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Seasonal and Spatial Distribution of Nematode Larvae of the Genera *Anisakis* and *Contracaecum* (Anisakidae) in Two Populations of *Mugil Cephalus* (Mugilidae) from Saloum and Senegal Rivers

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Abstract

Anisakis sp. and *Contracaecum* sp. are nematodes belonging to the family of Anisakidae. They are worldwide distributed and use a great number of fish species as intermediate, paratenic or final host. Despite their effects on fish and human beings, they play ecological role as biological tags to identify fish population or bioindicators of marine pollution. From January to December 2009, the helminthological examinations of 129 and 167 specimens of *Mugil cephalus* originating from Saloum and Senegal estuaries respectively revealed the presence of *Anisakis* sp. and *Contracaecum* sp. larvae. Generally, whatever the season or locality, the kidney lodges more larvae of *Anisakis* sp. than the liver or the general cavity. All *Contracaecum* sp. larvae were found in the mesentery of *Mugil cephalus* whatever the locality and season. In each locality, the prevalence of *Anisakis* sp. larvae was higher in rainy season than in dry season whereas the larvae of *Contracaecum* sp. were more frequent in dry season than in wet season. Mean intensity of the Anisakidae larvae showed significant differences according to the season in the estuary of Saloum and non significant variation in the estuary of Senegal. The mean abundance of these larvae varied significantly depending on the locality, the season or the site of infection. These results are explained by factors related to the host (fat content in infested organs), factors in relation to the environment (temperature, salinity) and the presence of birds, final hosts. In the Saloum estuary, *Anisakis* sp. was dominant in rainy season, while *Contracaecum* sp. dominates in dry season. However, in the estuary of Senegal, *Anisakis* sp. was dominant all the year. So we can say that *Anisakis* sp. is characteristic of the estuary of Senegal while *Contracaecum* sp. is characteristic of the Saloum estuary. Distribution of anisakidae larvae is more related to the locality, the season and the site of infection rather than the fish gender.

Keywords: *Mugil cephalus*, Distribution, Prevalence, Intensity, Abundance, Dominance, *Anisakis* sp., *Contracaecum* sp., Saloum, Senegal.

1. Introduction

Mugilidae or mugilids are abundant fishes in Saloum and Senegal estuaries and are well represented in the landings (Anonyme 2005, 2009; Bousso, 1991; Ndour, 2003). They are very important, based on consumption, economic and ecological point of view. As a real source of proteins, mugilids are consumed fresh or transformed by local populations. Since they are really appreciated by fishermen, they underwent an overexploitation these last years for needs of export.

The ecological interest of the mugilidae, especially that of *Mugil cephalus* lies in their use as indicators of the health status of estuaries and in their position at the food chain's base (Tantanasi et al., 2012).

It is with the knowledge of the important ecological values which the mugilids present that we were interested to investigate their nematodes of the genera *Anisakis* and *Contracaecum* belonging to Anisakidae family. Anisakidae has a worldwide distribution and more fish species act as intermediate (Bomker, 1982; Farjallah et al., 2006, 2008; Umehara et al., 2006; Abowei and Ezekiel, 2011; Quiazon and al., 2011; Rosas-Valdez et al., 2007; Tantanasi et al., 2012) or final hosts (De Oleivera Rodrigues et al., 1975; Nuchjangreed et al., 2006). Although they cause public health problems (Sher et al., 2003; Caballero et al., 2011; Daschner et al., 2011;

Gonz áles et al., 2005, 2010, 2011) and more or less important pathogenic effects on fishes (Bomker, 1982; Murphy et al., 2010; Abowei and Ezekiel, 2011), these parasites play significant roles. Infact, they are used as biological labels (genetic markers) to identify stocks or pelagic and demersal fish sub-populations (Mattiucci, 2006; Mattiucci et al., 2007; Rebecca et al., 2011). They also constitute biological markers of marine pollution since they have the possibility of accumulating 300 times more heavy metals than their hosts (Hogue and Peng, 2003; Pascual and Abollo, 2005).

Anisakid larvae are present in various organs of which muscles (Olivero-Verbel et al., 2005; Martins et al., 2005; Valero et al., 2006; Rello et al., 2009; Suzuki et al., 2010; Abattouy et al., 2011). Their presence in the flesh decreases the commercial value of the host and increases the risk of contamination by raw or badly cooked fish consumption.

In this study we discribe the distribution of *Anisakis* sp. and *Contracaecum* sp. larvae in two populations of *Mugil cephalus* caught in Saloum and Senegal estuaries.

2. Materials and Methods

2.1 Sampling Procedure

Saloum and Senegal estuaries constitute the localities chosen within the framework of this study. These ecosystems are characterized by a particular vegetation type, the mangrove swamp. The latter, formerly prosperous, offers in low Senegal the degraded aspect of a relict vegetation only, while the banks of Saloum, by their southernmost position, shelter a more flourishing vegetation.

In Saloum and Senegal rivers, samplings were done systematically per quarter. Three fish samplings were carried out in the dry season and one in the rainy season. On the whole 129 and 167 individuals of *Mugil cephalus* were caught in the estuaries of Saloum and Senegal rivers respectively from January to December 2009. The techniques of fishing used were the beach seines and the gill nets.

The abdominal cavity was openned by incision starting from the anus up to the mouth. After that, various fish organs (gills, oesophagus, stomach, caeca, intestine and their contents, general cavity, kidney, liver, gonads) were examined with the binocular magnifying glass. Nematodes were also searched in the flesh of fishes by dilacerating the muscles in water under a binocular magnifying glass. Those found were cleaned in water with brushes then preserved in 70% ethanol.

2.2 Determination of Ecological Parameters

To study parasitism, we considered the component populations of parasites (i.e. the parasitic populations which are found in host populations) and the component community (i.e. all parasites discovered in a host population). Each host fish is a measurement repeated like any quadrat on a forest ground, which will make it possible to calculate ecological parameters.

The ecological terms prevalence, mean intensity and mean abundance were calculated according to Margolis et al., (1982). In a host population, the prevalence of a parasitic species corresponds to its frequency.

2.3 Statistical Analysis

To determine the differences of mean abundance by site of infection, sex, season or locality, we used the nonparametric test of Kruskal-Wallis (KW) or that of Wilcoxon. The significance of these statistical analyses was established to 0.05. Dominance of each parasite species in the component community was also calculated.

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3. Results

3.1 Distribution of Anisakid Larvae in Populations of Mugil Cephalus According to Seasons and site of infection

Results obtained revealed that *Anisakis* sp. (photo 1) and *Contracaecum* sp. (photo 2) larvae are distributed in various organs according to the localities and the seasons. In the estuary of Saloum, in dry season, 34% of the individuals of *Anisakis* sp. infected the liver whereas 66% were found in the kidney. This difference is accentuated in wet season with 12% of the worms found in the liver and 88% in the kidney. The percentage of larvae infecting the kidney is higher in the wet season than in the dry season, while that of the larvae recorvered in the liver is higher in the dry season than in the rainy season (Figure 1).

In the estuary of Senegal, the percentage of parasitic individuals found in dry season in the kidney (70%) is higher than that of the individuals met in the liver (30%). Percentage of the individuals of *Anisakis* sp. collected in rainy season is also higher in the kidney (75%) than in the liver (23%) and the general cavity (2%). It is reported that the percentage of the individuals of *Anisakis* sp. in the liver is higher in dry season than in rainy season while for the kidney, it is higher in wet season than in dry season. Percentage of the individuals of *Anisakis* sp. living in the body cavity is also higher in rainy season than in dry season. Generally, whatever the season or locality, the kidney lodges more larvae of *Anisakis* sp. than the liver or the general cavity (Figure 1). However all *Contracaecum* sp. larvae were found in the mesentery of *Mugil cephalus* whatever the locality and season.

Photo 1: Larvae of *Anisakis* sp.

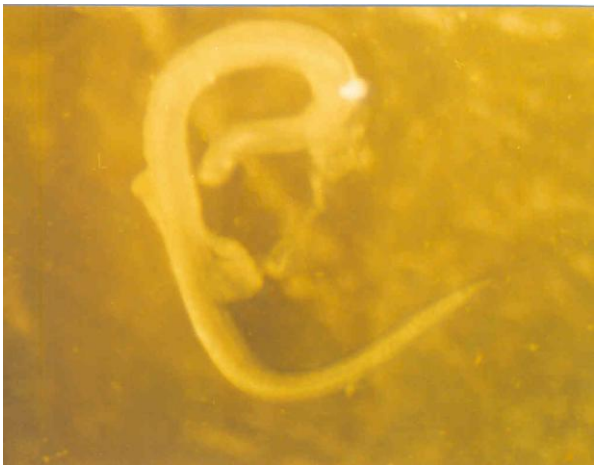


Photo 2: Larvae of *Contracaecum* sp.

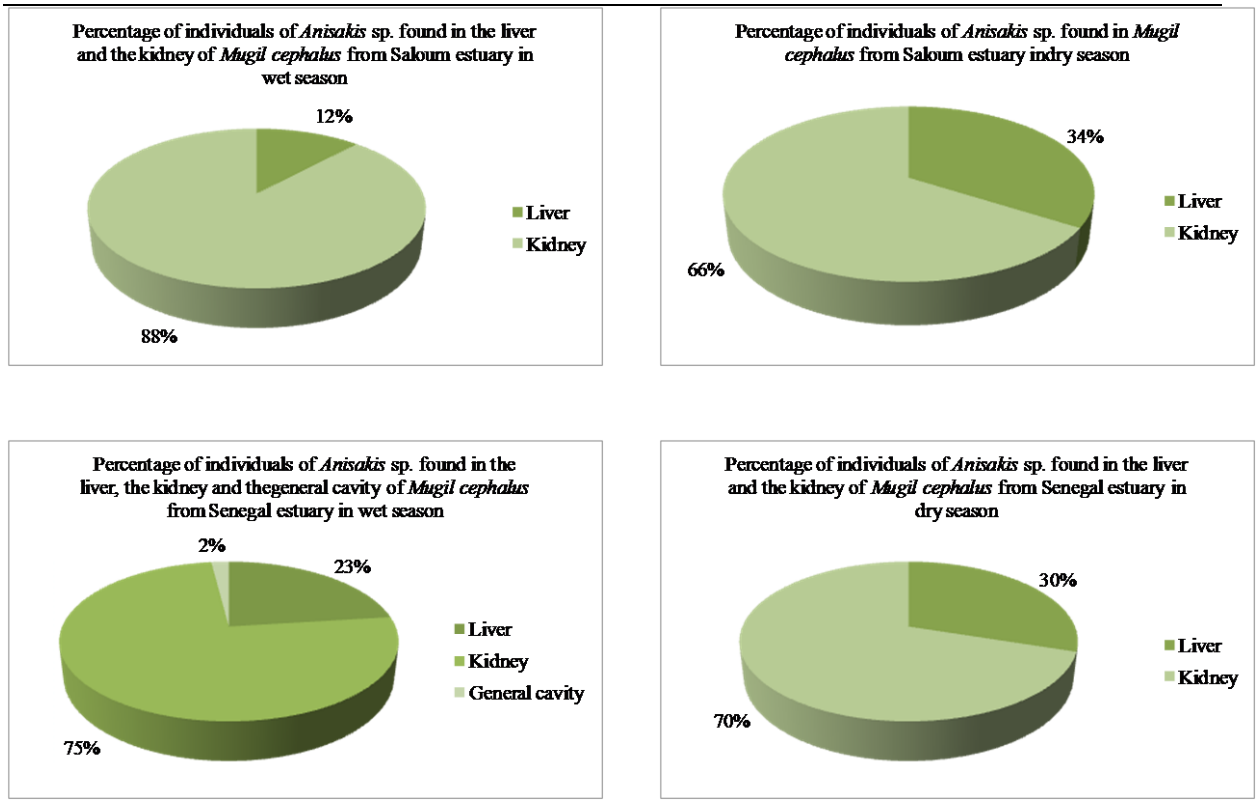


Figure 1. Percentage of individuals of *Anisakis* sp. according to the site of infection and the season in Saloum and Senegal estuaries.

3.2 Comparison of the Prevalence and the Mean Intensity of Anisakid Larvae in Populations of Mugil Cephalus in the Two Estuaries

In Saloum estuary, larvae of *Anisakis* sp. were found in the liver and the kidney of *Mugil cephalus* with a prevalence of 52.94% in rainy season and 39.33% in dry season. In the estuary of Senegal, this parasite presented a prevalence of 92.94% in the wet season and 77.61% in the dry season. Thus the prevalence of *Anisakis* sp. is higher in the estuary of Senegal than in the Saloum estuary and this whatever the season (Figure 2.a).

As for the prevalence of *Anisakis* sp., we noted a mean intensity higher in the estuary of Senegal than in the estuary of Saloum (p=0.000364). The same observation was done in both seasons. In the estuary of Saloum, the mean intensity was more significantly important in wet season (1.48 ± 0.64) than in the dry season (1.17 ± 0.38) (p=0.03178). In the estuary of Senegal, the mean intensity of *Anisakis* sp. noted in wet season (2.11 ± 1.4) was not significantly different from that recorded in the dry season (1.81 ± 1.14) (p=0.1860) (Figure 2.b).

Larvae of *Contracaecum* sp. were only found in the mesentery of *Mugil cephalus* with a prevalence of 22.45% in rainy season and 38.75% in dry season in the Saloum estuary while in the estuary of Senegal it was equal to 6.66% in rainy season and 25.23% in the dry season (Figure 2.c).

In each locality, the mean intensity of this parasite was higher in the dry season than in the wet season. In the estuary of the Saloum delta, its value was 12.48 ± 15.65 in the dry season and 3.09 ± 1.81 in the rainy season. The difference between these values is significant (p=0.000). In the estuary of Senegal, the parasitic load was of 5.93 ± 7.16 in the dry season and 3.5 ± 2.78 in wet season. These mean intensity values are not significantly different (p=0.633) (Figure 2.d).

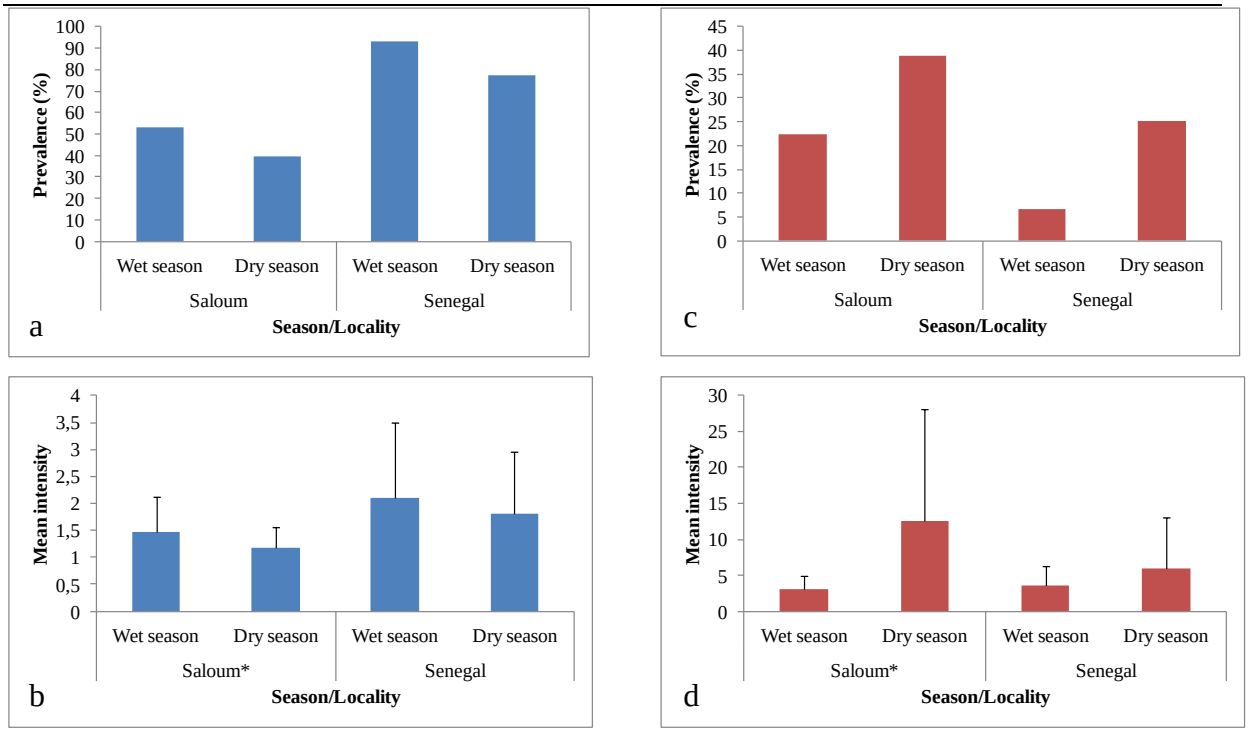


Figure 2. Prevalence and mean intensity of *Anisakis* sp. (a and b) and of *Contraceacum* sp. (c and d) according to the season and the locality. * = significant differences

3.3 Mean Abundance Variation of Anisakis Sp. and of Contracaecum Sp. in Populations of Mugil Cephalus

The variations of the mean abundance of *Anisakis* sp. and *Contracaecum* sp. were studied according to various variables in order to take into account the whole population of *Mugil cephalus* in the two estuaries.

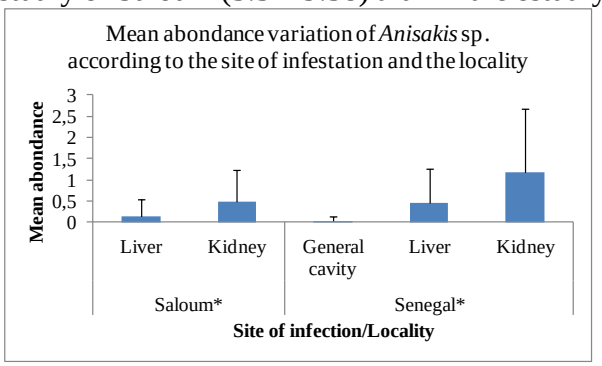
The examination of various organs of *Mugil cephalus* revealed, in the estuary of Saloum, the presence of *Anisakis* sp. larvae in the kidney and the liver with a higher mean abundance in the kidney (0.48 ± 0.74) than in the liver (0.15 ± 0.4) (Figure 3.a). The test of Kruskal-Wallis showed a highly significant difference between the above-mentioned sites (p=0.000). In the estuary of Senegal, the larvae were found in the kidney (1.51 ±1.57), the liver (0.59 ±0.87) and the general cavity (0.02 ±0.13) (Figure 3.a), with also a higher mean parasitic abundance in the kidney than in the other organs (p=0.000).

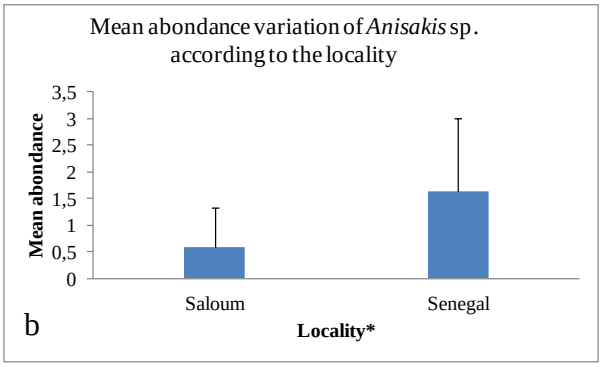
The mean abundance of *Anisakis* sp. was significantly higher in the estuary of Senegal (1.64 ± 1.36) than in the Saloum estuary (0.58 ± 0.74) (p=0.000) (Figure 3.b) whereas for *Contracaecum* sp., found in the mesentery only, the mean abundance was significantly higher in the estuary of Saloum (3.26 ± 9.25) than in the Senegal estuary (1.04 ± 3.64) (p=0,002) (Figure 3.e). This ecological parameter has known a reverse seasonal variation according to the parasite. Thus, *Anisakis* sp. has a mean abundance significantly higher in the rainy season (Saloum estuary: 0.78 ± 0.88; Senegal estuary: 1.96 ± 1.45) than in the dry season (Saloum estuary: 0.46 ± 0.62; Senegal estuary: 1.42 ± 1.25) with significant differences (Saloum estuary: p=0,038; Senegal estuary: p=0,004) (Figure 3.c). On the other hand, the mean abundance of *Contracaecum* sp. in the dry season (Saloum estuary: 4.84 ± 11.42; Senegal estuary: 1.50 ± 4.42) was higher than that obtained in the wet season (Saloum estuary: 0.69 ± 1.54; Senegal estuary: 0.23 ± 1.11) (Figure 3.f). The test of Wilcoxon showed significant differences in the estuary of Saloum (p=0,008) and in that of Senegal (p=0,003).

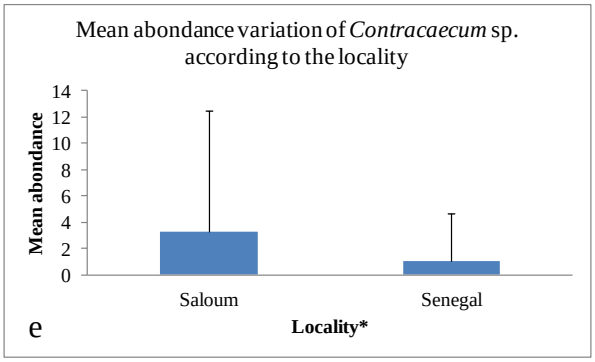
The population of *Mugil cephalus* caught in the Saloum estuary was composed by females and males, while those coming from the Senegal estuary were made up of males and individuals with indeterminate sex. In the estuary of Saloum, the mean abundance of *Anisakis* sp. in the females (1.25 ± 0.96) was higher than that obtained in the males (0.56 ± 0.73), but the difference was not significant (p=0,092) while for *Contracaecum* sp., it was the reverse (females: 2 ± 3.67; males: 3.3 ± 9.38), with also a non significant difference (p=0,636). In the estuary of Senegal, these parasites had a mean abundance higher in the males (*Anisakis* sp.: 1.67 ± 1.44; *Contracaecum* sp.: 1.65 ± 5.44) than the individuals with unspecified sex (*Anisakis* sp.: 1.61 ± 1.30; *Contracaecum* sp.: 0.64 ± 1.55) (Figures 3.d and 3.g). However, the test of Wilcoxon showed nonsignificant differences of the mean abundance of *Anisakis* sp. (p=0,918) and of that of *Contracaecum* sp. (p=0,507) between the males and unspecified sex individuals.

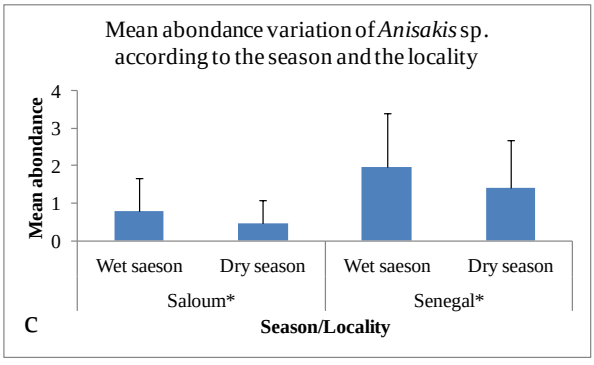
A comparison of parasitic mean abundance between individuals of the same sex from these estuaries will be impossible except for the males. The mean abundance of *Anisakis* sp. noted in the males of *Mugil cephalus* from the estuary of Senegal (1.67 ± 1.44) was higher than that obtained in the males caught in the Saloum estuary (0.56 ± 0.73) (Figure 3.d.), with a highly significant difference (p=0,000). For *Contracaecum* sp., mean abundance was significantly higher in the estuary of Saloum (3.3 ± 9.38) than in the estuary of Senegal (1.63 ± 5.4) (p=0,013) (Figure 3.g.).

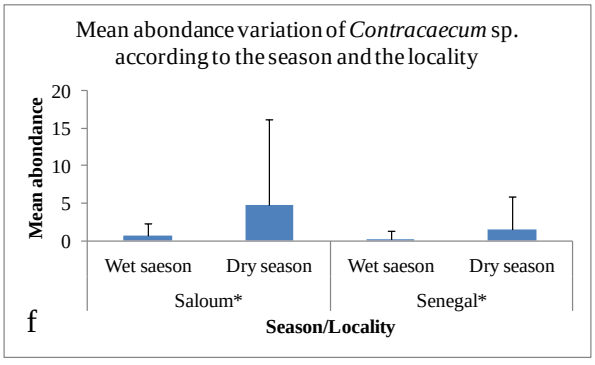
a

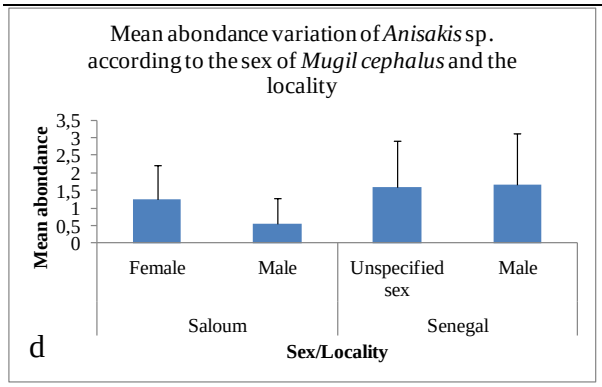












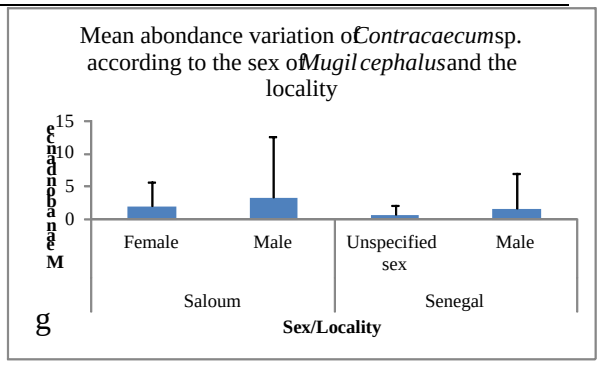
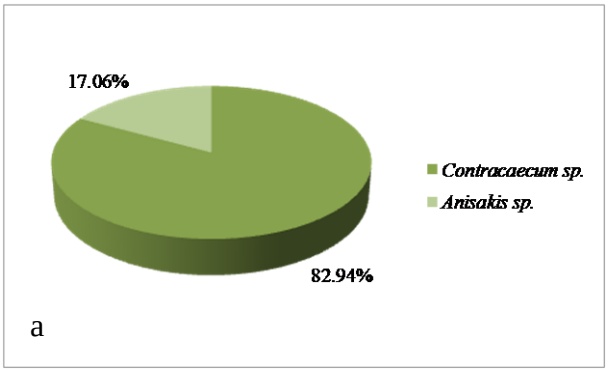


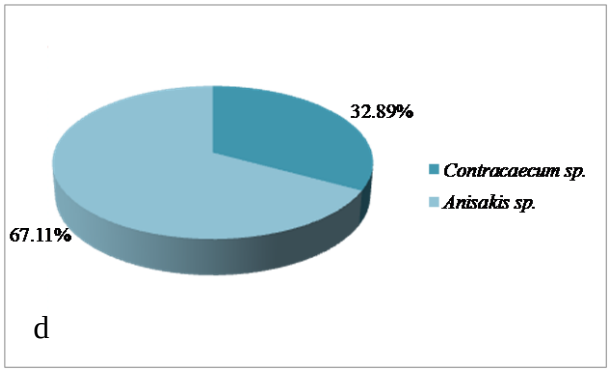
Figure 3: Mean abundance variation of *Anisakis* sp. (a, b, c and d) and of *Contracaecum* sp. (e, f and g). * = significant differences

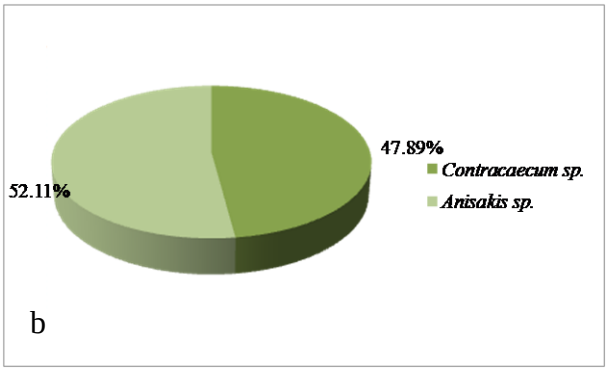
3.4 Dominance Index of Parasitic Species

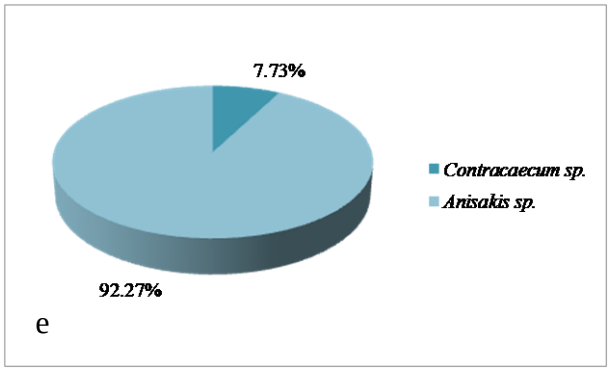
In the component community, the dominance of parasitic species varied according to the locality and the season. Indeed, in the estuary of Saloum, *Contracaecum* sp. was overall dominating (Figure 4.a). But considering the seasons, this nematode dominates *Anisakis* sp. in the dry season (Figure 4.c) while in the rainy season, *Anisakis* sp. becomes dominating (Figure 4.b).

In the Senegal estuary, *Anisakis* sp. was dominating throughout the year (Figure 4.d, e and f).









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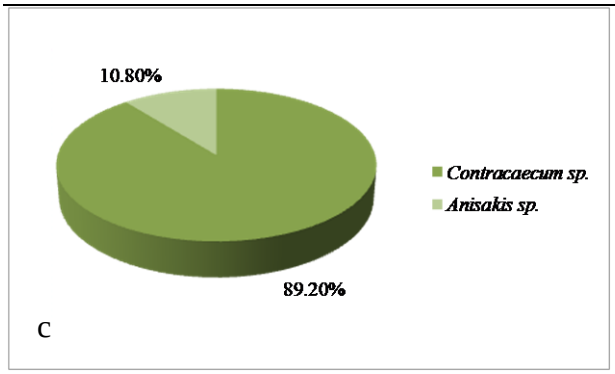

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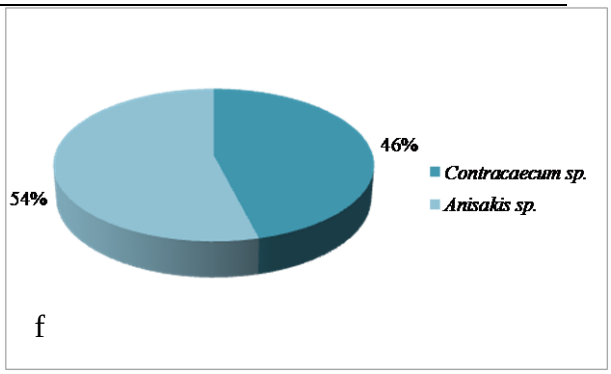

f

Figure 4: Spectrum of dominance in the estuaries of Saloum (a : all year, b: rainy season and c: dry season) and of Senegal (d : all year, e: rainy season and f: dry season)

4. Discussion

In the Saloum and Senegal estuaries, the percentage of *Anisakis* sp. in the kidney or the general cavity was higher in the wet season than in the dry season whereas in the liver, it was higher in the dry season than in the rainy season. Moreover, whatever the season or the locality, the kidney lodged much more worms than the liver or the general cavity. Larvae of *Contracaecum* sp. infected the mesentery of *Mugil cephalus* only with a low prevalence in the estuary of the Senegal estuary and more or less high prevalence in the estuary of Saloum depending on the season. Work on Anisakidae revealed that these worms have been more present in fatty tissue (Lymbery and al., 2002; Strømnes and Anderson, 2003; Abattouy and al., 2011). The presence of *Anisakis* sp. and *Contracaecum* sp. larvae in the general cavity, the kidney and the liver and in the mesentery of *Mugil cephalus* respectively showed that these vital organs have had an important lipid rate. Indeed, the mugilids are fatty fishes (Vallet, 2011) which have the characteristic to distribute their lipids in the fatty cells through all their body mainly in the gonads (lipidic globules of the ovaries), in hepatic tissue, in muscular tissue and in fatty visceral tissue (mesentery). The lipids are mainly stored in the form of triglycerides (Ackman, 1995).

The high prevalence of the larvae of *Anisakis* sp. in the populations of *Mugil cephalus* from Saloum and Senegal estuaries means that this nematode is frequent in these two host populations and it is even more frequent in the estuary of Senegal than in the estuary of Saloum. Moreover, *Anisakis* sp. is more frequent in the wet season than in the dry season in the two localities. Prevalence values of *Contracaecum* sp. generally lower than 40%, mean that this nematode is not frequent in the populations of *Mugil cephalus* from the two estuaries.

The values of the mean intensity and the mean abundance of the parasites were generally low in the two estuaries, but for *Anisakis* sp., they were higher in the estuary of Senegal than in that of Saloum while for *Contracaecum* sp., it was the reverse. In each locality, they were also higher in the dry season than in the rainy season for *Contracaecum* sp. whereas for *Anisakis* sp., the values of the mean intensity and the mean abundance found in wet season were higher than those obtained in the dry season. These results are in agreement with those of Juliana et al., (2012) who showed, for *Anisakis* sp., a mean intensity and a higher mean abundance in wet season, particularly in August.

Variations observed in the prevalence, the mean intensity and the mean abundance of *Anisakis* sp. and *Contracaecum* sp. between the two localities could be explained by certain physical (temperature) and chemical (salinity) conditions. In fact, the estuary of Senegal is a normal estuary, with an even worthless very low salinity in rainy season or bordering that of sea water in dry season. Whereas in the estuary of Saloum, salinity is slightly lower than that of sea water in wet season because of contributions of rain waters (Simier et al., 2004; Gning, 2008) and is much higher than that of sea water in the dry season. In the two estuaries, the temperature of water is higher in the wet season than in the dry season; with a maximum in august (Simier et

al., 2004) and it is all the more important in the estuary of Saloum. These ecological factors of the environment have an effect on the time of hatching and/or the lifetime of the larvae of *Anisakis* sp. (Højgaard, 1998). Indeed, the temperature has a significant effect on the time of hatching and the lifetime of the larvae i.e., the more the temperature increases, the more the time of hatching and the lifetime of the larvae decrease and vice versa. On the other hand salinity influences the lifetime of the larvae of *Anisakis* sp. which only increases with it. The highest prevalence, mean intensity and mean abundance of *Anisakis* sp. in the estuary of Senegal are thus related to the low salinity of water. This salinity also explains the high values of the prevalence of *Anisakis* sp. in the rainy season in Saloum and Senegal rivers. On the contrary, relatively high values of the prevalence, mean intensity and mean abundance of *Contracaecum* sp. in the population of *Mugil cephalus* from Saloum river are related to the strong salinity of water. Thus we can say that the more the salinity of water is raised, the more the prevalence of *Contracaecum* sp. is high and the less that of *Anisakis* sp. is large.

The prevalence of *Contracaecum* sp. in Saloum estuary could also be explained by the strong presence of birds (pelicans, cormorants, herons, flamingos, hibis, spatulas…) (Diagana and Dodman, 2006), final hosts of the parasite (Barson and Marshall, 2004; Farjallah and al. 2008; Mattiucci and al., 2008; Garbin and al., 2011; Kanarek, 2011). In fact, with a mangrove swamp flourishing, the Saloum estuary offers a large variety of niches for the birds contrary to the estuary of Senegal. These final hosts will spread and deposit the eggs of the parasite in the environment. These eggs hatch in 2 to 3 days with 24 ℃ or in 5 to 7 days with 21 ℃ (Al-Zubaidy, 2009). The L2 larvae will infest the first intermediate host which is usually a shellfish (Paperna, 1974; Al-Zubaidy, 2009) before arriving at fish via the food chain (Al-Zubaidy, 2009; Abowei and Ezekiel, 2011). The arrival of migratory birds in the dry season could also increase the number of emitted eggs and consequently, the infections of the intermediate hosts, which would explain the higher values of prevalence, mean intensity and mean abundance of *Contracaecum* sp. in the dry season in the estuaries.

High prevalence and low values of mean intensity and mean abundance would correspond to a broad dispersion of *Anisakis* sp. and *Contracaecum* sp. larvae in the populations of *Mugil cephalus* (Costa et al. 2004).

5. Conclusion

We can therefore conclude that the larvae of *Anisakis* sp. and *Contracaecum* sp. are more common in the estuaries of Senegal and Saloum respectively. Their mean intensity and mean abundance are in relation to the locality, the season and the site of infection. The analysis of the dominance index makes it possible to associate the geographic origin of the host to the dominating nematode genus i.e., a fish coming from the estuary of Senegal lodges more larvae of *Anisakis* sp. than larvae of *Contracaecum* sp. and if it comes from the estuary of Saloum river, it will lodge much more *Contracaecum* sp. than *Anisakis* sp. larvae. That makes it possible to say that the genera *Anisakis* and *Contracaecum* are respectively characteristic of Senegal and Saloum estuaries.

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