Chaetognaths in Boka Kotorska Bay

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ABSTRACT

Temporal and spatial distribution of pelagic chaetognaths was studied at six stations in Boka Kotorska Bay from March 2009 to June 2010. This work present for the first time detailed information of the ecology of cheatognaths in the specific ecosystem of Boka Kotorska Bay. Chaetognaths were more numerous in Kotor Bay and Parasagitta setosa *was dominant species at all stations. Salinity was the main hydrological factor which influence on chaetognath abundance. Small copepods and copepodites were the main food for the most abundant chaetognath species as for juvenile stages. Low population densities were noticed during ctenophore* Bolinopsis vitrea *bloom in 2009 indicating their important nutritional competition relationships.*

Key words: Chaetognaths, Boka Kotorska Bay,

INTRODUCTION

Chaetognaths have an important functional role in marine food webs, and within carnivorous zooplankton often dominated in their biomass (Reeve, 1970). As one of the main predators of copepods (Pearre, 1980; Stuart and Verheye, 1991), chaetognaths have an important role of transfer energy through marine food webs.

The first data for the Adriatic chaetognaths fauna was published by Graeffe (1905). During annual investigation of Gulf of Trieste, only *Sagitta* *setosa* was found present throughout the year. Detailed description of Adriatic chaetognaths fauna were given by Ritter-Zohony (1909) and Baldasseroni (1914) based on materials collected during “Pola” and “Ciclope” expeditions. The more compressive data for northern Adriatic chaetognaths distributions were presented by Scaccini & Ghirardelli (1941), Ghirardelli & Specchi (1965), Ghirardelli (1975). Gamulin (1979) and Gamulin & Ghirardelli (1983) presented perennial investigations of chaetognaths along eastern Adriatic coast. More detailed data about chaetognaths composition, abundance and behavior of open southern Adriatic waters were given by (Batistić *et al*., 2003; 2004; 2007).

Only one data about chaetognaths in Boka Kotorska Bay relate to their species description (Vukanić & Vukanić 2004), and the results were represented as a percentage contribution.

This work present for the first time detailed information of the ecology of cheatognaths in the specific ecosystem of Boka Kotorska Bay. The aim of this paper is to give information about their faunistic list, temporal variability and spatial distribution among Kotor, Tivat and Herceg Novi Bay. Furthermore, present study point out how physical parameters influence on abundance of chaetognaths, and how chaetognaths pressure on their potential prey.

MATERIAL AND METHODOLOGY

Study area

Boka Kotorska Bay, located in the southeastern Adriatic Sea, is a complex morphological structure, consisting of three regions: Kotor, Tivat and Herceg Novi Bay. Owing to the large amount of winter precipitation over its karstic drainage basin, Boka Kotorska is greatly influenced by the large influx of fresh water from streams and submarine springs. Moreover the bay can be considered one of the main freshwater inputs into the southern Europe area (Bellafiore *et al.*, 2011). Water exchange with open Adriatic depend primary on tidal variations and wind direction, with incoming currents near the bottom and outgoing currents on the surface.

The Boka Kotorska Bay is a moderately eutrophic shallow area and level of eutrofication increased toward the inner part and recurrent phytoplankton blooms have been documented in the Kotor Bay (Viličić 1989; Vuksanović 2003; Krivokapić *et al.*, 2011; Drakulović et al., 2012). Among zooplankton, small copepods and copepodites dominated in all area. 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).

Sampling

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.

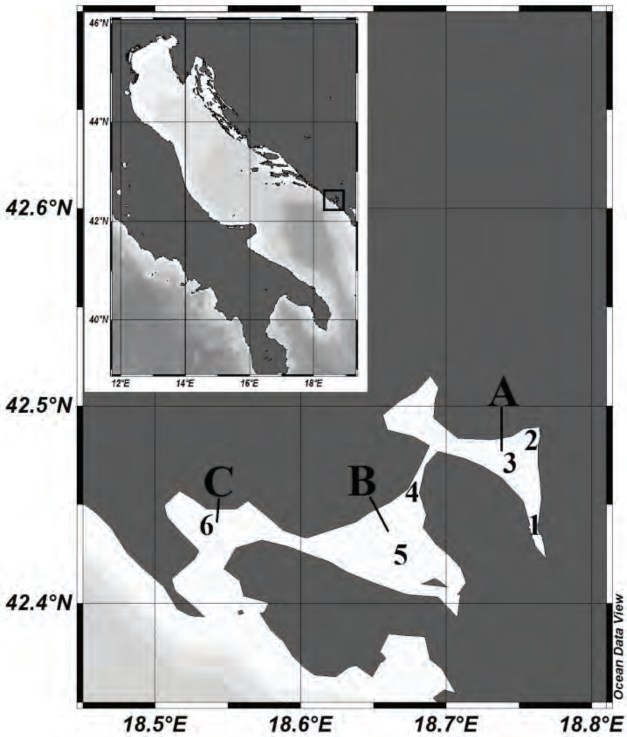


Figure 1. Map of sampling stations

Table 1. List of investigated stations with corresponding longitude and latitude as well as the working depth

Stations	Longitude N	Latitude E	Working depth (m)
A1	42º26,2'N	18º45,6'E	A 15
A2	42º29,2'N	18º45,7'E	A 15
A3	42º28,5'N	18º44,5'E	A 30
B4	42º27,5'N	18º40,5'E	B 15
B5	42º25,9'N	18º39,5'E	B 30
C6	42º26,3'N	18º32,7'E	C 40

Zooplankton sampling and measuring of temperature, salinity, and chlorophyll *a* concentration were carried out from spring 2009 to summer 2010 at different time intervals. Station A1 was observed every eighth day (mean) over the period from March 2009 to 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 (except February 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. Totally, 162 samples were examined. The collected zooplankton material was preserved in 4% formaldehyde seawater solution. All chaetognaths and mesozooplankton identifications were performed using a Nikon SMz1000/SMz800 stereomicroscope. Chaetognath nomenclature is consistent with that of Bieri (1991). Abundances of chaetognaths are expressed as number of organisms per cubic meter.

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).

The Spearman's rank-order correlation coefficient was used to compare densities of frequently observed and chaetognaths species to densities of small copepods and copepodites as their potential prey.

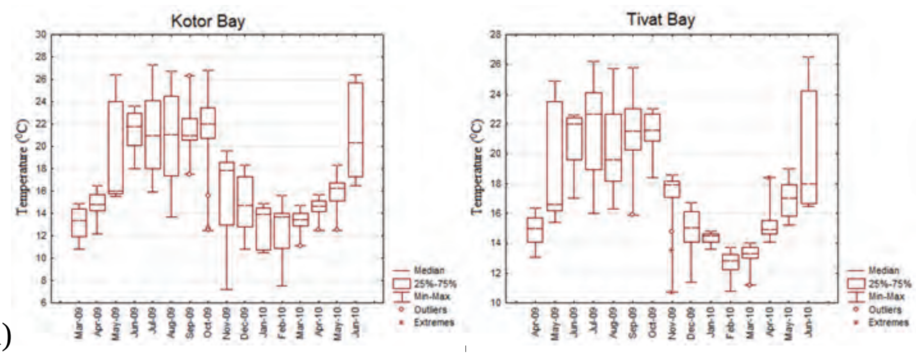
Principal Component Analysis was performed with STATISTICA 7 Software. PCA was used to identify which organisms of potential prey have major influence on chaetognaths abundance.

Analysis of similarity (ANOSIM) was used to determine the differences between chaethognath populations of investigated sites. ANOSIM generates a test statistic, R, and a magnitude of R is indicative of the degree of separation between groups, with a score of 1 indicating complete separation and 0 indicating no separation (Clarke 1993; Clarke & Green 1988).

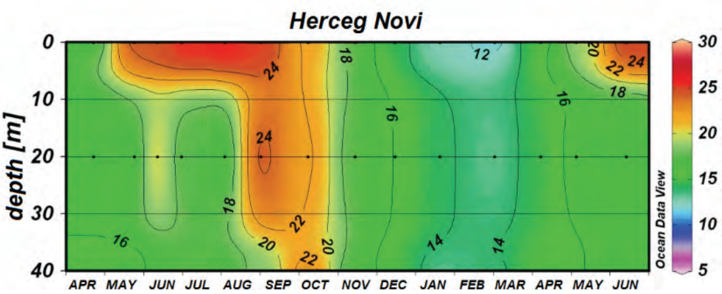
RESULTS

Hydrographic parameters

The highest fluctuations of temperature were recorded on the surface in Kotor Bay: from 6.3°C (November 2009) to 27.3°C (July 2009) (Figure 2a).



a)



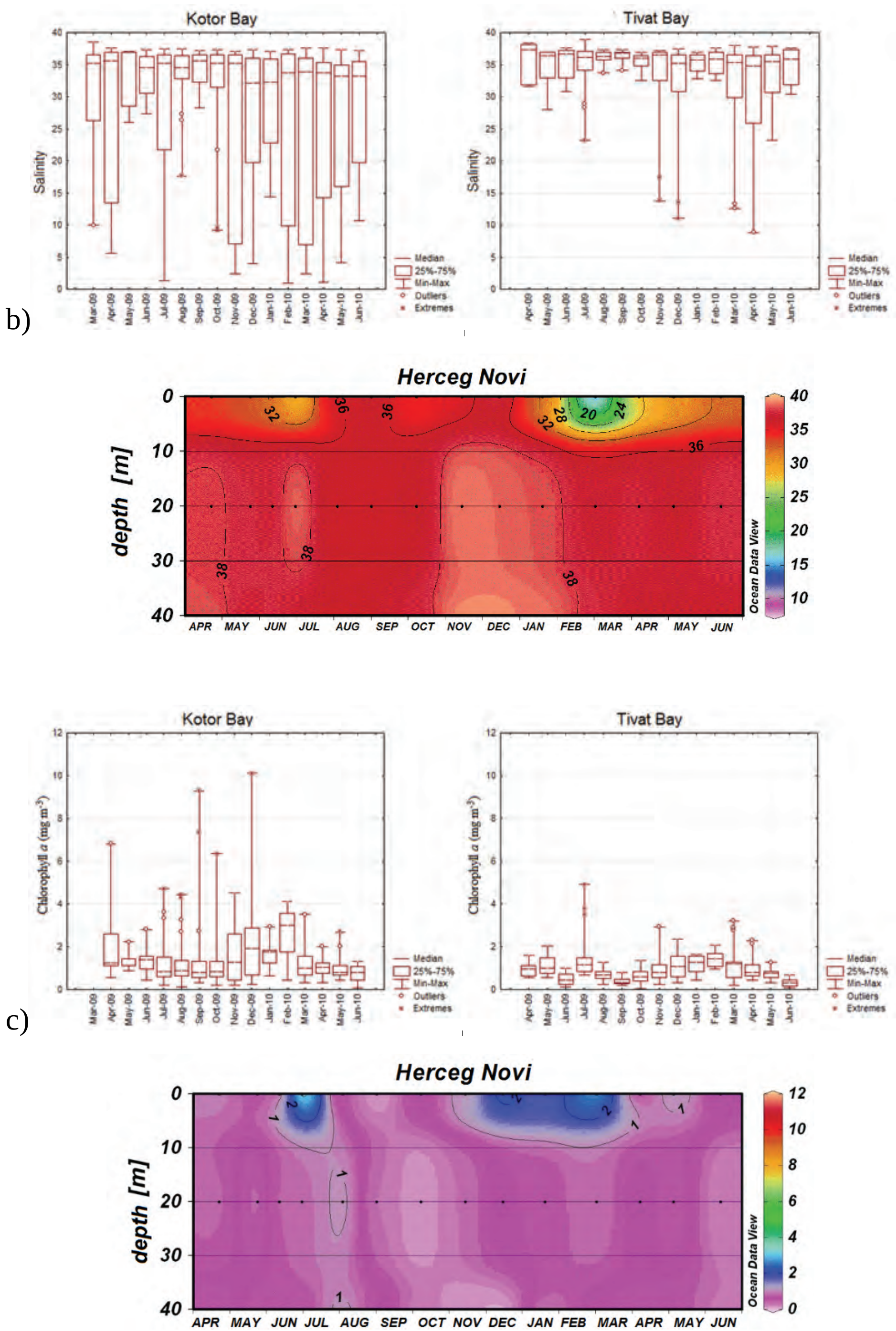


Figure 2. Temporal variability of a) temperature; b) salinity and c) chlorophyll *a* in Kotor, Tivat and Herceg Novi Bay

Pronounced salinity oscillations were presented in Kotor Bay in all seasons, while Tivat and Herceg Novi Bay were under less influence of fresh water during investigated period. Unexpected low value of salinity (1.8) in July in Kotor Bay (Figure 2b) was the consequence of rainy days before sampling and high influence of river Ljuta. Minimum value of salinity (1) was noticed in February 2010 in Kotor Bay. Temperature and salinity fluctuations in Tivat Bay were lower than in Kotor Bay, and already bellow 5m, salinity conditions are stable. Maximum salinity value was measured in Tivat Bay in July and reached 38.9 on the bottom.

Mean values of chlorophyll *a* showed regular gradient from inner stations towards Herceg Novi Bay. The maximum of 10.11 mg m^{-3} was recorded in December in Kotor Bay (Figure 2c) while the highest values of chlorophyll *a* concentration in Tivat and Herceg Novi Bay were noticed in July, 4.9 mg m^{-3} and 3.75mg m^{-3} respectively.

<u>Zooplankton community structure</u>

Dominant group of zooplankton in Boka Kotorska Bay were copepods with the mean contribution of 67%. The highest share of copepods (95%) in total zooplankton abundance was noticed in January in Herceg Novi Bay. Apart from copepods, cladocerans prevailed copepods number in September when they have 60% of contribution in total zooplankton abundance.

Maximum copepod abundance of 22 414 ind m^{-3} was noticed in August 2009 (Kotor Bay). Density increase of this group was also recorded in October and during winter season (December and January) i Tivat and Herceg Novi Bay The most abundant were genus *Oncea* and *Oithona nana*. Higher values of genus Oncaea was noticed in August and October in Kotor

Bay, and in December in Tivat and Herceg Novi Bay. *Oithona nana* was the most abundant in June 2009 in Kotor Bay, while maximum values for Tivat and Herceg Novi Bay were noticed during winter months, in December and January, respectively.

Chaethognath species composition and abundance

Three species of chetognaths were collected in Boka Kotorska Bay: *Flaccisagitta enflata* (Grassi, 1881), *Mesosagitta minima* (Grassi, 1881) and *Parasagitta setosa* (Muller, 1847). Total densities of chaethognaths over the investigated period showed an upward trend over the summer months 2009 and spring 2010 (Figure 3). Therefore, a maximum of 614 ind m^{-3} was recorded in August 2009 and the second higher value of chetognaths was 273 ind m^{-3} found in April 2010 (Figure 3). Medians show that difference in chaetognath abundance between stations was most intensive in period August-October 2009 as well as April-June 2010 (Figure 3).

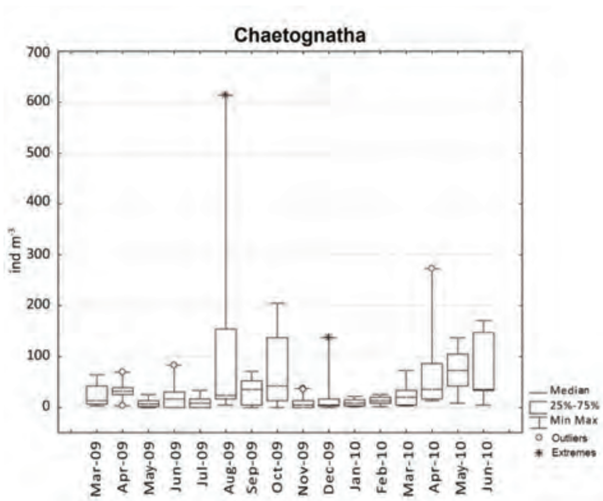
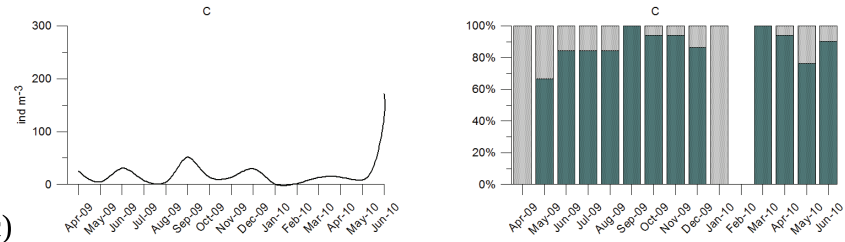


Figure 3. Box-plot diagram of the total value of chetognaths (ind m^{-3}) at all stations per month.

Mean monthly abundance of chaetognaths in Kotor Bay was 146 ind m^{-3} in August (Figure 4a). The highest values were registered Tivat Bay, ranged up 303 ind m^{-3} (Figure 4b). An increasing trend in abundance was continued from the beginning to the end of the study in this area. In Herceg Novi Bay, maximum abundance was noticed in June 2010 and reached 170 ind m^{-3}. Apparently, comparing period March-June 2009 with the same period in 2010 there are differences in chaetognath abundance. Lower abundances in 2009 are especially noticeable in Kotor and Tivat Bay. Juvenile stages were dominated in all bays with an average contribution of 66%, 62% and 75% in Kotor, Tivat and Herceg Novi Bay (Figure 4). In August 2009 in Kotor Bay, and June 2010 in Tivat Bay contribution of adults prevailed, and the proportion of juvenile animals were represented with only 33% and 37%, while in April 2009 and February 2010 adults reached 100% of contribution in Tivat Bay.



c)

Figure 4. Average monthly value and percentage contribution of juvenile and adult individuals in researched areas (A - Kotor Bay, B - Tivat Bay, C – Herceg Novi Bay)

The most dominant species in all areas was *Parasagitta setosa*. The highest percentage contribution of 83% was observed in Tivat Bay, while the maximum abundance was noticed in the Kotor Bay, up to 204 ind m^{-3} in August 2009 and April 2010 (Table 4).

Table 4. Species composition of chaetognaths, with their maximum abundance (max. ind m^{-3}), the mean value (mean+ SD; ind.m^{-3}), the mean percentage value of the total abundance (mean%) and frequency of occurrence (f%).

		Kotor Bay		
Species	***max***	***mean±SD***	***mean %***	***f %***
P. setosa	204	7.22+25.33	75.91	42
F. enflata	68	2.34+8.01	22.21	23
M. minima	17	0.43+2.19	1.87	13
		Tivat Bay		
Species	***max***	***mean±SD***	***mean %***	***f %***
P. setosa	68	9.03+17.20	83.22	32
F. enflata	34	3.50+8.91	16.34	22
M. minima	10	0.35+1.56	0.44	5
		Herceg Novi Bay		
Species	***max***	***mean±SD***	***mean %***	***f %***
P. setosa	13	1.69+3.38	63.59	8
F. enflata	25	2.40+6.73	31.92	8
M. minima	0.8	0.19+0.34	4.49	4

Statistically significant differences which were confirmed by Kruskal - Wallis's and Mann Whitney U test showed that the abundances in Tivat Bay were significantly different from other areas (Table 5). Another quantitatively important species of chaetognaths was *Flaccisagitta enflata*. Maximum value of 68 ind m^{-3} was found in Kotor Bay in April 2009. The highest percentage contribution was found in Herceg Novi Bay (31.92 %), and then in Kotor Bay (22.21 %) (Table 4). *Mesosagitta minima* is the least abundant species of chaetognaths. The maximum number of 17 ind m^{-3} was observed in the area of Kotor in March and April 2010, while the highest prevalence of only 4.5 % was recorded in the area of Herceg Novi bay .Kruskal - Wallis's and Mann Whitney U test showed that there were no statistically significant differences in abundances of *F. enflata* and *M. minima* in relation to the studied area.

Table 5. Kruskal-Wallis's and Mann Whitney U test of chaetognath species in researched area (A – Kotor Bay, B - Tivat Bay, C – Herceg Novi Bay).

Species	**Probability**	**Median**
Parasagitta setosa	**p=0,01	B>A=C
Flaccisagitta enflata	p>0,05	A=B=C
Mesosagitta minima	p>0,05	A=B=C

Table 6. ANOSIM analysis for three stations in researched area (A – Kotor Bay, B - Tivat Bay, C – Herceg Novi Bay).

	Global R	**Pair-wise test R**	**Significance level (%)**
Location	0.007		54.1
A-B		0.011	33.0
A-C		-0.052	70.8
B-C		-0.039	66.4

The general composition of chaethognaths of the Boka Kotorska Bay did not differ significantly between the three investigated stations (Table 6). As we expected, differences were the highest between Herceg Novi and Kotor Bay.

Using principal components analysis were analyzed data of chaetognaths and small copepods. The analysis showed that the first three axes describe the chaetognaths variability of 90.59 % (Table 7). According to factor PC1, describing 44.65 % of the total variability, grouped the species *F. enflata* and *M. minima*. PC2 is segregated juvenile stages, whereas the PC3 distinguished *P. setosa* (Table 7).

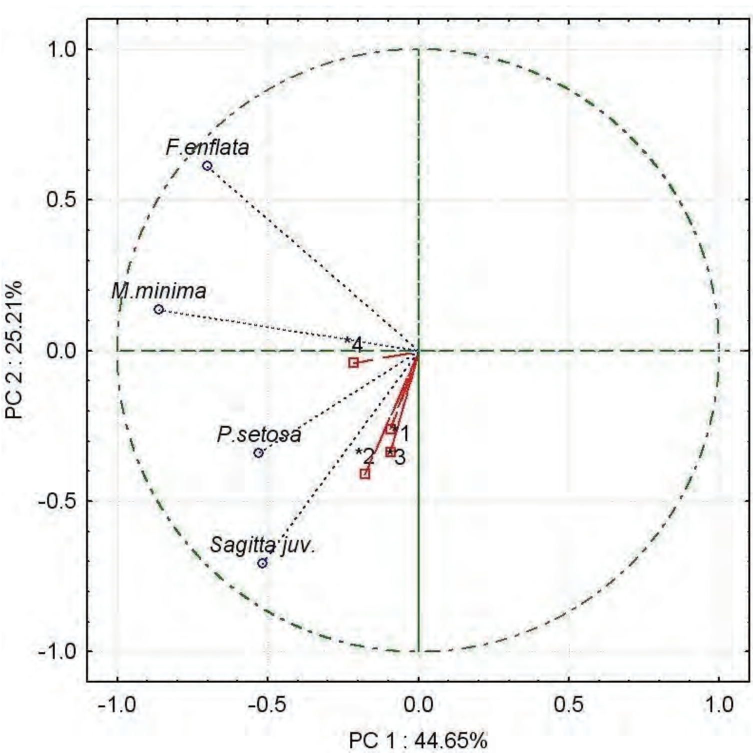


Figure 5. Principal component analysis (PCA) for main variables chetognath and supplementary variables (small copepods as potential prey)
1* calanoid copepodites; 2* cyclopoid copepodites; 3* Oithona nana;
4* Oncaeaidae; ○ active variable; □ supplementary variable

Table 7. The total variance (%) for the first three coordinates and the results of PCA analysis of species.

	PC 1 44,65%	*PC 2 25,21%*	*PC3 3 20,73%*
P. setosa	-0,529	-0,339	**0,777**
F. enflata	**-0,701**	0,612	-0,011
M. minima	**-0,862**	0,135	-0,213
Sagitta juv.	-0,518	**-0,707**	-0,423

Supplementary variables, small copepods, showed mostly positive correlation with juvenile stages of chaetognaths (0.257 < P < 0.314), whereas *O. nana* and cyclopoid copepodites positively correlated with *P. setosa* (R = 0.241 and r = 0.395) (Figure 5). The results of the PCA analysis of the relationship of Chaetognaths and their potential prey were confirmed by Spearman rank order correlations (Table 9). Statistical analysis (Spearman rank order correlation) revealed significant correlations between some species and hydrological parameters: positive correlation with temperature was observed with juvenile stages, while salinity was positively correlated with *P. setosa* and *F. enflata*. Species *M. minima* was not significantly correlated with these parameters (Table 8).

Table 8. Spearman rank order correlation of Chaetognaths with hydrographic and biological parameters (T-temperature, S-salinity, Cl*a*- chlorophyll *a*).

	T	*S*	*Chl a*
P. setosa	-0,017	**0,163***	-0,174
F. enflata	-0,049	**0,261****	-0,017
M. minima	0,056	0,048	0,034
Sagitta juv.	**0,251****	0,097	**-0,179***

*p<0,05; **p<0,01; p<0,001

Table 9. Spearman rank order correlation of chaetognaths and potential prey.

	M. minima	***P. setosa***	***F. enflata***	***Sagitta juv.***
cal. copepodites	-0.040	**0.287*** **	0.047	**0.213** **
cycl. copepodites	0.033	**0.313*** **	-0.029	**0.158* **
Oithona nana	-0.048	**0.226** **	-0.120	0.154
Cladocera	0.135	0.055	-0.042	**0.247** **
Appendicularia	0.157	**0.310*** **	-0.023	**0.167* **

*p<0,05; **p<0,01; ***p<0,001

DISCUSSION

According to data provided by the Alvariño (1965), 47 species of planktonic chaetognaths have been found in the world's seas and oceans, 17 of which are present in the Mediterranean and 10 in the Adriatic Sea (Ghirardelli &Specchi 1965). In shallow areas as North Adriatic Sea three species were registered frequently, while four appear only accidentally. In South Adriatic coastal area Batistić *et al*. (2007) found 8 species at all. In Boka Kotorska Bay we found three coastal species of chaetognaths (*Flaccisagitta enflata*, *Mesosagitta minima* and *Parasagitta setosa*) that correspondent with species composition of northern Adriatic (Ghirardelli, 1975). Unusually, (Vukanić & Vukanić, 2004) registered seven species in Boka Kotorska Bay.

Abiotic environmental factors are of special importance for chaetognaths distribution (Lough & Trietes, 1989). The water temperature varied during our study period, especially in surface layer. This is consistent with previous knowledge of hydrographic parameters of Boka Kotorska Bay (Krivokapić *et al*., 2009, 2011). Stratification of salinity was similar to the other estuaries of the eastern Adriatic coast (Cetinić *et al*., 2006; Burić *et al*., 2007): there is a layer of open seawater below the halocline, and brackish water layer above the halocline. These hydrography conditions are similarly for northern Adriatic (Artegiani et al., 1997) that corresponds with our chaetognaths species composition.

ANOSIM analysis showed significant difference of Herceg Novi Bay according to composition and abundance of this zooplankton group. The lowest average values for all of three species were found in Herceg Novi Bay. Maximum abundance of chaetognaths was recorded in the late summer (August) and the time of this occurrence coincides with the maximum values found in the coastal southern Adriatic Sea (Lučić & Onofri, 1990; Lučić & Kršinić, 1998; Batistić *et al*., 2003; Batistić *et al*., 2007). Noticeably lower abundances of total chaetognaths in spring 2009 comparing with spring 2010 could be caused by mass occurrence of ctenophore *Bolinopsis vitrea* in March and April 2009 (Lučić *et al*., 2012).

Parasagitta setosa was the most abundant species at all stations in Boka Kotorska Bay. This species is noticed as dominant in other neritic coastal areas (Lučić & Onofri, 1990; Lučić & Kršinić., 1998). Maximum value of *P. setosa*, 204 ind m^{-3} was higher than recorded abundances in Mali Ston Bay (Lučić & Onofri, 1990). However, in the eutrophicated Vranic basin (eastern Adriatic Sea) juvenile chaetognaths dominated but their abundances did not reach above 50 ind m^{-3} (Vidjak *et al*., 2006). Investigations of the Fladen Ground area (UK), show that *Parasagitta setosa* was more abundant at shallow areas with a higher influence of fresh water (Rakuše - Suszezewski, 1967), as seem as for northern Adriatic (Ghirardelli, 1975) and other bays along eastern coast (Gamulin, 1979; Lučić & Kršinić, 1998). In our investigation, this species was the most abundant in Kotor Bay but showed positive correlation with salinity because maximum value was noticed in summer season. Similar pattern was observed in another enclosed South Adriatic site (Mljet Lake) where this species inhabit deep more saline waters, with maximum density in October (Miloslavić, 2012).

Flacisagitta enflata and *Mesosagitta minima* are more present and abundant in Boka Kotorska Bay than in other neritic coastal areas where are rare (Lučić & Onofri, 1990; Lučić & Kršinić, 1998). High abundances of these species are similar to abundances noticed in coastal area in South Adriatic open sea (Batistić *et al*., 2003; Batisić *et al*., 2007). *F. enflata* showed significant positive correlation with salinity. This same pattern was observed in tropical estuary in southeastern Brasil (Fernandes et al., 2005). *M. minima* did not show any significant correlation with either temperature or salinity. It is well known that both species are more numerous in coastal areas with a higher salinity values (Batistić *et al*., 2007).

Apart from hydrological conditions, food availability also seems to influence chaetognath distribution (Fernandes *et al*., 2005). Chaetognaths are very important zooplankton predators in the sea (Feigenbaum & Maris, 1984; Baier & Purcell 1997). The main source of food represents a small fraction of a size copepods and copepodites (Feigenbaum & Maris, 1984; Feigenbaum, 1991; Duro & Saiz, 2000). Appendicularians, chaetognaths, cladocerans and larval fish occasionally participate in their diet (Alvariño, 1965; Rakuše - Suszczewski, 1967; Pearre 1974; Feigenbaum & Maris 1984; Kehayias *et al*., 2005). This study has found a significant correlation of *P. setosa* and juvenile chaetognaths with small sized fractions of copepods. The abundance of *P. setosa* is correlated with the total abundance of apendicularians. Similar relations predator - prey have been found in many other studies of the Adriatic (see Batistić, 1994). Pierrot-Bults (1996) mentioned that approximately 10 to 30% of the copepod biomass is transferred to the chaetognath biomass. The results obtained in this study are in accordance with those observations, since a close correlation of chaetognath occurrence and high copepod densities was observed (Pestorić, 2013).

CONCLUSIONS

In this work we present for the first time detailed information of the ecology of cheatognaths in the specific ecosystem of Boka Kotorska Bay. Species composition is in accordance with recent knowledge of their distribution in the Adriatic Sea. The highest abundance was noticed in the most productive Kotor Bay and these densities were slightly higher than were known for other productivity Adriatic bays. Kotor Bay can be used as representative station because of frequent sampling performed in this area. This is high dynamic system with extremely variable hydrological and consequently biological factors on annual scale. The most numerous species *Sagitta setosa* showed high variations in abundance and strong correlation with available food. Presence of competitive species and potential predator *Bolinopsis vitrea* could influence total chaetognaths abundance.

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