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FISH DIVERSITY AND RATE OF THEIR ABUNDANCE IN ITUNLA RIVER IN IGELE, OGUN WATERSIDE LOCAL GOVERNMENT AREA, OGUN STATE, NIGERIA

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ABSTRACT

Fish is a major source of high quality animal protein, and also provide several socio-economic values for man. Studies on fish diversity, physical and chemical parameters of water in Itunla River was carried between February to July, 2015 to ascertain fish yield and interaction with water quality. The fish were obtained from fishermen using gillnets fleets ranging from 1 inch mesh to 4 inch, sorted into taxonomic categories and weighed. The results showed that 6599 fish species sampled have eighteen fin fish species from twelve families. Bagridae family had the highest contribution (22.4% and 24.0%), Mormyridae (21.7% and 23.7%) and Mochokidae (11.9% and 10.7%) in abundance and weight respectively. Monthly contribution by abundance was highest in May and least in July. Simpson index was highest (0.91) and with low dominance value of (0.09) and evenness value (0.75). The value of physicochemical parameters assessed included temperature (28.87 ± 0.27 °C), dissolved oxygen (6.94 ± 0.15 mg/l), transparency (6.4 ± 0.48 m), water pH (7.24 ± 0.22), total dissolved solid (82.42 ± 5.88 mg/l), conductivity (161 ± 12.67 μ s/cm), nitrate (2.92 ± 0.27 mg/l) and phosphate (0.08 ± 0.01 mg/l) with no significant difference ($p > 0.05$) except in nitrate, phosphate and conductivity. The river contributes highly to the inland fishery of the State due to its high composition of fish species which is an evidence of its high productivity.

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1. INTRODUCTION

Fishing as means of livelihood is attracting a lot of focus because it contributes significantly to the world's proteins requirement (Moses, 1990). Fish is a high quality food, and its content of protein

matter is important. It is rich in vitamins and contains variable quantities of fats and calcium for human health (Moses, 1990). Nigeria is blessed with vast natural water bodies and abundant fish resources, and its freshwater bodies are the richest in West Africa in terms of fish abundance (Meye and Ikomi, 2008). The fish resources, apart from being a major source of high quality animal protein for man, provide several socio-economic values such as sources of job opportunities and raw material for some industrial activities as well as recreational purposes (Yakub, 2012). Most of Nigeria populations live near water bodies such as lakes, lagoons, reservoirs, rivers, swamps and coastal lagoons and majority of this populates depend heavily on the resources of such water bodies for their main source of animal protein and family income (Abubakar *et al.*, 2006). Ita (1993) reported 268 different species in 34 well known Nigerian freshwater rivers, lakes, reservoirs, which constitute about 12% of Nigeria's total surface area of about 98,185,000 hectares. However, according to Jamiu and Ayinla (2003), the yields of most of these inland waters are generally on the decline due to environmental degradation such as water pollution and improper or poor management of fisheries resources. The US Environmental Protection Agency (EPA) recommended species richness and relative abundance as ecological risk assessment in aquatic ecosystem (EPA, 2007).

Biodiversity concept seems to provide a scientific foundation for wide spread desires to preserve natural ecosystems in aqua regimes (Ehrlich and Wilson, 1991). Biodiversity is often misused or over used to describe population dynamics of a location or community, but in a real sense it is a measure of number of species that make up a biological community and is considered to be one of the most important aspects of community, organization and structure (Lawson and Olusanya, 2010). Species richness and relative abundance describe key elements of biodiversity. Species richness is the number of different species in a given area and this is the fundamental unit in which to assess the homogeneity of an environment and commonly used in conservation studies to determine the sensitivity of an ecosystem and their resident species. On the other hand, relative abundance describes how common or rare a species is relative to other species in a given community and are usually described for a single trophic level (Lawson and Olusanya, 2010). Diversity encompasses two different concepts of variety and variability, viz richness and evenness. These two concepts, in theory can be applied across a hierarchy of scales from genetic diversity through to ecosystem diversity (Burton *et al.*, 1992).

In Africa, fisheries resources are on the decline most especially in Nigeria due to over exploitation and inadequate management of Nigeria coastal water. For sustainability of these resources, an adequate knowledge of species composition, diversity and relative abundance of the water bodies must be understood and pursued vigorously (Lawson and Olusanya, 2010). This is to have a comprehensive understanding of the ichthyofauna composition and distribution of the water bodies. However, very few studies are available on the assessment of sustainable fisheries potentials of these water bodies.

To this end, the purpose of this study is to identify, investigate fish species composition, assess the fish species diversity, richness and rate of their abundance and determine water quality parameters and get information on the interaction between hydro biological condition and fish yield in Itunla River, Igele, Ogun Waterside Local Government Area, Ogun State of Nigeria in order to conserve biodiversity of the fish species as well as their yield.

2.3. Biological Diversity

According to (Harper, 1999) diversity is measured by two main components: species richness (the total number of species present), species evenness (how evenly distributed the species are in a designated community). Additionally, species composition (which particular species are present) is often measured. Simpson's Index of Dominance is a measure of both the richness and proportion (percentage) of each species (written as lower case l) Simpson (1949). It is calculated as follows:

$$\frac{\sum n(n-1)}{N(N-1)} \quad (2)$$

Dominance (D) = (ranges from 0 to 1); Shannon Index (entropy) H : Evenness = $e^{H/S}$ according to Ellenbroek (1987).

2.4. Measurement of Physical and Chemical Parameters of Water

Water samples were collected in sample bottles from the surface of the water in each location on each sampling visit. The following physical and chemical parameters were analyzed.

2.4.1. Temperature

The temperature of the water sample as well as that of the air was determined in situ using a dissolved oxygen analyser.

2.4.2. pH

The pH was measured using a multi-purpose pH-EC-TDS meter (HANNA HI 9810) meter.

2.4.3. Total dissolved solid

The total dissolved solid of the water sample was determined using a multi-purpose pH -EC-TDS meter (HANNA HI 9810).

2.4.4. Conductivity

The conductivity of the sample was measured by using a multi-purpose PH -EC-TOS meter (HANNA HI 9810).

2.4.5. Transparency

Transparency was determined using secchi disc (a flat rounded steel of alternate black and white) with a suspended rope of 5 m at the centre. It was suspended in the water and readings were taken at the disappearance and reappearance of the disc.

2.4.6. Dissolved oxygen

Dissolve oxygen was determined by using the BOD bottle to collect the sample in the river. Reagent used was 5ml of manganese sulphate, potassium iodide and concentrated H_2SO_4 (sulphuric acid) with the use of syringe rinsed after every reagent. The solution was taken to laboratory for proper readings by titration using Winkler (1888) method.

2.4.7. Phosphate

The concentration of phosphate in water was measured using Vanado-molybido-phosphatic acid calorimetric method (Ademoroti, 1996), in water sample collected. Activated carbon (0.2 g) was

added to 50ml of each sample to remove any excessive colour and allow settling for about 5 minutes. The mixture was filtered through white paper. The filtrate (25ml) was pipette into 50ml volumetric flask, 10ml of Vanadate-molybdate reagent was added to develop a yellow colour and diluted to distilled water. Distilled water (25ml) was substituted for sample solution and used as blank. Spectrophotometer was used to determine the absorbance level of the wavelength. The intensity of the developed yellow colour is directly proportional to phosphate concentration in water sample.

2.4.8. Nitrate

Sodium salicylate (colorimeter) method was used to measure the concentration of nitrates in water sample collected (Ademoroti, 1996).

3. RESULTS AND DISSCUSION

3.1. Fish Species Consumption

Eighteen (18) fish species belonging to twelve (12) families were observed in the study. The families were: *Bagridae*, *Characidae*, *Cichlidae*, *Clariidae*, *Gymnarchidae*, *Malapterudae*, *Mochokidae*, *Mormyridae*, *Ophiocephalidae*, *Osteoglossidae*, *Polypteridae* and *Schilbedae*. Table 1 shows the results of fish contribution by abundance and weight to fish production respectively. *Mormyrus rume* (15.73 %) has the highest contribution to the total fish catch by abundance followed by *Chrysichthys nigrodigitatus* (14.31%), *Synodontis nigrita* (11.8%), *Chrysichthys auratus* (8.06%), *Clarias gariepinus* (6.64%), *Mormyrus deliciosus* (5.97%), *Shilbe mystus* (5.61%), *Parachanna obsura* (5.43%), *Tilapia mariae* (5.34%), *Alestes leuciscus* (5.12%), *Coptodon zilli* (4.14%), *Oreochromis niloticus* (2.30%), *Calamichthys calabaricus* (2.29%), *Hydrocynus vittatus* (2.24 %), *Gymnarchus niloticus* (1.33%), *Malapterurus electricus* (1.29%), *Polyterus senegalensis* (1.23%) and *Heterotis niloticus* (1.05%) which has the lowest contribution in fish abundance. In weight, *Mormyrus rume* (15.96%) has the highest contribution followed by *Chrysichthys nigrodigitatus* (14.39%), *Synodontis nigrita* (10.74%), *Chrysichthys auratus* (9.63%), *Clarias gariepinus* (8.06%), *Mormyrus deliciosus* (7.75%), *Gymnarchus niloticus* (4.45%), *Parachanna obsura* (4.25%), *Heterotis niloticus* (3.95%), *Hydrocynus vittatus* (3.90%), *Shilbe mystus* (3.74%), *Tilapia mariae* (3.25%), *Tilapia zilli* (2.49%), *Alestes leuciscus* (2.22%), *Oreochromis niloticus* (2.22%), *Malapterurus electricus* (1.29%), *Polyterus senegalensis* (1.00%) and *Calamichthys calabaricus* (0.71%).

3.2. Diversity Indices of Fish Species Composition

Table 2, shows the result of diversity indices of sample fish catch. Highest species richness twelve (12) and individual (6599) Simpson index (0.91) and Evenness (0.75), Dominance value (0.09) were lowest. On monthly analysis as shown Table (3), fish taxa was highest in May and June, both months have (18) and least in July (12), Simpson index was lowest in July (0.87), Evenness was lowest in July. It was observed that May and June have similar dominance value (0.08) and February have highest dominance value (0.11). However, the study area as homogeneity unit recorded high diversity from 18 taxa and 6599 fish sampled. The month of May had highest record in terms of fish weight. The month of July recorded minimum weight of total fish catch during the study period. Fish abundance and weight are depicted in figure 2. Both catch abundance and weight were higher in the values. And monthly contribution to the total catch by abundance was least in July and highest in May. It was also observed that bigger fishes were caught in the months of May and June. Certain species such as *Calamichthys calabaricus* and *Polyterus senegalensis* were found in May, June and July and month of May has the highest in abundance and weight.

Table 1: Fin fish species composition of Itunla River, Igele, Ogun State

| SN | Fish species | Total | | Relative Percentage (%) | |
|----|------------------------------------|-----------|--------|-------------------------|--------|
| | | Abundance | Wt(kg) | Abundance(no.) | Wt(kg) |
| 1 | <i>Alestes leuciscus</i> | 338 | 29 | 5.12 | 2.22 |
| 2 | <i>Calamichthys calabaricus</i> | 151 | 9.2 | 2.29 | 0.71 |
| 3 | <i>Chrysichthys auratus</i> | 532 | 125.5 | 8.06 | 9.63 |
| 4 | <i>Chrysichthys nigrodigitatus</i> | 944 | 187.6 | 14.31 | 14.39 |
| 5 | <i>Clarias gariepinus</i> | 438 | 105 | 6.64 | 8.06 |
| 6 | <i>Gymnarchus niloticus</i> | 88 | 58 | 1.33 | 4.45 |
| 7 | <i>Heterotis niloticus</i> | 69 | 51.5 | 1.05 | 3.95 |
| 8 | <i>Hydrocynus vittatus</i> | 148 | 50.8 | 2.24 | 3.90 |
| 9 | <i>Malapterurus electricus</i> | 85 | 16.8 | 1.29 | 1.29 |
| 10 | <i>Mormyrus deliciosus</i> | 394 | 101 | 5.97 | 7.75 |
| 11 | <i>Mormyrus rume</i> | 1038 | 208.1 | 15.73 | 15.96 |
| 12 | <i>Oreochromis niloticus</i> | 152 | 29 | 2.30 | 2.22 |
| 13 | <i>Parachanna obscura</i> | 358 | 55.4 | 5.43 | 4.25 |
| 14 | <i>Polyterus senegalensis</i> | 81 | 13 | 1.23 | 1.00 |
| 15 | <i>Schilbe mystus</i> | 370 | 48.7 | 5.61 | 3.74 |
| 16 | <i>Synodontis nigrita</i> | 784 | 140 | 11.88 | 10.74 |
| 17 | <i>Tilapia mariae</i> | 356 | 42.4 | 5.39 | 3.25 |
| 18 | <i>Tilapia zilli</i> | 273 | 32.5 | 4.14 | 2.49 |
| | Total | 6599 | 1303.5 | 100 | 100 |

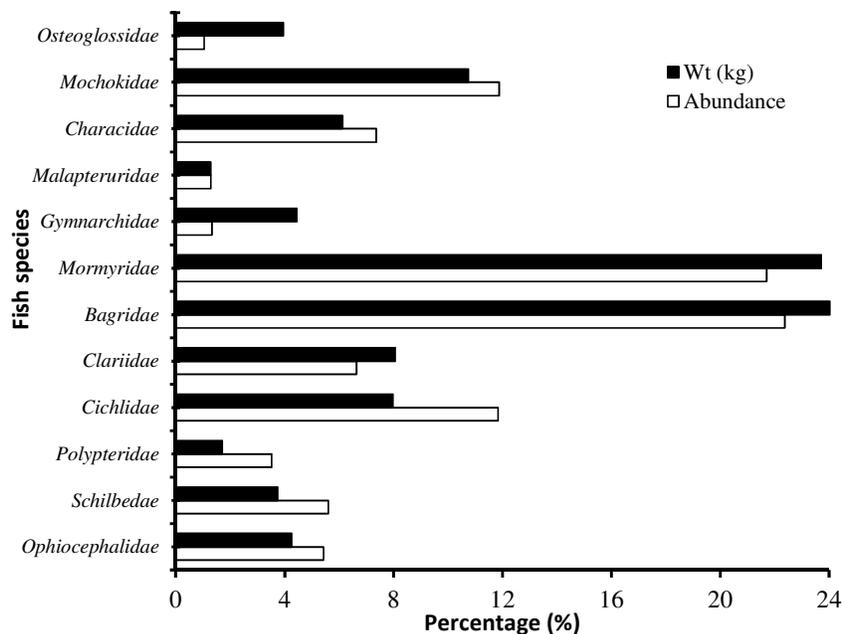


Figure 2: Fin fish production (Abundance and weight) in Itunla River, Ogun State

Table 2: Diversity indices of fin fish species in Itunla River, Igele, Ogun State

| Indices | Feb | Mar | Apr | May | June | Jul | Pooled |
|-------------|------|------|------|------|------|------|--------|
| Taxa | 14 | 16 | 16 | 18 | 18 | 12 | 18 |
| Individuals | 990 | 1117 | 1187 | 1651 | 1254 | 400 | 6599 |
| Dominance | 0.11 | 0.09 | 0.10 | 0.08 | 0.08 | 0.13 | 0.09 |
| Shannon | 2.39 | 2.53 | 2.51 | 2.69 | 2.67 | 2.18 | 2.61 |
| Simpson | 0.89 | 0.91 | 0.90 | 0.92 | 0.92 | 0.87 | 0.91 |
| Evenness | 0.78 | 0.79 | 0.77 | 0.82 | 0.80 | 0.74 | 0.75 |

3.3. Physical and Chemical Parameters of Itunla River

The results of the water parameters of Itunla River, Ogun water side local government, Igele, Ogun State during the study period are shown in Table 3 and Figures 3-11. Air temperature ranged from $29.0 \pm 0.0^\circ\text{C}$ in April to $30.1 \pm 0.90^\circ\text{C}$. Maximum air temperature was recorded in the month of February and minimum in the month of April and with no significant difference ($p > 0.05$). Water temperature was between the range of $28.3 \pm 1.05^\circ\text{C}$ and $30.0 \pm 0.85^\circ\text{C}$ and there was no significant difference ($p > 0.05$). The maximum and minimum water temperature was observed in the month of May and April respectively. Dissolved oxygen ranged from $6.5 \pm 0.05\text{mg/l}$ in June to $7.9 \pm 0.35\text{mg/l}$. Maximum dissolved oxygen was recorded in the month of May which coincides with the rainy season and there was no significant difference ($p > 0.05$). Water pH was recorded between the range of 6.2 ± 0.03 in May and 8.2 ± 0.25 in the month of March, with no significant difference ($p > 0.05$). Total dissolved solid fluctuated between the range of $59.0 \pm 1.00\text{mg/l}$ in February and $1.08\text{E}^2 \pm 4.5\text{mg/l}$, the maximum total dissolved solid was observed in May and minimum in February with no significant difference ($p > 0.05$). Conductivity ranged between $120 \pm 1.0\mu\text{s/cm}$ in February and $220 \pm 20.0\mu\text{s/cm}$ in May with no significant difference ($p > 0.05$). Nitrate concentration fluctuated between $1.4 \pm 0.19\text{mg/l}$ and $4.0 \pm 0.07\text{mg/l}$ and significantly higher ($p < 0.05$). Nitrate concentration was maximum in month of May and minimum in month of February. Phosphate ranged between $0.06 \pm 0.01\text{mg/l}$ and $0.08 \pm 0.01\text{mg/l}$ and significantly higher ($p < 0.05$). Maximum phosphate concentration was observed in the month of May and minimum in month of February and July. Water transparency ranged from $0.4.0 \pm 0.01\text{m}$ in February and $80.0. \pm 0.0\text{m}$ with no significant difference ($p > 0.05$).

The study revealed that *Mormyrus rume* dominated the catch as against *Chrysiichthys nigrodigitatus*. The dominance of *Mormyrus rume* in the study as observed corroborated that they are among the major river pelagic fish, most valuable and abundant in the artisanal fisheries in Nigeria. Ellenbroek (1987) opined that the dominant species in a community largely determine the structure and the functioning of the community. The highest fish production both in abundance as observed in Table 2 could be attributed to the highest concentration of fishermen using outboard engines in their fishing activities. The highest fish production both in number and weight was observed in the month of May which could be as result of availability of rain and abundant natural foods resulting from the nutrients in the water during the rainy season. During the period of February to April *Calamoichthys calabaricus* and *Polypterus senegalensis* were not found from the fish catch. But in month of May to July *Calamoichthys calabaricus* and *Polypterus senegalensis* were found among the fish catches, showing that the two fish species dwell in swampy area of the river and also at bottom of aquatic macrophyte which protect them from predators and since the fish are herbivorous in nature they also feed on the macrophyte. Also weaving basket trap made from palm frond are used to catch the two species unlike the others species in which gill net and cast net were used. The cluster methods of ecological analysis could only be regarded as providing possible initial pointers of important species.

The variation observed in species composition from this work is likely to be due to differences in time, the fishing gear used and climatic condition. High Shannon diversity and low evenness value might be as a result of more contribution of *Mormyrus rume* to the total catch in that month. Ecosystem diversity is often evaluated through measure of diversity of the component species.

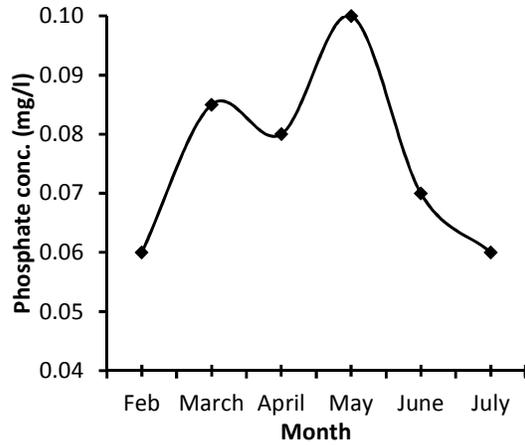


Figure 3: Monthly mean variation of phosphorus concentration in Itunla River, Igele, Ogun State

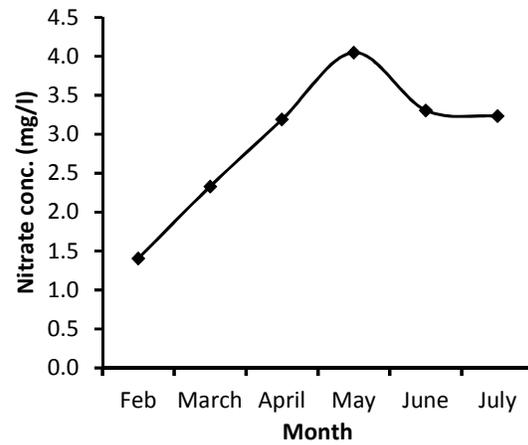


Figure 4: Monthly mean variation of nitrate concentration in Itunla River, Igele, Ogun State

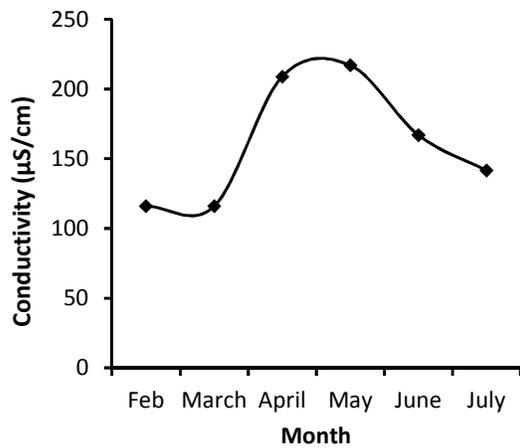


Figure 5: Monthly mean variation of electrical conductivity in Itunla River, Igele, Ogun State

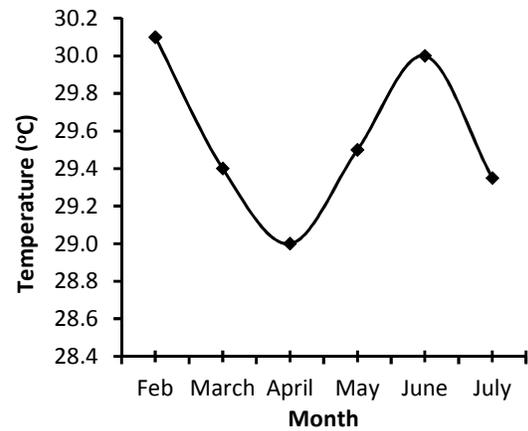


Figure 6: Monthly mean variation of air temperature in Itunla River, Igele, Ogun State

Water quality attributes are essential factors that influence fish survival, reproduction, growth and overall biological production. Water quality parameters play important roles on determining the suitability of the water for aquatic biota, their ranges and temporal regimes (Karr and Dubley, 1981). Nine water parameters were carried out for six months in the study area. Nitrate and phosphate have been known to be essential nutrients for plant growth. Nitrate and Phosphate were maximum in month of May in their concentration, which correspond with peak of rainy season and as a result of runoff washed into the river. Nitrogen is an essential element of phytoplankton growth in aquatic environment and is the main nutrient supporting fish production in the open ocean (Maruo *et al.*, 2006).

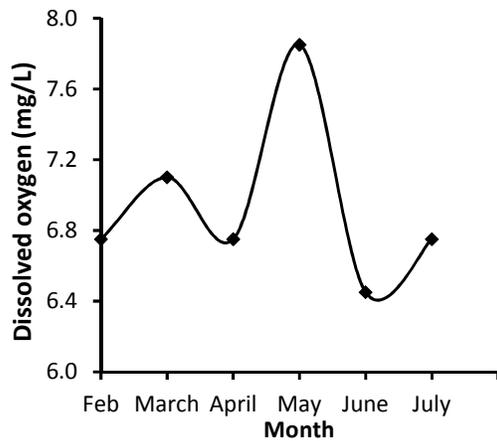


Figure 7: Monthly mean variation of dissolved oxygen concentration in Itunla River, Igele, Ogun State

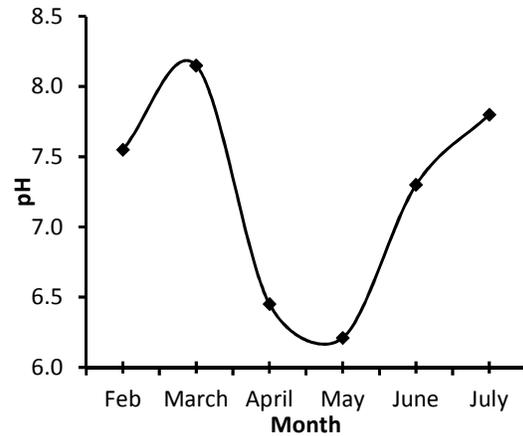


Figure 8: Monthly mean variation of pH concentration in Itunla River, Igele, Ogun State

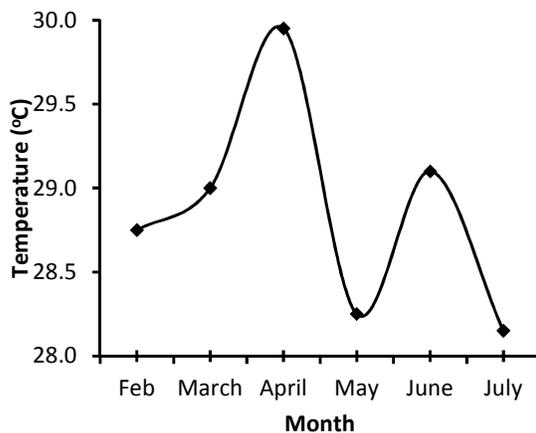


Figure 9: Monthly mean variation of water temperature in Itunla River, Igele, Ogun State

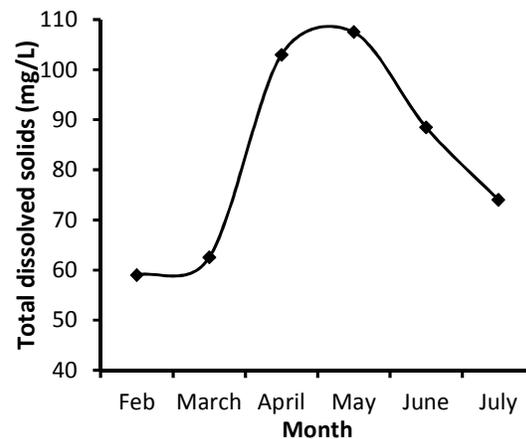


Figure 10: Monthly mean variation of total dissolved solids in Itunla River, Igele, Ogun State

Nitrate in excessive amount in water lead to illness and can lead to mortality in fish. Nitrates are measured in terms of mg/l and measured in the laboratory using reagents. Phosphorous occurs in waters and in wastewaters almost solely as phosphates. It is essential to the growth of fish and can enhance the primary productivity in water. Phosphates are measured in terms of mg/l in laboratory using standard methods.

Maximum dissolved oxygen value of 7.9 ± 0.35 mg/l observed in the study for the area might be as result of dynamic nature of the surface water. The amount of dissolved oxygen depends on temperature, atmospheric pressure, salinity and its relative solubility in water (UNEPGEM, 2006). Oxygen source in water are by diffusion of oxygen from the air into water, photosynthetic activity of aquatic autotrophs and inflowing streams. Dissolved oxygen is very important parameter for survival of fishes and other aquatic organisms. Too low concentrations of oxygen may not be enough to sustain life.

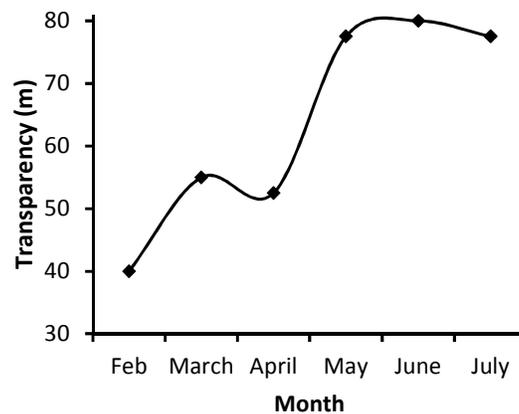


Figure 11: Monthly mean variation of water transparency in Itunla River, Igele, Ogun State

Table 3: Correlation matrix of water quality parameters of Itunla River Igele, Ogun State

| | Air temp | Water temp | DO | Transparency | pH | TDS | EC | Nitrate | Phosphorus |
|--------------|----------|------------|-------|--------------|-------|------|------|---------|------------|
| Air temp | 1 | | | | | | | | |
| Water temp | 0.31 | 1 | | | | | | | |
| DO | -0.38 | -0.59 | 1 | | | | | | |
| Transparency | -0.20 | -0.41 | 0.23 | 1 | | | | | |
| pH | -0.07 | -0.25 | -0.29 | -0.15 | 1 | | | | |
| TDS | -0.14 | 0.046 | 0.35 | 0.46 | -0.84 | 1 | | | |
| EC | -0.26 | 0.03 | 0.40 | 0.41 | -0.86 | 0.98 | 1 | | |
| Nitrate | -0.17 | -0.06 | 0.30 | 0.74 | -0.50 | 0.81 | 0.76 | 1 | |
| Phosphorus | -0.18 | 0.27 | 0.50 | 0.15 | -0.39 | 0.43 | 0.43 | 0.54 | 1 |

Water temperature is about 28°C in many tropical waters (Wickstead, 1976). Frotal *et al.*, (2004) reported surface water temperature between 24°C and 26°C in Abrolhos Bank in Brazilian coast. Changes in species diversity would occur over time as result of changes in temperature level (Spellerberg, 1991). Although the tolerance to individual species varies, it however exerts a major influence on the biological activities and growth. To a certain point, increase in temperature leads to greater biological productivity. Temperature influence water chemistry, such as solubility, dissolved oxygen, density, pH, conductivity etc. And some compounds are more toxic to aquatic organisms at higher temperature. The potential of hydrogen is the measure of concentration of hydrogen ions. It provides the measure of the acidity or alkalinity o water. UNEPGEM (2006) reported that pH values between 6.5-8.5 were indicator of good water quality. The maximum water pH of 8.2±0.55 of the river was supported by the finding of (Parson *et al.*, 1984) who reported that pH of water is usually in the range of 7.5-8.5 and any changes within the range are due to photosynthesis and respiration of organisms and also high pH could result as a result of precipitation of mucus on the gills of fish.

Total dissolved solids refer to the matter suspended or dissolved in water. Solids may affect water or effluent quality adversely in a number of ways, water with high dissolved solids generally are of inferior palatability.

There are many factors that could cause a change of nutrients concentration in water. These include terrestrial input such as river runoff, rain, vertical mixing and biological processes. *Bagridae*,

Mormyridae and *Mochokidae* were the most important fish resources in the study area in terms of abundance and weight. Among the fin fish species *Mormyrus rume*, *Chrysiichthys nigrodigitatus*, *Synodontis nigrita* were the major contributors. The month of May has the highest fish harvested compared with other months during the study, and month of July has the lowest fish harvested in terms of weight and abundance in the wet season. The study reveals that most of the fish species identified at Itunla river in Igele, Ogun water side local government of Ogun state are in good condition and many small sizes were observed in the catches of fishermen. Water quality of the river in the study area was good enough as it encouraged good growth of fish species. Species are the most practical and widely applicable measure of biodiversity. The most important aspect of biodiversity is the species composition.

Itunla River is an important river in Ogun Waterside Local government of Ogun State contributing highly to the inland fishery of the State. The research shows that there is high composition of fish species in the river which is an evidence of its high productivity. The presence of *Calamoichthys calabaricus* which can be used as ornamental fish further highlights the potential of the river. The wide coverage of the river across, Ondo, Ogun and Lagos States of Nigeria makes the river to be of particular interest and with proper management it could be developed for multi- purpose usage such as transportation, sport fishing and recreation. Problems observed in the study area included pollution of the water with agricultural chemicals, use of small sized mesh nets, lack of social amenities such as drinkable water and schools, human activities such as washing of clothes, logging and waste disposal into the water. These have negative impacts on the quality of the water and need to be addressed and corrected. Fishermen have a critical role to play in understanding and protecting diverse fish resources because if properly managed, the river will go a long way in boosting the economic status of the state as its existence is essential for man. It is hoped that the information gathered from this study will be very useful in formulating management policies that would be useful in future management of Itunla River:

4. CONCLUSION

Bagridae, *Mormyridae* and *Mochokidae* were the most important fish resources in the study area in terms of abundance and weight. Among the fin fish species *Mormyrus rume*, *Chrysiichthys nigrodigitatus*, *Synodontis nigrita* were the major contributors. The month of May has the highest fish harvested compared with other months during the study, and month of July has the lowest fish harvested in terms of weight and abundance in the wet season. The study reveals that most of the fish species identified at Itunla River are in good condition and many small sizes were observed in their catches. Water quality of the river in the study area was good enough as it encouraged good growth of fish species. Species are the most practical and widely applicable measure of biodiversity and its most important aspect is their composition.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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