

Occurrence of the rare microflagellates *Prorocentrum arcuatum* Issel and *Hermesinum adriaticum* Zacharias in the marine Lake Rogoznica (eastern Adriatic coast)

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*The marine Lake Rogoznica is a small, karstic habitat with potential anthropogenic influence, situated in the eastern-central Adriatic coast. In this naturally eutrophic, hypoxic and periodically anoxic lake, an investigation of the microplankton community was performed during the 1995-1998 period when the ecology of the lake was greatly influenced by strong stratification and the appearance of anoxic conditions in the entire water column. The microplankton community (maximum abundance 1.06×10^7 cells L^{-1}) was composed of 40 taxa, mainly diatoms (62.5%) and dinoflagellates (29%). The research provided evidence of exceptionally dense populations and the seasonally-recurrent appearance of two rare microflagellates: *Prorocentrum arcuatum* Issel (dinoflagellate) and *Hermesinum adriaticum* Zacharias (heterotrophic microflagellate). The development of *H. adriaticum* and *P. arcuatum* mostly increased during the summer under conditions of nitrate deficiency, while the co-dominant diatom *Chaetoceros curvisetus* Cleve appeared during the spring when higher nitrate concentrations were present. Canonical Correspondence Analysis (CCA) indicated the importance of temperature, oxygen, silicate and nitrate on the incidence of these dominant species. The investigation of these microflagellates has allowed for the gaining of insight into their ecophysiological characteristics as well as the monitoring of their distribution in the Mediterranean, which has possibly expanded due to climate changes.*

Key words: *Hermesinum adriaticum*, *Prorocentrum arcuatum*, flagellates, seasonality, marine lake, Rogoznica

INTRODUCTION

Isolated marine systems (CRISAFFI, 1955; SOROKIN & DONATO, 1975; BULJAN & ŠPAN, 1976) provide extreme conditions that do not change over longer periods of time, with reduced numbers of species and the occurrence of rare organisms (KRŠINIĆ, 2003). In those natural laboratories, repetitive field measurements of biological and physical attributes are possible, thus leading to some authors comparing them to chemostats (HAMNER & HAMNER, 1998; CER-RANO *et al.*, 2006).

The small, periodically stratified, marine Lake Rogoznica has very peculiar ecological characteristics, quite different from those of other coastal salt lakes in the Mediterranean (KRŠINIĆ *et al.*, 2000; CIGLENEČKI *et al.*, 2005). In the lake, allochthonous organic matter recycles microbiologically, resulting in oxygen consumption, anoxia in the bottom layer and the formation of a chemocline (CIGLENEČKI *et al.*, 1998; CIGLENEČKI *et al.*, 2005). Under certain conditions, hydrogen sulphide comes to the surface of the lake. Due to the extreme ecological conditions that prevail in this lake, phyto- and zooplankton populations are represented by a relatively small number of species. However, some of these populations are denser than those in the surrounding sea (KRŠINIĆ *et al.*, 2000; CIGLENEČKI *et al.*, 2005).

Prorocentrum arcuatum, a rare, photosynthetic dinoflagellate is known from the eastern Mediterranean (SCHILLER, 1933; RAMPI & BERNHARD, 1980), and according to recent research in the Adriatic Sea it has been detected only in the marine Lake Rogoznica (VILIČIĆ *et al.*, 1997). *Hermesinum adriaticum*, a heterotrophic and mixotrophic microflagellate (due to the presence of photosynthetic endosymbionts) (HARGRAVES & MILLER, 1974), belongs to Ebriales, a protistan group with uncertain taxonomic position, with an internal siliceous skeleton (DEFLANDRE, 1952; PREISIG, 1994). It was first described in the northern Adriatic Sea (ZACHARIAS, 1906) and is now known as a rare neritic, tropical and temperate species (SOURNIA, 1986). *Hermesinum adriaticum* has been found in the Black Sea (BODEANU,

1969) and south-eastern Mediterranean (HALIM, 1960; VILIČIĆ *et al.*, 1997; CAROPPO *et al.*, 1999), but not in the northern Mediterranean (TRAVERS & TRAVERS, 1968). It can be found in Pettaquamscutt River in Rhode Island (HARGRAVES & MILLER, 1974), the lower Chesapeake Bay (RHODES & GIBSON, 1981) and in the saline lake Salton Sea (TAPPAN, 1980). The greatest known abundance of *H. adriaticum* has been found in the mixohaline extension of the Narragansett Bay (25×10^4 cells L^{-1}) (HARGRAVES & MILLER, 1974) and in the Salton Sea, California (45×10^4 cells L^{-1}) (CARPELAN, 1961). The centric diatom *Chaetoceros curvisetus* is a common marine species, usually appearing in early spring-late autumn in the eastern coastal Adriatic (VILIČIĆ *et al.*, 1998).

The objective of this paper is to present the seasonal distribution of two common microflagellates (*P. arcuatum*, *H. adriaticum*) and one co-dominant centric diatom (*C. curvisetus*), as well as the peculiar environmental conditions that maintained such a specific microplankton community in the 1995-1998 period. The chosen investigated period is characterised by strong stratified conditions on one side of the lake and appearance of anoxia in the entire water column on the other side. Because the flagellates studied are rare in the surrounding oligotrophic coastal sea (unpublished data), Lake Rogoznica proves to be an ideal site to study their ecology. These two flagellates are of interest because of the current lack of knowledge concerning their physiological-ecological responses.

MATERIAL AND METHODS

Study area

The marine Lake Rogoznica is located on a small peninsula, in the vicinity of the village of Rogoznica and the city of Šibenik, in the central part of the eastern karstic coast of the Adriatic Sea (Fig. 1). The studied area is sparsely inhabited. The surface area of the lake is 10 300 m² and the maximum depth is about 15 m. Due to its weak connection with the sea through the surrounding porous karstic rocks, tides are

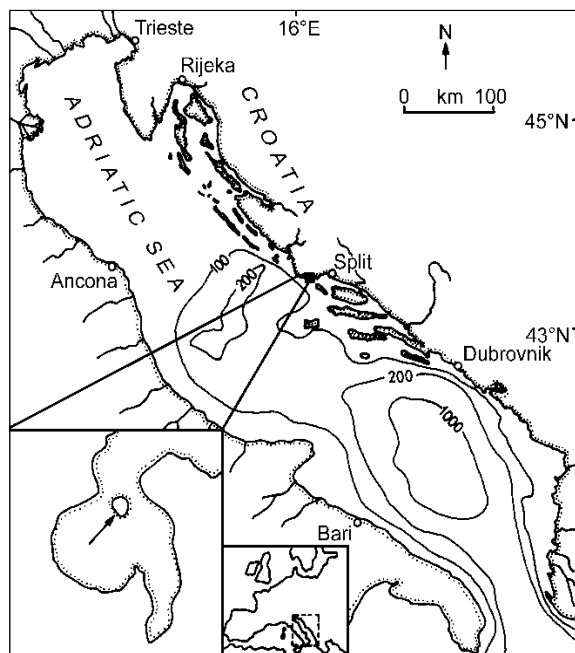


Fig. 1. Position of the marine Lake Rogoznica in the Eastern Adriatic coast

detectable in the lake. A certain delay of tidal maxima/minima of approximately two hours as compared with those in the nearby coastal sea was noticed in the lake. The position of the lake protects it from the effect of winds. Meteorological conditions, which include the quantity of rainfall and a cold or mild winter, greatly influence the stratification and mixing of the water column in Rogoznica Lake (CIGLENEČKI *et al.*, 1998; CIGLENEČKI *et al.*, 2005). The water column can be divided into an upper oxic zone (mostly above 9 m depth) and a lower anoxic zone (mostly below 9 m depth) rich in reduced sulphur species (up to 900 μM) (CIGLENEČKI *et al.*, 1996; CIGLENEČKI *et al.*, 2005). An increasing gradient of phosphate, nitrate, nitrite, ammonia, silicate, iodine and sulphur species concentrations has been detected in the layer of a sharp chemocline between 6 and 10 m (CIGLENEČKI *et al.*, 1996; STIPANIČEV & BRANICA, 1996; VILIČIĆ *et al.*, 1997; KRŠINIĆ *et al.*, 2000; CIGLENEČKI *et al.*, 2005). The saline characteristics of the deeper layers of the lake are influenced by the surrounding sea due to the porosity of the karst. During most of the year, vertical, physical and chemical stratification prevails, giving the lake meromictic characteristics.

Sampling and analyses

Water samples for the analyses of phytoplankton were collected approximately monthly in the period from October 1995 to December 1998 (25 field trips). Samples were collected in 5 L Niskin bottles, in the middle of the lake. Eleven discrete samples of the water column were collected on each sampling date (0.2, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 m). The samples were preserved in a 2% (final concentration) neutralised formaldehyde solution. Cell counts were obtained by the inverted microscope method (UTERMÖHL, 1958). Subsamples of 50 ml were analysed microscopically after sedimentation for 24 h. Microplankton (MICRO) cells (longer than 20 μm) were counted under a magnification of 400 X (1-2 transects), as well as 200 and 100 X (transects along the rest of the counting chamber base plate). The precision of the counting method was $\pm 10\%$.

Salinity was determined by argentometric titration, temperature by an inverted thermometer, and oxygen by Winkler titration. Nutrient concentrations were determined following standard methods (STRICKLAND & PARSONS, 1972; IVANČIĆ & DEGOBBIS, 1984) using the Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. Salinity, oxygen and nutrients were measured once for each depth per field survey.

The vertical averaged potential energy anomaly, Φ (SIMPSON *et al.*, 1982), was used as a bulk index of stratification. The value, Φ (J m^{-3}), is the energy input required to cause complete vertical mixing of the water column and is thus directly proportional to the strength of stratification. For data analysis Grapher 5, Surfer 6,7 and Statistica 7 programs were used.

Canonical Correspondence Analysis (CCA; (TER BRAAK, 1995)) was used to display environment – phytoplankton relationships in the period from July 1996 to December 1998. In the species data matrix, only dominant species relevant for this study were used (*P. arcuatum*, *H. adriaticum*, *C. curvisetus* and *Eunotia* sp.). A logarithmic transformation ($\log(x+1)$) was used on the phytoplankton abundance data to obtain the normal distribution. In the samples that had

no target species present, the log abundance value of 0.1 was used.

RESULTS

Temperature in the marine Lake Rogoznica ranged from 8 to 28.2 °C and salinity from 23 to 38.8 (Fig. 2). The index of stratification decreased during the June - October 1997 period (Fig. 3). Stratified conditions were most evident in 1996 (March-April, October), February-March 1997, and January and July 1998.

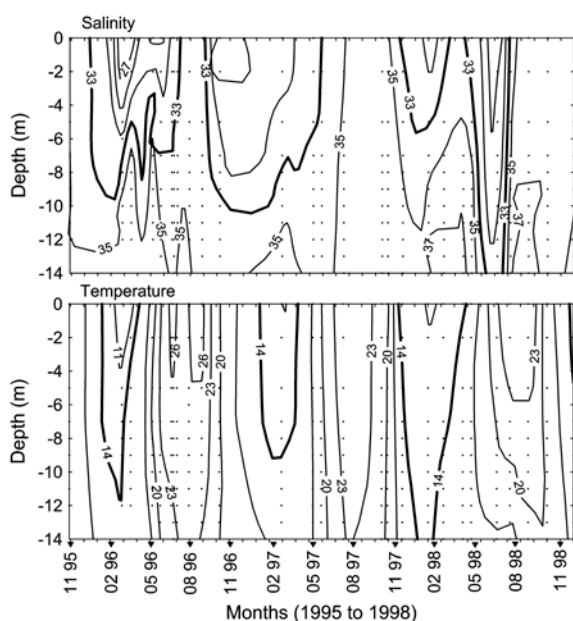


Fig. 2. Depth vs. time contour plots for salinity (PSU) and temperature (°C) in the marine Lake Rogoznica

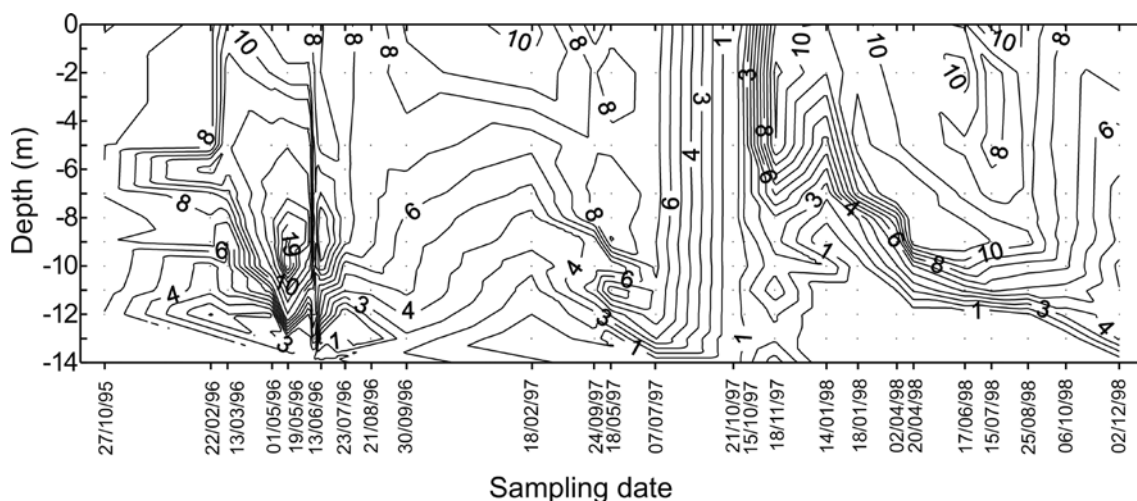


Fig. 4. Depth vs. time contour plots for oxygen concentrations (mg L^{-1}) in the marine Lake Rogoznica

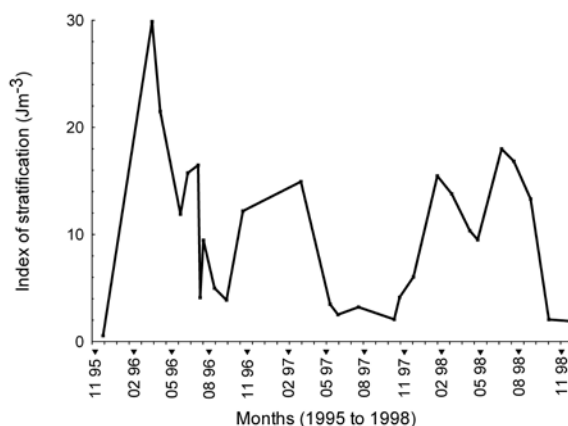


Fig. 3. Seasonal variations of the stratification indexes in the marine Lake Rogoznica

The maximum oxygen concentration of 21 mg L^{-1} was detected in May 1996 in the 9-10 m layer (Fig. 4), while anoxic conditions were frequently detected below 12 m depth. In October 1997, anoxia was detected throughout the entire water column.

The vertical distribution of nutrients was characterised by the positive gradient of orthophosphate, ammonia and silicate from the surface to the bottom, with a sharp increase in concentrations, mostly below the depth of 9 m (Fig 5). In contrast to orthophosphates, which exhibited strong concentration gradients through the water column (0.02-8.26 $\mu\text{mol L}^{-1}$ in May 1997), vertical concentration gradients of nitrates were lower (6.95-1.54 $\mu\text{mol L}^{-1}$ in September 1996). In November 1997 concentrations of nutrients,

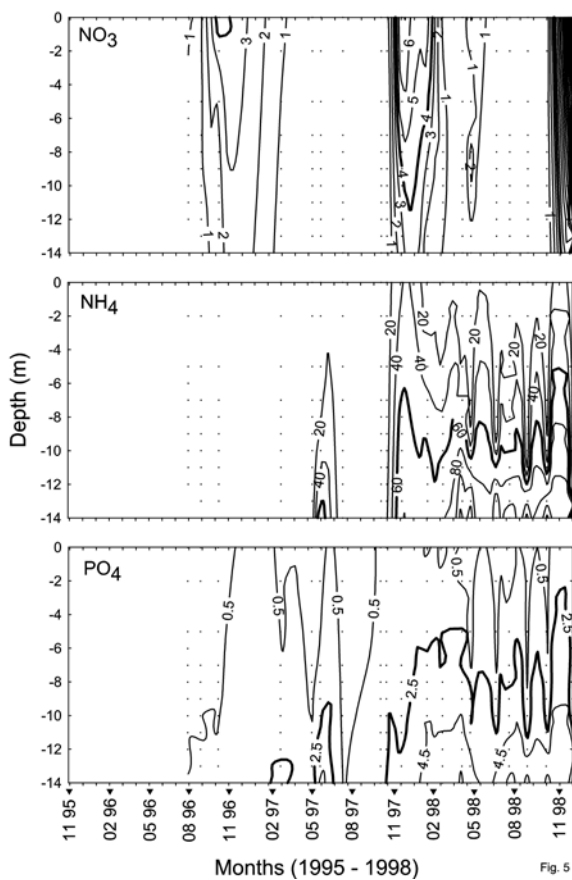


Fig. 5. Depth vs. time contour plots for nitrates, ammonia and orthophosphates ($\mu\text{mol L}^{-1}$) in the marine Lake Rogoznica

particularly ammonia and orthophosphates, were significantly higher ($p < 0.01$) than in October of the same year, and remained high during the rest of the study period.

N:P ratios showed minimum values in summer months while a maximum ratio value of 51 was measured in November 1997. Silicate concentrations ranged from 0.44 to 66.22 $\mu\text{mol L}^{-1}$ in the 0-5 m layer, 9.64 to 175.67 $\mu\text{mol L}^{-1}$ in the 7-11 m layer, and 4.9 to 301.57 $\mu\text{mol L}^{-1}$ in the 12-14 m layer.

The microplankton population was mainly composed of Bacillariophyceae (62.5% of the total taxa) and Dinophyceae (29%) (Table 1). Dominant species were *C. curvisetus*, *P. arcuatum* (Fig. 6) and *H. adriaticum* (Fig 7). The diatoms *Pseudo-nitzschia* spp. and *Eunotia* sp. were sporadically abundant (Table 1).

Higher microplankton abundances ($> 10^6$ cells L^{-1}) were generally detected in summer months (June 1996, July 1997) and autumn (October 1996) in the upper layer (< 9 m depth) of the water column (Fig. 8). After anoxic conditions in October 1997, to the end of studied period, a low abundance of microplankton was detected, with the exception of the upper layer where gymnodinoid species developed. Dominant species *Prorocentrum arcuatum* and



Fig. 6. LM of dinoflagellate *Prorocentrum arcuatum*

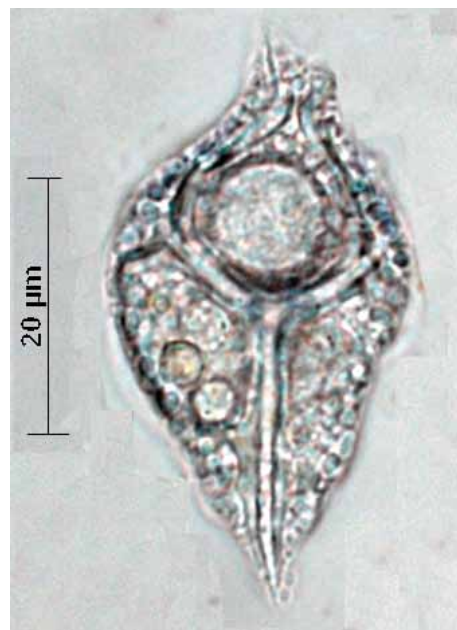


Fig. 7. LM of *Hermesinum adriaticum*

Table 1. List of dominant microphytoplankton taxa, with frequency of findings (F) $\geq 2\%$, and maximum cell abundance ≥ 1000 cells L^{-1} , in Rogoznica lake, in the period from 1995 to 1998. Number of samples = 278

Taxa	F	F (%)	Maximum abundance (cells L^{-1})	Date of recorded maximum
PRYMNESIOPHYCEAE				
<i>Caiyptrosphaera oblonga</i> Lohm.	10	3.6	4 000	OCT 27, 95
DINOPHYCEAE				
<i>Ceratium furca</i> (Ehrenb.) Claparede et Lachmann	14	5.0	3 600	FEB 18, 97
<i>Diplopsalis</i> "group"	10	3.6	2 400	JUN 17, 98
<i>Glenodinium</i> spp.	11	4.0	3 600	APR 29, 97
<i>Gyrodinium</i> spp.	8	2.9	220 000	JUN 13, 96
<i>Prorocentrum arcuatum</i> Issel	114	41.0	467 000	MAY 01, 96
<i>Scrippsiella</i> spp	16	5.8	30 530	APR 29, 97
Gymnodinoid cells	65	23.4	45 800	APR 29, 97
BACILLARIOPHYCEAE				
<i>Cerataulina pelagica</i> (Cleve) Hendey	10	3.6	205 400	JUN 13, 96
<i>Chaetoceros affinis</i> Lauder	16	5.8	12 900	FEB 22, 96
<i>Chaetoceros convolutus</i> Castr.	10	3.6	2 400	OCT 01, 97
<i>Chaetoceros curvisetus</i> Cleve	189	68.0	9 031 000	APR 02, 98
<i>Chaetoceros danicus</i> Cleve	27	9.7	169 800	JUL 07, 97
<i>Chaetoceros decipiens</i> Cleve	18	6.5	21 600	OCT 06, 98
<i>Cyclotella</i> sp.	17	6.1	146 000	OCT 27, 95
<i>Dactyliosolen fragilissimus</i> (Bergon) Hasle	55	19.8	3 890 000	JUN 13, 96
<i>Eunotia</i> sp.	40	14.4	7 432 000	AUG 21, 96
<i>Leptocylindrus danicus</i> Cleve	6	2.2	3 200	AUG 25, 98
<i>Nitzschia longissima</i> (Breb.) Ralfs	25	9.0	4 780 000	JUN 13, 96
<i>Pseudo-nitzschia</i> spp.	43	15.5	6 520 000	JUN 05, 97
<i>Synedra</i> spp.	33	11.9	101 700	FEB 18, 97
<i>Thalassiosira leptopus</i> (Grun.) Hasle et Fryxell	19	6.8	754 700	NOV 18, 97
<i>Thalassionema nitzschioides</i> Grun.	54	19.4	815 000	SEP 30, 96
EBRIALES				
<i>Hermesinum adriaticum</i> Zach.	85	30.6	203 800	JUN 17, 98

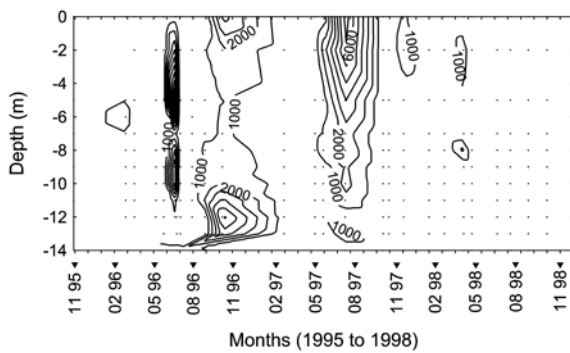


Fig. 8. Depth vs. time contour plots for microphytoplankton abundance (1000 cells L⁻¹) in the marine Lake Rogoznica

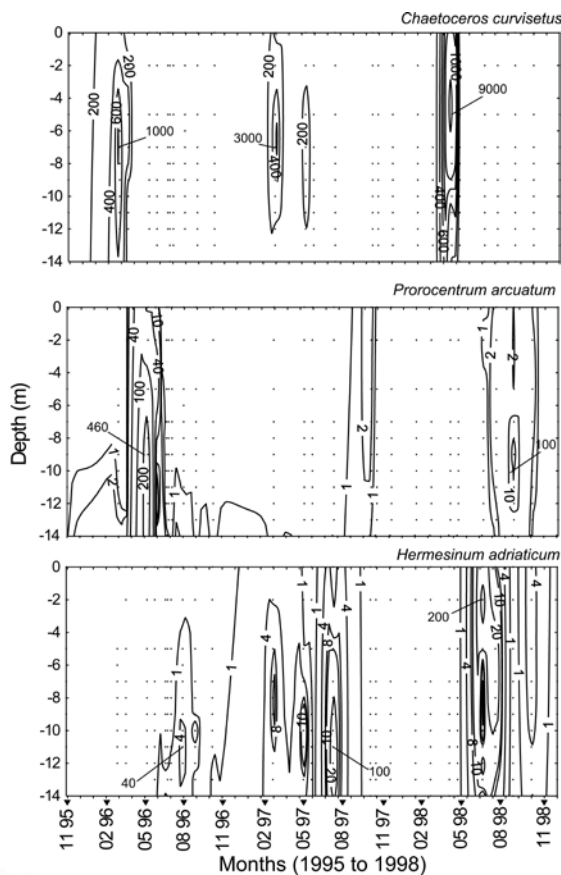


Fig. 9. Depth vs. time contour plots for abundance (1000 cells L⁻¹) of *Prorocentrum arcuatum*, *Chaetoceros curvisetus* and *Hermesinum adriaticum* in the marine Lake Rogoznica

Hermesinum adriaticum had a significant positive correlation ($r = 0.3$; $p < 0.01$) in blooms, while *Chaetoceros curvisetus* did not show a significant correlation. *C. curvisetus* bloomed in February (1996 and 1997), and *P. arcuatum* and

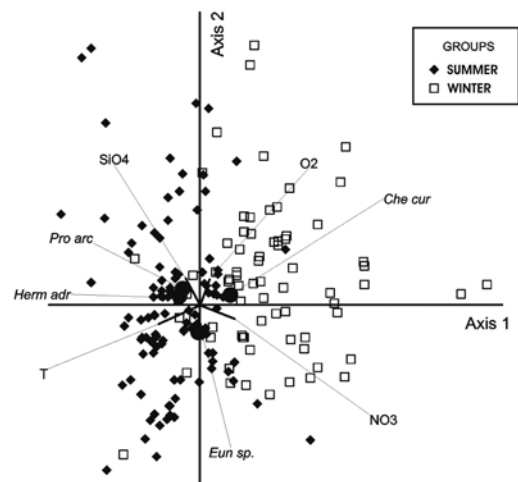


Fig. 10. Ordination diagram displaying first and second axis of CCA. Dominant phytoplankton species are shown in different symbols as can be seen in the legend. Summer period is combined by samples taken from May to October, and winter group by samples from November to April in investigated years. The environmental variables are displayed as black lines (T - temperature, O₂ - dissolved oxygen, NO₃ - nitrate, SiO₄ - silica). Species *Prorocentrum arcuatum* (*Pro arc*), *Hermesinum adriaticum* (*Herm adr*), *Chaetoceros curvisetus* (*Chae cur*) and *Eunotia sp.* (*Eun sp.*) are shown with big black dots

H. adriaticum were mostly co-occurrent in summer months (Fig 9). From October 1997 to April 1998, the seasonal development of dominant species was not recorded.

The CCA biplot indicated a higher influence of temperature on the distribution of dominant species (Fig 10). Oxygen, silicate and nitrate had a correlation greater than 0.100 with the first two axes (cumulative variance explained by first two axis = 35.2%). Salinity, nitrite, phosphate and ammonia had a correlation lower than 0.1 with first two axes, which indicates the low significance of the distribution of the species the biplot space, and therefore no important role on the occurrence of the dominant species in our samples. The diatom *C. curvisetus* showed a significantly negative correlation ($r = -0.22$, $p < 0.01$), while *P. arcuatum*, *H. adriaticum* and *Eunotia sp.* were significantly positively correlated ($r = 0.25$; $r = 0.26$; $r = 0.2$; $p < 0.01$) to temperature. Only one of the dominant species, *C. curvisetus*, was significantly correlated to oxygen ($r = 0.02$; $p < 0.01$).

DISCUSSION

The microflagellates *P. arcuatum* and *H. adriaticum*, as well as the diatom *C. curvisetus*, were found to be the most frequent and abundant microplankton species, and appeared recurrently during annual plankton succession in Lake Rogoznica. Specific environmental conditions which usually characterise Rogoznica Lake were even more pronounced during the investigated period, influencing the successful development of above mentioned species which might have excluded inferior competitive species.

The centric diatom *Chaetoceros curvisetus* in the CCA biplot is situated among samples from late autumn, winter and early spring (winter group) on the lower temperature gradient. Its position on the higher nitrate gradient also points to the dominance of this species in early spring-time, ensured by the larger amount of nitrates.

The abundant autotrophic dinoflagellate *P. arcuatum* and heterotrophic *H. adriaticum* mostly occurred in the higher temperature period. In the summer, a period characterised by nitrate deficiency, *P. arcuatum*, and *H. adriaticum* were found abundant, with the probable intake of suspended particulate matter as a source of nutrients.

Among nutrients, only nitrate showed a clear seasonal pattern while ammonia, orthophosphate and silicates did not show a yearly recurrent seasonal pattern. Silicates did not change as much as phosphorus and nitrogen during the research period. The increased N:P ratio in October 1997 was the result of exceptionally high ammonia concentrations throughout the water column. A high N:P ratio indicates that this system tends to P- rather than N-limitation, but P-concentrations must be low for P to actually become limiting. Since the PO_4^{3-} concentrations were still about 2 μmol in October 1997, the phosphorus limitation was probably not the reason for the decline in phytoplankton abundance.

In spring, *C. curvisetus* and *P. arcuatum* produced higher oxygen concentrations, indicating their important role in the photosynthetically active phytoplankton community. The appearance of high oxygen production during

relatively low microphytoplankton abundance in March-April 1996 may be interpreted as a result of picoautotrophic photosynthetic activity (ĆOSOVIĆ *et al.*, 2000). At the beginning of October 1997 concentrations of nutrients, particularly NH_4^+ and PO_4^{3-} , were significantly higher ($p < 0.01$) than in September and anoxia was determined throughout the entire water column. Exceptionally low marine phytoplankton abundance ($< 10^3$ cells L^{-1}) was found in that period. Although nutrient concentration remained high during the rest of 1997, and one month later in 1998, oxygen saturation increased with saturation achieving the usual values during subsequent months. The occurrence of extreme environmental conditions (anoxia and reduced sulphur compounds) throughout the water column in October 1997 was the result of mixing conditions which replaced the ordinary stratified (meromictic) conditions in the lake (BARIĆ *et al.*, 2003). The appearance of mixing conditions are a result of lake cooling followed by pycnocline dissolution which allows the upward transport of reduced sulphur species from the bottom to the surface. The considerable release of reduced sulphur species causes oxygen depletion (due to oxidation) and the appearance of anoxia in the whole water column, which further influences the mass mortality of phytoplankton and benthic organisms (CIGLENEČKI *et al.*, 2005).

Hydrogen sulphide (CIGLENEČKI *et al.*, 1996) and subsequently high nutrient concentrations (especially ammonia) in the post-anoxic period probably influenced the seasonality of the analysed microplankton species shortly after the anoxia. High nutrient concentrations near the bottom in the post-anoxia period (following October 15, 1997) indicated intensive microbial degradation of organic matter at the sediment-water interface. One month after the anoxia, in November, the ecological stabilisation of the water column resulted in the development of gymnodinoid species near the surface.

Moderate microphytoplankton abundance ($>10^6$ cells L^{-1}), high concentrations of inorganic nutrients and organic matter, as well as hypoxia, indicated that Lake Rogoznica is a naturally eutrophic system.

Accumulation of microplankton around the halocline has already been studied during the 1994 to 1995 period (VILIČIĆ *et al.*, 1997). Sub-surface accumulations were probably due to the sinking of cells and their retention along the halocline/pycnocline (RASMUSSEN & RICHARDSON, 1989; VILIČIĆ *et al.*, 1989; TISELIUS *et al.*, 1994), thermocline (CARRETO *et al.*, 1995) or nitracline (TREES *et al.*, 1986). The subsurface accumulation of *P. arcuatum* could be attributed to the availability of some nutrients stimulating growth, and swimming/sinking around the halocline. The halocline layer could constitute a refuge from predation. This is the layer where osmotic stress regulates the behaviour of zooplankton, resulting in their escape to more favourable living conditions above and below the halocline and decreased grazing in the halocline. Zooplankton was not abundant in the halocline (KRŠINIĆ *et al.*, 2000). According to the vertical distribution of zooplankton we can speculate that grazing was mostly greater in the 6–12 m layer (KRŠINIĆ *et al.*, 2000), i.e. mostly below the maximum abundance of *P. arcuatum*.

Due to the impact of stratification/mixing on phytoplankton succession during the investigated period, *P. arcuatum* and *C. curvisetus* occupied different domains with the dinoflagellate being favoured in stratified conditions and the diatom in mixed water columns. The same effect of stratification (and irradiance) on phytoplankton community structure has been previously recorded (SIMPSON *et al.*, 1982; JONES & GOWEN, 1990).

H. adriaticum is known to be a heterotrophic (herbivorous) species (TAPPAN, 1980; SANDERS, 1991). The fact that *H. adriaticum* accumulates (and possibly grows) at the chemocline requires further research to verify the nutrition of this species. Its occurrence during warmer months, in the same period as *P. arcuatum*, could be a result of the successful supply of nutrients. In summer, abundant endosymbionts (probably cyanobacteria) were noticed by phase contrast microscopy inside the cells of *H. adriaticum*. Such a commensalism may be indirect evidence how *H. adriaticum* cells might be supplied with additional nutrients by using photosynthetic

products produced by the endosymbiont (CARON, 2000). Commensalisms between *Hermesinum* and cyanobacteria have already been recorded (HARGRAVES & MILLER, 1974).

H. adriaticum is a species common in other stratified ecosystems such as the Black Sea (BODEANU, 1969) or Pettaquamscutt River (HARGRAVES & MILLER, 1974). Occasionally, this species can be found in the eastern Adriatic's highly stratified Krka and Zrmanja estuaries (VILIČIĆ *et al.*, 2002). *H. adriaticum* is not recorded on the northern Adriatic microplankton check-list (REVELANTE *et al.*, 1985), probably due to its scarce abundance in the seventies and eighties. This species has recently been migrating from the eastern Mediterranean into the Adriatic (VILIČIĆ *et al.*, 1997) during episodes of higher (average) salinity of the Adriatic Sea (MIKOVIĆ *et al.*, 2001).

CONCLUSIONS

Moderate microphytoplankton abundance, high concentrations of inorganic nutrients and organic matter, as well as periodic hypoxia and anoxia occurrence, indicated that Lake Rogoznica is a naturally eutrophic system.

Hydrogen sulphide and subsequent high nutrient concentrations (especially ammonia) in the post-anoxic period probably influenced the seasonality of the analysed microplankton species.

The most frequent and abundant microplankton species were *P. arcuatum* and *H. adriaticum*, as well as the diatom *C. curvisetus*. These species appeared recurrently during annual plankton succession in Lake Rogoznica. The development of *H. adriaticum* and *P. arcuatum* mostly increased during the summer under nitrate deficiency. Co-dominant diatom *C. curvisetus* appeared in early spring during higher nitrate concentrations.

Due to the impact of stratification/mixing on phytoplankton succession during the investigated period, *P. arcuatum* and *C. curvisetus* occupied different domains. The dinoflagellate *P. arcuatum* being favoured in stratified conditions and the diatom *C. curvisetus* in mixed water columns. *H.*

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The investigation of the relatively rare microflagellates *H. adriaticum* and *P. arcuatum* merits further experimental work, the next step being the gaining of some insight into the eco-

physiological characteristics of these species, as well as the monitoring of their distribution in the Mediterranean which might have expanded owing to climate changes.

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REFERENCES

- BARIĆ, A., B. GRBEC, G. KUŠPILIĆ, I. MARASOVIĆ, Ž. NINČEVIĆ & I. GRUBELIĆ. 2003. Mass mortality event in a small saline lake (Lake Rogoznica) caused by unusual holomictic conditions. *Sci. Mar.*, 67: 129-141.
- BODEANU, N. 1969. Cercetari asupra fitoplanctonului din zona de mica adncime de la litoralul romanese al Marii Negre (Research of phytoplankton in the Romanian coastal Black Sea). *Ecol. Marina*, 3: 65-147.
- BULJAN, M. & A. ŠPAN. 1976. Hidrografska svojstva mljetskih jezera i susjednog mora (Hydrographical properties of the Mljet »Lakes« and the adjoining sea). *Acta Adriat.*, 6: 1-224.
- CARON, D.A. 2000. Symbiosis and mixotrophy among pelagic microorganisms. In: D.L. Kirchman (Editor). *Microbial Ecology of the Oceans*. New York, Wiley-Liss, pp. 495-523.
- CAROPPO, C., A. FIOCCA, P. SAMMARCO & G. MAGAZZU. 1999. Seasonal variations of nutrients and phytoplankton in the coastal SW Adriatic Sea (1995-1997). *Bot. Mar.*, 42(4): 389-400.
- CARRETO, J.I., V.A. LUTZ, M.O. CARIGNAN, A.D. CUCCHI-COLLEONI & S.G. DEMARCOSS. 1995. Hydrography and chlorophyll *a* in a transect from the coast to the shelf-break in the Argentinian Sea. *Cont. Shelf Res.*, 15: 315-336.
- CARPELAN, L.H. 1961. Phytoplankton and plant productivity. In B. Walker (Editor). *The Ecology of the Salton Sea, California in Relation to the Sportfishery*. Calif. Fish Game, 113: 33-43.
- CERRANO, C., F. AZZINI, G. BAVESTRELLO, B. CALCINAL, M. PANSINI, M. SARTI & D. THUNG. 2006. Marine lakes of karst islands in Ha Long Bay (Vietnam). *Chem. Ecol.*, 22: 489-500.
- CIGLENEČKI, I., M. CARIĆ, F. KRŠINIĆ, D. VILIČIĆ & B. ČOSOVIĆ. 2005. The extinction by sulfide-turnover and recovery of a naturally eutrophic, meromictic seawater lake. *J. Mar.Syst.*, 56(1-2): 29-44.
- CIGLENEČKI, I., Z. KODBA & B. ČOSOVIĆ. 1996. Sulfur species in Rogoznica Lake. *Mar. Chem.*, 53: 101-110.
- CIGLENEČKI, I., Z. KODBA, D. VILIČIĆ & B. ČOSOVIĆ. 1998. Seasonal variation of anoxic conditions in the Rogoznica Lake. *Croat. Chem. Acta*, 71(2): 217-232.
- CRISAFFI, P. 1955. Un anno di ricerche fisico-chimiche continuative sui laghi di Ganzirri e Fano (Annual research of physical-chemical characteristics in Ganzirri Lakes near Fano). *Boll. Pesca, Piscic. Idrobiol.*, 19: 1-31.
- ČOSOVIĆ, B., I. CIGLENEČKI, D. VILIČIĆ & M. AHEL. 2000. Distribution and seasonal variability of organic matter in a small eutrophicated salt lake. *Est. Coast. Shelf Sci.*, 51(6): 705-715.
- DEFLANDRE, G. 1952. Classe des Ebriédiens (The Class of Ebriedins). In: P.P. Grasse (Editor). *Traité de zoologie (Zoology)*. Tome I. Masson, Paris, pp. 405-424.

- HALIM, Y. 1960. Observations on the Nile bloom of phytoplankton in the Mediterranean. *J. Cons.*, 26: 57-67.
- HAMNER, W.M. & P.P. HAMNER. 1998. Stratified marine lakes of Palau (Western Caroline Islands). *Phys. Geogr.*, 19: 175-220.
- HARGRAVES, P.E. & B.T. MILLER. 1974. The ebridian flagellate *Hermesinum adriaticum* Zach. *Arch. Protistenkd.*, 116: 280-284.
- IVANČIĆ, I. & D. DEGOBBIS. 1984. An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Res.*, 18: 1143-1147.
- JONES, K.J. & R.J. GOWEN. 1990. Influence of stratification and irradiance regime on summer phytoplankton composition in coastal and shelf seas of the British Isles. *Est. Coast. Shelf Sci.*, 30: 557-567.
- KRŠINIĆ, F. 2003. *Mesaikeras hurei* n.sp. (Copepoda, Calanoida, Mesaiokeratidae) from the Adriatic Sea. *J. Plankton Res.*, 25: 939-948.
- KRŠINIĆ, F., M. CARIĆ, D. VILIČIĆ & I. CIGLENEČKI. 2000. The calanoid copepod *Acartia italica* Steuer, phenomenon in the small saline Lake Rogoznica (Eastern Adriatic coast). *J. Plankton Res.*, 22(8): 1441-1464.
- MIOKOVIĆ, D., D. VILIČIĆ, M. CRNČEVIĆ & Z. BURIĆ. 2001. Unusual occurrence of the microflagellate *Hermesinum adriaticum* Zach. in the northern Adriatic Sea in 1998 and 1999. *Rapp. Comm. int. Mer. Médit.*, 36: 302.
- PREISIG, H.R. 1994. Siliceous structures and siliification in flagellated protists. *Protoplasma*, 181: 29-42.
- RAMPI, L. & M. BERNHARD. 1980. Chiave per la determinazione delle peridinee pelagiche mediterranee (Key for determination of the Mediterranean pelagic peridineans). CNEN, La Spezia.
- RASMUSSEN, J. & K. RICHARDSON. 1989. Response of *Gonyaulax tamarensis* to the presence of a pycnocline in an artificial water column. *J. Plankton Res.*, 11: 747-762.
- REVELANTE, N., M. GILMARTIN & N. SMODLAKA. 1985. The Effects of Po River Induced Eutrophication on the Distribution and Community Structure of Ciliated Protozoan and Micrometazoan Populations in the Northern Adriatic Sea. *J. Plankton Res.*, 7(4): 461-471.
- RHODES, R. & V. GIBSON. 1981. An annual survey of *Hermesinum adriaticum* and *Ebria tripartita*, two ebridian algae in the lower Chesapeake Bay. *Estuaries*, 4: 150-152.
- SANDERS, R.W. 1991. Trophic strategies among heterotrophic flagellates. In: D.J. Patterson & J. Larsen (Editors). *The biology of free-living heterotrophic flagellates*. London, Oxford, pp. 21-38.
- SCHILLER, J. 1933. *Dinoflagellatae*. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz (*Dinoflagellatae*. Rabenhorst's cryptogamyc flora of Germany, Austria and Switzerland). Leipzig, Akademische Verlagsgesellschaft, pp. 617.
- SIMPSON, J.H., P.B. TETT, M.L. ARGOTE-ESPINOZA, A. EDWARDS, K.J. JONES & G. SAVIDGE. 1982. Mixing and phytoplankton growth around an island in a stratified sea. *Cont. Shelf Res.*, 1: 15-31.
- SOROKIN, J.I. & M. DONATO. 1975. On the carbon and sulphur metabolism in the meromictic Lake Faro (Sicily). *Hydrobiologia*, 47: 241-252.
- SOURNIA, A. 1986. Atlas du phytoplancton marin In: *Introduction, Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées* (Atlas of the marine phytoplankton in: *Introduction, Cyanophyceae, Dictyochophyceae, Diniphyceae and Raphidophyceae*). Paris, Centre National de la recherche scientifique, pp. 219.
- STIPANIČEV, V. & M. BRANICA. 1996. Iodine spetiation in the water column of the Rogoznica lake (eastern Adriatic coast). *Sci. Total Environ.*, 182: 1-9.
- STRICKLAND, J.D.H. & T.R. PARSONS. 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Board Can.*, 167: 1-310.
- TAPPAN, H.N. 1980. *The Paleobiology of Plant Protists*. W. H. Freeman and Co. San Francisco.
- TER BRAAK, C.J.F. 1995. Ordination. In: R.H.G. Jongman, C.J.F. Ter Braak & O.F.R. Van Tongeren (Editors). *Data Analysis in*

- Community and Landscape Ecology. Cambridge University Press, pp. 91-173.
- TISELIUS, T., G. NIELSEN & T.G. NIELSEN. 1994. Microscale patchiness of plankton within a sharp pycnocline. *J. Plankton Res.*, 16: 543-554.
- TRAVERS, A. & M. TRAVERS. 1968. Les silicoflagellés du Golfe de Marseille (Silicoflagellates of the Marseille Bay). *Mar. Biol.*, 1: 285-288.
- TREES, C.C., R.R. BIDIGARE & J.M. BROOKS. 1986. Distribution of chlorophylls and phaeopigments in the northwestern Atlantic Ocean. *J. Plankton Res.*, 8: 447-458.
- UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.*, 9: 1-38.
- VILIČIĆ, D., T. LEGOVIĆ & V. ŽUTIĆ. 1989. Vertical-Distribution of Phytoplankton in a Stratified Estuary. *Aquat. Sci.*, 51(1): 31-46.
- VILIČIĆ, D., I. MARASOVIĆ & G. KUŠPILIĆ. 1997. The heterotrophic ebridian microflagellate *Hermesinum adriaticum* ZACH in the Adriatic Sea. *Arch. Protistenkd.*, 147(3-4): 373-379.
- VILIČIĆ, D., N. JASPRICA, M. CARIĆ & Z. BURIĆ. 1998. Taxonomic composition and seasonal distribution of microphytoplankton in Mali Ston Bay (eastern Adriatic). *Acta Bot. Croat.*, 57: 29-48.
- VILIČIĆ, D., I. MARASOVIĆ & D. MIKOVIĆ. 2002. Checklist of phytoplankton in the eastern Adriatic Sea. *Acta Bot. Croat.*, 61: 57 - 91.
- ZACHARIAS, O. 1906. Eine neue Dictyochide aus dem Mittelmeer, *Hermesinum adriaticum* n.g., n.sp (Phytoplankton counting technique). *Arch. Hydrobiol.*, 1: 394-398.

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Pojava rijetkih mikroflagelata *Prorocentrum arcuatum* Issel i *Hermesinum adriaticum* Zacharias u Rogozničkom jezeru (istočna Jadranska obala)

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SAŽETAK

Rogozničko jezero, smješteno u obalnom dijelu srednjeg Jadrana, je prirodni eutroficirani morski sustav s potencijalnim antropogenim utjecajem. U tom ekstremom okolišu, sa značajkama meromiktičkog i anoksičnog sustava, provedeno je istraživanje mikroplanktona u razdoblju od 1995. do 1998. godine. U mikroplanktonskoj zajednici (maksimalna abundancija 1.06×10^7 stanica L⁻¹) određeno je 40 taksona, većinom dijatomeja (62.5%) i dinoflagelata (29%). Zabilježene su iznimno visoke abundancije i sezonsko opetovano pojavljivanje dva rijetka mikroflagelata: *Prorocentrum arcuatum* Issel (dinoflagelat) i *Hermesinum adriaticum* Zacharias (hetrotrofan mikroflagelat). Maksimalan razvoj *P. arcuatum* i *H. adriaticum* zabilježen je ljeti u uvjetima nedostatka dušika. Kodominantna vrsta u zajednici bila je dijatomeja *Chaetoceros curvisetus* Cleve koja je imala maksimalan razvoj u proljeće u uvjetima bogatim dušikom. Statistička analiza ukazala je na važnost temperature, kisika, silikata i nitrata na razvoj navedenih vrsta. Ovim istraživanjem dobivene su nove spoznaje o ekofiziološkim svojstvima rijetkih vrsta, kao i spoznaje o njihovom prisustvu i razvoju u Mediteranu, koje bi moglo biti pod utjecajem klimatskih promjena.

Ključne riječi: *Hermesinum adriaticum*, *Prorocentrum arcuatum*, flagelati, sezonalnost, slano jezero, Rogoznica