probability of 0.01%).

Table – 3: ANALYSIS OF VARIANCE (ANOVA) FOR VARIATION IN GONADOSOMATIC INDEX (GSI) FOR FEMALE FISHES.

Female Clarias gariepinus	Simulated Fish (A)	Non-simulated Fish (B)	Mean	S
Gonadosomatic Index	19.047[a]	12.610[b]	15.828	**

*: - significant at P<0.05, **: - significant at P<0.01, ***: - significant at P<0.001

a,b; means in the same row with different superscript are significant (P<0.05)

4. DISCUSSION

The morphological changes associated with increased, peaking and dropping of gametogenic activities from June to November confirmed this period as a period of high gametogenic activity and as the main reproductive period of both simulated (A) and non - simulated (B) fish groups. This observed pattern was synonymous with catfishes and other tropical Teleosts that characteristically breed during the rainy season (May to October) in the tropical warm waters (Clay, 1979;Bruton 1979;Arockiaraj *et al,* 2004 and, Cek and Yilmaz, 2007). Clay (1979), reported that sexual activity in the African catfish is cyclical in nature. Van Oordt *et al* (1987), also reported that, the reproductive periods consisted of the breeding period, resting period and recrudescence period, as witnessed in both fish groups.

The high proportion of spent gonads seen in all fish groups in October indicated that spawning had taken place. Clay (1979) and Legendre *et al*, (1992) reported that *C. gariepinus* reproduces naturally from May to October. Van Oordt *et al*(1987) identified May to August, all in the wet season. Bruton (1979) reported spawning to be from June to September with a peak period in July and October when the rains were higher and littoral zones expanded. Tripathi (1996) reported that flooding and accumulation of rains facilitates immigration of fish into newly inundated fields/grasslands to prepare their nests.

From November to April of the next year (dry season), the gonadal activity of both fish groups differed distinctly. The drastic drop in gonadal development/mean GSI of the non-simulated fish indicated that this group of fishes had entered a period when gametogenic activities were dropping and the gonads were experiencing atresia after spawning. It can thus be inferred that, the non-simulated B group of fishes experienced inhibition of gonadal development. This however is synonymous with gonadal regression observed naturally in most Teleosts during this period of the year, the dry season (Clay, 1979 and Bruton, 1979). According to Van Oordt *et al,*

(1987) reproduction period consisted of the breeding period, resting period and a recrudescence period. Nagahama (1983) reported that there is an interplay of all the factors that stimulates the hypotalamus-hypophysis-gonad axis being essential for the regulation of reproduction in teleosts at this period.

The gametogenic activities of the non-simulated-B group were seen to drop sharply and the mean GSI fell sharply also. Similar observations were obtained by Van Oordt et al (1987) and Richer et al (1987) in their research in Israel. Bruton (1979) and Clay (1979) reported that gametogenic activity reduces and gonads go into the resting stage. In Nigeria, Nwadukwe and Ayinla (1993), similarly, reported that, the water temperature especially between February and April have a depressing effect on brood-fish development. This demonstrates a periodicity in gonadal maturation which parallels the cyclical changes in environmental factors in the study area. It could thus be said that, environmental stress accompanied by diminishing food organism was responsible for the ceasure of gametogenic activity.

The gradual rise from March to May of 2004, in mean GSI and maturity stages of gonads represented the onset of gametogenic activities or recrudescence period as similarly reported by Sikoki and Ejike (1997) when environmental conditions and subsequently nutrient situation were gradually improving and the rains were also approaching.

The non-simulated B fish group however could be said not to have gone into the resting phase, but recuperated and re-entered another phase as gametogenic activities, confirmed by the presence of a majority of mature stage III in November, fully matured stage IV to Running ripe stage V gonads in December and the presence of fingerlings in the ponds. This was further confirmed by the cyclic gonadosomatic index, showing a rise in gametogenic activities into another phase of full maturation and spawning irrespective of the season. This fish group could be said to have overcome the reproductive restrictions. Areerat (1987) carried out environmental and nutritional manipulation on *C. macrocephalus, C. hurruchus and C. batrachus* brood-fish in Asia and obtained high success rates.

Richter *et al* (1987); Nwadukwe and Ayinla (1993) and Legendre *et al* (1996) in similar works reported that sexually mature individuals can be found all year and round in ponds or enclosures if provided with unrestricted feeding.. Legendre *et al* (1996), further reported that, after reaching sexual maturity *C. gariepinus* seem capable of multiple spawning.

Clay(1979), also reported that, the presence of mature gonads all year round and bi-modal peak in reproduction indicates that multiple spawning could occur under favorable conditions. Freund *et al.* (1995) reported that *C. gareipinus* reproduction during the rainy season is related to increase in water level and flooding of grass lands. Tripathi (1996) reported that flooding and accumulation of rains facilitates immigration of fish into newly inundated fields/grasslands to prepare their nests.

5. CONCLUSION AND RECOMMENDATIONS

It is noteworthy that the environmental and nutritional manipulation successfully of enhanced gonaldal maturation, spawning and recrudescence of African catfish, *C. gariepinus*.

This simple, adaptive and organic method of seed multiplication is capable of encouraging large scale, *C. gariepinus* production by the subsistent/ artisanal aquaculturists predominant in the African fish production sector.

Also, this method is capable of reducing high loss in fishes sacrificed for pituitary semen and ova, for breeding purposes and reduces the use of inorganic hormonal compounds for the breeding of the African catfish.

To address the problem of cannibalism of fingerlings by brood-fish encountered and to, ensue higher fingerling production in grow-out systems, the eggs of the females after fertilization can be removed and put in incubators (Kund-Hanson et al, 1990) or juveniles be removed from the systems and put in incubators immediately after hatching and managed adequately.

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