Zooplankton in Montenegrin Adriatic Offshore Waters

Branka Pestorić, Dragana Drakulović, Davor Lučić, Nikola Đorđević, and Danijela Joksimović

Contents

Abstract This chapter includes a review of the recent results regarding zooplankton in offshore Montenegrin waters (since 2009) and a comparison of these results with earlier surveys. The highest abundance of total zooplankton was determined at station E, close to the river Bojana, in the summer period reaching $74,972$ ind m⁻³. A lower density of total zooplankton was observed during the winter. The abundance of total zooplankton increased toward the southern part of Montenegrin waters and from offshore to coastal stations. Contrary to the abundance, biodiversity grew from the coastal to the offshore sites as well as from the southern to the northern part of Montenegrin waters. Thirteen groups and 126 mesozooplankton taxa were determined. Analysis of the 12-month monitoring revealed that copepods were the most dominant group with an average contribution of 79% and a maximum share of 99%. Copepods were the most diverse group with 44 determined taxa. The most dominant taxa were Onceaidae-like species with 17% share, followed by: Oithona nana

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B. Pestorić (\boxtimes), D. Drakulović, N. Đorđević, and D. Joksimović

Institute of Marine Biology, University of Montenegro, Kotor, Montenegro e-mail: [brankap@ucg.ac.me;](mailto:brankap@ucg.ac.me) [dragana.dr@ucg.ac.me;](mailto:dragana.dr@ucg.ac.me) [nikoladj@ucg.ac.me;](mailto:nikoladj@ucg.ac.me) danijela.j@ucg.ac.me

D. Lučić

Institute for Marine and Coastal Research, University of Dubrovnik, Dubrovnik, Croatia e-mail: davor.lucic@unidu.hr

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(14%), Acartia (Acartiura) clausi and Euterpina acutifrons (10%), and Paracalanus parvus parvus (8.5%). Cladoceran species dominated during the summer period, but the average contribution was 10%. Noctiluca scintillans reached a maximum value of 6,417 ind m^{-3} in August 2009 at A3 station. Seventeen taxa of hydromedusae and 11 species of siphonophorae were collected during the survey period. Chelophyes appendiculata was noted for the first time in Montenegrin waters. High abundances of meroplankton larvae were recorded in this area throughout the investigation period, representing 2.7% of the total mesozooplankton. Bivalve larvae generally constituted the majority of this population.

Biodiversity indexes showed statistically significant difference by seasons and decreasing values from transect A to transect E but no statistical difference was found. Comparison of medians of coastal with offshore stations showed statistically significant difference for the Margalef's index.

The data presented here nevertheless suggest where limited sampling resources should be used to describe more confidently the functional role of the mesozooplankton community in the Montenegrin Adriatic offshore waters.

Keywords Net zooplankton, Open sea, South Adriatic, Spatial and temporal variability

1 Introduction

The distribution of plankton biomass and species abundance in relation to environmental conditions, i.e., physical, geochemical, and biological processes is an important aspect of the structure and function of marine plankton communities [[1](#page-18-1)–[3](#page-18-2)].

Zooplankton has been extremely well studied in marine ecosystems; its potential value as an indicator of changes in the marine environmental status still needs to be assessed [\[4](#page-18-3)]. Higher trophic levels in the marine plankton usually receive less attention in environmental monitoring [[5,](#page-18-4) [6\]](#page-19-0), although changes in the abundance, distribution, and succession of zooplankton organisms are indicative of changes in the environmental conditions [[7,](#page-19-1) [8](#page-19-2)].

High temperatures, the low primary production, and low chlorophyll a in many oligotrophic environments favor heterotrophic plankton communities [[9\]](#page-19-3). One such region, the phosphate-limited eastern Mediterranean Sea [[10\]](#page-19-4), has the predominant influence on the open waters of the southern Adriatic [\[11](#page-19-5)].

The South Adriatic (SA) is characterized by seasonal variability of upper-layer physical, chemical, and biological properties [\[12](#page-19-6)]. The current along the Eastern Adriatic coast comes from the central Mediterranean Sea (Ionian Sea), one of the most oligotrophic areas in the world [[13](#page-19-7)], and it transports the Ionian Surface Water along the eastern boundary northward into the Adriatic Sea [\[12](#page-19-6), [14\]](#page-19-8). In the Southeastern Adriatic Sea, the Buna/Bojana river with the largest single discharge (about 700 m³ s⁻¹) [\[14](#page-19-8)] has a significant influence on the plankton community in that area.

The water exchange with the Ionian Sea and the occurrence of strong winter vertical mixing of the water column have also large impacts on the phytoplankton biomass and hence on the primary production in the Adriatic Sea [[14](#page-19-8)–[16\]](#page-19-9).

Different methods are described to classify the trophic states of the Mediterranean Sea [\[17](#page-19-10), [18\]](#page-19-11) with satellite or laboratory measurements of chlorophyll a concentration. Based on these classifications, the obtained chlorophyll a concentration $> 1 \mu g L^{-1}$ indicates the zone of eutrophic character. According to these classifications, there are three eutrophic regions in the Adriatic Sea. One of these is in the Southeastern Adriatic Sea, along the coasts of Montenegro and Albania [\[14](#page-19-8)].

Detailed studies of net zooplankton in the SA began in the middle of the last century with studies on their annual production cycles, horizontal and vertical distributions, and diel vertical migration patterns [[19](#page-19-12)–[28\]](#page-20-0), instead, provided information on the composition, numerical abundance, and vertical structure of micro and mesozooplankton across the coastal and offshore waters of Albania. Interannual variation of zooplankton and zooplankton community structure during winter convection in the deep SA were described by [[29,](#page-20-1) [30\]](#page-20-2). Zooplankton blooms in open South Adriatic were described by [\[2](#page-18-5)]. Finally, the winter community structure of the mesozooplankton related to water-masses in the eastern SA was described by [\[31](#page-20-3)]. While the Boka Kotorska Bay was much more explored [[32](#page-20-4)–[36\]](#page-20-5), few studies were published on the Montenegrin waters: about cladoceran distribution [[37\]](#page-20-6), NiS in Adriatic ports encompassing the Port of Bar [\[38\]](#page-20-7) and plankton communities [\[39](#page-20-8)]. However, a detailed review focused on the zooplankton community composition in the Montenegrin waters is lacking.

The objective of this chapter is to review the main results of all previous studies of zooplankton in open sea sites of Montenegrin waters with particular emphasis on the results of more recent research activities. In addition, we intend to present some unpublished results related to changes in the zooplankton composition and abundance that have been recorded in the past few years in Montenegrin waters, and which could correlate with global warming phenomena.

2 Material and Methods

Mesozooplankton samples were collected in the time frame of different projects and time periods. All samples were taken at open sea locations in period 2009–2010 and 2018–2019. In the first sampling period (National monitoring 2009–2010) six sites were sampled (A2, A3, B, C, D, and E) from April to November 2009 (Fig. [1](#page-3-1)). Sites D and E were sampled in December 2009 and January and March 2010 additionally. In the period from July 2018 to April 2019 (National monitoring) mesozooplankton samples were collected at five following stations: A1, B, C, D, and E. In addition to the above sampling, in October 2019, a detailed sampling of offshore Montenegrin waters was performed at 17 sites arranged in five transects (a–e) (GEF Adriatic project) (Fig. [1\)](#page-3-1).

Zooplankton samples were taken by vertical hauls from the bottom to the surface with a Nansen plankton net, 0.55 m diameter and 125 μm mesh size. An exception

Fig. 1 Map of sampling at Montenegrin offshore stations. National monitoring (2009–2010): A2, A3, B, C D, and E; National monitoring 2018–2019: A1, B, C D, and E. One-time sampling: GEF Adriatic project (transects a–e, 17 stations)

was the sampling in October 2019, when samples were collected in two layers of the water column, upper and lower, and the boundary between the layers was determined based on the thermocline of the station. The collected zooplankton material was preserved in 2.5% formaldehyde seawater solution and analyzed using a Nikon SMZ800 stereomicroscope (Table [1](#page-4-0)).

Detailed methodology of sampling and counting of mesozooplankton samples are described by [\[28](#page-20-0), [40](#page-20-9)–[43\]](#page-20-10).

Data were contoured with graphical programs Grapher 7 (Golden Software) and Statistica 7 for Windows. Diversity was estimated, on species or genus level, calculating Margalef's species index (d) and Shannon-Wiener's diversity index (H') for each sample using PRIMER 6 for Windows software $[44]$ $[44]$.

3 Temporal and Spatial Distribution of Total Zooplankton Abundance

The spatial and seasonal distribution of total zooplankton abundances for national monitoring (2009–2010 and 2018–2019) is shown in Fig. [2.](#page-4-1) The highest range of total zooplankton abundances was determined at site E (652–74,972 ind m^{-3}) while

Table 1 Working depths of sampling sites

Fig. 2 Spatial (a) and seasonal (b) variability of total zooplankton abundance at the offshore sites of Montenegrin coast in period 2009–2010 and 2018–2019

the lowest ranges were found at A2 site $(261-3,703 \text{ ind m}^{-3})$. Median values did not differ significantly among the sites (Kruskal-Wallis test, $H = 8.3512$, $p > 0.05$), but according to total zooplankton abundance, there were significant differences

Fig. 3 Temporal variability of total zooplankton abundance at offshore sites in the Northern part of the studied area: A1, A2, and A3

between locations A and E (Mann Whitney, $p < 0.05$) and B and B and E (Mann Whitney, $p < 0.05$). A lower density of total zooplankton was observed during the winter. Increased values were recorded in the warmer part of the sampling period with maximum value in the summer. Analysis of medians showed significant differences ($H = 20.8729$, $p < 0.001$) related to seasons.

At the northern stations, A2 and A3 during the sampling period (April–November 2009) total zooplankton abundance ranged \lt 10,000 ind m⁻³ except in June 2009 at A3 site where determined total zooplankton abundance was 25,964 ind m^{-3} (Fig. [3\)](#page-5-0). Such a large abundance of total zooplankton was a consequence of the presence of copepod species Acartia (Acartiura) clausi in high number (>20,000 ind m⁻³). Acartia (Acartiura) clausi is a medium-sized copepod high ranking in spatial and temporal scales in the Adriatic Sea [\[4](#page-18-3)]. This species, classified as omnivore [[45\]](#page-20-12), represents an important heterotrophic pray in the nutrition of dominant copepods in

the coastal area $[46]$ $[46]$. In areas of high organic production, it participates with $60-85\%$ in total zooplankton [[47,](#page-20-14) [48](#page-20-15)]. In the same area, site A1 was sampled in the period from August 2018 to April 2019 but total abundance did not exceed 4,000 ind m^{-3} (Fig. [3\)](#page-5-0).

Analyzing the abundance of total zooplankton at site B, an extremely high number was noticeable in June 2009. The maximum value reached $25,171$ ind m^{-3} (Fig. [4](#page-7-0)). When compared with sampling in the period 2018–2019, such a high abundance was not noticed. Total zooplankton abundance ranged $214-4,404$ ind m⁻³ in this period at the site B.

High variability of total zooplankton abundance was noticed in both monitoring periods at site B. The highest abundance of 24,716 ind m^{-3} was reached in June 2009 and another peak was noted in September 2009 (14,413 ind m^{-3}). During the sampling period 2018–2019 abundance of total zooplankton ranged 375–8,841 ind m^{-3} .

The Southern part of the studied area showed the highest abundance of total zooplankton during the studied period. Two sites were sampled: D and E. The maximum value of total zooplankton abundance noted at E site in September 2009 reached 74,972 ind m^{-3} (Fig. [5\)](#page-8-0). At this station, another peak was noted in August 2018. Such a high abundance is a consequence of the presence of cladoceran species Penilia avirostris in high numbers. This species was present at D site causing maximum value in September 2009 too. Site D has a different biodiversity picture and the highest abundance was noted in June 2009 (Fig. [5](#page-8-0)).

Comparison of the abundance of total zooplankton during two sampling periods (2009–2010 and 2018–2019) showed statistically significant difference (Kruskal-Wallis, $H = 32.9805$, $p < 0.0001$). Significantly higher abundances were noted during the first sampling period at all sites (Fig. [6](#page-9-0)).

Analyzing the 12-month monitoring, copepods were the most dominant group with an average contribution of 79%. Its maximum contribution was 99% in June 2009 at site A3. Cladocerans were the second group in terms of abundance with an average contribution of 10%. The maximum contribution of this group was noticed at site E in September 2009 reaching 79% in total zooplankton abundance. Meroplankton organisms were present with an average contribution of 4% while the maximum was noticed in April 2009 at site D.

Based on a one-time study of net zooplankton by transect in Montenegrin offshore waters, a statistically significant difference in the total abundance was determined (Fig. [7\)](#page-9-1) (Kruskal-Wallis, $H = 13,8,553$, $p < 0.01$). The abundance of total zooplankton increased in the direction from transect "a" to transect "e".

In contrast to transects, observing coastal and the offshore sites, no significant statistical difference was found, although the values of total zooplankton were higher at coastal sites.

The highest abundance was recorded on transect "d", at the shallowest location above the thermocline, and was $4,820$ ind m⁻³ (Fig. [8\)](#page-10-1). Sites 4, 7, 11, and 15 are of a typically coastal character, and due to the small depth and the absence of a precisely defined thermocline; samples were taken in just one stretch of 2 m above the seabed to the surface.

Fig. 4 Temporal variability of total zooplankton abundance at offshore sites in the middle part of the investigated area: B and C

Copepods dominated zooplankton assemblages and generally represent the most numerous zooplankton organisms. Their share in the total number ranged from 56% to 93%. Following the copepods, the most numerous organisms group were cladocera species, especially on transects "d" and "c" above the thermocline

Fig. 5 Temporal variability of total zooplankton abundance at offshore sites in the Southern part of the studied area: D and E

(Fig. [8\)](#page-10-1). The share of juvenile stages of copepods (kalanoid and cyclopoid copepods) in the total number of copepods ranged from 36% to 68%. Also, in the total zooplankton, numerous were taxa of Onceaidae found in all samples as well as species of the genus *Calocalanus* sp. with an incidence rate of 97%, then *Oithona* similis 93%, and Coryceus sp. 86%. This taxa structure was observed on all transects except transect "d", where the most dominant species was cladocera Penilia

Fig. 6 Average contribution (%) of the most abundant groups in total zooplankton abundance by months

Fig. 7 Box plot diagram of the variability of total zooplankton by transects (October 2019)

avirostris (simper analysis). Penilia avirostris was found predominantly at sites of transects "d" and "e" with a maximum value of 921 ind $m⁻³$ at a typically coastal site 11 belonging to the "d" transect.

Fig. 8 Spatial variability of total zooplankton abundance at offshore stations

4 Diversity of Zooplankton Taxa

Owing to its geographical position, Montenegrin offshore area exhibits great species richness, similar to the richer areas in the Western and Eastern Mediterranean Sea [\[49](#page-21-0)–[51](#page-21-1)]. In total, we found 126 mesozooplankton taxa: 118 were recorded during National monitoring 2009–2010, and then 106 in National monitoring 2018–2019, and 71 in the frame of GEF Adriatic cruise. These data are in accordance with results obtained from coastal open sea site (up to 150 m) of South Adriatic [[31\]](#page-20-3).

Thirteen groups were determined: Protozoa, Hydromedusae, Siphonophorae, Ostracoda, Cladocera, Copepoda, Hyperidea, Pteropoda, Appendicularia, Chaetognatha, Mysidacea, Thaliacea, and Meroplankton.

Noctiluca scintillans reached a maximum value of 6,417 ind m^{-3} during the National monitoring in August 2009 at A3 site. Compared to the results obtained in the South Adriatic [[16\]](#page-19-9), the data noticed at A3 exceed the usual values that are considered high for the open sea. The high abundance of Noctiluca scintillans indicates that Open South Adriatic is not oligotrophic at certain times of the year. But, comparing with the data of the North Adriatic [\[52](#page-21-2)] the results obtained in Trašte are in accordance with those. Therefore, Trašte, as a semi-enclosed bay, with high coastal influence and trophic characteristics is more similar to the North Adriatic Sea and the Boka Kotorska Bay.

Seventeen taxa of hydromedusae and eleven species of siphonophorae were collected during the studied period. The most frequent species of hydromedusae was Aglaura hemistoma (37%) while the most abundant hydromedusae were *Podocorynoides minima* (68 ind m^{-3}). At the Central and the South Adriatic 28 species were found [[53\]](#page-21-3) during only one season (spring). This difference in species number can be explained with a limited sampling depth (the deepest site was 225 m). Medusae are important predators in marine ecosystems, so they have a very important role in its functioning. Long-term research [\[54](#page-21-4)–[56](#page-21-5)] has shown that there is an increase in abundance that can be related to climate change and its influence on plankton structure.

Among siphonophorae, *Lensia subtilis* was the most abundant species with a noticed maximum value of 68 ind m^{-3} . *Chelophyes appendiculata* was noted for the first time in the Montenegrin waters in the time frame of GEF Adriatic project reaching a value of 6.4 ind m^{-3} .

Six of eight known cladoceran species were found. The most abundant and the most frequent was Penilia avirostris. Its abundance was higher during the warmer period. It will be possible to consider these species as an ecological indicator of environment assessment because these species react with growth dynamics to environmental changes.

Copepods were the most diverse group with 44 determined taxa. The most dominant taxa were Onceaidae-like species with 17% share followed by: Oithona nana (14%), Acartia (Acartiura) clausi and Euterpina acutifrons (10%), and Paracalanus parvus parvus (8.5%) in total zooplankton. Copepods are the major component of the overall plankton in the South Adriatic [\[3](#page-18-2)]. Their share in total net zooplankton >90% was noted in the open waters of Albania too [[28\]](#page-20-0). Their contribution to total zooplankton can be reduced due to the high proportion of cladoceran species. Medium and small-sized species were more dominant. Their contribution to the total community is significant, especially in the oligotrophic seas [[57\]](#page-21-6). The annual cycle of copepod densities usually peaks in the spring [\[3](#page-18-2), [58,](#page-21-7) [59\]](#page-21-8).

Among six species of pteropods, *Creseis virgula* was the most abundant with the most frequent appearance with a maximum abundance of 410 ind m^{-3} . Oikopleura logicauda was the most dominant species in group of Appendicularia. The maximum value noticed reached 1,229 ind m^{-3} .

Chaetognaths were recorded throughout the studied period and were dominated by Flacissagitta enflata.

Fig. 9 Box plot diagram of Biodiversity indexes of National monitoring by seasons

High abundances of meroplankton larvae were recorded in this area throughout the studied period, representing 2.7% of the total mesozooplankton. Bivalve larvae generally constituted the majority of this population.

Other groups: Thaliacea, Ostracoda, Hyperiidae, and Mysidae were present with less than 0.1% share.

The Margalef's and Shannon-Wiener's indexes showed statistically significant differences by seasons ($p > 0.001$) in the analysis of the national monitoring data (Fig. [9\)](#page-12-0). The maximum value was recorded at A2 site in October 2009 reaching 6.708 for the Margalef's index and E site (2.816) in February 2019 for the Shannon-Wiener's index. The medians show that the biodiversity index values are generally higher during the colder period (autumn, winter) especially for the Shannon-Wiener's index (Fig. [8](#page-10-1)). In the spring, the most dominant species was Acartia (Acartiura) clausi with a 56% share in total zooplankton abundance. The share of Oithona nana in total zooplankton was 18%. During summer, Oithona nana (32%) was followed with typical summer species Penilia avirostris (29%) and Acartia (Acartiura) clausi (10%). In the autumn, six species contributed to 70% of total zooplankton abundance: Oithina nana, Onceaidae, Euterpina acutifrons, Paracalanus parvus parvus, Penilia avirostris, and Temora stilifera while simper analysis showed that dominant species in the winter were Onceaidae-like species (33%), Eurepina acutifrons (22%), Paracalanus parvus parvus (9%), and Coryceus spp (8%).

The analysis of the diversity index of sites sampled in the time frame of GEF Adriatic project by transects showed that the values decreased from transect A to transect E; however, no statistical difference was found. The maximum value of the Margalef's index of 5.45 and 5.46 was determined at sites 2 and 3, which are also the deepest sites in this area of research (115 and 225 m), while the lowest value of the index was recorded at site 15 and was 2.8. The Shannon-Wiener's index showed the same distribution, reaching a maximum value at transect A, site 1 (2.704). But, comparison of medians of coastal with offshore site, showed statistically significant difference for the Margalef's index and high difference, but not statistically significant, for the Shannon-Wiener's index (Fig. [10](#page-13-0)).

Simper analysis showed that in the coastal area the most abundant species was *Penilia avirostris* (avg abund. 305 ind m^{-3}) with 20% share. This cladocera was followed by Euterpina acutifrons (19%), Onceaidae (18%), Calocalanus sp (9%), and Paracalanus parvus parvus (5%).

At offshore sites, the most abundant taxa were *Onceaidae*-like species with an average abundance of 111 ind m^{-3} and a share of 30% in total zooplankton. Following these taxa, the most abundant were: Calocalanus sp (12%) and Penilia avirsotris (10%) taxa.

The highest difference between the two sampling groups was the consequence of abundance and distribution of taxa showed in Table [2](#page-13-1). The average dissimilarity

Fig. 10 Box plot diagram of Biodiversity indexes of GEF Adriatic project for coastal (1, 4, 7, 11, and 15) and offshore (2, 3, 5, 6, 8, 9, 10, 12, 13, 14, 16, 17) sites

Avg diss $= 66.28$	Coastal	Offshore				
	Av.	Av.	Av.	Diss/	Contrib	Cum.
Taxa	Abund	Abund	Diss	SD	$\%$	$\%$
Euterpina acutifrons	307.57	40.79	12.98	1.09	19.58	19.58
Penilia avirostris	305.04	141.26	12.47	1.44	18.82	38.4
Onceaidae	232.4	110.81	8.91	1.18	13.44	51.84
Calocalanus sp.	77.84	59.32	3.13	1.3	4.73	56.57
Corycaeus spp.	63.32	42.04	2.52	1.33	3.8	60.37
Clausocalanus furcatus	57.6	17.44	2.36	0.98	3.56	63.92
Paracalanus parvus	56.1	18.52	2.28	1.47	3.44	67.36
parvus						
Oithona nana	37.64	5.93	2.23	0.79	3.37	70.73
Oikopleura longicauda	45.77	18.97	2.01	0.75	3.04	73.76
Temora stylifera	27.00	21.53	1.71	0.83	2.59	76.35

Table 2 Simper analysis of dissimilarity among two sampling groups: Coastal and Offshore

among stations based on individual zooplankton species abundances computed through the SIMPER procedure for coastal and offshore sites was 66.28%. Further breaking down the average values into separate contributions from each species showed that 10 zooplankton taxa combined accounted for 76% of the total zooplankton abundance at all sampled sites.

5 Conclusion

The present study shows the first results of mesozooplankton community composition and abundance in the Montenegrin Adriatic offshore waters. High richness of species was noted. As in the other regions of the Mediterranean Sea, copepods were the major component of the overall plankton. The highest densities were found in the region with the high influence of freshwater. We can hypothesize that nutrient enrichment in this zone and the consequent phytoplankton development created conditions for the increased zooplankton abundance. At other sites, estimated abundances were similar to those reported for the epipelagic zone of other oligotrophic areas in the Mediterranean Sea. The mesozooplankton abundance distribution pattern also showed a classical decreasing trend from near coastal areas to deeper sites.

Copepods were the most dominant group in total zooplankton with 44 determined taxa. Cladoceran species dominated during the summer period, but their average share was 10%. Seventeen taxa of hydromedusae and eleven species of siphonophorae were collected during the sampling period. Chelophyes appendiculata was noted for the first time in Montenegrin waters. Biodiversity indexes showed statistically significant difference by seasons and decreasing values from transect A to transect E but no statistical difference was found. Comparison of medians of coastal with offshore sites showed statistically significant difference for Margalef's index.

The data presented here nevertheless suggest where limited sampling resources should be deployed to describe more confidently the functional role of the mesozooplankton community in the Montenegrin Adriatic offshore waters. Anyway, the coastal economy and social structure require additional attention aimed at a better knowledge of the total production of this area.

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Appendix

List of taxa determined in frame of National monitoring and GEF Adriatic project (avg ab = average abundance; freq% = frequency of appearance $(\%)$

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