

Parasite fauna associated with the Nile Tilapia *Oreochromis niloticus* L. (Cichlidae) from fish farms in Southern Benin (West Africa)

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Abstract

Aquaculture appears nowadays as the best strategy for filling the gap between people's demand for animal protein and current captures of wild fishes. Achieving this goal requires the breeding of suitable fishes, especially *Oreochromis niloticus* whose consumption is very common in Benin. Whereas success of this fish species production required convenient conditions for the growth of the fishes without parasite trouble. Parasites of *O. niloticus* were investigated in three fish farms located in Southern Benin, from August to December 2014. A total of 525 specimens of *O. niloticus* were examined for the occurrence of external and internal parasites. Fish were monthly sampling during this period and water from these farms were also sampled for physico-chemical analyses. A total of 18 parasites taxa were recorded including only one protozoan (coccidia) and four metazoan parasites namely myxosporea, monogenea, cestodea and acanthocephala. There was no significant difference between the prevalence of coccidian and myxosporea ($P > 0.05$). No significant differences were observed either between prevalence according to males and females or among fish size groups for parasite prevalence. Whereas influence of season on fish parasitism was not evident, analyses of the physicochemical parameters revealed significant differences only for conductivity on L1 and BOD5 at L3.

Keywords : Aquaculture, metazoan parasites, prevalence, protozoan parasites.

1. Introduction

Since many decades, fish has become the solutions for most overcoming protein shortage problem all over the world, due to its importance for nutritional balance and in human health. However, because of its low production of inland water fish, Benin depends on imported fish as a low cost protein source to supply the needs of its population (Anon, 2011). FAO (2012) reported that 134 million tons of fish were used for human consumption out of a global production estimated at 154 million tons in 2011. Moreover, only 60% of the world halieutic production in 2012 came from the wild production (FAO, 2014). This is the consequence of the decrease of the halieutic productivity of natural sources owing to populations raising pressures and changes in the environmental conditions.

Aquaculture appears to be necessary to increase fish production worldwide (Tidwell & Allan, 2001). Tilapia is the common name for nearly hundred species of Cichlid fish from the Tilapiine tribe. Tilapia represented mainly freshwater fish inhabiting shallow streams, ponds, rivers and lakes, and less commonly in brackish water (De Silva *et al.*, 2004). However, global tilapia production is dominated by three species in the genus *Oreochromis* namely: the mossambic tilapia *Oreochromis mossambicus*, the blue tilapia *Oreochromis aureus* and the Nile tilapia or Nile mouthbreeder *Oreochromis niloticus* which is considered as one of the best candidate for tilapia culture (FAO, 2012).

Froese & Pauly (2015) reported that *O. niloticus* reaches its sexual maturity at 3-6 months depending on temperature, when the fish weighed about 30 g, and reproduction occurs only when temperatures are above 20 °C with several yearly spawnings (every 30 days) and Grammer *et al.* (2012) assured its multi-year survival and establishment. *O. niloticus* has become a well-suited species for aquaculture for its wide range of trophic and

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ecological adaptations, and its adaptive life history characteristics that enable it to occupy many different tropical and sub-tropical freshwater niches. These meaningful features allowed *O. niloticus* to be widely introduced for aquaculture and augmentation of capture fisheries (Welcomme, 1988). In Benin as well as in many developed countries, *O. niloticus* has become one of the country's commercially most valuable fishes. Indeed, one of the social impact of tilapia aquaculture is the increase in household incomes from small farms and eateries associated with farms (CABI, 2015), thereby reducing poverty (Tallec & Kebe, 2006).

Parasites are an essential part of each aquatic community (Klimpel *et al.*, 2003). Therefore, they represent one of the numerous natural factors or anthropogenic factors that often reduce the abundance of fish populations (Sindermann, 1987) though some methods are engineering. In wild populations it is difficult to isolate and quantify the effects of any single factor, such as predation, or starvation, or disease, on fish stock size (Sindermann, 1987; Kabata, 1995; Harris *et al.*, 2000). But Goselle *et al.* (2008) asserted that parasitism was much more common and diversified in the wild fish populations than in the farms or ponds and hatcheries. However, the onset of disease conditions due to parasites has become a major constraint in aquaculture (Caruso, 2009; Akoll *et al.*, 2012). The reason is that when stress occurs, resistance of fish is often lowered and some parasites may greatly increase in abundance thereby affecting fish health. When it happens, affected fish become weak and more susceptible to predation, followed by subsequent increase of transmission of the parasite (Moore, 2002). Furthermore, fish parasites spoil the appearance of fish, thus resulting in consumer rejection (Gulelat *et al.*, 2013); consequently, despite the large fish population, the economic benefits remain marginal owing to the prevailing parasitic diseases and others. Ectoparasites such as protozoa, monogenea and digenetic flukes are the most dangerous groups affecting skin and gills and that induce liminess of the flesh, irritation, destruction of gills, anorexia and impaired breathing (Guo & Woo, 2009) while nematodes and cestodes may have detrimental effects on internal tissues and growth of fish (McClellan & 2002). The synergistic action of parasites causes severe fish mortalities (Sakiti *et al.*, 1991; Gbankoto *et al.*, 2003, Costello, 2009; Sanil *et al.*, 2011; Tossavi *et al.*, 2015).

Various parasites are associated with Tilapia species in the wild and cultured environments where they cause morbidity, mortality, and economic losses to aquaculture in various parts of the world (Subashinghe, 1995). Unfortunately, whereas many studies were performed on parasites of *O. niloticus* in many countries as noticed by Olurin & Samorin, 2006; Awharitowa & Ehigiator, 2012 and Olurin *et al.*, 2012, there is a severe paucity of data on the parasites of this fish species in the Republic of Benin. Hence the present study was initiated to improve our knowledge on the identity and infestation level of

parasites and to evaluate the relationship between the infection by protozoan and metazoan parasites and the sex or size of *O. niloticus*. The possible influence of the physicochemical parameters on the prevalence of parasites also was tested in order implementation adequate biosecurity measures.

2. Materials and methods

2.1. Sampling localities, animals collection and identification of parasites

Specimens of *O. niloticus* were collected from August to December 2014 in the southern region of Benin for parasitological research. The study area is located in a subequatorial climate region characterized by alternating dry and wet seasons with a long dry season (LDS) running from December to March, a long wet season (LWS) from April to July, a short dry season (SDS) from August to September, and a short wet season (SWS) running from October to November. The three farms sampled were IA Benin at Ouidah, Podji Les-Monts at Abomey-Calavi and Sogo at Sèmè-Kpodji representing the sampling localities (L) L1, L2 and L3 respectively, as presented on Figure 1.

Fish samplings were done once a month together with the measurements of the water quality parameters. Water samples were taken upstream and downstream in each of sites and the mean values were used. The water quality variables that were measured included some physical variables like temperature, conductivity and turbidity; chemical variables such as pH, Potentiel Redox, Dissolved Oxygen (O₂), Nitrate (NO₃) and Nitrite (NO₂), whereas the biological variables were Total Dissolved Substance (TDS) and Biological Oxygen Demand (BOD).

Monthly, 35 fishes were purchased per site and examined. In total, 525 fishes were collected. All the fishes collected were carried alive, in plastic containers with air conditioned to the laboratory at the University of Abomey-Calavi where they were processed. The body weight (g) and the standard length (mm) were measured for each fish and their sex was determined by observing the external genitalia or the gonads after dissection. Fishes were then inspected at the naked followed with an observation under the low-power stereomicroscope whereas the gut and other visceral organs were removed and examined in order to search for protozoan and metazoan parasites. The sorted metazoan parasites were inspected as fresh material under the low-power stereomicroscope. In order to get more detailed information on their morphological structure, they were examined under a phase-contrast light microscope or mounted in 4% buffered formalin or in ethanol (70 %) as long as necessary for further investigations.

2.2. Statistical analysis

Statistical analysis of data was performed on the groups of parasites within the community. In each fish farm,

prevalence was calculated as the ratio of the number of individuals containing at least one parasite to the total number of fishes examined. These data were compared among sampling sites, fish size, between sexes and among sampling months using a chi-square test and the Z-test whereas correlation between number of parasites and water parameters were tested with Spearman test. A one-factor analysis of variance was used to test the significance of the variation of physicochemical parameters of water among fish farms. All analyses were performed using the statistical program SPSS version 20 and results were considered significant at the 95% level ($P < 0.05$). The percentages of the same column followed by different letters differ significantly.

3. Results

A total of 525 specimens of *O. niloticus* were examined for the presence of parasites. Mean total weight and standard length described for each specimen were presented in Table 1. The parasite fauna associated with the fishes comprised of 18 taxa including protozoan (1 Coccidian taxa) and metazoa (10 monogenean taxa, 5 Myxosporean please taxa, 1 taxa of each of Cestode and Acanthocephala). Of the *O. niloticus* specimens examined, a total of 170 individuals were infested, which corresponded to a global prevalence rate of 32.38% (Table 1).

Table 1. Geographic coordinates of the fish farms (localities: L), mean size and mean total weight of the hosts and total prevalence according to locality

Locality	Geographic Coordinates	Mean total size (cm)	Mean total weight (g)	Infected Fish/Examined fish	Prevalence by locality (%)	Total prevalence (%)
IA Benin (L1)	N06°22'37.4"E002°05'46.3"	19.048 ± 1.73	78.2 ± 26.59	80/175	45.71	
Podji-les monts (L2)	N06°34'12.9"E002°22'11.8"	12.989 ± 1.39	75.15 ± 23.40	73/175	41.71	38.98
Djèrègbé (LP3)	N06°25'00.3"E002°38'35.9"	13.448 ± 1.56	84.56 ± 24.60	17/175	9.71	

Prevalence by sampling Locality (L) varied between 9.71 and 45.71% whereas prevalence by sex groups was 31.54% for male versus 38.98% for female *O. niloticus* (Table 2). Prevalence of monogenea differed significantly among sampling points (L). The highest infestation (42.85%) occurred at L1 while the lowest (7.42%) was recorded at L3 (Table 3). Prevalence of Cestoda and Acanthocephala varied significantly among L and none of these two groups were encountered at L1 and L3 (Table 3). As for Coccidia and Myxosporea, their prevalence did not differ among the three sampling localities (Table 3). Irrespective of the sex of the parasitized *O. niloticus*, prevalence was low for all the parasite groups, except for Monogenean where the infestation rate was 22.03% ($n = 13$) in females and 23.77% ($n = 112$) in males. Prevalence of Coccidia and Acanthocephala were 1.69% ($n = 1$) in females and 1.93% ($n = 9$) in males for each group. No females of *O. niloticus* were infested by Myxosporea whereas the prevalence of this group in male *O. niloticus* was 1.07% ($n = 5$). For Cestode the prevalence was 3.38% ($n = 2$) in females and 3.43% ($n = 16$) in males. No significant differences were observed between males and females with regard to the prevalence of parasites ($df = 1$; $P = 0.072$) according to the chi-square test. Fish individuals with size ranged from 80 to 180 mm were more infested by any of the parasite groups, except that individuals of 80 to 130 mm in size were not infested by Acanthocephala. Monogenean and Cestode exhibited the

highest infestation rates. Irrespective of the parasite group, there were no significant differences in their prevalence among the three size classes of *O. niloticus* (Table 4).

During the short dry season (August to September), only Monogenea were collected while prevalence of Coccidia, Myxosporea, Cestode and Acanthocephala tended to nil. In the short wet season (October to November), only Cestode and Acanthocephala were not recorded in October whereas Myxosporea were not recorded in November. At the beginning of the long dry season (December), coccidia, myxosporea, monogenea, cestode and acanthocephala were encountered. The most prevalent was monogenea whereas it did not differ significantly among sampling month, except with prevalence observed in August (Table 5). Infestations of fishes by Coccidia, Cestode and Acanthocephala varied significantly among sampling months and were the highest in November. Prevalence of Myxosporea did not differ significantly among sampling months (Table 5).

The coefficients and probabilities of the Spearman correlation between the number of parasites and the physicochemical parameters of the three fish farms (Table 6), shows that the correlation was significant and positive only between the number of parasites and the conductivity ($r = 0.90$, $P = 0.03$) at L1 and between the number of parasites and the BOD₅ at L3 ($r = 0.90$, $p = 0.03$).

Table 2. Prevalence according to the host' sex from each locality (L)

Localities	Males		Females	
	Examined	Infested	Examined	Infested
L1	165	72 (43.63 %)	10	8 (80 %)
L2	138	62 (44.92 %)	37	11 (29.72 %)
L3	163	13 (20.63 %)	12	4 (33.3 %)
Prevalence by sex	147/466 = 31.54 %		23/59 = 38.98 %	

Table 3. Prevalence (%) of the groups of parasites according to the locality showing the statistical difference. Prevalence are followed by various letters in the same column indicating the significant difference ($P < 0.05$). P = P-value delivered by Z-test.

Localities	Coccidia	Myxosporea	Monogenean	Cestode	Acanthocephala
L1	1.14a	1.14a	42.85a	0a	0a
L2	5.71a	0.57a	26.85b	9.71b	4b
L3	1.14a	1.14a	7.42c	0a	0a
P	0.068806	0.891811	0.0000007	0.000044	0.017180

L: locality and P: probability

Table 4. Total prevalence (%) of the taxonomic groups of parasites according to the size of fish. Prevalence are followed by various letters in the same column indicating the significant difference ($P < 0.05$). P = P-value delivered by Z-test.

Size (cm)	Coccidia	Myxosporea	Monogenean	Cestode	Acanthocephala
[08 – 13[1.64a	1.64a	22.95a	4.92a	0a
[13 – 18[2.2a	0.82a	26.92a	3.3a	2.2a
[18 – 23[1a	1a	21a	3a	1a
P	0.796438	0.849251	0.601611	0.743549	0.317831

Table 5. Prevalence of the groups of parasites according monthly sampling. Prevalence are followed by various letters in the same column indicating the significant difference ($P < 0.05$). P = P-value obtained by Z-test.

Months	Coccidia	Myxosporea	Monogenean	Cestode	Acanthocephala
August	0a	0a	8.57a	0a	0a
September	0a	0a	30.47b	0.95ab	0.95a
October	1.9ab	2.86a	23.8b	0a	0a
November	5.71b	0a	28.57b	11.43bc	6.66b
December	1.9ab	1.9a	31.43b	4.76b	0.95a
P	0.020152	0.103317	0.000742	0.000008	0.000891

Table 6. Coefficients (C) and probabilities (P) of the Spearman correlation between the percent infestation and the water physicochemical parameters. Values in bold are those which have statistical significance

Locality	pH	Conductivity	Turbidity	Dissolved Oxygen	Potential redox	TDS	Nitrate	Nitrite	BOD ₅	
L1	C	-0.80	0.90*	-0.50	-0.70	0.35	0.00	0.80	0.30	0.71
	P	0.10	0.03*	0.39	0.18	0.55	1.00	0.10	0.62	0.17
L2	C	-0.20	-0.05	-0.30	-0.40	0.20	0.50	0.20	-0.66	0.79
	P	0.74	0.93	0.62	0.50	0.74	0.39	0.74	0.21	0.11
L3	C	-0.30	-0.30	0.60	0.15	0.30	0.30	-0.30	0.20	0.90*
	P	0.62	0.62	0.28	0.80	0.62	0.62	0.61	0.74	0.03*

4. Discussion

The present study is certainly the first in Benin that provides insight of the species composition of the different external and internal parasites that infested cultured tilapia and on how they were affected by water quality. Our results showed that in the three fish farms investigated in Southern-Benin, the *O. niloticus* sampled were infested by 18 parasite species, including coccidia, myxosporea, monogenean, cestode and acanthocephala. Based on the total number of parasites by host, *O. niloticus* had a relatively high parasitic load. Indeed, studies on wild *O. niloticus* from Burkina-Faso (Kabré, 1997) and southeastern Nigeria (Opara and Okon, 2002) indicated the occurrence of only 7 and 5 parasite species, respectively, despite their longer sampling period, whereas in cichlids from fish ponds in Syria, Al-Sammanet *et al.*, (2006) recorded only 2 species. Our results support, in contrast, the findings by Akoll *et al.* (2012), Eissa *et al.*, (2011), Soliman & Ibrahim (2012) and Amare *et al.*, (2014) who reported similar observations from different parts of Africa.

In Benin, no study had yet focused on this fish parasites. Indeed, this study results provide new insight for understanding parasites generally pledged to *O. niloticus* in Benin. The total prevalence observed was thereby forecasting on how devastating parasitic infections can be in fish farms, especially while considering the stressful conditions linked to crowding and frequent deterioration of water quality (Mitchel, 1989; Meyer, 1991; Bondad-Reantaso *et al.*, 2005). This total prevalence was closed to the values recorded by Amare *et al.* (2014) in Ethiopia, and by Noor El-Deen *et al.* (2015) in Egypt, with 29% and 47% respectively. In contrast, our values are higher than the 6.94% recorded in Nigeria (Edema *et al.*, 2008) and lower than the 89% obtained in Uganda (Akoll *et al.*, 2012). Our values are also lower than the 83 to 100% recorded by Tossavi *et al.* (2014) for metazoan parasites regardless of the sex of the Clariid species and the 42% recorded from *O. niloticus* in lake Geriyo in Nigeria (Domo and Ester, 2015).

Our study did not reveal any difference between males and females with regards to their infestation by the parasites ($P > 0.05$). The uneven numbers of specimens examined from each sex may be an explanation for these results; however, literature on the relationship between sex and parasite prevalence is controversial (Olurin *et al.*, 2012). This is in contradictions with the findings by Emere (2000), who reported differences in the incidence of infestation between male and female individuals. This author attributed such differences to differential feeding habit developed by the two sexes, either in the quantity or in the quality of food eaten, or as a result of different degrees of resistance or infection (Gbankoto *et al.*, 2001; Opara and Okon, 2002; Tossavi *et al.*, 2015), but how this applies in cultured conditions is still open to investigations.

Our data shows that parasitism rate varied with fish size. For fish which size was more than 18 cm in length, the prevalence ranged between 1% and 3% for cestode, myxosporea and acanthocephala. These data are in agreement with those reported by Tossavi *et al.* (2015), but are in contradictions to those of El-Mansy and Bashtar (2002), Abdel-Ghaffar *et al.* (2008) and Morsy et al. (2012), who reported that high parasitism in fish was associated with a decrease in their length and weight. It is interesting to notify that some parasite taxa were found only at one site, as was the case for fishes from L1 and L3 that never harbored Cestode and Acanthocephala, while others such as Nematode were not recorded at all. Whereas, there is no geographical proximity of sampling areas that could be reflected in the similarity of parasite species assemblage, one plausible explanation for this difference among fish farms with regards to the presence of parasites could be explained the fact that L1 and SP3 are experimental farms used for several studies and were, therefore, more strictly monitored than L2. As a matter of fact, L2 is the most infested station considering the number of parasite groups recorded here.

The significant difference observed between the prevalence of Monogenea from one locality to another could depend on the differences in physicochemical status of the water which may be more or less favorable to parasite proliferation in the sampling environment. Since Monogenea have a direct life cycle, their propagation is mostly assured some host specimens become heavily parasitized (Balling and Pfeiffer, 1997). Moreover, Caruso (2009) suggested that a high fish density associated with a low water renewal could result in frequent occurrence of epizootic diseases. Hussen *et al.* (2012) stated that the prevalence and the intensity of helminth parasites in freshwater could depend on either the biology of the species or the nature of the host and its feeding habits, physical factors and hygiene of the water body (Mitchel, 1989; Meyer, 1991; Bondad Reantaso *et al.*, 2005). Finally, affected hosts can lose their feeding capacity, thereby causing delay of growth or loss of weight and possible direct mortality by starvation (Kennedy, 1974), thereby explaining, how parasitic infections can be devastating in fish farms.

As stated by Karvonen *et al.* (2013), prevalence of fish parasites is directly influenced by some environmental factors, including water temperature. Our data confirmed this statement as the prevalence of the different groups of parasites had significantly increased during the dry season with the highest prevalence in November when the highest temperature was also recorded. This result illustrates the need to consider the multi-stress context in which host-parasite interactions occur (Olias *et al.*, 2004, Sánchez *et al.*, 2015). Opara and Okon (2002) observed significant differences in the prevalence of monogenea between dry season and rainy season; thus confirming our findings herein where monogenean's prevalence reached 31.43% in November against 8.57% in the rainy season (August). The positively significant correlation

between infestation rates and some physicochemical parameters of the water such as conductivity at L1, and BOD₅ at L3, is in support to Ernst *et al.* (2005) and Khidr *et al.* (2012), who showed that the prevalence of fish parasites is, sometimes, correlated with some environmental factors depending on the season.

Conclusion

This study is the first conducted in Benin on the parasite fauna associated with the commercially cultivated fish species *O. niloticus*. Our data showed a wide spectrum of parasites of *O. niloticus* throughout the study area. Infestation of fish by monogenea was very high. The wide distribution of parasites reflected the indiscriminate movement of fishes from hatcheries to grow-out systems. So, special attention should be given to sensitize fish farmers about the negative impact of parasitic infestation in aquatic ecosystem, so as to prompt them using some biosecurity measures to protect their cultured fishes.

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