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Analysis on fish kill along University of Lagos Lagoon beach, Southwestern, Nigeria

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Abstract

Investigation on the mass mortality of fishes (*Sarotherodon melanotheron*) along the University of Lagos Beach Off Lagos Lagoon was carried out for six months (February to July 2016). Monthly collection of water and fish samples were done, Physio-chemical analysis of the water samples and biological analysis (mycological inclusive) of the collected fish samples were also carried out. The highest number of fish kills was recorded in February (35) and the lowest number (3) was recorded in July. The mean values for the physico-chemical characteristics in the three stations included: air temperature 24.7±0.55ºC; water temperature 27.1±0.3ºC; salinity 24.7±1.3‰; pH 6.7±0.1; dissolved oxygen 5.2±0.3mg/L; and hardness 10296±3962mg/L. The changes in water temperature (25.5ºC – 29ºC), D.O (2 – 6.6mg/L), salinity (19 ‰ – 29 ‰) and hardness (148 – 25000mg/L) affected the number of fish mortality in Lagos Lagoon. It was noted that the number of fish mortalities increased with increasing water temperature, salinity, and hardness. Mycological examination of infected fishes revealed the presence and loads of two fungal species namely: *Saprolegnia sp.* and *Pythium insidiosum.* The parasitic examination revealed the presence of the protozoan (*Trichodina sp.* and *Myxobolus sp.*) and platyhelminths parasites (*Diplyllobothrium latum*). *Diphyllobothrium latum* had the highest prevalence (0.9). It was concluded that there's need for further studies to elaborate these findings.

Key words: Fish mortality, Fungi, Parasites, Water chemistry.

Introduction

A fish kill can be described as any sudden and unexpected mass mortality of wild or cultured fish (Lugg, 2000). It can also be said to be the localized mass die offs of fish that can occur in marine, estuarine, or freshwaters (Meyer and Barclay, 1990). It is often characterized by a large number of fishes dying over a short period of time within a defined area (Kibria, 2011). When large number of fishes are found dead in natural waters it can indicate poor water quality and environmental health (Klemm *et al.,* 1993). A fish kill event could provide important information on the spatial and temporal distributions of pollutants and problems such as hypoxia in aquatic environments (Kibria, 2011).

Fish kill can be caused by a variety of environmental variables, including changes in salinity, temperature, acidity levels, dissolved oxygen levels, and severe algae development. Natural occurrences like these are a necessary component of the life cycle of the concerned species. Pesticides, pollutants, sewage, parasites, and pathogens all contribute to seasonal illness outbreaks in fish populations (Amiye and Erondu, 2010). Occasionally, fish are killed by mechanical injuries from turbines, pumps or underwater explosions which produce fatal internal

injuries and usually arise when fish are found in the immediate areas where the turbines are located or in the area of the blast in the case of explosions (La and Cooke, 2011).Three main factors that act synergistically in most fish kills are changes in water quality, disease pathogens and introduction of toxicants or pollutants. Although, a single factor in the absence of other factors could also result in a fish kill (Lugg, 2000).

Changes in water quality causes stress on fishes, very high level of stress reduces the immune system of the weaker fish and making the fish more susceptible to diseases (Coutant, 1990). Changes in the physical characteristics such as temperature could result in fish mortality. Fishes are obligate poikilotherms their body temperature fluctuates with that of their immediate environment (Coutant, 1990) so, rapid fluctuations or seasonal variations in water temperature could cause fish deaths. Rapid fluctuations can be due to human activities such as discharge of cooling waters from electric power generators. Thermal stress weakens fish and makes the m susceptible to diseases (Brett, 1971) it was further reported that fish could die as a result of low temperatures and in this case, it is usually termed 'cold kills'.

Changes in chemical characteristics could also be a contributor to fish kills. Depletion of oxygen can occur when there's excessive BOD (biological oxygen demand) and the COD (chemical oxygen demand) that exceeds the production from photosynthesis by aquatic plants diffusion from the atmosphere (Meyer and Barclay, 1990). Coldwater fish require 6 mg/L while warm water fish require 5 mg/L. When oxygen levels reduce to 1-2 mg/L for a few hours it can result in large fish kills (Cleveland and Grabble, 1998).
Despite all these reports, there's very little information on fish kills in Africa, especially in Nigeria particularly in relation to microbial loads (fungi) and parasites. The study is aimed at giving information on fish kill in relation to fungi, parasites attacks, and water characteristics in Lagos Lagoon.

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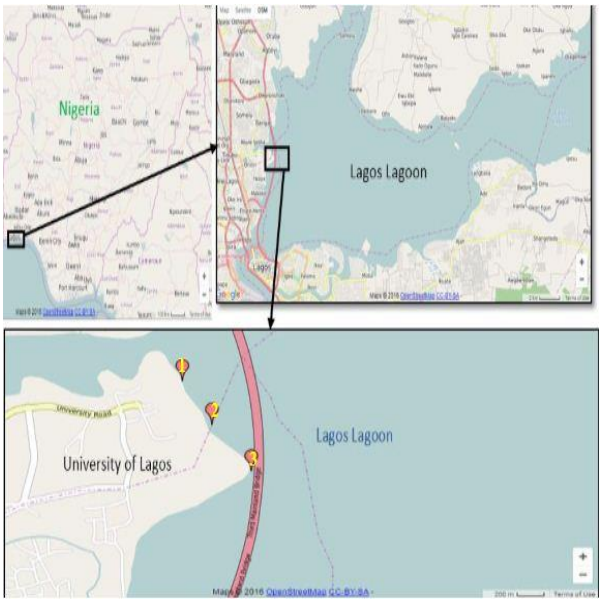
Materials and Methods

Description of study site

The University of Lagos Lagoon Beach (LLB) (Fig. 1) is part of the Lagos lagoon which is a continuous system of lagoons and creeks found along the coast of Nigeria from the border between Republic of Benin and Ondo State. It is found in the Northwestern parts of the Lagos lagoon on latitude 6°31'08.2 N and longitude 3.24'10.7" E (Solarin, 1998). Characteristically, being part of the Lagos lagoon, it has a seasonal fluctuation in salinity and high brackish water during the dry season (December – May), while freshwater condition exists in the rainy season (June – November) (Ugwumba and Kusemiju, 1992; Solarin, 1998). Lagos Lagoon receives freshwater from Lekki Lagoon via Epe Lagoon in the North-east, and discharges from Majidun, Agboyi and Ogudu creeks as well as Ogun River in the North-west (Soyinka, 2008; Lawal-Are *et al.*, 2010). The Research study was carried out in three sampling stations along University of Lagos beach of Lagos lagoon.



Source: http://www.geoplaner.com
Figure 1: An overview of University of Lagos Lagoon beach showing the sampling stations

Collection of samples.

Collection of water samples

The University of Lagos area of Lagos lagoon was sampled for six months (February – July 2016) from the three stations {1 ($6^0$31.247'N, $3^0$24.047'E), 2 ($6^0$31.074'N, $3^0$24.154'E) and 3 ($6^0$30.891'N, $3^0$24.289'E)}. Water samples were collected twice a month using 75 cl plastic containers between February and July 2016 at the study stations. Sampling was carried out between 6.30 and 8.30 hours on each sampling day. The use of sterile bottles at 1 m depth into the water, water samples were collected and taken to the laboratory for physical and chemical analysis using the method of APHA (1998).

Collection of fish samples

Floating dead and dying samples of *Sarotherodon melanotheron* were collected at the study stations for six months between February and July 2016. Fish samples were collected using a cast net (33mm mesh size) and scoop net (20mm mesh size). The fish samples were put in polyethene bags labeled appropriately before transferred to the laboratory for immediate analysis.

Determination of physico – chemical parameters of collected water samples

Air temperature and surface water temperature were determined in-situ using mercury-in glass thermometer and the value was recorded in degree Celsius (^{0}C). The pH values were determined using Griffin pH meter (model 400). Salinity was determined using Salinity Meter (Hanna Instrument HI 98203). Dissolved oxygen was determined using Winkler's method and the water hardness was determined by titration.

Biological analysis

Total length (TL) and standard length (SL) of each fish were measured in centimeters (cm) using a fish measuring board. The weight of each fish was measured in grams (g) using a weighing balance (Camry EK 5055). The condition factor was calculated as described by Oni *et al.*, 1983:

Condition factor (K) = $100W/L^3$; Where, W = weight in grams and L = total length (cm).

Mycological examination (Isolation of fungi from collected fish samples)

Cuts were made from infected areas corresponding to the active zone of the lesion from the skin, the gills, fins, the intestine and the body of fish. These cuts were then left in a solution of 40 percent hypochlorite for 60 seconds and washed with distilled water twice. The cuts from the infected fishes were allowed to drip off water before lightly embedded on the prepared sabround dextrose agar (SDA). The seeding culture plates were incubated at 30 ^{0}C (Robert and Pihet, 2008). These plates were examined daily for any morphological characteristics.

Identification of isolated fungi

Pure culture of each isolates was obtained by using an inoculating loop to transfer a little portion of it to a prepared SDA in aseptic environment as described by Rhodes *et al.* (2016). In order to accurately identify these isolate pathogenic fungi, each specific type of colony morphology by gross appearance (topography, texture, and pigmentation) is noted. A little portion of the growth colony was teased with an inoculation needle and mount in a drop of lactophenol cotton blue on a clean microscope slide. Covered with a cover slip, this was squash with the butt of the inoculation needle and the excess fluid then blot off. The preparation was examined under a light microscope with an attached camera (Motic McCamera [2000] 2.0 mega-pixel digital coloured camera) connected to a compound computer, for the microscopic photography of the Fungi.

DNA extraction of the isolated fungi

The DNA extraction procedure by Zymo Research Bacterial/Fungi kit by Inqaba Biotec (South Africa) was adopted.

Agarose gel electrophoresis

One gramme of Agarose powder was weighed and poured into a conical flask, it was then dissolved with 100ml of TAE (Tris base salt, Acetic acid and EDTA) buffer solution. The solution was melted in a microwave oven and cooled after 2-3mins. About 15µL of ethidium bromide was added and the solution was poured into an arranged gel caster. It was allowed to solidify, and the comb was removed after it has solidified. The TAE buffer was poured

into the gel tank, and the gel-caster was poured into the buffer-filled gel tank. The loading dye was used to mix the sample and the sample was carefully loaded into the wells created by the comb. The DNA standard was loaded and the electrophoresis power-pack was connected. The gel was run at 100V for about 1hr 30mins. The DNA bands were viewed under UV light.

Polymerase chain reaction (PCR) and DNA sequence of the isolated fungi

The extracted DNA samples were contracted out to Macrogen Corp. Great Seneca Hwy. Rockville, MD, USA for the PCR and DNA sequence analysis. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is used to amplify the internal transcribed spacer (ITS) region of the rDNA. The PCR was performed using the universal primer pair ITS1-ITS4. The thermal cycler was programmed for 4 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 2 min at 58 °C and 1.5 min at 72 °C. The nucleotide sequences were determined automatically using the cycle sequencing protocol by the Big-Dye Terminator v3.1 Cycle Sequencing Kit in the ABI PRISM 377-DNA Sequencer.

Generated sequences homology was done using NCBI-BLAST (Basic Local Alignment Search Tool). This was used to compare the generated nucleotide sequences data that was produced from the fungal DNA extracted to those deposited at the Genbank and determine the statistical significance of matches.

Examination of parasites on collected fishes

Fishes were collected from each sampling station for the duration of sampling. Sexes were identified. The sex ratio, parasite prevalence and Fulton Condition Factor (K) were estimated using the formulae below;

Sex Ratio = (No of Male individuals) / (No of Female individuals)

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Prevalence = (Number of infected fish) / (Total number of fishes examined)

Fulton's condition factor: K ¼ W/L3 T · 10^6 (Wilson and Najmudeen, 2020).

Gill examination for parasites

The gill of the sampled specimen was extracted and dissected using a dissecting scissors, the dissected sample was placed on a microscopic glass slide and a drop of distilled water was added. The sample was covered using a cover slip then viewed under a compound microscope for parasitic prevalence as described by Omeji *et al.*, (2010); Bubu-Davies *et al.,* (2021) and Emere and Egbe, (2006). All helminths parasites found on the gills were collected, counted, recorded and fixed in 70 % alcohol.

Intestinal examination for parasites

Fishes were dissected, the alimentary canals were removed and cut into parts then put into saline water for parasites recovery. The intestines were further carefully slit open longitudinally to aid the emergence of gastrointestinal helminth parasites. The recognition of the worms was enhanced by the wriggling movements when they were emerging. The collected helminth parasites were fixed in 70% alcohol, counted and recorded. Also, the intestines were scraped into 70% alcohol using a scraper for the examination and identification of protozoan parasites.

Results

Mortality of fishes

The number of fishes' mortalities in three stations between February and July are shown in Table 1. Relatively more mortalities occurred in the months of February and March with station 2 having the highest in all months except in July and the species of fish affected most was *Sarotherodon melanotheron* among the cichlids

Table 1: Frequency of fish kills in three stations along University of Lagos beach of Lagos Lagoon (Feb. – Jul. 2016).

Station\Month	Feb.	Mar.	Apr.	May	Jun.	Jul.
St.1	30	27	20	12	5	4
St.2	35	30	23	15	7	3
St.3	20	18	15	10	5	3

Physico-chemical parameters:

Monthly variations in the physical and chemical parameters at the three stations between February and July 2016 are presented in Figures 2, 3 & 4. The air temperatures for the stations were fluctuating during the period of study ranging between 22.5 ºC and 27 ºC. The lowest temperature was recorded in July (station 3) and the highest temperatures recorded in April (station 3) and June (station 2). The mean air temperatures and standard deviation values were 25±1.26 ºC; 24.5±1.39 ºC; 24.5±1.70 ºC for station 1, 2 and 3 respectively. The water temperature was fairly constant during the study period ranging from 25.5 ºC to 29 ºC with the highest recorded in April (Station 2) and lowest in June (Station 1). The Mean water temperatures were 26.75±0.82 ºC; 27.58±1.07 ºC; 27.2±0.71 ºC for stations 1, 2 and 3 respectively.

The salinity values ranged between 19 $^{0}/_{00}$ and 29 $^{0}/_{00}$, with the highest value recorded in March (Station 2) and lowest value was recorded in July (Station 3). The mean values were 24.58 (Station 1); 24.25 (Station 2); 25.17 (Station 3) and standard deviation values were ±2.97 (Station 1); ±3.02 (Station 2); ±3.66 (Station 1). The pH ranged between 6.25 and 7 with lowest recorded in April (Station 3) and the highest in July (Station 2). The Mean values were 6.73±0.17, 6.69±0.2 and 6.68±0.25 in station 1, 2 and 3 respectively. Dissolved oxygen ranged between 2 and 6.6 mg/L. The highest was recorded in May (Station 1) while the lowest was recorded in April (Station 3), with mean value of 5.6±0.51 mg/L, 5.43±0.52 mg/L and 4.48±1.86 mg/L in station 1, 2 and 3 respectively. Hardness ranged between 148 and 25000 mg/L. The highest was recorded in February (Station 2) while the lowest was recorded in July (Station 1), with mean values of 10043±9450 mg/L, 11023±10586 mg/L and 9822± 9203 mg/L in station 1, 2 and 3 respectively.

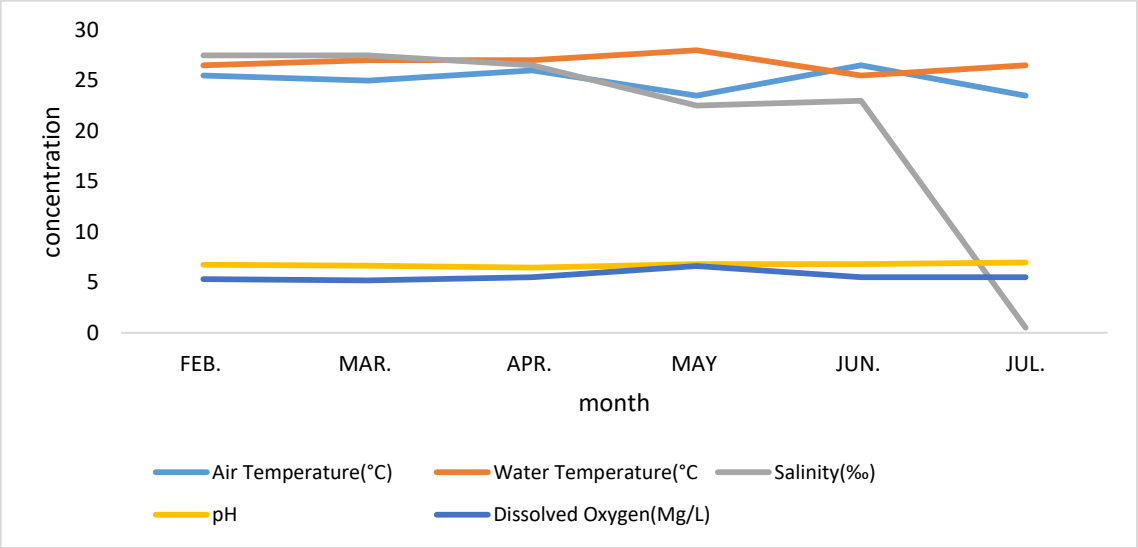

Fig. 2: Monthly variation of Physical and chemical parameters in station 1 in the Lagos Lagoon.

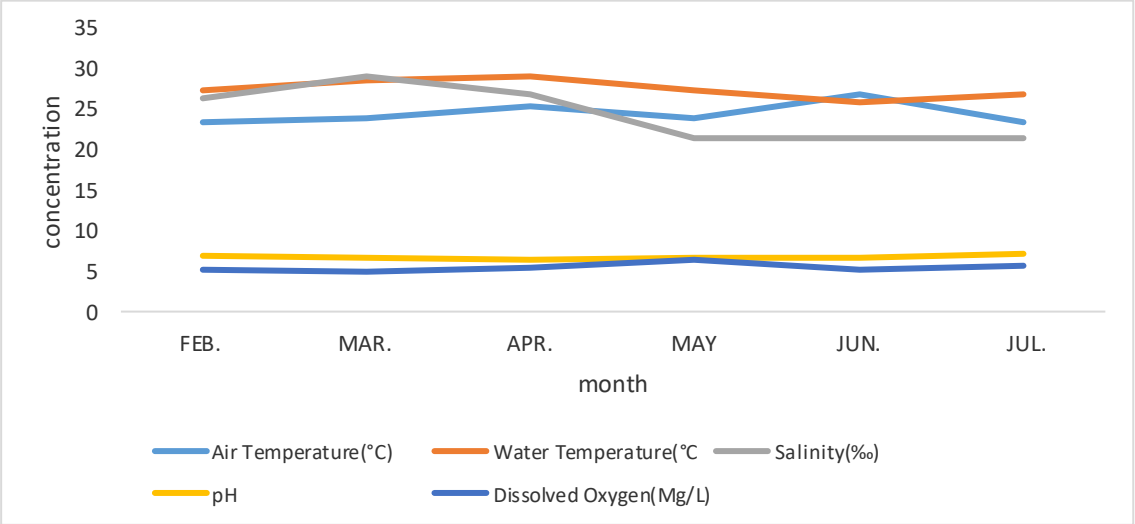

Fig. 3: Monthly variation of Physical and chemical parameters in station 2 in the Lagos Lagoon.

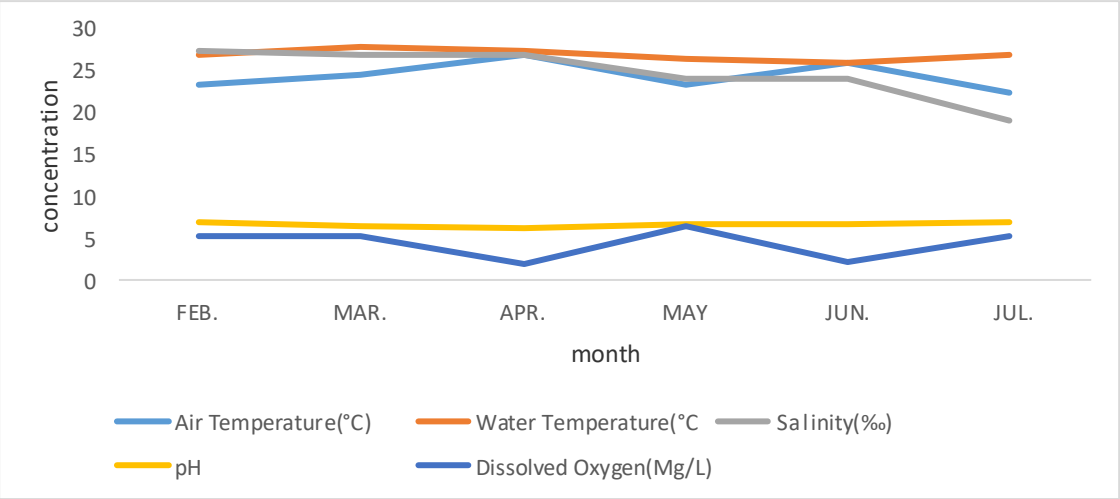

Fig. 4: Monthly variation of Physical and chemical parameters in station 3 in the Lagos Lagoon.

Fish Mortality and water quality in Lagos Lagoon

Mortality of fishes had a weak positive correlation with air temperature (r = 0.29), water temperature (r = 0.31), D.O (r = 0.31) and a strong positive correlation with salinity (r = 0.95), hardness (r = 0.97) while it showed strong negative correlation with pH (r = -0.64). `The relationship between the physico-chemical parameters and number of fish kills are presented in Table 2.

Biological analysis; Morphometric characteristics of fishes

The length of the fishes ranged from 6.3cm -15cm with an average weight of 46.15g (Table 3).

Table 2: Pearson correlation co-efficient (r) of physical and chemical parameters and number of fish kills along University of Lagos beach of Lagos Lagoon (Feb.–Jul. 2016).

PARAMETERS	1	2	3	4	5	6	7		PARAMETERS
1	0.81							1	Air Temp. (^{0}C)
2	-0.06	0.34						2	Water Temp. (^{0}C)
3	0.54	0.15	0.96					3	Salinity (‰)
4	-0.46	-0.23	-0.34	0.87				4	pH
5	-0.82	0.57	-0.15	0.53	0.38			5	DO (mg/l)
6	0.42	0.12	0.95	-0.67	-0.47	0.99		6	Hardness (mg/l)
7	0.29	0.31	0.95	-0.64	0.31	0.97	1.00	7	Number of Fish kills

Table 3: Mean monthly variation of length and weight of fishes.

Month	Total length(cm)	Standard length(cm)	Weight(g)
February	10.97	8.82	30.6
March	13.04	10.67	47.7
April	13.97	11.52	54.3
May	13.11	10.67	49.8
June	12.38	9.97	42.7
July	13.62	11.28	51.8

Mycological characterization of isolated fungi

After fourteen days of inoculation, two fungi species show some morphological features on the culture plates. Macroscopic features and microscopic character for identification; Macroscopically, the first fungal isolate is a slow growing fungus, with hard and elevated colony. This fungus is whitish at the initial stage but as it aged, turns brown which produce a kind of oil droplets as it aged and has a broad non-septate hypha. The second isolate, is also a slow growing fungus, with black pigmentation, the colony is elevated and stony. Filament is long with rounded ends, containing zoospores and coenocytic hyphae noted.

Extracted genomic DNA from the twelve fungal isolates showed intact bands which indicate extracted DNA is suitable for further downstream analysis. The purity of the extracted DNA was within the range of 1.8 - 2.0 as expected for pure DNA and agarose gels of PCR amplicons showed products ranging from 500 base pairs to 600 base pairs as expected for a successful amplification of the internally transcribed spacer regions of fungal species. The blasted ITS sequence data of the first isolated fungi in the NCBI-Genbank showed 99 % significant matched with *Pythium insidiosum* while the second isolates showed 98 % with *Saprolegnia species.*

Parasites in the affected fishes from Lagos Lagoon

Parasitic species recorded in 90 specimens analyzed are *Trichodina sp.* (protozoa) found on the gills, *Myxobolus sp.* (protozoa) and *Diphyllobothrium latum* (platyhelminths) found in the intestine as shown in Table 4. Out of the 90 specimen analyzed, a total of 50 (55.6%) specimens were infected with parasites. *Diphyllobothrium* was the most abundant parasite and was most prevalent in March (0.9). *Trichodina* was the least abundant and was least prevalent in April and May (0.1) as shown in Fig. 5. The condition factor (K) ranged from 3.64 - 4.67 in males and ranged from 3.1 - 4.92 in females as shown in Table 5.

Table 4: The species of parasites identified from specimens and the part of fish they were found

Species of parasite	Family of parasite	Site of infection
Trichodina sp.	Trichodinidae	Gill
Myxobolus sp.	Myxobolidae	Intestine
Diphyllobothrium latum	Diphyllobothriidae	Intestine

Table 5: Condition factor of fishes from Feb. - Jul. 2016

	Condition factor (K)		
Month	**Male**	**Female**	**Mean of combined sexes**
February	4.67	4.05	4.36
March	3.66	3.92	3.79
April	4.01	3.1	3.56
May	4.42	3.67	4.05
June	3.87	4.92	4.40

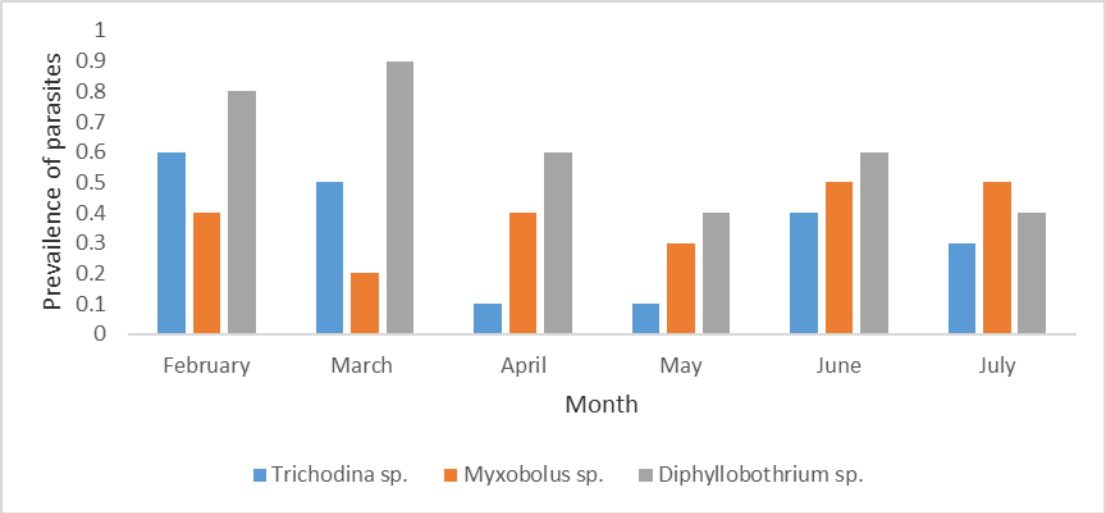

Fig 5: Prevalence of parasites with respect to months (Feb. – Jul. 2016)

Discussion

The physico-chemical changes observed indicates the influence of seasonal changes on the lagoon environment. The lowest values for air temperature, salinity, and hardness were recorded in the month of July while water temperature was lowest in June, which is the wet or rainy season in agreement with Nwankwo (2004). The highest values for air temperature and water temperature were recorded in April; salinity was highest in March, which is the dry season.

Air temperature values varied during the period of study. This is due to the season which is in agreement with Onyema (2008), that climatic conditions are key factors influencing air temperature in the tropics. The water temperature was fairly constant during the study period with the highest value (29ºC) recorded in April Station 2 and lowest value (25.5ºC) in June Station 1. This is also due to climatic factors and it agreed with the report of Onyema (2008) in the same environment. The salinity values were highest in March and lowest in July. According to Nwankwo (1996) the interaction between freshwater inflow and tidal seawater incursion determine the salinity of the Lagos lagoon environment every year. During the wet season according to Onyema *et al.*, (2003), there is increased river inflow which creates freshwater and low brackish water conditions in various parts of the lagoon. Also increased rainfall dilutes the water making it less saline (Nwankwo, 1993).According to Nwankwo (1984), the lagoon has a high pH which may be due to the buffering effect of the sea but in this study, the pH of the water was slightly acidic except in July at station 2 which has a neutral pH (7.0).

Dissolved oxygen ranged between 2 mg/L (May) and 6.6mg/L (April). Fish require a certain amount of oxygen in the water – at least 5 parts per million. Any lower than that, and they begin to have trouble; when dissolved oxygen levels fall below 2 ppm, an immediate fish kill will occur (Anderson, 2017). At

levels below 3 mg/L, most fish will not feed and will show signs of distress. They will often be at the surface of the water and appear to be pushing their noses out of the water (Anderson, 2017). Fish kills due to low oxygen are most common during hot, dry spells when algae grow and then die quickly. The organisms that decompose the dead algae may use so much oxygen that what remains is insufficient for fish (Swistock *et al.* 2006).
The highest value (25000 mg/l) for Hardness was recorded in February at Station 2 while the lowest value (148mg/l) was recorded in July at Station 1. Hardness was higher in dry than wet season as this may be attributed to reduced rainfall during this period. Water hardness affects fish health because it influences osmoregulation. Being open systems, fish are affected by the makeup of the surrounding water (Swistock *et al.,* 2006).

A weak positive correlation was recorded between water temperature and the number of fish kills. The water temperature didn't have much influence on the number of kills (Boyd and Tucker, 1998).
A weak positive correlation was recorded between DO and the number of fish kills. The relatively low DO level may have slightly influenced the number of fish kills. The optimum concentration for DO of warm waters is 5.0mg/l for fish (Lugg, 2000) and the average DO record was 5.27mg/l therefore the situation in the lagoon was not anoxic. Fish kills has been found to occur when water has a low pH (Kibria, 2011), the average pH was 6.7 which indicates mildly acidic environment. Different types of fish tolerate different pH levels but, in general, most fish will do better in ponds with a pH near 7.0 (Swistock *et al.,* 2006).

As levels of hardness decreases, mortality of fishes also decreased. Tovell *et al.,* (1974) showed that detergents were more toxic to goldfish and rainbow trout in hard water than soft water. According to Amaeze *et al.,* (2012) sewage and wastewater from the University, public toilets of neighboring communities are being discharged into the University of Lagos beach of the Lagos lagoon, and could be a source of detergents in the water. The toxicity of the detergents could have weakened the immune system of the fish. *Saprolegnia sp.* and *Pythium insidiosum* were fungi isolated from the species of fish *Sarotherodon melanotheron* in this study. It was noticed that beyond the month of March no fungal infection was observed, and fungi affected fish were only noticed on their skins. Species of *Saprolegnia* have been reported to cause mortality of fishes (Hatai and Hoshai, 1992). According to Chauhan, (2014) *Saprolegnia* infection of *Tilapia mossambicus* was responsible for mass mortalities in culture pond of the University campus in Bhopal. This agreed with the findings of this study as *Saprolegnia* infection caused the mortalities of the fish.

Pythium insidiosum is an oomycetes aquatic fungus that affects mainly tropical and subtropical waters (Gaastra *et al.,* 2010). Studies have revealed that some Pythium species have an impact on fish, but there is no evidence in the literature that *Pythium insidiosum* infects fish. According to studies, it can harm the cutaneous tissues of humans, dogs, and horses, leading to the potentially fatal disease pythiosis. (Mendoza *et al.* 1996) but this study identified it as a causative agent of fish kills in Lagos Lagoon. The DO was of optimum range, so it could have led to infestation of the fungi on fishes. Water quality has an impact on the diversity of fungus on fish (Hussein *et al.,* 2001). According to Sheila (2007), fungi use oxygen as they decompose dead organic matter in the stream. But the growth of *Saprolegnia* is promoted by poor water quality (Iqbal *et al.,* 2012).

During the period of study, the months where fungal infection were observed had the highest level of hardnesss which corresponds with the study by Barnes *et al.,* (2004) who observed that increased water hardness increased the growth of *Saprolegnia diclina* on *Cannabis sativa.* The condition factor (K) of the fishes ranged from 3.64 - 4.67 in males and ranged from 3.1 - 4.92 in females. According to Mireku *et al.,* (2016) the condition factor of *Sarotherodon melanotheron* in males ranged from 3.95 - 4.94 in males and ranged from 4.08 - 4.99 in females. The parasitic examination revealed the presence protozoan (*Trichodina sp.* and *Myxobolus sp.*) and platyhelminths parasites (*Diphyllobothrium latum*). This study showed that these parasites were more prevalent during the month of February and March which was the period of the kills. According Gbankoto *et al.*, (2001), increase in salinity makes fish host more sensitive to parasites by increasing

osmoregulatory cost. During the period of study, the parasites infestation was high during the dry season when salinity was high.

Conclusion

In conclusion, changes in water characteristics such as salinity and hardness cause stress to the fishes making them susceptible to the fungal attack. The increase in hardness could have also resulted in proliferation of fungal spores. The prevalence of parasites infestation weakened the immune system of the fish thereby reducing their resistance to fungal attack. The mass mortalities of fishes in February and March could likely may has been as a result of fungal attack.

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