

## Article

# Dynamics of Zooplankton along the Romanian Black Sea Coastline: Temporal Variation, Community Structure, and Environmental Drivers

Elena Bişinicu <sup>1,\*</sup> , Luminița Lazăr <sup>2,\*</sup>  and Florin Timofte <sup>3,\*</sup> 

<sup>1</sup> Ecology and Marine Biology Department, National Institute for Marine Research and Development “Grigore Antipa”, 300 Mamaia Blvd., 900581 Constanta, Romania

<sup>2</sup> Chemical Oceanography and Marine Pollution Department, National Institute for Marine Research and Development “Grigore Antipa”, 300 Mamaia Blvd., 900581 Constanta, Romania

<sup>3</sup> National Institute for Marine Research and Development “Grigore Antipa”, 300 Mamaia Blvd., 900581 Constanta, Romania

\* Correspondence: ebisinicu@alpha.rmri.ro (E.B.); llazar@alpha.rmri.ro (L.L.); ftimofte@alpha.rmri.ro (F.T.)

**Abstract:** Between 2013 and 2020, a total of 27 distinct zooplankton taxa, encompassing both holoplanktonic and meroplanktonic organisms spanning different classes and orders, were successfully identified along the Romanian Black Sea coastline. The seasonal dispersion patterns of these zooplankton taxa revealed a notable augmentation in species identification during the warmer periods, contrasting with reduced zooplankton diversity during the colder seasons. This phenomenon was underscored by the outcomes of SIMPER analysis, which highlighted that copepods, meroplankton, and cladocerans predominantly contributed to the community structure during warmer seasons. In contrast, during colder seasons, cladocerans were scarcely represented, and the density levels of copepods and meroplankton were diminished. From a quantitative perspective, the dinoflagellate *Noctiluca scintillans*, exhibited its highest density and biomass during the warm season, with a relatively lower representation during colder periods. The variation in the abundance of zooplankton was closely linked to changes in salinity and temperature, culminating in noticeable fluctuations, which were particularly evident during the cold season. During this period, the values of zooplankton abundance were notably lower compared to those observed in the warmer season. The comprehension of zooplankton dynamics stands as a critical facet for assessing biodiversity, monitoring ecosystem well-being, and guiding conservation endeavours.

**Keywords:** zooplankton density; analysis; taxa; season; salinity; temperature; biodiversity assessment; Black Sea



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## 1. Introduction

Zooplankton help to detect the extent of climate change by fixing carbon through the biological pump; but, at the same time, they are very susceptible to climate change [1]. Thus, zooplankton significantly mitigate climate change by aiding in carbon sequestration through the biological pump; yet, they face high vulnerability to climate change impacts, including rising temperatures, ocean acidification, and shifts in their food sources, which could disrupt their crucial role in carbon cycling and exacerbate climate change [2]. Different ecosystem components (trophic groups) respond phenologically to changes in environmental conditions (eutrophication and temperature rise), which can lead to mismatches between successive trophic levels and disrupt the synchrony between primary, secondary, and tertiary consumers. 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, any variation in zooplankton dynamics has implications for fishing and ecosystem services [1]. Unfortunately, all these have been observed in recent years in the Black Sea [3–6].

The dynamics of zooplankton populations, their reproductive cycles, growth, and survival rate are key factors influencing the recruitment of fish stocks. The latter role has made zooplankton ecology of interest for research.

Several 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 physicochemical parameters of seawater [7].

In the Black Sea, zooplankton have the role of a “key industry” by converting into organic matter not only a large amount of primary production but also dissolved organic substances. In this way, the matter and energy generated in the ecosystem and particulate or solvated organic substances from the water mass become accessible for organisms that are not planktivorous or do not have mechanisms for capturing organo-bacterial aggregates. Thus, zooplankton become the main food source for juvenile and adult pelagic fishes, species that in the Black Sea, are essential elements not only in the trophic base of cetaceans but also in the qualitative composition of industrial fishing [8].

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 intricately linked to plankton availability, with large fish stocks being found in regions with abundant planktonic biomass [9]. Owing 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 valuable in supporting research on fish production and changes in the ecosystem [9,10].

Assessment of zooplankton biodiversity is a crucial element in monitoring marine ecosystem processes and community responses to changes that may occur in the marine ecosystem [11]. The biodiversity of zooplankton is one of the most important ecological indicators of the environment, with each species having a specific role in the ecosystem [12]. Biological diversity plays a crucial role in the functioning of systems and in the many services they provide [13].

Zooplankton communities are sensitive to anthropogenic impacts, and environmental fluctuations and their study can be useful in predicting long-term changes [14–17]. Species diversity and zooplankton community composition may indicate environmental change or disturbance; it has been reported by several researchers that zooplankton can serve as an indicator of studies of trophic dynamics and the ecological status of aquatic ecosystems [16,18]. This holds significant relevance for the Romanian Black Sea, which experiences human impacts from factors like river input (Danube, Dniester, and Dnieper), ports, and industrial operations [19,20].

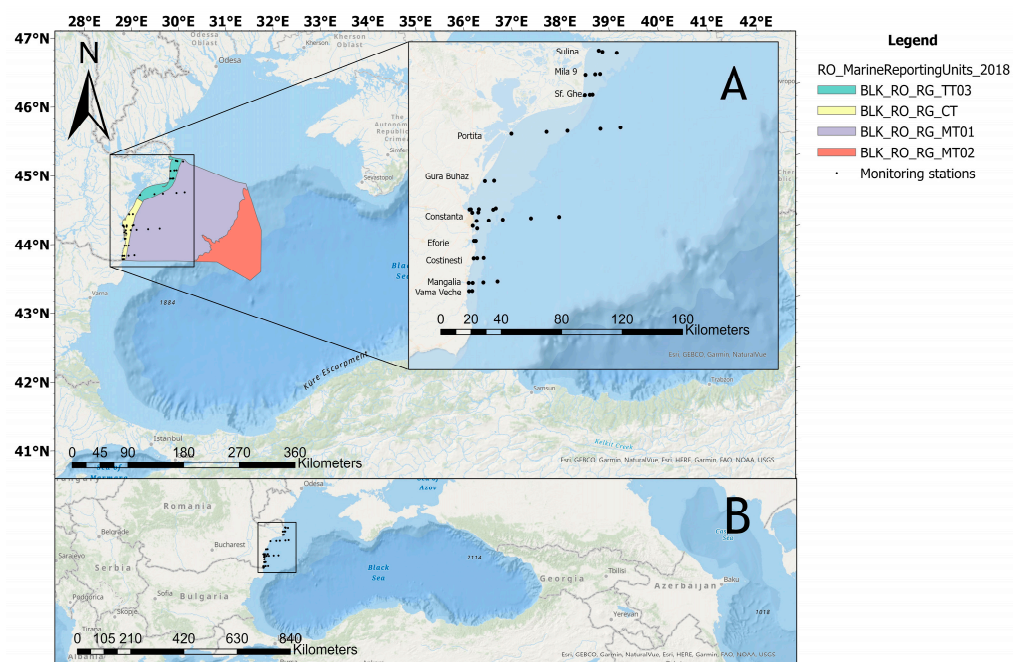
This study aims to comprehensively investigate the seasonal dynamics and composition of zooplankton taxa along the Romanian Black Sea coast from 2013 to 2020. Through taxonomic identification and analysis, the research seeks to elucidate how the distribution and diversity of zooplankton vary between warm and cold seasons. The study uses statistical analysis to identify key factors affecting the community structure in various seasons, with a particular focus on copepods, meroplankton, cladocerans, and the limited presence of cladocerans during the cold season. All the non-gelatinous and non-opportunistic species groups, such as Copepoda, Cladocera, meroplankton, and other groups that act as the primary prey for larger predators, are referred to as fodder zooplankton [3,5] in this paper and within the Black Sea riparian countries [3,5]. Furthermore, the research aims to quantify the density and biomass and explore between warm and cold seasons the variation in the *N. scintillans* dinoflagellate, which is an opportunistic species [3]; therefore, it is analysed as the non-fodder zooplanktonic component. By investigating the relationships between fodder zooplankton, salinity, temperature, and nutrients, the study aims to reveal distinct abundance patterns. This investigation also underscores the significance of

understanding zooplankton dynamics in the context of biodiversity assessment, ecosystem health monitoring, and the formulation of effective conservation strategies. This holds important significance, especially when considering that *N. scintillans* serves as the indicator for assessing blooms in the Romanian Black Sea as a part of the assessment of Descriptor 5—Eutrophication—under the Marine Strategy Framework Directive (Directive 2008/56/EC) implementation.

## 2. Materials and Methods

### 2.1. Description of the Study Area

Zooplankton samples were collected from stations located along the Romanian Black Sea monitoring network (Figure 1) in 2013–2020 in the warm (May–October) and cold (November–April) seasons. The warm season was characterised by a higher number of samples; while in the cold season, expeditions were only carried out in 2014, 2016, and 2017 (Table S2). The monitoring network, consisting of 45 sampling stations, extends over the entire continental platform of the Romanian Black Sea coast and consists of 13 profiles perpendicular to the coastline: Sulina, Mila 9, Sf. Gheorghe, Portița, Gura Buhaz, Cazino Mamaia, Constanța North, Constanța East, Constanța South, Eforie, Costinești, Mangalia, and Vama Veche. A total of 533 zooplankton samples and 387 water samples for evaluating physicochemical parameters were collected from 45 monitoring stations and analysed in NIMRD laboratories.



**Figure 1.** Map of the study area. The square shows the location of the sampling sites on the Romanian coastline (A) and the position in the Black Sea region (B).

### 2.2. Zooplankton Sampling and Analysis

Zooplankton samples were collected according to the methodology [21] using a Juday net (0.1 m<sup>2</sup> mouth opening area and 150 μm mesh size) equipped with a flow meter for estimating the volume of water filtered by vertically towing the net in the water column. In the warm season, zooplankton samples were collected from standard depths (10–0 m, 25–10 m, 50–25 m, and 100–50 m); while in the cold season, sampling was performed from the entire water column.

The quantitative and qualitative processing of the zooplankton samples were performed under an OlympusSZX10 stereomicroscope. When specifying the taxonomic affiliation of zooplankton and for species determination, we used proper manuals and

guides [22,23]; classification for identified taxa was conducted according to the World Register of Marine Species (WoRMS).

Not all the organisms could be determined at the species level; the meroplanktonic elements were identified only at the group level. In the subsample(s) examined in the Bogorov chamber, all the organisms were counted until at least 100 individuals for each of the three dominant taxonomic groups were recorded. For the estimation of large animal numbers, the whole sample was examined in a Petri dish. The number of individuals and mean individual weights were used for estimating the density in ind/m<sup>3</sup> and the biomass in mg/m<sup>3</sup> of wet weight, respectively, based on the tables of constant weight of the Black Sea zooplankters [21,24].

Additional data (qualitative and quantitative) from the scientific literature was used to analyse the long-term trend [8].

Seawater samples were collected at the same time as the biological ones from the same stations using Niskin bottles. A CastAway CTD multiparameter was properly calibrated and used for in situ temperature and salinity measurements. Dissolved nutrients (phosphate, silicate, inorganic forms of nitrogen-nitrite, nitrate, and ammonium) were analysed in the NIMRD laboratory using spectrophotometric methods according to *Methods of Seawater Analysis* [25].

### 2.3. Data Analysis

Multivariate and univariate analyses were conducted using PRIMER v. 7.0 [26], and statistical analysis was performed via XLSTAT [27] and Statistica<sup>®</sup> 14.0.1.25 (TIBCO Software Inc., Palo Alto, CA, USA) [28].

Data analysed with PRIMER v. 7.0 were square-root transformed. Multivariate analyses are characterised based on comparisons between two (or more) samples at the extent to which these samples share certain species at comparable levels of abundance. These facilitate a classification of samples into groups that are then mutually similar or an ordination diagram in which, for example, samples are “mapped” (usually in two or three dimensions) to reflect their relative differences in specific composition [26]. In this research, as a multivariate analysis, the Bray–Curtis similarity was used to evaluate the similarity of the zooplankton community between the analysed years and seasons.

As univariate analyses, diversity indices, shade plots, boxplots, and SIMPER analysis were used. Different diversity indices emphasise the species richness or equitability components of the diversity, the most common being the Shannon–Wiener diversity index ( $H'$ ), species richness, and Pielou’s evenness index ( $J'$ ) [26].

$$H' = -\sum P_i (\ln P_i)$$

where  $P_i$  is the proportion of each species in the sample, and  $a_i$  is the abundance of the  $i$ th species.

$$J' = \frac{H'}{H'_{max}} = \frac{H'}{\log S}$$

where  $H'_{max}$  is the maximum possible value of the Shannon diversity, i.e., that which would be achieved if all the species were equally abundant (namely,  $\log S$ ).

Shade plots are visual displays in the form of the data matrix itself [26], which were used for the identified zooplankton taxa. Boxplots provide a good graphical representation of the concentration of data, showing how far extreme values are from much information. This type of diagram was used to compare the distribution of the zooplankton community according to the taxonomic groups, taking into consideration the frequency of the identified taxa. SIMPER (similarity percentages) analysis was used for assessing the percentage contributions of species to the similarity and dissimilarity between groups, by season.

Statistica<sup>®</sup> was used for analysing the descriptive statistics of the environmental variables, including correlations. By default, the spreadsheet shows all the correlation coefficients that are significant at  $p < 0.05$  (two-tailed) in a different colour (highlighted



in red). Additionally, *t*-tests were used to compare the environmental characteristics between seasons by evaluating the differences in means between two groups (warm and cold seasons). The null hypothesis assumes that the two means are equal. Using the formulas, values are calculated and compared against the standard values. The assumed null hypothesis is accepted or rejected accordingly. The test returned the *p* values to check for statistical significance.

XLSTAT [27] was used to perform the Canonical Correspondence Analysis (CCA) to relate the abundance of zooplankton species to environmental variables. The included permutation test concludes that the species data either are or are not linearly related to the variable data at a 5% significance level. CCA uses linear combinations of environmental (explanatory) variables to optimally explain the species (response) variables.

ArcGIS Desktop 10.7 software (ESRI, 2019) [29] was used for creating distribution maps. The data interpolation was carried out using the IDW (inverse distance weighted) method, which determines the cell values using a linearly weighted combination of a set of sampling points. This method assumes that the variable decreases in influence with increasing distance from the sampled location. Therefore, for the maps of distribution, the untransformed data of the studied components were used.

### 3. Results

#### 3.1. The Environmental Parameters

The Danube's mouths areas exhibited the lowest temperatures and salinities during different seasons. In the colder season, temperatures decreased; whereas in the warmer season, the salinity dropped to extremely low levels due to the increased flow of the Danube, resulting in freshwater conditions (as shown in Tables S3–S5).

The oxygen saturation reached its minimum point within the marine water-stratified water column (0–30 m), accompanied by elevated nutrient levels. Additionally, the region influenced by the Danube displayed the highest nutrient levels.

Overall, the data indicate distinct statistical differences between the cold and warm seasons across all the parameters except for phosphate and nitrate concentrations, where the levels are similar. The warm season is characterised by higher temperatures, lower salinities, lower dissolved oxygen levels, and higher concentrations of nitrite and ammonium. The cold season, on the other hand, features lower temperatures, higher salinities, higher dissolved oxygen levels, and higher concentrations of silicate (Table S6).

#### 3.2. Zooplankton Composition

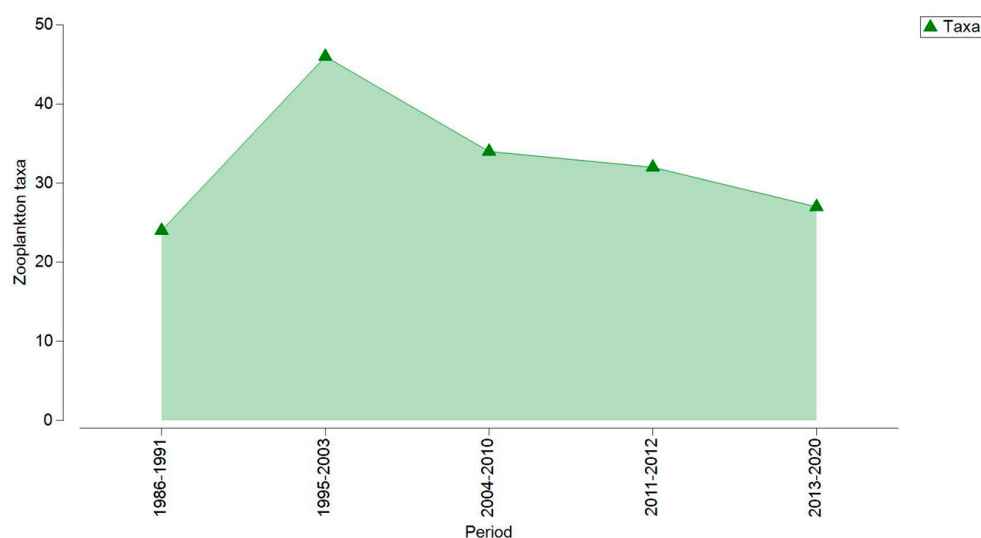
For analysing the zooplankton community from the Romanian Black Sea coastline, we distinguished between non-fodder zooplankton, represented by *N. scintillans*, and the fodder component, made up of copepods, cladocerans, meroplankton, and other groups.

Long-term data on the zooplankton community structure in the Romanian coastal waters showed fluctuations in species diversity (Figure 2). Upon examining the list of zooplanktonic species dating back to 1986 (Table S1), the most considerable count (45) was documented during 1995–2003, while a relatively consistent pattern is noted from 2004 to 2020.

During 2013–2020, 27 zooplanktonic taxa, of holoplanktonic and meroplanktonic natures, belonging to different classes and orders, were identified on the Romanian coastline (Table S2). Throughout the analysed timeframe, a reduction in the count of identified species is noticeable because, over the past few decades, certain species have vanished or grown scarce within the pelagic ecosystem [30].

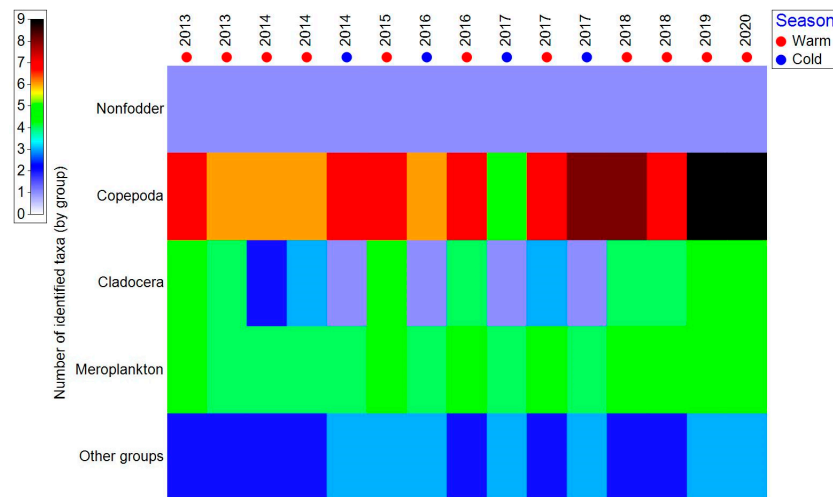
The non-fodder component (*N. scintillans*) was identified over the entire period, in both seasons (Figure 3). The group of copepods showed variations in the number of species, being best represented in 2019 and 2020, with a maximum of nine species (Figure 3). The core of the copepods (2013–2020) was represented by the species *Acartia clausi*, *Pseudocalanus elongatus*, *Oithona similis*, *Paracalanus parvus*, *Calanus euxinus*, and *Centropages ponticus*, which were identified in all the analysed samples. At the same time, a total of nine species

of cladocerans was identified on the Romanian coast (Figure 3); their appearance in such a large number in the plankton was quite rare and closely related to the influence of the freshwater input that the Danube exerts on marine waters. The high number of Cladocera is given by freshwater elements, such as *Chydorus sphaericus*, *Bosmina longirostris*, *Daphnia longispina*, and *Diaphanosoma brachyurum*, that occasionally appear in the marine waters near the Danube. *B. longirostris* was identified in 2013, 2015, and 2020; *C. sphaericus* and *D. longispina* appeared in samples only in 2015; and the species *D. brachyurum* was present only in samples from 2019 (Table S2). It is noted that *Pleopsis polyphemoides*, *Evadne spinifera*, *Penilia avirostris*, and *Pseudevadne tergestina* are the main representatives of the cladocerans; these species were constantly identified throughout the analysed period, especially in the warm season. The meroplankton did not show large variations in the number of taxa, recording a maximum of five taxa (Figure 3), namely, the larval stages of *Cirripectida*, *Decapoda*, *Gastropoda*, *Bivalvia*, and *Polychaeta* (Table S2). The other group category reached a maximum of three species (Figure 3) and is represented by the chaetognath *Parasagitta setosa*, the appendicular *Oikopleura dioica*, and the mysid *Mesopodopsis slabberi* (Table S2). Chaetognaths play an important role in zooplankton trophodynamics, as their diet includes small copepods (*Oithona* sp.) [31]; the species *P. setosa* was identified every year (2013–2020). *O. dioica*, a planktonic chordate with an exceptional filtering capacity, fast generation time, conserved early development, and a compact genome [32], is mainly distributed in coastal waters [33,34], occurs often in high abundance during phytoplankton blooms [26], and was identified throughout the analysed period. *M. slabberi* usually occurs in coastal ecosystems; and, on the Romanian coast, it was only identified in five years of the eight analysed (Table S2). The seasonal distribution of zooplankton species reveals that in the warm season, the number of identified species increases, while the diversity is lower in the cold season (Figure 3).



**Figure 2.** Number of zooplankton taxa identified on the Romanian Black Sea coastline (1986–2020).

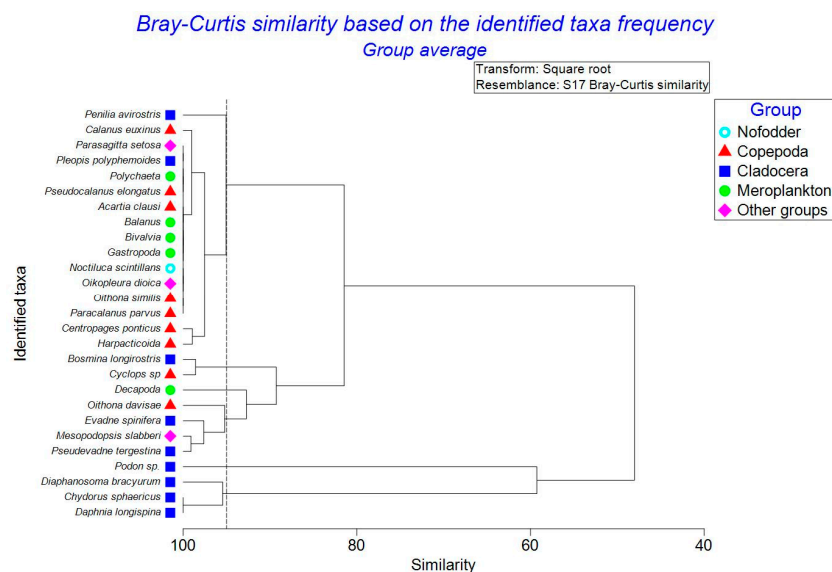
Following the discriminant analysis (DA) for significant differences among the groups by seasonal variation (Table 1), it was observed that of the 27 identified zooplankton taxa in 2013–2020, the most frequent were copepods *A. clausi*, *O. similis*, *P. parvus*, and *P. elongatus*; the cladoceran *P. polyphemoides*; meroplanktonic elements, such as *Balanus*, *Bivalvia*, *Gastropoda*, and *Polychaeta*; the non-fodder component represented by *N. scintillans*; the appendicular *O. dioica*; and the chaetognath *P. setosa*, representing the bulk of the zooplankton community and forming high-similarity clusters (Figure 4). Notably, it should be highlighted that the warm season exhibited the highest relative frequency (81.9%), attributed to the larger number of samples collected during 2013–2020 (Table 1).



**Figure 3.** Matrix of identified zooplankton taxa (taxonomic groups) in the Romanian Black Sea (2013–2020) by years and seasons.

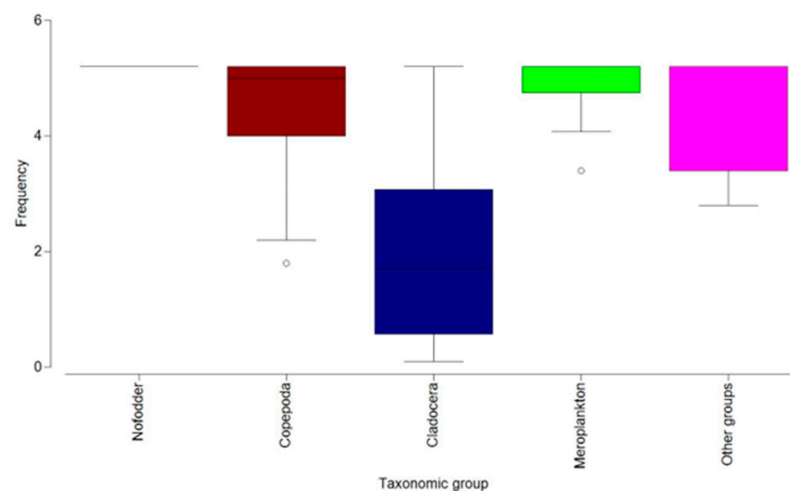
**Table 1.** Summary statistics (Qualitative data) by season (2013–2020).

Variables	Categories	Counts	Frequencies	Rel. Frequency per Category (%)	Taxonomic Groups
Season	Cold	1314	1314	18.1	
	Warm	5940	5940	81.9	
Taxa	<i>Acartia clausi</i>	379	379	5.2	Copepoda
	Balanus	378	378	5.2	Meroplankton
	Bivalvia	378	378	5.2	Meroplankton
	<i>Bosmina longirostris</i>	123	123	1.7	Cladocera
	<i>Calanus euxinus</i>	364	364	5.0	Copepoda
	<i>Centropages ponticus</i>	334	334	4.6	Copepoda
	<i>Chydorus sphaericus</i>	47	47	0.6	Cladocera
	<i>Cyclops</i> sp.	127	127	1.8	Copepoda
	<i>Daphnia longispina</i>	42	42	0.6	Cladocera
	Decapoda	249	249	3.4	Meroplankton
	<i>Diaphanosoma brachyurum</i>	36	36	0.5	Cladocera
	<i>Evadne spinifera</i>	179	179	2.5	Cladocera
	Gastropoda	377	377	5.2	Meroplankton
	Harpacticoida	350	350	4.8	Copepoda
	<i>Mesopodopsis slabberi</i>	202	202	2.8	Other groups
	<i>Noctiluca scintillans</i>	379	379	5.2	Non-fodder
	<i>Oikopleura dioica</i>	378	378	5.2	Other groups
	<i>Oithona davisae</i>	158	158	2.2	Copepoda
	<i>Oithona similis</i>	377	377	5.2	Copepoda
	<i>Paracalanus parvus</i>	378	378	5.2	Copepoda
	<i>Parasagitta setosa</i>	377	377	5.2	Other groups
	<i>Penilia avirostris</i>	305	305	4.2	Cladocera
	<i>Pleopis polyphemoides</i>	378	378	5.2	Cladocera
<i>Podon</i> sp.	4	4	0.1	Cladocera	
Polychaeta	378	378	5.2	Meroplankton	
<i>Pseudevadne tergestina</i>	199	199	2.7	Cladocera	
<i>Pseudocalanus elongatus</i>	378	378	5.2	Copepoda	



**Figure 4.** Dendrogram of cluster analysis using Bray–Curtis similarity, based on zooplankton taxa frequency in the Romanian Black Sea area, by taxonomic groups (2013–2020).

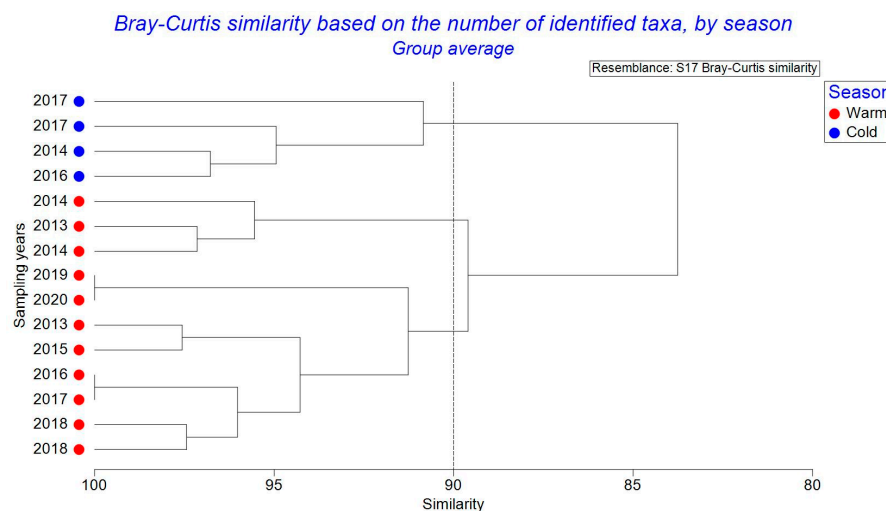
Distinct distributions were observed in the relative frequencies among taxonomic groups, with cladocerans displaying the lowest values. Conversely, the other zooplankton components were more prominently represented (Figure 5).



**Figure 5.** Boxplot of zooplankton frequency, by groups (2013–2020).

The constancy of zooplankton species is evident, with the copepod category maintaining dominance over the studied interval and displaying yearly fluctuations in the count of identified species. Similarly, the group of cladocerans, well-represented in qualitative terms, exhibits yearly fluctuations in species count, followed by the meroplanktonic segment. The release timing of meroplankton larvae varies among species, with temperature, nutrient availability, and meteorological conditions influencing egg production and larval emergence [35,36]. Other groups were less-well qualitatively represented throughout the study period (Figure 3). The Bray–Curtis analysis generated based on the number of zooplankton taxa indicates a very significant degree of similarity between the years, given the specific uniformity of the zooplankton (Figure 6). A very high degree of similarity was also recorded between the years 2019 and 2020 because the zooplankton component reached the highest number of species and had a similar structure in terms of the dominance of the identified species (Figure 6). The same situation was recorded for 2016 and 2017 owing to the same qualitative structure identified for the groups of copepods.





**Figure 6.** Dendrogram of cluster analysis using Bray–Curtis similarity, based on the number of zooplankton taxa identified in the Romanian Black Sea area, by seasons (2013–2020).

SIMPER analysis revealed that the main zooplanktonic groups that contributed to the community structure in the warm season are represented by copepods, meroplankton, and cladocerans (Table 2). Copepods were the most abundant in both seasons, while the taxa primarily responsible for the differences between the seasons were cladocerans. In the cold season, they were poorly represented and replaced by the presence of other groups (Table 2).

**Table 2.** Summary of the one-way crossed SIMPER results for the comparison of zooplanktonic groups between seasons (2013–2020).

Warm						
Average similarity: 96.14						
Taxonomic group	Av. Abund.	Av. Sim.	Sim./SD	Contrib. %	Cum. %	
Copepoda	2.69	27.36	27.5	28.46	28.46	
Meroplankton	2.16	22.32	19.19	23.21	51.67	
Cladocera	2.05	20.49	11.8	21.32	72.99	
Cold						
Average similarity: 96.83						
Taxonomic group	Av. Abund.	Av. Sim.	Sim./SD	Contrib. %	Cum. %	
Copepoda	2.54	28.35	18.77	29.27	29.27	
Meroplankton	2	23.9	35.29	24.68	53.95	
Other groups	1.73	20.69	35.29	21.37	75.32	
Groups Warm and Cold						
Average dissimilarity = 8.85						
Taxonomic group	Warm	Cold	Av. Diss.	Diss./SD	Contrib. %	Cum. %
Cladocera	2.05	1.1	5.33	3.45	60.16	60.16
Copepoda	2.69	2.54	1.44	1.29	16.24	76.4

Throughout the study, a clear fluctuation in species diversity was observed. The Shannon–Wiener diversity index ( $H'$ ) varied from 1 in 2015 to 2.3 in 2018, in the warm

season (Table 3). Pielou's evenness index values were the highest for the warm season of 2018 (0.8) and the lowest for the warm season of 2015 (0.4) (Table 3).

**Table 3.** Zooplankton diversity indices, by years and seasons (Shannon–Wiener legislation on the aquatic environment classified water quality as >4 = very good quality, 3–4 = good quality, 2–3 = moderate quality, 1–2 = poor quality, and <1 = very poor quality.).

Year	Species Richness (S)	Shannon–Wiener Index (H')	Pielou's Evenness Index (J')	Season
2013	25	1.8	0.6	Warm
2014	18	1.4	0.5	Warm
2015	18	1.0	0.4	Warm
2016	19	1.9	0.7	Warm
2017	18	1.2	0.4	Warm
2018	21	2.3	0.8	Warm
2019	25	2.0	0.6	Warm
2020	23	1.6	0.5	Warm
2014	16	1.5	0.5	Cold
2016	15	1.3	0.5	Cold
2017	18	1.7	0.6	Cold

### 3.3. Zooplankton Structure–Seasonal Variations

The mean total zooplankton density and biomass values in the Romanian Black Sea waters were the highest in the warm season and significantly declined in the cold one (Figure 7). The non-fodder component, represented by *N. scintillans*, was best represented in terms of density and biomass, recording the highest mean values (6929 ind/m<sup>3</sup> and 610 mg/m<sup>3</sup>, respectively) in the warm season, followed by copepods, at 4991 ind/m<sup>3</sup> in the same season (Figure 7). The meroplanktonic component and cladocerans were also better represented in the warm season; while in the cold season, the mean average density and biomass values for these two taxonomic groups were lower. In the cold season, copepods dominated only in terms of density, which indicates that smaller organisms dominated. On the other hand, the *N. scintillans* biomass dominated at lower densities (Figure 7).

### 3.4. Zooplankton Structure in Relation to Physicochemical Parameters

By analysing the non-fodder zooplankton component, represented by the dinoflagellate *N. scintillans*, in relation to the temperature in the warm seasons of 2013–2020, it is observed that the highest densities (80, 315–15, 1789 ind/m<sup>3</sup>) were recorded in the northern sector at temperatures between 19 °C and 20 °C (Figure 8). *N. scintillans* recorded similar values in all three sectors at temperatures between 23.6 °C and 24.9 °C. Regarding the salinity in the warm season, it is observed that at salinities between 16.99‰ and 18.94‰, *N. scintillans* recorded the lowest density values, with the dinoflagellate being much better represented quantitatively at salinities between 8.93‰ and 16.98‰ and the maximum density being reached in the northern sector at a salinity of 12.65–15.39 PSU (Figure 8).

The fodder zooplankton of the warm season recorded high densities. It is noted that the highest values (60.893–114,494 ind/m<sup>3</sup>) were recorded in shallow waters (Figure 9). The density of the fodder zooplankton in the warm season showed variations in relation to salinity, with the highest density being recorded in the southern and central sectors at salinities of 12.65–15.39‰ (Figure 9). It is observed that the lowest densities were reached in the offshore area, where the salinity was higher, between 16.99‰ and 18.94‰ (Figure 9).

Although it had much lower density values than those in the warm seasons, the dinoflagellate *N. scintillans* was also present in the cold seasons of 2013–2020. It is noteworthy that the species developed in all three sectors and in all the temperature ranges (Figure 10). The same pattern was observed for the salinity, where the species was found in all the salinity ranges in the cold season, even at 0.59–8.92‰, a range specific to the northern sector of the Romanian Black Sea coast under the direct influence of the Danube River (Figure 10).

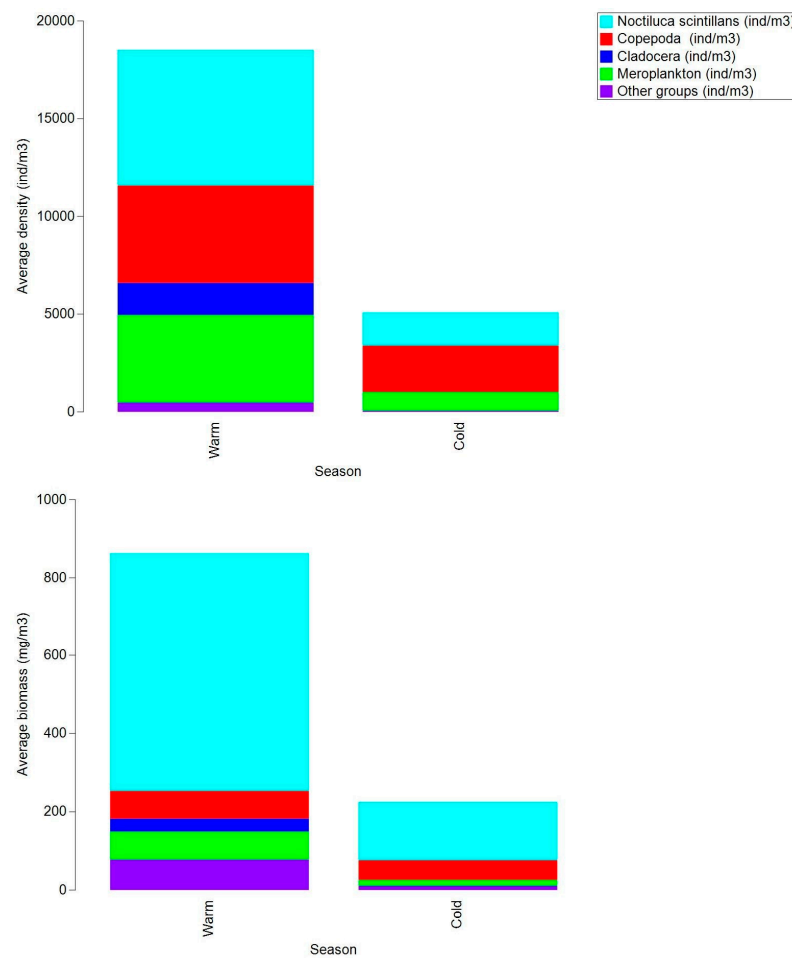


Figure 7. The bar plot of the zooplankton mean density and biomass values, by taxonomic groups and seasons (2013–2020).

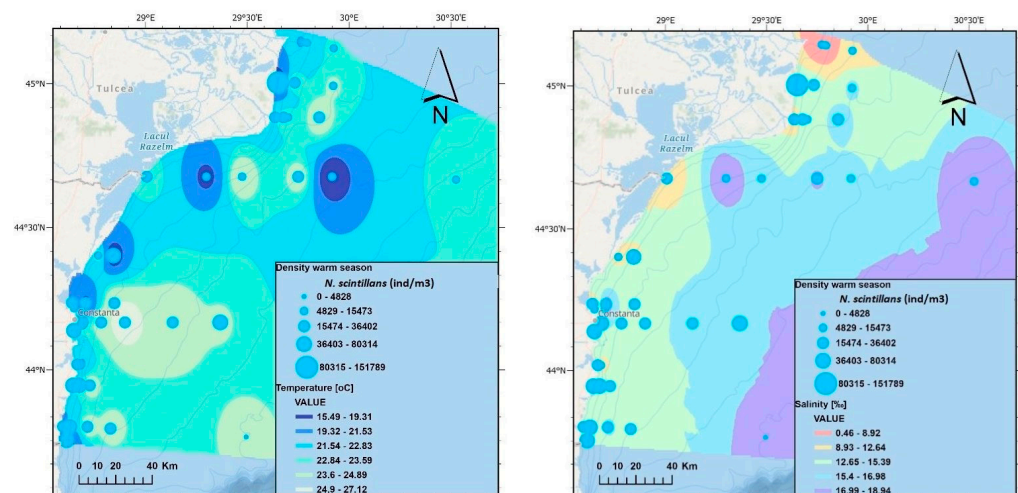


Figure 8. Spatial distributions of non-fodder zooplankton density, water temperature, and salinity on the Romanian coast—warm seasons, 2013–2020.

Variations in the zooplankton abundance are very clear in the cold seasons of the period 2013–2020, with the maximum density values being much lower compared to those in the warm seasons. It is observed that in the cold season, the zooplankton community recorded density values between 5164–25,028 ind/m<sup>3</sup>, regardless of the temperature and salinity values (Figure 11).

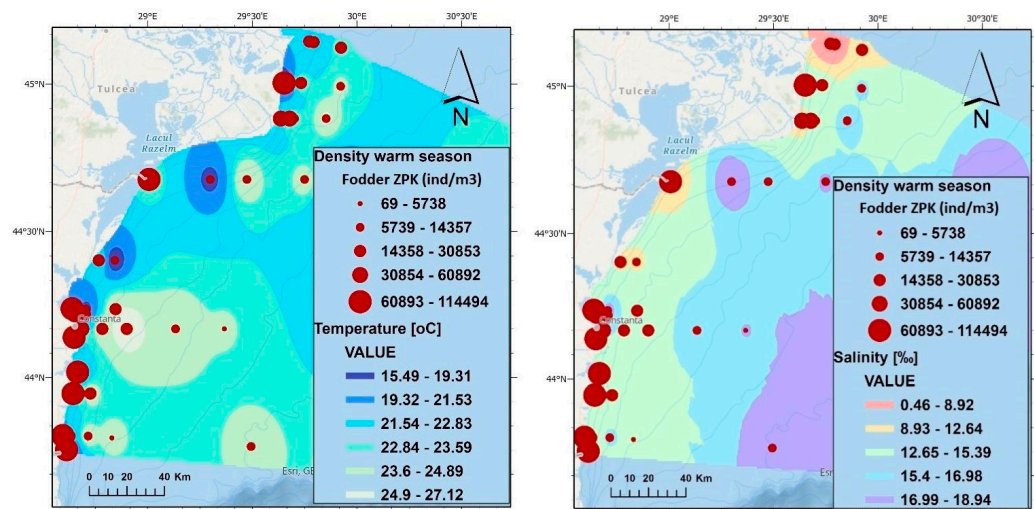


Figure 9. Spatial distributions of fodder zooplankton density, water temperature, and salinity on the Romanian coast—warm seasons, 2013–2020.

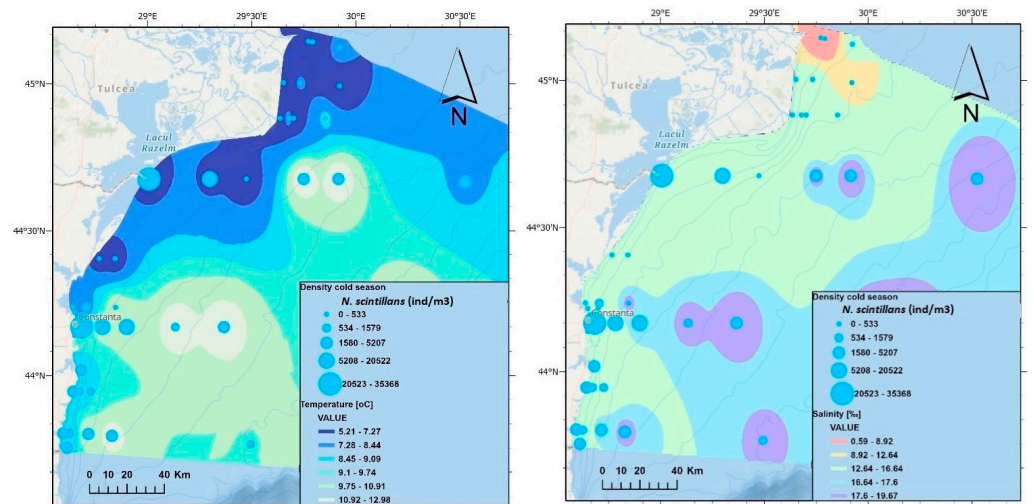


Figure 10. Spatial distributions of non-fodder zooplankton density, water temperature, and salinity on the Romanian coast—cold seasons of 2014, 2016, and 2017.

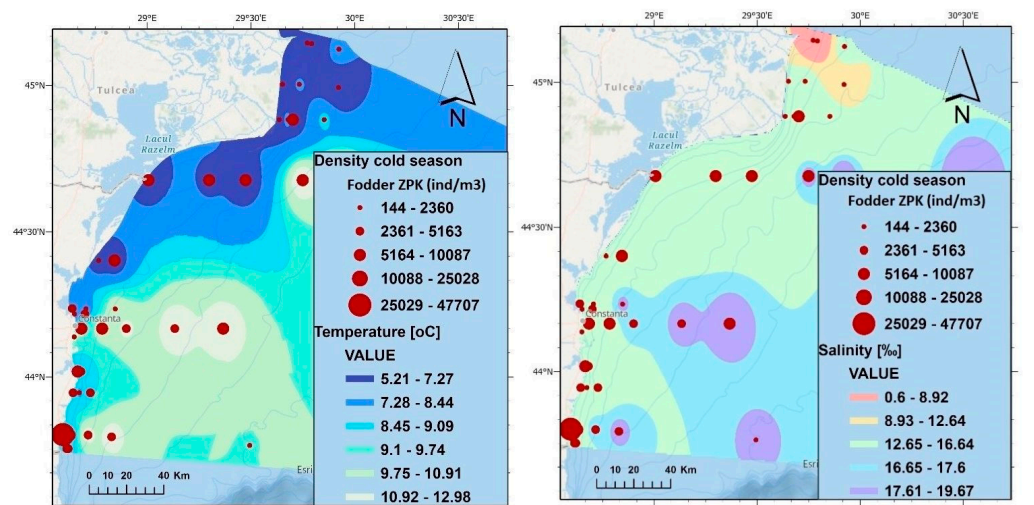


Figure 11. Spatial distributions of fodder zooplankton density, water temperature, and salinity on the Romanian coast—cold seasons of 2014, 2016, and 2017.



## 4. Discussion

### 4.1. Zooplankton Composition

Significant long-term changes in the zooplankton community occurred in the Black Sea between 1950 and 1970 and in 1995 owing to a remarkable increase in the anthropogenic impact on the ecosystem of the Black Sea, which noticeably affected the species composition of zooplankton assemblages [37]. The most important feature of the zooplankton community after the 1970s was the change in species composition; some species almost disappeared, whereas others (*N. scintillans*) increased in their abundance. These events affected mostly the northwestern part of the Black Sea, where the nutrient enrichment supplied by Danube, Dniester, and Dnieper runoffs was higher. The first signs of ecosystem rehabilitation appeared after 1994 because of pollution and eutrophication reductions and changes in the hydro-climatic regime in the warm climatic cycle [38].

Thus, according to Porumb (1977, 1994–1995) [39,40] the core of the holoplankton from the Romanian Black Sea consisted mainly of *N. scintillans*; calanoid copepods represented by *C. euxinus* (*ponticus*), *P. elongatus*, *P. parvus*, *C. ponticus*, and *A. clausi*; to which are also added cyclopoid copepods—*O. nana* and *O. similis*; Cladocera species, such as *P. avirostris*, *P. polyphemoides*, *E. spinifera*, and *P. tergestina*; the chaetognath *P. setosa* (*S. setosa*); and the appendicular *O. dioica*.

In the 1970s–1980s, the abundance of common species greatly decreased [41]. This change was further pronounced after the invasion of the ctenophore *Mnemiopsis leidyi*, which caused major changes in the structure and dynamics of pelagic communities in the Black Sea after 1988, reflecting the stressed state of the pelagic habitat dominated by the influence of the ctenophore [3]. *M. leidyi* flourished in the food web along the western coastal waters during 1989–1992 [42] owing to its wide prey base (e.g., ciliates, copepods of various sizes, nauplii, cirripeds larvae, bivalves, eggs, and fish larvae) [3]. The previously dominant cyclopoid *O. nana* and the calanoid *Labidocera brunescens* no longer occur in zooplankton samples [43]. In the northwestern Black Sea, the abundance of hyponeustonic copepods (Pontellidae) decreased between 1960 and 1980. In the same region, *P. parvus*, *C. ponticus*, *P. elongatus*, *C. euxinus* (*helgolandicus*), *P. setosa* (*S. setosa*), *P. avirostris*, and *E. spinifera* decreased in the same period [44]. All these changes observed in the pelagic system of the Black Sea seem to be due to pollution and heavy predation by *M. leidyi* on zooplanktonic organisms. The zooplankton community was drastically affected by the population outburst of the alien ctenophore species *M. leidyi* after 1988, causing changes in the water's physical properties by reducing transparency and, more significantly, the biological properties by causing a cascade effect on all the trophic levels, favouring phytoplankton growth and reducing food resources for planktivorous and predatory fishes. [45]. The introduction of its predator *Beroe ovata*, which came from either the Mediterranean Sea or the eastern coast of the North Atlantic through ballast waters in 1997, helped the later recovery of the ecosystem [45]. *B. ovata* was first encountered in the western shelf [46] and the northeastern basin in the summer of 1997 [47]. In addition, the entire planktonic component was affected by the severe climatic cooling regime in the 1980s, followed by a strong warming regime in the 1990s [48].

The physical and chemical environment regulates the size, structure, and taxonomic composition of plankton communities [10]. Most of the zooplankton in marine pelagic ecosystems usually consists of copepods, and these small crustaceans often constitute up to 80% of the zooplankton biomass [49]. In 2010, a new zooplanktonic species was identified for the Romanian waters of the Black Sea—the cyclopoid copepod *Oithona brevicornis*, a species reported for the first time in the waters of the Black Sea in 2001, in the Gulf of Sevastopol, and in the form of two isolated specimens in 2005 and 2006, which were observed in the same location but with much more abundant populations and in all the developmental stages [30]. This species was most likely brought to the Black Sea in the ballast water of ships [50], occupying the niche of *O. nana* [51], a species that was a valuable food source for fish larvae [52]. This species was also reported on the Romanian coastline, being first identified as *O. brevicornis* [53]. For the period 2013–2020, the copepod was



identified and considered as *O. davisae*, according to studies concluding that the cyclopid species recently recorded as new to the Black Sea was not *O. brevicornis* but *O. davisae* [51]. Species of a holoplanktonic nature that were very sensitive to environmental quality and eutrophication, such as *C. ponticus*, *P. avirostris*, and *P. setosa* (*S. setosa*), began to recover the populations [38]. As emphasised by this study, the mentioned species were prominently represented over the examined period.

The Shannon–Wiener diversity index ( $H'$ ) is a suitable indicator of water quality; high values of this index indicate greater species diversity or even the distribution of the species, while lower values indicate poor species composition. This index also determines the pollution status of a water body [54].

In the ongoing study, the diversity indices indicate that the environment exhibited non-uniformity throughout the research period. Consequently, the population density of the zooplankton has consistently fluctuated in response to the varying environmental conditions.

The pelagic environments of the Eastern Mediterranean and the Black Sea present some common features due to their connection through the Dardanelles and Bosphorus Straits. Similarities are recorded in flora and fauna compositions [54], the zooplanktonic communities in both regions are mainly represented by species of Atlantic origin. However, noticeable differences in hydrology, temperature, salinity, and biological production are found in these two distinct areas, driven by geographical and geo-morphological characteristics [55].

Limited species exchange and the lack of adaptation of Mediterranean species to the Black Sea even occur now under climate warming, habitat niche vacancy, and eutrophication. Similar peculiarities are also found in the Baltic Sea owing to biogeographical barriers to species expansions [56]. Therefore, both the Black and Marmara Seas have recorded low plankton and fish species biodiversities since the beginning of the post-glacial phase [55].

The low species biodiversity of the Black Sea (Table 3) is a sign of the ineffective bio-invasion process of Mediterranean species. This phenomenon is explained by strong differences in physical gradients and some physiological limitations along the colonization route of the Turkish Straits System (TSS), which make Mediterranean plankton species vulnerable to long-distance natural expansions. In conclusion, the Mediterraneanization of the Black Sea is an ongoing process under the prevailing physical dispersal and physiological limitations driven by peculiar horizontal and vertical variations in temperature, salinity, and dissolved oxygen concentration along the TSS. Climate change may have been a facilitating factor for a recent increase in new species observations in the TSS and the Black Sea [57].

#### 4.2. Zooplankton Structure–Seasonal Variations

The tendency of zooplankton evolution, both in terms of qualitative and quantitative structures, depends on the levels of nutrient input to the sea [58]. With the increase in water temperature, species of warm waters appear in the water mass, while those of cold waters migrate to the colder water layers [59]. The qualitative variety of zooplankton increases in the warm period, with copepods and Cladocera being the best represented both qualitatively and quantitatively [60], as shown in the present paper (Figure 3).

Abiotic factors, like water temperature, and biotic factors, such as food availability and predator-induced stress, influence the distribution of zooplankton [61]. Studies on marine copepods showed that across the temperature range from  $-1.7$  °C to  $30$  °C, the body size decreased by 43.9% [62]. This suggests that under the phenomenon of global warming, smaller copepod species are likely to dominate, with effects on fisheries and carbon sequestration [62–64]. The energetic demands of zooplankton are higher under warming conditions; therefore, nutrient limitation also increases and leads to smaller zooplankton body sizes, with negative effects on the community and carbon export [63,65]. Regional zooplankton communities present seasonal shifts between a cold and a warm season as a result of changes in environmental variables [66]. Seasonal changes in the zooplankton structure are also recorded in the central region of the Mediterranean Sea and

the Sea of Azov, with variations within similar limits. The range of seasonal changes in the quantitative indices of zooplankton in the central regions increases from the Mediterranean Sea to the Sea of Azov, corresponding to the increase in the range of seasonal variations in the temperature and quantity of phytoplankton [55].

#### 4.3. Zooplankton Structure in Relation to Physicochemical Parameters

The distribution of the zooplankton community depends on several factors, such as climatic conditions, water physical and chemical parameters, and biological interactions [67]. Zooplankton changes in response to changes in water quality and environmental parameters influencing the changes in zooplankton abundance [68].

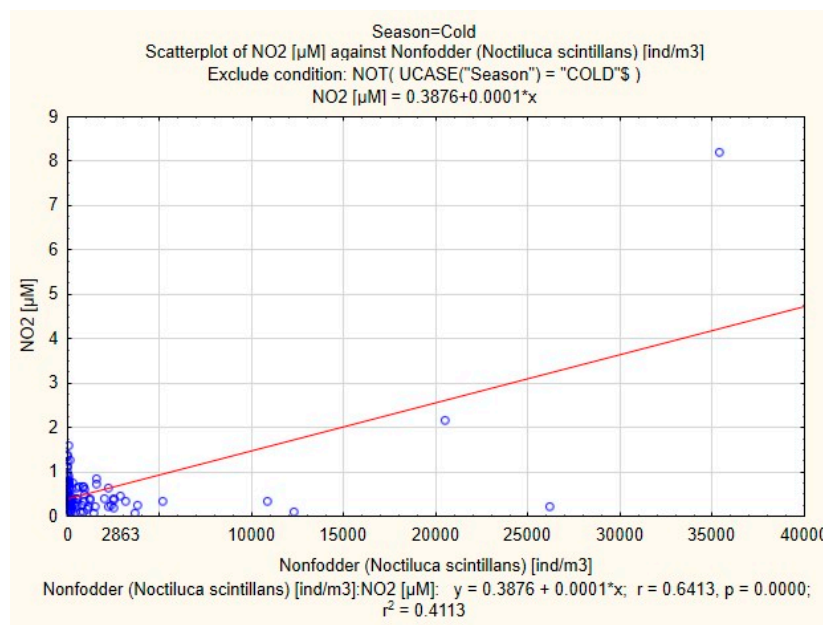
Seasonality, with significant changes in the temperature and salinity regime, influences the food and nutrients available to zooplankton and is an important driver of community distribution [69]. We observed that the fodder component records high abundances in the warm season. *N. scintillans*, the non-fodder component of the zooplankton community, is a dominant species in the Black Sea and is a voracious predator of phytoplankton, bacteria, detritus, microzooplankton, eggs, and naupliar stages of copepods [70]. It has almost no predator (except for *P. setosa* and a limited number of copepod species) owing to its low carbon and high ammonia contents [3]. Although the increase in the abundance of *N. scintillans* is affected by temperature and salinity, it is known to be an eurythermal and euryhaline organism [71]. Many previous research results have shown that the optimal temperature and salinity demands of *N. scintillans* are different in each ecosystem [72–74]. Therefore, it is difficult to discover only the effect of temperature and salinity on *N. scintillans* bloom formation [75]. Moreover, zooplankton, like all aquatic organisms, require oxygen for respiration. Adequate dissolved oxygen levels are essential for their survival and metabolic functions. Low dissolved oxygen levels (hypoxia) can lead to stress or mortality in zooplankton populations. Some species are more tolerant of hypoxic conditions than others. Hypoxic events can also disrupt zooplankton vertical migration patterns, impacting their feeding and predator avoidance behaviours [76,77]. Nutrient availability, especially nitrogen and phosphorus, can influence primary productivity in the water column, which subsequently affects zooplankton food sources (phytoplankton) [78]. High nutrient concentrations can lead to phytoplankton blooms, providing a surplus of food for zooplankton. This can result in population explosions known as “bloom and bust” cycles. Nutrient availability affects the nutritional quality of phytoplankton, which, in turn, influences the growth and reproduction of zooplankton that feed on them [79].

Thus, the dynamics of zooplankton populations in marine ecosystems are significantly influenced by a combination of environmental factors, including the temperature, salinity, and dissolved oxygen and nutrient concentrations [76,80,81].

The analysis of the most pronounced significant correlations, as presented in Tables S7–S9, underscores the substantial impact of temperature fluctuations across different seasons on the overall growth of the zooplankton population, encompassing both fodder and non-fodder species. Furthermore, the interplay of temperature and salinity variations emerges as a major influence on the development of “other groups” and meroplankton. Notably, “other groups” show a positive correlation with salinity, while meroplankton densities exhibit a negative one. The examination of correlations within individual seasons reveals that temperature and salinity continue to hold importance, with the warm season exhibiting stronger relationships. This is particularly pronounced given the heightened environmental parameter variability during this period, as indicated by the raised standard deviation (as detailed in Tables S3–S5). Climate change has led to significant alterations in seawater temperature and salinity patterns. Rising global temperatures are causing an increase in sea surface temperatures, impacting marine ecosystems. This warming is often accompanied by changes in salinity due to various factors, including altered precipitation and river flow patterns and changes in sea circulation. These shifts in temperature and salinity can affect zooplankton species/group distributions and influence the functioning

of marine ecosystems, underscoring the complex interplay between climate change and sea dynamics.

Additionally, an intriguing correlation emerged exclusively during the cold season, highlighting the connection between non-fodder zooplankton densities and nitrite concentrations (Figure 12). Notably, the presence of *N. scintillans* blooms serves as an indicator of eutrophication in this specific area of the Black Sea, aligning with the criteria set forth by the Marine Strategy Framework Directive implementation in Romania.



**Figure 12.** Scatterplot of NO<sub>2</sub> against *Noctiluca scintillans* densities in the cold seasons of 2014, 2016, and 2017—Black Sea.

Canonical Correspondence Analysis (CCA) examined the connection between the presence of five zooplankton species or groups and various environmental factors, such as temperature, salinity, dissolved oxygen, dissolved oxygen saturation, and nutrient levels (phosphate, silicate, nitrate, nitrite, and ammonium), considering the two seasons—warm and cold. The null hypothesis, which stated that there was no linear relationship between the species data and the environmental variables, was decisively rejected ( $p < 0.0001$ ) (Figure 13).

The significance of each environmental variable in influencing the factors is evident, with salinity and nitrate (F1) making a noteworthy contribution and salinity, nitrate, and temperature (F2) also playing a substantial role (Table 4). When considered together, F1 and F2 account for 86.45% of the variation in zooplankton abundance (Figure 14). Among these variables, copepods are predominantly affected by salinity and nitrate concentrations, while Cladocera and *N. scintillans* exhibit a stronger association with temperature and ammonium levels (Figure 14).

**Table 4.** CCA Regression coefficients.

	F1	F2	F3	F4
T (°C)	−0.017	0.512	0.506	−0.378
S (‰)	−0.704	0.887	−0.415	−0.400
PO <sub>4</sub> (µM)	−0.180	−0.311	0.236	−0.372
SiO <sub>4</sub> (µM)	−0.452	0.096	−0.394	−0.150
NO <sub>2</sub> (µM)	0.333	0.455	0.126	0.071
NO <sub>3</sub> (µM)	−0.506	−0.649	0.262	−0.207
NH <sub>4</sub> (µM)	0.094	0.156	−0.161	−0.046
O <sub>2</sub> (µM)	0.258	0.363	−0.459	−0.887

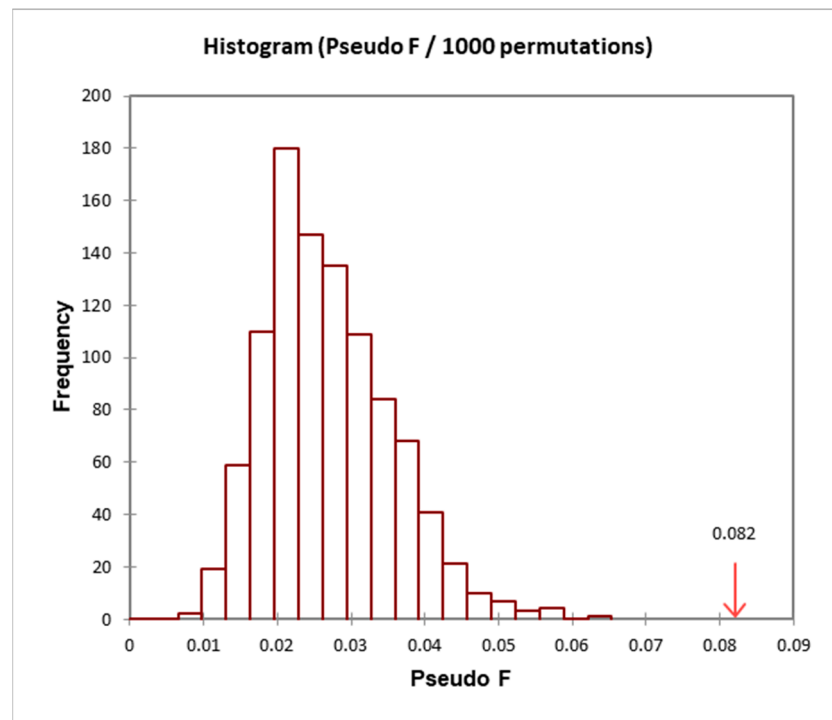


Figure 13. Permutation test (CCA) for zooplankton species groups related to environmental variables.

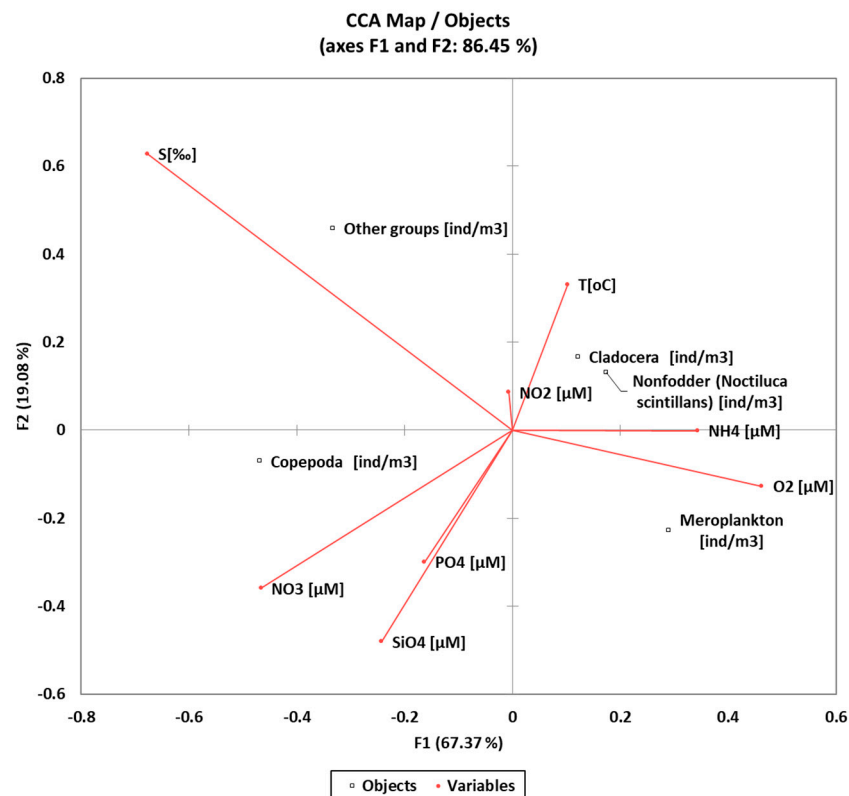


Figure 14. The Canonical Correspondence Analysis (CCA) map of zooplankton groups and environmental variables.

The results of our investigation highlight the dynamic nature of the environment during the study period, leading to fluctuations in zooplankton population density. Notably, salinity and nitrate (F1) emerge as key influencers; and, when combined with temperature in F2, they collectively account for a significant 86.45% of the variability in zooplankton

abundance (Figure 14). This underscores the importance of monitoring and understanding the interplay of these environmental factors in aquatic ecosystems. Furthermore, our findings reveal specific associations, such as the influence of salinity and nitrate on copepods and the impact of temperature and ammonium levels on Cladocera and *N. scintillans* (Figure 14). The findings are significant in the context of climate changes, which can result in altered precipitation patterns, leading to increased freshwater and nutrients input into coastal areas and in marine circulation changes, which can affect the water column stratification, leading to variations in temperature, salinity, and nutrient levels. Coastal areas stretching from the Mediterranean Sea to the Sea of Azov experience seasonal fluctuations in both zooplankton abundance and biomass. These variations are influenced by diverse factors, including temperature and the availability of food resources for zooplankton, which can vary under different conditions [5].

The seasonal shifts in the coastal areas of the Mediterranean Sea are a result of variations in both regional and local environmental conditions. To these changes, the influence of human activities on the ecosystem is further added [82]. In the Baltic Sea, salinity has been established as a crucial regulating factor for large neritic copepods, as demonstrated by Vuorinen et al. in 1998 and related studies. However, Hänninen et al., in 2000, revealed that there exists a significant time delay of approximately one year between freshwater runoff and salinity levels at a depth of 200 m [83].

## 5. Conclusions

The zooplankton taxa showed strong variability in species composition starting in the early 1970s; this fact was identified over the entire Black Sea basin and was mainly generated by eutrophication and anthropogenic impacts, to which the invasion of the ctenophore *M. leidy* was added. Starting in 1995, signs of improvement in the specific diversity of the zooplankton were observed, with species that were very sensitive to environmental conditions being frequently recorded. *M. leidy* invasion in the Black Sea occurred in an already disturbed ecosystem, with eutrophication leading to a reduction in zooplankton diversity and quantity values, especially for the fodder component, which has a trophic value for planktivorous fishes.

However, after 1995, the zooplankton community had a stable trend, with species that were scarcely represented becoming abundant. The community structure of the zooplankton in 2013–2020 had a significant seasonal pattern, with the occurrence of cladocerans being negligible in the cold season but appreciable in the warm one. The share of copepods that play a leading role in fisheries was quite similar in both seasons, with the highest values in the warm one.

The results of our investigation highlight the dynamic nature of the environment during the study period, leading to fluctuations in zooplankton population density. Notably, salinity and nitrate emerge as key influencers; and, when combined with temperature, they collectively account for a significant 86.45% of the variability in the zooplankton abundance. These underline the importance of monitoring and understanding the interplay of these environmental factors in aquatic ecosystems. Furthermore, our findings reveal specific associations, such as the influence of salinity and nitrate on copepods and the impact of temperature and ammonium levels on Cladocera and *N. scintillans*. This knowledge contributes valuable insights into the intricate relationships between environmental variables and zooplankton populations, which can inform future research and conservation efforts in aquatic ecosystems and the ecological implications of climate change for effective marine management strategies.

Understanding the distribution and abundance of zooplankton under the influence of physical and chemical processes may shape the effects of natural and anthropogenic pressures on the ecosystem structure and hold significant importance for the environment. As future research, a comprehensive understanding of how climate change affects zooplankton development and its broader ecological implications is of the utmost importance. Such knowledge is essential for informed decision-making, conservation efforts,



and the development of strategies to mitigate and adapt to the challenges posed by a changing climate.

To increase our current understanding of the changes in the Black Sea biodiversity through natural and human-mediated processes, future observational studies necessitate the development of a cross-disciplinary collaboration [57].

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15091024/s1>. Table S1: List of zooplankton taxa identified until 2020 along the Romanian Black Sea coastline (after Timofte, F., 2017 and personal observations); Table S2: List of zooplankton taxa identified along the Romanian Black Sea coastline in 2013–2020; Table S3: Descriptive statistics of physicochemical parameters under study, both seasons; Table S4: Descriptive statistics of physicochemical parameters under study, cold season; Table S5: Descriptive statistics of physicochemical parameters under study, warm season; Table S6: Statistical differences of physicochemical parameters under study, cold and warm seasons; Table S7: Correlations (all) between biological and environmental parameters; Table S8: Correlations (warm season) between biological and environmental parameters; Table S9: Correlations (cold season) between biological and environmental parameters.

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