

Parasite diversity and prevalence in the marine fish *Sardinella maderensis* (Teleostei : Clupeidae) in the nearshore waters of Benin (West Africa).

ABSTRACT

Aims: This study was designed to assess the diversity and the occurrence of parasites in *Sardinella maderensis* and the relationship between the prevalence of infestation and the fish length and sex.

Place and Duration of Study: Fish samplings were realized in the Artisanal fishing port of Cotonou between November 2019 and March 2020.

Methodology: During the study, 101 males and 64 females were sampled. They were kept on ice and transported into the laboratory. Each specimen was labeled and the length was collected. Then, they were first submitted to ectoparasites checking and secondarily dissected to get the internal organs for endoparasites examination. After parasite identification and counting, the prevalence, mean abundance, and mean intensity were assessed. Ecological indices for parasite community analysis such as Shannon Wiener index, Pielou equitability index, and Jaccard's Similarity index were determined. The chi-square test was performed to appreciate the probable significant variation between sex prevalence and length classes.

Results: A total of 742 individuals and eleven (11) species of the harvested parasites were distributed in five groups such as Monogenea, Digenea, Cestoda, Nematoda, and Copepoda. Nematoda were mostly represented in terms of number of recorded species and mean abundance. Total prevalence was 92.12%. No significant difference was recorded between prevalence in males and females ($\chi^2 = 1.168$; $P = 0.279$) on one hand and prevalence in fish length ($\chi^2 = 2.41$; $P = 0.66$). Cestoda's prevalence was more important while nematoda abundance was the highest. Nematoda has encountered 4 species whereas the two Trematoda were represented by only one species, each one. Ecological index record were representative of the equilibrium in parasite distribution between hosts sex.

Conclusion: Parasitism in *S. maderensis* was characterized by an important fauna of parasites mainly represented by Nematoda and Digenea. The prevalence of parasites was not related to the host's length or sex. These results, however, need further work to validate reliability.

Keywords: *Sardinella maderensis*, Occurrence, Ecological index, Parasitism, Mean abundance.

1. INTRODUCTION

World production of pelagic fish is around 39 million tons, representing almost a third of total catches (FAO, 2019). These pelagic fish belong to the Clupeidae, Carangidae, Engraulidae, and Scombridae of which the *Sardinella maderensis* and *S. aurita* are some of the most representative. Nunoo et al., (2015) have reported that *Sardinella maderensis* is more common in the catches along the west African coast. Therefore, it is presented as a relatively less expensive and a slice of appreciated meat by consumers in Africa in general and in Benin in particular (Hoto et al. 2020).

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Comment [S2]: Keeping fish in ice causes the separation of external parasites. In this situation, checking external parasites is almost impossible.

Comment [S3]: To identify the parasites, it is necessary to draw the parasites by the tube and then measure the key organs by the morphometric method, or to use the DNA sequencing method. This article does not mention any of them.

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For about a decade, research on flat *Sardinella* in Benin has focused on the study of the exploitation rate associated with the evaluation of the species' demographic parameters (Sossoukpè et al., 2016). A combined evaluation of the relationships between size, weight, and sex ratio was then initiated by Sohou et al., (2020) while the identification of morphological characteristics, the determination of stature-ponderal growth, and the condition factor were reported by Hoto et al. (2022). All this information are very useful for assessing the ecology and adaptation of the species in its living environment and the biology of its conservation. But to move towards sustainable fisheries management as suggested by Ouedraogo et al, (2015) and Mano et al., (2019), it is important to think like Hudson (2016) who suggested that parasites play important roles in influencing the ecosystem functioning, comprising the host community.

Given their small size, parasites have probably been given less consideration regarding their role in the ecosystem (Combes, 1995). The potential effects of parasitism on the host abundance (1), and the interactions between hosts within a community (2), are possibly to alter the biodiversity and the ecological functions relationships in the ecosystem (Frainer et al. 2018). Parasites can therefore increase or decrease ecosystem processes by reducing host abundance (Frainer et al. 2018, Brian et al., 2022) on one hand, while host diversity losses could induce parasite population declines (Wood et al. 2020) on the other hand. Regarding those parts of influence of management of *S. maderensis*, the present study is focused on assessing the diversity of parasites likely to infect fish to add to our knowledge of the biology of the relational life of the same species in Benin as it was underlying elsewhere.

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2. MATERIAL AND METHODS

2.1 Study area

The fish sampling was done from the "Port de Pêche Artisanal de Cotonou (PoPAC)" namely the Artisanal fishing port of Cotonou » located between 6° 21'4.212" N, and 2° 25'58.296" E in Benin, adjacent to the industrial fishing port (fig 1). PoPAC was developed in 1972 to facilitate the landing of pirogues. Covering an area of 14,800 m², it is bounded to the north by Avenue Jean-Paul II, to the south by the Atlantic Ocean, to the east by a jetty known as Quai C, and to the west by the road leading to the Cotonou tidal market. PoPAC is one of the three catching sites receiving 80% of fish landings (Sossoukpè et al., 2016); the two others are from Cotonou, unofficial and not under the control of PoPAC agents. Although the fishing trips to sea are monitored, the landings are not rigorously monitored and do not allow any significant data to be collected. Additionally, there was around 3 3-week period where specimens of *S. maderensis* were absent from the catches.

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2.2 Fish collection

From November 2019 to March 2020, 165 specimens of *S. maderensis* comprising 104 males and 61 females were randomly sampled from the regular fish catches in the morning kept on ice and carefully transported to the laboratory. Fish were labeled and bagged and the total length (cm) of each fish was measured using an ichthyometer. Each fish specimen was checked for ectoparasite along the external body comprising the mouth, eyes, gills, skin, and fins. For endoparasite, fish were first submitted to dissection. The internal organs such as the intestine, spleen, stomach, oesophagus, and gall bladder, were collected and put in Petri dishes containing the saline solution (0.9%). Vesical substances like bile was obtained by puncture in the gall bladder whereas kidney and spleen were observed in the form of squash.

The parasitological investigation was achieved when each organ was processed to a binocular magnifying glass (x4 to x40) and the collected parasites were observed under a binocular microscope with increasing magnification (x40 to x1000) as suggested by Claar et al. (2021). The parasite identification was based on the images captured and given according to genus level at least and counted. Selected parasite specimens were preserved in ethanol (70%) or buffered neutral formalin (10%).

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Comment [S9]: To identify the parasites, it is necessary to draw the parasites by the tube and then measure the key organs by the morphometric method, or to use the DNA sequencing method. This article does not mention any of them.

2.3 Data analysis

The morphometric data collected on the fish were entered into an Excel spreadsheet. The results of the observations (parasite species and number counted) are reported to determine indices for the ecological characterization of the communities. The parasite diversity was obtained by determining the total number of taxa found during the study (Aliaume et al., 1990). Assessing the community structure of parasite species, the Shannon-Wiener diversity index (H'), the Pielou equitability index (S), and the Jaccard

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similarity index (J) (Poulin, 2015), while the sub-communities structure is described using the basic parameters, prevalence (P), mean abundance (MA) and mean intensity of infection (IM) and abundance (Ab) employed by Bush et al. (1997). The frequency of dominance was calculated to assess the occurrence of each parasite group in the sub-community (Rhode et al., 1995).

The chi-square test of independence was used to assess the significance of the prevalence rates between the sexes and the sizes of the fish and their combinations. Data were processed using the Minitab software, version 18.

3. RESULTS

3.1 Global infection and parasite prevalence according to sex and length

The studied sample comprised 165 individuals of *Sardinella maderensis*, 104 males and 61 females, for a ratio of 1.70. Parasitological examination revealed a prevalence ranging from 90 to 95%. The chi-square test revealed no significant difference between prevalence according to sex (Table 1).

Table 1: Number of examined and prevalence according to sex

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Parameters	Sex		Total
	Males	Females	
Examined	104	61	165
Infected	94	58	152
Prevalence (%)	90.38	95.08	92.12
Chi-2 (P)	1.168 (0.279)		-

The fish length was varied between 10 and 35 cm. They were distributed into five classes of 5 cm unit spacing. Lengths belonging to the the small class (10-15) and high class (30-35) accounted for less than 1% of the sample, while those between 15 and 30 cm accounted for 99%. The classes represented by feable number were 100% infected, and the intermediates classes prevalence varied decreasing from 96.07% in [15-20[to 87.5% in [25-30[. However, no significant difference was recorded between parasite prevalence according to length ($\chi^2 = 2.41$; $p = 0.66$). A similar result was found considering the sex (Table 2) although it was important to note that females (100%) seem to be more infected than males (89.18%) within the class [15-20[.

Table 2 :Prevalence of *S. maderensis* infestation of individuals according to their size and sex.

Class of length	Summary		Males		Females	
	Parasites (infected/ examined)	Prevalence (%)	Parasited	Prevalence (%)	Parasited	Prevalence (%)
[10-15[4/4	100	3	100	1	100
[15-20[49/51	96.07	33	89.18	16	100
[20-25[84/93	90.32	50	90.9	34	91.89
[25-30[14/16	87.5	8	88.88	6	100
[30-35[1	100	0	0	1	100
Chi 2(P)	2.41(0.66)		0.44(0.93)		2.04(0.72)	

3.2 Parasites community, specific richness, and prevalence

The community of recorded parasites is composed of only helminths such as Monogenea, Digenea, Cestoda, Nematoda, and Copepoda. Fish were mostly infected by cestoda(45.45%), digenea(44.84%), and nematoda(43.63%). The difference was recorded within the groups ($\chi^2 = 150.61$, $p = 0.00$). The total number of parasites was 742 individuals. Nematoda, digenean, and Cestoda were the first three most harvested parasites with 334; 221, and 161 individuals, respectively, equaling 96.49% of the parasite infra community (Table 3) while monogenean and Copepoda represented an occurrence of 3.51%

Table 3: Data for the parasite group prevalence and frequency of domination in the infra-community

Parasites	Prevalence (%)	Counted individual of parasites	Contribution (%)
Monogenea	5.76	12	1.61
Digenea	44.84	221	29.78
Cestoda	45.45	161	21.69
Nematoda	43.63	334	45.01
Copepoda	4.85	14	1.88
TOTAL		742	100

3.3 Parasites identification and ecological and epidemiological parameters

The infra-community was composed of eleven species among which four (4) were identified namely, five were identified according to genera and two were named using their family. Nematoda was represented by 4 species, and Digenean by 3 species. The Cestoda encountered 2 species while monogenean and copepoda were represented by only one species, respectively. Table 4 shows that Cestoda (*Sphyriocephalus* sp.) have infested around 40% of fish while digena (*P. merus* and *Lepidapedongadi*) and nematoda (*P. laevionchus* and *C. ancyloides*) have infested 15 to 20% of the fish. The less prevalent parasite was *Anisakis* sp. with 1.12%. The first three highest mean intensities are those of *P. laevionchus* (7.5), *Rhabdochona* sp. (5.42), and the Gryporhynchidae (4.0). The lowest mean intensity (1.0) was recorded in the monogenea and *Anisakis* sp. Only *P. laevionchus* displayed a mean abundance relatively upper to 1.0 (Table 4).

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Table 4 : Data of prevalence, meanabundance, and meanintensityaccording forecorded parasite species

Parasitespecies	Prevalence (%)	Mean Abundance	Mean Intensity
Monogenea			
<i>Mazocraeoid</i> sp.	5.45	0.05	1
Digenea			
<i>Parahemiurus</i> merus	20	0.57	2.87
<i>Lepidapedongadi</i>	18.18	0.64	3.56
<i>Myxozenu</i> ssp.	6.66	0.12	1.81
Nematoda			
<i>Anisakis</i> sp.	1.12	0.012	1
<i>Procomallanus</i> leavionchus	18.18	1.36	7.5
<i>Camallanus</i> ancyloDIRUS	15.75	0.18	1.15
<i>Rhabdochona</i> sp.	8.48	0.46	5.42
Cestoda			
<i>Sphyriocephalus</i> sp.	39.4	0.73	1.86
Gryporhynchidae (family)	6.06	0.24	4
Copepoda			
<i>Clavellis</i> asp.	4.84	0.07	1.62

3.4 Ecological index of parasite diversity in *Sardinellamaderensis*

For the harvested parasite sample, the Shannon-weaver index value was $H' = 2.07$ and the Piélouequi-repartition value was $S = 0.6$ (Table 5). The similarity index of Jaccard (J) was 0.6. as almost all the different species of the various parasitic groups were found in both males and females, except for *Anisakis*sp. which was collected in males only (Table 5).

Table 5: Data for the ecological index of the parasite diversity in males and females.

Different index	All the community	Males	Females
Shannon-Weaner	2.07	1.9	2.18
PiélouEqui-distribution	0.6	0.55	0.66
Similarity index of Jaccard	-	0.9	

4. DISCUSSION

4.1 Morphometric and parasitic infectiondata

It appears that the total length and weight of the collected fish were slightly less than those recorded by Sossoukpè et al. (2016) and Ogbon et al. (2023) either in the same environment in Benin or in another sampling ecosystem in Cape Cost (Ghana). Wehye et al. (2017) have recorded *S. maderensis*with the possible lowest and highest length (5.5 to 42 cm). According to Camarena-Lurhs, (1986) and Samba, (2011), those variations could correspond to the sexual activity period followed by a modulated fry occurring during firstly November and December and secondly from May to August. During the present study, fish should spend much energy on reproduction and growing stages. Samba (2011) motivated his opinion by the fact that there are two reproductive periods for *S. maderensis*. Unfortunately, Diouf et al., (2010) explained that reproduction is in progress during the year for the same species. It would have been

important to consider the periods of possible abundance or to know whether *S. maderensis* is a migratory species as suggested by Thiao (2012).

4.2 Diversity, Prevalence, and abundance of the different parasite species of *Sardinella maderensis*.

Despite the relatively high prevalence recorded in this study, there was no sex influence on the degree of infection. The result suggested that habit use and diet are quite similar in both sexes in *S. maderensis*. Some authors assured that the prevalence and intensity of infection are linked to diet while qualitative and quantitative changes depend on fish length (T. N. Darius, Université Nationale d'Agriculture, Bénin, personal communication). However, it's important to notice that the sex-imbalanced sampling could disadvantage female prevalence. Anyway, this bias can lead to misinformation about the potential comparison variables. Therefore, the results would be better appreciated if the sampling sites were diversified.

4.3 Diversity and occurrence

The total prevalence of 20% observed during the present study was similar to Ogbon et al. (2023) working on *Sardinella maderensis* from Ghana (21%), approximately twice as low as that recorded on the same specimens from Benin (45%) but much lower than that presented by Feki et al. (2016) on *Sardinella aurita* while Ramdani et al. (2022) reported a 30% prevalence of *Glugea* infection in *S. aurita*. Although it has been shown that nematoda, cestoda and digeanea are numerically abundant, especially the species 1- *Paracamallanus laevionchus*, 2- *Sphyricephalus* sp. and 3- *Lepidapedon gadii* and *Parahemiurus merus*, respectively in the present study, the cestode *Sphyricephalus* sp. and the nematode *Procammallanus laevionchus* seem to have the highest parasite occurrences unlike the results of Ogbon (2023) where *P. merus* demonstrated the highest occurrence. Several authors have recorded *P. merus* in mackerels. That's in the cases of *Sardinella aurita* by Feki et al. (2016), *Sardina pilchardus* in the West Algerian Coast (Marzoug et al., 2012), *Sardinella cameronensis* (syn. *S. maderensis*) from the coastal waters in Ghana and Senegal (Fischthal et al. 1971, Ndiaye et al., 2012), *Sardinella aurita* in the Gulf of Gabès (Tunisia) and on the Algerian coast (Ramdani et al., 2020).

Endoparasites have been reported to infest the two sexes differently. According to Rohde (1993), male and female fish have different feeding habits. The parasites seem to affect the health of their hosts, despite the fact that the examined specimens were physically overweight. Aloo et al. (2004) revealed that in *S. crumenophthalmus* and *S. maderensis*, the nematodes were collected principally in the intestine and around the gonads. The same authors argued that the worms should have a preference for both organs and should probably have the possibility to derive some nutrients from them. Addressing the potential effects of parasites on their host, Hosan (2018) revealed that dietary factors including the quality or quantity of the food ingested have potentially impacted the outcome of host-parasite interactions, through a variety of mechanisms. Furthermore, it was indicated that intestinal parasites limited the digestive activity of the host and indirectly inhibited vitamin and blood sugar metabolism and growth; parasites in the liver affect glycogen metabolism and growth (Halvorsen, 1955). However, it is also important that the microbiota-pathogen interaction protects the host by creating colonization resistance. The microbiota competes for nutrients and space in the host (Medina-Felix et al. 2023). Beyond any analysis, it seems logical to recognize with Holmes (1990) that a relative helminth fauna was encompassed by *S. maderensis* and mainly represented by digeaneans and nematoda. The Cestode larvae that are collected from *S. maderensis* seem to have the same morphological characteristics as they could be brought closer to *S. viridis* Wagener, 1854 and *T. coryphaenae* (Ogbon et al., 2023).

P. laevionchus has already been recorded in several African countries in continental water fish (Mashego and Saayman, 1981; Barson, 2006) and recently in Benin (Tossavi et al. 2014, Togla et al. 2020, Houénou-Sèdogbo, 2019). Its presence in *Sardinella maderensis* would therefore be proof that it is widely distributed throughout the African continent, in both fresh and marine water. It was reported the level of infestation, parasitic fauna and the number of a particular parasite taxon in the marine environment depend on the diet of the host, its gregariousness (or density), intense swimming activity, and its size (Polyanski, 1961). Among the parasite species identified, *Clavellisa* sp. was reported for the first time in the Gulf of Bejaia in *S. aurita*, and is mostly abundant in the gills. Very rarely, specimens of this parasite have been collected from the digestive tract. This could be explained by the fact that these ectoparasites passed with the flow of water filtered by the gills to return to the intestine.

4.4 Ecological diversity of harvested parasite species.

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The Shannon diversity index (H') and the Pielou distribution index (J') were also performed to assess the diversity and distribution of parasites in *S. maderensis* respectively. Their values were 1.90 and 0.55 respectively, in males and 2.18 and 0.66 respectively, in females. Therefore, results according to H' suggested an evident diversity of parasites in the host species. J' takes a value between 0 and 1. It tends towards 0 when the parasites are concentrated in a single-sex, 1 being a complete equi-repartition between the sexes; it then tends towards 1 when all the species have approximately the same abundance. As J' is around .5 and 0.6, the community of parasites constantly fluctuates in the abundance of the various parasites, which could reflect that the fish examined have developed a certain specificity for the parasites collected. It can also be assumed that the availability of suitable host species may have been a factor limiting the colonization of hosts by parasites (Poulin, 1992). As for Jaccard's similarity index applied to sex, its value tends towards 1 ($S_j=0.9$) because almost all of the different species of the different parasite groups are found in practically all of the infected specimens, all sexes combined, with the exception of *Anisakis* sp. which was only collected from males, hence its status as a rare species. The value 1 represents the presence of parasites in both sexes. However, this is justified in the present study because the statistical tests do not impute the parasitic infestation observed to either the sex or the length of the fish. According to Rhode (1993), changes in parasite diversity may be associated with changes in size, since larger hosts not only reserve increasingly large microhabitats for parasites but also and above all consume greater quantities of food, thus being exposed to a greater possibility of infection.

CONSENT

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

NOT APPLICABLE

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Comment [S17]: Same as the last comment

Comment [S18]: The date of this reference in the text is given 2020. Which one is correct?

Comment [S19]: This reference not mentioned in the text

Comment [S20]: The date of this reference is not given

Comment [S21]: The date is inconsistent with the text

Comment [S22]: The date is inconsistent with the text

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Comment [S23]: ???

Comment [S24]: Scientific names must be italic

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