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Aspects of the biology and preliminary assessment of DNA quality and quantity of two cichlids from Lagos Lagoon, Nigeria

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ABSTRACT

Aspects of the growth pattern, reproductive biology, and molecular characteristics of two cichlids, Sarotherodon melanotheron and Coptodon zillii were investigated in Lagos Lagoon. A total of twenty-nine samples were collected from the Lagos Lagoon. Blood samples were obtained for DNA extraction and analysis from 9 specimens of each species. The morphometric data of S. melanotheron varied from 2.5-4.5cm (head length), 13.5-18.0cm (total length) and 35.00-135.00g (body weight), while that of C. zillii were 2.0-4.5cm, 12.0-21.5cm and 39.00-228.00g respectively. The growth pattern of S. melanotheron showed a positive allometric, b= 3.324, while that of C. zillii showed a negative allometric pattern (b= 2.715). The condition factors of S. melanotheron and C. zillii ranged from 4.15-4.55 and 4.12-5.07 respectively. The sex ratio for S. melanotheron (males, 11; females 2) was 1: 0.18, while C. zillii (males, 12; females 4) had a sex ratio of 1:0.33, and these were statistically significant (p< 0.05). From the molecular characterization analysis, the DNA concentration ranged from 374-765 ng/µl and 48-1265 ng/µl, while DNA purity ranged from 1.56-1.82 and 1.49-1.81, for S. melanotheron and C. zilli respectively. These findings form baseline information for conducting further investigations on the taxonomy, ecology, conservation of the genetic purity of these fisheries resources in Lagos Lagoon.

Introduction

Cichlids are a group of bony fishes belonging to the family Cichlidae, in the order Perciformes and suborder Labroidei (Trewavas, 1983). This family is very diverse with 2500 species of which 1300 species belonging to 220 genera have been scientifically identified (Kullander, 1998; Froese and Pauly, 2007). Much scientific interest has been shown in the cichlid fishes due to their rapid adaptive radiation and this has culminated into an elaborate ecological diversity, an indication of their great importance in tropical and subtropical aquaculture (Poletto *et al.,* 2010).

Several works of literature are available on aspects of ecology and biology of cichlid species (Fagade, 1982; Ikomi and Jessa, 2003) age, growth and mortality (Faunce *et al.*, 2002), condition factor (Fagade, 1983; Arawomo, 1982; Anene, 2005) among others. There are three genera of aquacultural importance: *Oreochromis niloticus*, *Sarotherodon melanotheron*, and *Coptodon guineensis*; and these inhabit fresh and brackish waters (Li *et al.,* 2011). Fish stocks have been identified with the use of biometric characters such as morphometric measurement and meristic count (Turin *et al.*, 2004) which are the easiest methods of identifying species. Of great significance in phylogenetics is the study of differences and variability in biometric characters of fish stocks and thus provides basic information for later studies on fish stock genetic improvement (Olufeagba *et al.,* 2015). With the establishment of techniques that specifically verify biochemical or molecular genetic variation, the orthodox methods still have an important role in identifying stock till the present times (Swain and Foote, 1999). However, for effective management of populations, there is a need to understand fish taxonomic identity and this has

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been approached using morphometric and meristic analysis (Pante *et al*., 1988; Kuton and Kusemiju, 2010; Muchlisin, 2013; Batubara *et al*., 2018); allozyme electrophoretic analysis (Macaranas *et al*., 1986; Lee and Kocher, 1996; Ahmed *et al*., 2004); serum protein analysis (Avtalion *et al*., 1976); immunology and agglutination assays, mitochondria DNA restriction analysis (Seyoum, 1989; Seyoum and Kornfield, 1992; Jamsari *et al*., 2010; Muchlisin *et al*., 2013; Farhana *et al*., 2018); karyotype analysis (Crosetti *et al*., 1988); DNA fingerprinting (Harris *et al*., 1991) and DNA microsatellite analysis (Lee and Kocher, 1998; McConnell *et al*., 2000; Bo-young Lee *et al*., 2005).

The goal of this study was to assess the morphological features of different cichlids, *C. zillii*, and *S. melanotheron,* in Lagos Lagoon by using a combination of both biometric and molecular techniques in characterizing the species; this will serve as a baseline study for future research on the cichlids.

Figure 1. Map of Lagos showing the Lagos Lagoon (Source: Soyinka et al., 2020).

Materials and Methods

Brief information on the area of study

The Lagos lagoon is part of a larger system of lagoons and creeks that run the length of Nigeria's coast, from the Republic of Benin's border to the Niger Delta. It is located at longitude 3°20' and 3°50' W and latitude 6°24' and 6°36'.N. It runs for about 257 kilometers from Cotonou, Benin Republic, to the western fringe of the Niger Delta. The Lagos lagoon is the biggest of the Gulf of Guinea's four lagoon complexes. (Emmanuel *et al*., 2008; Soyinka and Kassem, 2008) and also the largest of the ten lagoons that make up the lagoon systems in Nigeria. The lagoon supports artisanal fishing. It is more exposed to anthropogenic activities. This lagoon also functions as an estuary which is attributed to seasonal changes in salinity (Olaniyan 1957, Hill and Webb 1958). The lagoon is polluted by direct discharge of waste (industrial or domestic waste) and also the discharge of inland waters (rivers, creeks, streams, etc.) into the lagoon at different points.

Field studies

Collection of specimen

A total of 29 specimens of *C. zillii* and *S. melanotheron* (Figure 2) used for the study were obtained from Lagos Lagoon. The specimens were collected alive with cast nets at the Marine Sciences jetty, University of Lagos Lagoon Front. The blood of each specimen was extracted with a syringe and needle from the gill vessels under the right operculum; the blood samples were kept in EDTA bottles. Both the blood samples and the fish were kept in ice-chest to preserve them. The fishes were transferred later into the deep freezer (temperature –20°C) in the Marine Research Laboratory, University of Lagos, while the blood samples were preserved in a refrigerator for 24 hours and light was stable throughout.

Physical and chemical parameters

During the collection of specimens, the physical and chemical parameters of water were measured. The water temperature was determined with the aid of a mercury-in-glass thermometer; the dissolved oxygen was measured with Jenway DO Meter (Model4310), the salinity was measured with a salinity Refractometer (BIO MARINE Aqua fauna Model) and the hydrogen-ion concentration was determined using Jenway pH meter Hanna.

Figure 2. (a) *Coptodon zillii*, (b) *Sarotherodon melanotheron* from Lagos Lagoon.

Laboratory procedures

The fishes were removed from the deep freezer to thaw. Upon thawing some measurements were taken.

Length and Weight Measurement

The measurement of the fish's total weight and lengths was done using the methods described in Soyinka (2011).

Age and growth pattern analysis

Length-Frequency Distribution: This was utilized to know the age of the species. The percentage frequency was plotted against the size range of standard length.

Length-Weight Relationship: This was used to calculate the fish's rate of growth. It is represented by this equation:

$$W=aL^b$$

Where W= weight in grams (g); L= length (cm); a= regression constant; b= regression co-efficient.

According to Tesch (1968) cited by Froese (2006), the value of b is normally between 2 and 4 in most cases. When *b* is 3, the fish's growth is said to be isometric. When b is less than 3, there is negative allometric growth, and when b is larger than 3, there is positive allometric growth. The equation was further converted to a linear relationship:

$$\text{Log } W = \log a + b \log L$$

Condition factor (K)

The condition factor was determined according to Froese (2006) by the equation:

$$K = \frac{100W}{L^b} \text{ or } \frac{100W}{L^3}$$

Where K = condition factor; L = standard length (cm); W = weight (g); b = regression coefficient.

Reproduction studies

Sex Determination: This was done using the method described in Soyinka (2017)

Sex ratio: It is the male to female ratio population.

Sex ratio = M: F = M/M: F/M

Chi-square test: It is used to test if there is significant variation in observed differences in the sex ratio because there is an expected 1: 1 male to female ratio in nature.

$$\chi 2\ (1\ \text{d.f.}, 0.5\%) = \frac{(\text{male observed} - \text{male expected})^2}{\text{Male expected}} + \frac{(\text{female observed} - \text{male expected})^2}{\text{male expected}}$$

(As described in Soyinka, 2010)

Molecular studies

Sample Labeling: Identification of the fish used in DNA isolation is necessary since it helps to identify a particular fish and the location of the DNA sample during storage. This represents the numbers or alphabets commonly used in bioinformatics as a particular identifier given to a DNA or protein sequence record to give room for tracking or knowing the sample (Koonin and Galperin, 2003)

DNA extraction: This was done using quick-DNA™ Universal kit (2R D4068) manufactured by Zymo Research, following the procedures as specified by the manufacturer, following these six steps: 1) tissue disruption/homogenization, 2) cell lysis in DNA extraction buffer, 3) separation of DNA from other cellular components, 4) DNA precipitation, 5) DNA washing, and 6) DNA collection/resuspension for downstream processing.

Spectrophotometric Analysis: This involves measuring the concentrations and relative absorbance of the DNA solutions extracted from the fish. It is employed to ascertain the purity and concentration of a given DNA sample. A micropipette was used to measure 95µl of distilled water into a cuvette for blank checking, followed by the addition of 5 µl of DNA sample and mixed thoroughly without having bubbles. Then cuvette was placed in the Bio-photometer to determine the concentration of the sample, the relative absorbance at a different wavelength (230 nm, 260nm, 280 nm, and 340 nm respectively) and the absorbance ratio (at wavelength 260 nm to 280 nm) were determined and recorded for each of the samples.

Loading Dye and Buffer: One Tag Quick-Load EZ-VISION© Blue Light DNA Dye was used.

Gel Electrophoresis: This involves checking the quality of DNA samples and it was achieved using 1% Agarose gel.

Preparation of Agarose gel: 1% Agarose gel was prepared by measuring 1.00g of Agarose (CSL-AG 100) into a beaker. Add 100ml of 1X TBE buffer. Put it in the microwave to dissolve the solution for some minutes (1-2 minutes). Allow the solution to cool down before adding 4 µl of Ethidium Bromide and swirl to mix. The quantity of the DNA samples determines the quantity of Agarose gel to prepare.

Set-up of tanks and comb for electrophoresis

The gel was poured into a gel tray containing combs and allowed to stand for at least 30 minutes for the gel to solidify. Rubber stoppers were placed at each end to stop the liquid from pouring away. The combs and stoppers were removed after the gel has solidified and the tray was transferred into the electrophoresis tank flooded with 1X TBE buffer. A micropipette was used to measure 3 µl of loading dye and 5 µl of DNA sample on a parafilm paper. The mixtures of both the DNA and the loading dye were loaded into a well on the gel and this was run for 1 hour at 110 amps and 60 V. The trays are then moved to the UVidoc system. The quality of the DNA was then photographed under ultraviolet light using the UVI doc system.

Results

Age and growth pattern

For *S. melanotheron*, based on thirteen specimens from the Lagos lagoon, the standard length was from 9.5 to 14.0 cm. The length-frequency distribution is illustrated in Figure 3 and it reflected only one age group in the sampled population. The length-frequency distribution of the sixteen specimens of *C. zillii* with standard lengths varied from 9.4 to 16.5 cm is illustrated in Figure 4 and it reflected approximately two age groups in the sampled population.

The total weight of *S. melanotheron* ranged from 35.0 - 135.0 g. Table 3 and the log length – log weight relationship is shown in Figure 5, while that of *C. zillii*, with a total weight range of 39.0 - 228.0 g is shown in Figure 6.

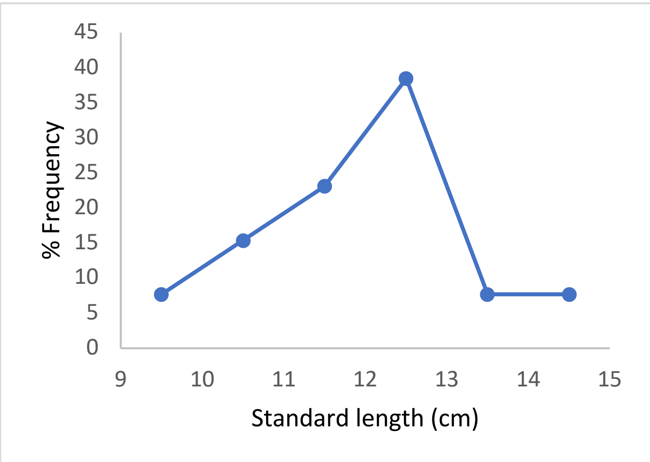

Figure 3. Standard-length frequency distribution of *S. melanotheron* in Lagos Lagoon

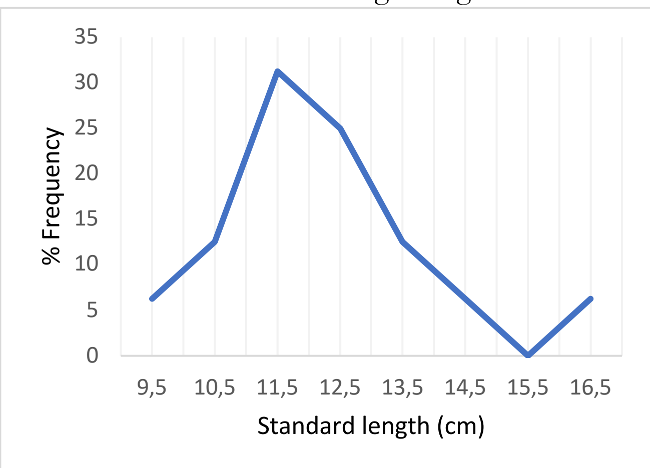

Figure 4. Standard Length -frequency distribution of *C. zillii* in Lagos Lagoon

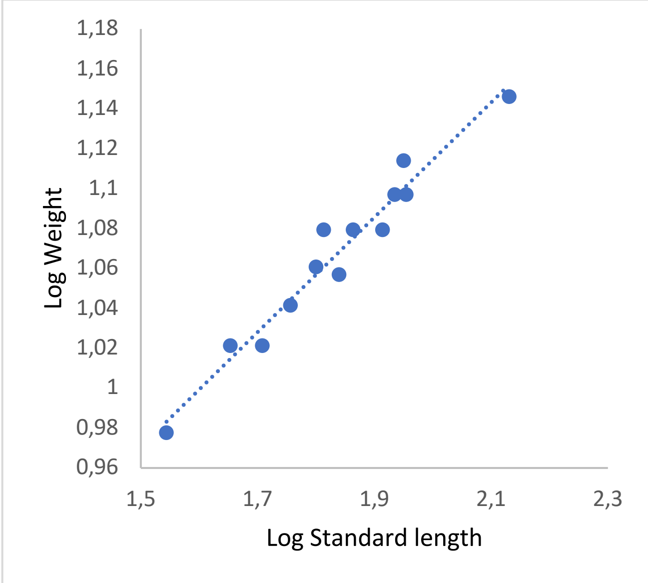

Figure 5. Log standard length-Log weight relationship of *Sarotherodon melanotheron* in Lagos Lagoon

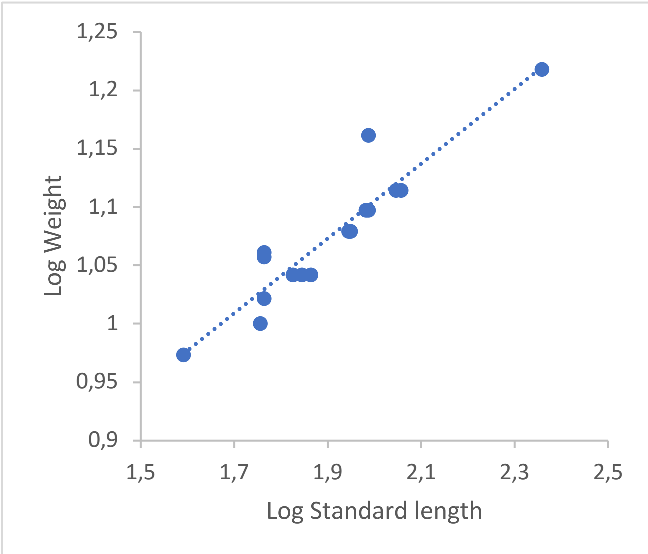

Figure 6. Log Standard length-Log weight relationship of *C. zillii* in Lagos Lagoon.

The equation of the Log length – Log weight relationship for the two cichlids were:

Log W = Log 1.7118 + 3.3244 Log L (n = 13; r = 0.9566) ....*S. melanotheron*

Log W = Log 1.0126 + 2.7149 Log L (n = 16; r = 0.8688) .... *C. zillii*

Condition factor

The condition factor of *S. melanotheron* was from 4.15 - 4.55, while that of *C. zillii* varied from 4.12 - 5.07 as shown in Table 1 and Table 2.

Table 1. Condition factor of *S. melanotheron* in Lagos Lagoon.

	Range (cm)	Average length (cm)	Average weight (g)	Condition factor (k)
Small-sized fish	8.5-10.4	10.17	43.67	4.15
Medium-sized fish	10.5-13.4	11.86	73.13	4.38
Large-sized fish	13.5-15.4	13.50	112.00	4.55

Table 2. Condition factor of *C. zillii* in Lagos Lagoon.

	Range (cm)	Average length (cm)	Average weight (g)	Condition factor (k)
Small-sized fish	8.5-12.4	10.73	60.00	4.86
Medium-sized fish	12.5-15.4	12.79	86.29	4.12
Large-sized fish	15.5-16.5	16.50	228.00	5.07

Table 3. Raw data showing DNA purity and DNA concentration of the cichlids from Lagos Lagoon

Sample	230	260	280	340	Purity 260/280	Conc.
TT1	1.061	0.458	0.259	0.021	1.77	504
TT2	0.705	0.291	0.161	0.003	1.81	320
TT3	0.697	0.951	0.562	0.076	1.69	1046
TT4	0.362	0.391	0.219	0.009	1.79	431
TT5	0.878	1.15	0.693	0.019	1.66	1265
TT6	0.12	0.044	0.029	0.009	1.49	48
TT7	0.511	0.695	0.382	0.004	1.82	765
TT8	0.558	0.857	0.551	0.1	1.56	943
TT9	1.069	0.34	0.197	0.007	1.73	374

Chi-Square Calculations

A chi-square test for both species indicated a significant difference ($p<0.05$) from the expected 1:1 ratio.

Chi-square calculation for *Sarotherodon melanotheron* was $\chi^2_{(1\ d.f.,\ 0.5\%)}$ cal = 6.2 (It is statistically significant)

Chi-square calculation for *C. zillii* was $\chi^2_{(1\ d.f.,\ 0.5\%)}$ cal = 4 (It is statistically significant).

DNA extraction

Table 3 below showed the different labeling assigned to the blood samples collected from different species which were eventually used for DNA isolation. TT1 – TT6 represent *C. zilli,* while TT7 – TT9 represent *S. melanotheron.*

Spectrophotometric analysis

DNA purity and concentration

DNA purity is the absorbance at 260/280 that is, A260/280, *S. melanotheron* and *C. zillii* from the Lagos Lagoon had a mean DNA purity ranged from 1.49-1.82 and a mean DNA concentration ranged from 48-1265ng/µl. Table 3 shows the DNA concentration and purity at various absorbance levels or wavelengths.

Gel electrophoresis: The quality of the DNA samples is shown in Figures 7 and Figure 8.

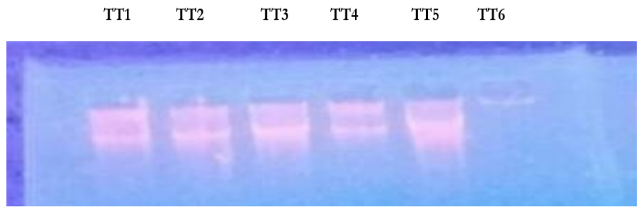

Figure 7. Gel profile of DNA samples of Cichlid, *C. zilli (*TT1- TT6).

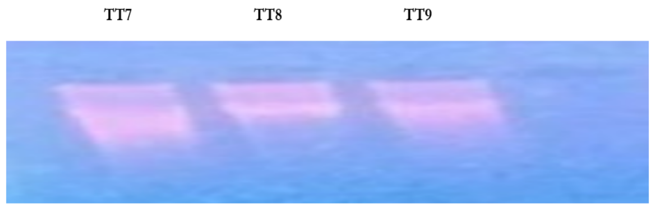

Figure 8. Gel profile of DNA samples of Cichlid, *S. melanotheron,* (TT7- TT9).

Discussion

The present investigation on aspects of the biology of the two common cichlids of interest in the Lagos Lagoon is a reflection of the endemic nature and the potentials of both species as a mainstay of the capture and culture fisheries occurring on the Lagos Lagoon

Although only one or two age groups were sampled from the population of *S. melanotheron* and *C. zillii* respectively, it is certain that both species are native residents in the Lagos Lagoon, occurring throughout the year. Several authors have reported their occurrences in the lagoon throughout the year (Fagade and Olaniyan, 1974; Ugwumba, 1988; Ayoola and Kuton, 2009; Soyinka *et al,* 2020). These cichlids though occupy a similar ecological niche, despite this, morphometric measurements differed by fish species, gender, and size. (Marcus, 1982; Ugwumba, 1990).

S. melanotheron had a positive allometric growth pattern while *C. zillii* had a negative allometric growth pattern in the present investigation. Soyinka and Ojo (2015) however recorded a negative allometric growth pattern for *S. melanotheron* in the same lagoon, while Famoofo and Abdul (2020) recorded positive allometric growth in *S. melanotheron* and *C. zillii* from Iwopin, Lekki Lagoon, Nigeria. According to Thomas *et al.* (2003), most aquatic species change shape as they grow, therefore divergence from isometric growth is common. The productivity of the organisms' immediate surroundings has a big influence on the direction and degree of deviation of b. Positive allometric growth is more common in highly productive zones, whereas negative allometric growth is more common in low productive zones, such as the deep-sea zone (Prasad, 2001; Thomas *et al.*, 2003). A negative allometric growth pattern in fish, according to Adeyemi *et al.* (2009), means that the weight increases at a slower pace than the cube of the body length.

The condition factor of two cichlids in the Lagos Lagoon was high. Stress, sex, size, season, availability of food, and other water quality elements can all contribute to differences in condition factors (Khallaf *et al.*, 2003); and these factors could has been the cause of the high and slight variations in the condition factor of these cichlids when compared with values ranging from 1.60 – 2.06 reported by some authors (Olopade, *et al.,* 2015). Braga and Gennari (1990) stated that a low K-factor indicates a period when accumulated fat is used for spawning, whereas a high K-factor indicates a period of significant rate of eating followed by a progressive increase in stored fat, indicating preparation for a new reproductive cycle. The K-factor was shown to be strongly linked to a fish reproductive cycle by Ugwumba, (1990) and Abaoba, (1993). Despite the reports indicating that the Lagos Lagoon has been under intensifying pollution with various contaminants such as sewage, industrial effluents, refuse, petrochemicals, and sawdust from adjourning lands, the two species thrive successfully and in good conditions as reflected in the high condition factors obtained in the present study. This also agreed with past reports on the species (Meye, 2012) and in which very high condition factor values as high as 30.25 were recorded (Famoofo and Abdul, 2020).

In both species, the male was significantly more than the female. The expected sex ratio in nature is 1:1. However, chi-square is used to calculate significant observed differences. The male specimens in both species were statistically more abundant than the females in the present study, but this could have resulted from the small sampled size of the population in the present study.

A population cannot evolve and adapt to environmental change without genetic diversity. In studying genetic diversity, good quality and pure DNA is important because it contains all the genes that the cell will ever need for making all the structures and chemicals necessary for life (Templeton *et al.*, 2001). Samples of good quality are expected to have absorbance ratio A260/A280 to lie in the range of 1.6 – 2.1 (Templeton *et al.*, 2001).

Ali *et al.* (2004) reported the usefulness of DNA polymorphism as a means of accessing genetic diversity of similarity in an aquatic organism. Omoniyi and Agbon (2008) suggested that a biochemical analysis of the species of *S. melanotheron* should be conducted to verify the genetic basis of differences observed in the Lagos Lagoon and freshwater populations before a selective breeding program for the species could be carried out. DNA purity is the absorbance at 260/280 wavelengths using a double-stranded DNA (dsDNA) in a spectrophotometer at 5-50µl. If the values are approximately 1.8, the DNA is said to be pure but if the values are less than 1.8, the DNA is said to be impure with contaminants (Wilfinger *et. al.,* 1997; Koetsier and Cantor, 2019). From Table 3 and based on the assertion of Templeton *et al.* (2001), it showed that most of the samples in this study have DNA purity values > 1.6 and are clean, with the exception of TT6 (1.49) being < 1.6 which is relatively impure probably due to the presence of impurities such as protein, phenols, or other contaminants that have strong absorbance near 280 nm (Wilfinger *et. al.,* 1997). The impure state of this sample is also evident in the gel picture of Plate 3.and will need to be purified further. However, the other pure extracts are good enough for further DNA analyses.

Typical yields are 3–30 ng/µl of high-quality DNA, depending on the samples used (Abdel-Latif *et al.*, 2017). The DNA yield in this study ranges from 48 to 1265 ng/µl (Table 3), which is well above the minimum standard. The implication here is, despite the contaminants in some samples, the quality and quantity of the isolated genomic DNA is still very high and will give better and reliable results after been subjected to different downstream analyses.

Conclusions

The phenotypic characteristics of the cichlid, *S. melanotheron,* and *O. niloticus*, the DNA quality, and quantity of the species provide baseline information for the monitoring of the Lagos Lagoon's fish stock. Further studies should be carried out on other cichlids in the lagoon to have comprehensive information on the genomics of the cichlid populations in the lagoon.

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