



The relative importance of physical-chemical factors in the brackish shallow lake Vrana (Croatia) as determinant of crustacean zooplankton community

DANIJELA PEROŠ-PUCAR¹
IVANČICA TERNJEJ²

¹Health Ecology Service,
Public Health Institute Zadar,
Kolovare 2, Zadar, Croatia

²Department of Zoology, Faculty of Science,
University of Zagreb, Rooseveltov trg 6,
HR-10000 Zagreb, Croatia

Correspondence:

Danijela Peroš-Pucar
Health Ecology Service
Public Health Institute Zadar
Kolovare 2, Zadar, Croatia
E-mail: dperos.pucar@zjz-zadar.hr

List of abbreviations:

APHA	American Public Health Association
RDA	Redundancy Analysis
DO	Dissolved oxygen
BOD	Biological oxygen demand
P1, P2, P3, P4	Names of stations

Key words: zooplankton temporal variation, brackish shallow lakes, multivariate analysis

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Abstract

Background and Purpose: Lake Vrana is shallow, Mediterranean, karstic lake, where water salinity is as an important ecological factor, because the lake is directly connected with the Adriatic Sea. Studies concerning zooplankton communities in these ecosystems are very scarce. To understand this very specific and unique ecosystem, the physical and chemical parameters and the makrozooplankton community structure in Lake Vrana were studied.

Material and Methods: The investigation was conducted monthly from January till December 2004 at four stations with different ecological characteristics (primarily depth, salinity, hydrology, nutrients supply). Chlorophyll *a* concentration was analyzed fluorometrically according to Method 445.0. Physical and chemical parameters (temperature, transparency, chlorinity, salinity, biological oxygen demand - BOD, dissolved oxygen - DO, ortho-phosphate, total phosphorus, chlorophyll *a*) were measured according to APHA. Data on environmental variables and makrozooplankton community were examined using Redundancy Analysis (RDA).

Results: Among the 13 different species which are found in macrozooplankton community of Lake Vrana, the dominant species were *Calanipeda aquedulcis* Kritchagin (Copepoda), and *Bosmina longirostris* O.F. Müller, (Cladocera) which were present in all four stations during study period. The same occurring had *Megacyclops gigas* Claus, *Alona* sp., *Alonella excisa* Fischer, and *Chydorus sphaericus* O.F. Müller while other species appeared sporadically.

The physical-chemical parameters showed temporal and spatial variations in Lake Vrana during study period resulting in different micro-habitat conditions on the studied sites. That is confirmed by the results of the RDA analysis that clearly shows the separation of stations due to the impact of studied indicators. According to multivariate analysis temperature, chlorophyll *a* and salinity have great influence on dynamics and structure of makrozooplankton in Lake Vrana.

Conclusions: Temperature, chlorophyll *a* and salinity are ecological factors which define the development as well as dynamics and structure of macrozooplankton community in the Vrana Lake.

INTRODUCTION

Zooplankton communities are dynamic systems in which the species composition changes with time. Spatial development, like the tem-

poral development, is the result of many physical and chemical processes interacting with several biological processes at a range of spatial scales, as described by the 'multiple driving force hypothesis' (1).

Along the eastern Adriatic coast there are several karstic lakes. Lake Vrana near Biograd is the biggest one. Lake is shallow and brackish, as a result of underground connection, proximity of the Adriatic Sea and long channel that connect lake and sea. Lake was investigated during last century, but most of the studies and papers dealt with fish stocking and problems about the influence of aquaculture on zooplankton community (2, 3, 4, 5, 6, 7, 8). There were only two studies concerning zooplankton communities in Vrana Lake (9, 10).

Our goal was to examine spatial and temporal structure in crustacean plankton in this shallow brackish lake and to establish possible relationship between makrozooplankton community and natural variation in the abiotic environment.

MATERIALS AND METHODS

Study site

Lake Vrana is situated at Eastern Adriatic coast, southern part of Croatia (Dalmatia) very close to the sea (Fig. 1) and with an area of 30 square kilometers is the largest natural lake in Croatia (10). It belongs to the group of lakes formed by karstic erosion in structurally determined valleys, which may lie in grabens against fault scarps, or in synclines, producing tectonic-karstic depressions, called 'polje' in Dalmatia (11). The limestone ridge wide 800-2500 m divides the Vrana Lake from the sea. The lake is supplied with water by several permanent freshwater springs (the biggest Zivaca) and two channels, main (Kotarka) and lateral channel. It is shallow lake with an average depth of 2 m, while the maximum depth is 5 m. Relatively shallow depth and conduction water flow due to waves caused complete vertical mixing of the water column and absens temperature stratification during the summer period. Since 1895, when an 800 m long channel was dug (to drain the Vrana valley), it has been connected with the sea. Salinity of the water varies from 8 ppm on the northwest side, to 20 ppm on the southeast (9).

The sampling stations are presented in Figure 1. Station P1 (43°54'27"N and 15°33'57"E) is in the middle of the lake, and has typical lacustric conditions. This is the deepest station with maximum depth of 3,75 m and minimum of 2,30 m during our investigation. Station P2 (43° 52'10"N and 15°38'03"E) is above underwater spring of fresh water. Station P3 (43°51'08"N and 15° 38'01"E) is near the channel which during low water level brings sea water in the lake. Station P4 (43°55'55"N and 15°33'16"E) is in front of lateral channel, which drains Vrana valley. The last three stations (P2, P3 and P4) are shallower and their depth don't exceeded 2,8 m during investigation period.

Water chemistry

Samples were collected monthly from January to December; at depth of 0.5 m. Samples for chemical analysis were taken simultaneously with plankton samples. Temperature was measured by mercury field thermometer and transparency was determined by 20 cm diameter Secchi disk, all *in situ*. Dissolved oxygen (DO) was measured in laboratory by iodometric method (Winkler method). Chlorophyll *a* concentration was analyzed fluorometrically according Method 445.0 (12). Other parameters (chlorinity, salinity, biological oxygen demand - BOD, orto-phosphate, total phosphorus, and alkalinity) were measured in laboratory according to APHA (13).

pH was measured pH meter in laboratory with a pH-meter (ORION model 420 A). Salinity was calculated as salinity – chlorinity relationship:

$$S \text{ (ppm)} = 0,03 + (1,8050 \times Cl \times \sigma_{20}^{-1})$$

Where is:

Cl= concentration of chloride ions mgL⁻¹

σ_{20} = water density at 20°C.

BOD (mg O₂L⁻¹) was measured by 5-day BOD test, where BOD is computed from the difference between initial and final DO. Total phosphorus (mgL⁻¹) was measured by persulfate digestion method. Ortho-phosphate (mgL⁻¹) was measured by stannous chloride method where molybdo-phosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue.

Alkalinity (mg CaCO₃ L⁻¹) was measured in laboratory by titration method and it is calculated as:

$$\text{Alkalinity (mg CaCO}_3\text{ L}^{-1}) = \frac{A \times N \times 50000}{V(\text{sample})[\text{mL}]}$$

Where is:

A = volume of standard acid used [mL]

N = normality of standard acid

Zooplankton sampling

Crustacean zooplankton was collected by vertical hauls of a plankton net (mesh size 60 μm) from the bottom to the surface, preserved in 4% formaldehyde (final concentration). Volume of filtrated water was calculated on the knowledge of haul depth and mouth area of the net. The plankton crustaceans were counted in a round glass Petri dish under a microscope (Option, 100 x magnifications). The entire volume of each sample was counted. Species identification was conducted according to Einsle (14, 15) for copepods and Margaritora (16) for cladocerans. Dry weight biomass was estimated by regression equations of length / weight relationships published by Dumont *et al.* (17), Botrell *et al.* (18) and Malley *et al.* (19).

TABLE 1

Means and standard errors (in brackets) of Environmental variables.

Variable	Abb.*	P1	P2	P3	P4
Koordinates		43°54'27"N 15°33'57"E	43°52'10"N 15°38'03"E	43°51'08"N 15°38'01"E	43°55'55"N 15°33'16"E
Temperature	Temp	14.260 (2.731)	14.280 (2.751)	14.410 (2.747)	14.370 (2.656)
Alkalinity	CaCO ₃	122.750 (8.752)	121.875 (10.374)	117.250 (8.979)	147.375 (14.437)
Dissolve oxygen	O ₂	11.049 (0.436)	11.070 (0.448)	10.957 (0.486)	11.396 (0.394)
Biological oxygen demand	BOD	0.991 (0.114)	0.923 (0.167)	0.931 (0.183)	1.462 (0.474)
Salinity	sal	1.330 (0.081)	1.316 (0.079)	1.383 (0.075)	1.086 (0.059)
Total phosphorus	UP	0.049 (0.024)	0.048 (0.022)	0.055 (0.022)	0.053 (0.022)
Orthophosphate	ortP	0.015 (0.003)	0.018 (0.003)	0.013 (0.004)	0.014 (0.004)
Chlorophyll <i>a</i>	Chla	2.366 (0.669)	2.548 (0.722)	2.724 (0.928)	2.304 (0.645)

*Abbreviations used in figure 4

Data analysis

A linear multivariate method (RDA) was performed using the program CANOCO 4.0 (20). The analysis was based on the species biomass in relation to data for the environmental variables. The species data matrix was log-transformed and centred prior to the analysis. In the crustacean species data matrix only those taxa were included which occurred in at least 5% of all samples. Thus, 10 of 13 enumerated taxa were met. The variables were also log-transformed to approximate normal distributions and standardized to zero mean and unit variance. The four sampling sites were coded as a dummy variable to repre-

sent all differences in microhabitats that were not covered by the environmental variables. The results are presented as a biplot where species and environmental variables course are plotted together.

RESULTS

Water chemistry

Environmental variables with their means and standard errors are presented in Table 1.

Temperature

Temporal distribution of the temperature was very similar in all station. The lowest values were measured in January: they varied from 1.6 to 2.2°C. From January water temperature is gradually rising, and in July it reaches maximal values. The highest summer temperature was measured in station P1 (26.2°C). From July water is gradually cooling until December when it reaches temperature between 6.5 – 9.0°C.

Alkalinity

Alkalinity values varied temporally and spatially. The highest values were measured in station P4 (195.0 mg CaCO₃L⁻¹). This maximum value was observed at the beginning of investigation, in January, followed by general trend of decreasing until August when minimum of 70.0 mg CaCO₃L⁻¹ was measured. During autumn alkalinity was increasing: its concentration at station P4 in November was again high (190.0 mg CaCO₃L⁻¹). In other station general pattern was slightly different. From January to April or May alkalinity is rising. In May the maximal values were measured, but they didn't exceed

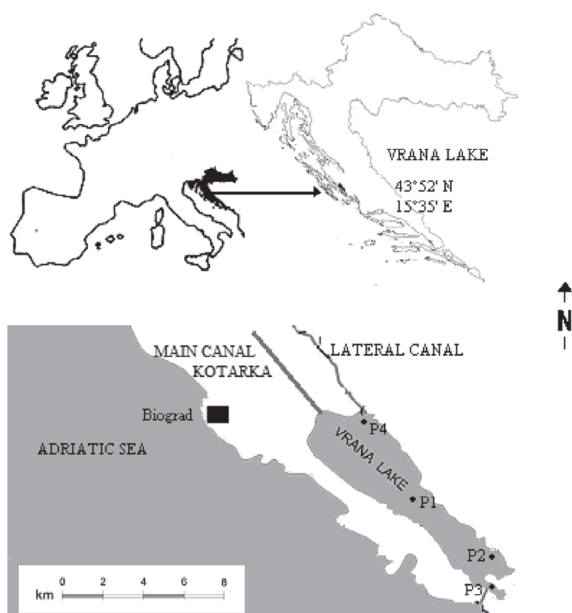


Figure 1. Investigated area –Lake Vrana (Dalmatia, Croatia).

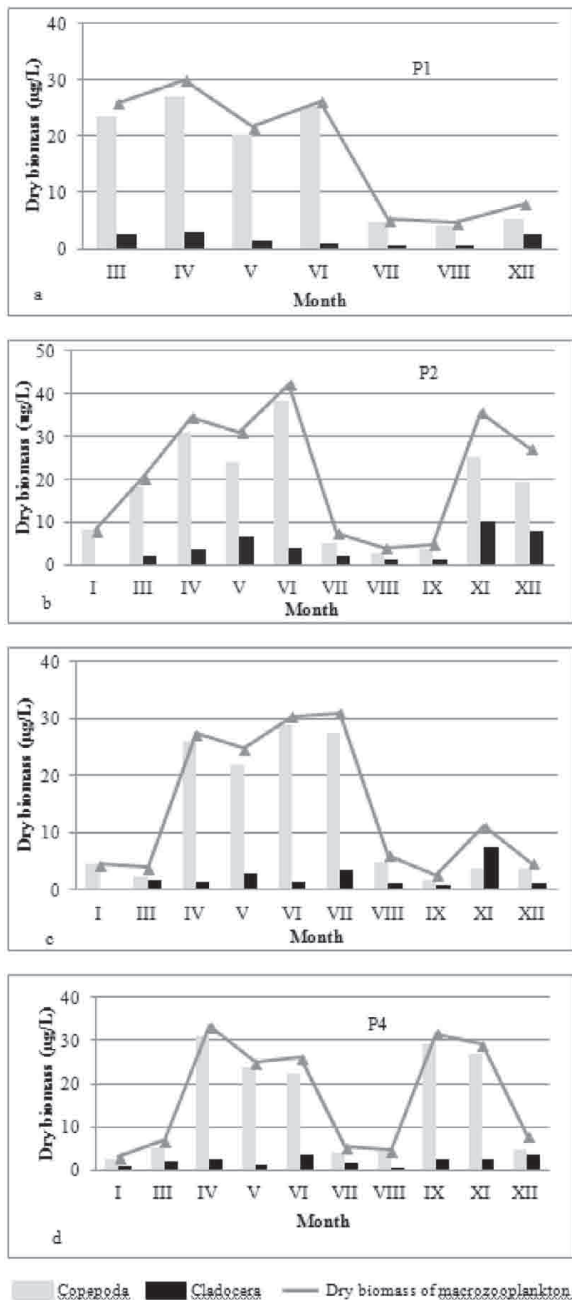


Figure 2. Macrozooplankton dry biomass (µg/L) in Lake Vrana during 2004.

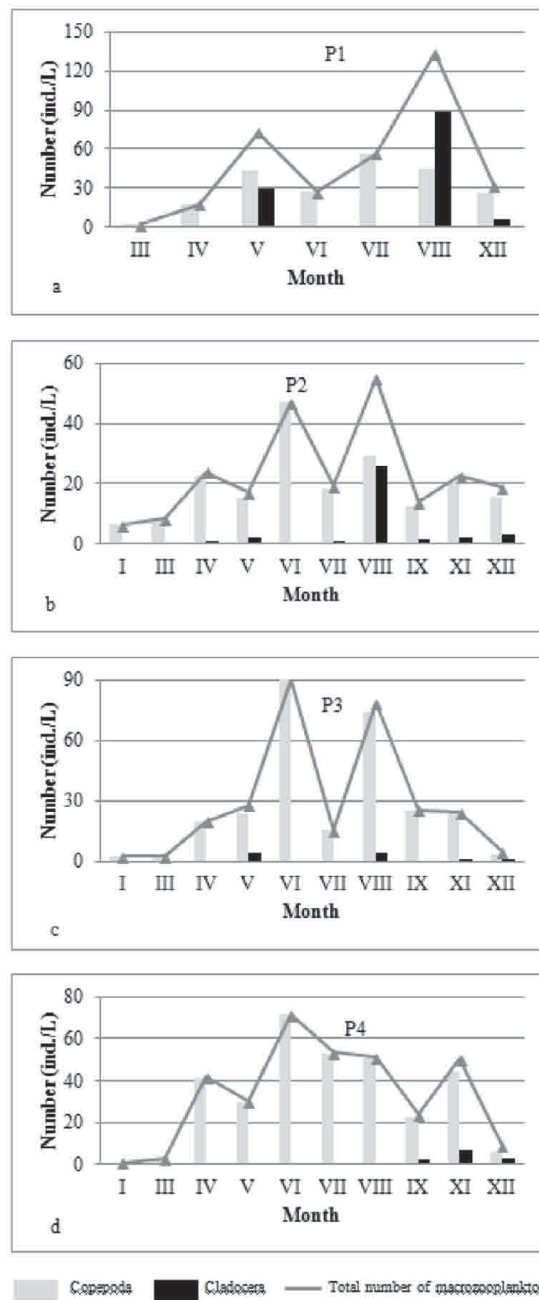


Figure 3. Macrozooplankton abundance (ind./L) in Lake Vrana during 2004.

165.0 mg CaCO₃L⁻¹. From July till August alkalinity is dropping, and then minimums were measured (80.0 mg CaCO₃L⁻¹). In autumn (August - December) alkalinity was again slightly increasing.

Oxygen

Oxygen concentration was complementary to the water temperature in all station. The lowest values (9.07 – 9.90 mg O₂L⁻¹) were observed during summer when water temperature was above 25°C. Maximal values were

measured during January - March period (temperature range 1.6 -5.4°C) and were characterized with range between 12.28 – 13.22 mg O₂L⁻¹.

BOD

Values of biological oxygen demand varied between 0.16 - 5.52 mg O₂L⁻¹. The highest value was measured in station P4. Indeed, this station had higher concentration of BOD, and different temporal pattern in compare to the other stations. Maximum is in January, but in March

BOD value dropped ($0.13 \text{ mg O}_2\text{L}^{-1}$), and was rising again until June. The rise wasn't profound (it didn't exceed $1.74 \text{ mg O}_2\text{L}^{-1}$). From July until December BOD value is between $0.55 - 0.98 \text{ mg O}_2\text{L}^{-1}$. In stations P1 – P3 two maximums were observed: in January and July, but values were lower than in station P4.

Salinity

Salinity was low. It varied between $0.80 - 1.93 \text{ ppm}$. Higher values were measured in January – March period, while during summer salinity dropped. Slight quantitative differences were observed between stations, but general pattern is the same.

Nutrients

Total phosphorus concentrations varied between $0.01 - 0.26 \text{ mg PL}^{-1}$. Several peaks were observed during a year. The first one is in January in all stations. In station P1 and P2 two more were noticed (July and December); while in station P3 and P4 total phosphorus values September maximum was also observed.

Orthophosphate had more or less similar cycle, with range between $<0.001 - 0.040 \text{ mg PL}^{-1}$. The lowest values were measured in station P2.

Chlorophyll *a* concentration in station P1 fluctuated. It showed three maximums: April, June, and September. In other stations only one maxima was observed (during June), but values varied: the highest being measured is $9.54 \text{ } \mu\text{gL}^{-1}$.

Crustacean plankton abundance and biomass

The annual cycle of biomass and abundance is presented in Figures 2 and 3. A total of 13 species in makrozooplankton community were determined during investigation (Table 2). Species *Calanipeda aquedulcis*, *Megacyclops gigas* Claus, *Alona* sp., *Alonella excisa* Fischer, *Bosmina longirostris* and *Chydorus sphaericus* O.F. Müller, were present at all studied stations and other species appeared sporadically. The dominant Copepod species was *C. aquedulcis* and cladoceran *B. longirostris*.

The total number of individuals varied between 0.96 and 133.50 No.L^{-1} . The highest abundance was noticed in station P1. In this station temporal distribution is char-

TABLE 2

Means and standard errors (in brackets) of Macrozooplankton dry biomass ($\mu\text{g/L}$) in Lake Vrana during 2004.

	Abb.*	P1	P2	P3	P4
CLADOCERA					
<i>Alona</i> sp.	Asp	0.127 (0.062)	0.378 (0.121)	0.647 (0.466)	0.249 (0.0799)
<i>Alonella excisa</i>	Aex	0.143 (0.093)	0.140 (0.071)	0.040 (0.040)	0.151 (0.077)
<i>Biapertura affinis</i> Lexdig		–	1.633 (0.864)	–	–
<i>Bosmina longirostris</i>	Blng	1.053 (0.188)	0.659 (0.097)	0.717 (0.108)	0.904 (0.100)
<i>Chydorus sphaericus</i>	Chsph	0.197 (0.133)	0.895 (0.322)	0.267 (0.139)	0.605 (0.286)
<i>Leydigia</i> sp.		–	–	0.204 (0.204)	–
<i>Macrotrix laticornis</i>	Mlat	–	0.104 (0.101)	0.005 (0.03)	0.009 (0.005)
<i>Pleuroxus aduncus</i> Jurine		0.149 (0.149)	–	–	0.033 (0.033)
<i>Pleuroxus</i> sp.	Psp	–	0.099 (0.099)	0.207 (0.161)	0.078 (0.053)
COPEPODA					
<i>Calanipeda aquedulcis</i>	Caqu	2.850 (0.221)	2.421 (0.520)	2.207 (0.435)	2.655 (0.329)
<i>Macrocyclus albidus</i>	Malb	–	1.174 (0.788)	–	0.826 (0.826)
<i>Megacyclops gigas</i>	Mgig	10.370 (3.715)	11.398 (3.365)	6.606 (3.177)	9.973 (0.370)
<i>Paracyclops fimbriatus</i> Fischer	Pfim	0.429 (0.429)	0.648 (0.435)	0.360 (0.360)	–
copepodits	kop	1.029 (0.042)	0.962 (0.027)	1.017 (0.031)	0.881 (0.099)
nauplii	naup	0.969 (0.024)	0.943 (0.019)	0.945 (0.022)	0.973 (0.017)

*Abbreviations used in figure 4

TABLE 3
Summary of redundancy analysis (first four axes).

Axis	1	2	3	4	Total variance
Eigenvalues	0.306	0.054	0.037	0.024	1.000
Species-environmental correlations	0.722	0.752	0.628	0.720	
Cumulative percentage variance:					
– of species data	30.60	36.0	39.8	42.10	
– of species-environmental relationship	66.40	78.1	86.2	91.0	
Sum of all unconstrained eigenvalues					1.000
Sum of all canonical eigenvalues					0.461
Variance explained (%)*	30.6	36.0	39.7	41.9	

*Total variance explained is calculated as the product of cumulative percentage for each axis and the sum of all canonical eigenvalues

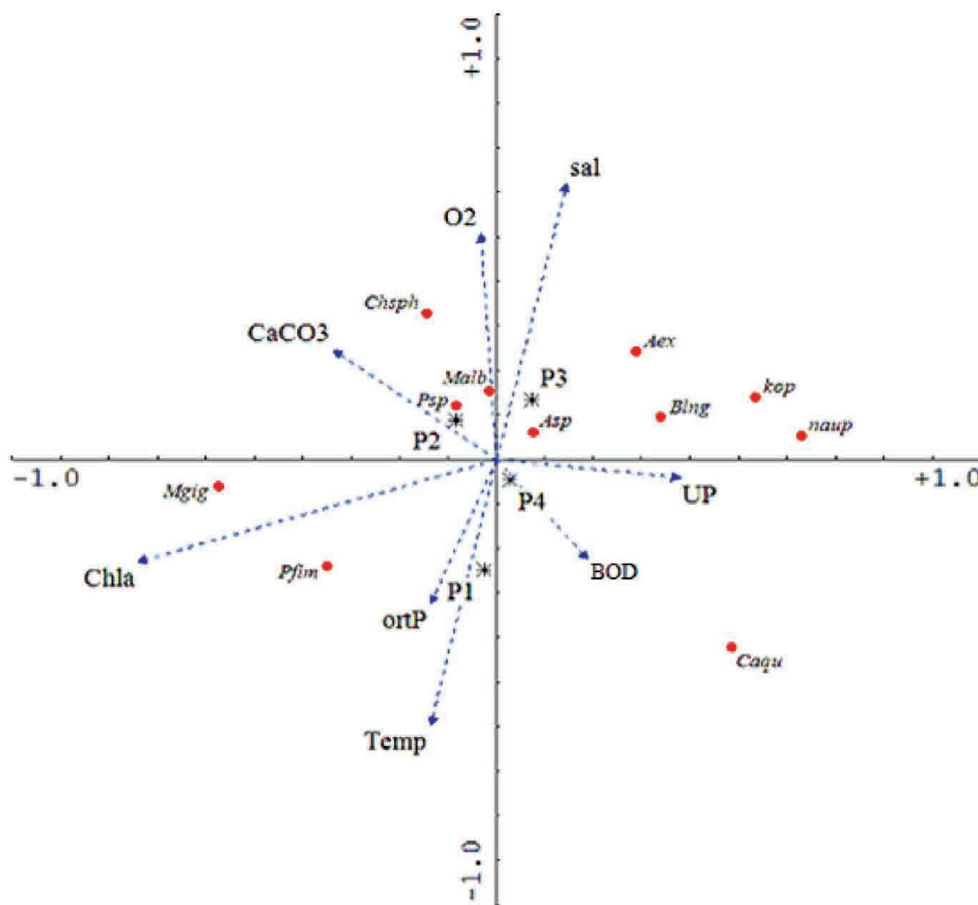


Figure 4. RDA biplot of species (circles) and environmental variable (arrows) scores on first two axes. Abbreviations as used in Tables 1 and 2.

acterized with maxima in May and August. During May makrozooplankton community was dominated by copepods but in August cladocerans prevailed. In station P2 macrozooplankton abundance was noticeably lower and ranged between 6.31 and 54.37 NoL⁻¹. Temporal distri-

bution showed fluctuations: first increase being in April, second in June, the third in August, and then again in June. During the whole investigation period copepods dominated but the magnitude of abundance was considerably lower than in previous station. Station P3

had the very similar temporal dynamic of macrozooplankton abundance as in station P1, Copepods prevailed, but total abundance doesn't exceed total of 90.27 NoL⁻¹. Macrozooplankton abundance in P4 had several peaks. First increase was in April, the second in June, and the final one in September. Copepods were also more numerous from cladocerans.

Biomass had different temporal and spatial distribution; it ranged between 2.65 and 35.44 µgL⁻¹. Each station had its own dynamic. In P1 crustacean biomass was around 25 µgL⁻¹ until June, then it dropped and stayed approximately at level of 5 µgL⁻¹ until the end of investigation. Fluctuations in station P2 were more profound. At the beginning of investigation biomass is rising until April (34.39 µgL⁻¹), dropped in May, and raised again in June (µgL⁻¹ 42.15). During summer crustacean biomass at station P2 was very low, but increased in autumn (in September) when it reached value of 35.44 µgL⁻¹. In station P3 almost same dynamic was observed, quantitatively different. From January till April it raised and reached approximately 30 µgL⁻¹. It stayed at that level until July. In August and September makrozooplankton biomass was below 6.0 µgL⁻¹, a slight increase was observed in November, when cladocerans dominated over copepods. Station P4 had the highest macrozooplankton biomass. Temporal dynamic was similar to the one observed in station P3.

Multivariate analysis

Total variance explained by RDA was 41.9 % of species-environmental relationship on first four axes (Table 3). The major environmental variables influencing macrozooplankton were chlorophyll *a*, salinity and temperature (Fig. 4). Species variance explained by model is presented in Table 3. Stations are segregated each in one quarter of plane (Fig. 4). The left upper part corresponds with station P2 and species *Pleuroxus* sp., *Macrocyclus albidus* Jurine, and *C. sphaericus*. In a gradient of higher oxygen and CaCO₃ concentrations these species find favorable conditions.

Higher salinity level corresponds with station P3 – the right up part. The most numerous in this station were copepod's larval stages (copepodites and nauplii) and cladocerans *B. longirostris*, *Alona* sp. and *A. excisa*. However, the nauplius larvae contributed to most of the copepod number in all stations. Juvenile copepods were abundant in summer and autumn. The populations of all three development stages alter during one-year cycle: adult peaks are followed by a nauplius peak, which is on the other hand followed by a copepodites peak.

Calanoids were numerous during the whole year, Cyclopoids are less abundant. They are present only occasionally, and with smaller populations: most cyclopoids were determined in station P1 during summer (lower left part). Adults of *C. aqueductalis* and cladoceran *Macrotrix laticornis* Jurine,

become abundant in late summer and autumn, when higher concentrations of phosphorous and BOD was measured. This situation corresponds with station P4.

DISCUSSION

The physical-chemical parameters show temporal and spatial oscillation in Lake Vrana. RDA analysis separated several microhabitats. The portion of total variance explained by multivariate analysis is relatively high in compare to similar studies (21, 22, 23). The model explained a total of 41.9 % variance of crustacean plankton and environmental data. According to multivariate analysis temperature, chlorophyll *a* and salinity have great influence on dynamics and structure of makrozooplankton in Lake Vrana.

Many biological processes depend on temperature (24, 25, 26). It is important factor which controls growth progress (development) of zooplankton (27, 28). Temperature also influences zooplankton-phytoplankton relationship (29). High abundance of plankton crustacean in Lake Vrana was noticed during warmer part of the year in all stations. However, in the same period there was also stagnation (even decrease) of makrozooplankton: in P1 in June; P2 and P3 in July; P4 in July and August. It might be a consequence of ecological conditions which are suitable for most of the species in the transitive season (11, 30, 31) especially during July which is a beginning of summer. Second reason might be a fish predation, which is the most intensive during the summer (32, 33). This investigation didn't include study on fish predation, but Treer and co-authors (34) cited that fish predation in the most important factor that influence on makrozooplankton population in Lake Vrana during summer.

Alkalinity and dissolve oxygen had impact on P2 which is near freshwater spring which is running through karstic field and brings water enriched with calcium carbonates.

Nutrient supply is another important factor controlling the relative abundance of phytoplankton, and eventually through it zooplankton abundance. The nutrient that varied most during seasonal cycle was phosphorus. The highest concentration of this nutrient was found at station P4. It is near the channel through water enriched with nutrients from surrounding agricultural field, enters the lake. Also the greater abundance and chlorophyll *a* in the spring coincided with the higher availability of nitrate and phosphate in the water column. Chlorophyll *a*, as primary production parameter, which is not very high (indicating mesotrophy) in this shallow lake, has a great impact on growth progress of zooplankton (35). One of the reasons for such primary production level and nutrient supply might be a buffering mechanism of macrophytes (21). The littoral area of the lake is covered by macrophytes. These plants may be the main consumer of nutrients.

Macro vegetation is very well developed in station P3, where a lot of small species and larval stages had the highest abundance. Most of them probably use macrophytes as shelter (36). Salinity is other important variable influencing macrozooplankton community. Sea water can drastically alter zooplankton composition of costal freshwater ecosystems (37). Salinity showed the greatest impact on station P3, which is near inflow of sea water. *B. longirostris* and *C. aquedulis* were found out as dominant species in this part of lake (P3 and P4 station). They are eurihaline and euryhaline species (8, 9) which can thrive in such conditions.

One of the major environmental variables (determined by RDA analysis) influencing macrozooplankton cycle was food supply (chlorophyll *a*). Chlorophyll concentrations showed the highest correlation with station P1 in the "cyclopoid" part of the lake. Nanophytoplankton is probably for their larval stages; their abundances seemed to follow chlorophyll *a* maxima.

CONCLUSION

In conclusion, the macrozooplankton abundance and biomass in Lake Vrana is primarily controlled by fluctuations in physical environment: particularly temperature (which causes high seasonality among samples), chlorophyll *a* (food supply) and salinity (connection with the sea).

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