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Macrophytic vegetation in the oligotrophic Mediterranean Lake Vrana (Island of Cres, Northern Adriatic) – New insight after 50 years

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ABSTRACT: Lake Vrana is a natural oligotrophic lake situated on the island of Cres (northeastern Adriatic Sea). Its macrophytic flora and vegetation were studied in 2010, nearly 50 years after the first comprehensive research on it. Vegetation of vascular plants, including belts of helophytes and submersed vegetation, dominates in the shallowest water. Charophytic vegetation starts to develop at the depth of approximately 8 m and is present in three clear zones dominated by *Chara aculeolata*, *Ch. virgata* and *Nitella opaca*, reaching a final depth of 30 m. The lake and its vegetation belong to habitat type 3140 (hard oligo-mesotrophic waters with benthic vegetation of *Chara* species), protected by the *Habitat Directive*. Due to the presence of several charophyte species endangered in the Balkans and Europe, the lake is an important site for the conservation of charophytes at the national and European levels. Great changes in the water regime over the last decades due to increase of water temperature and overexploitation of the lake's water are exerting a strong negative effect on this protected special reserve. A future comprehensive monitoring program, including climatological observation and surveillance of introduced alien fish populations, should focus on determining the cause of the observed retreat of vegetation in the lake.

Keywords: charophytes, macrophytes, helophytes, karst lake, stratification, Croatia

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INTRODUCTION

Stoneworts or charophytes (*Charophyta*), green algae of large size and complex structure, are represented in Croatia with all six genera (*Chara*, *Nitella*, *Lamprothamnium*, *Tolypella*, *Lychnothamnus* and *Nitellopsis*) and 36 species (Blaženčić *et al*. 2006). They grow in brackish or fresh water, in temporary ponds or deep lakes, as well as in both small streams and larger rivers (Wehr *et al.* 2015). Their communities are especially widespread in lakes, where they play an important ecological role. Various stonewort communities have been described in almost all large lake systems in Croatia – the Plitvice Lakes (Blaženčić & Blaženčić 1990-1991, 1992, 1994a, 1995, 1996; Blaženčić *et al.* 1991), Lake Vrana near Biograd in Dalmatia (Stanković & Alegro 2010), Lake Prokljansko

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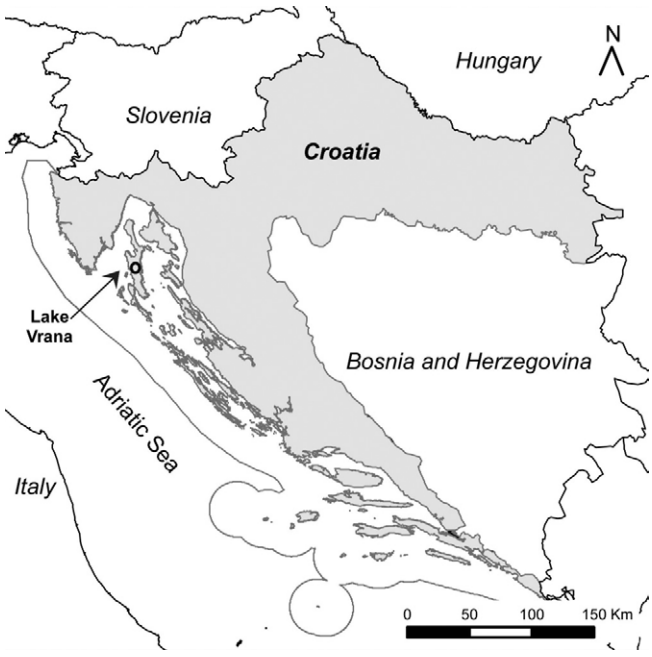

Fig. 1. Position of Lake Vrana on the island of Cres in the northeastern part of the Adriatic Sea.

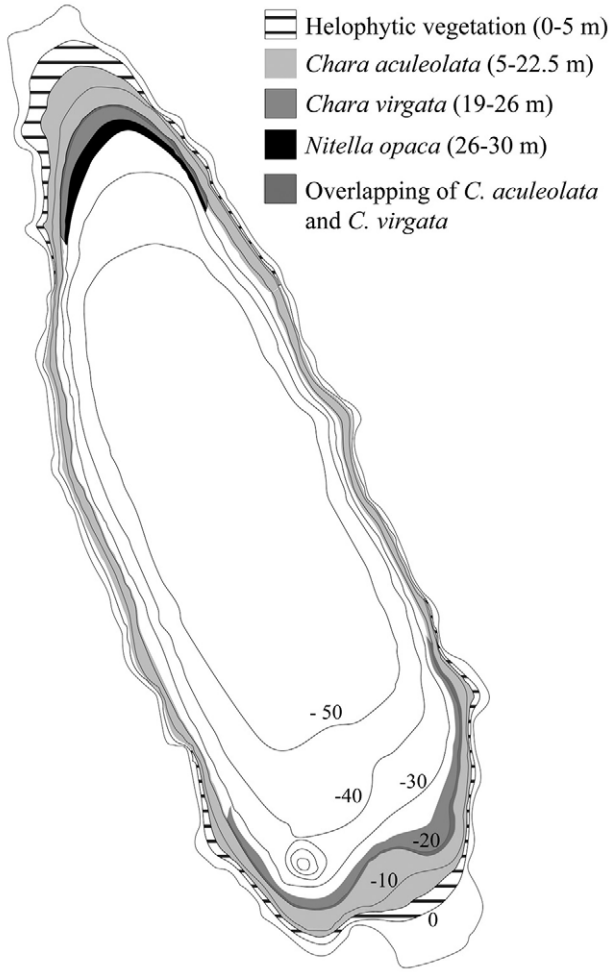

Fig. 2. Distribution of macrophytic vegetation in Lake Vrana.

in the Krka River (Blaženčić & Blaženčić 1990, 1994b), the Baćinska Lakes in Southern Dalmatia (Blaženčić 2006; Blaženčić & Blaženčić 2013) and Lake Vrana on the island of Cres (Golubić 1961a, b). Lake Vrana (Lake Vransko) is of special hydrological, ecological and environmental significance as a unique oligotrophic lake in the Eu-Mediterranean belt of Croatia and therefore an important reservoir of high-quality fresh water in this dry karst area.

Studies of stoneworts in Lake Vrana were started by Visiani (1872), who recorded *Chara intermedia* A. Braun and *Chara tomentosa* L. The collector was Stossich, who gave the plant material to the famous botanist from Trieste, Muzio Tommasini, and he in turn finally sent it to Visiani. Migula (1897) recorded only *Nitella hyalina* (De Candolle) C. Agardh. After more than 60 years without new data, Golubić (1961a, b) published the most comprehensive analyses on stonewort vegetation of Lake Vrana so far. He found several taxa - *Chara contraria* A. Braun ex Kützing, *Chara globularis* J.L. Thuiller f. *laxa* Desv., *Chara polyacantha* A. Braun (= *Ch. aculeolata* Kützing) f. *elongata* W. Migula, *Nitella confervacea* (Brébisson) A.Braun ex Leonhardi and *Nitella opaca* (C. Agardh ex Bruzelius) C. Agardh (with forms *laxa*, *elongate* and *simplex*) and described their distribution and zonation in the lake. Meanwhile, only Morton (1933) recorded the occurrence of some *Chara* species at a depth of 18 m.

Since no new data have been obtained on the macrophytic vegetation of Lake Vrana in the last 50 years and because a decreasing trend in the water level has been observed in recent times (Bonacci 2015), the main aims of the present work were: (i) to describe current vegetation of stoneworts (and other macrophytes) in Lake Vrana; and (ii) to assess the conservation status of the lake in terms of vegetation.

MATERIALS AND METHODS

Study area. Lake Vrana is a natural oligotrophic lake in the central part of the island of Cres, positioned in the northeastern part of the Adriatic Sea (Fig. 1). With Lošinj, Krk, Rab and some smaller islands, it is a part of the Kvarner Archipelago, within which Cres and Lošinj form a unique group of islands characterised by prominent karst, rocky pasture landscapes, along with reforestation processes in some areas (Magaš 2015). The island of Cres has an area of 405.7 km^2 and is almost completely composed of Cretaceous carbonate rocks with limestones and dolomites as the prevalent lithology (Bonacci 2015). Elongated in a NW-SE direction and mostly 40-50 m deep, Lake Vrana (Fig. 2) is a natural cryptodepression with an average water surface of 5.7 km^2 (Bonacci 1993, 2015; Kuhta & Brkić 2013). Formation of the lake was completed by the Holocene sea level rising, before which the lake was a karst field (Bonacci 2015). The lake's water originates mainly from

the atmosphere and its mean time of residence in the lake is 32 years (Hertelendi *et al*. 1997). The development of thermal stratification during the year (a thermocline was registered during June and August at a depth of 10-20 m) and formation of isothermal conditions at the end of winter (homeothermy was detected between 6.5 and 8.5°C) indicate that the entire water mass of the lake mixes once a year (Ternjej & Tomec 2005; Kuhta & Brkić 2013); thus, it is a monomictic lake. In terms of nutrient concentration and primary production, it can be classified as oligotrophic (Ternjej & Tomec 2005).

Survey of macrophytes. Study of the macrophytic vegetation of Lake Vrana was performed in August of 2010. In coastal parts of the lake (supralittoral and shallow zone of the littoral up to a depth of 1 m), macrophytes (vascular plants and stoneworts) were recorded and mapped from the banks and the water. The following methods were used to survey vegetation in water deeper than 1 m:

(i) The line transect method from a small boat employing a double-sided metal rake on a rope was the main method used (Stelzer & Schneider 2001; CENT/TC 230 2007). Seven transects 5 m wide (Schaumburg *et al.* 2007) perpendicular to the shoreline towards the centre of the lake were carried out in different parts of the lake. Stoneworts and other macrophytes were sampled repetitively with a depth change of 1 metre until the maximal depth of occurrence was reached. Positions of sampling spots were measured using a Garmin Oregon 550 GPS device. At each sampling spot, water transparency was measured with a Secchi plate, while depth was measured using an SM-5 Depthmate portable sounder (Speedtech Instruments).

(ii) The second survey method was scuba diving, which was performed according to CEN/TC 230 (2007). Scuba diving is highly recommended for better accuracy in the case of rare taxa (Raschke & Rusanowski 1984; Melzer & Schneider 2001; Schaumburg *et al.* 2007). Seven transects 5 m wide were made along with boat collection, and seven additional transects were carried out separately.

Stonewort samples were collected in plastic containers and preserved in a solution of 50% ethanol and 1% glycerine for species identification. Identification was made under a stereomicroscope using standard identification keys and manuals (Wood & Imahori 1965a, b; Moore 1986; Krause 1997; Schubert & Blindow 2004). The nomenclature follows The Plant List database (2016) for vascular plants and Characean Deutschland (Arbeitsgruppe Characeen 2016) for charophytes.

In order to prepare the vegetation map, all sampling spots with species lists were projected on a bathymetric map of the lake using the ArcGis 10.3 package. All dots representing the deepest occurrence of certain species were connected following the isobaths in order to delineate vegetation belts of the lake.

RESULTS

Based on direct observations from the banks, collecting by rakes from a boat and scuba diving, a quite clear picture of species composition and zonation of vegetation in the lake was acquired. Besides the supralittoral zone, which is made up of loose monodominant stands of *Vitex agnus-castus* L. encompassing the whole lake, the following vegetation zones can be recognised:

1. **Vegetation zone dominated by vascular plants**. It is developed in shallow water and reaches a maximal depth of 8 m. This zone is mostly restricted to the narrower northern and southern banks, which have gradual slopes and a muddy bottom that make colonisation of plants possible. It can be divided into two belts:
   1.1. **Belt of helophytic (emersed) vegetation (cl. *Phragmito-Magno-Caricetea* Klika in Klika et Novák 1941, all. *Phragmition australis* Koch 1926)**. It is developed from the shallowest water to a depth of 2.5 m. Nearest to the banks, loose to dense stands of *Phragmites australis* (Cav.) Trin. ex Steud. (ass. *Phragmitetum australis* Savič 1926) are developed and can be up to 5 m wide, rarely wider. They become looser towards the open water and gradually change to stands of *Schoenoplectus lacustris* (L.) Palla (ass. *Schoenoplectetum lacustris* Chouard 1924). Besides these two dominant species, *Juncus subnodulosus* Schrank and *Mentha aquatica* L. are also common here. The submerged species *Najas marina* var. *intermedia* (Wolfg. ex Gorski) Rendle, *Nitella hyalina*, and *Potamogeton pectinatus* L. are frequent, but do not form closed stands. *Chara aspera* C.L. Willdenow, *Chara hispida* L. and *Chara aculeolata* Kützning are rare, with only a few scattered individuals.
   1.2. **Belt of submerged vascular plant vegetation (cl. *Potametea pectinati* Klika in Klika et Novák 1941, all. *Potamion* (Koch 1926) Libbert 1931)**. This belt is developed at depths of from (2.5-) 3 to 5 (-8) m. *Potamogeton pectinatus* mixed with *N. marina* var. *intermedia* and sporadic but dense patches of *Myriophyllum spicatum* L. start to dominate from a depth of 3 m. *Myriophyllum spicatum* can be very vigorous, with stems longer than 3 m, in the NW part of the lake. Where the given belt is developed deeper than 5 m, it is composed of almost monodominant stands of *P. pectinatus*, which can reach even to a depth of 8 m. In this deeper zone, it is intermixed with *Ch. aculeolata*, which from a depth of 8 (or even 5 m in some parts) starts to be monodominant. Large surfaces of *P. pectinatus* populations are covered by pale, calcareous mud.
2. **Vegetation zone dominated by stoneworts (cl. *Charetea* Fukarek ex Krausch 1964, ord. *Charetalia***

***hispidae* Sauer ex Krausch 1964)**. It appeared to be the main and dominant vegetation zone of Lake Vrana. In contrast to the zone of vascular plants, which is restricted to the rather shallow and narrow parts of the lake, this zone encompasses the whole lake, up to a depth of 30 m. It is clearly divided into three belts, each dominated by certain charophyte species. The first two belts belong to the alliance *Charion fragilis* Krausch 1964 (= *Charion rudis-hispidae* Pietsch 1987), while the third one is classified as *Charion vulgaris* W. Krause 1981.

2.1. **Belt of *Chara aculeolata*** (= *Ch. polyacantha* A. Braun) **(ass. *Charetum aculeolatae* Dambska 1966 ex Gabka et Pelechaty 2003).** This is by far the widest and most widespread vegetation type of Lake Vrana. It is built of dense and monodominant stands of *Ch. aculeolata* occupying a depth range of from (5-) 8 to 24 (-28) m. While in the shallower zone, from 5 to 10 m of depth, it can be intermixed with *P. pectinatus*, in the deepest parts it does not form a continuous belt, e.g., it occurs sporadically. The belt has its optimum in terms of density and vitality of the algae at depths of 10-20 m. The length of whole algae and their internodes increases with depth, when they become more robust, but less encrusted by limestone. The species can sporadically occur even in the shallowest water, and it can therefore be designated as the one with the broadest ecological range and morphological plasticity in the lake. On the large stands, *Ch. aculeolata* lacks stem tips, due to grazing by fish.

2.2. **Belt of *Chara virgata* Kützing (ass. *Charetum virgatae* Doll 2012).** This belt occupies a depth range of from (19-) 22.5 to 25 (-29) m. Compared with the preceding belt, it is relatively narrow, but still well defined and composed of dense monodominant stands. Below 25 m, the belt is fragmented and built of discontinuous patches of *Ch. virgata*.

2.3. **Belt of *Nitella opaca* (C.Agardh ex Bruzelius) C.Agardh (ass. *Nitelletum opacae* Corill. 1957).** The last vegetation belt in the lake, it reaches a maximal depth of 30 m. Nevertheless, in some parts of the lake, even at depths of 26 m, no macrophyte vegetation occurs. We can therefore assert that *N. opaca* forms extended mats, but not a continuous belt.

Physical and chemical parameters of the lake's water were analysed within the framework of the National Water Quality Monitoring Programme and taken from the National Database for the period 2005-2010 (Table 1).

Table 1. Physical and chemical parameters of the lake's water for the period 2005-2010 (N=72).

Parameter	Range	Mean
Water temperature (°C)	6.5 - 29.2	16.6
pH	7.77 - 8.73	8.2
Conductivity (μS/cm)	376 - 512	422.2
Alkalinity (mg $CaCO_3$/L)	100 - 135	112.4
Oxygen (mg O_2/L)	8.2 - 12.3	10
Saturation (%)	84.3 - 123.6	101.3
BOD (mg O_2/L)	0.1 - 1.6	0.7
COD-Mn (mg O_2/L)	0.6 - 2.7	1.3
Total nitrogen (mg N/L)	0.06 - 0.4	0.196
Total phosphorous (mg P/L)	0.002 - 0.027	0.006
TOC (mg/L)	1.2 - 2.7	1.8
Secchi (m)	7 - 17	13
Chlorophyll *a* (μg/L)	0.2 - 2.6	0.7

DISCUSSION

As a deep oligotrophic lake situated in the Eu-Mediterranean zone, Lake Vrana is a quite unique ecosystem in Croatia (Ternjej & Tomec 2005; Gligora Udovič *et al.* 2015). Plant communities in the lake are well defined, discrete and arranged mainly along a gradient of depth and morphology of the lake floor. Thus, helophytic vegetation is restricted to the narrow southern and northern flanks of the lake, where depth increases gradually, permitting mud sedimentation and establishment of plant populations. In contrast to this, the elongated eastern and western flanks are conspicuously steep and rocky. For that reason, only submerged vegetation develops there, starting with the belt of *Potamogeton pectinatus*, spanning depths of from 3 to 6 m. However, deeper belts of the lake are covered exclusively by charophyte species (*Chara aculeoata*, *Ch. virgata* and *Nitella opaca*).

Charophytes are generally considered to be indicators of a good conservation status of water bodies, since the large ones (e.g., *Chara aculeolata*) are the first to disappear when pollution occurs (Blindow 1992; Blindow *et al.* 2002; Azzella *et al.* 2013). Their habitats are therefore protected by the *Habitat Directive*, and Lake Vrana is an excellent example of habitat type 3140 (hard oligo-mesotrophic waters with benthic vegetation of *Chara* species). The presence of *Chara aculeolata* and *Nitella hyalina* is of special importance, since *Ch. aculeolata*, the dominant species in Lake Vrana, is currently classified as *endangered* (EN) in the Balkans (Blaženčić & Blaženčić 2002; Blaženčić *et al.* 2006) and Switzerland (Joye & Schwarzer 2012) and *critically endangered* (CR) in Germany and Norway (Korsch *et al.* 2013). In the Balkan countries it is rare, known

only from three localities in Croatia and one in Greece. *Nitella hyalina* is very rare throughout Europe (Krause 1997) and is considered *critically endangered* (CR) in the Balkan countries (Blaženčić & Blaženčić 2002; Blaženčić *et al.* 2006) and Germany (Blaženčić *et al.* 2006; Korsch *et al.* 2013), *extinct* (EX) in Great Britain and Switzerland (Palmer 2008; Joye & Schwarzer 2012) and *potentially threatened* (PT) in Finland (Bjelke 2010). It has a wider area of distribution in the Balkans than *Ch. aculeolata*; however, in Croatia it is known only from four localities. *Chara virgata* is not on the proposed red list of charophytes in the Balkans; nevertheless, it is a rare species in Croatia, with only seven known localities. Only *Nitella opaca* occurs frequently in Croatia, with its centre of distribution in the Plitvice Lakes, where it forms abundant and widespread populations, reaching a depth of 20.5 m (Blaženčić & Blaženčić 1990-1991, 1992, 1994a, b, 1995, 1996; Blaženčić *et al.* 1991). In Germany *Nitella opaca* has been recorded from a depth of 33.4 m (van de Weyer & Krautkrämer 2009).

Lake Vrana, with five charophyte species found, can be considered an important site for the conservation of charophytes at the national and European levels. Its macrophytic vegetation is strongly dominated by charophytes, since they are able to use the dissolved carbonate as a source of CO_2, successfully thriving in lakes with high alkalinity and good transparency (Azzella 2013). Moreover, they perform an important functional role in ecosystems (Kufel & Kufel 2002; Rodrigo *et al.* 2007) by maintaining high transparency of the water (Blindow *et al.* 2002), counteracting the proliferation of phytoplankton (Mulderij *et al.* 2003; Berger & Schagerl 2004) and being a food source and habitat for many animal species (van den Berg *et al.* 1997; Noordhuis *et al.* 2002).

Due to its peculiar geomorphological, hydrological and biological features, Lake Vrana has been protected as a *special reserve*; however, various threats to this vulnerable ecosystem were identified during the last decades. From the mid-1980s, a decreasing trend in the mean annual water level of the lake has been recorded. Surprisingly, during this period there was no statistically significant decrease of annual precipitation. However, average annual air temperature, and consequently, average annual water temperature, have increased (Bonacci 2012, 2015). Lake Vrana is one of the largest freshwater reservoirs in the area and its overexploitation as a source of water supply for Cres and the surrounding islands, sharply increased with intensive tourism during the summer months, has been recognised as another factor affecting the lake's water regime (Bonacci 2015).

Comparing the composition and distribution of vegetation with the status in 1958 (Golubić 1961a, b), we see that species composition and vegetation belts have remained largely the same. However, 50 years ago (Golubić 1961a, b), *Nitella opaca* reached a depth of 38 to 40 m, while the maximal depth of its occurrence during our research appeared to be 30 m. Moreover, Golubić (1961a) recorded transparency of 12-23 m, while nowadays (during the period 2005-2010) it is 7-17 m. The observed retreat of the deepest vegetation zone along with decrease of the lake's transparency could be attributed to increase of turbidity and fish grazing. However, we neither measured turbidity nor studied fish populations; thus, this is no more than an assumption and a suggestion for further studies. Nevertheless, we observed that many *Potamogeton pectinatus* stands were covered by a fine coating of mud. It is known that benthivorous fish dig *Potamogeton* tubers, suspending the mud during the process of feeding. They also affect the next vegetation belt, stands of *Chara aculeolata*, with an optimum depth range of approximately 10 to 20 m. They are grazed over large areas and are therefore without stem tips. The effects of cyprinid overpopulation – resuspension of sediments and consequent mobilisation of nutrients, as well as direct destruction of macrophytes through grazing and digging - are well known from study of many lake ecosystems (Crivelli 1983; Kalbe 1984; Breukelaar *et al.* 1994; Roberts *et al.* 1995; Moss *et al.* 1996; Lougheed *et al.* 1998; Miller & Crowl 2006; Miller & Provenza 2007; Korsch *et al.* 2013; van de Weyer *et al.* 2015; Huser *et al.* 2016). The only native fish species in Lake Vrana is the eel (*Anguilla anguilla*) (Maloseja 1976; Stražičić 1980), while another three species (*Esox lucius*, *Tinca tinca* and *Scardinius erythrophthalmus*) were introduced, most likely during the first half of the 19th century. With the passage of time, they established stable and large populations, in spite of the lake's oligotrophy (Stražičić 1980). There would therefore seem to be a definite need for study of fish populations in the lake in order to adopt or reject hypotheses about their influence on macrophytic vegetation.

CONCLUSION

Lake Vrana, a natural oligotrophic lake, is a typical charophytic lake and a habitat of the protected type 3140 (hard oligo-mesotrophic waters with benthic vegetation of *Chara* species). Due to the recorded presence of five charophyte species, several of which are endangered in the Balkans and Europe, the lake is an important site for the conservation of charophytes at the national and European levels. Climate change (increase of air and water temperature and decrease of the water level) and strong pressure of water removal have been recognised as key factors powerfully affecting this, beyond any doubt, fragile ecosystem. It should therefore be regularly monitored and protected from excessive water loss. Further climatological observations as well as studies regarding the possible role of alien fish populations in the retreat of vegetation would be worthwhile.

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Botanica SERBICA

REZIME

Makrofitska vegetacija u oligotrofnom mediteranskom jezeru Vrana (Cres, severni Jadran) – Novi uvid nakon 50 godina

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Vransko jezero, prirodno oligotrofno jezero, smešteno je na ostrvu Cresu na severoistoku Jadranskog mora. Nakon 50 godina ponovno je istraživana njegova makrofitska flora i vegetacija tokom 2010. godine. Vegetacija vaskularnih biljaka, uključivši pojaseve helofitske i submerzne vegetacije dominira u najplićoj vodi. Vegetacija harofita počinje da se razvija na dubini od oko 8 m i deli se na tri jasne zone u kojima dominiraju *Chara aculeolata*, *Ch. virgata* i *Nitella opaca*, koja doseže maksimalnu dubinu od 30 m. Opisana vegeatcija pripada habitatnom tipu 3140 (Tvrde oligo-mezotrofne vode sa bentoskom vegetacijom *Chara* sp.), zaštićene Habitatnom direktivom. Zbog nekoliko vrsta harofita ugroženih na Balkanu i u Evropi, jezero predstavlja važno područje za harofite na nacionalnoj i evropskoj razini. Velike promene u vodnom režimu poslednjih decenija zbog rasta temperature vode i eksploatisanja jezerske vode snažno su negativno uticale na ovaj zaštićeni specijalni rezervat. Budući obuhvatan monitoring program koji bi uključivao klimatološka posmatranja i proučavanje populacija unesenih stranih vrsta riba u jezeru, trebao bi da se fokusira na rešenje geneze zapaženog uzmicanja vegetacije u jezeru.

Ključne reči: harofite, makrofite, helofite, karstno jezero, stratifikacija, Hrvatska