

## Pelagic cnidarians in the Boka Kotorska Bay, Montenegro (South Adriatic)

Branka PESTORIĆ\*, Jasmina KRPO-ĆETKOVIĆ<sup>2</sup>, Barbara GANGAI<sup>3</sup>  
and Davor LUČIĆ<sup>3</sup>

<sup>1</sup>*Institute of Marine Biology, P.O. Box 69, Dobrota bb, 85330 Kotor, Montenegro*

<sup>2</sup>*Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

<sup>3</sup>*Institute for Marine and Coastal Research, University of Dubrovnik,  
Kneza Damjana Jude 12, 20000 Dubrovnik, Croatia*

*\*Corresponding author: brankapestoric@t-com.me*

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*Planktonic cnidarians were investigated at six stations in the Boka Kotorska Bay from March 2009 to June 2010 by vertical hauls of plankton net from bottom to surface. In total, 12 species of hydromedusae and six species of siphonophores were found. With the exception of the instant blooms of *Obelia* spp. (341 ind. m<sup>-3</sup> in December), hydromedusae were generally less frequent and abundant: their average and median values rarely exceed 1 ind. m<sup>-3</sup>. On the contrary, siphonophores were both frequent and abundant. The most numerous were *Muggiaea kochi*, *Muggiaea atlantica*, and *Sphaeronectes gracilis*. Their total number was highest during the spring-summer period with a maximum of 38 ind. m<sup>-3</sup> observed in May 2009 and April 2010. *M. atlantica* dominated in the more eutrophicated inner area, while *M. kochi* was more numerous in the outer area, highly influenced by open sea waters. This study confirms a shift of dominant species within the coastal calyphores in the Adriatic Sea observed from 1996: autochthonous *M. kochi* is progressively being replaced by allochthonous *M. atlantica* in the coastal waters, especially in the eutrophicated areas. This study provides a detailed report on the composition and abundance of the planktonic cnidarians community in this region, and should be considered as a baseline for future studies on gelatinous zooplankton.*

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**Key words:** hydromedusae, siphonophore, gelatinous zooplankton, Mediterranean Sea

### INTRODUCTION

Planktonic cnidarians are conspicuous components of pelagic food webs that have gained attention in the last decade due to the enhanced frequency of massive proliferations events. Understanding of the ecological importance

of such pulses is of critical importance for the assessment of food web dynamics in the pelagic realm (CIESM, 2001; BOERO *et al.*, 2008). The current debate on the causes of such phenomena emphasize the role of synergistic effects of over-fishing, eutrophication, climate changes, translocation, and habitat modification as potential

underlying mechanisms triggering favourable conditions for jellyfish populations (MILLS, 2001; PURCELL, 2005; HAY, 2006; MOLINERO *et al.*, 2008; RICHARDSON *et al.*, 2009).

In the Adriatic Sea, research on planktonic cnidarians has been carried out for nearly 200 years (LUČIĆ *et al.*, 2009b; GAMULIN & KRŠINIĆ, 2000; KOGOVIŠEK *et al.*, 2010). Such long-term records, although not continuous, have shown that there is an increase in abundance of some hydrozoan medusa species in the last decade (BENOVIĆ *et al.*, 2005; LUČIĆ *et al.*, 2009a), which is probably associated with the increase of the average temperature and its subsequent influence on the plankton structure, although other factors, such as intensified sampling efforts should not be excluded (LUČIĆ *et al.*, 2009a).

The Boka Kotorska Bay is a eutrophic shallow embayment in the southern Adriatic Sea, where recurrent phytoplankton blooms have been documented (VILIČIĆ 1989; VUKSANOVIĆ 2003). In spring of 2009, LUČIĆ *et al.* (2012) noticed an intensive bloom of the ctenophore *Bolinopsis vitrea* in the inner area of the bay, which greatly impacted pelagic copepods and subsequently reduced grazing pressure on the phytoplankton. The result of such top-down forcing was an uncommon phytoplankton bloom (LUČIĆ *et al.*, 2012). Herewith, we present the observed cnidarian community structure during 2009 and 2010. These two years were characterized by a contrasting configuration shaped by the massive

proliferation of *B. vitrea* in 2009 and absence of *B. vitrea* bloom in 2010. Covering a two-year survey, this study aims to: 1) describe changes in planktonic cnidarian species composition and abundance; 2) describe their annual assemblages and seasonality, 3) understand the influence of environmental variables on structuring these assemblages.

## MATERIAL AND METHODS

### Study area

The Boka Kotorska Bay system is often referred to as a fjord, due to its dramatically steep mountain walls, although it is actually a submerged river canyon. Owing to a large amount of winter precipitation over its karstic drainage basin, it is greatly influenced by the large influx of freshwater from streams and submarine springs. Water exchange with other arms of the Boka Kotorska system and the open Adriatic Sea represent incoming currents near the bottom and outgoing currents on the surface. The whole system consists of four sub-bays: two inner bays, the Bay of Kotor, which is highly eutrophicated (VILIČIĆ, 1989), and the Bay of Risan, the mid situated Bay of Tivat, and the outermost Bay of Herceg Novi that directly communicates with the open sea waters.



Fig. 1. (a) Position of the Boka Kotorska Bay in the Mediterranean Sea. (b) Map of the Boka Kotorska Bay with sampling locations (A – Bay of Kotor; B – Bay of Tivat; C – Bay of Herceg Novi)

### Sample collection and data analysis

Zooplankton samples were collected at six stations in the Boka Kotorska Bay from March 2009 to June 2010. The stations A1, A2, and B4 are shallow near-shore stations, while A3, B5, and C6 represent central positions of the bays of Kotor, Tivat, and Herceg Novi, respectively. The position of sampling stations and corresponding geographical coordinates and depths are shown in Table 1 and Figure 1.

Table 1. List of sampling stations with corresponding latitude, longitude and maximum sampling depth.

Station	Latitude	Longitude	Depth (m)
A1	42°26.2'N	18°45.6E	15
A2	42°29.2'N	18°45.7E	15
A3	42°28.5'N	18°44.5E	30
B4	42°27.5'N	18°40.5E	15
B5	42°25.9'N	18°39.5E	30
C6	42°26.3'N	18°32.7E	40

Vertical profiles of temperature and salinity were obtained *in situ* by a HQ40d Multi-Parameter Digital Meter at 0 m, 5 m, 10 m, and 15 m at shallow stations, and at 10 m intervals at central positions in the sub-bays.

Water samples (1 L) for chlorophyll *a* measurement were pre-filtered through a 330 µm mesh net to remove large zooplankton. After filtration through a Whatman GF/F, pigment extraction was performed in 90% acetone, and chlorophyll *a* concentrations were determined by measurement of absorbance with a Perkin-Elmer UV/VIS spectrophotometer, and calculated according to JEFFREY *et al.* (1997).

Zooplankton sampling and measuring of temperature, salinity, and chlorophyll *a* concentration were carried out at different intervals. Station A1 was observed every eighth day (mean) over the period March 2009 – June 2010. Sampling and measuring at stations A2, A3, B4, and B5 were done twice a month, while sampling and measuring at station C6 were done monthly from April 2009 to June 2010.

Zooplankton samples were taken by vertical hauls from bottom to surface with a Nansen

plankton net, 0.55 m diameter and 125 µm mesh size. In all, 162 samples were examined.

The collected zooplankton material was preserved in 2.5 % formaldehyde seawater solution. All cnidarians and mesozooplankton identifications were performed using a Nikon SMZ1000/SMZ800 stereomicroscope. Each sample was sub-sampled in laboratory, depending on the abundance of individuals in the total sample. Zooplankton was counted from the representative sample of 1/64 of the total catch. After that, the entire material was carefully analysed in order to record any rare species. Siphonophoran abundance was expressed as the number of nectophores (polygastric only).

The Mann-Whitney *U* test (ZAR, 1974) was used to compare the differences in medusa and siphonophore abundance observed at the different areas of the Boka Kotorska Bay. A linear regression model was used to test correlations between the species abundance and sea temperature. The Spearman's rank-order correlation coefficient was used to compare densities of frequently observed and abundant siphonophore species to densities of small copepods and copepodites as their potential prey. The linear regression and Spearman's rank order correlation were performed with the STATISTICA 7 Software.

For the systematic classification of cnidarian species we used: LAND van der *et al.* (2001); BOUILLON *et al.* (2004); SCHUCHERT (2007) and NAWROCKI *et al.* (2010).

## RESULTS

### Environmental conditions

Water temperature and salinity showed a similar pattern during the sampling period, with lower values during the winter months. The highest temperature of 27.3°C was recorded in July 2010 at the surface of station A1. The minimum of 6.30°C was recorded in January 2010 at the surface of the same station (Table 2). A marked thermal stratification was noticed during the summer months. In October, sea surface temperature rapidly decreased and temperature

Table 2. Temperature, salinity and chlorophyll *a* concentration at six sampling stations in the Boka Kotorska Bay.

Position	Parameter	Min	Max	Mean	SD
		surf/bott	surf/bott		
A1	Temperature	6.3/13.4	28.9/21	16.70	4.05
	Salinity	2.36/34	31.8/38.5	28.09	10.93
	Chlorophyll <i>a</i>	0.09/0.56	9.30/4.53	1.88	1.80
A2	Temperature	10.5/12.9	26.4/23.4	17.67	4.54
	Salinity	1.00/32.1	32.3/37.3	27.22	12.02
	Chlorophyll <i>a</i>	0.19/0.44	6.82/4.14	1.44	1.33
A3	Temperature	7.2/12.6	26.7/20.9	17.25	3.78
	Salinity	3.9/35.8	33.8/37.6	31.86	8.75
	Chlorophyll <i>a</i>	0.22/0.22	5.46/1.84	1.26	1.10
B4	Temperature	10.8/12.8	26.2/22.7	18.27	4.14
	Salinity	8.86/34	36.4/37.3	32.49	6.52
	Chlorophyll <i>a</i>	0.10/0.04	4.90/1.70	1.06	0.82
B5	Temperature	10.7/13.1	26.5/21.9	17.47	3.60
	Salinity	11/36.1	35.7/38.9	34.71	5.04
	Chlorophyll <i>a</i>	0.22/0.21	3.78/1.32	0.87	0.60
C6	Temperature	11.4/13.2	25.7/22.8	17.87	4.02
	Salinity	14.26/36.3	37.7/39.3	35.71	4.36
	Chlorophyll <i>a</i>	0.10/0.04	3.53/1.09	0.78	0.68

inversion occurred in the Bay of Kotor during the winter months, while isothermal conditions were established in the bays of Tivat and Herceg Novi.

Salinity ranged from 1, at the surface of station A2 in February, to 39.3 at 40 m depth at station C6 in November. Surface salinity greatly varied during the investigated period, due to the influence of precipitation and freshwater discharge. However, salinity variations below the depth of 15 m were more stable, remaining higher or equal than 37.

The chlorophyll *a* concentration demonstrated a trophic gradient in the Boka Kotorska Bay. Mean values of chlorophyll *a* concentration were significantly higher in the Bay of Kotor (Kruskal-Wallis test,  $p < 0.001$ ) than in the other

bays. The maximum value of  $9.3 \text{ mg m}^{-3}$  was observed at station A1 in December 2009. High winter values were observed at other five stations as well, with maximum values in December at stations A2, B4, and B5.

### Hydromedusae

In total, 12 species of hydromedusae were found (Table 3). With the exception of instant blooms of certain species (Fig. 2), hydromedusae were generally not very abundant and their median values rarely exceed  $1 \text{ ind. m}^{-3}$  (Table 3; Fig. 2). In particular, small numbers were recorded in spring 2009, and compared with densities observed in the spring of 2010, they were significantly lower (Mann-Whitney *U* test,  $p < 0.001$ ).

Table 3. Composition of hydromedusa species in the Boka Kotorska Bay, with their maximum abundance values (max; ind. m<sup>-3</sup>), average abundances (av±SD; ind. m<sup>-3</sup>), mean percentage of the total hydromedusa abundance (mean %), and frequency of occurrence (f%)

Species	Bay of Kotor (A)				Bay of Tivat (B)				Bay of Herceg Novi (C)			
	max	av±SD	mean %	f %	max	av±SD	mean %	f %	max	av±SD	mean %	f %
<b>Anthomedusae</b>												
<i>Stauridiosarsia gemmifera</i> (Forbes, 1848)					1	0.05±0.18	0.27	9				
<i>Podocorynoides minima</i> (Trinci, 1903)	51	1.07±6.11	20.96	13	4	0.32±0.10	1.88	17	<1	0.06±0.122	2.20	21
<i>Hydractinia carica</i> Bergh, 1887	5	0.17±0.65	3.26	10	17	1.21±3.26	7.11	26	2	0.13±0.43	4.95	14
<b>Leptomedusae</b>												
<i>Obelia</i> spp. Péron & Lesueur, 1810	68	2.97±9.17	58.06	11	341	8.32±50.45	72.06	35	2	0.30±0.66	11.37	29
<i>Clytia</i> spp. Lamouroux, 1812	1	0.04±0.18	0.69	3	17	1.09±3.59	6.46	22	1	0.09±0.23	3.30	14
<i>Eirene viridula</i> (Péron & Lesueur, 1809)	2	0.06±0.30	1.23	6	4	0.19±0.67	1.12	20				
<i>Eutima gracilis</i> (Forbes & Goodsir, 1853)	1	0.02±0.12	0.31	2	17	0.66±2.79	3.92	20	<1	0.01±0.05	0.55	7
<i>Helgicirrha schulzei</i> Hartlaub, 1909	8	0.10±0.85	2.02	3	2	0.09±0.34	0.52	9	<1	0.01±0.05	0.55	7
<b>Trachymedusae</b>												
<i>Liriope tetraphylla</i> (Chamisso & Eysenhardt, 1821)	1	0.03±0.18	0.66	4	2	0.17±0.43	0.99	17	<1	0.01±0.05	0.55	7
<i>Aglaura hemistoma</i> Péron & Le Sueur, 1810	2	0.06±0.32	1.12	4	4	0.13±0.65	0.78	9	6	0.81±1.73	31.38	43
<i>Rhopalonema velatum</i> Gegenbaur, 1857					1	0.05±0.19	0.30	9	2	0.31±0.49	12.11	43
<b>Narcomedusae</b>												
<i>Solmaris</i> spp. Haeckel, 1879	34	0.76±4.08	14.90	8	17	0.84±3.11	4.85	9	6	0.80±1.83	30.83	29

Anthomedusae were represented with only three species: *Podocorynoides minima* and *Hydractinia carica* were frequently sampled, while *Stauridiosarsia gemmifera* was very rare, and found only in the Bay of Tivat (Table 3). This order of hydromedusae was the least abundant. Slightly higher values were found from May to August, with two extremes noted for *Podocorynoides minima* in August 2009 and June 2010, 52 ind. m<sup>-3</sup> and 34 ind. m<sup>-3</sup>, respectively.

In terms of both the number of individuals and the number of species, representatives of the order Leptomedusae dominated in the samples (Table 3). Very frequent were individuals of the genus *Obelia*, with an extreme maximum of 341 ind. m<sup>-3</sup> noted in the Bay of Tivat dur-

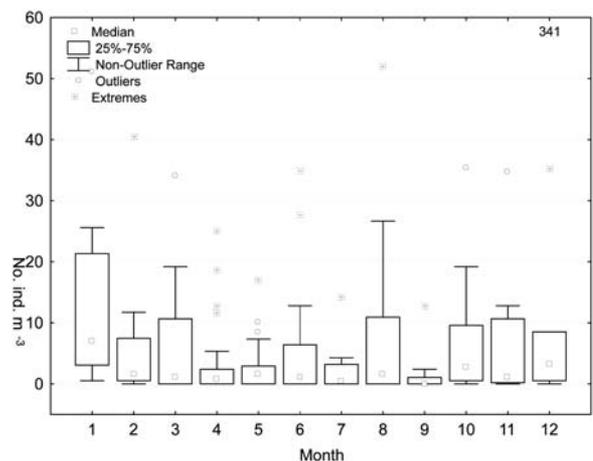


Fig. 2. Box plots of the numerical abundance of hydromedusae in the Boka Kotorska Bay



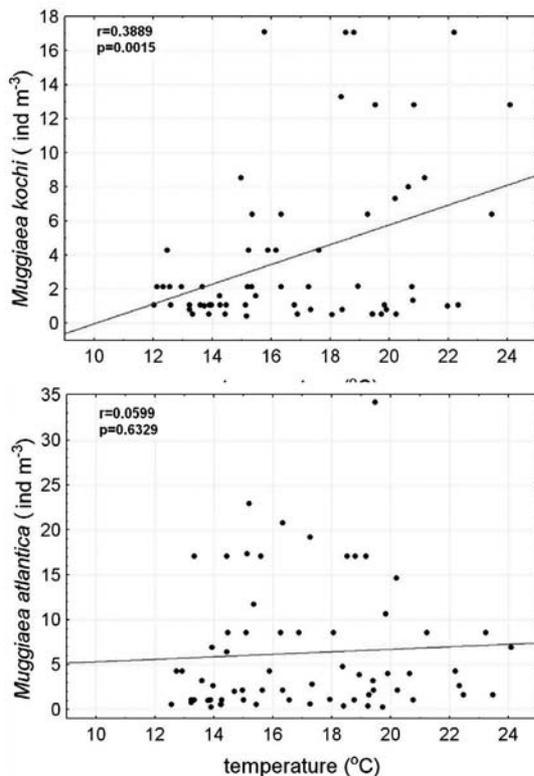


Fig. 4. Scatterplots and linear regression for average water temperature and abundance of siphonophore (a) *Muggiaea kochi* (b) *Muggiaea atlantica*

highest numbers were found in the inner Bay of Kotor, and in the winter season, only.

The linear regression of the abundance of *M. kochi* with water temperature revealed a strong positive relationship ( $r=0.388$ ;  $p=0.0015$ ), while for *M. atlantica* the correlation was not statistically significant ( $r=0.059$ ;  $p>0.05$ ) (Fig. 4).

The Spearman's rank order correlation was used to analyse the relationships of the common siphonophores and their potential prey. Significant positive correlations were found for *M. atlantica* and representative small copepods and

copepodites (Table 5) that numerically dominated at all stations (PESTORIĆ, unpublished data). *M. kochi* was positively correlated to abundance of calanoid copepodites, while no such correlations were observed for *Sphaeronectes gracilis*.

## DISCUSSION

A change in species composition and abundance of planktonic cnidarians, particularly meroplanktonic hydromedusae, could indicate long-term faunal or climatic changes (PURCELL, 2012). While outbreaks of medusae have been increasingly frequent, medusa species richness has declined in some regions (BENOVIĆ *et al.*, 1987, 2000; MILLS, 2001; PURCELL *et al.*, 2005; BOERO *et al.*, 2008). In view of that, we have found only 12 hydromedusa species (eight meroplanktonic), while VUKANIĆ (2006) recorded even less – 8 species (six meroplanktonic) in 84 samples taken along the entire Boka Kotorska Bay. These numbers are considerably smaller than historical findings for coastal central and southern Adriatic during 1939-1940, when BABNIK (1948) recorded 26 species. Although the abundance of hydrozoan species vary during their life cycles (BOERO *et al.*, 2008) and it is known that they can tolerate extreme environmental fluctuations, the ecological response in terms of the loss of these species may indicate irreversible ecosystem destruction processes (SANTHAKUMARI *et al.*, 1977; BENOVIĆ *et al.*, 2000). Hence, both our results and the findings of VUKANIĆ (2006) could indicate a decline in biodiversity of the Boka Kotorska Bay.

Meroplanktonic species of hydromedusae prevail in the inner parts of the bay, the Bay of Kotor and the Bay of Tivat, while holoplanktonic

Table 5. Spearman's rank-order correlation coefficients between frequently occurring and abundant siphonophore species and small copepods-copepodites in the Boka Kotorska Bay (\*  $<0.05$ ; \*\*  $<0.01$ ).

Potential prey	<i>M. kochi</i>	<i>M. atlantica</i>	<i>S. gracilis</i>
<i>Paracalanus parvus</i>	0.118275	<b>0.231866*</b>	-0.014638
Calanoida copepodites	<b>0.219230*</b>	<b>0.247684*</b>	-0.026341
<i>Oithona nana</i>	0.061862	<b>0.298886**</b>	-0.091944
Cyclopoida copepodites	0.171199	<b>0.327107**</b>	-0.112607

species were more abundant in the outer Bay of Herceg Novi, which has a direct communication with the open sea. This is in accordance with the established general pattern of hydromedusan distribution in the sea (GILI *et al.*, 1988).

In general, low abundance of hydromedusae was recorded, except for an extraordinary high number of *Obelia* spp. (341 ind. m<sup>-3</sup>) in the in Bay of Tivat during December 2009, which coincided with the high concentrations of chlorophyll *a*. The species of this genus are known as omnivorous, even able to concentrate bacteria in their gastric cavity (BOERO *et al.*, 2008). Such high abundance of *Obelia* species was not recorded before in the Adriatic coastal ecosystems. Only in the specific environment of the saline Mljet Island lake (South Adriatic), a high abundance (140 ind. m<sup>-3</sup>) of this species was recorded (BENOVIĆ *et al.*, 2000). Around the world, the abundance of this species rarely surpasses 5 ind. m<sup>-3</sup> (ALVARIÑO, 1968; VANNUCCI *et al.*, 1970; GOY, 1979; BOUILLON, 1995; PAGÈS & OREJAS, 1999; PALMA *et al.*, 2007). However, an unusual event of *Obelia* medusa bloom (maximum 1579 ind. m<sup>-3</sup>) was recorded in October 2003 along the Argentinean shoreline (GENZANO *et al.*, 2008). Sporadic blooms of certain meroplanktonic hydromedusae, as well as their low densities and small relative contribution to the total medusa abundance, could indicate the presence of environmental degradation in the area. Presently, it is accepted that increased populations of planktonic cnidarians may be a consequence of human activities in the coastal environment, which could lead to a top-down control of marine food webs by gelatinous predators (MILLS, 2001; BOERO *et al.*, 2008; PURCELL, 2012). Another evidence of such events is the bloom of the gelatinous species *Bolinopsis vitrea* (ctenophore) in the innermost part of the Boka Kotorska Bay (Bay of Kotor), which occurred in spring 2009 (LUČIĆ *et al.*, 2012). At the same time, particularly small numbers of hydromedusae were recorded, possibly as a consequence of competition for the same zooplankton prey with this voracious ctenophore species.

Three siphonophore species were present the whole year round in the Boka Kotorska Bay: *Muggiaea kochi*, *Muggiaea atlantica*, and

*Sphaeronectes gracilis*. We observed a significant correlation between water temperature and the abundance of *M. kochi*. This warm-water species prefers relatively high temperatures (ALVARIÑO, 1974). The complete life cycle of *M. kochi*, including the release of eudoxoids, occurs at temperatures between 18 and 24°C, while at a temperature of 13°C only the polygastric phase was observed (CARRÉ & CARRÉ, 1991). However, significant correlations between the abundance of *M. atlantica* and temperature was not observed during our investigations. On the other hand, BATISTIĆ *et al.* found that temperatures of about 14-18°C are optimal for the reproduction of this species. Since we analysed only the abundance of nectophores, this could explain the lack of significant correlation between water temperature and this siphonophore species in the present study.

Autochthonous Adriatic and Mediterranean species *M. kochi* was more numerous in the outer Bay of Herceg Novi. In the inner area, where zooplankton densities were higher in comparison with outer stations (PESTORIĆ, unpublished data), *M. atlantica*, a typical boreal Atlantic species, was more frequent and abundant, particularly during spring and summer. This species was present in low densities in the western Mediterranean prior to the 1980s (GAMULIN & KRŠINIĆ, 1993), and the expansion was recorded from the middle 1980s in the northwestern Mediterranean (GILI *et al.*, 1988). A shift of dominant species within the coastal calycophores, with *M. kochi* progressively being replaced by *M. atlantica*, was observed in the Adriatic Sea from 1996 (KRŠINIĆ & NJIRE, 2001; BATISTIĆ *et al.*, 2007). *M. atlantica* is known as an esurient predator, and its growth rate tends to increase with increasing density of its prey, crustacean developmental stages and small copepods (PURCELL, 1982). We observed a significant positive correlation between *M. atlantica* eudoxoides and their potential prey. These results may indicate a rapid reproductive response of this siphonophore associated with its potential prey densities.

Our study provides the first detailed report of the composition and abundance of the siphonophoran community for this region, and should

be considered as baseline for future studies on gelatinous zooplankton. Furthermore, we believe that future investigations of the hydromedusan pelagic and hydroid stages could supplement our hypothesis on decline in biodiversity of the Boka Kotorska Bay.

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## Planktonski žarnjaci Boka-Kotorskog zaljeva, Crna Gora (južni Jadran)

Branka PESTORIĆ\*, Jasmina KRPO-ĆETKOVIĆ<sup>2</sup>,  
Barbara GANGAI<sup>3</sup> i Davor LUČIĆ<sup>3</sup>

<sup>1</sup>Institut za biologiju mora, Dobrota bb, 85330 Kotor, Crna Gora

<sup>2</sup> Biološki fakultet, Sveučilište u Beogradu,  
Studentski trg 16, 11000 Beograd, Srbija

<sup>3</sup>Institut za more i priobalje, Sveučilište u Dubrovniku,  
Kneza Damjana Jude, 20000 Dubrovnik, Hrvatska

\*Kontakt adresa, e-mail: [brankapestoric@t-com.me](mailto:brankapestoric@t-com.me)

### SAŽETAK

Istraživanje planktonskih žarnjaka obavljeno je na šest postaja Boka-Kotorskog zaljeva od ožujka 2009. do lipnja 2010. Uzorci su sakupljeni vertikalnim potezima planktonske mreže od dna do površine. Ukupno je nađeno 12 vrsta hidromeduza i 6 vrsta sifonofora. S izuzetkom velikih nakupina vrste roda *Obelia* u prosincu 2009. (341 jed. m<sup>-3</sup>), prosječne vrijednosti hidromeduza rijetko su prelazile vrijednost od 1 jed. m<sup>-3</sup>. Za razliku od hidromeduza, sifonofore su bile češće i brojnije, osobito u proljeće i ljeto. Najbrojnije su bile *Muggiaea kochi*, *Muggiaea atlantica* i *Sphaeronectes gracilis*. Najveća gustoća od 38 jed. m<sup>-3</sup> utvrđena je u svibnju 2009 i ožujku 2010. godine. Vrsta *M. atlantica* je dominirala u eutroficiranom unutrašnjem dijelu zaljeva, dok je *M. kochi* bila brojnija u vanjskom dijelu zaljeva koji je pod jakim utjecajem otvorenog mora. Naša istraživanja su potvrdila dosadašnja saznanja za Jadransko more da u obalnim, osobito eutroficiranim područjima, novo pridošla sifonofora *M. atlantica* postaje dominantna u odnosu na autohtonu vrstu *M. kochi*. Ova istraživanja donose prve detaljne podatke o sastavu i brojnosti planktonskih žarnjaka Boka-Kotorskog zaljeva i predstavljaju osnovu za buduća istraživanja želatinoznog zooplanktona.

**Ključne riječi:** hidromeduze, sifonofore, želatinozni zooplankton, Sredozemno more