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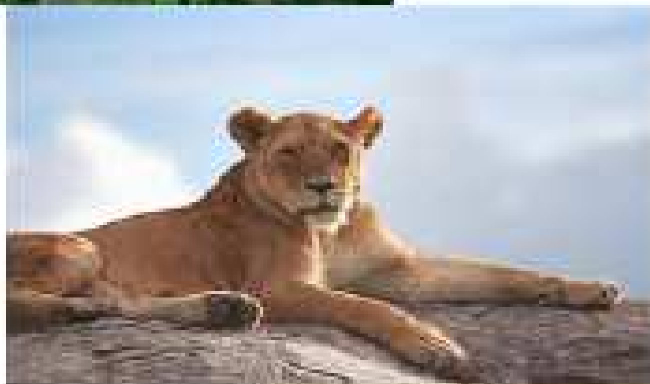

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HISTOLOGICAL STUDY OF THE PHARYNGEAL PAD OF THE AFRICAN CATFISH (*Clarias gariepinus* BURCHELL 1822)

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ABSTRACT

The pharyngeal pad located dorsally on the oro-pharynx was processed for light microscopy. The epithelium was of stratified mucous type containing taste buds, club cells and teeth. The micromorphology revealed the co-localization of teeth and taste bud. Developing, erupting and erupted teeth were also seen. The thin bone of cancellous type with a marrow seen provided point of origin and insertion for the skeletal muscle present. Osteoblasts were seen on the surface of the cancellous bone probably depositing bone matrix. The pad had a base of hyaline cartilage. Stratum adiposum was observed and may be site for nutrient storage and also function in reducing friction between sliding muscle fibres since it was sandwiched between muscle fibres. The micromorphology suggest an organ used in trituration and selection of food by gustation. The blood vessels in the bone marrow suggest haematopoietic function. The bone and cartilage present is for support.

Keywords: Pharyngeal pad, Histology, Food selection, Taste bud, African catfish

INTRODUCTION

The teleost digestive tract is simple comprising bucco-pharyngeal cavity, oesophagus, stomach, intestine and anus (Diaz *et al.*, 2008; Raji and Norouzi, 2010). These organs are involved in the breakdown of ingested food macromolecules into small absorbable macromolecules. These small molecules are necessary for the maintenance, growth and energy needs of the body (Johnson, 1994; Junqueira and Carneiro, 2005). The bucco-pharynx is the region that collects food from the environment. It bears a variety of specialized organs for specific functions. The organs include: lamella organ, buccal values, tongue, pharyngeal pads and epibranchial organ (Girgis, 1952; Kapoor, 1957; Khanna, 1959; Schmitz and Baker, 1969).

The pharyngeal pads located on both sides of the pharynx dorsally are round, flattened interiorly and sunken interposteriarily. It has a small pharyngeal teeth and taste buds on the entire surface. It is supported by thin bony care of cylindrical bones (Venkateswarlu, 1962; Kawamoto and Higashi, 1965). Taste buds in all vertebrates including teleosts are for gustation (Roper, 1989; Reutter and Witt, 1993). The excitation of the taste bud in bucco-pharynx has seen associated with food swallowing (Atema, 1971). Pharyngeal teeth in some teleosts are used for food processing which involves mastication and crushing before transporting to the

esophagus for deglutition (Sibbing, 1982; Claes and De Vree, 1991; Vandewalle *et al.*, 1994, 1995). The pharyngeal pad as an organ serves in food trituration or act as an effective filter or food selector by gustation in some teleost (Ezeasor, 1982; Linber *et al.*, 1998).

In this study, microanatomy of the pharyngeal pad in the domesticated African catfish was investigated since there is no information in available literature on its micromorphology. The result will provide baseline information and the functional significance of the organ is discussed.

MATERIALS AND METHODS

Fifteen adult African catfish (weighed 900 ± 56g and standard body length of 45 ± 5 cm) sourced from commercial fish farms in Umudike, Abia State, Nigeria were used for the study. The fish were immobilized by stunning. The oro-pharyngeal cavity was cut open through the membrane between the upper and lower jaws, and the pharyngeal pad dissected out. The pharyngeal pad was seen as mound rounded solid mass attached dorsally on the oro-pharyngeal wall very close to oesophageal inlet (Figure 1). It was excised, decalcified according to Gooding and Stewart (1932), before subjecting to routine histological procedure of dehydration in graded ethanol, clearing in xylene and embedding in paraffin wax. Sections 5μm thick were obtained with Leitz microtome model 1512. General tissue morphology was observed after haematoxlin and eosin (H&E) staining.

RESULTS

The epithelia were of stratified squamous containing mucous cells and taste buds (Figure 2). Club cells were seen but interspersed in between the epithelia. Pharyngeal teeth were seen erupting, erupted and some developing as tooth bud beside developed tooth (Figure 3). The teeth that were caudally pointing were attached to the cancellous bone by collagenous fibres in regular direction. Blood vessels were seen in the bone marrow. The skeletal muscle was seen originating from the connective tissue near the bone (Figure 3). Adipose tissues were present between the skeletal muscle bundles (Figure 3). Beneath the epithelia was a thick layer of collagenous fibres. The cancellous bone had marrow in between the species. Osteoblasts were seen at the surface of the bone (Figure 4). The presence of hyaline cartilage was observed at the base with thick collagen bundle surrounding it (the perichondrium). Regions of appositional growth were seen. The hyaline cartilage had chondrocytes in groups surrounded by homogenous matrix.

DISCUSSION

After the ingestion of food, it is processed and modified by mastication, crushing, or tearing prior to swallowing (Vandewalle *et al.*, 1995). Revelation by this microanatomy indicates the involvement of this organ – pharyngeal pad in this food processing. The epithelia of stratified squamous type are for protection of underlying tissue (Tamarkin, 2011). The presence of mucous cells indicates production of mucin for lubrication of the organ against mechanical abrasion since the teleost digestive tract lacks salivary gland (Elbal and Agulleiro, 1986; Micale and Mughia, 2011).

The co-localization of the taste buds and teeth on the pharynx of fish have been reported in several literatures (Reutter *et al.*, 1974; Ezeasor, 1982; Sibbing, 1982; Hobbler and Merchant, 1983; Northcott and Beveridge, 1988). This has been associated with ingestion or rejection of potential food item by gustation prior to swallowing (Linser *et al.*, 1998). Majority of taste buds observed are of type II since their receptor areas are located beneath the elevated epithelia papillae, but type III were also present since they have their apices slightly above the surface of corresponding epithelia (Reutter *et al.*, 1974).

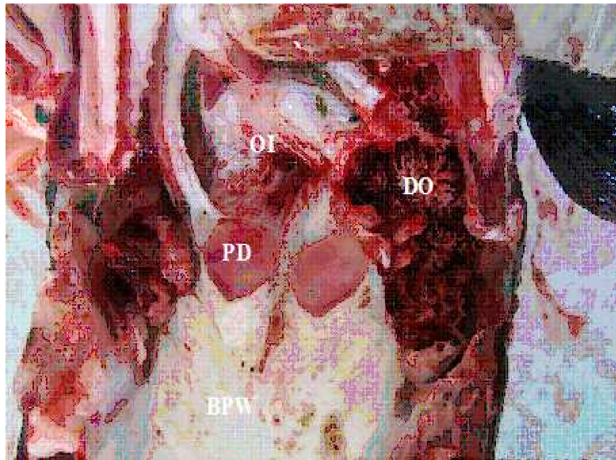

Figure 1: Topographic dissected section of adult maxillary part of buco-pharengeal cavity showing aditus oesophagus (OE),dendritic organ (DO), pharyngeal pad (PD), upper buco-pharyngeal wall (BPW). (H & E X 400)

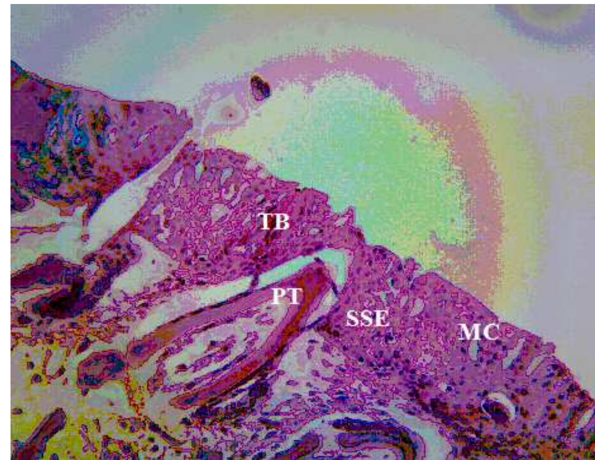

Figure 2: Adult pharyngeal pad showing taste bud (TB), pharyngeal pad tooth (PT), stratified squamous epithelia (SSE), mucous cell (MC). (H & E X 400)

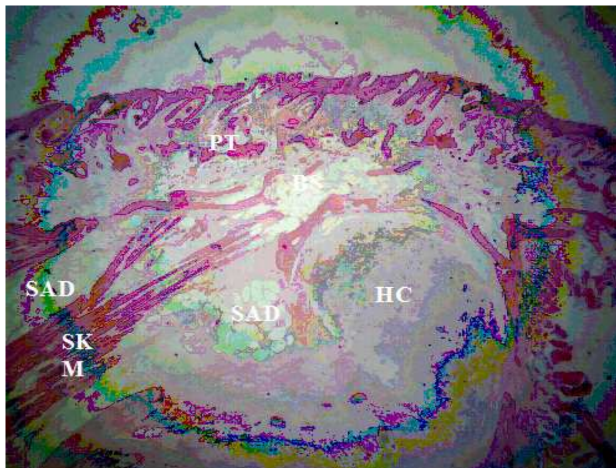

Figure 3: Section of adult pharyngeal pad showing pharyngeal tooth (PT), bone spicles (BS), skeletal muscle (SKM), Stratum Adiposum (SAD), hyaline cartilage, (HC). (H & E X 100)

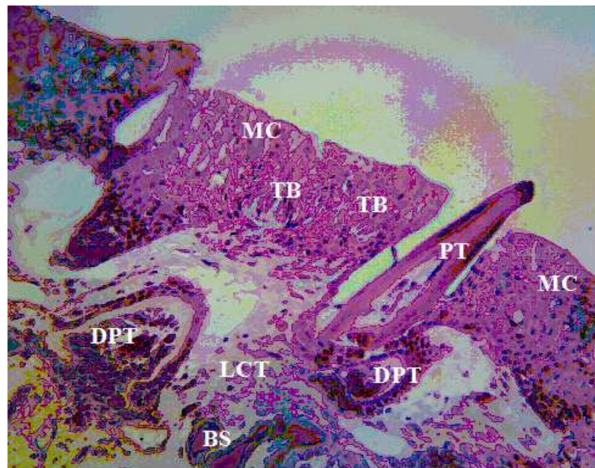

Figure 4: Section of adult pharyngeal pad showing taste bus (TB), mucous cell (MC), tooth (PT), developing tooth (DPT), bone spicles (BS) containing osteoblasts. Note loose connective tissue (LCT). (H & E X 400)

Type I taste buds were seen with prominently elevated receptor area above the epithelia (Ezeasor, 1982). The caudally pointing teeth may probably be used in crushing and directing the food towards the oesophagus. The presence of developing tooth bud beside each pharyngeal tooth and several unerupted teeth beneath the epithelium suggest that in the pharyngeal pad there is great turnover of teeth due to loss by mechanical action of regular crushing. It is also possible that the periodontal ligament is not firm enough to hold them in place for a long time, or the tooth which is of homodont dentition, is continually replaced, hence polyphydontia (Goth, 2009; John and Lisa, 2010).

The bone spicles with osteoblast present suggest a developing bore with continuous lay of bone matrix by the osteoblast (Baron, 2008;

Mellors, 2011). The presence of blood vessels in the bone marrow suggest haematopoietic function, or source of nourishment to this connective region. The bone which is of cancellous type may be providing point of origin and attachment for the skeletal muscle present. The skeletal muscle seen may probably be involved in voluntary regurgitation or selection of food (Al-Hussaini and Kholy, 1953; Barrington, 1957). The thick collagen fibre seen may be for support and strengthening of the epithelia. The stratum adiposum (Guerra *et al*., 2006) seen suggest metabolite storage, reducing frictional effect of the sliding and contracting skeletal muscle since it is present in-between the striated muscle layers and the bony core. The cartilaginous mass present is of hyaline type with homogeneous matrix and chondrocytes in lacunae. The presence of dividing chronblast in the perichondricum suggests appositional growth. This cartilaginous mass is for support (Lehner *et al*., 1989).

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PROXIMATE COMPOSITION OF *Oreochromis niloticus* SUBJECTED TO DIFFERENT PRESERVATION METHODS

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ABSTRACT

Twenty one (21) specimens of Oreochromis niloticus (total weight 400g) were collected from a market in Enugu metropolis and used to assess the effects of different preservation methods on proximate composition. The fish samples were stored for 28 days. Preservation and processing methods adopted were: refrigeration, deep-freezing, oven-drying, straight-smoking and salted smoking. The oven-dried, straight-smoked, and salted-smoked samples were stored in polythene bags at room temperature in the laboratory. Moisture, protein, fat, fiber and ash contents of all the sample were determined according to the Association of Official Analytical Chemists procedures. Free preservation on storage led to significant decrease (P<0.05) in the percentage ash, crude protein and fat contents of fish samples. The greatest reduction was observed in refrigerated samples while salted-smoked samples were the least affected. There was a significant difference (P<0.05) between the storage period and the free-fatty acid of the samples. The refrigerated samples showed the highest levels of rancidity while the salted-smoked method gave the highest level of stabilizing in the proximate composition on storage for 28 days.

Keywords: *Oreochromis niloticus*, Preservation methods, Proximate status

INTRODUCTION

In view of the increasing importance of tilapia (*Oreochromis niloticus*) as a cheap and affordable source of animal protein, the need to improve its handling, preservation and storage becomes necessary. Nigeria's annual demand for fish is rising astronomically and in order to meet this demand, measures must be taken to reduce heavy post-harvest losses through deliberate and conscious application of science and technology (Jones and Disney, 1967; Talabi 1977; Okpanefe, 1982).

The artisanal sector of Nigeria's fishery industry is faced with problems of spoilage and contamination of harvested fish. This is attributed to the fisher-folks' pattern of handling, processing and storage. Talabi and Igbinosun (1984) had estimated losses by weight of fish produced to be up to 45 %. The agents of spoilage (bacterial and autolytic enzymes) operate under optimum conditions. Bacteria require water to exist and are sensitive to heat, salt concentration and pH (Tobor, 1984). In Nigeria, temperature is very ambient for fish to get spoilt rapidly within 24 hours. This confirms Jones and Disney (1967) view that fish is a notorious perishable commodity. Microbial species primarily associated with fish spoilage belong to the genera *Pseudomonas, Micrococcus, Staphylococcus, Salmonella, Bacillus* and *Clostridium* (Ejike and Mohammed, 1982). Aribisalla (1978) noted that fish muscles are generally considered sterile when freshly caught, but when dead, the bacteria at the surface of the body, in the gills and intestines gradually penetrate into the sterile muscle. Ejike and Ezebialu (1983) investigated the effect of tissue water and temperature on bacteria load of deteriorating *Sarotherodon niloticus* and found that *Pseudomonas* and *Micrococcus* were the dominant organisms associated with

spoilage. When fish dies, a number of physical and chemical changes leading to spoilage take place in its body. Majority of quality changes that occur in fish result from protein denaturation and rancidity (Frazier and Westhoff, 1986).

Frazier and Westhoff maintained that fish oils seem to be susceptible to quick oxidative deterioration than most animal fats. Fatty fish is known to spoil quickly due to oxidation of fatty acids and lipid oxidation leading to rancidity. Reay *et al.* (1943) described the succession of external changes, in fish as it spoils and finally becomes putrid. They maintained that the bright characteristic colour of fish, fades giving rise to dirty-yellow or brownish discoloration. The most remarkable is the softening of the flesh and the exudation of juices when squeezed.

Owing to the prevailing temperature in Nigeria, fish preservation becomes a crucial aspect of fisheries commerce. Ikeme (1985) considered this problem and stipulated that only a negligible proportion of fish caught in the Nigerian rivers and lakes can be described as fresh since out of all flesh foods, fish is considered the most susceptible to autolysis. Oxidation and hydrolysis of fats as well as microbial spoilage, it becomes imperative that its preservation strategies must be prompt. Sorinmade *et al.* (1982) reported that refrigerated sea water at 4°C extended the storage life of *O. niloticus* for 18 – 28 days and maintained that all chemical parameters increased: thus minimizing the probability of nutrient losses during storage. Improvement of refrigeration facilities in both developed and developing countries has made refrigeration systems become more reliable and easier to use. Although some of the contaminating micro-organisms are killed in the process of freezing of fish (Cutting and Spencer 1968). Most of the psychrophilic micro-organisms associated with fish grow below 0°C.

Graham (1982) noted that when fish are correctly frozen after catch and stored in proper manner at the recommended temperature of 30°C, they do not keep indefinitely. Moisture control, primarily by drying provides an opportunity to prevent losses, which occur during harvesting, 'handling and storage. During smoking of fish, two distinct processes occur: i.e. drying which result in the characteristic texture and addition of smoke constitutes which results in the appropriate flavour in the product apart from its preservative effects (Foster and Simpson, 1961). The important aspects in the quality of smoked *O. niloticus* are concerned with freshness and manner of preparation of the raw material, the smoking process, the post-processing storage, transportation and retailing. Against this background, this study was aimed at evaluating the nutritional characteristics of *O. niloticus* preserved and stored under various conditions for a period of 28 days. The main focus was on the loss of nutrients arising from poor preservation and handling. Hence proximate composition of fish samples under various preservation methods were determined and compared. Assessments were made of the physico-chemical and fatty acid values of the fish samples.

MATERIALS AND METHODS

Fish: A total of twenty-one live specimens of *O. niloticus* (total weight 400g) used for this experiment were collected from the Artisan market, Ogui Road, Enugu, Nigeria. The fishes were descaled, degutted and thoroughly washed in clean water. Immediately after degutting, parts of the muscles of the fish samples were removed and divided into six sub-groups (15 g each). One group which served as the control was immediately taken for proximate chemical analysis, while the remaining five groups were subjected to five modes of processing and preservation viz: refrigeration, deep freezing, oven drying, straight smoking and salted smoking.

Processing and Preservation Techniques: Fish samples for the first group subjected to refrigeration were put in polythene bag, labeled and stored in a refrigerator at 0°C for 28 days, while the second group was wrapped in polythene bag, labelled and stored in a deep freezer at -10°C for 28 days. The third group was as well placed in a clean aluminum tray and

introduced into an isothermal oven pre-set at 105°C ± 5°C and dried for 4 hours. This was allowed to cool and transferred into a labeled polythene bag and stored at room temperature (25°C±4°C) in the laboratory for 28 days. A smoking Kiln (temperature 80°C – 85°C) was used to smoked another sample for 8 hours, after this the sample was allowed to cool at room temperature and packed in a labelled polythene bag and stored in the laboratory at room temperature (25°C ± 4°C) for 28 days. The last group of fish sample was soaked in 5 percent sodium chloride solution for an hour. The sample was smoked for 48 hours at 95°C ± 5°C, cooled at room temperature and stored at 25°C ± 4°C in a labelled polythene bag for 28 days.

Chemical Analysis: Proximate composition of each experimental fish sample was carried out to analyse protein, crude fibre, ash, moisture and fat composition using AOAC (1990). Metabolisable energy was determined using the bomb calorimetric method and carbohydrate by difference (AOAC, 1990). Free fatty acids were estimated using the lipid chromatographic method of Marrinetti (1967). Chemical analyses were done at day 0, 14 and 28.

Statistical Analysis: Data collected were analysed using analysis of variance (ANOVA) (Steel and Torrie, 1980) at 5% level of significance.

RESULTS AND DISCUSSION

The oven-dried, straight-smoked and salted-smoked samples showed significant differences ($P > 0.05$) from the refrigerated and deep-froze samples. The values for the various parameters also showed that the wet samples (control) had high moisture content and corresponding low fat values. The dried samples did not show very significant variations except the ash of salted-smoked treatment.

The pattern of distribution of values indicated that the percentage crude protein level of the treated samples and storage periods were significantly lower than those immediately after treatment at day 0. In all methods of preservation, the salted-smoked sample gave the highest crude protein on day 28 although straight-smoked sample had higher values on day 14 (Table 1).

The percentage fat content of fish muscles for the various storage methods showed that the refrigerated sample had the highest level of fat during storage, while the oven-dried and salted-smoked samples had the lowest level of fat, respectively (Table 1).

The changes in mineral composition as indicated by the ash values at day 0 were significantly higher ($P>0.05$) than values obtained at day 28. The salted-smoked sample had the lowest percentage of degradation in nutrients while the refrigerated sample had the highest nutrient loss.

The free fatty acid content of fish oil obtained during the various preservation methods showed progressive increase in free fatty values of each treatment up to day 14 followed by a decline in day 28, though at varying degrees (Table 1).

Fish undergoes appreciable deterioration in time not only at ambient temperature but even at cold storage temperatures of down to -30 °C. The quality of fish during storage is affected by the method of preservation. The observations of this research are in agreement with this view.

Results obtained from this study indicated that the different methods of preservation caused significant changes ($P<0.05$) in the proximate composition of the muscle tissues of *O. niloticus.*

All the fish wet preserved had high moisture content, while the dried samples had lower moisture values. This loss of moisture led to the different concentrations of the various nutrients in the resultant dried fish.

Estimation of ash indicated loss of nutrients during storage as shown by the decline in the percentage ash with highest losses obtained for samples under refrigeration. The salted-smoked and oven-dried fish samples produced the least variation in loss of minerals (ash) as compared to smoked, frozen and refrigerated samples.

Table 1: Some nutrient composition of *Oreochromis niloticus* subjected to various methods of preservation after 0, 14 and 28 days post preservation

Parameters	**Proximate composition (%)**								
Preservation methods	**Fresh sample**			**Refrigeration**			**Deep Freezing**		
Days	**0**	**14**	**28**	**0**	**14**	**28**	**0**	**14**	**28**
Carbohydrate	0.40	0.39	0.39	0.40	0.43	0.44	0.42	0.41	0.42
Moisture	70.59	70.60	77.61	76.82	76.84	76.80	77.10	77.21	77.18
Fibre	0.46	0.45	0.49	0.45	0.46	0.48	0.45	0.44	0.60
Protein	17.74	17.72	17.71	17.73	16.43	14.90	17.83	16.83	15.89
Fat	1.60	1.61	1.60	1.59	1.42	1.13	1.52	1.40	1.18
Ash	3.80	3.79	3.81	3.78	2.98	2.23	4.06	3.63	2.88
Fatty acids	3.70	3.71	3.66	3.65	5.34	4.56	3.58	4.81	4.39
	Straight Smoking			**Salted Smoking**			**Oven Drying**		
Carbohydrate	0.44	0.41	0.39	0.41	0.42	0.43	0.41	0.42	0.43
Moisture	18.60	18.62	18.62	21.60	21.59	21.60	17.58	17.60	17.62
Fibre	0.95	0.92	0.94	0.71	0.72	0.72	0.65	0.65	0.66
Protein	19.41	19.02	18.37	19.65	18.60	19.48	19.48	18.46	17.96
Fat	3.12	2.83	2.56	3.86	3.55	3.42	3.36	3.15	2.97
Ash	7.73	6.94	5.84	10.73	9.65	8.96	6.07	5.44	4.93
Fatty acids	4.23	5.85	5.42	4.57	5.76	5.42	4.46	5.24	4.50

This could be attributed to earlier observation made by Burges and Bannerman (1963) that drying and salting reduce the amount of water in the tissues available to spoilage by micro-organisms. This can further be explained by the findings of Igene (1983) that fish during smoking becomes impregnated with wood smoke and is thus given a distinctive flavour and becomes less liable to spoilage, since many components of the wood smoke act as antiseptic.

From the nutritional point of view, the most important quality parameter for fish is its protein content. Results obtained from this experiment indicated that the different methods of preservation led to significant decrease (P<0.05) in the percentage protein content of the muscle tissues of *O. niloticus.* Similarly decrease in crude protein was observed by Ufodike and Obureke (1989) when the effect of preservation techniques on quality of *O. niloticus* muscle was investigated. The workers reported that the gradual decrease in percentage protein level with storage time was probably due to hydrolysis of protein during autolysis in the fish tissue.

Ojobe *et al.* (1992) had made similar observations while working on crude protein levels of *Clarias gariepinus.* Reduction in crude protein level could be due to trimeihylamine and formaldehyde present in varying amounts in fish flesh (Dingle and Hynes, 1975). They stated that the amounts of these compounds some-times increase during storage while protein content decreases.

It was evident from the results obtained that crude protein deterioration was most pronounced in fish preserved by refrigeration (17.73 – 14.90). This was followed by deep-freezing (17.80 – 15.89) and oven-dried sample. Least reduction in crude protein were observed in straight-smoked (19.41 – 18.37) and salted-smoked sample (19.65 – 18.60). This suggested that salt-smoking is the best method for preserving *O. niloticus.*

Results obtained showec[1] variations in the fat content of the various methods of preservation. There were higher fat values in the dried samples, due to the loss of moisture in processing the fish muscle by drying. This trend was observed by Talabi and Igbinosun (1984) who contended that fish has inverse high or low water content depending on the fat content. It

is evident from this study that the refrigerated sample had the highest variation in value followed by deep-freezing samples.
Oven dried, straight and salted smoked samples had the least decline in percentage fat on storage. Similarly, the values for free fatty acids showed marked variations; with all the samples increasing in free fatty acids in the first two weeks and showing varying degrees of decline after four weeks. This may be explained by the observation made by Cutting and Spencer (1968) that the development of rancidity in fish during storage is due chiefly to the atmospheric oxidation assisted by certain tissue enzymes activated by the large proportion of highly unsaturated fat in fish

Conclusion: The authors wish to mention that results obtained from this research showed that salted-smoked fish gave least variation in proximate composition. It should therefore be employed as the best method for preserving fish among others. This becomes more appropriate because apart from the preservative effect of the method on the product, it adds flavour to fish tissue. Further research is recommended on the refrigeration of salted-smoked fish in order to compare its effectiveness in sustaining the nutritional quality of fish during storage.

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ASPECTS OF THE BIOLOGY OF *Hyperopisus bebe occidentalis* IN A TROPICAL FRESHWATER ECOSYSTEM

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ABSTRACT

Some aspects of the biology of Hyperopisus bebe occidentalis at Idah Area of River Niger were studied between October and December, 2010. A total of 129 fish samples were used for the study. The length-weight relationships were analyzed using the formula $W = aL^b$ and transformed to Log W = Log a + b Log L. Stomach contents were analyzed by frequency of occurrence method. The standard length for males, females and combined sexes ranged between 10.9 – 21.04 (cm), 11.3 – 22.5 (cm) and 10.90 – 22.70(cm) with b – values of 3.2953, 3.5015 and 3.4071. The condition factors for the male, female and combined sexes ranged from 0.56 – 0.98, 0.57 – 0.98 and 0.46 – 0.98, respectively. The result of the food and feeding habits showed that the fish feeds mainly on plant part (23.2%), larvae (14.4%), mollusk (24.0%), sand grain (17.6%), detritus (24.8%), Worm (10.4%), Algae (28.8%) and unidentified item (25.6%), respectively. H. bebe occidentalis in Idah area of River Niger could be referred to as an omnivore, feeding mainly on plant materials.

Keywords: Length-weight, Sexes, Stomach content, Feeding habits, Plant materials

INTRODUCTION

Fish is the critical food supply for the poor in the world, providing one billion people sustenance for their daily lives and 150 billion people employment in which 90% are in the artisan sector mostly in Africa. World wide per capita fish supply in 1997 stood at 16kg/year (World Fish Center 2005). The fishery sector is essentially in the economic development of many countries. The ability to meet world demand for fish from natural fish stock requires natural fisheries and genetically improved fast-growing fish species (Adeyemi *et al.,* 2011). The World Fish Center (2005) warned that exploitation of natural fish stocks is leveling off as population grows. Africa faces a major challenge to ensure fish supply to the estimated 200 million, mainly poor people relying on fish as a main part of diet.

The study of the biology of fishes could give important information necessary for fishery scientists in its management and sustenance. Several studies has been carried out on some aspects of the biology of some freshwater fish species across Nigeria (Oniye *et al.,* 2006; Malami *et al.,* 2007; Adeyemi *et al.,* 2009; Adeyemi, 2010) in order to generate useful information in positioning of the fishes in a food web in their environment and in formulation management strategy options.

Literatures on some aspect of the biology of mormyrids are vast (Nwani *et al.,* 2004; 2006 a, b; Oniye *et al.,* 2006; Malami *et al.,* 2007) among many others. This study is aimed at providing recent information on the length-weight relationship, condition factor and food and feeding habits of *Hyperopisus bebe occidentalis* a member of the family Mormyridae at Idah area of River Niger. This information is needful for sustainable management of River Niger mormyrid resources.

MATERIALS AND METHODS

Study Area: The study area (Figure 1) is Idah area of River Niger in Kogi State. The area is located on latitude 7 06'N and longitude 6 43'E of the Greenwich meridian in the Guinea Savannah vegetation zone of Nigeria. The study area experiences two weather conditions, dry season which starts from November to April and wet season which starts from April to October (Areola *et al*., 1992).

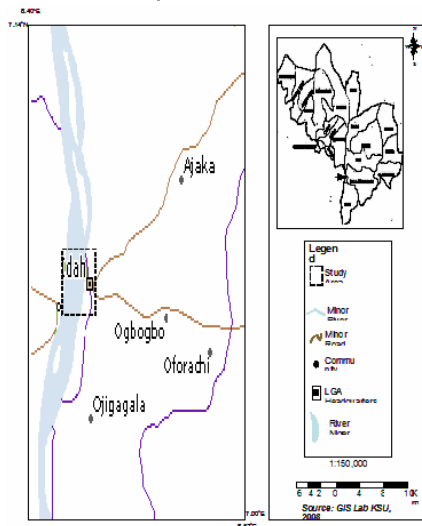

Figure 1: Map of Idah Area of River Niger, Kogi State, Nigeria

Sampling: A total of one hundred and twenty nine (129) samples of *H. bebe occidentalis* were purchased from fishermen at Idah area of River Niger between October and November, 2010 and identified (Idodo-Umeh, 2003; Olaosebikan and Raji, 2004) to species level. The fish samples were transported in plastic buckets to the Department of Biological Sciences Laboratory, Kogi State University, Anyigba for analysis.

Aspect of the Biology: The total length of the sampled fish were measured with an aid of measuring board from the anterior end of the fish snout (mouth closed) to the posterior extremity of the caudal fin, with the aid of a measuring board to the nearest 0.1 cm. The standard length was measured from the anterior tip of the snout to the end of the caudal peduncle for every fish in centimeters (cm). The body weight of each fish was also measured to the nearest 0.1g using top loading weighing balance in grams (g), respectively.

The sex of each fish was determined externally by the presence of a genital papilla (a corn-like projections of the genital aperture of the males which are absent in females). Each stomach was dissected and split open and the contents emptied into Petri-dishes containing 10% saline solution and observed under a compound microscope. The food items were counted and the stomachs were scored 0,25,50,70 and 100% according to its fullness as described by Bagenal (1978).

Data Analysis: For each fish sample, parameters such as length (L) in centimeters (cm) and weight (W) in grams (g) were used to estimate Length-Weight relationship (LWR) formula, i.e. $W = aL^b$ and transformed to $\text{Log } W = \text{Log } a + b \text{ Log } L$ through base 10 logarithm transformation. Allometric growth of the fish was recorded when the regression co-efficient 'b' was less than 3.0 or greater than 3.0 (Ama-Abasi, 2004; Paugy *et al.,* 2004).

The stomach contents were analyzed by frequency of occurrence as described by Hynes (1950) and Bagenal (1978). The occurrence of each food item was expressed as a percentage of all stomach with food. That is, $P = (b/a) \times 100$ where a = Total number of fish examined with food in the stomach; b = number of fish containing a particular food; P = percentage of occurrence of each food item.

RESULTS

One hundred and twenty nine (129) specimens were used out of which 63 were males and 66 were females. Male standard lengths ranged between 10.9 – 21.04 cm, while total length between 12.0 – 24.7cm. Female standard length ranged between 11.3 – 22.5 cm; with total length ranging between 12.5 – 24.6 cm. The combined sexes have standard length and total length ranging from 10.90 – 22.70 and 12.0 – 24.7 cm, respectively (Table 1). In terms of weight (g) males, females and combined sexes weight ranged between 10.0 - 74.0, 12.0 – 91.0 and 10.0 – 97.0g respectively. There was no significant difference (p>0.05).

Table 1: Body Measurement for *Hyperopisus bebe occidentalis* from Idah Area of River, Kogi State, Nigeria

Sex	Total No	SL(cm) Range	Mean SL (cm)	Wt (g) Range	Mean Wt (g)	a	b	r
Male	63	10.9 – 21.04	15.62±2.07	10.0 - 74.0	30.46±15.58	0.0033	3.2953	0.9629
Female	66	11.3 – 22.5	15.90±2.15	12.0 – 91.0	32.9±17.04	0.0019	3.5015	0.9788
Combined sex	129	10.90 – 22.70	15.90±2.4	10.0 – 97.0	44.92±15.70	0.0024	3.4071	0.9712

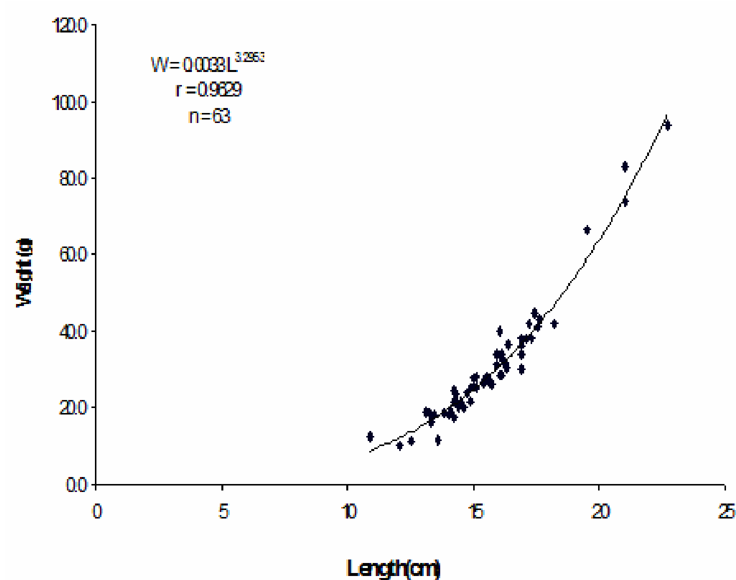

Figure 2a: Length-weight relationship of male *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

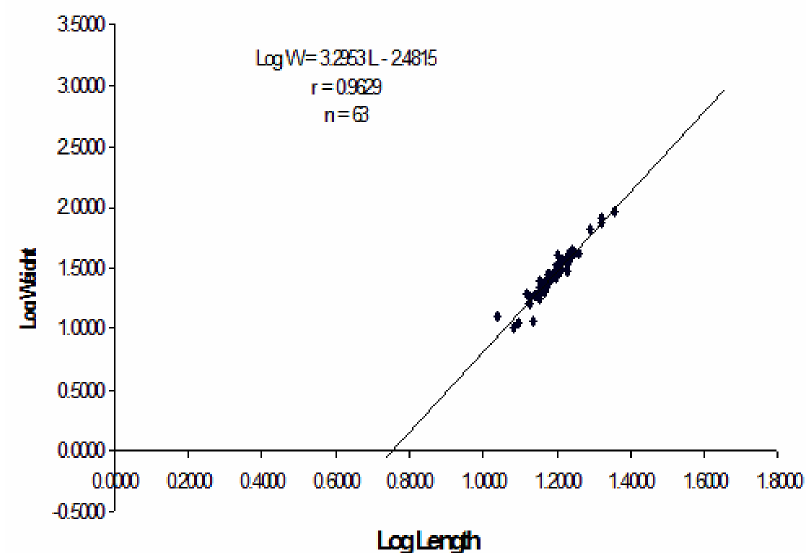

Figure 2b: Log length-weight relationship of male *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

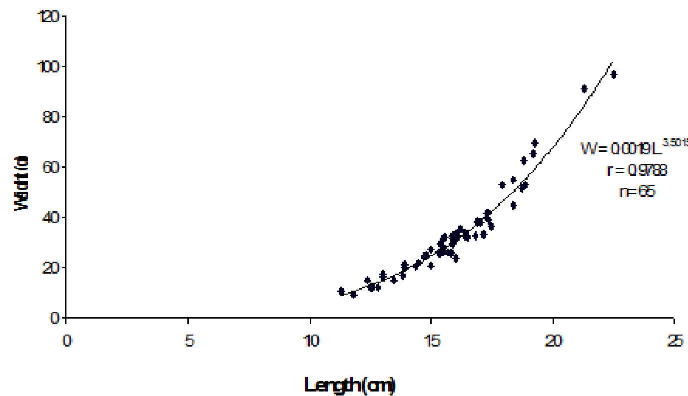

Figure 3a: length-weight relationship of *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

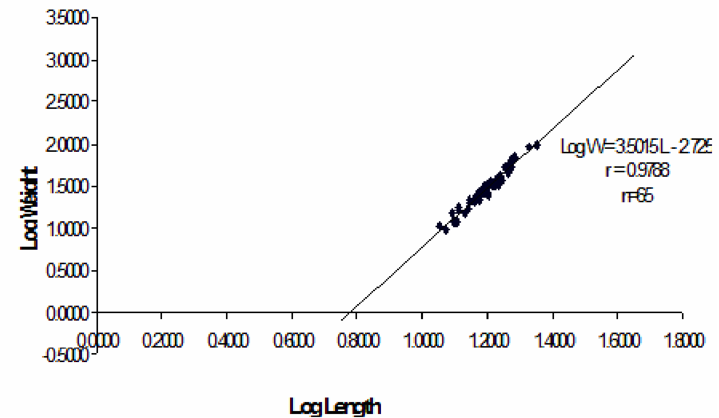

Figure 3b: Log length-weight relationship of *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

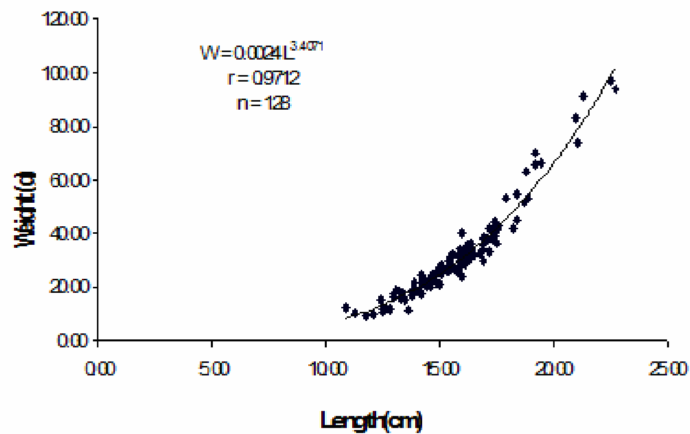

Figure 4a: Length-weight relationship for combine sex of *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

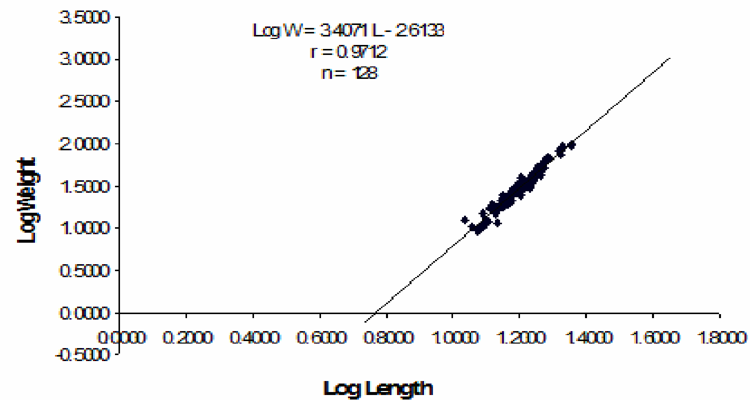

Figure 4b: Log length-weight relationship for combine sex of *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State, Nigeria

Figure 2a showed the length-weight relationship of male *H. bebe occidentalis* from Idah area of River Niger, Kogi state. This conforms to the curvilinear plot represented by the formula $W = aL^b$. The values of a, b and r are 0.0033, 3.2953 and 0.9629, respectively.

Table 2: Analysis of stomach contents of *Hyperopisus bebe occidentalis* at Idah Area of River Niger

Items	Frequency of Occurrence
Plant part	23.2
larvae	14.4
mollusk	24.0
sand grain	17.6
detritus	24.8
Worm	10.4
Algae	28.8
unidentified item	25.6

Table 3: Stomach content classification of *Hyperopisus bebe occidentalis* at Idah Area of River Niger, Kogi State.

Sex	Male n = 64	Female n = 65	Combined n = 129
% Stomach with food	37(28.6)	63(48.8)	100(77.5)
% stomach without food	27(20.9)	2(1.5)	29(22.4)
% Degree of fullness			
Full (4/4)	0(0)	0(0)	0(0)
Almost full (3/4)	15(11.6)	16(12.4)	31(24.0)
Half full (1/2)	37(28.6)	35(27.1)	72(55.8)
Almost empty (1/4)	8(6.2)	9(6.9)	17(13.1)
Empty (0/4)	2(1.5)	2(1.5)	4(3.1)

Table 4: Summary of Condition Factor for *Hyperopisus bebe occidentalis* from Idah Area of River Niger, Kogi State

Condition factor	Total Number	Range (cm)	Mean
Males	63	0.56 – 0.98	0.75±0.09
Females	66	0.57 – 0.98	0.75±0.09
Combined sex	129	0.46 – 0.98	0.75±0.09

This relationship is log transformed to give the plot (Figure 2b) with the formula Log W = Log 3.2953 - 2.4815 Log L. Figure 3a showed the length-weight relationship of female *H. bebe occidentalis*. The values a, b and r are 0.0019, 3.5015 and 0.9788, respectively. The log transformed relationship also gives the linear plot (Figure 3b) represented by the formula, Log W = Log 3.5015 - 2.725 Log L.

Figure 4a also showed the length-weigh relationship for the combined sexes of *H. bebe occidentalis* from Idah area of River Niger, Kogi State. The values a, b and r were 0.0024, 3.4071 and 0.9712, respectively. The log transformed plot (Figure 4b) is represented by the formula Log W = Log 3.4071 L - 2.6133 Log L.

Nine items were recorded from the stomach of the fish. These includes plant parts (23.2%), larva (14.4%), mollusk (24.0%), sand grain (17.6%), detritus (24.8%), worm (10.4%), algae (28.8%) and unidentified items (25.6%) (Table 2). The stomach fullness classification of *H. bebe occidentalis* based on degree of stomach fullness indicated that 37 (28.6%) had food in their stomach while 27 (20.98) had no food in their stomach. There was food in 63 (48.8%) stomachs of females while 2 (1.5%) had no food. There was no full stomach (0%) in the males, 15 (11.6%) almost full, 37 (28.6%) half full, 8 (6.2%) almost empty and 2 (1.5%) empty. There was significant difference ($p>0.05$) in the degree of stomach fullness of the fish except for full stomach ($p<0.05$) content (Table 3).

The values of condition factor for male, female and combined sexes range from 0.56 – 0.98, 0.57 – 0.98 and 0.46 – 0.98 for combined sexes respectively. The mean was 0.75 ± 0.09, 0.75 ± 0.09 and 0.75 ± 0.09, respectively (Table 4).

DISCUSSION

The b-values of 3.2953, 3.5015 and 3.4071 were observed for male female and combined sexes of *H. bebe occidentalis* is quite similar to the b-value of 3.051 reported by Konan *et al.* (2007) on *Labeo coubie* in the coastal rivers of South-Eastern Ivory Coast.

The condition factor parameter for *H. bebe occidentalis* reveals the males, females

and combined sexes to have mean condition factor of 0.75 ± 0.09, 0.75 ± 0.09 and 0.75 ± 0.09 respectively. These values are greater than 1. This means that the fish is in a good condition in the Idah area of River Niger.

This study showed that the items found in the diet of this species include plant part, larvae, mollusk, sand grain, detritus, worm, algae and unidentified items. Malami *et al.* (2007) reported substances of plant (69.8) animal origin (20.0%) and (4.4%), respectively. This suggests that the species is an omnivore feeding more on substances of plant and animal origin.

Growth in the species could said to be allometric. The condition factor also indicated that the species was thriving very well in the Idah area of River Niger. The findings of this study showed that 23.2% of items in the diet of *H. bebe occidentalis* were plant materials. *H. bebe occidentalis* at Idah area of River Niger, Kogi state could be referred to as an omnivore feeding on both plant and animal substances.

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SOME CHEMICAL PARAMETERS OF A FERTILIZED PRODUCTIVE POND

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ABSTRACT

Some chemical parameters of a rock water productive pond were determined. Chicken droppings were applied at the rate of 1.5kg per day to a pond of mean surface area of 300m$^{2:}$. The experimental period lasted for 10 months, with the first five months as the unfertilized period and the remaining five months as the fertilized period. All water quality parameters were studied titrimetrically and triplicated for accuracy and precision. There was no significant variation (P>0.05) in the conductivity (ionic content) of the pond water whether fertilized or unfertilized. The increased in the mean values of free carbon dioxide during the fertilized period was attributed to increased rate of decomposition of organic matter and a concomitant release of carbon dioxide. Both phosphate-phosphorus (P04-P) and nitrate-nitrogen ($N0_3$-N) were significantly different (P<0.05) when the pond was fertilized. This was attributed to the availability of nitrogen and phosphorus as part of the constituents of chicken droppings. From this study it was observed that the use of fertilizer has favourable effect on the chemical parameters of the pond.

Keywords: Free carbon dioxide, Phosphate-Phosphorus ($P0_4$-P), Nitrate-nitrogen (NO_3-N), Conductivity, Chicken droppings

INTRODUCTION

The manipulation of nutrients concentration in water, especially with organic fertilization, results in greater response in zooplankton abundance, which forms a substantial part of the diet of fish during early culture. Problems associated with excessive application of organic manure in ponds involve rapid depletion of oxygen especially at high temperatures and excessive production of carbon dioxide and hydrogen sulphide as a result of the activities of decomposing organisms. In China organic manure are left for 10 days in compost pits to ferment and reduce chances of pathogen transfer to fish under culture (FAO, 1980).

Low dissolved oxygen limits respiration and growth of aquatic animals (Jobling, 1981). It has been noted that the highest values of dissolved oxygen concentration coincide with periods of most intensive development of algae and vice versa. The most productive water has a pH range of 6.5 – 8.5 (Swingle, I960). However, Wokoma (1986) reported that the low pH tolerant tilapia *(Tilapia guineensis)* can survive for a long period at pH above 3.5, while at pH 3.2 can only survive for few hours. Against this background, the present study aims at determining the effect of fertilization with chicken manure on changes in some chemical parameters of rock water productive pond.

MATERIALS AND METHODS

The experiment was carried out at the University of Jos, Jos, Plateau State, Nigeria Senior Staff Quarters where a polyculture pond was sited. The pond has a mean surface area of 300 m and a depth of 1.2 m. The fish stocked were: *Oreochromis niloticus, Cyprinus carpio, Tilapia zilli* and Koi carp. Chicken droppings were applied at the rate of 1.5kg per day for

five months (July to November which represented the fertilized period of the experiment). The remaining five months (February to June) was the unfertilized period.

Weekly sampling of water from the pond was carried out to determine some selected parameters. Diurnal sampling was also done once a month at three-hourly intervals. The chemical parameters investigated were: dissolved oxygen (DO), electrical conductivity, free carbon dioxide, total alkalinity, phosphate-phosphorus, nitrate-nitrogen, hydrogen ion concentration (pH) and dissolved organic matter. All water quality parameter studied were analyzed in triplicates for accuracy and precision. Electrical conductivity was measured with a portable switch gear electrolytic conductivity meter (Model Mc-1 Makr V). To determine the hydrogen ion concentration (pH) of the water sample, a pocket pH-temperature meter (Yew Model 57) was dipped into water in an inclined position and allowed to stabilize for five minutes before readings were taken. The Winkler's titrimetric method (APHA, 1990) and Lind (1979) were applied for the determination of dissolved oxygen concentration. The concentration of oxygen in water samples was expressed in mgl^{-1} using the equation given by Boyd (1979) i.e. 0_2 mgl^{-l} = V(D) X N(D) X 8 X 100 / Volume of sample, where V = volume of sodium thiosulphate solution (0.025N $Na_{:2}$ S_2 O_2).

Free carbon dioxide concentration was determined using the method described by Lind (1979). 100ml water samples introduced into 250ml Erlenmeyer flasks with phenolphthalein as indicator, were analyzed for free carbon dioxide immediately on return from the field. This was to avoid changes in CO_2 concentration that may occur as a result of respiratory process. The concentration was expressed in mgl^{-1}, as: Titre value (ml) of N/44 NaOH X 10.

Dissolved organic matter (DOM) was determined titrimetrically using N/100 potassium permanganate ($KMn0_4$), 25N sulphuric acid (H_2SO_4) and ammonia oxalates solution. A blank titration was carried out using distilled water and the value of dissolved organic matter was computed as: Titre value (ml) of water sample-titre value (ml) of blank X 3.14.

Total alkalinity (mgl^{-1}) was measured through the determination of phenolphthalein and methyl orange alkalinities. Titrimetric procedure was applied for both determinations using 0.02N $H_2SO_{4,}$ 100ml water sample and an indicator depending on what alkalinity was being determined. Phenolphthalein was used as an indicator for the determination of methyl orange alkalinity and vice-versa. The titre value (ml) was multiplied by a factor of 10 on both cases. Total alkalinity was then computed as the sum of phenolphthalein and methyl orange alkalinities and expressed in mgl^{-1}.

Nitrate-Nitrogen (NO-N) was through the method of phenol disulphuric acid as described by Mackereth (1963). The resulting yellowish mixture was stirred and allowed to cool. The absorbance was measured with a calorimeter (Corning Model 252) at 410nm wavelength using distilled water as blank. The concentration of Nitrate-Nitrogen was extrapolated from a standard calibration curve.

The concentration of Phosphate-Phosphorus (PO_4-P) was determined using Deniges reagent method as described by Mackereth (1963), Linb (1979) and APHA (1990). The absorbance of the resulting bluish mixture was measured in 690nm wavelength using a calorimeter (Coming Model 252). Distilled water was used as a blank. The concentration of PO_4-P was then extrapolated from a standard calibration curve.

Statistical Analysis: The data obtained were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1990).

RESULTS AND DISCUSSION

Conductivity: Records, of the conductivity of the experimental pond are shown in Tables 1, 2 and 3. The highest mean value was recorded in February, while the lowest was in April. There was no significant variation (P>0.05) between fertilized and unfertilized period of the experiment. The lowest ionic content (conductivity) value was 36.30 ± 0.03 ohms cm^{-1} and the highest recorded was 48.12 ± 0.03 ohms cm^{-1}. Beadle (1981) reported that category 1 of African lakes have 40 ohms cm^{-1}

Table 1: Mean weekly values of some chemical parameters recorded in the pond February-April, 2006

Date	Conductivity ohms. Cm^{-1}	D.O (Mgl-^{1})	Free CO_2 (Mgl-^{1})	pH	Total Alkalinity (Mgl-^{1})	PO_4-P (Mgl-^{1})	NO_3-N (Mgl-^{1})	DOM (Mgl-^{1})
7/2/06	48.12±1.15	7.50±1.16	0.45±0.12	7.70±1.14	39.00±1.13	0.35±0.16	0.44±0.10	4.45±1.12
14/2/06	47.50±1.16	7.06±1.13	0.40±0.11	7.60±1.13	38.60±1.17	0.37±0.14	0.42±0.12	4.52±1.14
21/2/06	39.10±1.12	7.50±1.12	0.32±0.14	7.50±1.18	37.50±1.10	0.36±0.11	0.43±0.15	4.69±1.16
28/2/06	40.05±1.18	7.30±1.15	0.24±0.13	7.45±1.15	38.00±1.15	0.35±0.17	0.41±0.11	4.68±1.12
6/3/06	43.10±1.17	7.30±1.12	0.78±0.17	7.41±1.12	29.00±1.18	0.36±0.15	0.45±0.13	4.76±1.17
13/3/06	42.06±1.15	8.23±1.10	0.65±0.11	7.46±1.16	27.80±1.13	0.57±0.13	0.46±0.16	4.74±1.16
20/3/06	40.10±1.11	7.56±1.13	0.70±0.13	7.40±1.12	28.70±1.12	0.35±0.14	0.48±0.13	4.75±1.12
27/3/06	41.20±1.12	7.00±1.14	0.40±0.10	7.46±1.12	28.48±1.13	0.34±0.11	0.47±0.11	4.78±1.16
3/4/06	36.30±1.18	6.75±1.15	1.00±0.12	7.32±1.14	29.40±1.17	0.41±0.14	0.49±0.13	5.40±1.12
10/4/06	36.60±1.16	7.00±1.16	1.10±0.16	7.33±1.15	28.50±1.13	0.41±0.12	0.46±0.11	5.41±1.16
17/4/06	38.50±1.12	7.50±1.11	0.90±0.12	7.20±1.12	28.50±1.17	0.37±0.15	0.51±0.14	5.41±1.15
24/4/06	39.05±1.10	7.00±1.14	0.95±0.15	7.25±1.13	28.60±1.12	0.38±0.13	0.47±0.10	5.43±1.13

Table 2: Mean weekly values *of* some chemical parameters in the pond May-July, 2006

Date	Conductivity ohms. Cm^{-1}	D.O (Mgl-^{1})	Free CO_2 (Mgl-^{1})	pH	Total Alkalinity (Mgl-^{1})	PO_4-P (Mgl-^{1})	NO_3-N (Mgl-^{1})	DOM (Mgl-^{1})
1/5/06	42.01±1.12	6.55±1.13	0.80±0.11	7.80±1.13	30.50±1.14	0.39±0.10	0.49±0.11	5.42±1.13
8/5/06	43.00±1.10	6.80±1.11	0.60±0.10	7.90±1.12	31.00±1.15	0.40±0.15	0.50±0.13	5.41±1.14
15/4/06	41.00±1.13	6.95±1.10	0.90±0.12	7.50±1.15	31.00±1.16	0.50±0.10	0.41±0.12	5.40±1.13
22/5/06	43.00±1.14	7.00±1.11	0.87±0.14	7.60±1.13	31.00±1.13	0.50±0.12	0.57±0.13	5.50±1.11
29/5/06	43.00±1.11	7.00±1.18	0.89±0.13	7.80±1.13	32.00±1.14	0.60±0.11	0.49±0.14	5.50±1.12
5/6/06	44.00±1.13	7.00±1.13	0.88±0.14	7.80±1.13	32.01±1.15	0.62±0.13	0.48±0.15	5.51±1.13
12/6/06	42.00±1.15	7.90±1.17.	0.70±0.12	7.80±1.17	34.00±1.13	0.70±0.15	0.60±0.16	7.55±1.14
19/6/06	43.00±1.13	7.90±1.13	0.80±0.11	7.80±1.16	31.00±1.11	0.60±0.10	0.70±0.17	7.58±1.16
26/6/06	41.00±1.10	7.80±1.16.	0.70±0.10	7.00±1.15	35.00±1.12	0.65±0.14	0.85±0.18	7.56±1.15
3/7/06	41.00±1.13	8.90±1.15	0.70±0.12	7.90±1.14	31.00±1.14	0.70±0.15	0.90±0.10	7.78±1.14
10/7/06	42.00±1.11	8.90±1.13	0.87±0.14	7.80±1.13	36.00±1.13	0.75±0.13	0.90±0.19	7.84±1.13
17/7/06	43.00±1.13	8.50±1.14	0.89±0.13	7.00±1.12	37.00±1.15	0.76±0.11	0.85±0.11	7.82±1.12
24/7/06	42.01±1.10	8.70±1.12	0.87±0.15	7.60±1.11	37.00±1.16	0.75±0.12	0.78±0.14	7.90±1.13
31/7/06	43.00±1.13	8.80±1.11	85.00±0.10	7.50±1.13	38.00±1.17	8.00±0.11	0.86±0.13	7.82±1.12

Table3: Mean weekly values of some chemical parameters in the pond August-November, 2006

Date	Conductivity ohms. Cm^{-1}	D.O (Mgl^{-1})	Free CO_2 (Mgl^{-1})	pH	Total Alkalinity (Mgl^{-1})	PO_4-P (Mgl^{-1})	NO_3-N (Mgl^{-1})	DOM (Mgl^{-1})
7/6/06	43.00±1.00	9.00±1.10	0.70±0.14	7.80±1.10	37.60±1.13	0.80±0.10	0.85±0.10	7.92±1.16
14/6/06	42.50 ±1.13	8.50±1.14	0.90 ±0.17	7.80±1.13	38.00±1.14	0.90±0.11	0.82±0.11	7.94±1.17
21/8/06	41.50±1.11	8.60±1.11	0.75±0.15	7.50±1.15	39.00±1.15	0.83±0.17	0.84±0.12	7.96±1.14
28/8/92	44.00±1.10	8.75±1.13	0.82±0.10	7.90±1.14	37.80±1.10	0.90±0.16	0.85±0.15	7.94±1.12
4/9/06	43.00±1.11	8.00±1.10	0.90±0.12	8.00±1.13	36.90±1.12	0.58±0.11	0.77±0.17	7.50±1.12
11/9/06	41.02±1.15	9.00±1.12	0.85±0.10	7.00±1.18	37.50±1.11	0.79±0.13	0.85±0.13	7.55±1.13
18/9/06	41.30±1.10	8.00±1.13	0.88±0.14	7.60±1.13	39.00±1.15	0.81±0.10	0.85±0.15	7.52±1.11
25/9/06	42.40±1.14	8.00±1.03	0.90±0.11	7.50±1.11	0.92±1.13	0.92±0.14	0.89±0.11	7.54±1.12
2/10/06	43.10±1.11	8.50±1.11	0.70±0.13	7.40±1.10	34.00±1.12	0.79±0.15	0.87±0.10	7.70±1.10
9/10/06	41.20±1.10	8.60±1.10	0.90±0.15	7.50±1.13	35.00±1.10	0.78±0.10	0.88±0.17	7.72±1.10
16/10/06	42.30±1.15	8.40±1.14	0.70±0.13	7.30±1.14	36.00±1.13	0.61±0.14	0.74±0.11	7.74±1.12
23/10/06	41.20±1.12	9.10±1.11	0.90±0.10	7.60±1.11	37.00±1.10	0.79±0.17	0.81±0.16	7.65±1.14
30/10/06	42.10±1.14	9.20±1.13	0.80±0.12	7.40±1.14	36.00±1.15	0.81±0.11	0.81±0.13	7.70±1.11
6/11/06	43.00±1.15	8.30±1.14	0.90±0.13	7.40±1.12	35.00±1.12	0.78±0.16	0.81±0.11	5.59±1.10
3/11/06	41.00±1.10	8.40±1.03	0.70±0.10	7.40±1.17	34.00±1.14	0.81±0.10	0.71±0.17	5.60±1.13

value of conductivity. This implied that the value in this study is high. High value resulted from nutrient in-put which in turn resulted in more ionization.

Dissolved Oxygen: The highest and lowest values of dissolved oxygen were obtained in October (9.20 ± 0.05 mgl^{-1}) and May (6.55 ± 0.03 mgl^{-1}), respectively. The lower values were obtained during the fertilized period (Table 1 – 3). The values of dissolved oxygen recorded were conducive for most aquatic live (Chidobem, 1987). High dissolved oxygen resulted from higher photosynthetic rates, low algal and microbial respiration (Biswas, 1978). The lowest dissolved oxygen (DO) value recorded was 7.07 mgl^{-1} and the highest was 8.85 mgl^{-1}. Fish do not feed or grow well when DO levels remain continuously below 5 mgl^{-1} (Andrew, 1977). The value recorded in this experiment fell within a suitable range for aquatic life. Chidobem (1987) reported higher photosynthetic rate due to high biomass and a corresponding high level of dissolved oxygen.

Free Carbon Dioxide: Tables 1, 2 and 3 showed values obtained for the level of the carbon dioxide in the productive pond during the study. The highest value (1.10 ± 0.03 mgl^{-1}) and lowest value (0.24 ± 0.05 mgl^{-1} were rounded in April and February, respectively. There was increase in mean values of the above parameter during the fertilization period. This may be due to increase in the rate of decomposition of organic matter with the release of large amounts of carbon dioxide. Self shading of algal cells which led to low photosynthetic rate was also possible. In this pond, carbon dioxide released during respiration of aquatic organisms was not effectively utilized. A range of 0.24 ± 0.05 mgl^{-1} to 1.10 ± 0.03 mgl^{-1} free carbon dioxide was recorded. The mechanism of photosynthesis which required sunlight cannot occur in the absence of carbon dioxide. Generally, water supporting good fish populations have less than 5 ppm carbon dioxide (Mackereth, 1963). The low value of CO_2 obtained in this work came as a result of phytoplankton abundance arising from sufficient nutrients supplied by chicken droppings.

Hydrogen Ion Concentration (pH): There was slight variation in the values of pH between fertilized and unfertilized periods during the study, the increment probably result from the

neutralizing effect of total alkalinity, though carbon dioxide was also high. Highest value (8.00 ± 0.5 pH) was recorded in September while the lowest value (7.20 ± 0.03 pH) was obtained in April (Tables 1 – 3). The range of pH values recorded during the experiment was 7.2 – 8.0. Since the acid and alkaline death points according to Swingle (I960) are approximately 4 pH and 11 pH. It is clear from this study that the effect of fertilization on pH was slight. Therefore, pH was not a limiting factor for the production of plankton and fish.

Total Alkalinity: The mean values of total alkalinity values increased during the fertilized period. The increment in alkalinity level regulated the pH levels. Highest value was nonetheless recorded in February, while the lowest in March (Tables 1 – 3). The values obtained fell within the range conducive to the growth of fish and planktons (Akpan, 1991). The total alkalinity obtained fell within 28.48 – 39.00 mgl^{-1}. Likens (1975) associated high alkalinity values with high productivity for autotrophic waters. If alkalinity was low, the buffering capacity of water will be low for survival of fish. The alkalinity values obtained in this study fell within the limit of alkalinities for the survival of freshwater fish as observed by Mackereth (1963). Therefore, alkalinity was not a limiting factor in the productivity of the pond.

Dissolved Organic Matter: There were significant differences in the values of dissolved organic matter (DOM) during the fertilized and unfertilized periods. The increased value of dissolved organic matter possibly resulted from dead planktons as well as nutrients supplied by chicken droppings. The highest and lowest values were obtained in August and February, respectively (Tables 1 – 3).

Phosphate-Phosphorus (PO_4-P): The highest mean value of phosphate-phosphorus (PO_4-P) (0.90 ± 0.29 mgl^{-1}) and lowest (0.34 ± 0.02 mgl^{-1}) were recorded in August and March, respectively. Highest values were obtained during the fertilized period (July to November). This was probably due to the application of chicken droppings, which has phosphorus as part of its constituents. There was significant difference ($P<0.05$) between mean values obtained during fertilized and unfertilized periods. A range of 0.34 ± 0.02 mgl^{-1} to 0.92 ± 0.03 mgl^{-1} of phosphate phosphorus was recorded. The value of PO_4-P obtained in this study was very high. Mackereth (1963) reported that Lakes that consistently contain more than 0.I5 mgl^{-1} orthophosphate may experience more algal growth. The high orthophosphate level in this work resulted from the application of chicken droppings and about 0.54% phosphate may be present in the droppings (Omisore *et al.,* 2009).

Nitrate-Nitrogen (NO_3-N): The weekly mean values of nitrate-nitrogen are provided in Tables 1 – 3. The highest and lowest values were recorded in July and May, respectively. There was significant difference ($p<0.05$) between values during fertilized and unfertilized periods of the experiment. As mentioned in the case of PO_4-P, nitrogen is also part of the constituents of chicken droppings. Akpan (1991) suggested that lakes consistently containing more than 0.3 mgl^{-1} nitrogen may experience algal bloom. This implied that the value obtained in the work was high. Chidobem (1987) observed that nitrate was as important as phosphate in the productivity of plankton as well as the synthesis of pigment. This is attributed to 1.3% nitrogen composition of chicken droppings (Omisore *et al.,* 2009). Therefore the application of chicken droppings had effects on nitrate-nitrogen composition.

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PRELIMINARY CENSUS OF ZOOPLANKTONS AND PHYTOPLANKTONS COMMUNITY OF AJEKO STREAM, IYALE, NORTH CENTRAL NIGERIA

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ABSTRACT

The zooplankton and phytoplankton community of Ajeko Stream, North Central Nigeria were assessed between October and December 2010. Prior to sampling, Temperature, Transparency, Dissolve Oxygen and pH were evaluated. Zooplankton and phytoplanktons were sampled using plankton net of 20µm diameter with a collecting bottle attached at the base. Water samples were collected between 8 and 9am every forth nightly from three different points on the stream namely the Lower Course, Middle Course and Upper Course and labeled Stations A, B and C, respectively. Water samples obtained were brought to the Biological Sciences Laboratory of the Kogi State University Ayingba in sampling bottles for analysis of zooplanktons and phytoplanktons. 4% of formalin was used for preservation of zooplanktons and phytoplanktons. Each sample was concentrated to 250ml volume of water using pipette. 10ml was put to Petri-disc and the 1ml was quickly drawn with a wide-bore dropper. Samples were then introduced carefully into the counting chamber with a cover slip and observed under light microscope. The result revealed that zooplanktons were made up of Rotifera (44%), Cladocera (23%), Copepoda (20%) and Protozoa (13%) respectively. Phytoplanktons were made up of Chlorophyta (79%), Bacillariophyta (17%), Euglenophyta (2.54%) and Cryptophyta (2.00%) respectively. The status of the stream could said to be eutrophic as indicated by the diversity of zooplankton and phytoplanktons.

Keywords: Zooplankton, Phytoplankton, Temperature, Transparency, Dissolve oxygen

INTRODUCTION

Zooplanktons are the heterotrophic detritivorous component of the plankton that drifts in the water column of oceans, seas and freshwater bodies. They are microscopic organisms that are suspended in water. They include many kinds of protozoa, micro-crustaceans and other micro-invertebrates that are planktonic in water bodies (Omudu and Odeh, 2006).

Freshwater zooplankton is an important component in aquatic ecosystem whose main function is to act as primary and secondary links in the food chain. They are important link in the transfer of energy from producers to carnivores (Thurman, 1997). Zooplankton due to their large density, drifting nature, shorter life span, high group or special diversity and different tolerance are used as indicator agent for the physical, chemical and biological process in the aquatic ecosystem. Zooplanktons occupy a strategic trophic level in aquatic ecosystem. Apart from their ability to exert a tremendous influence on phytoplankton abundance and succession by means of selective grazing, they form an important source of food for carnivorous and omnivorous fish (Adeyemi *et al.,* 2009a).

Zooplankton communities often respond quickly to environmental changes because most species have short generation time (usually days to week in length) (Adeyemi *et al.,* 2010).

Zooplankton responds to a wide variety of disturbances including nutrient load, sediment input, contaminant densities and acidification. Jude *et al.* (2005) stressed that the specie assemblages of the zooplankton are indications of environmental quality and ecological changes.

Phytoplanktons are tiny autrophic and microscopic organisms that live in the water. The plant portion of this complex aquatic organism is called phytoplankton. Though they can not be seen without special equipment, once they are clustered together in large groups in water can appear to have a green coloration due to the chlorophyll present in their cells (Pearl and Tucker, 1995).

Phytoplankton species composition and diversity changes with environmental conditions such nutrients level, temperature, light and predator pressure etc. The relative importance of these factors varies considerably among different taxa under conditions of nutrient enrichment or eutrophication, the blue green algae are known to proliferate and form noxious blooms in freshwater environments. The development of phytoplankton blooms in eutrophic lakes and streams is attributed to their ability to accommodate reduced nitrogen to phosphorus ratios, low edibility due to their large colony sizes coupled with large herbivore regulation of other taxa (Adeyemi *et al.,* 2009b).

Limnologists have over the year's undertaken pre and post-impoundment studies of reservoirs, rivers, streams and lakes not only to describe the species of zooplankton and phytoplankton present but also to describe the species present and to monitor changes in species composition and seasonal abundance (Adeyemi and Ipinjolu 1997; Okayi *et al.,* 2001; Ado *et al.,* 2004; Adeyemi *et al.,* 2009c).

This study is aimed at determining the composition and abundance of zooplankton and phytoplankton, the limnological factors that supports the presence of zooplankton and phytoplankton and determines the productivity status of Ajeko stream.

MATERIALS AND METHODS

Study Area: The study area (Figure 1) Ajeko stream is located between Latitude $7^{0}36^{'}10^{'}$ North and Longitude $7^{0}13^{'}8^{'}$ East. The stream is 2 km North of Iyale Village in Dekina Local Government Area of Kogi State.

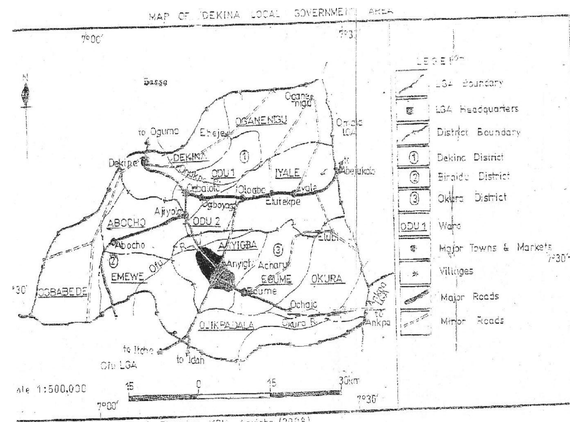

Figure 1: The study area showing Ajeko stream in Iyale Village, Dekina Local Government Area of Kogi State, Nigeria

It covers an area of $8,475^{2}$ meters with an approximate length of 150 m and breadth of 56.50 m. The stream has an average depth of 10 – 15 m depending on season, water enter the stream through various tributaries like Aji-Ite and Aji-Omachi during rainy season (Cartographic Laboratory, KSU 2010). The vegetation of the area is derived savanna which is characterized by grasses, shrubs and aquatic plants within the stream catchment's area. The average rainfall is between 850 – 16000 mm / annum. Iyale has two seasons viz rainy season which starts in April and ends in October and dry season which starts in November and end in March respectively.

Collection of Samples: With an aid of planktonic net of 20 µm diameter with a collecting bottle attached at the base water samples were collected between 8 and 9am every forth nightly from three different points on the stream namely the Lower Course, Middle Course and Upper Course and labeled Stations A, B and C, respectively, from October and December 2010.

Water samples obtained were brought to the Biological Sciences Laboratory in sampling bottles for analysis of zooplanktons and phytoplanktons. Physico-chemical parameters such as temperature and transparency were analyzed at the study site other parameters such as pH and dissolve oxygen were further analyzed in the laboratory and the mean reading recorded.

Determination of Physico-Chemical Parameters

Temperature: Water temperature was determined using mercury glass thermometer (range of 0^0 – 36^0C) which was calibrated at 0.2^0. The thermometer was immersed directly into the water for 5 minutes until a steady temperature was obtained.

Transparency: Transparency was determined using secchi disc as in Stirling (1985) with four graduant of alternate black and white on the upper surface and a long rope at the centre. Measurement was achieved by lowering the disc into the water the point of disappearance and re-appearance was noted and the distance was measured in a graduated rope and the results were recorded in centimeter.

Dissolved oxygen: Dissolved oxygen concentration of the sample area was determined using Winklers Titrimetric Method (Taylor *et al.,* 1996). Water samples were collected in a 250ml dissolve oxygen bottle, 2ml of manganese chloride and potassium iodide solution were added in order to fix the water. The bottle was carefully closed with a stopper to avoid air bubble and mixed thoroughly by shaking the bottle; this was done at the site. The precipitate formed was immediately transported to the laboratory for further analysis. In the laboratory 2ml of concentrated hydrogen chloride (HCl) acid was added and mixed thoroughly to dissolve. 50ml of this was titrated against (sodium thiosulphate solution) 0.0125 N_{a2}, S_2, 0_3. $5H_20$ inside a 50ml burette mounted on a tripod stand. 3 drops of starch solution was used as an indicator. The titration was repeated 3 times and the mean was determined this was calculated using the formula: $D0_2$ (cm^3/dm^3) = 0.056 x X x 100 at STP where X gives the volume of thiosulphate solution required for the titration of 50 ml samples and the dissolved oxygen in the water.

pH: Values were determined by the colorimetric method using the Lovibond comparator, with bromothymol blue as indicator.
Ten millilitres of each water sample was taken into each of the two glass tubes contained in the comparator. Ten drops of the indicator were added into the water sample contained in one of the tubes and thoroughly mixed. The indicator colour disc was then inserted in the comparator to compare with the colour in the tube containing the indicator and the water sample, the corresponding pH value was then read and recorded.

Sampling of Zooplankton and Phytoplankton: Planktonic nets were immersed below water surface and then towed through the water for qualitative plankton sampling. The content of the bottle were then poured into a sampling bottle of the same capacity and brought to the laboratory for further study, 4% of formalin was used for preservation of zooplanktons and phytoplanktons.

Enumeration of Zooplankton and Phytoplankton: Quantitative estimations were made using the new improved Naeubaur counting chamber. Before a quantitative enumeration of different organisms in each group was carried out, each zooplankton and phytoplankton sample was concentrated to a 250 ml volume of water using pipette. After shaking the bottle thoroughly, 10ml was put to Petri-disc and the 1 ml was quickly drawn with a wide-bore dropper. The sample was then introduced carefully into the counting chamber with a cover slip and observed under light microscope.

The count of 3 drops was averaged and the total number of each zooplankton and phytoplankton in the entire collection was

calculated per liter of water using the following formula: Number of organisms per liter = Organism in 1 ml of concentrate / Volume of water filtered X Volume of concentrate. Identification of zooplankton and phytoplankton species was carried out based on the keys provided by Palmer (1980).

RESULTS AND DISCUSSION

Physico-Chemical Parameters of Ajeko Stream: The result of the physico-chemical parameters of Ajeko stream, Iyale is as shown in Figures 2 – 4. The result showed that temperature range during the period of study was between 25.3^{0}C to 30.1^{0}C while transparency ranges from 10.7 to 50.0cm, dissolve oxygen is between 2.01 to 4.90 mg/l. pH was between 5.30 to 7.09, respectively.

The temperature range in Ajeko stream during the study period corresponded to the temperature range in the works of Grass *et al.* (1987) in River Nile. These conform to the temperature range adopted in the tropics (Alabaster and Lloyd, 1966).

Low transparency was recorded in the month of October due to rains which causes turbulence resulting in high turbidity; this has a corresponding low primary productivity, because turbidity reduces the amount of light penetration which in turn reduces photosynthesis and hence primary productivity (APHA, 1980). High transparency was recorded in the month of December due to dry season which in turn increase photosynthesis and hence primary productivity as a result of increase in light penetration into the water.

The value for dissolved oxygen content of the stream falls within the range of 0.51mg/l - 9.25mg/l. The range is in line with finding of Adeyemi *et al.* (2009b), which was 1.26mg/l - 3.1mg/l in their limnological investigation of Gbedikere Lake.

pH values recorded were between 5.30 - 7.09. This showed that the stream is a little acidic and a little alkaline.

The pH values recorded during the period of study was optima for fish and other aquatic organisms.

Zooplankton of Ajeko Stream: Figure 5 showed the variation in abundance of zooplankton in station A. The rotifers accounted for the highest number of total zooplanktons found in this station during the period of study, followed by the Cladocerans and copepods while protozoa have slight difference. This is also same in Figure 6 and 7 showing the variation in abundance of zooplanktons in station B and C, respectively.

Figure 8 showed the variation in abundance of zooplanktons between stations. The highest total number of zooplanktons were recorded in Station A followed by Station C and Station B respectively. A total of 15 species of zooplanktons were identified in Ajeko stream with four taxa of zooplanktons: Rotifera, Cladocera, Copepoda and Protozoa. Out of fifteen species six belonged to Rotifera and three each belonged to the Cladocera, Copepod and Protozoa. Species of the divisions Rotifera were the most dominant: they accounted for approximately 44% within the zooplankton community of the stream during the period of study followed by the cladocera 23% copepods 20% and the protozoa 13%. This compared favourably with the study of Omudu and Odeh (2006) in Agi stream who reported that total zooplankton abundance may increase with increasing eutrophication.

Phytoplankton of Ajeko Stream: A total number of fifteen species of phytoplankton were identified. The four major division of algal collected and identified during the period of the study include Chlorophyta, Bacillariophyta, Euglenophyta and Crytophyta. Chlorophyta dominated the total number of species in the community followed by Bacillariophyta, Englenophyta and Cryptophyta respectively.

Figure 9 – 11 showed the variation in abundance of phytoplanktons in station A. The Chlorophyta accounted for the highest number of phytoplanktons found in this station followed by the Bacillariophyta and with the Euglanophyta, Crytophyta having the lowest number of phytoplankton.

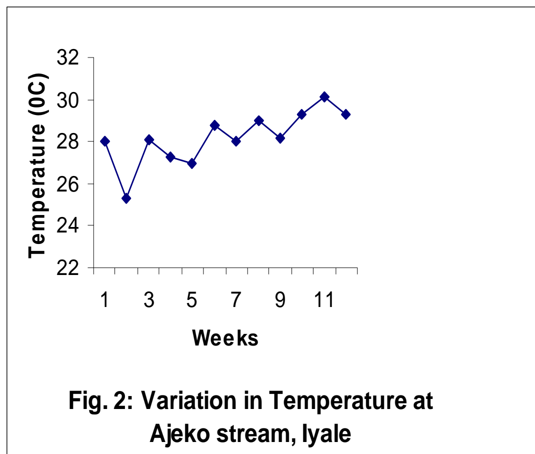

Fig. 2: Variation in Temperature at Ajeko stream, Iyale

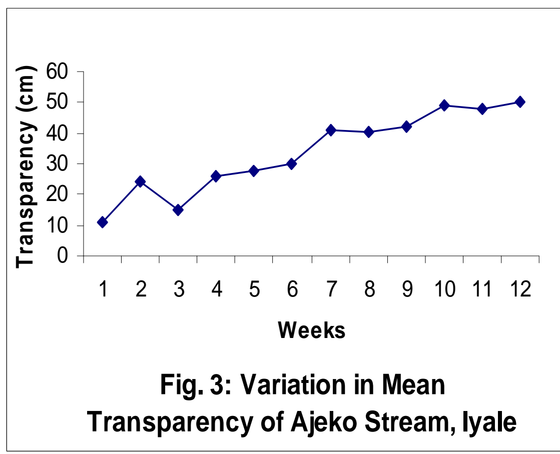

Fig. 3: Variation in Mean Transparency of Ajeko Stream, Iyale

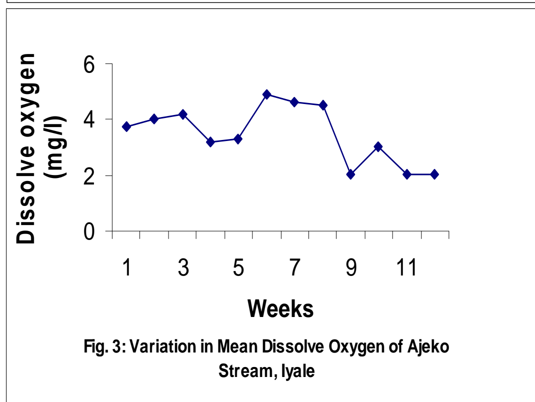

Fig. 3: Variation in Mean Dissolve Oxygen of Ajeko Stream, Iyale

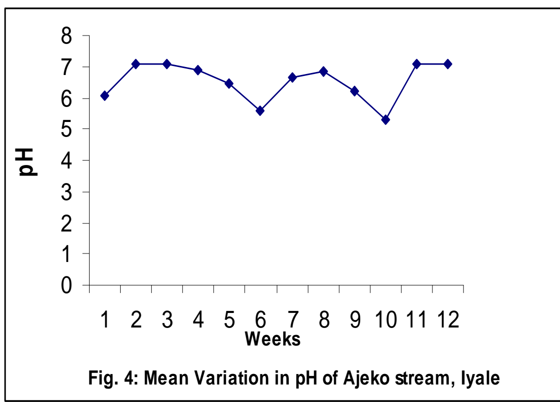

Fig. 4: Mean Variation in pH of Ajeko stream, Iyale

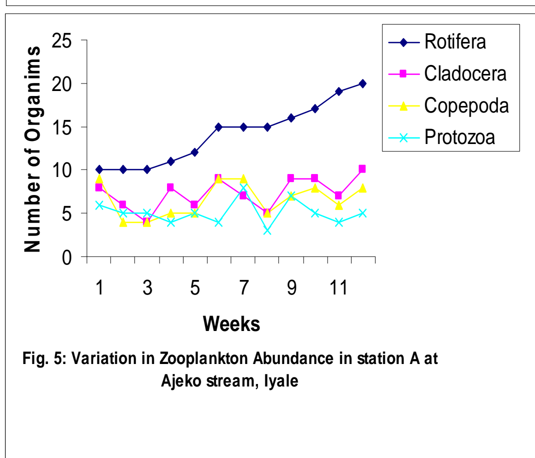

Fig. 5: Variation in Zooplankton Abundance in station A at Ajeko stream, Iyale

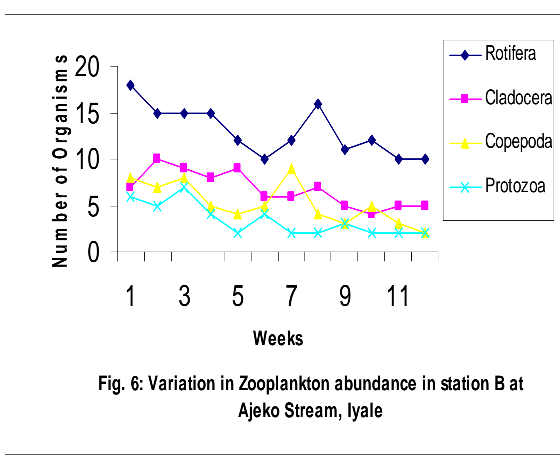

Fig. 6: Variation in Zooplankton abundance in station B at Ajeko Stream, Iyale

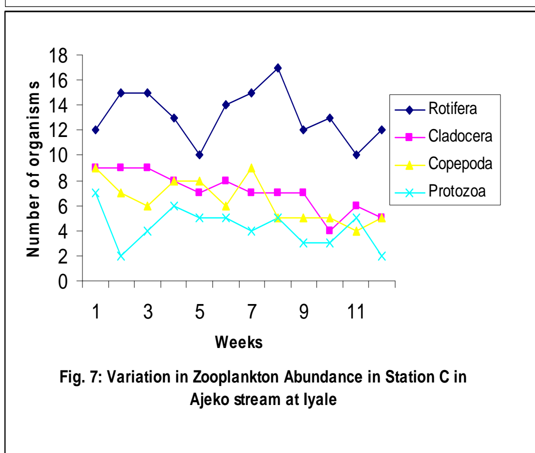

Fig. 7: Variation in Zooplankton Abundance in Station C in Ajeko stream at Iyale

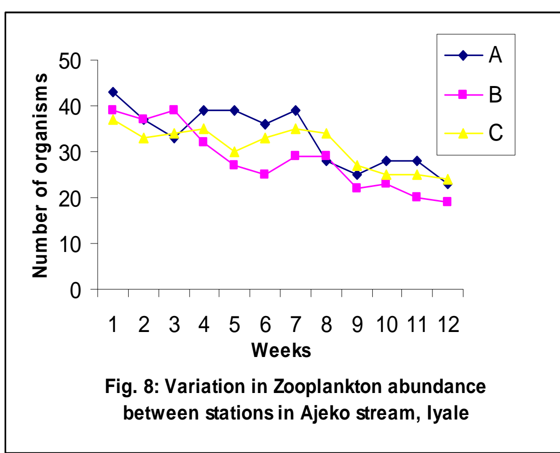

Fig. 8: Variation in Zooplankton abundance between stations in Ajeko stream, Iyale

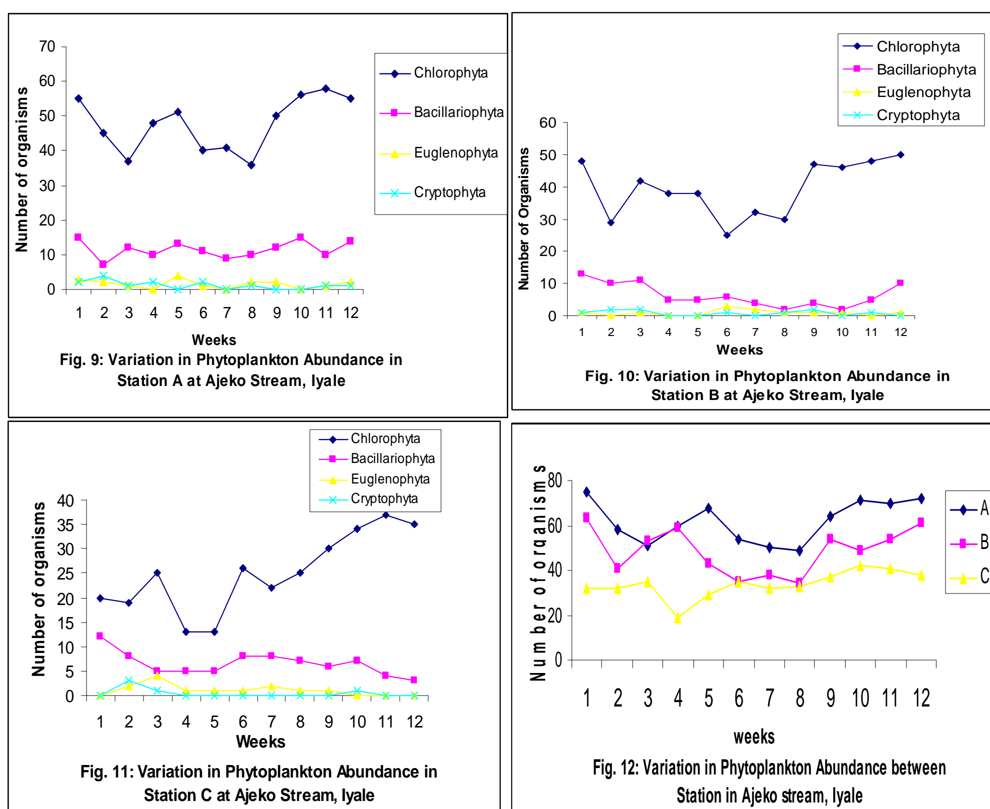

Fig. 9: Variation in Phytoplankton Abundance in Station A at Ajeko Stream, Iyale

Fig. 10: Variation in Phytoplankton Abundance in Station B at Ajeko Stream, Iyale

Fig. 11: Variation in Phytoplankton Abundance in Station C at Ajeko Stream, Iyale

Fig. 12: Variation in Phytoplankton Abundance between Station in Ajeko stream, Iyale

Figure 12 showed the variation in abundance of phytoplankton between the three stations, station A was recorded as the highest number of phytoplankton and the lowest number was recorded for station C. A total of 15 species of phytoplanktons were identified. The major divisions of algal including Chlorophyta, Bacillariophyta, Euglenophyta and Cryptophyta: Nine species belong to Cholorophyta, four species for Bacillariophyta and one species each for Euglenophyta and Cryptophyta. Species of the divisions Chlorophyta were the most dominant they accounted for approximately 79% within the phytoplanktons community of the stream during the period of study followed by the Bacillariophyta 17%, Euglenophyta 2% and the Cryptophyta 2%. The availability of these planktonic algae could be attributed to the physico-chemical parameters which are within tolerable limits. These conform favourably with the report of Adeyemi (2011) who state that physical and chemical factors are known to influence the growth and survival of plants.

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PLASMA AND ORGAN BIOCHEMISTRY OF *Clarias gariepinus* EXPOSED TO MONOAROMATIC, TOLUENE

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ABSTRACT

The effect of toluene on enzymes; Aspatate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in the plasma, organs (gills, kidney, liver) and muscle of Clarias gariepinus was assessed. The activity of AST in the gill was variable among the treated group and between the treated groups and the control with increase in all the concentrations (129.4 – 976%) above the value of the control (42.50 ± 28.72 IU/L). Activity of ALT in the gill was gradually raised from 50 ppm to a peak (187.50%) at 125 ppm. Although the ALP had the highest activity in the gill in comparison to other enzymes it was variable with inhibitions. Toluene caused excitation at 75 and 125 ppm were more than thrice and twice respectively that at the control in the kidney. It caused increase in the activity of ALT with more than 6x (25 ppm), 9x (75 ppm) and 15x (125 ppm) that of the control. However, the activity of ALP in the kidney was generally unaffected by the exposure at the other concentrations. There was general excitation of AST activity in the liver with higher levels of excitation, 25 – 40% at 25, 75 and 125 ppm. A raise in ALT activity was recorded in all the exposure. A concentration-dependent increase was observed in activity of ALP in the liver 299, 479 and 596% above the control value, 46.00 ± 34.84 IU/L. There was both excitation inhibition of AST activity in the muscle tissue of the fish. There was a sharp decline of 69 and 55% at 50 and 100 ppm in ALT activity and enhanced activity of 321, 179 and 227% at 25, 75 and 125 ppm, respectively above the control value. ALP activity was excited in the muscle in all the test concentrations (maximum, 90% at 25 ppm). There was a sharp decline in the activity of AST in the plasma with the least value at 75ppm, 64% lower than the control value. All concentrations toluene elicited the activity of ALT in the plasma.. The increase were 3.5x, 2.5x, 4.5x, 2.25x and 5x at the exposure concentrations (25, 50, 75, 100 and 125 ppm) respectively, relative to the control values. The relative activity of AST in the organs generally followed the pattern: muscle > liver > gill > kidney> plasma and that of ALT activity, muscle > kidney > liver > gill > plasma and ALP was kidney > muscle > liver > gill > plasma.

Keywords: Toluene, *Clarias gariepinus,* Aspartate transaminase, Alanine transaminase, Alkaline phosphatase

INTRODUCTION

Over the years there is constant contamination of the environment, particularly in the Niger Delta, Nigeria by petroleum hydrocarbons from numerous outlets by petroleum and allied products from and exploration and exploitation (Benson and Essien, 2009). This comes from anthropogenic sources which include offshore oil production, transportation, atmospheric and aerial depositions, direct dumping and accidental discharge among others (Abu-Hilal and Khordagui, 1994; NRC, 2000) which are capable of causing chronic effects in the water settlements. The yearly entry of crude oil into the aquatic environment is in the region of between 6 – 10 million barrels (Thorhang, 1992). Control of this problem in the aquatic environment is very difficult die to the large number of input sources, their geographic dispersions and the rate at which the products mixes with water and the resultant effect of their components on aquatic organisms (Patin, 1991; Dambo,1992).

Toluene is a low molecular weight aromatic monocyclic hydrocarbon. It is a major component of water soluble fractions of crude and refined petroleum products. It is released into the atmosphere principally from volatilization of petroleum fuels and toluene-based solvents and thinners, and from motor vehicle exhaust. Considerable emissions are from its discharge into waterways or spills on land, transport and disposal of fuels and oils; from its production from petroleum and coal, as a by product from styrene production and from its use as a chemical intermediate (EPA, 1994). The source further indicated that the amount of toluene released to land and water was totaled over 4 million Ibs from 1987 – 1993. Toluene released into water can take few days to several weeks to remove depending on the environmental condition. Like other volatile hydrocarbons, toluene is reported toxic to many aquatic life forms including fish with varying 96hr median lethal concentrations that were species, size and duration-dependent (Blacks *et al.,* 1982; Rice and Thomas, 1989; Kennedy *et al.,* 2006)

Most of the studies conducted on the physiological effects of oil pollution in aquatic fauna in Nigerian waters dealt with whole crude oil (Dambo, 1992; Ovuru and Mgbere, 2000), refined products (Ayalogu *et al.,* 2001; Gabriel *et al*., 2009), the water soluble fractions (Osuji and Mbata, 2004), but the effects of the low boiling points components (monoaromatic hydrocarbons) which are considered the most toxic constituents have received little of no attention. Petroleum oils and the aromatics have been shown to impact negatively on the physiology of important fish species (Dange and Masureka, 1981; Abu-Hilal and Khordagui, 1994; NRC, 2000). In the Niger Delta region which has heavy concentration of oil installations and oil-related activities and aquaculture production where the catfishes especially *Clarias gariepinus* is intensively cultured, minimal or no attention has been given to the possible effects of the monoaromatics on the physiology of the fish species despite the rampant spills recorded in the region. Therefore, this study was carried out to assess the possible effects of toluene on the enzyme activities in the blood (plasma) and organs of *C. gariepinus* under laboratory conditions.

MATERIALS AND METHODS

The experimental fish, *C. gariepinus* (mean total length, 166.01 ± 15.0 cm; mean weight, 30.15 ± 2.27 g) was procured from a private farm, Ellah Lakes, Obrikom, Rivers State, Nigeria and transported in unaerated aquaria to the Chemistry Laboratory, Rivers State University Science and Technology, Port Harcourt, Rivers State, Nigeria. Catfishes were acclimated individually in 10*l* water to laboratory condition in plastic aquaria for seven days. The fish were fed once daily on a 30% crude protein diet at one percent biomass. The aquaria were carefully washed and cleansed of uneaten food and feacal matters by siphoning after which the water also renewed daily.

At the end of the acclimation period, the fish was exposed individually in quadruplicates to graded levels of toluene (0.00, 25.00, 50.00,

75.00, 100.00 and 125 ppm), respectively for 21 days in a daily renewal bioassay. The fish were fed as in the acclimation period and toxicant was renewed daily. At the end of the experimental period, blood samples were collected from the kidney with a 21 G size needle and a 5 ml syringe into heparinised bottles for enzyme analysis. After this the fish were anaesthetized, dissected to expose the viscera and the organs (gill, kidney, liver) and muscle were excised from the fish. Samples (0.5g) of the organs were macerated and homogenised in a mortar with 5 ml of physiological saline for enzymatic studies. Both the blood and the organs samples were centrifuged at 3000 rpm for 10 minutes and the supernatant pipetted into plain bottles analysis of enzymatic activities.

Aspartate transaminase, AST (EC 2.6.1.1) and alanine transaminase, ALT (EC 2.6.1.2) activities were assayed using the procedures of Reitman and Frankel (1957). Alkaline phosphatase, ALP (EC 3.1.3.1) activity was assayed using the methods of Babson *et al*. (1996). The data obtained were subjected to a one way analysis of variance (ANOVA). Where differences existed in the parameters, Duncan's multiple range test (DMRT) was used to separate the means (Zar, 1984).

RESULTS

The activity of AST in the gill was variable among the treated group and between the treated groups and the control. There was increase of activity in all the concentrations (129.4 – 976%) above the value of the control, 42.50 ± 28.72 iu/l. The activity of ALT in the gill was gradually raised from 50 ppm to a peak (187.50%) at 125 ppm. ALP had the highest activity in the gill in comparison to other enzymes. However, the activity was not concentration dependent. The activity levels were variable with inhibition at 50, 100 and 125 ppm and excitation of 17.84 and 19.96% at 25 and 75 ppm, respectively (Table 1). In the kidney, exposure to toluene caused variable levels of excitation of AST activity in all the exposure concentrations above the control value. The excitation at 75 and 125 ppm were more than thrice and twice respectively that at the control. Exposure to toluene caused increased in the activity of ALT with more than 6 times at 25 ppm, 9 times at 75 ppm and 15 times at 125 ppm when compared with the control. However, the activity of ALP in the kidney was generally unaffected by exposure to the other concentrations (Table 2). There was general excitation of AST activity in the liver with higher levels of excitation, 25-40% at 25, 75 and 125ppm (Table 3). ALT activity was recorded in all the exposure concentrations with higher activity 369, 372 and 751% above the control value at 25, 75 and 125 ppm, respectively. A concentration-dependent increase was also observed in activity of ALP in the liver 299, 479 and 596% above the control value, 46.00 ± 34.84 IU/L (Table 3). There was both excitation inhibition of AST activity in the muscle tissue of the fish (Table 3). Excitation of 22 and 17% above control values were recorded at 25 and 75 ppm, respectively; while inhibition values of 11 and 4% were recorded at 50 and 100 and 125 ppm, respectively. There was a sharp decline of 69 and 55% at 50 and 100 ppm in ALT activity and enhanced activity of 321, 179 and 227% at 25, 75 and 125 ppm, respectively above the control value. ALP activity was excited in the muscle in all the test concentrations (maximum, 90% at 25ppm). However, the excitation decreased with increase the test concentration. The activities ranged from 4 – 90% in the reverse order (Table 4). There was a sharp decline in the activity of AST in the plasma with the least value at 75 ppm, 64% lower than the control value. All concentrations toluene elicited the activity of ALT in the plasma. The increase were 3.5x, 2.5x, 4.5x, 2.25x and 5x at the exposure concentrations (25, 50, 75, 100 and 125 ppm), respectively relative to the control values (Table 5). The relative activity of AST in the organs generally followed the pattern: muscle > liver > gill > kidney> plasma and that of ALT activity, muscle > kidney > liver > gill > plasma and ALP was kidney > muscle > liver > gill > plasma (Figures 1 – 3).

Table 1: Activities of enzymes (AST, ALT and ALP) the gill tissue of *Clarias gariepinus* after exposure to toluene for 21 day

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	42.50 ± 28.72b	100	10.00 ± 0.00a	100	511.25 ± 34ab	100
25	152.50 ± 82.61b	358.6	15.00 ± 7.07a	150	602.50 ± 23.27a	118
50	97.50 ± 23.63b	229.4	11.25 ± 2.5a	113	450.63 ± 81.56ab	88
75	575.00 ± 119.48a	130.3	18.33 ± 7.64a	183	613.33 ± 10.41a	120
100	61.25 ± 28.39b	144.03	18.75 ± 14.36a	188	346.67 ± 237.16b	68
125	500.00 ± 177.55a	1176	28.33 ± 32.75a	283	498.07 ± 124.25ab	97

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 2: Activities of enzymes (AST, ALT and ALP) in the kidney tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	131.25 ± 62.25b	100	15.00 ± 7.07c	100	542.5 ± 20.26a	100
25	243.75 ± 94.46b	185	97.50 ± 38.62b	650	498.25 ± 44.11a	91.8
50	230.00 ± 199.54b	175	17.50 ± 5.00c	117	548.75 ± 19.31a	101
75	440.00 ± 103.32a	335	146.67 ± 20.21b	998	605.00 ± 22.91a	112
100	156.00 ± 18.43b	119	25.00 ± 8.16c	167	556.25 ± 4.79a	103
125	325.00 ± 134.26ab	248	203.33 ± 75.12a	1556	486.67 ± 230.99a	90

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 3: Activities of enzymes (AST, ALT and ALP) in the liver tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	462.50 ± 317.29a	100	16.25 ± 6.29c	100	46.00 ± 34.84c	100
25	647.50 ± 44.44a	140	76.25 ± 34.73b	469	114.25 ± 36.65bc	248
50	468.75 ± 202.75a	101	17.50 ± 5.00c	108	156.13 ± 115.65bc	339
75	601.07 ± 27.54a	130	76.67 ± 20.21b	472	183.33 ± 58.53ac	399
100	501.25 ± 247.94a	108	23.75 ± 15.48c	146	266.13 ± 69.82ab	579
125	576.67 ± 68.98a	125	138.33 ± 45.09a	851	320.33 ± 185.91a	696

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 4: Activities of enzymes (AST, ALT and ALP) in the muscle tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	586.25 ± 132.5a	100	52.50 ± 29.01b	100	22.50 ± 9.77b	100
25	655.00 ± 150.11a	122	221.25 ± 84.30a	421	422.75 ± 12.92a	190
50	520.00 ± 0.00a	89	17.50 ± 5.00b	33	29.00 ± 16.28ab	129
75	688.33 ± 14.43a	117	146.67 ± 82.51b	279	28.50 ± 1.80ab	127
100	520.00 ± 0.00a	89	23.75 ± 15.48a	45	25.50 ± 6.48b	113
125	561.67 ± 165.86	96	171.67 ± 55.08	327	23.33 ± 4.86b	104

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 5: Activities of enzymes in the plasma tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	56.00 ± 69.19	100	2.00 ± 1.41	100	15.30 ± 4.41	100
25	26.75 ± 10.75	48	6.50 ± 3.32	325	13.85 ± 4.16	91
50	50.75 ± 2.31	91	4.50 ± 3.70	225	19.00 ± 14.54	124
75	20.33 ± 34.00	36	9.00 ± 6.08	450	18.33 ± 18.33	120
100	53.00 ± 12.53	95	4.25 ± 1.50	213	25.00 ± 8.39	163
125	28.00 ± 3.54	50	10.00 ± 6.25	500	18.33 ± 12.06	120

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

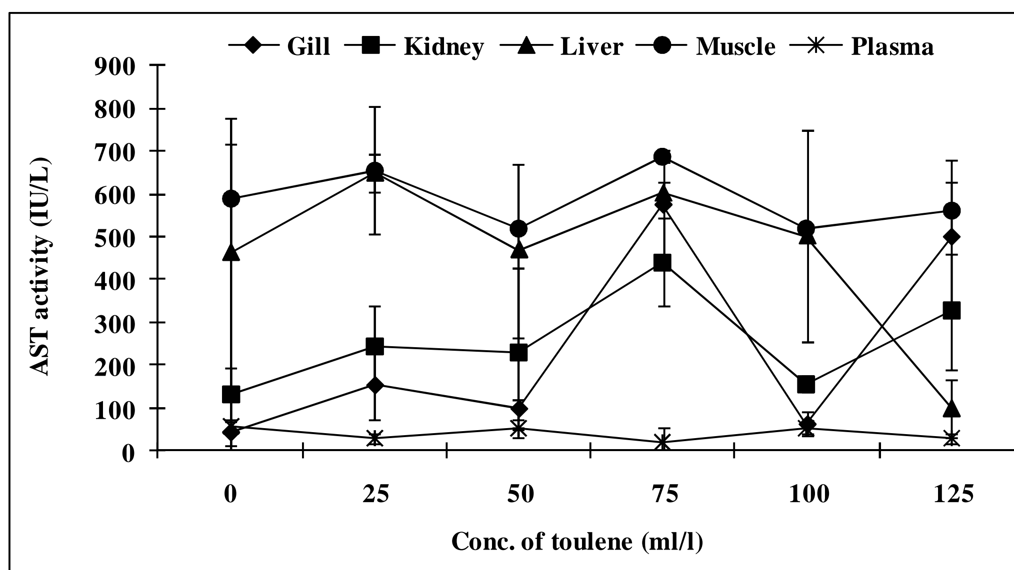

Figure 1: Relative activity of AST in selected tissues of *C. gariepinus* exposed to toluene for 21 days

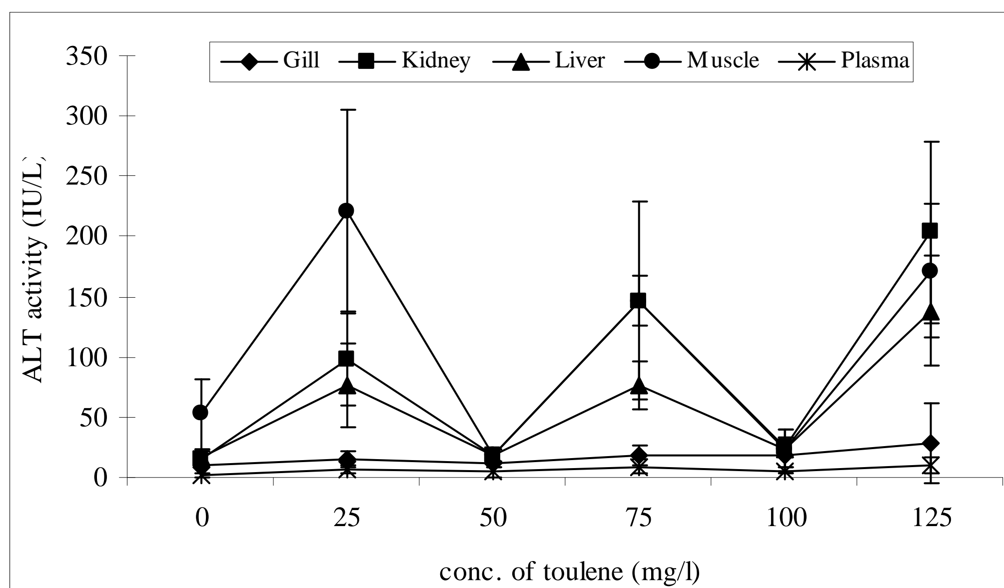

Figure 2: Relative activity of ALT in selected tissues of *C. gariepinus* exposed to toluene for 21 days

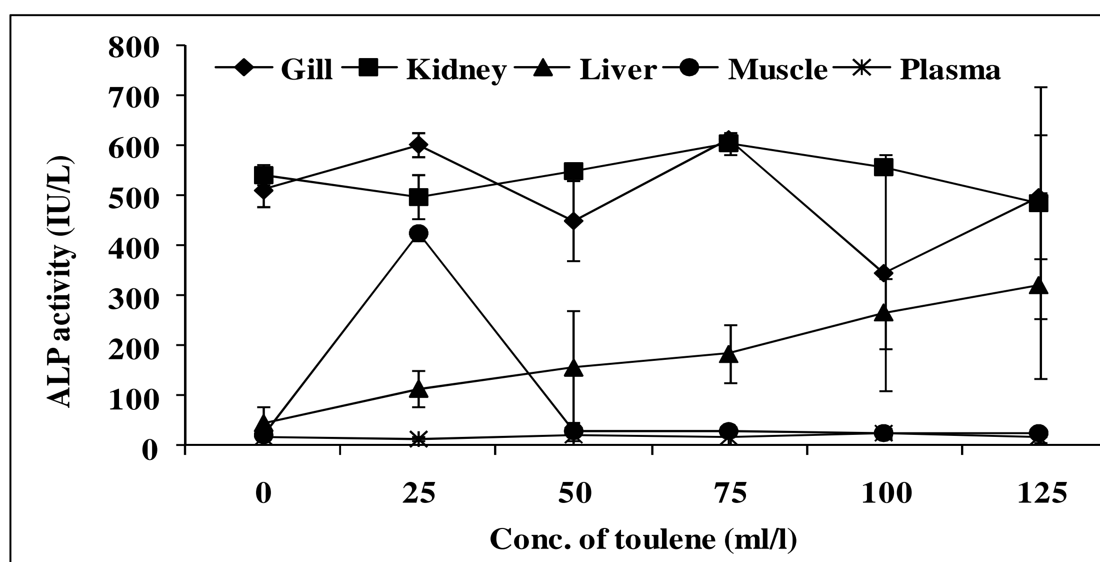

Figure 3: Relative activity of ALP in selected tissues of *C. gariepinus* exposed to toluene for 21 days

DISCUSSION

Disruption of the integrity of the cells of organisms is often measured by the changes in biochemistry and physiology of the organism (de la Torre *et al.,* 2000). Biomarkers such as enzymes (AST, ALT and ALP) are some of the most sensitive markers employed in the diagnosis of health condition of organisms under contaminant exposure because they are cytoplasmic in location and are released into circulation after cell damage (Pari and Murugavel, 2004). Treatment of the fish *C. gariepinus* with toluene generally increased the activities of the enzymes AST, ALT and ALP in all the organs (liver, gill, kidney and muscle) above the control value but decreased activities of these enzymes were recorded in the plasma of the fish. However, changes in activities of these enzymes have been observed in other studies (Ugwu *et al.,* 2008; Ozmen *et al.,* 2008).

The elevation of the transaminases is an indication of stress, a consequence of toluene

exposure reported for fish exposed to other environmental contaminants (Tiwari and Singh, 2004). The aminotransferases respond to stress or changes in physiological condition which in most cases leads to elevation of their activities (Natarajan, 1985). To overcome or combat stress, fishes need more energy to so that the demand for carbohydrate and its precursors could be maintained, therefore there is increasing step of the activities of the transaminases to cope with such energy requirement (Tiwari and Singh, 2004) and maintenance of the glycolytic pathway. Increase in the activities of these enzymes in the organs could be due to internal synthesis of the enzyme molecules, a pathway representing anaerobic tendencies of the tissues toward toluene toxicity or a counter adaptive measure to the assault of the toluene (Yakubu *et al*., 2001) leading to higher activities than in the control. Furthermore, the increased level of the transaminases after exposure to toluene may be due to changes in enzyme activity resulting from disturbance in the Kreb's cycle (Salah El-Deen and Rogers, 1993). However, the decreased activity in the plasma of all the enzymes (AST, ALT and ALP) suggests that the structural integrity of the cells membrane of the various organs were preserved and protected (Pari and Amali, 2005). The aminotransferases occupy a central position in the amino acid metabolism to form new amino acids during the degradation of amino acid and are also involved in the biochemical regulation of intracellular amino acid pool (Yakubu *et al*., 2005).

ALP is an important biomarker enzyme due to its involvement in adaptive cellular response to environmental toxicants (Lohner *et al*., 2001). ACP and ALP are hydrolytic enzymes involved in transphophorylation which are important in the transportation of metabolites, metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates, and synthesis of proteins (Srivastava *et al*., 1995). Since AP is responsible for the splitting of esters at an alkaline pH and mediates membrane transport, the rise in its activity will enhance transport, cell growth and proliferation. Also high activity of the enzyme in the liver can activate phosphorylase enzymes promoting glycogen production thereby providing needed energy for coping with the exposure stress from toluene toxicity (Heath, 1991). Increased activity of ALP in the organs following exposure to toluene infers an increase production of the enzyme to combat the effect of toluene (Yakubu *et al*., 2005). Severe acidosis was reportedly responsible for the inhibition of ALP activity in intoxicated liver of *Sarotherodon mossambicus* exposed to sevin which was an adaptation for anaerobic breakdown of glycogen to meet energy demand (Shaikila *et al.,* 1993). Conversely, an alkaline environment in the liver with high activity of ALP may have enhanced aerobic breakdown of glycogen to meet the energy demands of the fish. Another consequence of ALP overproduction is the adverse effect on the facilitation of the transfer of metabolites across cell membranes. Increased activity in the organs coupled with a decrease in the plasma indicates that the integrity of the organs has not been compromised there is absence of tissue damage (Pari and Amali, 2005; Adedeji, 2010). The results from this study indicated that toluene did not affect the activities of the enzymes in the plasma, suggesting the integrity of the organs of *C. gariepinus* were intact, but in the organs and muscle tissues it enhanced their activities. Hence, the assessment of these enzymes in the organs and muscle tissues could be used as good biomarkers of toluene toxicosis.

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SALINITY TOLERANCE OF LARVAE OF AFRICAN CATFISH *Clarias gariepinus* (♀) X *Heterobranchus bidorsalis* (♂) HYBRID

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ABSTRACT

Fourteen and twenty one day larvae were exposed to abrupt stepwise change in salinity (2, 4, 6, 8, 10 and 12 ppt) for 96 hours to determine mortality, median lethal mortality, MLS and median lethal time, MLT. The fourteen day-old fry that were exposed to 0 – 6 ppt recorded 90%, 87.5% 77.5% and 10% survival at the end of the 96 hours test period. Those that were subjected to 8 ppt and 10 ppt had 100% morality within 48 and 12 hours, respectively. The median lethal salinity at 96 hours (MLS-96) was 4.4 ppt which was about half the value for 6 ppt. The cumulative mortality of the graded salinities did not differ in 4 and 8 ppt but differed in 6 and 10 ppt, whereas the time intervals differed at 24 and 36 hours but did not differ at 48 – 96 hours, p<0.05. The 21 day-old fingerlings transferred into 8, 10 and 12ppt, recorded 100% mortality with 12, 3 and 2 hours, respectively. Those transferred into 0, 4 and 6 ppt had 100%, 80% and 10% survival, respectively at the end of 96hours test period. The median lethal salinity (MLS-96) for 6 and 12 hours were the same 7.19 ppt, while that for 48 hours was 4.79 ppt. The median lethal time (MLT_{50}) for 8 ppt was 8.55 hours, which was seven and half times the value for 4 ppt. The cumulative mortality the various salinity concentrations were not different in 10 and 12 ppt, but differ in 4 – 8 ppt, p<0.05. Mortality at various time intervals did not differ at 36 – 96 hours, but did at 3 and 6 hours, p<0.05.

Keywords: Catfish hybrid, Post larvae, Median lethal salinity, Median lethal time

INTRODUCTION

Clariid catfishes occur both in south-east Asia and in Africa and the highest generic diversity is on the African continent where some 14 genera have been reported (Teugels, 1984) against two in south-west Asia. In both continents, the clariids are of great economic important as food fish and for several years, they have been used in local fish culture where they proved to be fast growing protein source. They exhibit many qualities that make them suitable for culture. These include their hardy nature, disease resistance, tolerance to poor water quality, consumer acceptability, high fecundity, nutritional efficiency and attainment of large size within a short time (Hecht *et al.,* 1988; Haylor, 1989; Salami and Fagbenro, 1993).

Salinity is an important factor affecting the distribution of aquatic organisms. Depending on the concentration, it is known to affect the survival of the fish and other aquatic organisms by interacting with temperature, dissolved oxygen and other environmental factors (Eddy,

1981). The effects of physico-chemical parameters on cultured fish are of paramount importance and should be assessed in order to evaluate the optimum requirements and tolerances. The maximum and minimum levels of various environmental parameters such as: temperature, salinity, pH, dissolved oxygen (DO) concentrations, and ammonia among others are important in formulating culture practices of the organisms.

The osmotic pressure of water increases with increasing salinity (Boyd, 1982). Salinity therefore has been observed to influence growth, survival and production potentials of fish by its effect on osmoregulatory and metabolic activities of aquatic animals. At unfavourable concentrations, it can cause stress or even death of fish depending on the species (Eddy, 1981). Most estuarine and marine fish species which are euryhaline have optimal salinity range within which the scope for growth and other biological activities are optimal. Hence fish could be classified on the basis of their ability to tolerate environment differing in salinity. Thus, those that can tolerate wide range of salinity are termed euryhaline, while those with narrow range of salinity are stenohaline. Salinity tolerance tests with fish have been performed using different saline solution. Salinity tolerance tests with fish have been performed using different saline solutions for example, sodium chloride (Matern, 2001), diluted seawater (Chen and Chen, 2000) and synthetic seawater (Patridge and Jenkins, 2002). Study conducted indicated that mature catfish, *C. gariepinus* (0.6 – 1.5 kg) tolerated 10 ppt (25.6% seawater) for 100 hours with no sign of stress and with acclimatization the fish was able to tolerate 20ppt salinity (51.3%) seawater (Clay, 1977). Chervinski (1984) in his study of the salinity tolerance of young catfish (*C. gariepinus)* indicated that 95% of those on direct transfer tolerated 25% seawater (9.5%) but that no fish survived 30% seawater (11.7%), even though gradual increase. The medium lethal salinity (MLS_{50}) was 8 ppt using abrupt transfer and 10 ppt by gradual transfer (Iyagi, 1986). Britz and Hecht (1989) monitored the survival and growth of larvae of *C. gariepinus* between 0 and 5 ppt salinity.

The fingerlings of *H. bidorsalis* were found to resist changes from freshwater (0 ppt) up to 10 ppt (isosmotic) salinity without mortality (Fagbenro *et al.,* 1993). The present study was conducted to assess the tolerance of the post fry to salinity ranges which many occur in the natural habitat.

MATERIALS AND METHODS

Hybrid Procurement: Brood *C. gariepinus,* ♀ and *H. bidorsalis,* ♂ used for the study were obtained from a private fish farm at Aluu. The study was carried out in the hatchery and laboratory of African Regional Aquaculture Centre (ARAC) at Aluu, Port Harcourt from September to December, 2007. Gravid females of *C. gariepinus* (400 – 800 grams) were injected with ovaprim, a synthetic analogue of gonadotropin releasing agent. Dosage was calculated based on the manufacturer's recommendation of 0.5 ml/kg body weight of fish and administered intramuscularly in the dorsal muscle mass as described by Viveen *et al.* (1985). Ovulated eggs were stripped from the induced females into plastic bowl. Brood males of *H. bidorsalis* (0.6 – 1.3kg) were sacrificed and the testes dissected out. Incisions were made along the edges of the testes using a clean razor blade in order to extract the milt. The milt was squeezed out into a 0.9% saline solution. This was gently and thoroughly mixed. Fertilization was effected with addition of freshwater to the mixture of the eggs and milt, and by gently stirring with a plastic spoon.

The fertilized eggs were incubated in trays (30 x 45 cm^2) in a rectangular concrete tank containing freshwater. The hatched larvae were nursed in the concrete tank. The larvae were fed with *artemia* after the absorption of the yolk sacs up to the seventh day. The fry was then fed with ground and sieved Coppens feed (crude protein 45%) at 5% body weight for another week. Part of this was used for the salinity tolerance test for the fourteen day-old fry. The leftover in the concrete tank was fed with the same feed for another one week. These were used for the salinity tolerance test for the twenty one day-old fingerlings.

Saline Solution: Six salinity levels were prepared by weighting 2, 4, 6, 8, 10 and 12 g of sodium chloride with (Laptop balance Yamato LE 180, Yamato Scientific Company Limited, Tokyo, Japan) and dissolving each in a litre of freshwater to obtain 2, 4, 6, 8, 10 and 12 ppt, respectively. Freshwater was used as control (0ppt).

Salinity Tolerance test: Fingerlings each of fourteen day-old (1.22 ± 0.10 cm) and twenty one day-old (2.02 ± 0.2 cm) were introduced into each of the following test solutions 4, 6, 8, 10 and 12 ppt. The tolerance test was conducted in a five litre plastic aquarium containing three litre of the test solution. Each treatment level was replicated three times. The aquaria were covered with mosquito netting to prevent the fish from jumping out. Freshwater (0 ppt) was used as control. The fingerlings were not fed throughout the experimental period. Mortality was monitored at hourly interval for 96 hours. Dead fingerlings were removed, counted and recorded. Water temperature, dissolved oxygen and pH were determined twice a day. The test solution in each aquarium was changed daily. A fingerling was considered dead when opercular movement stopped and the fingerling did not react to gently prodding with a glass rod.

Water Quality: Mercury in glass thermometer was used to measure the water temperature during the salinity tolerance test for the fry and fingerlings of the African catfish hybrid. Hydrogen ion concentration (pH) was determined with handheld pH meter (Hannah Instruments Portugal, HA Model 191) which was calibrated against standard buffer solutions with pH values of 4, 7 and 10. Calibrations were carried done at each pH reading. Dissolved Oxygen was done with the Winkler method (APHA, 1985). Salinity was determined by a refractometer (ATAGOS/MILLE). The retractometer was first standardized with water from ARAC borehole to get reading of 0 ppt, before salinity of test solutions were determined.

Data Analysis: Probit analysis model (Finney, 1984) was used to determine the median lethal salinities (MLS) and median lethal times MLTs). Mortality at the various concentrations and duration were subjected to ANOVA and differences among means separated by Duncan multiple range test (Wahua, 1999). All analysis were done using Statistical Package for the Social Sciences, SPSS version 15 for Window.

RESULTS AND DISCUSSION

The water quality variables in the test aquaria were not different for the various salinity levels in the two life stages (Tables 1 and 2). The fourteen day-old fry that exposed to 0 – 6 ppt had 90%, 87.5% and 10% survival at the end of the 96 hour test period. Those subjected to 8 ppt and 10 ppt had 100% dead within 48 and 12 hours, respectively. The median lethal salinity at 96 hour (MLS_{50}) was 4.4 ppt which was about half the value for 6 hour (Table 3). However, the mortality at the exposure duration 48 – 96 hour was higher ($p<0.05$) from 3 – 36 hour for 14 day-old, whereas for 21 day-old mortality at 12 – 96 hour was similar but greater ($p<0.05$) than those at 3 – 6 hour (Table 3). The median lethal time (MLT_{50}) at 10 ppt was 11.65 hours which was about half the value for 6 ppt (Table 4). The percentage mortality of the 14 day-old larvae in this study declined with increased in salinity (Table 5). However, the survival at 48 – 96 hours were higher ($p<0.05$) compared to 3 – 36 hours (Table 5). For the 21 day-old, the 48 hour MLS_{50} was 4.79 (Table 6) and MLT_{50} at 8 ppt, 8.55 hour (Table 7). Percentage mortality increased with increase in the test salinity and at the exposure duration, and was similar at 12 – 96 hour and greater ($p<0.05$) than those at 3 – 6 hour (Table 8).

Salinity is one of the most important environmental factors exerting selective pressures on aquatic organisms and that organisms respond to varying salinity by either spending their life cycle in a single habitat where salinity is stable or variable; while others undergo ontogenic migrations with successive stages based on salinity regimes (Varsamos *et al.,* 2005).

The ability of each ontogenic stage to cope with salinity depends on the capacity to osmoregulate. Brett (1979) observed that observed that highest growth rates of various fish species relative to salinity clustered around 0 – 10 ± 2.00 or 28 – 35 ppt. These clusters correspond roughly to three ecological groupings: freshwater, stenohaline anadromous species; euryhaline and stenohaline marine species. The 21 day-old larva had higher survival when compared with the 14 day-old at 0 – 6 hour; however, at 8 and 10 ppt where 100% mortality was recorded in the 21 day-old it occurred at one-fourth and one-third respectively the time for that in the 14 day-old indicating the ontogenetic variation in salinity tolerance as reported in a number of fish species with increase in age (Murashige *et al*., 1991; Haddy and Pankhurst, 2004; Moustakas, *et al.,* 2004). However, the more rapid rate of death at 8 and 10 ppt in the 21 day-old is difficult to explain. The increased mortality ($p<0.05$) of the larvae in this study with increase in salinity was due to efflux of water out of the fish which has also been reported in larvae (Fashina-Bombata and Busari, 2003). The mortality at the exposure duration 48 – 96 hour was higher ($p<0.05$) from 3 – 36 hour for 14 day-old, whereas for 21 day-old mortality at 12 – 96 hour was similar but greater ($p<0.05$) than those at 3 – 6 hour and may suggest at that the larvae were able to attain internal homeostasis hence the rate of death did not differ as the exposure progressed with time.

Teleost embryos have developed strategies for coping with salinity challenge which includes formation of impermeable chorion and ion pump such as chloride cells, CC on the epithelium of embryos (Lin *et al.* 1999; Kaneko *et al.,* 2002). CC also played important roles in hydromineral and ionic homeostasis before tissues and organs for osmoregulation are developed (Ayson *et al.,* 1994*; Bone et al*., 1995). Besides, the 21 day-old may have developed tissues/organs in even miniature forms that have helped them cope better than the 14-day old. In some species such as *Mugil cephalus* (Lee and Menu, 1981), grouper-*Epinepalus coioides* (Yeh *et al.,* 1995) and black porgy- *Acanthopagrus schlegeli (*Chu *et al*., 1999) salinity tolerance seem to improve with age. The tolerance range of the larvae, 0 – 6 ppt with the optimum 2 – 4 and 2 – 6 ppt for 14 and 21 day-old, respectively indicated that the tolerance ranges were very narrow. The 96 hour MLS_{50} for the 21 day-old was 4.43 ppt; 48 hour MLS_{50} for 14 and 21 day-old were 4.52 and 4.79 ppt, a difference of 0.27 ppt suggesting the value was very narrow. Although the difference between the MLS_{50}, that between the MLT_{50} for 14 and 21 day-old larva (6.35 hour) at 8 ppt clearly showed that the older larvae was more tolerant. Decreasing tolerance with age is characteristic of freshwater stenohaline fishes possibly due to the physiological high energy cost of maintaining internal hydromineral and ionic balance in the face of increasing salinity (Kilambi, 1980). Hence several studies have confirmed that the early life stages of *Clarias and Heterobranchus* sp. hardly survive and grow for a long period beyond salinity above 10 ppt (Iyaji, 1986; Fagbenro *et al.,* 1993*).* Under salinity challenge it appears the body fluid osmolality of the larvae are raised relative to that of the external medium. Thus their tolerance is probably limited by the maximum osmotic pressure of the body fluid in which the cells can function (Maceina *et al*., 1980).

The fingerlings of the parent stocks, *H. bidorsalis* and *C. gariepinus* had been classified as freshwater stenohaline fishes (Fagbenro *et al.,* 1993; Oladosu *et al.,* 1999) which is true of larvae from their hybrid. A study by Odiete and Jacob (1985) indicated that the skin of the parents stock, *Clarias* sp. was non-keratinized with 25 – 32 layers of cells which make it permeable to Na^+ and Cl^- compared with that of another catfish, *Chrysichthys* sp. having keratinized layers that are impermeable to water and electrolytes. Hence, the fish tolerates wide salinity ranges even as was evidenced in fingerling *C. nigrodigitatus* that tolerated up to 22 ppt salinity (Anyanwu*,* 1991) unlike the clariids. The nature and functions of the integument of the larvae in this study may be similar to that of the parents and hence their response.

Laboratory studies on the effects of changing salinity on the early life stages of a number of freshwater fish species are meant to

Table 1: Water quality variables of the various test salinities for 14 day old *C. gariepinus* larvae

Salinity (‰)	Temperature (°C)		Dissolved oxygen (mg/l)		pH	
	Range	Mean	Range	Mean	Range	Mean
0	25.5-27.5	26.6±0.74	5.2-6.2	5.8±0.37	5.6-6.2	5.8±0.26
2	26.0-27.5	26.7±0.47	5.8-6.2	5.9±0.16	5.8-6.2	6.0±0.14
4	25.5-27.5	26.7±0.69	6.0-6.2	6.1±0.10	5.8-6.4	6.2±0.21
6	26.5-27.5	27.0±0.40	6.2-6.4	6.3±0.10	5.8-6.4	6.1±0.19
8	26.5-27.5	27.2±0.38	5.8-6.4	6.1±0.22	5.8-6.4	6.2±0.23
10	26.0-27.0	26.5±0.34	6.0-6.4	6.1±0.16	5.8-6.2	5.1±0.16

Table 2: Water quality parameters of the various test salinities for 21 day old *C. gariepinus* larva

Salinity (‰)	Temperature (°C)		Dissolved oxygen (mg/l)		pH	
	Range	Mean	Range	Mean	Range	Mean
0	26.0-27.0	26.5±0.46	6.0-6.2	6.1±0.10	5.6-6.2	5.9±0.22
4	25.5-27.0	26.1±0.68	5.8-6.2	6.1±0.17	5.8-6.2	6.1±0.17
6	25.5-27.0	26.5±0.76	5.8-6.2	5.9±0.16	5.6-5.8	5.7±0.10
8	26.5-27.0	26.2±0.34	5.6-6.2	5.8±0.21	5.4-6.2	5.8±0.29
10	26.5-27.0	26.5±0.44	6.2-6.8	6.4±0.24	6.2-6.8	6.4±0.0.24
12	25.0-27.0	25.8 ±0.68	6.2-6.6	6.4 ±0.14	6.2-6.6	6.4 ±0.16

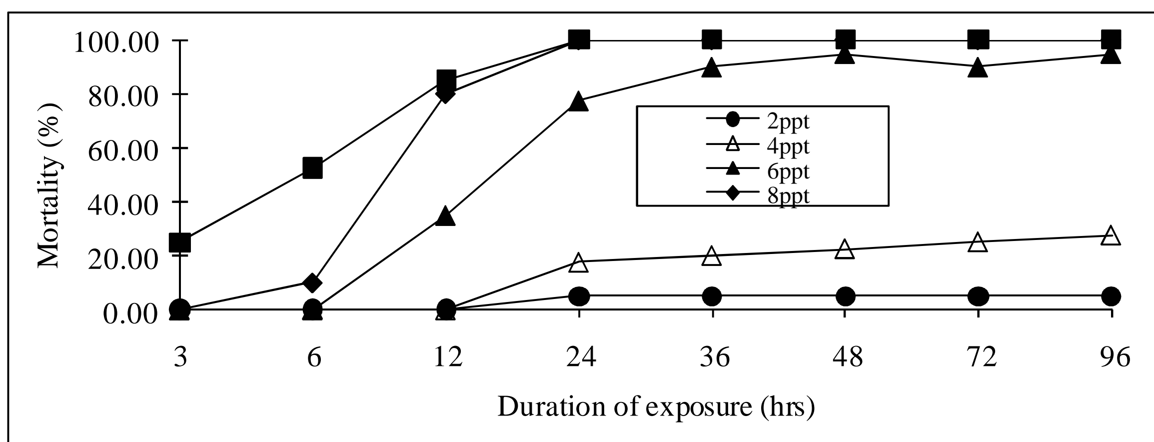

Figure 1: Mortality (%) of 14-day old larvae of *Clarias gariepinus* exposed graded levels of salinity under laboratory conditions

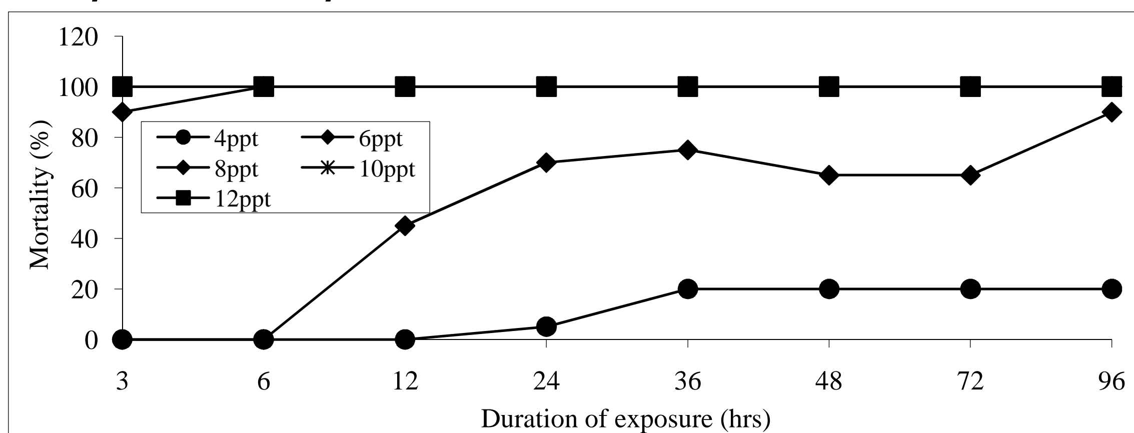

Figure 2: Mortality (%) of 21-day old larvae of *Clarias gariepinus* exposed graded levels of salinity under laboratory conditions

Table 3: Median Lethal salinity, MLS and associated 95% lower and upper confidence bounds (limits) of 14 day- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (hours)	MLS_5	MLS_{50}	MLS_{85}	MLS_{90}	MLS_{95}	MLS_{99}
6	7.85 (6.68-8.41)	9.80 (9.41-10.34)	11.02 (10.45-1125)	11.31 (10.67-12.72)	11.74 (10.99-13.43)	12.55 (11.58-14.77)
12	5.19 (4.30-5.75)	7.38 (6.99-7.77)	8.75 (8.30-9.43)	9.08 (8.58-9.85)	9.56 (8.98-10.49)	10.47 (9.72-72-11.70
24	2.96	5.36	6.87	7.23	7.76	8.76
48	2.06 (-2.02-3.30)	4.52 (241.6 (3.28-5.93	6.07 (4.97-9.23)	6.44 (5.26-10.12)	6.98 (5.67-11.47)	8.00 (6.38-14.04
72	2.00 (-1.42-3.17)	4.48 (3.36-5.71)	6.03 (5.00-8.68)	6.40 (5.30-9.12)	6.98 (5.67-11.47)	7.97 (6.44-12.98)
96	1.94 (-095-3.05)	4.43 (143-5.52)	6.00 (5.04-8.23)	6.37 (5.34-8.95)	6.92 (5J6-10.05)	7.95 (6.5042.14)

Table 4: Median lethal time, MLT and associated 95% lower and upper confidence bounds (limits) of 14 day- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (hours)	MLT_5	MLT_{50}	MLT_{85}	MLT_{90}	MLT_{95}	MLT_{99}
2	38.7 (13.55-58.96)	172.99 (124.97-394.41)	257.82 (177.34-640.69)	277.88 (189.59-699.06)	307.61 (207.71-785.64)	363.39 (241. 60-948.12)
4	24.60 (12.30-41.53)	132.77 (106.97-201.84)	200.93 (153.81-329.80)	217.05 (164.92-360.23)	240.94 (181.32-405.40)	285. 76 (211.99-490.22)
6	21.4	29.99	62.47	70.15	81.53	102.89
8	12.02 (9.34-13.89)	17.48 (15.77-19.37)	20.92 (19.07-23.57)	21.74 (19.79-24.63)	22.94 (20.84-26.21)	25.20 (22.73-29.25)
10	8.74	15.77	11.65	13.49	13.92	14.57

Table 5: Cumulative mortality associated 95% upper and lower confidence bounds of 14d-old larvae of catfish hybrid exposed to 9a) graded salinity at (b) various durations

(a) Salinity (‰)	Cumulative mortality	95%confidence bounds		(b) Duration	Cumulative mortality	95%confidence bounds	
		Upper	Lower			Upper	Lower
4	2.97[c]	1.98	3.96	3	1.20[d]	-0.10	2.50
6	1.47[b]	0.47	2.47	6	1.26[d]	0.02	2.48
8	3.50[c]	2.52	4.49	12	2.60[c]	1.30	3.90
10	8.03[a]	7.04	9.03	24	5.60[e]	4.34	6.84
4	2.97[c]	1.98	3.96	36	4.05[b]	2.79	5.31
6	1.47[b]	0.47	2.47	48	6.00[a]	4.74	7.26
8	3.50[c]	2.52	4.49	72	6.45[a]	5.19	7.71
10	8.03[a]	7.04	9.03	96	6.50[a]	5.24	7.76

Means with similar alphabets in the same are not significantly different 9p>0.05) for salinity and duration respectively.

Table 6: Median Lethal salinity, MLS and associated 95% lower and upper confidence bounds (limits) of 21 d- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (h)	MLS_5	MLS_{50}	MLS_{85}	MLS_{90}	MLS_{95}	MLS_{99}
6	5.72 (4.91-6.18)	7.18 (6.83-7.51)	8.09 (7.73-8.64)	8.31 (8.19-8.94)	8.6 (8.19-9.38)	9.24 (8.68-10.24)
12	4.74 (3.80-5.35)	7.19 (6.78-7.60)	8.73 (8.24-9.4)	9.09 (6.55-9.91)	9.63 (9.00-0.61)	10.64 (9.84-11.96)
24	4.53 (3.35-5.00)	5.66 (5.29-5.93)	6.38 (5.09-6.905)	6.55 (6.23-7.24)	6.80 (6.42-7.68)	7.27 (6.77-8.59)
48	2.25 (2.42-3.72)	4.79 (4.45-5.12)	5.79 (5.40-6.34)	5.99 (5.59-6.65)	6.33 (5.80-7.13)	6.97 (6.39-8.04)
72*						
96 *						

**Percentage responding in all salinities is the same.*

Table 7: Median Lethal Time, MLT and associated 95% lower and upper confidence bounds (limits) of 21 day-old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Salinity (‰)	MLT_5	MLT_{50}	MLT_{85}	MLT_{90}	MLT_{95}	MLT_{99}
4	19.05 (9.15 -26.34)	65.06 (59.57 -71.53)	96.06 (85.72-105.62)	100.92 (91.67-133.92)	111.08 (100.41-126.29)	130 (116.67-149.60)
6	29.79 (18.97-52.57)	34.10 (24.05 -87.83)	74.36 (47.11-423.31)	83.88 (53.72-512.87)	98.00 (62.09-164.46)	124.47 (78.21-898.21)
8	-11.13	8.55	18.6	21.40	25.45	33.01
10*						
12*						

**Percentage responding in all salinities is the same.*

Table 8: Cumulative mortality associated 95% upper and lower confidence bounds of 14 day old larvae of catfish hybrid exposed to 9a) graded salinity at (b) various durations

(c)	Salinity (‰)	Cumulative mortality	95% confidence bounds Upper	Lower	(d) Duration	Cumulative mortality	95%confidence bounds Upper	Lower
	4	1.00d	0.75	1.25	3	3.67e	3.38	3.95
	6	5.56c	5.32	5.81	6	4.83d	4.55	5.12
	8	8.88b	8.63	9.12	12	5.50b	5.21	5.79
	10	10.00a	9.75	10.25	24	6.25	5.96	6.54
	12	10.00a	9.75	10.25	36	6.50	6.21	6.79
	4	1.00d	0.75	1.25	48	6.83a	6.55	7.12
	6	5.56c	5.32	5.81	72	6.83a	6.55	7.12
	8	8.88b	8.63	9.12	96	6.83a	6.55	7.12

Means with similar alphabets in the same are not significantly different 9p>0.05) for salinity and duration respectively

assess the possibility of introducing such species into non-natural ecosystems especially with the salinization of rivers and wetlands which is a major environmental concern in many parts of the world (Williams, 1987). A most common approach to laboratory measure of salinity tolerance is the concentration that is lethal to 50% of the individuals (the LC_{50}) over a period of time usually 48 – 98 hours. Kefford *et al.* (2004) noted that the values are usually determined from three broad methods: direct transfer (direct LC_{50}), slow acclimation (slow LC_{50}) and early life stage or early LC_{50} which gives very large differences in the LC_{50}. Besides, the studies are conducted without other stressors, quite contrary to what obtain in natural ecosystems. The authors observed that "survival in laboratory experiment may not predict survival in nature, let alone the maximum salinity that could support a self sustaining population" Besides, although laboratory measurements of sublethal effects are possible, it is difficult to relate to the salinity at which a species can maintain a self sustaining population in nature (Brinkhurst *et al.,* 1983). Hence, although the larvae in this study had optimum salinity tolerance range at 0 – 4 ppt, in the natural environment with the vagaries in physico-chemical and other environmental factors the optimum range may be greatly influenced. The farmer intending to introduce the larvae to the optimum salinity in the natural environment should carry out a field validation study as suggested by Connell *et al.* (1999) so as avoid heavy losses that may discourage such attempts and the associated benefits.

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