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Occurrence of Myxosporidia of the Genus *Myxobolus* Bütschli, 1882 in the Gills and Intestines of Mugilidae Species from Lake Nokoue (Republic of Benin) and Lagos Lagoon (Nigeria)

ALADETOHUN, NIKE FUNMILAYO
Corresponding E-mail: adenike63@yahoo.com
Department of Fisheries Technology, Federal College of Fisheries and Marine Technology.

Abstract

The mullets (Mugiliformes: Mugilidae) have a worldwide distribution and inhabit tropical and temperate waters. Parasitic diseases of fish are very common all over the world. The mullet parasites studies about myxosporeans infecting worldwide mullets were considerably widened However, there have been reports of myxosporidea in different fish species there has not been much work done about *Myxobolus spp* in mullet, hence the study of myxosporea represents an important group of parasites infecting worldwide mullets. This study aims to examine the prevalence of Myxosporidia (*Myxobolusmyxosporean*gills and intestines of two Mugilidae (*Mugil Cephalus* and *Liza Falcipinnis*) from Lake Nokoue (Republic of Benin) and Lagos Lagoon (Nigeria). Three stations were selected for study within each area. Specimens of the two species of fish *Mugil cephalus* MC and *Liza falcipinnis* LF were collected from the Lagos lagoon (Nigeria) and Lake Nokoue lagoon (Republic of Benin) Fish samples were collected for nine months from June 2019 to February 2020 and examined for myxosporidean parasite, both in the dry and the rainy seasons. Data were analyzed using Chi-squared or Fisher's Exact test. The results obtained show Myxosporidia (*myxobolus sp*) infestations of gills and intestines in both species of the Mugilidae studied. The results revealed that the season and sex didn't impact the degree of LF and MC infestation by *Myxobolus sp* in Lake Nokoue and Lagos lagoon and Lake Nokoue and Lagos lagoon.

Keywords:
Mugilidae, Parasite, Lagos lagoon, Lake Nokoue, infestation

Introduction

Mullets are economically important species for aquaculture and commercial fisheries worldwide (Oren, 1981, Render *et al.*, 1995, Azien *et al.*, 2005). In Nigeria and the Republic of Benin, *Mugil cephalus* and *Liza falcipinnis* in the family Mugilidae, constitute an important proportion of the catches by artisanal or subsistence fishermen and are of high economic importance (Gnonhossou, 2006, Soyinka, 2008, Aladetohun *et al.*, 2013). Diseases and parasites affect fish in general by reducing their productivity and regeneration rate and causing mortality in fish farms and the natural environment, leading to economic loss

(De Kinkelin *et al*., 1985; Okaeme & Ibiwoye, 1989).

Myxobolus Bütschli, 1882 is the largest genus which contains over 450 of the 1700 species described in the phylum within the Myxospora (Myxozoa). Myxosporidians are pathogenic species that can cause high mortality in fish populations and are found in both wild and cultured fish. Note that the tissue damages of some species are pathways in the body of the fish for other pathogens such as viruses and bacteria that eventually kill the fish. Myxosporea represents one of the important groups of parasites infecting worldwide mullets (Kent et al. 2001). So far, a few revisionary studies of parasites infecting worldwide mullets have been conducted by Paperna (1975). Twelve species of Myxozoa were reviewed by Paperna and Overstreet (1981). The genera Sphaerospora, Henneguya, Myxidium, Myxosoma, Myxobolus, and Kudoa infecting mullets, were revisionary studied by Sitjà-Bobadilla and Alvarez-Pellitero (1994), Jajasri and Hoffman (1982), Landsberg and Lom (1991), Eiras 2002, Eiras *et al.,* (2005), and Moran et al. (1999). In the last decades, the geography of the mullet parasites studies and knowledge about myxosporeans infecting worldwide mullets were considerably widened However, there have been reports of myxosporidea in different fish species there has not much work done about *Myxobolus spp* in mullet

This study aims to examine the prevalence of Myxosporidia (*Myxobolus sp*) in the gills and intestines of two Mugilidae (*Mugil Cephalus* and *Liza Falcipinnis*) from Lake Nokoue (Republic of Benin) and Lagos Lagoon (Nigeria).

Materials and Methods

Lagos lagoon, the largest in the southern part of Nigeria is the largest of the lagoon systems of the West Africa sub-region. The lagoon lies between longitude 3°20’ and 3°40E and latitudes 6°154 and 6°40’N and has an area of 208km² (Soyinka, 2008). The Lagos lagoon extends eastward for about 200km from the Nigerian-Benin Republic border to the western limit of the transgressive mud coast Lagos lagoon (Fig. 1) has tropical climatic conditions with the rainy season from April through October and a dry season from November through March. Owing to the dynamics of river inflow and seawater incursion, the lagoon experiences brackish condition that is more discernible in the dry season. In the rainy season, the increased river inflow creates freshwater and low brackish conditions in various parts of the lagoon (Yakubu *et al.*, 2011).

Lac Nokoue (Fig. 2) is the largest lagoon in the Republic of Benin (Moreau, 2004). It is a shallow, sub-tropical coastal lagoon (60°25N, 2036E) with a surface of 150 km2 and stretches 20 km in its east-west direction by 11 km in the north-south direction (Laleye *et al.*, 2003). Lake Nokoue opens directly into the Atlantic Ocean through a channel at Cotonou which is about 24.5 km long. The name Nokoue Lake is the name given to the lagoon throughout history.

Three stations were selected within the Lagos lagoon for the study. The stations are Makoko (station 1) University of Lagos (station 2) and Mequin (station 3) (Fig. 1). The longitude and latitude of the sampling stations were measured using a Germain etrex G.P.S. device and the locations are presented in Table I.

Table I: GPS locations of sampling stations in Lagos lagoon

Lagos Lagoon	Longitude	Latitude
STATIONS		
Mequin	30°23’0.594”E	60°27’57.686”N
Makoko	30°23’56.829”E	60°29’34.733”N
Unilag	30°996”E	60°31’22.977”N

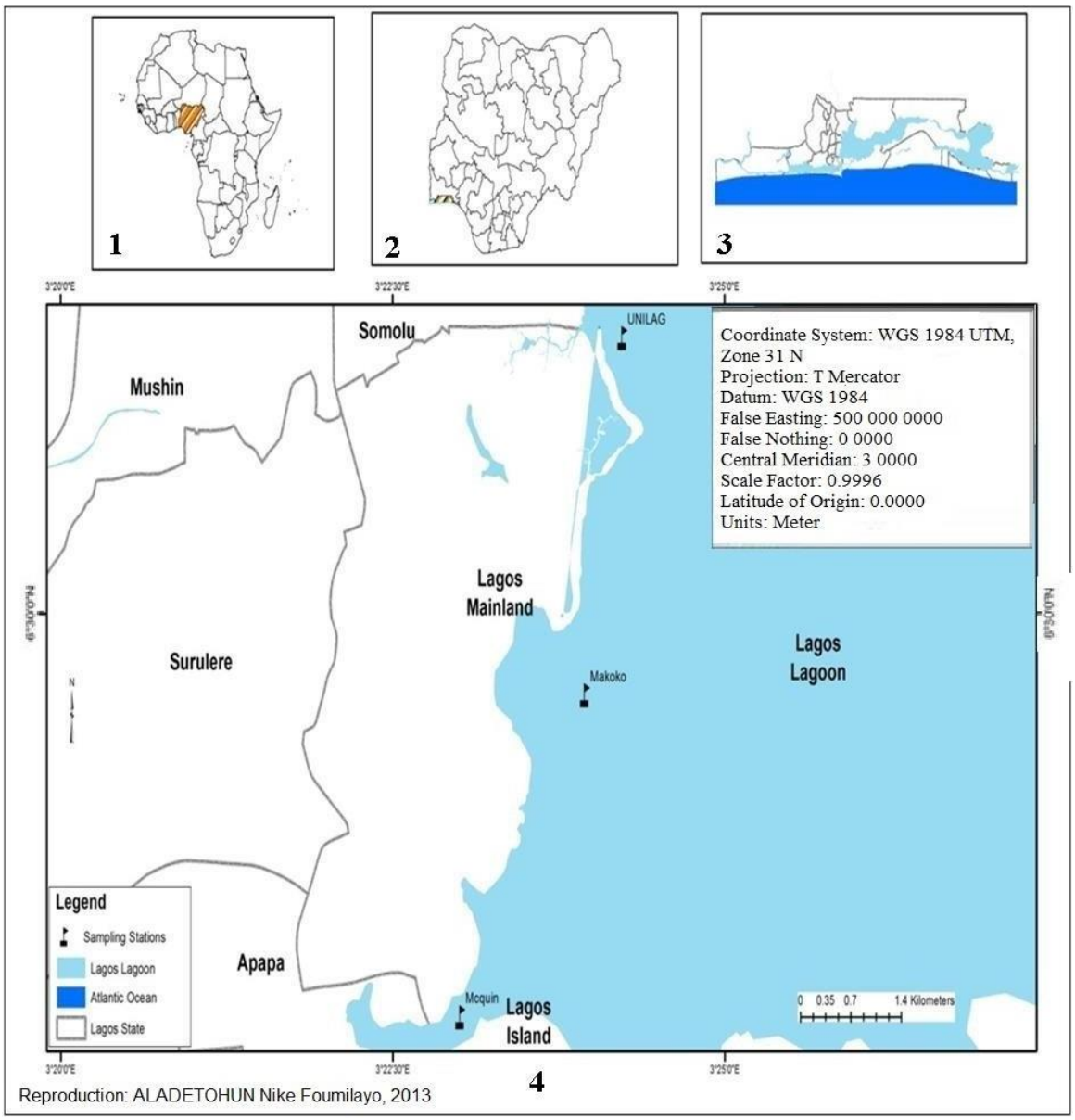

Figure 1: Map of Lagos lagoon (Nigeria), showing the sampling sites
Keys 1: Nigeria in Africa; 2: map of Nigéria; 3: river system in southern Nigeria; 4: study area in Nigeria

Site 2: Lake Nokoue (Republic of Benin)

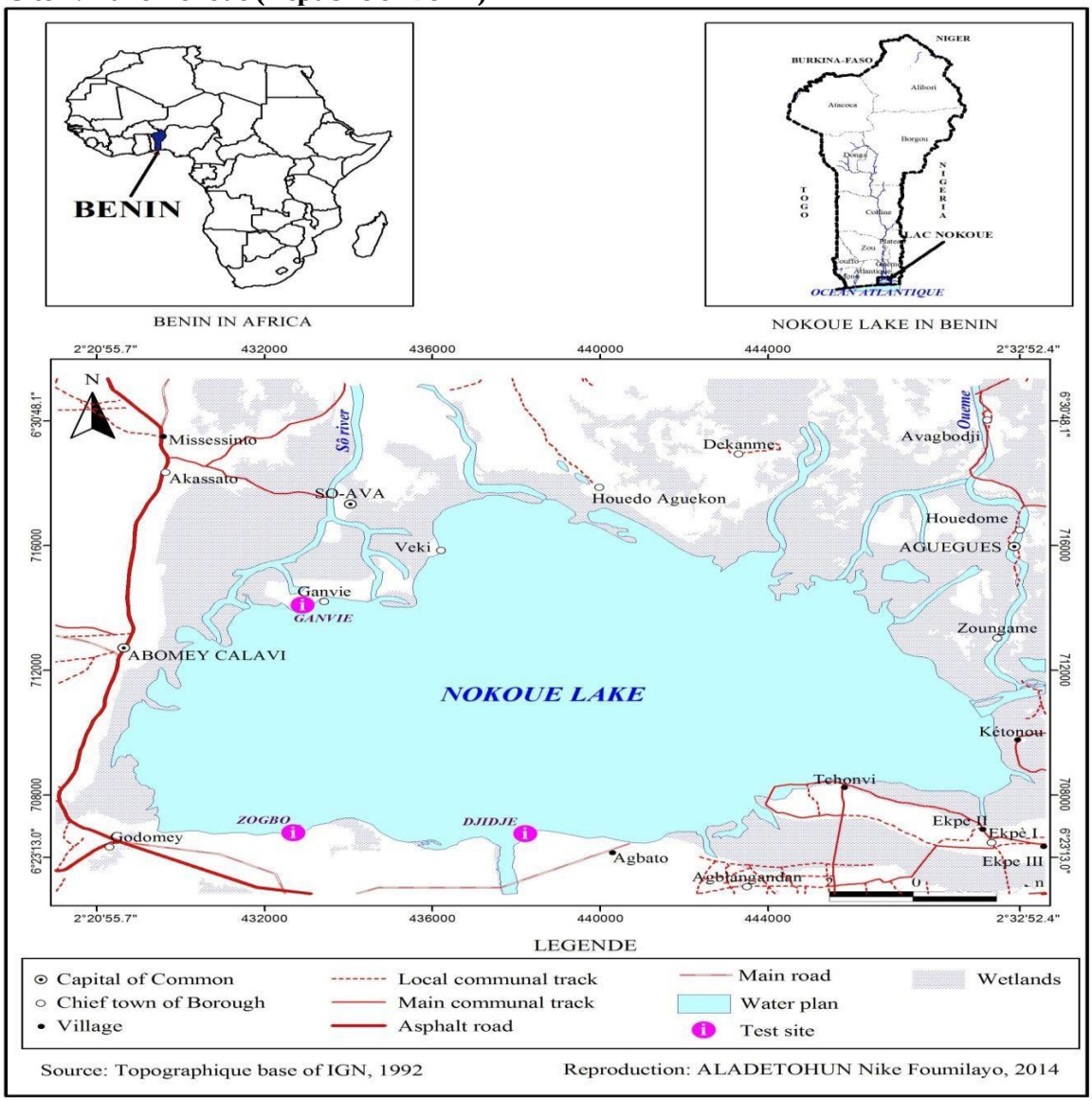

Figure 2: Map of Lake Nokoue lagoon
Keys: Republic of Benin, showing the sampling sites

Table 2: GPS locations Showing sampling stations of (Lake Nokoue, Republic of Benin)

Lagos Lagoon	Longitude	Latitude
STATIONS		
Djidje	20°25’8.393”E	60°23’11.077”N
Zogbo	20°24’7.512”E	60°23’40.162”N
Ganvie	20°24’32.615”E	60°27’33.77”N

Sampling of the fish specimens

Specimens of the two species of fish (*Mugil cephalus* and *Liza falcipinnis*) were collected from the Lagos lagoon (Nigeria) and Lake Nokoue lagoon (Republic of Benin) directly from the fishermen. Fish samples were collected for nine months from June 2019 to February 2020. In Lac Nokoue, a total of 363 of both fish species of fish (MC-149, LF-208) were investigated. In Lagos Lagoon, a total number of 330 of both species of fish (MC-139, LF-191) were examined. Two seasons were considered: the wet season (June-October2019) and the dry season (November 2019-February 2020).

The collected fish were transported in ice boxes to the laboratory and examined for parasites. The host species were identified using Fisher *et al*. (1987). Sex was determined according to Guerrero & Shelton (1974). The Weight (WT) of the fish was taken with a digital weighing balance and recorded. The total length (TL), standard length (SL) and forked length (FL) were measured with the aid of the meter rule.

Parasitological examination

Procedure for collection of gills

Gills were excised by cutting each gill at the upper and lower end, the extracted gills were put in a Petri dish. (Bykhovskaya-Pavlovskaya 1985) The gills were then examined for parasites myxosporidia parasite. The recovered parasites (cysts) were fixed and preserved in ethanol (70%) and then examined under a magnifying glass and later transferred on a glass slide for observation using a binocular microscope OPTIM 4 with an integrated video camera.

Procedure for collection of intestinal parasites

The abdominal cavity of the fish was opened from the anus and anteriorly and the intestinal organ was extracted according to Donets and Schulman (1973). and put in a Petri dish tangled intestines were untangled with fingers to avoid destruction of the organ. Saline water was poured into the intestinal tract and then cut open. The intestine was then examined for Myxosporidia parasites.

Microscopic examination

The extracted cysts of Myxosporidia are crushed between slide and cover slip in a drop of water or saline Ringer. The presence of spores of the microorganisms is indicated by light microscopy. The smears were stained by the method of May-Grunwald-Giemsa classic. Their observation allows identifying the nuclei of cellular structures, stages of development, and spore morphology.

Parasites species identification was based on morphological features according to Yamaguti (1963), Vassiliades (1975), Kabata (1979) Skryabin *et al.* (1984), Ben Hassine (1983), Dossou 1985, Sakiti *et al.* (1991), Kabre (1997), Kabre *et al.* (1997) and Moravec (2007).

Parasitological analysis

The parasitological terms follow Bush *et al.* (1997): prevalence (P) is the number of fish infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined (expressed as a percentage):

$$\text{Prevalence} = \frac{Number\ of\ hosts\ infested}{Number\ of\ hosts\ examined} \times 100$$

The intensity of infection (I) is the number of individuals of a particular parasite species in a single infected host (expressed as a numerical range); mean intensity (of infection, mI) is the average intensity or the total number of parasites of a particular species found in a sample divided by the number of infected hosts:

$$\text{Mean intensity} = \frac{Total\ number\ of\ a\ particular\ parasite}{Number\ of\ infected\ hosts}$$

Statistical analysis

Chi-squared and Fisher's Exact test was performed to assess expected links. Welch Two Sample t-test has been used to compare the

average mean intensity between the two Mugilidae inside each station of each study area on the one hand and for the same fish between the two countries on the other hand.

The one-way analysis of the variance (ANOVA) followed by the Tukey test has been performed to compare the average mean intensity among the stations inside each study area and for each species. All of these analyses have been done in R 2.15.3 statistical software.

Results

Of the 149MC and 208 LF from the Republic of Benin and 129 MC and 191 LF from Nigeria, the intestine and gills were parasitized by a specie of Myxosporidia forming whitish oval cysts. Light microscopic observations help to identify spores of Myxosporidia (Order: Bivalvulid; Family: Myxobolidae), genus *Myxobolus* Bütschli, 1882 and specie *Myxobolus sp* which *is* presented in Plate 1.

PLATE 1: *MYXOBOLUS SP*

1-3: Cysts of *Myxobolus sp* infecting the mullet, *Mugil cephalus* (scale: 0.63 mm).
1 and 2: Cysts (arrowheads) on the intestine (I)
3: Cysts (arrowheads) in gill filaments (GF) **4-8:** Spores (Sp) of *Myxobolus sp* (scale: 4.86µm).
4: Fresh spores showing some suture marks (arrowheads). S: Sporoplasm; IA: Intercapsular appendix; PC: Polar capsule
5: Stained spores (Sp) with the polar capsules (PC) and sporoplasme (S)
6 and 7: Abnormal stained (Fig 6) and fresh (Fig 7) spores. Look the position of the polar capsules in the sporal cavity
8: Spore with a discharged polar filament (DF)

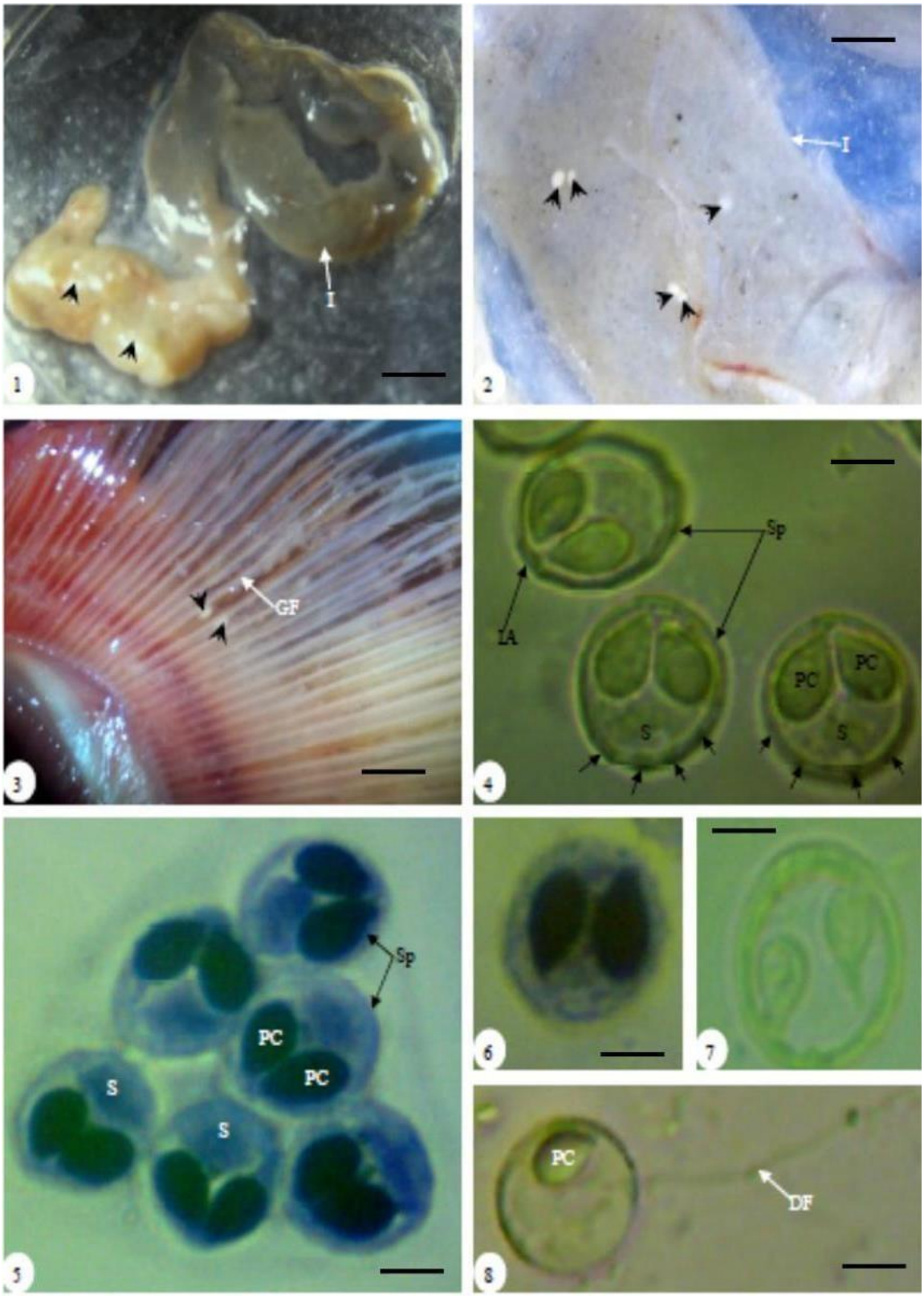

Plate 1: ***Myxobolus sp.***

Assessment of the influence of season, stage and sex on the prevalence rate of *Myxobolus sp* in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Lake Nokoue and Lagos lagoon

The results revealed that the season and sex didn't impact the degree of LF and MC infestation by *Myxobolus sp* in Lake Nokoue and Lagos lagoon. Meanwhile, for MC in Ganvie (Lake Nokoue), Unilag and Makoko (Lagos lagoon) the prevalence rate of *Myxobolus sp* is determined significantly by stage ($\chi2 = 6.84$, df = 2, P=0.03<0.05; $\chi2 = 13.43$, df = 2, P=0.00<0.05; $\chi2 = 17.85$, df = 2, P=0.00<0.05 respectively) . The fingerlings were more infested (20%) than adults (15.22%) and juveniles (14.29%) in Ganvie station. In Unilag, juveniles are more infested (17.50%) than adults (13.33%) and fingerlings are not infested (0.00%). However, in Makoko, adults are more infested (50.00%) than juveniles (22.58%) and fingerlings are not infested (0.00%). Regarding the degree of infestation of two fishes, two samples tested for equality of proportions showed that there is a high significance difference between the prevalence rate of *Myxobolus sp* in LF and MC (P < 0.05) in Lake Nokoue; LF is more infested than MC. In Lagos lagoon, there is not any significant difference between the prevalence rate of *Myxobolus sp* in LF and MC (P > 0.05).

The whole prevalence of *Myxobolus* on LF in Lake Nokoue is 5.29 % (11 infested on 208 examined) against 7.33% (14 infested on 191 examined) in Lagos lagoon. Two samples tested for equality of proportions showed that there is no significant difference between the two prevalences (Z-value = 0.40, P-value = 0.53). For MC, the whole prevalence in Lake Nokoue is 18.79 % (28 infested of 149 examined) against 19.42% (27 infested of 139 examined) in Lagos lagoon. Two samples test for equality of proportions showed also that there is no significant difference between the two prevalences (Z-value = 0, P-value = 1). All of these results are illustrated in figures 3-8 and tables 4-9.

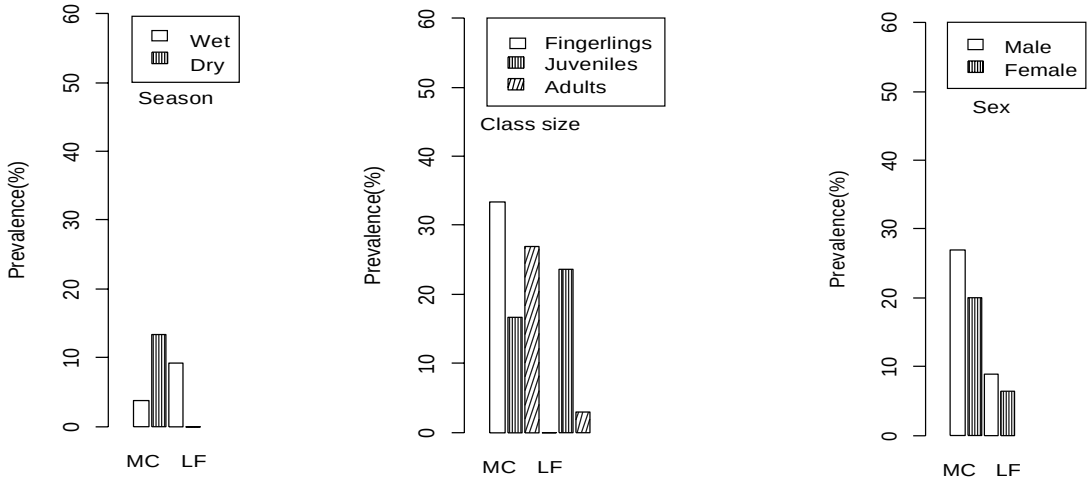

Figure 3: *Myxobolus sp* prevalence in Djidje

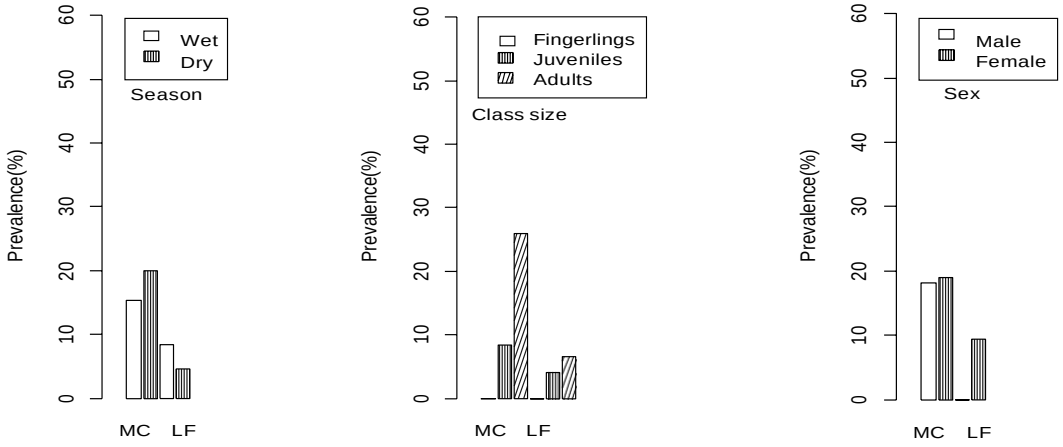

Figure 4: *Myxobolus sp* prevalence in Zogbo

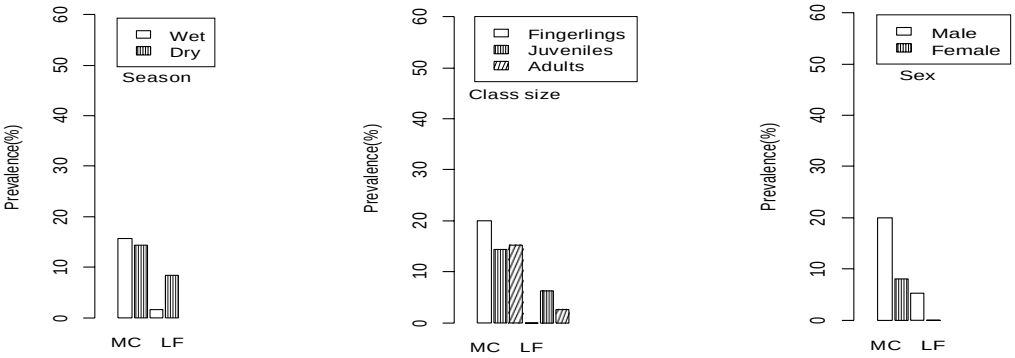

Figure 5: *Myxobolus sp* prevalence in Ganvie

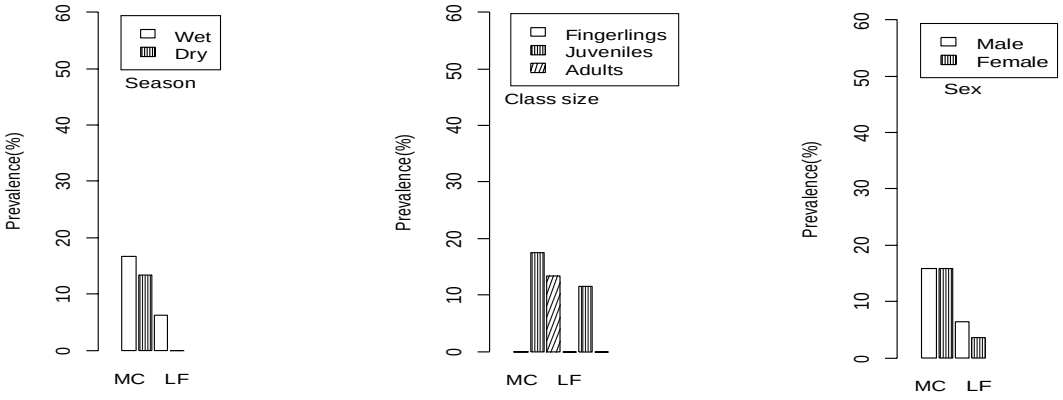

Figure 6: *Myxobolus sp* prevalence in Unilag

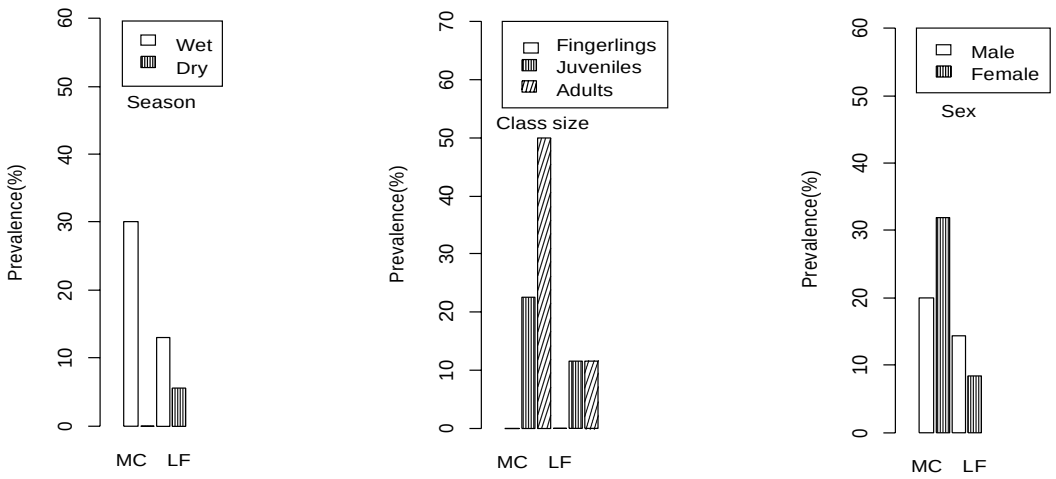

Figure 7: *Myxobolus sp* prevalence in Makoko

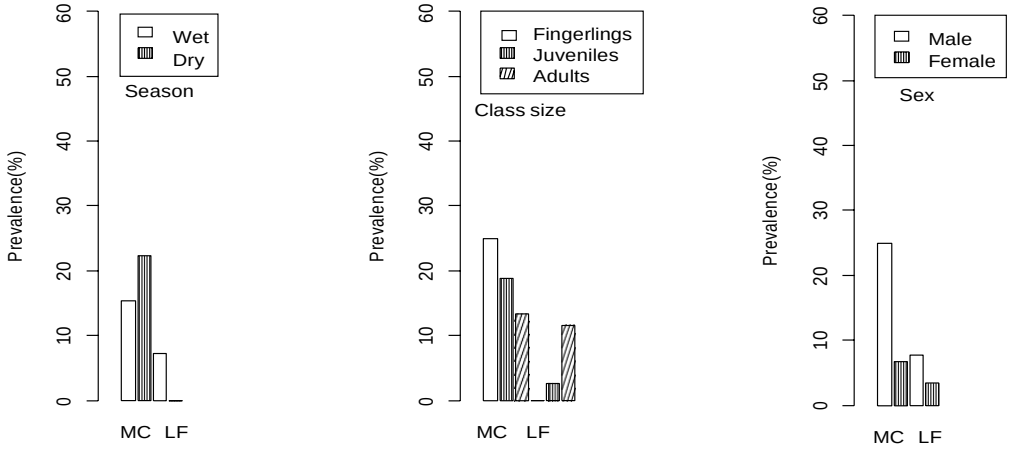

Figure 8: *Myxobolus sp* prevalence in Mequin

Table 3: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Djidje station in Lake Nokoue

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Total (%)	Z	P-Value
Season	**LF**	Wet	54	5	9.26	0.18	0.67	7.69	4.46	0.03
		Dry	11	0	0.00					
	MC	Wet	26	8	3.77	0.77	0.38	24.39		
		Dry	15	2	13.33					
Sex	**LF**	M	34	3	8.82	1.39	1.00	7.69	4.46	0.03
		F	31	2	6.45					
	MC	M	26	7	26.92	1.46	0.71	21.95		
		F	15	3	20					
Stage	**LF**	Fing	13	0	0.00	3.35	0.18	7.69	4.78	0.03
		Juve	16	4	25.00					
		Adul	36	1	2.86					
	MC	Fing	3	1	33.33	5.37	0.07	24.39		
		Juve	12	2	16.67					
		Adul	26	7	26.92					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Table 4: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Zogbo station in Lake Nokoue

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Total (%)	Z	P-Value
Season	**LF**	Wet	54	5	9.26	0.57	0.52	5.19	4.12	0.04
		Dry	11	0	0.00					
	MC	Wet	26	8	3.77	1.36	1.00	18.60		
		Dry	15	2	13.33					
Sex	**LF**	M	34	3	8.82	0.00	0.12	5.19	4.12	0.04
		F	31	2	6.45					
	MC	M	26	7	26.92	0.94	1.00	18.60		
		F	15	3	20					
Stage	**LF**	Fing	13	0	0.00	0.57	0.75	5.19	4.12	0.04
		Juve	16	4	25.00					
		Adul	36	1	2.86					
	MC	Fing	3	1	33.33	3.84	0.15	18.60		
		Juve	12	2	16.67					
		Adul	26	7	26.92					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Table6: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Ganvie station in Lake Nokoue

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Total (%)	Z	P-Value
Season	**LF**	Wet	54	1	1.58	0.21	0.33	30.30	4.61	0.03
		Dry	12	1	8.33					
	MC	Wet	51	8	15.69	0.00	1.00	15.38		
		Dry	14	2	14.29					
Sex	**LF**	M	38	2	5.26	0.26	0.61	30.30	4.61	0.03
		F	28	0	0.00					
	MC	M	40	8	20.00	0.90	0.34	15.38		
		F	25	2	8.00					
Stage	**LF**	Fing	12	0	0.00	2.86	0.24	30.30	4.61	0.03
		Juve	16	1	6.25					
		Adul	38	1	2.63					
	MC	Fing	5	1	20.00	6.84	0.03	15.38		
		Juve	14	2	14.29					
		Adul	46	7	15.22					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Table7: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Unilag station in Lagos lagoon

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Global (%)	Z	P-Value
Season	**LF**	Wet	48	3	6.25	0.00	93	5.08	2.52	0.11
		Dry	11	0	0.00					
	MC	Wet	42	7	16.67	1.29	1.00	15.79		
		Dry	15	2	13.33					
Sex	**LF**	M	31	2	6.45	1.84	1.00	5.08	2.52	0.11
		F	28	1	3.57					
	MC	M	38	6	15.79	1.00	1.00	15.79		
		F	19	3	15.79					
Stage	**LF**	Fing	3	0	0.00	4.26	0.11	5.08	2.52	0.11
		Juve	26	3	11.54					
		Adul	30	0	0.00					
	MC	Fing	2	0	0.00	13.43	0.00	15.79		
		Juve	40	7	17.50					
		Adul	15	2	13.33					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Table 8: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Makoko station in Lagos lagoon

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Total (%)	Z	P-Value
Season	**LF**	Wet	46	6	13.04	2.52	0.66	10.94	3.10	0.08
		Dry	18	1	5.56					
	MC	Wet	40	12	30.00	1.46	0.23	25.53		
		Dry	7	0	0.00					
Sex	**LF**	M	28	4	14.29	0.13	0.72	10.94	3.10	0.08
		F	36	3	08.33					
	MC	M	25	5	20.00	0.35	0.55	25.53		
		F	22	7	31.82					
Stage	**LF**	Fing	3	0	0.00	2.83	0.24	10.94	3.10	0.08
		Juve	26	3	11.54					
		Adul	35	4	11.43					
	MC	Fing	6	0	0.00	17.85	0.00	25.53		
		Juve	31	7	22.58					
		Adul	10	5	50.00					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Table9: *Myxobolus sp* prevalence rate estimation according to season, stage and sex in Mugilidae (*M. cephalus* and *L. falcipinnis*) from Mequin station in Lagos lagoon

			Ne	Np	Prev(%)	Chi2/Fisher's Exact statistic	P-value	Prev Global (%)	Z	P-Value
Season	**LF**	Wet	55	4	7.27	0.12	0.73	5.88	2.18	0.14
		Dry	13	0	0.00					
	MC	Wet	26	4	15.38	0.65	0.63	17.14		
		Dry	9	2	22.22					
Sex	**LF**	M	39	3	7.69	2.30	0.63	5.88	2.18	0.14
		F	29	1	3.45					
	MC	M	20	5	25	4.49	0.21	17.14		
		F	15	1	6.67					
Stage	**LF**	Fing	4	0	0.00	2.37	0.31	5.88	2.18	0.14
		Juve	38	1	2.63					
		Adul	26	3	11.54					
	MC	Fing	4	1	25.00	1.35	0.51	17.14		
		Juve	16	3	18.75					
		Adul	15	1	13.33					

Ne= number of fishes examined; Np= number of fishes parasited; Prev= Prevalence; M= Male; F=Female; Fing= Fingerlings; Juve= Jeveniles; Adul= Adults; Z are measures of standard deviation.

Discussion

The result shows that the myxosporidian found in this result belongs to the genus *Myxobolus*. They form some small white cysts on the gill filaments and the intestine wall. Though myxosporeans are generally considered harmless, they are reported to inflict high mortalities in cases of heavy infections and thereby cause heavy losses in Capture and Culture fisheries. Many a time these parasites may impair the growth of infected fish and reduce production (Reed, 2014).

The whole prevalence of *Myxobolus* on LF in Lake Nokoue is 5.29 % against 7.33% (14 infested on 191 examined) in Lagos lagoon, for MC, the whole prevalence in Lake Nokoue is 18.79 % against 19.42% in Lagos lagoon which is in line with the study done on Indian waters which varied between 1.75% and 25% by Kalavati and Anuradha, (1992) and Narasimhamurti *et al.*, (1980).

The study shows no significant difference in the prevalence of *Myxobolus sp* between the sex in Lagos Lagoon stations that is, they are parasitized equally which could be inferred that the infections would be mainly related with the type of diet and the habitat of the host, as indicated in the study done by Pardo-Gandarilla *et al.*, (2009) while in stations in Nokue there is significant difference between the prevalence and the sex of fish.

Conclusion and Recommendation

Since myxobolus sp. can infest all the stages of development (fingerlings, juveniles and adults) and any organ of the fish thereby affecting the physiological function of these fish and even kill, it is a very dangerous parasite.

Good management practices of wild and cultured mullets species

is very important. Pollution of the waterbody must be avoided or minimized There is a link between Lagos lagoon and Lac Nokoue therefore joint effort between the two countries to guard against disease infection of these economicallyimportant fish is very vital.

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