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## EFFECT OF DIETARY SUPPLEMENTATION OF INORGANIC PHOSPHORUS ON FEED INTAKE, PROTEIN INTAKE, FEED CONVERSION AND PHOSPHORUS GAIN/LOSS OF THE HYBRID AFRICAN CATFISH *Heterobranchus bidorsalis* (♂) X *Clarias gariepinus* (♀) FRY

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### ABSTRACT

*Sixteen experimental diets were formulated to include four groups of inorganic phosphorus (P) sources {monosodium phosphate (MSP), monopotassium phosphate (MPP), monocalcium phosphate (MCP), and dicalcium phosphate (DCP) at four levels {A(0.40%), B(0.60%), C(0.80% and D(1.20%)}. Two controls of a non-phosphorus supplemental diet (CD) and a purified diet (PD) were fed along with the other 16 experimental diets to the fry of *Heterobranchus bidorsalis* X *Clarias gariepinus* hybrid (mean weight, 1.5 ± 0.12 g) at 5% body weight per day for 70 days. The results showed that the feed intake (FI), the protein intake (PI), the food conversion ratio (FCR) and the phosphorus gain/loss (PGL) varies significantly among the 18 diets tested (P < 0.001). The effect of the inorganic P sources on FI, PI, FCR and PGL was also significantly different (P < 0.001). The MSP supplemented diets appeared to elicit better responses in the fish than any of the other P-supplemented (MCP, MPP and DCP) diets. A comparatively higher quantity of protein was consumed by the fish fed the MSP diets (15.28%) than other P-supplemented diets. A loss in the percent phosphorus content of fish flesh fed MSP diet was observed (-0.04%). Based on the above, MSP diets were the best for enhancing growth.*

**Keywords:** Inorganic phosphorus, *Heterobranchus bidorsalis* X *Clarias gariepinus*, Catfish hybrid, Protein Intake, Food Conversion Ratio.

### INTRODUCTION

Channel catfish (*Ictalurus punctatus*) utilize inorganic elements to maintain osmotic balance between fluids in their body and water. Essential minerals may be obtained from water by exchange across gill membrane or from body by absorption across the gut (Phillips, 1959). The salmonid requirements for calcium, magnesium, potassium, sodium and zinc can be met directly from the water (Podoliak, 1970). However, certain minerals such as chlorides, phosphates and sulphates are more efficiently obtained from feed sources (Phillips *et al.*, 1963). Dissolved phosphorus is poorly absorbed by trout (Phillips *et al.*, 1963), while dietary phosphorus is used to meet the majority of the salmonid requirement.

Phosphorus is one of the most important nutrients for growth and normal development of bones in fish (Shin and Ho, 1989). It represents

the third most expensive nutrient following protein and energy (Potchanakorn and Potter, 1987). Phosphorus is a component of a wide variety of organic molecules and it is a major constituent of animal protoplasm. It is also the most limiting nutrient for plant and algal growth in freshwater pond ecosystems (Dobins and Boyd, 1976). Pond ecosystems receiving small applications of phosphorus would respond more to increased phosphorus cycling than those receiving high applications of phosphorus (Lichtkoppler and Boyd, 1977). Diets containing high levels of animal protein may not require supplemental inorganic phosphorus (Phillips *et al.*, 1993). The supplementation of production diet containing 25% whole herring meal with inorganic phosphates produced no significant difference in weight gain, feed conversion and mortalities of rainbow trout (P > 0.05) (Reinitz *et al.*, 1978). Ketola (1975) demonstrated that mineral supplements are required when

soybean meal is substituted for fishmeal in diets fed to Atlantic salmon. Atlantic salmon fed a diet containing 0.70% phosphorus from plant sources required a minimum inorganic phosphorus supplement of 0.60% of the diet for normal growth and survival. The level of available dietary phosphorus required to maintain normal growth in rainbow trout was estimated to be 0.70-0.80% of the diet (Ogino and Takeda, 1978).

Phosphorus in channel catfish pond effluents can be a source of pollution in receiving waters (Schwartz and Boyd, 1994). Therefore, methods for reducing the amount of phosphorus in pond water and effluents have been suggested (Boyd and Tucker, 1985). These include reducing stocking and feeding rates, managing ponds to minimize or eliminate effluents, discharging effluents through settling basins or constructed wetlands, conservative feeding practices and the use of low phosphorus feeds. Boyd (1995) stated that methods to lower the amount of phosphorus added to feeds without reducing fish production should be the primary consideration of fish nutritionists. He maintained that providing high quality feed in a manner that ensures essentially complete consumption of feed by fish could lower phosphorus inputs in ponds. Gross *et al.* (1998) observed that phosphorus recovered from harvested fish from a fishpond tended to increase by approximately 18% as the phosphorus levels in diets increased. They further reported that there was a clear increase in phosphorus adsorption by pond bottom soils as phosphorus in diets increased.

Eya and Lovell (1997) reported that an all-plant commercial type diet with no phosphorus supplement and containing 0.20% available phosphorus was sufficient for maximum weight gain by channel catfish grown to marketable size in ponds. This was lower than 0.27% recorded for the same fish by Robinson *et al.* (1996). Both studies, however, agreed that the phosphorus requirement for the growth of large channel catfish in ponds was lower than that for small channel catfish (1.60-6.00 g) grown in aquaria.

This study was therefore designed to supplement locally formulated diets fed to *H. bidorsalis* x *C. gariepinus* hybrid with either of the four inorganic phosphorus (monosodium phosphate, monocalcium phosphate, monopotassium phosphate and dicalcium phosphate). The growth and survival of the hybrid catfish fry was evaluated. In this study, the phosphorus requirement (0.06 to 0.80%) of

the channel catfish fry (Robinson *et al.*, 1996; Eya and Lovell, 1997) was exceeded as a means of testing higher doses on the survival and growth of the hybrid African catfish fry.

## MATERIALS AND METHODS

Sixteen isonitrogenous experimental diets (CP=38.00%) were formulated to include four groups of inorganic phosphorus sources {monosodium phosphate (MSP), monopotassium phosphate (MPP), monocalcium phosphate (MCP), and dicalcium (DCP) at four levels {A (0.40%), B (0.060%), C (0.80%) and D (1.20%)}. Two controls {a non-inorganic phosphorus supplemental diet and a purified diet (Table 1)} were also formulated and used along with the 16 diets to feed 4-weeks old hybrid of *H. bidorsalis* x *C. gariepinus* (mean weight,  $1.5 \pm 0.12$ g) at 5% body weight per day for 70 days.

One thousand and eighty (1080) advanced fry of the hybrid were randomly stocked into 54 aerated aquaria (55 x 30 x 30 cm). The aquaria received a continuous supply of clean water and their filtration systems helped in the collection of faeces and other residues. Batches of 20 fry were introduced into each aquarium and fed the compounded diets at 4 hourly intervals starting from 8.00 hours. The temperature range throughout the experimental period was between 24°C and 28°C while the water pH was  $6.60-6.80 \pm 0.2$ . Cultured catfishes were weighed with the aid of a Mettler balance every 7 days. The diet was adjusted in accordance with the body weight of fish. Both the experimental fish and test diets were analyzed in the laboratory for their proximate compositions (AOAC, 1995) (Tables 2a, 2b, and 3).

The nitrogen contents of the specimens (fish and diets) were determined by the microkjeldahl technique of Fels and Veatch (1959) and converted to total protein equivalent by multiplying by 6.25. The crude fat was measured in a Soxhlet apparatus of lipid by petroleum ether (b.pt 40-60°C) extraction. The dry weight was calculated gravimetrically after drying at 105°C for 24 hours and ash by combusting in a muffle furnace at 550°C for 12 hours. The nitrogen free extract (NFE) was derived thus:  $NFE = 100 - \% \text{ ash} - \% \text{ moisture} - \% \text{ protein} - \% \text{ lipid}$ . The phosphorus gain/loss (PGL) was estimated thus:  $PGL = \text{final tissue phosphorus} - \text{initial tissue phosphorus}$ . Analysis of variance and least significant difference were used to compare treatment means (Steel and Torrie, 1990).

Table 1: Gross composition of experimental diets fed the African catfish hybrid fry for 70 days

Ingredient	Inorganic P - supplementation				Control diet 0% P	Purified diet	
	A 0.40% P	B 0.60% P	C 0.80% P	D 1.20% P			
Yellow maize	9.81	9.55	9.29	9.07	10.32	Casein	33.00
Soyabean meal	54.76	54.86	54.84	54.86	54.68	Dextrin	35.00
Fish meal	10.95	10.96	10.97	10.97	10.97	Corn starch	20.00
Palm oil	5.00	5.00	5.00	5.00	5.00	Cod –liver oil	3.00
Salt	0.25	0.25	0.25	0.25	0.25	Palm oil	3.00
Vitamin mix <sup>a</sup>	0.60	0.60	0.60	0.60	0.60	Carboxymethyl Cellulose	3.00
Calcium & phosphorus free mineral mix <sup>b</sup>	1.80	1.80	1.80	1.80	1.80	Vitamin mix <sup>a</sup>	1.20
Inorganic phosphorus Supplementation (MSP), MPP, MCP, DCP	0.40	0.60	0.80	1.20	0.00	Calcium & Phosphorus free Mineral mix <sup>b</sup>	1.80
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	

Key: MSP = Monosodium phosphate, MPP = Monopotassium phosphate, MCP = Monocalcium phosphate, DCP = Dicalcium phosphate.

a. Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): Thiamin, 10; riboflavin, 20; pyridoxin, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3000; niacin, 150; vitamin B<sub>12</sub>, 0.06; retinyl acetate (500,000 IU/g), 6; menadione bisulphate, 0.1; inositol, 400; biotin, 2; vitamin C, 200; ethoxyquin, 200; alphatocopherol, 50; cholecalciferol (1,000,000 IU/g).

b. Contained g/kg of premix, FeSO<sub>4</sub>·7H<sub>2</sub>O, 5; MgSO<sub>4</sub>·7H<sub>2</sub>O, 132; K<sub>2</sub>SO<sub>4</sub>, 329.90; KI, 0.15; NaCl, 45; Na<sub>2</sub>SO<sub>4</sub>, 44.88; AlCl<sub>3</sub>·0.15; CoCl<sub>2</sub>·6H<sub>2</sub>O, 5; CuSO<sub>4</sub>·5H<sub>2</sub>O, 5; NaSeO<sub>3</sub>, 0.11; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.07; and cellulose, 380.97.

Table 2a: Proximate composition of experimental diets

Nutrient (%)	Dietary Groups					Purified Diet
	A (0.40% P)	B (0.60% P)	C (0.80% P)	D (1.20% P)	Control Diet (0% P)	
Crude Protein (CP)	38.88	37.85	37.58	36.96	37.65	37.84
Ether Extract (EE)	4.64	5.02	5.68	4.64	5.48	5.32
Ash (AS)	10.56	10.65	10.48	12.32	10.54	5.55
Dry matter (DM)	11.70	11.50	11.80	11.21	11.65	11.70
Nitrogen Free Extract (NFE)	36.28	34.98	34.46	34.87	34.68	39.59
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

Key: Dietary Groups A (0.40% P) B (0.60% P) C (0.80% P) D (1.20% P)

1 2 3 4 -Monosodium phosphate (MSP)  
5 6 7 8 -Monopotassium phosphate (MPP)  
9 10 11 12 -Monocalcium phosphate (MCP)  
13 14 15 16 -Dicalcium phosphate (DCP).

Note: Diets with numbers in the same row were supplemented with the same inorganic phosphorus; Diets with numbers in the same column had the same inorganic phosphorus inclusion.

Table 2b: Phosphorus contents of experimental diets

Ingredient %	Dietary Group A 0.40% inorganic P- supplementation	Dietary Group B 0.60% inorganic P- supplementation	Dietary Group C 0.80% inorganic P- supplementation	Dietary group D 1.20% inorganic P- supplementation	Control Diet 0% inorganic P- Supplementation
Yellow maize (%)	0.12	0.12	0.12	0.12	0.12
Soyabean meal (%)	0.15	0.15	0.15	0.15	0.15
Fish meal (%)	1.90	1.90	1.90	1.90	1.90
Blood meal (%)	0.17	0.17	0.17	0.17	0.17
% Inorganic P-supplementation (i.e. MSP, MPP, MCP or DCP)	0.40	0.60	0.80	1.20	0.00
<b>Total % Dietary P concentration (TPC)</b>	<b>2.74</b>	<b>2.94</b>	<b>3.14</b>	<b>3.54</b>	<b>2.34</b>

Key: MSP = Monosodium phosphate, MPP = Monopotassium phosphate, MCP = Monocalcium phosphate, DCP = Dicalcium phosphate, TPC = Total phosphorus concentration.

## RESULT

Table 3 shows the proximate composition of the hybrid African catfish (*H. bidorsalis* X *C. gariepinus*) fry fed diets supplemented with four inorganic phosphorus sources. Fish fed diets supplemented with DCP had more crude protein deposited in their body tissue (15.28%) than those fed diets supplemented with MSP (15.28%), MCP (14.13%) and MPP (13.10%). The control diet (19.12%) had similar crude protein value as DCP (19.10%). The fish tissue ether extract (EE) recorded were MSP (7.05%), MPP (5.95%) MCP (4.39%) and DCP (3.50%). The EE of control diet (8.69%) and purified diet (8.66%) were higher than those obtained with the P-supplemented diets. Ash content (AS) also varied significantly among the test diets ( $P < 0.001$ ).

The daily feed intake (FI) of fish ranged from 0.37g in the purified diet (PD) to 0.54g in the DCP diet. These values varied significantly among the 18 test diets (Table 4) ( $P < 0.001$ ). The mean effects of the supplementary phosphorus on FI of fish were MCP (0.48g), DCP (0.47g), MSP (0.44g) and MPP (0.43g). The fish fed less of the purified diets (0.37g) than any of the P-supplemented diets and the control diet (0.43g). There were also significant effects of the dietary P sources on the daily consumption of feed by the fish (Table 4) ( $P < 0.001$ ). There were positive correlations between FI and FCR (0.84,  $P < 0.001$ ) and the weekly protein intake (0.87,  $P < 0.001$ ), while FI was negatively correlated with PGL (-0.20,  $P < 0.001$ ). The protein intake (PI) of the fish ranged from 1.01% in the purified diet to 1.47% in the MSP diet. These values also varied significantly among the 18 test diets (Table 4) ( $P < 0.001$ ). The effects of the supplementary phosphorus sources on PI were MSP (1.29%), MCP (1.28%), MPP (1.18%) and DCP (1.18%). Less protein was consumed by the fish fed the control (1.13%) and the purified (1.01%) diets than those fed the P-supplemented diets (Table 4). PI varied significantly with the dietary inorganic P sources ( $P < 0.001$ ). PI was positively correlated with FI (0.87,  $P < 0.001$ ) and FCR (0.84,  $P < 0.001$ ) but was negatively correlated with PGL (-0.19,  $P < 0.001$ ).

The food conversion ratio (FCR) ranged from 1.65 in the purified diet to 4.94 in the MPP diet. The values also varied significantly among the 18 test diets (Table 4) ( $P < 0.001$ ). The effects of the supplemented phosphorus on FCR

were MSP (3.51), MCP (3.72), DCP (4.04) and MPP (4.11). The FCR of fish fed the control (1.82) and the purified (1.65) diets were better than those fed the P-supplemented diets (Table 4). The FCR of fish also varied significantly among the P-supplemented diets and the controls ( $P < 0.001$ ).

The phosphorus gain or loss (PGL) of the fish ranged from -0.05% in the MSP diet to 0.03% in the MCP diet. These values also varied significantly among the 18 test diets (Table 4) ( $P < 0.001$ ). The effects of the supplementary phosphorus on PGL were MCP (0.02), MPP (0.01%), DCP (0.00%) and the purified (0.04%). Fishes fed the control (0.08%) and the purified (0.04%) diets gained more phosphorus than those fed any of the P-supplemented diets (Table 4). The PGL of fish varied significantly among the P-supplemented diets and the controls ( $P < 0.001$ ). PGL showed significant negative correlations with FI (-0.20), PI (-0.19) and FCR (-0.25) ( $P < 0.001$ ).

There were significant effects of duration (days) on FI, PI, FCR and PGL (Table 5) ( $P < 0.001$ ). Whereas protein intake increased progressively from day 7 (0.20%) to day 70 (2.45%), there seemed to be moderate variability of the fish tissue phosphorus as the study progressed from day 7 (0.07%) to day 70 (0.01%) (Table 5).

## DISCUSSION

Data on the hybrid African catfish fed supplemented phosphorus diets is scanty. Fish fed MCP-supplemented diets consumed more of this diet than those fed DCP, MSP and MPP. The significant differences in performance of the P-supplemented diets, the purified and the control diets on feed intake may be attributed to the levels of P-supplementation and sources of inorganic P in the diet (Table 4). The higher quantity of protein consumed by the fish fed with the MSP diet than any of the P-supplemented diets (Table 4) is consistent with the higher protein intake recorded by Andrews *et al.* (1973) for channel catfish, fed MSP diet when compared with those fed MCP and DCP diets. The range values of PI (1.30 – 1.40%) for this study also compared favourably with 1.02 – 1.72% reported for *Oreochromis aureus* fingerling (Wu and Jan, 1977). The significant but negative correlation between PI and PGL ( $P < 0.001$ ) may be an indication that increased protein intake by fish possibly had negative effect on the phosphorus gain/loss.

Table 3: Effect of inorganic phosphorus dietary supplementation on the proximate composition of the African catfish hybrid fry fed for 70 days

Proximate composition	Inorganic Phosphorus Source						S.E	L.S.D	Sign. Level
	A MSP	B MPP	C MCP	D DCP	CD	PD			
Crude Protein (CP)	15.28	13.96	14.13	19.10	19.12	19.00	14.75	0.19	0.42
Ether Extract (EE)	7.05	5.95	4.83	3.50	8.69	8.66	5.70	0.25	0.55
Ash (AS)	2.25	1.85	2.13	2.04	3.59	4.31	2.34	0.10	0.22
Moisture Content	7.12	68.46	65.36	66.12	66.34	65.53	67.16	1.14	0.56
Nitrogen Free Extract (NFE)	5.00	9.78	13.35	12.79	2.17	2.50	7.14	6.14	3.14
Total	100.00	100.00	100.00	100.00	100.00	100.00			

MSP = Monosodium phosphate, MPP = Monopotassium phosphate, MCP = Monocalcium phosphate Dicalcium phosphate, S.E. = Standard error, L.S.d = Least significant difference; \* \* \* = Significant at 0.1% ( $P < 0.001$ )

Table 4: Growth performance of the African catfish hybrid fry fed different inorganic phosphorus supplemented diets

Diet	Feed intake (FI-g)	Protein intake (PI-%)	Food conversion ratio (FCR)	Phosphorus gain/loss (PGL-%)
<b>Supplementation with monosodium phosphorus</b>				
Diet 1 (0.40%P)	0.39	1.31	4.33	-0.03
Diet 2 (0.60%P)	0.45	1.21	4.33	-0.04
Diet 3 (0.80%P)	0.46	1.17	3.52	-0.04
Diet 4 (1.20%P)	0.47	1.47	3.23	-0.05
Mean	0.44	1.29	2.95	-0.04
<b>Supplementation with monopotassium 3.51phosphate (MPP)</b>				
Diet 5 (0.40%P)	0.45	1.18	4.94	0.00
Diet 6 (0.60%P)	0.38	1.03	4.02	0.01
Diet 7 (0.80%P)	0.41	1.09	3.84	0.00
Diet 8 (1.20%P)	0.46	1.43	3.65	0.02
Mean	0.43	1.18	4.11	0.01
<b>Supplementation with monocalcium phosphate (MCP)</b>				
Diet 9 (0.40%P)	0.53	1.28	3.71	0.01
Diet 10 (0.60%P)	0.44	1.17	3.77	0.02
Diet 11 (0.80%P)	0.46	1.24	3.60	0.02
Diet 12 (1.20%P)	0.50	1.45	3.80	0.03
Mean	0.48	1.28	3.72	0.02
<b>Supplementation with dicacium phosphate (DCP)</b>				
Diet 13 (0.40%P)	0.46	1.17	4.32	-0.01
Diet 14 (0.60%P)	0.45	1.03	3.74	-0.01
Diet 15 (0.80%P)	0.41	1.12	3.49	0.04
Diet 16 (1.20%P)	0.54	1.40	4.61	-0.01
Mean	0.47	1.18	4.04	0.003
Diet 17 (control diet)	0.43	1.13	1.82	0.08
Diet 18 (purified diet)	0.37	1.01	1.65	0.04
Overall mean	0.45	1.22	1.61	0.01
S.E. of mean	0.007	0.012	0.001	0.001
L.S.D.	0.021	0.033	0.004	0.001
Significant level	***	***	***	***

Least significant difference; \* \* \* = Significant at 0.1% ( $P < 0.001$ ); S.E = Standard error.

This was evident from the negative values of PI recorded for the fish fed with MSP diets and which had better protein intake than those fed other P-supplemented diets. Lovell (1978) reported that the availability of phosphorus in a diet ensures the maximal utilization of nitrogen, which is a major component of protein

necessary for growth. This was not the case with the P-supplemented diets of this study.

The FCR of fish fed the control (1.82) and the purified (1.65) diets compared favourably with Donald and Robinson (1987) values of 1.60 – 1.90 for red drum (*Sciaenops ocellatus*).



Table 5: Effect of duration (days) on the growth performance of the African catfish hybrid fry fed different supplemental dietary phosphorus

Growth Parameter	7 Day s	14 Day s	21 Day s	28 Day s	35 Day s	42 Day s	49 Day s	56 Day s	63 Days	70 Days	S.E±	L.S.D	Sign. Level
Feed intake (FI) (g)	0.09	0.17	0.24	0.31	0.39	0.48	0.58	0.63	0.70	0.86	0.006	0.016	* * *
Protein intake (PI (g %)	0.20	0.44	0.66	0.84	1.05	1.29	1.56	1.69	1.88	2.56	0.009	0.024	* * *
Food conversion ratio (FCR)	0.38	0.79	1.16	1.46	1.84	2.22	5.62	6.34	7.17	9.13	0.001	0.003	* * *
Phosphorus gain /loss (PGL) (%)	0.7	0.05	0.04	0.05	0.08	0.04	0.34	0.01	0.05	0.01	0.001	0.003	* * *

However, the data on red drum contrasted with the FCR values obtained when the fish in this study were fed with the P-supplemented diets (Table 4). Robinson *et al.* (1996) recorded FCR values of 1.43 – 1.45 for channel catfish (*I. punctatus*) fed isonitrogenous diets supplemented with MSP, while Eya and Lovell (1997) reported FCR values of 1.60 – 1.90 for the same species. However the results of Donald and Robinson (1987), Robinson *et al.* (1996) and Eya and Lovell (1997) compared favourably with the FCR of control diets (1.60 – 1.82) of this study. Feeding fingerlings of *Oreochromis niloticus* with MSP-supplemented diets for 12 weeks, Robinson *et al.* (1996) reported higher FCR values (2.30 – 2.50) relative to the present control (1.82) and purified (1.65) diets. This range values (2.30 – 2.50) was lower than the values (3.51 – 4.04) recorded with the P-supplemented diets of this study (Table 4).

Furthermore, FCR decreased as the P-supplementation levels increased from 0.04 – 1.20 % (Table 4). This result agreed with Sakamoto and Yone (1978) who reported that a decrease of dietary phosphorus resulted in a decrease in glycogen (energy) content of liver, crude ash, calcium and phosphorus content of vertebrae.

The phosphorus gain/loss (PGL) was estimated as the weekly increase or decrease in percent phosphorus content of fish. Despite the higher quantity of protein consumed by fish fed the MSP diet (Table 4), there was a loss in the fish percent phosphorus compared the gains recorded with the MCP and control diet. The availability of phosphorus in a diet ensures maximal utilization of nitrogen, which is a major component of protein necessary for growth (Lovell, 1978). The significant negative

correlations between PGL and PI and FCR ( $P < 0.001$ ) suggested that the above parameters (i.e. feed intake, protein intake and feed conversion) affected the percent phosphorus gain in fish negatively.

The effect of duration (days) on feed intake (Table 5) showed that the quantity of food consumed increased as the age of fish increased. This increased food consumption relative to increasing size and age may be due to the interaction of factors affecting internal motivation or drive for feeding such as season, temperature, time and nature of last feeding, food stimuli perceived by the senses, lateral line system, hunger curiosity and gluttony (Lagler, *et al.*, 1977). As time progressed (7 – 70days), the fish species of this study must have developed a higher sense of perception of food stimuli, which enhanced their internal motivation to feeding. Furthermore, the protein intake increased with time from day 7 to day 70 (Table 5) in agreement with Lagler *et al.* (1988) who reported that increased food consumption relative to size and age is affected by time among other factors. Similarly, the food conversion ratio (FCR) values increased as the study progressed from day 7 to day 70 (Table 5). This implied that time affected the ability of the fish to convert a unit gram of diet consumed to a unit gram of flesh produced. It is possible that the development of the lateral line system and sense of perception of food stimuli of the fish with time increased their internal motivation to feeding (Lagler *et al.*, 1977) and consequent rate of food conversion

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## INFLUENCE OF MILKING FREQUENCY ON LACTATION CHARACTERISTICS OF RED SOKOTO GOATS

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### ABSTRACT

*The study was carried out to determine the consequences of milking frequency on total yield, average-daily yield, peak day, flow rate, dairy merit and persistency in Red Sokoto goats. Thirty lactating does were divided into three categories on milking frequencies, once a day, twice a day and thrice a day milking of 10 animals each. All the does were at their third lactation and were hand milked for a period of 120 days postpartum. Over the 120 days lactation period, total yield, average daily yield, peak yield, peak day, flow rate, dairy merit and persistency were  $55.5 \pm 2.95$  kg,  $0.466 \pm 0.025$  kg,  $0.791 \pm 0.042$  kg,  $33.8 \pm 4.01$  d,  $3.7 \pm 0.25$  g/d,  $10.6 \pm 0.21$  % and  $96.1 \pm 7.92$  %. Milking frequency significantly ( $P < 0.05$ ) influenced total yield, average daily yield, peak yield, and milk flow rate, but not peak day, dairy merit and persistency. Milk yield characteristics increased with milking frequency, but at a decreasing flow rate. In Red Sokoto goats milking frequency affected milk yield characteristics but not dairy merit and persistency. The high lactation persistency of these goats was an indication of their ability to maintain milk production throughout lactation.*

**Keywords:** Milking frequency, Milk yield, Dairy merit, Lactation persistency, Red Sokoto goats

### INTRODUCTION

The World Health Organisation (WHO) recommended protein requirement of an adult is 0.5 - 1.0 g per kg body weight per day (65 g per day) and (approximately 20 g) of this should come from animal protein source (Flachowsky, 1999). On the average about 25 g of animal protein is available per person per day and these ranges between 9 g in Africa and 65 g in North America (Flachowsky 1999). Thus indicating that protein of animal origin for Africa is far below the recommended 20 g per capita per day.

Animal milk is one good source that can be used to bridge this gap. Out of  $537403 \times 10^3$  metric tonne of total milk production from popular dairy animals (cow, buffalo, sheep and goats), goats contribute  $10144 \times 10^3$  metric tonne which is about 5% (FAO, 1997). In Africa, goats contribute  $2078 \times 10^3$  metric tonne which is about 9.2% of total milk production ( $22501 \times 10^3$  metric tonne) from dairy animals (FAO, 1997). A good dairy goat gives about 3.4 litres of milk daily which is 900 – 1800 kg milk in a 305 day lactation period (Haenlein, 1992). Compared to cows, goats will be nutritionally more economical because of their smaller size, require less food and of a type much cheaper

than cows. This implied that the potential production of milk from goats could be further stimulated to increase the overall milk supply from dairy animals. Above all, goat milk fat and protein are readily digested; and the constituent amino acids absorbed more efficiently than those of cows (Jandal, 1996). The mean fat and protein percentages of milk were 4.75 and 3.38% for Red Sokoto; and 6.9 and 3.9% for West African Dwarf goats (Ehoche and Buvanendran, 1983; Akinsoyinu *et al.*, 1977).

Accurate prediction of yield responses to increasing milking frequency is required for sound decisions by dairy producers to optimize economic returns (Erdman and Varner, 1995). Kiel *et al.* (1997) compared twice and thrice daily milking and found that milk yield was increased by 4.7 % and 7.3 %, respectively. Therefore, more frequent removal of milk enables a longer maximal secretion rate (Barpeled *et al.*, 1995). In Red Sokoto goats, both total milk yield efficiency of milk production were influenced positively by increasing milking frequency (Akpa *et al.*, 2001). The current study was therefore carried out to ascertain the influence of milking frequency on lactation characteristics of Red Sokoto goats.



## MATERIALS AND METHODS

**Study Area and Animal Management:** This study was conducted in Ahmadu Bello University Farm, Zaria, using 30 Red Sokoto goats that were at their third parity. The does were allowed to graze sown pasture of Gamba and Digiteria, together with other animals on the farm. In addition, they received concentrate mixture consisting of whole maize, wheat offal and cotton seed cake in the ratio of 1:1:1. Concentrate mixture was given at 3% body weight. The 30 does were grouped into 3 groups of 10 each: for hand milking once a day (OAD) in the morning, twice a day (TAD) in the morning and evening; and thrice a day (THAD) in the morning, afternoon and evening. Each doe was milked for 120 days.

### Data Collection and Estimations:

Measurements were made of each doe for body weight taken every 30 days and averaged over the 120 days in kg; milk yield, MY (kg); milking time, MT (seconds); Average daily yield, ADY (kg); Total yield, TY (Kg); peak yield, PY (kg); and peak day, PD (days). Flow rate (FR) was defined as MY/MT. Dairy merit (DM) was calculated using the measures as described by Rao and Nagaceekar (1979) as follows:

\*Fat corrected milk (FCM) = [(0.4 x milk yield (kg)) + [(15 x Fat yield (kg))]

\*FCM/kg W =  $\frac{FCM}{W}$  where W = body weight (kg)

\*FCM/kg MW =  $\frac{FCM}{W^{0.75}}$  where MW is the metabolic weight

\*FCN/day/kg MW =  $\frac{FCM / day}{W^{0.75}}$  \*Net energy

efficiency (NEE)(%) =  $\frac{750 \times FCM / day \times 100}{(750 \times FCM / day) + 70W^{0.75}}$

Where 750 = kilocalories of energy per kg of FCM and 70 = Basal metabolic rate,

\*Dairy Merit (DM) (%) =

$\frac{NEE \times FCM / day}{FCM / day + 0.173W^{0.75}}$  (Brody, 1945).

The average fat percent in milk obtained for the different animals was used for calculating FCM on the basis of lactation yield in 120 days. Total fat yield was calculated as % Fat x total milk yield.

The lactation persistency for each doe was calculated using the method of ratios

(Ludwick and Peterson, 1943). The total lactation period of 120 days was divided into four consecutive periods of 30 days each; and total milk yield in each period for each doe was recorded. The following ratios were obtained:

$$R_1 = \frac{X_2}{X_1}; R_2 = \frac{X_3}{X_3}; R_3 = \frac{X_4}{X_3}$$

Where  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$  refer to the total milk yield in the four consecutive periods. The ratios were added and weighting factors calculated as:

$$W_1 = \frac{R_1}{R_1 + R_2 + R_3}; W_2 = \frac{R_2}{R_1 + R_2 + R_3};$$

$$W_3 = \frac{R_3}{R_1 + R_2 + R_3}$$

By using  $W_1$ ,  $W_2$  and  $W_3$ , the persistency index (PI) of each doe was calculated as: PI = ( $W_1.R_1 + W_2.R_2 + W_3.R_3$ ) 100.

**Statistical Analysis:** Least squares means and standard errors for total yield, average daily yield, peak day, flow rate, dairy merit and persistency were estimated (SAS, 1989). These characteristics were subjected to analysis of variance using the estimated values for each of 10 does in the 3 milking frequency groupings. Significant means were separated using the Duncan's Multiple Range Test. (SAS, 1989).

## RESULTS AND DISCUSSION

Table 1 shows the effect of milking frequency on dairy characteristics of these goats. While milking frequency significantly ( $P < 0.05$ ) influenced total yield, average daily yield, peak yield and milk flow rate, did not affect peak day, dairy merit and persistency of these goats. The milk yield characteristics increased with increase in milking frequency. Conversely, increase in milking frequency decreased milk flow rate.'

The observed corresponding incremental effect of milking frequency on milk yield characteristics have been reported by several other workers (Eradman and Varner, 1995; Barpeled *et al.*, 1995; Klei *et al.*, 1997). As was observed by Barpeled *et al.*, (1995), the main effect of milking frequency on milk yield was directly related to the actual milk removal from the udder, regulated by the presence of protein in the milk that inhibits milk secretion and the feedback inhibitor of lactation (FIL). As milk accumulates in the udder between milking, secretion rate gradually decreases because of the action of FIL.

**Table 1: Least squares means of dairy characteristics by milking frequency**

Character	N	Once	Twice	Thrice	Overall
Total yield (kg)	10	39.6±3.01 <sup>b</sup>	60.1±3.59 <sup>a</sup>	66.8±2.58 <sup>a</sup>	55.5±2.95
Mean daily yield (kg/d)	10	0.3±0.03 <sup>b</sup>	0.5±0.03 <sup>a</sup>	0.6±0.02 <sup>a</sup>	0.5±0.03
Peak-yield (kg/d)	10	0.6±0.03 <sup>b</sup>	0.8±0.05 <sup>a</sup>	0.9±0.06 <sup>a</sup>	0.8±0.04
Peak day (d)	10	27.5±1.41	29.8±7.68	36.8±6.71	33.8±4.01
Flow rate (g/s)	10	4.4±0.48 <sup>a</sup>	3.8±0.45 <sup>ab</sup>	2.9±0.21 <sup>b</sup>	3.7±0.25
Dairy merit (%)	10	10.3±0.47	10.6±0.28	10.9±0.34	10.6±0.21
Persistency (%)	10	96.6±6.30	92.0±2.25	99.5±15.21	96.1±7.92

a, b means along the same row with different superscript differ significantly ( $P < 0.05$ )

More frequent removal of milk from the udder enables a longer maximal secretion rate than less frequent one, causing milk accumulate in the udder between milking, leading to gradual decrease in milking secretion rate due to the FIL effect. Therefore, milking frequency is extrinsic in action; thus explaining why it had no influence on peak day, dairy merit and persistency which are intrinsic properties of the does that are less subjected to extrinsic influences.

Since labour for milking harvest accounts for as much as 80% of animal milking cost (Blake and McDaniel, 1978), and over 50% of the routine operational requirement of dairy farms (Albright, 1964), labour for milk harvest may be reduced by adopting a system of milking that allows high milk yield at a faster flow rate. For economics of milk production therefore, twice a day (TAD) milking appears to provide optimum milking frequency for Red Sokoto goats in this study. This is because there was a 40% (200 g) increase in milk yield with a marginal reduction in flow rate of 14% (0.6 g/s) compared to once a day (OAD) milking. Although thrice a day (THAD) milking had a significant increase in milk yield of 50% (300 g), there was a wide reduction in milk flow rate of 52% (1.5g/s) when compared with OAD milking. All the same, THAD milking provided an increase of 10% (56 g) over TAD, but this was not significant ( $P > 0.05$ ) and cannot compensate for the reduction in the milk flow rate of 31% or 0.9 g/s. This suggests a better profit and economic margins for TAD since milking speed determines the dairy labour profit (Dodd and Foot, 1953)

**Conclusion:** In this study, milking frequency significantly influenced milk yield characteristics but not peak day, dairy merit and persistency in Red Sokoto goats. It appears that twice daily milking of these goats would provide a better profit and economic margin for the farmers.

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## PHENOTYPIC CHARACTERISTICS OF THE AFRICAN GIANT SNAIL, *Archachatina marginata* SWAINSON

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### ABSTRACT

*Observations were made on 'gigantism' and albinism in the giant snail, Archachatina marginata. Gigantic snails were initially about twice the size of normal snails of same age. However this growth superiority of 'gigantic' snails apparently slowed down with age. Albinism in the snails was expressed in form of creamy-white bodies instead of the normally brownish colour. Albino snails however retained normal shell colouration. All offspring of these albinos maintained these same characteristics. There was no difference in mortality rates of albino and normal snails. 'Gigantism' and albinism have serious implications for commercial snail farming.*

**Keywords:** Albinism, *Archachatina marginata*, Giant land snail, Gigantism

### INTRODUCTION

Giant land snails constitute an important protein source for many of the inhabitants of West Africa, especially the rainforest zone. Whether in the rural or urban areas in southern Nigeria, it is hard to find a market where giant land snails are not displayed for sale. The two main genera of giant land snails in W. Africa are *Achatina* and *Archachatina* both of which show preference for primary rainforest and moist secondary forest. The flesh of the giant snails is of remarkable nutritive value with high iron content (Ogbeide, 1974) and a protein content of 37.0 – 51.3 g/100g dry matter (Udedibie, 1989). Snail meat also has profound cultural and medicinal values in many rural W. Africa communities. Among the Igbo of south-eastern Nigeria, snail meat is an indispensable item in the diet of nursing mothers. Osemeobo (1992) listed 15 health conditions that are believed to be curable with the meat, fluid and shell of African giant snails.

Snail production in the wild has been on the decline due to the depletion of the rainforest, overharvesting of snails, bush burning and the increased use of agricultural pesticides. In many urban centres in Nigeria, the land snail has already attained the status of a highly priced delicacy within the reach of only a few. With growing awareness of the role of cholesterol in various heart and arterial diseases, the demand for low cholesterol meat

like snails has even become more acute. Interest is growing on commercial snail farming as a means of meeting the demand for snail meat. One of the greatest constraints to commercial snail farming is the slow growth rate of snails which may take as much as 8 – 15 months to reach market size/sexual maturity, depending on species and culture conditions. Considerable research effort on snails has therefore largely focused on growth.

Though previous studies have identified factors that enhanced growth in snails to include high levels of exchangeable cations like calcium and magnesium (Gomot *et al*, 1986), highly varied diet (Monney, 1994; Okorie, 2003) and humic acid (Elmslie, 1998), a lot more information on growth is needed to enhance the commercial viability of snail farming. This study is a preliminary report of two abnormal growth characteristics, namely, 'gigantism' and albinism in the giant land snail, *Archachatina marginata*, under culture conditions

### MATERIALS AND METHODS

Specimens of giant land snails, *A. marginata*, including few albino snails, collected from the wild were cultured indoors in wooden vivaria. Identification of snails was done with the aid of keys and descriptions from Bequaert (1950) and Hodasi (1984). The wooden boxes used for culturing the snails measured 30 cm x 60 cm x 35 cm with a 15 cm layer of humus soil in the

bottom. The snail in the cages was replaced with fresh soil every 8 weeks. The snails were fed *ad libitum* with a variety of local leaves and ripe fruits. The leaves fed the snails were paw-paw (*Carica papaya*), cocoyam (*Colocassia esculenta* and *Xanthosoma sagittifolium*), cassava (*Manihot utilissima*), banana (*Musa sapientum*) and plantain (*Musa paradisiaca*). Ripe fruits were paw-paw, banana and oil palm fruit (*Elaeis guineensis*). Humidity of the vivaria was maintained by daily sprinkling of water on the vivaria soil and keeping the culture houses fairly closed. Growth measurements were based on total shell lengths (Plummer, 1975) using vernier calipers, to the nearest 0.1mm. Other morphometric features taken were length of body whorl (L. bw), width of aperture (Wa), shell thickness (S.T), shell width (Wd) and length of aperture (La).

For purposes of comparing mortality records of normal and albino snails, the two populations were segregated into separate cages to avoid cross-breeding. The hatchlings from each clutch were isolated in a separate cage for future growth studies. For each clutch the number of surviving snails was noted at weekly intervals. The number of surviving snails was expressed as a percentage of total number of snails in each clutch per week. Altogether 10 clutches each of normal and albino snails were used for this study. In this way, the overall percent survival in the normal and albino snail populations was computed for a 48-week rearing period.

## RESULTS AND DISCUSSION

**Identification:** Based on the keys and descriptions provided by Bequaert (1950) the giant land snail identified in this study was *Archachatina marginata*. The distinguishing characteristic of the species is the presence of more or less strongly engraved subsutural lines on the shells. Based on the same identification keys, four subspecies were identified among the snails, namely, *A. marginata marginata*, *A. marginata suturalis*, *A. marginata grevillei* and *A. marginata eduardi*.

There is no mistaking the specimens for *Achatina* sp. While the shell of *Archachatina* sp is characterized by a wide, bulbous or dome-shaped apex, the shell of *Achatina* sp is broadly ovate and subglobular with regular conical spine and narrow apex (Hodasi, 1984). In addition, while *Archachatina* produces a few (rarely more than 10 in a clutch) and relatively large eggs,

*Achatina* produces numerous and usually small eggs.

**Gigantism:** Extraordinarily large young snails were observed on two occasions. In both cases the gigantic snails were the lone surviving hatchlings in their respective clutches. While the body and foot were more or less brownish like other snails, the shells of the gigantic snails were distinctly darker in colouration. Table 1 compares some morphological features in normal snails against gigantic snail. In terms of shell size, expressed as shell length, the gigantic snails were obviously larger than other snails of same age. On the second day of life, the shell of gigantic snail (A.1) was x 1.94 larger than the average shell length of the normal snails of same age. Similarly, a second gigantic snail (A.2) was x2.10 larger than the average size of snails of same age. However, with age, the size differences narrowed. By the 5<sup>th</sup> month, when A.1 died, it was only x1.43 larger than normal snails. Similarly, when A.2 died in 14 months, it was only x1.15 larger than the average normal snails of same age. Thus the growth superiority of the gigantic snails apparently slowed with age.

It is possible that the extraordinary snails were hybrids resulting from crosses between different subspecies in the vivaria. Such subspecific crosses have been reported (Stievenart and Backeljau, 1994) between *Achatina fulica hamelei* and *Achatina fulica rodatzki*, though there was no mention of any form of gigantism. It is also possible that the gigantic growth of the hatchlings was environmentally induced and has no genetic basis. Elmslie (pers. comm.) suggested that the early growth advantage was due to egg-cannibalism and does not affect the final body size. While acknowledging many instances of inbreeding depression in snails, the communication did not identify any instance of hybrid vigor in land snails. Elmslie was of the opinion that gigantism in young snails was as a result of egg-cannibalism. Elmslie's view on egg-cannibalism was opined from Baur (1988) who demonstrated that hatchlings of *Helix pomatia* (L.) possessed an innate propensity for egg-cannibalism. According to the study, hatchling *H. pomatia* first ate a hole in their own eggshell and then devoured it completely.

There is also a slim possibility that the gigantic snails were sterile, though the results here are far from conclusive: abnormal snail (A.2) had not laid any eggs by the time it died

Table 1: Morphological comparisons of normal snails against cases of 'gigantism' in *A. marginata*

Character	Range	Normal snails (84) * Means $\pm$ S.E	Abnormal snail, Abnormal snail,	
			A.1	A.2
S.L (mm)(at 2 days)	8.7-11.6	10.1 $\pm$ 0.77	19.6	21.2
S.L (mm)(at 5 mon)	43.2-52.4	47.00 $\pm$ 1.5	-	67.2
S.L (mm)(at 14 mon)	76.5 – 104.6	89.1 $\pm$ 1.4	102.5	-
L.bw/S.L	0.53-0.67	0.58 $\pm$ 0.003	0.57	0.58
Wd/S.L	0.55-0.64	0.59 $\pm$ 0.003	0.61	0.63
La/S.L	0.54-0.69	0.59 $\pm$ 0.005	0.59	0.60
Wa/S.L	0.30 – 0.38	0.34 $\pm$ 0.003	0.33	0.32
Wa/La	0.52-0.68	0.59 $\pm$ 0.005	0.58	0.57

S.L = Shell length, L.bw = Length of body whorl, Wa = Width of aperture, S.T = Shell thickness, Wd = Shell width, La = Length of aperture, \* Total number of snails.

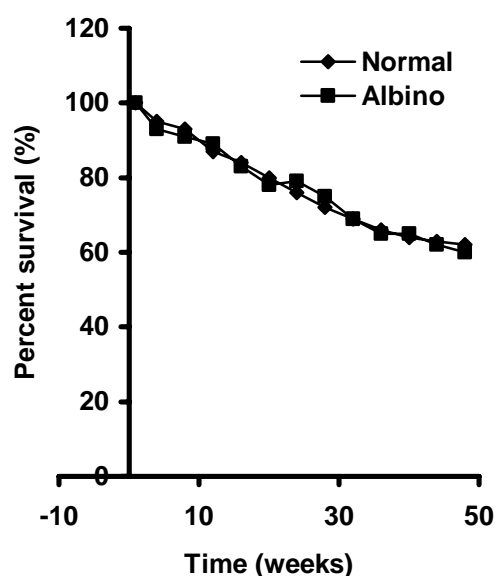


Figure 1: Survivorship curves of normal and albino snails

in the 14th month, just about the upper age limit of sexual maturity in these snails. In spite of these controversies, there is need for a comprehensive investigation of possible interbreeding of subspecies of *A. marginata*, as this may have profound implication for commercial snail farming where the slow growth of snails is the major constraint.

**Albinism:** Albino snails had entirely creamy-white body and foot. Only the eyes showed dark pigment. The shells, however, displayed the usual variability of shell pigmentation seen in normal snails. The albino snail in this study laid several batches of eggs and 100% of the offspring were albinos.

Similarly, Plummer (1975) reported the appearance of a colour mutant in a laboratory

colony of *Archachatina marginata* subspecies *ovum*. According to the report, there appeared to be a general trend towards a paler body colour in cultured snails. But there was one abrupt colour change which the study attributed to a recessive mutation.

In view of the aversion for light by land snails, it was speculated that albino snails may be subject to a higher mortality rate than normal snails. However, this study showed no appreciable difference in the mortality rates between the two groups. Figure 1 shows survivorship curves of albino and normal snails during their first 48 weeks of life. Perhaps, there would have been a disparity in the mortality rates if the snails were subjected to stressful culture conditions involving more exposure to sunlight.

There is also some controversy as to whether albinism in snails should be defined in terms of the colour of the shell or colour of the body. Unlike all the other ten (10) subspecies of *A. marginata* described by Bequaert (1950) which all display considerable variety in shell pigmentation, only *A. marginata grevillei* has uniformly straw-yellow shells. In local parlance, they are referred to as 'white snails' and often looked on as albinos. A close look at the shells shows that the shell apex, parietal wall and columella are extensively red. Furthermore, the snail body (i.e. head and foot) is coloured, showing the same more or less brownish colouration as in other subspecies.

Albino snails are of little or no food value, as their lack of pigmentation is attributed to fetish beliefs in typical W. African communities and only a few persons would dare consume them. However, they may be highly useful research tools in the study of albinism in man and other animals. Also, in view of their



beauty, they may be used as ornamentals in indoor glass vivaria.

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## EFFECT OF FEEDING *Hordeum jabatum* HAY SUPPLEMENTED WITH *Leucaena leucocephala* ON NUTRIENT DIGESTIBILITY IN SHEEP

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### ABSTRACT

*The fermentation profiles and nutrient digestibility of Leucaena leucocephala as a supplement to Hordeum jabatum hay was investigated using twelve castrated sheep averaging  $24.4 \pm 2.2$  kg body weight (BW). Six of the sheep were fistulated at the rumen and used for ruminal pH, ammonia and volatile fatty acid determination in rumen fluid. Dried leaves of Leucaena leucocephala were offered as supplement at two levels, 25% (diet 2) and 50% (diet 3) of dry matter intake (DMI), replacing Hordeum jabatum hay diet. The basal hay diet without supplementation was diet 1. Rumen liquor was sampled one hour before, and one, three and five hours after the morning feeding. The sheep were subjected to digestibility trial. Sheep on diet 3 had higher ( $P < 0.05$ ) ruminal pH than sheep on diets 1 and 2, respectively. The ruminal ammonia concentration of sheep on diet 2 was superior ( $P < 0.05$ ) to those on diet 1 but not with diet 3. Diet 1 had superior ( $P < 0.05$ ) volatile fatty acid concentration than diets 2 and 3, respectively. There were no differences ( $P > 0.05$ ) in the dry matter, organic matter, neutral detergent fibre, acid detergent fibre and hemicellulose intake among treatments. There were however, significant ( $P < 0.05$ ) differences in the digestibility of nutrients among treatments. It was concluded that dried leaves of Leucaena leucocephala has a forage potential for livestock farmers. It can be classified as a plant of moderate fodder value.*

**Keywords:** *Leucaena leucocephala*, Rumen parameter, Nutrient digestibility, Wethers

### INTRODUCTION

Browises, in the form of fodder trees and shrubs, form an integral part of farming systems in humid West Africa (Atta-Krah *et al.*, 1986). As their establishment and management require little effort, labour, time, technical know-how and resources, it should be easy to promote and intensify their use as animal feed. The multipurpose nature of browises as fuel wood, shade, food (fruits), poles, etc, as well as their potential to improve soil fertility and conservation, are added incentives. In terms of utilisation as animal feed, browises currently play an important albeit non-strategic, role, as animals under confinement often receive one type or another of browse, from fallow lands or around homesteads (Reynolds and Adediran, 1988). Efficient utilisation in a complementary way with grass forages and crop residues is what needs to be worked out through research, in order to exploit their potential nutritive value. Data in the literature (Reynolds, 1989; Ademosun, 1988) demonstrated the potential

complementary roles between browises and grass forages. Although the nutrient contents of some common browises indicate on the average that browises contain more crude protein and organic matter, but less fibre than tropical grasses, they nevertheless contain anti nutritional factors that limit their utilisation (Osakwe *et al.*, 1999; Osakwe, 2003). This study was therefore designed to examine the effect of *Leucaena leucocephala* supplementation with *Hordeum jabatum* hay on nutrient digestibility in wethers.

### MATERIALS AND METHODS

The study was conducted at the Institute of Animal Nutrition, (450), University of Hohenheim, Germany.

***Leucaena leucocephala:*** This leguminous tree of the Mimosaceae family, grows up to 15 metres tall and has its origin in the tropical regions of America. It is a classical fodder tree and as a legume plant, serves to improve soil

fertility. Its use as a fodder plant is restricted by its content of anti nutritional factors particularly mimosine. Leaves from mature *Leucaena leucocephala* from the humid zone of Cotonou (Benin) Republic were collected during the dry season, sun dried on a raised wooden platform at the experimental station of "Direction de la Recherche Agronomique", Cotonou. The dried leaves were then packed in plastic containers and transported to the University of Hohenheim, Germany for analysis and feeding trial.

**Hay:** The hay consisted of grasses harvested in mid-October at the Hohenheim University. Grass species composition was predominantly foxtail barley (*Hordeum jabatum*).

Dried leaves of *Leucaena leucocephala* were offered as supplements at two levels, 25% and 50% of dry matter intake, replacing *Hordeum jabatum* hay in the basal hay diet. The experimental diets were as follows:

Diet 1 (100% *Hordeum jabatum*), diet 2 (25% *Leucaena leucocephala* leaves + 75% *Hordeum jabatum*) and diet 3 (50% *Leucaena leucocephala* leaves + 50% *Hordeum jabatum*). In addition to the experimental diet, animals received a mineral premix supplement (10 g d<sup>-1</sup>). Feed was offered twice a day at 0800 and 1600 hr. Water was provided *ad libitum*.

**Rumen pH:** Rumen liquor was taken one hour prior to feeding and one, three and five hours after feeding directly by means of a vacuum pump with plastic tube thrust into the rumen compartment. Immediately after collection, pH was measured with Schott CG 840-pH Meter. The samples were then immediately freed of coarse particles by filtration through cheese-cloth and centrifuged at 2500g for 20 min under refrigeration.

**Rumen Ammonia:** Ruminal ammonia was determined using 5 ml of the rumen filtrate that was diluted with 45 ml of de-ionised water and then 0.5 ml of 10 mol/l NaOH added. The gas released was measured immediately using a gas sensitive electrode. A standard solution was used for the calibration curve for an ammonia electrode as described by Cammann (1979).

**Ruminal VFA Determination:** Volatile fatty acid pattern in ruminal fluid was determined in duplicate using 5 ml of the rumen filtrate that was vacuum distilled according to Zijlstra *et al.* (1977). Thereafter, gas-chromatography analysis was made using HP 5880A series gas-chromatograph with hp 7671A automatic sampler

**Animal Trial:** Twelve castrated sheep (average body weight 24.4 ± 2.20 kg) were used in a completely randomised design to determine nutrient digestibility of sheep fed *Hordeum jabatum* supplemented with *Leucaena leucocephala*. In trial 1, four sheep each (two fistulated and two non fistulated) were randomly assigned to diets 1, 2 and 3, respectively. The animals were adapted for 10 days to the experimental diets. This was followed by an 8 day collection period in which animals were kept in individual metabolism crates to measure food intake, and to collect faeces and urine outputs.

**Analytical Methods:** Feed samples were ground in a hammer mill to pass a 1mm mesh sieve for chemical analysis. Feed and faecal samples were analysed for moisture (934.01), ash (942.05), crude protein (988.05), fat (920.39), by procedures of AOAC, (1990). Neutral detergent fibre (NDF), Acid detergent fibre (ADF), and Acid detergent lignin (ADL) were determined as described by Van Soest *et al.* (1991). The difference between NDF and ADF was designated as hemicellulose, and between ADF and ADL as cellulose. Gross energy of feed and faeces was measured by bomb calorimeter using benzoic acid as a standard (26437 J/g). Analyses of extractable condensed tannins were carried out by the method described by Markkar *et al.* (1993). Ruminal ammonia was determined as described by Camman, (1979) and volatile fatty acid as described by Zijlstra *et al.* (1977).

**Statistical Analysis:** Analysis of Variance (ANOVA) was used to analyse the data using the General Linear Modelling Procedure (SAS, 1985). Significant treatment means were separated using Duncan's Multiple Range Test (Duncan, 1955).

## RESULTS

The chemical composition and gross energy content of the experimental diets and *Leucaena leucocephala* is presented in Table 1. *Leucaena leucocephala* has a high CP (30.1 %) and a relatively high GE content (21.4 kJ/g DM).

The effect of *Leucaena leucocephala* supplementation on ruminal pH is shown in Figure 1. There were significant ( $P < 0.05$ ) differences on the mean values of ruminal pH of sheep on the different diets. Sheep on diet 3 had higher ( $P < 0.05$ ) ruminal pH than sheep on diets 1 and 2, respectively.

Table 1: Composition of experimental diets and *Leucaena leucocephala* (% of DM)

Item	Diet 1	Diet 2	Diet 3	<i>Leucaena leucocephala</i>
CP	13.1	17.4	21.6	30.1
Ash	10.5	10.0	9.5	8.5
Ether extract	2.0	2.7	3.3	4.6
NDF	61.0	58.2	55.5	49.9
ADF	35.4	34.2	33.0	30.6
ADL	3.7	6.8	9.8	15.9
Cellulose	31.7	27.5	23.2	14.7
Hemicellulose	25.6	24.0	28.9	19.3
Condensed tannins <sup>1</sup>	n.a.	0.3	0.6	1.2
GE (kJg <sup>-1</sup> DM)	17.9	18.8	19.7	21.4
Mineral premix <sup>2</sup>	10.0	10.0	10.0	n.a.

<sup>1</sup>As leucocyanidin equivalent; n.a.: Not applicable; <sup>2</sup>Composition/kg: vit A 600,000 IU, vit D3 75,000 IU, vit E 300 mg, Zn 3,000 mg, Mn 480 mg, Co 12 mg, Se 10 mg.

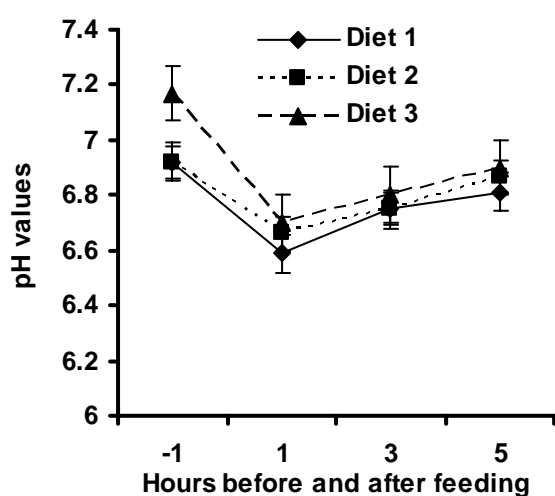


Figure 1: Effect of *Leucaena leucocephala* supplementation on ruminal pH

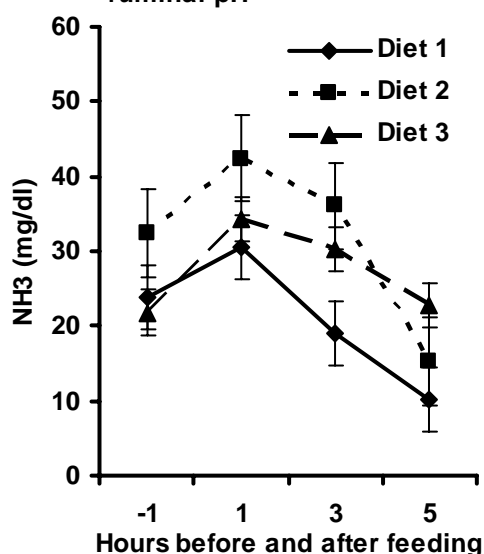


Figure 2: Effect of *Leucaena leucocephala* supplementation on ruminal ammonia concentration.

There was no difference in the ruminal pH of sheep on diets 1 and 2.

Mean values of ruminal ammonia concentrations of sheep supplemented with *Leucaena leucocephala* is shown in Figure 2. The ruminal ammonia concentrations of sheep on diet 2 was superior ( $P < 0.05$ ) to those of sheep on diet 1 but not with diet 3.

The effect of supplementation with *Leucaena leucocephala* on total volatile fatty acid (VFA) is presented in Figure 3. Diet 1 had superior ( $P < 0.05$ ) VFA concentrations than diets 2 and 3, respectively. Diets 2 and 3 are not significantly different in their VFA concentrations.

Total nutrient intake and digestibility coefficients of sheep supplemented with *Leucaena leucocephala* is summarised in Table 2. There were no differences ( $P > 0.05$ ) in the dry matter intake, organic matter, NDF, ADF and hemicellulose intake among the treatments. There were however, significant ( $P < 0.05$ ) differences in the digestibility of nutrient among treatments.

## DISCUSSION

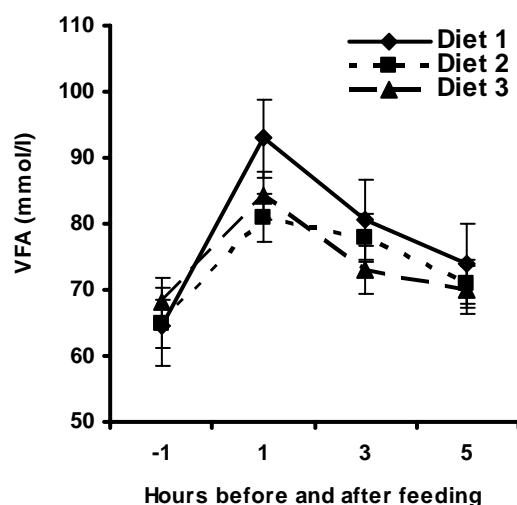
McLeod (1974) reported the effects of pH on complex formation of tannin and proteins. Condensed tannins (CT) can react and form complexes by H-bonding with carbohydrates and proteins, but at neutral pH form stronger bonds with proteins. Barry and Forss (1983) reported that complexes with low tannin concentration can be deaminated by rumen microorganism in the pH range of 6.5 to 7.0. The lower pH of diets 1 and 2 compared with diet 3 could indicate a higher rumen fermentation. The higher pH of diet 3 compared with diets 1 and 2 would suggest inhibition of fermentation.

The superior ruminal ammonia concentration of diet 2 compared with diet 1 would indicate a positive effect of supplementation with *Leucaena leucocephala* at 25% inclusion level.

Table 2: Total nutrient intake (g/d) and digestibility coefficients of sheep supplemented with *Leucaena leucocephala*

Items	Diet 1	Diet 2	Diet 3	SEM	P level
<b>Nutrient intake (g/d)</b>					
Dry matter	490.8	519.1	540.8	19.3	NS
Organic matter	439.3	467.3	489.4	17.4	NS
Ash	51.5	51.8	51.4	1.86	NS
Crude protein	64.2 <sup>c</sup>	90.5 <sup>b</sup>	116.6 <sup>a</sup>	4.1	***
NDF	299.4	302.0	300.0	10.8	NS
ADF	173.9	177.6	178.6	6.4	NS
ADL	18.3 <sup>c</sup>	35.5 <sup>b</sup>	53.0 <sup>a</sup>	1.8	***
Cellulose	155.6 <sup>a</sup>	142.1 <sup>ab</sup>	125.6 <sup>b</sup>	4.8	**
Hemicellulose	125.4	124.4	121.4	4.4	NS
<b>Digestibility (0-1)</b>					
Dry matter	0.703 <sup>a</sup>	0.653 <sup>ab</sup>	0.524 <sup>b</sup>	2.95	*
Organic matter	0.683 <sup>a</sup>	0.576 <sup>b</sup>	0.502 <sup>c</sup>	1.48	***
Ash	0.621 <sup>a</sup>	0.472 <sup>ab</sup>	0.313 <sup>b</sup>	3.65	**
Crude protein	0.711 <sup>a</sup>	0.616 <sup>b</sup>	0.579 <sup>b</sup>	0.94	***
NDF	0.653 <sup>a</sup>	0.564 <sup>ab</sup>	0.513 <sup>b</sup>	2.17	**
ADF	0.612 <sup>a</sup>	0.449 <sup>b</sup>	0.309 <sup>c</sup>	2.46	***
ADL	0.133 <sup>a</sup>	0.064 <sup>a</sup>	-0.240 <sup>b</sup>	5.95	**
Cellulose	0.669 <sup>a</sup>	0.543 <sup>b</sup>	0.540 <sup>b</sup>	2.89	*
Hemicellulose	0.710	0.728 <sup>ab</sup>	0.813 <sup>a</sup>	1.90	*

a,b,c Means in a row with different superscript differ significantly ( $P < 0.05$ ). \* = ( $P < 0.05$ ); \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ ; NS = Not significant



**Figure 3: Effect of *Leucaena leucocephala* supplementation on total volatile fatty acid concentration**

However, further increase in the supplementation level to 50% led to a decrease in the ruminal ammonia concentration. This reduction in ruminal ammonia concentration could be attributed to the inhibitory effects of tannins and mimosine on degradability of proteins by rumen microbes (Rodriguez *et al.*, 1975; Barry and Forss 1983).

The inferior total volatile fatty acid concentration of diets 2 and 3, respectively when compared to diet 1 would suggest an inhibitory

effect of condensed tannins and mimosine on digestibility of cell wall carbohydrates by rumen microbes. This observation is consistent with the reports of Reed *et al.* (1990).

The present study showed that there were no differences in the dry matter intake, organic matter, NDF, ADF and hemicellulose intake among treatments but digestibilities of organic matter, crude protein, ADF and ADL decreased with supplementation. Barry and Manley (1984) reported that tannins may reduce the digestion of cell-wall carbohydrates. The decrease in the digestibility of organic matter, crude protein, ADF and ADL with supplementation could indicate an inhibition of digestive enzyme activity by dietary tannins. This observation is in agreement with the findings of Griffiths and Jones (1977).

**Conclusion:** The results presented here support the use and integration of *Leucaena leucocephala* trees into the farming systems of sub-Saharan Africa. Its high crude protein (30.1 %), energy (21.4 kJ/g) and relatively low content of condensed tannins (1.2 %) DM has placed it among useful fodder trees for livestock feeding during the dry season. *Leucaena leucocephala* should not be fed as sole feed due to its poor digestibility and low metabolisable energy value. An inclusion level of 25% is therefore recommended.



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## LENGTH-WEIGHT RELATIONSHIP AND CONDITION OF FRESHWATER SHRIMPS *Atya gabonensis* AND *Macrobrachium felicinum* FROM THE MU RIVER, MAKURDI, NIGERIA

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### ABSTRACT

*Length-weight parameters (a and b) of the equation:  $W = aL^b$  were estimated for two freshwater shrimp species *Atya gabonensis* and *Macrobrachium felicinum* caught bimonthly from October 2001 to March 2002 using brush traps in the Mu river. The mean b values were  $2.989 \pm 0.328$  and  $3.003 \pm 0.318$  for *A. gabonensis* and *M. felicinum* respectively. The values did not differ significantly ( $P < 0.05$ ) from 3, showing that their growths were isometric, *M. felicinum* where in better condition than *A. gabonensis*.*

**Key words:** Length-weight, Relationship, Condition factor, *Atya gabonensis*, *Macrobrachium felicinum*, Mu river

### INTRODUCTION

In fisheries research, length-weight relationships are important for the estimation of weight where only length data are available and as an index of the condition of the fish (Pauly, 1993 and Goncalves, *et al.*, 1997). King (1996a) noted that only a few estimates of species length-weight relationship parameters are available for Nigerian fishes. Of the 149 species of fish in Nigeria's inland and coastal waters compiled by king (1996a, 1996b) from various studies, none of the papers contained information on the length-weight relationship of fin-fishes (shrimps) from the inland waters. Freshwater shrimp constitute one of the most desirable candidates for freshwater aquaculture in different parts of the indo-pacific region. Knowledge of the biology of these species is important, since they are highly demanded in both Nigerian domestic and export markets. They are therefore culture candidate in our local fresh and brackish water ponds.

This study presents information on the size distribution, length-weight relationship and relative conditions of *A. gabonensis* and *M. felicinum* in the Mu river, Fiidi-Makurdi.

### MATERIALS AND METHODS

A total of one hundred and fifty (150) *A. gabonensis* and fifty five (55) *M. felicinum* were collected from two sampling sites in the Mu river between October 2001 and March 2002 using brush traps placed in water along the riverbank.

Total length (cm) and body weight (g) were taken after draining water and blotting out excess water on the body (king, 1996b). For each species, the parameter a (proportionality constant) and b (exponent) of the LWR of the equation  $W = aL^b$  were estimated using base 10 logarithm transformation of L - W data pairs and ordinary least - square linear regression (i.e. log transformed versions of  $W = aL^b$ ) as  $\log W = \log a + b - \log L$ . The condition factor was calculated using Fulton's condition factor,  $K = 100W/L^3$  (Carlender, 1969), where L = length (cm), W = weight (g) and 3 derived from exponential b of  $W=aL^b$ .

### RESULT AND DISCUSSION

The length of *A. gabonensis* ranged from 5.0 to 12.2 cm, with a mean value of  $7.99 \pm 2.12$  cm and the weight ranged from 3.3 to 51.6 g with a mean value of  $13.98 \pm 10.36$  g. The length of *M. felicinum* ranged from 3.1 to 8.2 cm with a mean value of  $6.33 \pm 1.003$  cm while the weight ranged from 2.8 to 11.2 g with a mean value of  $6.6 \pm 1.67$  g. The length-weight relationship *A. gabonensis* ranging from 5.0 to 12.2 cm was  $W = 0.014L^{2.989}$ . The corresponding relationship for *M. felicinum* ranging from 3.1 – 8.2 cm was  $W = 0.0016L^{3.003}$ . These values indicated isometric relationship with 98 % of the variation in body weight being accounted for by changes in length. The length – weight relationship obtained for *A. gabonensis* and *M. felicinum* revealed that the values of the slopes (b) for

**Table 1: Length-weight relationship and condition of *Atya gabonensis* and *Macrobrachium felicinum* from Mu river, Fiidi, Makurdi, Nigeria**

Species	Non-linear regression				
	N	a	b	SE(b)	r <sup>2</sup>
<i>A. gabonensis</i>	150	0.014	2.989	0.329	0.990
<i>M. felicinum</i>	55	0.0016	3.003	0.318	0.998
	Length (TL cm)				
	Max	Min	Mean	SE	
<i>A. gabonensis</i>	12.2	5.0	7.99	2.12	
<i>M. felicinum</i>	8.2	3.1	6.33	1.003	
	Total Weight (g)				
	Max	Min	Mean	SE	
<i>A. gabonensis</i>	51.6	3.3	13.9	1.104	
<i>M. felicinum</i>	11.2	2.8	6.6	1.7	
	Condition Factor K = 100W/L <sup>3</sup>				
<i>A. gabonensis</i>			1.014		
<i>M. felicinum</i>			2.031		

both species were not significantly different from 3 ( $p < 0.05$ ) (Table 1). The two species in Mu river exhibited a positive isometric growth, which means that all parts of the shrimps grow at similar rates. The b values suggested that Mu river has a better ecological condition for the species thus supports higher biomass. Values of the condition factor (k) for the species show that *M. felicinum* had higher condition factor

and were therefore in better condition than *A. gabonensis* in the river.

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## ENDEMICITY OF MALARIA AMONG PRIMARY SCHOOL CHILDREN IN EBONYI STATE, NIGERIA

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### ABSTRACT

*A study was undertaken to determine the prevalence of malaria among primary school children in Ebonyi State. The degree of malaria parasite, infection and species of the parasite isolated were used to determine the level of endemicity of the disease. Out of one thousand and two hundred (1,200) primary school children aged between 5 –16 years sampled, the prevalence rate of 40.08 % was recorded. The species of the parasite associated with the disease was Plasmodium falciparum. It was observed that the rate of malaria parasitaemia was higher in younger (aged 5-10 years) than those of older (aged 11-16 years) children. A  $\chi^2$  test conducted on the infection rate according to ages of the pupils showed significant difference between the age groups indicating that infection was age dependent ( $P < 0.05$ ). Greater number of males (243) than females (238) were infected but the infection rate according to sex was found not to be significant and therefore not sex dependent ( $P > 0.05$ ). Among the Local Government Areas, Ivo had the highest prevalence rate. This was followed by Ishielu and Abakaliki local government areas of Ebonyi State. The results showed that malaria is endemic in the state and a major health problem for school children. The possible effect on academic performance was discussed.*

**Keywords:** Malaria, Prevalence, Children, Endemicity, Parasitaemia, Ebonyi State

### INTRODUCTION

Malaria remains one of the most pressing health problems in the world with an estimated 300-500 million cases annually of which 90 % occurs in Africa (Tarimo *et al*, 1998). It is by far the world's most important tropical parasitic disease and kills more people than any other communicable disease except tuberculosis.

According to Wenceslaus (2000), the malaria situation in sub-Saharan Africa is grim and the disease now constitutes a leading cause of poverty in the region. This is because sub-Saharan African region has the greatest number of people exposed to malaria transmission, greatest burden of malaria morbidity and mortality in the world (WHO, 1996; Snow *et al*, 1999). The problems associated with malaria treatment in Africa had substantially increased the rates of illness and death (Peter *et al*, 2000). It is estimated in Africa that, malaria is responsible for over one million deaths of infants and young children each year (Angyo *et al*, 1996). With regard to morbidity, people in areas of high endemicity usually go through several attacks every year. Such attack episode may last for 5 - 15 days often incapacitating the victim. Furthermore, in these areas, most cases of severe malaria occur among children aged between 1 and 3 years of age.

In many countries malaria exerts an enormous toll on lives, medical cost and daily labour cost. The daily labour cost coupled with cost of treatment and high mortality associated with the disease make malaria one of the main factors retarding development in Africa (Mutero *et al*, 1998). By adversely affecting people's health, strength and productivity, malaria further marginalizes and impoverishes them (David, 2000). In Nigeria, Malaria is hyper-endemic with stable transmission (Ofovwé and Eregie, 2001).

Malaria is an infection caused by the parasite *Plasmodium*. There are four species of the parasite that infect man. These are *Plasmodium falciparum*, *vivax*, *malariae* and *P. ovale*. *P. falciparum* and *P. vivax* are the most common. Mixed infections with two or more of the *Plasmodium* species are common. *P. falciparum* is responsible for the most severe, often fatal forms of malaria. It is deeply entrenched in tropical Africa. *P. vivax* is the commonest species in America and Asia while *P. malariae* and *P. ovale* rarely occur. The disease is transmitted by female *Anopheles* mosquitoes. The infection may be acquired wherever there are human hosts carrying the parasites and sufficient Anopheline mosquitoes, together with condition of temperature and humidity that

favours the development of parasite in the mosquitoes.

The diagnosis of malaria is made with certainty on identification of the malaria parasite in blood films of patients together with other symptoms associated with the disease. Due to the large and increasing number of children in attendance at health – centres, clinics and out-patient department of hospitals in areas where the disease is endemic, it is not always possible to investigate every suspected case even where sufficient laboratory equipment, chemicals and staff are available. Thus diagnosis of clinical malaria is commonly made in children who are feverish without other overt childhood diseases, and chloroquine is often given as therapeutic measure (Azuike, 1993).

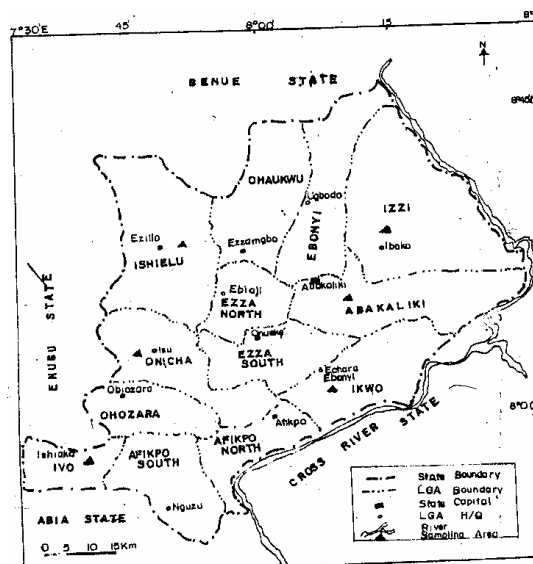
Prevalence surveys can give insights into the transmission patterns of diseases in any given area and act as useful tools for control purposes. In Ebonyi State, previous information on the prevalence of malaria parasitaemia among primary school children is lacking. This study was undertaken, therefore to determine the prevalence of malaria parasitaemia among primary school children in Ebonyi State, and as such provide baseline data for future malaria control programmes in the State.

## MATERIALS AND METHODS

**Study Area:** The study was carried out in Ebonyi State (Figure 1). This is one of the thirty-six States of Nigeria. It is in the South - eastern part of the country and is made up of thirteen Local Government Areas which are Abakaliki, Ebonyi, Izzi, Ishielu, Ezza North, Ezza South, Ikwo, Ohaukwu, Onicha, Ohazara, Ivo, Afikpo North and Afikpo South local government areas. It has a population of about 1.8 million and a total land area of about 5935 km<sup>2</sup> which gives a population density of over 300 persons per square kilometer (Anon, 1997).

The state is located between latitudes 7°30' East and 8° 30' East and longitudes 5°40' North and 6° 45' North within the rain forest zone of Nigeria characterized by high rainfall, run-off volumes and relative humidity. The annual rainfall is over 1600 mm while the mean daily rainfall is over 150 mm. The mean daily maximum and minimum temperatures are 32°C and 25°C respectively (Anon, 1997).

The fertile and rich soils of Ebonyi State encourage large scale agriculture. Available statistics indicate that agriculture provides productive employment to over 85 % of Ebonyians (Anon, 1997). This makes agriculture



**Figure 1: Map of Ebonyi State showing sampled areas**

the mainstay of the state's economy. In fact Ebonyi state is regarded as one of the food basket states of the federation (Anon, 1997).

There are primary health centres in all the local Government Areas, some comprehensive health centres operated under the supervision of medical doctors and free mobile health clinic. There is a Federal Medical Centre and a State's University Teaching Hospital in the state capital. There are also strong believes in the use of traditional medicine in many rural areas of the state.

**Selection of Schools:** Three senatorial zones of the state: (Ebonyi South, Ebonyi North and Ebonyi central) were selected for study. In each senatorial zone of the State, Local Government Areas that make up the zone were listed and two out of them were selected using simple random sampling. The six Local Government Areas selected were Onicha and Ivo from Ebonyi South, Ikwo and Ishielu from Ebonyi Central and Abakaliki and Izzi from Ebonyi North Senatorial zone. Primary schools in each of the selected Local Government Area were listed and four schools were selected from each Local Government Area using random sampling technique. In the random sampling for Local Government Areas and schools, table of random numbers was used.

**Blood Sample Collection and Analysis:** From each of the twenty-four schools selected, 50 pupils were sampled using lottery method of sampling. In this method, papers written "yes" and "no" were folded neatly and displayed to the pupils to pick. The lottery papers were



usually counted for the expected number of "Yes" donors in each school. Those that picked yes were separated from those that picked "No". At the end of this exercise, the age, sex, weight and height of the pupils that picked yes were recorded. A total of 1,200 pupils aged between 5-16 years were sampled.

Venous blood was collected from each pupil using disposable needles and syringes. Thick and thin blood films were smeared on the slides for malaria parasite (mp) and species identification. For mp, the smears were stained with Giemsa stain while those for species identification were stained with Leishman's stain. Slides were examined under the binocular light microscope using x 100 objective oil - immersion. The presence of malaria parasites, the degree of infection and the species of parasite were identified. (Shute and Maryon, 1966).

The intensity of parasitaemia was measured per microscopic field. Up to 5 microscopic fields were examined and the number of parasites per field counted. The average number of parasites per five microscopic fields was then taken.

**Statistical Analysis:** The data were analyzed statistically using chi- square ( $\chi^2$ ) test.

## RESULTS

A total of 1,200 primary school children aged between 5 to 16 years were examined for malaria parasite. Out of this number, 481 (40.08 %) were infected. The malaria parasite isolated was *Plasmodium falciparum*.

### Malaria Parasitaemia in Primary Schools:

The highest prevalence rate of 52 % was recorded in Central School, Okue in Ivo L.G.A and Eketube Enyigba Community School, Abakaliki L. G. A. These two schools are located in the rural villages of the state. These were followed by Central School, Ntezi and Ndibulofia Ominyi Community School, Izzi with 50 % prevalence rates. Other values recorded were Agalegu Amachi Community School in Abakaliki with 48 %, Ataragu Amagu community Primary School, Ivo, with 46 % etc. The least infection rate of 22% was recorded in Community Central School Amanator Isu in Onicha L.G.A (Table 1)

**Gender, Age and Malaria Parasitaemia in Ebonyi State:** The highest prevalence of 231 (47.05 %) was recorded in children between 5 - 7 years. This was followed by 179 (39.08 %) recorded in the age class 8 - 10 years.

The least prevalence of 3 (17.65 %) was recorded in 14 - 16 years class. A chi- square test carried on the data to know if the infection was age dependent was statistically significant ( $P < 0.05$ ). It can be observed from Table 2 that malaria parasitaemia was higher in children of younger age groups than in those of older age groups. Table 2 also highlights the prevalence of malaria parasitaemia due to sex. Out of 591 males and 609 females examined, 243 males and 238 females were infected. This difference in infection according to sex was not statistically significant ( $P > 0.05$ ). In the male and female, the highest prevalence was recorded in the 5-7 years age class, while the least prevalence was recorded in the 14-16 years age class. The parasitaemia in both sexes followed a similar pattern.

**Intensity of Infections:** The intensity in each age group is shown in table 3. In all the age groups sampled, children under the age class 8 - 10 years had the highest intensity of 11.59. Those under the age class 5 - 7 had 11.56 while the least intensity of infection was recorded in the age class 14 - 16 years.

## DISCUSSION

The parasite rate of 40.08 % in this study was quite high, indicating a high degree of malaria parasitaemia among primary school children in Ebonyi State. The species of malaria parasite was *Plasmodium falciparum*. The malaria infection in the state is severe because *Plasmodium falciparum* causes complicated form of malaria especially in young children.

Several studies have shown high parasite rates among primary school children in Nigeria. Salako *et al*, (1990) reported a parasite rate of 74 % in Nigeria while Ademowo *et al*, (1995) reported a lower percentage of 27 % among school children from a rural village in Western Nigeria. Furthermore, Adeyemo *et al*, (1999) recorded a parasitaemic rate of 80 % among primary school children in malaria endemic village of Erunmu in South- West Nigeria. The endemicity is high in rural than urban communities of Ebonyi State. For instance, the highest parasitaemic rate of 52 % was recorded in two rural schools of Okue and Eketube. This result suggests that the rural environment offers adequate conditions for breeding of mosquitoes. The socio-economic status of parents in rural communities also helps in the transmission of malaria.

**Table 1: School by school malaria parasitaemic rates among primary school children in Ebonyi State**

LGA	School	Number Examined	Number Infected	Infection Rate (%)
Ivo	Iyioji Comm. Pri. School, Akaeze.	50	19	38
"	Ndiobasi Comm. Pri. School, Ishiagu.	50	21	42
"	Central School, Okue.	"	26	52
"	Ataragu Amagu Comm. Pri. School.	"	23	46
Abakaliki	Eketube Enyigba Comm. School.	"	26	52
"	Agalegu Amachi Comm. School.	"	24	48
"	EBSU Staff Pri. Sch., Abakaliki	"	18	36
"	Ezikwo Rd Pri. School, Azuiyokwu.	"	18	36
1zzi	Ndibuolfia Ominyi Comm. School, Izzi	"	25	59
"	Ezzainymagu Comm. School Ndubia	"	21	42
"	Agbaja Central School, Izzi	"	20	40
"	Onuenyim Comm. School, Izzi	"	17	34
Ikwo	Comm. Pri. Sch. Agubia Ikwo	"	14	28
"	Urban Pri. Sch. Ndufu Echara	"	13	26
"	Comm. Cent. Sch. Echialike Ikwo	"	22	44
"	Comm. Pri. Sch. Ndiagu Amagu	"	22	44
Ishielu	Comm. Pri. Sch. Ezillo	"	19	38
"	Central School, Ntezi	"	25	50
"	Central School Umuhuali	"	20	40
"	Comm. Cent. Sch. Ohofia Agba	"	22	44
Onicha	Amokpara Comm. School, Oshiri	"	22	44
"	Amanator Onicha Comm. Pri. Sch.	"	20	40
"	Comm. Cent. Sch. Anamnator Isu.	"	11	22
"	Isuachara pri. School, Isu	"	13	36
<b>Total</b>		<b>1,200</b>	<b>481</b>	<b>40.08</b>

**Table 2: Sex and age – related malaria parasitaemia among primary school children in Ebonyi State**

Age Class (Years)	Male		Female		Total No Exam. In each class	Total Number & (%) infected in each class
	Number Examined	No & (%) infected	Number Examined	No & (%) infected		
5-7	248	117(47.18)	243	114(46.91)	491	231(47.05)
8-10	223	88(39.46)	235	91.(38.72)	458	179 (39.08)
11-13	115	37 (32.07)	119	31(26.05)	234	68 (29.06)
14-16	5	1 (20)	12	2 (16.67)	17	3 (17.65)
<b>Total</b>	<b>591</b>	<b>243 (41.12)</b>	<b>609</b>	<b>238 (39.08)</b>	<b>1200</b>	<b>481 (40.08)</b>

**Table 3: Age-related intensity of parasitaemia**

Age class (years)	Number of Parasites	Number of pupils infected	Intensity
5-7	2670	231	11.56
8-10	2075	179	11.59
11-13	760	68	11.18
14-16	30	3	10.00
<b>Total</b>	<b>5535</b>	<b>481</b>	<b>11.51</b>

Most of these parents cannot afford screening of their homes, insecticides and treated bed nets for mosquito control. Illiteracy and ethnic beliefs among the rural dwellers have further encouraged the transmission of malaria parasites. In some cases, the infected children are not fully treated with the correct doses of antimalaria drugs. Among the age groups sampled, children between the 5 - 7 years age group had the highest rate of parasitaemia and thus vulnerable to malaria attacks due to the fact that their immunity to malaria parasite had not been fully developed.

Angyo *et al.* (1996) in a similar study at Jos Nigeria reported a parasite rate of 70. 5 % among children of the same age group. In this study, children of 8-10 years age group had parasitaemic rate of 39.08 %, those of 11- 13 years age class had 29.06 % while those of 14 -16 years age class had 17.65 % parasitaemic rates indicating that the parasitaemic rate decreases with increasing age. Older children are therefore less susceptible to malaria attack because they seem to have developed their own active immunity against malaria parasite (Angyo, *et al.* 1996).

Immunity has important effects on the transmission of the disease by reducing the level of parasitaemia after infective bites and increasing ten folds the rate of clearance of parasitaemia (Ademowo, 1995). With repeated infections in areas of intense transmission, the level at which parasitaemia stabilizes falls and the threshold for symptoms rises. Consequently, parasitaemia becomes asymptomatic and the risks of unrestrained parasitic multiplication to lethal

burdens decline (Ademowo, 1995). Numerically, more males than females were infected despite the fact that more females than males were examined in the present study. This is comparable to the report of Okeahialam *et al*, (1972) and Uzoegwu and Onwurah (2003) for children and adults respectively. However, in this present study, the difference in males and females infected with malaria parasite was not statistically significant and therefore infection was not dependent on sex.

The intensity of parasitaemia was high. The fact that children with such parasite densities were healthy and able to go to school means that malaria is well tolerated in Ebonyi state just as in many other parts of the sub-Saharan Africa. This is in consonance with the report of Ogunrin, (2001) who noted that children in endemic areas might tolerate very high levels of parasitaemia without severe symptoms.

**Conclusion:** Efforts must be put up to undertake a massive attack on the parasite and its vectors in the state because it is evident that malaria parasitaemia is widespread among younger children. If the situation is not checked, more children will be vulnerable to malaria attack and soon most of the debilitating effects of malaria will surface. Therefore concrete steps should be taken quickly to avert an epidemic of malaria especially among children of school going age. Apart from chemotherapeutic control of malaria in the state, health education focusing on malaria control should be intensified both at school and at the community level to ginger up community participatory activities aimed at sustainable malaria control.

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## RELATIONSHIPS BETWEEN PHYSICAL BODY TRAITS OF THE GRASSCUTTER (RODENTIA: THRYONOMYIDAE) IN AKPAKA FOREST RESERVE, ONITSHA

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### ABSTRACT

*Physical body traits of nine grasscutters (Thryonomys swinderianus Temminck) were characterized using body weights (BW), body length (BL), heart girth (HG), and height-at-withers (H). Simple linear correlation matrix showed high, positive and significant values among the parameters studied ( $P < 0.01$ ). The highest coefficient was obtained for body weight and body length ( $r = 0.9956$ ). The very high associations for body length and heart girth ( $r = 0.9821$ ) and between body length and height ( $r = 0.9905$ ) indicate that frame size and absolute height were complementary. Selection for increased measurement in any of the parameter would mean positive significant influence on the other and would lead to increased skeletal stature with concomitant increases in other absolute body measurements. Regression equations from this study could be used to estimate live body weights of grasscutters aged between 2 -10 months.*

**Keywords:** Grasscutter, Physical Body Traits, Estimation, Correlation and Regression Models

### INTRODUCTION

The relationships existing among physical body traits provide useful information on productive performance and carcass characteristics of animals. Different linear body measures would be required to quantify body shape and size in different breeds and under different conditions of feeding (Ibe, 1989; Ibe and Ezekwe, 1994). Quantitative measures for size and shape are necessary for estimating genetic parameters in animal breeding programmes (Chineke, 2000). Much work has been done in this area with ruminants (Akpa, 2000, Alade *et. al.*, 1999, Ozoje and Herbert, 1997, Chineke, 1996, Orheruata and Olutogun, 1994) and rabbits (Chineke, 2000, Chineke, *et. al.*, 2000).

There is however a dearth of information on the interrelationships among physical body traits in captive grasscutters. The aim of this paper is to establish the relationships that exist among body weight and linear body measurements (body length, heart girth, and height-at-withers) in grasscutters reared in Akpaka Forest Reserve, Onitsha, Nigeria. This will also provide formulae for the estimation of live weights of grasscutters aged between 2 – 10 months.

### MATERIALS AND METHODS

Body weights and linear measurements of nine 2-months-old grasscutters from our grasscutter research station at the Akpaka forest reserve, Onitsha were assessed for 2 - 10 months. Body weight (BW) in kilograms of each animal was taken every month, by means of a Way Master precision scale. Body length (BL), heart girth (HG), and height-at-withers (H) were similarly taken in centimeters. Body length was taken as the distance from the point of the nose to the base of the tail. Heart girth was taken as the circumference of the chest while height-at-withers was measured as the distance from the surface of a platform to the withers.

Data were collated and the method of least square means was used for simple linear correlation and multiple linear regression analyses (Little and Hills, 1977). *Simple linear correlation*,  $r = (\sum yx_1) / \sqrt{(\sum y^2 \sum x_1^2)}$  etc., was applied to the paired-variables Body weight versus Body length ( $Y:X_1$ ), Body weight versus Heart girth ( $Y:X_2$ ), Body weight versus Height-at-withers ( $Y:X_3$ ), Body length versus Heart girth

**Table 1: Body weight and linear body measurements of grasscutters**

Observation (n)	Age (months)	BW (kg) Y	BL (cm) X <sub>1</sub>	HG (cm) X <sub>2</sub>	H (cm) X <sub>3</sub>
1	2	0.66	23.25	18.64	11.00
2	3	0.88	26.33	20.95	12.55
3	4	1.10	29.29	22.04	14.05
4	5	1.39	31.41	24.41	15.45
5	6	1.63	35.03	26.63	17.02
6	7	1.86	39.50	32.25	18.08
7	8	2.08	40.68	35.33	19.95
8	9	2.22	43.57	37.90	21.62
9	10	2.51	46.61	40.14	23.75
<b>Means</b>		<b>1.59</b>	<b>35.10</b>	<b>28.48</b>	<b>17.05</b>

Deviations  $y$ ,  $x_1$ ,  $x_2$ , and  $x_3$  from the means of variables  $Y$ ,  $X_1$ ,  $X_2$ , and  $X_3$  were used to compute products of all possible pair-combinations of the corrected sums of squares.

**Table 2: Sum of products of all possible pair-combinations of the corrected sums of squares**

n	Age (months)	$y^2$	$x_1^2$	$x_2^2$	$x_3^2$	$y x_1$	$y x_2$	$y x_3$	$x_1 x_2$	$x_1 x_3$	$x_2 x_3$
1	2	0.879	134.7	96.86	36.62	0.88	9.23	5.67	114.2	70.24	59.56
2	3	0.515	77.03	56.73	20.26	6.30	5.40	3.23	66.10	39.51	33.90
3	4	0.248	34.14	71.261	9.01	2.89	4.20	1.48	49.10	17.46	25.34
4	5	0.043	13.66	16.58	2.56	0.76	0.84	0.35	15.05	5.92	6.52
5	6	0.001	0.0001	3.42	0.001	-0.001	-0.05	-0.001	0.01	0.0002	0.059
6	7	0.068	22.05	14.19	1.05	1.23	0.98	0.26	17.69	4.84	3.87
7	8	0.232	31.05	46.89	8.39	2.68	3.30	1.39	38.16	16.15	19.84
8	9	0.465	71.62	88.69	20.86	5.77	6.42	3.11	79.70	38.65	43.02
9	10	0.831	132.3	137.1	44.86	10.49	10.67	6.10	134.6	77.04	78.42
<b>Σ</b>		<b>3.28</b>	<b>516.5</b>	<b>531.6</b>	<b>143.6</b>	<b>40.98</b>	<b>40.98</b>	<b>21.59</b>	<b>514.7</b>	<b>269.7</b>	<b>270.5</b>

**Table 3: Simple linear correlation among body traits in the grasscutter**

Variables	BW (Y)	BL (X <sub>1</sub> )	HG (X <sub>2</sub> )	H (X <sub>3</sub> )
Body weight (BW)	-			
Body length (BL)	0.9956	-		
Heart girth (HG)	0.9813	0.9821	-	
Height-at-withers(H)	0.9949	0.9905	0.9790	-

Calculated  $r$ -values were greater than the tabular  $r$ -values at these levels of significant. ( $P < 0.01$ ).

**Table 4: Simple linear regression equations for body traits in the grasscutter**

Paired variables	Slope, $b$ $b = (\sum yx) / \sum x^2$	Intercept, $a$ $a = (\text{Mean } Y) - b (\text{mean } X)$	Regression equation $\hat{Y} = a + b X$
BW: BL (Y:X <sub>1</sub> )	0.0793	- 1.1934	BW = - 1.1934 + 0.0793BL
BW: HG (Y:X <sub>2</sub> )	0.0796	- 0.6684	BW = - 0.6684 + 0.0796HG
BW: H (Y:X <sub>3</sub> )	0.1503	- 0.9726	BW = - 0.9726 + 0.1503H
BL : HG (X <sub>1</sub> :X <sub>2</sub> )	1.0000	- 3.3800	BL = 6 + HG
BL : H (X <sub>1</sub> :X <sub>3</sub> )	1.8786	3.0690	BL = 3.069 + 1.8786H
HG : H (X <sub>2</sub> :X <sub>3</sub> )	1.8837	- 3.6370	HG = - 3.637 + 1.8837H

**Table 5: Sum of cross products of all possible pair-combinations of corrected sums of squares of the k+1 variables**

	Dependent variable		Independent variables	
	Y (BW)	X <sub>1</sub> (BL)	X <sub>2</sub> (HG)	X <sub>3</sub> (H)
Y	3.28	-	-	-
X <sub>1</sub>	40.98	516.5	514.7	269.7
X <sub>2</sub>	40.98	514.7	531.6	270.5
X <sub>3</sub>	21.59	269.7	270.5	143.6

$k$  equations:

$$b_1 516.5 + b_2 514.7 + b_3 269.7 \pm 40.98 \dots\dots\dots(I),$$

$$b_1 514.7 + b_2 531.6 + b_3 270.5 \pm 40.98 \dots\dots\dots(II)$$

$$b_1 269.7 + b_2 270.5 + b_3 143.6 \pm 21.59 \dots\dots\dots(III),$$

Where  $b_1$ ,  $b_2$  and  $b_3$  are partial regression co-efficients of BL, HG and H respectively



**Table 6: Multiple linear regression analysis of body traits in the grasscutter**

ANOVA				
Source of variation	df	SS	MS	F <sub>cal</sub>
Regression	3	3.25	1.0833	216.66
Error	6	0.03	0.005	
Total	9	3.28		
<b>Regression equation:</b> $BW = -1.2179 + 0.0675BL + 0.0096HG + 0.0097H$ $R = 9953; R^2 = 0.9908$				

(X<sub>1</sub>:X<sub>2</sub>), Body length versus Height-at-withers  
 (X<sub>1</sub>:X<sub>3</sub>), Heart girth versus Height-at-withers  
 (X<sub>2</sub>:X<sub>3</sub>).

## RESULTS AND DISCUSSION

Average body weights and linear body measurements obtained from the grasscutters are presented in Table 1. Table 2 shows products of all possible pair-combinations of the corrected sums of squares. Simple linear correlation among body traits are presented in Table 3. Regression equations computed for the estimation of body traits in grasscutters are presented in Table 4. Products of all possible pair-combinations of corrected sums of squares of the k+1 variable are arranged in Table 5 to bring out the so-called normal or k equations. The result of multiple linear regression analysis is presented in Table 6. R<sup>2</sup> was significant, indicating that some portion of the variability in body weight can be explained by body length, heart girth and height-at-withers of the grasscutter.

Table 1 showed that the body weights of grasscutters aged 2 – 10 months ranged from 0.66 – 2.51 kg. The coefficients of correlation among the body weight, body length, heart girth and height-at-withers were high, positive and significant and ranged between 0.9790 and 0.9956 (see Table 3). The highest correlation between BW and BL ( $r = 0.9956$ ) shows that body length was the best predictor for body weight. Very high association between BL and HG ( $r = 0.9821$ ), BL and H ( $r = 0.9905$ ) is an indication that heart girth and height may be complementary. High correlation between heart girth and body size has long been recognized in livestock (Chineke, 2000). The high co-relationship that existed among the linear body traits indicates that any one of them studied was sufficient for the estimation of body length. This is in line with the findings in rabbits (Chineke, 2000). Selection for increased measurement in any of the parameters would mean positive significant influence on the other and would lead to increased skeletal stature with concomitant increases in other body measurements.

Simple linear regression analysis confirmed the existence of linear relationships between physical body traits in the grasscutter. Grasscutter producer and researchers could estimate the live weight in 2-10 months old grasscutters by substituting the values of linear measurements in any of the equations shown in Table 4. Heart girth (cm) could be easily converted to body length (cm) by adding 6cm to the measured circumference of the chest (i.e.,  $HG + 6 = BL$ ). The choice of any linear body measurement depends on the ease in which that characteristic was measured.

Partial regression coefficients determined in this study have shown that the combined effects of body length, heart girth and height-at-withers in the manner prescribed by the regression equation contribute significantly to the variation in body weight of the grasscutter. The coefficient of determination ( $R^2 = 0.9908$ ), also significant, is a strong indication that more than 90% of the variability in body weight was explained by linear body measurements through the regression equation,  $BW = -1.2179 + 0.0675BL + 0.0096HG + 0.0097H$  from this study.

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## EVALUATION OF TAMARIND (*Tamarindus indica*) SEED MEAL AS A DIETARY CARBOHYDRATE FOR THE PRODUCTION OF NILE TILAPIA *Oreochromis niloticus* (L)

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### ABSTRACT

*A feeding study was conducted to assess the value of Tamarind, Tamarindus indica seed meal as dietary carbohydrate in the diets of Nile Tilapia, Oreochromis niloticus. Tamarind seeds were used to replace maize at 0, 20, 40, 60, 80, 100 % substitution levels for treatments 1 to 6. Growth trial was conducted in outdoor concrete tanks for 56 days. The fishes were fed at 4 % body weight twice daily. There were no significant variations in the mean weight gain, specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) ( $p > 0.05$ ). The apparent digestibility coefficient (ADC) of protein and energy of the fishes fed diets 1 – 6 were similar ( $p > 0.05$ ). There were no significant differences in the blood total erythrocyte counts, (TEC), pack cell volume (PCV) and red blood cells count (RBC) ( $p > 0.05$ ). Based on the findings, complete replacement of maize with tamarind seed meal in the diets of O. niloticus is recommended.*

**Keywords:** Tamarind, Replacement, Maize, *Oreochromis niloticus*.

### INTRODUCTION

Plant protein and carbohydrate feedstuffs, particularly soybean (*Glycine max*) and maize (*Zea mays*), have been extensively used in fish feeds with nutritional, environmental and economic benefits (Tacon, 1993). As the use of soybeans and maize in human food and livestock feed increases, their costs have increased, and the economics of using them in fish feeds may become less favourable. Hence the evaluation of underutilized indigenous plant protein/energy-rich sources becomes imperative and remains a high research priority in Nigeria.

Vast quantities of forest seeds are discarded as wastes in Nigeria. There is a strong economic justification for their use either as protein or carbohydrate/energy supplements in low cost diets for fish. A national feedstuff survey revealed that the seed of tamarind, (*Tamarindus indica*) represents a good source of digestible dietary energy and is desirable in fish feeds because of its low cost and availability (Balogun 1990). Tamarind contains about 18 % crude protein, with amino acid profile comparable to maize, but with a higher methionine + cystine (met + cys) value than maize. There is a dearth of information on the nutritive value of tamarind seed meal as a feedstuff for Nile tilapia, *Oreochromis niloticus*.

*Oreochromis niloticus* is a fast growing and preferred aquaculture species in Africa and is capable of utilizing plant materials in its diets. The

present study was conducted to evaluate the nutritional and economic feasibility of replacing maize with tamarind seeds in practical diets for *O. niloticus*.

### MATERIALS AND METHODS

**Diet:** The ingredients used in this study were purchased from Pfizer (Livestock) feeds depot, Ibadan Nigeria, while tamarind seeds were obtained from Kainji lake area, New Busa, Nigeria. Tamarind seeds were mechanically dehulled. The dehulled seeds were soaked in warm water for 24 h to remove the inner coat before sun drying to a constant moisture of < 10 %, and milled into fine powder. Phytin and tannin contents of the tamarind seed meal (TM) were determined according to the methods of Sathe and Salunkhe (1981). The mineral content of TM was determined as described by Harris (1970), while the proximate composition was determined according to the methods of AOAC (1990) (Table 1).

Based on the proximate composition of the feedstuffs, six diets were formulated (Table 2). The control diet (diet 1) contained 30% of maize which was replaced with TM in diets 2, 3, 4, 5 and 6 at 20, 40, 60, 80 and 100% respectively. The feedstuffs were milled, blended, moistened, pelleted, sun-dried at 30°C between 1200 – 1600h for three days and stored in air-tight polythylene bags at ambient

temperature (28°C). Proximate analysis of the diets and the fishes, (moisture, crude protein (N 58. x 6.25), crude lipid, crude fibre and total ash) were conducted in triplicate samples according to AOAC (1990) methods. Gross energy of the diets was determined in triplicate samples by combustion in bomb calorimeter.

**Growth Trials:** Groups of 20 fingerlings of *O. niloticus* (6.15 ± .03g) having been acclimated for seven days were randomly stocked into 18 outdoor rectangular concrete tanks (1.8 x 1.8 x 1m) containing 800 L of water for growth trials. The mean pH, dissolved and temperature of the tank waters were 6.9 ± 0.2, 5.6 ± 0.4 and 26.8 ± 0.6 respectively. Each of the diets was fed to the fishes in triplicate tanks at 4 % body weight twice daily (0900–1000 and 1500–1600h) for 56 days. Fresh water was used to change the water in the experimental tanks once every two weeks. Total weight of fishes in each tank was taken bi-weekly to monitor growth responses and for feed adjustments. Mean weight gain (MWG) specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR) were estimated from bi-weekly weight data according to the methods of Olivera Novoa *et al.* (1990) as follow: Mean weight gain = final mean weight – initial mean weight; Specific growth rate =  $10^2 (\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}) / \text{culture period (days)}$ ; Food conversion ratio = Dry weight of feed fed (g)/ fish weight gain; Protein efficiency ratio = fish weight gain/protein fed.

**Carcass and Haematological Analyses:** At the beginning and end of feeding trials, six tilapia, randomly selected from each treatment group were oven dried at 48 °C for 48 h, blended into fine powder, packed in air-tight polythene bags and stored in a deep freezer (-20°C), prior to carcass analyses. Similarly, before and after the feeding trials three fish from each tank (9 fish/treatment) were removed, anesthetized using 10 mg/l tricaine methane sulfonate (MSS222, Sandoz). Blood samples were withdrawn from the caudal vein with heparinized syringes, and immediately centrifuged at 5,000 rpm for 15 mins to remove red blood cells. The blood parameters, total erythrocyte count (TEC), pack cell volume (PCV) and red blood cells (RBC) were determined according to the methods of Svobodova *et al.* (1991).

**Digestibility Studies:** Groups of 20 *O. niloticus* fingerlings (7.2 ± 0.8g) having been

acclimated for seven days were randomly stocked into 18 indoor 60 L rectangular glass tanks (75 x 40 x 40 cm) supplied with fresh water (flow rate 1L m<sup>-1</sup>). Each of the diets was fed to the fishes in triplicate tanks at 4 % body weight twice daily for 14 days. Faeces were collected from each of the tanks eight hours after each feeding. The faeces and unfed diets were pooled separately and their proximate composition determined according to the methods of AOAC (1990). The digestibility of unfed feed and the dried faeces per sample/treatment were determined by AIA method (Halver *et al.* 1993). These samples were ashed and digested by Acid in soluble Ash (AIA).  $\text{AIA\%} = 10^2 (\text{weight of Ash} - \text{weight of AIA}) / \text{weight of Ash}$ . Apparent digestibility coefficient (ADC) was determined from the formula,  $\text{ADC} = 10^2 - (10^2 \times (\text{Af/At} \times \text{Nt/Nf}))$  where, Af = AIA in feeds, At = AIA in faeces, Nf = Nutrient in feed and Nt = Nutrient in faeces.

**Statistical Analyses:** The one way analysis of variance (ANOVA) and Duncan's multiple-range tests (Zar, 1984) were used to compare differences between diet treatment means (p = 0.05).

## RESULTS AND DISCUSSION

Table 1 presents the mineral, proximate composition, and anti-nutrients in tamarind seeds; which showed potassium (K), calcium (Ca), and magnesium (Mg), as the most abundant minerals. The protein content is high (16.6%), with low fat and fibre contents.

**Table 1: Mineral, anti-nutrient and proximate composition of tamarind seed meal**

Items Analyzed	Proximate Composition (g/100g/DM)
<b>Mineral and Anti-nutrient (g/kg)</b>	
Iron (Fe)	0.10
Zinc (Zn)	0.13
Magnesium (Mg)	0.31
Sodium (Na)	0.23
Potassium (K)	0.43
Calcium (Ca)	0.40
Phytin	1.17
Tannin	0.02
<b>Nutrients</b>	
Crude Protein	16.6
Fat	0.35
NFE	69.8
Moisture	4.74
Fibre	5.82
Ash	2.70

Table 2: Ingredient and proximate composition of experimental diets

Items Analyzed	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
<b>Ingredients(g/100g/DM)</b>						
Fish meal	15	15	15	15	15	15
Soybean meal	45	45	45	45	45	45
Maize	30	24	18	12	6	0
Tamarind	0	6	12	18	24	30
Fish oil	6	6	6	6	6	6
Vitamin-Mineral Premix <sup>1</sup>	2	2	2	2	2	2
Carboxymethyl cellulose	2	2	2	2	2	2
<b>Proximate Composition (g/100g/DM)</b>						
Crude protein	30.0	31.0	31.8	32.0	32.9	33.1
Ether extract	8.0	8.06	8.57	8.70	8.99	8.78
Nitrogen free extract	43.4	42.8	42.0	41.7	41.0	40.1
Crude fibre	1.24	1.22	1.20	1.19	1.17	1.15
Ash	6.64	6.41	6.20	6.08	5.89	5.88
G. Energy (kcal/g/DM)	398.8	398.9	404.0	405.2	409.3	404.7
Protein – Energy Ratio	76.8	77.8	78.6	79.0	80.2	81.7

<sup>1</sup> supplied as (mg/kg diet): Ca-pantothenic acid, 40; pyridoxine, 10; riboflavin, 12; niacin, 20; folic acid, 2; choline chloride, 2000; thiamin HCl 2; D-biotin, 0.28; cyanocobalamin (B<sub>12</sub>), 0.04; menadione sodium bisulphate (vitamin K), 2; ascorbic acid (vitamin C), 100; DL- $\alpha$ -tocopherol acetate (vitamin E), 100; cholecalciferol (vitamin D), 4000IU; retinyl acetate (vitamin A), 5000IU; salt (NaCl), 6200; copper sulphate (CuSO<sub>4</sub>.5H<sub>2</sub>O), 56; ferrous sulphate (FeSO<sub>4</sub>.7H<sub>2</sub>O), 130; manganese sulphate (MnSO<sub>4</sub>.H<sub>2</sub>O), 178; zinc sulphate (ZnSO<sub>4</sub>.H<sub>2</sub>O), 120; potassium iodide (KI), 16; corn starch

Table 3: Growth, nutrient utilization and apparent digestibility coefficient ADC) of *O. niloticus* fed tamarind diets for 56 days

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Initial mean weight (g)	6.10 ±0.17	6.30 ±0.17	6.36 ±0.18	6.04 ±0.19	6.04 ±0.18	6.00 ±0.17
Final mean weight (g)	13.13±0.52	13.66±0.51	14.23±0.53	14.25±0.51	13.61±0.51	13.04±0.52
Weight gain (g)	7.03 ±0.47	7.30 ±0.46	7.87 ±0.48	8.21 ±0.46	7.57 ±0.46	7.04 ±0.48
SGR (g/day)	1.37 ±0.06	1.37 ±0.05	1.44 ±0.03	1.53 ±0.05	1.45 ±0.06	1.39 ±0.05
FCR	2.29 ±0.09	2.31 ±0.08	2.21 ±0.10	2.08 ±0.07	2.17 ±0.10	2.27 ±0.08
PER	3.67 ±0.24	3.76 ±0.25	3.96 ±0.25	4.10 ±0.24	3.69 ±0.25	3.41 ±0.24
ADC protein (%)	84.9 ±3.05	87.0 ±3.04	89.6 ±3.10	91.2 ±3.11	88.2 ±3.08	82.9 ±3.12
ADC energy (%)	82.4 ±3.41	84.9 ±3.40	87.9 ±3.43	89.7 ±3.42	86.4 ±3.43	80.6 ±4.13

Values along the same row are not significantly different ( $P > 0.05$ )

Table 4: Carcass composition and blood parameters of *O. niloticus* fed for 56 days

Fish Fed	Crude protein	Ether extract	Ash	TEC (million/il) <sup>a</sup>	PCV (%) <sup>b</sup>	RBC (10 <sup>4</sup> mm <sup>3</sup> ) <sup>c</sup>
Before feeding	36.3	6.25	27.6	3.0	30	180
Diet 1	53.5	11.2	21.5	3.0	29	181
Diet 2	53.6	11.1	21.5	3.1	31	179
Diet 3	53.7	11.2	23.1	3.2	30	182
Diet 4	53.8	11.2	23.9	3.0	31	180
Diet 5	54.0	11.2	23.8	3.1	29	181
Diet 6	54.1	11.6	24.4	3.2	31	180

a. Total erythrocyte count b. Pack cell volume c. Red blood cells

This protein content is higher than that of maize (10%). The major anti-nutrients found in tamarind seed meal were low values of phytin and tannin. Gross composition of the experimental diets, the proximate composition, gross energy (GE) and protein-energy ratio (P: E) were presented in Table 2. The mean value of the dietary fat

recorded from the present study is within the acceptable range recommended for fish culture (Teshima and Kanazawa, 1986). The low fibre contents of the experimental diets depict the high quality which can support high feed digestibility and good fish yield. The mean value of the GE obtained is close to the value of 467 kcal.g<sup>-1</sup> used



by Abdelghany (2000) for good tilapia production. Li *et al.* (1991) showed that P:E of diets influenced nutrient utilization and growth; and optimum P:E boosted the profitability of diets and protein sparing ability of the diets (Xiquin *et al.* 1994).

The mean P:E obtained from this study was high but lower than 100mg kcal<sup>-1</sup> recorded by Santiago and Laron (1991) as optimum for red tilapia fed 30 % crude protein diets. However, the value compared favourably with the mean value of  $86 \pm 1.6$  which supported good yield of *O. niloticus*, (Nwanna and Daramola, 2000). Results of the feeding trials (Table 3) showed no significant variations in the mean weight gain (MWG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) among the fishes fed the control diet and those fed diets supplemented with tamarind seed meal at 20, 40, 60, 80 and 100 % levels of substitution. The mean values of SGR and FCR obtained from this study was close to the mean values of  $1.28 \pm 0.17$  and  $1.77 \pm 0.33$  for SGR and FCR reported for *O. niloticus* fed cottonseed meal based diets Mbahinzireki *et al.* (2001). The SGR obtained was also similar to the mean value of  $1.53 \pm 0.06$  documented for *O. niloticus* fed compounded diets (Maina *et al.* 2002). Furthermore, the mean value of PER obtained from the present study compared well with the mean values of PER of  $2.64 \pm 0.46$ , and  $2.48 \pm 0.55$  reported by Hossain *et al.* (2002) and Ulloa and Verreth (2002) for *O. niloticus* fed dhaincha seed meal based diets and bacteria-treated coffee pulp meal based diets, respectively.

The digestibility of individual ingredients in compounded diet is one of the important factors affecting the growth of fish (De Silva *et al.*, 1996). Uys (1988) noted that rather than look at growth responses, the digestibility of nutrients and energy contents of feedstuffs could be used to assess the suitability and nutritive value of feedstuffs/diets in fishes. The apparent digestibility coefficient (ADC) for protein and energy (Table 3) obtained from the present study were high. There were no significant variations in the ADC for protein and energy of the fishes fed diets 1 – 6 ( $p > 0.05$ ). However, the ADC for protein and energy were marginally higher in fish fed 60 % of tamarind seed meal based diet than in fishes fed other diets ( $p > 0.05$ ). The high values of the ADC for protein and energy recorded from this study indicated that the diets were well digested. The mean values of ADC for protein and energy obtained are close to the ADC for protein (93.0%) and energy (83.0%) recorded by Degani *et al.* (1997) for hybrid tilapia (*O. niloticus*<sup>0</sup> x *O. aureus*<sup>0</sup>) fed corn meal. Furthermore, the ADC for protein recorded from the present experiment was similar to mean ADC

value for protein of  $(81.7 \pm 7.3)$  reported by Mbahinzireki *et al.* (2001).

The carcass composition and blood parameters of the fishes fed diets 1-6 for 56 days are presented in Table 4. Protein and fat deposition were similar in all the fishes fed diets 1–6, and there was no significant difference in ash content ( $p > 0.05$ ). The total erythrocyte count (TEC), pack cell volume (PCV) and the red blood cells (RBC) were high. There were no significant variations in TEC, PCV and RBC of the fishes fed all the diets ( $p > 0.05$ ). The RBC value in this study was close to the range of 700,000-2,000,000 cells mm<sup>3</sup> recommended by Saunders (1966) for healthy teleosts. The mean PCV value obtained from the present study was similar to the value of 32.1 % reported by Abdelghany (2000), and the mean value of  $36 \pm 0.5$  reported for *O. niloticus* by Mbahinzireki *et al.* (2001).

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## SEX DISCRIMINATION AMONG FOUR MORMYRID SPECIES OF ANAMBRA RIVER SYSTEM NIGERIA

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### ABSTRACT

*Sex discriminating characters of four mormyrid species caught from Anambra river basin, Nigeria were investigated. Sexual dimorphism occurred in only one transformed character – dorsal fin base length and in four raw morphometric characters namely total length, standard length, dorsal fin base length and anal fin base length. These characters are recommended as key characters in mormyrid taxonomy.*

**Key Words:** Mormyridae, sex dimorphism, Anambra river

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### INTRODUCTION

*Mormyrus rume*, *Hyperopisus bebe*, *Campylomormyrus tamandua* and *Gnathonemus petersii* are among the mormyrids inhabiting fresh waters of tropical Africa (Lowe-McConnell, 1975). They are very common, of commercial importance and are often seen in piles of smoke-cured fishes (Teugels *et al* 1992). They are used, along with *Clarias* species (Ezenwaji and Inyang, 1998), in preparing local delicacies for marriage and naming ceremonies. Despite their fisheries and importance, very little information exists on their morphometric characters and meristic counts. Venu and Kurup (2003) had noted the importance of morphometric characters for the differentiation of taxonomic units in fishes. Anyanwu and Ugwumba (2003) used morphometric parameters, meristic counts and electrophoresis traces to separate *Pseudotolithus senegalensis* caught from three zones in the Nigerian inshore waters. Morphometric characters and meristic counts have been used to delimit *Clarias* species in Anambra river (Ezenwaji, 1986; Eyo, 1997, 2003 a, b, Eyo and Inyang 2004) and to distinguish between *Heterobranchus bidorsalis* and *Heterobranchus bidorsalis* x *Clarias gariepinus* hybrid (Madu *et al.*, 1993). The present study aims at identifying specific differences in morphometric characters to establish sexual dimorphism in *M. rume*, *H. bebe*, *C. tamandua* and *G. petersii*.

### MATERIALS AND METHODS

Fish specimens were collected monthly at Otuocho and Ogurugu between October, 2000 and March, 2002 using gill nets, drag nets, surface drift nets and cast nets of various mesh sizes. Baskets, traps and hook and line were also used to catch the fish. Specimens were also bought from the major landing Anambra river port at Otuocho. The multiple sampling methods were employed to eliminate gear selectivity and ensure good representation of all sizes of the Fish. Individuals required for the morphometric studies were iced and transported to the Pure and Applied Project Laboratory, Department of Zoology, University of Nigeria, Nsukka where they were kept under refrigeration until used.

Identification of fish collected was done using the keys of Holden and Reed (1972), Lowe-McConnell (1972), Teugels *et al.* (1992) and Olaosebikan and Raji (1998). The sex of each fish was determined. Prior to the measurement of the morphometric characters, each frozen specimen was allowed to thaw completely after which the weight was taken to the nearest 0.01 gram using a Metler PC 2000 electronic balance. Fish were measured to the nearest 0.01 centimeters using a fish measuring board, Veneir caliper and a pair of dividers. The characters measured were:

- Standard length (SL): The length from the tip of the snout to the anterior base of the caudal fin.

**Table 1: Sex dimorphism in raw data among the mormyrid species in Anambra river**

Morphometric parameters	<i>Mormyrus rume</i>				<i>Hyperopisus bebe</i>			
	Males	Females	T-Value	2-Tail Prob.	Males	Female	T-Value	2-Tail Prob.
Standard length (SL)	37.38±0.55	45.03±0.85	-23.03	0.00*	27.59±0.64	29.55±0.74	-9.98	0.00*
Total length (TL)	40.08±0.77	45.02±0.85	-18.88	0.00*	29.81±0.81	32.55±1.59	-5.05	0.00*
Pectoral fin Height (PFH)	4.14±0.53	4.23±1.21	-0.28	0.79	2.75±0.41	2.86±0.41	-0.68	0.51
Pectoral fin Base length (PFBL)	1.49±0.31	1.54±0.46	-0.26	0.80	1.01±0.24	0.85±0.28	1.62	0.13
Pelvic fin Height (PeFH)	3.41±0.37	3.86±1.07	-1.54	0.15	2.20±0.26	2.47±0.19	-3.06	0.01*
Pelvic fin Base length (PeBL)	1.61±0.40	1.45±0.52	1.68	0.12	0.71±0.23	0.85±0.28	1.51	0.16
Dorsal fin Base Length (DFBL)	16.28±0.40	17.32±0.44	-6.01	0.00*	2.02±0.19	2.50±0.31	5.72	0.00*
Anal fin Height (AFH)	2.85±0.21	3.62±0.42	-6.02	0.00*	2.78±0.25	2.58±0.28	2.05	0.06
Anal fin Base length (AFBL)	2.80±0.35	3.25±0.35	-4.70	0.00*	10.91±0.41	12.99±0.65	-8.28	0.00*
Pelvic-Anal fin space (PeAFS)	5.82±0.74	7.47±0.51	-5.04	0.00*	5.01±0.33	5.04±0.46	-0.17	0.87
Pectoral-Pelvic fin space (PpeFS)	4.71±0.30	5.35±0.15	-6.32	0.00*	2.60±0.49	3.35±0.28	-4.12	0.00*
Morphometric parameters	<i>Campylomormyrus tamandua</i>				<i>Gnathonemus petersii</i>			
	Males	Females	T-Value	2-Tail Prob.	Males	Female	T-Value	2-tail Prob.
Standard length (SL)	27.40±0.36	28.82±0.63	-6.05	0.00*	34.02±1.72	39.55±3.81	-4.44	0.00*
Total length (TL)	28.86±0.52	30.11±1.49	-2.52	0.03*	37.58±0.29	41.44±0.37	-6.79	0.00*
Pectoral fin Height (PFH)	3.95±0.56	4.85±0.56	-5/76	0.00*	4.15±0.47	4.62±0.71	-2.44	0.03*
Pectoral fin Base length (PFBL)	1.27±0.20	1.56±0.27	-3.19	0.01*	1.69±0.34	2.02±0.39	-1.92	0.08
Pelvic fin Height (PeFH)	3.18±0.26	3.42±0.38	-2.11	0.06	2.45±0.54	2.60±0.64	-1.26	0.23
Pelvic fin Base length (PeBL)	1.10±0.21	1.99±0.37	-9.74	0.00*	1.14±0.39	0.53±0.37	-2.50	0.03*
Dorsal fin Base Length (DFBL)	7.12±0.25	8.85±0.33	-1.86	0.05*	3.15±0.56	4.17±0.73	-4.31	0.00*
Anal fin Height (AFH)	3.12±0.45	3.45±0.44	-1.87	0.09	3.37±0.36	3.70±0.35	-2.60	0.02*
Anal fin Base length (AFBL)	10.52±0.49	11.02±0.56	-2.60	0.02*	8.26±0.68	9.36±0.56	-3.99	0.00*
Pelvic-Anal fin space (PeAFS)	4.70±0.29	4.82±0.26	-0.99	0.34	4.32±0.52	4.35±0.53	-0.04	0.97
Pectoral-Pelvic fin space (PpeFS)	2.77±0.31	2.77±0.31	-1.92	0.08	3.16±0.41	2.72±0.30	2.91	0.01*

Key \*indicates significant difference @ P = 0.05

**Table 2: Sex dimorphism in ratio (percentage standard length) among the mormyrid species in Anambra river**

Morphometric parameters	<i>Mormyrus rume</i>				<i>Hyperopisus bebe</i>			
	Males	Females	T-Value	2-Tail Prob.	Males	Female	T-Value	2-tail Prob.
Total length (TL)	110.8±2.61	107.23±2.18	4.44	0.00*	108.12±4.72	110.18±5.24	-1.16	0.27
Pectoral fin Height (PFH)	11.08±1.41	11.09±1.17	-0.02	0.99	9.99±0.91	9.85±1.25	0.30	0.77
Pectoral fin Base length (PFBL)	3.99±0.64	3.75±1.25	0.53	0.60	3.68±0.75	7.68±1.29	-10.94	0.00*
Pelvic fin Height (PeFH)	9.12±0.07	10.20±1.02	-2.26	0.04*	7.98±1.05	8.35±0.68	-1.21	0.25
Pelvic fin Base length (PeBL)	4.45±1.15	3.56±1.29	3.32	0.01*	2.58±0.87	2.91±1.02	-0.99	0.34
Dorsal fin Base Length (DFBL)	45.51±1.44	42.32±1.75	1.57	0.02*	7.34±0.77	8.46±0.09	-3.73	0.00*
Anal fin Height (AFH)	7.64±0.56	8.86±1.19	-3.45	0.00*	10.07±0.93	8.72±0.82	3.89	0.00*
Anal fin Base length (AFBL)	7.95±0.88	7.49±0.96	1.63	0.13	39.55±1.35	43.96±2.28	-5.29	0.00*
Pelvic-Anal fin space (PeAFS)	18.26±1.48	15.58±0.50	3.05	0.01*	9.42±1.71	11.37±1.07	-2.88	0.01*
Pectoral-Pelvic fin space (PpeFS)	12.60±0.73	13.04±0.08	-1.92	0.08	9.46±1.74	11.37±1.07	-2.75	0.02*
Morphometric parameters	<i>Campylomormyrus tamandua</i>				<i>Gnathonemus petersii</i>			
	Males	Females	T-Value	2-Tail Prob.	Males	Female	T-Value	2-tail Prob.
Total length (TL)	105.32±2.22	105.67±2.67	-0.33	0.75	110.76±6.91	105.53±9.33	1.70	0.12
Pectoral fin Height (PFH)	14.41±1.61	16.41±1.98	-4.21	0.00*	12.36±1.43	11.71±1.70	1.27	0.23
Pectoral fin Base length (PFBL)	4.59±0.48	5.42±0.97	-2.43	0.03*	5.02±1.26	5.14±0.88	-0.25	0.81
Pelvic fin Height (PeFH)	11.51±1.05	11.90±1.59	-0.88	0.42	7.23±1.48	6.62±1.47	1.32	0.21
Pelvic fin Base length (PeBL)	3.98±0.77	6.93±1.33	-8.37	0.00*	3.45±1.04	3.88±0.85	-1.27	0.23
Dorsal fin Base Length (DFBL)	25.99±0.90	27.17±1.41	2.52	0.03*	9.30±1.85	10.48±1.79	-1.87	0.04*
Anal fin Height (AFH)	11.40±1.63	11.96±1.61	-0.36	0.41	9.95±1.22	9.43±1.17	1.08	0.30
Anal fin Base length (AFBL)	38.06±2.43	38.24±1.59	-0.22	0.83	24.37±2.36	23.85±2.53	0.44	0.67
Pelvic-Anal fin space (PeAFS)	10.08±1.15	10.25±1.36	-0.47	0.64	9.32±1.17	6.94±1.16	5.40	0.00*
Pectoral-Pelvic fin space (PpeFS)	10.10±1.13	10.18±1.44	-0.22	0.83	9.32±1.17	6.94±1.16	5.40	0.00*

Key \*Indicate significant difference @ P= 0.05

- Total length (TL): The length from the tip of the snout to the end of the caudal fin.
- Pectoral fin height (PFH): The length of the tallest pectoral fin ray.
- Pectoral fin base length (FBL): The basal length of the pectoral fin.
- Pelvic fin height (PeFH): The length of the tallest pelvic fin ray.
- Pelvic fin base length (PeFBL): The basal length of the pelvic fin i.e. the distance between the anterior base of the first pelvic fin ray to the posterior base of the last pelvic fin ray.

- Dorsal fin base length (DFBL): The distance between the anterior base of the first dorsal fin ray to the posterior base of the last dorsal fin ray.
- Anal fin height (AFH): The length of the tallest anal fin ray.
- Anal fin base length (AFBL): The basal length of the anal fin i.e. the distance between the first and last anal fin rays.
- Pelvic-anal fin space (Pe-AFS): The ventrobasal distance between the posterior end of the pelvic fin and the anterior end of the anal fin.
- Pectoral – pelvic fin space (Ppe-Fs): The ventrobasal distance between the posterior end of the pectoral fin and the anterior end of the pelvic fin.

## RESULTS AND DISCUSSION

Sex differentiating characters in the raw data among males and females of all the mormyrid species (Table 1) were detected in 4 (36.4 %) of the 11 studied characters. These include total length, standard length, dorsal fin base length and anal fin base length. In the transformed data (ratio data), the only sex differentiating character among males and females in all the mormyrid species studied was dorsal fin base length (Table 2). These characters are recommended as key characters in mormyrid taxonomy.

Among *M. rume*, sex discrimination occurred in 7 (63.6 %) raw and 6 (60 %) ratio morphometric characters. Four characters (total length, dorsal fin base length, anal fin height and pelvic – anal – fin space) showed sex discrimination both in the raw and transformed (ratio) data. For *H. bebe*, sex discrimination was recorded in 6 (54.6 %) raw and 6 (60 %) ratio characters. Three morphometric characters namely dorsal fin base length, anal fin base length and pectoral-pelvic fin space showed sex discrimination both in the raw and transformed data. Sex discrimination occurred in 7 (63.6 %) raw and 4 (40 %) morphometric characters in *C. tamandua*. Three characters (pectoral fin base length, pelvic fin base length and dorsal fin base length) showed sex discrimination both in the raw and transformed (ratio) data.

Considering *G. petersii*, sex discrimination was recorded in 3 (27.3 %) ratio and 8 (80 %) raw morphometric characters. Two characters namely dorsal fin base length and pectoral-pelvic fin space showed sex discrimination both in the raw and transformed (ratio) data.

Similar work in sexual dimorphism among the teleosts has been reported by Libosvsky and Bishara (1987). Their report demonstrated sexual differences in three characters in *O. niloticus* and seven characters in *T. zilli*. This finding is also similar to the report of Beacham *et al* (1988) who noted sexual dimorphism in four morphometric characters (head width, caudal peduncle depth, anal fin base length and dorsal fin height) between male and female pink salmon *Onchorhynchus gorbuscha* in British Columbia. This report also relates to the findings of Reist *et al* (1995) who demonstrated sexual dimorphism in pelvic and anal fin rays among male and female arctic char *Salvelinus alpinus* from lake Hazen. Furthermore, the present study is consistent with the report of Nwani (1998) who reported sexual dimorphism in four raw morphometric characters (fork length, anal fin height, pectoral – pelvic fin space and pelvic anal fin space) among *Distichodus* species of Anambra river.

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## LIPID COMPOSITION OF TWO MARINE FISHES – *Scomber scombrus* AND *Trachurus trachurus*

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### ABSTRACT

*Lipids from two species of marine fish – Scomber scombrus and Trachurus trachurus were investigated. Fish oil from Trachurus trachurus had higher oil yield than that of Scomber scombrus. The lipids contain high levels of triacylglycerol 228 – 250 mg%, cholesterol 160 – 235 mg%, and phospholipid 2.2 – 2.4 mg%. Saponification of the different oils yielded saturated and unsaturated fatty acids, such as palmitic, oleic linoleic acids. Hexane and methanol were found effective solvents for separation of fatty acids from the fish oil.*

**Keywords:** Fish oil, Nutrition, Industrial applications

### INTRODUCTION

Fish oils have long been a natural constituent of human diet, and have been reported to be hypocholesterolemic (Bang, 1990). They contain essential fatty acids especially oleic and linoleic acids as well as other polyunsaturated fatty acids (Sztem and Harris, 1991). The unique unsaturated fatty acids of marine oil particularly their long chain fatty acids C<sub>20</sub> – C<sub>22</sub> unsaturates make them desirable for a number of industrial and food applications (Visser and Meijer, 1990; and Wright, 1990). In the present study the composition of lipids obtained from two common marine fishes sold in a tropical Nigerian market, as well as fractionation of the constituent fatty acids was investigated to complement existing knowledge on their nutritional uses.

### MATERIALS AND METHODS

Fresh fishes were collected from the Nsukka market, Enugu State, Nigeria and their identity authenticated by the taxonomical section of the Department of Zoology, University of Nigeria, Nsukka. The fishes were dissected to remove the visceral content, and were oven dried at 55 ± 1 °C for 25 minutes to prepare them for milling. Total lipids for each fish species were extracted from the dried mill (100 g) using the method of Folch *et al.*, (1957). Triacylglycerol was estimated by the method of Gottfried and

Rosenberg (1973), Cholesterol by the method of Stadam (1975) and phospholipid by the method of Stewart (1980). Saponification of the oil and fractionation of fatty acids were performed by solvent crystallization method of El-Zanati and Khedr (1991). All the analysis were carried out in triplicates.

The oil (100 g) was saponified with alcoholic potassium hydroxide and the resulting soap hydrolysed with 1% dilute sulphuric acid to liberate the free fatty acids, which were then washed with warm water until free from mineral acids. Solvent crystallization was carried out to separate the fatty acids into fraction El-Zanati and Khedri (1991).

Several organic solvents (hexane, methanol, acetone and ethanol) were tried in order to determine the most suitable solvent for winterization and separation of fatty acids. A sample (50 g) of each fish was dissolved in the organic solvents at different oil to solvent ratio ranging from 1:1 to 1:5 (w/v). The solution formed were cooled at 56 ± 1 °C for 24 hours to complete the crystallization of the fatty acids. The crystals were recovered by filtration. Further fatty acids were obtained from mother liquor by distillation and chilling of the concentrates. The degree of unsaturation and saturation of the fatty acids were determined by evaluation of the iodine values using (AOAC, 1984).

**Statistical Analysis:** Mean values were compared using student T-test.

## RESULTS

The lipid constituent of the oils is presented in Table 1. From the table, the total oil yield of *Trachurus trachurus* was 17.10 % while that of *scomber scombrus* was 10.11 %. The Triacylglycerol levels ranged between 228 mg% in *scomber scombrus* and 250 mg% for *Trachurus trachurus*. The cholesterol levels found in the study was 168 mg% for *scomber scombrus* and 235 mg% for *trachurus trachurus*. The lecithin levels (phospholipids) was found to be between 2.2 mg% and 2.4 mg% for both species. The saturated and unsaturated fatty acids are presented on table 2.

**Table 1: Lipid composition of *Trachurus trachurus* and *Scomber scombrus***

Lipids	<i>Trachurus trachurus</i>
% yield	17.10 ± 0.14
Triacylglycerol mg%	250.0 ± 0.36
Cholesterol mg%	235.0 ± .46
Phospholipid mg%	2.4 ± 0.2
	<i>Scomber scombrus</i>
% yield	10.11 ± 0.15
Triacylglycerol mg%	228.0 ± 0.24
Cholesterol mg%	168.0 ± 0.34
Phospholipid mg%	2.2 ± 0.18

**Table 2: Percentage yield of saturated and unsaturated fatty acids using hexane and methanol as solvents**

Fish Species	Hexane	
	SFA	USFA
<i>Trachurus trachurus</i>	44.75	55.23
<i>Scomber scombrus</i>	47.14	52.85
	Methanol	
	SFA	USFA
<i>Trachurus trachurus</i>	40.67	59.38
<i>Scomber scombrus</i>	43.64	55.85

## DISCUSSION

The result of this study shows that marine fishes are good sources of oil. The oil yield from the two species averaged 13.9 %. The total lipid from these species differ from those found in some other marine fishes especially mackerel 5.23 % and those from fresh water fishes (5.8 %) (Viswanatha and Gopakumar, 1978). The actual reason for this variation in the total lipid content of different species is not clearly understood, but possibly the long spawning

migration as well as size of the edible portion might be plausible reason (Stansby, 1972). The triacylglycerol and cholesterol contents of the marine fishes were high and are consistent with earlier reports (Ackman and Eaton, 1972 and Stansby, 1972). The high levels of the triacylglycerol may be important in the physiology as well as energy needs of the fishes, as they are readily used during long spawning migrations (Stansby, 1991). Similarly, the cholesterol may be utilized for biosynthetic processes, especially in the synthesis of steroids and bile acids (Voet and Voet, 1990). In nutrition and health, fish oil has found tremendous use in the treatment of arthritis, multiple sclerosis, cancer, malaria, non-insulin dependent diabetes, renal diseases, and chronic liver diseases (Orvilla and Arba, 1992; Wright, 1990; Visser and Meijer, 1990). Although the fraction of the fish oil that achieve these therapeutic functions are the omega 3-fatty acids (Bang, 1990). In the present study we did not determine the omega 3-fatty acids rather we fractionated the oil into its saturated and unsaturated fractions. The results obtained show that hexane rather than methanol, ethanol and acetone could be used satisfactorily in separating fish oil into its constituent fatty acids. The percentage saturated fatty acids when compared to the unsaturated fractions were high and compares well with results obtained from another study (Stansby, 1972). Apart from vegetable oils, fish oils contain primarily polyunsaturated fatty acids which vary in the location of the double bonds within the molecules. Polyunsaturated fatty acids have long been reported to be protective against cardiovascular diseases (Engerberg, 1959). It therefore means that diet rich in fish oil will be protective against cardiovascular diseases, and possibly other diseases like cancer, arthritis (Stansby, 1991). Although fats rich in unsaturated fatty acids are prone to oxidation which may trigger off free radicals, the results obtained from this study as well as earlier results from other studies, shows that fish oil contains a lot of antioxidant vitamins, A & E as well as phospholipids (Aruoma, 1993). These vitamins and phospholipids protect the oil from oxidative reactions and hence prevent the production of the free radicals, which could lead to tissue damage. Similarly, the phospholipids extracted could be emulsified and used in drug formulation as an emulsifier (Ononogbu, 1988). From an industrial view point, the saturated fatty acid produced may have immediate applications, especially as fat blends in the

confectionery industries or used in the formulation of fat emulsions for parenteral nutrition (Akoh, 1995; Bimbo and Gowther, 1992). This may well substitute coconut oil or be used as blends with coconut oil, a common oil used in parenteral nutrition.

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## FACTORS AFFECTING GROWTH AND BODY MEASUREMENTS OF THE GRASSCUTTER (RODENTIA: THRYONOMYIDAE)

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### ABSTRACT

*Overall mean body weights ( $0.138 \pm 0.06$  and  $0.513 \pm 0.03$  kg), body lengths ( $17.24 \pm 0.30$  and  $21.68 \pm 0.65$  cm), heart girths ( $12.57 \pm 0.18$  and  $16.83 \pm 0.90$  cm), and height-at-withers ( $7.39 \pm 0.14$  and  $9.86 \pm 0.52$  cm) of the grasscutter (*Thryonomys swinderianus* Temminck) at birth and 60 days of age, respectively, were recorded. Litter size and sex significantly influenced body weight and linear body measurements in the grasscutter. Mean birth weight ( $0.173 \pm 0.02$  kg) of rats born singles was significantly different from that of twin births ( $0.135 \pm 0.08$  kg) and triplets ( $0.135 \pm 0.09$  kg) ( $P < 0.05$ ). Male grasscutters with a mean birth weight of  $0.148 \pm 0.01$  kg were heavier ( $P < 0.05$ ) than the females, which weighed  $0.128 \pm 0.02$  kg. Average daily weight gain for the first 60 days for males ( $0.007$  kg/d) was significantly different from that of females ( $0.005$  kg/d) ( $P < 0.05$ ). Parity had no significant effect on the rat's birth weight, weight at 60 days of age, and average daily weight gain for the first 60 days. Litter size, sex and parity did not have significant effect on the linear body measurements of the grasscutter at birth ( $P > 0.05$ ). However litter size and sex had significant influence on body length and heart girth of the grasscutter at 60 days of age ( $P < 0.05$ ). At 60 days the mean body length ( $23 \pm 0.28$  cm) and heart girth ( $18.13 \pm 0.23$  cm) of rats born singles were longer and larger than those of twins ( $21.66 \pm 0.89$  and  $16.82 \pm 0.76$  cm) and triplets ( $21.59 \pm 0.96$  and  $16.71 \pm 0.80$ ). Males also have longer body length and larger heart girth than females at that age.*

**Keywords:** Grasscutter, Growth, Body Weight, Linear Measurements

### INTRODUCTION

Correlation matrix has shown high, positive and significant values among body weight, body length, heart girth and height-at-withers in the grasscutter. Ikpeze and Ebeneke (2004) reported that any one of these linear measurements could be used to predict the grasscutter body weight at 2-10 months of age. However, genetic improvement is required to improve the meat yield of the grasscutter.

To achieve this goal proper measurement of grasscutter body traits is required. Equally important is the need to understand the factors that influence their growth and development. The aim of this study therefore is to estimate the possible effects of parity, the litter size and sex on the birth weight, growth rate and linear body measurements in the grasscutter. This will provide parameters as correction factors in estimating body traits for selection purposes.

### MATERIALS AND METHODS

Data used for this study were collected from 48 grasscutter rats given birth by 7 dams. The female grasscutters were identified. Their first three parities occurred between 2001 and 2003 at our grasscutter research station in Akpaka forest reserve Onitsha. The animals were reared under the floor-housing system of management (Ikpeze and Ebeneke, 2004). Combinations of grasses and pineapple crowns were provided *ad lib* as the main diets of the animals. Proximate analysis indicated high content of fiber in the feeds of the grasscutter. Grasses fed the animals included *Adropogon gayanas* (11.60 % CP, 21 % CF), *Panicum maximum* (5.65 % CP, 30 % CF), *Paspalum vaginatum* (14.0 %, 27.4 % CF) *Pennisetum purpureum* (7.35 % CP, 25 % CF) and pineapple crowns (*Ananas cosmosus* - CP 3.75 %).

Records kept on each dam, included parity, litter size, and sex of the resultant rats. Body weight of the rats at birth and at 60 days

was taken using a Way Master precision scale. The average daily weight gain (kg/day) was obtained by subtracting the birth weight (kg) from 60 day's body weight and dividing by 60. The body length (cm) was measured as the distance from the tip of the nose to the base of the tail. Heart girth (cm) was measured as the circumference of the chest, and height-at-withers (cm) was the distance from the surface of a platform to the withers. The rats were measured at birth and at 60 days of age. 48 sets of measurements were obtained for the nine variables considered.

Only rats with complete records from birth to 60 days of age were included in the statistical analyses. Least square means for body weight, average daily weight gains, linear body traits were estimated. One way analysis of variance was done to examine the possible effects of parity, litter size and sex of resultant rats on the measured variables.

## RESULTS AND DISCUSSION

Tables 1, 2 and 3 show the 48 sets of data obtained from the female grasscutters during their first three parities respectively. The seven females produced 17 grasscutter rats at their first parity, sixteen at second parity, and 15 during the third parity. Average litter size was 2 - 3, with an average sex ratio of one male to two female births. Mean values of the measurements obtained from the resultant grasscutter during the periods under study are summarized as shown in Tables 4 and 5. The effects of litter size, sex and parity on the birth weight, weight at 60 days of age, the average daily weight gain for the first 60 days are shown in Table 4. The mean values of body length, heart girth, and height-at-withers are presented in Table 5.

The average litter size of 2 - 3 recorded in this study compares favourably with Average of 2 - 4 reported by Ajayi (1983). Asibey (1974), Onadeko and Amubode (2002) also reported an average of 4 litter births. The extent to which dietary composition of feed, level of feeding, and housing condition influence litter size in captive grasscutter has not been fully investigated. Succulent grasses, pineapple crowns and oil palm fruits that were provided *ad lib* as the major feeds of the grasscutter were available in the study area throughout the seasons. Timibitei (1998) reported that the rabbit reared in floor system obtained about 18% of digestible crude fiber by eating the fibrous litter materials to balance their fiber

requirement. This instinctive behaviour may be in operation in the grasscutter reared in the floor system in this study. Elephant grass (*Pennisetum purpureum*) was highly utilized by the grasscutter in the wild (Afolayan and Anadu, 1980). Crowns of pineapple (*Ananas comosus*), nuts of oil palm (*Elaeis guineensis*), fruits of pawpaw (*Carica papaya*) were also reported to be cherished by the grasscutter (Adu, 1995). It was observed in this study that males feed more aggressively than the females; females usually started feeding when the males were satisfied. Litter size had significant influence on the birth weight of grasscutters.

Mean birth weights ( $0.173 \pm 0.02$  kg) of grasscutters born singles were significantly different from the twin births ( $0.135 \pm 0.08$  kg) and triplets births ( $0.135 \pm 0.09$  kg) (see Table 4). This is an indication that birth weight may be inversely related to litter size, but this requires further investigation. Male grasscutters with a mean birth weight of  $0.148 \pm 0.01$  kg were generally heavier than the females, which weighed  $0.128 \pm 0.02$  kg ( $P < 0.05$ ). The mean birth weight of 0.138 kg recorded in this study compares favourably with 0.134 kg and 0.128 kg for males and females grasscutters respectively (Yewadan and Schrage, 1992). Onadeko and Amubode (2002) had reported a mean birth weight of  $0.118 \pm 0.0027$  kg and  $0.100 \pm 0.0275$  kg for males and females respectively. There were significant differences in the mean daily weight gains for the first 60 days in single births ( $0.007 \pm 0.0004$  kg), twin births ( $0.006 \pm 0.0008$  kg) and triplet births were competition usually occurred among the littermates. Mean daily weight gain for the first 60 days in the male ( $0.007 \pm 0.0003$  kg/d) was significantly different from the female ( $0.005 \pm 0.0006$  kg/d), perhaps due to the aggressive feeding behaviour of the male. At 60 days, the mean body length ( $23 \pm 0.28$  cm) and heart girth ( $18.13 \pm 0.23$  cm) of rats born singles were longer and larger than those of twins ( $21.66 \pm 0.89$  and  $16.82 \pm 0.76$  cm) and triplets ( $21.59 \pm 0.96$  and  $16.71 \pm 0.80$ ). Males also have longer body length ( $22.49 \pm 0.58$ ) and larger heart girth ( $17.63 \pm 0.47$ ) than females ( $21.34 \pm 0.96$  and  $16.47 \pm 0.73$  respectively) at that age. There is an indication of sexual dimorphism in the grasscutter. Parity had no significant effect on the rat's birth, 60 days weight, and average daily weight gain for first 60 days. The effect of younger dams giving birth to rats of smaller birth weight may have been obscured by the presence of dams of similar ages across parities.

Table 1: Records obtained from female grasscutters during their 1<sup>st</sup> Parity at Akpaka Forest Reserve Onitsha

Reserve Offspring				Body measurements at Birth				Body measurements at 60 days				Daily weight gain for 1 <sup>st</sup> 60 days (kg/day)
Dams s/no.	Litter size	Rats s/no	Sex	BW (kg)	BL (cm)	HG (cm)	H (cm)	BW (kg)	BL (cm)	HG (cm)	H (cm)	
1	3	1	M	0.148	17.72	12.84	7.60	0.586	23.15	18.00	10.64	0.00730
		2	F	0.137	17.59	12.70	7.54	0.522	22.35	17.25	10.20	0.00640
		3	F	0.139	17.62	12.73	7.55	0.526	22.39	17.30	10.22	0.00645
2	1	4	M	0.192	18.25	13.35	7.92	0.592	23.20	18.07	10.68	0.00666
3	3	5	F	0.142	17.65	12.77	7.57	0.526	22.39	17.29	10.22	0.00640
		6	F	0.144	17.67	12.79	7.59	0.520	22.30	17.22	10.18	0.00626
		7	F	0.148	17.72	12.81	7.59	0.513	22.23	17.14	10.14	0.00608
4	2	8	M	0.151	17.75	12.80	7.65	0.593	23.22	18.07	10.68	0.00736
		9	F	0.146	17.70	12.82	7.60	0.548	22.66	17.59	10.37	0.00670
5	3	10	M	0.150	17.75	12.86	7.63	0.585	23.12	17.98	10.63	0.00725
		11	F	0.140	17.62	12.74	7.50	0.537	22.52	17.42	10.30	0.00661
		12	F	0.145	17.68	12.80	7.59	0.532	22.48	17.36	10.26	0.00645
6	3	13	F	0.143	17.66	12.78	7.58	0.526	22.38	17.29	10.22	0.00638
		14	F	0.141	17.64	12.78	7.58	0.529	22.42	17.32	10.24	0.00646
7	2	15	F	0.144	17.64	12.76	7.56	0.524	22.36	17.27	10.21	0.00633
		16	F	0.145	17.68	12.80	7.50	0.530	22.44	17.34	10.25	0.00641
		17	F	0.144	17.67	12.80	7.59	0.527	22.40	17.30	10.23	0.00638
Average				0.147	17.70	12.82	7.59	0.542	22.58	17.48	10.33	0.00658

Average litter size was 2.43 with a sex ratio of 4 male to 13 females (i.e., 1:3.25). Though the average birth weight was 0.147kg, it appears to be inversely related to litter size. Males were generally heavier than females at birth and at 60 days of age.

Table 2: Records obtained from female grasscutters during their 2<sup>nd</sup> Parity at Akpaka Forest Reserve Onitsha

Reserve Ontario												
Dams s/no.	Litter size	Rats s/no	Sex	Body measurements at Birth				Body measurements at 60 days				Daily weight gain for 1 <sup>st</sup> 60 days (kg/day)
				BW (kg)	BL (cm)	HG (cm)	H (cm)	BW (kg)	BL (cm)	HG (cm)	H (cm)	
1	2	18	M	0.131	17.10	12.48	7.29	0.554	22.19	17.40	10.13	0.00705
		19	F	0.126	17.00	12.42	7.26	0.442	20.84	16.10	9.38	0.00526
2	3	20	F	0.132	17.10	12.49	7.30	0.448	20.92	16.17	9.42	0.00526
		21	F	0.128	17.06	12.45	7.27	0.442	20.81	16.00	9.35	0.00523
3	3	22	F	0.118	16.94	12.33	7.20	0.429	20.68	15.95	9.29	0.00518
		23	M	0.138	17.20	12.6	7.35	0.566	22.34	17.57	10.21	0.00693
		24	M	0.140	17.62	12.70	7.48	0.559	22.25	17.48	10.16	0.00698
4	1	25	F	0.123	17.00	12.39	7.24	0.458	21.05	16.28	9.48	0.00558
		26	M	0.175	17.63	13.00	7.58	0.642	23.25	18.42	10.72	0.00778
5	3	27	M	0.141	17.60	12.72	7.50	0.572	22.41	17.61	10.25	0.00718
		28	F	0.133	17.13	12.50	7.30	0.496	21.49	16.72	9.74	0.00605
6	2	29	F	0.128	17.05	12.40	7.25	0.437	20.78	16.04	9.34	0.00515
		30	M	0.130	17.09	12.47	7.28	0.559	22.25	17.48	10.16	0.00715
		31	F	0.124	17.00	12.40	7.25	0.468	21.18	16.40	9.56	0.00573
7	2	32	F	0.128	17.06	12.45	7.26	0.443	20.86	16.11	9.38	0.00525
		33	F	0.122	16.90	12.37	7.23	0.421	20.59	15.85	9.24	0.00498
Average				0.133	17.70	12.51	7.31	0.496	22.58	17.48	10.33	0.0060

Average litter size was 2.28 with a sex ratio of 6 male to 10 females (i.e., 1:1.6). Though the average birth weight was 0.133kg, males were generally heavier than females at birth and at 60 days of age.



Table 3: Records obtained from female grasscutters during their 3<sup>rd</sup> Parity at Akpaka Forest Reserve Onitsha

Dams s/no.	Litter size	Rats s/no	Sex	Body measurements at Birth				Body measurements at 60 days				Daily weight gain for 1 <sup>st</sup> 60 days (kg/day)
				BW (kg)	BL (cm)	HG (cm)	H (cm)	BW (kg)	BL (cm)	HG (cm)	H (cm)	
1	1	34	M	0.138	16.98	12.42	7.29	0.605	22.54	17.78	10.40	0.00778
2	2	35	M	0.142	17.00	12.46	7.31	0.545	21.82	17.09	10.00	0.00671
		36	F	0.131	16.89	12.34	7.25	0.421	20.35	15.67	9.17	0.00480
3	3	37	M	0.140	17.00	12.45	7.30	0.517	21.49	16.77	9.82	0.00628
		38	M	0.138	16.98	12.42	7.28	0.525	21.56	16.86	9.87	0.00645
		39	F	0.130	16.88	12.32	7.23	0.416	20.29	15.61	9.14	0.00476
4	3	40	F	0.135	16.94	12.38	7.27	0.407	20.18	15.51	9.08	0.00453
		41	F	0.126	16.83	12.28	7.20	0.426	20.41	15.73	9.21	0.00500
		42	F	0.120	16.76	12.21	7.17	0.422	20.36	15.68	9.18	0.00503
5	1	43	M	0.188	17.54	13.00	7.62	0.647	23.04	18.27	10.68	0.00765
6	2	44	M	0.138	16.98	12.41	7.28	0.561	22.01	17.28	10.10	0.00705
		45	F	0.133	16.92	12.38	7.25	0.433	20.49	15.81	9.25	0.00500
7	3	46	F	0.125	16.82	12.27	7.20	0.411	20.23	15.55	9.10	0.00496
		47	F	0.115	16.70	12.15	7.14	0.400	20.10	15.43	9.00	0.00475
		48	F	0.122	16.80	12.23	7.18	0.424	20.38	15.70	9.20	0.00503
Average				0.134	16.93	12.38	7.26	0.477	21.01	16.31	9.54	0.00570

Average litter size was 2.4 with a sex ratio of 6 males to 9 females (1:1.5). Though the average birth weight was 0.134 kg, but males were generally heavier than females at birth and at 60 days old.

Table 4: Factors that affect birth weight, post-weaning weight, and average daily weight gain of grasscutters at Akpaka Forest Reserve Onitsha.

Factors	No. of rats	Birth wt. (kg)	Wt. at 60 days (kg)	DWG for 1 <sup>st</sup> 60 days (kg/day)
Litter size	48			
1	4	0.173±0.02 <sup>a</sup>	0.621±0.02 <sup>a</sup>	0.007±0.0004 <sup>a</sup>
2	14	0.135±0.08 <sup>b</sup>	0.503±0.05 <sup>b</sup>	0.006±0.0008 <sup>ab</sup>
3	30	0.135±0.09 <sup>b</sup>	0.492±0.05 <sup>b</sup>	0.005±0.0008 <sup>b</sup>
Sex	48			
Male	16	0.148±0.01 <sup>a</sup>	0.575±0.03 <sup>a</sup>	0.007±0.0003 <sup>a</sup>
Female	32	0.128±0.02 <sup>b</sup>	0.472±0.04 <sup>b</sup>	0.005±0.0006 <sup>b</sup>
Parity	48			
1 <sup>st</sup>	17	0.147±0.01	0.542±0.02	0.006±0.0003
2 <sup>nd</sup>	16	0.132±0.01	0.496±0.06	0.006±0.0009
3 <sup>rd</sup>	15	0.134±0.01	0.477±0.07	0.005±0.0010
Overall mean	48	0.138±0.06	0.513±0.03	0.006±0.0003

a, b Means in the same column under the same factor with different superscripts differ significantly ( $P<0.05$ ).

Table 5: Factors that affect linear body measurements in grasscutters at Akpaka Forest Reserve Onitsha

Factors	No. of rats	Linear body measurements at birth			Linear body measurements at 60 days		
		BL (cm)	HG (cm)	H (cm)	BL (cm)	HG (cm)	H (cm)
Litter size	48						
1	4	17.60±0.45	12.94±0.33	7.60±0.22	23.00±0.28 <sup>a</sup>	18.13±0.23 <sup>a</sup>	10.62±0.12
2	14	17.98±0.32	12.52±0.17	7.35±0.14	21.66±0.89 <sup>b</sup>	16.82±0.76 <sup>b</sup>	9.85±0.48
3	30	17.27±0.36	12.55±0.22	7.39±0.18	21.59±0.96 <sup>b</sup>	16.71±0.80 <sup>b</sup>	9.81±0.50
Sex	48						
Male	16	17.38±0.37	12.68±0.26	7.46±0.18	22.49±0.58 <sup>a</sup>	17.63±0.47 <sup>a</sup>	10.32±0.30
Female	32	17.23±0.36	12.67±0.93	7.36±0.16	21.34±0.96 <sup>b</sup>	16.47±0.73 <sup>b</sup>	9.67±0.48
Parity	48						
1 <sup>st</sup>	17	17.70±0.14	12.82±0.12	7.59±0.08	22.58±0.33	17.48±0.31	10.33±0.18
2 <sup>nd</sup>	16	17.15±0.23	12.51±0.16	7.31±0.10	21.49±0.79	16.67±0.78	9.73±0.44
3 <sup>rd</sup>	15	16.93±0.18	12.38±0.18	7.28±0.10	21.01±0.93	16.31±0.90	9.54±0.52
Overall mean	48	17.24±0.30	12.57±0.18	7.39±0.14	21.68±0.65	16.83±0.90	9.86±0.52

a, b Means in the same column under the same factor with different superscripts differ significantly ( $P<0.05$ ).

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## COMPARISON OF CLINICAL, PARASITOLOGICAL AND SEROLOGICAL DIAGNOSTIC METHODS FOR THE DEFINITIVE DIAGNOSIS OF ONCHOCERCIASIS IN NSUKKA SENATORIAL ZONE

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### ABSTRACT

*Clinical, parasitological and serological diagnostic methods were compared for definitive diagnosis of human onchocerciasis in three endemic communities of Nkpologu, Ukpabi and Obimo; located at differing distances from the bank of Adada river in Nsukka senatorial zone of Enugu State, Nigeria. The results revealed that 43.98%, 2.78%, 57.60% and 76.55% of the total number of volunteers tested were positive by most common and rare clinical symptoms, skin biopsy and Enzyme-linked immunosorbent assay (ELISA) respectively. Of those seropositive, 86.02% had microfilariae in their skin. Similarly, 67.28% and 91.91% of those who were positive by ELISA and skin biopsy respectively, displayed onchocercal nodules either on the head, trunk, groin, laps or near the knee. However, 96.76% of those with nodules had microfilariae in their skin. The results further showed that the incidence of onchocerciasis and worm burden in the three communities vary inversely with their respective distances from the river. Considering the relative significance of these methods in the diagnosis of onchocerciasis, we recommend the use of a combination of the most common clinical manifestations, skin biopsy and ELISA in the diagnosis of onchocerciasis, at least for epidemiological studies, until a single definitive diagnostic method is developed.*

**Keywords:** Human onchocerciasis, Clinical symptoms, Skin biopsy, ELISA

### INTRODUCTION

Human onchocerciasis is a major blinding disease in equatorial Africa, Central and South America (Guderian *et al.*, 1997), Yemen and Asia (Morroquin, 1981). The disease is also associated with other morbid and debilitating presentations such as visual impairment, blindness, dys-pigmentation, itching-skin rashes, skin keratinization, onchocercal nodules, elephantiasis, genital hydrocoele and muscular skeletal pains (Nwoke, 1992; Nwoke *et al.*, 1993; Abanobi *et al.*, 1994) and renal impairment (Ngu and Blackett, 1976).

Although skin biopsy is the main diagnostic method for human onchocerciasis (Toel *et al.*, 1998) and nodule palpation rated indicator of choice for clinical diagnosis, on account of its close correlation with the result of skin biopsy (TDR News, 1992), they cannot be used to detect low and prepatent infections (Anyia, 1981). Apart from not being very sensitive, skin biopsy has an additional defect such as being painful and its high risk of blood-borne infections such as human immunodeficiency virus (Hagan, 1998). The development of a single, sensitive and specific diagnostic test for onchocerciasis has remained a priority of the World Health Organisation (WHO). Previous unsuccessful attempts have been made

to resolve the problem associated with onchocerciasis diagnosis. Such attempts were the assessment of two or more clinical manifestations of the disease (Gemade and Utsalo, 1990; Kelly and Akogun, 1997), provision of large quantities of onchocercal antigens by cloning for serodiagnosis (Chandrashaker *et al.*, 1991, 1996), the use of polymerase chain reaction method to identify onchocercal specific complement DNA (Zimmerman *et al.*, 1994) especially in superficial bloodless skin scrapping (Toel *et al.*, 1998) and the monitoring of the elevated serum angiotensin (Ronday *et al.*, 1996). However, the search for a sensitive diagnostic tool has hitherto been rendered illusive by many varying clinical manifestations of the disease in patients (Cohen and Warren, 1982). The need therefore to solve the problems of the existing diagnostic tools and the dire need for a single more definitive diagnostic method for onchocerciasis informed this study. Consequently, the existing clinical manifestations for onchocerciasis, skin biopsy and ELISA were therefore assessed and compared for the definitive diagnosis of the disease in three onchocerciasis – endemic contiguous communities located at differing distances from Adada river in Nsukka Senatorial Zone of Enugu State, Nigeria.

## MATERIALS AND METHODS

**Study Area and Population:** This study was carried out in Nkpologu, Ukpabi and Obimo communities, located between longitude 7°08' and 7°20' East and latitude 6°46' and 6°49' North. The communities lie at the northern bank of Adada river, a well aerated fast-flowing river that flows south-westwards into Obinna river which, in turn empties into Anambra river, a tributary of river Niger. While Nkpologu community is located one (1) kilometer, Ukpabi and Obimo communities are located five (5) and six (6) kilometers from Adada river respectively. According to the Nigerian census of 1991, the total population of the three communities was 33,630 (males = 15938 and female = 17,692). The inhabitants of these communities are predominantly crop - farmers whose farmlands are as close as possible to the Adada river for purposes of improved farm yields. Many young male and female children are either in primary or secondary schools but actively take part in their seasonal farm work.

**Sample Size:** A total of 4120 inhabitants of the three communities (2834 males and 1286 females) were randomly tested in this study. A total of 1906 persons (1311 males and 595 females) were tested in Nkpologu, 886 (609 males and 277 females) were sampled in Ukpabi and 1328 volunteers (914 males and 414 females) were tested in Obimo communities. A total of 103 negative control subjects (77 males and 26 females) were selected from the University of Nigeria Nsukka community. These negative control subjects had no history of onchocerciasis nor had they any onchocercal infection by the time the samples were taken from them.

**Clinical Diagnosis:** Proforma was completed by or for each of the volunteers to obtain their sex, age, and information on intake of "banocide" (diethylcarbamazine, DEC), discovered to be widely administered to the onchocerciasis victims by patent medicine dealers in the area. The subjects were then palpated for the presence of onchocercal nodules and examined for both most common and rare clinical manifestations such as tygroid-leopard skin, tough lizard skin, itching-skin rashes, blindness, elephantiasis, genital hydrocoel and impaired vision, by the medical officers attached to the Cottage Hospital, Nkpologu, Uzouwani Local Government Area of Enugu State. No detailed ophthalmologic examination was performed. However, visual impairment was determined by counting fingers at varying distances. Inability to count fingers at 3 meters or less was regarded as blindness (WHO, 1996). An auxiliary measure for the clinical diagnosis was done by watching out for the development of

itching-rashes within a few hours after 2mg DEC per kilogramme body weight was administered, Mazzotti test (Mazzotti, 1951).

**Parasitological Diagnosis:** Skin snips were taken from iliac crest (Buck, 1974), calves and the back shoulder with a 1.5 mm corneo-sclerectomy punch (Storz, Instrument Comp, St Louis, USA). The sclerectomy punch was sterilized in absolute alcohol and then flamed over a spirit burner before using it on another subject. The operation was bloodless and painless. The skin snips, relatively of uniform size were weighed with torsion balance and then suspended in 150  $\mu$ l physiological saline solution (0.85 % w/v), contained in 1.5 ml volume screw-stopped cryotube. The suspensions were incubated for six hours to achieve maximum migration of the microfilariae (Mf) out of the skin snips (Tada *et al.*, 1973). The presence of Mf in the solution was identified under low power magnification of Olympus microscope (Japan). Positivity for *Onchocerca volvulus* microfilariae was based on the presence of at least one Mf in the suspension (Figure 1). The total number of Mf was then counted. Worm density (worm burden) was expressed as the mean number of Mf per milligramme skin (Mf/mg Skin<sup>-1</sup>).



**Figure 1: Identified *Onchocerca volvulus* microfilaria**

**Preparation of Sera:** Blood (5 ml) was drawn from each of the volunteers by venipuncture and poured gently into 10 ml plastic centrifuge tube to clot. Serum was then prepared by centrifuging the blood at 3000 x g for 10 minutes using Hettich universal bench centrifuge (model 1200 Tutilingen). The upper layer (serum) was collected and stored frozen in 1.5 ml plastic vials in aliquots of 200  $\mu$ l until used.

**Preparation of Microfilarial Antigen Homogenate:** Onchocercal nodules, excised from onchocerciasis patients by medical health officers (Figure 2) working at Cottage Hospital, Nkpologu, were freed of connective tissues and fatty materials, weighed and then immersed in normal physiological saline (0.85 % w/v) for washing. The nodules, contained in cold compartment, were transported to the Biochemistry Laboratory of the University of Nigeria, Nsukka, and stored frozen until used. Adult worms were isolated from the nodules according to the collagenase-enzyme digestion method of Schulzkey *et al.* (1977), and

then washed three times in RMPI buffer solution (Biolab) reconstituted as directed in the manufacturer's manual. Onchocercal antigen homogenate was then prepared from washed adult worms according to the modified method of Lobos *et al.* (1991) as summarized below. The worms were homogenized thoroughly, using glass homogenizer with Teflon piston in some quantity of extraction buffer. The extraction buffer contained 20 mM Tris-HCl, 2 % Deoxycholic Acid, 1mM ethylenediamine tetracetic acid (EDTA) and 1 mM phenylmethyl sulphonyl fluoride (PMSF, Sigma) in a ratio of 20:1:20:20 respectively. 2.5 mg each of L-5-amino-L-(P-toluenesulphonyl)-amidopentyl-chloro-methyl ketone (TLCK) and L-I-(toluenesulphonyl) – amido-2-phenyl ethyl-chloromethyl ketone (TPCK, Sigma USA) were added and the solution centrifuged at 26,000 x g for 30 minutes at 4 °C. The supernatant containing the crude soluble antigen was collected, the protein concentration determined according to the modified micro-lowry method of Sachaterle and Pollack (1973) as reported by Beechey *et al.* (1975) and then stored in 200µl aliquots at -20 °C until used.



**Figure 2: Excision of onchocercal nodules from onchocerciasis patient by medical health officer**

**Enzyme-Linked Immonosorbent Assay Procedure:** Enzyme-linked immonosorbent assay (ELISA) was performed according to the method described by Voller *et al.* (1977) as modified by Speiser (1980).

## RESULTS

**Clinical Symptoms:** Clinical examination of all the volunteers showed that, itching-skin rashes (57.94 %, 2387/4120), nodular presence (52.52 %, 2164/4120), tygroid-leopard skin (dyspigmentation) (36.84 %, 1518/4120) and tough, rough lizard skin (28.64 %, 1180/4120) were the most observable common symptoms, while blindness (0.02 %) elephantiasis (0.02 %), visual impairment (7.96 %) and hydrococle (0.02 %) were the less frequently encountered symptoms (Rare symptoms) among the people of all the communities. Although, goiter was

observed in 15.68 % (646/4120) of the whole population, only 0.29 % (12/4120) was positive for microfilariae by skin biopsy (Table 1). While onchocercal nodules (Onchocercomas) were predominantly located at the forehead, legs, trunk, groin and near the knee of the victims (Figures 3 and 4). Skin dyspigmentation was located mainly on the hands and legs (Figures 5, 6 and 7).



**Figure 3: Onchocercomas located at the forehead and trunk of a twelve year old onchocerciasis patient**



**Figure 4: Multiple onchocercal nodules located on the leg of a 67 year old farmer from Nkpologu community**



**Figure 5: Leopard skin on the hand of an onchocerciasis patient from Nkpologu community**

**Table 1: Prevalence of most common and rare clinical features of onchocerciasis in Nkpologu, Upkabi and Obimo Communities**

	No. Positive	Percentage Incidence (%)
Observable Most Common Clinical Symptoms		
Communities	Nkpologu	
Total No. Tested	1906	
Itching of skin rashes	1290	67.68
Nodular Presence	1364	71.56
Tygrid-Leopard Skin		
Tough, Rough Lizard	904	47.43
Skin	698	36.62
(AVERAGE)	1064	25.82
Communities	Ukpabi	
Total No. Tested	886	
Itching of skin rashes	530	59.82
Nodular Presence	400	45.15
Tygrid-Leopard Skin		
Tough, Rough Lizard	250	28.22
Skin	160	18.06
(AVERAGE)	335	37.81
Communities	Obimo	
Total No. Tested	1328	
Itching of skin rashes	567	42.70
Nodular Presence	400	30.12
Tygrid-Leopard Skin		
Tough, Rough Lizard	364	27.41
Skin	322	24.25
(AVERAGE)	413	31.10
All Communities	Total	
Total No. Tested	4120	
Itching of skin rashes	57.94% (2397/4120)	
Nodular Presence	52.52% (2164/4120)	
Tygrid-Leopard Skin		
Tough, Rough Lizard	36.84% (1518/4120)	
Skin	28.64% (1180/4120)	
(AVERAGE)	43.98 (1812/4120)	
Less frequently encountered (Rare) Symptoms		
Communities	Nkpologu	
Total No. Tested	1906	
Blindness	-	-
Elephantiasis	1	0.05
Hydrocoel	-	-
Visual impaired	103	5.40
Goiter	4	0.21
AVERAGE	36	5.66
Communities	Ukpabi	
Total No. Tested	886	
Blindness	-	-
Elephantiasis	-	-
Hydrocoel	1	0.11
Visual impaired	164	18.51
Goiter	2	0.23
AVERAGE	57	6.32
Communities	Obimo	
Total No. Tested	1328	
Blindness	1	0.08
Elephantiasis	-	-
Hydrocoel	-	-
Visual impaired	61	4.59
Goiter	6	0.45
AVERAGE	23	1.73
All Communities	Total	
Total No. Tested	4120	
Blindness	0.02%(1/4120)	
Elephantiasis	0.02%(1/4120)	
Hydrocoel	0.02%(1/4120)	
Visual impaired	7.96%(328/4120)	
Goiter	0.29%(12/4120)	
AVERAGE	1.63%(67/4120)	



**Figures 6 and 7: Depigmentation on the legs of a 66 and 70 year old onchocerciasis patients from Ukpabi and Obimo communities respectively. The 66 year old farmer carries one onchocercal nodule on one side of each knees (Figure 6)**

Ninety percent (90 %) of those with onchocercomas were positive for microfilariae by skin biopsy, while 62 % of those with tygid, leopard skin were positive by skin biopsy. Tough-rough lizard skin (Figure 8) was mainly observed in older patients, 93 % of whom were positive by skin biopsy.



**Figure 8: Rough scaly legs of a 79-year old onchocerciasis patient from Nkpologu community**

Rashes, accompanied by itching were located at all parts of the body (Figures 9 and 10). Ninety-six percent (96 %) of those with rashes were positive for microfilariae by skin biopsy. Elephantiasis (Figure 11) and genital hydrocoele were observed in one patient each and these patients were positive by skin biopsy and ELISA. Visual impairment was common in the residents of all the communities but only 15.20 % of them were positive by skin biopsy. Blindness was observed in only one of the subjects tested. Detailed investigation of the cause of the blindness at the University of Nigeria Teaching Hospital, Enugu, revealed accumulation of microfilariae in the eye, indicating that the microfilariae could be a possible cause of the blindness.

**Incidence of Onchocerciasis in Relation to Proximity to River Adada:** Tables 2 (A and B), show the incidence of onchocerciasis by skin

biopsy in the three communities, their relative distances from the river and the average worm-burden of the patients in each community. They indicate that the prevalence and the worm-burden increase as the distances of the communities from the river decrease.



Figures 9 and 10: Skin rashes on the hands and body of female onchocerciasis patients from Ukpabi community



Figure 11: Elephantiasis affecting the left leg of an onchocercal microfilariae-infected patient from Obimo community

Table 2: Prevalence of onchocerciasis in Nkpologu, Ukpabi and Obimo communities by skin snips (A) and ELISA (B)

(A) Skin Biopsy	Nkpologu	Ukpabi	Obimo	Total
No. Tested	1906	886	1328	4120
No. Positive	1270	542	561	2373
Percentage Positive (%)	66.63	61.17	42.24	57.60
(B) ELISA				
No. Tested	1906	886	1328	4120
No. Positive	1725	634	795	3154
Percentage Positive (%)	90.53	71.56	59.86	76.55
Distance From River (km)	1	5	6	4
Worm Burden (Mf/mg skin)	3.90	1.92	1.25	2.38

**Comparison of Incidences of Onchocerciasis Obtained from Different Diagnostic Methods:** Table 3 summaries the onchocerciasis diagnostic results of the communities.

Table 3: Prevalence of onchocerciasis based on clinical, parasitological and serological diagnostic methods

Diagnostic Methods	No. Tested	No. Positive	No. Positive Rate (%)
Most Common Clinical Symptoms	4120	1812	43.98
Less Frequently Observed Clinical Symptoms	4120	115	2.79
Skin Biopsy	4120	2373	57.60
ELISA	4120	3154	76.55

## DISCUSSION

A combination of onchocercal clinical manifestations, parasitological and serological diagnostic methods were evaluated and then the results were accessed and compared for their onchocerciasis diagnostic potentials in the three onchocerciasis endemic communities of Nkpologu, Ukpabi and Obimo in Nsukka senatorial zone of Enugu State, Nigeria. The results identified positive correlation between the most common clinical features (nodular presence, skin dyspigmentation, itching-skin rashes and tough, rough scaly lizard skin) on one hand and skin biopsy and ELISA on the other hand. Although significant positive correlation ( $p < 0.05$ ) exists between the most common clinical features, and ELISA, that between skin biopsy and ELISA was not significant ( $P > 0.05$ ) (Table 4).

Table 4: Correlation coefficient of prevalence of onchocerciasis based on clinical pathological and serological diagnoses

		Most common	Less frequent	Skin biopsy	ELISA
Most common	Pearson				
	Correlation	1.000	-.173	-.919	-.478
	Sig. (2-tail)	.	.781	.028	.416
	N	5	5	5	5
Less frequent	Pearson				
	Correlation	-.173	1.000	-.089	-.368
	Sig. (2-tail)	.781	.	.886	.542
	N	5	5	5	5
Skin biopsy	Pearson				
	Correlation	-.919*	-.089	1.000	.375
	Sig. (2-tail)	.028	.886	.	.534
	N	5	5	5	5
ELISA	Pearson				
	Correlation	-.478	-.368	.375	1.000
	Sig. (2-tail)	.416	.542	.534	.
	N	5	5	5	5

Correlation is significant at the 0.05 level (2-tailed)

The most common clinical symptoms and skin biopsy diagnostic methods could therefore be relied upon for the diagnosis of the disease



particularly where laboratory facility for ELISA is not available. The average incidence rate of the less frequently observed clinical symptoms (1.63%) is too low to be considered for use in the clinical diagnosis of onchocerciasis.

The average prevalence of 43.98 %, 57.60% and 76.55% for the most common clinical features, skin biopsy and ELISA respectively, classify the communities as being mesoendemic for onchocerciasis according to the classification adopted for onchocerciasis endemicity (WHO, 1973). This result is consistent with a similar one reported by Edingbola and Asaolu (1984), in their parasitological survey for onchocerciasis in the Babara district of Kwara State of Nigeria. The study also identified low rate of blindness 0.02 % (1/4120) caused by onchocerciasis, perhaps because blindness rate seldom exceeds 3 % in onchocerciasis patients in the forest zone of Africa, but is responsible for high blindness rates up to 10% in the Sudan Savanna zone (Sasa, 1976, Ogurinde, *et al*, 1999). The study area is situated at the forest zone of Nigeria. The apparent causes of the low blindness rate, visual impairment and low incidence of worm-burden among the people of the study communities were not investigated. However, one could implicate large consumption of diethylcarbamazine (DEC) by the inhabitants of the communities as revealed by the patients during the study. Massive DEC consumption could reduce blindness and visual impairment rates by killing microfilariae that could migrate into the eyeball where they cause opacity of the eye or often damage the optic nerve (Sasa, 1976). In addition, microfilariae could invade the anterior chamber of the eye, their death causes chronic iritis which is one of the consistent and common ocular involvements of onchocerciasis (Sasa, 1976). Microfilarial worm burden could be reduced by the death of onchocercal microfilariae. Dead microfilarial constituents could trigger off allergic reaction (Hensan, *et al.*, 1979) which could be responsible for the high incidence of itching-skin-rashes (56.75 %) observed in this study.

The observation of skin dyspigmentation (leopard, tygroid skin), in 34.35% of the total volunteers tested, mainly among onchocerciasis patients over 30 years of age (Figs. 5, 6 and 7) is similar to that reported by Edungbola *et al.* (1987) who observed the symptom in only patients over the age of 20 years. The age-dependence of this manifestation could implicate the duration and intensity of onchocercal infection. However the occurrence of this manifestation in some people not positive for onchocerciasis (Figure 12) could implicate other factors such as leprosy (Browne, 1960), treponematoses (Buck, 1974) in the generation of the manifestation. Tough, rough scaly lizard skin observed mainly in older onchocerciasis victims positive for *O. volvulus*

infection by skin biopsy and ELISA (Figure 7) could implicate long-standing onchocercal infection in the generation of the symptoms.



**Figure 12: Depigmentation caused by another infection on a 48 year old woman from Nkpologu community. The woman was not positive for *Onchocerca volvulus* by skin biopsy or by ELISA**

The highest incidence of onchocercal nodules (71.56 %) and worm-burden (3.90 mg/mg skin) were apparent in Nkpologu community among older onchocerciasis victims than among younger ones, except in a 13 year old boy with multiple onchocercal nodules located in many parts of his body (Fig. 3). This selective occurrence of nodules in these communities could implicate duration of infection and degree of exposure to the bites of infected *Simulium* vector among others, in the generation of onchocercal nodules.

Our results also revealed that the average incidence of onchocerciasis in all the communities as detected by ELISA (76.55%) is significantly higher ( $p < 0.05$ ) than the overall average incidence (57.60%) evaluated for skin biopsy. Going by this result, it is probably reasonable to speculate that ELISA method of onchocerciasis diagnosis is more sensitive than the skin biopsy method. This speculation is consistent with the observation of Voller *et al.* (1977). The higher sensitivity by ELISA is not surprising given that ELISA method is principally based on the detection of serum antibodies produced against soluble or surface onchocercal antigen and thereby can detect prepatent or occult onchocercal infection. However, cross-reactions with the antibodies against antigens of some other common African parasitic worms (Roffi *et al.*, 1982) could possibly

exaggerate the ELISA incidence. In addition, consumption of DEC, evident in these communities, could as well contribute to this sensitivity difference existing between these two diagnostic methods. Diethylcarbamazine can flush microfilariae from the skin into the urine or sputum within few hours of treatment (Bryceson *et al.*, 1977), thereby reducing the number in the skin and precipitating false lower incidence by skin biopsy method.

Despite close proximity of the communities, similar climatic conditions and local topography, there still existed wide differences in onchocerciasis prevalences in the three communities as manifested by the different diagnostic methods in this study (Table 3). These differences could be attributed to variations arising from the frequency, duration and degree of exposure to the bites of infected blackflies, the vector of *Onchocerca volvulus*, possibly imposed by differences in the distances of the communities from the breeding site of the vector. The role played by proximity is supported by the fact that differences in prevalence rates of onchocerciasis obtained by different diagnostic methods show inverse relationship with the respective distances of the communities from Adada river, the breeding site of *Simulium damnosum* as well as the worm-burden results in which Nkpologu, the nearest community to the river exhibited the highest worm-burden of 3.90 Mf/mg skin, while Obimo, the farthest community showed the least worm-burden of 1.25 Mf per mg skin. Alternatively, these differences could reflect the variations in the susceptibility of the residents to the infecting organism or the variations in the amount and regularity of drug treatment.

Going by the classification adopted for onchocerciasis endemicity by WHO (1973) and Tada *et al.* (1973), ELISA results depict hyperendemicity in the three communities while those of skin biopsy depict hyperendemicity in Nkpologu and Ukpabi and mesoendemicity in Obimo. This result can also support the higher sensitivity of ELISA when compared with skin biopsy. The most common clinical manifestation results exhibited mesoendemicity for Nkpologu and Ukpabi but hypoendemicity for Obimo, while those of minor clinical manifestations showed only hypoendemicity for all the communities. Although the incidences of the most common clinical manifestations considered in this study varied widely in the communities, they are still significant enough to be considered as relevant diagnostic tools for onchocerciasis as indicated by Nelson (1981). However, the incidences of the less frequently observed clinical manifestations obtained in these communities are too low to be considered relevant diagnostic tools.

Based on the high prevalence rate of this disease manifested by the most common clinical symptoms, parasitological and serological diagnostic methods in this study, we recommend, in support of other workers (Nwoke, 1992; Ovuga, *et al.*, 1992 and Abanobi, 1994) that a combination of the methods be employed in the diagnosis of the disease at least for epidemiological purposes, until a single, more definitive method is developed. The development of such a single definitive method is our priority.

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## THE BIOLOGY OF THE WEST AFRICAN CLARIID, *Clarias macromystax* GUNTHER, 1864 (OSTEICHTHYES: CLARIIDAE) IN A NIGERIAN RIVER BASIN

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### ABSTRACT

*The biology of the West African clariid, Clarias macromystax, was studied in Anambra river basin, Nigeria. The clariid occurred more abundantly and frequently in forest floodplain ponds than in other habitats, and was totally absent in the river systems. Length ranged from 9.7 to 30.2 cm TL and weight from 9 to 168 g; females were heavier, but not longer, than males. The b-values (2.4190-2.5209) of the total length-weight relationships exhibited negative allometric function. Mean relative condition,  $K_r$ , was better in females than males but showed a definite cycle in both sexes. Over 50% of both sexes were mature at 15.1-20.0 cm TL in their first year of life. Fecundity ranged from  $2.136 \times 10^3$  to  $37.250 \times 10^3$  (mean  $14.942 \times 10^3 \pm 11.248 \times 10^3$ ) and correlated highly and positively with length, body weight and ovary weight. Ovary weight was the best predictor of fecundity. Communal spawning involving *C. macromystax* and *C. agboyiensis* occurred. Feeding was carried out throughout the day with higher feeding intensity at night. Food of primary importance were *Caridina niloticus*, *Sudanonates africanus*, *Odonata naiad*, terrestrial Orthoptera, formicoid Hymenoptera, *Dytiscidae*, *Oreochromis niloticus*, *Parachanna obscura*, fruits and seeds, plant detritus and mud. Diet breadth was season-dependent. The clariid fed by foraging, shoveling and surface feeding. *E. clarias*, *Procamallanus laevichonchus* and a larval spiruroid parasitized various organs. *C. macromystax* is a new host record for these helminth parasites.*

**Keywords:** *Clarias macromystax*, Abundance, Reproduction, Food, Parasites, Anambra river basin, Nigeria.

### INTRODUCTION

Eleven *Clarias* species – *C. agboyiensis*, *C. buettikoferi*, *C. dialonensis*, *C. ebriensis*, *C. laeviceps*, *C. lamottei*, *C. longior*, *C. maclaremi*, *C. macromystax*, *C. salae* and *C. submarginatus* -- are endemic in the coastal forested areas of West Africa and each of them has very restricted distribution. For example, *C. macromystax* is restricted to the coastal basins from Benin to Nigeria. *C. macromystax*, *C. agboyiensis* and *C. ebriensis* occur in the guinean zone of Nigeria, but *C. macromystax* also inhabits the soudanean zone. Except for species of the subgenera *Dinotopteroides* and *Clarias*, there appears to be a marked tendency for increased speciation of species of other genera of *Clarias* in the waters of forest areas (Sydenham, 1980). *C. macromystax* is the least abundant of clariids of the subgenera *Clarioides* and *Anguilloclarias*, which occur in the Anambra river basin (Ezenwaji, 1992, 1993). The clariid is usually abundant in some floodplain ponds where it is cultured semi-intensively. Like other clariids, its flesh is very tasty and it contributes to the protein intake of the riverine people.

There is remarkable paucity of information on all aspects of the biology of *C. macromystax*. The purpose of this contribution is to fill this information gap and to investigate the distribution,

abundance, population structure, reproduction, food and parasites of *C. macromystax* in the Anambra river basin, southeastern Nigeria.

### MATERIALS AND METHODS

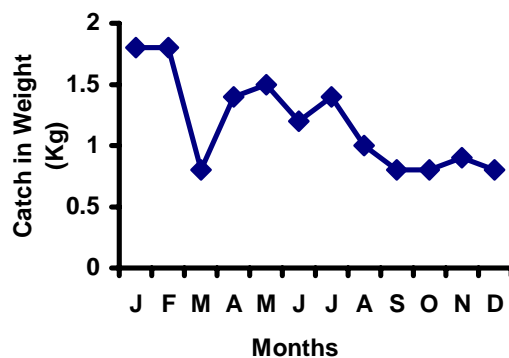
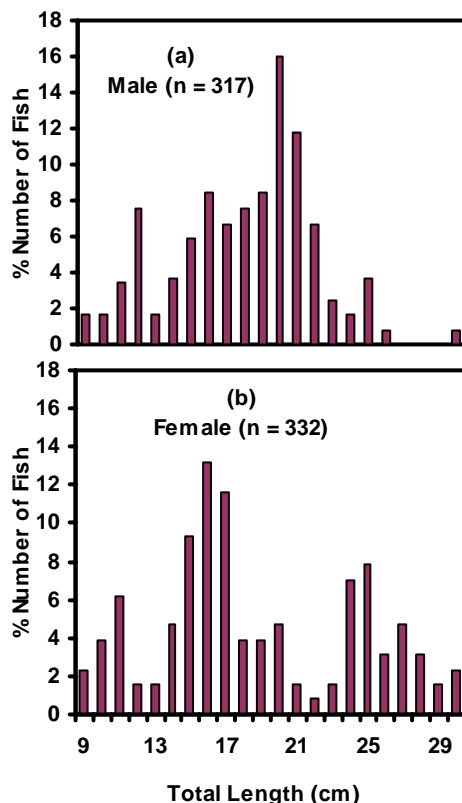
Samples of *C. macromystax* were collected from Nsugbe, Otuocha, Oroma-etiti, Enugwu-otu, Ogurugu and Ugwuoba in the Anambra river basin (Ezenwaji, 2002). Collections were made in these sampling locations from June, 1983 to September 1985, June to September 1986, January to May 1987 and May to December 1997 with 200 baited hook (No. 17) and line, 10 fishing baskets and 30 hoop fyke traps. Each sampling location was divided into four major habitats – forest floodplain pond (ffp), grassland floodplain pond (gfp), marshy area (m) and river (r). Each of the four sets of 200 lines set overnight (18.00 – 07.00 h) in each sampling location was taken as a unit of effort used to determine distribution and abundance of the clariid.

Standard (snout to end of caudal peduncle) and total lengths (SL and TL, to the nearest centimeter) and body weight (to the nearest gram) of each *C. macromystax* were measured and the sex of fish above 9.0 cm TL was determined by examining the genital papilla (pointed in males) and/or the gonads microscopically. The total length-standard length,

**Table 1: Abundance and percentage frequency of occurrence (%FO) of *C. macromystax* (n = 255) using experimental gear (200 baited hook and line)**

Location	No. and %FO in habitats (values in parentheses show weight (kg))								Location total
	ffp <sup>a</sup>		gfp <sup>b</sup>		r		m <sup>c</sup>		
	No.	%FO	No.	%FO	No.	%FO	No.	%FO	
Nsugbe	25(1.4)	96	9(0.5)	33	0(0)	0	0(0)	0	34 <sup>i</sup> (1.9)
Oroma-etiti	27(1.5)	100	16(0.9)	83	0 <sup>d</sup> (0)	0 <sup>d</sup>	8(0.4)	39	51 <sup>h</sup> (2.8)
Otuocha	13(0.7)	50	7(0.4)	29	0 <sup>e</sup> (0)	0 <sup>e</sup>	0(0)	0	20 <sup>j</sup> (1.1)
Enugwu-otu	21(1.2)	100	5(0.3)	28	0(0)	0	0(0)	0	26 <sup>g</sup> (3.9)
Ogurugu	31(1.7)	100	29(1.6)	100	0(0)	0	11(0.6)	56	71 <sup>g</sup> (3.9)
Ugwuoba	28(1.6)	100	16(0.9)	80	0 <sup>f</sup> (0)	0 <sup>f</sup>	9(0.5)	45	53 <sup>h</sup> (3.0)
Habitat Total	145(8.1)		82(4.6)		0(0)		28(1.5)		255(14.2)
% Habitat total <sup>+</sup>	56.9 <sup>k</sup> (57.0)		32.2(32.4)		0(0)		11.0 <sup>m</sup> (10.6)		
Habitat %FO		91		59		0		23	

<sup>+</sup> \*Figures in the % habitat total row and location total column with the same letter are not significantly different,  $P = 0.05$ ; <sup>a</sup> Forest floodplain pond; <sup>b</sup> Grassland floodplain pond; <sup>c</sup> Marshy area; <sup>d</sup> Ezechi river; <sup>e</sup> Anambra river, <sup>f</sup> Ezu river

**Figure 1: Changes in the monthly abundance of *C. macromystax* in the Anambra river basin****Figure 2: Length - frequency distribution of male (a) and female (b) *C. macromystax* in the Anambra river basin**

and length-weight, relationships were determined using the power curves,  $TL = aSL^b$  and  $W = aTL^b$  respectively. Relative condition factor ( $K_n$ ) was estimated as  $K_n = W/aTL^b$  (Le Cren, 1951).

The gonad maturity stages for this clariid were delimited as in Ezenwaji (2002) as follows: I – immature, II – developing; III – mature; IV – ripe; V – running; VI – spent. Gonad stages IV and V in the male were indistinguishable from stage III, so the three stages were grouped under stage III. Thus, four stages were recognized in the male. Size at maturity was determined as the size at which 50% of individuals were in gonad stage III. Absolute fecundity, defined as the number of ripening oocytes in the female ovaries prior to the next spawning period (Bagenal, 1978), was determined by counting all ripe oocytes in both ovaries. Regression analyses of fecundity (F) on SL, TL, body weight ( $W_b$ ) and ovary weight ( $W_o$ ), and of gonadosomatic index (GSI) on F were performed using the least squares method. Observations on spawning aggregation, migration and actual spawning were made from elevated platforms along the banks and on spawning grounds.

The relative volume of food in each fish stomach was determined irrespective of fish size. The degree of stomach fullness was estimated by an arbitrary 0 – 16 point scale as follows: 16 points for full, 12 for  $\frac{3}{4}$  full, 8 for  $\frac{1}{2}$  full, 4 for  $\frac{1}{4}$  full, 2 for  $\frac{1}{8}$  full, 1 for traces of food and 0 for empty stomach. Stomach fullness of 22 specimens caught at night (19.30 – 05.00 h) and 30 caught during the day (08.00-17.00 h) were used to evaluate diel-feeding activity. The vacuity index was calculated as the number of empty stomachs divided by the total number of stomachs examined multiplied by 100. Stomach contents were sorted into species/groups and analyzed for dry weight and relative frequency (Hyslop, 1980). The latter was calculated as the frequency of each food category expressed as a percentage of the sum of the frequencies of all food items (King, 1988) as

Table 2: The sex ratio of *C. macromystax* in Anambra river basin

Month	Overall		Sex ratio (M:F)	In gonad stages III-V		Sex ratio (M:F)	On spawning run		Sex ratio (M:F)
	Number collected			Number collected			Number collected		
	M	F		M	F		M	F	
January	32	26	1:0.8	6	16	1:2.7	-	-	-
February	29	24	1:0.8	11	6	1:0.5	-	-	-
March	15	20	1:1.3	4	10	1:2.5	-	-	-
April	22	22	1:1.0	16	14	1:0.9	-	-	-
May	33	39	1:1.2	27	32	1:1.2	-	-	-
June	37	51	1:1.4	29	44	1:1.5	14	19	1:1.4
July	29	38	1:1.3	4	14	1:3.5	2	5	1:2.5
August	21	34	1:1.6	4	10	1:2.5	2	2	1:1
September	14	18	1:1.3	6	1	1:0.2	-	-	-
October	24	19	1:0.8	9	4	1:0.4	-	-	-
November	31	28	1:0.9	5	6	1:1.2	-	-	-
December	30	33	1:1.1	8	10	1:1.3	-	-	-
Total	317	352	1:1.1	129	167	1:1.3	18	26	1:1.4

Table 3: TL – SL (a), SL – weight (b) and TL-weight (c) relationships of male, female and both sexes of *C. macromystax*

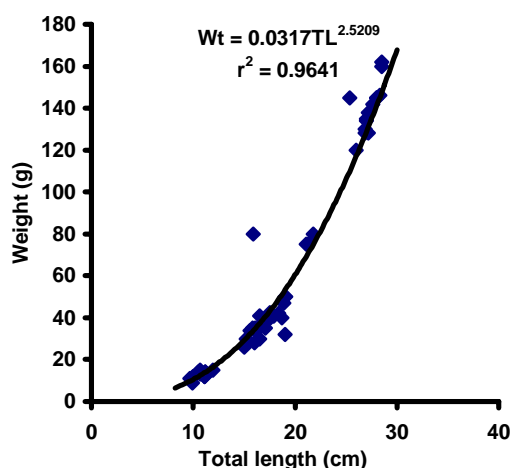
a)	Sex	n	SL (cm)		Mean (S.D)	TL-SL relationships		
			Min.	Max.		a	b	r <sup>2</sup>
	Male	49	8.5	26.9	15.7±1.6	1.1905	0.9813	0.997
	Female	55	8.4	26.7	15.4±5.8	1.2066	0.9800	0.997
	Both sexes	104	8.4	26.9	15.5±4.7	1.2060	0.9786	0.997

b)	Sex	n	SL (cm)		Mean (S.D)	SL-weight relationships		
			Min.	Max.		a	b	r <sup>2</sup>
	Male	49	8.5	26.9	15.7±1.6	0.0598	2.3678	0.947
	Female	55	8.4	26.7	15.4±5.8	0.0496	2.4804	0.969
	Both sexes	104	8.4	26.9	15.5±4.7	0.0545	2.4246	0.956

c)	Sex	n	TL (cm)		Mean (S.D)	TL-weight relationships		
			Min.	Max.		a	b	r <sup>2</sup>
	Male	49	9.9	30.2	18.8±1.2	0.0386	2.4190	0.955
	Female	55	9.7	30.1	18.3±6.2	0.0317	2.5209	0.964
	Both sexes	104	9.7	30.2	18.5±5.3	0.0345	2.4751	0.957

Figure 3: Length-weight relationship of female *C. macromystax* in Anambra river basin

follows:  $\% RF = 100(ai / \sum_{i=1}^n A)$  where  $ai$  =

the frequency of item  $a_i$ , and  $A$  = the frequency of the  $n^{th}$  item. The dietary importance of each food

item was then expressed as an index of food significance (IFS) as follows:

$$IFS = \% RF \times \% W_t / \sum (\% RF \times \% W_t) \times 100.$$

Food items with  $IFS \geq 1.5\%$  were considered primary, whereas those with  $IFS \geq 0.01\%$  but  $< 1.5\%$  were secondary food items. IFS data were used to compute diet breadth based on Shannon-Wiener function ( $\bar{H}$ ) derived from the equation:

$$\bar{H}_{(IFS)} = - \sum_{i=1}^s (ni/N) \log_e (ni/N)$$

where  $n_i$  = the IFS of each food item, and  $N$  = the total IFS of all food items.

Food richness was defined as the number of food items in the diet with  $IFS \geq 0.01\%$ .

The helminth parasites were investigated by examining the external and internal organs. Further treatment of the parasites was as in Ezenwaji and Inyang (1998).

Data for the same month in different years were pooled. Abundance data were tested for normality and analyzed using a two-way ANOVA. Relative condition and food composition were analyzed



**Table 4: Size at maturity of *C. macromystax* in Anambra river basin**

Length group (cm TL)	Male		Female	
	Sample size (breeding)	% Breeding	Sample size (breeding)	% Breeding
<10.0	24(0)	-	19(0)	-
10.1 – 15.0	19(5)	26.3	13(3)	23.1
15.1 – 20.0	39(26)	66.7	34(23)	67.6
20.1 – 25.0	11(9)	81.8	22(20)	90.9
25.1 – 30.0	7(7)	100	9(8)	88.9

\* Percentage of males and females in breeding condition (gonad stages III – V) by size groups are shown.

**Table 5: Regression equations for the relationships between fecundity and total length, standard length, body weight and ovary weight ( $F = aL^b$ ,  $F = \alpha + \beta X$  and GSI and fecundity ( $GSI = aF^b$ ) in *C. macromystax***

Variable	Unit	a	b	r <sup>2</sup>	p
<b>(a)</b>					
Fecundity	cm	0.049	3.983	0.855	<0.001
	mm	$5.66 \times 10^{-6}$	3.965	0.853	<0.001
Standard length	cm	0.135	3.812	0.860	<0.001
	mm	$2.1 \times 10^{-5}$	3.812	0.859	<0.001
Body weight	g	46.035	1.260	0.822	<0.001
	g	-	208.996	0.775	<0.001
Ovary weight	g	3998.709	0.987	0.995	<0.001
	g	933.149	0.987	0.995	<0.001
<b>(b) GSI</b>					
Fecundity		0.545	0.36	0.55	<0.001

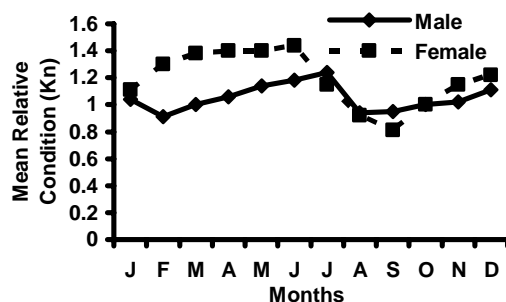


Figure 4: The mean monthly relative condition of *C. macromystax* in Anambra river basin (95 % confidence limits are indicated).

with Student's t-test, whereas sex ratio was analyzed using  $\chi^2$  test. Terminology of infection statistics (Margolis *et al.*, 1982) as modified by Bush *et al.* (1997) was employed in the analysis of parasite data. Differences were considered significant at 5% level of probability.

## RESULTS

**Distribution and Abundance:** *C. macromystax* was caught, but unevenly distributed, in the locations (Table 1). Its distribution in the habitats showed that the clariid preferred ffps, with a habitat frequency of occurrence of 91%, and was totally absent from the rivers.

The number of the clariid in Ogurugu was significantly higher than in other locations ( $P < 0.05$ ), followed by Ugwuoba and Oroma-etiti, which were not different from one another ( $P > 0.05$ ) (Table 1). Within the habitats, the clariid was most abundant in ffps. Peak catches occurred in January and February and from April to July, whereas low catches occurred from September to December and in March (Figure 1).

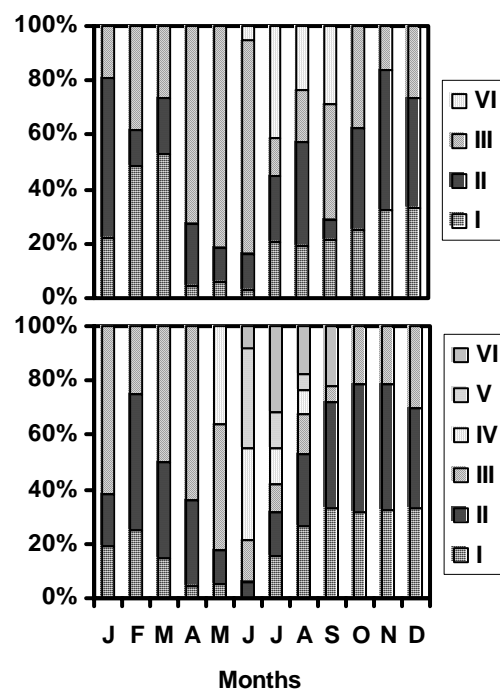


Figure 5: The monthly percentage distribution of male (a) and female (b) *C. macromystax* with gonads in different stages of maturity. Stages III - V are grouped under III in males

**Size Range and Population Structure:** The length of *C. macromystax* ranged from 9.7 to 30.2 cm TL. Males (range 9.9 – 30.2 cm TL, mean  $18.8 \pm 1.2$  cm TL) were not longer than females (range 9.7 - 30.1 cm TL, mean  $18.3 \pm 6.3$  cm TL) ( $P > 0.05$ ), but females (range 9 – 162 g, mean  $60.1 \pm 50.3$  g) were heavier than males (range 10 – 168 g, mean  $50.0 \pm 28.5$  g) ( $P < 0.05$ ). Both sexes had two modes but while the females had one big mode at 16 cm TL and a small one at 25 cm TL, males had the big mode at 20 cm TL and the small one at 12 cm TL (Figure 2). Females were clearly dominant from 24 – 30 cm TL. The monthly overall sex ratio (M, 317:F, 352;  $\chi^2 = 1.83$ ) and the ratio

on spawning run (M, 18 : F, 26;  $\chi^2 = 1.45$ ) (Table 2) did not differ significantly from 1:1 sex ratio ( $P > 0.05$ ), but the ratio in gonad stages III – V (M, 129:F, 167;  $\chi^2 = 4.88$ ) differed significantly.

Within the months females were dominant in August in overall, in January, March, June to August in gonad stages III – V and in July during spawning run. Males were predominant in February and September to October in gonad stages III-V.

**Table 6: The tropic spectrum and index of food significance (IFS) of the diet of *C. macromystax* in Anambra river basin**

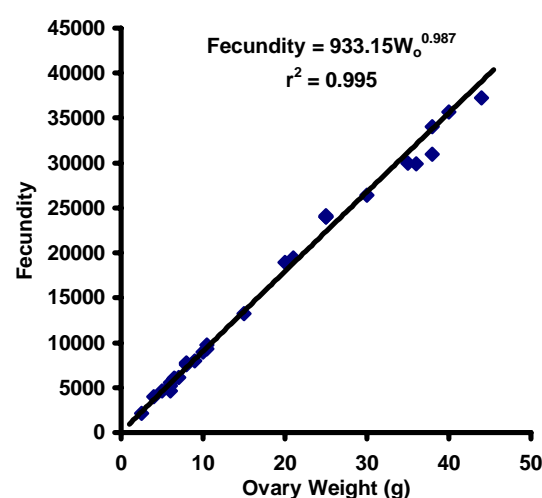
Food species/group	%RF	%Wt	IFS
<b>Rotifera</b>			
<i>Keratella cochlearis</i>	0.29	+	+
<b>Annelida</b>			
<i>Libyornis</i> sp	0.44	1.34	0.22
Leeches	0.58	1.91	0.41
<b>Arachnida</b>			
Water mite	0.44	+	+
<b>Crustacea</b>			
Ostracoda	7.84	0.07	0.20
<i>Bosmina longirostris</i>	3.48	0.01	0.01
<i>Daphnia</i> sp	4.79	0.02	0.04
<i>Thermocyclops</i> sp	1.02	+	+
<i>Caridina noloticus</i>	1.89	4.30	2.99
<i>Sudanonaues africanus</i>	1.45	4.46	2.38
<b>Insecta</b>			
<i>Povilla adusta</i>	4.21	0.1	0.15
Odonata naiad	2.32	2.31	1.97
Terrestrial Orthoptera	3.05	16.48	18.50
<i>Carixa</i> sp	2.18	0.02	0.02
<i>Gerris</i> sp	0.58	0.01	+
Peidae	0.44	+	+
Trichoptera larvae	1.45	0.04	0.02
Chironomidae	14.66	0.15	0.81
<i>Chaoborus</i> larvae	0.73	+	+
Mosquito larvae and pupae	4.79	0.06	0.11
Formicoid Hymenoptera	4.21	4.80	7.44
Dytiscidae	7.40	5.25	14.30
<b>Fish</b>			
<i>Oreochromis niloticus</i>	2.76	25.71	26.12
<i>Parachanna obscura</i>	1.16	18.47	7.89
<i>Phractolaemus ansorgii</i>	0.16	0.72	0.04
<i>Tilapia</i> fry	1.16	3.18	1.36
<b>Amphibia</b>			
Tadpole	0.44	0.21	0.03
<b>Algae</b>			
<i>Spirogyra</i> sp	3.34	+	+
<i>Zygnema</i> sp	0.58	+	+
<i>Microcystis</i> sp	0.29	+	+
<i>Scenedemus</i> sp	1.74	+	+
<i>Navicula</i> sp	1.02	+	+
<b>Fruits and seeds</b>	3.34	7.56	9.29
<b>Plant detritus</b>	7.11	1.17	3.06
<b>Mud</b>	5.08	0.84	1.57
<b>Sand grains</b>	3.63	0.80	1.07

**Morphometric Relationships:** The relationship between SL and TL ( $TL = aSL^b$ ) of male, female and both sexes of *C. macromystax* (Table 3a) showed that their b-values were not different from 1 ( $P > 0.05$ ) and that their correlation coefficients were high, positive and significant ( $r = 0.998$ ,  $P < 0.001$ ). For the SL-weight (Table 3b) and TL-

weight (Table 3c) (Figure 3) relationships, the intercept, a, and the b-values showed high homogeneity. The b-values revealed negative allometric LWR.

**Relative Condition,  $K_n$ :** The mean monthly relative condition ( $K_n$ ) was  $1.05 \pm 0.10$  (range  $0.91 \pm 0.04 - 1.24 \pm 0.04$ ) in the male, and  $1.19 \pm 0.21$  (range  $0.81 \pm 0.11 - 1.44 \pm 0.08$ ) in the female (Figure 4). The mean  $K_n$  rose steadily from October to a peak in June after which it gradually fell to the lowest value in September. A definite cycle was, therefore, established. Generally, females were in better condition than the males.

**Reproduction:** The monthly percentage distribution of male and female gonad maturity stages (Figures 5a and b) showed that immature, developing and mature gonads occurred all the year round. The high numbers of immature individuals of both sexes from July to March showed that the clariid was recruited into the artisanal fishery during this period. Gonad recrudescence lasted seven months (October – April). At this time, ripe, running or spent individuals were totally absent. The breeding season was from May to September when ripe and running individuals were present in the population.



**Figure 6: The fecundity - ovary weight relationship of *C. macromystax* in Anambra river basin**

Over 50 % of both sexes were mature at 15.1 - 20.0 cm TL in their first year of life (Table 4). The smallest mature female and male were 13.8 cm TL and 13.1 cm TL respectively. The fecundity of 31 ripe female *C. macromystax* ranged from  $2.136 \times 10^3$  to  $37.250 \times 10^3$  (mean  $14.942 \times 10^3 \pm 11.248 \times 10^3$ ). The regression equations for the relationships between fecundity and total length, standard length, body weight and

**Table 7: Monthly IFS of *C. macromystax* in Anambra river basin**

Food species/group	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
<b>Rotifera</b>												
<i>K. cochlearis</i>	-	0.01	-	-	-	-	+	-	-	-	-	-
<b>Annelida</b>												
<i>Libyodrilus</i> sp	-	-	-	-	-	-	-	0.31	18.12	-	-	-
Leaches	3.77	-	-	-	-	-	-	-	-	5.15	4.42	5.44
<b>Arachnida</b>												
Water mite	-	-	-	0.02	-	-	-	-	-	-	-	-
<b>Crustacea</b>												
Ostracoda	0.08	0.21	0.03	0.02	0.07	0.01	0.22	0.36	0.05	0.11	0.11	0.07
<i>B. longirostris</i>	0.01	-	0.06	-	0.02	0.01	0.01	0.02	-	-	-	0.03
<i>Daphnia</i> sp	0.01	0.01	0.01	+	0.09	0.02	0.05	0.02	0.02	0.03	0.03	0.08
<i>T. crassus</i>	-	0.03	0.01	-	-	-	+	-	-	-	-	0.01
<i>C. niloticus</i>	-	-	38.37	5.26	2.89	-	-	2.63	9.71	2.14	2.14	5.27
<i>S. africanus</i>	2.43	36.21	3.40	2.34	7.71	-	-	-	3.24	-	-	21.05
<b>Insecta</b>												
<i>Povilla adusta</i>	0.10	0.18	-	0.46	-	-	0.10	0.22	0.02	0.07	0.03	0.25
Odonata naiad	1.22	-	-	-	-	0.75	2.24	1.92	2.43	4.98	0.71	1.76
Terrestrial												
Orthoptera	5.48	13.60	7.67	10.52	8.67	30.45	14.01	4.42	7.29	7.48	38.43	7.90
<i>Corixa</i> sp.	-	0.03	0.03	0.14	0.07	-	0.01	0.03	-	-	-	-
<i>Gerris</i> sp.	-	-	-	0.01	-	-	0.01	-	-	-	-	-
Pleidae	-	-	-	0.05	-	-	-	-	-	-	-	-
Trichoptera larvae	-	0.06	0.03	0.24	0.20	0.03	-	-	-	0.10	-	-
Chironomidae	0.09	0.73	0.39	0.14	0.49	0.70	0.39	0.62	0.25	0.50	0.52	0.77
<i>Chaoborus</i> sp	-	-	-	-	-	-	-	0.01	-	0.01	-	-
Mosquito larvae and pupae	0.03	0.15	0.09	0.05	0.13	0.14	0.06	0.02	0.02	0.22	0.03	0.09
Formicoid												
Hymenoptera	5.48	1.36	10.65	2.63	4.33	-	9.07	7.49	0.73	2.36	0.64	2.37
Dytistidae	8.76	7.24	1.02	8.43	5.78	16.90	14.55	11.10	2.91	4.48	2.57	3.16
<b>Fish</b>												
<i>O. niloticus</i>	41.37	25.69	-	-	65.41	-	9.53	59.63	54.99	56.41	48.39	14.91
<i>P. obscura</i>	-	-	24.70	67.93	-	43.58	28.90	1.59	-	-	-	-
<i>P. ansorgi</i>	-	-	7.67	-	-	-	-	-	-	-	-	-
<i>Tilapia</i> fry	-	7.55	-	-	-	-	-	0.27	-	-	-	-
<b>Amphibia</b>												
Tadpole	-	-	0.77	-	-	-	-	0.20	-	-	-	-
<b>Algae</b>												
<i>Sprogyra</i> sp.	+	+	+	-	-	+	+	+	+	+	+	+
<i>Zygonema</i> sp.	-	-	+	-	-	-	+	-	-	-	-	+
<i>Microcystis</i> sp.	-	-	+	-	-	-	-	-	+	-	-	-
<i>Scenedemus</i> sp.	-	-	-	-	-	-	+	+	-	-	-	-
<i>Navicula</i> sp	-	-	-	-	-	-	-	0.01	-	-	-	-
<b>Fruits &amp; seeds</b>	24.33	-	-	-	-	4.14	16.91	5.70	-	6.64	-	22.80
<b>Plant detritus</b>	2.93	0.46	4.10	0.17	2.60	0.11	2.69	2.37	0.24	2.97	0.85	4.22
<b>Mud</b>	2.93	4.08	1.02	1.58	1.15	1.80	0.67	0.26	-	0.25	0.85	4.22
<b>Sand grains</b>	0.97	2.41	-	-	0.39	1.35	0.62	0.79	-	0.33	0.29	5.62
<b>Food richness</b>	17	18	18	17	16	14	18	23	14	18	15	19
<b>Diet breadth</b>	1.78	1.76	1.76	1.19	1.32	1.38	1.96	1.55	1.43	1.63	1.24	2.23
<b>No. examined</b>	14	14	15	16	14	17	31	30	13	16	17	15
<b>No. with food</b>	12	11	12	13	12	11	23	25	11	12	12	14

ovary weight ( $F = aL^b$ ,  $F = \alpha + \beta X$ ) showed that ovary weight had the best predictive value accounting for > 99.6 % of the variation in fecundity (Table 5a).

The GSI varied from 5 to 30.3 (mean  $16.8 \pm 6.0$ ) and related exponentially to fecundity ( $GSI = aF^b$ ) (Table 5b); the coefficient was high, positive and significant. Fecundity accounted for 74.2% of the variation in GSI.

The clariid spawned on the submerged grasses of adjacent areas of floodplain ponds from which it undertook short spawning run. It participated in communal spawning with *C.*

*agboyiensis* on 17 July, 1997, 17 h after a heavy downpour which lasted almost two days.

**Trophic Biology:** Of the 212 *C. macromystax* (9.7 - 30.2 cm TL) stomachs examined, 44 (20.75 %) were empty and 168 (79.25 %) contained food. Only 28 (16.67 %) of the stomachs with food were full, whereas 140 (83.33 %) were partially filled. The mean vacuity index was 20.75 %. There were fuller stomachs during the night (19.30 – 05.00 h) than the day (08.00 – 17.00 h) indicating that the clariid fed more at night than during the day.

Generally, stomachs were less than half-full (Figure 7a). The mean stomach fullness index was  $6.77 \pm 1.09$  (range 4.6 - 8.3); the index during the rains ( $6.92 \pm 1.31$ ) was not different from that in the dry season ( $6.62 \pm 0.92$ ) ( $P < 0.05$ ). The mean percentage empty stomach was  $20.3 \pm 7.7$  (range 6.7 - 35.3) indicating that stomachs generally contained food.

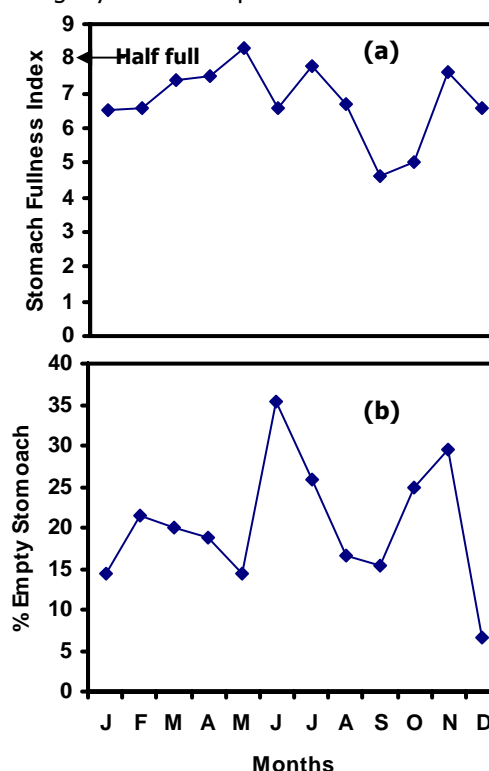
**Table 8: Seasonal variation in the IFS of *C. macromystax* in the Anambra river basin**

Food species/group	Season		P
	Dry	Rainy	
<b>Rotifera</b>			
<i>K. cochlearis</i>	+	+	ns
<b>Annelida</b>			
<i>Libyodrilus</i> sp	+	0.38	
Leaches	3.32	-	
<b>Arachnida</b>			
Water mite	-	+	
<b>Crustacea</b>			
Ostracoda	0.10	0.22	<0.05
<i>B. longirostris</i>	0.01	0.03	<0.05
<i>Daphnia</i> sp	0.03	0.03	ns
<i>T. crassus</i>	+	+	ns
<i>C. niloticus</i>	4.02	1.82	<0.05
<i>S. africanus</i>	9.37	0.38	<0.05
<b>Insecta</b>			
Povilla adusta	0.10	0.14	ns
Odonata naiad	1.34	1.76	ns
Terrestrial Orthoptera	16.89	14.39	ns
<i>Corixa</i> sp.	+	0.04	<0.05
<i>Gerris</i> sp.	-	+	
Pleidae	-	+	
Trichoptera larvae	0.02	0.01	<0.05
Chironomidae	0.67	0.60	ns
<i>Chaoborus</i> sp	+	+	ns
Mosquito larvae and pupae	0.13	0.07	<0.05
Formicoid Hymenoptera	5.15	6.58	ns
Dytistidae	6.03	15.16	<0.05
<b>Fish</b>			
<i>O. niloticus</i>	36.39	29.11	<0.05
<i>P. obscura</i>	0.98	17.86	<0.05
<i>P. ansorgi</i>	0.30	-	
<i>Tilapia</i> fry	0.17	0.04	<0.05
<b>Amphibia</b>			
Tadpole	0.03	0.03	ns
<b>Algae</b>			
<i>Sprogyra</i> sp.	+	+	ns
<i>Zygonema</i> sp.	+	+	ns
<i>Microcystis</i> sp.	+	+	ns
<i>Scenedemus</i> sp.	-	+	
<i>Navicula</i> sp	-	+	
<b>Fruits and seeds</b>	7.42	7.50	ns
<b>Plant detritus</b>	3.62	2.33	<0.05
<b>Mud</b>	2.58	0.83	<0.05
<b>Sand grains</b>	1.34	0.68	<0.05
<b>Food richness</b>	23	23	
<b>Diet breadth</b>	2.11	2.03	
<b>No. examined</b>	91	121	
<b>No. with food</b>	73	95	

Thirty-six different food items were ingested (Figure 6). Of these, only six constituted primary food items. The dominant food groups, in order of importance, were insects, fish, fruits and seeds, crustaceans and plant detritus. Even though

the diversity of insects in the diet was more than that of fish, the latter contributed to the diet in more months (9) than insects (3) (Table 7). Seven food items were ingested in all the months but only formicoid Hymenoptera was of primary importance. Food richness showed that the lowest number (5) of food items of primary importance occurred in June and November, whereas the highest (12) was in December. This latter month also had the highest diet breadth (2.23); the diet breadth of other months varied moderately.

The IFS of nine food items were higher in the dry season, and five during the rains (t-test,  $P < 0.05$  in each case) (Table 8). Ten food items were of primary importance in the dry season, whereas nine were of primary importance during the rains. Quantitative food composition was the same in both rainy and dry season. Diet breadth was slightly season-dependent.



**Figure 7: The monthly stomach fullness index (a) and percentage empty stomach (b) of *C. macromystax* in the Anambra river basin**

*C. macromystax* fed by foraging, shoveling and surface feeding. Many food items of primary importance were ingested employing the latter method of feeding in this clariid.

**Parasites:** Three helminth parasites were recovered from the 164 *C. macromystax* examined. These were the metacercariae of *Euclinostomum clarias* Dubois (Digenea), *Procamallanus laevichonchus* (Wedl) and a larval

**Table 9: The parasite spectrum and their overall prevalence (a) and the prevalence in relation to site in the host (b) in *C. macromystax* (n = 164) in Anambra river basin**

Parasite species	Sites in host	No. of fish infected	Total no. of parasites recovered	Prevalence	Mean intensity	Mean abundance
<b>a) Parasite spectrum and their overall prevalence</b>						
<i>Euclinostomum clarias</i>	Liver, gut mesentery, ovary and kidney	45	325	27.44	7.22	1.98
<i>Procamallanus laevichonchus</i>	Stomach and rectum	5	9	3.05	1.8	0.05
Larval spiruroid	Muscle, kidney and coelom	10	19	6.10	1.9	0.12
<b>b) Prevalence in relation to sites in host</b>						
<i>E. clarias</i>	Liver	29	248	17.68	8.55	1.57
	Gut mesentery	8	49	4.88	6.13	0.30
	Kidney	3	7	1.83	2.33	0.04
	Ovary	5	21	3.05	4.2	0.13
<i>P. laevichonchus</i>	Stomach	4	6	2.44	1.5	0.04
Larval spiruroid (f. Physalopteridae)	Muscle	5	10	3.05	2.0	0.06
	Kidney	4	8	2.44	2.0	0.05
	coelom	1	1	0.61	1.0	0.01

spiruroid (f. Physalopteridae) (Nematoda) (Table 9a). *E. clarias* was the most prevalent and parasitized the liver, gut mesentery, ovary and kidney of the host. The liver was more heavily parasitized than other sites (Table 9b). The number of oocytes in parasitized ovaries was not different from that of non-parasitized ones ( $P < 0.05$ ). In 45 hosts, seven (15.56 %) were simultaneously infected in the liver and gut mesentery by *E. clarias*, whereas one (2.22 %) was simultaneously infected in the liver and ovary. The prevalence, mean intensity and mean abundance of *E. clarias* and the larval spiruroid were significantly more in the dry than the rainy season ( $P < 0.05$ ). The mean abundance of *E. clarias* and the larval spiruroid were not different between the sexes.

## DISCUSSION

The total absence of *C. macromystax* from the river systems (Table 1) may be attributed to the hydrologic regime, unfavourable habitat and inability to disperse adequately, but it appears to be causally related to food supply and ichthyophagic predation pressure. In rivers, large, social hunting fish predators, such as *Clarias gariepinus* (Bruton, 1979), using juvenile *C. macromystax* as forage species (pers. obs), preclude the latter from the river habitat, as is the

case, to a lesser extent, with *C. agboyiensis* and *C. albopunctatus* (Ezenwaji and Inyang, 1998; Ezenwaji, 1999). In addition, abundant food supply in the floodplain ponds probably offers little incentive for the clariid to migrate. In fact, most food of primary importance ingested by this clariid, such as terrestrial Orthoptera, formicoid Hymenoptera, fruits and seeds, plant detritus, Odonata nymph and fish, are either allochthonous from the dense overhanging vegetation in forest floodplain ponds or autochthonous but abundant in these habitats. This rich food supply promotes rapid growth and good recruitment; thus, over 89% of the *C. macromystax* of this study came from floodplain ponds, which are almost predator-free. The virtual restriction of *C. macromystax* in floodplain ponds, unlike *C. albopunctatus* present in all habitats (Ezenwaji, 1999), is probably the reason why it is the least abundant, and *C. albopunctatus* the most abundant, of the seven *Clarias* species of the Anambra river basin (Ezenwaji, 1986, 1992).

The 1:1 sex ratio in *C. macromystax* on spawning run (Table 2) appears to have some relationship with communal spawning observed in *Clarias* species of the Anambra river basin (Ezenwaji, 1992). The synchrony of endogenous and exogenous factors in these clariids and the pairing of the sexes prior to spawning suggest a 1:1 rule observed for the clariids on spawning run. In the situation of communal spawning and where

sperms are released externally, the probability of hybridization becomes high. In fact, this may be responsible for the very high speciation of some clariids in forest coastal areas (Sydenham, 1980) and the observed difficulties in the precise identification of African *Clarias* species.

The food of *C. macromystax* is consistent with the food of other clariids of the subgenera *Clarioides*, *Anguilloclarias* and *Brevicephaloides* in its high content of insects (Jackson, 1961; Corbet, 1961; Welcomme, 1985; Bruton, 1979). Thus, terrestrial Orthoptera was the only food item of primary importance in all the months (Table 17). However, fish (*O. niloticus* and *P. obscura*) made the highest contribution energetically in most of the months. The dependence on food of allochthonous origin is probably why *C. macromystax* is much more abundant in forest floodplain ponds with overhanging canopies than in other habitats. The slightly higher diet breadth in the dry (2.11) than during the rains (2.03) indicates depressed resource abundance in the dry season. This is consistent with the optimal foraging theory (Schoener, 1971), which postulates that as diet breadth increases; resource abundance decreases, and vice versa. Studies in the topics, which also show lower resource abundance and higher diet breadth in the dry season are those of Zaret and Rand (1971), Welcomme (1979, 1985), Lowe-McConnell (1975) and King (1988). Conversely, the rainy season showed decreased diet breadth and increased resource abundance.

*C. macromystax* is a new host record for the helminth parasites reported in this study. The most important of these in terms of fisheries importance is *E. clarias* metacercariae, which parasitized the liver of *C. macromystax* much more than any other clariid of the basin (Ezenwaji and Ilozumba, 1992). As *E. clarias* has also been recovered from the body cavity of *Clarias angolensis* in Angola, recorded in *Clarias anguillaris* in Zaria (Shotter, 1980) and from many organs in *C. ebriensis*, *C. agboyiensis*, *C. albopunctatus* and *C. buthupogon* in the Anambra river basin (Ezenwaji and Ilozumba, 1982), it appears that the parasite may be more widely distributed in African *Clarias* species than was hitherto realized. This is the first report of the recovery of *E. clarias* in the liver, gut mesentery, kidney and ovary of *C. macromystax*. When infected with over 25 *E. clarias*, as in a 20.7 cm TL *C. macromystax*, the liver appeared diseased, the fish looked emaciated and metacercariae had escaped from their cysts and were free in the coelom. Unlike the nematode, *Philometra translucida*, which inhibits the eggs of *Pseudotolithus senegalensis*, *P. typus* and *P. elongatus* from growing to maturity and significantly lowers their fecundity (Anyanwu, 1983), *E. clarias* parasitizing the ovaries of clariids

does not appear to be normal ovarian parasite. Consequently, the fecundity of *C. macromystax* is apparently unaffected.

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## SOCIO-ECONOMIC AND WATER CONTACT STUDIES IN *Schistosomiasis haematobium* INFESTED AREA OF ANAMBRA STATE, NIGERIA

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### ABSTRACT

*A survey of urinary schistosomiasis in Agulu, Anambra state, Nigeria was carried out using primary school pupils. The relationship between family socio-economic status of the pupils and infection with Schistosoma haematobium and between water contact activities of the people with S. haematobium was investigated. Seven primary schools out of fifteen in the town had pupils with Schistosoma haematobium infection. Children whose parents were farmers and teachers had infection rates of 38.9% and 14.4% respectively. A greater number of children (7,000) (74.2%) than adults (2,438) (25.8%) perform different activities in different parts of the lake which bring them in contact with the water during the period of study. The highest number of people (3,324) (35.2%) were engaged in swimming while the lowest number 480 (5.1%) were found fishing.*

**Keywords:** Water contact, *Schistosoma haematobium*, Socio-economic

### INTRODUCTION

Knowledge of the pattern of exposure to infection is essential to an understanding of the epidemiology of *S. haematobium* infection. Human contact with water arises from four major basic needs. These include occupational, recreational, domestic and socio-cultural. Among the few studies on human water-contact patterns in tropical Africa are those by Dalton and Pole (1978) in lake Volta, Ghana, Tayo *et al* (1980) in Malumfashi in northern Nigeria. The demands of occupations like fishing or farming in which many in the rural areas are engaged bring them into regular contact with water. Thus, such people are constantly exposed to infection. The activities of women and children which involve washing of house hold utensils and fetching of water in the morning or evening also take them to water sites. These activities are seen to enhance transmission (Ukoli, 1990). Water-contact studies can therefore be a useful means of determining the principal human activities that create a high risk of exposure to schistosomiasis in areas where it occurs. They also serve as means of assessing possible reductions in human exposure to cercarial populations and subsequent worm burdens through control measures.

The socio economic background of school children taking into consideration their parents' or guardians' occupation could also influence the infection rate of schistosomiasis in areas where it occurs. So there is the need to study this aspect in endemic areas of the

disease among school children because they provide convenient base line data for the whole population (Forsyth, 1969, Wilkins, 1977).

### MATERIALS AND METHODS

**Urine Collection and Socio-economic Investigation:** Urine samples were collected from all the children in the seven primary schools in Agulu implicated in *Schistosoma haematobium* infection (Ekwunife, 2003) with wide mouthed screw cap containers. A simple centrifugal sedimentation procedure (5 min. at 5000 rpm) of 10ml aliquot urine sample drawn from each specimen (Oliver and Uemura, 1973) was used for analysis. *S. hametobium* ova in the sediment poured on a McMaster slide were counted using a binocular microscope (X 100). During the urine collection, inquiry about the occupation of the parent/guardian of each pupil was made and this was recorded against the person's identification number.

**Water Contact Studies:** For six months (October 2000 to March 2001) human activities in Agulu lake were observed and recorded. The ages of the people were also recorded. Observation was made on daily basis. This was done every evening between 4 pm and 6 pm except on Sundays. On Saturdays the human activities were observed from 8.00 am till 6.00 pm. These are periods when activities like washing of household utensils, fetching of water and fishing take them to the water sites.

**Table 1: Variation among individuals from families of various occupational groups infected with urinary schistosomiasis in the different schools**

School	Traders		Farmers		Teachers		Other civil servant		Others	
	No. Ex.	No. Inf.	No. Ex.	No. Inf.	No. Ex.	No. Inf.	No. Ex.	No. Inf.	No. Ex.	No. Inf.
Umuowelle Primary School	46	24	70	50	14	5	30	12	41	20
	(52.2)		(71.4)		(35.7)		(40.0)		(48.8)	
Ugwuaba Primary School	34	15	63	32	12	2	32	5	44	14
	(44.1)		(50.8)		(16.7)		(15.6)		(31.8)	
Community Primary School	61	23	77	35	12	2	29	5	40	11
	(37.7)		(45.5)		(16.7)		(17.2)		(27.5)	
Nneogidi Primary School	40	13	64	23	10	2	30	4	42	13
	(32.5)		(35.9)		(20.0)		(15.0)		(13.0)	
Practicing Primary School	142	32	185	59	25	3	81	11	99	23
	(22.5)		(31.9)		(12.0)		(13.6)		(23.2)	
Ifiteani Primary School	30	7	46	14	8	1	27	2	30	9
	(23.3)		(30.4)		(12.5)		(7.4)		(30.0)	
Obeagu Primary School	41	2	53	4	9	0	30	0	36	1
	(4.9)		(7.5)		(0)		(0)		(28)	
<b>Total</b>	<b>394</b>	<b>116</b>	<b>558</b>	<b>271</b>	<b>90</b>	<b>13</b>	<b>259</b>	<b>39</b>	<b>332</b>	<b>91</b>
	(29.4)		(38.9)		(14.4)		(15.1)		(27.4)	

**Table 2: Distribution of individuals by age and water contact activities**

Activity	No of children (0 – 9 yrs)		No of Adults (> 20 yrs)		Total
	Observed	/ %	Observed	/ %	
Domestic					
Washing cloths and Utensils	1,000	(62.5)	600	(37.5)	1,600(17.0)
Fetching water	2,080	(90.5)	218	(9.5)	2,298(24.3)
Processing breadfruits and fermenting cassava	806	(46.4)	930	(53.6)	1,736(18.4)
Recreational					
Swimming	2,914	(87.7)	410	(12.3)	3,324(35.2)
Economic					
Fishing	200	(41.7)	280	(58.3)	480(5.1)
<b>Total</b>	<b>7,000</b>	<b>(74.2)</b>	<b>2,438</b>	<b>(25.8)</b>	<b>9,438</b>

## RESULTS

Information on schistosomiasis infection in children with different socio-economic background from different schools are presented in Table 1. Urinary schistosomiasis was wide spread in children from families of different occupational groups in the endemic communities. Pupils from farming families had the highest infection rate of 38.9 % followed by those from trading families with 29.4 %. In all schools, except Ifiteani Primary School, children whose parents were teachers had the lowest infection rate.

The results of the six months observation of human activities on water sites (Agulu lake arms) are shown in Table 2. More children 1,000 (62.5 %), 2,080 (90.5 %), 2,914 (87.7 %) than adults 600 (37.5 %), 281 (9.5 %), 410 (12.3 %) were observed washing clothes/utensils, fetching water, and swimming in the lake respectively. Though more adults were seen performing activities like

processing breadfruit, fermenting cassava and fishing, on the whole a greater number of children 7,000 (74.2 %) than adults 2,438 (25.8 %) were seen performing different activities in different parts of the lake which bring them in contact with the water. The highest number of people 3,324 (35.2 %) were found swimming.

## DISCUSSION

The children's socio-economic background taking into consideration their parents or guardians occupation showed that those from farming family had the highest infection rate. This was probably because they joined their parents at farm during the holidays, for Okafor (1984) reported that farmers bath in the fresh water, streams and or pools near their farms after each day's work. In the study population, most farmers were ignorant of the mode of transmission of the disease and so they might not have known the implication of the

lake in the transmission of the disease and even few that know seemed to neglect it (Emejulu, 1994). Traders' children were next to farmers' with infection rate of 29.4%. Most members of this group were also ignorant and so may suffer the same fate as the farmers. The children of teachers and civil servants' were the least infected. This suggests that community motivated health education campaign on the mode of transmission of the parasite could be effective in reducing the disease infection rate in this study population.

The communal life in Agulu is such that domestic activities like washing of clothes and utensils and fetching of water are the sole responsibility of the children. Swimming carried the greatest risk followed by fetching of water. School children were also observed swimming in the lake. This was probably the cause of the high prevalence recorded for them. Wilkins (1987) stressed that recreational use of water for swimming and playing is usually of greater importance to younger children. He thus pointed out that for children, such activity might carry a high risk of infection as it often involves much of the body being in contact with water for long periods of time. Other studies (Dalton, 1976, Dalton and Pole 1978, Tayo *et al* 1980, Kvalsvig and Schutle, 1986) showed varying age groups to be at risk. Some workers observed that adult males are usually the group involved in occupational exposure in fishing and farming. Thus the diversity of human society and behaviour make it difficult to draw valid general conclusions about the results of these and other water contact studies. However, it has often been found that the majority of contact is domestic and recreational but that children have more contact than adults. It is thus established by this study that the level of water contact is usually lower in older people. Some researchers have suggested that acquired immunity may be responsible for the lower egg output in adults. Dalton and Pole (1978) stated however that there is no need to postulate some mechanism such as immunity as playing a significant role in the distribution of infection by age.

This study has also shown that the reduction in egg output which researchers like Wilkins (1987) attributed to immunity, was closely related to a lower level of water contact for older people, thus providing a simpler explanation than that of intrinsic immunity. By water contact observation it has been possible to identify which human activities carried the greatest risk. The elimination or reduction in intensity of these particular activities by control measures may provide the best means of reducing the output of eggs in human urine, and hence the worm burden of the population.

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## SCHISTOSOMIASIS INFECTION IN PRIMARY SCHOOLS IN AGULU TOWN OF ANAMBRA STATE, NIGERIA

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### ABSTRACT

*Investigation was made to reveal the state and level of Schistosomiasis haematobium infection in the whole of Agulu town in Anaocha local Government Area of Anambra State, Nigeria where a lake (Agulu lake) is implicated in the transmission of the disease. Urine sample was collected from 3029 children for Schistosoma egg identification. This was used to calculate the level of infection in the different schools. Schistosomiasis prevalence was highest (55.2%) in Umuowelle primary school and lowest (4.1%) in Obeagu primary school. Males had higher infection rate than females in the endemic schools. In Umuowelle, Community and Nneogidi primary schools, infection rates in males were 36.4%, 13.3%, 11.3% respectively while infection rates in females were 25.2%, 11.7% and 6.8% respectively. However, the sex differences were not statistically significant at 5% confidence level ( $t$ -test = 2.179,  $df$  = 12). Infection levels investigated in all the schools revealed that the age group 10-14 years recorded the highest level while 0-4 years had the lowest. There was also shifts in peaks of infection within the various age groups, for instance, in the 10 - 14 years age group of Ifiteani primary school, infection peak was in 14 years while in Nneogidi primary school it was in 13 year old pupils.*

**Keywords:** Agulu town, Agulu lake, Schistosomiasis, Sex, Age groups

### INTRODUCTION

The epidemiology of *S. haematobium* in man has been described in terms of age - specific prevalence. School children, who usually represent the age groups at greatest risk and greatest intensity of infections, have often been studied, thus providing convenient baseline data for the whole population (Forsyth, 1969, Wilkins, 1977). Stimmel and Scott (1956) observed that egg output is greatest between noon and 2 p.m. while Bradley (1963) observed that egg output is least variable between noon and 2 p.m. Experiences from field work have led many workers to examine a 10 ml aliquot from the entire urine passed at the peak period and usually data obtained have been used as an index of community egg output. There have been many schistosomiasis surveys in Nigeria since Ramsay (1934) studied intestinal schistosomes in Northern Nigeria. They include Blair (1956), Okpala (1961), Cowper (1973), Anya and Okafor (1986), Ejezie et al (1989), Ozumba et al (1989), Adewunmi et al (1990) etc. Emejulu et al (1994) had investigated into the prevalence of urinary schistosomiasis in the Agulu lake area of Anambra State, Nigeria. However, their research covered three communities (Agulu, Nri, Adazi nnukwu.) around the lake and random samples of persons were made during their

investigation. Emejulu et al (1992) also gave a comprehensive report on the intermediate snails hosts (*Bulinus globosus* and *Bulinus truncatus*) of urinary schistosomiasis found in Agulu lake.

In the present study, Agulu town was singled out and a comprehensive investigation which involved screening all the primary school children in the town was carried out. The objectives of the study were to show the level of endemicity in the different villages of the town, infection rates among sex, infection rates between and within age groups and to identify particular individuals with *S. haematobium* infection. Such a comprehensive record of a disease in a town will help in disease control and will allow comparison with other studies on urinary schistosomiasis from different regions. It is hoped that such comprehensive study will be repeated in the other two afflicted communities (Nri & Adazi nnukwu) around the lake

### MATERIALS AND METHODS

**The Study Area and Study Population:** Agulu town was purposively selected for the study because of previous knowledge of the presence of *S. haematobium* infection in the town (Emejulu 1994, Emejulu et al, 1994). Agulu which is in Anaocha Local Government Area is located between latitude

for greatest egg output (Stimmel and Scott, 1956; Bradley, 1963). The visitations were made to one school on two different days of every week and the urine samples taken straight to the laboratory for analysis that particular day. Visitation was however made to practicing school 5 times because it had over 500 pupils. A simple centrifugal sedimentation procedure (5 min. at 5000 rpm) of 10ml aliquot urine sample drawn from each specimen was used (Clivier and Uemura, 1973). *Schistosoma haematobium* ova in the sediment poured on a McMaster slide were counted under 10x-microscope eyepiece. The following calculations were made:

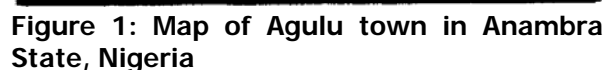
School Infection level = Total number infected in a school / Total number examined in the school x Total infected in all schools / Total number examined in all schools x 100.

Age group infection level= Average of specific age infection level of a particular age group.

Overall sex infection level= Total sex inf. in a school / Total sex examined x Total number infected in school / Total number examined in the school x 100.

**Schistosomiasis Infection in the Schools:** The level of infection of the disease for each school among all the schools in the whole town is shown in the Table 1. Among all the 15 schools in Agulu town, Umuowelle Primary School recorded the highest schistosomiasis infection level of 8.8% followed by Ugwuaba Primary School with 6.9%. Community, Nneogidi, Practicing, Ifiteani and Obeagu Primary Schools recorded 5.6, 4.7, 3.9, 3.7, and 0.7 infection level respectively. The remaining eight schools had no individuals infected with urinary schistosomiasis, thus 0% for each of them.

**Specific Ages in the various Age Cohorts:** The disease infection levels by sex and specific ages in the affected schools are shown in Figures 1 and 2. In all the age groups of various schools, the disease was higher in males than in females. In both sexes the highest age group infection level was recorded in the group 10 – 14 years. However, within and between each age group, there is shift in peaks of infection among the sexes. In all the schools, only Umuowelle and Community primary schools had individual of 0 - 4years with positive cases of urinary schistosomiasis. Within this age group, the peak of infection was in 4years individuals in both schools.



**Urine Collection and Analysis:** All the primary school children from the 15 schools in Agulu were involved in this study. Thus urine was collected from 3029 children. Wide mouthed screw cap containers with numbers for identification were used to collect urine samples from each person in the different schools on visitation. This was done during the dry season months for 16 weeks (November 1999 – February 2000). The time of collection of urine was between 12.00 pm and 2.00 pm. This is the period

Table 1: Agulu town schools schistosomiasis infection level

School	Villages located	No. Examined	No. Infected	% Infected	Infection level %
Agunkwo P/S	Amaorji	70	0	0	0
Cent P/S	Odidama, Obe	200	0	0	0
Chukwuka P/S	Uhueme, Ukunu	241	0	0	0
Community P/S	Umunowu	291	76	34.7	5.6
Ezenyanwu P/S	Odidama, Okpu Amaezike, Nneoha	223	0	0	0
Nwanchi P/S	Nwanchi	110	0	0	0
Obe P/S	Obe	223	0	0	0
Obeagu P/S	Obeagu	169	7	4.1	0.7
Onike P/S	Okpu	140	0	0	0
Practicing P/S	Nkitaku, Umubiala, Okpuifite, Amatutu	532	128	24.1	3.9
Udoka P/S	Ukunu, Isiamaigbo	189	0	0	0
Ugwuaba P/S	Umufite	185	80	43.2	6.9
Umuowelle P/S	Umuowelle	201	111	55.2	8.8
Ifiteani P/S	Ifiteani	141	33	23.4	3.7
Nneogidi P/S	Nneogidi	186	55	29.6	4.7
Total		3029	490	16.2	

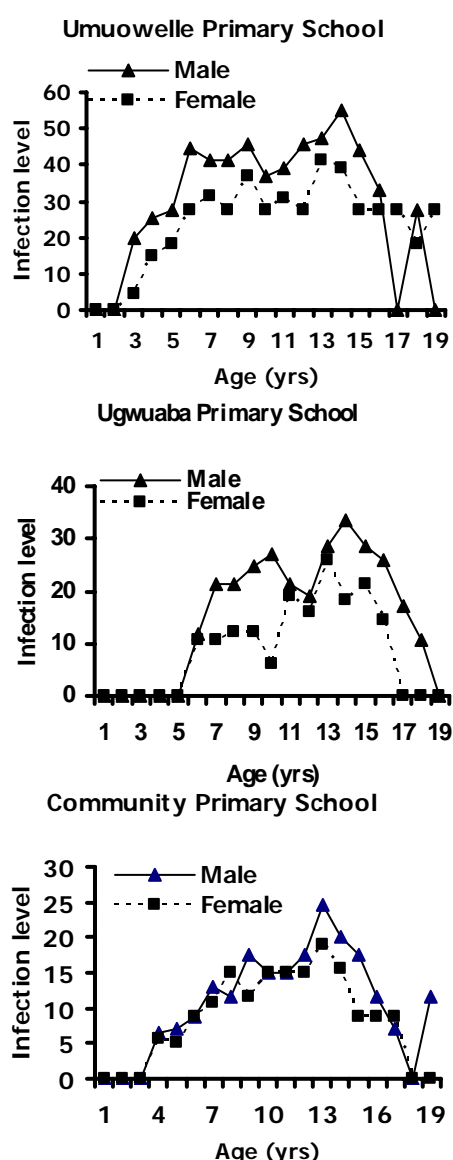


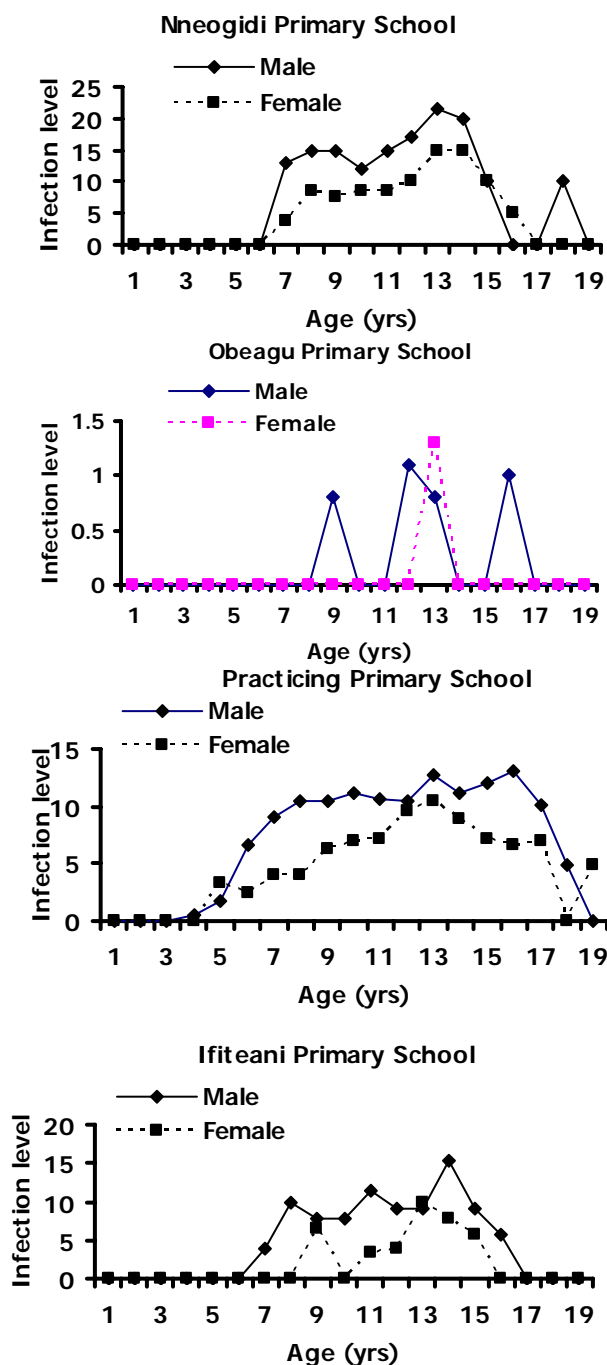
Figure 1: Schistosomiasis infection level by sex and specific ages in Umuowelle, Ugwuaba and Community Primary Schools

Among the 5 - 9 years group, all the schools recorded infection peak in 9 years individuals for both males and females except Ifiteani Primary School with male peak infection in 8 years pupils. In the 10 - 14 years group, Umuowelle, Ugwuaba and Ifiteani Primary Schools had male peak infection in 14 years and female peak infection in 13 years individuals. Community, Practicing and Nneogidi Primary Schools had both male and female age infection peak in 13 years individuals while Obeagu Primary School had its male peak in 12 years and female peak in 13 years pupils.

Among 15 -19 years age group, the male peaks were in 15 years individuals in Umuowelle, Ugwuaba, Community, Ifiteani and Obeagu Primary Schools. Nneogidi Primary School recorded its male peak in both 15 and 18 years individuals, while Practicing Primary School had its male peak infection in 16 years individuals. The female infection peaks were also in 1 years pupils in Ugwuaba, Nneogidi, Practicing, Ifiteani and Obeagu Primary Schools, while Umuowelle had its female peak in 15, 16 and 17 year pupils and Community Primary School had its infection peak in 17 years old pupils.

## DISCUSSION

Urine screening showed the presence of urinary schistosomiasis in 7 schools in the town. Observations on the infection in the 7 schools indicated that there was a significant variation in the rate of the infection between schools within same and different villages in the town. Umuowelle Primary School recorded the highest infection rate, followed by Community Primary School Umunowu Village. This could be attributed to their nearness to the lake. The overall school prevalence rate was higher than the figure reported by Ejezie and Ade-Serrano (1981) in Nigeria but lower than the figures established by Scott *et al.* (1982) and Okpala (1961). These variations may be due to the type and



**Figure 2: Schistosomiasis infection level by sex and specific ages in Nneogidi, Practicing, Obeagu and Ifiteani Primary Schools**

nature of water and human activities in relation to the disease transmission in the studied area.

More males were shown to have higher rate of infection than females in the endemic schools but the difference was not statistically significant. This is in agreement with the findings of Pugh *et al.* (1980) and Scott *et al.* (1982) in Northern Nigeria and Lake Volta Ghana respectively but is at variance with the findings of some other studies. For example, Anya and Okafor (1986) in parts of former Anambra State

of Eastern Nigeria and Okpala (1961) in Epe, Western Nigeria, reported higher prevalence of the infection among females. Such differences may be attributed to the degree of exposure to various transmission foci. Sexual differences in prevalence rates and intensity were not found to be significant by Forsyth and Bradley (1966) and Wilkins (1977), but Edington *et al.* (1970) found the reverse, recording the rate to be significantly higher in males than females in Ibadan, Western Nigeria.

The age group differences in infection rates with usually age group 10-14 being more susceptible to infection appears to be a common feature of urinary schistosomiasis (Okpala, 1961; Bradley and McCullough, 1973). Bradley and McCullough (1973) explained that people begin life uninfected and generally become infected as they expose themselves over the first 10 years of life. Then by the age of 10 years most children have been infected for a variable number of years and have acquired substantial concomitant immunity, so that the 10-14 age group are both heavily infected and protected from further infection. However, Anya and Okafor (1986) reported the age with peak infection as 15-19 years in males and 20-29 years in females. These groups they explained were farmers and were always in contact with small pools of infected water due to the nature of their work. The present study also showed that within an age group there were shifts in peaks of infection. This highlights the specific ages that are more infected than others. These high peaks of infection index among certain specific ages in an age group could also be attributed to more exposure to infected site. The lowest infection rate among the age group 0-4 years could be attributed to their not visiting the infected site. The few that are infected must have acquired it through infected water brought to the house. The specific age infection level also showed that even individuals of 4 years old in Umuowelle Primary School had higher infection than some 18 years individual. It can therefore be suggested that the 18 year individuals had acquired immunity to infection.

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## COMMON LEG ALIMENT OF POULTRY IN PLATEAU STATE, NIGERIA

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### ABSTRACT

*A study was carried out to investigate the occurrence and causes of leg ailment in poultry through questionnaire survey and farm visitation. Post mortem and laboratory examination were also conducted. The results showed that different species of poultry were affected with various types of leg ailments; out of which local chickens had the highest incidence 175(30.49 %), followed by broilers and commercial laying chickens 153(26.67 %) and least in ostrich 16(6.75 %). Swollen legs were recorded thus: local chickens 65(36.72 %), commercial layer chicken 51(28.81 %) and broilers 33(18.64 %). Incoordination, another common leg ailment were observed in broilers 40(33.33%), layer chickens 39(32.50 %), local chickens 24(20.0 %), and turkeys 4(14.81 %). Physical injuries due to trauma, burns, trampling (smothering) and accidents were recorded in broilers 28(30.77 %), commercial laying chickens 25(27.47 %), local chickens 21(23.08 %), cockerels 17(18.68 %), guinea fowls 15(39.47 %) and ostriches 2(5.26 %). Curled toes, mange, Newcastle disease, Mareks disease and Clostridia infections resulting in leg ailments were also observed.*

**Keywords:** Leg ailments, Poultry, Implication, Plateau State

### INTRODUCTION

In Nigeria, the rearing of poultry such as domestic chickens, guinea fowls, ducks, and turkeys and of recent quails and ostriches is well known. Most of these species of birds are managed either in the traditional (extensive) or the semi-intensive and intensive systems of management (Smith, 2001 and Nwagu, 2002).

However, irrespective of the management system practiced, the production of poultry in Nigeria is constrained by a number of factors among which are infectious diseases, parasitic infestations, poor nutrition and some physical injuries which may have direct or indirect effects on musculoskeletal system and consequently restrict movement or locomotion of the affected bird. Thus, leading to poor feed intake, growth retardation, decline in production, death and carcass condemnation. Abdu and Saidu (2002), stated that Newcastle disease in susceptible birds was associated with nervous signs such as incoordination, paralysis of the legs and wings, circling, backward movement, twisting of the neck, star gazing and somersaulting. Ayo and Minka (2004) from the study of some major constraints on ostrich

productivity in Northern Nigeria also reported that the most common cause of ostrich chick mortality is lower limb deformity (LLD), which affected up to 36.7 % of the chicks hatched.

However, the cause of leg ailments may be multifactor in nature, including nutrition, genetics, over feeding, lack of exercise and trauma to the lower limbs (Ritchie *et al*, 1994; Ayo and Minka, 2004). The implications of leg problems and their causes in poultry production cannot be over emphasized. Thus, this study surveys the common leg ailments of poultry in Plateau State, Nigeria.

### MATERIALS AND METHODS

Farm records and visitations to serve questionnaires and to obtain photograph of cases of leg problems in some randomly selected poultry farms and households in Jos, Plateau State and other places were used for the study. The questionnaire covers types of birds kept by the farmers, flock size, age of birds, type of management system (deep litter or battery cage), type of feed, type of leg problem, clinical signs observed, number sick and number dead. Post mortem and laboratory

analysis were also conducted using dead birds and bacterial culture of leg lesions seen. Data obtained were presented in simple descriptive form and also statistically analyzed using percentage and chi-square (Olawuyi, 1996).

## RESULTS AND DISCUSSION

Tables 1 and 2 showed the different types of leg ailments in the different species of poultry. Leg ailments occurs most commonly in local chickens 175 (30.49 %), followed by broilers and commercial laying chickens 153 (26.66 %) and least in cocks 93 (16.20 %) as shown in Table 1.

**Table 1: Occurrence of leg ailments in different types of chicken**

Problems	Local Chicken Number (%)	Broilers number (%)	Layers Number (%)	Cocks Number (%)
Swollen legs	65 (36.7)	33 (18.6)	51 (28.8)	28 (15.8)
Incoordination.	24 (20.0)	40 (33.3)	39 (32.5)	17 (14.2)
Curled toes	18 (32.4)	11 (19.6)	9 (16.1)	18 (32.1)
Paralysis/lameness	4 (37.7)	39 (34.2)	21 (18.4)	11 (9.7)
Physical injuries	21 (23.1)	28 (30.8)	25 (27.5)	17 (18.7)
Mange /mites.	4 (25.0)	2 (12.5)	8 (50.0)	2 (12.5)
<b>Total</b>	<b>175</b>	<b>153</b>	<b>153</b>	<b>93</b>

Swollen leg has the highest occurrence of 65 (36.72 %) in local chickens, followed by commercial laying chickens 51 (28.81 %) and broilers 33 (18.64 %) while incoordination was highest in broilers 40 (33.33 %), followed by laying chickens 39 (32.50 %). Curled toes were common in local chickens and cocks 18 (32.14 %) and paralysis/lameness was more in local chickens 43 (37.72 %) and broilers 39 (34.21 %) than any other type of poultry. The higher occurrence of leg ailments in local chickens may be due to their free-range nature of management, which exposes them to many hazards. Though, feed was not analyzed in this study, but Ritchie *et al* (1994) as well as Ayo and Minka (2004) reported that the cause of leg ailment may be multifactor in nature, including nutrition, genetics, over feeding, lack of exercise and trauma to the lower limbs. Furthermore, vitamin B<sub>2</sub> deficiency can cause curled toe paralysis while deficiencies in some minerals

such as potassium, phosphorus, choline, manganese and zinc has been reported to cause rickets, joint enlargement and other forms of bone deformities (McDonald *et al*, 1988; Ritchie *et al*, 1994; McDonald *et al*, 1995; Abdu and Saidu, 2002).

The high incidence of physical injuries due to trauma and burns in broilers 28 (30.77 %) and commercial layer chickens 25 (27.47 %) than in local chickens 21 (23.08 %) and cockerels 17 (18.68 %) may be due to their tender skin nature and the high stocking density under intensive system of management. Parasitic mange (mites) infestation had higher occurrence in commercial laying chickens 8 (50.00 %) and least occurrence in broilers and cocks 2 (12.50%). The higher incidence of mange parasites in commercial laying chickens may be due to their intensive nature of management, which concentrates the parasites and encourages their rapid multiplication.

Table 2 showed that leg ailments were high in ducks, turkeys, guinea fowls and ostrich. Paralysis/lameness and swollen leg had higher occurrences in ducks (32 and 27) and turkeys (23 and 15), representing about 48.5% and 34.8 %, respectively. In ostriches (Figure 1), tibia dyschondroplasia does occur as a result of bone deformities leading to death of the affected birds (Ritchie *et al*, 1994). Nutritional imbalances particularly protein and mineral deficiencies have been reported to be the cause of the bone problems in ostriches (Ritchie *et al*, 1994).

**Table 2: Occurrence of leg ailments in turkeys, ducks, ostrich and guinea fowls**

Problems	Turkeys Number (%)	Ducks number (%)	Ostrich Number (%)	G/fowl Number (%)
Swollen legs	15 (25.0)	27 (45.0)	4 (6.7)	14 (23.3)
Incoordination	4 (14.8)	11 (40.7)	6 (22.2)	6 (22.2)
Curled toes	12 (34.3)	14 (40.0)	3 (8.6)	6 (17.1)
Paralysis/lameness	23 (34.9)	32 (48.5)	1 (1.5)	10 (15.2)
Physical injuries	12 (31.6)	9 (23.7)	2 (5.3)	15 (39.5)
Mange /mites	6 (54.6)	5 (45.5)	0 (0.0)	0 (0.0)
<b>Total</b>	<b>72</b>	<b>98</b>	<b>16</b>	<b>51</b>

The absence of mange/mites in ostriches and guinea fowls may be due to the small number that are usually kept and the lack of attention given vis-à-vis external parasites investigation in these species of birds. Ectoparasite infestation such as burrowing mites or scaly leg mites (*Cnemidocoptes mutans*) can cause excessive scaliness of skin of the legs

leading to thickening and deformation of the legs (Merck, 1991).



**Figure 1: Swollen leg disease in ostrich**

Our result further revealed isolation of *Clostridium perfringens* from leg lesions of ostrich (Table 3). Generally, infectious diseases that can affect the legs of birds include infectious synovitis (*Mycoplasma synoviae*, *Staphylococcus aureus*), Newcastle disease, Mareks disease, fungal disease, Fowl cholera (*Pasteurella multocida*), and Clostridial infections (*Clostridium botulinum*). These conditions usually lead to swelling, abscesses, paralysis of affected legs and consequently lameness (Merck, 1991; Jensen and Skeeles, 1998; Anon, 1998; Abdu and Saidu, 2002).

**Table 3: Post mortem lesion and bacterial organisms isolated**

Type of poultry	Post mortem lesions observed	Microorganism isolated
Local chickens	Swollen leg (joint), curled toes, physical injury (e.g. wound, burn)	NA
Broilers	Swollen leg (joint), curled toes, physical injury (e.g. wound, mange)	NA
Layers	As above	NA
Cocks	As above	NA
Turkeys	As above	NA
Ducks	As above	NA
Ostriches	Swollen leg (hock joint), wound	<i>Clostridium perfringens</i>
Guinea fowls	As above	NA

Key: NA = Not Applicable (not tested)

Although, there was no sex restriction as to the occurrence of leg ailments in different species of poultry in this study, it was observed that most of the leg ailments occurred in the adults than young birds. Statistical analysis using Chi-square showed calculated value (47.31 and 35.12) greater than the tabulated

value (27.5). It was therefore concluded that significant difference exist between the four groups of chickens and other poultry species in relation to occurrence of leg ailments ( $P < 0.05$ )

**Conclusion:** Based on our results we conclude that the incidence of leg ailments is high in poultry species. The overall economic implication associated with these problems in poultry can add up to a huge loss of Naira. Strategies for the prevention and control of problems associated with leg ailments in poultry production are therefore necessary.

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