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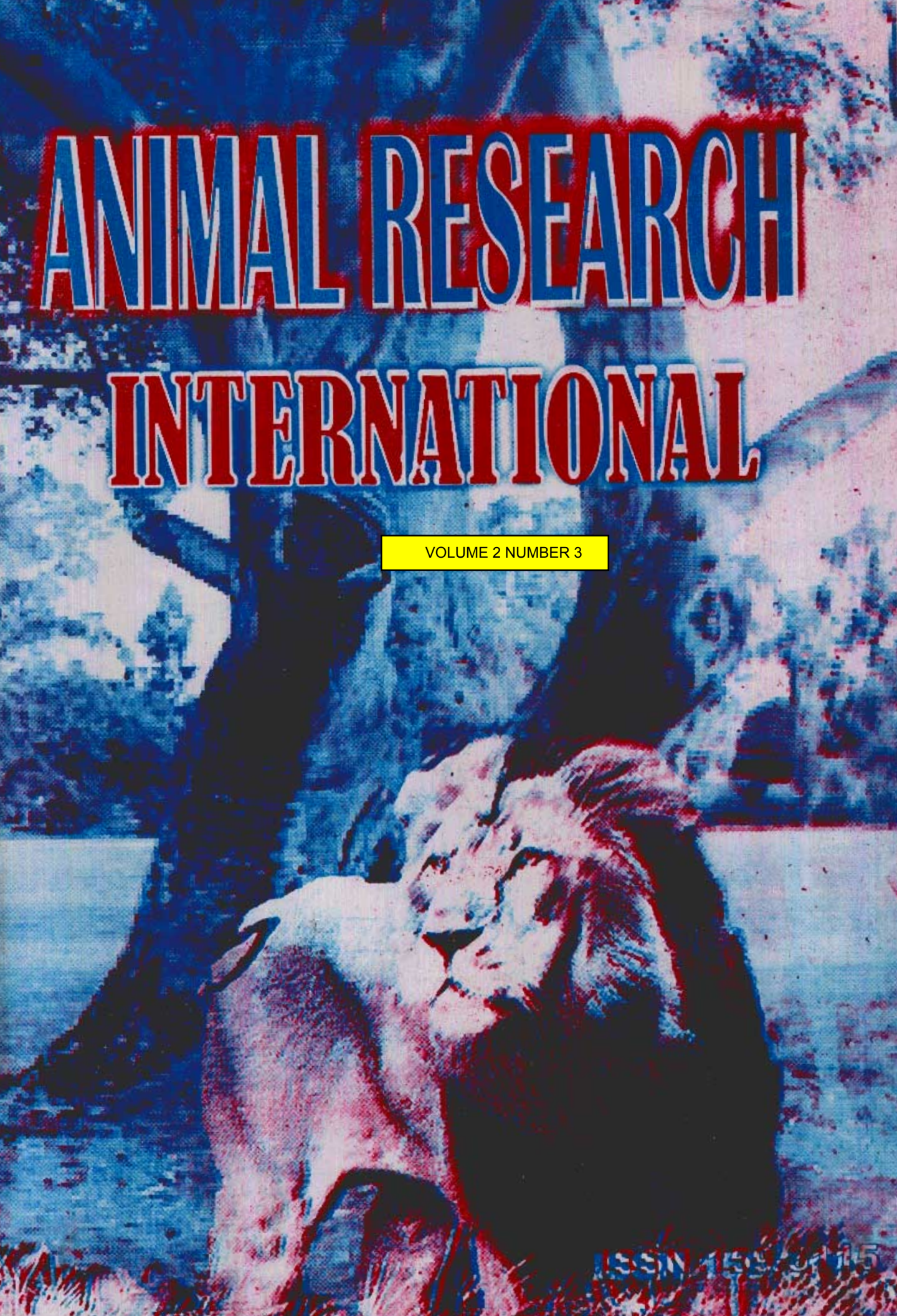

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EFFECT OF *Meloidogyne incognita* (ROOT- KNOT NEMATODE) ON THE DEVELOPMENT OF *Abelmoschus esculentus* (OKRA)

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ABSTRACT

Seedlings of Okra Abelmoschus esculentus were inoculated with different numbers of egg masses (0, 4, 8 and 12) of Meloidogyne incognita. The different inoculums elicited varied reactions on the Okra plants. Root galls increased progressively and significantly with increased levels of inoculum. At 0 (zero) inoculum level no root gall was observed. At low inoculum levels, 4 and 8 egg masses, the plants performed seemingly better than the control (in terms of plant dry weight, flower and fruit production). At high inoculum levels very low mean yields of the above parameters were recoded when compared to the control. The implications of enhanced performance observed at low inoculum levels on experimental crops are discoursed.

Keywords: *Meloidogyne incognita,* Root-knot nematode, *Abelmoschus esculentus,* Okra, Root galls

INTRODUCTION

Successful production of vegetables in Nigeria has been hampered to some extent by nematode pests, especially the root- knot nematodes *Meloidogyne* spp. (Ogbuji, 1983a, 1983b; Atu and Ogbuji, 1986 Enopka *et al.*, 1996; Agu and Ogbuji, 2001).

Thre*e* species of root-knot nematodes, *M. javanica, M. incognita* and *M. arenaria*, are found in Nigeria and they attack over 140 species of cultivated plants amongst which are important food crops and vegetables (Ezigbo, 1973; Idowu, 1981; Ogbuji, 1983a, 1984; Enokpa *et al*., 1996).

There have been reports on the effects of population densities of root-knot nematodes on growth and yield of vegetable crops in Nigeria. Ezigbo (1973) reported that *Meloidogyne* spp. induced dwarfing, withering, discoloration of leaves, flower abortion and in severe cases premature death in cowpea. Enokpa *et al.* (1996) also reported stunted growth in tomato plant treated with *Meloidogyne* spp. Reports of stunted growth, chlorotic and early senescence were reported in pepper (Capsicum *annuum)* inoculated with *Meloidogyne* Spp. (Ogbuji and Okarfor, 1984). In these examples authors reported that the *Meloidogyne* led to poor yields. The inducing of adventitious root formation in cowpea by root – knot nematode has also been reported (Ezigbo, 1973).

Abelmoschus esculentus commonly called Okra ranks high amongst the economical important vegetables of the world. The immature fruits of Okra, which are good sources of vitamin C, are used for the preparation of certain soups and sauces (Diouf, 1997). In the Tropics, *M.incognita* very frequently attack okra (Seck, 1990; Singh *et al.*, 1993; Khan and Khan, 1994; Khan *et al*., 1998). Kahn *et al.* (1994) reported that *M. incognita* elicited leaf browning, suppression in plant growth, fruit yield and photosynthetic pigments in okra.

Two species of root- knot nematodes, *M. incognita and M. javanica* very frequently attack *A. esculentus* in numerous farms in Nigeria (Caveness, 1976). In Nigeria, Okra is not only planted as the sole crop in farms but also used as a traditional intercrop planted with yams *(Dioscorea* spp.) (Ogbuji, 1986). Ogbuji (1986) further reported that this intercropping of okra with *Dioscorea rotunda* resulted in greater damage on the harvested tubers as a result of cross infestation of *M.incognita* from the Okra to the yams.

This paper reports the effects of *M. incognita* on the vegetative development of *Abelmoschus esculentus* in Nsukka, Nigeria.

MATERIALS AND METHODS

Seedlings of *Abelmoschus esculentus* were raised in black polythene sowing bags containing steam-sterilized soil. Three weeks old seedlings of the crops of about the same size were selected from the nursery and transplanted into each of 60 (sixty) experimental polythene bags. The bagged plants were arranged in a Complete Randomized Block Design and in three replicates to facilitate analysis of the results. Each replicate contained four rows with five plants per row totaling twenty (20) plants in each replicate.

Preparatory to the experiment**,** *M. incognita* originating from roots of field grown *Abelmoschus esculentus* were maintained on roots of tomato cultivars in special nursery bags. The species of the experimental nematode was confirmed from the examination of the perennial patterns (Ezigbo, 1973). Mass propagation of *M.incognita* was noticed on the tomato roots. In the experimental phases, egg masses of *M. incognita* of uniform size from the tomato root were inoculated thus:

- 0 egg mass per plant (control)

- 4 egg mass inoculum level (IL4) i.e. 4 egg masses per plant.
- 8 egg mass inoculum level (IL8) i.e. 8 egg masses per plant.
- 12 egg mass inoculum level (IL12) i.e. 12 egg masses per plant.

To inoculate each experimental plant, appropriate egg mass inoculum was added to a 3 cm depression ring in the soil around the roots of the three week old plants. The first row in each replicate were the control plants in which there was no infestation with nematode .The second row were those infected with four (4) egg masses per plant While the third and fourth rows were treated with 8 and 12 egg masses per plant respectively. The potted plants were duly tended and exposed to normal daylight. Dieldrex 20 (20 % dieldrin w/v) at 0.51 in 30 litres of water was sprayed weekly against insect attack. During harvesting, fruits were picked when they attained marketing quality (5.82 ± 0.19 cm). The first harvest took place seven weeks after planting. The numbers of aborted / dehisced fruits were also recorded. Once every week from week five (5) to six (6) and from week seven (7) to ten (10) when the experiment was terminated. Data collected for analysis were as follows:

5th to 8th week: the number of leaves, flowers and fruits per stand; 9th week: total number of fruits per plant, number of aborted fruits per plant; 10th week: dry weight of shoot per plant, dry weight of root per plant, length (cm) of shoot per plant, dry weight of fruits per plant, number of galls per plant. The dry weights were measured with a weighing balance to the nearest 0.05 grams.

RESULTS

Figure 1 shows the weekly mean shoot height of *A. esculentus* in relation to the inoculum levels of *M. incognita*. As shown on figure 1, inoculated plants were taller than the control at the 5th and 6th weeks. Subsequently the uninoculated planets were tallest. However from the seventh week to the end of the experiment control plants attained the tallest shoot heights followed closely by plants inoculated with 8 egg masses, while the plants inoculated with 12 egg masses recorded the lowest shoot height. These effects were shown to be significant ($P < 0.001$).

Figure 2 shows the effect of different inoculum levels of *M. incognita* on mean number of leaves of *A. esculentus.* At the 5th week the number of leaves on plants treated with 4 and 8 egg masses of *M. incognita* were a little more than those of control plants and plants treated with 12 egg masses. From the 6th week to the end of the experiment, plants treated with 8 egg masses of *M. incognita* clearly exhibited the highest number of leaves, followed by those four egg masses, the control plants and plants with 12 egg masses These differences in leaf number was not significant ($P > 0.001$). Highest number of leaves was produced at the 5th week and the leaves of the plants treated with 8 egg masses of *M.incognita* were the most luxuriant.

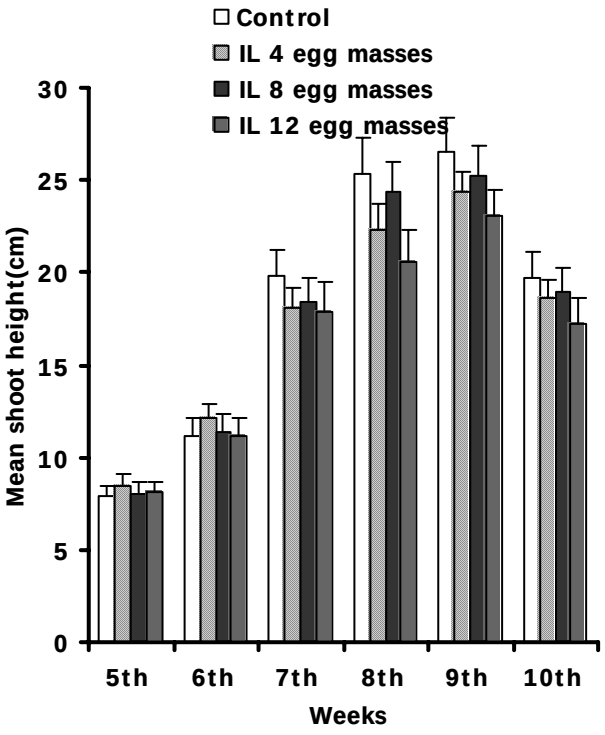

Figure 1: Weekly mean shoot height of Abelmoschus esculentus in relation to the inoculum levels of M. incognita

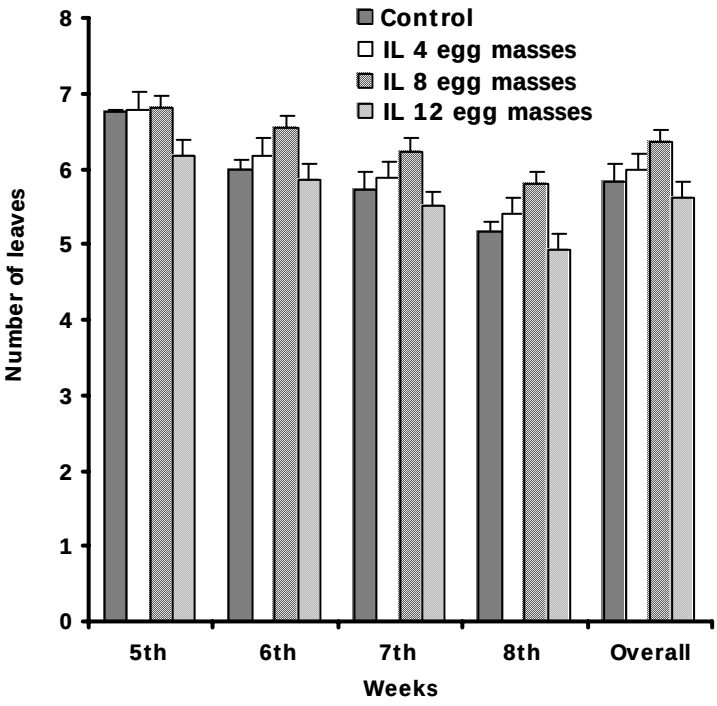

Figure 2: Weekly mean number of leaves (additional relative to time) of A. esculentus in relation to inoculum levels of *M. incognita*

The control plants were the only plants flowering in the 5th week, but from the 6th to 8th week the inoculated plants started flowering (Table 1). For both the control and inoculated plants, peak flowering was in the 7th week, with the plants inoculated with 8 egg masses producing the highest number of flowers (80), followed by plants inoculated with 4 egg masses (70), control plants (53) and 12 egg masses inoculated plants (30) respectively. Form the 6th week; the 8 egg masses inoculated plants produced the highest number of flowers at each time interval.

Table 1: Total number (additional relative to time intervals) of flowers per treatment and numbers actually flowering, (give in brackets) at different time intervals during the experiment

	Number of flowers and plants flowering/Treatment			
Week	**Control**	**IL 4 egg masses**	**IL 8 egg masses**	**IL 12 egg masses**
5th week	4(4)	0(0)	0(0)	0(0)
6th week	20(8)	28(13)	32(15)	21(2)
7th week	53(15)	70(14)	80(15)	30(10)
8th week	18(12)	16(8)	35(15)	15(8)
9th week	0(0)	0(0)	1(1)	0(0)

Stands in the IL8 egg masses produced the highest number of fruits, 116, while the lowest number of fruits 17, was produced by stands treated with IL 12 egg masses (Table 2).

Table 2: Total number (cumulative) of fruits formed at different time intervals during the experiment

	Number of fruits formed/Treatment			
Weeks	**Control**	**IL 4 egg masses**	**IL 8 egg masses**	**IL 12 egg masses**
7th week	41	20	26	7
8th week	48	55	70	13
9th week	65	78	116	17

The highest number of fruits 10, was aborted in plants treated with IL 12 egg masses, while the least fruit abortion 2, occurred in the control plants (Table 3).

Table 3: Summary of observations made on fruits maturation during the experiment

Treatment	**No. of aborted fruits**	**No. of Damaged fruits**	**No. of Undamaged fruits**	**Total no. of fruits formed**	**% wholesome fruits**
Control	2	3	60	65	92.3
IL 4 egg masses	8	40	30	78	38.4
IL 8 egg masses	4	55	57	116	49.13
IL 12 egg masses	10	4	3	17	17.64

The highest number of damaged fruits 55, was recorded in plants treated with IL 8 egg masses, while 40, 4 and 3 were recorded in plants treated with IL 4 egg masses, IL 12 egg masses and the control plants respectively (Table 3). When the number of fruits aborted and damaged per treatment were added, 92.3 % of total fruits in the control were normal, while for plants treated with IL8 egg masses, only 49.13 % of the fruits were normal (Table 3). Out of the 78 fruits yielded by the fruits treated with IL4 egg masses only 38.4 % were normal, while only 17.6 % of fruits produced by plants treated with IL 12 egg masses were normal (Table 3).

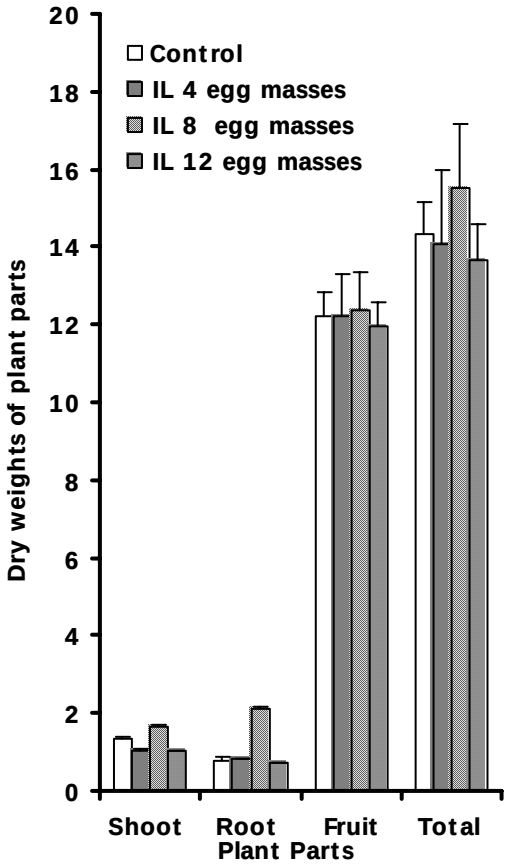

Figure 3: Effect of different inoculum levels of *M. incognita* on mean dry plant weight (g) of *A. esculentus*

Figure 3 gives the mean dry weight of the shoots, roots, fruits and total weight of the plant in grams. The mean dry shoot weight of the plants treated with IL8 egg masses was the highest (1.6 g), followed by the control plants (1.36 g). The least mean dry shoot weight occurred in plants treated with IL12 egg masses (1.017 g). The mean dry root weight was significantly different ($P < 0.05$) amongst treatments. The stands with IL8 egg masses had the highest mean dry root weight (2.1 g) while the IL4, control and IL12 egg masses had 0.8 g, 0.79 g and 0.3 g respectively. The mean dry fruit weight of the control and treated plants did not show any significant variation ($P > 0.05$). Table 4 illustrates the effects of *M. incognita* in galls formation on *A. esculentus.*

Table 4: effect of *Meloidogyne incognita* on mean number of galls on *A. esculentus*

Treatment	Mean number of galls
Control	0.00
IL 4 egg masses	50 ± 18.53
IL 8 egg masses	100 ± 65.99
IL 12 egg masses	120 ± 21.66

The control plants had no galls on them, while plants treated with IL12 egg masses had the highest number of galls (120 ± 21.66), followed by those treated with IL8 egg masses (100 ± 65.99). The least galls occurred in those treated with IL4 egg masses (50 ± 8.53). The mean number of galls for the treatments when tested statistically was significantly different ($P < 0.05$).

DISCUSSION

In this study, the occurrence of taller shoots in nematode infected plants than in the controls from week 5 to 6 after inoculation could be explained by the findings of Ezigbo, 1973. Ezigbo (1973), in his study of the effect of root- knot nematode on vegetables established that the first response to root - knot nematode stimulation is the formation of galls. Galls are induced by surface feeding without actual entry of the larvae into the roots. On galls formation, Ezigbo (1973) reported the formation of lateral roots in the region of the galls. These additional lateral roots, enhances the uptake of water and mineral salts by the treated plants and this enhancement manifested as increased shoot height in the treated plants, until the damage of root cells by the entry of the second stage infective larva. In this sturdy it is therefore assumed that from the seventh week to the end of the experiment when the control shoots were taller than the treated shoots, the second stage larvae may have eaten up part of the roots of the treated plants .The damage done was insufficient to hamper abundant flower and fruit production.

The control plants attained the tallest heights from the 7^{th} to the 8^{th} week .The finding was in line with the findings of Ezigbo (1973), Singh *et al.* (1993) and Enopka *et al.* (1996) . These authors in their various works on the effects of root-knot nematodes on vegetables observed some pathological changes in the inoculated plants. These pathological changes manifested in shoot heights, shoot weights, root weights, and most importantly in fruit development and maturation.

Among the nematode inoculated plants, 8 egg masses inoculated plants had higher shoot height than 4 and 12 egg masses inoculated plants. A convex interaction is demonstrated between the nematode and the host plant at various levels of inoculum.

Low nematode levels stimulating plant growth, food production and maturation have been reported by other workers Khan *et al.*, 1996 and Rao and Krishnappa, 1994). Khan *et al* (1996) infected cowpeas with various inoculums of *M. incognita* while Rao and Krishna (1994), infected chickpea with different inoculum densities of the same root knot-nematode; they found that growth stimulation occurred at low infection levels. At higher infection levels growth was suppressed. They concluded that at low inoculum levels of *M. incognita,* the production of lateral roots was stimulated and this accounts for the increased root weight of the plants and possibly increased nutrient uptake. This observation of Khan *et al*. (1996) and Rao and Krishnappa (1994), could be used to explain the occurrence of low flower and fruit production in plants inoculated with 12 egg masses. The findings of Khan *et al*. (1996) and Rao and Krishnappa (1994) were also supported by the findings in this study in which low inoculum levels of 4 and 8 egg masses gave the highest flower, food production and dry root weights of plants than the control. However plants treated with 4 egg masses having fewer flowers, lower shoot height and fruit yield than those treated with 8 egg masses, presupposes that the nematode *M. incognita* elicits a positive interaction though at different degrees at low inoculum levels. This assumption presupposes that at inoculum level 8 egg masses, *M. incognita* elicits a higher degree of positive interaction in A. *esculentus*.

Although more fruits were produced at low inoculum levels as shown with 4 and 8 egg masses inoculated plants, more marketable and healthier fruits were recovered from the control plants. This root stimulation seemingly advantageous would in the long run be detrimental to the plant in terms of fruit production, development and maturation. Increased inoculum levels lead to increased root galling in *A. esculentus.*

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THE EFFECT OF HOMOPLASTIC PITUITARY INJECTION OVERDOSE ON INDUCED SPAWNING OF AFRICAN CATFISH *Clarias gariepinus*, BURCHELL 1822

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ABSTRACT

Twelve pairs of male and female African catfish, Clarias gariepinus broodfish were monthly treated with graded doses of crude homoplastic pituitary injection. Different sets of pairs were used for each month, after certifying their gonadal maturity fitness for induced breeding. The first two pairs of spawners received one pituitary gland (3.8 – 5.7 mg) each from donors having equivalent body weight. The second two pairs received two glands (7.2 – 11.3 g), the third two pairs received three glands (10.1 – 16.2 mg) the fourth two pairs received four glands (13.5 – 22.2 mg) and the fifth two pairs received five glands (17.7 – 27.5 mg) the sixth two pairs (control) were not injected. Each spawning pair was kept in concrete spawning tank for 24 hours for natural spawning to take place. Administration of one pituitary injection failed to induce spawning, two and three glands yielded optimum results. Four and five yielded good spawning but all the hatchlings died after hatching. Death may be attributed to over secretion of thyroxin, thus leading to faculty vitellogenesis.

Keywords: Overdose, Homoplastic, Pituitary injection, *Clarias gariepinus*

INTRODUCTION

Since the origin of induced breeding, several authors have recorded varying successes in induced spawning of differing species of fish with varied techniques (Pickford and Atz, 1957; Dekimpe and Micha, 1971; Eyo, 1997; Ofor, 2001; Orji *et al*, 2002 and Yousuf *et al*, 2003). Harvey and Hoar, (1979) observed that since its inception, induced breeding has generated increased interest and solutions to the problem of piscine reproduction.

Recently, purified gonadotropins, hypothalmic releasing hormones, hormones of mammalian origin, sex steroids and such "extra-biologic" substances, such as antiestogen clomiphene, have been employed with various degrees of successes. Also various investigators have examined the effect of pituitary dosage administered (Ufodike *et al*, 1986). Zonneveled *et al*, (1988) and Carolfeld *et al*, (1988) had determined the optimum dosage required to ensure no hormonal wastage.

This work investigated the effect of pituitary overdose in the African catfish, *Clarias gariepinus.* Earlier, Clemens and Sneed (1971) stated that low dosages will not lead to spawning.

MATERIALS AND METHODS

Broodfish used for this study were raised from egg to maturity in an indoor hatchery and grow-out ponds. Broodfish weights were determined with a salter weighing balance after drying the fish with towel. Total and standard length measurements were determined to the nearest (mm). The weight of the pituitary was determined with a Mettler H30 balance after drying it with blotter and the dosage determined by grinding the appropriate number of glands in 2 m/s of distilled water with mortar and pestle.

The broodfish served as both spawners and donors for pituitary glands. Gonad stages, extraction of pituitary, preparation of pituitary homogenates and hormonal injections were carried out according to Hogendoorn, (1979 and Viveen *et al,* 1985). Assessment of female gonadal maturation was based on its exhibition of protruding reddish vent and swollen abdomen that oozed out brownish or greenish ripped eggs (0.9 – 1.2 mm) with slight manual pressure. Matured males exhibited reddish elongated, conical genital papillae. It was also observed that matured males had highly vascularized fins (dorsal, anal, pelvic and pectoral).

Twelve pairs of broodfish received graded doses of crude pituitary injections for four successive months (April to July 1998). Different sets of broodfish were used each month. The first two pairs of spawners received one gland (3.8 – 5.7 mg) of pituitary injection each, from donors of equivalent body weight; the second set of two pairs received two glands (7.2 – 11.2 mg) of pituitary infection each, the third set of two pairs received three glands (10.1 – 11.2 mg), the fourth set of two pairs received four glands (13.8 – 15.4 mg) each and the fifth set of two pairs received five glands (17.7 – 27.5 mg) each. The sixth set of two pairs (control) received no pituitary injection. Each injected male and female were kept in a concrete spawning tank for natural spawning, in a randomized block experiment. The methods of Hogendoorn (1979) were applied to determine the number of spawned eggs (relative fecundity), percent fertilization, percent hatching and percent fry survival.

RESULTS

Table 1 demonstrates the effects of overdose pituitary injection on induced breeding of *C. gariepinus*. Female spawners injected with one gland

Table 1: The Effect of homoplastic pituitary homogenate injection overdose on induced spawning of *Clarias gariepinus*

S/NO Injected	No. of Glands	Mean Weight of Glands	Mean % Fertilization	Mean % Hatch	Mean % survival
1	1	7.5	–	–	–
2	1	6.6	–	–	–
3	2	10.2	87	85	20
4	2	11.2	79	79	15
5	3	18.2	79	83	13`
6	3	19.0	74	59	07
7	4	26.1	85	83	–
8	4	25.1	83	81	–
9	5	37.2	85	91	–
10	5	32.0	86	80	–

did not spawn. Two and three glands gave optimum results, with mean percentage fertilization, percentage hatch and percentage fry survival ranges as 79 – 87 %, 79 – 85 % and 15 – 20 % respectively. For three glands, the ranges were 74 – 79 %, 59 – 83 % and 7 – 13 % respectively for percentage fertilization percentage hatch and percentage fry survival. For four and five glands the values for percentage fertilization and percentage hatched were 80 – 86 %, 80 – 91 % and zero for fry survival, as all the hatchlings died 24h after hatching. This response was repeated in each of the four months trials. The male and female sets paired without pituitary injections (control) failed to spawn.

DISCUSSION

The fact that female broodfish injected with one gland from donors of equivalent weights failed to spawn indicated that an insufficient dosage was administered to effect spawning. Clemens and Sneed (1971) conducted similar investigation with *Carpiodes velifera* pituitary which are relatively small (1 gland weighed 1 mg) compared with *C. gariepinus* (1 gland weighed 3 – 10 mg). They found no ovulation using a single pituitary homogenates.

When the number of glands increased from two to five for each male and female pair, relative fecundity, fertilization, hatching and fry survival of two to three glands were quite satisfactory, while for four and five glands, all the hatchlings died 24h after hatching. Clemens and Sneed (1971) observed that in almost all negative instances where nine or more glands were injected into a fish, blood exuded from the oviduct, when hand stripping was applied, suggesting an overdose for the fish. They concluded that the response was a physiological rather than pharmacological. However, matching the recipients' size with that of the donor was not reported, as such the case of injection overdose should not have been reported.

Pickford and Atz (1957) stated in their review that improper application of the pituitary injection during ovulation induction can yield inferior sex products. Inferior sex products refer to infertile eggs, or sperms, reduced viability, incidence of monsters and in the case of sturgeons, pathenogenic development of eggs. Clemens and Sneed (1971) attributed the effect of inferior sax products to extremely large dosage of pituitary homogenates, faulty techniques, state of pituitary gland in the donor species and the use of unripe or spent fish as recipient.

The larval mortalities within 24 h reported for the pair that received above three glands of pituitary in this study can neither be attributed to poorly developed or immature gonads nor pituitaries that contain toxic materials as suggested by Clemens and Sneed (1971). Since this response occurred repeatedly for four months, a more plausible explanation may be an over secretion of thyroxin resulting from overdose of pituitary homogenate injection. Hurlburt (1977) pointed out that low doses of thyroxin stimulated vitellogenesis in *Carasius auratus.* The above assumption is based on the fact that *C. gariepinus* fry could depend on their yolk for seven days after hatching before exploring for exogenous food, (Mgbenka and Orji 1997). If the endogenous food (yolk) was lacking or faulty due to faulty process of vitellogenesis the fry could die sooner than usual.

Davy and Chouinard (1980) also observed that excessive use of human chronic gonadotropin (HCG) could produce immunological effects. Be that as it may there is need for more investigation involving endocrinologist, nutritionist physiologist and fish biologist into the feed back mechanism responsible for the shut down of vitellogenesis due to overdose of pituitary homogenates in fish.

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RISK FACTORS ASSOCIATED WITH CANINE PARVOVIRUS ENTERITIS IN VOM AND ENVIRONS

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ABSTRACT

A study was carried out to assess the effects of age, sex, breed, location of cases and tick infestation on the prevalence of canine parvovirus (CPV) enteritis in dogs treated in the Veterinary Clinic of the National Veterinary Research Institute Vom between July 1999 and July 2002. A case control study design was used to assess the association between the risk factors and the disease. Out of 3075 dogs examined during the period, 87 had CPV enteritis (2.8%). Dogs between 0 to 5 months of age had elevated risk (OR = 25.14; 95% CI = 9.74, 67.26%). Other factors did not significantly affect the occurrence of the disease. The disease was most prevalent in May and June with a lesser peak in January. Age and seasonal variation should be considered in planning a control programme.

Keywords: Risk factors, Canine parvovirus enteritis.

INTRODUCTION

Canine parvovirus enteritis is gastroenteritis of acute onset and varying morbidity and mortality, caused by a parvovirus that was first reported in 1978 (MERCK, 1979). Houston *et al.,* (1996) reported that at the end of 1983, Canine Parvovirus infections had been reported in 50 countries around the world.

Initially, two common clinical forms of the disease were recognized. They are myocarditis and gastroenteritis. Myocarditis was seen in young puppies, leading to myocardial necrosis with either acute cardiopulmonary failure or scarring of the myocardium and progressing cardiac insufficiency. However, myocarditis is no longer seen because effective immunizations of bitches protect puppies during this early period of life (MERCK, 1998).

Gastroenteritis is more common in puppies 6-20 weeks old, that is, the period when maternal antibody protection falls and vaccination has not yet adequately protected the puppy against infection. Dogs with the enteric form suffer from an acute onset of lethargy, anorexia, fever, vomiting and diarrhea, with loose faeces, which may contain mucus or blood (MERCK, 1998).

A lot of work had been done on the risk factors associated with the disease in many parts of the world. Glickman *et al*, (1985) found that Doberman Pinschers, Rottweilers, English Springer Spaniels had significantly increased risk factor for CPV enteritis. In another work, Rottweilers, American Pit Bull Terriers, Doberman Pinschers, and German shepherd had significantly higher risk factor for CPV corresponding to age and sex. Sexually intact male dogs were more admitted with CPV enteritis in July, August and September compared with the rest of the months (Houston *et al*, 1996). Although a lot of work had been done on the risk factors associated with CPV enteritis in many parts of the world, no work had been done on the risk factors associated with the disease in Vom and its environs. The aim and objective of the study is to determine the relationship of age, sex, breed and seasonal predisposition on the prevalence of CPV enteritis in dogs examined in Vom and its environs, using the Epi info computer software to statistically check the association between each of the risk factors and CPV enteritis.

MATERIALS AND METHODS

Criteria for Selection of Cases and Controls: Data was obtained by going through clinical records in Veterinary Clinic, National Veterinary Research Institute, Vom from July 1999 to July 2002. The medical records were reviewed and the dogs with a history of foul smelling diarrhea and / or tentative diagnosis of CPV enteritis were selected. The rejected cases included a situation whereby enteritis, gastroenteritis or CPV enteritis was given as the tentative diagnosis but the history and clinical signs recorded had nothing to suggest the diagnosis of CPV enteritis. For all the cases, control dogs were selected and examined. The control dogs were clinically normal dogs brought to the clinic for vaccination or routine check up.

Data analysis: The data was analyzed to check for association between each risk factor and CPV enteritis using the Epi info software. Odds ratio (OR) of each variable was calculated and 95% confidence interval set up. A value of odds ratio greater than unity denotes association. The association is significant if the 95 % Confidence Interval (CI) does not include one. Seasonal distribution of the disease was

assessed by isolating seasonal indices for each month using the ratio- to- moving average method (Harnett and Murphy, 1974) and plotting the indices against the calendar months.

RESULTS

The analysis of the risk factor for CPV is presented on table 1. Of the 3075 dogs brought to the Vet Clinic, NVRI, Vom, 87 were diagnosed tentatively as CPV enteritis cases (prevalence rate of 2.8%). The result showed that the odds ratio (OR) for age was significantly elevated (OR = 25.14, 95% CI 9.74-67.26%). While the OR for the other risk factors considered like sex (OR = 1.45 CI 0.74-2.87%), breed (OR = 0.71 CI 0.31-1.64%), location (OR = 1.59 CI 0.61- 4.19%) and presence of ticks (OR = 1.27 CI 0.27-7.42%) were not significantly elevated (Table 1).

Table 1: Analysis of risk factors for the development of canine parvovirus enteritis in Vom

Risk factor	No. of Cases (n= 87)	No. of controls (n= 87)	Odds ratio	95% confidence interval
Age (months)				
0 - 5	75	22	25.14	9.74-7.26
6 and above	8	59	1.00	
Sex				
Male	48	46	1.45	0.74-2.87
Female	28	39	1.00	
Breed				
Local	59	69	0.71	0.31-1.64
Exotic	18	15	1.00	
Location				
A	68	60	1.59	0.61-4.19
B	10	14	1.00	
C	7	10	0.98	0.23-4.15
Presence of ticks				
Yes	29	3	1.27	0.27-7.42
No	38	5	1.00	

A = K/Vom, Vom and Kuru; B = Bukuru; C = Jos and other places

By plotting the average percentage index against the months (Table 2), it could be seen that the disease is more prevalent in the dry season months from December to June with a peak period in May. The disease is lower in July to August and absent in September to October (Figure 1).

DISCUSSION

The findings reported here indicate that CPV enteritis is a disease of the young animals. We also found that the disease has a seasonal pattern. However in our area (Vom), the disease is most prevalent (showing average percentage index of 476.5%) in May to June and lowest (0%) in September to October (figure 1). However, Houston et al, (1996) found in Canada that dogs were more likely to be admitted with CPV enteritis in July to September than in other months of the year.

Table 2: Monthly average percentage index of canine parvovirus enteritis in Vom

Months	Average % index
January	196
February	42.5
March	164
April	42
May	476.5
June	89
July	15
August	13.5
September	0
October	0
November	37
December	85

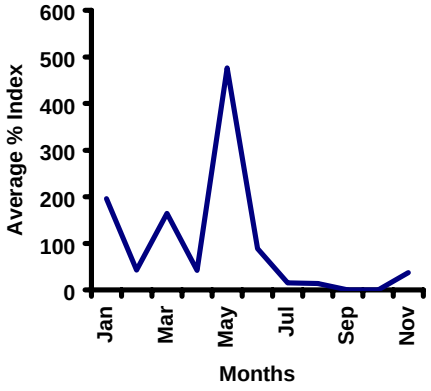

Figure 1: Monthly prevalence of CPV enteritis at Vet clinic, Vom

There was no significant association between CPV enteritis and breed probably due to the fact that most of dogs around Vom were of the same breed (Local breed). There was also no significant association between CPV enteritis and sex, as the disease affects both males and females in the study area (Vom). Houston *et al,* (1996) however, found that sexually intact dogs above 6 months of age were more likely to develop CPV enteritis, compared with neutered dogs. Furthermore, intact male dogs above 6 months of age were twice more likely to develop the disease than intact females.

There was no association between the presence of ticks and CPV enteritis. CPV enteritis is not known to be transmitted by ticks. Location did not play a significant role in the development of CPV enteritis. However, further work may need to be carried out on the relationship between sexually active dogs and CPV enteritis in Nigeria.

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METALS AND MINERAL NUTRIENT CONCENTRATION IN *Orechromis niloticus*, *Clarias gariepinus* AND *Chrysichthys furcatus* FROM BENUE RIVER, MAKURDI, NIGERIA

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ABSTRACT

Concentration of five metals and minerals, Iron (Fe), Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd), Sodium (Na), Potassium (K), Ammonia (NH_3), phosphate($P0_4$) were determined in three species, of fish from the Benue River (Orechromis niloticus, Clarias gariepinus and Chrysichthys furcatus), at four different sampling stations. The levels of metals and minerals were assayed from the muscle, liver, kidney, and intestine and gills of the three species. Differences in all means concentration of metals and minerals were analyzed using F-LSD and comparisons were made between stations and the fish species, significant difference were shown between values of iron and ammonia nitrogen amongst the species and between upstream stations and downstream stations respectively.

Keywords: Metals, Nutrients, Fishes, Benue river

INTRODUCTION

Freshwater fishes are often subjected to pollution especially near industrial or populated areas. Metals have been known to exert a wide range of effects on fishes. These effects may include metabolic, physiologic behavioural and ecological (Fostner and Wittmann, 1981). Specific metabolic and physiologic effect includes disturbances in osmoregulation, respiration, and tissue damage (Tuarala, 1983, Tort *et al* 1984, Annune and Olademeji 1994), reduced energetic resources (Health, 1984) and poor performance (Steele, 1983).

In Nigeria metals from industries are indiscriminately discharge into water bodies without regard to the health of the aquatic life. Metal from the aquatic environment has been studied in water columns and sediments (Ajayi, 1981 and Okoye *et al* 1991), Histopathological changes and tissue accumulation in some fishes (Onwusers and Oladimeji, 1990. Ofojekwu *et al* 1993). Commenting on the environmental implications of Sunshine Batteries Industry, at Ikot Ikpene, Udosen *et al* (1987) warned against gross pollution of streams by wastes and effluents of domestics, commercial and industrial sources. According to them, concentrations of metals in the Batteries industry effluents were not high enough to present serious pollution problem. Their concentration could increase in future if steps were not taken to check rising trend in the amount of untreated effluents that enter the streams. Kakulu *et al* (1987) reported high level of heavy metals in fish and shellfish of the Niger Delta. There is however no information on the metal and mineral nutrient concentration in fishes from the Benue River. The aim of this paper is to present metal and mineral nutrient level in some selected fishes from the Benue River and also to establish a relationship between tissue and water concentration.

MATERIALS AND METHODS

The fishes ware taken to the Laboratory for identification using the Anthony (1982) method. In the Laboratory Specimen where filleted and 5 g each of the tissue (liver, kidney, Intestine, gills and muscle) was weighed, homogenized and digested wilt a mixture of nitric and percholoric acid in the ratio of 2:1. The resultant solution was evaporated to dryness on a hot plate and the white residue formed dissolved in 10 ml of 20% nitric acid.

The Sample Solution was diluted with 30 ml of de-ionized water and analyzed on a Buck Scientific Model 210-VGP computerized Atomic Absorption Spectrophotometer (AAS). Metals such as Fe, Zn, Cu, Pb, Cd, Cr, Na, and K were determined. All analysis were carried out in triplicates and the resulting data analyzed using condiscriptive statistics and two-way analysis of variance.

RESULTS AND DISCUSSION

Physico-chemical Characteristics: Table 1 slows the physico-chemical characteristic of Benue river. Dissolved oxygen in the river shows a range between 3.7 mg/l and 6.8 mg/l during the sampling period with a mean value of 5.7±0.64 these indicated that the river was well oxygenated, though the mean pH value indicated slightly acidic water. The temperature and biological oxygen demand with values of 28.03± 2.06 ^{0}C and 2.55±0.54 are within the normal range for fresh-water environment. The level of iron in water ranged from 6.2-21.0(mg/l) with mean value of 12.48± 4.64,(mg/l) these value together with that of

zinc (0.01-3.8 mg/l) and mean (1.39±1.44)mg/l shows high values that are above acceptable limit for fresh water body.

Tables 2 and 3 shows metal and nutrients concentration in tissues of *C. gariepinus*, O. *niloticus* and *C. furcatus* sampled from the river while table 5 shows mean values from the pooled data.

Result shows high level of ammonia-nitrogen (NH_3 $^{-N}$) and iron (Fe) in the tissue of the three fish species studied, with *C. furcatus* having the highest concentration in tissues with mean kidney concentration of 95.66 ± 3.56 mg/g of ammonia-nitrogen and 47.8 ± 19.79 mg/g of iron. Respectively low concentration of ammonia-nitrogen and iron was found in the muscle of *C. gariepinus* with values of 2.67±0.21 mg/g and 1.59±0.49 mg/g respectively. The least concentration of lead was recorded in the gills of *C. gariepinus* with values of 0.004 ± 0.001 mg/g, in the liver of *O. niloticus* with values of 0.004 ± 0.0008 mg/g and in the intestine of *C. furcatus* with values of 0.006 ± 0.0003.

A two-way analysis of variance of metal and nutrient concentration in tissues of fish along the stations indicated that the concentration of all the metal and nutrient at down stream station B_3 and B_4 were significantly different from those observed at upstream station (B_1 and $B_{2)}$ ($P< 0.001$). This could result from the high concentration of human activities in as evidence in domestic sewage that predominate the down stream station which drain the main town of Makurdi. The values of metal and nutrient concentration in the liver and kidney generally showed higher concentration when compared with other tissues for all the fish species. The general high level of iron may be due to its high concentration in the sediments and water as reported by Okayi *et al* (200l). The mean concentration in zinc copper, lead and cadmium reported in this study are suitable and adequate for aquatic production as the value reported were below the standard set by the Australian Nation Health and Medical Research Council for metal concentration in aquatic food thus: Zn (1000.0 mg/g), copper (30.0 mg/g), lead (2.0 mg/g) and cadmium (2.0 mg./g) (Babington *et al.,* 1977). Metals and nutrient uptake in body tissue of the three-fish species were found to be in the order of the kidney >liver >gills >muscles for *C gariepinus* and kidney >gills > liver>muscle for *O. niloticus* and in the order of kidney >liver >intestine >gills >muscles for *C. furcatus*. This order was similar to the study of Annune *et al* (1993) on the accumulation of trace metals in tissues of freshwater fishes. Okoye *et al.* (1991) reported anthropogenic heavy metal enrichment of Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb and Zn in the Lagos lagoon and implicated land based urban and Industrial wastes sources. Pollution studies on 26 rivers in some southern and northern states of Nigeria (Ajayi and Osibanjo, 1981) showed that, with the exception of iron. The concentration of most trace metals in the surface waters and tissue of aquatic animals are generally lower than the global average levels for surface waters. Analyses of sediments and fish from the Nigeria delta area of Nigeria (kakulu and Osibanjo, 1987) revealed that the level of Cd, Cu, Fe, Mn, Pb and Zn were higher in shell fish than in finfish, with the exception of the lead level in some shellfish; levels of these metals were generally lower than WHO recommended limits in foods.

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Table 1: Physical Characteristic of Benue River Makurdi, Benue State

Parameters	No	Min.	Max	Mean and S.E.
Dissolved Oxygen (mg/g)	48	3.7	6.8	5.07±0.64
Temperature (°C)	48	24.0	31.0	28.03±2.06
PH	48	4.5	7.4	6.67±0.49
SDT (m)	48	0.16	0.81	0.42±0.22
BOD_5 (mg/g)	48	1.2	3.9	2.55±0.54
Alkalinity ($CaCo_3$ mg/1)	48	25	100	68.9±17.45
NH_3^{-N} (mg/1)	48	0.20	0.62	0.44±0.12
Iron (mg/1)	16	6.2	21.0	12.48±4.64
Zinc (mg/1)	16	0.01	3.8	1.39±1.44
Copper (mg/1)	15	0.12	1.20	0.64±0.25
Lead (mg/1)	16	20.01	1.45	0.78±0.48

Table: 2: Metal and nutrients concentrations in *Clarias garipinus,* Orechromis niloticus and Chrysichthys furcatus tissues from Benue River

Fish tissue	Trace metals and nutrient concentration (mg/g)								
	NH_3	PO_3	Fe	Zn	Cu	Pd	Cd	Na	K
					Clarias garipinus				
Muscle	2.67	0.02	1.59	0.021±0.005	0.02	0.012	0.002	0.04	0.02
	±0.21	±0.01	±0.49		±0.002	±0.006	±0.0004	±0.005	±0.002
Liver	15.06	0.06	8.05	0.17	0.0	0.07	0.022	0.21	0.12
	±3.96	±0.006	±2.26	±0.05	0.0	±0.03	±0.0008	±0.05	±0.03
Kidney	19.5	0.14	13.1	0.24	0.13	0.14	0.02	0.26	0.14
	±2.81	±0.009	±0.12	±0.02	±0.005	±0.016	±0.005	±0.04	±0.026
Intestine	18.46	0.13	10.33	0.03	0.0001	0.003	0.01	0.3	0.11
	±1.31	±0.08	±0.82	±0.002	±0.00004	±0.0004	±0.004	±0.026	±0.021
Gills	13.77	0.11	7.6	0.06	0.0002	0.005	0.004	0.16	0.15
	±1.44	±0.03	±0.86	±0.004	±000049	±0006	±0.001	±0.007	±0.012
					Orechromis niloticus				
Muscle	2.72	0.02	1.5	0.022	0.034	0.001	0.042	0.012	-
	±0.32	±0.002	±0.057	±0.005	±0.005	±0.0005	±0.002	±0.0012	
Liver	14.31	0.16	7.62	0.158	0.16	0.004	0.12	0.031	-
	±0.94	±0.05	±2.93	±0.046	±0.086	±0.0008	±0.04	±0.0014	
Kidney	25.5	0.12	11.8	0.28	0.18	0.01	0.36	0.18	-
	±1.40	±0.005	±1.04	±0.38	±0.017	±0.001	±0.09	±0.11	
Intestine	-	-	-	-	-	-	-	-	-
Gills	17.49	0.16	9.11	0.08	0.007	0.003	0.32	0.12	-
					Chrysichthys furcatus				
Muscle	3.14	0.02	1.96	0.01	0.046	0.003	0	0.042	0.02
	±0.21	±0.04	±0.31	±0.005	±0.02	±0.0004		±0.03	±0.007
Liver	23.94	0.16	16.21	0.21	0.27	0.012	0.005	0.306	0.2
	±0.79	±0.05	±9.54	±0.16	±0.024	±0.005	±0.0002	±0.13	±0.016
Kidney	95.66	0.26	47.8	1.03	0.4	0.133	0.03	0.68	0.4
	±3.55	±0.04	±19.79	±0.08	±0.06	±0.002	±0.0049	±0.04	±0.004
Intestine	15.92	0.09	9.33	0.06	0.0002	0.006	0.002	0.2	0.11
	±1.60	±0.002	±0.43	±0.008	±0.000012	±0.0003	±0.0005	±0.07	±0.03
Gills	11.53	0.13	6.89	0.06	0.0001	0.005	0.003	0.26	0.08
	±1.94	±0.03	±0.60	±0.004	±0.00004	±0.0004	±0.0004	±0.03	±0.001

Table: 3: Comparison of mean concentration of metals and nutrients in the tissues of *Oreochromis niloticus, Clarias gariepinus* and *Chrysicthis furcatus* from upstream and downstream stations using pooled data

	NH_3	PO_4	Fe	Zn	Cu	Cd	Pd
				O. niloticus			
Up stations	12.81*	0.065	6.01*	0.12	0.09	0.04	0.004
(B_1 and B_2)	7-25.5	0.018-0.12	1.56-11.8	0.021-0.28	0.02-0.18	0.003-0.13	0.001-0.01
Down station	10.52	0.08	3.68	0.09	0.13	0.01	0.003
(B_3 and B_4)	2.64-18.4	0.01-0.16	1.5-5.79	0.02-0.15	0.03-3.73	0.001-0.006	0.001-0.006
				C. gariepinus			
Up station	6.39*	0.04	3.68	0.043	0.046	0.023	0.006
	2.0-10.1	0.02-0.06	1.5-0.79	0.02-0.06	0.02-0.06	0.007-0.03	0.002-0.01
Down Stations	13.95*	0.07	8.33*	0.147	0.147	0.053	0.013
	2.35-19.5	0.05-0.14	1.59-13.1	0.002-022	0.002-022	0.003-0.14	0.006-0.02
				C. furcatus			
Up stations	7.49*	0.045	4018*	0.035	0.085	0.007	0.005
	3.14-11.85	0.02-0.07	1.6-6.6	0.01-0.05	0.04-0.12	0.002-0.012	0.005-0.005
Down Stations	55.85*	0.21	36.68*	0.704	0.33	0.133	0.031
	36.03-75.6	0.16-0.26	25.7-47.6	0.37-1.03	0.36-0.4	0.133-0.133	0.03-0.03

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MORPHOMETRIC VARIATIONS AMONG THREE *Distichodus* SPECIES OF ANAMBRA RIVER, NIGERIA

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ABSTRACT

Studies on the morphometric variations of three Distichodus species namely D. rostratus, D. brevipinnis and D. engycephalus from Anambra river were investigated from November 2002 to October 2003. Fish specimens were collected monthly at Otuocha and Ogurugu area using hook and line, traps, baskets, gillnets, dragnets, surface drift nets and cast nests of various mesh sizes. Specific differences among the Distichodus species occurred in 2 raw characters; pelvic fin height and pectoral-pelvic fin space and 6 ratio (transformed) characters notably pelvic fin height, anal fin height, pectoral-pelvic fin space, pelvic-anal fin space, head length and caudal peduncle depth. Sexual dimorphism occurred in two ratio characters namely pectoral-pelvic fin space and pelvic-anal fin space among Distichodus brevipinnis. These characters are recommended as key characters in the taxonomy of Distichodus.

Keywords: Anambra river, *Distichodus*, Taxonomy, Morphometric character

INTRODUCTION

Fish unlike crude oil is a renewable natural resource, which when conservatively managed could meet about 50 % of Nigeria's animal protein and other nutritional requirements (Olayide and Akinwumi, 1980). This applies also to most other developing countries currently ravaged by hunger and malnutrition. Fish exploitation in Nigeria is confined to the marine, brackish and inland waters. A major component of the inland waters in Eastern Nigeria is the Anambra river, and its drainage systems – Anambra river basin. The major occupation of the people in the area is fishing and farming (including fish farming). *Distichodus* species are among the major exploitable fish species of the Basin. Distichodus belongs to the family Distichodontidae with three species occurring in the Basin: *Distichodus rostratus* Gunther 1864, *D. engycephalus* Gunther 1864, and *D. brevipinnis G*unther 1864. Teugels *et al* (1992) reported that they are widely distributed in Nigeria, Nilo-Sudan, Niger, Volta, Chad and Nile basins. *Distichodus* species are extensively used in aquaculture on account of their good qualities, which according to Satia (1990) include high availability of seed for stocking, good adaptation to climate, ability to support high population densities, ability to feed on grasses and weeds in ponds and popularity among the consumers. In Nigeria *Distichodus* species are cultured in fish farms and numerous lentic water bodies because of their ability to feed on grasses and weeds.

Socio-culturally, dried *Distichodus* species are widely used in conjunction with other fishes like *Heterotis*, *Gymnarchus*, *Channa* etc. to prepare fish pepper soup used during traditional marriage ceremonies, cultural festivals and entertainment of special guests in the riverine states of Nigeria. Teugels et al. (1992) reported that the popularity among the consumers has made the fish to be of commercial importance and are often seen in piles of smoke-cured fishes. Reports on the taxonomy of *Distichodus* species, (Reed *et al.,* 1967; Holden and Reed 1972) were based on the number of scales on the lateral line and size of the adipose fin. The dependence on these characters for identification of *Distichodus* species may pose taxonomic problems due to overlapping number of the scales among the various *Distichodus* species. Moreover, the size of adipose fin is age, sex and size dependent thus not a foolproof character in delimiting *Distichodus* species. Morphometric and meristic features of many species have been used widely in separating different species of fishes. Ezenwaji (1986) stated that meristic counts and other measurements may be employed in separating different clariid species but warned that these measurements must be used with caution. Madu *et al.* (1993) used morphometric and meristic characteristics to distinguish between *Heterobranchus bidorsalis* and *Heterobranchus bidorsalis* vs. *Clarias anguillaris* hybrid. Similarly, Eyo (1997, 2002, 2003) discriminated members of the genus *Clarias* of Anambra river using biometrical variations. Otobo (1976) separated *Pellonula afzelusis* from *Sierathrisa leonensis* using meristic characters like number of fin rays, spines and sizes of the fins among others. Anyanwu and Ugwumba (2003) used morphometric parameters, meristic counts and electrophoresis techniques to separate *Pseudotolithus senegalensis* caught from three zones in the Nigerian Economic Exploitable Zone (EEZ) of the coast of Lagos. Ugbomeh (1989) developed a key for the identification of the Nigerian Grey Mullets (Mugilidae)

using meristic and morphometric characters and observed that the useful diagnostic characters were:

1. The number and form of the pyloric caeca.
2. The number of annuli on the cephalic scales
3. The size and number of scales on the lateral line of the body and
4. The number of anal fin rays.

This study therefore employs morphometric measurements as means of delimiting *Distichodus* species of Anambra river to ensure unmistakable identification.

MATERIALS AND METHODS

Fish samples were collected monthly at Otuocha and Ogurugu (Figure 1) from November 2002 to October 2003 using gill, drag, drift and cast nets of mesh sizes of between 70 to 120 mm. Baskets, traps and hook and line were also used. Fish specimens were also purchased from the local markets at Otuocha and Ogurugu to ensure adequate representation of all sizes of *Distichodus.*

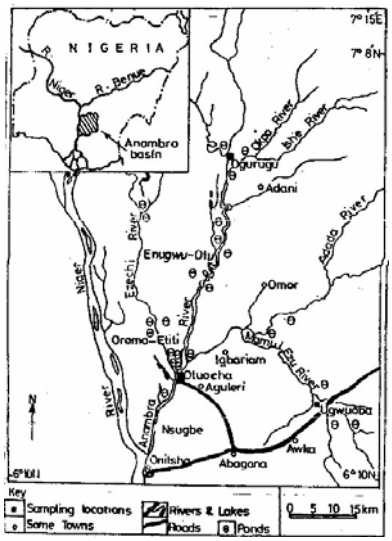
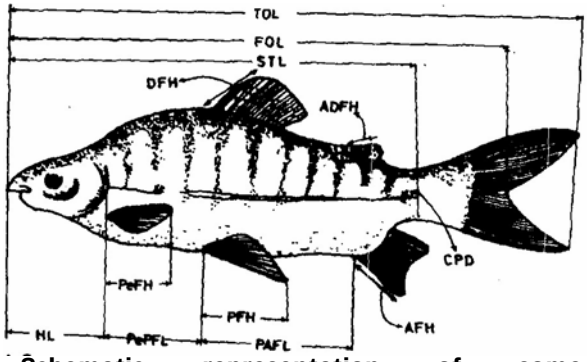


Figure 1: Map of Anambra river basin showing sampling locations

Fish were measured to the nearest 0.01 centimeters using venire Caliper, dividers and a fish measuring board. The fresh weight of the fish was taken to the nearest 0.01 gram using a mettler PC 2000 electronic balance. Identification of the fish collected was done using the keys of Holden and Reed (1972) and Lowe-McConnell (1972). The sex of the fish specimens was determined by examining their gonads after dissection. The determination of sexes in very young fish was problematic. In such cases, the excised gonads were pressed between two slides and examined under the microscope for immature eggs or sperm. Schematic representations of measured morphometric characteristics are shown in Figure 2.

All measurements were taken on the left side of the fish. The description of morphometric parameters used for the study are given below:



Schematic representation of some morphometric character measurements for Distichodus species of Anambra river, Nigeria. Lateral view [codes are explained in the text]

Standard Length (SL): The length from the tip of the snout to the anterior base of the caudal fin/posterior base of the caudal peduncle.

Total length (TL): The length from the tip of the snout to the end of the caudal fin.

Fork Length (FOL): The length from the tip of the snout to the shortest median caudal fin ray.

Head Length (HL): The length measured from the tip of the snout to the posterior end of the membranous margin of the gill opening of the body.

Dorsal fin Height (DFH): The length from the base of the adipose fin to the tip.

Pelvic fin Height (PFH): Taken as the length of the tallest pelvic fin ray.

Pectoral fin Height (PeFH): Taken as the length of the tallest pectoral fin ray.

Anal fin Height (AFH): The length form the base of the anal fin to the tip.

Pectoral Pelvic Fin Space (PPeFS): The ventro-basal distance between the posterior end of the pectoral fin and anterior end of the pelvic fin.

Pelvic Anal fin space (PAFS): The ventro-basal distance between the posterior end of the pelvic fin and anterior end of the anal fin.

Caudal Peduncle Depth (CPD): The dorso-ventral distance at the end of base of the caudal peduncle.

Data Analysis: Analysis of Variance (ANOVA) and Fisher's Least Significant Difference (F-LSD) (Steel and Torrie 1984) were employed to analyze the data.

RESULTS AND DISCUSSION

The size and weight ranges of *Distichodus* species in Anambra river are presented in Table 1. A total of 169 *Distichodus rostratus* made up of 84 males with size range of 11.0 – 32.0 cm total length and 45 – 576 weight and 85 females of 13.0 - 34.0 cm total length range and weight range of 50-675 g weight were collected. 167 *D. brevipinnis* consisting of 81 males of 12.0 - 34.0 cm total length and 53 – 831 g weight and 86 females ranging from 13.0 - 38.8 cm total length and 58 – 976 g weight were also collected. 79 males of 9.0 – 27.0 cm total length and 34 – 540 g weight and 84 females of 10.0 - 30.0 cm total length and 40-650 g weight of *D. engycephalus* were also used for the study.

Table 1: Size and weight ranges of *Distichodus* species in Anambra river

	D. rostratus	
Number	**M**	**F**
of fish	84	85
Size range	11.0-32.0	13.0 34.0
(mean)	(22.03)	(22.99)
Weight range	45-576	50-675
(mean)	(227.00)	(303.78)
Number	***D. brevipinnis***	
of fish	81	86
Size range	12.0 –34.0	13.0-38.8
(mean)	(23.57)	(24.57)
Weight range	53-831	58-976g (218.27)
(mean)	(332.00)	
Number	***D. encycephalus***	
of fish	79	84
Size range	9.0-27.0	10.0-30.0
(mean)	(20.23)	(21.62)
Weight range	34-540	40-650
(mean)	(218.27)	(213.73)

The specific difference in raw and ratio morphometric data among *Distichodus rostratus, D. brevipinnis* and *D. engycephalus* at P > 0.05 considering twelve morphometric measurements are shown in Table 2. Specific differences in raw morphometric character data occurred in 2 (16.67 %) of the studied characters namely the pectoral-pelvic fin space and pelvic fin height. Previous fisheries taxonomists notably Teugels (1982) in his key to the subgenera of the genus *Clarias,* Ezenwaji (1986) while reviewing the problems of *Clarias* taxonomy and Nwadiaro and Okorie (1985) in Biometric characteristics of *Chrysichthys filamentosus* (Pisces Bagridae) from Oguta lake did not recognize pelvic fin height and pectoral – pelvic fin space (raw data) as important diagnostic characters. However, Eyo (2003) noted that among four *Clarias* species (*Clarias ebriensis, C. albopunctatus, C. gariepinus* and *C. anguillaris),* congeneric differences occurred in 2 raw (pectoral fin base length and frontal width), 9 transformed (pelvic fin base length, Pectoral spine height, dorsal fin height, maxillary teeth band width, premaxillary teeth band depth, frontal, fontenelle length, internasal space, pelvic fin-anal fin space and prenasal barbell length) and 6 residual characters (Total Length, prepectoral length, pectoral fin base length, dorsal fin base length, outer mandibular barbel space and eye diameter).Specific differences among *Distichodus* species vis-à-vis the ratio data occurred in 7 (58.33 %) of the studied characters. The pelvic fin height, the dorsal fin height, the anal fin height, pectoral-pelvic fin space, pelvic anal fin space, head length and caudal peduncle depth were of significant taxonomic importance in discriminating all the studied *Distichodus* species. These characters are considered key characters for *Distichodus* taxonomy. Some of these characters like the ratio data of the pelvic fin height, dorsal fin height, anal fin height and the head length have been employed frequently but mainly for the taxonomy of *Clarias.* Other characters unexploited by some previous fisheries taxonomists but important in taxonomy are the percentage standard lengths of the pectoral pelvic fin space, pelvic-anal fin space, head length and caudal peduncle depth.

Observation from this study indicated that these character ratio data were heterogeneously distributed among the examined *Distichodus* and were significantly different at P > 0.05 in all the species thus indicating their valuability as key characters.

The sex dimorphic characters used to differentiate between male and female *Distichodus* species of Anambra river are presented in table 3. From the data, the ratios of adipose fin height, pectoral fin height, pelvic fin height, total length as well as anal fin height were statistically insignificantly different among males and females of all *Distichodus* species sampled (P > 0.05). An evaluation of the dorsal fin height ratios revealed the occurrence of significant difference among males and females of *D. brevipinnis* (P > 0.05). Similarly the fork length ratios were also significantly different between males and females of the species. There was also a significant difference in anal fin height between the males and females *D. engycephalus.* An assessment of sex dimorphism in pectoral-pelvic fin ratios indicated significant difference between males and females *D. brevipinnis.* Additionally, the pelvic-anal fin space ratios were significantly different among males and females of *D. rostratus* (P > 0.05) and *D. brevipinnis* (P > 0.05). Sex discriminating characters have been widely used by fish taxonomists in separating male and female species. Libovarsky and Bishara (1987) demonstrated sexual differences in three characters (snout length + eye diameter, predorsal length and maximum body depth) in *Oreochromis niloticus,* seven characters (standard Length, Snout length, Iris diameter, head length, pre-dorsal length, pre-pelvic length and prenasal length, in *O. aureus,* two characters (snout length and head length) in *sarotherodon galilaeus* and four characters (snout length, snout length + eye diameter, head depth and maximum body depth) in *Tilapia zilli*. Also Eyo (2002) reported that conspecific differences among males and female clariids inhabiting Anambra river systems occurred in 7, 11, 20 and 26, morphometric characters for *Clarias ebriensis C. albopunctatus, C. gariepinus* and *C. anguillaris.* This finding was

Table 2: Specific differences in raw and ratio morphometric data among *Distichodus* species of Anambra river Nigeria employing F-LSD

Morhometric character	Raw Data				Ratio Data			
	F-LSD Values	*D. rostratus*	*D. brevipinnis*	*D. engy cephalus*	F-LSD Values	*D. rostratus*	*D. beevipinnis*	*D. engy cephalus*
Adipose fin height (ADFH)	1.97	2.08ac	2.37ab	1.55bc	1.04	8.95ac	9.65ab	9.31bc
Pectoral fin height (PFH)	0.27	4.37ac	3.98ab	3.55bc	0.34	16.68ab	16.71b	16.43bc
Pelvic fin height (PFH)	0.24	4.56a	4.19b	4.33c	0.37	20.46a	17.48b	16.10c
Dorsal fin height (DFH)	0.32	5.10a	4.51b	4.30bc	1.00	22.47a	18.56b	20.66bc
Fork length (FOL)	0.44	25.29a	25.29ab	22.99c	1.82	113.72a	109.84b	108.66bc
Total length (TOL)	1.36	28.74a	35.47bc	34.99ac	3.00	130.01ab	127.71b	123.88c
Anal fin height (AFH)	0.21	3.02ab	3.31b	3.21c	0.65	18.36a	16.87b	15.71c
Pectoral-pelvic fin space (PPFS)	0.32	5.89a	5.46b	5.46c	0.59	27.01a	28.57b	25.38c
Pelvic-Anal fin space (PAFS)	1.40	4.55ac	5.16ab	3.83bc	0.48	19.50a	21.39b	18.14c
Head Length (HL)	0.42	6.14a	5.15b	4.83bc	0.70	26.71a	21.49b	23.70c
Caudal peduncle Depth (CPD)	0.63	3.99ac	3.50ab	3.50bc	1.97	17.66a	15.39b	13.59c
Standard Length (SL)	1.56	22.52ac	24.08ab	20.99bc	-	-	-	-

Key: a, b and c indicates significant corresponding means at P = 0.05 D.= Distichodus

Table 3: Sex Dimorphism in ratio data (Percentage standard length) among the *Distichodus* species of Anambra river, Nigeria

Morhometric character	*Distichodus rostratus*				*Distichodus brevipinnis*				*Distichodus engycephalus*			
	Males	Fe-males	T. Values	2 Tail Prob.	Males	Fe-males	T. Values	2 Tail Prob.	Males	Fe-males	T. Values	2 Tail Prob.
Adipose fin height (ADFH)	9.15± 3.67	8.76± 3.48	0.71	0.48	9.39± 3.03	9.90± 4.56	-0.78	0.44	9.96± 11.09	8.69± 3.22	1.00	0.32
Pectoral fin height (PFH)	19.51± 3.64	19.85± 3.56	-0.61	0.54	16.58± 3.03	16.84± 3.64	-0.49	0.63	16.47± 2.92	16.41± 2.42	0.15	0.88
Peivic fin height (PFH)	20.52± 3.55	20.40± 3.04	0.23	0.82	17.52± 3.88	17.44± 4.91	0.13	0.90	15.89± 2.98	16.30± 2.73	-0.91	0.37
Dorsal fin height (DFH)	22.36± 5.62	22.57± 5.82	-0.24	0.81	18.03± 5.18	19.06± 3.93	1.44	0.05*	21.40± 5.44	20.54± 6.26	0.93	0.93
Fork length (FOL)	114.87± 20.72	112.60± 22.10	0.69	0.49	107.54± 19.55	111.62± 13.00	1.41	0.05*	110.08± 10.73	107.30± 13.92	1.42	1.43
Total length (TOL)	128.75± 25.42	131.23± 17.81	0.73	0.46	127.93± 8.16	127.49± 8.21	0.34	0.73	124.41± 10.60	123.36± 12.60	0.42	0.67
Anal fin height (AFH)	18.16± 2.37	18.56± 2.97	-0.82	0.41	16.66± 3.35	17.06± 4.54	-0.65	0.52	15.16± 3.36	16.25± 3.60	1.99	0.05*
Pectoral-pelvic fin space (PPFS)	27.17± 4.10	26.85± 3.08	0.57	0.57	27.88± 5.65	29.21± 5.04	1.61	0.01*	26.26± 7.66	24.54± 7.11	1.48	0.04*
Pelvic – Anal fin space (PAFS)	19.04± 3.06	19.94± 3.69	1.72	0.04*	20.48± 4.36	22.25± 4.36	2.62	0.01*	18.37± 6.17	17.92± 5.47	0.49	0.62
Head Length (HL)	26.15± 8.09	27.24± 8.55	-0.85	0.40	21.74± 6.09	21.25± 6.43	0.51	0.61	23.57± 5.15	23.83± 4.84	-0.31	0.74
Caudal peduncle Depth (CPD)	17.67± 4.19	17.66± 4.29	0.02	0.98	15.73± 9.18	15.07± 4.96	0.59	0.56	13.13± 5.92	14.03± 5.11	-1.04	0.30

** Significant difference @ P = 0.05.*

supported by Nwani (2004) who reported sexual dimorphism in one transformed (dorsal fin base length) and four raw (Total length, standard length, dorsal fin base length and anal fine base length) morphometric characters among M*ormyrus rume, Hyperopisus bebe, Campylomormyrus tamandua* and *Gnathonemus petersii* occurring in Anambra river system.

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HOMESTEAD ARTIFICIAL PROPAGATION, GROWTH AND MORPHOMETRIC CHARACTERISTICS OF THE AFRICAN CATFISH (*Clarias gariepinus*, PISCES: CLARIIDAE)

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ABSTRACT

Thirty (30), eighteen months old gravid females (458.20 ± 2.256) of the African catfish, Clarias gariepinus were injected intramuscularly with different doses (0.00, 10.00, 30.00, 50.00 and 70.00 µg Kg^{-1}) of luteinizing hormone releasing hormone analog (LHRHa) at a water temperature of 25 ± 1.00^{0}C. Fifteen (15) mature males (453.97 ± 2.13g) received half the dose given to the females and they provided the milt (spermatozoa) used in the artificial fertilization of ovulated eggs from females. The hatched fry were randomly allotted to 15 indoor concrete tanks (0.70 x 1.50 x 0.50m), arranged in 5 rows with 3 replicates per row (5 x 3) and allowed to stay for 10 days. Twelve (12) concrete tanks (8.00 x 4.00 x 1.00m) were used outdoor for the feeding of the advanced fry on formulated ration (CP = 38%) at 5% body weight per day for 7 days. The results of the artificial inducement of the catfish with different doses of LHRHa (10-70 µg Kg^{-1}) indicate that there were significant variations in the percent ovulation (P < 0.01), spawn weight (P < 0.01), percent fertilization and survival (P < 0.05) of the fish. The mean body weight, head diameter, standard and total body lengths of the fry also varied significantly among the different hormonal doses (P < 0.05). These results signified that different doses of LHRHa affected the growth and morphometric indices of C. gariepinus fry.

Keywords: *Clarias gariepinus,* Luteinizing hormone, Artificial inducement

INTRODUCTION

Scarcity of fingerlings from the wild to stock existing ponds in tropical Africa, and the growing aquaculture industry have stimulated the propagation of culturable warm water fish species. Reports on induced spawning of fish using different hormonal materials (Hogendoorn and Vismans, 1980; Young *et al*., 1989; Ayson, 1991) are available. Successful trials have also been reported with carp pituitary (Janseen, 1985), human chorionic gonadotropin (HCG) (Legendre, 1986), progesterone, and leutinizing hormone releasing hormone analog (LHRHa) (Richter *et al*., 1987; Solar *et al*., 1990).

Advances made in aquaculture include the understanding and application of scientific knowledge relating to piscine reproduction (Harvey *et al*., 1993; Donaldson and Devlin, 1996; Donaldson, 2000, 2001; Lee and Donaldson, 2001; Zohar and Mylonas, 2001). The various contributions have enabled the sophistication of biochemical, physiological, endocrine and genetic technologies for the optimization of reproductive processes in cultured finfish. Endocrine techniques for the induction of ovulation and spermiation have advanced from the use of pituitary extracts to the use of gonadotropin releasing hormone (Gn RH) with or without dopamine antagonists (Donaldson, 2003). There have been advances in the methods of the administration of the hormones by injection, by implantation, and more recently by dietary administration.

In many developing countries of the world, the application of sophisticated techniques to piscine reproduction is not much. In Nigeria, Nwadukwe (1993) used locally available frog pituitary extract to spawn the African catfish (*Heterobranchus longifilis*) Mustafa *et al*. (1984) spawned the Asian catfish (*Heteropneustes fossilis,* Bloch) with the pituitary extract from the Indian frog (*Rana trigrina*, Daudin). Semi-natural or hormone induced propagation of *Clarias gariepinus* in ponds/tanks has not proved to be a reliable method for mass propagation of fry (Delince *et al*., 1987). Artificial propagation under controlled hatchery conditions has been adopted for the mass production of fry and fingerlings. The deliberate spawning of large numbers of tilapia became important in recent time due to the advances made in hybridization, genetic selection and the need to meet the growing demands of extension work (Delince *et al*., 1987).

The technicality involved in the artificial spawning of *C. gariepinus,* and the cost of constructing modern hatcheries has greatly hindered the mass propagation of this species in Nigeria. The present study was conducted to determine the effect of different hormonal concentrations of LHRHa on growth

and morphometric characteristics of *C. gariepinus* fry. The aim was to provide information on the use of simple realizable techniques to achieve artificial propagation of the species and to keep pace with the current advances in piscine reproduction by using the LHRH analog.

MATERIALS AND METHODS

Collection of *C. gariepinus* Broodfish: Thirty, eighteen months old gravid females (458.20 ± 2.25) and fifteen matured males (453.97 ± 2.13 g) of *C. gariepinus* were purchased from a private fish farmer at Ihiala, Anambra State, Nigeria. Identification of the individual brood fish was done following the method described by Reed *et al*. (1967). Selection of broodfish was based on ovarian biopsy of the oocytes as described by Legendre (1986). The selected fish were given 2 ppm potassium permanganate, prophylactic-tic treatment and stocked according to gender in two outdoor concrete tanks (1.80 x 1.20 x 0.80 m). Feeding was carried out twice daily at 3 % biomass for 14 days with locally formulated diet (CP = 38 %). The juveniles of *Oreochromis niloticus* (L.) were stocked in each tank to serve as natural food for *C. gariepinus*. In readiness for artificial spawning, the female broodfish (30) were scooped out of the concrete tanks in the evening and randomly introduced in 15 plastic containers (25 l) at 2 fish per container. The male broodfish (15) were left in the tank till the next morning when milt preparation was necessary.

Arrangement of Water Holding Facilities: Ten litres of dechlorinated tap water were introduced into 15 plastic containers (25 l) in 5 rows on elevated platforms in the mini-hatchery of the Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki, Nigeria. Each plastic container was stocked with 2 female broodfish of *C. gariepinus* and left for 6 hours before hormone injection. Fifteen indoor concrete tanks (0.70 x 1.50 x 0.50 m) were washed and disinfected with 0.02 ppm malachite green (fungicide). Thirty centimeters of water were put in each tank from a 500 litre plastic water tank, suspended on 1.00 m high table. Water was sprayed onto the concrete tanks with perforated plastic hoses (0.050 cm diameter) from a height of 0.05m. Water was drained from the tanks through turndown pipes installed to regulate water volume.

Preparation of Milt: One male fish per a pair of female fish was killed, dissected, and the milt sac removed one hour prior to artificial spawning. The sac was cut open with a sharp razor blade and the milt washed into a vial with 0.90% saline solution. For this study, 12 vials with milt were prepared to cater for fish spawned with triplicates of 4 doses (10, 30, 50 and 70 µg Kg^{-1}) of luteinizing hormone releasing hormone analog (LHRHa), i.e. 4 x 3 =. Fish under the control experiment were also injected in triplicates with physiological solution (0.90 % saline).

Artificial Spawning: All the 30 gravid females were weighed (458 ± 2.25 g) 6 hours before the commencement of hormone injection at 8 pm. Six fish specimens (2 fish/container) from each row of triplicate plastic containers were injected with 2 ml of LHRHa with concentrations 10, 30, 50 and 70; while the fish in the control (6) were injected in triplicates with 0.90 % physiological (saline) solution. Similarly, 3 male fish (1 fish/container) for each row of triplicate plastic containers were injected with half dose (1 ml) of the hormone. In all cases, infection was intramuscularly just below the dorsal fin. Water temperature (25 ± 1.0^0C) was measured with a Celsius thermometer. All the induced fish were covered with wooden boards and left for 11 hours.

One induced female fish per plastic container was dissected to recover the ovaries and estimate the percent ovulation (i.e. the percentage of the oocytes in the ovary that were ovulated after injection). Stripping and fertilization commenced at 7 am the next day in accordance with the method described by Hogendoorn and Vismans (1980). The remaining 3 concrete tanks earlier prepared for the study were left empty since no eggs were stripped of the control fish injected with 0.90 % saline solution.

The fertilized eggs (59.80 ± 1.50g) from each hormone treatment were scooped with a plastic spoon and sparsely spread on strands of polyethylene fibres (*kakabans*) submerged in water contained in 12 indoor concrete tanks (0.70 x 1.50 x 0.50m). The now sticky fertilized eggs were left to incubate for 27 hours at 25 ± 1.0^0C.

Hatching commenced after the incubation period. Dead whitish eggs were siphoned out to avoid bacterial and fungal infection. The *kakabans* were then removed. The sac fry were retained in the same concrete tank for 5 days for the yolk sacs to be completely reabsorbed. Mixed zooplankton obtained with No. 35 (10 mm) bolt silk plankton net were fed to the fry, 6 times daily for 10 days. The fry were subsequently transferred to 12 outdoor nursery tanks adequately protected with mosquito-mesh nets. Feeding was by the use of a mixture of palm kernel cake, groundnut cake, brewer's waste and ground crayfish; at 5 % body weight per day for 7 days.

Records of the percent fertilization and hatching, fish standard and total body lengths, as well as head diameter were taken for each hormone treatment. The data obtained were statistically analyzed using analysis of variance (Steel and Torrie, 1980).

Table 1: The results of artificial propagation of *C. gariepinus* hypophyzed with different doses of luteinizing hormone releasing hormone (LHRH) analog

Experimental parameters	Luteinizing Hormone Releasing Hormone (LHRH) analog dosages (µg Kg^{-1})							
	10.00	3.00	50.00	70.00	Control 0.00	Overall Mean	S.E±	Significant difference
Number of gravid females	6.00	6.00	6.00	6.00	6.00	6.00	-	-
Number of sexual mature males	3.00	3.00	3.00	3.00	3.00	3.00	-	-
Female broodfish weight (g)	455.00	460.00	458.00	456.00	460.00	458.20	2.25	n.s.
Weight of mature male (g)	450.50	456.40	460.20	452.40	450.36	453.97	2.13	n.s.
Spawn weight (g)	58.00	61.00	66.00	72.00	-	59.80	1.50	**
% Ovulation (Ov) (or % spawning)	68.00	73.00	82.00	85.00	-	77.00	-	**
% Fertilization (% surviving embryos 10h after fertilization)	71.00	72.00	77.00	78.00	-	75.00	-	*
% Hatching (HT)	56.00	65.00	73.00	75.00	-	67.00	-	**
%Survival (SV)(17 days old fry	68.00	70.00	75.00	86.00	-	75.00	-	**

*S.E± = standard error, * = significant at 5%, ** = significant at 1%, ns = not significantly different at 5%. LHRH analog has chemical structure of: L-Pyro glutamyl –L- Histidyl –L- Troptophyl-L-Seryl-L- Tryosyl-D Alanyl-L- Leucyl-L-Arginyl –L- Proline Ethyl amide supplied by ELISCO SCIENTIFIC EQUIPMENT LIMITED ENUGU, NIGERIA.*

Table 2: The results of growth and morphometric parameters of *C. gariepinus* fry hypophyzed with different doses of LHRHa[1]

Parameters	LHRHa dosage in µKg^{-1}						
	10.00	30.00	50.00	70.00	Control 0.00	Overall mean	Significant level (P =0.05)
Mean Body weight (MBW)	15.20 ± 0.08	15.95 ± 0.70	6.30 ± 0.93	16.50 ± 0.70	-	15.99 ± 0.78	*
Standard body length (SBL)	5.60 ± 0.20	6.70 ± 0.04	6.75 ± 0.40	6.90 ± 0.40	-	7.91 ± 0.16	*
Total body length (TBL)	7.40 ± 0.13	7.90 ± 0.16	8.06 ± 0.16	8.26 ± 0.18	-	7.91 ± 0.16	*
Head diameter (HD)	0.80 ± 0.02	1.75 ± 0.04	1.76 ± 0.03	1.85 ± 0.04	-	1.24 ± 0.03	**

*1. LHRa = lutenizing hormone releasing hormone analog with a chemical structure of: L-Pyroglutamyl-L- Histidyl –L- Tryptophyl –L- Seryl-L-trypsyl-D-Alanyl-L-of: L-Pyroglutamyl-l-Histidyl-L-Tryptophyl-L- Seryl-L-Trysoyl-D-Alanyl-L-Leucycyl-L-Arginyl-L- Proline Ethyl Amide; supplied by ELISCO SCIENTIFIC EQUIPMENT LIMITED, ENUGU, Nigeria. * = significant at 5%. ** = significant at 1%.*

Determination of Parameters: The spawn weight was determined by estimating the mean weight of eggs used to achieve percent (%) fertilization. The percent (%) ovulation was estimated from the weight of eggs released as a percentage of the total weight of the ovary. The percent fertilization was estimated from the surviving embryos 10 hours after fertilization. The percent (%) hatching was the number of hatched fry relative to the fertilized eggs; while the percent (%) survival was the number of surviving fry after 17 days of feeding with mixed zooplankton and artificial diets. The fish standard and total lengths, as well as head diameter, were measured with a metre rule fixed on a fingerling table.

RESULTS

The results of the homestead artificial propagation of *C. gariepinus* using different doses of luteinizing hormone releasing hormone analog (LHRHa) are shown in Table 1. Oocyte maturation and ovulation occurred in all females hypophyzed with 10, 30, 50 and 70 µg Kg^{-1} LHRHa within 11 hours of latency period, at a temperature of $25^0 \pm 1.0^0$C. No spawning was observed in any of the control sets that received saline injection. During stripping, the oocytes were extruded at the slightest pressure and they appeared transparent. When a few oocytes were placed in a Petri dish containing little water and examined under light, the cytoplasm appeared shifted to the periphery. In all hormonal treatments, dead eggs appeared whitish and opaque within 8 to 10 hours of fertilization. The eggs on the *kakabans* hatched after 27 hours of incubation at a temperature of 25^0C. The fry later aggregated at the dark corners of the tanks.

The range values of the mean weight of the female broodfish were 455.00g for fish induced with 10 µKg^{-1} LHRHa to 460.00g for fish induced with 30 µKg^{-1} and the control (0.00µ Kg^{-1}) (Table 1). Similarly, the male broodfish ranged from 450.50g for fish induced with half dose of 10µ Kg^{-1}LHRHa to 460.20g for fish induced with half dose of 50µ Kg^{-1} LHRHa. The range values of the spawn weight (SW) of eggs in the induced female *C. gariepinus* were 58.00g (10µ Kg^{-1} LHRHa) to 72.00g (70µ Kg^{-1} LHRHa). These values varied significantly among the different hormonal treatments ($P < 0.001$) (Table 1).

The range values of the percent ovulation (OV) also varied significantly as the hormonal dosage increased from 10µ Kg^{-1} to 70µ Kg^{-1} ($P < 0.01$) (Table 1). The percent ovulation ranged from 68% (10 µ Kg^{-1} LHRHa) to 85% (70 µ Kg^{-1} LHRHa) and the percent values increased with increasing LHRHa dosage. The percent fertilization (FT) of the eggs ranged from 71% (10µ Kg^{-1} LHRHa) to 78% (70µ Kg^{-1} LHRHa). There was also a significant difference in the values of percent fertilization as the hormonal dosage increased ($P < 0.05$). Both the percent hatching (HT) and percent survival (SV) of the fry (Table 1) followed the same pattern of increase as demonstrated by SW, OV and FT above. Both parameters (i.e. HT and SV) varied significantly with increase in hormonal dosage ($P < 0.01$). Fish under the control experiment, and injected with 0.90% physiological (saline) solution remained dormant to the artificial propagation technique applied and hence did not provide values for SW, OV, FT, HT and SV.

The growth and morphometric parameters of *C. gariepinus* fry namely: mean body weights (MBW), standard body length (SBL), total body length (TBL) and head diameter (HD) are shown in Table 2. MBW, SBL and TBL varied significantly as LHRHa dosage increased from 10µ Kg^{-1} to 70µ Kg^{-1} ($P < 0.05$) (Table 2); while HD was significantly different at 1% ($P < 0.01$). Generally, the growth and morphometric parameters considered in this study indicated that the values for MBW (15.20 ± 0.80g), SBL (5.60 ± 0.20 cm), TBL (7.40 ± 0.13G) and HD (0.80 ±0.20 cm) were least when the fish were induced with 10µ Kg^{-1} LHRHa (Table 2). These values increased progressively up to the hormonal dosage of 70µ Kg^{-1} LHRHa which recorded the highest values for MBW, SBL, TBL and HD (Table 2).

DISCUSSION

While Thalathiah *et al*. (1988) reported a 30% to 60% spawning in *Leptobarbus hoevenin* treated with a combination of 50 to 300 IU of human chorionic gonadotropin (HCG) and carp pituitary extract; Saidin (1986) reported a 50% to 70% spawning in catfish, *Clarias macrocephalus* treated with 4000 IU. HCG. A 68% to 85% spawning was recorded in this study for *C. gariepinus* induced with luteinizing hormone releasing hormone analog (LHRHa), with the best dosage for growth and morphometric characteristics recorded at 70.00µ Kg^{-1}. The difference between the percent spawning in this study and those recorded for other species by Saidin (1986) and Thalathiah (1988) could be due to generic/species differences and type of hormone applied.

The increase in percent fertilization (FT) of *C. gariepinus* eggs as LHRHa increased from 10.00 to 70.00µ Kg^{-1} (Table 1) in this study was consistent with the increase in FT recorded by Nwadukwe (1993) for *H. longifilis* as the dosage of frog pituitary extract applied increased from 30 to 300 mg µ Kg^{-1}. The range values of FT for *C. gariepinus* in this study (71.00 - 78.00 %), with an overall mean of 75.00 %, compared closely with the range values (59.00-86.00%), with an overall mean of 73.00%, recorded by Nwadukwe (1993) for *H. bidosalis.* Similarly, the study recorded a mean percent hatching of 67.00 % for *C. gariepinus* eggs and this value is comparable with the 63.00% percent hatching for *H. longifilis* eggs (Nwadukwe, 1993).

It was evident from this study that the choice of LHRHa to stimulate spawning (77 %), fertilization (75 %) and hatching (73 %) in *C gariepinus* superceded the results of earlier workers (i.e. Thalathiah *et al*., 1988; Saidin, 1986). Hence, the present results are consistent with Donaldson (2003) report that the use of aqueous extracts of either fresh piscine pituitaries, or acetone-dried piscine powder, or HCG has been superceded by the use of LHRH and GnRH analogs. The range of LHRHa dosage suggested by Donaldson (2003) was 5.00 - 100.00 µg Kg^{-1} and this covered the range of 10.00-70.00 µg Kg^{-1} used in this study.

It was observed that the inducement of *C. gariepinus* with LHRHa ranging between 10.00 to 70.00 µg Kg^{-1} resulted in progressive increases in SW, FT, HT and SV (Table 1). This implied that in order to obtain reasonable results from spawn weight (SW), %

fertilization (FT), and % hatching (HT) of the African catfish (*C. gariepinus)* broodfish, up to 70.00 µg Kg^{-1} LHRHa should be applied. This same deduction is applicable to the growth and morphometric characteristics of the *C. gariepinus* fry when MBW, SBL, TBL and HD are considered (Table 2).

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HETEROSEXUAL BEHAVIOUR OF IN-SCHOOL ADOLESCENTS IN OGBADIBO LOCAL GOVERNMENT OF BENUE STATE

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ABSTRACT

The heterosexual behaviour of secondary school adolescents in Ogbadibo Local Government Area was investigated to find out the sexual relationship of schoolboys and girls. The survey research design was utilized for the study and the instrument for data collection was the questionnaire. Data were collected from a sample of five hundred adolescents students. Five hundred copies of the questionnaire were distributed, out of which data from 450 heterosexual active respondents were used for the analysis. Simple percentage was used for the analysis of the data collected. The findings of the study showed that some adolescents experienced their first heterosexual intercourse before the age of twelve years, and had one life-time heterosexual partner. Some never used condom while most of them use condom during heterosexual intercourse. There were also findings on school adolescent's sex sales. Further more, the study revealed that age and school type influenced the adolescent's patterns of heterosexual behaviour. Some recommendations were also made.

Keywords: Heterosexual, Behaviour and Heterosexualism

INTRODUCTION

The problems and consequences of heterosexual behaviour of adolescents in secondary schools in the country particularly Ogbadibo Local Government of Benue state is alarming. For this obvious reason many developing countries including Nigeria had seen the need to include sex education in the school curriculum.

Heterosexual behaviour has been condemned by most religious and cultural groups. Onwuamanam (1982) noted that in Nigeria adolescents no longer adhere to the cultural regulations regarding sex and virginity at marriage which was traditionally regarded as a virtue.

Heterosexual behaviour according to Anderson *et al.* (1991) includes the possession of multiple sexual partners and engagement in unprotected sexual intercourse. Selling or buying of sex and early initiation of intercourse are also components of heterosexual behaviour.

Anderson (1980), considered adolescent as a stage when the desire of the opposite sex becomes an extremely powerful urge which results in tension consuming. According to Anderson, hunger for food and desire for sexual union are two of the strongest drives human being can experience.

Katz (1995) view heterosexuality as a sexual orientation characterized by romantic love or sexual desire exclusively for member of the opposite sex or gender, contrasted with homosexuality and distinguished from bisexuality and asexuality. In addition to referring to a sexual orientation, the terms heterosexuality or heterosexual may also refer to sexual behaviour or sexual activities between people of the opposite sex. Some people identify themselves as heterosexual even though they may engage in sexual activity with both men and women (whether occasionally or regularly). Most people in most societies around the world had mostly experienced heterosexual attraction and engaged in predominantly heterosexual behaviour.

Heterosexualism is sometimes used as a synonym for heterosexuality, that is, a sexual orientation or behaviour. However, heterosexualism (not heterosexuality) is also used in a different sense, to refer to heterosexism (the idea that heterosexuality is superior or normal).

"Heterosexual" was first listed in Merrian-Websters" New International Dictionary as a medical term for "morbid sexual passion for one of the opposite sex", but in 1934 in their second edition, unabridged it is "manifestation of sexual passion for one of the opposite sex; normal sexuality" (Katz, 1995).

Heterosexual behaviour is accompanied with various risks like contraction of diseases such as HIV/AIDS, gonorrhea, and syphilis. Numerous studies have found college students to possess relatively low level of knowledge concerning HIV/AIDS transmission risks and preventive techniques (Fennel, 1990; Anderson and Christenson, 1991; Dorman and Rienzo, 1991). Such low level knowledge has been implicated on the impairment of students' ability to undertake effective risk reduction behaviour (McDomott *et al.,* 1987).

Heterosexual behaviour also leads to teenage pregnancy, abortion or illegitimate children, poverty and dropping out of school. Since adolescents in secondary schools are sexually active, it is therefore worth while studying heterosexual behaviour of adolescent particularly that of Ogbadibo Local Government Area of Benue State.

MATERIALS AND METHODS

The study was designed to find out heterosexual behaviour adopted by secondary school adolescents. The study was specifically directed at:

1. Identifying the pattern of heterosexual behaviour common to secondary school adolescent.
2. Identifying age difference at their heterosexual behaviour.
3. Comparing the adolescences heterosexual behaviour according to school-type.
4. Identify possible problems arising from adolescents' first heterosexual intercourse.

Study Area: The study was carried out in Ogbadigbo local government area of Benue State. Five hundred students from Junior Secondary III and Senior Secondary II were sampled using closed ended questionnaire as instrument for data collection. All data were reported using percentages.

RESULTS

The data in Table 1 showed that the highest proportion of adolescent females (18.8 %) and males (16.8 %) had their first heterosexual intercourse when they were 16 and 13 years respectively. The result also showed that 5.8 %, 11.2 %, 5.4 % of adolescent female experienced their first heterosexual intercourse at the ages of 12, 15 and less than 12 years respectively.

Table 1: Age of Adolescents at their First Heterosexual Intercourse

Age	Girls' School (n = 224)		Boys School (n = 226)	
	F	%	M	%
Less than 12 years	12	5.4	30	13.3
12 years	13	5.8	38	16.8
13 years	27	12.1	42	11.5
14 years	37	16.5	26	15.9
15 years	25	11.2	36	11.1
16 years	42	18.8	27	5.8
17 years	38	16.1	13	5.8
18 years	30	13.4	12	5.3

The results further showed that 11.5 %, 11.5 %, 5.8 % and 5.3 % of adolescents male had their first heterosexual intercourse at the ages of 14, 16, 17 and 18 years respectively. The lowest proportion (5.4 %) females and 5.3 % males had the first intercourse at 12 and 18 years respectively.

Table 2 above indicated that (15.3 %) adolescent males and (13.3%) adolescent females had used condom during heterosexual intercourse. Furthermore, (20 %) male adolescents and (18.7%) female adolescents indicated that they never used condom during their first heterosexual intercourse. The table also showed that 20 % males and 22 % females indicated that they do not know if they used condom during their last Heterosexual intercourse. The table also showed that 13.3 % males and 16.7 % females respectively indicated they had not use condom at their first heterosexual intercourse. Furthermore, while 16 % males and 16 % females indicated they had used condom, 16.7 % males and 14.7 % females indicated they had not used condom during heterosexual intercourse.

Table 3 above showed that 26.7 % in school adolescents do collect money before, during or after heterosexual intercourse and 37.3 % do not collect materials reward before, during or after heterosexual intercourse. The table also showed that about 34 % do not know if they receive favour or promise, before, during or after heterosexual intercourse. The table also showed that 36.7 % adolescents receive favour or promise, 36.7 % collect material reward before, during or after heterosexual intercourse. Generally, the highest proportion (40 %) claimed they do not know whether they collected material rewards while the least proportion (26 %) claimed they do not know whether they collected money before, during or after heterosexual intercourse.

Table above showed that there are differences in the age at first heterosexual intercourse among the various age groups of school adolescents. There are also differences in the age groups in terms of the ages at which majority of them had their first heterosexual experience.

The result also showed that 16.1% of females and 16.1 % of males aged 12-14 years had their first heterosexuals intercourse at the age of 12 years. The table further showed that 6.4 % females and 7.1 % males of those aged 15-17 years had theirs at the age of 15 years. The results also showed that 9.4 % female and 7.4 % had their first heterosexual intercourse at 18 years and above. The result, however, remarkably showed that the least proportion (3.4 %) females and (2 %) males had their first heterosexual intercourse at the age of 12 and 18 years respectively.

The result in Table 5 revealed little or no age difference in the responses of the adolescents regarding collection of money for heterosexual intercourse. The data indicate that 12.5 %, 14.9 %, 11.5 % and 20.1 % of those aged below 12 years, 12-14 years, 15-17 years and 18 years and above respectively showed that they had not collected money for sex.

The table also indicated differences between the various age groups of female adolescents only in their responses regarding collection of material rewards for sex. The table also showed that 10.2 %, 11.5 % and 12.1 % of those aged. 12-14 years, 15-17 years, 18 years and above respectively received favour or promise of favour before, during or after sex. Even though the research work is studying both sexes, the table indicated that only female were responsible for the various forms of sex sale.

The results in table indicated age differences in the adolescents' responses on their age at first heterosexual intercourse based on their school type. The data showed that 14.2 % adolescent's girls had their first heterosexual intercourse at the age of t7 years. The table showed that a higher proportion of adolescent females and males of mixed school (7.6 % and 8.4 %) had their first intercourse at the age of 12 years and 18 respectively.

Table 2: Condom use Among Adolescent of Ogbadigbo LGA, Benue State

Condom use	Yes (n=150)				No (n = 150)				Do not know (n = 150)			
	M	%	F	%	M	%	F	%	M	%	F	%
Intercourse	23	15.3	20	13.3	30	20	28	18.7	10	6.7	23	15.3
During last Heterosexual Intercourse	28	18.7	31	21.7	20	13.3	25	16.7	35	23.3	19	12.7

Table 3: Sale of Sex Among Schooling Adolescents of Ogbadigbo LGA, Benue State

Forms of sex sale	Yes (n=150)				No (n = 150)				Do not know (n = 150)			
	M	%	F	%	M	%	F	%	M	%	F	%
Collecting of money before during or after heterosexual intercourse	-	-	40	26.7	-	-	50	33.3	-	-	39	26
Collecting of materials reward before, during or after heterosexual intercourse	-	-	55	36.7	-	-	56	37.3	-	-	60	40

Table 4: Age at first Heterosexual Intercourse Among the various Age Groups of Adolescents (n = 450)

Groups at first heterosexual intercourse	Below 12 Years (n=8)				12 – 14 years (n = 156)				18 years and above (n = 149)							
	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%
Below 12 years	4	50	4	50	10	7.3	11	8.0	14	9.0	12	7.7	10	6.7	9	6.1
12 years	-	-	-	-	22	16.1	22	16.1	12	7.7	11	7.1	5	3.4	5	3.4
13 years	-	-	-	-	25	18.2	24	17.5	11	7.1	14	9.0	10	6.7	11	7.4
14 years	-	-	-	-	12	8.8	11	8.0	12	7.7	11	7.1	10	6.7	3	2.0
15 years	-	-	-	-	-	-	-	-	10	6.4	11	7.1	12	8.1	10	6.7
16 years	-	-	-	-	-	-	-	-	9	5.8	9	5.8	11	7.4	14	9.4
17 years	-	-	-	-	-	-	-	-	10	6.4	10	6.4	7	4.7	7	4.7
18 years & above	-	-	-	-	-	-	-	-	-	-	-	-	14	9.4	11	7.4

Furthermore the mixed school adolescent boys and girls had their first heterosexual intercourse at the age of 17 years. The data in Table 7 showed that adolescent males (18.2 %) recorded the highest percentage with three life-time heterosexual partners. The highest number of lifetime heterosexual partners for females (10.7 %) and males (9.3 %) in mixed schools were six. The table also indicated that boys school adolescent (9.8%) males recorded lesser number "one" heterosexual partner. The data generally indicated that mixed school adolescent boys and girls had more number of heterosexual partners.

The results from the Table 11 showed the existence of some degree of differences in the responses of the school adolescents from the two-school type (boys' school and girls' school) on condom use. The results indicated that a higher proportion of the adolescent females (36.4%) and (34.2%) for males (34.2 %) in girls' schools and boys' schools respectively indicated higher use of condom during sexual intercourse.

The table also indicated that 33.8 % of male and 33.2 % female adolescent indicated not using condom in boy's school and girls' schools respectively.

Furthermore, 32.0 % males and 30.2 % females indicated they do not know if ever they used condom in boy's school and girl's school respectively.

The data from Table 9 revealed no wide difference between the responses of adolescents in girls' schools and mixed schools. The results showed that while 12 % of adolescents in girls' school reported collecting money for sex, 11.1 % adolescent females in mixed schools also indicated colleting money before, during or after intercourse. The results also showed that adolescents in the girls' schools were almost the same with those in the mixed schools on their responses to the collection of material rewards for sex. The results showed that while 11.1 % of those in the girls' schools reported collecting material rewards for sex, 11.6 % of those in mixed schools also reported collecting material rewards before, during or after sex.

Furthermore, the result showed that the high proportions (33.3 %) of mixed schools adolescent females indicated receiving favour or promise of favour, 8.9 % of those females in the same mixed school indicated that they do not know if they receive favour or promise of favour during heterosexual intercourse.

Table 5: Reported Sale of Sex Among the Adolescents According to the Age of Students n = 450

Forms of Sex Sale	Below 12 years (n = 8)												12 – 14 years (n = 137)											
	Yes				No				Do not know				Yes				No				Do not know			
	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%
Collection of money before during or after heterosexual intercourse	2	25.0	-	-	1	12.5	-	-	1	12.5	-	-	10	7.1	-	-	20	14.6	-	-	20	14.6	-	-
Collection of material reward before, during or after heterosexual intercourse	1	12.5	-	-	2	25.0	-	-	1	12.5	-	-	12	8.8	-	-	15	10.9	-	-	25	18.2	-	-
Receiving a favour or promise of a favour during or after heterosexual intercourse		-	-	-	-	-	-	-	-	-	-	-	14	10.2	-	-	15	10.9	-	-	6	4.4	-	-

Forms of Sex Sale	15 – 17 years (n = 156)												18 years and above (n = 149)											
	No				Do not know				Yes				N0				No				Do not know			
	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%
Collection of money before during or after heterosexual intercourse	-	-	18	11.5	-	-	15	9.6	-	-	10	6.7	-	-	30	20.1	-	-	15	10.1	-	-	-	-
Collection of material reward before, during or after heterosexual intercourse		-	14	9.0	-	-	19	12.2	-	-	22	14.8	-	-	10	6.7	-	-	25	16.8	-	-	-	-
Receiving a favour or promise of a favour during or after heterosexual intercourse	-	20	12.8	-	-	18	11.5	-	-	18	12.1	-	-	10	6.7	-	-	9	6.0	-	-	-	-	-

Table 6: The Adolescents Age at First Heterosexual Intercourse According to School

Age at first heterosexual intercourse	Girls school (n = 225)				Mixed school (n = 225)			
	F	%	M	%	F	%	M	%
Below 12 years	29	12.9	-	-	10	4.4	11	4.9
12 years	28	12.4	-	-	18	8	17	7.6
13 years	30	13.3	-	-	12	5.3	13	5.8
14 years	26	11.6	-	-	16	7.1	15	6.7
15 years	31	13.8	-	-	13	5.8	12	5.3
16 years	25	11.1	-	-	15	6.7	16	7.1
17 years	32	14.2	-	-	14	6.2	9	0.4
18 years & above	24	10.7	-	-	15	6.7	19	8.4

Table 7: Reported Number of Life-time Heterosexual Partners According to the Adolescents School Type

Age at first heterosexual Partners	Boys school adolescents (n = 225)				Mixed school adolescents (n = 225)			
	F	%	M	%	F	%	M	%
One	-	-	22	9.8	9	4	20	8.9
Two	-	-	34	15.1	21	9.3	10	4.4
Three	-	-	41	18.2	23	10.2	20	8.9
Four	-	-	25	11.1	18	8	15	6.7
Five	-	-	39	17.3	12	5.3	17	7.6
Six	-	-	27	12.0	24	10.7	17	7.6
Moe than six	-	-	37	16.4	6	2.7	9	4

Table 8: Condom Use Among the Adolescents According to their School Type

Age at first heterosexual intercourse	Boys school (n = 225)				Girls school (n = 225)			
Condom use	**F**	**%**	**M**	**%**	**F**	**%**	**M**	**%**
Yes	-	-	77	34.2	75	36.4	-	-
No	-	-	76	33.8	82	33.3	-	-
Do not know	-	-	72	32.0	68	30.2	-	-

Table 9: Reported Sale of Sex Among the Adolescents According to their School Types

Forms of Sex Sale	Girls' School students adolescent (n = 225)												Mixed school students adolescent (n = 225)											
	Yes				No				Do not know				Yes				No				Do not know			
	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%	F	%	M	%
Collection of money before during or after heterosexual intercourse	27	12	-	-	27	12	-	-	28	12.4	-	-	25	11.1	-	-	26	11.6	-	-	28	12.4	-	-
Collection of material reward before, during or after heterosexual intercourse	25	11.1	-	-	25	11.1	-	-	25	11.1	-	-	26	11.6	-	-	25	11.1	-	-	76	33.8	-	-
Receiving a favour or promise of a favour during or after heterosexual intercourse	22	9.8	-	-	23	10.2	-	-	23	10.2	-	-	75	33.3	-	-	74	32.9	-	-	20	9.9	-	-

Generally, it was noted that only female adolescents collect money, receive material reward or receive favour or promise of favour, before, during or after sex, while the female adolescents in mixed schools reported the highest sale of sex.

DISCUSSION

The finding of the study indicated that the highest proportion of the school adolescents' boys and girls had their heterosexual intercourse when they were 13 and 16 years respectively. Earlier studies actually indicated that young Nigerian's were heterosexually active but none indicated anything close to the proportion found in the present study. For instance, the Federal Office of Statistics (1992) indicated that nationwide, the median age at first heterosexual intercourse for women aged 30 – 40 years was 16.3 years, in an earlier study. Makinwa (1991) indicated that between 7 and 8 % of young Nigerian girls, had reported having their heterosexual debut before the age of fifteen years. It is, therefore, surprising how things had changed within such short a period of time with regard to the sex life of young Nigerians. However, knowing that the present study covered only twenty-five secondary schools in Ogbadibo Local Government Area, one may argue that the picture in this Local Governments may still look-alike as the one reported by the Federal Office of Statistics (1992) and Makinwa (1991).

Much of those reporting selling of sex were in the majority. In the same vein, Adedoyin and Adegoke (1995) had earlier observed that whenever young girls sell sex in any form they tend to lose the power to negotiate for condom use.

There were previous studies stratified according to school type, upon which to compare the present findings. The finding which compared girls' school with mixed school students indicated that mixed school students (boys and girls) experienced debut at the age of 17 years while girls school students experienced their heterosexual debut at the age of 18 years and above.

Galli (1978) had severally indicated that education and information per se could lead to the acquisition of knowledge, but the knowledge may not always translate to change in behaviour. The evolution of the intervention programme from an empirical study of the students' patterns of heterosexual behaviour was in line with the suggestion of WHO (1992).

Implication of the Study: One of the findings of this study was that the students exhibited certain behaviours which are common to them. They debuted heterosexually at a very tender age of about less than twelve years, kept one life-time heterosexual partner and some did not use condom. Others maintained that they sold sex. The implication of these in relation to sexual transmitted diseases (STD) and AIDS prevention is that intervention work needs to be done on this population. This is so because most of the behaviour listed above pose great danger to the students as far s STD/AIDS transmission is concerned. Their behaviours call for urgent intervention.

The findings that age and school type significantly influenced the adolescents' heterosexual behaviour patterns suggest the need to execute the STD/AIDS intervention programme designed in the course of the study with a lot of attention on the various independent variables as they affect the adolescents. For instance, it suggests the need to recognize that more young adolescents, that their older counterparts use condom so that in the course of executing the programme, extra effort would be made to get the younger adolescents change this pattern of behaviour

Conclusion: On the basis of the findings and discussion the following conclusions were reached:

1. Patterns of heterosexual behaviour common to secondary school adolescents' boys and girls are that they had heterosexual intercourse too early in life, they kept at least one life time heterosexual partner, use of condom with their sexual partner and selling of sex was common behaviour among them.
2. The school adolescent patterns of heterosexual behaviour differed significantly between the various age groups.
3. Remarkable differences existed among the school types in terms of their patterns of heterosexual behaviour.

Recommendations: There is need to introduce, to and intensify innovative sex education programme in primary to tertiary institutions to enable the young ones acquire appropriate knowledge and behaviour about sexual relationships so that they can escape reproductive and sexual problems.

1. A national campaign and series of advertisements should be carried out and such should strive to make adolescent aware of the dangerous consequences of early sexual intercourse.
2. There is need to avoid all those films that advertise on the sale and exhibition of pornographic materials since it encourages younger ones into sexual activity without appropriate knowledge of control.
3. Moral education should be one of the teaching subjects in primary and secondary schools to reinforce the traditional and religious norms regarding sexual behaviours.
4. Adults should serve as a model to the younger ones in their sexual behaviour.

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ACETYLSALICYLIC ACID AND CELLULAR DAMAGE IN KIDNEY OF METABISULPHITE TREATED RATS

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ABSTRACT

The effect of acetylsalicylic acid (ASA) as membrane stabilizers was investigated on the kidney of experimental rats treated with sodium metabisulphite. Administration of sodium metabisulphite has been shown to labilize the plasma membrane of some rat tissues. Sodium metabisulphite (10 mg/kg b.wt) acetylsalicylic while both chemical substances of same dose were both chemicals were concurrently administered to three group of rats for two weeks (14 days) while the fourth (4th) group of rats served as control and were given physiological saline alone. Two 'marker' enzymes, alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were spectrophotometrically determined to monitor the efficacy of acetylsalicylic acid in membrane stabilization. Following the initial administration of metabisulphite alone, immediate significant decreases ($p < 0.05$) in ALP activities were observed. The activity latter recovered towards control value by the tenth day. For ACP, the loss in activities was sustained throughout the experimental period. However, the difference showed no significant difference ($p > 0.05$). In acetylsalicylic acid administered rats the activities of ALP were higher than for the control group while the activities of ACP were not appreciably affected. The combined treatment gave values that were not significantly different from the control values ($p < 0.05$).

Keywords: Acetylsalicylic acid, Kidney, Metabisulphite, Cellullar damage

INTRODUCTION

Acetylsalicylic acid (ASA) is a member of the salicylate drugs earlier known in use to the Greeks and Romans (Roger *et al., 1981*). Substitution of the phenolic group of salicylic acid with acetic anhydride produced acetyl salicylic acid.

Acetylsalicylic acid is a non-steroidal anti-flammatory drug (Roger *et al; 1981*). It has earlier been reported that certain anti-inflammatory drugs are potent membrane stabilizers (Ignarro, 1971). These anti-inflammatory drugs probably act by inhibiting prastanoid synthesis by acetylation of fatty acid cyclooxygenase (EC. 1.14.99.1) (Durand *et al.*, 2002a). Acetylsalicylic acid thus irreversibly blocks cyclooxygenase (COX) (Durand *et al. 2002*), an effect short-lived in endothelial or smooth muscle cells due to resynthesis (Durand *et al.,* 2002b). the duration of cyclooxygenase blockade by ASA however depends on the type of cell studied (Hla and Bailey, 1989) and this difference in the duration of the effect of ASA is the rationale for the use of 50 – 1500 mg/day (Abou-Elenin *et al.,* 2002) of this drug with long inter – dose intervals in an attempt to inhibit thromboxane production in platelets (Patrono *et al.*, 1998). Baghat *et al.,* (1995) reported the possibility of ASA having longer effects *in vivo* than invitro depending on the type of cells studied. The capacity of the anti – inflammatory drugs to stabilize lysosomal membrane of rat liver under invitro conditions have been demonstrated (Ignarro 1971). The stabilization of the Kidney lysosomal membrane by ASA after its labilization by chloroquine has been reported (Ngaha and Akanji, 1982).

Through sodium metabisulphite and other sulphiting agents employed in food preservation have been generally regarded as safe, some recent toxicological findings tend to underscore their safety (Taylor *et al*; 1986).

The possibilities of toxic products resulting from interaction between sulphur dioxide, a compound readily generated by sodium metabisulphite (Wedzicha, 1984) and dietary components had earlier been studied (Baghat and Lockett, 1964).

Sulphites are known to have inhibitory action on some enzymes such as Lactate dehydrogenase and Malate dehydrogenase (Pfleiderer *et al.,* 1956). Sulphonation at the N^6 atom of flavin – active site of Flavo proteins, occurs through formation of chemical adducts with sulphites (Gunnison *et a.,* 1981).

In aqueous solution sodium metabisulphite like other sulphites from sulphurous acid, H_2SO_3 (William and Dennis 1995), the active compound in its preservative property. This acid has two dissociation constants at pH 2 and pH 7. At pH 7 the HSO_3^- and SO_3^{2-} are in about equal proportion. At pH 5 most of the compound is in HSO_3^-. At lower pH value protonation by bisulphate ion HSO_3^- results in molecular SO_2 (George, 2002).

Sulphites inactivate certain enzymes systems such as cytoplasmic membrane of cells thereby altering its permeability (George, 2002) as well as react with pyrimidine bases altering their properties.

Kaplan *et al.* (1975) reported induced oxidation in corn- oil emulsified in 1.5% polysorbate

solution by low concentrations (0.5 mM) of bisulphate. Unsaturated membrane lipids incubated with a large excess of bisulphate was reported to have different chromatographic pattern indicative of addition of bisulphate across double bonds. Such changes in membrane lipids could account for the irritant effect of sulphites (Akagyeran and Southerland, 1980).

Sodium metabisulphite is highly rich in oxygen and oxygen radicals. $O_2^{\cdot}$ is good nucleophile which could react readily with electrophilic sites on biological molecules (Halliwell, 1974). Nevertheless O_2 does not seem to be especially reactive but dismutation of $O_2^{\cdot}$ either spontaneously or by action of super oxide dismutase give hydrogen peroxide, (H_2O_2) whose reactivity is enhanced in the presence of transition metal ions. Transition metal ions break H_2O_2 into reactive radical species (Halliwell, 1978). Thus it was proposed that $O_2^{\cdot-}$ react with H_2O_2 to produce hydroxyl ions, hydroxyl radicals and oxygen.

$$H_2O_2 + O_2^{\cdot-} \longrightarrow {}^{\cdot}OH + OH^- + O_2$$

The non – enzymatic dismutation of $O_2^{\cdot-}$ has been reported to generate oxygen, in the singlet state Khan, (1970). Singlet oxygen is reactive enough to attack molecules such as alkenes and the polyunsaturated fatty acids found in membrane lipids (Halliwell, 1974). The toxicity of sodium metabisulphite through induction of oxidation of lipids of cell membrane because of its high content of polyunsaturated fatty acids chain have been reported (Halliwell, 1978).

Alkaline phosphatase, ALP (EC.3.1.3.1) is a 'marker' enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). It is found mainly in the liver and Kidney (Folley and kay, 1935; Kaplan, 1972). Acid phosphatase, ACP, (EC.3.1.3.2) is a 'marker' enzyme for lysomal membrane (de Duve *et al*; 1962). Found mainly in many animals tissues such as the prostate the seminal plasma (Wilkinson, 1963), showed intense activity in the convoluted tubules of rat Kidney.

These mono ester phosphohydrolases are active at pH 10.1 and pH 4.5 respectively. Their activities in the Kidney are monitored after being insulted with sodium metabisulphite to establish cell damage and the potency of acetylsalicylic acid in repairing such damaged cell membrane.

MATERIALS AND METHODS

Male white albino rats (150 – 200 g) were obtained from the Research Laboratory, Biochemistry Department, University of Ilorin, Nigeria. Sodium metabisulphite was purchased from May and Baker Ltd, Dagenham, England. Acetylsalicylic acid was obtained from Tega Laboratories, Chelsea, London. 4 – Nitrophenyl orthophosphate (disodium salt) was obtained from British Drug Houses (Chemicals) Ltd, Prole, England.

Animal Grouping: Forty rats weighing between (150 – 200 g) were divided randomly into four group of 10 rats each. The first three groups were experimental groups and the fourth the control group. Each group of animals was kept in separate metabolic cages and fed with rat cubes and water *ad libitum*. Each set up was replicated thrice.

Group 1 rats were administered daily with sodium metabisulphite (10 mg/kg body weight).

Group 2 rats were administered daily with solution of acetylsalicylic acid (10 mg/kg b.wt).

Group 3 rats were administered daily with solutions of the two chemical compounds concurrently while rats in the control group were administered with physiological saline alone.

Drug Administration: Solution of sodium metabisulphite (2 mg/ml) and acetylsalicylic acid (2 mg/ml) were prepared in distilled water. They were administered intraperitoneally to rats daily (24 hourly) as enumerated above for 15 days.

Animal Sacrifice: Rats from each group were sacrificed on alternate days (1, 3, 5, 10, 15) starting from the day when administration commenced. Day 1 represents rats that were given one daily dose of appropriate chemical compound or its combination and thereafter sacrificed 24 hours, while day 15 represents rats that were given 15 daily doses of the appropriate chemical compound or its combination and left thereafter for 24 hours before sacrifice (Akanji and Nlumanze, 1987). Rats in the control group administered with physiological saline were sacrificed 24 hours after the 15th dose.

Preparation of Tissue Homogenates: A desiccator containing cotton wool soaked in chloroform was used to anaesthetize the rats until they go unconscious. The rats were taken out and immediately dissected. The Kidney was removed decapsulated, washed, weighed (1 g) and cut into pieces for homogenization in ice – cooled 0.25 M sucrose solution (1.5 w/v) (as buffer to maintain the integrity of the organ) using a pre – cooled enamel mortar and pestle. Triton X-100 was added to a final concentration of 1% (Ngaha *et al; 1979*). The kidney homogenates was frozen over night. This allows unbroken cells to lyse being used for enzyme assay (Akanji and Ngaha, 1989).

Tissue Dilution: The tissue homogenates were diluted using 0.25 M sucrose solution as diluent, before being assayed for protein and enzyme activities. The dilution factors are presented on table 1.

Table 1: Dilution factors for kidney tissue

	Protein	ALP	ACP
Tissue (Kidney)	30	600	600

Enzyme and protein Measurements: The Biuret method of Gromal *et al* (1949) was used to determine protein concentration. The absorbance was read at 540 nm and extrapolated in standard protein curve. The absorbance obtained for each sample was used to obtain the corresponding protein concentration from the standard protein curve. Protein concentration (mg/ml) = C x F where

C = Protein concentration from standard curve, and F = Dilution factor.

The activities of the phosphatase were followed using the assay method described by (Wright *et al; 1972*). All measurements were carried out using spectronic 20, Bauch and Lamb Rochet.

Data Analysis: The data obtained were subjected to statistical analysis (ANOVA) to determine the level of significance.

RESULT AND DISCUSSION

Figures 1 and 2 illustrate the results obtained following the administration of the chemical compounds on the activities of the phosphatases (ALP and ACP) on rat Kidney respectively.

Figure 1 reflects the effect of daily administration of the chemical compounds on the activities of alkaline phosphatase of rat kidney. Following administration of metabisulphite, there was an immediate decrease of enzyme activities which persisted until after the fifth dose. Thereafter a recovery towards control value was obtained. By the 10^{th} day, values were not significantly different from control value ($P > 0.05$.).

Contrarily, administration of acetylsalicylic acid resulted in increased enzyme activity throughout the experimental period. Combination of the two chemical compounds gave values that are not significantly different ($P > 0.05$) from those obtained when acetylsalicylic acid alone was administered.

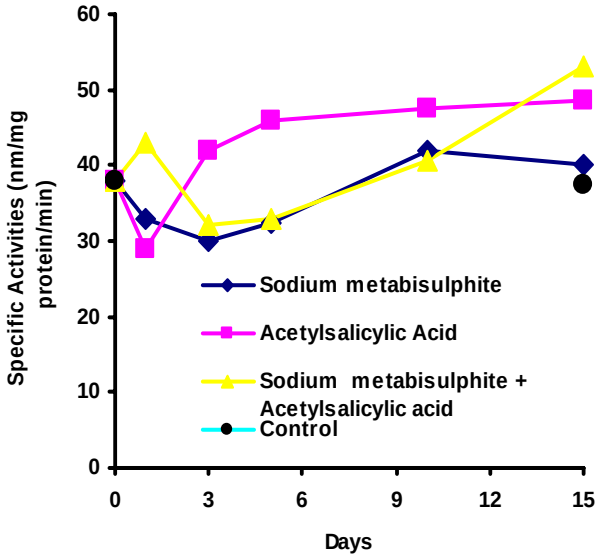

Figure 1: Effect of administration of acetylsalicylic acid on ALP of the kidney of metabisulphite treated rat

Figure 2 illustrated the effects of the individual chemical compounds and the combination treatment on the activities of acid phosphatase. Administration of metabisulphite resulted in loss of enzyme activity up to the fifth day. Thereafter there was recovery of activity and this lasted to the end of the experimental period. A continuous reduction in enzyme activity throughout the duration of experiment attended administration of acetylsalicylic acid. The same pattern was obtained when the two compounds were administered together although the magnitude of loss of activity was smaller.

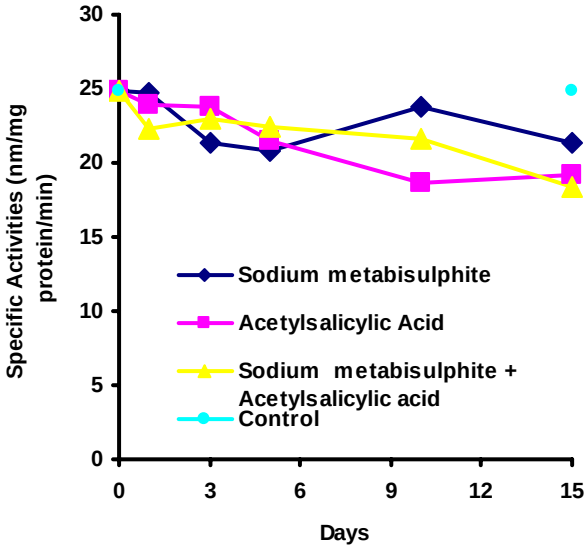

Figure 2: Effect of administration of acetylsalicylic acid on ACP of the kidney of metabisulphite treated rat

It has been shown that biochemical parameters like enzyme assay indicate tissue/cellular damage long before structural damage that can be picked up by conventional biological techniques (Ngaha, 1979 and Akanji 1986). Two 'marker' enzymes were assayed in the present study. These enzymes are found in specific regions of the cell. Alkaline phosphatase is a plasma membrane enzyme (Wright and Plummer, 1974), while acid phosphatase is a lysosomal enzyme (Shibko and Tappel, 1965). The activities of these enzymes before and after the tissue is insulted with chemical agents, can be monitored to deduce pattern and sequence of cell damage.

The organ studied was the Kidney, an organ involved with active absorption of substances (Schanker *et al.*, 1957; Clegg and Clegg, 1975).

In this work, administration of sodium metabisulphite resulted in a significant decrease ($P < 0.05$) in alkaline phosphatase activities in the kidney (figure 1). The loss on the activities of alkaline phosphatase on the tissue could be as a result of either or combination of the following reasons:

i. Damage to the cell plasma membrane resulting from the administration of metabisulphite.
ii. Inactivation of the enzyme in situ (Ngaha, 1982).
iii. Increase in synthesis of other cellular protein elicited by the compounds (Harkness and Roth, 1969).

The result of this study showed that the level of reduction in enzyme activities may not likely be due to inhibition of alkaline phosphatase, but may be as a result of damage to plasma membrane. The kidney plays vital primary functions in all organisms. It is involved in active transportation of molecules and ions across cell membrane (Akanji and Nlumanze, 1987).

The activities of acid phosphatase were not affected to any appreciable extent in the kidney following the administration of the compound. The values obtained showed no significant difference from the control value ($P > 0.05$). it may imply that the integrity of the lysosome where acid phosphatase is located was maintained despite the chemical result. A possible explanation for this might be that sodium metabisulphite has been completely eliminated before coming in contact with the organelles since they have their individual organelle membrane (Wright *et al*., 1979).

When acetylsalicylic acid alone was administered to rats and the kidney enzymes assayed, it was observed that the activities of the enzymes in the kidney were not appreciably affected (fig. 1 and 2). There were high fluctuations in enzyme activities around the control levels.

Acetylsalicylic acid seems to play a stabilizing role, and thus maintained the integrity of the cell membranes. This accounted for the stable activities of the enzymes throughout the period of the experiment.

The concurrent administration of sodium metabisulphite and acetylsalicylic acid to the animals produced distinct pathways from what was observed when metabisulphite alone was injected. The attendant decrease in alkaline phosphatase activities in the kidney when metabisulphite was administered were no longer observed.

Enzyme activities were not higher, but brought towards control level on administration of both metabisulphite and acetylsalicylic acid. Miller and Smith (1966) reported that acetylsalicylic acid stabilizes rat liver lysosomes in vitro while Ngaha and Akanji, (1982) have shown that acetylsalicylic acid stabilizes rat kidney lysosomal membrane after its labilization by chloroquine.

Acetylsalicylic acid has been demonstrated in plasma at level of 0.2 – 1.4 mg/100 ml, 30 minutes after oral administration of 1.2 g of acetylsalicylic acid. It was also been recovered in hydrolysed form in urine indicating that complete hydrolysis of acetylsalicylic acid in vivo does not occur (Miller and Smith, 1966). Thus, this concentration which has stabilizing effect on lysosomes invitro can be achieved *in vivo* following administration of a single dose of 1.2 g of acetylsalicylic acid orally (Miller and Smith, 1966).

Proposed Mechanism: Olagoke (1991) summarized the probable mechanisms to explain the mode of toxicity of metabisulphite in tissues in the following ways,

i. Oxidation of lipids of the cell membrane arising from high oxygen content of metabisulphite.
ii. Production of very reactive free radicals ($^{\cdot}SO_3$) by the compound, which disrupt the ordered lipid bilayer of the cell membrane.
iii. Production of sulphate radical anion $^{\cdot}SO_3$ in (ii) above can lead to further production of oxidizing radical $^{\cdot}O_2$ or $^{\cdot}OH$, which can eventually lead to peroxide formation.

The design of this study is such that it will use a block membrane stabilizer (acetylsalicylic acid) to show whether metabisulphite, disrupts affected membrane by creating gaps along the membrane walls.

Acetylsalicylic acid as a membrane stabilizer (Miller and Smith, 1966; Ngaha and Akanji; 1982) is expected to prevent the disruption of the membrane when it is in concurrent administration with sodium metabisulphite. The results (Figure 1) showed that the loss of alkaline phosphatase activities was prevented when acetylsalicylic acid was concurrently administered with metabisulphite to the experimental animals.

The stabilizing effect of acetylsalicylic acid in preventing the disruption of cell membrane induced by metabisulphite was demonstrated. This may be attributable to the ability of acetylsalicylic acid to lodge itself in spaces created between molecules on the membrane structure.

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SEASONAL TESTICULAR HISTOLOGY AND REPRODUCTIVE CYCLE OF THE RAINBOW LIZARD, *Agama agama agama*, L, (AGAMIDAE, REPTILIA) IN ILE-IFE, SOUTH WESTERN NIGERIA

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ABSTRACT

Seasonal histological features of the testis and epididymis were studied in male A. agama agama from July l990 to June, 1992 at Ile-Ife, Nigeria. Testis weights showed no significant difference (P > 0.05) in the dry and rainy seasons, but were generally low from August to January. Whereas seminiferous tubule diameter and epithelia heights showed no seasonal variation (p > 0.05), the epididymal tubule diameter and epithelia heights varied seasonally (P < 0.001). Although males in full breeding condition were caught all through the months, such were more prevalent from April to July. Females with eggs or enlarged ovarian follicles were caught all through the study period. Cases of multiple clutches were predominant from February to July. However vitellogenic activities decreased from August to January thus coinciding with the observed decrease in spermatogenic activity in the male. We propose that individual male Agama lizards maintain peculiar breeding patterns and that reproduction in Agama seems to be influenced by food availability as well as microclimatic conditions at oviposition sites.

Keywords: Tropical lizards, Agamidae, Histology, Testes, Spermatogenesis, Reproduction

INTRODUCTION

Various studies on testicular cycles of tropical lizard species indicate that spermatogenic patterns are peculiar to each species. Many tropical male lizard species exhibit continuous spermatogenic cycle with little or no evidence of variations in the testicular or seminiferous tubule size (Wilhoft, 1963; Somma and Brooks, 1976; Simbotwe, 1980; Vial and Stewart, 1985; Wikramanayake and Dryden, 1988). Others undergo cyclic changes in their testicular mass as an indication of their reproductive readiness (Wilhoft and Reiter, 1965; Marion and Sexton, 1971; Sexton *et al*., 1971; Sherbrooke, 1975). Most of the observed changes in testicular mass were found to occur mainly during the dry season at which time spermatogenesis also continued at a more or less reduced rate.

The roles environmental factors play in regulating lizard reproduction in the tropics have been variously reported (Licht, 1971, 1973; Gorman and Licht, 1974; Vitt, 1982). Generally, environmental factors exert indirect influence on reproductive strategies of tropical lizards. In tropical regions with clear cut wet and dry seasons, the lizard species have been reported to reproduce during the wet season which is generally regarded as a period of abundant proteinous food (Marshall and Hook, 1960; Janzen and Schoener, 1968; Sexton *et al*., 1971; Janzen, 1973). Lizards occupying tropical environments with non-thermal seasonality have been reported to manifest continuous breeding patterns (Sherbrooke, 1975; Vitt, 1982; Vial and Stewart, 1985). On the other hand, the influence of low temperatures on hatching potentials of eggs have been deduced as the major cause of variations in ovarian cycles of some Anoline lizard species in Puerto Rico (Gorman and Licht, 1974).

The Rainbow lizard, *A. agama agama* is the most common and widely distributed lizard species in Nigeria. It has an enviable tolerance for considerable range of climatic conditions (Harris, 1963, 1964). Information concerning reproductive activities of male *Agama* has been very limited. Sodeinde and Kuku (1989) reported a more or less general account of the presence or absence of spermatozoa in the lumen of the testes for some months. Ejere and Adegoke (2002) linked the presence of polyploid spermatocytes to the reproductive success of male *Agama* at Ile –Ife, South western Nigeria.

The present study was aimed at utilizing data accumulated for 24 month period for males and females of *A. agama agama* to: -

(i) describe the gross morphological and histological features of the testis and epididymis of the males;
(ii) establish the seasonal pattern of their spermatogenic and vitellogenic cycles, and;
(iii) compare the observed reproductive pattern with those described for similar *Agamid* species in other localities within and beyond Nigeria.

MATERIALS AND METHODS

Study Area and Meteorological Data: Data collection was carried out at Obafemi Awolowo University, Ile-Ife, Nigeria sited within the rain forest region of South Western, Nigeria (Harris, 1964). The campus is located at latitude 07^{o} 28^{1}N and longitude

04^{o} 33^{1}E with an altitude of 800 m above mean sea level (MSL).

The meteorological data collation and interpretation are in accordance with that reported earlier for the study area in respect of rainfall, relative humidity and temperature from July 1990 to June 1992 (Ejere and Adegoke, 2002; 2003). Two seasons were determined for the study area. The rainy season began in April and ended in October, while the dry season began from November and ended in March of the following year. The study area also showed little or no monthly temperature fluctuations. This regimen of fluctuating rainfall and lack of thermal seasonality is consistent with the definition of most tropical environments (Sexton *et al.*, 1971; Sherbrooke, 1975; Vitt, 1982; Vial and Stewart, 1985).

Field and Laboratory Methods: Male and female specimens of *A. agama agama* used were caught randomly on a biweekly basis for the 24-month period of the study. The animals used were manually trapped from the walls of houses, in gutters, and on grass lawns which minimized injury to the lizards. They were collected both during the day and night periods. The live lizards were taken to the laboratory within 24 hours of capture and anaesthetized with chloroform.

The snout-vent length (SVL) and intact tail length were obtained to the nearest millimeter using a meter rule. Anaesthetized lizards were then autopsied. For males, the left testis weight was obtained using a Mettler balance. Observations made on the condition of the testes and epididymides were recorded. The left testis and epididymis were then fixed in Bouin's fluid. Standard histological procedures including dehydration in series of graded ethanol, clearing in xylene, embedding in fresh molten paraffin wax, sectioning at 8μ, staining with Ehrlich's haematoxylin and counter-staining with Eosin (Humason, 1979) were employed.

Histological interpretation of the testis and epididymis, as well as the spermatogenic stages were in accordance with the technique of Mayhew and Wright (1970). Diameters of the seminiferous and epididymal tubules as well as their epithelial heights were read off from a microscope fitted with an ocular micrometer. These measurements enabled the determination of probable seasonal changes in the testis and epididymis. Simultaneously, the females were examined for the presence of oviductal eggs; yolking enlarged ovarian follicles, as well as evidence for multiple clutches (Vitt, 1977).

Data Analysis: Pairwise comparisons were made using the T-test while the relationship of variables was determined by linear regression analysis (Sokal and Rohlf, 1981). Results of all statistical tests were considered significant at $P < 0.05$ and highly significant at $P < 0.001$.

RESULTS

A total of 130 male *Agama* lizard species were sampled for the 24 months. All male *Agama* measuring 115.0 mm SVL and above had spermatozoa in the lumen of the seminiferous tubule and were considered as adults.

Testicular Weight Cycle: The mean left testis weights in *Agama* were 200.92 $\pm$ 113.879 mg. Whereas the left testis weights showed no significant difference ($p > 0.05$) between the dry and rainy seasons, some form of monthly variations were evident. The testis weights showed maximal increase from about the end of the dry season in March (298 $\pm$ 113.879 mg) through the beginning of the rainy season in April (340 $\pm$ 113.879 mg). The weights were lowest from about the end of the rainy season in October (52 $\pm$ 113.879 mg) through the beginning of the dry season in November (53 $\pm$ 113.879 mg). For the rest of the months, the testis weights exhibited more or less intermediate values. Secondly, the testis weights in *Agama* showed no correlation with the SVL ($P > 0.05$, $r = 0.038$, $n=72$).

Spermatogenic Cycle: Examination of the testicular histo-sections revealed that spermatogenic activity in adult male *Agama* lizards followed the same general pattern developed for lizard species (Mayhew and Wright, 1970). The proportions of male *Agama* exhibiting these various spermatogenic stages per month are illustrated in table 1. There was the absence of stage 2 tubules in adult male *Agama.* Whereas males with fully developed testes (stage 6) were obtained in all the months of the year, some kind of monthly variations occurred in the proportions. The proportion of male *Agama* possessing testes in full breeding condition was generally low from about the late rainy season (August to October) through the dry season (November to March). There was an increase in the proportion of males in full breeding condition from onset of the rainy season, April (92 %) such that by June and July, all the males caught had stage 6 testes.

There was no statistical difference in the seminiferous tubule diameter and epithelial heights between seasons in this lizard species ($p > 0.05$). However, the seminiferous tubule diameters correlated positively with the testis weights ($p < 0.05$, $r = 0.672$, $n = 72$). Similarly, no correlation was observed between the seminiferous tubule diameter and the SVL ($p > 0.05$, $r = 0.003$, $n = 72$).

Epididymal Cycle: The histological appearance of the epididymis in male *Agama* is in accordance with the descriptions of Mayhew and Wright (1970). They consisted mainly of an external basement membrane with a single row of cuboidal to columnar epithelial cells lining the lumen of the tubule.

Table 1: Monthly percentage of adult male *A. agama agama* exhibiting various spermatogenic stages

Organism	Stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
	1								14		31	37	9	
	2													
	3										8	13	9	
Agama	**4**			7					14					
	5	82	75	73					43	33	8	37	46	
	6	18	25	20	92	75	100	100	29	33	8	13	36	
	7				8	25				33	14			
	8										31			
Number		11	12	15	12	8	7	6	7	9	13	8	11	119

Table 2: Snout-vent lengths, clutch size, number of enlarged follicles and multiple clutches in female *A. agama agama*

Month	Snout-vent lengths (mm)		Oviductal eggs		Enlarged follicles		Proportion of females with evidence of multiple clutches
	Mean SVL	Range	Mean clutch size	Range	Mean	Range	
January	106.6	98-116	4.5 (2)	4-5	10.6 (5)	7-22	0
February	112.9	102-120	6.9 (7)	6-8	0	12(1)	5/8
March	108.6	99.5-122	7.1 (8)	6.9	0	0	4/8
April	110.3	100.5-123	6.0(7)	4-7	0	0	3/7
May	112.5	102-118	5.5(4)	4-7	0	0	¼
June	109.7	105-115	6.5(2)	6-7	0	22.0(1)	1/3
July	114.0	110-118	3.7(3-4)	3-4	0	0	2/3
August	118.5	118-119	0	4.0(1)	0	10.0(1)	0
September	115.0	112-121	5.8(4)	4-7	0	9.0(1)	3/5
October	110.3	103-121	4.7(3)	4-5	6.3 (3)	5-7	0
November	0	116.0	0	0	0	9.0(1)	0
December	116.0	110-125	0	0	12.5(4)	4-24	0

Sample sizes are in parentheses.

The epididymes of testes in stages 3, 4 and 5, had a somewhat mixture of columnar and pseudostratified epithelium. In the full breeding condition (stage 6) the epididymis had a columnar epithelium usually with prominent basophilic granules. The epididymides of testes in stage 7 were observed to possess purely pseudostratified cuboidal epithelium.

There was a steady increase in the epididymal tubule diameter during the breeding stage over that observed in the non-breeding stages. The mean tubule diameter of the epididymis which testes was in stages 3, 5 and 6 caught were 67.80 $\pm$ 26.82μ, 88.40 $\pm$ 26.82μ and 117.80 $\pm$ 26.82μ respectively. The epididymal epithelia heights also showed variations which were largely associated with the developmental stage of the testis in individual males. The mean epididymal epithelia heights of testes in stages 3, 5 and 6 were 11.80 $\pm$ 6.66μ, 19.30 $\pm$ 6.66μ and 23.90 $\pm$ 6.66μ respectively.

Morphologically normal spermatozoa were observed in the lumen of the epididymes irrespective of the stage of development of the testes. However the functional capability of the spermatozoa observed in the epididymes of non-breeding testes was not verified. Furthermore, a highly seasonal statistical significant difference, $p < 0.001$ was observed in the mean epididymal tubule diameters and epithelial heights. The mean epididymal tubule diameter (107.11 $\pm$ 26.992μ) and epithelia heights (21.98 $\pm$ 6.654μ) were highest during the rainy season.

Ovarian Cycle: Female *Agama* with oviductal eggs was caught throughout the year except in the months of November and December (Table 2). The percentage of such females with oviductal eggs was much more in February (87.50 %), March (100 %) and April (100 %). Generally, the percentage of females with oviductal eggs was higher in the rainy season (58.50 %) as against 41.50 % obtained in the dry season. Clutch size varied from 3 to 9 eggs with a mean clutch size of 6 eggs. A relatively high percentage of female *Agama* species exhibiting multiple clutches were observed in February, March, April, July and September. Females with enlarged follicles were observed monthly except in March, April, May and July. No correlation was observed between the SVL and clutch size ($p > 0.01$, $r = 0.166$, $n = 41$).

DISCUSSION

Reports on agamid reproduction in the rainforest region of Nigeria include those of Harris (1964); Ekundayo and Otusanya (1969); Sodeinde and Kuku (1989) and Sodeinde 1992) among others. None of these past studies had dealt in details with the seasonal changes in the morphology and histology of the male reproductive system as well as the state of the ovary in the females. The current study provides a comprehensive data on the gross morphological and histological features of the male reproductive system as well as the reproductive readiness of the female *Agama* lizard.

Spermatogenic activity in adult male *Agama* here-in reported, showed close similarities with those obtained for other adult male lizards (Mayhew and Wright, 1970) with minor variation. Our bi-weekly data showed that the transition from primary to secondary spermatocytes in male *Agama* was very rapid. The consistency of this phenomenon tends to suggest that it is adaptive and actually underscores the reproductive urgency in the adult male of this lizard species. Furthermore, the lack of seasonal variations in the testicular parameters fully indicates that males of this lizard were capable of reproductive activity in both seasons of the year. However, it seems plausible that each adult male is capable of exhibiting distinct spermatogenic pattern. This is more so because males in full breeding condition were observed in those months of the year when the *Agama* population experiences a reduction in reproductive activity (Table 1). This trend is consistent with the assumption that in adult tropical lizards exhibiting continuous spermatogenesis, a reduction in testis weight or size is an indication of reduced sperm production (Daniel, 1960; Sexton *et al*., 1971; Sherbrooke, 1975).

A base line always utilized in any attempt to fully explain reproductive strategies of Adult male lizards have remained the secretory activities of the epididymis and the associated ducts (Hahn, 1964; Wilhoft and Reiter, 1965). Presently, the most distinct feature perceivable in the testicular cycle of this lizard species remains our data on the secretory activities of the epididymis. The changes observed in its morphology were associated considerably with the stage of the breeding condition of individual animals. This constitutes an important indicator of the timing of their breeding activities (Ejere, 1997). Thus, it appears conceivable that much of sperm production, maturation and copulation do occur during the rainy season in male *Agama (Ejere* and Adegoke, 2002). This assertion is also consistent with our data on the proportion of males in full breeding condition (stage 6) (Table 1). Approximately, 64 % of male *Agama* caught during the rainy season was in full breeding condition as against the 23 % of such males sampled in the dry season. Nevertheless both sexes of the *Agama* species experience maximal breeding activity from February to July depicting an overlap between the dry and rainy seasons. This overlap ruled out an overall seasonal component in the breeding behaviour of the Rainbow lizard at Ile-Ife (Ejere and Adegoke, 2002). Daniel (1960) obtained similar results for the *africana* race of *Agama* species at Liberia. This synchrony is very interesting and suggests that the two races might be sub-species of same organism occupying more or less similar ecological setting which is quite distinct from that occupied by the *lionotus* race in Kenya which breeds only at the onset of the rainy season (Marshall and Hook, 1960).

Whatever life history pattern evolved by any lizard species at any point in time should be such which tends to maximise the sum of "present" reproductive success in addition to the probable "future" reproductive success (Stearns, 1976). Hence it is of utmost importance that the ultimate factors which condition the reproductive behaviour of *Agama* species at Ile-Ife, must be fully understood. Our data tend to support the notion that reproduction in *Agama* species is not directly associated with rainfall, but with food availability, soil texture and success of incubating eggs. Food availability for females and hatchlings is of utmost importance in the reproductive behaviour of lizard species (Abts, 1988; Wikramanayake and Dryden, 1988). Though the *Agama* lizard is catholic in its feeding behaviour, the bulk of its nutrition at Ile-Ife is insects (Ejere, 1997). Cases of cannibalism among this lizard species, as well as its subsistence on various food materials such as leaves, grasses, bread crumbs, biscuits etc. during the months of least rainfall (November-January) in the rain forest zone of Nigeria have also been elucidated (Cloudsley-Thompson, 1981). During such dry months, insect abundance is grossly inadequate than the situation during the early first rains and the rainy season when insect foods are abundant in tropical areas (Marshall and Hook, 1960; Janzen and Schoener, 1968; Janzen, 1973). There is a complete implication of dependence on resource availability judging from the fact that the *Agama* population at Ile-Ife experiences an increase in reproductive activities from the end of the dry season (February-March) through the early rainy season (April –July). This opinion is further strengthened by past reports that the oviposited eggs of this lizard species take approximately two months to hatch; 58 days (Sodeinde and Kuku, 1989); 60 days (Sodeinde, 1992). Our data showed an increase in oviposition of eggs by female *Agama* species from February to May (Table 2). Such eggs will hatch at the beginning of the increased rains (April-July) when small insect foods are usually abundant in the environment at Ile-Ife (Ejere, 1997). Females that lay eggs towards the end of the rainy season would have their offspring emerging during the very driest of the year (November-January) when animal protein will be grossly inadequate in the environment (Cloudsley-Thompson, 1981). Hence, it is conceivable that an important consideration in the evolution of life history traits of insectivorous lizard species in the tropics remains the rate of survival of hatchlings (Sherbrooke, 1975; Wikramanayake and Dryden, 1988). Secondly, the preponderance of females exhibiting multiple clutches from February to July, further suggests that the females of this lizard species in South Western Nigeria, have a short period between clutches. As such an individual female *Agama* lizard probably lays at least two or three clutches per year.

Past reports on oviposition by female *Agama* species in the rainforest region of Nigeria, occurred at those periods of the year when sunshine hours are high, resulting in soil temperatures being above the mean, as well as at sites with soft soil texture. Oviposition have been observed in January and March at Ijebu-Ode (Sodeinde, 1992); June at Port Harcourt (Romer, 1953); and February to October at Ibadan (Harris, 1964). That we obtained female *Agama* lizards with oviductal eggs from January to October is

therefore not surprising. Our data therefore, further attest to the fact that oviposition by female *Agama* lizards generally occur within these months in the rainforest zone of Nigeria. This is more so because the soil conditions during these months (January-October) in the rainforest zone have been reported to be favourable to the incubation process of this lizard's eggs unlike the case in the Sudan Savannah region of Nigeria (Harris, 1964; Sodeinde, 1992). Presently, our data seem to suggest that the reproductive behaviour of this lizard may have evolved under predictable environmental conditions. The extent to which this hypothesis holds true will only be unravelled by studies on more widely separated populations of the *Agama* lizard inhabiting varying ecological regions within Nigeria.

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