

Article

Baseline Assessment of Black Sea Food Web Integrity Using a Zooplankton-Based Approach Under the Marine Strategy Framework Directive

Elena Bisinicu *  and George Emanuel Harcota 

Ecology and Marine Biology Department, National Institute for Marine Research and Development
“Grigore Antipa”, 300 Mamaia Blvd., 900581 Constanta, Romania; gharcota@alpha.rmri.ro

* Correspondence: ebsinicu@alpha.rmri.ro

Abstract: This study presents the first scientific evaluation of food web integrity in the Black Sea under the Marine Strategy Framework Directive (MSFD) Descriptor 4 (Food Webs), utilizing zooplankton indicators to assess the environmental status during the warm season (May to October) from 2018 to 2023. The research aims to analyze trophic interactions, biodiversity patterns, and ecological stability by examining three key indicators: the Shannon–Wiener diversity index, Copepoda biomass, and *Mnemiopsis leidyi* biomass. The findings reveal that the Shannon–Wiener diversity index failed to achieve Good Environmental Status (GES) in any of the four Marine Reporting Units (MRUs), suggesting insufficient community diversity and potential ecological imbalances. Copepoda biomass met GES criteria only in marine and offshore waters, indicating spatial variability in copepod population stability across the Black Sea. Meanwhile, *M. leidyi* biomass remained within GES thresholds in all MRUs, suggesting that its population levels do not currently threaten the food web, despite its known invasive impact. This study provides critical baseline data on food web structure and dynamics in the Black Sea, offering a scientifically grounded framework for future ecological monitoring and management strategies. The results emphasize the need for targeted conservation efforts and adaptive management approaches to enhance the ecological health of the Black Sea in alignment with MSFD objectives.



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1. Introduction

The Black Sea, one of the largest semi-enclosed seas in the world, holds high ecological and socio-economic value for the six nations that border its waters [1,2]. Its unique environmental characteristics, such as the permanent stratification between oxygenated surface waters and anoxic deep layers, make it a distinct and sensitive marine ecosystem [3–5]. The oxygenated zone, rich in dissolved oxygen, extends from the surface to about 150–200 m in depth, depending on seasonal variations and local conditions. Below this, the anoxic zone begins, typically starting around 200–300 m, where oxygen is absent, and the waters are instead rich in hydrogen sulfide [6].

In the Black Sea, food webs have been affected by a combination of human activities and natural pressures. Over decades, the region has experienced significant disruptions, including widespread eutrophication caused by nutrient runoff from agriculture, overfishing of key species like mackerel and turbot, and the introduction of invasive species such as

the comb jelly *Mnemiopsis leidyi* [7–12]. These factors have destabilized trophic interactions, reduced biodiversity, and compromised the energy flows essential for a healthy ecosystem [7–9,13]. Climate change further compounds these challenges, influencing species distributions and altering food web dynamics [14–16].

The Black Sea's food web relies on a delicate balance between primary producers, zooplankton, and higher trophic levels. Small pelagic fish, such as anchovy and sprat, serve as a critical link between plankton and predators, while larger species like marine mammals and predatory fish regulate the food web from the top down [13]. Any disruption in these interactions can lead to cascading effects that weaken the ecosystem's functionality and resilience [8,10,17].

The Marine Strategy Framework Directive (MSFD) [18], formally known as Directive 2008/56/EC, was adopted by the European Union in 2008 with the primary goal of protecting the marine environment across Europe [19] and achieving Good Environmental Status (GES) in EU marine waters. The directive adopts an ecosystem-based approach to managing human activities that affect the marine environment, emphasizing sustainable use and preventing further degradation. It establishes 11 descriptors that Member states must assess and manage to safeguard marine health, promote biodiversity, and ensure the resilience of marine ecosystems. GES refers to the environmental condition where seas are ecologically diverse, clean, healthy, and productive, with sustainable use that supports ecosystem functions and human needs. GES is assessed through descriptors covering biodiversity (D1), non-indigenous species (D2), commercial fish and shellfish (D3), food webs (D4), eutrophication (D5), seafloor integrity (D6), hydrographical conditions (D7), contaminants (D8), contaminants in seafood (D9), marine litter (D10), and energy, including underwater noise (D11), all aimed at reducing human impact and promoting resilience.

Addressing Descriptor 4 under MSFD [18] in the Black Sea involves understanding and managing these complex trophic relationships. Monitoring efforts must focus on the abundance, diversity, and role of species at different trophic levels and the energy flows that sustain the ecosystem. This requires advanced modeling, comprehensive field studies, and long-term data collection to detect changes and guide management actions [20–22]. By focusing on Descriptor 4, the MSFD [18] aims to restore and protect the integrity of the Black Sea's food webs, ensuring their ability to support marine biodiversity and the livelihoods that depend on a healthy and productive sea. This approach is vital for the region's long-term ecological and economic sustainability.

Achieving GES for food webs in the Black Sea also requires mitigating human impacts. Efforts to reduce nutrient pollution, restore overexploited fish stocks, and prevent the spread of invasive species are essential [5,9,23]. Regional cooperation, such as through the Black Sea Commission and other international frameworks, is equally critical to ensure coordinated and effective management of the shared marine environment.

Zooplankton are a critical component of marine food webs due to their central role in transferring energy through the ecosystem. These microscopic organisms, which include a wide range of species such as copepods, and jellyfish, serve as an essential link between primary producers (phytoplankton) and higher trophic levels, including fish, marine mammals, and seabirds [24,25]. By feeding on phytoplankton, zooplankton converts the energy contained in primary production into biomass that is accessible to larger organisms in the food web [26–29]. Zooplankton also plays a key role in regulating nutrient cycling in marine ecosystems. Their feeding behavior helps control phytoplankton populations and maintain a balance between the primary producers [24,25]. Furthermore, when higher predators consume zooplankton, they pass on the energy accumulated from the lower trophic levels. Thus, zooplankton populations are essential for sustaining biodiversity and the overall productivity of marine ecosystems [24,28,30].

Fluctuations in zooplankton populations can affect food availability for higher predators, alter biodiversity, and influence nutrient cycling [24,25,27,31,32]. Although reduced predation from overfishing might theoretically allow zooplankton populations to increase, the removal of apex predators in the Black Sea ecosystem can trigger trophic cascades that boost the numbers of smaller planktivorous fish, increasing grazing pressure on zooplankton. Additionally, the intrusion of invasive species disrupts native trophic interactions. These combined effects may lead to a decrease in zooplankton biomass, ultimately reducing food availability for higher trophic levels, including commercially important fish. The presence of invasive species like *M. leidyi* further destabilizes the food web by imposing direct predation pressure on native zooplankton, thereby reducing their populations and altering trophic dynamics [8,33–36]. Thus, monitoring zooplankton populations and their biomass provides valuable insight into the health of marine ecosystems and the stability of food webs.

The composition and biomass of zooplankton populations are sensitive indicators of ecological changes because both environmental conditions and human-induced pressures influence them [24,28,37,38]. For instance, changes in water temperature, salinity, and nutrient availability can directly affect zooplankton growth, reproduction, and distribution [29,39,40]. These factors are often the result of broader environmental shifts, such as climate change or eutrophication, making zooplankton an early warning signal for ecosystem health [25,41,42].

Zooplankton are typically categorized into size fractions to facilitate ecological and biological studies, as their size influences their ecological roles, feeding behaviors, and interactions within the marine food web. These size-based classifications often reflect functional and trophic differences among groups, with larger zooplankton often serving as key prey for higher trophic levels, while smaller fractions contribute significantly to energy transfer within the microbial loop [43]. The size fractions of zooplankton generally include:

- Microzooplankton (20–200 μm): Small protozoans and early developmental stages of larger zooplankton;
- Mesozooplankton (200–2000 μm): Medium-sized organisms such as copepods, cladocerans, and meroplanktonic larvae;
- Macroplankton (>2000 μm): Larger gelatinous organisms.

This study focuses on the mesozooplankton and macroplankton size fractions, which are essential in marine ecosystems. Mesozooplankton plays a critical role in transferring energy from primary producers to higher trophic levels [25,29,39,40,44–48], while macroplankton contributes significantly to the marine food web by serving as prey for larger predators and participating in nutrient cycling and carbon export [9,48,49].

This study aims to provide the first comprehensive evaluation of food web integrity in the Black Sea under Descriptor 4 of the MSFD, using zooplankton indicators to assess the environmental status during the warm season of 2018–2023. The assessment focuses on three key indicators: the Shannon–Wiener diversity index for mesozooplankton's biomass taxa, Copepoda biomass, and *M. leidyi* biomass for the macroplankton component. The Shannon–Wiener diversity index for mesozooplankton reflects overall community diversity, the copepod biomass—representing a group with numerous species—serves as an indicator of energy flow stability, and the biomass of *M. leidyi* highlights the impact of invasive species on trophic interactions.

The hypothesis of this study posits those changes in zooplankton indicators—namely, the Shannon–Wiener diversity index for mesozooplankton, Copepoda biomass, and *M. leidyi* biomass—between 2018 and 2023 are correlated with disruptions in food web integrity in the Black Sea during the warm season (May to October). It is anticipated that these shifts will align with regions where Good Environmental Status (GES) under Descriptor 4 of the

Marine Strategy Framework Directive (MSFD) has not been achieved. The study aims to test this hypothesis by assessing the relationship between these indicators and food web dynamics during the warm season.

The results of this study provide essential baseline data for understanding the current state of food web dynamics in the Black Sea. By identifying areas where GES has been achieved and where challenges remain, this research contributes valuable insights into the ecological health of the Black Sea. Furthermore, it underscores the need for region-specific monitoring and management strategies to enhance food web resilience and align with MSFD objectives for sustainable marine ecosystem management.

2. Materials and Methods

2.1. Description of the Study Area

The Black Sea, a semi-enclosed sea connected to the world's oceans only via the narrow Bosphorus Strait, spans latitudes $40^{\circ}55'–46^{\circ}32'$ N and longitudes $27^{\circ}27'–41^{\circ}32'$ E. To the south, it connects to the Mediterranean through the Bosphorus (average width: 1.6 km, depth: 36 m, length: 31 km) and to the north, to the Sea of Azov via the shallow Kerch Strait (depth <20 m) [50]. It is bordered by six countries—Bulgaria, Georgia, Romania, Russia, Turkey, and Ukraine—and influenced by 17 basin countries, 13 capital cities, and ~160 million people. Major rivers such as the Danube, Dnieper, and Don significantly impact the marine ecosystem, draining nearly one-third of Europe's land area [49].

For the Romanian Black Sea coast, four MRUs were established [51]:

- Transitional waters: Northern marine waters influenced by the Danube, at depths ≥ 30 m;
- Coastal waters: Central to southern marine waters (Portița–Vama Veche) from the baseline to 30 m isobaths;
- Marine waters: Inner and outer shelf waters (30–100 m isobaths);
- Offshore waters at depths >100 m.

Between May 2018 and October 2023 (warm season), ten oceanographic expeditions were conducted using “Steaua de Mare” and “Mare Nigrum” research vessels across two station networks that covered all MRUs under the MSFD (Figure 1). In total, 565 mesozooplankton samples (Table S1) and 278 macroplankton samples (Table S2) were collected from 78 stations.

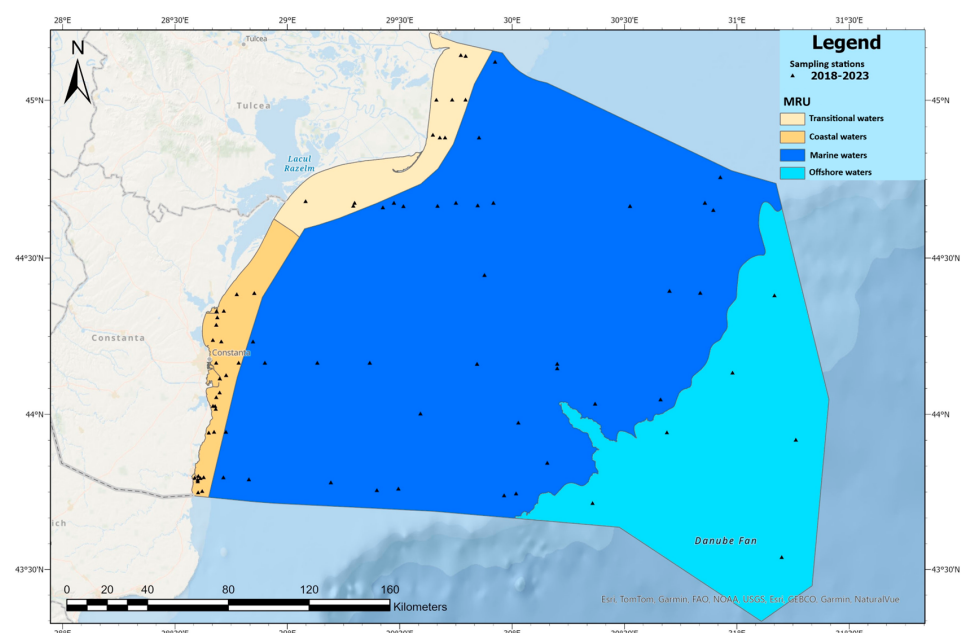


Figure 1. Map of Black Sea sampling stations, 2018–2023.

2.2. Zooplankton Sampling and Analysis

Mesozooplankton samples were collected following the methodology [52] using a Juday net (0.1 m² mouth opening, 150 µm mesh size) equipped with a Hydro-Bios flow meter (model 438,110) to quantify the volume of water filtered. The net was towed vertically through specific depth layers by lowering it to a set depth and retrieving it upwards, capturing samples across distinct water column strata to analyze vertical distribution patterns. Samples were processed both quantitatively and qualitatively under an Olympus SZX10 stereomicroscope (Evident Corporation, Tokyo, Japan). Taxonomic identification and species determination were performed using appropriate manuals and guides [53,54], while classification followed the World Register of Marine Species (WoRMS) [55], while meroplanktonic elements were identified only at the group level.

For each subsample analyzed in the Bogorov chamber, organisms were counted until at least 100 individuals from each of the three dominant taxonomic groups were recorded. Larger organisms were quantified by examining the entire sample in a Petri dish. Abundance (ind/m³) and biomass (mg/m³ wet weight) were estimated using the Black Sea zooplankton constant weight tables [52,56].

Macroplankton samples were collected using a Hensen net with a 70 cm diameter and a 300 µm mesh size. The biological material was collected by vertically towing the net through the water column (from the sea bottom to the surface) at a low speed (0.5–1 m/s) to prevent damage to gelatinous organisms [57]. After sampling, the net was gently rinsed with seawater to remove organisms or mucus from jellyfish. Macroplankton organisms retained in the collection cup were carefully transferred to a bucket, where they were immediately identified, measured, and counted. Larger specimens were rinsed with seawater over the container from which the sample had been taken. All organisms in the sample were measured, with specific dimensions recorded depending on the species (e.g., width, aboral length, or total length) [57].

The wet biomass of macroplankton was expressed in grams per cubic meter (g/m³), calculated following the guidelines for macroplankton [57].

2.3. Zooplankton Indicators

Under Descriptor 4 of the MSFD, we focused on three key zooplankton indicators, the Shannon–Wiener diversity index, Copepoda biomass, and *M. leidyi* biomass to assess the health and functioning of marine food webs (Table 1). Zooplankton biomass represents the food supply for higher trophic levels, including fish and marine mammals, and serves as a marker for shifts in environmental conditions and ecosystem productivity.

The threshold values for zooplankton indicators were defined in earlier reports and studies [39,57–59] focusing on marine ecosystem monitoring and management. These values (Table 1) are based on historical data, scientific research, and expert consensus. Based on the biomass values of each indicator and by comparing them to the established threshold value, a comprehensive assessment of the Black Sea's environmental status for Descriptor 4 within the MSFD framework was conducted. A Good Environmental Status (GES) classification for each MRU was assigned the following criterion: if more than 50% of the sampling stations recorded values exceeding the established threshold (Table 1), the MRU was classified as being in GES. This methodological approach ensured a robust evaluation of the zooplankton community's role in the Black Sea ecosystem and its alignment with marine environmental management objectives. The diversity of the trophic guild was evaluated using the Shannon–Wiener diversity index (H'), which measures species diversity based on species richness and evenness.

The balance of total abundance between trophic guilds was assessed using copepod biomass as the primary indicator. In addition to copepods, *M. leidyi* biomass was monitored as an indicator of the impact of invasive species on zooplankton communities.

The size distribution of individuals across the trophic guild was not assessed in this study. Similarly, productivity was not evaluated, and no specific indicators or thresholds were defined for this aspect of trophic dynamics.

Table 1. Zooplankton indicators and threshold values for Descriptor 4 (food webs) of MSFD.

Criteria Code	Type	Criteria	Indicators and Threshold Values	Indicator Objectives
D4C1	Primary	The diversity (species composition and relative abundance) of the trophic guild is not adversely affected due to anthropogenic pressures.	Shannon Wiener (H') for mesozooplankton biomass (mg.m ⁻³) >3—Transitional and coastal waters, >2—Marine waters and offshore [59]	High values of this index indicate greater species diversity or a more even distribution of species, while low values indicate a poor species composition.
D4C2	Primary	The balance of total abundance between trophic guilds is not adversely affected due to anthropogenic pressures.	Copepod biomass (mg.m ⁻³) >45—Transitional >65—Coastal >45—Marine and offshore [39,58] <i>Mnemiopsis leidyi</i> biomass ≤4 g.m ⁻³ for all MRUs [57,58]	The assessment of copepod biomass is important for determining the availability of food resources for fish. Copepods are the most important group in the zooplankton community as they serve as a food source for fish larvae and commercially important fish species. Fish body condition and weight have been reported to be positively correlated with copepod abundance/biomass. The increase in the biomass of the species <i>M. leidyi</i> directly affects the size and composition of the zooplankton population and, indirectly, plankton-feeding fish and primary producers in the food web.
D4C3	Secondary	The size distribution of individuals across the trophic guild is not adversely affected due to anthropogenic pressures.	N/A	—
D4C4	Secondary	The productivity of the trophic guild is not adversely affected due to anthropogenic pressures.	N/A	—

2.4. Data Analysis

The Shannon–Wiener diversity index (H) was calculated as follows:

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

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

The statistical analyses were conducted using Primer version 7.0 [60] and Statistica software version 14.0.1.25 [61]. To meet the assumptions of normality and homogeneity of variance, all biomass data were square root transformed before analysis. The square root transformation was applied because the raw biomass data exhibited a right-skewed distribution, which is common in ecological data. The transformation helped to stabilize variance, reducing the impact of high biomass values and making the distribution closer to normal. In Primer, shade plots were generated to visualize the underlying patterns in data distributions, providing an intuitive representation of variations across samples. Bray–Curtis dissimilarity was used to quantify differences in community composition across sites by measuring the compositional dissimilarity between sample pairs based on species

biomass. Euclidean distance was utilized as a quantitative metric to assess the dissimilarity in mesozooplankton species composition. This statistical approach offers an objective measure of multivariate dissimilarity by computing the geometric distance between samples in a multidimensional space, where each axis represents the abundance of a specific species. By incorporating abundance data, Euclidean distance effectively captures variations in community structure, facilitating a rigorous comparative analysis of mesozooplankton assemblages. SIMPER (Similarity Percentage) analysis was subsequently used to determine the primary contributors to dissimilarities between groups, enabling a more detailed understanding of ecological patterns and the variables driving these differences.

In Statistica, box plots were utilized to graphically depict the variability, distribution, and central tendencies of the data across different experimental conditions, offering a clear visual comparison among groups. A one-way analysis of variance (ANOVA) was performed to evaluate statistically significant differences in measured parameters among the MRUs. The ANOVA test was conducted to assess whether variations in the dataset could be attributed to differences among MRUs rather than random variability.

To refine comparisons between group means, Least Squares Means (LS Means) were calculated. This approach facilitated precise pairwise comparisons while accounting for potential imbalances in sample sizes. To assess the variability and precision of the statistical estimates, 95% confidence intervals were applied. A significance threshold of $p < 0.05$ was established to determine statistically meaningful differences among the groups. The “Effective Hypothesis Decomposition” method was employed as part of the ANOVA framework, enabling the partitioning of total variation into meaningful components associated with the factors under investigation. This method provided insights into the contribution of individual factors to the observed variance, enhancing the interpretability of the statistical results. The analyses allowed for a detailed comparison of mean Copepoda concentrations across the four regions, with results visualized to highlight differences and trends in the dataset.

Geographic Information System (GIS) ArcGIS Desktop software, version 10.7 [62] was utilized to analyze spatial patterns and distributions of the zooplankton component across the study regions. GIS enabled the integration and visualization of spatially referenced data, facilitating the mapping of sampling locations. This approach provided critical insights into the spatial variability of macroplankton distributions and their relationship with environmental factors, enhancing the understanding of ecological patterns across the study area.

3. Results

3.1. Mesozooplankton's Qualitative and Quantitative Structure

The composition and diversity of mesozooplankton varied across MRUs (Figure 2). Copepoda emerged as the dominant group, exhibiting the highest taxa richness, especially in transitional and offshore waters. Cladocera and meroplankton showed moderate diversity, based on the number of taxa observed within these groups. In contrast, Nonfodder (e.g., *Noctiluca scintillans*) and other groups consistently exhibited low taxa richness across all MRUs.

The results reveal distinct spatial patterns in zooplankton across the four marine units (Figure 3). *N. scintillans* exhibited a pronounced peak in the coastal zone, indicating a localized bloom, while copepods such as *Acartia* sp., *Calanus euxinus*, and *Centropages ponticus* were widely distributed but showed increased abundance in the marine and offshore regions, suggesting a preference for these environments. Cladocerans, particularly *Pleopsis polyphemoides*, were more prevalent in transitional and coastal zones, reflecting their association with estuarine or nearshore conditions. Meroplanktonic taxa, including larvae of polychaetes and bivalves, exhibited higher abundances in the transitional and coastal areas (Figure 3).

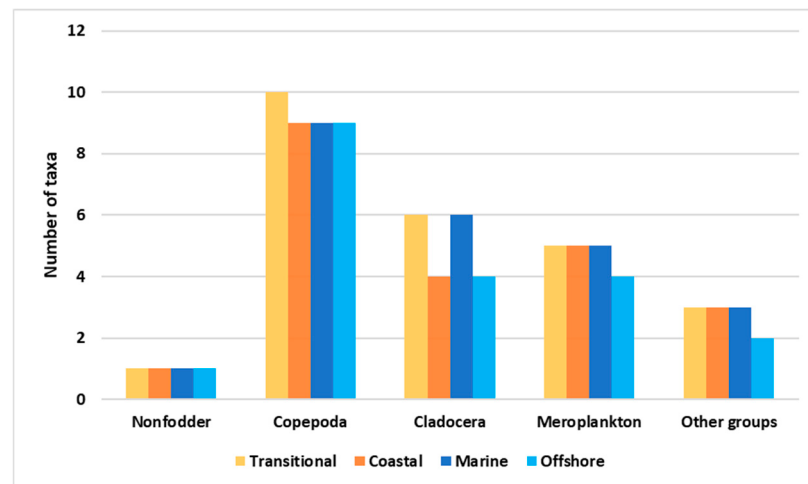


Figure 2. Distribution of mesozooplankton taxa across MRUs in the Black Sea, 2018–2023.

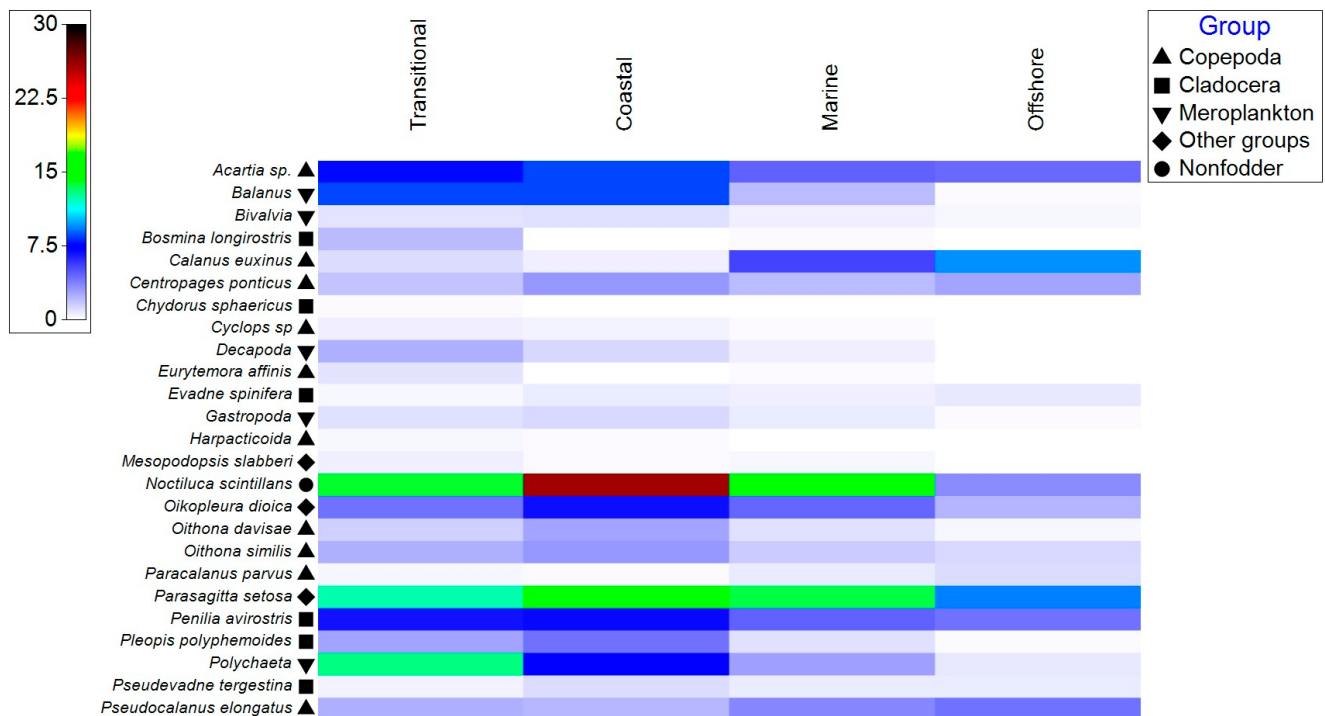


Figure 3. Shade plot of mesozooplankton taxa biomass (mg.m^{-3}), across MRUs in the Black Sea, 2018–2023.

The hierarchical clustering analysis using Bray–Curtis dissimilarity and complete linkage revealed a clear separation among the MRUs, with the coastal/transitional group displaying lower dissimilarity percentages, indicating shared biomass characteristics (Figure 4). In contrast, the marine/offshore group exhibited greater similarity within its cluster. However, the two major clusters—coastal/transitional and marine/offshore—showed a high dissimilarity percentage of nearly 60%, emphasizing the ecological separation between nearshore and offshore environments (Figure 4).

The analysis of mesozooplankton biomass across MRUs revealed significant dissimilarities driven by key taxa, as evidenced by hierarchical clustering (Figure 5) and SIMPER dissimilarity analysis (Table 2). The dendrogram highlights distinct groupings, with copepods, cladocerans, and meroplankton clustering based on ecological similarity, while *Noctiluca scintillans* and *Parasagitta setosa* appear as highly distinct species.

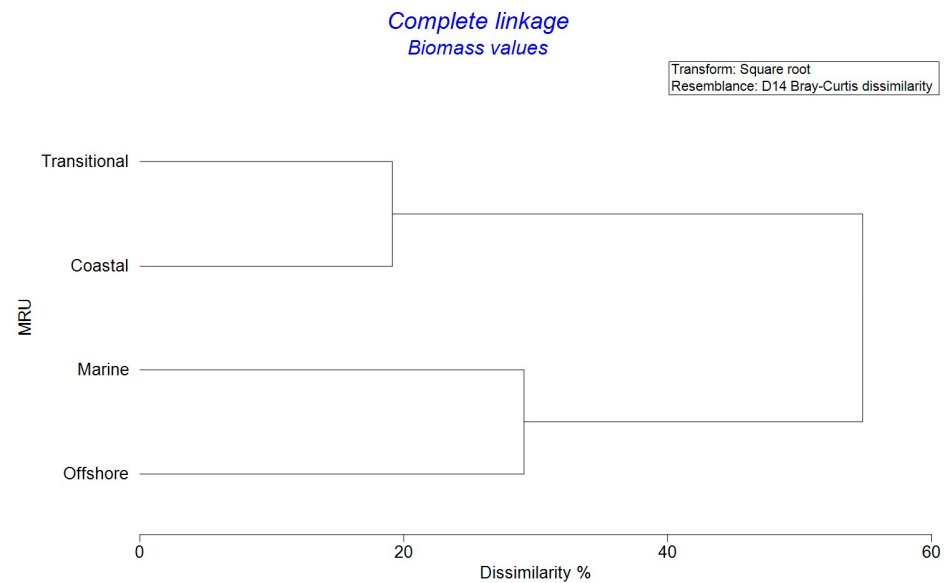


Figure 4. Bray–Curtis dissimilarity dendrogram illustrating mesozooplankton’s community structure across MRUs in the Black Sea, 2018–2023.

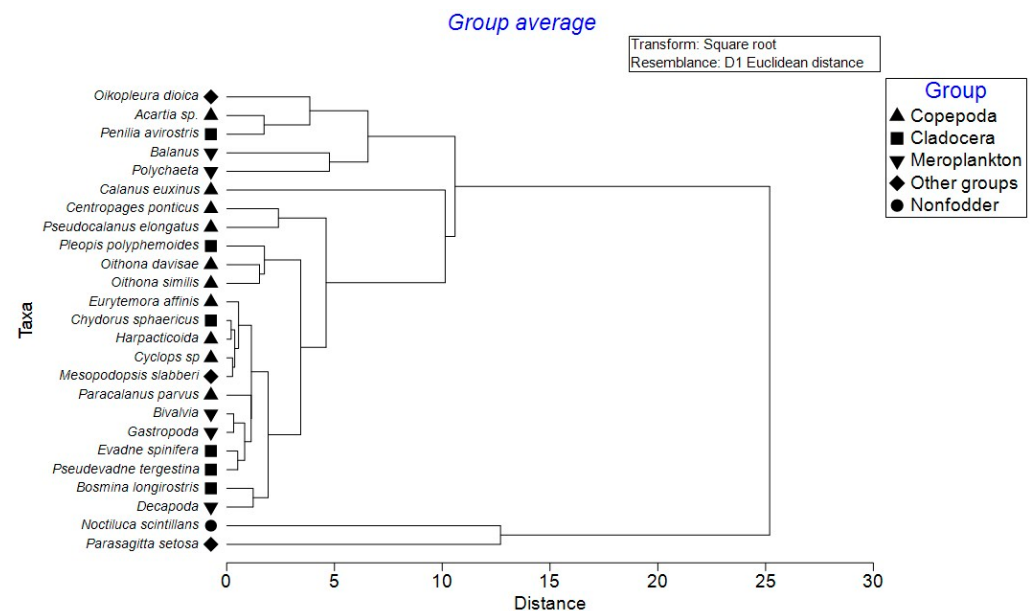


Figure 5. Cluster analysis dendrogram (group average linkage) based on D1 Euclidean distance of mesozooplankton taxa.

Table 2. SIMPER analysis for mesozooplankton taxa across MRUs in the Black Sea, 2018–2023.

Groups Transitional and Coastal					
Average dissimilarity = 19.14					
Group Transitional		Group Coastal			
Species	Av.Abund	Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Noctiluca scintillans</i>	13.86	25.79	6.43	33.57	33.57
<i>Polychaeta larvae</i>	12.94	7.28	3.05	15.91	49.48
<i>Oikopleura dioica</i>	4.06	6.78	1.47	7.66	57.14
<i>Parasagitta setosa</i>	12.25	14.64	1.29	6.73	63.87
<i>Bosmina longirostris</i>	2.01	0	1.08	5.65	69.51
<i>Pleopis polyphemoides</i>	2.7	4.09	0.75	3.93	73.45

Table 2. Cont.

Groups Transitional and Marine					
Average dissimilarity = 26.18					
	Group Transitional	Group Marine			
Species	Av.Abund	Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Polychaeta larvae</i>	12.94	2.84	6.69	25.57	25.57
<i>Balanus larvae</i>	8.43	1.97	4.28	16.37	41.94
<i>Calanus euxinus</i>	1.08	5.43	2.88	11.01	52.95
<i>Acartia</i> sp.	7.58	4.62	1.96	7.5	60.45
<i>Penilia avirostris</i>	6.71	4.6	1.4	5.35	65.8
<i>Bosmina longirostris</i>	2.01	0.11	1.26	4.81	70.61
Groups Coastal and Marine					
Average dissimilarity = 30.17					
	Group Coastal	Group Marine			
Species	Av.Abund	Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Noctiluca scintillans</i>	25.79	14.33	7	23.2	23.2
<i>Balanus larvae</i>	8.4	1.97	3.93	13.02	36.22
<i>Calanus euxinus</i>	0.5	5.43	3.01	9.97	46.19
<i>Polychaeta larvae</i>	7.28	2.84	2.71	8.98	55.17
<i>Acartia</i> sp.	8.45	4.62	2.34	7.75	62.93
<i>Pleopis polyphemoides</i>	4.09	0.93	1.93	6.41	69.34
<i>Penilia avirostris</i>	6.97	4.6	1.45	4.79	74.13
Groups Transitional and Offshore					
Average dissimilarity = 51.15					
	Group Transitional	Group Offshore			
Species	Av.Abund	Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Polychaeta larvae</i>	12.94	0.74	9.29	18.17	18.17
<i>Noctiluca scintillans</i>	13.86	3.36	8	15.63	33.8
<i>Calanus euxinus</i>	1.08	9.45	6.38	12.48	46.28
<i>Balanus larvae</i>	8.43	0.09	6.36	12.43	58.7
<i>Acartia</i> sp.	7.58	4.34	2.48	4.84	63.54
<i>Parasagitta setosa</i>	12.25	9.2	2.32	4.54	68.09
<i>Penilia avirostris</i>	6.71	4.13	1.97	3.85	71.93
Groups Coastal and Offshore					
Average dissimilarity = 54.87					
	Group Coastal	Group Offshore			
Species	Av.Abund	Av.Abund	Av.Diss	Contrib%	Cum.%
<i>Noctiluca scintillans</i>	25.79	3.36	15.56	28.36	28.36
<i>Calanus euxinus</i>	0.5	9.45	6.21	11.32	39.68
<i>Balanus larvae</i>	8.4	0.09	5.77	10.51	50.19
<i>Polychaeta larvae</i>	7.28	0.74	4.53	8.26	58.45
<i>Parasagitta setosa</i>	14.64	9.2	3.77	6.88	65.33
<i>Oikopleura dioica</i>	6.78	2.22	3.17	5.77	71.1

Table 2. Cont.

Groups Marine and Offshore					
Average dissimilarity = 29.31					
Species	Group Marine	Group Offshore	Av.Diss	Contrib%	Cum.%
	Av.Abund	Av.Abund			
<i>Noctiluca scintillans</i>	14.33	3.36	10.04	34.24	34.24
<i>Parasagitta setosa</i>	13.57	9.2	4	13.66	47.89
<i>Calanus euxinus</i>	5.43	9.45	3.68	12.57	60.46
<i>Oikopleura dioica</i>	4.46	2.22	2.05	6.98	67.44
<i>Polychaeta larvae</i>	2.84	0.74	1.92	6.55	74

Pairwise comparisons show that transitional and coastal zones exhibit 19.14% dissimilarity, primarily driven by *Noctiluca scintillans* and *Polychaeta larvae* (49%). Transitional and marine waters (26.18%) are differentiated mainly by *Polychaeta* and *Balanus larvae* (41.94%), while coastal and marine zones (30.17%) show *N. scintillans* and *Balanus larvae* as key differentiators (36.22%). The highest dissimilarity (54.87%) occurs between coastal and offshore waters, with *N. scintillans* (28.36%) and *Calanus euxinus* as dominant contributors, while marine and offshore waters (29.31%) are mainly distinguished by *N. scintillans* and *Parasagitta setosa* (48%). The clustering results align with these findings, as species contributing most to dissimilarity are also widely separated in the dendrogram, reinforcing their role in defining ecosystem transitions.

3.2. Analysis of Copepoda from 2018 to 2023

An analysis of Copepoda biomass distribution using interpolated data revealed significantly higher concentrations in marine and offshore regions. Biomass values peaked at 485.22 mg.m^{-3} , primarily in offshore zones, as indicated by the red and orange areas on the spatial distribution map. In contrast, coastal and nearshore areas exhibited considerably lower biomass levels (Figure 6). This pattern highlights the pronounced spatial variation in Copepoda biomass, with offshore regions consistently sustaining higher densities than coastal areas.

Variability in Copepoda biomass was observed across all MRUs, with distinct patterns emerging in each region (Figure 7). The transitional waters exhibited the lowest variability, characterized by a narrow range and fewer outliers. In contrast, the coastal region displayed the highest mean Copepoda concentration (110 mg.m^{-3}) and pronounced variability, with a wide range and numerous outliers, including some extreme values. The marine region exhibited the lowest mean concentration (70 mg.m^{-3}) and moderate variability, with a narrower range and fewer extreme outliers compared to the coastal region. The offshore region demonstrated the highest overall variability, with the broadest range and frequent outliers, including some extreme values. Its mean concentration (105 mg.m^{-3}) was slightly lower than the coastal region but exhibited wider 95% confidence intervals, indicating substantial fluctuations.

Statistical analysis (ANOVA) confirmed significant variation in Copepoda concentrations (mg.m^{-3}) across the four MRUs ($F(3, 561) = 4.4572, p = 0.00417$) (Figure 8). These results underscore the heterogeneity in Copepoda biomass distribution, with the most notable fluctuations occurring in the coastal and offshore waters.

The distribution of copepod species varied significantly across the MRUs, reflecting their ecological specialization and adaptability to different environmental conditions. In the transitional and coastal waters, *Acartia* sp. dominated, with additional contributions from species such as *Oithona similis* and *Pseudocalanus elongatus* in the coastal region. The

marine MRU exhibited a more variable species distribution, *Calanus euxinus* showing higher abundance, while other taxa remain less represented. In the offshore MRU, *C. euxinus* displayed a distinct peak, clearly dominating this region as most other species exhibited low abundance (Figure 9). These patterns highlight the habitat specificity of certain taxa and the influence of factors such as nutrient availability, salinity, and depth on copepod community structure across marine ecosystems.

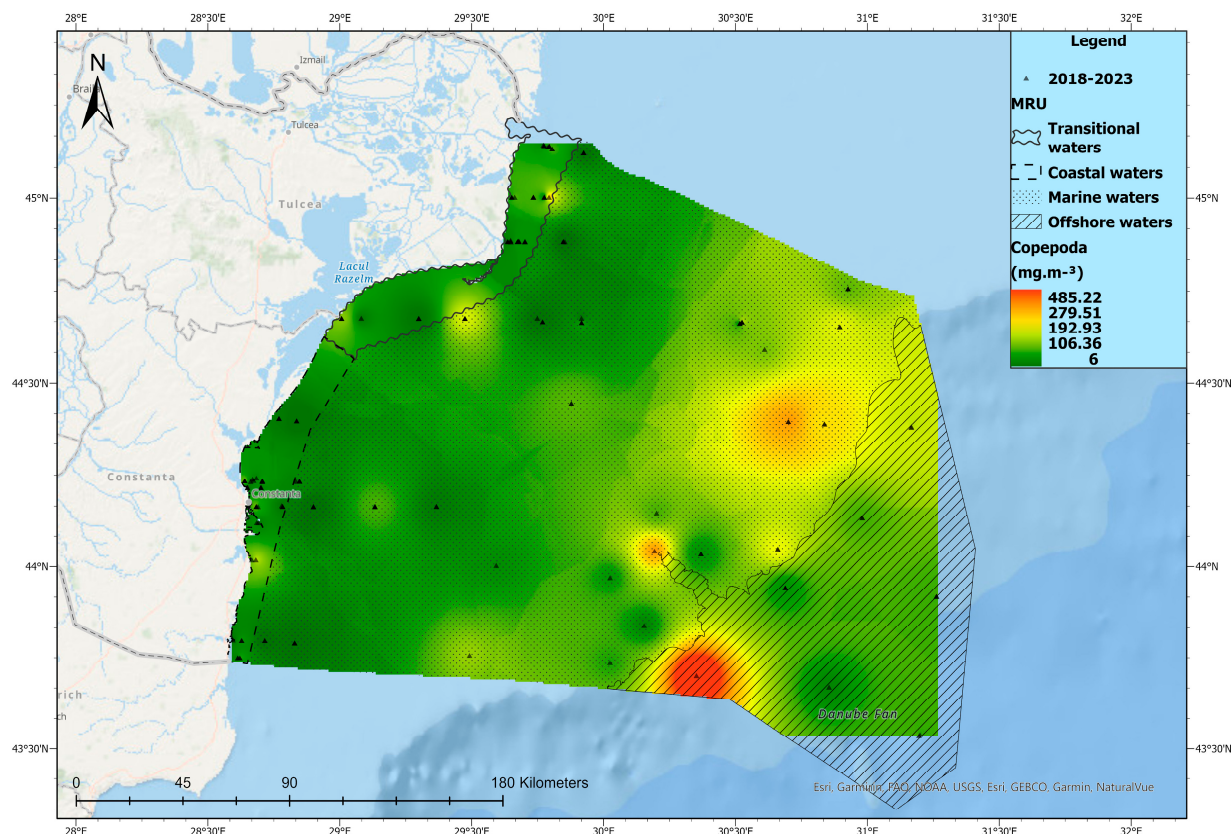


Figure 6. Spatial distribution of copepod biomass (mg.m^{-3}) in the Black Sea, 2018–2023; Danube Fan is an underwater sedimentary feature extending from the mouth of the Danube River into the offshore area of the Black Sea.

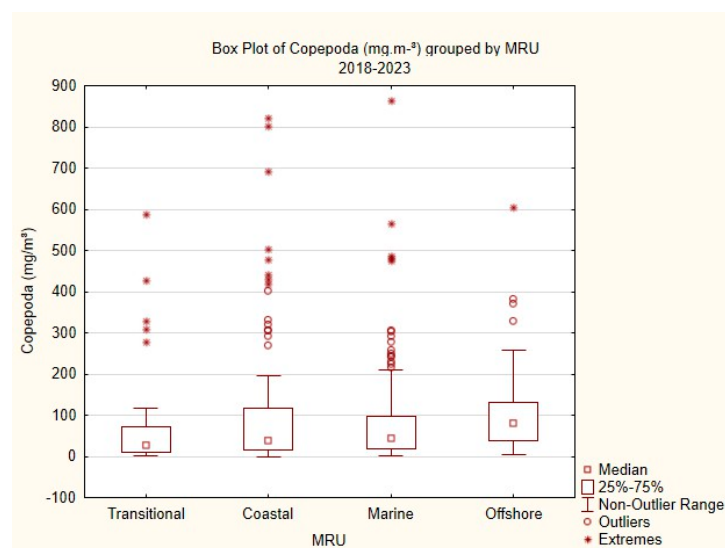


Figure 7. Box plot of Copepoda biomass (mg.m^{-3}) grouped by MRUs, in the Black Sea, 2018–2023.

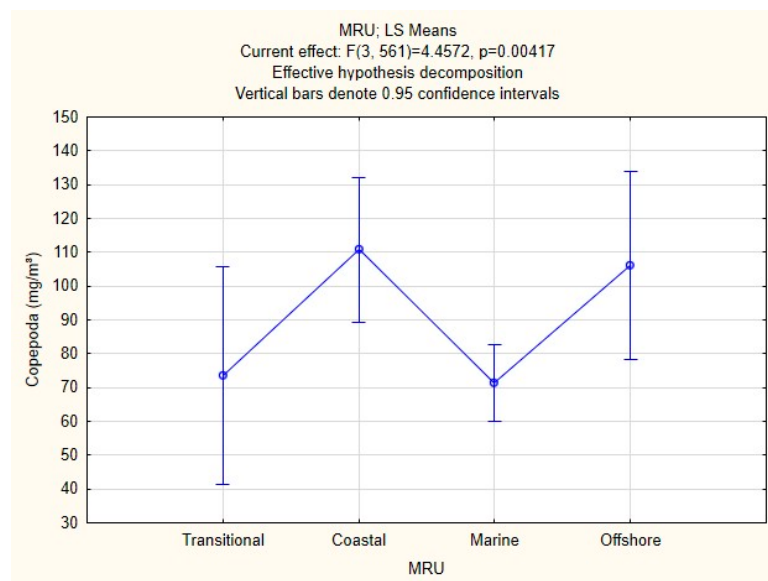


Figure 8. Mean Copepoda biomass (mg.m^{-3}) across the four MRUs in the Black Sea, 2018–2023.

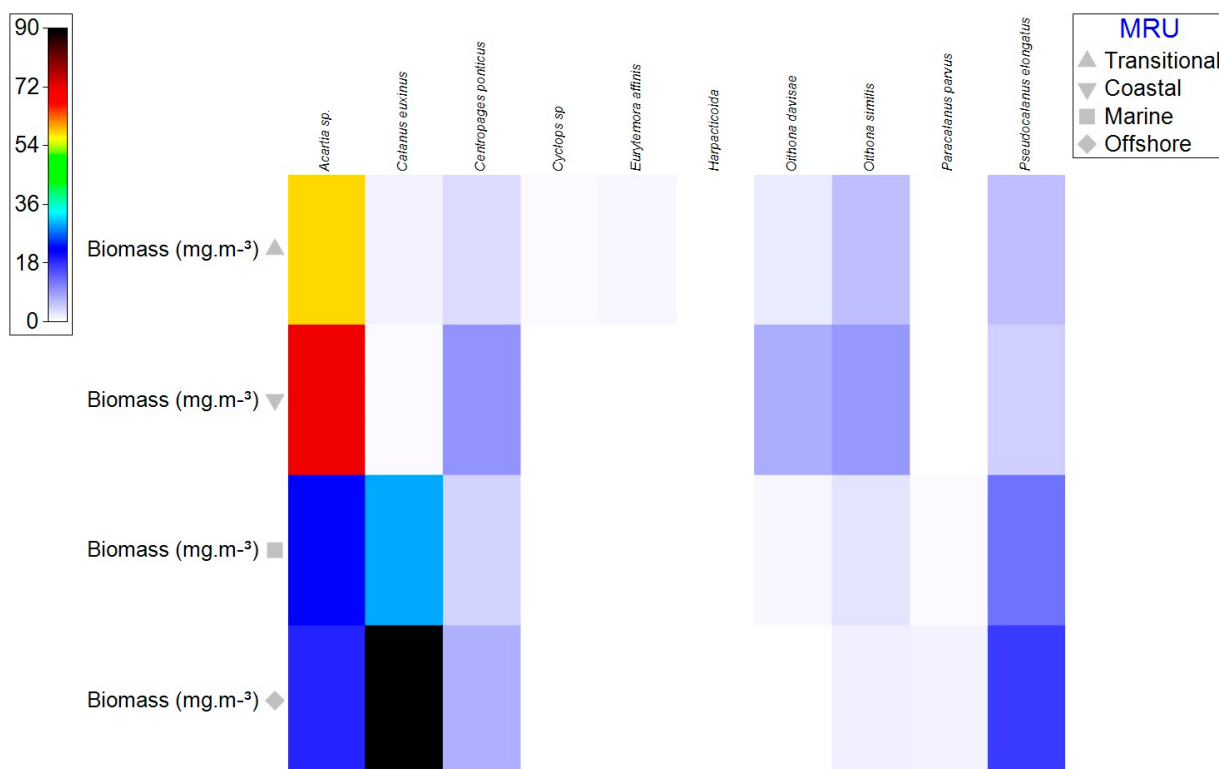


Figure 9. Biomass distribution (mg.m^{-3}) of identified Copepoda species across MRUs in the Black Sea, 2018–2023.

3.3. Analysis of *M. leidy* from 2018 to 2023

The biomass distribution map highlights significant spatial variation across the Romanian continental shelf. The highest biomass levels of *M. leidy*, ranging from 810 to 1220 g.m^{-3} , were concentrated in the central and southern offshore marine zones. Specifically, the central and southern sectors of the Romanian continental shelf exhibited the highest biomass concentrations, with values between 662 and 1220 g.m^{-3} (Figure 10). In contrast, much lower biomass levels, ranging from 0 to 90 g.m^{-3} were recorded in the coastal waters.

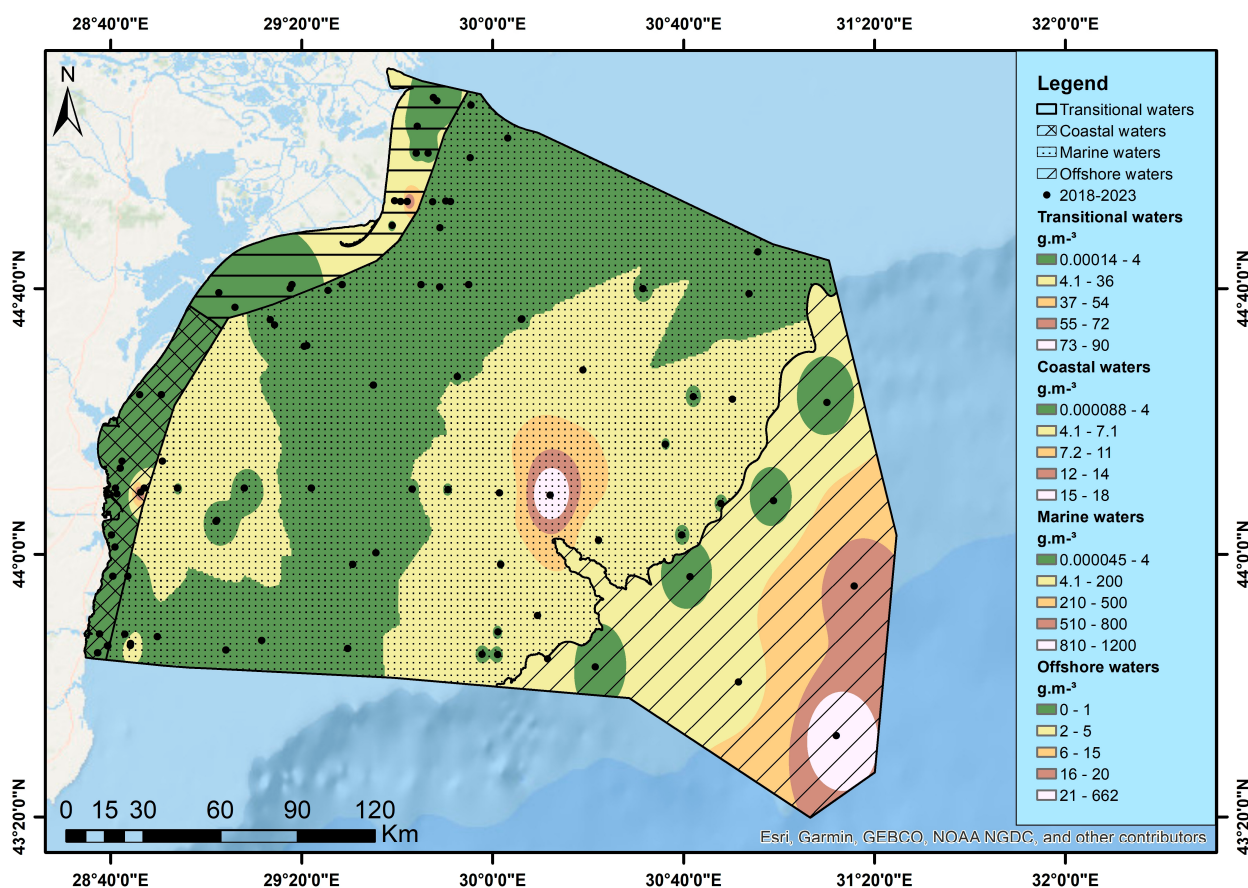


Figure 10. Spatial distribution of *M. leidyi* biomass (g.m^{-3}) in the Romanian Black Sea, 2018–2023.

The box plot analysis revealed that *M. leidyi* biomass was much more variable in marine waters, where it can experience substantial, sporadic surges, leading to higher average biomass. In contrast, biomass in transitional, coastal, and offshore waters remains consistently low, with only occasional, isolated spikes (Figure 11).

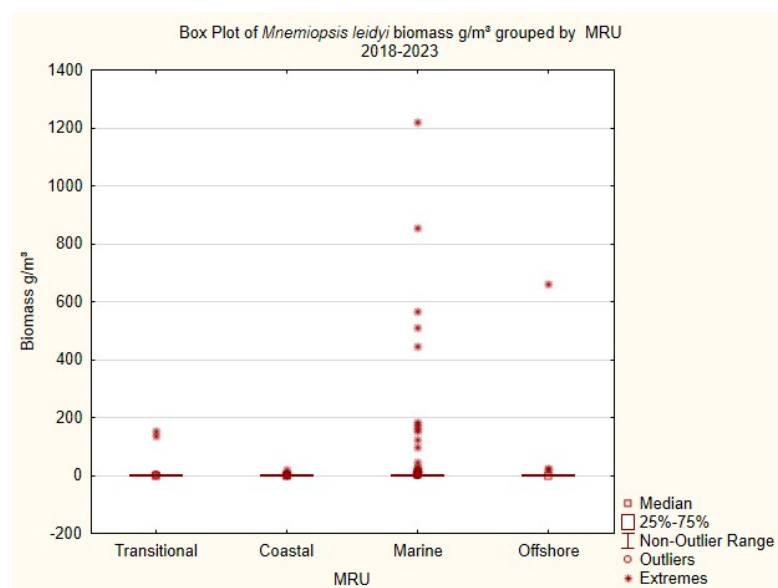


Figure 11. Box plot of *M. leidyi* biomass grouped by MRU in the Black Sea, 2018–2023.

The analysis of *M. leydyi* biomass distribution across different MRUs using ANOVA indicates no statistically significant differences among the transitional, coastal, marine, and

offshore zones ($F(3, 274) = 0.78764, p = 0.50168$). Although the mean biomass values show a slightly increasing trend from coastal to offshore areas, the large confidence intervals suggest high variability, particularly in the transitional and offshore zones. The overlapping confidence intervals further indicate that any observed differences are not statistically significant (Figure 12).

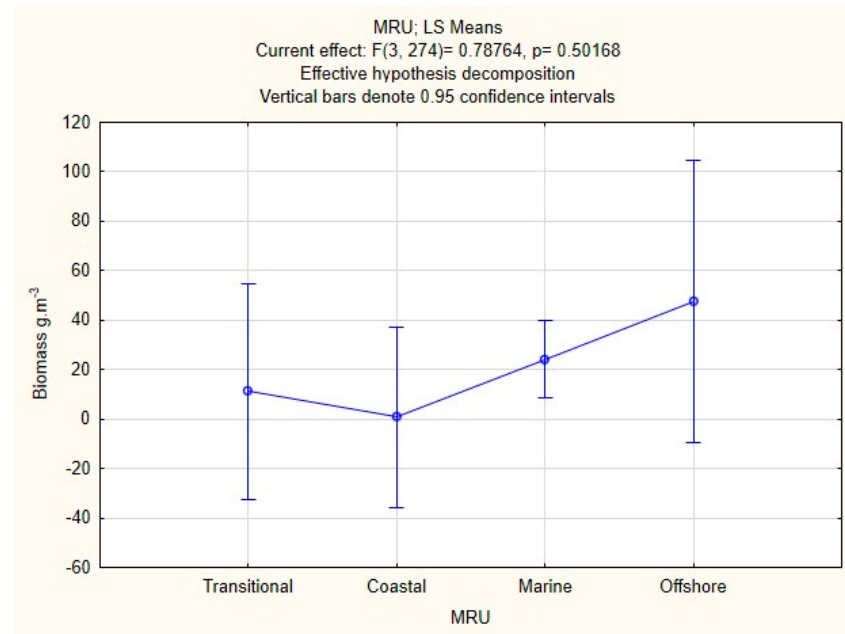


Figure 12. Mean biomass (g.m^{-3}) across different MRUs based on ANOVA results test in the Black Sea, 2018–2023.

The similarity and dissimilarity analysis of *M. leidyi* biomass across the four MRUs revealed notable spatial variations in biomass distribution (Table 3). The within-group similarity was highest in the Marine zone, with an average similarity of 21.13, driven by a high average biomass abundance of 24.04 g.m^{-3} . This indicated consistent and elevated biomass levels in the Marine region. The Transitional group exhibited moderate similarity at 7.23, with an average biomass abundance of 11.16 g.m^{-3} , suggesting moderate variability in this zone. The coastal and offshore zones showed low within-group similarity, at 6.28 and 4.38, respectively, reflecting greater variability and generally lower biomass levels in these regions. Notably, the offshore zone had the highest average biomass abundance (47.44 g.m^{-3}) despite its low similarity, indicating sporadic but substantial biomass peaks.

Between-group dissimilarity was substantial across all MRUs, reflecting significant differences in biomass distribution. The highest dissimilarity was observed between the coastal and offshore groups (94.68%) and the transitional and offshore groups (94.50%), primarily due to the large disparity in biomass abundance between these zones. Moderate dissimilarity was noted between the coastal and marine groups (90.01%) and the marine and offshore groups (90.76%), indicating distinct biomass patterns across these areas. The lowest dissimilarity was between the transitional and marine groups (88.13%), suggesting somewhat closer biomass dynamics between these zones compared to others.

Overall, the results highlighted that *M. leidyi* biomass was most consistently elevated in the marine zone, highly variable but abundant in the offshore zone, and relatively low and inconsistent in the coastal and transitional zones. The pronounced dissimilarity between groups emphasizes the spatial heterogeneity in *M. leidyi* distribution across different marine environments.

Table 3. SIMPER analysis for *M. leidyi* across MRUs in the Black Sea, 2018–2023.

Group Transitional						
Average similarity: 7.23						
	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
<i>M. leidyi</i> biomass g.m ⁻³	11.16	7.23	0.37	100	100	
Group Coastal						
Average similarity: 6.28						
<i>M. leidyi</i> biomass g.m ⁻³	0.81	6.28	0.34	100	100	
Group Marine						
Average similarity: 21.13						
<i>M. leidyi</i> biomass g.m ⁻³	24.04	21.13	0.73	100	100	
Group Offshore						
Average similarity: 4.38						
<i>M. leidyi</i> biomass g.m ⁻³	47.44	4.38	0.28	100	100	
Groups Coastal and Transitional						
Average dissimilarity = 92.95						
	Group Coastal	Group Transitional				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	0.81	11.16	92.95	4.71	100	100
Groups Coastal and Marine						
Average dissimilarity = 90.01						
	Group Coastal	Group Marine				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	0.81	24.04	90.01	3.96	100	100
Groups Transitional and Marine						
Average dissimilarity = 88.13						
	Group Transitional	Group Marine				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	11.16	24.04	88.13	3.62	100	100
Groups Coastal and Offshore						
Average dissimilarity = 94.68						
	Group Coastal	Group Offshore				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	0.81	47.44	94.68	5.64	100	100
Groups Transitional and Offshore						
Average dissimilarity = 94.50						
	Group Transitional	Group Offshore				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	11.16	47.44	94.5	5.85	100	100
Groups Marine and Offshore						
Average dissimilarity = 90.76						
	Group Marine	Group Offshore				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>M. leidyi</i> biomass g.m ⁻³	24.04	47.44	90.76	4.3	100	100

3.4. Zooplankton Indicators Assessment

The results of the Shannon–Wiener Index (H') analysis reveal low biodiversity levels across all assessed MRUs, with H' values ranging between 1.5 and 1.8.

The H' values for all MRUs remain within the Non-GES, signaling limited species diversity and evenness across the study period (Figure 13). Among the four MRUs, the transitional waters exhibited the highest mean diversity index, followed by a noticeable decline in biodiversity from coastal to marine and finally to offshore waters, where the lowest values are recorded. This steady decrease in H' values reflects a clear spatial gradient, with biodiversity diminishing progressively from nearshore to offshore environments.

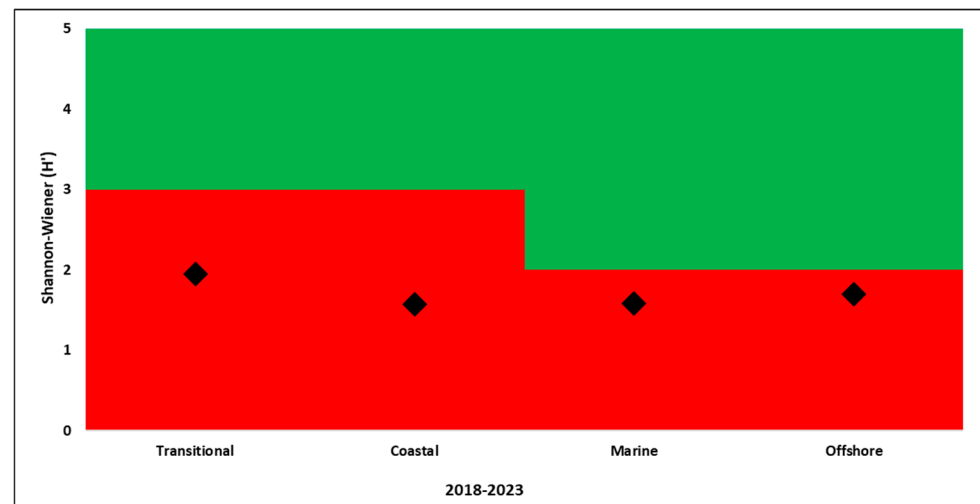


Figure 13. Shannon–Wiener diversity index (H') values in the Black Sea, 2018–2023. Red area—Non-GES; green area—GES.

The analysis of Copepoda biomass across the four MRUs revealed a gradient of environmental conditions, with varying levels of ecological health and stress (Figure 14). In the transitional waters, only 36% of the biomass achieved GES, with 64% classified as Non-GES, reflecting significant environmental stress likely caused by pollution, nutrient runoff, and habitat degradation. Similarly, the coastal zone shows poor conditions, with 42% of the biomass meeting GES and 58% falling into the Non-GES category, highlighting the intense pressures from human activities such as urbanization and industrial discharge. The marine waters exhibited moderate improvement, with 55% of the biomass achieving GES and 45% classified as Non-GES, indicating ongoing but slightly reduced stress compared to the nearshore zones. In contrast, the offshore waters demonstrated the healthiest conditions, with 75% of the biomass achieving GES and only 25% classified as Non-GES, reflecting the reduced influence of human activities in this more distant environment [4].

In transitional waters, 92% of the *M. leidyi* biomass falls within GES, leaving only 8% in Non-GES. This indicates that most of the biomass in this zone exists under favorable environmental conditions. Similarly, the coastal zone demonstrates the most favorable status, with 95% of the biomass categorized as GES and only 5% in Non-GES, making it the zone with the highest proportion of biomass in Good Environmental Status (Figure 15).

In marine waters, 87% of the biomass is under GES, while 13% is classified as Non-GES. Although the proportion of Non-GES is slightly higher than in the coastal and transitional waters, the marine zone still maintains predominantly favorable environmental conditions. Similarly, the offshore zone has 80% of its biomass categorized as GES and 20% as Non-GES. Despite the relatively higher proportion of Non-GES biomass compared to other zones, the majority of biomass in the offshore waters is still in Good Environmental Status, indicating generally favorable conditions.

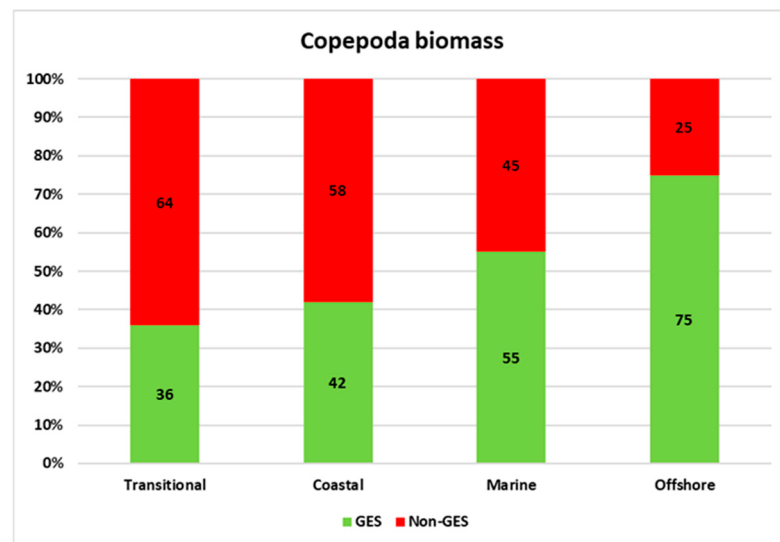


Figure 14. Ecological status of Copepoda biomass indicator in the Black Sea, 2018–2023.

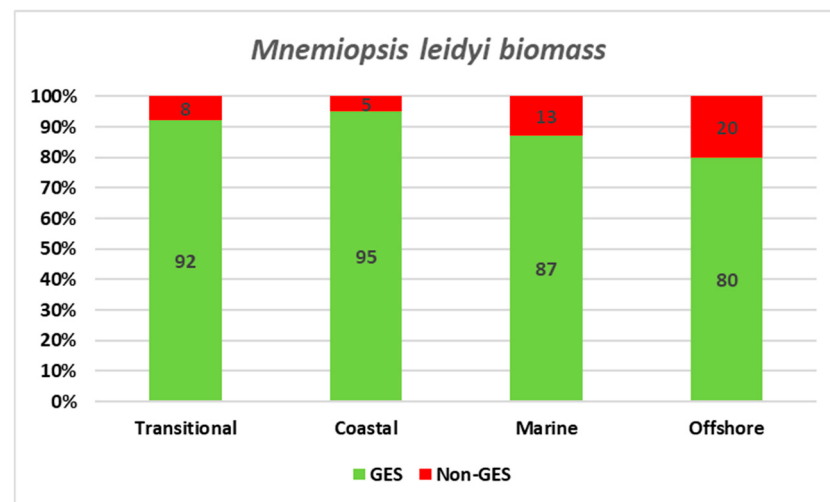


Figure 15. Ecological status of *M. leidyi* biomass indicator in the Black Sea, 2018–2023.

4. Discussion

The Black Sea ecosystem has undergone significant transformations due to anthropogenic pressures, environmental changes, and biological invasions, which have disrupted its food web structure and functioning [4,8,29,31]. In the 1960s, the Black Sea supported a diverse and balanced mesotrophic ecosystem, with large fish species and cetaceans at the apex of the food web [13]. However, nutrient enrichment led to eutrophication by the 1970s and 1980s, fueling excessive production and hypoxia, which collapsed benthic ecosystems [13]. This resulted in the dominance of small pelagic fish and gelatinous zooplankton, such as *Aurelia aurita*, signaling a shift in trophic structure [8,13,36].

The introduction of the invasive ctenophore *M. leidyi* in the 1980s exacerbated the disruption by consuming zooplankton and fish larvae, creating a trophic bottleneck and decoupling primary production from higher trophic levels [33–35,63]. This led to a simplified food web dominated by gelatinous organisms [8,64]. The arrival of *Beroe ovata* in the 1990s, a predator of *M. leidyi*, helped reduce its population, contributing to a partial recovery [34,65,66]. However, the ecosystem has not fully returned to its pre-eutrophication state, particularly in the northwestern shelf and coastal waters [4,9,67,68].

These transformations emphasize the importance of understanding food web dynamics for managing marine ecosystems. Overfishing and eutrophication triggered trophic cascades, while the invasion of *M. leidyi* further disrupted the food web [8,17,31,36]. The partial recovery highlights the role of trophic interactions, such as the control of *M. leidyi* by *Beroe ovata*, in ecosystem stability [8,34]. Despite this, the ongoing challenges of eutrophication and overfishing necessitate integrated management approaches to restore food web integrity.

4.1. Mesozooplankton's Qualitative and Quantitative Structure

The dominance of Copepoda in terms of taxa richness and abundance across all habitats has several implications for food web dynamics. As the primary consumers of phytoplankton, Copepoda plays a central role in transferring energy from primary producers to higher trophic levels. Their diverse and abundant populations in coastal and marine waters suggest that these ecosystems provide a rich food base for planktivorous organisms. The high biomass of Copepoda supports the productivity of higher trophic levels, including fish and other zooplankton, which rely on them as a key food source [45–47,69]. The moderate presence of Cladocera and Meroplankton further supports this flow of energy by providing additional prey for higher trophic levels, contributing to the stability and complexity of food webs in these areas. The findings emphasize that changes in Copepoda populations could have profound effects on the structure and functioning of food webs, influencing energy flow to higher trophic levels and potentially altering ecosystem dynamics, particularly in coastal and marine waters where Copepoda is most abundant.

The distribution patterns of mesozooplankton play a crucial role in shaping aquatic food webs. Mesozooplankton act as a vital link, transferring energy from primary producers, such as phytoplankton, to higher trophic levels, including fish and larger zooplankton. Notably, *N. scintillans* differ in its ecological role; it is not a primary food source for higher trophic levels due to its predatory nature, feeding on smaller plankton rather than serving as prey. Despite this, the high biomass of Noctiluca observed in coastal and marine waters suggests its potential for localized top-down control, significantly impacting smaller planktonic organisms. This could indirectly influence the community structure of primary producers and other mesozooplankton, thereby altering energy transfer pathways within the food web.

In contrast, the widespread distribution of species such as *A. clausi*, *C. euxinus* across transitional, coastal, marine, and offshore waters indicates their importance as a stable and continuous food resource for planktivorous organisms. Their presence ensures consistent energy flow and trophic connectivity across varying environments, supporting a diverse array of predators and contributing to overall food web stability.

The lower diversity observed in transitional waters likely reflects less favorable environmental conditions, such as fluctuating salinity and temperature, which may restrict mesozooplankton abundance and biomass. These challenging conditions can reduce prey availability for higher trophic levels, potentially weakening food web dynamics in these habitats. On the other hand, the prominence of *Pleopis polyphemoides* in offshore waters highlights the presence of specialized mesozooplankton communities adapted to offshore conditions. These specialized communities may play a key role in offshore food webs, providing critical prey resources for offshore predators.

The findings revealed that the mesozooplankton biomass and community structure are strongly influenced by habitat-specific environmental conditions, underscoring the pivotal role of localized ecological factors in shaping mesozooplankton assemblages. These patterns emphasize the intricate interactions within the marine food web, linking mesozooplankton

to their predators, such as fish and larger invertebrates, and their primary producers, including phytoplankton. The observed habitat-driven variations in mesozooplankton dynamics suggest that these organisms not only respond to but also influence the flow of energy and nutrients within their ecosystems. Understanding these dynamics is critical for anticipating the potential impacts of environmental changes, such as climate-induced fluctuations in temperature, salinity, or nutrient availability, as well as anthropogenic disturbances like pollution or habitat degradation. Such insights are essential for predicting shifts in marine ecosystem stability, biodiversity, and productivity, ultimately informing conservation strategies and sustainable resource management in the face of global change.

4.2. Analysis of Copepoda from 2018 to 2023

The variability in copepod species distribution has profound implications for the marine food web, as copepods serve as a critical link in transferring energy from primary producers, such as phytoplankton, to higher trophic levels, including fish, marine mammals, and seabirds. The dominance of specific species in different MRUs reflects their adaptation to unique environmental conditions, such as nutrient gradients, salinity, temperature, and depth. These adaptations influence not only biomass but also the nutritional quality and availability of copepods as a food source for predators.

Differences in species composition and abundance across MRUs can lead to variations in the efficiency of energy transfer through the food web. For example, *C. euxinus*, which dominated the offshore MRU, is known for its high lipid content, making it an energy-rich prey for higher trophic levels. In contrast, species like *Acartia* sp. and *O. similis*, prevalent in transitional and coastal waters, may differ in their nutritional value and seasonal availability. Such differences can influence predator foraging strategies, growth rates, and reproductive success, potentially affecting the population dynamics of higher trophic levels.

Moreover, the observed variability in copepod distributions highlights the susceptibility of marine ecosystems to environmental changes. Factors such as nutrient loading, temperature shifts, salinity changes, and anthropogenic impacts (e.g., pollution, and habitat alteration) can disrupt copepod community structures, leading to cascading effects throughout the food web. A reduction in the abundance or diversity of key copepod species could impair energy transfer efficiency, reduce the resilience of marine ecosystems, and compromise the stability of fisheries that depend on these trophic interactions.

Copepoda biomass concentrations were significantly higher in marine and offshore regions of the northwestern Black Sea. This distribution pattern may result from several ecological and oceanographic factors. One major factor is the nutrient-rich outflow from the Danube River, which delivers substantial amounts of organic matter and nutrients into the Black Sea. These nutrients can be transported offshore by currents and mixing processes, creating favorable conditions for phytoplankton blooms, the primary food source for copepods. Additionally, the Danube Fan, a significant underwater sediment structure, can influence local hydrodynamics, potentially enhancing upwelling and nutrient recycling in the deeper marine environment.

Offshore regions typically experience less anthropogenic disturbance compared to coastal zones, allowing for more stable ecological conditions that support zooplankton growth. Furthermore, the stratification of water masses and the presence of mesoscale eddies can enhance nutrient transport to surface waters, further boosting copepod productivity in these areas.

In contrast, lower biomass levels in nearshore and coastal waters, shown in green and yellow shades, could be attributed to increased human activities such as pollution, over-fishing, and habitat degradation, which may negatively impact zooplankton populations.

Additionally, coastal areas may experience more variable environmental conditions, such as temperature fluctuations and salinity changes, which can limit copepod abundance.

Overall, the observed pattern highlights the ecological importance of offshore zones in supporting high copepod biomass, likely driven by nutrient availability, physical oceanographic processes, and reduced human impact compared to nearshore environments.

4.3. Analysis of *M. leidy* from 2018 to 2023

The elevated biomass of *M. leidy* in the central and southern regions of the Romanian continental shelf suggests significant implications for the local marine food web. As an invasive ctenophore, *M. leidy* is a voracious predator of zooplankton, fish eggs, and larvae, which can lead to the depletion of key trophic resources for native fish species and other marine organisms. This disruption can result in a reduced recruitment of commercially important fish populations and alter predator–prey dynamics, ultimately destabilizing the ecosystem. The lower biomass levels observed in coastal and northwestern areas, potentially influenced by freshwater inputs from the Danube River, may create spatial variations in predation pressure and nutrient availability, further impacting the food web structure. The dominance of *M. leidy* in deeper waters highlights its role as a top-down regulator, potentially suppressing zooplankton populations and altering energy transfer efficiency within the marine ecosystem [68–70].

Distinct patterns of *M. leidy* biomass across marine zones reveal their varying influence. In the marine zone, consistently high and stable biomass indicates strong top-down control, leading to a more structured yet predator-dominated food web. The offshore zone, characterized by high biomass with significant variability, suggests sporadic surges in *M. leidy* populations. These surges may cause temporary disruptions in local food webs through intensified predation. The deep marine zones often have high concentrations of these prey species, making it an ideal environment for *M. leidy* to sustain large populations. Additionally, these areas tend to have fewer predators that would control the populations of comb jellies, further enhancing the potential for population growth. Conversely, the coastal and transitional zones exhibit low yet variable biomass, indicating a more transient and less pronounced influence, likely moderated by external environmental factors.

The distribution of *M. leidy* highlights the spatial heterogeneity of its ecological impact. Differences in nutrient availability, hydrodynamics, and environmental conditions across zones drive this variability. Such heterogeneity underscores the need to consider local ecological factors when assessing *M. leidy*'s role in marine ecosystems. Understanding these spatial dynamics is crucial for predicting the broader ecological implications and managing its impact on native biodiversity and fisheries.

The findings emphasize the importance of targeted management strategies to mitigate the impact of *M. leidy*. Monitoring its biomass in vulnerable regions, particularly during periods of high abundance, is essential. Strategies could also focus on managing nutrient inputs, which may exacerbate blooms, and protecting native fish populations from increased predation pressure [70–72]. Addressing these challenges requires a comprehensive understanding of *M. leidy*'s ecological role to ensure the sustainability of marine ecosystems and the fisheries that depend on them.

4.4. Zooplankton Indicators Assessment

The low biodiversity levels observed in all MRUs significantly affect food web dynamics. Reduced biodiversity often disrupts the structure and balance of food webs, leading to cascading effects across trophic levels. A decline in species diversity can weaken ecosystem stability, making it more susceptible to external disturbances such as climate change or invasive species [73–75]. In low-diversity ecosystems, key species that play vital

roles in the food web—such as primary producers, keystone predators, or essential prey species—are more likely to be impacted [76–78]. This can cause the collapse of trophic interactions, diminishing food resources for higher trophic levels and altering nutrient cycling processes [79]. For example, a decline in primary producers or herbivores could disrupt energy transfer to predators, potentially resulting in population declines or shifts in species composition [77,79]. Additionally, the dominance of a few resilient species in such systems can lead to homogenization, reducing the ecosystem's capacity to adapt to environmental changes and further destabilizing food web interactions [13,15,80].

The results of Copepoda biomass assessment across the MRUs reveal critical implications for the food web, highlighting potential disruptions in energy flow and ecosystem functioning. Copepods are a cornerstone of marine food webs, functioning as primary consumers that convert energy from phytoplankton into a form accessible to higher trophic levels, including fish larvae, small fish, and large marine predators [81–84]. Copepod grazing can play a pivotal role in regulating phytoplankton blooms, particularly in high-latitude regions where suppression of spring blooms is critical for seasonal carbon sequestration [85]. In contrast, in nutrient-limited low-latitude regions, zooplankton grazing may indirectly stimulate primary production by consuming microzooplankton and recycling essential nutrients [85]. When copepod biomass is reduced or fails to meet GES, as observed in transitional and coastal waters, the energy available for species at higher trophic levels diminishes. This could lead to cascading effects, such as reduced growth rates, reproductive success, and population declines in species that depend on copepods as a primary food source [39,84]. Such impacts are likely to be particularly acute in ecosystems already stressed by anthropogenic influences.

In transitional waters, where only 36% of copepod biomass achieves GES, the high proportion of Non-GES biomass (64%) suggests significant environmental stress, potentially disrupting predator–prey relationships. Similarly, in the coastal zone, with 42% GES biomass, the reduced availability of copepods may hinder the recruitment and survival of economically important fish species that rely on these organisms during their early life stages. The marine waters, with 55% GES biomass, show slightly improved conditions, but the remaining 45% classified as Non-GES indicate that stressors persist, potentially limiting the resilience of the food web in this area.

The offshore area, in contrast, supports a more robust food web, with 75% of copepod biomass achieving GES. This relatively healthy state ensures better energy transfer efficiency and stability for higher trophic levels, contributing to a balanced and functional ecosystem. However, even in offshore waters, there remains a risk of cascading effects of stressors, such as climate change, overfishing, or offshore resource exploitation, intensifying and affecting copepod populations.

The overall gradient of copepod biomass, from the stressed nearshore zones to the healthier offshore zones, underscores the vulnerability of marine food webs to anthropogenic pressures. The findings from Lazar et al. [4] on the spatial variability of eutrophication in the Black Sea closely aligned with our results, particularly about the gradient of copepod biomass observed from stressed nearshore zones to healthier offshore zones. In our study, copepod biomass varied in response to coastal waters with higher nutrient concentrations due to agricultural runoff, urbanization, and industrial discharge, which led to a significant decrease in copepod biomass. As Lazar et al. highlighted [4], coastal and transitional waters were characterized by higher nutrient loads, notably inorganic nitrogen and phosphorus, primarily driven by the Danube River and human activities. These nutrient enrichments created an environment conducive to harmful algal blooms, such as *N. scintillans*, which depleted oxygen levels and further disrupted the food web. This disruption at the base of the food web, where copepods played a crucial role as primary

consumers, significantly impacted the overall health of marine ecosystems. In contrast, offshore waters, which had lower nutrient concentrations due to processes like dilution, upwelling, and vertical mixing, showed higher copepod populations. This reflected the reduced stressors on the ecosystem in offshore regions, where the food web remained more resilient. While offshore waters still experienced phytoplankton blooms, they were less frequent and intense, allowing for a more stable and productive environment for copepods and other marine organisms.

Our results, which demonstrated the decline in copepod health in nearshore zones, underscored the critical link between nutrient loading, food web structure, and ecosystem resilience. The reduced health and abundance of copepods in the eutrophic coastal areas directly affected higher trophic levels, such as fish and other marine predators, further destabilizing the food web. Lazar et al. [4] similarly pointed out that nutrient reductions in coastal and transitional waters were necessary to improve environmental conditions, aligning with the urgent need for pollution control and habitat restoration to mitigate stressors in these zones.

Thus, both our findings and Lazar et al. [4] highlighted the vulnerability of the marine food web to anthropogenic pressures, stressing the importance of managing nutrient inputs and protecting ecosystems, especially in coastal and transitional zones where human activities had the most significant impact. Disruptions at the base of the food web—where copepods play a central role—can compromise the productivity and resilience of the entire ecosystem.

The distribution of *M. leidyi* biomass across MRUs, with the majority categorized as GES, indicates a generally favorable condition for the food web. The high proportion of GES biomass suggests that ecosystems in these zones are functioning well, with stable environmental conditions supporting a balance among species. This implies that while *M. leidyi* is present, it is not causing widespread disruptions to lower trophic levels, such as zooplankton populations, which are vital to the food web. In the marine and offshore waters, where 87% and 80% of biomass are in GES, respectively, the ecosystems appear resilient. The stable conditions suggest that natural regulatory mechanisms, such as predation by species like *B. ovata*, may be helping to control its population [34,66,86]. This balance is critical for ensuring that *M. leidyi* does not outcompete other planktivorous species or deplete zooplankton to levels that could disrupt the food supply for fish larvae and other key organisms in the food web [8,36].

However, areas with Non-GES biomass, such as 20% in the offshore zone and 13% in the marine zone, raise concerns about localized stress. Jellyfish exert a significant influence on phytoplankton populations through their high grazing rates, especially during bloom periods [85]. The trophic adaptability of omnivorous zooplankton enables them to modify their diet based on food availability, thereby stabilizing food web dynamics during fluctuations in phytoplankton biomass [85]. *M. leidyi* is a highly effective zooplankton predator, and its abundance in such areas could lead to reduced zooplankton availability [34,35,63,66,87]. This reduction may result in competition with fish larvae and other zooplankton-dependent species, potentially causing shifts in trophic interactions and biodiversity over time [24,25,88]. Although the overall conditions remain favorable, these localized imbalances warrant attention. Continuous monitoring and adaptive management are essential to ensure that the balance within the food web is maintained and that *M. leidyi* does not become a significant disruptor in the ecosystem, as it was in the past [29,33,34].

5. Conclusions

This study provides a comprehensive assessment of food web integrity in the Black Sea, employing zooplankton indicators to evaluate the ecological status during the warm

season of 2018–2023, in alignment with the Marine Strategy Framework Directive (MSFD) Descriptor 4. The results demonstrate significant spatial variability in ecosystem structure, with the Shannon–Wiener diversity index consistently exhibiting low values across all MRUs. This pattern suggests a potential reduction in species diversity, which may be indicative of trophic instability and ecological degradation, as reduced biodiversity can impair the resilience and functionality of marine food webs. Copepoda biomass was found to fall below GES thresholds in transitional and coastal waters, implying the presence of environmental stressors that may hinder the production and availability of primary zooplankton biomass. Such disruptions in copepod populations could have cascading effects, compromising energy transfer to higher trophic levels and potentially destabilizing the entire food web.

Conversely, *M. leidyi* biomass remained within the acceptable limits across all MRUs, indicating that, despite its invasive nature and potential ecological impacts, the current biomass of this species does not appear to significantly disrupt food web dynamics at present. This observation may reflect a period of stabilization of the *M. leidyi* population, where its presence, while not without ecological consequences, does not yet manifest as a major destabilizing factor in the Black Sea ecosystem.

However, the absence of phytoplankton data limits a more comprehensive analysis of primary production and energy transfer, which are crucial for understanding ecosystem function. Future studies should incorporate phytoplankton dynamics and long-term monitoring to refine food web assessments and improve ecological models. The use of zooplankton as bioindicators provides a cost-effective method for tracking ecosystem changes, allowing for early detection of trophic imbalances. These findings contribute to the broader understanding of marine ecosystem functioning and highlight the need for adaptive management strategies to mitigate stressors such as pollution and habitat degradation.

A key limitation of this study is the temporal scope of the zooplankton data analyzed. The dataset used covers 6 years; however, sampling was conducted exclusively during the warm season (May–October). This temporal restriction was driven by the availability of consistent and comparable data across the study area and time frame. As a result, the findings presented here reflect only the zooplankton community structure and spatial distribution characteristic of the warm season.

Another potential limitation of this study is the variation in sample sizes across MRUs, which may influence the interpretation and comparability of findings. Although measures were taken to ensure consistency in data collection and analysis, future research should strive for more balanced sampling to strengthen the reliability of the results.

Beyond the Black Sea, this research offers insights applicable to other marine regions with similar ecological characteristics, such as semi-enclosed seas influenced by anthropogenic pressures. The methodologies employed can be adapted to monitor food web integrity in diverse marine environments, contributing to global conservation efforts. Additionally, the MSFD framework, while developed for EU waters, provides a structured approach that can be integrated into international marine management strategies to enhance biodiversity conservation and promote ecosystem-based management practices.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jmse13040713/s1>, Table S1. Number of mesozooplankton samples collected across MRUs from May to October, covering the years 2018 to 2023. Table S2. Number of macroplankton samples collected across MRUs from May to October, covering the years 2018 to 2023.

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Abbreviations

The following abbreviations are used in this manuscript:

MSFD	Marine Strategy Framework Directive
MRU	Marine Reporting Units
GES	Good Environmental Status
Non-GES	Not in Good Environmental Status
WoRMS	World Register of Marine Species
SIMPER	Similarity Percentage
ANOVA	Analysis of Variance
ANOSIM	Analysis of Similarity
GIS	Geographic Information System

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