Water quality and macrophytes in the Danube River: artificial neural network modelling

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Abstract

Ecological assessment of large rivers such as the Danube is a challenging task. Eutrophication was reported as one of the main drivers of aquatic community structures in the Danube basin. Due to their sedentary nature, relatively slow growth/ long life spans, and engineering role in aquatic ecosystems, macrophytes are widely used in the detection of nutrient enrichment. In this study, macrophyte presence-absence data within the 3 km long reaches obtained from the JDS3 survey were used to predict the water quality of the Danube river and its main tributaries. For each water quality variable (dissolved oxygen, nitrate-nitrogen, and orthophosphates), a feed-forward artificial neural network model (ANN) with the backpropagation algorithm was constructed using the macrophytes as explanatory variables. Despite the limited number of samples along the wide trophic gradient of the

Danube river from the source to the mouth, the model showed good predictive performances for the main river channel (the prediction rate: 82.93% for the dissolved oxygen model, 74.80% for the nitrate-nitrogen model and 71.55% for the orthophosphates model). From 64 analysed macrophyte species, 28 were selected by sensitivity analysis as significant water quality indicators for at least one environmental variable. Indicator species mainly belonged to the eutrophic tolerant submerged or emerged species with broad ecological amplitude. The developed ANN architecture represents the modelling approach which could be applied to other lotic systems and biological quality elements.

Keywords: ANN; Aquatic Vegetation; Eutrophication; Joint Danube Survey.

1. Introduction

Ecological assessment of large rivers such as the Danube, the second-largest river basin in Europe, is a challenging task (Birk et al., 2012; Chapman et al., 2016, Milosevic et al, 2018). Monitoring of these systems requires the balancing of sampling efforts with available resources, given research programmatic goals and objectives (Flotemersch et al., 2006). The most comprehensive investigative programs in the Danube Basin is the Joint Danube Survey (JDS) (Liška et al., 2015). The key purpose of JDS is to produce reliable and comparable information on carefully selected elements of water quality for the length of the Danube River and its tributaries. According to the Joint Danube Surveys conducted during the 2007 and 2013 , increasing eutrophication was reported as one of the main drivers structuring aquatic communities in the basin (Birk et al., 2012; Chapman et al., 2016). While agriculture was recognized as a major source of nitrogen emissions, the urban settlements were reported as significant sources for phosphorus emissions (ICPDR, 2010). According to Chapman et al. (2016), observations of nitrate-nitrogen and phosphorus from JDS3 showed high comparability with the time-corresponding data (August–September) from the long-term ICPDR surveillance monitoring (Liška et al., 2015). Understanding and modelling of this complex human pressure are of high importance for reaching/ maintaining the good ecological status of the Danube. However, the relatively small number of JDS2/3 sampling sites (and even smaller in JDS4), compared to other relevant studies (Gebler et al., 2014; 2017; 2018), makes the modelling of this ecological processes challenging.

Water quality models are important for effective environmental management (Wang et al., 2013). Worldwide, hundreds of these models have been developed, including models for prediction of riverine eutrophication. Some of them aimed to predict dissolved oxygen and biochemical oxygen demand by other nutrients or basic physico-chemical parameters as explanatory variables (Singh et al. 2009; Atanasijević et al. 2013; Atanasijević et al., 2014), while others aimed to explore the relationship between nutrients and biological quality elements (Gebler et al., 2014; 2017; 2018; Milošević et al., 2018). The majority of these models were based on Artificial Neural Networks, which are advanced algorithms capable to extracting complex, nonlinear relationships among aquatic communities and eutrophication variables.

Due to their sedentary nature and relatively slow growth/ long life spans, macrophytes are widely used in the detection of nutrient enrichment (Pall and Moser, 2009). Compared to other aquatic organisms, macrophyte species could be with a few exceptions identified immediately on the field, which makes them suitable for *in situ* assessment of trophic status. River trophic conditions are simultaneously influenced by multiple other factors (Birk et al., 2012), including catchment land-use, hydromorphological features, water velocity, habitat degradation, erosion, shoreline modification, etc. These habitat characteristics may influence macrophyte vegetation as well (Demars and Edwards, 2009). On the other hand, macrophytes are considered as an important constituent of lotic ecosystems as they directly influence the hydrology and sediment dynamics (O'Hare et al., 2018). They can engineer fine-scale physical heterogeneity and hydrogeomorphological processes in riverine habitats (O'Briain et al. 2017; Hood, 2012). Through their large surface area for hosting nitrifying and denitrifying organisms, macrophytes may increase the quantity of nitrite-nitrogen produced (Hood, 2012). On the other hand, aquatic plants may obtain nitrogen and phosphorus from the sediment and then release these elements into the water. These plants function as a source for nutrients, by trapping fine organic and inorganic particles, enhancing mineralization of organic matter through oxidation of the sediments, and altering the localized environment, thus enabling phosphorus release through reducing conditions and increased pH and temperature (Thiébaut, 2008). Therefore, macrophytes are considered as indicators of complex habitat conditions (Gebler et al., 2017; Gebler et al., 2018), while the best indicating results provide together with other biological quality elements (Birk et al., 2012).

Macrophyte composition and quantitative indices were found to be effective predictors of habitat characteristics (Demars and Edwards, 2009; Thomasen & Chow-Fraser, 2012), as well as prognostic parameters for modelling of different management options (Baart et al., 2010). Gebler et al. (2017) demonstrated that non-linear pressure-impact relationships occurring in aquatic ecosystems can be analysed with good results through advanced data analysis methods using macrophytes. Gebler et al. (2018) showed that ecological assessment of rivers based on macrophyte metrics does not only reflect the water quality but also the hydromorphological status as well. Advanced models considering macrophytes and eutrophication variables were the topic of many previous studies (Gebler et al., 2014; 2017; 2018). Still, none of them attempts to predict a river water quality using macrophytes as explanatory variables.

Therefore, the aim of this study was to develop a predictive model, based on artificial neural networks, for the water quality of the Danube river and its main tributaries using macrophytes as model inputs. To realize the main goal of the study, the following tasks were set: 1) to build the model based on macrophytes and environmental data, 2) to test the contribution of each of variables in the developed model and define macrophyte taxa with the highest indicator potential, and 3) to explore the ecological amplitude and position of selected indicator species within the water trophic gradient.

2. Material and Methods

2.1 Study area and field survey data

The research included the Danube river and its main tributaries (Appendix A). The Danube is the second largest river in Europe, having a length of 2860 km and a river basin covering an area of approximately 817,000 km2.

This study included macrophyte and environmental data extracted from the Joint Danube Survey 3 database (Liška et al., 2015), obtained within the EU-funded SOLUTIONS project. The general objective of the JDS3 was to undertake an international longitudinal survey that would produce comparable and reliable information on water quality for the whole of the length of the Danube River including the major tributaries on a short-term basis. The JDS3 survey was carried out during 2013 and included assessment of macrophyte vegetation of the Danube main channel and some mouth sections of important tributaries (Morava (Hainburg), Drava, Sava, Tisza, Velika Morava, Olt, Arges, Braila, etc., Appendix A). In total, 68 sites were sampled along a 2581 km stretch of the Danube (Figure 1), 15 of which were located in the mouths of tributaries. Survey units were of 1 km length, covering 3 river km on each side of the river in the main channel, thus resulting in 6 river km (6 samples) sampled at each sampling site. Abundance assessment followed European Standard EN 14184, comprising the assessment of individual species and their relative abundance per sampling site (Kohler and Janauer, 1994). Water samples were collected directly from the river together with the biological samples (Liška et al., 2015). In this study, environmental data included nitrate nitrogen, dissolved oxygen, and orthophosphates.

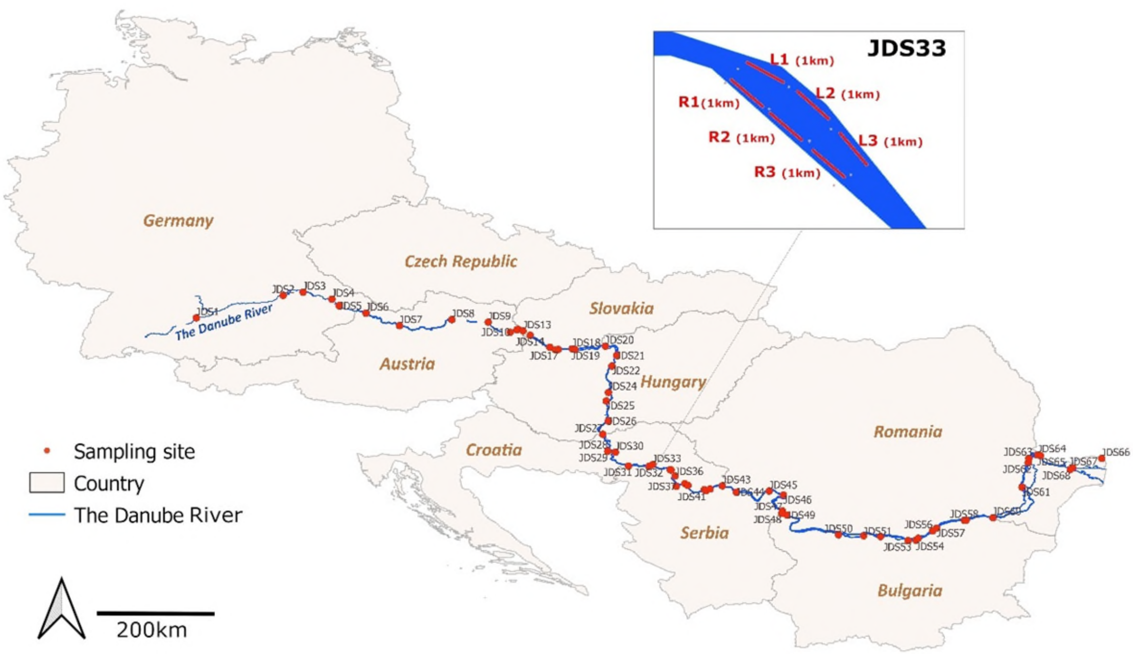

Figure 1. Map and scheme of the macrophyte sampling sites.

2.2 Data sets

Samples without recorded aquatic vegetation were omitted from the analysis. To eliminate statistical noise, invasive emergent and all semi-aquatic plant species were excluded from the data matrix. Invasive species were excluded due to broad tolerances to environmental changes which otherwise disturb the rest of the community (Holt and Miller, 2010). On the other hand, semi-aquatic species were excluded from the analysis because they are significantly influenced by the terrestrial environment. From 78 plant species recorded in the water during the JDS3 expedition, 64 macrophyte taxa (82%) were used for the model development. In order to increase the model prediction rate and accuracy, the number of null data was reduced by merging of 1 km survey units within the same sample site and the same river side into a single sample. Due to the impossibility of merging species abundance values, presence/ absence data were used in the final matrix. The final macrophyte data set included 123 samples and 64 macrophyte species.

Since there is no unique water quality classification system for the Danube river, for the purpose of this study, water quality classes were compiled considering the national water quality standards and boundaries of all Danube countries: Germany (Arle et al., 2014), Slovakia (Pekárová et al., 2009; Slobodnik et al., 2012), Austria (BGBl, 2006); Bulgaria (Sommerwerk et al., 2010), Ukraine (WHO, 2011), Croatia (Vlada Republike Hrvatske, 2019), Serbia (National Assembly of the Republic of Serbia, 2011); Hungary (Varga et al., 1990; Schiemer et al., 2004; Szilágyi et al., 2008); Romania (Apele Române, 2019); Moldova (Duca, 2014). The developed classification scheme included 7 quality classes for nitrate-nitrogen, dissolved oxygen, and orthophosphates (Table 1).

Table 1. Water quality classes (WQC) were compiled considering the national water quality standards and boundaries of all Danube countries

WQC	Dissolved oxygen [mg/l]	Nitrate-nitrogen [mg/l]	Orthophosphates [mg/l]
I	>9	0-0.019	0-0.019
II	7-8.9	0.02-0.9	0.02-0.039

III	5-6.9	1-2.9	0.04-0.09
IV	4-4.9	3-4.9	0.1-0.19
V	3 – 3.9	5-6.9	0.2-0.49
VI	2-2.9	7-10	0.5-0.8
VII	<2	>10	>0.8

2.3 Data analysis

The artificial neural network employs the model structure and working principle inspired by biological neural networks. It is a powerful computational technique for modelling complex non-linear relationships. The Backpropagation Neural Network (BPN) also called multi-layer feed-forward neural network or multi-layer perceptron is popular and is used more than other neural network types for a wide variety of tasks (Lek and Guégan, 1999). The architecture of the BPN is a layered feedforward neural network, in which the non-linear elements (neurons) are arranged in successive layers, and the information flows unidirectionally, from the input layer to the output layer, through the hidden layer(s). The signal passing through the neuron is modified by weights and transfer functions. The number of input and output units depends on the representations of the input and the output objects, respectively. In this study, three feed-forward neural networks with backpropagation learning were constructed for prediction of the river water quality classes (for dissolved oxygen, nitrate-nitrogen, and orthophosphates).

One artificial neural network model architecture was constructed and further generated and trained three times, for each environmental parameter separately (dissolved oxygen, nitrate-nitrogen, and orthophosphates). The artificial neural network model consisted of four layers: an input layer including 64 neurons, representing 64 macrophyte species; two hidden layers (12 and 8 neurons) and one output layer consisting of seven neurons, representing the seven water quality classes (Figure 2). The number of hidden layers and neurons in each layer was determined according to Keeni et al. (1999). Training started with many hidden units and then the network pruning was performed to the architecture with highest prediction rate (Equation 1) and minimal percentage of absolute errors (Equation 2).

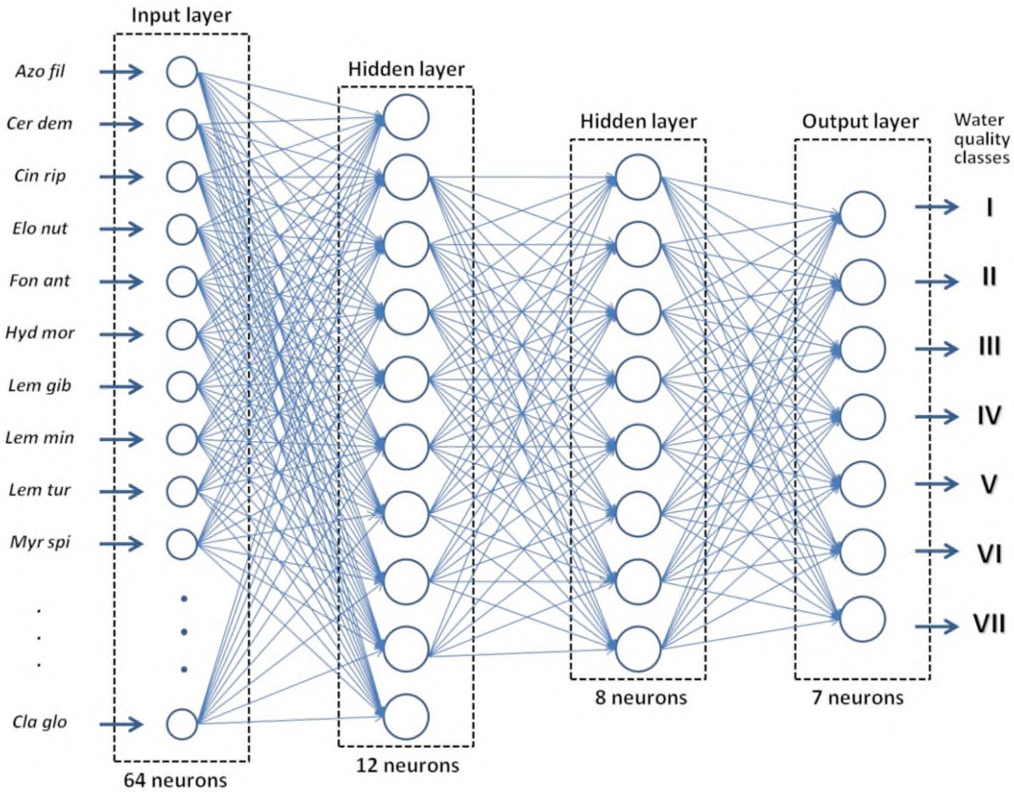

Figure 2. Diagram of the artificial neural network used in the modelling. Acronyms of aquatic macrophytes are listed within the input layer.

The Rectified Linear Activation Function (ReLu) was used for activation of the hidden layers, while the Sigmoid function was used for activation of the output layer. Currently, the ReLU is one of the most popular activation functions for deep neural networks (LeCun et al., 2015), while the sigmoid function is widely used activation function for ANNs (Acheampong and Boateng, 2015). Since the output layer consisted of 7 neurones, and only one is activated during each iteration, the Binary Cross-Entropy Function was used along the Adam Optimizer for the ANN model training.

Due to the limited number of samples, the Leave- one – out cross-validation approach was used to evaluate the model. In this approach, all samples except one is used to train the model. The model is then tested on that single sample that is left out. The process is then repeated for all samples (e.g., Wong, 2015). This implies that 123 iterations (one for each sample) with the training of the algorithm were performed for each environmental variable. In each iteration, the ‘most excited’ neuron in the output layer, corresponding to the particular water quality class, was considered as the output result. The results of the model cross-validation were summarized using the confusion matrix, constructed for each environmental variable.

Therefore, common approaches for the model accuracy evaluation, such as the mean square error, was not applicable in this study. Therefore, the Prediction rate (*Pr*), representing the overall accuracy of the model was calculated for each environmental variable, as a percentage of samples for which the predicted water quality class matched the observed ones:

$$Pr = \frac{Np}{N} * 100\% \qquad (1)$$

N is a total number of samples; *Np* is a number of samples for which the predicted water quality class matched the observed one.

The prediction rate shows how the model is successful in the prediction of exactly the right water quality class as it was measured in the field. The higher percentage rate value is, the better performances of the model is. However, in environmental management, when the model predicts one water quality class above or below the real values, it is in fact a better result compared to the situation when this discrepancy goes over two or more classes. In order to estimate this kind of discordance between predicted and observed values, the Percentage of absolute errors (Pa) was calculated as follows:

$$Pa = \frac{\sum_{i=1}^{N} \left(|ti^{e} - ti^{p} \ | \right)}{6N} * 100\% \qquad (2)$$

where $t_i^{\,e}$ is expected water quality class, and $t_i^{\,p}$ is predicted water quality. The lower value of the percentage absolute error is, the better the prediction accuracy is.

The agreement between observed and predicted water quality classes, as well as the degree to which this agreement can be attributed to chance, was estimated using the Kappa Index (Cohen, 1960). A

Kappa of 0 (or lower) is associated with a random classification result, while a Kappa of 1 indicates a perfect classification.

The sensitivity analysis was further performed to evaluate how ‘important’ the particular macrophyte species is for a model using a modified ‘Improved stepwise’ method (Gevrey et al., 2003). This approach is based on the construction of a new equivalent model consisting of one neuron less in the input layer compared to the original model (the model being tested by sensitive analysis). After that, the new equivalent model is generated and trained as many times as there are input variables in the original one, every time excluding another input. For each iteration, the model error is calculated. The variable that gives the largest error when eliminated is the most important. Hence, the model designed for sensitivity analysis in this research consisted of four layers: an input layer including 63 neurons, representing 63 macrophyte species; two hidden layers (12 and 8 neurons) and one output layer consisting of seven neurons, representing the seven water quality classes. For each environmental attribute, this additional ANN model was generated and trained 64 times, each time consisting of one species less. The decrease in *Pr* and increase in *Pa* values identified the most important species for the model. This allow species to be classified by order of their significance for the model. Based on these results, for each environmental parameter, a list of the best indicator species was created. The best indicator taxa were considered those showing at least 1.50% decrease of *Pr* value for all environmental variables and at least 1.45% increase of*Pa* value for orthophosphates and 0.66 % for dissolved oxygen and nitrate-nitrogen models. These thresholds represent the values at which the *Pr* and the *Pa* curves reach the plateaus when the species are ordered after the sensitivity analysis (Appendix A. Supplementary data 2. Figures 1-3). Modelling was carried out using the Python programming language and Keras library (Chollet, 2015).

Ecological amplitude and position of selected indicator species (species trophic ranks) within the water trophic gradient were further explored in order to get additional insights into the model applicability and its relationship with the existing macrophyte bioindication frame for rivers in Europe (Ellenberg, 1979; Ellemberg et al., 1992; Haury et al., 2006: Szoszkiewicz et al., 2010; Dawson et al., 1999; Schneider and Melzer, 2003). Trophic preferences of selected indicator species and those that the model found less significant were compared using the Mann-Whitney test ($P<0.05$) in the SPSS program package. The Mann-Whitney test was chosen due to the ordinal scale of data. Therefore, for each macrophyte trophic rank or ecological amplitude (Ellenberg, 1979; Ellemberg et al., 1992; Haury et al., 2006: Szoszkiewicz et al., 2010; Dawson et al., 1999; Schneider and Melzer, 2003) (Table 6), two group of species trophic values were compared: : i) nitrate-nitrogen indicators and the rest of species; ii) dissolved oxygen indicators and the rest of species; iii) orthophosphates indicators and the rest of species; and finally iv) all species which were identified as good indicators for any of environmental variables against the rest of species. This analysis allows to explore which ecological role (e.g., tolerance/ sensitivity to eutrophication, narrow/ wide trophic amplitude), underlying grouping of the indicator species.

The species trophic preferences which were analysed were: Ellenberg N value (Ellenberg, 1979; Ellemberg et al., 1992), the Macrophyte Biological Index for Rivers (IBMR) (Haury et al., 2006), the Macrophyte Index of Rivers (MIR) (Szoszkiewicz et al., 2010), the Mean Trophic Rank (MTR, the Species Trophic Rank -STR) (Dawson et al., 1999), the Trophic Index of Macrophytes -TIM (Schneider and Melzer, 2003). The Ellenberg N indicator values represent species requirements for nutrients and were designed on the basis of field experience for Central Europe (Ellenberg et al., 1992). The MTR system was developed for British rivers and was successfully applied across Europe (Brabec at al., 2006). In this system, Species Trophic Rank values are assigned to macrophyte species depending on their tolerance to eutrophication. The IBMR is an index created for estimating whether or not a river is affected by nutrient inputs (eutrophication) and/or heavy organic pollution in France (Haury et al., 2006). Moreover, the Macrophyte Index of Rivers (MIR) (Szoszkiewicz et al., 2010) is part of Polish national monitoring system which was found to correlate significantly with river trophic and hydromorphological conditions (Gebler et al., 2017; 2018), while the Trophic Index of Macrophytes -TIM (Schneider and Melzer, 2003) was developed from the same purpose in Germany.

3. Results

3.1 The model prediction performances and validation

One artificial neural network architecture was constructed and applied to each of water quality parameters. In total, three different models (dissolved oxygen, nitrate-nitrogen and orthophosphates), were generated, trained, tested and verified by the sensitivity analysis. The best prediction performances were obtained for the dissolved oxygen model, having a prediction rate of 82.93% and the percentage of absolute errors of 3.04%. The value of Kappa Index (*Ka* 0.61) for this model indicates a good agreement between measured and predicted water quality classes. The model for nitrate-nitrogen matched the correct water quality class for this parameter in 74.80 % of cases (*Pa*), with a relatively small percentage of absolute errors (5.42 %) and high the Kappa Index (0.64). Similar results were obtained for the orthophosphates model (*Pr* 71.55%, *Pa* 8.99%), but with the low level of agreement between observed and predicted water quality classes (*Ka* 0.17).

The Danube water quality according to JDS3 dataset and proposed classification scheme matched I-IV quality classes for dissolved oxygen, IV-VII classes range for nitrate-nitrogen content, and I and IV-V quality classes for orthophosphates. For the dissolved oxygen, the most frequent error of the model was the prediction of one water quality class higher compared to the observed values (Tables 2-4). The exceptions from this general pattern were the samples collected in tributaries or downstream from the tributaries mouth, where the model predicted lower-quality classes compared to the measured (Appendix A).

The predicted values for the nitrate-nitrogen model almost equally deviated above and below observed values. However, the orthophosphates model predicted lower quality class in 24 cases and higher class in 16 samples, than it was recorded on the field (Table 3).

The model errors for nitrate-nitrogen and orthophosphates quality classes didn't show any spatial pattern and were evenly distributed among all Danube reaches (Appendix A).

Generally, the highest discrepancy between observed and predicted water quality classes for all three environmental parameters together was obtained for the Danube tributaries, the Tisza and the Arges rivers (Appendix A). In these samples, the model mostly predicted lower (better) water quality classes compared to measured ones.

Table 2. Confusion matrix for dissolved oxygen water quality classes. The correctly predicted values are shown on the diagonal from the top left to the bottom-right of the matrix.

		Observed water quality classes						
		I	II	III	IV	V	VI	VII
Water quality classes predicted by the model	I	4	2	0	0	0	0	0
	II	6	86	5	1	0	0	0
	III	0	4	14	0	0	0	0
	IV	0	0	0	1	0	0	0
	V	0	0	0	0	0	0	0
	VI	0	0	0	0	0	0	0
	VII	0	0	0	0	0	0	0

Table 3. Confusion matrix for orthophosphates water quality classes. The correctly predicted values are shown on the diagonal from the top left to the bottom-right of the matrix.

		Observed water quality classes						
		I	II	III	IV	V	VI	VII
Water quality classes predicted by the model	I	5	0	2	9	1	0	0
	II	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0
	IV	7	0	4	77	10	0	1

	V	1	0	0	4	1	0	1
	VI	0	0	0	0	0	0	0
	VII	0	0	0	0	0	0	0

Table 4. Confusion matrix for nitrat-nitrogen water quality classes. The correctly predicted values are shown on the diagonal from the top left to the bottom-right of the matrix.

Observed water quality classes								
Water quality classes predicted by the model		I	II	III	IV	V	VI	VII
	I	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0
	III	0	0	0	0	1	0	0
	IV	0	0	0	33	8	0	1
	V	0	0	3	8	44	5	0
	VI	0	0	0	0	3	7	0
	VII	0	0	0	0	0	0	10

3.2 Sensitivity analysis and trophic ranks of indicator species

From 64 analysed macrophyte species, 28 were selected by the sensitivity analysis as significant water quality indicators for at least one environmental variable (Table 6). Only four species were found to be good predictors for all three water quality parameters together: *Amblistegium riparium*, *Agrostis* *stolonifera, Phragmites communis,* and *Myriophyllum spicatum.* Selected indicator species covered wide trophic range and included species tolerant and sensitive to eutrophication (Table 5).

Table 5. Trophic ranks of macrophyte species used in the analysis and indicator species selected by the sensitivity analysis

Species name	**Nitrate nitrogen**	**Dissolved oxygen**	**Orthophosphates**	**All variables**	**Ellen. N**	**IBMR (CSI)**	**IBMR (EI)**	**MIR (L)**	**MIR (W)**	**STR**	**TIM**
Agrostis stolonifera L.	+	+	+	+	5	10	1				
Alopecurus geniculatus L.					7			4	1		
Amblystegium riparium (Hedw.) Schimp.	+	+	+	+				1	1	1	
Azolla filiculoides Lam.					8	6	3			3	
Bidens frondosus L.					8						
Bidens tripartitus L.					8						
Butomus umbellatus L.					7	9	2			5	2.98
Carex acuta L.			+	+	5			5	1	5	
Carex elata All.					5						
Carex riparia Curtis					4			4	2	4	
Ceratophyllum demersum L.					8	5	2	2	3	2	3.18
Cinclidotus riparius (Host ex Brid.) Arn.						13	2				
Cladophora glomerata (Linnaeus) Kützing 1843			+	+		6	1	1	2	1	
Cyperus fuscus L.		+	+	+	4						
Cyperus michelianus (L.) Link	+			+	6						
Eleocharis acicularis (L.) Roem. & Schult.					2						
Eleocharis palustris (L.) R. Br.	+			+		12	2	6	2	6	
Elodea nuttallii (Planch.) H. St. John		+		+	7	8	2			3	2.75
Equisetum fluviatile L.		+		+	5	12	2	6	2	5	
Fontinalis antipyretica Hedw.			+	+		10	1	6	2	5	
Glyceria fluitans (L.) R. Br.		+		+	7	14	2	5	2		
Glyceria maxima (Hartm.) Holmb.	+			+	9			3	1	3	3.00
Gnaphalium uliginosum L.					4						
Hydrocharis morsus-ranae L.			+	+	6			6	2	6	
Iris pseudacorus L.		+		+	7	10	1	6	2	5	
Lemna gibba L.			+	+	8	5	3	1	3	2	
Lemna minor L.					6	10	1	2	2	4	

Lemna turionifera Landolt											
Lythrum salicaria L.					x						
Mentha aquatica L.			+	+	5	12	1	5	1		2.00
Mentha pulegium L.					7						
Myriophyllum spicatum L.					7	8	2	3	2	3	2.83
Najas marina L.	+	+	+	+	6	5	3				
Najas minor All.					4	6	3				
Nuphar lutea (L.) Sm.					6	9	1	4	2	3	3.15
Nymphoides peltata (S. G. Gmel.) Kuntze		+		+	7	10	2			2	
Persicaria hydropiper (L.) Delarbre	+	+	+	+	8	8	2	3	1		
Persicaria lapathifolia (L.) Delarbre	+		+	+	8						
Phalaroides arundinacea (L.) Rauschert					7	10	1	2	1		
Phragmites australis (Cav.) Steud.					7	9	2			4	
Potamogeton crispus L.	+		+	+	5	7	2	4	2	3	2.88
Potamogeton friesii Rupr.					6	10	1	3	2	3	2.68
Potamogeton gramineus L.					5	13	2	7	1	7	
Potamogeton lucens L.	+			+	7	7	3	4	3	3	2.65
Potamogeton natans L.					5	12	1	4	1	5	2.00
Potamogeton nodosus Poir.					5	4	3	3	2		3.10
Potamogeton perfoliatus L.					6	9	2	4	2	4	2.38
Potamogeton pusillus L.	+			+	5			4	2	4	2.40
Potamogeton trichoides Cham. & Schltdl.					4	7	2	2	2	2	
Ranunculus fluitans Lam.					8	10	2	7	2	7	3.00
Riccia crystallina L.											
Rorippa amphibia (L.) Besser			+	+	8	9	1	3	1	3	
Salvinia natans (L.) All.	+		+	+	7						
Schoenoplectus lacustris (L.) Palla					6					3	
Sparganium emersum Rehmann					7	13	2	4	2	3	2.78
Sparganium erectum L.					7	10	1	3	1	3	3.00
Spirodela polyrhiza (L.) Schleid.					6	6	2	2	2	2	
Stratiotes aloides L.					6			6	2		
Stuckenia pectinata (L.) Börner	+				8	2	2	1	1	1	
Trapa natans L.					8	10	3				
Typha angustifolia L.			+	+	7	6	2	3	2	2	
Typha latifolia L.					8	8	1	2	2	2	
Vallisneria spiralis L.					7	8	2				
Zannichellia palustris L.					8	5	1	2	1	2	2.93
Trophic index range (sensitive – tolerant)					1-9	20-0		10-1		10-1	1-4

Indicator weight value / Ecological amplitude(wide amplitude –narrow amplitude)							1-3		1-3		
Maximal - minimal (median) indicator values for all macrophyte species					2-9 (7)	2-14 (9)	1-3 (2)	1-7 (4)	1-3 (2)	1-7 (3)	2-3.18 (2.855)
Maximal - minimal (median) indicator values for selected indicator species								1-7 (5) ****	1-2 (1) **	3-7 (5) ***	
Maximal - minimal (median) indicator values for selected non-indicator species								1-5 (3) ****	1-3 (2) **	1-5 (3) ***	

Ellen. N - Ellenberg N value (Ellenberg, 1979; Ellenberg et al., 1992); IBMR (CSI) - the Macrophyte Biological Index for Rivers (Species Score); IBMR (EI) - the Macrophyte Biological Index for Rivers (Ecological Amplitude) (Haury et al., 2006); MIR (L) - the Macrophyte Index of Rivers (Species index value) (Szoszkiewicz et al., 2010); MIR (W) - the Macrophyte Index of Rivers (Species index weight) (Szoszkiewicz et al., 2010); TIM - the Trophic Index of Macrophytes - TIM (Schneider and Melzer, 2003); STR -the Species Trophic Rank -STR (Dawson et al., 1999). ** Values for nitrate-nitrogen indicator species. *** Values orthophosphates indicator species. ****Values for all indicator species.

Trophic preferences of selected indicator species and those that the model found less significant were compared for each environmental variable (Table 7). Significant differences (Mann-Whitney, $p<0.05$) were obtained for species trophic values (MIR(L)) and ecological amplitude (MIR(W)) according to the Polish Macrophyte Index for Rivers (Szoszkiewicz et al., 2010); and for species trophic ranks (STR) defined for UK rivers by Dawson et al. (1999).

The MIR(L) trophic values in the nitrate-nitrogen indicator group were significantly lower than for the rest of species (Mann-Whitney, U =57, p = 0.018). All species together which were identified as good indicators for any of the environmental variables showed significantly higher species MIR(W) weights (ecological amplitude), compared to the rest of the species (Mann-Whitney, U =116, p = 0.027). On the other hand, the species trophic ranks (STR) for orthophosphates indicator species were higher compared to the rest of species (Mann-Whitney, U =12, p = 0.024).

Table 7. Results of Mann-Whitney Test for the comparison of trophic ranks and ecological amplitude of selected indicator macrophytes against the rest of species. Only statistically significant results were presented (p<0.05).

Macrophyte trophic preferences	Test groups of species	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Asymp. Sig. (2-tailed)
MIR(W)_N	indicators	7	12.14	85.00	57	0.018
	non-indicators	33	22.27	735.00		
STR _P	indicators	4	19.50	78.00	12	0.024
	non-indicators	20	11.10	222.00		
MIR(L)_All	indicators	17	25.18	428.00	116	0.027
	non-indicators	23	17.04	392		

MIR(W)_N -comparison of species weights (ecological amplitudes) according to the Macrophyte Index of Rivers (Species index weight) (Szoszkiewicz et al., 2010) for nitrate-nitrogen indicator species against the rest of the species; STR _P - comparison of species trophic ranks according to Dawson et al., (1999) for orthophosphates indicator species against the rest of the species; MIR(L)_All -comparison of species trophic values according to the Macrophyte Index of Rivers (Species index weight) (Szoszkiewicz et al., 2010) for all indicator species against the rest of the species.

4. Discussion

The modelling approach applied in this study predicted the Danube trophic conditions with a high prediction rate using raw binary macrophyte data as explanatory variables. Modelling of habitat conditions by presence/ absence or abundance of indicator organisms is a basic task of bioindication (Schleiter et al., 2006). Since the variables may change in short time scales, biological indicators are adequate long-term probes for environmental quality. Schleiter et al. (1999) showed that environmental properties of lotic ecosystems, including dissolved oxygen, total phosphorus and nitrate-nitrogen could be successfully predicted by macroinvertebrate assemblages using artificial neural network. In a similar study, Schleiter et al. (2001), found that better predictive performances of the water quality model could be obtained using presence/ absence data compared to the abundance data.

In this study, the presence-absence data model was performed to reduce the number of null-data and to increase model performance. However, the long survey units of 3 river km may mask small scale habitat conditions and also weaken the ability of the data to detect linkages between local river conditions and the drivers of those conditions. Therefore, even better predictive performances of the developed models could be expected in the case of small sub-reaches sampling designs with multiple data points (Jusik et al., 2015). Nevertheless, the use of binary macrophyte data potentially eliminates the statistical noise of the water velocity, the important factor structuring the macrophyte assemblages in the Danube and the lotic systems at all (Janauer et al., 2010). To a certain extent, the water velocity may influence the aquatic plants abundance by simple physical removal of individuals, leaving species composition unchanged (Franklin et al, 2008). If water velocity exceeds 1 m s^{-1}, macrophytes are only present in negligible quantities or are completely absent (Franklin et al, 2008). This implies that the use of the developed predictive model is restricted to the river sections having water velocity below this threshold value.

Moreover, collecting the presence-absence macrophyte data is cost-effective and could be potentially performed on the Danube more frequently than JDS expeditions. Together with the fact that macrophyte species could be easily identified on the field, this may allow time-effective assessment of the river trophic state.

Some previous studies attempted to correlate water quality and habitat degradation along the Danube using various macrophyte metrics (Birk et al., 2012). Birk et al. (2012) demonstrated using the Joint Danube Survey 2 data that apart from macrophyte composition, macrophyte trophic metrics failed to reflect the Danube habitat conditions since the majority of macrophyte species belonged to eutrophication tolerant. Indicator species selected by sensitivity analysis in this study are mostly eutrophic tolerant as well, with broad ecological amplitude. Ecological amplitude (MIR_W), of nitrate-nitrogen indicator species, were relatively wider in comparison with the rest of the species. Moreover, species trophic values (MIR_L), classified all indicator species as eutrophic tolerant. In some previous studies, this Macrophyte Index of Rivers (MIR) (Szoszkiewicz et al., 2010) was shown to be a good predictor of various forms of nutrients, including nitrate nitrogen and orthophosphates (Szoszkiewicz et al., 2020). On the other hand, the orthophosphates indicator species group had a slightly higher STR value compared to the rest of the species, but still in the meso-eutrophic water quality spectrum. This implies that orthophosphates were better predicted with eutrophication sensitive species. This is in accordance with the conclusions of previous studies that macrophytes should be treated as indicators of river ecological degradation caused by complex trophic factors, not necessarily correlating among each other (Gebler et al., 2017; Gebler et al., 2018).

With a few exceptions, indicator species included submerged species such as *Potamogeton crispus, P. lucens*, and perennial emergent species (*Amblystegium riparium, Glyceria fluitans, Glyceria maxima, Mentha aquatica, Typha angustifolia*, etc.). Szoszkiewicz et al. (2017) demonstrated that on the lowland rivers in Poland, with wide trophic range, the most distinctive species, found exclusively in one trophic level, were predominantly emergent and amphibian species. Analysis of JDS2 data (Birk et al., 2012) obtained similar results for submerged pondweed species, which were found to characterise less disturbed river sections. Generally, submerged macrophytes have a strong ability to absorb phosphorus from the water column (Zhang et al., 2011; Christiansen et al., 2016), and clearly respond to changes of phosphorus concentrations in the water (Søndergaard et al., 2010). The percentage cover of these functional groups in the littoral zone of lakes was recognised as reliable and good performing water quality indicators (Kolada, 2014). While emergent species are more associated with waters having high nutrient and chlorophyll-*a* concentration, submerged species are good predictors of mesotrophic conditions (Kolada, 2014).

The highest prediction rate, which shows a model's ability to predict the right water quality class was calculated for the dissolved oxygen model. On the other hand, all three models showed good performances considering the percentage of absolute errors. For the main river channel, the model mostly showed equal distribution or errors around observed environmental values. The exceptions from this role were the samples collected in tributaries or downstream from the tributaries mouth, where the model predicted more frequently lower (better) quality classes compared to the observed once. This was pronounced for the orthophosphates, especially in the case of samples from the Tisza and the Arges rivers (Appendix A). In general, this prediction pattern is probably due to species sorting mechanisms, where these tributaries with better water quality (Liška et al., 2015) contributed to the Danube sample species pool (Heino et al., 2013). This also might be the reason for the lower value of the Kappa index for the orthophosphates model in comparison with the dissolved oxygen and nitrate nitrogen models.

5. Conclusions

In this study, macrophyte presence-absence data within the 3 km long Danube reaches obtained from the JDS3 survey were used to predict water quality classes. Instead of using macrophyte variables as the model outputs (dependent variables), an opposite approach was applied to develop ANN predictive model for the Danube trophic variables (dissolved oxygen, nitrate-nitrogen and orthophosphates). Despite the limited number of samples along the wide trophic gradient of the Danube river from the source to the mouth, the model showed good predictive performances for the main river channel. From 64 analysed macrophyte species, 28 were selected by sensitivity analysis as significant water quality indicators for at least one environmental variable. Indicator species mainly belonged to the eutrophic tolerant submerged or emerged species with broad ecological amplitude. This reflects the significance of the developed model for use on rivers significantly impacted by eutrophication such as the Danube. However, the use of the developed predictive model is restricted to the river sections with water velocity suitable for macrophytes growth. On the other hand, the developed ANN architecture represents the modelling approach which could be applied to other biological quality elements. Nevertheless, compared to other biological quality elements, macrophytes could be easily identified immediately on the field, allowing *in situ* assessment of river trophic conditions.

Credit authorship contribution statement

I. Krtolica: Formal analysis, Investigation, Writing - review & editing; D. Cvijanović: Conceptualization, Investigation, Methodology, Writing - original draft; Đ. Obradović: Software,

Methodology, Writing - review & editing; M. Novković: Formal analysis, Visualization, Writing - review & editing; Dj. Milošević: Conceptualization, Formal analysis, Methodology, Writing - review & editing; D. Savić: Methodology, Writing - review & editing; M. Vojinović-Miloradov: Supervision, Writing - review & editing; S. Radulović: Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

Role of the funding source

The funding source had no involvement in study design, collection, analysis and interpretation of data, writing of the report; and in the decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data 1.

Supplementary data 2.

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References:

Acheampong AO, Boateng EB: Modelling carbon emission intensity: Application of artificial neural network. *Journal of Cleaner Production* 2019, 225: 833-856.

Antanasijević D, Pocajt V, Perić-Grujić A, Ristić M: Modelling of dissolved oxygen in the Danube River using artificial neural networks and Monte Carlo Simulation uncertainty analysis. *Journal of Hydrology* 2014, 519:1895-1907.

Antanasijević D, Pocajt V, Povrenović D, Perić-Grujić A, Ristić M: Modelling of dissolved oxygen content using artificial neural networks: Danube River, North Serbia, case study. *Environmental Science and Pollution Research* 2013, 20(12):9006-9013.

Apele Române, Administraţia Naţională, Romania, Sediul Central al Aperol Romane. Available at: http//www.rowater.ro (21/11/2019)

Arle J, Blondzik K, Claussen U, Duffek A, Grimm S, Hilliges F, Hoffmann A, Leujak W, Mohaupt V, Naumann S: Water Resource Management in Germany-Part 2-Water Quality. Federal *environmental Aagency* 2014, 135.

Baart I, Gschöpf C, Blaschke AP, Preiner S, Hein T: Prediction of potential macrophyte development in response to restoration measures in an urban riverine wetland. *Aquatic Botany* 2010, 93(3):153-162.

BGBl. II. Verordnung des Bundesministers für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft über die Festlegung des Zielzustandes für Oberflächengewässer (Qualitätszielverordnung Chemie Oberflächengewässer – QZV Chemie OG), Nr. 96/2006, 2006.

Birk S, Van Kouwen L, Willby N: Harmonising the bioassessment of large rivers in the absence of near-natural reference conditions–a case study of the Danube River. *Freshwater Biology* 2012, 57(8):1716-1732.

Brabec K, Szoszkiewicz K: Macrophytes and diatoms—major results and conclusions from the STAR project. In: *The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods.* Springer; 2006: 175-178.

Chapman DV, Bradley C, Gettel GM, Hatvani IG, Hein T, Kovács J, Liska I, Oliver DM, Tanos P, Trásy B: Developments in water quality monitoring and management in large river catchments using the Danube River as an example. *Environmental Science & Policy* 2016, 64:141-154.

Chollet F: Keras: Deep learning library for theano and tensorflow. *URL: https://keras io/k* 2015, 7(8):T1.

Christiansen NH, Andersen FØ, Jensen HS: Phosphate uptake kinetics for four species of submerged freshwater macrophytes measured by a 33P phosphate radioisotope technique. *Aquatic Bbotany* 2016, 128:58-67.

Cohen J. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 1960, 20:37–46.

Dawson F, Newman J, Gravelle M: Assessment of the Trophic Status of Rivers Using Macrophytes: Supporting Documentation for the Evaluation of the Mean Trophic Rank: Environment Agency; 1999.

Demars BO, Edwards AC: Distribution of aquatic macrophytes in contrasting river systems: a critique of compositional-based assessment of water quality. *Science of the Total Environment* 2009, 407(2):975-990.

Duca G: Management of Water Quality in Moldova: Springer; 2014.

Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulißen D: Zeigerwerte von pflanzen in Mitteleuropa. 1992.

Ellenberg H: Indicator values of vascular plants in central Europe. *Indicator values of vascular plants in Ccentral Europe* 1974, 9.

Flotemersch JE, Stribling JB, Paul MJ: Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers: US Environmental Protection Agency, Office of Research and Development, 2006.

Franklin P, Dunbar M, Whitehead P: Flow controls on lowland river macrophytes: a review. *Science of the Total Environment* 2008, 400(1-3):369-378.

Gebler D, Kayzer D, Szoszkiewicz K, Budka A: Artificial neural network modelling of macrophyte indices based on physico-chemical characteristics of water. *Hydrobiologia* 2014, 737(1):215-224.

Gebler D, Szoszkiewicz K, Pietruczuk K: Modeling of the river ecological status with macrophytes using artificial neural networks. *Limnologica* 2017, 65:46-54.

Gebler D, Wiegleb G, Szoszkiewicz K: Integrating river hydromorphology and water quality into ecological status modelling by artificial neural networks. *Water Rresearch* 2018, 139:395-405.

Gevrey M, Dimopoulos I, Lek S: Review and comparison of methods to study the contribution of variables in artificial neural network models. *Ecological Modelling* 2003, 160(3):249-264.

Haury J, Peltre M-C, Trémolières M, Barbe J, Thiébaut G, Bernez I, Daniel H, Chatenet P, Haan-Archipof G, Muller S: A new method to assess water trophy and organic pollution—the Macrophyte Biological Index for Rivers (IBMR): its application to different types of river and pollution. In: *Macrophytes in aquatic ecosystems: Ffrom biology to management.* Springer; 2006: 153-158.

Heino J: The importance of metacommunity ecology for environmental assessment research in the freshwater realm. *Biological Reviews* 2013, 88(1):166-178.

Holt EA, Miller SW: Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge* 2011, 3(10):8.

Hood JLA: The role of submersed macrophytes in river eutrophication and biogeochemical nutrient cycling. 2012.

ICPDR. Water Quality in the Danube River Basin – 2007.TNMN – Yearbook 2007. Imprint. International Commission for the Protection of the Danube River, Vienna, 2010.

Janauer GA, Schmidt-Mumm U, Schmidt B: Aquatic macrophytes and water current velocity in the Danube River. *Ecological Engineering* 2010, 36(9):1138-1145.

Jusik S, Szoszkiewicz K, Kupiec JM, Lewin I, Samecka-Cymerman A: Development of comprehensive river typology based on macrophytes in the mountain-lowland gradient of different Central European ecoregions. *Hydrobiologia* 2015, 745(1):241-262.

Keeni K, Nakayama K, Shimodaira H: Estimation of initial weights and hidden units for fast learning of multi-layer neural networks for pattern classification. In *Proceedings of the International Joint Conference on Neural Networks (IJCNN '99)*. IEEE; 1999, 3:1652‑1656.

Kohler A, Janauer GA: Zur Methodik der Untersuchung von aquatischen Makrophyten in Fließgewässern. *Handbuch Angewandte Limnologie: Grundlagen-Gewässerbelastung-Restaurierung-Aquatische Ökotoxikologie-Bewertung-Gewässerschutz* 2014:1-22.

Kolada A: The effect of lake morphology on aquatic vegetation development and changes under the influence of eutrophication. *Ecological Indicators* 2014, 38:282-293.

LeCun Y, Bengio Y, Hinton G: Deep Learning. *Nature* 2015, 521 (7553): 436-444.
Lek S, Guégan J-F: Artificial neural networks as a tool in ecological modelling, an introduction. *Ecological Mmodelling* 1999, 120(2-3):65-73.

Liška, I., Wagner, F., Sengl, M., Deutsch, K., Slobodnik, J.. Joint Danube Survey 3, Final Scientific Report ICPDR. 2015 . Available at: http://www.icpdr.org/main/ activities-projects/jds3

Milošević D, Mančev D, Čerba D, Piperac MS, Popović N, Atanacković A, Đuknić J, Simić V, Paunović M: The potential of chironomid larvae-based metrics in the bioassessment of non-wadeable rivers. *Science of the Total Environment* 2018, 616:472-479.

National Assembly of the Republic of Serbia. Rulebook on parameters of ecological and chemical status of surface waters and parameters of chemical and quantitative status of groundwater. *Official Gazette of Republic of Serbia* 2011, 74/2011, Belgrade, Serbia

O'Briain R, Shephard S, Coghlan B: Pioneer macrophyte species engineer fine-scale physical heterogeneity in a shallow lowland river. *Ecological Engineering* 2017, 102:451-458.

O'Hare MT, Baattrup-Pedersen A, Baumgarte I, Freeman A, Gunn ID, Lázár AN, Sinclair R, Wade AJ, Bowes MJ: Responses of aquatic plants to eutrophication in rivers: a revised conceptual model. *Frontiers in Pplant Sscience* 2018, 9:451.

Pall K, Moser V: Austrian Index Macrophytes (AIM-Module 1) for lakes: a Water Framework Directive compliant assessment system for lakes using aquatic macrophytes. *Hydrobiologia* 2009, 633(1):83.

Pekárová P, Onderka M, Pekár J, Rončák P, Miklánek P: Prediction of water quality in the Danube River under extreme hydrological and temperature conditions. *Journal of Hydrology and Hydromechanics* 2009, 57(1):3-15.

Schiemer F, Guti G, Keckeis H, Staras M: Ecological status and problems of the Danube River and its fish fauna: a review. 2004.

Schleiter IM, Borchardt D, Wagner R, Dapper T, Schmidt K-D, Schmidt H-H, Werner H: Modelling water quality, bioindication and population dynamics in lotic ecosystems using neural networks. *Ecological Modelling* 1999, 120(2-3):271-286.

Schleiter IM, Obach M, Borchardt D, Werner H: Bioindication of chemical and hydromorphological habitat characteristics with benthic macro-invertebrates based on artificial neural networks. *Aquatic Ecology* 2001, 35(2):147-158.

Schleiter IM, Obach M, Wagner R, Werner H, Schmidt H-H, Borchardt D: Modelling ecological interrelations in running water ecosystems with artificial neural networks. In: *Ecological Iinformatics.* Springer; 2006: 169-186.

Schneider S, Melzer A: The Trophic Index of Macrophytes (TIM)–a new tool for indicating the trophic state of running waters. *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology* 2003, 88(1):49-67.

Singh KP, Basant A, Malik A, Jain G: Artificial neural network modeling of the river water quality—a case study. *Ecological Modelling* 2009, 220(6):888-895.

Slobodnik J, Mrafkova L, Carere M, Ferrara F, Pennelli B, Schüürmann G, von der Ohe PC: Identification of river basin specific pollutants and derivation of environmental quality standards: A case study in the Slovak Republic. *TrAC Trends in Analytical Chemistry* 2012, 41:133-145.

Sommerwerk N, Bloesch J, Paunović M, Baumgartner C, Venohr M, Schneider-Jacoby M, Hein T, Tockner K: Managing the world's most international river: the Danube River Basin. *Marine and Freshwater Research* 2010, 61(7):736-748.

Søndergaard M, Johansson LS, Lauridsen TL, Jørgensen TB, Liboriussen L, Jeppesen E: Submerged macrophytes as indicators of the ecological quality of lakes. *Freshwater Biology* 2010, 55(4):893-908.

Szilágyi F, Ács É, Borics G, Halasi-Kovács B, Juhász P, Kiss B, Kovács T, Müller Z, Lakatos G, Padisák J: Application of water framework directive in Hungary: development of biological classification systems. *Water Science and Technology* 2008, 58(11):2117-2125.

Szoszkiewicz K, Budka A, Pietruczuk K, Kayzer D, Gebler D: Is the macrophyte diversification along the trophic gradient distinct enough for river monitoring? *Environmental mMonitoring and Aassessment* 2017, 189(1):4.

Szoszkiewicz K, Jusik S, Pietruczuk K, Gebler D: The Macrophyte Index for Rivers (MIR) as an Advantageous Approach to Running Water Assessment in Local Geographical Conditions. *Water* 2020, 12(1):108.

Szoszkiewicz K, Zbierska J, Jusik S, Zgoła T: Macrophyte method for river assessment. *Poznan: Bogucki Wydawnictwo Naukowe* 2010.

Thiébaut G: Phosphorus and aquatic plants. In: *The Ecophysiology of Plant-Phosphorus Interactions.* Springer; 2008: 31-49.

Thomas FM, Yen JDL, Vesk PA. Using functional traits to predict species growth trajectories, and cross-validation to evaluate these models for ecological prediction. *Ecology and Evolution* 2019, 9:1554–1566.

Thomasen S, Chow-Fraser P: Detecting changes in ecosystem quality following long-term restoration efforts in Cootes Paradise Marsh. *Ecological Indicators* 2012, 13(1):82-92.

Varga P, Abraham M, Simor J: Water quality of the Danube in Hungary and its major determining factors. *Water Science and Technology* 1990, 22(5):113-118.

Vlada Republike Hrvatske. Uredba o standardu o kakvoći voda. Uredba. NN 96/2019 . Zagreb, Croatia

Wang Q, Li S, Jia P, Qi C, Ding F: A review of surface water quality models. *The Scientific World Journal* 2013, 2013.

WHO. Edition F: Guidelines for drinking-water quality. *WHO chronicle* 2011, 38(4):104-108.

Wong TT: Performance evaluation of classification algorithms by k-fold and leave-one-out cross validation. *Pattern Recognition* 2015, 48 (9): 2839–2846.

Zhang M, Cao T, Ni L, Xie P, Zhu G, Zhong A, Xu J, Fu H: Light-dependent phosphate uptake of a submersed macrophyte Myriophyllum spicatum L. *Aquatic Bbotany* 2011, 94(4):151-157.