ROMANIAN ACADEMY
INSTITUTE OF BIOLOGY BUCHAREST

SUMMARY OF THE PhD THESIS

Knowledge of the current diversity of the microzooplanktonic community from the Romanian Black Sea waters - populations of Tintinnids (Ciliophora)

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Contents

Introduction

In the last decades, simultaneously with the development of appropriate sampling and analysis techniques, more and more evidence has begun to emerge about the much higher importance of microzooplanktonic ciliates in the biological productivity of the marine ecosystem.

The lack of recent data on the population of ciliates, from the Romanian coast, as well as the publication of a relatively large number of scientific papers confirming the invasion in recent decades of non-indigene microzooplankton species in the Black Sea basin, motivated the beginning of research that was included in the thesis. The aim was to know the diversity of the populations of tintinnids (Ciliophora) from the Romanian Black Sea coast.

The thesis consists in 150 pages and it is structured into 8 chapters containing 27 tables (all original), 3 annexes and 128 figures, of which 27 are original photos, 24 maps made with ArcGIS 10.5., 74 graphs and diagrams. The bibliography includes 158 scientific paper from the country and abroad and 4 links with various supporting information.

The contribution of this thesis consists mainly in updating the scientific database with new data (2015-2018) on: diversity and distribution of the tintinnid community, newly identified species on the Romanian coast (12), new species reported for the Black Sea basin (2), seasonal distribution of tintinnids populations, correlations with different environmental factors, vertical distribution, morphometric characteristics of tintinnids.

The first chapter includes bibliographic information on the systematic position and ecological importance of the tintinnids group in the marine ecosystem. The tintinnids are systematically included in the Kingdom Chromista, Subkingdom Harosa, Infrakingdom Alveolata, Phylum Ciliophora, Subphylum Intramacronucleata, Class Oligotrichea, Subclass Oligotrichia, Order Choreotrichida, Suborder Tintinnina (WoRMS, 2020). The special role in the planktonic food chain is ensured by the fact that tintinnids represent a link between the microbial loop and the larger zooplankton. The Protozoans, especially the ciliates, "can sometimes have a much stronger impact on phytoplankton productivity than meso- and macrozooplankton" (Weisse & Scheffel-Möser, 1990) in that "only Tintinnids replace up to 20% of daily primary production and between 27-60% of the annual primary production in some marine environments "(Verity, 1985).

Chapter 2 includes a brief analysis of the literature on the history of research in the field, at the level of the entire Black Sea basin (subchapter 2.1) and at the level of the Romanian coast (subchapter 2.2), respectively.

In the first part of the last century the study of tintinnids consisted in the identification and description of several species by Mereschkovsky (1881), Lepşi (1920), Galadzhiev (1937), Paspalew (1942) (Lepși, 1965, Gavrilova & Dolan, JR, 2007, Kovalev, 1999).

In the second half of the last century, the diversity, distribution, and ecology of zooplankton as a component of the ecosystem were a target of scientific interest for researchers around the Black Sea basin. The first inventory of tintinnid species from the Romanian coast, consisting of 8 species, 2 varieties and 2 forms, was achieved in 1956 by Petran. A. (Petran, 1958). The first research on the abundance of the ciliates and determining the role of microzooplankton components in the pelagic community in the Black Sea basin was started in the late '60s by Zaika and Averina, Negre, Elian, Mărgineanu, Porumb. At the same time, the studies of taxonomy, biology, physiology of the constituent species are diversified according to the ecological events that take place in this area (eutrophication, the impact of invasive species, the impact of climate change).

All basic studies, from the north and northwest of the Black Sea, regarding the diversity, density, and distribution of ciliates populations were conducted in the late 1970s and early 1980s.

Pavlovskaya (1976) "demonstrates that the biomass of ciliates in the oxic zone makes a significant contribution to that of microzooplankton, compared to the nanoheterotrophs" (Sorokin, 2002). Mamaeva (1980), publishes a paper dedicated to the microzooplankton component in the neritic area of the Black Sea, in which he provides information on the weight of the component species.

In the early 2000s, Russian and Ukrainian researchers began a complex analysis of the ciliate populations in the northwestern part of the Black Sea. Following these studies, several non-native species are reported, new to the Black Sea basin (Polikarpov et al., 2003, Gavrilova, 2001, 2010, Kurilov, 2004, Selifonova, 2011a, 2011b).

From this chapter it results that the study of tintinnids in the Black Sea basin, including the Romanian coast, was carried out in general, as an integrated part in the mesozooplankton fraction. This led to the publications with general and fragmented information. The study of the microzooplankton fraction separately and more detailed began at the end of the last century, being now approached more and more complexly.

Chapter 3, structured in five subchapters, describes the methodology adopted, from establishing the areas of investigation to analyzing the data obtained in the laboratory.

Mangalia, Est Constanța and Portița profiles were chosen for analysis due to the highest frequency and constancy of testing between 2015-2018 but also due to the fact that they are located under various influences (in the northern sector of the coast predominantly under the influence of Danube waters, in the center and the south of the coast predominantly under the influence of anthropogenic factors) (Fig.1).

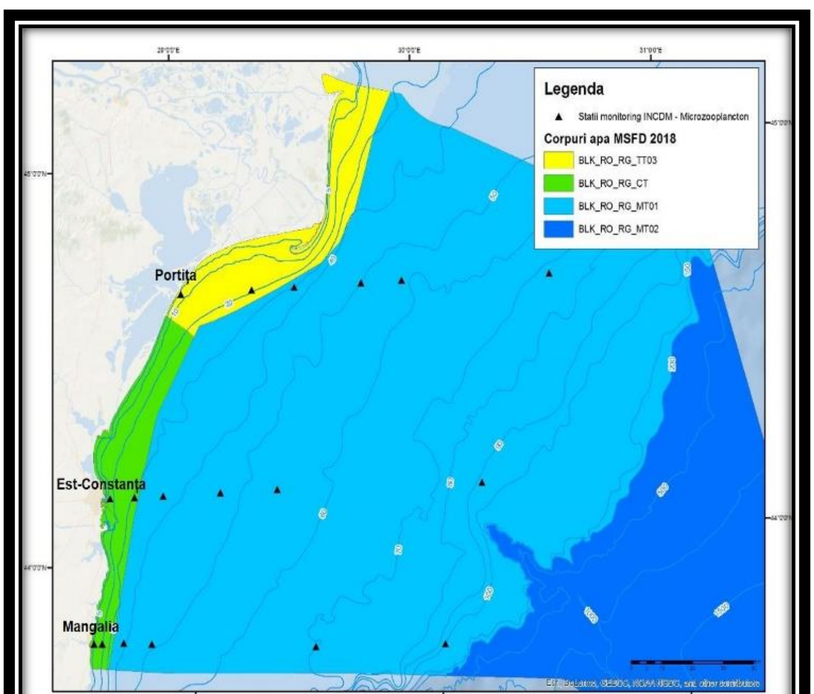


Fig. 1. - The stations network used for the study of tintinnids populations from the Romanian Black Sea coast, (source: INCDM "Gr. Antipa")

The samples (197) were collected over a period of four years, in seven scientific expeditions, carried out in two seasons (warm and cold).

The biological samples were collected from the surface horizons (0 and 10m respectively) using Niskin bottles. The minimum amount of seawater taken for each sample in order to fulfill "sufficient to provide a significant number of species" (Caspers, 1980) was 0.500 ml. Immediately after collection, the samples were preserved with 37% formaldehyde in a final sample concentration of 4% and stored in such a way that they were not moved for at least 24 hours (so as not to damage the biological material) (Harris et al., 2000).

In the laboratory, the samples were reduced to a final volume of 10 ml by repeated siphoning technique. For qualitative and quantitative analysis, the samples were fully analyzed, under an inverted microscope (Olympus XI 51). Taxonomic identification of tintinnids was performed according to the shape and size of the lorica, using the specialty literature: Petran, A., 1958, Abboud-Abi Saab, M., 2008, Al-Yamani, F.Y. et al., 2011, Trégouboff, G. & Rose, M., 1957. The empty lorica were not differentiated from those with protoplasm because studies have shown that "mechanical and chemical disturbances associated with collection and fixation procedures can cause cell detachment from lorica" (Thompson & Alder, 2005). Specimens whose taxonomic characteristics have been sufficiently affected not to provide an accurate taxonomic determination have not been considered.

The density of organisms was expressed as number of individuals per litre (ind /l). For the distribution maps, values of the densities were transformed to m^3 (ind/l $x10^3$) to be easily compared with the other components analyzed in the ecosystem (expressed in m^3). The volume of the lorica was estimated based on the individual measurements (total length, oral diameter and aboral diameter respectively) and subsequently converted (V_l) according to the various geometric shapes or combinations thereof, assumed depending on the species (Hillebrand et al., 1999). The biomass was calculated as the total biovolume (µgC/l). The biovolume of tintinnid lorica was expressed as carbon content. The calculation of the biovolume was made according to the volume of the lorica respectively the volume conversion factor: organic carbon established by Verity and Langdon (1984) for the biological material preserved, in our case, with formaldehyde.

The analysis of the data regarding the physico-chemical and bio-chemical parameters, necessary for the present study, were performed by colleagues from the Laboratory of Physico-Chemical Measurements and Analyzes, respectively the Phytoplankton Laboratory of the I.N.C.D.M. "Grigore Antipa".

Excel and PAST 3.20 programs, available online (Hammer et al., 2001), were used for the processing and statistical analysis of the data, and the ArcGIS 10.5 program was used for the preparation of the distribution maps.

The aim and objectives of the thesis are included in **Chapter 4**. The aim of this paper is to contribute, through a series of personal research results, to the knowledge of the current diversity and dynamics of Tintinnids (Ciliophora) populations from the Romanian Black Sea waters. The reasons for approaching this research topic are the lack of recent data on the Romanian coast, a series of scientific publications, which reported the introduction of new non-indigenous microzooplankton species in the Black Sea basin and last but not least the curiosity to see if the latter, have acclimatized or not to the conditions from the Romanian coast.

The first of the established objectives was to do a systematic and chorological catalog of the tintinnids identified at the Romanian Black Sea coast. This objective aimed at identifying the presence/absence of some species already mentioned in the literature as well as the possible enrichment of the microzooplankton component with new species and at the same time drawing up distribution maps for each identified species.

The second established objective was to obtain information on the seasonal distribution of tintinnids populations from the Romanian coast. This objective aimed at identifying associations of common / characteristic species for each analyzed season and the presence/absence of variations depending on the analysis period.

The third objective was to establish the relationships between seasonal environmental factors and the abundance and diversity of tintinnids populations from the Romanian coast. This objective aimed to identify those factors that may or may not constantly influence the dynamics of tintinnids populations.

The fourth objective was the analysis of the vertical distribution of tintinnids in the 0m and 10m horizons. This objective aimed to identify the associations of tintinnids species according to their distribution in the different depth layers.

All these data on the diversity, distribution, morphometry and ecology of tintinnid populations in the period 2015-2018, are intended to complete and update the scientific database but also to be a starting point for future research directions (e.g. trophic relationships, ecosystem energetics, etc.).

In the **5th chapter** the systematic and chorological catalog of tintinnids identified in the period 2015-2018, at the Romanian coast is presented. Following the investigations on the populations of loricate ciliates (tintinnids), 24 species were identified, belonging to 10 genera and six taxonomic families (Table 1).

Table 1 - List of tintinnids species identified along the Romanian Black Sea coast, in the period 2015-2018

No.	Order	Suborder	Family	Genus	Species
1	Choreotrichida	Tintinnina	Codonellidae	*Codonella*	*Codonella cratera* Leidy, 1887
2				*Tintinnopsis*	*Tintinnopsis baltica* Brandt, 1896
3					*Tintinnopsis beroidea* Stein, 1867
4					*Tintinnopsis campanula* Ehrenberg, 1840
5					*Tintinnopsis compressa* Daday, 1887
6					*Tintinnopsis cylindrica* Daday, 1887
7					*Tintinnopsis karajacensis* Brandt, 1896
8					*Tintinnopsis meunieri* Kofoid & Campbell, 1929
9					*Tintinnopsis minuta Wailes, 1925*
10					*Tintinnopsis parvula Jörgensen, 1912*
11					*Tintinnopsis tocantinensis* Kofoid & Campbell, 1929
12					*Tintinnopsis tubulosa* Levander, 1900
13					*Tintinnopsis urnula* Meunier, 1910
14			Codonellopsidae	*Codonellopsis*	*Codonellopsis schabi (Brandt, 1906) Kofoid & Campbell, 1929*
15				*Stenosemella*	*Stenosemella ventricosa* (Claparède & Lachmann, 1858) Jörgensen, 1924
16			Metacylididae	*Metacylis*	*Metacylis mediterranea* (Mereschkowsky, 1880) Jörgensen, 1924
17			Ptychocylididae	*Favella*	*Favella ehrenbergii* (Claparède & Lachmann, 1858) Jörgensen, 1924
18			Tintinnidae	*Eutintinnus*	*Eutintinnus apertus* Kofoid & Campbell, 1929
19					*Eutintinnus lusus-undae* (Entz, 1885)
20					*Eutintinnus pectinis* (Kofoid & Campbell, 1929)
21					*Eutintinnus tubulosus* (Ostenfeld, 1899) Kofoid & Campbell, 1939
22				*Salpingella*	*Salpingella decurtata* Jörgensen, 1924
23			Tintinnidiidae	*Leprotintinnus*	*Leprotintinnus pellucidus (Cleve, 1899)*
24				*Tintinnidium*	*Tintinnidium mucicola (Claparède & Lachmann, 1858) Daday, 1887*

Legend:
- (white) Common species to the Black Sea reported previous in the plankton from the Romanian coast
- (yellow) Common species to the Black Sea reported for the first time at the Romanian coast
- (blue) Non-indigenous species to the Black Sea reported for the first time at the Romanian coast
- (magenta) Non-indigenous species reported for the first time to the Black Sea and Romanian coast

One of the novelty elements brought by the present study is the completion of the list of zooplankton species, from the Romanian coast, with a number of 12 tintinnids of which, 5 common species of the Black Sea (*Tintinnopsis baltica, T. compressa, T. karajacensis, T. urnula* and

Tintinnidium mucicola) respectively 7 non-indigenous species (*Tintinnopsis tocantinensis, Codonellopsis schabi, Eutintinnus apertus, E. lasus-undae, E. pectinis, E. tubulosus* and *Salpingella decurtata*). It should be mentioned that two species, *Eutintinnus pectinis* and *Codonellopsis schabi*, are identified for the first time in the Black Sea, on the present study (Fig. 2 and 3). This fact is due on the one hand to the sampling method approached but also to the long period in which the component was not analyzed and which coincides with the penetration of non-indigenous species in the Black Sea (Gavrilova, 2005, Selifonova, 2011a, b).

Codonellopsis schabi was reported for the first time in the Black Sea, in the Romanian coast, on this study (Tabarcea et.al., 2019). Due to the similarity of morphological characters with those of the species *C. morchella*, *C. schabi* has long been synonymous with the first (Paulmier, 1997). *C. schabi* is reported to be present in the Marmara Sea and the Aegean Sea (Balkis & Tokai, 2014).

The non-indigenous species was found exclusively in the 10 m layer, in March 2017, in the 20 and 30 m isobath stations of the Mangalia profile (Fig. 2). Its presence at temperatures below those reported in most habitats inventoried by Gavrilova & Dovgal, 2018, can be exceptional, the temperature in the respective stations being 6.8 ° C and 7.2 ° C. Its abundances it was very low (2 ind/l). This situation requires a future monitoring of this species, to see if it will acclimatize or if the specimens found were most likely transported by ballast water of some ships. The conclusion is reinforced by the fact that the species was not subsequently identified in the collected samples (unpublished data).

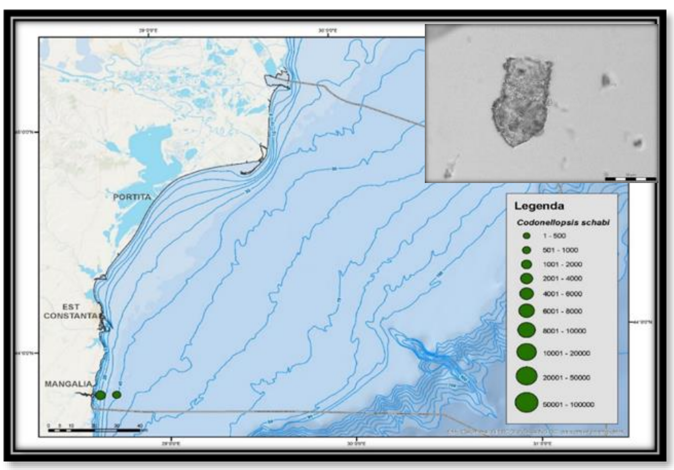


Fig. 2 - Distribution of the *Codonellopsis schabi* specie at the Romanian coast

Eutintinnus pectinis was first identified in the Black Sea, at the Romanian coast, during the present study (Boicenco et al, 2019). During the analyzed period, the species was identified only in August 2016, on all three investigated profiles (Fig. 3). The highest density values were recorded in the coastal station MG1 - 860 ind/l. The average value of the abundance of this species ranked it as a secondary species of the tintinnids community, after the native species *Tintinnopsis minuta*. From the vertical distribution point of view, a dominance was observed in the 0 m layer, in the coastal water body, respectively a dominance in the 10 m layer, in the other two bodies (marine and with variable salinity). The distribution of the species in the horizon 0-10 m, is in accordance with the data from the literature. Also, most of the individuals identified in the samples were parasitized by the parasitic dinoflagellate *Duboscquella cachoni*, the phenomenon being described in the literature as fatal to the tintinnid (Coats &. Heisler, 1989; Coats et al., 1994).

It should be mentioned that the species *E. pectinis* can be considered an acclimatized species in the Black Sea basin, being identified in large and numerous populations in 2020, along the Romanian coast (unpublished data).

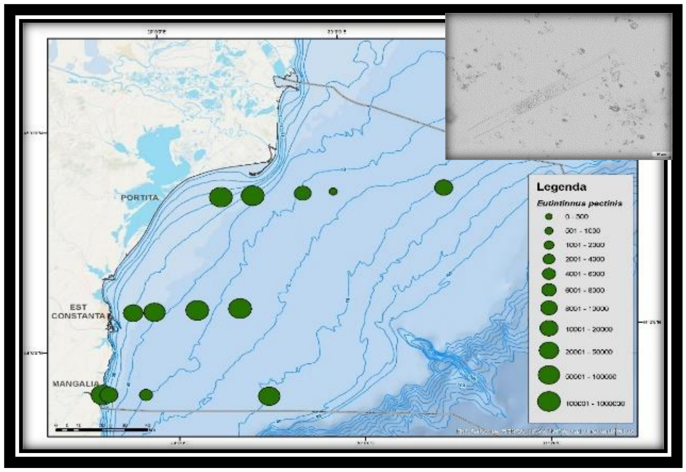


Fig. 3 - Distribution along the Romanian coast of the species *Eutintinnus pectinis*

Tintinnopsis tocantinensis was first identified on the Romanian coast, in August 2016, on Mangalia and Est Constanța profiles, in the present study (Boicenco et al. 2019). From the analyzed data it was observed the clear trend of exponential growth of abundances from Est Constanța profile to the south, on Mangalia profile (Fig. 4a). Maximum densities were recorded in the stations from coastal waters (28 ind/l) compared to those in marine waters (2 ind/l). In the period 2015-2018, the species was absent on the Portița profile, implicitly in the water body with variable salinity.

At the Romanian Black Sea coast, *Eutintinnus tubulosus* was first identified in October 2012, on all three profiles tested in this study, but was not analyzed quantitatively (unpublished data). In the period 2015-2018, the highest densities were found on the Est Constanța profiles (maximum, in the station from coastal water body, CT2 - 54 ind/l) and Mangalia (maximum, in the station from marine water body, MG5 - 63 ind/l) (Fig. 4b). *E. tubulosus* has been identified in samples collected in warm season, in all water bodies.

Eutintinnus apertus was first identified on the Romanian coast, in October 2012, on the Est Constanța profile but the quantitative analysis of the species begins with this study. In August 2016, the specie abundances in coastal and marine water of profiles Mangalia and Est Constanța, were registering low densities of 2 ind/l. In June 2018, the abundances and the distribution of the species in the same area, mentioned above, increased significantly, being found in 50% of the samples on the Mangalia profile (compared to 16.6% in 2016) and in 42.8% from the samples from Est Constanța profile, similar to 2016, but with greater abundances. During the analyzed period, the species *E. apertus* was not identified on Portița profile, being absent in the water body with variable salinity (Fig. 4c).

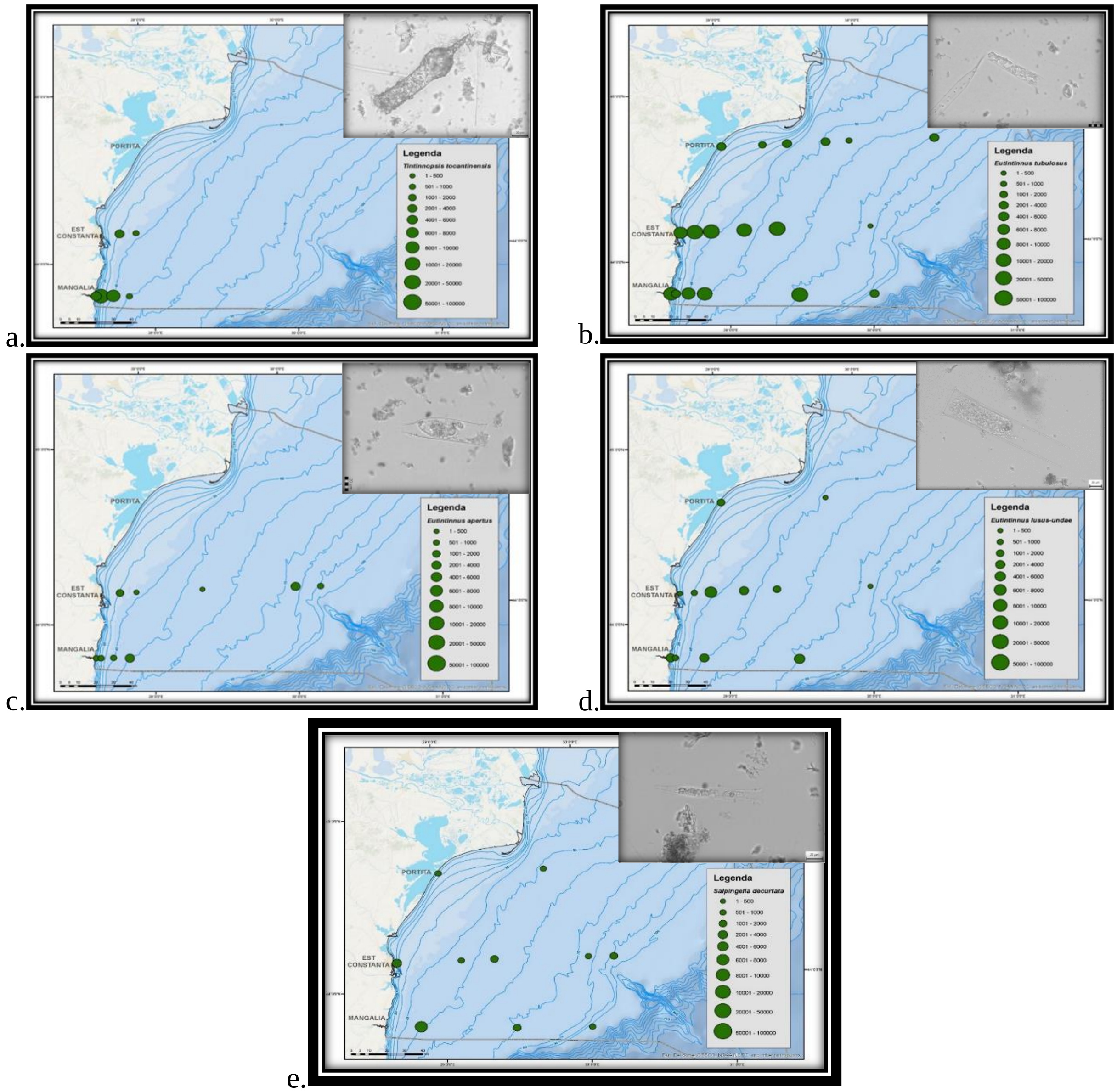


Fig. 4 - Distribution of non-indigenous species identified for the first time at the Romanian coast – a. *Tintinnopsis tocantinensis, b. Eutintinnus tubulosus,* c. *E. apertus,* d. *E. lasus-undae,* e. *Salpingella decurtata*

Eutintinnus lasus-undae, was first identified on the Romanian coast in October 2012, on Mangalia and Est Constanța profiles, but the quantitative analysis of the species begins on the present study (unpublished data). In August 2016, the species was identified in all water bodies. It is a species present in the warm season (June, July, and August). The average abundance, for the analyzed period, registered relatively high values and varied in wide limits (2-20 ind / l) from one station to another (Fig. 4d).

At the Romanian coast, the species *Salpingella decurtata* was identified for the first time in October 2012, on Mangalia, Est Constanța and Portița profiles, but is subject to analysis starting with the present study (unpublished data). During the period analyzed in the thesis, the species was present mainly in November 2017. Occasionally and in very low densities, it also appeared in

August 2016, in three samples from Mangalia profile. Probably, the relatively high water temperatures for November (12°C, on average) favored the development of the species, reaching during this period, an average density of 3ind /l, respectively a maximum of 14 ind/l, in MG4 station (Fig. 4d). Between 2015-2018, S. *decurtata* was present in all three tested water bodies.

Exept *C. schabi*, which is a stenothermic species (Gavrilova & Dovgal, 2018), the other six species identified above are cosmopolitan, with wide ecological values, which allows them to acclimatize more easily in a new ecosystem.

The species *Tintinnopsis baltica*, *T. compressa*, *T. karajacensis*, *T. urnula* and *Tintinnidium mucicola* are reported for the first time on the Romanian coast, on the occasion of the present study, although they are common species in the Black Sea basin. The identification in the present study may also be due to the sampling method adopted (Niskin bottles). Given that they are smaller in size, it is likely that they were not collected from the environment with previously used mesozooplankton nets. In terms of distribution, the species *Tintinnopsis baltica*, *T. compressa*, *T. karajacensis* were present on all three profiles investigated while the species *Tintinnopsis urnula* and *Tintinnidium mucicola* were not identified in the samples from the Mangalia profile (Fig. 5a a, b, c, d, e).

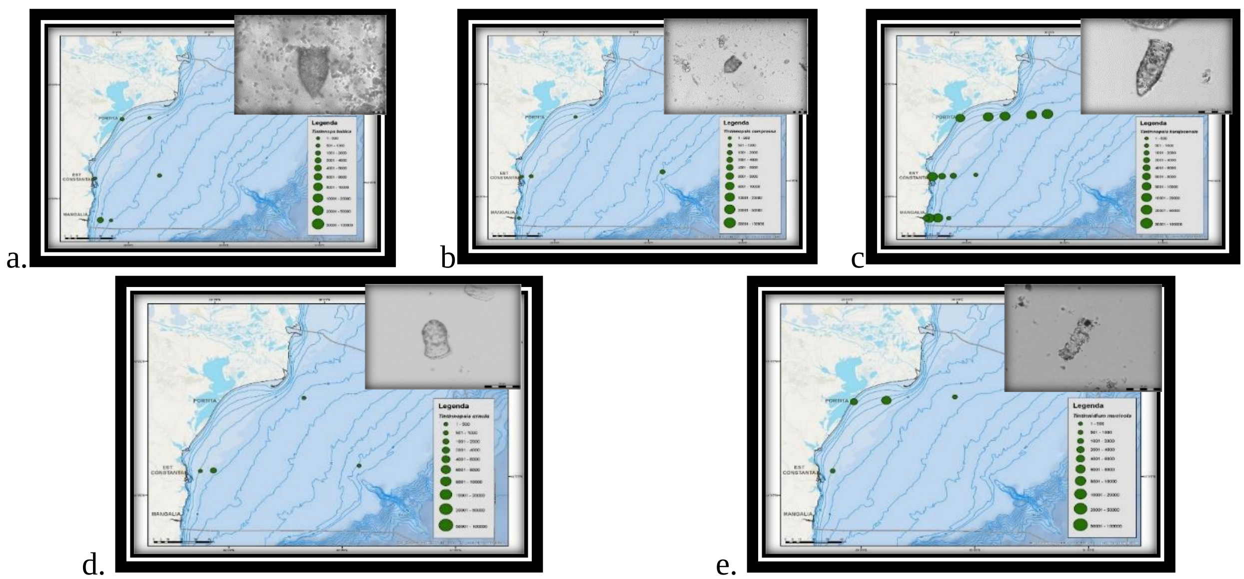


Fig. 5 - Distribution of native species identified for the first time on the Romanian coast – a. *Tintinnopsis baltica, b. T. compressa, c. T. karajacensis, d. T. urnula* și *e. Tintinnidium mucicola*

In the **6th chapter**, the qualitative and quantitative variation of the tintinnids populations in the two studied seasons (warm and cold) was analyzed. The present study demonstrates that both qualitatively and quantitatively, tintinnids populations have a wide variability in terms of distribution along the Romanian coast, on the one hand determined by seasonal annual fluctuations, on the other hand, by the physical gradients of the environmental parameters (temperature, salinity, currents) along the coast and from the shore to the coast.

The abundances of tintinnids in the warm season (June, July and August), between 2015 - 2018, varied widely, with a maximum of 1902 ind/l, in August 2016. The specific diversity of the tintinnid community varied between 6-12 species, depending on the analyzed period. Each period was characterized by the dominance of 2-3 species. In June 2015, 42% of the tintinnids abundances were represented by the non-indigenous species *Eutintinnus tubulosus*, unlike June 2018 when the same species did not reach more than 15%, being the second after *Tintinnopsis minuta*, with more than 82% in abundance (Fig. 6 a, d). In August 2016, the high contribution (41%) of the non-indigenous species *Eutintinnus pectinis* is highlighted, being the second dominant species after the native species *Tintinnopsis minuta* (47%) (Fig. 6b). *Metacylis mediterranea* was constantly found from 2015 to 2017, in June, July and August, with fluctuating populations, being the dominant species in July 2017 (47%), next to *Stenosemella ventricosa* (39%) (Fig. 6c). The dramatic change of dominance in July 2017, which recorded the massive presence of the latter species, generally characteristic of the cold season, is due to the upwelling phenomenon, which affected coastal waters near Est Constanta profile, by suddenly lowering the temperature to 10°C, on the one hand, and the increase of salinity (19 PSU), on the other hand.

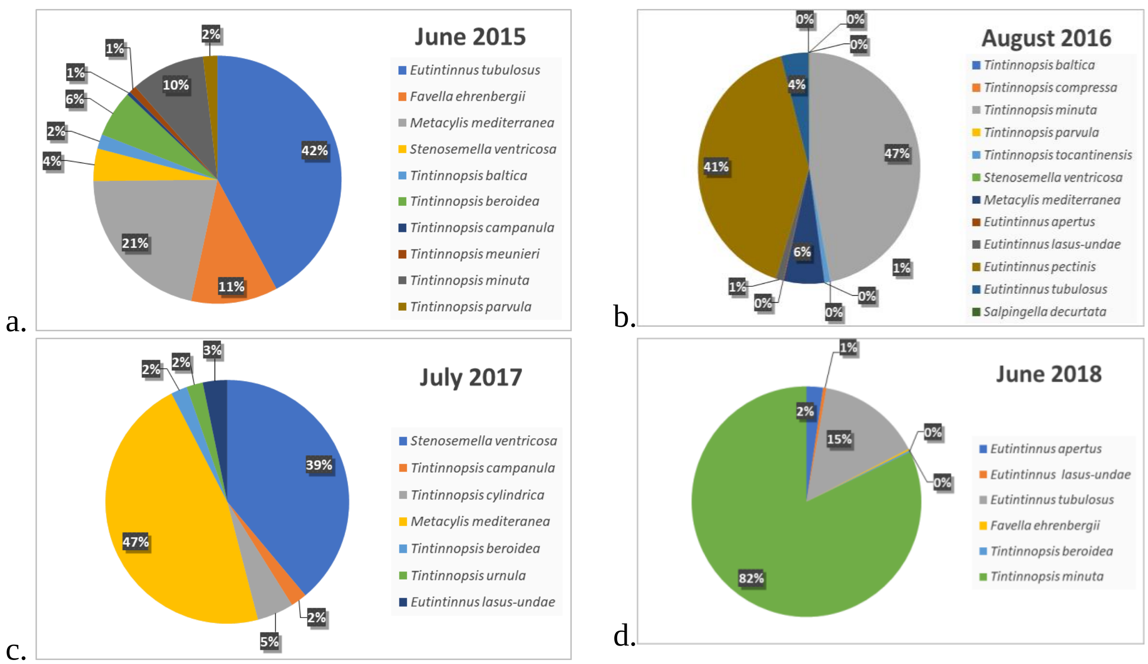


Fig. 6. Proportion of abundances of tintinnids species in the warm season (a, b, c, d), in each analyzed period

Overall, the warm season was characterized by the constancy and quantitative dominance of three species: the native species *Tintinnopsis minuta* (47.65%), respectively the non-indigenous species *Eutintinnus pectinis* (32.7%) and *Eutintinnus tubulosus* (7.4%) (Fig. 7a). The Bray - Curtis dendrogram made on the basis of the abundances of the species identified in the four periods, in the warm season, indicates a statistical differentiation between the periods - June 2018 and August 2016, respectively July 2017 and June 2015 (Fig. 8).

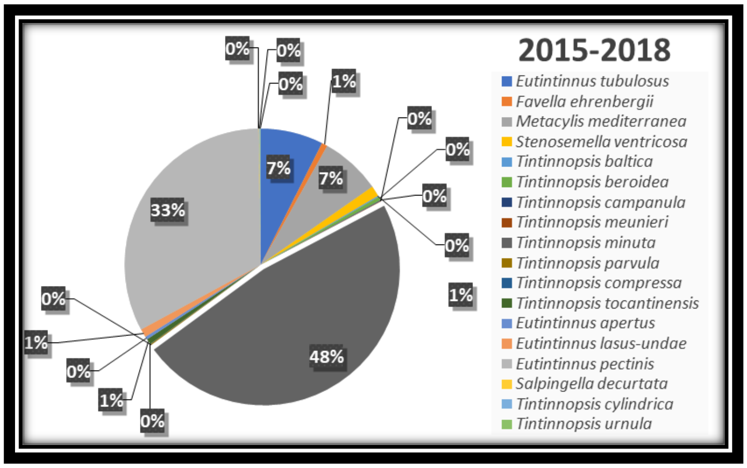


Fig. 7 - Quantitative dominance of tintinnids species in the warm season 2015-2018

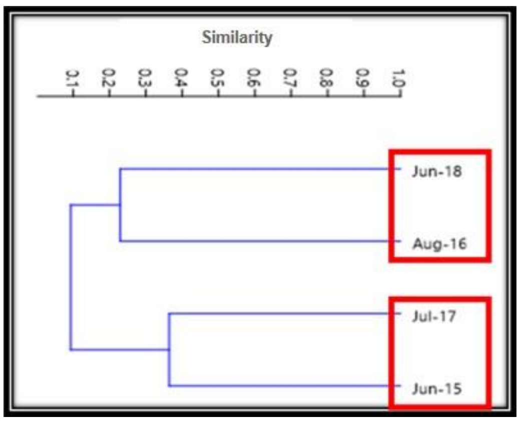


Fig. 8 - Bray-Curtis similarity between study periods, based on abundances (untransformed values) of tintinnids populations, in the warm season

In the cold season (March 2016, 2017 and November 2017), the abundances of the species were extremely small, with maximums of 284 ind/l, reached in March 2017. The specific diversity of the tintinnid community varied between 4-14 species, with the dominance of 3-4 species, depending on the analyzed period. Over 40% of the abundances were represented by *Tintinnopsis parvula* in March 2016 and 2017, greatly influencing the qualitative and quantitative structure of the community (Fig. 9 a, b). In November, the dominant species were *Favella ehrenbergii* (38.58%) and *Salpingella decurtata* (28.35%), species whose maximum development was generally reported in the summer - autumn season. Probably, the relatively high-water temperatures for November (12°C, on average) favored the development of the two species (Fig. 9 c).

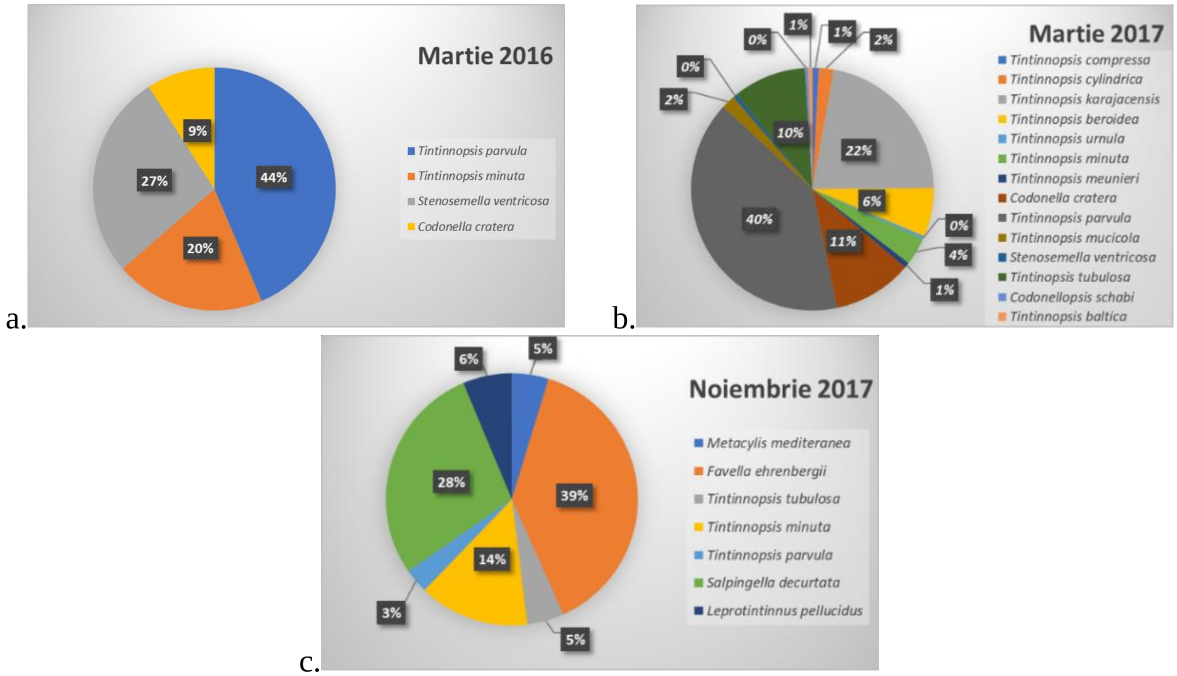


Fig. 9 - Proportion of abundances of tintinnid species in the cold season (a, b, c), in each of the analyzed period

The Bray - Curtis dendrogram made on the basis of the abundances of the species identified in the three periods, during cold season, indicates a reduced similarity between the periods - March 2016 and March 2017 and November 2017, respectively (Fig. 10). This is confirmed by the situation described above.

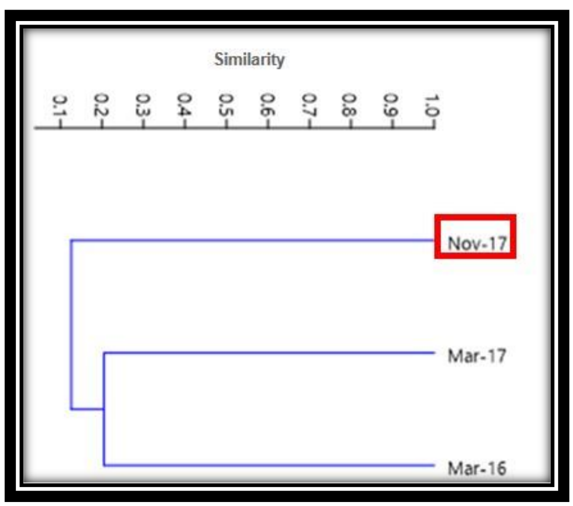


Fig. 10 - Bray-Curtis similarity between study periods, based on abundances (untransformed values) of tintinnids populations, during cold season

Following the seasonal analysis of species diversity, in the period 2015-2018, two associations of 18 species were identified for each analyzed season (warm and cold), of which 12 species common to both seasons (*Tintinnopsis baltica*, *T. beroidea*, *T compressa*, *T. cylindrica*, *T.*

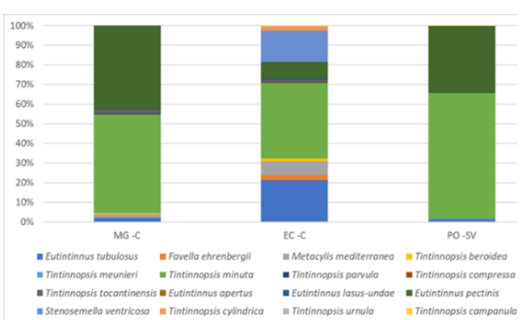
meunieri, *T. minuta*, *T. parvula*, *T.urnula*, *Stenosemella ventricosa*, *Metacylis mediterranea*, *Favella ehrenbergii*, *Salpingella decurtata*) and a number of 6 distinct species, specific to each season. In the warm season the characteristic species identified were *Tintinnopsis campanula*, *T. tocantinensis*, *Eutintinnus apertus*, *E. lusus-undae*, *E. pectinis* and *E. tubulosus* and in the cold season *Codonella cratera*, *Tintinnopsis karajacensis*, *T. tubulosa*, *Codonellopsis schabi*, *Leprot respectively Tintinnidium mucicola* (Tab. 2). The number of species within the same season, varied, depending on the analyzed period and the environmental conditions.

Table 2 - Seasonal associations of tintinnid species during 2015-2018

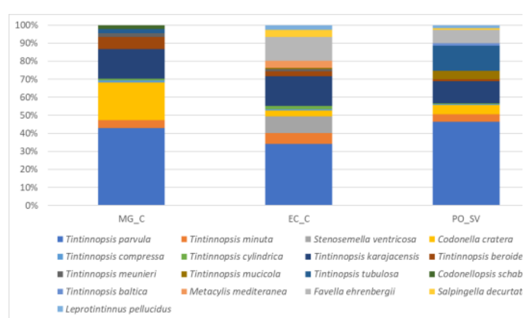
Specii	2015--2018	
	Cold season	Warm season
Codonella cratera		
Tintinnopsis baltica		
Tintinnopsis beroidae		
Tintinnopsis campanula		
Tintinnopsis compressa		
Tintinnopsis cylindrica		
Tintinnopsis karajacensis		
Tintinnopsis meunieri		
Tintinnopsis minuta		
Tintinnopsis parvula		
Tintinnopsis tocantinensis		
Tintinopsis tubulosa		
Tintinnopsis urnula		
Codonellopsis schabi		
Stenosemella ventricosa		
Metacylis mediterranea		
Favella ehrenbergii		
Eutintinnus apertus		
Eutintinnus lasus-undae		
Eutintinnus pectinis		
Eutintinnus tubulosus		
Salpingella decurtata		
Leprotintinnus pellucidus		
Tintinnidium mucicola		

Common species to both analyzed season
Characteristic species to the cold season
Characteristic species to the warm season

In water bodies with variable and coastal salinity, 16 species have been identified in the warm season. *T. minuta*, *E. pectinis*, and *E. tubulosus* constituting between 40 - 60% of the identified species (Fig. 11). In the cold season, 17 species were identified, the largest contribution being: *T. parvula* (over 40%), *Codonella cratera* (5 - 20%) and *T. karajacensis* (10 - 20%) (Fig. 14).



a.

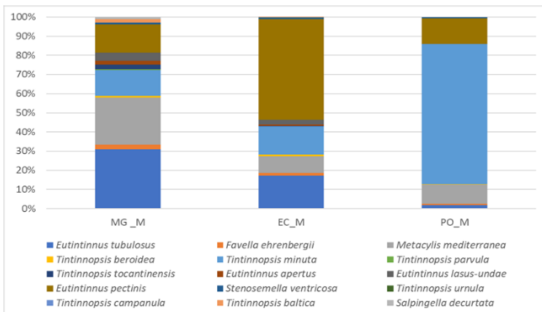


b.

Fig.11 - Proportion of total abundances of tintinide species in coastal water body and in the water body with variable salinity: a. In warm season, b. In cold season

During warm season, in the marine water body, 15 species were identified, most of them present on all three profiles. *Eutintinnus pectinis*, *E. tubulosus*, *Tininnopsis minuta* and *Metacylis mediterranea* are species with a significant contribution in the structure of tintinnid communities, with over 40% in abundance.

a.



b.

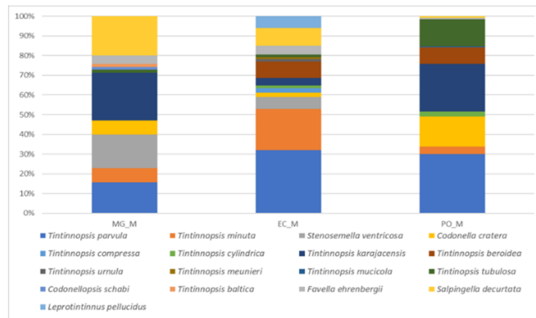


Fig.12 - Proportion of total abundances of tintinide species in marine water body:
a. in warm season, b. in cold season

The water body with variable salinity is the only one with a statistically significant difference in the structure of tintinnid communities compared to those in marine and coastal water bodies on Mangalia and Est Constanta profiles, a feature also revealed by the dendrogram based on the Jaccard similarity, the proportion of common species between the three profiles, respectively water bodies.

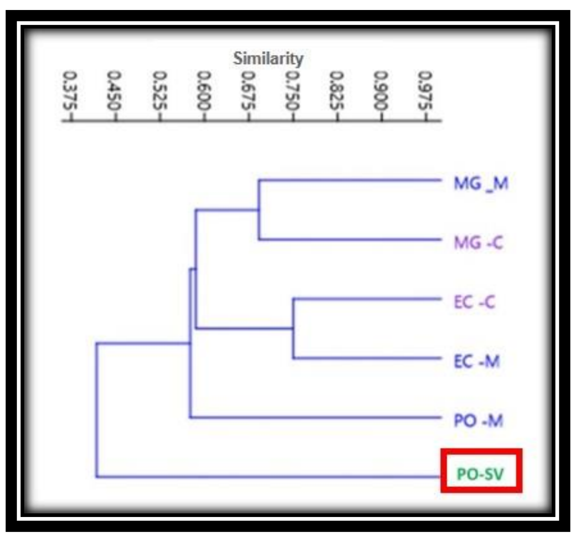


Fig. 13 - Similarity based on the Jacard coefficient between the tintinnid communities in waters with variable salinity (SV), coastal waters (C) and marine waters (M), on Mangalia (MG), Est Constanţa (EC) and Portiţa (PO) profiles

Chapter 7 analyzes the relationships between seasonal environmental factors and the abundance and diversity of tintinnids populations on the Romanian coast. In June 2015, density values of tintinnids populations on Mangalia profile correlated significantly positively (Pearson coefficient, r = 0.93; p <0.05) with temperature, suggesting the development of a community of thermophilic ciliate (Table 3). On Portița profile, population densities were positively correlated with chlorophyll (r = 0.88; p <0.05) and the pH negatively with salinity (r = -0.85; p <0.05) (Table 4).

Table 3 - Correlation (Pearson coefficient) between the tintinnids populations and environmental factors on Mangalia profile, in June 2015

MG June 2015	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	pH	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		0.21	0.11	0.98	0.02	0.20	0.35
Mean biomass (µgC/l)	0.68		0.87	0.87	0.46	0.96	0.83
Chl a (µg/l)	0.79	0.10		0.72	0.06	0.00	0.04
pH	-0.02	0.10	-0.22		0.77	0.52	0.30
T (t°C]	0.93	0.44	0.86	0.18		0.14	0.33
S (PSU)	-0.68	0.03	-0.98	0.39	-0.75		0.01
O2 (µM)	0.54	-0.13	0.90	-0.58	0.56	-0.95	

Table 4 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Portița profile in June 2015

PO June 2015	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	pH	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		0.14	0.02	0.03	0.15	0.03	0.68
Mean biomass (µgC/l)	0.67		0.35	0.16	0.10	0.14	0.27
Chl a (µg/l)	0.88	0.46		0.01	0.06	0.00	0.89
pH	0.85	0.65	0.93		0.02	0.00	0.94
T (t°C]	0.66	0.73	0.80	0.89		0.01	0.63
S (PSU)	-0.85	-0.67	-0.95	-0.95	-0.93		0.63
O2 (µM)	0.22	0.54	0.07	0.04	0.25	-0.25	

In August 2016, the density of tintinnids was significantly negatively correlated with salinity, both on Est Constanța and Portița (r = - 0.93, respectively r = -0.91; p <0.05) (Table 5). On Mangalia profile, they were significantly positively correlated with temperature (r = 0.99, respectively r = 0.97; p <0.05) (Table 6).

Table 5 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Portița profile, in August 2016

PO August 2016	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		0.12	0.07	0.31	0.001	0.73
Mean biomass (µgC/l)	0.70		0.63	0.82	0.064	0.95
Chl a (µg/l)	0.76	0.24		0.18	0.12	0.62
T (t°C]	-0.49	0.11	-0.62		0.32	0.45
S (PSU)	-0.91	-0.78	-0.69	0.48		0.81
O2 (µM)	0.17	0.028	-0.25	-0.38	-0.12	

Table 6 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Mangalia profile, in August 2016

MG August 2016	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		4.1E-05	0.84	0.00	0.14	0.43
Mean biomass (µgC/l)	0.99		0.94	0.00	0.17	0.44
Chl a (µg/l)	-0.11	-0.04		0.64	0.16	0.19
T (t°C]	0.99	0.97	-0,25		0.10	0.39
S (PSU)	0.68	0.64	-0,65	0.72		0.04
O2 (µM)	-0.40	-0.40	0.62	-0.43	-0.84	

In July 2017, the densities and biomasses of ciliates on Est Constanţa profile were significantly positively correlated with chlorophyll a (r = 0.96; r = 0.93) and negatively with temperature (r = - 0.79; r = - 0.82) while on Portiţa profile, the density was negatively correlated with salinity, due to its lower values (Tables 7 and 8).

Table 7 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Est Constanța profile, in July 2017

EC July 2017	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	pH	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		6E-05	5E-04	0,06	0,03	0,10	0,30
Mean biomass (µgC/l)	0.98		2E-03	0,06	0,02	0,05	0,20
Chl a (µg/l)	0.96	0.93		0.09	0.02	0.13	0.44
pH	-0.73	-0.74	-0.69		0.04	0.01	0.18
T (t°C]	-0.79	-0.82	-0.84	0.77		0.03	0.15
S (PSU)	0.68	0.76	0.62	-0.87	-0.80		0.12
O2 (µM)	0.50	0.58	0.35	-0.58	-0.60	0.64	

Table 8 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Portița profile, in July 2017

PO July 2017	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		0.12	0.08	0.31	0.01	0.73
Mean biomass (µgC/l)	0.70		0.63	0.82	0.06	0.96
Chl a (µg/l)	0.77	0.25		0.18	0.13	0.63
T (t°C]	-0.50	0.12	-0.63		0.33	0.46
S (PSU)	**-0.92**	-0.78	-0.69	0.49		0.81
O2 (µM)	0.18	0.03	-0.26	-0.38	-0.13	

In June 2018, significant correlations of tintinnids abundances with environmental parameters (except temperature) were observed especially on Mangalia profile (Table 9). On Portiţa profile, densities and biomasses were correlated only with oxygen (r = -0.96, respectively r = - 0.89) (Table 10).

Table 9 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Mangalia profile, in June 2018

MG June 2018	Mean density (ind/l)	Mean biomass (µgC/l)	T (t°C]	S (PSU)	O2 (µM)	pH	Chl a (µg/l)
Mean density (ind/l)		0.000	0.14	0.030	0.0003	0.0002	3.84E-05
Mean biomass (µgC/l)	0.99		0.14	0.012	0.0003	1,4E-05	8,2E-05
T (t°C]	0.67	0.67		0.449	0.06	0.128	0.1182
S (PSU)	-0.85	-0.90	-0.38		0.04	0.0147	0.027
O2 (µM)	0.98	0.98	0.77	-0.83		0.0001	0.00018
pH	-0.98	-0.99	-0.69	0.89	-0.98		0.00024
Chl a (µg/l)	0.99	0.99	0.70	-0.86	0.988	-0.98	

Table 10 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Portița profile, in June 2018

PO June 2018	Mean density (ind/l)	Mean biomass (µgC/l)	Chl a (µg/l)	pH	T (t°C]	S (PSU)	O2 (µM)
Mean density (ind/l)		0.11	0.07	0.91	0.10	0.55	0.01
Mean biomass (µgC/l)	0.80		0.51	0.44	0.15	0.77	0.05
Chl a (µg/l)	0.85	0.39		0.56	0.19	0.18	0.15
pH	0.07	0.46	-0.36		0.61	0.67	0.67
T (t°C]	0.81	0.74	0.70	0.31		0.46	0.03
S (PSU)	-0.36	0.18	-0.71	0.26	-0.44		0.63
O2 (µM)	-0.96	-0.89	-0.75	-0.26	-0.92	0.29	

In March 2016, neither of the two analyzed profiles (Mangalia and Est Constanţa) presented a statistical correlation between the density and biomass values of the tintinnids populations and environmental parameters. In March 2017, temperature, salinity, and pH significantly influenced tintinnids densities (Table 11).

Table 11 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on Est Constanța profile, in March 2017

EC March 2017	Mean density (ind/l)	Mean biomass (µgC/l)	T (t°C]	S (PSU)	O2 (µM)	pH	Chl a (µg/l)
Mean density (ind/l)		1,9E-05	9,E-03	0,03	0,12	7,E-03	0,09
Mean biomass (µgC/l)	0.99		0,01	0,05	0,09	0,02	0,10
T (t°C]	-0.88	-0,87		6,E-03	0,03	3,E-03	0,04
S (PSU)	-0.79	-0,75	0,90		0,15	6,E-03	6,E-04
O2 (µM)	-0.64	-0,68	0,80	0,60		0,07	0,40
pH	0.89	0,85	-0,92	-0,90	-0,72		0,05
Chl a (µg/l)	0.69	0.65	-0.77	-0.96	-0.38	0.76	

In November 2017, on Constanţa and Portiţa profiles, the densities, and biomasses of tintinnids were negatively correlated with temperature and salinity, on the one hand, and positively with oxygen and chlorophyll values (Table 12).

Table 12 - Correlation (Pearson coefficient) between the tintinnids population and environmental factors on the Est Constanța profile, in November 2017

EC November 2017	Mean density (ind/l)	Mean biomass (µgC/l)	T (t°C]	S (PSU)	O2 (µM)	pH	Chl a (µg/l)
Mean density (ind/l)		0.00	0.01	0.02	0.02	0.40	0.12
Mean biomass (µgC/l)	0.97		0.00	0.00	0.01	0.27	0.04
T (t°C]	-0.86	-0.91		0.00	0.01	0.07	0.01
S (PSU)	-0.83	-0.91	0.93		0.00	0.07	0.00
O2 (µM)	0.83	0.89	-0.89	-0.94		0.20	0.00
pH	-0.38	-0.48	0.71	0.72	-0.55		0.06
Chl a (µg/l)	0.65	0.79	-0.87	-0.94	0.91	-0.74	

The last chapter of the thesis (chapter 8) presents the results of the analysis of the vertical distribution, in the 0m and 10m depth layer, of the tintinnids community from the Romanian coast. In June 2015, on all three profiles, there is a great similarity between the specific diversity in the two layers. Except for two species, found only on the surface (*Tintinnopsis baltica* and *T. parvula*), the others appear almost uniformly distributed in the 0-10 m layer.

In August 2016, the differences between the two layers were insignificant, not being identified a pattern of the distribution of tintinnids populations, whether we refer to diversity or abundance.

In July 2017, on the Est Constanţa profile, unlike the Portiţa profile, there is a difference in the distribution of tintinnids species (Fig. 14). This is the result of thermal stratification in the coastal stations EC1 and EC2. Notable is the dominance of the species *Metacylis mediterranea* in surface waters, as well as the species *Stenosemella ventricosa*, in the waters of the 10 m horizon, in the waters of EC1 and EC2 stations, the latter being a typical cold water stenothermic species.

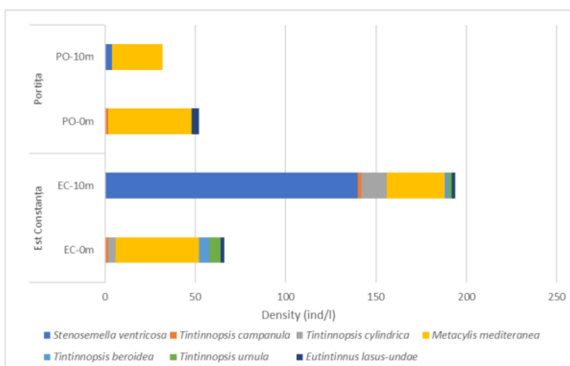


Fig 14 - Distribution of densities (ind./l) of tintinnids species in 0 and 10 m layers, in July 2017

In June 2018, the distribution of tintinids in the two layers differed only in terms of quantity, the most obvious being the situation encountered on the Mangalia profile, where both densities and biomass were 5-6 times higher in the 0m layer (Fig. .15).

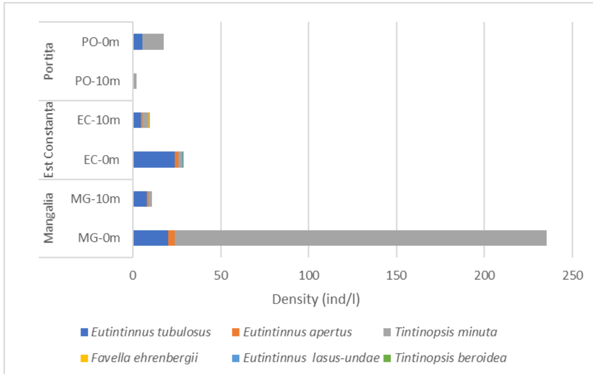


Fig 15 - Distribution of densities (ind./l) of tintinnids species in 0 and 10 m layers, in June 2018

In March 2017, the distribution of tintinids on deep horizons was similar on each profile, significant differences being recorded between Portiţa profile and the other two, in terms of densities and biomass (Fig. 16).

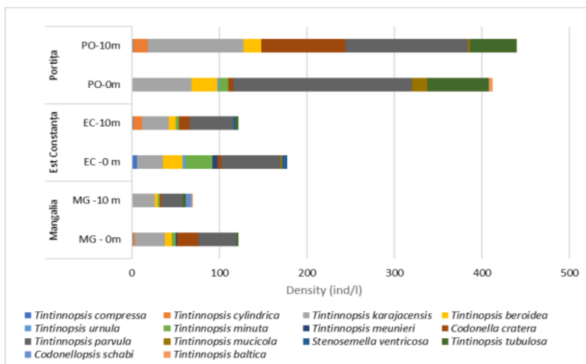


Fig 16 - Distribution of densities (ind./l) of tintinnids species in 0 and 10 m layers, in March 2017

In November 2017, the distribution of tintinnids populations in the two layers is noted by the higher abundances in the 10 m layer, the dominant species in the autumn plankton being *Favella ehrenbergii* and *Salpingella decurtate* as density and *L. pellucidus* as biomass (Fig. 17).

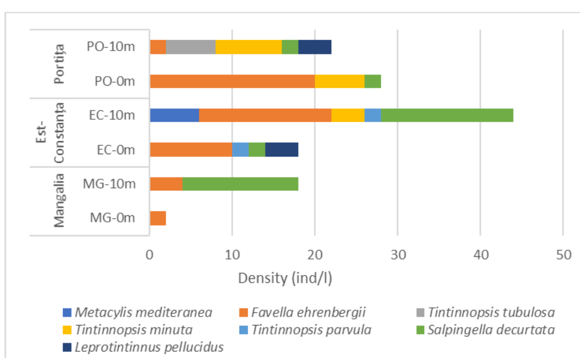


Fig 17 - Distribution of densities (ind./l) of tintinnids species in 0 and 10 m layers, in November 2017

In the 0m layer, in the period 2015-2018, the qualitative and quantitative composition of tintinnids varied, especially seasonally, differing, in general, 4 seasonal associations being established (similarity Ward linkage): August 2016; March 2016 and 2017; June 2018 and July 2017; November 2017 - June 2015 (Fig. 18). The distribution and structure of tintinnids populations were also dramatically different, in November 2017, on Mangalia and Portiţa profiles, compared to those of August 2016 and March 2017.

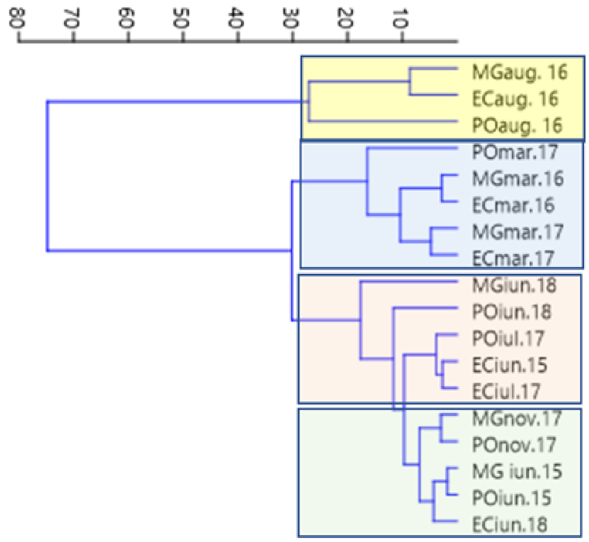


Fig. 18. Similarity between seasons and profiles based on Euclidean distance (Ward linkage)

In the 10m layer, during 2015-2018, in general, the abundances of tintinnids were significantly higher than in the surface layer (T test; $t = -2.78$; $p < 0.01$), noting the species *Metacylis mediterranea*, *Eutintinnus pectinis* and *E. tubulosus*. Mainly, 4 seasonal associations were highlighted, different from those in the 0m horizon: August 2016; March 2017; November 2017; June 2015 and 2018 (Fig. 19).

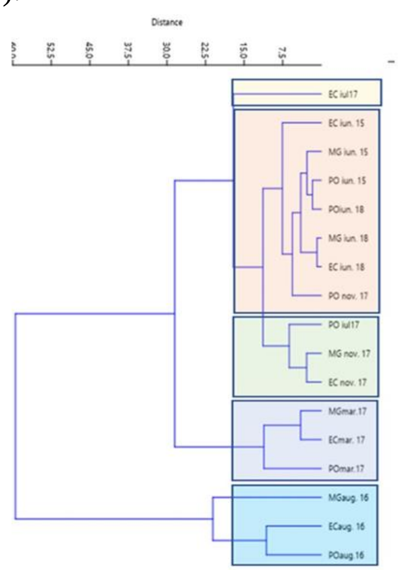


Fig. 19. The similarity between seasons and profiles based on Euclidean distance (Ward linkage)

Conclusions

1. The paper is based on 197 biological samples, collected from the 0m and 10m layers, during seven expeditions carried out between 2015 and 2018, along the Romanian coast.
2. The study led to the identification of 24 species, which belong to 10 genera and six taxonomic families.
3. The list of species from the Romanian coast was completed with 12 species:
   - two non-indigenous species, first reported in the Black Sea basin - *Eutintinnus pectinis* and *Codonellopsis schabi*
   - five non-indigenous species entered the Black Sea basin in the last two decades - *Tintinnopsis tocantinensis, Eutintinnus apertus, Eutintinnus. lasus-undae, Eutintinnus tubulosus, Salpingella decurtata;*
   - five species, common to the Black Sea basin, but which have not been previously mentioned on our coast - *Tintinnopsis baltica, Tintinnopsis compressa, Tintinnopsis. karajacensis, Tintinnopsis urnula* and *Tintinnidium mucicola*;
4. In the period 2015-2018, the species with the highest frequency in the investigated areas are *Tintinnopsis minuta* (86%), *Tintinnopsis parvula* (71%), *Stenosemella ventricosa* (71%), *Metacylis mediterranea* (57%), *Tintinnopsis beroidea* (57%) while at the opposite pole, with a frequency of 14% are *Tintinnidium mucicola, Tintinnopsis karajacensis, Tintinnopsis tocantinensis, Codonellopsis schabi, Eutintinnus pectinis, Leprotintinnus pellucidus.*
5. In each of the analyzed seasons (warm and cold), the populations of tintinids from the Romanian coast was characterized by 18 species, of which:
   - 12 common to both analyzed seasons - *Tintinnopsis baltica, Tintinnopsis beroidea, Tintinnopsis compressa, Tintinnopsis cylindrica, Tintinnopsis meunieri, Tintinnopsis minuta, Tintinnopsis parvula, Tintinnopsis urnula, Stenosemella ventricosa, Metacylis decenurt, Favella ehrenbergii, Salpingella decurtata*;
   - 6 species characteristic of warm season - *Tintinnopsis campanula, Tintinnopsis tocantinensis, Eutintinnus apertus, Eutintinnus lusus-undae, Eutintinnus pectinis, Eutintinnus tubulosus*;
   - 6 species characteristics of cold season - *Codonella cratera, Tintinnopsis karajacensis, Tintinnopsis tubulosa, Codonellopsis schabi, Leprotintinnus pellucidus, Tintinnidium mucicola.*
6. In the cold season, the maximum abundances of the species were about seven times lower than in the warm season
7. A correlation of tintinnids abundances was observed:
   - predominantly positive with temperature and chlorophyll respectively negative with salinity, in the warm season.
   - predominantly negative with temperature and salinity, in the cold season.
8. Regarding vertical distribution, 4 seasonal associations were generally highlighted:
   - in the 0m layer - August 2016; March 2016 and 2017; June 2018 and July 2017; November 2017 - June 2015.
   - in the 10m layer - August 2016; March 2017; November 2017; June 2015 and 2018.
9. During 2015-2018, the tintinnids populations from the Romanian coast, registered a wide variability both qualitatively and quantitatively, on the one hand determined by the seasonal annual fluctuations, on the other hand, by the physical gradients of the

environmental parameters (temperature, salinity, currents) along and from the shore to the coast.

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