Considerations on Parasite Influence on the Conservation State of Fish in the Romanian Natura 2000 Marine Sites *(T. Zaharia, E. Dumitrescu, V. Maximov, M. Cristea, M. Nenciu, A.Totoiu)*	**"Cercetări Marine" Issue no. 42** **Pages 173-183**	**2012**

CONSIDERATIONS ON PARASITE INFLUENCE ON THE CONSERVATION STATE OF FISH IN THE ROMANIAN NATURA 2000 MARINE SITES

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ABSTRACT

Fish populations are subject to various natural and anthropogenic factors reducing their abundance (Sinderman C., 2002). Parasites are one important factor, which might have a certain influence on the conservation state of fish. Parasitic diseases (invasive parasitoses) of marine fish are caused by animal parasites - protozoans, worms and crustaceans. Parasites can affect the entire body surface, the gills, the nose cavities, the digestive tube, the urinary, blood and breeding system, as well as fish muscles. In the past years, the research activities in the field of marine fish parasite fauna have developed greatly, parasites being used as biological markers of fish and indicators important for the study of fish circulation and natural stock assessment (Lester & MacKenzie, 2009). The paper herein is an outline of the influence of parasites identified during 2011 on the conservation state of fish collected from the Romanian Natura 2000 sites: ROSCI0269 - Vama Veche - 2 Mai, ROSCI0094 - Mangalia Submerged Sulphur Seeps, ROSCI0197 - Eforie North - Eforie South Submerged Beach, ROSCI0273 - Marine Area from Cape Tuzla and ROSCI0237 - Methanogenic Carbonate Structures from Sfantu Gheorghe.

KEY-WORDS: parasites, nematodes, conservation status, fish, Natura 2000, Romanian coastline

BACKGROUND

In wild fish populations, it is quite difficult to identify and quantify the effects or various factors on stock size, such as destruction by predators, lack of food or diseases. However, there is evidence indicating that some animal parasites can act as severe pathogenic agents, causing direct fatalities or increase vulnerability to other environmental or biotic stress factors.

The lethal effects of parasites can be determined statistically or by experience, yet the actual observation of fatalities is difficult. The sub-lethal effects (improper partial term, as many indirect effects cause fatalities) include muscular degeneration, liver malfunctions, eating disorders, heart diseases, nervous system affections, reduction of breeding capacities or mechanical interferences in laying roe, weight loss and influence on the entire body.

The study of fish in captivity or under rearing conditions provides information on the effects of parasites on survival rates. Epizooties and fatalities may occur, immunity can develop or not and sometimes parasites considered to be significant do not become as stressing as, for instance, high density and poor quality of water in the rearing environment.

There is a close connection, acknowledged by experts, between parasites and hosts, substantiated mainly by the fact that parasites increase the fatality rate of hosts. However, there are cases when balance sets between parasite and host. This balance depends, nevertheless, on the extent of parasitation, the immunity of the host to various parasites and general living conditions of the hosts.

Considering all these elements, various study models of the parasitic influence on hosts have been elaborated.

MATERIAL AND METHOD

The parasitation extent of fish, analyzed by the values of parasitation intensity (number of parasites/host) and parasitation extension (number of affected fish), was tracked by performing dissections on fresh biological material, collected during the physical-chemical and biological sample collection campaigns from the Natura 2000 marine sites: ROSCI0269 - Vama Veche - 2 Mai, ROSCI0094 - Mangalia Submerged Sulphur Seeps, ROSCI0197 - Eforie North - Eforie South Submerged Beach, ROSCI0273 - Marine Area from Cape Tuzla and ROSCI0237 - Methanogenic Carbonate Structures from Sfantu Gheorghe.

An assessment of nematode worms, the main parasites identified at fish in the above mentioned sites, was performed. Sample collection methods specific for parasitology were used and samples were subsequently analyzed under the microscope in order to identify parasites.

RESULTS AND DISCUSSION

In order to detect fatalities induced by parasites in natural fish populations, six methods are acknowledged and accepeted (Lester, R., J., G., 2010), as follows:

- Performing full disections on fish;
- Determining the frequency of infections known to be ultimately lethal;
- Observing the decrease of the long life cycle parasites' vantage or of lesions caused by parasites in relation to the host's age;
- Observing the decrease of the ratio between the parasite vantage/host's age;
- Comparing the frequency observed in a combination between two independent events with calculated occurrence probability;
- Comparing the distribution of parasite frequency with a designed frequency, based on data regarding the slightly infected fish. For this technique, the formulae are often created on truncated data and considering various points of view.

These methods do not necessarily offer final answers, they are indicative of the influence of parasites of fatalities and, in some cases, provide a probable estimation of it concerning the total mortality rate on the host.

The diversity and abundance of parasites vary widely within the poulations of the same fish species. These infection parameters are, to a certain extent, determined by the features of the host population or of the habitat (Bagge, A.M. et al., 2004).

Recent studies have supported predictions drived from epidemiologic models of the influence of the host population density, as follows: parasite abundance and parasite species richness are expected to increase along with the increase of the host population density, at least for parasites transmitted directly. However, this case is not applicable to all fish species or parasites, as other influencing factors may occur.

Various scientific papers examine the factors generating different dispersion models of parasite distribution within host populations (Anderson, R., M., Gordon, D., M., 1982). The increase and reduction of parasitic populations influences the various types of demographic processes of fish populations. It is known that distributions are dynamic, being generated by opposing forces, some acting to create great dispersion and other acting to generate sub-dispersion. The simulation experiences, based on the host populations' increase and decrease probability models, are used to study population dynamics. Special attention is focused on the role of parasites in inducing fatality to hosts.

It was demonstrated that, for certain host-parasite association types, the mean abundance convex curbs of parasites in relation to fish age (age - intensity curve), together with a decrease of the dispersion degree on older age classes of hosts, may prove the fatality of the host as being caused by the parasitic infection.

However, simulation studies pointed out the incidental technical drawbacks in establishing clear proof of host fatalities caused by parasites, by means of ecological studies of hosts and parasites in their natural habitats.

In light of the above and given the results of analyses performed to determine the parasites on fish in the five marine sites at the Romanian coast and their parasitation degree, an assessment of nematode worms, the main parasites identified in the fish, was carried out.

By means of dissections performed on the fish, nematode worms were identified, most often encountered as larvae, as well as the direct effects on the affected organs and tissues.

Contracaecum aduncum infests internal organs, encapsulated or free in the abdominal cavity, guts, piloric appendix and liver of fish. Once the fish ages, an accumulation of larvae occurs and they can reach up to several hundreds/fish (Fig. 1).

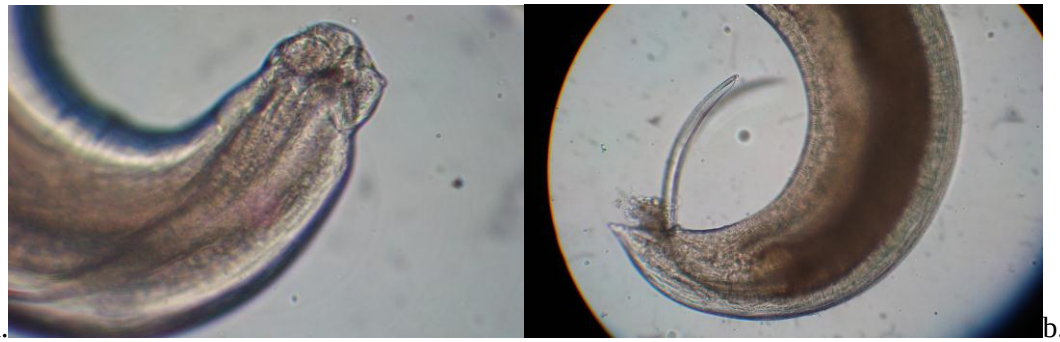
a.  b.

Fig. 1. *Contracaecum aduncum* (a - front side; b - rear side in male) (original photo/Dumitrescu E.)

Porocaecum sp., as larvae, sets in the muscles near the abdominal cavity of fish, the number of parasites increasing as the fish size grows (Fig. 2).

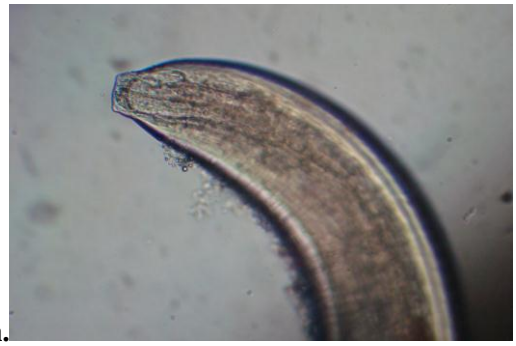
a.  **b.** 

Fig. 2. *Porrocaecum sp.* (a - front side; b - rear side) (original photo/Dumitrescu E.)

Anisakis sp., as larvae, nematode worms, infest encapasulated, in their immobile state, the liver, the piloric appendix and/or they migrate to various body organs, muscles, gonads, in their mobile state (Fig. 3).

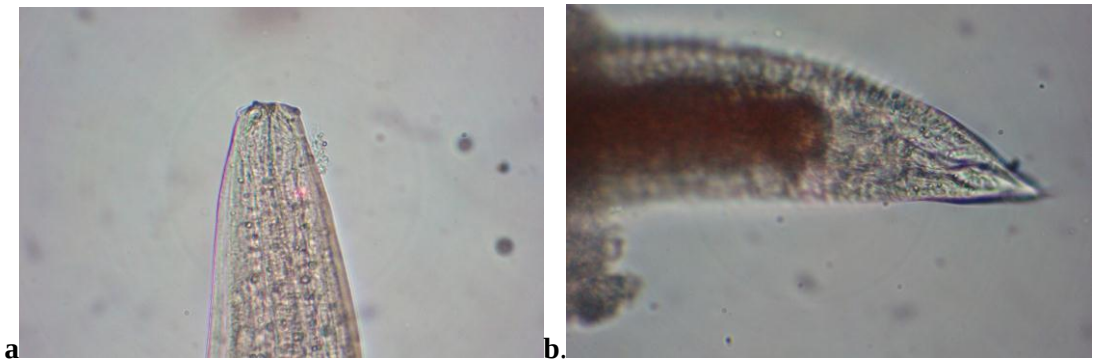


Fig. 3. *Anisakis sp*. (a - front side; b - rear side)
(original photo/Dumitrescu E.)

These parasites cause lesions and disturbances to the functions of the parasited organs, mainly the liver, as well as the breeding organs and the digestive duct. In case of scarce invasions, the fish bear parasitation, its effect being only a reduction of the fish growth rate. Severe infestations may cause the destruction of the parasited organs and death of the fish.

Nematode worms are generally encountered in marine clupeids, but in other fish species as well.

For an accurate assessment of nematodes' effects on fish, a number as high as possible of individuals must be analyzed. As such, the species for which analyses were performed for at least 50 individuals were selected, namely: sprat, anchovy and whiting.

With reference to the number of parasited individuals, in ***sprat***, parasitation was higher in the Vama Veche - 2 Mai site, where, out of a total of 50 fish analyzed, 25 (50%) were infested by nematode worms (Fig. 4).

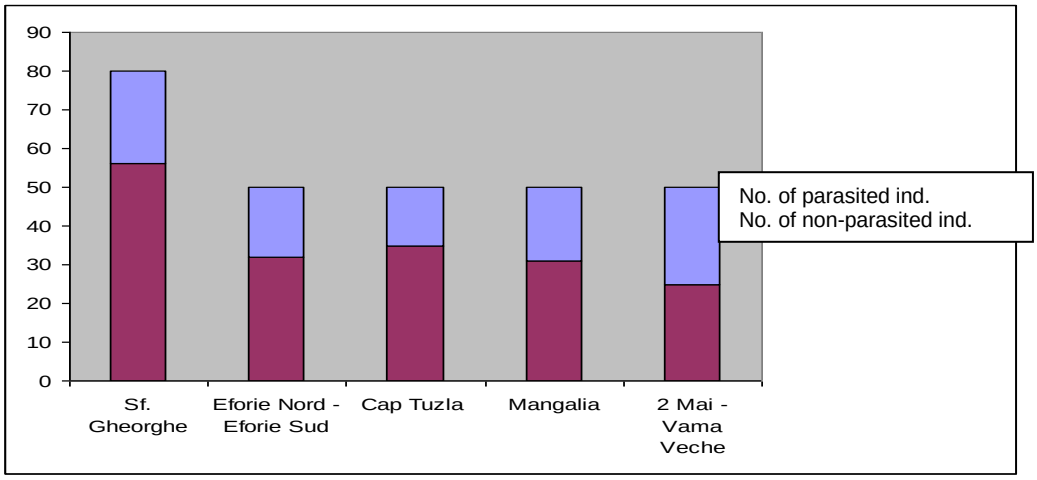


Fig. 4. Sprat parasitation extent by nematode worms
(no. of parasited ind. of the total analyzed)

In the other sites, the number of parasited sprat individuals ranged between 30-38% (30% in Sf. Gheorghe, 36% in Eforie North-Eforie South, 30% in Cape Tuzla and 38% in Mangalia) of the total number of individuals analyzed.

In ***anchovy***, the parasitation extent, expressed by the number of parasited individuals of the total analyzed was also highest in Vama Veche - Mai, 62 infested individuals out of 100 (Fig. 5).

In Sf. Gheorghe, the parasited fish reached 41% of the total analyzed fish, while in Eforie North-Eforie South 55%, in Cape Tuzla 55% and in Mangalia 38%.

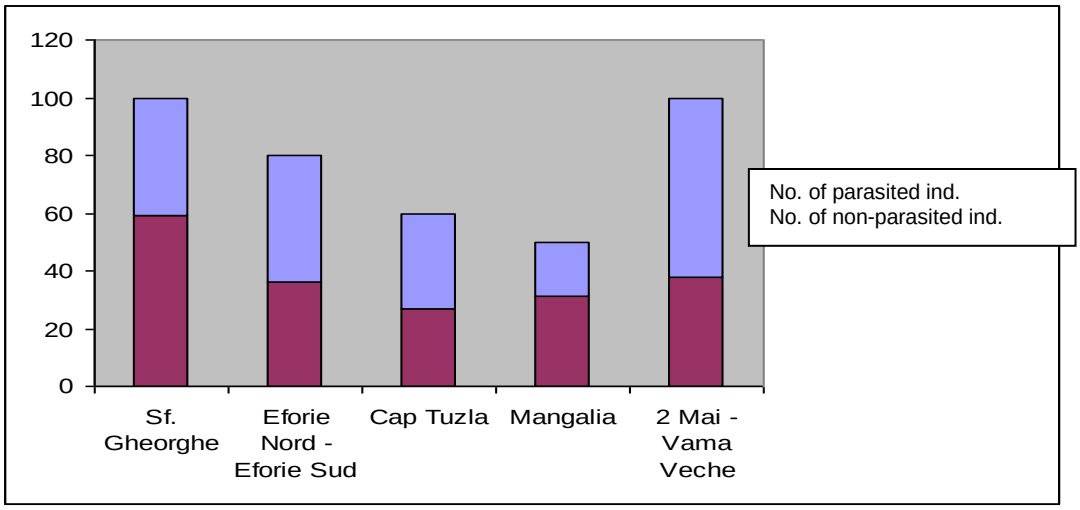


Fig. 5. Anchovy parasitation extent by nematode worms (no. of parasited ind. of the total analyzed)

The above mentioned findings point out that, compared to sprat, the number of anchovy individuals parasited by nematode worms was much higher, 30-62% compared to 30-50%.

Whiting recorded a nematode parasitation extent lower than sprat and anchovy, the number of infested individuals varying between 24% (in Sf. Gheorghe) and 34% (in Mangalia and Vama Veche - 2 Mai) (Fig. 6).

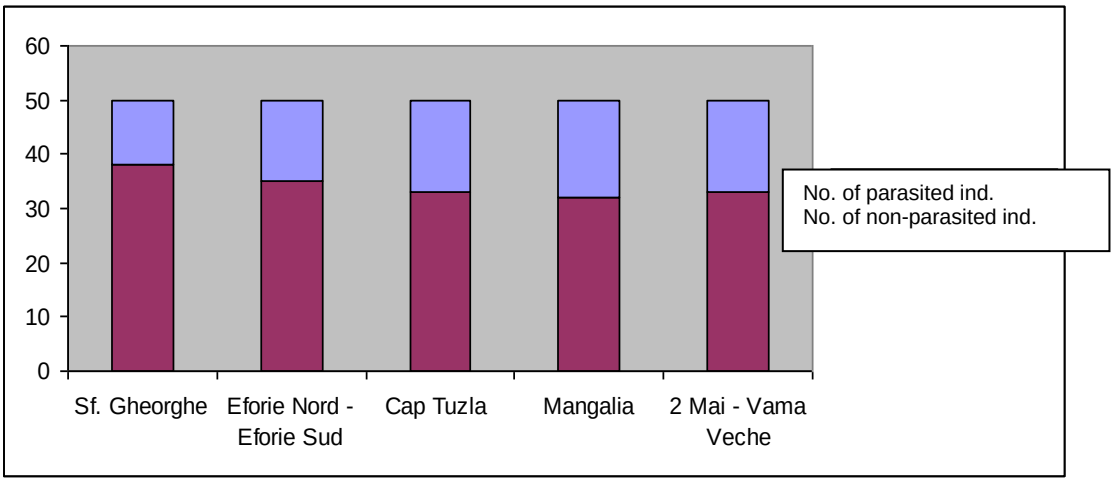


Fig. 6. Whiting parasitation extent by nematode worms (no. of parasited ind. of the total analyzed)

In the other sites, the whiting affected by nematodes represented 30-32% of the total number or analyzed individuals.

The analysis of the infestation intensity, given by the number of nematodes/host, considering the sizes of fish, reveals an increasing accumulation of nematodes in larger sized individuals; however, the number of such individuals was quite low.

In the smallest sized ***sprat*** in Sf. Gheorghe, 7-8 cm long, the number of nematodes was 1-3/host, while in individuals sized 9-10 cm, the parasitation intensity increased to 4-9 parasites/fish and in individuals sized 11-12 cm to 11-16 parasites/host.

In sprat caught in Eforie North-Eforie South, the intensity of parasitation was quite higher than in Sf. Gheorghe. Thus, in fish individuals 7-8 cm long, 2-4 parasites/host were identified and in individuals 9-10 m long, 11-16 parasites/host were found.

In the Cape Tuzla area, the sprat analyzed ranged between 8.5-9.5 cm, the intensity of parasitation ranging between 1 and 11 parasites per host.

In Mangalia, except for one sprat individual infested with the maximum number of 19 parasites/host, in most individuals the intensity of the invasion ranged between 5 and 10 parasites/fish.

Sprat caught in Vama Veche - 2 Mai, sized between 8 and 10 cm, carried forth a parasitation intensity of 4-16 parasites/host, the individuals infested by 4-8 nematodes/host were dominant.

In the ***anchovy*** caught in the five marine sites and analyzed to identifiy the variation of nematode infestation intensity, the same general trend of worms accumulating in larger individuals was reported.

In individuals 8-9 cm long in Sf. Gheorghe, the parasitation intensity was 2-6 parasites/host, and in 10-12 cm long individuals, dominant as number, the invasion intensity was much higher, summing up 7-19 nematodes/host.

A similar situation was reported in Eforie North-Eforie South as well, where the 9-10 cm long anchovy was parasited by 4-10 nematodes/fish and the 11-13 cm long fish were parasited by 11-19 parasites/hosts.

In Cape Tuzla, the large sized anchovy reached the highest infestation level. Thus, in a 13 cm long individual, the infestation intensity was maximum: nematode worms/host. Except for two anchovy individuals, in which the parasitation intensity was 30 and 36 parasites/host, respectively, in the other individuals, ranging between 11-13 cm in length, the number of parasites/fish individual was between 10 and 26. Anchovies ranging between 8 and 10 centimeters in length showed a lower parasitation degree, with 4-8 parasites/host. Overall, anchovy individuals parasited by 6-18 parasites/host were dominant.

The anchovy caught in Mangalia ranged between 9-11 cm in length, with an infestation intensity of 4-16 parasites/fish, the individuals with infestation levels of 7-12 parasites/host were dominant.

In the Vama Veche - 2 Mai area, at a maximum extension of parasited anchovy (62% of the total number of analyzed individuals), the nematode infestation intensity ranged between 4 and 12 parasites/host in fish individuals 8-9 cm long and 13-26 parasites/host in larger anchovy individuals, 10-12 cm in length.

In ***whiting***, the parasitation intensity was much lower than in sprat and anchovy.

Similarly to the other analyzed fish species, whiting also pointed out, even though less obviously, the same trend of nematode worm accumulation in large sized fish.

In Sf. Gheorghe, in 10-11 cm long whiting, the nematode parasitation intensity was 3-4 parasites/host and in 12-13 cm long individuals it was slightly higher: 5-9 parasites per host.

In Eforie South and Cape Tuzla, whiting was more intensely parasited, reaching, in larger individuals, 10-17 parasites/host and 10-20 parasites/host, respectively. To the south, in Mangalia and Vama Veche - 2 Mai, in 12-15 cm long individuals, the parasitation intensity was slightly lower: 7-11 parasites/host.

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Given the results presented above, it is pointed out that sprat and anchovy showed a significant degree of parasitation by nematode worms, from the genera *Contracaecum*, *Porrocaecum* and *Anisakis*, however not causing a major hazard by inducing fatalities on the natural populations due to the following reasons:

- the parasites, mainly as larvae, infested the abdominal cavity mostly free and less embedded in internal organs, which they can destroy especially by mechanical action, with degenerations, atrophies and reduction of functions;
- the small number of individuals in which the infestation intensity reached the peak figure of 40 parasites/host; generally, the parasitation intensity did not exceed 20 parasites/host;
- the trend of parasite accumulation once the fish size increases (the juveniles and early stages are less affected) invalidates the theory unanimously accepted by experts of mean parasite abundancy convex curves in relation to fish age (age - intensity curve), contemporary with a decrease of the older age classes dispersion degree of hosts, which may prove the host's cause of death as being induced by the parasitic infestation.

This consideration was made, however, subject to the fact that the fish analyzed were caught during a limited period (May-June 2011, with some additional frozen samples of fish caught in November 2010) and the number of analyzed individuals was quite low. We also considered the modifications induced by parasites, reported in individuals in which the parasitation intensities were higher than 30 parasites/host, namely

degenerations and atrophies in the liver and hemorrhagic lesions of the digestive duct. In case the number of fish recording such high parasitation intensities were significant and knowing that the effects of parasitation can be lethal, stock sizes could be seriously damaged.

An alarm should be triggered by the anchovy in the southern part of the Romanian coastline (ROSCI0197 - Eforie North - Eforie South Submerged Beach, ROSCI0273 - Marine Area from Cape Tuzla, ROSCI0269 - Vama Veche - 2 Mai), where 55%-62% of the analyzed fish were frequently parasited by 6-20 parasites/host and a maximum number or 40 nematode worms/fish. Under these circumstances, the organs affected by parasites have reduced functions, the fish manifesting significant disturbances of the feeding, breeding and growth functions.

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The ***shad***, extremely important species for Romanian fisheries, was highly parasited in the Eforie North-Eforie South and Vama Veche-2 Mai sites, where all analyzed individuals were infested by nematode worms belonging to the *Contracaeum, Porrocaecum* and *Anisakis* genera. The species *Contracaecum aduncum* was prevalent, parasiting in the adult state the digestive duct and as larvae the abdominal cavity and internal organs, up to 45 parasites/host. The parasited fish show obvious signs of exhaustion, with food lacking from the digestive system, degenerations and atrophies in the liver and intestine and fat content reduction. The small number of analyzed individuals did not allow the estimation of the influence of such parasites on the overall state of health of these fish and the way their stocks are affected.

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Few ***turbot*** individuals underwent parasitologic analyses, only 2-6 specimens/site. However, it was reported that large individuals, analyzed during the spawning period (2-5 kg/ind.), were highly parasited by the flat worm *Botriocephalus scorpii*. The stomach, totally lacking food, was actually full with parasites. Small specimens, 10-12 cm long, were lacking parasites. By appending themselves in the digestive duct, the parasites act mechanically causing its damaging and inflaming, exfoliation of the damaged mucous membrane, degenerations, necroses, conjuctive tissue proliferation.

The toxins released by the worms are absorbed and cause intoxication of the entire body. The prognosis is quite severe, given the fact that the number of parasites is high enough to occupy the fish stomach, inducing severe nutritional disorders, hemorrhagic lesions in the intestine walls, intoxications and even death of the fish.

Nevertheless, accurate estimations concerning the influence of parasites on turbot populations could not be made, given the small number of analyzed individuals. Due to the fact that the small specimens analyzed, yet insufficient as number, were not infested, it is possible that the influence of *Botriocephalus scorpii* parasitation is not major on turbot populations.

CONCLUSIONS

♦ In order to identify the parasite fauna of marine fish, 16 species were analyzed, as follows: *Squalus acanthias* - dogfish, *Sprattus sprattus* - sprat, *Alosa caspia nordmanni* - Azov shad, *Alosa pontica pontica* - Danube shad, *Engraulis encrasicholus* - anchovy, *Merlangus merlangus euxini* - whiting, *Belone belone euxini* - garfish, *Gaidropsarus mediterrneus* - shore rockling, *Atherina hepsetus* - sand smelt, *Trachurus mediteraneus ponticus* - horse mackerel, *Mullus barbatus ponticus* - red mullet, *Mesogobius batrachocephalus* - goby, *Psetta maeotica* - turbot, *Platichthys flesus luscus* - flounder, *Scorpaena porcus* - scorpion fish, *Solea nasuta* - sea sole (Zaharia T. et al., 2012).

♦ The analysis of the effects of nematode worms - main parasites identified in sprat, anchovy and whiting - on the conservation state of fish revealed that sprat and anchovy were highly parasited by species from the genera *Contracaecum, Porrocaecum* and *Anisakis*, which did not, however, severely endanger natural populations by causing significant fatalities.

This conclusion was substantiated by the fact that parasites were reported mainly as larvae, infested the abdominal cavity mostly free and less embedded in internal organs, which they can destroy especially by mechanical action, with degenerations, atrophies and reduction of functions. In addition, there was a small

number of individuals in which the infestation intensity reached the peak figure of 40 parasites/host; generally, the parasitation intensity did not exceed 20 parasites/host. The trend of parasite accumulation once the fish size increases (the juveniles and early stages are less affected) invalidates the theory unanimously accepted by experts of mean parasite abundancy convex curves in relation to fish age (age - intensity curve), contemporary with a decrease of the older age classes dispersion degree of hosts, which may prove the host's cause of death as being induced by the parasitic infestation.

♦ This consideration was made, however, subject to the fact that the fish analyzed were caught during a limited period (May-June 2011, with some additional frozen samples of fish caught in November 2010) and the number of analyzed individuals was quite low. We also considered the modifications induced by parasites, reported in individuals in which the parasitation intensities were higher than 30 parasites/host, namely degenerations and atrophies in the liver and hemorrhagic lesions of the digestive duct. In case the number of fish recording such high parasitation intensities were significant and knowing that the effects of parasitation can be lethal, stock sizes could be seriously damaged.

♦ An alarm should be triggered by the anchovy in the southern part of the Romanian coastline (ROSCI0197 - Eforie North - Eforie South Submerged Beach, ROSCI0273 - Marine Area from Cape Tuzla, ROSCI0269 - Vama Veche - 2 Mai), where 55%-62% of the analyzed fish were frequently parasited by 6-20 parasites/host and a maximum number or 40 nematode worms/fish. Under these circumstances, the organs affected by parasites have reduced functions, the fish manifesting significant disturbances of the feeding, breeding and growth functions.

♦ The shad was highly parasited in the Eforie North-Eforie South and Vama Veche-2 Mai sites, where all analyzed individuals were infested by nematode worms belonging to the *Contracaecum*, *Porrocaecum* and *Anisakis* genera. The species *Contracaecum aduncum* was prevalent, parasiting in the adult state the digestive duct and as larvae the abdominal cavity and internal organs, up to 45 parasites/host. The parasited fish showed obvious signs of exhaustion, with food lacking from the digestive system, degenerations and atrophies in the liver and intestine and fat content reduction. The small number of analyzed individuals did not allow the estimation of the influence of such parasites on the overall state of health of these fish and the way their stocks are affected.

♦ Given the results of the paper herein, we strongly suggest continuing the research on the parasite fauna of marine fish at the Romanian coast. In this respect, we advise performing the investigations on a number of fish individuals caught throughout an entire year as high as possible. The research shall be carried out in species already studied this year, as well as in other ecological and economic interest species. We particularly aim at adding up data on the parasite fauna in sprat, anchovy, whiting, as well as turbot and shad, given the fact that more data shall ensure a more accurate assessment of the parasite fauna influence on natural fish populations.

♦ Thus, the acquisition of additional data on the stock evolution for each target species, on the state of the marine living components representing intermediary hosts for parasites, on the general living conditions of fish, assessed in relation to the values of the physical-chemical and biological parameters of the marine environment shall contribute to a correct assessment of the health state of the main marine fish species and of the parasite fauna influence on their conservation state.

REFERENCES

1. Anderson R. M., D. M. Gordon, 1982 - *Processes Influencing the Distribution of Parasite Numbers within Host Populations with special Emphasis on Parasite-induced Host Mortalities*, Parasitology (1982), 85: pp 373-398;
2. Bagge A.M., Poulin R., Valtonen E.T., 2004 - *Fish Population Size, and not Density, as the Determining Factor of Parasite Infection: a Case Study. Parasitology*. 04/2004; 128 (Pt 3): 305-13;
3. Lester, R.J.G., MacKenzie, K., 2009 - *The Use and Abuse of Parasites as Stock Markers for Fish Fisheries Research*, Volume 97, Issues 1-2, April 2009, Pages 1-2;
4. Sindermann C., 1987, *Effects of Parasites on Fish Populations: Practical Considerations. International Journal for Parasitology*, 17, 371-382;
5. Zaharia T., Dumitrescu E., Maximov V., Cristea M., Nenciu M., 2012 - *Considerations on Parasite Influence on Fish Conservation Status in the Romanian Marine Natura 2000 Sites*, BENA Istanbul 2012, 21-24 June 2012, Sustainable Landscape Planning and Safe Environment, Istanbul Technical University, Conference Proceedings, Istanbul, 2012, ISBN 978-975-561-421-2: 501-510.