Environmental Processes (2024) 11:45
https://doi.org/10.1007/s40710-024-00723-5

RESEARCH

Determining the Ecological Status of the Dinaric Karst Natural Lakes in Croatia: Eight Natural Lakes, Seven Lake Types, and One Very Significant Equation

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Received: 27 February 2024 / Accepted: 12 August 2024

Abstract

The delicate balance of aquatic environments and communities living in them is closely linked to water quality, making its assessment a crucial factor in environmental management. Despite the progress driven by the European Water Framework Directive, challenges remain, especially in understanding and addressing the specificities of rare freshwater ecosystems, such as the Dinaric karst lakes in Croatia. We aimed to find a solution to two very uncommon issues in freshwater ecological classification and monitoring of lake ecosystems: (1) specific karst lake habitats, resulting in eight natural lakes being classified as seven specific lake types, and (2) a very narrow pressure gradient, as most lakes are in good or high ecological quality status or a near-natural state. A modified proportional stratified sampling approach was used, in which between 2 and 7 sampling sites were identified for each of the eight natural lakes based on various factors, including lake surface area and sediment composition. Environmental parameters and anthropogenic pressures were assessed for each lake. This process led to the creation of a stepwise linear regression model to estimate the reference conditions and a multimetric index to determine the final ecological quality ratios (EQRs) of the lakes. Through extensive sampling and analyses, the study contributes valuable insights to the assessment of water quality and proposes a model applicable to freshwater ecosystems with similar typological challenges and habitat specificities worldwide.

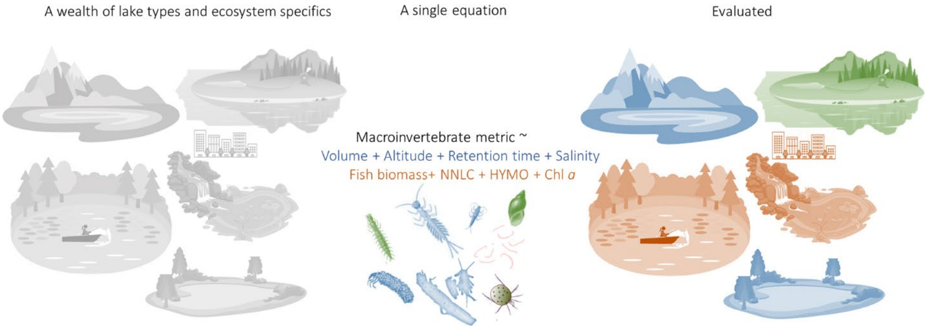
Highlights

- The ecological assessment of geologically and biologically specific lakes is presented.
- A regression model for hindcasting reference conditions is developed.
- Pressure-response relationships in lake macroinvertebrate communities are analyzed.
- The findings provide insights for the management of freshwater ecosystems worldwide.

Extended author information available on the last page of the article

Published online: 28 August 2024

Graphical Abstract



Keywords Water quality assessment · Dinaric karst lakes · Freshwater management · Macroinvertebrate bioassessment · Reference conditions

1 Introduction

The assessment of water quality is a crucial factor in understanding the delicate balance of aquatic ecosystems. Much progress has been made in this area since the implementation of the European Water Framework Directive (WFD, EC 2000/60), but there are still many challenges in implementing these strategies, especially in relation to specific and/or rare types of freshwater ecosystems (Miler et al. 2024; Poikane et al. 2015, 2016; Van Grinsven et al. 2016; Argillier et al. 2022). Given the increasing threats to freshwater resources from anthropogenic activities, hydromorphological alteration and environmental changes, the need for comprehensive assessment and management of these water bodies is becoming increasingly urgent (Boon et al. 2019; Poikane et al. 2020). The WFD proposes the use of Ecological Quality Ratios (EQRs). These are standardized quality metrics that assess the decline and improvement of ecological quality based on the responses of freshwater biota (Reyjol et al. 2014).

Among the diverse freshwater ecosystems, the natural lakes of the Dinaric western Balkan ecoregion, Ecoregion 5 (Illies 1978) (hereafter referred to as the Dinaric region) are characterized by a geological phenomenon that makes their hydrology and ecology very distinct and not comparable to other geographically close, but geologically distinct natural lakes. For this reason, the WFD requirements for the classification methods of these natural lakes are those that take into account a case where intercalibration (i.e., harmonization with the methods of other countries) is not possible (EC 2000/60). These lakes are embedded in a karst area characterized by soluble rock formations that create porous landscapes with underground drainage systems (Bonacci 2014; Petriki et al. 2020). The permeability of the geological deposits in the Dinaric region is precisely the reason why lakes are relatively rare in this area. The intricacies of karst hydrogeology contribute to a rich and complex aquatic habitat that makes these natural lakes reservoirs of biodiversity and ecological importance (Ivković and Plant 2015) where many endemic species occur (e.g., Andersen et al. 2016; Giłka et al. 2013; Kvifte and Ivković 2018). The complexity of the Dinaric karst lakes alone poses a challenge in creating a systematic classification that accurately reflects their diversity. However, an additional problem arises in the Dinaric region of Croatia where eight natural lakes are categorized into seven different lake types. Six of these types were identified in 2013 (Mihaljević et al. 2013; Mihaljević and Pozojević 2020) and recognized among seven natural lakes. In 2022, one lake previously identified as a transitional water body (i.e., with marine elements) was redefined as a natural freshwater lake and assigned to a new lake type.

Eutrophication driven by climate change and the increasing use of pesticides in agriculture are increasingly threatening the unique biodiversity of natural lakes worldwide. This emphasizes the need for comprehensive biomonitoring, which could help in future mitigation and restoration processes (Pilotto et al. 2015). Bioassessment of these ecosystems is an indispensable tool for analyzing the impact of these threats, but also for monitoring the progress of restoration efforts (Orzechowski and Steinman 2022; Woolway et al. 2020), and it is usually carried out by analyzing communities of different groups of organisms. It has been shown that macroinvertebrates are often one of the most reliable assessment groups, as they are highly dependent on the environmental conditions within these ecosystems (Lu et al. 2024). Nevertheless, they are often the most difficult to use for the ecological assessment of lakes due to their enormous diversity, heterogeneity of community composition, species-specific responses to pressures, but also due to the different benthic zones within the lakes (e.g., littoral vs. profundal) and the sampling approaches used in different countries (De Oliveira et al. 2024; Poikane et al. 2016).

This research attempts to fill the gap in our understanding of the dynamic pressure responses of macroinvertebrate communities in natural Dinaric karst lakes by introducing a novel approach to water quality assessment that utilizes this assessment group as a reliable tool. By exploring into the intricacies of seven natural lakes and their different types in the Dinaric region, this study aims to develop a singular equation (regression model) that encompasses the diverse ecological characteristics and reflects the reference state of these water bodies. The equation will serve as a unifying measure that allows for a more holistic assessment of the health and sustainability of the Dinaric karst lakes. More precisely, our objectives were: (1) to define macroinvertebrate based metrics suitable for the ecological assessment of natural lakes in the Dinaric region; (2) to develop an equation (regression model) for each of the selected metrics that would calculate their values in the reference state of specific lake types; (3) to test the EQRs derived from the calculated reference values against pressure gradients; and finally, (4) to test the equation tailored to karst lakes and the results derived from it on newly added lake type. By achieving these objectives, this study aims to make a valuable contribution to the broader field of water quality assessment and provide a model for understanding and managing the ecological dynamics of natural karst lakes and, by extension, freshwater ecosystems facing similar challenges worldwide. Furthermore, this study aims to provide an example on how comprehensive and well-rounded biomonitoring tools can be developed, even in the most difficult and diverse habitats, if sufficient taxonomic effort is applied.

2 Materials and Methods

2.1 Study Area

Between 2 and 7 sampling sites were defined for each lake, depending on the lake surface area and the composition of the lake sediments, but above all depending on the accessibility and heterogeneity of the shoreline. The more uniform the composition of the lake sediments and the shoreline, the fewer sampling sites there were in that lake (Fig. 1 and Supplementary Material (SM) Table SM1). The general characteristics of all eight natural lakes are listed in Table 1.

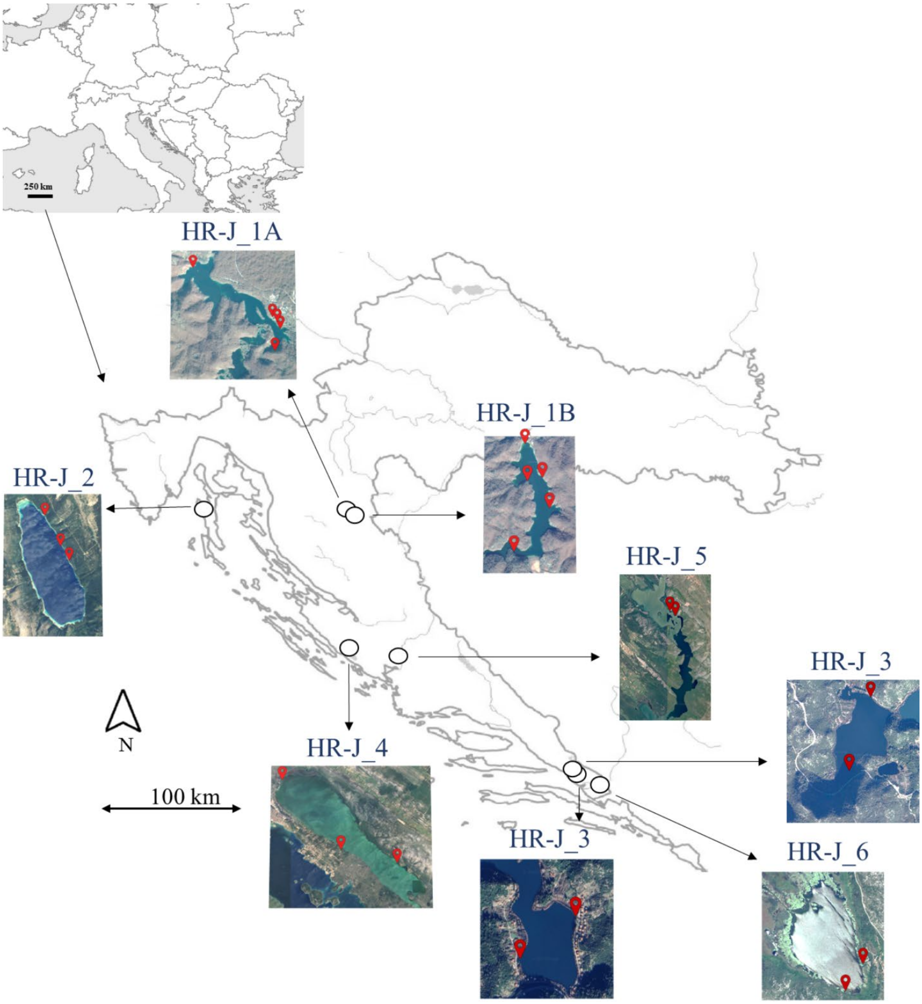


Fig. 1 Study area with 32 sampling sites from eight natural lakes belonging to seven lake types in the Dinaric Western Balkan Ecoregion in Croatia. The exact coordinates of the sites and sampling dates can be found in Table SM1 of the Supplementary Material

Table 1 General characteristics of natural lakes in the Dinaric Western Balkan Ecoregion in Croatia

Lake	Maximum depth (m)	Ecoregion/subecoregion	National type	Lake description	Depth profile
Kozjak (Plitvice Lakes)	48	Dinaric/Continental	HR-J_1A	carbonate substrate, dimictic, barrage lake	deep
Prošće (Plitvice Lakes)	38	Dinaric/Continental	HR-J_1B	carbonate substrate, dimictic, barrage lake	deep
Vrana Lake (on island Cres)	78	Dinaric/Mediterranean	HR-J_2	carbonate substrate, monomictic, cryptodepression	deep
Crniševo	31	Dinaric/Mediterranean	HR-J_3	carbonate substrate, monomictic, cryptodepression	deep
Oćuša	20	Dinaric/Mediterranean	HR-J_3	carbonate substrate, monomictic, cryptodepression	deep
Vransko Lake (near Biograd na Moru)	4–5	Dinaric/Mediterranean	HR-J_4	carbonate substrate, polymictic, cryptodepression	shallow
Visovac	28–30	Dinaric/Mediterranean	HR-J_5	carbonate substrate, monomictic, barrage lake	deep
Kuti*	4–5	Dinaric/Mediterranean	HR-J_6	carbonate substrate, polymictic, cryptodepression	shallow

*This lake was previously treated as brackish and was not included in the original equation. During continuous monitoring, only freshwater elements were found and the lake was included in the list of natural lakes with its own type

2.2 Macroinvertebrate Sampling and Processing

Sampling was carried out in summer 2018 and 2019 and for the additional lake type (HR-J_6) in late spring/summer 2022 and 2023 (sampling dates and coordinates are presented in Table SM1). A modified proportional stratified sampling approach was used (Urbanič et al. 2012). At each sampling site, a 25 m transect was established along the lake shore, extending 10 m toward the open water or until the water depth exceeded 1 m. At each location, 10 replicates were collected, considering microhabitat composition and the predefined water depth classes (0–0.25 m, 0.25–0.5 m, 0.5–0.75 m, and 0.75–1 m). Details of the sampling protocol are described in Urbanič et al. (2012). Replicates were stored individually in 90%

ethanol. The environmental parameters used in the analysis [Chl *a* (µg/L); Non-natural land cover; NNLC (%); Fish biomass (kg/ha); Hydromorphological degradation score - HYMO; Volume ($m^3 \times 10^6$); Altitude (m a.s.l.) and Retention time (days)] were obtained by Hrvatske vode (Croatian legal entity for water management). The assessment of hydromorphological degradation was carried out according to the 'Water quality – Guidance standard on determining the degree of modification of river hydromorphology' (DIN EN 156843 2010; Pavlek et al. 2023). All macroinvertebrates were separated in the laboratory, preserved in 70% ethanol and identified (Table 2).

2.3 Metric Selection and Calculation

The selection of metrics (calculated with the software Asterics, version 4.0.4, or manually calculated in the case of the percentage of Chironomini individuals in the community) followed the guidelines of Hering et al. (2006). The first criterion for excluding certain metrics was a sufficient amount of data. For some metrics, the database offered did not provide a sufficient amount of information or did not contain key indicator taxa, and they were not calculated for all lakes and stations analyzed. In the second metric selection criterion, all metrics that did not relate to the analyzed habitat type - standing water bodies were excluded. The values of the remaining metrics were tested for a normal and/or linear distribution in the Statistica 14.0 software package (TIBCO Software Inc. 2020) and excluded from further analysis if such a distribution was not confirmed. The metrics were then tested for significant correlations with environmental parameters and stressors (Chlorophyll *a* concentration, Non-natural land cover; Fish biomass and Hydromorphological degradation), leaving only those that showed significant relationships with at least one environmental parameter and one stressor. Finally, metrics were tested for autocorrelation with each other (using the Spearman correlation coefficient in TIBCO Software Inc. 2020), excluding one from the pair of metrics that had a correlation coefficient greater than ±0.8 and excluding metrics if more of them were from the same category: (1) Composition/abundance metrics; (2) Richness/diversity metrics; (3) Sensitivity/tolerance metrics and (4) Functional metrics.

Table 2 Identification level for macroinvertebrate groups in this study (and for regular monitoring purposes in Croatia)

Systematic group	Level of identification	Systematic group	Level of identification
Porifera	genera	Ephemeroptera	genera, species
Hydrozoa	genera	Trichoptera	genera, species
Bryozoa	presence	Odonata	genera, species
Turbellaria	genera, species	Megaloptera	genera, species
Oligochaeta	family, genera, species	Heteroptera	genera, species
Hirudinea	genera, species	Coleoptera	genera, species
Mollusca	genera, species	Diptera	family, genera, species
Crustacea	genera, species	Hydrachnidia	presence
Plecoptera	genera, species		

2.4 Development of Stepwise Linear Regression for each Metric

Environmental parameters and anthropogenic pressures were assessed for each lake (Table SM2). A stepwise linear regression of each selected metric (Table SM3) against environmental parameters and anthropogenic pressures was performed to ensure pressure-response relationships in Statistica 14.0. The reference conditions for each lake type were estimated using a hindcasting procedure based on stepwise linear regression. The final index was calculated as the average of the EQRs of the selected metrics. Data from all lake types were combined to develop a stepwise multimetric linear model for hindcasting the reference conditions. Most natural lakes in Croatia have good to high or near-natural status. In order to create a gradient of pressure variables, especially for sites with a lower ecological status, 21 sites from man-made lakes (reservoirs) in the same geographical region were included to improve the construction of the model (Table 3).

The reference metric values were predicted for each lake using a hindcasting procedure. The theoretical values of the metrics were estimated by minimizing the pressure values or setting them to zero (Table 4). Following Poikane et al. (2011), the reference concentration of chlorophyll a was set at 2.5 µg/L for shallow lakes and 1.8 µg/L for deep lakes. The maximum proportion of non-natural land cover was set at 8%, as defined by Ntislidou et al. (2016), who also established reference conditions for MED GIG lakes. The reference value for hydromorphological alteration was set at 1.5, which corresponds to undisturbed conditions (Poikane 2009). Reference fish biomass was calculated using reference values for total phosphorus concentration set at 0.01 mg/L (10 µg/L) for deep lakes and 0.02 mg/L (20 µg/L) for shallow lakes, as described by de Hoyos et al. (2014). The biomass calculation, based on Gassner et al. (2003), follows the formula:

$$\text{Fish biomass (kg/ha)} = 3.8148\,TP^{1.0940}$$

Table 3 Number of samples and range of independent and dependent variables used in the development of stepwise linear regression for hindcasting metric reference values

	Reservoir samples		Natural lake samples	
Number of samples (datasets)	21		42	
Variable ranges	min	max	min	max
Pressures				
Chl *a* (µg/L)	0	0.77	0.35	6.33
Non-natural land cover; NNLC (%)	0.05	99.98	0	66.19
Fish biomass (kg/ha)	10	240	30	225
Hydromorphological degradation score; HYMO	1.21	2.71	1	4
Environmental traits				
Volume ($m^3 \times 10^6$)	0.4	25.7	7	220
Altitude (m a.s.l.)	9	733	-0.16	636
Retention time (days)	1	278	27	11.68
Salinity (‰)	0.08	0.33	0.11	1.27
Metrics				
Diversity (Margalef Index)	3.18	7.96	2.29	8.71
EPT [%] (abundance classes)	1.02	20.16	4.10	30.21
% Chironomini	0.61	39.91	0	19.40
Number of Families	10	36	9	51

Table 4 Maximum pressure values determined for reference conditions in each lake type

Pressure:	Chl *a* (μg/L)	NNLC (%)	Fish biomass (kg/ha)	HYMO
Reference:	Poikane et al. (2011)	Ntislidou et al. (2016)	Gassner et al. (2003)	Poikane et al. (2009)
HR-J_1A	1.8	8	47.37	1.5
HR-J_1B	1.8	8	47.37	1.5
HR-J_2	1.8	8	47.37	1.5
HR-J_3	1.8	8	47.37	1.5
HR-J_4	1.8	8	47.37	1.5
HR-J_5	2.5	8	101.11	1.5
HR-J_6	2.5	8	101.11	1.5

where TP is the total phosphorus concentration in μg/L.

Finally, the reference metric values for the additionally added lake type (HR-J_6) were calculated using the calculated stepwise regression.

The EQRs for the individual metrics are calculated as follows:

$$EQR = \frac{metric\,value\ - lower\ anchor}{referece\,value - lower\ anchor}$$

The final EQR value from the multimetric index is equal to the average EQR value of all metrics.

3 Results

A total of 249 macroinvertebrate taxa, mainly determined to species or genus level, were identified in the natural lakes and along with data on their abundances processed into macroinvertebrate metrics. The initial number of potential metrics was 352. After checking the metrics in the elimination process: (1) sufficient amount of data – approximately 317 metrics available for most samples remained; (2) appropriate habitat type - all metrics not related to standing waters were excluded, leaving approximately 200 potential metrics; (3) significant relationships with environmental and pressure variables – approximately 90 metrics remained with about half showing only weak correlations; and finally, (4) normal and/or linear distribution - six metrics remained. Metrics dealing with species or taxa group richness showed overall better "performance", i.e., more of them passed the elimination process and generally showed stronger correlations to pressures. Therefore, two of these metrics were categorized as redundant: Number of Taxa and EPTCBO metric (number of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata) in favor of Number of families and Margalef diversity index, respectively. After the final elimination process, four metrics were selected: (1) percentage of Chironomini individuals in the community (% Chironomini); (2) Diversity (Margalef Index), (3) EPT [%] (abundance classes), and (4) Number of Families. All metrics were found to correlate significantly with at least one environmental variable and one stressor (Fig. 2).

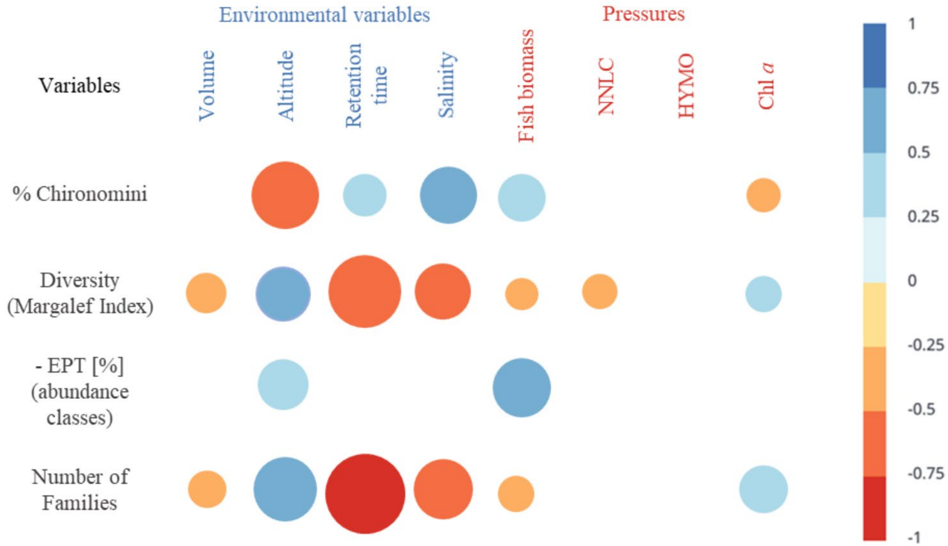


Fig. 2 Correlations between macroinvertebrate metrics and environmental characteristics (volume, altitude, retention time and salinity) and pressures (fish biomass, NNLC–non-natural land cover, HYMO–hydromorphological degradation and Chl *a* concentration). Only statistically significant correlations are given. The coloring and relative size of the circles indicates the direction (i.e., positive or negative) and the value of the correlation coefficient

The pressures considered in the linear model included chlorophyll *a* (Chl *a*, µg/L), non-natural land cover (NNLC, %), fish biomass (kg/ha), and hydromorphological degradation (HYMO degradation). The model parameters (multiple regressions) for the macroinvertebrate metrics against these environmental parameters and pressures are shown in Table 5. All metric regressions demonstrated significant correlations with at least one pressure variable.

A multimetric index was used to determine the final EQRs for the lakes. The multimetric index applies consistent metrics for all lake types, but uses different reference values for each type. An exception is lake type HR-J_2, where the Margalef diversity index is omitted from the ecological status assessment. The reference values for all metrics for all lake types, including HR-J_6, were derived from the stepwise linear regressions listed in Table 6.

The lower anchors for all metrics were set to the worst values observed in the entire dataset, including the reservoirs: % Chironomini −0.4, Diversity Margalef Index −2.28, EPT (%) abundance classes −1.02, and Number of Families −9. After calculating the individual metric EQRs and the final (multimetric) EQRs (Table 7), the pressure-response relationships were tested by linear regressions of the new final EQRs with pressure variables (Fig. 3 and).

4 Discussion

4.1 Specifics and Exceptions within the Karst Lake Habitats

For two specific lake types (HR-J_1B and HR-J_5), near reference values for the Ecological Quality Ratios (EQRs) are calculated, although the proportion of non-natural land cover (NNLC) in the catchment is above 20%.

The first lake type, exemplified by Prošće Lake (HR-J_1B), is located in the Plitvice Lakes National Park. This barrage lake is surrounded by extensive natural forest areas, which make up the entire forest cover of the catchment area. In addition, meadows and pastures within the national park make up 22% of the catchment area. These areas are an integral part of the historical landscape heritage and are subject to minimal agricultural activities, mainly limited to grass mowing (Miliša and Ivković 2023). Therefore, the authors justify the high EQR values observed at sites within this lake type.

The second lake type, Lake Visovac (HR-J_5), is located in the Krka National Park. This barrage (riverine) lake is surrounded by mostly natural land cover and is located downstream of Brljan Lake, which is subject to anthropogenic alterations. Despite the agricultural activities in the surrounding catchment area (12% extensive and 11% intensive farming), Lake Brljan acts as a sedimentation reservoir for organic matter and nutrients inflowing from upstream areas. Consequently, the environmental conditions in Visovac Lake (HR-J_5) are significantly more favorable compared to the calculated NNLC values, which may lead to misinterpretations (Mihaljević et al. 2001).

An exception to the multimetric index, which contains 4 metrics for other lake types, is the lake type HR-J_2 – Vransko Lake on the island of Cres. This lake is a very deep (max. depth: 78 m) cryptodepression located on an island (surrounded by the sea). Due to this apparent isolation, this ultra-oligotrophic lake, which has very low nitrate concentrations and is used as a drinking water supply (Tomec et al. 2002), generally has very low secondary production, as found in other oligotrophic lakes (Northington et al. 2010) and consequently

Table 5 The multimetric linear model used for hindcasting reference conditions for four metrics in Croatian natural lakes. Significance levels are denoted as "*" for $p<0.05$; "**" $p<0.01$ and "***" for $p<0.001$

		Environmental conditions				Stressor				Multimetric linear model	
Metric	Intercept	Volume	Altitude	Retention time	Salinity	Fish biomass	NNLC	HYMO	Chl a	R^2	F
% Chironomini	0.0019	-0.0009**	0	0	-0.0806**	0.001***	0.0014**	0.0115	-0.0124**	0.741	16.791
Diversity Margalef Index	7.6927***	0.0124	-0.002	-0.2105***	-1.5398*	-0.0147**	-0.0131	0.1632	-0.0738	0.621	9.631
EPT (%) abundance classes	12.2042***	-0.0049	0.0074*	0.0013**	-2.0193	0.0145	-0.0417	-0.7828*	0.9344**	0.548	7.135
Number of Families	37.1813***	0.0512	-0.0005	-0.0022**	-7.8707	-0.0435	-0.1579*	0.2428	1.0566	0.579	8.068

has a naturally low diversity (Schmidt et al. 2000). For this reason, the diversity index (Margalef diversity) is not considered when assessing the ecological status of this lake.

It is important to note that in Lake Kuti (HR-J_6), all samples collected in 2022 had lower EQR values (lake status) compared to those collected in 2023 at the same locations. This is due to the low precipitation rates and even drought that prevailed in most parts of Croatia (https://meteo.hr; Croatian Meteorological and Hydrological Service), causing low inflow of freshwater into the lentic ecosystems and deteriorated water quality due to internal nutrient recycling (Catalan et al. 2024). Unfortunately, as this lake type was added later, we do not have data on how the 2022 drought affected the other lake types analyzed in this study. However, it is known that this climate-induced change in precipitation rates affects freshwater communities (Dorić et al. 2024; Pozojević et al. 2023) and especially those of standing waters near the sea. This is the case for three of our lakes: Oćuša and Crniševo (HR-J_3) and Vransko Lake Biograd (HR-J_4), which are heavily salinized in periods without precipitation (Rubinić and Katalinić 2014). Within this research the increased salinity in certain sampling occasions in these lakes caused changes in the macroinvertebrate community, as also reported by Žganec et al. (2024), that resulted in lower EQR values in samples from the same sites compared to those with lower salinity. The authors strongly believe that these lake types are the most vulnerable to stress in view of the upcoming climate change predictions (Jeppesen et al. 2023) and should be monitored more closely to develop possible mitigation measures for the future.

4.2 Ecological Conditions and Quality Status of Karst Lake Habitats

Hydromorphological degradation (pressure) did not show a significant gradient in our studied natural lakes and (consequently) no significant correlations with relevant metrics. Nevertheless, we believe that the assessment of this pressure is crucial for the evaluation of the ecological status of lakes, as it has been repeatedly shown that macroinvertebrates are influenced by hydromorphology or - conversely - are a good indicator of hydromorphological pressure (Borics et al. 2018; de Hoyos et al. 2014; Poikane et al. 2011). In this study, the role of hydromophology is most evident in the macroinvertebrate community (and consequently EQR and status) of Site 1 of the later added lake type HR-J_6 (Lake Kuti). In all samplings, this site had lower EQR values than sites 2 and 3 of this small lake, which were sampled on the same day (i.e., the same environmental conditions prevailed). Compared to the undisturbed sites 2 and 3 (HYMO score 1), site 1 has concrete embankments that are proven to affect the community structure (HYMO score 3; Miler and Brauns 2020). Therefore, despite the low gradient and the lack of statistically significant relationships, we believe that the mentioned pressure was rightly included in the model for calculating the reference values of natural lakes.

As already mentioned, only a few lakes and reservoirs in this region of Croatia are affected by significant nutrient enrichment, and most of them are in good to high ecological status. All lakes have mean total phosphorus (Total P) concentrations below 30 µg/L during the growing season, which theoretically categorizes them as reference sites for this variable (de Hoyos et al. 2014; Borics et al. 2018). Similar trends are observed for total nitrogen concentrations in these lakes. Therefore, we selected Chl *a* concentration as the "pressure" variable, as we believe it best represents eutrophication pressure and expect a significant correlation with the macroinvertebrate metrics used. And indeed, Chl *a* concentration did

Table 6 Reference metrics values from seven Croatian lake types

National type	Metric	Reference value
HR-J_1A	% Chironomini	0.036
	Diversity Margalef Index	5.88
	EPT (%) abundance classes	16.80
	Number of Families	35.47
HR-J_1B	% Chironomini	0.036
	Diversity Margalef Index	5.60
	EPT (%) abundance classes	17.51
	Number of Families	35.07
HR-J_2	% Chironomini	0
	Diversity Margalef Index	not aplicable
	EPT (%) abundance classes	26.96
	Number of Families	20.59
HR-J_3	% Chironomini	0.035
	Diversity Margalef Index	6.78
	EPT (%) abundance classes	12.82
	Number of Families	34.92
HR-J_4	% Chironomini	0
	Diversity Margalef Index	5.77
	EPT (%) abundance classes	11.72
	Number of Families	30.95
HR-J_5	% Chironomini	0
	Diversity Margalef Index	7.90
	EPT (%) abundance classes	12.57
	Number of Families	39.90
HR-J_6	% Chironomini	0.076
	Diversity Margalef Index	5.75
	EPT (%) abundance classes	14.54
	Number of Families	32.46

correlate significantly with the final EQR values. However, it appeared that higher Chl *a* concentration indicated a higher ecological quality status in these lakes. This relationship, although frankly unexpected, is actually yet another testament to the oligotrophic nature of these karst lakes, where we conclude that small rates of eutrophication promote macroinvertebrate community diversity (also discussed in the case of the ultra-oligotrophic lake Vrana - HR-J_2).

Despite the great geological peculiarities and habitat heterogeneity of the karst lake systems in Croatia, the fish community of this region is naturally relatively species-poor, with the ichthyofauna in lake types HR-J_1A and HR-J_1B even being described as "A Wealth of Simplicity" (Buj et al. 2023). In general, the increased fish biomass is associated with allochthonous, more gamefish-like species, which have a negative impact on the lake ecological quality status, as was also proven in this research where a statistically significant negative relationship was found between fish biomass and final EQR values.

Finally, it is very important to point out that for these ecosystems, which have a very narrow gradient of environmental factors governing the macroinvertebrate community, it is extremely important to identify the invertebrates at lowest possible level (preferred species and genus), as different species of the same genus may have different preferences (as shown in Dorić et al. 2020). In these lakes, coarse taxonomic identification would lead to a false

Table 7 Final EQR values and ecological status of 32 sampled sites (49 samples) in eight lakes across seven Croatian lake types

Lake/Site/Year	Lake type	Final EQR value	Lake status
Kozjak/1/2018	HR-J_1A	1.00	High
Kozjak/1/2019	HR-J_1A	1.16	High
Kozjak/2/2018	HR-J_1A	1.17	High
Kozjak/4/2019	HR-J_1A	1.32	High
Kozjak/3/2018	HR-J_1A	1.28	High
Kozjak/5/2019	HR-J_1A	1.24	High
Kozjak/4/2018	HR-J_1A	1.23	High
Kozjak/6/2019	HR-J_1A	1.17	High
Kozjak/5/2018	HR-J_1A	1.28	High
Kozjak/7/2019	HR-J_1A	1.25	High
Prošće/1/2018	HR-J_1B	0.90	High
Prošće/1/2019	HR-J_1B	1.11	High
Prošće/2/2018	HR-J_1B	1.12	High
Prošće/2/2019	HR-J_1B	1.21	High
Prošće/3/2018	HR-J_1B	1.19	High
Prošće/3/2019	HR-J_1B	1.47	High
Prošće/4/2018	HR-J_1B	0.87	High
Prošće/5/2019	HR-J_1B	1.42	High
Prošće/5/2018	HR-J_1B	0.91	High
Prošće/6/2019	HR-J_1B	1.40	High
Vransko Cres/2/2019	HR-J_2	0.91	High
Vransko Cres/1/2018	HR-J_2	0.81	High
Vransko Cres/1/2019	HR-J_2	1.01	High
Vransko Cres/2/2018	HR-J_2	0.78	Good
Vransko Cres/3/2018	HR-J_2	0.82	Good
Vransko Cres/3/2019	HR-J_2	1.10	High
Crniševo/1/2018	HR-J_3	1.00	High
Crniševo/1/2019	HR-J_3	1.14	High
Crniševo/2/2018	HR-J_3	0.94	High
Oćuša/2/2019	HR-J_3	0.91	High
Oćuša/3/2019	HR-J_3	0.78	Good
Oćuša/1/2018	HR-J_3	0.90	High
Vransko Biograd/1/2018	HR-J_4	0.46	Moderate
Vransko Biograd/1/2019	HR-J_4	0.56	Moderate
Vransko Biograd/2/2018	HR-J_4	0.48	Moderate
Vransko Biograd/3/2019	HR-J_4	0.35	Poor
Vransko Biograd/3/2018	HR-J_4	0.84	High
Vransko Biograd/4/2019	HR-J_4	0.56	Moderate
Visovac/1/2018	HR-J_5	1.09	High
Visovac/3/2019	HR-J_5	1.08	High
Visovac/2/2018	HR-J_5	1.06	High
Visovac/4/2019	HR-J_5	0.99	High
Kuti/1/05-2022	HR-J_6	0.73	Good
Kuti/1/09-2022	HR-J_6	0.58	Moderate
Kuti/1/2023	HR-J_6	0.66	Good
Kuti/2/05-2022	HR-J_6	0.64	Good
Kuti/2/09-2022	HR-J_6	0.74	Good

Table 7 (continued)

Lake/Site/Year	Lake type	Final EQR value	Lake status
Kuti/2/2023	HR-J_6	0.87	High
Kuti/3/2023	HR-J_6	0.84	High

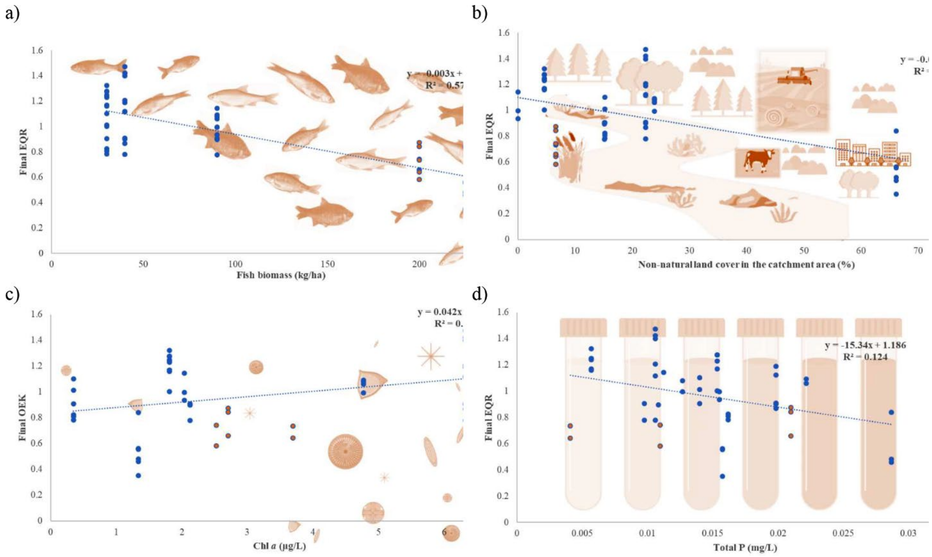


Fig. 3 Significant pressure-response relationships between the macroinvertebrate-based assessment method, i.e., the final ecological quality ratios (EQRs) against **a**) Fish biomass (kg/ha); **b**) Non-natural land cover (NNLC; %); **c**) Chlorophyll *a* concentration (chl *a*; µg/L); and d) Total phosphorus concentration (Total P; mg/L) in Croatian lakes. The samples of the subsequently added lake type (HR-J_6) are colored orange. All portrayed relationships are statistically significant

projection of low local diversity and, consequently, to a biased assessment of ecological quality. All the metrics we selected are highly dependent on high taxonomic resolution (% Chironomini; Diversity Margalef index; EPT (%) abundance classes; Number of families) and it is likely that the results would not reflect the true state of the lakes if a more robust determination was applied in this quality assessment method. We strongly recommend that future bioassessments focus both on the training of new taxonomists as well as the implementation of DNA barcoding and eDNA-based identification in order to obtain the most accurate bioassessments possible. This is because, as Ntislidou et al. (2023) noted, close collaboration between the two methods is still necessary, as all eDNA data still require a "classical taxonomist" for interpretation.

5 Conclusions

This study makes an important contribution to the assessment of water quality by considering the complexity of the of Dinaric karst lakes, which are characterized by their geological specificity and ecological importance. The developed regression model for macroinvertebrate metrics provides a holistic approach to assess the ecological status of these lakes, considering diverse types within the region. The results emphasize the importance of including specific metrics and understanding pressure-response relationships for an accurate assessment of water quality. The vulnerability of these lakes to climate change, particularly in terms of changing precipitation patterns and more frequent droughts, can have a significant impact on water quality and macroinvertebrate communities, so a customized assessment system for each lake type is a crucial steppingstone for future climate change mitigation efforts. The implications of the study extend to the broader context of freshwater ecosystem management, which faces similar challenges worldwide, and emphasize the need for tailored assessment models and continuous monitoring to ensure the maintenance of biodiversity and ecological balance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40710-024-00723-5.

Acknowledgements Hrvatske vode are thanked for funding and for providing land use and water physicochemical data. We would also like to thank Mirjana Dimnjaković and the many students who helped in sorting the material.

Author Contributions I.P and Z.M. did the manuscript conceptualization and statistical analysis. I.P. wrote the main manuscript text and M.Š. prepared figures. All authors were par of the field and lab work and invertebrate identification. All authors reviewed the manuscript.

Funding This research was funded by Croatian Waters (Hrvatske vode).

Data Availability Data is provided within the manuscript or supplementary information.

Declarations

Ethical Approval Non-applicable.

Consent to Participate Non-applicable.

Consent to Publish Non-applicable.

Competing Interests The authors declare no competing interests.

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