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SCREENING FOR ECTOPARASITISM ON PELAGIC AND DEMERSAL FISH FROM THE ROMANIAN BLACK SEA COAST

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Abstract

Ectoparasites affect the external surfaces of fish, specifically the skin, fins, eyes, and gills. This study analyzes biological samples of pelagic and demersal fish collected between 2016 and 2019 from the Romanian Black Sea coast. Six fish species were examined for ectoparasites: Sprattus spratus (sprat), Engraulis encrasicolus (anchovy), Trachurus mediterraneus (horse mackerel), Platichthys flesus (flounder), Pegusa lascaris (sand sole), and Scophthalmus maeoticus (turbot). Four ectoparasite species were identified: Trichodina domerguei and Cryptocaryon irritans (protozoa), Mazocraes alosae (flatworm), and Cystoopsis acipenseris (nematode). T. domerguei was the most common ectoparasite and had the highest level of infestation, particularly in turbot, which averaged 28 parasites per host, with 27-65% affected. M. alosae was found in sprat, while C. acipenseris and C. irritans affected horse mackerel and sand sole at low levels. Infestations caused by ectoparasites in fish do not have as great an impact as infections with other pathogens (such as viruses and bacteria). Still, it is one of the secondary causes of viral and bacterial diseases.

***Key words**: ectoparasites, fish, intensity of parasitism, screening.*

INTRODUCTION

Fish diseases are a major problem worldwide and can cause large losses in natural stocks and aquaculture systems. Parasitism in fish has often been considered an indicator of environmental health, representing an essential complement to maintaining a balance in marine ecosystems and aquaculture.

Some parasites use a complex web of interactions in the ecological chains of their hosts yet are vulnerable to any observed changes or disturbances to their habitats, even before larger taxa can be affected (Juarez-Estrada et al., 2023; MacKenzie & Abaunza, 2005)

Parasites play a complex role in the ecology of marine ecosystem diversity, influencing population sizes and altering patterns in both biodiversity and community structure (Niță et al., 2022, 2023).

Ectoparasites are defined as those that reside on the external surfaces of the host, including the gills, mouth, skin, and fins. Among the most significant external parasites affecting fish are protozoa, crustaceans, and monogenic trematodes (Kasse, 2019).

The pathological effects depend on the density of the parasite population and the nature of the tissue responses, which are closely related to the physiological state of the fish (Marques et al., 2011; Poulin et al., 2016). Stressful environmental conditions compromise the fish's ability to fight infection. Infection with ectoparasites in fish does not generate as great effects as infection with other pathogens such as viruses and bacteria, but it is one of the secondary effects that can cause viral and bacterial diseases (Catalano et al., 2014; Poulin et al., 2016). Ectoparasites affecting fish populations can affect fish survival, especially by inhibiting their growth (Nofyan et al., 2015). Although parasitism is common in fish, parasitic diseases only appear in environmental conditions that allow the multiplication of parasites and habitats that favor the transmission of parasites and the persistence of carriers or intermediate hosts (Bagge et al., 2004). Pelagic and demersal fish stock dynamics may be affected by parasitism; therefore, screening ectoparasites provides essential information for appropriate and sustainable resource management.

This study aimed to identify the ectoparasites present in *S. spratus* (sprat*), E. encrasicolus* (anchovy), *T. mediterraneus* (horse mackerel), *P. flesus* (flounder), *P. lascaris* (sand sole) and *S. maeoticus* (turbot) individuals and to determine the degree of parasitism of these ecologically and economically fish species from the Romanian Black Sea coast.

MATERIALS AND METHODS

The detection of ectoparasitoses identified in populations of *S. spratus* (sprat*), E. encrasicolus* (anchovy), *T. mediterraneus* (horse mackerel), *P. flesus* (floundr), *P. lascaris* (sand sole) and *S. maeoticus* (turbot) was carried out in two main stages, including the collection of fish samples and specific parasitological laboratory analyses.

Ectoparasites were determined by analyzing 750 pelagic and 90 demersal fish specimens between 2016 and 2019 (Table 1). The pelagic fish specimens were collected bi-monthly from the catch of pound nets from 6 sampling stations (Figure 1), and demersal species were fished with demersal trawls and turbot gillnets from stations located along the Romanian Black Sea coast (Figure 2).

Table 1. Fish species analyzed from 2016 to 2019

Year / Species	2016	2017	2018	2019	TOTAL
***S. spratus* (sprat)**	**60**	**65**	**50**	**75**	**250**
***E. encrasicolus* (anchovy)**	**65**	**50**	**100**	**60**	**275**
***T. mediterraneus* (horse mackerel)**	**55**	**50**	**70**	**50**	**225**
Total number of pelagic fish surveyed					**750**
***S. maximus* (turbot)**	**8**	**9**	**9**	**9**	**35**
***P. flesus* (flounder)**	**7**	**6**	**6**	**6**	**25**
***P. lascaris* (sand sole)**	**8**	**6**	**6**	**8**	**30**
Total number of demersal fish surveyed					**90**
Total number of fish surveyed					**840**

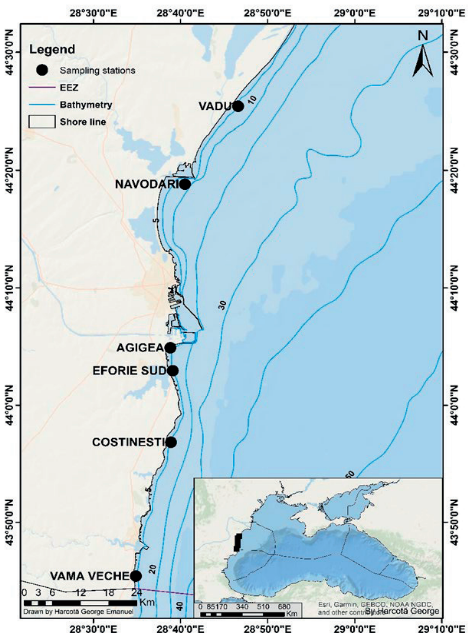


Figure 1. Distribution of pound nets/sampling points for pelagic fish (original)

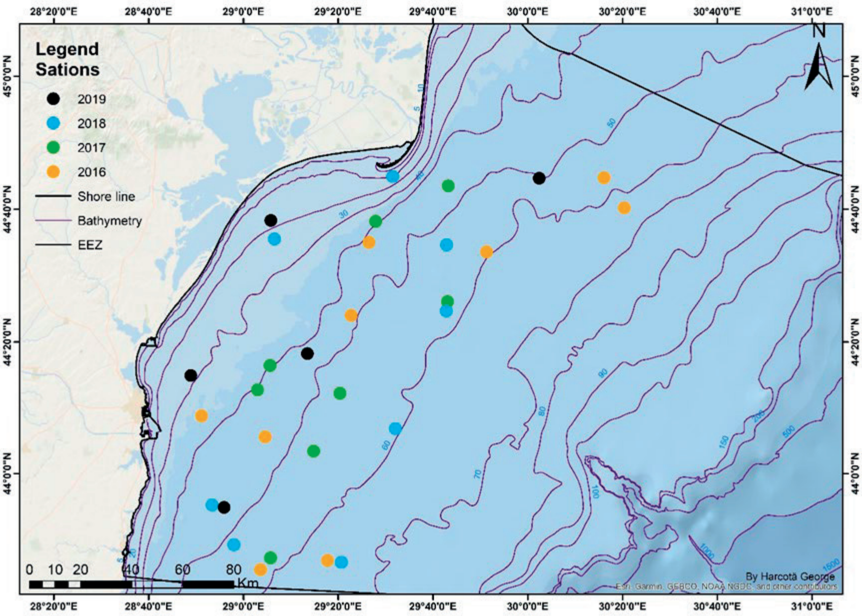


Figure 2. Distribution of trawling/sampling points for demersal fish (original)

The pound net, a large fishing gear, is typically set up at depths ranging from 5 to 12 meters. In larger nets, the concentration chambers (the net) and the catch chamber are positioned parallel to the shore and can extend up to 70 meters in length. Guiding the fish is carried out by wings made of mesh, which are 300 to 500 meters long and are situated perpendicular to the shore direction (Radu et al., 2008).

The demersal trawl is a truncated cone-shaped fishing gear equipped with an arming system, towed on the substrate level with the help of a vessel, using connecting elements (line, intermediate, bridle, etc.) (Radu et al., 2008).

The gillnet is a type of fishing gear that consists of a single net wall. It has reinforcing elements at the top (rafts) and bottom (leads) to keep it in a vertical position. This type of net is designed to catch fish by hooking and entangling those that move in the water or near the bottom of the water body (Radu et al., 2008).

For the identification of ectoparasites, each fish specimen was measured with the ichthyometer and weighed with the analytical balance in the ichthyopathology laboratory (Table 2).

To identify parasite infestations, frozen biological material from fish is examined both macroscopically and microscopically; to identify the parasites and the reactions they cause in the host (Bruno et al., 2006; Catalano et al., 2014).

Ectoparasite species were identified according to their specific characteristics related to size, external appearance, shape, color, and internal structure, determined by macroscopic and microscopic examination (Bruno et al., 2006; Gaevskaya, 2012).

Macroscopic examination involves using the naked eye and a magnifying glass to identify potential parasites and the changes they induce in the host's body. This includes observing the body's surface, eyes, and gills (Bruno et al., 2006; Gaevskaya, 2012).

For microscopic examination, samples are taken directly from the thawed fish. This involves scraping material from the surface of the area to be investigated, usually, the gills and skin, using a scalpel. The scraped material is then placed on a slide in a drop of water, covered with another slide, and examined microscopically.

Ectoparasites are identified using a Zeiss Axio Imagea A1 microscope equipped with a camera. Immersion oil and X20 and X40 objectives were used for viewing ectoparasites, with the eyepieces being X5 and X10.

To assess the impact of ectoparasites on fish, parasitological analysis should be conducted on as many fish individuals as possible. Parameters

analyzed include prevalence (percentage of infested fish) and mean intensity (average number of parasites per infested fish) (Bruno et al., 2006; Lester & MacKenzie, 2009).
Data processing and statistical analysis were performed using Excel and PRIMER v. 7.0 software packages (Clarke et al., 2014). Distribution maps for the sampling stations were generated using ArcMap 10.6.1 (Tom Sawyer Software, Berkeley, CA, USA).

RESULTS AND DISCUSSIONS

The parasitological investigations regarding the infection with ectoparasites were carried out on all six species of fish studied, with the length and weight according to Table 2. Four species of ectoparasites were identified, namely *Trichodina domerguei* (Wallengren, 1897), *Cryptocaryon irritans* Brown, 1951, *Mazocraes alosae* Hermann, 1782, and *Cystoopsis acipenseris* Wagner, 1867.

Table 2. The biological parameters of the six fish species studied between 2016 and 2019

Fish species	Length (cm)	Weight (g)
Pelagic fish species		
S. spratus (sprat)	7-10.4	2.2-9.5
E.encrasicolus (anchovy)	7-12.6	2.1-15.6
T. mediterraneus (horse mackerel)	9.1-16.3	6.4-39.3
Demersal fish species		
P. flesus (flounder)	16.7-32.2	10.4-382.2
P. lascaris (sand sole)	14.4-24.8	27.3-214.4
S. maeoticus (turbot)	43.1-65.6	1.380-4.460

1. *Trichodina domerguei*

It is a ciliate protozoan belonging to the family Urceolariidae, and it is shaped like a very flattened bell. In the central part, at the edge of the bell, there is a crown of cilia, with the help of which the parasite moves on the body of the fish (Figure 3) (Bruno et al., 2006; Gaevskaya, 2012; Xu et al., 2015).
The parasite was identified microscopically from scrapes made on the body and gills. It attaches itself to the gills and skin of the fish and feeds on mucus, the bacteria contained in it, and dead cells (Figure 3).

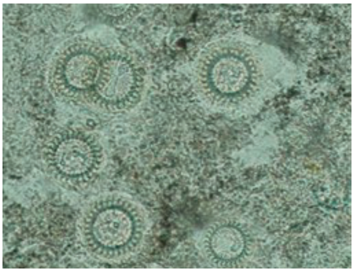


Figure 3. *Trichodina domerguei* (original photo)

2. *Cryptocaryon irritans*

It is a ciliated, ovoid protozoan of about 1 mm, slightly narrowed at the anterior part, where the cytostome is located. The cilia are arranged longitudinally across the body surface like meridians (Figure 4). Feeding on the host body, the ciliate reaches a certain size, after which it loses its mobility (cilia resorb), becomes surrounded by a cystic membrane, and becomes tomont (Bruno et al., 2006; Fridman, 2022; Gaevskaya, 2012)

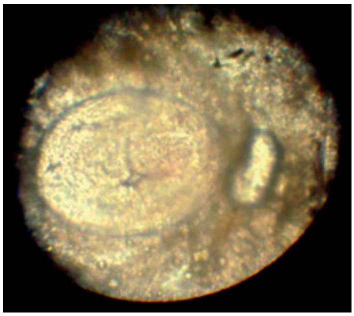
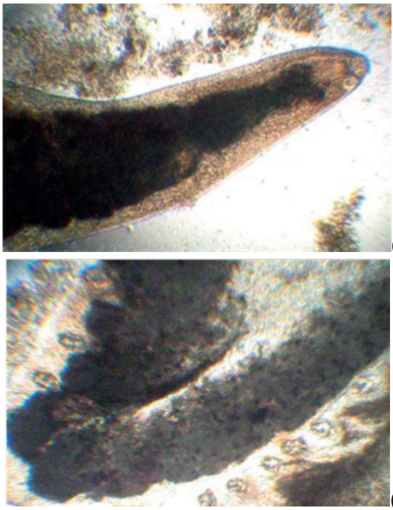


Figure 4. *Cryptocaryon irritans* (original photo)

3. *Mazocraes alosae*

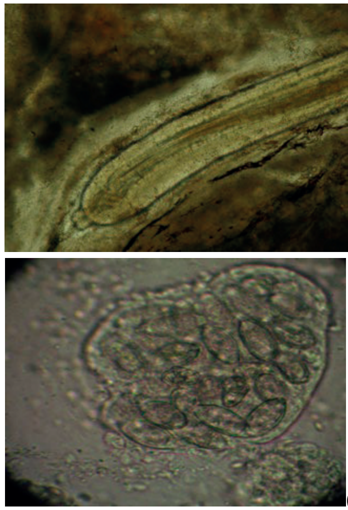
It is a flatworm, from the Monogenea class, that parasitizes the gills of marine fish. In the anterior part there is the prohaptor equipped with two dimples that communicate with the mouth, at the terminal part there is the opisthaptor, a rhomboidal fixation organ (Figure 5). The sources of infestation are infested fish and the water in which the eggs reach (Bruno et al., 2006; Gaevskaya, 2012). After their hatching, the free stage of the parasite emerges, which swims in time, and if it finds a host, it attaches itself to it and then migrates to the place of parasitism.



(a)

(b)

Figure 5. *Mazocraes alosae* - (a) anterior part; (b) posterior part (original photo)



(a)

(b)

Figure 6. *Cystoopsis acipenseris* - (a) male; (b) eggs (original photo)

4. *Cystoopsis acipenseris*

It is a long, cylindrical, sharp-pointed nematode worm that usually parasitizes the skin tissue of the sturgeon species. Males have much smaller bodies than females, with a single spike. Females have a filamentous anterior part and a spherical posterior part. The barrel-shaped eggs with caps at the ends have larvae inside (Figure 6) (Bruno et al., 2006; Gaevskaya, 2012).

The ectoparasites identified in the investigated fish populations showed two types of effects:

- mechanical - lesions caused to the integument and gills by their attachment organs and by the movements they make on the host body.
- inoculatory - inoculation at attachment sites of viruses, bacteria, and even other parasites.

The three demersal species studied (*S. maeoticus*, *P. flesus*, and *P. lascaris*) were affected by two species of ectoparasites (*T. domerguei* and *C. acipenseris*).

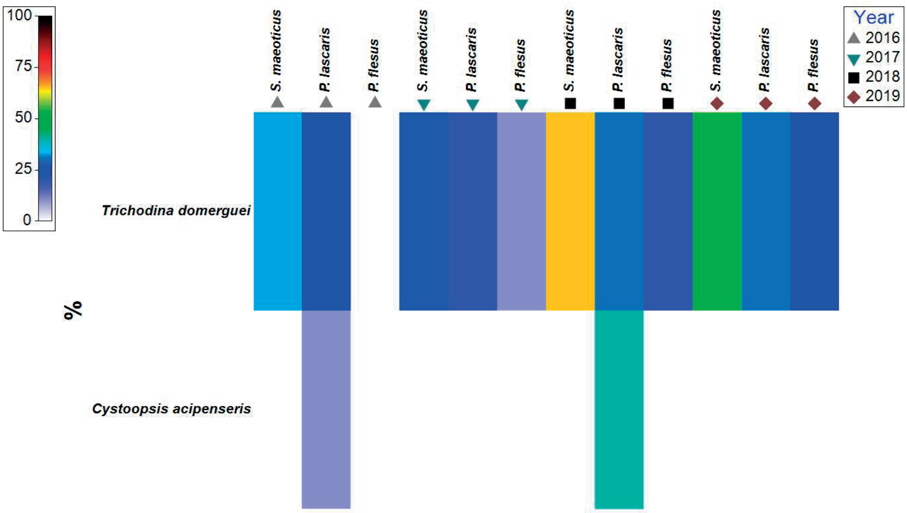


Figure 7. Prevalence of ectoparasite species (percentage of infested fish) in demersal species between 2016 and 2019

Turbot, the demersal species reported most frequently in catches, was infested throughout the period 2016-2019 only by the ectoparasite *T. domerguei,* located on the skin and gills. The degree of parasitism was high, reaching a maximum prevalence of 65% in 2018, and the minimum prevalence value was 27% in 2017. The maximum value of the average intensity of parasitism was 24 parasites/host in 2017 (Figure 7; Figure 8).

Flounder presented a low degree of parasitism, being parasitized only by *T. domerguei*, with a prevalence between 10-25% and an average parasitism intensity of 1-8 parasites/host. High parasitism values were reported in 2019 (Figure 7; Figure 8).

Sand sole was weakly infested by the protozoan *T. domerguei* and the nematode worm *C. acipenseris*, the maximum prevalence of these parasites being up to 40%, and the average parasitism intensities of 3-6 parasites/host. *C. acipenseris* was poorly reported in sand soles in 2016 and 2018 (Figure 7; Figure 8).

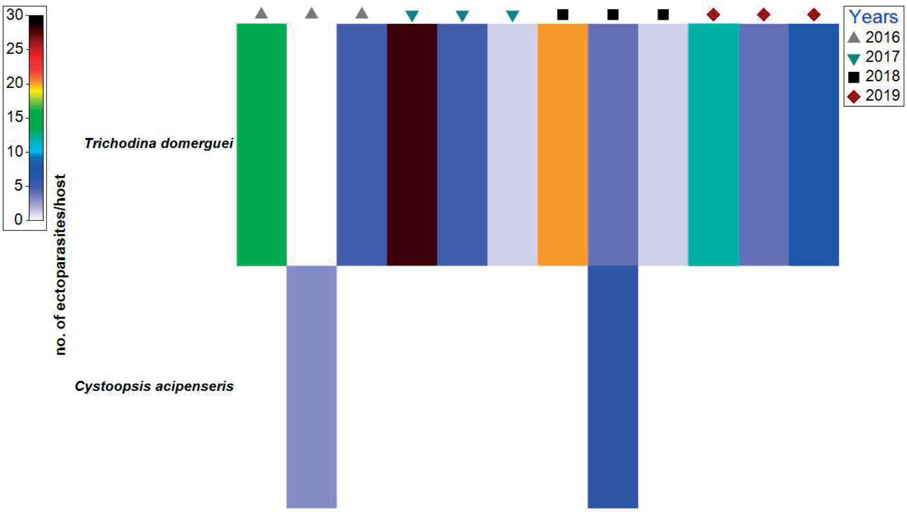


Figure 8. Average parasite intensity of ectoparasite species (parasites/host) in demersal species between 2016 and 2019

The pelagic fish species *(S. spratus, E. encrasicolus, T. mediterraneus*) were parasitized by three species of ectoparasites, namely: *T. domerguei*, *C. irritans*, and *M. alosae*.

Sprat was affected by parasites located on the gills and skin. During the screening, two species of parasites were identified, namely the protozoan *T. domerguei* and the monogenean worm *M. alosae*. Among them, the monogenean worm was highlighted, with an average parasitism intensity of 6-15 parasites/host throughout the period under study and a maximum prevalence of 33% of the fish analyzed in 2019. *T. domerguei* was weakly reported, the highest value of the average intensity of parasitism was recorded in 2016 (10 parasites/host) (Figure 9; Figure 10). *T. domerguei* affected fish outside the body, fins, and gills, and *M. alosae* affected only the gills.

T. domerguei was identified both on the gills and the skin of anchovy specimens in 2016 and 2019. The values of the average intensity of parasitism were between 5-20 parasites/host, and the prevalence was up to 30% (Figure 9; Figure 10).

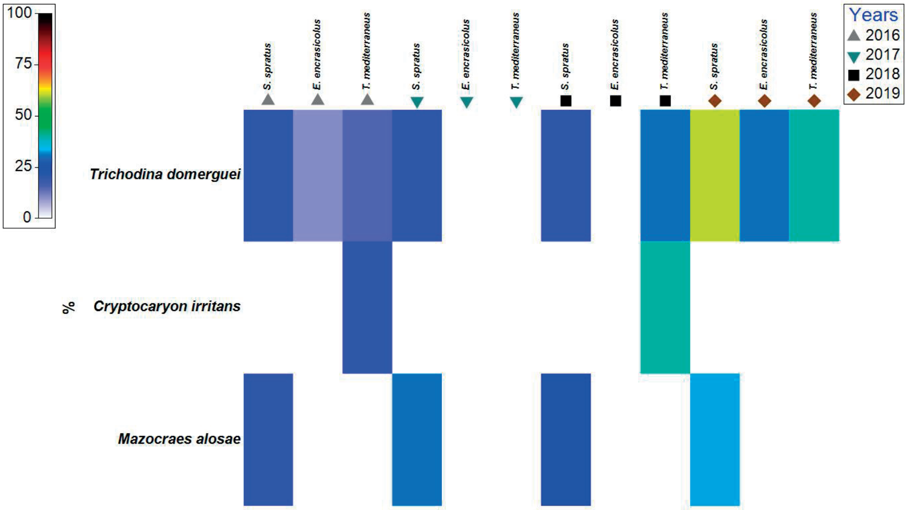


Figure 9. Prevalence of ectoparasite species (percentage of infested fish) in pelagic species between 2016 and 2019

The horse mackerel recorded relatively low parasitism, given by the two species of ectoparasites, *T. domerguei* and *C. irritans*, with average parasitism intensities of 6-10 parasites/host and prevalence of 15-40%.

In 2017, the presence of ectoparasites was not reported in the investigated horse mackerel specimens.

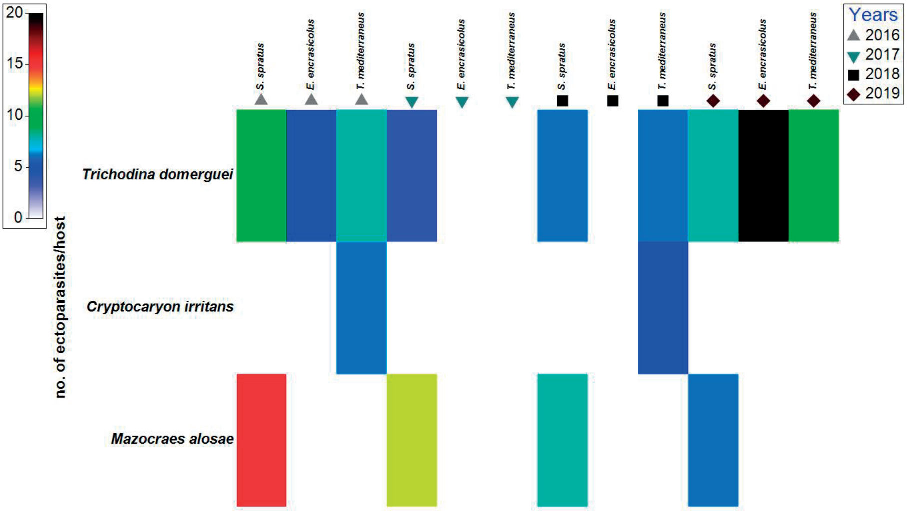


Figure 10. Average parasite intensity of ectoparasite species (parasites/host) in demersal species between 2016 and 2019

The highest degree of parasitism was recorded in pelagic fish individuals, which can be intermediate hosts, facilitating the transmission within the trophic chain (Figure 11). Parasites can induce significant changes in both the physical traits and behavior of their intermediate hosts. These changes often make hosts more susceptible to predation by definitive hosts(Poulin, 2011; Țoțoiu et al., 2024; Țoțoiu & Patriche, 2018).

In the demersal fish, one cause of parasitic diseases may also be the presence of other constitutional diseases, such as neoplasia (in turbot), pollution, and other biological elements (Țoțoiu et al., 2023). Fish populations are subject to many natural and anthropogenic factors that reduce their abundance (Demopoulos & Sikkel, 2015; Zaharieva et al., 2023).

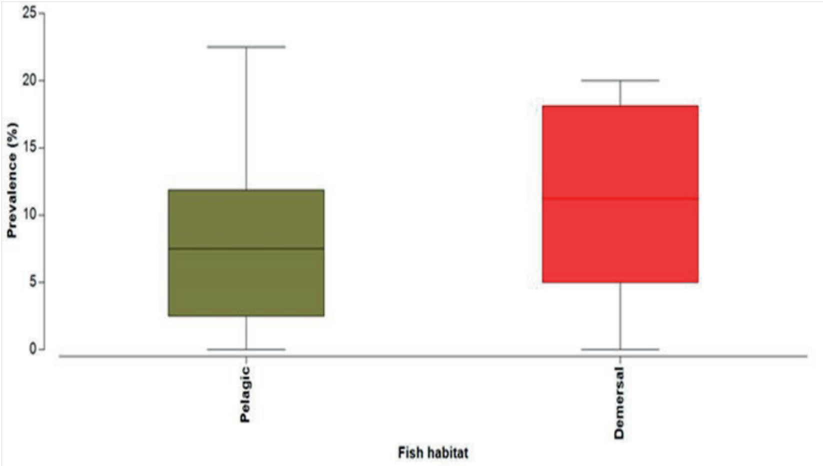


Figure 11. Average prevalence of ectoparasites in relation to the fish habitat (pelagic vs. demersal)

The degree of parasitism varied in the three analyzed areas, following a decreasing trend from north to center (Figure 12).

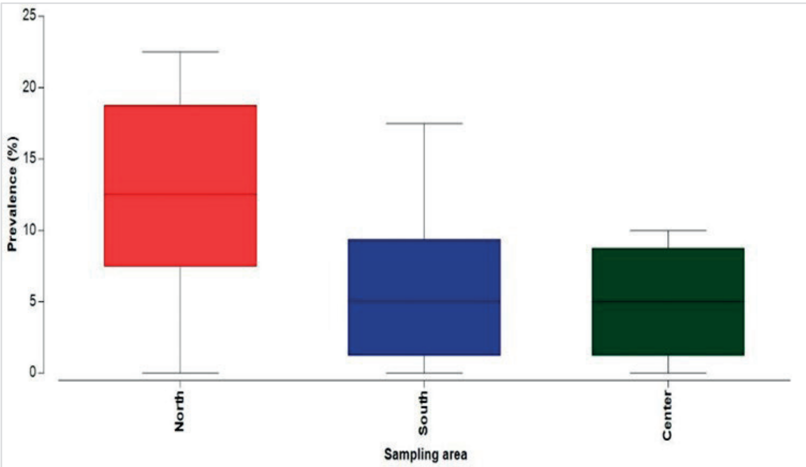


Figure 12. Average prevalence of ectoparasites in relation to the sampling area

Wild-caught fish usually host multiple species of parasites as this is a common feature for fish living under natural conditions (Scholz et al., 2018). Some species show a high degree of host specificity and sometimes have complicated life cycles involving different species of organisms as intermediate hosts and, as such, are not transmitted directly from fish to fish (Karlsbakk et al., 2014).

In wild populations, it is difficult to quantify the effects of any factor on stock size. However, evidence has accumulated supporting that some animal parasites, especially protozoa, can act as severe pathogens, causing direct mortality or increasing vulnerability to other environmental or biotic stressors (MacKenzie & Abaunza, 2014). In the northern area of the Black Sea, the highest degree of parasitism has been recorded, potentially due to various environmental pressures prevalent in the region. Eutrophication resulting from excessive nutrient runoff may be a significant factor contributing to these elevated parasitism levels. This process leads to algal blooms and subsequent hypoxic conditions (Lazar, Vlas, et al., 2024), which can stress marine organisms and make them more susceptible to parasitic infections. Additionally, pollution from agricultural and industrial sources introduces contaminants that may impair the immune systems of aquatic species, further increasing their vulnerability to parasites (Bisinicu et al., 2024; Grabner et al., 2023; Lazar, Spanu, et al., 2024; Zaharieva et al., 2023).

These environmental stressors collectively disrupt the ecological balance, facilitating the proliferation of parasitic organisms and intensifying host-parasite interactions in the Black Sea's waters.

The species of ectoparasites of the genus Trichodina can affect all marine species, they have a direct life cycle and are difficult to research. *T. domerguei* is a widespread species known for infesting a variety of fish hosts in freshwater, brackish, and marine habitats. Its low specificity allows it to thrive across different environments (Özer et al., 2013, 2015; Özturk & Özer, 2010). Species of this genus can cause high mortality, especially in areas with poor water quality (Bruno et al., 2006; Özturk & Özer, 2010)).

In this study, the anatomical-pathological lesions caused by ectoparasites in the infested specimens were slight erosions of the integument, inflammation of the gills, destruction of the gill epithelium, and the presence of an abundance of mucus. In the current study, the highest infestation rate with *T. domerguei* was 65%, and the average parasite intensity was 24 parasites/host. Özer and Kirca's (Özer & Kirca, 2013) the study reported a slightly higher prevalence of 56.2% and a lower average infestation intensity of 81.11 ± 36.56 (Özer & Kirca, 2013). Flatworms (monogenesis) are very specific to the host; the variation of specificity appears between the species of monogenesis, showing also a variation in the geographical distribution (Strona et al., 2010). Monogeneans have a direct, single-host life cycle. *M. alosae* lays eggs on the gills of fish, where post-oncomiracidia are also found. All organ systems of an adult are formed at about 15-20 days (Buchmann & Bresciani, 2006; Strona et al., 2010). *M. alosae* is a gill monogenean species specific to Clupaeidae all over the Black and Azov seas, following the host (Özer & Kirca, 2013).Parasites fixed on the gills cause irritation and breathing disorders in fish, leading to the fish reacting with a rich mucus secretion. Intense parasitism can cause fish asphyxiation (Plaksina et al., 2023; Wilber et al., 2016). Throughout the studied period, *M. alosae* was rarely reported in sprat, and the lesions it caused at the level of the gills were minor. Monogeneans primarily infect the exterior of fish, residing in specific areas such as the head, gills, fins, or the genitourinary system, where they feed on blood or cause tissue damage through parasite (Abo El-Ella et al., 2023; Buchmann & Bresciani, 2006; Kasse, 2019).

C. irritans is a ciliated, protozoan parasite that causes a disease known as marine “ich” or “white spot” disease in wild and aquacultured fish populations at temperatures between 15-30°C. *C. irritans* cause massive losses in the aquaculture industry, while in the marine environment, it is not as virulent and does not cause such high losses in fish populations (Fridman, 2022). It has been reported weakly in horse mackerel and anchovies fished in pound nets located in Năvodari and Eforie Sud, in 2016 and 2019. The intensity of parasitism did not constitute a danger for horse mackerel and anchovy specimens in the respective area.

C. acipenseris causes inflammation, purulent processes, and tissue destruction at the places where the worms attach. It was reported rarely, in sand sole (2016 and 2018) on the gills (eggs), skin, and fins, without affecting the general health of the fish.

The four ectoparasites produced superficial lesions on the skin, gills, and fins, however, these lesions can be a gateway for bacterial infections and other parasites.

Also, there is a growing acknowledgment of the vital role that parasites play in trophic pathways (Demopoulos & Sikkel, 2015). The integration of parasites into food webs has revealed heightened interdependencies among species (Amundsen et al., 2009), resulting in increased trophic efficiency (Arias-González & Morand, 2006). This expanded understanding highlights the intricate and interconnected nature of ecological systems. Even though the importance of parasites in marine ecosystems is clear, there is still much to be learned about the various effects that parasites have in different marine ecosystems.

CONCLUSIONS

Starting from the idea that diseases can act as severe pathogens, causing direct mortality or increasing the vulnerability of fish to stress factors (environmental and biotic), research was undertaken to identify ectoparasites in demersal and pelagic fish, in the period 2016 - 2019, belonging to the species *S. spratus*, *E. encrasicolus*, *T. mediterraneus*, *P. flesus*, *P. lascaris*, *S. maeoticus* from the Romanian coast of the Black Sea. Four species of ectoparasites were identified in the analyzed fish (the protozoa *T. domerguei* and *C. irritans*; the flatworm M. alosae, and the nematode *C. acipenseris*). The most common ectoparasite found in all analyzed fish species during the study period was *T. domerguei*. While it does not pose a major threat to the fish's life in their natural environment, it can serve as an entry point for other pathogens due to the diseases it causes. The monogenean worm *M. alosae* was reported in the sprat captured at the pound nets in the north of the Romanian coast and the one located at Eforie Sud, the other two species identified, *C. acipenseris*, *C. irritans*, sporadically affected fish populations (horse mackerel and sand sole), with low parasitism intensities. Pelagic and demersal fish stock dynamics may be affected by parasitism; thus, for appropriate and sustainable resource management, screening ectoparasites provides essential information and should be a continuous research endeavor.

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