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OCCURRENCE OF PARASITES IN FISH MARKETED IN THE INEZGANE WHOLESALE MARKET AND THE FISHING PORT OF AGADIR, MOROCCO

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ABSTRACT: Based on importance of animal products safety, the purpose of this work was to assess the extent of parasitism at the wholesale market level of Inezgane and the fishing port of Agadir in Morocco. For this purpose, fieldwork aimed at direct investigation of parasites involved 366 fish pieces. This study was conducted in the period between March and June 2021. The prevalence of parasitism was 20.76%. The total number of parasites collected is 2385 including 1959 nematodes, 318 xenomas, 92 cestodes, and 16 isopods. An abundance of 6.51 and an overall intensity of 31.38. These infestation parameters varied by species and location of origin. For the qualitative analysis of the parasites, the study revealed a predominance of L3 larvae of the *Anisakis* nematode with a percentage of 82.14%. Xenomas had a percentage of 13.33%. As for the cestodes of *Gymnorhynchus gigas*, the larvae were collected from the Atlantic pomfret (*Brama brama*) with a percentage of 3.86%. As a result of this study, a significant positive correlation of r=0.81 was shown between the total length of the fish and the number of anisakids. The results of this study revealed that the extent of parasitism seems to be less pronounced in some species, but there is still a presence of concern.

Keywords: Anisakis, Fish, Fishing port, Parasitism, Wholesale market level

INTRODUCTION

Morocco has a significant fishing potential, benefiting from favorable hydro-climatic conditions that give Moroccan waters diversity and a recognized marine biological richness. Morocco is a fishing-oriented country, located according to the latest FAO data from 2020 at the 17th worldwide (FAO, 2020). Parasites of fish are a potential contamination source, thus generating the main reason for discards of Moroccan fish products and a risk to public health (Youssir et al., 2017; Biary et al., 2021). Indeed, the consumption of raw fish and other almost raw or uncooked culinary fish preparations (semi-cooked fish, marinated, cold-smoked) increases the risk of contamination of consumers of seafood products by certain foodborne parasites (Klimpel et al., 2019). Among those, *Anisakis* is one of the most important nematodes in terms of public health (Klimpel et al., 2019; Fiorenza et al., 2020).

The evolutionary cycle of anisakids is an indirect cycle, characterized by 4 larval stages with a phase in the external environment, in this case in sea water. The definitive hosts are marine mammals and piscivorous birds, the intermediate hosts are crustaceans and fish are paratenic hosts which transport the larvae (Chai et al., 2005; EFSA, 2010), humans are an accidental host.

MATERIAL AND METHODS

Fieldwork

The number of pieces of fish examined in this study is 366 representing 27 species. These samples were taken at the Inezgane wholesale market (n=215) and the fishing port of Agadir (n=151), between March and June 2021 (Table 1).

Morphometric study

Each piece of fish was subjected to a freshness inspection, the determination of weight using a precision scale, and the determination of morphometric parameters, i.e, total length (TL) and fork length (LF) using an ichthyometer.

-Total length: is measured from the tip of the fish snout to the extremities of the longest rays of the caudal fin (cm);

-Fork length: is measured from the tip of the fish snout to the cartilaginous tip of the shortest ray or mid-ray of the caudal fin (in cm).

Detection of parasites

This step involves looking for visible parasites that have a dimension, color or texture that clearly distinguishes them from fish tissue. In order to collect the parasites, we first carried out a visual examination for the presence of the ectoparasites of the skin, the oral cavity and the gills. For internal parasites, the following methodology was used:

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- A first incision of the abdomen of the fish from the anus to the head;
- Visual and magnifying inspection under a light source of viscera on site and mesentery;
- Gastrointestinal tract and gonads collected and placed in petri dishes and examined;
- Existing parasites collection and counting;
- A second longitudinal incision of the musculature;
- Visual and light inspection of fish flesh;
- Existing parasites collection and counting;
- The number, type and anatomical location of each parasite are recorded.

Data processing

The results are treated by calculating the following parasitic infestation parameters: prevalence (P), intensity (I), abundance (A) for different fish species based on the three preceding parameters (Bush et al., 1997):

- Prevalence = Number of fish infested with parasites/Number of fish examined ;
- Intensity = Number of parasites detected/Number of fish infested with parasites ;
- Abundance = Number of parasites detected/Number of fish examined.

Table 1 - Fish species examined at Inezgane wholesale market level and the fishing port of Agadir

Scientific name	Species	Number of fish examined
Trachurus trachurus	Atlantic horse mackerel	50
Pagellus acarne	Axillary seabream	24
Scomber scombrus	Atlantic mackerel	32
Brama brama	Atlantic pomfret	8
Lepidopus caudatus	Silver scabbardfish	8
Merluccius merluccius	European hake	26
Phycis phycis	Forkbeard	9
Umbrina cirrosa	Shi drum	5
Zeus faber	John dory	2
Sarda sarda	Atlantic bonito	2
Engraulis encrasicolus	European anchovy	97
Sardina pilchardus	European pilchard	57
Gadus capelanus	Poor cod	8
Pomadasys incisus	Bastard grunt	1
Plectorhinchus mediterraneus	Rubberlip grunt	5
Scorpaena scrofa	Red scorpionfish	4
Cepola macrophthalma	Red bandfish	4
Scyliorhinus stellaris	Nursehound	2
Mullus barbatus	Red mullet	2
Pagellus bellottii	Red pandora	3
Spondyliosoma cantharus	Black seabream	3
Diplodus vulgaris	Common two-banded seabream	1
Diplodus sargus	White seabream	2
Dentex macrophtalmus	Large-eye dentex	5
Dentex dentex	Morocco dentex	2
Trachinus draco	Greater weever	2
Trachinus vipera	Lesser weever	2
Total		366

RESULTS AND DISCUSSION

Of the 366 fish specimens examined, 76 are infested with parasites (nematodes, cestodes and xenomas), which means an absolute prevalence of 20.76%, an abundance of 6.51 and an intensity of 31.38. Table 2 shows the repartition of parasites found in the different fish species. These results indicate a polyparasitism with a predominance of nematodes (Figures 1 and 2).

With regard to parasite associations, we detected three nematode-xenoma associations in silver scabbardfish and axillary seabream. External parasites are located primarily in the oral cavity and the gills of forkbeard. Viscera and muscle flesh are the sites frequently infested by internal parasites. Xenomas that are present with a percentage of 13.33%, was detected in the axillary seabream and in a single piece of silver scabbardfish (*Lepidopus caudatus*) is a tissue response of the parasite in response to microsporidian infestation of fish. This happens when the parasite seeks to take control of the cell's metabolism, it provides protection against the host's immune response in the form of whitish cystic structures «called xenoma» visible to the naked eye, of variable appearance, implanted in the flesh and easily detachable.

Two aspects of xenomas are retained in this study according to the form, regularly spherical cysts of whitish colour which contain a liquid substance, and cysts of elongated shape, creamy colour with a friable wall (Figure 3). In forkbeard, isopods and *Anisakis* nematodes were detected in addition from the oral cavity and gills, in the abdominal cavity and mainly in the stomach of examined fish (Figures 4-7).

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Table 2 - Repartition of parasites detected from fish specimens examined

Type of parasites	Number of parasites	%
Nematodes (*Anisakis*)	1959	82.14
Xenomas	318	13.33
Cestodes (*Gymnorhynchus gigas*)	92	3.86
Isopods	16	0.67
Total	2385	100

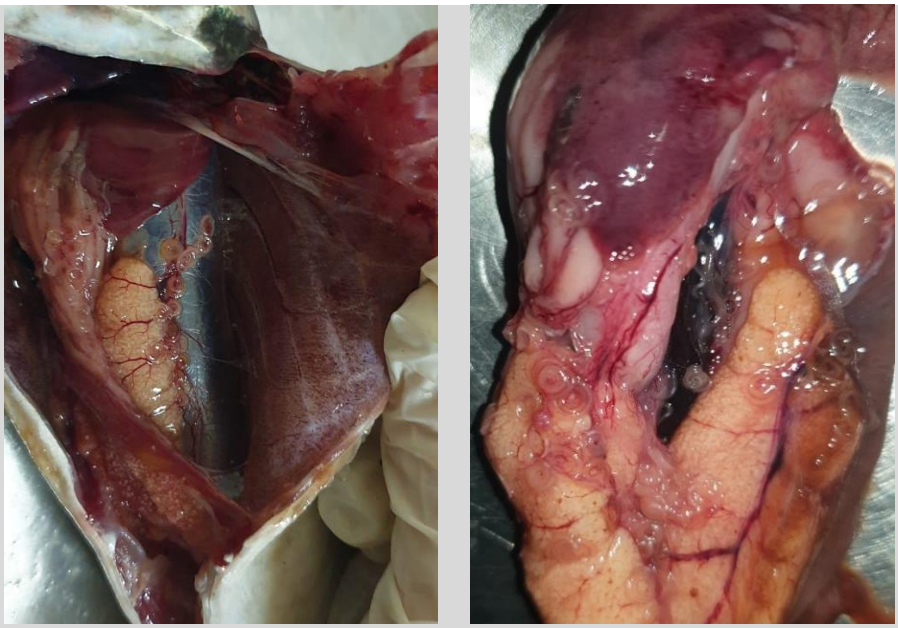

Figure 1 - Larvae of *Anisakis* in the viscera, liver and gonads of European horse mackerel (*Trachurus trachurus*)

Figure 2 - Larvae of *Anisakis* in silver scabbardfish intestine (*Lepidopus caudatus*)

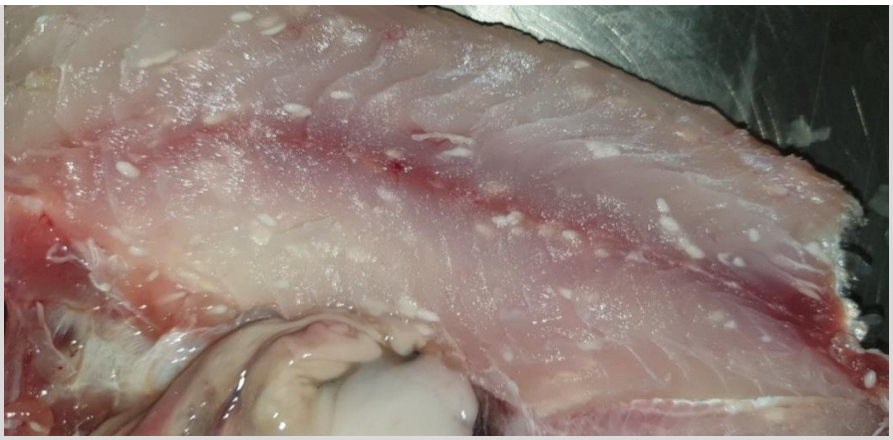

Figure 3 - Xenomas Housed in the chair of axillary seabream

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Figure 4 - Isopods in the buccal cavity of forkbeard (*Phycis phycis*)

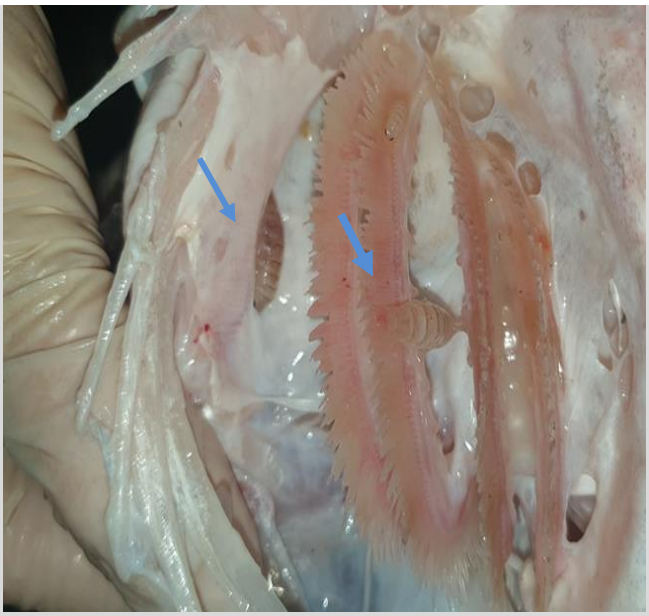

Figure 5 - Isopods in the gills of forkbeard (*Phycis phycis*)

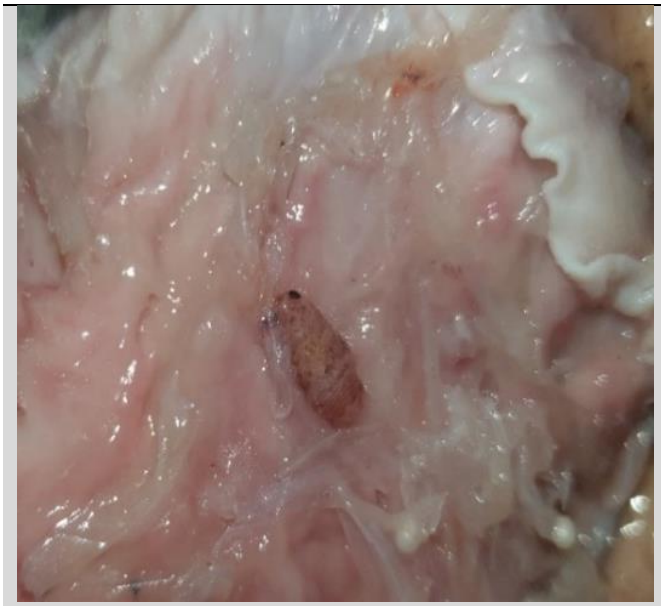

Figure 6 - Isopods in the abdominal cavity of forkbeard (*Phycis phycis*)

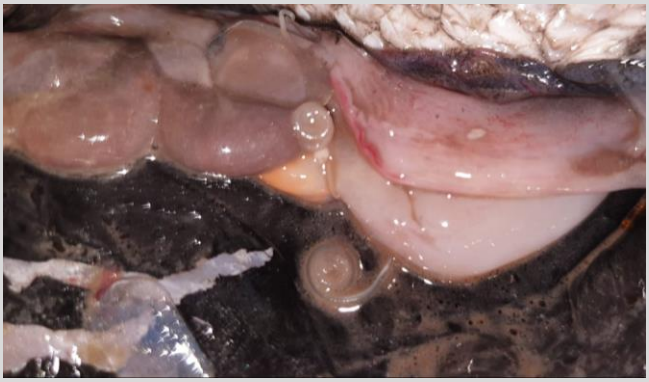

Figure 7 - Anisakis observed in the abdomen of forkbeard (*Phycis phycis*)

Study of the infestation parameters for the fish species examined

Of the 366 samples examined, 55 are infested with nematodes which mean a prevalence of 15.02%, an abundance of 5.35 and an intensity of 35.62. Fish sampled at the wholesale market level in Inezgane and the fishing port of Agadir has a maximum prevalence of nematodes of 100% recorded in silver scabbardfish. Table 3 presents the infestation parameters for the fish species examined. From the results obtained previously, we can classify fish species according to their prevalences into 6 categories (Table 4). 20 species of 27 examined (74.07%) were free of parasites.

For Atlantic mackerel, previous studies showed different prevalences, 67.9 (Abattouy et al., 2011), 60% (Lamane, 2013), and 30% (Benabbes and Boudakkou, 2019). These findings corroborate our current results, the Atlantic mackerel records a prevalence of 25% by classifying it as a low prevalence species. It can be concluded that parasitism in Atlantic mackerel has decreased on the Atlantic coast.

In this study, the European pilchard is shown to be free of parasites; this absence of parasites in this species has already been reported in previous studies on the Moroccan Atlantic coast (Lamane, 2013; Bainour, 2018; Ouakkouch, 2020). In the same context, a study by Biary et al. (2021) concluded a finding of absence of nematode parasites in European pilchard after visual inspection of samples. However, this same species is revealed in a previous study parasitized by isopods with a prevalence of 5.56% (Benabbes and Boudakkou, 2019). On the other hand, a study conducted in the northeastern Atlantic Ocean of Spain reported a prevalence of 2.5% of *anisakis* in European pilchard in the Cadiz zone and zero prevalence in the Isla Cristina zone in the same species (Molina-Fernández et al., 2015).

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Regarding anchovies, the prevalence of parasites is zero in this study, the same finding was made in the study conducted by Ouakkouch (2020), However one study reported a low prevalence in European anchovy (Bainour, 2018). These results can be explained by the fact that European pilchard and anchovies feed much more on copepods. They are only at risk of infestation after sufficient growth and feed on large plankton such as euphausiaceae (Lamane, 2013). Recently, one study reported the presence of *anisakis* larvae in commercially available anchovy oil products (Smaldone et al., 2020).

At the wholesale market level of Inezgane, the cestodes «*Gymnorhyncus gigas*» were found only in the atlantic pomfret «*Brama brama*» with a prevalence of 100% on 8 pieces of atlantic pomfret examined. The total number of parasites found in these 8 pieces of fish is 92. The 100% prevalence of cestodes in the atlantic pomfret is not a surprising result. Indeed, this result is corroborated by other results that also found a prevalence of 100% (Ouakkouch, 2020). A study reported a prevalence of 89.47% (Benabbes and Boudakkou, 2019). The 2013 study reported that Spanish researchers found that almost 100% of sea breams (*Brama brama*) were parasitized by this parasite (Lamane, 2013).

Of 24 pieces of axillary seabream examined, 13 pieces was infested with xenomas, a prevalence of 54.16%. The total number of parasites found in these 13 pieces of fish is 318.

Table 3 - Prevalence, abundance and intensity by fish species of nematodes

Species	Number of fish examined	Number of fish infested	Number of nemotodes	P (%)	A	I
Atlantic horse mackerel	50	25	1318	50	26.36	52.72
Atlantic mackerel	32	8	28	25	0.87	3.5
Silver scabbardfish	8	8	507	100	63.37	63.37
European hake	26	7	22	26.9	0.85	3.14
Forkbeard	9	5	72	55.5	8	14.4
Shi drum	5	1	8	20	1.6	8
John dory	2	1	4	50	2	4
Atlantic pomfret	8	8	0	-	-	-
Axillary seabream	24	13	0	-	-	-
Atlantic bonito	2	0	0	-	-	-
European anchovy	97	0	0	-	-	-
European pilchard	57	0	0	-	-	-
Poor cod	8	0	0	-	-	-
Bastard grunt	1	0	0	-	-	-
Rubberlip grunt	5	0	0	-	-	-
Red scorpionfish	4	0	0	-	-	-
Red bandfish	4	0	0	-	-	-
Nursehound	2	0	0	-	-	-
Red mullet	2	0	0	-	-	-
Red pandora	3	0	0	-	-	-
Black seabream	3	0	0	-	-	-
Common two-banded seabream	1	0	0	-	-	-
White seabream	2	0	0	-	-	-
Large-eye dentex	5	0	0	-	-	-
Morocco dentex	2	0	0	-	-	-
Greater weever	2	0	0	-	-	-
Lesser weever	2	0	0	-	-	-
	366	55	1959	15.02	5.35	35.62

P: Prevalence, A: Abundance, I: Intensity

Table 4 - Classification of fish species by prevalence of nematodes

Category	Prevalence (%)	Species
Very high prevalence	>80-100	Silver scabbardfish
High prevalence	>60-80	-
Moderate prevalence	>40-60	Forkbeard, atlantic horse mackerel, john dory
Low prevalence	>20-40	European hake, atlantic mackerel
Very low prevalence	>0-20	Shi drum
Zero prevalence	0	Axillary seabream, atlantic pomfret, atlantic bonito, european anchovy, european pilchard, poor cod, bastard grunt, rubberlip grunt, red scorpionfish, red bandfish, nursehound, red mullet, red pandora, black seabream, common two-banded seabream, white seabream, large-eye dentex, morocco dentex, greater weever, lesser weever

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Relationship between size and parasitism

The difference in the prevalence of parasitism in the fish examined leads us to the existence of a possible relationship between the size of fish and the number of *Anisakis* infesting the fish. Analysis of the sizes and numbers of anisakids collected from the examined fish of Atlantic horse mackerel reveals a significant correlation of R^2=0.44, which means the presence of a positive linear relationship between fish size and parasitic load (Figure 7). That is, parasitism increases with the size of the fish. This finding of increasing intensity of anisakid infestations gradually with fish size has also been reported in other studies (Bouchriti et al., 2015; Dahani et al., 2019; Ouakkouch, 2020). This is explained by the cumulative effect of parasites in the host during its lifetime (Abattouy et al., 2011). Another study reported that the direct relationship between infestation level and age or length in Atlantic horse mackerel is a widespread phenomenon in many fish species (Shawket et al., 2017).

Relationship between weight and intensity

In addition to fish size, it is interesting to review the number of parasites per kilogram of fish examined. Weight is also a factor in the variation of the parasitic risk, a positive correlation (R^2= 0.26) is shown between the weight of the fish (Atlantic mackerel) and the number of parasites isolated (Figure 8). Weight is also a risk variation factor impacting the infestation, corroborated by previous results which also reported a positive correlation between weight and parasitic intensity r=0.59 (Ouakkouch, 2020). A probability is raised of the increase in the intensity of anisakids in the muscle with the increase in weight, an explanation could be that the increase in weight is often related to the increase in fat, to which the larvae of anisakis migrate (Abattouy et al., 2011; Mo et al., 2021).

Comparative analysis with previous studies

Table 5 shows a comparison of the different work carried out on the study of the extent of coastal parasitism since 2013. The difference in infestation parameters is mainly due to the number of samples examined for each study.

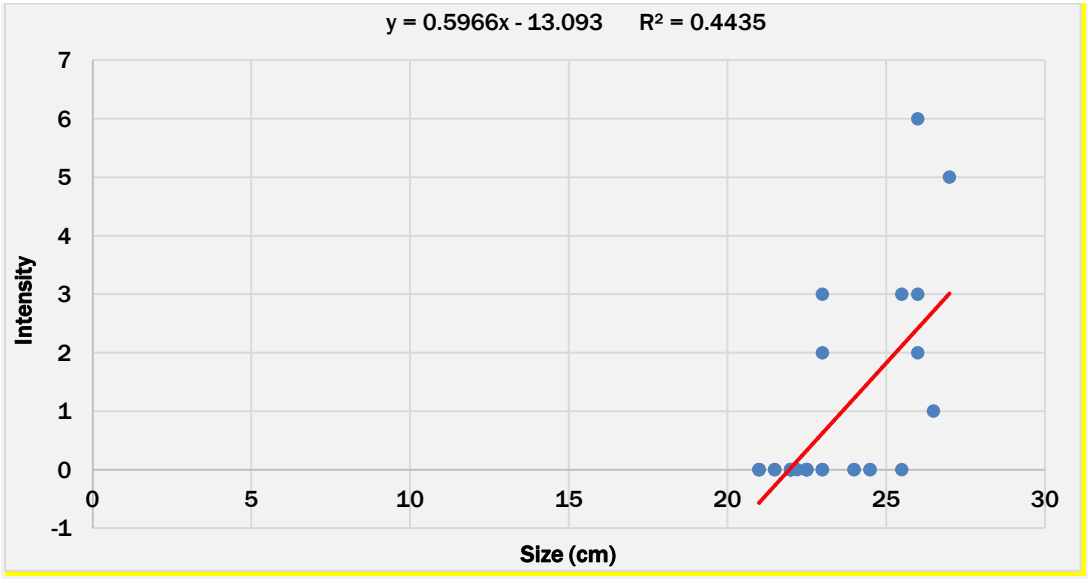

Figure 7 - Trend curve between fish size and nematode intensity.

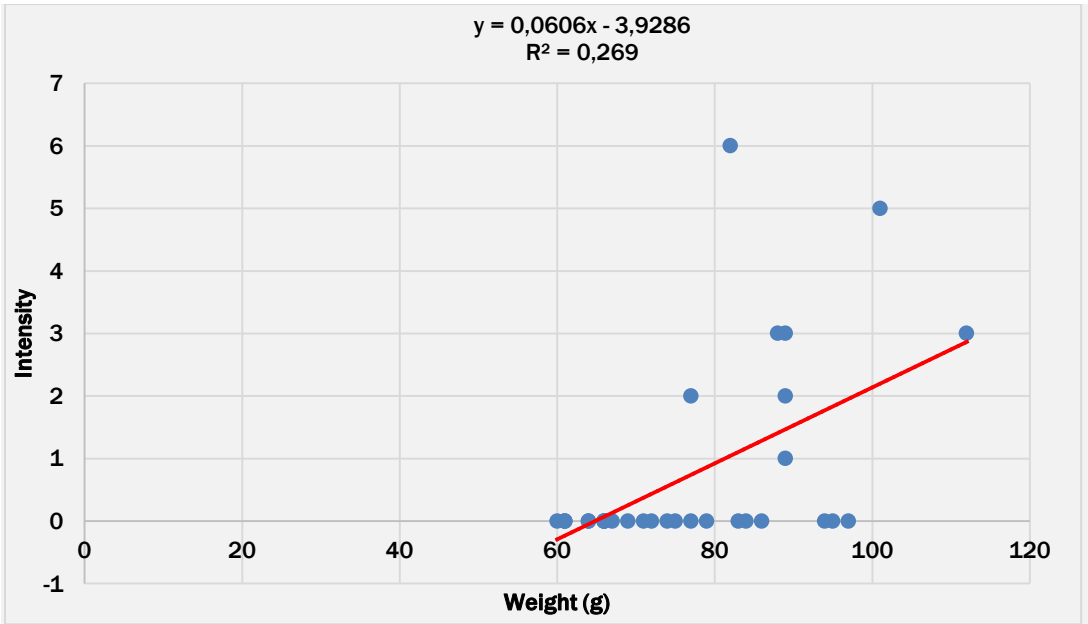

Figure 8 - Trend curve between weight and number of anisakids.

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Table 5 - Comparative analysis with previous studies

References \ Infestation parameters	Prevalence (%)	Abundance	Intensity
Present study	20.76	6.51	31.38
Ouakkouch (2020)	13.36	3.77	28.24
Benabbes and Boudakkou (2019) Atlantic	18.80	3.49	18.58
Benabbes and Boudakkou (2019) Mediterranean	24.8	0.79	3.2
Bainour (2018)	14.69	0.51	3.51
Lamane (2013)	38.16	8.67	22.73

***** The number of fish samples differs during the year according to availability.

CONCLUSION AND RECOMMENDATIONS

The study of parasitism at wholesale market of Inezgane and the fishing port of Agadir found that the markets had an absolute prevalence of 20.76% and 74.07% of the species examined were free of parasites. The species considered to be highly parasitized are the Atlantic pomfret and the silver scabbardfish, which is the most risky species by nematode infestation. The Atlantic pomfret has a prevalence of 100% of cestodes. The intensity of parasitism is correlated with the size and weight of the fish. Given the results of this study, it would seem interesting in the future to explore the following points: The xenomas detected in the axillary seabream require a more specific study in order to identify the infestation of parasites. The Atlantic pomfret requires further research as it is the species most affected by the cestodes "*Gymnorhynchus gigas*".

The results of this study are of great importance for the competent risk management authorities as well as for the processing establishments of fishery products which are invited to further strengthen the search for parasites in highly parasitized species, namely Atlantic pomfret and the silver scabbardfish. It is recommended to plan a study at the national level which will take into account several parameters, in particular the variations of parasitism according to the fishing zones and according to the seasons and the results of this study will allow the risk managers to take more management measures adapted even from a regulatory point of view, namely the prohibition of certain highly parasitized fish species in certain periods of the year or in certain fishing areas.

DECLARATIONS

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Author participations

All the authors contributed to the examination of fish, the results analysis and the writing of the final manuscript.

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Conflict of interest

The authors have declared that no competing interest exists.

Ethical regulation of Study

Not applicable. We have worked on marketed fish.

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