

## ON-LINE SUPPLEMENTAL MATERIAL

Šimunović M., Kulaš A., Žutinić P., Goreta G., Gligora Udovič M.: Phytoplankton metrics for trophic and ecological status assessment of a natural karstic lake. Acta Bot Croat, DOI: 10.37427/botcro-2022-016.

**On-line Suppl. Tab. 1.** Class boundaries of the ecological status for the Croatian national lake type HR-J\_5 (Official Gazette 2019). HLPI – Hungarian Lake Phytoplankton Index, SD – Secchi depth, NO<sub>3</sub><sup>-</sup> – nitrate, TP – total phosphorus.

Ecological status	HLPI	SD (m)	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	TP (mg L <sup>-1</sup> )
High	0.80 – 1.00	≥ 4.5	0.25	0.02
Good	0.60 – 0.79	≥ 2.5	0.40	0.05
Moderate	0.40 – 0.59	–	–	–
Poor	0.20 – 0.39	–	–	–
Bad	< 0.20	–	–	–

**On-line Suppl. Tab. 2.** Class boundaries of the trophic status (OECD 1982, \*Brettum 1989). SD – Secchi depth, TP – total phosphorus, Chl-*a* – Chlorophyll-*a*.

Trophic status	SD (m)	TP (μg L <sup>-1</sup> )	Chl- <i>a</i> (μg L <sup>-1</sup> )	Phytoplankton biomass (mg L <sup>-1</sup> ) *
Ultra-oligotrophic	> 12	< 4	< 1	< 0.12
Oligotrophic	12 – 6	4 – 10	1 - 2.5	0.12 - 0.4
Oligo-mesotrophic				0.4 - 0.6
Mesotrophic	6 – 3	10 – 35	2.5 - 8	0.6 - 1.5
Eutrophic	3 – 1.5	35 – 100	8 - 25	1.5 - 2.5
Polyeutrophic				2.5 - 5
Hypereutrophic	< 1.5	> 100	> 25	> 5

**On-line Suppl. Tab. 3.** Physical and chemical parameters in Lake Visovac during 2016 and 2019. O<sub>2</sub> – oxygen concentration, T – temperature, SD – Secchi depth, EC – electrical conductivity, TP – total phosphorus, NO<sub>3</sub><sup>-</sup> – nitrate, NO<sub>2</sub><sup>-</sup> – nitrite, NH<sub>4</sub><sup>+</sup> – ammonium, TN – total nitrogen.

	O <sub>2</sub> (mg L <sup>-1</sup> )	T (°C)	SD (m)	pH	EC (μS cm <sup>-1</sup> )	TP (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )
<b>2016</b>										
<b>April</b>	11.00	14.8	5.5	8.51	543	0.010	0.162	0.035	0.052	0.488
<b>May</b>	10.40	14.6	6.0	8.12	532	0.004	0.302	0.074	0.809	0.680
<b>June</b>	10.50	18.4	7.5	7.96	514	0.010	0.147	0.128	0.123	0.753
<b>July</b>	9.50	24.0	5.0	8.36	526	0.011	0.147	0.106	0.069	0.305
<b>August</b>	7.80	18.6	5.0	7.93	534	0.013	0.275	0.112	0.057	0.449
<b>September</b>	7.10	18.5	4.0	8.29	558	0.001	0.224	0.137	0.033	0.408
<b>2019</b>										
<b>April</b>	10.71	13.8	6.5	7.91	416	0.072	0.240	0.001	0.006	0.280
<b>May</b>	10.50	14.3	9.0	7.91	414	0.022	0.220	0.001	0.005	0.230
<b>June</b>	10.88	15.2	5.8	7.79	413	0.028	0.200	0.001	0.004	0.250
<b>July</b>	9.33	16.8	3.0	7.85	436	0.024	0.034	0.002	0.008	0.180
<b>August</b>	6.85	17.7	5.1	7.87	453	0.021	0.047	0.002	0.011	0.230
<b>September</b>	5.35	16.6	4.6	7.82	448	0.033	0.036	0.004	0.012	0.190

**On-line Suppl. Tab. 4.** Relative variance explained and factor coordinates of the variables for the first two principal components (PC1 and PC2) of the Principal Component Analysis (PCA). O<sub>2</sub> – oxygen concentration, T – temperature, SD – Secchi depth, EC – electrical conductivity, TP – total phosphorus, NO<sub>3</sub><sup>-</sup> – nitrate, NO<sub>2</sub><sup>-</sup> – nitrite, NH<sub>4</sub><sup>+</sup> – ammonium, TN – total nitrogen.

Variable	PC1	PC2
Variation (%)	41.4	25.8
Cumulative variation (%)	41.4	67.2
Eigenvalues	4.14	2.58
O <sub>2</sub>	-0.055	0.476
T	-0.207	-0.433
SD	0.018	0.474
pH	-0.350	-0.059
EC	-0.466	-0.135
TP	0.382	0.154
NO <sub>3</sub> <sup>-</sup>	-0.230	0.408
NO <sub>2</sub> <sup>-</sup>	-0.436	-0.112
NH <sub>4</sub> <sup>+</sup>	-0.262	0.276
TN	-0.395	0.245

**On-line Suppl. Tab. 5.** Summary of the Canonical Correspondence Analysis (CCA) results with relative variance explained and factor coordinates of the variables for the first four canonical axes.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.1044	0.0695	0.0590	0.0279
Explained variation (cumulative)	32.76	54.55	73.07	81.83
Pseudo-canonical correlation	0.9999	0.9874	0.9998	0.9906
Explained fitted variation (cumulative)	33.53	55.84	74.80	83.76

**On-line Suppl. Tab. 6.** Summary of the Spearman's rank correlation coefficient ( $r$ ,  $N = 12$ ) between the environmental variables, Chlorophyll-*a* and HLPI (Hungarian Lake Phytoplankton Index). Correlations in red indicate statistical significance of  $p < 0.05$ . O<sub>2</sub> – oxygen concentration, T – temperature, SD – Secchi depth, EC – electrical conductivity, TP – total phosphorus, NO<sub>3</sub><sup>-</sup> – nitrate, NO<sub>2</sub><sup>-</sup> – nitrite, NH<sub>4</sub><sup>+</sup> – ammonium, TN – total nitrogen.

Environmental variables	O <sub>2</sub> (mg L <sup>-1</sup> )	T (°C)	SD (m)	pH	EC (μS cm <sup>-1</sup> )	TP (mg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )
Chl- <i>a</i>	<b>-0.839</b>	<b>0.678</b>	<b>-0.872</b>	-0.224	0.273	-0.091	-0.448	0.372	0.070	-0.347
HLPI	<b>0.734</b>	-0.462	<b>0.928</b>	-0.021	-0.497	0.133	0.259	-0.382	-0.133	0.256

**On-line Suppl. Tab. 7.** Trophic and ecological status of Lake Visovac in 2016 and 2019 using different metrics. EU – eutrophic, MESO – mesotrophic, OLIGO-MESO – oligo-mesotrophic, OLIGO – oligotrophic, ULTRA-OLIGO – ultra-oligotrophic, SD – Secchi depth, TP – total phosphorus, NO<sub>3</sub><sup>-</sup> – nitrate, Chl-*a* – Chlorophyll-*a*.

ECOLOGICAL STATUS												
	2016						2019					
Metric	April	May	June	July	August	September	April	May	June	July	August	September
SD	HIGH	HIGH	HIGH	HIGH	HIGH	GOOD	HIGH	HIGH	HIGH	GOOD	HIGH	HIGH
TP	HIGH	HIGH	HIGH	HIGH	HIGH	HIGH	MODER- ATE	GOOD	GOOD	GOOD	GOOD	GOOD
NO <sub>3</sub> <sup>-</sup>	HIGH	GOOD	HIGH	HIGH	GOOD	HIGH	HIGH	HIGH	HIGH	HIGH	HIGH	HIGH
HLPI	GOOD	GOOD	HIGH	GOOD	GOOD	GOOD	GOOD	HIGH	GOOD	GOOD	GOOD	GOOD
TROPIC STATUS												
	2016						2019					
Metric	April	May	June	July	August	September	April	May	June	July	August	September
SD	MESO	MESO	OLIGO	MESO	MESO	MESO	OLIGO	OLIGO	MESO	EU	MESO	MESO
TP	MESO	ULTRA- OLIGO	MESO	MESO	MESO	ULTRA- OLIGO	EU	MESO	MESO	MESO	MESO	MESO
Chl- <i>a</i>	OLIGO	OLIGO	OLIGO	MESO	MESO	MESO	OLIGO	OLIGO	OLIGO	MESO	MESO	MESO
Biomass	OLIGO	OLIGO	OLIGO	MESO	OLIGO- MESO	MESO	OLIGO	OLIGO	MESO	OLIGO- MESO	OLIGO- MESO	MESO