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A preliminary investigation of phytoplankton of karstic pools (Dugi otok island, Croatia)

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Phytoplankton in the karstic pools of Croatian islands has not been investigated in recent times. This paper describes phytoplankton and environmental conditions in three karstic pools on Dugi Otok island (Dalmatia). The pools are small (ca 20 m^2) and shallow (max. depth 2 m), with Charophytes and vascular plants covering the bottoms. Eighty-five taxa of net phytoplankton were recorded. These included 22 Cyanobacteria, 14 Chlorophyceae, 17 Charophyceae (Zygnematales), 15 Euglenophyceae, and 17 Bacillariophyceae. Low species richness is owed to the predominance of Cyanobacteria. Despite their similar physicochemical regimes, the pools had different phytoplankton communities. The data presented here contribute baseline information concerning biological diversity, essential for evaluation of environmental changes in the future. Conservation of karstic pools is one way to preserve the overall biodiversity of karstic islands.

Key words: Phytoplankton, karst, pool, Dugi Otok, island, Croatia

Introduction

Dalmatia is a karstic region characterized by an average annual rainfall of 1,500 mm, and a general lack of surface water. Owing to the porous nature of karstic rocks, complex sub-surface water circulation patterns appear (MAGDALENIĆ 1991, BONACCI 1999). In such circumstances, sporadically emergent surface pools, typical small microenvironments of the Dinaric karst, are formed.

Such pools are found on many Dalmatian islands where, in the past, they provided the only source of water for island communities (CAR 2002). These pools contribute to the biodiversity of the islands. In recent times, they have become increasingly endangered by anthropogenic activities.

The present paper presents data on phytoplankton diversity in karstic pools on the largest of the northern Dalmatian karstic islands, Dugi Otok. Located 17 km from the Croatian mainland, the island is 52 km long and 1–4 km wide (Fig. 1), and enjoys a typical Mediter-

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ranean climate (BERTOVIĆ 1975). Fertile flat-floored depressions in karst limestones (poljes) are located between a ridge of limestone peaks, of which Vela Straža (338 m) is the highest point. Some of the karstic poljes flood occasionally, but during dry periods they are covered with vineyards and vegetable gardens, while the slopes have olive groves. The vascular flora of the island has been well investigated (TRINAJSTIĆ 1991), but nothing yet has been published on its phytoplankton.

The objective of this research was to determine for the first time the phytoplankton composition and the environmental characteristics of the karstic pools on Dugi Otok island.

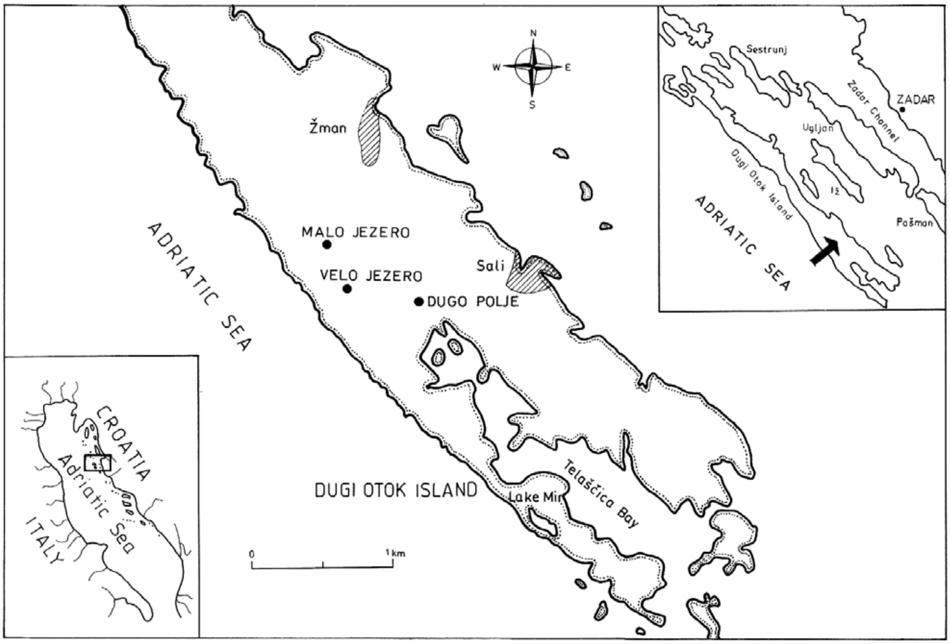

Fig. 1. Location of threee karstic pools (Malo jezero, Velo jezero and Dugo polje) in poljes on Dugi Otok island (Dalmatia, Croatia).

Materials and methods

In 2000, samples were taken from three pools in poljes in Dugi Otok: on June 22nd in Dugo Polje and on October 26th in Malo Jezero and Velo Jezero.

The pool in Dugo Polje is small (ca 20 m^2), shallow (maximum depth of 1 m), and has a bottom covered with *Chara* spp. and *Potamogeton* spp.

The pools in Malo Jezero and Velo Jezero (surface area ca 15 m^2) – located in poljes that occasionally flood – are shallow crypto-depressions (maximum depth is 2 m) with shorelines overgrown by hydrophilous and hygrophilous plants (TRINAJSTIĆ 1991 and reference therein).

Water samples for physico-chemical and phytoplankton analyses were taken with 5-L Niskin bottles. Temperature was measured with an inverted thermometer. Salinity and oxygen were measured using standard titration methods (STRICKLAND and PARSONS 1972). Nutrient concentrations (NO_3, NO_2, NH_4, N_{tot} – total inorganic + organic nitrogen, PO_4, P_{tot} – total inorganic + organic phosphorus, SiO_4) were measured using APHA methods (1989).

Samples for phytoplankton cell density analysis were fixed in 2.5% neutralized formaldehyde. Sub-samples (50–100 ml) were permitted to settle for 24–48 hours in the counting chambers. Cells subsequently were counted with an Olympus IX 71 inverted microscope (UTERMÖHL 1958), using phase-contrast, at 400x. Cells covering the entire bottom of the chamber were counted.

Results are expressed as cell density of phytoplankton (including cyanobacteria) per liter. Each cell of filamentous algae (and cyanobacteria) was counted as a single cell. Net phytoplankton samples were used for qualitative analysis of plankton. We used 20-cm diameter, 55-mm mesh- size net, horizontal tows, 50 cm beneath the surface.

Diatoms were identified following standard techniques described by BATTARBEE (1986). Relevant references were used in the identification of algae (HUSTEDT 1930; LIND and BROOK 1980; ETTL 1983; KRAMMER and LANGE-BERTALOT 1986, 1988, 1991a, b; POPOVSKÝ and PFIESTER 1990; CANTER-LUND and LUND 1996). Cyanobacteria were identified using GEITLER (1932). The taxonomy of higher-level categories was adjusted according to LEE (1999).

Margalef's species richness index (MARGALEF 1965) was used to characterize species richness:

$$D = \frac{S-1}{\log N},$$

where D is the index; S is the number of species; and N is the total number of individuals. (If only one species is present, S-1 is zero and thus D is undefined). The index was estimated using PRIMER v5 software packages (CLARKE and GORLEY 2001).

The similarity index of Jaccard (JI) (JACCARD 1908), based on the presence/absence of a species, rather than on its actual number, was used to quantify species associations in net plankton samples. It is symbolized as S (not to be confused with S in the Margalef expression) and calculated as

$$\mathrm{S} = 100\left[\frac{a}{a+b+c}\right],$$

where a is the number of species present in both samples (pools); b is the number of species present in sample 1, but absent in sample 2; and c is the number of species present in sample 2, but absent in sample 1. The multiplier 100 expresses JI as a percent. Double absences were not considered.

Results

Physicochemical parameters

Water temperature was 16.5 °C (June 2000) in the pools of Dugo Polje and 13.8 °C (October 2000) in those of Malo Jezero and Velo Jezero. All salinities were below 1. Oxygen and nutrient concentrations are shown in Table 1. The highest nutrient concentrations, excluding NO_3 and NO_2, were found in Malo Jezero, where conditions were anoxic. Maximum NO_3 was found in the Velo Jezero pool.

Tab. 1. The main physico-chemical parameters and total phytoplankton abundance in three karstic pools of Dugi otok Island (O_2 – mg L^{-1}; nutrients – μmol L^{-1}; N_{tot} – total inorganic + organic nitrogen; P_{tot} – total inorganic + organic phosphorus; PHYTO – phytoplankton abundance – cells L^{-1}). The maximum values for each parameter are noted in bold.

Pools	O_2	NO_3	NO_2	NH_4	N_{tot}	PO_4	P_{tot}	SiO_4	PHYTO
Dugo Polje (June 22, 2000)	–	0.12	**0.15**	–	15.46	0.17	0.85	40.84	**3.65 x 10^6**
Malo Jezero (Oct. 26, 2000)	0.83	4.02	0.06	**1.98**	**18.17**	**0.51**	**0.87**	**204.00**	0.931 x 10^6
Velo Jezero (Oct. 26, 2000)	**16.16**	**5.00**	0.03	0.99	15.84	0.09	0.62	121.20	0.076 x 10^6

Phytoplankton abundance

Maximum phytoplankton abundance (3.6 x 10^6 cells L^{-1}) was recorded in a pool in Dugo Polje (Tab. 1). The most abundant cyanobacteria were *Oscillatoria tenuis* (1.5 x 10^6 cells L^{-1}) and *Dactylococcopsis* sp. (2.1 x 10^6 cells L^{-1}) which contributed 99% to total phytoplankton abundance. Other taxa occurred at much lower levels (80–8000 cells L^{-1}).

The dominant taxa in Velo Jezero (October 2000) were *Gomphosphaeria aponina* (1.5 x 10^4 cells L^{-1}), *Oedogonium* sp. (1.0 x 10^4 cells L^{-1}), and *Mougeotia* sp. (3.2 x 10^4 cells L^{-1}). These accounted for 76% of total phytoplankton abundance.

Cyanobacteria *Oscillatoria limosa* (2.4 x1 0^5 cells L^{-1}), *Oscillatoria* sp. (3.9 x 10^5 cells L^{-1}), and *Lyngbya martensiana* (1.3 x 10^5 cells L^{-1}) were the dominant taxa in the pool in Malo Jezero, contributing 83% of total abundance.

Margalef's species richness index was 1.51 in Dugo Polje, 1.24 in Velo Jezero, and 2.40 in Malo Jezero.

Net phytoplankton composition

A total of 85 taxa of net phytoplankton were recorded in the three pools (Tab. 2). These included 22 Cyanobacteria, 14 Chlorophyceae, 17 Charophyceae (Zygnematales), 15 Euglenophyceae, and 17 Bacillariophyceae. In the pools in Dugo Polje, Malo Jezero, and Velo Jezero, 39, 34, and 28 taxa were noted, respectively. Only one (*Oocystis solitaria*) was common to all three pools.

Similarity coefficients (Jaccard index) between the flora found in the pools of Dugo polje and Malo Jezero, Dugo polje and Velo Jezero, and Malo Jezero and Velo Jezero were, respectively: 5.9, 5.1 and 11.1%.

Discussion

The present study has revealed a diversified algal community in the pools of Dugi Otok. From the standpoint of community structure, the similarity of phytoplankton taxa among

Tab. 2. Net phytoplankton taxa found in three karstic pools of Dugi otok Island (Adriatic Sea).

	Dugo Polje	**Malo Jezero**	**Velo Jezero**
Number of taxa	39	34	28
Division: Cyanobacteria			
Class: Cyanophyceae			
Anabaena flos-aquae Bréb. ex Bornet et Flahault	.	.	+
Aphanocapsa endophytica G.M. Smith	.	.	+
Chroococcus limneticus Lemm.	+	.	.
Chroococcus minor (Kütz.) Nägeli	+	.	.
Chroococcus minutus (Kütz.) Nägeli	+	.	.
Dactylococcopsis acicularis Lemm.	+	.	.
Gomphosphaeria aponina Kütz.	+	.	+
Gomphosphaeria lacustris Chod.	.	.	+
Lyngbya martensiana Menegh.	.	+	+
Microcystis aeruginosa (Kütz.) Kütz.	+	.	+
Microcystis elabens Kütz.	.	.	+
Microcystis Kütz. sp. 1	+	.	.
Nostoc Vauch. ex Bornet et Flahault sp. 1	.	+	.
Oscillatoria aponina C. Agardh et Gom.	+	.	.
Oscillatoria irrigua Gom.	.	+	+
Oscillatoria limosa C. Agardh	.	+	+
Oscillatoria tenuis C. Agardh	.	+	+
Oscillatoria Vauch. sp. 1	+	.	.
Oscillatoria Vauch. sp. 2	+	.	.
Oscillatoria Vauch. sp. 3	.	+	.
Raphidiopsis F.E.Fritsch et F.Rich sp. 1	+	.	.
Synechocystis aquatilis Sauv.	+	.	.
Division: Chlorophyta			
Class: Chlorophyceae			
Chlamydomonas Ehrenb. sp. 1	+	.	.
Coelastrum microporum Nägeli	+	.	.
Eudorina elegans Ehrenb.	+	.	.
Gonium pectorale O.F. Müller	+	+	.
Oedogonium Link. sp. 1	.	.	+
Oedogonium Link. sp. 2	.	.	+
Oocystis solitaria Wittr.	+	+	+
Pediastrum boryanum (Turp.) Menegh.	.	+	.
Pediastrum duplex Meyen	.	+	.
Pediastrum tetras (Ehrenb.) Ralfs	+	.	.
Scenedesmus bicaudatus (Hansg.) Chod.	+	.	.
Scenedesmus dimorphus (Turp.) Kütz.	.	+	.
Scenedesmus ecornis (Ehrenb. ex Ralfs) Chod.	.	+	.
Tetraedron minimum (A. Braun) Hansg.	.	+	.

Tab. 2. – continued

	Dugo Polje	Malo Jezero	Velo Jezero
Class: Charophyceae (Zygnematales)			
Closterium ehrenbergii Menegh. ex Ralfs	.	.	+
Closterium moniliferum (Bory) Ehrenb. ex Ralfs	.	.	+
Cosmarium granatum Bréb.	+	.	.
Cosmarium humile Nordst.	.	.	+
Cosmarium impressulum Elfv.	+	.	.
Cosmarium meneghinii Bréb.	+	+	.
Cosmarium punctulatum Bréb.	+	.	.
Cosmarium reniforme Arch.	+	.	.
Cosmarium subtumidum Nordst.	+	.	.
Cosmarium turpinii Bréb.	+	.	.
Cosmarium vexatum West.	+	.	.
Cosmarium Corda sp. 1	+	.	+
Mougeotia C. Agardh sp. 1	+	.	.
Spirogyra link. sp. 1	+	.	+
Staurastrum dilatatum Ehrenb.	.	+	+
Staurastrum muticum Bréb.	.	.	+
Staurastrum paradoxum Meyen	.	+	.
Division: Euglenophyta	.	.	.
Class: Euglenophyceae	.	.	.
Euglena acus Ehrenb.	+	+	.
Euglena caudata Hübn.	.	+	.
Euglena gracilis Klebs	.	.	+
Euglena limnophila Lemm.	+	.	.
Euglena pisciformis Klebs	+	.	.
Euglena variabilis Klebs	.	+	.
Euglena velata Klebs	+	.	.
Euglena Ehrenb. sp. 1	+	.	.
Phacus acuminatus Stokes	.	+	.
Phacus aenigmaticus Drez.	.	+	.
Phacus horridus Pochm.	.	+	.
Phacus longicauda (Ehrenb.) Dujard.	+	.	.
Phacus Dujard. sp. 1	+	.	.
Trachelomonas hispida Defl.	+	.	.
Trachelomonas planctonica Svir.	.	+	.
Division: Heterokontophyta			
Class: Bacillariophyceae			
Amphora ovalis (Kütz.) Kütz.	.	+	.
Anomoeoneis sphaerophora (Kütz.) Pfitzer	.	.	+
Cocconeis placentula Ehrenb.	.	+	.
Coscinodiscus lacustris Grun.	.	.	+

Tab. 2. – continued

	Dugo Polje	Malo Jezero	Velo Jezero
Cyclotella meneghiniana Kütz.	.	.	+
Cyclotella (Kütz.) Bréb. sp.1	+	+	.
Diatoma hiemale Lyngb. var. *mesodon* (Ehrenb.) Grun.	.	+	.
Denticula tenuis Kütz. var. *crassula* (Nägeli) Hust.	.	+	.
Epithemia Bréb. sp. 1	.	+	.
Eunotia pectinalis (Dillw.) Rabenh. var. *minor* (Kütz.) Rabenh.	.	.	+
Eunotia Ehrenb. sp. 1	.	.	+
Fragilaria Lyngb. sp. 1	.	+	.
Gomphonema acuminatum Ehrenb.	.	+	.
Gomphonema truncatum Ehrenb.	.	+	+
Meridion circulare (Grev.) C. Agardh	.	+	.
Pinnularia microstauron (Ehrenb.) Cleve var. *brebissonii* (Kütz.) Hust.	.	.	+
Rhopalodia gibba (Ehrenb.) O.F. Müller	.	+	.

the pools – characterized by the Jaccard index – was relatively low. The lower species richness in Dugo Polje and in Velo Jezero is owing to the predominance of a few species, mainly Cyanobacteria. The most important were *Oscillatoria* spp. BERG et al. (1986) found that species of the same genus dominated eutrophic freshwaters. Unfortunately, the results on nutrient concentrations were scanty for a reliable determination of the trophic status of the pools.

According to SCHREURS (1992), dominance of filamentous species is related to shallow lake depth. There are two reasons that might explain this. First, they may not be grazed by zooplankton either because of their toxic compounds (LINDHOLM et al. 1989) or because the length of their filaments mechanically interferes with zooplankton feeding (CYR and PACE 1992). Secondly, recruitment from the sediments (TRIMBEE and HARRIS 1984) and oxygen depletion in the water column (TRIMBEE and PREPAS 1988) may have controlled Cyanobacteria dominance in these pools.

Further, different thermal regimes might explain differences in phytoplankton diversity and abundance among the pools on the island. Higher temperatures favor Cyanobacteria during summer, owing to their generally higher optimum temperature than other algae (e.g. ROBARTS and ZOHARY 1987).

The highest number of taxa in all pools, besides Cyanobacteria, belonged to chlorophytes (Chlorophyceae and Charophyceae). In three pools of the northern Adriatic Krk island, PEVALEK (1929), identified 73 taxa, more than one-half of which consisted of Charophyceae (Zygnematales). This agrees with the more recent findings for the same localities (GLIGORA and PLENKOVIĆ-MORAJ 2003). In total, 13 taxa (*Anabaeana flos-aquae*, *Gomphosphaeria aponina*, *G. lacustris*, *Coelastrum microporum*, *Tetraedron minimum*, *Cosmarium granatum*, *C. humile*, *C. impressulum*, *C. meneghinii*, *C. reniforme*, *C. vexatum*, *Mougeotia* sp., and *Trachelomonas hispida*) were common to pools on Krk and Dugi Otok.

Bacillariophyceae were common in pools in Malo Jezero and Velo Jezero. FORTI (1901) and PLENKOVIĆ-MORAJ (STILINOVIĆ and PLENKOVIĆ-MORAJ 1995) noted 22 to 36 taxa of Bacillariophyceae in the pools of Krk island. Only four species (*Amphora ovalis*, *Cocconeis placentula*, *Gomphonema acuminatum*, and *Rhopalodia gibba*) were present on both islands. There were several tychoplanktonic species in the phytoplankton (mainly Bacillariophyceae and Charophyceae). Macrophytes provide a suitable substrate for epiphytic species (CATTANEO et al. 1998, CAPUT and PLENKOVIĆ-MORAJ 2000).

The differences in the plankton flora of the Krk and Dugi Otok islands may be explained, at least in part, by the differently sized water bodies, the origin and dynamic water flow of the karstic springs that feed these lakes and/or pools. Clearly, further studies on phytoplankton are required to increase the accuracy of predictions.

Analyses in this study are based on the larger »net« plankton. Thus, although smaller algae (nanophytoplankton) were found in the net samples, they likely to be under-represented; and some rarer species probably were missed entirely. The use of a plankton mesh less than 55 mm probably would have collected additional taxa. Nanophytoplankton and picophytoplankton should be the subject of future studies in the pools.

Data presented herein contribute baseline information essential in the long-term evaluation of the karstic environments of the Croatian islands.

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