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EFFECT OF FOOD SUPPLEMENTATION ON THE WHITE BLOOD CELLS COUNT AND DIFFERENTIAL LEUCOCYTES COUNT OF TRYPANOSOME-INFECTED PREGNANT RATS

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ABSTRACT

Trypanosomiasis is an important livestock disease in sub-Saharan Africa. Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and it also influences the rate of recovery. Earlier researchers demonstrated that dietary supplement of selenium and vitamin E enhanced immune response in white rats. It has also been reported that during pregnancy, immune response is depressed. Leucocytes count has been recognized as a measure of immune response. This research was therefore conducted using chicks' marsh fortified with 80 mg of vitamin E and 0.3 mg of selenium as control (Diet 1) to determine the effect of dietary supplementation of moderate protein (combination of 250 g of corn meal, 240 g soyabean meal and 10 g of crayfish) in the chicks' mash (Diet 2), high dietary protein (combination of 400 g of caseinogen and 300 g of soyabean meal) (Diet 3), and high dietary carbohydrate (combination of 400 g dextrose and 300 g cornmeal) (Diet 4) in the chicks' mash on the white blood cells count and differential leucocytes count of trypanosome-infected pregnant rats. Dies 1 – 4 were given to rats in Cage A – D, respectively. The rats were infected with trypanosomes within 10th to 14th day of pregnancy. Each experimental set up was replicated three times. On comparing the total white blood cell counts of all the rats fed different diets, there was no significant difference (P > 0.05) between the rats in Cage A (fed Diet 1) and rats in Cage B (fed Diet 2), and similarly between rats in Cage C (fed Diet 3) and rats in Cage D (fed Diet 4). There was, however, significant difference (P < 0.05) between rats in Cage A and rats in Cages C and D, and also between rats in Cage B and those in Cages C and D. Diet 2 with a moderate (20.01%) protein level and a balance of other nutrients produced the highest leucocytes count. Diet 2 therefore produced the highest immune response in the pregnant trypanosome-infected rats.

Keywords: Trypanosomiasis, Nutrition, Leucocytes count, Immune response

INTRODUCTION

Phenotypically, every organism is a product of its genes and environment. The most critical period for this interaction and one which has the most profound implications for life long health and well being occur before birth (Haggarty, 2002). There is evidence that natural variation in genetic make up has a direct effect on an organism's development, and that genotype interacts with nutrient intake and their effect can be modulated by nutritional status (Haggarty, 2002). Trypanosomiasis is one of the most important livestock diseases in sub-Saharan Africa (Morrison *et al.*, 1981). Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and it also influences the rate of recovery (Katungka-Rwakishaya, 1996).

Nutrition and disease are the major factors that affect reproduction and also determine the health condition of pregnant animal and the feotus. Many outcomes of pregnancy are affected by the balance of different nutrients. Nutrient may have an effect on different critical development and it may also be simultaneously beneficial for one outcome and detrimental for another (Haggarty, 2002). Physiological changes in pregnancy calls for extra nutrients and energy to meet demands of an expanding blood supply, the growth and development of maternal tissues before birth and preparation for lactation (Ladipo, 2000). Good maternal nutrition is vital for the health and reproductive performance of women and the health, development and survival of their children (Mora and Nestel, 2000). It has been suggested that a brief period of malnutrition may result in permanent alterations in development of organs that may be translated into pathology in later life (Barker, 1995). Leucocytes count during stress and infectious disease is a measure of immune response (Hardie *et al.*, 1991; Dufva and Allander, 1995). It has been reported that during pregnancy, immune response is depressed (Purtilo *et al.*, 1972) involving reduced leucocytes count. Earlier researchers demonstrated that supplemental vitamin E enhances animal immune response (Haeger, 1974; Tengerdy and Brown, 1977). Also, similar results had been reported on dietary supplement of selenium (Nockel, 1986). Mgbenka and Ufele (2004) showed

that combined supplementary selenium and vitamin E enhances trypanotolerance in rats.

In line with this background, using chicks' marsh fortified with 0.3 mg of selenium and 80 mg of vitamin E as control, this research was conducted to determine the effect of dietary supplementation of moderate dietary protein (combination of 250 g of corn meal, 240 g soyabean meal and 10 g of crayfish) in the fortified chicks' mash, high dietary protein (combination of 400 g of caseinogen and 300 g of soyabean meal) on one hand and high dietary carbohydrate (combination of 400 g dextrose and 300 g cornmeal) in the fortified chicks' mash on the other hand on the white blood cell and differential leucocyte counts of trypanosome-infected pregnant rats.

MATERIALS AND METHODS

Twenty 120-day-old female rats of were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house. The rats were placed five per cage. The cages were labeled A to D, corresponding to four diets given to the different groups of rats. Diet 1 was given to rats in Cage A (Treatment 1). Diet 2 was given to rats in Cage B (Treatment 2). Diet 3 was given to rats in Cage C (Treatment 3) and Diet 4 was given to rats in Cage D (Treatment 4). Each experimental set up was replicated three times. The rats had unlimited supply of clean water.

The rats were fed with diets containing different levels of protein and carbohydrate and constant levels of vitamin E and selenium. The diets were: Diet 1 (control), Diet 2, Diet 3 and Diet 4. The ingredient and proximate composition of the diets are shown in Table 1. Male and female reproducing rats were paired. The female rats were naturally impregnated by male rats. The pregnancy was detected by the presence of pan plug at the feacal pan which was released when pregnancy occurred after natural mating (Cukierski, *et al.*, 1991). The pregnant rats were infected with 8000 trypanosomes per ml of blood within 10^{th} to 14^{th} day of pregnancy.

Since the white blood cells count is a measure of immune response of the rats to the trypanosomes (Dufva, R. and Allander, K., 1995), at the end of the experiment, the total white blood cells count and the differential leucocytes count were taken following the method Mgbenka and Ufele (2004). The data were analysed for significant differences by descriptive statistics and analysis of variance (ANOVA) using SPSS computer package. Multiple comparisons of significant differences were done using the least significant difference (LSD) and the Duncan's Multiple Range Test post hoc tests.

RESULTS

There were significant differences ($P < 0.05$) among all the groups of rats in their total white blood cell count, polymorphonucleated cells and mononucleated cells (Figure 1).

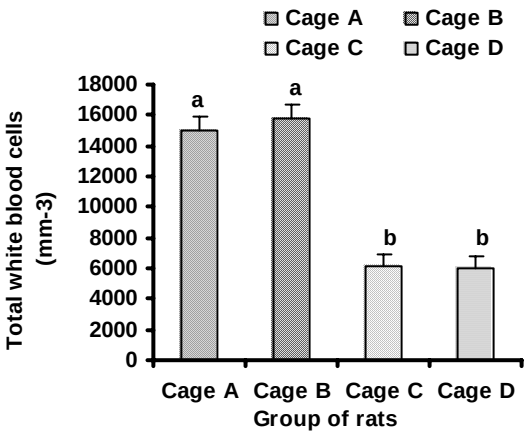

Figure 1: Mean of total white blood cells. Bars with the same letters on top are not significantly different (P > 0.05).

On comparing the total white blood cell counts of all the rats fed different diets, there was no significant difference ($P > 0.05$) between the rats in Cage B (fed with Diet 2) and rats in Cage A (fed with Diet 1), and also between rats in Cage C (fed with diet three) and rats in Cage D (fed with diet four). There was, however, significant difference ($P < 0.05$) between rats in Cage A and rats in Cages C and D and also between rats in Cage B and those in Cages C and D. Comparing the polymorphonucleated cells, there was no significant difference ($P > 0.05$) between the polymorphonucleated cells of rats in Cage A and the rats in Cage B, and also between rats in Cage C and that in Cage D (Figure 2).

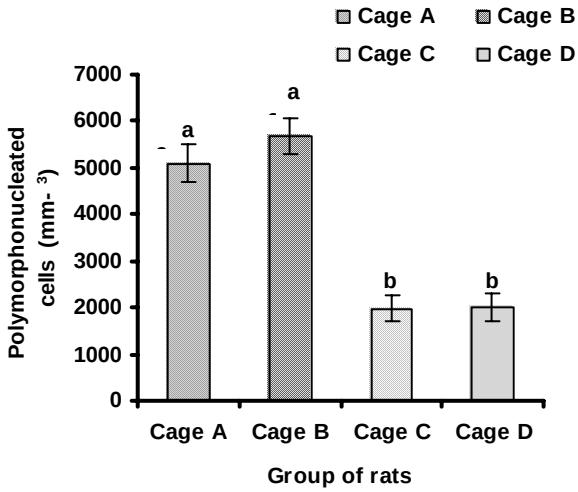

Figure 2: Mean of polymorphnucleated cells. Bars with the same letters on top are not significantly different (P > 0.05)

On the other hand, there was significant difference ($P < 0.05$) between the rats in Cage A and those in Cages C and D, and also between the rats in Cage B and those in cages C and D. Comparing mononucleated cells, there was no significant difference ($P > 0.05$) between the mononucleated cells of rats in Cage A and the rats in Cage B, and between rats in Cage C and that in Cage D (Figure

3). There was, however, significant difference ($P < 0.05$) between rats in Cage A and those in Cages C and D. Also, there were significant differences ($P < 0.05$) between rats in Cage B and those in Cages C and D. Figure 1 showed that rats fed with Diet 2 (Cage B) had the highest total white blood cells (15826 ± 882 mm^3), followed by rats fed with Diet 1 (Cage A) (15014 ± 894 though the values are not significantly different (0.05), while rats fed with Diets 3 and 4 (Cages C and D) were on the same range (6105 ± 750 mm^3 and 6057 ± 762 mm^3) respectively.

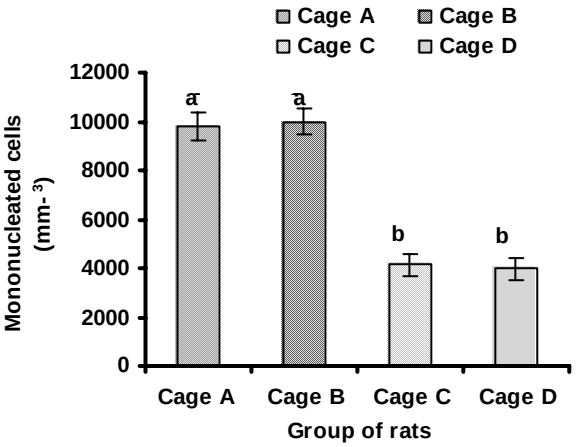

Figure 3: Mean of mononucleated cells. Bars with the same letters on top are not significantly different (P > 0.05).

It was observed that rats fed with Diet 2 (Cage B) had the highest polymorphonucleated cells (5682 ± 397 mm^3), though not significantly different from the value of the rats fed with Diet 1 (5100 ± 386 mm^3), while rats fed with Diets 3 and 4 (Cages C and D) were on the same range (1981 ± 286 mm^3 and 1997 ± 296 mm^3) (Figure 2). It was observed that rats fed with Diet 2 (Cage B) (9994 ± 510 mm^3) had the highest though not significantly different ($P > 0.05$) mononucleated cells to the value of the rats fed with Diet 1 (Cage A) (9813 ± 549 mm^3) (Figure 3). The rats fed with Diets 3 and 4 (Cages C and D) were on the same range (4124 ± 474 mm^3 and 3980 ± 466 mm^3) respectively.

DISCUSSION

From the above results, it was observed that rats fed with Diet 2 had the highest total white blood cell count, polymorphonucleated cells and mononucleated cells, when compared with Diet 1, Diet 3 and Diet 4. Our results of the experiments indicated that adequate nutrition enhanced trypanotolerance. Katungka-Rwakishaya (1996) observed that improvement on host's nutrition was important in modulating the severity of patho-physiological effect of trypanosomiasis and also influences the rate of recovery. It has been reported that during pregnancy, immune response are depressed (Purtilo, *et al.*, 1972). Our results indicated that balanced diet enhances the total white blood cell count, differential leucocytes count and hence immune response of pregnant rats. Furthermore it was observed that good maternal nutrition was vital to health and reproductive performance of pregnant rats and the health, survival and development of their offsprings (Mora and Nestel, 2000).

The high mean values of total white blood cell count, mononucleated cells and polymorphonucleated cells in rats fed with Diet 2, indicate that balanced diet with moderate protein level (20.01%) has positive influence on the immune response of trypanosome-infected rats. This agrees with the suggestion that the degree of trypanotolerance is greatly affected by the nutritional status of the host animal (Murray, 1988; Agymang *et al.*, 1990) and that supplementary diet enhances trypanotolerance in rats (Mgbenka and Ufele, 2004). The nutritional status of animals including rats influences trypanotolerance and reproduction. Since it is well known that the number and proportions of different types of leucocytes reflect the health status of individuals, as these cells quickly respond to stress and infectious diseases, leucocytes count, is a measure of immune response (Hardie *et al.*, 1991; Dufva and Allander, 1995). Vitamin E and selenium, together with dietary protein and carbohydrate, reduce depression of immune system in the pregnant trypanosome-infected rats. Agnew *et al.* (2005) found that *Echinacea* intake induced an immune response through altered expression of leucocyte hsp70, increased white cell counts and improved erythrocyte antioxidant. It is therefore inferred that Diet 2, a balanced diet with intermediate level of protein (20.01 %) produced the highest (though not significantly different from Diet 1) leucocytes counts, the best immune response to trypanosomes in the pregnant trypanosome-infected rats**.**

REFERENCES

AGNEW, L. L., GUFFOGG, S. P., MATTHIAS, A., LEHMANN, R. P., BONE. K. M. and WATSON, K. (2005). *Echinacea* intake induces an immune response through altered expression of leucocyte hsp70, increased white cell counts and improved erythrocyte antioxidant defences. *Journal of Clinical Pharmacy and Therapeutics*, 30 (4): 363 – 369.

AGYEMANG, K., DWINGER, R. H., TOURAY, B. N., JEANNIN, P., FOFANA, D. and GRIEVE, A. S. (1990). Effects of Nutrition on degree of aneamia and live weight changes in N'Dama cattle infected with trypanosomes. *Livestock Production Science,* 26: 39 – 51.

BARKER, D. J. P. (1995). Fetal origins of coronary heart disease. *British Medical Journal* 311: 171- 174.

CUKIERSKI, M. A., SINA, J. L., PRAHALADA, S. and ROBERTSON, R. T. (1991). Effects of seminal vesicle and coagulating gland ablation on fertility in rats. *Reproduction and Toxicology* 5: 347 - 352.

DUFVA, R. and ALLANDER, K. (1995). Intraspecific Variation in plumage coloration reflects immune response in great tit (*Parus major*). *Functional Ecology*, *9 (5)*: 785 – 789.

HARDIE, L. J., FLETCHER, T. C. and SECOMBES, C. J. (1991). The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture, 95(3-4):* 201 - 214.

HAEGER, K. (1974). Long-term treatment of intermittent caudication with vitamin E. *American Journal of Clinical Nutrition*, *27:* 1179 - 1181.

HAGGARTY, P. (2002). Gene - nutrient interactions in pregnancy. *Expression of Interest*, 1: 1 - 6.

KATUNGKA-RWAKISHAYA, E. (1996). Interaction between animal nutrition and parasites, studies with experimental trypanosomiasis in sheep. Pages 1 – 9. *In:* LEBBIE, S. H. B. and KAGWINI, E. (Ed.). *Small Ruminant Research and Development in Africa*. ILRI Nairobi Kenya.

LADIPO, O. (2000). Nutrition in pregnancy: mineral and vitamin supplement. *American Journal of Clinical Nutrition*, 72: 280 - 290.

MGBENKA, B. O. and UFELE, A. N. (2004). Effect of dietary supplementation of vitamin E and selenium on blood parameters of trypanosome-infected rats (*Rattus rattus*). *Bio-Research 2 (1)*: 8 - 17.

MORA, J. and NESTEL, P. S. (2000). Improving prenatal nutrition in developing countries: Strategies, prospects and challenges. *American Journal of Clinical Nutrition*, *71:* 1353 - 1363.

MORRISON, W. I., MURRAY, M. and McINTYRE, W. I. M. (1981). Bovine trypanosomiasis. Pages 469 – 497 (ed.). *In; Disease of cattle in Tropics.* Martinus Nijhoff Publishers, The Hague.

NOCKEL, C. F. (1986). Nutrient modulation of the immune system. Pages 177 – 192. *In:* RISSTIC, M., MCINTYRE, W. I. M. HARESIGN, W. AND COLE, D. J. A. (Eds.). *Recent Advances in Animal Nutrition*, Butterworth, London.

PURTILO, D., HALLGREN, H. M. and YUNIS, E. J. (1972). Depressed maternal lymphocytes response to PHA in human pregnancy. *Lancet*, *1*: 767 - 771.

TENDERDY, R. P. and BROWN, J. C. (1977). Effects of vitamin E and vitamin A on humoral immunity and phagocytosis in *E. coli* infected chicken. *Poultry Science*, *56:* 957 – 963.

TEIGER, J., TOLLERSRUDS, L. A. and LARSEU, J. (1982). Swine dysentery, the influence of Dietary vitamin E and selenium on the clinical and pathological effects of *Treponema hydysenteriae* infection in pigs. *Research in Veterinary Science*, *35:* 95 - 100.

TENDERDY, R. P. and BROWN, J. C. (1977). Effects of vitamin E and vitamin A on humoral immunity and phagocytosis in *E. coli* infected chicken. *Poultry Science*, *56:* 957 – 963.

TENGERDY, R. N., HEINZERLING, R. H. and NOCKELS, C. F. (1972). Supplemental Vitamin E in Disease condition. *Infection and Immunity*, *5:* 987 - 989.

COLEOPTERAN FAUNA OF AGROECOSYSTEMS IN AWKA, NIGERIA

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ABSTRACT

A study was carried out to investigate the coleopteran fauna of two agroecosystems (cultivated farmland and fallow plot at the Permanent Site of Nnamdi Azikiwe University, Awka) for a twelve-month period using the pitfall technique. Eight pitfall traps made up of plastic containers with mouth diameters of 9.80 cm and 6.20 cm deep were set monthly at random in the two sampling sites. The traps, which were filled to one-third with 5 % formalin, serving a preservative, were inspected every twenty-four hours and the insects caught sorted and counted under a dissecting microscope. Species of Coleoptera obtained from the cultivated plot were Macrocheilus labrosus, Hyparpalus sp., Carpophilus fumatus, Podagrica uniforma, Tetragonothorax sp., Chlaenius sp., Pheropsophus parallus, Silidas apicalis, Tenebroides mauritanicus, Heteroderes sp., and Heterorynchus licas while only Hyparpalus sp., and Mylabris sp., were obtained from the fallow plot. The result of Fisher's Least Significance Difference (F-LSD) test shows that the pitfall catches of Coleopterans from the two sampling sites were significantly different at p-value of 0.0053 and mean difference of 2.500. The heterogeneity of the coleopteran species at the cultivated plot was traced to cultivation. The role of certain coleopteran families as faunal indicators was highlighted. Other factors, which influenced the Coleopteran species at the farmlands were also discussed.

Keywords: Coleoptera, Fauna, Agroecosystems, Pitfall traps

INTRODUCTION

The insects are strategic in the welfare of man through their activities. The coleopterans (beetles), which constitute about two-thirds of all known insects and about one-third of all known animal species invariably, participate in various activities, resulting in several changes in the ecosystems. The coleopterans like other insects, often evolve and exists as components of communities of plants and other animals. Most of the species are terrestrial even though some are aquatic. In terms of food and feeding habits, many coleopterans are plant eaters; some are predacious with others being scavengers, while some of them are wood-borers. In terrestrial ecosystems many of these herbivorous forms constitute serious pests of crops and causing significant damage either directly or even transmitting diseases, even though some are known to be beneficial herbivores.

With destruction of natural habitats by man and in particular destruction of vast areas of forests for industrial, agricultural and urbanization purposes (Boorman, 1981), these coleopterans therefore constitute an interesting group to study. The study of the coleopteran species in a cultivated and a fallow farmland will no doubt provide useful information on their distribution and abundance.

MATERIALS AND METHODS

Site Description: The investigation was carried out in two rather contrasting study sites a cultivated farmland and a secondary regrowth forest, all of which are located at the Permanent Site of the Nnamdi Azikiwe University, Awka. Awka is the capital of Anambra State of Nigeria and located in the lowland rain forest zone of Southern Nigeria (Keay, 1965; Charter, 1970).

The cultivated plot, which measures 800 cm^2 in area is located between latitude 6.23782^O N and longitude 7.12884^O E. At the time of investigation and apart from the cassava, *Manihot esculenta* Kranz, planted in mounds, the plot had a variety of weeds which *Sida acuta* Burm, *Aspilia africana* (CD), *Euphobia hirta (*L.), *Chromolaena odorata* (L.), *Emilia sonchifolia* (L.), *Tridax procumbens* (L.), *Mariscus alternifolus* Vahl., *Commelina benghalensis* (L.), and *Axonopus compressus* (S.W.) Also present was a shrub *Phyllanthus amarus* Schum and Thom. On the other hand, the fallow farmland lies between latitude 6.25054^ON and longitude 7.12078^OE. The plot has been left fallow for twelve years after the previous cultivation and therefore was overgrown with plants associated with fallows. Identified herbaceous plants included *Chromolaena odorata* (L.), *Aspilia africana* (C.D), T*ridax procumbens* (L.), *Axonopus compressus* (Sw.) Beauv., *Mariscus longibracteatus* Cherm., *Sida acuta* Burm. f., *Panicum maximum* Jacq. and *Veronia ambigua* Kotchsky and Peyr. Trees found at the plot included *Pentaclethra macrophyla* (Bentham), *Chlorophora excelsa* (Welw.) Benth., *Mangifera indica* L., *Combretum molle* R. Br., *Eleais guineensis* Jacq., *Newbouldia laevis* (P. Beauv.), *Terminalia ivorensis* A. Chev. and., *Anthonata macrophylla* (P. Beauv.). The fallow farmland which is sandy loam and over 1000

m^2 in area is separated from the cultivated farmland by a tarred road leading from the first gate of the Permanent Site of the Nnamdi Azikiwe University, Awka.

Sampling Method: Eight pitfall traps made of plastic containers, with mouth diameters of 9.80 cm and 6.2 cm deep were set in the two study sites, on each sampling occasion (i.e. every month). The traps were filled to one-third with 5 % formalin. The traps were inspected every twenty-four hours, and the insects caught were sorted identified and counted under a dissecting microscope.

Rainfall data was collected during the sampling period using the rain-gauge while bulb thermometer was used to measure aerial and soil temperature on each sampling occasion. The readings of those temperatures were taken twice in each case both at the time of setting the traps and during their collection.

The insects and their larvae were identified using Insects of Nigeria - Check List and Bibliography by Medler (1980). The identification of the specimens was verified in the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The voucher specimens were also kept as reference point for further studies. The data was analysed using Fisher's Least Significant Difference (F-LSD) test, to ascertain whether or not statistical difference existed between the pitfall catches of coleopteran species, obtained from the fallow plot and the cultivated farmland.

RESULTS

A total of 46 beetles were trapped using the pitfall technique during the twelve-month sampling of both agroecosystems. The cultivated farmland had 17 coleopterans, which included *Macrocheilus labrosus, Pheropsophus parallus, Chlaenius* sp., and *Hypapalus* sp., which belong to the carabid family. Single species collected from the cultivated farmland include *Carpoplilus fumatus* (Nitulidae), *Tetragonothorax* sp. (Curculionidae), *Silidius appicalis* (Cantharidae), *Heteroderes* sp. (Elatridae), *Heterorynchus licas* (Scarabacidae) while a single species of *Hyparpalus* was collected from the fallow plot. Other seven species of Coleoptera were collected from the cultivated plot while other nine species were collected from the fallow plot. The result of Fischer's Least Significance Difference (F-LSD) also showed the pitfall catches of the coleopterans from the cultivated plot and the fallow farmland were significantly different ($p<0.05$), with more catches obtained from the cultivated plot.

DISCUSSION

Homogeneity in the distribution of the beetle species between the fallowed and cultivated sites is related to the efficiency and capture rate of the wandering species. Out of eight families of Coleoptera trapped, Carabidae, Nitudilidae, Curculionidae, Cantharidae, Ostomatidae, Elatridae, Scarabacidae and Staphylinidae were more abundant in the cultivated plot than in the fallow plot (Table 1).

Table 1: Pitfall catches of coleopterans obtained from the fallow plot and cultivated farmland at Awka, Nigeria

Beetle family	Genus and Species	Beetle Populations Sampling Sites *	
		A	B
Carabidae	*Macrocheilus labrosus*	1	-
	Pheropsophus parallus	1	-
	Chlaenius sp.	2	-
	Hyparpalus sp.	11	1
Nitudilidae	*Carpophilus fumatus*	1	-
Curculionidae	*Tetragonothorax* sp.	1	-
Cantharidae	*Silidius apicalis*	1	-
Ostomatidae	*Tenebroides mauritanicus*	2	-
Elatridae	*Heteroderes* sp.	1	-
Scarabacidae	*Heterorynchus licas*	1	-
Staphylinidae	*Mylabris* sp.	-	7
Unidentified Coleoptera		7	9
Mean Difference (Sites A and B)		**2.500**	
Critical Differences		**1.719**	
Probability (P) Value		**0.0053+**	

** Sampling sites: A – cultivated plot; B = Fallow plot; + P – value significant at 5% probability level*

In an earlier study, Ewuim (2004) associated members of Carabidae family with cultivation and the complex relationship between wandering beetle, abundance and the frequency of vegetation cover (weed) have been established (Spreight and Lawton, 1976, Ewuim 2004). The higher number of the beetles especially at the cultivated plot may be associated with the nature of the vegetation.

In earlier studies the relative abundance of the ground beetles was associated with nature of vegetation (Greenslade, 1964; Ewuim, 2004), while the curculionids have been associated with flower visiting and pollination (Gakai *et al*., 1998; Ewuim, 2004). Weevils are plant eaters and thus are serious agricultural pests. The lower catches of beetles at the fallow plot might also be associated with dense vegetation associated with the fallow plot which might have markedly impeded the locomotor activity of the beetles and thus their poor trapping. These observations are similar to those of Spreight and Lawton (1976), who observed that strip of vegetation offered resistance to movement of ground beetles.

It has been observed that adult beetles are herbivorous during their surface life and constitute the most influential grazers (Hinds and Rickard, 1973) hence their increased number in the cultivated farmland than the fallow plot. This also explains the trend in the result of the F-LSD carried out in which there was significant difference in the trapped coleopterans with more trapped in the cultivated plot when compared with the fallow plot (Ewuim, 2004). The alteration of vegetation structure in the non-forested plots studied therefore possibly influenced the spatial and temporal (spatiotemporal) variations in these species studied since in general, temporal dynamics of insect populations invariably take place within a spatial context. In the long run evidence

abound from this study that the least stable and perhaps the least efficient community is the highly diverse one as observed for the cultivated plot.

In the final analysis, the significant difference observation in the trapping of the coleopteran species with a higher population density for the cultivated plot is also a strong indication that the beetle families were particularly sensitive indicator taxa of land use (Rivers-Moore and Samsway, 1996; Ewuim, 2004) as confirmed by the increased density of the coleopteran species in the cultivated agro-ecosystem.

ACKNOWLEDGEMENTS

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REFERENCES

BOORMAN, J. (1981). *West African Insects.* Longman Group Ltd. Essex. 88 pp.

CHARTER, J. R. (1970). *Vegetation Ecological Zones.* Federal Department of Forest Research, Ibadan. Nigeria.

EWUIM, S. C. (2004). *A study of insect fauna of the Permanent Site of Nnamdi Azikiwe University, Awka.* Ph.D. Thesis, Nnamdi Azikiwe University, Awka. 269 pp.

GREENSLADE, P. J. M. (1964). Pitfall trapping as a method for studying populations of Carabidae (Coleoptera) *Journal of Animal Ecology, 33*: 301 - 310.

HINDS, W. T. and RICHARD, W. A. (1973). Correlation between climatological fluctuations and a population of *Philolithus densicollis* (Horn). (Coleoptera: Tenebrionidae) *Journal of Animal Ecology,* 42: 341 – 351.

KEAY, R. W. J. (1965). *An outline of Nigerian vegetation.* Federal Ministry of Information, Lagos, Nigeria. 46 pp.

LASEBIKAN, B. A. (1974). Preliminary communication on microarthropods from a tropical rainforest in Nigeria. *Pedobiologia.* 14:402-411.

RIVERS-MOORE, W. A. and SAMSWAY, M. J. (1996). Game and Cattle trapping and impacts on human dwelling on arthropods at a game park boundary. *Biodiversity a*nd Conservation, 5(12): 1545 - 1556.

SAKAI, KUNIYASU, M; TAKAKAZU, Y; MAKOTO, K and TAMIJI, I. (1998). Pollination of *Shorea parvifolia* (Section Mutika, Dipteraocarpae) in general flowering period in Sarawak, Malaysia. Abstracts from forest canopies 1998. Global Perspectives, Sarasota, USA.

SPREIGHT, M. R. and LAWTON, J. H. (1976). The efficiency of weed-cover on the mortality imposed on artificial prey by predatory ground beetles in cereal fields. *Oecologia* (Berl.), 23: 211 – 223.

PROPERTIES OF LIPASE (EC 3.1.1.3) FROM DIFFERENT VARIETIES OF MAIZE

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ABSTRACT

Lipase activity was studied in four varieties of Corn (Zea mays) namely: Local yellow (LY), Western yellow (WY), Western white (WW) and Pop corn (POP). Using emulsified olive oil as substrate, lipase was found to be present in the dry seeds of maize. Lipase activity increased with germination and reached it's peak on the first day of germination for WY and LY, third day for POP and sixth day for WW. Lipase activity was tested for its ability to hydrolyze different emulsified oils such as groundnut oil, palm kernel oil, and soybean oil. The highest activity was with soybean oil. This high activity was correlated with high specificity of corn lipase on linoleic acid. Thermal inactivation studies showed that the enzyme was stable up to 50^{o}C and showed rapid inactivation above this temperature. Its optimum temperature was 50^{o}C and the optimum pH, 8.0.

Keywords: Lipase, Enzymes, Maize, Thermal stability, Substrate specificities

INTRODUCTION

Lipase (EC 3.1.1.3) catalyses the hydrolysis of triacylglycerides to a mixture of diacylglycerides and monoacylgycerides. During seed germination, lipases are active in lipid storage organelles, called lipid bodies (oliosomes, oil bodies, spherosomes or oil droplets), where the storage triacylglycerides are localized (Lin *et al.,* 1982). The lipid body should be a prime candidate for subcellular location of lipase, since for lipolysis to occur, enzyme has to come in contact with the substrate (Huang *et al.,* 1983). A unique feature of seed lipases is that they are relatively specific for the characteristic triacylglycerols of a species. Thus corn lipase is most active on triacylglycerols of linoleic and oleic acid, which are the major fatty acid constituents of corn (Lin et al., 1986).

In this work, effort has been made to study lipase in different varieties of maize with a view to understanding their properties.

MATERIALS AND METHODS

Maize: Maize (*Zea mays*); POP, WY, LY and WW were purchased from the local market at Nsukka, Enugu state Nigeria. A grinding buffer was prepared from 0.5 M glycine, 0.6M sucrose, 10 mM KCl, I mM EDTA, I mM β-mercaptoethanol and I mM $MgCl_2$ and the pH of the buffer adjusted to pH 7.6 with NaOH. Different varieties of maize seeds were steeped in water for 24h after which they were spread on moist jute bags and allowed to germinate in the dark. Lipase assay was carried out every 24 h. At the end of the 24h of germination, the plumules and radicals of the germinating seeds were removed and 50 seeds (from each variety) were selected and crushed in the grinding buffer using pestle and mortar. After extraction, the homogenate was filtered using cheese cloth (8 folds). The filtrate was introduced in a centrifuge tube and a second version of the buffer containing 0.5M sucrose instead of 0.6M was layered on top and centrifuged at 10,000 x g for 15 min. The upper layer (lipid bodies) was removed and mixed with the water soluble fraction and kept as the crude enzyme on the basis of a modification of the purification method according to Huang and Lin (1983).

Enzyme Assay: The method of assay was a modification of Williamson *et al.* (1999). Activity with triacylglycerols was measured by the Olive oil emulsion method. The reaction mixture consisted of olive oil emulsion [50 % in 5 % Gum-Arabic solution], 27mM Tris–HCl pH 8.0; 0.013% sodium azide, and enzyme in a total volume of 3.75 ml. After vortex mixing, the mixture was incubated at room temperature for 1h. The reaction was stopped by adding 3.25 ml of 30 %ethanol. Controls were produced using the same reaction mixture but adding ethanol at the beginning of the 1 hour incubation. Thymophthalein (3 drops) was added to the mixture and the free fatty acids released by lipase action were titrated with NaOH (0.05M) until the colour of the solution turned a slight but definite blue. One unit of enzyme activity is defined as the release of 1 µmol of product per minute under the conditions of each assay.

Protein Determination: Protein was determined using the method of Lowry *et al.* (1951).

Lipids Hydrolysis: Emulsion of olive oil, groundnut oil, vegetable oil, palm kernel oil and soybean oil were used for the assay. Oil emulsion was prepared by a mixture of 0.5 ml of oil and 0.5 ml of 5 % Gum Arabic solution. This was vortexed at high speed for 30 seconds.

Thermal Inactivation: 10 ml of the crude enzyme were each added in a conical flask and heated in a

water bath at different temperatures (40, 50, 60, 70 and 80^{o}C) for the time interval between 0 – 8 h.
0.75 ml of the enzyme was withdrawn after every 60 sec and used to assay for residual activity. The method of assay was as earlier stated, except that the period of incubation was 15 min. Each activity was an average of three determinations (in triplicates).

pH Optimum and Stability: The method was a modification of Yamamoto and Fijiwara (1988). Enzyme assay was carried out using the following buffer systems: 0.025M Glycine-NaOH buffer (pH 5.5-9.0); 0.025 M potassium phosphate buffer (pH 5.5-9.0); 0.025 M Tris-HCl buffer (pH 6.5-9.0) and 0.025 M acetate buffer (pH 4.0-6.0) respectively. For stability test, the enzyme was incubated in different buffers (as above) at 29^{o}C for 24h. The residual enzyme activity was determined as earlier stated.

RESULTS AND DISCUSSION

Lipase activity was detected in the dry seeds of maize as has already been reported by Huang and Moreau (1978). However, maximum activity was observed on the first day of germination for WY and LY, on the 3rd for pop corn and on the 6th day for WW. Activity was least in pop corn, and highest in western white (WW) (Figure 1).

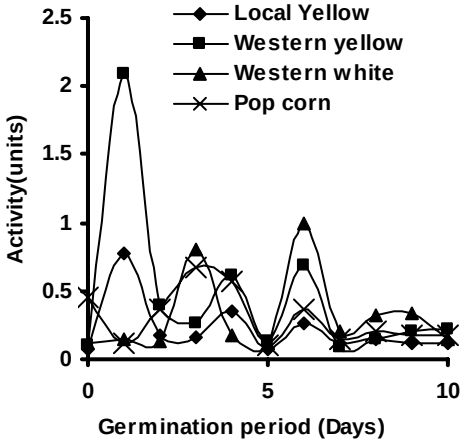

Figure 1: Variation of lipase activity with the period of germination for four varieties of maize.

In early seedling growth of oil seeds, the reserve triacylglycerides in the storage tissues are rapidly mobilized. The triacylglycerides are hydrolyzed to fatty acids are metabolized by β-oxidation. The acetate generated is processed in the glyoxylate cycle (Muto and Bevers, 1974; Huang and Mareau 1978; Lin *et al.,* 1982: Lin *et. al.,* 1983). Generally, lipolysis is most active in day 2 – 6 of germination but gradually drops as the storage triacylglycerides were depleted. Earlier reports on maize lipase indicate that there was no activity in the dry ungerminated seeds and that the peak of activities is between 5 – 6 days of germinations (Lin *et al.,* 1986). Lipase from a specific plant species displays highest specific activity with the major endogenous seed storage triacylglycerides. Thus castor bean lipase is most active on triricinolein, but is also fairly active on all other triacylglycerides containing saturated and unsaturated fatty acids. Corn lipase has highest activity on all other triacylglycerides of linoleic acid and oleic acid, rape seed lipase on trierucin and elm lipase on tricaprin (Lin *et al.*, 1986; Hope and Theimer, 1996). Such specificity is of physiological significance since each plant species produces very specific lipases for much more efficient catalysis as the fatty acid composition in each species is genetically determined and well defined. In this work, corn lipase showed highest activity with soybean oil (Figure 2) as the substrate. Soybean oil contains a high proportion of linoleic acid (50 – 55 %) and oleic acid (19 – 25 %).

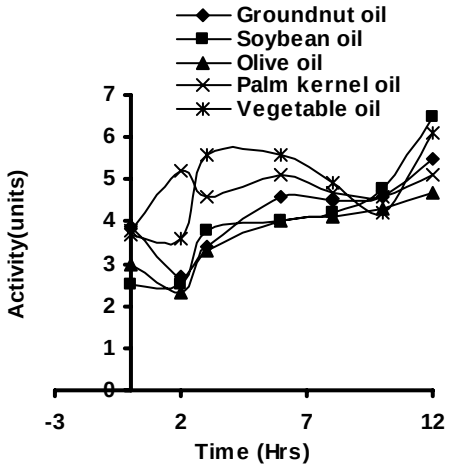

Figure 2: Lipase activity in the different substrates

Also groundnut oil with oleic acid content of 25 – 45 % is hydrolyzed by corn lipase. This is explained on the basis of specificity of the enzyme. There is only a few published reports on thermal inactivation of plant lipases. Hou *et al.* (1999), reported that fatty acid esterase from yam is stable below 50^{0}C. Lipase from micro-organism, *Pseudomonas* spp were shown to be thermostable, retaining sufficient activity when incubated at 50^{0}C for 24h. Inactivation studies of corn lipase showed that the enzyme is highly stable with a monophasic inactivation curves at various temperatures studied (Figure 3). This stability depends on the pH of the medium and the purity of the enzyme. For these organisms, the optimum temperature was 65^{o}C (Nisho *et al.,* 1987). The optimum temperature for maize lipase was 65^{o}C (Figure 4). The pH optimum of the enzyme was 8.0 and the enzyme was stable in the alkaline region of 8 – 9.5 suggesting that the enzyme is an alkaline enzyme.

In conclusion, during germination, the enzyme activities change as the day of germination progresses due to the mobilization of triacylglycerides from the storage tissue.
There is a linear correlation in change in activity with days of germination until a peak is reached which

eventually marks the depletion of triacylglycerides in the storage tissues. The fact that lipase activity in the scutellum appears due to *de novo* synthesis of lipase in post-germinative growth of maize kernel supports this linear proposition.

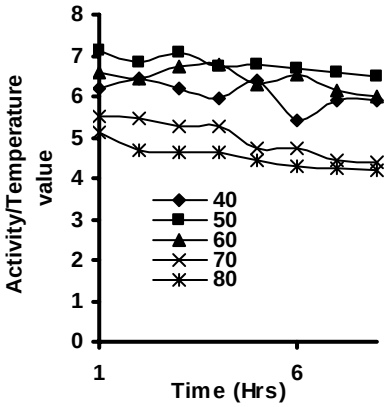

Figure 3: Heat inactivation of Lipase at different temperatures of 40^0 C, 50 ^{0} C 60^{0}C, 70^{0}C and 80^{0}C

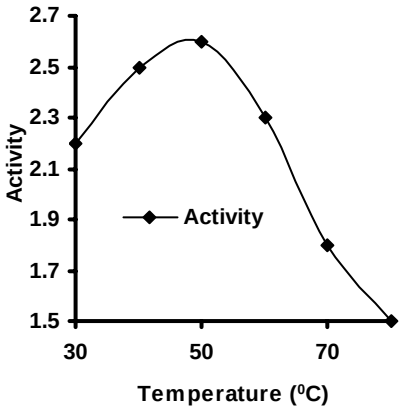

Figure 4: Temperature optimum for lipase activity

Maize lipase has the highest activity in Western white (WW); and show high specificity when oils of high percentage linoleic acid were used.

REFERENCES

HOPE, A and THEIMER R. R. (1997). Enzymes for lipolysis and fatty acid metabolism in different organelle fractions from rape seed cotyledons. *Planta, 202:* 227 – 234.

HOU, W. C., CHEN, H. J., CHANG, C. F. and LIN, Y. H. (1999). Purification and properties of fatty acid esterases from yam (*Dioscorea batatas* Decne). *Botanical Bulletin, 40:* 305 – 310.

HUANG, A. H. C. and MOREAU, R. A. (1978). Lipases in the storage tissues of peanut and other oil seeds during germination. Planta, 141: 111 – 116.

HUGNG, A. H. C. and LIN, Y. H. (1983). Lipase in lipid bodies of cotyledons of rape and mustard seedling. *Archives of Biochemistry and Biophysics, 225:* 360 – 369.

LIN, T.H., MOREAU R, A. and HUANG A. H. C. (1982). Involvement of glyoxysornal lipase in the hydrolysis of storage triacylglycerols in the cotyledons of soybean seedlings. *Plant Physiology, 70:* 108 – 112.

LIN, Y. H., WIMER, L. T. and HUANG, A. H. C. (1983). Lipase in the lipid bodies of corn scutella during seedling growth. *Plant Physiology, 73:* 460 – 463.

LIN, Y. H., YU. C. and HUANG, A. H. C. (1986). Substrate specificities of lipases from corn and other seeds *Archives of Biochemistry and Biophysics, 244:* 346 – 356.

LOWRY O. H; REOSENBROUGH N. R., FARR A. L. and RANDALL R. J. (1951). Protein measurement with the folinphenol reagent. *Journal of Biological Chemistry,* 193: 265 – 275.

MUTO, S. and BEAVERS, H. (1974). Lipase activities in castor bean endosperm during germination. *Plant Physiology, 54:* 23 – 28.

NISHIO, T., CHIKANO, T. and KAMIMURA, M. (1987). Purification and some properties of lipase produced by *Pseudomonas fragi* 22. 39B. *Agricultural and Biological Chemistry, 51(1):* 181 – 186.

YAMAMOTO, K. and FUJIWARA, N. (1988). Purifications and some properties of a castor-oil-hydrolyzing lipase from *Pseudomonas sp*. *Agricultural and Biological Chemistry,* 52(12): 3015 – 3021.

A COPROLOGICAL SURVEY OF GEOHELMINTH INFECTIONS AMONG SCHOOL CHILDREN IN RURAL EBONYI STATE, NIGERIA

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ABSTRACT

A cross-sectional coprological survey involving 420 primary school pupils of both sexes aged 6-14 years, was conducted in 8 primary schools at different locations in Ishielu Local Government Area (L.G.A.) of Ebonyi State, Nigeria, to determine the prevalence and intensity of infection of the geohelminth (soil-transmitted helminth) parasites. The dominant geohelminth parasites identified were Ascaris lumbricoides (32.9%), Trichuris trichiura (20.5%), and hookworms (3.8%). Altogether 240 respondents composed of 124 (29.4%) males and 116 (27.6%) females were infected with one or more of the parasites thus establishing an overall prevalence of 57.1%. In 7/8 (87.5%) of the study primary schools, over 50% of the children harboured the ova of the parasites. The distribution of the infections were not gender-dependent, and the between-sex prevalence was not statistically significant (p > 0.05). The prevalence of the infections appeared to be generally age-dependent but not over- dispersed as there was a marked association between age of the pupil and the infectious process. Concomitant infection involving 13.3% of all the infected pupils was recorded. Generally, the intensity of the geohelminthiasis was of the light category for A. lumbricoides (≤ 4999 eggs per gram faecal matter(epg); T trichiura (≤ 999 epg); and hookworm (≤ 999 epg). Moderate infections (1000 – 9999 epg) of trichiuriasis was recorded in all the study schools. A deworming programme of the infected schools and the at–risk population is recommended.

Keywords: Coprological survey, Geohelminth, Prevalence, Intensity of infection, Concomitant infection

INTRODUCTION

Despite the availability of suitable anthelmintic drugs, the human intestinal helminths continue to represent an undesirable global scourge in the developing countries of Africa, Asia and Latin America where their prevalences continue to increase. Humans are known to serve as hosts for over 300 species of helminths about 200 species of which have been reported from the alimentary tract and its associated ducts and organs. By for the most abundant and widely distributed species of human helminths are the nematodes (*Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus),* and whipworm (*Trichuris trichiura).* The two species of the hookworm together with *A. lumbricoides and T. trichiura* make up the quartet of major soil-transmitted helminths or geohelminths. These four nematode species undergo monoxenous (direct) life cycle pattern and transmission to human depends on faecal contamination of the soil and environment. While *A. duodenale* and *N. americanus* are transmitted via their soil-inhabiting filariform larvae that actively penetrate the skin of their hosts, *T. trichuris* and *A. lumbricoides* are transmitted to man directly through the accidental ingestion of their ova.

Recent estimates suggest that more than one billion people are infected globally with human geohelminth-induced disease while the at-risk population is over 2 billion people, (Montressor *et al.,* 1998; Brooker *et al.,* 1999). Morbidity due to geohelminthiasis is usually pronounced in children and pregnant women because it influences the nutritional status causing growth retardation in children and decreases work capacity and fitness in women (Crompton and Savioli, 1999). It further reduces cognitive development and increases absenteeism from school (Adams, 1994; Nokes *et al.,* 1992; Chan, 1997). The most serious consequences of geohelminth infections stem from chronic infection during the vulnerable years of childhood. It has been estimated that *N. americanus* is responsible for a mean (± SD) blood loss of 0.03 ± 0.012 ml per day. Similarly *A. duodenale* has been estimated to cause a mean blood loss of about 0.08 ± 0.02 ml per day (Awogun *et al.,* 1995; WHO 1994). These measurements of feeding activity explain the pathogenicity of hookworm and account for their contribution to the development of iron-deficiency (hypochromic) anaemia.

Over the past four decades various studies have been conducted in different parts of Nigeria to elucidate principally, the epidemiological profiles of intestinal and other human infections. Okpala (1961), Okpala and Njoku-Obi (1978) carried out investigations on the prevalence of intestinal helminths in Lagos and Nsukka areas respectively while Onubogu (1978) and Udonsi (1984, 1992) conducted epidemiological investigations in parts of old Anambra, Imo and Rivers States on features of intestinal nematode infections.

There is a dearth of published data on geohelminth epidemiological surveys in parts of Ebonyi State since the state was excised out of the former Enugu and Abia States in 1996.

Understanding the environmental limits of infection can aid the targeting of national control strategies. The gathering of information on the prevalence of infection and morbidity is an essential prerequisite for control implementation. For effective control strategies parasitological surveys have to be area-specific since nation-wide prevalence statistics do not say much (Montressor *et al.*, 1998).

This preliminary survey is aimed specifically at establishing the status of, and providing the baseline information on the prevalence and intensity of the soil-transmitted infections among school children within the study localities. The data derived from the study could be incorporated into the Primary Health Care (PHC) delivery programme currently receiving attention by the three tiers of government.

MATERIALS AND METHODS

The Study Area: Ishielu Local Government Area (LGA) where the study was conducted is one of the thirteen local government areas of Ebonyi state and is located within longitudes 7° 45' E to 8° 00' East, and latitudes 6° 15' N to 6° 39' N. It lies about 37 km west of Abakaliki, the state capital. The LGA is bounded by Benue State to the north, Onicha LGA to the south, Enugu State to the west while to the east lie Ohaukwu and Ezza-North Local Government Areas. Ishielu LGA is composed of 17 autonomous communities, namely Obogu, Egedege, Emazu, Umuhuali, Nkalagu, Edema, Ezillo, Abonyi, Isinkpuma, Ntezi, Okpoto, Lobo, Ogboji, Agba, Egbuho, Ohafia Agba and Ezzagu. The estimated population of the LGA is 153,846 based on the 1991 Nigerian national population census figures and a WHO annual population growth rate of 2½ %. The LGA is also the location of a cement factory at Nkalagu and a state-owned agricultural farm at Ezillo. In terms of soil and climate the area is ecologically homogeneous and the vegetation is of the typical guinea savanna mosaic type with gently undulating rocky lowland composed mostly of limestone (Ofomata, 1978).

The water supply system of the LGA is generally poor. The Ebonyi river with its major tributary, the Ora river system meanders in a north-south direction and roughly bisects the local government. In many communities the United Nations International Children's Emergency Fund(UNICEF)-assisted bore-hole hand pumps and the Japanese International Cooperation Agency (JICA)-built water mini reservoirs have broken down, compelling the affected villagers to resort to the seasonal streams, stagnant pools and ponds created by road construction and quarrying activities for their water needs.

Farming is the major occupation of the inhabitants of the study area. Farm produce include cassava, yam, rice, groundnut, mangoes, oranges, tomatoes and vegetables, most of which are grown in farms around the homesteads. Compost and human wastes are routinely used as farm manure to compensate partly for inadequate availability of commercial fertilizers and other farm inputs.

To a large extent, health care facilities are provided by health clinics, patent medicine dealers, drug peddlers and traditional herbal homes. Toilet facilities are generally discharged indiscriminately in lands surrounding the human habitation where vegetables and other crops are grown. This practice creates favourable environment for the development and transmission of the soil-transmitted helminth parasites.

Study Design and Population: Before the commencement of the survey, the local government education secretary was consulted on the purpose of the study and his permission and support were obtained. Prior to the enrolment of the pupils into the study, the head-teachers, teachers and parents/guardians of the pupils were briefed comprehensively on the benefits of the study to the population and the relevance of the diseases investigated. This was done to dispel any misconception and ensure community cooperation.

The target population of the survey was primary school children because the peak prevalence of geohelminth (except hook worms) is usually found in this age-group (Bundy *et al.*, 1987). Secondly, primary schools are accessible and there is generally good compliance from pupils and parents. Thirdly, data derived from primary school children can be used to assess not only whether geohelminthiasis threatens the health of school-age children, but also as a reference for evaluating the need for community intervention.

As the study area is ecologically and environmentally homogeneous, and in order to achieve the objective of the study, a sample size of 420 pupils was used drawn from 8 schools. The study was conducted in the months of May and June – the period of high humidity and temperature.

Selection of Schools and Data Collection: A list of primary schools and the number of pupils enrolled in each was obtained from the local government education authority at Ezillo – the Local Government headquarters. The eight (8) schools were selected using the simplified method for cluster sample utilizing sampling with probability proportional to size, to ensure coverage of the entire local government area, and which allows every school listed the chance of being selected. Schools were eligible for selection if there were more than 150 pupils enrolled and if there were enough pupils to be studied in the different age categories. Pupils in primaries 1 to 6 were eligible for study. Eligible pupils were excluded from the survey if they did not obtain parental/guardian permission to participate or did not provide stool samples, or had severe diarrhoea or had of recent been transferred to the school from outside the local government area.

Every eligible pupil was provided with a clean stool specimen container with provision for name, age, sex and locality in which to bring a fresh stool sample the following day. 10 % formalin was added to each specimen in order to preserve the ova of the helminths. Samples of equal numbers of male and female pupils in the age groups were randomly selected for study in each school.

Faecal Examination: Parasitological diagnosis of the parasites was carried out by analysing stool samples for presence of the ova using the Kato-Katz technique (WHO, 1994) as follows.

1. Cellophane strips 25 x 30 mm or 25 x 35 mm were soaked in 50 % glycerol-malachite green (or methylene blue) solution for 24 hours before use
2. A small amount of faeces was transferred onto a piece of newspaper
3. A plastic screen of 60 – 105 mesh was pressed on top of the faecal sample.
4. Using a flat-sided applicator stick, the upper surface of the screen was scrapped to sieve the faecal sample.
5. A plastic template was then placed on a clean microscopic slide
6. A small amount of the sieved material was transferred into the hole of the template and the hole was carefully filled and leveled with the applicator stick.
7. The template was then carefully removed making sure that all the faecal material was left on the slide and none was left sticking to the template.
8. The faecal sample on the slide was covered with glycerol-soaked cellophane strip wiping off excess glycerol if present on the upper surface of the cellophane with toilet tissue.
9. The microscope slide was then inverted and the faecal sample pressed against the cellophane on a smooth surface to spread the sample evenly.
10. The slide was then removed by gently sliding it sideways and holding the cellophane firmly.

Quality Control and Data Analysis: The slides were examined within one hour of preparation to avoid over clarification of the hookworm eggs. A random sample of 10 % of the faecal smears was read by two different parasitologists to evaluate the accuracy of the diagnosis and the precision of the egg counts. Slides were re-examined if the quality control showed a>10 % difference in egg counts.

As the sensitivity of the Kato-Katz method could be influenced by the intensity of infection, analysis of variance was used to compare the logarithmically transformed egg counts and estimated as ($\sum$ log (epg + 1) / n) – 1, where $\sum$ log (epg + 1) is the sum of the logarithm of each individual epg. A value of 1 is added to each egg count to permit calculation of the logarithm in case the number of eggs per gram (epg) was zero.

Data from the parasitological examination together with the name, age and sex of the pupil examined were recorded on a form and entered on computer software for statistical and epidemiological analysis using the EpiInfo software package. χ^2 and students't – test were used to asses differences in the prevalences and intensity of infection respectively. Results were considered statistically significant at $p < 0.05$.

Table 1: Thresholds for the classes of intensity for different helminthiases in stools

Geohelminth	Light Intensity Infection	Moderate Intensity]Infection	Heavy Intensity Infection
Ascaris lumbricoides	1 – 4,999 epg	5,000 – 9,999 epg	≥ 50,000 epg
Trichuris trichiura	1 – 999 epg	1,000 – 9,999 epg	≥ 10,000 epg
Hookworm	1 – 1,999 epg	2,000 – 3,999 epg	≥ 4,000 epg

Intensity of Infection: At individual level the intensity of infection was measured indirectly as eggs per gram of faecal matter (epg). The intensity of infection allows for the quantification of the proportion of individuals suffering severe consequences and was classified as follows, based on the thresholds proposed by WHO (1987) (Table 1).

RESULTS

The gender-related prevalence of geohelminthiasis among primary school children in different localities of Ishielu Local Government Area (LGA) is shown in Table 2. Out of the 420 pupils of both sexes screened for the presence of the ova of the parasites in 8 localities, 240 children composed of 29.4% males (n = 124) and 27.6 % females (n = 116) were infected with one or more of the parasites indicating an overall prevalence of 57.1 %. The geohelminth (soil-transmitted) parasites identified were *Ascaris lumbricoides* (32.9 %), *Trichuris trichiura* (20.5 %) and hookworm (32.9 %). In 7/8 (87.5 %) of the schools surveyed over 50 % of the pupils were infected with one or more of the parasites.

Infection of both sexes was generally widespread. School- specific prevalence ranged from 44 % at Nkalagu Junction primary school to 63.3 % at the community primary schools of Umuhuali in the north-west and Agba in the south of the LGA. Generally, the distribution of the infections appears not to be gender-dependent and the between-sex prevalence rate was not statistically significant ($P > 0.05$). The results also indicate that schools located along the major roads, (at Nkalagu, Ezillo and Ntezi) recorded lower prevalence rates than those situated further in the hinterland (Ohafia Agba, and Umuhuali). The association is significant statistically ($P < 0.05$).

Table 2: Gender-Specific Prevalence of Geohelminths in Different Primary Schools of Ishielu Local Government Area, Ebonyi State, Nigeria

Locality of school	Number examined (n = 420)	Soil-transmitted helminthes (%)			Total (%)
		Ascaris lumbricoides	*Trichuris trichiura*	Hookworm	
Umuhuali	Male [30]	11(18.3)	7(11.3)	3(5.0)	21(35.0)
	Female [30]	7(11.7)	9(15.0)	1(1.7)	17(28.3)
Nkalagu	Male [25]	8(16.0)	4(8.0)	12.0)	13(26.0)
	Female [25]	5(10.0)	4(8.0)	0(0.0)	9(18.0)
Ezillo	Male [25]	7(14.0)	4(8.0)	1(2.0)	12(24.0)
	Female [25]	11(22.0)	5(10.0)	1(2.0)	17(34.0)
Isinkpuma	Male [25]	6(12.0)	7(14.0)	3(6.0)	17(34.0)
	Female [25]	6(12.0)	5(10.0)	0(0.0)	11(22.0)
Ntezi	Male [25]	6(12.0)	3(6.0)	2(4.0)	11(22.0)
	Female [25]	9(18.0)	5(10.0)	1(2.0)	15(30.0)
Okpoto	Male [25]	12(24.0)	5(10.0)	1(2.0)	18(36.0)
	Female [25]	918.0)	2(4.0)	0(0.0)	11(24.0)
Ohafia Agba	Male [25]	7(14.0)	4(8.0)	0(0.0)	11(22.0)
	Female [25]	12(24.0)	8(16.0)	0(0.0)	20(40.0)
Agba	Male [30]	13(21.7)	9(5.0)	1(1.7)	23(46.0)
	Female [30]	9(15.0)	5(8.3)	1(1.7)	15(30.0)
Total	**420**	**138 (32.9)**	**86(20.5)**	**16(3.8)**	**240(57.1)**

Table 3 shows the age-and locality-specific distribution of the soil-transmitted helminth parasites (geohelminths) among school children aged 5 to 14 years in the 8 study primary schools of Ishielu LGA. The infections were not over dispersed but appeared to be widespread in all the various age groups studied. The infections also appeared to be age-dependent as there was a marked association between age of the pupil and the prevalence of the infections by the various geohelminth parasites. In all the different study areas children aged 5 – 6 years had a lower rate of infection than other age groups, ranging from 2 (4.0%) at Okpoto, Ntezi, and Nkalagu schools to 6 (12.0%) in Ohafia Agba community primary school. Pupils aged 7-10 years (n = 98) had an infection rate of 23.3% while others, aged 11-14 years (n = 113) recorded the highest prevalence rate of 26.7%. Between-age difference was not statistically significant ($p > 0.05$). The age-related prevalence of specific geohelminths in the different study localities is shown in Table 3: The dominant geohelminth species, *Ascaris lumbricoides* was non-randomly distributed in all the various age groups in all the study areas affecting 137 (32.6%) of the study population. The infection and its distribution was not age-dependent. As with *A. lumbricoides* infection, the hookworm infection was non-randomly distributed. However, the distribution of the hookworm parasites in the study population was age-dependent as 82.3% (n = 14) of all hookworm cases detected in all the localities affected pupils in the age group (11-14) years. Generally, the hookworms affected teenagers in the (13-14) years age bracket more than any other age group within the scope of the study.

Trichuris trichiura was also not over dispersed affecting generally all age categories. At Umuhuoli in the north-west, Agba and Ohafia Agba in the south, every age category was infected with *T. trichiura.* The between-age difference in prevalence was not statistically significant ($p>0.05$) in trichuriasis. Slight within-age variations in the distribution pattern of trichuriasis were also recorded in the various age categories. The highest prevalence of trichiuriasis (5.0%) was recorded for pupils (n=21) aged 11-12 years, followed by 4.5% (n=19) for respondents in the 9-10 years age group. The lowest prevalence rate of 3.1% was recorded for pupils in the 5 - 6 age group.

The number of individuals of a particular parasite species in a single infected school pupil, that is, the intensity of the parasite species within a given host is shown in Table 5. Generally the mean intensity of infection of the observed geohelminths (*Ascaris*, *Trichuris*; and hookworm) were of the light category ($\leq$ 4999 epg for the *Ascaris lumbricoides;* ($\leq$ 999 epg for *Trichuris trichiura;* and $\leq$ 1999 epg for hookworm). Out of the 137 ascariasis-positive children from the study schools 48.2% (n = 66) showed mean egg count $\geq$ 1000 gram faecal matter; 10.2% (n = 14) had mean intensity of infection $\geq$ 900 ova per gram faecal matter, and 41.6% (n = 57) egg count $\geq$ 900 per gram faeces. The heaviest ascariasis infection was recorded for the 22 pupils of Agba primary school, mean intensity 1148 epg (range 864 - 1488). At Okpoto and Umuhuali the mean intensities of ascariasis infection were 1051 epg count (range 840-1320) and 1019 epg (range 240 - 1920) respectively. The lightest infection, mean epg 655 (range 288 - 960) was recorded for 13 infected pupils at Nkalagu Junction primary school.

Light infections of trichuriasis ($\leq$ 999) epg were recorded in all the study primary schools. The least mean intensity of infection of 707 epg (range 528 - 912) was recorded for 9 pupils of Ezillo primary school, followed in ascending order of mean epg by Nkalagu Junction primary school, mean epg 771, (range 624 - 912); Umuhuali primary school mean epg 780 (range 624 - 980); Agba primary school mean epg 804 (range 768-900); Isimkpuma primary school, mean epg 811 (range 534-920); Ntezi primary school, mean epg

Table 3: Age and Locality Related Distribution of Geohelminth Infections Among School Children in Ishielu Local Government Area, Ebonyi State, Nigeria

SCHOOL	Age Group (Year)	Number Examined (N = 420)	Number Infected (%)
Community primary school, Unuhuali	5 – 6	12	5(8.3)
	7 – 8	12	10(16.7)
	9 – 10	12	8(13.3)
	11 – 12	12	8(13.3)
	13 – 14	12	7(11.7)
	Sub-total:	**60**	**38**
Junction Primary School, Nkalagu	5 – 6	10	2(4.0)
	7 – 8	10	4(8.0)
	9 – 10	10	4(8.0)
	11 – 12	10	6(12.0)
	13 – 14	10	6(12.0)
	Sub-total:	**50**	22
Community Primary School Ezillo	5 – 6	10	4(8.0)
	7 – 8	10	6(12.0)
	9 – 10	10	7(14.0)
	11 – 12	10	7(14.0)
	13 – 14	10	5(10.0)
	Sub-total:	**50**	29
Community Primary School, Isinkpuma	5 – 6	10	3(6.0)
	7 – 8	10	3(6.0)
	9 – 10	10	8(16.0)
	11 – 12	10	6(12.0)
	13 – 14	10	7(14.0)
	Sub-total:	**50**	**27**
Community Primary School, Ntezi	5 – 6	10	2(4.0)
	7 – 8	10	4(8.0)
	9 – 10	10	6(12.0)
	11 – 12	10	7(14.0)
	13 – 14	10	7(14.0)
	Sub-total:	**50**	**26**
Central School Okpoto	5 – 6	10	2(4.0)
	7 – 8	10	4(8.0)
	9 – 10	10	7(14.0)
	11 – 12	10	7(14.0)
	13 – 14	10	9(18.0)
	Sub-total:	**50**	**29**
Community Primary School, Ohafia Agba	5 – 6	10	6(12.0)
	7 – 8	10	6(12.0)
	9 – 10	10	7(14.0)
	11 – 12	10	5(10.0)
	13 – 14	10	7(14.0)
	Sub-total:	**50**	**31**
Central Primary School, Agba	5 – 6	12	5(10.0)
	7 – 8	12	7(14.0)
	9 – 10	12	7(14.0)
	11 – 12	12	9(18.0)
	13 – 14	12	10(20.0)
	Sub-total:	**60**	**38**
Total		**420**	**240(57.1)**

822 (range 672 - 988); Ohafia Agba primary school, mean epg 838 (range 840 - 898) and Okpoto primary school, mean epg 881 (range 648 - 908). Moderate infections of trichuriasis (1000 - 9999) epg were also observed in all the study schools with the exception of schools at Ezillo and Nkalagu. At Ntezi primary school a single male pupil was recorded to have moderate infection of trichuriasis of 1010 epg.

The intensity of hookworm infection, as with other geohelminth parasites encountered, was of the light category ($\leq$ 1999) epg in the schools studied except at Ohafia Agba primary school where zero ancylostomiasis was recorded. A peak mean intensity of 168 epg (range 144 - 192) was recorded for 2 pupils at Okpoto primary school, followed by 156 epg (range 120 – 192) for 2 other pupils at Agba primary school. The lowest intensity of ancylostomiasis infection was obtained from the only pupil observed to be infected at Nkalagu junction primary school with 53 epg.

DISCUSSION

The overall prevalence of 57.1 % geohelminthiasis recorded in the present study not only establishes the endemicity of the geohelminth parasites but also indicates that *Ascariasis*, *trichuriasis* and human hookworm are the principal geohelminth nematode infections among primary school children in Ishielu Local Government Area of Ebonyi State. The study further indicates that the major soil-transmitted nematode parasites infecting susceptible pupils in primary schools and by implication the population in the ecologically homogeneous communities of north-western Ebonyi State are *Ascaris lumbricoides, Trichuris trichiura,* and the human hookworms (Table 2). The explanation revolves around various environmental, behavioural, and social factors. Of the external factors affecting the population dynamics of the geohelminths, the diet of the host is one of the most basic. It has been established that almost all of the entire parasites within a definitive host are present because the host ingested an infective stage of the parasite. Thus the distribution of *A. lumbricoides, T. trichiura,* and the human hookworm parasites (all of which possess direct life cycle patterns) depend, to a large extent on the dietary habits and the associated personal food hygiene of the affected Ishielu people. Although large quantities of fruits and vegetables are produced in the study areas and therefore constitute major sources of diet, the relative scarcity of good water supply in the study communities has affected the general standard of sanitation and personal hygiene and significantly impacted on the transmission dynamics of the parasites. The widespread prevalence of the parasites in both sexes is indicative of the general ignorance of the modes of transmission of the parasites. While the dynamics of *A. lumbricoides* infection are essentially the same as those of *T. trichiura,* both being acquired by the population through accidental ingestion of the soil contaminated eggs, the hookworm infection, on the other hand is acquired by the natives through the soil-dwelling filariform larvae that actively penetrate the skin of their bare-footed susceptible hosts.

The notion of susceptibility of the host to the acquisition of the geohelminths may be associated with the non-genetic factors such as personal hygiene, indiscriminate defaecation practices, water usage resources and socioeconomic status.

Table 4: Age-related Prevalence of Specific Geohelminths in Different Localities of Ishielu Local Government Area, Ebonyi State, Nigeria.

Locality	Number examined (n = 420)	Number infected (n=240) (%)	Age group (years)	geohelminths			Concomitant infection
				A. lumbricoides (%)	*T. trichiura* (%)	Hookworm (%)	
Umuhuali	60	38(63.3)	5 – 6	4(6.7)	2(3.3)	0(0.0)	0
			7 – 8	5(8.3)	2(3.3)	1(1.7)	3
			9 – 10	4(6.7)	3(5.0)	0(0.0)	3
			11 – 12	3(5.0)	4(6.7)	1(1.7)	2
			13 – 14	2(3.3)	5(8.3)	2(3.3)	2
Nkalagu	50	22(44)	5 – 6	2(4.0)	0(0.0)	0(0.0)	0
			7 – 8	3(6.0)	1(2.0)	0(0.0)	1
			9 – 10	3(6.0)	1(2.0)	0(0.0)	0
			11 – 12	24.0)	2(4.0)	1(2.0)	0
			13 – 14	3(6.0)	4(8.0)	0(0.0)	0
Ezillo	50	29(58)	5 – 6	4(8.0)	1(2.0)	0(0.0)	1
			7 – 8	4(8.0)	0(0.0)	0(0.0)	1
			9 – 10	5(10.0)	3(6.0)	1(2.0)	0
			11 – 12	2(4.0)	3(6.0)	0(0.0)	0
			13 – 14	3(6.0)	2(4.0)	1(2.0)	0
Isinkpuma	50	27(54)	5 – 6	3 (6.0)	2(4.0)	0(0.0)	0
			7 – 8	3 (6.0)	4(8.0)	1(2.0)	2
			9 – 10	2(4.0)	3(6.0)	0(0.0)	0
			11 – 12	1(2.0)	3(6.0)	1(2.0)	1
			13 – 14	3(6.0)	0(0.0)	1(2.0)	0
Ntezi	50	26(52)	5 – 6	2(4.0)	1(2.0)	0(0.0)	0
			7 – 8	3(6.0)	0(0.0)	1(2.0)	2
			9 – 10	4(8.0)	2(4.0)	0(0.0)	1
			11 – 12	2(4.0)	2(4.0)	1(2.0)	1
			13 – 14	4(8.0)	3(6.0)	1(2.0)	0
Okpoto	50	29(58)	5 – 6	4(8.0)	2(4.0)	1(2.0)	2
			7 – 8	5(10.0)	1(2.0)	0(0.0)	1
			9 – 10	3(6.0)	2(4.0)	0(0.0)	2
			11 – 12	3(6.0)	2(4.0)	0(2.0)	0
			13 – 14	5(10.0)	0(0.0)	1(0.0)	0
Ohafia Agba	50	31(62)	5 – 6	4(8.0)	3(6.0)	0(0.0)	1
			7 – 8	3(6.0)	3(6.0)	0(0.0)	2
			9 – 10	3(6.0)	2(4.0)	0(0.0)	1
			11 – 12	48.0)	3(6.0)	0(0.0)	0
			13 – 14	5(10.0)	1(2.0)	0(0.0)	0
Agba	60	38(63.3)	5 – 6	5(8.3)	2(3.3)	0(0.0)	1
			7 – 8	6(10.8)	4(6.7)	0(0.0)	1
			9 – 10	5 (8.3)	3(5.0)	0(0.0)	0
			11 – 12	3(5.0)	2(3.3)	0(0.0)	1
			13 – 14	3(5.0)	3(5.0)	2(3.3)	0
TOTAL	**420**	**240 (57.1)**		**137(32.6)**	**85(20.5)**	**17(4.0)**	**32(13.3)**

Any of these factors could influence the transmission dynamics of these soil-transmitted parasites in such a way as to create the impression of susceptibility. The longevity of *A. lumbricoides* ova also contributes to the infectious dynamics of that parasite species as it has been shown that the eggs kept for 10 years in the soil could still be infective (Brudastor *et al.,* 1971). As a result of such longevity it is difficult to prevent infection and re-infection when hectares of farmland surrounding human habitations as found in the study have been polluted with *A. lumbricoides* ova.

Within the narrow age-limits of the study, the infectious geohelminth parasites were widespread, gender independent and not over dispersed (Tables 2, 3 and 4). The results are in conformity with those of earlier studies which established that young children are more vulnerable to many enteric infections than are adults and are the more responsible for contaminating the environment and transmitting the infections (Albonico *et al.,* 2002; Agbolade *et al.,* 2004; Adeyeba and Akinlabi, 2002).

Table 5: Intensity of infection of geohelminth parasites among primary school children in Ishielu area of Ebonyi State, Nigeria

Locality	Umuhuali		Nkalagu		Ezillo		Isinkpuma	
	No Infected	Mean epg (range)	No Infected	Mean epg (range)	No Infected	Mean epg (range)	No Infected	Mean epg (range)
Helminth Infection								
Ascaris lumbricoides	18		13		18		12	
Light 1-4,999 epg	18	1019 (240-1920)	13	655 (288-960)	18	848(600-1104)	12	808 (600-1200)
Moderate 5,000 - 9,999 epg	-	-	-	-	-	-	-	-
Heavy ≥ 10,000 epg	-	-	-	-	-	-	-	-
Trichuris trichiura	16		8		9		12	
Light 1- 4,999 epg	14	780 (624-980)	8	771 (624-912)	9	707 (528-912)	7	811 (534-920)
Moderate 5000-9,999 epg	2	1007 (1002-1012)	-		-		5	1020 (1006-1220)
Heavy ≥ 10,000 epg	-	-	-	-	-	-	-	-
Hook Worm	4		1		2		3	
Light 1-1,999 epg	4	84 (54-128)1	1		2	48 (24-72)	3	120 (48-192)
Moderate 2000 - 3,999 epg	-	-	-	-	-	-	-	-
Heavy ≥ 4000 epg	-	-	-	-	-	-	-	-
	Ntezi		**Okpoto**		**Ohafia Agba**		**Agba**	
Helminth Infection								
Ascaris lumbricoides	15		20		19		22	
Light 1-4,999 epg	15	968 (792-1104)	20	1051 (840-1320)	19	971 (720-1152)	22	1148 (864-1488)
Moderate 5,000-9,999 epg	-	-	-	-				
Heavy ≥10,00 epg	-	-	-	-				
Trichuris trichiura	8		7		12		14	
Light 1-999 epg	7	822 (672-988)	5	881 (648-908)	4	838 (840-898)	7	804 (768-900)
Moderate 1000-9,999 epg	1		2	1102 (1101-1103)	8	1004 (1002-1022)	7	1202 (1002-1020)
Heavy ≥ 10,000 epg	-	-	-	-	-	-	-	-
Hook Worm	3		2		0		2	
Light 1-1,999 epg	3	126 (96-168)	2	168 (144-192)	0		2	156 120-192
Moderate 2000-3,999 epg	-	-	-	-	-	-	-	-
Heavy ≥ 4000 epg	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-

epg= eggs per gram of faeces.

Even within the narrow age range, the results indicate that children in the 5 – 6 year age group generally showed the lowest infection rate while the older ones aged 11-14 years were the most heavily infected suggesting that even among the susceptible population of children, the infection is age dependent with a marked association between age and prevalence (Table 3) – a situation attributable to exposure to the risk factors.

In investigations involving entire local populations (young children and adults), *A. lumbricoides, T. trichiura* and hookworm parasite infections are commonly over dispersed, with a small segment of the population harbouring infections of high intensity. The small number of infected individuals seem to be predisposed to infection (Chan *et al.,* 1992; 1994; Kightlinger *et al.,* 1995). In the current study, the investigation centers specifically around the more susceptible segment of the local population; viz. infants and teenage primary school pupils aged ≤ 15 years. The age of the study population has consequently impacted on the non-random nature of the over dispersion frequency. Like susceptibility, predisposition of the children to the geohelminth parasites may also be due to behavioural, social and environmental factors acting alone or in combination. Some evidence of predisposition has suggested genetic susceptibility (Holland, *et al.,* 1992) but others have suggested that genetic factors, if any, are overwhelmed by environmental or behavioural characteristics of the host (Chan, *et al.,* 1994*).* Indiscriminate defaecation practices, inadequate personal hygiene therefore appear to be important factors in creating the different frequency distribution patterns of the three geohelminth parasites. Defaecating indiscriminately, particularly near human dwellings usually seeds the soil with the *Ascaris* and *Trichuris* ova that remain viable for considerable long periods of time. On the other hand hookworm parasites appear to have an age-dependent pattern of infection in that older teenage pupils who are more intensively exposed to infection by tilling the soil polluted with the geohelminth ova, gathering of fruits and vegetables and participation in other household chores that bring them more into contact with the soil, appear likely to have more hookworm than their younger colleagues. Although the intensity of the geohelminths infection was generally light, moderate infection of *T. trichiura* (100 – 9999) eggs per gram faecal matter was recorded in 6/8 (75 %) of the study primary schools. In terms of intensity of infection *Trichunis trichiura* is the dominant species encountered. The light intensity of infection recorded may be attributed to a number of factors including the estimating technique, and the internal environmental factors within the host affecting parasite densities and fecundity including interactions among parasite populations within species and between species. The technique of estimating the parasite density relying on the faecal egg counts as used in this study has the primary limitation in relation to the variability in the number of ova discharged by specific parasite per day over a given period of time. The internal environmental factors that may affect the geohelminth parasite densities in the study areas are complex because several phenomena such as behaviour, host and parasite genetics, natural and acquired resistance, among other factors, are involved and these factors frequently act in concert. Among the internal environmental factors possibly involved in the present study are density-dependent constraints created by interspecific parasite competition which causes the variability in daily egg output. It has also been established that egg out put by some enteric helminth parasites such as *Ascaris* sp. and *Trichuris* sp. will increase as parasite densities increase but only until a threshold is reached and then it may decline or fluctuate (Holland *et al.,* 1988; Elkins and Haswell-Elkins, 1989). From the results obtained on the intensity of infection of the geohelminths, it may be inferred that the number of parasite ova per individual host determines the risk of morbidity. It would also be inferred that in the absence of clinically overt infection, the intensity of egg output is an indicator of latent morbid state, because the greater the egg counts the greater the number of female helminth parasites present.

In conclusion the results advocate for a deworming programme to avert possible impact on the physical, mental and cognitive development of the at-risk children in the endemic localities of rural Ebonyi State.

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REFERENCES

ADAMS, E. J. (1994). Physical activity and growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved after treatment with albendazole. *Journal of nutrition*, *124*: 1199 -1206.

ADEYABA, O. A., and AKINLABI, A. M (2002). Intestinal parasitic infections among school children in a rural community, south west Nigeria. *Nigerian Journal of Parasitology, 23:* 11 – 18.

AGBOLADE, O. M., AKINBOYE, D. O., and AWOLAJA, A. (2004). Intestinal helminthiasis and urinary schistosomiasis in some villages in Ijebu North, Ogun State, Nigeria. *African Journal of Biotechnology, 3(3):* 200 – 209

ALBONICO, M., RANSAN, M., WRIGHT, V., JAPE, K., HAJI, H. J., TAYLOR, M., SAVIOLI, L., and BIEKLE, Q. (2002). Soil-transmitted nematode infections and mebendazole

treatment in Mafia Island school children. *Annals of Tropical Medicine and Parasitology, 96(7):* 717 - 726.

AWOGUN, I. A., OKWUREKWU, F. E., OYAWOYE, O. A., and BELLO, A. O. (1995). Helminthic and anaemia among pregnant women attending antenatal clinic in Ilorin, Nigeria. *Bioscience Research Communiqué, 7*: 14 – 45.

BROOKER, S., PESHU, N, WARN P. A., MOSOBO, M., GUYATT, H., MARSH, K. and Snow, R. W. (1999). The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 33*: 240 – 246.

BRUDASTOR, A. N., LEMELER. V. R., KHOLNUKHANEDOR, S. K., and KRASNOR; L. N. (1971). The clinical picture of the migration phase of ascariasis in self infection *Medskay Parasitology, 40*: *165 – 168*.

BUNDY, D. A. P; COOPER, E. S., THOMPSON, D. E., DIDIER, J. M., ANDERSON, R. M., and SIMMONS, I. (1987). Predisposition to *Trichuris trichiura. Epidemiology and Infection, 98:* 65 – 71.

CHAN, L., BUNDY, D. A. P., and KAN, S. P. (1994). Genetic relatedness as a determinant of predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* infection. *Parasitology, 108:* 77 – 80.

CHAN, L; KAN, S. P., and BUNDY, D. A. P. (1992). The effect of repeated chemotherapy on age-related predisposition to *Ascaris lumbricoides* and *Trichuris trichiura. Parasitology, 104:* 371 – 377.

CHAN, M.S. (1997). The global burden of intestinal nematode infections-fifty years on. *Parasitology Today, 13;* 438 – 443.

CROMPTON, D. W. T., and SAVIOLI, L. (1999) Intestinal parasitic infections and urbanization. *Bulletin of the World Health Organization, 71:* 1-7.

ELKINS, D. B., and HASWELL-ELKINS, M. (1989) The weight/length profiles of Ascaris lumbricoides within a human community before mass treatment and following reinfection. *Parasitology. 99:* 293 – 299.

HOLLAND, C. V., CROMPTON, D. W. T., ASAOLU, S. O., CRICHTON, W. B., TORIMIRO, S.E. A.,and WALTERS, D. E. (1992). A possible genetic factor influencing protection from infection with *Ascaris lumbricoides* in Nigerian children *Journal of Parasitology, 78:* 915-916.

HOLLAND, C. V., TAREN, D. L., CROMPTON, D. W. T., NASHIEM, M. C., SANJUR, D., BARBEU, I., TUCKER, K., TIFFANG, J., and RIVERS, G. (1988). Intestinal helminthiasis in relation to the socioeconomic environment of Paramanian children. *Social Science and Medicine, 26:* 209 – 213.

KIGHTLINGER, L. K., SEED, J. R, and KIGHTLINGER, M. B. (1995). The epidemiology of *Ascaris lumbricoides. Trichuris trichiura* and hookworms in children in the Ranomofana rainforest, Madagascar. *Journal of Parasitology, 81;* 159 – 169.

MONTRESSOR, A; CROMPTON, D. W. T., HALL, A., BUNDY, D. A. P. and SAVIOLI, L. (1998). Guideline for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. Geneva, World Health Organization (unpublished document WHO/CTD/SIP. 98.1).

NOKES, C; GRANTHAM-MCGREGOR, S. M., SAWYER, A. W., COOPER, E. S., ROBINSON, B. A., and BUNDY, D. A. P. (1992). Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. *Parasitology, 104:* 539 – 547.

OFOMATA, G. E. K. (1978). Nigeria in Maps. Eastern States. Ethiope publishing house. Benin city. 146 pp.

OKPALA, I (1961). A Survey of the Incidence of Intestinal Parasites among government workers in Lagos, Nigeria. *West African Medical Journal*, *5 (4):* 167 – 170.

OKPALA, I., OBI-NJOKU, I. C. (1978). A Survey of the incidence of intestinal helminth parasites in primary school children in Nsukka Area, Anambra State, Nigeria. *Nigerian Society of Parasitology* 2nd Annual Book of Abstracts pp 3 – 4.

ONUBOGU, V. U. (1978). Intestinal parasites of school children in Urban and Rural Areas of Eastern Nigeria. *Zentralblalt fur Bakterio-logie and Hygiene. 242:* 121 – 131.

UDONSI, J. K. (1984). Studies on the concurrence of two species of human hookworms in a riverine community in Nigeria. *Tropenmedizin and Parasitologie 35:* 37 – 40.

UDONSI, J. K. (1992). The human environment, occupation, and possible water-borne transmission of human hookworm, *Necator americanus* in endemic coastal communities of the Niger Delta, Nigeria. *Public Health, 106:* 63 – 71.

WHO. (1987). Prevention and control of intestinal parasitic infections. Report of a WHO Expert Committee (WHO Technical Report Series No 749). Geneva. World Health Organization.

WHO (1994). Report of the WHO Informal consultation on hookworm infection and anaemia in girls and women. Geneva, World Health Organization.

WHO. (1994). Bench aids for the diagnosis of intestinal parasites. Geneva, World Health Organization.

EFFECT OF DIFFERENT DIETARY ITEMS ON THE GROWTH OF AFRICAN CATFISH HYBRID *Heterobranchus bidorsalis* (♂) X *Clarias gariepinus* (♀)

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ABSTRACT

This paper describes the growth response of the developmental stages of hybrid catfish resulting from the crossing of Heterobranchus bidorsalis (male) and Clarias gariepinus (female), fed with different feed rations. Hatchlings of the hybrid catfish were cultured in plastic aquaria set up at the fish hatchery complex, Nnamdi Azikiwe University, Awka. The hatchlings were fed and observed for 91 days. Different food items such as artificially compounded feed, zooplankton / live organisms, Moina sp and Brachionus sp, and a mixture of zooplankton (Moina sp and Brachionus sp) and artificially compounded feed were administered. The mixture gave the best result with a mean weight of 7.92g ± 2.73g, followed by those fed Moina sp (1.93g ± 0.27) and Brachionus sp (1.80g ± 0.32) respectively. Hatchlings fed only artificially compounded feed exhibited the poorest growth. A mixture of live food organisms and artificially compounded feed is thus recommended for better growth response of catfish hybrid, (H. bidorsalis ♂ x C. gariepinus ♀).

Keywords: Catfish hybrid, Hatchlings, Juveniles, Diet, Growth

INTRODUCTION

The fish species *Clarias* and *Heterobranchus* (Family: Clariidae) are very common and widely distributed throughout Africa. They can grow to large sizes of over 10 kg and are in high market demand as table fish, being tasty and scaleless. *Clarias* do not grow as large or as quickly as *Heterobranchus*. *Heterobranchus* on the other hand, do not have the same survival rate as *Clarias*. The hybrid catfish referred to locally as "*Heteroclarias"* combines fast growth and high survival, thus, considerable attention is being given to the catfish hybrid, especially in intensive and semi-intensive pond culture.

A major pre-requisite for successful fish farming enterprise is a reliable and consistent source of fish seeds (fingerlings) of the commercially important species (Nwuba and Aguigwo, 2002). The surest and most reliable source of supply is to produce the fingerlings under a controlled system, usually in a hatchery. Not only is reproduction controlled, but the survival of hatchlings is maximized through adequate care and management (Madu and Ita, 1991). One of the major challenges in hatchery management is the provision of adequate and appropriate food for the fish hatchlings. This is because most formulated feed come either in pellets or sizes not small enough for the hatchlings to swallow; imbalanced or insufficient nutrient content, and probably, the adaptation of the fish gut to solid food from plant origin. As a result, there is usually high mortality rate when fish hatchlings change from endogenous to exogenous feeding after the first three to four days of their life. Growth may be retarded as development of sensory and motor capabilities and development of physiological characteristics occur during the early life stage and affect survival and competitive ability. According to Hyatt (1979), the first few months of life are perhaps the most critical for the survival of juvenile fish. Presently, most hatcheries are trying to make use of live zooplankton to meet the challenge of feeding fish hatchlings. According to Piggot and Tucker (1989), fish can swallow live prey of much larger size than dry formulated feed due to the elastic nature of live feed. Nwuba and Aguigwo (2002), however, reported that any single diet of either artificially compounded feed only or zooplankton only, could not sustain *Clarias anguillaris* hatchlings beyond a certain age but that addition of supplemental feed to live food offers better growth to growing hatchlings of the fish species. This paper investigates the effect of different food items on the growth response of the developmental stages of the catfish hybrid, *Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀**.**

MATERIALS AND METHODS

Hybrid: Hatchlings of hybrid catfish were produced through hormone induced breeding at Aquafish farms, Ihiala, and transferred to the fish hatchery complex, Zoology Department, Nnamdi Azikiwe University, Awka on the fourth day of life after hatching. The hatchlings were stocked in twelve forty (40) litre plastic aquaria with twenty (20) litres of water each and eighty (80) hatchlings per aquarium. The aquaria were divided into four groups according to the number of test diets, with each group having three replicates. The aquaria and groups were labeled accordingly, Diet I, II, III, and IV. Diet I was formulated diet, Diet II and III live organisms of *Moina sp* and *Brachionus sp* respectively and Diet IV a mixture of the formulated diet and the live organisms.

The appropriate food items were administered in the various aquaria from the fifth day of life and the effect of the food items on the growth of the hatchlings was monitored for ninety-one (91) days.

Artificial Diet: Artificial diet was prepared to contain 40 % crude protein (Table 1). The mass of individual feed ingredients used was calculated using the Pearson's square method (Pearson, 1976). The feed ingredients were milled and finely sieved. Each ingredient was separately weighed out, to a total of 200g of ingredients. The feedstuffs were thoroughly mixed together, with 80 ml of water added to form homogenous and well-kneaded dough. The dough was pressure cooked to help gelatinization of the feed and aid the release of necessary nutrients. The cooked dough was extruded as semi-moist pellets using a hand cranked pelletizer. The pellets were sun-dried, milled again, finely sieved and stored in sealed polythene bags.

Table 1: Weights of dietary ingredients and proximate composition of formulated diet

Ingredients	Weight(g)
Crayfish	63.0
Soya bean	63.0
Corn meal	72.0
Vitamin/Mineral Premix	1.0
Salt	0.5
Oil	0.5
Proximate composition	
Food class	**% composition**
Protein	39.81
Fibre	2.40
Ash	6.12
Moisture	10.83
Fat	11.90
Carbohydrate	28.92

The milled feed was dispensed manually into the water in the appropriate aquaria at 5 % body weight of the fish in the aquaria once daily. The proximate composition of the formulated diet fed to the hybrid catfish hatchlings was as shown in Table 1.

Live Diet: Zooplankton, *Moina sp* and *Brachionus sp* were isolated and cultured in plastic aquaria using slight modifications of techniques reported by Adeniji and Ovie (1986) and Ovie *et al* (1993). The live diets used were identified by viewing water samples under an Olympus Tokyo (HSB 376700) microscope and using the identification key given by Jeje and Fernando (1988). *Moina sp* was harvested from the culture aquaria very early in the morning and fed to the fish hatchlings twice daily at an estimated rate of five hundred (500) organisms per litre of water. *Brachionus sp* was also harvested in the morning and fed to the fish hatchlings twice daily at the rate of six hundred (600) organisms per litre of water. The density of harvested zooplankton was estimated using the volumetric method: Density of organisms = X/W x V; where X = number of organisms in a drop of culture water, W = volume of drop of water and V = total volume of water fed to aquarium. Another method of Escritor and Javallana (1981) as described by Ovie and Fali (1989) was used to determine feeding volume of harvested zooplankton in which: N = X /Y x V; where: N = volume of water (with zooplankton) to be fed the aquarium, X = actual count / density at source and V = volume of water in aquarium.

A beaker was used to take up the zooplankton and water, which was then poured gently into the appropriate aquaria when feeding.

Fish hatchlings were counted every five (5) days to minimize stress due to excessive handling

The length and weight of hatchlings were also monitored. Measurement of fish length started on the thirty-first day from when feeding started. A graduated test-tube was used for measuring the length of the fish. Measurement of fish weight started on the forty-first day.

The water in the aquaria were initially changed daily (for the first ten days) and then every other day. The aquaria were washed every five days, when the hatchlings were weighed. Water parameters such as temperature, pH and dissolved oxygen were also monitored.

Data Analysis: The data for hatchling growth were analyzed using a one-way Analysis of Variance (ANOVA) and Fisher's Least Significant Difference (F-LSD) at P = 0.01 and 0.05.

RESULTS

The growth performance of hybrid catfish hatchlings fed different diets for 91 days is shown in Tables 2 and 3. The length and weight were used as index of growth. Fish fed a mixture of zooplankton and artificial diet with a final mean weight of 7.92g ± 2.73g exhibited better growth, over those fed single diets of only zooplankton or artificial diet. The mean daily temperatures were similar for all the aquaria and stood at 25.2^{O}C ± 0.024^{O}C. Average pH for treatment aquaria stood at 6.80 ± 0.01 for *Moina sp* and *Brachionus sp*; 6.92 ± 0.018 for mixture of artificial feed and zooplankton and 6.95 ± 0.03 for artificially formulated feed. Dissolved oxygen (DO) stood at 5.84 ± 0.014 mg / litre on the average.

DISCUSSION

The growth performance recorded in this study supports the view held by Tacon (1993) that it is erroneous to mislead researchers and farmers into believing that the only economic way of feeding fish is by using a high quality "complete" pelleted diet. Feeding of hybrid catfish hatchlings with live organisms, improved the growth performance over feeding with complete artificial diet as opined by Ovie and Fali (1989), Jeje (1992) and Bone *et al* (1995). The final mean weights of fish fed *Moina sp* (1.93g ± 0.27) and those fed *Brachionus sp* (1.80g ± 0.32) were not statistically different, suggesting that as long as the live organisms (zooplankton) is of acceptable size, the species of natural zooplankton used for feeding may not matter. Any freshwater zooplankton species could suffice, barring any defensive adaptations, although hardier and easier to propagate species like

Table 2: Growth in weight of *H. bidorsalis* x *C. gariepinus* hybrid juveniles fed different live and artificial diet combinations

Diets	Days										
	41	46	51	56	61	66	71	76	81	86	91
***Moina* sp**	0.174	0.225	0.299	0.386	0.496	0.621	0.803	0.987	1.233	1.544	1.933
***Brachionus* sp**	0.198	0.252	0.321	0.406	0.497	0.618	0.759	0.929	1.156	1.407	1.803
Artificial	0.043	0.046	0.047	0.049	0.050	0.051	0.052	0.053	0.053	0.054	0.056
Mixture	0.371	0.537	0.755	1.079	1.541	2.238	3.150	3.933	4.937	6.397	7.924

Table 3: Growth in length (cm) of *H. bidorsalis* x *C. gariepinus* hybrid juveniles fed different live and artificial diet combinations

Diets	Days												
	31	36	41	46	51	56	61	66	71	76	81	86	91
***Moina* sp**	1.56	1.73	1.83	2.02	2.18	2.61	2.93	3.49	3.68	4.21	4.33	4.96	5.09
***Brachionus* sp**	1.29	1.47	1.57	1.81	1.94	2.32	2.72	3.36	3.57	3.97	4.23	4.68	5.00
Artificial	1.03	1.07	1.08	1.12	1.15	1.28	1.40	1.65	1.77	1.78	1.78	1.79	1.82
Mixture	2.40	2.50	2.66	2.74	2.91	3.38	4.11	4.86	5.25	5.56	6.04	6.48	6.99

Brachionus sp (Ezechi, 2005) may however be preferred. The use of live zooplankton only, however, may not be the best feeding technique, as the result of this study shows that supplementary feeding is still very important. The best performance exhibited by hybrid catfish hatchlings fed a mixture of both zooplankton and artificially compounded feed indicate that mixing artificially compounded feed and live zooplankton in the diet of the developmental stages of the hybrid catfish would be a better practice in fish farm practice. This supports the observations of Nwuba and Aguigwo (2002).

According to Smith (1989), the maintenance cost of the animal (fish) has priority and must be met before any energy is available for tissue synthesis and growth. This may explain the result obtained in this study. Since zooplanktons are naturally more proteinous, the problem of consumption of enough easily digestible energy (DE) to support the fish's maintenance cost and tissue synthesis may have arisen. This may have been a major factor in the enhanced performance of fish fed a mixture of live zooplankton and artificially compounded feed over those fed only live organisms (zooplankton). The integration of the formulated feed into the diet of the fish as supplement, along with the natural live freshwater organisms, may have increased the digestible energy (DE) / metabolizable energy (ME) available to the fish, thus leaving more energy for tissue synthesis and growth after the maintenance cost of the fish was met. Feeding with both live organisms and compounded feed is thus recommended for hybrid catfish hatchlings for enhanced growth performance.

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REFERENCES

ADENIJI, H. A. and OVIE, S. I. (1986). Commercial production of freshwater fish food. Pages 232 – 240. *In: Proceedings of the 5th Annual Conference of the Fisheries. Society of Nigeria* (FISON), Ilorin, September 22nd – 25th, 1986.

BONE, Q., MARSHALL, N. B. and BLAXTER, J. H. S. (1995). *Biology of Fishes* (2nd Edition). Chapman and Hall, London. 332 pp.

ESCRITOR, F. and JAVALLANA, S. (1981). Culture of Algae and Plotting of growth curve. *Training course on growing food organisms for fish hatcheries.* SEAFDEC Aquaculture Department, Natural food project, Tigbuanan, Iloilo, Philippines.

EZECHI, C. U. (2005). *Comparative study of the effect of different diets on the survival and growth rate of Heteroclarias hatchlings.* M.Sc Project Report, Nnamdi Azikiwe University, Awka, Nigeria. 79 pp.

HYATT, K. D. (1979). Feeding Strategy. Pages 71 – 119. *In:* W. S. HOAR, D. J. RANDALL and J. R. BRETT, eds., *Fish Physiology*, Volume 7. Academic Press, New York.

JEJE, C. Y. (1992). Post-larval feeding of *Clarias gariepinus* (Burchell, 1802) on cultured Zooplankton and Artemia diets. Pages 129 – 134. *In: Proceedings of the 10th Annual Conference of the Fisheries Society of Nigeria* (FISON), Abeokuta. 16th-20th Nov. 1992.

JEJE, C. Y. and FERNANDO, C. H. (1988). *A practical guide to the identification of Nigerian Zooplankton. (Cladocera, Copepoda and Rotifera).* Kainji Lake Research Institute, Kainji, Nigeria. 142 pp.

MADU, C. T. and ITA, E. O. (1991). Simple Techniques for large-scale production of fingerlings. *Proceedings of the Fourth*

Annual Seminar. NIFFR Annual Report 1991, 7 pp.

MADU, C. T. and ITA, E. O., MOHAMMED, S. (1999). *Fishery Business.* African Farming and Food Processing, Jan / Feb. 11-14.

NWUBA, L. A. and AGUIGWO, J. N. (2002). Studies on the effects of different food items on the survival of Hatchlings of *Clarias anguillaris*. *Journal of Aquatic Science*, 17(2): 121 – 124.

OVIE, S. I., ADENIJI, H. A. and OLOWE, D. I. (1993). Isolation and Growth Characteristics of freshwater zooplankton for feeding early larval and fry stages of fish. *Journal of Aquaculture in the Tropics*. *8:* 187 – 196.

OVIE, S. I. and FALI, A. I. (1989). Preliminary investigation into the use of cow-dung and rice straw mixture in the propagation of zooplankton. Pages 140 – 144. *NIFFR Annual Report*, 1989.

PEARSON, D. (1976). *The chemical analysis of foods*. 7th Edition, Churchill Livingstone, London.

PIGGOT, G. M. and TUCKER, B. W. (1989). Special feeds. Pages 653 – 679. *In:* HALVER, J. E. (Ed), *Fish Nutrition*, Second Edition, Academic Press Inc. San Diego, California.

SMITH, R. R. (1989). Nutritional Energetics. Pages 1 – 22. *In:* HALVER, J. E. (Ed), *Fish Nutrition*, Second Edition, Academic Press Inc. San Diego, California.

TACON, A. C. J. (1993). Aquaculture nutrition food for thought. *Aquaculture Newsletter*, *3:* 2 – 6.

FORMICID FAUNA OF CONTRASTING TROPICAL RAINFOREST AGRO-ECOSYSTEM AND THEIR ENVIRONMENTAL IMPLICATIONS

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ABSTRACT

The pitfall technique was used to study ants in a secondary re-growth forest and a fallow plot at the Permanent Site of Nnamdi Azikiwe Awka from January to December, 1998. The selected environmental variables including mean soil temperature, mean relative humidity and rainfall. Species of ant obtained from the two sampling sites were Acantholepsis sp. Camponotus acvapimensis, C. perrisi, Myrmicaria striata, C. maculatus, Dorylus affinis Paratrechina sp., Megaponera foetans, Pheidole sp., Crematogaster sp. and Oecophylla longinoda. Statistical differences existed only in the distribution of Camponotus acvapimensis and C. perrisi with less catches recorded at the forest than the fallow plot. There was positive correlation coefficient between the density of Dorylus affinis and mean soil temperature (r = 0.84) at the forest while at the fallow plot negative correlation value (r= -0.61) was obtained between the population density of Acantholepsis sp. Populations of Acantholepsis also recorded a positive correlation (r = 0.54) with monthly mean soil temperature and mean relative humidity. These results do not only suggest a reflection of response of these ant species to these environmental variables during their foraging activities but their implications during the exploratory activities of these ants carried out in relation to temporal organization of the foraging systems, with these ants species exhibiting centrifugal polytheism associated with ant societies. The environmental implications of the trapping of these formicid species in the habitats studied were also discussed.

Keywords: Formicid fauna, Tropical rainforest, Agro-ecosystem, Environmental variables

INTRODUCTION

The pitfall technique has been found effective and used extensively in trapping crawling animals especially arthropods in various habitats. In studying arthropods these traps have been installed with or without preservatives (Greenslade, 1976; Ewuim, 1996, 1997; Ewuim and Nwuba, 2002). Studies on the use of the pitfall traps in studying ant fauna inhabiting the litter of forest and agro ecosystems in Nigeria include those of Ewuim, 1996, 1997; Ewuim and Ezenwugo, 1997; Ewuim *et al*. 1997). Earlier studies on litter arthropod species include those of Lasebikan (1974), Lasebikan *et al.* (1985) and Badejo and Van Straalen (1993), which focused on various aspects of the ecology of the collembolan fauna of forests and cassava plot in Ile-Ife. Other similar earlier studies on litter arthropods fauna (Lasebikan, 1977; 1985) dwelt more on Acarina and Collembola than any other group of litter arthropods (Ewuim *et al*., 1997).

The formicids (ants) are a distinct group among the arthropods with an outstanding degree of eusociality in the structural organization. These ants are noted for their co-existence, resource partitioning and population stability while being highly abundant and widespread in distribution, with unparalleled effects on all organization (Caroll and Janzen, 1973; Torres, 1984; Ewuim, 1996, 1997. These formicids in the tropics are the most successful insects on the planet earth, having evolved to secure a wide range of dissimilar ecological niches as herbivores, predators, fungus grazers, seed-harvesters, leaf-cutters, aphid-tenders (Boorman, 1981). In spite of the fact that species are characterized by the detailed fit of their form and function in relation to their way of life and environment by adaptive complexity. (Bourke and Franks, 1995), the population density of these species and their distribution are usually influenced either directly or indirectly by their pattern of interaction with one another within the given ecosystem. The foraging workers (of ants) as a result of reproductive altruism are involved in foraging activities in order to promote the survival and reproduction of the brood they rear (Bourke and Franks, 1995).

In this paper therefore, the species composition and the relative abundance of foraging ants on the forest floor and a fallow plot in a tropical rainforest zone in Nigeria will be studied using the pitfall trap and in relation to the influence of selected environmental variables on some of the species. The possible environmental influence of some of these species will also be highlighted. It is envisaged that this investigation will help upgrade the available information on formicid fauna in the tropics.

MATERIALS AND METHODS

Site Description: The investigation was carried out in two contrasting sites - a fallow farmland, and a secondary regrowth forest, all of which are located at

the Permanent Site of the Nnamdi Azikiwe University, Awka. Awka, is the capital of Anambra State of Nigeria, is located in the lowland rain forest zone of Southern Nigeria (Keay, 1965; Charter, 1970).

The fallow farmland lies between latitude 6.25054^{O} N and longitude 7.12078^{O} E. The plot has been left fallow for twelve years after the previous cultivation and therefore was overgrown with plants and common weeds of fallows. Identified herbaceous plants included *Chromolaena odorata* (Kings and Robinson), *Aspilia africana* C.D. Adams), T*ridax procumbens* (L.), *Axonopus compressus (*Beauv.), *Mariscus longibracteatus* (Cherm.), *Sida acuta* (Burm). *Panicum maximum (*Jacq.) and *Veronia ambigua (*Kotchsky and Peyr.) Trees found at the plot included *Pentaclethra macrophyla* (Bentham), *Chlorophora excelsa* (Welw.) *Mangifera indica* (L.), *Combretum molle (*R. Br.), *Eleais guineensis* (Jacq.), *Newbouldia laevis* (P. Beauv.), *Terminalia ivorensis* (A. Chev.) and., *Anthonata macrophylla* (P. Beauv.). The fallow farmland which is sandy loam and over $1000m^2$ in area is separated from the cultivated farmland by a tarred road leading from the first gate of the Permanent Site of the Nnamdi Azikiwe University, Awka.

Similarly the forest under study can be described as a secondary regrowth forest in an area of forest – agricultural mosaic (Lasebikan, 1974). The study area lies between latitude 6.25774^{O} N and longitude 7.11275^{O} E. Alternatively it is located south east to east of the School of Postgraduate Studies and general south east of Rufai Garba Square with an approximate bearing of 125^{O} and a distance of 200m from the centre point of the Square. The size of the sampling plot is about 2000 m in area.

The herbaceous plants found at the fringe of the forest included *Chromolaena odorata* (L.) and *Panicum maximum (*Jacq.) In addition, shrubs like *Mallotus oppositifolius* (Giezel), and trees *Newbouldia laevis* (P. Beauv.), *Alstolia boonei (*de Wild), *Diallum guineensis* (L.), *Alchornea cordifolia* (Schum and Thonn.), *Alstonia bonei* (de Wild), *Ceiba pentandra* (Linn.) Gaertn., *Chlorophora exelsa (*Welw.) *Harungana madagascariensis (*Lam and Pols), *Newbouldia laevis* (P. Beauv)., *Mormda lucida* (Benth.), *Pterocarpus milbraedii (*Harrns.), *Ricinodendron heudelotti* (Bail)., *Rauvolfia vomitoria* (Afyel) and *Fagara macrophylla (*Engl.) were found.

Sampling Method: Eight pitfall traps made of plastic containers, with mouth diameters of 9.80 cm and 6.2 cm deep were set in all the study sites on monthly, for a twelve month period. The traps were filled to one-third with 5 % formalin. The traps were collected after twenty-four hours and the insects caught were sorted and counted under a dissecting microscope.

Rainfall data was collected during the sampling period using the rain gauge, while mercury in bulb thermometer was used to measure aerial and soil temperature on each sampling occasion. The readings of those temperatures were taken twice in each case both at the time of setting the traps and during their collection. Relative humidity was measured three times (with their average taken) on each sampling occasion using the whirling hygrometer. The relative humidity was obtained from the reading of wet and dry bulb thermometers of the whirling hygrometer by reference to an accompanying and usually laminated hygrometrical (conversion) table.

The insects and their larvae were identified using insect of Nigeria – Check List and Bibliography by Medler (1980). The identification of the specimens was verified in the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria Nigeria. The voucher specimens were also kept as point for further studies. The t-test was used to compare the forest and the fallow plot. Linear correlation test was carried out between selected environmental variable and the ant species sampled from the two contrasting habitats to assess any closeness of relationship. The site location was carried out using the Global Positioning System (GPS).

RESULTS

The results of the monthly pitfall catches of ant species from the forest and the fallow plot are shown in Table 1. A total number of ten species belonging to eight genera were recorded during the twelve-month sampling period. From the statistical analysis of the data using the Student t-test all the species failed to show any significant differences in their trapping except *Camponotus acvapimensis* and *C. perrisi* which showed significant difference at a t-value of 2.564 and 2.131 respectively, with less catches obtained at the forest than the fallow plot. Table 2 shows the physical variables – mean soil temperature, mean relative humidity and monthly rainfall. The mean soil temperature in the forest was relatively lower at the forest than in the fallow plot. On the other hand the monthly mean relative humidity was consistently higher at the forest than the fallow plot. The highest monthly rainfall was experienced in May 1998 while the months of January, February and December failed to experience rainfall. Table 3 shows the correlation coefficient values (r) obtained when the pitfall catches of some of the species were correlated with selected physical variables - mean soil temperature, mean relative humidity and rainfall. Significant positive correlation values were obtained for *Camponotus perrisi* and *Dorylus affinis* at the forest with r values of 0.64 and 0.84 respectively at probability level ($p \leq 0.05$), when the pitfall catches were correlated with mean soil temperature. At the fallow plot however the relative populations of *Acantholepsis* correlated with mean soil temperature correlated with monthly mean soil temperature ($r = 0.54$)) at $p \leq 0.10$ but negatively correlated with monthly mean relative humidity ($r = -0.61$) at $p \leq 0.05$).

Table 1: Monthly pitfall catches of ants species from the forest and the fallow plot

Ant Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
***Acantholepsis* sp.**	2	-	-	-	-	-	-	-	-	-	-	6	8
	(13)	-	-	-	(7)	-	(1)	-	-	-	(3)	-	(24)
Camponotus acvapimensis	-	2	1	1	3	-	-	-	2	-	-	3	10
	(7)	-	-	(3)	(5)	(2)	-	(1)	(2)	(6)	(6)	(4)	(36)
C. *perrisi*	3	-	-	-	2	-	-	-	1	-	-	-	6
	-	(2)	(2)	(5)	(2)	-	(1)	(2)	-	-	(2)	(4)	(20)
C. maculatus	-	-	-	-	-	-	-	-	-	-	-	-	0
	-	(5)	-	-	-	-	-	-	-	-	(1)	-	(6)
Dorylus affinis	16	-	-	-	-	-	-	-	-	-	-	-	16
	-	-	-	-	-	-	-	-	-	-	-	-	(0)
Megaponera foetans	-	-	1	-	-	1	-	1	2	1	-	-	6
	(11)	(1)	-	-	-	-	-	-	-	(1)	-	-	(13)
***Pheidole* sp.**	-	-	14	-	2	4	-	2	-	3	6	10	41
	-	-	(1)	(1)	-	-	-	(1)	(1)	(3)	(2)	(11)	(24)
***Crematogaster* sp.**	-	1	-	-	-	-	-	-	-	-	-	-	1
	-	-	-	-	-	-	-	-	-	-	-	-	(0)
Oecophylla *ionginoda*	-	-	-	-	-	-	-	-	-	-	3	-	3
	-	-	-	-	-	-	-	-	-	-	-	-	(0)
Myrmicaria striata	1	-	-	-	-	-	-	-	-	-	-	-	1
	-	-	-	-	-	-	-	-	-	-	-	-	(0)

Number of ants in fallow plot in parenthesis

Table 2: Monthly means meteorological parameters obtained at the two study sites during sampling

Month in 1998	Mean soil temperature		Mean relative humidity		Rainfall (daily average)	
	Fallow Plot	Forest	Fallow Plot	Forest	Fallow Plot	Forest
January	35.50	33.50	52.00	78.00	-	-
February	35.75	28.50	53.50	63.00	0.6	0.60
March	37.00	29.00	44.50	57.00	1.20	1.20
April	30.75	27.50	76.50	77.00	4.00	4.00
May	31.25	28.00	74.50	83.00	13.60	13.60
June	29.00	28.00	84.50	87.00	11.00	11.00
July	29.25	26.50	80.50	87.00	12.60	12.60
August	33.50	26.00	88.50	96.00	3.20	3.20
September	30.00	28.50	79.00	85.50	9.80	9.80
October	29.00	31.00	68.00	63.00	12.50	12.50
November	26.00	30.00	65.50	74.00	-	-
December	27.50	29.00	73.00	72.50	-	-

Table 3: Linear Correlation coefficient values between selected environmental variables and some ant species sampled at the forest and the Fallow Plot

Ant Species	Selected environmental variables at the plots					
	Mean soil temperature		Mean relative humidity		Rainfall (daily average)	
	Forest	Fallow plot	Forest	Fallow plot	Forest	Fallow plot
***Acantholepsis* sp.**	**-**	0.54++	**-**	-0.61+	**-**	-0.34
Camponotus perrisi	0.64+	**-**	0.19	**-**	**-**	0.09
Dorylus affinis	0.84+	**-**	0.02	**-**	-0.32	**-**
Megaponera foetans	-	0.39	-	0.45	-	-0.31
***Pheidole* sp.**	0.001	-0.49	-0.59+	-0.22	-0.46	0.09
Oecophylla ionginoda	-0.11	-	-0.01	-	-0.09	-

+ Significant at $p \leq 0.05$; ++ Significant at $p \leq 0.10$; - Absence of relevant information

At the forest while the pitfall catches of Ca*mponotus perrisi* correlated positively with monthly mean relative humidity (r = 0.64), the pitfall catches of *Pheidole* sp. showed a negative correlation (r = -0.59) with the monthly mean of the same environmental variable at $p \leq 0.05$.

DISCUSSION

The ants trapped from both the forest and the fallow plot under study largely represent the foraging ants. The non significant differences in the trapping of these foraging ant species except for *Camponotus acvapimensis* and *C. perrisi* is an indication that the fallow environment also favoured the nesting and

foraging activities of this species and indeed other members within the genera (Ewuim, 1996). In addition it has been reported that *Camponotus acvapimensis* are wholly ground nesting apparently over a wide area (Tailor and Adedoyin, 1978; Ewuim, 1997, 2004a).

The relatively higher monthly mean temperature at the forest than in the fallow plot is in agreement with the observation by Whitmore (1998); Ewuim *et al*. (2004) and Ewuim (2006) that the forest provided an internal microenvironment different from the general climate outside the canopy. This also explains the relatively higher monthly mean relative humidity prevalent in the forest interior as opposed to the lower monthly mean relative humidity in the fallow plot which is censored by the observation of Ewuim *et al*. (2004) and Ewuim (2004b) that the forest is humid in nature.

The significant positive correlation obtained for the population of *Camponotus perrisi* and *Dorylus affinis* when correlated with monthly soil temperature was indicative of the importance of this environmental variable to the species. The significant correlation of the densities of ground foraging *Camponotus perrisi* and *Dorylus affinis* with mean soil temperature does not only confirm the ability of these ant species to adapt to these temperature and also explore space efficiently (Bourke and Franks, 1995) but emphasize the importance of soil activities carried out in relation to temporal and spatial organization of the foraging systems (Holldobler and Wilson, 1990), with these ant species exhibiting centrifugal polyethism (or tendency of the old workers to work outside nest) (Bourke and Franks, 1995) associated with ant societies. It has also been observed that *Dorylus* is subterranean in habit building temporary nests, which are abandoned after some (Olaniyan, 1978; Ewuim, 2004b) which also strengthens the importance of soil temperature to *Dorylus affinis.*

The significant positive correlation of the population of *Acantholepsis* with monthly mean soil temperature is an indication of the influence of this physical variable on the species at the fallow plot. The significant negative correlation observed for *Acantholepsis* with monthly mean relative humidity is an indication of the negative influence of this environmental variable with the species at the fallow plot.

The significant positive correlation of *Pheidole* species with monthly mean relative humidity at the forest is also indicative of the importance of this environmental to the foraging activities the species. The fallow plot evidently favoured the foraging activities of *Pheidole* which have been described as harvesters since they feed on plant seeds, like those of grasses abundant in the fallow plot (Wilson, 1959, Ewuim, 1997).

In terms of the environmental implications of the ants sampled from the agro-ecosystem, ants are known to exert remarkable influence on ecosystems. In a heterogeneous environment where patches offer different conditions for growth, or have been disturbed at different times in the past, completive exclusion is likely to be very slow and might never reach completion (Palmer, 1994). More heterogeneous environments would then be expected to support greater number of species (Williams, 1964, Bell *et al*., 2000), with the ant species constituting predators, pathogen vectors, pests and of beneficial value.

The eight genera and ten species of ants taken in the pitfall traps with these ants belonging to the family Formicidae were represented in both habitats. Ant species (in addition to termites and earthworms) have been referred to as ecosystem engineers (Jones *et al*., 1994; Jones *et al*., 1996) in relation to their role in habitats. These ants are not only responsive to human impact but are important within the below ground process, not only through alteration of the physical and chemical environment, but through their effects on plants and micro-organisms (Folgarait, 1998).

Pheidole can also serve as bioindicators in habitats where they are found together with other species (Anderson, 1997). *Pheidole* is also implicated as a predator in tropical terrestrial ecosystem (Way and Khoo, 1992) with the exhibition of polyphenism, which allows the production of different castes in relation to colony needs and thus influencing their number in these habitats (Wheeler and Nijhout, 1983; 1984). *Pheidole* can serve as pests under synanthropic conditions. The predaceous, *Oecophylla longinoda* reputed to be the most aggressive insect, lives and nests in trees (De Pury, 1968). These tailor ant utilizes silk from larvae approaching metamorphosis to fasten the leaves of their nests together (Prudhomme *et al.,* 1985). By implication, therefore, *O. longinoda* is regarded as a nuisance pest capable of making harvesting of crops difficult, while reducing the photosynthetic efficiency of the leaves bound together in the course of building these nests (NFMANR and ODABG, 1996).

Crematogaster sp. are also tree nesting and have been classified as scavengers (Wilson 1959; De Pury, 1968) involved in tending honey dews produced by other insects. *Crematogaster* also produce phenolic compounds such as 3-pentylphenol from their metapleural gland with antibiotic properties for defense against pathogens (Chapman, 2000). By implication it is being suggested that their activities in their habitat may implicate them as pests under certain conditions. *Crematogaster* sp. including *C. gambiense* have been reported as a nuisance and a synanthropic formicid (ant) serving as pests of food stuff like crayfish in homes (Emosuairue, 1998). Species of *Crematogaster* occasionally damage cocoa and coffee (Le Pelley, 1968; Entwistle, 1972). *Crematogaster* sp. and *Oecophylla* sp. are arboreal (May, 1973; Bolton, 1973; Ewuim *et al.,* 1997), hence their low trapping in these pitfall traps e.g. (Ewuim *et al.,* 1997). It is therefore not surprising

that even though *Oecophylla* were observed in their nests on some tree especially at the forest, their relative abundance was not reflected in the pitfall traps as opposed to the numbers of the ground nesting *Camponotus* species. These ground-nesting *Camponotus* species under indoor or environment can constitute a nuisance, as opposed to their asynanthropic conditions in the plots.

Predation is widespread among the *Camponotus* sp., *Dorylus affinis* and *Megaponera foetans* thus making them liable for use as control agents for noxious species. *M. foetans* has been used for control of certain termite species (Skaife, 1953). These observations are in agreement with the report that ants have a major influence on other organisms in the tropics where some important predatory species serve as control agent (Caroll and Risch, 1983; Way and Khoo, 1992). *Dorylus* sp. can however constitute a serious pest to crops (Viswanath and Veeresh, 1988).

Finally *Camponotus perrisi,* and other *Camponotus* sp. were also taken in the traps. *Camponotus* sp. have pest status in these habitats. *Camponotus* sp. for example can strip bark off the roots of plants species (Le Pelley, 1968). The camponotine ants in these two agro- ecosystems can play beneficial role, largely carnivorous and usually ground-nesting and can help to reduce termite populations where they are found (Skaife, 1953) and a strategy in their ecosystem engineering services (Folgarait, 1998).

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REFERENCES

ANDERSON, A. N. (1997), Using ants as bioindicators: multiscale issues on ant community ecology. *Conservation Ecology, (1):* 1 – 8.

BADEJO, M. A. and VAN STRAALEN, N. M. (1993). The seasonal abundance of springtails, in two contrasting environments. *Biotropica, 25(2):* 222 - 228.

BELL, G., LECHOWCZ, M. J. and WATER, M. J. (2000). Environmental heterogeneity and species diversity of forest sedges. *Journal of Ecology,* 88: 67 - 87.

BOLTON, B. (1973). The ant genera of West Africa, A synonomic synopsis with keys (Hymenoptera: Formicidae). *Bulletin of British Museum and Natural History and Entomology, 27:* 319 - 368.

BOLTON, B. (2003). *Synopsis and classification of Formicidae.* Memoirs of the American Entomological Institute. Volume 71, Gainesville Florida, 370 pp.

BOORMAN, J. (1981). *West African Insects*. Longman Group Ltd., Essex. 81 pp.

BOURKE, A. F. G. and FRANKS, N. R. (1995). *Social evolution in ants.* Princeton University Press, New Jersey. 529 pp.

CARROL, C. R. and JANZEN, D. M. (1973). Ecology of foraging ants. *Review of Ecology and Systematics, 4:* 231– 257.

CARROL, C. R. and RISCH, S. J. (1983). Tropical Annual Cropping Systems: Ant Ecology. *Environmental Management,* 7: 51 – 57.

CHAPMAN, R. F. (2000). *The insects: Structure and Function.* 4th edition. Cambridge University Press, UK. 770 pp.

CHARTER, J. R. (1970). *Vegetation and Ecological Zones.* Federal Department of Forest Research, Ibadan, Nigeria.

DE PURY, J. M. S. (1968). *Crop Pests of East Africa.* Oxford University Press, London, 227 pp.

EMOSAIRUE, S. O. (1998). Plant derived oils as short term protectants of stored crayfish against infestation of the synanthropic formicid, *Crematogaster gambiensis* (And.) (Hemiptera: Formicidae). *Entomological Society of Nigeria Occasional Publication,* 31: 267 – 271.

ENTWISTLE, P. F. (1972). *Pest of Cocoa.* Longman, London 799 pp

EWUIM, S. C. (1996). Use of Pitfall trapping in sampling ants from two contrasting farmlands in Awka, Nigeria. *Journal of Science, Engineering and Technology,* 3(1): 331 – 338.

EWUIM, S. C. (1997). A comparative study of ant species sampled from a tropical rainforest and a fallow farmland using pitfall technique. *Journal of Science, Engineering and Technology* 4(1): 496 – 702.

EWUIM, S. C. (2002). A study of the arthropod fauna of a cultivated plot and a fallow farmland in Awka with particular reference to the insects using pitfall technique. *Journal of Multidisciplinary Studies* 8(1): 64 – 68.

EWUIM, S. C., (2004a). A study of insect fauna of the Permanent Site of Nnamdi Azikiwe University, Awka. PhD. Thesis, Nnamdi Azikiwe, University, Awka. 269 pp.

EWUIM, S. C. (2004b). A comparative study of the efficacy of selected preservatives used in pitfall and water traps for sampling arthropods in Awka. *African Journal of Science,* 5(1): 1003 – 1013.

EWUIM, S. C. (2006). A study of the relationship between pitfall trap parameters and catches in two habitats in Ile-Ife, Nigeria. *A Multidisciplinary Journal for Advancement of Scholarship,* 1(1): 149 – 154.

EWUIM, S. C., EGWUATU R. I. and NWANA, I. E. (2004). A comparison of epigaeic insect

populations from two habitats in Awka. *World Journal of Biotechnology,* 5: 815 – 819.

EWUIM, S. C. and EZENWUGO, M. (1997). Formicid fauna of three contrasting habitats in the Nnamdi Azikiwe University, Awka, Nigeria. *Journal of Science, Engineering and Technology,* 4(2): 771 – 779.

EWUIM, S. C., BADEJO, M. A. and AJAYI, O. O. (1997). Ants of forest and fallow plots in Nigeria. *Biotropica*, 29(1): 93-99.

EWUIM, S. C. and NWUBA, L. A. (2002). A comparison of the efficacy of ethanol in relation to three killing agents used in pitfall trapping in a fallow farmland in Awka. *Journal of Multidisciplinary Studies. 9(1):* 37 - 43.

FOLGARAIT, P. J. (1998). Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiversity and Conservation*, *7(9):* 1221 – 1244.

FRANKS, N. R. and BOSSERT, W. K. (1083). The influence of swarm raiding army ants on the patchiness and diversity of tropical leaf litter and communities. Pages 51 – 163. *In:* SUTTON, E. L. , WHITMORE, T. C. and CHADWICK, A. C. (EDS.) *Tropical rainforest: Ecology and Management.* Blackwell Scientific Publication Ltd., Oxford, England.

GREENSLADE, P. J. M. (1976). The meat ant and *Iridomyremex purpureus* (Hymenoptera: Formicidae) as a dominant member of an communities. *Journal of Austrian Entomological Society*, *15*: 237 - 240.

HOLLDOBLER, B. and WILSON, E. O. (1990). *The ants.* Harvard University Press, Cambridge

JONES, G. G. LAWTON, J. H. and SHECKAK, M. (1994). Organisms as ecosystems engineers. *Oikos, 69:* 373 - 386.

JONES, I. G., LAWTON, J. H. and SHACKAK, M. (1996). Organisms as ecosystems management, Springer – verlag, New York.

JONES C. G. LAWTON, J. H. and SHACK, M. (1994). Organisms as ecosystems engineers. *Oikos, 63:* 373 – 386.

KEAY, R. W. J. (1965). *An outline of Nigeria vegetation*, Federal Ministry of information, Lagos, Nigeria. 46 pp.

LABEIKAN, B. A. (1974). Preliminary to communications on micro arthropods from tropical rainforest in Nigeria. *Pedebiologia*, *14:* 402 – 411.

LASEBIKAN, B. A. (1977). Arthropod fauna of a decaying log of an oil palm tree. *Elaeis guineensis* Jacq in Nigeria. *Ecological Bulletin.* 25: 530 - 533.

LASEBIKAN, B. A. (1985). Forest litter decomposition and changes in population size of microarthropods. Pages 40-48. *In:* STRIGONOVA, A. (Ed.) *Soil Fauna and Soil Fertility.* Proceeding of 9th International Colloquium on Soil Zoology, Moscow, Russia, August 1985.

LASEBIKAN, B. A., BADEJO, M. A. and PRABHOO, N. R. (1985). The collembola fauna of two contrasting habitats at the University of Ife Campus, Nigeria. Pages 754 - 758. *In:* A. Strigonova (Ed.). Soil fauna and soil fertility. Proceedings of the 9th International Colloquium on Soil Zoology, Moscow, Russia, August 1985.

LASEBIKAN, B. A. (1974). Preliminary communication on micro arthropods from a tropical rain forest in Nigeria. Pedobiologia, 14: 402 – 411.

LE PELLEY, R. H. (1968). *Pests of Coffee.* Longman, London. 590 pp.

MAY, R. M. (1973). *Stability and complexity in model ecosystem.* Princeton University Press, Princeton.

NFMANR and ODABG (1996). Nigerian Federal Ministry of Agriculture and Natural Resources and Resource Development Administration of the British Government (1996). *Insect pests of Nigerian Crops: Identification, Biology and Control.* Natural Resource Institute Chatham, U.K. 253 pp.

OLANIYAN, C. J. O. (1978). *An introduction to West African Animal Ecology*. Heinemann Educational Books Limited, Ibadan. 170 pp.

PALMER, M. W. (1994). Variation in Species richness: towards a unification of hypothesis. *Folia Geobotanica Phytotaxaonoica,* 29: 511 - 530.

PRUDHOMME, J. C., COUBLE, P., GAREL, J. P. and DAILLIE, J. (1985). Silk synthesis. Pages 571 - 594. *In:* KERKUT, G. A. and GILBERT, L. I. (Eds). *Comprehensive Insect Physiology*, Biochemistry and Pharmacology, Volume 10, Pergamon Press, Oxford.

SKAIFE, S. A. (1953). *West African Life.* Longman, London, 387 pp.

TAYLOR, B and ADEDOYIN, S. F. (1978). The abundance of litter specific relations of common ant species (Hymenoptera: Formicidae) on cocoa farms in Nigeria, *Bulletin of Entomological Research.* 68(1): 105-122.

TORRES, J. A. (1984). Niches and co-existence of ant communities in Puerto Rico. *Biotropica, 16(4):* 284 – 295.

WAY, M. J. and KHOO, K. C. (1992). Role of ants in pest management. *Annual* Review *of Entomology*, *37:* 479 - 503.

WHITMORE, T. C. (1998). *An introduction to tropical rainforest.* 2nd Oxford University Press, New York pp. 282

WHEELER, D. E. and NIJHOUT. H. F. (1983). Soldier determination in *Pheidole bicarinata:* effect of methoprene on caste and size within casts. *Journal of Insect Physiology.* 29: 847 – 854.

WHEELER, D. E. and NIJHOUT, H. E. (1984). Soldier determination in *Pheidole bicarinata:* inhibition

of adult soldier. *Journal of Insect Physiology,* 30: 127 – 135.

WILLIAMS, G. (1959). The seasonal and diurnal activity of the fauna sampled by pitfall traps in different habitats. *Journal of Animal Ecology* 28: 309 – 330.

WILLIAMS, C. B. (1964). *Patterns in the balance of nature,* Academic Press, New York.

WILSON, E. O. (1959). Some ecological characteristics of ants. *In:* New Guinea Rainforest, Ecology 48(3): 437 – 447.

VISWANATH, B. N. and VEERESH, G. K. (1988). Ants. *In:* VEERESH, G. K. and RAGAGOPAL, D. (Eds). *Applied Soil Biology and Ecology.* 2nd Edition. Oxford and IBH Publishing Company. PVT Limited. New Delhi. 375 pp.

QUALITATIVE STUDY OF ANOPHELES SPECIES IN KONDUGA LAKE AREA OF BORNO STATE, NIGERIA

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ABSTRACT

The investigation on Anopheles species in Konduga lake area, Borno State of Nigeria, was carried out to identify various Anopheles species prevalent in the area and to determine their relative population densities. Six Anopheles species were recorded, namely, A. gambiae, A. funestus, A. ziemanni, A. squamosus, A. pharoensis, and A. maculipalpis. The relative population densities of various species were higher during rainy season than during the dry season. The population densities of female Anopheles were higher than those of the males. With the exemption of A. maculipalpis, all the other Anopheles species recorded during the study are known transmitters of human malaria. A. gambiae of the A. gambiae complex was dominant in the study area. The periodic occurrence of Anopheles explains periodicity of malaria epidemiology in the study area. The high population of the female Anopheles predisposes inhabitants of the study area to incessant contact with the malaria vectors. Lake Konduga and its environments seem to satisfy the basic requirements of Anopheles mosquitoes growth and survival.

Keywords: Tropical lake, Anopheles species, Malaria vectors

INTRODUCTION

Mosquitoes are perhaps the best known of all insects in the tropical and temperate regions of the world because of their aggressiveness not only to humans but to other animals as well. This is due to their painful bites and to the fact that they are vectors of causative agents of several dangerous diseases of man and his domestic animals. The most important man-biting mosquito species belong to the genera *Anopheles, Aedes, Gules, Haemogogus, Mansonia, Psorophora* and *Sabethes* (Service, 1980).

Breading sites of mosquitoes vary depending on species. The sites include freshwater, such as edges of streams, permanent still water bodies, such as gutters, discarded containers, tree holes, leaf axils and others.

Generally, *Anopheles* species thrive well in habitats where nice and deep freshwater, with good amount of vegetation of provide food and shelter is available. They thrive best in habitats where they are protected from extreme heat. The adults are, thus, adapted to definite ranges of temperature and variations in humidity. Konduga lake seems to possess these qualities for breeding of anophelines.

Anopheles is a gnus which is, among other mosquito genera, easily identified, *Anopheles* adults possess, in common, speckled wings with dark and pale-coloured scales, and the scutella are single-lobed, among other distinguishing features. Female palps are almost as long as the proboscis. When resting or biting the abdomen of an *Anopheles* is usually held up at an angel from the surface on which it is resting, forming a straight line with the proboscis. Cohen (1982) observed that *Anopheles* females tend to fly quietly and bite less painfully, and so their approaches are seldom noticed.

Female anophelines are important vectors responsible for the maintenance and spread of causative agents of human and animal malaria. Besides spreading malaria and other diseases, they cause irritation and annoyance by their bites and buzzing sound. Generally, when a disease is spread by a vector, it is simpler, cheaper, and more cost effective to attack the vector rather than the pathogen, thus a study of the ecology of the vector is necessary. This study is designed to contribute towards solving the problem in Konduga lake area of Borno State.

The study mainly attempts to provide qualitative data on relative population densities of *Anopheles* species incident in Konduga lake area. It also attempt to provide baseline information for subsequent assessment of probable cause of malaria epidemiology in Konduga and so ensure the proper planning of control measures.

MATERIALS AND METHODS

Study Area: Sampling was carried out in Low Cost Housing Estate of Borno State Housing Corporation, Konduga. Konduga is the headquarters of Konduga Local Government Area in Borno State, Nigeria. Konduga lies on latitude 11°40" N and longitude 13°15" E and is located about 32 kilometers from Maiduguri Urban and along Maiduguri-Bama road.

Mosquito Sampling: Indoor and outdoor resting adult mosquitoes were randomly collected once weekly with an oral aspirator between the hour of 4.00 and 7.00 am for a standard period of one hour

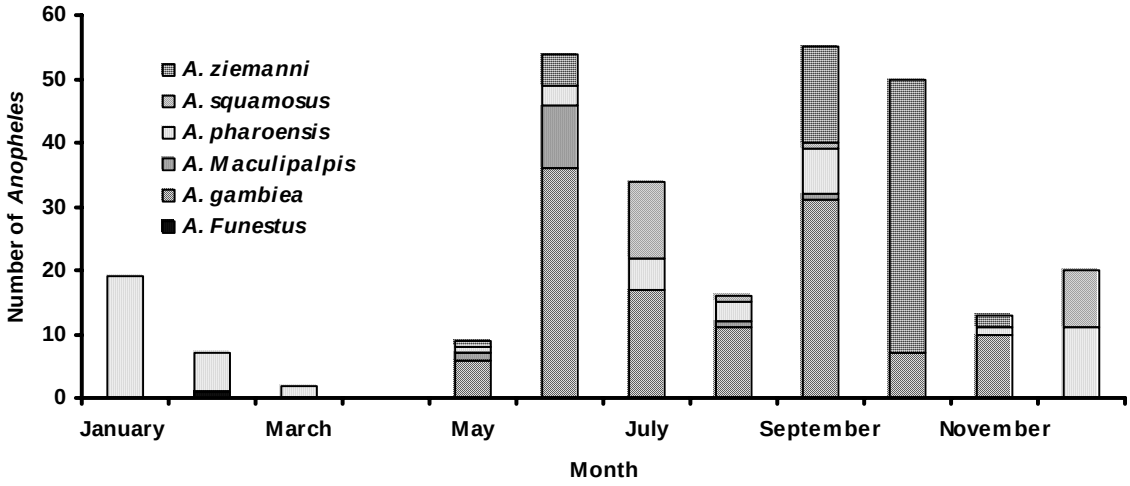

Figure 1: Monthly disribution of *Anophles* species in Konduga lake area

per sampling. Captured mosquitoes were gathered alive in large specimen glass tube with finely perforated cover to allow aeration, and taken to the laboratory on the same day. In the laboratory, the mosquitoes were paralysed with chloroform on top of the perforated specimen tube cover and allowed to stand for some time. The paralysed mosquitoes were, thereafter, identified using a dissecting microscope into culicines and anophelines, then the later were further identified into species and sexes, all in line with identification of mosquitoes by Service (1980). Collection of mosquitoes was carried out for a period of twelve months.

Climatic Data Collection: Atmospheric temperatures in Konduga lake area were recorded thrice daily throughout the survey period using a "maximum and minimum thermometer". Readings were taken in the morning by 4.00 to 7.00 am, at noon by 2.00 to 3.00 pm, and in the night by 8.00 to 9.00 pm. Relative humidities were calculated thrice daily. With the maximum temperature (Tmax) and minimum temperature (Tmin) readings of the thermometer, wet-bulb depression (WBD) was calculated by applying the equation. Tmax – Tmin = WBD. Using the Tmax and WBD values, Relative Humidities were read off from Psychromatic tables.

Data Analysis: Chi-square statistic was used to compare the monthly anopheline mosquito populations while analysis of species catches was done by using student's t-test.

RESULTS AND DISCUSSION

A total of 1481 adult mosquitoes were collected during the twelve months study period. This number was made up of 1197 culicines and 284 anophelines. Six *Anopheles* species, namely, *A. funestus, A. gambiae, A. maculipalpis, A. pharoensis, A. squamousus,* and *A. ziemanni,* occurred in the study area. Monthly and annual abundance of individual species indicated that *A. gambiae* was the most abundant. This result corroborates with the findings of Mafiana *et al.* (1998) and Amusan *et al.* (2003) in Abeokuta, Nigeria. *A. funestus* was the least abundant species. Out of the 284 anophelines caught 202 were females representing 71.1& of the *Anopheles* population.

Figure 1 is a graphic representation of the monthly occurrence of the various *Anopheles* species recorded during the survey period. The six species that occurred in the area showed monthly and seasonal variations in population densities. Populations were high during rains and low in dry months. This results conforms with Vas *et al.* (2004) and Jude *et al.* (2007) observations in Assam, India, and Central South-West Cameroon respectively. June of the survey period marked the on-set of rains while rains progressed through July and August but ended in September during the period. High *Anopheles* populations were recorded in June, September, and October of the study period with a population peak observed in September; five out of the six recorded *Anopheles* species occurred in this month. Whereas four species occurred in June and August, three occurred in July and October, which was the on-set of dryness, recorded two species. While complete absence of *Anopheles* was observed in April, other dry months witnessed low population densities. The various Anopheles species exhibited diversity in monthly occurrence. In January and March of the study period only *A. pharoensis* occurred. This same species occurred in ten out of the eleven months in which *Anopheles* were captured, being absent in October only. Meteorological records obtained in Konduga during the period of the survey indicate that April months was the warmest. Table 1 shows the monthly mean atmospheric temperature and relative humidities during the period.

The mean relative humidities of 71.9 percent and 70.7 percent recorded in July ad September respectively implicated these months to be most humid during the period, student t-test shows no

Table 1: Monthly mean temperatures and relative humilities at Konduga during the study period

Month	Mean Temperature (°C)	Mean Relative Humidity (%)
January	22.6	38.4
February	24.5	29.6
March	31.6	28.1
April	34.8	31.1
May	33.5	48.0
June	28.2	65.9
July	27.5	71.9
August	28.4	55.0
September	30.8	70.7
October	31.5	57.3
November	29.1	45.5
December	29.5	46.1

correlation between the mean temperature and relative humidities during the study period (P>0.05). However, there was positive correlation between mean relative humidity and monthly *Anopheles* population whereas there was no correlation between mean temperatures and monthly Anopheles populations.

The study area, being a typical arid zone, is known for lengthy dry season with associated dry winds lasting for about eight months per annum. During the present study the dry and windy period was observed from January to May then October to December during which period a few of the *Anopheles* species occurred with their attendant low population densities probably due to unfavourable breeding conditions caused by dryness of environments. June to September constituted the period of rains. The number of species and their populations apparently increased during this period probably due to favourable breeding conditions. With on-set of rains in June, there was sharp rise in population of *Anopheles* in the month with *A. gambiae* being the most abundant thereby confirming Mattingly (1969) finding that this species appears very rapidly with the on-set of rains.

Gadzama (1980) found that repeated rainfall causes flooding resulting in the temporary flushing of breeding places, especially for *A. gambiae* complex, thus, reducing the population of vector in the area, but the population becomes re-established when favourable conditions are restored. July and August of the study period witnessed repeated rainfall resulting in fluctuations in the populations of the various species, especially of *A. gambiae*. *Anopheles* population peak was recorded in September when favourable conditions were restored. These results conform with Gadzama (1977) finding that early October witnessed the on-set of cold, dry, windy harmattan period in the study area. As the harmattan intensified through December and January the population of *Anopheles* sharply dropped testifying the fact that population of the mosquitoes genus decline in the dry months and increase during the wet months. Exceptions to this variation patterns were *A. pharoensis* and *A. funestus* during the dry and wet months of the study. A similar observation was made by Gadzama (1983) when he studied mosquito vectors of the Sahel savanna.

The density of a vector species depends much on climatological factors favourable or unfavaourable for its breeding as well as adult survival. For instance, a heavy or repeated rainfall may be favourable for the development of a number of species and yet be detrimental to others. The reduction in population of *A. gambia* and the increases in populations of *A. squamosus, A. pharoensis* and *A. maculipalpis* in the months of repeated or heavy rainfall (July and August) during the present study conform with these facts.

Horsfall (1995) posited that temperatures stimulate mosquito populations in all situations and that tolerance to temperature varies generally from mosquito species to species. The adverse effects of temperature on *Anopheles* populations were clearly observed in dry months of the study period. From Table 1, it could be seen that mean temperature for the month of April was 34.8°C and the month was observed at the warmest or hottest month of the period. The absence of *Anopheles* of all species in this month (Figure 1) may be due partly to the disappearance of breeding places for the genus caused by rapid evaporation of breeding sites, including Konduga lake, induced by high temperature; and partly to the hibernation or aestivation of members of the genus. Mattingly (1969) reported that climate operates directly in determining the seasonal availability of breeding places and the requirement for hibernation or aestivation on the incidence of various mosquito species. The lowest mean temperature during the study period was recorded in January and only *A. pharoensis* occurred in the month. This species seems to tolerate temperature as low as 22.6°C, and as high as 33.5°C. *A. gambiae* seems to tolerate temperature ranges between 28°C and 33.5°C. The study result reveals that majority of the Anopheles species tolerate temperature ranges between 27.5°C and 33.5°C.

High population of female of the genus recorded during the survey indicates that the breeding places for the *Anopheles* were far away from the sampling sites. Konduga lake, located about 1.5 kilometers away from the sties, is probably the breeding place for most of the *Anopheles* species that occurred in the study area.

REFERENCES

AMUSAN, A. A. S., MAFIANA, C. F., IDOWU, A. B. and OKE, O. A. (2003). A survey of Adult Mosquitoes in the hostels of the University of Agriculture, Abeokuta, Ogun State, Nigeria. *Nigerian Journal of Parasitology, 24:*167 – 172.

COHEN, S. (1982). Malaria. *British Medical Bulletin, 28:* 126 – 148.

GILLETT, J. D. (1972). Common African Mosquitoes and their Medical Importance. William Heinemann Medical Books Ltd, London. 106 pp.

GADZAMA, N. M. (1977). The distribution of adult mosquitoes at Ahmadu Bello University, Zaria, Nigeria. *Savanna: 6(2):* 179 – 189.

GADZAMA, N. M. (1980). Comparative studies on mosquitoes of Zaria and Maiduguri. *Man and Biosphere. Proceeding of a Workshop held at Kainji lake Research Institute, New Bussa, Nigeria.*

GADZAMA, N. M. (1983). Mosquito vectors of the Sahel Savanna and Environmental Development. *Annals of Borno, 1:* 99 – 103.

HORSFALL, W. R. (1955). *Mosquitoes: their bionomics and relation to disease.* The Ronald Press Company. New York. 723 pp.

HORSFALL, W. R. (1962). *Medical Entomology.* The Ronald Press Company, New York. 467 pp.

JUDE, D. B., LUCIEN, M., VINCENT, P.K.T., MAUREEN, C. and ROSE G. F. L. (2007). Malaria vectors and transmission dynamics in Coastal South-Western Cameroon. *Malaria Journal, 6(5):* 1475 – 1875..

MATTINGLY, P. F. (1969). *The biology of mosquito borne disease.* George Allen and Unwin Ltd. London.

MAFIAN, C. F., ANAEME, L. and OLATUNDE, G. O. (1998). Breeding site of larval mosquitoes in Abeokuta, Nigeria. *Nigerian Journal of Entomology, 15:* 136 – 143.

VAS, DEV., SOBBHAN, P., VINOD, P. S. and SURAJI, P. A. (2004). Physiographic and entomological risk factors of malaria in Assam, India. *American Journal of Tropical Medicine and Hygiene, 71(4):* 451 – 456.

SERVICE, M. W. (1980). *A Guide to Medical Entomology.* Macmillan Press, London. 346 pp.

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MACROBENTHIC FAUNA OF A HUMID TROPICAL WATER RESERVOIR, ABIA STATE, NIGERIA

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ABSTRACT

The macrobenthic fauna of a humid tropical zone water reservoir was investigated from January to December 2002. Benthos was obtained using an improvised Surber Stream Bottom Sampler. Three phyla of macrobenthos (Arthropoda, Mollusca and Annelida) prevail in the reservoir, with quantitative variations. A total of 1,279 macrobenthic animals were recorded. The Arthropoda was represented by larvae of five genera of insects, with a total of 644 (50.4 %), while Mollusca was represented by two genera with a total of 165 (12.9 %), and Annelida by three genera, with a total of 470 (36.7 %) of the benthos. There was significant difference (P<0.05) among the phyla populations of the benthos.

Keywords: Macrobenthic, fauna, Humid, Tropics, Water reservoir

INTRODUCTION

The water reservoir is located in the National Root Crops Research. Institute (NRCRI), Umudike. This Institute is situated 8 kilometers south east of Umuahia, in Abia State. The reservoir was impounded in 1965 from a tributary of the river Qua-Iboe, which passes through the Institute, for irrigation of farms during dry seasons. As a result of the long existence of the reservoir, and erosion of the banks, it has become a large body of water, with an estimated surface area of 15.5 km^2. (Avoaja, 2005) The climate of Umudike is typical of the humid tropics, with fairly even and uniform temperatures throughout the seasons of the year (Iloeje, 1980). The rainy season usually starts from March and ends in October, with relatively constant temperature, frequent rains, and high humidity (Iloeje, 1980).

Both qualitative and quantitative studies on the benthos of lenthic and lotic waters abound in literature (Williams and Hynes, 1971). Poor taxonomic knowledge of African freshwater faunas in general, allows few groups to be identified beyond the generic level, thus limiting detailed analysis of benthic communities. (Williams and Hynes, 1971) According to Vareschi and Vareschi (1984), the benthic fauna of lake Nakuru (Kenya) was remarkably poor in species, which consisted two chironomid species. Lake Chad in contrast had 47 chironomids (Dejoux, 1968). Nematodes were found only very occasionally and the undersides of stones along the western shores of the lake were populated by the coleopteran, *Helochares species (Hydrophiliidae).* Characteristic benthic species of other lakes e.g. Oligochaetes, Chaoberides, Ostracods or Molluscs were completely absent in lake Nakuru (Vareschi and Vareschi, 1984). In a tropical flood river basin, the common invertebrate taxa were crustaceans, insect and gastropod mollusks (Ezenwaji, 1982; Okafor, 1990; Eyo and Ekwonye, 1995). Banerjee and Banerjee (2005) identified five types of macrobenthic fauna (Oligochaeta, Polychaeta, Crustacea, Gastropoda, and Bivalva) in three Indian estuaries.

Imbevbore and Bakare (1970) reported that most benthos and macroinvertebrates fed on the debris that settled at the bottom of water, and in turn served as food for a wide range of fish. Similar observations have been reported (Moss *et al.,* 1987; Eyo and Ekwonye, 1995). The identification of the benthos of this reservoir would give an insight into the natural fish food of the water body. The present paper embodies a study of macrobenthic fauna of the reservoir in Umudike, which adds to the knowledge of distributions and abundance of macrobenthic fauna in tropical freshwater ecosystem.

MATERIALS AND METHODS

Investigation was carried out in the reservoir from January to December 2002. The reservoir was divided into six sampling stations by a transact method. The six sampling stations were approximately two kilometers apart. That gave a stratified sampling station in which sub-divisions of the stations were sampled using a random sampling technique.

Benthos were sampled using an improvised Surber Stream Bottom Sampler, (a net 0.5 cm mesh, with an area of 900 sq. cm, on a metal frame). A sampling site in each of the stations was selected. The net was dipped inside the water (about 0.5 m depth) collecting mud with the edge of the square metal, into the net. The procedure was repeated five times, and each sample was put into different jars. The jars were labeled indicating the number of replicates, and then carried to the laboratory.

Table 1: Monthly macrobenthos collections from the reservoir

Months	Annelida			Mollusca		Arthropoda					Total	
	Nais batata	*Nais simplex*	*Glossiphonia sp.*	*Lymnaea sp.*	*Physella sp.*	*Chironomus sp larvae*	*Dysticus sp. larvae*	*Copelatus sp. larvae*	*Letes sp larvae*	*Ecdyonurus sp. larvae*		No. / M^2
Jan.	7	8	14	5	5	12	8	8	13	10	90	18
Feb.	11	8	10	9	5	12	11	10	11	12	99	19
Mar.	12	14	16	11	4	7	13	8	9	10	104	21
Apr.	13	15	17	10	5	10	8	11	13	14	116	23
May	11	15	14	14	8	8	8	6	11	12	107	21
Jun.	11	11	27	7	7	8	4	9	14	13	111	23
July	13	13	22	6	5	3	8	14	11	14	109	23
Aug.	7	6	32	8	7	13	12	9	13	15	122	23
Sept.	10	12	35	9	7	6	7	7	9	8	110	22
Oct.	7	6	19	6	5	15	17	9	15	14	113	23
Nov.	8	7	13	5	5	13	15	13	13	14	106	21
Dec.	8	7	11	8	4	10	11	10	11	12	92	20
Year	**118**	**122**	**130**	**98**	**67**	**117**	**122**	**114**	**143**	**148**		
Total Phyla		**470**			**165**			**644**				
Total % age		**36.7%**			**12.9%**			**50.4%**				

Table 2: Benthos distribution in stations in the water reservoir

Phylum	Class	Order	Family	Species	Stations					
					1	**2**	**3**	**4**	**5**	**6**
Annelida	Oligochaetae	Naidida	Naididae	*Nais Simplex*	-	-	+	+	+	+
				Nais batata	-	-	+	+	+	+
"	Hirudinea	Gnaltodbella	Glossiphoniidae	*Glossiphonia complonata*	+	+	+	+	+	+
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus sp.(larvae)*	+	+	+	+	+	+
Arthropoda	Insecta	Coleoptera	Dytiscidae	*Dysticus marginalis* (larvae)	-	+	+	+	+	-
"	"	"	"	*Copelatus* sp. (larvae)	-	+	+	-	-	-
"	"	Odonata	Lestidae	*Lestes* sp. (larvae)	+	+	+	+	+	+
"	"	"	"	*Ecdyonurus* sp. (larvae)	+	+	+	+	+	+
Mollusca	Gastropoda	-	Lymnaeiidae	*Lymnaea natalensis*	-	-	+	+	+	-
"	"	-	Physidae	*Physella sp.*	-	-	+	+	+	-

Key: *+ Present, - Absent*

In the laboratory, the contents of each jar were poured into a white enamel pan. The material from each enamel pan was then poured into a fine sieve (a no. 40 U.S. series sieve) to remove water, sand and debris. The organisms in the sieve were sorted out manually, and separated into taxonomic groupings for final identification and enumeration. The procedure was carried out for each of the six stations. The number of benthos per square meter was computed using Lind (1979) thus: umber of benthos per meter = Total no per month / Total area sampled (m^2) where, Total area sampled =area of net x No of replicates.

The animals recovered were put into specimen bottles, labelled, and preserved in 4 % formaldehyde solution for further examination. These specimens were identified using keys (Edmondson, 1966).

Statistical Analysis: SPSS version 10 employing simple percentiles and graphs were used in analyzing

the population of macrobenthic fauna of the reservoir.

RESULTS AND DISCUSSION

The benthic fauna was represented by three animal phyla, Annelida, Mollusca, and Arthropoda. The annelids were Oligochaetes, represented by *Nais simplex* and *Nais batata* and Hirudinea represented by *Glossiphonia complonata*. The Molluscans were the Gastropods, represent by *Lymnaea natalensis* and *Physella* sp. The Arthropods were mainly larvae of insects, represented by the larvae of *Chironomus sp.*; *Dysticus sp.*; *Copelatus sp.*; *Lestes* sp. and *Ecdyonurus sp*. (Table 1). A total of 1,279 benthic specimens were collected during the year. The minimum and maximum benthoses per square metre were recorded. 18 and 23 respectively. The record showed that in terms of number, Arthropoda was the highest with 644 (50.4 %), followed by the Annelida, 470 (36.7 %), and the least was the Mollusca, 165 (12.9 %). Table 2 shows the benthic distribution in the stations.

The Gastropods were found in stations 3, 4 and 5. The insect larvae were found almost in all the stations but stations 1 and 6 recorded no *Dysticus sp*. larvae, and stations 1, 4, 5 and 6 recorded no *Copelatus* larvae. There were significant differences ($P < 0.05$) between the phyla of the macrobenthos of the reservoir.

Benthic organisms play several important roles in the aquatic community. They are involved in the mineralization and recycling of organic matter produced in the open water or brought from external sources (Moss *et al;* 1987). They are important second and third links in the trophic sequence of aquatic communities (Moss *et al;* 1987). Many benthic insect larval forms are major food sources for fishes (Lind, 1979). These insect larvae identified in this study were probably food for both the young and adult fishes in the reservoir. Few animals which have the ability to exploit the benthic mud region of lakes and reservoirs, because of low oxygen tension arising from decay of organic matter, survive. Therefore, the degree of specialization required from the organisms in order to survive in the mud region means that the number of species will be limited (Brown, 1971).This probably, accounted for the few molluscans and Annelids recovered in this study.

REFERENCES

AVOAJA, D.A. (2005). *The Ecology and fish fauna of the Umudike water reservoir in Abia State Nigeria. PhD Thesis,* University of Nigeria Nsukka. 182 pp.

BANERJEE, P. and BANERJEE, S. (2005). Macrobenthic fauna in relation to soil and water quality of a portion of Hooghly Estuary from Konagar to Bally, West Bengal. *Environment and Ecology* 23(3): 687-690.

BROWN, A. L (1971). *Ecology of fresh water*. Heinemann Educational Books Limited, London. 129 pp

DEJOUX C. (1968). Contribution a L'etude des insects aquteques du Tchad. Catalogue des Chironomidae, haoboridae, Odonates, Trichoptere, Hemiptere, phemeropteres. Cah. ORSTOM Ser. *Hydrobiology, 11(2):* 51 – 78.

EDMONDSON, W. T. (1966). *Freshwater Biology* 2nd Ed. New York, London Sydney. 1248 pp.

EYO, J. E. and EKWONYE, U. C. (1995).The macroinvertebrate fauna of pool in the floodplain (Fadama) of the Anambra River, Nigeria. *Freshwater Forum, 5(3):* 160 – 162.

Ezenwaji, H. M. G. (1982). *Aspects of the Biology of Clarias albopunctatus in the Anambra River basin, Nigeria*. M.Sc. Thesis, University of Nigeria Nsukka. 173 pp.

ILOEJE, N. P. (1980). *A New geography of Nigeria*. Longman Nigerian Limited, Lagos. 200 pp.

IMEVBORE, A. M. A. and BAKARE, O. (1970). The food and feeding habits of non-cichlid fishes of the River Niger. Pages 49 – 64. *In: The Kanji Reservoir Area. Volume I, Ecology.* University Press, Ibadan.

LIND, O. T. (1979). *A hand book of common methods in Limnology,* 2nd Edition. C.V. Mosby Company, London. 197 pp.

MOSS, D., ARMILAGE, P. G. and GUNN, R. T. (1987). The use of prediction to assess macro-invertebrate fauna of unpolluted running water sites in Great Britain, using environmental data. *Freshwater Biology, 17:* 41 – 52.

OKAFOR, F. C. (1990). Distribution of freshwater gastropods in the lower River Niger and Cross River basin of South-eastern Nigeria, with reference to their trematode infections. *Batr. Tropical Landwirtsch Veterinary Medicine, 28:* 207 – 216.

VARESCHI, E. and VARESCHI, A. (1984). The ecology of Lake Nakuru (Kenya) IV. Biomass and distribution of consumer organisms. *Oecologia, 61:* 70 – 82.

WILLIAMS, T. R. and HYNES, H. B. N. (1971). A survey of the fauna of streams on mount Elgon, East Africa, with special reference to the Simuliidae (Diptera). *Freshwater Biology, 1:* 227 – 248.

MOLLUSCAN POPULATION OF AN AFRICAN ARID ZONE LAKE

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ABSTRACT

The species composition, abundance and distribution of molluscs population together with some physico-chemical variables from five different stations in the littoral region of lake Alau, Maiduguri; Borno state, were studied from October 2001 to September 2002. Three patterns of seasonal abundance were found, maximal abundance during the rainy season (July -September), moderate abundance during the harmattan season (November – February) and minimal abundance in the dry hot season (March - June). Significant differences in species composition and abundance of the molluscs were found between stations studied. The total number of organisms recorded was 3368 comprising of 1924 Bivalves and 1544 Gastropoda. 8 families were recorded which includes Bithyniidae, Hydrobiidae, Lymnaeidae, Physidae, Valvatidae, Viviparidae, Sphaeridae and Unionidae while 15 species were observed in this study. The predominant families in terms of total number collected from all stations Sphaeridae and Unionidae recording 1006 and 933 organisms with the percentage compositions of 29.86% and 27.7%. The least was from the families Lymnaciidae with total number of 116 and 3.44% as the percentage composition. The abundance of the molluscs was positively and significantly correlated at 5% confidence with temperature (r = 0.675), dissolved oxygen (r = 0.832), phosphate (r = 0.528).

Keywords: Molluscs, Littoral Region, Abundance, Unionidae

INTRODUCTION

The Phylum Mollusca inhabits permanent water bodies across a large range in Africa. Previous work includes studies by Appleton (1974, 1977) on the Mollusca composition of pond and river in South Africa, Babiker *et al.* (1985) on the snails of irrigation system in Sudan, Woolhouse and Chandiwana (1989) on River Zimbabwe snail composition, Akuforgwe *et al,* (1995) Mollusca of Jos dam, Imafidon, (1991); and Omudu and Iyough (2005) on River Benue Mollusca. These studies suggest that the population dynamics of the phylum was greatly affected by the water chemistry.

Idowu (2004) observed that shallow Lake often with a well developed littoral vegetation are often more productive than deep lakes and particularly in the tropics, they are important source for fisheries and other aquatic fauna products. The biology of molluscs has a status which allows a great deal of environmental exploitation because of its relationship with its environment such as being commercial important as source of income, nutrients for certain organism, as well its ability to exist as host of certain parasites of man (Dussart, 1977). These and certain other relationship have greatly necessitated the need for study of the organism. In this paper ecological studies of population dynamics of molluscs in Lake Alau are reported. The data include the study of some physical chemical characteristic as well as relationship between molluscs abundance and these variables.

MATERIALS AND METHODS

Lake Alau is one of the several tropical lakes in Africa, and it is the second largest lake in Borno state, Nigeria. Lake Alau was created in 1987 by damming river Ngadda about 22 km from Maiduguri, along Bama road. It is located between latitude 13^{o} N and 14^{o} N, and longitude 12^{o} E and 13^{o} E. It has a total surface area of 56 km^2 (CBDA, 1986, Bankole *et al.,* 1994). Being located in the north – east arid zone, the climate is Sahelian with three distinct seasons. The rainy season starts from June to October, the harmattan season with dry cold wind from November to February and dry hot season with extreme temperature from March to May. It has a mean depth of 9.5 m, temperance of about 0.48 m, total alkalinity of 38.4 mg/l (Idowu, 2004; Idowu *et al.,* 2004).

The study was carried out for over a 12 months period from October 2001 to September 2002. Five stations were chosen for this study based on accessibility, fishing activities, irrigation and drinking spot for animals. The description of each station is as descried in Idowu *et al*., (2004).

The stations were sampled fortnightly using 0.2 x 0.2 m scoop with a mesh size 2 x 2 mm. Fabricated serrated edge cylindrical bucket of diameter 50 cm and 30 cm height were also used in collection of samples. Each station was sampled in blocks of 10 m lengths. The samples were sorted in the Department of Biological Sciences Laboratory, University of Maiduguri into macro and micro samples using a sieve of 9 meshes per cm.

Each specimen was identified to species level using the keys of Mocas (1959), Brown (1970), Pennak (1978), Fitter and Manuel (1986). The relative abundance and dominance of each group was obtained by direct ratio comparisons using Sorenson diversity index (Margalef, 1982); Shannon (1948) Index as modified by Wilhm (1975) was used to characterize the integral richness and the eaves of distribution.

Qualitative and quantitative data on selected water quality were measured during each visit to the sites. These include water temperature, depth, dissolved oxygen, pH, water current, biochemical oxygen demand (BOD) and conductivity (Boyd, 1979; Apha, 1989). All data on the physical, chemical and biological studies were assessed for normality and homogeneity of variance. Correlation coefficient in relation to gastropod distribution was determined. Data collected for gastropod were subjected to two - way analysis of variance (ANOVA) and F - LSD

RESULTS

The mollusc population in Lake Alau comprised of 2 classes (Gastropoda and Bivalva), 8 families and 15 species (Table 1). Gastropoda had 6 families and 9 species, while Bivalva had 2 families and 6 species respectively. Sphaeridae and Unioniidae had the highest species composition. Hydrobiidae and Valvatidae had 2 species each. All other families i.e. Bithyniidae, Physidae, Vivparidae has a species each.

Most of these species were distributed in all the station except *Lampris radiates, viviparous species,* and *Bithynia tentaculata*, that were not found in stations 1 and 2. *Valvata sincera* was also absent in station 2 and 3. All other families were represented in all the stations, except Bithyniidae, Vivpariidae and Unionidae (Table 1).

The percentage composition calculated for each family shows that Sphaeridae had 29.86%, followed by Uniondae (27.7 %), Bithyniidae (10.90 %) and Physidae (9.85%) respectively. There were no significant difference ($P > 0.05$) between Sphaeridae and Unionidae. No significant difference ($P < 0.05$) was also observed between Bithyniidae and Physidae.

The distribution of molluscan population in relation to stations (Table 2) showed that station 4 had the highest number of species composition (15 species) and all the species were present in this station. The percentage composition in relation to stations showed that station 4 had 48.9% followed by station 5 with (17.5 %), with 14 species. Station 2 had 8.7% with 11 species (Table 1 and 2).

The Sorenson's index of similarity between stations showed the degree of similarity in this order, station 4 (89.96) higher than station 5 (76.66) and stations 3, 1 and 2 with 69.28, 58.50 and 52.72 similarities respectively. No significant difference ($P > 0.05$) was observed between molluscan similarities of stations.

The monthly variation in the population and abundance of Mollusca classes showed that Gastropoda were the most abundant group, and were dominant in all the months except between July and September.

Highest population abundance was observed between July and September (rainy season) which was significantly different ($P < 0.05$) from all other months. A drastic decline in total abundance of both classes was observed between February and May (dry hot season). However, there was an increase in the total numbers collected from October to January (Harmattan season). The seasonal variations showed 3 periods i.e. population increase (October to January). Maximal abundance (June to September) and population decrease (February to May). Gastropoda and Bivalva were found to exhibit the same seasonal periodicity, with marked differences in the relative abundance of species in the various months.

The physicochemical parameters of the five sampling stations are summarized on Table 3. Water temperature varied between 25.05 ± 0.4^{o} C and 27.24 ± 0.12^{o} C, the current was between 19.62 ± 0.30 cm/sec. The highest mean value for transparency was 0.42 ± 0.03 m, while 0.26 ± 0.01 m was the lowest. The dissolved oxygen varied between 5.15 ± 0.03 mg/l and 6.35 ± 0.05 mg/l.

The result of the correlation coefficient calculated between selected physicochemical characteristics and molluscs abundance showed a significant positive correlation with water temperature (0.675), dissolved oxygen (0.832). pH (0.710), current 0.528 and conductivity 0.899 (Table 4).

DISCUSSION

The seasonal variations of molluscs population may provide hints as to the extent of environment perturbation as the populations were proportionally higher in the rainy season and harmattan season. The same strong and pronounced seasonality of macroinvertebrates was observed by Dejoux *et al*, (1971) and Mbagwu (1993) in lake Chad and Tiga respectively. Also, discernable seasonal changes have been recorded in sub tropical Lake Sibaya (Hart, 1993), and Okomu forest reserve sanctuary in Nigeria Ogbeibu *et al.* (1995).

Climatic regime has long been known to explain variation in distribution pattern of aquatic invertebrates including molluscs, and thus community structure at global taxa. It was observed that mollusc fauna were higher in the rainy season especially for Gastropoda. It is likely that the rainy periods which give organisms more opportunity to colonize different habitats contribute to the abundance. The cooler and wetter environment may simply be a more suitable environment for the species

The abundance of gastropod in lake Alau may be due to the types of aquatic habitats in and around the Lake. It is also possible that Gastropoda abundance is a consequence of difference in protection and avoidance from the predators. It is unlikely that fish affect Gastropod distribution in lake Alau.

The overall composition and abundance of mollusc family and species in this study varied both spatially and temporally in response to selected physical chemical factors of the aquatic environment.

Table 1: Molluscan distribution in relation to stations in Lake Alau

Phylum	Class	Family	Species	Station				
				1	2	3	4	5
Mollusca	Gastropoda	Bithynidae	*Bithynia tentaculata*	-	-	+	+	-
		Hydrobiidae	*Potamopyrgus jenkinsi*	+	+	-	+	+
			Hyfrobiidae immatures	+	+	+	+	+
		Lymnaciidae	*Lymnae truncatula*	+	+	+	+	+
			Lymnae palustris	+	+	+	+	+
		Physidae	*Physelia species*	+	+	+	+	+
		Valvatidae	*Valvata lewisi*	+	+	+	+	+
			Valvata sincera	+	-	-	+	+
		Viviparidae	*Viviparous species*	-	-	+	+	+
	Bivala	Hydrobiidae	*Pisidium casternum*	+	+	+	+	+
		Physidae	*Pisidium nitidum*	+	+	+	+	+
		Sphaeridae	*Sphaerum nitidum*	+	+	+	+	+
		Unionidae	*Unio species*	+	+	+	+	+
			Elliptia campalanata	+	+	+	+	+
			Lampris radiate	-	-	-	+	+
Total	2	8	15	12	11	12	15	14

Key: - Absent, + Present

Table 2: The total abundance and percentage composition of Mollusca families collected in relation to station in Lake Alau

Taxa	Stations					Total Collected	Percentage composition
	1	2	3	4	5		
Bithynidae	-	-	106	260	-	366	10.90
Hydrobiidae	63	50	25	100	45	283	8.40
Lymnacidae	10	13	10	48	35	116	3.44
Physidae	46	30	22	200	34	332	3.85
Valvatidae	10	15	18	55	35	133	3.95
Viviparidae	-	-	46	88	65	199	5.9
Sphaeridae	136	168	150	390	162	1006	29.86
Unionidae	39	20	162	500	212	399	27.70
Total	304	296	539	1641	588	3368	100
% /station	**9.02**	**8.78**	**16.0**	**48.7**	**17.5**		**100**

Table 3: Physico-chemical parameters in relation to stations in lake Alau

Parameters	1	2	3	4	5
Temperature (°C)	25.25 ± 0.18^{b}	25.05 ± 0.14^{b}	27.24 ± 0.19^{b}	27.24 ± 0.12^{a}	25.13 ± 0.00^{b}
Current (cm/sec)	26.71 ± 0.30^{b}	25.46 ± 0.27^{b}	25.08 ± 0.36^{b}	25.10 ± 0.28^{b}	19.62 ± 0.22^{a}
Transparency (m)	0.36 ± 0.01^{b}	0.33 ± 0.02^{b}	0.35 ± 0.01^{b}	0.42 ± 0.03^{a}	0.26 ± 0.01^{c}
PH	6.79 ± 0.05^{b}	6.97 ± 0.02^{b}	6.83 ± 0.02^{b}	7.29 ± 0.05^{b}	6.59 ± 0.01^{b}
Dissolved oxygen (mg/l)	6.15 ± 0.05^{a}	6.35 ± 0.05^{a}	5.18 ± 0.02^{b}	6.32 ± 0.01^{a}	5.15 ± 0.03^{b}
Biochemical Oxygen Demand(mg/l)	4.34 ± 0.32^{a}	4.30 ± 0.28^{a}	4.45 ± 0.50^{q}	5.03 ± 0.33^{a}	5.31 ± 0.25^{a}
Conductivity (ohms/cm	131.45 ± 0.75^{b}	128.45 ± 0.52^{b}	119.42 ± 0.83^{a}	115.47 ± 0.75^{a}	118.47 ± 0.16^{a}

Table 4: Correlation Coefficient for selected physical and chemical parameters and mollusc abundance in lake Alau

Physical/ chemical Parameters	Correlation Coefficient "r" $P < 0.05$
Water temperature	0.675
Dissolved oxygen	0.832
Phosphate	0.648
Ph	0.710
Current	0.528
Conductivity	0.899
BOD	

The overriding influence of the temperature, current, dissolved oxygen, conductivity, biochemical oxygen demand (BOD) in distribution and abundance can explain the significant lower numbers observed in all stations between February and June. The effect of the rainy season between July and September may have increased feeding habitat and access to breeding. The various ecological requirements and also water quality parameters affected the spatial distribution and abundance. Various physical chemical factors collectively have an effect on the abundance of molluscs under condition. Okafor (1990) explained how rainfalls affect the quality of the habitat making it suitable or unsuitable for the molluscan population and abundance.

The seasonal pattern of the total mollusc abundance is consistent with the observation of Obureke (1980), Okafor (1990), Omudu and Iyough (2005). This seasonal dynamic is attributed to seasonal periodicity of the quality and quantity of edible, competition, resumption of normal metabolic activities, by those that have gone through period of adverse conditions, interaction as well as climatic changes in the natural environment. The distribution and abundance of the molluscan population in lake Alau may also be attributed to the availability of food, shelter and oviposition sites. This agrees with Whitton 1975, Omudu and Iyough (2005) that water bodies rich in organic and silt matter are known to support thriving populations of macroinvertebrates. The dominat species encountered in this study i.e. *Hydrobidae immature*, *Lymmae palustrism Physelia species.; Valvata lawisi, Pisidium casertanum, Pisidium nitidum Sphaerum nitidum, unio species* and *Eliptia campalanata* were encountered in all the station surveyed.

The correlation coefficient value between the selected physical and chemical parameters and the molluscs abundance showed significant correlation. This agrees with the findings of Imafidon (1991), Agi (1995), Okafor and Ngang (2004) that fresh water molluscan populations thrive well on environment with good high water qualities.

In conclusion, the temperature appears to be the most important factor influencing the development and distribution of molluscs in the lake Alau. Its effect may be direct in presenting optimum conditions for chemical activities for molluscan population, and indirect in distribution within the habitats. Arad *et al*. (1992, 1993), Okafor (1991), Idowu *et al.* (2005) suggested that climate and microhabitats are the main determinants of species resistance to desiccation, availability, distribution and abundance of molluscs in fresh water environment. The present study suggests that these factors may likewise play important roles in their distribution.

REFERENCES

AGI, P. I. (1995). Survey of Freshwater Snail Vectors of Schistosomiasis and study of Physico chemical parameters of the water bodies in Ogoni Communities, River State, Nigeria. *Acta Hydrobiologica, 21:* 3 – 36

AKUFORGWE, P. F., DONDJI, B., OKWUOSA, U.N., DAKUL, D. A and NTONIFOR, H. N. (1995). Observed disparity on Schistosome infection rates in field *Biomphalaria pfeifferi* between two areas of the Jos metropolis, Nigeria. *Parasites,* 2: 89 – 91.

APHA, A. (1979). *Standard Methods for the examination of water and waste water,* 17th Edition. American Public Health Association, Washington D.C. N.Y. 1134 pp.

APPLETON, C. C. (1974). The population fluctuation of five freshwater snails in the eastern Transverse loweld and their relationship to known bilharzias transmission patterns, *South African Journal of Science, 70:* 114 – 150.

APPLETON, C. C. (1977). The influence of temperature on the life cycle and distribution of *Biomphalaria pfeifferi (*krauss, 1948) in South-Eastern Africa. *International journal of Parasitology,* 7: 335 – 345.

ARAD, Z., GOLDENBERG, S., and HELLER, J. (1992). Intraspecific variation in resistance to desiccation and climate gradients in the distribution of the land snails *Xeropicta vestalis. Journal of Zoology,* 226*:* 643 – 656

ARAD, Z., GOLDENBERG, S. and HELLER, J. (1993). Intraspecific variation in resistance to desiccation and climate gradients in the distribution of the land snails Trochoidae Simulata. *Journal of Zoology, 229*: 249 –265

BABIKER, A., FENWICK A, DAFFALLA, A. A. and AMIN, M. A. (1985). Focality and Seasonality of *Schistosoma mansoni* transmission in the Gezira Irrigated Area, *Sudan. Journal of Tropical Medicine and Hygiene, 88:* 57 – 63

BANKOLE, N. O., SULE, O. D., OKWUUNDU, E. C. and AMADI, M. (1994). Preliminary Investigation into the fish and Catch assessment survey of Alau Lake. *Annual Report of National Institute for Freshwater fisheries researc*h (NIFFR) 28 pp.

BOYD, C. E. (1979). *Water quality in warm water fish pond.* Craft Master prints Incorporated, Auburn, Alabama. 353 pp.

BROWN, A. L. (1970). *Key to pond organisms.* Nuffield Advanced Science Penguin Books Limited, England.

CBDA (1986). A resettlement plan for the Lake Alau dam and Jere bowl scheme. Agricultural survey and background studies. *Report submitted to Chad Basin Development Authority by Askon Nigeria Limited*. 59 pp

DEJOUX, C., LAUZANE, L. and LEVEQUE, C. (1971). Nature des fords et reportitiondes organisms benthiques don la region de bal (Lac T. Chad) Cahiers ORSTOM series. *Hydrobilogia, 5:* 213 – 223

DUSSART, G. B. J. (1977). The ecology of *Potamor pygus jenkinsi* (smith) in North West England with a note on Marstoniopsis Schottzi (Schmidt). *Journal of Molluscan Studies,* 43: 208 – 216

FITTER, R. and MANUEL R. (1986). *Field guide to fresh water life.* William Collins and Son Toronto. 382 pp.

HART, H. I. (1993). A national approach to river. *Search, 24:* 125 –130

IDOWU, R. T. (2004). *Limnological Studies of Lake Alau, Borno State, Nigeria*, PhD Thesis, Department of Zoology, University of Nigeria Nsukka, Nigeria 189 pp.

IDOWU, R. T., INYANG N. M, and EYO, J. E. (2004).The physical chemical parameters of an African Arid Zone man made Lake. *Animal Research International. 1(2):* 113 – 119

IDOWU, R. T., INYANG N. M., and MGBENKA, B. O. (2005). Macroinvertebrate studies of the littoral region in an arid Zone Lake Alau, Maiduguri, Borno State. *Pecop Resources,* 11*:* 13 –18.

IMAFIDON, E. U. (1991). Ecological studies of Fresh water snails in Ibadan, Nigeria, *Nigerian Journal of Parasitology,* 12: 59 – 63.

IMAFIDON, E. U. and IYOUGH, A. (2005). Ecological Studies of fresh water snails in Ibadan, Nigeria. *Nigerian Journal of Parasitology, 12:* 59 – 63.

MACAN, T. T. (1959). *A guide to freshwater Invertebrate animals.* Longman England. 406 pp.

MARGALEF, R. (1982) Phytoplankton composition and distribution a san expression of properties of reservoirs. *Canadian Water Resources Journal,* 7: 26 50.

MBAGWU, I. G. (1993). Vertical distribution of macrobenthos invertebrates in the profundal sediments of the impoundment of the dammed river Dambe; *Australian Journal of Aquatic Science, 5:* 19 – 25.

OBUREKE, G. T. (1980). *Studies on the feeding biology anatomical variation and ecology of vector of Schistosomiasis and other fresh water Snails in South western Nigeria.* PhD Thesis, University of Ibadan 370 pp.

OGBEIBU, A. E., EGBORGE, E. and VICTOR, R. (1995). Hydrobiological studies of water bodies in the Okomu forest reserve (Sanctuary) in the Southern Nigeria; distribution and diversity of the invertebrates fauna. *Tropical Freshwater Biology,* 4: 1 – 27.

OKAFOR, F. C. (1990). *Schistosoma haematobium* cercariae transmission pattern in Freshwater systems of Anambra State Nigeria, *Angewandle Parasitologic, 31: 159 –166.*

OKAFOR, F. C. (1991). The effect of temperature on embroyonic development and reproduction of *Bulinus* (Physopsis) *globosus* (Morelet) and Lymnae natalensis (Krauss) (Gastropoda, Pulmonate). *Journal of Aquatic Science, 6*: 7-11

OKAFOR, F. C. and NGANG, I. (2004). Freshwater Snails of Niger–cem, Nkalagu Eastern Nigeria, Observations on some Demographic aspects of the Schistosome-transmitting Bulinids. *Animal Research International,* 2: 120 – 124.

OMUDU, E. A. and IYOUGH A. (2005). Ecological Studies of the Gastropod fauna of some minor tributaries of River fauna. Makurdi, *Nigeria Animal Research International, 2:* 306 – 310.

PENNAK, R. W. (1978). *Freshwater Invertebrate of the United States* 2nd Ed. Wiley Inter, Science, New York 265 pp.

SHANNON, C. E. (1948). A mathematical theory of communication. *Bell Systematic technical journal* 27: 379 – 423.

WOOLHOUSE, M. E. J. and CHANDIWANA, S. K. (1989). Spatial and temporal heterogeneity in the population dynamic of *Bulinus globosus* and *Bomphalaria pfeifferi* and the epidemiology of their infection with schistosomes, *Parasitology, 98:* 21- 34.

WHITTON, B. A. (1975). Zooplankton and Macro invertebrates. Pages 87 –118 In: WHITTON, B.A. (Ed) *Studies in River Ecology* vol. 2. Publisher Limited London.

WILHM, J. L. (1975). Biological indicators of Pollution. Pages 375 – 403. *In:* WHITTON, B. A. (Ed) Studies in River ecology Volume 1. Blackwell Scientific Publication, Oxford.

EVALUATION OF SNAIL MUCIN DISPERSED IN *Brachystegia* GUM GEL AS A WOUND HEALING AGENT

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ABSTRACT

Snail mucin was obtained from the mucilage of Archachatina marginata (Family Arionidae). The wound healing effect of the snail mucin was evaluated with special attention to the effect when combined with honey in Brachystegia eurychoma gel preparation. Brachystegia eurycoma gum, snail mucin and honey were combined in different concentrations in the treatment of wound made by excision model in rats. It was observed that mucin when combined with honey and in the Brachystegia eurycoma gel heals faster than when used alone. Brachystegia eurycoma gum was also observed to effect fast healing of the wounds when used alone. Complete healing was observed in 15 days post treatment. Honey in combination with mucin as well as the Brachystegia eurycoma gel should be harnessed in pharmaceutical formulations for the treatment of wounds. Brachystegia eurycoma gum in right combinations with mucin and honey for wound healing, prevents bacteria infection, scar formation and promotes regeneration of hair follicles.

Keywords: Snail mucin, Honey, *Brachystegia eurychoma* gum, Gel, Wound healing

INTRODUCTION

Wound healing is an important process involving tissue repair and regeneration. A wound is described as a break in the continuity of tissue from violence or trauma and is regarded as healed if there is a restoration of the wound site or inflamed tissue to normal condition (Adikwu and Ikejiuba, 2005). Tremendous advancements have been made in understating the process of wound healing. The cell types and the order in which they appear in the wound have been established. Many growth factors and their functions have been elucidated. Despite the advances in understanding the science of wound healing, many more steps are yet to be discovered.

An incision wound created by scalpel, trauma resulting from a bullet, or tissue death caused by a myocardial infection all undergo a similar and predictable reparative process. Understanding how the body repairs damaged tissue and what factors influence the wound healing process helps the surgeon ensure an acceptance outcome from surgery (Alvarez and Gilbreath, 1982).

The 3 categories of wound healing are primary, secondary and tertiary Primary wound healing involves healings or closure of a wound within hours of its creation. Secondary wound healing involves no formal wound closure or healing; the wound closes spontaneously by contraction and reepithalization. Tertiary wound healing or closure, are also known as delayed primary closure, involves initial debridement of the wound for an extended period and then formal closure with suturing or by another mechanism (McCarty, 1998).

The use of honey in the treatment of wound has been rediscovered, is becoming increasing interest as more effect of its effectiveness has been proved (Zumla and Lutat, 1989). The clinical observations recorded are that infection is rapidly cleared, inflammation, swelling and pain are quickly reduced, odour is reduced, sloughing of necrotic tissue is induced, granulation and epithelisation are hastened, and healing occurs rapidly with minimal scaring. The antimicrobial properties of honey prevent microbial growth in the moist healing environment created. Unlike other topical antiseptics, honey causes no tissue damage. In animal studies it has been demonstrated histologically that it actually promotes the healing process. It has a direct nutrient effect as well as drawing lymph out to he cells by osmosis. The stimulation of healing may also be due to the acidity of honey. The osmosis creates a solution of honey in contact with the wound surface which prevents the dressing sticking, so there is no pain or tissue damage when dressings are changed. There is a controlled clinical trial that have proven honey more effective than silver sulfadiazine and a polyurethane film dressing for the treatment of wound made be burns. Honey can also be used for wound dressing (Zumla and Lutat, 1989).

Many procedures have been used, but in most of the reports it was use to clean the wound first. Many described honey as having a cleansing and deriding action on wounds. The necrotic tissue is being removed, before dressing wounds with honey. Some used rigorous cleansing procedures, scrubbing with a soft toothbrush followed by hydrogen peroxide, saline rinse (Wadi *et al.,* 1987), betadine and another saline rinse, dilute Dakin solution on wound bed and alcohol on the surrounding skin (Subrahmanyam, 1993), or the wound was cleaned with Eusol or aqueous 1 % chlorhexidine (Obaseki-Ebor *et al.,* 1983). Some reported cleaning the wounds before dressing. One cleaned with gauze.

Snail mucin is used for wound healing (Adikwu and Ikejiuba, 2005).

It is rich in glycosamongycans which has been shown to possess wound healing properties (Glade, 1990). It is also used in the removing of keloid scars. Snail mucin is extracted from land snails. The compound acts as biological activator of the elimination of dead and damaged skin cells and the renewal of healthy cells. It also controls bacteria (Kim *et al*, 1996).

Snail mucin prevents, diminishes and eliminates stretch marks (*Striae atrophica*, *Striae distensea*) and scars. Snail mucin utilizes biological activators of mammalian skin growth factors. It dissolves damaged collagen skin cells, triggers the renewal of collagen, elastin and the production of glycosaminoglycans (GAGS) and proteoglycans from within the deep layers of the skin (Kim *et al*., 1996).

GAGS are complex polysaccharides (sugar chains) that participate in the regulation of physiological processes through their interactions with proteoglycans and with a wide variety of proteins. The loss of glycosaminoglycans from the skin weakens the supportive inter-cellular skin (Kim *et al*., 1996).

GAGS and poteoglycans have large water holding capacity, occupy a large space in the extracellular matrix and fill most of the intercellular space between the collagen and elastin fibres. They play a critical role as shock absorbents and provide binding, hydrating and swelling pressure to tissues enabling them to withstand compressional force and prevent tearing and scaring of the deep layers of the skin during pregnancy outgrowth, growth spurts during adolescence, overstretching by body building (in association with steroids) or over stretching by more than average weight gain. They also play a vital role in cell proliferation, migration and adhesion. Proteoglycans and GAGS are found to be prominent molecules during wound healing through their influential role in cell – cell and cell – matrix interactions (Kim *et al*, 1996).

The majority of gels are complex and the detailed chemical composition of some of them has not yet been elucidated. In general, they are high molecular weight carbohydrate polymers formed around a central unit of *D*-galactose of *D*-galacturonic acid linked together by sugar units (Hutchins and Singiser, 1955). Substances frequently called gels are hydrocarbons of high molecular mass, petroleum products, rubber latex, synthetic polymeric gums, balms and resins, which have sticky or gelly nature.
Dispersion of polysaccharides, acids and gels containing them present characteristics which render them suitable for application as laxatives agents for treatment of hypoacidity, vehicles for masking the taste of alkaloid preparations, bases for medicated jellies (Hutchins *et al.,* 1955). Besides their inherent emulsifying and stabilizing properties, some gels like, gel Arabic, their demulcent and emollient characteristics led to a number of uses, from the stabilization of emulsions, suspensions, to the formation of tablets and pills. However, gels such as tragacanth and agar are known to reduce the bacterial effect of incorporated preservatives (Taub *et al*., 1958).

Gels are also used in dairy product manufacture; for example, in ice creams as stabilizers. In ceramic industry, gels may be used for binding, thickening and as a fixing agent for enamels and porcelains. In the textiles industry, gels find use as pigment dispersing aid and above all as a thickening gent for colour printing pastes. The Brachystegia gum used here in the formulation of gels has been evaluated in the formulation of various pharmaceutical products.

MATERIALS AND METHODS

Chemicals: Acetone (Merck), diazepam injection (10 mg/2 ml, Tiajin Medicines and Health Products), methylated spirit (Hardis and Dromedras) and distilled water was obtained from an all glass still. The mucin was obtained from a batch prepared in our laboratory following earlier established procedures (Adikwu, 2005). Purified honey was obtained from the local market and diluted with sterile, distilled water to obtain a viscosity grade that was equivalent to that stated in the Pharmaceutical Codex (1979). Original honey was also purchased from commercial sources and prepared to meet pharmaceutical standards.

Animals: Albino rats of either sex weighing between 184 – 230 g were used during the study. Animals were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Before and after the surgery, the animals were housed individually in a partition metal cage and they were fed *ad libitum* on standard commercial pellet diet and water.

***Brachystegia eurycoma* gum:** *Brachystegia eurycoma* seeds were purchased from Ogigie market, Nsukka, Enugu State, Nigeria. The seeds were identified by Mr. Ozioko of Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka.

Twelve kg of *Brachystegia* seeds, was weighed out, roasted, and soaked in water. The effect of soaking was to increase the moisture content of the seed thereby increasing the swelling index. It was allowed to stand for 12 hours. The outer coat was then peeled off and the cotyledons were washed with tap water and dried for 12 hours in the oven (Manesty F) set to a temperature of 40 ^{0}C.

The dried seeds were collected and milled using a hammer mill (Retsh GMBH 5657 Ham) fitted with sieve no. 0.5. This was to reduce the particle size. The powder material obtained was further passed through sieve no. 60 aperture size and the fines (1.4 kg) were collected and these were used in the precipitation process after dispersion in 2 litres of water. The powder dispersion gave a good viscous mixture which hydrated properly within 24 hours and gave enough gum on precipitation. The dispersion was aided with a glass rod stirrer and then homogenized using a Silverson mixer.

The mixture was then stored for 24 hours to aid hydration of the powder. The dispersion was centrifuged and the supernatant was collected and reserved while the sediment was discarded. The introduction of acetone into the collected supernatant in the ratio of 1:1 resulted in the complete precipitation of the gum. The mixture obtained was centrifuged, the supernatant decanted while the sediment which is the brachystegia gum was collected.

The precipitated gum was further washed in acetone for 10 min, and then dried to a constant weight at a temperature of 40 ^{0}C in an oven. The dried flakes of the gum were reduced in size using an end runner mill (Pascal Engineering H) and were passed through sieve of aperture size 0.355 mm. The pulverized gum was weighed. A yield of 30 % was recorded.

Formulation of Gel: The gels were formulated according to the general formula (Table 1). Snail mucin was poured into a beaker containing 50 ml of distilled water which was stirred with a rod until the mixture was a viscous gel. Then brachystegia gum powder and honey were also added to the mixture and the stirring continued until a uniformly mixed gel was obtained. The gel was then transferred into sterilized plastic containers with cover. This same procedure was used in all the groups according to their various components (Table 1).

Table 1: Composition of the gel

Groups	Quantity of *Brachystegia* gum (g)	Quantity of snail mucin (g)	Quantity of honey (ml)	Volume of distilled water (ml)
A	200	100	7.4	50
B	200	200	14.8	50
C	200	300	22.2	50
D	200	-	-	50

Wound Healing Studies: A total of twenty rats were used for these studies. The animals were divided into five groups of four animals each, and were caged in a partition cage. The animals were anaesthetized with diazepam injection intramuscularly at the dose of 5 mg/kg body weight respectively, and the wound healing was carried out following the excision wound model. The formulations were applied on the inflected wound using a cotton bud. Then, wound areas were measured at 2 days interval up to 15 days. The animals were fed regularly and their drinking water was changed on daily bases. The wound areas on subsequent days were compared with the wound areas on the first day and the percentage contraction were calculated thus: Percentage wound contraction % = $W_{A0} - W_A \times 100 / W_{A0}$ Where: W_{A0} = Wound area on day 0 and W_A = Wound area on subsequent days.

Data Analysis: The data obtained were analyzed using the Student's *t*-test at the 5 % level of significance. Standard deviations of the results were also calculated.

RESULTS AND DISCUSSION

Wound healing process generally has 3 phases. They are the inflammatory phase, the proliferative phase and the maturational phase. The inflammatory phase is characterized by homeostasis and inflammation. Collagen exposed during wound formation, activates the clothing cascade (both the intrinsic and extrinsic pathways) initiating the inflammatory phase (Mazzotta, 1994).

After injury to tissue occur, the cell membrane damaged from the wound formation, releases thrombozane A2 and prostaglandin 2 – alpha, a potent vasoconstrictor. This initial response helps to limit haemorrhage. After a short period, capillary vasodilatation occurs secondary to local histamine release, and the cells of inflammation are able to migrate to the wound bed. The timeline for cell migration in a normal wound healing process is predictable. When the gel containing snail mucin is applied, it helps in arousing the immune system leading to various immune reactions and processes leading to protein proliferation at the point of injury hastening the wound closure. This is because the immune properties of the body at the point of the wound saw snail mucin as foreign.

Platelets, the first response cell, release multiple chemokines, including epidermal growth factor (EGF), fibronectin, fibronogen, histamine, platelet – derived growth factor (PDGF), serotonin, and von Willebrand factor. All these are closely related to the immune processes that can be encouraged by the presence of snail mucin. These factors help stabilize the wound through clot formation. These mediators control bleeding and limit the extent of injury. Platelet deggranulation also activities the complement cascade, specifically Csa, which is a potent chemoattractant for neutophils.

The inflammatory phase continues, and more immune response cell migrate to the wound. Neutrophils are the next substances to migrate to the wound and it is for debris scavenging. Neutrophils, along with the mucin and honey, kill bacteria and decontaminate the wound from foreign debris.

The next cells present in wound are leukocyte and the macrophages (monocytes). The macrophage referred to as orchestrator, which is essential for wound healing, numerous enzymes and cytokines are secreted by the macrophage. These are colagenases, which deride the wound, interleukins and tumors necrosis factor (TNF) which stimulate fibroblasts (produce collagen) and promote angiogenesis and transforming growth (TGF) which stimulates keratinocytes.

The second stage of wound healing is the proliferative phase. Epithelization, angiogenesis, granjulation tissue formation and collagen deposition are the main steps in this anabolic portion of wound healing, Epithelization occurs early in wound repair, if the basement membrane remains intact.

Table 2: Mean wound healing size (cm)*

Groups	Day 3 (cm)	Day 5 (cm)	Day 7 (cm)	Day 9 (cm)	Day 11 (cm)	Day 13 (cm)	Day 15 (cm)
A	1.8	1.5	1.1	0.5	0.3	0.08	0.0
	±0.03	±0.03	±0.09	±0.09	±0.05	±0.05	±0.0
A	1.8	1.6	1.3	0.6	0.4	0.23	0.025
	±0.05	±0.05	±0.05	±0.06	±0.07	±0.09	±0.03
C	1.9	1.8	1.5	0.9	0.7	0.43	0.15
	±0.03	±0.03	±0.05	±0.06	±0.05	±0.08	±0.06
D	1.6	1.3	0.7	0.2	0.05	0.0	0.0
	±0.03	±0.05	±0.05	±0.05	±0.03	±0.00	±0.00
E	2.0	1.9	1.9	1.6	1.5	1.1	1.2
	±0.03	±0.06	±0.06	±0.06	±0.11	±0.1	±0.10

**All the animals in each group have the same diameter of wound which is 2 cm.*

Table 3: Mean percentage wound healing contraction on days post-surgery

Groups	Day 3 (%)	Day 5 (%)	Day 7 (%)	Day 9 (%)	Day 11 (%)	Day 13 (%)	Day 15 (%)
A	12.50	25.00	43.75	76.25	86.25	96.25	100.00
	±1.44	±2.04	±4.27	±4.27	±2.39	±2.39	±0.00
A	11.25	21.25	36.25	71.25	80.00	88.75	98.75
	±2.39	±2.39	±2.39	±3.15	±3.54	±4.27	±1.25
C	5.00	12.50	26.25	5375.9	66.25	63.00	92.50
	±2.04	±1.44	±2.39	±3.15	±2.39	±18.81	±3.23
D	18.75	33.75	63.75	88.75	097.50	100.0	100.00
	±1.25	±2.39	±2.39	±2.39	±1.44	±0.00	±0.00
E	1.25	5.00	7.50	20.00	26.25	46.25	25.50
	±1.25	±2.89	±1.44	±2.89	±5.54	±5.15	±4.79

The epithelial cell migrates upwards in the normal pattern. The epithelial progenitor cells blow the wound and the normal layers of epidermis are restored in 2 – 3 days. If the basement membrane has been destroyed, then the wound is reepithelized from the normal cells in the periphery and from the skin appendages, if intact (e.g., hair follicles and sweat glands). The adhesive gel, containing the mucin and honey, helps in providing an additional layer of coverage that could prevent wound infection. This leads to the higher level of healing noted for the three combinations as shown in Tables 2 and 3. The results obtained for Brachystegia gum alone was also significant ($p< 0.05$).

Angiogenesis, stimulated by TNG-alpha is marked by endothelial cell migration and capillary formation. The new capillaries deliver nutrients to the wound and help maintain the granulation tissue bed. The migration of capillaries into the wound bed is critical for proper wound healing. The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure for this to occur result in a chronically unhealed wound.

The final phase of wound healing is the maturational phase. Fibroblasts differentiate and produce ground substance and then collagen. The ground substance is deposited into the wound bed; collagen is then deposited as the wound undergoes the final phase of repair. Many cytokines are involved in prolifertive phase of wound repair, which include insulin like growth factor (IGF). The wound undergoes contraction, intimately resulting in a smaller amount of apparent scar tissue. The entire process is a dynamic continuum with an overlap of each phase and continued remodelling. Collagen deposition continues for a prolonged period, but the net increase in collagen deposition plateaus after 10 days. This depends on the size and depth of wound. The gel, containing the mucin helps in keeping the wound moist, enabling all the biochemical processes to take place. The gum gel particularly helps to maintain contact of the mucin and honey with the wound surface due to its adhesive property.

Proper wound healing involves complex interactions of cell cytokines working in concert. In recent years, more chemical mediators integral to this process have been identified. The sequential steps and specific processes have not been fully differentiated. When examining the process of wound healing, one should identify the major steps and know the important mediators. The mucin in the preparation is reported to contain glycosaminogycans which have been reported to be of value in wound healing and repair (Glade, 1990; Kim *et al.,* 1999). Apart from the mucin, honey too has been reported to possess wound healing properties.

Honey was common form of wound dressing in ancient times (Forest, 1982). Excessive heating of honey should be avoided because the glucose oxidase enzyme in honey which produces hydrogen peroxide, a major component of the antibacterial activity of honey, is very readily inactivated by heat. Honey can be made very fluid by warming at 37 ^{0}C if vigorous stirring is not sufficient (Armon, 1980).

It has been reported from various studies on the usage of honey as a dressing for infected wounds that the wounds become sterile in 3 – 6 days. Others have reported that honey is effective in cleaning up infected wounds. It has also been reported that honey dressing halt advancing necrosis (Bloomfield, 1973).

Honey has also been found to act as a barrier preventing wounds from becoming infected (Seymour and West, 1951). It prevents cross-infection, and allows burn wound tissue to heal rapidly uninhibited by secondary infection (Yang, 1944).

It has been observed that under honey dressings sloughs, necrotic and gangrenous tissue separated so that they could be lifted off painlessly, and others have noted quick and easy separation of sloughs and removal of crust from a wound (Bose, 1982). Rapid cleansing and chemical or enzymic debridement resulting from the application of honey to wounds have also been reported, with no scar forming on burns. Several other authors have noted the cleansing effect of honey on wounds. It has also been noted that dirt is removed with the bandage when honey is used as a dressing, leaving as clean wound (Mcinerney, 1990). Honey has also been reported to give deodorization of offensively smelling wounds. These properties of honey when combined with those of mucin (Wei and Bobeck, 2005) can have very positive consequences on wound healing. The rapid wound healing noted in this study may suggest a synergistic effect of the honey and the mucin.

Conclusion: It could be concluded from the above results that Brachystegia gum when combined with snail mucin and honey in low concentrations gave appreciable results in terms of wound heading. Finally, from the results, it can strongly be advised that *Brachystegia* gum mixed with mucin and honey should be used in the preparation or formulations of topical drugs in the treatment of wounds. The advantage of this combination is that the components are all natural products with little or no known side effects but has high cicatrizant activity.

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REFERENCES

ADIKWU, M. U. and IKEJIUBA, C. C. (2005). Some physicochemical and wound healing properties of snail mucin. *Bolletino Chimico Farmceutico, 144:* 1 – 8.

ADIKWU, M. U. (2005). Evaluation of snail mucin motifs as rectal absorption enhancer for insulin in non-diabetic rat models. *Biological and Pharmaceutical Bulletin, 28(9):* 1801 – 1805.

ALVAREZ, G. M. and GILBREATH, R. L. (1982). Effect of dietary thiamine on intermolecular collagen cross-linking during wound repair: a mechanical and biochemical assessment. *Journal of Trauma*, 22: 20 – 24.

ARMON, P. J. (1980). The use of honey in the treatment of infected wounds. *Tropical Doctor, 10: 9.*

BLOMFIELD, R. (1973). Honey for decubitus ulcers. *Journal of American Medical Association*, 224: 906.

BOSE, B. (1882). Honey or sugar in treatment of infected wound? *Lancet,* 329 (April 21): 963.

FORREST, R. D. (1982). Early history of wound treatment. *Journal of the Royal Society of Medicine,* 75: 198 – 205.

GLADE, M. J. (1990). Polysulpahted glycosaminoglycans accelerates net synthesis collagen and glycosaminoglycans by arthritic equine cartilage tissues and chondrocytes*. American Journal of Veterinary Research,* 51: 779 – 785.

HUTCHINS, H. H. and SINGISER, R. E. (1955). New gums for pharmaceutical jellies. *Journal American Pharmaceutical Association, Practical Pharmaceutical Education*, 16: 226 – 230.

KIM, Y. S. Y. Y., CHANG, I. M., TOIDA, T., PARK, Y. and LINHARDT, R. J. (1996). A new glycosaminoglycan from the giant African snail *Achatina fulica*. .*Journal of Biological Chemistry,* 271: 11750 – 11755.

MAZZOTTA, M. Y. (1994). Nutrition and wound healing. *Journal of American Podiatric Medical Association* 84: 456 – 462.

MCCARTY, M. F. (1998) Glucosamine for wound healing. *Medical Hypotheses*, 47: 273 – 275.

Mcinerney, R. J. F. (1990). Honey a remedy rediscovered. *Journal of the Royal Society of Medicine,* 83: 127.

OBASEKI-EBOR, E. E., AFONYA, T. C. and ONYEKWELI, A. O. (1983). Preliminary report on the antimicrobial activity of honey distillate. *Journal of Pharmacy and Pharmacology, 35:* 748 – 749.

PHARMACEUTICAL CODEX (1979). The Pharmaceutical Press, London, 415 pp.

SEYMOUR, F. L. and WEST, K. S. (1951). Honey – its' role in medicine. *Medical Times*, *79:* 104 – 107.

SUBRAHMANYAM, M. (1993). Honey impregnated gauze versus amniotic membrane in the treatment of burns a prospective randomized study. *British Journal of Plastic Surgery, 46(4):* 322 – 232.

TAUB, A., MEER, W. A. and CLAUSEN, L. W. (1958). Conditions for the preservation of gum tragacanth jellies. *Journal American*

Pharmaceutical Association, Practical Pharmaceutical Education, 47: 235 – 238.

WADI, M., AL-AMIN, H., FARAOUQ, A., SASHEF, H. and KHALED, S. A. (1987). Sudanese bee honey in the treatment of suppurating wound. *Arab Medico*, 3: 16 – 18.

WEI, G. X. and BOBEK, L. A. (2005). Human salivary mucin MUC7 12-mer-L saliva, and 12-mer-D peptides:, enhancement of activity with protease inhibitor cocktail or EDTA, and cytotoxicity to human cells. *Antiimicrobial Agents and Chemotherapy*, *49:* 2336 - 2342.

YANG, K. L. (1944). The use of honey in the treatment of nonspecific ulcers, and small wounds. *Chinese Medical Journal*, 62: 55 – 60.

ZUMLA, A and LUTAT, A. (1989). Honey a remedy rediscovered. *Journal of the Royal Society of Medicine.* 82: 384 – 385.

BLOOD ERYTHROCYTIC AND LEUCOCYTIC COMPONENTS OF *Heterobranchus bidorsalis* JUVENILES STOCKED IN WATER POLLUTED WITH CRUDE OIL FRACTIONS

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ABSTRACT

The blood erythrocytic (RBC) and leucocytic (WBC) components of Heterobranchus bidorsalis juveniles exposed to different concentration of crude oil fractions were studied. Two study periods namely the toxicity and recovery periods were adopted. Lubricating engine oil (LBO), Bonny-light crude oil (BLCO), Kerosene (DPK) and premium motor spirit (PMS) were respectively applied at four concentrations 1.00, 2.00, 4.00 and 8.00 ml L^{-1},. Lower numbers of RBCs were recorded in fish samples exposed to the crude oil fractions than in the control fish. The comparatively high RBCs in fish during the 4 days toxicity period than during 42 days recovery periods is attributed to the greater destruction of RBCs during the toxicity period. The 4.00 ml L^{-1} LBO and BLCO concentration were apparently preferred by the fish to maintain a higher number of RBCs than with 1.00, 2.00, and 8.00 ml L^{-1} concentrations. Similarly, the 8.00 ml L^{-1} concentrations of DPK and PMS gave better values of RBCs than the 1.00, 2.00 and 4.00 ml L^{-1} concentrations. Reduced number WBCs in the fish blood during the toxicity period at concentrations of 1.00, 4.00 and 8.00 ml L^{-1} LBO; was recorded likely the phagocytic action of the WBCs.

Keywords: Heterobranchus bidorsalis, Erythrocyte, Leucocyte, Toxicity, Crude oil fractions

INTRODUCTION

Fish species are widely used to biologically monitor variations in environmental levels of anthropogenic pollutants (White *et al*., Flammarion, *et al*., 2002; Schmitt, 2004). The warm water fish such as the common carp was chosen as a test organism because it occupies a central position in freshwater food chain especially in inland water systems, which are situated near industrial areas and are influences by various xenobiotics (Schmitt, 2004).

Biomarkers are defined as physiological, biological, or histopathological alterations which occur in organisms as a result of exposure to environmental pollutants (Mayer *et al*., 2004). The potential utility of biomarkers for monitoring both environmental quality and health of organisms inhabiting polluted system has received increasing attention during recent years (Lopes *et al*., 2001; Smecka and Kempers, 2003; Gautheir *et al*., 2004). The effects of xenobiotics contamination in an ecosystem can be estimated through analysis of biochemical changes in organisms inhabiting the systems. (Norris *et al*., 2001; Brewer *et al*., 2001). The responses of aquatic organisms to pollution are noticed through expression of several key enzymes, especially those of biotransformation systems (Nwamba *et al*., 2006).

Heterobranchus bidorsalis juveniles are very delicate and sensitive to aquatic pollutants including crude oil and its fractions. An understanding of the effects of crude oil on development, growth, feeding energetic and swimming activity of fish is essential in assessing the impact of oil pollution on fish populations (Anderson *et al*., 1974). Although the uptake of crude oil and compounds from water is very rapid and bioaccumulations do occur, much is not known about what happens to these compounds within the fish (Stageman and Sabo, 1976). Fish has oxidative enzymes for metabolic detoxification of xenobiotics, including aromatic petroleum hydrocarbons (Payne and Penrose, 1975). Little is known about the metabolism of crude oil compounds in *H. bidorsalis.* The high demand of the giant African catfish (*H. bidorsalis),* due to its good flavor, informs the need to investigate aspects of hematological parameters and survival of this choice fish species in polluted water. Owing to incessant oil spills in Nigeria, resulting in environmental degradation, deprivation and spoilage of valuable food fish, the need to study the impact of crude oil fractions on the hematology of *H. bidorsalis* becomes imperative. Therefore, this investigated the effect of the oil fractions on the blood erythrocytic and leucocytic components of *H. bidorsalis* juveniles. The aim was to ascertain the dynamics of these blood components in the face of crude oil pollution and the consequences of this xenobiotic contaminants on fish survival.

MATERIALS AND METHODS

Six hundred (600) juveniles of *Heterobranchus bidorsalis* Geoffrey St. Hilaire, 1809 (means weight, 13.07 + 0.26g) were purchased from Phinomar Fish farm, Emene Enugu, Nigeria and transported in five plastic containers (25l) to the Fisheries Laboratory, Enugu, State University of Science and Technology, Enugu. The fishes were acclimatized for 14 days on a 38% crude protein diet at 5% body weight per day ($bw.d^{-1}$). Subsequently, they were stoc0ked by a completely Randomized Block Design (CRBD) into 60 plastic containers (25l) with 24L of dechlorinated tap water at 10 fish per container.

Crude Oil Fractions: Four samples of crude oil fractions were used for the study namely; Bonny-light crude oil (BLCO), premium motor spirit (PMS), dual purpose kerosene (DPK) and lubricating engine oil (LBO). Aliquots of these four oil samples were introduced in triplicates to 48 plastic containers with fish at the rate of 1.00, 2.00, 4.00 and 8.00 $m1L^{-1}$: twelve (12) plastic containers with fish were not contained with any oil samples (0.00 mlL^{-1}) and were left as the controls.

Experimental Periods: Two experimental periods were adopted for this study namely; the toxicity period which lasted 4 days (96 hours) and the recovery period (42 days). At the end of the toxicity period, the surviving fish were introduced into unpolluted fresh water (clean tap water) to allow for the 42 days recovery period to commence. Blood samples were collected at 4 days (toxicity period) and on fortnightly basis for the 42 days recovery period. The fish were fed 38 % crude protein diet (Table 1) at the rates of 3 % $bw.d^{-1}$ for the toxicity period and 5 % $bw.d^{-1}$ for the recovery period. Records of the mortality/survival rates were taken, while the feeding and swimming activities of the fish were observed. The water temperature (25 ± 1.50° C) and pH (6.65 ± 0.05) were recorded with the aid of a maximum–minimum thermometer and a pH meter (Model ph-J-201-1) respectively.

Blood Samples: Blood samples of fish from each triplicate treatment of the crude oil fractions (BLCO, PMS, DPK and LBO) and the control were collected with the aid of 2.50 ml capacity syringes and hypodermal needles. The collection of blood was via the dorso-anterior musculature, just below the dorsal fin and around the operculum. Anti-coagulant (EDTA) fluid was used to condition the syringes and needles prior to blood collection. Analysis of the blood samples were done within 12 hours at the Bronilla Diagnostic Laboratory, Enugu, Nigeria.

At the laboratory, triplicate samples of blood from fish of each triplicate treatment of the crude oil fractions and control were subjected to analysis of their erythrocytic (red blood corpuscles) (RBC) and leucocytic (white blood corpuscles) (WBC) components with the aid of a high powered microscope (Model LS-3AC). Analysis of the RBC and WBC were done through the estimation of the number of each type of blood cells present in $1mm^3$ concentration of blood samples within 12 hours.

Statistical Analysis: The data obtained was subjected to analysis of variance to establish if there were significant differences among treatment means and partitioned with the Duncan's Multiple Range Test (Steel and Torrie, 1990).

RESULTS

Table 1 shows the gross and proximate composition of the experimental diet. The RBC counts in fish exposed to different concentrations of the crude oil fractions and control indicated that the numbers of RBCs in the control fish blood were higher than in those exposed to the crude oil fractions (Table 2) for both the toxicity and recovery periods of the study. 4.00 mlL^{-1} LBO and BLCO concentrations recorded higher numbers of blood RBCs during the toxicity period at 137 ± 6.00 (for BLCO) than the other oil concentrations (1.00, 2.00 and 8.00 mlL^{-1}) (Table 2).

Table 1: Gross and proximate composition of experimental diet

Feed Ingredient	%
Yellow maize	9.29
Soybean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	2.40
Total	100.00
Nutrient	
Crude protein	37.58
Ether extracts	5.68
Ash	10.48
Dry matter	11.80
Nitrogen free extract	32.84
Crude fibre	1.80

This trend was also exhibited by the fish during the 42 days recovery period; that is at days 14, 28, and 42. Similarly, the numbers of RBCs of fish exposed to 8.00 mlL^{-1} concentrations of DPK (1210 ± 6.00) and PMS (1400 ± 6.00) were higher than those exposed to the other oil concentration (1.00, 2.00 and 4.00 $m1L^{-1}$). This trend was also shown in both the toxicity and recovery periods of the study. Significantly, the exposure of the fish to 4.00 mlL^{-1} concentrations of LBO and PMS resulted in higher numbers of the blood RBC of the oil treated fish relative to the control (Table 2). Generally, the numbers of RBCs in *H. bidorsalis* blood at both the toxicity and recovery periods varied significantly ($p < 0.05$).

The blood WBCs in fish exposed to 2.00 $m1L^{-1}$ LBO concentration (7,900 ± 30.00 WBCs) were higher than the WBC values recorded for 1.00, 4.00 and 8.00 ml L^{-1} LBO concentrations during the 4 days toxicity period (Table 3). This trend was also demonstrated as the fish recovered from day 14 to day 42. The highest numbers of blood WBCs were also recorded in fish exposed to BLCO, DPK and PMS

Table 2: Red blood corpuscles (RBC) count of *H. bidorsalis* juveniles mm^{-3} concentration of blood sample within 12 hours of collection[1]

Period	Duration (days)	Crude oil fraction	Oil concentrations (m1 L^{-1})					Overall mean
			0.0 (Control)	1.00	2.00	4.00	8.00	
Toxicity	4	LBO	1280 ± 6.00^{e}	870 ± 5.00^{a}	780 ± 4.00^{b}	1370 ± 4.00^{c}	790 ± 4.00^{d}	1018±5.00
		BLCO	1280 ± 6.00^{e}	978 ±6.00^{a}	980 ± 5.00^{b}	1180 ± 5.00^{c}	610 ± 3.00^{c}	1005±5.00
		DPK	1280 ± 6.00^{e}	1190 ±7.00^{a}	980 ± 6.00^{b}	970 ± 5.00^{c}	1210 ± 6.00^{d}	1126±6.00
		PMS	1289 ± 7.00^{e}	820 ±4.00^{a}	1200 ± 7.00^{b}	1300 ± 7.00^{b}	1400 ± 6.00^{d}	1200±6.00
	14	LBO	1536 ± 8.00^{e}	1044 ± 6.00^{a}	936 ± 5.00^{b}	1644 ± 8.00^{c}	948 ± 5.00^{d}	1222±6.00
		BLCO	1538 ± 8.00^{d}	1171 ± 7.00^{a}	1176 ± 6.00^{a}	1416 ± 8.00^{b}	732 ± 3.00^{c}	1207±6.00
		DPK	1542 ± 9.00^{e}	1428 ± 8.00^{a}	1176 ± 6.00^{a}	1164 ± 6.00^{c}	1452 ± 7.00^{d}	1352±7.00
		PMS	1540 ± 8.00^{e}	984 ± 5.00^{a}	1440 ± 8.00^{b}	1560 ± 7.00^{c}	1680 ± 7.00^{d}	1441±7.00
Recovery	28	LBO	1690 ± 8.00^{d}	1149 ± 6.00^{a}	1030 ± 5.00^{b}	1808 ± 8.00^{c}	1043 ± 6.00^{a}	1344±7.00
		BLCO	1689 ± 7.00^{e}	1288 ± 7.00^{a}	1294 ± 6.00^{b}	1558 ± 7.00^{c}	805 ± 5.00^{c}	1327±6.00
		DPK	1696 ± 8.00^{d}	1571 ± 8.00^{a}	1294 ± 7.00^{b}	1280 ± 6.00^{b}	1579 ± 7.00^{c}	1488±7.00
		PMS	1694 ± 9.00^{e}	985 ± 6.00^{a}	1320 ± 7.00^{b}	1716 ± 8.00^{c}	1848 ± 8.00^{d}	1513±8.00
	42	LBO	17755 ±10.00^{e}	1207 ± 7.00^{a}	1032 ± 5.00^{b}	1898 ± 9.00^{c}	1095 ± 6.00^{d}	1411±8.00
		BLCO	1774 ± 9.00^{d}	1352 ± 7.00^{a}	1369 ± 6.00^{b}	1636 ± 7.00^{b}	845 ± 4.00^{c}	1393±6.00
		DPK	17815 ± 9.00^{e}	1650 ± 8.00^{a}	1289 ± 7.00^{b}	1344 ± 6.00^{c}	1677 ± 8.00^{d}	1564±8.00
		PMS	1779 ± 10.00^{e}	1034 ± 6.00^{a}	1386 ± 6.00^{b}	1802 ± 9.00^{c}	1940 ± 9.00^{d}	1588±8.00

[1]LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit. Means in the same row with different superscripts differ significantly (P < 0.01).

Table 3: White blood corpuscles (WBC) count of *H. bidorsalis* juveniles mm^{-3} concentration of blood sample within 12 hours of collection[1]

Period	Duration (days)	Crude oil fraction	Oil concentrations (m1 L^{-1})					Overall mean
			0.0 (Control)	1.00	2.00	4.00	8.00	
Toxicity	4	LBO	1,500 ± 8.00^{e}	7.700 ± 30.00^{a}	7,900 ± 35.00^{b}	1,110 ± 7.00^{c}	1,400 ± 8.00^{d}	3,922 ± 15.00
		BLCO	1,500 ± 7.00^{c}	1.300 ± 5.00^{a}	1,500 ± 6.00^{a}	2,000 ± 3.00^{c}	300 ± 2.00^{d}	1,320 ± 4.00
		DPK	1,500 ± 10.00^{c}	1.200 ± 7.00^{a}	6,000 ± 3.00^{b}	800 ± 4.00^{c}	7.00 ± 5.00^{d}	960 ± 5,00
		PMS	1,650 ± 8.00^{e}	1,435 ± 6.00^{a}	1,200 ± 3.00^{b}	1,320 ± 9.00^{b}	1,210 ± 8.00^{d}	1,363 ± 7.00
	14	LBO	1,652 ± 8.00^{e}	8,470 ± 33.00^{a}	8,690 ± 39.00^{b}	1,210 ± 8.00^{c}	1,542 ± 9.00^{d}	4,313 ± 19.00
		BLCO	1,664 ± 7.00^{e}	1,430 ± 6.00^{a}	1,650 ± 7.00^{b}	2,202 ± 4.00^{c}	335 ± 2.00^{d}	1456 ± 5.00
		DPK	1,500 ± 10.00^{e}	1.300 ± 7.00^{a}	600 ± 3.00^{b}	800 ± 4.00^{c}	7.00 ± 5.00^{d}	960d ± 5,00
		PMS	1,650 ± 8.00^{e}	1,435 ± 6.00^{a}	1,200 ± 3.00^{b}	1,320 ± 3.00^{b}	1,210 ± 8.00^{d}	1,363 ± 7.00
Recovery	28	LBO	1735 ± 8.00^{e}	8,694 ± 36.00^{a}	8,892 ± 41.00^{b}	1,271 ± 9.00^{c}	1,619 ± 10.00^{d}	4,442 ± 21.00
		BLCO	1747 ± 6.00^{e}	1,506 ± 7.00^{a}	1,733 ± 8.00^{b}	2,312 ± 4.00^{c}	352 ± 2.00^{d}	1,529 ± 5.00
		DPK	1734 ± 8.00^{e}	1,386 ± 8.00^{a}	693 ± 4.00^{b}	930 ± 6.00^{c}	812 ± 6.00^{d}	1,111 ± 6.00
		PMS	1,733 ± 6.00^{e}	1,507 ± 6.00^{a}	1,260 ± 3.00^{b}	1,380 ± 10.00^{c}	1,271 ± 9.00^{d}	1,431 ± 7.00
	42	LBO	1838 ± 6.99^{e}	9,228 ± 35.00^{a}	9,414 ± 4.00^{b}	1,347 ± 7.00^{c}	1,716 ± 8.00^{d}	4,708 ± 20.00
		BLCO	1838 ± 5.00^{e}	1,698 ± 8.00^{a}	1,738 ± 6.00^{b}	2,450 ± 4.00^{c}	373 ± 4.00^{d}	1,502 ± 7.00
		DPK	1838 ± 7.00^{e}	1,469 ± 6.00^{a}	735 ± 6.00^{b}	986 ± 5.00^{c}	860 ± 5.00^{d}	1,176 ± 6.00
		PMS	1837 ± 8.00^{e}	1,521 ± 6.00^{a}	1,336 ± 4.00^{b}	1,469 ± 9.00^{c}	1,347 ± 6.00^{d}	1,502 ± 7.00

[1]LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit. Means in the same row with different superscripts differ significantly (P < 0.01).

Table 4: Percentage mortality and survival *H. bidorsalis* juveniles exposed to different concentrations of crude oil fractions[1]

Period	Duration (days)	Crude oil fraction	% Mortality Concentration of crude oil and fractions (ml L^{-1})					% Survival Concentration of crude oil and fractions (ml L^{-1})				
			0.0 (Control)	1.00	2.00	4.00	8.00	0.0 (Control)	1.00	2.00	4.00	8.00
Toxicity	4	LBO	0.00	10.00	0.00	40.00	50.00	100.00	90.00	100.00	60.00	50.00
		BLCO	0.00	0.00	0.00	20.00	30.00	100.00	100.00	100.00	80.00	70.00
		DPK	0.00	0.00	0.00	40.00	50.00	100.00	100.00	100.00	60.00	50.00
		PMS	0.00	0.00	10.00	50.00	70.00	100.00	100.00	90.00	50.00	30.00
	14	LBO	0.00	8.00	6.00	32.00	40.00	100.00	92.00	94.00	68.00	60.00
		BLCO	0.00	5.00	4.00	16.00	24.00	100.00	95.00	96.00	84.00	76.00
		DPK	0.00	3.00	2.00	33.00	42.00	100.00	97.00	98.00	67.00	58.00
		PMS	0.00	4.00	3.00	42.00	55.00	100.00	96.00	97.00	58.00	45.00
Recovery	28	LBO	0.00	2.00	1.00	24.00	36.00	100.00	98.00	99.00	76.00	64.00
		BLCO	0.00	2.00	1.00	12.00	18.00	100.00	98.00	99.00	88.00	82.00
		DPK	0.00	1.00	1.00	26.00	34.00	100.00	99.00	99.00	74.00	66.00
		PMS	0.00	1.00	1.00	35.00	42.00	100.00	99.00	100.00	65.00	58.00
	42	LBO	0.00	1.00	0.00	16.00	26.00	100.00	99.00	100.00	84.00	74.00
		BLCO	0.00	0.00	0.00	6.00	14.00	100.00	100.00	100.00	94.00	86.00
		DPK	0.00	0.00	0.00	18.00	24.00	100.00	100.00	100.00	82.00	76.00
		PMS	0.00	0.00	0.00	25.00	36.00	100.00	100.00	100.00	75.00	64.00

[1]*LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit.*

during the toxicity period at concentrations of 4.00ml L^{-1} (2000 ± 6.00 WBCs), 1.00 ml L^{-1} (1200 ± 7.00 WBCs) and 1.00ml (1,300 ± 6.00 WBCs) respectively (Table 3). Whereas the blood WBC number in *H. bidorsalis* exposed to 2.00ml L^{-1} BLCO (1500 ± 6.00 WBCs) paralleled that in the control fish, the WBCs in fish exposed to 1.00 ml L^{-1} LBO (7,700 3.00 WBCs and 2.00 ml L^{-1} LBO (7,900 ± 35.00 WBCs) were higher than those in the control fish both for the toxicity and recovery periods of the study (Table 3). The blood RBCs and WBCs in the fish blood for both the toxicity and recovery periods varied significantly ($P < 0.05$) as *H. bidorsalis* juveniles were exposed to the different concentrations of LBO, BLCO, DPK and PMS and the control.

The fish exposed to 4.00 and 8.00 mlL^{-1} of the crude oil fractions (LBO, BLCO, DPK and PMS) recorded higher percent mortality and lower survival than those exposed to the other oil concentrations and the control (Table 4). This state of affairs was prevalent both at the toxicity and at the recovery periods of the study. Lower percent mortality of fish was recorded as the fish recovered on a fortnightly basis than for the 4 days toxicity period (Table 4). In the same vein, higher percent survivals were recorded during the recovery period than during the toxicity period (Table 4). From these results it was evident that the 4.00 and 8.00 mlL^{-1} concentrations of LBO, BLCO, PMS and PMS caused the fish to die more and survive less during the toxicity and recovery periods of the study.

DISCUSSION

Stone *et al*. (2002) stated that about 5 million (RBCs) were present in each cubic millimeter (mm^3) of the blood in vertebrates, while the white blood corpuscles (WBCs) are less numerous than the RBCs (that is, 5000 -10,000 m^{-3}). The uptake and translocation of crude in fish may be through the gills or the intestinal wall (Roubal *et al*., 1977). The parent compounds readily solublize in cell membranes and are probably carried via the erythrocytes to the general circulation of blood. The lower numbers of RBCs recorded in fish samples exposed to different concentrations of crude oil fractions than in the control fish (Table 2) might be due to the destruction of RBCs in fish exposed to the oil fractions. This result is consistent with the report of Neff and Anderson (1981) who stated that the exposure of fish to crude oil compounds resulted in the destruction of WBCs important for the immune response of fish.

Incidentally for this study, except for the control fish, the destruction of the RBCs was higher in the fish exposed to oil pollutants during the 4 days toxicity period than in those recuperating from the oil stress during the 42 days recovery period (Table 2). The lower numbers of RBCs in the fish exposed to 2.00 and 8.00 mlL^{-1} LBO and BLCO than in 4.00 ml L^{-1} of the oil pollutants during the toxicity period implies that the later oil concentration (4.00 ml L^{-1}) was preferred by the fish to maintain a comparatively high number of RBCs in its blood relative to the control in terms of physiological adaptation. This trend was extended to the recovery period while the fish recuperated from the stress of oil pollution. This reason could also be attributed to the higher numbers of RBCs in fish exposed to DPK and PMS 8.00 ml L^{-1} than at the other oil concentrations applied. There were increases in the mortality rate and changes in the haemoglobin content of *H. bidorsalis* juveniles exposed to crude oil higher than 2.00 mlL^{-1}.

The reduced numbers of WBCs (Table 3) recorded in the blood of fish samples during the toxicity periods, and when the four oil types were applied at 1.00, 4.00 and 8.00 mlL^{-1} LBO; 1.00, 2.00 and 8.00 ml L^{-1} BLCO, 4.00, 2.00 and 8.00 DPK and 4.00, 2.00 and 8.00 PMS might be due to the phagocytic action of the WBCs (Stone *et al*., 2002) in the face of an infiltration of crude oil compounds into the blood. It could be that the WBCs engulfed the crude oil compounds with such intensity that resulted in their mortality and consequent reduction in number. The results of this study also show that the WBC in the fish blood started to build up with time (Table 3) especially as the fishes recuperated from the oil pollution stress. The effect of the contamination of the fish blood by the crude oil compound was probably reduced by the oxidative enzymes which metabolically detoxified xenobiotics especially the aromatic hydrocarbons (Payne and Penrose, 1975).

The higher percent mortality and the lower percent survival of *H. bidorsalis* juveniles when exposed to 4.00 and 8.00 mlL^{-1} concentrations of LBO, BLCO, DPK and PMS than when exposed to the other oil concentrations (Table 4) imply that the effect of the oil contaminants on the test fish was most pronounced between 4.00 and 8.00 mlL^{-1} oil concentrations. Neff and Anderson (1981) enumerated some ailments and metabolic malfunctions that resulted in fish exposed to crude oil compounds to include: alteration of liver metabolism, adrenal tissue damage, congested lungs, intestinal damage and hemorrhaging. These physiological problems must have been highly expressed when the fishes were exposed to 4.00 and 8.00 mlL^{-1} oil concentrations and consequently resulted in high mortality and low survival.

REFERENCES

ANDERSON, J. W., NEFF, J. M., COX, B. E. and HIGHTOWER, G. M. (1974). Characteristic of dispersions of water solute extracts of crude oil and refined oils and their toxicity to crustaceans and fishes. *Marine Biology, 27:* 75 – 88.

BREWER, S. K., LITTLE, E. E., DELONAY, A. J. BEAUVAIS, S. L., JONES S. B. and ELLERSIECK, M. R. (2001). Behavioral dysfunctions correlate to altered physiology in rain bow trout (*Oncorynchus mykiss*) exposed to cholinesterase- inhibiting chemicals, *Archives of Environmental Contamination and Toxicology, 40:* 70 -76.

FLUMMARION, P., NOURY, P. and GARRIE, J. (2002). The cholinesterase activities as a biomarker in chum (*Leuciscus cephalus*): the fish length should not be ignored. *Environmental Pollution, 120:* 32 5 - 330.

GAUTHIER, L., TARDY, E., MOUCHET, F. and MARTY, J. (2004). Biomonitoring of the genotoxic potential (Micronucleus assay) and detoxifying activity (EROD induction) in the River Dadou (France), using the amphibian *Xenopus laevis*. *Scientific Total Environment, 325:* 47 – 61.

LOPES, P. A., PINHEIOR, T., SANTOS, M. C., MATTHIAS, M. L., COLLARES- PEREIRA, M. J. and VIEGAS- CRESP, A. M. (2001). Responses of antioxidant enzyme in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. *Scientific Total Environment, 280:* 153 – 163.

MAYER, F. L., VERSTEEG, D. J., M. J., FOLMAR, L. C., GRANEY, R. L., MSCUME, D. C. and RATTER, B. A. (1992). Physiological and nonspecific biomarkers. Pages 5 – 85. *In:* J. R. HUGGET, R. A. KIMERLE, P. M. MEHRLE J. R. AND H. L. BERGMAN (Eds). *Biomarkers, Biochemical Physiological and Histopathological Markers of Anthropogenic Stress.* Lewis Publishers, Costa Rica.

NEFF, J. M. and ANDERSON, J. W. (1981). Response of marine animals and specific petroleum hydrocarbons. Pages 58 – 63. *In:* Neff, J. M. and Anderson, J. W. (Eds.). Applied Science, London.

NORRIS, D. O., CAMP, J. M., MALDONADO, T. A. and WOODLING, J. D. (2001). Some aspects of hepatic functions in feral brown trout, *Salmo trutta*, living in maul-contaminated water. *Comparative Biochemistry and Physiology, 127:* 71 – 78.

NWAMBA, H. O., MGBENKA, B. O., UGWU, L. L. C. and CHIFOMMA, A. N. (2006). The exposure of *Heterobranchus bidorsalis* juveniles to different concentrations of to different concentrations of Bonny-light crude oil and their effects on amylase and cretinine kinase activities. *Animal Research International, 3(3):* 516 – 520.

PAYNE, J. K. and PENROSE, W. R. (1975). Induction of aryl hydrocarbon (Benzo a Pyrene) hydroxylase in fish by petroleum. *Bulletin of Environmental Contamination and Toxicology, 14:* 112 – 226.

ROUBAL, W.T., COLLIE H. T. K. and MALINS, D. C.(1977). Accumulation and Metabolism of Carbon – 14 labeled benzene, naphthalene and anthracene by young coho salmon (*Oncorhynchus* sp.). *Archives of Environmental Contamination and Toxicology*, *5:* 515 – 529.

SAMEEKA, C. A. and KEMPERS, A. J. (2003). Biomonitoring of Water pollution with *Elodea canadensis*. A case study of three small polish rivers with different levels of pollution. *Water Air Soil Pollution, 145:* 139 – 153.

SCHMITT, C. A. (2004). Concentrations of arsenic, cadmium, copper, lead, selenium and zinc in fish from the Mississippi River basin, 1995. *Environmental Monitor and Assessment, 90: 289 – 321.*

STEEL, R. G. D. and TORRIE, J. H. (1990). *Principles and procedures in statistics.* McGraw-Hill New York. 451 pp.

STONE, R. H. COZENS, F. B. and NDU, F. O. C. (2002). Blood and the materials it carries. Pages 221 – 222. *In*: *New Biology (revised Edition)*. Longman Nigeria PLC, Lagos, Nigeria.

WHYTE, J. J., JUNG, R. E., SCHMITT, C. J. and TILLITT, D. E. (2000) Ethoxyresorufin-O deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Critical Review in Toxicology*, 30: 247 – 570.

ANTIDIABETIC EFFECT OF *Sarcocephalus latifolus AQUEOUS* ROOT EXTRACT IN EXPERIMENTAL RAT MODEL

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ABTRACT

Investigations were carried out to evaluate the blood sugar lowering activity of the aqueous extract of Sarcocephalus latifolus roots (SLA) in normal and streptozotocin-diabetic rats. The extract (250 mg/kg body weight; p.o) caused 76 % reduction in blood glucose within 6h in fasted diabetic rats. However, the extract at the same dose showed no effect in normorglycaemic rats. Acute toxicity studies of the extract in mice gave an oral LD_{50} value greater than 5000 mg/kg. Phytochemical tests revealed that SLA tested positive for alkaloids, tannins, saponins, terpenoids, reducing sugars, carbohydrates, sterols and glycosides. The results show that the aqueous root extract of S latifolus has blood glucose lowering effect, which is consistent with the use of the root in folklore diabetes management.

Keywords: *Sarcocephalus latifolus* roots, Streptozotocin, Antidiabetic

INTRODUCTION

Diabetes mellitus (DM), a very common and serious endocrine disorder which interferes with the metabolism of carbohydrates, lipids and proteins is caused by the complete or relative insufficiency of insulin secretion and / or resistance to insulin action (Balkau *et al*., 2000).

The key clinical manifestation of this disorder is chronic hyperglycemia (Scoppola *et al*., 2001) which causes glycation of body protein and so leads to secondary complications affecting the eyes, kidneys, nerves and arteries Kameswara *et al*., 1999) as well as micro/macro vascular complications and death (Nagappa *et al*., 2003). Though some non-insulin dependent diabetes mellitus (NIDDM) patients can be managed by diet alone, most require oral hypoglycemic agents and/or insulin therapy. Oral hypoglycemic agents and/or insulin therapy afford relatively effective glycaemic control, but they are not very ideal because of their numerous side effects (Rang and Dale, 1991).

Therefore, there is a great need for the development of newer alternative agents that meet the requirement of an ideal antidiabetic compound with little or no adverse side effects. In recent years research interests have shifted to the search for alternative and natural hypoglycemic agents, especially from plant sources (Krishna *et al*., 2004; Pepato *et al*., 2003).

Sarcocephalus latifolus belongs to the Rubiaceae family and is commonly called the 'pin cushion' tree. It is a shrub native to tropical Africa and Asia. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus. The plant is also used for the treatment of such other diseases as malaria (Kokwaro, 1976; Akabue and Mittal, 1982; Boye, 1990), gastrointestinal tract disorders (Madubunyi, 1995) and hypertension; the stems of this plant are also recommended as a chewing stick (Asubiojo *et al*., 1982). The antihyperglycemic potential of the leaves has also been demonstrated (Gidado *et al*., 2005). The present study was undertaken to evaluate the hypoglycemic effect of aqueous extract of the roots of *S latifolus* in normal and streptozotocin induced diabetic rats. In Abakaliki community of Ebonyi State, Nigeria, a decoction of the roots of *S latifolus* is popularly used for the treatment of diabetes. Usually 1 glass of the decoction of the root of this plant is taken twice daily for the treatment of diabetes.

MATERIALS AND METHODS

Collection of Plant Materials: Fresh roots of *Sarcocephalus latifolia* were collected in December 2005 from Oba, Enugu State, Nigeria and authenticated by Mr. A. Ozioko of the Bioresources Development and Conservation Programme Centre (BDCP), Nsukka, Enugu State, Nigeria.

Plant Extract Preparation: The roots were cleaned, cut into smaller pieces dried under the shade at room temperature and ground into coarse powder using a mechanical grinder. About 500g of the powder was boiled in 2L of distilled water for 45min with two changes of solvent. The extract was then cooled, filtered and evaporated to at 40^{O}C, to obtain 57.62g of crude residue.

Animals: Ten to twelve weeks old outbred healthy Sprague-Dawley male albino rats (*Rattus novergicus*) were obtained from the animal facility of the Department of Biochemistry, University of Nigeria, Nsukka, Nigeria. Six to eight weeks old healthy outbred Swiss albino mice *Mus musculus* were obtained from the same source. All the animals were housed in an environmentally controlled room with a

12 h light/dark cycle and fed with standard Pfizer pellets and water *ad libitum*. The animals were acclimatized for 7 days.

Phytochemical Analysis: The extract was subjected to phytochemical analysis for constituent identification using standard procedures (Evans, 1989; Harborne, 1998).

Acute Toxicity Test: The acute toxicity (LD_{50}) of the extract was determined in mice by the method of Lorke (Lorke, 1983) using the oral route.

Experimental Design: The study was carried out on normoglycemic and streptozotocin-induced diabetic rats. The animals were fasted for 16h before each experiment, and blood samples were collected from the caudal vein of the rats.

Hypoglycemic Study: The initial fasting blood glucose concentration was estimated in fasted rats, after which, the extract (SLA) was administered orally via an intubator at a dose of 250 mg/kg. Blood glucose concentrations were then determined hourly for 6 h, using a One-Touch ultra glucometer (Lifescan Inc. U.S.A).

Induction of Diabetes/Antihyperglycemic Study: Diabetes was induced by intravenous injection of 60 mg/kg of streptozotocin (STZ) (Sigma Chemical Co, St Louis, Mo, USA), freshly dissolved in citrate buffer (0.01M, pH 4.5). Control rats received only the buffer. Seventy two hours after these injections the animals were fasted and their blood glucose levels tested; animals with glucose levels higher than 200 mg/dl were used for the study. The experimental animals were divided into three groups; group 1 received the extract (SLA), group II received a standard antidiabetic drug (glibenclamide) while group III was treated with distilled water After these treatments the blood glucose concentrations of the animals were determined at one hour interval for 6 h. Percentage glycemic change was then calculated as a time function using the formula: % induced glycemia = $G_X - G_O$ / $G_{O;}$ where G_O= the initial glucose level (Gupta *et al*., 1984), G_X= glucose level at 1, 2, 3, 4, 5 and 6 h respectively.

Statistical Analysis: Results were analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM. The Data were further subjected to Fischer LSD post hoc test and differences between means were regarded significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 showed that the yield of the crude aqueous extract (SLA) was 11.52 %. Phytochemical analysis of SLA extract was positive reactions for carbohydrates, glycosides, reducing sugar, alkaloids, saponins, tannins, terpenoids and sterols but showed negative for flavonoids and resins (Table 1).

This finding is in consonance with the findings of Hotellier *et al*. (1979), Abreu and Pereiera (2001) as well as Morah (1995), who reported independently that *S latifolus* contains alkaloids and terpenes respectively. The oral LD_{50} of the aqueous extract (SLA) estimated in mice, was > 5000 mg/kg which falls within acceptable safety limits.

Table 1: Phytochemical constituents of the extract

Constituent	SLA
Carbohydrates	+
Glycosides	+
Reducing sugars	+
Alkaloids	+
Flavonids	–
Saponins	+
Tannins	+
Terpenoids	+
Resins	–
Sterols	+

(Yield % = 11.52 of the starting material) Key; +=present; - = absent

The fasting blood glucose levels as well as the percentage change in blood glucose levels of normal rats at different time intervals after oral administration of 250 mg/kg *S. latifolus* root extracts, glibenclamide and water is shown in Table 2. The extract elicited a slight but insignificant increase ($p<0.05$) in blood glucose levels of the rats from the first hour of administration; while glibenclamide significantly lowered the blood glucose levels ($p<0.05$) by the first three hours. The reduction continued till the sixth hour although the reduction was not significant.

Changes in fasting blood glucose levels and percentage glycaemic change of diabetic rats after administration of SLA, glibenclamide or water are shown in Table 3. When compared with control rats treated with distilled water, the aqueous extract SLA (250 mg/kg) significantly lowered blood glucose levels of streptozotocin diabetic rats ($P < 0.05$). The aqueous extract of the roots of *S latifolus* caused a 29.26% decrease in blood glucose levels of the diabetic rats within 1h of administration. A maximal reduction of 76.2 % was attained at 6h, this compared with the maximal reduction for glibenclamide at the 5 h. Treatment of non diabetic rats with the same dosage, for the same duration, did not however, show the same hypoglycemic activity. The hypoglycemic activity was not similar with that of glibenclamide, an oral hypoglycemic agent that lower blood glucose in both normal and diabetic animals (Gupta *et al*., 1984; Subramanian *et al*., 1996; Prince *et al*., 1999 and Okyar *et al*., 2001).

In conclusion, the aqueous root extract of *S latifolus* was found to exhibit a hypoglycaemic activity in streptozotocin diabetic rats and the activity was consistent up to the sixth hour. However, studies are in progress in our laboratory to elucidate the possible mechanism of action of this extract as well as the hypoglycaemic principle(s) of the plant.

Table 2: **Effect of the extract on blood glucose levels of normoglycaemic rats (h)**

Treatment	Dose	Fasting Blood glucose at time after treatment						
		0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
S. latifolus **Extract SLA**	250 mg/kg	55.75 ± 4.30	66.50 ± 1.55 (+19.6)	62.75 ± 2.25 (+12.5)	65.50 ± 3.6 (+17.85)	68.50 ± 2.92 (+23.21)	65.25 ± 2.92 (+16.07)	60.5 ± 13.39 (+8.92)
Gliben clainde	2 mg/kg	68.50 ± 4.87	62 ± 5.02 (-10.14)	48.75 ± 3.22 (-28.98)	41.25 ± 1.75***** (-40.57)	44.25 ± 1.49***** (-36.23)	50.25 ± 1.75 (-27-53)	53.50 ± 2.95 (-18-84)
Control Distilled water	5 mg/kg	62.20 ± 4.18	63.0 ± 3.50 (+2.25)	58.80 ± 3.78 (-5.46)	57.60 ± 3.58 (-7.39)	65.00 ± 3.27 (+4.50)	63.40 ± 3.65 (+1.92)	61.2 ± 3.15 (-1.61)

*Values are means ± SD; n=5 (One way ANOVA, Fischer LSD Post Hoc test) ±SEM; n = 4 -5 *P<0.05 compared to control values in parenthesis represent % glycaemic change.*

Table 3: Effect of the extract on fasting blood glucose levels of streptozotocin – diabetic rats

Treatment	Dose	Fasting Blood glucose (mg/dl)at time(h) after treatment						
		0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
S. latifolus **extract**	250mg/kg	311.00 ± 4.26	220.00 ± 3.36* (-29.26)	175.00 ± 24.95* (-43.72)	120.8 ± 7.02* (-61.09)	98.60 ± 10.07* (-68.16)	92.20 ± 9.27* (-70.41)	73.60 ± 2.73* (-76.20)
Gliben clainde	2mg/kg	247.25 ±5.64	161.75 ± 8.07* (-34.41)	123.60 ± 8.68* (-49.72)	84.75 ± 6.15* (-65.99)	80.50 ± 6.65* (-70.04)	66.00 ± 10.80* (-73.27)	85.50 ± 2.39* (-65.18)]
Control Distilled water	5mg/kg	293.60 ± 4.73	300.20 ± 3.85 (+2.24)	289.60 ± 25.24 (-1.36)	285.60 ± 6.07 (-2.72)	295.20 ± 3.61 (+0.34)	280.40 ± 6.75 (-4.76)	276.60 ± 25.27 (+1.02)

*Values are means ± SEM; n = 4-5; *P<0.05 compared with control (One way ANOVA, Fischer LSD Post Hoc Test) Values in parenthesis represent % glycaemic change.*

REFERENCES

ABREU, P. and PEREIRA, A. (2001). New Indole Alkaloids from *Sarcocephalus latifolius. Natural Products Letters*, *15(1):* 43 – 48.

AKABUE P. and MITTAL G. C. (1982). Clinical Evaluation of a Traditional Herbal Practice in Nigeria: A preliminary Rep. *Journal of Ethnmopharmacology*, *6(3):* 355 – 359.

ASUBIOJO, O. J., GUINN, V. P. and OKUNUGA, A. (1982). Multi-element analysis of Nigerian chewing sticks by instrumental neuron activation analysis. *Journal of Radiology and Analytical Chemistry*, *74:* 149 – 156.

BALKAU B., CHARLES M. A. and ESCHIWEGA, E. (2000). Discussion epidemiologique des nouveaux criteres du diabete. *Mt Endocrinologie, 2:* 229 - 234.

BOYE, G. L. (1990). Studies on antimalarial action of *Cryptolepis sanguinolenta* extract. Pages 243 – 251. *In: Proceedings of the International Symposium on East-West Medicine*. Seoul, Korea.

EVANS, W. C. (1989). *Trease and Evans Pharmacognosy.* 13th Edition. Bailliere Tindall, London.

GIDADO, A., AMEH, D. A. and ALAWODI, S. E. (2005). Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan induced diabetic rats. *African Journal of Biotechnology*, 4 (1):

GUPTA, M. P., SOLIS, N. G., ESPOSITO, M. and SANCHEZ, S. (1984). Hypoglycaemic activity of *Neurolaena lobata* (L) R. *British Journal of Ethnopharmacology, 10:* 323 – 327.

HARBORNE, J. B. (1998). *Phytochernical methods. A guide to modern techniques of plant analysis.* Chapman and Hall Limited, London.

HOTELLIER, F., DELAVEAU, P. and POUSSET J. L. (1979). Alkaloids and glycoalkaloids from leaves of *Nauclea latifolia. Planta Medica*, 35: 242 – 250.

KAMESWARA R., KESAVULU, M. M., GIRI, R. and RAO, C. (1999). Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook, fruit powder in alloxan diabetic rats. *Journal of Ethnopharmacol*ogy, *67:* 103 – 109.

KOKWARO J. O. (1976). *Medicinal Plants of East Africa*. East African Literature Bureau, Nairobi.

KRISHNA B., NAMMI, S., KOTA, M. K. and KRISHNA RAO R. V. (2004). Evaluation of hypoglycaemic and antihyperglycaemic effects of *Datura metel* Linn seeds in normal and alloxam-induced diabetic rats. *Journal of Ethnopharmacology*, 9: 95 – 98.

LORKE, D. (1983). A New approach to acute toxicity testing. *Archives of Toxicology, 54:* 275 – 287.

MADUBUNYI I. I. (1995). Anti-hepatotoxic and trypanocidal activities of the ethanolic extract of *Nauclea latifolia* root bark. *Journal of Herbs Spices and Medicinal Plants*, *3(2):* 23 – 53.

MORAH F. N. (1995). Naucledal and epinaucledal from an antiviral preparation from *Nauclea latifolia. Global Journal of Pure and Applied Science, 1(1 – 2):* 59 – 62.

OKYAR, A., CAN, A., AKER. N., BAKTIR, G. and SUTLUPINAR, N. (2001). Effect of *Aloe vera* leaves on blood glucose levels in type I and type II diabetic rat models. *Phytotherapy Research*, *15:* 157 – 161.

PEPATO M. T., BAVIERA A. M., VENDRAMINI R. C., PEREZ, M. P., KETTELHUT, I. C. and BRUNETTI, I. J. (2003). *Cissus sicyoides* (Princess wine) in long term treatment of streptozotocin-diabetic rats. *Biotechnology and Applied Biochemistry*, *37:* 15 – 20.

PRINCE, P. S. M., MENON, V. P. and GUNASEKHARAN, G. (1999). Alloxan diabetic rats. *Journal of Ethnopharmacology*, 64: 53 – 57.

RANG, H. P. and DALE, M. M. (1991). *The Endocrine System Pharmacology*, 2nd Edition, Longman, Harlow.

RAO, B. K. and RAO, A. (2001). Hypoglycemic and antihyperglycaemic activity of *Syzygium alternifolium* (Wt) walp seed extracts in normal and diabetic rats. *Phytomedicine, 8(2):* 88 – 93.

SCOPPOLA, A., MONTECCHI, F. R., MEZINGER, G. and LALA, A. (2001). Urinary mevalonate excretion rate in type II diabetes; role of metabolic control; *Atherosclerosis,* 156: 357 – 461.

SUBRAMANIAN, A., PUSHPAGANDAN, P., RAGASEKHARAN, S., EVANS D. A., LATHA P. G. and VALSARAJ, R. (1996). Effects of *Artemisia pallens* wall on blood glucose levels in normal and alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, *50:*13 - 17.

PARASITIC INCIDENCE IN CULTURED *Clarias gariepinus*

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ABSTRACT

A sample of 27 fish was collected from the fish breeding centre of the Ministry of Agriculture and Natural Resources. Agodi, Ibadan. This sample was examined for parasitic incidence and the results showed 77.7 % parasitic prevalence while infection was not sex dependent (P > 0.05). Parasitic prevalence within the sample increases as length of fish increases. The prevalence of parasites encountered were Henneguya (3.7%), Dactylogyrus sp (11.1 %), Capillaria sp (7.4 %), unidentified larvae(7.4 %),unidentified cysts(40.7%), Dibothriocephalu sp (3.7 %), Argulus 7.4 %), Ichthophthirius (3.7 %) and Tricodina (3.7 %).

Keywords: Parasites, Prevalence, *Clarias gariepinus*, Infection

INTRODUCTION

Incidence of heavy parasitic infection in fish has been reported globally because fish serves as reservoir and intermediate host to most stages of parasites ranging from protozoan to metazoans (Hoffman and Meyer, 1967; Kabata, 1970; Pal and Ghosh, 1985). Several authors have worked on parasitic incidence of fish in Nigeria (Ukoli, 1963; Alfred-Ockiya, 1985; Awa, *et al,* 1988; Okaeme, 1991 and Adeyemo, *et al*, 2003) and discovered that in the natural environment healthy individuals co-exist with diseased ones and in most parasitic infections host may not be killed unless the parasitic burden is high. But usually, growth rate and market value of fish may be reduced while infection may also be of public health importance. For public health concerns, it is necessary to identify disease reservoirs in order to have adequate knowledge of the transmission mechanism. This will help to develop an effective method of preventing the access of pathogens and their reservoirs to healthy facilities or individuals.

One of the culturable fish species in Nigeria is *Clarias gariepinus;* it is widely accepted for consumption and rearing. The study of parasitic incidence on this species will further help to understand its adaptation for culture purpose. This study will also add to the current knowledge on parasitic infections of cultured fish in Nigeria.

MATERIALS AND METHODS

The parasitic investigation was carried out on *C. gariepinus* that were sampled from the Fish Breeding Centre of the Ministry of Agric and Natural Resources, Agodi, Ibadan. The centre is located between latitude $7^{o}25^{l}$ N and longitude 3^{o} 33^{1}W. Sampling was carried out using the method of Ossiander and Wedemeyer (1973). A total of 27 live fishes was collected during the raining season period between the months of May – July. They were examined for parasites using the identification outline of Amlacher (1966), and Pal and Ghosh (1985). The individual fish were demobilized by pithing, while the organs were carefully examined using hand lens. Scrapping from the skin and gills were placed in Petri dishes with 2 drops of normal saline for examination, while the squash preparations from the organs were also examined under the low power (X10) magnification of a binocular microscope.

Prevalence and intensity were calculated using the indices of Margolis *et al.* (1982). Length range frequency in relation to prevalence within the sample was analyzed. The dependence of infection on sex was statistically determined using chi^2 analysis. The condition factor (K) was calculated and defined as $K = 100w/L^3$ where w is the weight and L as total length in cm (Bakare, 1970).

RESULTS AND DISCUSSION

Parasitic prevalence of 3.7 % was recorded for *Henneguya sp* a sporozoan parasite, which was found, encysted on the air sac of *C. gariepinus* (Table I) .The parasite was discovered when the cyst was teased to release the content. Meanwhile, other unidentified cysts were also found at the prevalence rate of 40.7 % in the musculature, on the skin, ovary and kidney of the fish. These cysts could be explained to arise through connective tissue reaction to sporozoan infection and sometimes could be larvae of helminthes being encapsulated by connective tissue, which may eventually calcify. (Amlacher, 1966). The importance of these cysts lies in quarantine procedure, since after 3 – 4 weeks of quarantine, the cyst may burst to release the pathogenic exudates or helminthes. Prevalence rate of 11.1 % was recorded for *Dactylogyrus sp a* monogenetic trematode. This trematode was described by Hendrix (1994), to be cosmopolitan in nature and could be ecto or endoparasite. Awa, *et al.* (1988) also reported incidence of monogenetic worms on *Sarotherodon galileans* at the Ikoyi fish farm, Lagos.

Table I: Parasitic incidence on cultured *Clarias gariepinus*

Parasites Found	Number of fish examined	Number of Infected fish	Parasite Burden	Prevalence (%)	Intensity of infection	Site infected
Heneguya sp.	27	*1	High	3.7	High	Air Sac
Unidentified Cysts	27	*11	50	40.7	4.7	Muscles, Skin, body cavity, Ovary , kidney
Dactylogyrus sp	27	*3	8	11.1	2.6	Intestine
Dibothriocephalus sp	27	1	1	3.7	1	Intestine
Capillaria sp	27	2	3	7.4	1.5	Stomach, body cavity
Unidentified larvae	27	2	5	7.4	2.5	Body surface
Argulus sp	27	2	2	7.4	1	Body surface
Ichthophthirius sp	27	1	Low	3.7	Low	Skin body surface
Tricodina sp	27	1	3	3.7	3	Stomach

** Parasite was found in more than one fish examined*

Table 2: Sex ratio analysis of infected *Clarias gariepinus*

	Female	Male	Sex ratio
Number of fish examined	13	14	1:1.01
Number of fish infected	9	12	1:1.02
Prevalence	69	86	1:1.3

Table 3: Length-weight in relation to infection and condition factor (k)

Length Range (cm)	Weight average (gm)	No within the group	No of infected Fish	Prevalence Within the Group (%)	Condition Factor(k)
22-23.5	103.33	6	5	83	0.8
24-25.5	120.90	11	7	63	0.7
26-27.5	191.66	6	5	83	0.9
28-29.5	152.25	2	2	100	0.6
30-31.5	265.00	2	2	100	0.8
Total		27	21	77.7	

The dominant species among these worms are namely *Dactylogyrus* sp and G*yrodactylus sp.* D*actylogyrus vastator* had been known to cause heavy mortalities of fry and fingerlings (Sarig, 1971). The occurrence of fish mortality at Nagpur, India was found to be due to infection by cestode as reported by Pal and Ghosh (1985). This present study recorded the prevalence of *Dibothriocephalus sp* at 3.7 %, this tapeworm was described by Needham and Wooten (1978) as broad fish tapeworm of man, which is known to occur in a variety of fresh water fish species. It is also of public health importance, because tapeworm of *Diphyllobothrium* sp has been found to be of zoonotic relevance. (USDHEW, 1973).

The unidentified larvae, *Capillaria sp* and *Argulus sp* were found at 7.4% prevalence rate respectively. These were known to cause skin irritation, which favours secondary infections and may lead to transmission of bacterial hemorrhagic septicemia (Moore *et al,* 1984). The two common protozoan, *Ichthophthirius sp* and *Tricodina sp* were found from the skin scrapings and stomach contents respectively, but there was no lesions observed on any of the samples. The parasitic infection on *Clarias sp* in this centre was found not sex dependent ($P > 0.05$) because dependence on sex was not statistically significant using chi^2 analysis. Sex ratio analysis of infected fish and the infection prevalence according to sex is shown in Table 2. Prevalence of infection according to length increases within the sample as length of fish increases while the overall prevalence was 77.7 %, also the effect of infection on fish condition factor was low as shown in Table 3. The condition factor (K) measures the well being of the fish and is usually close to value 3 for a healthy fish, in this study the K values were below value 1 indicating that all specimens were not healthy considering the high (77.7 %) parasite prevalence at the center.

Conclusion: This report would not have succeeded in identifying all the parasites that may likely be found on cultured *Clarias species*. It however advocates for more work on diseases and parasites of fish especially culturable fish species. Since temperature regime in our ecological waters favours rapid multiplication and cycling of both fish and parasites. Meanwhile, aquaculture is developing progressively at a high rate in the country. Wild fish that are sometimes stocked or strayed into rearing ponds may bring about incidence of transferred infection, fish mortalities and consequent loss of production. It will also attract public health concern when infected fish are improperly prepared for consumption.

REFERENCES

ADEYEMO, A. O., AGBEDE, S. A., TAIWO, V. O. and ADEDEJI, B. O. (2003). Prevalence, abundance and intensity of *Clinostomum*

tilapiae on cultured *Oreochomis niloticus. Tropical Vetenarian, 21(3):* 129 – 133.

ALFRED-OCKIYA, J. F. (1985). Preliminary survey of parasites of *Clarias* sp. in the Delta area of Nigeria. Pages 210 – 215. *In:* ITA, E. O. (Ed). *Proceedings of the 4th Annual Conference of Fisheries Society of Nigeria (FISON).*

AMLACHER, E. (1966) *Textbook of Fish Diseases.* Oliver and Boyd Limited, Aberdeen. 310 pp.

AWA, J. N., ANYANWU, P. and EZENWA, B. (1988). Incidence of parasites infection of pond raised *Tilapia sp* and some cultivable fish species from three ecological areas of Lagos State. Pages 20 – 21. *In:* Nigerian Institute for Oceanography and Marine Research (NIOMR) 1988 Annual Report.

BAKARE, O. (1970). Bottom deposits as food of inland fresh water fish in Kainji a Nigeria Man-made lake. Volume 1, Pages 65 – 86. *In:* VISSER S. A. (Ed). The Nigeria Institute of Social and Economic Research.

HENDRIX, S. S. (1994). *Marine Flora and Fauna of the Eastern United States*. National Oceanic and Atmospheric Administration (NOAA) Technical Report. National Marine Fisheries Services (NMFS) 121. US Department of Commerce, US. 106 pp.

HOFFMAN, G. L and MEYER, F. P. (1967). *Parasites of freshwater fishes: A review of their control and treatment.* TFH, New Jersey. 224 pp.

KABATA, Z. (1970). Crustacean as enemy of fishes. *In:* Sniezko, S. F and Axel, R. (Eds.). *Diseases of fishes.* TFH, New Jersey.

MARGOLIS, L., ESCH, G. W., HOLMES, J. C., KURRIS, A. M. and SHAD, G. A. (1982). The use of ecological terms in parasitology. *Journal of Parasitology, 68:* 131 – 133.

MOORE, B. R., MITCHELL, A. J., GRIFFIN, B. R. and HOFFMAN, G. L. (1984). Parasites and diseases of pond fishes. Pages 177 – 205. *3rd Annual Report of United States Department of Interior Fisheries and Wildlife Services.*

NEEDHAM, T. and WOOTEN, R. (1978). Parasitology of Teleost. Pages 144 – 182. *In:* ROBERTS R.J (Ed). *Fish Pathology.* Cassell Limited. Great Britain.

OKAEME, A. N. (1991). Helminthes fauna of tilapia of lake Kainji in pre and post impounded conditions. *Journal of Aquaculture, 6(1):* 1 – 8.

OSSIANDER, F. and WEDEMEYER, G. (1973). Computer programme for sample sizes required to determine disease incidence in fish populations. *Journal of the Fishery Resources Board of Canada, 30(9):* 1383 – 1384.

PAL, R. N. and GHOSH, A. K. (1985). *Identification and control of commonly occurring diseases in fresh water aquaculture.* Aquaculture Extension Manual Series, Volume 9, Central Inland Fish Institute, India. 23 pp.

SARIG, S. (1971). *Prevention and treatment of warm water fish diseases under sub-tropical conditions with special emphasis on intensive fish farming.* TFH, New Jersey.

UKOLI, F. M. A. (1963). Preliminary reports on the helminthes infection of fishes in River Niger at Shagumu. Pages 70 – 73. *In:* White E. (Ed). *1st Scientific Report of Kainji lake Biological Research Team.*

USDHEW (1973). *Epidemiological aspects of some of the zoonoses.* Centre for Disease Control Public Health Service of United States Department of health, Education and Welfare (USDHEW). No CDC 75-8182.

HARNESSING AQUATIC PHYSICOCHEMICAL PARAMETERS INFLUENCING MACROINVERTEBRATE FAUNA IN ANAMBRA RIVER BASIN FOR SUSTAINABLE FISH PRODUCTIVITY

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ABSTRACT

The management-oriented background for harnessing aquatic physicochemical parameters influencing macro invertebrate fauna of Anambra River basin for sustainable fish productivity was studied. The intra seasonal variability in the water quality of the river revealed mean transparency of 1.79 cm, Conductivity of 28.81 µscm^{-1}, nitrate-nitrogen of 3.23 mgl^{-1}, total hardness of 6.43 mgcaco$_3$L^{-1}, biological Oxygen Demand (BOD) of 3.62 mgl^{-1}, No$_3$-N/Po$_4$-P 0.54 ratio and Co$_2$/DO of 0.81 were significantly higher in the dry season than all other parameters which were significantly higher in the wet season. A total of 1808 individuals (mostly adults) belonging to 97 species of macro-invertebrate fauna were collected. The overall composition of the fauna in the river basin was dominated by coleopterans and the hemipterans. The estimated annual fish yield of the river basin was 72.91 kg/ha based on a morphoedaphic index. The water quality of the River can be harnessed by controlling and/or prohibiting the discharge of municipal effluents and domestic garbage into the river as well as the continuous use of the riparian zone for agronomy. The maintenance of peripheral 50/60 m of thick riparian vegetation can act as buffer strip to check bank erosion is suggested.

Keywords: Macro invertebrates, Physicochemical Parameters, Harnessing, Fish yield

INTRODUCTION

The invertebrate fauna are important food items for the young and some adult of many freshwater fishes (Ovie, 1993). As such their roles are significant and are extensively used in the rearing of larvae and fry of commercially important fishes. They are also indispensable in the food chain of fishes as animal food which supplies amino-acids, vitamins and mineral salts, (Watanabe, 1978; Alam *et al.,* 1989). The abundance and growth of macro fauna depend upon bacteria, yeast, organic, inorganic nutrients and other small and microscopic organisms and water properties (Hirayama and Watanabe, 1973; Hirata 1977; Ali *et al.,* 1986). Boyd (1982) stated that fish production from aquatic media can be enhanced by adopting rationale management strategies, including the curbing of anthropogenic perturbations of the physicochemical characteristics of water. This is based on the rationale that water quality attributes are prime factors that influence fish survival, reproduction, growth performance and overall biological production (Boyd and Lichtkoppler, 1979). They also affect aquatic biotic integrity by directly causing mortality and/or shifting the equilibrium among species due to subtle influences such as reduced reproductive rates or alterations in competitive ability.

In Nigeria, definitive information on aquaculture based on water quality parameters of lotic and lentic system is useful and many investigations have been conducted to assess the physicochemical and macroinvertebrate (Eyo and Ekwonye, 1995; Odo *et al.,* 2007) status of aquatic ecosystems as guidelines for rational fisheries management and resource conservation.

The present study was designed to provide management-oriented information on the physio-chemical integrity of the Anambra River Basin in Anambra State, Nigeria. This was intended to provide the background data for harnessing the macroinvertebrate fauna that are important food items for the young and some adult of many freshwater fishes. The results are discussed in the light of water quality standards conducive for fish production in tropical aquatic ecosystems. Perspectives in effective water quality improvement, checklist of macroinvertebrate fauna, fisheries potential, management and conservation of the river ecosystem were also provided.

MATERIALS AND METHODS

The Study Area: The Anambra river (Figure 1) is the largest tributary of River Niger below Lokoja, and is often regarded as a component part of the lower Niger lowlands. The source of the river is Ankpa hills where it flows in a southerly direction through a narrow trough that gradually broadens as it courses down. It crosses the Kogi/Anambra State boundary a bit north of Ogurugu, and then meanders through the Ogrugu station to Otuocha and Nsugbe. From there it flows down to its confluence with the Niger river at Ontisha.

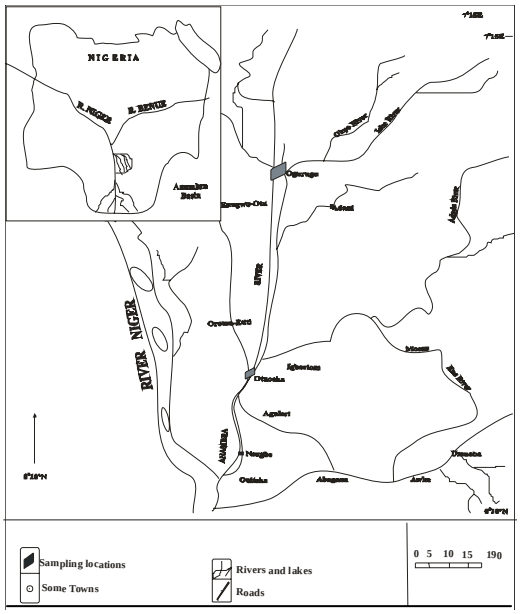

Figure 1: Map of Anambra river showing the sampled stations

The river is about 230 km in length and has an extensive basin which is Ca. 14, 010km^2. The basin lies between latitude 6^o 10" and 7^o 20', longitude 6^o 35' and 7^o 40', east of the Niger river into which the Anambra river empties. The Anambra river system is known to support a rich and thriving fishery (Awachie, 1976). Riparian vegetation, ecology and productivity of the river basin have been extensively studied (Awachie, 1976). Agriculture and fishing thus form the dominant occupations of the local people, and these major economic activities are geared to the two seasons of the year.

Physicochemical Characteristics: Eighteen physicochemical parameters (Table 1), were determined monthly (from January, 1998 to October 1999 inclusive) based on records and samples taken from three stations (Figure 1). All in situ determinations and collection of surface water samples were conducted during mid-morning (10 – 11 am).

Each station water level was determined by means of a graduated wooden pole. Surface temperatures were measured in situ by a 2 minutes immersion of 0 – 50^o C mercury in glass thermometer and transparency, with a 25 cm diameter secchi disc. The concentration of suspended solids (SS) was estimated by filtering (under suction) of 1 litre of water sample through a pre-weighted GF/C What man filter paper and oven-drying at 120^o C for 12 h; it was re-weighed after cooling in a desiccators to obtain the amount of non-filterable residue. The coefficient of coarseness (CC) of suspended solids was estimated by dividing the concentration of suspended solids (SS) by Transparency.

Hydrogen ion concentration (pH) was measured with a glass electrode of a battery powered pH J-250-F Griffin meter. Conductivity (CD) was determined with a Gensway digital meter. Analytical methods for all other parameters are described in (APHA, 1979). Dissolved oxygen was fixed in the field and the concentration estimated in the laboratory by Winkler's method. Water samples for BOD were collected from each station using 250 ml reagent bottles. These bottles were painted black to prevent light penetration. After incubating the sample for five days at 20^o C then it was fixed with Winkler's solutions A and B before adding two drops of concentrated H_2S0_4 to dissolve the precipitate Titration. Titration for DO was then carried out and the difference between the initial and final DO values was the BOD value.

Free $C0_2$ was determined in the field titrimetrically using 0.0027N NaOH and phenolphthalein indicator. Total alkalinity was estimated by titration with 0.02N H_2S0_4 using phenolphthalein and methyl orange indicator.

The No_3-N and Po_4-P were determined calorimetrically using a Gallencamp calorimeter in Analytical Chemistry Laboratory of Soil Science Department, University of Nigeria, Nsukka.

The current (Ms^{-1}) was estimated at the different aforementioned stations by noting the time taken when a piece of lead fixed cork was allowed to drift from one predetermined point to the other.

Macroinvertebrates: Three stations, Ogurugu, Otuocha and Nsugbe were chosen as sampling points because of accessibility as well as their strategic locations on the various stretches of the Anambra river and fishery activities. Samples were taken during the last week of each month from each of the three stations for 22 months. Samples were equally taken at Iyi-Eri, Ozele and Ojo ponds as well as in Adada and Igbariam occasionally. Macroinvertebrates were collected from each station monthly using Ekman grap which was normally lowered into the water body with a graduated rope which also measures the dept of the water. The samples collected were later emptied into wide mouthed plastic container for sorting.

The plankton net made of bolt silk number 10 meshes (154 cm) with a plastic bottle at the end was used to collect drifting organism along water current and other limnitic (pelagial) regions. The plankton samples were collected by sinking the plankton net beneath the surface of water and towering with a silk along the opposite direction of the flowing river.

Hand scoop net of mesh size 74 cm was used to collect macroinvertebrates at biotopes and littoral regions. Benthic samples were collected from the bank root biotope of the three sampling stations by kick sampling techniques. This technique has earlier been described (Hynes, 1970; Peterson and Fernado, 1970; Towns, 1979; and Berton, 1980).

An area of 0.3 m^2 was carefully marked out each sampling station. On every sampling occasion the substratum and emergent vegetation were vigorously disturbed for about five to ten minutes so as to dislodge the organisms. A benthic hand net made of bolting silk No. 10 mesh (154 $^u/_m$) with a plastic bottle at the end of it was used to sweep

organisms along water current. This was later emptied into a wide-mouthed plastic container. This technique was repeated three times at each sampling station.

On some sampling occasions samples were taken to the laboratory without fixing. The aim of this exercise was to see the moving organisms. This was useful for identification purpose, and a better method of recovering very small macro benthic invertebrates.

Macro invertebrate samples collected from the different stations were sieved through a 0.25 mm mesh sieve before sorting was done. Sorting was done both with the unaided eyes and under the Olympus, binocular dissecting microscope (model 570, 0.7 to 4-2x). Organisms were sorted into taxa, each taxon representing distinct morphological entity. The number of individuals was counted and recorded.

Representative specimens were photographed while temporary slides of representative specimens were prepared by mounting in polyvinyl lacto phenol, tented with lignin pink between slides and cover slips and then sealed with nail vanish for examination.

Large specimens were mounted directly without slide preparations. Specimens were identified using only the external taxonomic features. Specimens were preserved in vial bottles with 70 % alcohol mixed few drops of glycerin to keep them from dehydration.

Identification of species was by use of a wild MLL binocular microscope and relevant texts used to aid identification included Needham and Needham (1962), Mellamby, (1963), and Smith (1984). Also some notable aquatic Entomologists and Limnologists including Prof. Madubinyi of Entomology Unit, Faculty of Veterinary Medicine and Prof. M .C .Eluwa both of University of Nigeria, Nsukka and Prof Landis Hare of University of Waterloo, Ontario Canada were consulted for the identification of the macroinvertebrates.

Temporal Variability and Potential Fish Yield: Temporal variabilities in each water quality parameter were evaluated on annual and seasonal scales by the coefficient of variation (% CV = standard deviation x 100 / mean) (Lowentin, 1966) with the expectation that the amplitude of variation would be high under "poor" water quality condition and vice-versa under "better" condition (Karr *et al*., 1981). Potential fish yield from the river was estimated from the morphoedaphic index (Welcome and Henderson, 1976) using the formula: Y = 14.3136 (MEI); where Y = Potential fish yield (kg yr $^{-1}$); MEI = morphoedaphic index (i.e. annual mean conductivity/mean depth of river in metres).

Anthropogenic Perturbations: Non-quantitative field observations were conducted on the major forms of anthropogenic perturbations of the river basin that are likely to influence its suitability for aquatic organisms and fish productions.

Data Analysis: Ninety-seven macro invertebrate species were collected during the survey. The numeric and biomass data matrices consisted of eight sampling cruises covering three sampling stations each with five replicate. Numeric density was expressed as individuals/1000 m^2 and biomass in grams (wet weight). Species richness, biomass, individual numeric abundance, and species diversity (Shannon-Wiener H) were computed for each station using pooled data. Temporal variability of each water quality parameter were evaluated on annual and seasonal scales by the coefficient of variation (% CV = standard deviation x 100/mean; Lowentin, 1966) with the expectation that the amplitude of variation would be high under "poor" water quality condition and vice versa under "better" condition (Karr *et al.,* 1981). Potential fish yield from the river basin was estimated from the morphoedaphic index (Welcomme and Hendson, 1976). Non-quantitative field observations were conducted on the major forms of anthropogenic perturbations of the river basin that are likely to influence its suitability for fish production.

RESULTS

Physicochemical Characteristics: The annual means and ranges of the physicochemical parameters of the river are presented in Table 1. Means of the river basin were calculated for 22 months. The minimum and maximum records of water level showed that the amplitude of annual fluctuation was moderate and represented only 2.95 fold variation. The river has a relatively stable thermal regime with a surface water temperature difference of 6.4^o C between the extreme values. Water transparency displayed a 2.4 fold annual variation in the concentration of suspended solids. This lowest water transparency and highest concentration of suspended solids registered indicate that there were periods of very low light penetration.

Although average water pH was approximately neutral, the minimum and maximum estimates respectively depicted slight acidity and alkalinity. Annual fluctuation was minimal and moderate for fish production with a difference of 1.01 between the extreme ends (Table 1). The Anambra river was well oxygenated, with mean DO of less than 6 mg1^{-1}.

The average concentration of Co_2 was relative and the range corresponded to a 1.96-fold annual variation The Co_2/DO_2 ratios revealed that the levels of Co_2 exceeded those of DO_2 except in their minimal values in the month of (Jun and July) which were slightly less than a half unity. Total alkalinity was mainly of the bicarbonate type, with high values recorded during the wet season. The ranges in the concentrations of nitrate-nitrogen and phosphate-phosphorus revealed relatively moderate magnitudes of fluctuations (2.94 fold for No_3-N and 4.4 fold for PO_4-P).The nitrate- nitrogen exceeded those of phosphate-phosphorus during the months of the study

The mean conductivity of the River Basin revealed a relatively moderate dissolved electrolyte content.

Table 1: Physicochemical attributes of Anambra river basin and optima levels for fish production in tropical aquatic ecosystems

		Levels in Anambra River Basin			Optima levels for fish production		
		Means	Mini.	Maxi.	Mini.	Maxi.	Ref.
1	Dept. (m)	5.97	3.76	11.1			
2	Temperature (^{i}C)	28.89	25.7	32.1	25.0	32.0	1
3	Transparency (cm)	1.79	1.1	2.6	30.0	60.0	1
4	Coefficient of Coarseness of SS	3.01	1.1	6.0			
5	Suspended Solids Mgl^{-1}	3.03	1.3	5.6	25	80	2
6	Conductivity μscm^{-1}	29.81	16.9	40.5	40.7	61.8	4
7	PH	6.68	6.19	7.2	6.5	9.0	1
8	Nitrate-Nitrogen Mgl^{-1}	3.23	1.8	5.3			
9	Phosphate-phosphorus Mgl^{-1}	7.53	3.0	13.2	12.5	60.0	4
10	Free Carbon dioxide $MgCaCo_2l^{-1}$	3.33	2.3	4.5		5.0	1
11	Total Hardness $MgCaCo_3l^{-1}$	6.43	4.1	11.01		5.0	
12	Dissolved o_2 Mgl^{-1}	5.18	3.85	7.13		5.0	
13	Biological o_2 demand Mgl^{-1}	3.62	2.20	5.9			
14	Total alkalinity $MgCaCo_3l^{-1}$	33.28	25.34	48.36	20	300	1
15	Current ms^{-1}	2.02	1.6	2.6			
16	No_3-N/P04- Ratio	0.54	0.19	1.77		4.0	3
17	Co_2/o_2 Ratio	0.81	0.42	1.31			
18	Chemical o_2 demand Mgl^{-1}	4.17	1.81	6.8			

1 = Boyd and Lichkropler (1979), 2. = FWPCA (1968) 3. = Kutty (1987), 4. = King (1998)

Table 2: Conductivity (μscm^{-1}) and pH of some Africa river systems (Welcomme, 1985)

S/N	Rivers	Conductivity	pH	Reference
1	Ubangui	19.4-56	6.2-6.7	Micha, 1974
2	Oshun	57.96		Egborge, 1971
3	Niger	31-7-	6.7-7.2	Daget, 1957
4	Sokoto		6.9-8.1	Welcomme and Henderson, 1976
5	Chari	22-73	6.9-7.7	Welcomme, 1972
6	Black Volta	41-124	6.5	Welcomme, 1972
7	Idodo	27.9-54.1	6.04-7.39	Anibeze 1995
8	Anambra	16.9-40.5	6.19-7.2	This Study

Potential fish yield of the Anambra River = $Y = 14.3136 (MEI)^{0.4681}$ MEI = Conductivity/Dept MEI = Morphoedaphic index (i.e. annual mean conductivity/mean depth of the River. Annual mean conductivity = 29.44, Annual mean depth = 5.78, $Y = 14.3136 (2944/72.91$ kg ha^{-1} based on a morphoedaphic index.) $0.4681 = 72.905257^{0.4681}$

The range of conductivity (Table 2) was moderate represented only a 2.4 fold changes during the period of study

Macroinvertebrate: A total of 1808 individuals (mostly adults) belonging to 97 species of macro invertebrate fauna were collected. The composition of the river basin strongly dominated by Coleopteran, and Hemiptera. The coleopteran group consisted of Hydrophiledae, Dytiscidae, Gyrinidae**,** and Chrysomelidae (Table 3).

Among the species Gyrinidae ranked highest in abundance, followed by Decapods and the least were the species of the Simuliidae (Table 3). In the fauna is the monthly variation was A total of 1808 individuals (mostly adults) belonging to 97 species of macro-invertebrate fauna were collected. The overall composition of the river basin is strongly dominated by coleopteran and Hemiptera. The coleopteran groups consisted of Hydrohilidae, Dytiscidae, Gyrinidae and Chrysomelidae. Among the species Gyrinidae ranked highest in abundance, followed by Decapods and the least were the species of the Simuliidae (Table 3). The monthly variation of fauna was highest in the dry season months of November to January and low in September and October, wet season months.

Temporal variability and Potential Fish Yield: The estimated annual fish yield of the river basin was 72.91 kg ha^{-1} based on a morphoedaphic index. Temporal dynamics in the physicochemical parameters of Anambra river basin as indexed by coefficients of variation are presented in Table 4.

Amplitudes of annual variability were generally low for the lotic media (CV 30 %). The most variable parameters (CV 30 %) were suspended solids, Nitrate-Nitrogen, phosphate-phosphorus, total hardness, No_2-N/PO_4-P ratio, and Co_2/DO_2.Table 5.

Intra seasonal variability in the water quality of the River revealed that Transparency, conductivity, Nitrate-Nitrogen, Total hardness, biological oxygen demand, No_2-N/PO_4 ratio and Co_2/DO_2 were more variable in the dry season while all other attributes were more variable during the rains. Overall river quality was more stable in the dry season than in the wet season. Biological oxygen demand displayed a 2.7 fold annual variation and this indicated that the river was slightly polluted.

Anthropogenic Perturbations: About 50 – 60 % of the primary riparian vegetation of the River Basin has been cut to make way for predominantly subsistence crop agriculture.

Table 3: The composition, distribution, density (No/1000m^2) and diversity of macroinvertebrate fauna in the study stations of Anambra river basin for 22 months

Taxonomic	Ogurugu Station 1		Otuocha Station 2		Nsugbe Station 3		Total	
	No. of Taxa	No. of Indiv.	No. of Taxa	No. of Indiv.	No. of Taxa	No. of Indiv.	No. of Taxa	No. of Indiv.
1.*Coleoptera:*								
Hydrophilidae	3	67	10	80	1	9	14	156
Dytiscidae	4	89	7	101	2	14	13	204
Gyrindiae	6	184	8	270	3	91	17	545
Chrysomelidae	4	19	1	10			5	29
2. Decapods	2	110	4	215	1	40	7	365
3. Nematodes			1	2	1	1	2	3
4.*Hemiptera:*								
Nepidae	3	90	15	171	2	58	10	319
Velidae	1	5	3	87	2		6	126
5. Lepidoptera	2	5	3	6		3	5	11
6.*Mollusca*:								
Lymnaeidae			2	8	1	6	3	11
7. *Oligochaeta:*								
Lumbriculidae			3	10	2		5	16
8. *Dipterans:*								
Chironomidae	2	4	2	7			4	11
Simuliidae			1	2			1	2
9. *Ephemeroptera:*								
Baetidae			2	5			2	5
Caenidae			2	3	1	2	3	5
Total:	28	625	55	983	14	166	97	1808
Taxa richness		9.66		18.04		5.86		
Shannon div (H)		0.86		3.15		0.21		
Equitability.		0.09		0.32		0.03		
Faunal Similarity		0.69		0.64		0.64		
Community dominance		47.04		49.69		53.1		

Table 4: Temporal variability in the physicochemical attributes of Anambra river basin

S/N	Physicochemical Attributes	Coefficient of Variation %		
		Means	DS	WS
1	**Dept**	5.78	29.58	37.34
2	**Temperature**	28.91	4.98	6.30
3	**Transparency**	1.75	30.86	24.57
4	**Coefficient of Coarseness of suspended solids**	2.85	36.53	48.07
5	**Suspended Solids**	3.13	34.50	40.26
6	**Conductivity**	29.44	28.63	26.56
7	**Hydrogen ion Concentration**	6.69	5.38	6.28
8	**Nitrate-Nitrogen**	3.7	31.08	26.76
9	**Phosphate-phosphorus**	7.72	35.49	39.12
10	**Free Carbon dioxide**	3.68	19.57	21.20
11	**Total Hardness**	7.23	33.20	27.80
12	**Chemical oxygen demand**	4.27	15.69	20.80
13	**Dissolved oxygen demand**	5.14	12.54	16.28
14	**Biological O_2 demand**	4.06	11.69	10.98
15	**Total alkalinity $MgCaCo_3l^{-1}$**	3.60	22.51	39.62
16	**Current ms^{-1}**	2.07	25.60	25.76
17	**$No_3^-N/P04-$ Ratio**	0.66	83.33	53.77
18	**Co_2/o_2**	1.00	54.00	34.64

Values for calculation were used as from January-Dec. Dry season values were from Jan.-March and Nov.-Dec. Wet season = April—Oct.

This riparian land use has exposed the basin to non-point source input of surface run-off which peaks during the wet season. This has resulted in the silting up of the River banks and the concomitant destruction of fish micro-habitats, spawning areas and benthic food items.

Additionally, there are many discharge points of untreated municipal effluents into the river basin. The effluents enter the basin throughout the year with peaks in the wet season.

Moreover some of the river banks serve as dumpsites for city garbage which are constantly washed into the river via surface run-off. The rice mills are typical illustration of this effluent discharge into the river and anthropogenic perturbations

DISCUSSION

The potential impacts of the observed anthropogenic water shed perturbations include inter alias, water

Table 5: Monthly variations in Co_2/Do_2 and No_3-N/Po_4-P ratio in the river

S/N	Months	Co_2/Do_2	No_3-N/Po_4-P
1	**January**	1.30	1.77
2	**February**	1.05	1.07
3	**March**	1.28	0.78
4	**April**	0.80	0.57
5	**May**	0.64	0.43
6	**June**	0.58	0.33
7	**July**	0.59	0.27
8	**August**	0.58	0.23
9	**September**	0.64	0.40
10	**October**	0.89	0.25
11	**November**	0.91	0.48
12	**December**	1.19	0.67
13	**January**	1.21	0.98
14	**February**	1.31	0.83
15	**March**	0.97	0.74
16	**April**	0.67	0.57
17	**May**	0.61	0.46
18	**June**	0.46	0.35
19	**July**	0.42	0.30
20	**August**	0.50	0.26
21	**September**	0.54	0.22
22	**October**	0.70	0.19

quality degradation and/or high temporal plasticity in the physicochemical parameters (Karr *et al*., 1981). These can adversely influence the survival, growth and reproduction of aquatic biota including fish. The optima levels of physicochemical parameters for fish production in the tropics are presented in (Table 1). Surface temperature readings were well within the limits conducive for aquatic biota as well as for fish growth and production (Boyd and Lichkroppler, 1979). Mean transparency was lower than the optimum for lotic fish production; this low light penetration is probably responsible for the poor growth of aquatic macrophytes of the Anambra river which can enhance the growth of phytoplankton, an important food for aquatic biota and fish.

The levels of suspended solids in the Anambra river fall short of the FWPCA (1968) ranges for the maintenance of good or moderate fisheries. Since the suspended solids of the river comprise mainly silt/clay particles, its low concentration may not be a limiting factor based on the premise that it is only when suspended solids are composed of largely plankton that the low levels are limiting to aquatic biota and fish production (Boyd and Lictkoppler, 1979). However, this does not rule out the ecophysiological impacts of very high concentrations of suspended silt/clay on aquatic biota and fishes (Karr and Schlosser, 1981).

Mean river pH and DO_2 were within desirable ranges for fish production (Boyd and Lichkroppler, 1979). The low Co_2 content of the River Basin is suitable for aquatic biota and fish production as it may not be lethal to fish or culminate in the exhibition of a variety of distresses (Kutty 1987).

The river alkalinity indicates that the “soft water” is unlikely to support appreciable phytoplankton productivity (Boyd and Lichkroppler, 1979). The low primary productivity of Anambra River is corroborated by the low conductivity, concentrations of nitrate-nitrogen, and phosphate-phosphorus, all of which are nutrient determinants of phytoplankton productivity and hence fish production (Wetzel and Likens, 1979). Moreover, the mean No_3-N/Po_4-P ratio in the river is below that suggested by Kutty (1987) for the maintenance of a healthy aquatic ecosystem.

The high variability in the river volume indicates that the inputs of the composite of precipitation, surface run-off and municipal effluents, are in volumes that have an impact on the overall water levels of river throughout the year. The absence of marked variability in surface temperature is in consonance with the reports of Armitage (1984) for tropical streams and Abohweyer (1990) for the Kigera Reservoir, Nigeria since fishes have poor tolerance for sudden short-term temperature fluctuations (Boyd and Lichtkoppler, 1979), the stability in the ambient temperature of the river basin is thus an asset for fish production.

The stability of the Anambra river pH is attributable to the fact that most of the hydrogen ions are autochthonous; thus the river pH is unresponsive to cycles in the input of precipitation, surface run-off, municipal effluents and garbage’s. This low variability in pH which is comparable to that of Kigera (Abohweyere, 1990) is an index of good biotic integrity. The low variability in conductivity contradicts the high variability recorded in the Kigera (Abohweyere, 1990) and connotes regular mineralization of nutrients and allochthonous loadings.

The annual variability in DO was probably occasioned by regimes in the levels of CO_2 and inputs of municipal effluents and organic garbage’s. High variability in D0 concentration reduced the growth rate of fishes (Tsadik, 1984), but the level of DO is conducive for fishes in Anambra river, its variability appears to be favourable to fish growth. The broad variation in the level of suspended solids is in accordance with the findings of Karr and Dubley (1981) in modified headwater streams in Eastern North America and Abohweyere (1990) in the Kigera reservoir, Nigeria. The variations in the concentrations of nitrate-nitrogen and phosphate-phosphorus contradict the latter author.

The higher dry season variability (vis-à-vis the wet season) in nitrate-nitrogen, phosphate-phosphorus and CO_2/DO ratio are considered to reflect the effects of subtle intrinsic factors predominating in the dry season. Conversely, the higher variability of all other parameters in the wet season is perhaps related to the impacts of extrinsic factors e.g. precipitation, influx of surface run-off from riparian agro ecosystem and municipal effluents which were more predominant during the wet than dry season. Stochastic (CV = 30 % annually and/or per season) in most of the water quality parameters (Table 2) can adversely influence the well-known tropical seasonality regimes in the reproductive and feeding strategies of fishes (Lowe-McConnell, 1978).

The distribution and abundance of specific taxa could be of use in assessing the levels of impact in the study stations. Nematodes although considered as meiobenthos were used here because of their role

as indicators of siltation. Their abundance was however low in stations 2 and 3. The oligochaeters respond to organic pollution by increase in abundance (Egborge, 1995; Eyo and Ekwonye, 1995; Odo *et al.,* 2007). Their presence in stations 2 and 3 could be attributed to organic enrichment and growth of macrophytes. Decapods are common taxa in this river. Their presence in all the station 3 indicates that they are tolerant to the anthropogenic perturbations. Victor (1996) reported that coleopterans are useful indicators of organic pollution. They were the most abundant of the invertebrate fauna of the river.

This means that the aquatic ecosystem is favourable to them. The dipterans, notably the family chronomidae, have been found to dominate aquatic invertebrates communities (Hynes, 1970; Eyo and Ekwonye, 1995) and show no habitat restriction but was absent in station 3. The importance of water current in the distribution of the dipterans was further reinforced by their disappearance from station 3. The Hemiptera was second most abundant fauna of the River. They were recorded in all the 3 stations but was more abundant in Otuocha, the station 3. The high abundance could be as a result of its tolerance to municipal effluent, levels of physiochemical parameters, agro ecosystem perturbations and organic pollution.

The density and diversity indices vary both spatially and temporally. The pattern of temporal dynamics in the density of fauna was affected by the variability of the physicochemical parameters of the river. The overall diversity is the product of all spatial and temporal changes affecting the community. The higher variability in diversity indices that were observed in stations 2 and 1 are reflection of community instability in these stations. This is a further proof of the documented change in species composition, community structure, density and measures caused by agro ecosystem perturbations, municipal effluents, anthropogenic influences as well as physicochemical parameters, of lotic environments.

The potential fish yield from the Anambra River is high when viewed vis-à-vis the average estimates from other waters bodies e.g. the Cross river, Nigeria, Y = 57.44kg ha^{-1}, (Moses, 1987) and Kigera reservoir, Y = 19.5kg ha^{-1}, (Abohweyere, 1990). The high potential fish yield from the River is attribution to some of the aquatic physicochemical that are favourable to aquatic biota and macro invertebrates that serve as food for fishes.

From the foregoing, some of the water quality parameters are unsuitable for aquatic biota and fish production. The various methods of adjusting water quality parameters (Boyd and Lichtkoppler, 1979; Boyd, 1982; Kutty, 1987) can be employed prior to harnessing Anambra river for intensive fish production. The water quality of the river can be improved further by controlling and/or prohibiting the discharge of municipal effluents and domestic garbage into the river as well as the use of the riparian zone for crop agriculture. The maintenance of a 50 – 60 m of thick riparian vegetation can act as buffer strip to check erosion by acting as sediment break/filter.

REFERENCES

ABOHWEYERE, P. O. 1990). Study of Limnological parameters and potential fish yield in Kigera reservoir (extensive system) in Kainji, New Bussa, Nigeria. *Journal of Aquatic Sciences 5:* 53 – 58.

ALAM, M. J., HABIB, M. A. B. and BEGUM, M. (1989). Effect of water properties and dominant genera of phytoplankton on the abundance available genera of zooplankton. *Pakistan Journal of Science,* 32: 194 – 200.

APHA (1976). *Standard methods for examination of water and waste water* (14th Edition). America Public Health Association, Washington, DC. 1448 pp.

ARMITAGE, P. D. (1984). Environmental changes induced by stream regulation and their effect on lotic macroinvertebrates communities. Pages 139 – 165. *In:* LILLEHAMMER, A and SALTCEIT, S. J. (Eds). *Regulated rivers.* Oslo University Press, Oslo.

ALI, S., RAHMATULLAH, M. and HABIB, M. A. B. (1986). Interspecies correlations in some dominant species of phytoplankton. *Bangladesh Journal of Microbiology, 3:* 29 – 36.

ANIBEZE, C. I. (1995). *Aspects of the ecobiology of Heterobranchus longifilis (val. 1840) in Idodo river Basin (Nigeria) and their application in aquaculture.* PhD thesis, Department of Zoology, University of Nigeria, Nsukka.

AWACHIE, J. B. E. (1976). Fish culture possibilities on the flood plain of the Niger-Benue drainage system. *CIFA Technical Paper, Number 4 (supplementary 1):* 251 – 258.

BOYD C. E. (1982). *Water quality management for pond fish culture.* Elsevier Science Publishers, Britain. 318 pp.

BOYD, C. E. and LICHTKOPPLER, F. (1979). *Water quality management in pond fish culture.* International Center for Aquaculture. Agricultural Experiment Station, Auburn University Research and Development. Series No. 22 Project AID/DSANG. 1039 pp.

EGBORGE, A. B. N. (1995). The chemical hydrology of Osun river, Western State, Nigeria *Freshwater Biology, 4:*177 – 191.

EYO, J. E. and EKWONYE, U. C. (1995). The macroinvertebrate fauna of pool in the floodplain (fadama) of the Anambra River, Nigeria. *Freshwater Forum, 5(23):* 160 – 162.

FWPCA (1968) *Water quality criteria.* Federal Water Pollution Control Administration (FWPCA); National Technical Advisory Committee Report, Washington DC, 234 pp.

HIRATA, H. (1977). Zooplankton cultivation and prawn seed production in an artificial

ecosystem. *Helgolander wire mecresunters, 30:* 220 – 242.

HIRAYAMA, K. and WATANABE, K. (1973). Fundamental studies on physiology of rotifera for its mass culture (IV): Nutritional effect of yeast on population growth of rotifera. *Bulletin of Japanese Society of Science and Fishery, 39:* 1129 – 1133.

HYNES, H. B. N. (1970).*The ecology of running waters.* University of Toronto Press, Toronto. 555 pp.

KARR, J. R. and DUBLEY, D. R. (1981). Ecological perspective on water quality goals. *Environmental Management, 5:* 55 – 68.

KARR, J. R. and SCHLOSSER, I. J. (1981). Ecological perspective on water quality goals. *Science,* 201: 229 – 234.

KARR, J. R., TOTH, L. A. and GARMAN, G. D. (1981). *Habitat preservation for Midwest stream fishes: principles and guidelines.* US Environmental Protection Agency EPA-600/55-83-006, 120 pp.

KUTTY, M. N. (1987). *Site selection for aquaculture: Chemical features of water.* African Regional Aquaculture Centre, Port Harcourt, Nigeria ARAC.87.WP.2 (9), 53 pp.

KING, R. P. (1998). Physiochemical indices of the fisheries potential of a Nigerian rainforest pond. *Journal of Aquatic Sciences, 13:* 49 – 54.

LOWE-McCONNELL, R. H. (1978). *Ecological studies in tropical fish communities.* Cambridge University Press, Cambridge. 382 pp.

LOWENTIN, R.C. (1966). On the measurement of relative variability. *Systematic Zoology, 15:* 141 – 142.

MICHA, J. C. (1974). First guidelines for the culture of *Clarias lazera* in Central Africa. *Aquaculture, 4:* 227 – 248.

MOSES, S. (1987). The influence of flood regime on fish catch and fish communities of the Cross River floodplain ecosystem, Nigeria. *Environmental Biology of Fish, 18:* 51 – 65.

NEEDHAM, J. G. and NEEDHAM, P. R. (1962). *A guide to the study of freshwater biology.* Holder-Day Incorporated, San Francisco. 180 pp.

ODO, G. E., INYANG, N. M., EZENWAJI, H. M. G. and NWANI, C. D. (2007). Macroinvertebrate fauna of a Nigerian freshwater ecosystem. *Animal Research International, 4(1):* 611 – 616.

OVIE, S. I. (1993). Contribution to zooplankton community studies of some water bodies in Nigeria. *End of Course Report of the International Training Course, Zooplankton: A Tool in Lake Management.* 26 pp

PETERSON, D. and FERNANDO, R. Y. (1970). Macroinvertebrate communities in the erosional biotope of an urban stream in Nigeria. *Tropical Zoology, 4:* 49 – 59.

SMITH, T. Y. (1984). Community structure of macroinvertebrate fauna and water quality of a saline river system in Southwestern Australia. *Hydrobiologia, 248:* 143 – 160.

TOWNS, Y. (1979). Macroinvertebrate communities of a perturbed stream in Southern Nigeria. *Journal of Tropical Ecology, 4:* 1 – 10.

TSADIK, G. G. (1984). *Influence of oxygen on feeding and growth of cichlid fish, Oreochromis niloticus (Linnaeus)* M. Tech (Aquaculture) Thesis. African Regional Aquaculture Centre/Rivers State University of Science and Technology, Port Harcourt, Nigeria. 66 pp.

WATANABE, T. (1978). Nutritional quality of living feeds used in seed production of fish. Pages 49 – 57. *In: Proceedings of 7th Japan Soviet joint Symposium on Aquaculture.*

WETZEL, R. G. and LIKENS, G. F. (1979). *Limnological Analysis.* WB Saunders Company, Philadelphia, 357 pp.

WELCOMME, R. L. (1972). River fisheries. *FAO Fishery Technical Paper, 262:* 1 – 330.

WELCOMME, R. L. and HENDERSON, H. E. (1976). Aspect of the management of inland waters for fisheries. *FAO Fishery Technical Paper, 161:* 1 – 36.

VICTOR, R. (1996). The effects of urban perturbations on the benthic macroinvertebrates of a southern Nigeria stream. Pages 223 – 238. *In: Perspectives in Tropical Limnology.*

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