DUNĂREA DE JOS UNIVERSITY OF GALAŢI

DOCTORAL SCHOOL OF ENGINEERING

PhD Thesis

-Summary -

The spatio-temporal dynamics of the mesozooplankton community on the Romanian Black Sea coast and its contribution to the feeding of pelagic fish species with commercial value

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INTRODUCTION

The pelagic area is the largest habitat in the world and is of great ecological importance, as most organisms and fish species spend at least part of their lives in this area, either as larvae or as adults.
The zooplankton community is represented by animal organisms that float in the water body without actively moving in the water column and that are usually found in the pelagic area. These communities are very important in marine food webs, being the main consumers of phytoplankton and a source of fish feed, thus controlling phytoplankton production and modelling processes within pelagic ecosystems (Harris et al., 2000). Zooplankton can be divided into size classes, this doctoral thesis focusing on the mesozooplankton fraction (between 0.2-20 mm), being an important part of zooplankton in pelagic trophic web.
These organisms are crucial in marine trophic web, not only because of their sheer abundance and great diversity, but also for the vital functions of the trophic ecosystem they perform - especially in the pelagic trophic network - whether they are major consumers of phytoplankton or as prey, for many fish and other higher trophic levels.
Zooplankton help shape the extent of climate change by fixing carbon through the biological pump, but paradoxically they are very susceptible to climate change. Different components of the ecosystem (trophic groups) respond phenologically to changes in environmental conditions (eutrophication, temperature rise), this can lead to mismatches between successive trophic levels and can disrupt the synchrony between primary, secondary and tertiary producers. Pelagic fish are also directly and indirectly influenced by climate change, as rising water temperatures cause changes in fish migrations and lead to changes in the presence and development of their zooplankton prey. Therefore, zooplankton is a potential indicator for water pollution (Ismail et al., 2016). Any variation in zooplankton biomass has implications for fishing and ecosystem services (Caroppo et al., 2013).
Thus, because eutrophication causes changes in the qualitative and quantitative structure of zooplankton, its use as an indicator of changes that may occur at trophic level and ecological conditions caused by nutrient dynamics has a very important role (Jurczak et al., 2019).
The dynamics of zooplankton populations, their reproductive cycles, growth and survival rate are important factors influencing the recruitment of fish stocks. The latter role has made zooplankton ecology of particular interest for research.
A number of marine organisms feed on plankton either at some point in their life cycle or throughout their lives. Therefore, the importance of plankton in the transfer of energy to higher trophic levels through different food chains cannot be underestimated, as it is the basis of the marine food web and therefore plays a key role in fishing. Primary productivity and plankton growth are closely related to the physico-chemical parameters of seawater (Drira et al., 2018).
In the Black Sea, zooplankton has the role of "key industry", converting into organic matter a large amount of primary production, but also non-living protein material, in this way, the matter and energy generated in the ecosystem by photosynthetic autotrophs, on the one hand , but also the particulate or solvated organic substance from the water mass becomes accessible for organisms that are not phytophagous or that do not have mechanisms for capturing organo-bacterial aggregates (Stugren, 1994). Thus, zooplankton become the main food source for juveniles and adults of plankton-eating pelagic fish, species that in the Black Sea are essential elements in the trophic base of dolphins, but also in the qualitative composition of industrial fishing (Onciu et al., 2006).
Zooplankton play a significant role in the production of any aquatic ecosystem. To a confirmed extent, fishing failure and success - especially in the case of pelagic species - are closely linked to plankton availability, large fish stocks being found in regions with abundant planktonic biomass.
Due to the key role of zooplankton in the early stages of fish development, changes in species composition and abundance have significant implications for fish recruitment and dynamics. Therefore, understanding the abundance, species composition, and distribution of zooplankton

is very valuable in supporting research on fish production and changes in ecosystems (Liu et al., 2014; Richardson, 2008). In the larval and juvenile stages, fish consume zooplankton as food, the synchrony between the peak of mesozooplankton abundance and fish larvae being crucial in determining the recruitment and dynamics of fish populations (Liu et al., 2017).

OBJECTIVES AND PURPOSE OF THE WORK

The general aim of this doctoral study is to expand and update the knowledge about the mesozooplankton community (0.2-20 mm) from the Romanian Black Sea coast in 2013-2020 and to characterize its trophic role for pelagic fish.

Specifically, the study focuses on the following objectives:

1. Identification of the mesozooplankton's qualitative composition from the Romanian Black Sea coast
2. Assessment of the dynamics, temporal, and spatial distribution of mesozooplankton's population
3. Evaluation of environmental factors that influence the mesozooplankton component
4. Assessment of water bodies on the Romanian Black Sea coast according to EU Marine Strategy Framework Directive (MSFD) based on established mesozooplankton indicators
5. Development of distribution maps for pelagic fish agglomerations -*Sprattus sprattus* (Linnaeus, 1758) and *Alosa tanaica* (Grimm, 1901) and for the density of the fodder mesozooplankton community
6. Consolidation of knowledge on the diet of pelagic fish species by stomach content analysis in *Sprattus sprattus* (Linnaeus, 1758) and *Alosa tanaica* (Grimm, 1901)

The scientific value of this doctoral thesis is that it analyzed not only the structure of the mesozooplankton community on the Romanian coast (temporal and spatial details), but also the dynamics of clupeids in relation to the abundance of food in the water column by making distribution maps and availability prey by analyzing the stomach contents of two pelagic fish of commercial value.

The doctoral thesis is written in a volume of 252 pages and a total of 268 figures and graphs and 21 tables. The bibliography contains 206 bibliographic titles. The doctoral thesis is structured in eight chapters, the first two chapters containing the study of the literature, and the other six chapters dealing with the original contributions.

CHAPTER 1. Black Sea zooplankton

The first chapter of the thesis presents information on zooplankton in general, with reference to its ecological characteristics (**subchapter 1.1**), its importance in the ecosystem (**subchapter 1.2**), and a brief history of zooplankton research in the Romanian coast (**subchapter 1.3**).

Zooplankton are classified by size or stage of development (Figure 1), size categories (Harris et al., 2000) including:

• picoplankton, which measures less than 2 micrometers,
• nanoplankton (between 2-20 micrometers),
• microzooplankton (between 20-200 micrometers),
• mesozooplankton (between 0.2 - 20 millimeters)
• macrozooplankton (between 20-200 millimeters)
• megaplankton (measuring over 200 millimeters).

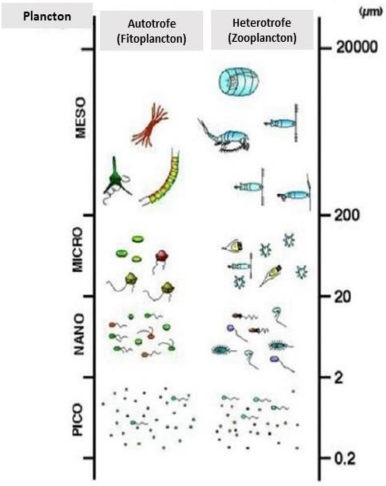

Figure 1. Dividing plankton into size classes, functional groups (Alcaraz et al., 2003)

CHAPTER 2. Pelagic fish from the Romanian Black Sea coast

The chapter begins with information on pelagic fish, emphasizing their role in the ecosystem, then describing the fish species of the Order Clupeiformes, present in the Black Sea (subchapters 2.1 and 2.2).

CHAPTER 3. Materials and methods

3.1. Mesozooplankton community

The collection of mesozooplankton samples was performed by towing vertically the net in the water mass, with a speed of 0.5-1 m / s , on standard horizons (10-0 m, 25-10 m, 50-25 m, 100 -50 m) in the warm period and in the entire water column, in the cold season.

The stations network with a number of 45 sampling points, is extended over the entire continental shelf of the Romanian coast and consists of 13 profiles perpendicular to the coastline: Sulina, Mila 9, Sf.Gheorghe, Portița, Gura Buhaz, Casino Mamaia, Constanța Nord, Est Constanța, Constanța Sud, Eforie, Costinești, Mangalia, Vama Veche(Figure 2).

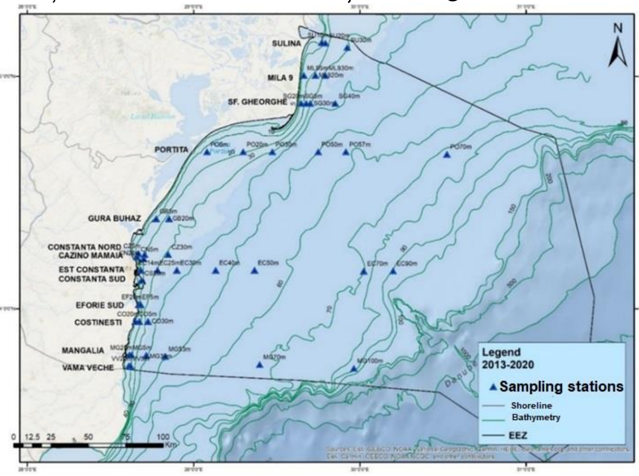

Figure 2. Map with mesozooplankton sampling stations in 2013-2020

3.2. Pelagic fish

The sampling of pelagic fish was carried out with the help of a 36 m long pelagic trawl, by surface trawling (0-5m), at a vessel speed of 2.8-3.2 Nd, 30 minutes trawl duration, the horizontal opening of 24 m trawl, 12 m vertical.
Sampling stations for sprat were at depths between 10 m (Zaton) and 60-70 m (Mangalia), in the period 2013-2020 (Figure 3) and for azov shad trawlings were carried out only in the northern sector of the coast, at depths up to 40m, in the period 2019-2020 (Figure 3).

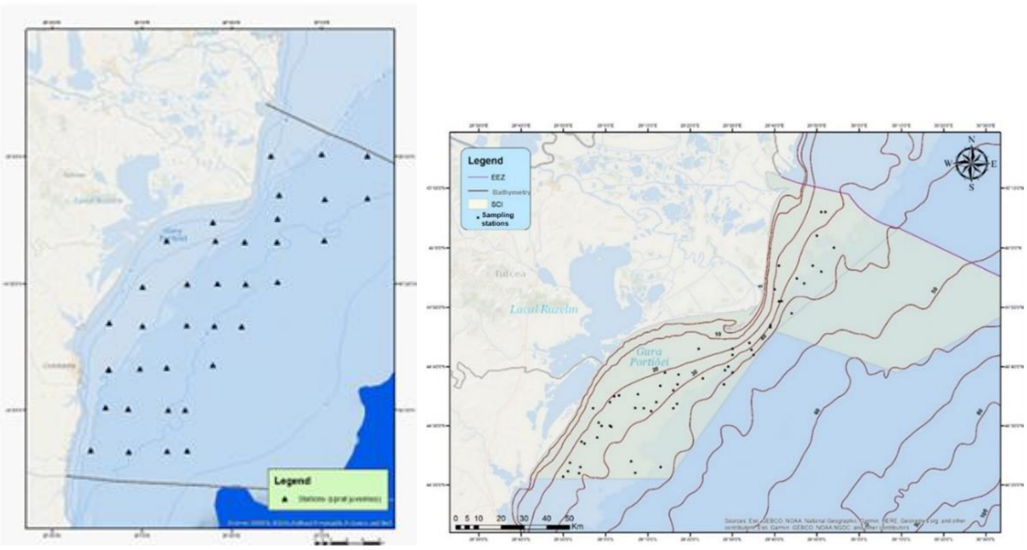

Figure 3. Map with sampling stations for sprat (left) and azov shad (right) (INCDM

3.3. Stomach content analysis

To identify the stomach content of pelagic fish, two fish species were studied: sprat - Sprattus sprattus (Linnaeus, 1758) and Alosa tanaica – azov shad (Grimm, 1901). In order to establish the contribution of the mesosoplankton component in the nutrition of the studied pelagic fish, the stomach content analysis method was used. The samples were collected from the continental shelf of the Black Sea, the sprat sampling covering the entire continental shelf (Figure 4), for azov shad, the samples being collected exclusively from stations located in the Northern sector of the coast, up to 40m isobath (Figure 4).

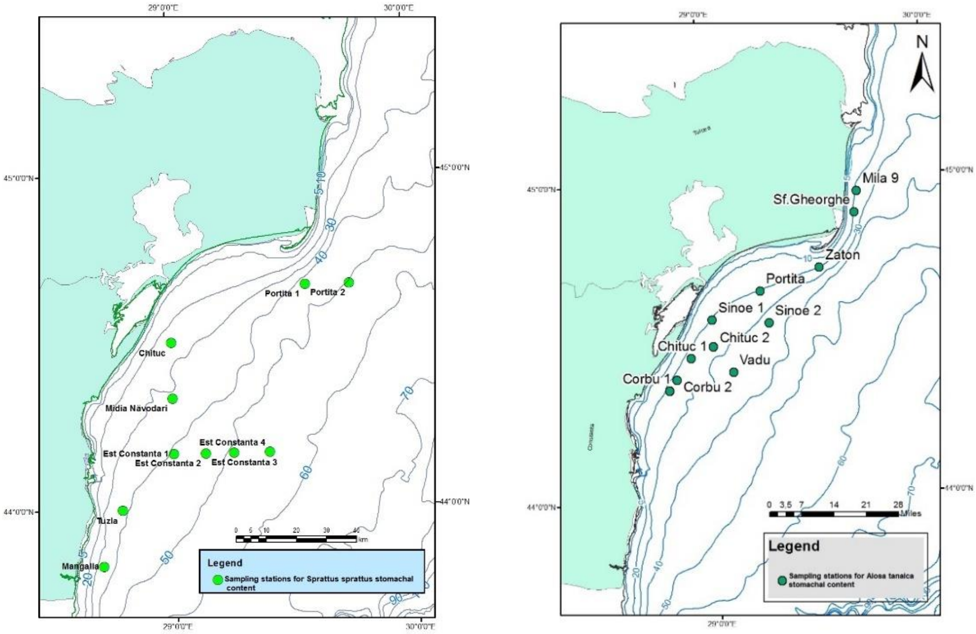

Figure 4. Map with the sampling stations from which *Sprattus sprattus* (Linnaeus, 1758) and *Alosa tanaica* (Grimm, 1901) individuals were collected to analyze the stomach content

Fish samples were measured and weighed, and the study of stomach content was performed by analyzing the contents and determining as accurately as possible the type of food found in the stomach, by determining the groups. The stomachal contents were determined by abdominal dissection of the fish specimen, followed by stomach extraction . The stomach was dissected, weighed, and the constituent food items were separated and analysed under a microscope (Figure 5).

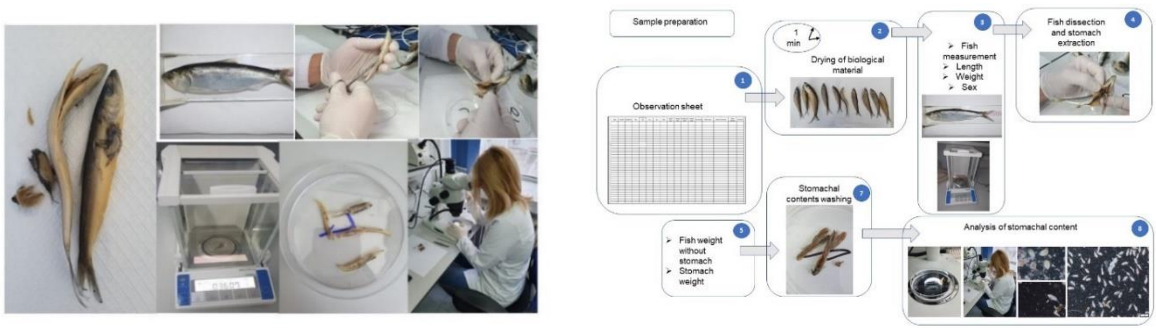

Figure 5. Analysis of stomach content in pelagic fish species

3.4. Methods of data analysis, processing and interpretation

STATISTICS 14.0.0.15 (TIBCO) was used to analyze the interactions between the abiotic component and the mesozooplankton community.

The data were also processed using the PRIMER 7 program (v.7.0.17) (Clarke, 2014) for the analysis of spatial and temporal variations of mesozooplankton data and for the stomach content of fish in all years of investigations.

ARCGIS PRO - professional mapping software is the newest professional (desktop) GIS (Geographic Information System) application from Esri, with which the distribution maps were created.

Caracteristic ecological indices that define the ecological status of the marine ecosystem have been applied based on the biological element mesozooplankton, in accordance with the requirements of the Marine Strategy Framework Directive -MSFD.

CHAPTER 4. The evolution of the mesozooplankton community from the Romanian Black Sea coast in 2013-2020

In this chapter the qualitative variation of mesozooplankton was analyzed (subchapter 4.1), the identified mesozooplankton species were described (subchapter 4.1.1), the quantitative analysis of mesozooplankton in 2013-2020 (subchapter 4.2) and the seasonal distribution and dynamics of the mesozooplankton community (subchapter 4.3) was described.

4.1. Qualitative analysis of the mesozooplankton population on the Romanian Black Sea coast

During 2013-2020, 27 mesozooplanktonic taxa, of holopanctonic and meroplanktonic nature, belonging to different classes and orders, were identified on the Romanian coast.
Regarding the qualitative structure by ecological groups (Figure 6), it is observed that throughout the study period, the largest share was held by copepods (between 33% and 43%), followed by the group of cladocera and the meroplanktonic component. The other group category recorded lower percentage values and the nonfodder component recorded the same value of 5% given the fact that it is represented by a single species, the dinoflagellate *Noctiluca scintillans*.

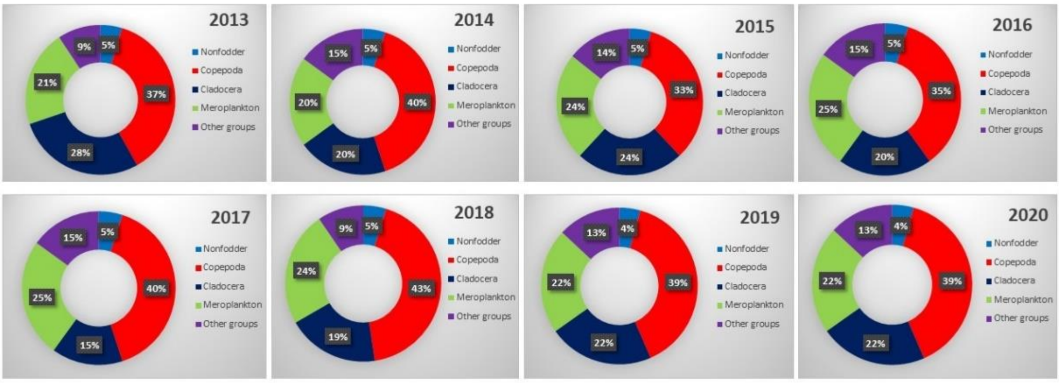

Figure 6. Qualitative structure of total mesozooplankton in 2013-2020

4.2. Quantitative analysis of mesozooplankton in 2013-2020

Mesozooplankton dynamics is influenced by Danube's nutrient supply, salinity, and the transport directions of the mesozooplankton community. Due to the variability of environmental conditions on the Romanian coast, a division into three sectors was considered: **Northern**

sector -N (Sulina, Mila 9, Sf. Gheorghe, Portiţa profiles) - area strongly influenced by the Danube, **Central** sector - C (profiles Gura Buhaz, Casino, Constanța Nord, East Constanța Constanţa Sud), **Southern** sector- S (profiles Eforie Sud, Costinesti, Mangalia, Vama Veche). From the box plot diagrams for the average mesozooplankton densities it is observed that there are variations between the three analysed sectors (Figure 7) which makes it opportune to analyse this component depending on the sectors.

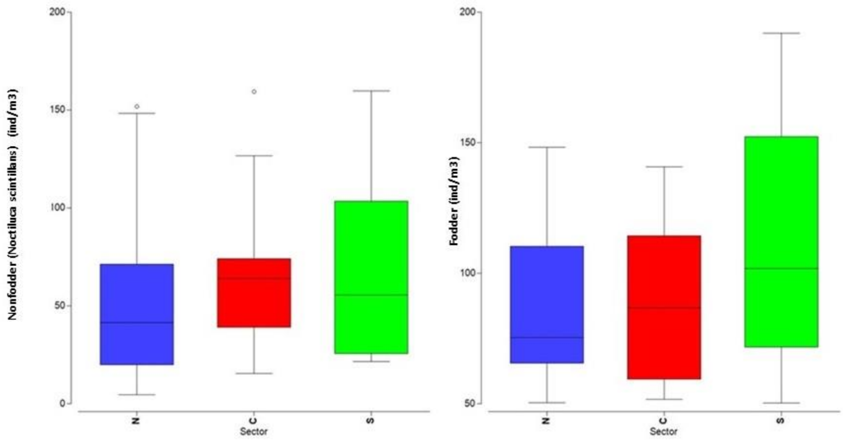

Figure 7. Box plot diagrams for average mesozooplankton density values (fodder and nonfodder) by sectors in 2013-2020

4.3. Distribution and seasonal dynamics of mesozooplankton in 2013-2020

The SIMPER analysis for the warm and cold season highlights the large contribution that the nonfodder component represented by Noctiluca scintillans had in the warm season, in the cold season being less represented in terms of density but dominating in terms of biomass (Table 1). Acartia clausi recorded higher average values in both warm and cold seasons, dominating in the cold season (Table 1). The meroplanktonic elements Balanus and Bivavlvia contributed in the warm season to the mesozooplankton community, and in the cold season only Bivalvia contributed to the average density values (Table 1).

Table 1. SIMPER analysis based on the average densities of mesozooplankton taxa, by seasons, in 2013-2020

Warm season					
Taxa	Average density (ind/m^3)	Average sim	Sim/SD	Contrib%	Cum%
Noctiluca scintillans	68,12	12,39	1,49	19,92	19,92
Acartia clausi	45,82	8,97	2,73	14,43	34,35
Balanus	39,78	7,57	2,16	12,18	46,52
Bivalvia	37,77	7,34	2,69	11,8	58,33
Pleopis polyphemoides	24,7	5,31	3,24	8,54	66,87
Pseudocalanus elogatus	15,76	3,87	3,41	6,22	73,09
Cold season					
Acartia clausi	39,41	15,66	3,72	26,78	26,78
Noctiluca scintillans	36,4	12,22	4,01	20,9	47,68
Pseudocalanus elogatus	23,67	9,82	5,29	16,8	64,48
Bivalvia	14,56	4,84	6,82	8,28	72,76

Analyzing the dominance of total mesozooplankton in the warm and cold season, it is observed that in the warm season there were variations for the fodder and nonfodder average density values. Thus, in some stations the dominance of fodder component is noticeable and in others that of nonfodder (Figure 8). In the cold season, the mesozooplankton fodder component of was better represented, reaching the highest average values, the nonfodder component being less developed in terms of quantity (Figure 8).

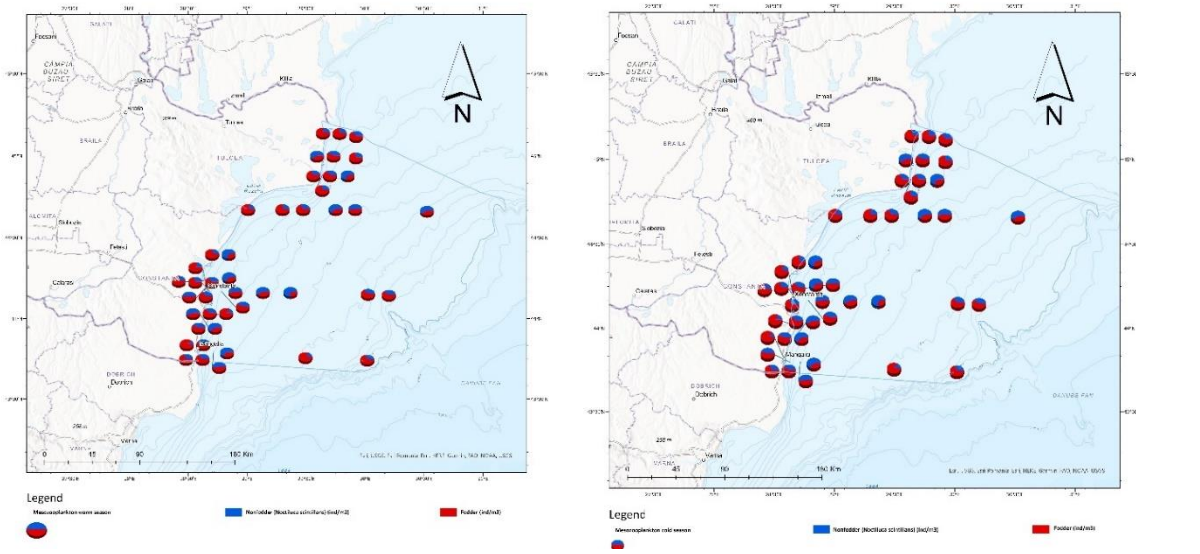

Figure 8. Spatial distribution of average mesozooplankton density in warm and cold season of 2013-2020

CHAPTER 5. Interactions between environmental factors and the mesozooplankton community

Climate change, whether natural or anthropogenic, can alter local ecological conditions of pelagic habitat, affecting species abundance and the composition of biological communities (Beaugrand et al., 2018).
Because the distribution of mesozooplankton communities is closely related to the evolution of abiotic environmental factors) (Peterson et al., 1979) we analyzed the variation of the main physico-chemical parameters (temperature (T), salinity (S) and dissolved oxygen (O2) as well as nutrients), phosphates (PO4), silicates (SiO4), nitrates (NO3), nitrates (NO2) and ammonium (NH4) dissolved in seawater, 2013-2020 (Table 2), in the warm season (May-October) and cold season (November-April).

Table 2. Descriptive statistics of environmental factors on the Romanian coast, 2013-2020

Variable	N	Average	Median	Min.	Max.	25th percentile	75th percentile	Std.Dev.	Coefficient of variation (%)
T [°C]	387	18,58	20,28	5,20	27,12	12,98	23,79	6,35	34,2
S [‰]	387	14,66	15,58	0,11	19,91	13,52	17,36	3,80	25,9
O2 [µM]	392	319,0	314,6	119,2	495,6	284,8	354,8	49,4	15,5
PO4 [µM]	391	0,34	0,24	0,01	3,04	0,10	0,44	0,38	111,8
SiO4 [µM]	391	14,22	6,49	0,05	168,32	3,07	15,58	20,77	146,1
NO2 [µM]	391	1,84	0,41	0,01	50,85	0,14	1,06	4,97	270,1

NO3 [µM]	391	5,21	2,53	0,01	69,23	1,49	5,59	7,46	143,2
NH4 [µM]	391	7,71	5,45	0,12	64,41	1,93	11,02	7,94	103,0

Principal Component Analysis (PCA) (Figure 9) explains, by the first two factors identified, 54.5% of the variation of environmental factors (PC1 - 31.8%, PC2 -22.7%). PC1 includes the effect of low salinity (-) and high content (+) of phosphorus and silica in river input [1] while PC2 takes into account the increase in temperature [2].

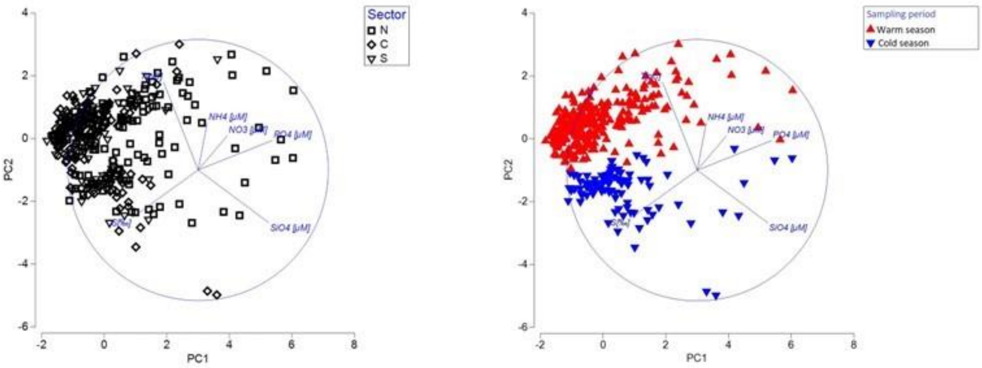

Figure 9. Analysis of the main components (PCA) for the environmental factors from the Romanian coast, 2013-2020

Temperature and salinity are the combination of significant factors (r = 0.302) that best explain the density of Black Sea mesozooplankton in 2013-2020 (Figure 10).

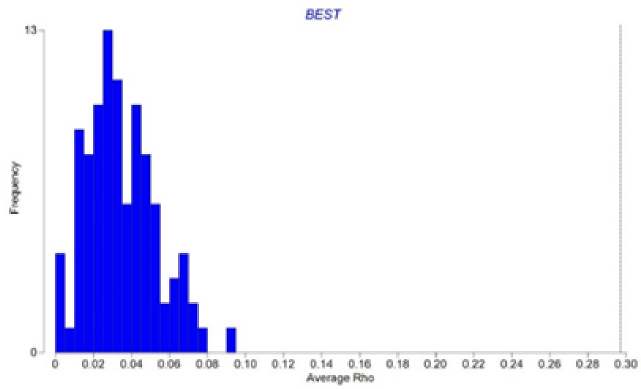

Figure 10. BEST histogram - environmental factors with mesozooplankton density - 2013-2020

Analyzing the nonfodder mesozooplankton represented by the dinoflagellate *Noctiluca scintillans* in relation to the the warm season temperature in 2013-2020, it is observed that the highest densities (80315-151789 ind / m3) were recorded in the Northern sector at temperatures between 15.49-19.31 0C and in the South at temperatures of 19.32-21.53 0C (Figure 11). *Noctiluca scintillans* recorded similar density values in all three sectors at temperature values between 23.6-24.89 0C. Regarding the salinity in the warm season, it is observed that at salinities between 16.99-18.94 PSU, *N.scintillans* recorded the lowest density values, the species being better represented from the quantitative point of view, at salinities between 8.93 PSU and 16.98 PSU, the maximum density being reached in the Northern sector at a salinity of 12.65-15.39 PSU (Figure 11).

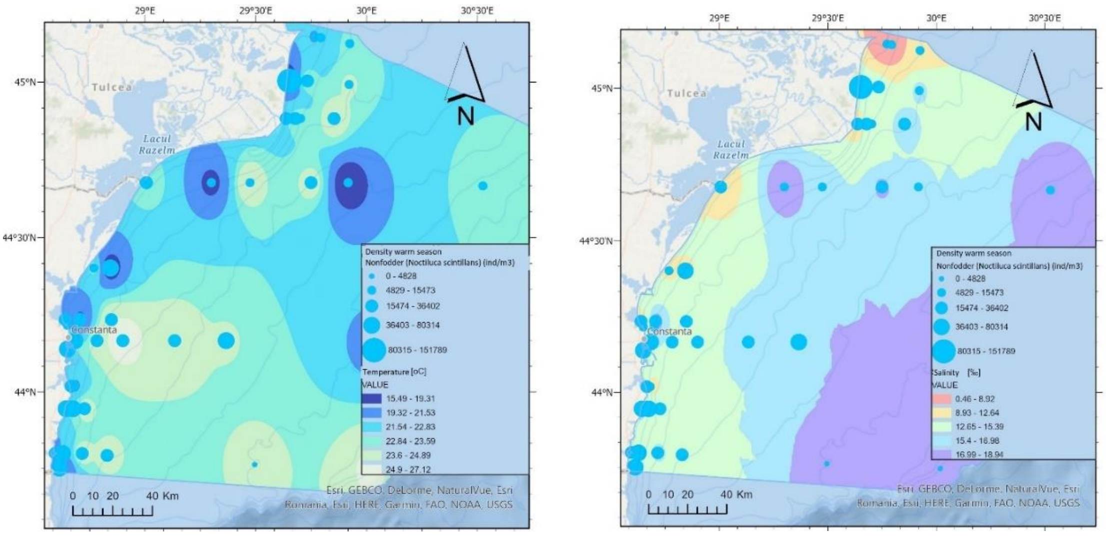

Figure 11. Spatial distribution of nonfodder mesozooplankton density, water temperature and salinity on the Romanian coast - warm season, 2013-2020

The fodder mesozooplankton in the warm season was well represented in terms of quantity, recording high density values. It is observed that the lowest values (60893-114494 ind / m3) were recorded in the shallow stations (Figure 12). The depth distribution of mesozooplankton is mainly influenced by abiotic factors such as water temperature and biotic factors such as food availability and predator stress (Hembre et al., 2003).
The density of fodder mesozooplankton in the warm season showed variations in relation to salinity values, the highest values of density being recorded in the Southern and Central sector, at salinities of 12.65-15.39 PSU (Figure 12). It is observed that the lowest densities were reached in the offshore area where the salinity ranged between 16.99-18.94 PSU (Figure 12).

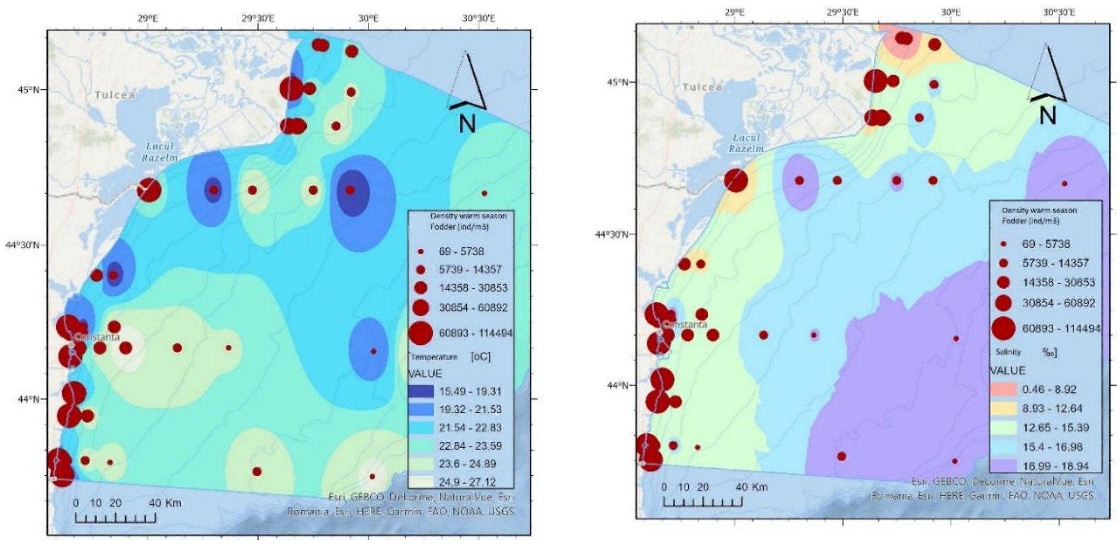

Figure 12. Spatial distribution of fodder mesozooplankton density, water temperature and salinity on the Romanian coast – warm season, 2013-2020

Although with lower density values than in the warm season, the dinoflagellate *Noctiluca scintillans* was also present in the cold season of 2013-2020. It is noteworthy that the species developed in all three sectors in all temperature ranges recorded (Figure 13), *Noctiluca* *scintillans* occurring in large temperature ranges, from temperatures below 0-30 ° C (Ollevier et al. , 2021). The same situation is encountered in the case of salinity, where the species was found in all salinity intervals in the cold season, even at 0.59-8.92 PSU, interval specific to the Northern sector of the Romanian Black Sea coast (Figure 13). Although the increase in *N. scintillans* abundance is generally affected by temperature and salinity, it is known to be a euriterm and eurihalin organism (Elbrachter et al., 1998). Many previous research results have shown that the optimum temperature and salinity requirements of *N. scintillans* are different in each ecosystem (Huang et al., 1997; Tada et al., 2004; Miyaguchi et al., 2006).

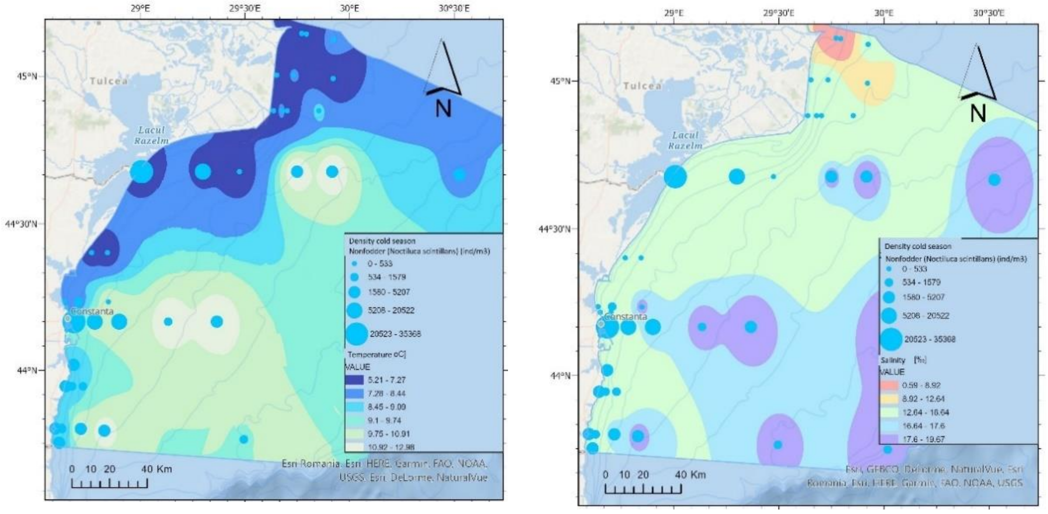

Figure 13. Spatial distribution of nonfodder mesozooplankton density, water temperature and salinity on the Romanian coast - cold season, 2013-2020

Changes in mesozooplankton abundance are very clear in the cold season of 2013-2020, maximum density values being lower compared to the warm season. It is observed that in the cold season, the mesozooplankton community recorded density values between 5164-25028 ind / m3, regardless of temperature and salinity value s(Figure 14).

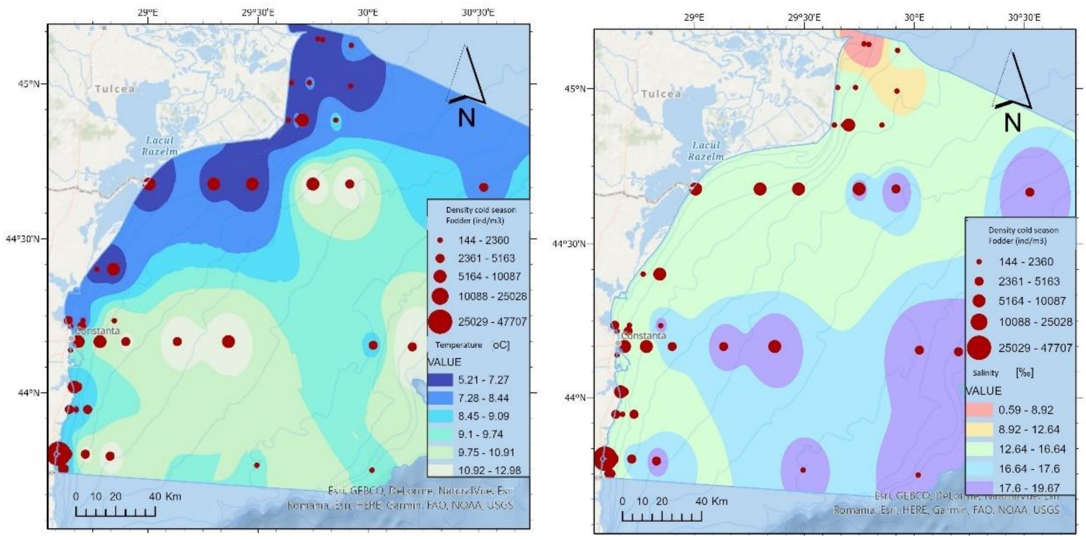

Figure 14. Spatial distribution of fodder mesozooplankton density, water temperature and salinity at the Romanian coast - cold season, 2013-2020

CHAPTER 6. Mesozooplankton community as an indicator of the ecological status of the marine environment in accordance with the principles of the Marine Strategy Framework Directive

The objective of the Marine Strategy Framework Directive 2008/56 / EC (DCSMM) is "to protect and restore European's seas and oceans and to ensure that human activities are carried out in a sustainable manner so that present and future generations can enjoy and benefit from them, biologically dynamic, safe, clean, healthy and productive oceans and seas.
For the characterization of pelagic habitats from the point of view of the mesozooplankton community, a number of two indicators for Criterion D1C6 and an indicator for Criterion D5C3 were established.

Mesozooplankton biomass (mg·m^{-3}) -D1C6

In the cold season, water bodies with variable salinity, coastal and marine have reached good ecological status in high percentages, as follows: waters with variable salinity have reached GES in proportion of 88%, coastal waters have reached a percentage of 82% and the marine waters reached 94% good ecological status (Figure 15).
In the warm season, good ecological status was not reached in any of the three water bodies , poor ecological status being dominant (Figure 15). Waters with variable salinity reached poor ecological status in proportion of 72%, coastal ones with a percentage of 52% and the marine ones with 56% (Figure 15).

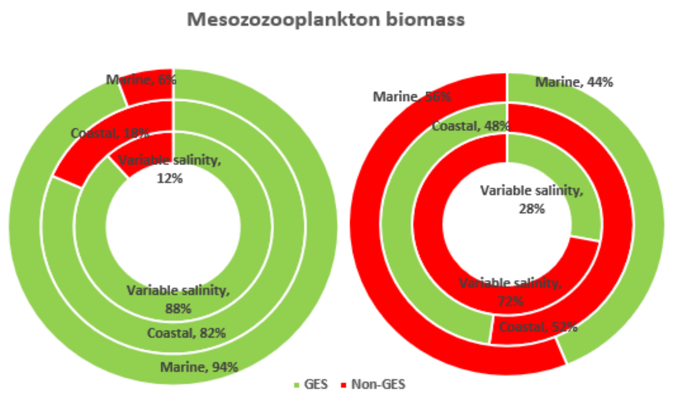

Figure 15. Ecological status of water bodies based on the indicator Mesozooplankton biomass in the cold season (left) and warm season (right) in 2013-2020

Copepoda biomass (mg·m^{-3})-D1C6

In the cold season of 2013-2020, based on Copepoda biomass indicator, good ecological status was achieved in all three water bodies. In waters with variable salinity, GES reached a 73%, in coastal waters it reached 72% and in marine waters the highest percentage for GES-92% was recorded (Figure 16). In the warm season, the poor ecological status was recorded in all three water bodies. In waters with variable salinity the poor ecological status was 65%, in coastal waters 59% and in marine waters 61% (Figure 16).

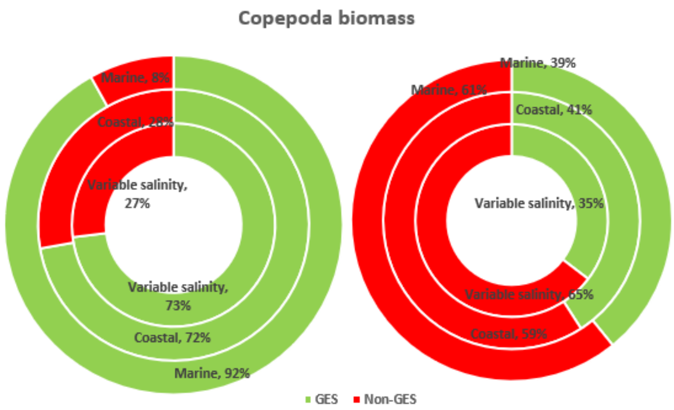

Figure 16. Ecological status of water bodies based on the indicator Copepoda biomass in the cold season (left) and warm season (right) in 2013-2020

Noctiluca scintillans **biomass (mg·m^{-3}) - D5C3**

In the cold season of 2013-2020, good ecological status was reached in all three water bodies, in waters with variable salinity, good ecological status being reached in proportion of 100%, in coastal waters in proportion of 75% and in marine waters in proportion of 54% (Figure 17). In the warm season, based on the indicator *Noctiluca scintillans* biomass, the good ecological status of the three water bodies was reached. In waters with variable salinity the percentage of GES was 72%, in coastal waters 58% and in marine waters it reached a value of 65% (Figure 17).

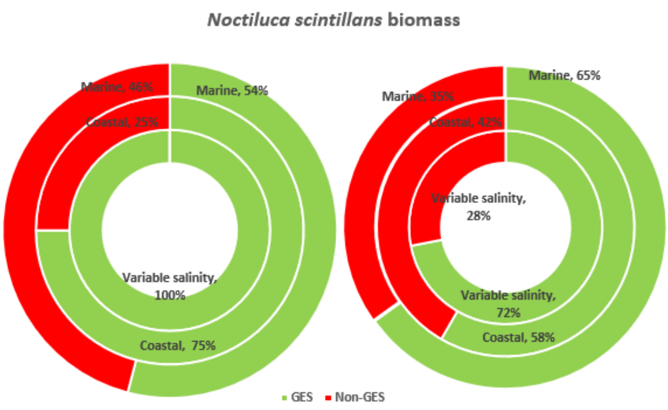

Figure 17.Ecological status of water bodies based on the indicator *Noctiluca scintillans* biomass in the cold season (left) and warm season (right) in 2013-2020

CHAPTER 7. Dynamics of pelagic fish in relation to fodder mesozooplankton from the Romanian coast in 2013-2020

In some ecosystems, pelagic fish play a prominent trophodynamic role, exercising both top-down control over zooplankton and being an abundant prey resource for other fish species (Peck et al., 2012).

7.1. Dynamics of *Sprattus sprattus* (Linnaeus, 1758) in relation to fodder mesozooplankton from the Romanian Black Sea coast in 2013-2020

Trophic dynamics of mesozooplankton and small pelagic fish occupy a significant position in marine pelagic trophic networks (Shannon et al., 2010). Mesozooplankton abundance can be taken as a good indicator of the availability of fishery resources (Nair et al., 1980). Copepods are a major component of marine zooplankton and thus are the main source of food for fish larvae (Poulet et al., 1991), promoting the survival, growth and development of juvenile fish (Hansen, 2017).

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in the spring of 2013

In the spring season of 2013, the sprat population recorded the highest catch value in the Northern and Central Black Sea stations, with the Southern sector being characterized by lower catch values (Figure 22. Analyzing mesozooplankton density values in the central and northern sectors it is observed that the highest values of abundance were recorded, especially in the shallow areas, in these conditions of adequate food provided, the sprat migrates to the shore. Copepods, part of the fodder mesozooplankton component and major prey of juvenile fish in the natural habitat due to their adequate nutritional value (Gamboa et al., 2019) were very well represented quantitatively, reaching maximum density values even in areas where formed agglomerations, which indicates a good trophic base.

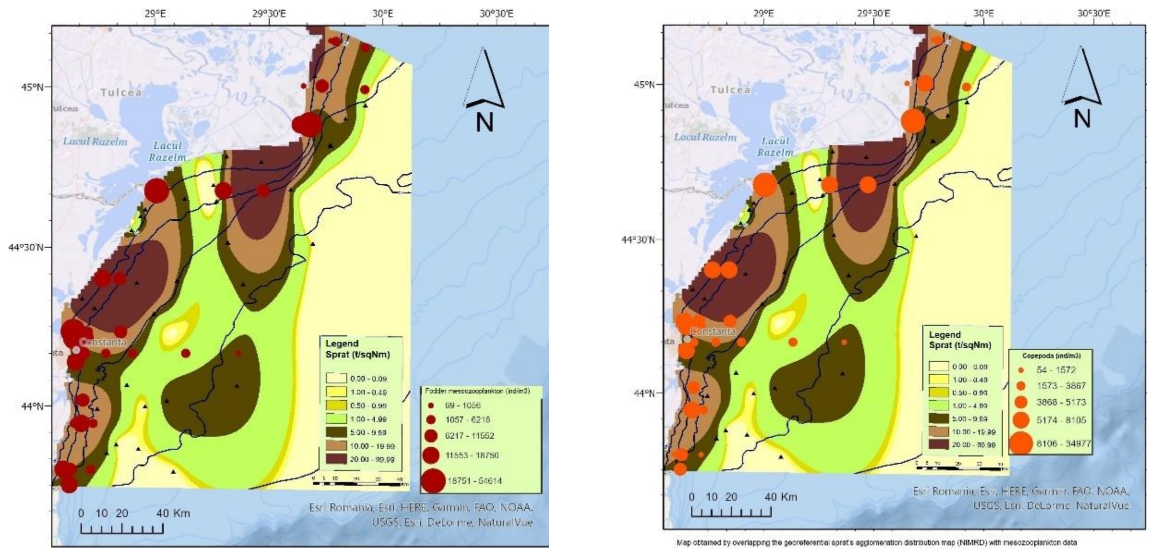

Figure 18. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2013

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in the summer of 2014

The sprat population in the summer of 2014 was characterized by high values of catches in the central and northern part of the coast, the maximum value of the catch being recorded in the northern sector (Figure 19). The quantitative differences that are registered in the sprat agglomerations from the three sectors of the Romanian coast are due to the biology of the sprat, a cryophilic fish that does not tolerate high temperatures.
With regard to fodder mesozooplankton, it is observed that its density has reached high values in the central and northern sector, in the shallow stations, where the sprat agglomerations have also reached high catch values. The group of copepods was best represented in the northern and central areas, which indicates a food availability.

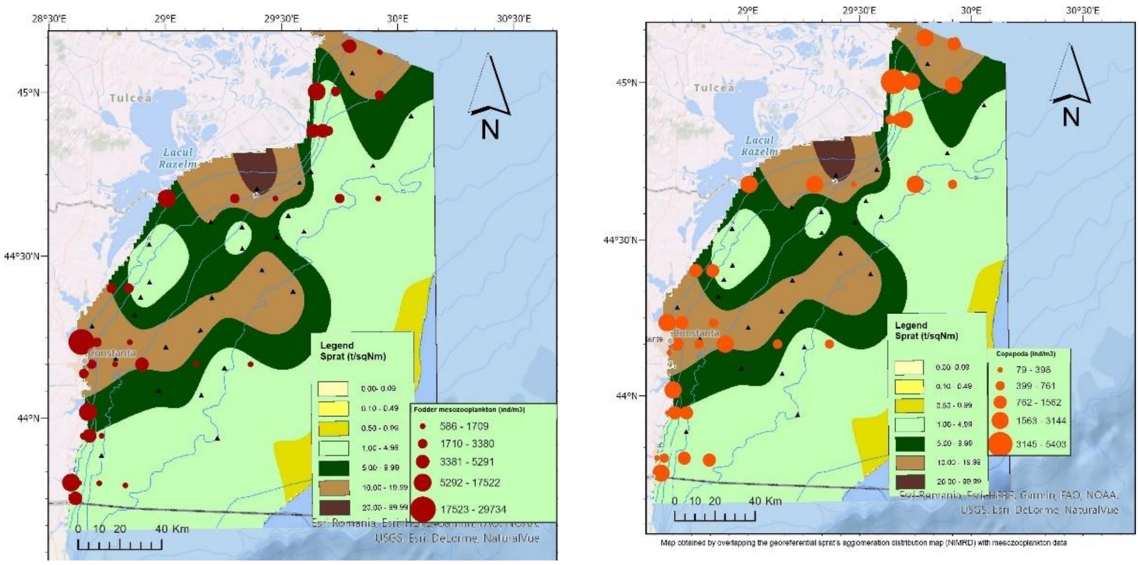

Figure 19. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in summer 2014

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in the spring of 2015

In the spring season of 2015, the sprat population varied quantitatively, registering high values in the central part of the Black Sea. In the southern sector, catch values for sprat ranged from 0.50-0.99 t / km2, except for one station where the value of catches was higher (10-19.99 t / km2) (Figure 20). Fodder mesozooplankton reached high density values in the shallower stations located in the central and southern sector, in the northern sector being less represented in terms of quantity (Figure 20). Regarding the quantitative structure of copepods, it is observed that they reached high values of density in all three sectors, being better represented quantitatively in the central sector (Figure 20) where the sprat population was also best developed, forming large agglomerations that could be generated by the abundance of food in the water column.

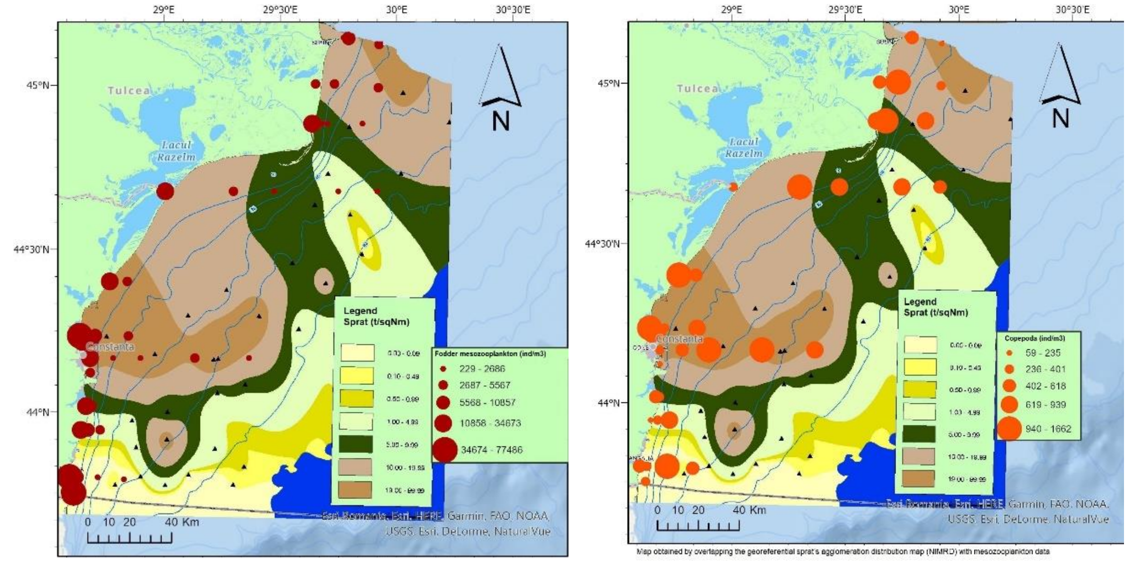

Figure 20. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2015

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2016

The spring sprat population varied quantitatively, with the highest catch values in the northern sector of the continental shelf (Figure 21). In the northern sector, the movement of shallow water to deepr depths is almost permanent (due to the action of the river current), the ascent of deep water masses to the shore being more accentuated. Therefore, the sprat catches in the warm season are higher in the northern sector, as opposed to the central and southern ones where the temperature drops in the surface layer are quite rare and for a short period of time (Cautiș, 1958). It is noteworthy that this year, the sprat population recorded approximately equal catch values in the central and southern sectors in the stations located up to 40m isobath, in the deeper waters the population being less represented (Figure 21). Fodder mesozooplankton showed a uniform distribution in the three sectors, with density values ranging from 3935-8359 ind / m3, the same relatively uniform distribution was also for copepods that recorded densities between 2114-4842 ind / m3 in most stations (Figure 21), the abundance of prey for the sprat population being optimal.

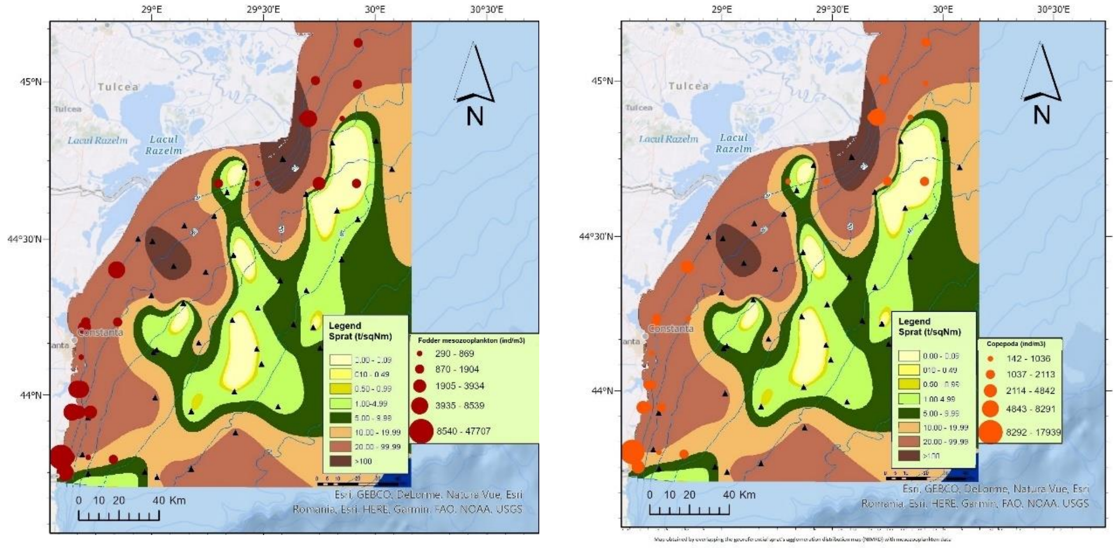

Figure 21. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2016

The sprat of autumn 2016 reached the highest values in the stations located in the southern sector (3 stations) and in the northern sector (1 station) (Figure 22). It is observed that in the central sector the sprat population was poorly represented. Fodder mesozooplankton recorded values between 4356-8593 ind / m3, being better represented in a single station in the southern sector and copepods reached higher density values in the stations located in the central and northern sector (Figure 22). It should be noted that in areas where sprat was better represented quantitatively, namely in the offshore areas, food availability was lower, the fodder mesozooplankton component and copepods being present in smaller quantities (Figure 22).

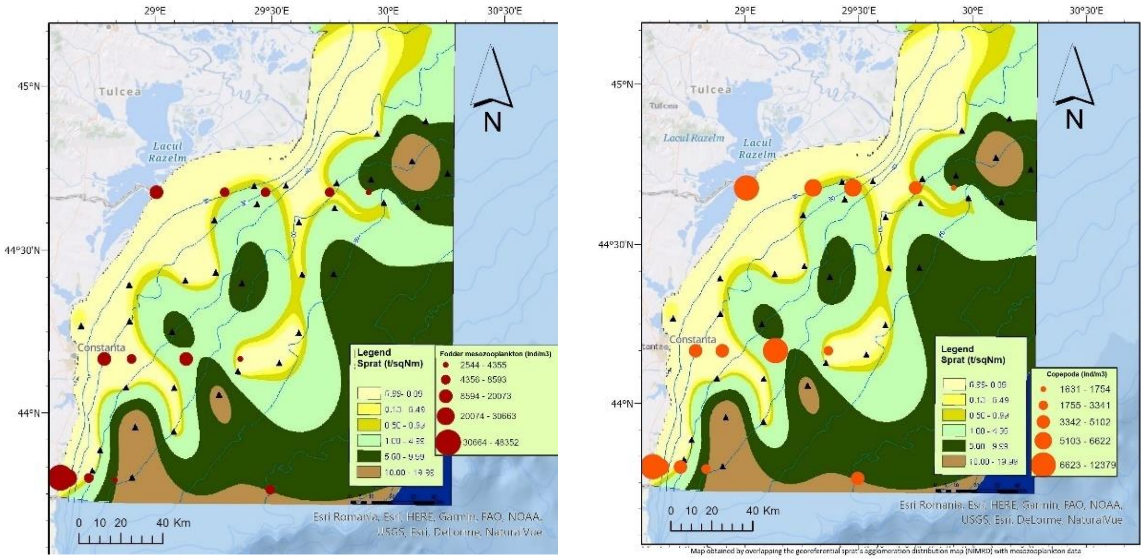

Figure 22. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in autumn 2016

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2017

In spring, sprat was characterized by the highest catches values in the northern and central part of the Romanian continental shelf. It is noteworthy that high catch values on the 30m isobath were reached in the stations located in the central and northern sector, the southern sector being characterized by low values for sprat agglomerations (Figure 23). The mesozooplankton fodder base was well represented quantitatively, the highest values for the fodder component including copepods being reached in the shallower stations, where the sprot catches were higher (Figure 23).

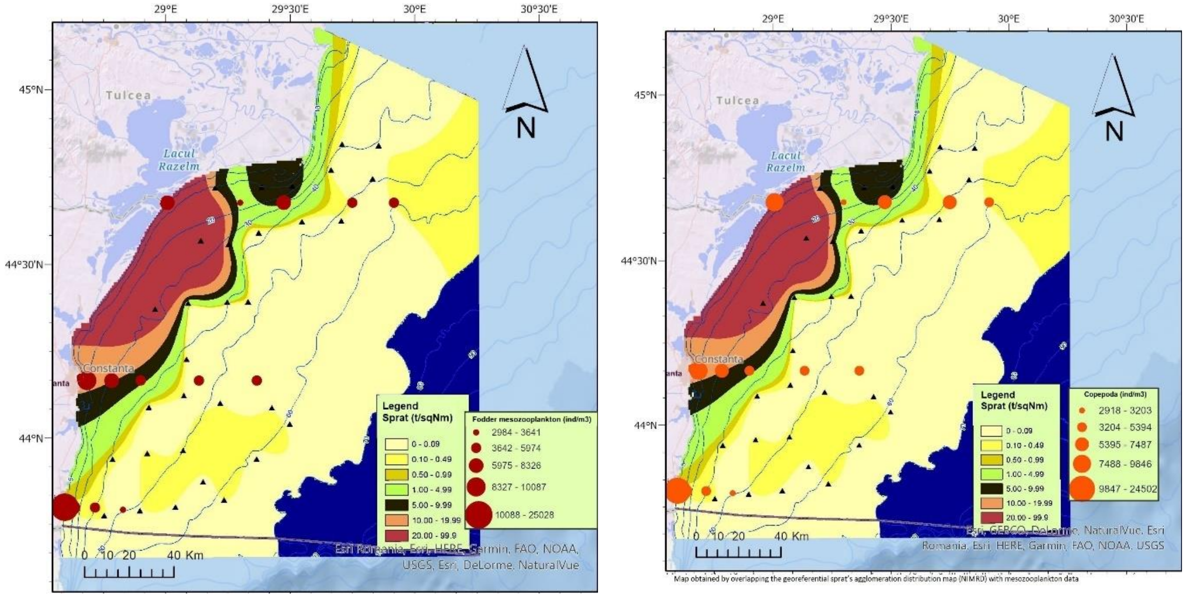

Figure 23. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2017

The sprat was well represented in terms of quantity in the fall of 2017, in all three sectors of the Romanian coast, recording catch values of 5.00-9.99 t / km2. The highest value of the catch (20.00-99.9 t km / 2) was reached in a single station located in the southern sector. It is observed that in the shallow stations the lowest values of catches were recorded (0-0.99, 0.10-0.49, 0.50-0.99 t / km2). The mesozooplankton fodder component was poorly represented quantitatively, with higher densities in only a few stations near the shore, where the sprat population was poorly represented. The same situation was recorded for copepods that have developed quantitatively only in a few stations located in shallower waters.

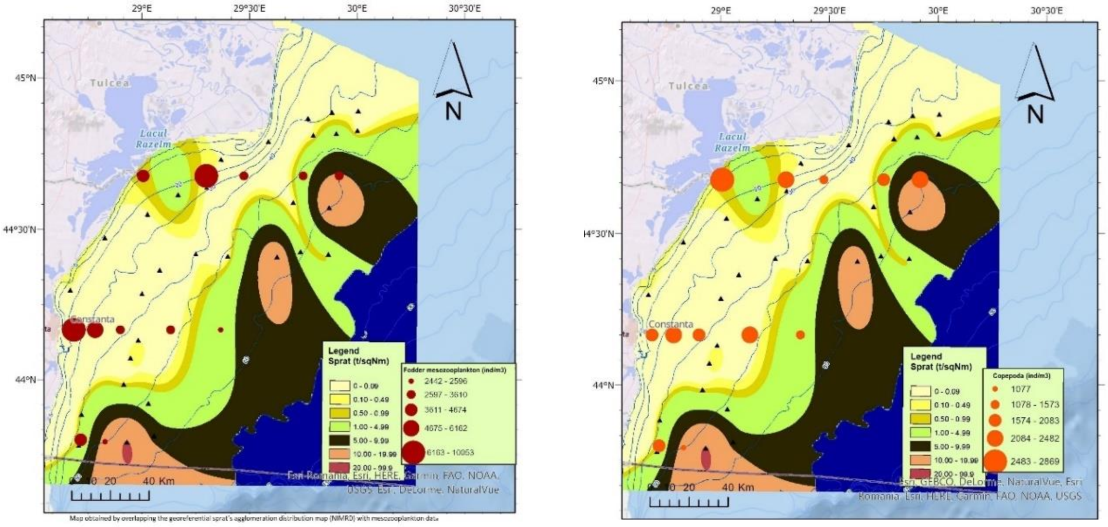

Figure 24. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in autumn 2017

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2018

This year, the spring sprat population was best represented quantitatively in the northern sector where it reached the maximum value of catches (20.00-99.99 t / km2) in most stations located in this sector. Values between 5.00-9.99 t / km2 were reached in the stations located in the central and northern sector, the southern sector being characterized by the lowest values for sprat catches (Figure 25). Fodder mesozooplankton reached high values of density in the same stations in which the sprat was best represented, the copepods being the trophic elements well represented quantitatively, most likely contributing to the formation of large agglomerations of sprat (Figure 25).

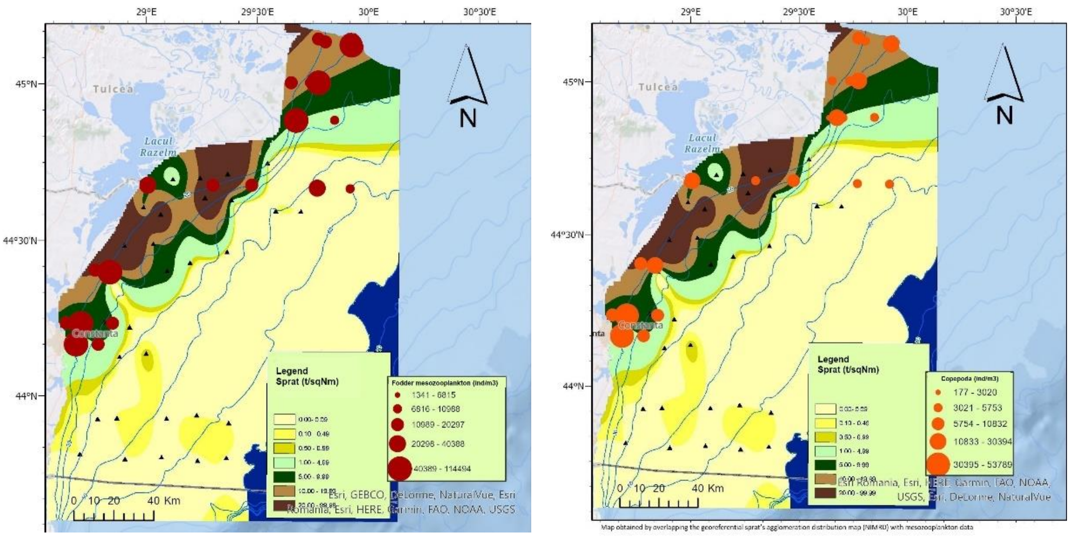

Figure 25. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2018

In the autumn of 2018, the sprat population recorded the maximum development in stations located in the northern sector, with a catch value of 20.00-99.99 t / km2. Sprat agglomerations were also higher in stations located in the southern and central sector, recording catch values of 10.00-19.99 t / km2 and 5.00-9.99 t / km2 (Figure 26). It is noteworthy that very high catch values have been reached in deeper waters. The sprat, a species par excellence cryophilic, moves away from the coast when the water temperature exceeds 18oC and returns only in the case of the existence of cold bottom currents (Cautiş, 1958, Şerpoianu, 1964). The fodder mesozooplankton structure during this period was not conducive to sprat, which is located in the deep areas where the mesozooplankton also reaches lower density values.

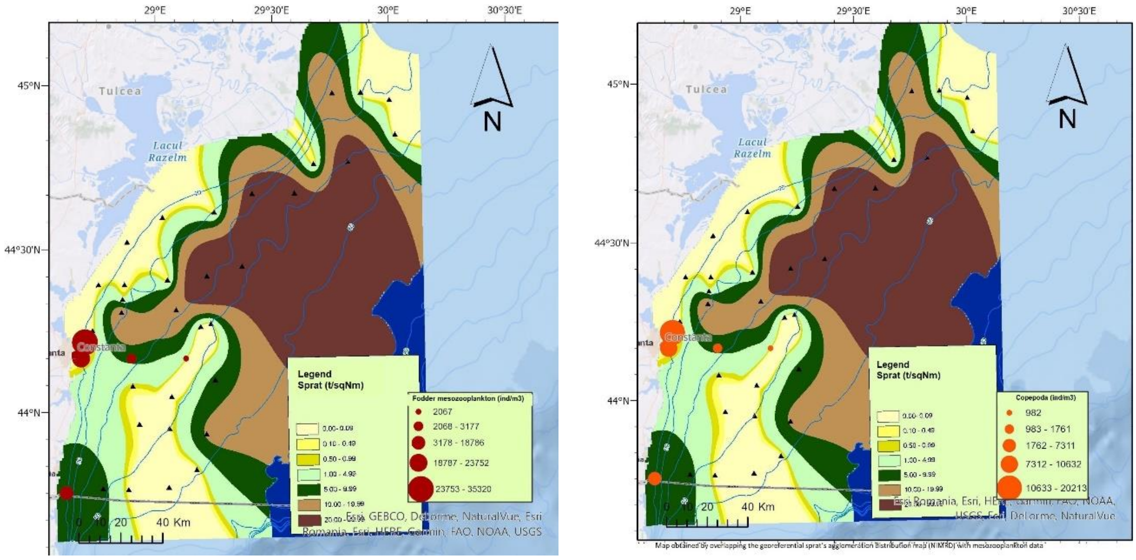

Figure 26. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in autumn 2018

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2019

The sprat agglomerations in the autumn of 2019 reached the maximum value (20.00-99.99 t / km2) in the northern sector, within a single station. The central sector was characterized by high values for the sprat population (5.00-9.99 t / km2) while in the southern sector the sprat was much less represented (Figure 27). It is observed that the fodder mesozooplankton trophic base was well represented quantitatively, the highest values of density being reached in the central and northern stations, where the sprat was in high quantities (Figure 27). The copepods reached the maximum density values in the stations located in all three sectors (Figure 27), constituting an adequate trophic base for sprat.

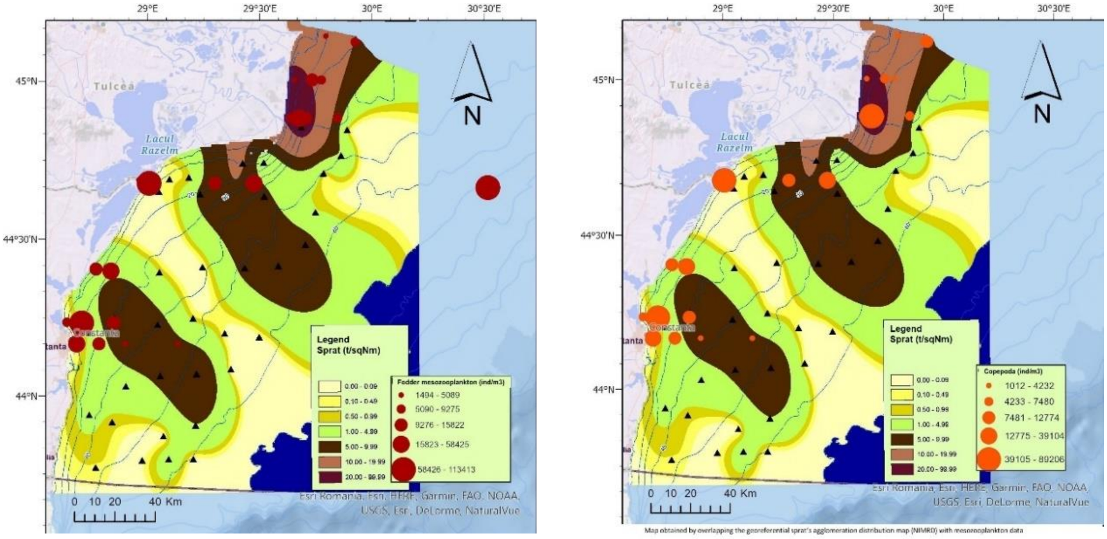

Figure 27. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in autumn 2019

Sprat dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2020

The sprat population showed a relatively uniform distribution of catches in the spring season, being found in high quantities in all three sectors, with the maximum recorded in a single station located in the northern sector. In the central sector, the sprat population reached catch values of 5.00-9.99 t / km2, a similar situation in the southern sector (Figure 28). Fodder mesozooplankton density was higher in the northern sector (Figure 28). Regarding the density values for copepods, it is observed that they were higher in the central and northern sector where the sprat also dominated (Figure 28) which could indicate a selectivity of the sprat in terms of food elements, copepods being preferred.

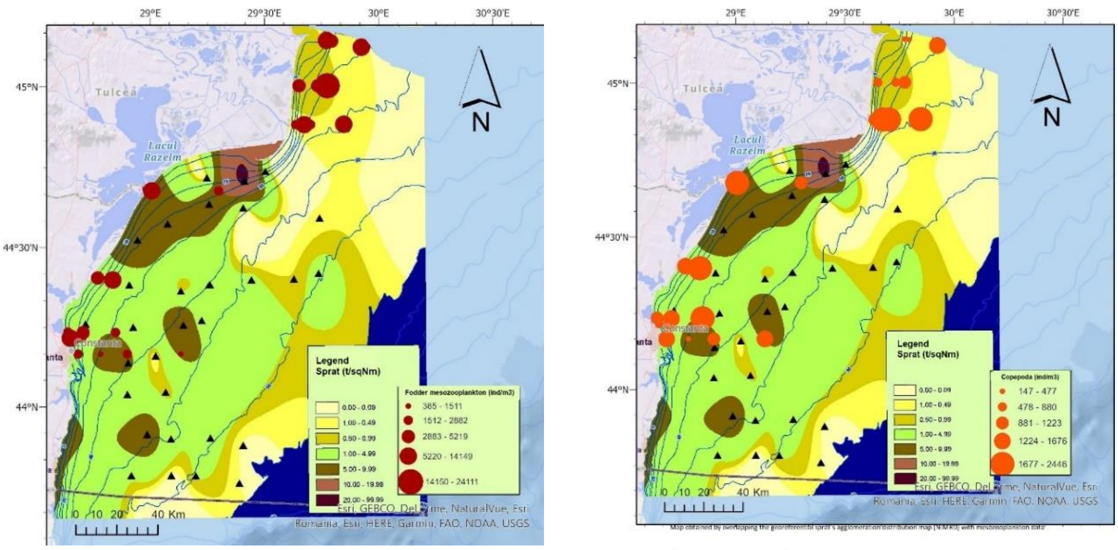

Figure 28. Distribution of sprat stocks (source: Romanian Annual Report on the National Data Collection Program for Fisheries), fodder mesozooplankton density and copepods in spring 2020

7.2. *Alosa tanaica* (Grimm, 1901) dynamics in relation to the fodder mesozooplankton from the Romanian Black Sea coast in 2019-2020

Azov shad, although present in small quantities, is frequently collected and appears in the trawls carried out in the Danube Delta area, in the northern sector of the Romanian Black Sea coast. In the fall of 2019, this species recorded low catch values but it is observed that where the fish population was better represented quantitatively, the fodder component of the mesozooplankton population and copepods recorded high density values, thus forming a good trophic base for feeding (Figure 29).

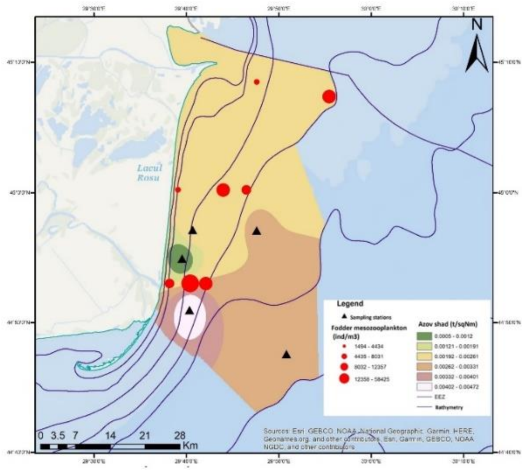

Figure 29 . Distribution of Azov shad stocks, fodder mesozooplankton density and copepods in autumn 2019

Azov shad in the spring of 2020 was again poorly represented quantitatively but the mesozooplankton fodder component and the group of copepods reached high values of density, which indicates that the fish in that area had large amounts of food, thus supporting the development of fish, having access to a good trophic base (Figure 30).

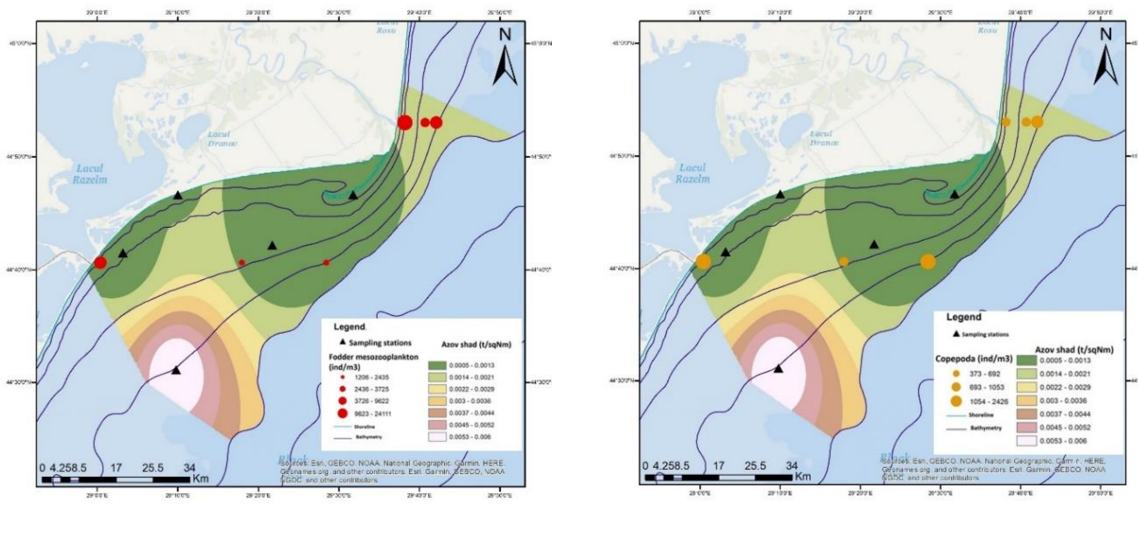

Figure 30. Distribution of Azov shad stocks, fodder mesozooplankton density and copepods in spring 2020

CHAPTER 8. Stomach content analysis at *Sprattus sprattus* (Linnaeus, 1758) and *Alosa tanaica* (Grimm, 1901)

An understanding of the relationship between fish and their food organisms, especially preferential foods, and their distribution can help to locate potential feeding places and also provide clues about the prediction and exploitation of fish stocks. Environmental protection has attracted the attention of a large section of people around the world and has now become a global issue among scientists and researchers working in this field. The affected biota component may also be responsible for changing the feeding behavior of fish (Krishna et al., 2016).

8.1. Stomach content analysis at *Sprattus sprattus* (Linnaeus, 1758) in 2018-2020

Analyzing the number of mesozooplankton elements identified in the sprat stomach content, it is observed that in all three years meroplankton and copepods were most consumed in most of the analyzed stations, cladocerans and other groups being preferred as a food source occasionally (Figure 31). It should be noted that in 2019 and 2020 the sprat population consumed copepods in very large quantities, unlike 2018 when copepods were consumed to a lesser extent. Meroplankton consumption was relatively constant over the three years, being more intensely consumed in 2020 but in only a few stations analyzed (Figure 31).

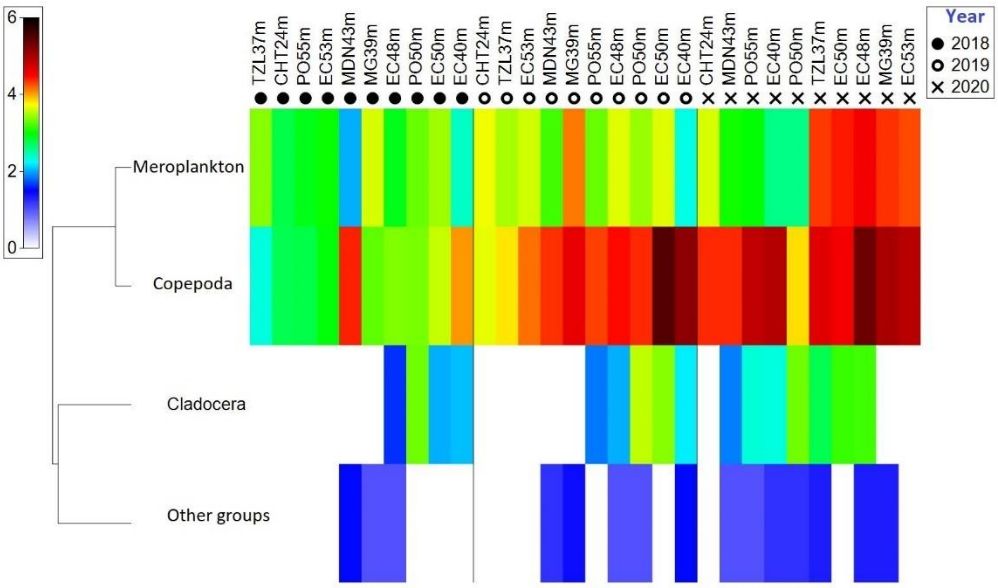

Figure 31. Matrix of mesozooplankton elements consumed by sprat in 2018-2020

There were 80% similarities between all years analyzed, forming clusters based on the number of meroplanktonic elements identified in the analyzed sprat specimens. It is worth mentioning the formation of the cluster consisting of CHT24m-EC53m-EC50m-TZL37 in 2018 due to the consumption of meroplankton and copepods, 2018 being the year in which the sprat population consumed fewer copepods and meroplanktonic elements (Figure 32).

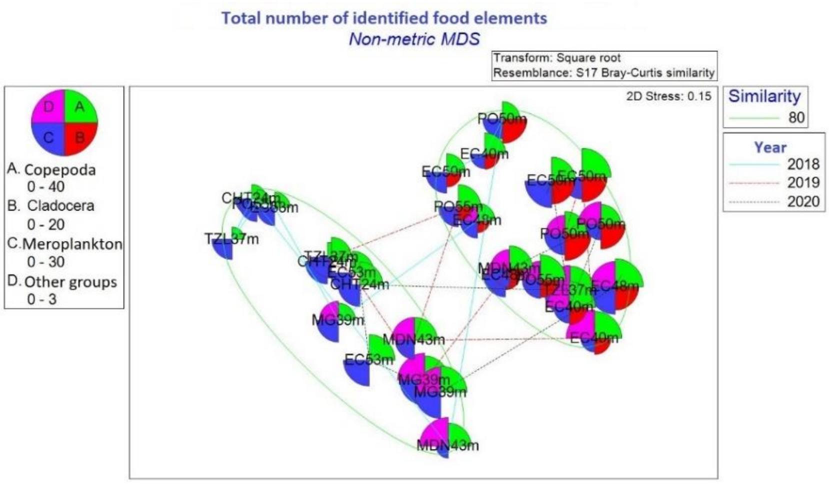

Figure 32 . NMDS analysis of the total number of mesozooplankton elements (by groups) consumed by sprat in 2018-2020

8.2. Stomach content analysis at *Alosa tanaica* (Grimm, 1901) in 2019-2020

It is observed that Azov shads consumed copepods in 2020, with the maximum in SIN20m and CRB30m stations as well as meroplanktonic elements in similar quantities in most stations (Figure 33). In 2019, however, Azov shads consumed copepods in high quantities in a single station (SF.GH20m) meroplanktonic elements were preferred as food, Decapoda mysis being the main element identified in the stomach contents (Figure 33).

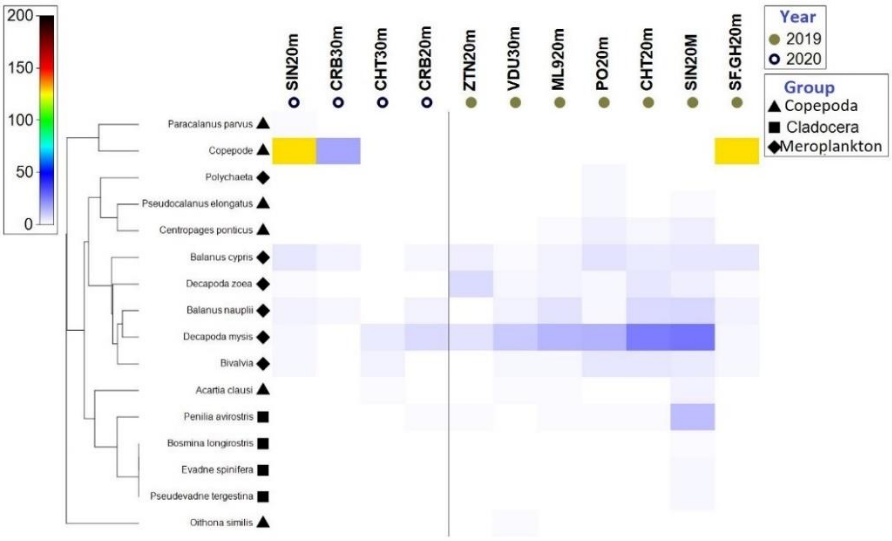

Figure 33. Matrix of the number of mesozooplankton elements consumed by Azov shad in the 2019-2020

The fact that there are differences in feeding between the two years analyzed also emerges from the NMDS diagram, forming two clusters of 60% similarity between the 2019 stations, the 2020 stations not being included in the clusters (Figure 34).

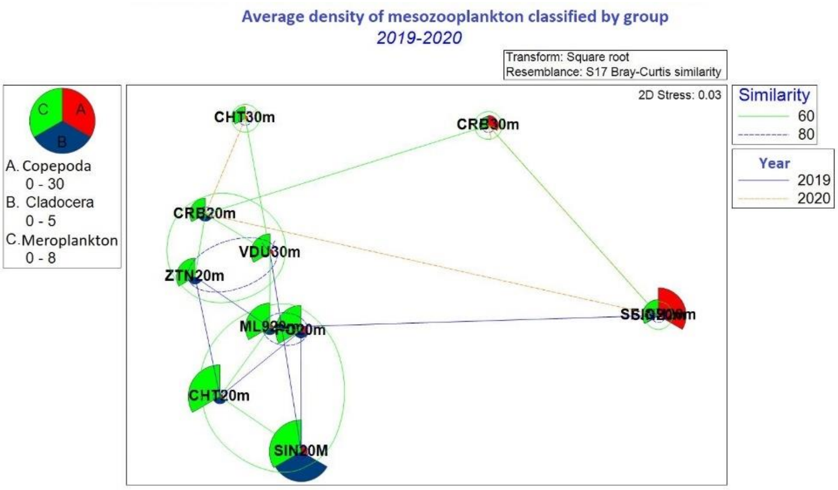

Figure 34. NMDS analysis of the total number of mesozooplankton elements (by groups) consumed by Azov shad in 2019-2020

8.3. Comparative analysis of food elements at *Sprattus sprattus* (Linnaeus, 1758) and *Alosa tanaica* (Grimm, 1901)

Pelagic species may have different ecological niches, thus minimizing competition for resources. This may be caused by the distinct vertical and seasonal distributions of fish (Zavala-Camin, 1982).

Using the ANOSIM function in PRIMER, on the Bray-Curtis similarity matrix, we tested the differences in the diets of the two species of pelagic fish, resulting in a value of 0.5 for R which indicates, from a statistical point of view, significant variability regarding the preferred mesozooplankton elements (Figure 35).

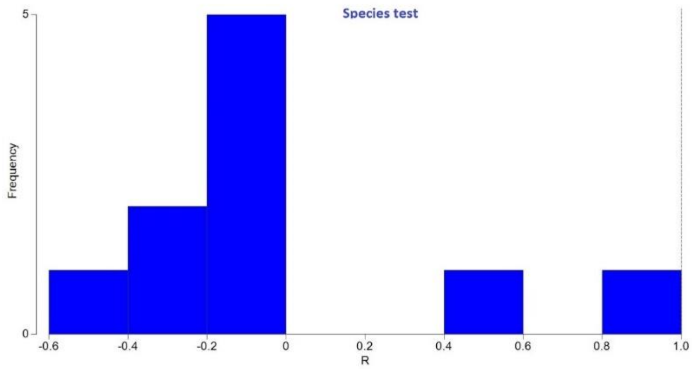

Figure 35. Multivariate analysis of similarities (ANOSIM) between sprat and Azov shad on the number of food items consumed

Analyzing the trophic spectrum of the two species, it is observed that, unlike sprat, Azov shad consumed only taxa belonging to the group of copepods, cladocerans and meroplankton. Sprat preferred as a food source species belonging to the category Other groups (Figure 36).

Regarding the consumption of mesozooplankton elements from a quantitative point of view, it is observed that during the years studied, sprat consumed mainly copepods followed by meroplankton elements, cladocerans and other groups being consumed to a lesser extent. Azov shad showed variations in feeding, in 2019 consuming mainly taxa belonging to meroplankton and cladocerans, in 2020 meroplankton being again dominant, followed by species belonging to the group of copepods (Figure 36).

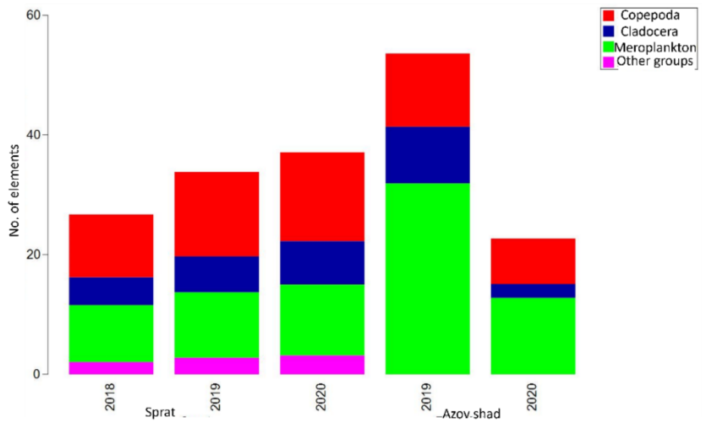

Figure 36. The number of mesozooplankton elements consumed by the two species of clupeids

The differences in feeding between the two species are also shown in the NMDS diagram, which shows that for sprat there is a cluster of 80% similarity between 2018-2019-2020 generated by the abundance of food elements identified in the stomach content, 2019 and 2020 for Azov shad did not form any similarity cluster (Figure 37).
It should be noted that the variability in feeding can also be generated by the fact that, for the sprat population, samples were collected and analyzed for three years, as opposed to Azov shad where the analysis was performed over two years and for a smaller number of fish individuals.

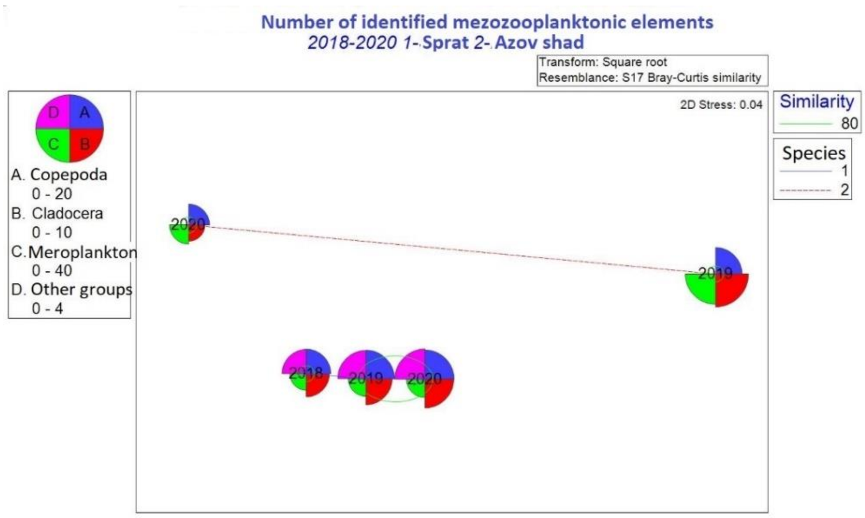

Figure 37. NMDS analysis of the total number of mesozooplanktonic elements (by groups) consumed by clupeid species

CONCLUSIONS

The mesozooplankton component has an important role for the marine environment, its monitoring being an absolutely necessary process, which provides information on the ecological status of the marine environment and the processes that take place in the marine food web (interaction with pelagic fish). Thus, following the research conducted and the interpretation of the obtained results, the following conclusions were formulated:

1. In 2013-2020, 27 mesozooplanktonic taxa, of holopanktonic and meroplanktonic nature, were identified on the Romanian Black Sea coast. There has been a decrease in the number of species compared to 1986-2010, this decrease being associated with anthropogenic impacts on the Black Sea ecosystem.
2. From the qualitative point of view, the group of copepods dominated throughout the period, with annual variations in the number of identified species. Another well-represented group was that of cladocerans, with annual variations in the number of species, followed by the meroplankton component, the category of other groups being less represented throughout the study period. The nonfodder component represented by the dinoflagellate *Noctiluca scintillans* was present in 2013-2020, for the entire analyzed period.
3. Throughout the study period, the largest share was held by copepods (between 33% and 43%), followed by the group of cladocerans and the meroplankton component. The other group category recorded lower percentage values and the nonfodder category recorded the same value of 5% given that it is represented by a single species, the dinoflagellate *Noctiluca scintillans*.
4. Quantitative analysis of the mesozooplankton community from 2013-2020 revealed that there were variations in density and biomass values for both fodder and nonfodder components, including variations in density and biomass for identified taxa. These variations are due to the natural variability of the component, to which is added the number of collected samples, the collection period but also the sectors studied, where the environmental conditions are different, thus exerting a series of pressures on the community.
5. The seasonal distribution of mesozooplankton species revealed a higher number of taxa identified in the warm season (25), the cold season being characterized by a maximum of 15 taxa.
6. From a quantitative point of view, the dinoflagellate *Noctiluca scintillans* - representative of the nonfodder component developed quantitatively in the warm season, when it reached the highest values of density and biomass, in the cold season being characterized by lower values of density and biomass.
7. From the mesozooplankton fodder category, the copepods *Acartia clausi* and *Pseudocalanus elongatus* were best represented quantitatively in both cold and warm seasons and the cladocerans recorded high values for density and biomass only in the warm season. The meroplankton component reached its maximum development also in the warm season, this situation being also identified for the Other groups category.
8. The biomass and density values recorded by total mesozooplankton (fodder and nonfodder) generated the formation of similarity clusters (80%) for the years 2013, 2016 and 2019 warm season and for 2020, 2014, 2017 warm season and cold season of 2017. The 80% similarity between the warm and cold season 2017 is generated by the high-water temperature values recorded in 2017. The year 2014 is notable, the cold season due to the very low average densities and biomasses recorded by the fodder and nonfodder mesozooplankton component.

9. The PCA analysis of environmental factors revealed that salinity and temperature were the main factors identified that influence the development of the mesozooplankton community.
10. Mesozooplankton groups responded differently to the environmental factors analyzed. Other groups was negatively correlated with the temperature at depths, as well as the group of cladocerans but at depths of 5 and 50m. The group of copepods correlated positively with salinity also in the offshore area and the dinoflagellate *Noctiluca scintillans* developed in areas with large variations in salinity. A correlation with salinity also showed the meroplanktonic elements but in shallower waters.
11. The assessment of the pelagic habitat according to the MSFD showed that for the indicators Mesozooplankton Biomass and Copepoda Biomass in the warm season the good ecological status of the water bodies was not reached, the GES status being reached only in the cold season. The values for the indicator *Noctiluca scintillans* Biomass in both warm and cold seasons have placed the three water bodies in good ecological condition (GES).
12. The sprat was very well represented in terms of quantity, the catches from 2013-2020 recording high values of biomass.
13. The distribution maps of sprat catches in relation to the mesozooplankton' s fodder density and copepods (fodder mesozooplankton category) in 2013-2020 showed that the sprat population had an abundant trophic base with a high nutritional value, generated by copepods. Thus, throughout the analysed period it was observed that in the area where the sprat recorded high catches values , the fodder component and the copepods recorded high values of density, thus supporting the optimal development of fish stocks.
14. *Alosa tanaica* (Grimm, 1901) is less captured, recording low biomass values and being found exclusively in the northern part of the Romanian Black Sea coast.
15. Distribution maps of Azov shad agglomerations in relation to fodder mesozooplankton and copepods have shown that in both 2019 and 2020, the population of *Alosa tanaica* (Grimm, 1901) had a well-represented source of food, thus favoring the optimal development of this clupeid.
16. The analysis of the stomach content in sprat highlighted the preference of this species for copepods and meroplankton as the main food source, taxa belonging to the group of copepods being intensely consumed in 2019 and 2020, the meroplankton elements being consumed equally during the three years of study. The sprat also consumed Cladocera which were represented by the species *Pleopis polyphemoides* and the tunicate *Oikopleura dioica* belonging to the Other groups category, these two species having a lowwer frequency occurrence in the stomach contents of the sprat.
17. The stomach content of *A. tanaica* showed an intense consumption of copepods, cladocerans and meroplankton, the number of mesozooplankton taxa identified in the stomach content varying during the two years of study, in 2019 the feeding intensity of this species being higher.
18. In 2020, *A. tanaica* consumed copepods and meroplanktonic elements in similar quantities at most stations. In 2019, however, Azov shad consumed copepods in higher quantities in only one station, the meroplanktonic food elements being consumed in higher quantities, Decapoda mysis being the main element identified in the stomach contents.
19. Analyzing the trophic spectrum of the two species, it is observed that, unlike sprat, Azov shad consumed elements belonging to three mesozooplankton groups -

copepods, Cladocera and meroplankton. Unlike Azov shad, sprat consumed and preferred as a source of food species belonging to the category Other groups.

20. The feeding differences between the two species were given by the number of mesozooplanktonic elements identified in the stomach contents of the two clupeids, the sprat preferring copepods followed by meroplanktonic elements. Azov shad consumed mainly taxa belonging to meroplankton and cladocerans, copepods being consumed to a lesser extent. It was noted that there are differences in the number of species belonging to the cladocera, the Azov shad consuming several species belonging to this group - Penilia avirostris, Bosmina longirostris, Evadne spinifera, Pseudevadne tegestina, as opposed to the sprat which consumed only the *cladoceran Pleopis polyphemoides*.

PERSONAL CONTRIBUTIONS

Within a marine ecosystem, mesozooplankton is one of the most important links. The qualitative and quantitative structure of mesozooplankton are conditioned by both biotic and abiotic factors, which in turn determine the composition and development of ichthyofauna, forming the basis for most commercial pelagic fish with commercial value.

The importance of mesozooplankton in fish feed and in the productivity of the aquatic ecosystem leads to the need to determine the reserves of exploitable fish in relation to the mesozooplankton trophic base in those areas, the evolution of the mesozooplankton community influencing fisheries production.

The contribution of this thesis is the provision of recent data on the diversity and distribution of mesozooplankton taxa, the quantitative assessment of the mesozooplankton community and commercial pelagic fish, and the updating and consolidation of information on the relationship between fish and their food elements. The novelty of this thesis is the assessment of the ecological status of the marine ecosystem according to MSFD as well as updating of information on the important role of the mesozooplankton trophic base in the nutrition of the two commercially valuable pelagic fish species.

Based on field and laboratory research, the specific objectives of the doctoral thesis were outlined, namely:

1. Establishing the current qualitative composition of the mesozooplankton community on the Romanian Black Sea coast
2. Analysis of the quantitative distribution of mesozooplankton in the period 2013-2020
3. Assessing and establishing the influence of abiotic factors on the mesozooplankton population
4. Analysis of the ecological status of the marine environment in terms of mesozooplankton indicators
5. Assessment of pelagic agglomerations in relation to the existing trophic base
6. Establishing the role of mesozooplankton in the life of pelagic fish by analyzing the stomach contents in sprat and Azov shad.

All these major objectives, together with the results obtained, represent the scientific basis for the determination of exploitable fish stocks in relation to the mesozooplanktonic trophic base.

FURTHER RESEARCH DIRECTIONS

The data and observations presented in the doctoral thesis showed that mesozooplankton taxa play a major role in the nutrition of pelagic fish of commercial value, but there are still a number of issues needed to be elucidated in future studies, such as :
- Further monitoring of industrially caught and researched pelagic fish species in this doctoral thesis
- Identification and nomination of other commercial species from the Romanian Black Sea coast in order to analyze the stomach content

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