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DIVERSITY AND DYNAMICS OF TINTINNID COMMUNITIES FROM ROMANIAN BLACK SEA, IN 2021

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ABSTRACT

Tintinnids are tiny ciliates from microzooplankton that are very important due to their implication in the microbial food web and for the fact that in the last two decades a considerable number of non-indigenous species have entered and acclimatized in the Black Sea basin. The paper presents the species diversity and dynamic of microzooplanktonic tintinnids' abundance as a result of two cruises carried out in May-June and August-September 2021, along the Romanian Black Sea coast. We identified 23 tintinnid species in total, with a higher diversity in the surface layer compared to those of depth. The species *Eutintinnus sp*. and *Salpingella decurtata* were found exclusively in the 10 m layer. *Tintinnopsis cylindrica* represented 74% of the mean biomass of tintinnid's community from May-June, the best quantitative representation recorded in the Romanian coast's northern sector. *Amphorellopsis acuta* represented 91% of the mean biomass of tintinnid's community in August-September. The biotic component was also correlated with temperature and salinity to give more information regarding spatial and temporal variations from one area to the other. The findings contribute to the knowledge of microzooplankton communities in the Romanian Black Sea region. The data on species composition, abundance distribution, and environmental correlations enhance our understanding of the ecological dynamics and potential ecological implications of tintinnid communities. This study brings valuable information to the understanding of the microzooplankton component in the Black Sea ecosystem.
Keywords: diversity, dynamics, variations, tintinnids, Romanian Black Sea

AIMS AND BACKGROUND

Tintinnids are ubiquitous ciliates in the marine and estuarine waters. They are loricate planktonic ciliates that live mainly in surface waters (Kim *et al.*, 2012).

The roles of planktonic protists, such as ciliates, in the microbial loop are to consume bacteria that are too small to serve directly as major prey items for most zooplankters and after to be themselves utilized by the larger zooplankters (Okuda *et al*, 2014). More than that, the tintinnid ciliates are an important trophic intermediate in the planktonic food web due to their high productivity and ability to feed on the small, highly productive phytoplankton (Middlebrook *et al,* 1987). Tintinnids are prey for many zooplankters, suspension-feeding benthic invertebrates, and fish larvae (Dolan *et al*., 2013).

Sea ports and bays act as acclimatization areas for the different ciliate and other invasive species which are transported through the ships' ballast water (Selifonova, 2018). The first abundance outbreak of non-indigenous tintinnid species (*Eutintinnus sp., E. lusus-undae, E. tubulosus* and *E. apertus*) in Black Sea was reported in the Novorossiysk Harbour in August-September of 2001 (Gavrilova, 2005). Since then, several non-indigenous species have been identified, including: *Salpingella decurtata* - 2002, *Amphorellopsis acuta* and *Tintinnopsis tocantinensis* – 2010 (Selifonova, 2012a, 2012b), *Dartintinnus alderae* – 2012, *Rhizodomus tagatzi* – 2015, *Tintinnopsis mortensenii* – 2017(Gavrilova, 2017), *Eutintinnus pecti*nis - 2016 (Boicenco *et al*., 2019). The reporting of an important number of tintinnid species as well as their acclimatization in the Black Sea basin requires careful monitoring of this microzooplankton component.

Most tintinnid species occurred for a limited period during the year, and the period of occurrence differed from species to species (Kamiyama,1996).

The objective of this paper is to examine the seasonal variations in the structure, distribution, and abundance of the tintinnid community, with a focus on investigating the potential influence of environmental parameters such as temperature and salinity.

EXPERIMENTAL

Sampling was performed from 63 stations along the Romanian Black Sea. Of these, 17 were in coastal waters, 10 in waters with variable salinity, 29 in marine waters and 7 were in offshore waters (Fig. 1). We mention that the first survey of the year was made with the “Steaua de Mare” vessel, in May-June period, and included coastal, marine, and variable salinity waters, while the second survey was made with the “Mare Nigrum” vessel, in August-September and included only marine and offshore waters.

The temperature and salinity were measured for each sampling point with a reversible thermometer and CastAway CTD multiparameter. Salinity samples from August-September were analysed in the NIMRD’S laboratory, according to the Mohr-Knudsen method for seawater analysis (Grasshoff *et al.*,1999).

For microzooplankton analyses, the water was sampled with Niskin bottles from the surface (0 m), 10 m and DCM (deep chlorophyll maximum), stored in plastic bottles (500 ml) to which formalin was added.

In the laboratory, samples were concentrated to a final volume of 20 ml by repeated sedimentation. The final volume was fully analysed under the inverted microscope (Olympus XI 51) using 200x and 400x magnification factors.

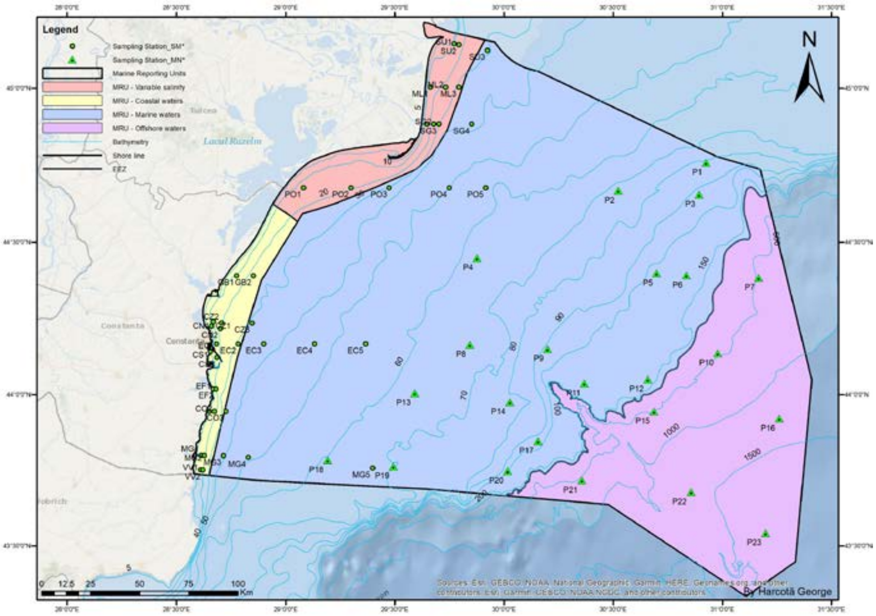


Fig.1. The map of the sampling locations

The taxonomic identification of tintinnids was made according to the shape and dimensions of the lorica, according to literature (Petran, 1958 (b), Gavrilova, 2005, Abboud-Abi Saab, 2008). Both empty tintinnids and those with protoplasm were considered for qualitative and quantitative analysis because the mechanical and chemical disturbances associated with collection and fixation procedures have been demonstrated to cause cell detachment (Thompson *et al*., 2005). The density of organisms was expressed as the number of individuals per litre (ind./L). The lorica volume was calculated according to the total length and aboral diameter of the lorica and to the geometric form assumed for each species. Biomass was expressed as carbon biomass (µgC/L) using the specific biovolume conversion formula for formalin conserved material (Verity *et al.*, 1984).

Statistical analysis of the environmental parameters and tintinnids community structure was carried out using the PRIMER version 7 program (Clarke & Gorley, 2015). The maps were made with the ArcGIS software package.

RESULTS AND DISCUSSION

As environmental variables, the temperature and salinity were taken into discussion. Tintinnids are highly sensitive to their environment, and temperature is considered one of the crucial abiotic factors that affect their growth, distribution, and behaviour. Seawater temperature changes are recorded seasonally both at the surface and in-depth, which makes it one of the most important abiotic influencing factors on tintinnids (Dolan *et al*, 2013). Some species can withstand different temperature ranges, being thus adapted to local environments, so the measurements from one region may not be applicable to others. These are the reasons why the distribution of the tintinnids species is followed in relation to this parameter. The table below includes the minimum, maximum and average values of the temperature in each investigated marine reporting unit, in the 2021 analysed season (Table 1).

Table 1. Environmental variables at the Romanian Black Sea in 2021

Period	Layer	MRU - Waters type	Temperature			Salinity		
			Min	Max	Average	Min	Max	Average
May-June	0 m	Variable salinity	16.95	20.44	19.42	4.17	16.76	10.77
		Coastal waters	16.08	19.82	18.68	13.27	18.00	15.59
		Marine waters	16.79	18.97	18.41	13.20	18.37	16.17
	10 m	Variable salinity	8.17	12.91	10.09	17.96	18.53	18.17
		Coastal waters	14.02	18.16	16.63	14.88	18.11	16.93
		Marine waters	10.63	18.41	16.43	15.25	18.36	17.05
August-September	0 m	Marine waters	21.80	25.69	23.93	16.23	18.52	17.97
		Offshore waters	22.69	25.60	24.52	18.45	18.71	18.55
	DCM	Marine waters	21.80	25.56	23.66	16.23	18.54	18.02
		Offshore waters	22.67	25.54	24.49	18.44	18.71	18.55

In spring, the surface water temperature had values between 16.08 °C and 20.44 °C with an average of 18.76 °C and a standard deviation of 0.90 °C. At the end of summer, it increased, varying in the range of 21.80-25.69 °C, average of 24.11 °C, standard deviation 1.37 °C. Thus, the two seasons are extremely statistically significantly distinguished (t-test, $p<0.0001$, df=58).

Salinity at the surface had heterogeneous values in the spring between 4.17 PSU and 18.37 PSU (mean 14.62 PSU, standard deviation 3.08 PSU). The minimum value was recorded on the profile of Sf. Gheorghe in the Danube's influence area. Since no more samples were taken from the northern area in September, the values are much more homogeneous, in the range of 16.23 - 18.71 PSU (average 18.15 PSU, standard deviation 0.76 PSU). In this case, the values from the two sampling periods are significantly different (t-test, p<0.0001, df=58), too.

A total of 23 species belonging to 9 genera were identified in 2021 (Table 2). The taxonomic composition of tintinnids was characterized by differences from one investigated season to another. The agglutinated genus *Tintinnopsis* was the most diverse in both analysed periods of 2021. A number of 13 species from the total of 23 it was present in both investigated seasons but with different abundance values from one season to another.

Table 2. List of tintinnid species encountered at the Romanian Black Sea in 2021

Order	Genus	Species
Choreotrichida	*Codonella*	*Codonella cratera*
	Tintinnopsis	*Tintinnopsis baltica*
		Tintinnopsis beroidea
		Tintinnopsis campanula
		Tintinnopsis compressa
		Tintinnopsis cylindrica
		Tintinnopsis karajacensis
		Tintinnopsis lobiancoi
		Tintinnopsis minuta
		Tintinnopsis parvula
		Tintinnopsis tocantinensis
		Tintinnopsis tubulosa
		Tintinnopsis urnula
	Stenosemella	*Stenosemella ventricosa*
	Metacylis	*Metacylis mediterranea*
	Favella	*Favella ehrenbergii*
	Eutintinnus	*Eutintinnus sp.*
		Eutintinnus lusus-undae
		Eutintinnus pectinis
		Eutintinnus tubulosus
	Amphorellopsis	*Amphorellopsis acuta*
	Salpingella	*Salpingella decurtata*
	Tintinnidium	*Tintinnidium mucicola*

In spring, May-June, a total of 20 tintinnid species, belonging to 7 genera (*Codonella*, *Tintinnopsis*, *Stenosemella*, *Favella*, *Eutintinnus*, *Salpingella*, *Tintinnidium*) were identified. The genus *Tintinnopsis* was the most diverse and represented by 12 species, being the dominant genus of the community in this season. *T. cylindrica* was the common species (as occurrence frequency and abundance) recorded in all investigated areas along the surface layer, while *Eutintinnus pectinis*, *Favella ehrenbergii*, *Tintinnopsis sp.*, *T. compressa*, and *T. urnula* were observed at a single station each and just in 0 m layer. The species *Tintinnopsis baltica*, *T. compressa*, *T. karajacensis*, *T. parvula*, *T. tocantinensis*, *T. tubulosa* and *Tintinnidium mucicola* were identified only in this season.

In the late summer, August-September, a total of 16 tintinnid species, representing 8 genera, were identified (the 7 listed for the previous period plus the genus *Amphorellopsis*). The genus *Tintinnopsis* was again the most diverse and represented by 6 species. *Amphorellopsis acuta* and *Salpingella decurtata* were the most common species, whereas *Codonella cratera*, *Favella ehrenbergii*, *Stenosemella ventricosa*, *Tintinnopsis sp.*, *T. beroidea*, *T. campanula*, *T. cylindrica*, *T. lobiancoi* and *T. urnula* were observed in a single station (rare species). The species *Amphorellopsis acuta*, *Eutintinnus sp.* and *Metacylis mediterranea* were identified only in this period.

In spring, tintinnid abundance ranged from 2 to 818 ind/L and 2 to 14 ind/L in 0 m and 10 m respectively. Their biomass ranged from 0.002 to 3.092 µg C/L and 0.005 to 0.033 µg C /L in 0 m and 10 m layer respectively. The higher abundances are observed at the surface in the Danube's influence area but also around Mangalia and the Constanta South areas, with strong anthropic influence (Fig. 2).

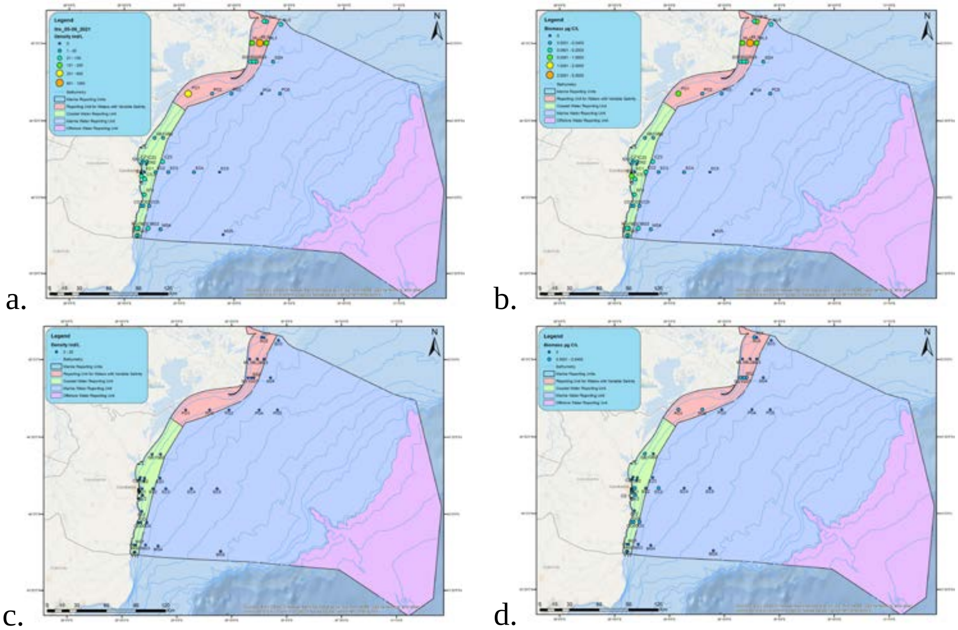
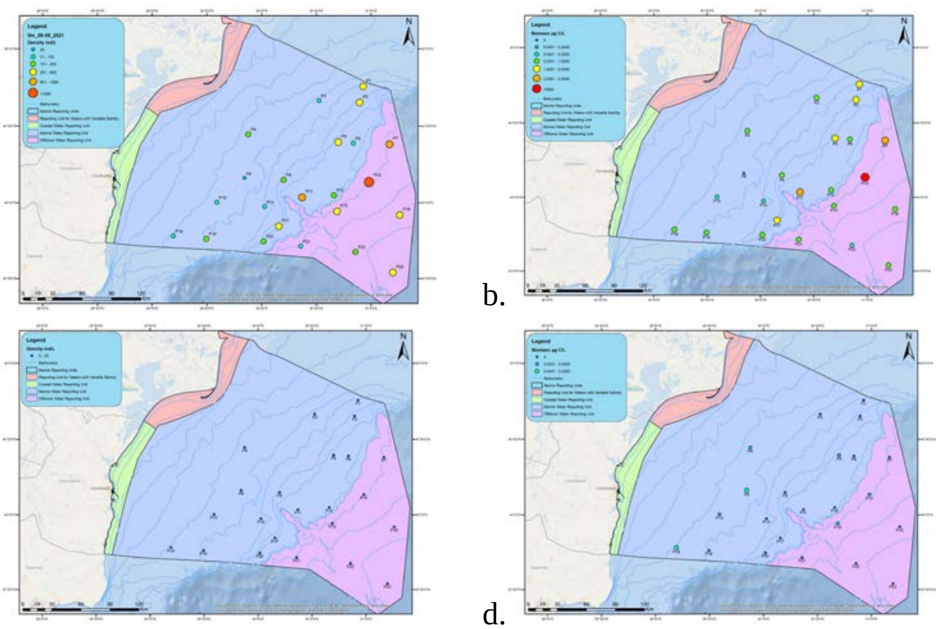


Fig. 2. Distribution of the abundance (a, c) and biomass (b, d) of tintinnids in May-June (spring) in the surface layer (above) and 10 m layer (below)

In August-September, tintinnid abundance ranged from 20 to 2968 and 2 to 12 ind/L in 0 m and DCM layer respectively. Their biomass ranged from 0.030 to 13.634 and 0.003 to 0.078 µg C/L in surface and DCM layer respectively. The higher abundances were observed in the offshore marine station P10 (Fig. 3). This season was dominated by *Amphorellopsis acuta* (93.3 frequency of occurrence and 2770 ind/L of maximum abundance in P10 station. The dominance of the non-indigene species *A. acuta* is justified by the higher temperatures during this period, being classified from the point of view of the biogeographical type as a warm water oceanic species (Fig 3a).



a. b. c. d.

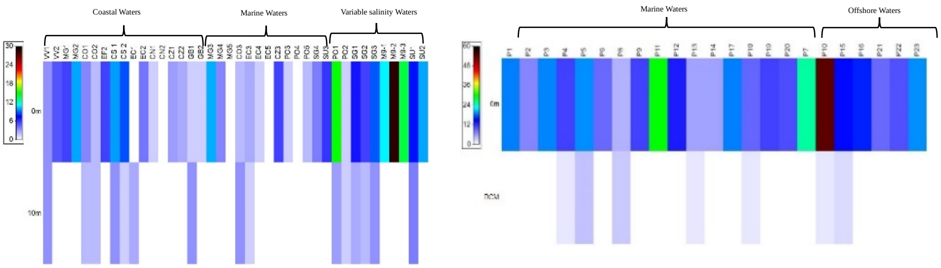
Fig. 3. Distribution of the abundance (a, c) and biomass (b, d) of tintinnids in August-September (late summer) in the surface layer (above) and DCM layer (below)

Analysing the vertical distributions in all investigated periods, both qualitative and quantitative dominance of tintinnids was observed in the 0 m layer (Fig. 2-4).

Observing the ratio between indigenous and non-indigenous species in the tintinnid population, a dominance of the number of indigenous species was observed in both analysed seasons. From a quantitative point of view, the situation differs from one season to another, so that in the spring indigenous species dominate (94% of the total density), while in the summer there is a clear dominance of the non-indigenous species (98% of the total density). Quantitative dominance of non-indigenous species in the second analysed season is in accordance with the ecology of these species, registering great developments in this period, and being cosmopolite, is not limited to the neritic zone.

Concerning the distribution in marine water units in May-June a quantitative dominance was evident in the body with variable salinity water, the average values of density and biomass being 167 ind/L and 0.5863 µg C/L.

In August-September, the offshore water was best represented registering the average values of density and biomass of 200 ind/L and 0.8972 µg C/L (Fig. 5). From a quantitative point of view, in the late summer, the abundance recorded was about three times higher than in the spring, this being directly correlated with the increase in temperature but also with the ecology of the species composition.



a. b.

Fig. 4. Shade plots (Primer 7) representation of the tintinnids' abundance and sampling sites – a. May-June, b. August-September.
Shading intensity indicates the square root transformed relative abundance of tintinnids from each analysed layer.

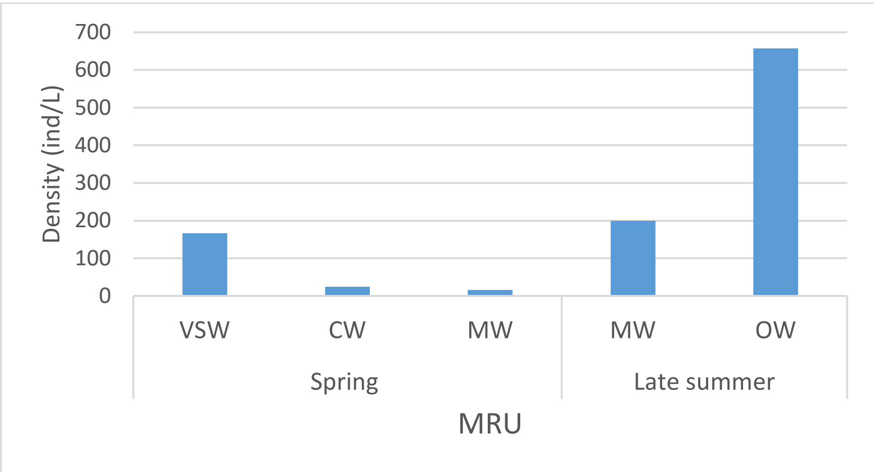


Fig. 5. Distribution of the density (ind./l) of tintinnids in different Marine Reporting Units (MRU) in 2021

CONCLUSIONS

In the present paper, we examined the seasonal variations in the structure, distribution, and abundance of the tintinnid community, with a focus on investigating the potential influence of environmental parameters such as temperature and salinity.

The values of temperature and salinity from the two sampling periods are significantly different, so we can talk about two different seasons: spring and late summer.

The 2021 tintinnid community analysis led to the identification of 23 species. Of these, 13 were common for the analysed season, while the remaining 10 species varied from one season to another. *Tintinnopsis cylindrica* was the dominant species in the spring while the *Amphorellopsis acuta* dominated in the late summer. It was observed that the spring season was characterized by a higher diversity of species but a lower density than the late summer season. The densities, about three times higher in the late summer season, were favoured on the one hand by the increase in temperature and salinity and on the other hand by the ecology of the constituent cosmopolite species (tolerance to greater temperature and salinity variations, maximum development period).

In the spring season, the highest abundance was observed in the Danube mouths, where higher average temperatures and lower average salinities were recorded. In the late summer, the highest abundance was observed in the offshore waters where higher averages of temperature and salinity were found.

The analysis of the indigenous/non-indigenous species ratio highlighted a quantitative dominance of the indigenous species in the spring and of the non-indigenous species in the late summer season, respectively.

Because the input and acclimatization of some non-indigenous tintinnid species require careful monitoring, preferably with a higher frequency, future research will be addressed to a long-term analysis of the acclimatization of these species and changes in the structure of the indigenous community over time.

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