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ASSESSING THE NEMATODE INFESTATION DEGREE OF COMMERCIAL CLUPEIDS AT THE ROMANIAN COAST

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ABSTRACT

This paper presents the results of parasitology research on the nematode infestation of the main commercial fish species at the Romanian coast belonging to the family Clupeidae: *Alosa tanaica* Grimm, 1901 (Azov shad), *Alosa immaculata* Bennett, 1835 (Danube shad) (synonym *Alosa pontica pontica* Eichwald, 1838) and *Sprattus sprattus* Linnaeus, 1758 (sprat).

The investigations were performed during 2012-2013 on fish caught along the Romanian coast from Sulina to Vama Veche, recording the following nematode worm species *Contracaecum aduncum*, *Contracaecum sp.*, *Anisakis sp.*, *Porrocaecum sp.*, infesting the abdominal cavity of fish, free or fixed on internal organs, mainly the liver. *Contracaecum aduncum* was also identified as an adult in a low number inside the intestine. The dominant species were *Contracaecum aduncum*, *Contracaecum sp.* and *Porrocaecum sp.*, along which, sporadically, *Anisakis sp.* was also identified.

The parasite infestation degree, assessed by the values of parasitization intensities (number of parasites/fish) and parasitization extensions (% of fish infested of the total analyzed), was rather high (maximum values up to 20-50 parasites/host in 80-100% of the analyzed fish).

The Danube shad was the most parasitized, actually all analyzed individuals were infested by nematode worms, with intensities ranging between 10 and 50 parasites/host. The Azov shad showed parasitization extensions of 20-50% and sprat 20-60%, with intensities below 30 parasites/host, larger individuals being generally more parasitized than the smaller ones.

KEYWORDS: parasites, nematodes, clupeids, commercial species, Romanian coast

AIMS AND BACKGROUND

Fish, as well as other aquatic and terrestrial animals, may be affected by infectious diseases and parasitization. Parasitic diseases are caused by animal parasites - protozoans, worms and crustaceans.

Nematode worms are large group of parasites, being mostly encountered in marine fish. Some nematode species infest fish as adults, while others as larvae; for the latter, the final hosts are various fish-eating vertebrates (predator fish, amphibians, reptiles, birds, mammals). Fish are either the intermediate host of nematodes, or the secondary and accumulation host (Munteanu & Bogatu, 2003).

Nematodes are spread by fish carrying such parasites through their feeding and breeding migrations, through intermediate hosts, water (in which parasites often survive and breed), as well as through direct contact (Sinderman, 1987).

The occurrence of nematode infestations is also determined by environmental conditions which may act both on the worms and the hosts, large agglomerations, food and fish migrations (Anderson & Gordon, 1982; Sinderman, 1987, Bagge et al., 2004).

There is a tight connection between parasites and host, based mainly on the fact that parasites cause the increase of fatalities in hosts (Munteanu & Bogatu, 2003, Lester, 2010). There are, however, cases in which a balance sets between parasites and hosts, but it depends on the intensity of parasitization, immunity of the hosts to parasites and the general living conditions of the hosts.

Nematode worms are encountered in almost all marine species, among which clupeids are one leading group. Starting with the 1970s, research was performed in the North Sea, which pointed-out high infestation degrees of some fish species by nematode worms. Thus, the death of sprat at a parasitization intensity of 300 worms of the species *Contracaecum sp./host* (Gaevscaia & Kovaleva, 1975) was reported. Relevant research showed the occurrence of nematodes, in significant intensities, in clupeids in the Black Sea and the Caspian Sea (Barzegar et al., 2012, Zaharia et al., 2012). In fish infested mainly by nematode larvae, severe liver illnesses were reported, as well as the significant reduction of size, hemorrhages, reduction of the fat content in the liver, weight loss and reduction of the weight gain coefficient of fish.

MATERIAL AND METHODS

In order to identify the nematodes, 3 clupeid species were studied (*Alosa tanaica* Grimm, 1901 - Azov shad, *Alosa immaculata* Bennett, 1835 - Danube shad (synonym *Alosa pontica pontica* Eichwald, 1838) and *Sprattus sprattus* Linnaeus, 1758 - sprat), fished during the warm seasons of 2012-2013, along the Romanian coast, from Sulina to Vama Veche. The samples were represented by 10 fish individuals for each species.

The examination of fish was made both macroscopically and microscopically, aiming at the same time to identify the parasites and the reactions they cause in the hosts (Amlacher, E., 1981; Munteanu & Bogatu, 2003).

The macroscopic examination was made by naked eye, on the oral cavity, gills and abdominal cavity, where each organ was studied to emphasize potential necrosed areas, cysts, parasites, color changes and other modifications visible by naked eye.

The microscopic examination, which is the most important in order to identify nematode infestations, was made with a Zeiss Axio Imagea A1 microscope, equipped with a photo camera. The parasites were viewed with a 10 lens and the 5 and 10 oculars. For the microscopic examination, *full preparations* were used (small portions of tissues and organs), as well as *crushed preparations (squashes)* between the blade and the lamella, making the film formed translucent and as thin as possible, allowing the sighting of potential parasites.

The parasitization degree of fish was determined by the values of parasitization intensities (number of parasites/host) and parasitization extension (number of infested fish), for each fish sample and the overall samples analyzed.

RESULTS AND DISCUSSION

In the three clupeid species analyzed during 2011-2012, sprat, Azov shad and Danube shad, the following nematode species were identified: *Contracaecum aduncum* Rudolphi, 1805, *Contracaecum sp.* Railliet et Henry, 1012, *Porrocaecum sp.* Railliet et Henry, 1912 and *Anisakis sp.* Dujardin, 1845 (***, 1975).

Contracaecum aduncum Rudolphi, 1805, is a nematode worm belonging to the family Anisakidae, filament-shaped, creamy-white in color. The head end is hexagonal and surrounded by 3 lips, the stomach is provided with two blind appendices, the stomach one facing towards the rear part and the intestinal one facing towards the front part (Photo 1).

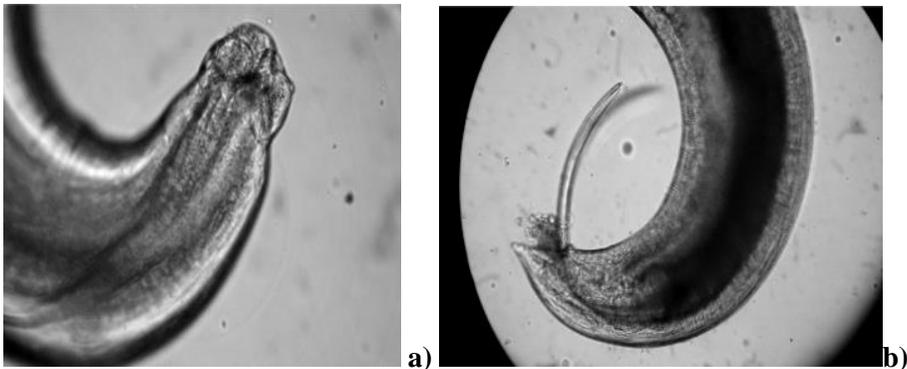


Photo 1 - *Contracaecum aduncum* (a - front part; b - rear part in male)
(original photo)

The pathogenic character of this parasite consists in the obstruction and damage of the digestive duct, harming and deranging the functions of organs parasitized by larvae. In case of weak invasions, fish can bear the infestation, its effect consisting in slowing-down the growth rhythm of fish. Severe infestations may cause the death of fish.

Contracaecum sp. Railliet et Henry, 1012, is a creamy-white nematode, with a slightly rounded head end, provided with primitive lips, and a conical rear part, ending with a small spine; the two stomach appendices are rather equal (Photo 2).

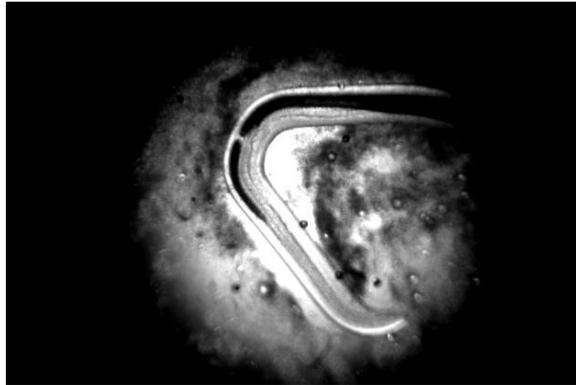


Photo 2 - *Contracaecum* sp. (original photo)

Contracaecum aduncum infests the viscera, encapsulated or free in the abdominal cavity, intestine, pyloric appendices and liver of fish.

Porrocaecum sp. Railet et Henry, 1912, is a nematode worm with a length usually ranging between 10-30 mm, brownish, truncated in the rear part, conical and ended with a small spine in the rear part. *Porrocaecum* sp. is located in the muscle tissue close to the abdominal cavity of fish, the number of parasites increasing along with the size of fish (Photo 3).



Photo 3 - *Porrocaecum* sp. (a - front part; b - rear part) (original photo)

Anisakis sp. Dujardin, 1845 usually parasitizes fish as larvae, which are easily recognizable due to their grayish, slightly transparent color. Their front ending is rounded, while the rear part is conical, ending with a sharp spine. The head ending is protected by a conjunctive shield, armed with a small penetration “tooth“, placed obliquely (Photo 4).

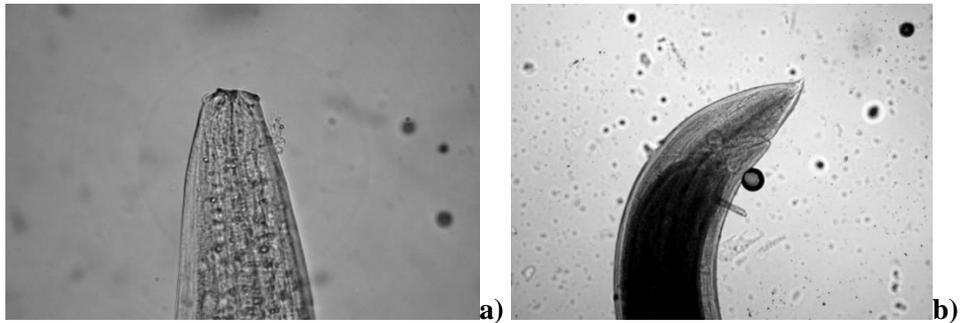


Photo 4 - *Anisakis* sp. (a - front part; b - rear part) (original photo)

Immobile nematode worms parasitize encapsulated in the liver, the pyloric appendices and/or mobile, migrating in various organs of the body, in muscles, gonads etc.

The nematode infestation degree by these parasite species, followed by the values of parasitization intensity (number of parasites/host) and the parasitization extension (number of parasitized fish) varied from one species to the other (Table 1).

Table 1 - Nematode infestation degree of clupeids

Fish species/ No. of analyzed individuals/year	Parasite species	Invasion extension % infested fish			Invasion intensity No. of parasites/host		
		Min.	Max.	Mean	Min.	Max.	Mean
1	2	3	3	5	6	7	8
Sprat / 100 / 2012	<i>Contracaecum aduncum</i>	0	20	6	0	4	2
	<i>Contracaecum</i> sp.	20	60	40	2	12	6
	<i>Porrocaecum</i> sp.	10	60	30	2	8	4
	<i>Anisakis</i> sp.	0	10	4	1	4	2
Sprat / 50 / 2013	<i>Contracaecum</i> sp.	30	60	40	1	8	2
	<i>Porrocaecum</i> sp.	10	40	20	1	2	1
Azov shad / 50 / 2012	<i>Contracaecum aduncum</i>	10	40	20	2	8	4
	<i>Contracaecum</i> sp.	20	100	50	5	12	8
	<i>Porrocaecum</i> sp.	30	60	45	2	10	6
	<i>Anisakis</i> sp.	0	10	2	1	4	2
Danube shad / 20 / 2012	<i>Contracaecum aduncum</i>	40	40	40	1	8	6
	<i>Contracaecum</i> sp.	80	80	80	5	30	14
	<i>Porrocaecum</i> sp.	60	60	60	4	20	8
	<i>Anisakis</i> sp.	10	10	10	2	2	2
Danube shad / 10 / 2013	<i>Contracaecum aduncum</i>	20	20	20	1	5	3
	<i>Contracaecum</i> sp.	60	60	60	5	25	15
	<i>Porrocaecum</i> sp.	40	40	40	4	16	8

Sprat

Sprat, the fish species most often reported in catches at the Romanian coast, was infested in 2012 by nematode worms of the genera *Contracaecum*, *Porrocaecum* and *Anisakis*, located in the abdominal cavity. The parasitization degree was high, reaching a maximum extension of 60% for parasitization by *Contracaecum sp.* and *Porrocaecum sp.* and a maximum intensity of 20 parasites/host, while the species *Contracaecum aduncum* and *Anisakis sp.* were less reported. In 2013, the infestation degree was lower, only *Contracaecum sp.* and *Porrocaecum sp.* being identified.

Azov shad

The Azov shad was affected by the nematode worms *Contracaecum aduncum*, *Contracaecum sp.*, *Porrocaecum sp.*, *Anisakis sp.* the parasitization degree was higher for infestation by *Contracaecum sp.*, the mean extension reaching 50% and the mean intensity 8 parasites/host.

Danube shad

In 2012, the Danube shad was highly parasitized by the nematode worms *Contracaecum aduncum*, *Contracaecum sp.* and *Porrocaecum sp.*, *Anisakis sp.*, located in the gut of fish and in the abdominal cavity, free or fixed on internal organs, mainly on the liver. All Danube shad individuals analyzed were parasitized by nematodes, the maximum number of total parasites reaching 50 parasites/host. The infestation extension, on parasite species, ranged between 40% and 80%, the maximum intensity being up to 30 parasites/host. In 2013, parasitization was slightly lower, with maximum values of intensity and extension of 25 *Contracaecum sp.* parasites/host in 60% of the analyzed fish.

The analysis of nematode parasitization extension of sprat, on length classes, pointed-out an increase of the infestation along with the increase of fish size (Fig. 1).

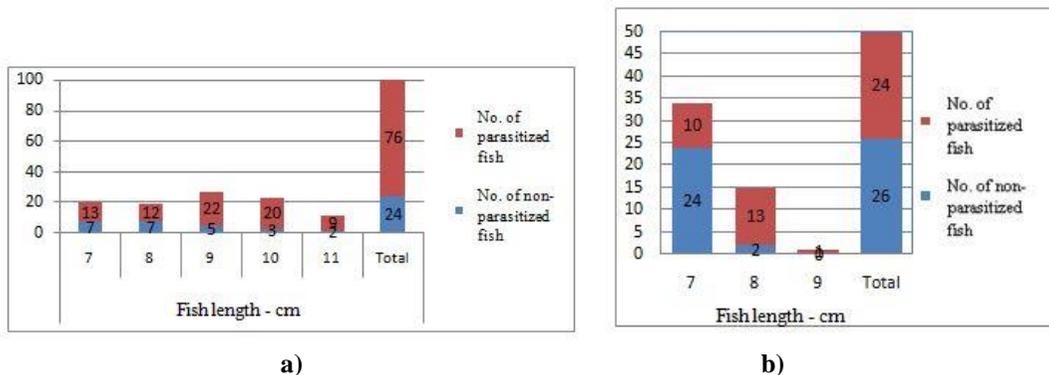


Fig. 1 - Nematode infestation of sprat in 2012 (a) and 2013 (b)

For such reason, the higher parasitization degree in 2012 was due to the fact that sprat was larger in 2012 than in 2013, emphasizing the trend of increase of infested fish and the number of parasites/host along with the size growth of fish.

In the Azov shad, in 2012, the same trend of increasing number of infested fish along with the growth in size of the fish was also reported (Fig. 2).

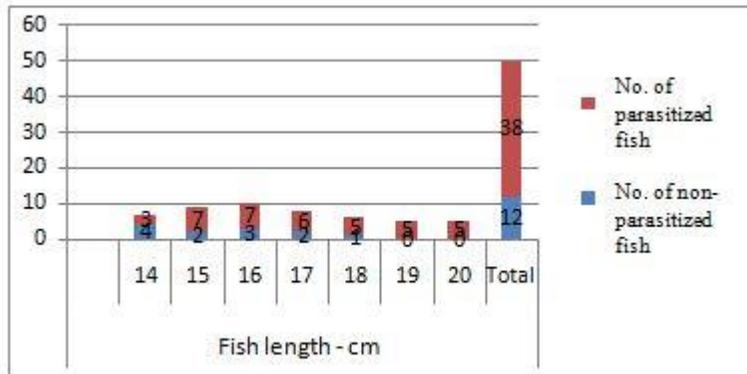


Fig. 2 - Nematode infestation of Azov shad in 2012

For the length classes ranging between 15 and 17 cm, the number of infested individuals was 2-3 times higher than the number of non-parasitized individuals, while 19-20 cm individual were 100% infested.

The clupeids covered by the study, with a high nematode infestation degree (more than 30 nematodes/fish), showed obvious signs of exhaustion and illness, with lack of food in the digestive duct, bleeding lesions, degenerations and atrophies in the liver and intestine and reduction of fat content.

CONCLUSIONS

- The nematode infestation degree was studied in three clupeid species often encountered in fish catches at the Romanian coast, namely: *Alosa tanaica* Grimm, 1901 - Azov shad, *Alosa immaculata* Bennett, 1835 - Danube shad (synonym *Alosa pontica pontica* Eichwald, 1838) and *Sprattus sprattus* Linnaeus, 1758 - sprat, in which four nematode worm species were identified: *Contracaecum aduncum*, *Contracaecum sp.*, *Porrocaecum sp.* and *Anisakis sp.*,
- Sprat and Azov shad were infested by all 4 nematode worm species, *Contracaecum aduncum*, *Contracaecum sp.*, *Porrocaecum sp.*, *Anisakis sp.*, located in their abdominal cavity, free and/or fixed on internal organs, mainly the liver. The infestation degree was maximum in case of parasitization by *Contracaecum sp.* and *Porrocaecum sp.* (extension of 40-60% and intensity of 8-12 parasites/host in sprat and 60-100% and 10-12 parasites/host, respectively, in Azov shad).

- The Danube shad was 100% infested in 2012, the maximum intensity reaching 50 parasites/host, while in 2013 the extension was 80%, with up to 40 parasites/host, the dominant nematodes belonging to the genera *Contraecaecum* and *Porrocaecum*.
- The species *Anisakis* sp. was identified in very few fish, with an intensity of up to 4 parasites/fish in sprat and Azov shad.
- The clupeids with a high nematode infestation degree (more than 30 nematodes/fish), showed obvious signs of exhaustion and illness, with lack of food in the digestive duct, bleeding lesions, degenerations and atrophies in the liver and intestine and reduction of fat content. These changes were found in larger individuals, in which the extension also increased up to 100%.
- Due to the small number of analyzed fish, it is absolutely necessary to continue pathological investigations, in order to be able to make an accurate assessment of the nematode infestation degree of marine fish at the Romanian coast.

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